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ACADEMY of HIGHER EDUCATION

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# **BRICS Workshop Biophotonics - 2024**

**October 03 - 05, 2024**

**Organized by**

**Department of Atomic and Molecular Physics**

**Manipal Academy of Higher Education**

**Saratov State University, Russia**

**Hainan University & Huazhong University of Science and  
Technology, China**

**University of São Paulo, Brazil**

**University of Johannesburg, RSA**



# **BRICS WORKSHOP ON BIOPHOTONICS - 2024**

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**Department of Atomic and Molecular Physics, MAHE, Manipal, India  
Saratov State University, Russia  
Hainan University & Huazhong University of Science and Technology, China  
University of São Paulo, Brazil  
University of Johannesburg, RSA**

**Venue: Silver Jubilee Hall, Manipal School of Life Sciences (MSLS) Annex,  
Silver Jubilee Block, MAHE, Manipal – 576 104**

## ***Chairs:***

**Dr. Santhosh Chidangil, MAHE, Manipal, India  
Dr. Valery V. Tuchin, Saratov State University, Russia  
Dr. Qingming Luo, Hainan University, China  
Dr. Vanderlei Salvador Bagnato, University of São Paulo, Brazil  
Dr. Heidi Abrahamse, University of Johannesburg, RSA**

## ***Co-Chairs:***

**Dr. Nirmalya Ghosh, IISER, Kolkata  
Dr. Elina A. Genina, Saratov State University, Russia  
Dr. Dan Zhu, Huazhong University of Science and Technology, China  
Dr. Cristina Kurachi, University of São Paulo, Brazil  
Dr. Michael Hamblin, University of Johannesburg, RSA**

## ***Secretaries:***

**Dr. Jijo Lukose, MAHE, Manipal, India  
Dr. Polina A. Timoshina, Saratov State University, Russia  
Dr. Dongyu Li, Huazhong University of Science and Technology, China  
Dr. Natalia M. Inada, University of São Paulo, Brazil  
Dr. Sathish Kumar, University of Johannesburg, RSA**

## PROGRAMME SCHEDULE

DAY -1: 3 <sup>rd</sup> OCTOBER (THURSDAY)-2024		
08.00 AM - 08.45 AM	<b>Registration</b>	
08.45 AM - 09.25 AM	<b>Breakfast</b>	
09.30 AM- 09.35 AM	Welcome Address by <b>Dr. Santhosh Chidangil</b> Convener and India Chair, BRICS workshop on Biophotonics - 2024	
09.35 AM- 09.40 AM	Overview of the BRICS Conference by <b>Dr. Heidi Abrahamse</b> South Africa Chair BRICS workshop on Biophotonics - 2024	
09.40 AM- 09.50 AM	Inauguration and Inaugural Address by <b>Lt. Gen. (Dr.) M. D. Venkatesh</b> Vice Chancellor, MAHE, Manipal	
09.50 AM- 09.55 AM	<b>Messages from BRICS Chairs</b>  <b>Biophotonics Pioneers from India</b>	
09.55 AM- 10.00 AM	Vote of Thanks by <b>Dr. Sajan D. George</b> Convener, BRICS workshop on Biophotonics - 2024	
10.00 AM – 10.40 AM	<b>Keynote Address by:</b>  <b>Dr. Heidi Abrahamse</b> University of Johannesburg, South Africa  <b>Chair:</b> <b>Prof. Murukeshan Vadakke Matham,</b> NTU Singapore	Phthalocyanine-Based Probes for Alleviating or Evading Tumour- Hypoxia for Enhanced Photo- and Sono-Mediated Therapy
10.40AM-11.00AM	<b>Tea break</b>	
<b>Session 1</b>		
<b>Session Chair: Dr. Satish Rao</b>		
11.00 AM – 11.40 AM	Invited Lecture by <b>Dr. Samir Kumar Pal</b> S N Bose National Centre for Basic Sciences Kolkata	“Probing” Spectroscopic Probes for Non-invasive Simultaneous Disease Diagnosis

11.40 AM – 12.20 PM	Invited Lecture by <b>Dr. Sangeeta Kale</b> Defence Institute of Advanced Technology, Pune, India	Non-Invasive Optical Sensor Setup for Health Monitoring: A Device Perspective
12.20 PM – 01.00 PM	Invited Lecture by <b>Dr. Dalip-Singh Mehta,</b> IIT Delhi, India	Optical Biopsy Assisted with AI/ML: Multimodal and Multispectral Optical Techniques for Real-time Screening

01.30 PM – 02.30 PM	<b>Lunch Break</b>	
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## Session 2

**Session Chair: Dr. Samir Kumar Pal**

02.30 PM – 03.10 PM	Invited Lecture by <b>Dr. Chandrabhas Narayana</b> Rajiv Gandhi Centre for Biotechnology Thiruvananthapuram, India	Protein structure-function, drug discovery and diagnostics with Raman spectroscopy
03.10 PM – 03.50 PM	Invited Lecture by <b>Dr. Blassan George</b> University of Johannesburg, South Africa	Pheophorbide-a Mediated Photodynamic Therapy in breast and lung cancer cells in vitro
03.50 PM – 04.20 PM	Invited Lecture by <b>Dr. Anine Crous</b> University of Johannesburg, South Africa	Photobiomodulation for Enhanced Differentiation of Adipose-Derived Stem Cells into Brain Organoids and Osseous Tissue
04.20 PM – 04.35 PM	Tea Break	
04.35 PM – 05.15 PM	Invited Lecture by <b>Mr Brendon Roets</b> University of Johannesburg, South Africa	Progressing Stem Cell Regenerative Therapy via Photobiomodulation to Facilitate Tenocyte Differentiation.

**Program of the 3rd BRICS Workshop on Biophotonics October 03 –05, 2023, Manipal, INDIA**

**Online session (Moscow time (UTC+3)/Brazil time/India time/RSA time/China time**

**Day 1:**

<https://zoom.us/j/92134283523?pwd=TPa3EHSvwlQd8JNqAgUoiwlatthkAP.1>

**Passcode: 835556**

**Session Chair: Dr. Qingming LUO, Hainan University, China**

**Dr. Santhosh Chidangil, Manipal Academy of Higher Education, India**

15.25-15.45/9.25-9.45 /17.55-18.15 15.25-15.45/20.25-20.45	Welcome words from <b>Prof. Valery V.Tuchin</b> , Institute of Physics and Science Medical Center, Saratov State University and <b>Dr. Qingming LUO</b> , Hainan University, China.	
15.45-16.05/9.45-10.05 /18.15-18.35 14.45-15.05/20.45-21.05	Invited Lecture <b>Dr. Yao He</b> , Key Laboratory of Optic-Electric Sensing and Analytical Chemistry for Life Science, MOE, Soochow University, China	Fluorescence imaging for precision diagnosis and treatment of diseases
16.05-16.25/10.05-10.25 /18.35-18.55 15.05-15.25/21.05-21.25	Invited Lecture <b>Dr. Valery V. Tuchin</b> , Institute of Physics and Science Medical Center, Saratov State University, Institute of Precision Mechanics and Control, FRS "Saratov Scientific Centre of the RAS", Saratov, Russia; Laboratory of Laser Molecular Imaging and Machine Learning, Tomsk State University, Tomsk, Russia	Biophotonics has acquired windows of transparency of biological tissues from UV to THz waves

**DAY-2: 4<sup>th</sup> OCTOBER (FRIDAY)-2024**

<b>8.00 AM-9.00 AM</b>	<b>Breakfast</b>	
<b>Session 1</b>		
<b>Session Chair: Dr. Chandrabhas Narayana</b>		
09.00 AM – 09.40 AM	Invited lecture by <b>Dr. Gautham K Samanta</b> , Photonic Sciences Laboratory Physical Research Laboratory (PRL), Ahmedabad-380009, India	Quantum imaging of biological sample using Hong-Ou-Mandel interferometry
09.40 AM – 10.20 AM	Invited Lecture by <b>Dr. Vincent Mathew</b> , Central University Kerala, India	Topological Photonics: Concepts and Applications
10.20 AM – 11.00 AM	Invited Lecture by <b>Dr. Hari M Varma</b> Indian Institute of Technology Bombay India	A novel approach based on stochastic calculus for laser speckle imaging

11.00 AM - 11.15 AM	<b>Tea Break</b>	
<b>Session 2</b>		

<b>Session Chair: Dr. Krishna K Mahato</b>		
11.15 AM – 11.55 PM	Invited Lecture by <b>Dr. C. Murali Krishna</b> ACTREC, Mumbai, India	Serum Raman Theranostics: Perspectives and Outlook
11.55 AM – 12.35PM	Invited Lecture by <b>Dr. Sajan George</b> Vellore Institute of Technology India	Generation of Functional Neurons by Photobiomodulation
12.35 AM – 1.15 PM	Invited Lecture by <b>Dr. AVR Murthy</b> Defence Institute of Advanced Technology (DIAT), Pune, India	Construction of light sheet fluorescence microscope (LSFM) for biophotonic imaging applications
1.15 PM – 02.15 PM	<b>Lunch Break</b>	
<b>Session 3</b>		
Chair: <b>Dr. M.K. Satheesh Kumar</b>		
02.15 PM- 02.55 PM	Invited Lecture by <b>Dr. Santhosh Chidangil</b> Manipal Academy of Higher Education Manipal, India	Single cell spectroscopy of blood components using micro-Raman combined with optical tweezers.
02.55 PM – 04.00 PM	<b>POSTER SESSION</b>	
04.00 pm- 04.15pm	<b>Tea break</b>	
<b>DAY-2, 4<sup>th</sup> OCTOBER (SATURDAY)-2024</b>		
<b>ONLINE SESSION</b>		
<b>Day 2:</b> <a href="https://zoom.us/j/92416948601?pwd=oTi9HatmTF8lmwWygSiL2j7HvSQ4kp.1">https://zoom.us/j/92416948601?pwd=oTi9HatmTF8lmwWygSiL2j7HvSQ4kp.1</a> <b>Passcode:</b> 542568		
<b>Session Chair: Valery V. Tuchin</b> , Saratov State University, Russia <b>Santhosh Chidangil</b> , Manipal Academy of Higher Education, India		
13.45-14.05/7.45- 8.05 /16.15-16.35 12.45-13.05/18.45- 19.05	Invited Lecture <b>Dr. Dan Zhu</b> , Wuhan National Laboratory for Optoelectronics, Huazhong University of Science, China	Tissue optical clearing imaging: from in vitro to in vivo

14.05-14.25/8.05-8.25 /16.35-16.55 13.05-13.25/19.05-19.25	Invited Lecture <b>Dr. Xuantao Su</b> , School of Integrated Circuits, Shandong University, China	Intelligent imaging flow cytometry for label-free analysis of single cells and exosomes
14.25-14.45/8.25-8.45 /16.55-17.15 13.25-13.45/19.25-19.45	Invited Lecture <b>Dr. Ping Xue</b> , Department of Physics, Tsinghua University, China	Multifunctional OCT for intraoperative tumor diagnosis and rapid pathology
14.45-15.05/8.45-9.05 /17.15-17.35 13.45-14.05/19.45-20.05	Invited Lecture by <b>Dr. Siwen Li</b> , State Key Laboratory of Natural Medicines, China Pharmaceutical University, China	Multimodal collaborative tumor precision therapy based on phototherapy
15.05-15.25/9.05-9.25 /17.35-17.55 14.05-14.25/20.05-20.25	Invited Lecture <b>Dr. Wei Chen</b> , School of Mechanical Science and Engineering, Huazhong University of Science and Technology	High spatiotemporal resolution multiphoton microscopy for brain imaging
15.25-15.45/9.25-9.45 /17.55-18.15 14.25-14.45/20.25-20.45	Invited Lecture <b>Dr. Irina V. Semenova</b> , Ioffe Institute of the Russian Academy of Sciences, St. Petersburg, Russia	Potentials of QPI techniques in analysis of cells' response to photodynamic treatment
15.45-16.05/9.45-10.05 /18.15-18.35 14.45-15.05/20.45-21.05	Invited Lecture <b>Dr. Andrei E. Lugovtsov</b> , Laboratory of Biomedical Photonics, Faculty of Physics, Lomonosov Moscow State University, Moscow, Russia	Interaction of erythrocytes with endothelium in microfluidic channels studied by optical techniques
16.05-16.25/10.05-10.25 /18.35-18.55 15.05-15.25/21.05-21.25	Invited Lecture <b>Dr. Victoria V. Zherdeva</b> , Bach Institute of Biochemistry, Research Center of Biotechnology of the Russian Academy of Sciences, Moscow, Russian Federation	Combining MRI and fluorescence imaging for monitoring polyester copolymers' degradation in vivo

**ONLINE SESSION DAY-3 (Continue)**

**Day 3:**

<https://zoom.us/j/96482950308?pwd=btYUaJ5dhU6rRGYv7SXsTLMpGEpypE.1>

**Passcode:** 690827

Session Chair: <b>Dr. Vanderlei Salvador Bagnato</b> , University of São Paulo, Brazil		
<b>Dr. Santhosh Chidangil</b> , Manipal Academy of Higher Education, India		
6.20-6.40/00.20-00.40 (05.10.24)/8.50-9.10 5.20-5.40/11.20-11.40	Invited Lecture  <b>Dr. Ayan Banerjee</b> Department of Physical Sciences, Center of Excellence in Space Sciences, India (CESSI), IISER, Kolkata– 741246, India	Microbubble lithography: using laser manipulated microbubbles towards patterning 'everything' mesoscopic
6.40-7.00/00.40-1.00 /9.10-9.30 5.40-6.00/11.40-12.00	Invited Lecture  <b>Dr. Basudev Roy</b> , Department of Physics, Indian Institute of Technology Madras, Chennai, India	Study of out of plane rotations in optical tweezers and subsequent applications in soft and biological matter systems
7.00-7.20/1.00-1.20 /9.30-9.50 6.00-6.20/12.00-12.20	Invited Lecture  <b>Prof Mike Hamblin</b> Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa.	New Applications of Transcranial Photobiomodulation
7.20-7.40/1.20-1.40 /9.50-10.10 6.20-6.40/12.20-12.40	Invited Lecture  <b>Ms. Yulia Svenskaya</b> Science Medical Center, Saratov State University, Russia	Biodegradable vaterite carriers for the delivery of glucocorticoids into hair follicles
7.40-8.00/1.40-02.00 /10.10-10.30 6.40-7.00/12.40-13.00	Invited Lecture  <b>Ms. Yuzhakova V. Diana</b> Research Institute of Experimental Oncology and biomedical technologies, Privolzhsky Research medical University, Nizhny Novgorod, Russia	Optical bioimaging in personalization of cancer treatment
8.00-8.20/2.00-2.20 /10.30-10.50 7.00-7.20/13.00-13.20	Invited Lecture  <b>Dr. Alexander V. Priezzhev</b> Laboratory of Biomedical Photonics, Faculty of Physics, Lomonosov Moscow State University, Moscow, Russia	Application of laser-optical methods for studying microcirculation and microrheology of blood in vivo and in vitro
8.20-8.40/2.20-2.40 /10.50-11.10 7.20-7.40/13.20-13.40	Invited Lecture  <b>Dr. Alexander P. Savitsky</b> A.N. Bach Institute of Biochemistry, Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, Moscow, Russia	The role of the trehalose transporter in the photoinactivation of Mycobacterium tuberculosis by near- infrared dye conjugated with trehalose
8.40-9.00/2.40-3.00 /11.10-11.30 7.40-8.00/13.40-	Invited Lecture  <b>Mr. Evgeny Shirshin</b> ,	Optical spectroscopy in surgery guidance from laboratory to the clinics



14.00	Lomonosov Moscow State University, Moscow, Russia	
9.00-9.20/3.00-3.20 /11.30-11.50 8.00-8.20/14.00- 14.20	Invited Lecture <b>Mr. Boris Yakimov</b> , Sechenov University, Moscow, Russia	Blood plasma spectroscopy for biomedical diagnostics: recent advances
9.20-9.40/3.20-3.40 /11.50-12.10 8.20-8.40/14.20- 14.40	Invited Lecture <b>Mr. Denis Davydov</b>  Lomonosov Moscow State University, Moscow, Russia.	Body composition analysis with a portable NIR device: hydration, fat and muscles
9.40-10.00/3.40-4.00 /12.10-12.30 8.40-9.00/14.40- 15.00	Invited Lecture <b>Dr Sathish Sundar Dhillip Kumar</b>  Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa	The Synergistic Impact of Aloin- Infused Biologically Active Film and Photobiomodulation for Wound Healing
10.00-10.20/4.00- 04.20 /12.30-12.50 9.00-9.20/15.00- 15.20	Invited Lecture <b>Dr Rahul Chandran</b> Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa	Hypocrellin: A natural photosensitizer in the Photodynamic therapy of Breast and Skin cancer'
10.20-10.40/4.20- 4.40 /12.50-13.10 9.20-9.40/15.20- 15.40	Invited Lecture <b>Dr Lelo Simelane</b> Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa	Targeted photodynamic therapy treatment on colorectal tumour spheroids
10.40-11.00/4.40- 5.00 /13.10-13.30 9.40-10.00/15.40- 16.00	Invited Lecture <b>Dr Nkune Nkune</b> Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa	Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa
11.00-11.20/5.00- 5.20 /13.30-13.50 10.00-10.20/16.00- 16.20	<b>BREAK</b>	
Session chair <b>Dr. Heidi Abrahamse</b> , University of Johannesburg, RSA <b>Dr. Santhosh Chidangil</b> , Manipal Academy of Higher Education, India		
11.20-11.40/5.20- 5.40 /13.50-14.10 10.20-10.40/16.20- 16.40	Invited Lecture <b>Mr. Alex Chota</b>  Laser Research Centre, Faculty of Health Sciences, University of Johannesburg, South Africa	Nanoparticles Loaded With Photosensitizer for Enhanced PDT Effects In Breast Cancer Cells
11.40-12.00/5.40- 6.00 /14.10-14.30	Invited Lecture	Investigation of the dynamics of the

10.40-11.00/16.40-17.00	<b>Mr. Victor Chuchin</b> Institute of laser technologies, ITMO University, Russia	skin reflection spectrum as a result of its heating by visible or infrared laser radiation
12.00-12.20/6.00-6.20/14.30-14.50/11.00-11.20/17.00-17.20	Invited Lecture <b>Dr. Vanderlei Salvador Bagnato</b> São Carlos Institute of Physics, University of São Paulo, Brazil	Photodynamic Therapy in Brazil: From cancer to microbiological control
12.20-12.40/6.20-6.40/14.50-15.10/11.20-11.40/17.20-17.40	Invited Lecture <b>Dr. Natalia Mayumi Matheus Kurachi</b> São Carlos Institute of Physics, University of São Paulo, Brazil.	Antimicrobial photodynamic therapy – challenges and strategies for achieving inactivation in biofilms and infected tissues
12.40-13.00/6.40-7.00/15.10-15.30/11.40-12.00/17.40-18.00	Invited Lecture <b>Dr. Kate Blanco</b> São Carlos Institute of Physics, University of São Paulo, Brazil.	Antimicrobial Resistance: Exploring Photodynamic Therapy as a Solution
13.00-13.20/7.00-7.20/15.30-15.50/12.00-12.20/18.00-18.20	Invited Lecture <b>Dr. Natalia Mayumi Inada</b> São Carlos Institute of Physics, University of São Paulo, Brazil.	High-grade squamous intraepithelial lesion (hsil) treatment with photodynamic therapy
13.20-13.40/7.20-7.40/15.50-16.10/12.20-12.40/18.20-18.40	Invited Lecture <b>Dr. Mirian Denise Stringasci</b> São Carlos Institute of Physics, University of São Paulo, Brazil.	Murine melanoma treatment effects using photodynamic therapy and radiotherapy combination
13.40-14.00/7.40-8.00/16.10-16.30/12.40-13.00/18.40-19.00	Invited Lecture <b>Dr. Alessandra Ramos Lima</b> Environmental Biophotonics Laboratory, São Carlos Institute of Physics, University of São Paulo, Brazil.	Advances in photonic supplementation in plant cultivation: perspectives and challenges in agriculture
14.00-14.20/8.00-8.20/16.30-16.50/13.00-13.20/19.00-19.20	Invited Lecture <b>Dr. Denise Maria Zezel</b> Laboratory of Biophotonics, Center for Lasers and Applications – Nuclear and Energy Research Institute, IPEN/CNEN-SP, São Paulo- Brazil.	Hyperspectral imaging pathology shining light on diseases
14.20-14.40/8.20-8.40/16.50-17.10/13.20-13.40/19.20-19.40	Invited Lecture <b>Dr. Anderson Rodrigues Lima Caires</b> Optics and Photonics Group, Institute of Physics, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil.	Chlorophyll fluorescence spectroscopy: basics and applications
14.40-15.00/8.40-9.00/17.10-17.30	Invited Lecture	Photodiagnosis in Latin America: Some

13.40-14.00/19.40-20.00	<p><b>Dr. Cicero Cena</b></p> <p>Optics and Photonics Group, Institute of Physics, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil</p>	solutions based on Optical Spectroscopy and Machine Learning
15.00-15.20/9.00-9.20/17.30-17.50/14.00-14.20/20.00-20.20	<p>Invited Lecture</p> <p><b>Dr. Michelle Barreto Requena</b></p> <p>São Carlos Institute of Physics, University of São Paulo, Brazil</p>	Optimizing photodynamic therapy for skin cancer using microneedles: a step closer to clinical trials
15.20-15.40/9.20-9.40/17.50-18.10/14.20-14.40/20.20-20.40	<p>Invited Lecture</p> <p><b>Dr. Lucas Danilo Dias</b></p> <p>Universidade Evangélica de Goiás (Brazil)</p>	Development and Application of Photoantimicrobial Films: Potential Use in Packaging and Coating for Medical Devices
15.40-16.00/9.40-10.00/18.10-18.30/14.40-15.00/20.40-21.00	<p>Invited Lecture</p> <p><b>Mr. M.Sc. Matheus Garbuio</b></p> <p>Environmental Biophotonics Laboratory, São Carlos Institute of Physics, University of São Paulo, Brazil</p>	Photodynamic inactivation against Aedes aegypti larvae.
16.00-16.20/10.00-10.20/18.30-18.50/15.00-15.20/21.00-21.20	<p>Invited Lecture</p> <p><b>Dr. Pavan Kumar</b></p> <p>Department of Physics, Indian Institute of Science Education and Research, Pune</p>	Optothermal Tweezers: Dynamic Assembly and Pattern Formation
16.20-16.40/10.20-10.40/18.50-19.10/15.20-15.40/21.20-21.40	<p><b>Valedictory function</b></p> <p><b>Group photo</b></p>	

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# Keynote Address

## Phthalocyanine-Based Probes for Alleviating or Evading Tumour-Hypoxia for Enhanced Photo- and Sono-Mediated Therapy

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Cancer remains one of the leading causes of morbidity and mortality worldwide. Conventional treatment options, including chemotherapy, radiation, and surgery, often have significant drawbacks such as invasiveness, lack of controllability, and the development of resistance. To address these issues, sono-photodynamic combinatorial therapy (SPDT) has emerged as a promising alternative, offering a non-invasive and controllable approach to treatment. SPDT integrates sonodynamic therapy (SDT), which employs ultrasound, with photodynamic therapy (PDT), which uses light to activate sensitizers and initiate cancer destruction. Phthalocyanines (Pcs) are gaining attention as sensitizers for SPDT due to their ability to induce toxicity under both SDT and PDT. This presentation explores the structural prerequisites of Pcs that may affect their overall effectiveness in SPDT for cancer therapy. We will also discuss strategies to enhance the therapeutic efficacy of Pc-based probes in photo- and sono-dynamic therapies, particularly in hypoxic conditions. Key design strategies include modifying the central metal, adjusting substituent positions, and examining the impact of adjuvants or nanoparticles (NPs) on the therapeutic activity of Pcs. Different mechanisms of cell death resulting from the compositions of Pcs-probes will be analyzed, with a focus on oxygen (O<sub>2</sub>)-dependent mechanisms. These mechanisms include methods to enhance O<sub>2</sub> concentrations in the tumor microenvironment to boost PDT or SDT efficacy. Additionally, we will consider O<sub>2</sub>-independent mechanisms that circumvent hypoxia by activating anticancer processes that do not rely on O<sub>2</sub>, such as the Fenton reaction or thermal ablation effects.

