MINI-CONFERENCE

Raman Nanotheranostics

Next Generation Healthcare Technology

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UKRI Engineering and Physical Sciences Research Council

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University of Exeter, UK

5 – 7 SEP 2022

Schedule

&

Abstract Booklet

Monday 5th September

13.00 – 14.00 **Registration / Lunch**

14.00 – 14.15 **Welcome & Introduction – Prof. Nick Stone (RaNT Director)**

Session 1:
A Bright Future – Tuning Biocompatible Nanoparticles

Co-Chairs: Dr. Renata Lang Sala & Dr. Megha Mehta

14.15 – 14.55	INVITED	**Prof. Hatice ALTUG** *Nanophotonics for Optical Biosensing, Bioimaging and Spectroscopy*
14.55 – 15.15		**Dr. Renata Lang SALA** *Kinetically arrested colloidally-stable plasmonic assemblies*
15.15 – 15.35		**Dr. Periklis PANTAZIS** *Biodegradable Harmonophores for Targeted High-Resolution In Vivo Tumour Imaging*
15.35 – 16.15	INVITED	**Dr. Carl Emil ESKILDSEN** *Raman spectroscopy for biomedical applications*
16.15 – 16.25		**Ryan D. MELLOR** *Development of Bio-Functionalized, Raman Responsive, Ultrasmall-in-Nano Gold Constructs*
16.25 – 16.50		**Refreshment Break**
16.50 – 17.30	INVITED	**Dr. Bhavya SHARMA** *Advances in Raman Spectroscopy for Neurochemical Sensing*
17.30 – 17.50		**Dr. Megha MEHTA** *Comparison of resonant and non-resonant reporter for the selection of brightest gold nanoparticles for Surface-enhanced Raman spectroscopy*
17.50 – 18.10		**Prof. Nicholas W. TURNER** *Molecularly Imprinted Polymers: Nanoscale Biomimetics*
18.10 – 18.30		**Prof. Dr. Alexander GLASSMANN** *Enhanced Specific Drug Delivery Using Polyoma-Virus Deduced Engineered Protein Nanoparticles*
18.30 – 18.50		**Wenjing HU** *Soft templating of copper-based nanoparticles for photothermal and photodynamic therapy*
18.50 – 19.30		**Optional Lab Tours & Break for the Day**

Tuesday 6th September

09.00 – 09.30 **Arrival Refreshments**

Session 2:
In-Depth Issues – Detection, Therapy & Monitoring of Disease

Chair: Dr. Sara Mosca

09.30 – 10.10	INVITED	**Dr. Sanathana KONUGOLU VENKATA SEKAR** *Phantoms to fast-track device development and standardization in Biophotonics*
10.10 – 10.50		**Prof. Peter WEIGHTMAN** *The application of machine learning and infrared aperture SNOM to cancer diagnosis*
10.50 – 11.20		Refreshment Break
11.20 – 12.00	INVITED	**Prof. Paola TARONI** *Diffuse optical spectroscopy and imaging for breast cancer management*
12.00 – 12.20		**Jan VALIS** *Classification pipeline for in vivo Raman spectra of colorectal tissue for cancer detection*
12.20 – 12:40		**Dr. Michelle BAILEY** *Brillouin spectroscopy as a probe of biomechanics*
12:40 – 13:00		**Dr. Sara MOSCA** *Estimating the depth of inclusion and the optical properties of biological tissues using Spatially Offset Raman Spectroscopy*
13.00 – 14.00		Lunch

Session 3:
Cellular Insights – Understanding Accumulation of Nanotheranostics

Co-Chairs: Dr. Alexandra-Geanina Văideanu & Dr. Chun-Chin Wang

14.00 – 14.40	INVITED	**Prof. Ji-Xin CHENG** *New Advances in Coherent Raman Scattering Microscopy*
14.40 – 15.00		**Dr. Chun-Chin WANG** *Multimodal molecular imaging of functionalised nanoparticles uptake by cancer cells and tissues*

15.00 – 15.20	**Dr. Martin LEE** *Alkyne tagging as a strategy to image nanoparticles in vitro and ex-vivo by stimulated Raman scattering*
15.20 – 15.40	**Nicole SLESIONA** *Four Wave Mixing imaging for the detection of gold nanoparticle internalisation into living cells at the single particle level*
15.40 – 16.00	Refreshment Break
16.00 – 16.40	INVITED **Prof. Warren C.W. CHAN** *Nanoparticle delivery to solid tumours*
16.40 – 17.00	**Dr. Alexandra-Geanina VĂIDEANU** *Total mass balance of gold nanomaterials in vivo*
17.00 – 17.20	**Dr. Nathanne Cristina Vilela ROST** *Post-publication peer review of publications reporting nanoparticle-based SERS intracellular sensing*
17.20 – 17.40	**Dr. William H. SKINNER** *Surface-enhanced Raman scattering (SERS) micro-sensors for pH measurements in patient-derived airway organoids*
17.40 – 19.00	Poster Session / Exhibition / Drinks Reception & Working Dinner

Wednesday 7th September

09.00 – 09.30 Arrival Refreshments

Session 4:
Theranostics in the Clinic – Tales of Healthcare Technology Translation

Chair: Dr. Benjamin Gardner

09.30 – 10.10	INVITED **Dr. Holly BUTLER (or Dr. Matthew BAKER)** *A Tale of Clinical Translation: Developing a Spectroscopy-Based Medical Device for Cancer Diagnostics*
10.10 – 10.30	**Dr. Benjamin GARDNER** *TBC*
10.30 – 10.50	**Dr. Dimitrios TSIKRITSIS** *Standardisation for stimulated Raman scattering microscopy applications*
10.50 – 11.20	Refreshment Break

11.20 – 12.00	INVITED	**Prof. Paul BEARD** *The clinical translation of a novel photoacoustic imaging technology: from first principles to first-in-human*
12.00 – 12.30		**Dr. Sam MABBOTT** *Point-of-Care Diagnostics for Underserved Populations*
12.30 – 12.50		**Jennifer HASKELL** *High Wavenumber Raman Spectroscopy of Breast Tissue for Tumour Margin Assessment*
12.50 – 13.10		**Diana FRIMPONG** *Demystifying NHS Ethics Applications*
13.10 – 13.25		**Closing Remarks – Prof. Nick Stone (RaNT Director)**
13.25 – 14.30		**Lunch & Finish**

INVITED SPEAKERS

Hatice ALTUG

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Nanophotonics for Optical Biosensing, Bioimaging and Spectroscopy

Emerging healthcare needs and initiatives, including global health care, personalized medicine, and point-of-care applications are demanding breakthrough advancements in diagnostic tools. Biosensors play an essential role in bioanalytics, but traditional methods are limited in precision, affordability, integration or portability. Furthermore, they require long detection times, sophisticated infrastructure, and trained personnel. Our research group addresses these challenges by developing next-generation optical biosensors, spectroscopy and bioimaging technologies with nanophotonics, nanofabrication, microfluidics, surface chemistry and data science. Using nanophotonics we engineer nanostructures that can confine light below the fundamental diffraction limit and generate strong electromagnetic fields at important spectral ranges including visible, near-infrared and mid-infrared. Through nano-scale optical effects, we increase light-matter interaction to achieve high sensitivity, accuracy, throughput, rapid response, on-chip integration and miniaturization. We introduce wafer-scale nanofabrication methods for low-cost manufacturing of nanophotonic substrates. We integrate our nanophotonic chips with microfluidics for efficient sample handling and employ surface functionalization and biopatterning approaches for operation in complex samples. We use smart data science tools to achieve higher device performance. In this talk, I will present our recent effort in these directions.

Paul BEARD **University College London**

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The clinical translation of a novel photoacoustic imaging technology: from first principles to first-in-human

Photoacoustic imaging is an emerging biomedical imaging modality based on the use of laser-generated ultrasound. It is a hybrid technique that combines the high contrast and spectroscopic-based specificity of optical imaging with the high spatial resolution available to ultrasound. As a consequence, it overcomes the limited penetration depth/spatial resolution of purely optical imaging techniques such as light microscopy or diffuse optical tomography due to the overwhelming optical scattering exhibited by tissue. At the same time, it retains their high contrast and spectral specificity enabling visualisation of anatomical features indistinguishable with other modalities such as ultrasound imaging. Photoacoustic image contrast is dominated by optical absorption. This makes the technique particularly well suited to visualising vascular anatomy on account of the strong absorption of haemoglobin. As a consequence, photoacoustic imaging has potentially broad clinical application encompassing the assessment of breast and skin cancers, cardiovascular disease, and abnormalities of the microcirculation implicated in diabetes and skin conditions.

At UCL we have developed a novel photoacoustic imaging technology based an optical ultrasound sensor. Over the last two decades we have taken the technology from first principles at component level to engineering practical clinical imaging instruments. A number of first-in-human clinical studies are now currently underway to assess its suitability for assessing inflammatory arthritis, peripheral vascular disease, the diabetic foot and guiding liver cancer and plastic surgery. This talk will discuss the trials and tribulations of this journey and the broader outlook for the clinical translation of photoacoustic imaging.

Holly BUTLER **Dxcover Ltd.**

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A Tale of Clinical Translation: Developing a Spectroscopy-Based Medical Device for Cancer Diagnostics

H. Butler[1]

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The ultimate goal for biomedical vibrational spectroscopy is to integrate an effective technology into health services worldwide, improving current clinical practices and thus providing tangible benefits to patients each and every day. A wealth of promising proof-of-concept studies and revolutionary technical developments have allowed progression towards this end goal; however, as yet, there is still no single technology that has achieved this feat. This could be attributed to the multitude of hurdles along this pathway to translation including prototype development, clinical feasibility, regulatory approval, and of course, ever elusive funding.

Here we describe the development of an infrared spectroscopy-based blood test for the earlier detection of cancer. The Dxcover platform utilizes infrared spectroscopy and machine learning to detect early-stage tumors. The company has generated compelling clinical data from 3,000 patients across 8 cancers and is developing Organ Specific tests and Multi-Cancer Combination tests. The Dxcover Platform is advancing towards regulatory approvals and aims to be commercially available by 2024 following successful clinical studies. In this tale of translation, key milestones along the pathway towards commercialisation will be described, as well as the trials and tribulations associated with medical device development.

Warren C.W. CHAN **University of Toronto**

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Nanoparticle Delivery to Solid Tumours

Warren C.W. Chan[1]

[1]University of Toronto, ,Donnelly Centre for Cellular & Biomolecular Research, 164 College St., Room 407, Toronto, ON, M5S 3G9

The delivery of medical agents to a specific diseased tissue or cell is critical for diagnosing and treating patients. Nanomaterials can transport drugs, contrast agents, immunotherapies, and gene editors to diseased sites. However, less than 1% are delivered to solid tumours, impacting their use and clinical translation for cancer applications. In this presentation, I will discuss the challenge of delivering of medical agents and nanoparticles to solid tumours. The seminar will discuss the impact of nanoparticle design and biology on the in vivo transport process. It will finish with a discussion of strategies to overcome the delivery problem with nano-bio interaction studies, machine learning, and computational analysis.

Ji-Xin CHENG **Boston University**

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New Advances in Coherent Raman Scattering Microscopy

Ji-Xin Cheng[1]

[1]*Boston University, Photonics Center, 8 Saint Mary's Street, Boston, MA 02459*

Coherent Raman scattering (CRS) microscopy utilizing fingerprint vibrational spectroscopic signals opens a new window to map the chemical contents in living cells temporally and spatially. Such capacity opens a new window to visualize the orchestra of molecules and/or biological structures inside living systems. Cheng and his research team have been dedicated to pushing the boundary of coherent Raman scattering microscopy through developing novel instrumentation and data science approaches, discovering molecular signatures in diseases, and commercializing the technology for molecule-based precision diagnosis. Here, Cheng will present his team's most recent advances, including plasmonic CRS towards ultrasensitive chemical imaging[1], computational SRS microscopy to break the trade-off between speed and signal to noise ratio[2]; SRS-FISH to bridge single cell metabolism and identity[3]. Cheng will also present most recent applications towards precision medicine, including Rapid AST[4], drug action mechanism[5], and multi-color SRS histology. Finally, latest progress in miniaturization and commercialization of SRS microscope will be discussed.

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[1] Cheng Zong, Ranjith Premasiri, Haonan Lin, Yimin Huang, Chi Zhang, Chen Yang, Bin Ren, Larry Ziegler, and Ji-Xin Cheng*, "Plasmon-enhanced stimulated Raman scattering microscopy with single molecule sensitivity", Nature Communications, 2019, 10:5318.

[2] Haonan Lin, Hyeon Jeong Lee, Nathan Tague, Jean-Baptiste Lugagne, Cheng Zong, Fengyuan Deng, Tian Lei, Wilson Wong, Mary Dunlop and Ji-Xin Cheng*. "Fingerprint Spectroscopic SRS Imaging of Single Living Cells and Whole Brain by Ultrafast Tuning and Spatial-Spectral Learning", Nature Communications, 2021, 12:3052.

