WOMEN IN PHOTONICS

JUNE 1 – 5, 2025 // JENA // GERMANY

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OVERVIEW

Sunday, June 1, 2025 // Restaurant Scala	
18:00	**Get-Together & Registration**
Monday, June 2, 2025 // Leibniz Institute of Photonic Technology	
9:00	**Welcome Note** | Chair: Jürgen Popp | Leibniz Institute of Photonic Technology, Jena, Germany
9:20	**The Optics and Photonics Industry in Jena and Thuringia – Introductory Talk** | Anke Siegmeier, OPTONET, Jena, Germany
9:40	Coffee Break
10:10	**Career Workshop Part I** | by Curiositas
12:15	Lunch Break
13:30	**Career Workshop Part II** | by Curiositas
15:00	Coffee Break
15:30	**Career Report I** | Zsuzsanna Heiner, Humbold University Berlin, Germany
16:00	**Poster Session**
Tuesday, June 3, 2025 // Leibniz Institute of Photonic Technology	
9:00	**Session I – Biomedical Diagnostics and Translational Research** | Chair: Anuradha Ramoji, Leibniz Institute of Photonic Technology, Jena
10:30	Coffee Break
11:00	**Session II – Photonics, Fiberoptics, and Microtechnologies** | Chair: Regina Gumenyuk, Tampere University, Finland
11:00	**Career Report II and Introduction to the V4F Project** | Regina Gumenyuk, Tampere University, Finland
12:30	Lunch Break
13:30	**Career Report III** | Mihaela Žigman, Max Planck Institute of Quantum Optics, Garching, Germany
14:00	**Career Opportunities in Industry and Academia** | ams OSRAM International GmbH, Fraunhofer IOF, ZEISS, Jenoptik, Leibniz IPHT
14:50	Coffee Break

15:20	**Session III – Photodynamic and Laser Therapies** | Chair: Anja Silge, Leibniz Institute of Photonic Technology, Jena
16:20	**Session IV – AI and Computational Photonics** | Chair: Shuxia Guo, Leibniz Institute of Photonic Technology, Jena
18:00	**Networking Evening** | Zeiss-Planetarium

Wednesday, June 4, 2025 // Leibniz Institute of Photonic Technology

9:00	**Session V – Optical and Vibrational Spectroscopy Advances** | Chair: Linda Zedler, Leibniz Institute of Photonic Technology, Jena
10:30	Coffee Break
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15:20	Coffe Break
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Thursday, June 5, 2025 // Leibniz Institute of Photonic Technology

9:00	**Lab Tours at Leibniz IPHT**

DEAR PARTICIPANTS,

Welcome to Jena – the "City of Light" – and to the Leibniz Institute of Photonic Technology. We are pleased to welcome you to the latest edition of the Women in Photonics career workshop.

This year, we are particularly pleased with the great interest and impressive number of registrations from early career women researchers from a wide range of photonics disciplines. Photonics remains a dynamic and forward-looking field, providing key innovations and solutions to some of today's most pressing global challenges – in medicine, health, security, the environment and energy. However, the path to leadership in science and technology still presents particular hurdles for women. With this workshop, we aim to create a space where young women scientists can connect, share experiences, and gain practical tools to navigate their careers with confidence and determination. I am particularly looking forward to welcoming four accomplished women from academia and industry who have already forged their own successful paths: Mihaela Žigman (Max Planck Institute, Garching), Zsuzsanna Heiner (Humboldt University, Berlin), Karin Wieland (CHASE Competence Center, Vienna), and Julie Sheridan Eng (Chief Technology Officer, Coherent Corp.). They will generously share their insights, stories and perspectives on building a career in photonics.

We wish you an inspiring and empowering workshop experience – and a wonderful time here in Jena! Our sincere thanks to all the sponsors and supporters who have made this event possible.

Best regards,
Juergen Popp

Scientific Director, Leibniz IPHT

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BEUTENBERG CAMPUS

*Currently there is a construction site in front of the main entrance of Leibniz IPHT. The main entrance can still be used. Please use the separate footpath at the side.

GENERAL INFORMATION

Venue Address:

Leibniz-Institute of Photonic Technology // Albert-Einstein-Straße 9 // 07745 Jena // Germany

Social Program Addresses:

Restaurant SCALA // JenTower // Leutragraben 1 // 07743 Jena
Planetarium Jena // Am Planetarium 5 // 07743 Jena

Hotel Addresses:

Hotel Eulensteins // Oberauengasse 26 // 07743 Jena // Germany
B&B Hotel Jena // Am Anger 32 // 07743 Jena // Germany

WiFi at Leibniz IPHT:

Network: ipht-gast // **Password:** guest_wifi@ipht!

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AGENDA

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Thursday, June 5, 2025 // Leibniz Institute of Photonic Technology

9:00	**Lab Tours at Leibniz IPHT**

Additional Poster

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WELCOME RECEPTION AT THE SCALA RESTAURANT SUNDAY, JUNE 1, 2025

We welcome all participants with a spectacular view over Jena at a height of 120 meters on the 29th floor of the so called "JenTower", the town's highest building. We cordially invite you to a Get-Together at this special location. Enjoy the evening with a snack and drink. You also have the opportunitiy to pick up your workshop documents here.

Venue Address:

Scala Restaurant // Leutragraben 1 // 07743 Jena // Germany // www.scala-jena.de

How to get there:

The restaurant is located at the top level of Jenas highest building, the "JenTower". You cannot miss it, it is right in the city centre. When you stand in front of the tower, just make sure you do not use the entrance to the shopping mall but to the restaurant. It has written "Scala" over the entrance.

- from Hotel Eulensteins: 5 min walk (350 m)
- from B & B Hotel: 13 min walk (1000 m)

NETWORKING DINNER AT ZEISS-PLANETARIUM TUESDAY, JUNE 3, 2025

The Zeiss-Planetarium in Jena, Germany, opened on July 18, 1926, and is the world's oldest planetarium still in operation. It was developed by the Carl Zeiss company, using a revolutionary optical-mechanical projector designed by Walther Bauersfeld. The planetarium introduced the concept of projecting a realistic night sky onto a dome, creating a new way to experience astronomy. Over the years, it has been modernized with advanced digital technology while preserving its historical significance.

Join us for the Networking Dinner at the Zeiss-Planetarium on Tuesday evening. Enjoy a short film projected in the dome, dinner, drinks and nice conversations with your fellows in the nice atmosphere of the 1920s style restaurant "Bauersfed" right next to the Planetarium.

Venue Address:

Zeiss-Planetarium // Am Planetarium 5 // 07743 Jena // Germany //
www.planetarium-jena.de

How to get there:

The Planetarium is within walking distance from the city centre.

- from Hotel Euensteins: 8 min walk (600m)
- from B & B Hotel: 6 min walk (500 m)

Program:

17:45: Arrival
18:00: Welcome and Short movie “Into the Microverse”
18:30: Drinks & Dinner at the Restaurant

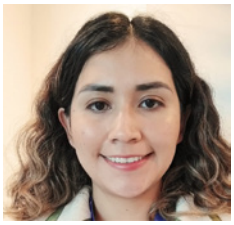
ABSTRACTS

Early Breast Cancer Detection Based on Blood-Serum and Bio-Markers Using Raman Spectroscopy

Lariab Akhtar
University of Guadalajara,
Jalisco, Mexico

Breast cancer causes millions of deaths annually and is the second most reported cancer in women. The early detection of breast cancer can help us to escalate the struggle towards discovering, developing, and optimizing biomarkers that can improve its diagnosis and treatment outcomes. In our study, we obtained the blood serum samples from 11 breast cancer, 2 early-stage breast cancer, and 5 healthy patients and also 4 samples of proteins Alpha-1 Antitrypsin (A1AT), Peptidyl-Prolyl cis-trans Isomerase A (PPIA), PeroxiReDoXin 2 (PRDX2) and TriosePhosphate Isomerase 1 (TPI1). Biochemical research has shown that these proteins are good biomarkers of early-stage breast cancer. We study these blood serum samples using Raman spectroscopy and principal component analysis (PCA). We compare the spectra of these blood serum samples to obtain suitable biomarkers for breast cancer diagnosis. We saw clear differences in the peaks of spectra from breast cancer and healthy samples, which correspond to phospholipids, amino acids, and amide (I, III). Our results showed a difference in one Raman peak between early-stage breast cancer and breast cancer spectra, which could correspond to the glutathione molecule. We also compare the spectra of four proteins with breast cancer and get a similar peak difference corresponding to the glutathione molecule. These preliminary results show that this Raman band corresponding to glutathione could be a good candidate for early-stage breast cancer detection. In the future, we will have more samples and by using machine learning we could confirm the result presented in this work.

Innovation in Diagnosis and Monitoring: Remote Photoplethysmography for the Assessment of Physiological Parameters



Sarai Dominguez
Centro de Investigaciones en Optica, Leon, Mexico

Since the COVID-19 pandemic, the use of remote technologies for disease detection and vital sign monitoring has increased. Remote photoplethysmography (PPGr) is a non-contact method that uses a camera to record skin images and analyze pixel intensity variations. These variations reflect blood volume fluctuations between the systolic and diastolic phases of the cardiac cycle. The main advantage of these technologies is that physiological information on the cardiac cycle can be obtained without physical contact with the patient. However, the full potential of PPGr remains underutilized due to the challenges associated with signal acquisition. One of the main difficulties is that the signal is embedded in significant noise from various sources, including motion artifacts, sensor type, measurement conditions, skin tone, and other factors.

We propose a novel method to address the aforementioned challenges, enabling the acquisition of high-resolution, high-quality PPG signals from remote photoplethysmography. These signals allow for the calculation of physiological parameters such as heart rate, blood pressure, and intraocular pressure. Among these, intraocular pressure measurement stands out as one of the most innovative yet largely unexplored areas in the field.

We use a conventional camera to record videos of the forehead and eye surface, which are then converted into images to extract PPG signals. Our approach effectively reduces noise, enabling the acquisition of high-quality, high-resolution PPG signals. This method offers the advantage of being non-contact, operating under uncontrolled (non-laboratory) conditions, and utilizing a standard camera. As a result, rPPG becomes a valuable tool in scenarios involving contagious diseases, minimizing the risk of transmission. Additionally, it presents a novel and effective alternative to existing methods for measuring intraocular pressure.

Supervised Learning of Infrared Spectral Images for the Diagnosis of Different Breast Cancer Subtypes

Hayat EL TAHECH[1], Seydou KANE[1], Cédric LEREVEREND[2], Caroline FICHEL[2], Jawaheer HASSANI[1], Sandrine TREVOUX[1], Valérie UNTEREINER[3], Jacqueline LEHMANN-CHE[4], Eva BRABENCOVA[5], Vincent VUIBLET[1,6], Olivier PIOT[1], Stéphane POTTEAUX[2], Cyril GOBINET[1]

[1]University of Reims Champagne-Ardenne, BIOSPECT, Reims, France // [2]University of Reims Champagne-Ardenne, IRMAIC, Reims, France // [3]University of Reims Champagne-Ardenne, URCATech, PICT, Reims, France // [4]Pathophysiology of Breast Cancer Team, University of Paris, INSERM U976, HIPI, Paris, France // [5]Institut Godinot, Reims, France // [6]Departement of Nephro-Pathology, Pathology Unit, CHU Reims, Reims, France

Hayat El Tahech
Université de Reims Champagne-Ardenne, Reims, France

Introduction: Breast cancer is the most diagnosed cancer in women and the leading cause of cancer-related deaths among the female population. Therefore, early diagnosis of breast cancer is crucial to improve patient care and limit its progression. The aim of this study is to explore the potential of infrared spectral imaging and machine learning for the early diagnosis of breast cancers, with a particular focus on the differentiation of two of its subtypes, i.e. Human Epidermal Growth Factor Receptor 2 (HER2+) and Triple-Negative Breast Cancer (TNBC) given that they are the most aggressive and associated with the poorest prognosis.

Materials and Methods: Infrared spectral images were acquired at a spatial resolution of 6.25 µm/pixel, within the spectral range of 900 to 1800 cm^{-1}, from 8 µm serial tissue sections from 33 cancer patients (18 HER2+ and 15 TNBC) and 12 benign patients who underwent curative surgery without chemotherapy.

In a first part, the spectral images were corrected from the contributions of water vapor and carbon dioxide, then denoised by a Savitzky-Golay filter, and finally preprocessed by Extended Multiplicative Signal Correction (EMSC) to neutralize baseline effects and lipid contributions by normalizing all spectra around a reference spectrum, typically the average tissue spectrum.

In a second part, the tissue regions in each image were automatically selected using the

clustering algorithm Kmeans. Then, recent and popular supervised learning algorithms, i.e. deep learning and LightGBM, were trained and optimised first to differentiate benign versus cancer samples (8 benign and 11 cancer patients in the train set, and 4 benign and 22 cancer patients in the test set), then to differentiate the HER2+ and TNBC subtypes among the cancer samples (11 HER2+ and 9 TNBC patients in the train set, and 7 HER2+ and 6 TNBC patients in the test set). This classification was performed both on the highly tumorous regions of the sample and on the entire sample.

Results and Discussion: For each classification task, 10 different models were optimized from a different random distribution of patients in the training and validation sets. For model optimization, cross-validation was used by calculating the number of accurately classified patients for each parameter combination. Specifically, for a given patient in the validation set, the model's prediction is calculated, and the patient is assigned to the class where the majority of its spectra (pixels) are predicted. The test set remains fixed and unchanged for all 10 models. In order to evaluate the performance of the models and in order to exploit the diversity of these 10 models, ensemble learning by majority voting was implemented in this work in order to classify the patients in the test set. A patient is classified into the class that is most frequently predicted across the 10 models. This methodology was tested for classifying benign and cancer patients, as well as for distinguishing between TNBC and HER2+.

For the classification between benign and cancer patients, a sensitivity of 100% and a specificity of 100% were achieved on the test set, proving the efficiency of infrared spectral imaging combined to recent supervised classification methods for the diagnosis of breast cancer from suspect benign samples.

For the TNBC versus HER2+ classification on the highly tumorous regions, HER2+ test samples were correctly identified in 86% of cases and TNBC test samples in 66%. And for the classification on the entire sample, HER2+ test samples were correctly identified in 86% of cases and TNBC test samples in 83%. These results demonstrated the ability of infrared spectral imaging to exploit the tiny spectral differences between these two breast cancer subtypes. It is more effective on the entire sample, which can reflects the importance of tumor microenvironment and simplifies the treatement procedure by removing the step of selecting cancer regeions.

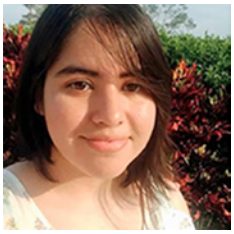
Whatever the used supervised classification algorithm, i.e. deep learning or LightGBM, both gave the same results on the test set. However, deep learning being much more time-consuming, LightGBM seems to be a better choice for this kind of application.

Conclusions: The combination of infrared spectral imaging and recent and powerfull supervised classification algorithm is able to automatically identify cancer samples from benign ones, and more interestingly to differentiate HER2+ from TNBC samples. In a future work, this methodology will be extended to the identification of another breast cancer subtype, named luminal tumor. However, this more complex problem will require the construction of a more consequent dataset, by the acquisition of numerous spectral images or by implementing data augmentation strategies.

