NOVEL 515nm DPSS-LASER STIMULATES FLUORESCENCE MICROSCOPY

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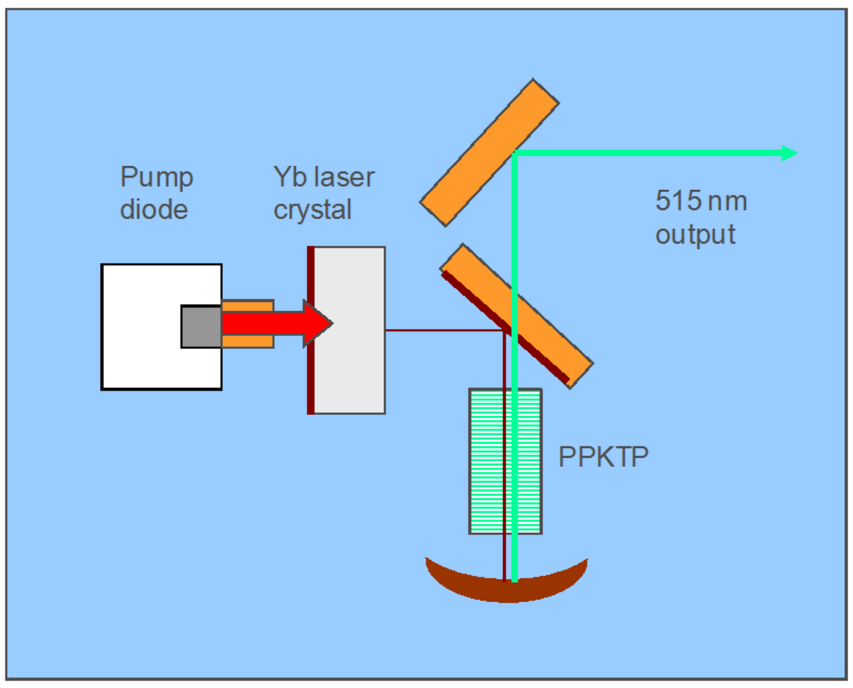
A novel diode-pumped solid-state (DPSS) laser emitting at 515 nm is closing an important gap in the area of compact all solid-state lasers. Especially biological applications such as live cell imaging using confocal microscopy, FRET, FRAP and TIRF microscopy as well as flow cytometry are benefitting from this new technology.

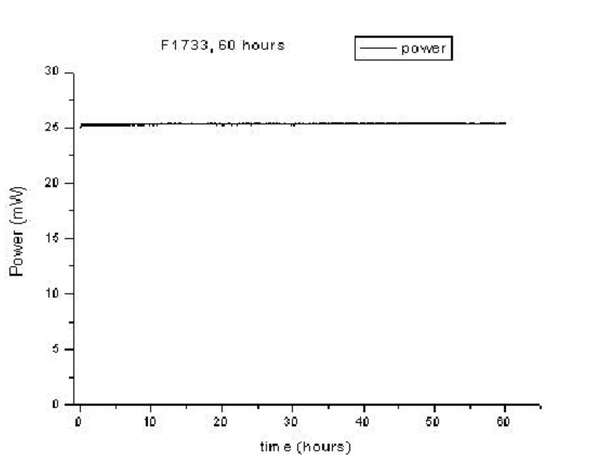
The use of solid-state lasers as excitation sources in bioanalytical instrumentation technology, as alternatives to gas lasers, has grown increasingly popular over the last few years. The reasons are a multitude of advantages in comparison to gas lasers, such as compact size, less heat generation and power consumption, no vibrations or noise, high beam stability and longer lifetime. Diode lasers are available in UV and deep-blue (e.g. 375, 405, 445 nm) and in red/IR (e.g. 635, 780 and 830 nm), whereas typical DPSS laser lines are 488, 532 and 561 nm. Until now there was one important gap, however, around 515 nm; a wavelength around which many fluorochromes (fluorescent markers or "tags" attaching to the microscopic objects to be studied) can be excited.

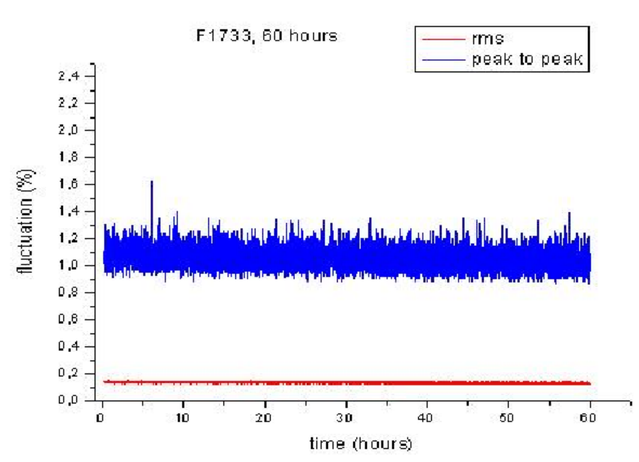
For these applications traditionally argon-ion lasers have been used, in lack of alternatives and despite all of its disadvantages. Here we describe the technical background and applications for an innovative DPSS laser, which with its 515 nm emission now can replace the argon laser.

