Flow Cytometry with a 580 nm Fiber Laser

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Abstract: A fibre laser (MPB Communications) emitting at 580 nm was integrated into a flow cytometer for the excitation of a variety of yellow-excited fluorescent probes. Texas Red, allophycocyanin and other probes were easily detectable using this laser source.

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Yellow laser excitation (ranging from 560 to 600 nm) has been difficult to achieve in benchtop flow cytometry due to limits on laser technology. Nevertheless, this wavelength range is potentially very useful for the excitation of a variety of fluorochromes. DPSS 561 nm lasers have been previously shown to give excellent excitation of PE, DsRed, lissamine rhodamine and Alexa Fluor 568, but their wavelength is too short for optimal excitation of yellow- and red-excited fluorochromes such as Texas Red, Alexa Fluor 594, APC and the APC tandem conjugates. Yellow HeNe lasers have been used in the past for exciting these fluorochromes, but their inherently low power level has limited their usage in flow cytometry.

Recent advances in solid state laser technology have provided several new options for yellow excitation with increased power levels. This study evaluateed a recently developed solid state yellow lasers source, a Yb-doped fibre laser with a PPLN frequency doubler emitting at 580 nm (MPB Communications, Inc.). A prototype of this laser was mounted on a cuvette-based flow cytometer (LSR II, BD Biosciences), and evaluated for its ability to excite a variety of yellow- and red-excited fluorescent probes, including Texas Red, Alexa Fluor 594, allophycocyanin and its tandem conjugates, and the fluorescent protein DsRed.

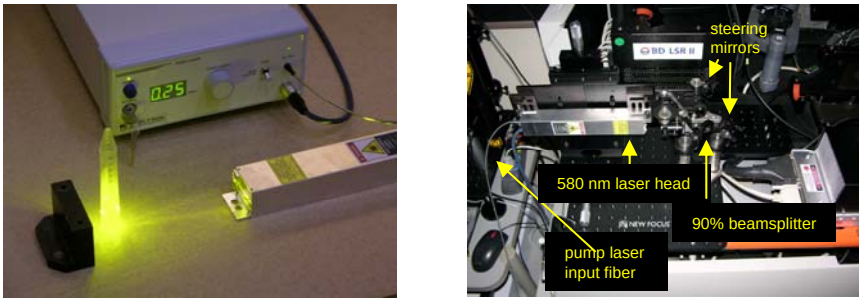

Figure 1. Left, the 580 nm fiber laser. Right, the laser unit mounted on a BD Biosciences LSR II flow cytometer. The unit emitted at >500 mW; a dichroic beamsplitter was used to attentuate the laser power below 100 mW.

Cells (EL4 mouse lymphoma) labeled with antibodies against a cell surface receptor coupled to a variety of fluorescent probes were then analyzed using the 580 nm laser source. The yellow excited probes Texas Red and Alexa Fluor 594 were well-excited at 580 nm (Figure 2). Allophycocyanin and several low molecular eight red-excited probes (ncluding Cy5, Alexa Fluor 633 and 647) were also easily detectable using 580 nm excitation. Sensitivity for Texas Red and Alexa Fluor 594 was better than with a HeNe 594 nm source, and sensitivity for APC was comparable to that observed with a traditional HeNe 633 nm source (data not shown).

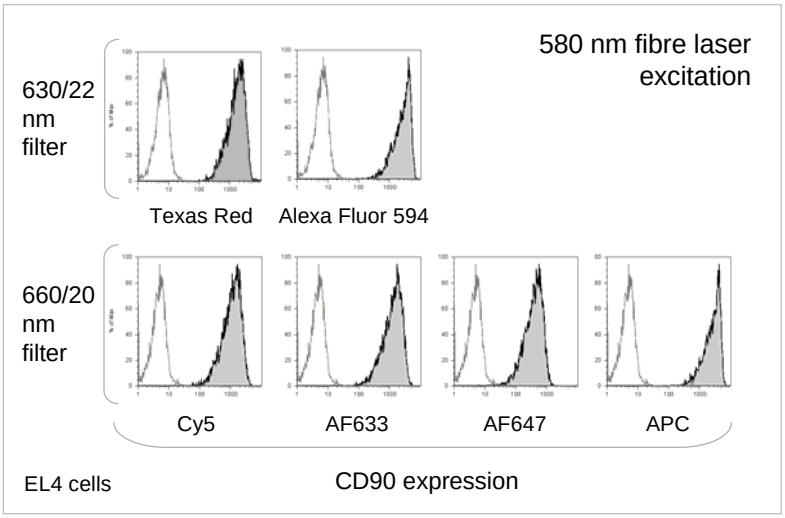

Figure 2. Analysis of EL4 mouse lymphoma cells labeled with Texas Red, Alexa Fluor 594, APC, Cy5, Alexa Fluor 633 and Alexa Fluor 647. The bandpass filters used are indicated on the left. Unfilled histogram traces represent unlabeled cells (background fluorescence).

The expressible fluorescent protein DsRed is a critical fluorescent marker for gene expression in mammalian cells. Excitation of DsRed expressing cells was excellent using the 580 nm laser source, with considerably better sensitivity signal-to-noise ratio than with a 488 nm laser source (traditionally used to excite this protein

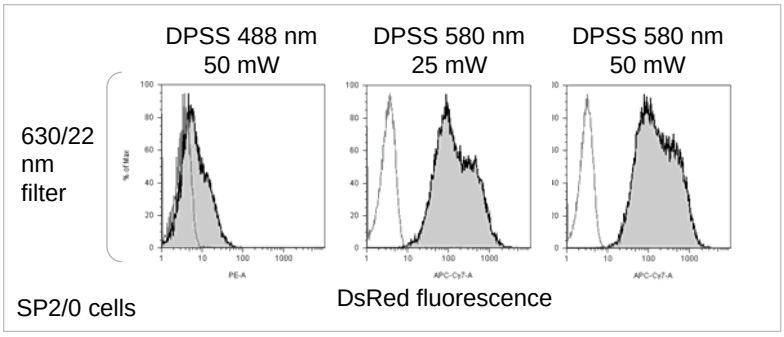

Figure 3. DsRed analysis using DPSS 488 nm (left) and 580 nm sources (middle and right). SP2/0 cells that constituitively expressed DsRed were analyzed using the indicated laser source. Unfilled histogram traces indicate unlabeled cells (background fluorescence).

The 580 nm fibre laser therefore proved to be an useful excitation source for flow cytometry, allowing the use of a variety of yellow-and red-excited fluorochromes