Lab-based phase-contrast micro-CT for osteochondral tissue at ~1 µm resolution

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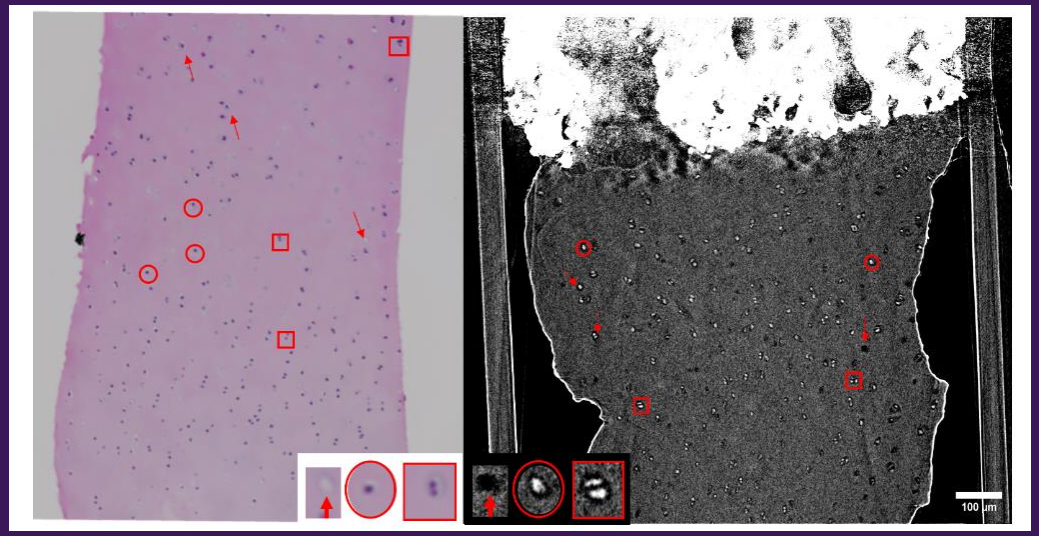


i4health

UKRI Engineering and Physical Sciences Research Council

The Francis Crick Institute

NXCT National X-Ray Computed Tomography

AXIM ADVANCED X-RAY IMAGING

UCL

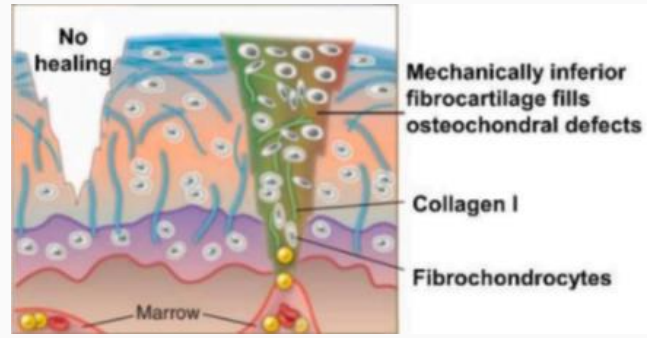
Introduction

- Osteochondral tissue (OC): Load-bearing composite structure found in joints comprising **cartilage**, **Calcified cartilage** and the underlying **subchondral bone**

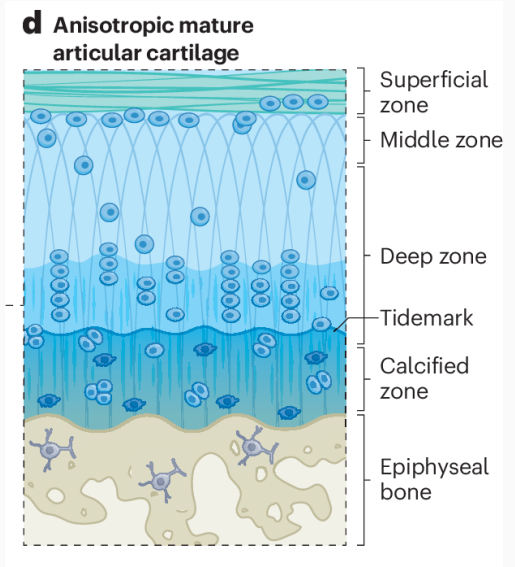
- Often subjected to trauma or degradation leading to **osteoarthritis** ([~303.1 million prevalent cases])

- The limited regenerative capacity of **avascular, aneural and alymphatic** cartilage poses a major challenge after injury or disease.

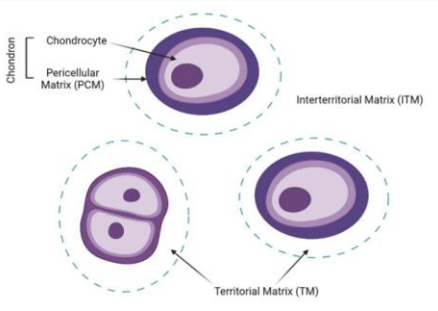
- In its natural state, OC tissue presents **well-defined cellular and extracellular matrix architecture** that has not been successfully restored using conventional treatment



(Niu et al., 2023)



(Pueyo Moliner et al., 2025)

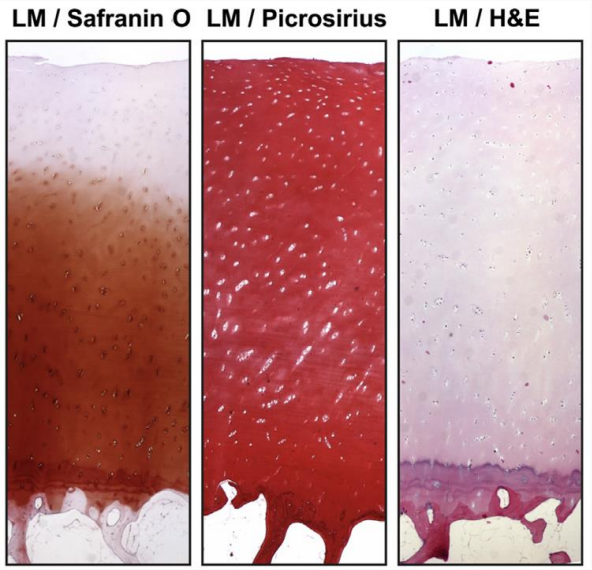


(Davis S.L., 2025)

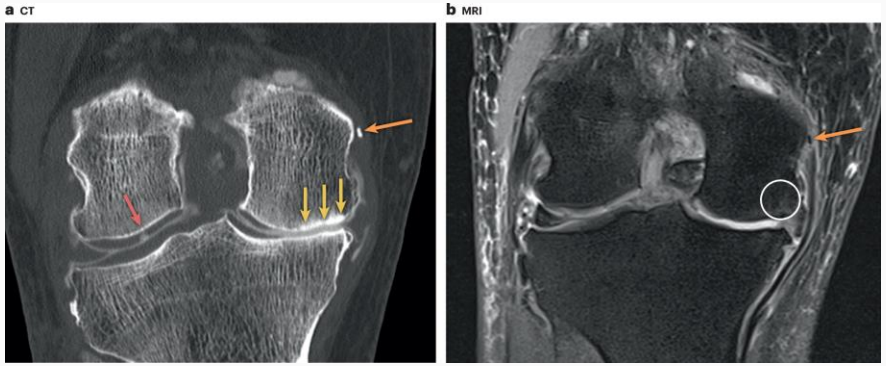
Need & aim

- The study of OC tissue has largely relied on **2D imaging techniques** that require destructive sample preparation (Histology is “gold standard”)
- 2D imaging fails to capture the relation between cell **shape**, **size** and **distribution** with the maintenance of the **ECM**
- Volumetric, non-destructive techniques have emerged, mainly **MRI and X-ray CT**, for the assessment of natural or engineered tissue.
- However, a **lack of contrast** (cartilage) and a **limited resolution** (mm-cm) have limited its application for the OC tissue.

