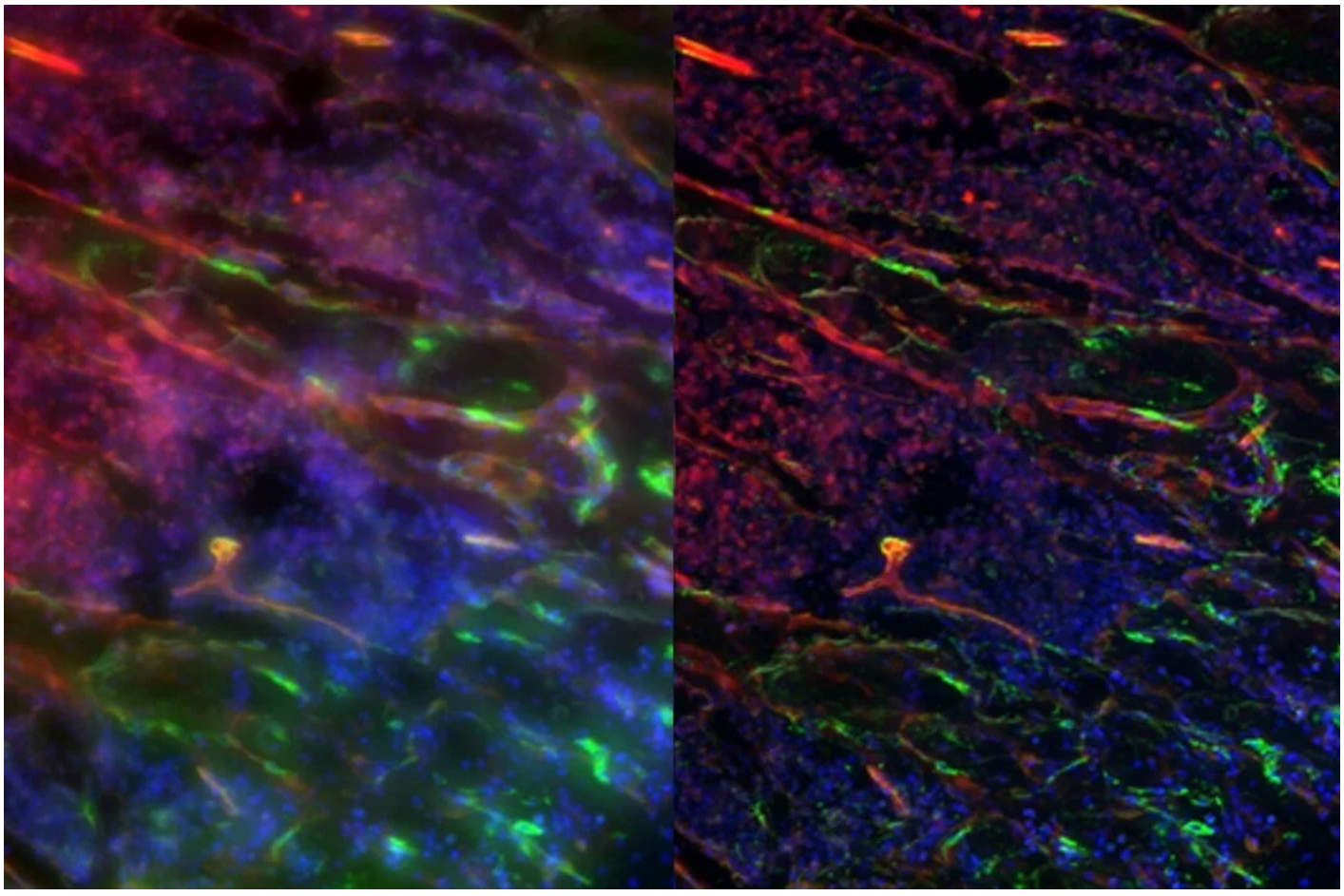
From Eye to Insight

Leica MICROSYSTEMS

# LOCALIZING BONE STEM CELLS IN VIVO

## Rapid, high-contrast optical imaging of undecalcified mouse bone




Authors

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**Abstract**

This article demonstrates how undecalcified mouse bone samples can be investigated in detail more easily and rapidly with a cryohistological method used in combination with a THUNDER Imager Tissue and Instant Computational Clearing (ICC). The goal is to identify osteoprogenitor stem cells in their native microenvironment. Dysfunction of these bone stem cells can result in various bone diseases. Compared to conventional widefield microscopy, THUNDER imaging provides sharper images without the out-of-focus blur or haze, while maintaining high-throughput imaging.

