Oil Objective

Introduction

The Lionheart FX automated imager is compatible with high numerical aperture oil immersion objectives. These objectives offer magnification up to 100X and significantly improve the optical resolution of the system. Cellular details, as small as a few hundred nanometers, can be resolved on the Lionheart FX using oil-immersion microscopy. Here we describe how to use oil immersion objectives on the Lionheart FX and best practices for producing high quality fluorescent images.

Hardware

There are two Olympus oil immersion objectives available for use with the Lionheart FX automated cell imager which can deliver magnifications of either 60X or 100X (Figure 1). As the name suggests, these highly specialized objectives must be used with oil. A small drop of immersion oil is deposited between the front lens of the objective and the coverslip “linking” them together. The transparent oil has a high viscosity that produces an uninterrupted liquid column between the two surfaces. Moreover, the oil is optically similar to glass with a refraction index of 1.515. This increases the maximum angle of the cone of light that can enter the front lens of the objective which is a characteristic that is described by the objective’s numerical aperture (NA). The greater the NA of an objective, the more light it is able to collect. Additionally, objectives with large NA values can resolve greater detail in a sample. Oil immersion objectives have much larger NA values compared to “dry” objectives.

Figure 1. Oil-immersion objectives available for use with the Lionheart FX.

Detailed specifications of these objectives can be found in Table 1. An important specification to keep in mind when using oil-immersion is the objective's working distance-- the distance between the front lens of the objective and the surface of the coverslip. The oil-immersion objectives used in the Lionheart FX have extremely short working distances (<200 microns). Therefore, great care must be taken to prevent damaging the front lens by collisions with the coverslip.

Importantly, the short working distance of oil-immersion objectives make them incompatible with thick plastic bottom microplates (or labware) and must be used with 0.17 mm thick coverslips. Cell culture vessels typically have bottom thickness that range from 0.5 mm to 2.0 mm. Due to the short working distance of oil immersion objectives, attempting to image cells through thick bottom vessels is impossible with these short working distance objectives, as the front lens of the objective would collide with the culture vessel before the sample could be brought into focus. Although the front end of both the 60x and 100x oil objectives are spring loaded to minimize costly damage from inadvertent collisions, they should never be used with incompatible culture vessels.

Magnification	100x	60x
Numerical aperture	1.40	1.42
Working distance	0.13 mm	0.15 mm
Depth of field	.28 µm	.27 µm
Coverslip thickness (mm)	0.17	0.17
Optical correction	Plan Super-Apochromat	Plan Apochromat
Mfg.	Olympus	Olympus

Table 1. Oil-immersion objectives available for the Lionheart Fx.

Procedure: Using Oil Immersion Objectives with the Lionheart FX

Instructions for installing oil-immersion objectives can be found in the Lionheart FX Operator's manual. Note, oil-immersions objectives are not calibrated during setup and are only compatible with manual mode imaging.

1) Define the bottom elevation of your vessel.

Before entering manual mode, it is important to define the bottom elevation of your slide, glass bottom plate or any other vessel you intend to image. Instructions for defining the bottom elevation of a vessel can be found in the Gen5 help menu or on page 39 of the Cell Imaging Training Guide. You will need to use an air ("dry") objective to complete this process.

2) Find the area and/or objects of interest with a lower power air objective.

As usual in microscopy, it is most efficient to begin with a lower magnification to find your objects of interest before switching to a high magnification objective. Using a lower power air objective (such as the 20X) will make it easier to find an area of interest because of its large field of view. Use either the joystick or the navigation buttons in Gen5 to center objects of interest before moving on.

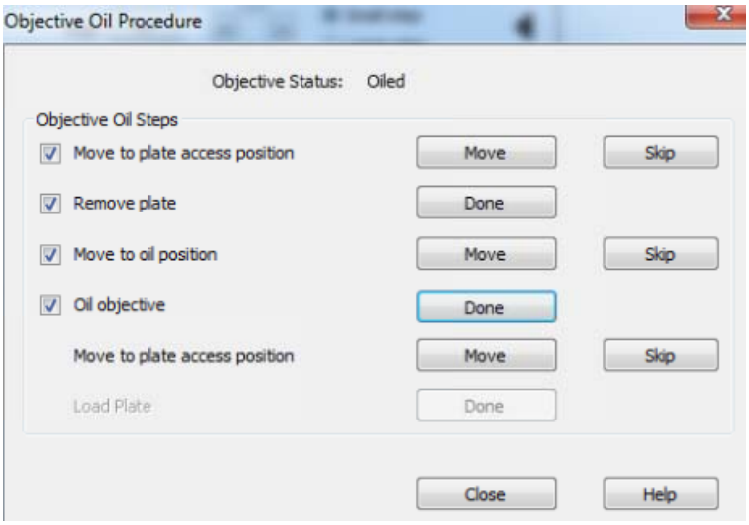
3) Lower the objectives 500 microns BEFORE switching to the oil-immersion objective.

The oil-immersion objectives used in the Lionheart FX are not calibrated during instrument setup. Unlike the non-oil objectives installed in the instrument, focal height may not be maintained once you switch from a lower power objective to an oil-immersion objective. It is recommended that you lower the z-height before switching objectives to reduce risk of collisions.

4) Change to the oil-immersion objective and follow the oiling procedure.

