SCiLS™ Scope: a new viewing experience for spatial omics via a sharable platform

Abstract

SCiLS™ Scope is a software viewer for analyzing MALDI HiPLEX-IHC data for targeted proteins within a pathological context. With the easy-to-use interface it is ideal for sharing data with collaborators who may be unfamiliar with the MALDI Imaging domain and want to focus on examining spatial distribution images.
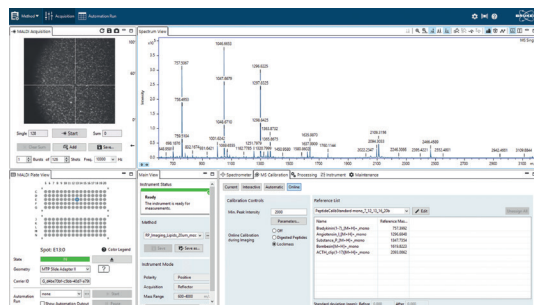
Introduction

MALDI Imaging is well established for spatial analysis of complex biological systems, such as tissue, with accessible molecular classes that range from metabolites, lipids, glycans, and more recently, to targeted proteins.
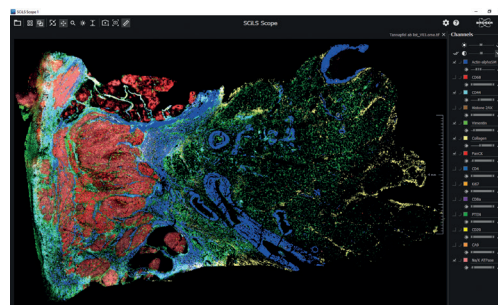
The MALDI HiPLEX-IHC workflow, which uses technology developed by AmberGen [1], allows for high resolution spatial analysis of targeted proteins within cellular architectures in a multiplexed way. Expanding MALDI Imaging techniques to targeted proteins has the potential to impact disease-based research in proteomics, tissue pathology, tissue diagnostics, therapeutics, precision medicine, and more.

For optimal support of collaborative researchers a simple method of data acquisition and visualizing is required. Here, the SCiLS™ autopilot workflow for creating OME-TIFF files and the subsequent visualization in SCiLS Scope software are described. The example shown here is specifically for MALDI HiPLEX-IHC, but the utility of SCiLS Scope extends to any targeted workflow with a list of ions of interest.

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Data acquisition

Visualization in SCiLS Scope

Automatic steps:
- Processing
- Creation of OME-TIFF files

Figure 1

Workflow for acquisition of MALDI HiPLEX-IHC data on the neofleX instrument and visualization in SCiLS Scope.
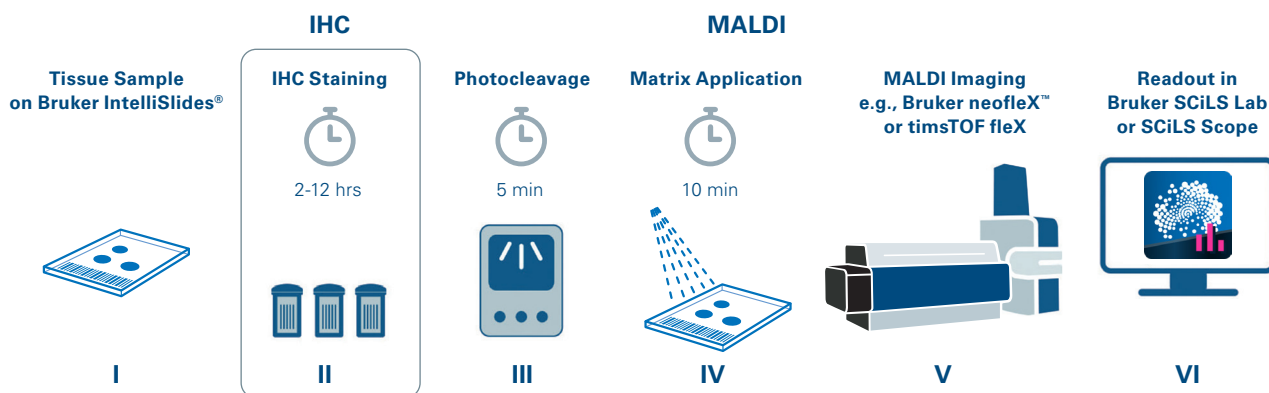

IHC

MALDI

Tissue Sample on Bruker IntelliSlides®

IHC Staining

2-12 hrs

Photocleavage

5 min

Matrix Application

10 min

MALDI Imaging e.g., Bruker neofleX™ or timsTOF fleX

Readout in Bruker SCiLS Lab or SCiLS Scope

I · II · III · IV · V · VI

Figure 2

Overview of the MALDI HiPLEX-IHC workflow.

