X-ray Microtomography Study of Carious Dentine and a Comparison of its Removal by Three Techniques

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Thesis submitted for the Degree of Doctor of Philosophy, Faculty of Science

July 2010
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I also declare that I have not used extensive quotations or close paraphrasing and that I have neither copied from the work of another person, nor used the ideas of another person, without proper acknowledgement.

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Title of Work submitted:
X-RAY MICROTMOMOGRAPHY STUDY OF CARIOUS DENTINE AND A COMPARISON OF ITS REMOVAL BY THREE TECHNIQUES

Examination: A thesis submitted for the degree of Doctor of Philosophy, University of London.

Signature:               Date:
- The Opening -

In the name of Allah, the Most Gracious, the Most Merciful

Praise be to Allah, The Lord of the Universe

The Most Gracious, The Most Merciful

King of the Day of Judgement

You alone we worship and You alone we ask for help

Guide us to the straight Way;

The way of those whom you have blessed, not of those who have incurred your anger,

Nor of those who have gone astray.

Amen

- For Mummy and Daddy -
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Abstract

The wisdom on mechanical removal of carious dentine based on hardness has been challenged and chemo-mechanical technique has been proposed as a more conservative method. However, the extent and comparison of excessive removal of sound dentine and insufficient removal of carious dentine have not been studied. The aims of the present study are to use X-ray microtomography (XMT) to determine the mineral concentrations of sound and carious dentine, and the excavated dentine using a hand excavation (HE) technique, a hand excavation technique aided by Caries Detector Dye (CD) and a chemo-mechanical removal technique using Carisolv (CSS). Comparison of the three techniques with respect to the mineral concentrations of the excavated dentine caries and the volumes of sound dentine removed were investigated. It was aimed to identify the boundary between “infected” and “affected” using the X-ray linear attenuation coefficients (LAC) from the XMT results and the ultrastructural images obtained from the Back Scattered Electron (BSE) imaging and Atomic Force Microscopy (AFM).

Thirty eight deciduous molars with open carious cavities were sectioned in half. One half of each molar had the carious tissue removed by HE and the other by CSS or hand excavation plus CD. XMT images were taken before and after caries removal. From the data set, an assessor, who was ‘blind’ to which technique was used, used LAC histograms to assess the efficacy of the excavation techniques. The volumes of sound dentine removed by the 3 techniques were calculated and compared. Detailed analyses were carried out using XMT slices to investigate the mineral concentration of removed and residual dentine. Remineralisation experiments of residual dentine were performed
after caries removal. Backscattered electron (BSE) microscopy and atomic force microscopy (AFM) were used to investigate the ultrastructure of the carious dentine.

The results showed that CSS was a better technique; conforming to the principles of minimally invasive dentistry. Volume analyses showed that both CSS and CD were effective in removing less sound dentine than conventional hand excavation. It was shown for the first time that the partially demineralised layer of dentine in a natural carious lesion, which was maintained by the CSS technique, had the potential to remineralise up to 80% of the mineral level for sound dentine. Combining XMT results with AFM and BSE images, ultrastructural changes were found at the boundary around a LAC value of 0.8 cm$^{-1}$ which corresponded to a KHN of 7.66 kgmm$^{-2}$.

It was concluded that carious dentine removal up to a hardness level of 7.66 kgmm$^{-2}$ could be recommended in order to preserve dentine that has potential to remineralise.
Table of contents

Acknowledgements ..................................................................................................................... 4
Abstract ......................................................................................................................................... 6
Table of Contents ........................................................................................................................... 8
List of Figures ................................................................................................................................. 13
List of Tables ................................................................................................................................ 21
List of Abbreviations ........................................................................................................................ 22

1 Background and Introduction ................................................................................................... 23
Part I: Literature Review and Aims ............................................................................................... 26

2 Dentine ...................................................................................................................................... 26

2.1. Dentine overview .................................................................................................................. 26
2.2. Dentine-pulp complex ............................................................................................................. 27
2.3. Structure and Physiology of Dentine ...................................................................................... 28

2.3.1. Dentine tubules .................................................................................................................. 28
2.3.2. Intratubular/Peritubular dentine ......................................................................................... 29
2.3.3. Sclerotic/translucent dentine ............................................................................................. 31
2.3.4. Dead tracts ......................................................................................................................... 31
2.3.5. Intertubular dentine ........................................................................................................... 32

2.4. Dentinogenesis ....................................................................................................................... 32
2.5. Mineralisation in dentine ......................................................................................................... 33

3 Dentine caries ............................................................................................................................ 35

3.1. Structure and morphology of dentine caries ......................................................................... 35

3.1.1. Mineral dissolution ............................................................................................................. 35
3.1.2. Degradation of collagen .................................................................................................... 36
3.1.3. Dentine caries process ....................................................................................................... 37

3.2. Microbiology ......................................................................................................................... 39

3.2.1. Bacteria and Adherence ................................................................................................... 39
3.2.2. Acid production ................................................................................................................ 40

3.3. Remineralisation of affected carious dentine ......................................................................... 41

4 Minimally Invasive Dentistry .................................................................................................... 42

4.1. Pulpal response to dentine caries .......................................................................................... 43
4.2. Rationale and Treatment approach to Minimally Invasive Dentistry .................................. 43

4.2.1. Stepwise excavation of dentine caries ................................................................................ 43
4.2.2. Complete vs. partial removal of carious dentine before restoration ................................. 44

5 Indicators of caries ...................................................................................................................... 45

5.1. Hardness ................................................................................................................................ 46
5.2. Caries detector dye ................................................................................................................ 48
5.3. Colour ..................................................................................................................................... 49
5.4. Autofluorescence .................................................................................................................... 50
5.5. Bacterial analysis ...................................................................................................................... 51

6 Caries Excavation ...................................................................................................................... 52

6.1. Mechanical carious tissue removal techniques ....................................................................... 52

6.1.1. Excavators, handpieces and burs ...................................................................................... 52
6.1.2. Air abrasion ....................................................................................................................... 52
Part II: XMT study of dentine caries and its removal by three techniques

7 X-ray microscopy ..................................................................................... 63
  7.1. X-rays .......................................................................................... 63
    7.1.1. Introduction ........................................................................... 63
    7.1.2. Nature of X-rays .................................................................. 64
    7.1.3. X-ray production .................................................................. 65
  7.2. X-ray interactions with matter ......................................................... 70
    7.2.1. Attenuation mechanisms ......................................................... 70
  7.3. Factors affecting the X-ray spectrum ............................................. 72
  7.4. Beer’s law .................................................................................... 74
  7.5. Attenuation coefficients .................................................................. 74
    7.5.1. Linear attenuation coefficients (LAC) ..................................... 74
    7.5.2. Mass attenuation coefficients ............................................... 75
  7.6. X-ray beam attenuation .................................................................. 76
    7.6.1. Beam hardening .................................................................... 76
  7.7. Transmitted X-ray beam and subject contrast .................................. 77
    7.7.1. Image geometry .................................................................... 77
    7.7.2. Subject contrast .................................................................... 78
  7.8. X-ray detectors .............................................................................. 78
    7.8.1. Characteristics of X-ray detectors .......................................... 78
    7.8.2. Analogue X-ray detectors ...................................................... 79
    7.8.3. Digital X-ray detectors .......................................................... 79
    7.8.4. Dynamic range ...................................................................... 81
    7.8.5. Image quality and accuracy .................................................... 81

8 X-ray Tomography .................................................................................. 82
  8.1. Introduction .................................................................................... 82
  8.2. Sectional Imaging: X-ray CT .......................................................... 83
  8.3. Development of X-ray Microtomography ...................................... 86
  8.4. Ring Artefacts ................................................................................ 86
  8.5. Advantages of using XMT ............................................................. 87
  8.6. Comparison of MuCat system and the commercial XMT systems ..... 88
  8.7. Applications of XMT ................................................................. 88
  8.8. XMT studies of mineralised tissues .............................................. 89

9 General Aims and Objectives ................................................................ 91
Part II: XMT study of dentine caries and its removal by three techniques .... 94

10 Comparison of XMT systems ............................................................. 94
  10.1. MuCat XMT system .................................................................... 94
      10.1.1. Description of MuCat XMT system ..................................... 94
11.8.4. Discussion ................................................................. 169
11.9. Remineralisation study of teeth after caries removal .................. 170
   11.9.1. Introduction and Aims .............................................. 170
   11.9.2. Materials and Methods ........................................... 170
   11.9.3. Results ............................................................... 171
   11.9.4. Discussion ......................................................... 185
11.10. XMT and Colour of the carious dentine lesion ........................ 187
   11.10.1. Introduction and Aims ............................................ 187
   11.10.2. Materials and Methods ......................................... 187
   11.10.3. Results ............................................................ 188
   11.10.4. Discussion ....................................................... 207
11.11. Conclusions ................................................................ 209

**Part III: Correlation of XMT with other techniques** .......................... 211

12 **Backscattered Electron Microscopy** ........................................... 211
   12.1. Introduction and Aims ................................................ 211
   12.2. Materials and Methods ................................................ 214
       12.2.1. Sample preparation .............................................. 214
       12.2.2. BSE imaging technique .......................................... 214
   12.3. Results ..................................................................... 216
       12.3.1. Sample 1 - LB sample ........................................... 216
       12.3.2. Sample 2 – AB sample ........................................... 230
       12.3.3. Decaying constants .............................................. 230
   12.4. Discussion .................................................................. 239
   12.5. Conclusions .................................................................. 241

13 **Atomic Force Microscopy (AFM)** ............................................. 242
   13.1. Introduction .................................................................. 242
       13.1.1. AFM – Theory and Background .................................. 242
       13.1.2. AFM components .................................................. 247
       13.1.3. Images acquired ................................................... 250
       13.1.4. The Force-distance curve ....................................... 252
       13.1.5. AFM studies on dentine ......................................... 253
   13.2. Investigation of natural carious dentine lesion with AFM ........... 254
       13.2.1. Investigation using Veeco Explorer ............................. 254
       13.2.2. Investigation with Veeco Dimension 3100 .................... 262
       13.2.3. Investigation using Photothermal Microspectroscopy (PTMS) 265
       13.2.4. Effect of Carisolv solution on collagen ....................... 273
       13.2.5. AFM images of the collagen sheet ............................. 274
       13.2.6. Discussion of AFM studies ...................................... 276
       13.2.7. Conclusions of AFM studies .................................... 278

**General Conclusions** .................................................................. 278

**Future Work** ........................................................................ 279

**References** ............................................................................. 280

**Appendices** ............................................................................ 296

**Appendix A – Ethics Approval** .................................................. 297

**Appendix B – Basic results** ....................................................... 298
Appendix C – Presentations and Publications……………………………………………………302
Appendix D – CD containing pdf file of images and graphs for each dataset (at the back)
List of Figures

Figure 2-1 Schematic diagram showing the microstructure of the dentine in the tooth [from SmartBur website, www.smartburs.com, accessed March 2005]..........................28

Figure 2-2 AFM image of dentine showing highly mineralised intratubular dentine......29

Figure 2-3 Schematic diagram showing the pre-eruptive (A), post eruptive (B) and late (C) stages in the deposition of coronal dentine.................................................................33

Figure 7-1 The EM spectrum in terms of wavelength, frequency or energy, highlighting X-ray region ..................................................................................................................64

Figure 7-2 An X-ray is a transverse electromagnetic wave, where the electric and magnetic field is perpendicular to each other and to the direction of propagation...........65

Figure 7-3 A simple diagram of an X-ray tube plus circuit ............................................66

Figure 7-4 (a) Continuous spectrum depicts a continuous range of photon energies produced by electrons being decelerated in the target. (b) Line spectrum depicts a limited number of precise characteristic photon energies.................................................68

Figure 7-5 Attenuation mechanisms (a) Photoelectric effect; (b) Simple scatter; (c) Compton scatter .................................................................................................................71

Figure 7-6 Factors affecting the X-ray spectrum. (a) Changing the tube voltage changes the X-ray spectrum; (b) Effect of tube current on the X-ray spectrum; (c) Effect of target material on the spectrum; (d) Adding a filter changes the shape of the X-ray spectrum.73

Figure 7-7 Different attenuation mechanisms at different photon energies.................75

Figure 7-8 Example of an image of a tooth sample with beam hardening and with beam hardening correction............................................................77

Figure 8-1 Evolution of geometries of X-ray CT scanners (a) First generation scanner, where the pencil X-ray beam is translated and rotated to cover the object being imaged. (b) Second generation scanner, where the diverging fan beam and detector array are translated and rotated. (c) Third generation scanner which has a fan beam source and detectors rotate together. (d) Fourth generation detector which has a rotating fan beam source and a stationary ring of detectors.........................................................85

Figure 8-2 Example of ring artefacts in XMT reconstructed images..............................87

Figure 8-3 Graph showing the LAC of 100% hydroxyapatite (HAP) and Aluminium with varying energy.................................................................89
Figure 10-1 (a) A schematic diagram of the 4th generation XMT scanner developed by Davis and Elliott, (b) MuCat system at Queen Mary
Figure 10-2 Example of a circle being imaged and how the CCD reads out the data
Figure 10-3 Time sequence showing TDI CCD read out of the captured image
Figure 10-4 Schematic diagram of bird’s eye view of XMT system showing the difference between scanning a sample (a) without and (b) with moving slits
Figure 10-5 Seven-step aluminium step wedge
Figure 10-6 New adopted aluminium step wedge to overcome scattering effects
Figure 10-7 Sample mounting of the sample for the comparison of excavation techniques experiment
Figure 10-8 SkyScan XMT scan imaged at 7.32 µm resolution. (a) greyscale, (b) colour1, (c) colour2, (d) binary
Figure 10-9 MuCat XMT scan imaged at 8.7 µm resolution. (a) greyscale, (b) colour1, (c) colour2, (d) binary
Figure 10-10 Comparison of line profiles through HAP sample using Skyscan and MuCat XMT systems
Figure 10-11 MuCat XMT image highlighting true structure (slice 157 (brightness 104, contrast 445)) (TView software from SkyScan)
Figure 10-12 SkyScan XMT image highlighting ring artefacts (slice 996, brightness 104, contrast 445) (TView software from SkyScan)
Figure 10-13 MuCat XMT image highlighting ring artefacts (slice 157)
Figure 10-14 2D greyscale XMT images of carious tooth samples imaged with (a) SkyScan, (b) MuCat system at 15 µm resolution and (c) 30 µm resolution
Figure 10-15 3D surface rendered image of carious tooth sample using the SkyScan software, CTan
Figure 10-16 Greyscale images of where the line was taken through the carious tooth sample
Figure 10-17 Line probes through the carious tooth datasets to compare the SkyScan and MuCat XMT systems. The MuCat system (purple and blue lines) can pick up subtle features such as the natural gradient of enamel and dentine, where as with the SkyScan
system, it is difficult to distinguish the natural gradient of enamel and dentine from the noise. .................................................................118

Figure 10-18 SkyScan and MuCat analysis of lineprobes through the aluminium wire 119

Figure 10-19 SkyScan images of the root of the carious tooth with the ring artefact reduction applied at (a) value 1 and (b) value 15 ..............................................................120

Figure 10-20 Effect of beam hardening correction on the SkyScan XMT image .......121

Figure 10-21 Comparison of single 2D slice of an embedded carious tooth sample imaged with MuCat and MuCat2 ..................................................................................122

Figure 10-22 Line profiles through embedded tooth sample comparing both MuCat and MuCat2 XMT systems .........................................................................................124

Figure 11-1 Sample mounting of the sample for the comparison of excavation techniques experiment ...........................................................................................................130

Figure 11-2 XMT images of a carious tooth viewed in three different planes: (a) XY plane, (b) XZ plane and (c) YZ plane .................................................................134

Figure 11-3 3D image of a carious tooth with colour scale (LAC scale) ...............135

Figure 11-4 (a) Scanned images of the two halves of a carious tooth. (b) is a photograph of the sample before caries removal and (c) is after caries removal .........................136

Figure 11-5 Histogram for the carious tooth before excavation (No. of voxels against the XMT values) ..............................................................................................................136

Figure 11-7 Histogram for before and after excavation and the difference for Section A .................................................................................................................................141

Figure 11-8 Histogram for before and after excavation and the difference for Section B .................................................................................................................................141

Figure 11-10 Difference of the before and after scans ........................................144

Figure 11-11 Difference of the before and after scans using the more accurate alignment program ..............................................................................................................145

Figure 11-12 Mineral content contours superimposed on the image of the removed tissue .......................................................................................................................146

Figure 11-13 LAC histogram after subtraction of the ‘before’ and ‘after images .......146
Figure 11-14 Line probe through the carious lesion (med 1)..........................148

Figure 11-15 Line probe plot before and after excavation for Section A ............149

Figure 11-16 Line profile plot before and after excavation for Section B (med 18) ....149

Figure 11-17 LAC histograms of a caries free tooth (Sample med 47).................153

Figure 11-18 Leaf and stem plot showing the volume of sound dentine removed by various technique. .................................................................156

Figure 11-19 Scatter plot to show the relationship between volume of the carious lesion and amount of sound dentine removed according to removal techniques ..........157

Figure 11-20 Sample med 46 before and after application of Carisolv (with and without scraping using instruments), Caries Detector dye and 1% sodium hypochlorite........168

Figure 11-21 Subtracted image of sample med 46 highlighting misalignment artifacts (Slice 94).................................................................168

Figure 11-22 Speckled subtraction image of sample med 46 indicating the effects of chemicals: Carisolv (with and without scraping using instruments), Caries Detector dye and 1% sodium hypochlorite (Slice 114) .........................................................169

Figure 11-23 Comparison of LAC histogram for water and remineralising solution....172

Figure 11-24 Remineralisation experiment for sample med 28: (a) 2D grayscale image of sample med 28 before immersing in remineralising solution (slice 114). (b) 2D grayscale image of sample med 28 after immersing in remineralising solution. (c) 2D grayscale image of subtracted dataset to illustrate addition of material. (d) Colour map of LAC of added material of the subtracted dataset..................................................174

Figure 11-25 Line profile through the “remineralised” region ‘A’ in the subtracted image of Figure 10.24(c).................................................................175

Figure 11-26 Line profiles through region excavated with Carisolv (region ‘A’) at 0 and 12 weeks.................................................................176

Figure 11-27 Line profiles through region excavated with HE (region ‘B’) at 0 and 12 weeks in remineralising solution........................................177

Figure 11-28 The rate of mineral accumulated over the 12 weeks for sample med 28 .178

Figure 11-29 Line profile through dentine caries (region ‘C’) at 0 and 12 weeks in remineralising solution.........................................................179
Figure 11-30 Line profile through enamel region at 0 and 12 weeks in remineralising solution

Figure 11-31 2D greyscale images (slice 141) of sample med 40 before and after immersion in remineralising solution with corresponding subtraction images

Figure 11-32 Line profile through tooth before and after placing in remineralising solution for section A (0 and 8 weeks), which had caries removed by Carisolv technique

Figure 11-33 Line probe through tooth before and after placing in remineralising solution for section B (0 and 8 weeks), which had caries removed by Carisolv hand instruments only

Figure 11-34 Optical image of med 39 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B

Figure 11-35 XMT and optical line profile through carious lesion for med 39 A with corresponding exponential decay curve fit

Figure 11-36 XMT and optical line profile through carious lesion for med 39 B with corresponding exponential decay curve fit

Figure 11-37 Optical image of med 31 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B

Figure 11-38 Line profile through carious lesion for med 31 A with corresponding exponential decay curve fit

Figure 11-39 Line profile through carious lesion for med 31 B with corresponding exponential decay curve fit

Figure 11-40 Optical image of med 10 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B

Figure 11-41 XMT and optical colour line profile through carious lesion for med 10 A with corresponding exponential decay curve fit

Figure 11-42 XMT and optical colour line profile through carious lesion for med 10 B with corresponding exponential decay curve fit

Figure 12-1 Scanned colour image of PMMA embedded carious tooth sample LB using a flatbed scanner. The tooth sample consisted of two lesions which were analysed using BSE and XMT. The imaging were divided into two regions – Region 1 and Region 2.
Figure 12-2 BSE image of PMMA embedded carious dentine lesion (Sample LB – region 1) at 33x magnification with corresponding XMT image at 15 µm resolution. In the BSE image the dentine lesion consists of three layers: Blue, green and yellow. Line probe was obtained from A to B which is shown in Figure 12.3

Figure 12-3 Line profiles (A to B) obtained from the XMT and BSE images for sample LB – region 1 (See Figure 12.2). The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine from the XMT study (Chapter 11)

Figure 12-4 BSE images of Sample LB – region 1. (A) Image obtained at 33x magnification (2700µm x 2700µm) – taken at the periphery of the dentine carious lesion. (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation (Distance from Figure 12.3 was 0.20mm). At the periphery of the lesion, the infected structure has a surface layer which is highly mineralised. (C) Image obtained at 500x magnification (180µm x 180µm) where the dentinal tubules are filled with highly mineralised material.

Figure 12-5 Colour image of sample LB – region 2 (A) with BSE image (B) and corresponding XMT grayscale and false colour images (C and D respectively) (Dimensions 307, 165, slice 308). Line profiles a, b and c are shown Figures 13.6, 13.8 and 13.10

Figure 12-6 Line profiles (A to B) obtained from the XMT and BSE images for position a in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine

Figure 12-7 BSE images of Sample LB – region a in region 2. (A) Image obtained at 33x magnification (2700µm x 2700µm). (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation. (Distance from Figure 12.6 was 0.30mm) (C) Image obtained at 500x magnification (180µm x 180µm) showing collapsed dentine structure and also filled dentinal tubules.

Figure 12-8 Line profiles (A to B) obtained from the XMT and BSE images for position b in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine

Figure 12-10 Line profiles (A to B) obtained from the XMT and BSE images for position c in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine

Figure 12-11 BSE images of Sample LB – (A) region c in region 2 obtained at 33x (2700µm x 2700µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation (Distance from Figure 12.10 was 0.35mm) and (B) image obtained at 500x magnification (180µm x 180µm).
Figure 12-12 Scanned colour image of PMMA embedded sample AB using a flatbed scanner .................................................................231

Figure 12-13 BSE images (greyscale and colour – A and B) of PMMA embedded carious dentine (Sample AB) with corresponding XMT images (C and D) showing sclerotic dentine tract clearly .................................................................232

Figure 12-14 Line profiles (A to B) obtained from the XMT and BSE images for Sample AB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine ........................................................................................................233

Figure 12-16 BSE image of sample AB obtained at 33x magnification (2700µm x 2700µm) (A) together with (B) a BSE image obtained of the sclerotic dentine tract region and adjacent to sound dentine at 200x magnification (450µm x 450µm) ........235

Figure 12-17 Decay fit to the XMT and BSE line profile plots (sample LB – region 2 (a) – Figure 12.6) ..................................................................................................................................................236

Figure 12-18 Decay fit to the XMT and BSE line profile plots (sample LB – region 2 (b) – Figure 12.8) ..................................................................................................................................................237

Figure 12-19 Decay fit to the XMT and BSE line profile plots (sample AB – Figure 12.14) ..................................................................................................................................................238

Figure 13-1 Graph showing the relationship of the Force against the tip-sample separation .............................................................................................................................................................................243

Figure 13-2 Schematic diagram showing the configuration of the AFM ...............245

Figure 13-3 A typical AFM cantilever ..........................................................................................................................247

Figure 13-4 Tripod scanner piezo configuration ..........................................................................................................249

Figure 13-5 Feedback circuit ............................................................................................................................................250

Figure 13-6 An example of a force-distance curve .........................................................................................................252

Figure 13-7 Image of Veeco Explorer ..........................................................................................................................254

Figure 13-8 (a) Schematic diagram of the where the tooth sample was cut (b) a picture of a typical tooth sample for using in the AFM ............................................................................................................255

Figure 13-9 AFM image of the dentine surface (leveling has been applied using the SPMLab software) ..................................................................................................................................................257
Figure 13-10 A topographic AFM image of dentine together with a 3D depiction (SPIP software) .......................................................... 258

Figure 13-11 Topographic AFM images of (a) sound and (b) carious dentine .......... 259

Figure 13-12 A topographic image of dentine with a Force distance graph ............ 259

Figure 13-13 Force modulation images together with topographic data (Example 1) - (a) Topographic, (b) Force modulation .............................................................................................................. 261

Figure 13-14 Force modulation images together with topographic data (Example 2) - (a) Topographic, (b) Force modulation. The peritubular dentine which is more stiff than the intertubular dentine appears as defined dark rings around the dentinal tubules. ....... 261

Figure 13-15 Image of Veeco Dimension 3100 ........................................................................................................................................... 262

Figure 13-16 (a) Image of longitudinal and (b) transverse sections of carious deciduous molars embedded in PMMA ............................................................................................................. 263

Figure 13-18 Schematic diagram of the photothermal FTIR micro-spectroscopy technique. The microscope is mounted inside the sample compartment of the FTIR spectrometer. The schematic diagram is together with the upper part of the Veeco Explorer ........................................................................................................................................ 266

Figure 13-19 IR spectrometer – part of the PTMS configuration .......................... 267

Figure 13-20 (a) Schematic diagram of thermal probe made from Wollaston process wire, (b) Image of thermal probe ............................................................................................................. 269

Figure 13-21 PTMS image of dentine .............................................................................................................................. 271

Figure 13-22 Zoom in PTMS image focusing on the dentinal tubules with corresponding 3D topographic image of the same region ....................................................................................... 271

Figure 13-23 Schematic diagram of dentine cut perpendicular to the dentinal tubules emphasis on the collagen contribution to the dentine composite ................................................................. 272

Figure 13-24 Digital image of holes formed by using thermal probe ................. 274

Figure 13-25 3D depiction of the hole before application of Carisolv .................. 275

Figure 13-26 Comparison of ‘hole’ before and after Carisolv application. Inset shows a zoom in image (5 µm x 5 µm) ............................................................................................................. 276
List of Tables

Table 7-1 Comparison of factors affecting the X-ray spectrum ........................................73
Table 11-1 Results from the 1\textsuperscript{st} analysis in judging the ‘ideal’ technique .......................151
Table 11-2 Results from the 2\textsuperscript{nd} analysis in judging the ‘ideal’ technique .......................151
Table 11-3 Amount of sound dentine removed during excavation with indication whether pulp was involved ..........................................................154
Table 11-4 Clinical and XMT description of carious dentine lesions clinically.............191
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACP</td>
<td>amorphous calcium phosphate</td>
</tr>
<tr>
<td>ADC</td>
<td>analogue-to-digital converter</td>
</tr>
<tr>
<td>AF</td>
<td>Autofluorescence</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscopy</td>
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<tr>
<td>Al</td>
<td>Aluminium</td>
</tr>
<tr>
<td>BSE</td>
<td>Backscattered Electron</td>
</tr>
<tr>
<td>Ca</td>
<td>Calcium</td>
</tr>
<tr>
<td>CCD</td>
<td>Charge Coupled Detector</td>
</tr>
<tr>
<td>CD</td>
<td>Caries Detector Dye</td>
</tr>
<tr>
<td>CE</td>
<td>Conventional excavation</td>
</tr>
<tr>
<td>CsI</td>
<td>Cesium Iodide</td>
</tr>
<tr>
<td>CSS</td>
<td>Carisolv</td>
</tr>
<tr>
<td>CT/CAT</td>
<td>Computed (axial) Tomography</td>
</tr>
<tr>
<td>d</td>
<td>cantilever’s motion</td>
</tr>
<tr>
<td>$D_i$</td>
<td>initial diameter of the dentinal tubule</td>
</tr>
<tr>
<td>$D_o$</td>
<td>observed diameter of the dentine tubule</td>
</tr>
<tr>
<td>DEJ</td>
<td>Dentine-enamel junction</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>Maximum energy</td>
</tr>
<tr>
<td>EFM</td>
<td>Electric Force Microscopy</td>
</tr>
<tr>
<td>EM</td>
<td>Electromagnetic</td>
</tr>
<tr>
<td>Er:YAG</td>
<td>Erbium doped Yttrium Aluminum Garnet</td>
</tr>
<tr>
<td>Er, Cr:YSGG</td>
<td>Erbium, Chromium doped Yttrium Scandium Gallium Garnet</td>
</tr>
</tbody>
</table>
F                 Force
FACE             Fluorescence assisted caries excavation
FW               Prof. Ferranti Wong
HA/HAP           Hydroxyapatite (unit-cell formula Ca$_{10}$(PO$_4$)$_6$(OH)$_2$)
HD               High definition
HED              Hand excavation/Caries detector dye
HES              Hand excavation/Carisolv
I                Transmitted Intensity
I$_0$            Initial intensity
IAP              Ionic activity product
k                Spring constant
K$_{sp}$          Solubility product
K$_{sp}$(HAP)     Solubility product for HAP
KHN              Knoop Hardness Number
LFM              Lateral Force Microscopy
MFM              Magnetic Force Microscopy
MMA              Methylmethacrylate
N                Sample diameter
NMG, GK101       N-monochloroglycine
NMAB, GK101E     N-monochloro-D,L-2-aminobutyrate
Pb               Lead
PID              Proportional, Integral and Derivative gain
PO$_4$           Phosphate
<table>
<thead>
<tr>
<th><strong>Abbreviation</strong></th>
<th><strong>Full Form</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
</tr>
<tr>
<td>PTMS</td>
<td>Photothermal Microspectroscopy</td>
</tr>
<tr>
<td>PTR</td>
<td>Photothermal Radiometry</td>
</tr>
<tr>
<td>rpm</td>
<td>revolutions per minute</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
</tr>
<tr>
<td>SPM</td>
<td>Scanning Probe Microscopy</td>
</tr>
<tr>
<td>t</td>
<td>traverse time</td>
</tr>
<tr>
<td>t_x</td>
<td>exposure time</td>
</tr>
<tr>
<td>TDI</td>
<td>Time Delay Integration</td>
</tr>
<tr>
<td>UMIS</td>
<td>Ultra-Micro Indentation System</td>
</tr>
<tr>
<td>W_T</td>
<td>width of the intratubular dentine</td>
</tr>
<tr>
<td>(x)</td>
<td>thickness of the medium/length of the sample</td>
</tr>
<tr>
<td>XMT</td>
<td>X-ray Microtomography</td>
</tr>
<tr>
<td>Z</td>
<td>Atomic number</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>wavelength</td>
</tr>
<tr>
<td>(\mu/LAC)</td>
<td>Linear attenuation coefficient</td>
</tr>
<tr>
<td>(\mu_m/MAC)</td>
<td>Mass attenuation coefficient</td>
</tr>
<tr>
<td>(\rho)</td>
<td>density</td>
</tr>
</tbody>
</table>
1 Background and Introduction

Dental caries is a global health problem in both industrialised and developing countries. An estimated five billion people worldwide have experienced dental caries [Petersen, 2003; Porter, 2004]. Literature on dental and oral infections goes back to the ancient Romans, Greeks and Egyptians [Ismail, 2001]. However it was only in the late 19th and 20th centuries that the field of dentistry was revolutionised, G. V. Black, father of modern operative dentistry, combined his clinical skills with experimental research [Ismail, 2001]. Also during this time, the aetiology of dental caries and its progression were proposed but its details are still being researched today.

Caries is a disease that results from the action of bacteria which produce acids that dissolve the enamel and dentine of the tooth. Caries is a dynamic process in which demineralisation is followed by periods of remineralisation. However, if demineralisation is predominant, there will be a net loss of mineral from the tooth surface; the tooth tissue begins to dissolve. This net mineral loss results in incipient lesions (white spot lesions). Without intervention the lesion continues to progress, and eventually it will extend into the dentine, and possibly the pulp. Treatment of caries lesions are based on G. V. Black’s principles, one of which is ‘extension for prevention’ [Banerjee et al., 2000d]. G. V. Black suggested that it was necessary to remove all trace of demineralised enamel and dentine from the floor, walls and margins of the cavity [Mount and Hume, 2005]. This concept of treatment is now regarded as too invasive and destructive.
New scientific developments in cariology, dental materials and diagnostic systems have changed dentistry’s approach to the diagnosis and management of dental caries towards Minimally Invasive Dentistry, where the aim is to try to remove as little tooth tissue as necessary [Burke, 2003]. Despite efforts to implement this, the application of Minimally Invasive Dentistry to dentine caries is still very subjective. This subjectivity results in either removing too much healthy tissue leading to unstable tooth structure, or too little unhealthy tissue removal leading to recurrent caries. Therefore, in order to remove dentine caries effectively, the basic structural elements of sound and carious dentine, their distribution and properties need to be known for the excavation procedure. This thesis will address the problems of determining carious dentine and compare the extent of its removal by three different techniques. The techniques used for studying dentine caries, and the attempt of correlating other techniques with XMT will be discussed within each experimental technique chapter.
Part I

Literature Review
Part I: Literature Review and Aims

2 Dentine

Introduction

Caries is a dynamic process which is fairly well understood in terms of its aetiology, and the literature on caries is extensive. However, specifically to dentine caries, especially surrounding the excavation procedure, clinical dentistry has failed to develop in accordance with the science. Even with the current “biological” approach, the excavation procedure is still a subjective process which relies on the clinician’s judgement to remove the correct amount of carious dentine in order to preserve the tooth’s integrity. Hence it is necessary to develop an objective marker for carious dentine to help the clinician to adhere to the concept of Minimally Invasive Dentistry. In order to achieve this aim, firstly, the structure of sound dentine and its related properties, as well as how these are changed/altered by caries will be reviewed. Also the current tissue removal techniques will be discussed in this literature review.

2.1. Dentine overview

The following review of dentine structure is based on Chapter 13 – Dentine and Pulp in the book Illustrated Dental Embryology, Histology and Anatomy [Bath-Balogh and Fehrenbach, 1997].

