GE Healthcare

Life Sciences

DeltaVision OMX™

Customer Instructions

Ring TIRF Option

DeltaVision OMX™ Ring TIRF Option

- *Describes TIRF and Ring TIRF imaging techniques*
- *Illustrates the advantages of the DeltaVision OMX Ring TIRF Option*
- *Includes Ring TIRF-specific laser safety information*
- *Describes how to use Ring TIRF on the DeltaVision OMX*

What is TIRF?

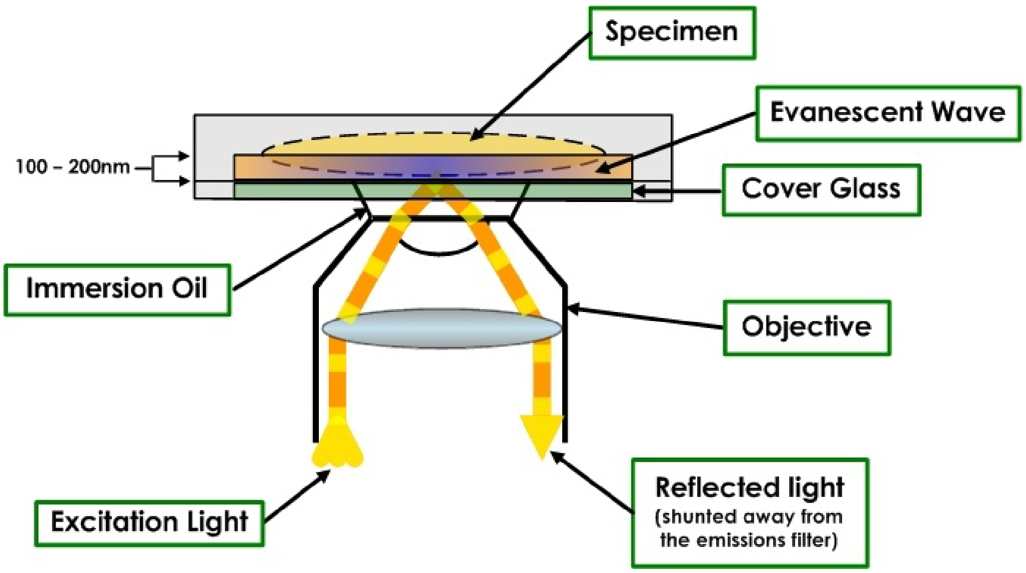
TIRF (Total Internal Reflection Fluorescence) is an optical sectioning technique that limits fluorescence imaging to the thin area at the surface of a specimen, closest to the cover slip/mounting medium surface, and typically limited to a depth of 100 - 200nm. This limitation results in an enhanced signal-to-noise ratio and increased imaging contrast.

TIRF uses a simple law of physics to improve biological imaging. When light passes from a medium of high refractive index to a medium of low refractive index and the angle of incidence is greater than or equal to the critical angle, the light will reflect off of the interface and not actually enter the second medium. Under these conditions, an electromagnetic wave traveling perpendicular to the interface is created. This electromagnetic wave, known as an evanescent wave, has the same wavelength as the light that created it and decays very rapidly in the direction of the optical axis such that most of the energy is lost within 200 nanometers of the interface.

The Evanescent Wave

The following illustration describes the electromagnetic wave created when TIRF is used. This wave is known as an evanescent wave and it illuminates the specimen to a depth of only 100 - 200nm from the coverslip.

