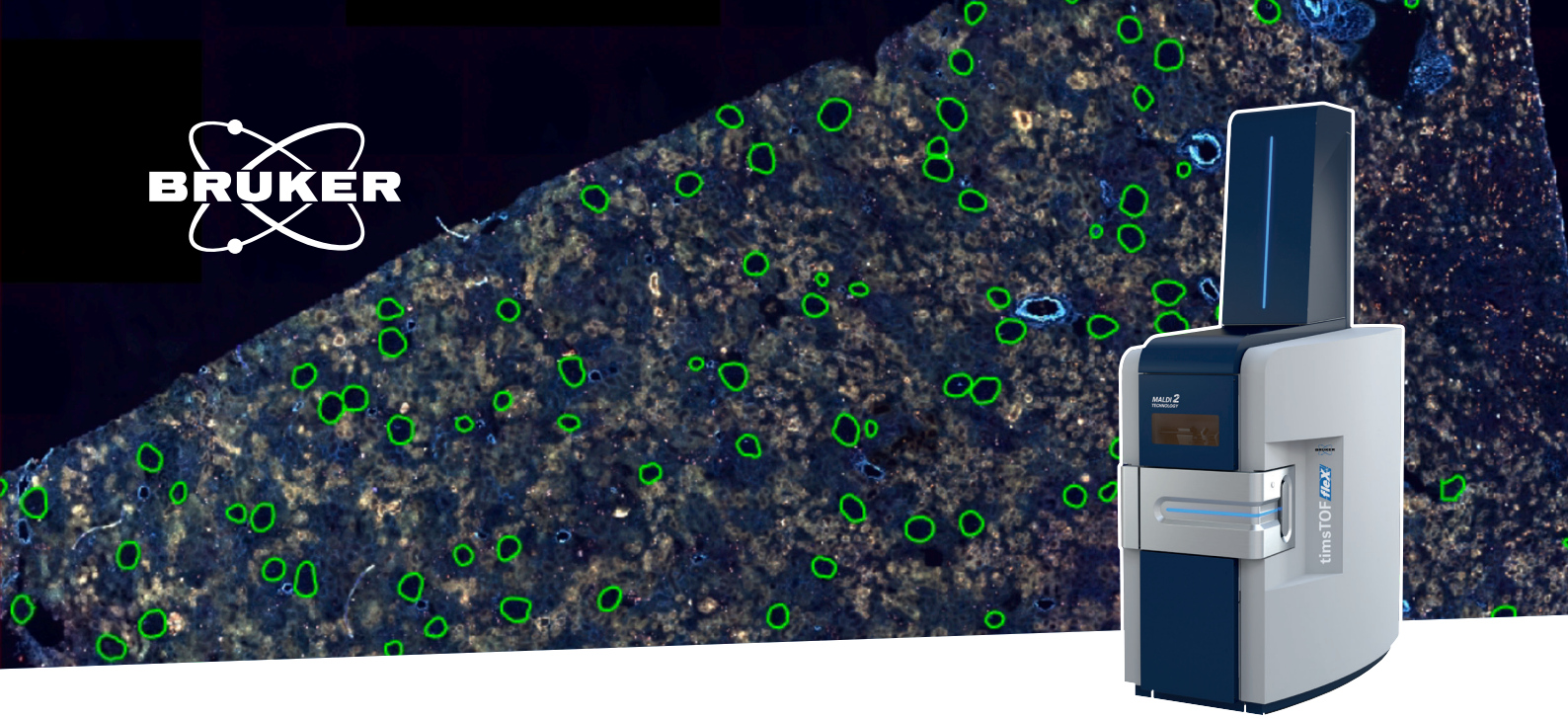

QuPath Integration for Targeted MALDI Imaging: A Streamlined Workflow for Microscopy-Driven Data Collection

Targeted MALDI Imaging experiments driven by microscopy annotations significantly improve throughput and reduce acquisition time by focusing data collection on specific regions of interest (ROIs).

Abstract

The integration of QuPath, an open-source digital pathology software [1], with Bruker's flexImaging provides a streamlined workflow for defining measurement regions based on histological features. This Application Note demonstrates how to leverage QuPath's annotation capabilities to create targeted MALDI Imaging experiments using the GeoJSON file format, eliminating the need for custom scripts or manual ROI drawing in instrument software.

