

OSTEOARTHRITIC SUBCHONDRAL BONE MARROW HISTOLOGY. LESSONS FROM CHANGES IN ALKAPTONURIA.



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Introduction

It has been previously shown that studies of the most severe osteoarthritis (OA) lesions in alkaptonuria (AKU) have led the way to understanding things happening in 'regular' OA. We have recently developed a new polarized light microscope (PLM) system with automated acquisition of multiple images during rotation of crossed polarizing filters. This permits interrogation of thin sections of bones, either decalcified or undecalcified, to map the 3D orientation of birefringent structure. We applied this new technology to an age-old problem.

Methods

With full patient and ethical review committee consent, tissue from femoral heads removed at arthrodesis was studied after fixation in neutral buffered formaldehyde, dehydration in ethanol, with any storage prior to tissue processing in either 70% ethanol or glycerol. For light routine light microscopic (LM) histology, tissue was decalcified, dehydrated, substituted with xylene, and paraffin wax: 5 to 10µm thin sections were mounted in DPX unstained or after H&E. For initial study with backscattered electron scanning electron microscopy (BSE-SEM), undecalcified tissue was embedded in PMMA and block surfaces finished flat and carbon coated for mineral concentration-dependent imaging: then later re-polished and stained with iodine to visualize cells, osteoid and cartilage matrix by BSE-SEM. Some ~ 10µm sections were prepared from the PMMA block face by laser ablation microtomy (Boyde, J Microscopy 2018) and studied by both PLM and SEM.

Commonest PLM procedure

Six images at 15° rotations of crossed polars are merged using ImageJ software (Red, Yellow/2, Green, Cyan/2, Blue, Magenta/2): the division by two for the intermediate colours is necessary because of the way ImageJ works. For example, Yellow = 100% Red plus 100% Green. Contrast enhanced using ImageJ if necessary. Widths of fields using 4X objective = 3.05mm; 10X = 1.22mm; 20X = 0.605mm; 40X = 0.302mm.

Results

The new PLM method is exquisitely sensitive and able to map collagen orientation even at low concentrations and in non-mineralized tissue.

We confirmed our earlier findings in AKU:-

(A) up to complete removal of articular calcified cartilage (ACC), leading to regions where there is no attachment via ACC to subchondral bone (SCB) such that bone trabeculae make direct contact with pigmented hyaline articular cartilage.

(B) adipocyte moulded additions of bone to trabeculae to form trabecular excrescences.

(C) High density mineralized infill (HDMI) in cracks in SCB and ACC and extension of the same into HAC to form high density mineralized protrusions (HDMPs).

New findings shown on this poster:-

(D) Prior 'bone marrow space' regions may be occupied by cartilage, woven bone or fibrous connective tissue and the latter is frequently dense and structured. It may in turn be attached to trabeculae via Sharpey fibers inserted therein, suggesting that the fibrous marrow is mechanically loaded.

Conclusions

- Rare bone diseases often provide clues to fundamental disease processes by reaching or exceeding an extreme.
- Here we show radical changes in the bone marrow space compartment in extreme OA by using a highly sensitive new methodology.
- We anticipate that similar findings may be found in more common OA in the future.