Use lab-based phase contrast µCT to develop a non-destructive imaging methodology that provides a reliable and easily accessible assessment tool for natural or engineered OC tissue with sufficient resolution and contrast to visualize cells within the extracellular matrix in 3D



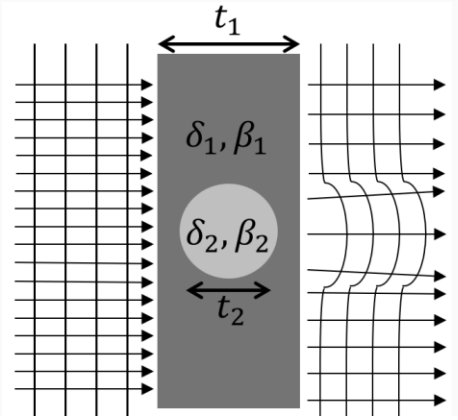
(Nieminen H.J, et. al., 2017)



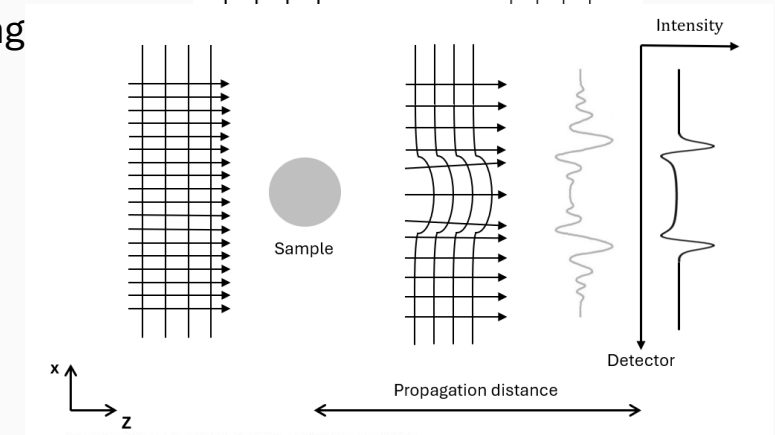
(Guermazi, A., Eckstein, F., Gold, G. *et al.*., 2026)

Inline phase-contrast imaging

- In contrast to attenuation-based imaging, phase-contrast exploits the **phase-shift** experienced by the X-rays as they interact with the sample to generate imaging contrast



- Leveraging the complex refractive index $n = 1 - \delta + i\beta$ to generate **contrast** in the imag offers an opportunity to **increase SNR**, especially when dealing with weakly attenuating samples



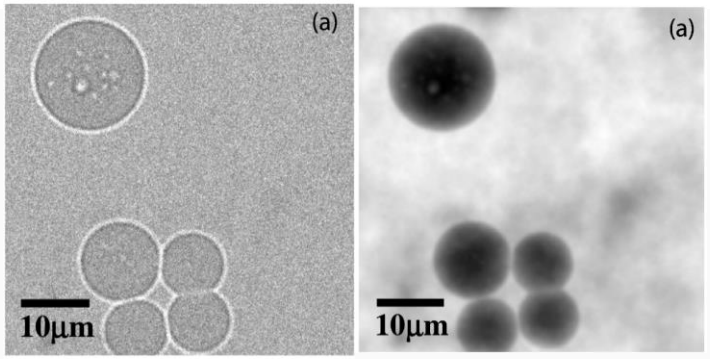
- For propagation-based PC: the refraction process induces an **intensity modulation pattern** that corresponds to a **mixture** between **δ and β**

$$I(x,y;M,\lambda) = \frac{I_0}{M^2} e^{-2T(x,y,\lambda)}\left[1 + \frac{R_2\lambda}{M2\pi}\nabla_\perp^2\phi(x,y;R_1,\lambda)\right] \Rightarrow J = I * \mathrm{PSF} * \left[S\frac{z_{od}}{z_{so}}\right]$$

$$T(x,y,\lambda) = \frac{2\pi}{\lambda}\int_o \beta(x,y,z,\lambda)dz$$

$$\phi = -\frac{2\pi}{\lambda}\int_{object} \delta(x,y,z;\lambda)dz$$

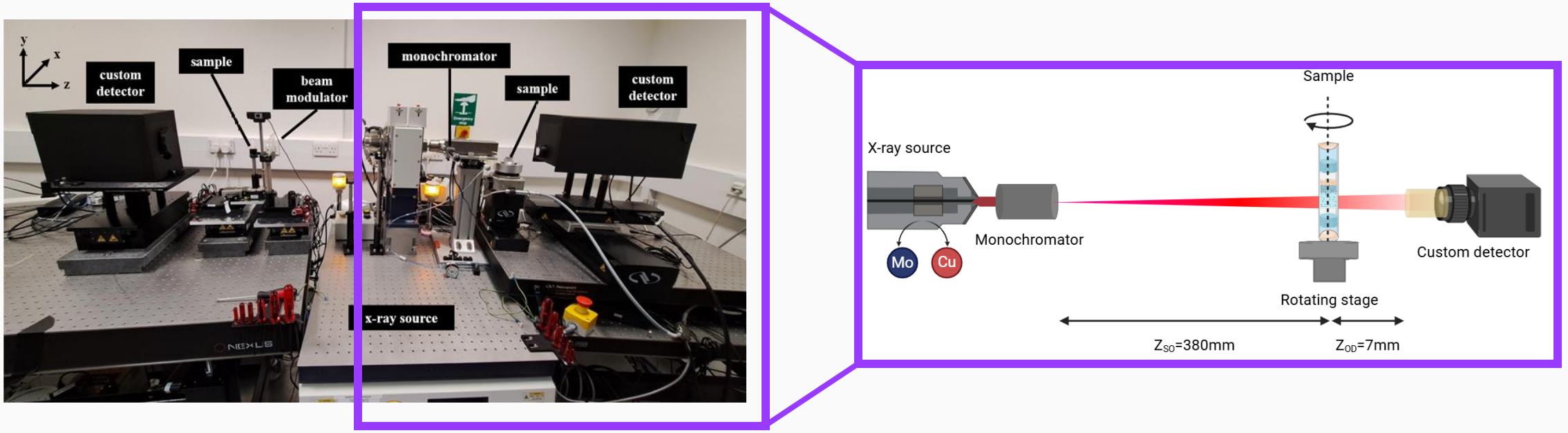
- To extract phase and amplitude information from the image a **phase-retrieval algorithm** must be applied: **Paganin's single-image method** (Non-quantitative)



(Paganin, et.al, 2002)

- The phase-retrieved projections can be reconstructed into 3D volumes to describe internal structure of the object with proper tomographic reconstruction method

μCT at UCL (NXCT)



- Detector pixel size: 4.5μm (3200x2200)

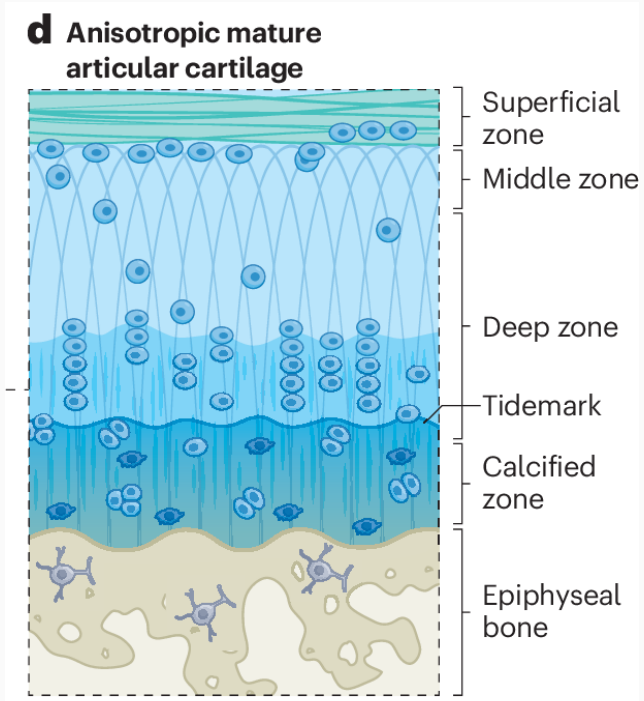
Optical magnification	Effective pixel size	FOV
5x	0.9μm	~2mm
10x	0.45μm	~1mm
20x	0.225μm	~0.5mm

FIRST STAGE: is it possible?

Could the LB-XPCi-uCT effectively be used for assessing OC tissue?