Keywords:
MALDI Imaging, QuPath, timsTOF fleX, Targeted Sampling, Multimodal Imaging, ROI Selection

Introduction

High spatial resolution MALDI Imaging experiments often face challenges related to extended acquisition times, large file sizes, and increased experimental costs. Traditional approaches require researchers to manually define regions of interest within instrument software, a time-consuming and error-prone process for large-scale studies.

QuPath is an open-source tool designed for digital pathology and bioimage analysis that offers sophisticated annotation capabilities. The latest version of flexImaging (v7.5) includes native support for importing GeoJSON annotation files, enabling seamless integration between QuPath annotations and MALDI Imaging data acquisition on the timsTOF fleX platform.

This workflow tackles key limitations in targeted MALDI Imaging by streamlining and accelerating the entire process. It eliminates the need for manual measurement region selection in the instrument software and enables automated segmentation of hundreds of features, saving valuable time and reducing user bias. Acquisition time is cut by up to 95% compared to traditional full-area scanning, while still supporting both manual annotations

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and advanced segmentation algorithms. The workflow also integrates seamlessly with histopathology, enabling powerful multimodal analysis for deeper biological insights.

Methods

Sample Preparation

- Tissue Mounting: Mount tissue sections on ITO-coated glass slides, e.g. IntelliSlides®
- Reference Imaging: Acquire autofluorescence or brightfield microscopy images before matrix application
- Matrix Application: Apply matrix using standard protocols
- Image Registration: Align microscopy images to sample carrier position ("teaching" process)

QuPath Annotation Workflow

Manual Annotation Method

- Image Import: Load microscopy images into QuPath
- ROI Definition: Use QuPath's annotation tools
  - Polygon tool for irregular shapes (e.g., vasculature)
  - Brush tool for complex regions
  - Wand tool for intensity-based selection
- Export: Save annotations as GeoJSON files

Automated Segmentation Method

- Cell Detection: Use QuPath's automated Cell Detection feature
- Custom Algorithms: Implement machine learning models for specific tissue structures
- Batch Processing: Process multiple samples automatically

MALDI Imaging Data Acquisition

- Import Measurement Regions*: Load GeoJSON files into flexImaging v7.5 using the "Import Regions" feature
- Method Setup: Configure acquisition parameters on timsTOF fleX
- Targeted Acquisition: Run MALDI Imaging experiment on QuPath annotated regions only
- Data Analysis: Process results using SCiLS™ Lab

* Regions can be imported as "Measurement" or "ROI". "ROI" in flexImaging refers to a subregion information for subsequent data analysis and does not implement the measurement of this subregion.

