Appendix A: The Immersion Oil Kit

The immersion oil kit is a collection of oils with refractive indexes that range from 1.500 to 1.534. Use of the correct immersion oil decreases the spherical aberration in the image data.

• For DeltaVision, the immersion oil kit includes eighteen oils that range from 1.500 to 1.534, in increments of 0.002.

Note For personalDV, the immersion oil kit includes six oils that range from 1.512 to 1.522, in increments of 0.002.

Many factors influence the optimum refractive index of the immersion oil, including specimen preparation, temperature, humidity, and atmospheric pressure.

The Oil Calculator

In order to calculate the desired refractive index, softWoRx is equipped with the Lens Information function. This function is located in the Utilities menu in softWoRx. It can also be accessed from Resolve 3D by clicking the Info button. The following parameters are explained here to help you enter the appropriate information and use the resulting calculations.

Distance from Coverslip to Specimen (microns)
Establishes the distance from the surface of the coverslip to the desired focal plane.
**Temperature**
Defines the temperature of the specimen and the immersion medium.

**Specimen Refractive Index**
Defines the refractive index of the specimen, which is usually that of the mounting medium. In some cases, the specimen itself contributes significant refraction.

**Recommended Refractive Index**
Displays the resulting optimal refractive index of the immersion oil. Actually experimenting with oils with refractive indexes very close to this value is the best way to select the optimal oil.

**Resolution Ratio**
Displays the ratio between the Z resolution and the XY ratio. This serves as a reference to the degree of Z elongation.

**Maximum XY Pixel Size**
Displays the maximum recommended XY pixel size for deconvolution.

**Recommended Z Step**
Displays the smallest possible Z step for this objective. Choosing a smaller Z step will add to the size of the image file, but will not improve image quality.
Appendix B: Troubleshooting

This appendix was designed to help you diagnose and correct the most common problems encountered on the *DeltaVision* system. Two types of troubleshooting tasks are covered:

- **Diagnosing System Problems**
- **Analyzing Reasons for Poor Image Quality**

If you are unable to correct a problem, fill out the *DeltaVision* Problem Report Form at the end of this appendix and either e-mail it to hotline@api.com or fax it to 425-557-1055, attn: Bio Service Hotline.

**Diagnosing System Problems**

**Troubleshooting the Controller**

The following table shows the most common Instrument Controller problems and their resolutions.
### Table B-1: Controller Troubleshooting Chart

<table>
<thead>
<tr>
<th>Indication</th>
<th>Cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encoder Error when initializing stage.</td>
<td>Poor cable connection.</td>
<td>Power down system, including IC/MIC.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reseat X, Y, and Z motor cables.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reseat motor cables on excitation module.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Power up system.</td>
</tr>
</tbody>
</table>

### Troubleshooting the Workstation

Other system troubles are indicated by messages or readings in the software. The Resolve 3D message window displays Resolve 3D activity. Observe the messages in this window when troubleshooting. This table shows possible problems and corrective actions.

### Table B-2: Workstation Troubleshooting Chart

<table>
<thead>
<tr>
<th>Indication</th>
<th>Cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;File system full&quot; message when trying to save images.</td>
<td>There is no more storage space for image data.</td>
<td>Delete unwanted files.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Save image files to CD or DVD or LAN.</td>
</tr>
<tr>
<td>&quot;Camera not found&quot; message.</td>
<td>Power up sequence was incorrect.</td>
<td>Shut down the system and then restart it using the steps described in Chapter 9: Maintenance.</td>
</tr>
<tr>
<td>Resolve 3D settings are not updating changes made using the keypad or joystick (for example, the filter selection, or Z position).</td>
<td>Lack of communication between the Instrument Controller and Workstation.</td>
<td>Shut down the system and then restart it using the steps described in Chapter 9: Maintenance.</td>
</tr>
<tr>
<td>At login, user name not recognized.</td>
<td>User has not been added.</td>
<td>Add user. See the softWoRx Imaging Workstation User's Guide.</td>
</tr>
</tbody>
</table>

### Analyzing Reasons for Poor Image Quality

The following table documents the most common acquisition difficulties and abnormalities in image data.
<table>
<thead>
<tr>
<th>Indication</th>
<th>Cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dim images or long exposure times.</td>
<td>Poor illumination.</td>
<td>Fully open field aperture. Ensure that filter cube turret is locked in position on rail mount. Ensure shutter on polychroic filter wheel is open. Ensure slider behind microscope is seated in an open position. Ensure proper filter cube is in position and seated in detent.</td>
</tr>
<tr>
<td>Dim illumination. When fiber optic cable and focusing lens are removed, the projected light does not form a circle.</td>
<td>Filter wheel(s) out of alignment.</td>
<td>Shut down and start up as described on Pages 35 and 48 or unplug and plug in the Eyepiece filter wheel. This will reset the home position of the filter wheels and align filter wheel position. Calibrate filter wheels following instructions on Page 232.</td>
</tr>
<tr>
<td>Dark, out of focus spots on image.</td>
<td>Dust interference.</td>
<td>Clean polychroic filter, emission filter, and camera window using low-pressure air. Do not use canned air. See Page 246 for further recommendations regarding cleaning system components.</td>
</tr>
<tr>
<td>Image is distorted around edges or throughout. Occlusion seems to creep in toward center.</td>
<td>Condensation on camera window, possibly due to improper camera temperature.</td>
<td>Clean camera window using low-pressure air. Do not use canned air. See Page 246 for further recommendations regarding cleaning system components. Check camera temperature in Resolve3D. Consult camera documentation for proper setting.</td>
</tr>
<tr>
<td>Brightness of Z section images varies greatly</td>
<td>A broken Photo sensor cable</td>
<td>Disconnect the Photo sensor cable and the EX module cable. Connect the Photo sensor cable to the EX module. Set the Excitation filter to FITC or some other visible light. Open the EX shutter. Bend the cable and examine it for light leaks. If you observe a light leak, replace the cable. Direct the light to a wall. If you observe inconsistencies in the light output as you bend the cable, replace the Photosensor cable.</td>
</tr>
</tbody>
</table>
### Table B-3: Image Quality Troubleshooting Chart (cont'd)

<table>
<thead>
<tr>
<th>Indication</th>
<th>Cause</th>
<th>Correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Image has a traveling light or bubble.</td>
<td>Air bubble in immersion oil.</td>
<td>Clean front and back surfaces of objective and coverslip. Reapply immersion oil and restart experiment.</td>
</tr>
<tr>
<td>Interference in image data.</td>
<td>Possibly dirt, dust, oil, or air bubble.</td>
<td>Clean front and back surfaces of objective and coverslip.</td>
</tr>
<tr>
<td>Very bright image or camera saturation message.</td>
<td>Camera saturation.</td>
<td>Use lower exposure time and/or higher neutral density filter.</td>
</tr>
<tr>
<td>Zseries shows uneven or off center illumination.</td>
<td>Poorly aligned illumination.</td>
<td>Align xenon lamp and fiber optic cable. See Chapter 9, Maintenance.</td>
</tr>
<tr>
<td>No image when <strong>Acquire</strong> is pressed.</td>
<td>Knob at base of microscope is directing light to the eyepiece.</td>
<td>Move knob to direct light to camera.</td>
</tr>
<tr>
<td>Zseries out of focus and incomplete.</td>
<td>Stage was not centered within sample at start of experiment.</td>
<td>Position stage in center of the sample and run experiment again.</td>
</tr>
</tbody>
</table>
## DeltaVision Problem Report Form

<table>
<thead>
<tr>
<th>Research Facility:</th>
<th>System Serial Number:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Person:</td>
<td>softWoRx version: (use Help → Software Versions to display)</td>
</tr>
<tr>
<td>Phone:</td>
<td></td>
</tr>
<tr>
<td>E-mail:</td>
<td>Date:</td>
</tr>
</tbody>
</table>

### Problem Encountered:
Please write a detailed description, answering as many of the following questions as possible.

#### Questions:

1. When did this first occur?
2. Was there any recent change or update to the system prior to the problem occurring?
3. What sequence of operations produces the problem?
4. What other programs were running when you encountered the problem?
5. What error messages, if any, were shown?
6. Is the failure the same each time or does it show different symptoms?
7. Does it occur consistently or is it random?
8. Does it go away after the workstation is re-booted?
9. Does it go away after the instrument controller is re-booted?
10. How often do you re-boot the workstation and instrument controller?

### Additional Comments:
(See Page 2 for additional clarification issues)

Please supply the following log files:

**Workstation:** (/home/username/softworx-logs/softworxlog.txt)

**Instrument Controller:** (c:\ic540_dv\log\IC540_dv.log)

Please e-mail this form to hotline@api.com or Fax to: 425-557-1055, attn: Bio Service Hotline
Appendix C: Acquiring a PSF

This appendix shows how to acquire a Point Spread Function (PSF) and convert it to an Optical Transfer Function (OTF).

- **Acquiring a PSF** shows how to measure a Point Spread Function.
- **Converting PSF to OTF** shows how to convert the Point Spread Function to the Optical Transfer Function that is required to process images.

**Before You Start**

Before you attempt to measure a PSF, check the OTF library included with softWoRx to find out if the library provides an OTF for your objective.

**Acquiring a PSF**

To measure the point spread function (PSF), you need to optically section a fluorescent bead. Since the properties of the objective lens are the most important elements of determining a PSF, it is necessary to have the proper PSF whenever new lenses are added to your microscope. The deconvolution software adapts to the PSF (actually the OTF) wavelength, so it is unnecessary to measure the PSF at more than one wavelength.
**Note** If you do not have the tools necessary to acquire a PSF, softWoRx includes a utility that allows you to calculate a theoretical OTF based on the numerical aperture of the camera lens, index of refraction, and emission wavelength. If the aperture of the lens is lower than 0.75 N.A., the calculated OTF may work as well, or even better, than a measured OTF. However, if the aperture is greater than 0.75 N.A., a measured OTF will generally give you better results. For information about calculating an OTF, see the softWoRx online Help.

A well-measured PSF is a key to successful deconvolution. For this reason, make sure that you:

- Thoroughly check all imaging conditions.
- Take the time you need to get a good signal-to-noise ratio in the image.
- Find a bead that is completely isolated from others in XYZ. (Use the field stop aperture to block fluorescence if needed.)
- Completely scan the bead.

**Tools**

This procedure requires the following tools:

- A clean and aligned DeltaVision system
- A bead slide with 0.1\( \mu \)m, or smaller, fluorescent beads
- A grid slide or other microscopic ruler
- An immersion oil set, if appropriate (see *Selecting the Correct Oil* on Page 259.)

The following steps describe how to calculate pixel size, measure the PSF, and obtain the corresponding OTF.

**To calculate pixel size:**

1. Place the new objective in the Objective Turret and set the maximum image size, best camera speed, and auxiliary magnification slider at 1X.
2. Use the Eyepiece Filter Wheel to select the FITC eyepiece filter.
3. In the Resolve3D window, select the following values:

<table>
<thead>
<tr>
<th>In this Field</th>
<th>Select</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation</td>
<td>TRITC</td>
</tr>
<tr>
<td>Emission</td>
<td>FITC</td>
</tr>
<tr>
<td>%T</td>
<td>2%</td>
</tr>
</tbody>
</table>
4. Place the silicon target grid on the stage and focus it (9.995 \mu m/square). Align the grid image to the vertical and horizontal axis and maximize the image.

5. Switch the Port Selector to Camera and click Acquire. Leave Data Collection Window 21 open.

6. In the Image window, choose Tools | Measure Distances. Then set the Units to Pixels in the Measure Distance window.

7. Draw a line across the grid slide image from a point on the top left square to a point in the same relative position on the top right square.

\[ \text{Note} \] Your measurement will be more accurate if you choose points on the vertices of the squares.

8. If the vertical delta is more than four pixels, re-align the slide. Repeat this process at the middle and bottom. Then count and record the number of grid elements (3 in the above image) and record the distance in pixels.

9. Repeat Steps 7 and 8 in the vertical direction.

10. Calculate the pixel size for each of the six measurements (top, middle, bottom, left, center, and right) as follows:

\[
\text{Pixel Size (\mu m)} = \frac{9.995 \mu m/box}{\text{Measured distance (in pixels)/(Number of grids per measurement)}}
\]

Your calculation should be accurate to four decimal places.

11. Average the six pixel sizes to obtain the correct pixel size.

12. Repeat these steps for each camera attached to your DeltaVision system.

**To add an objective to the microscope configuration:**

If you are adding a new objective to the microscope, follow these steps:

1. To obtain the objective lens ID number, select Conversions | Convert PSF to OTF from the softWoRx main menu. The PSF to OTF Conversion window is displayed. The current lens identification number is shown in the Lens ID field.

