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ARTICLE

CONFOCAL & LSFM Imaging: White laser VS. Laser Diodes

Confocal microscopy, most frequently confocal laser scanning microscopy (CLSM), is an optical imaging technique for increasing optical resolution and contrast of a micrograph by means of adding a spatial pinhole placed at the confocal plane of the lens to eliminate out-of-focus light.

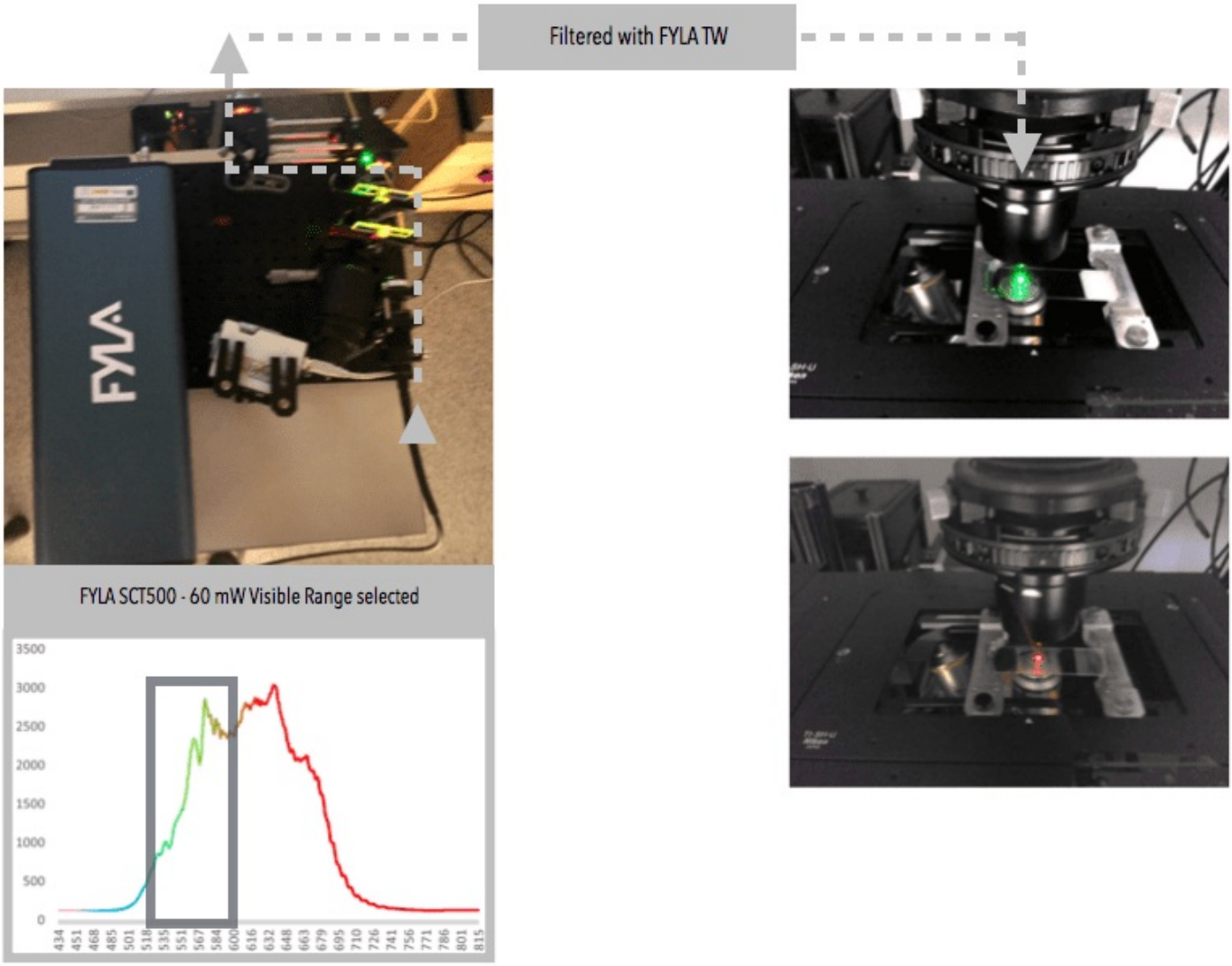
Fine detail is often obscured by the haze and cannot be detected in a non-confocal, fluorescent microscope. The confocal microscope has a stepper motor attached to the fine focus, enabling the collection of a series of images through a three dimensional object.

A real limitation of lasers is their monochromy, which requires a whole battery of lasers to be combined in order to perform multi-parameter fluorescence measurements in simultaneous mode, a method expected as a standard by biomedical researchers. These batteries will be replaced by a all fiber single SCT500 FYLA laser.

Lasers have been critical to the advancement on confocal microscopy, and the white light laser (WLL) offers particular advantages.Finessing WLL output for bioimaging is a complex task, though, and traditional approaches retain key limitations. But supercontinuum sources are not directly applicable to bioimaging

because of the delicate nature of specimens. Now, their adaptation has taken a step forward.

