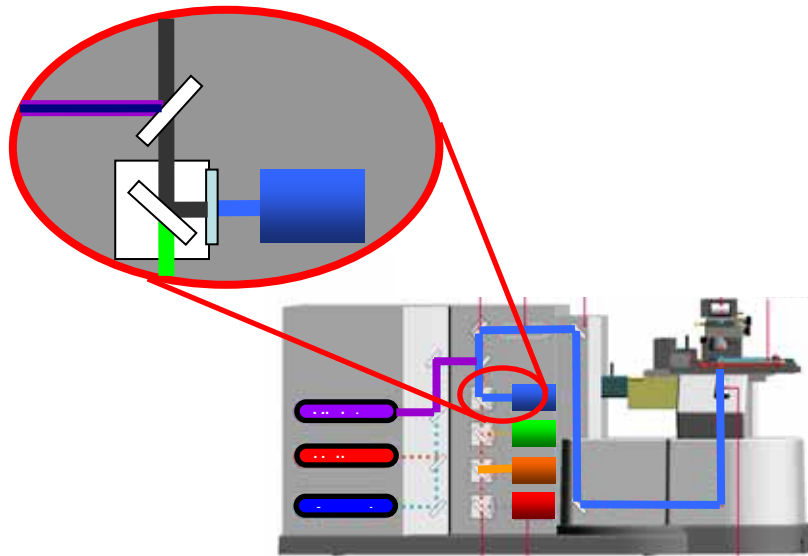


# Enhanced Blue Cube Upgrade



for

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

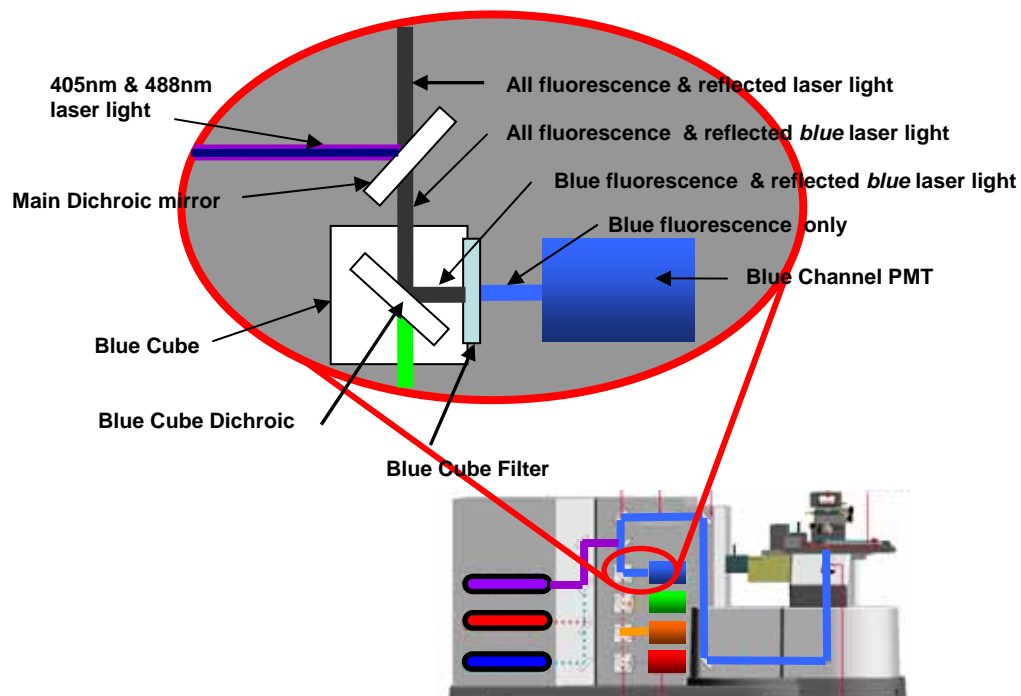
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The Enhanced Blue Cube allows scans with the 405nm (Violet Diode) and 488nm (Argon) lasers to be performed in a single pass, halving the scan time required for assays using both Violet and Argon laser-excited dyes.

The Enhanced Blue Cube is available as an upgrade for all iCyte® and iCys® Imaging Cytometers.

