Publishable JRP Summary Report for JRP NEW02 Raman Metrology for Raman Spectroscopy

Background:

In the last three decades, there has been a steep rise in publication of research involving Raman scattering, yet to the metrologists Raman spectroscopy is a relatively new technique. Raman scattering has a unique place as a label-free, non-destructive, contact-less, fast, optical method for identification, quantification and mapping the distribution of chemicals and micro-to nano-scale structures in 2 or 3D in ambient air or in aqueous medium.

There has been a surge in use of Raman spectroscopy in the last two decades and a number of novel techniques have evolved to:

- Increase the sensitivity *e.g.* surface enhanced Raman scattering (SERS), coherent anti-Stokes Raman scattering (CARS), stimulated Raman scattering (SRS), and
- Increase spatial resolution *e.g.* tip-enhanced Raman scattering (TERS).

However the metrology for enhanced Raman techniques has never been addressed in a structured way leading to a lack of traceability and underpinning metrology tools, which has led to inadequate standards.

Need for the project:

Raman spectroscopy is primarily used as a qualitative tool in the pharmaceutical, healthcare, biotechnology, nanotechnology, medical technology and forensic science sectors to identify and map distribution of substances in 2 or 3D. However Raman spectroscopy lacks traceability so it is not accepted by the regulatory bodies for approval of drugs. Although standards exist, the instrument manufacturers and users do not use them as they not cater to their needs. Instrument manufacturers categorically mention the need for spatial resolution, depth resolution and confocality measurement standards which do not currently exist.

There are strong technological needs for full chemical and structural identification of species at higher spatial resolution. In addition high temporal resolution is also needed to investigate dynamics of chemistry and structure of the species.

The JRP aims to address the regulatory needs of Raman spectroscopy related to: conventional Raman spectroscopy, tip-enhanced Raman scattering, and multi-photon Raman scattering. The JRP will improve measurement reliability, establish traceability, develop reference samples and prepare related documentary standards.

Scientific and technical objectives: The JRP will make Raman spectroscopy quantifiable by

- Establishing traceability of amount of substance measurements to the mole by conventional Raman scattering and surface-enhanced Raman scattering (SERS)
- Establishing spatial and depth resolution measurements traceable to the metre (target uncertainty sub-micrometre resolution in the XY plane) using confocal Raman microscopy and Tip-Enhanced Raman Scattering (TERS).
- Developing methods for 3D chemical imaging at high speed using multi-photon Raman scattering, (Stimulated Raman Scattering (SRS) and Coherent Anti-Stokes Scattering (CARS))
- Investigating light-matter interactions in Raman scattering using finite difference time domain (FDTD) calculations, to establish mathematical models to underpin traceable measurements
- Improving the repeatability of Raman measurements and establishing robust uncertainty budgets for Raman spectroscopy.

EMRP European Metrology Research Programme Programme of EURAMET
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Expected results and potential impact:

The JRP will maximise the impact of scientific developments through close stakeholder engagement. This will ensure the relevance and usability of developed standards. The JRP currently has 11 stakeholders and 4 collaborators engaged with the project. Through the JRP biochemical imaging of tissue samples could provide substantial impact. Some of the stakeholders are considering using the facilities to characterise skin tissue and plant tissue. These are usually difficult samples to image in their native environments. The visualisation technique will make significant impact in assessing efficacy of consumer health products as well as agrochemical products.

Three reference samples are currently being prepared by the JRP so that a pre-normative study can be conducted in order to make them ready for ISO standardisation. One of the collaborators, NIST, is involved in ISO229 where these standards can be considered. To date there is only one reference sample for intensity calibration of the detector; however, there are no ISO standards for quantification of Raman signals. In this process, the JRP-Consortium gets an opportunity to keep the stakeholders involved.

Dissemination of project results at conferences is going very well as the number of presentations required to be given has already been surpassed.

JRP-Participants have attended the Surface Analysis Working Group (SAWG) meeting that was held as part of the CCQM (Consultative Committee for Amount of Substance – Metrology in Chemistry) meeting in Paris, France, in April (2014). This contributes towards the project's input to standards bodies.

The JRP has organised the first metrology session on Raman scattering in the international conference on Raman spectroscopy (ICORS). One reference sample, the first in the world, has been published, one is being written, and one is being tested.

The JRP has demonstrated its excellence in advancement in quantitative multi-photon Raman scattering techniques by publishing a novel method of suppressing resonance background in the Raman signal which will facilitate quantification of the signal.

Guidance training on Pitfalls and Metrology in Raman Spectroscopy was completed in Brazil.