**Keywords:** Sonodynamic therapy, Photodynamic therapy, Combinatorial therapy, Phthalocyanines, Sensitizer, Cancer, Hypoxia

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# ABSTRACTS OF INVITED LECTURES

## **Photodynamic therapy in Brazil: from cancer to microbiological control**

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Photodynamic therapy in Brazil has been an excellent option for the treatment of skin cancer, cervix and also for the control of infections, especially those resistant to antibiotics. In this presentation we will explain how we have been acting with the development of protocols that today reach a success rate of 95% of tumor elimination as well as the approval of the treatment by the unified public health system. The advantages of using photodynamic therapy for countries with an emerging economy should be discussed. In the microbiological control part, we will explore the problem of bacterial resistance as well as the opportunities created by photodynamic inactivation in different types of infections, including pneumonia. Breaking down bacterial resistance to antibiotics will also be addressed.

**Keywords:** Photodynamic action, Skin cancer, Cervical cancer, HPV lesions and Microbiological control.

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## **Antimicrobial photodynamic therapy – challenges and strategies for achieving inactivation in biofilms and infected tissues**

Cristina Kurachi

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Antimicrobial photodynamic therapy (aPDT), is a relevant option for the use of antibiotics in the infectious disease treatment. aPDT has already been proven effective in inactivating bacteria, fungi, and viruses, but the results are highly impaired when investigating biofilms or infected tissues. In biofilms, due to the production of the extracellular matrix (ECM), the microorganisms are protected from physical, chemical and biological hazards. In the infected tissues, the presence of biological fluids changes the photosensitizer dynamics, interactions with microorganism cells, and then the photodynamic action. In the majority of the infectious diseases, the microorganisms are in the biofilm form, especially when considering only the chronic infections, in 80% the

pathogen is organized in a biofilm [1]. In this talk we will discuss different combination methods to improve the PDT inactivation response in bacterial and fungal biofilms based on the more efficient delivery of the porphyrin (Gang Zheng's porphyrins) [2] or with the association of mechanical treatment (ultrasonics) and chemical (surfactant, inorganic salts) or biological agents (enzymes) [3,4]. Recent experimental results will also be presented with the treatment of the bacterial biofilm using the photoproducts of a photodynamic reaction with methylene blue in the presence of potassium iodine, bringing other alternative inactivation mechanism based on iodide reactive species [5].

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## Antimicrobial Resistance: Exploring Photodynamic Therapy as a Solution

Kate Cristina Blanco

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This presentation delves into the critical issue of antimicrobial resistance (AMR) and investigates photodynamic therapy (PDT) as a promising solution. We will explore the mechanisms of AMR, the limitations of current treatment options, and how PDT utilizes photosensitizing agents and light to effectively target and kill resistant microorganisms. The potential benefits, challenges, and future prospects of implementing PDT in clinical settings will also be discussed.

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## High-grade squamous intraepithelial lesion (HSIL) treatment with photodynamic therapy

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Cervical cancer is the third most common cancer in women in Brazil and fourth globally. High-grade squamous intraepithelial lesion is a squamous cell abnormality associated with human papillomavirus (HPV). It encompasses the previously used terms cervical intraepithelial neoplasia grades 2 and 3 (CIN 2 and CIN 3), moderate and severe

dysplasia, and carcinoma in situ. Excisional techniques such as high-frequency surgery are recommended by the National Cancer Institute (INCA) in women with high-grade CINs and aged over 25 years. These excisional therapies are effective but can carry some risks to the women's health who want to get pregnant. This clinical trial offers an individualized and conservative treatment with Photodynamic Therapy (PDT). We have an expertise of over than ten years treating women with HPV-induced lesions. I will be presenting the results of the evolution of the clinical protocols and the establishment of a new project with the personalization of treatments. Thirty-one women with CIN 2/3 were randomized in three different protocols. The most promising results were obtained in a protocol with two PDT sessions twenty-one days apart and HPV hybrid capture 120 days after the second treatment. Our study suggests that PDT is a promising alternative to excisional therapies for high-grade cervical dysplasia, as it is less invasive, more conservative, and more affordable. Nowadays we are correlating each case individually with the response to therapy following by colposcopy, cytology, PCR and the immunohistochemical markers p16 and Ki67.

**Keywords:** High-grade squamous intraepithelial lesion, Cervical Intraepithelial Neoplasia, HPV, Photodynamic Therapy, Cervical cancer, CIN 2/3.

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## **Murine melanoma treatment effects using photodynamic therapy and radiotherapy combination**

Mirian Denise Stringasci<sup>1</sup>, Natalia Mayumi Inada<sup>1</sup>, Vanderlei Salvador Bagnato<sup>1,2</sup>

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Melanoma lesions, specifically, present resistance mechanisms to both radiotherapy (RT) and photodynamic therapy (PDT); however, this combination of techniques has shown promising results. RT generates free radicals that react with cellular macromolecules such as DNA, RNA, proteins and membranes, causing dysfunction and cell death. This damage can favor the outcome of a consecutive application of PDT, either by allowing greater penetration of the photosensitizer into the cells or by weakening the defense mechanisms. On the other hand, PDT causes direct damage to the cell that can activate an immune response against tumor cells, which can optimize the effect of another technique applied in sequence, such as RT. In this study, in vivo trials are being performed to evaluate the effects of RT and PDT with the B16F10 melanoma cell line. Different combinations of techniques are being performed to obtain the best treatment strategy with additive or synergistic effects.

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## **Advances in photonic supplementation in plant cultivation: Perspectives and challenges in agriculture**

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Photonic supplementation and plant cultivation are emerging as significant trends in precision agriculture. The use of artificial light through photobiomodulation aims to enhance agricultural quality and productivity by bio-stimulating plants and optimizing their development. However, understanding new approaches to integrated management and bioecology, along with improved photobiomodulation, still presents a challenge for the scientific community. Overcoming these challenges is crucial to addressing the expansion of agriculture and the interaction with biotic and abiotic factors in the plant's development environment. This presentation aims to integrate basic sciences, exploring the benefits and challenges related to the environment, agriculture, and photonic supplementation of plants with artificial light.

**Keywords:** Environmental biophotonics, Agriculture, Photonics, Photobiomodulation.

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## **Hyperspectral imaging pathology shining light on diseases**

Denise Maria Zezel

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The interaction between light and biological tissues has formed the foundation for numerous scientific advancements. Over the past thirty years, the Center for Lasers and Applications at IPEN-CNEN/SP in Brazil has played a pivotal role in pioneering new diagnostic and therapeutic techniques in both Dentistry and Medicine, in close partnership with the University of São Paulo's School of Dentistry and Faculty of Medicine. These efforts have centered on leveraging Photonics to explore and innovate in these fields.

One key area of progress has been the exploration of the spectroscopic properties of biological tissues, which has proven to be a powerful diagnostic tool for identifying various diseases and tracking their progression. My research group has delved into the analysis of numerous biological tissue biopsies using Fourier Transform Infrared Spectroscopy (FTIR) and Optical Coherence Tomography (OCT). Our research has contributed to the development of several clinical procedures, including methods for preventing dental caries and diagnosing the different stages of dental enamel lesions. Notably, we've also devised a clinical protocol that combines Nd:YAG and Er:YAG laser irradiation, offering a successful treatment for sleep apnea without the need for anesthesia in patients.

Furthermore, by applying machine learning and deep learning techniques, I will present findings that demonstrate the ability to differentiate between normal and tumorous tissues—including those from the thyroid, lungs, skin, breast, and oral cavity—using micro-FTIR hyperspectral imaging analysis.

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## **Chlorophyll fluorescence spectroscopy: Basics and applications**

Anderson Rodrigues Lima Caires

Optics and Photonics Group, Institute of Physics, Federal University of Mato Grosso do Sul (UFMS), Campo Grande, MS, Brazil.

Chlorophyll fluorescence is a powerful non-invasive technique used to assess photosystem II (PSII) activity and plant responses to various stress conditions, including biotic and abiotic factors such as mineral deficiencies, soil salinity, and pathogenic diseases. This presentation aims to provide an in-depth look at the principles and applications of chlorophyll a fluorescence, highlighting the importance of this technique for understanding photosynthetic mechanisms and monitoring plant responses to environmental changes, emphasizing its growing relevance in contemporary crop research and ecology.

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## **Photodiagnosis in Latin America: Some solutions based on Optical Spectroscopy and Machine Learning**

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There is a growing need for fast and accurate diagnostic methods, particularly in Latin America, where optical spectroscopy techniques can play a significant role. Techniques such as near-infrared (NIR), Fourier Transformed Infrared (FTIR), and Raman spectroscopy offer rapid, non-destructive, and reliable alternatives to traditional laboratory methods for disease diagnosis. This is especially important in resource-limited settings where access to advanced medical facilities is limited. Portable optical spectroscopy sensors can enable point-of-care testing and real-time health condition monitoring, which is crucial in rural and underserved areas lacking centralized healthcare facilities. Combining optical spectroscopy with machine learning techniques can further enhance the speed and accuracy of diagnostics. At SISFOTON-UFMS, our Optics and Photonics Lab has explored this approach, demonstrating many proof-of-concept applications for human, plant, and animal diagnosis. In our presentation, we will share significant results achieved in our laboratory, highlighting the potential for new studies and collaborations. We will focus on addressing the demand from Latin America, particularly Brazil, for advancements in the diagnosis of neglected diseases and the animal production sector

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## Photodynamic inactivation against *Aedes aegypti* larvae

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The *Aedes aegypti* mosquito is a vector of several arboviruses, including dengue, Chikungunya, yellow fever and Zika. Control of this vector is therefore a public health priority. Traditionally, control methods for *Ae. aegypti* have focused on eliminating breeding sites, using chemical insecticides, and applying larvicides and adulticides. However, these methods face limitations, such as the selection of insecticide-resistant mosquito populations and adverse environmental impacts. Photodynamic inactivation (PDI) is a promising technique against the proliferation of the *Ae. aegypti* vector. This method uses light at an appropriate wavelength, a photosensitizer (PS) and molecular oxygen. The combination of these three factors generates reactive oxygen species that are highly reactive and toxic to the target organism. Curcumin, a dye extracted from the rhizome of *Curcuma longa*, popularly known as Turmeric, has been demonstrated as a potential photolarvicidal agent. Following the guidelines of the World Health Organization (WHO), it is essential that this technique is rigorously tested and evaluated at each stage of its implementation, ensuring that the benefits observed in the laboratory translate into effective and sustainable results in the field. The adoption of innovative methods such as this is crucial to overcome current challenges and protect global publichealth in an effective and sustainable manner.

**Keywords:** Environmental Biophotonics, Vector control, Photolarvicidal, Curcumin.

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## Tissue optical clearing imaging: from in vitro to in vivo

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Biomedical optical Imaging techniques provide powerful tools for observing biomedical tissue structural and functional information. However, the high scattering of biological tissues limits the penetration of light, and decreases imaging resolution and contrast as light propagates deeper into the tissue. Fortunately, novel tissue optical clearing technique provide a way for solving the above problem. This presentation will introduce our progress in tissue optical clearing imaging, i.e., in vitro tissue optical clearing methods for whole organs imaging; in vivo skull/skin optical clearing window for imaging structural and functional of cutaneous / cortical vascular and cells.

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# Mueller matrix microscopy for digital pathology

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Mueller matrix microscopy is becoming an increasingly attractive digital pathology technique for characterizing complex biological tissues. A Mueller matrix image (MMI) contains rich optical and microstructural information in all the pixels, comprehensively encoded in the high-dimensional polarization feature space. Since the contrast mechanism does not rely on absorption and is sensitive to scattering features down to a fraction of wavelength, the technique is label-free and non-invasive, capable of cross-scale and quantitative measurements and ideal for probing biomedical samples including tissues and cells. Despite all the apparent advantages of Mueller matrix microscopy for quantitative characterization of the microstructural properties, how to extract polarization features of pathological significance remains challenging for clinical applications [1-3]. In this report, we propose polarization super-pixels approach to exploit pixel-level polarization features for pathological applications.

For MMIs, polarization features are encoded in the density distribution of pixels in polarization feature space. Using clustering techniques, we can group together pixels of similar polarization properties to obtain a collection of polarization super-pixels (PSP), which are characterized by their centroid position, standard deviation and pixel population [4]. The set of PSPs provide an approximate representation of the MMIs in polarization space,

For pathological slides of liver cancer tissues, we took MMIs for multipole region-of-interest (ROI) and calculated 1024 PSPs for each MMI. Then for all the PSPs of the ROIs, we applied UMAP for dimension reduction followed by hierarchal clustering. Different clusters correspond to different pathological components of the liver tissues, such as normal and malignant nuclei, cytoplasm and fibrous structures. The decomposition method helped doctors to identify more specific indicators for pathology auxiliary diagnosis.

If pathological features are spatially labelled, we can compute the relative-contributions of individual PSPs to the labelled area and assign weight coefficients. The set of PSPs with weights forms a polarization feature template, or PFT, which can be used for label spreading by highlighting pixels of similar polarization features to those within the labelled areas. Results demonstrate that we can construct the PFT from small patches of labelled lung adenocarcinoma, and spread the label to the entire field of view. PFT essentially functions as the linkage, connecting polarization features to the spatial pattern of the specimen. Leveraging PSP and PFT the approach provides the capability to identify specific pathological features for assisted diagnosis, effectively reducing labor and time costs for pathologists.

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## **Diffractive-Optics-Based Structured Illuminations in Biomedical Microscopy**

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By shaping laser beams in biomedical microscopes, structured illumination enhances performance for biomedical detection. Our diffractive-optics-based structured illumination microscopy uses diffractive optical elements (DOEs) for deeper, faster, and more precise imaging [1, 2]. This allows for high spatiotemporal resolution three-dimensional (3D) in vivo microscopy and ultra-high throughput multi-parameter single-cell imaging. Two notable examples developed by our team are the imaging flow cytometer (iFCM) [2] and a needle-shaped beam (NB) for extended-depth-of-focus [1]. We developed a spectral imaging flow cytometer that profiles 5,000 cells per second in terms of molecular composition and morphology [2]. This technology generates label-free scattering images and 32/64-channel fluorescence images of each cell. Innovations include the linear-array-spot-excitation (LASE) imaging method using DOEs, microfluidic chips for high-speed 3D hydrodynamic focusing, a MEMS sorter utilizing spark cavitation single bubbles, and a high-speed, high-sensitivity fluorescence spectrometer. This system can analyze 1 million cells every three minutes, aiding cell atlas creation, drug discovery, blood analysis, synthetic biology, cell and gene therapy, liquid biopsy, molecular breeding, and marine biology.

To address the trade-off between high resolution and deep depth of field in biomedical microscopy, we developed a needle-shaped beam (NB) [1,3,4,5]. This method extends the beam length by 83 times and improves 3D imaging efficiency in biological samples by 14 times compared to conventional Gaussian beams. The theoretical model uses a random spatial multiplexing strategy to convert the input beam into hundreds of axial foci, allowing for precise control over focal depth, beam diameter, spatial position, segment number, and energy distribution while suppressing sidelobe noise. We also developed fabrication protocols for 4-inch quartz wafer DOEs with 5nm precision and created metasurfaces containing 100 million nanocolumns using photolithography. The NB has broad applications in biomedical microscopy, including NB optical coherence

tomography (NB-OCT) for skin cancer diagnosis, NB photoacoustic tomography (NB-PAT) for histology and angiography [6], and NB two-photon (NB-2P) for observing brain neuron activities.

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## Multifunctional OCT for intraoperative tumor diagnosis and rapid pathology

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The precise recognition of tumorous margin in brain tumor resect operation is vital for increasing tumor resection rate, suppressing relapse rate and improving prognosis. However, a real-time accurate intraoperative imaging pattern is still absent. Optical coherence tomography (OCT), a noninvasive label-free and high-resolution 3D optical imaging technology, can obtain 3D morphological characteristics and visualize the 3D network of microcirculation [1]. The feasibility of applying OCT in brain tissue imaging is demonstrated in this research. The difference of the OCT signal between normal and tumor brain tissue is also clarified. We utilize a multi-scale multi-functional ultrahigh resolution OCT imaging system, as called *iBrain-OCT*, and assort real time image processing software to judge the margin of brain tumor for the first time. More objective and precise references are also offered by *iBrain-OCT* via comparing the parameters 3D structure and micro-circulative hydrodynamics in region of tumorous tissue, pre-tumorous and normal brain tissue. We believe that such a real-time label-free ultrahigh-resolution intraoperative imaging pattern sheds light on a neo-index of pathological brain tumor margin diagnosis in situ during brain tumor resection operation. Pathological features are the gold standard for tumor diagnosis, guiding treatment and prognosis. However, standard histopathological process is labor-intensive and time-consuming [2]. Here, we present an active phase modulation-assisted dynamic full-field OCT (APMD-FFOCT) to improve the imaging stability and contrast [3] and further employ an unsupervised generative adversarial network to convert APMD-FFOCT images into virtual hematoxylin and eosin (H&E) stained images. Three-dimensional virtual H&E-stained images have been obtained at a scanning rate of 1 frame per second. Furthermore, we also demonstrate that this novel technique has successfully applied in cancer diagnosis for human central nervous system and breast. We believe that this new method will play a new unique and important role in intraoperative histology.

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## **Multimodal collaborative tumor precision therapy based on phototherapy**

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Chemotherapy, radiotherapy and surgery are the main treatments in the field of tumor therapy, but they all have their own limitations. Phototherapy, including photodynamic therapy (PDT) and photothermal therapy (PTT), which relies on the conversion of light energy into chemical and thermal energy by phototherapeutic moieties to kill tumors, has been widely used in clinic as a non-invasive oncologic therapeutic modality. Besides, the biological effects in vivo of phototherapy can be combined with other strategies to achieve the purpose of synergistic treatment. In this study, we constructed a nanobiomaterial drug carrying system for multimodal combined precision treatment of solid tumor, which combined immunotherapy, gene therapy, chemotherapy and phototherapy to make each treatment cooperative and enhance tumor treatment including effective inhibition of tumor development, metastasis and recurrence. In vitro and in vivo experiments have shown that these tactics may provide a promising and pragmatic platform for clinical applications.

**Keywords:** Phototherapy, Nanobiomaterials, Synergistic therapy, Tumor targeting.

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## **Intelligent imaging cytometry for label-free analysis of single cells and exosomes**

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Flow cytometry measures the physical or chemical information of single cells or particles in a fluidic stream, which has wide applications in many fields such as medicine and pharmacy. Recently, the development of imaging flow cytometry has enabled both the imaging and high throughput measurements for single cells. Artificial intelligences have been widely adopted for the analysis of cytometry data, especially for label-free cell analysis. Exosomes, which are nanoparticles around 100 nm, have been widely studied in recent years in many interesting areas such as cancer detection and drug delivery. It is worthy to explore the imaging cytometric method for the analysis of exosomes.

In this presentation, we will introduce our recent developments of the imaging cytometry with artificial intelligence for the analysis of label-free single cells or exosomes. The combining of deep learning with light scattering imaging will be introduced for label-free cell or particle analysis. The intelligent imaging flow cytometry has been demonstrated for the analysis of clinical cervical cancer cell samples. We will also introduce our intelligent imaging cytometric method for the analysis of exosomes, which includes the development of a deep-learning based small extracellular vesicles analyzer (DeepEVAnalyzer), a scattering image-spectro-microscopy (SISM) for exosomes analysis, and the study of exosomes with dark-field light scattering imaging method in cell microenvironments.

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# Long-Term Fluorescence Imaging Analysis and Targeted Detection of Eye Disease

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Fluorescein angiography (FA) is a standard imaging modality for evaluating vascular abnormalities in eye diseases, which is recognized as the major cause of vision loss [1]. Long-term and real-time fluorescein angiography is of great importance in preclinical research, nevertheless remaining big challenges up to present. On the other hand, Infectious keratitis that would lead to corneal opacities is one globally major cause of vision impairment and blindness. Whereas the pathogens have been proved to include bacteria, fungi, viruses and protozoa, proper diagnosis (i.e., infections are definitively confirmed and causative organisms are identified) is critical to initiate aggressive treatment [2]. The prevailing diagnostic techniques are currently Gram stain and culture, typically time-consuming, hours to days, and tedious in manipulation. Nevertheless, infectious keratitis recommended as a medical emergency usually implies an acute and rapid disease progression (e.g., *Staphylococcus aureus* (*S. aureus*)-induced keratitis can even cause the loss of vision within days as the complete destruction of corneal stroma is induced), and hence time is absent in this sight-threatening disease. Consequently, a substantial room is reserved for developing novel strategies, those are suitable for rapid, non-invasive, and sensitive diagnosis of infectious keratitis. In this presentation, we introduce new kinds of nanoprobe featuring high fluorescence intensity (photoluminescence quantum yield, robust photostability, begin biocompatibility, good water dispersibility as well as small size [3,4]. Furthermore, the nanoprobe are employed for long-term immunofluorescent cell imaging, imaging and treatment of ocular neovascularization, imaging and photoactive killing of Gram-negative and Gram-positive bacteria. Typically, we show that long-term fluorescence imaging of vessels is enabled through a kind of fluorescent photostable probes, which feature strong fluorescence, robust photostability, lengthened blood residency and negligible toxicity. In particular, the presented nanoprobe are capable of imaging retinal capillaries in 10 min, which is around 10-fold longer than that (1 min) of fluorescein sodium (FS, known as the most widely used contrast agents for FA in clinic). Based on rational surface modification of the probes, targeted fluorescence detection of neovascularization could be readily realized [5, 6]. On the hand other, we further introduce a new kind of SiNPs-based nanotheranostic agents for bacterial keratitis prepared through modifying fluorescent SiNPs with vancomycin (Van) molecules (SiNPs-Van). Notably, the glycopeptide antibiotic of Van is capable of forming a strong five-hydrogen bond with the D-Ala-D-Ala dipeptide on the cell wall of Gram-positive bacteria (e.g., *S. aureus*), enabling it to prevent bacterial infection-relative eye diseases.

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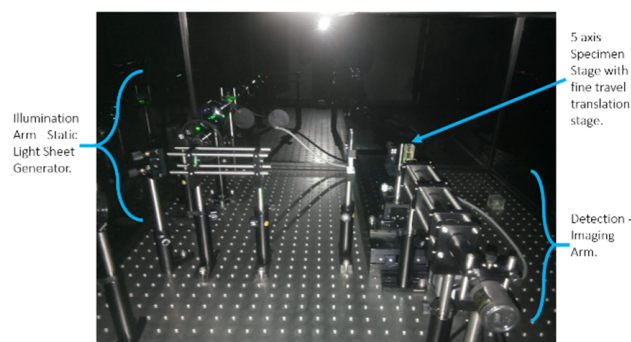
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## Construction of Light Sheet Fluorescence Microscope (LSFM) for Biophotonic Imaging Applications

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In this paper, we present the complete construction of home-built Light Sheet Fluorescence Microscope (LSFM) along with image processing for tomography applications. This will enable the selective visualization of photo-sensitive biological samples by a low photo-bleach and low photo-damage and offer a variety of in-vivo biophotonic applications. Introduction of Zebra-fish, in 1981 by George Streisinger to study genetic mutations affecting nervous system development and proven to be a boon to humanity as its genome has a 75% match with human disease genes, which makes it a model to mimic diversity of diseases and conditions like cancer, cardiovascular diseases, neurodegenerative diseases, etc. Imaging is one of the fundamental ways for observation and obtaining qualitative as well as quantitative data of any specimen, microscope is one such instrument that has been used for centuries to do so. Fluorescence microscopy is an advancement which enables selective visualization of organelles inside cell, molecules inside biochemical assays, etc. Photo bleaching of fluorophores and photo damage to light sensitive biological samples are problems encountered commonly in diascopic and episodic microscope imaging, which paved way for development of light sheet fluorescence microscopy (LSFM) which due to its unique orthogonal illumination technique is a low photo-bleach and a low photo-damage instrumentation technique used for fast imaging of specimen, obtaining a scan of 2D images which later can be stacked together to create 3D image where all the features of specimen appear in-focus. Here, we also present the details of image processing software GUI which we have developed for image tomography applications.



