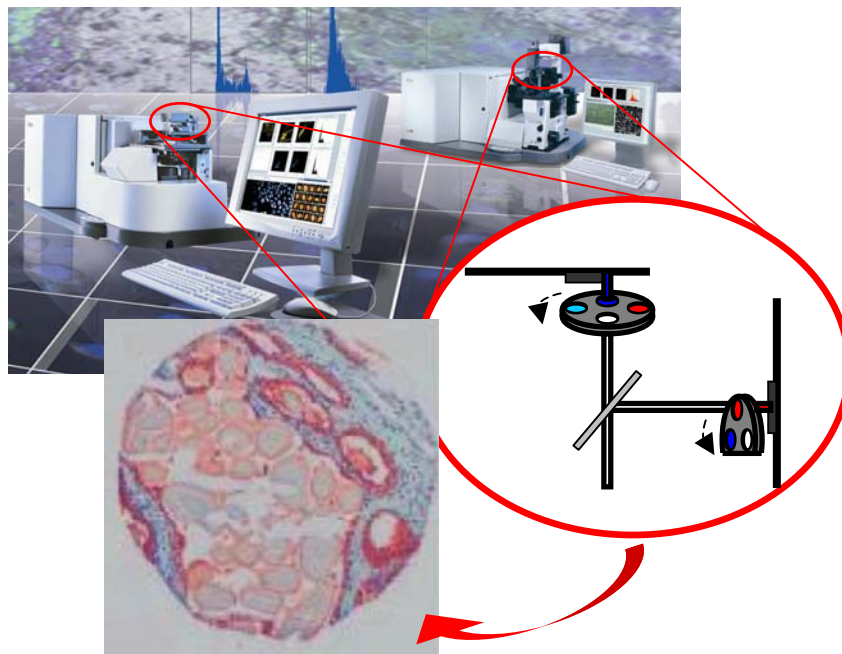


# Dual Channel Laser Light Scatter & Laser Light-Loss Detector

for

**iCyte<sup>®</sup> & iCys<sup>®</sup> Imaging Cytometers**



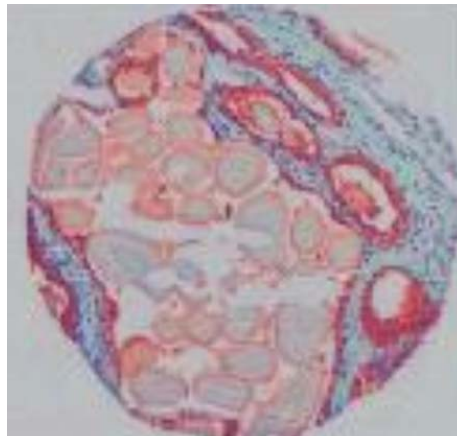
DOC 191-0017-001 Revision B

**CompuCyte's Dual Channel Laser Light Scatter & Laser Light-Loss Detector Assembly allows simultaneous capture by two channels of scatter and/or light-loss data, reducing throughput time by more than 50% for certain assays and providing enhanced data sets.**

The Dual Channel Assembly is available as an option for the iCys<sup>®</sup> Research Imaging Cytometer, and can be retrofit onto existing iCyte and iCys instruments equipped with the single detector assembly.

### **Benefits of the Dual Channel Assembly**

- Quantify chromatic dye light-loss with two chromatic stains in half the time: When the two channels are both set for light-loss measurement (see Configuration Options, below), it is possible to quantify chromatic dye absorption for two dyes in a single pass. This reduces the scan time required for this type of analysis to less than half the time needed using a single light-loss detector.



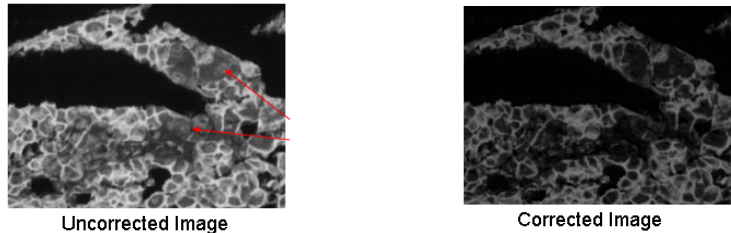
**Figure 1: CompuColor image combining the 488 and 633 light-loss images**

- Obtain richer data in a single pass: When one channel is set for shaded relief (scatter) and the other for light-loss, shaded relief and light-loss images are captured simultaneously. This allows quantification of chromatic dye levels from the light loss channel, coupled with the morphology information from the shaded relief image.

