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AI-based benchmark to test the potential of 3-photon excited fluorescence in intraoperative lung cancer detection with multiphoton microscopy

Bronchoscopy, Diagnosis

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Introduction: Higher harmonic generation microscopy (HHGM) enables intra-operative tissue analysis of lung biopsies, producing images for automated analysis (Van Huizen et al., Mod Pat 2025;38(1)100633). Our microscope includes detection channels for 2- and 3-photon excited fluorescence (3PEF) enabling imaging of flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NADH) showing metabolic activity. The autofluorescence lifetimes of FAD and NADH decrease in cancer. The free to protein-bound NADH lifetime ratio increases in cancer (Wang et al., BBA Clinical 2017;8:7-13).

Aims and objectives: This study aims to establish an AI-based benchmark to evaluate HHGM components in lung cancer detection, demonstrating the benefit of 3PEF.

Methods: HHGM images from navigation bronchoscopy biopsies of 143 patients, 124 images after exclusion, were collected. To detect tumor, a neural network was trained with self-supervision to extract domain-specific features before training a binary tumor classifier. The performance was evaluated using 10-fold cross-validation and the area under the receiver operating characteristic curve with equivalence tested via the Wilcoxon signed-rank test.

Results: An AI-based benchmark was built to test lung cancer detection with HHGM hypotheses. Preliminary experiments show that including 3PEF did not alter model outcomes (p=0.23).

Conclusions: While further research is needed to validate our findings on the added value of including 3PEF for tumor detection, we introduced an AI-based validation framework that can be extended to test other tumor detection hypotheses or endpoints such as segmentation or prognosis.