*Construction of Light Sheet Fluorescence Microscopy*

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## **The Optical Measuring Of Concentration Of Hemoglobin: Invasive And Noninvasive Methods**

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Optical measurement methods provide low-cost different ways to monitor biological signals such as neuronal and hemodynamic signals. These methods can be used to check the health status of people in medical centers. Recently, Near-infrared (NIR) and visible spectroscopic methods based on scattering and absorption of photons inside the biological tissue have been presented to measure the hemodynamic signals including oxy- and deoxyhemoglobin. The scattering and absorption of photons in NIR and visible spectral range can be correlated by spatial map of refractive index of tissue and concentration of chemical chromophores in the region of interest (ROI). Here, two deferent spectroscopic ways, invasive and noninvasive methods, for measuring the concentration of hemoglobin are studied. The mechanisms and recent clinical trials of both method are investigated. The accuracy, reproducibility, and stability of results obtained by these methods depend on our knowledge about photon migration in biological tissues. In addition, using optical clearing solution improve the optical depth of measurement and open a new way for functional in-vivo optical imaging in the human brain and skin. Finally the perspective of these methods in clinical treatments are discussed.

**Keywords:** Hemoglobin, Absorption, Scattering, Photon migration, Invasive measurement, Tissue optical clearing, Clinical trial.

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## **Microbubble lithography: using laser manipulated microbubbles towards patterning 'everything' mesoscopic.**

Ayan Banarjee

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## **Protein structure-function, drug discovery and diagnostics with Raman spectroscopy**

Chandrabas Narayana

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## **Optical Biopsy Assisted with AI/ML: Multimodal and Multispectral Optical Techniques for Real-time Screening and Diagnosis of Common Cancers: A point-of-care Approach**

Dilip Sing Mehta

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## **Quantum imaging of biological sample using Hong-Ou-Mandel interferometry**

Samanatha

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## **A novel approach based on stochastic calculus for laser speckle imaging**

Hari

We have recently introduced a novel approach to model speckles intensity in dynamic turbid medium like tissue using stochastic differential equations. A calibration phantom for speckle based imaging systems was designed and tested based on the above model. We have further extended the above work to implement a stochastic optimization algorithm to generate blood flow images from speckle measurements in biological tissue. The developed models and systems were validated using tissue mimicking phantoms and in vivo human experiments.

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## **Serum Raman Theranostics :Perspectives and Outlook**

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Optical-diagnostics alternatively or interchangeably, also, referred to as optical-pathology, optical-biopsy, spectral-diagnosis describe applications of spectroscopic and/or optical-based methods in disease diagnosis and management. Conventionally, diseases are diagnosed by clinical examination followed by relevant biochemical/microbiological/pathological/imaging examinations, which rely on symptomatic manifestations, often lead to late diagnosis and poor prognosis. Since biochemical changes precede morphological/symptomatic changes, optical spectroscopies which are sensitive alterations chemical compositions are emerging as potential alternatives/adjuncts. Raman spectroscopy, due to attributes such as sensitivity to biochemical changes precede morphological/symptomatic changes, rapidity, no external labelling or sample processing, objectivity, and most importantly in vivo/in situ on-line diagnosis, has widely been explored. The present talk appraises latest development of serum Raman spectroscopy towards disease diagnosis and therapeutic monitoring, i.e., theranostics.

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## **Emerging polarised light methods for probing nanostructural anisotropy**

Nirmalya Ghosh

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## **Generation of Functional Neurons By Photobiomodulation**

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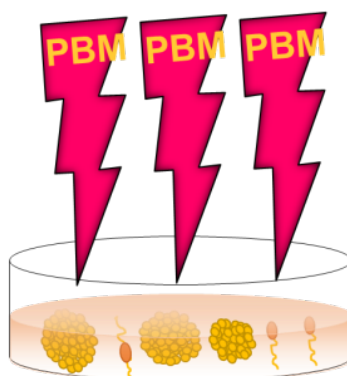
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Adipose-Derived Stem Cells (ADSCs) harvested from body fat could potentially be used for the replacement therapies of nervous system injuries and diseases. However, it still faces challenges in establishing neuronal functionality in vitro and extrapolating its therapeutic effects in vivo. Thus far, little progress has been made in transferring this technology from the culture dish to human clinics. We have shown that the photobiomodulation on differentiating ADSCs can improve the yield of functional neurons up to 10%. (Figure) The yield of differentiated neurons can be improved on bio-mimetic scaffolds and brain organoids in the presence of laser or light [1,2]. Still, electrophysiological analysis of these differentiated neurons is a major challenge in

determining their functionality *in vitro*. Techniques such as light-mediated patterning and electrochemicals are alternate options for the generation of functional neurons and the research is ongoing. Moreover, rigorous quality control measures have to be adopted on newly differentiated neurons before they are deemed to be fit for preclinical evaluations in animal models. This talk scientifically analysis how far we are yet to go in translating the benefits of photobiomodulation for neuronal differentiation from laboratory bench to bedside.

### Near Infrared laser



*Photobiomodulation of Adipose-Derived Stem Cells in vitro*

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## **“Probing” Spectroscopic Probes for Non-invasive Simultaneous Disease Diagnosis**

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Non-invasive diagnosis of human diseases rely on the detection of molecular marker (probe) in a painless manner. Although the molecular markers oftenly used are extrinsic and intrinsic, the intrinsic disease probes (molecular markers) are preferable, given the fact that the probes are omni-present in our body and deviation of their concentration from normal limit clearly indicates anomaly in human bodies i.e. disease. Here, we report non-invasive spectroscopic measurements of total hemoglobin, bilirubin, and the

ratio of oxy and de-oxy hemoglobin as disease markers of anemia, jaundice, and oxygen deficiency respectively using a meticulously designed optical fibre probe. The challenges associated with the designing of the fibre probe for the simultaneous non-invasive detection including optical power, spectral density of the probing light and resolution of the spectrometer were found to be critical in the measurement. We have also developed prototype and performed human clinical trial for the diagnosis of the diseases and compared with the conventional techniques (blood tests).

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### **Manipulations of structure-property relationships of MAXene systems using doping and etching approaches**

Sangeetha Kale  
DIAT Pune

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### **Breaking the resistance of bacteria to antibiotics: From the fundamentals to the proposal to treat pneumonia using Photodynamic**

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Bacterial resistance to antibiotics is one of the most serious problems related to the control of human infections. Many of the attempts to create new molecules with Artificial Intelligence or even using PHAGES to combat infection have not produced the desired results. In this presentation, we will show the fundamental experiments carried out demonstrating the breaking of bacterial resistance to antibiotics using photodynamic flashes. Checking basic aspects of this resistance breakdown, making bacteria susceptible again to traditional antibiotics, constitutes a very viable alternative to the problem. We will be discussing the basic experiments as well as their application in pharyngotonsillitis and the case of resistant pneumonia. Various aspects of these applications will be discussed, and the most recent results presented. Photonic techniques for detecting resistant bacteria will also be discussed. Work with the participation of collaborators: K. Blanco, J. Machado, N. Inada, V. Yakovlev, C. Kurachi, C. Patino.

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## Optical Bioimaging In Personalization of Cancer Treatment

Diana Yuzhakova<sup>1</sup>, Anna Izosimova<sup>1</sup>, Darya Sachkova<sup>1,2</sup>, Konstantin Yashin<sup>3</sup>, Irina Shumskaya<sup>1,4</sup>, Elena Kiseleva<sup>1</sup>, Artem Mozherov<sup>1</sup>, Vladislav Shcheslavskiy<sup>1</sup> and Marina Shirmanova<sup>1</sup>

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In the era of personalized medicine, it is necessary to identify the individual cancer patients who will respond well to the therapy and achieve maximum clinical benefit and those who require modifications in treatment regimen. One of the approach to make individual prognosis is to explore biological properties of patient-derived cells. However, this requires reliable methods for assessment of the response of patient-derived cells to treatment. Fluorescence lifetime imaging (FLIM) is a promising instrument to obtain information about cellular metabolism on a label-free basis, which is sensitive to therapeutic interventions. In the present study we demonstrated the abilities of FLIM of metabolic coenzyme nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) for assessing the response of cells and tissues to the cancer treatment in patient-derived models. An LSM 880 fluorescence confocal microscope (Carl Zeiss, Germany) with a TCSPC FLIM module (Becker Hickl GmbH, Germany) and an confocal macro-FLIM system (BeckerHickl GmbH, Germany) were used. First, we investigated the potential of FLIM microscopy to assess the individual sensitivity of patient-derived tumor cells to the in vitro treatment for prediction of the therapy effectiveness for patients or testing the new therapeutic agents. The original protocol of the evaluation of the sensitivity of patient-derived glioma cells to temozolomide was developed. Our results demonstrate that the more-responsive cell cultures displayed an increase in the protein-bound NAD(P)H fraction  $a_2$  and the NAD(P)H mean lifetime  $t_m$ , associated with a more oxidative metabolism and reduced proliferative activity [1]. We developed the original 3D patient-derived glioma models based on a spheroid or explant culture and optimized the FLIM protocol, which may become a platform for individual drug screening and development of new therapies for glioma patients [2]. In addition to FLIM of cancer cells, we have performed metabolic imaging of immune cells. For the first time, we showed on mouse melanoma model that FLIM microscopy has a potential to monitor the early response of immune cells in the fresh sections of mouse lymph nodes to the in vivo therapy by immune checkpoint inhibitors (ICI) [3]. Responsive mice showed a higher relative contribution of the free fraction of NADH  $a_1$ , associated with a shift towards glycolysis to maintain cytotoxic functions, an extended lifetime of the bound component of NADH  $t_2$ , associated with an increase in the total pool of NAD(P)H molecules in the cell due to increased metabolic activity. Our future work will be focused on the development of approach to predict the efficiency of ICI for individual melanoma patients using FLIM of isolated lymphocytes. Finally, we explored the ability of FLIM of NAD(P)H on a macro level for the intraoperative differentiation of the tumor tissue which is crucially important for brain tumors. For this, we developed the new highly invasive fluorescent/bioluminescent



patient-derived intracranial model of glioblastoma in mice and showed the high potential of macro-FLIM to accurately differentiate the glioblastoma tissue from the surrounding white matter [4].

Therefore, FLIM of NAD(P)H may be a powerful tool for personalized cancer therapy.

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## Single-cell spectroscopy of blood components using Micro-Raman combined with Optical Tweezers

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Raman spectroscopy is an analytical method used to examine molecular structures and their functions. It is a non-destructive technique that requires minimal sample quantity and preparation. Its application in the biomedical field offers promising insights, as biological samples are Raman-active, and its aqueous environment does not influence analytical results; hence, this technique is an excellent option for the analysis of biological samples. For studies involving single or live cells, Raman spectroscopy can be combined with an optical tweezers system. The "Raman tweezers" method enables the analysis of live biological cells in their physiological medium. The technique can provide detailed information of live cells and their interaction with externally applied stressors. In this study, we explored interactions between red blood cells and external agents and observed changes in RBCs under disease conditions by analyzing Raman spectra. The analysis of Raman spectra of single live platelets has also been explored. The Raman spectra of red blood cells (RBCs) treated with Bisphenol-A, and various intravenous fluids exhibit differences in band intensities, particularly in the oxygenation and deoxygenation marker regions. For iron deficiency anemia, a significant reduction in intensity is observed in the porphyrin breathing mode around the 752  $\text{cm}^{-1}$  region. Raman spectroscopy can effectively distinguish between iron deficiency anemia and beta-thalassemia. Additionally, this technique has been used to differentiate between neutrophils, lymphocytes, and monocytes in white blood cells.

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# **Biophotonics Has Acquired Windows Of Transparency Of Biological Tissues From UV To THz Waves**

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The results of measuring the optical properties of biological tissues in a wide spectral range with the aim of discovering new transparency windows and using these windows in biophotonics are presented. The method of immersion tissue optical clearing (TOC), which ensures the creation of new virtual and expansion of known transparency windows of various tissues in a wide range of wavelengths from deep UV to THz waves is developed and successfully applied in numerous urgent biomedical areas, including reliable differentiation of healthy and pathological tissues and enhanced phototherapeutics.

The principles and advances of TOC based on the temporary and reversible suppression of light scattering in tissues using biocompatible optical clearing agents (OCAs) will be discussed [1]. Delivery of the appropriate OCA into living tissue ensures its transient transparency in a wide spectral range with higher imaging depth and better contrast in optics, CT and MRI.

New areas of biomedical applications of this technology will be demonstrated for optical imaging, drug delivery monitoring, effective antitumor and antimicrobial phototherapy, optical communication and charging of smart implants in the human body, as well as laser therapy and microsurgery.

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# Combining MRI And Fluorescence Imaging For Monitoring Polyester Copolymers' Degradation In Vivo

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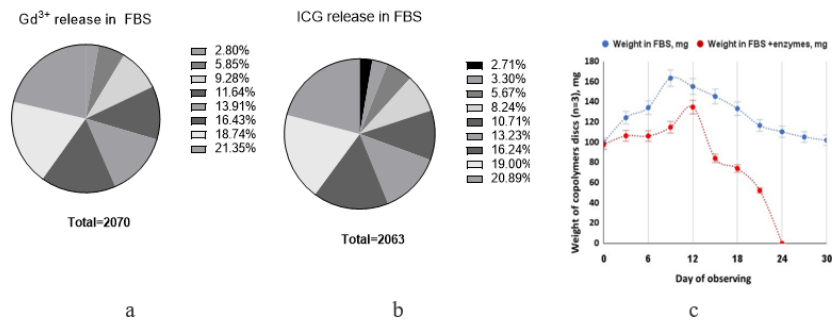
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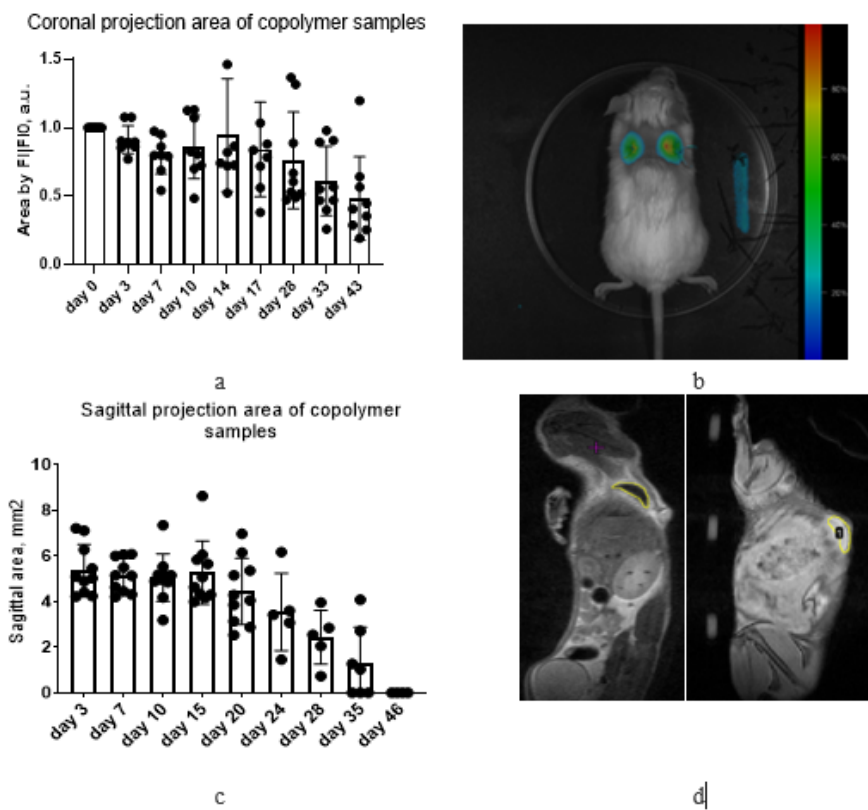
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Currently, the challenge for modern biomedicine of implants is to developing implantable materials of high grade of biocompatibility and resorption, thus omitting the necessary of surgical removing of the implant. Biodegradable synthetic polymers can be not only a good basis for self-resorbable scaffolds and implants, but also for creating multifunctional controllable modular devices. The development of methods for visualizing such materials in vivo, the rate of their resorption, and study of tissue effects should help in understanding of both general mechanisms of resorption and individual characteristics. Here, we proposed a comprehensive imaging method for biodegradable polyester copolymers based on the 1,3-propanediol, 1,5-pentadiol, succinic acid and citric acid labeled with fluorescent label (indocyanine green (ICG)) and MR contrast label (gadolinium citrate(Gd3+)) using fluorescence imaging (FI) and magnetic resonance imaging (MRI) correspondently [1]. These results include in vitro modeling of degradation estimated by the fluorescence and MRI (Fig.1a,b) following by in vivo imaging of implanted copolymers in mice (Fig.2). We demonstrated that the degradation of polyester polymers in vitrowas described by two-phase hydrolysis model in fetal bovine serum (FBS) (Fig.1c).



*Fluorescence and paramagnetic labels release from the copolymers samples in vitro in FBS supplied by the normal lipase activity (about 50-150 M.E., pH 7,2-7,4) during 24 days of incubation(a) Gd-citrate release from the copolymers samples at 0,3,6,9,12,15,18,22,24 days of incubation (n=3-4);(b) ICG release from the copolymers samples 0,3,6,9,12,15,18,22,24 days of incubation(n=3-4);(c) gravimetric assay of copolymer samples' weight loss (%) in FBS with lipase (red)compared to FBS (blue).*

The profile of the change in fluorescence intensity and MR intensity of polyester copolymers in vivo at certain stages from 0 to 46 days of observation correlated with the samples size changes. The coronal projection of copolymers' size was detected by the fluorescence imaging in depicting ROI following the optical clearing with 70% of water mixing of 0,7 Glycerol and 0,05 DMSO (Fig.2a,b). The MRI in the region of interest(ROI) in T2w FSE detected the sagittal projection of copolymers' size(Fig.2c,d). The MRI in depicting ROI in T1w GRE indicated the areas of Gd citrate relaxation reducing as for copolymers samples as for the inflammation around it(Fig.2c,d).Resorption of polyester copolymers in vivo had the signs of a two-phase degradation model of in vitro, and was, presumably, strengthened by the cellular immune response.



*Fluorescence and paramagnetic labels release from the copolymers samples in vivo in BALB mice during 46 days of incubation(a) Area change detected by the reduce of FI signal in the copolymers samples at 0-46 days of observaton(n=8-12);(b)An example of a fluorescence imaging of a mouse acquired at 10th day (iBox,(USA) ex.650nm, em 747nm; (c)Area change detected by the reduce of MR-intensity signal in the copolymers samples at 0-46 days of observaton (n=8-12); (d)An example of a sagittal MRI of a mouse acquired at 10th day in T2wFSE (left) and T1wGRE (right).*

We presented in vivo studies of resorption of polyester copolymers implanted into mice supplied by in vitro degradation modeling based on labeled copolymers. The behavior of the copolymers in vivo corresponded to the two-phases dergradation revealed in vitro like swelling and hydrolysis, but additionally mechanism like cellular immune response should taking into account to depict the resorption more completely. These results push the further investigation in order to understand the individual reactions on polyester copolymers and

other kind of implantable materials in order to prevent some side effects.

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## **Interaction Of Erythrocytes With Endothelium In Microfluidic Channels Studied By Optical Techniques**

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In this work, the intercellular interaction of erythrocytes and endothelial cells was studied in vitro using optical methods. Using laser tweezers, the adhesion forces of erythrocytes to the endothelium were measured, as well as the aggregation forces of erythrocytes upon addition of various compounds (L-Arginine, L-NAME) affecting the interaction of these cells to blood plasma or whole blood. The aggregation of erythrocytes in the blood flow in the presence of endothelial cells was studied using the laser aggregometry technique. The results demonstrate a decrease in the aggregation forces of erythrocytes in the presence of the endothelium upon stimulation of nitric oxide release. In the flow, erythrocyte aggregation also decreases in the presence of endothelial cells. The adhesion forces of erythrocytes to the endothelium do not change or decrease slightly (in some cases) with an increase in the concentration of nitric oxide.

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## **Potentials Of QPI Techniques In Analysis Of Cells' Response To Photodynamic Treatment**

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An extended family of phase-sensitive techniques combined under the name of Quantitative Phase Imaging (QPI) became a powerful nondestructive methodology in research of cells and tissues, see e.g. [1]. QPI does not require any exogenous labels and utilizes low-power radiation. The traditional QPI techniques, off-axis digital holographic microscopy (DHM) and tomography (DHT) operate with coherent radiation and the results obtained often suffer from errors caused by coherent noise. Recently the low-coherent inline QPI techniques, spatial light interference microscopy (SLIM) [2] and transport of intensity equation (TIE) [3], proved to be promising for application in biomedical research. Besides elimination of coherent noise, these techniques allow for installation on the inverted biological microscope and for combination with fluorescent and FLIM measurements. In this paper we consider advantages and disadvantages of the

QPI methods on a particular example of the analysis of the response of cell samples in vitro to photodynamic treatment.

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## The Role Of The Trehalose Transporter In The Photoinactivation Of Mycobacterium Tuberculosis By Near-Infrared Dye Conjugated With Trehalose

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One of the most dangerous diseases caused by mycobacteria is tuberculosis. The complexity of treating this disease is associated with the emergence and fairly widespread of antibiotic-resistant strains. Therefore, chemotherapy of such an infection has limited effectiveness. In this work, we propose for the first time to use tricarbocyanine dyes conjugated with trehalose for photodynamic inactivation of *Mycobacterium tuberculosis*, which has special transport proteins for trehalose in its cell wall. This leads to effective accumulation of the tricarbocyanine dye conjugate with trehalose and 99.99% efficiency of photodynamic activation of *M. tuberculosis* and *M. Smegmatis*. At the same time, other mycobacteria Gram-positive *Micrococcus luteus* and the Gram-negative *Escherichia coli* are significantly less sensitive to such a conjugate. Thus, in our work we demonstrated the use of a photosensitizer conjugate with trehalose for targeted transport into mycobacteria and their effective inactivation by light in the near infrared range, which opens up new prospects in the treatment of tuberculosis, especially antibiotic-resistant strains [1].

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## **Biodegradable Vaterite Carriers For The Delivery Of Glucocorticoids Into Hair Follicles**

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Topical glucocorticosteroids (GCS) represent the basis for therapy of a wide range of inflammatory dermatoses, including psoriasis, atopic dermatitis, alopecia areata, lichen planus, etc. However, the improvement for their penetration through the stratum corneum and intradermal accumulation in the sites of GCS receptors remains actual. Low dermal bioavailability causes the need for repeated applications of conventional topical GCS formulations that contributes to the development of skin atrophy. The GCS delivery to the hair follicles opens up the possibility to localize and enhance its therapeutic effect, since a large number of GCS receptors are located in the outer root sheath of the follicle, as well as in the areas of the sebaceous glands and sweat ducts.

We report on a novel approach towards the GCS encapsulation and delivery to the pilosebaceous reservoir, which can improve the topical application of such hormonal drugs. It involves the use of vaterite particles, which are biodegradable and biocompatible. We demonstrate the possibility of effective immobilization of various GCSs into the porous vaterite matrix. The obtained carriers displayed a good cellular uptake together with the low cytotoxicity when studied in fibroblasts in vitro. Efficient intrafollicular accumulation of the carriers after their US-assisted topical application in vivo in rats provided the delivery of the drug molecules to targeted receptors. Gradual degradation of the vaterite matrices inside the hair follicles granted in situ liberation of the payload. The resulting enhancement of a local corticosteroid's concentration in skin appendages could provide the lowering of the dose and frequency of the GCS application. Thus, it might contribute to the reduction of the side effects associated with its administration.

