New method for quantitative polarised light microscopy of laser-ablation machined sections of bones and joints

LBP11

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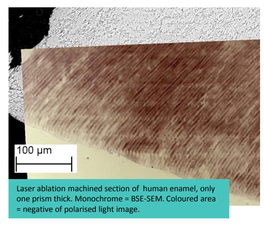
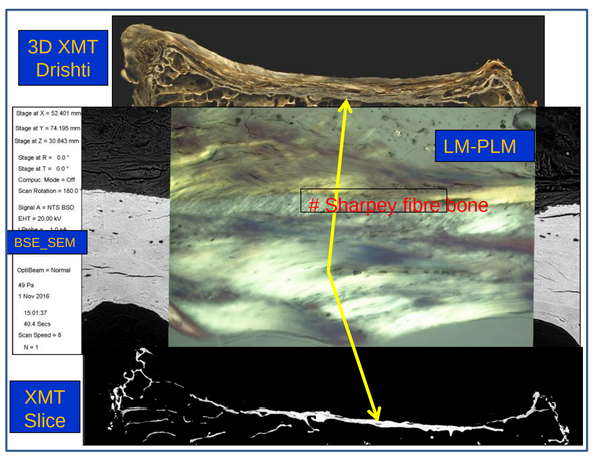
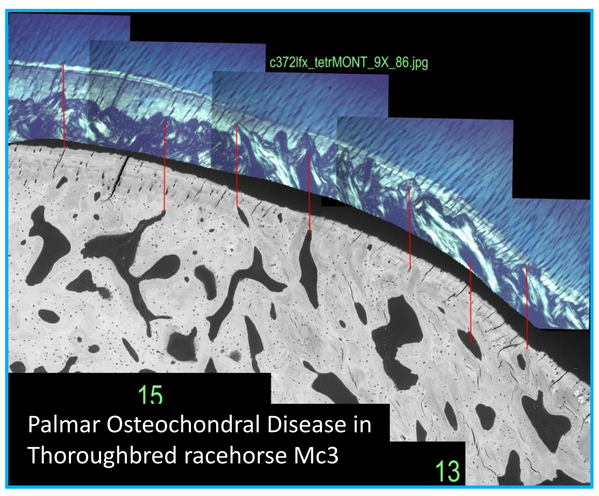


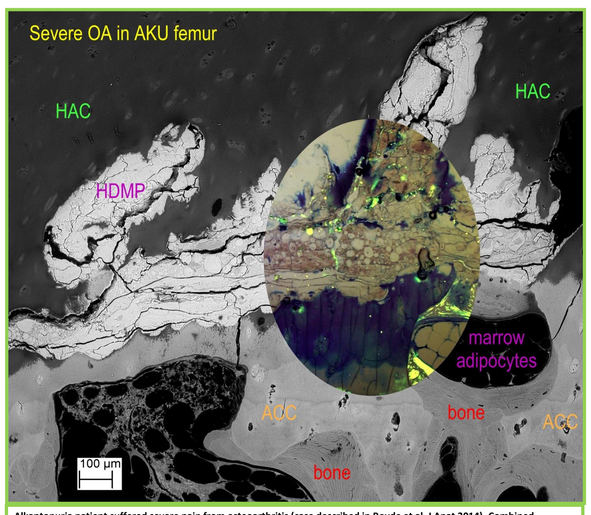
Laser ablation machined section of human enamel, only one prism thick. Monochrome = BSE-SEM. Coloured area = negative of polarised light image.

We have recently prepared very thin sections from the front face of bone blocks embedded in PMMA - which had previously been studied by backscattered electron scanning electron microscopy (BSE-SEM) and x-ray microtomography - by the new technique of laser-ablation machining: ABOVE. This method has been applied to an array of large and small bone and joint samples.

Examples of correlative light, BSE-SEM and micro-CT (XMT) using Rowiak 'TissueSurgeon' sections below.

