Paulo Sampaio*, Davide Scandella, C.H. Lucas Patty, Heather DiFazio, Pablo Márquez-Neila, Irene Centeno Ramos, Martin Wartenberg, Federico Storni, Brice-Olivier Demory, Daniel Candinas, Aurel Perren, Raphael Sznitman

Fast and user-friendly multi-spectra Mueller matrix polarimeter for fresh tissue biopsy imaging

https://doi.org/10.1515/cdbme-2025-0144

Abstract: Mueller Matrix polarimetry (MMP) characterizes changes in light polarization after interacting with a medium, providing insights into tissue microstructure. Combined with multispectral (MS) imaging cameras, MS-MMP offers a novel way to quickly and safely acquire tissue surface information. Machine learning methodologies enable new diagnostic methods by automating tasks on fresh tissue biopsies, though this requires extensive and diverse data. To achieve this, we propose a user-friendly MS-MMP imager with a simple interface and fast acquisition time operated by laboratory technicians and residents. We show that our system, when operated by laboratory staff over several months, yields high-quality data in large amounts and with positive feedback of its inclusion in a clinically compliant workflow. This positive outcome is promising for such systems to be used for large data collection initiatives.

Keywords: Polarimetry, tissue biopsy, pathology, machine learning

1 Introduction

Optical polarimetry (*i.e.,* measuring the rotation and amplitude of the light wave's electric field) has shown potential for biomedical applications, enabling the analysis of biological materials using light intensity alone. The state of polarization of a light wave is sensitive to the microscopic structure of a material. Polarimetry thus provides a way to distinguish multiply scattered photons (*e.g.*, in tissues) from those that experience nominal scattering events [1]. Therefore, one can use an unpolarized light source pointed at a tissue specimen and analyze the polarization state of the light scattered off a tissue to probe its cell structure non-invasively [2], [3]. Specifically, polarimetry is sensitive to the anisotropic organization of fibrous structures [4], [5], is innocuous, and alleviates the need for processing (tissue staining/labeling) for cancer diagnosis [6].

Polarimetric, *ex-vivo* imaging studies for different anatomies have been achieved in the past two decades for cancer diagnosis and staging, such as skin [2], laryngeal [7], colon [8], rectum [9], pancreas [10] or cervix [11], [12], [13], [14]. By measuring how the sample modifies the state of linear and circular polarization of incident light, the Mueller Matrix (MM) -- a 4×4 matrix representation of how the polarization state of light changes when it passes through or reflects off a material – can capture structural changes in tissues non-invasively and image them effectively without modification [15].

Despite this evidence in the characterization power of Mueller Matrix Polarimetry (MMP), virtually no commercial system exists capable of snapshot imaging of large tissue specimens (i.e., centimeter-sized). With the prospect of using MMP for diagnostic purposes or the ability to access tissue quality, the combination of MMP imaging with modern machine learning methodology appears particularly well suited given the tensor nature of the MM that be collected at a pixel level. Yet, machine learning models require large amounts of data to be trained, in particular, if one wants them to be resilient and generalizable.

To this end, we propose and demonstrate the development of a novel MMP imager that is both fast and user-friendly for tissue biopsy imaging. Our compact system enables imaging to be performed by non-technical staff with minimal training. Experimental results show that laboratory residents and

***Corresponding author: Paulo Sampaio:** ARTORG Center, University of Bern, Bern, Switzerland, e-mail: paulo.sampaio@unibe.ch
Davide Scandella, C.H. Lucas Patty, Raphael Sznitman, Heather DiFazio, Pablo Márquez-Neila: ARTORG Center, University of Bern, Bern, Switzerland
Brice-Olivier Demory: ARTORG Center, University of Bern, Bern, Switzerland; Center for Space and Habitability, University of Bern, Switzerland
Martin Wartenberg, Irene Centeno Ramos, Aurel Perren: Institute of Tissue Pathology and Medicine, University of Bern, Berm, Switzerland
Federico Storni, Daniel Candinas: Department of Visceral Surgery and Medicine, Bern University Hospital, Bern, Switzerland

technicians can acquire high-quality, reliable data. After several weeks of experimentation in a pathology laboratory, we demonstrate that our system can effectively image a wide range of tissue types.

2 Material and methods

We introduce a new MMP imaging system that is composed of an (i) imager and (ii) a software solution. We detail these in the following subsections, along with the data collection process we followed. Our system is shown in Figure 1.

