A Hyperspectral Imaging System for Meningioma Grade Discrimination

Pietro Ricci[a,b,*], Camilla Bonaudo[c], Ivan Ezhov[d], Anam Toaha[a,b], Dorotea Nardini[a,b], Louis Chessel[e], Luca Giannoni[f], Ilias Tachtsidis[f], Francesco S. Pavone[a,b,g]

a Department of Physics and Astronomy, University of Florence, Florence, Italy; b European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy; c Neurosurgery, Department of Neuroscience, Psychology, Pharmacology and Child Health, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy; d Klinikum rechts der Isar, Technischen Universität München, Munich, Germany; e Polytech Lyon, Villeurbanne, France; f Department of Medical Physics and Biomedical Engineering, University College London, London,UK; g National Institute of Optics, National Research Council, Sesto Fiorentino, Italy.
**Corresponding Author: pietro.ricci@unifi.it*

Abstract: Histopathology is the gold standard for meningioma grading but is limited by processing time and subjectivity. We present a hyperspectral imaging system for real-time, label-free analysis, demonstrating the potential to enhance efficiency in meningioma grading.
Keywords: hyperspectral imaging, meningiomas, tumor grade discrimination

1. Introduction

The histopathological analysis represents the gold standard in post-surgical oncology but is limited by lengthy processing times and diagnostic subjectivity. Hyperspectral imaging (HSI) has emerged as a promising alternative, enabling label-free, biochemical tissue assessment through spectral analysis [1]. By capturing spatial and spectral data, HSI allows for rapid, quantitative evaluation of tissue composition. Recently, we introduced the HyperProbe [2], a compact, transportable HSI system capable of imaging fresh surgical biopsies of glioma, providing detailed biomarker mapping without sample alteration. While our initial focus was on gliomas, this approach holds great potential for studying other types of brain tumors, paving the way for broader applications in oncological diagnostics. In this regard, Meningiomas are the most common primary intracranial tumors, accounting for 37.6% of all primary central nervous system (CNS) tumors and 53.3% of benign CNS tumors. These extra-axial neoplasms originate from arachnoid cap cells and are typically benign, slow-growing, and non-infiltrating [3]. The 2021 WHO classification categorizes meningiomas into three grades based on histopathological and molecular features: Grade I (benign), Grade II (atypical), and Grade III (malignant). Grade I meningiomas are the most common (≈80%), have well-defined borders, grow slowly and show minimal cellular atypia. Grade II tumors (15–20%) exhibit higher mitotic activity, increased recurrence rates, and a tendency for brain invasion [4]. Grade III meningiomas, though rare (1–3%), are highly aggressive, characterized by rapid growth, high mitotic activity, and frequent local recurrence, making them the most difficult to manage. For meningiomas, surgical resection remains the cornerstone of diagnosis and treatment, with the extent of removal classified by the Simpson grading system. Gross total resection (Simpson Grade I), involving removal of the tumor, underlying bone, and dura, is associated with lower recurrence rates, while subtotal resection (Simpson Grade IV) carries a higher risk of recurrence. Postoperative management of Grade II and III meningiomas requires a multidisciplinary approach, integrating surgery, radiotherapy, and, in select cases, chemotherapy. For inoperable or recurrent tumors, radiosurgery or fractionated radiotherapy (RT) may be considered, depending on tumor size and proximity to critical structures. Combined treatment strategies, including surgery followed by adjuvant RT, are increasingly utilized, though debate continues regarding the optimal timing, type, and dosing of radiotherapy.
So far, the currently available intraoperative technologies do not provide information about the histological grading of Meningiomas. Here we show how HyperProbe can be used to qualitatively investigate histological sample molecular composition, and to determine meningioma grading. From the analysis of molecular and metabolic features, Hyperprobe outcome can pave the way to an optimization of the surgical and clinical management of this pathology.

2. Materials and Methods

HyperProbe (HP) is a hyperspectral imaging (HSI) system based on spectral scanning, where the target is sequentially illuminated at selected wavelengths, and a full-frame image is acquired at each step. The spectral frames are then stacked to form a 3D hypercube of the target [1]. Fig. 1 illustrates the HP setup and system components.