Dentine is a living, hard tissue that makes the bulk of the tooth. The composition of dentine is: ~ 50% vol. mineral, ~ 30% vol. organic material and the rest is fluid, (respectively 70, 20 and 10 wt %). Structurally, it is a complex hydrated composite consisting of four structural features: (1) oriented tubules surrounded by (2) a highly
mineralised peritubular zone embedded in an intertubular matrix consisting of (3) mainly Type I collagen with embedded apatite crystals, and (4) dentinal fluid [Marshall, Jr., 1993]. With regard to mechanical properties, dentine can be viewed as a two phase system, a mineral phase and an organic phase. Like most biological hard tissues, the mineral phase consists of an impure form of hydroxyapatite (HAP), with a unit-cell formula of Ca\(_{10}\)(PO\(_4\))\(_6\)(OH)\(_2\). It is less mineralised than enamel (~95% vol. mineral). The size of the apatite crystal for dentine (30 Å wide, 200-1000 Å long) is also smaller than that of enamel (400 Å wide on average, 1600-10,000 Å long) [Scott and Symons, 1982].

The organic phase consists mostly of collagen and proteins such as proteoglycans, phosphoproteins and phospholipids. 90% of the total organic dentine matrix is composed of collagen, primarily of Type I collagen. This organic matrix sets a framework for mineralisation. Therefore the resilient dentine (lower elastic modulus) mechanically supports the overlying brittle enamel.

2.2. Dentine-pulp complex

Coronal dentine is protected by enamel from the oral surroundings. Root dentine is covered by cementum which anchors the tooth to the alveolar bone via connective tissue fibres. Whilst enamel has an ectodermal origin, dentine and the pulp develop from the dental papilla, i.e. they are of mesenchymal origin. The close relationship of these two tissues starts from the prenatal development stage and are interdependent to form what is known as the ‘dentine-pulp complex’. Dentine and pulp must be considered as one functioning unit, so if dentine is affected by pathological factors, both tissues are vulnerable; but the same living unit can react to external stimulus to provide a defence mechanism.
2.3. Structure and Physiology of Dentine

2.3.1. Dentine tubules

Odontoblast cells line the pulp periphery. These cells retreat towards the pulp, as dentine is laid down. Each odontoblast leaves behind a long cytoplasmic process known as the odontoblastic process. Each process then becomes embedded in the dentine matrix forming dentinal tubules (Figure 2.1).

![Dentine Tubules](image)

**Figure 2-1 Schematic diagram showing the microstructure of the dentine in the tooth** [from SmartBur website, www.smartburs.com, accessed March 2005]

These tubules connect the dentine to the pulp and neural plexus, hence giving dentine its sensitive nature. In dental textbooks, images of dentine tubules show a lot of complex structure and this differs depending on the orientation in the dentine tissue. They are not smooth, uniform tubes, they are S shaped and the tubules have irregular walls with many lateral branches which connect neighbouring tubules [Mjor and Nordahl, 1996]. The diameter of the tubules is larger near the pulp (2.5 µm) and gets smaller (900 nm) approaching the dentine-enamel junction (DEJ). The number density of the tubules also
varies with location. In the coronal part of a molar tooth, the values are between 59,000 and 76,000 per mm$^2$ near the pulp. Approaching the DEJ, the number density is halved.

The dentinal tubules are lined by an organic sheath called the lamina limitans, and the tubules themselves consist of the odontoblast process and dentinal fluid. All these components together not only make dentine highly permeable but they work in a complex network in connection with the pulp to respond to external stimuli e.g. against caries attack.

2.3.2. Intratubular/Peritubular dentine

Transverse dentine sections show a hypermineralised ring around the tubule known as peritubular dentine. Imaging techniques such as electron and light microscopy have illustrated this ring. A beautiful depiction of the peritubular dentine was obtained by Marshall et al. [1997b] using the atomic force microscope (Figure 2.2).

![AFM image of dentine](image)

**Figure 2-2** AFM image of dentine [Marshall, Jr. et al., 1997b; Kinney et al., 2003b] showing highly mineralised intratubular dentine
Despite its name, this ring of dentine is inside the dentinal tubule. A more appropriate terminology would be intratubular dentine. This hypermineralised dentine (which may imply a higher crystalline content) is 40% more mineralised than intertubular dentine [Ten Cate and Nanci, 1994]. The width of the ring is dependent upon its location in relation to the pulp; it is the widest near the DEJ (750 nm) and is the narrowest at the pulpal end (44 nm). During dentinogenesis, the original diameter of the dentinal tubule before it has been occluded by intratubular dentine can be calculated by:

\[ D_i = D_o + 2W_I \]

Equation 2-1

where \( D_i \) = initial diameter of the dentinal tubule

\( D_o \) = observed diameter of the dentine tubule, and

\( W_I \) = width of the intratubular dentine.

Equation 2.1 shows the initial diameter of the dentinal tubule is \(~2400\) nm near the DEJ and \( 2588 \) nm near the pulp. Hence, the intratubular dentine could be accountable for the difference in the observed diameters in matured dentinal tubules between the DEJ and the pulp regions.

In deciduous teeth, the intratubular dentine is hardly present, but with age the intratubular dentine becomes more well-defined in permanent teeth. This indicates that intratubular dentine requires a long time to be deposited and its mineralisation continues throughout its life until it reaches its maximum potential. However it can be argued that the formation of intertubular dentine is due to purely odontoblast activity or it has been suggested that the intratubular dentine is a result of physiochemical reactions involving
redistribution of material (Ca, PO$_4$) of the oral environment fluid [Ten Cate and Nanci, 1994].

2.3.3. Sclerotic/translucent dentine

This type of dentine can be found around the apical region related to ageing processes [Solheim, 1989] or under a carious lesion [Johnson et al., 1969; Levine, 1974]. The area of dentine appears translucent when the section is viewed by transmitted light and dark with reflected light. It is an optical phenomenon due to intratubular mineral deposition occluding the dentinal tubules. As a result, the dentine is more homogeneous and the light passing through this region is less scattered [Kidd et al., 2003]. Its function was believed to be a pulpal response to mild stimulus (e.g. slowly progressive carious lesion). However, it has been shown that translucent dentine can also be formed by physiochemical process without cellular involment [Daculsi et al., 1987; Shellis, 1994].

2.3.4. Dead tracts

Dead tracts are areas of dentine which appear to be black when the specimen is viewed in transmitted light and white in reflected light [Scott and Symons, 1982]. The optical phenomenon is due to empty dentinal tubules. When the odontoblastic process is completely destroyed by strong stimuli (e.g. rapidly progressive carious lesion). A layer of irregular secondary dentine is usually found at the pulp surface. Sometimes, in dried ground sections, the appearance of dead tracts are found in normal dentine because of shrinkage of the odontoblastic processes and the tubules are filled with air. A true dead tract can be distinguished by the presence of the irregular dentine at its pulp end.
2.3.5. Intertubular dentine

Intertubular dentine is located between the dentine tubules. This is the main secretion product of the odontoblasts during dentinogenesis. It consists of a tightly interwoven network of collagen fibrils in which apatite crystals are deposited. The fibrils are randomly arranged perpendicular to the dentinal tubules and the orientation of the long axes of the apatite crystals are parallel to the collagen fibrils. The ground substance of intertubular dentine consists of phosphoproteins, proteoglycans, $\gamma$-carboxyglutamate containing proteins, glycoproteins and some plasma proteins.

2.4. Dentinogenesis

The formation of dentine is a highly regulated and controlled course of actions in which several constituents of both cellular and extracellular nature play a role. The following is an account of the sequence of events in the formation of the coronal dentine [Symons, 1968]:

During the period of the formation of the primary dentine, intratubular dentine is first deposited as a thin layer within the dentinal tubules, and it begins to appear at the same time as the mineralisation of the intertubular dentine (A). By the time the tooth has erupted into the mouth cavity, the intratubular dentine has greatly increased in thickness in the tubules, but stops a short distance from the predentine-dentine junction at the pulpal region. After eruption at this junction a narrow zone of intertubular dentine is present which shows a marked content of polysaccharide material, including acid mucopolysaccharide. This zone tends to increase slowly in thickness, and becomes more mineralised than the earlier formed primary intertubular dentine. This zone, the later formed intertubular dentine and adjacent intratubular dentine, represents the
physiological regular secondary dentine (B). Sometimes, secondary dentine that is formed later has an irregular structure which is less mineralised, this is not related to destruction or damage to the dentine. Intratubular dentine continues to form within the physiological secondary dentine, but as a thin layer with a different organic matrix compared to that formed within the primary dentine (C).

Figure 2-3 Schematic diagram showing the pre-eruptive (A), post eruptive (B) and late (C) stages in the deposition of coronal dentine [Scott and Symons, 1982]

2.5. Mineralisation in dentine

The predentine is an approximately 10-30 µm wide unmineralised zone between the mineralised dentine and odontoblasts, where dentine mineralisation sharply occurs at a specific mineralisation front [Ten Cate and Nanci, 1994]. At the start of dentine formation, odontoblasts synthesize and secrete Type I collagen and proteoglycans and other significant constituents to the predentine layer. In predentine, collagen molecule
fibres amass together with their long axes in parallel into fibrils, which further arrange into bundles, probably under the influence of proteoglycans [Ten Cate and Nanci, 1994]. Two types of dentine are formed before tooth eruption, namely the mantle and primary dentine. The mantle dentine is an outer layer of dentine underneath the enamel. The odontoblasts secrete large collagen fibrils which are mineralised via the matrix vesicles, as this is the first dentine to be mineralised. Hydroxyapatite comes in the form of single crystals which then grow quickly and burst, spreading to form clusters of crystallites which go on to fuse with other clusters to make up the inorganic matrix.

Primary dentine, which makes up most of the tooth, is produced at a fast rate during the development of the tooth. The odontoblasts secrete fine fibred collagen which then becomes mineralised to form this dentine. Calcium ions are transported to the mineralising front by transcellular means. The associated proteins are brought together to form a mineral nucleus which starts off the formation of the apatite crystals. They bind to the collagen fibre surface which helps the fibres to bind to calcium ions, which in turn leads to mineral deposition.

After eruption, dentine remains as a living hard tissue. Throughout the life of the tooth, the odontoblasts continue to lay down dentine, known as secondary dentine, whose rate of deposition is slower in comparison to primary dentine. Structurally, secondary dentine has a less regular tubular pattern than primary dentine and staining techniques have shown that the organic matrix is slightly different for these two dentine types, which
consequently affect the mineralisation; secondary dentine is less mineralised in comparison to primary dentine.

There is a link between the mineralisation of dentine and the rate of dentine formation. In fast growing primary dentine, the hydroxyapatite crystals grow and fuse together to form the mineral matrix. This fast growth may produce areas of uncalcified material in which the large globular clusters have not fused completely. These areas are called interglobular dentine.

As shown above, there are various forms of dentine, so it should be regarded as a heterogeneous composite, having a natural gradient [Kishen et al., 2000]. As the formation of dentine varies according to the time of development, dentine research should consider the age of the tooth and its location to fully appreciate the dentine functions and its response to insults such as caries.

3 Dentine caries

3.1. Structure and morphology of dentine caries

The following is a review of the stages that take place in the dentine caries process.

3.1.1. Mineral dissolution

The dentine mineral matrix is mainly composed of HAP, and so it seems reasonable to apply the thermodynamics and kinetics of HAP dissolution to model the demineralisation of dentine. The solubility product, $K_{s(HAP)}$ of HAP is

$$
K_{s(HAP)} = \left[Ca^{2+}\right]_{eqm} \left[PO_4^{3-}\right]_{eqm} \left[OH^-\right]^{\frac{2}{3}} m_{eqm}, \text{ where } X_{eqm} = \text{ the concentration of ion X in an}
$$
equilibrium solution. When HAP is immersed in an undersaturated solution, as the ionic product is less than $K_{s(HAP)}$, the HAP will dissolve until equilibrium is achieved.

The effect of the $H^+$ ion from an acidic solution is to reduce the ionic product so that:

$$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 8H^+ \rightarrow 10\text{Ca}^{2+} + 6\text{HPO}_4^{2-} + 2\text{H}_2\text{O}$$

This model is often used to describe the demineralisation of enamel or dentine by bacterial acid in caries progression.

However, this simplified model of mineral dissolution does not take into account that dentine is a living tissue. Although dentine does not have a defence mechanism to fight against the acid attack (in terms of neutralising the acid or producing more calcium phosphate), it defends itself at the pulpal end by laying down more tertiary dentine. This layer of dentine can be either known as reactive dentine (where tertiary dentine matrix is secreted by surviving post-mitotic odontoblasts in response to an appropriate stimulus), reparative dentine (where a tertiary dentine matrix is secreted by a new generation of odontoblastic-like cells in response to an appropriate stimulus after the death of the original post-mitotic odontoblasts responsible for primary and secondary secretion) or irregular secondary dentine. Tertiary dentine, produced as part of the pulp-dentine unit, defends against harmful stimuli such as caries or a restorative dental procedure, so that the acid does not reach the odontoblasts to destroy them.

### 3.1.2. Degradation of collagen

Dentine caries is a process which involves HAP dissolution only, it also involves denaturing of the organic materials. Proteolytic and hydrolytic enzymes are needed to break up the collagen matrix. Organic acids, also produced by micro organisms, play an
important role in demineralising the hard tissue, thus enhancing bacterial penetration and destroying the organic materials by proteolytic enzymes [Hojo et al., 1994]. Once the collagen fibres are exposed (either totally or partially) by the loss of the attached apatite crystals, they are broken down to release the remaining crystals. At this point the odontoblast processes and the intratubular dentine in the tubules are replaced by bacteria or loosely scattered crystals. As there are no vital odontoblasts or sound collagen fibres, the apatite crystals have no foundation to attach to, hence cannot be physiologically recalcified [Ogushi and Fusayama, 1975].

3.1.3. Dentine caries process

It is understandable that enamel caries and dentine caries are described as two separate subjects since the origins of the two tissues differ. However, changes in the dentine during caries progression cannot be understood without taking into account the spread of the enamel lesion and the dentine reactions to it.

The following is a description of the set of events that take place in dentine during the caries process based on the paper by Silverstone & Hicks [1985].

3.1.3.1. Small dentine lesion deep to an intact enamel surface

With the enamel surface still intact, the lesion spreads through the enamel to reach the dentine-enamel junction (DEJ). When the DEJ is reached, caries spreads laterally as this is the plane of least resistance. Consequently this undermines the sound enamel surface. In earlier research, Silverstone & Hicks [1985] claimed that this is a relatively early
stage in dentine caries, bacteria have not penetrated the tissue to reach the dentine until a cavity is formed in the enamel. However, since the carious enamel is high in porosity, acids are able to diffuse to the underlying dentine. This triggers a reaction from the dentine-pulp unit giving rise to two zones, the translucent zone and the body of the lesion. On the contrary, recent studies found that bacteria can be found in the dentine under non-cavitated enamel lesions [Seppa et al., 1985; Parolo and Maltz, 2006].

3.1.3.2. The lesion of dentine caries after cavitation of the enamel

Once the enamel lesion cavitates, bacteria are able to penetrate into the tissue and the rate of progression of the dentine lesion increases. Dentine is more vulnerable as there is a direct pathway for the microorganisms to the pulp via the dentinal tubules. The first wave of bacteria infecting the dentine is mainly acidogenic. The acidic by-products of the bacteria diffuse deep and in advance of the organisms causing demineralisation. It has been suggested by McKay [1976] that the bacterial invasion of dentine occurs in two waves. First, lactobacilli occupy the tubules towards the edge of the lesion. The second invasion causes the expansion of the occupied tubules causing damage to the surrounding dentine.

3.1.3.3. Two layers of dentine caries

Fusayama & Terachima [1972] found that dentine caries consisted of two layers which differ as follows:

1) A superficial first layer, characterised by extensive decalcification, degenerated collagen fibres and no odontoblastic processes; this is physiologically unrecalcifiable.
2) An underlying second layer characterised by intermediate decalcification, sound collagen fibres and living odontoblastic processes is physiologically recalcifiable [Kuboki et al., 1977].

It has been revealed that bacterial invasion occurs in the first layer but not in the second layer [Ogushi and Fusayama, 1975; Sato and Fusayama, 1976]. As the inner layer has potential to be recalcified, confirmed in human teeth by a study by Miyauchi et al. [1978] on vital teeth, it was concluded clinically this part of the dentine caries should be preserved.

3.2. Microbiology

Caries is a bacterial infection, and so the microflora needs to be understood in order to prevent or arrest this disease. The following is a brief summary of the role of bacteria in dental caries.

3.2.1. Bacteria and Adherence

Adherence of a microbe to an oral surface is a need for colonisation and is the initial step in the path leading to subsequent infection or invasion of tissue. *Streptococcus mutans* is associated with the initiation of the caries process. It is not the main cause of caries because of the complexity of the oral microflora, which contains several hundred species of bacteria and millions of cells growing on a single tooth surface, no single bacterial species can predict caries development in a particular person [Selwitz et al., 2007]. Lactobacilli are the pioneer organisms in the advancing front of the caries process, especially in dentine caries [Samaranayake, 2002]. The importance of adhesions in the
cariogenic process is shown by the finding that only *S. mutans* adhered specifically to Type I collagen and so are capable of invading dentine tubules [Love *et al.*, 1997].

3.2.2. Acid production

Organic acids in caries lesions play important roles in initiation and progress of dental caries. Hojo *et al.* [1994] showed that there was a clear relationship between clinical classification of dentine caries and acid profile and pH, suggesting that both factors are important in dentine caries etiology. Carious dentine contains a variety of acids, however the major acids associated with demineralisation are lactate, acetate and propionate.

Emphasis is given to the importance of acid profiles and concentration, not on pH alone during demineralisation. Therefore variations in acid profile may relate to the progression stage of caries in dentine as well as in enamel. In active caries, lactate is dominant, whereas in arrested caries, acetate and propionate are dominant. Interestingly although in comparison to carious dentine, sound dentine has hardly any acid, acetate and propionate are the dominant acids. Loesche & Syed [1973] and Kneist *et al.* [1989] reported that some carious dentine contains high numbers of lactic acid producing bacteria such as streptococci and lactobacilli. So in active lesions, such bacteria can be expected to produce lactic acid when sugar is available in large amounts which promote an acidic environment. Therefore the environment of active type carious lesions can be characterised by (a) the large number of streptococci and lactobacilli and (b) the high permeability of carbohydrate and acids into the lesion.
3.3. Remineralisation of affected carious dentine

Remineralisation is the process in which restoration of partially decalcified tooth substance to its normal or near normal structure takes place by re-precipitation of calcium phosphate in the decalcified area. Partially demineralised apatite crystals can grow to their original size as a result of exposure to a solution that is supersaturated with respect to apatite [Kidd et al., 2003]

The use of microradiography as the endpoint for determining the success of remineralisation [Kawasaki et al., 1999; ten Cate, 2001] has recently been challenged by Kinney et al. [2003a] as heterogeneous deposition of interfibrillar calcium phosphate minerals alone does not result in a highly mineralised collagen matrix. They argued that the remineralisation experiment conducted may not be considered as true remineralisation.

Although remineralisation of enamel lesions is predictably achieved, the ability to remineralise dentine is still problematic [Wei et al., 1968; Kinney et al., 2003a]. In caries affected dentine some demineralisation occurs but the collagen matrix remains intact allowing reconstitution of a fluoride containing apatite in dentine. Strontium and fluoride ions from glass ionomer cement restorations have been detected in infected dentine consistent with remineralisation. The nature and composition of the remineralised tissues will depend upon the ions present and the extent of degradation of the supporting collagen matrix [Ngo et al., 2006]. The hardening of carious dentine may be compared to skin scar tissue [Knight et al., 2010]. Carious dentine may contain structurally altered collagen of different thicknesses, as the boundary between ‘infected’
and partially demineralised carious dentine is not concrete. This has a hierarchal effect on apatite remineralisation, as the order between gap zones and collagen molecules is irreversibly altered in denatured collagen (i.e. gelatine), which is different to intact mineralised collagen [Veis et al., 1961; Zeugolis et al., 2008; Mai et al., 2010].

4 Minimally Invasive Dentistry

The term minimally invasive dentistry has been used broadly to include preventative procedures. Minimally Intervention Dentistry should be used for prevention and early caries detection to help control the disease from progressing further and avoid invasive treatment. If treatment is necessary then the least invasive option should be used to control the disease process.

At present most practitioners seem to subscribe to the physical removal of the highly infected tissue itself and then restoring the integrity of the tooth surface. The primary aim to eradicate only the highly infected, irreversibly demineralised and denatured biomass is made all the more difficult by the fact that the boundary between the superficial zone of dentine requiring excavation and the deeper affected but repairable tissue is not obvious. The inherent subjectivity in detecting this boundary results in clinically significant differences in the quality and quantity of dentine removed by different operators and possibly by the same operator on different occasions [Banerjee et al., 2000d; Willmott, 2003].
4.1. Pulpal response to dentine caries

Serial sections of carious deciduous molar teeth were examined and it was found that inflammation of the pulp occurred when the average thickness of remaining dentine between the most deeply penetrating bacteria and the pulp was 0·6 mm, and the maximal width of dentine between the pulp and the most deeply penetrating bacteria associated with pulpitis was 1·8 mm [Shovelton, 1968]. The comparable figures for permanent teeth noted by Shovelton [1968] were 0·3 mm and 0·8 mm respectively. This indicates that the deciduous pulp responds more rapidly to the effects of dentine caries than does the permanent pulp, and emphasises the importance of prevention in young children, the need for early detection of caries and providing early restorative care [Rayner and Southam, 1979].

4.2. Rationale and Treatment approach to Minimally Invasive Dentistry

4.2.1. Stepwise excavation of dentine caries

Bjorndal & Kidd [2005] discuss the concept of a modified and less invasive stepwise excavation to preserve pulp vitality. There are two steps to the restorative procedure: firstly the aim is to make a change within the cariogenic/oral environment as well as leaving just enough pulpal dentine to reduce chance of pulp exposure. Microbiological and clinical studies have shown that the number of bacteria decreases during stepwise excavation procedures, and results in the lesion arresting clinically. Secondly, when the residual, active, soft-yellowish, demineralised dentine turns into a darker, harder and drier demineralised dentine, resembling a slowly progressing lesion, it can easily be removed at the final visit because of its distinctive colour. The final excavation stage has two aims: (i) to verify that arrestment has taken place clinically and (ii) to remove the
slowly progressing but still slightly infected discoloured demineralised dentine, before the permanent and final restoration is placed.

4.2.2. Complete vs. partial removal of carious dentine before restoration

The role of operative dentistry in caries management is to prevent/arrest spread of the disease (i.e. caries) and to restore the integrity of the tooth surface so that the tooth remains functional.

Conventional concept states that all infected carious dentine tissues must be removed because any bacteria that are left can multiply and grow, causing further spread of the lesion. Recent literatures show that the composition of biofilm is an important factor in caries process and progression [Banerjee et al., 2000c; Kidd, 2004; Knight et al., 2010]. A change in the biofilm’s ecology may change the balance of forces between caries progression and its arrest or even regression (e.g. remineralisation). Under a restoration, the environment is different from the original carious lesion and it has been proposed that this change is enough to make any remaining bacteria non-viable and reduce in number [Handelman et al., 1976]. Thus carious lesion would not progress. A clinical study by Mertz-Fairhurst et al. [1998] showed that sealed occlusal restorations which had active caries inside had similar longevity as those restorations which had complete caries removal and also showed that caries infected dentine may stabilise underneath a restoration [Mertz-Fairhurst et al., 1998]. A recent Cochrane review [Ricketts et al., 2006] also concludes that partial removal of caries does not result in more progression of caries or shorter longevity of restorations in permanent teeth. Also the presence of
arrested root caries shows that the tooth has ability to heal itself by the remineralisation of carious tooth structure [Chow and Vogel, 2001]. For primary teeth, the Hall technique which restores carious primary molars by cementing a preformed metal crown on the unprepared tooth with no caries removal has been proposed. A 2 year randomised controlled trial [Innes et al, 2006] shows that the ‘Hall crowns’ had 20% and 50% of major and minor failures respectively compared to the respective 15% and 46% for conventional restorations [Ricketts and Pitts, 2009].

These above studies indicate that it is not important to have a bacteria free environment under the lesion. However, it is still not clear how much carious tissue can be left behind to preserve the integrity of the tooth and the pulp without the danger of secondary spread of infection. At present there is no international consensus on whether carious tissue should be left under a restoration. This is clearly demonstrated when comparing cariology and restorative dentistry with endodontic literature. Cariology research focuses on the possibility that a deep carious lesion can be arrested, and endodontic research contests the treatment concepts of permanently leaving diseased tissue under a restoration.

5 Indicators of caries

This section will include conventional method of caries detector and caries removal agents as well as use of hardness, colour and bacteria as indicators of the extent of caries progress or regress.
5.1. Hardness

The following is a description of the key mechanical properties of dentine (hardness and Young’s modulus) and how these contribute to the micromechanics model of dentine. This is based on the review of the mechanical properties of dentine [Kinney et al., 2003b].

Hardness tests measure the resistance of the dentine to deformation caused by penetration of an indenting stylus. The Knoop indenter has been frequently used in the studies of dentine. The long aspect ratio (7.11 times longer in one dimension) allows for accurate measurements of area, even with shallow indentations making this Knoop indentation extremely useful for small, thin specimens typical of studies of mineralised tissues.

Hardness is defined in units of pressure, or force per unit area of indentation. Unlike Vickers or Brinell methods, which use the contact area of the indenter stylus, the Knoop method uses the projected area, $A_p$, in the calculation of hardness (KHN):

$$KHN = \frac{P}{A_p} = 14.22 \frac{P}{l^2}$$  \hspace{1cm} \text{Equation 5-1}

where $P$ is the applied load (in kg) and $l$ is the length (in mm) across the long axis of the remnant impression. In SI units, $1 \text{ kgmm}^{-2} \approx 9.8 \text{ MPa}$ (where 1 kg = 1 kilogram force = 9.8 N).
Many investigators have determined that hardness depends on mineral concentration. Featherstone et al. [1983] developed an analytic expression relating Knoop hardness to the volume percent of mineral ($V_m$) for enamel and sound dentine:

\[ KHN = (0.197V_m - 0.24)^2 \quad \text{Equation 5-2} \]

However Equation 5.2 may not be applicable for low concentrations of mineral, nevertheless the expression appears to fit the experimental data over a range of mineral concentrations associated with normal and carious dentine. Kielbassa et al. [1999] confirmed the same relationship between KHN and percentage mineral content (i.e. KHN is proportional to $V_m^2$) but their constants differ:

\[ KHN = ((V_m - 21.19)/3.66)^2 \quad \text{Equation 5-3} \]

The works of Ogawa [1983] and Wang and Weiner [1998] showed that the mantle dentine immediately subjacent to the enamel ($KHN \approx 60 \text{ kgmm}^2$) is softer than the underlying primary dentine ($KHN \approx 70 \text{ kgmm}^2$). Also, hardness gradually decreases with proximity to the pulp, falling steeply in the inner layer of dentine of about 0.5 mm thickness surrounding the pulp ($KHN \approx 30 \text{ kgmm}^2$). This implies that $V_m$ for dentine must be lower near the pulp is lower than that in the primary dentine. This decrease in hardness may be related to the increase in density of tubule lumens near the pulp [Pashley et al., 1985]. However Kinney et al. [1996] showed that most of the decreased hardness near the pulp could be explained by a decrease in the hardness of the intertubular dentine matrix. Thus, it is likely that the intertubular dentine matrix near the pulp is less mineralised.
Using an ultra-micro indentation system, Angker et al. [2004b] investigated the relationship of hardness with mineral content and found that the relationship followed an exponential expression:

\[ H = 0.005e^{0.126\text{min}} \]  

where \( H \) is the hardness, min is mineral content in vol\%.

5.2. Caries detector dye

After Fusayama found that dentine caries consisted of two distinctive layers [Fusayama and Terachima, 1972], he developed a dye containing 0.5% basic fuchsin or 1% Acid Red 52 in propylene glycol [Fusayama, 1988], to make visible the boundary between inner and outer dentine, based on the binding to collagen cross links, and to stain only the outer irreversibly damaged area. However there are conflicting opinions on whether caries detector dyes do actually aid the clinician to remove caries effectively. Van de Rijke [1991] found that the dye was an appropriate aid at differentiating the two layers of dentine, and the caries removed eliminated the bacteria. Contrary to this, Kidd et al. [1993] found that the stain actually stained the altered organic matrix of the demineralised dentine, not the bacteria. So there would naturally be a lag between what was stained and the bacterial front, meaning that the staining was that of reduced mineral content. This was confirmed by Yip et al. [1994] as they found that the caries detector also stained healthy circumpulpal dentine and around the EDJ, regions which have lower mineral content compared with normal healthy dentine. This is also highlighted in undergraduate dental students textbooks like ‘Clinical Problem Solving in Dentistry’
In an attempt to overcome excessive dentine removal, Hosoya et al. [2007] evaluated the clinical efficacy of a new caries detecting dye for primary and permanent carious dentine. This new dye (Caries Check, Nippon Shika Yakuhin, Shimonoseki, Japan) contains 1% acid red in polypropylene glycol instead of propylene glycol. The molecular weight is higher in polypropylene glycol (MW=300) than in propylene glycol (MW=76). The authors concluded that this new dye is more efficacious in removing just the infected dentine and avoiding excessive removal of sound and affected dentine in primary and permanent teeth.

5.3. Colour

Colour has been used by dentists to assess the activity of a lesion, as well as the age of the lesion. But it is dependent on many factors such as lighting and the hydration of the tooth. Brown staining of dentine is indicative of the decayed tissue. This brown staining was postulated to be due to Maillard reaction between sugar and protein [Armstrong, 1964; Kleter et al., 1998]. When dentine is demineralised, the protein matrix is exposed, subjecting the dentine matrix to the Maillard reaction [Kleter et al., 1998]. The colour change is also believed to be due to action of bacterial enzymes. Therefore the aim of dentists was to remove all stained tissue in order to eradicate bacteria. It was later found by Kidd et al. [1996] that this removal of stained tissue did not eliminate all bacteria. It has also been shown that using colorimetric parameters as an indicator for caries removal may remove healthy dentinal tissue which under appropriate conditions would be capable of remineralisation [Cartagena and Ayarzun, 2001]. Nevertheless, arrested caries appears to be black in colour as sulphur salts become incorporated into the remineralising tissue [Knight et al., 2010].
5.4. Autofluorescence

Fluorescence occurs when a sample absorbs incident light of one wavelength and re-emits it as a light of a longer wavelength. It has been shown that carious dentine has its own characteristic fluorescence, known as autofluorescence (AF) [Alfano and Yao, 1981; Sundstrom et al., 1985]. It has been found that the autofluorescence is produced by a chromophore residing in the altered carious dentine matrix, created by an interaction between bacteria and the dentine matrix deep within the lesion. Banerjee et al. [1999] found that AF from carious dentine that terminated at a level adjacent to the translucent zone is correlated to the soft dentine, which dentists excavate. So AF signal detection could offer an objective marker for distinguishing between the infected and affected dentine.

Using an ultraviolet light source, Lennon et al. [2009] differentiated the sound dental hard tissue by its green fluorescence to the carious tissue by its orange-red fluorescence in order to aid caries removal and called it FACE (Fluorescence Aided Caries Excavation). This change in fluorescence is due to the porphyrins, a bacterial by-product. These authors compared the efficacy of caries removal using FACE techniques to that with caries detector dye, chemomechanical and conventional caries removal using staining method to analyse the remaining bacteria. They found that the best combination of excavation time and successful removal of infected dentine was achieved by FACE.
Instead of using ultraviolet light, lasers can also be used to excite autofluorescence. A commercially available product, DIAGNOdent (KaVo, Biberach, Germany) has been investigated by Iwami et al. [2004] who found that there was a relationship between the DIAGNOdent values and rates of bacterial detection assessed by polymerase chain reaction (PCR) method of bacteria DNA.

AF has also been used for diagnosis of early caries lesion as bacteria are present in non-cavitated lesions. The sensitivity to detect D2 lesions in permanent teeth ranges from 0.42 [Shi et al., 2000] to 0.87 [Lussi et al., 1999] depending on the ‘gold standard’ method and the conditions under which it was assessed. The respective specificity range from 0.72 [Lussi et al., 1999] to 0.95 [Shi et al., 2000].

5.5. Bacterial analysis

Many microbiologists have tried to analyse and identify the bacteria or bacterium responsible for dental caries. The studies involve using culturing methods to detect and quantify the type and proportions of bacteria within the lesions. However a disadvantage of this technique is that the cultivable fraction of bacteria is not representative of the oral infection, i.e. the number and distributions are oversimplified. Banerjee et al. [2002] showed that the bacterial count as a function of lesion depth decreases as the distance from the advancing front of the lesion increases. Current restorative concepts challenge the need to remove all bacteria in the carious lesion [Oliveira et al., 2006]. It proposes that if the seal of the overlying restoration is perfect, the bacteria will be “starved” of nutrients to grow.
6 Caries Excavation

Since the invention and application of rotary instruments, the operative treatment of carious lesions has often resulted in considerable removal of tooth structure. More recently, newer innovative techniques for the removal of carious dentine have been developed in an attempt to minimise this excessive tissue loss. A review by Banerjee et al. [2000b] classified the methods as mechanical and non-mechanical, rotary and non-rotary, including: dental handpieces/burs, manual excavators, air abrasion, air polishing, ultrasonication, sono-abrasion, chemo-mechanical methods, lasers and enzymes (the underlined techniques will be reviewed in this section). Some claim to remove infected or demineralised dentine selectively whereas others are not able to differentiate between the two.

6.1. Mechanical carious tissue removal techniques

6.1.1. Excavators, handpieces and burs

This mechanical method is universally used by dentists. The rotating bur easily cuts through carious dentine to eventually open up healthy tubules deep in the tissue. In current practice, dentists gain access to the carious dentine using the high-speed air turbine handpiece (100,000 – 350,000 rpm) and bur and the slow-speed (3,000 – 25,000 rpm) or hand excavator for carious dentine excavation. The hand excavator generally allows more sensitive tactile feedback during the removal of softened tissues.