Tissue samples undergo the following process: (**I**) section on IntelliSlides, (**II**) staining with photo-cleavable mass-tag antibodies from AmberGen (**III**) cleave mass-tags that are conjugated to the antibody, (**IV**) apply MALDI matrix, (**V**) analysis with the neofleX™ or timsTOF fleX, (**VI**) visualize data in SCiLS Scope software.

Experimental

End-to-end software automation from data acquisition to creation of image files is described in Figure 1, with detailed information following. For the MALDI HiPLEX-IHC workflow, all necessary file components are provided. Users who want to expand to other targeted workflows must manually edit the *.tlst file for target ions.

Sample preparation

Sample preparation on IntelliSlides® (required for SCiLS autopilot functionality) was performed according to standard protocols from AmberGen. The workflow is represented in Figure 2. Briefly, the workflow includes tissue preparation with antigen retrieval and staining with antibodies of choice with photocleavable mass-tags. Next, photocleavage in a UV-light box followed by matrix application. Mass spectrometry measurements were conducted on a neofleX axial TOF instrument.

Data acquisition

After sample preparation, data acquisition was done using flexControl and flexImaging on the neofleX. A general outline for acquisition follows: A default acquisition method is provided in the flexControl software. Main parameters are:

- Positive reflector mode
- m/z range 800–2200
- Online calibration activated
- Peak detection centroid

- For online calibration, the masses of the mass-tag are used.
- MALDI Imaging measurements are set up using SCiLS autopilot, a software feature performing all relevant performance checks prior to measurements which is integrated in flexImaging.
- During the setup of the measurement, a target list allows automatic processing and the creation of an OME-TIFF file with mass traces for each mass-tag.

Compatibility

Software

OME-TIFF files are created in an automatic manner by flexImaging 7.4 and SCiLS Lab 2024b. Data acquisition is performed by flexControl 5.0 or timsControl 5.1. In addition, detailed review of the raw data (*.mis) and a manual OME-TIFF export is also possible via SCiLS Lab 2024b.

Hardware

While the focus here is on neofleX and the MALDI HiPLEX-IHC workflow, note that SCiLS Scope is supported for other OME-TIFF files, e.g. from timsTOF fleX.

Visualization

SCiLS Scope offers essential functionality for quickly assessing acquired MALDI HiPLEX-IHC data. Each mass-tag released from an antibody is represented by a channel that can be visualized individually or in combination. In the illustrated example (see Figure 3), 14 channels shown in the selection window on the right side of the Graphical User Interface (GUI) have been analyzed within the context of a colorectal cancer FFPE section, and 6 of these channels are visually displayed.

Additional software features are shown in Figures 4 and 5 and are described below. For a more detailed investigation, the channels for the mass-tags, which are automatically displayed in SCiLS Scope upon the automatic OME-TIFF export, can be viewed separately (Figure 4A).
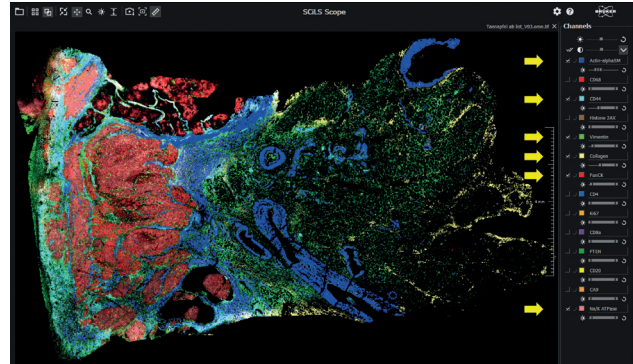

