DeltaVision DV Elite and PersonalDV Imaging Systems

Standard Components

TruLight Illumination System
Applied Precision has developed the TruLight Illumination System, a radical new design for the DeltaVision fluorescence illumination light path that delivers outstanding signal-to-noise capability and increases light transmission to the sample by 5 fold.

Solid State Illumination: The *InsightSSI*™ unit is a solid state light engine utilizing a variety of light source technologies to optimize the output for each color. The *InsightSSI* has several key advantages over traditional light sources such as LEDs, arc lamps and lasers. Those systems are hindered by source instability, insufficient light output, limited wavelengths and the generation of unwanted heat and vibration. However, the *InsightSSI* produces stable monochromatic light with little heat and at low lifetime cost relative to other light sources. The exposure times are driven by TTL signaling enabling more stringent control than traditional shutters. Unlike mechanical shutters, TTL drivers do not suffer from mechanical failure due to wear over time.

***InsightSSI* 100 microsecond switching time**

- Drastically reduce non-productive cell illumination
- Decreased photobleaching
- Increased cell viability
- Only uses illumination life when the shutter is open
- Near-simultaneous multi-color live cell imaging

Traditional arc lamps suffer from variations in intensity over time and the absolute position of the arc can change in 3-dimensional space. These variations in intensity and position result in errors in the analysis of acquired 2D and 3D volumes data. TruLight dramatically minimizes spatial and temporal variations in light intensity and improve data collection. These integrated components of TruLight are critical to the success of generating uniformly illuminated 2D and 3D images.

Integrated Photosensor
The Integrated Photosensor measures light intensity delivered to each individual image. All images acquired are normalized and balanced to minimize variations in light intensity and generate consistent and quantitative results.

Automated Viewing and Imaging Modes
The Viewing Mode of TruLight focuses light to the back of the objective (Köhler illumination) and enables the user to see the full of view through the eyepieces. This mode is useful for rapid sample selection but imaging this mode exposes large regions of the sample to nonproductive illumination. This can lead to photobleaching and phototoxicity of the sample. The Imaging Mode of TruLight focuses the light at the sample plane (known as critical illumination). This mode doubles the power of the light delivered to the sample, improves imaging of low signals, decreases photobleaching and improves cell viability in live cell imaging. TheTruLight system can be set to automatically switch between Viewing and Imaging mode.

LED Trans Illumination

Bright field applications (such as DIC and Phase) utilize transmitted light to illuminate the field of view. DeltaVision systems use a white LED to create even illumination across the sample. This is superior to the typical halogen bulb which is bright in the center and appears gray at the edges of the field of view. The intensity and speed of the LED source are controlled through the softWoRx software.

Summary

The TruLight Illumination System exclusive to DeltaVision Imaging Systems provides:

- Powerful solid state illumination and ultrafast wavelength switching
- Optimized transmission increases light to the sample 5 times
- Seamless switching between viewing and imaging modes for superior image quality

Applied Precision Flexure XYZ Stages (patent pending)

Standard Stage: 25 mm(X) x 50 mm (Y) stage
Microtiter Stage available at additional cost (not available on PersonalDV)

Every DeltaVision stage is calibrated using a laser interferometer. Laser interferometer positioning systems provide precise positional information for motion control or dimensional measurements. When built into manufacturing and inspection equipment, a laser interferometer system reports the position product platform with more accuracy than any other method. Each motor (X, Y and Z) moves independently of the others to ensure linear motion in each axis; this is a critical component of repeatable imaging. When comparing the advanced DeltaVision stage to other competitive stages, it is critical to remember that the DeltaVision stage has always been a three-dimensional (3D) stage and only the DeltaVision stage has the accuracy and precision necessary for the highest levels of parallel (Point Visiting) live cell experiments.

X and Y axes
Travel: 25 mm(X) x 50 mm (Y)
Step Resolution: 20 nm
Absolute Accuracy: < 10 um per 25 mm

Z axis
Travel: 1 mm
Step Resolution: 5 nm
Linearity error: <0.6 um per 13 um travel range

Eyepiece Filter Wheel

This wheel includes filters for visible wavelengths for observation through the eyepieces of the microscope. This allows the user to determine the fluorescent image to be captured by the camera attached to the side port. The unique feedback circuitry allows the user to control wavelengths by moving Eyepiece Filter Wheel for improved workflow and effiency.

Interline Camera

The cooled interline CCD chip is aligned at the intermediate focal plane of the side-port of the microscope with no additional optics to degrade the image. Additional optics cause light loss and potential degradation of axial chromatic registration resulting in poor image quality.

Qualified Objectives: Applied Precision hand selects PSF quality 40x, 60x and 100x objectives for each system. Objectives are tested for astigmatism, spherical and chromatic aberration. No other system in the market offers such assurances with respect to objective performance.

softWoRx Software

softWoRx by Applied Precision is an intuitive, user-friendly software interface that enables the user to set up experiment design and features post-processing tasks and data analysis tools. Highlights include:

Point Visiting allows a user to repeatedly visit a series of points on a coverslip. The point-to point accuracy, precision, and total travel of the 3D Flexure stage are unmatched in the industry. Easy-to-use *softWoRx* software allows programming of up to a thousand points on a single slide. These point coordinates can be stored and retrieved for subsequent analysis. The high speed Nanomover motors ensure that minimal time is lost moving between different imaging sites Point Visiting is a critical tool for advanced Live Cell imaging as it allows multiple sites to be imaged in a single experiment. In practice, the number of sites is limited only by the minimum acceptable time interval between each time point at a single site. This makes time-lapse imaging much more efficient, and allows enough data to be collected easily to generate statistically significant results.