6.1.2. Air abrasion
Air-abrasion was originally developed by R.B. Black in 1945 [Black, 1945; Black, 1950]. The tooth surface is bombarded with high velocity particles (aluminium oxide) carried in a stream of air. Naturally the effect of this technique is dependent on the abrasive used. However it was found that air-abrasion is also very efficient in removing healthy dentine and enamel. To reduce the amount of damage, a number of options can be altered such as changing the air pressure; the distance between the nozzle and the tooth surface and the length of cutting. Early patient surveys by Goldberg [1952], Morrison and Berman [1953] and Gabel [1953] show that patients and dentists preferred this technique. However the clinical use was stopped in the late 1950’s but the interest has re-emerged as a selective cutting technique, targeting only softened diseased tissue [Banerjee et al., 2000a].

Paolinelis et al. [2006] assessed the effects of air abrasion by investigating the microhardness of dentine. They concluded the dentist should be aware that air abrasion systems using alumina particles removed healthy dentine more efficiently than carious dentine, with the associated implications that excessive dentine is removed clinically. Therefore a less abrasive bioactive glass has been investigated and was shown to be potentially more selective in removing carious dentine than alumina [Paolinelis et al., 2008].

6.1.3. Sono-abrasion

Unlike air abrasion, sono-abrasion does not involve any loose particles. Instead, a specially designed diamond coated tip is attached to a sonic air-scaler piece, which allows removal of dental hard tissues. It has been proposed to be an alternative
instrument to conventional rotary instruments in preparing cavities [Hugo and Stassinakis, 1998].

6.1.4. SmartBur/SmartPrep

Smartbur (previously known as SmartPrep, SSWhite) is a novel polymer rotary instrument which recently came on to the market. Due to its self limiting properties, its edges become rounded when it comes into contact with harder, healthy dentine. It is a conservative approach in selective dentine caries removal, which purportedly will safely and effectively clear away decayed dentine leaving behind healthy dentine intact, and the company claims it reduces the need for anaesthesia in comparison to carbide burs [Allen et al., 2005]. The SmartPrep has a Knoop Hardness Number (KHN) of 50 whereas the hardness for dentine and dentine caries are 70-90 KHN and 0-30 KHN respectively. Unfortunately the bur is now out of circulation, as the product was not commercially viable in its current form. At present the burs are being re-designed so that they can be used at a higher rpm with better durability and will then be relaunched.

6.2. Chemomechanical caries removal techniques

This alternative method to traditional drilling involves the chemical softening of carious dentine followed by gentle excavation. A chemomechanical caries removal reagent (CMCR) causes further degradation of the already partially degraded collagen in the outer layer of dentine caries but leaves behind the inner carious dentine where the collagen fibrils are still intact. The two main CMCRs are Caridex and Carisolv, and both of these products were reviewed by Beeley et al. [2000] and Maragakis et al. [2001] which are summarised as follows.
6.2.1. Caridex

Goldman [1976] reported the possibility of removing carious material chemically using N-monochloroglycine (NMG, GK101). After modification, the Caridex system, which was marketed in the US in the 80’s, was developed containing N-monochloro-D,L-2-aminobutyrate (NMAB, GK-101E). It consists of two solutions: Solution one contains sodium hypochlorite (a non-specific proteolytic agent) and solution two contains glycine, aminobutyric acid, sodium chloride and sodium hydroxide. Early studies by Anusavice & Kinchloe and Zinck et al. [1988] found the technique to be advantageous especially for clinical use: increased patient compliance and reduced need for local anaesthesia. Brannstrom [1980] showed it to be a successful way of removing soft carious dentine without significant damage to the underlying healthy dentine. Barwart [1991] showed no beneficial effects of Caridex in excavating carious dentine in comparison with a control system of water and saline. In deciduous teeth, the addition of urea to the solution significantly improved carious dentine excavation compared with the same solution without urea [Yip et al., 1995].

The mechanism of NMG and NMAB are still uncertain, and knowledge of the chemistry of chlorination of amino acids and their effects is still sparse. However it was thought that the procedure involved chlorination of the partially degraded collagen in the carious lesion and the conversion of hydroxyproline to pyrrole-2-carboxylic acid. Another suggestion involves cleavage by oxidation of glycine residues, which disrupts the collagen fibrils making it friable so it can be easily removed [Beeley et al., 2000].
There are limitations in using this system: Large volumes of solution and a special applicator system were required, as well as rotary and/or hand instruments still needed for the removal of tissue other than degraded dentine collagen.

6.2.2. Carisolv

Carisolv came onto the market in 1998 and was developed by MediTeam Dental AB (Sweden), which has now changed its name to OraSolv AB (Sweden). It is a successor of Caridex, which also has two components, one is sodium hypochlorite and the other consists of a carboxymethylcellulose (gel based) with three amino acids, glutamate (acidic), lycine (basic) and leucine (hydrophobic). It also comes with specially designed excavators to remove the softened carious dentine (Figure 6.1). The company claims Carisolv offers pain reduced treatment and better preservation of teeth compared to e.g. drilling. With the Carisolv method of removing caries, both drilling and local anaesthesia can be minimized or completely avoided, making treatment less painful for the patient [Anusavice and Kincheloe, 1987]. It is a well-documented method for tissue-preserving caries removal with more than 150 scientific references. The studies so far have shown Carisolv to be effective, but some studies have differed on the opinion that Carisolv is time consuming (which is disliked by clinicians) and so would prefer to use a rotary bur due to the shorter preparation time [Yazici et al., 2003]. However, it has been claimed that the new clear Carisolv gel is 25% faster compared with the previous gel. Caries removal can now be completed in 5.2 minutes, which is less than 30% of average total treatment time. Hence the time factor is no longer clinically significant [MediTeam,
The concentration of sodium hypochlorite has been increased from 0.5 % to 0.95 % in the new Carisolv gel.

Figure 6.1 Hand instruments supplied with Carisolv

This chemo-mechanical technique also claimed to minimize the need for local anaesthesia and is a good alternative to the use of rotary instruments, eliminating the effect of heat and pressure. Comparing the conventional hand excavator to Carisolv technique, Flückiger et al. [2005] showed (using microhardness) that both excavate the same amount of carious tissue efficiently. Using Carisolv tended to leave some carious dentine, however 3D determination of residual caries could not be gained. Hahn et al. [2004] used microCT to assess chemo-mechanical caries removal and also found that residual caries was left behind but it was unclear whether it was carious or not. It has been reported that patients preferred the Carisolv technique as it was pain free [Rafique et al., 2003]. There are, reportedly, no adverse effects on composition of sound dentine, and the possibility of remaining residual softened dentine was minimal [Hossain et al., 2003]. However, the mechanism of Carisolv is still not clear and studies comparing Carisolv with sodium hypochlorite on its own Tonami et al. [2003] and Dammaschke et al. [2005] found that sodium hypochlorite softens both sound dentine
and affected dentine. Carisolv, apparently does not have this harsh effect on removing sound dentine because of the three amino acids, and so the distinctive mechanism is the ability to dissolve denatured dentine only, which is consistent with the study by Hannig [1999].

Despite not knowing the exact mechanism of Carisolv, the following is the standard postulate of the mechanism [MediTeam, 2007]: When the two components are mixed together the amino acids bind chlorine and form chloramines at a high pH. The three amino acids are differently charged (positively charged, negatively charged and neutral) which allows for an electrostatic attraction to different areas of proteins in the carious dentine. The chemical result of these processes is the breakdown of degraded collagen found in demineralised part of carious dentine. The degraded collagen has an open structure and is more susceptible to further breakdown by chloramines. The porous nature of demineralised dentine allows for penetration of Carisolv. The unaffected collagen is more resistant to degradation but the framework of degraded collagen in porous mineral is broken down and can easily be scraped away.

Haak et al. [2000] demonstrated that chemomechanical caries removal has no adverse effect on bonding of modern adhesive systems to dentine. In addition smear layer dissolving or modifying bonding systems could potentially benefit from chemomechanical pre-treatment.
Kirzioglu et al. [2007] investigated the status of restorations after application of Carisolv at 3, 6, 9 and 12 months. They concluded that Carisolv system is effective in the removal of caries and causes minimum level of pain. Carisolv system seems to be a promising restorative approach to remove occlusal caries in primary molar teeth. However studies of longer duration are needed to confirm these findings.

6.2.3. Enzymes

In 1989, Goldberg and Keil successfully removed soft carious dentine using bacterial Achromobacter collagenase, which did not affect the sound layer of dentine beneath the lesion [Goldberg and Keil, 1989]. Another enzyme used recently is called pronase [Nordbo et al., 1996]. Further laboratory research is needed for validation of the use of enzymes for selective dentine caries removal.

6.3. Lasers

Since the development of the first laser in 1960, many new types have become available such as Er:YAG (Erbium doped Yttrium Aluminum Garnet) laser. This was recently investigated for the use of excavating dentine caries effectively [Eberhard et al., 2005]. The efficacy of lasers will depend upon factors including the wave characteristics, pulse energy, repetition rate and the optical properties of the incident tissue [Wigdor et al., 1995; Seka et al., 1996].

Er, Cr:YSGG (Erbium, Chromium doped Yttrium Scandium Gallium Garnet) laser technique in removing dentine caries was compared with Carisolv in a study by [Kinoshita et al., 2003] and they concluded that the laser method was the more effective
caries removal technique. In addition to caries removal, studies have shown that in the presence of a suitable photo-sensitizer, lower power laser light has the ability to destroy *Streptococcus mutans* [Burns et al., 1995].

As porphyrin, a bacterial by-product, emitted red fluorescence when excited by a laser light [Lennon et al., 2002], a feedback system using the level of autofluorescence to control the cutting power of the Er:YAG laser beam was designed to remove the infected dentine only. A randomised clinical trial using this fluorescence control Er:YAG laser was conducted by Dommische et al. [2008]. The samples were processed for culturing *S. mutans* and lactobacilli, based on assessment of the number of CFU at different threshold levels. Levels 7 [U] and 8 [U] is comparable to caries removal with a conventional bur based on similar microbiological outcomes. In laser fluorescence studies, an autofluorescence relative unit of 7 [U] is usually used to demarcate the level between sound and infected dentine clinically. However, bacteria could still be found in residual dentine even when carious dentine with a fluorescence unit of 9 [U] was removed [Lennon et al., 2003]. Nevertheless, the number of bacteria forming colonies was <100 CFU ml$^{-1}$, indicating that the fluorescence controlled laser treatment is at least as effective as dentine being removed by conventional bur treatment.

More investigations are needed on the control of lasers as an excavation procedure and the possible alteration/destruction of the adjacent sound tissue.

6.4. Comparative studies
The following comparative studies show that restorative dentistry has moved away from a ‘drill and fill’ philosophy to a minimally invasive approach.

Lennon et al. [2006] compared the efficiency of 4 caries excavation methods: FACE, caries detector dye (CD), chemomechanical excavation (CS) and conventional excavation (CE). They measured the efficiency for the caries removal techniques as the time taken to excavate and successfully remove bacterially infected dentine. The gold standards used to determine the completeness of the excavation included microhardness and light microscopy. They found that FACE required the shortest time in removing bacterially infected dentine, comparable to conventional excavation. They also found that bacteria in the residual dentine were significantly fewer in the FACE and CE group compared to CD and CS group.

The same group [Lennon et al., 2007] also compared the quantity of remaining bacteria and cavity size after excavation with FACE, caries detector dye and conventional excavation in vitro and concluded that FACE is more effective in removing heavily infected dentine without significantly increasing cavity size compared to conventional methods.

Banerjee et al. [2000b] evaluated the effectiveness and efficiency of five caries removal techniques in permanent teeth. They concluded that Carisolv technique was slowest but removed adequate amount of infected dentine. Sono-abrasion tended to leave too much infected dentine whereas air-abrasion and hand excavation were similar with hand excavation being the best combination of efficiency and effectiveness.
Celiberti et al. [2006] investigated the performance of four dentine excavation methods in deciduous teeth: 1) Round carbide bur, 2) Er:YAG laser, 3) hand excavator and 4) polymer bur. The efficacies of the techniques were assessed using confocal imaging and autofluorescence. They concluded that the polymer bur left the largest area of infected dentine whereas steel bur resulted in largest overpreparation area. Overall, hand excavation seemed to be the most suitable dentine caries removal technique in deciduous teeth.

6.5. Summary

Clinically, assessment of dentine caries indicators is subjective and therefore dependent on individual operators. The laboratory investigations on carious indicators that relate the carious dentine to mechanical properties or bacterial infiltration are usually confined to 2 dimensions. Also, most investigations are cross-sectional, i.e. the same specimen is not followed through, and therefore, the volume and type of tissue removed cannot be related to original structures (previous investigators mainly used adjacent sections to indicate the original structures) for assessment of over- and under preparations. The golden aim of removing only the “infected” and leaving the “affected” dentine has also been challenged. However, there is consensus to remove the “soft” dentine so that restorations can be placed on a firm base. Nevertheless, there are no agreed parameters to determine the boundary between the soft and hard dentine to guide the clinicians’ in cavity preparations.

The physical criteria used as a guide by dentists to remove infected demineralised dentine are based on the hardness and texture of the tissue. But dentists may also use colour or caries detector dyes as a guide. Despite these criteria, the guides are not
accurate and as a consequence the quality and quantity of the dentine removed differ
during the excavation process. As a result, the clinical implications of different sized
cavities is the health of the pulp and the strength of the remaining tooth structure.

7 X-ray microscopy

7.1. X-rays

7.1.1. Introduction

Ever since Roentgen discovered X-rays in 1895, they have played a vital role in our
everyday lives: they make the unseen in our bodies visible; they are part of security
measures at airports; they allow non-destructive testing of a wide variety of materials. In
over a hundred years since the discovery of X-rays, the range of current techniques now
available for scientific analysis is extensive. Because of the different properties of X-
rays, they have been selectively utilised to investigate a wide range of medical,
industrial, and scientific problems, as well as applications in foods and cosmetics. In
industry they are valuable for diagnosis and non-destructive testing of products for
defects. This could involve detecting small cracks in rockets or other large machines or
just a routine X-ray inspection used to examine metal parts for internal stress defects.
Another X-ray technique, X-ray diffraction, is used throughout the physical sciences.
Through X-ray diffraction and spectroscopy it is possible to probe matter at atomic level
to determine lattice structures as well as chemical formulas, bond lengths and angles.
The most significant and earliest exploitation of the penetrating character of X-rays is its
use as an imaging tool in the field of medicine and dentistry to aid clinical diagnosis.
The development of “non invasive” imaging techniques especially Computed
Tomography (CT) has been a main contributor to the enormous progress of medicine
and dentistry with the increase in digital technologies allowing more extensive use of image processing [Hendee, 1995]. For microscopic investigation, especially in mineralised tissue research, techniques such as X-ray microtomography (XMT), a miniaturised version of medical CT and microradiography have been developed. The following review will look at the production of X-rays and their properties, interaction with matter and detection. This will form the basis for understanding the principles of XMT.

7.1.2. Nature of X-rays

X-rays are electromagnetic radiation, and as such they are characterised either by wavelength, frequency or energy (Figure 7.1).

![EM spectrum diagram](image)

**Figure 7-1 The EM spectrum in terms of wavelength, frequency or energy, highlighting X-ray region [Seibert, 2004]**

A monochromatic beam of X-rays can be described as a transverse EM wave, consisting of two components, electric and magnetic waves which are perpendicular to each other and to the direction of propagation (Figure 7.2). X-rays have wavelengths in the region of an Ångstrom ($10^{-10}$m). However quantum mechanically, a monochromatic beam of X-rays is shown to be quantised into photons (packets of discrete energy) with an energy
value of the order of keV. So not only do X-rays show wave properties, which is confirmed by diffraction and interference phenomenon, X-rays also show particle like traits demonstrated by the photoelectric effect. So an X-ray photon can interact and remove electrons bound to an atom i.e. the interaction is collisional. This is the dual nature of X-rays. Like all the other EM radiation, X-rays must be considered as both particles and waves.

![Graphic](image)

**Figure 7-2 An X-ray is a transverse electromagnetic wave, where the electric and magnetic field is perpendicular to each other and to the direction of propagation [Seibert, 2004]**

7.1.3. X-ray production

The production and scattering of X-rays provide additional examples of the quantum nature of electromagnetic radiation. X-rays can be produced when rapidly moving electrons that have been accelerated through a potential difference of the order of $10^3$ to $10^6$ V strike a metal target.

**7.1.3.1. Impact sources**
X-ray generation achieved by an electron impact source, in the form of an X-ray tube, is generally used as a source of X-rays in research laboratories. Since X-rays are produced whenever high-speed electrons collide with a metal target, any X-ray tube must contain (a) a source of electrons, (b) a high accelerating voltage, and (c) a metal target. Also, since most of the kinetic energy of the electrons is converted into heat in the target, a cooling system is usually required to prevent melting.

X-ray filament tubes which are commonly used consist of a filament which, when heated, emits electrons via thermionic emission (Figure 7.3). These electrons are then accelerated across the evacuated X-ray tube, under the influence of a large voltage, the filament forming the cathode and the target being the anode.

![Diagram of an X-ray tube plus circuit](http://www.bh.rmit.edu.au/mrs/subject/mr100/prodxtube.html#)

**Figure 7-3 A simple diagram of an X-ray tube plus circuit**
Two kinds of filament tubes exist: sealed and demountable. A sealed tube is evacuated
and sealed under vacuum at the factory. It is much easier to use since no high-vacuum
pumping equipment is necessary. However, it is expensive, and the life of the tube is
determined by the life of the filament. In demountable tubes (most often used for special
purposes) both the filament and target are accessible for replacement. However, it must
be pumped continuously during operation.

7.1.3.2. Bremsstrahlung radiation

When electrons are decelerated in the target, part or all of their kinetic energy is
converted directly to a continuous spectrum of photons, including X-rays (Figure
7.4(a)). The term given to this process of producing this radiation is called
Bremsstrahlung, meaning braking radiation.

7.1.3.3. Characteristic X-rays

The impact process gives peaks in the X-ray spectrum at characteristic frequencies that
depend on the target element. Electrons, if they have enough kinetic energy, can transfer
that energy either partly or completely to individual atoms within the target. These
atoms are left in excited levels; when they decay back to their ground levels, they emit
X-ray photons. Since each element has a unique set of atomic energy levels, each also
has a characteristic X-ray emission spectrum (Figure 7.4(b)).
Figure 7-4 (a) Continuous spectrum depicts a continuous range of photon energies produced by electrons being decelerated in the target. (b) Line spectrum depicts a limited number of precise characteristic photon energies [Pope, 1998]

7.1.3.4. Synchrotrons

When charged particles are forced to accelerate in a circular orbit (maintained by bending magnets), photons are emitted. When the particles are travelling at relativistic velocities (near to the speed of light) these photons are emitted in a narrow cone in the forward direction, i.e. tangential to the orbit. In a high energy electron or positron storage ring these photons are emitted with energies ranging from infra-red to X-rays. This is known as synchrotron radiation. It has many advantages:

- Synchrotron radiation is extremely intense and highly collimated i.e. hundreds and thousands of times more intense than that from conventional X-ray tubes, which allows rapid data collection. With the aid of insertion devices such as wigglers and undulators, periodic arrays of magnets which are designed to produce a series of deflections, the intensity of the beam can be further accentuated.
- Synchrotron radiation is highly polarised.
- Synchrotron radiation is coherent.
- Synchrotron XMT has the capability of providing images with high contrast resolution and high spatial resolution.

It is the combination of the above properties that make synchrotron radiation a unique and powerful source for a wide range of scientific and technical applications. The high intensity, natural collimation and continuous spectrum make possible the production of intense, tuneable, highly monochromatic beams of radiation [Lewis, 1997]. The selection of a specific wavelength from a broad white radiation spectrum is achieved using single crystal monochromators (Si or Ge). The monochromatic beam produced in turn gives accurate quantitation of LACs without the need for the calibration and beam hardening corrections required for laboratory X-ray sources (beam hardening and calibration will be discussed further in sections 7.6.1 and 10.1.2).

Synchrotron XMT has been previously used to measure mineral concentrations at micrometre-scale resolution within bone [Nuzzo et al., 2002], carious dentine [Kinney et al., 1994], root dentine [Kinney et al., 2005], sound enamel and brown spot lesions [Dowker et al., 2004] and human dental fissure enamel [Dowker et al., 2006]. But there are also many disadvantages such as their limited availability, expense and inconvenience of use especially in relation to the study of tooth samples. The small number of studies of dental mineralised tissues is a reflection of this [Dowker et al., 2004; Dowker et al., 2006]. Also as the maximum X-ray energy is generally lower than that of laboratory sources, this consequently limits maximum bone/tooth sample size.
and/or the atomic number Z of other samples than for laboratory sources [Elliott et al., 1997].

7.2. X-ray interactions with matter

As a beam of X-rays travels through a material, the beam becomes attenuated; in other words its intensity decreases.

Two ways in which this can happen are by scattering and absorption:

- When an X-ray beam passes through an object, the photons can get scattered causing the beam to deflect.
- Or the X-rays might get absorbed by the object, giving energy to the object.

How much the X-rays are attenuated is dependent on:

- The energy of the X-rays
- The density of the material
- The atomic number(s) of the material

7.2.1. Attenuation mechanisms

The attenuation mechanisms in general for any object are described as follows:

7.2.1.1. Photoelectric effect

An incoming photon gives all its energy to the inner electron, causing it to be ejected from the atom. This excited atom then returns to its ground state giving rise to the emission of its characteristic photon. The energy is quite low and so it is rapidly absorbed by the material itself. The ejected photoelectron ionises more atoms in its path until all the kinetic energy has dispersed (Figure 7.5(a)).
7.2.1.2. Rayleigh scattering

Simple scatter: As can be seen in Figure 7.5 (b), the incoming photon has an energy which is not enough to knock the electron out of the atom, as it is less than the binding energy. As a result the photon just gets deflected; the photon is elastically scattered.

7.2.1.3. Compton scattering

Compton scatter: The incoming photon has an energy greater than the binding energy of the electron in the atom, but only part of this energy is given to the outer electron (this electron can be considered free and unbound). The end result is that the outgoing photon has changed its direction with reduced energy and the electron (known as the recoil electron) distributes its energy via ionisation (See Figure 7.5(c)).

7.2.1.4. Pair production

Pair production: Pair production takes place when the incident X-ray has an energy greater than 1.02 MeV. The interaction of the incident photon with the electric field of the nucleus produces an electron-positron pair.

Figure 7-5 Attenuation mechanisms (a) Photoelectric effect; (b) Simple scatter; (c) Compton scatter [Pope, 1998]
7.3. Factors affecting the X-ray spectrum

There are many variables that effect the final X-ray spectrum, and these can be visualised in the plots in Figure 7.6.

This is further summarized in Table 7.1. It can be seen that the maximum energy ($E_{\text{max}}$) can only be increased by an increase in voltage. The total intensity (or brightness) increases with increases in all the factors except for adding a filter. The average photon energy is independent of the current but increases with the other factors. Finally all the factors except for the current have an effect on the characteristic lines of the X-ray spectrum.
Figure 7-6 Factors affecting the X-ray spectrum. (a) Changing the tube voltage changes the X-ray spectrum; (b) Effect of tube current on the X-ray spectrum; (c) Effect of target material on the spectrum; (d) Adding a filter changes the shape of the X-ray spectrum [Pope, 1998]

Table 7-1 Comparison of factors affecting the X-ray spectrum [Pope, 1998]

<table>
<thead>
<tr>
<th>Increasing</th>
<th>( E_{\text{max}} )</th>
<th>Total intensity</th>
<th>Average photon energy</th>
<th>Characteristic lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voltage</td>
<td>increases</td>
<td>Increases rapidly</td>
<td>increases</td>
<td>more may appear</td>
</tr>
<tr>
<td>Current</td>
<td>Same</td>
<td>increases</td>
<td>same</td>
<td>same</td>
</tr>
<tr>
<td>Z (target)</td>
<td>Same</td>
<td>increases</td>
<td>may increase</td>
<td>Shift to higher energies</td>
</tr>
<tr>
<td>Filtration</td>
<td>Same</td>
<td>decreases</td>
<td>increase</td>
<td>Some lower energy lines may ‘disappear’</td>
</tr>
</tbody>
</table>

\( Z \) is defined as the atomic number, \( E_{\text{max}} \) is the maximum energy
7.4. Beer’s law

A monochromatic X-ray beam consists of photons with one energy only whereas a polychromatic X-ray beam consists of a range of photon energies. When a monochromatic beam passes through a medium, the transmitted intensity $I$, is given by the following relationship, known as Beer’s law:

$$ I = I_0 \exp \left( -\mu x \right) $$  \hspace{1cm} \text{Equation 7-1}

where $I_0$ is the initial intensity of the beam

$\mu$ is the total linear attenuation coefficient (LAC).

If the scanned object is composed of a number of different materials, then equation 7.1 will become:

$$ I = I_0 \exp \left[ \sum_i -\mu_i x_i \right] $$  \hspace{1cm} \text{Equation 7-2}

where each increment $i$ reflects a single material with attenuation $\mu_i$ over a linear extent $x_i$.

7.5. Attenuation coefficients

7.5.1. Linear attenuation coefficients (LAC)

The linear attenuation coefficient for an object is denoted by the symbol, $\mu$. It is defined as the logarithmic decrease in the intensity of the radiation per unit thickness of the absorber. It conventionally has units of cm$^{-1}$. Tomographic images can be considered as maps of spatially varying LAC of the tissue being scanned.
7.5.2. Mass attenuation coefficients

At a given photon energy, the linear attenuation coefficient can vary significantly for the same material if it exhibits differences in physical density. However for a given energy, $E$ and atomic number, $Z$, the LAC is proportional to density, $\rho$, according to the following relationship:

$$\mu_m = \frac{\mu}{\rho}$$

Equation 7-3

where $\mu_m =$ mass attenuation coefficient.

This describes the attenuation per unit area density of material, and has units of $m^2 kg^{-1}$ but is normally expressed as $cm^2 g^{-1}$.

In Figure 7.7 the effects of the attenuation mechanisms at different photon energies is depicted. As pair production is concerned with higher energies, in regards to XMT which is associated with relatively low energies, this mechanism can be discarded.

Figure 7-7 Different attenuation mechanisms at different photon energies [Pope, 1998]
If we look at photon energies between 30 and 40 keV (which is the typical photon energy in XMT, dependent on the specimen) the first three mechanisms contribute to the total attenuation of the object. In regards to this project, the main aim is to distinguish and differentiate between different hard tissues. Photoelectric effect/absorption is the main attenuation mechanism which is responsible for giving the best contrast in the image especially in hard tissues. This is because the photoelectric effect has an approximate $Z^3$ dependence.

7.6. X-ray beam attenuation

7.6.1. Beam hardening

The previous section gave a relationship of a monochromatic beam between the incident and the transmitted beam passing through an object. In reality, X-rays produced with impact sources are polychromatic i.e. X-rays are produced with a range of photon energies. The attenuation of X-rays is related to the wavelength ($\lambda$) and the atomic number of the material: $\mu \propto Z^4\lambda^3$, where $\lambda$ is inversely proportional to the photon energy ($E$). This relationship is observed in the low energy range. When a polychromatic beam passes through a medium, a higher proportion of lower energy photons are absorbed by the medium, whilst photons with higher energies are more likely to pass through. This leads to beam hardening, which means that the transmitted X-ray spectrum shifts towards the higher energies as the thickness of the object increases. Beam hardening depends on the physical characteristics of the attenuator. For example, absorbers such as hard tissue, which have high average atomic number (because of the calcium content) cause more beam hardening than soft tissue. As a result of beam hardening, reconstructed 3D XMT images may exhibit “dishing” artefacts i.e. the middle
of a homogeneous specimen appears to have a lower LAC in the middle (Figure 7.8). Therefore correction is needed. Aluminium filters are used to help reduce beam hardening effects. Methods of correcting this will be discussed further in Section 10.1.2.

![Figure 7-8 Example of an image of a tooth sample with beam hardening and with beam hardening correction](image)

7.7. Transmitted X-ray beam and subject contrast

7.7.1. Image geometry

X-rays are emitted from the focal spot of the X-ray tube. A single X-ray point source produces a diverging X-ray beam, which results in the magnification of objects that are positioned some distance from the detector. The focal spot can be considered as a distributed array of point sources, each producing its own view of the object, with the summation of all views creating the projection image. This results in geometric blurring. This blurring is evident at the edge of an object, (the penumbra or edge gradient is often
used to measure the amount of blurring) and causes a loss of object resolution even before X-rays are detected. Geometric blurring can be reduced with less magnification or with a smaller focal spot.

7.7.2. Subject contrast

This describes the ability to differentiate between two adjacent features of an image. However this is affected by the way the image has been produced:

- In diagnostic radiology, contrast is due to the differences in both the LAC and the path length through an object or region of the object.
- In X-ray CT it is caused by the differences in LACs alone.

7.8. X-ray detectors

7.8.1. Characteristics of X-ray detectors

X-ray contrast is recorded by the detection of the transmitted X-ray distribution and its conversion to a two dimensional image. In an ideal world, an X-ray detector would capture with 100% efficiency, without any loss of information producing a perfect snapshot from the locally absorbed X-ray energies for visualisation and analysis. Unfortunately most 2D X-ray detectors are energy integrators which are not capable of distinguishing between individual photon events based on their photon energy. This is due to:

- the fact that polychromatic radiation is used and
- mainly the fluence of the X-rays is very high which would overwhelm detectors with energy discrimination (detector elements must be read after each single event).
However efficient X-ray absorption can be achieved by using detectors with high atomic number elements and high density to increase the probability of photoelectric absorption.

7.8.2. Analogue X-ray detectors

The term “analogue” is given to data that are continuous, such as the brightness variations induced in an X-ray intensifying screen and the recording of the variations based on the continuous response of film (from a macroscopic point of view). The screen-film cassette is the most widely used form of analogue X-ray detector. Screen-film cassettes are comprised of a phosphor screen (phosphor material releases visible light when X-rays are absorbed), a light sensitive film, and a cassette holder. Incident X-rays are absorbed and converted to light. A light sensitive film emulsion in direct contact with the screen produces a latent image, which is subsequently rendered visible by chemical processing. The result is a greyscale image encoded by the spatially dependent build-up of black silver grains in the film emulsion. Image viewing is done by transmitting a uniform light through the developed film, producing a 2-dimensional pattern of light in which the brightness corresponds to the attenuation [Seibert and Boone, 2005].

7.8.3. Digital X-ray detectors

The major difference between analogue and digital X-ray detectors is not the detection and conversion of X-ray photons, but the digitization of the continuous output signal by discrete spatial sampling and quantisation at the detector by an analogue-to-digital converter (ADC). ADCs are specified in terms of the sampling rate (how many samples
can be converted per second), which affects the readout and display speed of the digital device and also the dynamic range (usually expressed in number of bits). Spatial sampling is the division of the X-ray detector area into discrete element areas, over which X-ray information is summed. As digital information is discrete, there is loss of information during digitization.

Direct read-out electronic X-ray detectors can be divided into either direct-conversion detectors or indirect-conversion detectors. The difference is that direct-conversion detectors have an X-ray photoconductor, such as amorphous selenium, that directly converts X-ray photons into an electric charge, whereas indirect-conversion detectors have a scintillator that converts the X-rays into visible light first which is then converted to an electric charge via photodetectors such as amorphous silicon photodiode arrays or CCDs. In addition, indirect-conversion detectors require optical coupling methods such as direct contact, fibre optic coupling or lenses.

Digital imaging has many advantages such as providing the ability to separate the acquisition, display, and archive of images. With digital images, the images can be digitally processed on a computer. Under- or overexposed images may be corrected (within limits) by scaling the data. Image resolution and contrast can be altered by image processing, and quantitative information is easily extracted using appropriate software and display tools. Digital detectors are now coming into place, due to technological advantages, plus they are becoming cheaper and use up less space [Seibert and Boone,
Furthermore, less dose is required, which is advantageous from a medical point of view.

7.8.4. Dynamic range

CCD cameras are being used as detectors due to their high dynamic range. This means that the exposure time can be increased, which in turn increases the signal to noise ratio of the images produced. According to Davis [2004] there is a fourth order relationship between definition (the number of volume elements across the specimen) and the X-ray exposure required to maintain a given signal to noise ratio (SNR) in a tomographic image. The SNR is defined as:

\[ \frac{\mu}{\sigma} \approx \sqrt[n_0]{p} \frac{d}{d} \]

\[ \text{Equation 7.4} \]

where \( \mu = \text{LAC} \)

\( \sigma \) is the noise at the centre of the image

\( n_0 \) is the incident number of X-ray photons per projection

\( p \) is the number of projections

\( d \) is the definition.

7.8.5. Image quality and accuracy

The ultimate quest in X-ray imaging techniques is to use basic science to achieve perfect three dimensional X-ray attenuation maps. After applying reconstruction algorithms the final image obtained is an approximate three dimensional representation. Understanding errors and artefacts, and trying to reduce them helps to improve image quality and accuracy. Davis [1999] defined image quality and accuracy as:
- Image quality is associated with the signal to noise ratio and resolution
- Image accuracy is concerned with errors caused by insufficient data for reconstruction and/or inexact reconstruction methodology.

Both the quality and accuracy of tomographic images depend not only on the scanner and reconstruction methodology, but also on the nature of the specimen itself. Noise is the statistical uncertainty in the image, primarily due to the finite number of X-ray photons detected and will vary with the X-ray source and detection system as well as the specimen.

8 X-ray Tomography

8.1. Introduction

Medical X-rays for diagnostic imaging have been used for over a century, soon after the published discovery by Roentgen in 1896. The underlying basis for medical applications of X-rays remains the same; it depends on the differential attenuation of X-rays when interacting with the object. An X-ray beam incident on an object interacts with the object, producing a corresponding transmitted X-ray flux that is dependent on the attenuation along the beam paths i.e. the proportion of scattered or absorbed X-rays. This produces a superimposed “shadow” of the internal structure. An X-ray–sensitive detector captures the transmitted fraction and converts the X-rays into a greyscale projection image to depict the data obtained. More recently, contributions from engineers and physicists introduced the ability to provide a true 3-dimensional representation of an object by the acquisition of multiple, angular-dependent projections to create tomographic images with computer algorithms. CT revolutionized the use of X-
rays in diagnostic medical imaging and propelled the use of computerized image acquisition for medical diagnosis [Seibert, 2004].