2. From the softWoRx main menu, choose Utilities | Revise Microscope Configuration. Then enter the root password to open the RESOLVE3D.SYS file.

3. In the RESOLVE3D.SYS file, under the Microscope Specifications section:
a. Increase the number of lenses next to `MS_Number_Lenses:` by 1.

b. Add the name of the objective to `MS_Lens_Names:` (e.g., 100Xoil, 60Xwater).

c. Add the lens ID to `MS_Lens_ID_Numbers`.

d. Enter the pixel size for the new lens.

For example:

If the desired lens is 40X/1.35 with ID=10403 (the third lens in the list), then the pixel size is 0.1656.

| MS_Lens_Names: 10X 20X 40X 60X 100X |
| MS_Pixel_Size_1: 0.6680 0.3313 0.1656 0.1103 0.06631 (for Coolsnap HQ2) |
| MS_Pixel_Size_2: 0.01 0.01 0.01 0.01 0.01 (for Evolve EMCCD – conventional mode) |
| MS_Pixel_Size_3: 0.01 0.01 0.01 0.01 0.01 (for Evolve EMCCD – EM mode) |
| MS_Lens_ID_Numbers: 10105 10205 10403 10602 10002 |

4. To apply the new information, save and close `Resolve3D.sys`, then close and restart `softWoRx`.

**To acquire a PSF:**

It is easiest to find beads in a very dark room. Bead slides from Applied Precision include 1μm beads and 0.1μm beads. Both fluoresce brightly at 617 nm. Coarsely focus on the slide by positioning the lens near the slide. Scan the slide while looking for fluorescent haze from the 1μm beads. When you focus on the fluorescence haze from the 1μm beads you should also find the 0.1μm beads.

**Note**

Although a replacement bead slide is included in the Slide kit, bead slides have a limited shelf life. To purchase bead slides from Applied Precision, contact us at the appropriate number or address listed in Chapter 1: Getting Started.

1. Mount a bead slide on the microscope and focus on the beads to obtain the maximum intensity. Find a bead that is located by itself.

2. Use the **Center Object** tool to center a single bead in the X and Y directions. (It is helpful to collect large images, such as 1024×1024.)

3. Now use a 256×256 image.

4. Adjust the CCD exposure time so that the maximum intensity at the plane of best focus is at least 2000 counts. Make sure that the camera does not saturate at the plane of best focus.
5. Ensure there is only one bead in the field of view and that, as you go out of focus, no rings from other nearby beads enter the image. If necessary, use the field stop aperture to block out undesired fluorescence.

6. Verify that your microscope and software are accurately configured for lens and auxiliary magnification.

7. Execute the Standard PSF Measurement Macro described in the online Help to measure the standard point spread function (or run a Z series through the bead consisting of 128 sections acquired in 0.1 μm Z increments).

8. Run the softWoRx PSF to OTF program that converts the optical sections into an OTF. (Refer to Converting PSF to OTF later in this appendix.)

**Selecting the Correct Immersion Oil**

Accurate PSF measurements depend on the selection of the correct immersion oil. Our experience has shown that the oils recommended by microscope manufacturers are often not ideal for 3-D microscopy. We recommend that PSFs are measured with a minimal amount of spherical aberration. Inappropriate immersion oils yield asymmetric PSF measurements as a result of spherical aberration. In the case of Olympus microscopes, an index of refraction equal to 1.518 is ideal for measuring beads that are mounted in glycerol using #1.5 coverslips. There are many variables that can affect the selection of the correct immersion oil. The softWoRx Lens Information program can help you select the proper oil.

**Note** An oil kit is included with your DeltaVision system. To purchase replacement oil, please contact Applied Precision.

**To confirm that you are selecting the correct immersion oil:**


2. Move the blue crosshair to the center of the bead in the viewer. To better see the shape of the PSF, it is helpful to do an exponential scaling—an exponent of 0.3 usually works well.

3. Look for symmetric flare in the resulting image. Symmetry indicates that the oil is correct, and in virtually all situations, the most symmetric PSF along the Z axis is also the smallest and has the highest intensity. In other words, symmetry corresponds with the highest resolution.

4. Repeat the process with different oils until you determine the optimal immersion oil.
The following figure demonstrates how image flare can be affected by the use of different immersion oils.

**Flare from Immersion Oils (Orthogonal Views)**

**Converting PSF to OTF**

The PSF to OTF program converts a measured point spread function (PSF) to an optical transfer function (OTF). Essentially, the OTF is the Fourier transform of the PSF. The pixel size of the resulting image is given in cycles/μm. To reduce problems associated with measurement noise, the PSF is radially averaged during the conversion and, as a result, the 3D PSF image becomes a 2D OTF image.

The horizontal axis of the OTF represents axial (Z) frequency and the vertical axis represents radial (XY) frequency. The brightness of the OTF image elements, on a scale of 0 to 1, represents the frequency response of the microscope system at the corresponding radial and axial frequencies.
Appendix C: Acquiring a PSF

Sample OTF Image

Each option in PSF to OTF Conversion is described briefly below. For additional information regarding these options, refer to the online Help.

**PSF File**
Defines the name of the PSF image file to be converted to an OTF.

**OTF File**
Displays the name of the resulting axially symmetric OTF. (For your convenience, the OTF filename is created by appending "_.otf" to the PSF filename.)
**X Range**
Defines the start and end pixel numbers in X.

**Y Range**
Defines the start and end pixel numbers in Y.

**Z Range**
Defines the start and end pixel numbers in Z.

**T Range**
This field is not used for PSF to OTF conversion.

**Wavelengths**
Determined by PSF wavelength.

**Lens ID**
Specifies the lens identification number (e.g., 12004).

**Sub-Image: Center**
Specifies the central XYZ coordinates of the point spread.

**Sub-Image: Size**
Specifies the XYZ image dimensions about the central coordinates. (The standard softWoRx point spread measurement is 256×256×128.)

**Additional Parameters: Border Rolloff (voxels)**
Specifies the number of voxels to roll off at the edge of the image. This reduces edge effects resulting from the Fourier Transform used in the PSF to OTF conversion.

The procedure for converting a PSF to an OTF is very simple. After the PSF file has been identified in PSF to OTF Conversion, softWoRx assigns default settings to the rest of the options in the window. In almost every instance, these settings will be appropriate to use for the conversion.

**To convert a PSF to an OTF:**

1. Click **Conversions** on the main menu bar of softWoRx.
2. Click **Convert PSF to OTF** in the **Conversions** menu. PSF to OTF Conversions will appear.
3. Use one of the following options to enter the PSF file to convert.
   - Drag the appropriate PSF file from the File Manager into the **PSF File** text box.
   - Click **PSF File** to display a small version of the File Manager and then choose the PSF file that you wish to convert.
• Type the desired path and filename into the **PSF File** text box.

4. Click **Do It**.

**To place OTF into OTF Library**

If the objective used is in addition to those already present, you’ll need to modify `softWoRx` to use the new objective by adding the file to 
`/usr/local/softWoRx/config/system.swrc` as follows:

1. Log in to Linux as **root**.

2. Navigate to `/usr/local/softWoRx/config/system.swrc`

3. Find the section labeled, “Lens-to-OTF matching” and follow the instructions provided for the OTF file.

The following is an example of this section of the file:

```bash
# Lens-to-OTF matching. These are of the form LENS_<lensIDNumber>_OTF
# and are defined to be the file name in the OTF directory of the OTF that is
# to be used for this lens ID.
LENS_12_OTF 60X140_sample.otf
LENS_10602_OTF 60X140.otf
LENS_10003_OTF 100X135.otf
LENS_10403_OTF 40X135_sample.otf
LENS_10603_OTF 60Xw_120.otf
LENS_10205_OTF 20X0.75c.otf
```
Appendix D: Reference Information

The appendix includes the following topics:

- **Standard Filename Extensions** lists the filename conventions used by *DeltaVision*.
- **Standard Fluorescence Filters** shows the excitation and emission peaks of the standard filters for both fixed and live cell experiments included with *DeltaVision*.
- **Reference List** includes references for microscopy, Linux, image processing, optics, microscopy, and sample preparation.

### Standard Filename Extensions

The following is a list of filename conventions used by *DeltaVision*.

<table>
<thead>
<tr>
<th>Filename Extension</th>
<th>Type of File</th>
</tr>
</thead>
<tbody>
<tr>
<td>*.dv</td>
<td>Standard DeltaVision image</td>
</tr>
<tr>
<td>*.otf</td>
<td>Optical Transfer Function</td>
</tr>
<tr>
<td>*_R3D.dv</td>
<td>Resolve3D image</td>
</tr>
<tr>
<td>*_D3D.dv</td>
<td>Deconvolved image</td>
</tr>
<tr>
<td>*_VOL.dv</td>
<td>Volume Rendered image</td>
</tr>
</tbody>
</table>
Standard Fluorescence Filters

Depending on which broadband light source the system uses, the DeltaVision imaging system provides a variety of different filter sets for both fixed and live cell imaging, a polarizer, and multiple sets of optional filters. These filters are designed to be used with many common fluorescent probes. If you are using fluorescent probes that are not well matched with the standard DeltaVision filters, contact Applied Precision for assistance.

InsightSSI Module

The 4-color Fixed InsightSSI includes four of the most common sets of filters used for fixed cell imaging.

InsightSSI 4-color Fixed

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Excitation</th>
<th>Emission</th>
<th>Appropriate Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>UV, 381-401nm</td>
<td>Blue, 409-456nm</td>
<td>DAPI, Hoechst, Coumarin, Alexa 350&lt;sup&gt;®&lt;/sup&gt;, Alexa 405&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>FITC</td>
<td>Blue Green, 464-492nm</td>
<td>Green, 500-523nm</td>
<td>Fluorescein, GFP, Cy3, Alexa 488&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>TRITC</td>
<td>Yellow Green, 531-556nm</td>
<td>Yellow, 564-611nm</td>
<td>Rhodamine, Texas Red, Phycoerythrin, Alexa 568&lt;sup&gt;®&lt;/sup&gt;, Alexa 594&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cy5</td>
<td>Red, 619-644nm</td>
<td>Infrared, 652-700nm</td>
<td>Cy5, Alexa 647&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The 4-color Live Cell InsightSSI includes four of the most common sets of filters used for live cell imaging.

InsightSSI 4-color Live Cell

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Excitation</th>
<th>Emission</th>
<th>Appropriate Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFP</td>
<td>Blue, 400-454nm</td>
<td>Blue Green, 463-487nm</td>
<td>Cyan FP (CFP)</td>
</tr>
<tr>
<td>GFP</td>
<td>Blue Green, 425-495nm</td>
<td>Green, 500-550nm</td>
<td>E-GFP</td>
</tr>
<tr>
<td>YFP</td>
<td>Green, 496-528nm</td>
<td>Yellow Green, 537-559nm</td>
<td>Yellow FP (YFP)</td>
</tr>
<tr>
<td>mCherry</td>
<td>Yellow, 555-590nm</td>
<td>Orange, 600-675nm</td>
<td>mCherry, tdTomato, mRFP, DsRed</td>
</tr>
</tbody>
</table>
The 7-color Live Cell InsightSSI includes seven of the most common sets of filters used for fixed and live cell imaging.

**InsightSSI 7-color (for Fixed and Live Cell Imaging)**

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Excitation</th>
<th>Emission</th>
<th>Appropriate Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>UV, 381-401nm</td>
<td>Blue, 409-456nm</td>
<td>DAPI</td>
</tr>
<tr>
<td>CFP</td>
<td>Blue, 400-454nm</td>
<td>Blue Green, 463-487nm</td>
<td>CFP</td>
</tr>
<tr>
<td>FITC-GFP</td>
<td>Blue Green, 425-495nm</td>
<td>Green, 500-550nm</td>
<td>Fluorescein, E-GFP, Alexa 488®, Cy3</td>
</tr>
<tr>
<td>YFP</td>
<td>Green, 496-528nm</td>
<td>Yellow Green, 537-559nm</td>
<td>Yellow FP (YFP)</td>
</tr>
<tr>
<td>TRITC</td>
<td>Yellow Green 531-556nm</td>
<td>Yellow 564-611nm</td>
<td>Texas Red, Rhodamine</td>
</tr>
<tr>
<td>mCherry</td>
<td>Yellow 555-590nm</td>
<td>Orange 600-675nm</td>
<td>mCherry, tdTomato, mRFP, DsRed</td>
</tr>
<tr>
<td>Cy5</td>
<td>Red 619-644nm</td>
<td>Infrared 652-700nm</td>
<td>Cy5, Alexa 647®</td>
</tr>
</tbody>
</table>

**Xenon Arc Lamp**

The excitation and emission peaks of the DeltaVision filters are provided in the following table.