Modelling the sample

- As rule-of-thumb one should aim for 25-75% of X-ray transmission



(Pueyo Moliner et al., 2025)

Cartilage

Bone

"Rule of mixtures" kind of law

$$\mu/\rho = \sum_i w_i (\mu/\rho)_i$$

%O	%C	%H	%N	%P	%S	%Na	%Cl
74	9.9	9.6	2.2	2.2	0.9	0.5	0.3

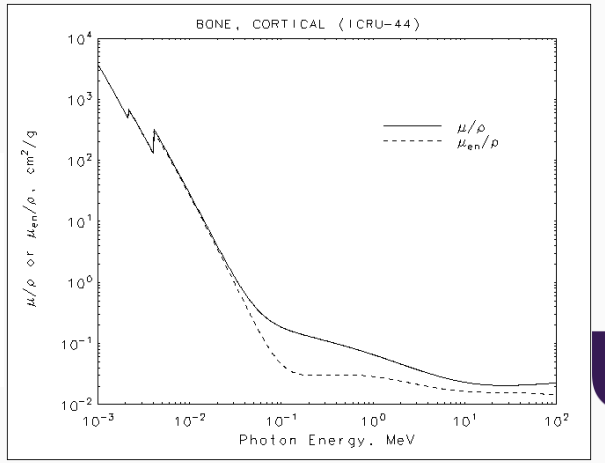
Linear attenuation coefficient

$$I_1 = I_0 e^{-\mu x}$$

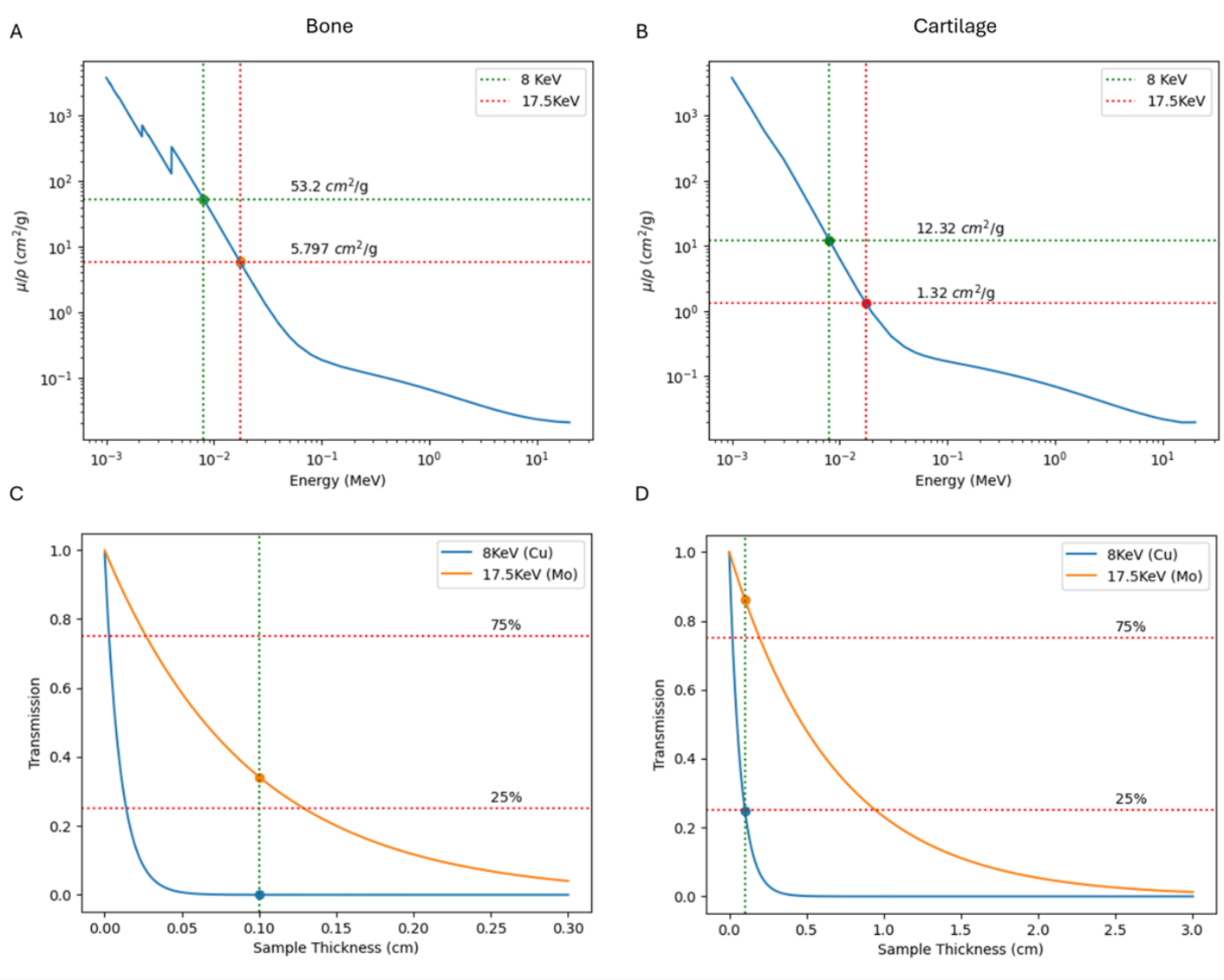
X-Ray Mass Attenuation Coefficients

NIST Standard Reference Database 126

Last Update to Data Content: July 2004 | NISTIR 5632 | Version History | Disclaimer |
DOI: https://dx.doi.org/10.18434/T4D01F