[3] Xiaowei Ge, Fatima C. Pereira, Matthias Mitteregger, David Berry, Meng Zhang, Michael Wagner, and Ji-Xin Cheng, SRS-FISH: high-throughput platform linking function to identity at single cell level", PNAS, 2022, in press.

[4] Zhang, M., Hong, W., Abutaleb, N. S., Li, J., Dong, P.-T., Zong, C., Wang, P., Seleem, M. N., Cheng, J.-X., Rapid Determination of Antimicrobial Susceptibility by Stimulated Raman Scattering Imaging of D_2O Metabolic Incorporation in a Single Bacterium. Advanced Science. 2020, 2001452

[5] Pu-Ting Dong, Cheng Zong, Zeina Dagher, Jie Hui, Junjie Li, Yuewei Zhan, Meng Zhang, Michael K. Mansour, Ji-Xin Cheng*, "Polarization-sensitive stimulated Raman scattering imaging resolves amphotericin B orientation in *Candida* membrane", Science Advances, 2021, 7: eabd5230.

Carl Emil ESKILDSEN *(He/Him)* **Imperial College London**

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Raman spectroscopy for biomedical applications

Carl Emil Eskildsen[1] and Molly M. Stevens[1]

[1]*Imperial College London, Faculty of Engineering, Department of Materials, The Stevens Group*

This presentation will focus on four examples, which provide an overview of the research conducted in the area of Raman spectroscopy for disease detection by The Stevens Group at the Imperial College London. (1) We will show how self-assembled monolayers (SAMs) are used to functionalize Au-nanopillar substrates for surface-enhanced Raman spectroscopy (SERS). We discriminate between lysed HS578T breast carcinoma cells and Hs578Bst normal fibroblast-like cells using SERS with multiple SAM-functionalized surfaces[1]. (2) We present the Single Particle Automated Raman Trapping Analysis (SPARTA) technology, which allows for high-throughput characterization of single nanoparticles[2]. We will demonstrate how the SPARTA technology can characterize the composition of extracellular vesicles and how this approach can be used in cancer diagnostics[3]. (3) We will show how confocal Raman spectroscopy is used for *In vivo* biomolecular imaging of zebrafish embryos (an important model organism) to visualize complex biological processes such as wound responses[4]. (4) Last, we will show a comprehensive framework for higher-throughput molecular imaging via deep-learning-enabled Raman spectroscopy, which can be applied to Raman imaging in biomedical sciences. The framework consists of denoising (resulting in higher signal-to-noise spectra) and a neural network for robust spatial super-resolution of hyperspectral Raman images, which preserve molecular cellular information. This framework speeds up Raman imagining, enabling good-quality cellular imaging with a high resolution and a high signal-to-noise ratio at a reduced time cost[5].

[1] *Nat. Commun.* 2020, 11, 207
[2] *Nat. Commun.* 2018, 9, 4256
[3] *ACS Nano*, 2021, 15, 18192-18205
[4] *Nat. Commun.* 2020, 11, 6172
[5] *Anal. Chem.* 2021, 93,15850-15860

Sanathana KONUGOLU VENKATA SEKAR
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Phantoms to fast-track device development and standardization in Biophotonics

Phantoms play a critical role in the testing, characterisation, calibration and development of Biophotonics devices. The recent success of translational research in Biophotonics emphasises the requirement for standardized tools to accelerate device development. In this talk, a wide range of phantoms that can fast-track device development from lab to clinic will be presented. An example of a standardized approach for performance assessment and quality-control of a biomedical medical device based on optical phantoms will be presented. This approach is tailored to meet the requirements of the Medical Device Regulation and is extendable to other biophotonics devices.

Bhavya SHARMA **University of Tennessee**

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Advances in Raman Spectroscopy for Neurochemical Sensing

Diagnosis of neurological disease is difficult, particularly in the early stages of disease progression, where patients present few clinical symptoms. Once symptoms are present, methods used for neurochemical sensing are often invasive, and require complex sample preparation steps and long collection times. For neurological diseases, such as Parkinson's Disease, the presence of symptoms indicates irreversible neurodegeneration, which results in pharmaceutical intervention being aimed towards managing symptoms, as opposed to treating or reversing neurodegeneration. There is an urgent need for the development of neurochemical sensing methods for early disease detection that are rapid, label-free, selective, and involve little to no sample preparation. Our group is developing Raman spectroscopic methods for *in vitro* and *in vivo* detection of neurochemicals. For both *in vitro* and *in vivo* detection, we utilize surface-enhanced Raman spectroscopic (SERS)-based nanoprobes. We are also driving the development and application of spatially-offset Raman spectroscopy (SORS) combined with SERS (SESORS) for non-invasive detection of neurochemicals in the brain. Here, I will discuss recent advances in SERS and SESORS for neurochemical detection.

Paola TARONI **Politecnico di Milano**

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Diffuse optical spectroscopy and imaging for breast cancer management

Paola Taroni[1]
[1]Politecnico di Milano, Department of Physics

Diffuse optics can be used for the non-invasive in-depth optical characterization of highly diffusive media, such as biological tissues. Operating in the time domain, picosecond light pulses are injected into the medium and the reemitted pulses are collected at a known distance. Interpreting the effects of photon migration in the medium with a suitable theoretical model (typically the diffusion approximation to the radiative transport theory), both absorption and scattering properties can be retrieved from a single measurement. When measurements are performed at several wavelengths in the red-near infrared spectral range, tissue composition (in terms of lipids, water and collagen concentrations) and physiological information (blood volume and oxygenation level) can be estimated from the absorption properties, while scattering provides information on the microscopic structure.

Broadband time domain diffuse optics will be introduced. Potential advantages and limitations for its use in *in vivo* diagnostics will be highlighted outlining, as a paradigmatic example, its application to breast cancer management, including:

I. Improvement of breast screening specificity (through the discrimination between benign and malignant lesions, based on optical assessment of tissue composition, which can also be complemented by information on morphology and stiffness obtained from ultrasound-based imaging).
II. estimate of breast density, strong independent risk factor for developing breast cancer. Currently, it becomes known typically only at the age of 50 (age of first mammography). Optical assessment can make the information available at any young age. Apart from potential preventive interventions, its knowledge would allow the design of earlier, more effective, and personalized breast cancer screening and monitoring.
III. monitoring and prediction of pathologic outcome of neo-adjuvant chemotherapy, which presently cannot be effectively achieved with conventional imaging techniques, while diffuse optics seems to be obtaining promising results.

ORAL PRESENTATIONS

Michelle BAILEY *(She/Her)* **University of Exeter**

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Brillouin spectroscopy as a probe of biomechanics

M. Bailey*[1], F. Palombo[1]

[1] *School of Physics and Astronomy, University of Exeter, UK*

KEY WORDS: Brillouin microscopy, Raman spectroscopy, micromechanics

Brillouin spectroscopy is an all-optical technique, providing information on micromechanics through the scattering of light from acoustic waves, or phonons. The mechanical properties within the biological environment are crucial to the health and vitality of the system, and alterations in mechanics can thereby indicate disease. Biological applications of Brillouin spectroscopy have ranged from the measurement of live cells[1] and organisms,[2] to tissues[3] and fibrous proteins,[4] demonstrating potential for diagnosis of pathology and characterisation of biomechanics.

Here, the application of Brillouin spectroscopy to probe the viscoelastic properties of a range of biologically relevant samples will be covered. These include tissue-mimicking hydrogels (Figure 1),[5] to elucidate the origin of Brillouin signals, and pathological changes in tissue mechanics.[6] The introduction of Raman spectroscopy as a correlative technique will also be discussed, which enables the chemical composition to be determined simultaneously to the mechanical properties probed by Brillouin spectroscopy, thus providing a comprehensive assessment of the sample.

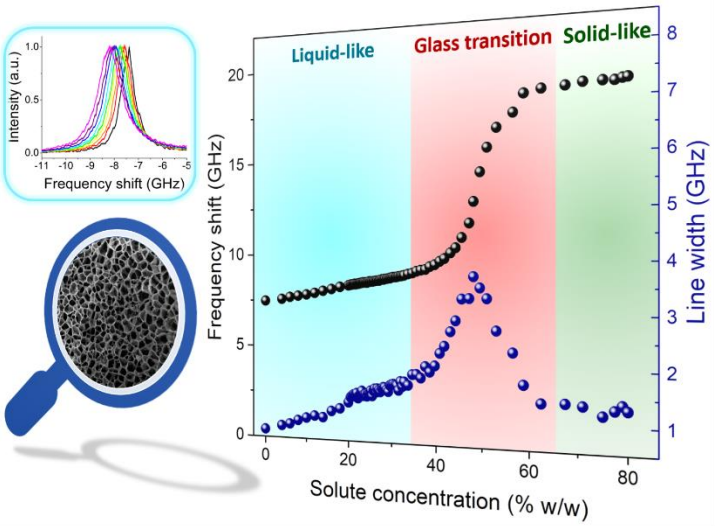

Figure 1: Glass-like transition as a function of solute concentration, observed through the evolution of Brillouin frequency shift and linewidth. Left-hand side: evolution of the Brillouin peak across a range of gel concentrations and cryo-SEM image (diameter ~85 μm, 400x, 5 kV) of a 4% gel.

[1] G. Scarcelli et al., Nat. Methods, 12(12), 1132-1134, (2015).
[2] I. Remer et al., Nat. Methods, 17(9), 913-916, (2020).
[3] G. Scarcelli & S. H. Yun, Opt. Express, 20(8), 9197-9202, (2012).
[4] F. Palombo et al., J. R. Soc. Interface, 11(101), 20140739, (2014).
[5] M. Bailey et al., Sci. Adv. 6(44), eabc1937, (2020).
[6] F. Palombo et al., J. Biophotonics, *9*(7), 694-700, (2016).

Diana FRIMPONG **University of Exeter**

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Demystifying NHS Ethics Applications

D. Frimpong[1,2]

[1]University of Exeter, College of Medicine and Health, Exeter, Devon EX1 2LU, United Kingdom; [2]University Hospitals Bristol and Weston NHS Trust, Bristol, BS2 8EG, United Kingdom

The NHS ethics application process can be (but need not be!) a daunting experience for all researchers, but particularly for first-time applicants. Besides the detailed application document, the coordination and feedback between institutions can often be frustratingly lengthy. This talk focuses on tips and tricks from a fellow early career researcher and their experience of applying for NHS ethics. We will discuss making the most of your local ethics and governance officers, the importance of a detailed study protocol, and understanding the role of the ethics committee.

Alexander GLASSMANN *(He/Him)*

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Enhanced Specific Drug Delivery Using Polyoma-Virus Deduced Engineered Protein Nanoparticles

Drug delivery using nanomaterials has attracted notice focus for biomedical applications. Expected benefits are improved tissue specificity and reduced side effects of the drugs. Comparing to artificial, chemical nanomaterial, nanoparticles based on viral capsid proteins (protein nanoparticles; PN) show the advantage of biocompatibility and biodegradability. While occupying the cell-penetrating features of the corresponding virus, these particles are the genome-free counterparts. As a convenience protein nanoparticle have got the intrinsic capability to build shells after recombinant expression and enable the incorporation of different payloads after *in vitro* dissociation and reassociation processes. To broaden the distinct cell tropism of PN which correspond to their parental virus, a wide range of genetic and chemical engineering methods have been established that allow the generation of engineered protein nanoparticles (EPN) with defined cell tropisms and new formulations to carry different payloads. Here, we describe the engineering and modification of protein nanoparticles based on the polyoma capsid proteins of polyoma-viruses.

Jennifer HASKELL *(She/Her)* **University of Exeter**

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High Wavenumber Raman Spectroscopy of Breast Tissue for Tumour Margin Assessment

Jennifer Haskell[1,2], Mr Thomas Hubbard[1,2], Dr Claire Murray[2], Dr Tanwen Wright[2], Miss Charlotte Ives[2,1], Mr Douglas Ferguson[2,1], Prof Pavel Matousek[3] and Prof Nick Stone[1,2]

[1]Physics and Astronomy College of Engineering, Mathematics and Physical Sciences, University of Exeter, Exeter, Devon, United Kingdom; [2]Royal Devon University Healthcare NHS Foundation Trust, Exeter, Devon, United Kingdom; [3]STFC Rutherford Appleton Laboratory, Didcot, Oxfordshire, United Kingdom.

Key Words: Breast cancer, High wavenumber Raman spectroscopy, Water

Surgery is the standard first line of treatment for breast cancer, and breast conserving surgery, unlike mastectomy, allows tumour removal while maintaining the majority of breast tissue. While this is aesthetically and psychologically beneficial for the patient, re-excision operations are often required due to positive tumour margins. To address this, there is the need for the development of an intraoperative margin assessment tool, which would indicate any positive margins during the first operation, allowing their removal there and then. This work aims to investigate the ability of high wavenumber Raman spectroscopy of tissue water content to discriminate tumour vs non-tumour breast tissue as a basis for the development of such a tool[1] [2].

