

**Contribution aux exercices de prospective 2020-2030**  
***Contribution to the 2020-2030 prospective reflection***

**Sciences Nucléaires et Vivant**  
*Nuclear Science and Health*

**Description détaillée de la contribution**  
*Detailed description of contribution*

***Please indicate science objectives (2 pages max. including figures)***

The main goal of surgical resection is to achieve maximum safe removal of malignant tissue from patients with primary and metastatic brain tumors<sup>1,2</sup>. Such an approach requires the identification of tumors margins defined by the boundary between tumor and surrounding normal tissue. In practice, these margins are determined based on the surgeon's palpation and visual assessment through the operating microscope. One approach to preserve normal tissue has been to map cortical functional pathways intraoperatively using brain mapping with direct electrical stimulations under awake surgery<sup>3,4</sup>. A common approach to better guide resection is the use of 3D ultrasound imaging or Magnetic Resonance Imaging (MRI) intraoperatively. Despite these solutions however, delineating tumor margins remains challenging as none of these techniques have sufficient spatial resolution to identify the microscopic boundary of malignancy. The sub-optimal spatial resolution and sensitivity of intraoperative imaging also limits the surgeon's ability to discriminate tumor infiltration from surgically-induced brain tissue alterations (contusion, ischemia or edema) at the cellular level<sup>5,6</sup>. Although the assessment of tumor margins at the cellular scale can be achieved using intraoperative pathology techniques, practical and repeat use is limited due to the time constraints and the under-sampling technique dictated by surgery. The extent of resection is significantly associated with prolonged survival. Since most patients succumb to brain cancer, the goal of gross total resection is thus not only to improve time to tumor progression, but rather to optimize the patient's quality of life<sup>7,8</sup>. This is possible if maximum safe resection is achieved, where normal functional tissue is preserved<sup>7,8</sup>. In fact, a prospective study showed that maximum safe resection prevented the decay in functional well-being as well as neurocognitive function, which is otherwise typically observed in patients as time progress. The main challenge in neurosurgery is therefore to accurately identify tumor margins.

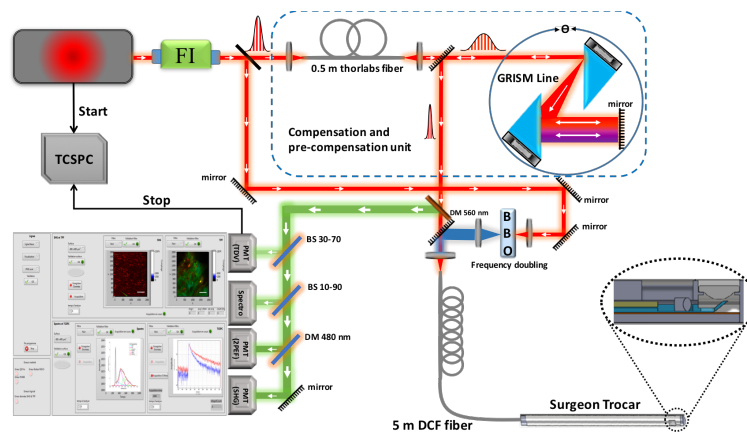
Intraoperative fluorescence imaging on the other hand has been shown to be a valuable adjunct to neurosurgery, where tumors are highlighted in real-time. The most common form of fluorescence-guided surgery is mediated by the preferential overproduction of the fluorophore protoporphyrin IX after an oral dose of 5-Aminolevelunic Acid (ALA). Clinical studies have shown an increased extent of resection and improved overall survival using ALA<sup>9,10</sup>, however, its sensitivity can be improved, especially in infiltrative areas<sup>11,12</sup>. Moreover, any dye has contraindications and side effects. The limitations described above and the need to preserve functional tissue render conventional neurosurgery inadequate for maximal safe resection motivating the need to develop an imaging modality capable of real-time assessment of brain tumor margins with sub-micrometer resolution.

To this end, we propose a label-free advanced optical imaging modality capable of extracting multiple tissue properties that characterize different tissue types based on their respective molecular, cellular, and morphological signatures. While initial attempts at label-free optical imaging date back to 1990's, active research is still undergoing yet with no major progress towards its clinical translation<sup>12-16</sup>. Over the last 20 years, a more advanced label-free optical imaging technique based on nonlinear optics has emerged<sup>13,14</sup>. Nonlinear optical microscopy uses 'higher-order' light-matter interactions involving multiple photons for contrast generation such as two-photon fluorescence (TPF) or second harmonic generation (SHG). This technique is superior to classical linear optical microscopy such as confocal microscopy<sup>15-17</sup> due to its ability to image several endogenous contrasts, without the need to introduce

exogenous dye. Additionally, the near infrared (NIR) excitation source probes tissue at larger depths with minimal photon-induced tissue damage. Recently, two photon microscopy (TPM) has been shown to be valuable for the intraoperative assessment of various pathological features with the added advantage of reducing surgical biopsies along with the time required for histopathological processing<sup>18,19</sup>. However, TPM is still limited to research based applications, where its main application is centered on fixed or *ex-vivo* human samples and or on preclinical *in vivo* imaging. In fact, a recurrent request from the neurosurgeons has been<sup>20</sup> to convert the linear endomicroscope into a nonlinear optical imaging tool providing several complementary means of endogenous contrasts at sub-cellular resolution. Due to its flexibility and its deep penetration capabilities, multiphoton endomicroscopy has the potential to complete the classical surgical procedure providing an “optical biopsy” in real-time along with additional relevant morphological, physiological and quantitative information. The design of a multiphoton endomicroscope should overcome several major challenges<sup>21,22</sup> (efficient excitation with short pulse duration, miniaturization of the distal end optics and scanning system, multimodal acquisition, large FOV and sub-cellular resolution), which has not been combined by any research group to this date.

In this project, we present a solution addressing all these needs in the same instrument. Specifically, **we propose the development and optimization of a miniaturized multimodal non-linear endomicroscope able to achieve preoperative real time diagnosis**. The overall design of the endomicroscope is superior to other configurations currently under development as it can be seamlessly integrated with the surgeon’s work flow corresponds to what is actually done in clinics particularly during stereotactic biopsy. In fact, our endomicroscope has unique physical **dimensions that are compatible with the routinely used surgical trocars and offer the possibility to image on the side edge**. The side-viewing probe is defined by five main components: a Micro Electro Mechanical System (MEMS) chip, a miniature optics lens, a customized small-core double-clad photonic crystal fiber (DC-PCF), an FPCB and a probe mount. This endomicroscope is able to detect four different endogenous contrast allowing a quick, reliable, rigorous and exhaustive response.

Aside from the instrumental development, we aim to **construct a multimodal multiscale database of optical information from freshly resected human samples**. This series of specific multimodal “optical signatures” linked to different types of tissues will help to (i) define the better excitation and collection parameters, (ii) calibrate the endomicroscope for human *in vivo* analysis, and (iii) give new insights into brain morphology. This multimodal optical analysis is expected to overcome shortcomings of each technique used separately, thereby providing additional clinically useful information on human brain tumors.



**Figure 1** Schematic of the endomicroscope under development with multiwavelength excitation and emission capabilities and equipped with a miniaturized MEMS scanning device. Image of a surgeon trocar with the fiber Tip.

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