Smart Optical Biopsy with Polarized Light (SmartOpsy)

Igor Meglinski[1*] and Anabela Da Silva[2]

[1] University of Oulu, Opto-Electronics and Measurement Techniques Unit, Oulu, 90014, Finland
[2] Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, Marseille, France
*Corresponding author: igor.meglinski@oulu.fi

ABSTRACT

The non-invasive probing of biological tissues at the cellular level is a shared objective for physicians, biologists, physiologists and pharmacists. In this project the fundamental properties of laser light are used to develop novel highly sensitive non-invasive optical diagnostics of cells and biological tissues. With the systematic investigation of influence of cell structure malformation on the spin angular momentum of light and its changes due to multiple scattering two robust experimental systems/approaches suitable for routine clinical applications have been introduced.

Keywords: circularly polarized light, correlation, fractal and statistical analysis, biological tissues, biopsy, twisted light

1. INTRODUCTION

Our project aims to investigate a very new technology, developed by us, for which we hope the research will foreshadow the development of a clinically relevant *in vivo* cytological diagnostic capability that could be used in real time for the detection of cancer, with the potential to revolutionize the present need to take a cancer tissue biopsy for pathology. Cancer detection plays an increasingly important role in global cancer control initiatives, and in this regard the field of cancer diagnostics is rapidly expanding. Although effective to a greater or lesser extent, each diagnostic test has shortcomings associated with time, cost, reliability, or patient discomfort. Currently, the 'gold-standard' and most widely used methodology for precise cancer diagnosis is histological analysis that utilizes exhaustive microscopy investigation. However, despite the fact that the field of anatomic pathology is rapidly expanding, and a number of innovative diagnostic tests become available, the rate of conclusive diagnosis by histological analysis for a range of the most dangerous chronic diseases is only 65-75% [1,2]. Therefore, there is clearly an immediate vital requirement for improving the diagnostic and imaging modalities utilized in the current practice of anatomic pathology.

This proposal represents the next strategic step with respect to explore the opportunity of using the fundamental properties of light, namely circular and/or elliptical polarization – spin angular momentum (SAM) of light, for advanced cell cultures diagnosis and tissue samples screening.

The project enabled the creation of new, low cost measurement technology that has the potential to indirectly reduce the healthcare costs by detecting the disease earlier, and assisting in the selection of a tailored treatment plan that will increase the survival rate and quality of life of the patients.

The Stokes vector-based experimental approach and Mueller matrix (MM) imaging were developed and used routinely in the study. The Monte Carlo (MC) based computational model for imitation of the detected SAM, phase, degree of polarization and their variations due to interaction with cells and biological tissues is developed, validated and applied for the further investigations of polarized light propagation in complex tissue-like scattering medium. Realistic tissue phantoms with the controlled opto-mechanical properties are developed and extensively used for testing, validation of MC and optimization of the experimental systems. The Artificial Neural Networks (ANN) algorithm is developed for automatic analysis of tissue screening data. The major results obtained in frame of SmartOpsy Phase 1 are published and/or submitted to the peer-reviewed scientific journals: 12 papers and few monographs; up to 10 more papers and conference presentations are expected to be prepared and published/delivered.

2. STATE OF THE ART

The field of anatomic pathology is rapidly expanding, and as the imaging and diagnostic technologies are improved, so does the ability to detect and identify the many different types and sub-types of cancer. Today, a number of diagnostic tests are available for determining the presence of cancer. These tests include: surgical biopsy; protein sequence analysis (PSA); DRE tests; computed axial tomography (CAT or CT scans); magnetic resonance imaging (MRI) scans; ultrasound scans; bone scans; positron emission tomography (PET) scans; bone

marrow testing; barium swallow tests; endoscopy; cytoscopy; T/Tn antigen tests; mammography; and other tests.

