# Intravital Imaging Facility

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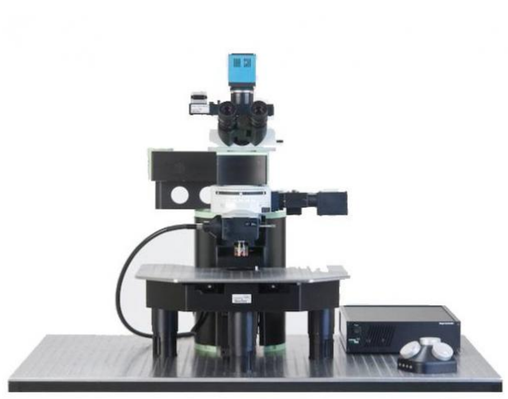
The In Vivo Imaging Facility provides comprehensive, affordable support to investigators interested in utilizing two-photon laser scanning microscopy in their experiments. It offers operator-assisted imaging as well as the surgical preparation and anesthesia of mice for intravital imaging on a fee per service basis. This microscopy facility can be used to image fluorescently labeled cells or structures within intact tissue at much greater depths than a standard confocal microscope. In addition to 3D renderings of large tissue volumes, time resolved data sets within living anesthetized mice can also be acquired. This methodology can often provide investigators with information that is otherwise difficult to deduce through the sole use of in vitro assays or static histology.



- Upright laser scanning microscope (LaVision Biotec)
- Tunable Titanium-Sapphire two-photon laser (Chameleon Vision II, Coherent)
- Long working distance, high NA objectives (20x and 40x) compatible with infared excitation
- Automated in vivo imaging stage with integrated xyz translation that enables tiling
- Restraining platforms for intravital imaging of rodents as well as excised tissue within perfusion chambers
- Cloud scanner permits more rapid frame acquisition rates than standard raster scanners and has enhanced ROI (Region of Interest) and line scanning options
- Sensitive multi-plexed non-descanned detectors (NDDs), capable of simultaneously detecting up to 4 colors, are optimally located just above the objective
- Emitted light is collected through a customizable arrangement of dichroic and bandpass filters that are easily customizable.

**Contact Us**

For additional information and a consultation prior to initiating a project please write or call with any questions.

In Vivo Imaging Facility

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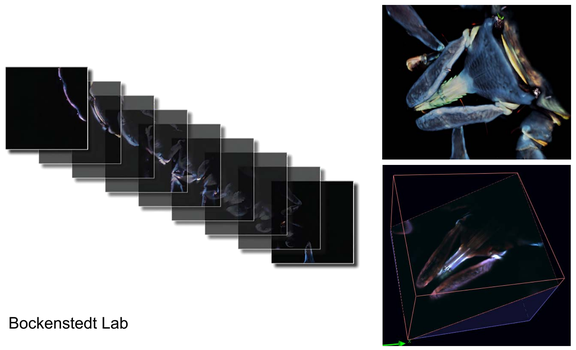
Our facility is located in The Anlyan Center, TAC S-614.

**3D Reconstructions**

High definition 3D renderings of any excised tissue type is possible by the acquisition of large stacks of optical sections. This approach can be used to better define a cellular context within the overall architecture of the imaged tissue volume and can often be used with tissue types that are difficult to cut for histology. A facility technician is available on a fee-per-service basis who can operate the microscope or train those who wish to do this themselves.

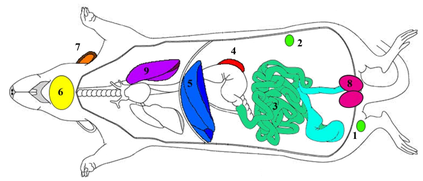


Bockenstedt Lab

These images are a representative sampling from a 340µm z-stack captured of the head of an *Ixodes scapularis* tick. Each individual image represents the auto-fluoresence from a single 1um section of the tick's head. These individual images were combined in Bitplane's Imaris software to produce a high definition 3D-rendering.

**4D Intravital Imaging**

Time resolved intravital imaging allows one to directly visualize and quantify dynamic cellular behaviors, movements and interactions of fluorescently labeled cells in vivo. These intravital studies can be conducted within any tissue type, limited only by the ability to bring the desired tissue close to the objective without compromising native tissue perfusion or function.



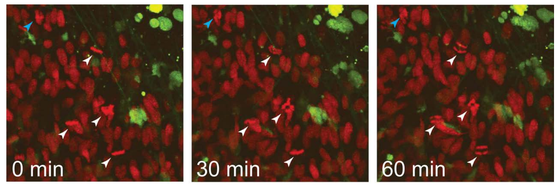

Currently we can successfully perform intravital imaging of:

1. Popliteal lymph node
2. Inguinal lymph node/flank
3. Intestine
4. Spleen
5. Liver
6. Calvarium
7. Skin
8. Testicles
9. Lung

A highly skilled technician is available who can surgically prepare mice, maintain anesthesia and operate the microscope during intravital imaging. All surgical procedures, anesthesia and imaging are offered on a fee-per-service basis. Training in established techniques is also available for those who prefer to do this independently.