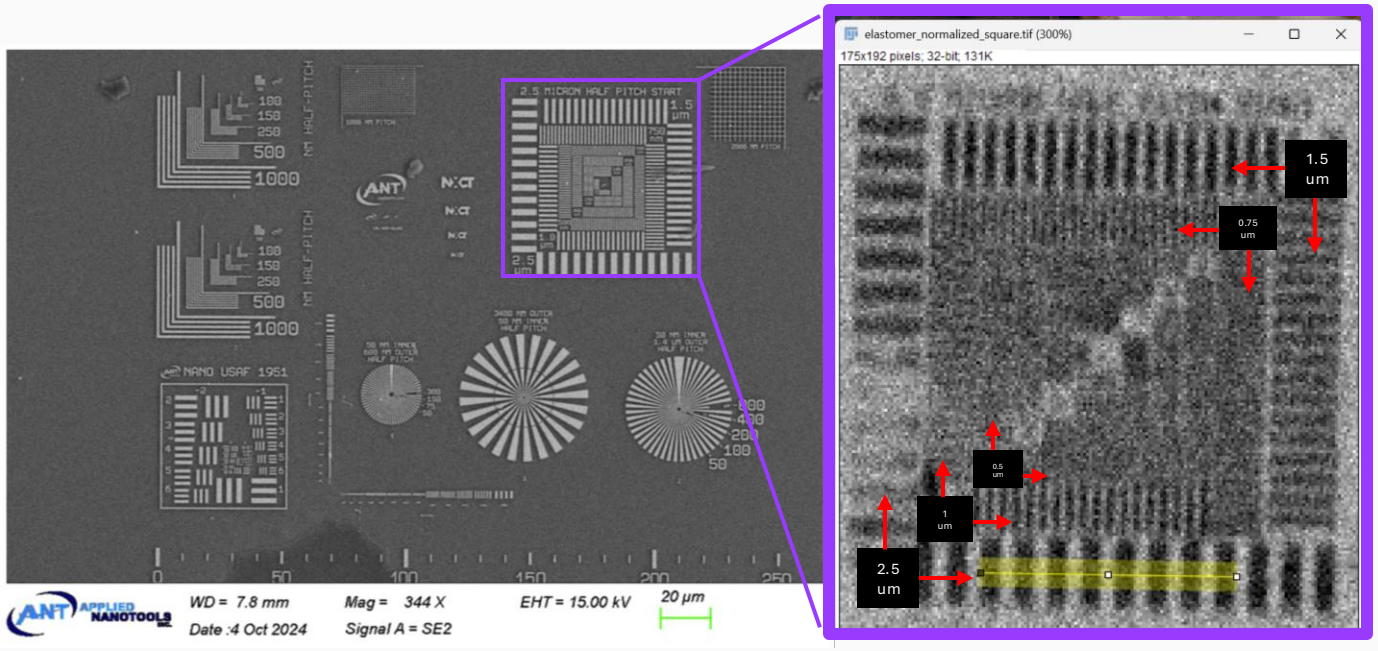
Modelling the sample

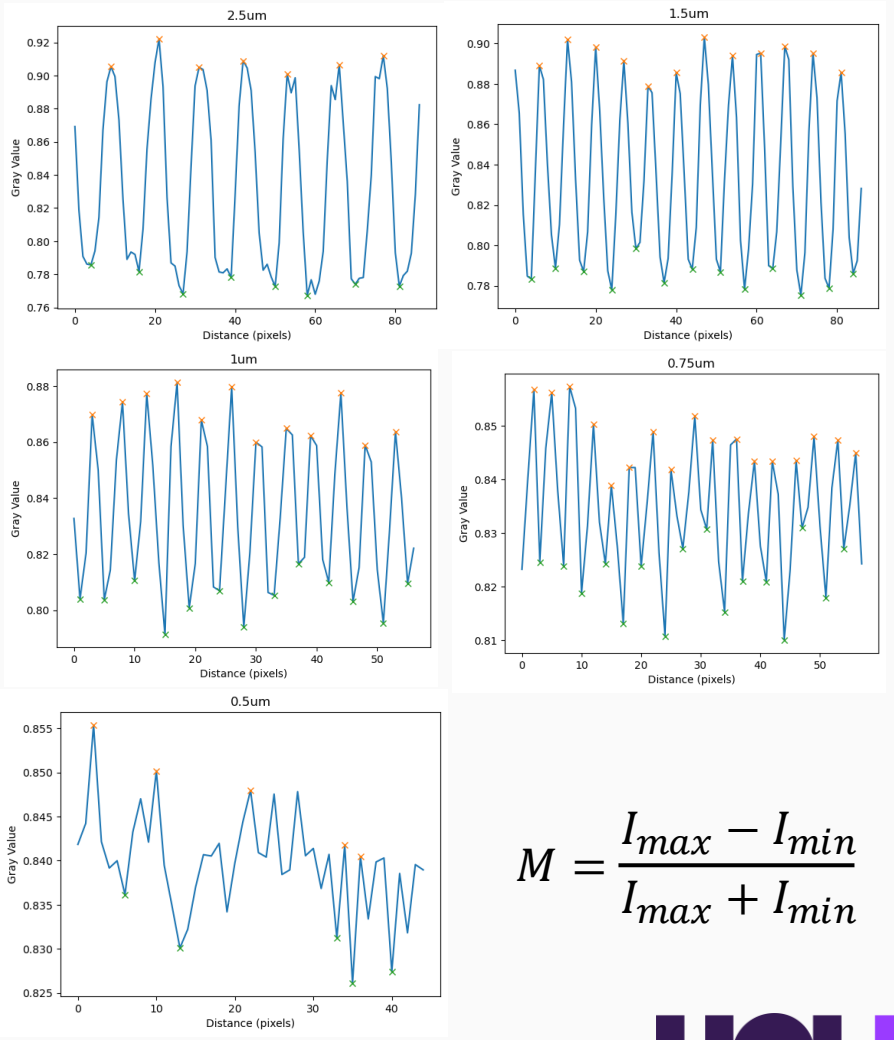


Only for a 1 mm thick sample both the cartilage and bone showed transmission ~[25%-75%] for either Cu or Mo

Resolution test

- Could the system resolve ~10µm features (cell)
- Calculate the MTF

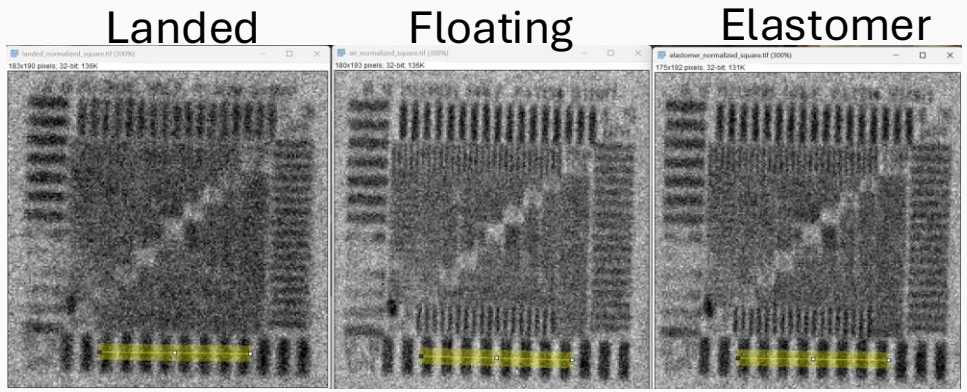


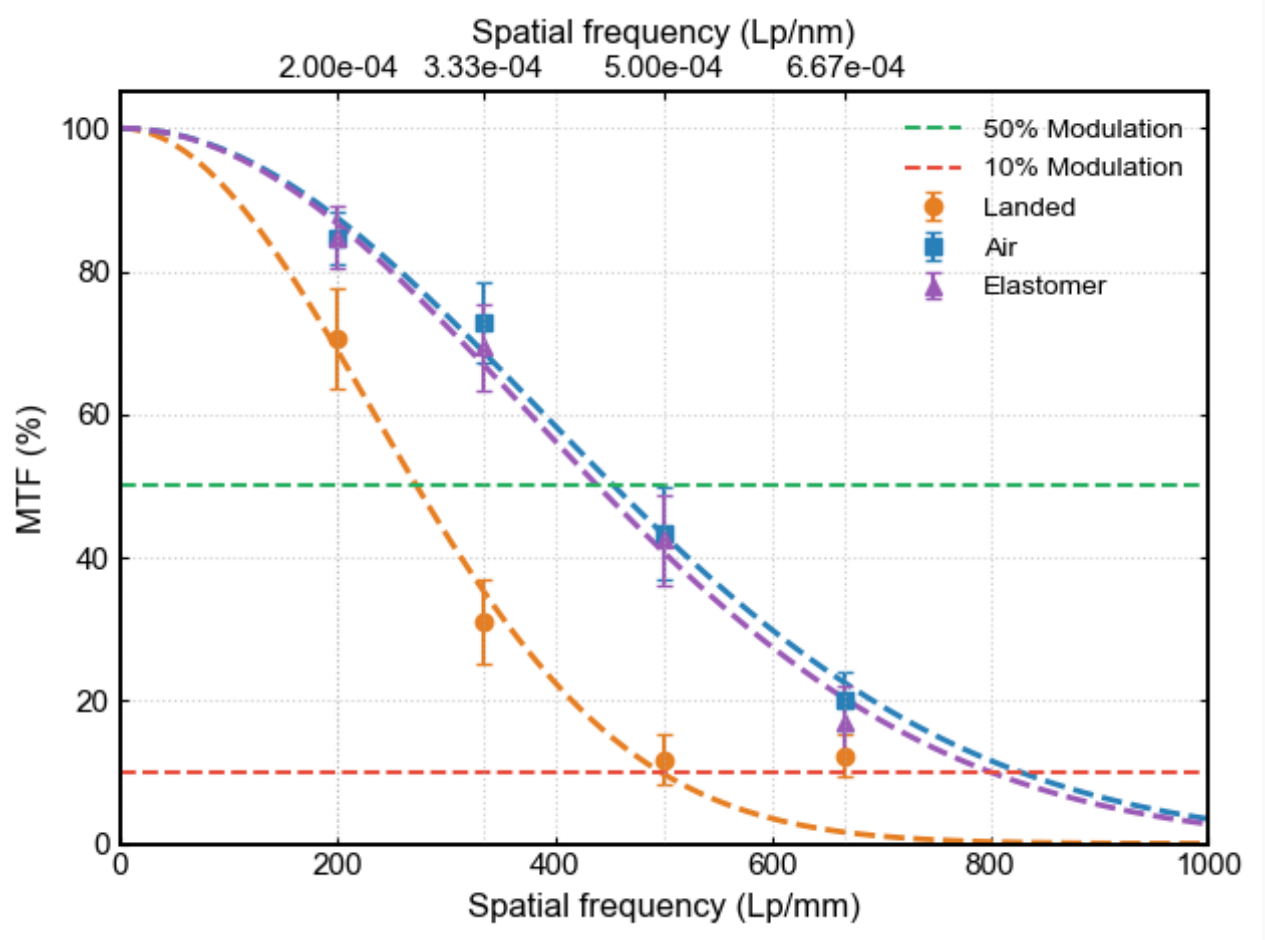


$$M = \frac{I_{max} - I_{min}}{I_{max} + I_{min}}$$

Image quality does not only depend on the detector…but also on the stability of the system