In the last decade, consistent and successful innovations have been achieved in the field of lasers and optics, collectively known as 'photonics', founding new applications in biomedicine, including biopsy [3]. The non-invasive photonics-based imaging and diagnostic modalities are rapidly expanding, and with their exponential improving there is a high potential to develop a practical instrumentation for automatic detection and identification of different types and/or sub-types of danger chronic diseases at the very early stage. Photonics-based diagnostics offers the great advantage that a routine clinical diagnosis can be performed non-invasively in real time on tissue in situ. Early optical biopsy systems involve irradiating tissue with UV, visible, or infrared light, and attempts to characterize the resulting reflectance or fluorescence emission or Raman spectra [4]. A number of other optical modalities, such as optical coherence tomography (OCT), non-linear microscopy, diffuse optical spectroscopy, coherent back-scattering (CBS), photo-acoustic tomography (PAT), tissue polarimetry and other were tried and tested for optical biopsy [5,6]. Unfortunately, in current stage of the photonic-based techniques development an identification of tissue malformations associated with a particular dangerous chronic disease is achieved at a relatively late stage, hence the relatively high mortality rates.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

The Photonics21 Multiannual Strategic Roadmap (2021–2027) specifically highlighted that Photonics provides vital components to medical technologies for the instant diagnosis of major diseases and will be essential during the digital transformation and Industry 4.0. While the global photonics market has reached €600 billion, only 20% of the potential power and benefits of light technologies have been unlocked so far. The headline outcome of the SmartOpsy is a pioneering a radically new line of photonics-based technology by establishing Proof-of-Concept utilizing an emerging paradigm in physics, studying new fundamental concept in light-tissue interaction and transferring the developed technology to current practice of histopathology and advanced microscopy, as well as in relevant applications in material science. An early detection of the diseases is the key factor for increasing the survival rate and the quality of the life. In fact, the core of the project research represents not an incremental studies but a real breakthrough for the understanding of fundamental physics laws and creating a prototype of the instrument for non-invasive optical biopsy.

4. PROJECT RESULTS

While coherence and polarization are the fundamental properties of light that have been attracting great attention due to an extensive use of lasers in various practical applications from space (by NASA) to modern biology and medicine, as well as consumer goods/wearable devices (e.g. Apple Watch). When light interacts with the matter its SAM/degree of polarization is changed. The degree of polarization of linearly, elliptically or circularly polarized light has long (since 1800s) been used to characterize material surfaces, thin films and transparent media. Our ATTRACT-based studies have shown that the phase shift of polarized light backscattered from samples of biological tissue carries important information about the presence of cervical intraepithelial neoplasia [7], whereas circularly polarized light in frame Stokes vector formalism is also able to distinguish the successive grades of cancer (Fig.1). MM imaging is very effective for biopsy and quantitative characterization of human tissues and alterations associated with cancer [8]. Both approaches demonstrate a high potential for evaluation of progression of amyloid-beta plaques with Alzheimer's disease [9] (see Fig.1).

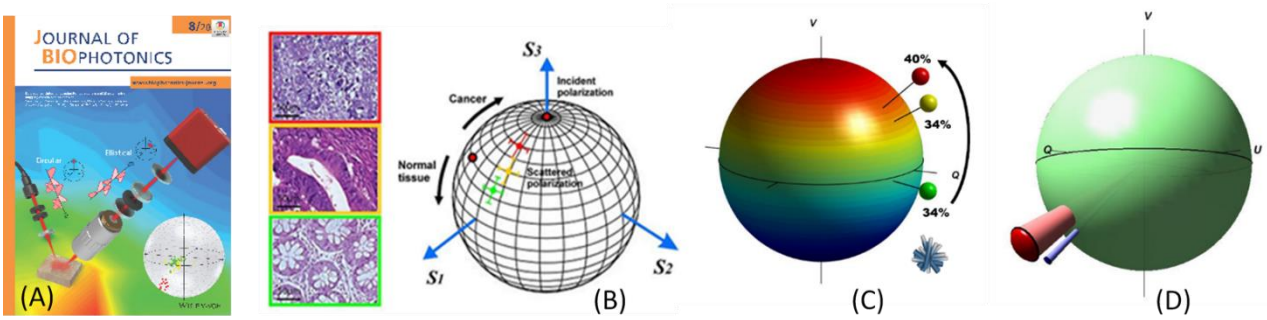

Fig. 1. Schematic presentation of the experimental set up used for the screening of paraffin-embedded tissue blocks (A), recognized front cover placing [7]. Position of the Stokes vector on the Poincaré sphere correlates with successive grades of colorectal cancer (grade 1 – green; grade 2 – yellow, and grade 3 – red) confirmed by microscopy investigation (B). Locus of the Stokes vector on the Poincaré sphere measured with an increase density of amyloid-beta plaques (C) [9] and epilepsy (D) [10].