References

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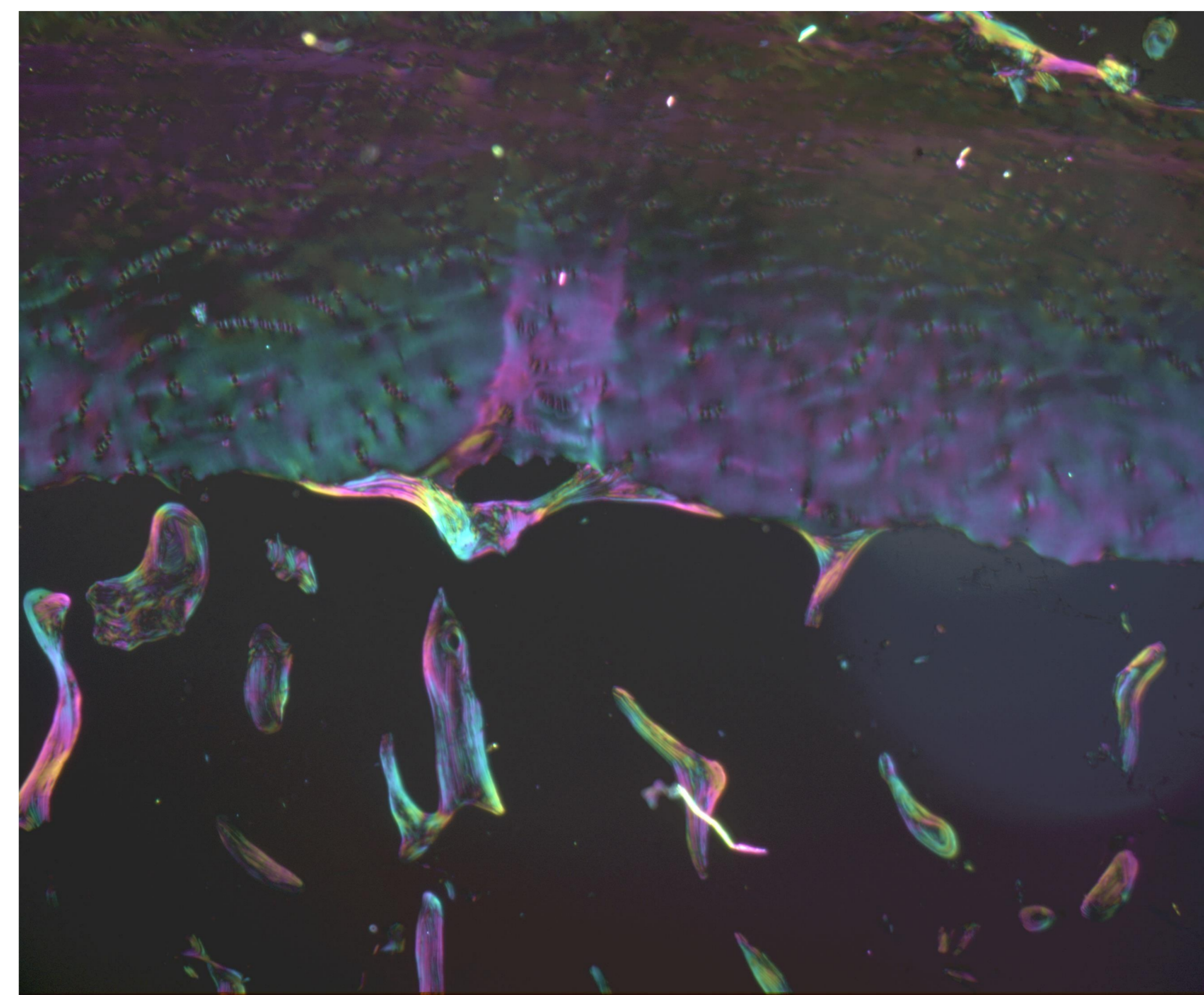
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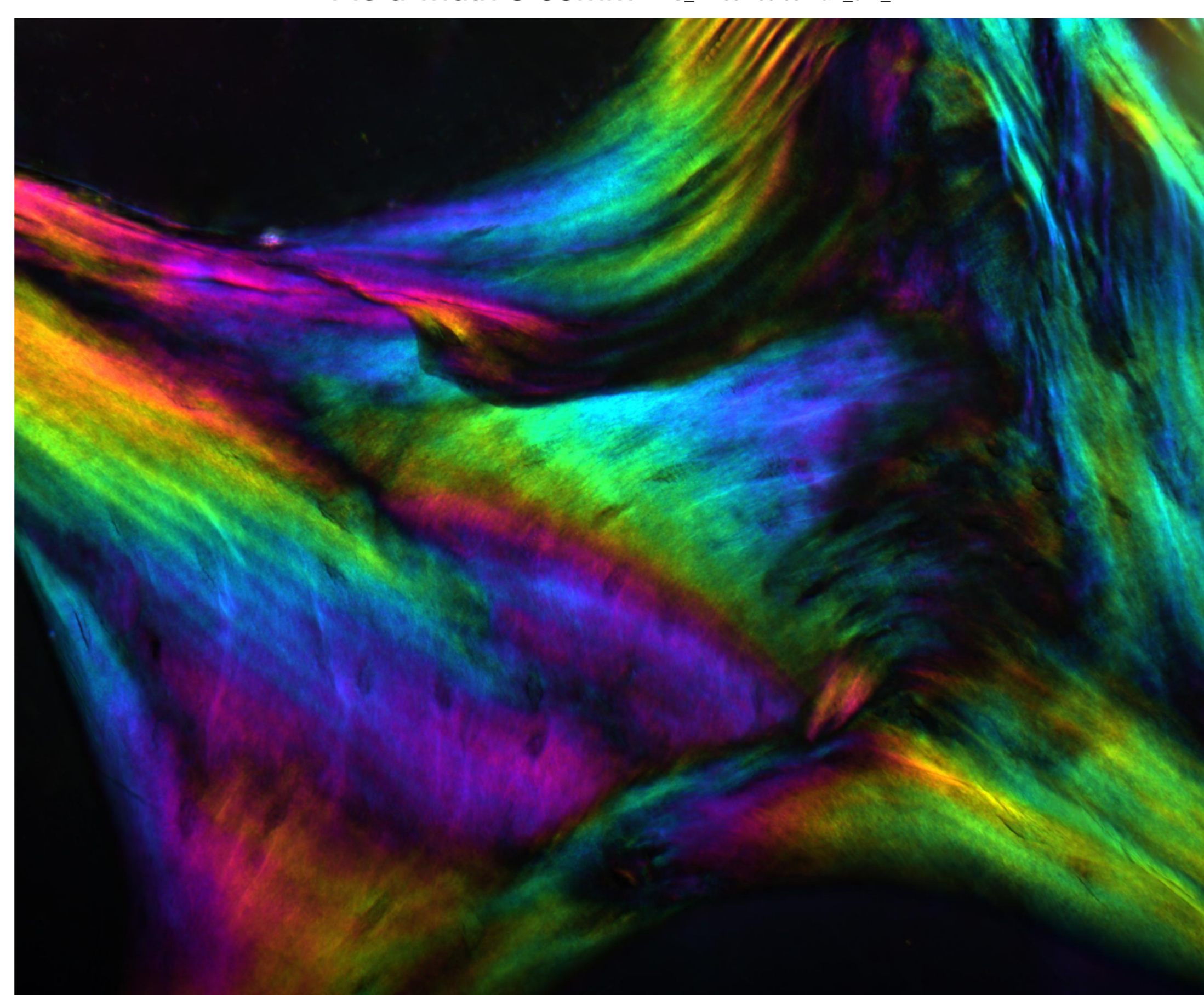
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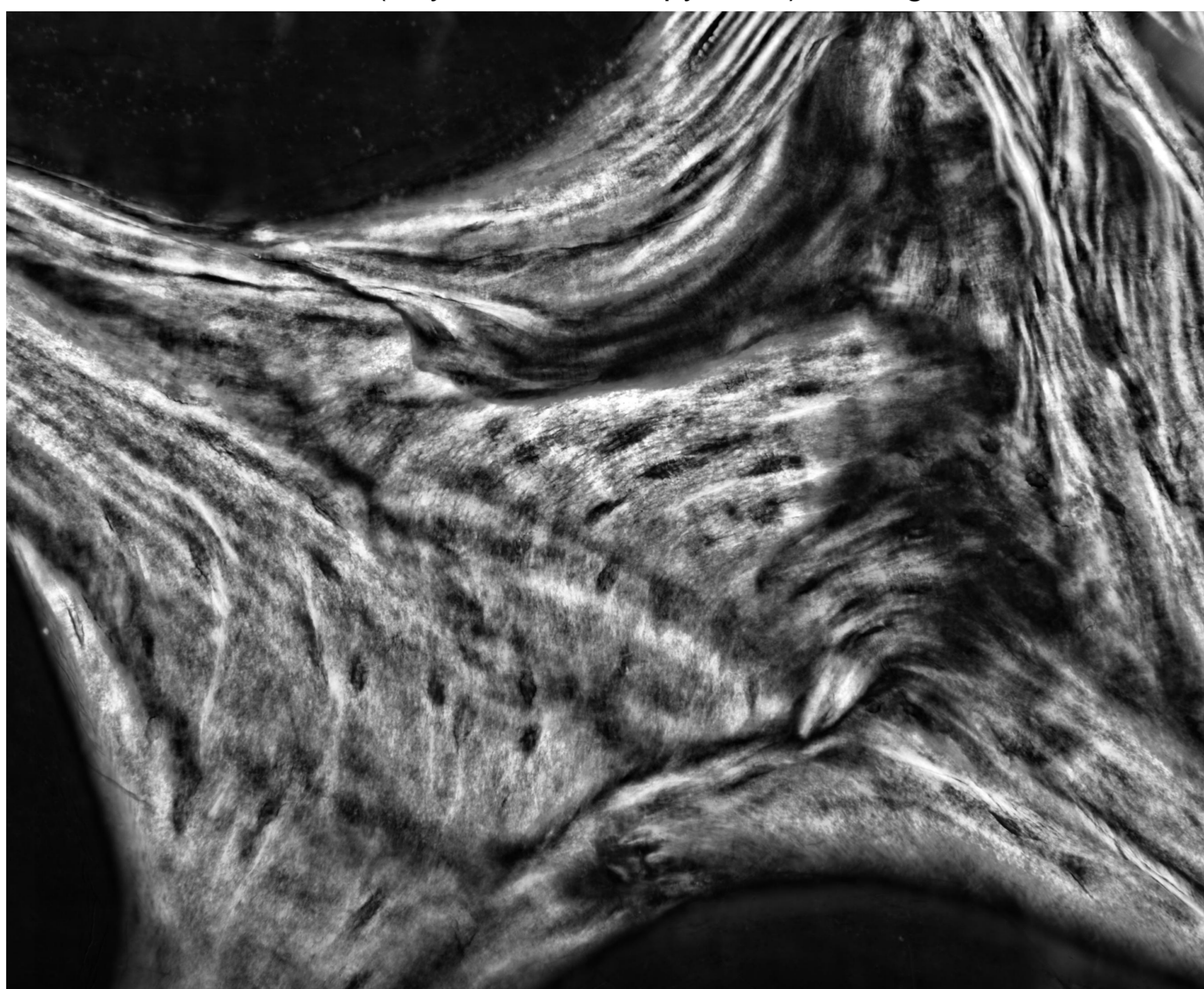
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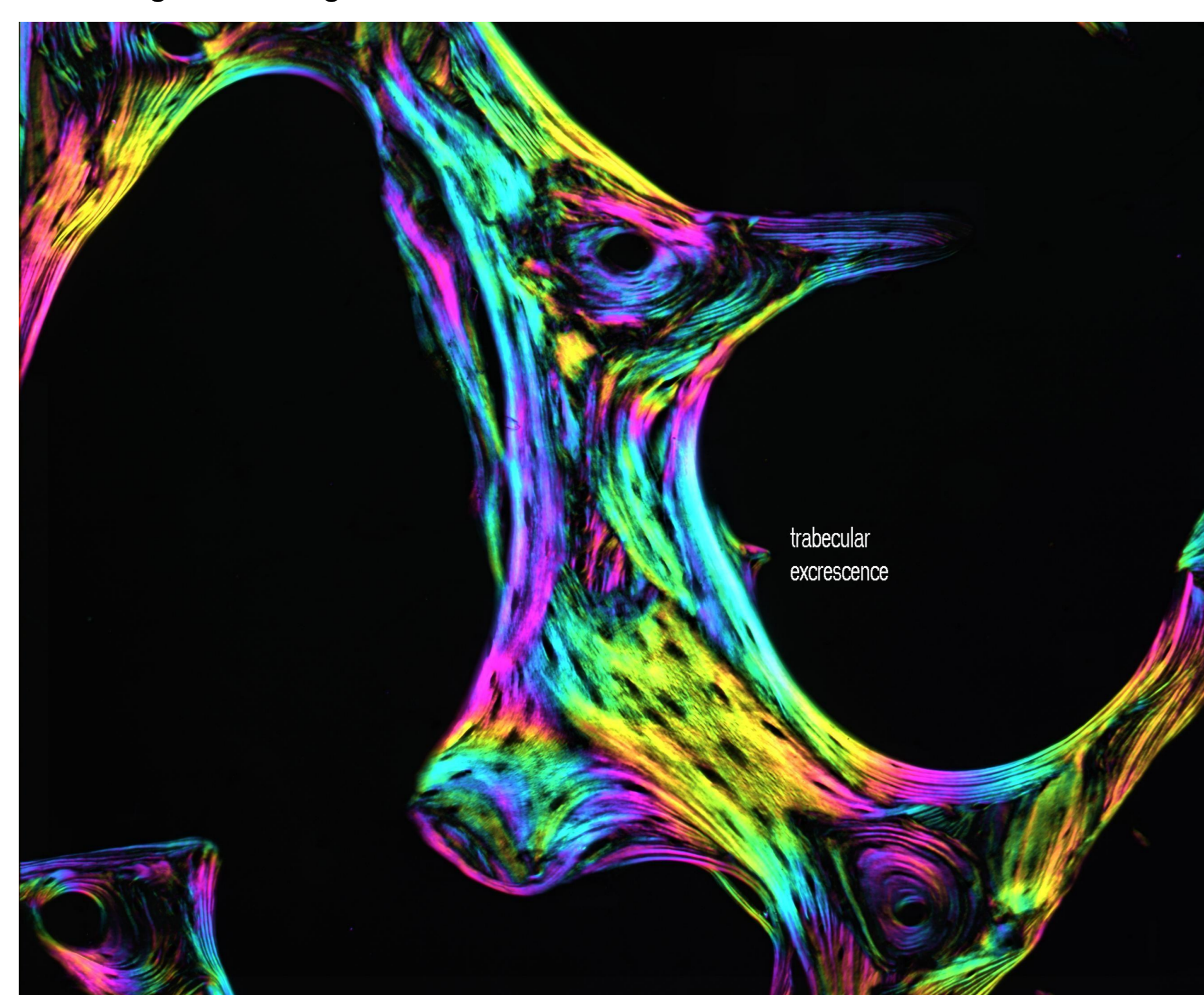
Decalcified H&E section showing absence of SubChondral Bone Plate, Field width 3.05mm AKU_44-08-150-08-H&E_04x_1