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## **Investigation Of The Dynamics Of The Skin Reflection Spectrum As A Result Of Its Heating By Visible Or Infrared Laser Radiation**

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Diode lasers are widely used in modern medicine for heating biological tissues. The efficiency and selectivity of laser heating is determined not least by the wavelength of

the radiation. Laser radiation with a wavelength of 980 nm is effectively absorbed by water, and radiation with a wavelength of 450 nm is absorbed by blood. In this regard, for effective selective heating of blood-filled tissues, it is preferable to use radiation with a wavelength of 450 nm. However, the issue of comparing the influence of the radiation parameters of the above-mentioned lasers on the transformations occurring in the blood has not been fully studied, especially in the context of the development of laser systems with feedback. Feedback mechanisms in laser systems enable real-time adjustments of laser parameters to achieve the desired outcomes efficiently [1]. Among these, thermal feedback is the most prevalent, analyzing the glow of the heated fiber optic tip to maintain a constant temperature [2, 3]. When tissue is subjected to elevated heat, proteins within the tissue begin to denature and undergo irreversible structural changes. Thus, it is crucial to identify markers indicating the temperature reached [4]. However, temperature alone does not fully capture the changes occurring in biological tissue. Optical methods, on the other hand, can detect chemical and structural alterations in the tissue during and after thermal exposure, allowing real-time monitoring of these changes induced by laser impact. An optical feedback method can be based on monitoring the intensity of the signal reflected from the skin at wavelengths where the optical properties of the skin change most significantly due to laser heating.

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### **Application Of Laser-Optical Methods For Studying Microcirculation And Microrheology Of Blood *In Vivo* And *In Vitro***

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In our laboratory of biomedical photonics, we have been developing new methods of quantitative assessment of microrheologic properties of blood and microcirculation



parameters. These methods include diffuse light scattering, laser tweezers and vital digital capillaroscopy [1-3]. Currently we work on combining these methods with laser speckle contrast imaging (LSCI) technique, which we use for mapping the blood flow speed in the laboratory small animals [4-5]. Microcirculation parameters are studied using the Kapilyaroscan-1 device (Russia) implementing artificial intelligence for digital image processing, which allows for estimating the number of red blood cells (RBC) in the capillaries [6]. We characterize the microrheologic properties of blood by the intrinsic properties of RBC to reversibly aggregate and to deform in shear flow by measuring such parameters as aggregation index, aggregation rate, hydrodynamic strength of aggregates, paired aggregation and disaggregation forces, RBC deformability indices as functions of shear stress [7]. These measurements are performed in vitro in the samples of blood freshly drawn from healthy donors or patients suffering from socially important diseases. Digital capillaroscopy and LSCI measurements are performed in vivo.

Three groups of patients were formed for the study with the main diagnoses of atrial fibrillation (AF), coronary heart disease (CHD), and chronic heart failure (CHF). Statistical differences in parameters for different groups of patients were analyzed using the nonparametric statistical Mann-Whitney test ( $p < 0.05$ ). The correlation between capillary blood velocity (CBV) with the number of RBC aggregates in the capillaries was calculated according to the Pearson coefficient of linear correlation. The results of the study showed a statistically significant correlation of measured parameters for the AF and CHF groups of patients. In percentage terms, the ratio of CBV in capillaries without aggregates to CBV in capillaries with aggregates for different groups of patients equals to 39.6% (AF); 46.9% (CHD); 46.4% (CHF). Based on these data we can conclude that there is a significant correlation between the presence of aggregates in the capillaries and a decrease in CBV in the examined patients. The analysis of the relationship between the number of aggregates in capillaries in patients suffering from AF and CHD has shown that the impairment of microcirculation is associated with an increase in the number of RBC aggregates in the capillaries of patients of both groups.

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## **Application Of Laser-Optical Methods For Studying Microcirculation And Microrheology Of Blood *In Vivo* And *In Vitro***

Diana Yuzhakova<sup>1</sup>, Anna Izosimova<sup>1</sup>, Darya Sachkova<sup>1,2</sup>, Konstantin Yashin<sup>3</sup>, Irina Shumskaya<sup>1,4</sup>, Sergey Gamayunov<sup>4</sup>, Elena Kiseleva<sup>1</sup>, Gaukhar Yusubalieva<sup>5</sup>, Vladimir Baklaushev<sup>5</sup>, Artem Mozherov<sup>1</sup>, Varvara Dudenkova<sup>1</sup>, Vladislav Shcheslavskiy<sup>1</sup>, and Marina Shirmanova<sup>1</sup>

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In the era of personalized medicine, it is necessary to identify the individual cancer patients who will respond well to the therapy and achieve maximum clinical benefit and those who require modifications in treatment regimen. One of the approach to make individual prognosis is to explore biological properties of patient-derived cells. However, this requires reliable methods for assessment of the response of patient-derived cells to treatment. Fluorescence lifetime imaging (FLIM) is a promising instrument to obtain information about cellular metabolism on a label-free basis, which is sensitive to therapeutic interventions. In the present study we demonstrated the abilities of FLIM of metabolic coenzyme nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) for assessing the response of cells and tissues to the cancer treatment in patient-derived models. An LSM 880 fluorescence confocal microscope (Carl Zeiss, Germany) with a TCSPC FLIM module (Becker Hickl GmbH, Germany), an confocal macro-FLIM system (BeckerHickl GmbH, Germany) and a BD FACSAria III cell sorter (USA) were used.

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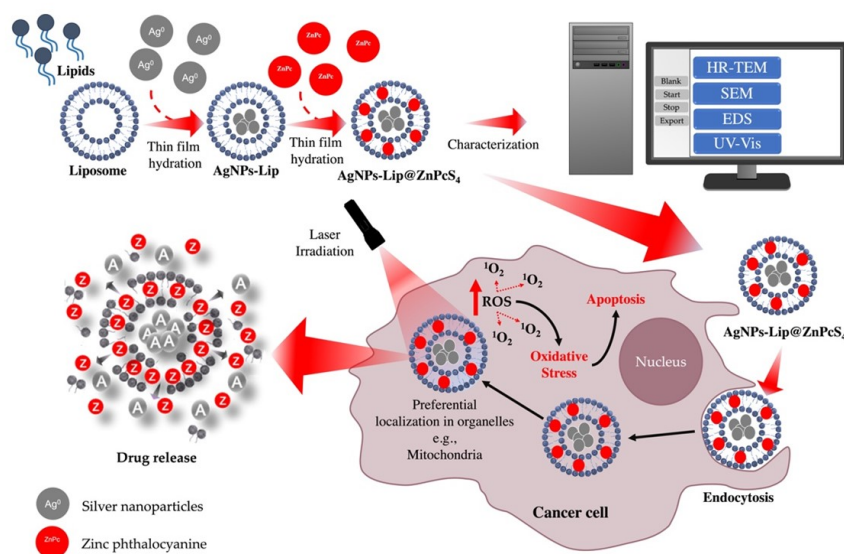
# **Green Synthesized Nanoparticles Loaded With Photosensitiser For Enhanced Photodynamic Therapeutic Efficacy Against Breast Cancer Cells**

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Breast cancer remains a formidable challenge in oncology despite significant advancements in treatment modalities [1]. Conventional therapies such as surgery, chemotherapy, radiation therapy, and hormonal therapy have been the mainstay in managing breast cancer for decades. However, a subset of patient's experiences treatment failure, leading to disease recurrence and progression [2]. Therefore, this study investigates the therapeutic potential of green-synthesized silver nanoparticles (AgNPs) using an African medicinal plant (*Dicoma anomala* methanol root extract) as a reducing agent for combating breast cancer. AgNPs were synthesized using the bottom-up approach and later modified with liposomes (Lip) loaded with photosensitizer (PS) zinc phthalocyanine tetra-sulfonate (Lip@ZnPcS4) using thin film hydration method. The successful formation and Lip modification of AgNPs, alongside ZnPcS4, were confirmed through various analytical techniques including UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), high-resolution transmission electron microscopy (HR-TEM), scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Following a 24 h treatment period, MCF-7 cells were assessed for viability using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT viability assay), cell death analysis using mitochondrial membrane potential (MMP) ( $\Delta\Psi_m$ ), enzyme-linked immunosorbent assay (ELISA) cytochrome c, Annexin V-fluorescein isothiocyanate (FITC)-propidium iodide (PI) kit, and caspase- 3, 8 and 9 activities. The experiments were repeated four times ( $n = 4$ ), and the results were analyzed using SPSS statistical software version 27, with a confidence interval set at 0.95. The synthesized nanoparticles and nanocomplex, including AgNPs, AgNPs-Lip, Lip@ZnPcS4, and AgNPs-Lip@ZnPcS4, exhibited notable cytotoxicity and therapeutic efficacy against MCF-7 breast cancer cells. Notably, the induction of apoptosis, governed by the upregulation of apoptotic proteins i.e., caspase 8 and 9 activities. In addition, caspase 3 was not expressed by MCF-7 cells in both control and experimental groups. Given the challenging prognosis associated with breast cancer, the findings underscore the promise of liposomal nanoformulations in cancer photodynamic therapy (PDT), thus warranting further exploration in clinical settings. An overview of the study is represented in Figure.



*A graphical overview of the synthesis and characterization of metallic silver nanoparticles and AgNPs-Lip@ZnPcS<sub>4</sub> nanoformulation as a hybridized therapeutic solution for breast cancer treatment through photodynamic therapy. After administration, exposure, and laser treatment, this nanocomplex induced drug release and intracellular production of cytotoxic reactive oxygen species (ROS), thereby triggering apoptosis activation.*

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## Illuminating Neural Transformation: Harnessing Photobiomodulation To Guide Stem Cell Differentiation For Neural Regeneration

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This study aimed to highlight the role of embryoid bodies (EBs) in regenerative medicine and stem cell research, while investigating the potential of photobiomodulation (PBM) as a non-invasive approach to influence cellular behavior and guide stem cell differentiation. The research also sought to evaluate how specific PBM parameters—such as wavelength and energy dose—affect the transdifferentiation of adipose-derived stem cells (ADSCs) into neural embryoid bodies (NEBs), with implications for tissue regeneration and neural engineering. EBs, multicellular structures that emulate early embryonic development, are

vital tools in regenerative medicine, while PBM has shown promise in modulating cellular activities. This study examined the impact of PBM at wavelengths of near-infrared (NIR) 825 nm, green (G) 525 nm, and a combination of NIR and G wavelengths, with doses of 5 and 10 J/cm<sup>2</sup>, on the transdifferentiation process. Cellular characteristics including morphology, viability, proliferation, cytotoxicity, and gene expression were analyzed, and statistical methods were employed to assess the results. PBM significantly influenced NEB formation, particularly at the 10 J/cm<sup>2</sup> dosage, leading to marked changes in cellular activities. Notably, CD105 expression decreased at 5 J/cm<sup>2</sup>, suggesting differentiation into mature neural cell types. Early neural commitment was evidenced by increased PAX6 expression, while downregulation of Nestin confirmed the establishment of neural identity at both 5 and 10 J/cm<sup>2</sup>. SOX2 expression revealed the complexity of neural differentiation, especially at 5 J/cm<sup>2</sup>. Statistical analyses confirmed the significance of these findings, with p-values indicating substantial changes in cellular behavior and gene expression. Overall, this study underscores PBM's potential to regulate cellular processes and promote ADSC differentiation into neural lineages, offering valuable insights for future applications in regenerative medicine and neural tissue engineering.

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## **Pheophorbide A-Mediated Photodynamic Therapy In Breast And Lung Cancer Cells In Vitro**

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Lung cancer is the second leading cancer type diagnosed and the leading cause of cancer related death which originates from lungs. Breast cancer is the most diagnosed cancer and second leading causes of cancer related death in females next to lung cancer. Photodynamic therapy (PDT) is one of the localized, minimally invasive and clinically approved treatments for a range of diseases including cancer. Green nanotechnology is a promising therapeutic option that is adopted in cancer research. *Dicoma anomala* (*D. anomala*) is an African medicinal plant used for the treatment of various medical conditions including cancer. Some phytochemicals are also capable of photosensitizing action. About 5-10% of anticancer compounds from plants were reported to show phototoxic properties. In this study, silver nanoparticles (AgNPs) were synthesized using *D. anomala* (DA) MeOH root extract. We further evaluated the anticancer efficacy of the synthesized AgNPs as an individual treatment as well as in combination with pheophorbide a (PPBa) mediated PDT. UV-VIS spectroscopy, high-resolution transmission electron microscopy (HR-TEM), Scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS) were used to confirm the formation of

nanoparticles. Post 24 h treatment, A549 lung and MCF-7 breast cancer cells were evaluated for ATP proliferation, morphological changes supported by LIVE/DEAD assay, and caspase activities. The results revealed a dose-dependent decrease in cell proliferation in both individual and combination therapy of PPBa-mediated PDT and DA AgNPs on A549 lung cancer cells with significant morphological changes. Additionally, the LIVE/DEAD assay displayed a significant increase in the number of dead cell populations in individual treatments (i.e., IC50's treated A549 cells) as well as in combination therapy. Cytotoxicity using MTT assay of AuNPs, PPBa and Lipo@AuNPs@PPBa nano complex was investigated on A549 spheroid cells. Lipo@AuNPs@PPBa in combination with 660 nm laser irradiation induced significant cell cycle arrest at G2/M phase. PPBa also induced cell death in MCF 7 wild type and doxorubicin resistant breast cancer cells. In conclusion, the findings from this study demonstrated the anticancer efficacy of green synthesized AgNPs, nanocomplex as a mono-therapeutic drug as well as in combination with a chlorophyll derivative PPBa in PDT. Taken together, the findings highlight the therapeutic potential of green nanotechnology in medicine.

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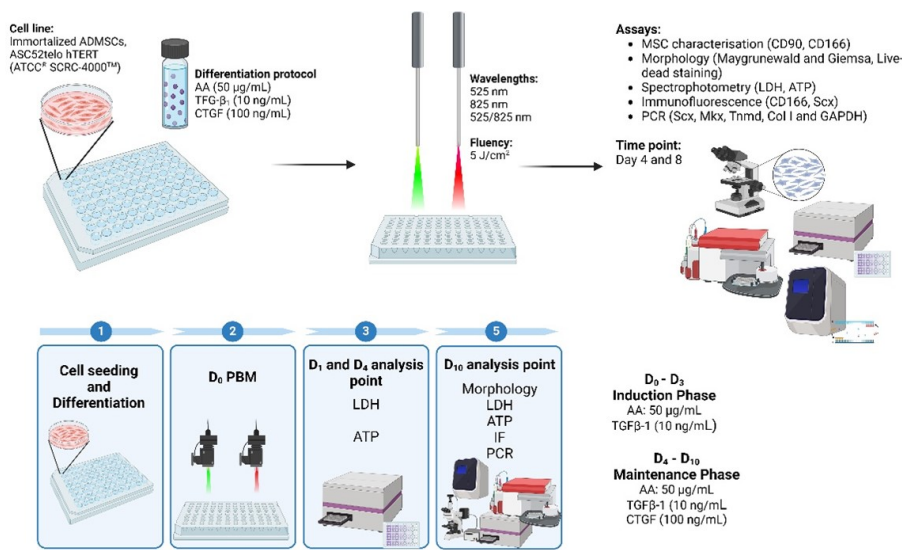
## **Progressing Stem Cell Regenerative Therapy Via Photobiomodulation To Facilitate Tenocyte Differentiation**

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Tendons are fibrous connective tissue structures that attach muscle to bone, to facilitate movement [1]. These structures are frequently damaged due to acute injuries or degenerative changes. Natural healing often results in ineffective repair, re-injury and fibrosis due to the hypocellularity of tendons and low metabolic activity of tenocytes [2]. Photobiomodulation (PBM) is a form of light therapy that stimulates endogenous chromophores to enhance cellular processes, such as proliferation, viability and differentiation [3]. The aim of this study was to investigate the potential application of PBM to enhance the proliferation, viability and differentiation of immortalized adipose-derived mesenchymal stem cells (iADMSCs) into tenocytes. iADMSCs were irradiated using 525 nm, 825 nm and their combination wavelengths, with a 5 J/cm<sup>2</sup> fluency. The iADMSCs were characterized as stem cells using CD90 and CD166, before irradiation. Upon completion of the differentiation protocol the morphology was observed (Maygrunewald-giemsa), cell viability (live-dead staining, cytotoxicity) and proliferation (Adenosine triphosphate) was recorded and tenogenic differentiation was evaluated using PCR (Scleraxis, Tenomodulin and Collagen I) and immunofluorescence (CD166 and Scleraxis). Stem cell markers were expressed, no significant morphological alterations were observed, cell viability and proliferation were enhanced, with augmentation of differentiation markers. These findings suggest that PBM should be considered as a potential augmentative tool for the development of tenogenic differentiation protocols.

**Keywords:** Photobiomodulation, Tenogenic differentiation, Tenocytes, iADMSCs.



*Methodology outline.*

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## New Applications Of Transcranial Photobiomodulation

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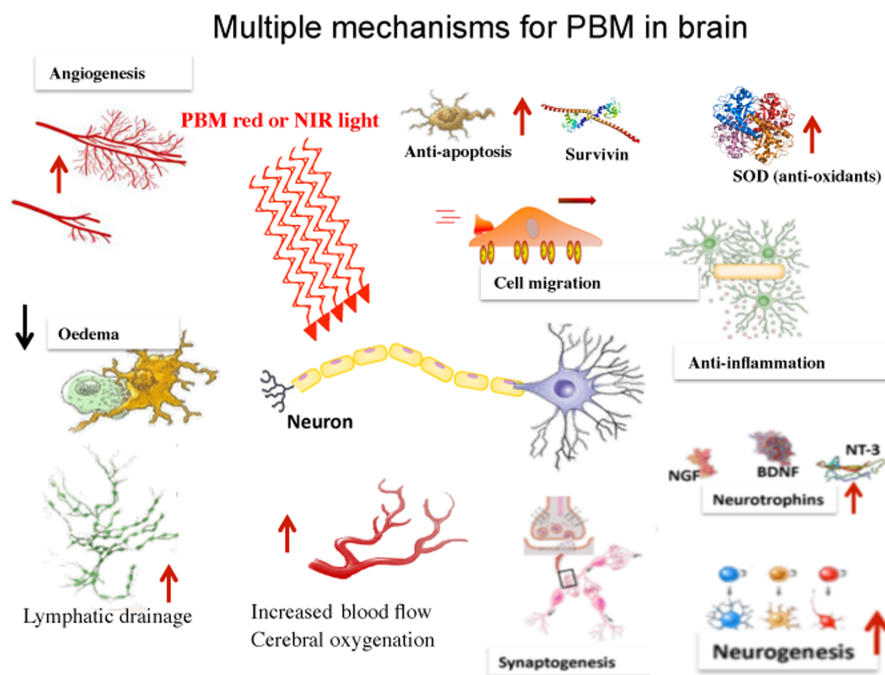
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Photobiomodulation therapy (PBMT) is a rapidly growing approach to the healing, stimulation, protection, and regeneration of many human organs and tissue types. PBMT started in the 1960s as low-level laser therapy for wound healing, but since then the introduction of light emitting diodes (LEDs) has dramatically increased the number of applications and reports of positive results. PBMT generally uses red (620-700 nm) and/or near-infrared (NIR, 780-1270 nm) wavelengths of light at an intensity that causes no tissue heating, and its activity is based on well-established biological and cellular mechanisms [1]. While laser therapists continue to use various types of laser in their office practice, LEDs are ideally suited for home use devices because they are completely safe and without any known significant adverse effects. Among the various body parts on which PBMT has been shown to exert beneficial effects, the brain stands

out as perhaps the most promising overall. PBMT has been shown to reduce neuroinflammation, while increasing mitochondrial function, oxygen consumption and blood flow within the brain [2]. Moreover, PBMT can stimulate the processes of synaptogenesis, neurogenesis, and neuroplasticity thus helping the brain to heal itself. PBMT has neuroprotective activity and can prevent brain damage in the acute phase after traumatic brain injury or stroke, because it inhibits apoptosis and upregulates the expression of anti-apoptotic proteins, as well as improving brain metabolism and oxygenation. In the chronic phase, PBMT can improve memory, cognitive function, mood and sleep quality. In degenerative brain disorders (dementia, Alzheimer's and Parkinson's disease) PBMT can improve motor, cognitive and social functioning (at least for some time). In a range of psychiatric disorders (depression, anxiety, autism spectrum disorder, opioid addiction) PBMT can lead to significant improvements [3]. This presentation will outline the mechanisms of action of PBMT on cells and tissues, and summarize the wide range of current applications to the brain and spinal cord, while proposing some new directions in psychiatry. It was discovered some time ago that the principal chromophores (light absorbing molecules) are located within the mitochondria. Cytochromes are involved in several units of the respiratory chain (including cytochrome c oxidase), and they can absorb red/NIR light thus increasing electron transport and the mitochondrial membrane potential (MMP) to increased ATP production. The raised MMP leads to a brief burst of reactive oxygen species (ROS) in normal cells, but in dysfunctional mitochondria, the normalization of the MMP reduces the generation of ROS and mitigates oxidative stress. There are also increases in nitric oxide and intracellular calcium, along with activation of numerous transcription factors [1]. Other chromophores have been proposed, including light and heat sensitive transient potential ion channels, water surrounding ATP synthase, and direct excitation of oxygen by 1064 nm and 1270 nm lasers, but all of these mechanisms are also proposed to affect the mitochondria. The activation of mitochondrial metabolism by PBM can be characterized as a switch towards oxidative phosphorylation (OXPHOS) away from glycolysis. This switch has two important consequences. Firstly stem cells living in their hypoxic niche mainly rely on glycolysis for their energy requirements. However when their mitochondria are activated by PBM they must leave their niche to go in search of sufficient oxygen to support OXPHOS. Once outside the niche the stem cells become progenitor cells, and can be influenced by any cues that might be released from areas of tissue damage in order to differentiate into somatic cells where they can then repair the brain. The second consequence involves macrophages and microglia. These cells can be polarized into a M1 pro-inflammatory phenotype, which also mainly rely on glycolysis for energy production. However the counterpart M2 phenotype is anti-inflammatory, and the cells rely more on OXPHOS for their energy production. This M1-M2 switch accounts for the pronounced anti-inflammatory effects of PBM [4]. Figure 1 illustrates the wide range of cellular and tissue mechanisms that could play a role in the benefits of tPBMT. All of them have been shown to operate in at least some circumstances, while for each type of brain disorder some mechanisms may be more important than others. Because PBM mainly affects the mitochondria, it is not surprising that organs and tissues that are rich in mitochondria are some of the most popular targets for PBMT. These tissue targets include neurons, brain, spinal cord, retina, muscles, liver and kidney. However these mitochondria-rich tissues are generally located deep within the body, so



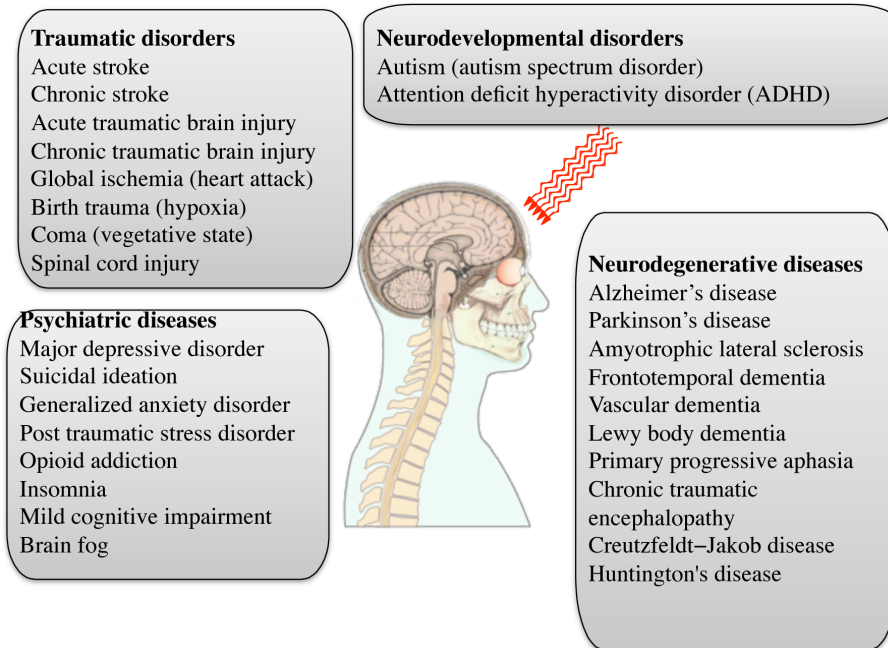
it has been claimed that the light must be able to penetrate sufficiently through tissue to have any effect. This is an intriguing question because over the years it has become clear that in addition to the local effects of PBMT, there is also a pronounced systemic effect or action at a distance. In other words light delivered to one part of the body can have beneficial effects on tissues and organs elsewhere. It is logical that these beneficial effects must be transported via the bloodstream, and the recent discovery of cell free respiratory competent circulating mitochondria in blood might provide an explanation of this effect [5]. Because we are interested in the brain it is worth mentioning that the amount of light penetration through the human scalp and skull that reaches reach the cortical surface has been estimated at 1-2% depending on the wavelength and skull thickness [6]. Nevertheless it must be realized that when light is shone on the head it might be absorbed by the blood flowing within the scalp, or by the bone marrow located in the calvarial bone of the skull, so “transcranial PBMT” might work without actually being physically transcranial. There have been reports that PBMT can be effective in animals when the light is applied to remote areas of the body, or when the mice are simply allowed to run around under LED light delivered from the top of the cage.