We use the Poincaré sphere to represent the state of polarization of light scattered within biological tissues [11]. Abnormalities induced in tissues by cancerous changes include an increased nucleus to cytoplasm ratio and an overall increase in the volume density of cells [12,13]. These two effects impact greatly on the state of polarisation of light propagated through the tissue [14]. An increase in nuclear size leads to a higher forward scattering of light. Therefore, if the state of polarisation of the scattered light is closer to the state of incident right-handed circular polarized light, i.e. to the North Pole on the Poincaré sphere, then the tissue sample is either neoplastic potentially malignant or neoplastic malignant. If the state of polarisation of the scattered light is close to the equator of the Poincaré sphere then the tissue sample is normal (see Fig.1-B).

We pioneered the use of statistical, correlation and fractal analysis for the MM image post-processing, which is very effective for quantitative characterisation of biological tissues and alterations associated with cancer progression [8]. This technique was extensively used for the screening of the polycrystalline structure of fibrillary biological tissues [15]. Jones-matrix and Stokes-correlometry approaches were utilised, respectively, to diagnostics of polycrystalline films of biological fluids and for quantitative polarisation images of histological sections of optically anisotropic biological tissues with different morphological structures and physiological conditions [16].

5. FUTURE PROJECT VISION

Thus, the SAM-based experimental systems developed in this project could be very useful in the detection of pre-cancerous changes in human tissues. However, they are only suitable for probing the structure of cells at or adjacent to the surface of the tissue, as the linear polarised light loses its polarisation due to multiple scattering in turbid media such as biological tissues. Therefore, there remains a need for further, relatively inexpensive, optionally non-invasive methods and systems for screening patients, or tissue samples from patients, to diagnose conditions such as the presence of cancerous lesions.

5.1. Technology Scaling

In fact, the structure of light can be more ‘complex’ – twisted [17], i.e. in addition to SAM the light beams can be radially or azimuthally polarized and carry orbital angular momentum (OAM). In the last decade the applied research in this field achieved high recognition in generation and characterization of the exotic twisted light (TL) beams [18,19], improving telecommunication technologies [20], and optical trapping of cells and particles [21,22].

Our initial pilot studies (should be considered as **TRL 1-3 in Phase 1**) suggest that TL could be up to ~10^3 times sensitive to structural changes that it has a potential to revolutionize the current practices of screening and prognosis monitoring. With the combined use of SAM and OAM, as well as with addition of the degree of twist, coherence, and self-torque, it will be possible to identify any disruption to the organized layered structure of tissues, particularly those that result from pre-cancer, Alzheimer and other diseases. Further studies (**TRL 5-7 in Phase 2**) of these new fundamental physical phenomena will provide valuable guidance for the design and assembling of an innovative TL-based biopsy, and finally lead to the development of a new TL-based biopsy platform for real-time non-invasive stand-alone multiscale screening and characterization of biological tissues. The TL-based biopsy approach promises to be so specific that it will potentially revolutionize procedures currently used in histological clinical tests, with the potential to completely avoid the need to take tissue biopsy samples for pathology.

5.2. Project Synergies and Outreach

Achieving TRL 5-7 can only succeed with close collaboration between the diverse fields of photonics, laser physics, optical engineering, theoretical studies and computer modelling, machine learning, cellular physiology and histopathology. To achieve this, the proposal brings together a consortium of experts in the respective fields to go beyond current mainstream collaboration configurations in joint science-and-technology research, by bringing together a consortium of experts in photonics, light-tissue interaction, single cell functional investigation, clinical histopathology, and biomedical imaging. Namely: University of Oulu (Finland), CNRS (France), and Aston University (UK). Based on our previous conversations we also consider as primary partners the POSEIDON and NXGTDC ATTRACT teams for further improvement of the technology in terms of cost-efficiency and optimization. A crucial factor that greatly increases the likelihood of success is that different members of the consortium have a history of successful collaboration: Oulu and CNRS through ATTRACT Phase 1, Aston and Oulu H2020 FET NEUROPA project, Aston and CNRS through the joint conference organizing.

5.3. Technology application and demonstration cases

The Phase 2 research contains intensive theoretical and experimental studies. First, to understand how TL interacts with scattering media, which will both shine the light on the new fundamental physics phenomena and deliver the valuable guidance for the design of innovative instrument, the numerical and phenomenological models will be developed. Next, the final stage of the project, we will create a low-cost, mobile platform that will deliver

OAM beams that utilize a subset of spin-orbit interactions, for use in clinical applications. The second phase research will be performed during 3 years in consortium of six members: Oulu, CNRS, Aston, POSEIDON, NXGTDC, and ZEISS; required 2 million Euros funding.

