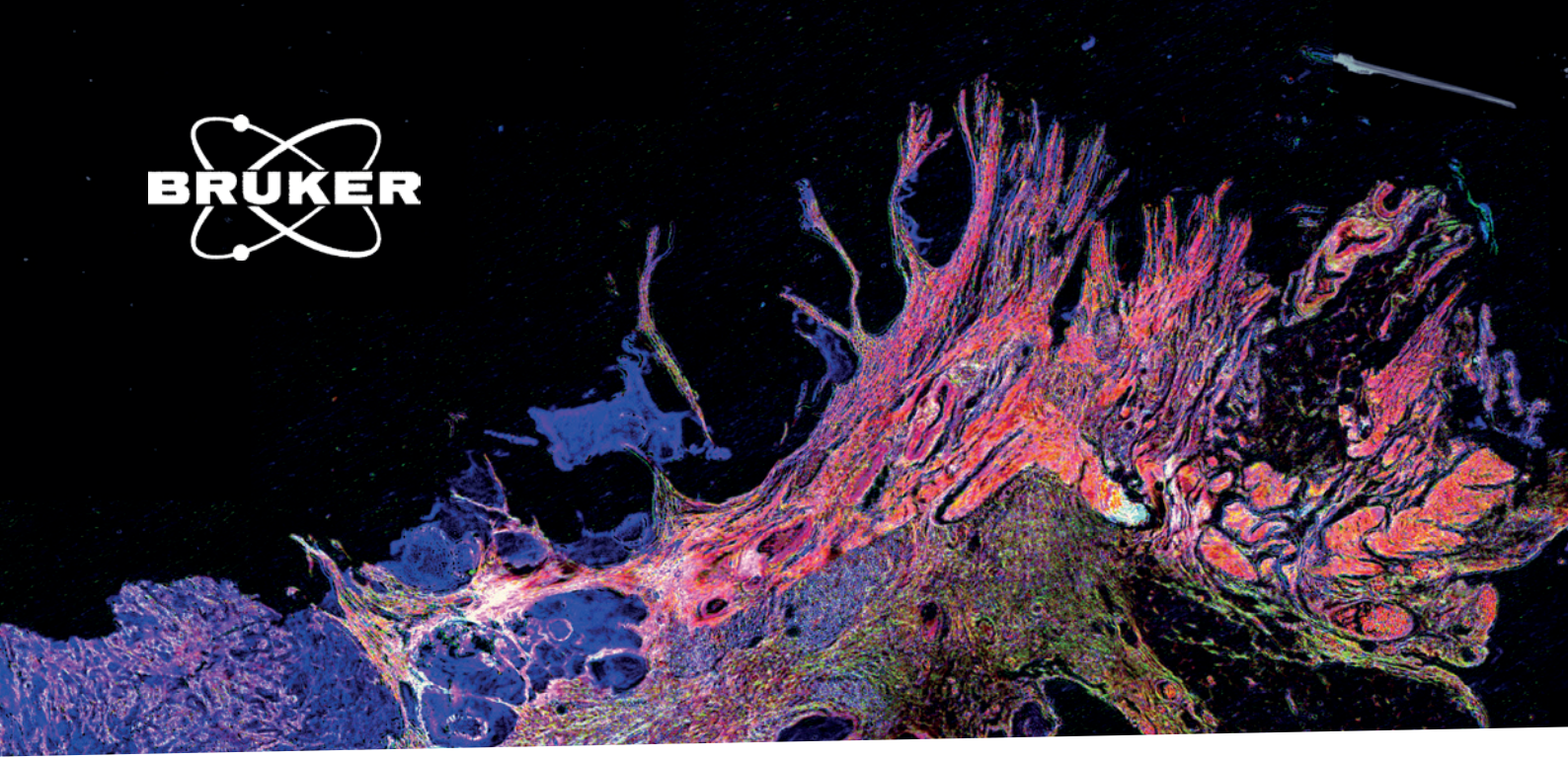


IR LASER IMAGING MICROSCOPES

The TissuePlus™ Workflow

Making Whole Slide IR Imaging Faster and Simpler

IR Laser Imaging for Tissue Analysis

Infrared Laser Imaging (ILIM) enables label-free spatial and molecular profiling of biological tissue sections within minutes. The high sample throughput allows to generate comprehensive training repositories for clinically relevant artificial intelligence (AI) models in biomedical research and spectral pathology. As a non-destructive analytical technique, ILIM also seamlessly integrates into multimodal workflows – such as IR Guided MALDI Imaging – enhancing bioanalytical depth and expanding the scope of molecular investigations.