2.1 MMP imager

We designed a custom dual-rotating retarder polarimeter in a 16-degree reflection position, consisting of a light source, a polarization state generator (PSG), a polarization state analyzer (PSA), and a multispectral camera. The PSG generates polarization states by letting light from an LED light source pass through a fixed linear polarizer and a rotating quarter-wave plate. The light then interacts with the sample positioned on its holder. The reflected light then passes through the PSA, which is comprised of a quarter-wave plate and a linear polarizer. Finally, the light reaches the detector, a multispectral camera (Photonfocus, Lachen, Switzerland) with 16 passbands in the spectral region from 460nm to 598nm. These filters are arranged in a 4 by 4 mosaic with a full-width half-maximum of 15 nm. The spatial resolution of the sensor is 512 x 272 per spectral band, with a pixel size of 140μn. The acquisition time for a 512x272 pixel image across 16 wavelengths is approximately 35 seconds.

Calibration of the optical system is performed using an ND filter placed at the imaging focal point using a 3D-printed holder that can be placed manually. The entire MMP is operated by a dedicated computer that runs all software locally (see next section) with a touchscreen interface. Images acquired are saved locally on a dedicated storage unit of the computer.

2.2 Software

Our hardware system is operated using a dedicated software application that controls the motor positions of the polarimeter and the camera. From this, the application contains two specific routines: (1) calibration and (2) acquisition. In both cases, initially, the operator was responsible for selecting the optimal exposure time. After initial feedback from the operators, one optimal exposure time was determined, considering the ambient light, and fixed to simplify daily operation.

The application itself consists of a Python backend with a QML GUI interaction that allows users to create new acquisitions and log them with unique identifiers.

2.3 Data collection and procedure

Our system was deployed in the Tissue Biobanking unit of the Institute of Tissue Medicine and Pathology (ITMP) at the University of Bern from January to March 2025 (3 months). The local technicians and resident staff were instructed using a 1-page instruction sheet for how the device had to be used during a 15-minute training session. All data acquired were from patients who had signed general consent forms, and the Bern cantonal ethics commission approved this study.

Calibration was performed once per day when the system was turned on. All subsequent acquisitions, as well as the calibration, were performed by the laboratory staff. Tissue specimens were imaged in the histology cassette and placed on a 3D printed holder to ensure positioning at the focus point of

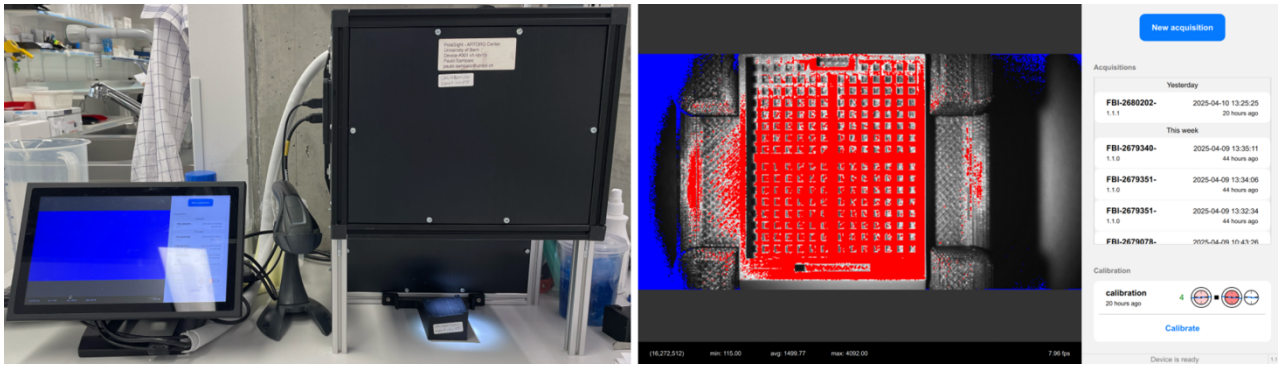

Figure 1: (left) Our custom-designed MS-MMP imager is at the Institute of Tissue Medicine and Pathology, Bern University. (right) Example image of acquisition interface.

the camera. Each sample is logged into the software via a barcode reader with its unique identifier.

3 Results

A total of 137 samples were collected using our system over the course of 10 weeks. Of these, 20 were discarded due to incorrect light exposure by the operator, yielding a total of 117 usable acquisitions (85.4% yield). This occurred in the initial weeks prior to the decision to fix an exposure time. As illustrated in Table 1, a range of different tissue types, or topologies, were collected, whereby Uterus, Brain, and Lung were the top three tissue types collected. There is no apparent relation between the tissue topology and the quality of the acquisitions.