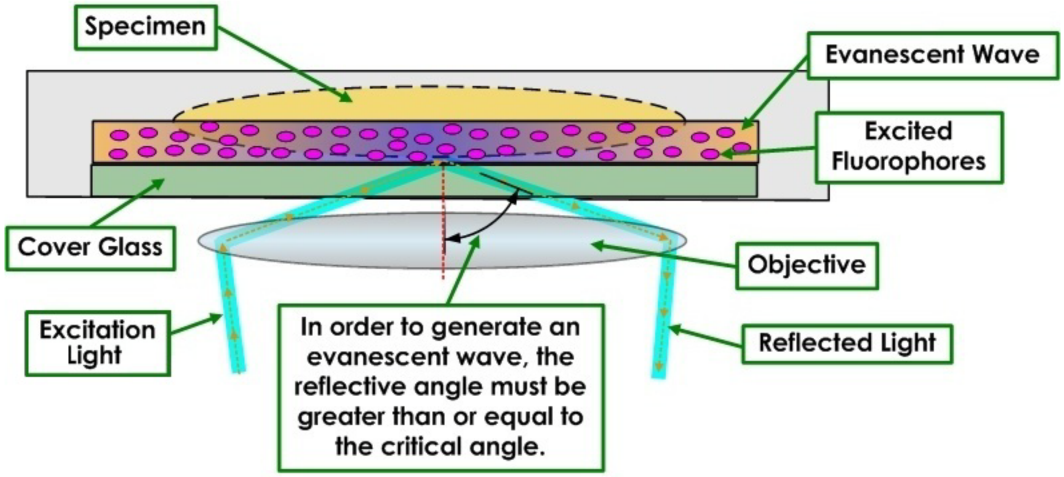
Figure 1. TIRF's Evanescent Wave



If there are fluorescent molecules in close proximity to the coverslip surface, and these molecules are capable of absorbing the wavelength of the evanescent wave, then these molecules (known as fluorophores) will become excited and fluoresce. Since the evanescent wave decays rapidly, molecules that are more than about 200nm from the surface of the coverslip will not be sufficiently excited to fluoresce. These molecules contribute to neither the signal nor the background fluorescence (noise).

For TIRF slides, the sample is usually grown on a glass cover slip and then mounted in a water-based buffer with a refractive index of approximately 1.33. The glass cover slip typically has a refractive index of about 1.515. When light is introduced from the cover slip to the buffer at an angle that is greater than about 61°, the light reflects off the glass-water interface and establishes an evanescent wave that will travel into the buffer. Fluorophores close enough to the interface and that can be excited by the evanescent wave will generate fluorescence that is then transmitted through the objective lens and detected by the camera.

Figure 2. TIRF Reflective Angle

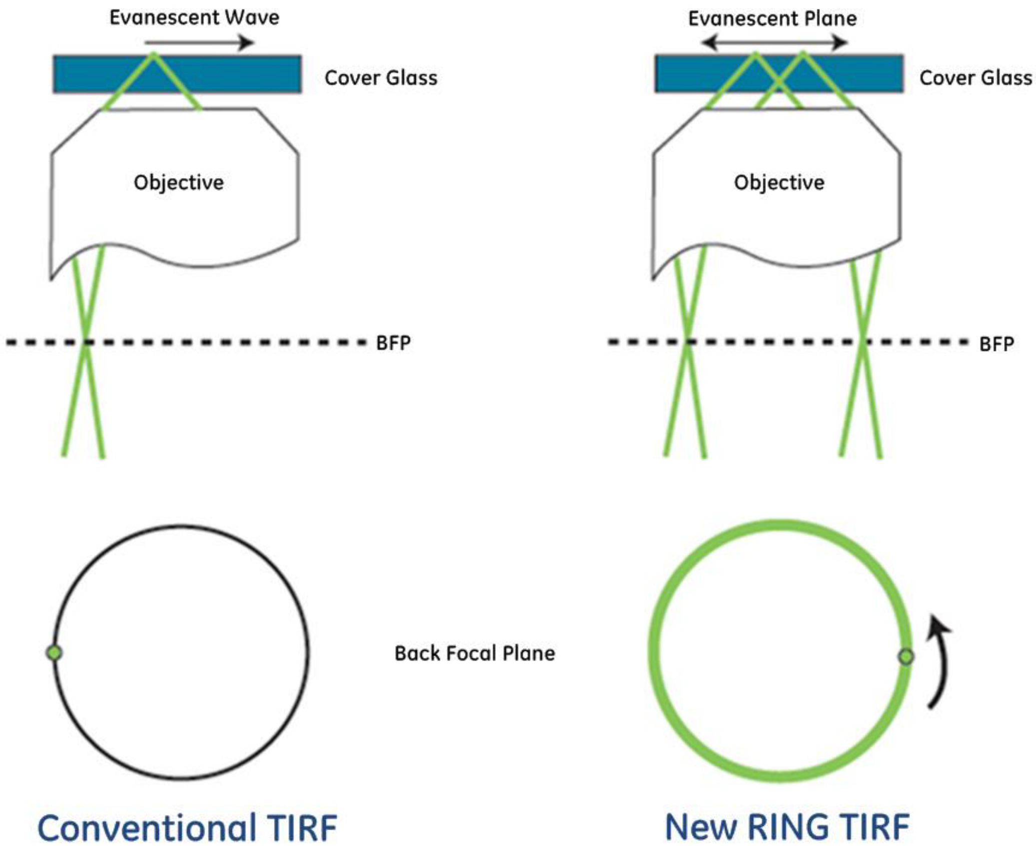


What Makes Ring TIRF Different?

