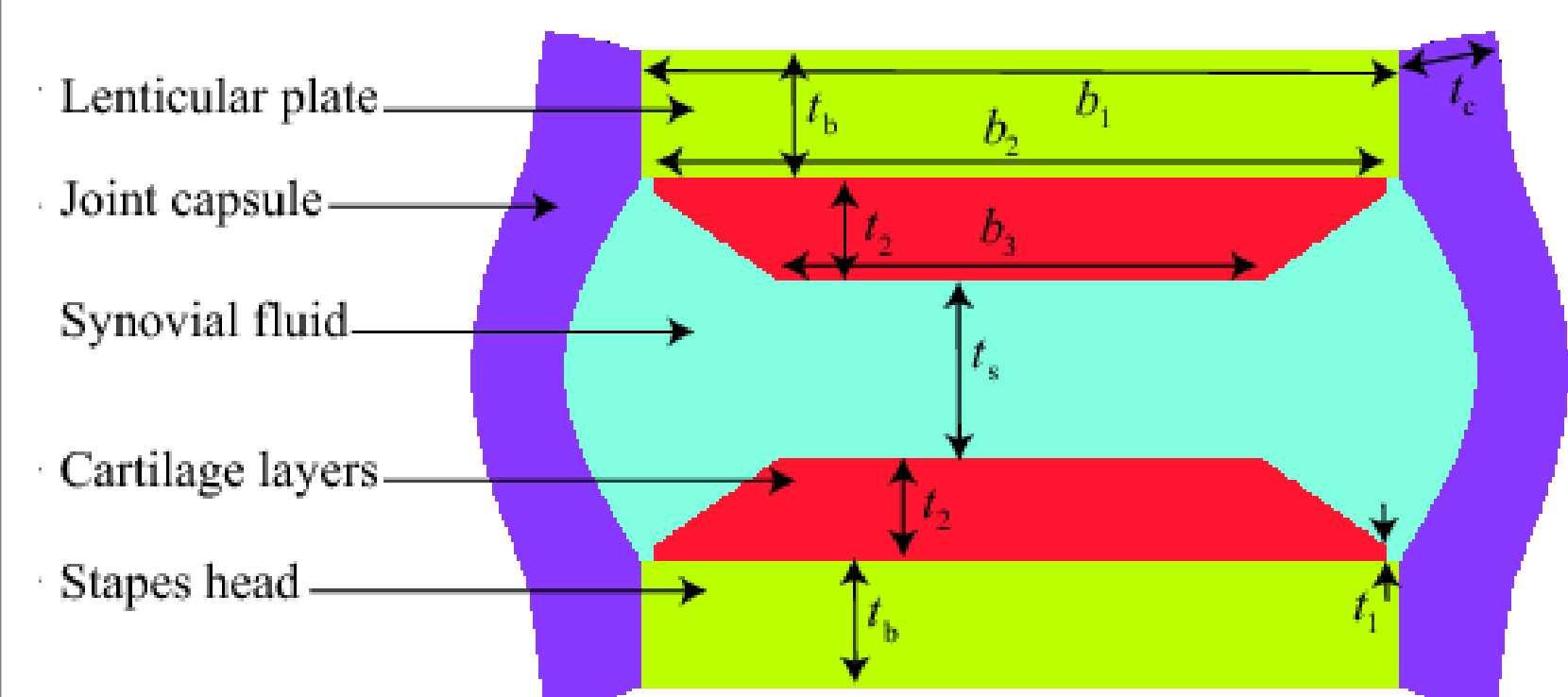
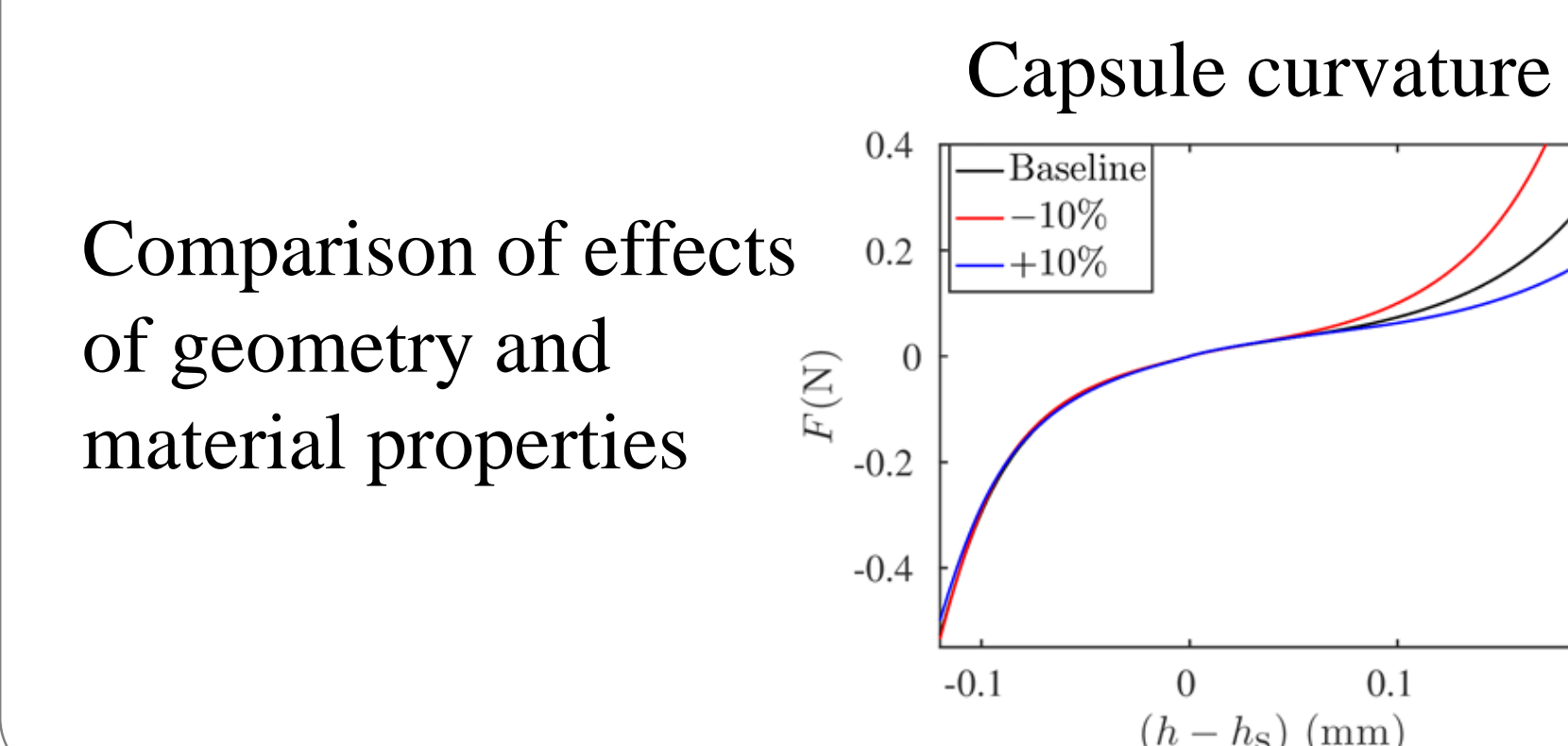