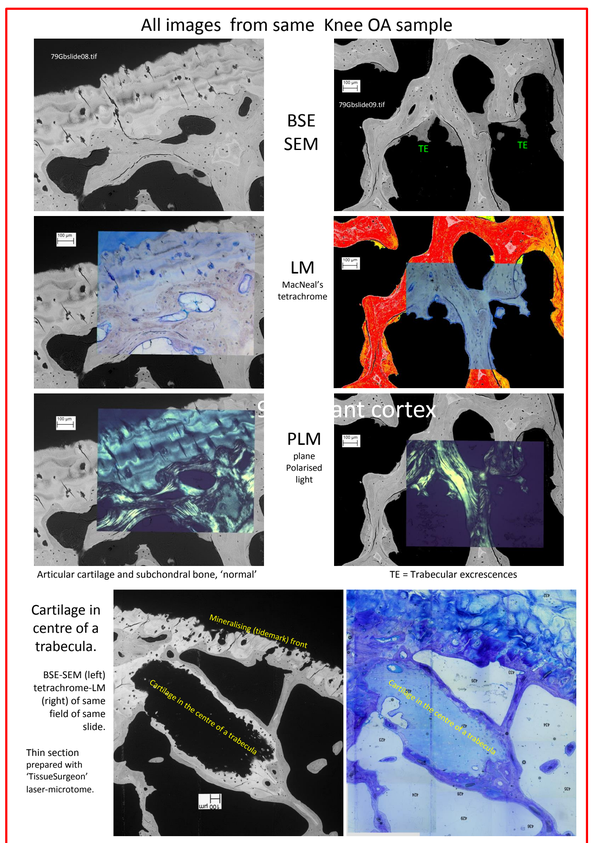






Alkaptonuria patient suffered severe pain from osteoarthritis (case described in Boyde et al J Anat 2014). Combined Tetrachrome-stained and polarised light image within oval area, aligned and superimposed on BSE SEM image of iodine stained PMMA block face from which the section was cut using the Rowiak 'TissueSurgeon' laser microtome. HDMP = high density mineralised protrusion. HAC = hyaline articular cartilage. ACC = articular calcified cartilage.

All images from same Knee OA sample



Cartilage in centre of a trabecula.

BSE-SEM (left) tetrachrome-LM (right) of same field of same slide.

Thin section prepared with 'TissueSurgeon' laser-microtome.

These new laser cut 'ground' sections justify the development and use of new 3D high resolution light microscopic methods.

In conventional polarised light microscopy (PLM), positively birefringent crystals such as hydroxyapatite and/or negatively birefringent arrays of oriented molecules such as collagen appear brightest if they lie both in the plane of the section and at 45º to the axes of the crossed polarising filter elements. Birefringent elements appear black if they lie parallel to either polariser or analyser (or perpendicular to the plane of section). This situation prevents us from seeing the whole scene at once, because nothing can be seen in the dark sectors of the *'Maltese cross'*.

New PLM Method: We have overcome this problem by combining three grey-level PLM images.

Digital images are recorded using green light with the polariser and analyser rotated 30º between each and used as red, green and blue components in a composite image.

