

Evaluating the utility of axial TOF MALDI Imaging–based HiPLEX IHC in CNS tumor analysis

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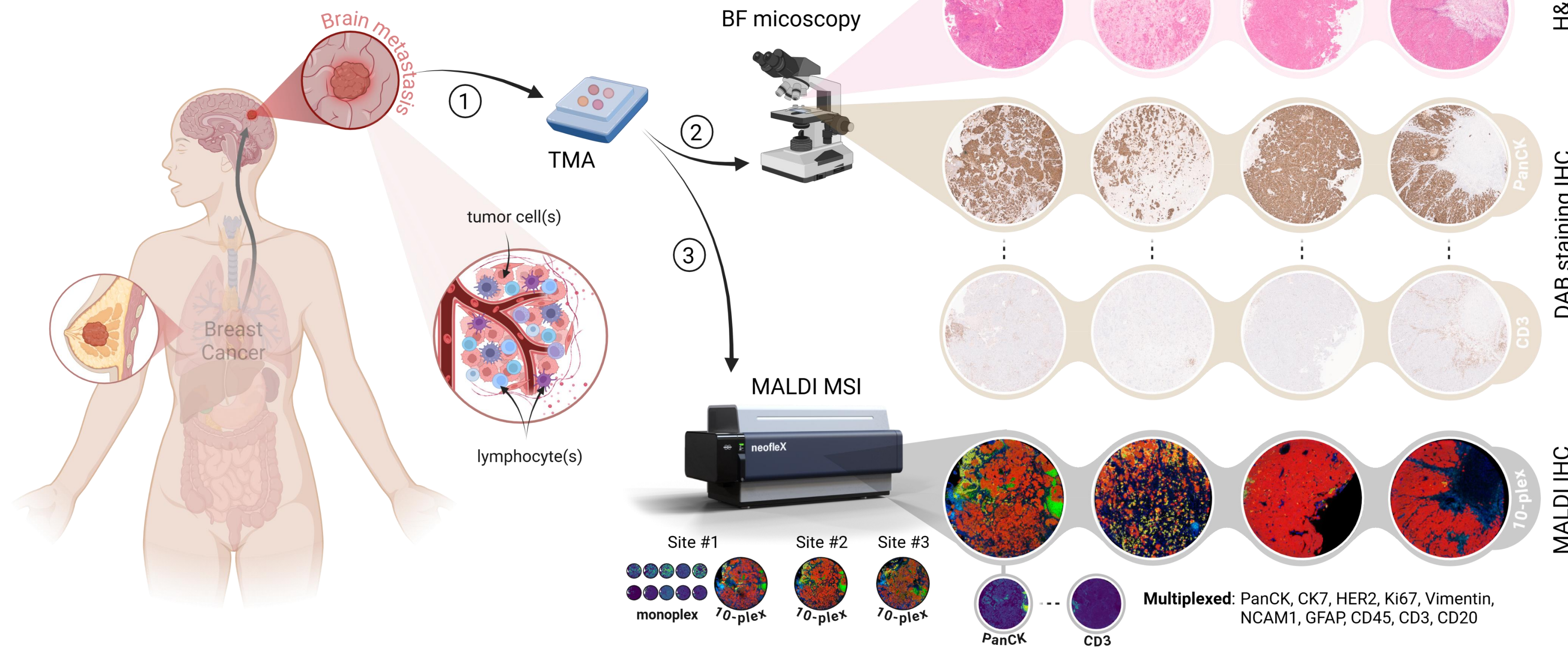
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Motivation & Methods

