Issue 2 – June 2025

THE VOXEL

SPEAKING OF MICROSCOPY AT UMSOM

https://www.medschool.umaryland.edu/cibr/core/confocal

IN THIS NEWSLETTER...

Welcome to The Voxel, Issue 2!

Light microscopy continues as a central technology enabling critical discoveries in basic, translational, and clinical science. To aid the large and growing microscopy user community at UMB, we have established this newsletter, The Voxel. We hope it will help keep our researchers informed about the state-of-the-art microscopes on campus and highlight new developments in microscopy that we believe will impact research here. The Voxel will also be a forum to feature the exciting discoveries of microscopists across UMB and get researchers in touch with others with helpful technical expertise.

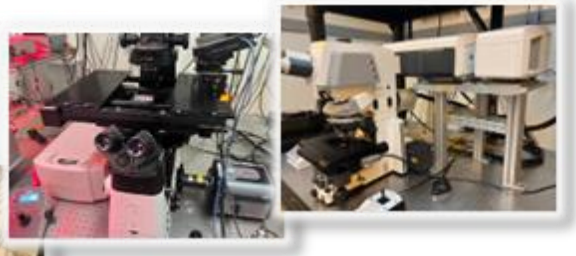
The Confocal Core is excited to announce the launch of **Image Analysis for Life Scientists**, a series of recurring classes. Starting in **Fall 2025**, these sessions are designed to equip researchers and students in the life sciences with practical skills to analyze microscopy and other biological imaging data using modern tools and techniques. The class will cover topics such as image segmentation, quantification, colocalization, automation, visualization and rendering. The classes will provide hands-on experience with widely-used software like ImageJ and Imaris. The workflows will be tailored for biological applications. Whether you're new to image analysis or looking to broaden your skills, these classes offer an accessible and supportive learning environment. Stay tuned for the detailed schedule and registration details! *Do you have a topic you'd like to see presented in a class or workshop format? Let us know!* sdkumar@som.umaryland.edu

NEW MICROSCOPES to come online SOON!

Your days of difficulty finding time on your favorite Confocal Core microscope are over:
We have THREE MICROSCOPE SYSTEMS coming to the Core!

Three systems

- Nikon X1 spinning disk + A1R confocal
- Zeiss LSM 880 upright Airyscan confocal
- Nikon N-STORM + A1R confocal

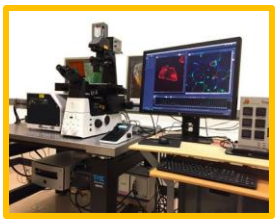


Our big thanks to Dr. Jon Lederer, Professor of Pharmacology and Physiology, for transitioning these systems to the Core.

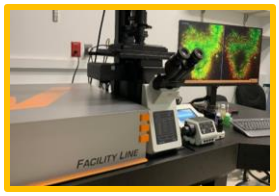
Key features

- Increased capacity, especially spinning disk
- Two new forms of super-resolution imaging
- 4th floor of Howard Hall—coming to iLab soon!