Figure 3
SCiLS Scope Graphical User Interface after opening an OME-TIFF file from a colorectal cancer tissue.
The displayed channels are highlighted by yellow arrows: Actin-alpha SM, CD44, Vimentin, Collagen, PanCK, Na/K ATPase (sample courtesy of A. Tannapfel and J. Christmann, Institute of Pathology, University of Bochum, Germany).
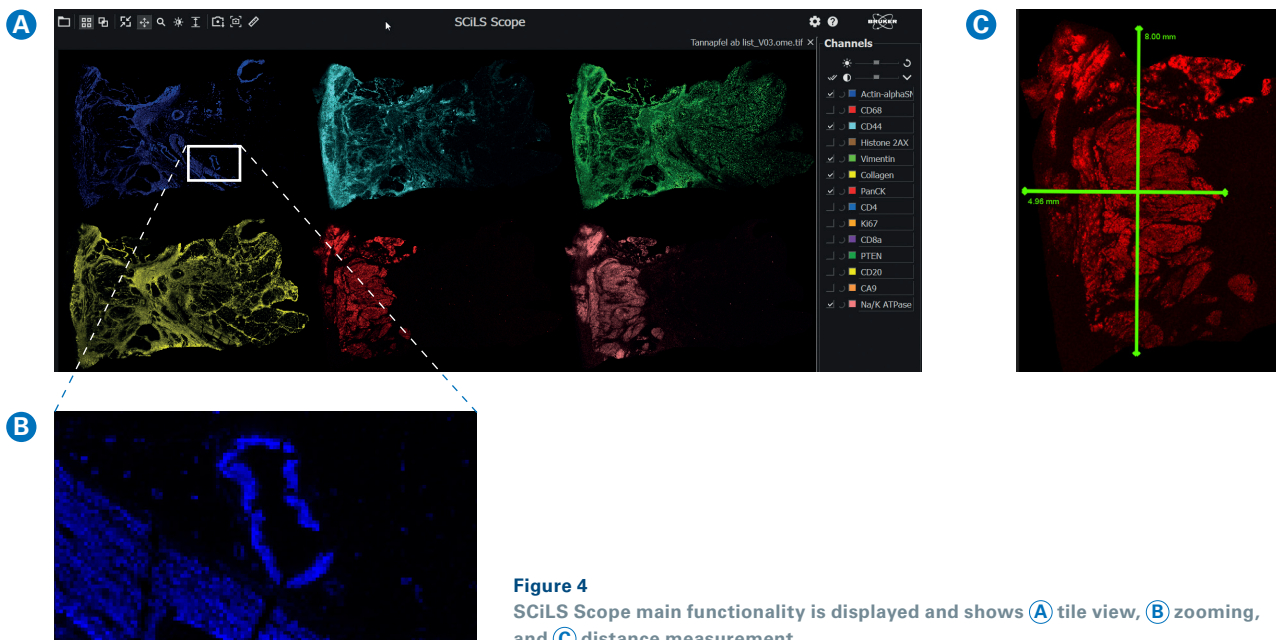

Figure 4
SCiLS Scope main functionality is displayed and shows (A) tile view, (B) zooming, and (C) distance measurement.

- Stepless zooming enables detailed exploration, as demonstrated for α-Smooth Muscle Actin and Collagen-1A1 (Figure 4B).

- Distances can be easily measured. As shown in Figure 4C, PanCK is visualized and the overall area shown is 5.8 mm x 8.0 mm. This feature is unique to SCiLS Scope and is not present in SCiLS Lab.

- Screenshots can be exported by copying to the clipboard or saving as PNG or TIFF files.

- Brightness and contrast adjustments can be applied globally to all channels, or individually for each channel to accommodate for different signal and noise levels in the image (see Figure 5 as compared to Figure 3 which has no adjustments).
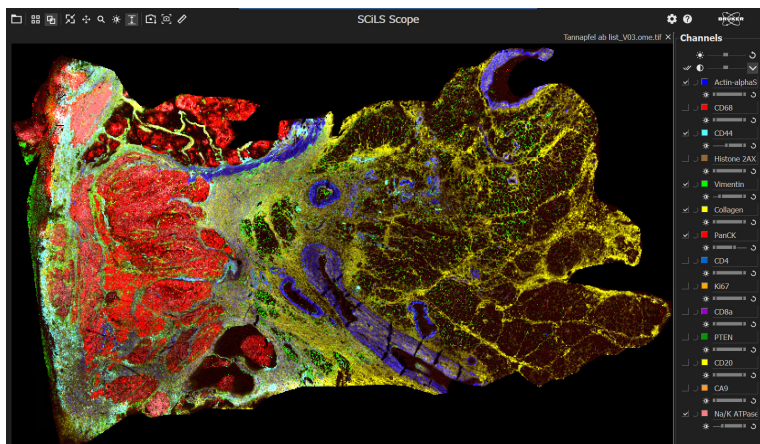

Figure 5
SCiLS Scope main functionality showing brightness and contrast features.

Summary

SCiLS Scope enables easy image overview, including basic image adjustment and analysis, that can be used to exchange with collaborative researchers. The ability to easily visualize features from a targeted mass list enables easy entry to MALDI Imaging.

References

[1] Yagnik L, Rothschild L, (2021). *Highly Multiplexed Immunohistochemical MALDI-MS Imaging of Biomarkers in Tissue.* Anal. Chem. **32**,977-988, doi: 10.1021/jasms.0c00473

[2] Bruker Application note MSI-23 SCiLS autopilot

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