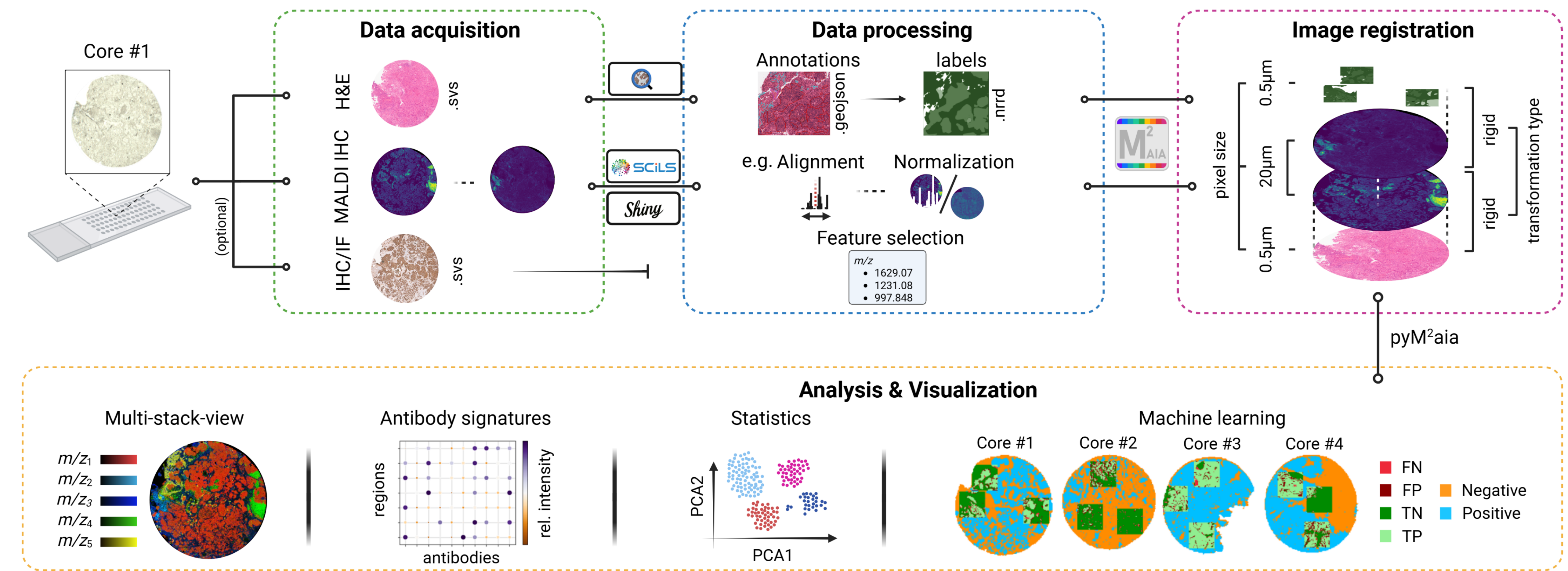
Multi-site study of MALDI IHC in brain metastasis of breast cancer in the context of **Cancer of Unknown Primary**.



[1] Pouygiourou, M., et al. "Rethinking cancer of unknown primary: from diagnostic challenge to targeted treatment." *Nature Reviews Clinical Oncology* 22.10 (2025): 781-799.
[2] Müller, Elisabeth, et al. "Exploring the A β plaque microenvironment in Alzheimer's disease model mice by multimodal lipid-protein-histology imaging on a benchtop mass spectrometer." *Pharmaceuticals* 18.2 (2025): 252.

