# Yellow Lasers for Flow Cytometry: An Update

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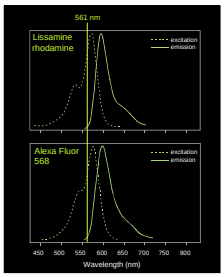
## Abstract

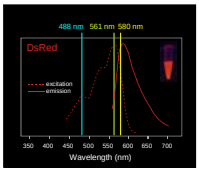
Yellow laser excitation (ranging from 560 to 600 nm) has been difficult to achieve in benchtop flow cytometry due to limits on laser technology. Nevertheless, this wavelength range is potentially very useful for the excitation of a variety of fluorochromes. DPSS 561 nm lasers have been previously shown 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.

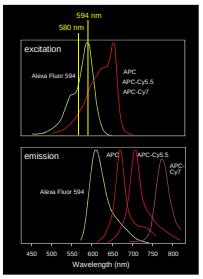
Recent advances in solid state laser technology, however, have provided several new options for yellow excitation with increased power levels. This report evaluates several recently developed solid state yellow lasers sources, including Yb-doped fibre laser with a PPLN frequency doubler emitting at 580 nm, and a diode-pumped solid state 593 nm laser. Both of these units provided good excitation of Texas Red and Alexa Fluor 594, while still giving APC and APC tandem conjugate excitation that was comparable to red laser sources. This combined technology is therefore closing a significant gap in the laser wavelengths available for benchtop flow cytometry.

Yellow laser excitation (between 560 and 600 nm) is potentially very useful for flow cytometry. Excitation in the 560 to 580 nm range should give excellent excitation of lissamine rhodamine, Alexa Fluor 568, and the fluorescent protein DsRed. Longer wavelength yellow light can excite Texas Red and Alexa Fluor 594, while still providing good excitation of APC and its tandem conjugates.

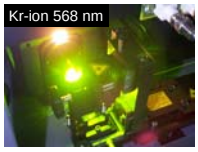



Excitation/emission spectra for lissamine rhodamine and Alexa Fluor 568 *(left)*, DsRed *(below)* and Alexa Fluor 594, APC and the APC tandem conjugates in a potential four color labeling scheme *(right)*. Proposed laser lines are indicated.

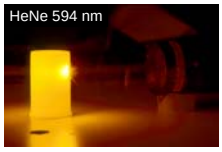



Yellow laser light has nevertheless been technologically difficult to obtain using traditional laser sources. Krypton-ion sources produce a 568 nm line, but can only be accommodated on large-scale cytometers. Yellow HeNe lasers emitting at 594 nm have been available for years yet are relatively low in power (<8 mW). Dye head lasers (using a high-power argon-ion pump laser and a dye head running R6G) produce a range of yellow and orange lines, but are difficult to maintain and are also restricted to larger cell sorters.



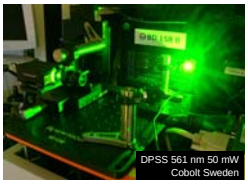


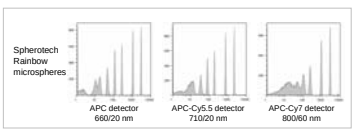



Recent advances in solid state laser technology have produced several laser sources in this wavelength range that are useful for flow cytometry.

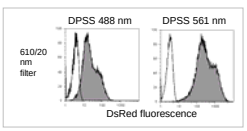
## High-power DPSS 561 nm

DPSS 561 nm lasers have been previously shown to give excellent excitation of PE and DsRed, as well as the low molecular weight fluorochromes lissamine rhodamine, Alexa Fluor 568, Texas Red and Alexa Fluor 594. The power level available at this wavelength has since increased to 50 mW.