The consortium will aim to publish the main achievements of the project in non-mutually exclusive open-access peer-reviewed journals, and/or make available the research data via e.g. the project's website, after appropriate measures for IP protection have been put in place (when and where relevant). As indicated in the relevant guideline , various approaches will be utilized:

• "Green" open access, online self-archiving into the BIOTWID website repository;

• "Gold" open access, scientific papers will be immediately provided in open access mode by the publisher.

The primary target audiences will range from academic, business, and governmental organizations.

The research program will place significant emphasis on the multi-disciplinary research teamwork, as well as on the development of individual initiative and provide an excellent basis for EU/national and international collaboration with the world-known experts in the field of optical instrumentation and polarized light. Through our partnership with ZEISS, the SmartOpsy project has true potential for strengthening European "Innovation Union" by contributing to the EU's scientific excellence and leadership in future hot topic technology, such as advanced biopsy-diagnostic-imaging technologies. The project impact on future EU scientific and industrial leadership will be ensured by involving the young postdoctoral researchers and PhD students along with visiting internationally leading scientists in the field into the workshops associated with the key stages of SmartOpsy project. Existing young researchers at Oulu, CRNS and Aston are earmarked for prominent roles.

5.4. Technology commercialization

We already discuss the possible collaboration with ZEISS, MobileODT, and LUMICS. ZEISS is a leading producer of surgical microscopes expressed a potential interest in applying the technology utilizing TL with OAM, developing in our project, for the differentiation of healthy from malignant tissue during surgery.

5.5. Envisioned risks

The biomedical applications of TL has a strong potential to become so specific that it will revolutionize current practice of cells diagnosis and tissue biopsy. But TL has not yet been explored in tissue biopsy. There are no background theoretical and experimental studies on sensitivity of TL to the small tissue alterations by cancer or any other diseases. To mitigate this risk, the project includes a quantitative comparison between the measurements made in tissue and cells phantoms using light beams with and without OAM. Together with the implementation of TL adaptive OAM biopsy, permits optimization of the measurement strategies to discard methods or approaches that do not improve the specificity of optical biopsy. This checkpoint, together with the further implementation of "adaptive biopsy twist", will permit optimizing the measurement strategies and discard the methods or approaches that do not enhance the specificity of the optical diagnosis. Other emerging risks will be addressed by communication and cooperation between partners and the use of their respective state of the art facilities. This will be facilitated by the participants, who in addition to leading expertise in polarized light, light-tissue interaction, also have a strong competence in cells biology and biomedical imaging, which will enable coordination between partners and disciplines to solve the emerging issues. There are no gender specific issues relevant to or to be accounted for related to the research proposed in the SmartOpsy. The studies and technology developed are suitable for use without gender differentiation. SmartOpsy consortium will benefit from reasonable gender balance (presently it's equal 50/50%) and lessons, which can be drawn from it.

5.6. Liaison with Student Teams and Socio-Economic Study

Second Phase of SmartOpsy research project will contribute to the training and development of interdisciplinary expert young researchers at the intersect of interdisciplinary fields, including fundamental physics, modern laser physics, biomedical optics, cells biology, and histopathology.

The project will also target specific MSc Level student teams that track new healthcare technology, particularly those concerned with extensive histology and histopathology studies, e.g. The Royal Microscopical Society, European Microscopy Society. Peer-reviewed publications, specialist web sites and scientific conferences will form the primary information channels of the research community. There is also a need to communicate to the wider public at large, providing education and spreading enthusiasm for science and technology. Popular newspapers, magazines, radio, electronic journals and Social Media (Facebook, LinkedIn and Twitter) will be used to raise public awareness and informing opinion. The most appropriate tools for dissemination of the SmartOpsy results, and a selection of these are presented below:

• Project website & social networks: The project web site will be the most important front-end, i.e. the public image, of the project. The site will allow users to readily collect information about the project and about issues, which might be of interest to stakeholders. Targets for web-site hits & social media pages visited will be set.

• A brochure with SmartOpsy public summary and contact information, will be printed in English within the first year of the project. It will be handled out at all events. Additionally, an online version will be available for download from the website. Distribution targets for it will be set and monitored.

