Intra-Vital Microscopy

Stephen Lockett

1) Basic Concept
2) Technology and example images
3) Typical experimental outline and expertise / resources
4) Dr. Amiran Dzutsev: “Surveillance of the gut lumen by colonic dendritic cells in vivo.”
**Basic Concept: Fluorescence**

- Detect specific molecules (immunocytochemistry, in situ hybridization, fluorescent proteins)
- Must keep the specimen transparent.
- Detect multiple molecular species at the same time

**Jablonski Diagram**

- Absorption of a Photon
- Emission of a Photon
- Ground State
- $S_1$

*Dr. Yien Che Tsai, NCI*
Basic Concept: Multi-Color Fluorescence
Basic Concept: Two photon excitation

Advantages of Two Photon
1. Much less photodamage to specimen
2. Can image to greater depths ($\approx 500 \, \mu m$), because less scattering ($\propto \lambda^{-1}$)
3. Only excitation near the focal point

The intensity of the infrared light ($\lambda = 800 \, \text{nm}$) increases towards the focal point. Near focus, two infrared photons act as one blue photon. Thus only fluorescent molecules near the focal point are excited.
Benefits of Two Photon Microscopy

(http://micro.magnet.fsu.edu/primer/techniques/fluorescence/multiphoton/multiphotonintro.html)

(Leica Inc.)
Two Photon Microscope

microscope

animal

Additional stage to support animal

objective lens inverter

objective lens

another lens

animal

Additional stage to support animal

microscope
Outline of a Typical Experiment

1) Obtain approval for animal experiment
2) Sedate animal
3) Perform surgery on animal
4) Inject fluorescent dye into animal
5) Move animal to microscope
6) Maintain / monitor animal
7) Acquire images
8) Animal housed until the next imaging session
9) Repeat (4), (5), (6), (7) and (8) as necessary
10) Euthanize animal
11) Interpret results from images
12) Repeat experiments (back to (2))
Two Photon Example

Presumably autofluorescence detected by second harmonic generation.
Two Photon Example

(In collaboration with Dr. Sunny Jansen, Laboratory of Dr. Terry Van Dyke)
Existing Resources

1) Tunable from 700 to 1060 nm, with adjustment of pre-chirping.

2) Water immersion lenses suitable for several hundred microns into tissue. 20X 1.0 NA dipping (1.8 mm working distance), 40X (with a cover glass, 1.1 NA water), 63X 1.4 NA oil, 10X 0.45 NA water, 10X 0.45 NA dry.

3) Either upright or inverted imaging. Upright imaging is achieved using an objective lens inverter. One inverter enables a detector to be placed directly above objective lens

4) Two non-descanned detectors for GFP (500 – 550 nm emission) and a red emitting dye. (565 – 610 nm emission).

5) Heating blanket and temperature probe.

6) Manually adjustable xyz stage for animal. (A motorized stage is considered essential in order to increased the field of view while retaining high spatial resolution.)

7) Broad range of one photon confocal capabilities.

8) Second harmonic generation imaging.
Required Expertise for Experiment

1) Principal Investigator (NCI):
   a. Design experiment, solicit ACUC approval for study / interpret results

2) Postdoc / technician in PI’s lab (NCI):
   a. Ensure that all equipment and materials are in place
   b. Likely perform surgery on animal
   c. Technique development/validation and troubleshooting. Includes ex vivo imaging, finding the best wavelength for excitation, checking the SNR, histological/molecular validation, finding the right concentration for injected dyes or cells etc.
   d. Conduct experiment and interpret results

3) Animal technician (LASP): monitor health of animal, feed and clean mice, may perform surgery ($45 / hour). An experiment may incur costs of $250 - $500. However, some labs have their own animal techs.

4) Animal veterinarian (LASP): approve protocol. Check health of animal if needed. No charge

5) Microscopy Lab (OMAL) No charge:
   a. Provide physical infrastructure
   b. Provide advice about which protocols investigators need to get approved
   c. Provide advice and expertise about how to prepare animal for imaging
   d. Participate in acquisition of images.
   e. Participate in analysis of images and interpretation of results.