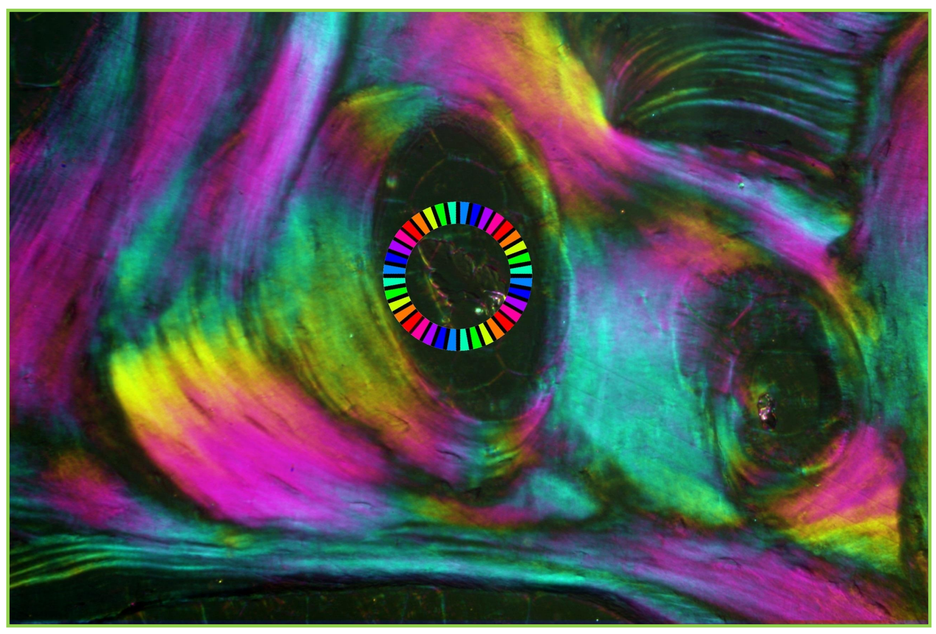
Colour maps the in-plane direction of the oriented molecular arrays irrespective of whether they are too small to be resolved.

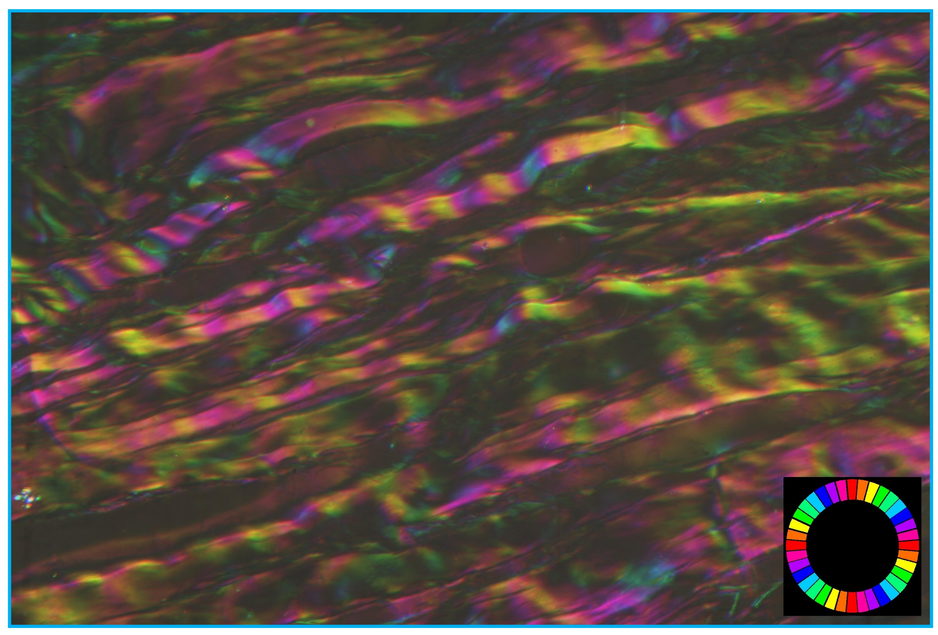
Intensity of the colour indicates the 'strike' of the molecules, i.e., the angle that they make to the plane of the section, brightest being parallel.

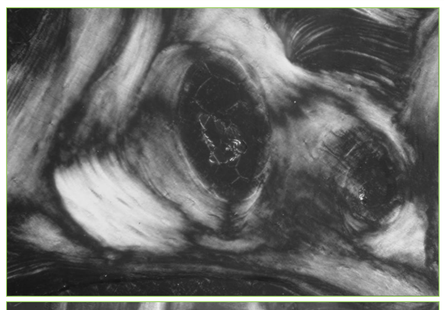
Laser ablation microtomy produces high quality, thin sections of both hard, mineralised and dense fibrous connective tissues of any sort – even of single thin bone trabeculae – which can be studied with any light microscopic method as well as by BSE-SEM and correlated with XMT (uCT) of the whole block.