News on Pricing and Updates for Hardware and Software



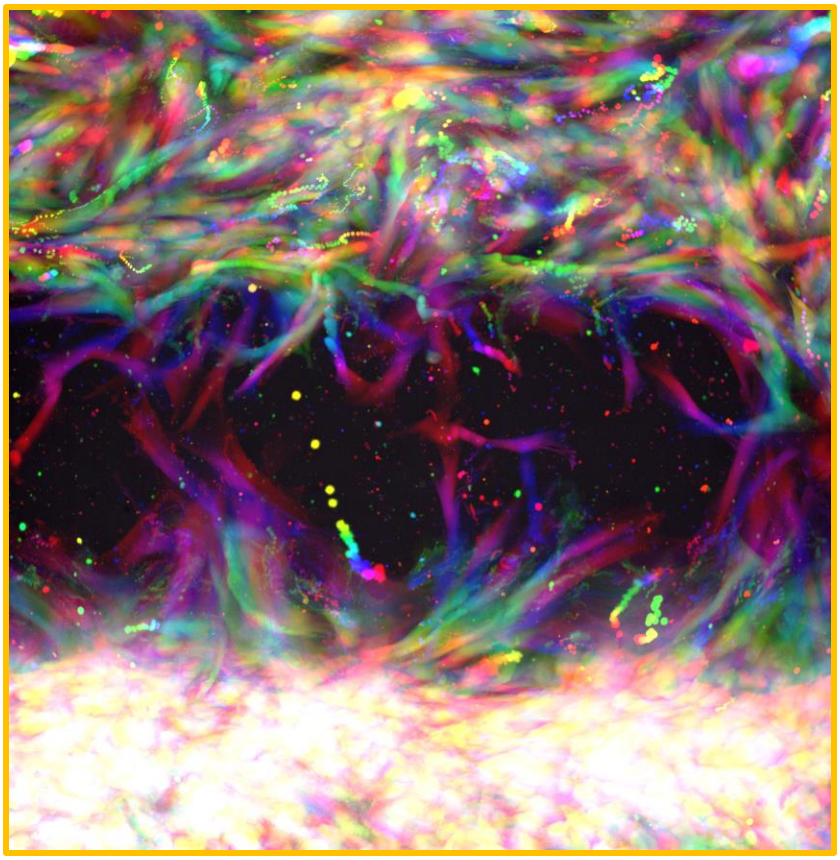
The Nikon W1 spinning disk has now received key updates to the software and a graphics card. These upgrades have exponentially increased the processing speed of W1, for both acquisition and for computationally extensive processes like denoising and deconvolution. To take advantage of these new game-changing capabilities, reach out to us right away!



The hourly price for the **Abberior STED Microscope** has been adjusted to $60/ hour ($30 off-hours) to accommodate the investment we've made to get this system on campus. To get your **super resolution** experiments started on the STED and other microscopes, please visit: CIBR Confocal Core iLab webpage

The Bitplane Imaris license has now been upgraded to version 10.2. This version provides higher 3D rendering speeds and improved AI segmentation modules with pixel classification. Book your time on **iLab** to explore Bitplane Imaris 10.2 and try it out with your dataset. The software now costs $10 per hour on weekdays between 9am – 6pm and $5 off-hours and weekends. **We can help train and get you started using Bitplane.**

Image of the Month: Light Paint Microscopy



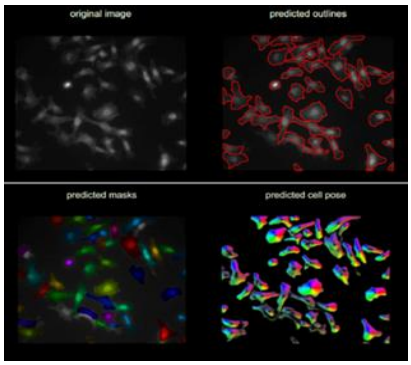
This image depicts the migration patterns of fibroblasts visualized over 24 hours via live-cell microscopy (spinning disk). Each time point was assigned a unique color along the visible spectrum and then projected into a single image, creating a similar effect to long exposure techniques used in time lapse photography.

Credit: Beth Pattie, PhD Candidate in Phil Iffland's lab Program in Neuroscience

Have an image to submit for the Image of the Month? Submit to jmauban@som.umaryland.edu!

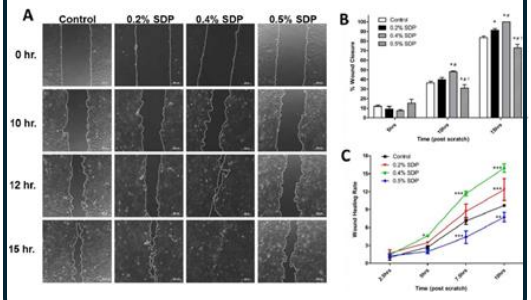
DO YOU HAVE DATA READY FOR ANALYSIS AND QUANTIFICATION?