Resolution test

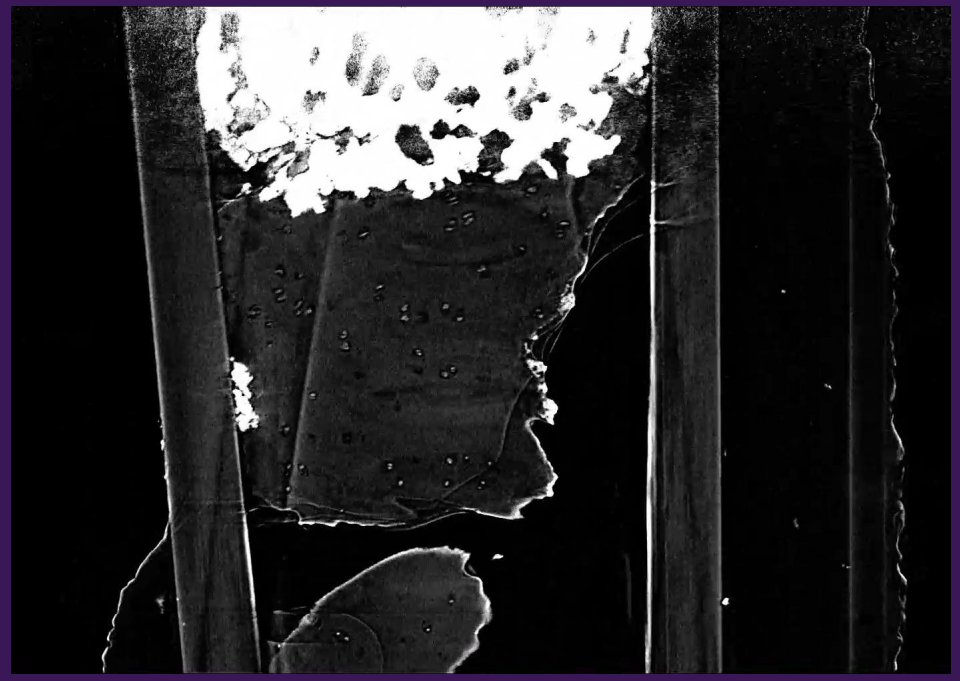




For both the floating and elastomer configurations the system exhibits an MTF50 at 500 LP/mm, corresponding to an effective spatial resolution of <1 μm

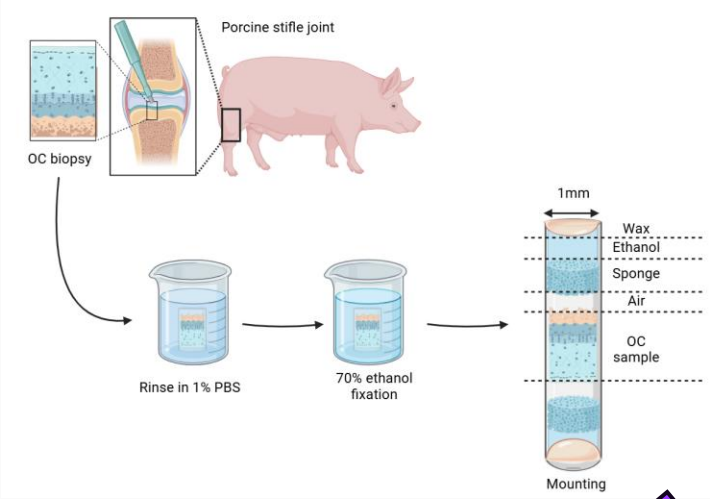
Second STAGE: Pilot study

Can LB-XPCi-uCT effectively visualize individual chondrocytes (~10um) within cartilage

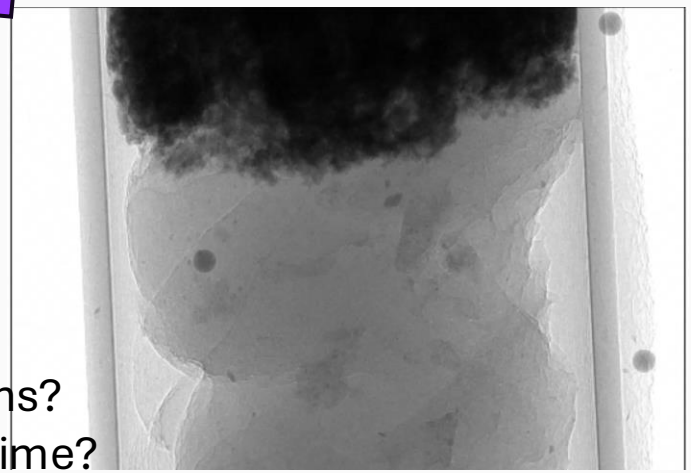


Methods

Sample preparation protocol

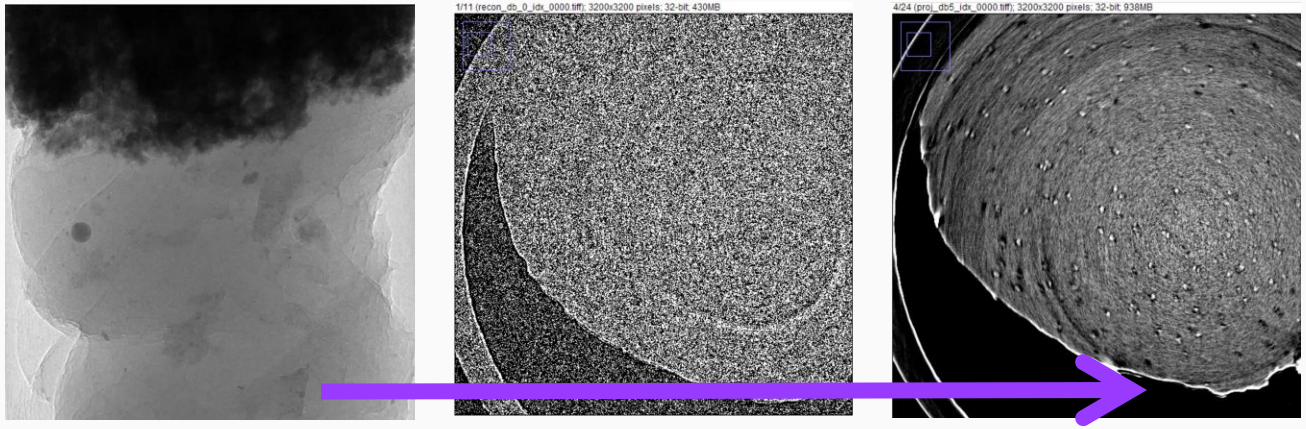


Acquisition



#projections?
Exposure time?