Introduction

Developing an accurate mechanical model of the behaviour of a biological system requires understanding of both the geometry and the material properties of its components. Gaining structural information about the incudostapedial joint (ISJ) will be valuable for developing more realistic models of middle-ear mechanics. There have been a number of anatomical and modelling studies of human, cat and gerbil ISJ's (e.g., Funnell et al., 2005, Karmody et al., 2009, Buytaert et al., 2011, Decraemer et al., 2015, Soleimani et al., 2018a,b). Simplified geometries have been used for modelling and we have found that the responses of models of the joint are very sensitive to its geometry. We are doing a post-mortem study of the anatomy of the joint in gerbils within three hours after sacrifice, using different imaging modalities: stereoscopic microscope, X-ray nanoCT, and light-sheet microscope.



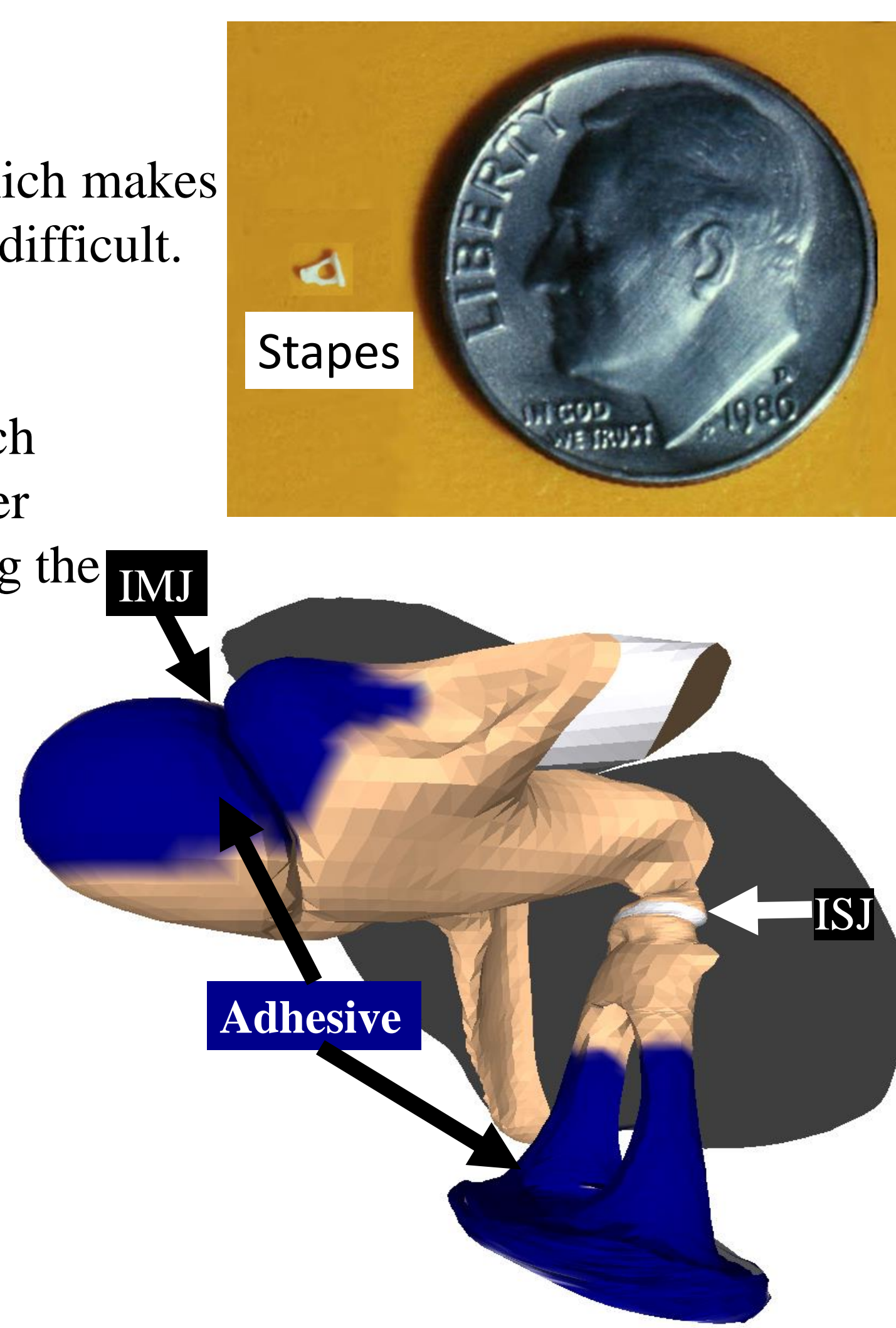
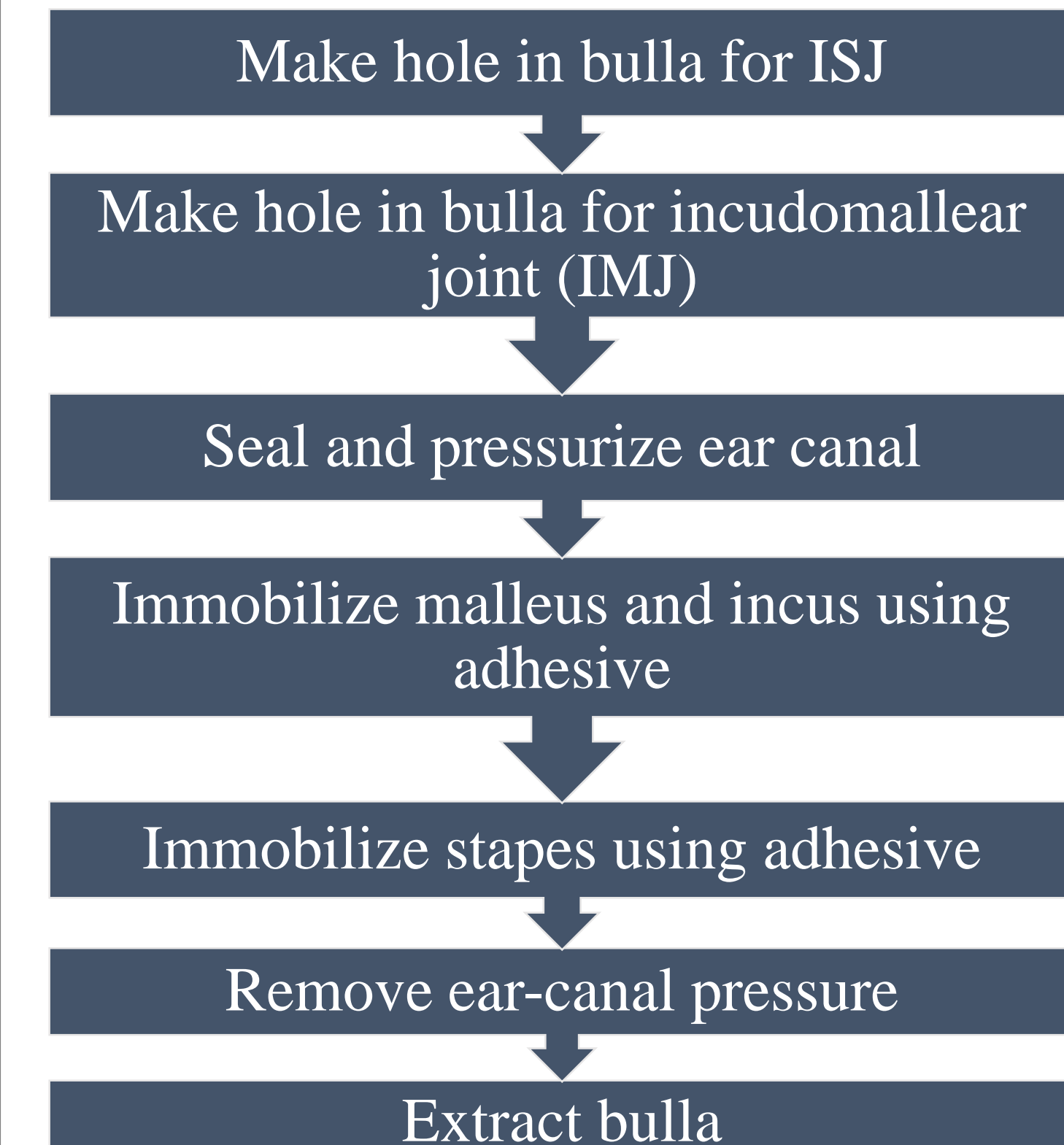
Human ISJ model of Soleimani et al. (ms. in preparation)