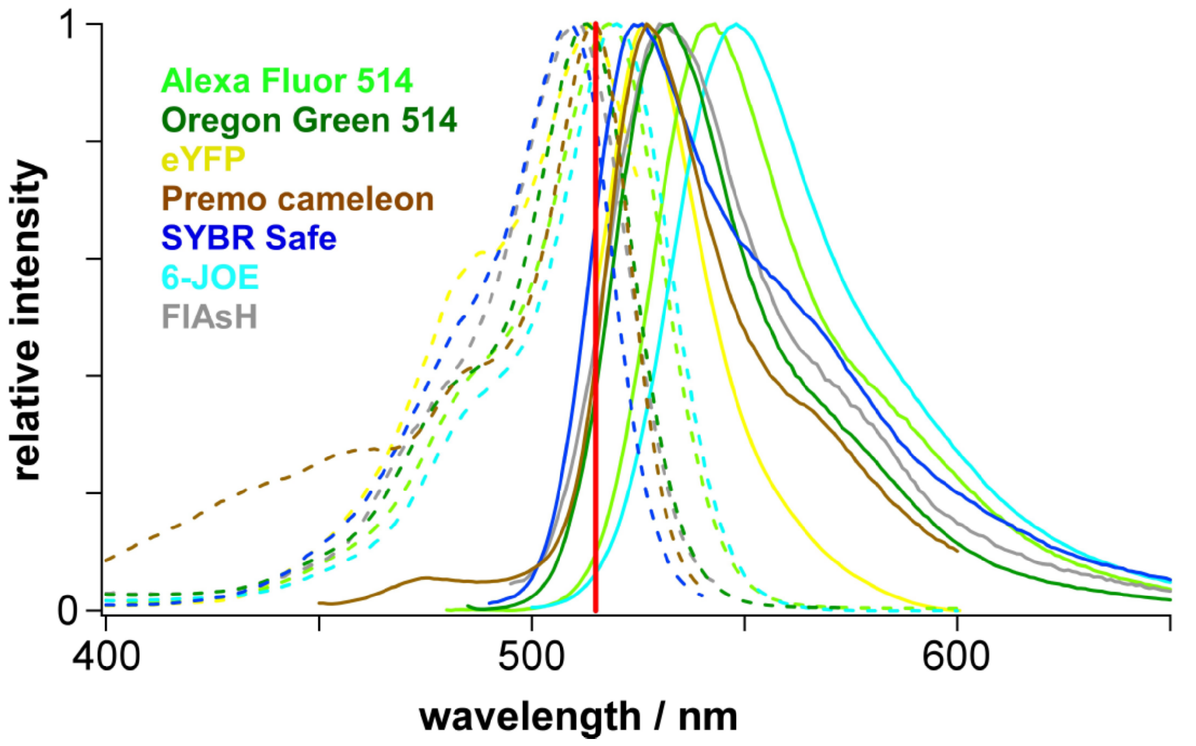


The laser cavity is enclosed in a small and robust hermetically sealed package, which provides a very high degree of resistance to varying ambient temperature and humidity conditions as well as to mechanical shocks and vibrations. Standard broad-striped edge-emitting laser diodes are used as pump sources and the laser concept is inherently power scalable.

The Cobolt Fandango™ is a continuous-wave diode-pumped solid-state lasers operating at a fixed wavelength of 515 nm. The gain medium is an Yb-doped laser crystal emitting at 1030 nm and Cobolt's proprietary PPKTP technology is used for efficient intra-cavity frequency conversion. Spectral filters make the laser operate in single longitudinal mode (<30 MHz bandwidth), which results in very low noise and stable output power over a wide temperature range. The use of a separate curved cavity mirror ensures a very high transversal mode quality in the output beam at all output power levels.