Light Against Gloomy Bacteria: Application of Fourier Transform-Infrared Raman Spectroscopy and Machine Learning Algorithms to Accelerate the Identification of Hyper-Biofilm Producers of Porphyromonas Gingivalis

Rosario del Carmen Flores-Vallejo[1], Alida C. M. Veloo[1], Mariam Ahmed2, Dongyi Xu[1], Jan Maarten van Dijl[1]*

[1]Department of Medical Microbiology and Infection Prevention, University Medical Centre Groningen, Hanzeplein 1, 9700 RB. Groningen, the Netherlands. 2) Bruker Nederland B.V. Elisabethhof 15. 2353 EW. Leiderdorp, The Netherlands.
[*]Presenting author: r.c.flores.vallejo@umcg.nl



Rosario del Carmen Flores-Vallej
University Medical Center Groningen, The Netherlands

Background: Porphyromonas gingivalis (Pg) is dark pigmented pathobiont, often regarded as keystone in the development of periodontitis. Periodontitis affects ~20% of the global population with a cost of USD$298 billion/year **[1]**. Although tissue debridement and prophylactic administration of antibiotics serve as common treatments **[2]**, infections with Pg are difficult to diagnose and eradicate in a prompt manner for different factors, including: 1) its intrinsic slow growth speed; 2) high diversity of virulence phenotypes amongst clinical samples; 3) need of special equipment and training to grow it under anaerobic conditions; 4)

increased resistance to antibiotics when it grows under biofilm conditions in the oral cavity; and 5) restricted availability of publicly available biological databases and datasets from clinical specimens **[3-5]**. The application of Fourier Transform-Infrared Raman Spectroscopy (FT-IRS) in combination with Machine Learning Algorithms (MLAs) are emerging as a comprehensive, non-destructive and cost-efficient tools in the field of bacterial Phenomics to speed up the identification bacteria for different clinical and therapeutic purposes **[6]**. Currently, there are no available reports and methods that characterized clinical isolates of anaerobic bacteria through FT-IRS, in particular Pg. **Research objectives and hypothesis:** Considering all the above, in this investigation, our aims were: (i) to standardize a protocol to obtain the FT-IR spectra of the anaerobe Pg under two different culture conditions; (ii) to obtain the spectra of selected isolates that show marked phenotypic differences in their virulence traits (particularly biofilm production); (iii) generate an open source FT-IR spectra database of Pg clinical isolates together with a computational model through MLAs that can help to accelerate the discrimination of hyper-biofilm producers of Pg. Our hypothesis is that the molecular fingerprint obtained from the FT-IR spectra and the application of MLAs can help to discriminate accurately hyper-biofilm producer strains of Pg and reduce the turn-around time (TaT) for clinical diagnostic of such phenotypic variants. **Methods:** For this purpose, a collection of 81 clinical isolates of Pg was screened for their biofilm production capacity in vitro and the results were analyzed statistically. From this screening a group of eight specimens of high, medium and low biofilm producers were selected and grown anaerobically under two different culture conditions using “diagnostic” media (DI) or “biofilm producing”(BP). media Then, the standardized protocol for processing the anaerobic bacterial biomass was implemented and the samples were applied into a 96-spot silicon microplate. The FT-IR spectra were obtained with a IR Biotyper® from Bruker. All specimens were grown in three biological replicates and samples were analyzed in five technical replicates (n=720). **Results:** So far, a standardized and reproducible protocol was established to obtain the FT-IR spectra of the anaerobe Pg. Then, based on a preliminar Linear Discriminant Analysis (LDA) it was observed that the Pg isolates were clustered in two groups based on the type of media that they were grown at. Also, LDA analysis enabled the discrimination of high, medium and low groups of biofilm producers **(Fig. 1)**. Currently, we are working on the development of an MLA model to test its discriminant capacity in an outer group of Pg clinical isolates. We consider these results as a promising starting point to build a FT-IR Pg database to accelerate the accurate diagnosis and improve the treatment of infections related to different virulent phenotypes of Pg.

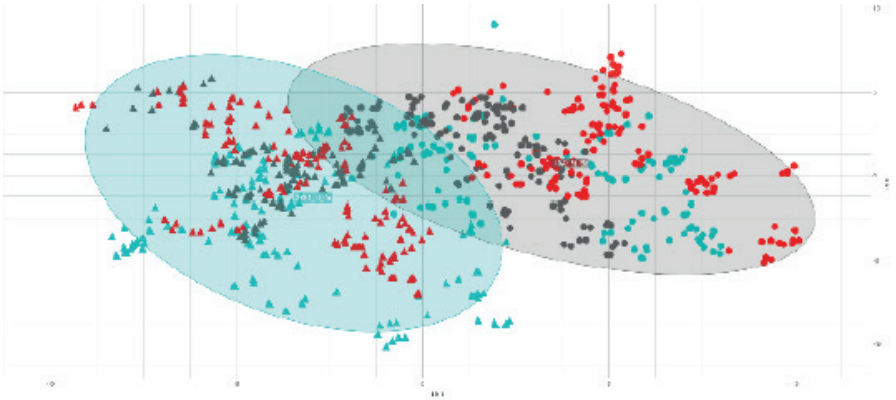


Fig. 1. Discrimination of Pg clinical isolates with different biofilm producing phenotypes through FTIR-spectral molecular fingerprinting. Bidimensional plot of Linear Discriminant Analysis (LDA) (CI: 0.95/20) based on default splicing method. Variance: LDC1 (38.10%) and LDC2 (20.78%). Triangles (Δ) represent samples grown on BP media and circles (o) represent samples grown on DI media. Samples in red represent High Biofilm producers, grey represent Medium Biofilm producers and light blue represents Low Biofilm producers. Light blue ellipse

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Drug-Target-Interactions of Antimalarials in Infected Red Blood Cells

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Malaria, caused by Plasmodium spp. and transmitted by Anopheles mosquitoes, remains a significant global health challenge, particularly in sub-Saharan Africa[1]. Achieving the WHO's target of a 90% reduction in malaria cases and mortality by 2030 requires enhanced prevention, surveillance, and treatment strategies1. Understanding the molecular interactions between antimalarial drugs and their targets is key to combat drug resistance and develop more effective therapies. In this study, specific laser excitation wavelengths were selected in order to examine the interactions of established and

novel antimalarial agents directly within infected red blood cells by means of resonance Raman microspectroscopy was employed2. Advanced data analysis combined with the selective enhancement of the crystallization-sensitive vibrational bands of the malaria pigment hemozoin provided molecular insights into the molecular mode of action of chloroquine, artemether, and the novel drug candidate trioxaquine DU-1302 on the drug target hemozoin. For the first time, the hemozoin inhibition mechanisms of artemether and trioxaquine were observed in situ in infected erythrocytes2. Additionally, differences in the drug interaction with hemozoin across different parasite developmental stages were identified[2].

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Raman Imaging as a Tool for the Investigation of Glycogen in Pompe Disease

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Pompe disease, also known as glycogen storage disorder (GSD) type II, is a rare, inherited muscle disorder known for more than 80 years. The decrease in activity of ∂-1,4-glucosidase (GAA), a lysosomal enzyme, is causative for the disease. Several types of tissue, mainly cardiac and skeletal tissue are affected by an irreversible accumulation of glycogen. In consequence, patients suffer from a wide range of symptoms, for example, muscle weakness, wheelchair dependency, or breathing difficulties. Although Pompe disease has been known for such a long time, there is still no cure for the disease. Moreover, the currently used enzyme replacement therapy only leads to a slowing down in progress. Thus, more research is needed to gain further knowledge about Pompe disease.[1,2,3]

The aim of this project is to complement already existing biological methods for glycogen measurements, like PAS staining, with the advantages of Raman imaging on a single-cell level. Here, myoblasts, a precursor form of muscle tissue, are used, to examine glycogen, proteins, and lipids within single cells. Our approach is to measure primary muscle cell cultures in a non-fixated way. We use a homemade closed measurement chamber, which additionally ensures nutrient supply and a constant environment throughout the whole measurement time. Samples include primary muscle cell cultures affected by Pompe disease, as well as control cell lines. The results show that areas dominated by glycogen can be clearly detected within single myoblasts. Moreover, a clear difference in glycogen content between control cells and cells affected by Pompe disease can be made. Affected cells show a significant increase in the amount of glycogen within the cells. These accumulations can mainly be found homogenously distributed in the cytoplasm, surrounding lysosomes and nuclei.

It is possible through Raman Imaging to get a deeper insight into the distribution and localization of glycogen in primary cells of patients diagnosed with Pompe disease. As biological methods often lack either specificity or single-cell resolution, Raman spectroscopy can help investigate muscle disorders like Pompe disease.

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Breast Cancer Management Through Time Domain Diffuse Optical Spectroscopy

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Breast cancer is the second most common cancer worldwide and the first among women [1]. Effective diagnostic imaging tools are crucial, yet standard techniques have limitations: X-ray mammography is less effective for dense breasts and has side effects, US interpretation depends on the operator experience, MRI/PET scans are costly and time-consuming.

Diffuse Optical Breast Imaging is an emerging technique that could answer to such unmet clinical needs [2]: it is time- and cost-effective, non-invasive, highly sensitive on dense breasts, and provides quantitative physiological information about the breast tissue composition and microstructure. It is an application of Diffuse Optics, that describes photon migration in turbid media in the red and near-infrared range, that proved useful for different stages of the clinical path of a woman with breast cancer: risk assessment, lesion diagnosis, therapy monitoring, the prediction of its outcome and surgical resection margin identification.

This work illustrates the experimental activity we performed in laboratory and in clinics on 3 instrumentations for Diffuse Optical Breast Imaging, for different applications.

The first instrumentation is a time-resolved system based on a supercontinuum laser, used to perform measurements at different geometries (reflectance and transmittance), subject's positions (seated and lying), probing locations (various breast quadrants), and source-detector distances. We tested it on 11 healthy volunteers in preparation of a future clinical trial [3], with the aim to design a simplified device for a routine assessment of breast density (associated breast cancer risk) in screening programs.

The second instrumentation is the first hand-held probe combining 8-wavelength (640 – 1050 nm) time-resolved Diffuse Optical Tomography, US, Shear Wave Elastography and Colour Dop-

pler sonography for the multiparametric non-invasive diagnosis of breast cancer (Horizon 2020 project SOLUS [4]). Preliminary analysis of 21 benign and 12 malignant lesions using machine learning shows a sensitivity of 97%, specificity of 57%, and balanced accuracy of 77%. Adding US-based predictors improves these to 100%, 77%, and 88%, respectively. [5].

The third instrumentation is a 7-wavelength (635 – 1060 nm) time-resolved optical mammograph that acquires projection images of the breast to monitor neoadjuvant chemotherapy (NAC) and possibly predict its outcome. Early results on 10 patients indicate that collagen (here evaluated for the first time for this application) is the only biomarker distinguishing complete from partial responders within 2-3 weeks of NAC initiation (p=0.0423 vs. p=0.1012) [6].

Even if encouraging, findings are not conclusive: the clinical trials are still ongoing. Future analysis then could highlight even more the potential of Diffuse Optical Breast Imaging.

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Fast and Cost-Effective Detection of VRE Using Multiplex Real Time PCR and RPA

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Keywords: Enterococcus spp, resistance-genes, PCR, RPA, VRE

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Vancomycin resistant enterococci (VRE) are one of the leading causes of antibiotic resistant infections in the hospital setting worldwide and this has become a major issue because most of the infected patients are difficult to treat. Multiplex real time polymerase chain reaction (RT PCR) is an advantageous technique that can amplify multiple targets in a single reaction and can be used to quickly detect specific targets in VRE within two hours starting from suspected colonies of bacterial cultures without sample preparation.

In this study, we selected the glycopeptide/vancomycin resistance genes that are most common in clinical settings vanA and vanB in combination with species markers ddl_faecium and ddl_faecalis for the most common VRE species – Enterococcus faecium and Enterococcus faecalis. Primers and probes were then designed for these targets and commercially produced. DNA from forty clinical VRE strains was prepared using a fast and economic heat lysis method and multiplex real time PCR assay was optimized and carried out subsequently. Results were in concordance with the results from recombinase polymerase amplification (RPA) of the same VRE samples. Multiplex RT PCR and RPA for VRE detection proffers a second method for confirmation of vancomycin resistance and it can be developed as a fast screening assay for patients before admission into high risk settings.

In Vivo Phenotyping of the Tumor Immune Microenvironment to Predict Melanoma Outcomes

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Tumor outcomes (progression, response to immunotherapies) are influenced by tumor-intrinsic and the tumor-immune microenvironment (TiME) factors and the dynamic interplay and crosstalk between them. To overcome the existing limitations of phenotyping tumors using single-time, single-site ex vivo biopsies in predicting tumor behavior, we propose an integrated dynamic in vivo approach for phenotyping TiME by combining inflammation, vasculature/angiogenesis and tumor-intrinsic features through dynamic high-resolution reflectance confocal microscopy (RCM) in patients. Melanoma patients (n=35) were imaged to characterize density and spatial distribution of tumor, inflammation, vasculature/angiogenesis and leukocyte trafficking. Unsupervised clustering (hierarchical clustering analysis, HCA and principal component analysis, PCA) was performed on TiME features to derive phenotypes. Phenotypes were correlated with immune cells (T-cells, B-cells) and tertiary lymphoid structures (TLS), and response to topical toll-like receptor agonist imiquimod immunotherapy. On a subset of data, we also correlated treatment response with immune markers on RT-PCR and CODEX multiplexed immunostaining (MxIF). We demonstrate three main phenotypes, $Inflam^{HIGH}Vasc^{HIGH}$, $Inflam^{HIGH}Vasc^{LOW}$ and $Inflam^{LOW}Vasc^{HIGH}$ in HCA and PCA such that top contributors to PC1 were inflammation-related while to PC2 were vasculature-related features. Tumor-intrinsic features had a relatively minor contribution to the phenotyping. No correlation of TiME phenotypes with tumor stage, age, gender or sun exposure was observed. Biological correlation in a subset of cases show highest T-cell and B-cell infiltration ($p<0.01$), and TLS presence (ns) in the $Inflam^{HIGH}$ phenotype. Response to imiquimod therapy in patients (n=21) was correlated with phenotypes and individual TiME features. Overall, lower lichenoid inflammation and number of vessels were found to be hallmarks of non-responders. On

spatial proteomics using MxIF, we found increased numbers of CD4+ helper T cells, CD8+ cytotoxic T cells, and B lymphocytes in responders while endothelial cells were enriched in non-responders. Similarly, on gene expression analyses, upregulation of pro-inflammatory and cytotoxic markers in responders while upregulation of immune-suppressive features (IDO1, IL10, COX2) was observed in non-responders. Our preliminary results support presence of unique TiME phenotypes in melanoma that correlate with underlying immune states, and response to topical TLRA immunotherapy. Response was associated with higher pro-inflammatory signatures on gene and protein expression. Quantitative models will be tested using both imaging and gene/protein biomarkers to predict melanoma progression and response to immune checkpoint therapies in subsequent studies.

Advancing Neurophotonics: The Development and Applications of PDMS-Based Flexible Optical Waveguides

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The field of optogenetics increasingly requires the use of flexible optical tools to accommodate dynamic neural environments. This study focuses on the development and detailed characterization of polydimethylsiloxane (PDMS)-based flexible optical waveguides, which are specifically designed for neural interfacing. In contrast to conventional rigid fibers, these PDMS waveguides provide superior biocompatibility and flexibility, essential for interacting with soft neural tissues. The manufacturing process employs a three-layer configuration, incorporating a nanoparticle-doped core surrounded by pure PDMS, which ensures effective light confinement and high transmission efficiency at crucial neural interfacing wavelengths. Additionally, the careful optimization of nanoparticle dispersion within the core reduces scattering losses, thereby improving the waveguides' functionality. Initial biocompatibility tests suggest minimal cytotoxicity, endorsing their suitability for in vivo use in neurophotonics and optogenetic stimulation. This research highlights the advantages of PDMS-based waveguides in addressing the limitations posed by traditional optical fibers and demonstrates their potential for integration into advanced neuro-optical interfaces, facilitating new possibilities in optogenetic therapies.