Traditional TIRF, as described earlier in this topic, is achieved by aligning a laser near the edge of the back focal plane of a TIRF objective lens with a high NA (numerical aperture). This allows light to hit the coverslip at a very high incident angle, higher than the critical angle of the interface between the coverslip and the sample surface. The excitation light then reflects within the coverslip, creating an evanescent wave that propagates along the surface of the glass. One challenge with this approach is that any defects in the light path, including dirt or dust on the coverslip, can result in an uneven TIRF illumination field, resulting in poor sample excitation.

Ring TIRF, in comparison, uses a pair of high-speed, galvanometer-controlled mirrors that steer the laser spot in a circle at the back focal plane of the lens at a rate of about 600 revolutions per second. This revolving circle, or “ring,” of laser light creates an evanescent wave plane that is generated from multiple sides almost simultaneously, producing a more even TIRF field and improved sample illumination. Ring TIRF’s improved sample illumination represents a significant improvement over traditional TIRF as it removes interference fringes and provides fast, multi-angle illumination that allows 3D illumination of the cell structure. Ring TIRF also permits the TIRF field’s depth of illumination to be easily adjusted by modifying the diameter of the circle, allowing the researcher to optimize the TIRF field depth for different excitation wavelengths.

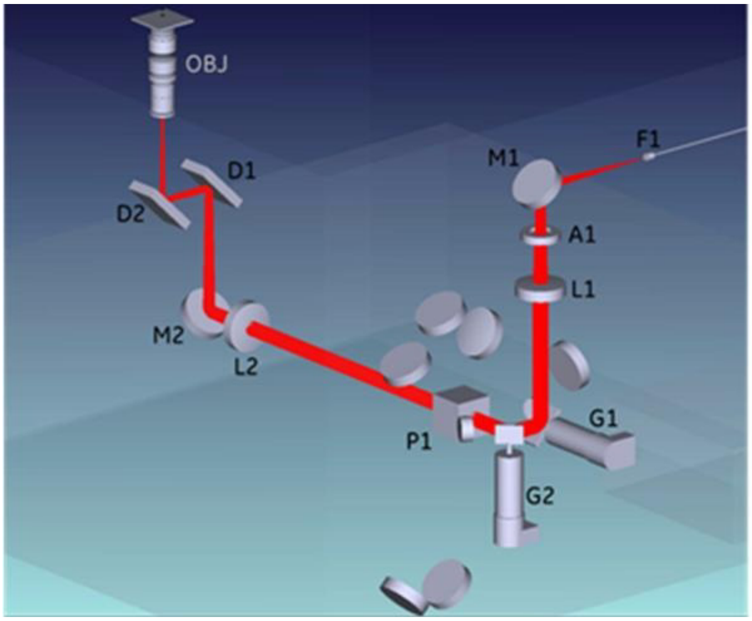
Figure 3. TIRF and Ring TIRF Compared



The DeltaVision OMX Ring TIRF hardware also incorporates a by-pass system that allows light to be delivered to the sample for PK (Photokinetic/Photobleaching) applications, such as FRAP (Fluorescence Recovery After Photobleaching). As the same mirror system is used to control both the TIRF and PK beams, it is possible to bleach areas of different size and configuration depending on how the PK beam is directed.

The following figures illustrate both the Ring TIRF and PK light paths.

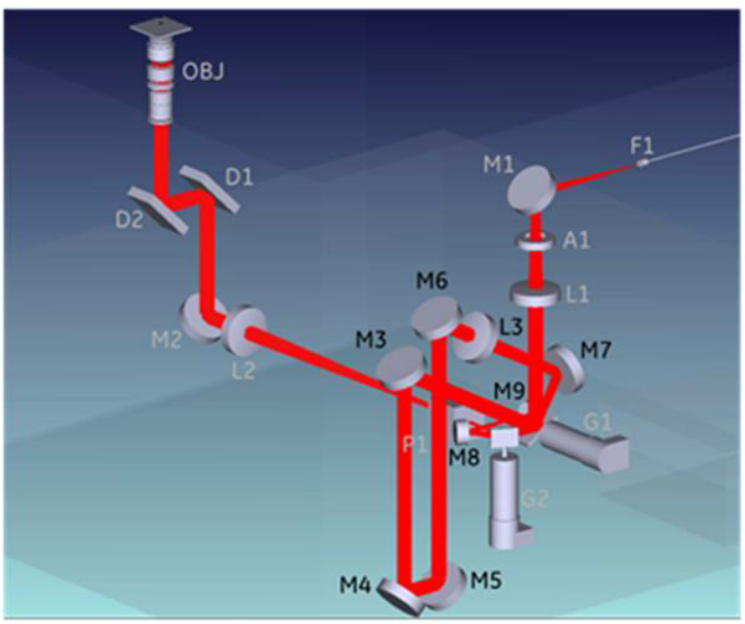
Figure 4. Ring TIRF Module TIRF Light Path