The illumination system consists of a plasma light source (PLS) (Isteq, XWS-65) and a flexible wavelength selector (FWS) (Spectrolight, FWS-Poly-CUS-10), allowing selection of wavelength steps (min. 3 nm), bandwidths (3-15 nm), and switching times (10-100 ms). Light is delivered via a 1-mm core diameter optical fiber (Avantes, FC-IR1000-1) and collected using an apochromatic macro-objective (Olympus, SDF PLAPO 1X PF), providing a field of view (FOV) of 0.65 x 0.65 cm². A dual filter wheel (Cairn Research, Dual OptoSpin32) filters the reflected signal

Translational Biophotonics: Diagnostics and Therapeutics IV, edited by Zhiwei Huang, Lothar D. Lilge, Proc. of SPIE-Optica Vol. 13934, 139342F · 2025 Published by SPIE-Optica · 1605-7422 · doi: 10.1117/12.3098477

before image acquisition with a CMOS camera (PCO, pco.panda 4.2) featuring a 2048 × 2048 pixel sensor, 6.5 µm pixel size, and 40 fps readout rate.

Meningioma samples were obtained from routine neurosurgeries at Azienda Ospedaliero-Universitaria Careggi (Florence) under ethical approval (Studio ID: 23672 - 23672_BIO) and patient-informed consent. A total of eight (n = 8) samples were analyzed, each classified by histopathological screening according to the 2021 WHO grading system (I-III) [5]. Samples (2-3 cm in size) were washed in phosphate-buffered saline (PBS) to eliminate blood and other unwanted residuals, covered with a thin glass coverslip for uniform focus, and imaged within 1 h post-excision. A dark absorbing material was placed underneath to prevent back-reflections. Each hypercube consisted of 125 wavelengths (5 nm steps, 5 nm bandwidth), with acquisition times under 25 s to prevent tissue degradation. Multiple FOVs per sample were acquired to investigate specimen variability. Reflectance hypercubes were normalized using a white reference (Labsphere, Spectralon® 5″) and corrected for dark counts. Every dataset was acquired while any ambient light was switched off. A spectral unmixing approach based on the modified Beer-Lambert law (MBLL) was applied to determine molecular compositions, following Ezhov et al. [6]. The analysis (510–900 nm) included endmembers such as haemoglobin (HbO_2, HHb), cytochrome-c-oxidase (oxCCO, redCCO), cytochromes-b/c (Cyt-B, Cyt-C), water, and fat. Mean spectral values were computed per FOV, with compositions expressed in (mM/cm) and (cm^{-1}), as a unitary pathlength (1 cm) was used in the experiments. These values were used to differentiate meningioma grades (I-III), providing a quantitative basis for classification.