**Xenon Standard Fixed Cell Filter Set**

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Excitation</th>
<th>Emission</th>
<th>Appropriate Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>UV, 325-375nm</td>
<td>Blue, 438-478nm</td>
<td>DAPI, Hoechst, Coumarin, Alexa 350®, Alexa 405®</td>
</tr>
<tr>
<td>FITC</td>
<td>Blue Green, 481-502nm</td>
<td>Green, 506-543nm</td>
<td>Fluorescein, GFP, Cy3, Alexa 488®</td>
</tr>
<tr>
<td>TRITC</td>
<td>Green, 547-563nm</td>
<td>Yellow/Orange, 576-630nm</td>
<td>Rhodamine, Texas Red, Phycoerythrin, Alexa 568®, Alexa 594®</td>
</tr>
<tr>
<td>Cy5®</td>
<td>Red, 636-656nm</td>
<td>Infrared, 667-719nm</td>
<td>Cy5, Alexa 647®</td>
</tr>
</tbody>
</table>
The optional Xenon Live Cell filter wheel module includes four of the most common sets of filters used for live cell imaging.

**Xenon Live Cell Filter Set**

<table>
<thead>
<tr>
<th>Filter Name</th>
<th>Excitation</th>
<th>Emission</th>
<th>Appropriate Probes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFP</td>
<td>Blue, 415-445nm</td>
<td>Blue Green, 455-485nm</td>
<td>Cyan FP (CFP)</td>
</tr>
<tr>
<td>YFP</td>
<td>Green, 490-510nm</td>
<td>Yellow Green, 520-550nm</td>
<td>Yellow FP (YFP)</td>
</tr>
<tr>
<td>mCherry</td>
<td>Yellow, 555-590nm</td>
<td>Orange, 600-675nm</td>
<td>DsRed, tdTomato, mCherry, mRFP</td>
</tr>
<tr>
<td>GFP</td>
<td>Blue Green, 425-495nm</td>
<td>Green 500-530nm</td>
<td>E-GFP</td>
</tr>
</tbody>
</table>

**Additional Reading Material**

Material for further reading is available on the following pages. Contact Applied Precision for the most recent list. If you notice omissions from the list, please inform Applied Precision.

**Microscopy**


**Linux Operating System**


**Image Processing**


**Optics**


Appendix E: Resolve3D and Keypad Options

This chapter describes the following:

- *The Resolve3D Window* is the main window for data acquisition.

- *The Design/Run Experiment Window* provides tools to select, design, edit, and execute experiment macros.

- *The Settings Window* used to control how images are displayed, select camera settings, and specify file output.

- *Keypad/Joystick Operation* is a reference for the buttons on the keypad. Many of the Resolve3D functions are also available on the keypad and joystick.
The Resolve3D Window

The Resolve3D window is the main data acquisition window. In addition to providing many of the acquisition options and controls, it provides access to the other windows that are used for data acquisition.

**To open Resolve3D:**

- From the softWoRx menu, choose File | Acquire (Resolve3D). The Resolve3D window is displayed.
The Resolve3D Menu

The Resolve3D menu has the following menu items:

- **The File menu** includes commands to acquire images and to open the key windows for setting up and running experiments.

- **The View menu** includes commands to manage marked points and to create a blank screen.

- **The Options menu** includes commands to open the Settings window (where you can set display and image options) and to save settings.

- **The Calibration menu** opens the Calibration tool.

- **The Help menu** provides options to show or hide ToolTips and to get Help.

The File Menu

Use the following Resolve3D File menu commands to acquire images, create scratch files, and open the Design/Run Experiment window.

**Acquire Image**
Collects and displays an image from the microscope. This image is only displayed in the Data Collection window. It is not saved to a disk file for later use.

**Continuous Acquire**
Opens the Continuous Acquire window that you can use to collect and display images continuously. These images are only displayed; they are not saved to a disk file.

**Snapshot**
Launches a tool to let you collect a single 2-D, multi-wavelength "snapshot" image. If you need to collect a Z series, time-lapse image, or other complex scheme, you
will need to design an experiment with the Resolve3D Experiment Designer. You can also do an OAI as part of your snapshot.

**Scratch File**
Creates a "scratch file" to which you can save individual image frames for later use. After a file is opened, clicking **Save Current Image** saves the most recently collected image frame. Clicking **Close Scratch File** or **Done** closes the file. Note that the image size cannot be changed while a scratch file is open.

**Experiment**
Opens the Design/Run Experiment window. You can use Design/Run Experiment to select a previously created experiment, to design a new experiment, or to open the Experiment Macro Editor to create or edit an experiment macro.

**Quit**
Closes the Resolve3D window.

### The Resolve3D View Menu
Use the View menu to manage points that you have marked, to clear the history of the path that you took while exploring your sample, or to create a black screen for light-sensitive conditions.

**Point List**
Opens the Points List window. This window helps you manage a list of points (with X, Y, and Z coordinates) that you want the system to remember. These points can be interactively "visited" at any time and they can be used in experiments.

**Clear Stage Trails**
Clears the Stage Trails history. (The system maintains a history of the paths of motion that you take while exploring your sample. These paths are displayed as "Stage Trails" on the Stage View.)

**Clear Stage Thumbnails**
Clears all of the thumbnail images currently displayed on the Stage View.
Blank Screen
Turns the computer screen blank. This is useful when you are imaging under very light-sensitive conditions. Clicking anywhere on the screen restores it.

Command Line Interface
Opens the Command Line window that provides advanced users with the ability to issue individual Resolve3D commands to the system.

⚠️ CAUTION: The Command Line Interface should be used carefully because it can put the system in an unstable state.

The Resolve3D Options Menu
Use the Options menu to open the Settings window (where you can set display and image options) and to save configuration settings and state information.

The Resolve3D Options Menu

Zero the Stage Z
Selecting Zero the Stage Z from the Options menu provides a protocol for moving the stage in the Z direction to the middle of its working range.

Center the Stage XY
Selecting the Center the Stage XY from the Options menu provides a protocol for moving the stage in the XY direction to the middle of its working range.

Settings
Opens the Settings window which allows you to control display, imaging, and file output options.

Save Settings
Saves the configuration settings and information (current filters, image size, etc.) to be used the next time Resolve3D is opened.

The Resolve3D Calibration Menu
Use the Calibration menu to make calibration tables, read calibration tables, and designate which calibration tables are active.
The Resolve3D Calibration Menu

**Make**
Opens the Calibration tool used to create flat-field calibration tables and optional tables of "bad" pixels. These tables may be applied to images, either when they are acquired or at a later time. (To apply the tables to images after they are acquired, use the Calibrate utility available from the Process item on the main softWoRx menu.)

**Read**
Opens the Read Calibration Files window that you can use to read calibration tables.

**Manage**
Opens the Manage tool that you can use to designate which calibration tables are active and to remove tables from the system's session memory.

The Resolve3D Help Menu

Use this menu to turn ToolTips on or off, get help on the Resolve3D window, or find out which version of softWoRx you are using.

The Resolve3D Help Menu

**Turn ToolTips On/Off**
Turns tool tips on or off. Tool tips display “pop-up” information about buttons on the interface. They open when the mouse pointer is held over a button for a few seconds.

**On Window**
Opens Help for the Resolve3D window.

**Version**
Displays version numbers for the softWoRx components.
The Resolve3D Toolbar

Use the buttons on the Resolve3D toolbar to acquire images, open the Experiment Designer/Run window that allows you to set up and run experiments, and open the Settings window that allows you to control display, imaging, and file output options.

Acquire
Collects and displays an image from the microscope with the current settings. This image is only displayed in the Data Collection window. It is not saved in a file.

Experiment
Opens the Design/Run Experiment window that you can use to select a previously created experiment, to design a new experiment, or to open the Experiment Macro Editor to create or edit an experiment macro.

Settings
Opens the Settings window that allows you to control display, imaging, and file output options.

Image Control Fields
Use the Resolve3D Image Control fields to:

- Select the Excitation, Emission, and Neutral Density filters.
- Select exposure time.
- Determine whether to calibrate the image.
- Select the desired shutter.
- Select the image size.
- Select the lens and get lens information, including the oil calculation.
- Select whether auxiliary magnification is used.
- Determine binning parameters.
- Get pixel size.
Select the polychroic.

The **Resolve3D Image Control Fields**

**Excitation**
Specifies an excitation channel. When this field is changed, the following parameters are set to the last values that were used for that excitation channel.

- Emission filter
- Exposure time
- Neutral Density value
- Active illumination shutters
- Number of frames to average
- Target intensity
- Camera gain

You can choose **Save Settings** from the Options menu to store the configuration. The wavelength/bandwidth of the selected filter is indicated to the right of the filter choice box.

**Emission**
Specifies an emission filter. The wavelength/bandwidth of the selected filter is indicated to the right of the filter choice box.
Polychroic
Specifies a polychroic to place into the active position. The selections available are displayed in the drop-down list in this field. Your choices will completely depend upon which polychroic filters are installed on your system.

Illumination
Indicates which light source will be used. Your choices are as follows:

- For broadband illumination, select SSI.
- For transmitted light, select TRANS.
- For laser light, select LASER.

%T
Specifies a Neutral Density value. The relative illumination intensity is indicated in the menu.

Exposure
Specifies camera exposure time (in seconds). The minimum and maximum exposure times allowed for this field depend upon the camera type.

Find
Opens the Target Intensity window which allows you to find the exposure time necessary to reach a Max intensity near the Target Intensity Value. For dim samples, 300-900 counts are acceptable. For bright samples, 200-2500 counts provide a high dynamic range without saturation. This tool is not recommended for use with live cells.

Calibrate
Calibrates images when they are collected from the camera. If you use this option, you must load and activate a calibration table that fits the current imaging parameters (wavelength, image size, objective, bin choice) before you acquire images.

Image Size
Specifies image size (pixels or CCD detector elements) for acquired images. The pull-down list contains predefined sizes for convenience. You can enter special sizes by choosing Other... from the pull-down list. (Image size must be a multiple of four.)

Lens
Specifies the objective lens name. The pull-down list contains the lenses that are known to be part of the microscope system.

Info
Opens the Lens Information window which displays information about the current objective lens, including the oil calculator.
**Bin**
Specifications the number of CCD detector elements to add together to form one image element. Binning is applied in both the X and Y directions. It increases intensity, but it decreases resolution. Pixel size is a function of binning.

**Aux. Mag.**
Specifies whether the microscope's 1.6x manual auxiliary magnification is in use. (On IX70 stands, the manual auxiliary magnification is 1.5x. For EMCCD camera systems, the auxiliary magnification is 2.0x.)

**Pixel size**
Displays pixel dimensions in microns/pixel. The value is calculated from the Lens, Bin, and Auxiliary magnification settings.

### Stage Position Control Fields and Buttons

The Resolve3D Stage Position Control area allows you to control the microscope's X, Y, and Z stage positions. The Center, Mark, and Visit tools and the Stage Motion Controls are shown below.

#### Stage Control and Display Tools

**Center Object**
Centers the stage on an object selected in an Image Window. The pixel size must be correct in order for object centering to work properly, which means that the
correct lens and auxiliary magnification setting must be selected. An image will be acquired after the object is centered.

**Mark Point**
Marks the current X, Y, and Z stage coordinates as points to be visited later.

**Mark Top of Sample**
Marks the current stage Z position as the "top" of your sample. Because the scanning process always moves the stage toward the objective, this position also represents the point where the stage is closest to the objective (the most negative Z value). This also represents the focal plane that is the closest to the slide side of a sample. Use this along with the **Mark Bottom of Sample** button to establish the thickness of the sample. You can use these marked positions to aid with the Z sectioning setup.

**Note**
All scans that are set up using the Experiment Designer scan in Z using relative coordinates. The **Mark Top of Sample** and **Mark Bottom of Sample** buttons are most helpful in determining the thickness of the sample to be scanned. When an experiment is started, the scan region is determined by the current focus point and the thickness of the sample. So, for example, if you have marked three points to visit in an experiment and they all have different middle-Z positions, the experiment will calculate the scan based on these different Z positions and the fixed thickness.

**Visit Top**
Moves the stage to the position marked as the top of the sample.

**Mark Bottom of Sample (end of scan)**
Marks the current stage Z position as the "bottom" of your sample for a potential scan. This corresponds to the positive stage Z coordinate value or the coverslip side of the sample.

**Visit Bottom**
Moves the stage to the position marked as the bottom of the sample.

**Visit Middle**
Visits the point in the middle of the defined scan region.

**Plate Viewer**
Displays the Microtiter Plate Viewer window. You must have the microtiter stage option installed on your *DeltaVision* system in order to scan microtiter plates.