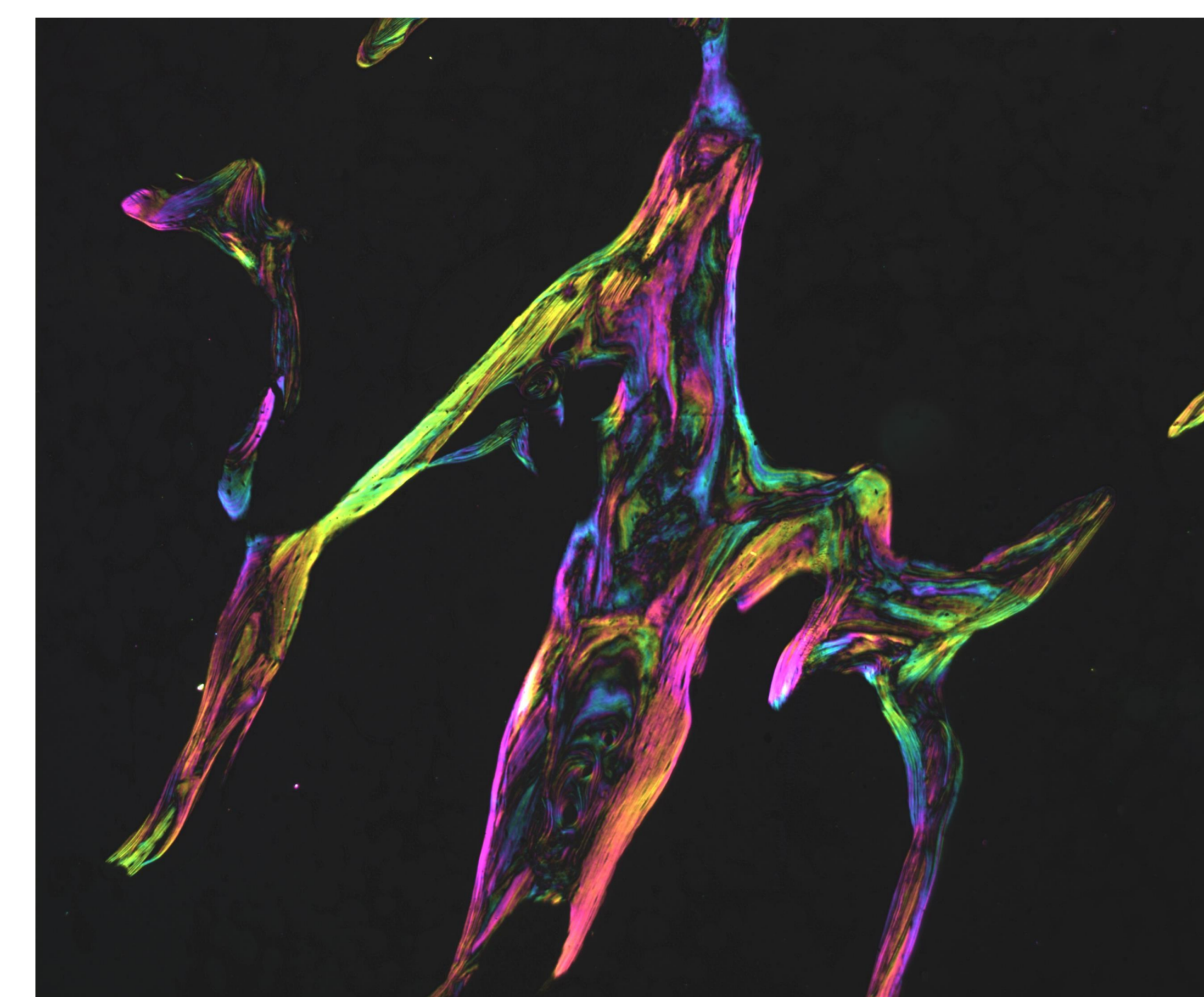
10 micron undecalcified section prepared by laser ablation microtomy, Fieldwidth 0.302mm (Boyde J Microscopy 2018). 3 images at 30° > RGB.