Brightfield/ Fluorescence image segmentation



Time-lapse microscopy: Wound-healing assays, object tracking

https://doi.org/10.1167/iovs.16-19957



Colocalization analysis

https://doi.org/10.3390/biom12030456

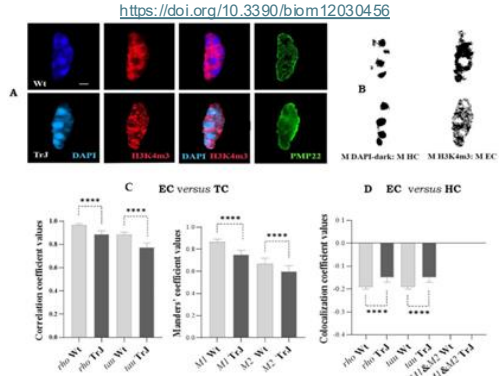
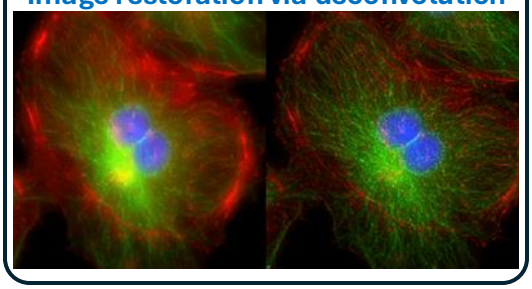
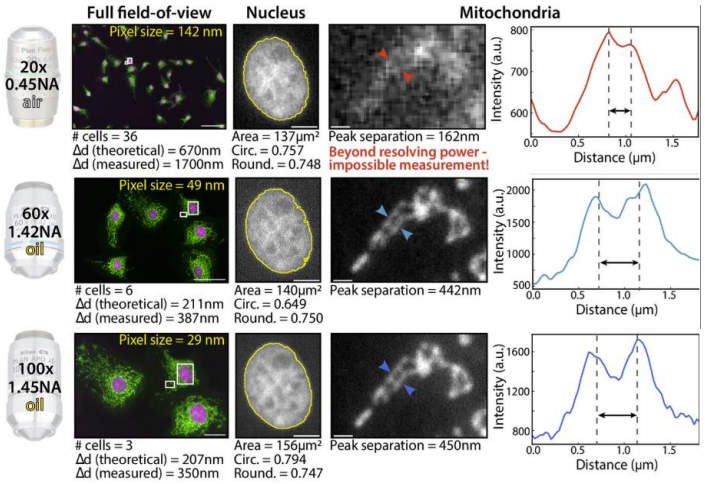


Image restoration via deconvolution



Cell count and morphological measurements

https://doi.org/10.1111/jmi.13208



CIBR Confocal Core offers comprehensive image analysis services to support researchers acquiring data on our advanced imaging systems. Whether you're conducting confocal, widefield, lightsheet or super-resolution microscopy, we can help in quantifying fluorescence intensity, colocalization, morphological measurements, 3D reconstructions, time-lapse analysis, and more.

We provide state-of-the-art commercial software tools such as

- **Bitplane Imaris,**
- **Nikon Elements,**
- **Zeiss Arivis**
- **Thermofisher Scientific Amira.**

We also provide assistance with custom open-source software for high-throughput or specialized analyses.

- **ImageJ/ Fiji,**
- **CellProfiler**
- **Cellpose etc.**

From raw image processing to statistical interpretation, we ensure your imaging data is accurately and efficiently translated into meaningful biological insights.