### **Additional features**

Several features, available now with the single channel scatter/ light-loss assembly, become even more important with two scatter/ light-loss channels:

- Spectral Overlap Correction: Application of spectral overlap correction to two chromatic dyes may be employed for a single pass scan with the Dual Channel Assembly, because both channels of data may be acquired simultaneously. Compensation for spectral overlap between the dyes can be employed, as is routinely done with fluorescence analysis. The tissue sample shown below is stained with DAB and hematoxylin:



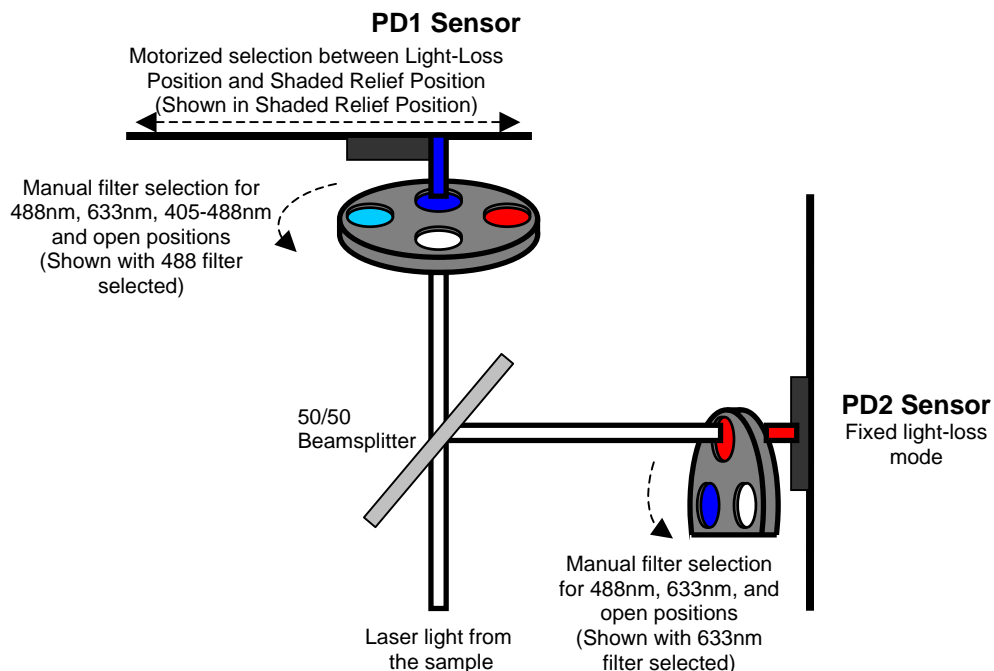
**Figure 2: DAB- and hematoxylin-stained tissue. In the image on the left, some of the hematoxylin staining is seen in the Blue (primarily DAB) channel. In the image on the right, the hematoxylin signal has been subtracted from the image.**

- Combined fluorescence and chromatic analysis: Because the absorption measurement uses dedicated detectors that are separate from the fluorescence-measuring photomultiplier tubes, chromatic and fluorescence measurements may be made simultaneously. This allows correction of the chromatic dye images for autofluorescence when appropriate.

### Technical Description of the Dual Channel Assembly

Laser light is transmitted through the sample, enters the new Dual Channel Assembly, and is divided into two separate beams by a 50/50 beamsplitter. Each beam is directed to one of two filtered photodiode (PD) sensors. The mode of PD Sensor #1 is controlled by a motor that allows the sensor to be moved between the “*Shaded Relief*” (scatter) position (as shown in Figure 3, below) and the “*Light-Loss*” (absorption) position. The filter set in front of PD Sensor #1 may be manually set to pass either 488nm blue light (as shown,) 633nm red light, 405 & 488 nm light or light from all three lasers (the open position).

The position of PD Sensor #2 is fixed in the “*Light-Loss*” (absorption) position. The filter set in front of PD Sensor #2 may be manually set to pass either 488nm blue light (as shown). 633nm red light or light from all three lasers (the open position).



**Figure 3: A schematic of the Dual Channel Assembly**

### Configuration options for the Dual Channel Assembly

The Dual Channel Assembly can be configured by the user in a variety of ways as described in the table below. The numbered configurations in the table are explained in detail in the **Highlighted configurations** section.