Image optimization: Phase retrieval & CT reconstruction



Results: Image optimization and reconstruction

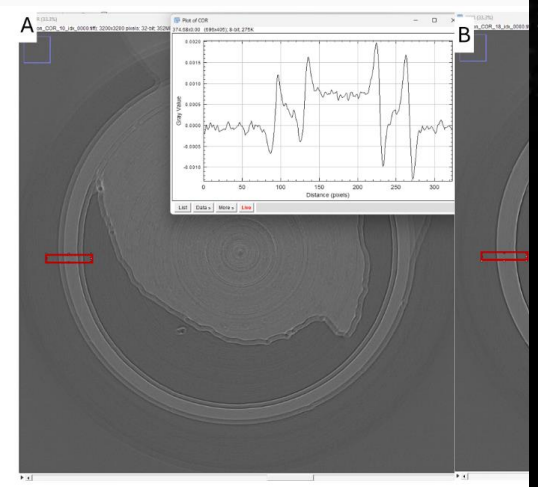
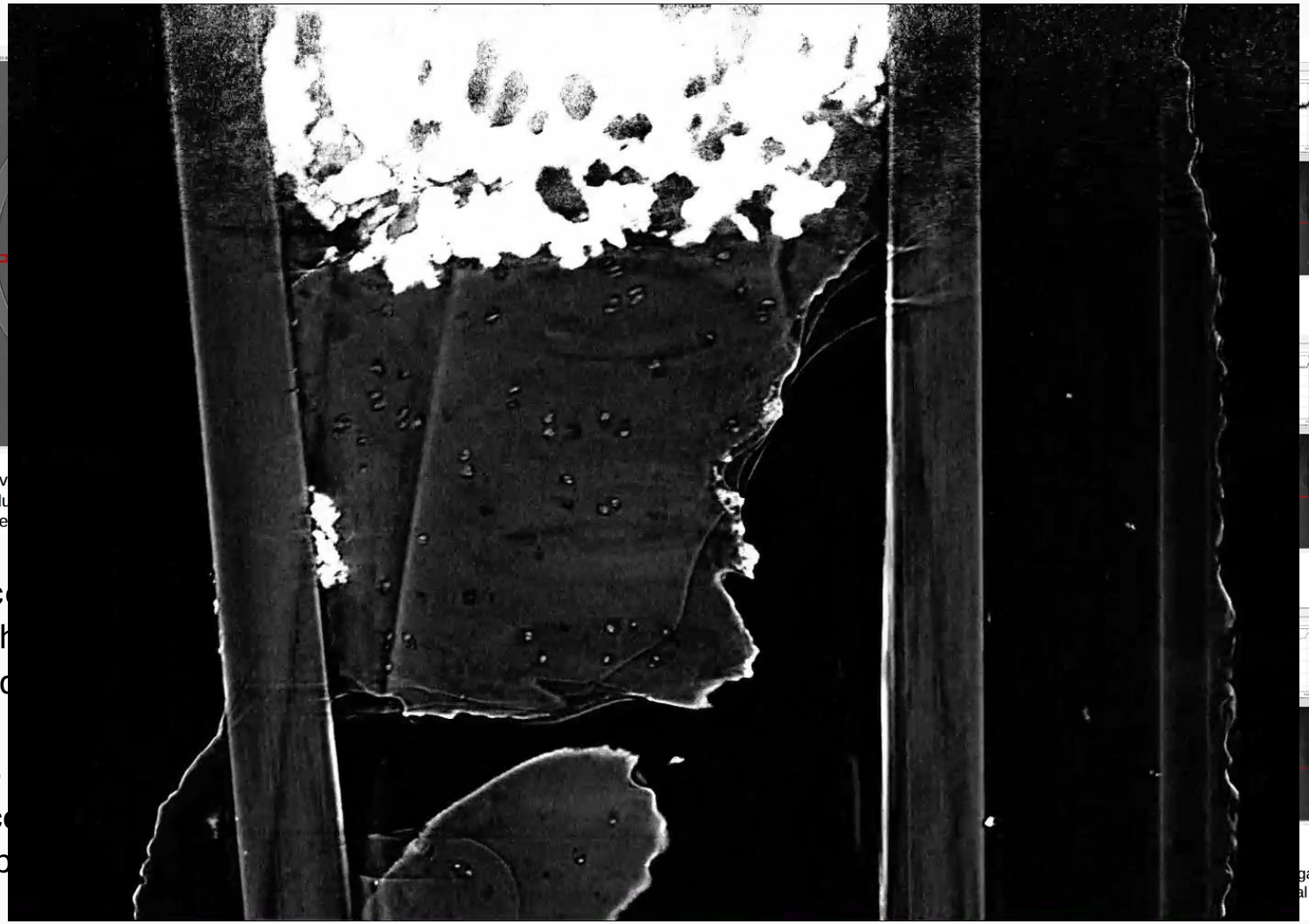
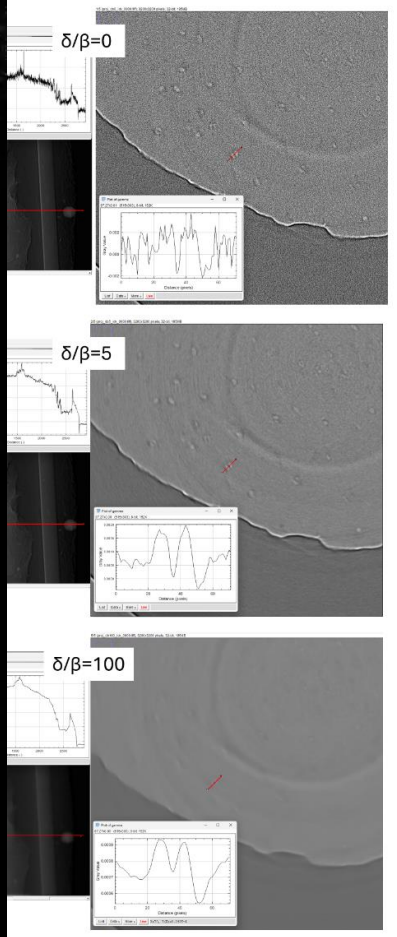


Figure 4.6: COR selection for a middle slice in the OC v
COR alignment, showing double edges and degraded resolu
removing double edges and displaying phase-contrast fringe

- COR and ring artefact c
  optimized to improve th
  cells without inducing
  image
- Optimisation of the δ/β
  ECM boundary **enhanc**
  and improves noise sup





ganin phase retrieval: (A) Low δ/β producing low contrast.
l value enhancing cellular visibility; (C) High δ/β resulting

Results: Image optimization and reconstruction

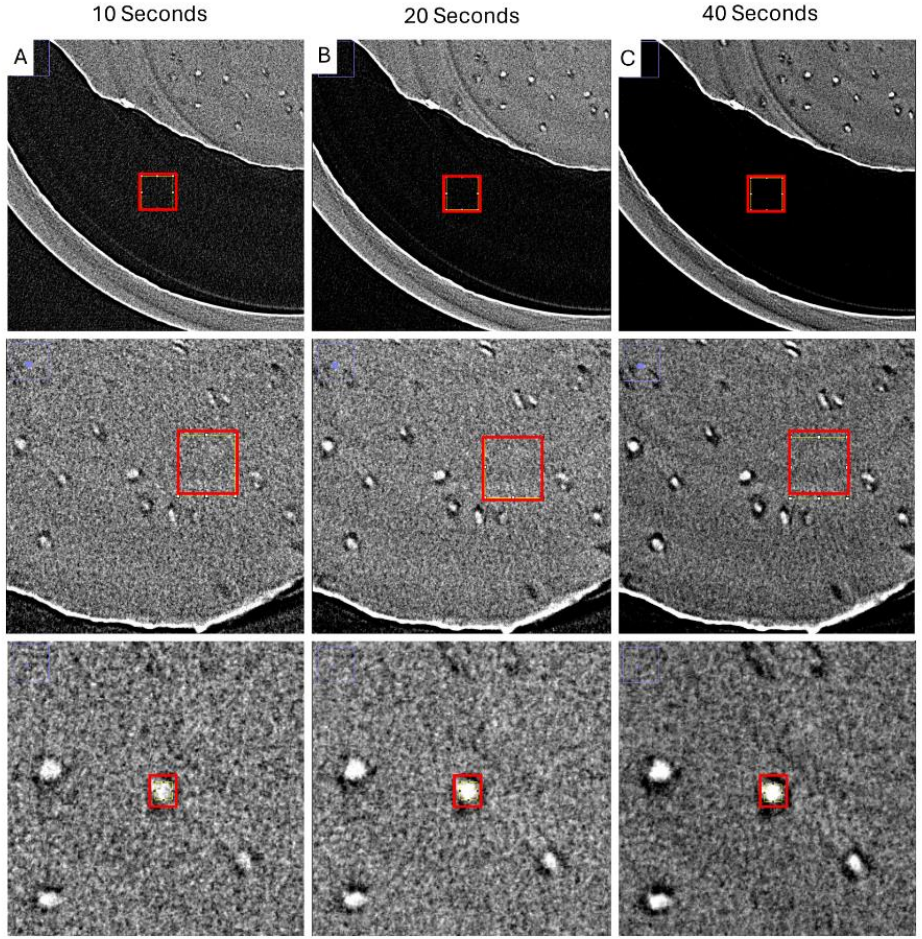


Figure 4.12: Comparison of reconstructed regions of interest from volumes acquired at different exposure times: (A) 10s, (B) 20s, and (C) 40s per projection. Despite the reduction in exposure time, cellular features such as individual chondrocytes remain clearly visible, even at 10 s exposure.

Table 4.1: Summary of noise and intensity measurements, as well as SNR and CNR values, for different exposure times.