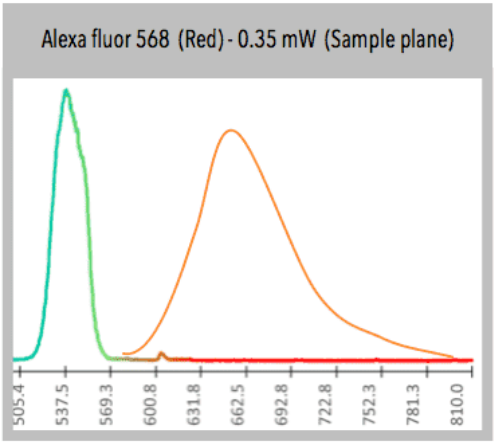
The light requirements for confocal imaging are intensity of a few milliwatts (otherwise, the sample would burn away immediately) along with small bandlets of spectral range that can specifically excite the desired fluorochromes. WLL provides an intensity of some watts, so for this application the white emission is typically filtered with a dynamic device (FYLA TW)



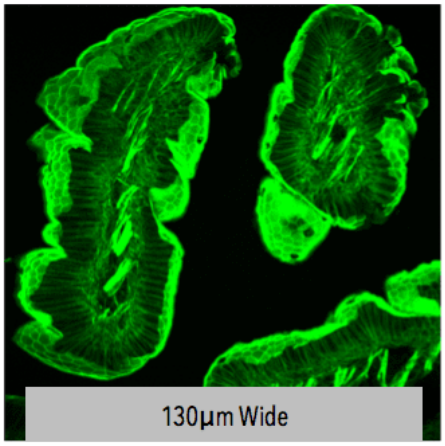
During the fluorescence process, molecules are promoted from the ground state to an excited state by absorption of a photon. The

excited state rapidly relaxes into the lowest vibrational state, from which energy is then emitted by a photon with less energy than the excitation (Stokes shift). This emission is temporally delayed, typically by a couple of nanoseconds. The average time a molecule stays in that excited state correlates to the chemical compound and is referred to as fluorescence lifetime.

BELOW. Emission/Excitation pattern

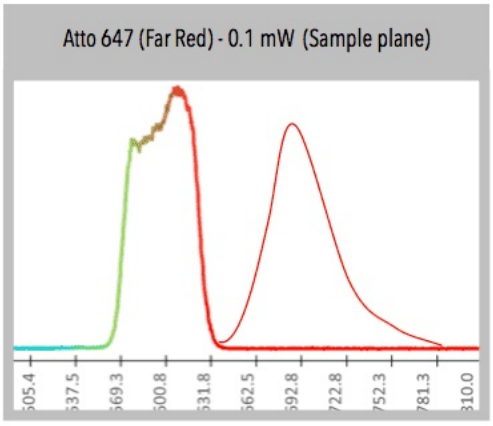


BELOW. Confocal images: 512x512, Pinhole: 1.1 A.U., 1s 1s frame rate, 20 gain (256 max)

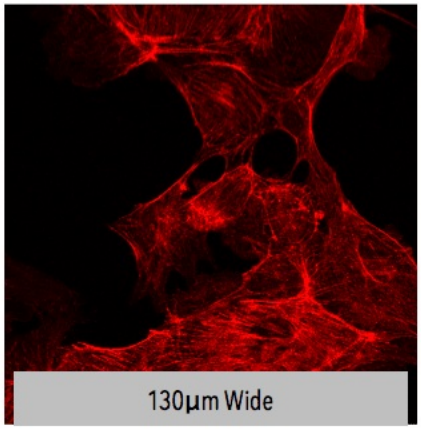


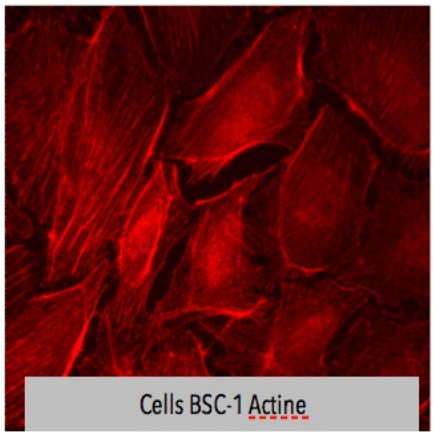
SCT Laser is pulsed , and the pulsed excitation allows yet another approach to acquire data in a true confocal microscope system. In combination with a sensor device that is fast enough to allow photon counting at high rates and has sufficient dynamic range to cover the typical intensities in fluorescence imaging, a “pulsed detection” is accessible. Here, the signal is collected only if the collection gates are open; if not, it is suppressed, allowing In Vivo Imaging.

BELOW. Emission/Excitation pattern



BELOW. Confocal images: 512x512, Pinhole: 1.5 A.U., 1s 1s frame rate, 60 gain (256 max)





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