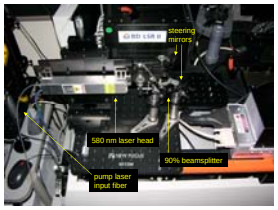

***Left***, 561 nm excitation of Spherotech Rainbow microspheres with the indicated emission filter.



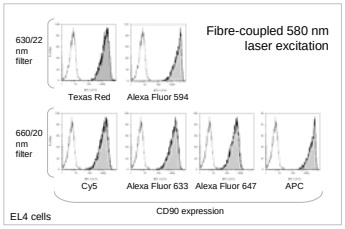

***Above***, DsRed fluorescence in stably transfected SP2/0 cells, at 488 nm (left) or 561 nm (right).

## DPSS 580 nm

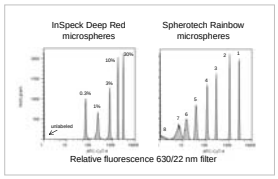
Solid-state 580 nm fibre lasers are now available (MPB Communications, Inc.). This unit emitted at >500 mW but could be attentuated using a beamsplitter to power levels appropriate for cytometry. This wavelength excited Texas Red, Alexa Fluor 594 and DsRed extremely well, and gave adequate excitation of APC and its tandems.



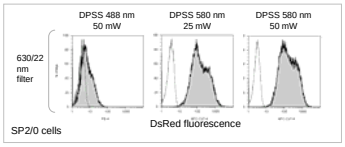

***Above***, the emission cavity of the DPSS 580 nm laser mounted on a BD LSR II. Pump laser input fiber is visible on the left.




***Above***, EL4 cells labeled with biotin-anti-CD90 followed by the indicated fluorochrome-conjugated streptavidin. Texas Red and Anexa Fluor 594 were detected through a 630/22 nm filter.



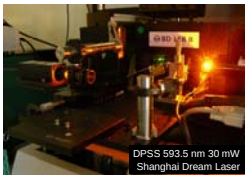

***Above***, MPI InSpeck Deep Red and Spherotech Rainbow microspheres excited with the DPSS 580 nm source.

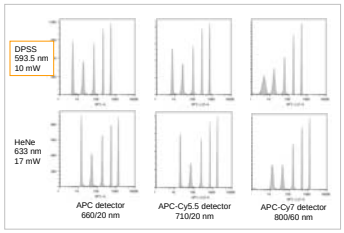



***Above***, DsRed analysis using DPSS 488 nm (left) and 580 nm sources (middle and right). Power levels are indicated

## DPSS 593.5 nm

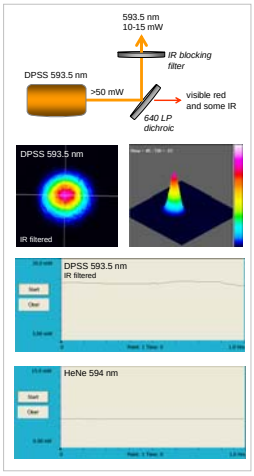
DPSS 593.5 nm lasers are not yet available from North American or European companies, but are obtainable from Chinese suppliers. This unit (spec'd at 30 mW, low noise) had significant red and infrared emission, requiring multistep filtering, with a final 593.5 nm output between 10 and 15 mW. The laser beam profile was good, and the unit could be implemented on a the BD LSR II. However, power level was still slightly unstable over long periods.

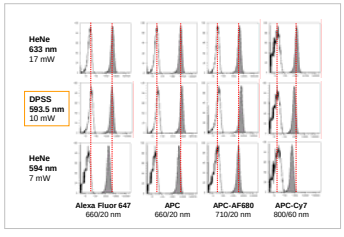



***Above***, MPI InSpeck Deep Red microspheres excited with the DPSS 593.5 nm or a HeNe 633 nm laser for comparison. Emission filters were as indicated.

***Below***, Optical scheme to remove red and IR emission from the 593.5 nm beam. The filtered beam profile was good, but power level over extended operation was unstable (here compared to a HeNe 594 nm laser, at 5 sec intervals over 1 hour.




***Above***, EL4 cells labeled with biotin-anti-CD44, followed by the streptavidin conjugated to the indicated fluorochrome. Excitation with the DPSS 593.5 nm, with HeNe 633 and 594 nm lasers for comparison.