8.2. Sectional Imaging: X-ray CT

A major breakthrough was accomplished in 1972 with the introduction of X-ray transmission computed (axial) tomography (abbreviated as CT or CAT). This 3D imaging technique was brought to clinical medicine by Godfrey Hounsfield and Allan Cormack, and both shared the 1979 Nobel Prize in medicine [Hendee, 1995]. Tomography is a technique for digitally cutting a specimen open using X-rays to reveal its interior structure (“Tomos” is the Greek word for “slice”). A CT image is created by directing X-rays through the slice plane from different orientations and measuring their resultant decrease in intensity. A specialised algorithm is then used to reconstruct the distribution of X-ray attenuation in the slice plane. Many slices can be stacked up to form a 3D replica of the object, analogous to a loaf of bread being reconstructed by piling up all of its slices.

The first CT scanners used a single collimated X-ray source and detector. The source and detector mapped out projection data in a translate-rotate geometry, one X-ray path at a time. However first generation scanners were time consuming, as it took several minutes to acquire enough data to reconstruct one slice (See Figure 8.1(a)) [Hendee, 1995].
In order to acquire data faster, the next set of generation CT scanners were developed which consisted of fan beam scanners. The type of scanner shown in Figure 8.1(b) also used a translate-rotate geometry. Figure 8.1(c) shows a 3rd generation system with a collection of detectors that only have rotational geometry and Figure 8.1(d) shows a 4th generation medical CT system. These types of CT systems obtain data for a slice in seconds.
Configuration of X-ray CT systems:

Figure 8-1 Evolution of geometries of X-ray CT scanners (a) First generation scanner, where the pencil X-ray beam is translated and rotated to cover the object being imaged. (b) Second generation scanner, where the diverging fan beam and detector array are translated and rotated. (c) Third generation scanner which has a fan beam source and detectors rotate together. (d) Fourth generation detector which has a rotating fan beam source and a stationary ring of detectors [Hendee, 1995]
8.3. Development of X-ray Microtomography

X-ray microtomography (XMT) was first developed by Elliott and Dover [1982]. It is a miniaturised version of CT scanning with the ability to image samples with a resolution of micrometres. It is a non destructive technique, so an advantage is that samples can be re-scanned after any mechanical or chemical intervention. Like a CT system, the XMT configuration has three basic components; an X-ray source, an X-ray detector and a sample manipulator. However, unlike medical CT, the X-ray source and detector are fixed and the specimen rotates.

A conventional impact X-ray source is used, as it is not always feasible to use a synchrotron source, but with that come many disadvantages such as beam hardening and low S/N ratio as mentioned previously. However the image quality can be improved by designing a laboratory scanner with a high dynamic range CCD X-ray camera, which has a time delay integration (TDI) readout to remove ring artefacts [Davis and Elliott, 2003].

8.4. Ring Artefacts

The most common type of non-random errors are ring artefacts which are caused by slight differences in the sensitivities of adjacent detecting elements. This causes streaks in each back-projection which reinforce as rings (Figure 8.2). To reduce this effect, the detector array can be ‘randomly’ shifted by a few pixels between projections [Davis and Elliott, 2003]. The TDI technique removes this effect completely.
8.5. Advantages of using XMT

In biological studies the main advantage of XMT is its ability to quantify the mineral content of hard tissues in three dimensions at the microscopic level. Two important applications are the study of bone loss in osteoporosis and the loss of mineral content in teeth due to caries. These are not directly fatal conditions but they cost a lot in medical care worldwide, making research in these areas very important. For example, the annual cost of dental treatment in 2006 to the NHS in the UK was about £1.5 billion. [http://www.dpb.nhs.uk/archive/nhs_statistics/latestdata.shtml]
8.6. Comparison of MuCat system and the commercial XMT systems

The main differences between the MuCat XMT scanner developed at Queen Mary, University of London, and other commercial XMT systems are the improved signal to noise ratio and calibration procedures. The time-delay integration scanning method enables high dynamic range images at the expense of data acquisition time. Although correction for beam hardening is fairly straightforward, the commercial suppliers do not devote resources in correcting it since accurate greyscale calibration is not needed for most applications.

8.7. Applications of XMT

The wider applications of XMT are applied to geosciences which include studying the interiors of fossils or meteorites; textural analysis of igneous and metamorphic rocks; quantification of porosity and permeability in rocks and soils [Ketcham and Carlson, 2001]. In material sciences, XMT has been used to investigate inorganic matrix composites, transport in media and fatigue crack closures [Stock, 1999]. Recently, a fascinating application of XMT is to study foods such as analysing air bubbles in dairy products and pores in rice kernels [van Dalen et al., 2003]; or visualisation of ice crystal structures formed during freezing of a number of foods such as meat, fish, chicken, potato, cheese and carrot [Mousavi et al., 2007].
8.8. XMT studies of mineralised tissues

The SMD (School of Medicine and Dentistry) group at Queen Mary, University of London, apply XMT to study hard tissues, mainly bone and tooth [Davis and Wong, 1996].

All the studies by the XMT group at Queen Mary use an aluminium step wedge for calibration (which will be discussed in Section 10.1.2). As shown in Figure 8.3 the LAC vs. Energy plots for aluminium and hydroxyapatite are similar in the low energy range. In relation to dental hard tissues, many studies have been carried out ranging from in vitro endodontic studies [Dowker et al., 1997] to the mineral concentration gradient of enamel of mandibular rat incisors [Wong et al., 2000].

![LAC vs. Energy plot for HAP and Aluminium](image)

**Figure 8-3** Graph showing the LAC of 100% hydroxyapatite (HAP) and Aluminium with varying energy
Recent works in the department are briefly discussed as follows:

1) Wong et al. studied the morphology of dentine caries, showing in 3D that the pulp receded as a result of bacterial attack [Wong et al., 2006].

2) Kawabata et al. utilised XMT to investigate diffusion in dentine [Kawabata et al., 2008].

3) Ahmed et al. used XMT to follow local failure in trabecular bone after applying different strains. In addition a full femoral head was scanned at 25 µm to visualise and quantify the distribution of microcallus patches [unpublished work].

4) Atar et al. used the XMT to study diabetic rodents [Atar et al., 2007]

Another group which uses XMT to study biological hard tissues is the group at UCSF. Kinney et al. used a synchrotron X-ray source to study the mineral concentration of dentine caries at a high resolution, with the ability to image individual dentinal tubules [Kinney et al., 1994]. Recently they published a paper on age-related transparent root dentine [Kinney et al., 2005]. The group is well established in bone studies and calcified tissues such as an in vivo study of osteoporosis in rats [Kinney et al., 1995b], fracture healing, kidney stone disease and autoimmune diseases such as arthritis [https://www.llnl.gov/str/Kinney.html].
9 General Aims and Objectives

In the previous chapters, it was concluded that the boundary/level to which a dentist should excavate is unclear. Conventional techniques, although they are capable of high resolution, are mainly destructive and cannot follow through a sample after chemical or mechanical intervention. Quantitative XMT has been identified as a technique that has the capability in quantifying mineral concentration accurately and following the changes in the same sample in interventional studies. Hence the general aims of the present study were to use XMT:

- To investigate the mineral concentration of natural carious lesions and to determine the boundary/level a clinician should excavate to;
- To evaluate the volume of over- and under-excavation of dentine caries using various techniques;
- To correlate the microstructure of carious dentine with other microscopic techniques with higher resolution such as BSE and AFM.

The general objectives were:

- To investigate and compare the volume of tissue removed using chemomechanical and conventional excavation procedures;
- To measure the LAC of dentine to which each method is excavated to;
- To investigate the LACs of the carious dentine that was removed; To determine the mineral concentration gradient of the carious lesion;
- To relate the gradient to caries progression;
• To explore the relationship of XMT images with BSE and AFM images and colour changes visually.

In order to fulfil the general aims, small experiments were conducted and their specific aims and objectives will be presented in the following chapters.
Part II

X-ray Microtomography (XMT) study of dentine caries and its removal by three different techniques
Part II: XMT study of dentine caries and its removal by three techniques

10 Comparison of XMT systems

Introduction and Aims

There are many commercialised microCT/XMT systems available in the market. The Department of Physical Dental Sciences at Queen Mary, University of London (QMUL), has developed an ‘in-house’ system called MuCat which claims to be more quantitative. The aim of this section is to compare the performance of a commercial XMT system, SkyScan 1072, with the QMUL ‘in-house’ system, MuCat. A further aim was to compare the original MuCat system with a later QMUL developed high definition (HD) MuCat 2 system.

Materials and Methods

10.1. MuCat XMT system

10.1.1. Description of MuCat XMT system

XMT scanners have gone through many developmental stages from single-beam detector system (1st generation) to the cone beam area detector system. In this study a 4th generation XMT scanner (not to be confused with the 4th generation of medical CT) developed by Davis and Elliott [2003], was used to eliminate ring artefacts (Figure 10.1).
This system uses a cooled slow scan CCD camera (Astrocam, Ltd, UK, now part of Perkin Elmer Inc.) with a 1:1 lens coupling to 70 µm thick columnar CsI (Cesium Iodide) scintillator (Applied Scintillation Technologies Ltd, UK). In order to eliminate ring artefacts, the scanner uses the TDI (time delay integration) CCD readout method. This averages out the characteristics of all the detector elements in each row (CCD column) of the projection. The camera moves through the X-ray beam and simultaneously the CCD is read out (Figure 10.2). The camera motion and the readout are timed such that the relative motion of charge along the CCD surface matches that of the focused image (Figure 10.3). Each recorded image pixel is derived from the accumulation of charge as it is shifted along the CCD such that the sensitivities of all the recorded pixels in each row of the image are the same.
Figure 10-2 Example of a circle being imaged and how the CCD reads out the data
Figure 10-3 Time sequence showing TDI CCD read out of the captured image
The CCD detector has 1152 x 770 square pixels of dimension 22.5 µm which are binned 3 x 3, giving 384 x 256 square binned pixels of dimension 67.5 µm. The geometric magnification, gives a sample voxel size down to 8 µm. The X-ray generator (X-Tek Ltd., UK) has a spot size down to 5 µm, and so in theory it is possible to obtain even higher resolution. For dimensional stability of the system during data acquisition, the air temperature inside the X-ray enclosure is controlled to within 0.1ºC.

At the start of every scan, a dark reference image is taken, with the CCD shuttered, and then a light reference scan is taken without the sample in the X-ray beam.

Another feature of the XMT design is its moving slits. The slits move in synchrony with the detector which reduces the scattering of the X-ray beam (Figure 10.4 to see the difference between scanning a sample without and with moving slits). As a result this improves the contrast and the LAC measurements.
Figure 10-4 Schematic diagram of bird’s eye view of XMT system showing the difference between scanning a sample (a) without and (b) with moving slits (page 79 [Wassif, 2007])
10.1.2. Calibration

In order to correct for beam hardening due to polychromatic radiation, the following calibrating technique was employed.

Originally a symmetrical seven step wedge (thickest at the centre) made of 99.98% Al, 1.2 mm thick sheets, was used for calibration (Figure 10.5). It was scanned periodically at each combination of voltage and filter thickness used.

![Figure 10-5 Seven-step aluminium step wedge](image)

However, there are problems linked to using this wedge design. Due to the wedge being thickest at the centre, scattering effects can influence the intensity and in turn affect the true LAC of the sample. So another aluminium step wedge design (Figure 10.6) was adopted to overcome the scattering effects. The thickness of the individual sheets was 0.54 mm. For the vertical wedge each step is scanned individually, thus no radiation is scattered from the thinner steps into the shadows of the thicker steps.
The reason why aluminium was used is because its attenuation coefficient versus energy relationship is similar to that of HAP and both have no absorption edge within the energy range of the present XMT system. The requirement is that the calibration material should behave similarly to that of the scanned object with regards to attenuation vs. energy. Plus high purity Aluminium is easy to obtain in foils. For studying dental hard tissues, thus Al is suitable.

In addition, a piece of aluminium wire was mounted beside the tooth samples (99.999% purity, Alfa Aesar) to be scanned together as an internal check, giving ‘fine tuning’ to the overall data set. The LACs of the tooth in the final reconstructed image are calibrated to the measured Al LAC at 40 keV. For each CCD column (corresponding to
image rows), a 5\textsuperscript{th} order polynomial is fitted to the measured attenuation against the calculated attenuation for a chosen monochromatic energy which was 40keV in the present experiment (X-ray acceleration voltage 90 kV). This is because 40keV is the energy at which the LAC of Al gives 50\% attenuation with approximately the same thickness as with the 90 kV polychromatic radiation.

10.1.3. Data collection

To calculate the minimum number of projections needed for a sample is:

\[
\text{Number of projections} = \frac{\pi}{2} x
\]

Equation 10-1

where \( x \) is the diameter of the sample (assuming the sample is approximately cylindrical) in voxels [Kak and Slaney, 1987]. An odd number of projections is used to give angular interlacing, at least for the central ray, over the first and second 180\(^\circ\) of rotation [Davis and Elliott, 2006b]

The total time for each scan is equivalent to the number of projections times the time for each projection. The time for each projection includes the fly back time (which depends on the specimen size, but is typically 3 - 4 seconds) and the traverse time (\( t \)). The traverse time is expressed as follows:

\[
t = t_x \left( 1 + \frac{N + 64}{384} \right)
\]

Equation 10-2

where \( N \) is the sample diameter, \( t_x \) the exposure time which is 10 seconds minimum, the value 64 comes from the 32 pixels on either side of the specimen used for measuring \( I_0 \) (initial intensity), and the value 384 comes from the width of the CCD detector in binned pixels. The resolution of the data could be of either 8.7, 15 or 30 µm. The latter
resolution was used for the present experiment to reduce scanning time because the higher resolution was not needed for clinically relevant accuracy.

10.1.4. Linearisation

The first stage of image reconstruction is pre-processing the data, which converts intensity to \( \ln \frac{I_0}{I} \) values and applies polychromatic correction. When the data is processed the dark reference is deducted from the light reference and the log is taken. For each image row a parabola is fitted. Then for the rest of the projections the dark reference is subtracted and the log is taken for each projection. This is then subtracted from the appropriate reference parabola and an offset adjustment is made in relation to the mean log of the 32 pixels on both sides of the projection, which is why the scanned image length is set so that there is always a minimum of 32 pixels either side of the sample at each projection. The polynomial created in the calibration process is then applied to provide ‘correct’ values for the attenuation at 40 keV, as if monochromatic radiation was used. The pre-processing stage produces a ‘.cra’ metadata text file and ‘.con’ floating point data file.

10.1.5. Reconstruction

These corrected projections are then reconstructed using a modified Feldkamp cone-beam back projection algorithm [Feldkamp et al., 1984; Davis and Elliott, 2006b]. The files produced are loaded, and for the central slice, the projected centre of mass is located for each projection. From these, the centre of rotation is calculated. However the
TDI introduces a slight bias in this value and so the central slice is reconstructed first, which is done 11 times with different centre of rotation offsets (± 5 pixels) with edge enhancement. The sharpness is determined from the sum of squares of the edge enhanced image. A parabola is fitted to the three highest sharpness values and the location of the peak of this parabola is taken as the centre of rotation. Having determined the centre of rotation, the complete 3D volume is reconstructed and stored in a ‘.bin’ file, containing reconstructed LAC data, as floating point values. This output file is ‘trimmed’ to a reduced size file to a minimum cuboid volume around the sample or region of interest (using IDL®, Research Systems Inc., Colorado, USA) and converted to 256 grey levels (maximum-minimum levels) to produce a final reconstructed data file, ‘.tom’ file. Further to this, the data is normalised to alter the LAC of all the data points so that the Al wire beside the sample gives the calculated value of 1.5336 cm$^{-1}$ for Al at 40 keV. The mean LAC of the Al wire can be calculated from the ‘trimmed’ file and then the floating point to 8-bit conversion is repeated with an appropriate adjustment factor.

10.1.6. Visual display and analysis

IDL®, Amira™ (TGS Template Graphics Software Inc., USA), and VG Studio Max (Volume Graphics, Germany) and Drishti are software packages used for visualisation of the XMT data set. They have similar algorithms and each has different advantageous features. The Amira™ program allows visualisation of single slices as well as surface and volume rendered images enabling viewing of a sample from any angle. VG Studio Max was used to view and manipulate the reconstructed images of the sample as it can
manipulate large sample sizes. Drishti performs high speed volume rendering using the graphics card and has the feature of using gradient information to identify partial volume effects [Limaye, 2006].

10.2. Comparison of MuCat XMT system and commercial system SkyScan

10.2.1. Introduction

There are commercial XMT systems now widely available on the market. This experiment was designed to compare the MuCat XMT system to the SkyScan 1072, using a HAP disc and a carious tooth as samples for investigation.

10.2.2. Description of SkyScan apparatus

The following is a brief description of the SkyScan 1072 (SkyScan, Aartselaar, Belgium):

The SkyScan 1072 is a compact desktop system for X-ray microscopy and microtomography. It consists of a combination of X-ray shadow microscopic system and computer with tomographic reconstruction software. The equipment contains an X-ray microfocus tube with high-voltage power supply, a specimen stage with precision manipulator, two-dimensional X-ray CCD-camera connected to a frame-grabber and a Dual Pentium computer with colour monitor. For the SkyScan 1072, the X-ray microfocus tube has a focal spot size of 7 μm which operates at 20-100 kV / 0-250 μA. The X-ray CCD-camera is based on a high resolution (1024 x 1024 pixels) cooled CCD-sensor with fibre optic taper coupling (3.7:1 image reduction) to the X-ray scintillator.
The X-ray shadow projections are digitized as 1024 x 1024 pixels with 4096 brightness gradations (12 bit) for cooled camera or 256 gradations (8 bit) for analogue camera. The reconstructed cross-sections have a 1024 x 1024 pixels (floating point) format. Typical data for reconstruction contains shadow image acquisitions from 200 to 400 views over 180 or 360 degrees of the object.

10.2.3. Reconstruction

For the reconstruction of a three-dimensional object one can use a series of cross sections (raw data) obtained at different orientations using software NRecon (version 1.4). For the reconstruction of the carious tooth, the central slice was reconstructed using the available options to improve the beam hardening effect (options are given as a percentage) and ring artefacts (options are 0-20). Once the parameters are chosen this is followed by an "off-line" reconstruction of the complete three-dimensional object in a resolution of 1024 x 1024 x 1024 layers. After the serial reconstruction, one can display cross-sections of the object onto the screen as well as construct a realistic view of the 3D-object with possibilities to "rotate" and "cut".

10.2.4. Visual display and analysis (CTan)

CTAn is the name of the application for deriving quantitative parameters and construction of visual models from scanned data sets obtained with SkyScan 1072.
10.2.5. Sample preparation

10.2.5.1. HAP sample

A piece of hydroxyapatite (HAP) with 20% porosity (Plasma-Biotal Ltd) was cut and then mounted on to the specifically designed stage for the SkyScan using BluTack (Bostik), ensuring that the specimen was centered. The scan was imaged at the best resolution possible without saturation which was 7.32 µm. The piece of HAP was then mounted on to the aluminium plate for the MuCat XMT with sticky wax (Griffin & George Ltd) and scanned at a resolution of 8.7 µm (the highest resolution on the MuCat system), as this would allow comparisons to be made between the two XMT systems.

10.2.5.2. Carious tooth sample

In order to ensure that the same parameters and conditions were reproduced during scans obtained with MuCat and the SkyScan systems, the following approaches were adopted. Firstly a whole tooth was mounted onto an aluminium plate designed specifically for the MuCat XMT system with Polymorph (See Figure 10.8). The tooth sample was then scanned at a resolution at both 15 and 30 microns. Afterwards, just the top part of the mounting with the carious tooth sample was cut and placed onto the mounting piece specific to the SkyScan system (See dashed section of Figure 10.7).
As a copper foil filter (99.995% purity, thickness 0.05 mm, Advent Research Materials Ltd, Oxford, UK) is used in the MuCat XMT, a copper filter was made for the SkyScan system also, with dimensions 35 mm x 40 mm. The tooth sample was then scanned at a resolution of 14.66 microns (Image pixel size 14.65 microns). The source voltage was 90 keV and the source current was 110 µA. The rotation step size was 0.45°. The object to source distance was 204 mm.

10.3. Comparison of MuCat and MuCat2 systems

The main differences between MuCat and MuCat2 are the scintillator to CCD coupling and the size of the CCD detector. Optical lens coupling is used in the MuCat system, which was found in a study by Davis and Elliott [2006a] to be approximately 60% efficient (in terms of equivalent number of X-ray photons detected). In MuCat2 the scintillator is coupled to the CCD by means of a parallel fibre-optic faceplate. In comparison to the lens coupled system, the fibre-optic coupled scintillator showed a

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**Figure 10-7 Sample mounting of the sample for the comparison of excavation techniques experiment**

_cpus Figure 10-7 Sample mounting of the sample for the comparison of excavation techniques experiment

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marked improvement in efficiency, but because of this, the detector has a greater tendency to saturate thus limiting the maximum exposure time and/or beam current.

MuCat2 has a larger detector than MuCat (4k x 4k pixels), giving an image size of 60 mm x 60 mm. The device was read out in TDI mode with 2 x 2 binning in the chip, and a further 2 x 2 binning carried out in software, giving 4 x 4 binning in total. This gives a final working image pixel size of 60 µm, compared to 67.5 µm with the lens coupled system. By increasing the size of the detector array, the data acquisition time can be reduced without increasing the X-ray flux or sacrificing the signal to noise ratio [Davis and Elliott, 2006a]. In addition, in the MuCat2 system, a variable resolution can be chosen down to 8 µm, unlike choosing one of either 30 µm, 15 µm or 8 µm for the older MuCat system.

Another difference between the two systems is the calibration procedure. For MuCat system, the method of calibration is to fit a polynomial curve to the calibration data from the step wedge. Davis et al. [2008] described the method of beam hardening correction/calibration for the MuCat2 system. It is a modelling approach which makes use of a priori information. In addition to knowledge of the specimen, it makes use of the X-ray generator settings (voltage and take-off angle) and scintillator type. The purpose of the model is to predict how attenuation through the sample differs from that of the calibration material and to be able to extrapolate this attenuation beyond the range of the step wedge. A polynomial is fitted to the derived attenuation curve (maintains compatibility with existing beam hardening correction software). Once the spectrum is
determined from the model, a “virtual step wedge” can be made from any material using published X-ray attenuation data. This model was written in IDL (ITT Visual Information Solutions), using the “amoeba” multidimensional minimisation function to find the optimal tungsten and scintillator thickness, which minimises error. This model approach was developed recently and so could not be utilised for the data obtained for this project.

10.4. Results

10.4.1. Comparison of MuCat system with SkyScan

10.4.1.1. HAP pellet sample

The Sky Scan image of a HAP sample at 7.32 µm is shown in Figure 10.8 and the MuCat XMT image of the same sample at a resolution of 8.7 µm is shown in Figure 10.9. Using the TView software (Skyscan dataset viewer) the dataset can be visualised in original, greyscale, a choice of two pseudocolour scales and binary. The greyscale and colour scales were used to highlight features and allow comparisons to be made between the two XMT systems.

As seen, the SkyScan images are extremely noisy due to the fast acquisition; this has a profound effect on the contrast resolution of the reconstructed image i.e. losing the ability to pick up subtle features (Figure 10.8). In comparison, the MuCat images are less noisy (Figure 10.9), showing the uniformity of the structure of the HAP.
Figure 10-8 SkyScan XMT scan imaged at 7.32 µm resolution. (a) greyscale, (b) colour1, (c) colour2, (d) binary (TView software from SkyScan)
Comparing the line profiles through the HAP sample (Figure 10.10), the SkyScan data plot depicts ‘dishing’ artefacts which are not found in the XMT plot. The MuCat XMT dataset was corrected, giving an average LAC value of 1.965 cm$^{-1}$ (greyscale value 196.5). As there are no set protocols for correcting the ‘dishing’ artefacts in the SkyScan data, no correction was applied.

Another difference that can be seen between the two XMT systems is that the MuCat images show more ‘true’ structure as voids and bright spots can be visualised and therefore quantitative measurements can be studied, such as porosity (Figure 10.11). These features could not be detected in the SkyScan image due to noise.
Comparison of lineprobe through HAP sample using SkyScan and MuCat XMT systems

Figure 10-10 Comparison of line profiles through HAP sample using Skyscan and MuCat XMT systems

Figure 10-11 MuCat XMT image highlighting true structure (slice 157 (brightness 104, contrast 445)) (TView software from SkyScan)
Although ring artefacts could be seen in the MuCat scans, they were not as consistent throughout the sample as the SkyScan images (Figures 10.12 and 10.13). These faint rings from the MuCat system are typically caused by specks of material on the X-ray output window.

Figure 10-12 SkyScan XMT image highlighting ring artefacts (slice 996, brightness 104, contrast 445) (TView software from SkyScan)

Figure 10-13 MuCat XMT image highlighting ring artefacts (slice 157) (In-house software, TomView)
### 10.4.1.2. Carious tooth sample

The following is a comparison of the three datasets acquired from SkyScan and MuCat of a carious tooth. The SkyScan dataset was imaged at 14.6 µm (Figure 10.14 (a)) and the MuCat datasets were imaged at 14.79 µm and 29.55 µm (Figure 10.14 (b) and (c) respectively). The acquisition time and reconstruction time for both systems are summarised in Table 10.1:

**Table 10-1 Table comparing the acquisition and reconstruction times for both SkyScan and MuCat XMT systems**

<table>
<thead>
<tr>
<th></th>
<th>SkyScan 1072</th>
<th>MuCat system</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acquisition time</strong></td>
<td>4 hrs 57 mins at 14.6 µm resolution</td>
<td>11 hrs 13 mins at 14.79 µm resolution</td>
</tr>
<tr>
<td><strong>Reconstruction time</strong></td>
<td>~ 1 hr</td>
<td>~ 30 mins</td>
</tr>
</tbody>
</table>

Comparing the reconstruction slices of the two systems, clearly the MuCat XMT scans show less noisy images than the SkyScan images (Figure 10.14).

**Figure 10-14 2D greyscale XMT images of carious tooth samples imaged with (a) SkyScan, (b) MuCat system at 15 µm resolution and (c) 30 µm resolution**
Figure 10.15 shows a 3-D surface rendered image of the SkyScan dataset using their image software CTan. Surface rendering consists of contouring lines over datapoints. This image demonstrates that a surface rendered image will give a final representation of the dataset; however this hides the noise depending on the threshold applied. Surface rendering is not ideal for visualising noisy datasets. Volume rendering is preferred as different opacities and colours, therefore different materials/phases can be highlighted as it is a ray tracing technique, observed by the viewer. It is considered a direct visualisation of any dataset.

Figure 10-15 3D surface rendered image of carious tooth sample using the SkyScan software, CTan
For a quantitative comparison of the two XMT systems, line profiles were taken through sound enamel and sound dentine (Figure 10.16), as the natural gradient of sound tissue is not as varied as carious dentine or enamel tissue. Figure 10.17 shows the comparison for the three datasets. As the MuCat system has the ability to pick up subtle changes in LAC, the natural gradient in both enamel and dentine can be seen, and the low resolution MuCat scan appears smoother in comparison to the higher resolution. The SkyScan plot does not depict the natural gradient as the variation caused by the noise hides the subtle details.

Figure 10-16 Greyscale images of where the line was taken through the carious tooth sample
Figure 10-17 Line probes through the carious tooth datasets to compare the SkyScan and MuCat XMT systems. The MuCat system (purple and blue lines) can pick up subtle features such as the natural gradient of enamel and dentine, whereas with the SkyScan system, it is difficult to distinguish the natural gradient of enamel and dentine from the noise.

Line profiles were taken through the aluminium wire, to see the differences between the XMT systems (Figure 10.18). Even after applying the beam hardening correction, the aluminium wire displays dishing artefacts with the Sky Scan system. With the MuCat system, after applying the calibration and normalising the data, the lineprobe through the wire is consistent and free of dishing artefacts.
For improvement of the SkyScan images, at the reconstruction stage varying levels of beam hardening correction and ring artefact reduction could be applied. Figure 10.19 shows the effect the ring artefact reduction value had on the carious tooth image. Even at the value of 15 which is an arbitrary way of reducing artefacts, ring artefacts could still be detected in the image. For the correction of beam hardening, the user chooses a percentage value to improve the overall image. Figure 10.20 shows a plot of the effect of the 40%, 50%, 60%, 70% and 80% beam hardening correction on the XMT image. It could be seen that at 80% correction, the gradient in enamel on the right side of the graph disappeared. However, it would be difficult to justify whether this has corrected
for the ‘dishing’ effect; or it has over-corrected to mask the actual gradient that is usually found in enamel (e.g. comparing the graphs using the MuCat in Figure 10.17). Usually, it was recommended to use a value of 70% for beam hardening correction and 5 for ring artefact reduction. It could be seen that the ‘dishing’ effect appears reduced with increase in percentage. For reconstruction the beam hardening correction and ring artefact reduction was based on visual judgement; 70% beam hardening correction and value 5 for the ring artefact reduction. It should be noted that, since there is a true radial gradient in mineral concentration, this subjective method of beam hardening correction adjustment is unreliable and likely to give erroneous results.

Figure 10-19 SkyScan images of the root of the carious tooth with the ring artefact reduction applied at (a) value 1 and (b) value 15
Effect of beam hardening correction on XMT image

Figure 10.20 Effect of beam hardening correction on the SkyScan XMT image

10.4.2. Comparison of MuCat and MuCat2 systems

Figure 10.21 shows a comparison of a tooth sample embedded in PMMA imaged using MuCat and MuCat2. The image obtained with the MuCat2 system has a higher contrast in comparison to the MuCat. The zoom in images in Figure 10.21 are of features in the datasets, to highlight the subtle differences between the XMT systems. The crack in the enamel is more clearly defined in the MuCat2 image than the MuCat image, even though the MuCat image was obtained at a better resolution. This was confirmed by Davis and Elliott [2006a].
Figure 10-21 Comparison of single 2D slice of an embedded carious tooth sample imaged with MuCat and MuCat2.

(a) **MuCat1_compare**: (744, 614, 212) at 14.79 µm – 1501 projections – slice 136
(b) **MuCat2_compare**: (744, 614, 348) at 15.00 µm – 1501 projections – slice 269
A line profile taken through the sample was taken to qualitatively see if there are any differences in the ability to pick up subtle features such as the natural gradient within enamel (Figure 10.22). This showed that both systems are exactly the same in terms of detecting the subtle gradient in enamel. However looking more closely, MuCat2 depicted what looks like a noisier profile, but this may be due to the difference in exposure times for the systems (for the MuCat2 scan, the frame average was two).
Comparison of MuCat and MuCat2 systems

Figure 10-22 Line profiles through embedded tooth sample comparing both MuCat and MuCat2 XMT systems
10.5. Discussion

10.5.1. Comparison of MuCat system with commercial SkyScan system

The results shown for comparing the MuCat and SkyScan systems proved that the MuCat system was the better system for the present study. The system was specifically designed for accurate quantitative analysis, with better S/N ratio and an emphasis on beam hardening correction. With the SkyScan system, structural characterisations of biological systems/materials are better suited as it costs less and data acquisition is faster. A key example illustrating the difference in suitability of the two systems is the natural gradient in enamel and dentine. The noisy images (Figure 10.12 and 10.14 (a)) obtained with the SkyScan and lack of precise beam hardening correction make it a system incapable of clearly defining the gradient through tooth tissues [Clementino-Luedemann, 2007]. The SkyScan images are extremely noisy due to the fast acquisition; this has a profound effect on the contrast resolution of the reconstructed image i.e. losing ability to pick up subtle features. In comparison, the MuCat images show less noise, showing the uniformity of the structure of the HAP (Figure 10.11).

HAP and Carious tooth

A key advantage of TDI feature of MuCat XMT system is the elimination of ring artefacts [Davis and Elliott, 1997]. However ring artefacts were shown in images obtained by both XMT systems (Figure 10.12 and 10.13). The SkyScan images displayed more frequent and more intense rings in comparison to the MuCat systems. However the ring artefact reduction feature on the SkyScan software seemed to be arbitrary, as the ring artefacts reduction percentage parameter was increased, the
‘ringing’ effect became more emphasised. But the final parameter value decision is based on visual appearance.

According to SkyScan (personal communication with Xuan Liu at SkyScan), the explanation of the ring artefact reduction was provided: NRecon (SkyScan reconstruction software) corrects small non-uniformity using the sum of all projection images in a scan. This sum image reflects the detector uniformity during the scanning and is usually very smooth. For each detector pixel, the relative difference between the pixel and its neighbours (the value of its neighbours are obtained using a median filter: the ring correction level selected by the user sets the half-width of the median filter) is assumed to be the fluctuation of the detector pixel, and is therefore used to correct the pixel at each projection. This works very well for small deviations. For detector pixels with large variation from the neighbours, in extreme cases, a completely or partially dead pixel or an extremely bright pixel (can be observed in the sum image, and sometimes also in the individual projection image), the method mentioned above might fail. In the later NRecon versions, the values of these “defect” pixels are simply ignored and replaced by those of their “good” neighbours using interpolation. This works adequately in most cases. This method cannot correct differences in non-linearities between adjacent detector elements, such as might arise from slight differences in scintillator thickness.

10.5.2. Comparison of MuCat and MuCat2

The images obtained with the MuCat2 system have better contrast resolution in comparison to the original MuCat scanner. The original system used a relay lens comprising two 50mm f1.2 camera lenses mounted face to face. At full aperture, there
was some loss of contrast and definition. MuCat2 uses a fibre-optic faceplate to couple light from the scintillator to the CCD. This faceplate incorporates enhanced statistical extra mural absorbers to absorb scattered light, giving the best possible image contrast (an early version of the MuCat 2 scanner had used an inferior faceplate requiring deconvolution to compensate for scattered light [Davis and Elliott, 2006a]).