Impact of PSD Bandwidth Limitations on Recording High-Speed Lissajous Patterns of MEMS-mirrors for Endomicroscopic Probes

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2D resonant Micro-Electro-Mechanical Systems (MEMS) mirrors' small size and fast response capabilities make them a viable option for endomicroscopic probes. Here, these mirrors contribute to achieve high-resolution imaging by precisely controlling the movement of the light beams. One of the methods for obtaining resonant scanning involves driving MEMS mirrors in Lissajous patterns, which are generated when the two axes of a MEMS mirror are moved with sine signals of slightly different frequencies that are tuned to natural resonant frequencies of the mirror's axes for optimal deflection. However, when driven in these patterns, the mirrors can display non-linear behavior, including crosstalk between axes: with movement on one axis interfering with the other. Hence, the light spot movement is tracked externally by position sensitive devices (PSD) before tissue imaging to generate an accurate look-up table for image reconstruction. In Lissajous scanning, the light spot moves at a dynamic speed: moving faster at the center than at the edges of the scanning. This dynamic speed across the entire field of view (FOV) hinders PSD recording when the pattern size is magnified from the object plane of the probe to the PSD to benefit from higher signal-to-noise ratio (SNR) signals. With magnification, the light spot may move more quickly since it follows the same path, but the produced pattern is expanded in size as the magnification coefficient increases. It can be problematic for areas with the fastest movement which signals are subject to bandwidth limitations of the PSD. Hence, recorded trajectories can be smoothed, distorted, and phase-shifted resulting in poor images. Therefore, it is needed to study this case, so that the scanning pattern can be reproduced in a look-up table as correctly as possible. This work aims to simulate such a case and to identify a functioning correction procedure.

Design and Manufacturing of High-Precision ZnSe Lenses for Advanced FLIR Applications

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Zinc selenide (ZnSe) is widely used in optical components, including lenses, mirrors and thermal imaging systems, owing to its medium refractive index and broad infrared transmission range (0.6 to 21 microns). However, its soft-brittle nature presents challenges in achieving the nanometric smooth finishes required for precision manufacturing. This study introduces a single-step precision manufacturing process for ZnSe and demonstrates its application in a plano-convex lens integrated into FLIR thermal imaging systems for heat detection in integrated circuits. Although diamond turning of brittle materials has been practiced for decades, systematic studies on the nanometric cutting behaviour of chalcogenide glasses comprising sulfur, selenium, and tellurium remains limited. Systematic investigations are essential to comprehensively understand their cutting behaviour and to validate their suitability for specific applications.

In this study, face turning trials is conducted to optimize the experimental cutting parameters, which is then applied to successfully fabricate a spherical plano-convex lens with an aerial surface roughness (Sa) of 3.98 nm. The fabricated lens is subsequently integrated into thermal microscopy for detecting heat in integrated circuits. The results demonstrate that single-point diamond turning can serve as a high-throughput, single-step manufacturing process for producing usable ZnSe lenses without the need for post-machining processing.

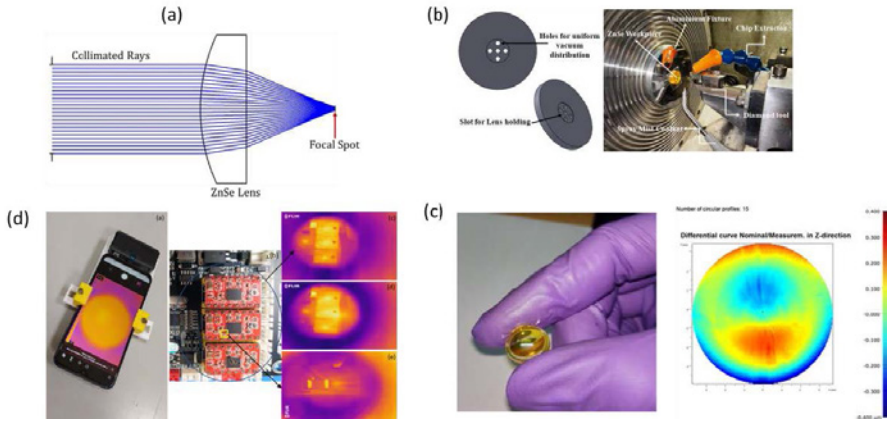

Figure 1: (a) Ray Simulation of ZnSe lens (b) Fixture & Diamond Turning Experimental Setup (c) Spherical plano-convex lens of ZnSe with Sa of 3.9 nm and form error of PV 0.222 µm (d) Setup for acquisition of images using ZnSe lens and acquired images

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Experimental Analysis of Thulium-Doped Fiber Amplifier and Raman Amplifier in Long Haul Coherent Transmission

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In this work, we experimentally compared the performance of two different S-band amplifiers, a thulium-doped fiber amplifier (TDFA) and a distributed Raman amplifier. We characterized these two amplifiers by measuring the gain and noise figure from 1470 nm to 1520 nm of wavelengths. Then, we conducted an experimental comparison of the performance of these two S-band amplifiers within a long-haul coherent transmission system. We used 30 GBaud dual-polarization 16 quadrature amplitude modulation (DP-16QAM) signals over 1050 km of standard single-mode fiber (SSMF).

The gain and noise figure were measured at -4 dBm signal launch power. For distributed Raman amplifier, four laser pumps with different wavelengths at 1365 nm, 1385nm, 1405 and 1425 nm were combined by the pump combiner, which were merged by a wavelength division

multiplexing (WDM) coupler. We used backward pumping configuration for the distributed Raman amplifier, where the pumps were put at the end of the transmission fiber. 75 km of SSMF used as the gain medium for distributed Raman amplifier. The spectrum was captured by optical spectrum analyzer (OSA).

The TDFA obtained the highest gain of 19.5 dB at 1520 nm, while the distributed Raman amplifier achieved the highest gain of 18.4 dB at 1485 nm. The distributed Raman amplifier obtained the lowest noise figure of -3.2 dB at 1510 nm because backward pumping configuration reduced the amplified spontaneous emission (ASE) noise from the amplifier. The TDFA obtained the lowest noise figure of 4.1 dB at 1485.

The distributed Raman amplifier demonstrated superior performance with the SNR of 14.2 dB compared to the TDFA with 12.9 dB of SNR over 1050 km of transmission distance. This is due to the lower noise figure of the distributed Raman amplifier.

Liquid Sensing Inside Anti-Resonant Optical Fibers

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Optofluidics merges photonics and microfluidics, enabling innovative applications such as highly sensitive optical sensors. The precise monitoring of liquid properties is essential across industries, from environmental monitoring to biomedical diagnostics. Optical fiber sensors, particularly anti-resonant optical fibers (ARFs), have emerged as particularly promising for liquid sensing applications due to their robustness, high sensitivity, and ability to function in harsh environments. Unlike conventional fibers, ARFs guide light via interference effects rather than total internal reflection. Their hollow-core structure is especially suitable for liquid sensing, allowing analytes to flow directly through the fiber and enabling real-time measurements. This design aligns with ongoing research into fluid-filled waveguides and liquid-core fibers [1-4].

In this work, we explore the use of ARFs for sensing inorganic salt concentrations using an absorption-based setup. Our experimental system employed a supercontinuum laser coupled into an ~8 cm ARF via a single-mode fiber. The transmitted light was collected by a multi-mode fiber and analyzed with a spectrometer. Liquid samples were introduced into the hollow core using a microfluidic pump, enabling continuous analyte flow with minimal sample volumes. The setup is compact, adaptable, and easily integrable into other sensing platforms.

We measured $Cu(NO_3)_2$ absorption spectra in the ARF and compared them with reference spectra obtained using a standard quartz cuvette. The ARF spectra aligned well with those from the reference system, and from the data, we derived the molar attenuation coefficients for both systems. Although maintaining a uniform analyte concentration inside the fiber remains a challenge, our findings demonstrate the promising capabilities of ARF-based sensors for precise liquid analysis. This work highlights the potential for further development and application of ARFs in diverse sensing environments.

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Combining Interferometric Nanoparticle Tracking Analysis (iNTA) with Fluorescence Detection to Distinguish Nanoparticle Sub-Populations with Single-Molecule Precision

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Background: The characterization of biological particles, such as extracellular vesicles (EVs), is crucial for understanding their roles in biological processes and their diagnostic potential. However, their heterogeneous composition poses challenges on the quantification of their biophysical and biochemical properties.

Methods: Recently, we presented interferometric nanoparticle tracking analysis (iNTA) as a label-free method for characterizing nanoparticles such EVs. Here, we show that by combining iNTA with sensitive fluorescence detection, one can not only characterize the EVs but also their biomolecular content. Additionally, we use a flow system for the sample loading process in order to mitigate bleaching of fluorescence signals. This approach enables us to simultaneously determine the size, refractive index, and intensity of the fluorescent labels of single particles.

Results: As a proof of principle, we have shown that we can differentiate nanoparticles in the presence of different fluorescence by using a mixture of 30nm gold nanoparticles, 40nm FluoSpheres (505/515) and 100nm FluoSpheres (580/605). We examined the fluorescence response of our setup by measuring liposomes labeled with two different lipid dyes, MemBright (for 488nm excitation) and R18 (for 561nm excitation). We quantified the fluorescence intensity of each liposome, finding it to be proportional to the liposome size and the amount of the dye used. Finally, we labeled EVs derived from HEK293 cells with anti-CD9 and anti-CD81 fluorescent antibodies, enabling us to quantify the percentage of EVs expressing these biomarkers.

Conclusion: Our findings suggest that our multimodal analysis approach can significantly enhance one's ability to distinguish multiple populations within heterogeneous samples, thus, contributing to the advancement of technologies for biomarker discovery.

Interferometric Nanoparticle Tracking Analysis

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Characterization of the size, material properties and concentration of particles in liquid suspensions is in high demand, for example, for the analysis of extracellular vesicles (EVs) in bodily fluids or quality control of nanoparticle-based drug formulation. However, existing methods are often limited in sensitivity and cannot handle a high degree of polydispersion.

We present interferometric nanoparticle tracking analysis (iNTA), which combines nanoparticle tracking analysis (NTA) with interferometric scattering (iSCAT) microscopy to achieve higher sensitivity than traditional NTA instruments [1]. We show that iNTA can measure the size and refractive index of individual nanoparticles with unprecedented precision. Furthermore, we demonstrate that the concentrations of different subpopulations in polydispersions can be determined from the trajectory numbers [2].

We benchmarked iNTA with synthetic nanoparticles and showed that gold nanoparticles as small as 10 nm can be measured. Afterwards we show that iNTA can extract the size and internal structure of liposomes and EVs. In addition, we demonstrate that iNTA can differentiate EVs from lipoproteins in blood plasma-derived samples, which is not possible with conventional NTA [3]. Finally, we show that iNTA can differentiate viruses from EVs in SARS-CoV2 infected cell supernatants.

We have shown that iNTA is a useful tool not only for characterization of particle size and refractive index, but also for quantitative assessment of particle concentration, even when sizes overlap. This is very useful for the quantification of EVs where co-isolates are often present in the samples.

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Photo-biomodulation Therapy for Effective Treatment Solitary Rectal Ulcers

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Solitary rectal ulcer syndrome (SRUS) is an inflammatory condition, often linked to chronic constipation leading to bleeding and straining during bowel movement. The conventional treatments have shown a limited effectiveness in terms of symptomatic response and promoting lesion healing. In this study, Photobiomodulation Therapy (PBMT) was explored as a novel therapeutic approach due to its anti-inflammatory and antioxidant properties, aiming to address the underlying pathology and alleviate symptoms of SRUS.

A prospective randomized controlled trial has been conducted at the Center for Liver and Digestive Diseases, Holy Family Hospital, Rawalpindi, Pakistan. PBMT was administered by illuminating the affected rectal tissue with a 635 nm red laser at 300 mW power, through a cylindrical diffuse length of 2.5cm delivering a total dose of 180 J in each session. Statistical analysis was performed using SPSS 25.0 (SPSS Inc, IBM, USA), and $p < 0.05$ was considered statistically significant.

The study included 36 patients (22 male, 14 female) from diverse localities, with an average age of 21 years. The PBMT treatment group included 20 patients, while 16 patients received

conventional therapy. Each patient underwent a pretreatment sigmoidoscopy, followed by 14 PBMT sessions administered 48 hours apart. Symptomatic responses were monitored throughout, and post-treatment sigmoidoscopies were performed to evaluate lesion healing.

The PBMT group demonstrated significant improvements, with 99% achieving symptomatic relief and an average ulcer size reduction of 78%, compared to 45% in the control group ($p < 0.05$). By targeting inflammation and promoting mucosal healing, PBMT presents a promising noninvasive alternative for managing SRUS, enhance quality of life, and reduce recurrence.

A case Report has already been Published from this study in Photobiomodulation (Effective Treatment of Solitary Rectal Ulcer Syndrome by Using Photobiomodulation Therapy: A Case Report)

Influence of Lapatinib on Photodynamic Therapy in Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is the most common and aggressive type of primary brain tumor. The standard of care for this disease consists of maximum safe tumor resection followed by a combination of chemotherapy with the alkylating agent Temozolomide (TMZ), irradiation, and Tumor-treating field therapy. However, heterogeneity of GBM tumors could reduce or prevent the beneficial effect of these interventions.

Photodynamic therapy (PDT) relies on an intracellular photosensitizer activated by light of a given wavelength. This generates lethal amounts of reactive oxygen species (ROS) that cause cell death. In GBM, a selective accumulation of the naturally occurring photosensitizer protoporphyrin IX (PpIX) can be observed. This accumulation can be enhanced by the oral administration of the precursor molecule 5-aminolevulinic acid (5-ALA) which can be further metabolized to PpIX in the mitochondria. The minimally invasive

nature of PDT, its penetration depth, and its natural selectivity for tumor cells make it a promising therapeutic approach for the difficult-to-treat tumors of GBM. Several investigations are still mandatory to optimize the procedure and further elucidate the underlying mechanisms. More so, the efficacy of a combination of 5-ALA-mediated PDT with an inhibitor of ABCG2 transporters has been proposed. Thus, Lapatinib, a tyrosine kinase inhibitor, is used in breast cancer treatment and possesses an ABCG2 inhibitory effect.

An In-vitro cell culture system of four Glioblastoma cell lines (U87MG, TMZ-rU87, U-251MG, and T98G) was established in our lab. We investigated TMZ resistance using a drug sensitivity cell viability assay. The effect of Lapatinip on PpIX accumulation was measured by fluorometry using 5-ALA as a prodrug of the photosensitizer PpIX. Also, we evaluated the effect on PDT after irradiation with light of 635 nm, measured cell viability and production of reactive oxygen species (ROS) in the different cell lines. From the results obtained, Lapatinib showed a dose-dependent enhancement of PpIX accumulation in the cell lines. This demonstrates the possibility of Lapatinib supporting 5-ALA mediated-PDT in both sensitive and resistant cell lines, thus suggesting a potential synergistic effect.

Overall, this potential therapeutic strategy positions the 5-ALA mediated-PDT and Lapatinib combination for enhanced oxidative stress-mediated cancer cell death.

Key words: glioblastoma multiforme, protoporphyrin IX, reactive oxygen species, photodynamic therapy, 5-ALA, Lapatinib.