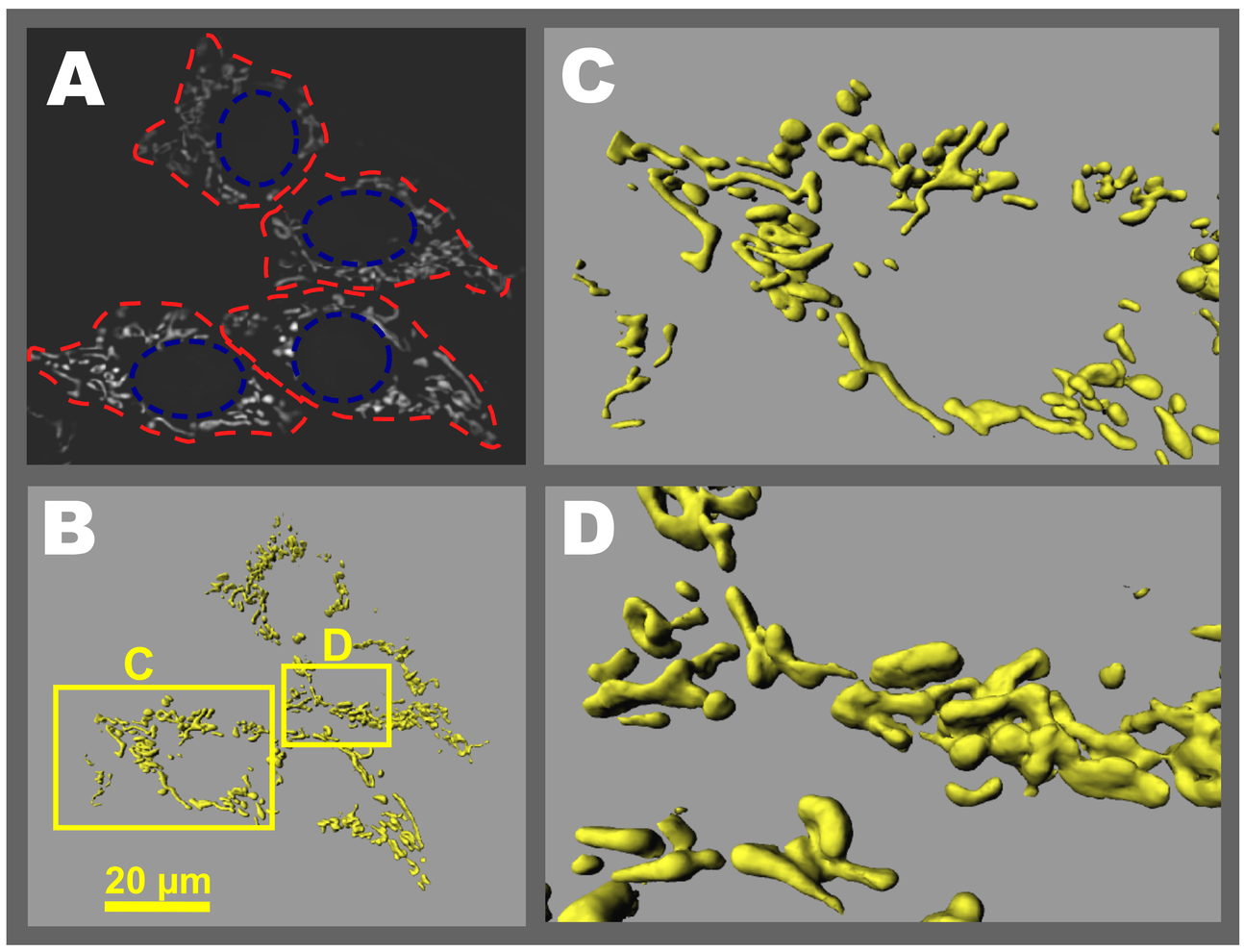




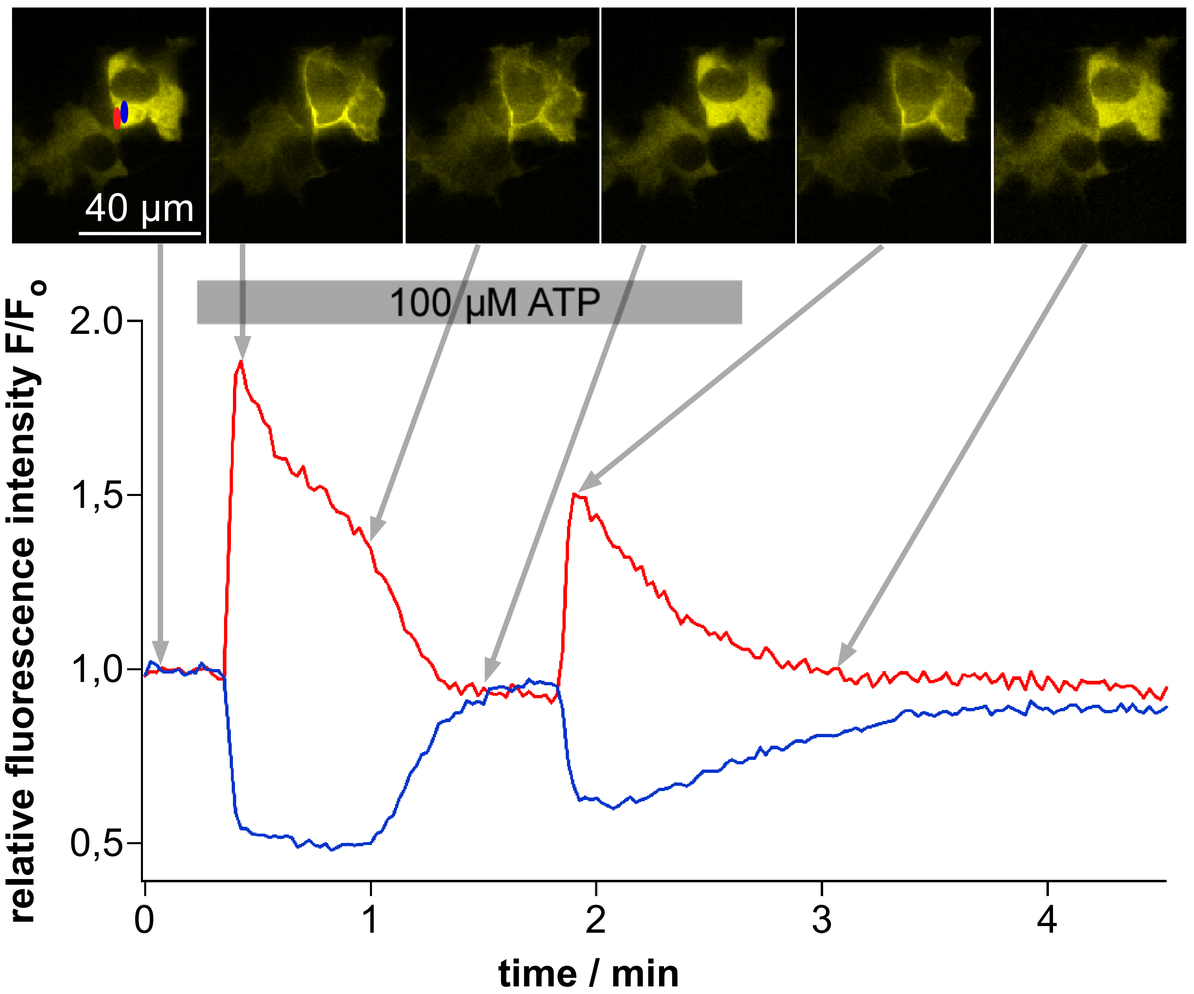


Many fluorescent dyes and fluorophores have their absorption maximum around the 514.5 nm argon-ion laser line. These include e.g. anti-body markers like Alexa Fluor 514 and Oregon Green 514, the yellow fluorescent protein eYFP as well as fluorescent protein-based fusion proteins such as the Calcium calcium indicator Premo Cameleon. Further markers with absorption maximum close to 515 nm are e.g. SYBR Safe, a substance, which can replace the toxic Ethidium ethidium bromide for DNA gel staining, 6-JOE, used in automated DNS DNA sequencing, or FlAsH, a minimally invasive tetracystein-based protein tag. In modern biomedical research, high-resolution fluorescence microscopy for the study of living cells plays an increasingly important role. This accounts for structural and morphological investigations as well as for dynamical processes all the way to the visualization of biochemical reactions. Several of these signal processes, especially in the brain and in heart muscles are so fast that an frame imaging rate of several hundreds to thousands images per second is necessary. Conventional confocal microscopes are usually not able to reach such imaging speeds and thus multibeam approaches, in which several many parallel laser beams are used simultaneously, as well as lineslit-scanning confocal microscopes have since some years become more and more common.

