# Examining the Bone Marrow Niche in a Fracture Healing Model with the Use of Multiplex Imaging and Transcriptomics Technologies

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**Background:** In the US, 6.3 million fractures occur annually. Additionally, 5-10% of fractures do not heal without additional interventions. The bone microenvironment is comprised of cells such as osteoblasts (OBs), megakaryocytes (MKs), and endothelial cells (ECs). Traditional technologies, such as flow cytometry, immunofluorescence (IF), and qPCR have limitations that prevent studying the bone microenvironment as a whole.

**Project Methods:** Some of the advanced multiplexed technologies that can be useful tools in studying a complex microenvironment such as bone are the PhenoCycler™, previously known as CODEX (CO-Detection by indEXing) and Nanostring nCounter which use fluorescent probe hybridization to either visualize the tissue or quantify gene expression, respectively. Phenocycler addresses the limitations of IF by imaging up to 60 cell markers, thus, allowing for better identification of cells within the bone microenvironment. This complex information can then be used for image analysis with the HALO image analysis software, providing the spatial context as well as functional aspects of cell interactions during homeostatic, disease, and injury states. Nanostring nCounter maps out hundreds of genetic pathways without requiring amplification or the need to convert mRNA to cDNA. This process is highly reproducible and decreases variability.

**Results:** In the current study, we are standardizing and optimizing protocols for both of these technologies for bone which are more difficult to process than soft tissues. Regarding Nanostring, samples have been submitted to the SNRI Biomarker Core and are awaiting processing.

**Potential Impact:** We anticipate that both the multiplexed technologies will allow us to determine which interconnected pathways, such as angiogenesis, inflammation, and immune response are differentially regulated during normal fracture repair, repair using new therapies, and repair in aged or diseased animal models.