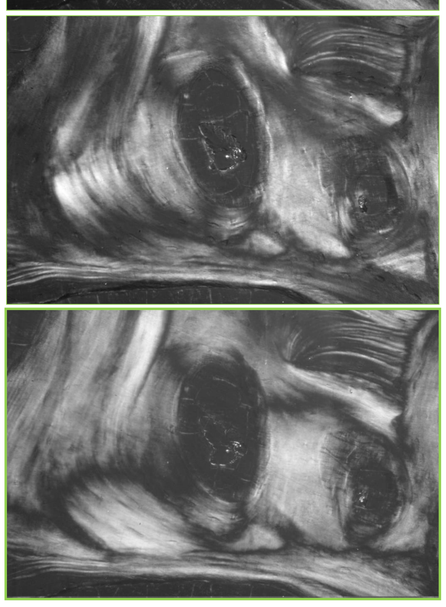
The novel 3x30º pseudocolour PLM images refine our understanding of bone, cartilage, calcified cartilage, Sharpey fibre bone, ligament, calcified ligament, tendon, calcified tendon and fibrous periosteum structure in bones.

Inset diagrams show the colours for different orientations.





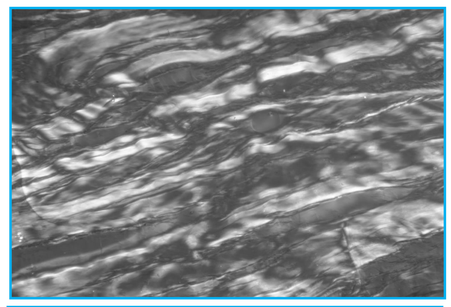


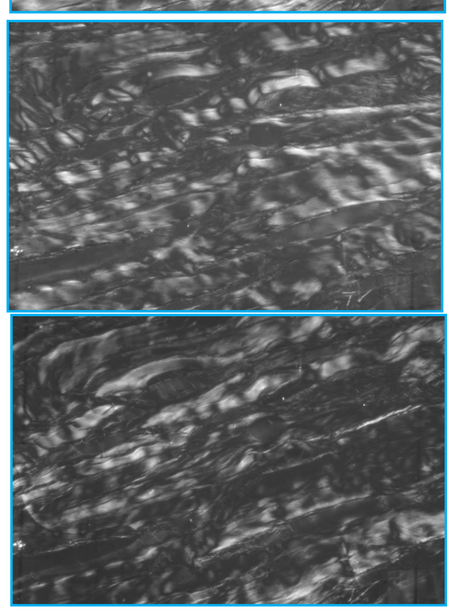


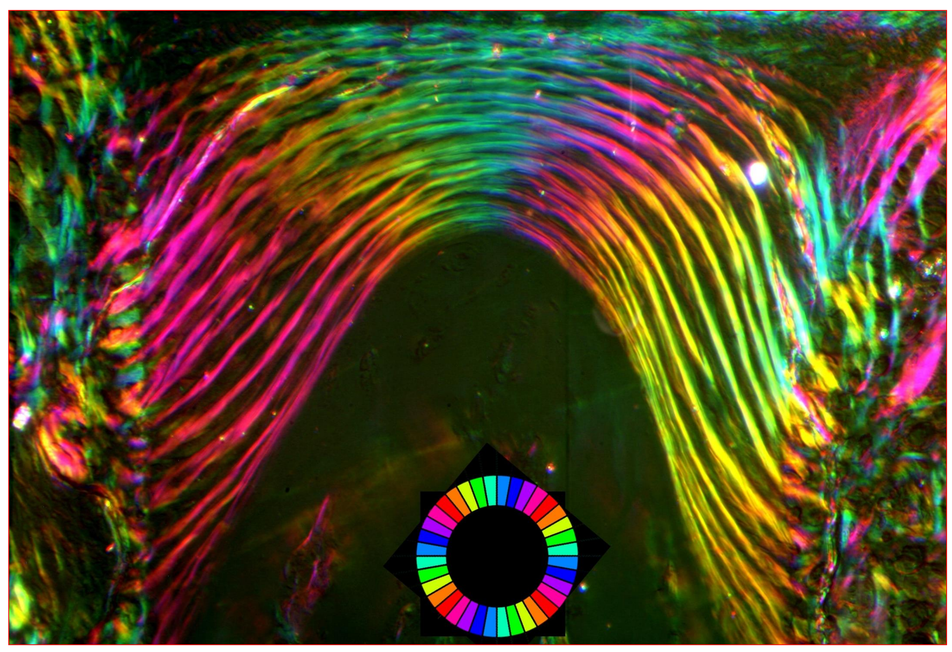
Femoral head removed at arthroplasty, subchondral trabecular bone, case of severe osteoarthritis in AKU. Thin