A Raman probe was used to measure mastectomy specimens from 30 patients. To allow direct access to tumour tissue, each specimen was sliced open by the surgeon to bisect the tumour. Point measurements were taken on 'tumour' and 'normal' tissue and indicated accordingly for confirmation by pathology. A further line of point measurements were taken along the length of the specimen slice. Spectra from tumour tissue showed significantly higher water content than spectra from normal tissue, and inputting the data into a multivariate PCA-LDA model achieves 75.0 % sensitivity and 94.6% specificity. This indicates that a high wavenumber Raman is capable of discerning between normal and tumour breast tissue, and highlights the potential of this method for further development into an intraoperative margin assessment tool.

[1] T. Hubbard, et al., Analyst, 2019, 114, 6479-6496

[2] E. Barroso, et al., Anal. Chem., 2015, 87, 2419–2426

Wenjing HU **King's College London**

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Soft templating of copper-based nanoparticles for photothermal and photodynamic therapy

Wenjing Hu*[1], Mark. Green[2], Cecile. Dreiss[1]

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KEY WORDS: Copper sulfide nanoparticles, soft templating, photo-therapy

Copper sulfide nanoparticles ($Cu_{2-x}S$ NPs) have aroused researchers' interest for applications in photodynamic/photothermal therapy, because of their low toxicity and their absorption in the near-infrared (NIR) region caused by the localized surface plasmon resonance effect (LSPR). We have adopted a "soft templating" approach to synthesize and assemble $Cu_{2-x}S$ NPs, where we grow NPs within the structures of flexible, elongated surfactant aggregates, namely, wormlike micelles (WLM). Through in-situ synthesis, the Cu2-xS NPs generated are effectively organized into long filaments, achieving a structural transcription from soft templates to well-defined nanostructures.

We use $CuCl_2$ ions as the metal precursor, cetyltrimethylammonium bromide (CTAB) as the surfactant, and thiourea (Tu) as the sulfur source[1]. Sodium thiosalicylate is used as a co-surfactant, which, by screening the repulsive electrostatic interactions between the CTAB headgroups, effectively induces micellar elongation into WLM.

The resulting constructs have been fully characterised. UV spectroscopy measurements confirm a maximum absorption in the NIR region. Transmission electron microscopy (TEM), and atomic force microscopy (AFM) images show that the NPs synthesized are assembled into elongated aggregates, reminiscent of wormlike micelles. X-ray powder diffraction (XRD) measurements confirm the existence of covellite (CuS). The CuS NPs generated exhibit excellent photothermal conversion efficiency. They can also produce reactive oxygen species, which makes them promising candidates for photothermal and photodynamic therapy.

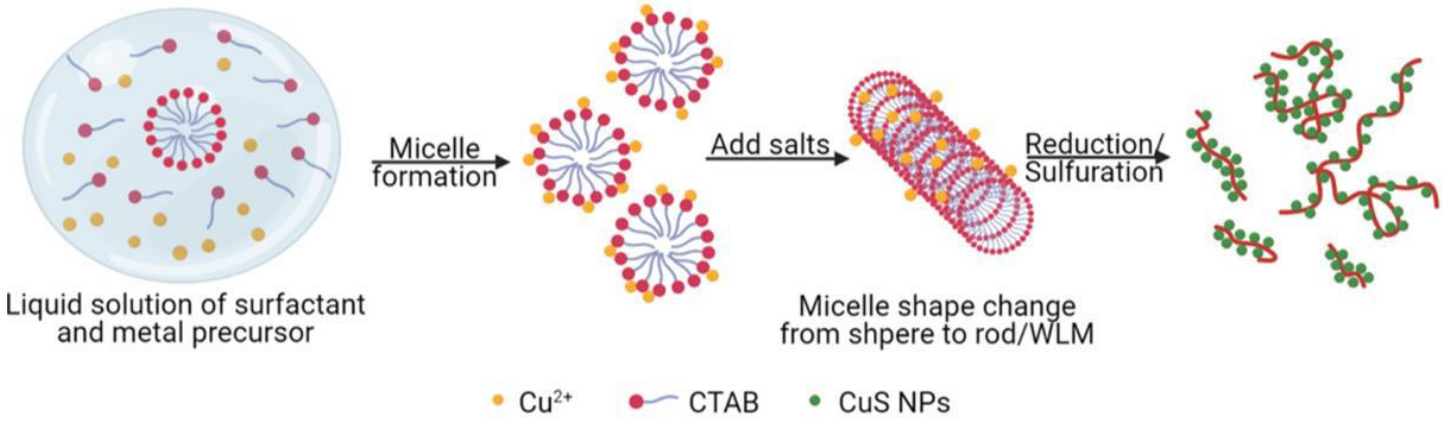

Fig. 1 Scheme of soft templating

[1] Y. Zhou and H. Zeng. J. Am. Chem Soc., 136 (2014) 13805-13817.

Martin LEE *(He/Him)* **University of Edinburgh**

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Alkyne tagging as a strategy to image nanoparticles in vitro and ex-vivo by stimulated Raman scattering.

Martin Lee*[1], Sally Vanden-Hehir[2], Manasa Punaha-Ravindra[2], Stegan A Cairns[2], Lida Zoupi[3], Michael P Shaver[2], Valerie G Brunton[1], Anna Williams[3], Alison N Hulme[2].

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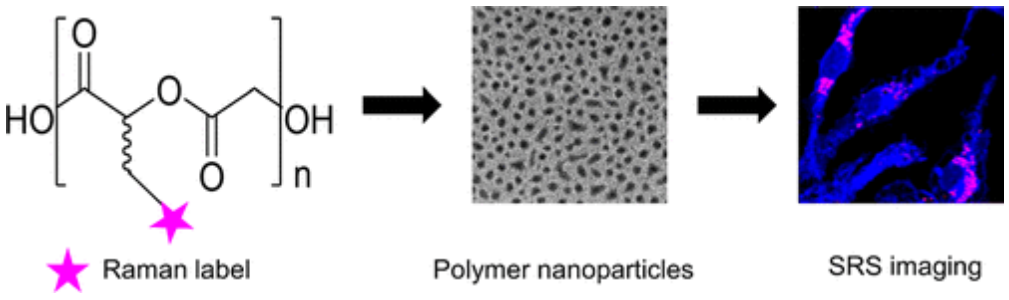
[2]*EaStCHEM School of Chemistry, University of Edinburgh, David Brewster Road, Edinburgh, EH9 3FJ, United Kingdom*

[3]*MRC Centre for Regenerative Medicine, The University of Edinburgh, 5, Little France Drive, Edinburgh, EH16 4UU, United Kingdom*

KEY WORDS: Nanoparticles, Stimulated Raman Scattering, Microglia

Polymeric nanoparticles (NPs) are attractive candidates for the controlled and targeted delivery of therapeutics *in vitro* and *in vivo*. However, detailed understanding of the uptake, location, and ultimate cellular fate of the NPs is necessary to satisfy safety concerns, which is difficult because of the nanoscale size of these carriers. In this work, we show how small chemical labels can be appended to poly(lactic acid-co-glycolic acid) (PLGA) to synthesize NPs that can then be imaged by stimulated Raman scattering microscopy, a vibrational imaging technique that can elucidate bond-specific information in biological environments, such as the identification of alkyne signatures in modified PLGA terpolymers. We show that both deuterium and alkyne labeled NPs can be imaged within primary rat microglia, and the alkyne NPs can also be imaged in *ex vivo* cortical mouse brain tissue. Immunohistochemical analysis confirms that the NPs localize in microglia in the mouse brain tissue, demonstrating that these NPs have the potential to deliver therapeutics selectively to microglia[1].

We further look at strategies to fine tune alkyne Raman peaks using ring substitutions on to an aryl capped diyne motif to shift the alkyne stretch frequency potentially allowing for multiplex imaging studies to be conducted.



Polymeric nanoparticles can be labelled with small alkyne additions allowing for imaging the uptake and localization with stimulated Raman scattering

[1] S. Vanden-Hehir, S. A. Cairns, M. Lee, L. Zoupi, M. P. Shaver, V. G. Brunton, A. Williams, and A. N. Hulme, *Biomacromolecules* **20** (2019), 4008-4014. DOI: 10.1021/acs.biomac.9b01092.

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Point-of-Care Diagnostics for Underserved Populations

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In 2017, the Precise Advanced Technologies and Health Systems for Underserved Populations (PATHS-UP) Engineering Research Center (ERC), funded by the National Science Foundation (NSF), was founded at Texas A&M university in collaboration with partners from the University of California at Los Angeles, Rice University, and Florida International University. The vision of the PATHS-UP ERC is to change the paradigm for the health of underserved populations by developing revolutionary and cost-effective technologies and systems at the point-of-care. Research amongst the partners is centered on creating technologies designed to help with chronic diseases, such as diabetes and cardiovascular disease, which are leading causes of morbidity and mortality worldwide but also cause a disproportionate burden amongst underserved communities in the United States. During my talk, I will give a more detailed overview of the center, its structure, and its translational efforts. I will also discuss how my group's research integrates with the center's mission.

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Comparison of resonant and non-resonant reporter for the selection of brightest gold nanoparticles for Surface-enhanced Raman spectroscopy

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KEY WORDS: surface-enhanced Raman spectroscopy, surface-enhanced resonance Raman scattering, multiplexed imaging.

The choice of Raman reporter is a significant aspect for improving the imaging sensitivity and multiplexing capabilities of SERS nanoparticles. In this study, we have investigated resonant reporters, IR 125, IR-820 and two non-resonant reporters (2-bi-(4-pyridyl) ethylene and 4-mercaptobenzoic acid (MBCN) bound to gold nanoparticles of different morphologies – nanospheres and nano-raspberries. We have synthesised negative surface charged gold nano-raspberries (AuNRBs) using green chemistry method[1] of reduction of gold ion by 2-[4-(2-hydroxyethyl)-1-piperazyl] ethane sulfonic acid (HEPES) and positive surface charged AuNRBs using chitosan[2] as a stabilising and capping agent. Both methods were carried out limiting the need for extensive post-synthesis routines of biofunctionalization to improve sensitivity. The appropriate reporter concentration, and volume ratio of reporter to nanoparticle concentration parameters were analysed to provide the valuable assessment of the reporter molecule that gives maximum SERS enhancement. We have used 785 nm laser excitation wavelengths to find the brightest 'Raman reporter – gold nanoparticle' combination for further multiplexed imaging. We have demonstrated that negatively charged AuNRBs provide significant SERS enhancement with better sensitivity for Raman resonant reporters due to strong label binding affinity of dye to gold surface as compared to non-resonant dyes. It also explains inherently stronger signals generated by surface-enhanced resonance Raman scattering (SERRS), as opposed to surface-enhanced Raman scattering (SERS). These simple, scalable and tunable size AuNRBs are excellent candidates for predicting which Raman reporters could improve sensitivity and be used for multiplexed imaging.

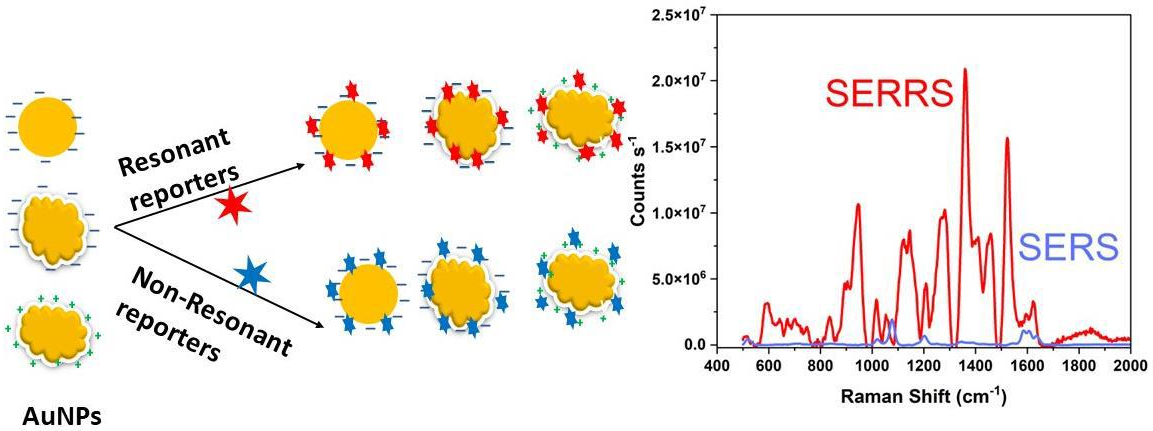

Figure 1. Schematic representation of different morphology gold nanoparticles after tagging with resonant and non-resonant reporter showing strong SERRS enhancement than SERS.

[1] J. Johnston, E. Taylor, R. Gilbert, and T. Webster, *Int J Nanomedicine,* **11** (2016) 45-53.