Comparison of effects of geometry and material properties

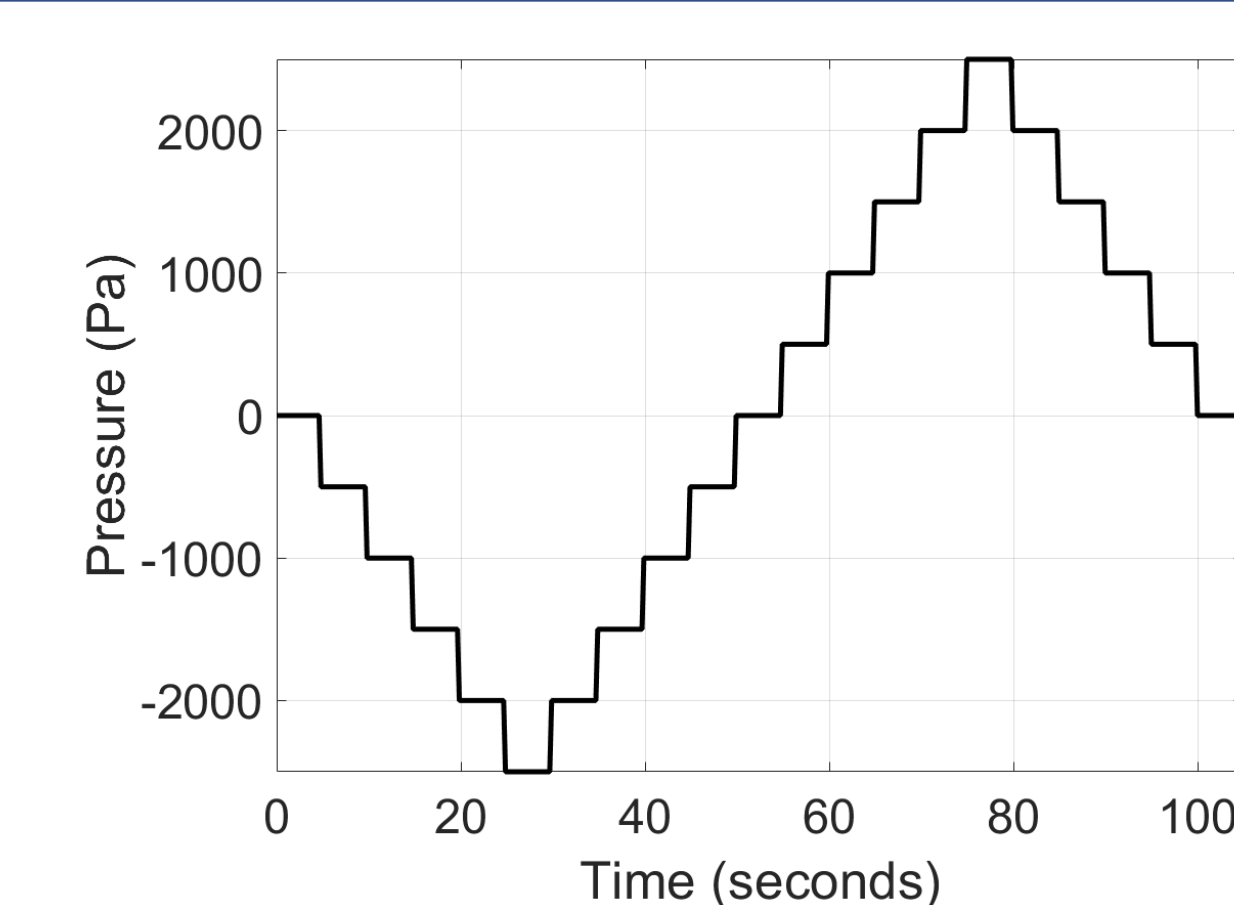
Methods: Auditory bulla dissection

- The joint is very small and delicate, which makes extraction and sample preparation very difficult.
- We developed a dissection method which preserves the geometry of the joint under different static pressures while preparing the sample for imaging



- Stapes, incus and malleus are attached to the walls of the bulla using All-Purpose Instant Crazy Glue.
- Changes of the joint are captured using a stereoscopic microscope while ear canal is being pressurized.

