# DeltaVision OMX™

## Customer Instructions

## Photokinetics

# DeltaVision OMX™ Photokinetics

- *Discusses important use and safety information*
- *Introduces Photokinetics*
- *Describes the Ring TIRF/PK Hardware Module*
- *Describes how to acquire and analyze PK (FRAP and FRET) data*

## Important user information

**WARNING**
Using controls, making adjustments, or performing procedures other than those specified in the DeltaVision OMX Imaging System's documentation can result in hazardous exposure to high voltage, laser radiation, or moving parts. Exposure to these hazards can cause severe personal injury. Do not operate the DeltaVision OMX Imaging System in any other way than described in the user documentation.

**IMPORTANT!**
Prior to reading this document, you must read and understand the safety and operating information described in the *DeltaVision OMX Operating Instructions* and the *DeltaVision OMX Getting Started Guide*.

Do not operate the DeltaVision OMX system in any way other than described in the user documentation. If you do, you may be exposed to hazards that can lead to personal injury and you may cause damage to the equipment.

### Intended Use

Use of the DeltaVision OMX Imaging System assumes that you are familiar with the basics of fluorescence microscopy. In addition, an understanding of image processing basics will help you use the system to its full potential. To manage the computer systems, familiarity with Linux workstations and Windows-based personal computers is also helpful.

## About Photokinetics

Photokinetics (PK) refers to the reactivity of fluorescent molecules while they are in an excited state. Photokinetics can be used to study the interactions of molecules within living cells. Photo-bleaching, FRET, and photo-activation are examples of PK reactions.

The following table shows photokinetic experiment methods and the biological applications that can be studied with each of these methods.

**Table .1 Photokinetic Methods and Applications**

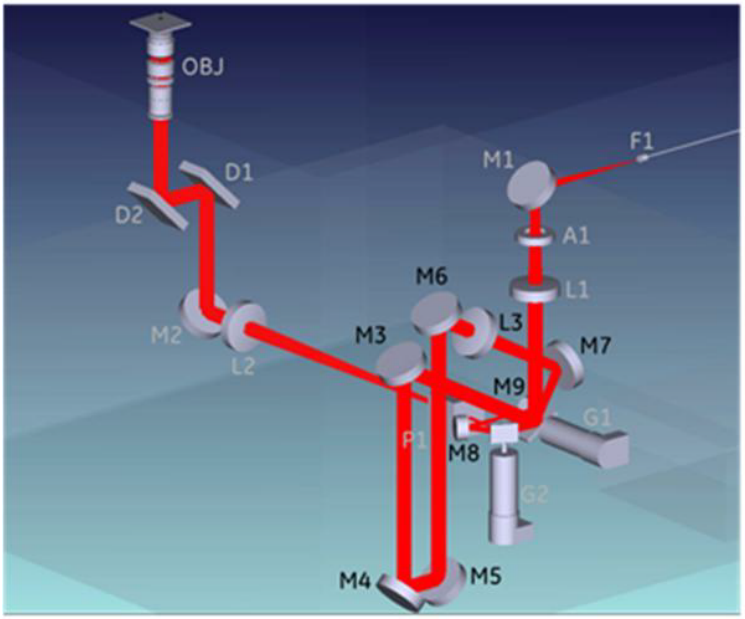
| Photokinetic Methods | Biological Applications |
|---|---|
| FRAP (Fluorescence Recovery After Photo-Bleaching) - Single-Point and Multi-Point | Affinity, Biomolecular Cycling, Biomolecular Environment, Structural Kinetics |
| Pattern Bleaching | Compartmental Analysis, Biomolecular Cycling, Transport |
| FLIP | Compartmental Analysis, Biomolecular Cycling, Transport, Structural Visualization |
| Background Reduction | Structural Visualization |
| Combinations:<br>- FRAP/FRET<br>- Repeat during cell cycle<br>- Rapid Repeat | Compartmental Analysis, Biomolecular Cycling, Transport<br>Affinity, Biomolecular Cycling, Biomolecular Environment |
| FRET:<br>- Sensitized emission<br>- Donor Photo-bleaching<br>- Acceptor Depletion | Affinity, Biomolecular Environment |
| Photo Activation | Compartmental Analysis, Affinity, Biomolecular Cycling, Biomolecular Environment, Transport, Cell Fate, Structural Kinetics, Structural Visualization |

# The Ring TIRF/PK Module

Light is delivered to the sample for PK applications using a by-pass system incorporated into the DeltaVision OMX Ring TIRF Module. The same mirror system is used to control both the TIRF and PK beams, making it possible to bleach areas of different sizes and configurations, depending on how the PK beam is directed. The following figure illustrates the PK light path.