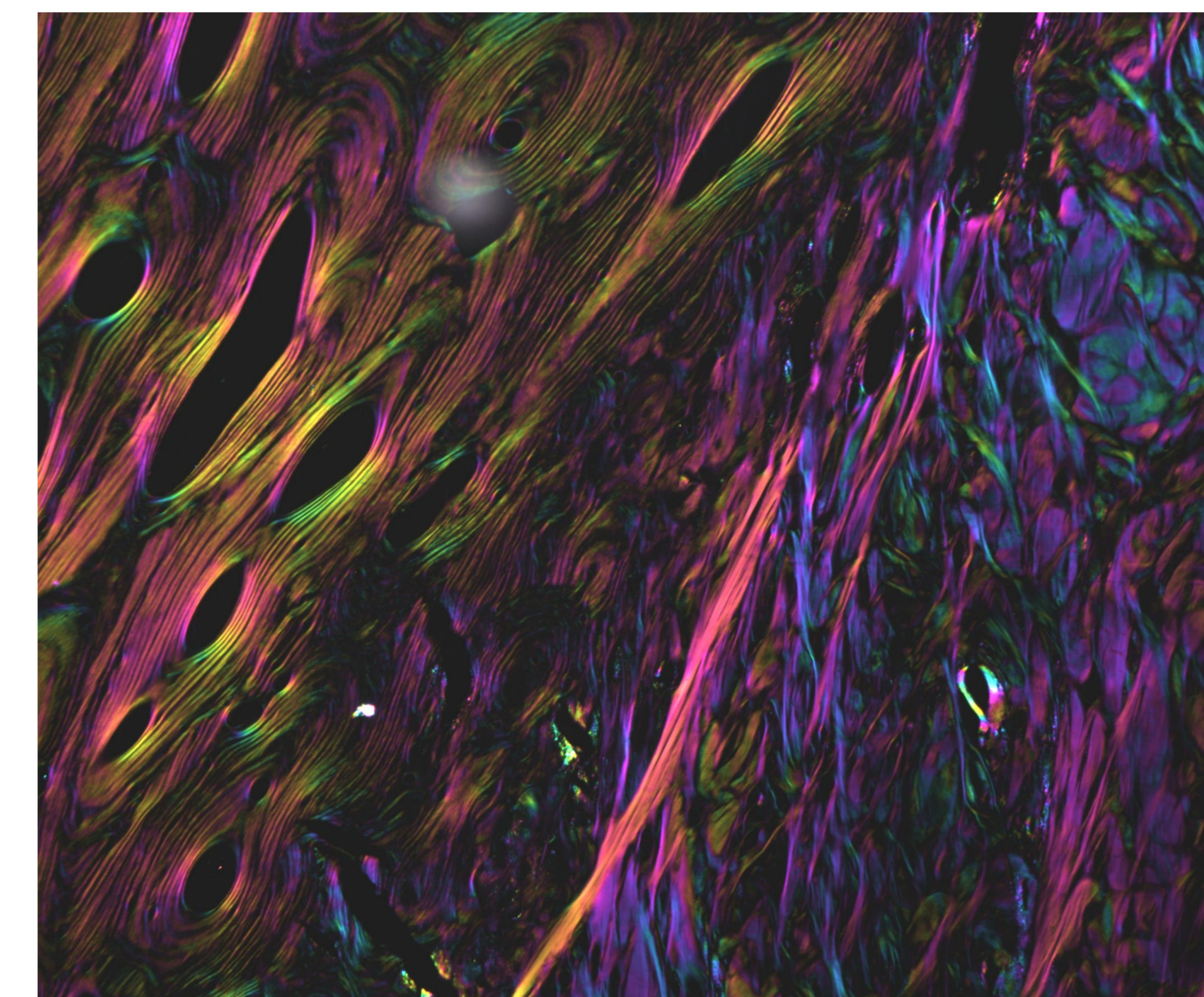
10 micron undecalcified section prepared by laser ablation microtomy. 18 images at 5 degree rotations, maximum. Fieldwidth 0.302mm 2018a