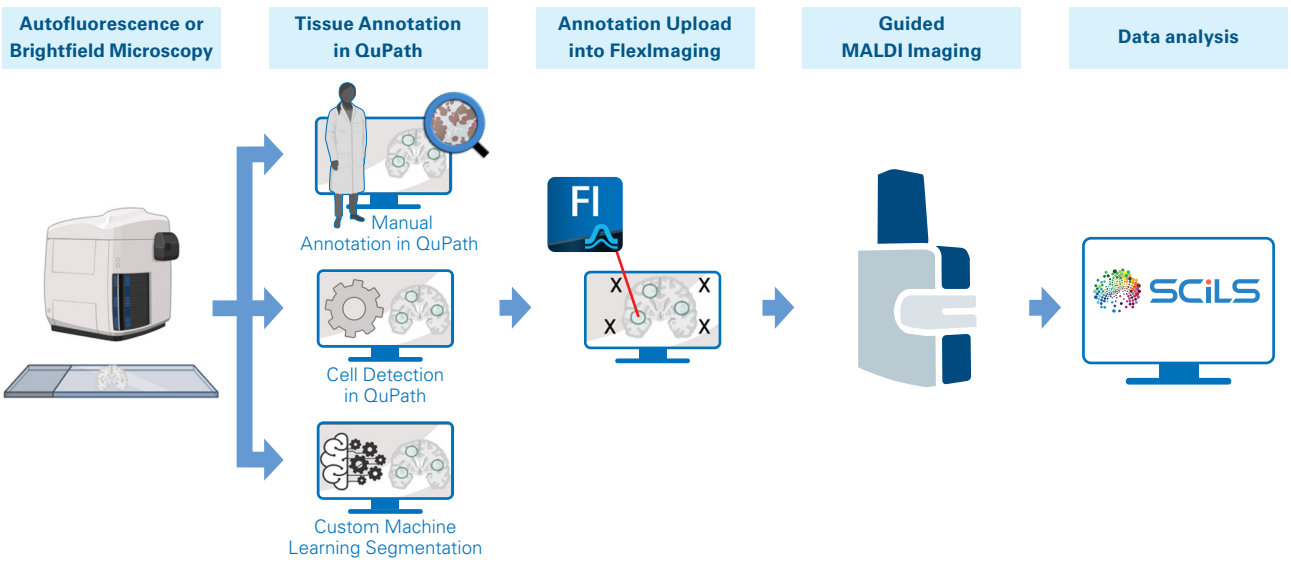

Figure 1. Workflow overview.
Autofluorescence/Brightfield microscopy provides reference images, which are annotated in QuPath in various ways. Annotations are exported to flexImaging to initiate microscopy guided MALDI Imaging. Final data analysis is performed in SCiLS Lab.

Results and Applications

Technical Advantages

Workflow Benefits

Universal Format:	GeoJSON standard ensures compatibility across software platforms
No Custom Scripting:	Direct import eliminates need for in-house development
Quality Control:	Post-acquisition microscopy validates targeting accuracy
Scalability:	Supports studies from single cells to whole-slide imaging

Performance Improvements

Time Reduction:	Up to 95% decrease in acquisition time
Cost Efficiency:	Significantly reduced instrument usage
Data Quality:	Higher signal-to-noise ratios in targeted regions
Throughput:	Enables large-scale multimodal studies

Software Integration

QuPath Integration:	Seamless export to GeoJSON format
flexImaging Compatibility:	Native region import in v7.5
SCiLS Lab Analysis:	Complete data processing and visualization
Multimodal Support:	Co-registration with immunofluorescence and histology

Case Study 1: Cerebral Vasculature Analysis

Application: Study of cerebral amyloid angiopathy (CAA) in Alzheimer's disease research

Workflow Implementation

- Manual annotation of 17 vasculature ROIs using QuPath polygon tool (Figure 2A)
- 95% reduction in acquisition pixels (73,181 vs. 1,711,697)
- Successful targeting validated by post-imaging microscopy

Key Findings

- Specific lipid distributions (e.g., PA 36:1) localized to vasculature regions (Figure 2B)
- High correlation between MALDI Imaging data and immunofluorescence validation
- Expert pathologist-guided ROI selection supports targeted neuropathology studies

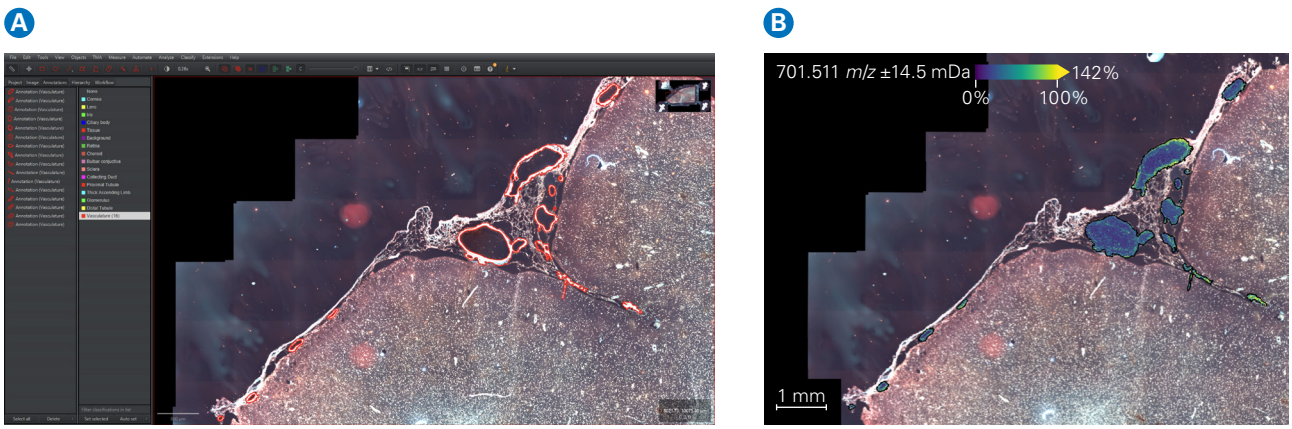

Figure 2. Vasculature in the frontal cortex of the human brain.
Ⓐ The autofluorescence image of the tissue section was used to manually annotate 17 vasculature ROIs in the tissue section using the polygon tool in QuPath. Ⓑ Ion image of PA (36:1) (*m/z* 701.512, -1.1 ppm) within the annotated vasculature regions overlayed onto the autofluorescence image demonstrates high heterogeneity.