Terahertz Spectroscopy Sheds Light on Real-Time Exchange Kinetics Occurring Through Plasma Membrane During Photodynamic Therapy Treatment

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Methods to follow in real time permeabilization of live cells is a complex and increasingly addressed issue, whether it is for medical application, or in lab research protocols where a constant necessary effort is made to reach more realistic investigation conditions in biological system. Cell permeabilization is characterized by increased molecule transfers through the cell membrane.

The terahertz region has been shown to have potential in biomedical applications, including the possibility to spectroscopically address complex systems such as cells and even accessible tissues or small organs [1-4]. Recent works have shown the ability to follow the permeabilization of a cell layer in physiological condition, without any marker nor sampler preparation, using attenuated total reflection (ATR) in the terahertz domain [5-8]. Here, we show that terahertz spectroscopy reveals early events in plasma membrane alteration generated during a photodynamic therapy protocol, events which are not observable in any other conventional biological techniques performed in parallel as comparison [9]. The photodynamic process was examined in MDCK1 cells using Pheophorbide a (Pheo) photosensitizer alone or alternatively encapsulated in poly(ethylene oxide)-block-poly(e-caprolactone) micelles for drug delivery purpose. Terahertz spectroscopy revealed that plasma membrane permeabilization started simultaneously with illumination and was stronger when photosensitizer was encapsulated (see fig.1). In parallel, the exchange of biological species was assessed. Over several hours, this conventional approach demonstrated significant differences between free and encapsulated Pheo, the latter leading to high penetration of propidium iodide, Na^+ and Ca^{2+} ions, and a high level of leakage of K^+, ATP and LDH. THz spectroscopy provides, in a single measurement, the relative number of defects per membrane

surface created after PDT, which is not achieved by any other method, providing early, sensitive real-time information. Supplementary experiments have shown that the terahertz sensor is able to differentiate the dynamics of permeabilization induced by photodynamic therapy when the photosensitizer is encapsulated with different types of polymers.

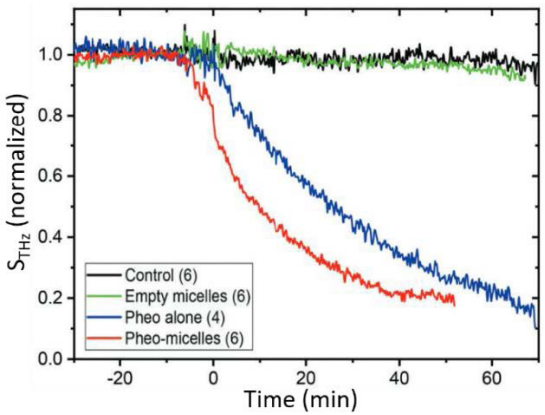

Fig. 1. Terahertz signal during photodynamic therapy protocol in different configurations.

This study validates the original and innovative application of terahertz spectroscopy, a physical technique that is applicable to biological and medical issues. It is a powerful, promising and complementary technique to other biological approaches to monitor changes in cell permeability upon a photochemical stress, especially thanks to its high sensitivity and capacities to enable the early detection of membrane permeabilization processes in real-time on living cells. The terahertz sensor has proven to be a very powerful tool for studying the effectiveness of copolymer-based nanovectors as an agent for drug delivery.

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Escaping AMR: 213 nm Laser Light to Combat ESKAPE Pathogens

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Introduction: ESKAPE pathogens *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa and Enterobacteriaceae* are multi-drug resistant and linked to critical infections. With the rise of antimicrobial resistance, estimated to cost USD$1 trillion and kill more people than cancer by 2050, there is an urgent and unmet need for new antimicrobial strategies. Ultraviolet (UV) light between 245-450 nm has long been employed for surface disinfection, however, investigation into UV therapy in humans is limited due to toxicity. Far-UV light between 200-230 nm has been demonstrated to be antimicrobial and non-toxic, offering a promising alternative strategy to combat multi-drug resistant pathogens implicated in life-threatening infections.

Hypothesis/Aim: I hypothesised that a far-UV 213 nm laser has antimicrobial properties. Our aim was to establish the efficacy of 213 nm laser light against 6 examples of ESKAPE pathogens.

Methods: *E. faecium 19434, S. aureus 25923, K. pneumoniae 13883, A. baumannii 19696, P. aeruginosa PA01, and E. cloacae 13047* (ATCC) were used to demonstrate laser antimicrobial efficacy against ESKAPE pathogens.

Planktonic bacteria were spread on Tryptone Soya Agar, dried, and treated with the 213 nm laser for increasing doses (exposure times 5 ns-300 s). After overnight incubation (37°C), the area where bacterial growth was absent was measured.

Results: 213 nm laser light was highly effective against planktonic ESKAPE pathogens with growth inhibition after 1 s of exposure in all species.

Conclusion: Far-UV 213 nm laser light has demonstrated antimicrobial efficacy against ESKAPE pathogens, laying the foundation for more in-depth testing against key antimicrobial resistant pathogens in a range of models as well as safety testing in ex vivo skin samples. Our findings suggest that 213 nm lasers hold potential as a novel tool to combat antimicrobial resistant infections.

Pushing the Limits of Laser Surgery: Precise Biological Tissue Ablation Using Deep Ultraviolet Femtosecond Laser Pulses

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Ultrashort laser pulses enable precise ablation of biological tissues with minimal thermal and mechanical side effects. Deep ultraviolet (deep-UV) femtosecond lasers can non-thermally ablate tissue by breaking molecular bonds, offering the potential for surgical precision beyond existing tools. However, while infrared femtosecond and deep-UV nanosecond lasers are established in medicine, femtosecond deep-UV laser ablation remains largely unexplored.

In this talk, I will present my research on soft biological tissue ablation using femtosecond deep-UV (206 nm) laser pulses. I will discuss how key laser parameters – pulse energy, spot size, and fluence – affect the ablation process, and how these parameters can be optimised to maximise precision while avoiding unintended effects. Additionally, I will demonstrate the application of this technique on a clinically relevant brain tissue model, highlighting its potential for neurosurgical procedures.

Optimising laser parameters is critical for advancing deep-UV laser-based therapies. My findings suggest that ultrashort deep-UV lasers could provide a valuable tool for precise tissue removal, with potential applications in neurosurgery, such as complete brain tumour resection.

Osteosarcoma Chemosensitivity Signatures with Holographic Dynamic-Contrast OCT

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Abstract: Patient-derived xenograft (PDX) models are often used to screen for drug-sensitive patients. The procedure involves disaggregating human tumor tissue and implanting it into immunocompromised mice. The method of growing the tumors, implanting them and conducting chemotherapy testing can take months [1]. Additionally, the PDX model has an altered microenvironment as the cells are regrown in an alien species with different genetic and physical characteristics. Holographic Dynamic-Contrast Optical Coherence Tomography (OCT), also called biodynamic imaging (BDI), measures the intracellular dynamics of tissues responding to chemotherapeutics with the potential to provide a chemotherapy assay result within 48 hours performed on tumor biopsies from the patient. A key question is how a PDX drug response compares to a primary biopsy drug response. As a first step to answering this question, we perform BDI on osteosarcoma PDX samples to test the effect of BET inhibitors, a class of novel anticancer drugs.

Biodynamic imaging is a dynamic contrast optical coherence tomography (OCT) technique that employs off-axis digital holography [2]. It captures coherent and dynamic speckle generated by light scattering off living tissue biopsies, revealing Doppler frequency shifts linked to intracellular motion. Cellular dynamics operate at nanometers per second, while organelle or vesicle transport moves at microns per second [3], producing intracellular Doppler spectra that span from 10 mHz to 10 Hz.

Osteosarcoma (OS), the most prevalent primary bone cancer in adolescents, constitutes approximately 2% of pediatric cancers. While front-line chemotherapy increases patient survival to some extent, many patients still face fatal outcomes, in part, due to chemotherapy resistance.

To address chemotherapy resistance, BDI conducts chemosensitivity assays on BETs (BRD2,3,4) which are epigenetic reader proteins that recognize and bind to acetylated histones to induce gene transcription that contributes to osteosarcoma pathogenesis. BET inhibitors (BETi) can increase replication stress via transcription-replication imbalances in osteosarcoma that augment genomic instabilities within this disease.

Thus, BET inhibitors can be exploited as therapeutic targets in osteosarcoma to exacerbate replication stress and induce cell death. This study utilizes BDI to perform BET inhibitor (BETi) assays on OS PDX, demonstrating drug effectiveness across multiple cell lines from diverse human patients.

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How Intracavity Soliton Dynamics Accelerate Ultrafast Spectroscopy

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Ultra-short light pulses are relevant in various physical and technical fields, from laser surgery in medicine to material investigation in industry. This contribution explores the dynamics and interactions of ultra-short light pulses, known as solitons, in laser cavities to optimize mode-locked lasers and develop innovative material sampling techniques.

For this, we clarify system-specific intra-cavity soliton interactions, such as attraction, repulsion, or binding, through simulations

based on the main pulse shaping effects. We present two distinct laser systems demonstrating contrasting soliton interaction regimes: stepwise attraction and repulsion.

In a Ti:sapphire solid-state laser, higher-order Kerr effects combined with gain dynamics lead to soliton attraction and the formation of short-range bound states due to Raman oscillations [1]. Conversely, in an erbium-doped fiber laser, long-range coupling through slow gain dynamics, along with the interplay of a fast saturable absorber, results in soliton repulsion and harmonic mode-locking.

This new understanding of intracavity dynamics provides insights into the origins of noise sources, allowing them to be minimized. Additionally, it enables external control of soliton trajectories by modulating their intensity. This facilitates multi-kHz optical scanning of temporal delays without the need for external mechanical stages or dual cavities [2]. As proof of concept, a pump-probe measurement of an InGaAs sample is shown in Fig. 1. The inset displays a schematic of the measurement technique. Thus, intracavity soliton dynamics can be utilized for ultrafast spectroscopy.

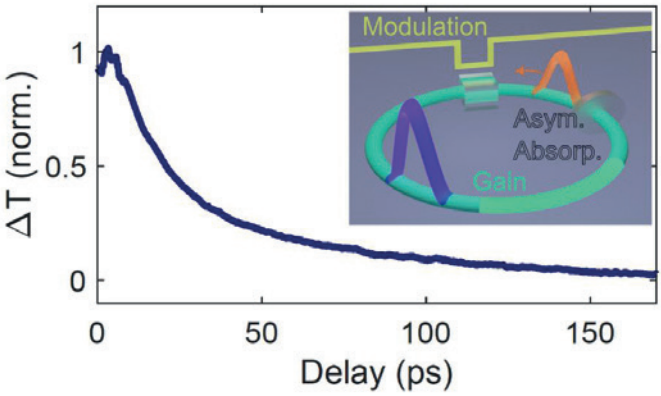

Fig. 1: 1550 nm degenerate pump-probe measurement of an In0.53Ga0.47As sample with a lifetime of 21 ps at a fluence of Φ=2.4 mJ/cm². Insert: Schematic of intracavity modulation.

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Analytical Techniques to Study the Formation and Structure of Homogentisic Acid Derived Pigment

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Alkaptonuria (AKU) is a rare, recessive genetic disorder caused by mutations in the gene encoding for the homogentisate 1,2-dioxygenase (HGD) enzyme. This halts the tyrosine and phenylalanine metabolic pathway leading to an accumulation of homogentisic acid (HGA). Consequently, an alternative metabolism takes place forming a dark brown or black HGA-derived pigment. This pigment is central to the disorder and is deposited into connective tissues in a process called ochronosis. This causes tissues to become hard and brittle leading to ochronotic osteoarthritis which is painful and debilitating. There is currently no cure although a recently approved drug 'Nitisinone' can halt pigmentation and slow disease progression.

Owing to being a rare disease, there are many unanswered questions surrounding AKU. One major question is the metabolism of HGA to form HGA-derived pigment. Structural similarity between HGA and hydroquinone has led to the conclusion that pigment forms through oxidation of HGA to BQA which reacts to form HGA-derived pigment. However, there is little evidence to support this. Instead, there is increasing evidence that metabolism occurs via a radical reaction. Additionally, the structure of pigment is unknown. Pigment has always been concluded as a polymer, due to similarities to melanin, with little evidence to prove or disprove this theory. HGA-derived pigment is central to the disorder so increasing understanding could improve disease management and help in working towards a cure.

Increasing pigment knowledge could also help with answering one of the other biggest questions of AKU which is the mechanism by which pigment is deposited into tissues. Increasing understanding of this interaction could lead to new therapeutic targets that aim to prevent or remove pigmentation which causes the poor quality of life for AKU patients.

This presentation highlights the progress made in studying HGA-derived pigment. Spectroscopic techniques including Fourier Transform infrared spectroscopy and electron paramagnet-

ic resonance spectroscopy have been combined with other analytical techniques such as gel permeation chromatography. Together, these techniques have enabled an increased understanding of pigment structure and its reaction.

Structural and Vibrational Spectroscopic Studies of a Molecular Complex

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Abastract: This research project investigates the molecular structure and vibrational spectra of a synthesized molecular complex through Density Functional Theory (DFT) calculations, focusing on pyrazinamide (PZA) and 2,5-dihydroxybenzoic acid (25DHBA), compounds with significant medical applications in treating tuberculosis (TB) and bacterial infections. The study aims to provide detailed insights into the molecular structure and properties of this complex, given its biological importance in the medical field.

The work involves comparing experimental Fourier Transform Infrared (FTIR) spectra with simulated spectra obtained via DFT/B3LYP calculations. Vibrational assignments are made based on animated modes to elucidate key spectral features and interactions within the complex. The investigated PZA-25DHBA co-crystal demonstrates stabilization primarily through strong hydrogen bonding interactions, such as H-N-H---O and C=O---H-O bonds, which play a crucial role in influencing the structural and vibrational behavior of the complex.

A comparison of molecular structure parameters derived from X-ray diffraction (XRD) data with those optimized using the B3LYP method shows excellent agreement. The presence of strong hydrogen bonding significantly affects the frequencies of critical functional groups, particularly the OH, NH2, and C=O stretching modes, as well as out-of-plane deformations. Understanding these interactions is essential for better characterizing the structural properties that may influence the biological activity of the complex.

Theoretical vibrational spectra closely align with experimental data, validating the accuracy of the DFT/B3LYP approach in modeling molecular structures. Some deviations are noted, likely due to the inherent limitations of the theoretical approach and the differences between the experimental solid-phase spectrum and the simulated gas-phase (vacuum) data.

By providing a comprehensive analysis of the molecular structure and vibrational properties of the PZA-25DHBA complex, this study enhances our understanding of its role in medical applications, particularly its potential in the treatment of TB and bacterial infections.

Characterization of Microfluidics for High-Throughput Field-Resolved Infrared Spectroscopy of Particles in Flow

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Keywords: infrared spectroscopy, imaging, microfluidics, flow cytometry In the last decade field-resolved infrared spectroscopy (FRS), a laser based spectroscopic technique covering a vast part of of mid infrared (mid-IR) range, was developed and is now approaching the ultimate detection sensitivity, resulting in high dynamic range and signal to noise ratio of the FRS systems [1]. This makes FRS technology suitable for investigation of biological samples with high water content and in their natural aqueous environment despite the strong attenuation of mid-IR light by water [2]. Combining FRS with flow cytometry enables the possibility for label-free investigation of a great number of individual particles (e.g., cells) in a short time, potentially exceeding thousands of cells per second [3]. However, for a reliable FRS measurement of particles in flow it is essential that the particles pass through an IR-compatible microfluidic chip in a corridor of a certain width, the so-called lamella, in a very repeatable manner.