section prepared by laser ablation. LEFT: Three monochrome images recorded with three 30 degrees different orientations of crossed polars used as RGB input for composite image ABOVE.

Iliac crest autopsy, dense fibrous connective tissue of periosteum (case of Fibrogenesis Imperfecta Ossium). Thin section prepared by laser ablation. RIGHT: Three monochrome images recorded with three 30 degrees different orientations of crossed polars used as RGB input to make composite image ABOVE.

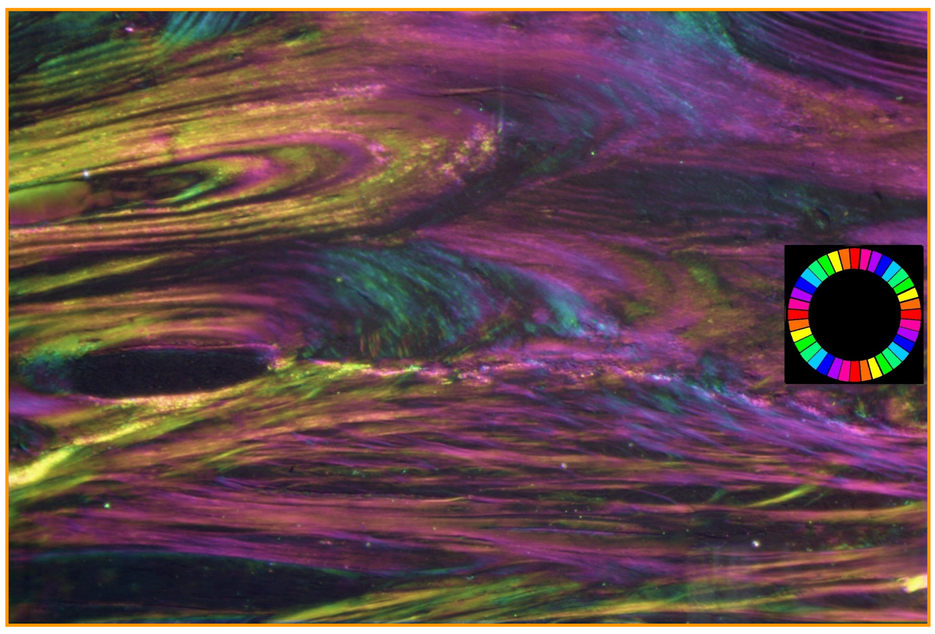
All fields ↙←↖↑↗→↘↓ are 738 microns wide

