

Fluorescence Microscope

For Highly Targeted Test Placement Using Fluorescence

Nanoscale materials characterization inherently requires high-precision positioning capabilities to ensure areas of interest are accurately identified, targeted and subsequently tested. Nanomechanical test positioning using Hysitron's industry-leading instrumentation suite, highlighted by its patented *in situ* SPM imaging capability, has been further enhanced with the addition of a **Fluorescence Microscope** option for the **TI Series TriboIndenter®**.

Fluorescence microscopy is a commonly utilized technique in many areas of research due to enhanced imaging capabilities that are not available with other traditional optical microscopy modes. Hysitron's Fluorescence Microscope enables fluorescing microscopic structures to become clearly visible against the non-fluorescing background, enabling the testing of highly specific regions of inhomogeneous materials and hierarchical structures that are not identifiable using standard imaging techniques. Fluorescence microscopy can be used in conjunction with any material that absorbs excitation light at a given wavelength and emits light at a longer wavelength. The fundamental goal is to bind fluorophores or fluorochromes to molecular structures of interest, such that the spatial distribution of these molecules can be accurately identified and tested.

Hysitron's Fluorescence Microscope option replaces the standard optics column in the **TriboIndenter Series** of nanomechanical test instruments and is capable of performing both standard bright-field and fluorescence imaging. A high intensity and broad-spectrum light source, high-sensitivity camera, and easily interchangeable filter sets provide the ability to reliably excite and image a wide range of fluorescing materials. Conventional nanomechanical test instruments require fluorescence imaging and nanomechanical testing to be performed on separate platforms. This approach makes it nearly impossible and extremely time consuming to accurately place tests on micron-scale regions of interest. Coupling fluorescence with nanoindentation circumvents need

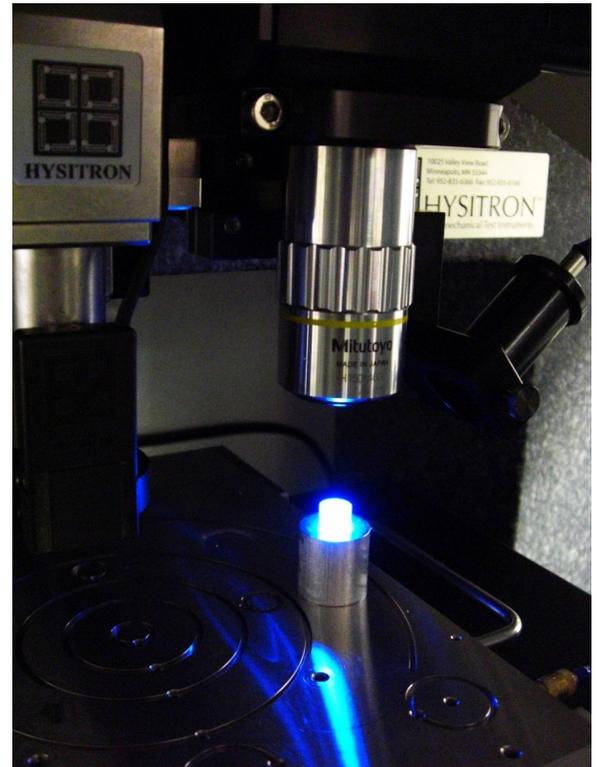


Figure 1: Fluorescence Microscope option inside the **TI 950 TriboIndenter®**.

for image registration or reliance on fiducial markers for indent placement, while ensuring fast, reliable, and unparalleled test positioning accuracy on the most complex material systems. State-of-the-art staging combined with *in situ* SPM imaging ensures accurate and repeatable test placement on areas marked with fluorochromes.

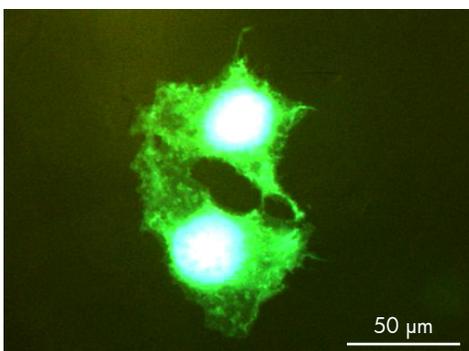
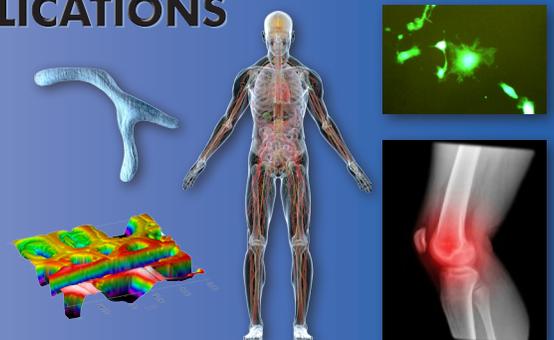


Figure 2: Cells derived from African green monkey kidney transfected by GFP. Fluorescence image was taken at blue excitation light and green emission.