10.6. Conclusions

For this study MuCat/MuCat2 is more suitable as it was designed for longitudinal studies and the ability to pick up small differences in the linear attenuation coefficient locally. In comparison to commercial Skyscan XMT system, the MuCat systems achieve better contrast resolution (at the expense of time), produce less artefacts and because of the beam hardening correction and calibration used, the LAC values obtained can be directly related to mineral concentration.
11 XMT study of Caries Removal Techniques

11.1. Comparison of sound dentine removed between Carisolv, Hand Excavation, and Hand Excavation with Caries Detector Dye techniques

Introduction and Aims

Hand Excavation is a conventional method of excavating dentine caries based on tactile assessment of hardness. Caries Detector is commercially available product which claims to stain bacteria, thus giving a visual aid for the dentist to identify the boundary between “infected” and sound dentine. Carisolv is a commercially available chemo-mechanical method which claims to remove infected carious dentine only. The main aim of this section was to compare the efficacy of these three excavation techniques (section 11.1 - 11.7). Other factors which might affect the volume of sound dentine removal were also investigated. Further small experiments were carried out to investigate:

(1) the effect of the Carisolv solution on dentine (section 11.8);

(2) the potential for remineralising residual dentine after caries removal (section 11.9);

(3) the effect of remineralising solution on a natural carious lesion (section 11.9);

(4) the relationship between colours and LACs from XMT images (section 11.10).

Null hypotheses

In order to investigate whether chemochemical removal technique, or hand excavation technique aided with caries indicator is better than conventional hand excavation technique in preserving sound dentine, the following null hypotheses were formulated:
There is no difference in the volume of sound dentine removed by conventional hand excavation and chemomechanical excavation caries removal techniques;

There is no difference in the volume of sound dentine removed by conventional hand excavation and hand excavation aided with Caries Detector.

11.2. Materials and Methods

11.2.1. Sample preparation

Extracted human deciduous molars with opened carious cavities were obtained from patients with consent for this experiment (REC Ref. No: 06/Q0605/82 – See Appendix A). These teeth were stored in 70% ethanol. They were longitudinally cut in half through the centre of the cavitated carious lesion using a rotary saw (Microslice 2, Malvern, England). The surfaces of the two halves were scanned using an optical colour scanner (Perfection 1640SU Scanner, Epson). The two halves were reassembled separated by a ~ 2 mm gap. In early experiments, their root portions were mounted in Polymorph (Middlesex University Teaching Resources (MUTR) Ltd.) with the cavities exposed. Polymorph is a commercial polymer with the characteristics of a tough ‘engineering’ material yet it fuses and becomes easily mouldable at just 62°C with either hot water or a hairdryer and is easily moulded by hand. The tooth sample mounted in the Polymorph was then mounted onto an aluminium plate that was specifically designed for the MuCat system (Figure 11.1). One of the requirements for the mounting of the tooth sample is that the two halves are securely reassembled, and that minimum movement occurs during the excavation process. However, the preliminary results showed that with the Polymorph, there was slight rotation of the sample after excavation. Eventually, Epoxy
putty (RS Components, UK) was found to be more secure and so was used as the mounting compound for later experiments.

Figure 11-1 Sample mounting of the sample for the comparison of excavation techniques experiment

In order to closely simulate clinical conditions, the sample needed to be kept moist. Also, dryness causes samples to shrink, causing an erroneous increase in linear absorption coefficient within a voxel. Hence, drops of de-ionised water were placed on the sample which was wrapped tightly in cling film to prevent dessication during scanning. In between the scans, the specimen was immersed in de-ionised water.

11.2.2. Excavation methods

After an initial base line scan the tooth was taken to a clinician (FW) who was instructed to excavate all the caries in one half of the lesion using a new spoon excavator (Excavator No.127/8 (Part No 9790923) – DE Healthcare Products, Gillingham, UK) until the dentine felt hard, thus following the conventional method of caries removal using tactile feedback. This acted as the control. On the other half, caries was removed
using either Carisolv (MediTeam/OraSolv, Sweden) system or with the aid of Caries Detector Dye (Kuraray Medical Inc., Tokyo, Japan). The allocation of the excavation technique to which half of the tooth was assigned randomly. The clinician followed the instructions which came with the products. The whole procedure was set up such that it closely resembled a typical clinical situation as far as possible. It was carried out in a dental operatory under dental lighting conditions. For Carisolv, the gel was freshly mixed every time and was applied onto the dentine lesion for one minute. The manufacturer’s hand instruments were used to ‘scrape’ the soft dentine. The lesion was then washed and the procedure was repeated until no noticeable amount of dentine could be removed (CSS technique). For Caries Detector Dye (CD), the dye was applied to the carious dentine for 15 seconds, the lesion was washed with water and a hand excavator was used to remove the red stained dentine. The procedure was repeated until the dentine could not be or only faintly stained by the CD. After the excavation procedure was completed, the tooth was rescanned with XMT and then both scans were reconstructed and analysed. After the main study of comparing the 3 excavation techniques, a small experiment was carried out to compare the CSS techniques to using Carisolv hand instruments only without the gel.

11.3. Comparison of sound dentine removed by the three techniques

The comparison of the efficacy of the three excavation techniques were carried out in two ways:

1. Qualitative approach - to identify which technique adheres to the ideal principle;
2. Quantitative approach - to test the null hypotheses of volume of sound dentine removed by the 3 techniques.

*Qualitative approach* - An ideal technique of caries removal was regarded as one that removed all the grossly demineralised dentine, left a layer of the partially demineralised dentine intact and removed the least amount of sound dentine. In order to test which technique would lead to this ideal result, the present investigator was blinded to which technique was used on which half of the tooth. The investigator had to use the results from the analysis to determine which half of the tooth had the better preparation according to the above “ideal”. Afterwards the technique was disclosed by FW. The agreement between preparation method and result was analysed using chi squared statistics.

*Quantitative approach* – Using the XMT data, comparison of the volume of sound dentine removed were measured and compared between each half of the tooth. Paired t-tests were used to test the null hypotheses.

11.4. Results

Before the results of the three caries removal techniques could be compared, it was necessary to determine the LACs for carious and sound dentine from the XMT data. The following sections describe the strategies that were used to analyse the data in 2-D and 3-D.
11.4.1. General analysis of teeth before excavation using 3-D data

A typical scan time for a tooth was about 6.5 hours for 651 projections. The data was acquired with a filter which consisted of 1.2 mm Al and 50 µm Cu. After reconstruction, which usually took 15 minutes, the extent and the shape of the dentine caries could be seen in three orthogonal planes (XY, XZ and YZ) as XMT slices (Figure 11.2 for a test tooth sample). The XMT slices show that the carious lesion had a ‘dark’ body, i.e. the bulk of material in the cavity, and a demineralising front advancing into the sound dentine. These appear to correspond to the brown lesions and the pale white front respectively as seen visually in the optically scanned image and the photographs (Figure 11.4). The whole data set could also be volume rendered to show the lesions in 3-dimensions (Figure. 11.3). In this 3-D rendered image, the body of the lesion was made red to show that the base of the lesion was ‘bowl’ shape. The demineralising front was very close to the pulp horn. The demineralising front (or ‘affected’ dentine) was highlighted green.

The histogram of the whole dataset (Figure 11.5) show that the LACs for the body of the lesion was 0.35 - 0.6 cm\(^{-1}\), for sound dentine 1.4 - 1.75 cm\(^{-1}\), for sound enamel 2.3 – 2.75 cm\(^{-1}\). Between the body of the lesion and the sound dentine, there was a small volume of demineralised dentine, having LACs 0.8 - 1.4 cm\(^{-1}\). From the XMT slices, the remnants of the pulp tissue had similar LACs to the body of the lesion. The figure also shows that the histogram for the different regions was not Gaussian, e.g. the sound dentine had a longer tail in the lower LAC side. Hence, instead of using the mean LAC value within each region as the representative measurement, the modal value was used.
In this tooth sample, the modal LACs for the body of the lesion, the sound dentine and the sound enamel were 0.5 cm\(^{-1}\), 1.60 cm\(^{-1}\), 2.5 cm\(^{-1}\) respectively. Pooling all the samples together, the mean (s.d.) modal LAC for the body of the lesion was 0.43 (0.12) cm\(^{-1}\) (using histograms) or 0.45 (0.11) cm\(^{-1}\) (using subtraction histograms), and for sound dentine, 1.56 (0.04) cm\(^{-1}\).

Figure 11-2 XMT images of a carious tooth viewed in three different planes: (a) XY plane, (b) XZ plane and (c) YZ plane
The two layers in dentine caries can be seen qualitatively.

Figure 11-3 3D image of a carious tooth with colour scale (LAC scale) (Drishti software [Limaye, 2006])
Figure 11-4 (a) Scanned images of the two halves of a carious tooth. (b) is a photograph of the sample before caries removal and (c) is after caries removal.

Figure 11-5 Histogram for the carious tooth before excavation (No. of voxels against the XMT values)
Conversion of LAC to mineral concentration for carious dentine

Assuming the mineral phase of the dental tissue is pure HA with a density of 3.15 g cm\(^{-3}\), the mineral concentration of enamel can be calculated using:

\[
C_E = \frac{\mu_E}{\mu_{\text{mhap}}} \quad \text{Equation 11-1}
\]

Where:

- \(C_E\) = the mineral concentration of enamel (g cm\(^{-3}\))
- \(\mu_E\) = the linear attenuation coefficient of enamel (cm\(^{-1}\))
- \(\mu_{\text{mhap}}\) = the mass attenuation coefficient of HAP (0.99 cm\(^2\)g\(^{-1}\) at 40 keV)

For dentine caries, because of its organic content, further adjustment was needed. The organic phase was assumed to be collagen with mass fractions of 53%, 7%, 1%, 17% and 22% for carbon, hydrogen, sulphur, oxygen and nitrogen respectively. Therefore, the mineral concentrations for sound and carious dentine were calculated from the measured LAC using Equation 11.2:

\[
C_D = \frac{\mu_D - (\mu_{\text{mcol}}C_{\text{col}})}{\mu_{\text{mhap}}} \quad \text{Equation 11-2}
\]

Where:

- \(C_D\) = the mineral concentration of dentine or caries
- \(\mu_D\) = the LAC of the dentine or caries (cm\(^{-1}\))
- \(\mu_{\text{mcol}}\) = the mass attenuation coefficient of collagen (0.24 cm\(^2\)g\(^{-1}\) at 40 keV)
\( C_{\text{col}} = \) the concentration of collagen in dentine \((0.54 \text{ g cm}^{-3})\) [Wong, 1995; Willmott et al., 2007]

So the corresponding values for mineral concentration for carious dentine and sound dentine are as follows:

Carious dentine = 0.30/0.32 g cm\(^{-3}\) (obtained from histograms and subtraction histograms respectively).

Sound dentine = 1.44 g cm\(^{-3}\).

11.4.2. Qualitative analysis of XMT slice using 2-D data

One typical XMT slice (Slice No. 114 of sample med 18), before and after caries removal, is shown in Figure 11.6. Qualitatively, it could be seen that the carious lesion in dentine extended underneath the enamel (white arrow in Figure 11.6(A)). As the integrity of the enamel was compromised, the undermined enamel, although non-carious, broke away in the caries removal process. Subtle grey level differences could be detected comparing the outer dentine near the EDJ and the inner dentine near the pulp. This may indicate the natural mineral content gradient in sound dentine [Kishen et al., 2000], or it may be due to the difference between primary and secondary dentine. After excavation, in the lower half of the image in Figure 11.6(b), a rim of demineralised dentine was left, corresponding to the base of pre-excavated carious lesion. This rim of demineralised dentine was not evident in the upper half of the slice except at the corner just below the undermined enamel (white arrow in Fig. 11.6(b)).
11.4.3. Quantitative analysis of removed dentine caries

11.4.3.1. Alignment errors between scans

A way of quantifying the amount of carious dentine that was excavated was by comparing the LAC histograms of the XMT scan data before and after excavation. The data were trimmed to include just the region around the carious lesion to reduce the peaks for the background, sound dentine and enamel. Consequently, the peak for the body of the lesion was enhanced, making it easier to analyse the volume removed to facilitate the comparison of the two excavation methods (Figure 11.7 and 11.8). In theory, by subtracting the histogram of the post-treatment scan from the initial scan, the LAC of the removed dentine could be shown as a histogram from which the volume could be calculated. However, in practice, comparing the histograms of sound dentine

Figure 11-6 Typical XMT slice (114 of tooth sample med 18) (a) before excavation; (b) after two different excavation techniques. The white arrows denoted the undermined enamel.
before and after caries removal (Figure 11.7 and Figure 11.8), it was found in some areas that the volume of sound dentine appeared to have increased after excavation. As this would be impossible in real life, after investigation, it was concluded that there was a slight specimen movement between the two scans. This movement was likely to be caused by the pressure exerted on the tooth during excavation despite the use of hard setting epoxy resin. Furthermore, it was discovered that the two halves moved independently to each other. In order to overcome this misalignment error, the tooth sample data was split into two separate datasets, each consisting one half of the tooth only (Sections A and B in Figure 11.6). An in-house image analysis programme was developed to give direction and the number of voxel shift in the three orthogonal planes, using features that are remote from the carious lesion between the two datasets. This information was used to reconstruct 2\textsuperscript{nd} scan data to match that of the 1\textsuperscript{st} scan. After adjustment, the LAC histograms from the two scans showed that the peaks for sound dentine and enamel had less volume from the post-treatment scan in section A (confirmed by the peaks in sound dentine and enamel region in the subtraction histogram in Figure 11.7). However despite this adjustment, misalignment still exists in section B shown by more sound dentine after caries removal (shown by a trough in the subtraction histogram) in Figure 10.8.
Figure 11-7 Histogram for before and after excavation and the difference for Section A

Figure 11-8 Histogram for before and after excavation and the difference for Section B
11.4.3.2. Image subtraction

After alignment, the XMT images from the initial scan could be superimposed onto those from the post-treatment scan for investigation of material that was removed. In Figure 11.9, the LAC contours of the tooth before excavation were superimposed onto the XMT image of the tooth after excavation. The highest contour threshold was set to be 2.5 cm\(^{-1}\) which corresponded to the LAC of sound dentine. It could be observed that some un-supported enamel was removed (also shown in Figure 11.10). It could also be seen that a large area of sound dentine was removed in section A (white arrow) but not in section B. However, in section B, some demineralised dentine with low LACs (black arrow) was left behind.

Furthermore, the post-treatment XMT dataset was subtracted from the pre-treatment dataset to form a new subtracted dataset for analysis. Figure 11.10 illustrates the subtracted image of the same slice shown in Figure 11.6. The unsupported enamel that was chipped off was clearly shown. It was also shown that after alignment, there were still thin lines in parts of the tooth remote to the carious lesion. This may be due to slight dimensional changes because of humidity, or it may be due to rotational misalignment that had not been corrected.
Figure 11-9 The contours of the variation of mineral content of the carious lesion superimposed on the XMT image after excavation.
Figure 11.10 Difference of the before and after scans

Figure 11.11 is the subtracted images after using the more accurate subtraction program (written by Dr Davis, 2007). Unlike the previous subtraction program, the before and after datasets were aligned more accurately, as the program allows adjustments for four out of the six degrees of motion (x, y, z axes and 1 rotation axis (yawing)). This is shown in Figure 11.12 where the two images (Tooth Sample med 18) were aligned by moving 2, -2 and 0 pixels for Section A, and -2, -1, 0 for Section B in the X, Y, and Z planes respectively. Plus the adjustment in the rotation axis was 11 and 13 (x10) respectively. Comparing the subtraction images for the two different programs (Figures 11.10 and 11.11), it can be seen that the misalignment artefacts were reduced after using the more accurate subtraction program.
Using LAC line contours to highlight the mineral distribution of the excavated lesions (Figure 11.12), it could be observed that excavated caries in Section B had lower LACs, especially near the base of the lesion, than that in Section A. In section A, some of the excavated materials had LACs of up to 1.2 cm$^{-1}$ (orange colour). Using histogram to analyse the bulk of the excavated material (Figure 11.13), it was shown that no materials having LACs larger than 1.1 cm$^{-1}$ were excavated in the dentine region in section B, whereas in section A, materials with LACs up to 1.5 cm$^{-1}$ could be found.
Figure 11-12 Mineral content contours superimposed on the image of the removed tissue

Figure 11-13 LAC histogram after subtraction of the ‘before’ and ‘after’ images
11.4.3.3. Line profile through the carious lesion

Since the subtraction histogram did not allow detailed analysis of the partial demineralised regions, arbitrary 2D line profiles, measuring LAC along them, were used. Due to the bowl shaped nature of the carious lesion, the line profile was made to pass the middle of the lesion. The typical LACs along the line from inner sound dentine to outer carious dentine are plotted in Figure 10.14. This shows that between the body of the lesion and sound dentine, there is a layer of partially demineralised dentine of 632 μm thick. It has been mentioned in Willmott’s MSc dissertation [unpublished data] that the mean of the lowest LACs at the partially demineralised dentine region was 0.49 cm\(^{-1}\). This constant fell within the region shown as a circle in the figure, demarcating the boundary between the body of the carious lesion and partially demineralised dentine.

Typical line profiles were plotted for the coronal slices to show the LACs before and after excavation (Figures 11.15 and 11.16). For both sections, there were gentle declines of the LACs in the partially demineralised layer in the pre-treatment profiles. In the post-treatment profiles, the steep decline of LACs (arrowed) showed that the carious material had been removed. In section A, it is clearly shown that the sharp decline started at the sound dentine region, whereas in section B, the sharp decline started in the partially demineralised dentine.
Figure 11-14 Line probe through the carious lesion (med 1)
Figure 11-15 Line probe plot before and after excavation for Section A

Figure 11-16 Line profile plot before and after excavation for Section B (med 18)
11.5. Comparison of sound dentine removed by the three techniques

11.5.1. Qualitative Analysis for Ideal excavation techniques

From the above analysis and results using bulk histograms and line profiles of LACs, image subtraction and LAC line contours, it could be concluded that Section A had more sound dentine removed than Section B. Also, in Section B, a layer of partially demineralised dentine was preserved. Therefore, the technique that was used to excavate caries in Section B could be regarded as a better or ‘ideal’ technique according to the ideal criteria for minimally invasive dentistry. In the 1st analysis, the present investigator, who was blind to the techniques used for excavation, used initially the bulk histogram and line profiles methods of analysis to grade which half of the tooth excavation was closest to the ideal method. After the grading, the actual techniques were disclosed. A chi-square test was used to test which of the three excavation techniques was graded closest to the ideal technique (Table 11.1). A few months later, this exercise was repeated with the additional aid of image subtraction method and LAC line contour methods (2nd analysis). The latter results are shown in Table 11.2.

Forty-four tooth pair samples were studied. For the Carisolv / Hand Excavation pairs, 14 out of the 21 tooth samples that were judged to have caries removed by the ‘better’ technique belonged to the Carisolv group and Carisolv was statistically shown to be the better technique than Hand Excavation (p=0.03 and p<0.001 for 1st and 2nd analyses respectively). For the Caries Detector / Hand Excavation pairs, the aid of the caries detector did not show significant improvement in removing caries to the ideal level (p=0.30 and p=0.09 for 1st and 2nd analyses respectively). When Carisolv technique was
compared to the technique using only the Carisolv hand instruments without chemical agents, no significant difference was found (p=1 for both analyses).

Table 11-1 Results from the 1\textsuperscript{st} analysis in judging the ‘ideal’ technique

<table>
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<th>Ideal</th>
<th>Non-ideal</th>
<th>p (Chi square)</th>
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<td>14</td>
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<td>7</td>
<td>0.3035</td>
</tr>
<tr>
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<td>10</td>
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</tr>
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<td>3</td>
<td>1</td>
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<tr>
<td>Carisolv hand instruments</td>
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Table 11-2 Results from the 2\textsuperscript{nd} analysis in judging the ‘ideal’ technique

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</tr>
</thead>
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<tr>
<td>Carisolv hand instruments</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

11.6. Quantitative analysis in comparing the volume of sound dentine removed by the three caries removal technique

Examining the histograms of the whole tooth, it was easy to identify the LAC values at the peak of the sound dentine histogram. However, due to partial volume effects and partially demineralised dentine, the lowest cut-off value was obscured. Hence, in order to quantify the amount of sound tissue removed the following strategy was used. The dataset of a deciduous molar (sample med 47; this sample is not part of the main XMT
study), was selected to the region of interest, to include sound dentine only. A histogram was obtained for sound dentine, pulp and pulpal dentine for the trimmed dataset (Figure 11.17). A ratio was calculated by dividing the LAC value at peak (A) by that at the lower end (B) of the histogram. This ratio was then applied to all the highest values of the sound dentine peak for all the samples to give a corresponding lowest cut-off value for the sound dentine. This was then located on the subtraction histogram for the halves for each sample. The volume of removed dentine was calculated by summing all the voxels from this cut-off LAC to LAC of 2.0 cm$^{-1}$ (this value excluded any enamel removed during excavation). This volume was then expressed as a percentage of the total volume of all the tissues removed excluding the contribution of water which had a LAC lower than 0.25 cm$^{-1}$. The results are summarised in Table 11.3. The leaf and stem plots (Figure 11.18) showed that CSS and CD had less sound dentine removed (mean % = 2.1 ± 1.76 and 2.6 ± 2.78 respectively) than using hand excavators (mean % = 4.0 ± 3.14 and 4.9 ± 3.57 respectively). A paired student t-test comparing the techniques on the same tooth showed that the difference of sound dentine removed was significant at p= 0.011 for CSS/HES and 0.008 for CD/HED.
Figure 11-17 LAC histograms of a caries free tooth (Sample med 47). The blue histogram was for the whole trimmed dataset (enamel peak was cropped). This showed that the contribution of the pulp and pulpal dentine increased in tail size in the lower end of the sound dentine peak due to noise levels and partial volume effects. The pink histogram was for a block of sound dentine excluding the pulpal dentine, showing that the histogram was approximately Gaussian with clear cut off point in the lower end. The ratio was calculated by dividing the LAC values at A by that at B. The yellow histogram showed the LAC for pulp and pulpal dentine.
Table 11-3 Amount of sound dentine removed during excavation with indication whether pulp was involved. HES= hand excavation/Carisolv, CSS=Carisolv, HED= hand excavation/Caries detector dye, and CD=Caries detector dye.

<table>
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<th>Sound dentine removed (voxels)</th>
<th>Pulp involvement</th>
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Figure 11-18 Leaf and stem plot showing the volume of sound dentine removed by various technique. HES – hand excavation in CSS group, CSS – Carisolv®, HED – hand excavation in CD group, CD – Caries detector dye.
11.6.1. Other influencing factors on volume of sound dentine removed during excavation procedures

*Volume of the carious lesion* – A scatter plot diagram (Figure 11.19) was drawn to observe any relationship between the amount of sound dentine removed and the volume of the carious lesion. The linear regression lines suggested that there was a trend between the volume of sound dentine removed and the volume of the carious lesions for HED, HES and CD. However, for CSS, the volume of sound dentine removed appeared to be independent of the volume of the carious lesion.

![Plot of sound dentine removed vs. total volume of lesion](image)

*Figure 11-19* Scatter plot to show the relationship between volume of the carious lesion and amount of sound dentine removed according to removal techniques.
Pulpal involvement – It was shown that the volume of sound dentine removed was not dependent on whether the lesion has extended into the pulp (Table 11.3). However, it was noted that in four CD/HED pairs, pulp exposures were present in the HED halves but not in the CD halves.

11.7. Discussion

11.7.1. Sample preparation

In order to ensure accurate relocation between XMT scans, the mounting compound needed to be strong enough to prevent sample movement during the experiment. Although the epoxy putty proved to be the better mounting material in comparison to the Polymorph, movement of the tooth still occurred between “initial” and “after” scans. This was dependent on the different variations of the root anatomy. If the roots were far apart then the mounting material had more surface area to adhere to and the sample was stable. For short rooted teeth, despite the strength of the epoxy putty, sample movement still occurred (Figure 11.6). For this reason, an in-house subtraction program was written to correct movement of the tooth specimens. However this subtraction program could only correct for movement in the x, y, z and one rotational axis in the orthogonal plane thus allowed correction in 4 out of the 6 degrees of freedom (Figure 11.10). The small movement due to rotation in either of the other two planes was not corrected. Nevertheless this small misalignment should not have a significant effect on the results of the main study.
Another factor which might cause misalignment was hydration of the sample during the scans. Too much water could cause slight expansion which might affect the subtraction dataset as shown in Figure 11.22, where the subtracted image appears very speckled.

11.7.2. Relationship between LAC and mineral concentration in dentine

The dentine component that contributes to the LAC value includes mineral, collagen, non-collagenous matrix, water and air. The LAC for air is low and therefore can be ignored. In sound dentine, the concentration of collagen can be assumed to be constant at 0.54 g cm\(^{-3}\) at 40 keV (mass fraction of collagen is 53%). Its mass attenuation coefficient (MAC), \(\mu_{\text{mcoll}}\) at 40 keV is 0.24 cm\(^{-2}\) g\(^{-1}\). The MAC for water is 0.268 cm\(^{-2}\) g\(^{-1}\) [Hubbell, 1982]. The samples were kept hydrated throughout the XMT scans. As the values for water and collagen are comparable, the difference can be ignored in relation to its contribution it has on the overall mineral content. Using Equation 10.2, according to Willmott et al. [2003; 2007], the LAC can be converted to mineral concentration easily. However, for carious dentine, it is difficult to obtain the concentration for collagen, especially with proteolysis of collagen and infiltration of bacteria. Therefore the actual mineral concentration is impossible to calculate. To avoid confusion, the LAC values were mainly used in our results and discussion, avoiding any assumptions about the composition of the organic component. Nonetheless, for comparison purposes the mineral concentration range calculated from the average LAC for the bulk of the carious lesion was 0.30 g cm\(^{-3}\) - 0.45 g cm\(^{-3}\) at 40 keV using histograms and subtraction histograms respectively in this study. The mineral concentration of sound dentine in this study was 1.44 g cm\(^{-3}\) which agrees with the averaged estimates for mineral.
concentration of dentine quoted as 1.4 g cm\(^{-3}\) by Kinney et al. [1994] who used a synchrotron source to measure the LAC. However the value for carious dentine in this study was lower in comparison to the value of 0.55 g cm\(^{-3}\) from the study by Kinney et al. [1994].

11.7.3. Affected and infected dentine

The resolution of the XMT technique was typically 30 µm which is not high enough for investigation at the cellular level i.e. the collagen network. Therefore it can be argued that XMT is not an ideal technique in distinguishing between the infected and affected layers of carious dentine. The boundary between infected and affected dentine is not absolute, but is gradual. There is a transitional change in mineral content at this phase and the difficulty is in deciding the threshold level of the XMT images to provide a "cut-off" zone. However, even with high resolution light microscopy, the boundary between infected and affected dentine is not well defined. Banerjee [2002] showed that bacterial infiltration could be detected inside the tubules of sound dentine. Hence in this study, instead of defining the boundary between infected and affected dentine, emphasis of investigation was focused on the partially demineralised front. The aim was to find a boundary or level of mineral concentration above which the demineralised dentine has potential to remineralise. The thickness of the partly demineralised dentine remaining after excavation was determined on the basis of single line scans instead of utilising the whole dataset for the total lesion volume. The reason for this is due to the geometry of the lesion. As the lesion is bowl shaped, if a line probe is placed such that it is always along the direction towards the pulp, this is representative of the whole lesion.
It has been suggested that the transparent zone corresponds to the ‘affected’ dentine proposed by Ogawa et al. [1983] and is recalcifiable, therefore, it would be beneficial to keep part of the partially demineralised dentine in order to preserve tooth tissue.

11.7.4. XMT study of comparison of Carisolv, Caries Detector Dye and hand excavation

For the main study a ‘blind’ analysis method was adopted to eliminate bias when deciding which technique is better. Line profiles and histograms (whole and subtraction datasets) were used to assess which excavation technique adheres to the principle of minimally invasive dentistry. The investigator asked the clinician for this project (FW) to comment on his perception of (1) using Carisolv and Caries detector dye compared to hand excavation and (2) using the Carisolv technique without the application of the solution (i.e., using the Carisolv hand instruments only):

Of the three excavation techniques, FW reported that Carisolv was comfortable and easy to use because no excessive force was needed. The action of removal was a scraping action. He found that with a hand excavator it was difficult to discriminate and remove diseased tissue only. He felt that excavation with a new sharp excavator appeared to go deeper and exposed the pulp easily. With the Caries detector dye, it was difficult to remove all the red stained dentine.

Comparing the excavator to Carisolv hand instruments, he felt that the blunt ended Carisolv instruments did not allow him to excavate deeper and remove any more tissue once a certain depth of dentine was reached.
Commenting on the time taken for cavity preparation, FW reported that the hand excavator was the fastest. For Caries detector dye and Carisolv, at least double the time was needed for the solution to work.

With regard to using the Carisolv hand instruments without the solution, FW felt that more initial force was required. He commented that this force was similar to that of using a blunt hand excavator. To extrapolate the laboratory experience to clinical application, FW commented that patients may prefer the Carisolv technique because some children tend to equate pressure with pain.

“Blind” method – If the criteria for removing carious dentine to the ideal level which is at the boundary between infected and affected dentine are well established, it would be easy to compare results from the three excavation techniques to those criteria. However, as discussed, to search for the boundary between the affected and infected dentine appears to be a difficult task to accomplish. Hence, in this study, it was only possible to compare, in pairs, in order to find which technique was “better” according to the criteria mentioned in the Methods Section (11.3). This qualitative assessment showed that the Carisolv technique was markedly better in preserving tooth tissue than hand excavator (Table 11-1). This result was further confirmed when the assessment was enhanced by the digital subtraction program (Table 11-2). This finding is in agreement with the manufacturer’s claim and those reported in vitro research and clinical research studies listed on the MediTeam/OraSolv website [MediTeam, 2007]. However, there were at least 5 teeth that the Carisolv technique was judged to be poorer than conventional hand
excavation. The reason for this was not clear but the close proximity to the pulp and the volume of the carious lesion were suggested but this was not proved by the present results.

Although Caries Detector Dye has shown a tendency to be a better technique than hand excavation, the results did not reach a significant level (Tables 11-1 and 11-2) using this qualitative assessment. This does not support the manufacturer’s claim. In the literature, it has been shown that the dye is not able to differentiate between infected, affected and sound dentine [Kidd et al., 1993] especially when it is in close proximity to the pulp. However, it can be argued that this qualitative assessment was not sensitive enough. When a more sophisticated method of comparison (discussed in the following section) was used, it showed that the Caries Detector Dye was as effective as the Carisolv technique in preserving sound dentine, and was better than the hand excavator.

*Volume of sound dentine removed* – XMT is probably the only method that can measure and locate the volume of sound dentine removed because of its non-destructive nature. Other laboratory techniques are mainly destructive and do not have the original sample to compare with the sample after the experimental procedure. Other radiographic techniques such as plain film radiography or scanning microradiography are 2-dimensional or for thin sections only. In order to calculate the exact volume of dentine removed, the scanning parameters, and the location of the specimen must be the same for the initial and after scans. In order to avoid spurious spectrum change which may happen due to X-ray filament change or generator stability, the time between the initial
and after scan was kept to a minimum, typically 5-7 hours to allow for reconstruction time and the time required to take the specimen to the clinic for excavation procedures. Small movement of the sample was corrected by the subtraction program. This program produced a dataset which was the difference between the initial and after scans. From this dataset, the amount of sound dentine could be determined if the LAC threshold was known. To this end, the method by Willmott et al. [2007] was used. They selected a volume of sound dentine from a tooth, obtained a histogram of its LAC and found the LAC at its lower end tail of the sound dentine peak. However, since each tooth might have a different sound dentine mineral concentration, to apply one value for all the teeth may give erroneous results. Hence, a method using ratio (sound to unsound dentine) was adopted and applied to each tooth in order to determine the lowest LAC value for dentine (Section 10.2.4.1).

Before comparing the volumes of sound dentine removed by the three techniques, it was thought that other parameters may influence its removal. Firstly, it was shown that the larger the carious lesion, the more sound dentine would be removed (Figure 11.19). Therefore, the sound dentine volume was normalised to the total carious volume and expressed as a percentage. Secondly, it was thought that the less destructive techniques would preserve a layer of dentine to avoid pulpal exposure. This was not apparent in the experimental results. The pattern appeared to be random in Table 11.3. However, it was interesting to note that the four paired CD vs. HED teeth, where only one half had pulpal exposure, that half belonged to the HE technique (Table 11.3). This indicates that the
CD technique may help the clinician in preventing pulpal exposure during caries excavation.

In contrast to the “blind” method, both CSS and CD techniques showed significantly less sound dentine was removed than the HE technique. Hence the null hypotheses were rejected. In the “blind” method, the present investigator, apart from comparing LAC histograms, visualised XMT slices for the presence of a retained partially demineralised layer. It is likely that the dye would stain the partially demineralised dentine, leading to its removal, but it did not stain sound dentine (in agreement with the manufacturer’s claims). Hence, in this quantitative volume analysis, it was shown that it was as effective as the CSS technique in preserving sound dentine.

In the present study, bacterial investigation was not carried out on the partially demineralised dentine. However, it has been shown that bacteria exist even in sound tissue [Banerjee et al., 2002], therefore the traditional idea of removing all the bacteria would result in removing sound dentine. Current concepts suggests that the bacteria might not be viable if a hermetically sealed restoration is placed in the cavity. Hence total eradication of bacteria in sound dentine is no longer required. Furthermore, Innes et al. proposed the Hall Technique, a simplified method of managing carious primary molars by sealing the cavity with a preformed metal crowns (PMCs) which are cemented with no local anaesthesia, caries removal or tooth preparation [Innes et al., 2006; Innes et al., 2007]. The two year results showed the Hall PMCs have more favourable
outcomes for pulpal health and longer restoration duration than conventional restorations.

