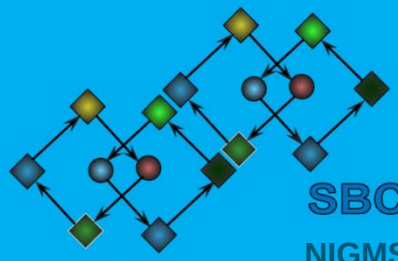


NIGMS funded Center

Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

Marc Birtwistle, PhD
Department of Pharmacology & Systems Therapeutics
Fall 2014, Week 6, Live-Cell Imaging

Icahn School of Medicine at Mount Sinai

Outline

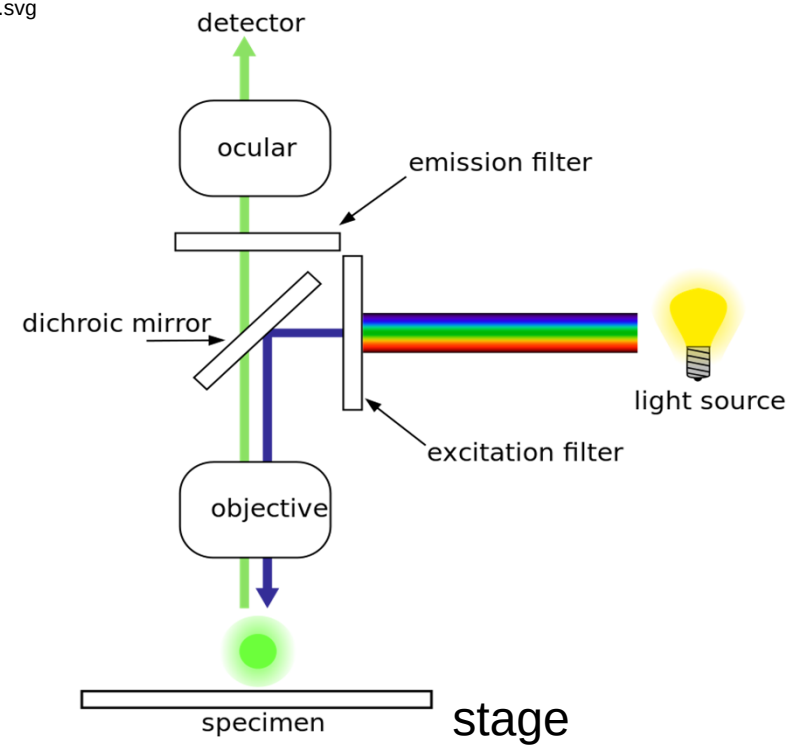
- Fluorescence Microscopy—Instrumentation
  - Components of a fluorescence microscope
  - Keeping cells “happy”—environmental control
  - Types of imaging
  - Acquisition properties
- Fluorescent Probes
  - Small molecules
  - Genetically-encoded sensors
- Quantification

- A note about
  - super resolution—relatively new technique that allows imaging past the diffraction limit but we won’t cover it here.
  - single molecule imaging—it is possible with fluorescence microscopy in live cells but we will not go into detail on it. Useful for modeling stochastic phenomena.
  - Fluorescence recovery after photobleaching (FRAP)—usually done in live cells to estimate diffusivity of tagged proteins but typically not repeatedly…we won’t cover it here

Fluorescence Microscopy—Instrumentation

General Components of a Fluorescence Microscope

http://en.wikipedia.org/wiki/Fluorescence_microscope#mediaviewer/File:FluorescenceFilters_2008-09-28.svg



