



## Olympus BX51 and IX71 with Image Pro

(1/18/06)

**Login** at terminal for scope of choice.

Fill out paper log book

Start computer (if not on)

Login guest, guest

Turn on Mercury lamp if doing fluorescence

Turn on Halogen lamp for transmitted or reflected light

### **BX51 Microscope with Roper Cool Snap Camera**

Check light path on top of microscope. If knob is up the mercury lamp light path is open. If down, the reflected light path (halogen lamp) is open. When using the mercury lamp, you must have filter position 2-4 in place before looking into the eyepieces.

**With filter 1 you will expose your eyes to UV light.**

To decrease the intensity of the mercury lamp use the 25%ND filter (top left).

The other filter next to it is a diffusion filter for the halogen lamp.

You can add the top polarizer (on right) to reduce the intensity even more.

**Shutter** is just above the filter wheel. Always close shutter after acquiring an image or when not viewing specimen.

To send light to camera, pull out knob on right just below camera.

#### **Image Pro Plus**

FIRST Turn on camera

Start Image Pro Plus

Camera icon in top menu

In advanced mode check 2 things:

Setup—check 8 bits (12 bits if left unchecked). Images are always greyscale

Image—choose New Image unless you are doing a time course

Then choose **Basic Dialog** (this one is easier to use)

Hit **More** to expand

#### **Basic Dialog Settings**

Lock Exp Pvw and Exp Acq times to use the same for both

**Preview**—gives you a live image

Click Workspace Preview to get a larger image

Adjust focus, X-Y, and exposure time (just guess at the time)

**Stop** to stop preview

**Snap**—to acquire an image you can save

**Always close shutter after snap**



### **Other options**

**Capture Area**—keep locked. Set both to full frame to get the largest area of your sample. Image size is 1394 x 1040 pixels.

**Gain**—use 1 or 2.. Keep locked.

**Binning**—see below 1x1 binning gives the highest resolution.

## **IX71 Microscope with DP70 Color Camera**

Open shutter behind microscope for fluorescence or reflected light.

Check light path on top of microscope. If knob is up the mercury light path is open. If down, the reflected light path (halogen lamp) is open. When using the mercury lamp, you must have filter position 2-4 in place before looking into the eyepieces.

**With filter 1 you will expose your eyes to UV light.**

**Shutter** is just above the filter wheel on the right. Always close shutter after an acquiring image or when not viewing specimen.

**To send light to camera**, turn upper knob on front of scope down to camera icon. This does not send all of the light to the camera. Do **not** use the knob at the top of the scope (like on the BX51).

### **Image Pro Express**

Camera is always on

Start Image Pro Express

**Camera icon** in top menu

In Advanced mode choose the **Image** Tab

Choose New Image unless you are doing a time course

Use sequence and multiple images for a time series

Then choose **Basic Dialog** (this one is easier to use)

Hit More to expand

Lock Exp Pvw and Exp Acq times to use the same for both

**Preview**—gives you a live image

Do **not** check Workspace Preview

Adjust focus, X-Y, and exposure time (just guess at the time)

**Stop** to stop preview

**Snap**—to acquire an image you can save

**Always close shutter after snap**

### **Other Options**

#### **Resolution**

Pvw Resolution – Normal (bin 1x1) see Binning, below

Acq Resolution -- 1360 x 1024 pixels is plenty for most things. You can use 680 x 512.

For very high resolution, you can go larger.



**Capture Area**—Do NOT lock

Pvw Area can only be 680 x 512

Acq Area—Reset to full frame to get the largest area of your sample

**Capture Depth**

For real color, bright field images, or real color fluorescence images, use **24 bit color**.

For single color fluorescence images that you intend to merge, use **8 bit monochrome**.

If you are collecting separate red and green images with separate filter cubes and you want to merge them, you cannot collect them in color. You can colorize them later.

A 16 bit image (65,365 'grey' levels per color) will be 8MB in size. You don't need this.

**Gain**—can be 1-4

**Gamma**—changes the linearity of image brightness with sample brightness. Keep at 1 if you are doing fluorescence and want to compare brightness of images.

**Offset**—mostly for fluorescence, affects background contrast.

Lock all these.

**Binning**—see below

Lowering your resolution is the same as binning for acquisition.

## Both versions of Image Pro

**Saving images**

Make a folder under C:\user images e.g. C:\user images\myname

**Window Menu**

Close all—Tile—Cascade

F2 or F3 to go to next or previous image (open images only)

**Enhance**

Works with 8-bit or 12-bit or 16-bit images

**Display range**—Move upper and lower ends of range—if you convert to 8 bit these changes are permanent

Contrast enhancement—use for Gamma

Apply—makes Contrast enhancement. Gamma or LUT permanent

**Merging or adding Color**

Process--Color Channel—to merge RGB

Choose image for each color—Merge

To colorize an image, just choose one image.

**Other Acquisition Options**

Integration—Use **Dynamic** to build image



Choose Black Level  
Saturation warning ON  
If all blue, black level is too high  
Keep exposure short  
Use large # of images  
Stop when bright enough  
Integration—use **Sequential** to save intermediate images  
No black level adj  
Set # images and total time, calculate time/image  
Save best image  
Turn off integration when not required

### **Converting to 8 bit**

File—convert (?)  
Go to end of files and click up  
Convert to tif grey scale  
or  
**Edit—Convert**—choose 8 bit or whatever—one image at a time

## **Optimizing Image Quality**

### **8 bit vs 12 bit vs 16 bit Images**

For most work, 8 bits (256 gray levels is adequate).