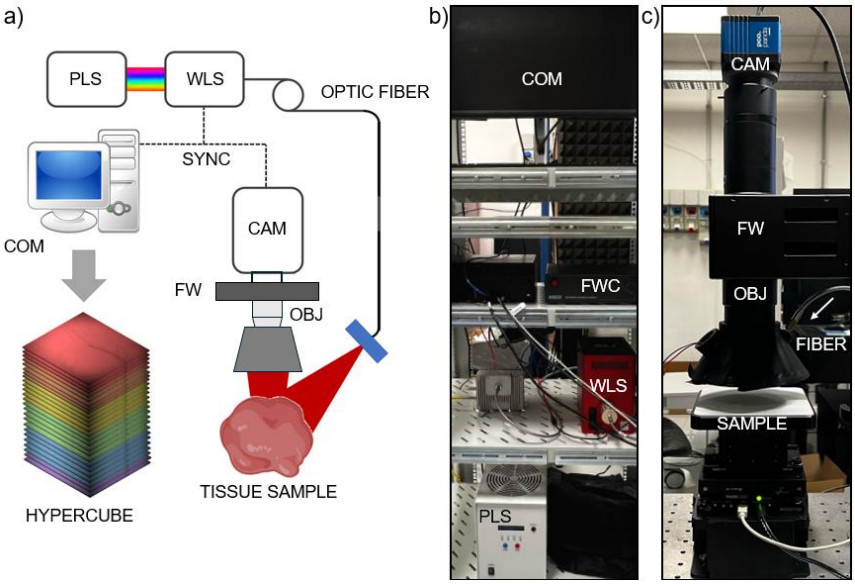

Fig. 1. a) Schematic of the HyperProbe with all its components. In red, the light from the optical fiber, selected as output as example. The Hypercube stack is multicolored as result of multi wavelength illumination. b) and c) Pictures of the back and front panel of HyperProbe, respectively. PLS: plasma light source; WLS: wavelength selector; OBJ: objective; FW: filter wheel; FWC: filter wheel controller; CAM: camera; COM: computer.

3. Results and Discussion

The HP system enables qualitative and quantitative content analysis for meningioma evaluation. Fig. 2a shows the reconstructed sample using stitched fields of view (FOVs) for a comprehensive assessment, with the inset providing a real photograph as a macroscopic reference. With this system we can easily investigate on brain tumor contents, such as lipid, haemoglobin difference (Hbdiff=HbO2-HHb), which reflects hemoglobin oxygenation variations and provides insights into vascularization and haemodynamics; and cytochrome-c-oxidase difference (diffCCO=oxCCO-redCCO), that indicates CCO redox concentration variations, revealing metabolic activity. Fig. 2b investigates the sample composition through spectral analysis of its content, highlighting intersample variability in hemoglobin oxygenation states: the first two regions reveal oxyhaemoglobin, while the third corresponds to deoxyhaemoglobin.

Moreover, HP can be used for precise meningioma grading classification. Fig. 2c and 2d present the mean distributions of Hbdiff and diffCCO versus mean lipid distribution. The results clearly distinguish meningioma samples into three distinct regions, corresponding to different tumor grades, confirmed through histopathological analysis. This study provides promising preliminary evidence for the HyperProbe system in neuro-oncology, enabling meningioma grading based on biochemical signatures. Future work will refine classification algorithms and validate findings in larger cohorts.

