## Bi227 Assignment #2 FALL 2007

## Goals for this week

**Image thin fixed samples.** Examine the differences between air and oil objective lenses, imaging parameters, and acquire multicolor images.

| **Dates:** | | |
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| | 22 October 2007, 3-4 pm | Assignment & Demo |
| | 22 October – 28 October | Students sign up for scope and acquire images |
| | 2 November 2007, 2-4 pm | Presentations, Q&A |

## Things to watch for

1) Be *VERY* careful not to get oil onto an air immersion objective lens, especially when switching from oil to air objectives as there is usually enough residual oil on the sample slide to corrupt the air lens. Before switching from an oil to an air objective, be sure to clean the sample slide with an OpticPad cleaning pads (lens paper with isopropanol).
2) *NEVER* clean any objective lens with a Kim wipe or OpticPads. Use only lens paper. Clean objective lenses with lens paper by the "drop and drag" method, and never apply direct pressure to the front surface of the objective lens.

## Samples

1) Carolina sample slides (pollen, stems, …). **Clean off oil with OptiPad when done**.
2) Fluorescent beads slides
3) *Drosophila melanogaster* embryos labeled with Histone H3 antibody with an AlexaFluor 488 (green) secondary antibody, and Dorsal antibody with an AlexaFluor 555 (red) secondary antibody. **DO NOT CLEAN OFF OIL AFTER USE!**

## Assignments

### A) Single Channel Imaging of Fixed Carolina Slides

For this part you only need to acquire single channel images, but be sure to try both the 488nm and 543 lasers, as they will have different optimal parameters (especially laser attenuation). Use Carolina Slides (blue box) and fluorescent beads slides for this part.

### A1) Compare differences in brightness and resolution between air and oil objectives.

Using one of the **Carolina slides**, take your best image using the **40x air objective**, recording the settings. Now, switch to the **40x oil objective** and collect an image using the same settings as for the 40x air lens. Then adjust the settings and take the best possible image with the oil lens.
***For Friday presentation:*** What adjustments did you need to make to improve the image taken with the 40x oil? How does the objective's NA fit in to the differences between these imaging parameters?

### A2) Try different scan speeds.

What gives the best image?
***For Friday presentation:*** Compare an image collected at a very fast scan speed with an image of the same region collected with a scan speed $\geq 8$.
**Use a 40x oil objective.**

### A3) Try different averaging schemes.

What gives the best image?
***For Friday presentation:*** Compare images of the same sample collected with or without averaging (try averages of 4, 8, 16).
**Use a 40x oil objective.**

**A4) Explore different pinhole diameters**. Take an image of a sample with the pinhole totally open and then a series of images with a smaller and smaller pinhole. What happens to the image as you decrease the pinhole? ***For Friday presentation:*** Compare an image of the same sample collected with the pinhole of
~ 1.2 airy unit with an image collected with larger and larger pinhole settings.
**Use a 40x oil objective.**

**A5) Collect images at varying Zoom settings.** How does image quality compare at 1x, 2x, 4x?
***For Friday presentation:*** Compare an image of the same sample collected with the pinhole of
~ 1.2 airy unit at Zoom settings = 1x, 2x, 4x. **Use a 40x oil objective**

### B) Two-Channel Imaging of Fixed *Drosophila melanogaster* Samples

You will be imaging with both channels to capture the localization of two labeled proteins: Histone H3 and Dorsal. **Samples: TA prepared slides found in the freezer (excite with 488nm and/or 543nm lasers).** Since there are a limited number of slides please be careful not to drop them.

**B1)** Working with a single channel at a time, collect images from the green channel **(ex 488nm => GFP emission)** or the red channel **(ex 543nm => Alexa568 emission)**. What settings are the best for you? Did the sample fade or bleach? **Use a 40x oil objective.**

Test the effects of
1) Laser attenuation
2) Scan speed
3) Pinhole size
4) Averaging schemes

**B2)** Employing the information you learned in above, collect two-channel images of the TA-prepared sample. **Use a 40x oil objective**.

***For Friday presentation:*** Collect two-channel images using various pinhole settings, scan speed settings and averaging schemes. Save your best images for presentation and discussion.

## General Notes about Friday Presentations

Each group prepares a presentation with a selection of images illustrating the topics covered in the assignment**.** Discuss any problems and successes you had during imaging–the goal of these sessions is mainly to troubleshoot your images.

Aim for a 10-minute presentation. Be prepared to show your images and the settings you used to collect them. If you have too many slides, save some for the Q&A session at the end of the presentation.

You can open and view your images with ImageJ, which works on most computer platforms and is freely downloadable (http://rsb.info.nih.gov/ij/). Most students use PowerPoint for their presentation.

**Please upload your presentation to the Mac computer in Church Room 68 prior to Friday's class meeting so that we can quickly move through all the presentations without changing computers for each group.**