[2] N. Gandra, C. Portz, S. Z. Nergiz, A. Fales, T. Vo-Dinh, and S. Singamaneni, *Scientific reports,* **5** (2015), 10311

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Development of Bio-Functionalized, Raman Responsive, Ultrasmall-in-Nano Gold Constructs

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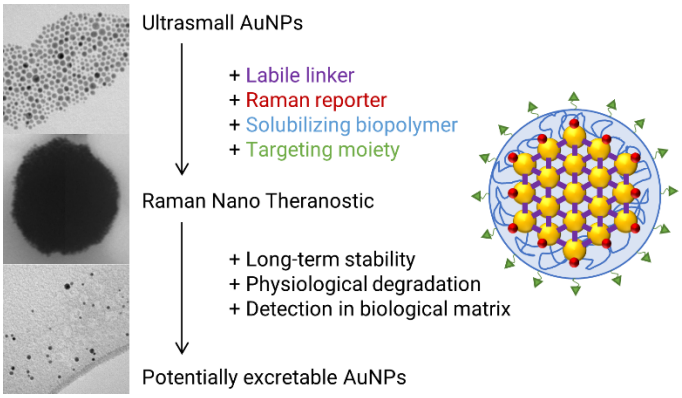
Gold nanoparticles (AuNPs) are used experimentally for non-invasive *in vivo* Raman monitoring because they show a strong absorbance in the phototherapeutic window (650–850 nm), a feature that is accompanied by a particle size in excess of 100 nm. However, these AuNPs cannot be used clinically because they are likely to persist in mammalian systems and resist excretion. Raman Nano Theranostic (RaNT) constructs aim to address these issues by reversibly clustering excretable ultrasmall AuNPs (sub-5 nm) to a size range where particle's optical properties become tuned for non-invasive *in vivo* detection and monitoring. Labelling these nano particles with a biphenyl-4-thiol (BPT) Raman tag allows for a specific Raman response in plasma. Further encapsulation within a chitosan derived amphiphilic polymer affords a plethora of potential bio-functionalization including PEGylation for extended circulation, and aptamer-labelling for tumor targeted delivery.



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Estimating the depth of inclusion and the optical properties of biological tissues using Spatially Offset Raman Spectroscopy

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KEY WORDS: SORS; Turbid media; Non-invasive target detection.

In a clinical context, it is beneficial to identify physical-chemical information and the depth of a buried object in biological tissues. Spatially offset Raman spectroscopy (SORS) allows the chemical characterisation of biological tissues at depths. Here we propose a new method for estimating the depth of inclusion within turbid media (e.g ex vivo. biological tissues) using SORS with the aid of external calibration data only. This new approach facilitates a fully non-invasive methodology potentially applicable for *in vivo* medical diagnosis without any *a priori* knowledge of the sample.

The concept of depth prediction is based on relative changes in Raman band intensities of the inclusion that are directly related to the pathlength of Raman photons travelling through the medium, thereby encoding information on the depth of the inclusion[1,2]. Monte Carlo simulations of photon propagation were used to gain an insight into the relationship between the spatial offset and the photon pathlengths inside different tissues, enabling one to derive a general scaling factor to be used in SORS measurements for depth prediction. The approach was validated by predicting the depth of surface-enhanced Raman scattering (SERS) labelled nanoparticles (NPs) acting as an inclusion inside ex vivo porcine tissue with an average root mean square error of prediction of 7.3 % of the overall tissue thickness[3].

These results pave the way for future non-invasive deep Raman spectroscopy *in vivo* by enabling, for example, the localisation of cancer lesions or biomarkers for diagnosis and targeted treatment.

[1] Mosca, S.; Dey, P.; Tabish, T. A.; Palombo, F.; Stone, N.; Matousek, P. Anal. Chem. 2019, 91 (14), 8994
[2] Mosca, S.; Dey, P.; Tabish, T. A.; Palombo, F.; Stone, N.; Matousek, P. J. Biophotonics 2020, 13 (1), 1–7.
[3] Mosca, S.; Dey, P.; Salimi, M.; Palombo, F.; Stone, N.; Matousek, P. Analyst 2020, 145 (23), 7623–7629.

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Biodegradable Harmonophores for Targeted High-Resolution *In Vivo* Tumour Imaging

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KEY WORDS: biodegradable nonlinear imaging probe, self-assembling peptides, cancer detection

Optical imaging probes have played a major role in detecting and monitoring a variety of diseases. In particular, nonlinear optical imaging probes, such as second harmonic generating (SHG) nanoprobes, hold great promise as clinical contrast agents, as they can be imaged with little background signal and unmatched long-term photostability[1]. As their chemical composition often includes transition metals, the use of inorganic SHG nanoprobes can raise long-term health concerns. Ideally, contrast agents for biomedical applications should be degraded *in vivo* without any long-term toxicological consequences to the organism. Here, we developed biodegradable Harmonophores (bioharmonophores) that consist of polymerencapsulated, self-assembling peptides that generate a strong SHG signal[2]. When functionalised with tumour cell surface markers, these reporters can target single cancer cells with high detection sensitivity in zebrafish embryos *in* vivo (**Fig. 1**). Thus, bioharmonophores will enable an innovative approach to cancer treatment using targeted high-resolution optical imaging for diagnostics and therapy.

[1] Pantazis, P., Maloney, J., Wu, D. & Fraser, S. E. Second harmonic generating (SHG) nanoprobes for in vivo imaging. Proc Natl Acad Sci U S A 107, 14535-14540, doi:10.1073/pnas.1004748107 (2010).

[2] Sonay, A. Y. et al. Biodegradable Harmonophores for Targeted High-Resolution In Vivo Tumor Imaging. ACS Nano, doi:10.1021/acsnano.0c10634 (2021).

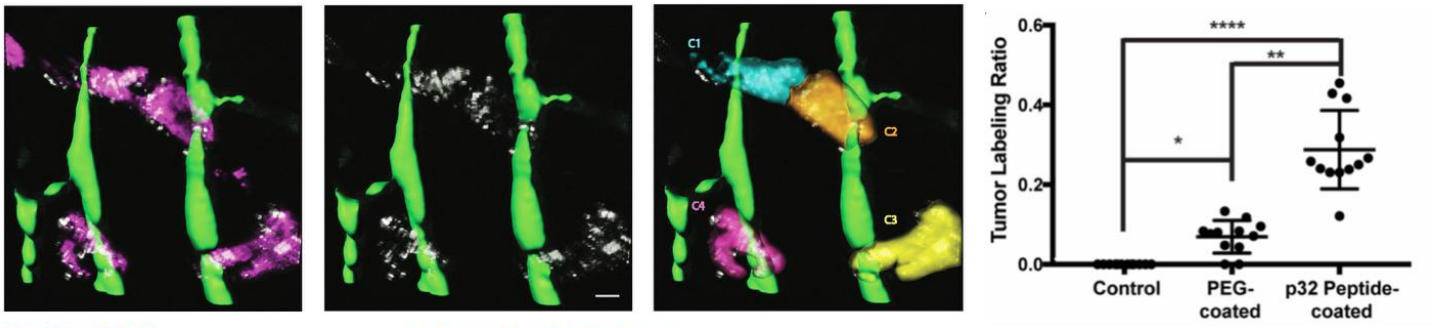

Figure 1: Bioharmonophores can be specifically targeted to single cancer cells *in vivo.* (left) Closeup image of one of the tumour site reveals DsRed-labelled single cancer cells (magenta), adjacent to the eGFP-labelled vasculature (green) and labelled with targeted bioharmonophores (white). Note that cellular bioharmonophore distribution can in most cases predict cancer cell morphologies. Scale bar, 15 μm. (**right**) Quantification of the fraction of SHG-labelled tumours as the ratio of labelled tumours to all tumours in a given zebrafish embryo after PEG- and p32 peptide-coated bioharmonophore injection, respectively. Each data point signifies one zebrafish. Note that active targeting with p32-coated bioharmonophores significantly increases the labeling efficiency (approximately 4-fold). Mean ± s.d. ****, $P < 0.0001$, **, $P = 0.0063$, *, $P = 0.0470$ (nonparametric Kruskal–Wallis test with Dunn's post hoc multiple comparison). $N = 12$, pooled from 3 independent experiments.

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Post-publication peer review of publications reporting nanoparticle-based SERS intracellular sensing

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KEY WORDS: Post-publication peer review, SERS, intracellular imaging.

The scientific literature includes errors. Although science is supposed to be self-correcting, in practice misconceptions can persist for many years. The NanoBubbles project funded by the European Research Council promotes the synergy of engineering, philosophy and history of science, nanoscience, sociology and linguistics to study "how, when and why science fails to correct itself.[1]" Part of this initiative will involve the replication of experiments that rely on the ability of nanoparticles to cross the cell membrane or somehow reach the cell cytosol. NanoBubbles also involves the post-publication peer review of published articles following specific evaluation criteria.[2] We write reviews of published articles and we post them on the website PubPeer.com thus enabling authors and other interested scientists to join the discussion. Some of the articles annotated so far report nanoparticle-based SERS intracellular sensing. In this presentation, we will present the first results of this annotation project and invite early-career researchers (and others) to join us in this community approach, which we hope could contribute to accelerate the correction of errors, and help identify sound methodology for imaging and sensing inside cells.

[1] "NanoBubbles – ERC Synergy Grant project". https://nanobubbles.hypotheses.org/ (accessed on August 9th, 2022).

[2] NanoBubbles website, "What are our evaluation criteria?, https://nanobubbles.hypotheses.org/post-publication-peer-review/what-are-our-evaluation-criteria". https://nanobubbles.hypotheses.org/post-publication-peer-review/what-are-our-evaluation-criteria (accessed on August 9th, 2022).

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Fingerprinting of (Cancer) Cells using Integrated Nanophotonics and Plasmonic Nanopore Sensing

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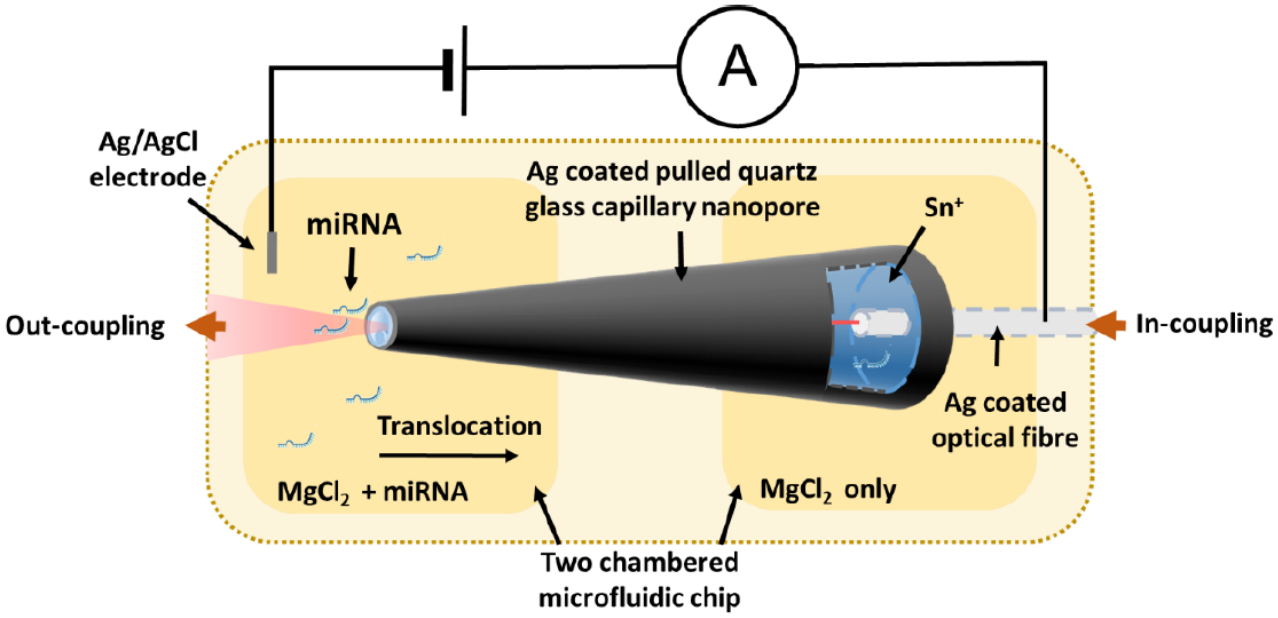
KEY WORDS: SERS, nanopore sensing, early-stage cancer detection.

MiRNAs are a powerful biomarker for early-stage cancer detection as they are widely implicated in the molecular biology of neoplasia. Since they are tissue and cell lineage specific they may enable classification of cancer into prognostic groups[1]. However, various challenges for their detection exist, such as their size, homogeneity and the presence of pre-miRNAs requiring both sensitivity and selectivity[2]. Among current methods there is typically a trade-off between quantification ability, sensitivity, specificity and ability to detect novel miRNAs. Emerging technologies based on plasmonic nanopores show potential in overcoming this trade-off. However, the geometry and fabrication methodology must be carefully chosen to maximise reproducibility, lifetime of device and field enhancement.