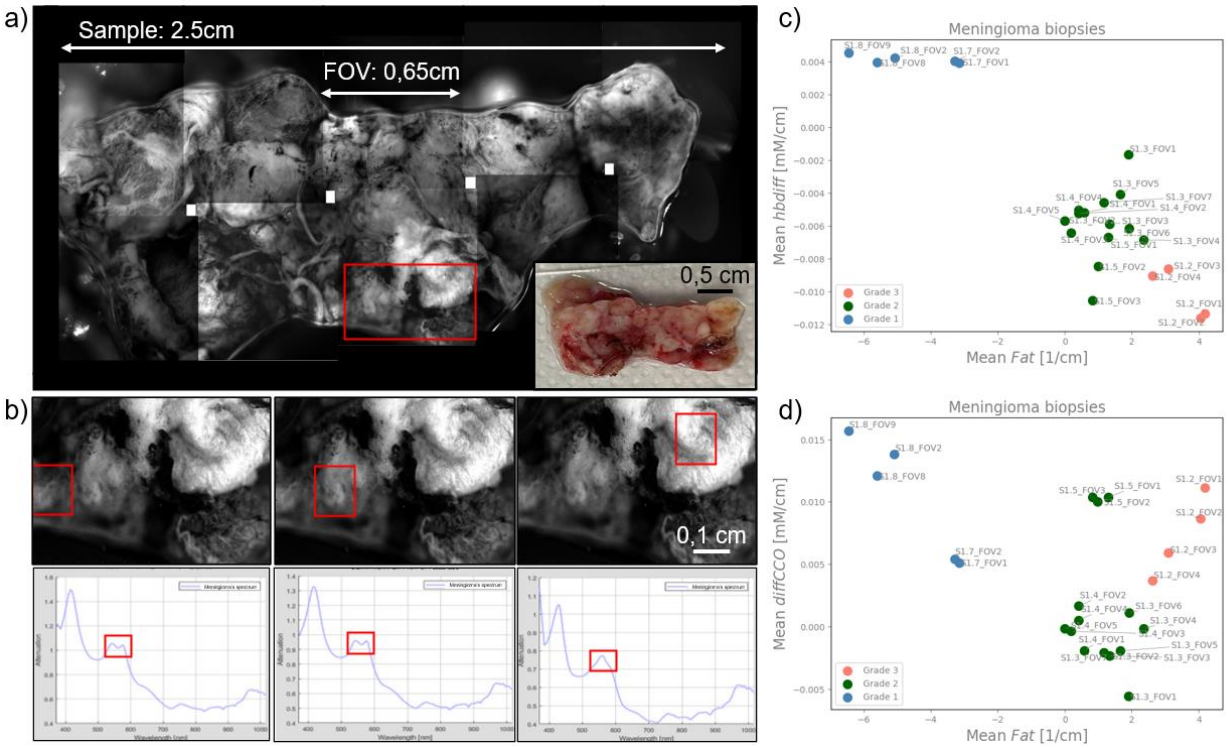

Fig. 2. a) Picture of a meningioma sample realized by stitching together sequential FOVs acquired with HyperProbe. In inset, a real photograph of the sample. b) Zoom-in of the red rectangular area highlighted in a), where three different ROIs are spectrally analyzed (top). Red rectangles in plots shows differences in haemoglobin states (bottom). c, d) Distribution of the mean spectral values of hbdiff (c) and diffCCO (d) concentrations (y-axis) as a function of the lipid content (x-axis). The means are computed over the full FOV of 8 meningioma samples for several FOVs. In blue, I-grade meningiomas; in green, II-grade meningiomas; in pink, III-grade meningiomas.

4. Data Availability and Disclosures

The datasets generated during the current study are available from the corresponding author upon reasonable request. The authors declare no conflict of interest.

5. Acknowledgments

The HyperProbe consortium and project have received funding from the European Union's Horizon Europe research and innovation program under grant agreement No 101071040 – Project HyperProbe. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them. IL from UCL is supported by the UK Research and Innovation (UKRI) (Grant No. 10048387).

References

1. L. Giannoni, F. Lange, and I. Tachtsidis, "Hyperspectral imaging solutions for brain tissue metabolic and hemodynamic monitoring: Past, current and future developments," Journal of Optics (United Kingdom) **20**, (2018).
2. L. Giannoni, M. Marradi, K. Scibilia, I. Ezhov, C. Bonaudo, A. Artemiou, A. Toaha, F. Lange, C. Caredda, B. Montcel, A. Della Puppa, I. Tachtsidis, D. Rückert, and F. S. Pavone, "Transportable hyperspectral imaging setup based on fast, high-density spectral scanning for in situ quantitative biochemical mapping of fresh tissue biopsies," J Biomed Opt **29**, (2024).
3. V. Yarabarla, A. Mylarapu, T. J. Han, S. L. McGovern, S. M. Raza, and T. H. Beckham, "Intracranial meningiomas: an update of the 2021 World Health Organization classifications and review of management with a focus on radiation therapy," Front Oncol **13**, 1137849 (2023).
4. A. Okano, S. Miyawaki, Y. Teranishi, K. Ohara, H. Hongo, Y. Sakai, D. Ishigami, H. Nakatomi, and N. Saito, "Advances in Molecular Biological and Translational Studies in World Health Organization Grades 2 and 3 Meningiomas: A Literature Review," Neurol Med Chir (Tokyo) **62**, 347–360 (2022).
5. D. Louis, A. Perry, P. Wesseling, … D. B.-N., and undefined 2021, "The 2021 WHO classification of tumors of the central nervous system: a summary," academic.oup.comDN Louis, A Perry, P Wesseling, DJ Brat, IA Cree, D Figarella-Branger, C Hawkins, HK NgNeuro-oncology, 2021•academic.oup.com (n.d.).
6. I. Ezhov, K. Scibilia, L. Giannoni, F. Kofler, I. Iliash, F. Hsieh, S. Shit, C. Caredda, F. Lange, B. Montcel, I. Tachtsidis, and D. Rueckert, "Learnable real-time inference of molecular composition from diffuse spectroscopy of brain tissue," J Biomed Opt **29**, (2024).