SRS histopathology (SRHisto)

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ABSTRACT

The SRS hitopathology project aims at developing a novel technology able to generate histology images that are used by medical doctors to detect and diagnose cancer tissues. The technology is based on the stimulated Raman effect, a nonlinear optical technique that can visualize the chemical bonds present in tissues. In this project, we have developed the building blocks (laser source and detection system) and demonstrated the ability to generate histology images in few minutes only, as compared to the 12h-24h commonly required by standard histology protocols. These results open the road further develop the technology for its transfer and evaluation in the hospital.

Keywords: Stimulated Raman imaging, nonlinear microscopy, laser sources, histology, oncology

1. INTRODUCTION

Every year, approximately 14 million cancers are diagnosed and 8 million people die of cancer worldwide (13% of all deaths every year). This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world. Histology plays a vital role in the diagnosis, staging, and treatment of cancers and is the 'gold standard' method recognized by the medical community and legal authorities. Histology is a labor intensive technique that requires the removal of small regions of suspect tissues (biopsies) that are later sectioned and stained with hematoxylin, eosin and saffron (HES) [1]. Standard HES protocol requires the excised samples to undergo several preparation steps (dehydration, fixation, wax, staining, slicing, mounting) that are time consuming, typically in the range of 10 hours up to 3 days. There is an urgent need for an intra-operative diagnostic technique that avoids HES staining and delivers immediate high quality histology images in the operatory room to assist in surgical and decision making.

Histopathologic diagnostic requires the visualization of cell nuclei and cell cytoplasm. Although many imaging approaches have been developed to address histologic needs such and confocal reflectance [2], fluorescence [3] or multiphoton [4, 5] microscopies, none of them provide direct and unambiguous tissue components identification based of their chemical signature. An elegant approach to identify these components from their chemical signature, in a label free manner and at high resolution is to use coherent Raman scattering (CRS) [6]. CRS encompasses coherent anti-Stokes Raman scattering (CARS) and stimulated Raman scattering (SRS). Both are optical nonlinear wave mixing processes [7] that can probe the presence of CH_2 *and* CH_3 *chemical bonds found in cell cytoplasm and cell nuclei* using their distinctive bond vibrations, respectively. This ability of SRS was shown very recently to provide label free microscopic images of healthy and cancer tissues sections in near-perfect concordance with standard histology [8, 9]. This major step forwards opens the new field of label free **stimulated Raman histology (SRH)** for real-time intra-operative surgical cancer tissue resections and treatments. Figure 1 shows SRH microscopy images (Fig. 1 a), obtained by Institut Fresnel [9], of a human colon tissue section when combining the CH_2 (2845 cm^{-1}) and CH_3 (2930 cm^{-1}) chemical bonds images. The SRH image can be readily compared to the standard histology H&E image (Fig. 1 b) and reveals the same structural features (cell nuclei, cell body, extra-cellular matrix organization). Fig 1.c shows the overlay between the SRH image (dashed red box) and the standard histology H&E image.

In the ATTRACT SRHisto project APE GmbH (DE) and Institut Fresnel (FR) have implemented the building blocks (laser, detection systems, microscope) of a novel technology able to acquire SRH images in few minutes only over mm^2 areas whereas the standard histology protocol requires 12h to 24h to achieve the same results. **This breakthrough brings the SRH technology competitive to perform real time diagnostic of freshly excised tissues in the operatory room with immediate benefit for the patients.** In a forthcoming ATTRACT step 2, we wish to further developed the technology to build a demonstrator that can be transferred and evaluated in the hospital by medical doctors.

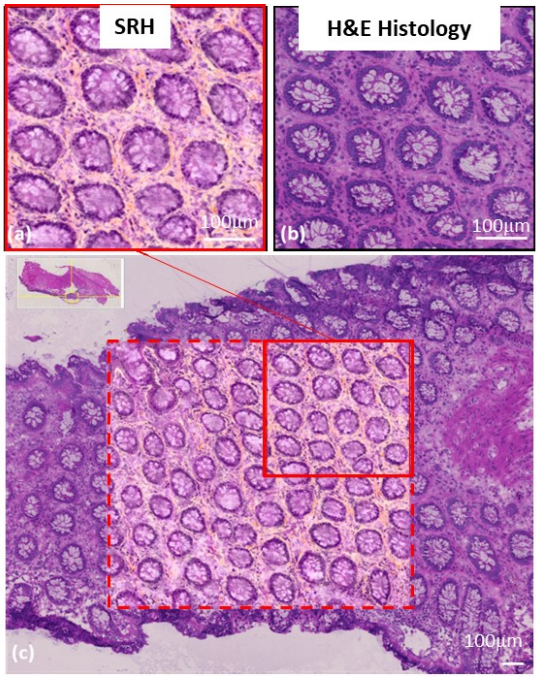

Figure 1: Stimulated Raman histology (SRH) in human colon tissue (Institut Fresnel work [9]). (a) SRH image obtained in 5 minutes, (b) standard H&E histology image, (c) overlay between the SRH image (red dashed box) and the standard H&E histology image.