The need for mechanical retention is less with modern adhesive materials. Unlike amalgam, there is no need to remove excessive sound tissue to create mechanical retentive cavities. The activity of a deep carious lesion in dentine can be preferentially modified, by sealing in the dentine caries. This allows reparative pulp-dentine complex reactions to take place (Stepwise excavations) [Ricketts, 2001]. Absence of leakage of a restoration means any remaining bacteria will die off or become non-viable because of the lack of nutrient supply [Mertz-Fairhurst et al., 1998]. What is more important is the binding of the material to the dentine and the creation of a pro-remineralising environment with either fluoride [Savarino et al., 2004] or amorphous calcium phosphate (ACP) [Rahiotis and Vougiouklakis, 2007].
11.8. Effects of chemical agents on sound dentine

11.8.1. Introduction and Aims

It is uncertain what effect the Carisolv and Caries Detector Dye has on sound dentin. Hence this experiment aimed to investigate and compare the effect of Carisolv, Caries Detector Dye and Sodium hypochlorite (one of the key ingredients in Carisolv) on the mineral concentration of sound dentine.

11.8.2. Materials and Methods

Four holes were drilled to expose dentine in a tooth sample (not part of any previous study), an initial XMT scan was taken to confirm lack of caries. The tooth sample was taken to a clinician (FW) who then applied the following chemical reagents on the drilled holes.

1) Carisolv was applied, no scraping
2) Carisolv was applied, with scraping using the provided hand instruments
3) Caries detector dye was applied for one minute and then washed off
4) sodium hypochlorite (1%) solution was applied for a minute and then washed off.

Afterwards the tooth sample was re-scanned with XMT.

11.8.3. Results

Figure 10.20 shows the before and after XMT images of a tooth sample with four artificial holes. The chemical interventions for the four holes were Carisolv (with and without scraping using instruments), Caries Detector dye and 1% sodium hypochlorite. Brightness and contrast have been altered to highlight the artefacts (Figure 11.21).
These figures show that after application of the chemicals, sound dentine has not been removed.

Figure 11-20 Sample med 46 before and after application of Carisolv (with and without scraping using instruments), Caries Detector dye and 1% sodium hypochlorite

Figure 11-21 Subtracted image of sample med 46 highlighting misalignment artifacts (Slice 94)
11.8.4. Discussion

Carisolv, apparently does not have this harsh effect on removing sound dentine because of the three amino acids, and so the distinctive mechanism is the ability to dissolve denatured dentine only, which is consistent with the study by Hannig [1999]. Comparing the effect of Carisolv gel on sound dentine with and without the scraping using the specialised Carisolv blunt instruments, no difference between the two was found. This suggests that the biochemical effect of the gel is self-limiting even if a force is applied with a blunt instrument.

Figure 11-22 Speckled subtraction image of sample med 46 indicating the effects of chemicals: Carisolv (with and without scraping using instruments), Caries Detector dye and 1% sodium hypochlorite (Slice 114)
Caries detector dye (1% acid red in propylene glycol) has no effect on sound dentine. However, its dyeing ability may not be specific for carious dentine but also stains hypomineralised non carious dentine.

Tonami et al. [2003] and Dammaschke et al. [2005] found that sodium hypochlorite softens both sound dentine and affected dentine. In the present study, the sodium hypochlorite was not applied long enough for the harsh effect to take place.

11.9. Remineralisation study of teeth after caries removal

11.9.1. Introduction and Aims

The remineralising potential of natural carious and residual dentine after caries removal has not been investigated previously. Therefore the aim of this experiment was to investigate the change in mineral concentration of carious and residual dentine after immersing the specimen in a remineralising solution.

11.9.2. Materials and Methods

The protocol for this experiment followed the ‘recipe’ for remineralisation of dentine using a saliva-like remineralising solution [Tantbirojn et al., 2006]. This solution contained 1.5 mmol/L calcium chloride (CaCl₂), 0.9 mmol/L potassium dihydrogen phosphate (KH₂PO₄), 130 mmol/L potassium chloride (KCl), and 20 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), adjusted to pH 7.0 with 1 mol/L KOH [Mukai et al., 2001]. First, one plastic cylinder containing this
remineralising solution, and another one containing distilled water only were XMT scanned.

Two samples which had already been scanned using the XMT from the study in Section 11.2.2 were immersed into 75 ml of the saliva-like solution which was changed every two weeks. Sequential XMT scans were carried out every 2 weeks. These samples were:

(1) a tooth in which excavation had been carried out by hand instrument or standard Carisolv techniques (Sample med 28). This tooth also had a carious lesion on the other side to act as a control for investigation of the effect of remineralising solution on gross caries. This sample was immersed in the solution for up to 12 weeks.

(2) a tooth in which excavation had been carried out by standard Carisolv technique and by Carisolv hand instrument alone (Sample med 40). This tooth was immersed in the solution for up to 8 weeks.

11.9.3. Results

11.9.3.1. Comparison of Carisolv and hand excavation techniques (Sample med 28)

The LAC of the remineralising solution was shown to be higher than that of water with a modal value of 0.29 and 0.26 cm\(^{-1}\) respectively at 40 keV (Figure 11.23).
After 12 weeks in remineralising solution, accumulation of mineral could be detected along the edge of the partially demineralised dentine in the half tooth that had caries removed by the Carisolv technique (labelled ‘A’ in Figure 11.24(a)). This additional material had a maximum LAC of 0.43 cm\(^{-1}\) (Figure 11.25). Additional material was also found in the natural carious lesion (labelled ‘C’ in Figure 11.24(a)) but its maximum LAC (≈0.25 cm\(^{-1}\)) is less than that in the ‘A’ region. In the half tooth in which caries was removed by hand excavator, no additional material was detected in the sound dentine layer (labelled ‘B’ in Figure 11.24(a)). These features were more clearly illustrated in the line profiles of the three different regions. The line profile in region ‘A’ showed that mineral was added to the partially demineralised dentine with an LAC above 0.8 cm\(^{-1}\) (Figure 11.26) after immersion in remineralising solution for more than 2 weeks. The
gradient of the post immersion line became more similar to that for sound dentine in region ‘B’ where no additional mineral was deposited (Figure 11.27). The line profiles in ‘C’ region showed that some mineral were added but to a lesser extent in the low LAC region (Figure 11.29). An interesting feature was found in the line profile for enamel where a small amount of mineral was deposited on the surface (arrowed in Figure 11.30). Following the LAC at one point of the sample throughout the remineralisation period, the rate of aggregation of additional mineral at one point was shown to be $1.19 \times 10^{-4} \text{ g cm}^{-3} \text{ h}^{-1}$ for the Carisolv half (Figure 11.28).
Figure 11-24 Remineralisation experiment for sample med 28: (a) 2D grayscale image of sample med 28 before immersing in remineralising solution (slice 114). (b) 2D grayscale image of sample med 28 after immersing in remineralising solution. (c) 2D grayscale image of subtracted dataset to illustrate addition of material. (d) Colour map of LAC of added material of the subtracted dataset.
Figure 11-25 Line profile through the “remineralised” region ‘A’ in the subtracted image of Figure 10.24(c)
Figure 11-26 Line profiles through region excavated with Carisolv (region ‘A’) at 0 and 12 weeks
Figure 11-27 Line profiles through region excavated with HE (region ‘B’) at 0 and 12 weeks in remineralising solution.
Figure 11-28 The rate of mineral accumulated over the 12 weeks for sample med 28
Figure 11-29 Line profile through dentine caries (region ‘C’) at 0 and 12 weeks in remineralising solution.
Figure 11-30 Line profile through enamel region at 0 and 12 weeks in remineralising solution
11.9.3.2. Comparison of Carisolv and Carisolv hand instruments only techniques (Sample med 40)

Additional materials were shown to accumulate in the partially demineralised layers irrespective of whether the sections were excavated by the Carisolv technique (‘A’ in Figure 11.31) or by using the Carisolv hand instruments alone (‘B’ in Figure 11.31) after immersion in remineralising solution after 8 weeks. The line profiles for both regions showed that additional minerals were deposited to dentine with an LAC above 0.8 cm⁻¹ (arrowed in Figure 11.32 and 11.33). The rate of deposition at one point was shown to be $1.26 \times 10^{-4}$ g cm⁻³ h⁻¹ and $1.71 \times 10^{-4}$ g cm⁻³ h⁻¹ for the Carisolv half and Carisolv hand instrument only half respectively.
Figure 11-31 2D greyscale images (slice 141) of sample med 40 before and after immersion in remineralising solution with corresponding subtraction images
Figure 11-32 Line profile through tooth before and after placing in remineralising solution for section A (0 and 8 weeks), which had caries removed by Carisolv technique.
Figure 11-33 Line probe through tooth before and after placing in remineralising solution for section B (0 and 8 weeks), which had caries removed by Carisolv hand instruments only.
11.9.4. Discussion

Fusayama proposed that if the ‘affected’ demineralised dentine is left behind after caries removal, this layer of dentine is remineralisable but this concept has not been proven experimentally. Although previous studies show that artificially carious dentine lesion created by in vitro demineralisation can remineralise [Mukai et al., 2001; Bertassoni et al., 2009], no previous studies were found by the author demonstrating that the layer of ‘affected’ dentine in natural caries lesion can remineralise, whether in vivo or in vitro. This may be due to the limitations of most microscopic techniques, which are destructive. In previous sections, it was discussed that although it is impossible to identify a layer of bacteria free ‘affected’ dentine, the partially demineralised layer preserved with the CSS technique should be the level up to which caries removal reaches. It was shown that this partially demineralised layer could be remineralised, under experimental condition, up to 80.1% of that of sound dentine. Hence, it could be inferred that the partially demineralised layer, with a LAC value above 0.8 cm\(^{-1}\), still retained the organic, possibly collagenous, structures for mineral to be deposited. This is the first time that natural carious lesion, which has been subjected to hostile oral environment, has a potential to remineralise. Therefore, in clinical situation, it could be inferred that partially demineralised dentine could be left to remineralise if favourable conditions are provided. Future studies such as an in situ experiment using slab with affected dentine could be designed to prove this concept further.

In the previously described experiment (Section 11.9.2) repeated XMT scans were taken every fortnight in the initial period. This showed that remineralisation reached its
maximum at about 4 weeks, indicating relatively rapid remineralisation. One future experiment will be to scan the lesion at more frequent intervals, which will shed further light on the rate of remineralisation.

It is also of interest to note that sound dentine in the HE section (Figure 11.27) could not gain extra mineral. This indicates that all the available space for mineral has been occupied. Clinically, it could be inferred that if Carisolv is used to remove carious dentine to preserve tooth tissue, and a glass ionomer cement is used as a lining, the partially demineralised layer will be remineralised to form an almost sound dentine base for restoration.

The additional material found in the bulk of the natural carious lesion (image in Figure 11.24 – Section C and line probe shown in Figure 11.29) is of interest. There is a surface layer of mineral precipitation of infected dentine, which could be due to alignment artefacts. However mineral has also precipitated deep in the partially demineralised dentine of the carious lesion. The infected dentine was previously regarded to have no potential for remineralisation. This experiment shows that the porous infected dentine, assumed to have no structural integrity, may have residual organic framework to hold mineral. However, the increase was only up to 26.2% of sound dentine. This may be too soft to be of good use clinically as a base for a restoration.
11.10. XMT and Colour of the carious dentine lesion

11.10.1. Introduction and Aims

Although colour change of carious dentine is also one of the most used parameters to remove infected/affected tissues [Cartagena and Ayarzun, 2001], there are conflicting views in regards to caries removal using colorimetric parameters. Using colour as an indicator may eliminate healthy dentinal tissue which under appropriate conditions would be capable of remineralisation [Cartagena and Ayarzun, 2001]. A cross-section through a typical carious dentine lesion clearly show colour gradations. However, what these colour changes mean in terms of the demineralisation process and the level of infectivity is still not entirely clear [Banerjee et al., 2000d].

The aim of this part of the project was to try to use the XMT greyscale dataset and relate this to colour, from the images obtained with the optical scanner.

11.10.2. Materials and Methods

Qualitatively, FW described the clinical appearance of the carious lesion from the images of the optical scan. The XMT slices closest to the cut surface were assessed and described by the author.

Quantitatively, six samples were used. For each tooth sample, a colour image together with the corresponding XMT slice was obtained. Then a line profile was taken through the lesion in the XMT image using amiraTM imaging software. For the optical images,
ImageJ was used to convert the file into 255 grey levels. A line profile was obtained in approximately the same region.

11.10.3. Results

11.10.3.1. Qualitative measurement

The following is a table to show the clinical and XMT description of the carious dentine lesions. The comparisons of clinical description and XMT grey levels were summarised in Table 11.4. A tooth with typical shallow carious lesion (med 39), a tooth with typical deep carious lesion (med 31) and a tooth with an atypical lesion (med 10) are described below.

In a tooth with relative shallow carious lesion (med 39), where excavation did not result in exposing the pulp, dark brown colour of caries faded to become yellow colour from the depth of the cavity towards the sound dentine (Fig 11.34). The faint yellow colour could be detected extending beyond the carious lesion into sound dentine (Section B, Fig 11.34). In the XMT image, the demineralised lesion did not seem to extend as far compared to the faint yellow colour. In a tooth with more extensive carious dentine where excavation resulted in exposure of the pulp (med 31, Fig. 11.37), the brown lesion extended to the pulp. In the XMT image, the pulp has reacted and a thin barrier was present. In these teeth with typical lesions, the XMT images show that the body of the lesion had homogenous low mineral concentration. In sample med 10, the colour images were not distinctive to any other lesions but its XMT images show that there was a hypermineralised surface layer in Section A and there were 2 hypermineralised sub-layers in Section B (Figure 11.40). The LACs for these 2 bands were 1.25 and 1.45 cm$^{-1}$. 
From Table 11.4, some teeth were observed to have translucent dentine from the optically scanned pictures, and these features appeared as bright bands in the XMT images, indicating having higher mineral concentrations. However, no direct relationship could be deduced between the colour and whether the pulp had reacted to caries. The pale yellow colour could be seen in both demineralised and sound dentine in corresponding XMT images.

### 11.10.3.2. Quantitative analysis

Three corresponding line profiles obtained from XMT slices and ImageJ are shown in Figure 11.35, 11.36, 11.41 and 11.42. These figures demonstrated that there was no direct correlation between the colour intensities and LAC values.

### 11.10.3.3. Gradient of partially demineralised layer

Apart from directly correlating colour to mineral concentration, Possible correlation of the colour to the gradient of demineralisation was also explored. From the line profiles, a dissolution curve pattern was fitted to the partially demineralised region by an equation:

\[
LAC = a + b \exp(-\alpha d)
\]

**Equation 11-3**

where a, b are constants, \(\alpha\) is the gradient, d is the distance in millimetres and LAC is the linear absorption coefficient.

The fitted curves for the six XMT slices with corresponding optical scan images are shown in Figures 11.35, 11.36, 11.38, 11.39, 11.41, and 11.42). Table 11.5 shows the
summarised results with an overall gradient mean (s.d.) of 1.78 (1.53) and a large range from 0.27 – 5.61. Apart from large variations between sample teeth, the variation in gradients with the same tooth could be as much as 2.6 fold (med 39).
Table 11-4 Clinical and XMT description of carious dentine lesions clinically (Bolded titles represented samples investigated with ImageJ)

<table>
<thead>
<tr>
<th>Sample Title</th>
<th>Clinical description</th>
<th>XMT description</th>
</tr>
</thead>
<tbody>
<tr>
<td>med 1</td>
<td>Dark brown superficial layer (about 0.5mm) with yellowish brown in the body of the lesion and yellow advancing front extending towards the pulp horn.</td>
<td>Homogeneous demineralised lesion in the body with a partially demineralised advancing front. Pulp has not receded. Thick sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 2</td>
<td>Not available</td>
<td>Very deep dentine lesion. Pulp has not reacted. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 3</td>
<td>Mainly dark brown layer throughout the body of the lesion with a faint yellowish pulpal advancing front (about 0.1mm)</td>
<td>Homogeneous demineralised lesion in the body extending into the pulp horn. Pulp has not reacted. No sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 4</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body extending into the pulp. The lesion has a hypermineralised layer in the ‘infected’ dentine.</td>
</tr>
<tr>
<td>med 5</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. A thick sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 6</td>
<td>Dark brown lesion extending into the pulp horn in the open cavity. A separate yellowish occlusal layer under an occlusal fissure.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 7</td>
<td>Mainly dark brown lesion throughout the lesion with an exposed pulp</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 8</td>
<td>Dark brown lesion in the body of the lesion with a pale yellowish pulp front. There is a translucent layer of dentine around the pulp horn under the carious lesion.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. There is a partially demineralised advancing front. A distinctive sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 9</td>
<td>Brown lesion extending all the way to the pulp.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded following the shape of the cavity. There is a partially demineralised advancing front. A distinctive sound tissue barrier between the lesion and the pulp. Two bright hypermineralised bands following shape of dentinal tubules – indicating sclerotic dentine.</td>
</tr>
<tr>
<td>med 10</td>
<td>Dark brown lesion extending all the way to the pulp, especially in Section B.</td>
<td>Deep dentine lesion where pulp has receded. In Section A, there is a hypermineralised superficial layer. In Section B, there are 2 distinctive hypermineralised sub-layers.</td>
</tr>
<tr>
<td>med 11</td>
<td>Dark brown lesion extending all the way to the pulp. White bands following the shape of dentine tubules.</td>
<td>Deep dentine lesion where pulp has receded. Sound tissue barrier between the lesion and the pulp. Bright features following shape of dentinal tubules - possibly sclerotic dentine.</td>
</tr>
<tr>
<td>med 12</td>
<td>Brown lesion in the body of the lesion with a yellowish sub layer extending to the pulp</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Distinctive sound tissue barrier between the lesion and the pulp. There is partially demineralised advancing front. Bright features below the lesion - possibly sclerotic dentine.</td>
</tr>
<tr>
<td>med 13</td>
<td>Brown layer extended into the pulp with presence of pulpal necrotic tissue</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin or no sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 14</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Clear bright features under the lesion along the pulp indicating pulpal reactions to caries.</td>
</tr>
<tr>
<td>med 15</td>
<td>Dark brown layer in the body of the lesion with a yellowish/translucent front towards the pulp. There is a small dark brown lesion at the pulpal horn.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Clear bright features under the lesion along the pulp indicating pulpal reactions to caries.</td>
</tr>
<tr>
<td>med 16</td>
<td>Dark brown lesion extending into the pulp. Presence of pulpal necrotic tissue</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. No sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 17</td>
<td>Brown lesion extending into the pulp.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Clear bright features under the lesion along the pulp indicating pulpal reactions to caries.</td>
</tr>
<tr>
<td>med 18</td>
<td>Brownish body of the lesion with yellowish pulpal front. There is a small layer of translucent dentine around the pulp horn.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Bright features following shape of dentinal tubules - possibly sclerotic dentine.</td>
</tr>
<tr>
<td>med 19</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 20</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Distinctive sound tissue barrier between the lesion and the pulp. Clear bright features under the lesion along the pulp indicating pulpal reactions to caries.</td>
</tr>
<tr>
<td>med 21</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. No sound tissue barrier between the lesion and the pulp. There is a partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 22</td>
<td>This lesion is deep and extends into the pulp. The colour of the lesion is white. No other dentine zone beneath the lesion is detected.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. No sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 23</td>
<td>Brown lesion extending into the pulp</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 24</td>
<td>Light brown body of the lesion. There is a thin layer of translucent dentine around the pulp horn</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp. There is a partially demineralised advancing</td>
</tr>
<tr>
<td>med 25</td>
<td>Brown lesions extending into the pulp</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Partially demineralised</td>
</tr>
<tr>
<td>med 26</td>
<td>Dark brown body of the lesion with a translucent layer of dentine around the pulp horn. Evidence of receding pulp.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Distinctive sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 27</td>
<td><strong>Brown body of the lesion which become paler towards the pulp</strong></td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 28</td>
<td>Not available</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 29</td>
<td>Brown lesion extended into the pulp</td>
<td>Large homogeneous demineralised lesion in the body extended to the pulp. Pulp has receded. No sound tissue barrier between the lesion and the pulp. Presence of demineralised advancing front.</td>
</tr>
<tr>
<td>med 30</td>
<td><strong>Dark brown body of the lesion with yellowish brown dentine extending toward the pulp</strong></td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Bright features following shape of dentinal tubules - possibly sclerotic dentine Translucent zone.</td>
</tr>
<tr>
<td>med 31</td>
<td><strong>Yellow brown lesion with pale yellow dentine towards the pulp. A thin barrier between the floor and the pulp.</strong></td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded following the shape of the floor of the lesion. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 32</td>
<td>Brown lesion throughout the lesion with transparent dentine around the pulp horn.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Bright features below the lesion - possibly sclerotic dentine.</td>
</tr>
<tr>
<td>med 33</td>
<td>Dark brown body of the lesion with pale brown dentine sublayer. A thin layer of transparent dentine around the pulp horn</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Thick sound tissue barrier between the lesion and the pulp. Thin hypermineralised layer at the bottom of the cavity.</td>
</tr>
<tr>
<td>med 34</td>
<td>Yellowish brown dentine throughout the lesion with</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Thick sound</td>
</tr>
<tr>
<td>Image</td>
<td>Description</td>
<td>Observation</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>med 35</td>
<td>Yellowish brown dentine throughout the lesion with a layer of transparent dentine around the pulp horn. Evidence of receding pulp under the lesion.</td>
<td>Homogeneous demineralised lesion in the body extending to the pulp. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 36</td>
<td>Dark brown lesion throughout the lesion extending close to the pulp.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 37</td>
<td>Brown lesion throughout with pulpal exposure</td>
<td>Homogeneous demineralised lesion in the body extending to the pulp horn. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 38</td>
<td>Dark brown lesion throughout with a layer of transparent dentine around the pulp horn.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 39</td>
<td>Dark brown body of the lesion with yellow dentine sub-layer towards the pulp.</td>
<td>Shallow homogeneous demineralised lesion in the body. Pulp has receded in Section A but not B. Thick sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 40</td>
<td>Brown lesion throughout with pulpal exposure</td>
<td>Homogeneous demineralised lesion in the body. Pulp has receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 41</td>
<td>Dark brown lesion with yellow dentine sublayer towards the pulp.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 42</td>
<td>Brown lesion towards the pulp and a layer of yellow dentine layer surrounding the lesion. There is a smaller yellow lesion on the opposite side of the experimental cavity.</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp.</td>
</tr>
<tr>
<td>med 43</td>
<td>Brown body of lesion with a small yellow layer pulpal front</td>
<td>Homogeneous demineralised lesion in the body. Pulp has not receded. Thick sound tissue barrier between the lesion and the pulp. Thin calcified surface layer in the ‘infected’dentine. Bright features following shape of dentinal tubules below the lesion - possibly sclerotic dentine.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>med 44</td>
<td>Dark brown lesion in the body. There is a thick layer of translucent zone between the lesion and the pulp</td>
<td>Shallow homogeneous demineralised lesion in the body. Pulp has not receded. Thick sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
<tr>
<td>med 45</td>
<td>Dark brown lesion throughout with pulpal exposure in the open cavity. A separate lighter brown lesion with pale yellow dentine in the opposite side.</td>
<td>Deep Homogeneous demineralised lesion in the body extended closed to the pulp. Pulp has not receded. Very thin sound tissue barrier between the lesion and the pulp. Presence of partially demineralised advancing front.</td>
</tr>
</tbody>
</table>
Figure 11-34 Optical image of med 39 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B.
Figure 11-35 XMT and optical line profile through carious lesion for med 39 A with corresponding exponential decay curve fit.
Figure 11-36 XMT and optical line profile through carious lesion for med 39 B with corresponding exponential decay curve fit
Figure 11-37 Optical image of med 31 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B
Line profile through carious lesion for med 31 A

Figure 11-38 Line profile through carious lesion for med 31 A with corresponding exponential decay curve fit
Figure 11-39 Line profile through carious lesion for med 31 B with corresponding exponential decay curve fit
Figure 11-40 Optical image of med 10 with corresponding ‘before’ and ‘after’ excavation XMT images for sections A and B
Line profile through carious lesion for med 10 A

![Graph showing LAC (cm⁻¹) vs Distance (mm)]

Optical line profile

![Graph showing Greyscale value vs Distance (pixels)]

Figure 11-41 XMT and optical colour line profile through carious lesion for med 10 A with corresponding exponential decay curve fit
Line profile through carious lesion for med 10 B

Figure 11-42 XMT and optical colour line profile through carious lesion for med 10 B with corresponding exponential decay curve fit
Table 11.5 Table showing the values for the constants a, b, and α for the six datasets

<table>
<thead>
<tr>
<th>Dataset No. (med)</th>
<th>a</th>
<th>b</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>-0.13</td>
<td>0.92</td>
<td>0.72</td>
</tr>
<tr>
<td>Section A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Section B</td>
<td>-1.54</td>
<td>2.91</td>
<td>0.27</td>
</tr>
<tr>
<td>30</td>
<td>-0.09</td>
<td>1.21</td>
<td>1.32</td>
</tr>
<tr>
<td>Section A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Section B</td>
<td>-0.09</td>
<td>1.23</td>
<td>1.17</td>
</tr>
<tr>
<td>31</td>
<td>-0.06</td>
<td>1.24</td>
<td>1.54</td>
</tr>
<tr>
<td>Section B</td>
<td>-0.08</td>
<td>1.35</td>
<td>1.26</td>
</tr>
<tr>
<td>39</td>
<td>-0.16</td>
<td>6.49</td>
<td>2.15</td>
</tr>
<tr>
<td>Section B</td>
<td>0.02</td>
<td>36.76</td>
<td>5.61</td>
</tr>
<tr>
<td>10</td>
<td>-0.44</td>
<td>4.09</td>
<td>0.64</td>
</tr>
<tr>
<td>Section B</td>
<td>-0.51</td>
<td>3.72</td>
<td>0.48</td>
</tr>
<tr>
<td>11</td>
<td>-0.02</td>
<td>1.99</td>
<td>2.79</td>
</tr>
<tr>
<td>Section B</td>
<td>-0.03</td>
<td>2.04</td>
<td>3.41</td>
</tr>
<tr>
<td>Mean (s.d.) gradient</td>
<td></td>
<td></td>
<td>1.78 (1.53)</td>
</tr>
</tbody>
</table>
11.10.4. Discussion

Aligning the XMT slice to the optical scan image was a challenge. Firstly, the scan images were 2-D reflected images of the 3-D tooth surfaces. Therefore, the images included the background and were not exactly representative of the cut surface. Secondly, the X-ray beam may not pass parallel to the cut surfaces. Therefore, the first full XMT slice of the lesion from the cut surface was used for the comparison. Hence, this comparison may be confined to qualitative description. Furthermore, the colours were converted to grey levels which may cause further errors in attempting to correlate XMT values to colour. However, even with qualitative descriptions, the correlation between colour and XMT values are weak except for the translucent sclerotic dentine.

Dentine discolouration is commonly used by clinicians as an indicator for caries, and more specifically the rate of caries progression. A soft light-brown colour is believed to be more rapidly progressing than the dark brown colour [Topping and Pitts, 2009]. However, in the present study, no relationships were shown between colours and the gradient of mineral concentration (α value in Table 11.5). In fact, even within the same tooth (med 39), the colour on the cut surfaces of each half should be uniform, but the gradient difference between the two halves was 2.6 fold. These two XMT slices are 200-300µm apart and they are usually regarded as mirror images of one another. If their gradients indicate rate of caries progression, it can be concluded the rate of mineral dissolution has large variation within one lesion, thus using mirror half tooth as a control to study demineralisation process might not be justified.
In the literature, it has been shown that hardness is related to colour [Fusayama et al., 1966]. As hardness is related to mineral content [Angker et al., 2004b], it is expected colour should have a relationship with LAC. Hence, further work is needed to describe or measure colours digitally so that further analysis could be made.

It has been stated that in the body of carious lesion, the collagen has been denatured, so the dentine has lost its potential to remineralise. Therefore, it is interesting to note that in sample med 10, bands of hypermineralised layer with a LAC up to 93% of that for sound dentine (1.56 cm$^{-1}$). Hypermineralised surface layers have been observed in carious root dentine [Nyvad and Fejerskov, 1982; Wefel et al., 1985] which could be reproduced under experimental conditions [Nyvad et al., 1989]. Subsurface hypermineralised layer of carious root dentine was also reported by Schüpbach et al. [1990]. It has been shown recently that dentine with denatured collagen could have aggregation of apatite crystals around the PVPA-bound gelatine [Mai et al., 2010] when the specimen was placed in a biomimetic remineralising solution. These authors stated that the denatured collagenous lesion is unlikely to remineralise to the same hierarchical order to that with intact collagen. In section 11.9, it was shown in vitro, that although remineralisation was found in the body of the lesion, the potential was much less than in the partially mineralised layer where intact collagen was expected to be found. Hence, the remineralisation process in vivo might be very different to that in vitro because the in vivo remineralised layer had a mineral concentration close to that of sound dentine.
11.11. Conclusions

In this part, QM MuCat XMT system was used to investigate caries removal by three techniques. The findings are summarised as follows:

1. Carisolv technique tended to leave a layer of partially demineralised dentine which was not found in the Hand Excavation and Caries Detector technique.

2. The volume of sound dentine removed was significantly reduced in the CS and CD techniques compared to the HE technique.

3. Carisolv technique did not have any effect on sound dentine.

4. Carisolv hand instrument had a limiting cutting efficiency that would leave a layer of partially demineralised dentine.

5. The layer of partially demineralised dentine had the potential to remineralise.

6. The body of the lesion with denatured collagen could be remineralised in vitro but its potential was greatly reduced. However, a tooth with a remineralised lesion in the body of the lesion found naturally had a mineral concentration close to that of sound dentine.

7. Dentine with a LAC of 0.8 cm$^{-1}$ should not be removed as it has good potential to remineralise to 80% of sound dentine.

8. The use of dentine colour to indicate rate of demineralisation has not been justified.
Part III

Correlation of XMT with other techniques
Part III: Correlation of XMT with other techniques

In this present study, XMT provided good quantitative data based on LAC at 30 µm resolution. However some microscopic structures such as dentinal tubules were not visible. Hence it would be of interest to relate mineral concentration to microscopic features. The following is an account of exploring two microscopic techniques, Backscattered Electron microscopy (BSE) and Atomic Force Microscopy (AFM), to correlate with the XMT data.

12 Backscattered Electron Microscopy

12.1. Introduction and Aims

Methods for magnifying surface features go back as far as the late 18th century, with magnifying lenses and optical microscopy. Naturally, optical instruments are limited by the wavelength of visible light and can resolve objects down to approximately 0.5 microns. During the 20th century, methods for magnification based on electron and ion beams were developed. The scanning electron microscope (SEM) can resolve features to the level of approximately 30 Ångstroms if the specimen’s atomic number contrast is high.

When an electron hits a tissue, it interacts with its elements and sometimes, the electron may be deflected (scattered) back out of the surface of the tissue. If the tissue is composed of elements with high atomic numbers, the chance of the electron being absorbed is higher and the number of electrons which escape through the surface is
reduced. Therefore, BSE microscopy can be used to measure the mean atomic number of a tissue.

BSE is capable of producing an image with sub-micron resolution but the resolution is dependent on the penetration and the reaction of the electrons in the superficial layer of the specimen. Samples need to be sectioned and the images obtained are two-dimensional. For biological tissues embedded in PMMA (polymethylmethacrylate), the mean atomic number is related to the fractional volume concentration of mineral, and that is related to density. Therefore BSE can be used to correlate microscopic differences with levels of tissue mineralisation [Wong and Elliott, 1997] by calibrating its grey levels to mineral concentrations.

Compared to light microscopy and microradiography, atomic-number-contrast BSE imaging is virtually free of projection effect errors resulting from the superimposition of multiple tissues due to section thickness [Bachus and Bloebaum 1992]. The resolution of BSE imaging depends on the penetration of electrons into the specimen, which is approximately 5 µm (for an electron beam of 20–30 keV) in bone [Bachus and Bloebaum 1992] and can go down to 0.5 µm for an electron of 10 keV [Howell and Boyde, 2003]. The interpretation of the grey level in BSE images has been a topic of discussion. In theory, the grey level is directly related to the mean atomic number of the tissue. In practice, because the mean atomic number is related to the fractional volume concentration of mineral in biological hard tissue, which in turn is related to density [Skedros et al., 1993a; Skedros et al., 1993b], BSE can be used to assess the microscopic differences in tissue mineralisation [Reid and Boyde 1987]. Since the
introduction of BSE microscopy to investigate biological tissues [Boyde and Jones, 1983a; Boyde and Jones, 1983b], appropriate standards have been sought to cover the grey level range of mineralized tissues so that an absolute value of mineral concentration can be determined. XMT has been used to calibrate the grey level in BSE [Boyde et al 1990] by taking a BSE image of a transverse cross section of a rat femur, and afterwards an XMT image of the same section. A calibration curve was derived by comparing histograms of the grey level in the BSE image and the LACs in the XMT image [Mechanic et al., 1990; Davis and Wong, 1996]. More recently, dibutyl methacrylate has been used as the calibrating standards for BSE imaging [Boyde et al., 1996].

BSE imaging has been recommended as a tool to quantify the mineralisation state of calcified tissues and is commonly used in bone studies [Angker et al., 2004a]. It has also been used to characterise the mineral content of sound and carious dentine [Angker et al., 2004a] and correlate to mechanical properties obtained from microindentation technique for carious dentine.[Angker et al., 2004b].