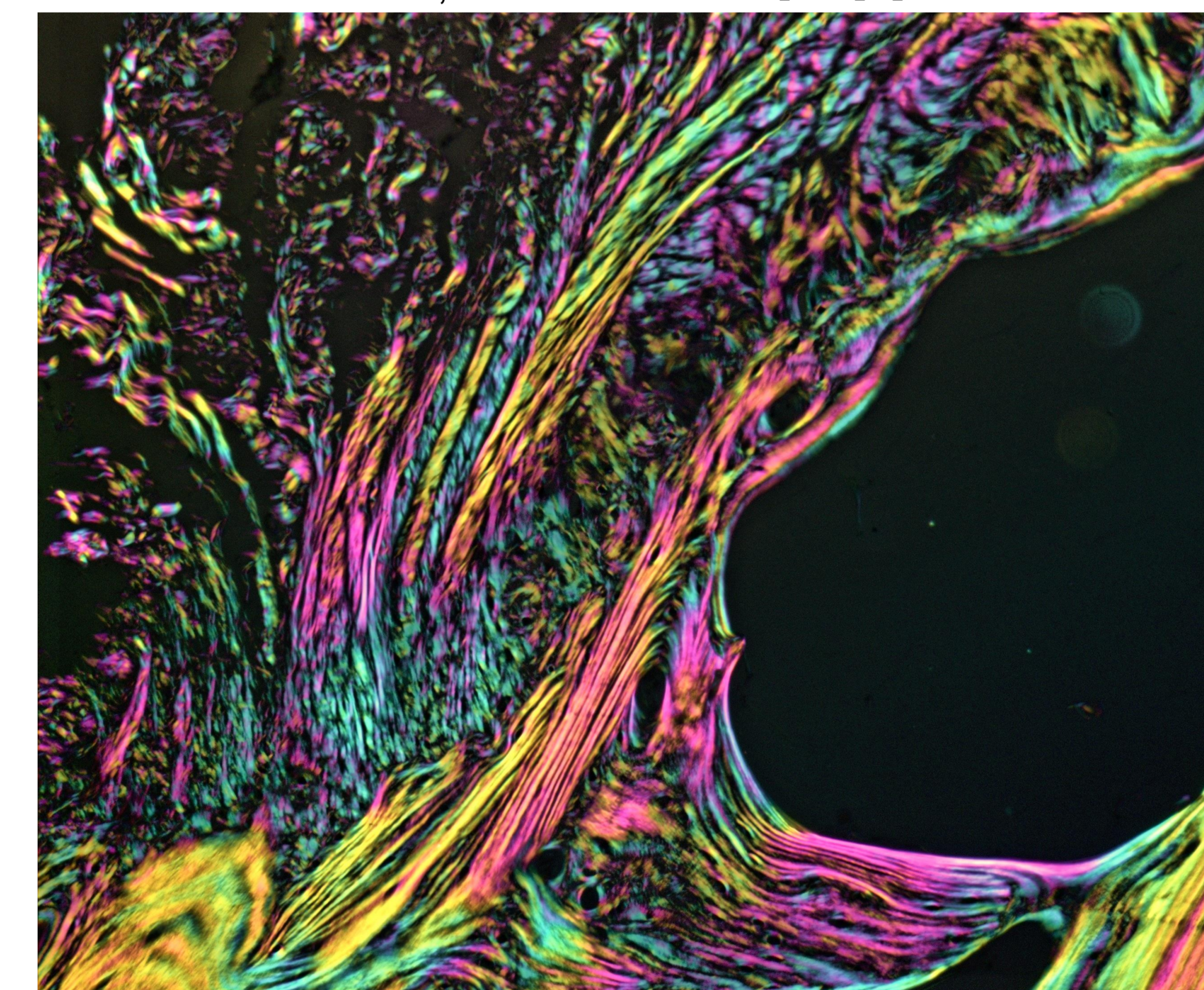
Decalcified H&E stained section showing trabecular excrescence in subchondral bone Fieldwidth 1.22mm AKU30_08-5-H&E_10x_1