Methods & Data Analysis

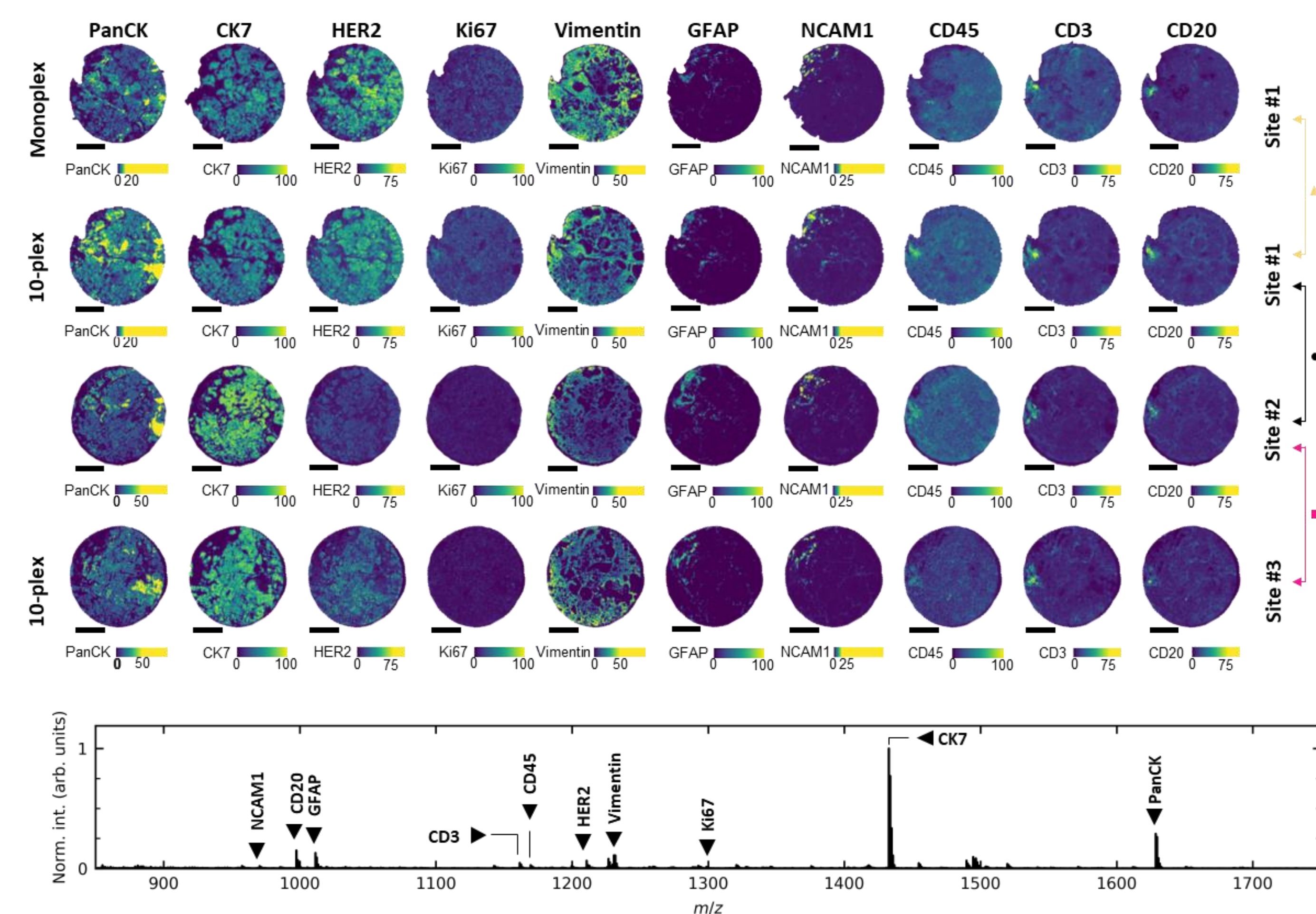
Workflow for correlative analysis of multiplex MALDI-IHC data using the computational framework of M²aia.



[2] Cordes, J., et al. "M2aia—Interactive, fast, and memory-efficient analysis of 2D and 3D multi-modal mass spectrometry imaging data." *GigaScience* 10.7 (2021): giab049.
[3] Cordes, J., et al. "pyM2aia: Python interface for mass spectrometry imaging with focus on deep learning." *Bioinformatics* 40.3 (2024): btac133.

