

INTEGRATING HISTOLOGICAL GROUND TRUTH FOR DATASET GENERATION AND VALIDATION OF POLARIMETRIC IMAGING

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As the definitive ground truth for tissue characterization, histology plays a central role in developing and validating the performance of emerging optical imaging techniques. Here, we propose two complementary strategies for leveraging histological data to support and evaluate optical imaging approaches, using wide-field polarimetric imaging of brain tissue as a representative example.

First, we developed a custom sample preparation protocol and an image processing pipeline which together enable accurate comparison between histological annotations and polarimetric maps [1]. We analyzed 45 polarimetric measurements of brain samples processed with the new protocol. Co-registration with annotated histological slides revealed distinct polarimetric signatures across tissue types, including reduced linear retardance and pronounced randomization of the azimuth of the optical axis in tumor-labeled regions. The application of this protocol to diverse samples enabled the generation of a large database of histologically annotated polarimetric data. However, a key limitation is that fresh specimens rarely contain both tumor and adjacent tumor-free tissue.

To address this, we developed an image processing pipeline to construct a database of spatially co-registered polarimetric and histological images from formalin-fixed whole-brain sections [2], containing both tumor and tumor-free tissue. Because the regions of interest were substantially larger than the field of view of both polarimetric and histological imaging systems, we implemented a tile-based reconstruction and alignment workflow. This enabled integration of polarimetric maps with high-resolution histological images across large tissue areas, supporting the training and evaluation of segmentation algorithms for brain tissue classification. Moreover, polarimetric features observed in fresh tumor samples, such as reduced retardance and increased randomization of the azimuth of the optical axis, were also detected in formalin-fixed tumor regions.

Taken together, these strategies demonstrate how histology can serve not only as a validation tool for optical biomarkers but also to generate high-quality, spatially annotated datasets that power the training of machine learning models, paving the way for future intraoperative applications.

[1] É. Gros et al., "Characterization of Polarimetric Properties in Various Brain Tumor Types Using Wide-Field Imaging Mueller Polarimetry", *IEEE Trans. Med. Imaging*, 43(12), 4120–4132 (2024).

[2] É. Gros et al., "Alignment of Histological and Polarimetric Large-Scale Imaging for Brain Tissue Characterization", *J. Biomed. Opt.*, (under review)