MULTIMODAL IMAGING OF OPAQUE MUSCULOSKELETAL TISSUES

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The spectrum of musculoskeletal (MSK) injuries and diseases is large, increasing and affects people of all ages. Research into injury and disease mechanisms as well as treatment and prevention, on a cellular level can be limited due to the highly opaque nature of bone, cartilage and other MSK tissues. Visualising the micro and nanostructure of MSK tissues in 3D is nontrivial and often requires destructive and time-consuming processing that limits researchers reusing the sample. For instance, MicroCT allows for the physical microstructure, of radiopaque tissues (i.e. bone) to be discerned. This information can then be combined with biochemical assays or histological observations using standard lab-based and/or light microscopy techniques. Unfortunately, visible light does not penetrate these opaque samples well, and thus these samples cannot be restained to look at other targets.

Here we show how a recent advance in tissue clearing termed "passive clarity technique" (PACT) [1] yields a transparent bone sample suitable for imaging by confocal & light sheet microscopy, which provides structural and biological data from the same sample in a non-destructive and less time-consuming way . This allows for whole mount fluorescent staining and 3D imaging of intact bone samples using confocal & light sheet microscopy. After imaging PACT cleared samples, remain compatible with further histological sectioning/staining. Allowing additional targets to be investigated. Second harmonic generation (SHG) can also be used in combination with the above for label free visualisation of collagen, before and after clearing adding another dimension to the characterisation of the tissue.

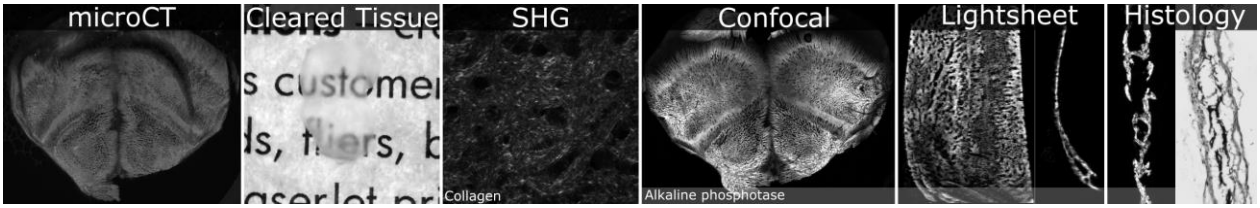

Figure 1: *Example data obtained from various imaging methods of a single P5 mouse calvaria.*

By reducing the high level of light scattering inherent in bone tissue, it is possible to visualise fluorescent targets within intact 3D samples. These same samples can then be further processed to investigate standard histochemical staining targets. MicroCT and SHG imaging then provides structural information. This multimodal approach to the analysis of MSK tissue samples greatly increases the information yielded from a single sample and will assist researchers to push the field of orthopaedic research forward.

[1] Treweek, J.B., et al., Whole-body tissue stabilization and selective extractions via tissue-hydrogel hybrids for high-resolution intact circuit mapping and phenotyping. Nature Protocols, 2015. 10: p. 1860. doi: 10.1038/nprot.2015.122