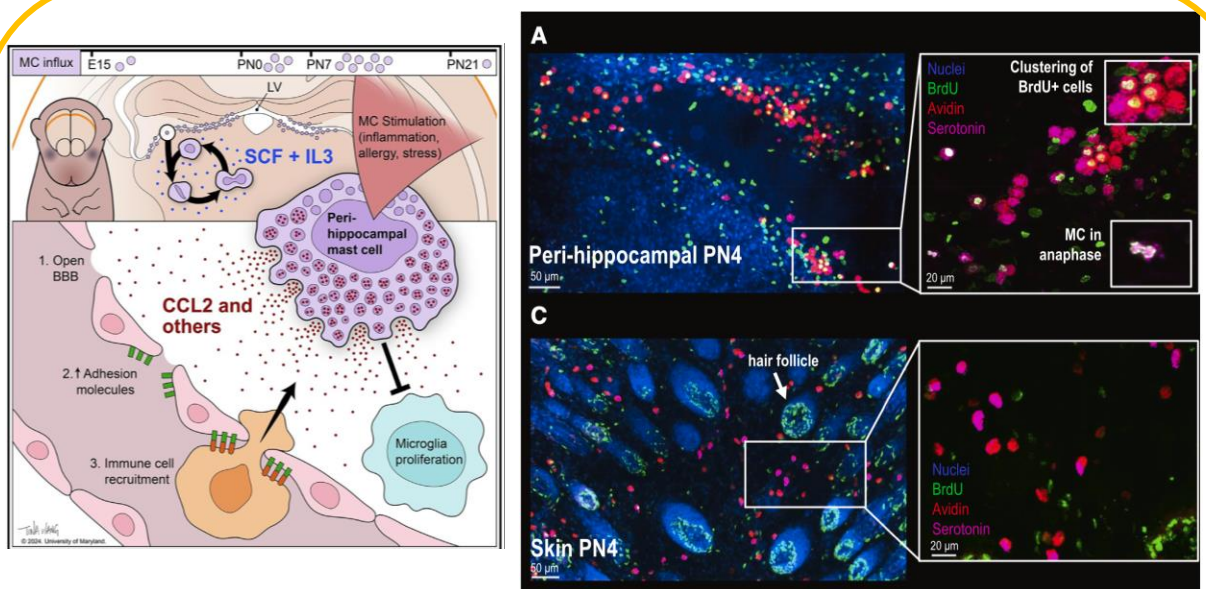
If you have data that is ready for analysis, do not wait! Please contact Joseph Mauban or Shilpa Dilip Kumar for any analysis questions! (jmauban@som.umaryland.edu), or sdkumar@som.umaryland.edu)

Featured Publication from Confocal Core Users

Mast cells proliferate in the peri-hippocampal space during early development and modulate local and peripheral immune cells

Alexa C. Blanchard, Anna Maximova, Taylor Phillips-Jones, Matthew R. Bruce, Pavlos Anastasiadis, Christie V. Dionisos, Kaliroi Engel, Erin Reinl, Aidan Pham, Sonia Malaiya, Nevil Singh, Seth Ament, and **Margaret M. McCarthy.**

Developmental Cell, 24 March 2025, Vol 60: 6, 853-870.e7. DOI: 10.1016/j.devcel.2024.11.015



The study by Blanchard *et. al*. discovered and interrogated a new population of mast cells in the developing rat brain. Peri-hippocampal mast cells and skin mast cells replicate vigorously *in situ* during the first 2 postnatal weeks. The images were acquired with the **Nikon CSU-W1 spinning disk microscope**.

Brain development is a non-linear process of regionally specific epochs occurring during windows of sensitivity to endogenous and exogenous stimuli. The study by Blanchard *et. al*. identifies an epoch in the neonatal rat brain defined by a transient population of peri-hippocampal mast cells (phMCs) that are abundant from birth through 2-weeks post-natal but absent thereafter. The phMCs are maintained by proliferation and harbor a unique transcriptome compared with mast cells residing in the skin, bone marrow, or other brain regions. Pharmacological activation of this population broadly increases blood-brain barrier permeability, recruits peripheral immune cells, and stunts local microglia proliferation. Examination of the post-mortem human brain demonstrated mast cells in the peri-hippocampal region of a newborn, but not an older infant, suggesting a similar developmental period exists in humans. Mast cells specifically, and early-life inflammation generally, have been linked to heightened risk for neurodevelopmental disorders, and these results demonstrate a plausible source of that risk.