A t-SNE plot of the collected data shows that topology can be clustered in a non-linear embedding space with a reasonable level of separability, as shown in Figure 2, where we show this for the two different subsets of tissue subgroups. Example images acquired of different specimens can be seen in Figure 3, where we show the MM as well as the projection of stokes vectors on the MM to depict linear and circular responses captured by our system.

Table 1: Statistics of collected biopsy specimens from non-technical staff members in the first 3 weeks, using user-selected exposure time.

Topology	Collected samples	Acceptable samples
Uterus	11	5
Brain	9	4
Lung	5	5
Ductus deferens	4	0
Thyroid	3	2
Others (15 types)	29	23

4 Discussion and conclusion

We presented a self-contained, portable, production-ready MS-MMP solution for fresh tissue biopsy imaging, along with its successful deployment and seamless integration into a clinical environment. The simple user interface and fast acquisition time allow it to be operated by non-technical staff with minimum impact on current workflow. Over the course of 10 weeks, the system demonstrated robust performance and consistent outputs across various tissue types. Continuous feedback from operators helped improve usability, increasing buy-in and, ultimately, data throughput. Projection of the MMP data over the different samples using t-SNE show separability of tissue types in a non-linear embedding space, suggesting that tissue classification using machine learning approaches would yield some moderate-to-high performance capabilities.

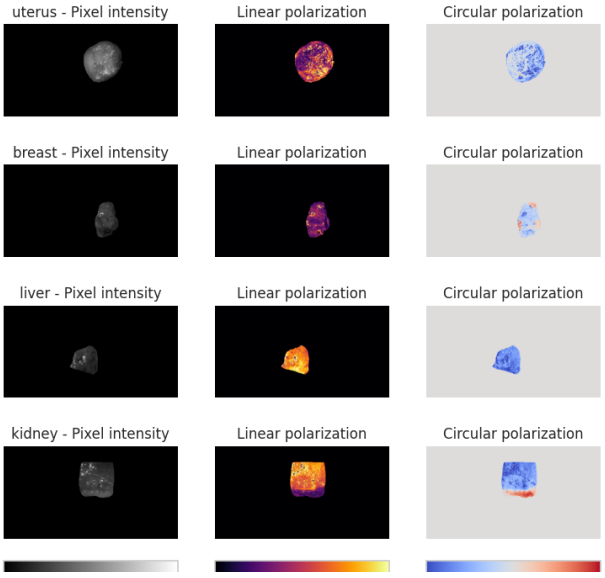

Figure 2: Examples of pixel intensities, linear and circular projections. The projections are the output stokes vector obtained after shining a purely linear and purely circular polarized light.

Figure 3: t-SNE from sampling 1000 pixels from the tissues collected in the first 3 weeks with at least two acceptable samples

Our solution is easily scalable to other clinical centers, enabling multi-center data collection campaigns. The volume and variability of such datasets data are essential to robust, generalizable machine learning applications. This is promising for future machine learning applications of MMP data and its high potential. We believe this work is an important step to explore the full potential of combining MS-MMP with machine learning, laying the groundwork for developing innovative data-driven medical imaging methods.

The work presented however has its limitations. In particular, the imager itself is a compromise in terms of wavelength captured and imaging resolution. As Figure 2 illustrates, macroscopic structures of tissue are indeed captured. It is very much possible that 140μm fails to capture critical information within samples. This comes to the benefit of having 16 different imaging wavelengths.

In the future, we plan to explore how the data collected from such a system can be correlated to traditional histology methodology (e.g., H&E slide histology) to match tissue type delineations at a finer resolution. This would allow for the analysis of specific tissue types at a scale that allows deeper scientific questions on tissue pathology to be investigated.

Author Statement

Research funding: This work was partially funded by the University of Bern, the Found'action contre le cancer and FreeNovation. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations and institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors the institutional review board or equivalent committee.

References

[1] S. Alali and A. Vitkin, “Polarized light imaging in biomedicine: emerging Mueller matrix methodologies for bulk tissue assessment.,” *J Biomed Opt*, vol. 20, no. 6, p. 61104, Jun. 2015, doi: 10.1117/1.JBO.20.6.061104.

[2] S. L. Jacques, J. C. Ramella-Roman, and K. Lee, “Imaging skin pathology with polarized light.,” *J Biomed Opt*, vol. 7, no. 3, pp. 329–340, Jul. 2002, doi: 10.1117/1.1484498.