**Introduction**

The structures that form the skeleton arise very early in embryonic development, continue to grow during childhood, and are actively remodeled throughout our lives. Mesenchymal stem cells (MSCs) derive from the embryonic mesoderm and give rise to the connective tissues throughout the body. Lineage restricted derivatives of these cells persist through post-natal growth, and function in maintenance and repair of connective tissues throughout our life span. Whether these MSC's persist post-natally as truly, multipotent 'stem cells' is unresolved due to a lack of sufficiently specific molecular markers, which identify these cells in situ. There is a need to better understand how these MSC develop into bone forming osteoblasts and how they interact with other cells in their microenvironment, including bone resorbing osteoclasts, hematopoietic and vascular cells. These interactions, along with input from systemic endocrine stimuli, collaboratively regulate bone integrity. Perturbation of MSC maturation and signaling can lead to a variety of structural and metabolic bone diseases, such as osteoporosis, and can result in fractures. Greater understanding of MSC biology and their role in the bone microenvironment may translate to new strategies to prevent or correct bone disease.

Common histological methods used for studying musculoskeletal tissues are laborious and can require weeks to fix, decalcify, section, stain, and image the specimen. To achieve efficient and high-throughput analysis of a variety of molecular signals within a bone specimen, there is a need for improved histological techniques that can associate multiple molecular signals to specific cells. The results from a rapid cryohistological method for preparing mineralized, undecalcified mouse bone sections [1] followed by rapid high-contrast THUNDER imaging of the sections are shown in this article.

**Challenges**

When imaging undecalcified bone samples, a solution that can quickly image them and achieve sharp, high-contrast 3D imaging, where important details are clearly resolved, is most practical. Conventional widefield microscopy is fast and offers detection sensitivity, but unfortunately images of thick specimens often show an out-of-focus blur or haze which reduces the contrast [2].

**Methods**

Undecalcified bone sections from a LepRCre x Rosa26 mT/mG mouse expressing GFP and tdTomato [3] labeling osteoprogenitor MSC were prepared according to the protocol of Dyment et al. [1] and stained with Hoechst 33342 to indicate DNA. A 38 µm Z-stack of images was acquired with a THUNDER Imager Tissue using a 25× / 0.95 NA (numerical aperture) objective. Instant computational clearing (ICC) [2] was applied to the image stack followed by a maximum intensity projection.

**Results**

The THUNDER technology enables visualization of more details of the undecalcified bone by the removal of out-of-focus light from the image when compared to imaging with conventional widefield microscopy.

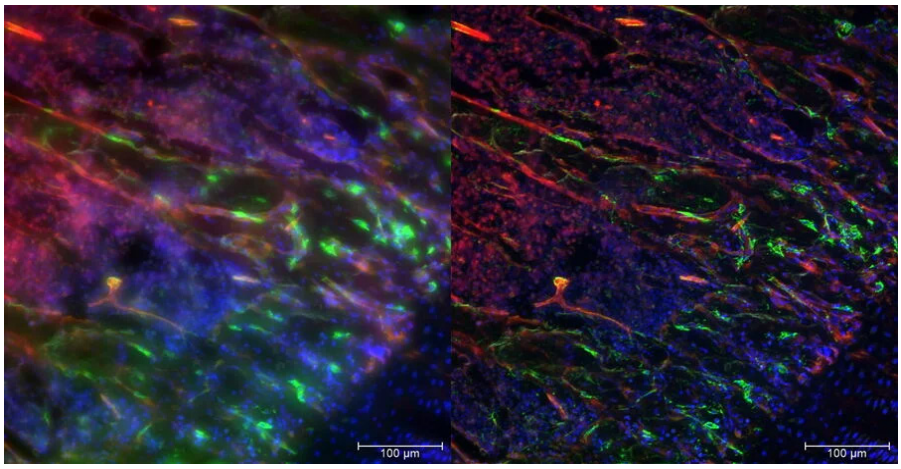


Fig. 1: Maximum intensity projection of undecalcified mouse bone tissue expressing GFP (green) and tdTomato (red) and stained with Hoechst 33342 (blue). Imaged using a THUNDER Imager Tissue: A) raw data and B) with ICC. Scale bar is 100 µm.

## Conclusions

The THUNDER technology Instant Computational Clearing (ICC) [2] significantly enhances the contrast when imaging undecalcified mouse bone compared to conventional widefield microscopy, allowing more details to be resolved rapidly. It may help researchers gain more insights into intercellular signaling in bone.

## References


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