Choose the oil-immersion objective you wish to use and click the oil can icon () to open the Objective Oil Procedure wizard. Follow the instructions on-screen and click close when you have completed all the steps.

Important: Do not add an excessive amount of oil to the front lens of the objective. One drop should be sufficient to cover the entire lens. Adding too much could cause oil to run down the side of the objective and damage sensitive components in the instrument.

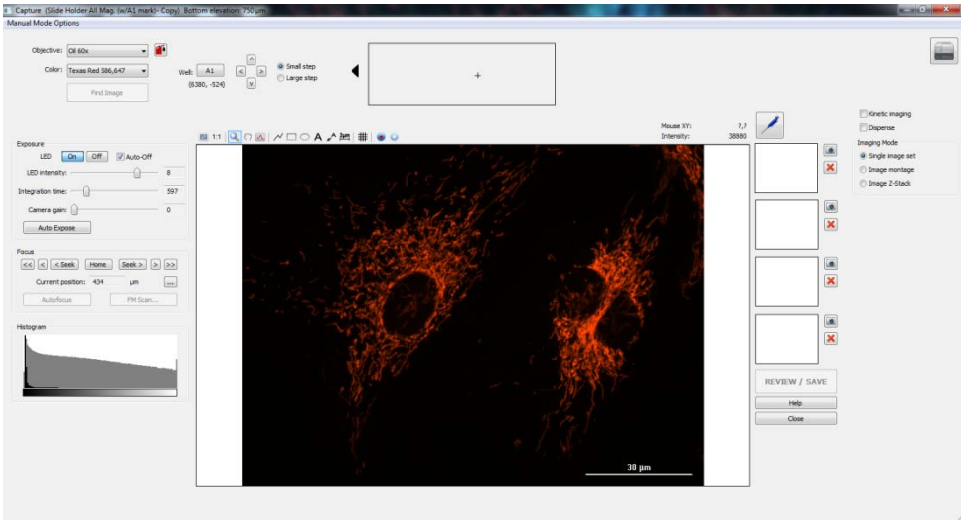


5) Slowly raise the objective until your object of interest comes into sharp focus.

Once the Objective Oil Procedure wizard is closed, Gen5 will resume the manual mode session. Slowly increase the focus position by using either the joystick or the focus arrows in the manual mode window.

6) Center the objective of interest and adjust exposure settings.

Use the directional arrows to center your object of interest in the field of view. Adjust the exposure by clicking the auto expose button. You can also manually define your exposure settings using the sliders in the Manual Mode window.



7) Clean the objective before exiting Gen5 or changing objectives.

Gen5 considers an objective to be “dirty” after completing the steps of the Objective Oil Procedure. Before exiting Gen5 or changing objectives, you will be prompted to clean the objective. Properly removing any residual oil from the front lens of the objective is critical to prevent costly damage to the instrument or the objective itself.

To clean the objective, follow the instructions in the Objective Clean-Up window and click close when you are done.

Warning: Only clean the objective with lens paper. Using other cleaning products such as multi-purpose lab wipes or paper towels will scratch the lens of the objective causing irreparable damage. Never ‘polish’ or push hard on the front lens with dry lens paper. Instead, gently wipe the oil off and use multiple sheets of lens paper if needed.

Best Practices and Tips

Below is a list of best practices and tips for imaging with oil-immersion objectives. Following these recommendations will allow you to make the most of these high magnification objectives and ensure the capture of high quality images.

1) Image your sample through #1.5 (0.17 mm thick) cover slips.

As mentioned above, the oil immersion objectives available for use in the Lionheart FX can only image samples through glass 0.17 mm thick. Using coverslips of the correct thickness is crucial to obtaining high quality images and to prevent damage to your objective. Note: Since the Lionheart FX is an inverted microscope by design, microscope slides must be loaded with cover slip facing down.

2) Only use Olympus® Immersion Oil Type-F supplied with your objective.

Objective manufacturers design immersion oil based on the optical properties of their objectives. Using immersion oil from another manufacturer can have significant effects on image quality due to small variations of the oil’s refractive index. Importantly, if you intend to use a slide previously imaged on another microscope, make sure that the coverslip has been cleaned thoroughly. Even a small amount of residual oil from another manufacturer can degrade image quality when mixed with the proper oil.

3) Lower the LED intensity to minimize the effects of photobleaching.

Keep in mind that using high magnification oil-immersion objectives will photobleach your sample much faster than an objective with a lower NA. Objectives with high NA collect more light from the sample and therefore are more sensitive to dim signals. However, this also means that your sample is exposed to more light as the objective acts as the source of illumination in fluorescence microscopy. When using these objectives it is good practice to lower the LED intensity while focusing or searching for objectives of interest and turn the LED off when not in use.

4) Be patient while focusing.

Quickly raising the objective can result in damaging collisions between the objective’s front lens and the coverslip. Slowly bring your specimen into focus using the fine focus arrows or, if available, by gently twisting the joystick.

5) Always secure your slide to the holder.

Use the clips on the slide holder to secure the sample slide in place. As the objective is brought closer to the sample the high surface tension of immersion oil can push the slide out of its holder if not properly secured.

6) Clean your objective with pure ethanol as needed.

If required, pure ethanol can be used to clean excessive oil from the objective. As described above, only use non-abrasive lens paper when cleaning an oil-immersion objective to prevent scratches.