**Autofocus**
Automatically focuses using a contrast-based software method.

**Acquire Image**
Collects and displays an image from the microscope. The current Resolve3D image control settings are used to collect the image. This image is only displayed in the Data Collection window. It is not saved in a file for later use.
**XY Stage Controls**
Moves the stage in the X and Y axis. The left and right arrows move the stage in the X axis in the increment set in the \( dX \) field and the up and down arrows move it in the Y direction in the increment set in the \( dY \) field.

**Z stage Motion Controls**
Moves the stage in the Z axis in the increment set in the \( dZ \) field.

---

**Stage Movement Controls**

**Pan**
Places the stage view in Pan mode. Click and drag the current view to pan it. Note that this tool is "sticky." To disable Pan mode, click the **Pan** tool again.

**Spiral Mosaic**
Starts a preview collection pattern centered on the current stage location and using the active Resolve3D wavelengths. The pattern begins with the current stage location and then continues acquiring thumbnail images directly adjacent to it, spiraling outward in a counter-clockwise rotation so that the entire area centered around the initial stage position is previewed in the Resolve3D stage view. Thumbnail collection continues until either the preset spiral mosaic size (set in Resolve3D | Settings | Misc tab) is reached or you click the **Spiral Mosaic** button again. The stage is always returned to the initial position regardless of how the collection ends.

**Clear Stage Trails**
Clears all of the stage trail lines from the stage view.

**Clear Thumbnails**
Clears all of the thumbnail images currently displayed on the stage view.
Marked Points list
Opens the Point List window to manage the list of marked points.

Zoom Tool
Controls zoom of the stage view. Drag the thumbwheel down to zoom in; up to zoom out. The button under the thumbwheel resets the zoom to 1:1.

Z Slider
Moves the stage up or down. Click and drag the blue horizontal bar to move a maximum of ± 5μm.

dX
Specifies the X step size, in microns.

dY
Specifies the Y step size, in microns.

dZ
Specifies the Z step size, in microns.

Stage Trails Window
The blue box represents the current stage location. Drag the box to move the stage in X and Y.

Image Intensity and Scale Values
The Resolve3D image intensity and scale show the intensity values of the data collection window in numerical and graphical formats. The scale of the image is also shown.
The Resolve3D Image and Intensity and Scale View

Min, Max, Mean
Displays the minimum, maximum, and mean intensity values of the most recently acquired image. For a 12 bit CCD camera, these values range between 0 and 4095. A value of 4095 indicates camera saturation, unless image calibration is in effect. (If you are using 0.5X Gain, the saturation is less than 4095 counts.)

Histogram
Shows the intensity distribution for the most recently acquired image. The vertical blue bars indicate the Scale min and Scale max and can be dragged interactively with the mouse to change how the data collection window display is scaled. The X-axis represents intensity and the Y-axis represents the number of pixels.

Scale min/Scale max
Specifies the settings for the minimum and maximum display values. These numbers can be changed manually or by moving the histogram threshold bars.

Io
Indicates valid (or invalid) photo sensor values or saturation. The indicator is green if the photo sensor value is valid. It is red before the first image is acquired. After the first scan, a red color indicates that the most recently acquired image has an invalid photo sensor value. A red indicator can also signify unreasonable saturation or an improperly functioning photo sensor device.

The Message Pane
The Resolve3D Message pane reports the status of various Resolve3D activities. Use the scroll-bar to view messages that have scrolled off the top of the pane.

Resolve3D Shortcuts
You can right-click anywhere in the Resolve3D window to open a shortcut menu that allows you to acquire images, mark points, and create a blank screen (for imaging under very light-sensitive conditions).
Appendix E: Resolve3D and Keypad Options

Resolve3D Shortcut Menu

**Acquire**
Collects and displays an image from the microscope. The current settings are used to collect the image. This image is only displayed in the Data Collection window. It is not saved in a file for later use.

**Snapshot**
Launches a tool to let you collect a single 2-D, multi-wavelength "Snapshot" image. (The current settings are used to collect the image.)

**Mark point**
Marks the current X, Y, and Z stage coordinates as a point to be visited later.

**Blank Screen**
Turns the computer screen black for imaging under very light-sensitive conditions. Clicking anywhere on the screen restores it.

The Design/Run Experiment Window

The Design/Run Experiment window provides tools to select, design, edit, and execute experiment macros. (Experiment macros are "scripts" of commands that guide the DeltaVision system to collect images.)

**To open the Design/Run Experiment window:**

- From the Resolve3D window, click **Experiment** and click the **Run Experiment** tab.
The Design/Run Experiment Window

Note The Design PK Experiment tab is available only for systems equipped with a laser module.

The Design Experiment Tab

The Design Experiment tab is used to generate experiment command macros.

To open the Design Experiment tab:

- From the Resolve3D window choose Experiment. The Design/Run Experiment window opens with the Design Experiment tab selected.
Appendix E: Resolve3D and Keypad Options

Design/Run Experiment Window

Experiment name
Specifies the name of the experiment macro.

Enable Fast Acquisition
Enables fast acquisition experiments (see Page 294).

Sectioning Tab
Specifies sectioning for 3D images (see Page 294).

Channels Tab
Specifies channels and exposure time (see Page 296).

Time-lapse Tab
Specifies criteria for time-lapse experiments (see Page 297).

Points Tab
Specifies a list of marked points (see Page 300) and options for UltimateFocus and Autofocus.

Panels Tab
Allows you to set up panel collection that you can use to acquire a large area of a slide to stitch together to form a single image (see Page 302).

Plate Tab
Provides tools for setting up and running microtiter plate scanning experiments when your DeltaVision system is equipped with the Microtiter Stage option.
Actions Tab
Lets you designate new Laser, Autofocus, and UltimateFocus events, add Pause and Wait times, alter Time-lapse intervals and image status methods, and other specific actions to occur during the experiment.

Experiment Name and Enable Fast Acquisition

Experiment name
Specifies the name of the file that is generated by the Experiment Designer. A file extension of .exp will be added to the name.

Enable Fast Acquisition
Enables fast image acquisition of 2D images. (This should not be used with cameras that require a shutter.)

This data acquisition mode should be used carefully. Fast Acquisition uses a single command to set up a data stream to the Instrument Controller.

There is an upper memory limit for data collection that is based on the size of RAM memory for the Instrument Controller (typically about 350-400 MB). If you run into this limitation, turning Fast Acquisition off allows you to collect larger data sets.

If the system is unstable after collecting a large data set with Fast Acquisition, restart the workstation or stop using Fast Acquisition unless it is strictly necessary. Sometimes Fast Acquisition isn’t much faster than standard acquisition, and more is risked than gained by using it.

Note You can set Fast Image Acquisition options to control the scan sequence, the shutter open mode, the camera readout mode, and the starting Z location for the scan. (See the online Help for more information.)

Sectioning Setup
Once your microscope is focused near the middle of the vertical zone of interest of your sample, you can use the parameters in the Design Experiment Sectioning tab to control the Optical Sectioning procedure.

Note The standard scan direction moves the objective lens towards the specimen.
Focus point when scan starts
Specifies the Z location of the sample when the experiment starts. The recommended selection is Middle of Sample.

Optical section spacing
Specifies the spacing (in microns) between each optical section. The focal point will be changed by this value after each image is collected.

Number of optical sections
Specifies the number of sections to collect (for each wavelength) for the experiment. softWoRx automatically calculates and displays this value if the Sample thickness and Optical section spacing are entered. If you specify this value, the Sample thickness value is changed.

Sample thickness
Displays the Number of optical sections multiplied by the Optical section spacing.

Get Thickness
Retrieves the Sample thickness, based on the locations defined with the ↑ and ↓ buttons in the Resolve3D window.

OAI
Acquires a 2D Z projection of the interval defined by the marked top and bottom of the sample. The optical section spacing and the number of optical sections are determined automatically, based on the depth-of-field of the objective and the exposure time.
Channels Setup

Use the Design Experiment Channels tab to select wavelengths (filters) and to specify an exposure time for each filter.

Design Experiment Channels Options

Refresh exposure conditions
Updates the filter and exposure settings to those last used in the main Resolve3D window.

Active Wavelength Toggle Buttons
Enable the exposure time, filters, and display settings for specific wavelengths. Select the buttons that activate the wavelengths that you want to collect. (You must select one button for each wavelength.) If no wavelengths are selected, the exposure and filters that are set in the Resolve3D window are used.

Exp
Specifies the exposure time (in seconds) to be used when acquiring an image for the selected wavelength. If left blank, the value specified in Resolve3D will be used when the experiment is run.

EX Filter
Specifies the excitation filter to use for this experiment or image. When it is changed, the currently “paired” emission filter is automatically selected.
EM Filter
Specifies the emission filter to use. This filter may be selected independently of the EX Filter setting.

%T Filter
Specifies the Neutral Density value to use. The % value indicates light transmission. A value of 100 % indicates that no light is blocked.

Ex Source
Specifies the excitation (illumination) source to use for each channel of the image.

Polychroic
Specifies the polychroic filter to use for the experiment. If you have the optional motorized mirror turret installed, it will automatically move the selected polychroic into the light path. The polychroic is moved when the experiment is run.

Note
This field is available only if the polychroic turret is motorized.

Reference Image
Specifies to use an alternate filter or the transmitted light to acquire a reference image that can be combined with other images. This option is useful for Differential Interference Contrast (DIC) analysis. It can also be useful for other types of reference images. Only one reference image per Z stack is collected.

Time-lapse Setup
Use the Design Experiment Time-lapse tab to specify the number of time points, the time periods, and the total time for a time-lapse experiment.
Design Experiment | Time-lapse Setup Options

**Time-lapse option**
Specifies running a time-lapse experiment.

**Time-lapse**
Specifies the hours, minutes, and seconds between each time period.

**Total Time**
Displays the total time of the experiment, which can also be calculated as follows:

\[
\text{Total time} = (\text{The number of time points} - 1) \times \text{time-lapse}
\]

Specifying a total time will update the **Time Points** field.

**Time Points**
Specifies the number of time samples to collect in a time-lapse experiment. Changing this value will update the **Total Time** field.

**Enable Cell Tracking**
Moves the stage laterally to follow cells as they move during a time-lapse experiment. With the **Enable Cell Tracking** option selected, DeltaVision automatically keeps cells in the field of view.

**Cell Tracking Options**
Opens the Cell Tracking Options window that allows you to set the parameters for cell tracking. For more information on using the tools on this window, see Tracking Cells on Page 90.
Maintain Focus with UltimateFocus

Specifies focus should be maintained during the time-lapse experiment. If this experiment does not involve point visiting, a calibration (characterizing the UltimateFocus response at the current location) is performed when the experiment is started.

Notes
#1 The initial focus is essential to the success of focus maintenance.
#2 The actual focus maintenance process happens just before each time-lapse event.

UltimateFocus Options

Opens the UltimateFocus Options window, in which you can indicate a specific Move Threshold and the Maximum (number of) Iterations to move within the threshold before re-running UltimateFocus.

On the UltimateFocus Action Options window:

- In the Move Threshold field, enter the desired maximum measured focus error (in μm) before a corrective action should be taken.
- In the Maximum Iterations field, enter the maximum measure/move sequences for this action to reach the calibrated focus point.

Note
More iterations will take longer, but may provide better focus.

- Click the Done button.

Image-based Autofocus before imaging

Using the software-based Autofocus function, automatically focus the camera before each time point. For point visiting experiments, the camera is automatically focused every time that a point is visited.
**Autofocus Options**

Opens the Resolve3D Settings window to the Autofocus tab, in which you can manipulate the settings for the software-based Autofocus function.

![Resolve3D Settings Window | Autofocus Tab](image)

**Point Visiting Setup**

Use the Design Experiment Points tab to specify a list of marked points to visit during the experiment.

**Tip** Before you specify these points, make sure that there are active points in the point list.
**Visit Point List**
Specifies the list of points (described by number) to visit during the experiment. All sectioning and wavelength procedures are repeated at each of the listed points. A point list can be entered as a series of numbers separated by commas or dashes. Separating two numbers with a dash ‘-’ (as shown in the following example) indicates that all point numbers in between should also be visited.

For example:

1, 2, 5, 7-10

**Note** When you use Point Visiting with Z sectioning, the microscope uses the Z value of the current focus of each point as the initial reference for that point. Values specified for Z sectioning are incremented relative to that point.

**Maintain Focus with UltimateFocus**
Specifies focus should be maintained during the point visiting experiment. A UltimateFocus calibration (characterizing the UltimateFocus response at the current location) is performed when the point is initially marked.

