Low noise orange DPSS lasers for red fluorophore excitation

Elizabeth Illy, Håkan Karlsson & Jonas Hellström
Cobolt AB
Vretenvägen 13, Solna, Sweden.
info@cobolt.se

Background

Recent advances in diode pumped solid state (DPSS) laser technology have increased the number of wavelengths that can be practically incorporated into flow cytometers. 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; however, these applications have not been able to migrate to cytometry. 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.

Results & Conclusion

In this paper, we present a compact DPSS laser operating at 594 nm which has up to 100 mW output power, extremely good power stability, very low intensity noise (rms < 0.3%), and a nearly perfect TEM_{00}-mode and low-divergent beam (M^2<1.1). These are all performance characteristics that are required for good results in cytometry. The laser is based on a frequency mixed design using proprietary PPKTP technology for optimum flexibility and efficiency. Furthermore, the laser is manufactured into a hermetically sealed and compact package using proprietary HTCure^{TM} technology for extreme robustness, which facilitates integration into bench-top life science instruments.

Data showing that the laser is perfectly suited to exciting the newly developed red fluorophores such as mPlum, TagFP635 (scientific name mKate) and TurboFP635 (scientific name Katushka) is shown in Figure 1a & 1b (co Dr William G. Telford, NIH, Rockville, USA). The 594 nm laser not only enables excitation of red fluorescent proteins but can also be used to excite proteins typically excited by 640 nm diodes such as APC and APC-Cy7. Thus with one laser source the can researcher access the spectrum from 561 nm – 640 nm.

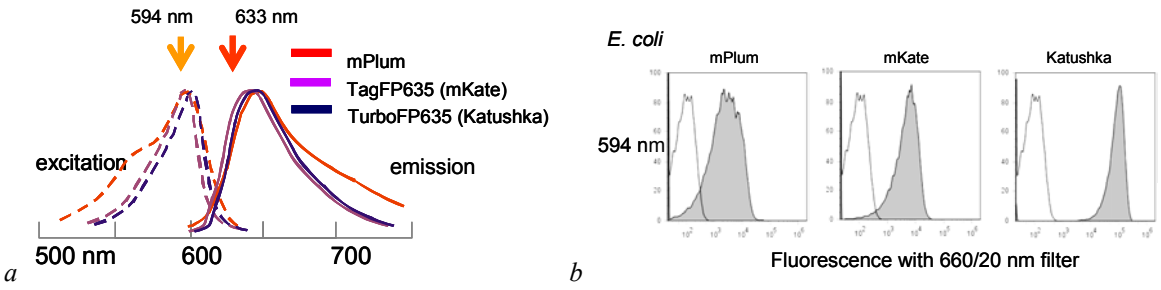

Figure 1: a. Excitation and emission spectra of the fluorescent proteins mPlum, TagFP635 (mKate), or TurboFP635 (Katushka) & b. SP2/0 cells stably transfected with and expressing mPlum, TagFP635 or TurboFP635. Open peaks show untransfected cells, filled peak show transfected cells.