2. STATE OF THE ART

Up to now the SRH imaging technology has used noisy fiber laser sources that required complex balanced detections scheme [8], or bulky laser systems [9]. Furthermore, these systems acquired sequentially the two images corresponding to the CH_2 and CH_3 chemical bonds that are necessary to generate SRH images. This sequential acquisition limits the acquisition speed of SRH images on freshly excised tissues that are prone to move during the image acquisition. A dual-colour approach enable to acquire simultaneously the CH_2 and CH_3 incorporating a grating together with an acousto-optic deflector employed as angle-to-wavelength pulse shaper was recently proposed [10]. As a disadvantages the largest part of the fs-laser is wasted. Detecting two non-neighbouring Raman bands (off- or in-resonance), spectral focusing [11] in combination with two different time delays was implemented for dual-color SRS imaging [12]. However, any time delay between pump and Stokes pulses affect the optimal temporal overlap of the pulses and leads to a reduced signal generation. Alternatively, and to circumvent major drawbacks of the previously mentioned techniques, Institut Fresnel recently introduced a dual-color SRS-microscopy approach combining the output of a fiber-laser at 1030 nm and two optical parametric oscillators (OPOs) which are modulated at two distinct frequencies to enable cross-talk-free SRS-images at two independently tunable Raman-shifts within the range of $500cm^{-1}$ – $5000cm^{-1}$ [13]. The performance of the latter is compromised by the utilization of a pump fiber-laser that is 6 dB(W) above the shot-noise limit resulting in the reduction of the acquisition speed by a factor of approximately 4 compared to a suitable shot-noise limited system.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

In the ATTRACT SRHisto project we have breakthrough these limitations and built, for the first time, a low noise compact laser system able to acquire the CH_2 and CH_2 images simultaneously. Contrary to [13], the system is shot noise limited and shows x4 reduction of the footprint. We have also developed an optimized detection system and a H&E virtual colouring software that provides SRH images over mm^2 areas in few minutes only. We have demonstrated the ability of the developed SRH imaging system to perform SRH imaging in the gastric and brain systems (Fig. 2), both for healthy and cancerous samples. The technology has been validated by medical doctors that confirm that SRH must be further developed in a fully integrated and compact imaging system to be transferred to the hospital for its intra-operative evaluation.

4. PROJECT RESULTS

The project has been developed along several directions that are briefly presented below.

Laser source system: We have designed, assembled and built a Lab demo of a novel laser system that is able to address simultaneously the CH_2 ($2845\ cm^{-1}$) and CH_3 ($2930\ cm^{-1}$) chemical bonds to produce SRH images. The system is shot noise limited, compact and can be easily interfaced to laser scanning microscope systems.

Laser scanning microscope: We have interfaced the novel laser source system to a custom CRS laser scanning microscope and developed the control softwares.

Virtual SRH colouring: based on the simultaneously acquired CH_2 and CH_3 images, we have developed a software able to generate instantaneously SRH images.

Figure 2 shows the SRH images obtained with our novel SRH imaging system on freshly excised human tissues over mm^2 areas in the liver, kidney and brain. These images have been obtained in 5 minutes only and Fig. 2 present their direct comparison with standard histology H&E images prepared from the same samples (after they have been imaged by SRH). Figure 2 reveals the exquisite agreement between SRH images and their H&E counterpart, opening the road towards intra-operative histology.