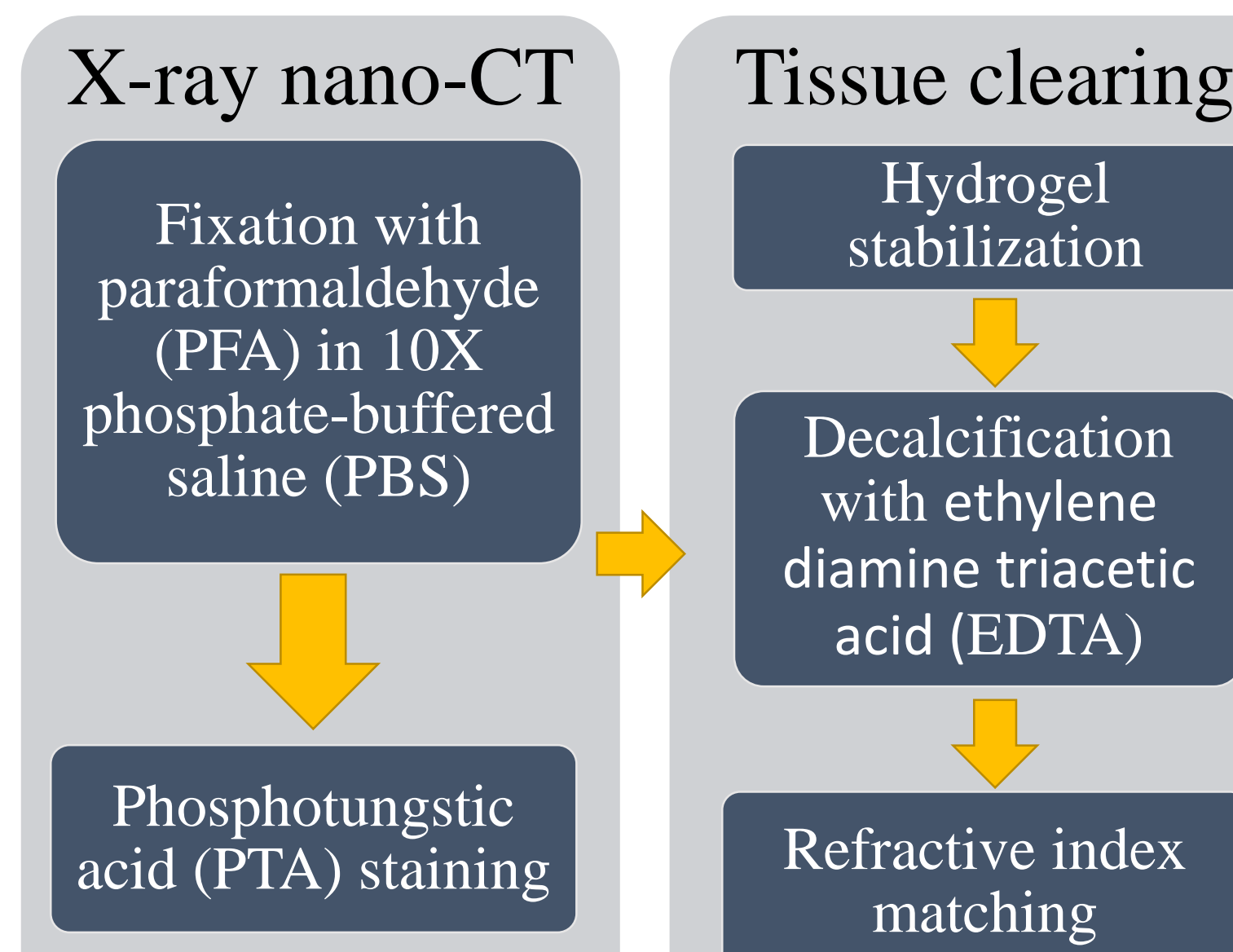
Methods: Pressurization



- Input pressures applied to ear canal (multiple cycles)
- Pressure steps of 500 Pa for duration of 5 seconds per step