**NOTE** Based on the system hardware configuration, the light path may vary from that shown in the following figure.

Figure 1. Ring TIRF Module - PK Light Path




**Optical Components**

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

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

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

## Ring TIRF/PK Hardware

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

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

Figure 2. Ring TIRF/PK Module Mounted on DeltaVision OMX System Block (Yellow-Bordered Area)

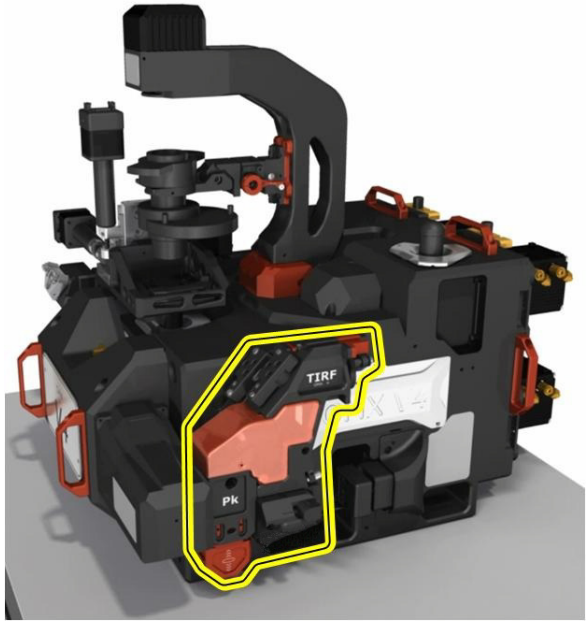



Figure 3. Ring TIRF/PK Module (Close Up)

# Ring TIRF/PK Laser Safety Considerations

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

**WARNING!**
The DeltaVision OMX Imaging System is a Class I laser system. No access to laser radiation is permitted during operation or maintenance. During service, however, Class IV radiation is accessible.

Due to the potential for personal injury, particularly to the eyes, service on the DeltaVision OMX Imaging System should ONLY be performed by GE personnel or persons trained by GE specifically for this purpose. Unauthorized service by any other personnel may violate the warranty.

**WARNING! (Service Only)** When servicing the TIRF/PK system, use extreme caution that the emitted light is not directed into anyone's eyes. Appropriate laser safety goggles selected for the specific wavelength being tested are mandatory.

# Running PK Experiments

This section describes how to run a basic photokinetic experiment, such as FRAP or FRET.

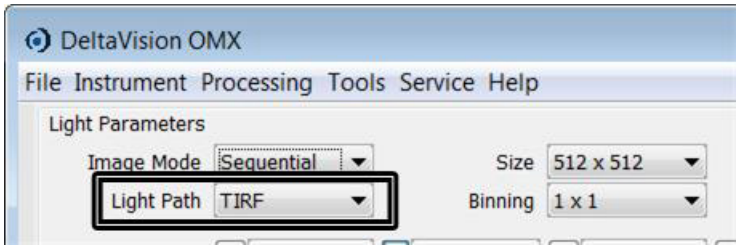
### To run a basic PK experiment:

1. Mount the sample slide and focus on the sample using the appropriate camera and excitation settings for your experiment.

   **Tips**
   **#1** Selecting a "Conventional" ***Light Path*** setting may help you focus on the sample.
   **#2** Raising the stage brings the coverslip more closely into focus so, to focus near the coverslip, raise the stage to the highest position at which the sample remains in focus.

