# Imaging Facilities Newsletter

Featured Researcher: Kody Moore

My name is Kody Moore, and I am currently a junior in the Immunology and Medical Microbiology undergraduate program. I have been conducting my research in Dr. Edwin Wan's neuroimmunology lab. We study the cellular mechanisms and pathways in various neurological disorders. We have been specifically interested in better understanding the role of monocytes in the brain following ischemic stroke. It is currently known that monocytes can aid in blood-vessel repair in post-stroke recovery however, there are no good models to show how the cells interact with the brain vasculature.

We have developed a model of ischemic stroke, where we can produce both three-dimensional images of the vasculature as well as visualize the activity of the monocytes in three-dimensions. Using the MIFs Light Sheet Microscope, we have imaged whole processed brain tissue and have looked at multiple fluorescent reporter signals within the same sample. With our model, we have observed monocytes directly interacting with the brain vasculature three-days after stroke. The monocytes exhibit a phenotype in which they align and wrap themselves along vessel branches. Using our same model, we have also looked at other immune cells in the brain and how they behave following stroke. Overall, the Light Sheet imaging model we developed will allow for three-dimensional fluorescent imaging across a wide range of cell types in whole cleared tissue samples.
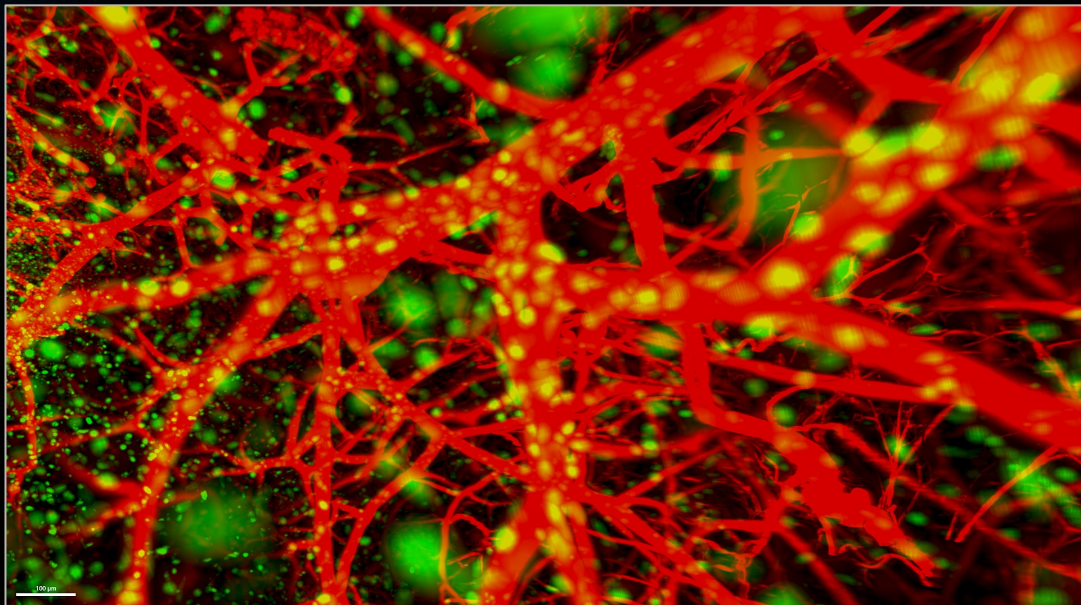
Three days following ischemic stroke, CCR2+ monocytes (green) can be seen infiltrating into the brain where the stroke occurs. Monocytes specifically can be seen in close proximity to and aligning themselves with vascular branches (red) in the brain. This phenotype has been exclusive to monocytes and is not present in models with other immune cells. This is one of the first methods to investigate the activity of immune cells in the brain three-dimensionally.

We are also currently investigating multiple sclerosis (MS) using a relapsing remitting MS (RRMS) murine model. With the help of the AMIF and their Comprehensive Lab Animal Monitoring (CLAMS) system we have generated data that is allowing us to investigate how an RRMS disease course may alter or permanently change the activity of metabolism in the body.

