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

Siem de Jong[1], Annick J. H. E. van der Kroef[2], Marie Louise Groot[3], Roel L. J. Verhoeven[2], Erik H. F. M. van der Heijden[2], Francesco Ciompi[1]

[1]Radboud university medical center, Department of Pathology, Nijmegen, The Netherlands,
[2]Radboud university medical center, Department of Pulmonary Diseases, Nijmegen, The Netherlands,
[3]LaserLab Amsterdam, Department of Physics, Faculty of Science, VU Amsterdam, Amsterdam, The Netherlands

Graphical Abstract

Suspected lung cancer patients (n=179) underwent bronchoscopy and biopsies (235) were included

Acquired rapid on-site HHGM images of biopsies

Developed AI pipeline to detect lung cancer in HHGM images

Used AI pipeline to test model hypotheses

(e.g. “does 3-photon excited fluorescence, 3PEF, improve cancer detection performance?”)

Abstract

Introduction: Higher harmonic generation microscopy (HHGM) enables intra-operative tissue analysis of lung biopsies, producing images for automated analysis[1,2]. 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[3].

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.

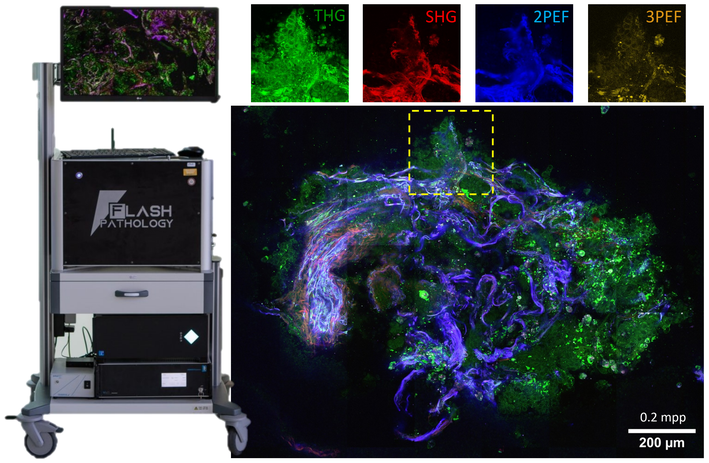
Methods: HHGM images from 235 navigation bronchoscopy biopsies from 179 patients, were collected. To detect tumor, a neural network was trained with self-supervision to extract domain-specific features before training a binary tumor classifier. The performance was evaluated using 8-fold cross-validation 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=0.74).

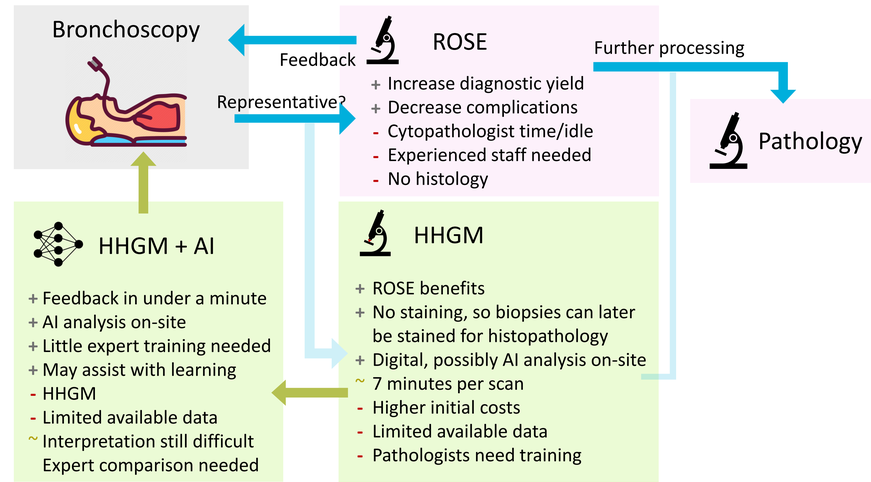
Conclusions: While further validation is needed regarding 3PEF's added value for tumor detection, we introduced an AI-based validation framework extensible to other tumor detection hypotheses and endpoints including segmentation and prognosis.

We developed an adaptable AI framework to detect lung cancer in bronchoscopy biopsies with label-free microscopy. Experiments on our data show comparable performance with and without 3-photon excited fluorescence.