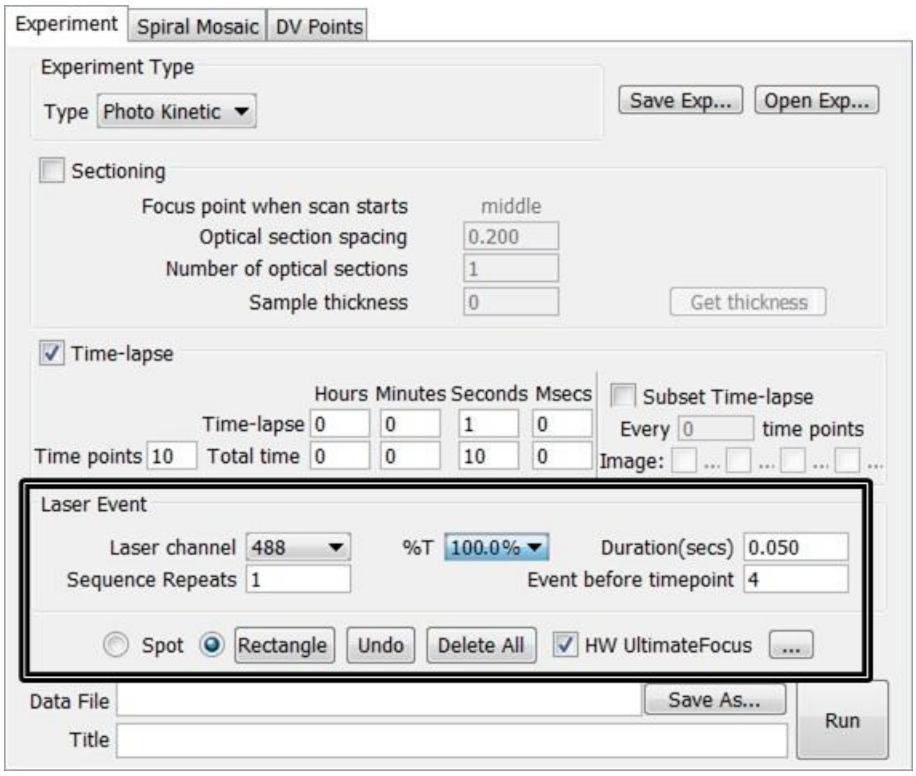
2. In the **Image Mode** field, select either the "Simultaneous" or the "Sequential" imaging mode. For multi-channel PK experiments, select "Simultaneous" for faster imaging after the PK event.
3. In the **Light Path** field, select "TIRF" or "Conventional."

Figure 4. Select the TIRF or Conventional Light Path



4. Set the **%T** and the **Exposure** fields to produce imaging conditions at the desired signal level.
5. On the Experiment tab on the main program window, select the "Photo Kinetic" experiment ***Type***. (The PK-specific fields will appear at the bottom of the tab, in a section titled Laser Event, as shown in the following figure.)

Figure 5. Photo Kinetic Experiment Tab




6. Set up the ***Sectioning*** parameters as desired for your photokinetic experiment. If sectioning ***is not*** enabled, the laser event will occur at the imaging plane. If sectioning ***is*** enabled, the laser event will occur at the top, middle, or bottom of the sample, as defined in the ***Focus point when scan starts*** field.
7. Set up the ***Time-lapse*** parameters as desired for your photokinetic experiment.
8. In the Laser Event section, select the ***Laser channel*** to use for the laser event.
9. Adjust the ***%T*** and ***Duration (secs)*** fields as required for your experiment.

   **NOTES**
   **#1** When setting up these fields, we recommend that you use alternative (sacrificial) cells for testing the bleaching/activation effects.
   **#2** If you are imaging in TIRF mode and using the same laser line for both illumination and the laser event, the ***%T*** setting will remain at the same level for both imaging and the PK event.
10. Specify how often the photokinetic event sequence repeats in the ***Sequence Repeats*** field.