Moving forward we also intend to use the MIFs Multi Photon Microscope to perform live cell imaging in live animals to study the behavior of immune cells in real time, investigating both MS and stroke.

Microscope Imaging Facility News

Since our most recent newsletter, a new director, Dr. Neil Billington, joined the MIF from his previous position at NIH. "I'm very excited to continue the great work done by the MIF" said Dr. Billington. "I've already been fortunate to meet and work with many of you and look forward to helping you all with the imaging and analysis you need for your research" he added.

Meanwhile, Imaging Specialist Kelsey Wright departed in July to take up her studies at the WVU School of Medicine. We wish her the best as she embarks on her future career.

Animal Models & Imaging Facility News

Dr. Amanda Stewart was appointed as manager of the Animal Models and Imaging facility in October of last year. Amanda has over 20 years of experience in animal research and is looking forward to developing MRI and multimodal imaging techniques in addition to enhancing AMIF's current capabilities in metabolic monitoring.

Workstations for both MIF and AMIF image processing have been rehoused in the new Image Processing Center on Erma 2. The image processing center features several PCs with both commercial and non-commercial image analysis software. Training and expertise in using the software is available on request.

The Imaris Workstation, which runs the newest version of the software, was upgraded in September. The new hardware is optimized to allow handling of very large 3D image datasets. Workstation 3 now includes VivoQuant and Imaris Viewer for MRI analysis, Oxymax and Clax for CLAMS analysis, and Amersham Imager as an alternate location for Western blot analysis. A recorded training for VivoQuant is available to users, and in-person training is available for all platforms by appointment.
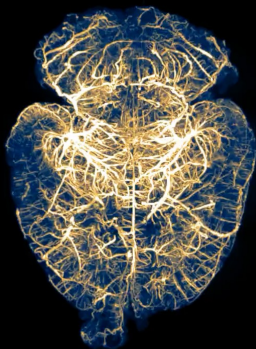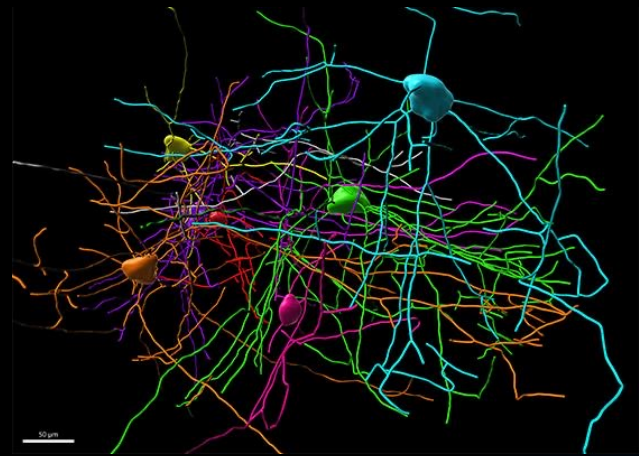
IMARIS 10

Version 10 of Imaris is installed on the new Workstation. A number of updates are included to improve workflow :-
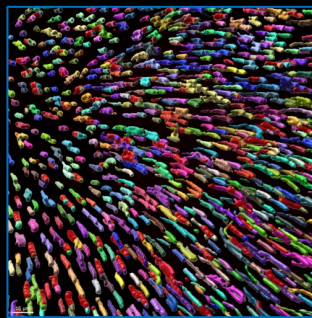
- AI Powered Filament Tracer

Machine learning completes filament tracing based on user selected examples.
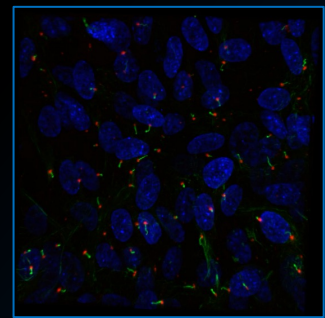
- Faster calculation

- Faster rendering

- Gesture based selection

Stitching & 3D rendering
Mouse brain, Wan Lab

AI assisted segmentation
Mouse photoreceptors, Deng lab

Volumetric Analysis
RPE cilia, Rong Liu lab

In November MIF hosted an Imaris workshop given by Tomas Silva Santisteban of Oxford Instruments.