Channel 1 \ Channel 2	405 - 488nm		488nm		633nm		Open	
	Light-Loss	Shaded Relief	Light-Loss	Shaded Relief	Light-Loss	Shaded Relief	Light-Loss	Shaded Relief
488nm LightLoss	✓	✓	---	Config. 2	✓	✓	✓	✓
633nm LightLoss	Config. 4	✓	Config. 1	Config. 3	---	✓	✓	✓
Open LightLoss	✓	✓	✓	✓	✓	✓	---	✓

### Highlighted Configurations

- **Configuration 1 - 488nm and 633nm light-loss:** Use this configuration to capture two channels of light-loss images. Channel 1 images will show absorption of the 488nm laser line and Channel 2 images will show absorption of the 633nm laser line. This configuration is a good choice when analyzing tissue stained with two chromatic dyes that absorb in different areas of the spectrum, such as DAB and hematoxylin. These images may be combined and colored with CompuColor as shown in Figure 1 above.
- **Configuration 2 - 488nm shaded relief and 488nm light-loss:** Use this configuration to simultaneously generate CompuCyte's patented laser scatter images using Channel 1 and light-loss images with Channel 2. The Channel 2 images will show absorption of the 488nm laser line; therefore this configuration is a good choice when using chromatic dyes that absorb blue, such as DAB (which visually appears red because of its ability to absorb blue light). The shaded relief/light-loss combination could be useful in looking at live cells, beads, and other whole objects.
- **Configuration 3 - 488nm shaded relief and 633nm light-loss:** Use this configuration to combine scatter images using Channel 1 with red-absorbing light-loss images using Channel 2. The Channel 2 images will show absorption of the 633nm laser line. This configuration is a good choice when using chromatic dyes that absorb red (such as hematoxylin, which visually appears blue because of its ability to absorb red light).
- **Configuration 4 - 3-color light-loss:** Use this configuration for automated two-pass scanning. Use the 405nm and 633nm lasers for the first pass scan and the 488nm and 633nm lasers for the second pass scan. Because the short pass (405-488nm) filter will pass 405 or 488nm light, the filter wheel will not need to be manually adjusted between the first and second pass scans.
- **Adjusting the configuration of the Dual Channel Assembly:** The selection of Shaded Relief or Light-Loss mode for Channel 1 is controlled from a dialogue box in the application software. This allows for precise control and repeatability for the Shaded Relief (scatter) position. The Channel 1 mode selection can be defined differently for each scale of a multi-scale analysis when using the iNovator Application Development Toolkit. Filter selection for both Channel 1 and Channel 2 is controlled manually on the assembly itself.

### Combining the Dual-Channel Absorption / Scatter Detector with the Enhanced Blue Cube

The Enhanced Blue Cube for the iCyte and iCys allows the use of the 405nm and 488nm lasers in a single pass by blocking reflected 488 laser light from the blue PMT. as a result, the blue channel PMT measures only fluorescence light generated by 405nm laser excitation.



## iGeneration Dual Channel Laser Light Scatter & Laser Light-Loss Detector

- Configuration 1, 2 or 3 with single-pass 405nm and 488nm excitation: Combining the Dual-Channel Absorption / Scatter Detector with the Enhanced Blue Cube allows the acquisition of 488 shaded relief or light-loss during a two-laser scan by filtering out the 405nm component. The second PD may be used to simultaneously acquire one of the following: 488nm light-loss or shaded relief or 633nm light-loss or shaded relief.

More information on the Enhanced Blue Cube Upgrade and other new products from CompuCyte is available on the [New Releases](#) section of our website.

### **Ordering Information**

Order the part number for your particular system:

<b><u>Part Number</u></b>	<b><u>Description</u></b>
250-1057-001	iCyte <sup>®</sup> Dual-Channel Absorption / Scatter Detector Upgrade (Note: Requires Version 3.2.2 iCyte <sup>®</sup> Cytometric Analysis Software)
250-1047-000	iCys <sup>®</sup> Dual-Channel Absorption / Scatter Detector Upgrade (Note: Requires Version 3.2.2 iCys <sup>®</sup> Cytometric Analysis Software)
250-1207-000	Enhanced Blue Cube Upgrade (for iCyte <sup>®</sup> or iCys <sup>®</sup> )