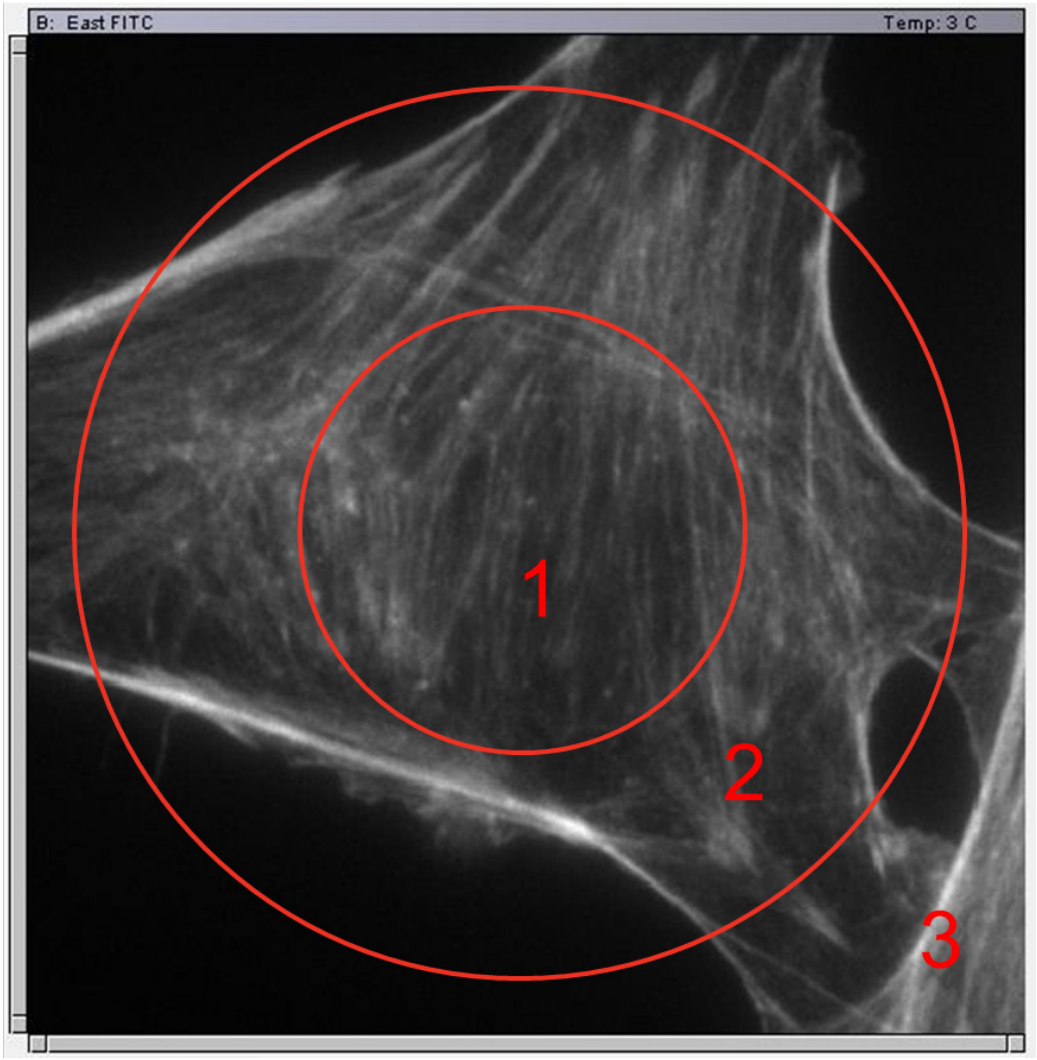
    **NOTE** Typical FRAP experiments will use a Sequence Repeats value of "1". If another value (***x***) is used, the entire timelapse sequence will be repeated ***x*** times. For example,

if ***Sequence Repeats*** was set to "3" in the experiment above, a total of 30 timepoints would be acquired with the laser events occurring three times.

11. Specify the number of time points before the laser event occurs in the ***Event before timepoint*** field. For the experiment shown in the previous figure, the laser event will occur between time points 3 and 4.
12. Laser events can be a single spot or rectangle, multiple spots or rectangles, or a combination of both.

    **NOTE** The PK targeting accuracy is best near the middle of the field of view and will decrease as you move outwards. For best results, position the areas to bleach (activate) near the center of the image. For reference, expected accuracy for the image below (512x512 field of view) is about 0.5 µm in area 1, about 1 µm in area 2, and about 2 µm in area 3,