A recorded version can be found on the G drive of the Imaris workstation.

2023 Publications

Aboaziza E, Feaster K, Hare L, Chantler PD, Olfert IM. Maternal electronic cigarette use during pregnancy affects long-term arterial function in offspring. J Appl Physiol (1985). 2023; 134(1): 59-71. PMCID: PMCPMC9762967.

Arsiwala TA, Blethen KE, Wolford CP, Panchal DM, Sprowls SA, Fladeland RA, Kielkowski BN, Pritt TA, Wang P, Wilson O, Carpenter JS, Finomore V, Rezai A, Lockman PR. Blood-tumor barrier opening by MRI-guided transcranial focused ultrasound in a preclinical breast cancer brain metastasis model improves efficacy of combinatorial chemotherapy. Front Oncol. 2023; 13: 1104594. PMCID: PMCPMC9950566.

Blethen KE, Sprowls SA, Arsiwala TA, Wolford CP, Panchal DM, Fladeland RA, Glass MJ, Dykstra LP, Kielkowski BN, Blackburn JR, Andrick CJ, Lockman PR. Effects of whole-brain radiation therapy on the blood-brain barrier in immunocompetent and immunocompromised mouse models. Radiat Oncol. 2023; 18(1): 22. PMCID: PMCPMC9896731.

Bumgarner JR, Walker WH, Quintana DD, White RC, Richmond AA, Meléndez-Fernández OH, Liu JA, Becker-Krail DD, Walton JC, Simpkins JW, DeVries AC, Nelson RJ. Acute exposure to artificial light at night alters hippocampal vascular structure in mice. iScience. 2023; 26(7): 106996.

Durr AJ, Korol AS, Hathaway QA, Kunovac A, Taylor AD, Rizwan S, Pinti MV, Hollander JM. Machine learning for spatial stratification of progressive cardiovascular dysfunction in a murine model of type 2 diabetes mellitus. PLoS One. 2023; 18(5): e0285512. PMCID: PMCPMC10166525.

Grund M, Choi SJ, Powell L, Lukomski S. Intranasal immunization with a Bucl8-based vaccine ameliorates bacterial burden and pathological inflammation, and promotes an IgG2a/b dominant response in an outbred mouse model of Burkholderia infection. Front Immunol. 2023; 14: 1177650. PMCID: PMCPMC10399622.

Heenatigala Palliyage G, Samart P, Bobbala S, Rojanasakul LW, Coyle J, Martin K, Callery PS, Rojanasakul Y. Chemotherapy-induced PDL-1 expression in cancer-associated fibroblasts promotes chemoresistance in NSCLC. Lung Cancer. 2023; 181: 107258. PMCID: PMCPMC10330668.

Hostetler RE, Hu H, Agmon A. Genetically Defined Subtypes of Somatostatin-Containing Cortical Interneurons. eNeuro. 2023; 10(8). PMCID: PMCPMC10414551.

Lacinski RA, Markel JE, Pratt HG, Reinbeau RM, Stewart A, Santiago SP, Lindsey BA. Optimizing the synthesis of interleukin-12-loaded PLGA nanospheres (rmIL-12ns) via ultrasonication for treatment of metastatic osteosarcoma. J Orthop Res. 2023; 41(7): 1565-81. PMCID: PMCPMC10232680.

2023 Publications (cont)

Lahiri A, Walton JC, Zhang N, Billington N, DeVries AC, Meares GP. Astrocytic deletion of protein kinase R-like ER kinase (PERK) does not affect learning and memory in aged mice but worsens outcome from experimental stroke. J Neurosci Res. 2023.