Collecting images with 16 bits (65,365 gray levels) or 12 bits (4096 gray levels) is useful when you are doing quantitative work, complex image processing, or when you have very low light levels or high background.

When converting to 8 bit, you can choose the 256 levels anywhere within the larger scale.

**Color Images** are 24 bit, 36 bit or 48 bit, which is 8, 12 or 16 bits per color (RGB).

A 24 bit RGB image (8 bits per color) is a normal image.

36 and 48 bit images are very large, up to 8 MB and are mostly unnecessary.

### **Enhance-Display Range**

After acquisition, use the end bars to adjust the white and black levels. (This is just like Photoshop using Image-Adjustment-Levels.) The left bar chooses the level of grey that will be absolute white and the right bar sets the grey level below which everything will be black. You lose some of your grey levels but if you start with 4096 or 65,365, this is generally OK. Do not adjust so far that you have significantly less than 256 levels left. Use a longer exposure when this happens. When you convert to 8-bit, these adjustments become permanent and you will have only 256 grey levels.

You can also adjust the **gamma** (under **Contrast Enhancement**) but use this with care as it makes the relationship between brightness and fluorophore concentration non-



linear. Always use gamma=1 if you want to make any brightness comparisons between images.

### **Image Intensity**

You should always avoid saturating pixels, as information is lost and can never be retrieved. Keep your exposure time short enough so you are not saturating pixels. When you adjust your 12 or 16 bit images, do not make them too bright and also do not make them too black. The area outside the bars is lost when you convert to 8-bit and can never be retrieved (unless you save the 12 or 16 bit images—always a good idea). Further adjustment of the 8-bit image is possible with Photoshop.

**Saturation Warning**—makes any pixel that is off scale (over-exposed or saturated) red. Pixels that are too black turn blue

### **Gain**

This is an electronic boost to the signal. Your image will be brighter without increasing exposure time. Increasing gain also adds noise to the image. This can be muted with a median filter.

### **Binning**

1x1 binning gives the highest resolution. A 2x2 will make one pixel the size of 4 pixels and you will collect fewer pixels. On the DP70 camera, this is the same as lowering the resolution.

The large pixel will have a brightness equal to the sum of the 4 pixels. This is useful if your exposures are very long and high resolution is not required.

You can use binning for the preview so that the image refreshes faster. More than 500 ms preview time is very tedious for focusing. Then use 1x1 binning for the acquisition. In this case, do not lock the binning. If you check Adjust exp for binning, the exposure time for Acquisition will change even if it is locked.

## **Miscellaneous**

### **Scale Bar**

Measure—Calibration- Spatial

Name is the objective magnification

Apply—to active image

Mark—to add scale bar

On image is permanent but has fewer options

Non-Destructive is not saved unless you use:

Measure—Snap measurements, a new file is created and the bar is permanent.

Follow instructions carefully, the non-destructive is easier to use, the text and bar move separately, colors change together, you can redo it.

### **Measuring Fluorescence intensity**



**Measure—Histogram**

This measures the entire image, gives a few stats (under Report-Statistics), and puts all data to create the histogram into the Excel file. To see a subset of the data as a histogram, do Report-Range/Area. It is supposed to measure only the Area of Interest (AOI) if there is one on the image but this option does not seem to work.

**Process --Segmentation** (is the same as Threshold)

Allows you to highlight a range of grey levels

This can be used for counting or measuring morphological characteristics, but doesn't seem to be usable for measuring intensity

**Area of Interest (AOI)**

To be continued

**Specifications**

**Olympus BX51 upright microscope**

Roper Cool Snap HQ camera (black and white)

Objectives	Immersion	NA	Cover slip	Working Distance
U Plan Fluorite 10x	Dry	0.30	No	10 mm
U Plan Fluorite 20x	Dry	0.50	#1.5	1.6 mm
U Plan Fluorite 40x	Dry	0.75	#1.5	0.51 mm
U Plan Fluorite 100x Imm	Oil	1.3	#1.5	0.10 mm
LMPlanFI 20x	Dry	0.40	No	12 mm
LMPlanFI 50x	Dry	0.50	No	10.6 mm

**Filters in BX51 Microscope**

Filter position	Name	Ex	Dichroic	Em	Chroma #
1	BF	Bright field			U-MBFL3
2	DAPI	360/40 (340-380)	400	460/50 (435-485)	31000
3	GFP	470/40 (450-490)	495	525/50 (500-550)	41017
4	TRITC	545/30 (530-560)	570	605/75 (572-648)	41002B
5	Cy 5	620/60 (590-650)	660	600/75 (667-737)	41008



## Olympus IX71 inverted microscope

Olympus DP70 color camera

Mag	Name	NA	Working Dist. (mm)	Phase Setting	Cover slip	Olympus#	Cap / Collar*
4x	U PL FL Ph	0.13	17	PHLU	No	UC522	No
10x	U PL FL Ph	0.3	10	PH1U	No	UC523	No
20x	C PL FL Ph	0.4	6.75-7.55	PH1U	yes	UC345	yes
40x	LWD U FL	0.6	2.1-2.8	not	yes	UC377	yes
60x	LWD C PL FL Ph	0.7	1.1-1.89	PH2U	yes	UC351	yes

PH=phase

LWD=long working distance

\*Correction cap for plastic 1.1mm – take off for plastic dishes

Collar for vessel thickness +/-0.5 – keep at -0.5 for normal use

## Filters in IX71 Microscope

Position	Name	Ex	Dichroic	Em	Chroma #
1	BF	Bright field			U-MBF3
2	HQ: FITC	480 / 40	505	535 / 50	41001
3	HQ: TR	560 / 55	595	645 / 75	41004
4	B/G	FITC/Texas Red			51006