Here we present the performance of the IR-compatible microfluidics in terms of the lamella width and its central position stability with a self-built optical imaging setup compatible with our FRS system. The central position of the lamella should coincide with the center of the IR focus and should be stable. The latter is the performance characteristic of the microfluidics and needs to characterized and optimized. The former can be aligned within the FRS system by positioning the microfluidic chip on a translation stage. The tests for the lamella stability was first conducted by using a dyed solution instead of particle dispersion in a transparent buffer. By monitoring the position of the dark band in the microfluidic chip (i.e., lamella) the

stability of the position of the lamella can be determined. The result of such characterization for an example of a dissatisfactory performance of the microfluidics is shown in Figure 1.

Furthermore, we will show our efforts toward optimized performance of the microfluidic system for high throughput measurements, particle tracking across frames, as well as the development of standard operating procedures for a stable and repeatable running of microfluidics to avoid particle spill-over from one measurement campaign to the other.

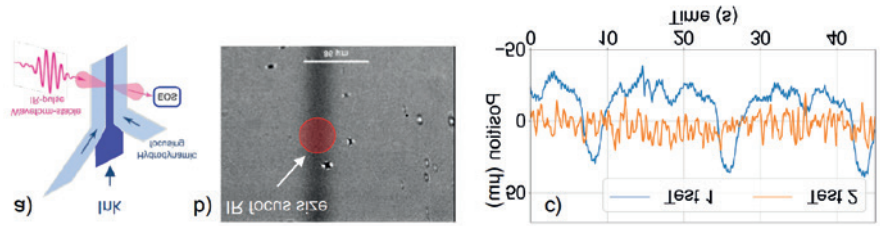

Figure 1. Investigation of the stability of the lamella position. a) For this investigation ink was used to form the lamella. b) Image of the lamella (dark band) with the IR focus depicted in red, for size comparison and indication of the lamella movement in a duration of 45 seconds. c) Tests done to observe the relative position of the lamella with respect to the measurement time.

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Evaluation of the Diagnostic Potential of Vibrational Spectroscopy in the Context of Different Medical Questions with an Unmet Clinical Need

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Introduction: Despite significant improvements in medical diagnosis this last decade, some medical questions are left without answers or with unsatisfactory solutions. In this context, vibrational spectroscopy, a label-free technique that provides a biochemical fingerprint of a sample, is emerging as a potential method for aiding in the diagnosis or for the exploration of molecular mechanisms involved in the evolution of a disease. The objective of our studies is to assess the potential of infrared and Raman spectroscopies to fulfil such roles. First, we investigated the ability of mid-infrared imaging to provide diagnostic markers of Hirschsprung's disease. The same technique was also employed to study the differences between cholangiocarcinoma (CCA) and hepatocellular carcinoma (HCC) tumours in comparison with combined hepatocellular-cholangiocarcinoma (cHCC-CCA) tumours. Secondly, Raman imaging was used to investigate the interactions between adipocytes and tumour cells in breast cancers.

Methods: For infrared analysis, two successive 8 µm sections were cut from FFPE samples: one was deposited on a calcium fluoride (CaF_2) slide for infrared imaging, and the second on a glass slide for hematoxylin-eosin (HE) staining which marks the tissue structures. For the Hirschsprung analysis, 6 patients were included. For each patient, two samples were available: one at the functional region of the colon, and one at the non-functional and pathological region. For the CCA, HCC and cHCC-CAA tumour samples, 5 patients were included for each condition, with only one sample per patient. The infrared spectral images of the tissues were acquired over the spectral range 4000-800 cm^{-1} with a spectral resolution of 4 cm^{-1}, 4 accumulations per spectrum, and with a pixel size of respectively 6.25x6.25 and 25x25 $µm^2$.

For the Raman analysis, two modalities were used. Raman imaging was used to analyse breast cancer samples from patients of various BMI (body mass index) by focusing on the invasive tumour front. Additionally, single-point spectra were acquired to study an in vitro co-culture model that was developed to mimic the interactions of adipocytes and cancer cells. The human samples were deposited on CaF2 slides and the coculture were grown over glass slides, and then fixed before acquisition. The Raman spectra were acquired over the spectral range 3100-550 cm-1 with a spectral resolution of 2 cm-1, 2 accumulations per spectrum, and a step size of 2 µm in X and Y directions for the spectral images.

The spectral images were pre-treated by Extended Multiplicative Signal Correction and processed by common multivariate methods such as Principal Component Analysis and Kmeans clustering to highlight spectral features which carry useful information in the biomedical issues studied here.

Results and Conclusion: Using infrared micro-imaging, we demonstrated the possibility to discriminate healthy from pathological regions of Hirschsprung's colon samples by focusing on the muscularis, while no morphological differences were visible within this structure with the gold-standard. Infrared imaging also proved itself to be an efficient technique to help differentiate CCA, HCC and cHCC-CCA tumour from healthy tissue and to characterise the differences between these tumoral types. In addition, Raman spectroscopy permits to characterise the biochemical nature of lipids involved in the adipocytes/breast cancer cells interactions. Further experiments are ongoing to comprehend the impact of obesity in these exchanges. Our experiments contribute to promoting vibrational spectroscopy as a tool of interest in various medical questions, with the advantage to be possibly incorporated in the routine diagnostic workflow.

From Development to Detection: Dendritic Nanostructures in SERS for Advanced Biomolecular Analysis

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Surface-enhanced Raman spectroscopy (SERS) is a powerful technique that has shown great promise in the field of biomolecular detection for various applications in medical diagnostics and research [1, 2]. Conventional SERS substrates, typically fabricated from silver nitrate, suUer from oxidation and degradation, restricting their long-term utility. By using silver sulfate as a precursor instead of silver nitrate, we developed dendritic nanostructures with sensitive SERS detection capabilities. Silver sulfate acts as a growth agent and a mild capping agent, which simplifies the fabrication process and improves the stability of the substrates. The substrates demonstrated excellent sensitivity and stability, eUectively detecting 4-mercapto benzoic acid (4-MBA) at sub-femtomolar concentrations. Thus, these findings highlight their potential for use in tracing small

concentrations of biomolecules. The newly developed SERS substrate was also tested with a range of drugs, including 6-thioguanine, methotrexate, erlotinib, doxorubicin, and moxifloxacin, with detection limits down to the sub nanomolar range, demonstrating its potential in drug monitoring. Furthermore, to simulate clinical applications in relevant matrices, the drugs were spiked into human plasma from healthy donors. Despite the complexity and interference of the plasma matrix, the drugs were detected in the concentration range required for therapeutic monitoring. These results demonstrate the significant potential of sulfate ion-directed dendritic structures for therapeutic drug monitoring in clinical diagnostics.

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Resolving Biological (T)issues with Vibrational Raman Tags

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Topic: Raman microspectroscopy, triple bond tagging, fluorescence imaging, small molecule detection

Abstract: The analysis of biological systems by means of Raman spectroscopy is often associated with its ability to provide chemical contrast in a label-free fashion. While this approach effectively reveals the overall composition and morphochemistry[1], selectively detecting specific small molecular targets in complex environments remains challenging due to the non-unique bond patterns commonly present. In that regard, the implementation of Raman labels can provide

an effective solution for specialized research questions focusing on the fate of certain small molecules. Unlike fluorescent labels, Raman labels are very small (only a few bonds in size) and thus designed to induce only minimal modifications to the original molecule, preserving its faithful structure and biological properties as best as possible. Along with their uniqueness in a biological sense (biological orthogonality), spectroscopic orthogonality is enforced. Triple bond moieties (alkyne, nitrile ...) so as isotopic deuterium labels fulfill these requirements, providing bands in the wavenumber silent region of the Raman spectrum. While tags with outstanding spectroscopic properties are already established [2], this presentation focuses on their application in addressing biological riddles and the invention of efficient tag structures driven by their desired application of custom molecules. We will explore the use of tags for small molecule localization through spontaneous and coherent Raman techniques in simple biological systems, including human and plant cells as well as nematodes, thereby highlighting both the future potential but also associated challenges of vibrational tag design and application. Findings from the projects highlighted in this presentation suggest insights that could advance in biomedical research and the environmental sector.

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This work was additionally supported by the TAB (Thüringer Aufbaubank) and the Thüringer Innovationszentrum für Medizintechnik-Lösungen (ThIMEDOP) funded by the European Union within the framework of the European Regional Development Fund (Grant Number IZN 2018 0002) and the Deutsche Forschungsgemeinschaft (Project-ID 460889961).

Using Surface-Enhanced Raman Scattering and Multivariate Analysis for the Simultaneous Multiplex Detection and Quantification of Malodorous Thiols

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Odour is widely known to be due to the microbial conversion of odourless molecules produced from the apocrine glands to volatile organic compounds (VOCs), which includes thiols.[1,2] These thiols strongly contribute to the odour but are only found at low detectable quantities. Additionally, these molecules are highly volatile and small, making them difficult to sample and analyse, including by gas chromatography-mass spectrometry.[3] This leads to the loss of information which is needed for understanding the formation of odour. In this study, colloidal surface-enhanced Raman scattering (SERS) combined with chemometrics has been utilised to simultaneously detect and quantify these thiols individually and in multiplex solutions containing target thiols. To calculate the limit of detection (LoD), both univariate and multivariate methods were used to compare between these methods. Partial least squares regression (PLS-R) was used as the multivariate technique. The LoD values calculated appeared to agree between the two methods, with LoDs calculated to be between 0.0227 ppm and 0.0153 ppm using PLS-R for the target thiols. The target thiols were then examined and quantified simultaneously in 120 mixtures using PLS-R. These models showed high linearity (Q^2 values between 0.9712 and 0.9827 for both PLS-1 and PLS-2) and low values of root mean squared error of predictions (RMSEP) (RMSEP values between 0.0359 ppm and 0.0459 ppm for PLS-1 and PLS-2). To prove that these models worked and SERS could detect unknown concentrations, these models were then applied to 15 new blind test samples. These experiments were performed two weeks after the initial 120 samples. The results proved that these PLS-R models and SERS could be used to predict the unknown concentrations of these thiols in a mixture. In conclusion, these results display the usability of SERS for the simultaneous multiplex detection of analytes and its potential for future development for use in detecting gaseous thiols produced from the apocrine glands.

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Refining the SERS Protocol for Urine Analysis in Cancer Diagnostics

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The clinical utility of surface-enhanced Raman Spectroscopy (SERS) as a diagnostic tool is hindered by the absence of standardized protocols. This study aims to contribute to the optimization of SERS acquisition protocol to maximize spectral information and reliability from urine samples while preserving the simplicity of SERS method, which makes it more accessible than other spectroscopic techniques.

We analyzed a control urine sample using SERS under different conditions, including pH (4, 5, 6, 7, 8, 9), dilution (10x, 100x, 250x, 500x, 1000x), cations supplementation (Ca^{2+}, Mg^{2+}, Cu^{2+}, Al^{3+}, Mn^{2+}, Pb^{2+}, Zn^{2+}) and citrate and chloride surfactant of the silver nanoparticles (AgNPs). The optimized protocol was then applied to 18 early-stage breast cancer patients and 10 control urine samples. Urine SERS spectra were acquired in clinical environment, using a portable Raman spectrometer with a 532 nm excitation laser. The pH of urine samples was adjusted to 5, 7 and 9 by adding HNO_3 and NaOH. Data pre-processing included baseline correction and vector normalization, followed by principal component analysis (PCA). For classification of breast cancer and control samples we employed logistic regression (LR) model with Lasso regularization.

Among the tested cations for adsorbing molecules onto the silver surface, Ca2+ showed the best signal enhancement. Surfactant also played a crucial role, with chloride-capped AgNPs leading to the best signal-to-noise ratio (SNR) in the SERS spectrum of urine. Interestingly, the SNR and the bands intensity did not increase with concentration, but otherwise. We

found that a dilution of 250x of the urine sample lead to the best SNR results. Next, we investigated the influence of urine pH on the SERS spectra. The SERS bands of urine at various pH were similar (primarly corresponding to uric acid and hypoxanthine), with SNR incresing with pH. Notably, at pH 9, creatinine band also appeared, allowing for spectral normalization to its band and thereby minimizing the effect of urine dilution. A similar behaviour was observed in a mixture of uric acid, hypoxanthine, xanthine and creatinine showing the competitive adsorption of these metabolites onto the silver surface, which however, can be modulated by adjusting the pH of the solution. The optimized protocol was applied to classify breast cancer and control samples. PCA was used to reduce data dimensionality and the first five principal components (PCs) serving as input variabiles for the classifier. The LR model achieved a classification accuracy of 85.7%.

Concluding, the study shows that urine sample dilution and pH significantly affect the SERS spectra, making their normalization among samples crucial. The optimal dilution was found to be 250x, with the best results at pH 9. The most effective SERS protocol utilized hya-AgNPs activated by Ca^{2+}, which was applied to urine samples for breast cancer diagnosis. Normalization to creatinine at pH 9 enhanced the reliability of SERS analysis, leading to a classificaton accuracy of 85.7%. These findings support further validation in larger cohorts, paving the way for SERS-based liquid biopsy tools in cancer diagnostics.

VOC Detection Using Biophotonics for Healthcare Applications

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Abstract: Volatile Organic Compounds (VOC) have high vapor pressure and low boiling point at room temperature. VOCs are biogenic, the primary source of emission from plants, use and production of fossil fuel, compressed aerosol goods, biofuel use, biomass combustion, etc. In the context of healthcare, most of these VOCs show adverse effects on human health and may lead to long-term chronic diseases [1,2]. For instance, human breath consists of hundreds of VOCs, which serve as biomarkers for disease diagnosis. In recent years, the detection of VOC attained a great deal in all human-associated fields. The detection and quantification of VOCs by optical technologies account for 25% of existing sensing technolo-

gies [3]. Surface-enhanced Raman Spectroscopy (SERS) is one of the most attentive biophotonics technologies due to its cost-effectiveness, high sensitivity, and ability to detect multiple analytes. Fabrication of hybrid SERS substrate composed of bimetallic (Ag/Au) nanoparticles and Si nanowires [4] can form effective hotspots and is beneficial for increased analyte-capturing moiety and enabling target molecule adsorption. FESEM [Fig. 1(a)], and TEM [Fig. 1(b)] reveal the morphological and structural information. XPS provides the binding energy of elements [Fig. 1(c)] present in the substrate. The capability of the hybrid substrate was tested extensively by detecting acetone, ethanol, and isoprene vapors, which have been linked to lung cancer, diabetes, skin, liver, and COVID-19 [5].

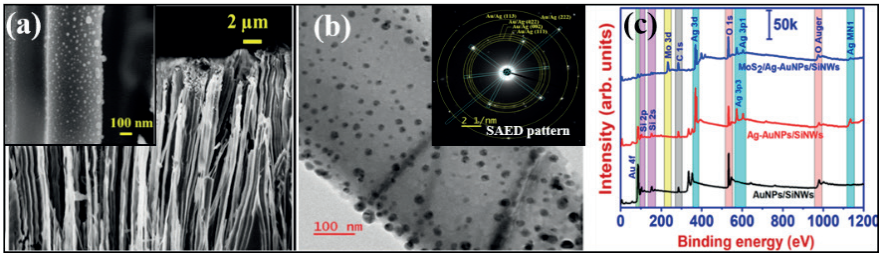

Figure 1. (a) FESEM image of Ag-AuNPs decorated SiNWs (inset: higher magnification), (b) Bright-field image with the corresponding SEAD pattern as inset (c) XPS data of AuNPs/SiNWs.