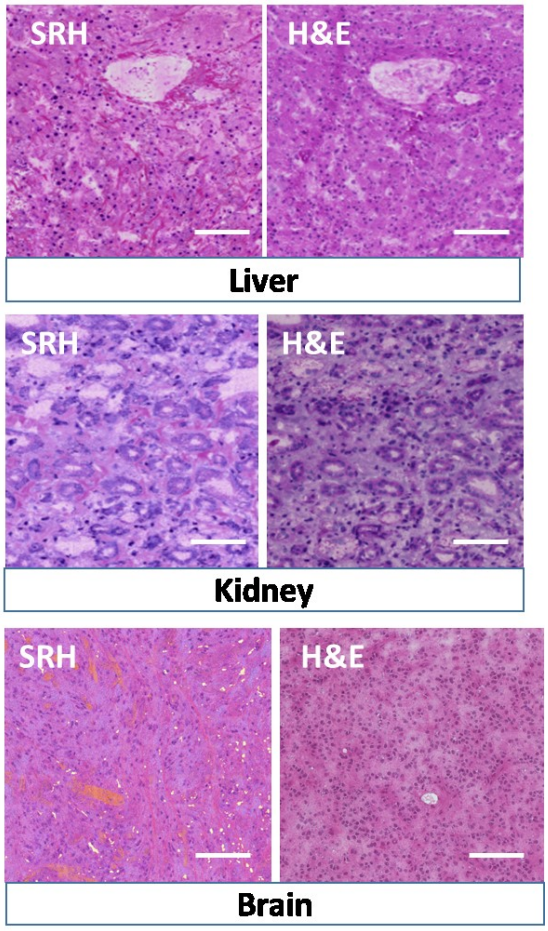

Figure 2: Validated SRH images and their direct comparison with H&E classical histology images. SRH acquisition time 5 minutes; scale bar: 100µm. The samples are coming from AP-HM and IPC hospitals in Marseille.

5- FUTURE PROJECT VISION

The aim of the SRHisto ATTRACT phase 2 project **is to develop new optical imaging instruments using label free stimulated Raman histology (SRH) to generate images of histological quality from freshly excised biopsies**, in real time, without labelling or any sample preparation. The device will be a **compact microscope including the ATTRACT phase 1 laser source** and detection schemes that can be used close to the operatory room to examine sections of freshly excised tissue to evaluate their cancerous nature in few minutes only. The microscope will be transferred to partner hospital for its direct evaluation by medical doctors (histo-pathologists and surgeons).

5.1. Technology Scaling

In SRHisto ATTRACT phase 2 we aim at bringing the developed technology to TRL 7-8 and build an integrated SRH microscope system ready to be evaluated in the hospitals. Figure 3 presents the system that we want to build and disseminate to the medical end-users. It is composed of three parts, (1) a compact laser source system (outcome of ATTRACT phase 1) coupled to (2) a laser scanning microscope enabling the real time acquisition of SRH images thanks to (3) a dedicated colouring software. The system is controlled through a user friendly software and has a footprint compatible for its operation in the operatory room. It can be easily operated by non-specialists to generate virtual histology images in format compatible with the hospital workflow.

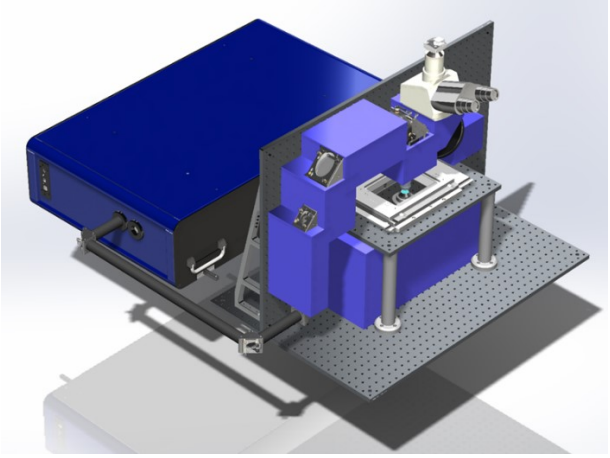

Figure 3: The envisioned SRH microscope system that will be designed and assembled during the ATTRACT phase 2 project. The system will be designed to be transferred to hospitals that will act as direct application partners in the project.

5.2. Project Synergies and Outreach

The SRHisto ATTRACT phase 2 project is built to bring label free fast stimulated Raman histology (SRH) to the hospital and to evaluate its ability to image fresh healthy and cancer tissue samples from the central nervous (CNS) and gastro-intestinal (GI) systems. To conduct the phase 2 project, we will bring together a multidisciplinary consortium intended to (1) design and built the SRH microscope, (2) develop the software control and SRH image generation and (3) to evaluate the technology in the hospital. The consortium will gather experts in the field of optical engineering, electrical engineering, software developers together with hospital medical patricians (histopathologists, endoscopists, surgeons from major hospitals in France and Germany). The AP-HM Marseille will validate the technology for brain oncology whereas the Institut Paoli-Calmettes (Marseille) will evaluate the technology in the gastric system. We will also add another partner hospital from Germany in another application field (for instance urology or lung cancer).

