McGill
Department of Bioengineering

BIEN 530
Imaging and Bioanalytical Instrumentation

Midterm Exam 2

due 10 April 2020 by midnight.

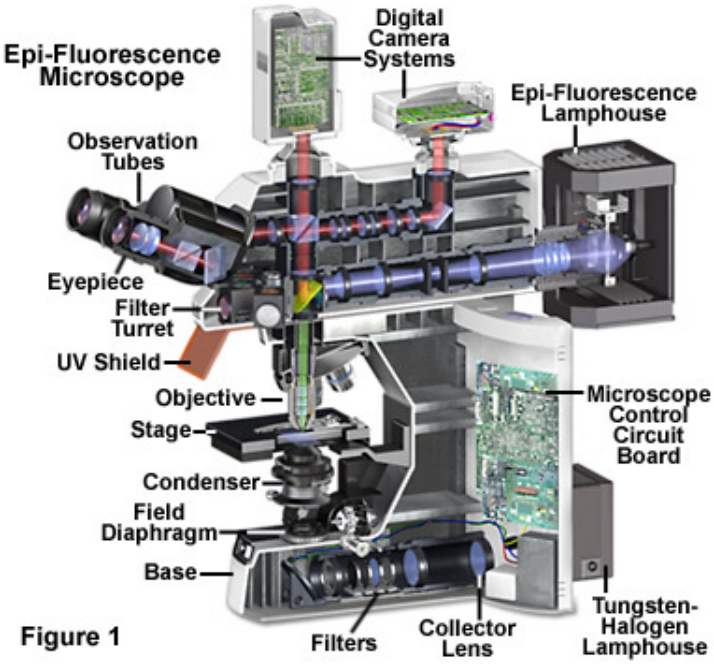
For each problem, generate a single figure (with multiple panels) and legend as though you were preparing a journal paper that explains the experiment and results, complete with scale bars, etc. Include a "Methods" section where you describe the analysis. You are welcome to discuss the problems with me and your classmates, but all of the data and analysis that you present should be your own. Include an "Acknowledgements" section to note any contributions from classmates.

1. Design a fluorescence microscopy experiment. We would like to examine if our protein of interest (mcgillin) interacts with the endoplasmic reticulum. Design a live-cell experiment to characterize mcgillin's interaction with the endoplasmic reticulum using the fluorescence microscope below. The sample will be illuminated using a broad-spectrum LED such as the Lumencor SOLA light engine. What fluorophores will you use? Choose fluorophores that are bright (brightness is the product of the extinction coefficient and the quantum yield) and photostable. How will you label mcgillin and the endoplasmic reticulum? What excitation, emission, and dichroic filters will you use for each fluorophore? Use either one of the tools below to choose filters and plot the spectra for the fluorophores and filters. How will you limit spectral overlap between the two fluorescence channels? What imaging conditions (exposure time, magnification, light intensity) will you use? What analysis steps will you perform? What challenges and potential pitfalls might you encounter, and how will you address them?

- Semrock searchlight (https://searchlight.semrock.com)
- Chroma spectraviewer (https://www.chroma.com/spectra-viewer)

Helpful information on fluorophores:

- Properties of fluorescent proteins. See Lambert (2019) Nature Methods.
- See the resources on mycourses under "3-fluorescence"



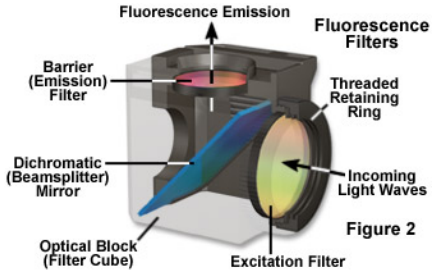


Figure 1: Fluorescence microscope [taken from www.microscopyu.com]. Light from the lamp is filtered by the excitation filter and reflected into the objective. The fluorescence signal is collected by the objective, passes through the dichroic filter, and is filtered by the emission filter.