*Illustration of cellular and tissue mechanisms that have been reported to occur with PBMT in the brain.*

There is a large body of work showing that PBMT is highly effective in treating models of various disorders of the brain and spinal cord created in laboratory animals, as well as some genetically engineered mouse models such as Alzheimer’s (AD) or Parkinson’s disease (PD) [2]. Due to space limitations these reports cannot be discussed in any great detail. However, it is worth mentioning that animal models of a sudden brain insult, e.g. traumatic brain injury (TBI), stroke, neonatal hypoxia-ischemia, as well as spinal cord injury (SCI) can often be treated with a single exposure of the head to the light at a few

hours after the insult. Degenerative diseases such as AD or PD usually require a repeated course of exposures at 1-2 day intervals for several weeks to show significant improvement in cognitive and motor performance. Figure 2 shows a broad overview of the wide range of brain conditions that have been treated with PBMT, classified into traumatic, neurodegenerative, neurodevelopmental, and psychiatric disorders.



*Brain disorders that could be treated with PBMT using red/NIR light delivered to the head.*

There was a major effort to investigate the benefits of PBMT in patients who had suffered an acute ischemic stroke, when it was applied as a single treatment to the head within 24 hours of the stroke, as suggested by the good results from animal models. Despite encouraging results from the phase 1 and 2 trials, the large NEST-3 trial was discontinued early due to “futility” [7]. Several reasons have been put forward to explain the failure of NEST-3 in terms of the trial design being sub-optimal. Up to the present time there have been only a few attempts to demonstrate the effectiveness of PBMT in rehabilitation of patients who were suffering from chronic stroke, despite several theoretical considerations suggesting it should be helpful. Similarly although PBMT has been shown to be very effective in animal models of SCI, there have been few reports of its effectiveness in humans who have been unfortunate enough to suffer an SCI. TBI in humans has also been treated successfully with PBMT both in the acute phase as well as the chronic phase. The chronic TBI patients required regular applications of tPBMT to maintain their initial improvements in memory and executive function. Chronic traumatic encephalopathy (CTE) was also treated with PBMT administered by transcranial and intranasal routes in a case series of four ex-American football players. Cognitive improvements were confirmed by objective measurements of functional connectivity (resting state fMRI) and metabolism (magnetic resonance spectroscopy) [8]. There are several efforts underway to show the efficacy of tPBM in treating dementia and AD in humans, after impressive success was shown in mouse models of AD.

Recently a randomized, double-blind, sham-controlled trial was reported using a combination of LED helmet and abdominal belt in 57 mild-moderate AD patients who received 40 treatment sessions lasting 25min each over 8 weeks [9]. The PBM treated patients showed lower ADAS-Cog subscores, higher forward verbal spans, and lower TMT-B execution times.

Parkinson's disease has been treated with PBMT in Australia [10]. The light has often been applied to the head as well as to other parts of the body such as the abdomen or the nose at the same time. PD symptoms including tremor, akinesia, gait, difficulty in swallowing and speech, impaired facial animation and fine motor skills, sense of smell and social confidence, showed 75% overall improvement after 30 min of PBMT daily for 10 days. Autism spectrum disorder (ASD) in adults as well as children appears to respond well to PBMT [11]. One 8-week open-label study assessed the tolerability, safety, and efficacy of transcranial LED therapy in adult patients with ASD. An 830 nm LED array was applied to the forehead twice a week for 8 weeks. The patients that completed the study showed significant improvements in several scales related to ASD severity. Psychiatric disorders such as major depression, generalized anxiety disorder, drug addiction, and insomnia have all been beneficially treated with PBMT administered to the head [12]. Treatment resistant depression would probably be the best indication at the present time. It is important to distinguish between PBMT and bright light therapy, which are both used for treating depression, but have very different mechanisms of action. Bright light therapy works via specific photoreceptors in the eyes, and signaling in the brain related to circadian rhythms. The pronounced effects of PBMT in improving neuroplasticity and functional connectivity suggest that it may prove helpful in additional future applications. These could include post-traumatic stress disorder, obsessive-compulsive disorder, attention deficit hyperactivity disorder, eating disorders (anorexia, bulimia), addictions (drugs, alcohol, gambling), chronic insomnia, and even schizophrenia. Some pilot trials for these indications would appear to be warranted in the near future.

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## **Evaluation Of Photodiagnosis And Targeted Photodynamic Therapy On Metastatic Melanoma Tumour Spheroids**

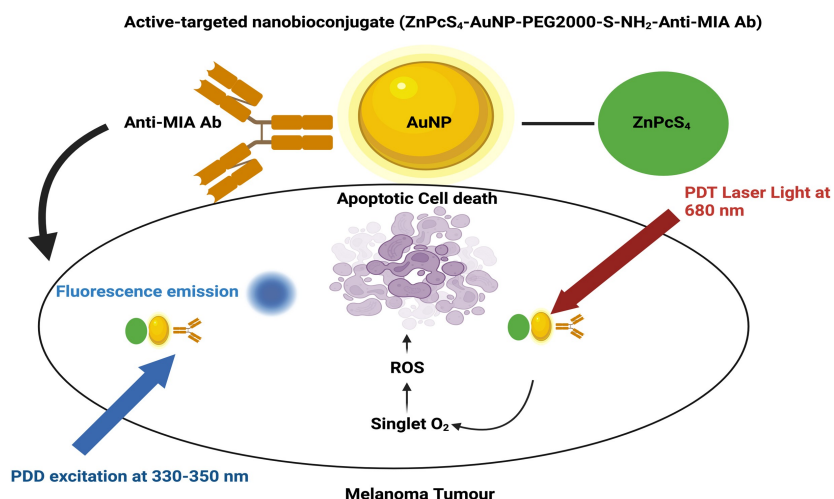
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Melanoma is the most aggressive and deadly form of skin cancer, accounting for alarming mortality rates globally due to metastasis. The efficiency of current therapeutic approaches can be decreased once the cancer has metastases, thus early detection is vital. Additionally, it remains imperative to further investigate alternative therapeutic strategies that can detect, target, and treat metastatic melanoma (mm) in order to enhance prognosis and increase patient survival rates. Novel photodynamic diagnosis (PDD) and photodynamic therapy (PDT) techniques exploit the photodynamic properties of photosensitizers (PSS) for photodetection or photochemical treatment of cancer upon excitation with a particular wavelength of light. PDD is based on the detection of the fluorescence emitted by administered PS, which has exceptional tumour specificity with negligible off-target localization. The emitted fluorescence is non-toxic to the cells, but it allows for an accurate diagnosis, whereas PDT produces reactive oxygen species (ROS), which destroy cancer cells. This study reports for the first time the phototoxic effect and PDD abilities of an active-targeted nanobioconjugate (NBC) composed of zinc phthalocyanine tetrasulfonic acid (znpcs4) conjugated to gold nanoparticles (AUNPS) with melanoma-specific targeting antibodies, anti-melanoma inhibitory activity ab

(anti-mia ab), attached to their surface, in order to improve PS bioavailability and affinity for 3-d tumour spheroid models. The nbc, znpcs4-aunps-anti-mia ab, was found to be stable and showed increased cellular uptake in mm spheroids for improved PDD and PDT outcomes in comparison to normal human cells. Additionally, the NBC showed a higher apoptotic rate (75%\*\*\*) upon photoactivation compared to free znpcs4 (51%\*\*\*). These results suggest that the attachment of a targeting biomolecule specific for mm onto the PS nanocarrier system can enhance both the PDD and PDT outcomes.



*Graphical abstract of the hypothesised active-targeted nanobioconjugate (NBC) (AuNP-PEG2000-S-NH<sub>2</sub>-ZnPcS<sub>4</sub>-Anti-MIA Ab) for PDD and PDT applications in melanoma.*

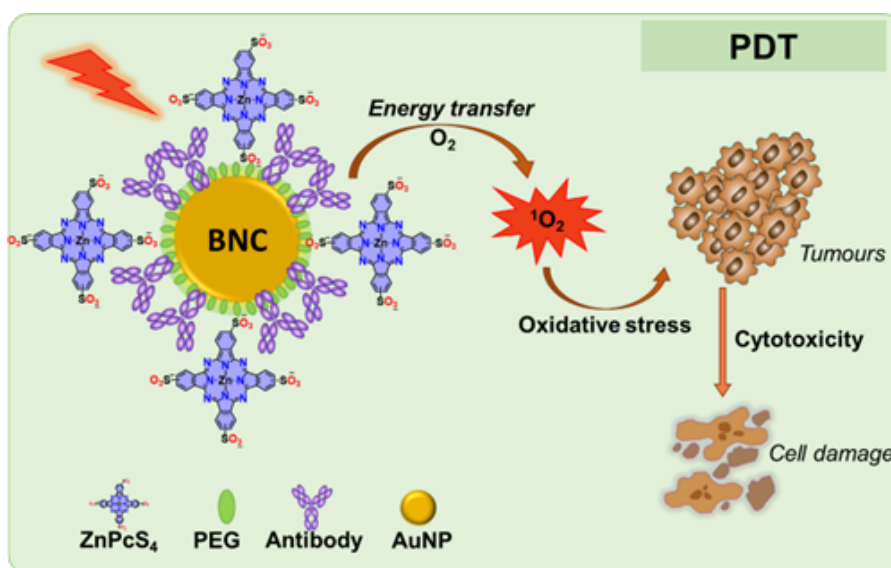
## **Targeted Photodynamic Therapy Treatment On Colorectal Tumour Spheroids**

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Colorectal cancer (CRC) is a persistent health problem and the conventional therapeutic approaches that are currently in use have limitations. Photodynamic therapy (PDT) is a minimally invasive technique that uses laser light and photosensitizers (PSs) to eradicate cancer cells. The use of tailored targeted PS delivery nanoplatfroms has the potential to enhance PDT efficacy. Three-dimensional multicellular tumour spheroids (MCTS) have the ability to closely replicate in vivo avascular tumour features and provide a more realistic insight of architecture, which makes them useful tools for the screening of novel antitumour agents. Therefore, in this study, a photodynamic treatment approach involving gold nanoparticles (AuNPs) as nanocarriers, were modified with CRC specific targeting antibodies, Anti-GCC mAb along with the photosensitizing agent zinc phthalocyanine tetrasulfonate (ZnPcS<sub>4</sub>) to selectively target and eradicate Caco-2 human

colorectal spheroids. The synthesized ZnPcS<sub>4</sub>-AuNPs -Anti-GCC underwent characterization through TEM and UV-VIS analysis. Cellular response was evaluated using microscopy assessment, LDH and MTT viability assay, and cell death analysis was performed using live/dead assay in Caco-2 MCTSs. TEM micrographs showed spherical shaped nanoparticles. LDH assay showed that when Caco-2 MCTSs were exposed to (IC<sub>50</sub>) of ZnPcS<sub>4</sub> (3 μM) combined with laser irradiation (637 nm, fluence 10 J/cm<sup>2</sup>), they exhibited 50% cytotoxicity. ZnPcS<sub>4</sub>-AuNPs-Anti-GCC bionanoconjugate (BNC) exerted greater inhibitory effects in Caco-2 MCTSs (20%\*\*\*) compared to controls. Additionally, apoptosis was the predominant mode of cell death in treated MCTSs, as evidenced by the reduction in live cells population, though a lesser extent was observed in 3D spheroids suggesting that MCTSs showed more resistance to PS drug. The findings from this study show that the BNC can significantly reduce cell viability and enhance PDT effectiveness. The photoactive nanocomposite system has the potential to be developed as an effective photosensitizer delivery system in MCTSs phototherapy.



*Photoactive nanocomposite system.*

## **Hypocrellin Mediated Photodynamic Therapy Against Skin, Lung And Breast Cancers**

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Natural photosensitizers (PSs) from natural extracts are gaining interest over synthetic compounds due to relatively low toxicity. Natural extracts and compounds from plants, microbes, and other sources are being studied extensively to discover novel chemical compounds with. It has been demonstrated that novel photosensitizer Hypocrellin B (HB) is a pigment isolated from insect parasite Hypocrellin bambusae has potent

anticancer activity and can be employed in photodynamic therapy. It has several advantages, including simple manufacture, purification, a high yield of ROS, strong phototoxicity, and rapid clearance from normal tissues. These properties make them ideal candidate as a PS in PDT of cancer. The study was designed to investigate the therapeutic impacts of HB and HB-PDT on human melanoma (A375), lung (A549) and Breast cancer (MCF-7) cells. The cells were grown under ideal culture conditions. To assess the post-PDT impacts of HB, the cells were irradiated at 470 nm at an energy density of 5 J/cm<sup>2</sup>. Post irradiation effects on A375, A549 and MCF-7 cells, were determined using MTT, ATP proliferation and LDH assays. Moreover, we conducted an intracellular reactive oxygen species (ROS) assay for HB and HB-PDT using DCF fluorescent dye on these cells. This comparative study revealed different dose responses of HB on all the cell lines studied. Our findings indicated that HB-PDT led to decreased cell viability as observed in the MTT assay and increased cytotoxicity levels in the LDH assay when compared control and HB group alone. The cells also showed decreased ATP proliferation following HB-PDT. Furthermore, it is noteworthy that HB-PDT treatment resulted in increased generation of ROS in cells compared to HB treatment alone. The investigation on these cell lines proved to be beneficial in understanding the role of Hypocellin on these cancers, although further investigations are underway to understand HB-based PDT cell death mechanisms. Hence, identifying such natural PSs could promote interest in compounds that has wide action against different cancer types. considerable promise as an accurate and minimally invasive therapeutic method for tackling lung cancer.

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## **The Synergistic Impact Of Aloin-Infused Biologically Active Film And Photobiomodulation For Wound Healing**

Sathish Sundar Dhilip Kumar, Sivakumar Singaravelu, and Heidi Abrahamse

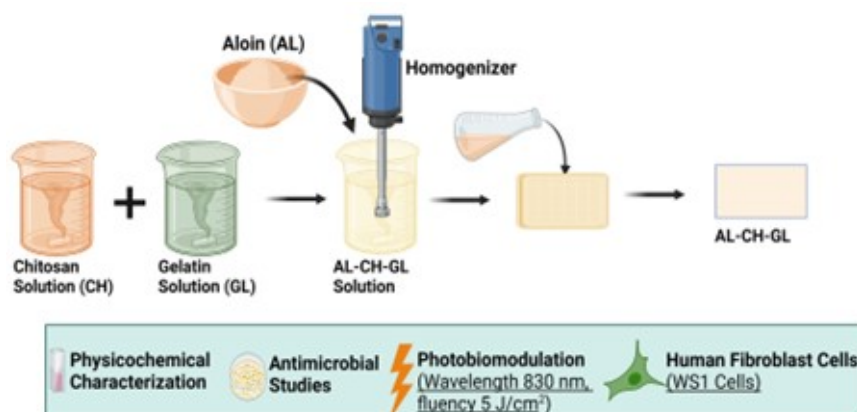
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The global biomaterial market is projected to experience a compound annual growth rate (CAGR) of 7.3%, increasing from its current valuation of 45.2 billion in 2024 to 64.2 billion by 2029. Some key application areas, such as wound healing and plastic surgery, have been identified in an industry trend analysis that supports this growth trajectory. In these sectors, biomaterials are essential for soft tissue restoration, reconstruction, and wound management due to their safe and natural integration into the human body. The increasing demand for biomaterials across various applications underscores their significance in medicine. This trend can be attributed to their demonstrated efficacy in enhancing healing processes and reducing infection rates. Gelatin and chitosan biomaterials have attracted considerable attention as dressings in formulations intended for chronic wound treatment. Incorporating aloin, derived from the Aloe genus, into chitosan dressings shows promise for enhanced wound healing, given aloin's diverse pharmacological properties. This study aims to evaluate the effects of varying concentrations of aloin, gelatin, and chitosan on the physicochemical properties of the



resulting films. Utilizing a casting technique, the biomaterials were systematically prepared and subjected to comprehensive characterization, including assessments of color parameters, morphology, barrier, and mechanical properties, as well as thermal analysis. The results indicated significant changes in the color parameters of the biomaterials with the inclusion of aloin, alongside modifications in barrier properties, increased fluid handling capacity (FHC), and decreased water vapor permeability (WVP). Notably, while aloin concentration exhibited minimal effects on the overall biomaterial properties, its presence substantially enhanced stability at temperatures below 200°C, reflecting chitosan's behavior in isolation, and maintained resilience like that of gelatin-chitosan biomaterials at temperatures exceeding 400°C. These findings suggest the possibility of a crosslinking or complexation interaction between gelatin-chitosan and aloin, leading to a synergistic enhancement of physicochemical properties that are favorable for their use as effective wound dressing materials.



*Schematic representation of the preparation of Aloin-infused film for wound healing applications.*



# ABSTRACTS OF POSTERS

## Probing of Amyloid $\beta$ oligomers by SERS, aiming early diagnosis of Alzheimer's Disease

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One of the hallmarks of Alzheimer's Disease (AD) is the accumulation of fibrillar aggregates (plaques) of Amyloid  $\beta$  ( $A\beta$ ) in the brain. Current diagnostics of AD (e.g. MRI, CT scans, and PET) probe the extent of neurodegeneration and/or plaque formation, which provides only limited therapeutic possibilities. We are in urgent need of developing alternative sensitive techniques that can flag the possibility of having AD, much early in the timeline of the disease progression.  $A\beta$ -aggregation initiates with the formation of small-soluble oligomers having antiparallel  $\beta$ -sheet structure. These oligomers, via their interaction with the plasma membrane, can cause large-scale cytotoxicity. Therefore, the concentrations of the oligomers in samples are expected to correlate well with the severity of the disease. Thus, probing the presence of  $A\beta$  oligomers in body fluids provides an attractive strategy for developing early diagnostics for AD. One major challenge, however, is that such oligomers are expected to be present in extremely low concentrations. Therefore, their detection would require highly sensitive techniques. Also, these oligomers will be present in an added mixture of several other types of biomolecules, which demand the detection technique to have the required selectivity. Surface-enhanced Raman spectroscopy (SERS) is an extremely sensitive technique. However, the strength of SERS signals is strongly dependent upon the analyte-nanoconstruct distance, which allows experiments to be designed to have the required selectivity. Here, we aimed to develop SERS-based detection platforms for probing  $A\beta$ -oligomers in solution. We functionalized monodisperse silver nanoparticles (AgNPs) with an amyloid-oligomer-specific dye, Rose-Bengal (RB). The RB molecules were grafted on the AgNPs via linker molecules of different lengths. The interaction of the  $A\beta$ -oligomers with RB reoriented the dye molecules with respect to the AgNP surface, resulting in the alteration of the SERS signal originating from RB as a function of  $A\beta$ -oligomer concentration.

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## **Study of properties of Thiamine and its detection techniques**

Clint Mathew, Ajaya Kumar Barik, Jijo Lukose, and Santhosh Chidangil\*

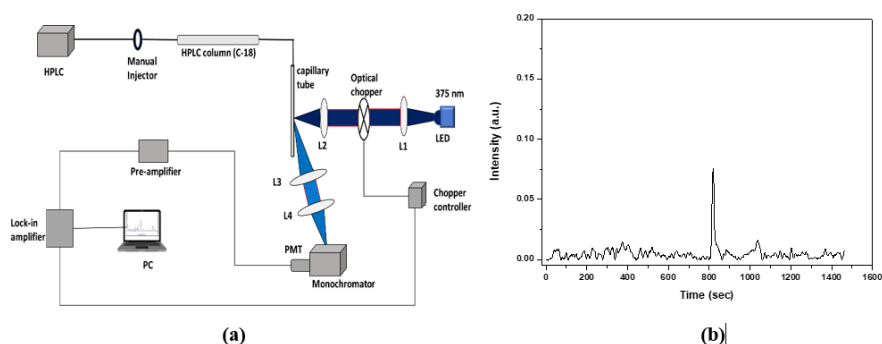
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Thiamine, also known as Vitamin B1, is a water-soluble nutrient found in various food items such as fish, meat, nuts, seeds, beans, soy products, etc. It is one of the eight essential B vitamins for the regular functioning of the central and peripheral nervous systems. It helps the body's conversion of food (fat, carbohydrates, and protein) into energy [1]. The structural formula for thiamine is 2-methyl-4-aminopyrimidine linked to a thiazole ring by a methylene group.

The detection of thiamine is mainly done by the fluorescence. Even though thiamine is a weakly fluorescing molecule, it can be oxidized to form thiochrome, which is strongly fluorescing, using suitable derivatizing agent. Potassium ferricyanide is the most commonly used derivatizing agent [2]. Thiochrome is unstable, degraded when exposed to light and heat. So, the experiments have to be done in dark and temperature-controlled environments. The stability and fluorescence intensity of thiochrome also depends on the pH. Here we are studying the properties of thiamine using spectroscopy.

HPLC methods are generally used for the detection and quantification of metabolites including thiamine. Pre-column derivatization and fluorescence detection is the common method [3]. We propose HPLC LED-IF technique for the quantification of thiamine. We were able to detect up to 10nM concentration of TDP using the developed system.



(a) Schematic diagram of HPLC with fluorescence detection, (b) Chromatogram of 10 nM thiamine diphosphate

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## Rapid detection of faecal indole in women with and without thyroid dysfunction: A UV-visible spectroscopic approach

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Introduction: The relationship between thyroid and gut health is complex and poorly understood. The gut bacterial product, indole, is a valuable biomarker for gut health and function. Growing research suggests that indole levels are elevated in gut dysbiosis. Specific assays to quantitate indole are complex and require expensive equipment and a

high level of training. The spectroscopic technique using the Hydroxylamine method is a simple, cost-effective, and rapid approach to the detection of indole.

**Method:** A prospective observational study from 2020 to 2023 was conducted in the Departments of OBG and the Department of Reproductive Medicine and Surgery, in a secondary and tertiary care centre in coastal Karnataka. Thirty women (15 each with and without thyroid dysfunction) were recruited. The stool sample was collected, transported to the laboratory, and kept at  $-80^{\circ}\text{C}$  until analysis. Faecal indole concentration was determined using the Hydroxylamine method. The absorbance was measured at 530nm and was compared to a standard curve of known indole concentration (0 - 300  $\mu\text{M}$ ). The standard graph for indole had an  $r^2$  value of  $\geq 0.99$ .

**Results:** For women with and without thyroid dysfunction, the mean (SD) age was 40(6) and 39 (8) years, respectively. The mean (SD) BMI ( $\text{kg}/\text{m}^2$ ) was 24.9(3.5) and 23.9(2.1), respectively. For the two groups, the mean (SD) of indole( $\text{mg}/\text{mL}$ ) were 47.9(18.7) and 45.6(15.4) respectively and was not statistically significant ( $p > 0.05$ ).