**UltimateFocus Options**
Opens the UltimateFocus Options window, in which you can indicate a specific Move Threshold and the Maximum (number of) Iterations to move within the threshold before re-running UltimateFocus.
On the UltimateFocus Action Options window:

- In the **Move Threshold** field, enter the desired maximum measured focus error (in μm) before a corrective action should be taken.

- In the **Maximum Iterations** field, enter the maximum measure/move sequences for this action to reach the calibrated focus point.

<table>
<thead>
<tr>
<th>UltimateFocus Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Move Threshold (μm)</td>
</tr>
<tr>
<td>Maximum Iterations</td>
</tr>
</tbody>
</table>

| Note | More iterations will take longer, but may provide better focus. |

- Click the **Done** button.

**Image-based Autofocus before imaging**

Using the software-based Autofocus function, automatically focus the camera at each visited point before acquiring an image.

**Autofocus Options**

Opens the Resolve3D Settings window and the Autofocus tab, in which you can manipulate the settings for the software-based Autofocus function.

**Panel Collection Setup**

Use the Design Experiment Panels tab to create panel collection macros. These macros are useful when you want to scan a large area with a relatively high magnification lens. You can use the panels as a means of reviewing a large area of a slide, or as data that you want to stitch together to form a single, large image.

| Note | Panel collection macros are sensitive to microscope settings such as image size and magnification. Because the number of panels required depends upon many factors, it is usually not a good idea to reuse panel collection macros. |
Appendix E: Resolve3D and Keypad Options

Design Experiment | Panel Collection Setup Options

**Collect Panels**
Specifies to use a panel collection macro.

**Overlap (pixels)**
Specifies the amount of overlap (in pixels) between adjacent panels.

**Start Coordinates**
Specifies the XYZ coordinates at which to start collecting panels. Use the **Get Start** button to obtain the current XYZ stage coordinates.

**End Coordinates**
Specifies the XYZ coordinates at which to finish collecting panels. Use the **Get End** button to obtain the current XYZ stage coordinates.

**Note**
These coordinates will be included at minimum. Based on CCD size, the final collection area may be larger than specified.

Design PK Experiment Tab

The TIRF/PK Module provides the ability to add a laser beam into the optical path of the *DeltaVision* microscope. A laser beam is introduced into the back aperture of the microscope objective to provide a focused illumination spot in the center of the optical field. With lasers attached (like the *DeltaVision X4 Laser Module*), users can design photokinetic experiments and TIRF experiments. TIRF is an imaging method that offsets the laser excitation light slightly off-axis using a special TIRF-
based objective. The excitation light emits from the objective at a shallow angle, enters into the cover slip, and bounces inside the cover slip creating an evanescent field of fluorescence. The TIRF/PK Module is optional for the DeltaVision system and is offered in three varieties: TIRF/PK, TIRF only, and PK only.

The Design PK Experiment tab provides options for designing the nature of a photokinetic event, setting up the event timing, setting image conditions to associate with the event, and defining the locations of photokinetic events.

Running Photokinetic (PK) Experiments

The Resolve3D Design/Run Experiment window contains basic photokinetic experiments such as FRAP (fluorescence recovery after photobleaching).

To run a basic PK experiment:

8. Click the Experiment button in the Resolve3D main menu to open the Design/Run Experiment window.
9. Select the Design PK Experiment tab.
10. On the Laser Events tab, select the desired laser for the drop-down menu.

11. To generate multiple bleach events, the stage moves the sample to the site of laser activity. You can optimize this motion for either speed or position. When Optimize for Position is selected, the stage goes through an LMC (Lost Motion Compensation) move to achieve the most accurate position. This move takes more time than when Optimize for Speed is selected.
12. Bleach events occur at either the center point of the field of view or in a specified position. Select **Use Bleach Event Specification** in the **Event pattern** field.

13. In the Create Bleach Events section of the window, select an event pattern by choosing the spot, line, or polygon tool.

14. Click on the image in the Data Collection window to generate the selected bleach pattern.

15. Click on the **Imaging** tab to select the **Channel Setup** (exposure time, wavelength, and illumination source settings) and **Time Course Specification** conditions.

16. When you select the Time course style, the drop-down list presents three choices:
   - **Adaptive Time Intervals** – allows you to select the total number of images to be collected. Images are collected faster after the bleach event and then slower over time.
   - **Uniform Time Intervals** – allows you to select the total number of images collected, as well as the time interval between images. The value in the **Experiment Duration** field is calculated based on these factors. Also, if you change the value in the **Experiment Duration** field, the **Post-event images** field automatically updates.
   - **As Fast As Possible** – collects the images in rapid succession with no time-lapse interval. For this style, you enter only the total number of **Post-event images**.
The Run Experiment Tab

Use the Run Experiments tab options to access tools that allow you to select and execute experiment macros. There are also controls to monitor and interact with a running experiment.

![Image of the Run Experiment Tab]

**Note** The Design PK Experiment tab is available only for systems equipped with a laser module.

**The Green Arrow Button**
Starts the selected macro to run an experiment.

**Image file name**
Specifies the file name to use for the image in this text field. If you do not provide a file name, you will be asked to provide one when the experiment starts running. If you have the Auto-increment file names setting (in the Settings window) turned on, you will only need to provide a name once for each session. The names will have incrementing numbers appended to them automatically.

**Image title**
Specifies text to save in the header of the image file created by the experiment. The title can be viewed later using the Header Labels button of the Image window's Image Information window.
**Add note to log**
Inserts a note in the experiment's log file at any time before or while an experiment is running. (You will need to click **Do It** to insert the note.)

**Change next time lapse**
Changes the time lapse value while an experiment is executing. (You will need to type the desired time, in seconds, in the text field and click the **Do It** button to change the value.) This value only affects experiment macros that contain a TLAPSE command. It does not permanently change the macro.

| Note | This value does not change the current time lapse. |

**Show images during acquisition**
Displays images in a Data Collection window as they are collected. In cases where acquisition speed is important, deselecting this option may increase performance.

**Show PK Progress graph**
Opens a new window to display and update a preliminary results graph of a photokinetic experiment.

**Launch viewer after experiment**
Launches an Image window when the experiment is finished. Note that if more than two files are created in a point-visiting experiment, the Image windows will not launch even if this option is selected.

**Show Intensity Graph**
Provides three choices for displaying an intensity graph: **None**, **Maximum Intensity**, and **Mean Intensity**.

| Note | This option is available only for time-lapse images. |

**Schedule Post-Acquisition Processing Tasks**
Displays the Post Processing Options window.
Refer to the section “Scheduling Post-Acquisition Processing Tasks,” in the softWoRx Imaging Workstation User’s Manual for complete information on the options available from this window.

**Images acquired/requested:**
Displays the current number of images acquired, compared to the total number requested. (During fast acquisition, the reported number of acquired images may not be updated regularly.)

*Note* The request portion of this is updated as the experiment is designed.

**Disk space required**
Displays the estimated size of the experiment (i.e., the size of the image file). Resolve3D checks the disk space to make sure that enough space is available on the selected volume before it runs the experiment.

*Note* The disk space required value is updated as the experiment is designed.

**Elapsed time:**
Displays the elapsed time of the experiment, in seconds.

**Estimated Finish**
Displays the estimated clock time in which a running time-lapse experiment will finish.

**Current command:**
Reports each macro command as it is executed.
The Cancel Scan Button
Terminates a running experiment. (The images that are collected before the experiment is cancelled are automatically saved.)

Help
Opens the online Help for the Design/Run Experiment window.

The Settings Window

Use the Resolve3D Settings window to control how images are displayed, select camera settings, and specify file output.

To open the Settings window:

- From the Resolve3D Window, click the Settings button.

The Settings Window | Display Options

Note The Lasers tab is available only for systems that have lasers installed.

Settings Window Display Options
The Settings window Display tab options allow you to control how the images are displayed and what information about them is available.

Image Display Mode
Specifies which window and wave are used for image display. Five modes are currently available:
### Mode Description

<table>
<thead>
<tr>
<th>Mode</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>Displays images in the current window.</td>
</tr>
<tr>
<td>Scratch</td>
<td>Displays all images in the default Data Collection window (Window 21).</td>
</tr>
<tr>
<td>Auto Grayscale</td>
<td>Displays images in a separate window for each emission filter.</td>
</tr>
<tr>
<td>Auto Color</td>
<td>Displays images in color as they are collected. When using Point Visiting, images are automatically displayed in separate windows for each point. This option should be used only when you are running an experiment.</td>
</tr>
<tr>
<td>Point Track</td>
<td>Opens a separate window for each visited point in a point-visiting experiment.</td>
</tr>
</tbody>
</table>

**Window**

Specifies the number of the data collection window, sometimes referred to as the “Scratch” window. Select a new window to change where the next image will be displayed. (Temporary windows are numbered 21 or higher.)

**Wave**

Specifies the number of the wave (channel) that will receive the next image in the Data Collection window. Select a new wave to change which display window channel to use.

**Calculate statistics**

Calculates image intensity statistics. The typical reason for disabling this feature is to improve readout speed.

**Calculate histogram**

Calculates and displays the image intensity histogram in the Resolve3D status area during image collection. (This option does not set scaling.)

**Auto histogram range**

When enabled, automatically scales the Resolve3D status area histogram width for each image that is acquired, so that the histogram display ranges from the minimum to the maximum intensity. When disabled, the status area histogram is scaled to the full range of a typical CCD.

**Display images**

Displays images when the Acquire button is clicked or an experiment is running. Deselecting this option can increase performance.

**Deconvolve preview images**

Displays instantly processed 2D image previews that closely resemble images processed with advanced 3D image restoration techniques.
**Auto intensity scale**
Provides automatic scaling of the image intensity between the minimum and maximum brightness. (This switch applies only to the appearance of the displayed image, not the actual data.)

**Acquire after point visit**
Automatically acquires an image when the Visit Point option is selected.

**Show alignment target (crosshairs)**
Displays a crosshair over the data collection window indicating the location of the image center.

**Show Alignment Target**

**Settings Window Files Options**
Use the Settings window File options to set the image output directory, set the directory where experiment macros are stored, and select auto incrementing for file names.
Data Folder
Specifies the destination directory for Resolve3D output images. If you supply a directory that you do not have permissions to use, you will be warned. If you provide a directory name that does not exist, you will be presented with the option of creating the directory. (You can also change the destination directory by changing the global softWoRx Image Data Directory in the User Parameters tool.)

Experiment macros folder
Specifies the directory where your experiment macros are stored.

Data folder is temporary
Specifies that any directory entered in the Data Folder field only applies to the current Resolve3D session. In subsequent Resolve3D sessions, the Data folder value reverts back to the global softWoRx Data Directory as specified in User Parameters.

Use this option in environments where several users are running the microscope and using a single system login. Data folders can be created for each user under a common parent folder. As users start Resolve3D, they can choose to use their own folders.

In most cases, you should not use this option if each user has their own system login in your environment. With the option deselected, the Data folder assignment modifies the global softWoRx Data Directory definition and the system remembers the directory used in the last Resolve3D session.
**Auto-increment file names**
Creates a new file name by appending a serialized number to the base file name each time you run an experiment.

**Convert to 2 byte signed integer**
If enabled, Resolve3D saves all images as signed 16-bit (the softWoRx default). This setting is designed to increase compatibility with older software. Note that this setting cannot be enabled for EMCCD cameras.

**Settings Window Imaging Options**
The Settings window Imaging options allow you to select camera settings.

The Settings Window | Imaging Options

**Camera**
Specifies which camera to use. For the EMCCD camera, this list also specifies whether to use Conventional or Electron Multiplication mode.

**Frames to average**
Specifies the number of successive camera images to average into a result that is displayed in the window, increasing signal to noise. (This is similar to the AVG macro command.)

**Gain**
This field allows you to specify a gain value for the selected camera.

**Transfer speed**
Specifies a transfer rate for the selected camera (in kHz). Higher speeds may boost performance at the expense of image noise.
**Target temperature**
This field displays the cooled CCD camera's target temperature. This can be changed only by modifying the appropriate configuration file on the Instrument Controller computer.

**Note**  Not all cameras can be temperature controlled.

**Current temperature**
This field displays the current temperature. Resolve3D monitors the temperature and updates this value occasionally. If you want to force a request for the current temperature, click the **Refresh** button.

**Use photosensor**
Enables use of the photosensor.

**Settings Window Illumination Options**
Use the options on the Resolve3D Illumination tab to set up illumination parameters.

**“EX 1” and “EX 2” bulb age (hours)**
These fields provide figures for the current number of hours of use on each xenon bulb.