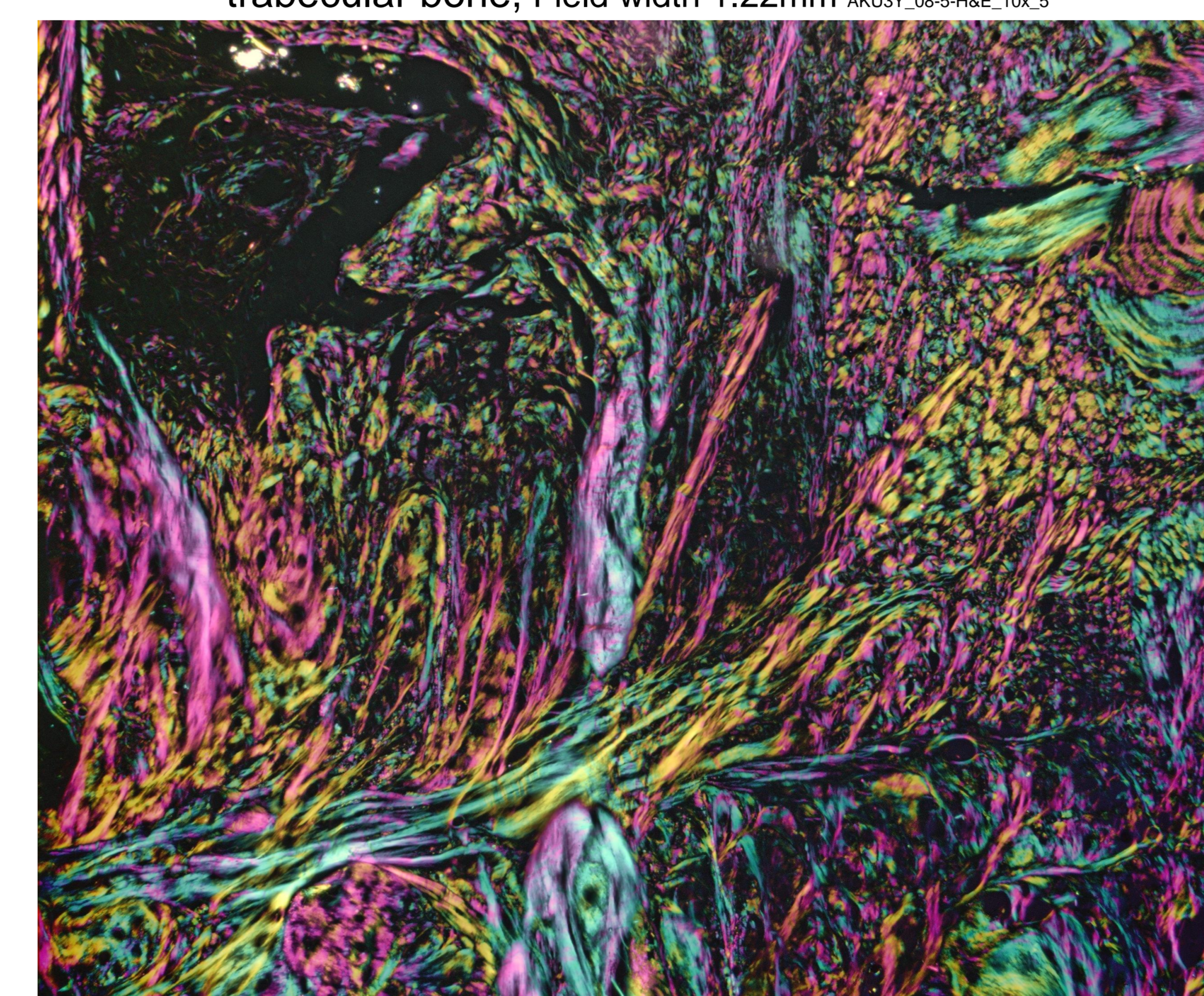
Decalcified section, unstained, subchondral trabecular bone, Field 3.05mm AKUSU_08-4-H&E4_04x



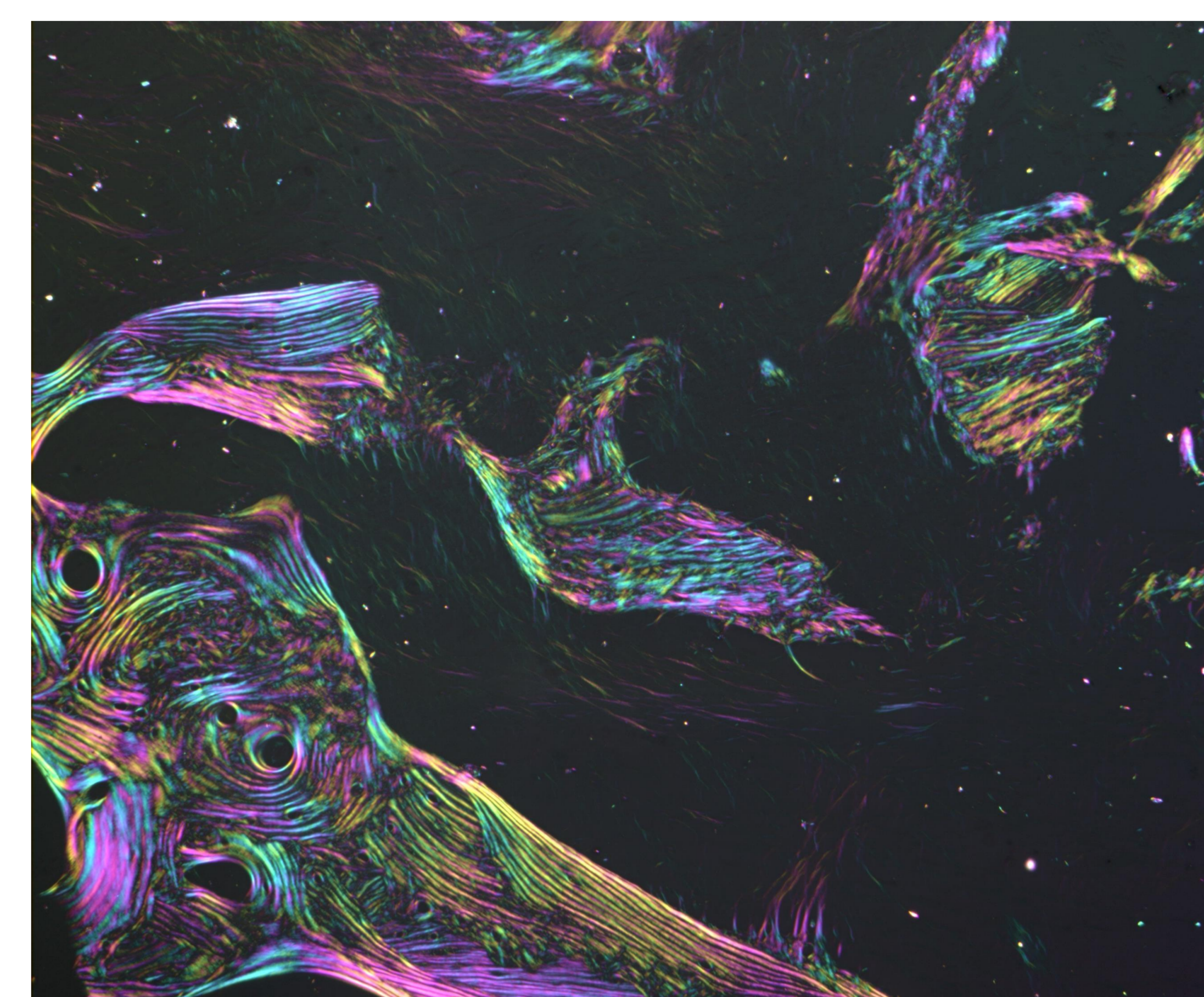
Decalcified section, unstained, cortex, junction of periosteum and bone, Field 1.22mm AKUSO_08-3-US_10x_3



