The unique broadband spectrum of the **venteon power** for Differential Coherent Anti-Stokes Raman Scattering (D-CARS).

Abstract

In this whitepaper, we highlight how a team of scientists at Cardiff University[1] used the unique broadband properties of the 5fs pulses generated by Laser Quantum's venteon power to simultaneously undertake Differential Coherent Anti-Stokes Raman Scattering (D-CARS), Second Harmonic Generation (SHG) and Two-Photon Fluorescence (TPF), to provide cell and tissue images with added chemical specificity.

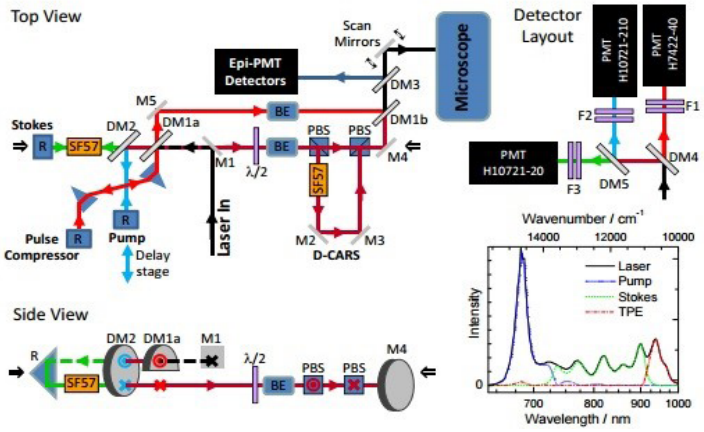

Figure 2: Sketch of the microscope setup. M: mirror; DM: dichroic mirror, SF57: glass blocks; R: reflecting prism; λ/2: half-wave plate; BE: beam expander; PBS: polarizing beam splitter; F: filter. The side view of the optics between by the two indicated arrows shows the beam

Introduction

CARS microscopy is a well-known 3rd order nonlinear process which provides both chemical specificity and 3D spatial resolution without the need to label the living cells[2].

In the CARS process, the pump and Stokes optical fields combine to coherently drive the molecular vibrations. These vibrations are then probed by a third field to generate anti-Stokes Raman Scattering, as depicted in figure 1. In this highlighted case, a second pump pulse is used as the probe field, simplifying the setup.

CARS is ideally suited to cell and tissue imaging providing video rate acquisition speeds at moderate laser powers. This is possible due to the constructive interference of the light scattered by the coherently driven vibrational modes, amplifying the CARS signal. The fast acquisition time also has the benefit of minimising cell exposure, helping to maintain cell viability during live cell and time-lapse imaging. In addition to standard CARS, this work utilises Differential-CARS, a technique whereby the non-resonant CARS background is easily removed by way of simultaneously exciting two different vibrational resonances and measuring the difference in intensities. With the added benefit that two different chemical components can be distinguished from a single image.

Whilst picosecond lasers, specifically Erbium fibre lasers, have shown potential for CARS experiments, the current focus is on femtosecond systems based on Ti:Sapphire oscillators. This is due to many considerations including the availability and cost of Ti:Sapphire lasers, compared with the alternatives; the increased average output powers available; the ease of use and, possibly of most interest, the broadband spectrum which can be "split" into regions for CARS and TPF/SHG. By using one laser for the three techniques, significant cost savings can be realised whilst simplifying the experimental setup and increasing the experimental dataset recorded per analysis.

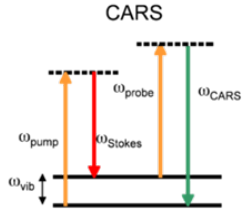

Figure 1: Diagam showing the priciple of CARS

Experimental data

Further details can be seen in figure 2 of the experimental set up, full details can be found in the publication by Pope et al. The system could only be achieved by utilising a broadband pulse such as that generated by the highly stable, customised **venteon power**. Pulses with a duration of 5fs, spectral width of 310nm at 10% of maximum intensity, a repetition rate of 80MHz and high average power of 600mW were generated.

The current turn-key system also benefits from Laser Quantum's proprietary self-start and exceptional lifetimes of the Laser Quantum **finesse pure** pump source which has a mean time to failure of over 40,000 hours.

Results

By splitting the pulse spectrum into the 3 areas of interest, the team in Cardiff imaged simultaneously with D-CARS, TPF and SHG techniques. Figure 3 shows a false colour image of a mouse tail tissue sample which perfectly highlights the advantages of combining the techniques.

The overall image benefits from the spatial resolution and chemical specificity of each of the constituent techniques, whilst allowing a broad range of species to be investigated simultaneously. This combination reduces the time required, and therefore specimens needed, for experimental imaging work. Furthermore, the simultaneous acquisition means colocalisation, between the different techniques, can be unambiguously shown even in live, dynamic samples.

Conclusion

The multimodal hyperspectral CARS/TPF/SHG microscope was developed utilising the Laser Quantum **venteon power** laser (figure 4). This provided a significantly large enough pulse spectrum for CARS covering the entire spectral range from the fingerprint to the CH/OH stretch region, without the need for a dual system. In addition the higher wavelength region, >910nm, has been effectively utilised for TPE. With no laser tuning, or synchronisation of multiple lasers required, the multimodal system was successfully integrated into an inverted microscopy setup. This provided a reliable and easy to use imaging platform.

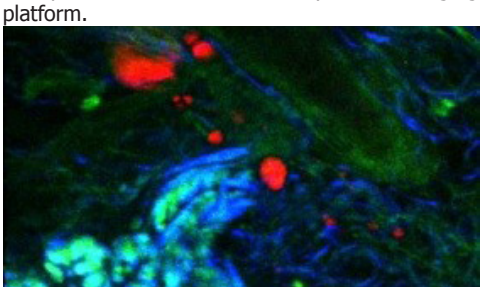

Figure 3: TPF (green), CARS from subcutaneous lipid deposits (red) and SHG from collagen (blue) of tissue from a mouse tail.

Figure 4: The Laser Quantum **venteon power** laser

Acknowledgements:
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References:
[1] I. Pope, W. Langbein, P. Watson and P. Borri, "Simultaneous hyperspectral differential-CARS, TPF and SHG microscopy with a single 5 fs Ti:Sa laser" Optics Express. Vol 21, No 6. 2013.
[2] M. Muller and A. Zumbusch, "Coherent anti-stokes raman scattering microscopy" Chem. Phys. Chem. 8, 2156-2170 (2007).