

## Raman atomic force microscopy – further studies

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### Introduction

Over the last 15 years the LSF has been responsible for many developments in the field of Raman spectroscopy, this includes the development of the first picosecond 400 nm pumped OPA to provide fully tunable wavelengths to support time resolved resonance Raman studies, the development of the Kerr gate technology to remove fluorescent problems, the latest innovation SORS (spatially offset Raman spectroscopy) for non-invasive probing of diffusely scattering media and recent investigations into comparing pulsed and CW laser systems for SERS studies. In this light it is therefore highly appropriate that the LSF should develop expertise in the use of surface enhanced Raman using a scanning atomic force microscope tip. This newly emerging technique, also referred to as tip enhanced Raman spectroscopy (TERS), adds a major factor to existing AFM techniques by enabling molecular specific information to be obtained theoretically down to 10 nm spatial resolution and this potential is being developed in several laboratories<sup>1,2</sup>. This report continues our investigations from last year<sup>3</sup> and is based on a 3 week run that had several aims:-

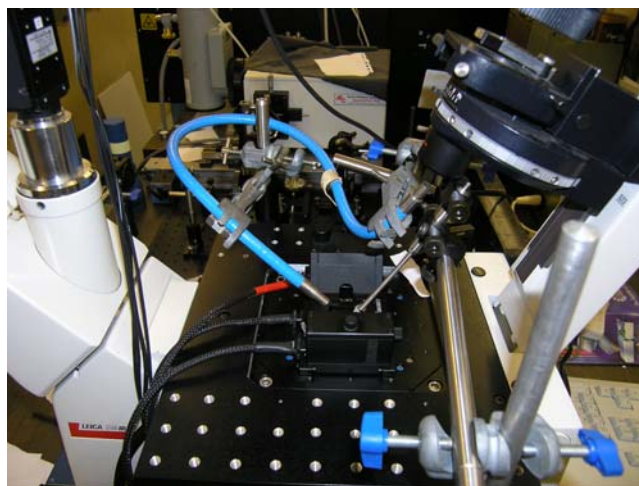
a) Incorporate an AFM instrument into our existing simple inverted microscope set-up.

b) Convert the illumination from below the sample, as used previously<sup>3</sup> to a side illumination. This should provide a substantially larger tip-enhancement factor due to larger electric component available along the z-axis of the AFM tip<sup>2</sup>.

c) Test newly developed gold AFM tips, both commercial and home made.

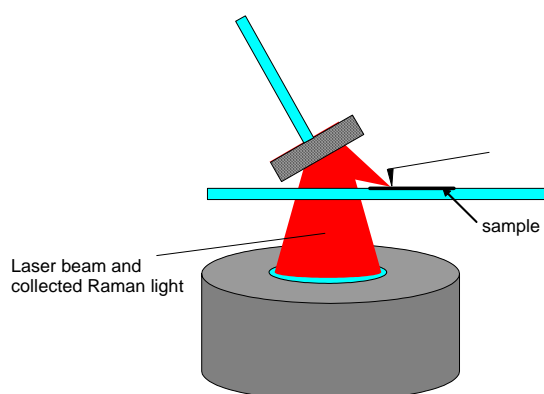
### Experimental

The main components were, a Leica DM-IRB microscope with x40 objective lens (NA = 0.5), the AFM head was a Nanonics AFM. To optimise the spectra two excitation sources were used either a 514.5 nm cw Coherent Innova 90-5UV Argon-ion laser or a 632 nm Melles Griot He-Ne. The 632 nm gave the better signals. Backscattered Raman light was passed through a Kaiser Optics holographic notch filter matched to the laser wavelength and then collected using an Acton Research Corporation SpectraPro 500 spectrograph with a Princeton Instruments LN/CCD-1024 CCD camera. A variety of AFM tips were used. Commercially available tips with gold or silver spheres were provided by Nanonics free of charge and all tips showed a good and very reproducible enhancement effect (unlike last year). Therefore, the major obstacle, the problem of sourcing reproducible Raman-active tips has been solved. Sharp gold tips were prepared in-house by a method described below. The experimental set up is shown in Figure 1.



**Figure 1.** Photograph of the side illuminated LSF-TERS set-up. The blue liquid light guide was used to illuminate with the housed microscope lamp to view the sample and AFM tip for visual alignment purposes.

The main challenge was to develop side-illumination geometry compatible with the inverted microscope and the commercial AFM head. The novel approach used a small mirror to reflect the laser beam, coming from the microscope objective from below and onto the sample located to the side. The collected Raman light was coupled back into the microscope via the same mirror. This simple modification, depicted in Figure 2, enabled us to maximise the Raman enhancement effect whilst maintaining the simplicity of the layout and compatibility with the existing commercial instrumentation.