**Conclusion:** There was a slight increase in the indole concentration among women with thyroid dysfunction however, it was not significant. Further research using additional samples in a controlled environment may be necessary to reach a definitive conclusion.

**Keywords:** Faecal indole, Hydroxylamine method, Spectrophotometry, Thyroid Dysfunction.

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## Optothermal Lateral Manipulation Of Single Cell Across Printed Plasmonic Nanostructures

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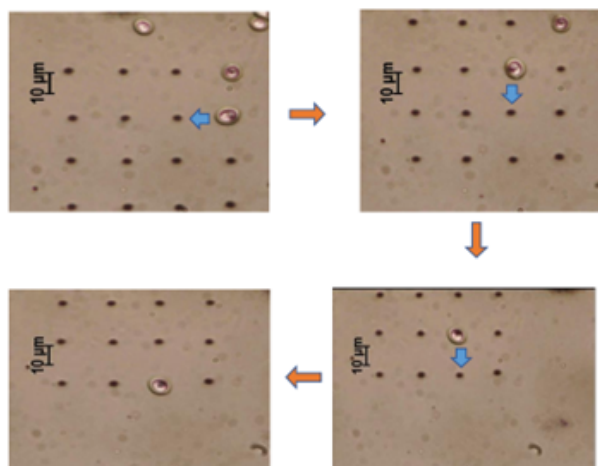
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Optothermal manipulation is a versatile emerging technique in biophotonics and cell biology that leverages light-induced heat generation to precisely control microscopic biological entities. By employing low-power lasers, this method can manipulate biological cells without causing significant damage, making it a promising tool for various applications such as particle/cell sorting, studying intercellular interactions, and positioning cells in specific microenvironments. This technique is particularly useful for isolating rare cell populations, analysing cellular genetics, and investigating drug efficacy(1). While previous studies have successfully trapped cells using optothermal methods, lateral manipulation between plasmonic trap sites has remained a challenge. This capability is crucial for studying two-particle interactions, which are fundamental to understanding biological processes such as protein-protein interactions(2), gene regulation, and cell signalling. In our experiments, we demonstrated optothermal manipulation by fabricating a plasmonic substrate composed of silver nanoparticles. Yeast cells were then introduced onto the substrate and illuminated with a laser. Due to plasmonic interaction, the laser energy was absorbed by the silver nanoparticles, leading to localized surface plasmon resonance and heat generation. This heat created a temperature gradient, inducing a fluid flow that trapped the yeast cell on the illuminated spot. By sequentially illuminating different spots, we achieved lateral manipulation of

the single yeast cell (Figure).



*Lateral manipulation of yeast cell.*

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## Effect Of Optical Clearing In Tissue Autofluorescence: A Pilot Study

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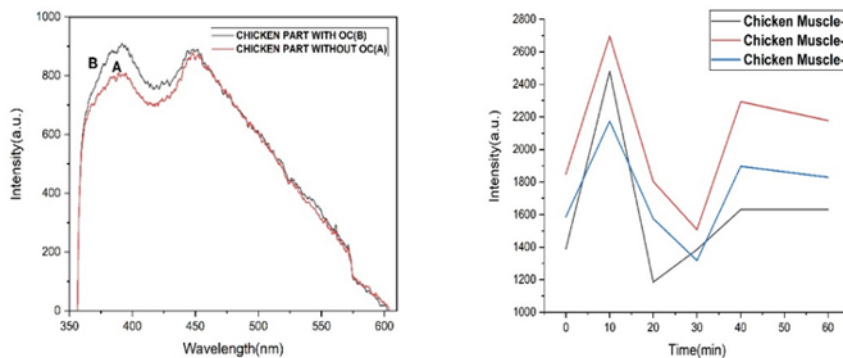
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Optical Clearing is a method used to minimise the refractive index mismatch between the tissue surface and the medium through which the incident light travels and hence making the tissue more transparent [2][3], this is achieved through dehydration of tissue by using hyperosmotic agents called optical clearing agent(OCA's). Glycerol was used an OCA and the effect on tissue (Chicken muscle) autofluorescence was studied. The fluorescence was measured using a home-built Laser induced fluorescence (LIF) set up employing a He–Cd laser (325 nm excitation) with 190 MW power. Two major fluorophores were observed such as structural protein collagen (385nm) and Co-enzyme

NADH (460nm). The Figure shows the comparison of the tissue fluorescence with and without optical clearing. The Figure 2 shows The fluorescence was found to be enhanced in case of collagen and there is no much difference for NADH. The increase in collagen content is justified as in the optically cleared tissue, the collagen in the stroma was more exposed. After the preliminary test, the chicken tissues were tested without OC then with the three different OC at different time intervals, first test was done till 60 minutes with time interval of 10 minutes, example of chicken muscle shown in Figure. Then second test was done till 80 minutes with only chicken muscle and chicken liver with time interval of 5 minutes. For both the tests Time (minutes) vs Intensity graphs were plotted.



*Chicken Muscle without OC and after OC and Chicken Muscle Time vs Intensity Plot.*

## **Investigating diffuse reflectance and fluence distribution in normal and diabetic foot: a seven-layered skin tissue model-based Monte Carlo simulation**

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Timely diagnosis, monitoring, and severity assessment of diabetic foot ulcers (DFUs) are important to ameliorate its long term adverse outcomes. Diffuse reflectance spectroscopy (DRS) has the ability to non-invasively diagnose and evaluate the severity of DFU by detecting variations in reflectance spectra due to changes in optical properties (absorption ( $\mu_a$ ), scattering properties ( $\mu_s$ ) and anisotropy factor ( $g$ )) that occur during ulcer progression [1]. To use the DRS method to timely diagnose DFU, one must have prior knowledge of fluence rate ( $\Phi$ ) and diffuse reflectance ( $R_d$ ) in the normal and diabetic foot. In this context, Monte Carlo (MC) simulation could be a suitable option, as it is one of the most versatile and powerful tools that has been used to understand the light distribution of different diseased states of biological skin tissue [2]. Therefore,

herein, a seven-layer skin tissue model has been adopted [3] and modified according to the human foot to understand  $\Phi$  and  $R_d$  in normal and diabetic foot using MC simulation. The MC model takes into account layer-wise variations in thickness, refractive index, blood vessel diameter, spatial distribution of blood volume fraction, water, melanin, reduced/-oxyhemoglobin and oxygen saturation as per the literature. Simulation results show that  $\Phi$  is more in normal foot compared to diabetic foot. Photon absorption in the diabetic foot increases by 10.5% to 12.5% compared to the normal foot at the Q bands (542 nm and 577 nm). In contrast, a significant decrease was found in the simulated  $R_d$  spectra of diabetic foot compared to the normal foot. In the wavelength region 530 nm to 584 nm i.e., in the neighborhood of Q band, the change in  $R_d$  spectra of normal and diabetic foot varies from 9.06% to 15.05%. Furthermore, the simulations also conclude that a detector-source distance of approximately 0.8 mm to 3 mm is optimal in a fiber-optic probe for  $R_d$  measurements from normal and diabetic foot. Thus, the presented foot skin model may be used for the development and advancement of DRS techniques for timely diagnosis and severity assessment of DFU.

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## Study of protein profile of blood plasma for cervical cancer using HPLC-FLD

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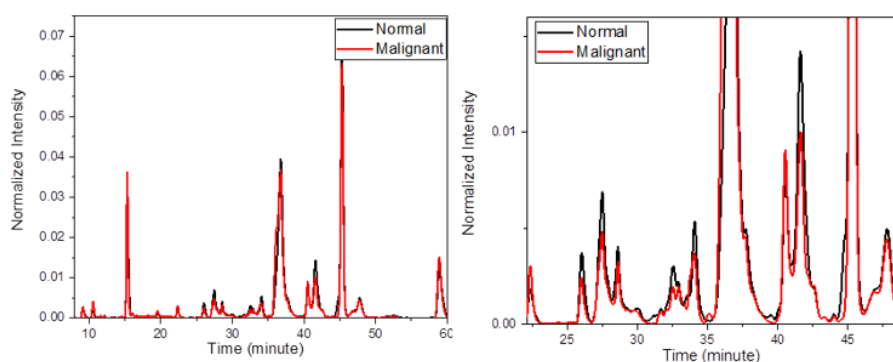
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Cancer is a key global health problem, and the world is expected to face approximately 26 million new cancer cases and 17 million deaths per annum by 2030. Early diagnosis and immediate treatment can reduce these trends. Optical techniques are promising diagnostic methods that can replace expensive diagnostic modalities like CT and PET, but they are still in the research stage at several research labs around the globe. I have studied the protein profile analysis of blood plasma sample collected from cervical cancer patients and control samples using the HPLC-FLD (high performance liquid chromatography-fluorescence detector) system. The primary proteins found in blood plasma and serum are human serum albumin (HSA), immunoglobulins, and transferrin. Nonetheless, it is important to examine the less abundant proteins as well. Traditional

methods typically use affinity resins to remove high molecular weight proteins such as albumin and immunoglobulin G. However, this method often leads to the loss of lower molecular weight proteins. Recognising the significance of removing high molecular weight proteins, the acetonitrile (ACN) precipitation method was implemented for the blood plasma samples. This approach effectively eliminates high molecular weight proteins while facilitating the recovery of lower molecular weight proteins. The blood plasma was collected and centrifuged at 3000 RPM for 5 minutes, and the supernatant was collected and stored at -80 o C for the study. The plasma contains major proteins such as human serum albumin (HSA), immunoglobulins, and transferrin. The solvents are Solvent A : water + 0.1% TFA and Solvent B: Acetonitrile + 0.1% TFA. The blood plasma was mixed with chilled acetonitrile in a 1:1 ratio and then mixed using the vortex mixer. The sample was centrifuged at 5000rpm for 5minutes and the supernatant was collected. The collected supernatant was diluted in 1:4 ratio with HPLC grade water before injecting the sample. The blood plasma samples from the normal and malignant samples were recorded using the gradient mentioned in Table 1 with the flow rate of 0.4ml/min. The average chromatogram form 41 normal and 25 malignant samples is shown in Figure 1. The difference is chromatogram was observed at 28, 32, 34 and 43 min.



*Average chromatogram of normal and malignant blood plasma sample, and Average chromatogram of normal and malignant blood plasma sample in 20 min to 50 min range.*

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# Modeling Spatial Correlations In Laser Speckle Patterns For Bioimaging Applications

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Laser speckle imaging is a well-established technique for evaluating blood flow in various research and clinical settings. Traditionally, this method was limited to assessing superficial blood flow at depths of less than 1 millimeter. However, recent advancements have extended its applicability to deeper tissues, reaching several centimeters [1,2]. There is a growing interest in developing algorithms to simulate speckles with applications either in the superficial or deep tissue laser speckle contrast imaging. Building on our previous work, where we employed stochastic differential equation (SDE) to model temporal evolution of speckle intensity [3], we present a method to include the spatial correlation of speckles over a fixed time interval resulting in a complete speckle intensity simulation method. Our approach begins with the construction of a theoretical correlation matrix derived from pixel distances. By applying Cholesky decomposition to this matrix, we generate pixel patterns with predefined spatial correlations. We validated this methodology by analyzing the correlation and covariance matrices of the generated speckle patterns, which were found to conform to a gamma distribution. We have further conducted the analysis of the variations in spatial correlation as influenced by different distance functions. This approach eventually enables us to explore and characterize the spatiotemporal dynamics and statistical properties of bio speckle patterns and hence leading to significant improvements in the analysis and interpretation of laser speckle imaging data.

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# Gold Nanoparticle In-situ Reduced Pdms Contact Lens For Color Blindness Management

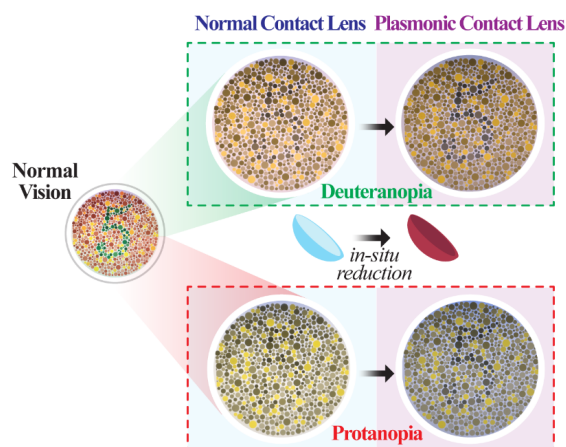
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Color vision deficiency or color blindness is an ocular condition in which individuals have difficulty distinguishing between certain colors. While there is currently no cure for this condition, various wearables can be used to improve the color perception of those affected. The most common wearables used are color-filtering glasses and lenses, which filter out the problematic wavelengths. The most prevalent form of color vision is more commonly known as red-green color blindness. In this study, gold nanoparticles are in-situ reduced onto contact lens material, forming plasmonic contact lenses targeted for red-green color blindness management. The absorption of the plasmonic particles that peak around 533 nm filter out wavelength to significantly enhance the color perception of both deuteranopia and protanopia. The study also presents an approach of imaging through the plasmonic lenses followed by the color blindness vision simulation to replicate the colorblind individual's vision. When combined with the Ishihara test, this approach proves to effectively improve color perception with the use of plasmonic contact lenses. The study presents a facile method for creating stable hydrophilic plasmonic contact lenses to manage color blindness. While also offers a unique way to simulate the impact of color filtering on the vision of individuals with color blindness.



# Tissue Stiffness Characterization Using Optical Coherence Elastography: Computational Estimation And Analysis

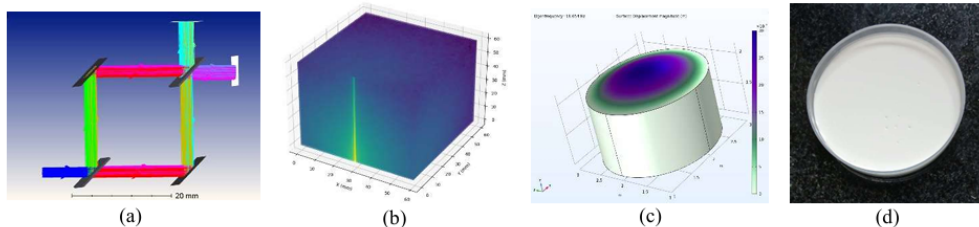
Arjun Bhattacharya and Renu John

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Tissue stiffness analysis with robust boundary estimation is essential for clinical diagnosis and evaluation of treatment efficacy. This requirement has introduced the domain of biomechanical characterisation of tissues, notably via elastography techniques. Elastography encompasses several approaches to introduce excitation and deformation in the targeted tissue. An umbrella of modalities can be employed to compile elastic responses and get mapped to an elastogram. However, Optical Coherence Elastography (OCE) is a non-destructive system that utilises OCT high-resolution imaging to generate significantly sensitive elastograms for diverse applications. The prevalent wave-based OCE method introduces many wave perturbations in the applied media to examine elastic responses.

In this context, the work proposes a comprehensive analysis framework using various tissue models computationally examined by non-invasive elastography techniques underlying realistic assumptions. The devised interferometer setup configuration gets optimised using simulated solvers for practical sample analysis. Moreover, the study entails light transport with matter interactions investigated using Monte Carlo (robust numerical approach) methods and estimates for optical fluence. Additionally, a collection of 2D and 3D optical phantom models with numerous scatterers, absorbers and principal matrix (bulk material) are employed to mimic natural tissue properties. For wave-based OCE, the nature of palpitations gets evaluated using finite element analysis and mathematical (matrix) models of diverse media.

Furthermore, this work explores the optics-based parameters, such as anisotropy, refractive index, absorption and scattering coefficients, that influence photon transport within clinical structures. The elastogram infers diversified biomechanical behaviours, including stiffness, stress-strain, viscoelasticity, damping, wave propagation and boundary interfaces having tissue-mimicking properties. Therefore, this methodology produces a well-established and validated workflow to optimise the imaging setup, model layer properties, and perturbation techniques to reconstruct elastograms. This approach ensures detectability with reliable feature resolution for clinical diagnosis.



(a) Simulation of Mach-Zehnder Interferometer using the Zemax Optic Studio. (b) Monte Carlo Simulation of Homogenous Phantom [Size: 6mm x 6mm x 6mm]. (c) Surface displacement simulation of Silicon-based cylindrical model perturbed by mechanical vibration in COMSOL Multiphysics. (d) Agar-based phantom with TiO<sub>2</sub> scatterer.

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## **Sensing The Bactericidal And Bacteriostatic Antimicrobial Mode Of Action Using Raman Deuterium Stable Isotope Probing (DSIP) In Escherichia Coli**

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The mode of action of antibiotics can be broadly classified as bacteriostatic and bactericidal. The bacteriostatic mode leads to the arrested growth of the cells, while the bacteriocidal mode causes cell death. In this work, we report the applicability of deuterium stable isotope probing (DSIP) in combination with Raman spectroscopy (Raman DSIP) for discriminating the mode of action of antibiotics at the community level. *Escherichia coli*, a well-known model microbe, was used as an organism for the study. We optimized the concentration of deuterium oxide required for metabolic activity monitoring without compromising the microbial growth. Our findings suggest that changes in the intensity of the C–D band in the high-wavenumber region could serve as a quantifiable marker for determining the antibiotic mode of action. This can be used for early identification of the antibiotic's mode of action. Our results explore the new perspective that supports the utility of deuterium-based vibrational tags in the field of clinical spectroscopy. Understanding the antibiotic's mode of action on bacterial cells in a short and objective manner can significantly enhance the clinical management abilities of infectious diseases and may also help in personalized antimicrobial therapy.

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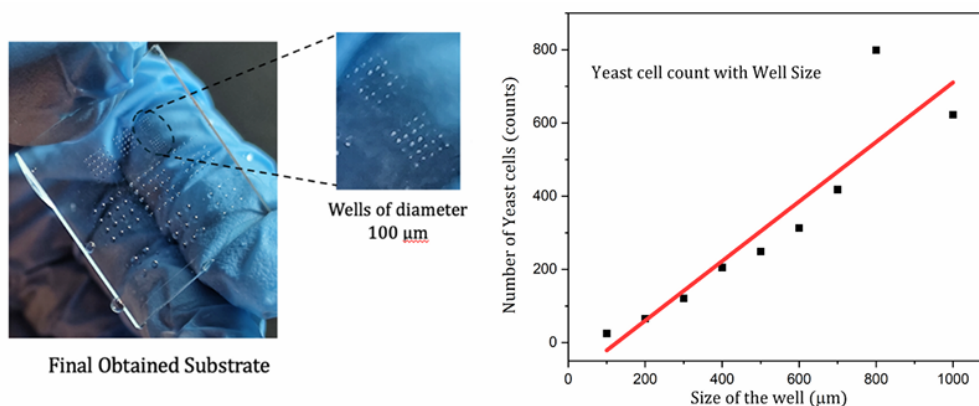
# Development Of A Wettability Contrast Substrate For Controlled Cell Seeding Towards Biomedical Applications

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A microwell array platform is a versatile tool that enables precise, high-throughput screening in biology, biotechnology, and medicine by offering control over cell and molecular environments at a microscale level. These wells are extensively used in the field of single cell analysis, drug screening, diagnostics and biosensing (1). In this work, we demonstrate a simple wettability contrast surface with hydrophilic spots of varying size to obtain surfaces that mimic microwells that can accommodate desired number of cells on splitting yeast cell solution. The wettability contrast surface is created by oil grafting the substrate containing pillar like structure of positive photoresist AZ5214E, which was fabricated using photolithography process. Once grafting is completed, the substrate is ultrasonicated in acetone to remove the photoresist from substrate to obtain hydrophilic regions. This substrate is then used to split the yeast solution and Acridine Orange dye. The fluorescent images of yeast cell trapped in wells of size ranging from 100  $\mu\text{m}$  to 800  $\mu\text{m}$  are captured using fluorescence microscope and analysed. It is observed that, the number of yeast cells can be controlled by varying the size of the well. In our future work, we will be integrating with spectroscopic techniques for single cell studies.



*Image of the developed wettability contrast substrate(left), and the variation of the yeast cell count with the size of the well (right).*

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## Band Gap Properties Of 2D Square Lattice Photonic Crystal

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Photonic crystals are materials with a periodic structure on the scale of the wavelength of light, allowing them to manipulate and control the propagation of electromagnetic radiation. Photonic crystals can be categorized into one-dimensional (1D), two-dimensional (2D), and three-dimensional (3D) structures according to their periodicity. Photonic crystals exhibit photonic band gaps (PBG), which are specific wavelength or frequency ranges that prevent light propagation through the structure. The engineering of photonic band gaps unlocks a wide range of applications in both biomedical and technological fields. In this work, we investigated the band gap properties of a 2D square lattice photonic crystals with circular and square rods using the plane wave expansion method. It is observed that the band gap can be very well tuned by varying the radius of the circular rod, length of the square rod and permittivity of the materials

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## Laser Induced Fluorescence From Optically Cleared Bio Samples

Alaka Pramod, Sanoop Pavithran M, C. S. Suchand Sandeep, and Santhosh Chidangil

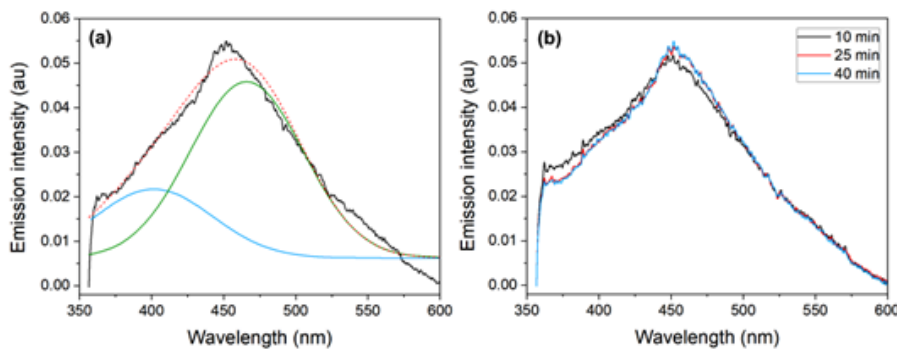
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Fluorescence imaging is a non-invasive technique widely utilized in the biomedical sciences to investigate biological samples with high-resolution and molecular specificity at the cellular level. However, achieving effective imaging in deeper tissue layers presents significant challenges due to light scattering caused by various structures present in the sample. Tissues are made up of various components such as collagen, fibers, organelles, and ribosomes, which predominantly scatter light [1]. Therefore, optical imaging into deeper layers of tissues remains challenging because scattering limits the depth of penetration of light, making it difficult to achieve deep tissue imaging. Tissue optical clearing was introduced as a solution to tackle this problem and to improve light penetration into the sample [1,2]. This approach reduces tissue heterogeneity, minimizes light scattering, and improves the depth of light penetration [2]. The primary objective of this work is to investigate the efficacy of optical clearing techniques in enhancing the transparency in chicken tissue samples for improved fluorescence signal using laser-induced fluorescence (LIF) spectroscopy.

Preliminary results include LIF spectroscopy measurements performed on chicken muscle tissue samples at an excitation wavelength of 324 nm. A fiber-coupled LIF measurement setup was used for the measurements, which included a He-Cd laser

connected to a fiber optic probe consisting of a bifurcated fiber bundle. The emission path of the fiber bundle was connected to a spectrograph equipped with a thermoelectrically cooled Andor CCD camera [3]. Autofluorescence spectra from the samples were recorded and Gaussian deconvolution of the spectra was carried out to identify the spectral contributions from different components in the tissue (Fig.a). The emission peaks are attributed to the autofluorescence of collagen and enzyme NADH. For optical clearing of the samples, glycerol was used as the optical clearing agent and the changes in the autofluorescence spectra were analysed by recording the spectra over an optical clearing period of 40 minutes, with measurements taken every 5 minutes of optical clearing (Fig. b).



