Use the simplest technology that will answer the scientific question!

- Fluorometer
  - Low light digital imaging
    - Confocal microscopy
      - Two photon microscopy
Use the simplest technology that will answer the scientific question!

Population response adequate
cell/cell heterogeneity not crucial

Cell:cell heterogeneity important
intracellular heterogeneity not crucial

Samples up to 50 µm thick,
cell:cell or subcellular resolution needed

Thick specimens up to several hundred µm thick,
cell:cell or subcellular resolution needed
Selecting a system

The decision points

speed

depth of focus

resolution

sensitivity
Selecting a system

The cleanest triage point: speed

If you need detailed image information at greater than 3 frames/second, think about the Perkin-Elmer/Solamere fast scanner.

But first ask…

- Can you do your experiment with a subset of the image? Maybe you can use other systems…

- Do you need simultaneous dual or triple emissions? Maybe you should use other systems…

- Are you just thinking you MAY need to go fast, but for 90% of what you do another system is of better use?
Selecting a system

The next cleanest triage point: **depth**

If you need to image into specimens thicker than 60 µm, think about multi-photon microscopy.

But first ask…

- Do you know if you can get your reporter molecule in that deep?
- Could you just look at the sample from the other side as well?
- Does your tissue happen to be a really great confocal sample or a really lousy two-photon sample?
Selecting a system

The lousiest triage points: **resolution and sensitivity**

Extremely dependent on how you set up your experiment. Personal rather than absolute. Often comes down to available options or sample preparation.

Fortunately, simple tests to see if any given microscope gives adequate results.

**STRATEGY:** Ask if the device can give good results with YOUR sample that YOU want to study, not some test specimen that is completely irrelevant.
Selecting a system

Define sensitivity by ability to minimize cell damage

Get the sample that everyone hates. The one that photobleaches before you can even find the cell you are interested in.
  fixed: FITC without antifades (antioxidants)
  live: BCECF

1. Set your detectors as sensitive as possible.
2. Set your light source as low as possible.
3. Increase light and/or average the noisy image until you get an image that you can live with (compulsive people can estimate Signal/Bkground noise ratios)
4. Collect a series of ~30 images at 1/sec, then at 1/10 second
Selecting a system

Define sensitivity by ability to minimize cell damage

IF percent fluorescence loss is a constant with each time point, then it is due to photobleaching.
Selecting a system

Define sensitivity by ability to minimize cell damage

IF fluorescence loss does not change as a function of sampling rate, then it is due to dye leakage/diffusion with no evident photobleaching. This is a more sensitive system, able to image without damage.
Selecting a system

Invite vendors in for extended demos

Replace color glossy brochures with your own experience

Get your hands involved
  Don’t let them run the device all the time
  You need to see how user-friendly it is

Make sure you understand the features and capabilities
  Even better, try them out to see if they work

Work with lousy samples
  Dim
  One label is massively brighter than another
  Bad pairing of colors with lots of bleed over
Funding a system

NSF: Multi-User Equipment and Instrumentation Resources Grant (MUE)

first Monday in October

up to $400,000
minimum $40,000 instrument (30% cost share required)
no “imaging facility” bundle of instruments

goal: further NSF-funded, or NSF applicable research with SOATs
need minimum of 3 independent investigators
“Some” users should have active NSF funding
Funding a system

NIH: Shared Instrumentation Grant (S10)
once a year deadline, March

up to $500,000
minimum $100,000 instrument (cost share)
no “imaging facility” bundle of instruments

goal: further NIH-funded research with SOATs
need minimum of 3 NIH-funded investigators
suggest 4-6 in user group, not 16

Biological question not under fire
feasibility and sensibility of equipment use under review
preliminary data crucial to demonstrate experience and feasibility
Funding a system

NIH: Shared Instrumentation Grant (S10)
grant pieces important: read the instructions

User group and instrument should be a tight fit.

requested options should be used by 3 or more investigators
  justify everything: no justification, no lens

make it clear how the instrument…
  improves your efficiency and progress
  gets around existing bottlenecks (time, availability, capability)
  provides new areas for exploration

Make sure the expertise is available to help users
  technician should be trained
  optical expertise should be evident

Place in a core facility and outline how sharing works
  leave time for outside users
  captive instruments tick off the reviewers
How to get preliminary data

NIH/NCRR Biomedical Technology Resources


Scientists at these centers ensure that biomedical investigators who have NIH-supported projects may gain access to the newest and most advanced technologies, techniques, and methodologies.

**Laser Applications**
- Center for Fluorescence Spectroscopy (Baltimore, MD)
- Laboratory for Fluorescence Dynamics (Urbana, IL)**
- Laser Biomedical Research Center (Cambridge, MA)
- Laser Microbeam and Medical Program (Irvine, CA)**
- National Flow Cytometry and Sorting Research Resource (Los Alamos, NM)
- Ultrafast Optical Processes Laboratory (Philadelphia, PA)**

**Microscopy**
- National Center for Microscopy and Imaging Research (La Jolla, CA)**
- National Resource for Automated Molecular Microscopy (La Jolla, CA)
- Resource for the Visualization of Biological Complexity (Albany, NY)
- Three-Dimensional Electron Microscopy of Cells (Boulder, CO)
- Three-Dimensional Electron Microscopy of Macromolecules (Houston, TX)
How to get preliminary data
How to get preliminary data
Establishing a microscopy facility

Prepare the space
  - Power
  - Cooling
  - Air flow
  - Wet lab/surgery facilities

Prepare the skills and peripheral tools
  - Training staff and ongoing user training
  - Sign up strategies for equitable sharing
  - Off-line analyses

Prepare for disaster
  - Fiscal survival
  - Replacements/repair
  - Penalties
Post-delivery depression

Living with the system after purchase

- Monitoring performance/ Standardize response
  “..but I’m sure it was more sensitive last week”
- Fixed hour for PM/cleanup weekly

Living with the users after purchase

- Data archiving
  Get users off the microscope for data analysis and printing images
  “Tell me when it’s broken or I can’t fix it”