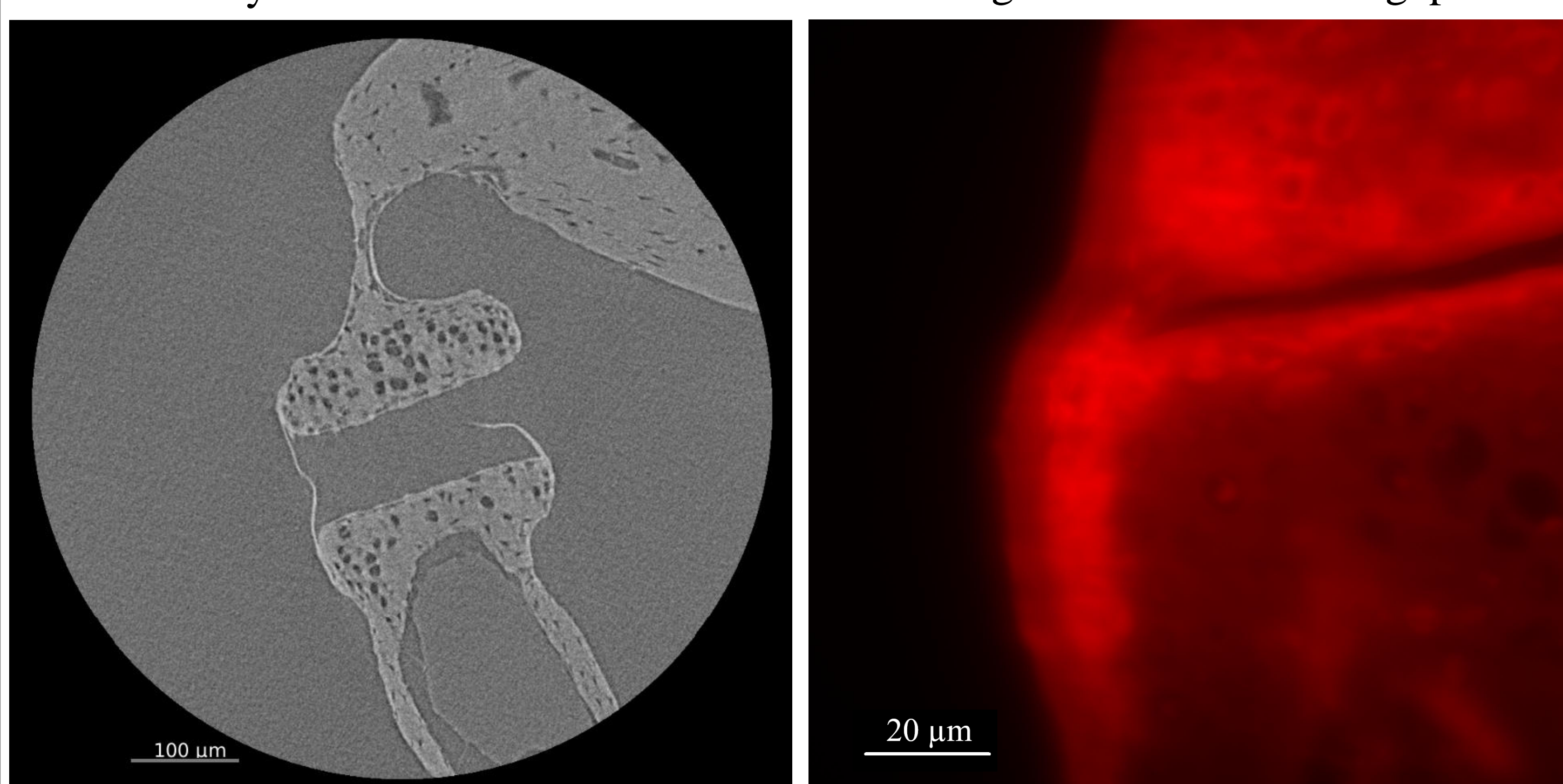
Methods: Sample preparation and imaging

- Different sample preparation methods are used to image various tissue types for each sample
- Zeiss Xradia 520 Versa X-ray nanoCT microscope and Zeiss Z.1 light-sheet microscope are used
- Process starts with preparing the dissected sample for nanoCT imaging, followed by tissue clearing for light-sheet microscopy