Optical Components

F1: Fiber Launch
M1: Mirror 1
A1: Field Aperture
L1: Collimating Lens
G1: X-Galvo
G2: Y-Galvo
P1: Polarizing Cube
L2: Focusing Lens
M2: Mirror2
D1: Polychroic Mirror
D2: HWAF Dichroic Mirror
OBJ: Objective Lens

Figure 5. Ring TIRF Module PK Light Path



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OBJ: Objective Lens

M3: PK Mirror 3
M4: PK Mirror 4
M5: PK Mirror 5
M6: PK Mirror 6
M7: PK Mirror 7
M8: PK Mirror 8
M9: PK Mirror 9
L3: Focusing Lens

NOTE Based on the hardware configuration of your system, the light paths may vary from those shown above.

The Ring TIRF system utilized on the DeltaVision OMX system was developed by researchers at Yale University and is exclusively licensed to GE Healthcare Company.

Ring TIRF Hardware

The Ring TIRF module is mounted on the OMX block, as shown in the following figures.

NOTE Based on the configuration of your system, the hardware may vary from that shown in the following figures.

Figure 6. Ring TIRF Module Mounted on OMX Block (Yellow-Bordered Area)

Figure 7. Ring TIRF Module (Close Up)

Ring TIRF-specific Laser Safety Considerations

Due to the Ring TIRF illumination optics, the light being emitted from the DeltaVision OMX objective is collimated and has high power density. The TIRF system also has the ability to direct this light to sharp off-axis angles relative to the objective axis.

WARNING! When servicing the TIRF system, use extreme caution that the emitted light is not directed into the user's eyes. Appropriate laser safety goggles selected for the specific wavelength being tested are mandatory.

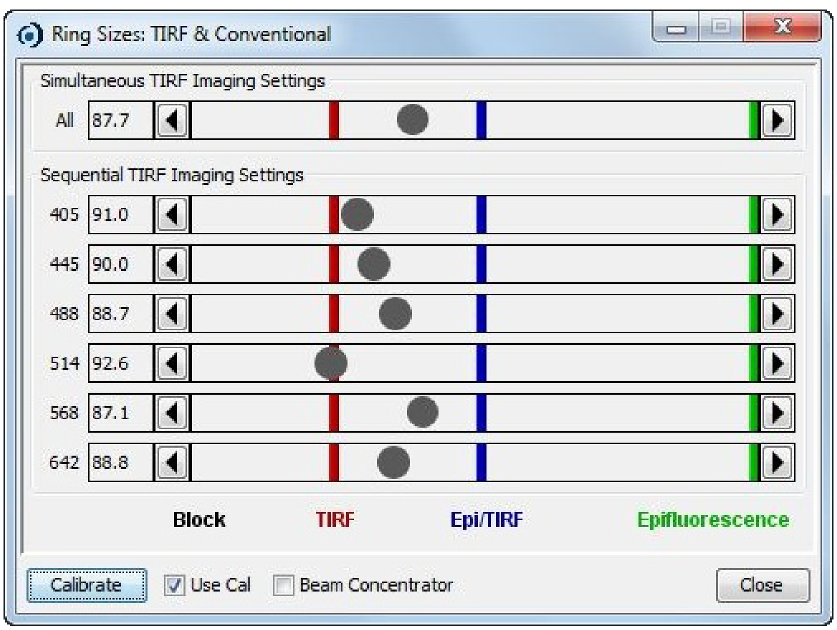
Using Ring TIRF

The first section in this topic describes the primary Ring TIRF dialog box in the DeltaVision OMX software, including the options used to calibrate the system for use with non-flat coverslips. The second section describes how to set up and use the Ring TIRF option.