**Proliferation & Apoptosis**

By imaging tissue over time you can observe and quantify cellular events such as proliferation and apoptosis. Below is an example of a rapidly growing induced tumor, expressing nuclear H2BRFP, two weeks after injection into the flank of a CD11cYFP reporter mouse. During this brief 1hr time lapse you can see both proliferation (white) and apoptotic (blue) events along the border of the tumor.



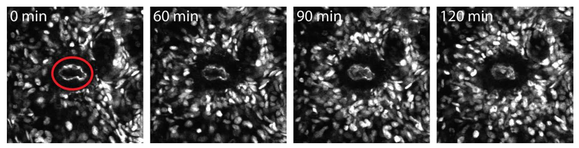

William Damsky, Bosenberg Lab

**Using the Laser as a Tool**

The laser itself can be utilized as a tool to:

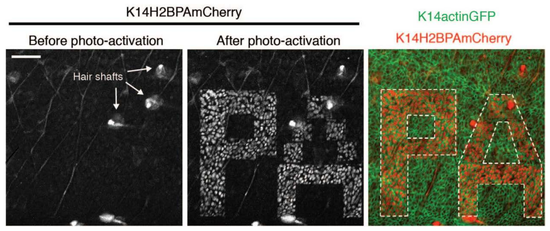
- Induce a wound
- Ablate individual or groups of cells within tissue
- Photoconvert fluorophores
- Photoactivate fluorophores
- Photobleach fluorophores

Below is an example of a laser induced wound in the epidermis. The Red circle highlights the site of the ablation. Over time you can appreciate the extent of immune cell recruitment to the site as shown by an ActinH2BGFP fluorescent reporter depicted here in grayscale.




Mesa K et al., Cell Stem Cell 1;.23:1-10 (2018)

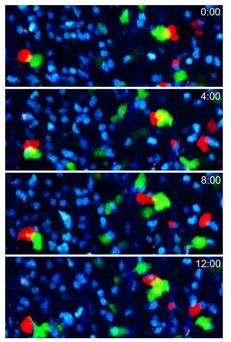
While there are several varieties of photoactivatable fluorescent proteins available, below is an example of a photoactivatable mCherry fluorescent reporter in the epidermis of an animal expressing ActinGFP under the keratin 14 promoter. You can see the field of view both before and after photoactivation of the fluorescent protein, which can allow for such things as short-term lineage tracing experiments as well as migration assays.




Rompolas P et al., Science 352(6292),1471-4 (2016)

**Cellular Interactions**

Time lapse imaging will also allow you to observe and track cell-cell interactions. During immune responses, activated B and T lymphocytes form long lasting cognate interactions. Shown here, RFP+ and GFP+ antigen specific T and B cells form immunological synapses in the popliteal lymph node 2 days after immunization. Such long lasting interactions can be quantified.

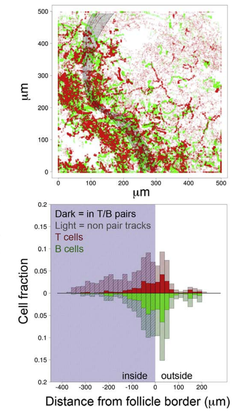



S. M. Kerfoot et al., Immunity 34, 947-960 (2011)

**Data Analysis**

A workstation is available for data analysis using Bitplane's Imaris software. This software can be used to generate 3D volume renderings or movies of time resolved images. It can also be used to track the direction of cell movement, determine motility parameters and define the characteristics of fluorescent cells such as size and shape. Analysis can be done for you on a fee-per-service basis, but training is also available.

Shown here are tracks of activated B and T lymphocyte cognate pairs at the border of the B cell follicle (top panel, bold tracks) overlaying tracks of individual B and T cells that are not in contact (lighter tracks), 3 days after immunization. A histogram of the mean distance for individual T and B cell tracks relative to the follicle border is plotted with the cognate pairs overlaying.




S. M. Kerfoot et al., Immunity 34, 947-960 (2011)

**Current User Fees**

| Service | Rate |
|---|---|
| 2P Microscope Imaging Independently | $40/hr |
| 2P Microscope Imaging w/Technician | $64/hr |
| 2P Microscope Training | $64/hr |
| Facility Workstation Use (Imaris) | $7/hr |
| Technician Assisted Data Analysis (Imaris) | $35/hr |
| Imaris Software Training | $35/hr |
| Skin Preparation | $65/prep |
| Intestine Preparation | $90/prep |
| Spleen Preparation | $90/prep |
| Liver Preparation | $90/prep |
| Calvarium Preparation | $65/prep |
| Testicular Preparation | $65/prep |
| Lung Preparation | $90/prep |
| Popliteal Lymph Node Preparation | $65/prep |
| Inguinal Lymph Node Preparation | $65/prep |