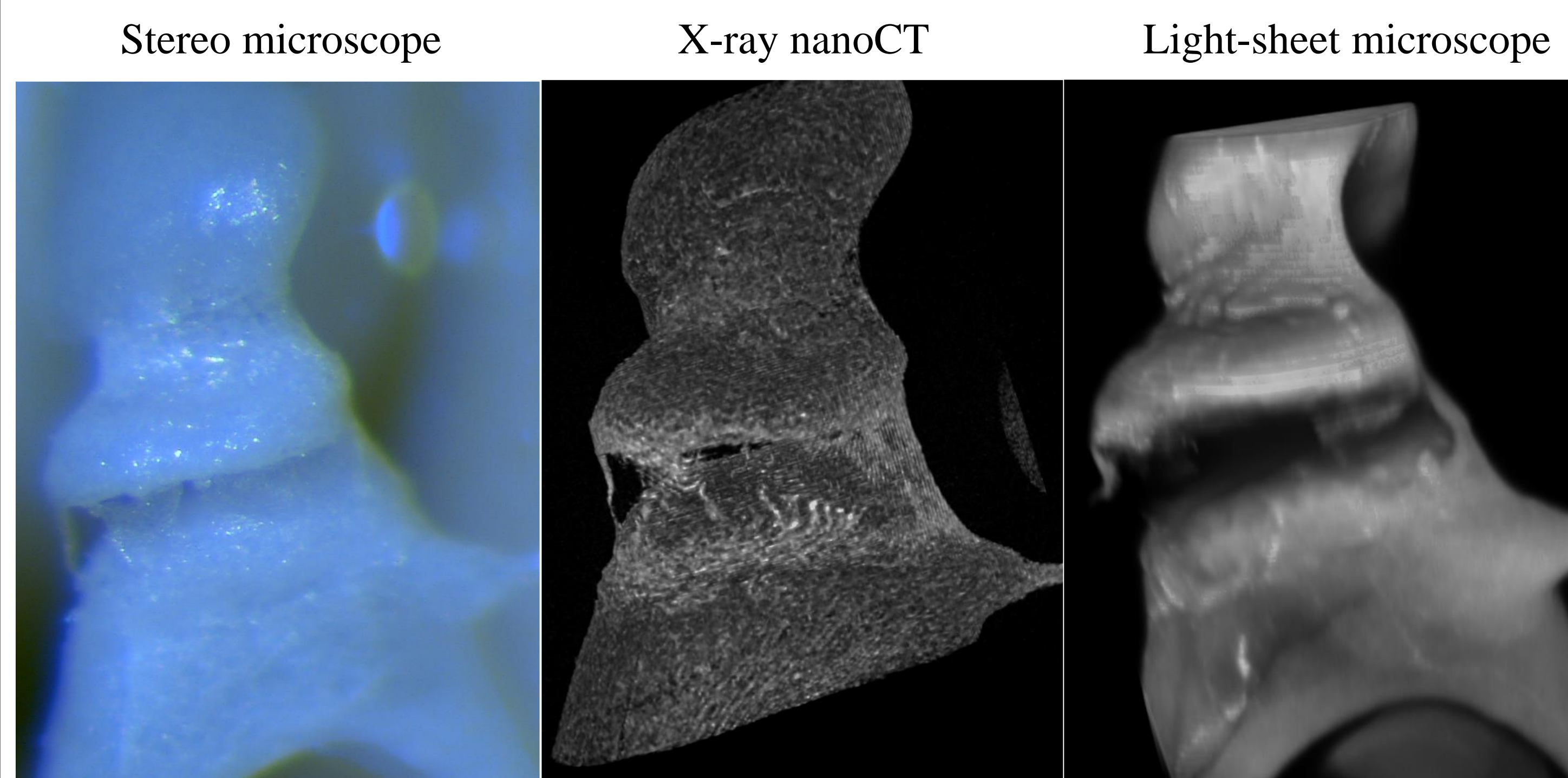


Results: Examples of different imaging modalities

- X-ray nanoCT of a broken
- Light-sheet scan of ISJ gap

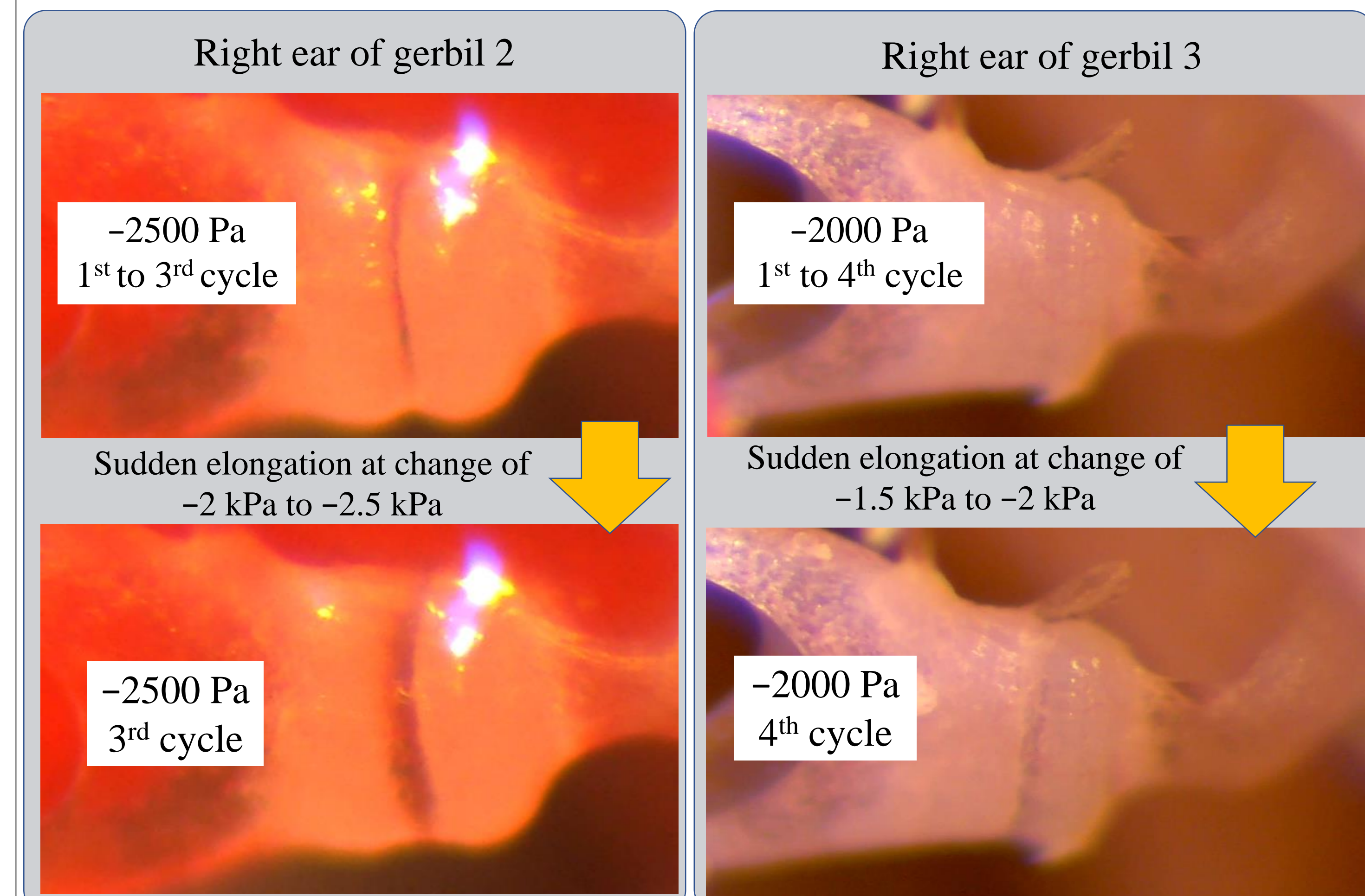


- 3-D viewing of broken ISJ using different imaging modalities

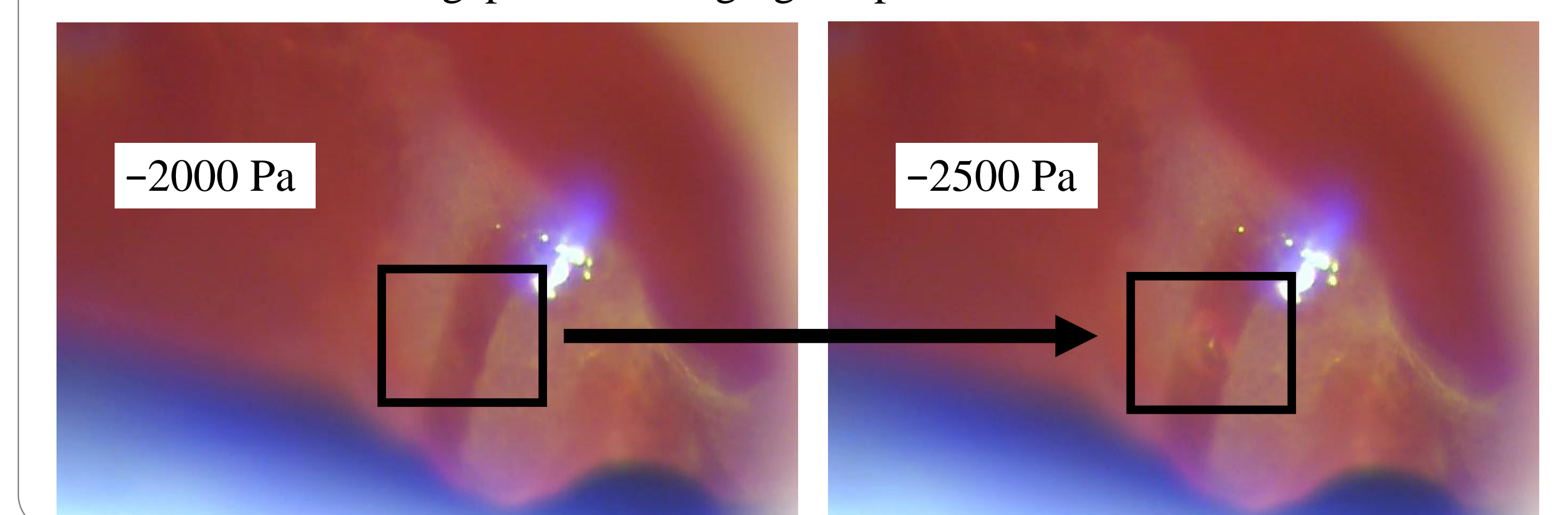


Results: Effect of pressurization

- After a few full pressure cycles, a sudden change in the ISJ gap was observed in three animals at high negative pressures (-2000 Pa and -2500 Pa) in which the gap length had a very high elongation.
- After the sudden change, behaviour of the joint was significantly altered in subsequent cycles.



- Continuing the pressurization after the sudden change caused the appearance of an air bubble in the ISJ gap when changing the pressure from -2000 Pa to -2500 Pa.



Conclusion

- We used different imaging modalities to obtain detailed information about diverse tissues in the ISJ
- The joint may be damaged after repeated pressurization cycles in post-mortem animals

Acknowledgements

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