Here we propose a biomedical platform for the simultaneous electro-optical detection of cancer biomarkers for diagnostics. Combining photonics, optofluidics and nanopore sensing - the proposed nanophotonic sensor is grounded on the translocation of miRNA molecules through nanopores and their electro-optical detection and identification. The aim is to extend nanopore sensing methodologies beyond their label-free, single-molecule and ultra-low sensing capabilities, to achieve chemical specificity and molecular fingerprinting ability using Surface Enhanced Raman Spectroscopy (SERS).



Proposed sensing platform to enable simultaneous SERS and ionic nanopore measurements.

[1] Lu, J. et al., 2005. Nature, 435(7043)

[2] de Planell-Saguer, M. and Rodicio, M., 2013. Clinical Biochemistry, 46(10-11).

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Kinetically arrested colloidally-stable plasmonic assemblies

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KEY WORDS: self-assembly, Au NPs, kinetic arrest, thermosensitive polymers, SERS

Colloidally stable gold nanoparticle (AuNP) aggregates are of great interest of nanophotonics, (photo)catalysis as well as sensing and nanotheranostics. However, colloidal stabilization of NP aggregates remains challenging as, once induced, self-assembly usually continues until the nanoparticulate components are consumed, yielding crystals, complex solids or amorphous precipitates. Formation of these terminal thermodynamic structures can be prevented by addition of chemical entities adhering to interfaces of emerging metastable aggregates, leading to colloidally-stable kinetically arrested phases[1].

Herein, we demonstrate a synthetic strategy leading to colloidally-stable AuNP aggregates that can be both (i) disassembled on demand using selected small molecules or (ii) further assembled into larger aggregates by gentle heating (Figure 1). In the first system, thiol terminated polyethylene glycol (PEG-SH) chains (0.5-6.0 kDa) act as effective assembly modulators in cucurbit[7]uril (CB[7])-triggered aggregation of AuNP (14–80 nm) through kinetic arrest, allowing the rapid formation (within seconds) of colloidally stable hybrid aggregates. The resultant hybrids can be further disassembled by using physiological concentrations of chemical entities being components of extra- and intracellular fluids, *i.e.* cystine, cysteine, gluthatione. The life-time of these aggregates is controlled by both number and length of the PEG-SH chains used for the structures stabilisation. In the second approach, the thermosensitive poly(N-vinylcaprolactam) (PNVCL) is added to the pre-assembled CB[7]-AuNP cluster. Once heated (40-50 °C), the initial hydrate polymer chains collapse and aggregate, promoting further assembly of the preformed AuNPs aggregates with specific sizes. Both of the demonstrated hybrid colloids efficiently harvest small molecules from a surrounding aqueous environment, which can be then detected through the incorporated surface-enhanced Raman pectroscopy–active plasmonic compartments. This strategy paves the way for widespread use of analogous hybrids in sensing and theranostics, where they first can be delivered to a target location, used for diagnostic and treatment and finally disassembled on demand to potentially extractable individual nanoparticulate units.

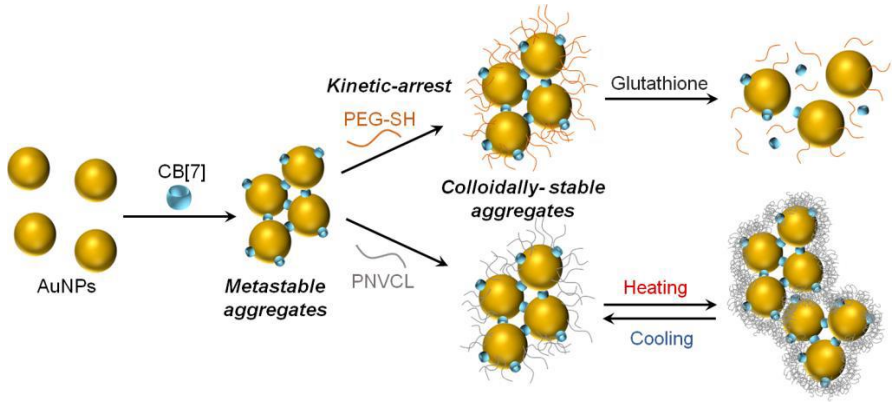

Figure 1. Synthetic strategy leading to colloidally-stable AuNP aggregates.

[1] Sokołowski, K., Huang, J., Földes, T., McCune, J.A., Xu, D.D., de Nijs, B., Chikkaraddy, R., Collins, S.M., Rosta, E., Baumberg, J.J. and Scherman, O.A. *Nature Nanotechnology*, **16**(10), (2022), 1121.

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Surface-enhanced Raman scattering (SERS) micro-sensors for pH measurements in patient-derived airway organoids

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KEY WORDS: SERS, organoids, pH

We applied SERS micro-sensors to probe pH in the lumen and extracellular matrix (ECM) of airway organoids (AOs). The lumen of AOs model the surface of the airway epithelium and present an opportunity to study the (hotly debated) role of pH in the respiratory disease cystic fibrosis[1]. However, because the lumen of AOs is low-volume and enclosed by epithelial cells its pH is rarely sampled. We developed surface-enhanced Raman scattering micro-sensors (SERS-MS) (Figure 1.A) and explored their application as pH sensors for the lumen of patient-derived AOs. SERS-MS were fabricated by assembling monolayers of gold nanoparticles on polystyrene micro-spheres, which were then functionalised with pH-sensitive reporter molecule 4-mercaptobenzoic acid (MBA). We added SERS-MS to AO cultures grown from non-cystic fibrosis donors and collected optical pH measurements by analysing pH-dependent changes in the SERS spectrum of MBA[2]. We characterised how the pH sensitivity of SERS-MS changed in hydrogels and used optical pH measurements to track the metabolic activity of AOs. Using the high spatial accuracy of SERS-MS pH measurements, we found the metabolic activity of AOs established extracellular pH gradients in the AO cultures. Finally, we observed that some SERS-MS were spontaneously engulfed by AOs and resided in the organoid lumen (Figure 1.B). This allowed us to measure pH in the AO lumen without the need for intrusive probe microinjection[3]. This work laid the foundation for studying lumen pH in cystic fibrosis AOs and could also be applied to monitor lumen pH temporally following drug exposure.

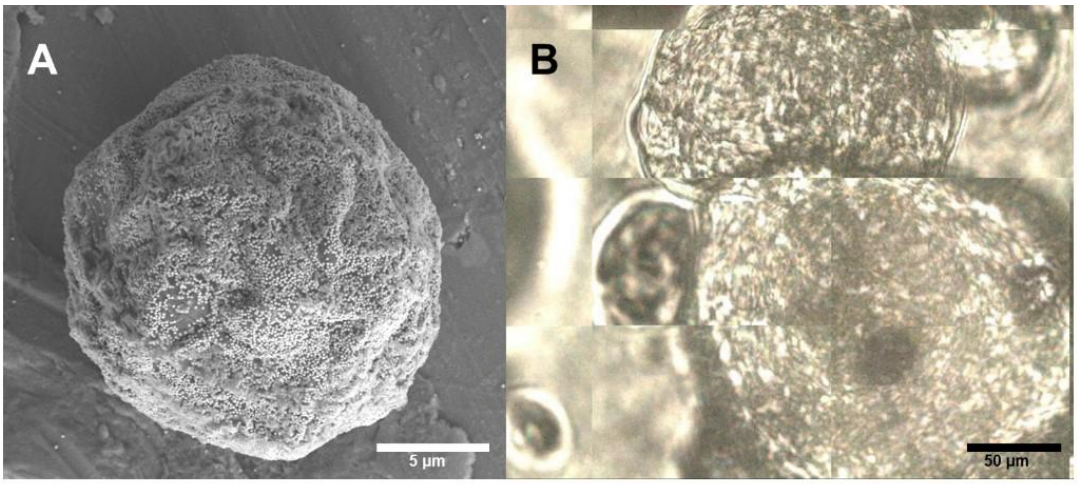

Figure 1: A) SEM image of SERS-MS. B) SERS-MS in the lumen of an AO.

[1] A. Schultz, R. Puvvadi, S.M. Borisov et al., *Nat Commun*, **8** (2017) 1409.
[2] S.W. Bishnoi, C.J. Rozell, C.S. Levin, M.K. Gheith, B.R. Johnson, D.H. Johnson, and N.J. Halas, *Nano Lett.*, ***6*** (2006) 1687-1692.
[3] K. W. McCracken, E. Aihara, B. Martin, C. M. Crawford, T. Broda, J. Treguier, X. Zhang, J. M. Shannon, M. H. Montrose and J. M. Wells, *Nature*, **541** (2017) 182-187.

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Four Wave Mixing imaging for the detection of gold nanoparticle internalisation into living cells at the single particle level

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KEY WORDS: Four-wave mixing, gold nanoparticles, imaging, live cells, endocytosis

Metallic nanoparticles are promising targeting and labelling agents in the research fields of drug delivery and biomedical detection. To further their use in nanotherapeutic applications, it is important to understand the internalisation processes they induce upon interaction with living cells at the single particle level. Gold nanoparticles (GNPs) can be used as labels and/or drug delivery vehicles owing to their linear as well as nonlinear responses to electromagnetic waves. Previously, direct imaging of GNPs was only possible when conjugated with a fluorescent label or by using sufficiently large particles to detect them via absorption/scattering of incident light.

In our group, we have recently developed transient resonant Four Wave Mixing microscopy (FWM), a coherent nonlinear optical microscopy technique that allows the detection of GNPs in complex conditions as found in cells and tissues, without suffering from background originating from autofluorescence or other scatterers present in the focal volume. Due to the nonlinearity of the GNP's response to multiphoton excitation, low excitation powers (<100 kW/cm^2) are possible while still returning a high signal to noise image without harming live cells[1, 2, 3].

In this work, we show the capability of our FWM set up to detect individual 20 nm diameter GNPs functionalised with transferrin, in live HeLa cells as they are being internalised. We show binding of the GNP and their internalisation into the cell to reveal new possible strategies to bring forward a new class of targeted nanotherapeutic agents.

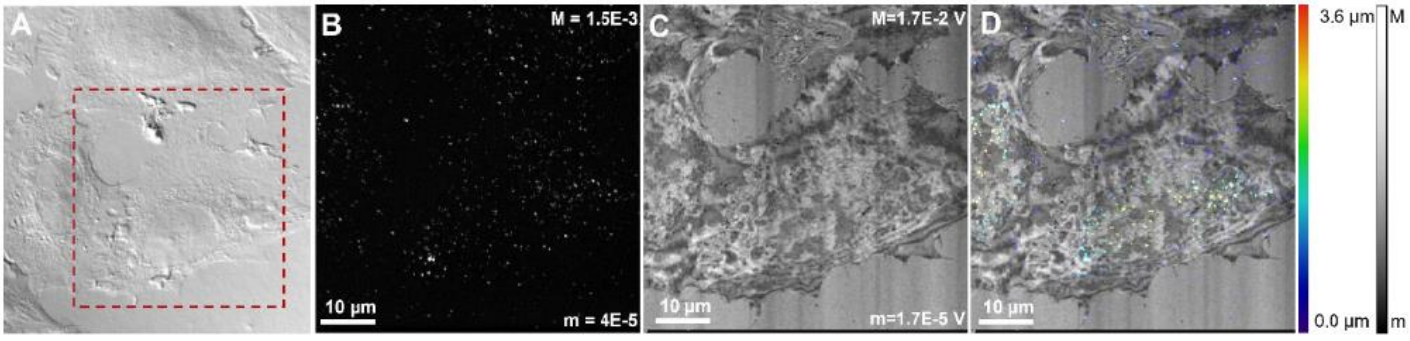

Figure 1 FWM imaging in fixed HeLas. (A) DIC image of HeLa cells. **(B)** FWM field amplitude as maximum intensity projection over an axial stack of 3.6 µm. **(C)** Amplitude of the reflected probe field. **(D)** Hyperspectral merge of C (grey) and B (spectral).

[1] F. Masia et al., Opt. Lett. 34, 1816 (2009)
[2] G. Zoriniants et al., Phys. Rev. X 7, 041022 (2017)
[3] N. Giannakopoulou et al., Nanoscale, 12, 4622-4635 (2020)

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Standardisation for stimulated Raman scattering microscopy applications

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KEY WORDS: Metrology, measurement standardization, Stimulated Raman scattering

Molecular imaging for digital pathology is a rising field that is encapsulating several techniques that can be acting in corroborating or complementary manner. Data reproducibility and consistent inter and intra-instrument response are fundamental for their exploitation in digital pathology. Recently diagnostic tools based on coherent Raman scattering microscopies have been developed. Their extensive utilization in the biomedical field is due to their ability to generate 3D label-free chemical images in a non-destructive and minimally invasive manner. Stimulated Raman scattering offers the possibility of chemical quantification since the Raman signal intensity is linearly proportional to the concentration of molecules excited.