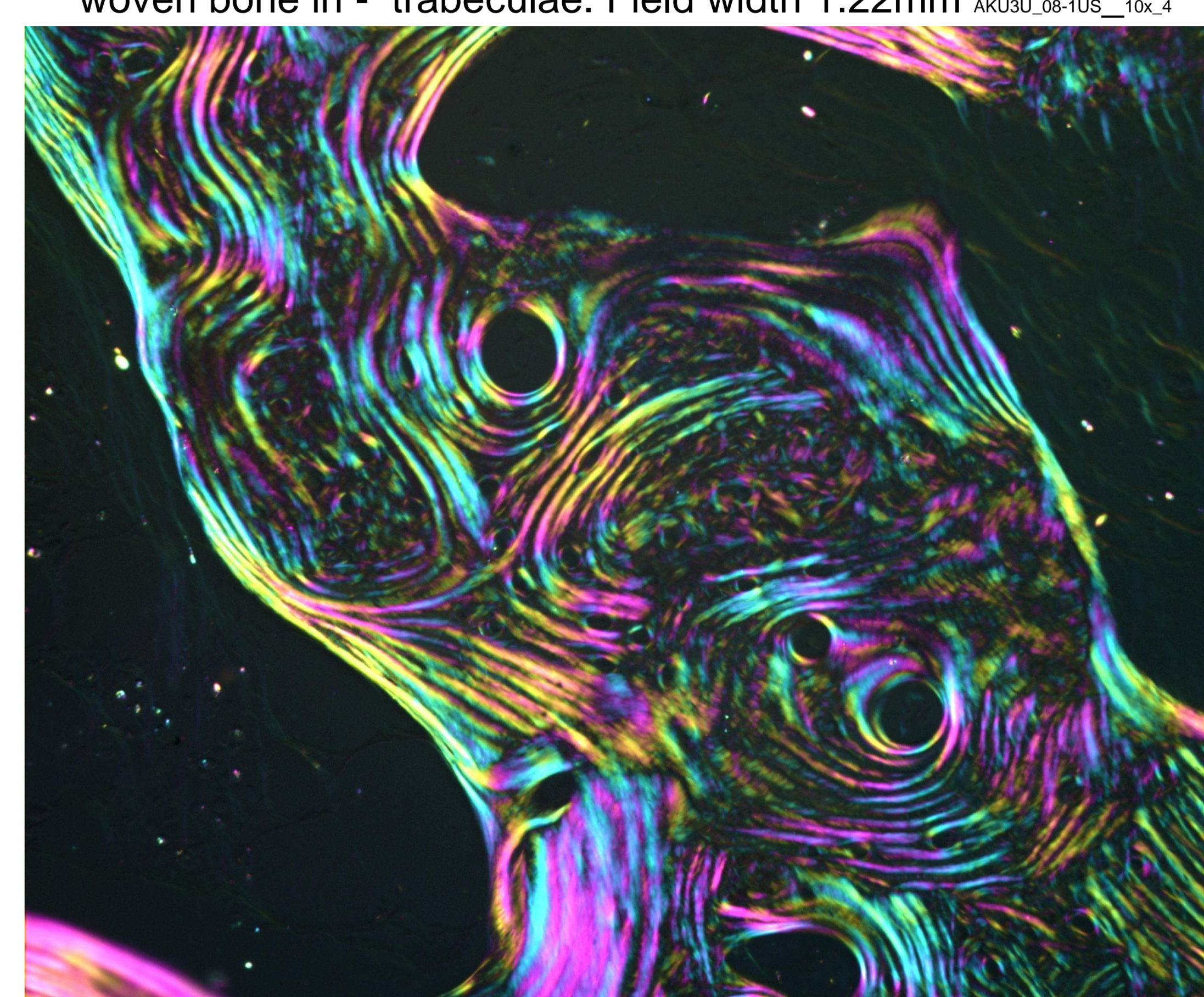
Decalcified section, H&E, junction of fibrous marrow (top) and trabecular bone, Field width 1.22mm AKU3Y_08-5-H&E_10x_5



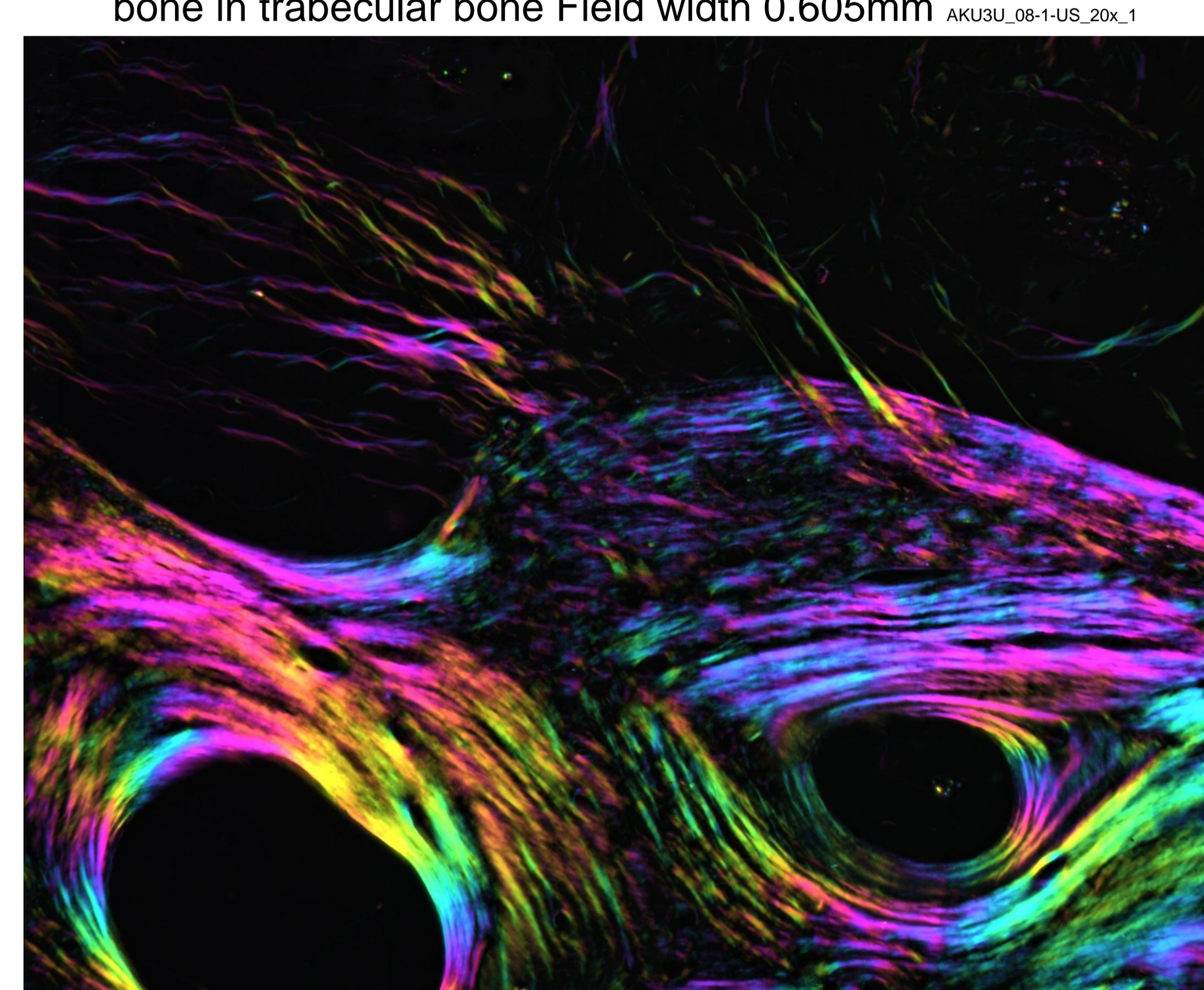
Decalcified section, unstained, dense fibrous marrow. Field width 1.22mm AKU3X_08-2-US_10x_4



