Center for Musculoskeletal Research

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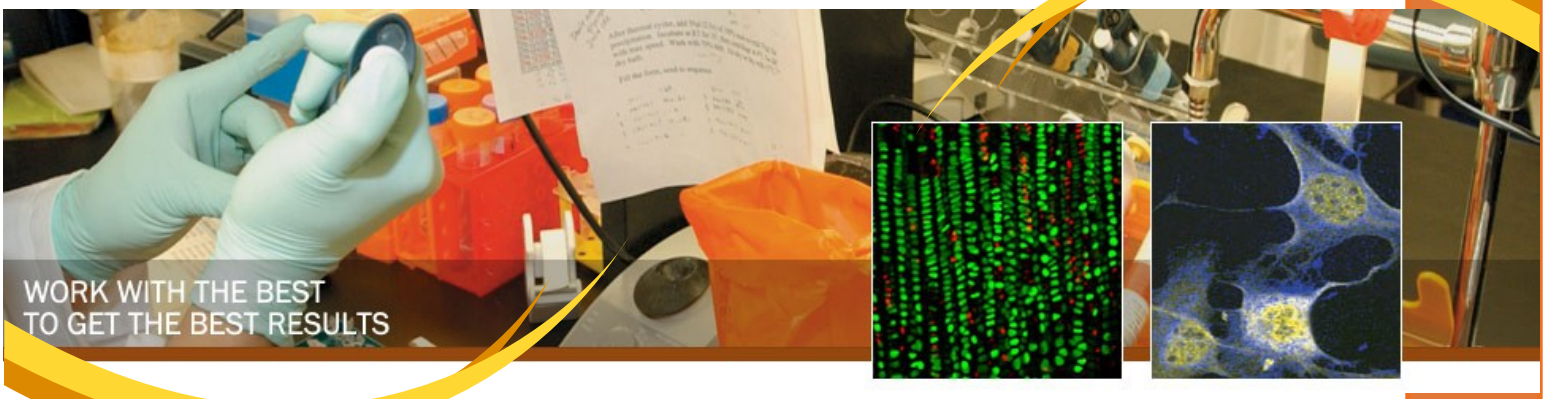
http://musculoskeletalcore.wustl.edu/



In Situ Molecular Analysis

Lis DeLassus and John Freeman coordinate the fluorescence microscopy with deconvolution system.

WHY DO YOU NEED THE DECONVOLUTION PROCEDURE?

When acquiring fluorescence images with a standard fluorescence microscope, the entire field of view is illuminated with exciting light, and fluorescence images are acquired (at different light wavelengths) thru the appropriate dichroic filters. However, each fluorescing point in the sample acts as a light source which can scatter light into neighboring fluorescing points thus blurring the image. This, in effect, creates a "haze" in the recorded fluorescence image (Figure 1). The "haze" must be removed from the fluorescence image .

HIGH RESOLUTION, Z-STACK IMAGES

We obtain fluorescence images with multiple fluorophores at different focal planes within the sample with our Nikon E800, upright fluorescence microscope. This microscope has a computer controlled imaging camera (Retiga 2000R) and a Prior digital Z drive for varying the height of the focal plane within the sample. For both data collection and deconvolution of the fluorescence images, we use Metamorph software (64 bit).

Use of the system can be arranged by contacting John Freeman at freeman@wudosis.wustl.edu

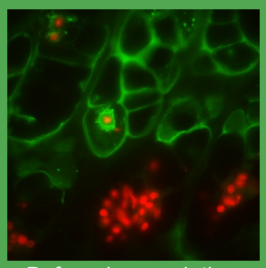
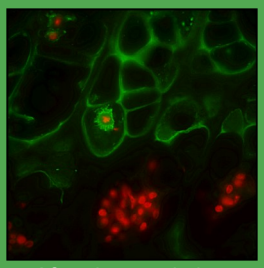

Before deconvolution

Figure 1
***Rabbit growth plate*: Image acquired with a 60 X oil immersion using both FITC stain (green frame) and DAPI stain for the nucleus. The DAPI image collected with 365 nm UV excitation/ blue emission BUT the blue is re-colored RED for enhanced visual contrast.**


After deconvolution

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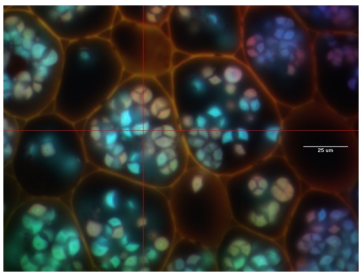
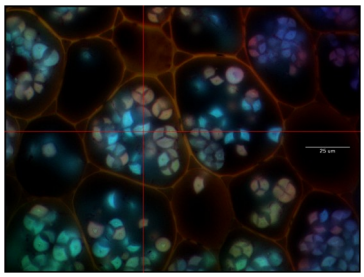
For more information on the Cores, please click on the links below:
Core A—Administrative Core
Core B—Structure and Strength Core
Core C—In Situ Molecular Analysis Core
Core D—Mouse Genetics Models Core

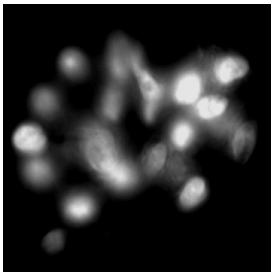
Remember to include reference to support from the Center in your abstracts and publications. **Cite Grant # P30AR057235** from the National Institute Of Arthritis And Musculoskeletal And Skin Diseases.

In Situ Molecular Analysis (cont.)

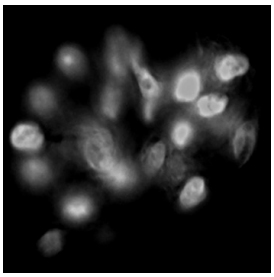


Z stack, 60X 4 um Z intervals
3 flurophors including UV/Blue
Orthogonal Cross Sections for
interior of cells



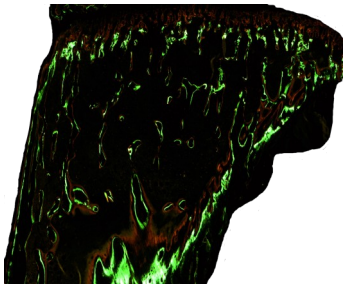


High magnification images of
DAPI stained nuclei-blue image
colored grey for better
definition of detail



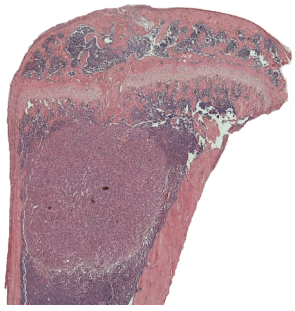
Core News

Core C– In situ Molecular Analysis



During the next billing cycle, your bills for histology services will actually go DOWN! Higher efficiency has allowed us to decrease the price of many services, most notable the special stains (now ½ price). As many of you have discovered, Crystal is a true expert, and her stains are the most beautiful on campus. Although you may be able to do some of the staining in your own lab, the cost in time and materials for troubleshooting the procedures is likely much greater than our charges. The advantage of having a well utilized, well equipped, well supported core is that you can focus on interpreting your slides, not producing them, saving time and money.

The histology lab in core C is also planning an expansion. As we work towards a fully free-standing facility in the new building next year, we are also looking for a second histology technician to join Crystal, and the opening has now been posted in HR. The additional manpower should allow us to expand our services, including immunohistochemistry, and further improve the turnaround time on your projects.



As always, you can contact me with comments or questions Deborah Novack: (novack@wustl.edu).

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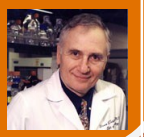

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