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Setup of Oil Objectives – ImageXpress Micro

General Recommendations for Working with Oil Lenses on the ImageXpress Micro: Setting Up the Lens/Oil/Sample Interface

- Using the functions in Plate Acquisition & Control dialog, step up Z until you find the point at which the oil makes contact with the plate/slide, then do not drop the objective lower. (You can pull open the side doors to see this point on the IXM.)

- Work in very small movement ranges. Due to the small movement ranges, you will have to select "Skip Find Sample" (see next slide, Autofocus Tab in Plate Acquisition Setup).

(Y	Z	Load Settings	Summary	
		Save Settings	Setup	
	Z: 0.00	Experiment base name: Experiment 1 Wavelength: W1 - unselected		
Well: A01				
Go To well: AI	Step size. 10			
Go To A1	Find Sample	Snap Current	Show Live	
Eject Plate	Autofocus	Preview	Acquire Plate	
9)				



General Recommendations for Working with Oil Lenses on the ImageXpress Micro: Using Image Autofocus

We have created two journals (that can be implemented so that IAF is possible when imaging with oil (see screenshot below).

The first journal "FocusOffsetsforOil_Start_of_Plate.jnl" will prompt you (at start of plate imaging) to set up focus offsets for up to 7 wavelengths. This is useful if you are using the 100X Oil Plan Fluor lens, which is not color corrected. However, you should also run this with the 60X Oil PA lens, just enter "0" for your desired wavelengths – this will prevent the system from performing a new focus search for all WL subsequent to WL1. You should calculate any Z offsets manually during setup (see previous slide).

Modify the "Find imagebased focus_v3_Before_Each_Image.jnl" (find focus, set the range appropriately for your lens/sample) according to your needs and enable it "before each image" when using IAF for oil imaging. It will reference the offsets in the start of plate journal

MX5.1 SB - 10/01/2013- Hook this journal up before each image. It will perfrom an image based autofocus of 5 um IF Screen.Status.WaveNum=1 THEN ELSE The focus(5, 0.5, NOBACKLASH) END IF *** End of Journal ***	Algorithm: Standard Range, current +/-: 5 Accuracy: um(s): 0.5 Number of z moves: 6	
Keep in mind that image based focus exposes your sample to excitation light at every step, so photobleaching may occur if extensive focus ranges are used.	Current position = 0 Backlash compensation Display image being acquired Find Focus More >> Close	



General Recommendations for Working with Oil Lenses on the ImageXpress Micro: Using Image Autofocus (Cont'd)

Before you begin imaging, you should also check the Acquire dialog (under Control > Acquire > Acquire) and ensure that it is set to use Current Shutter.

Acquire	Image: 🛃 Shading Image
Save Image	Save to: CMXXVAcquired001.tif Set Save
Save w/Sequence	Display Acquire Correct Deconvolution Annotate Special
xposure Time:	Auto-Expose Settings:
5 🔶 [ms 🔻]	Target Intensity: 191
AutoExpose	% of Max [4095]: 4.7
lipping: 1 🚔	Maximum Exposure: 30 🚖 sec 👻
Camera Area:	External Shutter Linked to Camera:
Full Chip	Illumination: Cy5
-> Center Quad.	[None] Preferences: [Current Shutter]
Use Active Region	Amount to ac DAPI
Show Live	Reflectance
ive Bin: 1 🚖	Zoom live TL TRITC
Temp:-20.7 c	
	Custom Field of View:
etting [Modified]:	Enable Custom Field of View Width: 10 🚔 % He
zyla no binning 👻	



Strategy for Imaging Multiple Sites/Wells with Oil

When imaging multiple sites per well, keep the sites close to each other to minimize "dragging" of oil across intervening areas. Settings can be configured on the "Sites to Visit" tab in Plate Acquisition setup.

eriment- Experiment1	Site Acquisition Op	otion		
Names and Description	O Single site	Fixed number	or sites U Adaptive acquisition	
Objective and Camera- 100X Oil	Site layout in well:	Spacing (um):	Image size: "Emp: Image calibration units	
Plate- Costar 96-well Plastic	Columns: 3	U 💌	Custom field of view (%) X: 85 + X85 +	
Wells to Visit- 15 of 96	Rows: 3	0	Well size: 7.00 x 7.00 mm	
Sites to Visit- multi-site	Minimum to vis	it 2 👘	Image spread: "Error: Image calibration	
Timelapse- 1 time point(s)	Total Sites: 9			
Acquisition Loop	Tile sites	Fit sites to well		
Autofocus				
W1 DAPI				
Journals- 0 selected				
Display Settings			Site locations:	
Post Acquisition				
Summary				
_	Save Settinge	Summan	Previous Next Close	

- General guidelines:

- For 96 well plates: 1 drop should cover most of a single well (especially if sites are directly adjacent)

- For 384 wells: 1 drop for ~2 wells
- When moving long distances (well-to-well move for 384 and 96 well plates) you might consider dropping the objective between moves to prevent oil being wasted. Oil will be left on the objective and may be enough to image an additional well.



General Recommendations for Working with Oil Lenses on the ImageXpress Micro: Use of Laser Autofocus (LAF)

- On a plate: LAF can be used, however you will want to search for one surface only (bottom of well). Do NOT use the LAF wizard – you will need to adjust the settings manually.

- On a slide: LAF can be challenging to set up, review the diagram in the IXM Slide Taskbar handout. You will have to increase exposure time to see two surfaces, coverslip/mounting media and mounting media/slide. Don't try to see the top surface (slide/air) as that will be too high for the WD of the objective. Whether it is best to search for one surface or two depends on the specific sample.



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