(a) Gaussian deconvolution of the LIF spectra. (b) LIF from samples that underwent optical clearing for different durations.

These results confirm that the fluorescence properties of the chicken tissue, particularly intensity and peak positions, are preserved after optical clearing treatment of the sample. Continuation of the research aims to identify optimal optical clearing duration, quantification of optical clearing depth and investigate LIF characteristics across different optical clearing agents. The findings from these measurements are envisaged to contribute valuable insights into enhancing optical imaging procedures for deeper tissue analysis that could have broader implications for biomedical applications.

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# Diabetes And Cancer Detection Using Whispering Gallery Mode Based Microring Resonator Sensor

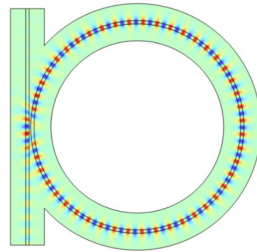
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With 450 million individuals suffering from diabetes and 20 million people diagnosed with cancer annually, early detection of both diseases is essential for effective management and improved outcomes. Timely diagnosis of cancer and diabetes can significantly reduce complications, enhancing the quality of life for millions globally. This study presents a whispering gallery mode (WGM)- based optical sensor designed to detect cancer cells in blood samples and diabetes markers in tear samples, operating at a wavelength of  $1.55\mu\text{m}$ . The WGM-based approach significantly enhances modern optical sensing, leveraging its high-quality factor and rapid sensing capabilities. The sensor features a micro-ring structure with a radius of approximately  $10\mu\text{m}$ , coupled to a waveguide with a width of about  $2\mu\text{m}$ , offering compact design advantages for portable device integration. The sensor's sensitivity and quality factor are theoretically evaluated using the finite element method, showcasing its flexibility and versatility in diagnostic applications. This innovation holds promise for the efficient detection of both cancer and diabetes biomarkers in a compact, efficient manner.



*Schematic diagram and electric field profile of the whispering gallery mode (WGM)-based optical sensor, illustrating the micro-ring structure coupled to the waveguide and the distribution of the electric field within the resonator.*



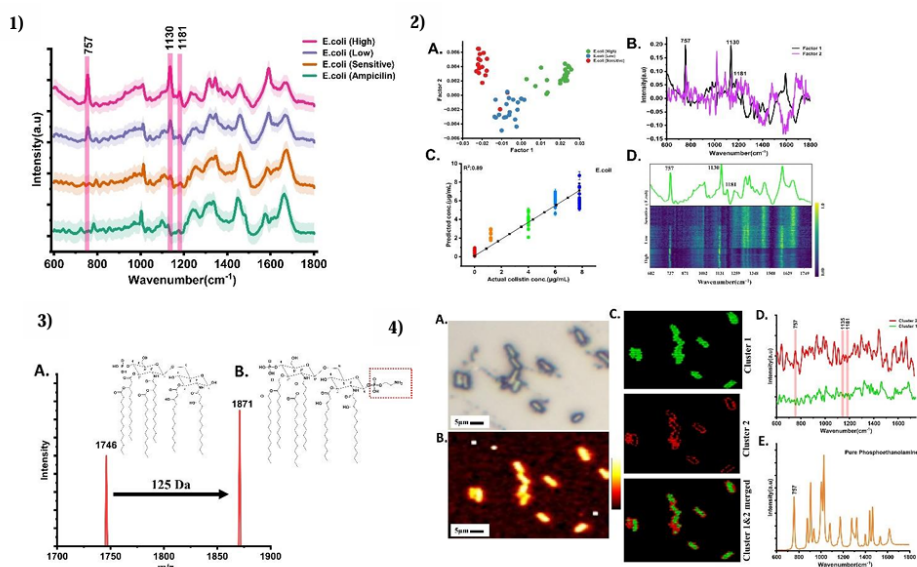
# **Machine learning assisted Raman spectroscopic validation of phosphoethanolamine modification as a potential marker of colistin resistance**

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Colistin antibiotic is regarded as the final line of defense for treating infections caused by gram-negative bacteria. The combination of Raman spectroscopy (RS) with diverse machine learning methods has helped to unravel the complexity of various microbiology problems. This approach offers a culture-free, rapid, and objective tool for identifying antimicrobial resistance (AMR). In this study, we employed the combinatorial approach of machine learning and RS to identify a novel spectral marker associated with phosphoethanolamine modification in lipid A moiety of colistin resistant gram-negative *Escherichia coli*. The visible spectral fingerprints of this marker have been validated by partial least square regression and discriminant analysis. The origin of the spectral feature has been confirmed by hyperspectral imaging and K-means clustering of a single bacterial cell as shown in the figure. The chemical structure of the modified lipid A moiety has been also verified by gold standard MALDI-TOF mass spectrometry. Our findings support futuristic applicability of this spectroscopic marker in objectively identifying colistin-sensitive and resistant.



Average spectra obtained from *E. coli* cells ampicillin resistant, colistin-sensitive, resistant to low concentration colistin and resistant to high colistin concentration, 2) (A). Partial least square discriminant analysis (PLS-DA) and corresponding coefficients (B), (C) Comparison between actual and predicted colistin concentrations through PLS regression, (D) Color coded heat map showing average spectra from all the spectra acquired from bacteria under different group. The top shows the regression vector from the prediction model. The vector plot was found to mimic the prominent spectral peaks from the colistin resistant microbes, 3) Lipid-A structural difference between sensitive (A) and resistant strain (B) using MALDI-TOF-MS, PEA addition in the resistant strain indicated by the red rectangle. 4) Localization of resistant specific spectral signatures: (A) The white light image of the bacterial cells, (B) Visualization and understanding of the overall bacterial map using signal at 2954  $\text{cm}^{-1}$  (C) K-means clustering analysis from Raman signal.

## Label-Free Optical Biopsy: A Multi-Modal Microscopic Approach For Oral Cancer Diagnosis

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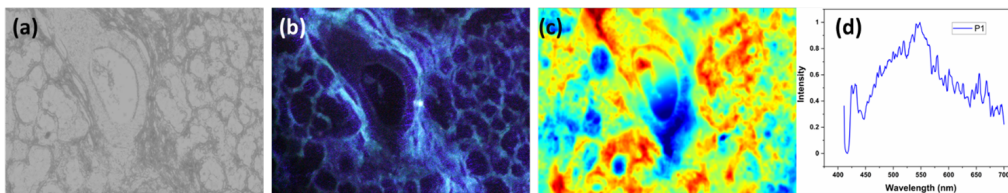
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The present study demonstrates the design, development, and comprehensive testing of a novel multimodal optical system for simultaneous auto-fluorescence imaging, spectroscopy, and quantitative phase microscopy within the same field of view (FOV). The system combines the strengths of autofluorescence, which provides molecular-level insights into metabolic activities, with quantitative phase imaging (QPI), offering nanometer-scale sensitivity to cellular morphology and refractive index distribution. Autofluorescence of biological tissues serve as a natural biomarker, detecting abnormal cellular changes often associated with early stages of cancer development, thereby

offering a non-invasive approach to identifying malignant transformations [1]. QPI provides detailed structural information by probing refractive index-dependent variations in cellular morphology, which can be indicative of cancerous progression. There are several QPI techniques which is generally associated with recording a high contrast interferogram which is then processed computationally to retrieve the phase map of the system. However, the instability and complex optical alignment associated with the interferometric measurements limits the potential of QPI for multi-model techniques [2, 3]. Further, the need for coherent light source for illumination also gives rise to coherent noise in the phase retrieval which poses challenges for multi-model integration of the system. The use of a non-interferometric quantitative phase imaging technique has the potential to overcome several inherent challenges of interferometric QPI, such as susceptibility to mechanical instability and coherent noise, ensuring a more robust and stable phase retrieval process. In the present paper, a single microscopic objective (MO) is used to collect the autofluorescence and BF image of the sample within the FOV. Defocused images of the sample are recorded at  $\pm \Delta z$  positions, which are then processed computationally using transport of intensity equation to obtain the phase map of the sample. The integration of such system enhances both the spatial and molecular diagnostic capabilities, allowing for a more comprehensive assessment of cellular health. The versatility and the potential of the setup is demonstrated on oral cancer tissues.



*Multi-model analysis of oral cancer tissue (a) BF image (b) AF image (c) Phase map (d) AF spectra*

The ability to simultaneously obtain biochemical and morphological data from the same tissue section significantly improves the accuracy and specificity of label-free cancer diagnostics. This approach offers the potential to revolutionize early cancer detection and monitoring by providing a powerful, minimally invasive and highly sensitive tool for clinicians and researchers, with broad applications in oncology and biomedical imaging.

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# Exploring The Spectroscopic Interactions Between Folic Acid And Citrate-Stabilized Gold Nanoparticles: NSET Study

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Energy transfer from folic acid to metal nanoparticles is a significant process with potential applications in sensing, diagnostics, or other nanoscale technologies. Using photoluminescence excitation and emission spectroscopy, we investigated the interaction of citrate-stabilized spherical gold nanoparticles (Acceptor) of various sizes (5 nm, 10 nm, 20 nm, and 40 nm) with water-soluble vitamin folic acid (Donor), also known as vitamin B9. As the concentration of Gold Nanoparticles (AuNPs) increased (from 0.0156 nM to 1 nM at a wavelength of 444 nm), the intensity of the fluorescence of the donor molecule diminished. The efficiency of energy transfer increases significantly as the metal nanoparticle's size increases. Time-resolved fluorescence spectroscopy demonstrated a reduction in the fluorescence lifetimes of folic acid from 3.95 ns to 2.9 ns, 2.6 ns, 2.5 ns, and 1.7 ns upon addition of gold nanoparticles of varying sizes. Our findings suggest that Nanoparticle Surface Energy Transfer (NSET) is the predominant mechanism accountable for the quenching of Photoluminescence (PL) by AuNPs, providing crucial insights for the advancement, analysis, and utilization of nanobiosensors that depend on photoluminescence quenching by AuNPs.

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## Photodynamic Therapy As A Promising Tool In The Fight Against *Pseudomonas Aeruginosa*

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Bacteria have developed resistance to numerous drugs worldwide [1]. One such multidrug-resistant (MDR) microbe is *Pseudomonas aeruginosa*, a gram-negative opportunistic bacteria [2] that causes acute or chronic infections in immunocompromised individuals with a 40% mortality rate [3]. Chronic colonization by bacteria is associated with high-density biofilm formation and matrix-enclosed aggregates that are difficult to eliminate [4]. Under certain environmental conditions, *P. aeruginosa* is reported to

disperse from the initial infection site to widespread infection, leading to systemic dissemination [4].

Photodynamic therapy (PDT) is emerging as an antibacterial strategy because of its noninvasiveness, low adverse effects, and inability to cause the development of drug resistance [5]. Photodynamic therapy combines light of a specific wavelength, a substrate, and a photosensitizer (PS) to release free radicals and reactive oxygen species (ROS) while inhibiting biofilm formation and eradicating target cells [1]. After conducting a literature review of studies from the past nine years, we found PDT to be an effective alternative to antimicrobials for controlling *P. aeruginosa* infections by inhibiting biofilm formation via the QS pathway [6].

Recent advances in PDT have focused on optimizing photosensitizer molecules for better selectivity, enhanced ROS generation and deeper tissue penetration [7]. Macrophage-directed photodynamic therapy [3] and nanotechnology-based delivery systems [2] are also being explored for targeted delivery and selectivity. It has emerged as a more viable alternative to conventional clinical treatments in counteracting the increasing prevalence of MDR *P. aeruginosa* [8].

The application of the aPDT approach has been reported in vitro, but to understand its better usage, many clinical trials are necessary [4]. Finally, the current challenges of PDT and future direction of organic photosensitizer-based phototherapy for clinical antimicrobial applications are presented in this review. .

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## **Bioresorbable hydrogel laden with curcumin-encapsulated surface engineered nano frameworks for biomedical applications: Physiochemical and *in vitro* study**

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Tissue engineering is recognized as a constantly evolving domain since past two decades. It bridges technical innovations from fields such as material science, nanoscience, cell and molecular biology. The, promising progresses in these field has resulted in novel nano drug carriers, smart biomaterials and 3D printed tissue modelling with precise control and stimuli responsive micro environments for cell growth and differentiation. Nanomaterial, zeolitic imidazolate frameworks-8 (ZIF-8), is a type of metal-organic frameworks (MOFs), that displays large surface area with pores, that can hold massive amounts of drugs. They have an extremely, tunable chemical functionality, which makes them highly versatile materials for various applications including drug delivery. In this work, silver doped curcumin loaded zeolitic imidazolate frameworks-8 (Ag@C-ZIF-8) were synthesized via single step reaction and subsequently loaded into bioresorbable k carrageenan/egg albumen (KC/EA) based hydrogels. Spectroscopic, analysis of nanocages revealed that, absorption peak shift of ZIF-8 in Ag@C-ZIF-8, indicates curcumin encapsulation within ZIF-8 cages. Micrographs of Ag@C-ZIF-8 showed a regular rhombic dodecahedron shape, with a particle size of approximately  $222 \pm 55$  nm, which is quite larger than ZIF-8 nanocrystals ( $158 \pm 21$  nm) due to the successful encapsulation of curcumin. Additionally, the synthesized nanocomposite was encumbered into KC/EA hydrogels synthesised via inotropic gelation technique. Functional group analysis by FTIR revealed that, there was no specific chemical interaction neither chemical bond formation among nanoparticles and hydrogel matrix, signifying nanoparticles where loaded within the voids of hydrogel matrix, making the releases of curcumin effortless, as a consequence supports a sustained release. The general cytotoxicity of the scaffolds was determined using MTT assay against the 3T3 mouse fibroblast cell line. The results indicated over 90% cell viability over both Ag@C-ZIF-8 loaded and unloaded KC/EA hydrogels. These preliminary findings represented significant implications for future applications of the metal doped ZIF-8 particles as curcumin carriers, also displayed a novel combination of biopolymers for tissue engineering applications with an antimicrobial feature.

**Keywords:** Scaffolds, Curcumin, Metal-organic frameworks, Polymer, Biocompatibility.

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# Machine learning based crop health monitoring in hydroponic lettuce using svm classification of hyperspectral data

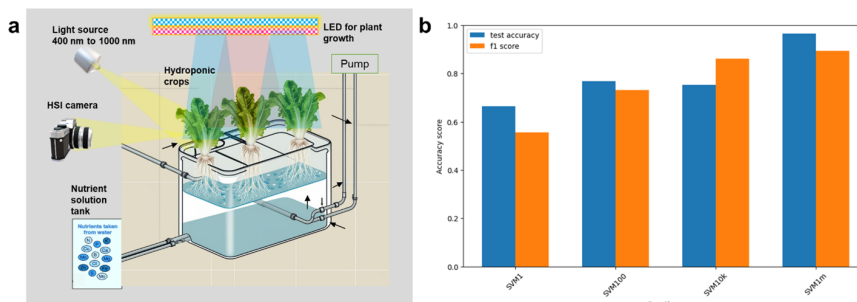
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Effective crop health monitoring is crucial for ensuring year-round crop production and quality in hydroponic farming [1]. Recent advancements in imaging technology have positioned hyperspectral imaging (HSI) as a promising tool for plant research, offering non-destructive, rapid, and highly automated methods for agricultural monitoring [2, 3]. By integrating HSI with machine learning (ML), solutions are emerging for key agricultural challenges, including stress detection, crop monitoring, yield estimation, and nutrient management [4, 5]. Among the various ML techniques, Support Vector Machines (SVM) and Random Forests (RF) are gaining considerable attention for HSI analysis. However, despite their potential, the application of these methods for early-stage nutrient deficiency detection in crops remains relatively underexplored [6]. In this context, this research proposes an SVM-based model for early-stage detection of nutrient deficiencies in hydroponic crops. The model utilizes features extracted from hyperspectral datacubes to train SVM classifiers. Among the investigated models, SVM with 100 iterations (SVM100) demonstrated a balance between performance time and accuracy of 4.41 minutes and 76.87% respectively. The SVM model with 1 million iterations (SVM1m) achieved the best test accuracy of 96.42% and the highest f1 score of 89.29% on the cross-validation dataset. The proposed non-invasive imaging system is envisaged to revolutionize automated monitoring in indoor hydroponic farms, contributing to a sustainable future.



(a) Schematic diagram of the experimental setup used, (b) SVM binary model outcomes in terms of accuracy and f1 score

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# Investigation Of Microplastics From Pet Bottles Using Raman Optical Tweezers

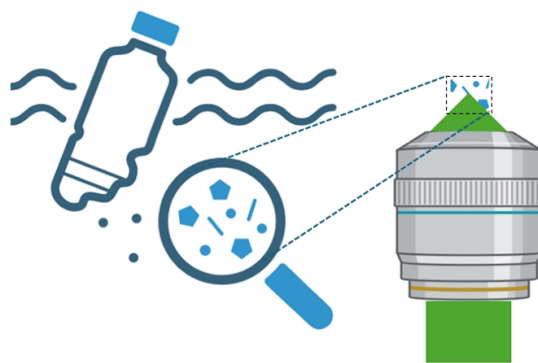
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Microplastics (MPs) have percolated into food chain through drinking water, tea bags, packaged food, fishes etc. Although the effects of microplastic bioaccumulation for humans are not fully understood, there have been reports of oxidative stress, inflammatory lesions, and increased risk of neoplasia.[1] The detection and quantification of MPs are governed by techniques like light scattering techniques, Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS).[2] We investigate the presence of MPs using Raman optical tweezers (optical tweezers coupled with spectroscopy), as an analytical tool for identification. Single particle analysis helps us to differentiate MPs from sediments like slit clay and organic matter, unlike the conventional spectroscopic technique that account for the ensemble average. Since this is an imaging technique, we can simultaneously visualize the Fig. Optical trapping of leached microplastics MP along with the spectroscopic data. Single use plastic bottles and PET bottles are expected to show differences in terms of fate and distribution of microplastic. Therefore, this modality has the potential to impact future landscape of environmental plastic pollution.



*Optical trapping of leached microplastics*

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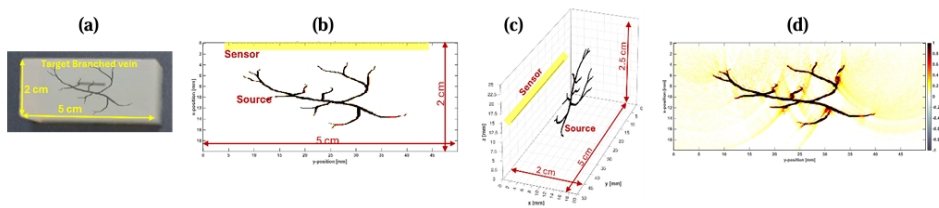
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# Preliminary Tutorial On In-silico Photoacoustic Imaging Using Tissue Mimicking Phantoms

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Photoacoustic imaging (PAI) is an emerging non-invasive hybrid imaging modality combining optical excitation and ultrasound (US) detection [1]. PAI relies on photoacoustic (PA) effect, where US waves are generated by thermoelastic expansion when the sample is illuminated by nanosecond pulsed laser. PAI provides a high optical contrast along with highly scalable resolution at deep penetration depths of up to centimeters. PAI can provide molecular, structural and functional information by using endogenous contrast agents like water, hemoglobin or a variety of exogeneous contrast agents, or both. PAI has shown potential in a wide range of clinical applications, including vasculature characterization, breast imaging, dermatologic imaging and many more. With the advancement of PAI, computational modelling will play a significant role in enabling clinical translation by simulating real-world performance in a variety of biological and device scenarios [2]. The incident light can be modelled using the Monte Carlo model of tissue light transport. The 2D and 3D acoustic wave propagation can be modelled using k-wave toolbox [3]. The magnitude of the PA signal generated is directly related to the optical fluence and the optical absorption of the target (source). In this paper, an introductory tutorial is presented to generate PA signals, where basic light transport model is used to get optical fluence. The generated PA signals are detected using ultrasound transducer (UST) modeled in k-wave toolbox. The in-silico model mimics the in-house made phantom and the UST is modelled to mimic a 128 element UST with 0.3 mm element pitch. These acoustic models are also created in 3D to better demonstrate the wave propagation in real 3D phantom. The detected PA signals are used to reconstruct the images (Fig). Modelling tools can help optimize device design and assess PAI system performance by deepening our understanding of complex physical processes.



*in-silico phantom inspired from in-house made tissue mimicking phantom: (a) in-house phantom of size 2cm x 5cm x 2.5cm, (b, c) 2D and 3D computational grid of same dimensions as the in-house phantom respectively, (d) reconstructed image*

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## **Micro-spectro-endoscope: A Cancer Screening Device**

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and Dalip Singh Mehta<sup>1</sup>

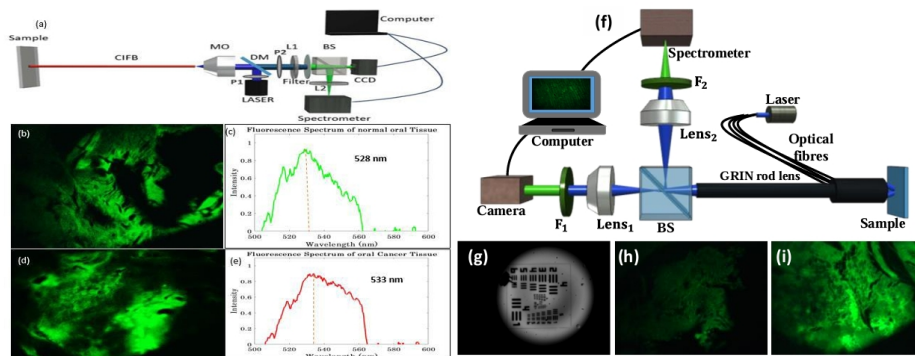
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Oral cancer is one of the most common cancers among all cancers worldwide [1]. The development of oral squamous cell carcinoma (OSCC) from mucosal precancerous is the main reason for mortality. Precancerous changes might be noticeable in the form of white and red lesions, such as leukoplakia and erythroplakia, respectively, but the progression of OSCC can't be macroscopically visible [2]. Microscopic changes with morphological textures can be probed with the help of endoscopic microscopy with fluorescence technique [3-5]. Fluorescence provides molecular-level transitions for a particular excitation, which can result in chemical composition within normal and cancerous tissues. In addition to this, the fluorescence technique might be used for the progression of OSCC from the precancerous stages [6]. Fluorescence microscopic images can give quantitative statistical parameters like mean fluorescence intensity, standard deviation, skewness, kurtosis, etc., and fluorescence spectra can give the fluorescence intensity, peak maximum, and full-width half maximum. Depending upon these parameters, one can distinguish between OSCC, normal, and early-stage or precancerous-stage tissues. Early-stage screening could be done with the help of optical techniques and can lead to low fatality. The present manuscript deals with endoscopic microscopy of OSCC using fluorescence modality with simultaneous spectra. A GRIN-lens-based and imaging fibre bundle-based micro-spectro-endoscope (MSE) are developed for the fluorescence endoscopic, microscopic imaging and spectroscopy of OSCC tissue. Imaging fibre bundles allow for one-to-one mapping of OSCC tissue. Fluorescence imaging gives the statistical quantitative parameters based on the fluorescence contrast, which is entirely different for OSCC and normal tissue, and gives information about heterogeneity present in cancerous and normal tissue. The figure shows the developed MSE. Details about the development of MSE using GRIN lens and coherent fibre bundle will be discussed. Experimental results of cancerous, precancerous, and normal proteinuria will be demonstrated. The MSE device has the potential for in-vivo microstructural imaging and fluorescence spectroscopy of oral

cancer patients. It is low-cost and field portable.



(a) Schematic of the developed experimental setup of MSE using imaging fibre bundle. (b) Fluorescence image of normal oral tissue. (c) Fluorescence spectrum of normal oral tissue (d) Fluorescence image of oral cancer tissue. (e) Fluorescence spectrum of oral cancer tissue. (f) Micro-spectro-endoscope with GRIN rod lens, laser, fibre bundle, tube lens emission filter, CCD, and computer. (g) USAF chart showing a resolution of 4.38 microns. (h) Fluorescence image of normal oral tissue. (i) Fluorescence image of OSCC tissue.