Exposure Time	σ_{Air}	$\hat{I}_{ECM}$	σ_{ECM}	$\hat{I}_{Cell}$	SNR_{ECM}	SNR_{Cell}	CNR_{Cell}
10 seconds	0.00027	0.000799	0.00030	0.00166	2.96	6.25	2.92
20 seconds	0.00019	0.000788	0.00022	0.00169	4.13	8.89	4.17
40 seconds	0.00014	0.000787	0.00016	0.00171	5.57	12.14	5.81

- SNR and CNR improve with the integration time following a $\sqrt{n}$ **relation** (Poisson statistics)
- Even at 10s exposure the cellular structures were observed **reducing imaging time to ~4hours**

Results: General Assessment

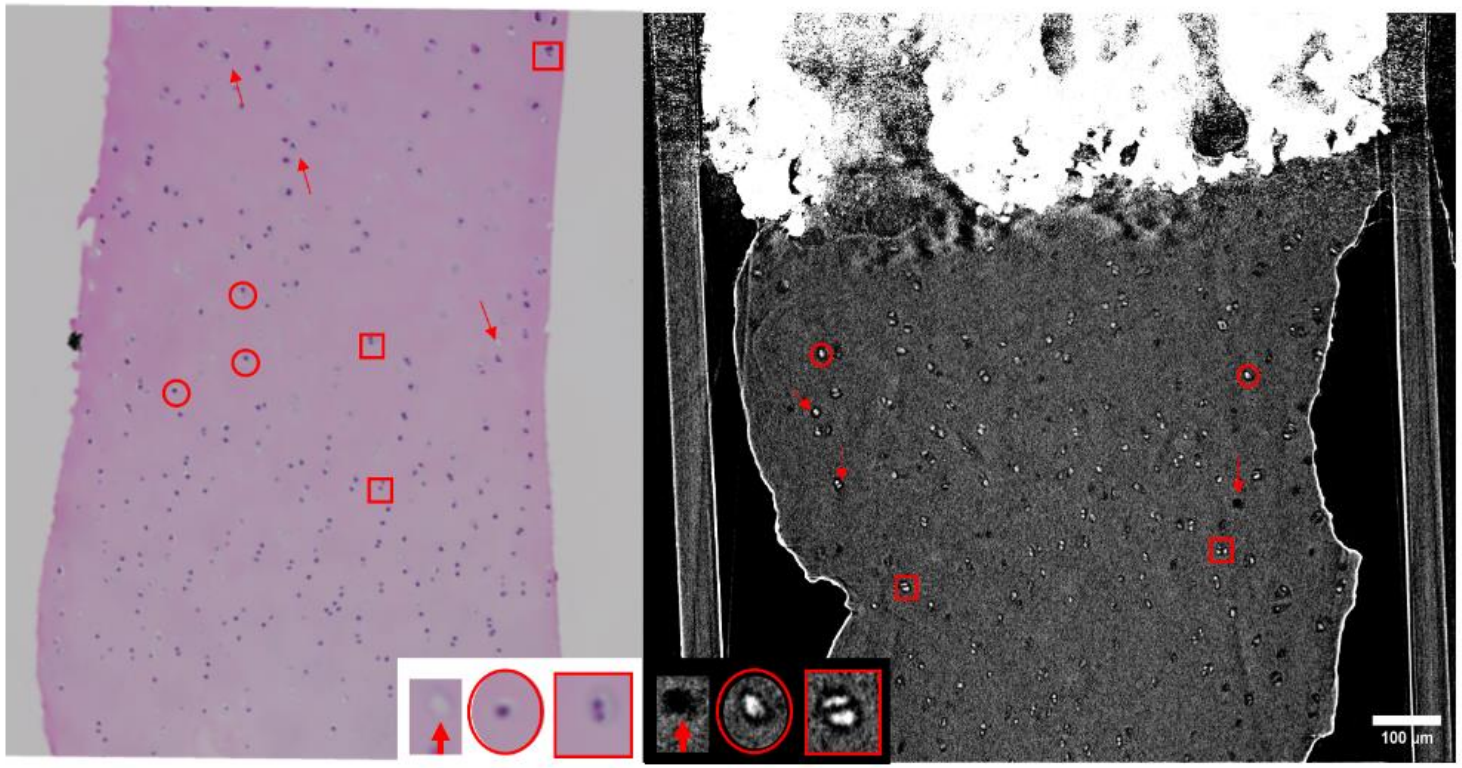
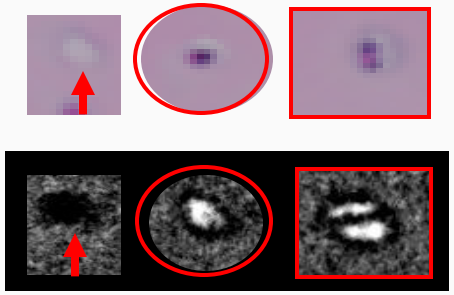


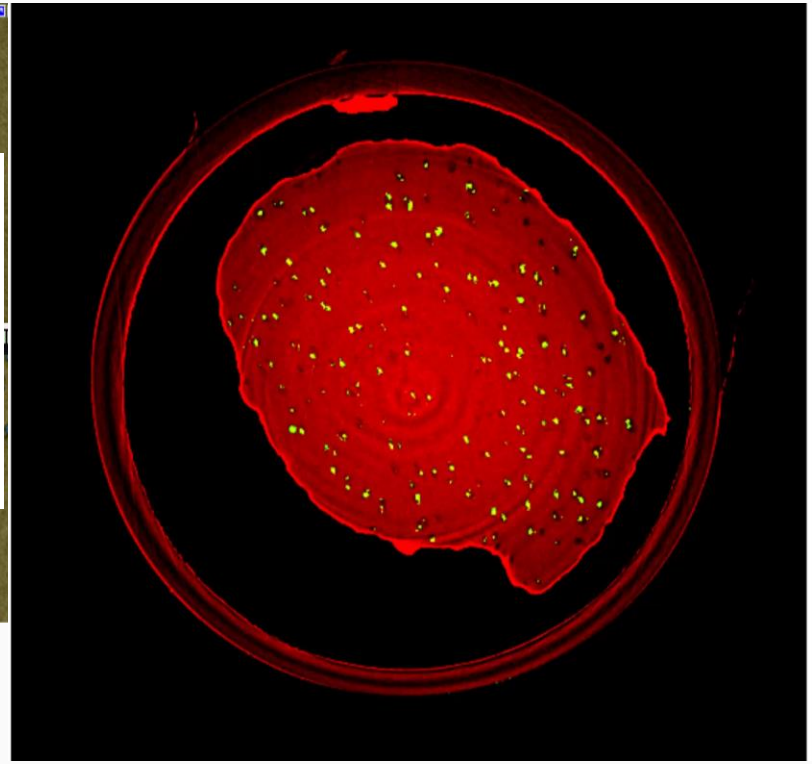
Figure 4.11: Comparison of X-ray phase-contrast imaging and histology (H&E staining). Circles indicate chondrocytes, squares highlight isogenous groups, and arrows denote empty or filled chondrocyte lacunae.



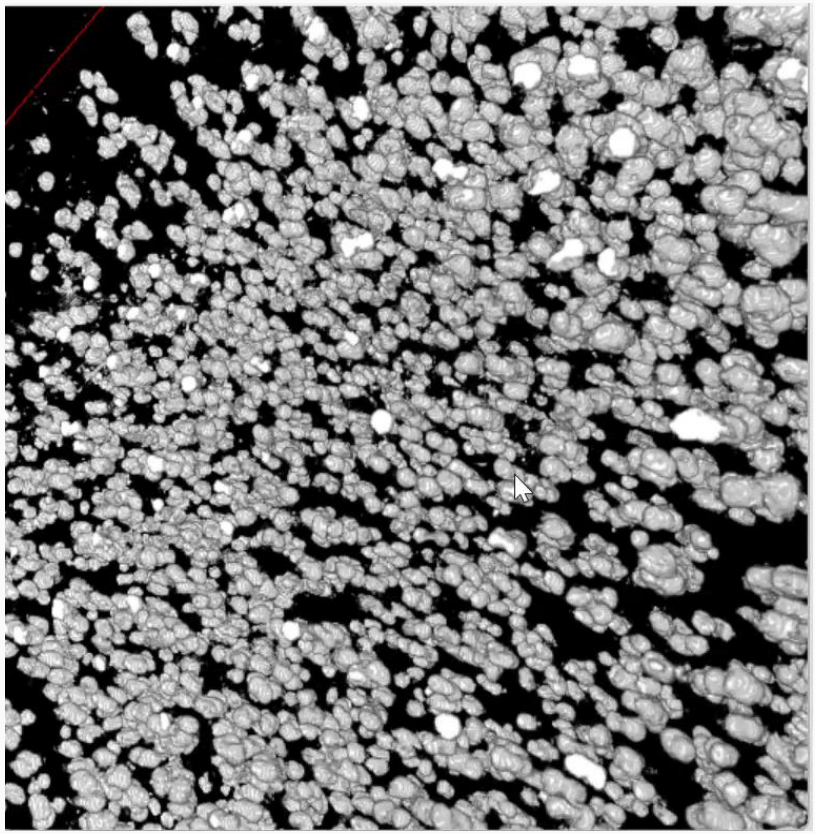
- Mean **chondrocyte diameter** measured 10.34 ±1.84 µm is consistent with literature (~10µm)
- Structural features are **comparable** between imaging modalities

Results: Volumetric segmentation

- **Pixel-wise** 3D volume segmentation
- Feature selection in the classification model (Intensity, texture, edges)



- Visual examination shows **good agreement** between **chondrocyte segmentation** and images.



