Chromacity

Application Note 04

Second-Harmonic Generation Imaging using the Chromacity Spark 1040

Second-harmonic generation imaging microscopy (SHG Microscopy, also known as SHIM) offers several advantages for live cell and tissue imaging. The ultrafast pulsewidth of the Chromacity Spark 1040 is ideal for generating a second-harmonic response from a wide range of biological samples. This application note illustrates the suitability of the Chromacity Spark 1040 for generating SHG images in starch granules and collagen fibres.

Second harmonic generation imaging

In recent years, SHG microscopy has proven its capability in the study of crystallized bio-molecules such as starch, collagen and myosin. Unlike fluorescence-microscopy, SHG microscopy does not involve the creation of excited electronic states, so cell viability issues associated with heating and photo-bleaching are reduced. By using near-infrared wavelengths it enables the construction of 3-D images of specimens by imaging deeper into thick tissues. It enables the direct visualization of tissue structure (in situ) as it relies only on species present in the sample to provide a contrast. Imaging with external markers/labels normally only infer structural aspects as it relies on absorption whereas SHG microscopy signals stem from an induced polarization of tissue samples whose structural organization and molecular orientation are non-centrosymmetric, such as collagen and starch.

Collagen imaging

The non-centrosymmetric molecular structure of collagen makes it an ideal sample to image with SHG microscopy. Using a simple setup illustrated in Fig. 2(a). images of collagen fibres could be acquired in both the forward and backward directions. Fig. 1 demonstrates the typical images that can be generated by SHG Microscopy when using the Spark 1040.

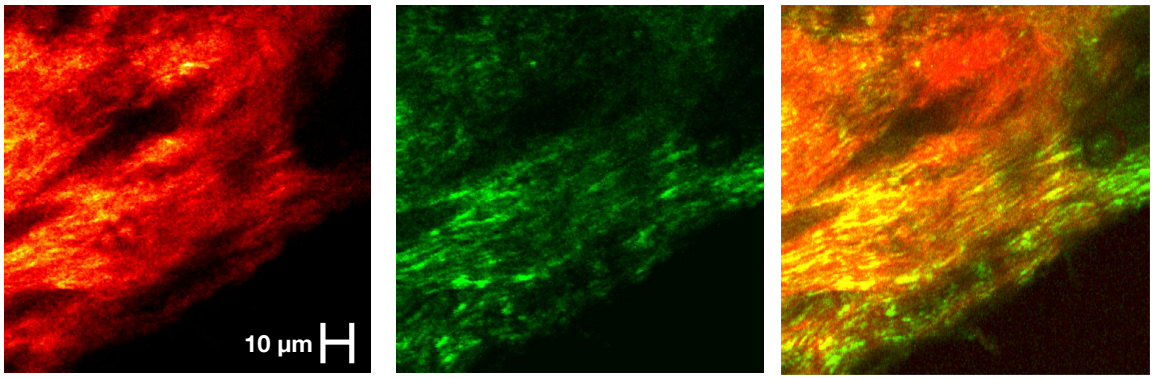

Fig. 1. SHG images of collagen fibres. Images a) and b) are images of the same sample collected in the forward and backward directions respectively. Image c) is a composite showing the detail of the fibres. To acquire these images 300 mW of laser light was incident on the galvo-scanning mirrors.

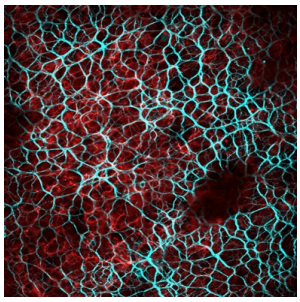

Fig. 2. SHG image of collagen fibres on liver.

Fig. 2 illustrates the imaging of collagen fibres overlaid on a liver sample which has been imaged using multiphoton microscopy. The SHG method enables accurate structural information to be detected using the Spark 1040 as an excitation source. The liver has been imaged using mT:mG and two-photon microscopy with a two channel detection system enabled the simultaneous acquisition of SHG images of the collagen and the two-photon fluorescene signal of the liver, before recombining as a single image.

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Experimental Setup and Starch Imaging

Starch, which is an important food source and a promising future energy candidate, has been shown to exhibit strong SHG response and is a relatively new tool for plant research and other applications.

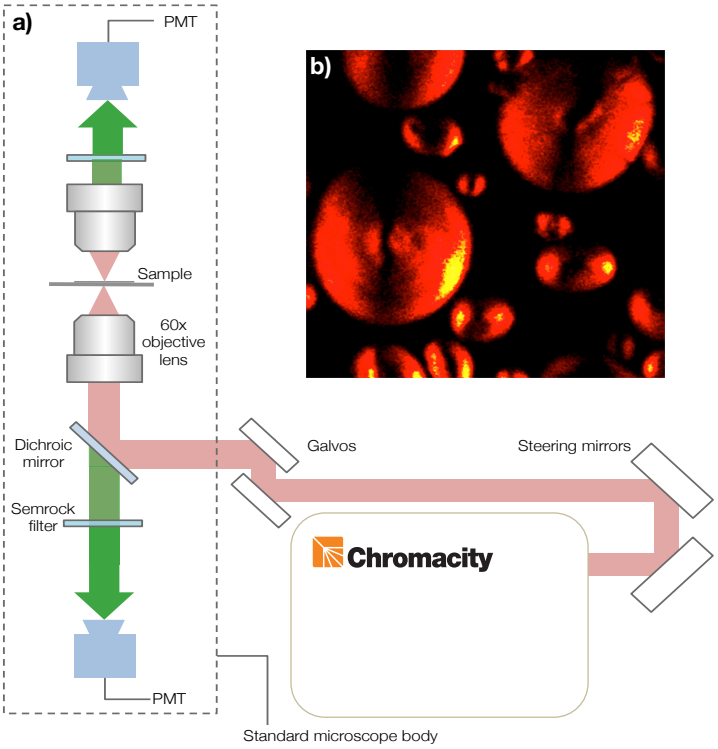

Fig. 3(a) Schematic representation of microscopy setup; (b) second harmonic generation observed in a sample of starch molecules. The field of view for this image is 100 x 100 µm. Only 300 mW of laser light was necessary to acquire these images.

Fig. 3(a). illustrates how the Spark 1040 was used to generate an SHG signal in a solution of starch molecules. Unlike solid-state lasers, which can produce beams with an elliptical cross-section because of astigmatism in the laser cavity, the laser's beam originates from a single-mode fibre, so it is perfectly symmetric making it ideally suited for coupling into commercial laser-scanning microscopes.

into which the beam was introduced via a pair of galvo-scanning mirrors. A Nikon x60 plan Apochromat oil immersion objective (NA 1.4, working distance 0.21 mm) was used to image the starch samples. A suitable filter was used to reject all wavelengths except the SHG signal, which was collected using a photo-multiplier tube (PMT).

Fig. 3(b) illustrates typical images acquired from the system in the forward direction. The power levels from the Spark were more than adequate for generating these fluorescence images, which were recorded with 300 mW incident on the galvo-scanning mirrors.

Summary

The Chromacity Spark 1040 is an ideal source for an SHG Microscopy system, allowing users to generate exceptionally clear, high-resolution images, a result of the Spark's excellent beam quality and high average power levels.

Please contact us for advice on achieving your SHG microscopy requirements.

Multi-photon microscopy is also possible with the Chromacity Spark 1040 femtosecond laser, and is described in Applications Note #3.

Acknowledgement

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MRC Medical Research Council

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