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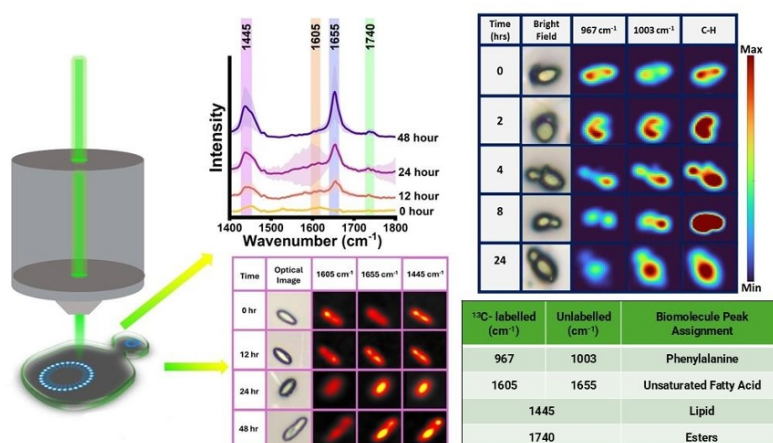
# Extraction-free approach for monitoring and imaging microbial metabolism in action: Advancing Raman spectroscopy-based metabolomics

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Single cells are complex dynamic systems which require advanced multiplexing tools for monitoring and imaging biomolecules and their synthesis as well as turnover. Raman spectroscopy and imaging is a non-invasive vibrational imaging platform for the spatio-temporal monitoring and distribution of multiple biomolecules such as amino acids, nucleic acids, proteins, lipids and others simultaneously at the single cell level. [1–3] In our study, we have used carbon -13 ( $^{13}\text{C}_6$ ) stable isotopes as vibrational tags which show a distinct shifted peak assigned to biomolecules in Raman spectra when compared to their unlabelled counterpart. We have grown  $^{13}\text{C}_6$  labelled microbial cells in the carbon-free culture medium with an unlabelled ( $^{12}\text{C}_6$ ) carbon source and acquired the Raman spectra and image of a single microbial cell at different time points. The single-cell Raman spectra in the early hour show a highly intense signal distribution from the  $^{13}\text{C}_6$  labelled biomolecule peak position whereas a diminished signal intensity from the unlabelled biomolecule peak position. As incubation time progresses, signal intensity distribution from the  $^{13}\text{C}_6$  labelled peak weakens and the nascent unlabelled peak becomes more pronounced. These finding shows the applicability of Raman spectroscopy and imaging to monitor multiple biosynthesis pathways in action simultaneously and generate spatial maps of single cells to visualize the turnover distribution of newly synthesized biomolecules. Further, it also demonstrates the rapid and sensitive applicability of Raman spectroscopy for qualitative and quasi-quantitative monitoring of microbial metabolism in action.



*Raman images showing time-dependent dynamics of different metabolites in the single microbial cell in situ.*

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## Synthesis and Characterization of Copper Tin Sulfide (CTS) Thin films by SILAR Method

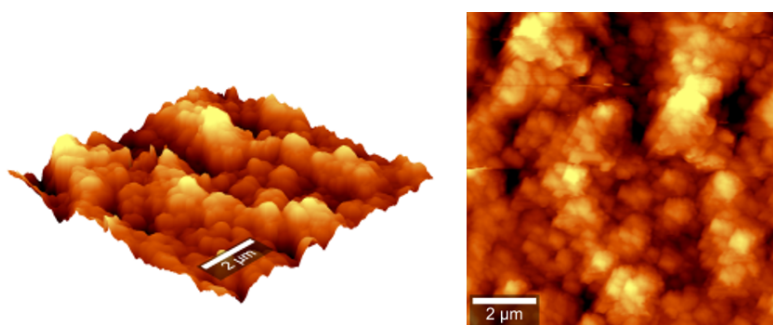
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Copper Tin Sulfide (CTS) thin films were successfully synthesized using the Successive Ionic Layer Adsorption and Reaction (SILAR) method. Initially, glass substrates were cleaned in acetone, methanol, and deionized water. The deposition process began with the immersion of the substrate in a mixture of 0.02 M CuSO<sub>4</sub> solution and 0.01 M SnCl<sub>2</sub> solution for 15 seconds, followed by rinsing 2S with deionized water. Then it is immersed in 0.03 M Na<sub>2</sub>S solution for the sulfur layer formation followed by rinsing. The deposition process was repeated for 40 cycles to achieve the desired film thickness. The resulted films were characterized using X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM). XRD analysis revealed the presence of a crystalline orthorhombic phase. AFM imaging showed a smooth and uniform surface morphology. The results demonstrate the effectiveness of the SILAR method in producing high-quality CTS thin films with potential applications in photovoltaic devices. The study highlights the importance of characterization techniques in understanding the structural and morphological properties of thin films.

**Keywords:** CTS, SILAR, thin film, Photovoltaic cells.



*AFM images of CTS thin film synthesized by SILAR method.*

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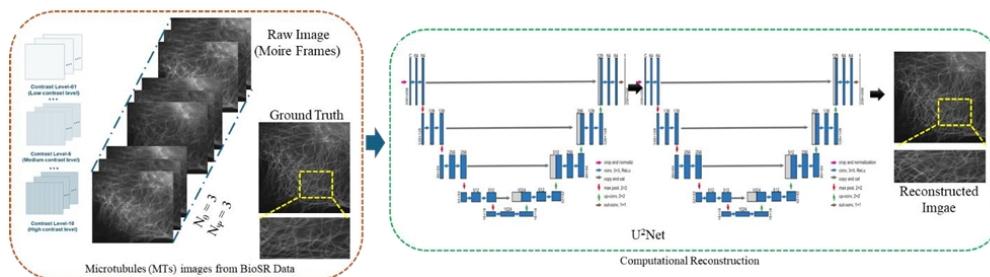
# Quantifying The Effect Of Light Contrast On Structured Illumination Microscopy Imaging

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Structured illumination microscopy (SIM) surpasses the diffraction limit, offering enhanced resolution through multiple image acquisitions under varying illumination patterns. However, conventional SIM demands high-contrast patterns, stable light sources, and precision optics. To address these limitations, we propose a deep-learning approach for contrast-enhanced low-light SIM. SIM leverages patterned illumination to extract high-resolution information via post-processing. While high contrast patterns are essential for accurate reconstructions, low-contrast conditions hinder image quality. Recent studies demonstrate deep learning's potential for improving low-contrast SIM images through adaptive illumination compensation [1].

A deep learning-based approach for SIM image reconstruction is presented to improve the quality of SIM images, even under low-light imaging conditions. The BioSR [2] open-source dataset of different contrast stripe patterns was used to train a deep neural network to enhance SIM image quality, particularly in low-contrast scenarios. High-quality ground truth images paired with raw 2D SIM images of varying contrast levels (9 Levels) were included in this dataset. A U-Net and U2-Net architecture with an encoder and decoder was employed. The network's loss function was iteratively learned from these pairs, resulting in improved image quality. Fine features were extracted from the raw SIM images by the encoder, which acted as an analyst. These features were then passed to the decoder, which functions as an image enhancer to refine the features and create a high-quality final image. Finally, a super-resolution image was reconstructed from the raw SIM data by the decoder utilizing the processed features, leading to a significantly improved image compared to the original low light input.



Block diagram of proposed approach.

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## **Optical trapping of *E-Coli*, *S. Aureus* and *Cyanobacteria***

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Understanding of cell mechanics is essential for diagnosis of diseases and healthy life cycle. Adhesion of bacterial cells on different substrates leads to infection. *E. coli* can cause gastroenteritis, infections in the urinary tract, and neonatal meningitis. In rare cases, these pathogenic strains are also responsible for uremic hemolytic syndrome. *S. Aureus* causes skin infections, bloodstream infection and lung infection. Cyanobacteria release toxins which is harmful to ecosystem; water quality is assessed on the percentage of Cynaobacteria (blue green algae). Stiffness of the bacterial cell depends on the wall of the bacterial cell. Silica bead is considered as a role model for position calibration and force calibration. Bacteria was trapped by focused IR laser (975nm ) using Thorlab's Modular Optical Tweezer. Dynamic fluctuation changes in trapped bacteria is monitored on CCD and QPD (Quadrant photodetector). Trap force is calculated by measuring the trap stiffness ( $K_{\text{trap}}$ ) of the bacterial cell wall using back focal plane interferometric based Power spectrum method. Corner frequency( $f_c$ ) and trap stiffness( $K_{\text{trap}}$ ) are key parameters to investigate force acting on the cell. In this paper, measurement of Optical forces of geometrically different bacterial cells are reported using Optical Tweezer Technique.

**Keywords:** Bacteria, Power spectrum, Stiffness, Cell wall, Optical Tweezer

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## **Improving Temporal Resolution Of Laser Speckle Imaging System Using Deep Learning Based Approach**

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Laser speckle-based techniques is a wide-field and non-invasive optical technique which is being adopted for micro vascular blood flow studies at superficial and deep tissue level [1]. The accuracy of blood flow estimation is significantly improved by the acquisition of multi-exposure and wide dynamic range of speckle contrast data [2-3]. As the prior information of the blood flow is unknown, choosing an appropriate range of camera exposure time (T) is critical. Due to limited dynamic range and signal-noise ratio (SNR) of the imaging system, selection of range of camera exposure time and number of exposure (N) is demanding. This results with longer data acquisition time and overall increase the temporal resolution of the imaging system. The tradeoff associated with

temporal resolution and accuracy in quantifying the blood flow has been addressed based on data imputation using deep learning (DL) network. In particular, generative adversarial network (GAN) [4] has been used to generate denser sample of multi-exposure speckle contrast data from the coarsely acquired dataset. The performance of the GAN based data imputation approach has been verified using simulation. From the results and its analysis, it is evident that the GAN helps to estimate the moving scatterer decay time without compromising its accuracy and also reduce the need of capturing denser speckle contrast data. At present, the GAN efficiently impute speckle contrast data at the wider range of exposure time and also enhancing the temporal resolution of the imaging system by 3 fold.

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## Olfactory Sensing In Humans Using Diffuse Correlation Spectroscopy (DCS) System

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In this work, we present a novel study on olfactory sensing in healthy human subjects using an in-house developed Diffuse Correlation Spectroscopy (DCS) system, with potential for clinical translation. In this study we used DCS to show relative Cerebral Blood Flow Changes (rCBF) in response to olfactory stimulus in real-time. Over a five-minute period, we observed shifts in rCBF in the pre-frontal cortex following odour exposure, as compared to baseline measurements. These preliminary results in olfactory sensing using DCS pave the way for more detailed explorations into olfaction like responsivity to various types of odours in healthy as well as patients with anosmia (loss of sense of smell) and hyposmia/microsmia (weakened sense of smell).

We further demonstrate the robustness of this system enroute by conducting head-of-bed positioning experiments on 6 healthy subjects. Real-time monitoring revealed changes in blood flow in the pre-frontal cortical region corresponding to two positions—supine and standing—over a five-minute duration per subject. The observed  $-31.3 \pm 4.23\%$  change in relative Cerebral Blood Flow (rCBF) from supine to standing position aligns with

findings from existing studies, validating the accuracy of our system. This novel study in olfaction highlights the sensitivity of our Diffuse Correlation Spectroscopy (DCS) system to changes in deep tissue blood flow, suggesting its potential value for research involving viral or neurodegenerative conditions often associated with olfactory deficits. Overall, our results from both these functional studies demonstrate the system's promise for clinical application, with potential for understanding neurovascular implications of various health conditions and supporting early diagnosis and treatment monitoring.

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## **Spatial Coherence Of Optically Pumped Dye-Doped Nematic Liquid Crystals Random Laser**

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Over the past three decades, there have been extensive experimental and theoretical investigations worldwide on random lasers—a special type of laser that has distinct characteristics from conventional lasers [1]. In random lasers (RLs), the optical feedback for lasing stems from recurrent multiple scattering events in a disordered gain medium, at the expense of many key properties of a conventional laser, such as spatial coherence, directionality, beaming nature, and spatial profile. To date, several efforts have been devoted to the demonstration and characterization of RLs in a plethora of disordered and diffusive systems, including semiconducting nanoparticles, metallic nanostructures, polymers, biological tissues, liquid crystals, and many more. When paired with suitable emitters and optically pumped near the resonance wavelength, nematic liquid crystals (NLCs) can random lase with high conversion efficiency [2, 3]. NLC-based RLs provide several degrees of freedom to fine-tune the lasing properties. Dye-doped NLCs can be confined in thin cells, capillaries, and hollow-core optical fibers, exploiting their thermal and electro-optic stimuli responses to tune the lasing threshold, efficiency, and emission wavelength.

Spatial and temporal coherence are two signature characteristics of a laser [4]. The temporal and second-order coherence of random lasers has been thoroughly investigated. However, the spatial coherence properties are not well explored, as they strongly depend on the scattering feedback mechanism exhibited by the random media. Hence, each RL possesses a varying degree of spatial coherence, as it is determined by the internal scattering process. A laser with high spatial coherence can generate a directional light beam with small divergence, but it can induce coherent artifacts in imaging. NLC-based RLs possess ultra-low lasing thresholds, a high degree of polarization, numerous lasing modes, periodicity in the emission spectrum, rich spatial structure, and high directionality, which are lacking in common RLs. Owing to these properties, NLC-based RLs could serve as a potential light source for various applications, including imaging, displays, wavefront shaping, and more. However, information on their spatial coherence is still lacking in the literature.

Herein, we investigate the spatial coherence properties of dye-doped NLC random laser confined in a planar cell. Our RL material consists of a mixture of commercially available E7 (Synthon Chemicals) doped with 0.3 weight % of Pyrromethene 597 (PM 597) dye (Merck). The planar cell is made up of two parallel indium tin oxide (ITO) coated borosilicate glass slides with a thickness of 0.5 mm. The inner surface of the glass plates is also treated with polyimide and mechanically rubbed in parallel directions to ensure homogeneous alignment of the liquid crystal molecules. Mylar spacers of 100  $\mu\text{m}$  thickness are inserted between the glass plates to define the thickness of the fabricated cell. The spatial coherence of the fabricated random laser is examined using Young's double-slit interference experiment and by measuring the speckle contrast ratio. We observed that the average value of the mutual coherence function from the fringe profiles obtained from Young's double-slit interference experiment and the speckle contrast ratio obtained for the NLC random laser are significantly lower than those of the conventional pump laser. This is highly significant for imaging as well as display applications.

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## Exploring label-free RBC morphometrics using brightfield microscopy and explainable AI with an evolving AI technology

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Red blood cells (RBCs) are vital for transportation of gases in blood and regulating blood pH. Analyzing RBC morphology is crucial for understanding blood disorders. While advanced techniques such as flow cytometry and Raman spectroscopy exist, brightfield microscopy is commonly used in smaller labs and medical facilities due to its practicality [1]. Traditionally, this technique necessitated the staining of RBCs. However, with the advancements in Artificial Intelligence (AI), it has become feasible to

conduct RBC morphometric studies without the need for labelling. In this study, images of label-free red blood cells (RBCs) were captured using three distinct brightfield microscopes: Motic 2.0, BX43 Olympus, and Nikon Eclipse Ni-L. Subsequently, the images were subjected to morphometric analysis. A total of 12,550 RBCs, encompassing various morphologies such as discocytes, echinocytes, spherocytes, stomatocytes, codocytes, and elliptocytes, were identified and annotated post cell segmentation utilizing the RedTell Tool. Additionally, overlapping cells, partial cells, artifacts, and folded cells were categorized as separate groups. Employing the RedTell Tool, 74 morphometric features were extracted, comprising object shape-based, intensity-based, and texture based characteristics [2]. A Random Forest Classifier AI model was trained and tested with 1369 different types of RBCs, achieving an accuracy rate of 97.58% and an F1 score of 0.91. To evaluate the model's performance and predictive capabilities during RBC classification, SHAP and LIME explainable AI methods were employed [3]. The results underscore the potential of straightforward brightfield microscopy techniques to enable robust RBC morphometric analysis, further bolstered by the expanding capabilities of AI technology.

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## Fabrication Of A Microvasculature Mimicking Phantom Suitable For Multimodal Perfusion Imaging

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In this work we introduce a novel microfluidic-based perfusion phantom fabrication method tailored for imaging microvascular flow and perfusion studies.

We have adapted the lifted-Hele Shaw Cell (IHSC) technique, a lithography-less method, to create masks with fractal-like geometry, which are then used to fabricate polydimethylsiloxane (PDMS) phantoms via soft lithography. We have modified the technique to produce various masks, including those mimicking vascular-tree structures with channel widths ranging from 200 to 800 microns. A comparative study of the flow profiles in these phantoms with actual mouse brain blood vessels revealed a reasonable match, indicating high structural similarity. Next, we employed a guided anisotropy method with the IHSC technique to mimic the structure of certain blood vessels from a

broader region of interest of a mice brain, resulting in phantoms that closely resemble the desired vasculature. Additionally, we developed a mask to replicate the retinal vasculature as seen in the coronal section of the human eye.

Overall, we propose a versatile and scalable phantom that can be customized to mimic vascular structures from various regions, including the brain, eye, and others, as per user requirements. Such phantoms have the potential to be utilized for standardizing various optical imaging modalities such as Laser Speckle Contrast Imaging (LSCI), Diffuse Correlation Spectroscopy/Tomography (DCS/DCT), Diffuse Optical Spectroscopy/Tomography (DOS/DOT), Speckle Contrast Optical Spectroscopy/Tomography (SCOS/SCOT), Laser Doppler Flowmetry (LDF) and Optical Coherence Tomography (OCT) systems.

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# Plasmonic Slippery Surface For Surface-Enhanced Raman Spectroscopy And Protein Adsorption Inhibition

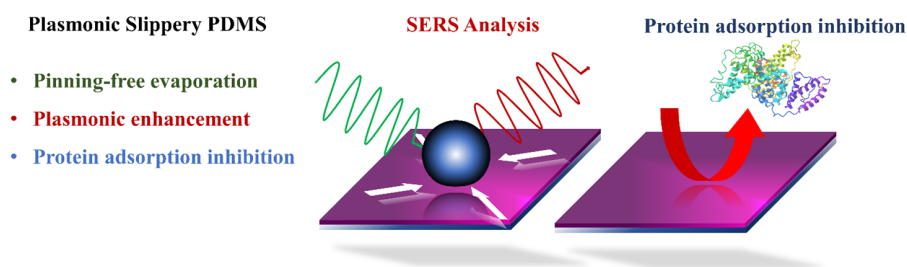
Swithin Hanosh<sup>1</sup>, Monisha K<sup>1</sup> and Sajan D. George<sup>1,2</sup>

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Slippery liquid-infused porous surfaces (SLIPS) are a class of surface that offers low contact angle hysteresis and low tilt angle for water droplet shedding. This property also endows the surface with pinning-free evaporation which in turn has been exploited for analyte concentration enrichment for Surface Enhanced Raman Spectroscopic (SERS) applications and anti-biofouling. Herein, we demonstrate a facile approach to creating SLIPS with low contact angle hysteresis and low tilt angle for water shedding by coating the equal-volume mixture of polydimethylsiloxane (PDMS) and silicone oil. By exploiting the in-situ plasmonic particle reduction capability of the PDMS, the surface is converted to plasmonic SLIPS surfaces, which illustrates its potential as a sensitive analytical platform via surface-enhanced Raman spectroscopy. The Raman spectroscopic studies using crystal violet as a reference sample show a limit of detection of 76 pM. Further, we have demonstrated that the fabricated plasmonic substrate is found to be more efficient in inhibiting proteins (bovine serum albumin) onto the surface as compared to pristine PDMS surfaces. Our fabricated plasmonic surface can find applications in ultrasensitive molecular detection for applications related to analytical chemistry, diagnostics, environmental monitoring, and national security and more importantly can control the non-specific adsorption of proteins.



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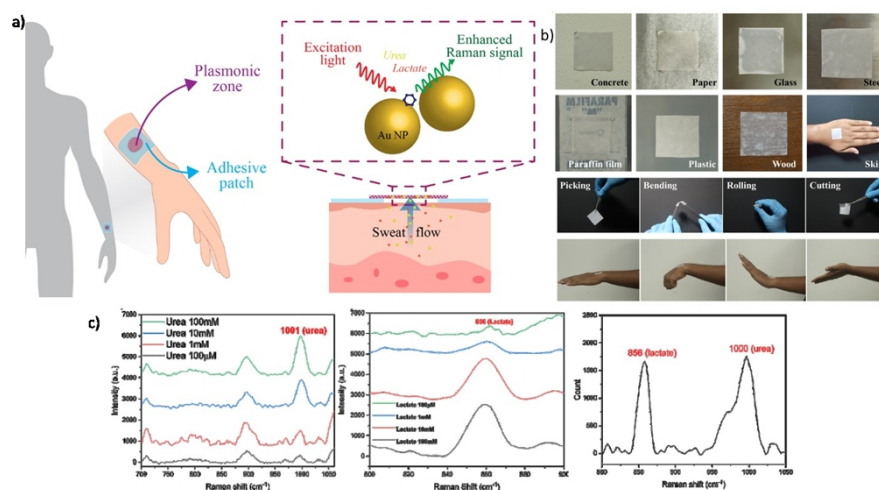
# A Biocompatible Wearable Adhesive Patch For The SERS Based Sensing Of Sweat

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The development of flexible and wearable patches made from biocompatible materials for molecular fingerprinting of body fluids is an emerging area of research in the field of healthcare devices [1,2]. Here, a surface-enhanced Raman scattering sensor is designed and developed using a two-layer paper-based substrate in which the first layer is mixed with polydimethylsiloxane and oleic acid for skin adhesion and the second layer comprises a filter paper with in-situ reduced nanoparticles for Raman signal enhancement of the sweat components. The design of the patch avoids the direct exposure of nanoparticles, as well as the exciting laser to the skin. The volume ratio of polydimethylsiloxane to oleic acid in the adhesive mixture is optimized for maximum adhesion to various substrates with oil residue. The plasmonic paper employed here exhibited an excellent limit of detection of 0.21 mM and 0.36 mM for sweat components such as urea and lactate, respectively. By utilizing the skin adhesive patch, multiplexed detection of urea and lactate directly from the sweat using surface-enhanced Raman spectroscopy. The developed SERS patch can be potentially utilized as a wearable healthcare sensor for molecular fingerprinting of body fluids and open avenues for the development of sensors in the field of wearable personalized healthcare devices.



a) Schematic of the biocompatible adhesive wearable patch for SERS based sweat sensing b) photographs showing the good adhesion to different materials mechanical flexibility and stability of the patch under dynamic wrist movements c) Raman spectroscopy based multiplex detection of sweat components urea and lactate.

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## **Surface Texture Assessment Of Tomato Using Speckle Pattern Analysis**

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Laser speckle imaging (LSI) is a versatile technique for evaluating surface properties and dynamic behaviours, particularly in non-invasive monitoring applications. It utilizes the formation of speckle patterns—random variations in intensity that occur when coherent light, such as a laser beam, interacts with a rough or textured surface. These speckles contain valuable information about the surface's characteristics, such as texture, roughness, and movement. By analysing the statistical properties of these speckle patterns, LSI enables detailed insights into the surface's structural and dynamic features. LSI has emerged as a promising technique for assessing crop quality, particularly in terms of surface texture. In this study, the laser speckle patterns of tomato were captured using a CCD camera on the first and seventh days of observation. Both first-order and second-order statistical methods were applied to process the captured speckle images and extract relevant texture information. The histogram-based first-order statistical method was employed to analyse the intensity distribution of the speckle patterns. Key statistical parameters such as contrast, skewness, and kurtosis were computed from the histogram, providing quantitative insights into the texture of the tomato surface. Additionally, the grey-level co-occurrence matrix (GLCM) method was utilized for a more detailed analysis of the speckle pattern. Analysis of speckle pattern can reveal the extent of decay or damage in a tomato, making it possible to distinguish between a fresh, ripening, or deceased tomato through changes in its surface properties and texture.

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