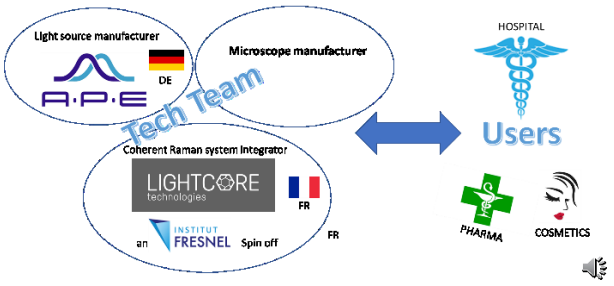

Figure 4: SRHisto ATTRACT phase 2 envisioned consortium

The identified technology partners are APE (lead of the SRHisto project) specialized in laser systems manufacturing for coherent Raman, Lightcore Technologies, a spinoff of Institut Fresnel, that was created during the ATTRACT phase 1 project to develop the SRH technology. We aim also to bring together a microscope manufacturer and possibly a software developer/machine learning expert to help in the virtual slide colouring.

The project will be part of the 'Canceropôle PACA' (https://canceropole-paca.com/) national French initiative that intends to disseminate novel technology to the hospital to detect and treat cancers. 'Cancerôple centers' have open public platform and initiative to disseminate the information and promote the projects towards the general audience. Note that the SRHisto project has also received funding from the 'Canceropôle PACA'. The SRHisto project phase 2 will be also part of the initiative and benefit from the dissemination platform and outreach activities of 'Canceropôle centers'.

5.3. Technology application and demonstration cases

The primary SRHisto application field is oncology; the developed SRH machines (Fig. 3) will be used to study healthy and cancer tissues coming from the neuro-oncology and pathology department at Assistance Publique Hôpitaux de Marseille (AP-HM) and the gastrointestinal oncology and pathology department at the institute Paoli-Calmettes Marseille (IPC). At the end of the project the developed SRH microscope will be transferred to AP-HM and IPC to be immersed in the hospital workflow for their direct evaluation by histology patricians, endoscopists and surgeons. **Because the SRH technology provides 'HES quality like images' that can be readily interpreted by histopathologists this novel technology is likely to be immediately accepted by the medical doctor community.**

We also want to explore the interest of the SRH technology in the pharma industry for the mapping of pharmaceutical tablets as we demonstrated recently [14]. We have also demonstrated that SRH is interesting to study drug penetration in skin for the cosmetic industries [15, 16]. We envisioned to bring to the SRHisto phase 2 consortium major pharmacological and cosmetic companies to explore these application markets.

5.4. Technology commercialization

Because the developed SRH technology outperforms the standard histology H&E in terms of speed, we have created the novel Lightcore Technologies company during the ATTRACT phase 1 project. This company is intended (1) to integrate the technological bricks (laser source, microscope, software) coming from tech partners and (2) to transfer the SRH technology to the hospital and demonstrate its relevance for intra-operative fast cancer detection. The creation of Lightcore Technologies is a concrete initiative towards the commercialization of the technology. The company is based on private funding and has already attract the interest of investors for its forthcoming capitalization. Clearly the ATTRACT phase 2 project will be a booster to bring the SRH technology to life.

5.5. Envisioned risks

We are confident to bring the technology to a TRL 7-8 level during the ATTRACT phase 2 project. There is no major risk concerning the technology as all the building blocks have been demonstrated during the ATTRACT phase 1 stage. APE, Lightcore Technologies, together with a microscope company will bring the SRH technology ready to be evaluated in the hospitals.

The main challenge is the marketing of the technology to the hospital to convince medical institution to invest in this novel technology. We have already worked along this direction with medical doctors in Marseille and the director of the Institut Paoli-Calmettes (the 2nd cancer center in France) that are very supportive. However, we are conscious that the journey will be long to reach the broad acceptance and dissemination of the SH technology to the hospital. Note that SRH is providing an image that is similar to histology and can be readily interpreted by pathologists, this is a key point that should ease its broad acceptance.

Because the medical market will take a while to establish itself, we will explore the pharma and cosmetic markets that are easier to penetrate and that will give quicker feedbacks and financial incomes.

5.6. Liaison with Student Teams and Socio-Economic Study

The SRHisto project is closely linked to the Fresnel Institute and to the Aix-Marseille University. Most importantly the Europhotonics master program (https://sciences.univ-amu.fr/en/europhotonics) from Aix-Marseille Univ has already provided master students during the SRhisto ATTRACT phase 1 to work on the project. This will be continued during phase 2 and Institut

Fresnel will open several MSc training position every year on the SRHisto project. It is likely that PhD research topic will follow, they will be funded through Aix-Marseille Univ or using the CIFRE mechanism open for small companies in France.

Lightcore Technologies is closely followed by the Marseille SATT (http://www.sattse.com/qui-sommes-nous/), SATT is intended to promote, help and follow startup companies in the south-east of France. SATT provides a full service to startup companies in terms of initial funding, marketing, public relationships. During the ATTRACT phase 2 project Lightcore Technologies will allocate a SATT representative to help in the dissemination and the referencing of the technology impact.

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