**Note**  The **EX 1** and **EX 2 bulb age (hours)** settings are displayed only if your DeltaVision system is using a xenon lamp for broadband illumination.
Field Stop Aperture (Closed <-> Open)
This field allows you to set the field stop aperture to Closed, Open, or any percentage in between. You can either enter a percentage in the field or use the slider to set the percentage.

| Note | When the setting is set to Closed, the field stop aperture is about 20µm in diameter. |

Critical/Köhler Illumination Switching
On an Applied Precision Fluorescence Illumination Module, there are three choices for the motorized Critical/Köhler illumination position:

- **Automatic** – *DeltaVision* automatically adjusts the position of the critical/Köhler illumination when the EX shutter is opened using the keypad (typically when the eyepieces are being used). The position is automatically returned to critical illumination when an image is requested, either via Resolve3D or by running an experiment.

- **Always Critical** – *DeltaVision* moves the critical/Köhler motor to critical illumination and leaves it in its position regardless of the current shutter states.

- **Always Köhler** – *DeltaVision* moves the critical/Köhler motor to Köhler illumination and leaves it in its position regardless of any new image requests or experiments running.

Settings Window Autofocus Options
Use the options on the Resolve3D Autofocus tab to set up Autofocus parameters.
Automatically determine parameters
Autofocus parameters are determined from the depth of field of the objective lens, which is calculated from the lens’ numerical aperture. You must select the proper objective lens in order to get the appropriate Autofocus settings. When the Automatically determine parameters checkbox is activated, the remaining fields in this window are set automatically.

Channel for Autofocus
This setting indicates the wavelength to use for Autofocus.

Contrast calculation method
This setting determines the polarity of the contrast calculation. There are three choices for image contrast calculation methods:

- **Auto** – The instrument controller usually can determine which contrast calculation method to use, but not always.
- **Fluorescence** – for light objects on a dark background
- **Brightfield** – for dark objects on a light background

Autofocus Z test step (μm)
This option sets the step size used for Autofocus within the maximum Z range.

Maximum Z test range (μm)
This setting indicates the maximum range that the Autofocus will search.

Post-autofocus Z offset (μm)
This setting can provide a constant offset after the Autofocus position determines the best plane of focus. Often times Autofocus will find a plane that is consistently different from the desired plane.

Calibrate UltimateFocus for Plate Scanning
This button displays the UltimateFocus Plate Calibration window.
This window provides a procedure for plate calibration. The **Home Stage Z** and the **Calibrate Focus** buttons at the bottom of the window are used in the calibration procedure. You can also set the **Z Sweep Minimum**, the **Z Sweep Maximum**, and the **Z Step Size** from this window.

When working with microtiter plates, many factors affect the performance of UltimateFocus, such as stage angle, plate flatness, plate angle and more. These types of issues are mitigated by characterizing UltimateFocus response over a larger Z range in a single well. This characterization (or calibration) is then used during the course of an entire plate scan. The boxes at the bottom of the calibration tool are used to limit the calibration Z range (**Z Sweep Min/Max**) and the **Step Size** which is used between UltimateFocus calibration measurements. Typically, the entire Z range is used with a step size of 0.20 μm. Using step sizes smaller than this can cause calibration to take longer to finish and may not increase performance.

**Settings Window Lasers Options**

Use the options on the Resolve3D Lasers tab to set up parameters for the attached lasers.

---

**Note** If no lasers are installed on your DeltaVision imaging system, the Laser tab is not displayed.
Laser Status
This field displays the peak wavelength for each laser currently installed on the DeltaVision system. Activate or deactivate the individual checkboxes to turn laser emission on or off.

Status
Each field shows the current status of the associated laser. The fields will display Off, Stabilizing, or Ready for each installed laser.

Power (%)
Each field displays the percentage of power currently applied to each laser.

Laser to test
Use the drop-down list in this field to select a specific laser on which to run PK tests.

Show spot alignment target
Activate this checkbox to include a target alignment reticle on the displayed test image as shown below.
**PK Laser Tests | Image The Spot**

**Image The Spot**
Selecting this button opens the laser shutter and acquires a single image of the laser spot. The event duration is set using the Exposure field in Resolve3D.

**Continuous**
Selecting this button opens the laser shutter and acquires continuous images of the laser spot. Select Stop Imaging to discontinue the imaging process.

**Do Test Bleach**
Perform a bleach test using the current Power and PK Duration settings.

**% Bleach**
The value entered in this field displays a before and after comparison of the intensities at the bleach spot: 100x (Signal After/Signal Before).

**Background**
The value in this field provides a measurement of the signal (intensity value) after the bleach event.

**Multi-line TIRF Settings**
Selecting this button opens the Multi-line TIRF Settings window.
Multi-line TIRF Settings Window
From the Multi-line TIRF Settings window, the following actions are available:

- **TIRF Depth** – allows you to select a laser to adjust its evanescent wave depth.

- **Adjust channels independently** – selects whether to independently adjust the TIRF Depth sliders. With the checkbox activated, you can adjust the depth setting for each laser separately.

  **Note** Keep in mind that adjusting these separately means a significant time penalty for imaging.

- **Block** – sets the TIRF depth adjuster to a position that prohibits illumination of the sample.

- **Epifluorescence** – sets the TIRF depth adjuster for epifluorescence (the highest value of the TIRF depth slider).

- **Restore TIRF Depth** – restores the TIRF depth adjuster to the last-used TIRF position.

- **Back Aperture Focus for Current Lens** – focuses the laser on the back aperture of the objective resulting in a collimated illumination laser and an even TIRF depth across the field of view. This value should be set once for each installed objective. To make sure this position is set correctly for imaging, verify the proper objective is selected from the Resolve3D dropdown menu. The **Locked** checkbox (activated by default) keeps the slider from being inadvertently moved.
- **Laser Path Splitter** – sets the percent of laser light to be directed to the TIRF illumination path versus the PK path.

**Settings Window Misc Options**

Use the options on the Resolve3D Misc tab to select or clear Lost Motion Compensation or to select a filter wheel configuration (if your system is configured to use alternate filter wheels).

![The Settings Window | Misc Options](image)

**Allow Lost Motion Compensation (LMC)**

Enable Lost Motion Compensation to remove the effect of hysteresis in the stage. This option should be selected for most applications. When the option is turned off, the Z focal plane may shift depending on the direction of approach. Clear this option if you want to improve speed at the expense of position repeatability.

**Spiral Mosaic**

Sets the size for the Spiral Mosaic preview collection pattern centered at the current stage location. The pattern begins at the center and then continues acquiring thumbnail images directly adjacent to it, spiraling outward in a counterclockwise rotation so that the entire area centered around the initial stage position is previewed in the Resolve3D Stage View. Thumbnail collection continues until either the preset spiral mosaic size (set here) is reached or you click the **Spiral Mosaic** button again. The stage is always returned to the initial position regardless of how the collection ends.
**Show stage trails**
Display the path of stage movement on the Resolve3D Stage View.

**Show stage thumbnails**
Display a thumbnail image of each image on the Stage View as the image is acquired.

**Show point numbers**
Display the number of each point in a point list on the Stage View.

**Filter Wheel Sets**
Use these fields to switch between active filter sets.

**Activate Filter Sets**
This button reinitializes the filter wheels after changes have been made in any of the above fields. Finalizes any new selections made to the Filter Wheel Sets fields and may include reinitializing filter wheels.

**Action Buttons**

**Done**
The Done button closes the Resolve3D Settings window.

**Save Settings**
The Save Settings button preserves the current options for your next Resolve3D session. In addition to options in the Resolve3D Settings window, current state information such as currently selected filters and exposure time is saved.

**Help**
The Help button opens the online Help for the Settings window.

**Keypad/Joystick Operation**

Many of the functions accessible through Resolve3D are also available on the keypad/ joystick. This section describes each key on the keypad/ joystick.

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**Note** Some buttons on your keypad may not be active.
RESET
Clears communication buffers, closes shutters, stops all motors, and clears encoder errors. Use the RESET button when you suspect that the workstation and controller are not synchronized.

CONTROL MODE
Toggles the controller mode between local mode and remote mode. Local mode enables control by the keypad and joystick. The remote mode disables the joystick and the keypad buttons (except for CONTROL MODE and a few other buttons) and shifts control to other internal components.

LMC RESET ON/OFF
Executes a Lost Motion Compensation (LMC) move.

Tip You can disable LMC on the Resolve3D Settings window. (For more information, see the online Help.)

REMOVE TRAIL
Clears the stage movement history from memory.

KEYLIGHT ON/OFF
Turns the keypad backlight on and off.
**BLANK SCREEN**
Suspends (or activates) the monitor’s light display (BLANK SCREEN is a toggle button). Use this feature when viewing dim samples or performing light sensitive experiments.

**SLOW/MEDIUM/FAST**
Adjusts the joystick stage movement speed.

**DISABLE MOTION KEYS**
Disables the eight keys below the joystick. This prevents accidental input from the keys when using the joystick.

**ACQUIRE MODE ARROWS**
Changes the acquisition mode as set in Resolve3D. (This includes the Excitation filter, exposure time, shutter configuration, and many of the options defined in the Settings window.)

**JOYSTICK**
Moves the stage in X and Y.

**POINT ARROWS**
Scrolls through the list of marked points. Press **ACQUIRE IMAGE** to view the image for a selected marked point.

**ND and EX ARROWS**
Changes the neutral density (ND) or excitation (EX) selection up or down the list.

**ACQUIRE IMAGE**
Commands the system to collect image data. This key function is identical to clicking **Acquire** in Resolve3D. Use this key when you are scanning through your sample and using the eyepiece to find a region of interest, or when you want to get a quick look at the specimen on the monitor.

**SAVE IMAGE**
Saves the last acquired image to the currently open file.

**STEP DECREASE/INCREASE**
Changes the step size of the movement controlled by the arrow keys located beneath the joystick. Pressing either button many times will change the step size to the minimum/maximum. There are 8 possible step sizes: 50nm, 100nm, 200nm, 400nm, 800nm, 2250nm, 4500nm, and 9000nm.

**X AND Y ARROW KEYS**
Move the stage in the direction shown.

**Z ARROW KEYS**
Move the stage in the direction shown.
**Z1 MARK/Z2 MARK**  
Mark the bottom (Z1) and the top (Z2) of the focal plane for your sample.

**EX SHUTTER**  
Toggles the excitation shutter between open and closed.

**TRANS SHUTTER**  
Toggles the transmitted light source between On and Off.

**POINT MARK**  
Adds the stage position to the marked points list.
Appendix F: Lasers and Safety Issues

This appendix describes the necessary precautions to take when working with instruments containing lasers, including required safety labeling and label locations. The appendix is divided into two main sections.

- **DeltaVision X4 Laser Module Safety** provides laser safety information for the *DeltaVision X4 Laser Module* when used in conjunction with the Olympus Fluorescence Illumination Module.

- **API Fluorescence Illumination Module Safety** provides laser safety information for the *DeltaVision X4 Laser Module* when used in conjunction with the Applied Precision (API) Fluorescence Illumination Module.

Much of the information presented is redundant from one section to the next, but not all. Please read the sections carefully to determine the proper safety measures to take for your particular *DeltaVision* laser configuration.
DeltaVision X4 Laser Module Safety

This section describes the hazards and precautions to take when using the *DeltaVision X4* Laser Module in conjunction with the Olympus Fluorescence Illumination Module. These hazards and precautions must be fully reviewed and understood, and proper safety protocols followed during any use of the *DeltaVision X4* Laser Module.

The *DeltaVision X4* Laser Module is a Class 3B laser system.

**Important Safety Recommendations**

The X4 Laser Module contains up to three 50mW lasers and one 100mW laser, and is considered a Class 3B device. This means that radiation from all installed lasers can exit the device at the same time. The power level is high enough to cause damage to the human eye instantaneously.

OSHA regulations require (via ANSI Z136.1) and IEC 60825-1 recommends that a Laser Safety Officer be identified who will be responsible for the safe use of Class 3B lasers. This includes training users, installing all necessary warnings and controls in the laser area, and other duties.

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**WARNING:**

Given the inherent exposure possible with an inverted frame microscope stand, users of the system must be trained in laser safety before using this instrument. Contact your lab administrator for information about Laser Safety training at your institution. Training is also available online at:

www.kentek-laser.com/edu/lasercrs.htm

The International Electrotechnical Commission (IEC) and the FDA recommend that Class 3B and Class 4 lasers be used only in restricted areas.

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**Safety Features**

The Laser Module provides several features that control access to the device and improve its safety.

- The Key Lock switch on the front panel of the Laser Source Chassis must be in the LASER ON position to enable use of the lasers. The key can be removed only when this switch is in the LASER STAND-BY position.
Laser Source Key Switch

- A Port Selector Interlock prevents laser output unless the Port Selector knob on the microscope stand is set to the Camera (left side port) position. This device prevents exposure to the beam while looking through the eyepieces.