To enhance productivity and robustness, especially for non-spectroscopists, we introduce TissuePlus™ as an end-to-end workflow dedicated to whole slide IR imaging.

This innovative software solution adresses the **unmet need** for a streamlined software solution and automates data acquisition across multiple tissue sections per slide, converting raw data into meaningful insights using integrated onboad analysis. TissuePlus™ maximizes sample throughput while minimizing the required user input and ensures reproducible high-quality data within and between large-scale studies.

System, Setup, and Methodology

The TissuePlus™ workflow is designed for IR Laser Imaging Microscopes. For this study, we utilized the following setup:

- **Tissue Sections**
  Formalin-fixed and paraffin embedded (FFPE) colon tissue sections (10 µm thickness) mounted onto polyethylene terephthalate (PET) frameslides were kindly provided by Prof. Dr. Klaus Gerwert and Dr. Frederik Großerüschkamp from Bochum University (ProDi).

- **Mesaurement Setup**
  The LUMOS II ILIM in transmission mode and the software package TissuePlus™ were used to perform automated whole slide IR imaging. The laser wavelength was tuned with a spectral step size of 4 cm^{-1} across the MIR fingerprint region (950 to 1800 cm^{-1}).

- **Data Analysis Tool**
  With activated RGB Creator and Adaptive Chemical Imaging (ACI) plugins, TissuePlus™ automatically processed raw data into RGB integration overlays and k-means clustering maps upon acquisition completion.

Creating Work Items

The TissuePlus™ main menu (Figure 1A) allows users to define experimental parameters and select plug-ins to start generating work items. These are created by assigning prepared workflows to sample IDs (Figure 1B). The work items appear under 'Done' or 'Planned' tabs based on status, where they can be accessed, loaded, or restarted anytime (Figure 1C).

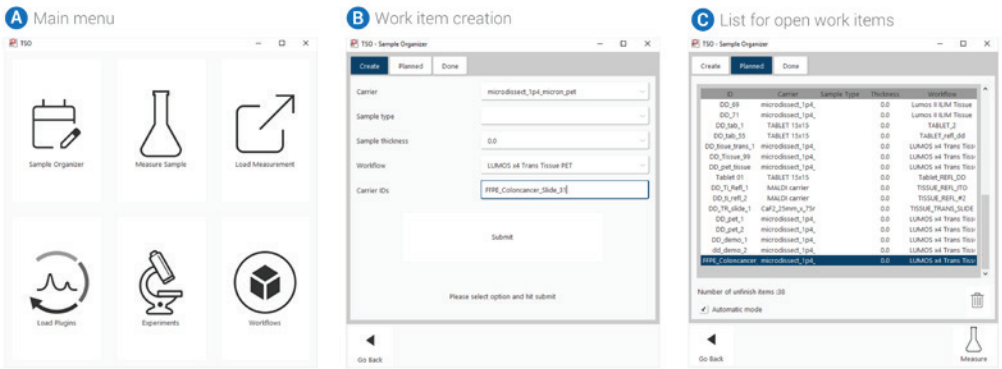


Fig. 1
Creating work items in TissuePlus™.

Imaging the Complete Slide

After starting a work item, the reference position for the background measurement (Figure 2A, RP: clean off-sample area of the sample substrate) and at least three focusing points (Figure 2A, FP) along the slide have to be defined using the live IR view (Figure 2B). The FOV of the IR live view is 2.2 x 2.0 mm, and the tuning wavenumber can be adjusted within the range of 950 to 1800 cm^{-1}. For optimal contrast between the tissue sample and substrate, the wavenumber 1650 cm^{-1} was selected.