Did you author a publication using the instruments at the Confocal Core? Please report your publication using this link: Confocal Core Publications or by scanning the QR Code:

Microscopy Questions

Dear Joe…

Hey, take a look at these images! They look fantastic. I can see aggregations in the lung tissue. I was staining lung tissue looking for tumor cells, and I think I found them. We should be able to count and quantify the cell aggregations. It all fits perfectly into our hypothesis. I have one issue, though: some of these features can be seen in samples that were not treated with any antibodies. So, what is all this signal? If it is there in this condition, won't it be there in all our experimental conditions, too? This could be a serious problem for our interpretations of our experimental images. What should we do?

My reply: You need imaging controls my friend!

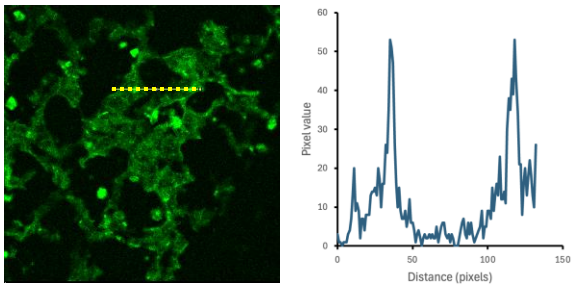


Figure 1. Signal without staining. left) Confocal image of lung tissue section using 488 nm excitation and emission of 500 to 550 nm. right) Pixel intensities along the line indicated on the image. The image shows intricate tissue detail and great contrast, revealing the strong and complex signals that can arise even without antibodies or any deliberate labeling.

What are controls in the context of imaging?

Controls are prepared under identical sample preparation conditions and microscope acquisition settings. They may be **positive controls** that help make sure you can detect a true signal or **negative controls** that do not contain the target of interest. Controls therefore help the investigator determine that a signal present in the experimental sample corresponds to the 'desired specific' signal. Our new microscopes have incredible sensitivity and new fluorophores are brighter and less prone to photobleaching than ever before, but these improvements mean you're better able to see background fluorescence and non-specific signals better than ever, too. Proper controls are an essential part of every imaging experiment.

When are they needed?

Always. Let me say it again: Always!

As Figure 1 shows, our images are always the sum of several factors beyond what we are trying to measure: The sometimes-complex fluorescent 'tissue background' as well as all the brightness and noise associated with the imaging system itself. **Only relative to imaging controls can investigators identify and measure the specific true signals.**

How are controls prepared?

There are many ways to prepare controls, just as many as there are experimental designs. Most commonly, for immunohistochemistry experiments, an important negative control is a sample prepared without a primary antibody, but treated with the secondary antibody carrying its usual fluorophore and imaged exactly the same as in the cases treated with the primary. You'll be surprised by how much signal sometimes remains in these conditions, but hopefully you can find staining and imaging conditions to minimize it. Then the primary can be applied with the secondary antibody to stain knockout or knockdown samples, which are critical negative controls showing specificity of the primary in your conditions. **(Don't count on other people's data when it comes to the specificity of your primary!)** These negative controls allow the researcher to truly determine the 'background' signal of a sample. Thus, the 'specific' signal can be deduced via a differential comparison between the sample and the negative controls.

Positive controls are critical to determine that your assay is working—that your labeling is appropriate and that you can detect and measure your structure of interest. For example, a different cell type that has high expression of a protein may be used to validate that the labeling system can provide specific labeling of a particular protein and determine its localization. Again, the images need to be collected with identical processing and acquisition parameters. And for all your controls, make sure to blind yourself at the time of acquisition, during analysis, or both!