- **Morphological** examination and **quantitative** information has yet to be obtained

Conclusions

- **Can LB-XPCi-uCT effectively visualize individual chondrocytes (~10um) within osteochondral tissue/scaffolds?**
  - Yes, it can! It is even possible to segment the cells throughout the volume opening the possibility for quantitative assessments
  - The volume showed comparable morphological features to histological imaging

BUT…

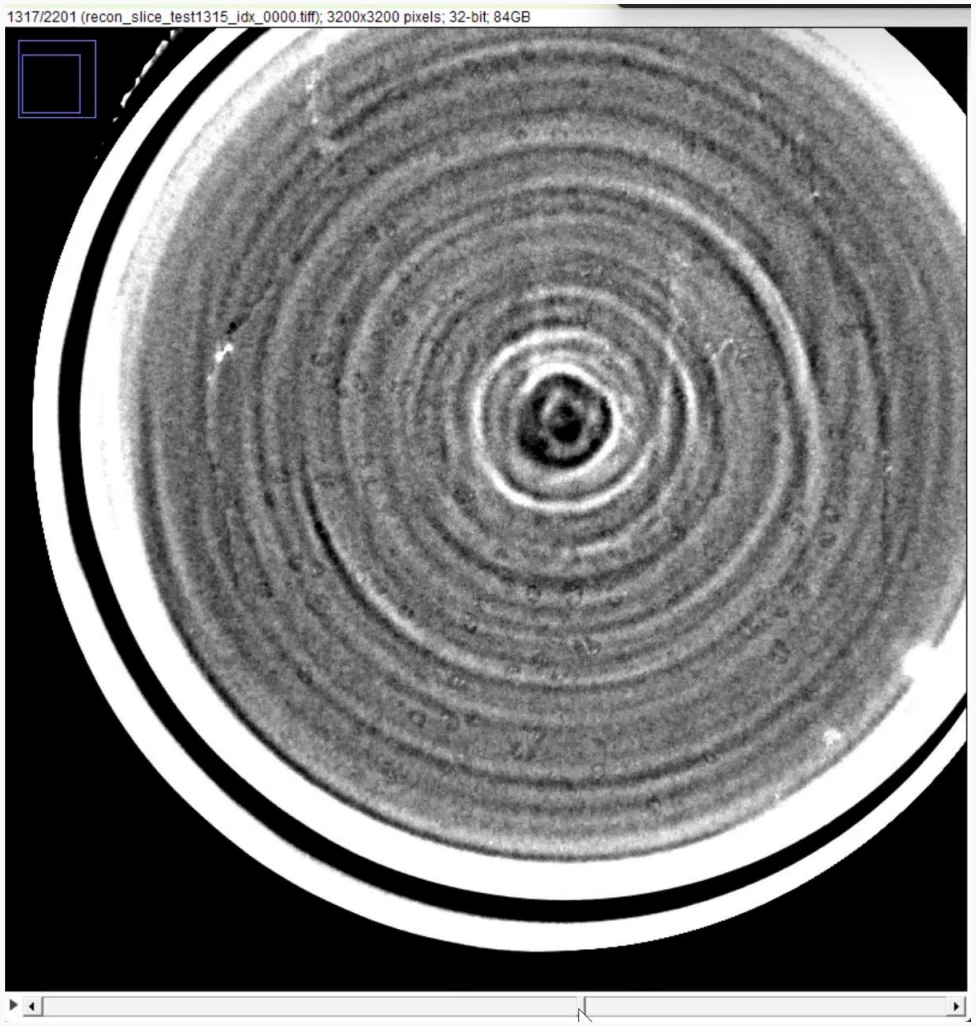
- Although not “destructive” the sample preparation protocol followed still required fixation/dehydration which may change both the morphological and mechanical characteristics of the OC tissue…

Third stage: let’s challenge the methodology

Is it possible to visualize the same features in non-fixed & hydrated tissue resembling physiological conditions?

Imaging hydrated samples

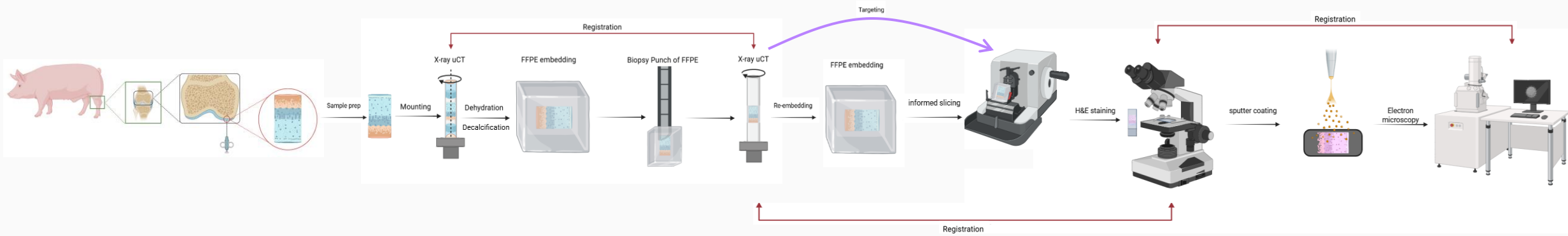
- ~ 16000 projections * 10sec =~ 44 hours (non-ideal)



- Although this protocol has not yet been optimized, we are still able to observe cellular features in **non-fixed, non-stained, hydrated samples**
- Opens the possibility of evaluating what are the **implications** of sample preparation protocols and assess the morphological characteristics of cells in a near-physiological state

Proposal: Sample preparation and Correlative validation

- Implement a “Daisy chain” registration strategy to evaluate morphological changes in the sample during destructive sample preparation protocols
- Correlative analysis between XPC-CT-histology-EM

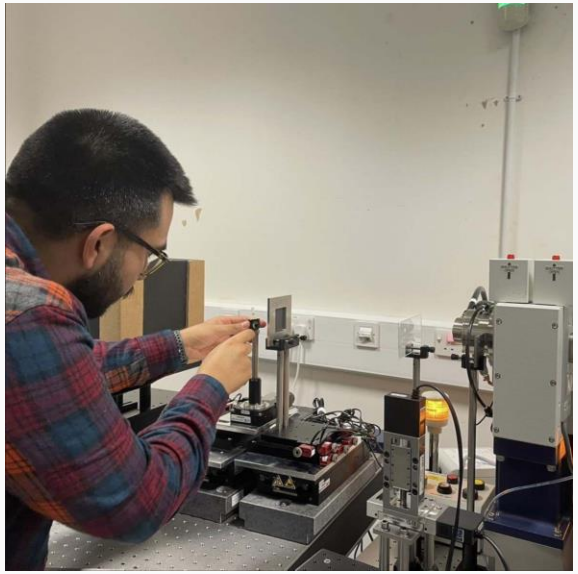


Conclusion

- Lab-based Phase-contrast µCT **can visualize individual chondrocytes (cells) in 3D**
- **Cell segmentation is possible** without destructive histology
- **Imaging hydrated tissue in the lab** is very challenging **but feasible**
- **Correlative imaging** will help quantify sample preparation induced changes

Future work

- **Quantitative Morphological Analysis:** Finalize the extraction of **quantitative metrics** (shape, size, and distribution) from segmented 3D volumes to move beyond qualitative visual examination
- **"Daisy Chain" Correlative Validation:** Complete the registration strategy to correlate **XPC-µCT data with histology and electron microscopy**, allowing for a precise evaluation of morphological changes induced by traditional sample preparation



Gracias