The aim of this part of the project was to use BSE to characterise the ultra-structure of carious dentine in relation to its LAC values from XMT. An exploration to correlate BSE values to LACs was also attempted.
12.2. Materials and Methods

12.2.1. Sample preparation

Two teeth with large carious lesions were used for this part of the project. They were embedded in PMMA (Sample AB) according to the recommended embedding procedure described below:

*Embedding method procedure*

A small amount of MMA (methylmethacrylate (Merck)) solution with an added catalyst (Azo-iso-butyronitrile (Merck)) was put into a glass test tube, which was put into an oven to set, in order to create a platform that is flat for the tooth to be deposited on. The tooth samples were firstly dehydrated in 100% alcohol followed by xylene for a day. Then they were submerged in MMA solution for 24 hours to allow full infiltration of the MMA solution and catalyst. Finally each tooth was submerged in the test tube with the MMA platform. MMA solution and catalyst were added and the whole thing was tightly sealed to prevent evaporation of the MMA mixture. Once the sample was set (which could take from a couple of days to just over a week), it was ground down to the region of interest using a rotary polisher with platens and polishing cloths (Metaserv), and then finely polished to a 6 micron finish using a water based diamond polishing paste (Metprep).

12.2.2. BSE imaging technique

The PMMA embedded tooth samples (samples LB and AB) were carbon coated and qBSE images were obtained in the Zeiss DSM 962 SEM. Montages of the embedded tooth sample were made at 33x magnification, 17 mm working distance and 20 kV
accelerating voltage. Regions of interest, i.e. dentine carious lesions were located (identified) and imaged at 200x and 500x magnification to explore whether there was structural differences between ‘infected’ and ‘affected’ carious dentine.

Mono-iodinated and mono-brominated-PMMA standard density objects were used to calibrate atomic number contrast. Zeiss IMG format images were converted to TIFF images and the image grey levels stretched so that the BSE coefficients of the mono-Br-PMMA standard image grey was set to 0 and that of the mono-I-PMMA to 255.

After BSE imaging, XMT scans of the two samples were obtained at 15 µm resolution. The samples were scanned so that the surface of the sample was in line with the central ray in order to avoid geometric distortion of the divergent rays.

**Greyscale for BSE images**

The backscatter current is proportional to the mean atomic number of the sample of interest. The analogue current signal is digitized into 256 bins, which are subsequently given grey values for visualization and analysis. More densely mineralised tissues have higher average atomic numbers (Z) and therefore have higher digital bin values. Comparison of specimen backscatter current to known standard backscatter objects allows the normalization of BSE current readings to a quantitative scale.

Halogenated methacrylates have been used as effective standards for bone and calcified tissue as they have backscatter coefficients just greater and lesser than mineralised bone
and cartilage. This is in contrast to elemental backscatter standards as Al and C, whose backscatter coefficients are well beyond those typically found in mineralised tissues and in which channeling contrast due to crystallographic grain orientation occur and may override the Z contrast. Histogram peaks within BSE images of backscatter standards yield two standard grey levels. Pixel grey values within the sample image \( p \) are normalized \( p_n \) by subtracting the grey value of the lower standard \( b \), multiplying by the bin range \( r \) (255 for 8-bit images) and dividing by the difference between the higher \( i \) and lower standard grey value (Equation 12.1). Standards used for this part of the project were polymers of monobrominated-dimethacrylate and monoiodinated-dimethacrylate [Doube, 2007].

\[
p_n = \frac{(p - b)r}{i - b} \tag{Equation 12-1}
\]

12.3. Results

12.3.1. Sample 1 - LB sample

Both XMT and BSE datasets were cropped to the two region of interest (Figure 12.1) to the same dimensions, Region 1 shown in 12.2 and Region 2 shown in Figure 12.5. The clinical descriptions of the lesion in Region 1 and Region 2 were yellowish brown dentine throughout the lesion and yellowish brown dentine throughout the lesion with a layer of opaque (hypermineralised) dentine around the pulp horn. To pinpoint the structural features in the high resolution BSE image at a particular LAC value, line profiles (A to B) for both BSE and XMT images were obtained (Figure 12.3). It was
noted that the BSE values had the same trend as the LAC values, but they were noisier due to its higher resolution. The LAC value of 0.8 cm$^{-1}$, the proposed boundary to which caries should be excavated to (Chapter 11) corresponded to the grey level value of 64 in Figure 12.3 (illustrated by the black arrow). Using this value as the assumed boundary between the ‘infected’ and ‘affected’ dentine, and using the distance obtained from the curve to locate the regions in the BSE images, the ultra-structural differences between the two types of carious dentine could be studied.

Figure 13.4 shows greyscale and false colour BSE images of Region 1 at three different magnifications: 33x, 200x and 500x (grid size = 2700µm x 2700µm, 450µm x 450µm, 180µm x 180µm respectively). At the highest resolution individual dentinal tubules were clearly imaged. The carious lesion consisted of three layers in the coloured BSE image: Blue, green and yellow. Enlargement of dentinal tubules were observed in the body of the carious ‘infected’ dentine region (blue/green regions). Below this, in the ‘affected’ carious dentine (green/yellow coloured regions), red colour (indicating higher mineral concentration) was observed within the dentinal tubules. Also at the cavity floor, along the surface of the carious lesion, a layer of tissue with high mineral content was observed which was not found in the XMT images.
Figure 12-1 Scanned colour image of PMMA embedded carious tooth sample LB using a flatbed scanner. The tooth sample consisted of two lesions which were analysed using BSE and XMT. The imaging were divided into two regions – Region 1 and Region 2.
Figure 12-2 BSE image of PMMA embedded carious dentine lesion (Sample LB – region 1) at 33x magnification with corresponding XMT image at 15 µm resolution. In the BSE image the dentine lesion consists of three layers: Blue, green and yellow. Line probe was obtained from A to B which is shown in Figure 12.3
Figure 12.3 Line profiles (A to B) obtained from the XMT and BSE images for sample LB – region 1 (See Figure 12.2). The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine from the XMT study (Chapter 11)
Figure 12-4 BSE images of Sample LB – region 1. (A) Image obtained at 33x magnification (2700µm x 2700µm) – taken at the periphery of the dentine carious lesion. (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation (Distance from Figure 12.3 was 0.20mm). At the periphery of the lesion, the infected structure has a surface layer which is highly mineralised. (C) Image obtained at 500x magnification (180µm x 180µm) where the dentinal tubules are filled with highly mineralised material.
The following is a visual analysis of Region 2 of Sample LB using BSE and XMT images shown in Figure 12.5. The images obtained using the two techniques were cropped to the region of interest with the same dimensions. The same coloured layers could be seen in the carious dentine region for both techniques: blue, green and yellow (Figure 12.5 A and C). Also structural changes could be seen going from the body of the carious lesion to the ‘affected’ dentine based on the distances extracted from the line profiles shown in Figures 12.6, 12.8 and 12.10. The blue/green region is magnified in Figure 12.7 and 12.9 at 500x magnification. The dentinal tubules were enlarged to the extent that the structural integrity was destroyed and large ‘patchy’ holes could be visualised in the BSE images. Amongst the large ‘patchy’ holes, filled dentinal tubules can also be visualized with highly mineralised material. The assumed boundary between the ‘infected’ and ‘affected’ dentine was located in the BSE images. This was in the region at the interface of the blue/green coloured levels (Figure 12.7 (B), 12.9 (B) and 12.11 (A)). In region around line profile c, the magnified BSE image at 500x showed that the sound dentine directly below the advancing front of the lesion had dentine tubules occluded with highly mineralised material.
Figure 12-5 Colour image of sample LB – region 2 (A) with BSE image (B) and corresponding XMT grayscale and false colour images (C and D respectively) (Dimensions 307, 165, slice 308). Line profiles a, b and c are shown Figures 13.6, 13.8 and 13.10.
Figure 12-6 Line profiles (A to B) obtained from the XMT and BSE images for position a in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine.
Figure 12-7 BSE images of Sample LB – region a in region 2. (A) Image obtained at 33x magnification (2700µm x 2700µm). (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation. (Distance from Figure 12.6 was 0.30mm) (C) Image obtained at 500x magnification (180µm x 180µm) showing collapsed dentine structure and also filled dentinal tubules
Figure 12-8 Line profiles (A to B) obtained from the XMT and BSE images for position b in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine.
Figure 12-9 BSE images of Sample LB – region b in region 2. (A) Image obtained at 33x magnification (2700µm x 2700µm). (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation. (Distance from Figure 12.8 was 0.30m) (C) Image obtained at 500x magnification (D) Image obtained at 500x magnification (180µm x 180µm). Images C and D show collapsed dentine structure.
Figure 12-10 Line profiles (A to B) obtained from the XMT and BSE images for position c in Section 2 – Sample LB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine.
Figure 12-11 BSE images of Sample LB – (A) region c in region 2 obtained at 33x (2700µm x 2700µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation (Distance from Figure 12.10 was 0.35mm) and (B) image obtained at 500x magnification (180µm x 180µm).
12.3.2. Sample 2 – AB sample

An optically scanned image of Sample AB obtained with a flatbed scanner is shown in Figure 12.12 showing a mainly dark brown lesion and hypermineralised tubules extending into the pulp. Figure 12.13 shows BSE and XMT datasets cropped to the region of interest. The same trends were seen in this sample as with sample LB. The same coloured layers could be seen in the carious dentine region for both techniques: blue, green and yellow (Figure 12.13 B and D). This tooth had an arrested dentine carious lesion and hypermineralised sclerotic dentine tracts were observed in both the BSE and XMT images. Again line profiles were obtained from the XMT and BSE images (Figure 12.14). From these line profiles, the assumed boundary between the ‘infected’ and ‘affected’ dentine was located. This was in the region at the interface of the blue/green coloured levels (Figure 12.15 B). In comparison to the previous sample, more dentine tubules in the blue region had accumulated mineral of a higher mineral concentration (Figure 12.15 C and D). However, as shown in the 200x BSE image, the sclerotic dentine was captured showing filled dentine tubules but the adjacent sound dentine did not have any filled dentine tubules (Figure 12.16).

12.3.3. Decaying constants

Using Equation 11.3 in Section 11.2.7, decay fit was applied to both the BSE and XMT line profile data. The decay fits are shown in Figures 12.17, 12.18 and 12.19. As BSE had a better resolution in comparison to XMT, the data were noisier, thus the correlation coefficients \( r^2 \) of the fit were smaller. For sample LB, the gradient for BSE seemed to
be of the same order of magnitude as that for the XMT line profiles. However for sample AB, the gradient for BSE was much lower than that for XMT.

Figure 12-12 Scanned colour image of PMMA embedded sample AB using a flatbed scanner
Figure 12-13 BSE images (greyscale and colour – A and B) of PMMA embedded carious dentine (Sample AB) with corresponding XMT images (C and D) showing sclerotic dentine tract clearly
Figure 12-14 Line profiles (A to B) obtained from the XMT and BSE images for Sample AB. The arrow indicates the assumed boundary between ‘infected’ and ‘affected’ dentine.
Figure 12-15 BSE images of Sample AB: (A) Image obtained at 33x magnification (2700µm x 2700µm). (B) Image obtained at 200x magnification (450µm x 450µm) with marked lines indicating the assumed depth of the tissue which should be removed during excavation. (C) Image obtained at 500x magnification (180µm x 180µm) (D) Image obtained also at 500x magnification
Figure 12-16 BSE image of sample AB obtained at 33x magnification (2700µm x 2700µm) (A) together with (B) a BSE image obtained of the sclerotic dentine tract region and adjacent to sound dentine at 200x magnification (450µm x 450µm)
Figure 12-17 Decay fit to the XMT and BSE line profile plots (sample LB – region 2 (a) – Figure 12.6)

XMT parameters \( r^2 = 0.95; \ a = 6.06; \ b = 134.53; \ c = 3.19 \)

BSE parameters \( r^2 = 0.79; \ a = -15.71; \ b = 238.53; \ c = 1.22 \)
Figure 12-18 Decay fit to the XMT and BSE line profile plots (sample LB – region 2 (b) – Figure 12.8)

XMT parameters – $r^2 = 0.97$; $a = 13.53$; $b = 267.96$; $c = 3.27$
BSE parameters – $r^2 = 0.85$; $a = 20.52$; $b = 197.66$; $c = 2.16$
Figure 12-19 Decay fit to the XMT and BSE line profile plots (sample AB – Figure 12.14)

XMT parameters – $r^2 = 0.98$; $a = 36.35$; $b = 1587.56$; $c = 4.18$

BSE parameters – $r^2 = 0.84$; $a = -113.27$; $b = 1772.25$; $c = 0.61$
12.4. Discussion

Theoretical correlation of LAC from XMT and mean atomic number from BSE has been discussed by Wong and Elliott [1997]. An attempt was made using the line profile data from BSE and XTM images to calibrate both techniques at a point-to-point level. However, because of the high noise level from BSE, a direct comparison is not possible. Therefore spatial correlation was used for comparison purposes to relate LAC values to histological structures from BSE images.

Using LAC value of 0.8 cm\(^{-1}\) (Greyscale value of 64) the corresponding cut-off point was in the interface of the blue/green levels shown in the BSE images. The structures of the dentine towards the cavity floor from this level showed breakdown of the dentine mineral phase. Dentinal structures beyond this level towards the pulp also showed accumulation of mineral inside the tubules. Repair processes in dentine are clearly shown as occluded tubules (Figure 12.4 (C) and 12.7 (C)) and remineralisation of the outer infected dentine (Figure 12.4 (B)). The substance may be reactionary intratubular dentine. Hence, it can be concluded that the LAC value of 0.8 cm\(^{-1}\) can be used to determine the level to which dentists’ should excavate to.

Naturally different teeth could react differently to caries attack. Sample AB differed from Sample LB in presence of sclerotic dentine tracts, a defence mechanism against caries attack [Richardson, 1966]. In these regions, the pulp reacts to the carious attack by blocking the tubules with mineral. This reaction may or may not involve cellular activities [Daculsi et al., 1987; Shellis, 1994]. However, these hypermineralised tracts
are not present in all the lesions even within the same tooth. This demonstrates that the pulpal reactionary responses are localised.

There is no literature relating LAC values to hardness. Angker et al. [2004a] demonstrated an exponential relationship between hardness and mineral content determined from BSE data. Bembey et al. [2005] showed that the elastic modulus of dentine, unlike enamel, is less dependent on connectivity but more dependent on volume fraction of the mineral, which can be determined from LAC values. Therefore it can be concluded that the local variation in LAC values measured by XMT in this project had a direct relationship to hardness in dentine. Using Equations 5.2, 5.3 and 5.4 and finding the volume percent of mineral from the measured LAC using Equation 11.2, the LAC of 0.8 cm\(^{-1}\) could be converted to a KHN. According to Equation 11.2, \(C_D\) can be calculated for the given measured LAC. As the density of HAP is 3.15 g cm\(^{-3}\), the \(V_m\) of dentine can be calculated as:

\[
V_m = \frac{C_{Dmin}}{\mu_{mHAP}} \tag{Equation 12.2}
\]

Therefore, the measured threshold LAC 0.8 cm\(^{-1}\) can be converted into a volume % of 21.50%. Substituting this value into Equation 5.4, which relates hardness with mineral content, the hardness for this threshold value is 7.66 kgmm\(^{-2}\) (where 1 kgmm\(^{-2}\) \(\approx\) 9.8 MPa). Paolinelis et al. [2006] quoted that the values for carious dentine were in the range KHN 5.5 and 14. The KHN value derived from the LAC value in the present study is half way between those quoted by Paolinelis et al. [2006]. The assumption for this calculation is that demineralised dentine is replaced with material e.g. water and bacteria which has a negligible contribution to LAC. However, if Equations 5.2 and 5.3 were...
used, the corresponding KHN values would be 16 and 0.007 respectively. This indicates that Equation 5.4 is more appropriate for correlating mineral concentration to hardness.

12.5. Conclusions

This study used the relationship between LAC and BSE information to investigate the ultrastructure of carious dentine at particular LAC levels. The LAC value of 0.8 cm$^{-1}$ is a reasonable value to mark the level where the ultrastructure of carious dentine changes from a disintegrated mass to dentine which has evidence of reactionary accretion of minerals. The corresponding KHN value of 7.66 kgmm$^{-2}$ could be used to design the cutting efficiency of excavating instruments.
13 Atomic Force Microscopy (AFM)

13.1. Introduction

Atomic Force Microscopy (AFM), a type of Scanning Probe Microscopy (SPM), is a recent, innovative and rapidly growing form of microscopy. It has the advantage of resolving surface features in three dimensions without damaging the sample surface. As this technique could, in theory, give surface stiffness/toughness values, it should complement the XMT studies in higher resolution and could help to search the boundary to which caries should be excavated to, like the BSE study in the previous chapter. However, many difficulties have been encountered, especially on the softness of the gross carious dentine. Hence, the original aim to correlate the XMT and AFM had to be abandoned. Nevertheless, an account of the investigation/exploration is present in this section to highlight the difficulties for future studies. The account of this exploration included the use of conventional AFM and collaborative work with Dr Laurent Bozec [Bozec and Horton, 2007], at the Bone and Mineral Centre, UCL, to use the novel Photothermal Microspectroscopy (PTMS) to investigate carious dentine.

13.1.1. AFM – Theory and Background

Atomic force microscopy allows scanning of any, non-conductive or conductive, samples. For AFM the most common long-range interactions associated with the deflection of the cantilever are: van der Waals, electromagnetic or capillary forces (resulting from the presence of a water layer between the tip and sample). The graph of Force versus tip-to-sample separation is shown in Figure 13.1.
At the right side of the curve (Figure 13.1) the atoms are separated by a large distance. As the atoms are gradually brought together, they first weakly attract each other (point (i)). This attraction increases until the atoms are so close together that their electron clouds begin to repel each other electrostatically (point (ii)). This electrostatic repulsion progressively counteracts the attractive force as the interatomic separation continues to decrease. The force goes to zero when the distance between the atoms reaches a couple of angstroms, about the length of a chemical bond (point (iii)). When the total van der Waals force becomes positive (repulsive), the atoms are in contact (point (iv)).

The slope of the van der Waals curve is very steep in the repulsive or contact regime. As a result, the dominant repulsive van der Waals force balances attractive forces. In AFM
this means that when the cantilever pushes the tip against the sample, the cantilever bends rather than forcing the tip atoms closer to the sample atoms. Using the inter-molecular force curve, the three main classes of interaction are contact (repulsive mode), non-contact, and tapping-mode.

13.1.1.1. Contact AFM

In the contact AFM method, the probe tip (which is mounted to the end of the cantilever) scans across the sample surface, coming into direct physical contact with the sample. As the probe tip scans, the changes in topographic features consequently cause the tip and cantilever to deflect. It is comparable to a record player; the stylus follows the contours of the record. A light beam from a small laser is bounced off of the cantilever and reflected on to a four-section photodetector (See Figure 13.2). The amount of deflection of the cantilever, which is essentially the force it applies to the sample, can then be calculated from the difference in light intensity on the sectors. Hooke’s Law gives the relationship between the cantilever’s motion, $d$, and the force required to generate the motion, $F$:

$$F = -kd$$

Equation 13-1

where $k$ is the spring constant which differs for all tips. The amount of motion of the cantilever, or the force it applies to the sample, can then be used in a feedback loop to control the Z piezo, maintaining constant cantilever deflection (also known as constant force mode). In this way, topographic data is obtained. AFM is a technique which is fast, high force, gives best resolution of hard samples e.g. atomic resolution of Si.
245

Figure 13-2 Schematic diagram showing the configuration of the AFM

13.1.1.2. Non-contact AFM

As the cantilever moves across the sample surface in contact scanning, the lateral motion of the cantilever may cause damage to soft or fragile samples such as biological specimens or polymers. Because of this phenomenon as well as a number of other factors, non-contact scanning is sometimes preferable. In non-contact operation, the cantilever is oscillated at its resonant frequency. In this mode, what is being detected is changes in force between the tip and sample, even though they are not in contact. These force changes are also referred to as the force gradient. As the probe gets closer to the sample surface, the force gradient changes, thus changing both the oscillation amplitude and phase of the vibrating cantilever. Either the change in amplitude or the change in phase can be detected and used to control the tracking of the probe over the surface (i.e., the feedback-control loop).
13.1.1.3. Tapping-Mode AFM

This mode measures the topography of a surface by tapping the surface with an oscillating probe tip. This reduces shear forces which can damage soft samples and reduce image resolution. This is an improvement on conventional contact AFM, in which the cantilever just drags across the surface at constant force and can result in surface damage. Tapping mode is gentle enough even for the visualization of supported lipid bilayers or adsorbed single polymer molecules (for instance, 0.4 nm thick chains of synthetic polyelectrolytes) under liquid medium.

13.1.1.4. Force Modulation

Force modulation imaging is an AFM technique that maps out the stiffness of the sample surface. This technique is used to distinguish different constituents of the sample as in the case of dentine. This technique is an extension of contact mode AFM. In contact mode AFM, the scan is done in an x-y raster pattern. In force modulation the tip moves with a small vertical oscillation which is significantly faster than the raster scan rate. This modulates the force on the sample, meaning that the average force is equal to the force in contact mode. This modulation, together with the tip in contact with the sample, allows the resistance to the oscillations by the surface which as a result causes the cantilever to bend. For example, a surface with a low stiffness will deform more easily than a surface with higher stiffness as the stiffer surface area is more capable of resisting the vertical oscillation of the cantilever causing greater bending of the cantilever. What is displayed is a relative stiffness map of the surface with the units being the cantilever deflection amplitude at the frequency of modulation. This technique has been applied to
sound dentine, and a force modulation image has been obtained together with a topography image by Marshall *et al.* [1997b].

Modulated force AFM scanning maps variations in the hardness/compliance of the sample. Like lateral force information, modulated force data reveals less topography than standard SPM modes, but it is always collected simultaneously with topographic data.

### 13.1.2. AFM components

The following is a description of the AFM configuration as described by the manufacturer (Thermoscopes, Veeco, UK).

*AFM cantilevers* - AFM cantilevers (Figure 13.3) are fabricated from silicon nitride (Si$_3$N$_4$) or silicon (Si). A V-type cantilever was used as these tend to have a smaller spring constant.

![V-type cantilever](image)

*Figure 13-3 A typical AFM cantilever*
Piezoelectric ceramics - Control of the SPM probe over extremely small distances is made possible by the use of piezoelectric ceramics. Because of the properties of these materials, changing the applied electric potential across electrodes on opposing faces causes a corresponding change in their physical dimensions — as shown in Figure 13.4, where the piezoelectric cylinder becomes elongated and the diameter shrinks when voltage is applied. Piezoelectric ceramics are fabricated into a variety of shapes and configurations. Each type of ceramic has a unique expansion coefficient that allows calculation of physical distortion upon the application of a potential. The coefficients range from 1 Å/volt up to 3000 Å/volt. As a result, these ceramics permit highly accurate positioning of the probe tip.

Tripod scanners are made from three discrete pieces of piezoelectric ceramic, mounted orthogonally. These create positive or negative scan motion in the X, Y, or Z directions as voltage is applied to the appropriate piezo. As the X and/or Y piezo are activated, they push against the base of the Z piezo, causing it to pivot laterally over a stationary pivot point. Applying voltage to the Z piezo creates Z movement. In the tripod scanner piezo configuration, the sample is mounted at the top of the Z piezo and rastered beneath the stationary tip/laser assembly. Because the X, Y, and Z piezos are physically and electrically decoupled, the configuration creates less distortion in large-area scanning applications.
Figure 13-4 Tripod scanner piezo configuration

*Laser light lever AFM sensor* - In contact AFM, the probe tip scans across the sample surface, coming into direct physical contact with the sample. As the probe tip scans, topographic features cause deflections of the tip/cantilever. A light beam from a small laser is reflected from the cantilever and is reflected on to a four-section photodetector. The amount of deflection of the cantilever, and the force it applies to the sample, can then be calculated from the change in light falling on the sectors of the photodetector. The resulting change in the detector current signal (internal sensor current) is converted into an image based on surface height.

*Feedback* - An electronic feedback circuit uses the output of the probe/sensor to drive the Z piezoelectric ceramic to create the Z-positioning mechanism (Figure 13.5). A PID (proportional, integral, and derivative) feedback control system is used. The feedback response is based on an equation which combines terms proportional, integral, and derivative of the error signal to set the Z position of the probe to maintain a constant sensor signal. The error signal is the difference between the sensor signal and a user-defined set point. The optimum settings for the PID coefficients depend on sample
properties, scan rate, and the probe tip geometry. Therefore, they need to be determined experimentally.

![Feedback Circuit Diagram](image1)

**Figure 13-5 Feedback circuit**

When the interaction between the tip and sample increases i.e. increase in force for contact AFM, the output of the sensor also increases. A differential amplifier compares the increased value from the sensor to the reference value ($V_s$) and outputs a correction voltage which drives the piezoelectric ceramic to pull the sensor away from the surface, restoring it to its original output level. This feedback circuit operates continuously to maintain constant tip/sample separation, or in contact AFM, constant force.

13.1.3. Images acquired

The instrument allows two methods of obtaining surface information in the form of images: *internal sensor* and *topography*. Each method has particular advantages and disadvantages. However, both detection methods can also be used simultaneously, to acquire separate topography and internal sensor images.
Internal sensor - The internal sensor detection method creates images with the greatest resolution of topographic detail and contrast. Data is gathered by detecting the sensor current (change in cantilever deflection) which can then be converted into surface height information (in contact AFM). However, accurately measuring Z height with this method requires that the PID values be set to zero (eliminating Z feedback control movement from the equation). A zero PID setting limits the instrument’s ability to accurately measure large changes in Z height.

Topography - The second, more common method of acquiring image data is the topography method. It provides the greatest level of accuracy in measuring and imaging larger-scale features and topography changes. This method involves optimizing the P, I, and D feedback coefficients and taking the voltage reading directly from the Z piezo feedback signal during the scan. The movement of the Z piezo is controlled by the feedback loop (PIDs).

Proportional, Integral and Derivative (PID) - The variable proportional, integral and derivative gain values (PIDs) control the tip response time of the Z piezo feedback loop. Together with the scan rate, PID gain settings are the most important adjustments when optimizing the feedback loop. If PID settings that are too low or too high; or a scan rate that is too high this can result in poor-quality images. The parameters can be varied whilst scanning.
13.1.4. The Force-distance curve

During AFM data acquisition, it is possible to utilize the *force-distance curve* function to measure the adhesion between two surfaces or the hardness of a sample surface. This curve represents a measurement of the force exerted on the AFM cantilever (with force constant, $k$) by the sample surface (Figure 13.6).

![FORCE-DISTANCE CURVE](image)

**Figure 13-6 An example of a force-distance curve**

Single force-distance curves can provide the information discussed above, but repeated measurements at the same sample position can provide additional information, especially where plastic or slowly responding elastic (viscoelastic) deformations are present.
13.1.5. AFM studies on dentine

Combining AFM and nano-indentation, mechanical properties such as hardness, Young’s modulus and viscoelasticity of dentine have been obtained [Kinney et al., 1996; Balooch et al., 1998; 2003b]. Usually the AFM contact mode is used to investigate the surface morphology, e.g. the surface change of dentine by demineralising or conditioning agents [Marshall, Jr. et al., 1995; Kinney et al., 1995a; Marshall, Jr. et al., 1997a; Marshall, Jr. et al., 1998; Balooch et al., 1998; Marshall, Jr. et al., 2004]. Higher quality AFM images have also been obtained using AFM in Tapping mode to investigate the acid conditioning effect on dentine surface [Eliades et al., 1997; Silikas et al., 1999]. However, no AFM study was found in natural lesions of dentine.

As dentine is a hard tissue, contact mode would be the best mode and imaging in a liquid environment is preferable to keep dentine hydrated. This mode generally allows a wide variety of samples to be scanned non-destructively to the sample surface.
13.2. Investigation of natural carious dentine lesion with AFM

13.2.1. Investigation using Veeco Explorer

A standard AFM system, Veeco Explorer (Figure 13.7) had three-piezo scanners with a maximum planar range of 100 μm in each of the planar \((x, y)\) directions and a maximum vertical \((z)\) range of 10.5 μm, with a CCD camera. The images were obtained using contact mode because it is the simplest AFM method, allowing high-resolution (~ 0.1 Å) topographic data to be obtained, and it is recommended for hard substrates, applicable to dentine. Standard V-type cantilevers, made of non conductive silicon nitride were used because they tend to have a smaller spring constant, allowing them to be stable whilst scanning.

![Figure 13-7 Image of Veeco Explorer](image)

13.2.1.1. Sample preparation

Transverse cross sections of one deciduous molar with gross carious lesion was cut using a rotary saw (Microslice II, Malvern Ltd). The thickness of the samples was 1 mm. They were mounted on thin sheets of steel using general purpose adhesive, so that the sample was stable whilst scanning (Figure 13.8).
The sample was polished with Alumina powder (0.3 micron size particles). The polishing machine used was an OMP-1 (Research and Industrial Instrument Co.) with a 150 mm diameter plate spinning at 50 rpm. It was covered with a MasterTex synthetic short nap cloth (Buehler U.K.). The alumina was made into a paste with distilled water. Prior to scanning, the sample was ultrasonicated in water to get rid of unwanted debris. The sample was then securely held by the magnetic sample holder on the base of the AFM. Marking the sample was quite difficult but once a reference point was found (e.g. a hole was visible on the surface which was due to the way the tooth sample was cut, the cut was made right through the top of the pulp) this point could be relocated via the CCD video camera connected to the AFM apparatus with great ease.

![Figure 13-8](image)

Figure 13-8 (a) Schematic diagram of the where the tooth sample was cut (modified image from [Yang et al., 2005]) (b) a picture of a typical tooth sample for using in the AFM
13.2.1.2. AFM images of dentine using Veeco Explorer

The typical AFM image of sound dentine is shown in Figure 13.9. It can be observed that the dentinal tubules (1-2 µm in diameter) are surrounded by peritubular dentine (~ 1 µm thick). The peritubular dentine appears to have a height greater than the intertubular dentine (shown by the brighter yellow in Figure 13.10). This may be due to their differences in hardness. As peritubular dentine is harder, during polishing, more intertubular dentine was abraded than peritubular dentine. Hence the peritubular dentine appears to be prouder on the surface.

Comparing the processed AFM images (levelling has been applied using the SPMLab software) of carious dentine to sound dentine (Figure 13.11), some of the tubules in carious dentine merged together to form large gaps. This suggests that there was a loss of structural integrity causing the tubules to collapse.

Point spectroscopy was used to acquire Force distance curves at four data points on the scanned surface. Figure 13.12 demonstrates a typical force distance curve, which is related to the approaching and retracting of the tip from the sample surface. In theory, this should be related to the hardness of the sample. However although the curve can provide relative qualitative information in regards to the hardness of the sample, it is not possible to extract absolute hardness measurements from the plot.
Figure 13-9 AFM image of the dentine surface (leveling has been applied using the SPMLab software)
Figure 13-10 A topographic AFM image of dentine together with a 3D depiction (SPIP software)
Figure 13-11 Topographic AFM images of (a) sound and (b) carious dentine

Figure 13-12 A topographic image of dentine with a Force distance graph
**Force Modulation AFM images**

Processed topographic images together, with Force Modulation images are shown in Figures 13.13 and 13.12. In the force modulation images it can be observed that the peritubular dentine is much stiffer than the intertubular dentine, which appears as dark rings around the openings of the dentinal tubules (Figure 13.12(b)). Also the intertubular dentine exhibits a variation of stiffness whereas the peritubular dentine appears to be homogeneous. It has been suggested that this variation is due to the distribution of apatite on the collagen matrix [Marshall, Jr. et al., 1997b].
Figure 13-13 Force modulation images together with topographic data (Example 1) - (a) Topographic, (b) Force modulation

Peritubular dentine

Figure 13-14 Force modulation images together with topographic data (Example 2) - (a) Topographic, (b) Force modulation. The peritubular dentine which is more stiff than the intertubular dentine appears as defined dark rings around the dentinal tubules.
13.2.2. Investigation with Veeco Dimension 3100

The Dimension 3100 (with Dimension DAFMLN head) is a higher resolution AFM system than Veeco Explorer (Figure 13.15). The Dimension DAFMLN head could scan up to 90μm in X-Y and up to 6μm in Z. This head included a piezoelectric tube scanner. Standard contact mode cantilevers (tipless) mounted specifically for the Dimension (Veeco, Europe) was used. They were V-type cantilevers, made of non conductive silicon nitride.

![Image of Veeco Dimension 3100](image)

13.2.2.1. Sample preparation

Four tooth samples (two longitudinal sections and two transverse sections) were embedded in PMMA and were ground to sections of approximately to 5 mm by 5 mm (Figure 13.16). (See Section 12.2.1 for embedding procedure).
Figure 13-16 (a) Image of longitudinal and (b) transverse sections of carious deciduous molars embedded in PMMA

13.2.2.2. AFM images of dentine using Veeco Dimension 3100

In the region about the boundary between sound and carious dentine, the raised peritubular dentine around the tubules was clearly seen (Figure 13.18 (a)). In Figure 13.18(b), at a high magnification a dentinal tubule, which was located close to the bulk of the carious lesion, was imaged. The D-banding of collagen fibrils in the intertubular dentine could be visualised (Figure 13.18(c)), confirming that the fibrils were perpendicular to the direction of the dentine tubules and the collagen framework around sound/carious dentine boundary was intact. Attempts were made to image the body of the lesion but it was too soft for the AFM probe. Instead of the probe being deflected when it was brought into contact, the probe made a dent in the sample. Several attempts were made in other embedded samples, including the use of non-contact mode. The same difficulties were encountered. Therefore, no further investigations were carried out.
Figure 13-17 (a) Topographic AFM image of embedded transverse sections of carious dentine. (b) AFM image of one dentine tubule with (c) a zoom-in image of collagen fibrils in demineralised carious dentine. (Courtesy of Dr Laurent Bozec – Collaborative Project)
13.2.3. Investigation using Photothermal Microspectroscopy (PTMS)

It is desirable not only to observe topographical data, but also compositional changes within samples [Bozec et al., 2006]. Although methods for chemical characterisation at the sub-micron scale exists, there is a current need, especially in biosciences, to develop techniques that integrate imaging with chemically specific measurements at a scale beyond the diffraction limit and using the entire mid-infrared spectral range [Bozec et al., 2001].