Port Selector Knob for Laser Module

- Indicator lights on the system illuminate when the lasers are powered on and the interlocks are closed. The lasers can be fired at any time when the system is in this state, so the lights warn users to take any necessary precautions. A pair of indicator lights is visible on the front of the Laser Source Chassis. Another pair of indicator lights is visible on the Port Selector switch. All lights should be OFF or ON at the same time in proper operation.
A User Interlock Connector on the back panel of the Laser Source Chassis allows you to install a door interlock to prevent laser output when the door is open. When the door interlock is active, the lasers turn ON but cannot emit light past the shutter.

If you are not using a remote interlock, you must insert Interlock Jumpers into the User Interlock Port and the Fiber1 Interlock Port. These jumpers must be in place for the lasers to emit light.

For the lasers to operate, all of the following safety devices must be set as follows:

- The Key Lock switch must be ON.
- The Port Select knob must be set to the Camera position.
- The User Interlock Connector must be closed.
- At least one of the fiber interlocks must be closed.

If any of these devices are not set as described above, the shutters between the laser heads and the fibers are prevented from opening.

**Avoiding Specific Hazards**

The hazard of being exposed to the laser beam through the objective turret when no objective is in place can be mitigated by turning off the key lock switch on the front of the Laser Source Chassis prior to any system reconfiguration or maintenance.

Specific hazards during alignment or system maintenance include:

- Exposure to the beam when disassembling the Fiber Optic Module from the microscope.
- Exposure to the beam when disassembling the Laser Optics Module from the Fiber Optic Module.
Exposure to the beam when disassembling the optical fiber from the Laser Optics Module or the Laser Source Chassis.

Exposure to the beam while the polychroic beam splitter turret is removed from the stand.

Radiation can be emitted as follows:

- **Through the objective.** The beam that comes through the objective is emitted during imaging experiments. It can be as powerful as 26mW and includes all wavelengths of lasers available in the Laser Source Chassis. The beam is highly divergent and depends on the objective used; using various API-approved objectives, the divergence angle can be as low as 45 degrees or as high as 140 degrees. With the maximum power on all lasers, using the lowest-NA objective, the Nominal Optical Hazard Distance (NOHD) is less than 10cm (4 inches).

- **From the objective turret when no objective is in place.** This beam is only visible during maintenance (for example, when aligning the Laser Optics Module) or when the lasers are triggered accidentally without an objective being in place. This beam is collimated and small (a few mm across). The beam power may be as high as 30mW.

- **From the fluorescence illuminator when no polychroic is in place.** This beam is only visible during maintenance, when adding or removing cubes from the polychroic turret. This beam is collimated and small (a few mm across). The beam power may be as high as 45mW.

Whenever you use the X4 Laser Module, it is critical to keep your own safety and the safety of those around you a top priority. This is particularly important when maintaining the system, such as aligning the Optics Module or focusing the beam. *Some maintenance tasks involve potential exposure to dangerous levels of laser radiation.* According to ANSI Z135.1 (which is the standard the United States government uses for the safe use of lasers), the operator of a laser is responsible for the safety of everyone in the area, so you must be aware of the risks and keep others in the area safe. Some basic precautions will dramatically reduce the potential for injuries and damage.

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**WARNING:**  
**CAUTION - USE OF CONTROLS OR ADJUSTMENTS OR PERFORMANCE OF PROCEDURES OTHER THAN THOSE SPECIFIED HEREIN MAY RESULT IN HAZARDOUS RADIATION EXPOSURE.**

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**WARNING!** EVEN IF ALL OF THESE PRECAUTIONS ARE TAKEN, A RISK OF INJURY CAN STILL EXIST.
**DURING USE:**

- **Make sure the system is ready for use before enabling the lasers.** An objective should be in place and the polychroic turret should be installed. The indicator lights on the system (two small lights on or near the Port Selector Interlock, depending on whether or not the system includes the API FI Module) should be either all ON or all OFF.
- **Do not lean close to the objective** to view the sample or make adjustments to parts of the system, etc. while the laser is on.

**BEFORE BEGINNING MAINTENANCE:**

- **Whenever possible, work with the lasers disabled,** for example, by turning the key to the “LASER STAND-BY” position.
- **When lasers must be used, begin work by reducing the beam power** as much as possible. Only increase the beam power if the work cannot be performed with lower laser power settings.
- **Do not turn the laser on until the entire beam path is safe.** Determine where the beam is going to go before turning on the laser and make sure the beam is blocked as soon as possible. Clear all reflective surfaces from the beam path—a reflected beam can be dangerous for several meters in many cases.
- **Be extremely vigilant about putting items in the beam path.** Tools, watches, rings and microscope samples can all make excellent reflective surfaces, and when inserted into the beam path, they can steer the beam in dangerous and unpredictable ways.
- **If anyone is in the room, brief them on the procedures to be performed and what hazards will be present.** Reiterate that the area may be dangerous and that they must comply with any instructions you give regarding safety.

---

**WARNING:** CAUTION - USE OF CONTROLS OR ADJUSTMENTS OR PERFORMANCE OF PROCEDURES OTHER THAN THOSE SPECIFIED HEREIN MAY RESULT IN HAZARDOUS RADIATION EXPOSURE.

---

**TIRF-specific Laser Safety Considerations**

Due to the TIRF illumination optics provided by the TIRF Module and its laser component, the light being emitted from the DeltaVision 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. Appropriate laser safety goggles selected for the specific wavelength being used are recommended.
X4 Laser Module Safety Labeling

Standard Configuration

Avoid Exposure Caution Label

CAUTION
CLASS 3B VISIBLE AND INVISIBLE LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO BEAM.

Avoid Exposure Label

AVOID EXPOSURE.
VISIBLE AND INVISIBLE LASER RADIATION IS EMITTED FROM THIS APERTURE.

Avoid Exposure Label

CAUTION
CLASS 3B VISIBLE AND INVISIBLE LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO BEAM.

Optional Configurations

405nm Laser Option Label

Maximum Output = 100 mW
Wavelengths Emitted = 405 nm
IEC-80825-1:2001-08

405nm Laser Option

\[
\lambda \quad \text{max. power}
\]

405nm 100mW

445nm Laser Option Label

Maximum Output: 40mW
Wavelengths Emitted: 445nm
IEC-80825-1:2007-03

445nm Laser Option

\[
\lambda \quad \text{max. power}
\]

445nm 40mW
**488nm Laser Option Label**

Maximum Output = 50 mW  
Wavelengths Emitted = 488 nm  
IEC-60825-1-2007-03

<table>
<thead>
<tr>
<th>λ</th>
<th>max. power</th>
</tr>
</thead>
<tbody>
<tr>
<td>488nm</td>
<td>50mW</td>
</tr>
</tbody>
</table>

**514nm Laser Option Label**

Maximum Output = 50 mW  
Wavelengths Emitted = 514 nm  
IEC-60825-1-2007-03

<table>
<thead>
<tr>
<th>λ</th>
<th>max. power</th>
</tr>
</thead>
<tbody>
<tr>
<td>514nm</td>
<td>50mW</td>
</tr>
</tbody>
</table>

**561nm Laser Option Label**

Maximum Output = 50 mW  
Wavelengths Emitted = 561 nm  
IEC-60825-1-2007-03

<table>
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</tr>
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<tbody>
<tr>
<td>561nm</td>
<td>50mW</td>
</tr>
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</table>

**640nm Laser Option Label**

Maximum Output = 100 mW  
Wavelengths Emitted = 640 nm  
IEC-60825-1-2007-03

<table>
<thead>
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<th>λ</th>
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</tr>
</thead>
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<tr>
<td>640nm</td>
<td>100mW</td>
</tr>
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</table>

**X4 Safety Label Locations**

Laser Safety labels and notifications should be installed on the X4 Laser Module product as illustrated below. In the event that a label is not installed or installed improperly, please notify Applied Precision by contacting hotline@api.com or calling 800.862.5166.

**X4 Laser Source Chassis**

The following labels are placed on the front of the Laser Source Chassis.

**Note:** Only safety labels that correspond to the laser(s) installed in the Laser Source Chassis will be present.
The following photos show the locations for the safety labels above to be placed on the front of the Laser Source chassis.
Laser Module Key Switch Label Location

Note: Safety labels on the Laser Source Chassis will vary depending on which lasers are installed in your system.
X4 Laser Optics Module

The X4 Laser Optic Module connects to the DeltaVision Fiber Optic Module on non-API FI systems. The Laser Optics Module has the following laser safety label attached in two places on the module as shown.

One safety label is attached to the beam cover of the Optics Module as shown.

The second safety label is attached to the upper portion (opposite side) of the Optics Module as shown.
DeltaVision Stage

The following safety label is placed on the edge of the DeltaVision stage as shown.

Optics Module Safety Label (Location 2)

Laser Safety Label Location - DeltaVision Stage
**Polychroic Beam Splitter Removal Screw**

The following label is placed on the side of the DeltaVision, next to the screw for removing the Polychroic Beam Splitter.

![Polychroic Beam Splitter Removal Warning]

**Polychroic Beam Splitter Removal Warning**

This label warns users that, with the turret removed, laser radiation can come out through the fluorescence illuminator and be accessible. The label is attached to the DeltaVision as shown.

![CE Label]

**CE Label**

The CE Label for the X4 Laser Module is attached to the back of the Laser Source Chassis as shown.
API Fluorescence Illumination Module Safety

This section describes the hazards and precautions to take when using the API FI Module with lasers installed. These hazards and precautions must be fully reviewed and understood, and proper safety protocols must be followed during any use of the module, particularly those components which contain lasers.

Important Safety Recommendations

A DeltaVision system configured with the API FI Module can incorporate up to three 50mW lasers and one 100mW laser, and is considered a Class 3B device. This means that laser radiation from the installed lasers can exit the device at the same time. The power level is high enough to cause damage to the human eye instantaneously.

The UltimateFocus™ Module complies with CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007 IEC 60825-1, 2007-03.

OSHA regulations require (via ANSI Z136.1) and IEC 60825-1 recommends that a Laser Safety Officer be identified who will be responsible for the safe use of Class 3B lasers at your site. This includes training users, installing all necessary warnings and controls in the laser area, and other duties.

WARNING:
Given the inherent exposure possible with an inverted frame microscope stand, users of the system must be trained in laser safety before using this instrument. Contact your lab administrator for information about Laser Safety training at your institution. Training information is also available online at:
www.kenteklaserstore.com/
The International Electrotechnical Commission (IEC) and the FDA recommend that Class 3B and Class 4 lasers be used only in restricted areas.

Safety Features

The Laser Module component of the API Fluorescence Illumination Module provides several features that control access to the device and improve its safety.

- The Key Lock switch on the front panel of the Laser Source Chassis must be in the LASER ON position to enable use of the lasers. The key can be removed only when this switch is in the LASER STAND-BY position.
Appendix F: Lasers and Safety Issues

Laser Module Key Switch

- A Port Selector Interlock prevents laser output unless the Port Selector knob on the microscope stand is set to the Camera (left side port) position. This device prevents exposure to the beam while looking through the eyepieces.

Port Selector Knob

- Indicator lights on the system illuminate when the lasers are powered on and the interlocks are closed. The lasers can be fired at any time when the system is in this state, so the lights warn users to take any necessary precautions. A pair of indicator lights is visible on the front of the Laser Source Chassis. Another pair of indicator lights is visible on the Port Selector switch. All lights should be OFF or ON at the same time in proper operation.
A User Interlock Connector on the back panel of the Laser Source Chassis allows you to install a door interlock to prevent laser output when the door is open. When the door interlock is active, the lasers turn ON but cannot emit light past the shutter.

If you are not using a remote interlock, you must insert Interlock Jumpers into the User Port and the Fiber1 Interlock Port. These jumpers must be in place for the lasers to emit light.

For the lasers to operate, all of the following safety devices must be set as follows:

- The Key Lock switch must be ON.
- The Port Select knob must be set to the Camera position.
- The User Interlock Connector must be closed.
- At least one of the fiber interlocks must be closed.

If any of these devices are not set as described above, the shutters (between the laser heads and the fibers) are prevented from opening.

**Avoiding Specific Hazards**

The hazard of being exposed to the laser beam through the objective turret when no objective is in place can be mitigated by turning off the key lock switch on the front of the Laser Source Chassis prior to any system reconfiguration or maintenance.

Specific hazards during alignment or system maintenance include:

- Exposure to the beam when servicing the Fiber Optic Module from the microscope.
- Exposure to the beam when servicing the Laser Optics Module from the Fiber Optic Module.
Exposure to the beam when servicing the optical fiber from the Laser Optics Module or the Laser Source Chassis.