However, data reproducibility and measurement robustness rely on several hardware parameters that may fluctuate (for instance the laser source stability, detector response and sensitivity, settings on lock-in amplifier etc) and affect the reproducibility of a quantitative measurement. Elucidation of quantitative data can also be compromised by the presence of parasitic signals, which are commonly observed for materials containing strong absorbers, such as metallic particles or pigments. In addition, for larger 3D specimens, corrections are needed to account for signal attenuation with depth due to scattering and absorption.

To increase reproducibility, data transfer and comparability across different platforms (commercial and custom-made systems) calibration samples are proposed as references to allow enable data harmonization.

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Molecularly Imprinted Polymers: Nanoscale Biomimetics

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KEY WORDS: Sensing, Molecular Imprinting, Labelling

Molecular Imprinting (MI) is a technique which allows for the creation of target specific recognition sites in a wide range of materials and formats. Capable of specifically targeting anything from cells and proteins to small bioactive molecules these "artificial antibodies" have shown great promise in a number of fields, including biotechnology, pharmacy, chemistry and environmental science. As such, MI has garnered significant interest in the past few years with a near exponential growth in published work.

In this short presentation Professor Turner will give a brief overview of the technique, and describe the current work in his lab, developing novel imprinted nanoparticle materials. These free-standing nanoparticles, generated using a solid phase method, can be used for sensing[1], labelling[2], or actively modulating protein activity[3].

The potential for these materials to be used for imaging, or as therapeutic carriers will be discussed.

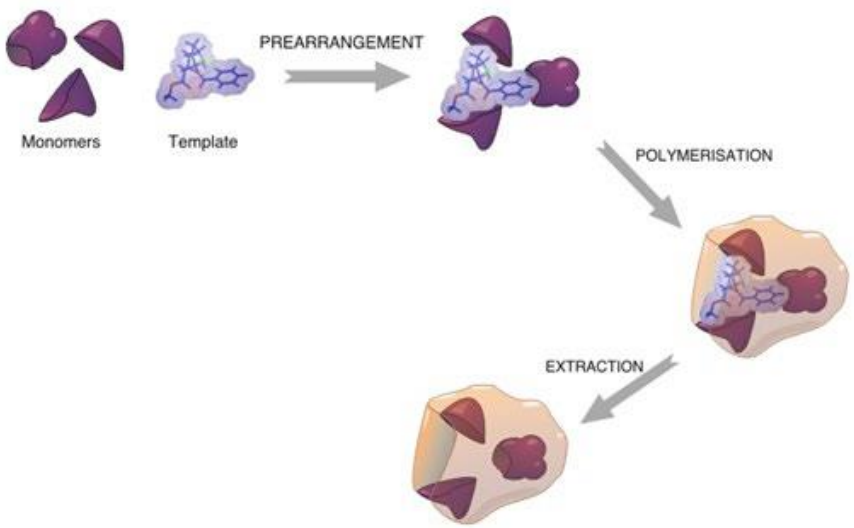

Figure 1: Basic Principle of Molecular Imprinting. A target (template) is introduced to selected monomers that complex around the template. This complex is entrapped within a polymer matrix through a simple polymerisation reaction. Subsequent removal of the template leaves a binding pocket akin to the recognition site of a receptor or enzyme, one that is sterically and chemically complimentary to the original template.

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[1] Sullivan, M., Henderson, A., Hand, R.A., & **Turner N.W.** (2022) nanoMIP based Surface Plasmon Resonance sensing for Antibiotics in Milk and River Water – *Analytical and Bioanalytical Chemistry* – DOI: 10.1007/s00216-022-04012-8

[2] Garnier, M., Sabbah, M, Menager, C. & Griffete, N. (2021) Hybrid Molecularly Imprinted Polymers: The Future of Nanomedicine? *Nanomaterials* – DOI:10.3390/nano11113091

[3] Piletsky, S., Bedwell, T.S., Paoletti, R., Karim, K., Canfarotta, F., Norman, R., Jones, D.J.L., **Turner, N.W.** & Piletska, E.V. (2022) Modulation of acetylcholineesterase using molecularly imprinted polymer nanoparticles. *Journal of Materials Chemistry B* – DOI: 10.1039/d2tb00278g

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Total mass balance of gold nanomaterials *in vivo*

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KEY WORDS: gold, total dose, *in vivo*

While gold nanomaterials are of great interest in nanomedicine, one important aspect to address when considering their translation to the clinic is their biocompatibility. While gold (0) is considered chemically inert, some reports have identified and quantified reactivity of gold in biological matrices[1]. The use of such materials for the targeting of solid tumours has significant promise if the challenge of low accumulation in tumours[2] could be overcome. To date, the lack of accessible bioanalytical methods and robust methodology has limited the availability of comparable quantitative results between studies. Furthermore, while the impact of dose on nanoparticle distribution has been demonstrated[3], most studies do not account for total doses administered. Given the significant amount of gold that may be distributed off target and the potential interactions this may have in organisms, this may also severely impede the success of gold nanomedicines in the clinic; a quantitative reproducible workflow for the *in vivo* total mass balance of gold would help to address these challenges. In this talk, we discuss the limitations of quantitative gold nanoparticle distributions and present our efforts in overcoming these using a fully validated Inductively Coupled Plasma-Mass Spectrometry bioanalytical method as well as additional methodological improvements and tools with the goal to account for the entire administered dose in animal models. We offer examples of total mass balance of gold nanomaterials routinely used in the nanomedicine field and demonstrate the replicability of such studies, as well as preliminary results for the biodistribution of Raman Nanotheranostics (RaNT) relevant nano-constructs.

[1] A. Balfourier, N. Luciani, G. Wang, G. Lelong, O. Ersen, A. Khelfa, D. Alloyeau, F. Gazeau, and F. Carn, *Proc. Natl. Acad. Sci. U. S. A.*, 2020, *117*, 103.
[2] S. Wilhelm, A. J. Tavares, Q. Dai, S. Ohta, J. Audet, H. F. Dvorak, and W. C. W. Chan, *Nature Reviews Materials*, 2016, *1*, 1.
[3] B. Ouyang, W. Poon, Y.-N. Zhang, Z. P. Lin, B. R. Kingston, A. J. Tavares, Y. Zhang, J. Chen, M. S. Valic, A. M. Syed, P. MacMillan, J. Couture-Senécal, G. Zheng, and W. C. W. Chan, *Nat. Mater.*, 2020, *19*, 1362.

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Classification pipeline for *in vivo* Raman spectra of colorectal tissue for cancer detection

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KEY WORDS: colorectal cancer, in-vivo, Raman spectroscopy

Colorectal cancer is amongst the leading causes of death in oncological patients[1], with its diagnosis usually involving biopsy. *In vivo* Raman spectroscopy, a less invasive examination method, has shown great potential to discriminate between normal and cancerous tissue[2]. However, the complex, often manual, processing of Raman spectra and the absence of a suitable instant classifier are the main obstacles to its adoption in clinical practice. Here, we look to remedy these issues by developing a real-time automated classification pipeline (Fig. 1) coupled with an application for use by non-spectroscopists. First, in addition to routine colonoscopy, *in vivo* measurements of Raman spectra of healthy and cancerous lung tissue were conducted using a custom-made microprobe. The spectra were then loaded into the pipeline and pre-processed in several steps, including standard normal variate transformation and finite impulse response filtration. The quality of the pre-processed spectra was checked using signal-to-noise ratio before the suitable spectra were decomposed and classified using principal component analysis and random forest, respectively. In addition, an application with a graphical user interface was developed to facilitate the use of our data pipeline by non-experts in a clinical environment. Overall, the combination of supervised/unsupervised machine learning with algorithmic preprocessing of *in vivo* measured Raman spectra appears to be a viable way of reducing the relatively large number of biopsies currently needed to definitively diagnose colorectal cancer.

Acknowledgement: This work was supported by grant no. NU20-09-00229 provided by the Ministry of Health of the Czech Republic.

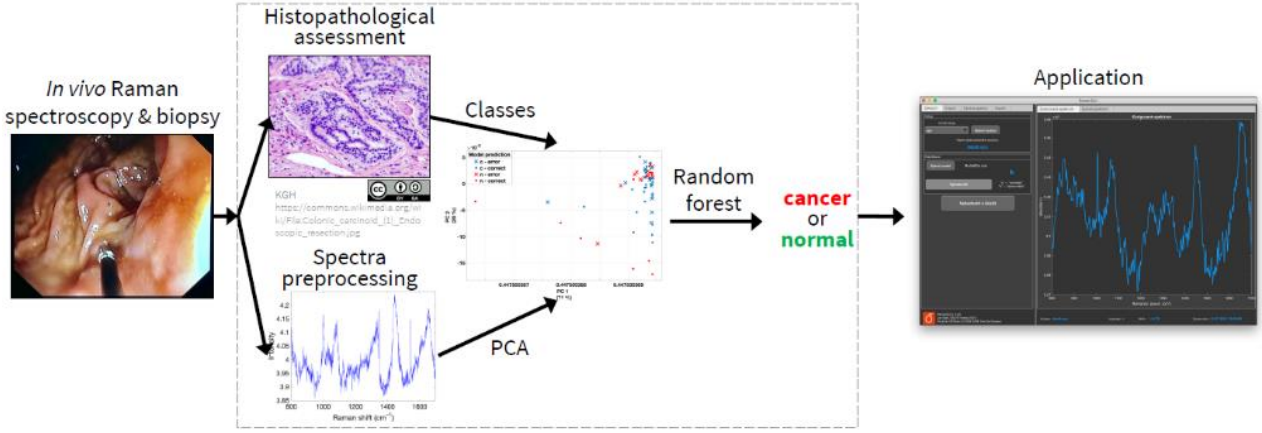

Fig. 1 Classification pipeline and application for in vivo Raman spectra of colorectal tissue for cancer detection

[1] Sung H., Ferlay J., Siegel R. L., et al.: CA: A Cancer Journal for Clinicians 71, 209 (2021).
[2] Wang W., Zhao J., Short M., Zeng H.: J Biophotonics 8, 527 (2015).

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Multimodal molecular imaging of functionalised nanoparticles uptake by cancer cells and tissues

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KEY WORDS: SERS cellular imaging, cellular uptake, nanoparticle localisation

Probing gold nanoparticles (AuNPs) in living systems is essential to reveal the interaction between AuNPs and biological tissues. Moreover, by integrating nonlinear optical signals such as stimulated Raman scattering (SRS), two-photon excited fluorescence (TPEF), and transient absorption (TA) into an imaging platform, it can be used to reveal biomolecular contrast of cellular structures and AuNPs in a multimodal manner. In this study[1], we present a multimodal nonlinear optical microscopy and apply it to perform chemically specific imaging of AuNPs uptake by cancer cells and tissues. This imaging platform provides a novel approach for developing more efficient functionalised AuNPs and determining whether they are within vasculatures surrounding the tumor, pericellular, or cellular spaces.

ACKNOWLEDGMENTS: This research was supported by EPSRC Grants: Raman Nanotheranostics (EP/R020965/1) and CONTRAST facility (EP/S009957/1).

[1] C.C. Wang, J.C. Mansfield, N. Stone, and J. Moger, *Journal of Visualized Experiments*, **183** (2022), e63637.

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The application of machine learning and infrared aperture SNOM to cancer diagnosis

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KEY WORDS: Machine Learning, SNOM

We have developed a methodology for relating discriminating infrared (IR) biomarkers found by the application of a machine leaning algorithm (MLA) to Fourier transform IR (FTIR) spectral images of cancerous tissue to the molecular structure of the tissue with high spatial resolution.

While MLA techniques have had a significant impact on the study of cancer the mechanisms by which they function are often opaque. Recently an MLA has been developed for the analysis of FTIR spectral images which provides some insight into the discriminating spectral characteristics of cancer cells[1]. This MLA assess the contribution of "metrics", ratios of intensities at pairs of wavenumbers, in discriminating between tissue types. When only a few metrics are required to discriminate between tissue types then these spectral intensity ratios can provide insight into the chemical differences between tissues. However, FTIR spectral images are diffraction limited and images of the same tissue were obtained at higher spatial resolution using an aperture scanning near-field optical microscope (SNOM)[2,3]. The FTIR spectral images were acquired using an Agilent Cary 620 FTIR microscope coupled to an Agilent Cary 670 FTIR spectrometer[1-4]. The experiments were conducted on archival formalin-fixed, paraffin embedded tissue obtained from patients with informed consent.

In one study a single metric, the ratio of the intensities of FTIR spectral images obtained at 1252 cm^{-1} and 1285 cm^{-1}, was found to discriminate between oral squamous cell carcinoma nodal metastases and surrounding lymphoid tissue with sensitivities and specificities of 98.8 ± 0.1% and 99.78 ± 0.02% respectively[2]. The SNOM images confirm the importance of these two wave numbers and reveal insight into the chemical structure of the tissue in the region of the metastatic core. In a second study[4] the MLA was able to *predict* which oral lesions would become malignant with an accuracy of 80%.