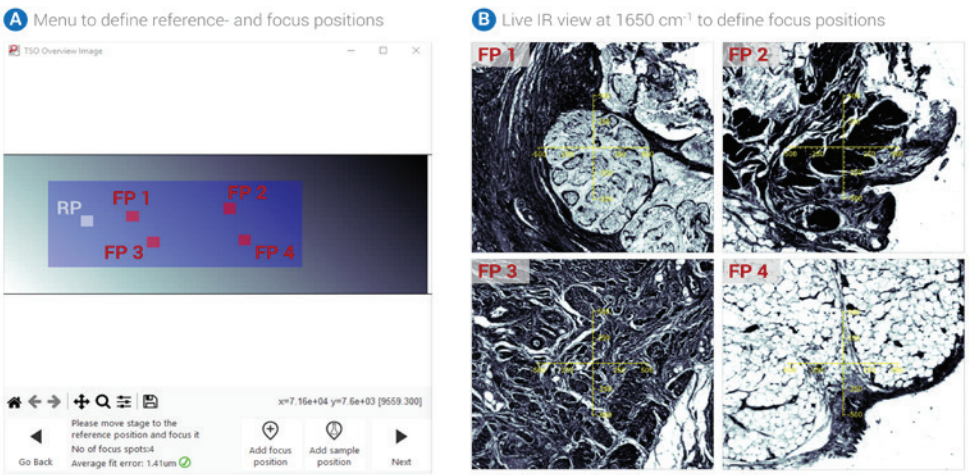


Fig. 2
Overview image acquisition for the whole sample substrate.

Based on the defined focusing positions, an optimized plane along the z-axis is calculated to compensate for height variations caused by intrinsic sample tilts.

This feature ensures consistent laser focus and fluence across the whole slide. Based on the selected substrate in the workflow configuration, an overview image at 1650 cm^{-1} is automatically acquired for the substrate's whole PET-area (Figure 2A, blue).

Next, the SampleFinder plugin automatically detects the boundaries of the tissue sections, sets-up the sampling grids, and starts the hyperspectral acquisition automatically (Figure 3A). For the colon cancer tissue section shown here, 58 measurement tiles (each 2.2 x 2.0 mm) entailing 14.5 million full fingerprint IR spectra were acquired in just seven minutes (Figure 3B).

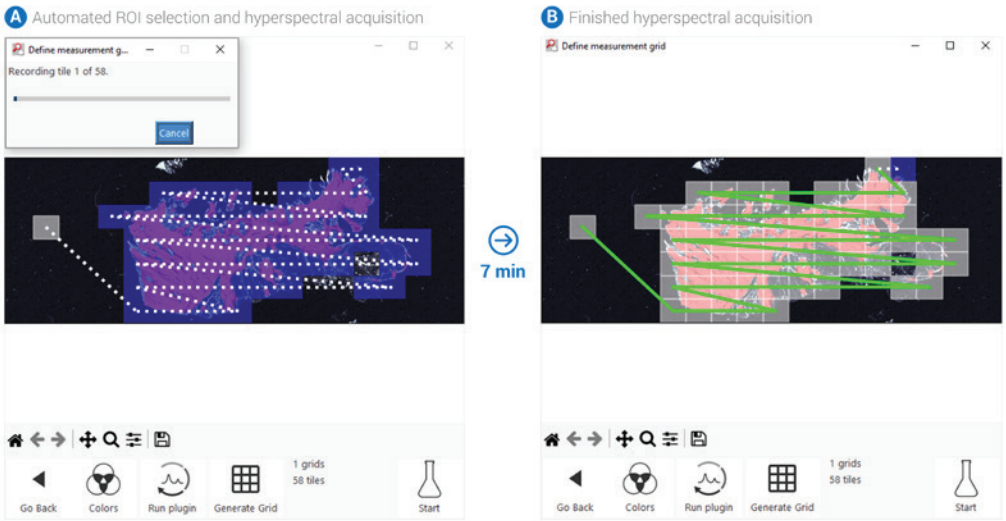


Fig. 3
Automated hyperspectral acquisition for the tissue section.

Onboard Data Analysis Tools for Immediate Insights

Using TissuePlus™, raw data is immediately converted into meaningful insights through integrated data analysis plugins. Here, RGB Creator and ACI were automatically employed upon finished acquisition. All plugins come with user interfaces (UI's) for intuitive and straightforward configuration (Figure 4).