Exposure to the beam while the polychroic beam splitter turret is removed from the stand.

Radiation can be emitted as follows:

- **Through the objective.** The beam that comes through the objective is emitted during imaging experiments. It can be as powerful as 26mW and include all wavelengths of lasers available in the Laser Source Chassis. The beam is highly divergent and depends on the objective used; using various API-approved objectives, the divergence angle can be as low as 45 degrees or as high as 140 degrees. With the maximum power on all lasers, using the lowest-NA objective, the Nominal Optical Hazard Distance (NOHD) is less than 10 cm (4 inches).

  **WARNING!** When using low NA air objectives (anything below 0.45 NA), the UltimateFocus™ laser beam does not diverge as much as it does with higher NA objectives. The beam, a Class 3B Invisible Laser light up to 2.2mW, is nearly collimated and is emitting straight up through the objective. Whenever the Laser Emission Indicators show that UltimateFocus™ is on, users must not look down the objective turret.

- **From the objective turret when no objective is in place.** This beam is only visible during maintenance (for example, when aligning the Laser Optics Module) or when the lasers are triggered accidentally without an objective being in place. This beam is collimated and small (a few mm across). The beam power may be as high as 30mW.

- **From the fluorescence illuminator when no polychroic is in place.** This beam is only visible during maintenance, when adding or removing cubes from the polychroic turret. This beam is collimated and small (a few mm across). The beam power may be as high as 45mW.

Whenever you use any of the DeltaVision lasers, it is critical to keep your own safety and the safety of those around you a top priority. This is particularly important when maintaining the system, such as aligning the Optics Module or focusing the beam. Some maintenance tasks involve potential exposure to dangerous levels of laser radiation. According to ANSI Z135.1 (which is the standard the United States government uses for the safe use of lasers), the operator of a laser is responsible for the safety of everyone in the area, so you must be aware of the risks and keep others in the area safe. Some basic precautions will dramatically reduce the potential for injuries and damage.

**WARNING: CAUTION - USE OF CONTROLS OR ADJUSTMENTS OR PERFORMANCE OF PROCEDURES OTHER THAN THOSE SPECIFIED HEREIN MAY RESULT IN HAZARDOUS RADIATION EXPOSURE.**
**WARNING!** EVEN IF ALL OF THESE PRECAUTIONS ARE TAKEN, A RISK OF INJURY CAN STILL EXIST.

### DURING USE:
- **Make sure the system is ready for use before enabling the lasers.** An objective should be in place and the polychroic turret should be installed. The indicator lights on the system (two small lights on the Port Selector Interlock and two more on the Laser Source Chassis) should be either all ON or all OFF.
- **Do not lean close to the objective** to view the sample or make adjustments to parts of the system, etc. while the laser is on.

### BEFORE BEGINNING MAINTENANCE:
- **Whenever possible, work with the lasers disabled,** for example, by turning the key to the “LASER STAND-BY” position.
- **When lasers must be used, begin work by reducing the beam power** as much as possible. Only increase the beam power if the work cannot be performed with lower laser power settings.
- **Do not turn the laser on until the entire beam path is safe.** Determine where the beam is going to go before turning on the laser and make sure the beam is blocked as soon as possible. Clear all reflective surfaces from the beam path—a reflected beam can be dangerous for several meters in many cases.
- **Be extremely vigilant about not putting items in the beam path.** Tools, watches, rings and microscope slides can all create excellent reflective surfaces, and when inserted into the beam path, they can steer the beam in dangerous and unpredictable ways.
- **If anyone is in the room, brief them on the procedures to be performed and what hazards will be present.** Reiterate that the area may be dangerous and that they must comply with any instructions you give regarding safety.
- **Always wear appropriate laser safety goggles.**

---

**WARNING: CAUTION - USE OF CONTROLS OR ADJUSTMENTS OR PERFORMANCE OF PROCEDURES OTHER THAN THOSE SPECIFIED HEREIN MAY RESULT IN HAZARDOUS RADIATION EXPOSURE.**

---

**TIRF-specific Laser Safety Considerations**

Due to the TIRF illumination optics provided by the TIRF/PK Module and its laser component, the light being emitted from the *DeltaVision* 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. 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.
Safety Labeling

Standard Configuration

Avoid Exposure Caution Label

CAUTION
CLASS 3B VISIBLE AND INVISIBLE LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO BEAM.

Avoid Exposure Label

AVOID EXPOSURE.
VISIBLE AND INVISIBLE LASER RADIATION IS EMITTED FROM THIS APERTURE.

Avoid Exposure Label

CAUTION
CLASS 3B VISIBLE AND INVISIBLE LASER RADIATION WHEN OPEN.
AVOID EXPOSURE TO BEAM.
Optional Configurations

405nm Laser Option Label
- Maximum Output = 100 mW
- Wavelengths Emitted = 405 nm
- IEC-60825-1-2001-08

405nm Laser Option
\( \lambda \) max. power
- 405nm 100mW

445nm Laser Option Label
- Maximum Output = 40mW
- Wavelengths Emitted = 445 nm
- IEC-60825-1-2007-03

445nm Laser Option
\( \lambda \) max. power
- 445nm 40mW

488nm Laser Option Label
- Maximum Output = 50 mW
- Wavelengths Emitted = 488 nm
- IEC-60825-1-2001-08

488nm Laser Option
\( \lambda \) max. power
- 488nm 50mW

514nm Laser Option Label
- Maximum Output = 50 mW
- Wavelengths Emitted = 514 nm
- IEC-60825-1-2007-03

514nm Laser Option
\( \lambda \) max. power
- 514nm 50mW

561nm Laser Option Label
- Maximum Output = 50 mW
- Wavelengths Emitted = 561 nm
- IEC-60825-1-2007-03

561nm Laser Option
\( \lambda \) max. power
- 561nm 50mW

640nm Laser Option Label
- Maximum Output = 100 mW
- Wavelengths Emitted = 640 nm
- IEC-60825-1-2007-03

640nm Laser Option
\( \lambda \) max. power
- 640nm 100mW

Safety Label Locations

Laser Safety labels and notifications should be installed on the API Fluorescence Illumination Module product as illustrated below. In the event that a label is not installed or installed improperly, please notify Applied Precision by contacting hotline@api.com or calling 800.862.5166.

Laser Source Chassis

The following labels are placed on the front of the Laser Source Chassis.

\( \text{Note: Only safety labels that correspond to the laser(s) installed in the Laser Source Chassis will be present.} \)
Appendix F: Lasers and Safety Issues

Laser Module Key Switch Safety Label

Primary Laser Safety Label

640nm Laser Safety Label

Maximum Output = 100 mW
Wavelengths Emitted = 640 nm
IEC-60825-1-2007-03

561nm Laser Safety Label

Maximum Output = 50 mW
Wavelengths Emitted = 561 nm
IEC-60825-1-2007-03

514nm Laser Safety Label

Maximum Output = 50 mW
Wavelengths Emitted = 514 nm
IEC-60825-1-2007-03

488nm Laser Safety Label

Maximum Output = 50 mW
Wavelengths Emitted = 488 nm
IEC-60825-1-2007-03

445nm Laser Safety Label

Maximum Output = 40 mW
Wavelengths Emitted = 445 nm
IEC-60825-1-2007-03
405nm Laser Safety Label

The following photo shows the locations for the safety labels above to be placed on the front of the Laser Source chassis.

**Note:** Safety labels on the Laser Source Chassis will vary depending on which lasers are installed in your system.
Laser Safety Labels with API FI

Depending on how the API Fluorescence Illumination Module is configured, it can result in adding several different visible and invisible laser wavelengths to the standard DeltaVision system. Since the different options available with this module are so many and varied, you should consult the following table to determine the location and type of Laser Safety Labels that should be attached to a particular system.

The following table shows the location and type of Laser Safety Labels, depending on the various options installed, that should be attached to a system equipped with the API FI Module. Use this table in conjunction with the illustrations immediately following, in which the label positions are called out by number.

<table>
<thead>
<tr>
<th>Options and Label Locations</th>
<th>X4</th>
<th>Ultimate Focus™</th>
<th>X4 and Ultimate Focus™</th>
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<tbody>
<tr>
<td>Visible Laser Warning</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Invisible Laser Warning</td>
<td>9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of Laser Safety Labels on DeltaVision System]

![Image of Visible Laser Warning Label]

![Image of Invisible Laser Warning Label]
<table>
<thead>
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<th>Visible and Invisible Laser Warning</th>
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</thead>
<tbody>
<tr>
<td>Class 3B Invisible when Open Laser Warning</td>
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</tr>
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<td>Class 3B Visible when Open Laser Warning</td>
<td>1, 4, 5A, 6, 7, 10, 11A</td>
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<tr>
<td>Invisible Laser Radiation, Class 3B</td>
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</tr>
<tr>
<td>Class 3B Visible and Invisible when Open</td>
<td>3</td>
</tr>
</tbody>
</table>
Appendix F: Lasers and Safety Issues

Port Select Laser Safety Label

- Label is also present underneath this panel.
- Label is present only when the Ultimate Focus Module is not installed.

Laser Safety Label Locations (as viewed from the right rear of the system)

Laser Safety Label Locations (as viewed from the left front)
**DeltaVision Stage**

The following label is placed on the edge of the *DeltaVision* stage as shown.

**CO₂ Cell Box / TIRF Cover**

The following label is placed on the CO₂ Cell Box / TIRF Cover as shown.
**Polychroic Turret Removal**

The following label is placed on the right side of the *DeltaVision*, next to the screw for removing the Polychroic Turret.

This label warns users that, with the turret removed, laser radiation can be accessible coming out through the fluorescence illuminator. The label is attached to the *DeltaVision* as shown.

**Compliance Labels**

The CE Label for the X4 Laser Module is attached to the back of the Laser Source Chassis as shown.
X4 Laser Module - CE Label

The Compliance Label for the UltimateFocus™ Module is attached to the back of the DeltaVision Microscope as shown.
Appendix G: China_RoHS

This symbol indicates the product contains hazardous materials in excess of the limits established by SJ/T11364-2006 Marking for Control of Pollution caused by Electronic Information Products. The number in the symbol is the Environment-friendly Use Period (EFUP), which indicates the period during which the toxic or hazardous substances or elements contained in electronic information products will not leak or mutate under normal operating conditions so that the use of such electronic information products will not result in any severe environmental pollution, any bodily injury or damage to any assets, the unit of the period is "Year". In order to maintain the declared EFUP, the product shall be operated normally according to the instructions and environmental conditions as defined in the product manual, and periodic maintenance schedules specified in Product Maintenance Procedures shall be followed strictly. Consumables or certain parts may have their own label with an EFUP value less than the product. Periodic replacement of those consumables or parts to maintain the declared EFUP shall be done in accordance with the Product Maintenance Procedures.

This symbol indicates that this electronic information product does not contain any toxic or hazardous substances or elements above the maximum concentration value established by the Chinese standard SJ/T11363-2006, and can be recycled after being discarded, and should not be casually discarded.
O: 表示该有毒有害物质在该部件所有均质材料中的含量均在SJ/T11363-2006标准规定的限量要求以下
X: 表示该有毒有害物质至少在该部件的某一均质材料中的含量超出SJ/T11363-2006标准规定的限量要求

由于缺少经济上或技术上合理可行的替代物质或方案,此医疗设备运用以上一些有毒有害物质来实现设备的预期临床功能,或给人员或环境提供更好的保护效果。

O: Indicates that this toxic or hazardous substance contained in all of the homogeneous materials for this part is below the limit requirement in SJ/T11363-2006.
X: Indicates that this toxic or hazardous substance contained in at least one of the homogeneous materials used for this part is above the limit requirement in SJ/T11363-2006.

• Data listed in the table represents best information available at the time of publication
• Applications of hazardous substances in this medical device are required to achieve its intended clinical uses, and/or to provide better protection to human beings and/or to environment, due to lack of reasonably (economically or technically) available substitutes.

<table>
<thead>
<tr>
<th>部件名称</th>
<th>铅 (Pb)</th>
<th>汞 (Hg)</th>
<th>镉 (Cd)</th>
<th>六价铬 (Cr6+)</th>
<th>多溴联苯 (PBB)</th>
<th>多溴二苯醚 (PBDE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printed Circuit Board Assemblies</td>
<td>X</td>
<td>O</td>
<td>O</td>
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<td>O</td>
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<tr>
<td>Arc Lamp and Filter Assembly</td>
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<td>X</td>
<td>O</td>
<td>O</td>
<td>O</td>
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<td>Power Supplies</td>
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