

DeltaVision®

Quantifiable Laser Module (QLM)

INTRODUCTION

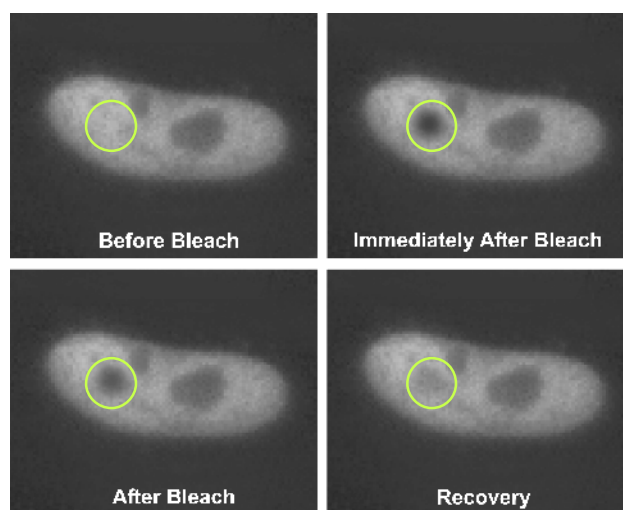
The Quantifiable Laser Module (QLM) is an optional DeltaVision system component that adds a laser beam into the back aperture of the microscope objective to provide a focused illumination spot in the center of the optical field.

The QLM is designed for photokinetic experiments that involve the interactions of light with biomolecules and fluorochromes. A photokinetic event is an event within an experiment in which the sample is illuminated with a laser. It can be as simple as photobleaching a single diffraction-limited location in a cell. Or it can be as complex as repeatedly activating a pattern of points using one laser and then switching to a different laser to photobleach a smaller region within that pattern. Whether this event causes photobleaching, photoactivation, or some other phenomenon is largely dependent on the molecules that are present and the parameters of the photokinetic event (e.g., laser wavelength, laser power, and spot size).

For live-cell dynamics, the optional Quantifiable Laser Module features the industry's shortest switching times between photobleach and image acquisition in an integrated commercial package—all without compromising acquisition spot or area size, for fast detection of rapid molecular dynamics. Fast FRAP is a unique feature on the DeltaVision system. Fast Frap allows the user to image almost instantaneously after the photobleach event (< 2 ms compared to most confocal FRAP applications).

The QLM provides simple-to-set up and simple-to-use laser-based applications, FRAP, PA-GFP and colocalization (laser-FRET and FLIP), all in a single integrated system. Acquisition and analysis software enables real-time results allowing faster data interpretation.

The simple-to-use Laser Experiment Designer enables complete control over the 410, 488 and 532 nm lasers including power control and spot sizes ranging from approximately 0.5 μm to over 5 μm enabling fast, dynamic results.



A point of interest (indicated by the green circle) is photobleached and monitored in this Fluorescence Recovery After Photobleaching (FRAP) experiment. The return of fluorescent signal in the bleached area indicates mobility of the fluorescent molecules.

APPLICATIONS

The QLM, along with the DeltaVision system, enables substantial flexibility and control for a wide variety of applications that can be used for many types of studies.

Applications	Types of Studies
Fluorescence Recovery After Photobleaching (FRAP)	Affinity, biomolecular cycling, biomolecular environments, and structural kinetics studies
Fluorescence Loss in Photobleaching (FLIP)	Biomolecular cycling, transport, structural visualization, and compartmental studies
Photoactivation (PA-GFP)	Affinity, biomolecular cycling, biomolecular environments, transport, cell fate, structural kinetics, structural visualization, and compartmental studies
Laser Fluorescence Resonant Energy Transfer (FRET)	Affinity and biomolecular environment studies

Combinations of these techniques can be used for novel approaches to a variety of studies including: subcellular localization, biomolecular cycling, transport, affinity, and subcellular environments.

Several configurations are available, from a base system (a single laser) to a complete laser package (3 lasers).

Configuration	Laser	Sample Applications
Base System	488nm	Fast FRAP (GFP, YFP, Alexa488) FLIP
Option 1 (PA-GFP) Additional Laser	410 nm	Photoactivation (PA-GFP) Fast FRAP (CFP, Lucifer Yellow Alexa436)
Option 2 (FRET) Additional Laser	532 nm	Laser FRET (YFP) Fast FRAP (Ds-Red, Rhodamine, TexasRed™, Alexa532)
Complete Laser Package	488nm 410nm 532nm	All of the above

*DeltaVision FRAP has < 2 ms between the end of photobleaching and the beginning of image acquisition.

A retractable beam splitter provides full excitation light when lasers are not used and 50% of excitation light when lasers are used.

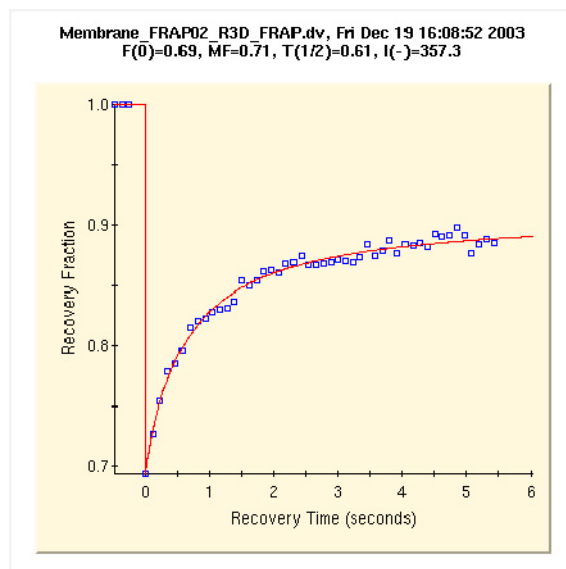
CONTROL, ACCURACY, QUALITY

All three lasers fit into the DeltaVision equipment rack (if the system has a single camera). Each laser has a separate power control that can be adjusted with the DeltaVision Laser Experiment Designer. The QLM provides a high level of control and accuracy:

- The 4-position beam expander delivers near diffraction-limited spot size (depending on the objective lens and the beam expander position).
- Spot sizes range from 0.5µm to over 5µm depending on objective NA and sample preparations.
- The Laser Optic module that delivers the laser light has 4 degrees of alignment to permit beam centration without loss of beam quality.
- Fast laser shutters (5ms ± 2ms minimum) enable a high level of control.

ANALYSIS

The Fluorescence Recovery graph shows the recovery of mobile fraction fluorescence in the bleached area.



The Fluorescence Recovery Graph

Key FRAP parameters include:

- 1) Mobile Fraction (MF), the fraction of fluorescent molecules that are free to move within the bleached region,
 - 2) Half-time for Recovery ($t_{1/2}$), the time required to recover half of the mobile fraction, and
 - 3) Diffusion coefficient (D), the rate (in µm/sec) of two-dimensional diffusion of the fluorescent molecules.
- Other results are also provided.

	VISIBLE AND INVISIBLE LASER RADIATION. AVOID EXPOSURE TO BEAM. CLASS 3B LASER PRODUCT.								
	<table border="1"> <tr><th>λ</th><th>max. power</th></tr> <tr><td>400-680 nm</td><td>50 mW</td></tr> <tr><td>780-820 nm</td><td>20 mW</td></tr> <tr><td>980-1060 nm</td><td>50 mW</td></tr> </table>	λ	max. power	400-680 nm	50 mW	780-820 nm	20 mW	980-1060 nm	50 mW
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