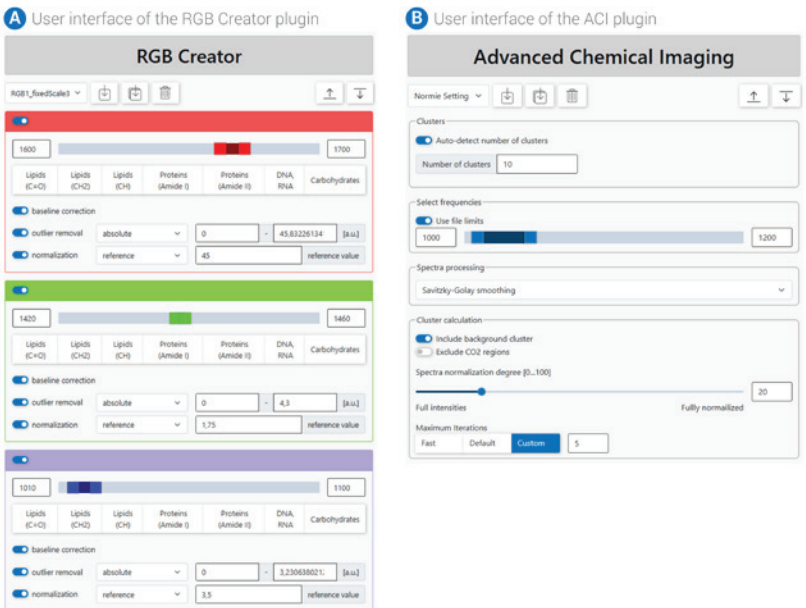


Fig. 4
GUI of the data analysis plugins RGB Creator and ACI.

ACI performs machine-learning driven k-means clustering to generate segmentation maps based on spectral similarity. Tissue regions and -types with similar chemical fingerprints are clustered together and assigned a specific pixel color (Figure 5A), with the average cluster spectra providing deeper analytical insights (Figure 5B).

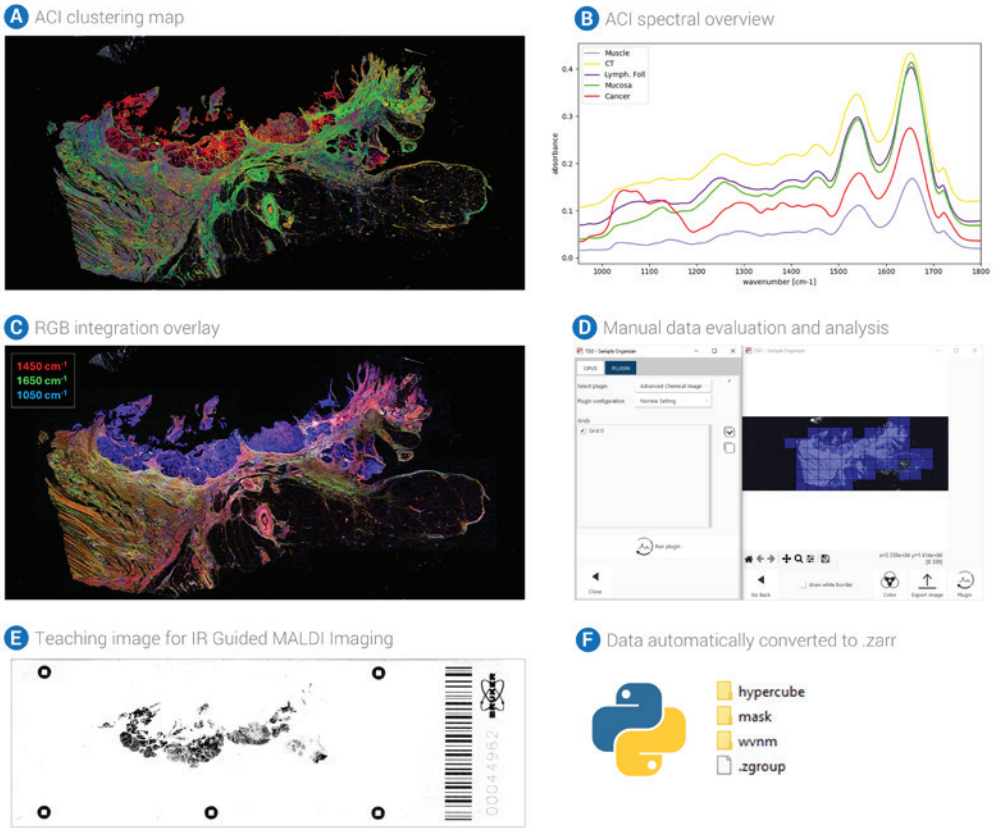


Fig. 5
Results overview for whole slide IR imaging of colon cancer tissue.