[1] J. Ingham et. al. *Infrared Physics and Technology.* **102** (2019) 103007
[2] B. Ellis et. al. *Analyst.* **146** (2021) 4895
[3] S. Al Jedani et. al. *Anal. Methods.* **12** (2020) 3397
[4] B. Ellis et. al. *PLoS One.* **17** (2022) e0266043

POSTER PRESENTATIONS

Poster #1

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Ambient Soft Landing of Gold Nanoparticles by Electrospray deposition for Surface Enhanced Raman Spectroscopy

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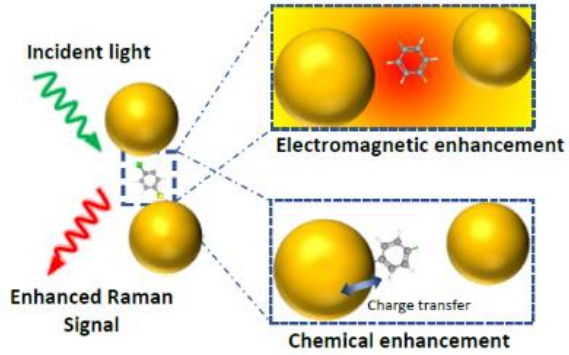
KEY WORDS: SERS, Electrospray ionisation, SERS mapping

Surface-enhanced Raman spectroscopy (SERS) is widely used as a powerful tool for sensitive detection of surface adsorbed species[1]. Numerous analytical applications in a wide variety of fields, including electrochemistry, catalysis, biology, medicine, art conservation and materials science, have been reported using SERS because of the rich vibrational spectroscopic information it provides[2,3,4].

Conventional SERS substrates are commonly fabricated by three methods: (i) mixing plasmonic nanoparticles (NPs) with a dilute solution of analytes, (ii) spin/drop-casting of analyte solution on a solid dried film of NPs, or (iii) incubating particles with a dilute solution of analyte(s) for a given period of time for better adsorption. In these approaches, the preferred adsorption of NPs at specific sites does not happen efficiently within a short period. Even there are other ways of preparing SERS substrates using templated nanostructures on surfaces which are stable, reproducible and convenient to handle, but fabrication procedures and associated instrumentation have rendered them considerably more expensive and uncommon. A possible alternative approach for the preparation of SERS substrates is by ambient electrospray deposition (ESD). Here, we designed a novel focusing ion funnel combined with an electrospray emitter to deposit gold NPs onto a metallic substrate. When we compare our results with a spin-drop casted sample, our results show a 100-fold signal enhancement whilst consuming $< 50\%$ gold NPs colloidal solution. Moreover, initial Raman mapping results (400 μm^2 area) indicate that the uniformity of our ESD substrate exhibits $\sim 50\%$ less variance compared to a spin-drop cast substrate.



[1] Fleischmann, M., Hendra, P. J., & McQuillan, A. J. *Chemical physics letters*, (1974) ***26*(2)** 163-166.
[2] P. Makam, R. Shilpa, A. E. Kandjani, S. R. Periasamy, Y. M. Sabri, C. Madhu, S. K. Bhargava and T. Govindaraju, *Biosens. Bioelectron.*, (2018) **100** 556–564.
[3] D. Cialla-May, X. S. Zheng, K. Weber and J. Popp, *Chem. Soc. Rev.*, (2017) **46** 3945–3961.
[4] A. B. Zrimsek, N. Chiang, M. Mattei, S. Zaleski, M. O. McAnally, C. T. Chapman, A. I. Henry, G. C. Schatz and R. P. Van Duyne, *Chem. Rev.,* (2017) **117** 7583–7613

Poster #2

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Reproducibility study of gold nanorods synthesis when using the seed-mediated growth method

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KEY WORDS: Gold nanorods synthesis, seed-mediated growth method, reproducibility.

Early diagnosis of cancer is important to enable successful treatments to be achieved[1]. Nowadays, less invasive and more reliable tumour diagnosis strategies are being developed using molecular diagnosis tools, as they enable one to monitor biochemical changes corresponding to hallmarks of cancer. Moreover, it is hoped these approaches will allow a better definition of tumour margins, which is crucial for successful surgery.

For our project, we decided to create plasmonic nanoparticles that would 'spot' cancer cells by functionalizing them with a targeting moiety, such as a peptide, and labelling them with a molecule displaying a high signal for Raman spectroscopy on its surface, usually referred to as Raman reporter. The localized surface plasmon resonance of the metallic nanoparticle will highly intensify the Raman reporter signal compared to the signal from the biomolecules in the tissues, giving the localization of the nanoparticles targeting the cancer cells using Raman spectroscopy[2]. We decided to use gold nanorods, as they are biocompatible, easy to functionalize and their localised plasmon resonance is activated by NIR light[3,4]. In this range of wavelengths, the light can penetrate deeply in tissues, as the absorption from biological molecules is low[5]. Because the optical properties of nanorods depends on their morphology, the first step of our project is to adapt our synthesis protocol to make it as reproducible as possible, to avoid any change of properties between the nanorods batches. A range of different parameters, such as how the glassware was cleaned, the stirring method or the quality of reagents, was investigated to understand their impact on the reproducibility of the synthesis.

[1] N. J. Massat, A. Dibden, D. Parmar, J. Cuzick, P. D. Sasieni and S. W. Duffy, *Cancer Epidemiol. Biomarkers Prev.*, **25**, (2016), 455–462.
[2] L. A. Lane, X. Qian and S. Nie, *Chem. Rev.*, **115**, (2015), 10489–10529.
[3] E. C. Dreaden, A. M. Alkilany, X. Huang, C. J. Murphy and M. A. El-Sayed, *Chem. Soc. Rev.*, **41**, (2012), 2740–2779.
[4] X. Yang, M. Yang, B. Pang, M. Vara and Y. Xia, *Chem. Rev.*, **115**, (2015), 10410–10488.
[5] R. Weissleder, *Nat. Biotechnol.*, (2001), 316–317.

Poster #3

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Cofunctionalisation of Silica Nanoparticles with Coumarin and PEG towards Photocrosslinkable Supramolecular Hydrogel Composite

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KEY WORDS: Photocrosslinking, Hydrogel, Supramolecular

Studies on supramolecular polymer colloidal composites (SPCCs) have shown that this new class of material ossesses interesting properties for drug delivery, catalysis, and plasmonic materials that are not seen from regular composites. The use of macrocyclic hosts such as cucurbit[8]uril (CB[8]) as supramolecular handcuffing motifs within these systems has been shown to crosslink different nanoarchitectures including gold nanoparticles[1], silica nanoparticles (SNP)[2], graphene[3] and cerium nanowires[4], to polymeric backbones to create supramolecular composites. Additional functionality can be imparted within these composites through the introduction of a responsive guest moiety. Coumarin (COU) is a photo switchable molecule that has been established to form homoternary complexes with CB[8][5] however, it has never been investigated as a motif within these CB[8]-crosslinked SPCCs. Coumarin is of particular interest due to the ability to form a reversible covalent crosslink through a photocatalysed [2+2] cycloaddition. However, direct functionalisation of SNP with coumarin results in aggregation, and homogenous distribution of nanoparticles in the SPCCs. Here, we demonstrate a two-step co-functionalisation route to coumarin functionalised SNPs that do not undergo self-aggregation. Partial functionalisation of the SNP with polymer chains (mPEG) increases the particle size and reduces the accessible surface area of the SNP. Precise tuning of the PEG : COU ratio and length of the PEG chains results in stable SNP COU-mPEG systems. DLS and zeta potential studies of SNP COU-mPEG in the presence of CB[8] shows that host-guest complexation between CB[8] and COUI on the surface of the nanoparticle is maintained without aggregation of the SNPs. Development of COU decorated SNPs will allow for studies that bridge colloidal, non-colloidal, supramolecular and covalent hydrogel in a highly comparable system. We anticipate this study will establish design principles for CB[8] mediated SPCCs with reversible covalent crosslink towards smart materials for different applications such as drug delivery and theranostics.

[1] R. J. Coulston, S. T. Jones, T.-C. Lee, E. A. Appel and O. A. Scherman, *Chem. Commun.*, 2011, **47**, 164-166.

[2] Y. Wu, D. U. Shah, C. Liu, Z. Yu, J. Liu, X. Ren, M. J. Rowland, C. Abell, M. H. Ramage and O. A. Scherman, *Proc. Natl. Acad. Sci.*, 2017, **114**, 8163-8168.

[3] V. K. Rana, A. Tabet, J. A. Vigil, C. J. Balzer, A. Narkevicius, J. Finlay, C. Hallou, D. H. Rowitch, H. Bulstrode and O. A. Scherman, *ACS Macro Lett.,* 2019, **8**, 1629-1634.

[4] C. Liu, G. Xiang, Y. Wu, S. J. Barrow, M. J. Rowland, D. E. Clarke, G. Wu and O. A. Scherman, *Polym. Chem.*, 2016, **7**, 6485-6489.

[5] A. Tabet, R. A. Forster, C. C. Parkins, G. Wu and O. A. Scherman, *Polym. Chem.*, 2019, **10**, 467 —472

Poster #4

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The medical device regulatory pathway

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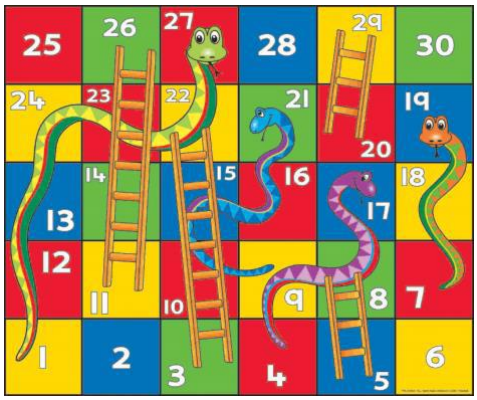
KEY WORDS: Regulation, medical device, clinical trial

The medical device industry, like the pharmaceutical industry is, understandably, highly regulated and requires substantial work before ideas can find their way anywhere near a patient. Regulations have advanced along with the technology, but careful documentation has always remained key. Most of the work centres around risk management and risk mitigation. Implementing procedures for assessing risk at every stage of planning, from the device components themselves through to the design of the study. Document control and risk assessments are essential to ensuring the pathway is safe for gaining approval for any device's eventual use.

Despite a move towards harmonisation, each country/customs union has its own set of rules[1,2]. Many countries classify their devices according to the risk that they might pose, for example an implant that remains for decades, or one that is in contact with the central nervous system is identified as having the highest risk and hence have the largest number of regulations. Conversely, something that is only temporary and touching intact skin has the lowest risk and the smallest regulatory framework. Increased risk also scales with increased level of documentation and time to prepare the application, down to the last nut or screw.

From the experience gained by preparing the application of two clinical investigations (first in human trials) of medical devices for the UK market we will demonstrate an overview of the regulatory process, from the initial planning stages of a study, to assessing risk, through the preparation and submission of an application, as well as the surveillance of the product once placed on the market.

Funded by NIHR.



Snakes and ladders boardgame

[1] U.S. Food & Drug Administration, Classify Your Medical Device, https://www.fda.gov/medical-devices/overview-device-regulation/classify-your-medical-device, (accessed August 17, 2022).

[2] The European Parliament and of the Council, REGULATION (EU) 2017/745, https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32017R0745&from=EN, (accessed August 17, 2022).

Poster #5

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Can we afford a slight delay prior to extracting plasma for Raman Spectroscopy?

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KEY WORDS: biofluids, plasma, spectral analysis, DCDRS, Raman

Introduction

Assessment of biological fluids using molecular spectroscopic techniques is a rapidly advancing field which has shown significant promise for diagnostics[1]. Great care is taken in planning scientific methodology for the processing of these samples to ensure high quality interpretable data. Biofluids such as blood commence degenerative changes once collected suggesting that one should be mindful of time delay from collection to measuring or storage[2]. In clinical settings, there is seldom an ideal lab environment to allow for immediate measuring of samples. In this project, we have explored the impact of whole blood storage prior to plasma extraction for Raman spectroscopy.

Methods

Blood was collected in EDTA tubes and plasma extracted immediately, after six hours, 12 hours and 24 hours of storage in a fridge or at room temperature respectively. All plasma were snap frozen immediately for batch transport. Raman spectra were collected from dried drops of plasma deposited on stainless steel using the Renishaw InVia spectrometer with an 830nm laser.

Results

There was no observed shift in spectra or loss of spectral features. The highest signal to noise ratio of the mean spectra was observed in the 24 hour room sample however there was excellent signal and spectral quality across all the samples with the lowest SNR being 401. There was however a lot of variance in the data noted in the 12 hour and 24 hour samples as demonstrated in the first scattered mean centered principal component analysis scores. There also appears to be a time grouping of the immediate and six hour samples, and then the 12 hour and 24 hour samples.

Conclusion

Scores grouping with the immediate samples and reduced variation in the spectra suggests that delaying plasma extraction from whole blood for up to 6 hours does not have a significant effect on the quality of Raman spectra. There appears to be a closer relationship between the six hour fridge sample PC1 scores to the immediate sample.

