Using the **ventus** in Laser Tweezers Raman Spectroscopy (LTRS)

Abstract

One in two people born after 1960 in the UK will be diagnosed with some form of cancer during their lifetime. Analysis of cancer tissue at the bulk level means individual cell attributes are averaged, and therefore single cell methods are of interest, both in a diagnostic setting and in the context of drug-cell interaction studies. In recent years, significant progress has been made into the label-free detection and discrimination of individual cancer cells using Laser Tweezers Raman Spectroscopy (LTRS). However, LTRS methods have invariably involved a high degree of manual intervention, and before this technique can be translated into a clinical setting a greater degree of automation is required.

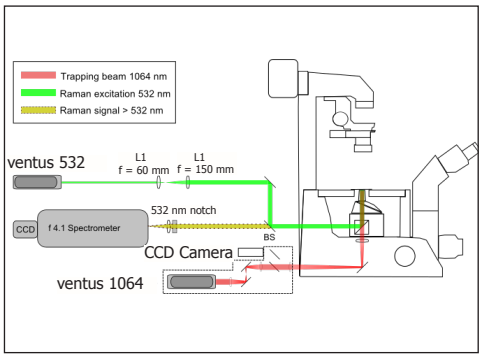

Figure 2: The experiemental set-up

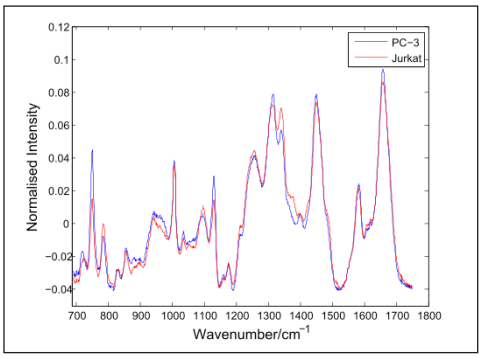

Figure 3: The average PC-3 Raman spectrum, showing increased intensities representative of cytochrome-c in addition to enhanced protein/ lipid bands

First observed in 1928 by Raman and Krishnan, Raman scattering (RS) is the inelastic scattering of light from a sample, which reveals information relating to the distribution of vibrational states (Figure 1). The non-destructive, label-free nature of RS makes the technique particularly attractive for the analysis of biological samples. However, the low efficiency at which Raman scattering occurs compared to fluorescence is a fundamental limitation, making the choice of substrate and sample preparation paramount. With typical signal collection times in the order of seconds, a method of immobilising samples during the acquisition of a spectrum is required, and the majority of biological Raman studies involve single cells either cultured or spun onto substrates.

An alternative method of maintaining the position of micron-sized objects including individual cells is optical trapping, first demonstrated by Ashkin in 1986. Optical trapping of cells has been achieved with both a single beam (laser tweezers, LT) and using two counter propagating beams, in both cases requiring the generation of a high intensity optical gradient, resulting in forces of the order of pico Newtons. For a single laser beam focussed by a high numerical aperture lens, these forces can be sufficient to hold an individual cell above a substrate. As such, LT provide two key advantages for single cell analysis: first, cells can be isolated away from a Raman active/fluorescent substrate, and second, it is possible to manipulate a trapped object, allowing cells of interest to be isolated from a larger population.

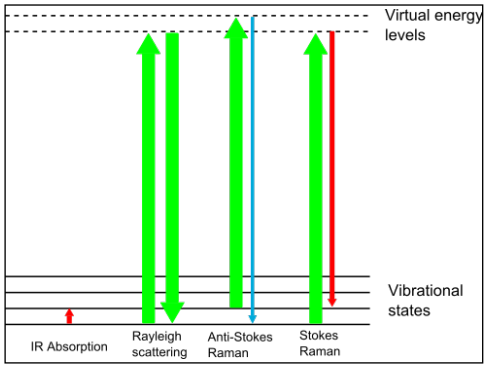

Figure 1: Energy level diagram showing vibrational states probed by Raman spectroscopy

The combination of LT and RS in a single experimental arrangement is straightforward, since the low signal levels found in RS generally require the use of a microscope objective and the means to couple laser radiation to a sample. The LTRS arrangement outlined utilises a **ventus 1064** together with a **ventus 532** for optical trapping and Raman excitation respectively. The use of a low cost microfluidic flow chamber controlled via a syringe pump allows individual cells to be automatically trapped while a Raman spectrum is acquired, and greatly reduces the amount of operator input required compared to traditional methods of optical trapping.