Figure 6. PK Targeting Accuracy



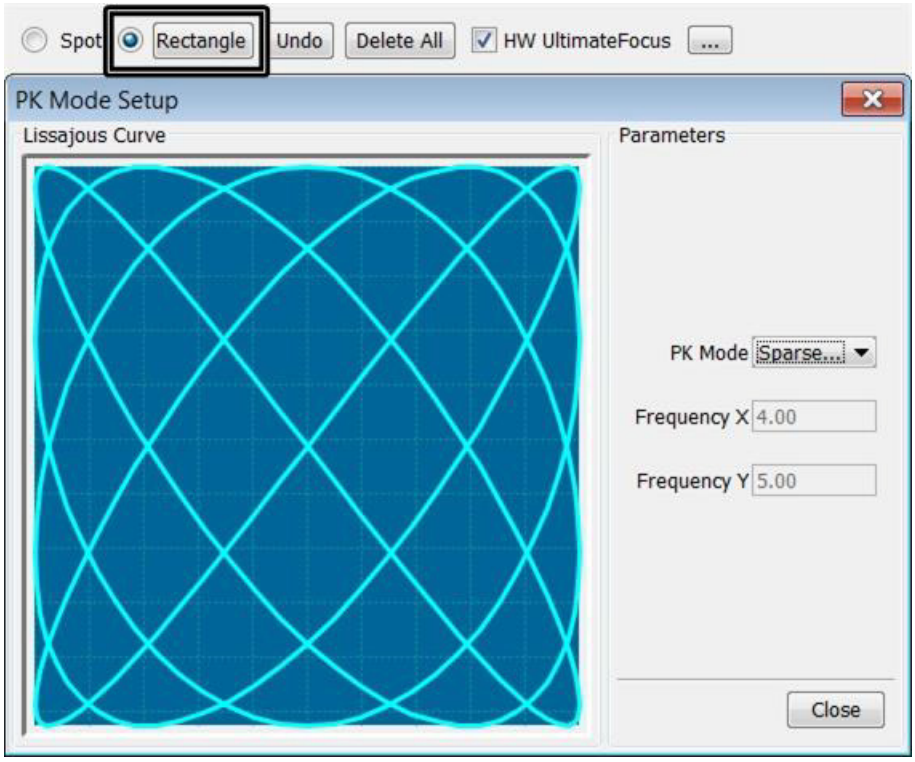

For spots, click the ***Spot*** radio button then click on the image where you want the bleach event to occur. The spot is roughly 0.5 microns in diameter and will change based on the objective used. The bleach spot is formed by moving the galvos to the defined position while the stage remains static throughout the laser event.

For rectangles, click the ***Rectangle*** radio button and then click and drag the mouse cursor on the image to draw a rectangle of the desired size. The bleach rectangle is formed by continuously moving the galvos during the laser event to form a Lissajous pattern. The density of the Lissajous pattern is defined by clicking the ***Rectangle*** button (which appears when you select the ***Rectangle*** radio button). In the PK Mode Setup dialog box that appears, make the appropriate ***PK Mode*** selection based on the following definitions:

- **Dense.** Provides a denser fill for larger rectangles.
- **Sparse.** Provides a sparser fill for smaller rectangles.
- **Manual.** Allows you to specify the X and Y frequency for the pattern. The ideal settings are when Y = X - 1 or X + 1.

As the Lissajous parameters are changed, an estimation of the laser pattern is drawn in the window. If necessary, click the ***Undo*** or ***Delete All*** buttons to remove the bleach patterns and start over.

Figure 7. Defining a Rectangular Event Pattern



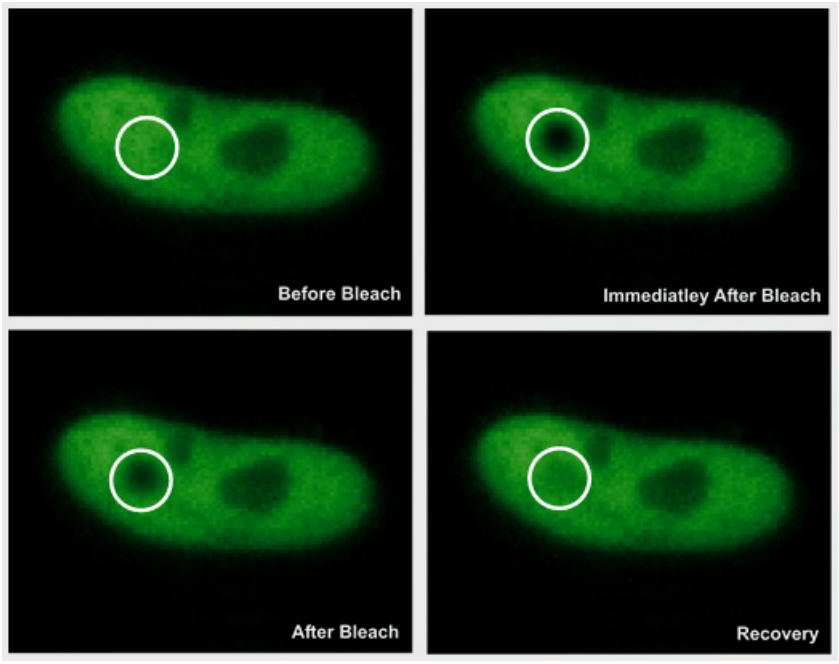

13. If you would like to use ***HW UltimateFocus***, select the check box and click the **...** button to set up the parameters.
14. Enter a ***Data File*** name and ***Title*** (if desired) and then click ***Run*** to start the experiment.