Conclusion

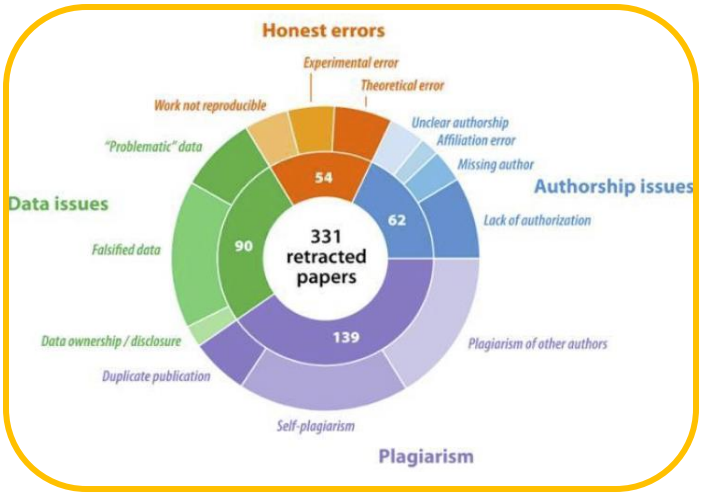
Controls are needed now, more than ever. Microscopes have evolved as precise, hypersensitive instruments and the demand for scientific precision and rigor are ever present. To maintain the highest level of scientific certainty, every researcher must always factor in controls for their imaging experiments. There are, alas, no shortcuts. **Science will always demand its controls**.

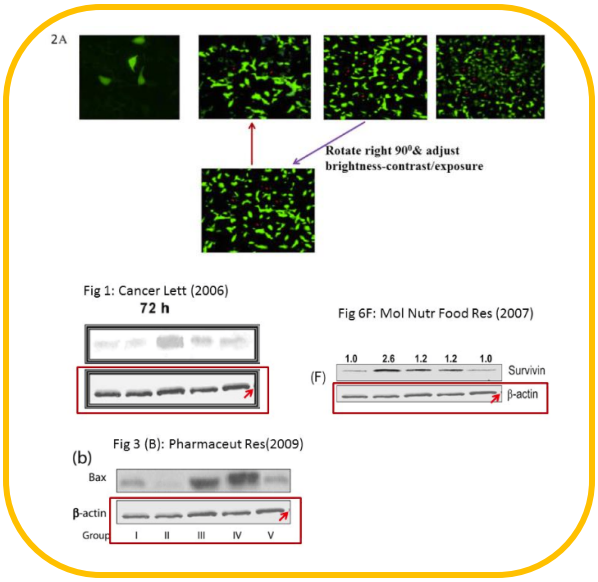
Analysis Questions

Dear Shilpa..

I want to analyze my images and present my work to my colleagues. But I have some background and non-specific signals that I want to remove. How can I make sure I am handling this ethically?

Scientists receive extensive training on how to collect data, but less work goes into teaching them how to showcase the information in publications, presentations and grant applications. For microscopy applications, data collected is completely digital, which makes data collection and handling easier but also makes image manipulation very easy. Often, inaccurate manipulations are the result of lack of understanding of the regulations and guidelines on appropriate handling of the images. However, there have been increasing evidence found of image falsification and unethical manipulations which have resulted in publications being retracted. The advent of generative AI tools have further contributed to difficulties in identifying fraud and misconduct. It becomes important for researchers to learn about the guidelines about ethical handling of data.





The Nature Portfolio journals have published their policies and guideline for image integrity and standards: **Image integrity and standards**

Few of the important ethical guidelines that need to be followed when working with scientific images are listed below (Avoiding Twisted Pixels: Ethical Guidelines for the Appropriate Use and Manipulation of Scientific Digital Images):

1. Digital images should be acquired in a way that they convey the important information and do not intend to deceive the viewer allowing for alternative interpretations of the data. Images should present the variability in the specimen.
2. Manipulation of digital images should only be performed on a copy of the unprocessed image data file (always keep the original data file safe and unchanged!).
3. Simple adjustments to the entire image are usually acceptable.
4. Digital images that will be compared to one another should be acquired under identical conditions, and any post-acquisition image processing should also be identical.
5. Manipulations that are specific to one area of an image and are not performed on other areas are questionable.
6. Cloning or copying objects into a digital image, from other parts of the same image or from a different image, is very questionable.
7. Intensity measurements should be performed on uniformly processed image data, and the data should be calibrated to a known standard.