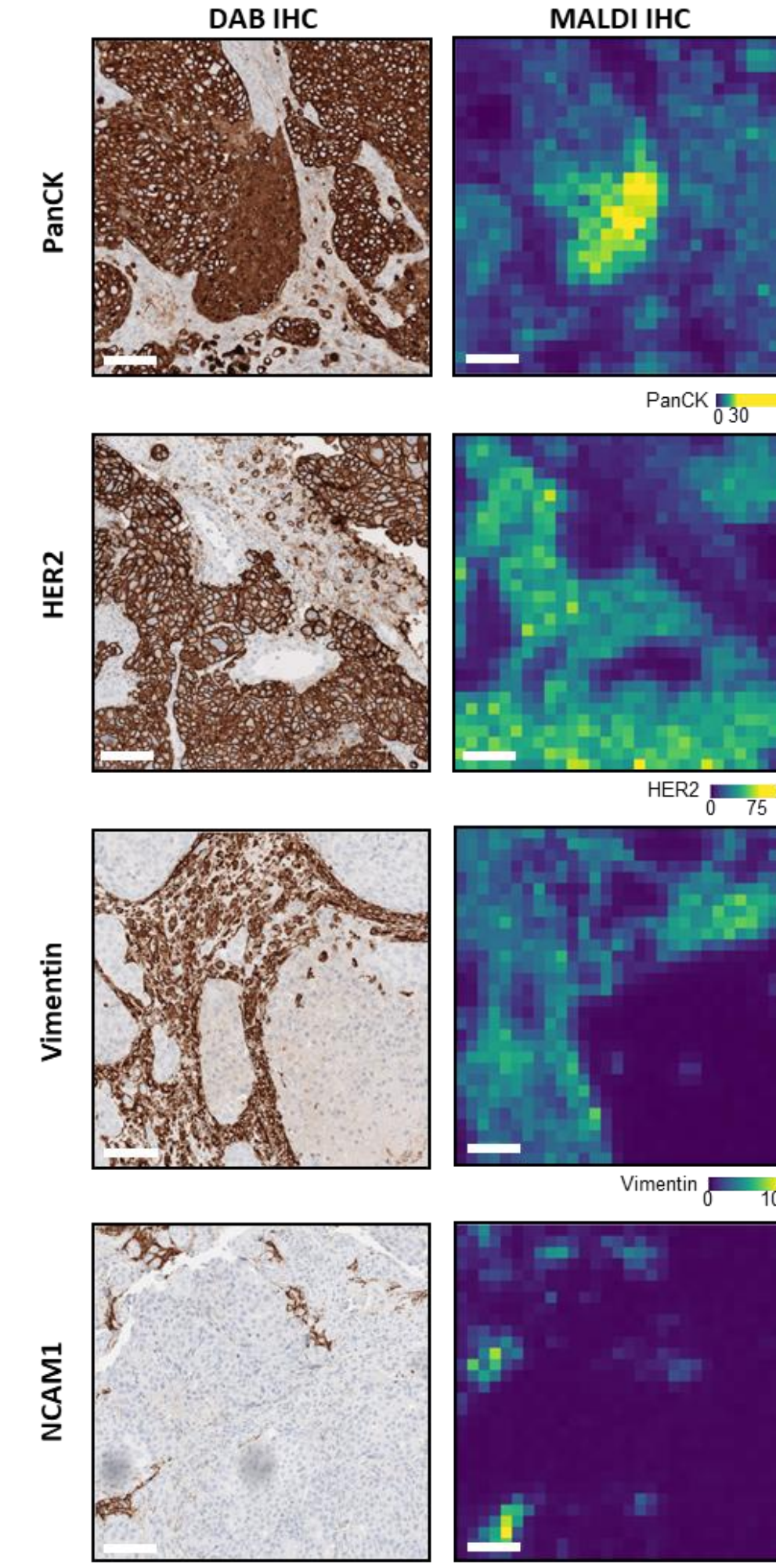
Results

Multi-site comparison: good site-to-site reproducibility



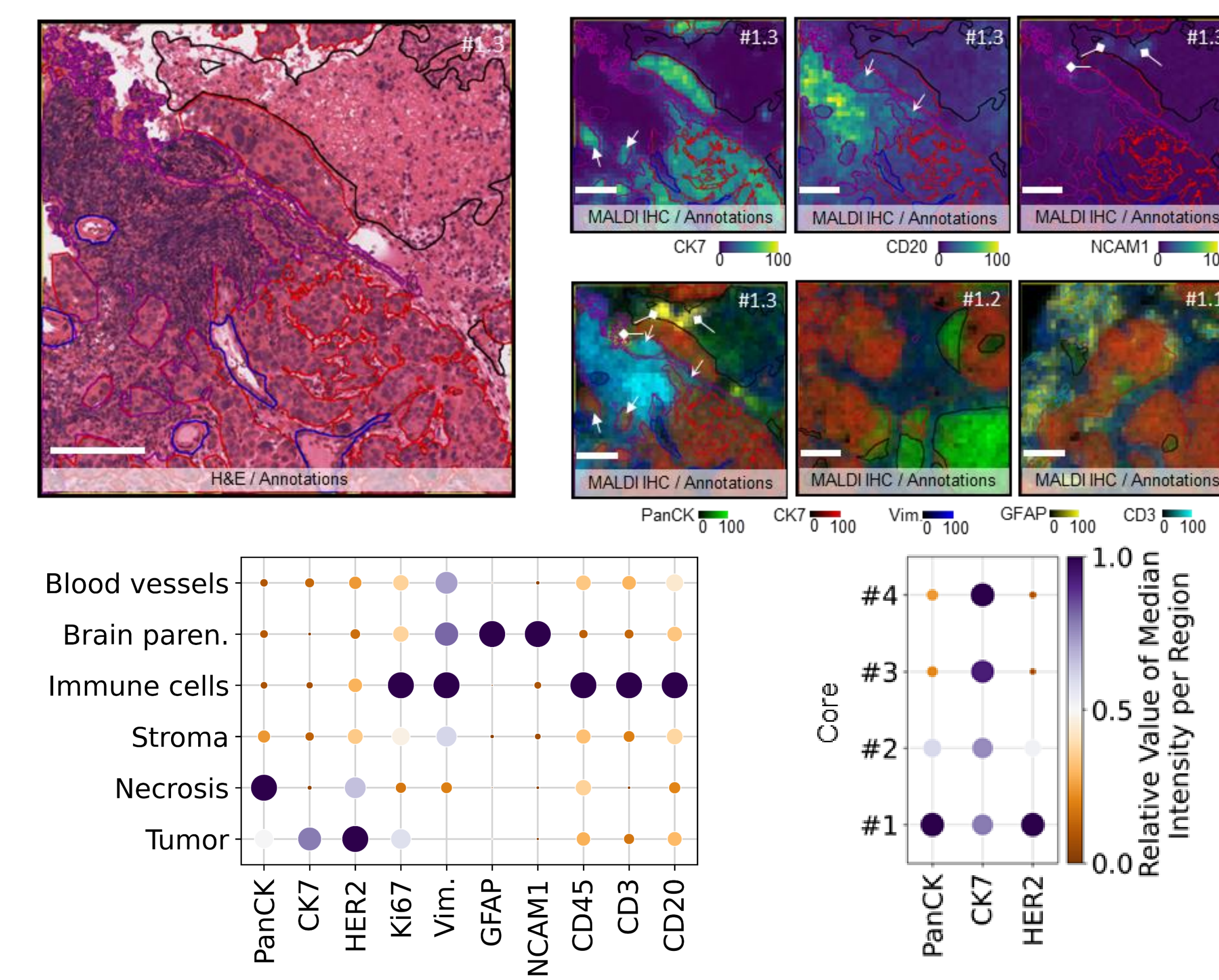
Upper part: Comparison of MALDI-MSI data for monoplex and multiplex (10-plex antibody staining) measured at different sites.
Lower part: Average mass spectrum (10-plex data, site #2) highlighting the spectral position of the mass tags for the various antibodies. Clear separated peaks across the entire mass range of the multiplex-imaging data set were obtained.

DAB IHC & MALDI IHC



MALDI-IHC antibody distributions throughout tissue agree with conventional DAB-IHC.

Tissue type-specific antibody signatures



Upper part: H&E-based Annotations: tumor (red), necrosis (black), immune cells (purple), brain parenchyma (cyan), blood vessels (blue) and tumor stroma (yellow) on