[3] N. Ghosh and I. A. Vitkin, “Tissue polarimetry: concepts, challenges, applications, and outlook.,” *J Biomed Opt*, vol. 16, no. 11, p. 110801, Nov. 2011, doi: 10.1117/1.3652896.

[4] L. v Wang, G. L. Coté, and S. L. Jacques, “Special Section Guest Editorial: Tissue Polarimetry,” *J Biomed Opt*, vol. 7, no. 3, 2002.

[5] P. G. Ellingsen, L. M. S. Aas, V. S. Hagen, R. Kumar, M. B. Lilledahl, and M. Kildemo, “Mueller matrix three-dimensional directional imaging of collagen fibers.,” *J Biomed Opt*, vol. 19, no. 2, p. 26002, Feb. 2014, doi: 10.1117/1.JBO.19.2.026002.

[6] T. Novikova, A. Pierangelo, A. de Martino, A. Benali, and P. Validire, “Polarimetric Imaging for Cancer Diagnosis and Staging,” *Opt Photonics News*, vol. 23, no. 10, p. 26, Oct. 2012, doi: 10.1364/OPN.23.10.000026.

[7] J. Qi, T. Tatla, E. Nissanka-Jayasuriya, A. Y. Yuan, D. Stoyanov, and D. S. Elson, “Surgical polarimetric endoscopy for the detection of laryngeal cancer,” *Nat Biomed Eng*, 2023, doi: 10.1038/S41551-023-01018-0.

[8] A. Pierangelo, A. Benali, M.-R. Antonelli, T. Novikova, P. Validire, B. Gayet, and A. de Martino, “Ex-vivo characterization of human colon cancer by Mueller polarimetric imaging,” *Opt Express*, vol. 19, no. 2, p. 1582, Jan. 2011, doi: 10.1364/OE.19.001582.

[9] A. Pierangelo, S. Manhas, A. Benali, C. Fallet, J.-L. Totobenazara, M.-R. Antonelli, T. Novikova, B. Gayet, A. de Martino, and P. Validire, “Multispectral Mueller polarimetric imaging detecting residual cancer and cancer regression after neoadjuvant treatment for colorectal carcinomas.,” *J Biomed Opt*, vol. 18, no. 4, p. 46014, Apr. 2013, doi: 10.1117/1.JBO.18.4.046014.

[10] P. Sampaio, M. Lopez, F. Storni, J. Wicht, G. Sokeland, M. Wartenberg, P. Marquez Neila, D. Candinas, A. Perren, B. Demory, and R. Sznitman, “Müller matrix polarimetry for pancreatic tissue characterization,” *Nature Scientific Reports*, Sep. 2023.

[11] A. Pierangelo, A. Nazac, A. Benali, P. Validire, H. Cohen, T. Novikova, B. H. Ibrahim, S. Manhas, C. Fallet, M.-R. Antonelli, and A.-D. Martino, “Polarimetric imaging of uterine cervix: a case study,” *Opt Express*, vol. 21, no. 12, p. 14120, Jun. 2013, doi: 10.1364/OE.21.014120.

[12] P. Shukla and A. Pradhan, “Mueller decomposition images for cervical tissue: Potential for discriminating normal and dysplastic states,” *Opt Express*, vol. 17, p. 1600, Jan. 2009.

[13] J. Rehbinder, H. Haddad, S. Deby, B. Teig, A. Nazac, T. Novikova, A. Pierangelo, and F. Moreau, “Ex vivo Mueller polarimetric imaging of the uterine cervix: a first statistical evaluation.,” *J Biomed Opt*, vol. 21, no. 7, p. 71113, Apr. 2016, doi: 10.1117/1.JBO.21.7.071113.

[14] J. Vizet, J. Rehbinder, S. Deby, S. Roussel, A. Nazac, R. Soufan, C. Genestie, C. Haie-Meder, H. Fernandez, F. Moreau, and A. Pierangelo, “In vivo imaging of uterine cervix with a Mueller polarimetric colposcope.,” *Sci Rep*, vol. 7, no. 1, p. 2471, Dec. 2017, doi: 10.1038/s41598-017-02645-9.

[15] T. Pissas, P. Márquez-Neila, S. Wolf, M. Zinkernagel, and R. Sznitman, “Masked Image Modelling for Retinal OCT Understanding,” in *Ophthalmic Medical Image Analysis. OMIA 2024*, Springer, Oct. 2024, pp. 115–125. doi: 10.1007/978-3-031-73119-8_12.