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Surface-Enhanced Raman Spectroscopy (SERS) for the Detection, Characterization, and Identification of Microorganisms

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One of the crucial challenges in the field of Raman spectroscopy is the weak signal from certain samples such as biological samples, which is often overshadowed by intense fluorescence. Metallic nanoparticles can be used to create so-called Surface-enhanced Raman spectroscopy (SERS) to amplify the Raman signal. Metallic nanostructures exhibit plasmon resonance, a coherent oscillation of conduction electrons induced by the interaction of visible radiation with those structures. The Raman signal is enhanced when the analyte, often gold or silver, is adsorbed onto the surface of metallic nanoparticles [1,2].

SERS substrates exist in various forms, including nanoparticles (e.g., gold nanorods, AuNRs), nanostructured planar layers, and many more. The SERS method allows the rapid identification and differentiation of a wide range of chemical and biological samples, including various types of microorganisms. However, variations in the chemical composition of bacteria may be too insignificant to reliably distinguish closely related species [1,2].

To achieve species-specific identification, diagnostics can be combined with so-called SERS-tags, a technique that involves using specific antibodies. SERS-tags are gold nanoparticles with a surface modified with a Raman reporter, which provides a strong and distinct spectral signal enhanced by the SERS effect. Additionally, the nanoparticle surface is functionalized with an antibody that selectively binds to a specific bacterial strain based so the SERS-tags used are based on antigen-antibody affinity. The presence of the Raman reporter signal in the sample spectrum validates the presence of the target bacteria. These SERS-tags can be modified for labeling any bacterial species by modifying their surface with strain-specific antibodies [1].

SERS can be directly used to analyze bacterial metabolism under stress conditions. Bacteria can be applied directly onto gold nanoparticles and afterward measured. The ob-

tained signal may originate from the bacterial surface or external metabolites. For example, one of the stress markers of Staphylococcus aureus is adenine (see Figure 2) [2].

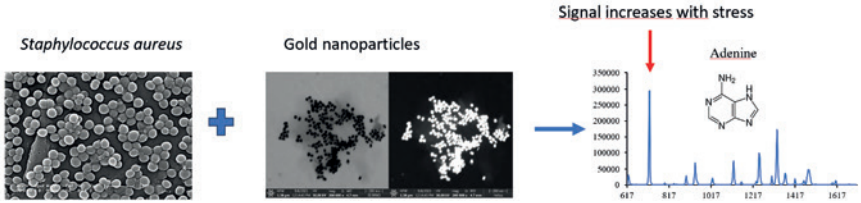

Figure 2: The SERS measurement of the Staphylococcus aureus using gold nanoparticles to enhance signal of the external metabolites such as adenine.

The presented real-time (RT) setup also has the potential to be adapted into a portable version, enabling on-site analysis of microbial processes (MPs).

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Raman Spectroscopy Tools Supporting a Sustainable Pigments Extraction from Shrimp Tissues

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The blue bioeconomy promotes sustainable utilization of aquatic waste, with crustacean shells (e.g., crab, lobster, shrimp) being a key focus due to their rich mineral and organic composition. These materials serve as valuable sources for innovative upcycling products and processes of a high economic and biomedical importance [1,2]. Among the bioactive compounds present, astaxanthin, a highly valued carotenoid pigment, is recognized for its substantial economic and biomedical significance due to its antioxidant properties [3,4]. However, conventional extraction methods rely on harsh solvents such as acetone and ethanol, raising environmental concerns. As a greener alternative, acetic acid has demonstrated efficacy in carotenoid extraction, as confirmed through our Raman-tracked demineralization of crustacean shells. [5].

This study investigates the carotenoid concentration trends during extraction, explore the effects of storage conditions on carotenoid profiles, and demonstrate the potential of acetic acid as a green, sustainable alternative for carotenoid recovery from waste biomaterial derived from shrimp tissues. Resonant Raman Spectroscopy effectively identifies the onset of astaxanthin extraction within one hour of immersing the intact biomaterials in the acetic acid solution. Hourly spectral monitorization enables the development of extraction kinetics curves for hard and soft tissue stocks, revealing a plateau in extraction yield after three hours.

Time-dependent analysis of the extracts is further conducted using Surface-Enhanced Raman Spectroscopy (SERS) and UV-Vis Spectroscopy, providing additional insights into the carotenoid profile of the extracts. Both SERS and UV-Vis analysis revealed the presence of multiple carotenoids in the extracted solution. Our study supports the broader effort to valorise shrimp shell waste, contributing to waste reduction in the seafood industry while promoting a circular bioeconomy.

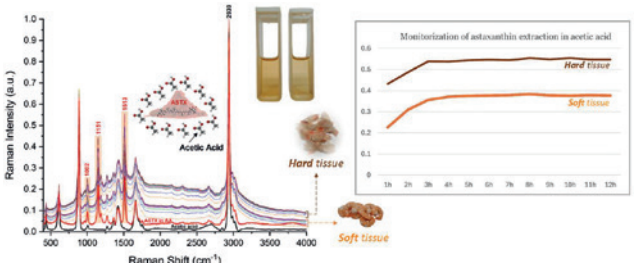

Fig. 1. Raman Spectroscopy monitoring the 12-hour extraction of astaxanthin from shrimp hard and soft tissues in acetic acid. Excitation laser line: 532 nm. The kinetics curves revealed the relative intensity of the 1513 cm^{-1} C=C band from the Resonance Raman spectra of extraction bath reported to the 2939 cm^{-1} band of acetic acid.

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From Simulation to Imaging: Exploring Nonlinear Photodamage Origins in Multiphoton Light-Sheet Microscopy

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High speed imaging of multiphoton fluorescence microscopy provides a window of opportunity to study fast biological processes in vivo, such as neural activity or the dynamics of a beating heart [1]. Light-sheet illumination using orthogonal geometries is particularly advantageous in strategies to increase the speed of image acquisition. In comparison to conventional approaches, parallelized illumination of weakly focused beam enables faster imaging with reduced peak intensity, lower average power, and longer pixel dwell times [2]. Surprisingly, our measurements revealed a counterintuitive result: the maximum peak laser intensity threshold for avoiding nonlinear optical damage in living embryos is significantly lower in multiphoton light-sheet microscopy than in standard point-scanning multiphoton microscopy, which employs a highly focused beam.

In this research, we studied how the weakly focused beam used in light-sheet microscopy could unexpectedly contribute to nonlinear photodamage in vivo. In fact, we observed that cells can act as micrometer-scale lenses, locally focusing the illumination beam and enhancing the local peak intensity beyond expected levels. The phenomenon of cellular lensing is related to the photonic jet produced by dielectric microspheres. We investigated this in vivo cellular lensing effect through live imaging of zebrafish embryonic hearts using multiphoton light-sheet microscopy. We extracted a set of experimental parameters for numerical simulations of image formation using Biobeam [3] or finite-difference time-domain (FDTD) methods [4]. Finally, we utilized and compared these simulations to investigate the effects of beam parameters and cell morphology on the strength of cellular lensing. This study indicates that cellular lensing may represent a source of nonlinear photodamage during live imaging with multiphoton light-sheet microscopy.

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AutoMitoNetwork: Software for Quantitative Mitochondrial Network Analysis in Label-Free Autofluorescence Images

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Mitochondria are key cellular organelles responsible for cellular respiration and metabolism through the generation of adenosine triphosphate (ATP). These dynamic organelles constantly undergo fusion and fission events, which are crucial for mitochondrial repair and the removal of damaged mitochondria, respectively. Mitochondrial impairment is linked to various conditions such as neurodegenerative diseases, metabolic disorders, cancer, aging,

and musculoskeletal disorders. The morphology of mitochondrial networks provides valuable physiological insights into cell metabolism.

Current studies investigating mitochondrial networks through fluorescence imaging typically use stains or labels, which have limitations such as long preparation times and potential toxicity. Imaging endogenous fluorophores, like NAD(P)H and flavins, offers a useful method for analysing mitochondria without these drawbacks. However, these studies often focus solely on intensity-based features, neglecting morphological information. Additionally, machine learning and deep learning have proven effective for accurate cell classification but are often inaccessible to researchers lacking programming skills.

In this study, we introduce AutoMitoNetwork, an open-source software tool for the image-based assessment of mitochondrial networks in label-free autofluorescence images. This tool characterises mitochondrial networks and detects mitochondrial impairment in retinal cells treated with rotenone or iodoacetic acid. We leverage the endogenous fluorescence of NAD(P)H, a co-enzyme predominantly found in mitochondria and essential for cellular energy production, for label-free imaging.

AutoMitoNetwork processes autofluorescence mitochondrial network images to generate a range of interpretable morphological, intensity, and textural features. These features, which are not easily discernible to the naked eye, are relevant to biological and metabolic mechanisms. We applied multi-dimensional feature analysis combined with a linear classifier to distinguish between healthy cells and those treated with rotenone or iodoacetic acid, achieving an area under the ROC curve of 0.82±0.01 and 0.84±0.02, respectively. Morphological features proved more informative and provided better classification compared to intensity and texture features alone. We hypothesize that this is due to the relationship between mitochondrial morphology and cell metabolism. This label-free, non-destructive method shows potential for use in mitochondria-based cell diagnostics, treatment monitoring, and drug development.

Label-Free Multispectral Autofluorescence Imaging for Melanoma Detection Using Feature-Based Machine Learning

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Melanoma is the most aggressive form of skin cancer and a leading cause of skin cancer-related deaths. Despite advances in treatment, prognosis for advanced-stage melanoma remains poor. Early detection is key, with 5-year survival rates exceeding 90% when diagnosed in early stages. However, current screening tools like dermoscopy rely heavily on pigmentation and clinical experience, making hypopigmented lesions such as amelanotic melanoma particularly difficult to diagnose. This presents a strong clinical need for reliable, non-invasive, and operator-independent diagnostic techniques.

In this study, we present a label-free multispectral autofluorescence imaging approach combined with machine learning to distinguish melanoma from healthy cells. Autofluorescence arises from endogenous fluorophores such as NAD(P)H and flavins that reflect the metabolic state of cells and are altered in cancer. By capturing these biochemical signatures, this method provides diagnostic signatures without the need for dyes or labels.

Immortalized fibroblasts (142BR), two melanoma cell lines (A375 – amelanotic; COLO679 – pigmented), and clinical patient cells were imaged using a custom-built microscope capturing 28 excitation-emission spectral combinations (excitation: 340–510 nm; emission: >391 nm). Preprocessing included background subtraction, device calibration, and smoothing. Over 3,000 features per cell were extracted across intensity, texture, and morphology categories using SMIAL, our in-house feature analysis software.

Classification models achieved excellent performance (AUC > 0.99) using seven features. To simulate fast, clinically feasible imaging, we reduced the feature set to two features based

on four channels, maintaining high accuracy (AUC > 0.96). These features included the optical redox ratio, a key indicator of metabolic state. Even without image preprocessing, classification remained strong (AUC > 0.9), demonstrating the method's robustness under simulated real-world conditions.

The approach was especially effective in identifying amelanotic melanoma (AUC > 0.96), which is often missed in conventional screening. By reducing imaging complexity and eliminating preprocessing, this technique supports development of compact, handheld devices for early melanoma detection.

Future work includes validating on primary tissues and additional subtypes, and integrating with other imaging modalities. This method holds strong potential for improving diagnostic accuracy, accelerating early intervention, and supporting personalized skin cancer care.

Azobenzene-Based Probe for Cell Imaging via Surface-Enhanced Resonance Raman Scattering Phenomenon

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Abstract: Resonance Raman (RR) probes have emerged as powerful tools for live-cell imaging, leveraging the strengths of Raman spectroscopy. Traditional Raman techniques often face challenges with overlapping spectral signals, complicating the precise identification of specific cellular components. To address this limitation, we developed an external RR probe based on azobenzene, featuring a tuneable absorption band and a low fluorescence quantum yield. These characteristics make the probe highly specific, sensitive, and versatile, enhancing detection sensitivity through resonance effects. Using a 532 nm excitation laser, an enhanced RR signal can be observed while reducing interference from native cellular components. This approach also supports simultaneous biomolecular mapping via spontaneous Raman scattering. Moreover, the choice of core-shell nanoparticles plays a significant role due to the ease of tunability of its plasmons, which overlap with the synthesized Raman probe as well as the

laser. We have achieved highly specific, rapid, and stable imaging of intracellular structures and molecular interactions by integrating appropriately chosen core-shell nanoparticles, a well-designed RR probe, and a precisely tuned Raman laser. The effectiveness of the proposed RR probe in combination with core-shell NPs was validated for Rat Brain Glioma cells, leading to the identification and imaging of cells through the Surface-Enhanced Resonance Raman scattering (SERRS) phenomenon.

Miniature Three-wavelength SFDI Endoscope for Early Pancreatic Cancer Detection

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Abstract: There is a clinical need for a cost-effective imaging device capable of detecting pancreatic cancer at early stages [1]. Here, we present a modified design for a miniature (3 mm diameter) endoscope prototype that uses Spatial Frequency Domain Imaging (SFDI) at three wavelengths (450, 515 and 660 nm) to achieve enhanced image contrast [2]. Spatial Frequency Domain Imaging (SFDI) is a low-cost optical imaging technique that works by projecting fringes over a sample and measuring the diffuse reflectance, from which optical properties that serve as cancer indicators can be calculated: the absorption and reduced scattering coefficients [3].

Previous work has miniaturised these devices to a level suitable for endoscopic deployment with two wavelengths (515 nm and 660 nm) [2]. However, the fringe patterns can only be modulated manually which creates relatively slow and unreliable performance, making it unfit for a real-time clinical usage. Here, we have integrated the novel fiber array project design into an automated system geared toward clinical use. The system is made up of a projector component consisting of a two-dimensional pitch-reducing optical fiber array with diameter of 0.125 mm, and a micro camera module of size 1x1 mm and resolution of 320x320 pixels, with pixel size of 2.4 µm. To automate the system, we introduce a Spatial Light Modulator (SLM) device (60 Hz frame rate), and we develop software to allow it to swap between patterns in < 1s. We also introduce a third wavelength (450 nm) to perform a second imaging technique called Narrow-Band Imaging (NBI), which enhances image contrast of blood vessels by providing differential contrast at two wavelengths of light (450 nm and 515 nm)

that probe absorption peaks of oxy- and deoxy-haemoglobin[4]. To validate the system's performance, we test the prototype on 16 calibrated BioPixS phantoms (10 mm thickness, 25 mm diameter) that span over a range of absorption (0.006 – 0.051 mm-1) and reduced scattering (0.116 – 0.506 mm-1) coefficients, and we use these findings to make an empirically derived Look-Up-Table to calibrate the system. These developments show the potential of the prototype to produce near real-time measurements of cancer indicators and provide improved imaging contrast in endoscopic applications.