Circulating Tumour Cells (CTCs) are cells which are shed into the blood system from a primary tumour. In the case of prostate cancer, prognosis is strongly dependent on whether cancer has spread to secondary sites, with outlook particularly poor for individuals with secondary bone metastases. It has been suggested that, for late stage (androgen independent) prostate cancer, detecting the number of CTCs in a sample of peripheral blood may also have some diagnostic potential, both in terms of establishing the current staging of the disease and in assessing the effectiveness of treatment. To verify the potential for LTRS to be used to differentiate between CTCs and white blood cells, the metastatic PC-3 prostate cell line and Jurkat cells were analysed using the LTRS system outlined in Figure 2.

As shown in Figure 3, the average PC-3 Raman spectrum was found to display increased intensities representative of cytochrome-c in addition to enhanced protein/lipid bands. The Jurkat cells were found to display increased intensities indicative of nucleic acids. Using Linear Discriminate Analysis, classification rates in excess of 95% were obtained for the two cell lines. Furthermore, using only the ratio of Raman bands representative of cytochrome-c and nucleic acids, the two cells lines could be successfully classified at acquisition times as short as 2 seconds.

Following the classification of PC-3 and Jurkat cells, a fully automated method of trapping and Raman analysis was explored using a second microfluidic flow chamber consisting of two individual channels. By alternating the position of the microfluidic flow cell relative to the focussed beams, time-dependant changes in levels of cytochrome-c were identified for a single cell line exposed to different conditions.

LTRS arrangements can utilise a single laser for both trapping and Raman excitation, or two separate wavelengths. Trapping of individual cancer cells was achieved using Laser Quantum's **ventus 1064** laser. The **ventus 1064** outputs a Single Transverse Mode (TEM_{00}) with an M^2 value of <1.4 as required for the generation of an intense optical gradient. The optically trapped cells are then illuminated by Laser Quantum's **ventus 532** laser. Illumination at 532 nm results in enhanced (resonant) Raman scattering by cytochrome-c, a heme protein involved in cellular respiration, allowing the two cell lines to be distinguished.

An important factor for Raman spectroscopy is the linewidth of the excitation laser, which will impact on how well closely-spaced Raman bands may be resolved. Typically, Raman linewidths within single-cell spectra are of the order of 10 cm^{-1} and as a result the 30 GHz linewidth of the **ventus 532** (equal to 1 cm^{-1}) is ideal. For any dual wavelength LTRS systems, a further consideration is the pointing stability of the two beams. Both the **ventus 1064** and **ventus 532** have excellent pointing stability (<10 µrad/°C), ensuring the two beams remain focussed at the same position over extended timeframes.

The combination of a low cost microfluidic flow chamber and dual wavelength LTRS system has greatly reduced the level of operator input needed to acquire single cell Raman spectra, whilst supplying invaluable data for cancer research. The experiments outlined reveal the potential for moving toward a single automated system, requiring simultaneous control of spectrometer, syringe pump, microscope translation stage and lasers. Both the **ventus 1064** and **ventus 532** lasers can be controlled via an RS232 interface or using **LabVIEW** drivers, allowing lasers to be fully integrated into a single experimental set-up.

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Reference: S. Casabella et. al. Automated analysis of single cells using Laser Tweezers Raman Spectroscopy. *Analyst*, 2016, 141, 689