For the RGB overlay, three distinct IR bands are integrated, each assigned to a color channel and visualized in a heat map (Figure 5C). Both approaches revealed different tissue types and structures, including mucosa, muscle and cancer tissue.

For instance, the cluster related to cancer tissue showed increased abundance at 1050 cm^{-1} (C–O stretching related to glycosidic bonds), which indicates increased carbohydrate accumulation due to aerobic glycolysis. This observation was also determined by integrating the carbohydrate band at 1050 cm^{-1} (Figure 5C, blue color channel). Manual and/or further data analysis can always be performed on completed work items (Figure 5D).

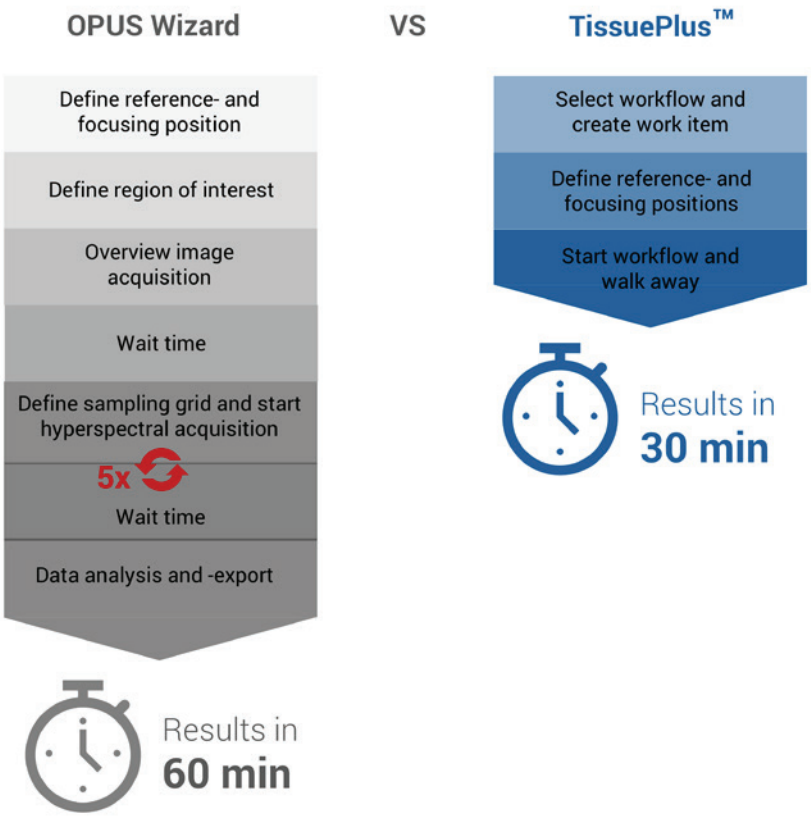
TissuePlus™ allows for the overlay of selected regions of interest (ROIs) or individual clusters onto the whole slide overview image for seamless interfacing with other imaging modalities. Here, the cluster associated with cancer tissue was translated into a measurement region for IR Guied MALDI Imaging using the acquired Teaching Image (Figure 5E).

To ensure smooth operation with in-house data analysis pipelines, raw data is automatically organized and stored in the open-source .zarr format for quick and straightforward import. A well-documented Python API facilitates effortless conversion of custom algorithms into plugins, enhancing TissuePlus™ as a highly flexible tool for whole slide IR imaging.

Conclusion

TissuePlus™ values time and maximizes productivity for whole slide IR imaging by delivering:

- **Routine ready data acquisition** characterized by high automation and minimal user input
- **Meaningful insights** provided by onboard data analysis tools
- **Maximized sample throughput** and reproducible high-quality data within and between projects across different operators – including non-IR experts.
- **Smooth data operation** due to open-source .zarr format and a Python API for customized plugin solutions.



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