6. ACKNOWLEDGEMENT

This project has received funding from the ATTRACT project funded by the EC under Grant Agreement 777222.

7. REFERENCES

[1] Brown, S.L. & Eisenhardt, K.M., 1998, Competing on the Edge: Strategy as Structured Chaos, Harvard Business School Press, Boston, MA, USA. (Book style)
[1] Sirota, R.L., "Error and error reduction in pathology", Arch. Pathol. Lab. Med., 129: 1228–1233 (2005).
[2] Sirota, R.L., "Defining error in anatomic pathology", Arch. Pathol. Lab. Med., 130: 604–606 (2006).
[3] Flotte, T.J., "Pathology Correlations with Optical Biopsy Techniques", Ann. NY Acad. Sci., 838: 143-149 (1998)
[4] Evers, D.J., Hendriks, B., Lucassen, G. and Ruers, T., "Optical spectroscopy: current advances and future applications in cancer diagnostics and therapy", Future Oncol., 8(3): 307-20 (2012).
[5] Ferrer-Roca, O., "Telepathology and Optical Biopsy," Int. J. Telemed. Appl., 2009: 740712 (2009).
[6] Vasefi, F., MacKinnon, N., Farkas, D.L. and Kateb, B., "Review of the potential of optical technologies for cancer diagnosis in neurosurgery: a step toward intraoperative neurophotonics", Neurophotonics, 4(1): 011010 (2016)
[7] Ivanov, D., et al., "Colon cancer detection via Poincaré sphere representation and 2D polarimetric mapping of *ex vivo* tissue samples", J. Biophoton., 13(8): e202000082 (2020).
[8] Trifonyuk, L., et al., "Differential Mueller-matrix imaging of partially depolarizing optically anisotropic biological tissues", Lasers Med. Sci. (2020) // doi: 10.1007/s10103-019-02878-2
[9] Borovkova, M., et al., "Evaluating β-amyloidosis Progression in Alzheimer's Disease with Mueller Polarimetry", Biomed. Opt. Exp., 11(8): 4509 – 4519 (2020).
[10] Borovkova, M., et al., in: Optical Biopsy XVII: Toward Real-Time Spectroscopic Imaging and Diagnosis, BiOS, SPIE Photonics West, San Francisco, USA, 2-7 February (2019).
[11] Borovkova, M., et al., "Role of scattering and birefringence in phase retardation revealed by locus of Stokes vector on Poincare sphere", J. Biomed. Opt., Vol.25, No.5, 057001 (2020).
[12] Webster, M., et al., "Sizing up the nucleus: nuclear shape, size and nuclear-envelope assembly", J. Cell Sci. 122 (10): 1477-1486 (2009)
[13] Masui, Y., "Towards understanding the control of the division cycle in animal cells", Biochem. Cell. Biol. 70 (10-11): 920-945 (1992).
[14] Ghosh, N., Vitkin, I.A., "Tissue polarimetry: concepts, challenges, applications, and outlook", J. Biomed. Opt. 16, 110801 (2011).
[15] Borovkova, M., et al., "Mueller-matrix-based polarization imaging and quantitative assessment of optically anisotropic polycrystalline networks", PLOS One, 14(5): e0214494 (2019).
[16] Ushenko, A., et al., "Stokes-Correlometry Analysis of Biological Tissues with Polycrystalline Structure", IEEE J. Sel. Top. Quantum Electron., 25(1): 7101612 (2019).
[17] Shen, Y., et al. "Optical vortices 30 years on: OAM manipulation from topological charge to multiple singularities", Light Sci. Appl. 8, 90 (2019).
[18] Cai, X., et al. "Integrated compact optical vortex beam emitters", Science, 338, 363-365 (2012).
[19] Rego, L., et al. "Generation of extreme-ultraviolet beams with time-varying orbital angular momentum", Science, 364, eaaw9486 (2019).
[20] Wang, J., et al. "Terabit free-space data transmission employing orbital angular momentum multiplexing", Nat. Photon., 6: 488–496 (2012).
[21] Zhu, R., et al. "Optical Tweezers in the studies of red blood cells", Cells, 9(3): 545 (2020).
[22] Avsievich, T., et al., "The advancement of blood cell research by optical tweezers", Reviews in Physics, 5: 100043 (2020).