0000000143 – SURE SRH

PhD Research and Training proposal

1. EXCELLENCE (4 pages max)

1.1. Pre-proposal's context, positioning and objective(s)

The proposed research topic is to design and developed algorithms simultaneously performing spectral unmixing and image denoising in the context of Stimulated Raman histology (SRH) image enhancement. Our goal is to enable proper interpretation and diagnosis by physicians but also to use the enhanced images as interesting feature for SRH deep learning applications such as aided-diagnosis [1] , automatic segmentation [2] or virtual staining [3].

SRH has recently emerged as a rapid (few minutes), preparation-free, microscopy technique that offers high quality histology images of frozen or freshy excised millimeter size biopsies. SRH is based on stimulated Raman scattering (SRS) microscopy [4], a label free imaging technique. SRH images are acquired with a Stimulated Raman Scattering (SRS) microscope configured to detect two Raman shifts 2845 cm^{-1} and 2930 cm^{-1} corresponding to CH2 and CH3 chemical bond vibrations. The resulting images are then two-channel images: one channel associated with CH2 chemical bonds (mainly located in cell bodies) and the other associated with CH3 chemical bonds (predominantly located in cell nuclei). Firstly, demonstrated in the brain [5], SRH has been extended to the gastro-intestinal tract [6,7,8], and the prostate [9].

Visualization of cell nuclei in histological sections is a crucial step for cancer diagnosis: their numbers, concentrations, and relative arrangements are key features in oncology. In SRH, cell nuclei are currently highlighted by performing a simple subtraction between both CH2 and CH3 image channels followed by a virtual colorization in eosin/hematoxylin colormap [7]. However, SRH images require more sophisticated processing to be correctly interpreted by physicians (e.g. to reveal measurements details that are heavily masked by the subtraction CH2 - CH3). Unmixing approach such as multivariable curve resolution [10], independent component analysis [11], vertex component analysis [12] and spectral phasor analysis [2] has been successfully applied to SRS microscopy. The previous approaches assign a meaningful spectral signature to each pixel based on Raman spectroscopy, helping to highlight important biological features. Spectral unmixing considers each pixel like a linear mixture of pure spectra. The problem is generally solved either using estimation of pure spectra or by considering statistical assumptions on these latter. In other words, spatial information and image content are not used in the previous unmixing methods.

SRS microscopy often suffers from low signal-to-noise ratio (SNR). Low SNR may arise from laser source noise, low concentrations of target molecules in the sample and/or scattering losses in deep-tissue imaging [13,14]. Image denoising, now widely studying, has been naturally applied to SRS microscopy. For instance, in [15], authors proposed a model based denoising algorithm for SRS images using total variation as regularization function. This implies that expected reconstructed images are piecewise constant. Data driven denoising approach such as deep learning algorithms has shown impressive results. In [16], the established U-Net architecture was used in a fully supervised manner. The input data to denoise were acquired with a low power laser while the output images were acquired with a high-power laser. In [17],

authors implement an autoencoder architecture to denoise SRS hyperspectral images in the spectral domain. This unsupervised strategy requires only one image for the training step since the training samples are the hyperspectral pixels. In [18], a self-supervised “noise to noise” deep learning approach is used to denoise hyperspectral SRS images. The idea is to use neighboring images in the Raman spectrum as input and output of the neural network.

In the present project, we aim to design and develop new algorithms simultaneously increasing the statistical independence between the image channels and performing image denoising. The algorithm has to take into account the raw images content so that the reconstructed image can be exploited for cancer diagnosis. Model based regularization such total variation [15] or wavelet domain sparsity [19] will be firstly considered. Plug-and-play (PnP) approaches [20] are also an interesting research direction for image denoising. The idea behind (PnP) methods is to use denoising algorithms within reconstruction algorithms. Denoising algorithms can be a model-based algorithm [21] or a data driven algorithm such as trained neural networks [22]. Eventually unrolled neural networks [23] will be investigated. Unrolled neural networks allow to directly learn priors from the data in each iteration of the reconstruction algorithms.

The proposed project presents two main challenges related to SRH. First, only two points are sampled in the Raman spectra for SRH images, which makes spectral unmixing complicated. Second, since SRH images are medical data from an emerging imaging modality, it is complex to build a database large enough to train neural networks.

1.2. Interdisciplinary dimension of the project

The success of this PhD project relies on the strong complementarity of the supervisors and partners involved, each bringing essential expertise crucial for achieving the scientific objectives.

The two official PhD supervisors provide highly complementary and indispensable skills. Hervé Rigneault (HDR), CNRS Research Director at the Fresnel Institute, is an internationally recognized expert in nonlinear optical microscopy, with a unique and long-standing expertise in Stimulated Raman Scattering (SRS) microscopy. He developed an in-house SRS microscope at the Fresnel Institute. His knowledge is essential for the acquisition, optimization, and physical understanding of SRH images. Rémi André, Associate Professor at Aix-Marseille University and the Fresnel Institute, is an expert in signal and image processing as well as data science. His research focuses on blind unmixing problems and deep learning approaches for biomedical image reconstruction and segmentation.