# Analyzing FRAP Data

The FRAP experiment method consists of photo-bleaching a point (or points) of interest and then observing the recovery of the fluorescence in the bleached area.

An example of a single-point FRAP experiment is shown in the following figure.

Figure 8. A Point of Interest Is Photo-Bleached and Monitored




There are two types of FRAP data:

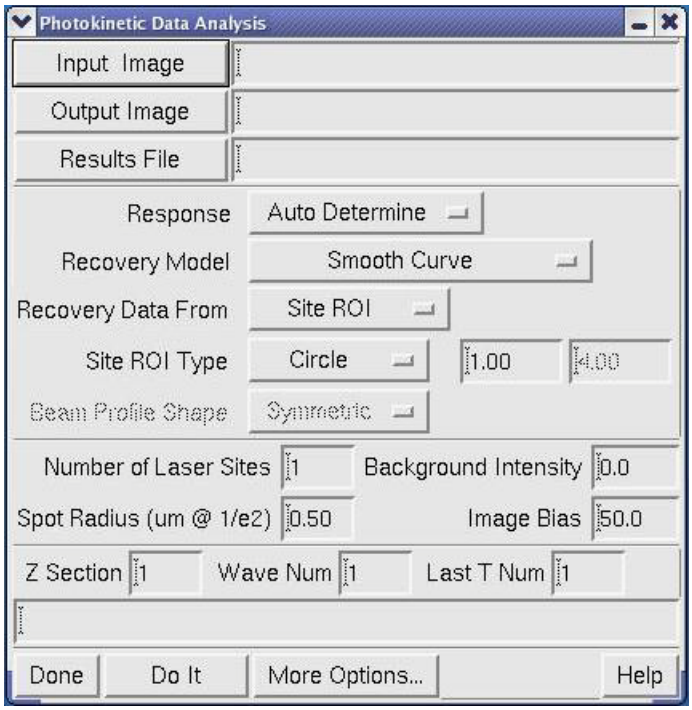
- Single-Point FRAP data is collected in experiments that monitor a single location or that monitor several locations in a sequential fashion.
- Multi-Point FRAP data is collected in experiments that monitor several locations in the sample at the same time.

The following procedure describes how to analyze FRAP data using the softWoRx Imaging Workstation.

### To analyze FRAP data:

1. Open an image in the Image window. From the softWoRx main menu, choose ***Measure:PK Analysis*** to display the Photokinetic Data Analysis window.

   Figure 9. PK Data Analysis Window



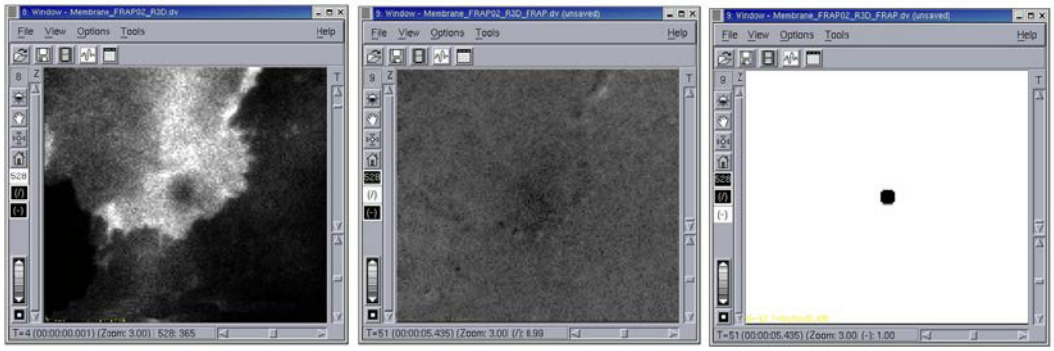

   **NOTE** You can use the Photokinetic Data Analysis window to specify a recovery model, type of ROI, beam profile shape, and number of sites. You can also use it to remove background intensity, select which Z sections and wavelengths to include in the analysis, and specify other options.