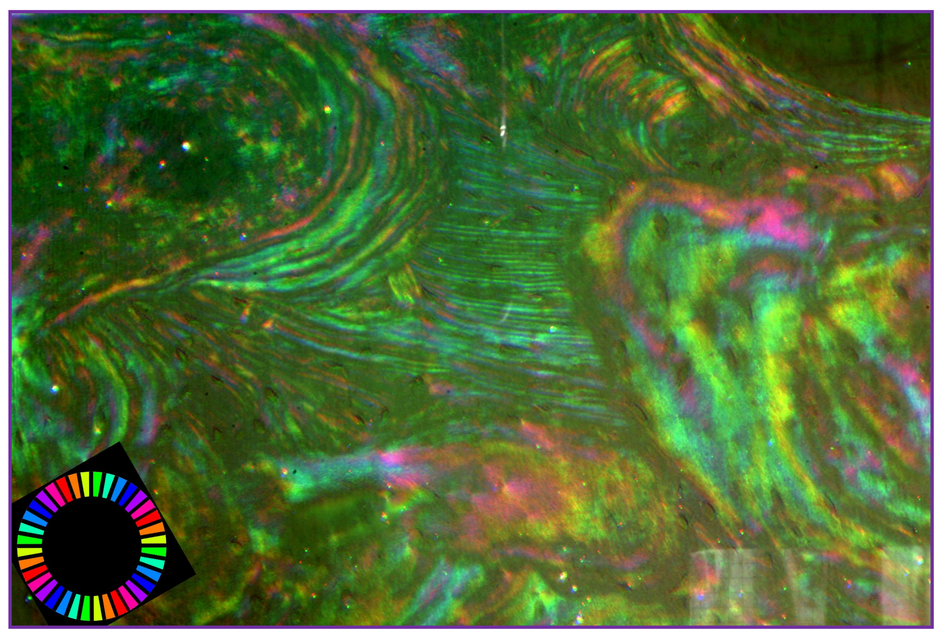


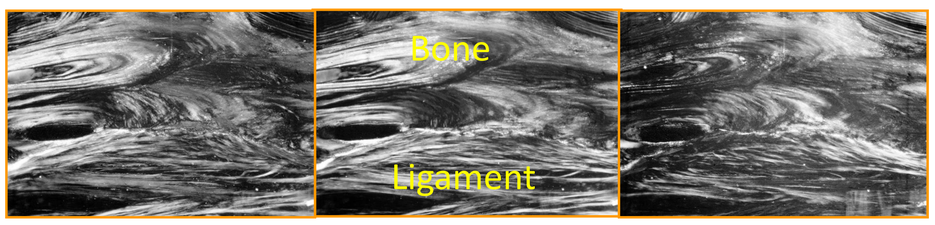




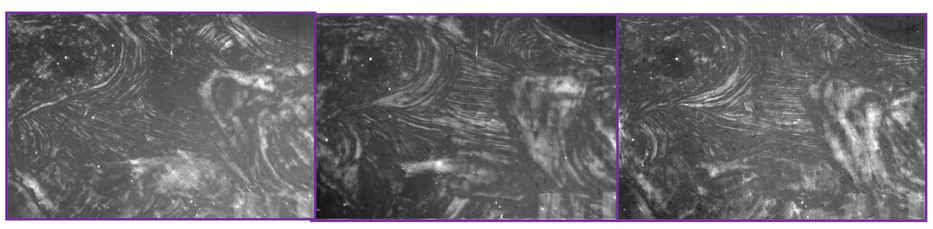
NodScid mouse tail, intervertebral disc, annulus fibrosus. Thin section prepared by laser ablation. Three monochrome images were recorded with three 30 degrees different orientations of crossed polars and used as RGB input for this composite image.







Anterior cortex in vertical section from front face of PMMA embedded L4 vertebra from 72y Male. Thin section prepared by laser ablation. BELOW: Nine images were recorded with 10 degree rotations of crossed polars. Each group of three was averaged (median) to make the three images used as input in RGB composite TOP.



Subchondral trabecular bone, distal condyle of third metacarpal bone of Thoroughbred racehorse with palmar osteochondral disease (POD OA). Thin section prepared by laser ablation. BELOW: Three monochrome images recorded with three 30 degrees different orientations of crossed polars used as RGB inputs for this composite image.