an H&E-stained image. Lower part: Antibody signatures for the annotated regions expressed as the relative value of the median intensity per region (global scaling). Localization and intensity of antibody binding match with the expectations for the different tissue types.

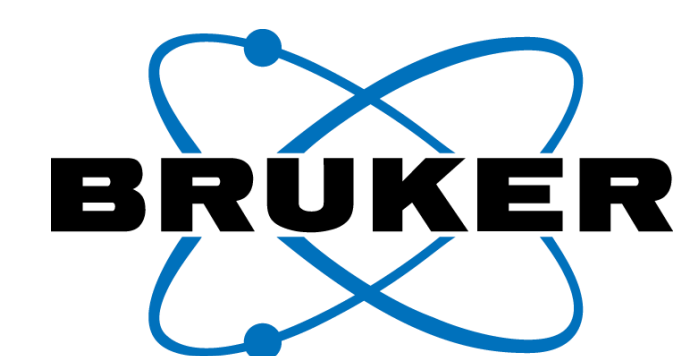
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Conflict of interest statement

J.O., N.T. and Co.He. are employees of Bruker Corporation. Bruker manufactures and sells analytical instrumentation including mass spectrometers and software used in this study. M.L. and G.Y. are employees of AmberGen Inc. The Miralys™ photocleavable mass tag imaging probes plus their high-plex, multiomic, and multimodal oriented workflows are patented by AmberGen Inc.



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