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Silicon Nanowire Arrays covered with Metal Nanoparticles for SERS Sensing and Photocatalysis

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Metal nanoparticle/silicon hybrid structures are canonical substrates for photonic applications due to their high potential for interaction with light. In this work, enhanced electric fields around hexagonally arranged SiNWs interact with the electric fields around metal NPs at their surface. [1, 2] AuNPs are used to study such hybrid substrates for their efficiency in surface-enhanced Ra-man spectroscopy (SERS). The self-assembly of AuNPs synthesized via wet-chemistry on functionalized Si is a simple and cost-effective approach to prepare such substrates. High control over the deposition process of the AuNPs on flat (2D) or nanostructured (3D) Si sub-strates (vertically aligned SiNW arrays) is crucial for obtaining homogeneous surfaces. The large number of parameters that can be adjusted during the Si functionalization and the self-assembly steps have hindered the reliable and efficient use of this synthetic route. We will

report on the critical parameters that influence NP coverage, aggregation, and assembly sites in 2D and 3D to prepare substrates with homogeneous optical properties and SERS activity. In particular, we report the reproducible synthesis of nearly-touching (separated by nanoscale gaps) AuNP monolayer aggregates providing a uniform and large increase in Raman signal due to the high density of hot spots. The directed assembly of the AuNPs at specific locations along the SiNWs is demonstrated, along with its influence on the resulting SERS activity. We also report the self-assembly of AuNPs on VA-SiNW arrays, where the SiNWs form dimer-like structures providing even higher Raman signal enhancement. [1, 2, 3, 4] Additionally, we report increased hot electron generation rate in AuNPs/SiNW hybrid substrates by following the in-situ dimerization of 4-nitrothiophenol with Raman spectroscopy. Finally, we observed increased photoactivity of RuNPs/SiNW dimer arrays for CO2 methanation under light irradiation compared to flat Si and arrays composed of RuNPs/SiNW monomers. We attribute this to the enhanced E-field generated within and at the surface of the RuNPs/SiNW dimer arrays. [3, 4, 5, 6]

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Nanoparticle-Enhanced SERS for Rapid Detection of DNA Methylation for Cancer Diagnostics

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Label-free surface-enhanced Raman scattering (SERS) offers a promising approach for the rapid analysis of DNA methylation markers, with application in cancer screening. However, a deeper understanding of the factors influencing DNA adsorption onto metal surfaces and the identification of reliable SERS bands indicative of DNA methylation levels remain essential.

In this study, we first demonstrated that cations such as Ca^{2+}, Mg^{2+}, Be^{2+}, Zn^{2+}, and Al^{3+} play a key role in facilitating DNA adsorption onto silver nanoparticles (AgNPs). These cations reduce the electrostatic repulsion between the negatively charged DNA and the nanoparticles, allowing the SERS spectrum of DNA to become detectable. Through SERS analysis, we identified a strong positive correlation ($R_{Pearson}$ = 0.94, p = 0.005) between the intensity of the cytosine SERS band at 790 cm^{-1} and the level of 5-methylcytosine (5mC) in genomic DNA samples from both benign and malignant cell lines. This relationship enabled the distinction of cancerous DNA through its characteristic global hypomethylation profile. Furthermore, we demonstrated the ability to monitor methylation levels based on the 790 cm^{-1} SERS band in a 180 bp DNA sequence from the SEPT9 gene promoter, a clinically validated methylation marker for colorectal cancer.

Additionally, we tracked DNA adsorption onto AgNPs using SYBR Green, a fluorescent molecule that binds to unbound DNA. Our observations indicated that hypermethylated genomic DNA exhibited a stronger affinity for the metal surface compared to unmethylated DNA. Consequently, DNA derived from malignant cells, which in the most cases present a lower global methylation level, show a lower adsorption affinity onto AgNPs than DNA from nonmalignant cells. The differential adsorption behavior highlights the potential of using AgNPbased platforms to distinguish between malignant and benign genomic DNA based on their methylation profiles.

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Study of Highly Potential SERS-Active Substrates for Detection of Biomolecular Substances

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Abstract: The growing need for reliable and robust biochemical detection methods, especially for human health and safety, continues to drive advancements in sensor technologies. Biological, spectroscopic, and imaging techniques are commonly employed to detect biomolecules. Over the past two decades, surface-enhanced Raman spectroscopy (SERS) has emerged as a promising technique for ultrasensitive analysis in biomedical and agri-food applications. SERS enables the detection of molecules at extremely low concentrations, including proteins, amino acids, blood components, and bacteria. Additionally, it facilitates the identification of single cells and contributes to disease diagnosis by providing detailed structural information about biological analytes. However, there remains a critical need for optimized nanostructured surfaces that enhance plasmonic resonance effects, significantly amplifying the scattered light signal by several orders of magnitude compared to conventional Raman spectroscopy.

In this study, we investigate the plasmonic effects of an innovative, high-performance SERS substrate composed of carbon and metallic nanostructures. This substrate exhibits a SERS enhancement factor approximately four orders of magnitude higher than those reported in the literature. The enhancement of SERS intensity, and consequently the reliable detection of target biomolecules, is achieved through the interaction between the analyte, nanostructures, and the surface, generating "hot spots" where the Raman signal is significantly amplified.

Building upon these findings, we will explore the potential of the SERS technique for advanced applications, including the detection of amino acids, peptides, and proteins, to identify diseases such as cancer, cardiovascular disorders, and dental conditions. Furthermore, the-

oretical simulations will be employed to complement and validate the experimental results, facilitating the interpretation of complex spectra.

Keywords: SERS, Raman, nanoplasmonics, biomolecules, nanotechnology, detection.

Advancing Correlative Cell Imaging within the EU-project NanoVIB: Integrating Multi-Modal Microscopy for Nanoscale Resolution

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Abstract: NanoVIB, an EU-funded research initiative, pioneers the integration of three advanced cell imaging modalities to achieve unprecedented spatial, metabolic, and biochemical insights at the nanoscale. This approach combines super-resolution MINFLUX Nanoscopy with Stimulated Raman Scattering (SRS) microscopy and Two-Photon Excitation (TPE) Autofluorescence Imaging. Together, these techniques enable comprehensive cellular analysis within morphological context while achieving protein localization at a few nanometres resolution. In this study, we present the successful establishment of all three imaging modalities and demonstrate their first correlative application on fixed lung epithelial cells (A549) (Figure1). The SRS modality provides label-free biochemical imaging (Figure1B), while TPE autofluorescence (Figure1C) reveals metabolic states through intrinsic NAD(P)H fluorescence. Incorporating a time-resolved photon counting module within the TPE modality, we also obtained fluorescence lifetime imaging (FLIM) (Figure1E), further correlating cellular metabolism with biochemical features. Furthermore, by TPE-scanning of the same cell at two different speeds, photo-induced dark state transition rates (TRAST) in NAD(P)H could be correlatively imaged (Figure1D). Thereby, we can integrate TPE-TRAST and FLIM data with SRS biochemical maps and MINFLUX-resolved protein distributions (Figure1G) at specific fields of view within the same cellular context. Our results thus establish correlative multi-modal imaging which can help in elucidating intricate cellular processes at nanometre resolution.

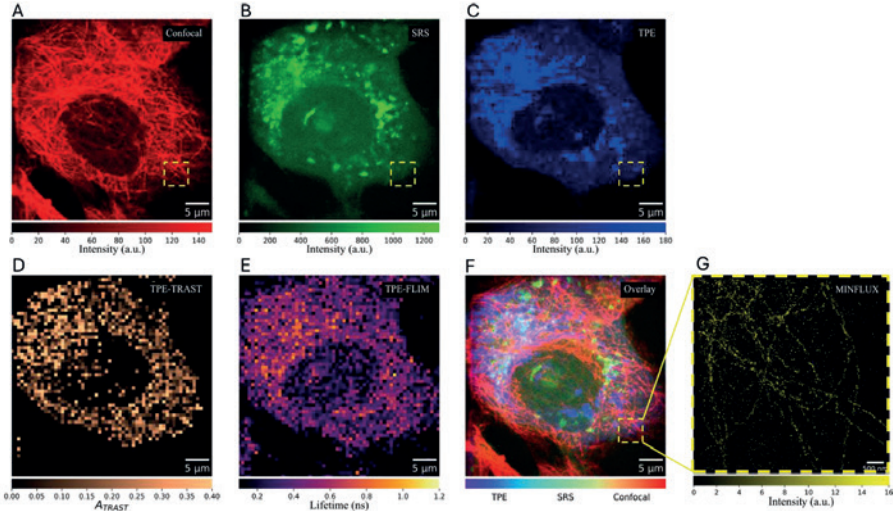

Figure 1: Correlative imaging of lung epithelial (A549) cells, combining confocal (A), SRS (B), TPE (C), TPE-TRAST (D), TPE-FLIM (E) images. Overlay image of confocal, SRS and TPE images (F). The MINFLUX image of the selected ROI (G), indicated by a yellow rectangle in A, B, C and F.

Orbital Angular Momentum Light in Complex Media: Propagation, Preservation, and Applications

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Abstract: We investigate twisted light propagation in turbid media, demonstrating phase memory retention despite microscopic scattering. Simulations and experiments reveal high refractive index sensitivity (10^{-6}) and a novel phase preservation framework, benefiting biomedical imaging, secure optics, and precision applications.

1. Introduction: The propagation of structured light in complex media remains a key challenge in modern optics [1]. Orbital Angular Momentum (OAM) light, with its helical wavefront, offers promising applications in optical sensing and information encoding, yet its behavior in turbid media, particularly phase preservation, remains underexplored. This study examines OAM stability in tissue-like scattering environments.

OAM has gained interest in biophotonics and biomedical optics for tissue diagnostics, imaging, and advanced microscopy [2], while also impacting optical communication [3], micro-particle manipulation [4], and optical tweezers [5,6]. However, scattering media like biological tissues introduce nanoscale refractive index variations [7], causing wavefront distortions that challenge conventional models such as effective medium theory [8] and radiative transport theory [9].

Using a custom Monte Carlo (MC) modelling approach [10], we analyze OAM beam propagation, focusing on coherence, polarization, interference, and wavefront distortions. Our findings enhance understanding of structured light behavior in scattering environments, advancing applications in optical communication and biomedical sensing.

2. Materials and methods: Our experimental configuration implements a modified Mach-Zehnder interferometer utilising a coherent light source of 633 nm. Laguerre-Gaussian (LG) beams with varying topological charges are simulated via a spatial light modulator [11]. Custom-designed tissue phantoms with controlled optical properties, including scattering coefficient (μs), absorption coefficient (μa), anisotropy factor (g) and refractive index (n) formulate the scattering medium.

Numerical modeling of light propagation in turbid media, including the atmosphere, fluids, and biological tissues, is challenging due to multiple scattering effects. MC method remains the gold standard for accurate simulations [12,13].

In its essence, by iterative tracking of photon packets' parameters such as position, direction, statistical weight, polarization, and orbital angular momentum (OAM) [14-16], this framework provides a highly detailed and realistic depiction of light-tissue interactions. Its advanced capabilities make it a robust tool for applications such as photodynamic therapy [17], coherent back-scattering [18,19], biomedical imaging and polarized and complex light propagation in

tissue-like scattering media [20-24]. However, traditional implementations often demand substantial computational resources, relying on multiple GPUs and consuming significant energy to achieve the required accuracy, making them less suitable for OAM simulations. To overcome these limitations, we enhanced our in-house simulation framework with advanced features to improve accuracy and efficiency [25]. It now includes full polarization tracking, phase evolution modeling, and coherent interference effects, enabling highly detailed light transport simulations in turbid media. The framework also models spatial coherence and polarization state evolution, providing a comprehensive view of light dynamics, including intensity, phase, and polarization changes.

A key innovation is its energy-efficient implementation, optimized for modern hardware like Apple's M-family chips, which offer high performance with low power consumption. Additionally, it accurately simulates phase relationships between scattered photons, crucial for modeling complex photon transport.

This framework matches the accuracy of traditional MC methods while significantly reducing computational time and energy consumption. By minimizing reliance on energy-intensive GPUs, it provides a sustainable, high-performance alternative for photon transport simulations, advancing efficient stochastic modeling techniques.

3. Results and discussions: Our results establishes that OAM light preserves its phase structure despite the presence of highly scattering media and this preservation is particularly demonstrated in the axial annular region, wherein the initial phase modulation remains detectable within the resulting speckle patterns (as depicted in Fig. 1 – left). The key observations include the maintenance of helical phase structure in low scattering regimes ($d/l^* \approx 1$ and <5), partial phase preservation in multiple scattering environments ($d/l^* > 5$), distinct phase memory effects in the axial annular region and correlation between initial and preserved phase patterns. Here, d and l^* are, respectively, the thickness of scattering medium and mean free photon path within the medium. The observed phase preservation (see Fig.1 – right) can be attributed to rotational symmetry of photon trajectories, coherent addition of scattered fields and spatial correlation maintenance in the axial region [11,12].

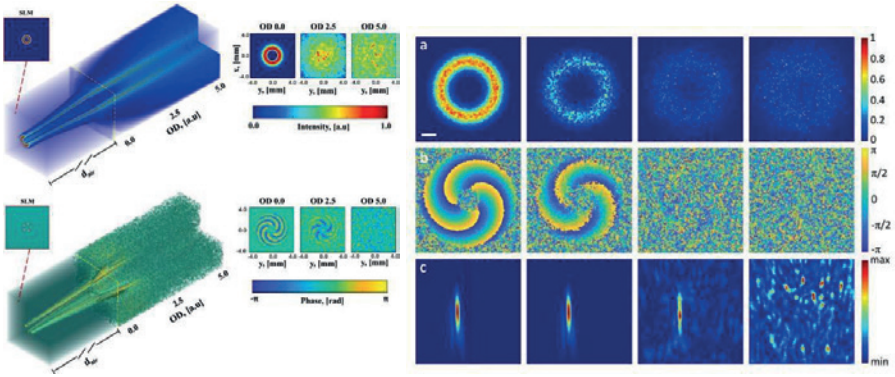

Fig. 1. Left: propagation of LG beam with minimal distortion and preserving the original OAM state: Intensity (top) and phase (bottom) distributions of LG^5 beam along propagation by spatial light modulator (SLM) into the complex scattering medium [12]. Right: Intensity (a) and phase (b) distributions of LG^3 beam after propagation through a complex scattering medium with 1 mm thickness and specified scattering coefficients μs= 2,4,6,10 mm^{-1}; absorption coefficient μa= 0.01mm-1 and anisotropy factor g=0.8. Row (c) depicts intensity output of the OAM [12]. Dotted red line on left figure indicates position of the intensity peak which corresponds to the Gaussian beam without OAM. The scale bar in (a) represents 0.5 mm, which applies to all images.

Our findings demonstrate significant potential in biomedical imaging for non-invasive diagnostic capabilities with enhanced penetration depths and potential for glucose monitoring and improved tissue characterization. Additionally, the field of optical communications can be enhanced with robust information transfer via scattering media, better security via phase encoding and maintained signal intensity in complex environments. Furthermore, sensing technologies can have high-precision refractive index measurements, structural change detection and early disease diagnosis potential.

4. Summary and Conclusions: In this study, we examined the propagation and phase preservation of OAM light in turbid, tissue-like scattering media. Using an in-house Monte Carlo method combined with experimental validation, we demonstrated that OAM light maintains phase memory even in highly scattering environments at the microscopic scale. This innovative approach to phase preservation analysis revealed remarkable sensitivity to refractive index variations at this scale, providing new insights into light-matter interactions in complex media [26].

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Enhanced In Vivo Imaging via Implantable Micro-Optics on a Miniaturized Imaging Window

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In the realm of biomaterial, small-molecule, and drug testing, intravital fluorescence microscopy facilitates in vivo quantification of immune responses, thereby reducing the number of laboratory animals needed for statistically significant validation. Despite advancements in non-linear microscopy technologies, in vivo fluorescence microscopy encounters challenges such as limited tissue penetration due to light scattering and optical aberrations caused by surrounding animal tissue1. To address these issues,

we developed a miniaturized integrated imaging window (Fig.1B), consisting of an implantable system of micro-optics coupled with microscaffolds to guide cell and tissue integration (Fig.1A, C). Using fluorescence beacons, this microstructured device functions as an advanced in situ microscope objective aimed at correcting spherical aberrations, thus overcoming in vivo imaging limitations and enabling longitudinal high-resolution observations within the same animal. The integrated device is fabricated using a novel one-step procedure involving Multi-Photon Polymerization (MPP) of a biocompatible photoresist named SZ20802. This method includes the initial microfabrication of the microscaffolds, followed by the continuous MPP of the microlens outer surface followed by the UV bulk polymerization of their inner core, significantly enhancing manufacturing efficiency and precision3. Preliminary characterization of the imaging capabilities of our implantable optical system on live cells was followed by in vivo validation through implantation in living chicken embryos. By coupling the device with low magnification objectives, we demonstrated the ability to perform magnified, high-resolution, and high signal-to-noise ratio acquisitions of integrated tissue through the microlenses. This enabled collagen-I formation and tissue integration visualization via non-linear excitation microscopy through the implanted optics in healthy chick embryos (Fig.1D, E). These remarkable results clear the path for intravital evaluation of capillaries in the context of angiogenesis and in response to administered vascular drugs if using drug-treated embryos model (e.g., doxorubicin). In conclusion, our innovative approach enhances speed and accuracy, providing an effective method for creating advanced microscale optical systems for improved in vivo imaging. This paves the way for the development of next-generation implantable diagnostic and therapeutic platforms.