If you have any questions on ethical handling of your data, please contact Shilpa Dilip Kumar.

News from other Imaging Cores:

New to CTRIM! Dixon MRI for Fat Quantification

The Dixon method is an MRI-based chemical shift imaging technique that **separates fat and water signals** by exploiting their distinct frequencies.

This allows ***in vivo* visualization and quantitative assessment of fat content**, with high uniformity and few artifacts, across a range of biological and clinical models.

At CTRIM, Dixon MRI is now being applied in two exciting ongoing studies: Quantifying Fat Infiltration in Rabbit Calf Muscles and Investigating VIPR1's Role in T Cell Phenotype on Body Metabolism.

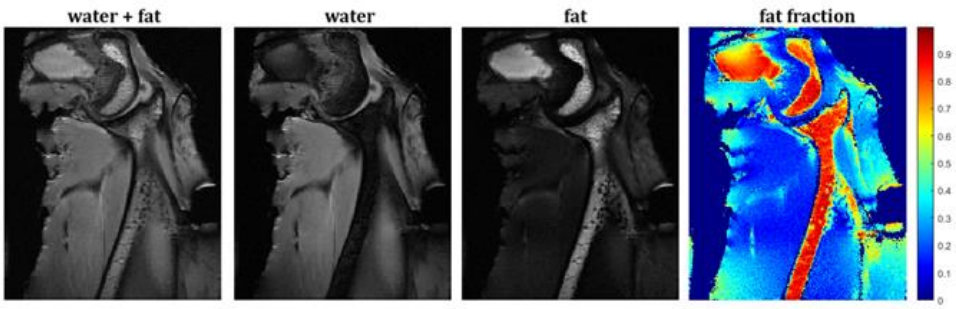
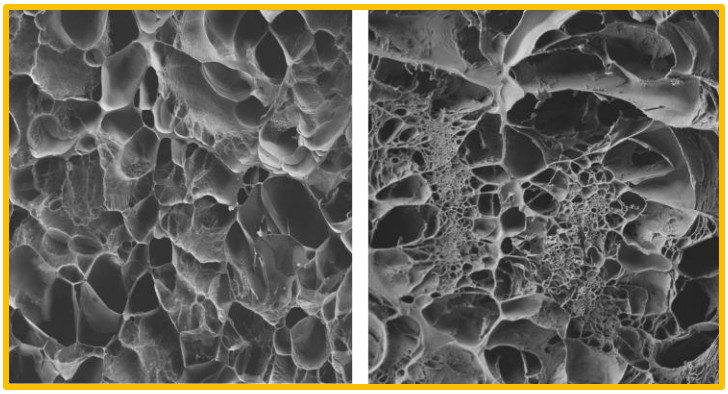


Fig 1. Fat quantification in rabbit calf with 2D fast spin echo Dixon Method

Other potential research and clinical applications for MRI fat imaging may include liver fat quantification, adipose tissue remodeling in obesity and diabetes models, and quantification of marrow fat content.

If you are interested in applying Dixon methods or employing other imaging modalities in your research quests, please reach out to CTRIM core. Please contact Jiachen Zhuo (jzhuo@som.umaryland.edu) for more information.

New to EMCIF: Capabilities to image hydrogels in 3D



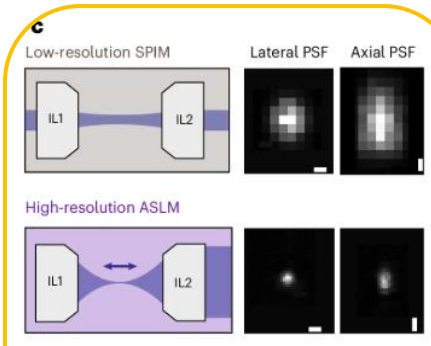
The Electron Microscope Core Imaging Facility's (EMCIF) new **Thermo Scientific™ Volumescope 2™ Scanning Electron Microscope** is in action and generating high-resolution images. The EM Core is now equipped with new capabilities to image hydrogels in the SEM. The image shown is of a hydrogel acquired by the cryo-SEM.

