Towards Cellular Epidemiology of Degenerative Diseases Using Multibeam SEM and Machine Learning Approaches

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INTRODUCTION: The paucity of rapid throughput imaging technologies that allow for seamless bridging of structure-function relationships across length scales (10^{-2} to 10^{-9} m) has stymied the study of degenerative processes associated with ageing and disease. The combination of geospatial approaches using multibeam scanning electron microscopy (multiSEM) and the Google Maps JavaScript API, have enabled cutting-edge cellular connectomics studies of ageing human tissues, from bone to brain [1-4].

METHODS: As a proof-of-principle study to elucidate the relationship between bone and cell health in the human femoral neck with osteoarthritis (OA), the Google JavaScript API was used to enable labelling of landmarks on a navigable map (Figure 1). A blinded observer marked blood vessel edges, viable and pyknotic osteocytes. This coordinate-based mapping enabled testing of specific hypotheses related to bone health in terms of osteocyte viability, transport path distances, and network relationships. While initially adequate, this manual method is not conducive to the pipeline scalability necessary for the magnitude of current datasets (>10TB). Automated object detection algorithms such as the You Only Look Once (YOLO) neural network [5] facilitate rapid throughput diagnostic assessment of imaging datasets, also mitigating the effects of observer bias. Initially, YOLO was trained for automated osteocyte detection, using 629 annotated cells, which were further augmented to 10^6 examples through variation by rotation, scale and contrast. Unseen images were then processed with YOLO and automatically detected objects were identified by bounding boxes. Latest testing of the YOLO algorithm has proved successful in detecting osteocytes in a complete cross-section of the human femoral neck in <100hrs, and early hypotheses have begun testing.

RESULTS SECTION: Initially, interest in the relationship between the path distance between all osteocytes detected using the YOLO algorithm. Previously [2], manual pinning using the Google Maps JavaScript API had revealed a relationship between osteocytes and the nearest healthy osteocyte. This investigation was furthered to include the relationship from each osteocyte (whether healthy or pyknotic) to all others per sample. Results demonstrate strong homogeneous networks of cell connectivity in this sample, implicating cell network connectivity in osteocyte survival in OA. Compared to viable-viable osteocyte connections, viable-pyknotic connections were significantly increased, whilst pyknotic-pyknotic connections were significantly decreased (Figure 2).

DISCUSSION: These results demonstrate, for the first time to our knowledge, a highly significant relationship in proximity to a viable network of osteocytes in aged human femoral neck tissue. Combining multiSEM with new machine learning techniques enables large-scale network health assessment in mesoscopic tissue and ultimately organ sized samples. This paves a path forward for more implementation of network analysis tools, such as clustering and central node analysis. Future work mining the vast datasets acquired with multibeam SEM and rendered with Google Maps API will enable epidemiological investigations of cellular inhabitants within and between individual patient's tissues and organs, as well as cohorts of such patients. These datasets and resulting epidemiological insights are expected to spawn next generation theranostic as well as diagnostic device design.

SIGNIFICANCE/CLINICAL RELEVANCE: Findings of homogeneous cellular network differences and similarities across populations with OA may lead to discovery of the cellular epidemiology of OA as well as leading to the future design of diagnostic devices. Additionally, the methods used in this work may be applied for the use in other degenerative conditions, such as osteoporosis and Alzheimer's Disease.

REFERENCES: [1] Knothe Tate. Science 2017; [2] Knothe Tate *et al*. Adv. Healthcare Mat. 5, 1840-1843, 2017; [3] Pereira *et al*. PLoS Comp. Biol. 12, 1-18, 2017; [4] Eberle *et al*. J. Microscopy 259, 114-120, 2015; [5] Redmon *et al*. Proc IEEE Conference Computer Vision Pattern Recognition 2015

IMAGES AND TABLES:

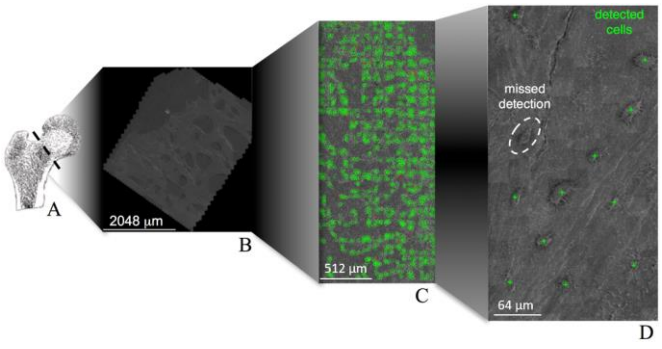

*Figure 1. Pipeline utilizing a trained neural network for automatic osteocyte detection in human femoral neck tissue from patients undergoing hip arthroplasty (**A**). This largest-to-date imaged multiSEM dataset (**B**) comprises 11 TB of images acquired with the 61-beam Zeiss MultiSEM 505, allowing for automatic detection of over 200,000 osteocytes (**C,D**).*

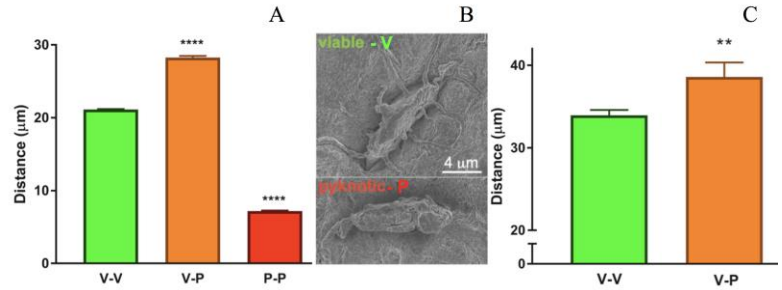

*Figure 2. Machine learning algorithms enable automatic detection of cells and their health states. This scales up to enable rapid throughput detection of cells and testing of significant relationships among cells, i.e. cellular epidemiological studies of mesoscopic tissue and organ samples. (**A,B**) Mean distance to the nearest osteocyte is depicted for viable - viable (V-V) osteocyte connections (n=158,905), viable-to-pyknotic connections (V-P, n=12,986) and pyknotic-to-pyknotic (P-P) connections (n=34,288). Cells were identified and categorised using the trained YOLO neural network. Kruskal-Wallis with Dunn's multiple comparisons test was performed. (**B,C**) The same analysis was performed on a previous, manually pinned data set, comparing healthy-to-healthy connections (n=619) and healthy-to-pyknotic connections (n=89). Mann-Whitney t-test was performed. **p<0.005, ****p<0.0001 compared to healthy-healthy connections. Data are expressed as mean ± SEM.*