2. Drag the window number into the ***Input Image*** field.
3. Select the desired ***Response*** type, ***Recovery Model***, and ***Recovery Data From*** options.
4. If you are analyzing a Multi-point FRAP data set, enter the number of laser sites into the ***Number of Laser Sites*** field.
5. If you are using background subtraction, enter a background value into the ***Background Intensity*** field.
6. To determine a number to enter into the ***Spot Radius*** field, use the Measure Distances tool to make an approximate measurement of the bleach spot.
7. Click ***Do It*** to run the analysis.

The software will generate the following files:

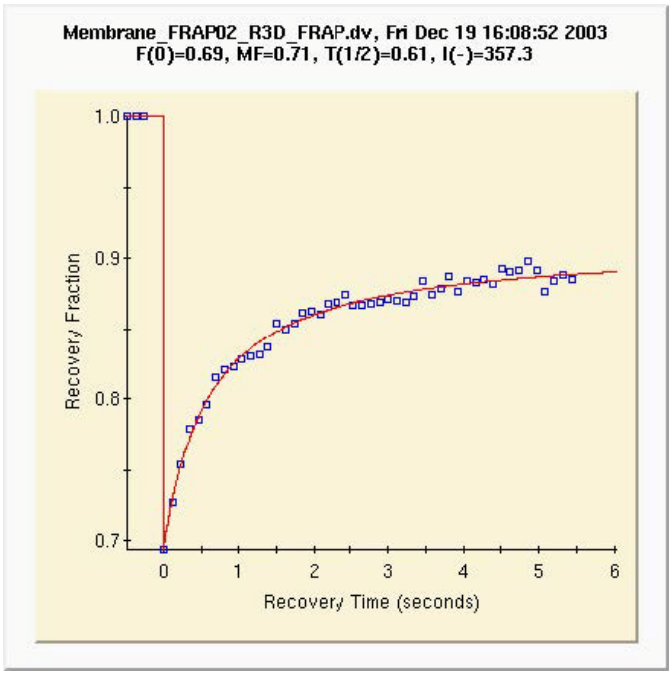
- A three channel output image file containing 1) the original time lapse image, 2) the ratio of the current time point to average pre-bleach time points, and 3) the ratio data at the location used for analysis.

Figure 10. Three Channel Image Generated by the Photokinetic Data Analysis Tool



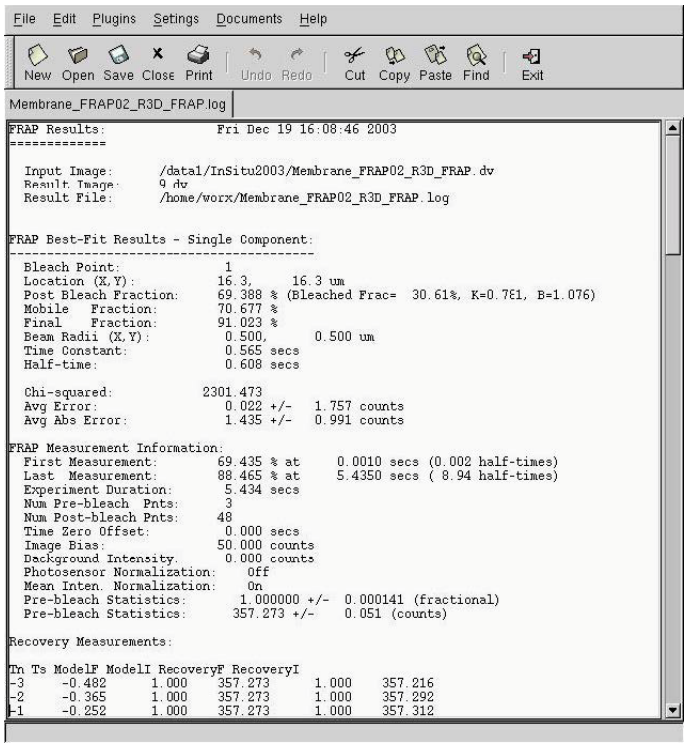
- A JPEG file containing a recovery graph that plots the fluorescence intensity before and after the event.

Figure 11. Fluorescence Recovery Graph



- A log file containing the analysis results.

Figure 12. FRAP Analysis Log File



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

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