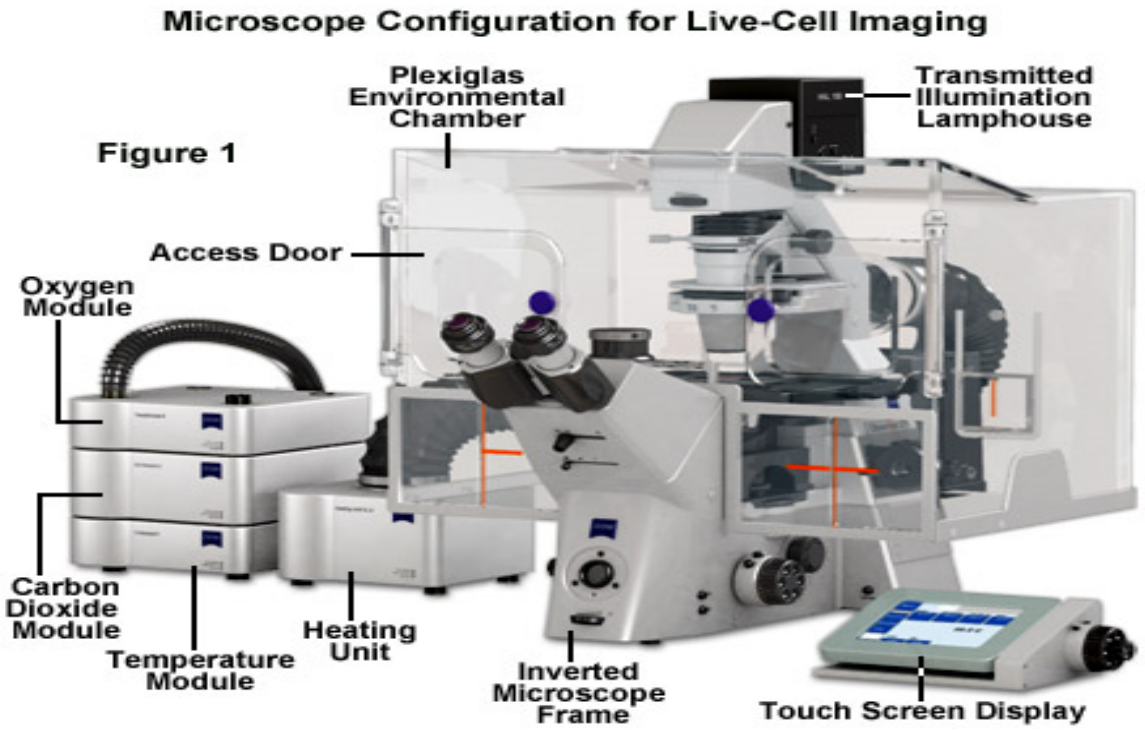
- Light source
  - Lasers
    - Defined wavelengths
    - "White" lasers
  - Broad coverage lamps
    - Xenon
    - Mercury
  - LED
    - Defined wavelengths
    - "White" LEDs
- Excitation filter
  - Allows only certain wavelengths of light through
  - Bandpass or longpass
- Dichroic Mirror
  - reflects excitation light but lets through emission light
  - Can also be polychroic
- Emission filter
  - Similar to excitation filter, but lets a different set of wavelengths through
- The excitation filter, dichroic mirror and emission filter are often contained together in a filter cube. They can be separate though.
- Objective
  - Lens that magnifies the image of the specimen
- Stage
  - Where the specimen sits, can be fixed or movable by hand or motorized
- Ocular
  - The eyepiece followed by the detector being one's eye
  - Can also be a camera
- This schematic is an upright microscope, but for live cell imaging inverted microscopes are typically used (objective below spec imen)

- Successful live-cell imaging experiments depend on the ability to maintain the cells in a healthy state and functioning normally on the microscope stage while being illuminated in the presence of synthetic fluorophores and/or fluorescent proteins.

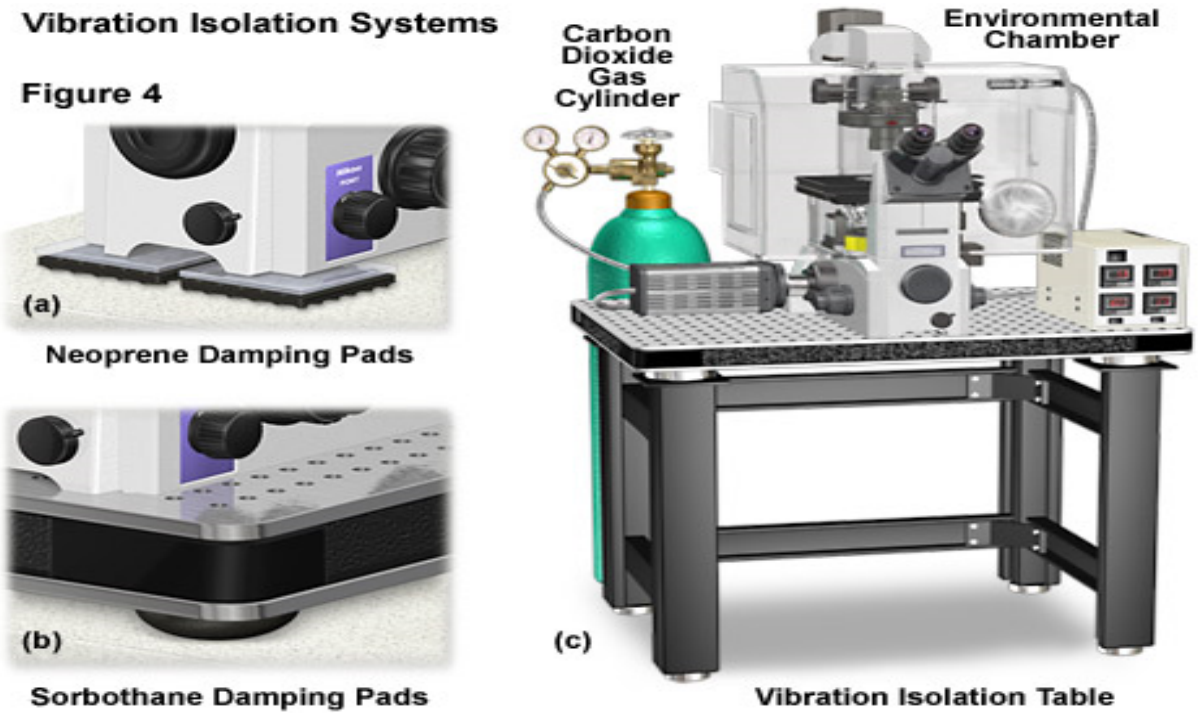
Environment: Keeping cells alive

- Media (pH, osmolarity)
- Temperature
- Atmospheric conditions (gas mixture and humidity)

Incubation chambers can maintain cells under desired conditions for long periods of time.



http://zeiss-campus.magnet.fsu.edu/articles/livecellimaging/imagingsystems.html



http://www.microscopyu.com/articles/livecellimaging/livecellmaintenance.html