To test the Cobolt Fandango 515 nm laser in fluorescence microscopy, the laser was directly coupled into a VTinfinity (Visitech, UK) multibeam confocal microscope set up for live cell imaging. In such instruments, the exit beam from the laser is divided split into several thousand beams and therefore a laser output power level of at least 20 mW is needed.



Images of the mitochondrial network in cells from a kidney cell line from the African green monkey (COS-1). The fluorescence comes from the eYFP, which was fused to the mitochondrial specific sequence from the subunit VII of the cytochrom C oxidase, and thus exclusively will be expressed in the mitochondria. From the 3D reconstruction details, it is obvious that the mitochondria - contrary to the typical textbook illustrations - are not always egg-shaped, but that they can have many different and partly highly complex, shapes.



Dynamic translocation of the signal molecule protein kinase C, coupled to eYFP, after a physiological stimulus with ATP, is shown. The host cells, expressing the fusion protein, were COS-1 cells.
The interpretation and relevance of this process go beyond the scope of this poster (for more information see e.g. Reither et al., 2006). It is anyway clear from this demonstration, that dynamic processes in life living cells can now successfully be analyzed in systems using a DPSS laser optimised for eYFP.

Far more important applications for this laser though, than these examples using single staining of YFP, will be for quantitative parameter determination, using constructs with the cyan fluorescent protein CFP and based on the now widespread Fluorescence Resonance Energy Transfer (FRET) microscopy method.