### What does the Blue Cube do?

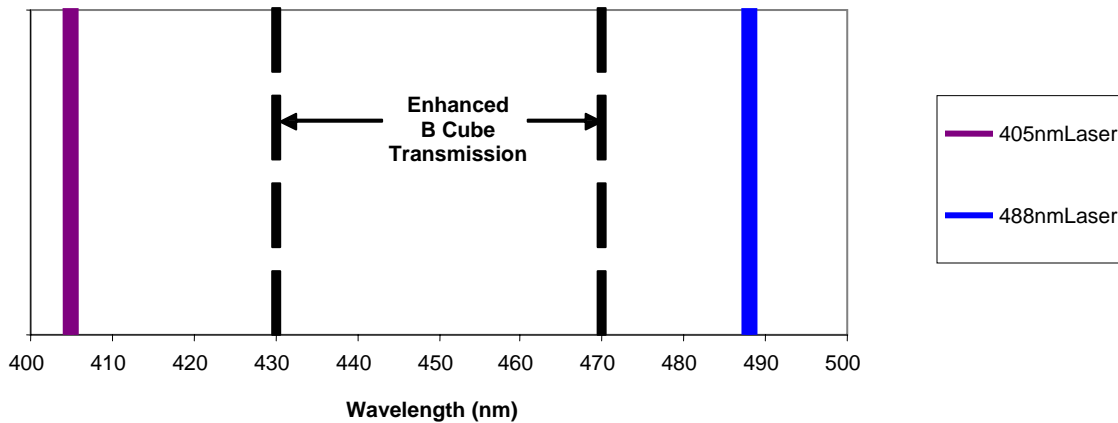
In a standard iCyte or iCys configuration, the Blue Cube controls the fluorescent light (generated by 405 nm laser excitation) that is measured by the first PMT. Specifically, the Blue Cube limits the wavelengths of light to which the first PMT is exposed to within a narrow blue band of emitted light (430 – 470 nm). Figure 1 shows the position of the Blue Cube within the system. Details of its configuration are shown in the inset.



**Figure 1: The blue cube within the Cytometer. Inset: Laser light is reflected by the main dichroic mirror upward to the sample. Fluorescently emitted light and some reflected laser light returns from the sample and passes through the dichroic mirror to the Blue Cube. The Blue Cube dichroic separates blue from longer wavelength light and directs the blue light to the right toward the blue channel PMT. The Enhanced Blue Cube filter further narrows the wavelength band that reaches the PMT, excluding the 488nm laser light and allowing only blue emitted light to be measured.**

### Benefits of the Enhanced Blue Cube

The Enhanced Blue Cube has a slightly reduced (and shorter) bandwidth of 430–470nm for its blue filter, compared to the 438–488nm bandwidth of the previous Blue Cube. This change does not significantly alter the level of fluorescently-emitted blue light measured. However, the Enhanced Blue Cube filter completely eliminates the 488nm laser line interference, allowing the 405nm and 488nm lasers to be run in the same pass. This effectively reduces by more than half the throughput time of assays combining 405nm-excited and 488nm-excited dyes.



**Figure 2: Enhanced Blue Cube filter bandpass and 405nm & 488nm excitation lines**

### Assay examples benefiting from the Enhanced Blue Cube

In cell cycle assays where (1) DNA content and chromatin condensation are used to determine the cell cycle phase and (2) other biomarker expression levels are also assessed, the ability to measure fluorescence excited by both the 405nm and 488nm lasers in a single pass can more than halve the scan time required. Some of the dye combinations benefiting from this technology are listed in the following table:

Laser	Detector	Dye	Target
405	Blue	DAPI	DNA – fixed cells
405	Blue	Hoechst	DNA - live cells
488	Green	Alexa 488	secondary
488	Orange	Phycoerytherin	secondary
488	Long Red	Pe/Cy5	secondary

### Potential for spectral overlap

With broad spectrum dyes such as DAPI, some of the emitted fluorescence will be detected in channels other than the primary channel for that dye. This is commonly referred to as spectral bleed-over or spectral overlap. Specifically for DAPI, fluorescence from this dye can be seen in the iCyte or iCys Green channel. When DAPI is used with a primarily green-emitting dye such as Alexa 488 in a single-pass, two-laser scan with the Enhanced Blue Cube, the raw Green channel signal will be a combination of DAPI and Alexa 488 signals. The DAPI contribution can be eliminated by employing *compensation* on the Green channel signal using the *Virtual Channel* feature of the iCyte or iCys application software. The process for employing compensation in these cases is described in the Release Notes accompanying the Enhanced Blue Cube.

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

Combining the Dual-Channel Absorption / Scatter Detector with the Enhanced Blue Cube allows the acquisition of 488 shaded relief during a two-laser scan by filtering out the 405nm component. Either 488nm or 633nm light-loss may be acquired along with the 488 shaded relief. More information on the Dual-Channel Absorption / Scatter Detector Upgrade and other new products from CompuCyte is available at the [New Releases](#) section on our website.



## Enhanced Blue Cube Option

### Ordering Information

Order the part number for your particular system:

<u>Part Number</u>	<u>Description</u>
250-1207-000	Enhanced Blue Cube Upgrade (for iCyte <sup>®</sup> or iCys <sup>®</sup> )
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)