Additional experts play a key role in reinforcing the interdisciplinary nature of the project. Julien Wojak, research engineer at Aix-Marseille University and the Fresnel Institute, is an expert in variational methods applied to image processing and brings complementary methodological expertise that strengthens the algorithmic developments carried out in the project. Romain Appay, MD, PhD, from AP-HM, is a specialist in Pathological Anatomy and Cytology. His clinical expertise is essential to ensure the medical relevance of the developed methods and to guide the interpretation and validation of the enhanced SRH images from a diagnostic perspective.

The project also benefits from a strong industrial partnership with Lightcore Technologies, a company specializing in the development of SRS microscopes dedicated to SRH imaging. Founded in 2019 as a spin-

off of the Fresnel Institute, Lightcore Technologies acquired in 2025 the exclusive rights to a virtual staining software for SRH images that we registered with SATT. This partnership is a key asset for the technological transfer and real-world deployment of the project outcomes. Patents and protected softwares will be filled under the Institution names (AMU, CNRS) and eventually licensed to Lightcore Technologies as we did in 2025 for the SRH virtual staining software.

Overall, the PhD candidate will be supervised by an expert in nonlinear optical microscopy, two experts in image and signal processing, and a hospital physician. These three areas of expertise are strictly necessary for the success of the project, whose ambition is to provide enhanced SRH images for reliable cancer diagnosis using artificial intelligence and advanced image/signal processing methods. Continuous interactions between the fellow and the different partners are expected to foster fruitful discussions on spatial and spectral resolution, imaging modalities, and relevant biological structures.

The two academic supervisors are located within the same laboratory, the PhD will then spend most of their time at the Fresnel Institute. Nevertheless, due to the close collaborations with AP-HM and Lightcore Technologies, which confer a strong intersectoral dimension to the project, the fellow will also interact regularly with the hospital environment and the healthcare industry. This interdisciplinary and intersectoral framework is essential to maximize both the scientific impact and the societal/economic relevance of the research.

2. IMPACT (2 pages max)

2.1. Expected impact of the project on the candidate's career

The candidate will work in the field of artificial intelligence applied to healthcare. Pursuing a PhD in artificial intelligence applied to healthcare can have a decisive impact on a student's career, both scientifically and professionally. The candidate will be at the intersection of two strategic and rapidly growing fields (AI and healthcare) making it a particularly valuable and sought-after profile.
From an academic perspective, a PhD in AI for healthcare enables the development of advanced expertise in state-of-the-art methods (machine learning, deep learning, biomedical image analysis) while also providing a deep understanding of clinical, biological, and ethical issues. The student learns to work with complex and sensitive data, collaborate with healthcare professionals, and produce results with real-world impact. This dual skill set significantly strengthens the doctoral candidate's scientific credibility and facilitates access to research positions, whether in academia or in specialized research institutes.
From a non-academic point of view, the impact is equally significant. Profiles capable of handling AI for healthcare are highly sought after by technology companies, digital health startups, pharmaceutical laboratories, and public organizations. A PhD in this field therefore opens up a wide range of career opportunities, including research and development, data science, medical innovation, consulting, and entrepreneurship. It also demonstrates the student's ability to manage complex projects, solve concrete problems, and work in multidisciplinary environments.

2.2. Expected impact for the thematic axis

Our project is a part of the axis "Artificial Intelligence and its applications". Enhanced SRH images with IA contribute to improve cancer diagnosis. Indeed, SRS microscopy enables to image a tissue sample in few minutes while classical histology technique can take from 24 to 72 hours.
SRH is then a highly promising tool for accelerating cancer diagnosis, particularly in time-critical settings such as intraoperative decision-making. However, the widespread clinical adoption of SRH remains limited by challenges related to image quality, including noise, limited contrast, optical artifacts, and variability across acquisition conditions.
The proposed research project aims to address these limitations by developing advanced image processing algorithms specifically tailored to SRH data. By enhancing contrast and signal-to-noise ratio, while preserving diagnostically relevant morphological and biochemical features, this work seeks to significantly improve the interpretability and reliability of SRH images.
The scientific impact of this project lies in the development of novel computational methods that leverage the unique physical and biochemical properties of SRH signals. The proposed algorithms will be designed to ensure a visual quality corresponding to meaningful biological information. This will contribute to the advancement of image processing methodologies for label-free optical imaging and expand their applicability in biomedical research.
From a clinical perspective, improving SRH image quality has direct implications for diagnostic accuracy and confidence. Enhanced images will facilitate interpretation by pathologists, reduce ambiguity in tissue classification, and improve the detection of subtle morphological features associated with malignancy. This, in turn, can lead to faster and more reliable diagnoses, supporting real-time clinical decision-making.

Moreover, high-quality SRH images may help for the effective deployment of artificial intelligence and machine learning models for automated tissue classification, automatic segmentation and virtual staining. By standardizing and improving image quality, this project will enable more robust training and generalization of AI-based diagnostic tools, further amplifying the impact of SRH.