On-the-fly deconvolution allows users to monitor the progress of experiments by viewing instantly processing with advanced 3D image restoration techniques.

Restoration 3D deconvolution is a quantitative method to increase signal-to-noise ratio, image contrast and image quality. This improvement in image quality is very useful in with low light or low contrast samples.

Optical Axis Integration (OAI) is exclusive to softWoRx to quickly data in the *z* axis. It is used to collect moving diffraction-limited objectives that might be lost in the time it takes to capture individual *z* planes. The fast data collection requires less light exposure, improving cell viability.

Contrast-based AutoFocus can be used with brightfield or fluorescent signal to maintain focus during experiments. This feature can be turned on and off as needed and can easily be combined with point visiting, time lapse, and integrated cell tracking experiments. Autofocus is especially useful for long time-lapse experiments. It allows users to acquire focused images without closely monitoring experiments.

Cell Tracking automatically moves the stage to follow cells as they move during a time-lapse experiment. The user specifies a region of interest around the cell. In subsequent images, the software recenters the cell within this ROI on-the-fly. DeltaVision allows you to adjust the following cell tracking parameters: tracking method, threshold, reference channel and ROI percent. Cell Tracking is enabled with a single mouse click.

Colocalization Analysis program with chromatic correction option allows easy calculation of Pearson coefficient of correlation to determine if two particles colocalize.

FRAP Analysis software automatically finds bleach/activation point and extracts data using one of four curve-fitting algorithms to determine the half times of recovery and diffusion constants.

FRET Calculator guides a researcher through the difficult task of analyzing the seven images required to make one sensitized emission FRET measurement. Speeds data analysis times by automatically calculating crosstalk values and creating intensity map of netFRET and FRET efficiency values. The module creates output data table of user-defined ROIs.

Instrument Controller/Microscope Interface Chassis (IC/MIC)
Server Class Computing Workstation

- Dual Processor, Dual Core Opteron
- Service Class Architecture

- Multi-threaded Deconvolution and Rendering
- 7 TTL ports to control external devices
- CentOS EL4.0
    - Enhanced stability
    - USB support
- 24" flat panel monitor LCD
- Backlit Keyboard
- Laser Mouse

Oil Matching

DeltaVision offers an oil calculator to match the refractive indices of the sample and the oil. This is crucial for spherical aberration correction. This solution does not require additional optics which may decrease the intensity of the sample. The DV Elite system is supplied with 18 different oils to match the specimen and mounting materials. The PersonalDV has a smaller kit of six oils.

Additional Options

Patent Pending *Ultimate Focus*™ Hardware AutoFocus (not available on PersonalDV)
Focus Maintenance
The *Ultimate Focus*™ module is a hardware-based autofocus option that automatically maintains the Z focal plane of the sample during imaging. This mechanism is integrated into the macro commands of the experiment to eliminate Z drift during an experiment. The mechanism does not involve exposing the sample to additional light thereby improving cell viability.
Focus Assist
The Focus Assist feedback loop of *UltimateFocus* determines the distance between the objective and the coverslip. It guides the user to bring the objective into area of focus without using the eyepieces or a camera.

Microtiter Plate Stage (not available on PersonalDV)
The Microtiter Stage can image 96 and 384 well plates in X, Y and Z. Slides and dishes can be imaged using the adapter insert. The software allows the user to select combinations of rows, columns or individual wells to design a plate scan and define how the wells are to be sampled. The user can specify how many fields of view are collected in each well and the spacing between each field. The Plate Review tool plays back the images collected without requiring the user to open each individual file.

***X4* Laser Module Option** (not available on PersonalDV)
The *X4* Laser Module combines up to four lasers that are simultaneously active for increased performance. The increased laser line selection allows greater flexibility in the use of fluorescent dyes and proteins. Lasers can be added to the base module in any combination:

Configuration	Laser	Sample Applications
Base System	488nm	fast FRAP, GFP, YFP, Alexa488, FLIP
Option 1	405 nm	Photoactivation (PA-GFP), fast FRAP, CFP, Alexa436
Option 2	445 nm	CFP
Option 3	515 nm	Acceptor Depletion FRET, YFP
Option 4	561nm	mCherry
Option 5	640 nm	Cy-5

Photokinetic (PK) Optics Option (not available on PersonalDV)
A PK event within an experiment is created when the sample is excited with a laser to measure a process. This can be as simple as photobleaching a spot in a cell or 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. The PK Optics focus a sub-diffraction sized laser beam into the back aperture of the microscope objective to generate a focused excitation spot in the center of the optical field.

Multi-line TIRF Option (not available on PersonalDV)
The novel Multi-line TIRF combines up to four laser lines with a unique Chromatic Correction Element. This element focuses each laser line to the same penetration depth, eliminating individual alignment of each laser line for user-friendly TIRF imaging.

Environmental Control
Enclosing the microscope stabilizes the components to reduce stage drift caused by thermal fluctuation and air currents. Temperatures can be controlled from ambient to 40°C. Included components for humidity and gas regulation by user defined and supplied gas flow (CO_2).

Photometrics Evolve EMCCD Camera 512 x 512
Excelon technology is available for an additional cost and may delay camera delivery.

Training

Installation and training included with the DeltaVision Imaging System.
A free three day advanced training course at Applied Precision headquarters in Issaquah, WA is included with the purchase of a DV Elite. Customer is responsible for travel and accommodation expenses.

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