Orange lasers for red fluorophore excitation

Recent advances in diode pumped solid state (DPSS) laser technology have increased the number of wavelengths that can be practically incorporated into instruments for life science research, in particular in flow cytometry and confocal microscopy. DPSS 532 nm and 561 nm lasers are becoming more common fixtures on these instruments, allowing biomedical investigators to use a new variety of fluorescent probes that require green or yellow excitation. However, the gap between yellow 561 nm and red HeNe or diode lasers (630 nm-640 nm) has been more difficult to fill.

Orange HeNe lasers emitting at 594 nm have been available for some time, but are very low in power and thus rarely incorporated into flow cytometers. Orange HeNes have been integrated into confocal microscopes, enabling the use of orange-excited fluorescent probes by microscopy. The ability to excite in the 590-595 nm range would indeed be very useful as there are a number of fluorescent probes, including some recently developed expressible fluorescent proteins, that are optimally excited by this wavelength range. Orange laser light has been the last major gap in flow cytometric excitation capabilities.

An example of flow cytometric analysis using a 594 nm module is shown in Fig.2. A Cobolt Mambo 50 mW module was integrated into a BD Biosciences LSR II flow cytometer (co William G. Telford, National Institutes of Health, Rockville, USA).

The 594 nm DPSS laser module is perfectly suited for exciting standard fluorescent probes such as Texas Red and Alexa Fluor 594 (Invitrogen, Carlsbad, CA), along with the more recently developed red fluorescent proteins such as mPlum, TagFP635 (formerly mKate), TurboFP635 (formerly Katushka) and CellTracker Red (Invitrogen).
As shown in the data in Fig. 3 the 594 nm wavelength indeed provides excellent excitation of these probes.

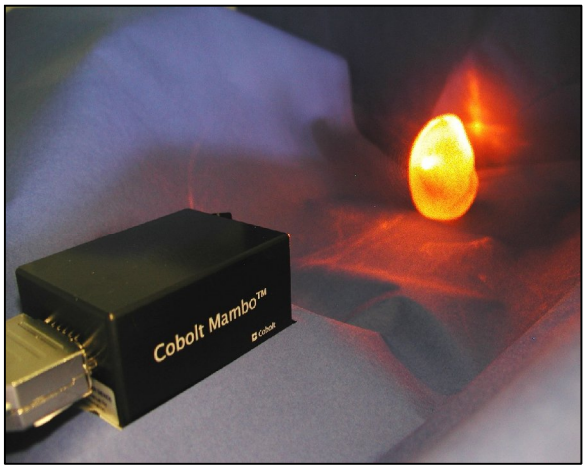


Figure 1. Cobolt Mambo™ 594 nm.

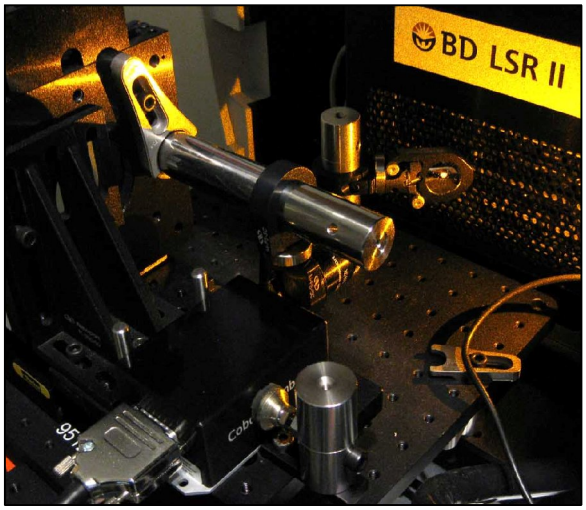


Figure 2. Cytometric analysis using Cobolt Mambo™ 594 nm.

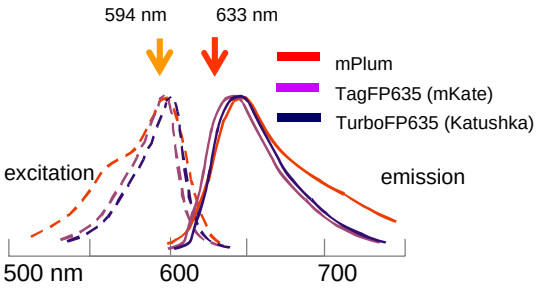


Figure 3. Excitation of red fluorescent proteins

Cobolt Headoffice, Sweden
www.cobolt.se
Phone: +46 8 5 45 912 30

The 594nm laser source not only enables excitation of red fluorescent proteins but can also be used to excite proteins typically excited by 640nm diodes such as APC and APC-Cy7. Thus with one laser source can the researcher access the spectrum from 561 nm – 640 nm (Fig.4).

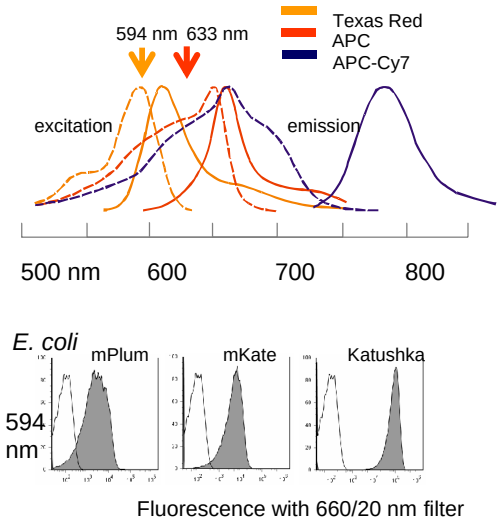
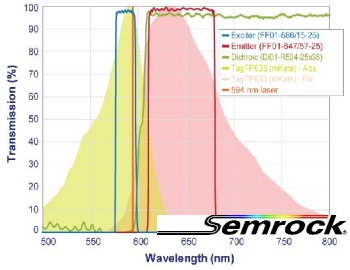


Figure 4. Excitation of proteins typically excited by 640nm diodes

In order for researchers and instrument manufacturers to be able to take advantage of these new excitation possibilities in the orange, it is also of critical importance that suitable filters are available. Hard-coated optical filters specifically designed for a 594 nm laser are now available from Semrock, Inc (Fig.5).

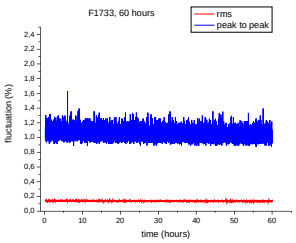


Figures 5. Hard-coated optical filters

Cobolt Mambo™ 594 nm

The Cobolt Mambo™ is a compact orange DPSS laser offering up to 100 mW CW at precisely 594 nm from a hermetically sealed package, with very low intensity noise, and in a high quality TEM00-mode and low-divergent beam.

Typical noise performance



Typical noise performance of the Cobolt Mambo™ shows peak to peak noise <3% and rms noise <0.3%. This kind of single-mode DPSS laser is particularly attractive for use in demanding fluorescence analysis applications such as flow cytometry, as it provides extremely good power stability, very low intensity noise (rms<0.3%), and a nearly perfect TEM00-mode low-divergent beam (M^2<1.1). These are all performance characteristics that are required for good results.

Cobolt Headoffice, Sweden
www.cobolt.se
Phone: +46 8 5 45 912 30