Please contact Christine Brantner (cbrantner@umaryland.edu) for more information on using the VolumeScope and other instruments at the EMCIF.

Imaging News from Around the World

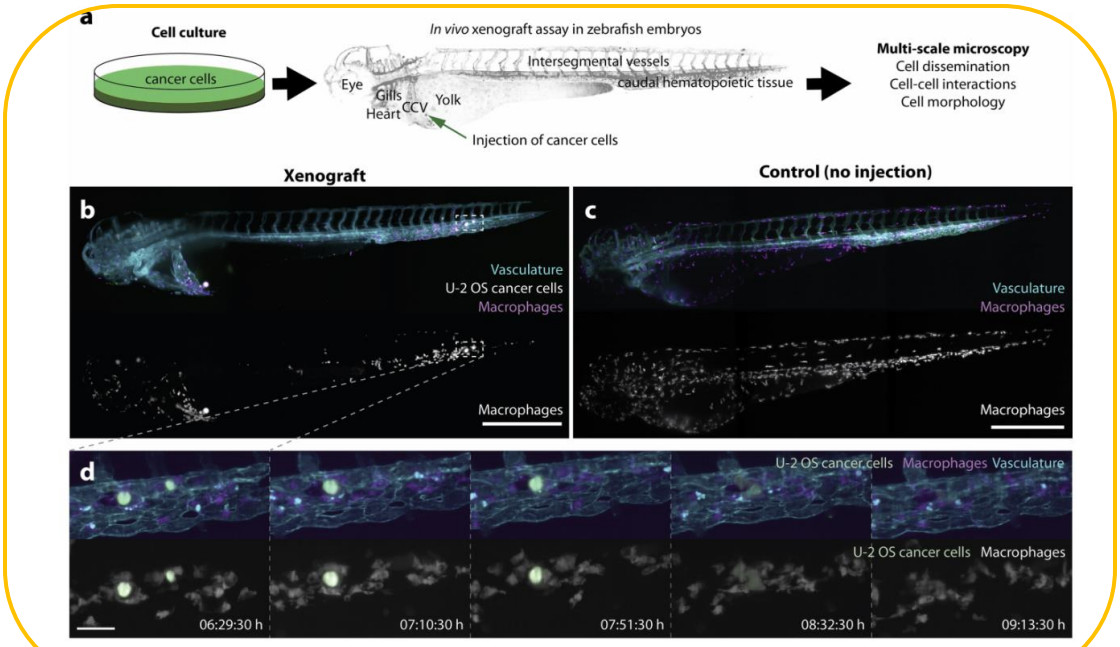
Imaging of cellular dynamics from a whole organism to subcellular scale with self-driving, multiscale microscopy

Stephan Daetwyler, Hanieh Mazloom-Farsibaf, Felix Y. Zhou, Dagan Segal, Etai Sapoznik, Bingying Chen, Jill M. Westcott, Rolf A. Brekken, Gaudenz Danuser & Reto Fiolka
Nature Methods **volume 22**, pages569–578 (2025) https://doi.org/10.1038/s41592-025-02598-2



Schematic top-view light-sheet profiles of dual-sided multidirectional SPIM (top, brown) for low-resolution imaging and single-sided ASLM for high-resolution imaging (bottom, violet).

Simplified rendering of the multiscale microscope.



In vivo zebrafish xenograft assay to study immune cell–cancer cell interactions *in situ*.

The study *"Imaging of cellular dynamics from a whole organism to subcellular scale with self-driving, multiscale microscopy"* presents an innovative light sheet microscopy system that autonomously captures dynamic biological processes across multiple spatial scales. By integrating adaptive imaging strategies with machine learning, the system can track and zoom into areas of interest, from entire organisms down to individual subcellular structures, in real-time. This self-driving approach significantly enhances the efficiency and resolution of biological imaging, enabling researchers to observe complex, dynamic events such as cell migration, division, and organ development with unprecedented detail and minimal manual intervention.