Monaghan KL, Zheng W, Akhter H, Wang L, Ammer AG, Li P, Lin JX, Hu G, Leonard WJ, Wan ECK. Tetramerization of STAT5 regulates monocyte differentiation and the dextran sulfate sodium-induced colitis in mice. Front Immunol. 2023; 14: 1117828. PMCID: PMCPMC10157487.

Purazo ML, Ice RJ, Shimpi R, Hoenerhoff M, Pugacheva EN. NEDD9 Overexpression Causes Hyperproliferation of Luminal Cells and Cooperates with HER2 Oncogene in Tumor Initiation: A Novel Prognostic Marker in Breast Cancer. Cancers (Basel). 2023; 15(4). PMCID: PMCPMC9954084.

Spahr EJ, McLaughlin SL, Tichinel AM, Kasson MT, Kijimoto T. Staining and Scanning Protocol for Micro-Computed Tomography to Observe the Morphology of Soft Tissues in Ambrosia Beetles. Bio Protoc. 2023; 13(1): e4584. PMCID: PMCPMC9901460.

Spahr EJ, Wasef F, Kasson MT, Kijimoto T. Developmental genetic underpinnings of a symbiosis-associated organ in the fungus-farming ambrosia beetle Euwallacea validus. Sci Rep. 2023; 13(1): 14014. PMCID: PMCPMC10462615.

Ul Banna H, Mitchell B, Chen S, Palko J. Super-Resolution Ultrasound Localization Microscopy Using High-Frequency Ultrasound to Measure Ocular Perfusion Velocity in the Rat Eye. Bioengineering (Basel). 2023; 10(6). PMCID: PMCPMC10295416.