EXAMPLE APPLICATIONS

- Bone, Teeth
- Cartilage, Tissues
- Microorganisms
- Wood Adhesives
- Pharmaceuticals



EXAMPLE APPLICATION

Monitoring Bone Tissue Age and Mechanical Properties in Mice

Discerning bone tissue properties based on age or treatment is important for understanding the effects on bone quality. Figures 3 and 4 show fluorescence and bright field images of a cross-sectioned mouse femur, respectively. The mouse was injected with calcein labels to mark the progression of bone mineralization as a function of time. Visualizing formation markers during nanoindentation provides a direct means of measuring tissue properties as a function of age and treatment.

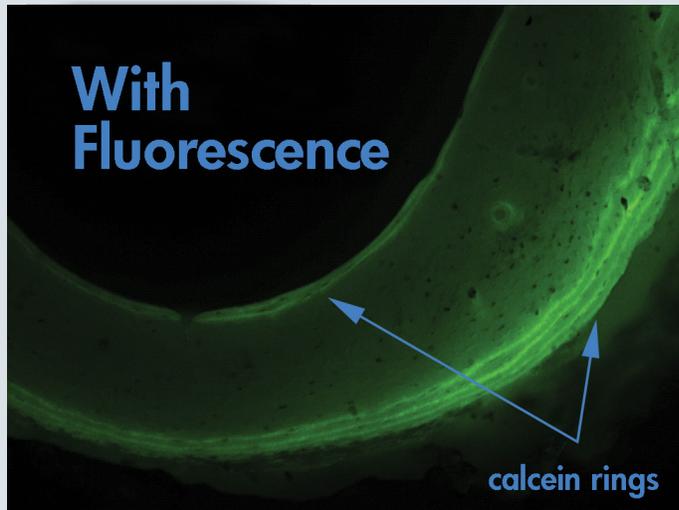


Figure 3: Fluorescence image of a mouse femur cross-section injected with calcein fluorochrome to monitor tissue age. The fluorescent rings present in the image correspond to areas of recent and older tissue deposition.

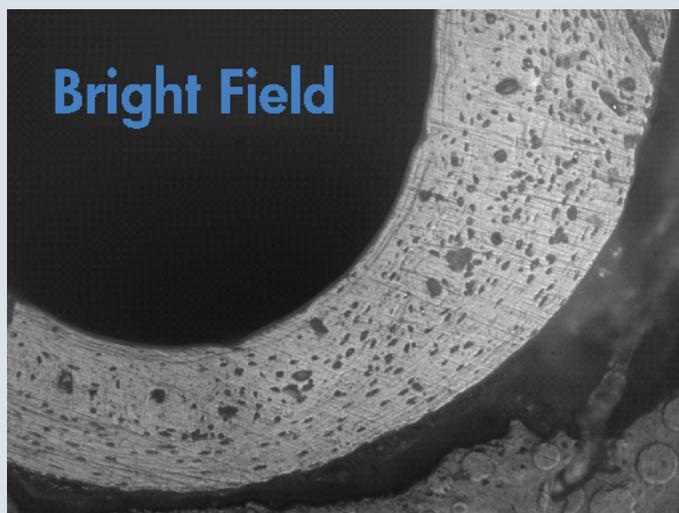


Figure 4: Bright field image of mouse femur shown in Figure 3 above. Notice the rings marked with calcein fluorochrome cannot be identified using bright field image microscopy.

Specimens prepared by Dr. Ken Kozloff, University of Michigan, Department of Orthopaedic Surgery.

HIGHLIGHTS

- Provides the ability to reliably and seamlessly position nanomechanical tests on specific areas of the sample that have been identified using fluorescence imaging
- Accurately identify specific components of inhomogeneous and hierarchical structures for testing
- Available on all new and existing **TI 900** and **TI 950 TriboIndenter** systems
- Capable of both fluorescence and bright field microscopy
- Easily interchangeable filter sets to accommodate a wide range of fluorochromes
- High-resolution color CCD camera
- Broad spectrum light source with high excitation intensity
- Compatible with all Hysitron testing techniques
- Able to resolve $\sim 1 \mu\text{m}$ features
- Replaces optical microscope color camera system in base **TI Series TriboIndenter** system
- Provides the ability to precisely measure tissue properties as a function of age and/or treatment