Photothermal Microspectroscopy (PTMS) is based on Nano-Infrared AFM spectroscopy, developed by Hammiche and Pollock at the University of Lancaster [Hammiche et al., 1996]. This technique has been used to differentiate between native and denatured collagen in rat tails [Bozec et al., 2005] and historical parchments [deGroot et al., 2005].

13.2.3.1. Photothermal Microspectroscopy configuration

The Vector 22 AFM - combined microscope (Figure 13.18 and 13.19) consists of a planar mirror and a concave mirror, machined in a block of aluminium, which acts as condensing optics to focus the flux at the sample surface. The sample is mounted on a stage attached to an X-Y-Z micrometer-based translator. With the probe attached to the scanner, the microscope can be moved so that the probe tip is positioned at the focal point of the IR radiation. As the sample absorbs the IR radiation, it heats up. The associated temperature rise is measured by the probe whose amplifier output is fed into the external input of the spectrometer. A Fourier transform algorithm is performed on this signal after digitisation. Peaks in the resulting spectrum represent peaks of
absorption by the sample across the wavelength range of the IR radiation. Coherent averaging using coadding is used for signal to noise improvement.

Figure 13-18 Schematic diagram of the photothermal FTIR micro-spectroscopy technique. The microscope is mounted inside the sample compartment of the FTIR spectrometer. The schematic diagram is together with the upper part of the Veeco Explorer.
13.2.3.2. The Wollaston thermal probe

The PTMS technique utilizes a probe which acts as an ultra-miniature electrical resistor [Hammiche et al., 2004a; Hammiche et al., 2004b]. The present configuration employs the probe design described by Dinwiddie and Pylkki [Dinwiddie et al., 1994; Pylkki et al., 1994; Reading et al., 2001] (Figure 13.20). This is fashioned from Wollaston process wire which consists of a 75 μm diameter silver wire surrounding a 5 μm diameter core of platinum (Pt)/10% rhodium (Rh) alloy. The wire is bent to form a sharp loop and secured into shape with a bead of epoxy resin. The silver layer is then etched away at the apex to reveal the platinum filament which forms the major electrical resistance element.
(approximately 2 Ω) in the assembly and acts as a temperature sensor and heater. A reflective mirror is glued on the wires to serve as a target for a laser spot which forms part of optical lever deflection feedback system of the microscope. The finished assembly is mounted on a carrier for mechanical and electrical connection to the piezoelectric scanner of the microscope.

The resistive probe is capable of performing three functions: it exerts a force on the sample surface; it acts as a highly localized heat source, either constant, modulated or ramped; and it measures heat flow [Pollock and Hammiche, 2001].
The resistive probe is capable of performing three functions: it exerts a force on the sample surface; it acts as a highly localized heat source, either constant, modulated or ramped; and it measures heat flow [Pollock and Hammiche, 2001].
13.2.3.3. PTMS images of dentine

One embedded sample from section 13.2.1 was imaged by PTMS. The difference in colour in Figures 13.21 and 13.22 corresponded to the different thermal conductivities of the dentine matrix, the higher the contrast the higher the thermal conductivity (i.e. the sample absorbs more heat). The more mineralised dentine was darker than that with the exposed collagen. The peritubular dentine is darker than the intertubular dentine because peritubular dentine is more mineralised. These images are similar to those by Sheard and Boyde [1989] who used Photothermal Radiometric (PTR) microscopy [Nordal and Kanstad, 1979] and found that less mineralised regions showed higher signal levels. The zoom in PTMS image in Figure 13.22 is strikingly similar to the proposed dentine structure by Scott and Symons as shown in their schematic diagram of dentine cut perpendicular to the dentinal tubules in Figure 13.23. Attempts were made to use PTMS to image the bulk of the caries lesion. It was also found that the grossly carious dentine was too soft for the probe. Hence, further investigation on dentine had to be abandoned.
Figure 13-21 PTMS image of dentine

Figure 13-22 Zoom in PTMS image focusing on the dentinal tubules with corresponding 3D topographic image of the same region
Figure 13-23 Schematic diagram of dentine cut perpendicular to the dentinal tubules emphasis on the collagen contribution to the dentine composite [Scott and Symons, 1982]
13.2.4. Effect of Carisolv solution on collagen

Carisolv solution is claimed by the company to dissolve only the denatured collagen part of infected carious dentine even though there are other residual organic materials present through, for example, partial hydrolysis by enzymes or Millard reactions of the organic component. As gelatine, which is denatured collagen, is found in carious dentine [Mai et al., 2010], it could be used as a simplified model to investigate the effect of Carisolv solution. Although it was not possible to use AFM to image carious dentine in the body of the lesion, it can be used to investigate the morphological change. Hence, a small experiment was carried out on a compressed collagen sheet to investigate the morphological change of pure and denatured collagen when the Carisolv solution is applied.

On a fabricated Type I collagen compressed sheet [Brown, 2005], five holes were created by the thermal probe. The focus was on the middle hole (Figure 13.24). In the middle hole, Carisolv was applied, the effect of which was observed by comparing the AFM images before and after application.
13.2.5. AFM images of the collagen sheet

Figure 13.25 shows a 3D depiction of the hole. The high temperature that was applied to the sample with the Wollaston probe not only pierced the sample surface but also gelatinised the surrounding area around the hole. This gelatinous region can be regarded as the denatured collagen.

Before application of Carisolv, the gelatine region was shown to have a glass like surface (Figure 13.26). After application of Carisolv, the image clearly shows that there was no change in the collagen matrix except in the region where collagen was changed to gelatine region in the peripheral region of the hole; some of the gelatine was removed. A line profile across the hole before and after Carisolv application showed that the width of the trough of the hole enlarged from 12 µm to 14 µm after application of the Carisolv solution, but the outer perimeter remained the same.
This small experiment confirms that the Carisolv solution is selective in removing denatured collagen only.

Figure 13-25 3D depiction of the hole before application of Carisolv
13.2.6. Discussion of AFM studies

The aim of this section was to exploit the ability of the AFM and correlate the findings with mineral concentration using the XMT data. However, this was not possible due to technical problems with the very low stiffness of the lesion; the AFM probe sank into the sample. Nevertheless some interesting details were observed using AFM. The images obtained using the Veeco Explorer were simple confirmation of what has been observed in the literature previously by Kinney et al. [2003b] (Figures 13.9 - 13.14).

Figure 13-26 Comparison of ‘hole’ before and after Carisolv application. Inset shows a zoom in image (5 µm x 5 µm)
High resolution images obtained also confirmed collagen fibrils in the bulk of the carious lesion seen as the D-banding of the collagen fibrils could be resolved. This suggests that this image was taken in the ‘affected’ carious dentine region (as it was difficult to obtain any images in the ‘infected’ dentine region) where the mineral has dissolved leaving behind an intact collagen structure which has potential to remineralise. The PTMS technique was used to try to obtain another way of differentiating between native and denatured collagen. The preliminary results demonstrated its capabilities, although further work is required for any clinical relevance.

In interpreting the photothermal images, what is being imaged is the spatial distribution of heat absorption depending on the different phases within the sample of interest. The thermal conductivities (K) of HA and collagen:

\[
K_{(HA)} \approx 2 \text{ W/m/k} \quad \text{[Dyshlovenko et al., 2004]}
\]

\[
K_{(coll)} \approx 0.6 \text{ W/m/k} \quad \text{[Bhattacharya and Mahajan, 2003]}
\]

As \( K_{(HA)} \gg K_{(coll)} \), the HA should be brighter on the images. However many factors need to be taken into consideration such as diffusivity and heat capacity. In Figure 13.22, PTMS zoom in image of dentine; the bright structures around the dentinal tubules could be interpreted as polishing artefacts or the structure of the embedded material, PMMA. However the striking resemblance of the PTMS image of the dentinal tubules (Figure 13.22) with the schematic diagram of dentine by Scott and Symons [1982] (Figure 13.23) suggests that the strand like structures seen within the intertubular dentine are collagen fibrils. This shows that the PTMS technique is sensitive and able to resolve the fine collagen structure based entirely on its thermal properties.
The PTMS technique was used for a preliminary study which was conducted to investigate the effects of the Carisolv product, if any, on a heat created gelatine sample. The results showed that a chemical reaction had taken place in 30 seconds of application of Carisolv on the artificially created holes by the thermal probe (Figure 13.26). The possible reason for the topographic changes could be due to the oxidising effect of the sodium hypochlorite within the product. Further experiments are needed to investigate the possibility of utilising thermal properties to differentiate between denatured and native collagen within carious dentine.

13.2.7. Conclusions of AFM studies

The low resolution AFM did not provide any additional information which could be correlated with XMT. High resolution AFM provided images to confirm that the collagen matrix is still intact in the partially demineralised region of dentine caries. Preliminary PTMS work shows that it could provide further insight into differentiating between infected and affected dentine. The effect of Carisolv gel on pure gelatine was clearly seen in changes in topographic detail.
General Conclusions

1. The mineral concentrations of dentine caries removed by 3 techniques were quantified using XMT techniques and the ultra-structures of carious dentine were investigated using BSE and AFM.

2. Carisolv was the most conservative in preserving sound dentine during caries removal, probably due to the lower cutting efficiency of its hand instruments.

3. A LAC value of 0.8 cm\(^{-1}\) (40 keV equivalent) with the corresponding KHN 7.66 kgmm\(^{-2}\) could be regarded as the boundary between “infected” and “affected” dentine.

4. Partially demineralised dentine at the advancing front of natural carious lesions was shown for the first time to have ability to remineralise up to 80% of sound dentine.

5. Carisolv gel aided the removal of denatured collagen

6. It was not possible to correlate optical colours of carious dentine to mineral concentration.

7. It was not possible to correlated BSE value to LAC at a point to point level due to the high noise level of BSE images.

8. It was not possible to obtain AFM images in the body of carious dentine due to its softness.
Future Work

- More investigation is needed for the partially demineralised dentine layer. An *in-situ* study can be used to investigate its remineralising potential.
- New hand-instruments with less cutting efficiency than the currently available excavators are needed to preserve sound dentine. Their efficacy can be investigated using the XMT technique.
- The approach in this study to investigate the efficacy of the Carisolv technique needs to be applied to permanent teeth.
- The superimpositions between scans need to be improved to include another degree of freedom of rotation and to be automated.
- The potential of PTMS is to be explored to differentiate native and denatured collagen at different levels of carious dentine.
References


Appendices
Appendix A – Ethics Approval

BLUE QUEEN MARY UNIVERSITY OF LONDON

BARS AND THE LONDON NHS TRUST

FINAL R&D APPROVAL

Dr. Ferranti Wong
Paediatric Dentistry
Dental Institute
New Road
Whitechapel
London
E1 1BB

31 August 2006

Dear Dr. Wong,

Re: X-ray microtomography study of carious teeth and comparison of its removal by various techniques

Thank you for sending confirmation of your approval from the ethics committee. I am now happy to inform you that the Joint R&D Office of Barts and The London NHS Trust and Queen Mary, University of London has arranged full indemnity cover for your study against any negligence that might occur during the course of your project.

Please note that all research with an NHS element is subject to the Research Governance Framework for Health and Social Care 2016. If you are unfamiliar with the standards contained in this document or the BLT and CMU1 policies that reinforce them, you can obtain details from the Joint R&D Office, tel: 0207 882 7200 or go to http://www.dh.gov.uk/PolicyAndGuidance/ResearchAndDevelopment/ResearchAndDevelopmentA2Z/ResearchGovernance/Inves

You must stay in touch with the Joint R&D Office during the course of the research project, particularly if:

- There is a change of Principal Investigator;
- The project finishes;
- Amendments are made, whether minor or substantial;
- Serious Adverse Events have occurred (must be reported within 24 hours of becoming aware of the event);

This is to ensure that your indemnity cover is valid. Should any unforeseen events occur it is essential that you contact the Joint R&D Office immediately. If patients or staff are involved in an incident, you should also contact the Clinical Risk Manager on 0207 882 4152.

I hope the project goes well, and if you need any help or assistance during its course, please do not hesitate to contact the Office.

Yours sincerely,

[Signature]

Gerry Leonard
Head of Research Resources
## Appendix B – Basic Results

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A - Total volume of dataset (voxels)
B - LAC of Dentine (modal value)
C - Lower cut-off point for sound dentine
D - Volume of sound dentine from subtraction dataset
E - Sound dentine removed (D/A)
F – Perceived better excavation
G - Actual excavation methods
Appendix C - Presentations and Publications

ORCA - Budapest, Hungary – 1\textsuperscript{st} - 4\textsuperscript{th} July 2009  
\textbf{Comparison of volumes of sound dentine removed by three caries removal techniques}  
F S L Wong\textsuperscript{*}, M Ahmed, G R Davis

IAPD - Munich, Germany – 17\textsuperscript{th} - 20\textsuperscript{th} June 2009  
\textbf{X-Ray Microtomography Study of Remineralisation After Caries Removal By Two Techniques}  
F S L Wong\textsuperscript{*}, M Ahmed, G R Davis

ORCA - Groningen, The Netherlands – 25\textsuperscript{th} - 28\textsuperscript{th} June 2008  
\textbf{X-Ray Microtomography Study of the Efficacy of Carisolv\textsuperscript{TM} in Removing Carious Dentine}  
F S L Wong\textsuperscript{*}, M Ahmed, G R Davis,

IADR - Thessaloniki, Greece – 26\textsuperscript{th} - 29\textsuperscript{th} September 2007  
\textbf{Three-dimensional Analysis of Efficacy of Chemo-mechanical vs. Mechanical Caries Removal}  
M Ahmed, G R Davis, F S L Wong

BSPD – London, UK – 12\textsuperscript{th} - 14\textsuperscript{th} September 2007, Oral Presentation  
\textbf{Three-Dimensional study of efficacy of Carisolv\textsuperscript{TM} for carious dentine removal}  
M Ahmed, G R Davis, F S L Wong

IAPD – Hong Kong - 14\textsuperscript{th} - 17\textsuperscript{th} June 2007, Oral Presentation  
OS085 - \textbf{High-definition X-ray microtomography of dental caries and developmental defects}  
F. S. L. WONG\textsuperscript{*}, M. AHMED, J. M. FEARNE & G. R. DAVIS  

BSDR/NOF – Durham, UK - 3\textsuperscript{rd} - 5\textsuperscript{th} April 2007, Poster Presentation  
\textbf{Three-Dimensional Analysis of Caries Removal by Excavator and Carisolv\textsuperscript{TM}}  
M Ahmed, G R Davis, FSL Wong
PEF IADR – Dublin, Ireland September 2006, Oral Presentation
X-ray Microtomography Evaluation of Mechanical vs. Chemo-mechanical Caries Removal
M. AHMED, G.R. DAVIS, and F.S.L. WONG
http://iadr.confex.com/iadr/pef06/techprogram/abstract_84278.htm

Atomic Force Microscopy Technique for imaging sound dentine and carious dentine
Atomic Force Microscopy Technique for imaging sound dentine and carious dentine

M. Ahmed, G. R. Davis, F. S. L. Wong

Biophysics, Institute of Dentistry, Queen Mary, University of London

Dental caries is the most common infectious disease affecting humans.

Dentists use physical properties of the tooth to differentiate between sound and carious parts of the tooth in order to treat it effectively.

Dentine is a hard tissue that makes the bulk of the tooth and is protected by enamel from the oral surrounding.

The surface structure of both dentine and carious dentine was imaged using Atomic Force Microscopy (AFM). It is a powerful technique which allows samples to be imaged in a liquid environment.

Images obtained by AFM showed that sound dentine had regular structure. However carious dentine depicted gaps which suggests that there is loss of structural integrity causing the tubules to collapse.

Also Force modulation mode was used to image the stiffness of the dentine surface. The force modulation depicted that the peritubular dentine is much stiffer than the intertubular dentine as found by Kinney et al. (1993). In addition the intertubular dentine exhibits a variation of stiffness whereas the peritubular dentine shows homogeneity.

AFM is very good for providing relative information. Using it solely is not good enough to use in clinical situations. So this technique will be calibrated with other quantitative methods like X-ray microtomography and micro/nano-indentation. This will lead to
improved models consisting of details of the properties of dentine and the distribution of the basic structural elements of dentine.

**Appendices**

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**Atomic Force Microscopy study of Imaging Sound Dentine and Carious Dentine**

**M. Ahmed, G. R. Davis, F. S. L. Wong**  
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Biophysics, Institute of Dentistry, Queen Mary, University of London  
http://www.smd.qmul.ac.uk/dental/bip/

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**Introduction**

5 billion people worldwide suffer dental caries. It is the most common infectious disease affecting all populations. Dentists use physical properties of the tooth to differentiate between sound and carious parts of the tooth in order to treat it effectively. Deterioration of the properties or structure is indicative of caries.

The surface structure of both dentine and carious dentine was imaged using Atomic Force Microscopy (AFM). It is a powerful technique which allows samples to be imaged in a liquid environment.

**Dentine Microstructure**

Dentine is a hard tissue that makes the bulk of the tooth and is protected by enamel from the oral surrounding. Root dentine is covered by cementum.

Dentine is a complex hydrated composite of oriented tubules surrounded by highly mineralised peritubular zone embedded in an intertubular matrix consisting mainly of type I collagen with embedded apatite crystals and dentinal fluid.

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**Results and Discussion**

**Contact Mode AFM**

Image (a) is that of sound dentine and image (b) is that of carious dentine. In these processed images the dentinal tubules can be seen. The image of carious dentine shows that some of the tubules have merged together making large gaps. This suggests that there is loss of structural integrity causing the tubules to collapse.

**Force Modulation Mode**

The above shows a processed topographic image together with Force Modulation Image. In image (a) the peritubular dentine thickness is larger. The force modulation image (b) is a processed image in which it can be seen that the peritubular dentine is much stiffer than the intertubular dentine. Also it can be seen that the intertubular dentine exhibits a variation of stiffness whereas the peritubular dentine shows homogeneity. This variation is due to the distribution of apatite on the collagen matrix.

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**Methodology**

**Samples**

Cross sections of deciduous teeth were cut using a rotary saw. The thickness of the samples were 1mm and were mounted on a thin sheet of steel, so that the sample was stable whilst scanning. Then the sample was polished using Alumina powder (0.3microns). Prior to scanning the sample was ultrasonicated in water, to help get rid of any unwanted debris.

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**AFM**

The instrument used was an Explorer (Thermomicroscopes). The mode of operation was Contact Mode AFM (liquid cell). In addition Force Modulation Mode was also used to image the stiffness.

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**Further Work**

AFM is a powerful technique with very high spatial resolution, but the information is relative. This is not concise enough to be used in clinical situations. So AFM will be calibrated with other quantitative methods such as X-ray microtomography and micro/nanindentation. This will further improve models consisting of details of the dentine properties and the distribution of the basic structural elements of dentine.

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**References**

WHO Report 2003

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**Acknowledgements**

The authors thank the MRC (Project No. OGDB1A2R). Also thanks to Ken Scott and Mark Baxendale

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**Poster presented at the Veeco SPM Conference and users meeting**
Three-Dimensional Analysis of Caries Removal by Excavator and Carisolv™

M Ahmed, G R Davis, FSL Wong

Key words: Caries removal, Carisolv™, X-ray Microtomography

Objectives:
The objectives of the study were to:
(1) Analyse dentine caries removed by hand excavator and Carisolv™ in three-dimensions using X-ray Microtomography (XMT).
(2) Identify and compare regions of the carious lesion such as the body of the lesion (infected dentine), demineralised front (affected dentine) and sound dentine before and after both caries removal techniques.
(3) Compare the effectiveness of these caries removal techniques.

Method:
Ten deciduous molars were bisected through open carious cavities. The two halves of each tooth were scanned using a high definition XMT scanner at 30 microns resolution. Following the initial scan, dentinal caries were removed for each pair of the same tooth samples using an excavator and Carisolv™. Each tooth sample was then re-scanned. The three-dimensional XMT data sets were then cropped to the volume of the carious lesion. Linear Attenuation Coefficients (LAC), values proportional to the mineral concentration were associated with each voxel (volume element). Histograms of the LAC were obtained for the volume of the caries lesions before and after removal. A ‘blind method’ analysis was used whereby the assessor did not know which half of the lesions were removed by hand excavation/Carisolv™ for each of the samples. Qualitative analysis of the histograms was performed to see whether there is a differentiation between the two techniques.

Results:
Of the ten tooth samples, eight were judged to have better caries removal on the half treated with Carisolv™. Assessment was based on removal of the lesion body and retention of sound and partially demineralised (affected) dentine.

Conclusion:
Caries removal by hand excavation and Carisolv™ are shown to have distinctive features in the LAC histograms. These features were identified qualitatively. This preliminary study suggests that caries removal with Carisolv™ is more effective, although quantitative analysis will need to be carried out.
Three-dimensional Analysis of Caries Removal by Excavation and Carisolv™

M. Ahmed, F. S. L. Wong and G. R. Davis
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Appendices

Background

Carious dentine removal is still subjective despite efforts to implement the revolutionary minimally invasive dentistry approach.

Cariolv™ has been proposed to be an objective aid to remove only the required amount of carious dentine (infected dentine), leaving uninfected dentine intact. Previous studies on its efficiency and efficacy were mainly based on techniques that were destructive and limited to 2 dimensions, making it difficult to compare the same studied samples before and after caries removal. This led to Fildes-Miller stating that the “granular lesions” affect Cariolv™ caries removal is still unclear, and 3-dimensional (3D) investigation on the extent of residual caries is needed.

Aim

The objectives of the study were:
1. To measure and analyze the mineral concentration of carious dentine removed by hand excavation and Cariolv™ in 3D using X-ray microtomography (XMT).
2. To identify the Linear Attenuation Coefficient (LAC), a value proportional to the mineral concentration, of the two zones of carious lesions (affected and infected dentine) and sound dentine before and after both caries removal techniques.
3. To compare the effectiveness of the two techniques by identifying which zone of carious lesion or sound dentine each technique removed.

Methodology

Specimen Preparation

Ten deciduous molars were bisected through the center of the caries lesions and the cavities were filled with an aluminum foil for internal calibration (Fig. 1A & B). The two halves of each tooth were scanned using a high-definition XMT scanner at 30 micron resolution.

XMT, 4th generation XMT scanner

The specimens were then re-scanned. The 3D-XMT data sets were then loaded into the virtual nest system Cariolv™. The software was used to perform a qualitative analysis of the caries lesion before and after treatment. The software was used to measure the mineral concentration of the carious dentine and the sound dentine before and after treatment. The software was used to compare the effectiveness of the two techniques by identifying which zone of carious lesion or sound dentine each technique removed.

Conclusions

Caries removal by hand excavation and Cariolv™ are shown to have distinctive features in the Linear Attenuation Coefficient (LAC) histogram. These features were identified independently. This preliminary study suggests that caries removal with Cariolv™ is more effective. Further research will be carried out to determine whether the demineralized layer which is proposed to be ‘affected’ dentine is infected with bacteria and whether this layer can be remineralized.

References


Acknowledgements

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ORCA 2008

X-Ray Microtomography Study of the Efficacy of Carisolv™ in Removing Carious Dentine.

F.S.L. Wong*, M. Ahmed, G.R. Davis

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Aim - To use a high definition X-ray Microtomography (XMT) scanner to study the mineral concentrations ($C_{\text{min}}$) of infected and affected carious dentine in deciduous molars, and the efficacy of Carisolv™ in removing the infected dentine.

Methods - Carious Deciduous molars with open cavities were bisected. The two halves were mounted on a kinematic stage and were scanned using a MuCat XMT scanner (developed at Barts and The London Dental School). This scanner uses a novel aluminium step wedge to calibrate the polychromatic X-ray beam to one with an effective energy of 40 keV and a novel scanning methodology to improve contrast ratio. After scanning, in one half of the tooth, carious dentine was removed using a new spoon excavator until the dentine was ‘felt’ clinically hard. On the other half, carious dentine was removed using the Carisolv™ technique. Then, the specimen was re-scanned. Analysis was carried out comparing the Linear Absorption Coefficient (LAC) of the XMT slices from the two scans. The mineral concentrations ($C_{\text{min}}$) of the dentine removed were measured, determined from LACs assuming the mineral is pure $\text{Ca}_{10}$$\text{(PO}_4\text{)}_6$$\text{(OH)}_2$ and the organic phase pure collagen.

Results - Twenty-one pairs of deciduous molars were scanned. The $C_{\text{min}}$ for the body of the carious dentine was ~0.37 gcm$^{-3}$ and for the demineralised zone, 0.57-1.07 gcm$^{-3}$. Dentine with higher $C_{\text{min}}$, up to 1.37 gcm$^{-3}$, was removed using the excavator whereas dentine with $C_{\text{min}}$ up to 0.87 gcm$^{-3}$ was removed using the Carisolv™ technique.

Conclusion – The Carisolv™ technique removed less sound dentine, leaving a layer of partially demineralised dentine which might correspond to the affected dentine. This efficacious method may be a useful tool within the current concept of minimally invasive dentistry.
ORCA 2009
Comparison of volumes of sound dentine removed by three caries removal techniques

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Aim - To measure and compare the volumes of sound dentine removed by hand excavator alone, hand excavator with the aid of caries detector dye, and by the Carisolv™ technique.

Methods - Carious deciduous molars with open cavities were bisected and scanned using a high definition X-ray microtomography scanner (MuCat, developed at Barts and The London Dental School). After initial scanning, in one group of the paired half tooth (HED/CD), one half had its carious dentine removed using an excavator until the dentine was ‘felt’ clinically hard, whilst in the other half, caries was removed with the aid of a caries detector dye (Kuraray Medical Inc.). In another group (HES/CS), caries was removed in one half using an excavator whilst the other half using the Carisolv™ technique. Afterwards, the specimen was re-scanned. The data from the second scan was subtracted from that from the first to reveal the tissue removed, using an in-house 3-D image subtraction program. The volume of sound dentine, expressed as a percentage of the total volume of tissue removed was calculated. A paired t-test was used to investigate whether there was a significant difference (p<0.05) in the volume of sound dentine removed between the techniques.

Results - Seventeen and twenty-one pairs were in the HED/CD and HES/CS groups respectively. The mean±sd volume percentage of sound dentine removed by HED and CD techniques were 2.6±2.78 and 4.9±3.57, and by HES and CS, 4.0±3.14 and 2.1±1.76 respectively. Significantly less dentine was removed by CD (p=0.008) and CSS (p = 0.011) techniques comparing to HED and HES respectively.

Conclusion - Both CD and CSS techniques are effective aids in reducing the amount of sound dentine removed during caries removal.

Summary – Sound dentine could be preserved by the aids of caries detector dye or Carisolv™ during caries removal.
Appendix D

Appendix D.1 – Scanned colour image of tooth samples with corresponding XMT images

Sample med 1 (Upper, Lower)
Sample med 2 (Upper, Lower)
Sample med 4 (Upper, Lower)

Sample med 5 (Upper, Lower)
Sample med 7 (Lower, upper)
Sample med 8 (Upper, Lower)
Appendices

Sample med 9 (Lower, Upper)
Sample med 10 (Upper, Lower)
Sample med 11 (Lower, Upper)
Sample med 13 (Upper, Lower)

Sample med 14 (Upper, Lower)
Sample med 15 (Upper (slice 14, xz plane), Lower (slice 156, xz plane))
Sample med 16 (Upper (slice 28, 18), Lower (slice 60))
Sample med 17 (Lower (slice 151), Upper (Slice 43))
Sample med 18 (Upper (slice 28), Lower (slice 167))
Sample med 19 (Upper (slice 34), Lower (slice 115))

Sample med 20 (Upper (slice 33), Lower (slice 131))
Appendices

Sample med 21 (Upper (slice 58), Lower (slice 179))
Sample med 22 (yz plane – Right (slice 41), Left (slice 154))
Sample med 23 (Lower (slice 158, 177), Upper (slice 53))
Sample med 24 (Lower (slice 70), Upper (slice 32))
Sample med 25 (Lower (slice 108), Upper (slice 54))
Sample med 26 (Upper, Lower)
Sample med 27 (Lower (slice 127), Upper (slice 43))
Sample med 28 (Upper, Lower)
Sample med 29 (Upper, Lower)
Sample med 30 (Upper, Lower)
Sample med 31 (Upper, Lower)
Sample med 32 (Upper, Lower)
Sample med 33 (Lower, Upper)
Sample med 34
Appendices

Sample med 35 (Lower, Upper)
Sample med 36 (Upper, Lower)
Sample med 37 (yz plane (right, left)
Sample med 38 (Upper, Lower)
Sample med 39 (Lower, Upper)
Sample med 40 (Upper, Lower)
Sample med 41 (Upper, Lower)

Sample med 42 (Lower, Upper)
Sample med 43 (Upper, Lower)
Sample med 44 (Upper, Lower)
Sample med 45 (Upper, Lower)
Appendix D.2 - Central slice for each dataset

Sample med 1
Sample med 2
Sample med 3

Sample med 4
Sample med 5

Sample med 6
Sample med 7

Sample med 8
Sample med 9
Sample med 10

Sample med 11
Sample med 12
Sample med 13

Sample med 14
Sample med 15
Sample med 16

Sample med 17
Sample med 18
Sample med 19

Sample med 20
Sample med 21

Sample med 22
Sample med 25

Sample med 26
Sample med 27

Sample med 28
Sample med 29

Sample med 30
Sample med 31

Sample med 32
Sample med 35

Sample med 36
Sample med 39

Sample med 40
Sample med 41
Sample med 42

Sample med 43
Sample med 46
Appendix D.3 - Before and after histograms
Appendices
Appendices
<table>
<thead>
<tr>
<th>LAC (cm(^{-1}))</th>
<th>Number of Voxels</th>
</tr>
</thead>
<tbody>
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<td>0.00E+00</td>
<td>2.00E+04</td>
</tr>
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</tr>
<tr>
<td>1.80E+05</td>
<td>2.00E+05</td>
</tr>
</tbody>
</table>

LAC (cm\(^{-1}\)) and Number of Voxels

- **med 15A**: purple line
- **med 15A\(_r\)**: blue line
Appendices
Appendices

![Graph showing LAC (cm⁻¹) vs Number of Voxels for med 22B and med 22B_r](image)

- **Blue line**: med 22B
- **Pink line**: med 22B_r
Number of Voxels

LAC (cm$^{-1}$)

- med 25A
- med 25A_r
Appendices

![Graph showing the number of voxels versus LAC (cm⁻¹)](image-url)

- **med 25B**
- **med 25B_r**
Appendices
Appendices

LAC (cm\(^{-1}\)) vs. Number of Voxels

- **med 35A**
- **med 35A_r**

**Legend:**
- med 35A
- med 35A_r
Appendices

![Graph showing the number of voxels against LAC (cm$^{-1}$)]

- **med 37A**
- **med 37A_r**
Appendices

The diagram shows the number of voxels as a function of LAC (cm$^{-1}$) for two different samples, labeled as med 43A and med 43A_r. The x-axis represents LAC (cm$^{-1}$) ranging from 0 to 3, and the y-axis represents the number of voxels ranging from 0.00E+00 to 2.00E+05.
Appendix D.4 - Subtraction Histograms

Subtraction histogram - med 1
Subtraction histogram - med 2

Number of Voxels

Section A  Section B

LAC (cm\(^{-1}\))

0.00E+00  5.00E+03  1.00E+04  1.50E+04  2.00E+04  2.50E+04  3.00E+04  3.50E+04  4.00E+04  4.50E+04  5.00E+04
0  0.2  0.4  0.6  0.8  1  1.2  1.4
Subtraction histogram - med 3

LAC (cm$^{-1}$)
Number of Voxels
Section A Section B
Subtraction histogram - med 4

LAC (cm\(^{-1}\))

Number of Voxels

Section A
Section B
Subtraction histogram - med 5

![Graph showing LAC (cm$^{-1}$) vs. Number of Voxels for Section A and Section B.](image-url)
Subtraction histogram - med 7

LAC (cm$^{-1}$)

Number of Voxels

Section A

Section B
Subtraction histogram - med 8

LAC (cm$^{-1}$)

Number of Voxels

Section A  Section B
Subtraction histogram - med 9

LAC (cm$^{-1}$)

Number of Voxels

Section A

Section B
Subtraction histogram - med 13

LAC (cm$^{-1}$) vs. Number of Voxels

Section A

Section B

Legend:
- Section A
- Section B
Subtraction histogram - med 17

LAC (cm$^{-1}$)

Number of Voxels

Section A

Section B
Subtraction histogram - med 19

LAC (cm\(^{-1}\)) vs. Number of Voxels for Section A and Section B.
Subtraction Histogram - med 25

Number of Voxels
Section A Section B

LAC (cm$^{-1}$)
Subtraction histogram - med 26

Number of Voxels

LAC (cm\(^{-1}\))

Section A
Section B
Subtraction histogram - med 27

LAC (cm\(^{-1}\))

Number of Voxels

Section A  Section B
Subtraction histogram - med 28

LAC (cm\(^{-1}\))

Number of Voxels

Section A

Section B

Appendices
Subtraction histogram - med 28

Number of Voxels

LAC (cm$^{-1}$)

Section A

Section B
Subtraction histogram - med 29

Number of Voxels

LAC (cm\(^{-1}\))

Section A

Section B
Subtraction histogram - med 31

LAC (cm$^{-1}$)

Number of Voxels

Section A  Section B
Subtraction histogram - med 32

LAC (cm$^{-1}$)

Number of Voxels

Section A Section B
Subtraction histogram - med 33

Number of Voxels vs. LAC (cm$^{-1}$) for Section A and Section B.

- Section A
- Section B

Data range:
- LAC: 0 to 1.5 E+04
- Number of Voxels: 0 to 5 E+04

Graph shows two curves, one for each section, indicating the distribution of LAC values with corresponding number of voxels.
Subtraction histogram - med 35
Subtraction histogram - med 38
Subtraction histogram - med 40

LAC (cm\(^{-1}\))

Number of Voxels

Section A
Section B
Subtraction histogram - med 43

![Graph showing subtraction histogram with LAC (cm$^{-1}$) on the x-axis and Number of Voxels on the y-axis. Two curves are plotted, one for Section A and one for Section B.](image)