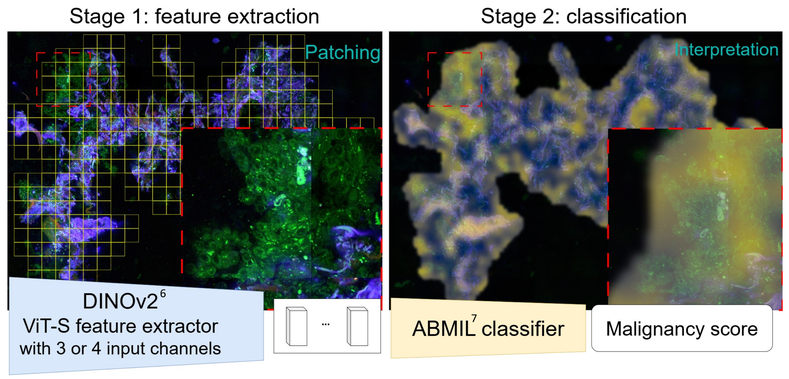
1 Higher harmonic generation microscopy (HHGM) images cells and interfaces, collagen, elastin and cellular fluorophores, and flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide (NADH)[4]. The portable microscope can be transported to the operating room.



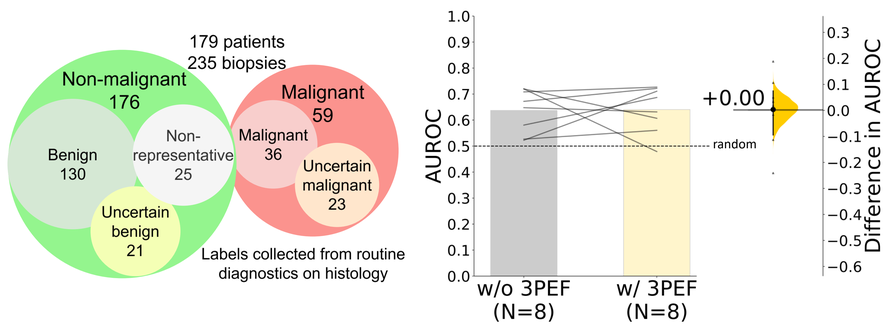
2 HHGM has the potential to improve rapid on-site evaluation (ROSE)[5] through label-free histology imaging and the capability for on-site AI-assisted analysis.



3 The 2-stage AI framework consists of extracting features and detecting cancer. Heatmaps provide interpretability.



4 We trained models with and without 3PEF on 235 biopsies using 8-fold cross-validation and measured area under the receiver operating characteristic curve (AUROC) and found no difference (p=0.74)



IMAGIO — IMAGING AND ADVANCED GUIDANCE FOR WORKFLOW OPTIMIZATION IN INTERVENTIONAL ONCOLOGY

FLASH PATHOLOGY

VU VRIJE UNIVERSITEIT AMSTERDAM

innovative health initiative

Funded by the European Union

Radboudumc university medical center

[1]L. M. G. van Huizen, M. Blokker, J. M. A. Daniels, T. Radonic, J. H. Von Der Thüsen, M. Veta, J. T. Annema, M. L. Groot, Mod. Pathol. 38(1), 100633 (2025).
[2]S. de Jong, M. L. Groot, R. L. J. Verhoeven, E. H. F. M. van der Heijden, F. Ciompi, In Medical Imaging with Deep Learning (2024).
[3]M. Wang, F. Tang, X. Pan, L. Yao, X. Wang, Y. Jing, J. Ma, G. Wang, L. Mi, BBA Clinical, 8, 7–13 (2017).
[4]L. M. G. van Huizen, T. Radonic, F. van Mourik, D. Seinstra, C. Dickhoff, J. M. A. Daniels, I. Bahce, J. T. Annema, M. L. Groot, Transl Biophotonics, 2(4), e202000009 (2020).
[5]S. E. P. Kops, L. J. W. van der Burgt, S. Vos, L. J. M. van Zuijlen-Manders, R. L. J. Verhoeven, E. H. F. M. van der Heijden, Cancer cytopathol., 132(4), 233–241, (2024).
[6]M. Oquab, T. Darcet, T. Moutakanni, H. Vo, M. Szafraniec, V. Khalidov, P. Fernandez, D. Haziza, F. Massa, A. El-Nouby, ..., P. Bojanowski, arXiv:2304.07193 [cs.CV] (2023).
[7]M. Y. Lu, D. F. K. Williamson, T. Y. Chen, R. J. Chen, M. Barbieri, F. Mahmood, Nat Biomed Eng 5, 555–570 (2021).

This study was funded by the European Union under the IMAGIO grant number 101112053.