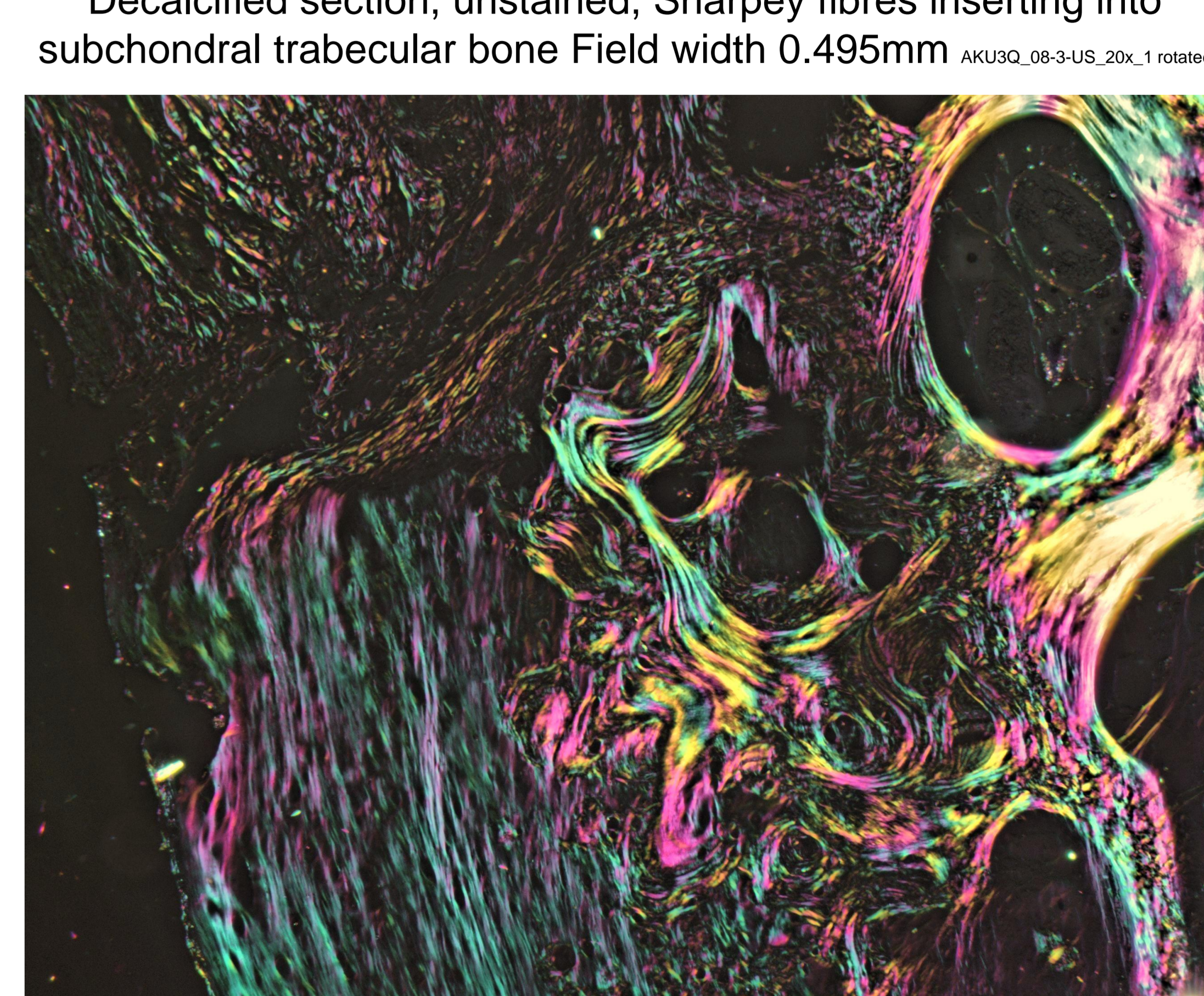
Decalcified section, unstained, Sharpey fibres inserting in – and woven bone in - trabeculae. Field width 1.22mm AKUSU_08-1US_10x_4



Decalcified section, unstained, mixture of woven and lamellar bone in trabecular bone Field width 0.605mm AKUSU_08-1-US_20x_1



Decalcified section, unstained, Sharpey fibres inserting into subchondral trabecular bone Field width 0.495mm AKU3Q_08-3-US_20x_1 rotated



Decalcified section, unstained, dense fibrous marrow joining trabecular bone Field width 1.22mm AKU3X_08-2-US_10x_3