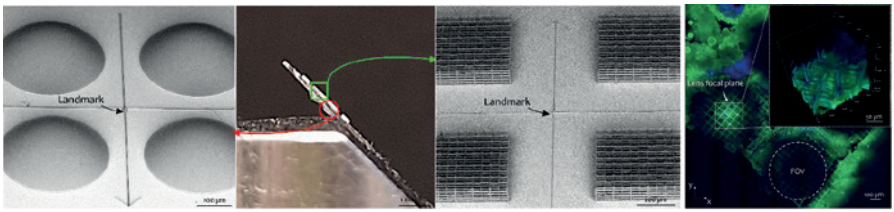

Figure 1. Set of three images of the double sided microstructured device. The central figure (B) shows a picture of the integrated device where are clearly visible both the constructs: four parabolic microlenses built on top of a microscaffold each. (A) SEM image of four parabolic microlenses; (C) SEM image of four microscaffolds; D) Stitched TPEF image of the whole microstructured area. TPEF wide field image collected at the objective focal plane where the black shadows highlight the presence of the parabolic microlenses above. TPEF image collected thorugh the parabolic microlens focal plane is superimposed as max projection to the groud (white dotted square); E) 3D reconstruction of the microstructure volume and the integrated tissue through the parabolic microlenses. Collagen Type I, in blue (400/40 nm), while in green (535/50 nm), tissue autofluorescence.

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Fiber Optic Sensors for Real-Time Measurements

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Technological advances in physics, electronics, computer science, materials science, and engineering have enabled the development of optical sensors specifically designed for industrial, medical, and research applications. Among these emerging technologies, optical fibers – cylindrical dielectric waveguides that transmit information as light through a core surrounded by cladding – have profoundly impacted the sensor community. Optical fibers offer several advantages, including flexibility, compact size, low weight, and immunity to electromagnetic interference. These features and their ability to support point, distributed, and quasi-distributed real-time monitoring have garnered significant interest.

In this work, optical fiber Fabry–Pĕrot interferometers were investigated and employed to develop in-line, compact, and easy-to-fabricate sensors for real-time applications. Novel configurations are proposed and experimentally demonstrated for measuring strain, room temperature, relative humidity, and refractive index with enhanced performance.

Confined Spaces Made by two Photon Polymerization for Cancer Cell Behavior Evaluation

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Cancer cell invasion through confined spaces leads to cell-specific morphological deformations, strongly influencing the migration mechanisms during cancer metastasis processes. Herein, we propose polymeric tissue-like scaffolds consisting of narrow confined micropores by two photon polymerization (TPP), suitable for testing the invasive behavior of melanoma cancer cells. Using a Nanoscribe GmbH platform with 780 nm femtosecond laser and 80 MHz repetition rate, we could fabricate woodpile-like 3D scaffolds with variable in-between lines dimensions in micrometer-range in SU8 photoresist. These scaffolds were further coated with collagen to better mimic the bioenvironment for testing the invasiveness potential of melanoma cancer cells in constrictive spaces (Figure 1(a)). Fluorescence microscopy revealed the cellular adhesion and invasiveness within the scaffold that were quantitatively evaluated by analyzing cellular nuclei, cytoskeleton and focal adhesion points (Figure 1(b)). We noticed two times higher cellular affinity around collagen-coated scaffolds based on the number of cell nuclei and number of focal adhesion points anchoring around the borders or inside the scaffolds. Additionally, we aimed to correlate the cancer cell invasiveness potential in confined spaces with their motility in unrestricted environment by performing brightfield time lapse microscopy for cell trajectories evaluation. Increased spreading area as well as mean migration velocity were found for melanoma cancer cells grown on collagen coated samples. Our studies have evidenced a higher cellular motility on collagen-coated substrates that correlates with the cell invasiveness within collagen-coated scaffolds.

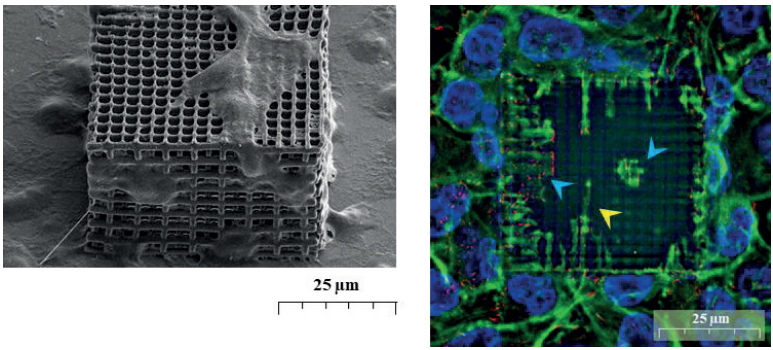

Figure 1 (a) Scanning electron microscopy image of woodpile-like 3D scaffold fabricated by two photon polymerization interacting with melanoma cells which develop (b) multiple cytoskeleton filopodia and invade the submicrometer narrow spaces

Combined Study of Retinal Pigment Epithelium (RPE) Using Nanoscale Mid-Infrared Spectroscopic Imaging and High-Resolution Fluorescence Microscopy

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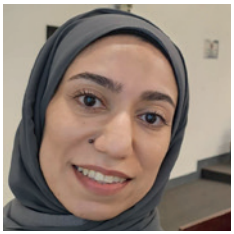

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Mid-Infrared Photo-Induced Force Microscopy (PiF-IR) combines powerful IR illumination with non-contact atomic force microscopy, achieving high spectral and unprecedented spatial resolution (< 5 nm).[1] Several unguided chemometric approaches have been developed for the analysis of PiF-IR hyperspectral datasets revealing the surface chemistry of biological and biomedical samples. The main chemical variation within the high-dimensional PiF-IR data is obtained using Principal Component Analysis (PCA) and compared to clusters extracted using Hierarchical Cluster Analysis (HCA). Additionally, Topological Data Analysis (TDA) has been trained as a novel approach for extracting chemical information from the data sets.

We applied PiF-IR to cross-sections of human retinal pigment epithelium (RPE) from an 80 years old donor who suffered of Age-related Macular Degeneration (AMD), a condition whose

underlying chemistry is not fully understood. We found a pronounced variation in the protein content on the surface of cell organelles at different positions in the highly polarized RPE cells. A previous study has classified the types of autofluorescent pigment organelles in three groups containing a total of nine subgroups by applying Structured Illumination Microscopy (SIM) to RPE flat mounts and cross-sections of human donors.[2] Within our project (HiRe-si4RPE, 542825796) funded by the German Research Foundation (DFG), we will combine PiF-IR with SIM and high-resolution Fluorescence Lifetime Imaging Microscopy (FLIM) and compare the results with clinical investigation aiming at new insights into the chemical modifications causing cell death leading to the loss of sight in AMD.

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Optimization of Fast Light Sheet Fluorescence Lifetime Imaging Microscopy for Metabolic Imaging

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Metabolic imaging is essential for understanding dynamic biological processes including cellular development and diseases [1,2]. However, traditional approaches used to measure and quantify metabolism rely on NADH fluorescence lifetime estimation and are thus limited by factors such as slow acquisition speed or poor signal-to-noise ratio (SNR) [3,4]. In our lab, we aim to develop non-linear optics imaging techniques to measure the metabolic transitions at cellular level with enhanced speed and performance in general [5-7].

Fluorescence lifetime imaging microscopy (FLIM) and two-photon light sheet microscopy techniques have both been commonly used to study dynamic processes in living biological systems cue to their ability to visualize and quantify different metabolic species and dynamic changes with improved acquisition speed and low photodamage in a label-free, noninvasive experimental condition. In this work, we focus on developing and optimizing fast FLIM with light sheet microscopy system for metabolic imaging applications in living zebrafish embryos and organoids. To achieve the most optimized performance of the system, we conduct a systematic numerical simulation analysis to evaluate the trade-offs between imaging speed, SNR, relative errors of the measurements and temporal resolution. By varying gating configurations in our time-gated FLIM setup, photon counts, and data analysis methods, we identify optimal parameters to precisely and efficiently extract metabolic information. Next, we show preliminary experimental results on fluorescent beads with various fluorescence lifetimes to evaluate and discuss the capability of the setup to separate distinct fluorophores.

Our work provides a robust framework for optimizing fast FLIM with light sheet microscopy, offering new insights into studying metabolic processes in vivo in living organisms in the future.

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3D Printed Encapsulated Microlens for Endoscopic Optical Coherence Tomography

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Spinal cord injuries disrupt communication between brain and body, causing permanently irreversible impairments in motor function, sensation, and autonomic regulation below the site of injury. High-resolution and real-time structural imaging may improve capabilities to assess damage and treatment effects, and to guide therapeutic interventions aimed at preserving neurological function and enhancing recovery.

However, imaging the spinal cord and its surrounding structures is challenging due to their diminutive size and the surrounding bony anatomy. Optical coherence tomography (OCT) has emerged as promising, minimally invasive in vivo diagnostic tool, capable of acquiring micron-scale, depth-resolved images of tissue. OCT endoscopic probes have the potential to assist in diagnosis and treatment guidance by enabling the visualization of anatomical spaces within the spinal canal, including nerve rootlets and the spinal cord. While conventional endoscopic OCT probes have limited resolution, mechanical stability, and depth of focus (DOF), two-photon 3D printing can produce high-quality microoptics with exceptional precision, e.g., arbitrary freeform optics with improved DOF. In particular, side-looking freeform optics enable the acquisition of 3D radial images by rotating the endoscopic probes in various anatomical regions. Such designs commonly exhibit a high degree of asymmetry, resulting in rotational instability and increased fragility upon mechanical stress. A common method to protect the microlens on the fiber tip involves encasing the miniaturized optics in optically transparent tubes. However, this introduces multiple additional interfaces, which can reduce signal strength and increase optical aberrations. We present a novel OCT-probe, featuring a 3D printed encapsulated microlens onto the tip of an optical fiber. It enhances robustness of the miniaturized

optics, avoids introducing additional interfaces, features high stability and rotational symmetry, and achieves high optical performance. The microlens design is aberration-corrected for the catheter sheath and generates a large DOF (970 µm) while maintaining a high lateral resolution (13 µm). This enables real-time imaging of the tissue microstructure.

We demonstrate the 3D printed endoscopic device in vivo in the spinal intrathecal space of an anaesthetised pig and were able to image anatomical structures using a rotational pull-back procedure, including the vertebral body, spinal cord, and nerve roots. We further expect its advanced design to enable superior performance in minimally invasive diagnostic in vivo applications, setting the stage for more precise interventions in clinical settings.

Thermal Effects on the Quality Parameters of Extra Virgin Olive Oil using Fluorescence Spectroscopy

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Mirpur University of Science and Technology, Mirpur, Pakistan

Extra virgin olive oil is one of the superlative due to its health benefits. In this work, the Fluorescence spectra of extra virgin olive oil (EVOO) from different olive growing regions of Pakistan and Al-Jouf region from the Kingdom of Saudi Arabia (KSA) were obtained. The emission bands depicted relative intensity variations in all non-heated and heated EVOO samples. Prominent emission bands at 385, 400, 435 and 470 nm represent oxidized products of fatty acids, bands at 520 and 673 nm has been assigned to beta carotene and chlorophyll isomers respectively. All EVOO samples collected from Al-Jouf region, KSA and from Pakistan (Loralai Baluchistan, Barani Agricultural Research Institute, Chakwal and Morgha Biodiversity Park, Rawalpindi) regions showed thermal stability. Other EVOO samples from Chaman Baluchistan and one sample from wild species (Baluchistan) bought directly from farmers showed denatured spectra even without heating. Chemical characteristics of all EVOO samples changed significantly at 200 °C. Relatively, EVOO samples from Al-Jouf showed more thermal stability which might be due to geographical distribution, environmental effects, genetic background and processing or storage conditions. These results demonstrated fluorescence spectroscopy as a quick, cost-effective and reliable approach to assess the quality and thermal stability of EVOO. These characteristics of fluorescence spectroscopy may lead to the development of portable device for the onsite monitoring of EVOO.

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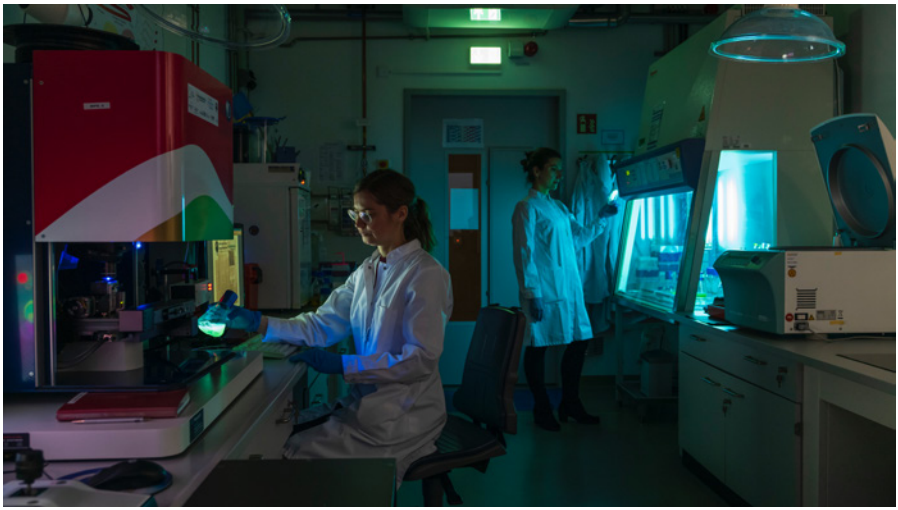

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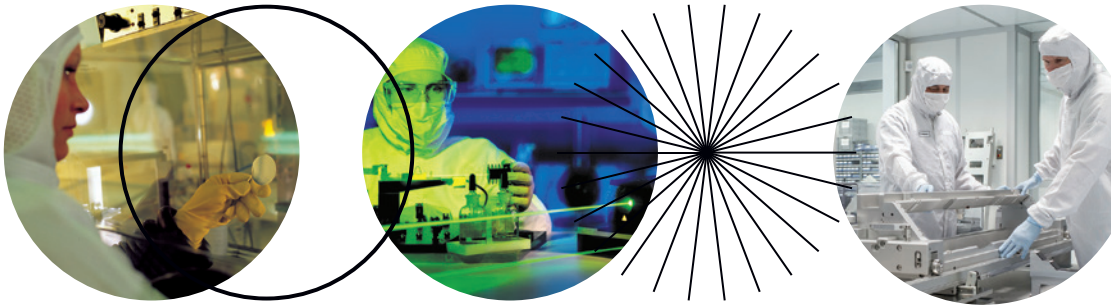

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