2.3. Dissemination, exploitation and communication activities planned

The project will generate new knowledge at the interface of stimulated Raman histology (SRH), image processing and Artificial Intelligence. A comprehensive dissemination and exploitation strategy will be implemented to ensure that the results reach a wide range of scientific, industrial, clinical, and societal stakeholders, and that their impact is maximized.

The primary dissemination channel will be peer-reviewed scientific publications in high-impact journals in the fields of biomedical imaging and medical image analysis. Results will also be presented at international conferences and workshops, such as those focusing on Raman imaging and image processing. These activities will ensure rapid dissemination of methodological advances and foster scientific exchange with peers.

To promote reproducibility and transparency, selected algorithms and evaluation protocols will be shared with the research community through open-access repositories or dedicated on-line tools where researchers from other laboratories can up-load their own SRS CH2 and CH3 images and benefit from the developed AI enhanced diagnostic. This will facilitate reuse, benchmarking, and further development by other academic teams.

The project is highly relevant to multiple stakeholders beyond academia. As previously said, close interaction with clinicians and healthcare industry will be encouraged through seminars, demonstrations, and interdisciplinary meetings, ensuring that the developed image enhancement methods address real clinical needs and constraints.

Lightcore Technologies, a spin-off of the Fresnel Institute (https://lightcore-technologies.com/fr/), commercializes a microscope that is able to capture the CH2 and CH3 chemical bond images in tissue. Lightcore has purchased a licensed of a recently developed virtual coloring software that have been developed by our group (André, Wojak, Rigneault) at the Fresnel Institute. It is likely that the outcomes of this PhD work will also lead to some software protections that might also lead to a licensing agreement between Lightcore Technologies and AMU/CNRS.

3. IMPLEMENTATION (2 pages max)

The work plan is carefully structured to ensure that both research and training objectives are fully achieved within the 36-month duration of the PhD.
The project is organized into coherent and interdependent tasks that progressively build toward the final objectives. Initial tasks focus on understanding SRH image formation, data characteristics, and current limitations, which are essential to guide the development of tailored image processing approaches. These foundational tasks interact closely with subsequent algorithmic development, ensuring that methodological choices are grounded in physical and biological reality.
The objectives of the project are realistic and achievable within the 36 months of the PhD. The work plan follows a gradual increase in complexity, starting from data characterization and baseline methods, moving toward advanced algorithmic development, and culminating in validation and dissemination. Each phase produces intermediate outputs that de-risk subsequent steps.

Project Organization and Milestones
The work project is structured into work packages:

- **WP1 (Months 0-6) – Data characterization and state-of-the-art analysis**
  Deliverables: Comparison study of existing unmixing and denoising algorithms for SRH images.
  Milestone: identification main limitations for image interpretation by physicians.

- **WP2 (Months 6-24) – Development of SRH-specific image processing algorithms**
  Deliverables: Prototype algorithms for noise reduction, contrast enhancement, and artifact correction, representative datasets.
  Milestone: Demonstration of improved image quality on representative datasets.

- **WP3 (Months 24-32) – Validation and clinical relevance assessment**
  Deliverables: Quantitative evaluation results; expert-based validation reports.
  Milestone: Confirmation of diagnostic relevance and robustness.

- **WP4 (throughout the project) – Dissemination, training, and valorization**
  Deliverables: Scientific publications; conference presentations; outreach materials.
  Milestone: Submission of at least one first-author journal article.

International Dimension
The Fresnel Institute maintains active collaborations in the field of stimulated Raman histology with leading European laboratories. Notably, the recently established EU 'Futur Histo' consortium—comprising the University of Vienna (AT), Maastricht University (NL), and Aix-Marseille University (FR)—aims to develop next-generation technologies for real-time histology without sample preparation.

Within this consortium:

- Aix-Marseille University and Maastricht University focus on stimulated Raman histology, directly aligning with the proposed PhD research.

- The University of Vienna specializes in virtual histology, using acridine orange (to stain cell nuclei) and two-photon fluorescence for deep tissue imaging.

As part of this PhD project, the candidate will spend time at these international partner institutions to apply developed software tools for image enhancement of tissue samples studied across the consortium.

4. ETHICS SELF-ASSEMENT

The image samples necessary for the AI automatic cancer diagnostic development will come from 'Assistance publique des hôpitaux de Marseille' (AP-HM) with which the Fresnel Institute has a collaborative agreement. The AP-HM which is a certified national French hospital that is conducting a policy regarding human material in adherence with the fundamental ethical principles.

- The principle of respect for human dignity and the principles of non-exploitation, non-discrimination and non-instrumentalisation;
- The principle of individual autonomy (entailing the giving of free and informed consent, and respect for privacy and confidentiality of personal data);
- The principle of justice (the equitable distribution of burdens and benefits of research);
- The principle of beneficence and non-maleficence, namely with regard to the improvement and protection of health;
- The principle of proportionality (including that research methods are necessary to the aims pursued and that no alternative more acceptable methods are available);

All images will be anonymized and cannot be considered as personal data.

It should be emphasized that no conversational or generative AI systems will be used in the proposed research. Deep learning techniques will be employed solely as image processing methods, and all neural networks will be developed and trained in-house.

5. REFERENCES

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