[1] K. Kong, C. Kendall, N. Stone, I. Notingher. Raman spectroscopy for medical diagnostics — From in-vitro biofluid assays to in-vivo cancer detection. *Advanced Drug Delivery Reviews*, **89** (2015) 121-134

[2] Boyanton BL Jr, Blick KE. Stability studies of twenty-four analytes in human plasma and serum. *Clin Chem.* 2002 Dec; **48**(12):2242-7

Poster #6

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Quantifying gold at parts per billion or lower concentrations in solution by ICP-MS

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KEY WORDS: gold quantification, ICP-MS

The analysis of gold has historically been associated with the determination of its concentration in geological materials for economic purposes. Such analyses were traditionally performed by pre-concentrating the gold prior to analysis (fire-assay). More recently, the inert nature of gold in the human body has led to its development as a treatment for inflammatory conditions (e.g. rheumatoid arthritis), as implants and uses for immunogold electron microscopy. Gold nanoparticles are widely used in advanced biomedical applications due to their excellent physicochemical properties[1] and are now being used as biomarkers for a wide range of applications. However, to quantify the concentration of gold in biological experiments the digestion and analysis of specific tissues are required, typically by Inductively Coupled Plasma-Mass Spectrometry, at potentially very low concentrations. Modern ICP-MS instruments can routinely detect concentrations lower than parts per trillion (ppt) levels and despite gold being stable in hydrochloric acid solutions at relatively high concentration (parts per million (ppm) or more), there are notable destabilising effects at concentrations in the ppm range or lower.

Our work has investigated the effect of the adsorption of gold onto plastic vessels at concentrations <100 parts per billion (ppb) to improve the accuracy and precision of analysis. We demonstrate that gold is unstable at concentrations less than 100 ppb in a 4%(v/v) hydrochloric acid solution and will adsorb to plastic within minutes of the solution being prepared. We outline areas where gold is unstable and suggest a preferred method to stabilise gold at these low concentrations.

[1] S.A. Bansal, V. Kumar, J. Karimi, A.P. Singh, S. Kumar, *Nanoscale Advances*, **2** (2020) 3764.

Poster #8

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From Mars to Humans: Interactive Raman Spectroscopy-based Outreach Activities

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From Mars to Humans is an interactive educational outreach project, developing the fundamentals needed to understand Raman spectroscopy and its many applications on earth and beyond. This interdisciplinary demonstration includes several devices and models developed by the Biophotonics and Imaging research group at the University of Southampton. We will cover the design and function of “Dr Raman” and the “Raman for Life Rover (R4L)”, two interactive activity devices that have been developed using state-of-the-art spectroscopy technology. These devices help translate the understanding of light-matter interactions to real-life applications, focusing on current popular media topics, public health and interplanetary discovery. With an additional aim to inspire a new generation of scientists, utilising how the underpinning science is leading new transformative technologies and advancing human endeavour. From Mars to Humans activity was deployed for the Southampton Science and Engineering Festival (SOTSEF 2022) and received excellent feedback from visitors.

Poster #7

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Spectrally focused Coherent Raman Imaging at the CONTRAST facility

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The CONTRAST facility is an imaging facility dedicated to Coherent Raman Imaging applications, which contribute to biomedical science and clinical diagnosis. In this abstract we focus on the narrowband spectrally focused Coherent Raman Imaging system and present some of the example applications we are investigating at the facility. The system is powered by a dual output InSightX3 fs laser, which provides a tuneable output between 680-1300nm and a fixed wavelength output at 1045nm. The beams are both spatially and temporally overlapped in the SF-TRU spectral focusing unit which also provides the intensity modulations in the Stokes beam necessary for SRS and chirps the beams from fs to ps pulses. This is then coupled to a modified confocal inverted microscope (Olympus Flouview3000 and IX81). The light is focused on the sample with a 1.2NA water objective. For stimulated Raman scattering (SRS) transmitted light is collected on a photodiode connected to a lockIn amplifier. However for thicker samples there is also an option to collect backscattered (Coherent anti-Stokes Raman scattering) CARS in an epi channel. For some application we simultaneously combine the coherent Raman with Second Harmonic Generation (SHG) and Two Photon fluorescence (TPF), which will provide complementary information about the collagen structures and endogenous fluorophores or added fluorescent labels.

We have applied the system to investigating a range of different applications. SRS imaging provides excellent contrast for lipids in biological tissues, and we apply this to investigate changes in lipid which occur in brain and spinal cord tissue which are associated with neurodegenerative diseases. Imaging sections from both wild-type and transgenic mice, shows changes in tissue structure and cellular lipids which occur with disease. The system is also being used to investigate the effects of fungal infection in mice models, where we were able to image fungal spores within the lungs of mice and detect lipids within the lung cells and within the fungal spores.

When SRS is combined with SHG and TPF imaging the system is able to provide important information on both the tissue structure and biochemistry. Many diseases including cancer are associated with changes in both the cellular composition and changes in elastin and collagen fibres within the tissue. Coherent Raman provides information on the cells, while SHG and TPF provide information on the collagen and elastin networks respectively. We have applied this combined imaging to look at both sarcoma and breast cancer biopsies, adipose tissue and changes in skin which occur in scleroderma.

Poster #9

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Surface-Enhanced Coherent Raman Scattering (SE-CRS) for Application in Cancer Diagnosis

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KEY WORDS: Surface-enhanced Raman scattering, gold thin films, coherent Raman scattering

The aim is to develop a laser-based imaging technique for medical diagnostics based on surface-enhanced Raman scattering (SERS). I will investigate the feasibility of using nonlinear excitation of surface plasmons (SP) to enhance weak optical effects from molecules at nanostructured metallic surfaces. Hence nanoparticles can be tethered to the metal surface where the local fields are several orders of magnitude higher than the driving field. The long-term goal is to successfully develop novel coherent Raman Scattering (CRS) tools to heighten the discrimination of molecular signals for application in cancer diagnostics.

Poster #10

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Overcoming Barriers to Translation; Minimising Impact of Optical Effects when Measuring Highly Fluorescent Samples with Raman Spectroscopy

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Raman spectroscopy is a highly chemically specific, non-invasive, real-time technique with a proven ability to highly accurately distinguish between disease states in a vast array of different tissue types[1]. However, one persistent barrier to clinical adoption is the presence of fluorescent background signals when measuring biological samples[2,3]. Whilst there are an array of different techniques to reduce the impact of these signals (such as utilising NIR excitation sources), even when these are applied this increased background can coalesce with an optical effect known as etaloning to detrimentally affect diagnostic capabilities.

Etaloning is an oscillatory effect wherein incoming photons are not absorbed within the photosensitive layer of the CCD and instead reflect before interfering both constructively and destructively with incoming photons. The result of this effect is a ringing pattern within the obtained spectra which serve to obscure the true chemical signals and complicate the differentiation of different tissue types[4]. As increased broadband signal levels increase the amplitude of the ringing pattern, there is significant interplay between the magnitude of the etaloning effect and the observed fluorescent background of a sample. There are a number of applications where Raman spectroscopy is particularly amenable to incorporation with the existing in vivo diagnostic pathway, but the tendency of the tissue to fluoresce strongly combines with this effect to partially obfuscate the chemical signals related to disease state. Examples include prostate[5], lymph[6] and breast cancer[7].

Thus it is important for clinical translation that the impact of etaloning on the obtained data be minimised. In this work, the etaloning performance of various CCDs was tested and characterised. The characterisation of these CCDs enables the determination of which camera demonstrates the lowest levels of etaloning and therefore maximises the potential for collection of high quality data.

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[1] I. Pence and A. Mahadevan-Jansen, "Clinical instrumentation and applications of Raman spectroscopy," Chem. Soc. Rev., vol. 45, no. 7, pp. 1958–1979, Mar. 2016.
[2] S. P. Mulvaney and C. D. Keating, "Raman Spectroscopy," Anal. Chem., vol. 72, no. 12, Jun. 2000.
[3] D. Wei, S. Chen, and Q. Liu, "Review of Fluorescence Suppression Techniques in Raman Spectroscopy," http://dx.doi.org/10.1080/05704928.2014.999936, vol. 50, no. 5, pp. 387–406, May 2015.
[4] Andor, "Optical Etaloning in Charge Coupled Devices," Oxford Instruments; Learning Centre. [Online]. Available: https://andor.oxinst.com/learning/view/article/optical-etaloning-in-charge-coupled-devices. [Accessed: 30-May-2019].
[5] R. E. Kast, S. C. Tucker, K. Killian, M. Trexler, K. V. Honn, and G. W. Auner, "Emerging technology: Applications of Raman spectroscopy for prostate cancer," Cancer Metastasis Rev., vol. 33, no. 2–3, pp. 673–693, Feb. 2014.
[6] J. C. C. Day and N. Stone, "A Subcutaneous Raman Needle Probe," Appl. Spectrosc., vol. 67, no. 3, pp. 349–354, Mar. 2013.
[7] R. E. Kast et al., "Raman spectroscopy can differentiate malignant tumors from normal breast tissue and detect early neoplastic changes in a mouse model," Biopolymers, vol. 89, no. 3, pp. 235–241, Mar. 2008.

Poster
#11

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Fluorescence Suppression in Raman Nanotheranostics using Low-Wavenumber Anti-Stokes Scattering

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KEY WORDS: Raman nanotheranostics, anti-Stokes Raman, surface-enhanced Raman spectroscopy.

Raman nanotheranostics is a process that combines the technique of Raman spectroscopy with the light-enhancing powers of gold nanostructures. The method demonstrates a huge potential for the detection and treatment of cancer aiming to overcome the number of drawbacks with the current methods of cancer diagnosis and treatment. However, fluorescence noise and high absorption at large depths often result in Raman signals of interest being relatively weak. This has been a significant challenge for biomedical Raman spectroscopy, particularly when using spatially offset Raman spectroscopy (SORS) which sacrifices signal strength for improved depth penetration. Here, I will outline a potential method of overcoming these barriers by using low-wavenumber anti-Stokes Raman scattering to suppress fluorescence and enhance the Raman signal. This will include demonstrations using SORS, surface-enhanced Raman spectroscopy (SERS) and surface-enhanced spatially-offset Raman spectroscopy (SESORS).

Poster
#12

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Plasmonic Magnesium Nanoparticles: a Promising Material for Photothermal Biomedical Therapies

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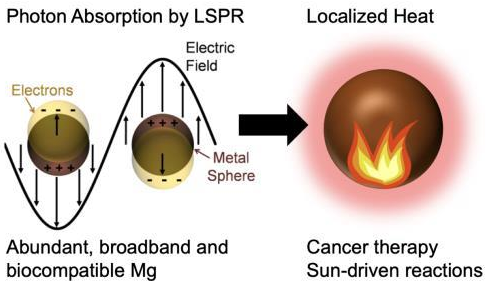
KEY WORDS: photothermal therapy, nanophotonics, biomedical applications

Understanding and guiding the influence of light on nanomaterials can have immeasurable impact on society by facilitating the development of more efficient, sustainable, and cost-effective technologies. One such emergent nanotechnology which has amassed excitement in the past fifteen years is nanoparticle-induced photothermal hyperthermia. Gold is overwhelmingly the most common material used for these applications due to its remarkable ability to convert light into heat. This energy conversion results from the nonradiative decay of the localized surface plasmon (LSP). An LSP is the geometry-specific oscillation of free electrons in a metallic nanoparticle. The heat produced by LSP decay can be used for hyperthermia therapies, including treating cancer and bacterial infections. While gold is the dominant material, it is prohibitively expensive and its long-term toxicity is unknown. Magnesium (Mg) is inexpensive and will not accumulate in the body (as gold does) because there exists pathways for processing this metal[1]. These attributes make it a much more attractive candidate material for photothermal therapies. However, its ability to transduce light into heat has been unexplored until this work.

Herein, I demonstrate that Mg nanoparticles can generate appreciable heating *via* LSP decay. By approximating a collection of Mg nanoparticles as electromagnetically and thermally coupled point dipoles, I show that a Mg nanoparticle colloid can produce temperatures sufficient for biomedical applications. Furthermore, I simulate the thermal response of the thermodynamically- and kinetically-accurate nanoparticle geometries. Each nanoparticle geometry possesses different LSP properties which offers wavelength tunability. Lastly, I perform photothermal transduction experiments on Mg nanoparticle colloids and gold nanoparticle colloids. By measuring the temperature increase upon laser illumination, I show that Mg nanoparticles can produce temperatures comparable to those produced by gold. A collective-heating model, single-particle simulations, and photothermal transduction experiments demonstrate that Mg nanoparticles are a promising new class of biocompatible photothermal nanotechnologies.



LSP-supporting Mg nanoparticle.

[1] Hopper, E., et al. Opportunities and Challenges for Alternative Nanoplasmonic Metals: Magnesium and Beyond. *J. Phys. Chem. C*, **126**, 26, 2022.