West Virginia University

Miicroscope Imaging Facility

Zeiss Airyscan

Zeiss Confocal

Nikon A1R Confocal

20 μm

Nikon Multiphoton

Miltenyi Lightsheet

Olympus Slide Scanner

Nikon A1R SIM

5 μm

Olympus MVX10

Animal Models & Imaging Facility

IVIS Spectrum CT

SkyScan Micro-CT

Vevo F2

Left Ventricle

Aortic outflow

CLAMS

M7 MRI

SkyScan Micro-CT

Vevo F2

Portal Vein

Right Lobe Liver

Right Kidney

Inferior Vena Cava

Adrenal Gland

XenX
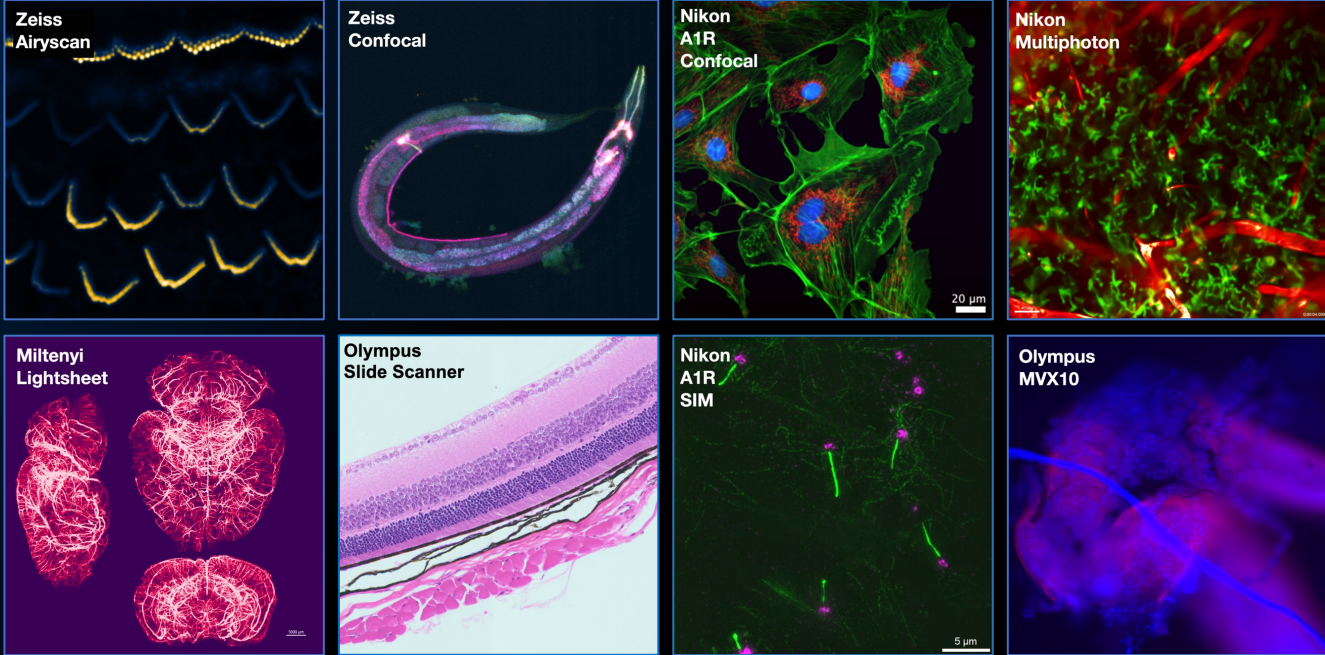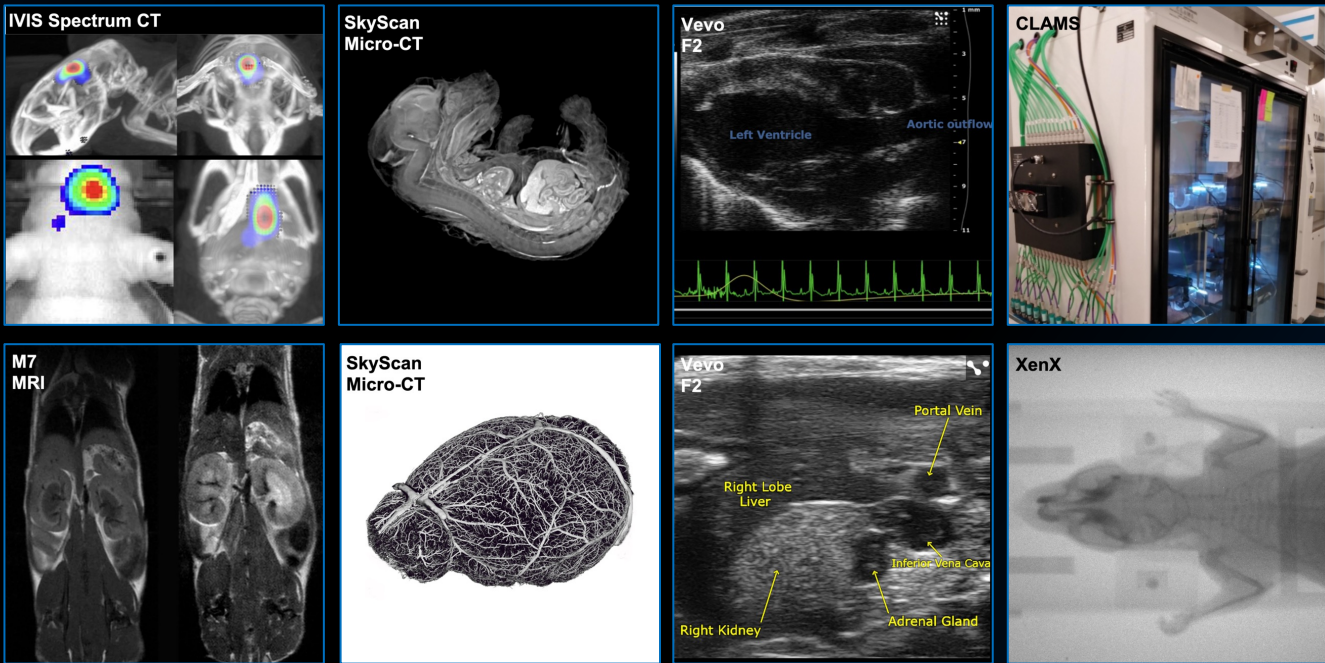
Please contact us to arrange training or discuss imaging options

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