The Ring TIRF Dialog Box

To open the Ring Sizes: TIRF & Conventional dialog box, click **Instrument | TIRF Setup**. The software will display the following dialog box.

Figure 8. Ring Sizes: TIRF & Conventional Dialog Box



As shown in the previous figure, a single slider defines the TIRF diameter for simultaneous TIRF imaging; however, when setting the individual TIRF diameters for sequential imaging, separate sliders define each laser.

The gray circle on each slider represents the diameter of the laser ring in the back aperture of the objective. Moving the gray circle to the right end of the slider decreases the diameter of the laser ring while moving the circle to the left end of the slider increases the diameter. The red and blue vertical bars on each slider represent the transition points, where illumination transitions from TIRF to epifluorescence (blue bar) or from TIRF to block (red bar). Keep in mind that TIRF illumination is blocked when the laser is beyond the TIRF critical angle.

The ring diameter can be adjusted using any one of (or combination of) the following methods:

- Using your mouse to drag the gray circle to the left or right on the slider.
- Entering a percent value into the data-entry field located on the left side of the slider.
- Clicking on the left or right arrow on either end of the slider bar. This moves the gray circle in the direction of the arrow..

Note Typical TIRF experiments do not require the Beam Concentrator, so the check box should remain unchecked. When checked, the Beam Concentrator provides a denser TIRF illumination, but limits the field of view. (Depending on the system's hardware configuration, the **Beam Concentrator** check box may not be available.)

Calibrating Non-Flat Cover Slips

The lower-left corner of the TIRF Ring Sizes dialog box contains the two controls used to calibrate Ring TIRF imaging for use with non-flat coverslips.

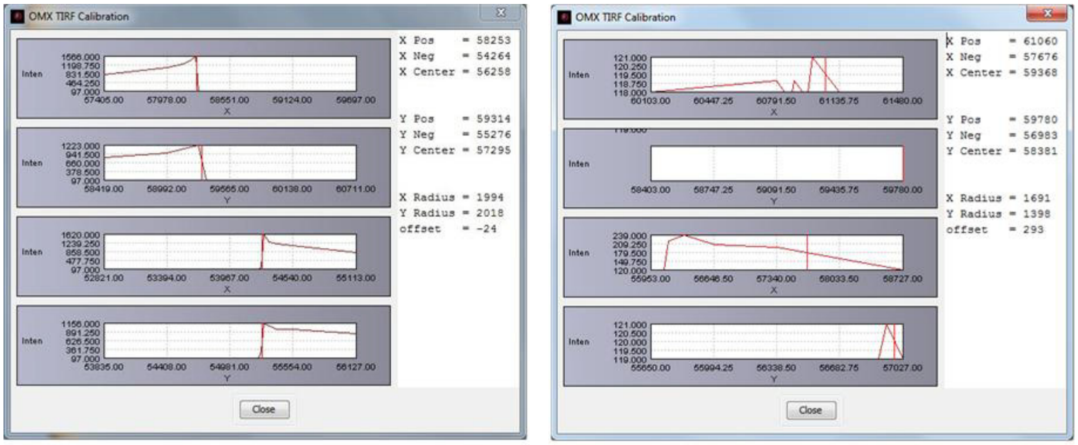
IMPORTANT! The non-flat Ring TIRF calibration performs image-based analysis on your sample, so ensure that your sample is in focus prior to running the calibration. As sample bleaching may occur during the calibration, choose a field of view that you do not plan to image later.

Figure 9. Ring TIRF Calibrate Controls

When a cover slip is mounted to the slide at a slight angle, the Ring TIRF "circle" becomes an "ellipse" that is oriented at an angle. Clicking the Ring TIRF **Calibrate** button initiates a calibration routine that updates the center of the ellipse and offsets that angle based on the collected data. The calibration is run using the currently loaded slide and the new parameters are calculated on the cover slip mounted on that slide.

When you click the **Calibrate** button, the system runs the calibration and displays the results. Results from two sample calibrations are displayed in the following figure. The results on the left are acceptable. Notice the steep edge in each of the four plots. The vertical line in each plot shows the point the software determined to be the middle of the "edge" and that position is displayed in the text on the right. The example on the right side of the figure shows "bad" plot results. Notice the low intensity values and how the data jumps around in the top and bottom graphs. In the middle graphs either the software could not find an edge and so defaulted to the last point or, even though there was an edge, the software was unable to find it because it was so far out.