2. Analysis of Interference reflection microscopy images. Microtubules exhibit dynamic instability, stochastically switching between periods of assembly and disassembly. Dynamic microtubules were imaged using Interference Reflection Microscopy (see Mahamdeh et al., 2018, J. of Microscopy). IRM is a label-free technique that takes advantage of interference between the light reflected from the glass-water interface and the light reflected from the water-sample interface to generate contrast. While the ability to image molecules without a label is a strength, the disadvantage of this technique is that it is sensitive to sources of noise (dust, small aggregates of protein, non-uniform illumination, etc. Thus, the raw images must be processed to visualize the sample. Generate an algorithm to process raw IRM images. In the figure, sketch the steps of the analysis and show results from the data set provided. Submit an ImageJ macro or matlab/python script that can be applied to other images.

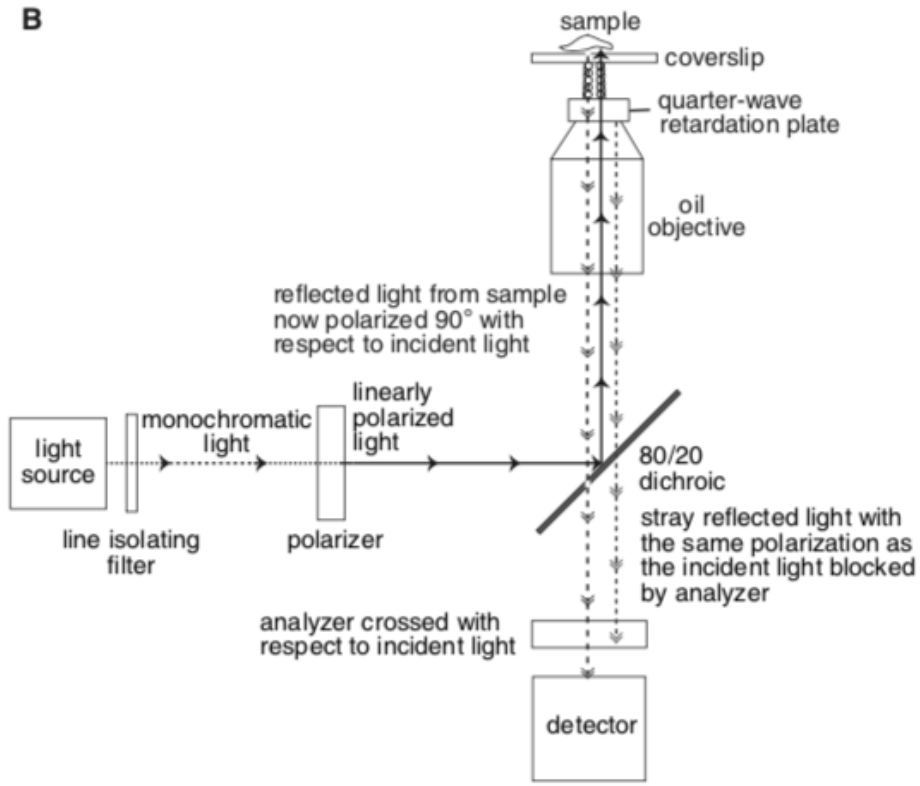


Figure 2: **Inferference Reflection Microscopy (IRM).** The [image from Barr and Bunnell (2009) Current Protocols in Cell Biology]

Files: download here.
•IRM-raw.tif: A tiff stack of IRM imaging of dynamic microtubules. Microtubules are attached to the coverslip. The microtubules stochastically switch between periods of assembly and disassembly. 926 frames; 10 frames/s; 107 nm/pixel.
•translational_background.tif: Single image obtained by acquiring images of a field of view while moving the stage laterally, and computing the median of that stack.

A few operations that might be useful:

TIFF virtual stacks:
Virtual stacks can be used when manipulating large data sets as only the current image in the stack is loaded into memory.
1. "File - Import - TIFF Virtual Stack"

Substack:
I suggest developing and testing your algorithm using a small substack, then applying it to the whole movie.
1. "Image - Stacks - Tools - Make Substack"

Maximum projection:
1. "Image - Stacks - Z project".
2. Select frames to project and projection type, click "OK".

Making a macro:
1. Record operations: "Plugins - Macro - Record". Click "Save" when finished.
2. Run: "Plugins - Macro - Run".

Spatial filters:
1. "Process - Filters -". 2. "Process - FFT - Bandpass Filter".

Mathematical operations:
1. "Process - Calculator Plus".

To include a scale bar:
1. "Analyze - Set Scale"
2. "Analyze - Tools - Scale bar"