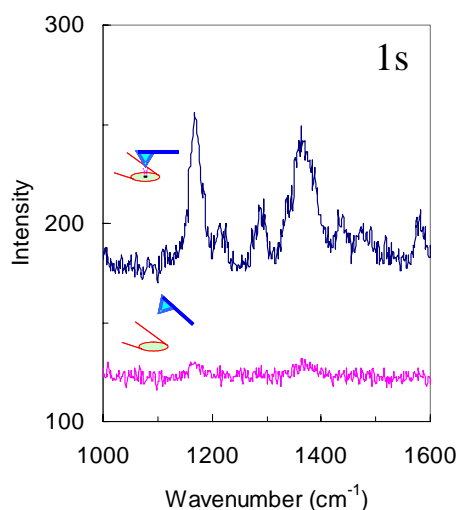
**Figure 2.** Schematic showing how laser light was directed onto the sample under the AFM tip from the inverted microscope and Raman scatter collected back along the same optical path.

Gold AFM tips were prepared by direct current electrochemical etching of gold wire in fuming HCl/ethanol solution as described in reference<sup>1)</sup>. The etching solution was prepared by mixing fuming HCl (Sigma-Aldrich, 36.5 %) and ethanol (Sigma-Aldrich, 95 %) in equal quantities. An 8 mm diameter cathode was formed using gold wire (Advent Research Materials Ltd,  $\varnothing = 1.0$  mm, 99.99 %) and placed flat on the surface of the etching solution and immersed by approximately 0.5 mm ensuring some of the electrode remained proud of the solution. The anode was formed from approximately 5 cm of gold wire (Advent Research Materials Ltd,  $\varnothing = 250$   $\mu\text{m}$ , 99.99 %) and its tip immersed to a depth of 3 mm inside the cathode ring.

A voltage of 2.4 V was applied using a potentiostat and the etching of the anode monitored using a CCD camera equipped with  $\times 25$  microscope objective and monitor. When the lower section of the anode dropped off forming a sharp tip visible on the monitor the etching was stopped. This method produced tips and this will be tested in due course.

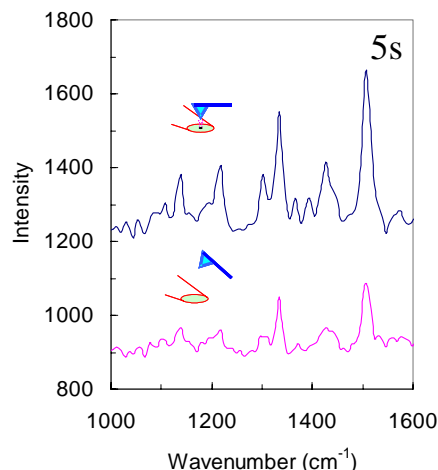
### Results

The instrument was tested at various laser wavelengths and with a wide range of molecules. The first sample tested was malachite green isothiocyanate (MGITC) chosen for two reasons, it has a large Raman cross section and has been used previously in TERS studies<sup>4)</sup> and therefore served as a simple comparison of the capability of our system. Samples were made by placing a few drops of a saturated solution of MGITC in ethanol onto a silver coated coverslip and allowing it to evaporate. The recorded spectra are shown in Figure 3.



**Figure 3.** TERS spectra of malachite green isothiocyanate. The lower spectrum is without the AFM tip on the sample and the upper spectrum is with the AFM tip engaged. The accumulation time was 1 s using a 632 nm laser, 0.5 mW.

Overall, the magnitude of enhancement observed for MGITC was c. 11 times. However, in comparing TERS enhancement it is important to consider that the area illuminated is much less than that covered by the AFM tip. By taking into account these factors the overall enhancement corresponds to more than 1000 times and this is in agreement with previous work<sup>4)</sup>. In line with the same publication we also observed fast photo-degradation of MGITC, this made it unsuitable for system alignment and testing the reproducibility of the set-up.



**Figure 4.** TERS spectra of zinc phthalocyanine. The lower spectra is the without the AFM tip on the sample and the upper spectrum is with the AFM tip engaged. The accumulation time was 5 s using a 632 nm laser, 0.5 mW.

In a search for a more robust molecule for this purpose we have discovered that zinc phthalocyanine exhibits good TERS properties. Another feature is that this molecule is extremely photostable and highly symmetric making it an excellent Raman sample. Again saturated solutions in ethanol were made and evaporated onto a silver coated microscope cover slip. This molecular system showed an enhancement of  $\sim 4$  (400 after the correction for tip/beam sizes). The TERS spectra are shown in Figure 4. The molecule is also biologically relevant as it is used in photodynamic therapy and may form a basis of future investigations in this area.

Finally, an ideal AFM Raman system should be able to perform a conventional AFM scan and then go to a point of interest to perform tip on and tip off Raman acquisitions or scan the vicinity of the interesting site. To test this a 2D scan was attempted briefly but these were severely hampered by software and hardware incompatibilities with this approach. The findings have been feedback to the manufacturer as suggestions for potential future developments.

### Conclusions

This relatively brief study demonstrates that we are able to perform TERS studies using an existing Raman microscopy instrumentation.

### References

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