Case Study 2: Renal Glomeruli Segmentation

Application: Kidney disease research requiring analysis of functional tissue units

Workflow Implementation

- Automated segmentation of 268 glomeruli using machine learning algorithms (Figure 3A)
- Custom models for detecting nephron components
- 89% reduction in total acquisition pixels

Key Findings

- Automated processing completed in minutes vs hours for manual annotation
- Successful identification of glomeruli-specific lipids (e.g., PS (36:1), SM(34:1;O2) and PE(O-38:5)) (Figure 3B)
- Scalable approach for whole-slide imaging studies

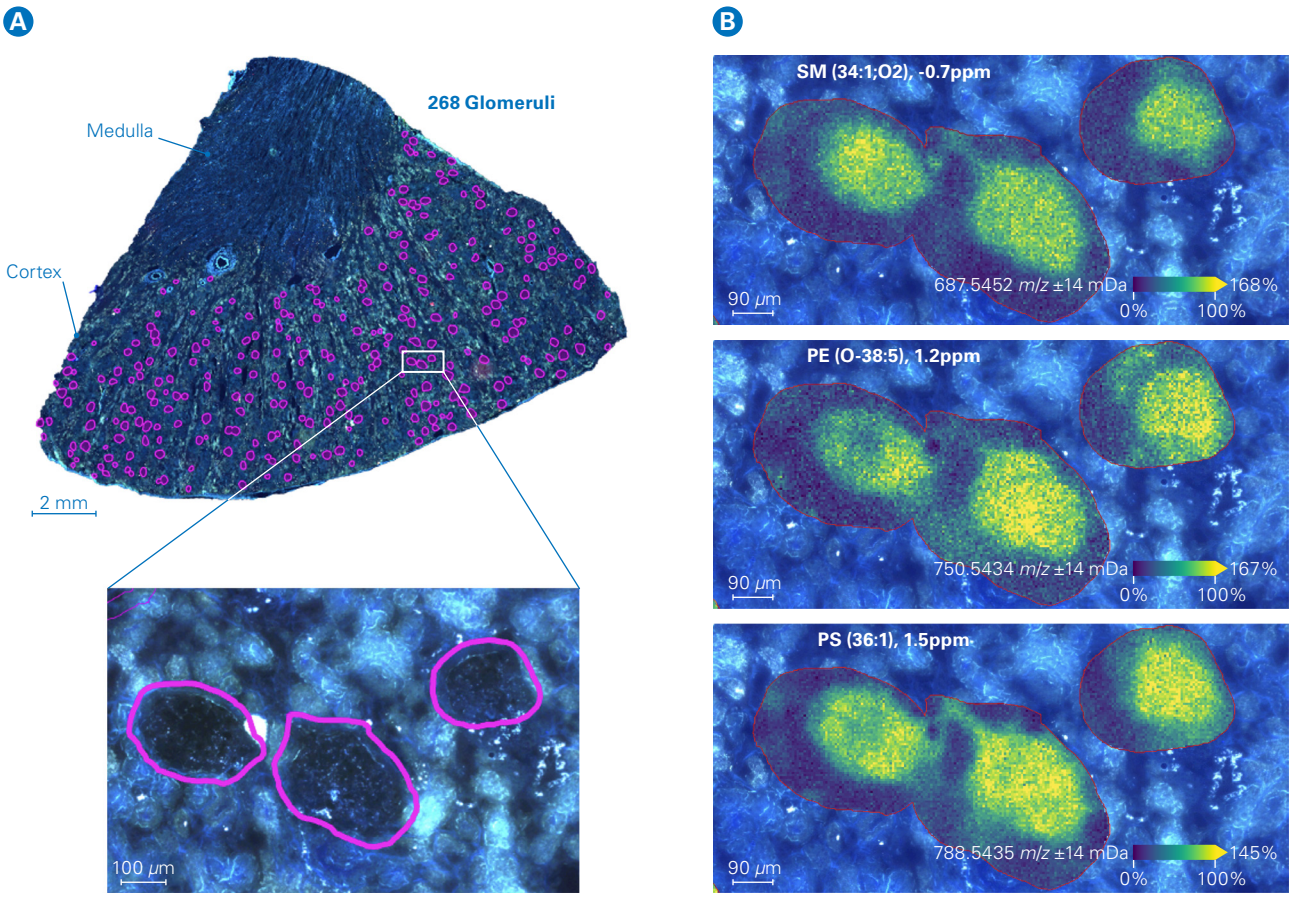

Figure 3. **Specific targeting of glomeruli in human kidney.**
Ⓐ The autofluorescence image of the tissue section was used with a custom segmentation model to annotate 268 glomeruli in the tissue section with QuPath. These annotations served as measurement regions for MALDI Imaging. Ⓑ Exemplary ion images of three different lipids colocalized with glomeruli from the MALDI Imaging experiment.