There have been many technical highlights during the JRP so far:

- Following the development of a measurement procedure Raman depth profiles were measured on prototype specimens. Some modifications were identified to minimise the user-influence on the measured depth profiles and a standardised procedure was developed.
- Several batches of solid Surface-Enhanced Raman Scattering (SERS) active substrates were prepared and SERS test measurements were made using several analytes.
- A model for focused beam excitation techniques like micro-Raman has been tested on various incident field conditions, polarisations and studied material geometries.
- Test specimens made of mono- and multilayer polymer films have been fabricated and characterized.
- GERS substrates were fabricated and delivered to JRP-Partners, along with the software macro and instructions written to enable them to identify the number of graphene layers. A protocol for the fabrication of markers-provided interchange samples was successfully tested and developed.
- SWCNT samples and TERS tips were fabricated and provided to the JRP-Partners for transmission mode measurements. The SWCNT samples from INRIM have been characterised by AFM and confocal Raman. The SWCNTs were uniformly distributed all over the glass coverslip and were well separated from each other.
- It was found that SWCNTs were an unsuitable material for a spatial resolution measurement. Therefore an alternative lateral and axial resolution reference sample was developed.
- Datasets on temporal resolution vs signal to noise ratio and spatial resolution vs signal to noise ratio, both using 100 nm polystyrene beads, have been collected.
- Models for different source conditions used in real experiments using micro Raman and TERS-III geometries were implemented. A dataset has been collected from FDTD calculations which model

the response from the focused beam interacting with the multilayer polymeric structure at various depths. The possibilities of the FDTD software to treat arbitrary geometry tips of any shape have been extended.

- Tissue imaging capability using stimulated Raman scattering has been developed. The technique is being exploited for measuring homogeneity of active ingredients in a topical cream and in pig skin.
- The project was the first to carry out a laboratory comparison on Raman depth profiling. The results should encourage users to have a more critical view on Raman depth profiling data in general and emphasize the need for a careful calibration of the instrument´s z-axis and confocality.
- An efficient network of scientific collaboration on Tip-enhanced Raman Spectroscopy (TERS) has been established for the first time among metrological and research institutes. Due to the particular complexity of the technique, this network is essential to allow breakthrough of TERS into more advanced and practical application fields.
- New pyramidal probes have been developed and successfully tested in scanning tunnelling microscopy (STM) TERS. A process for cantilever type gold pyramids has been developed.
- Quantitative measurements using isotope-diluted surface enhanced Raman spectroscopy (ID-SERS), collaborative work by NPL and INRIM on tip-enhanced Raman spectroscopy (TERS) and development of video-rate Raman imaging capability at NPL using stimulated Raman scattering and the release of the GSvit software within the consortium.
- An ID-SERS approach for protein quantification in the low nmol concentration range works very well for quantification of biological macromolecules such as hemoglobin. The application can be expanded to other small and large proteins. Density function theory (DFT) calculation of Raman spectra could predict the actual isotopic shift occurring for various isotopologues of a biomolecule which allows for the preparation of customised spike material.
- To further improve chemical imaging capability of spontaneous Raman spectroscopy, a chemometric software has been developed and tested on 4 different types of dye-loaded micro beads. It shows successful differentiation between the different beads. It is currently being tested on more physiological particles such as hydroxyl-apatite and drug such as paracetamol.
- Measurements on SWCNTs as a reference sample for spatial resolution, longevity of TERS tips, interlaboratory study of the bilayer reference sample and measurements on biomolecules and cell sections has been completed.
- NPL has developed a software for laser scanning imaging that is capable of fast SRS, CARS and fluorescence imaging at a frame rate of >1frames per second. This powerful tool allows real time monitoring of chemical and biological processes such as phase separation, permeation and diffusion of molecules. NPL has utilised this capability to study oxidative stress in living skin tissue, distribution of drugs (ibuprofen) and permeation of propylene glycol (PG) in pig skin. A study is in progress to screen a set of drugs which can be used to understand drug metabolism and pharmaco kinetics. NPL has been exploiting the TERS facility to study defects in graphene and 2D materials at an unprecedented 20 nm spatial resolution.
- CMI has released the FDTD solver GSvit within the consortium. The software now includes more accurate models of dispersive materials, namely metals and to introduce automated reduction of staircasing effect in FDTD calculations. There was also an effort to simplify the parameter file creation and training of other partners for performing FDTD calculations on their experimental setups. Software for simulation of scanning by micro Raman instrument has also been developed.

The recent developments together improve the confidence in label-free measurements of biomolecules and other substances at concentrations ranging from nano-molar to mili-molar and spatial resolution from 20 nm to micrometres. The new capability of video rate imaging will facilitate investigating dynamical processes in complex mediums. These combined capabilities are unique in Europe.

The collaborator NIST is working with all JRP-Partners. They will provide experience in dissemination and representation at standards committees and are a valued asset to the JRP-Consortium. This will ensure the results from the JRP extend from Europe to the rest of the world.

A strength of the JRP is that it is working with a commercial company, NMI, to prepare reliable commercially viable TERS tips. An interlaboratory study will allow NMI to assess quality, enhancement factor and shelf life of the tips.

JRP start date and duration:	01 August 2012 (36 months)
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