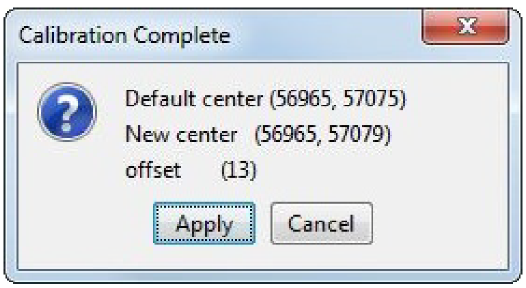
Figure 10. Calibration Results



Acceptable Results **Unacceptable Results**

Once the calibrations are complete, click **Close** and you will be given an opportunity to click either **Apply** (to accept) or **Cancel** (to cancel) the results.

Figure 11. Ring TIRF Calibration Complete



The **Use Cal** check box allows you to switch between the default calibration values stored in the configuration file that was defined upon system installation and the last calibrated values. Activate the **Use Cal** check box to use the last calibrated values; deactivate the check box to use the default calibration values stored in the configuration file.

Procedure - Using TIRF

1. If not already installed, install the TIRF objective onto the objective mount located on the OMX optics block.
2. Set the collar on the objective to the correct coverslip thickness.

> **Note** Most TIRF experiments require #1.5 coverslips, so the collar should be set to 0.17. (This value refers to the 0.17 micron thickness of the #1.5 coverslip.)

3. Ensure the required lasers are turned on.

4. Mount the sample slide and focus on the sample at the coverslip using the appropriate camera and excitation settings in the OMX software.

Tips

#1 Selecting "Conventional" as the **Light Path** setting may help you focus on the sample at the coverslip.

#2 Raising the stage brings the coverslip more closely into focus, so raise the stage to the highest position at which the sample remains in focus.

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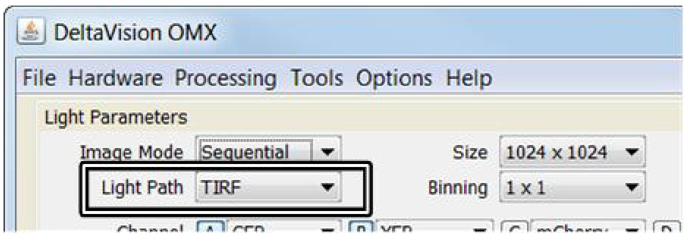
5. In the **Image Mode** field, select either the "Simultaneous" or the "Sequential" imaging mode.
6. In the **Light Path** field, select "TIRF."

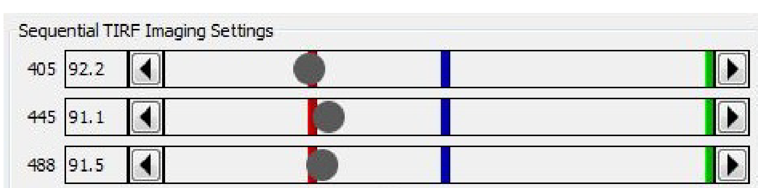
Figure 12. Select TIRF Light Path



7. Set the **%T** and the **Exposure** fields to produce imaging conditions for a reasonable signal level.
8. In the main menu, click **Instrument | TIRF Setup** to open the Ring Sizes: TIRF & Conventional dialog box.
9. Choose one of the following:
   - If **Image Mode** was set to "Simultaneous" in Step 4, adjust the position of the gray circle on the top slider bar to a location close to the red TIRF indicator bar, as shown below.

OR

   - If **Image Mode** was set to "Sequential" in Step 4, adjust the position of the gray circle on the slider bar for each of the selected excitation filters to a location close to the red TIRF indicator bar, as shown below.



NOTE The Continuous Acquire feature uses the "Simultaneous TIRF Image Settings," regardless of the current **Image Mode** setting. If continuous acquisition is being used to determine the TIRF ring size, only changes to the "Simultaneous TIRF Image Settings" will show effects on the continuous acquisition.

As you increase the ring diameter into the TIRF zone, the signal being generated from the portion of the sample located right at the coverslip will remain, while signal being generated from above the coverslip will fade away. When you reach TIRF, the background will be very dark and the signal will be the most intense (in comparison to epifluorescence).

10. Once you have determined your ideal TIRF setting(s), proceed to run your TIRF experiment(s).

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Notice to purchaser
DeltaVision OMX Imaging Systems are for research use only – not for use in diagnostic procedures.

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