Case Study 3: Single Cell Analysis

Application: HeLa cell analysis for single-cell MALDI Imaging

Workflow Implementation

- QuPath Cell Detection tool identified ~1000 individual cells (Figure 4A)
- Automated circular ROI generation around each cell
- Dramatic reduction in non-cellular pixel sampling

Key Findings

- Only 2.1% of pixels contained cellular information in rectangular acquisition
- Reduced matrix-associated chemical noise
- Improved signal-to-noise for biologically relevant ions
- Experiment completed in 90 minutes for 999 cells

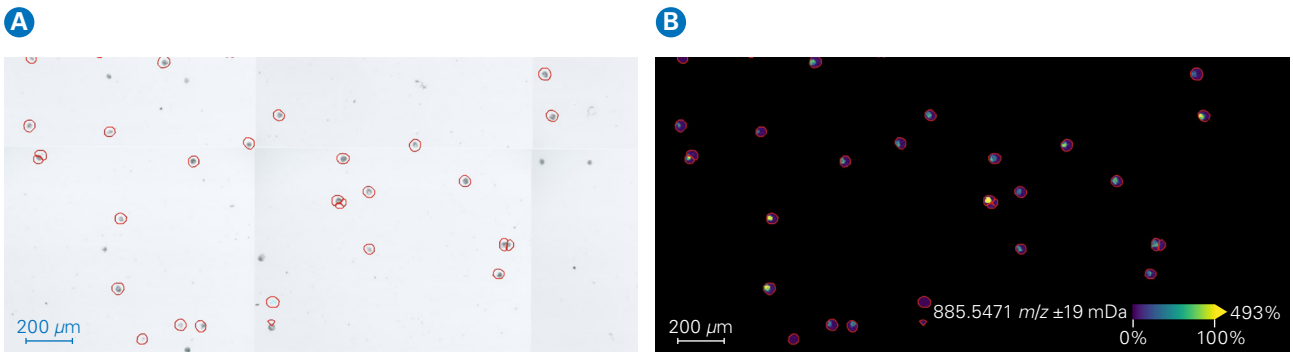

Figure 4. Targeted acquisition of HeLa cells.
Ⓐ The QuPath Cell Detection feature was used on a brightfield image to define approximately 1000 ROIs on the slide. Here only a small section is shown. Ⓑ MALDI ion image of PI (38:4) (*m/z* 885.547, 3.2 ppm) localized to only the HeLa cells (spatial resolution: 5µm).

Conclusion

The QuPath-to-flexImaging workflow represents a significant advancement in targeted MALDI Imaging capabilities. By leveraging open-source digital pathology tools and standardized file formats, researchers can now perform sophisticated, microscopy-driven MALDI Imaging experiments without requiring custom software development.

Key advantages include:

- Streamlined workflow accessible to the broader research community
- Dramatic improvements in experimental efficiency and cost-effectiveness
- Support for diverse biological applications from tissue analysis to single-cell studies
- Seamless integration with neofleX™ and timsTOF fleX series

This approach enables more sophisticated experimental designs that address specific biological questions while making high spatial resolution MALDI Imaging practical for large-scale studies.

References

[1] Bankhead P, Loughrey MB, Fernández JA, Dombrowski Y, McArt DG, Dunne PD, McQuaid S, Gray RT, Murray LJ, Coleman HG, James JA, Salto-Tellez M, Hamilton PW (2017). Scientific reports. **7**:16878

Learn More

For additional information about QuPath integration and targeted MALDI Imaging workflows watch the tutorial video or visit: www.bruker.com/timstofflex, SCiLS Software | Bruker

Software Requirements

- QuPath (open-source, available at qupath.github.io)
- flexImaging v7.5 or later
- SCiLS Lab (recommended for data analysis)

File Format Support

- Input: Standard microscopy formats (TIFF, PNG, JPEG, etc.)
- Region export/import: GeoJSON standard format

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