

Introduction

Drug discovery is crucial for advancing human health, yet it faces significant hurdles, including high attrition rates and the need for more effective strategies to understand drug distribution and target engagement within biological systems. Innovative analytical techniques such as quantitation by matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-qMSI) offers a powerful approach. Early methods for calibration in qMSI studies include the use of tissue mimetics and handheld spotting of standards which have demonstrated varying degrees of success. Although acoustic dispensing is not new, we aim to improve upon these early protocols. Herein, we describe the development of a workflow for precise deposition of nanoliter volumes of drug compounds using echo acoustic liquid handlers, enabling their subsequent analysis by MALDI-qMSI.

Background

Drug Imaging by MSI

Provides a tool to evaluate drug efficacy and toxicity while also eliciting answers to drug localization and quantitation.

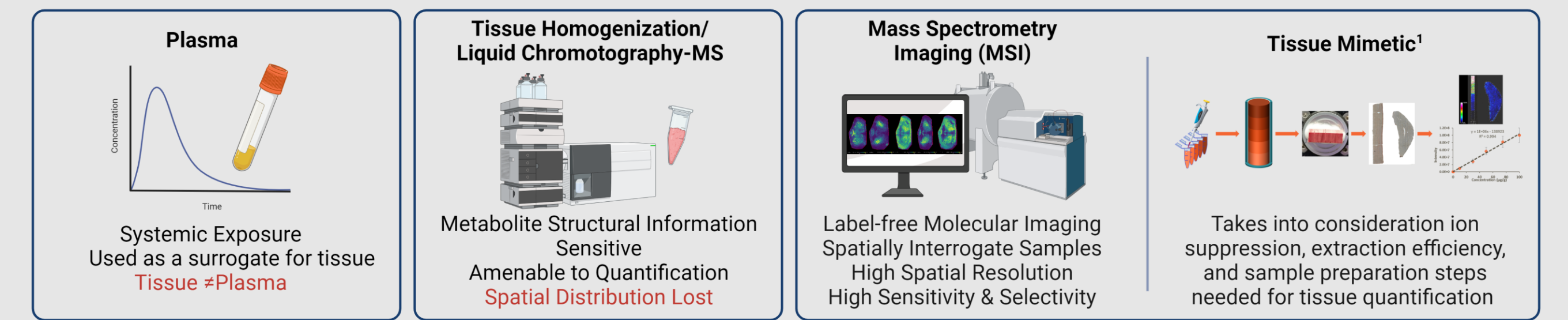


Fig. 1: Comparison of pharmacokinetic evaluations

Inspiration for Study

Previous reports show the viability of both acoustic and piezoelectric liquid handlers to either spot tissues or pre-spot slides with calibration curves for qMSI.^{2,3}

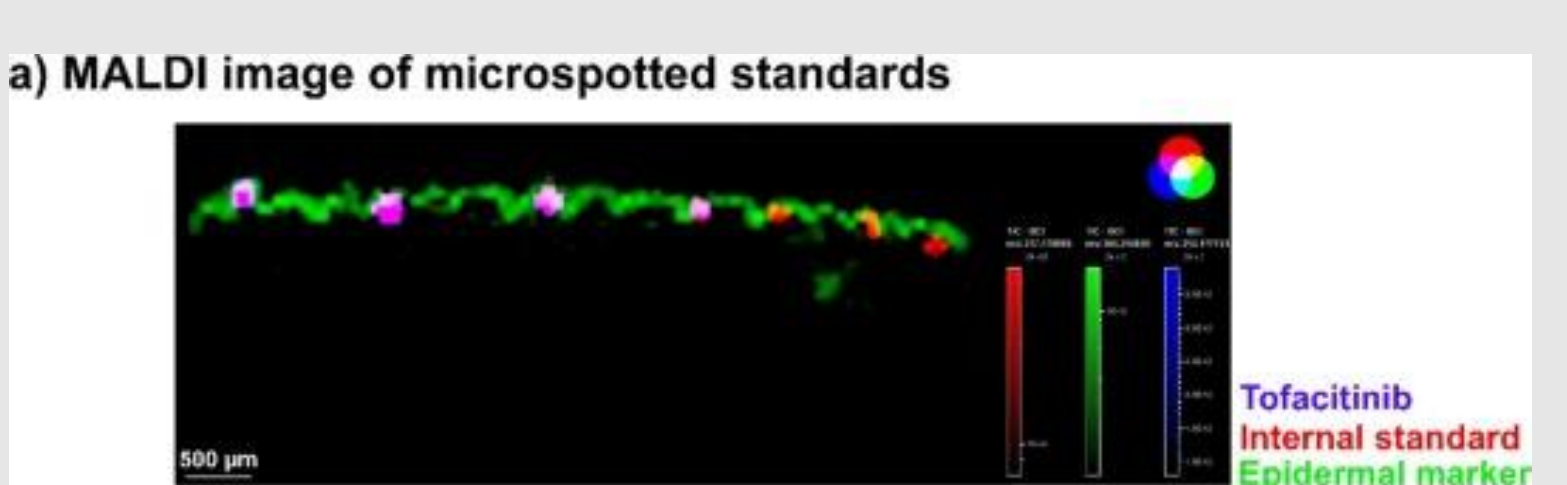


Fig. 2: Drug standards spotted on skin by acoustic robot spotter for generation of calibration curve.²

Methods

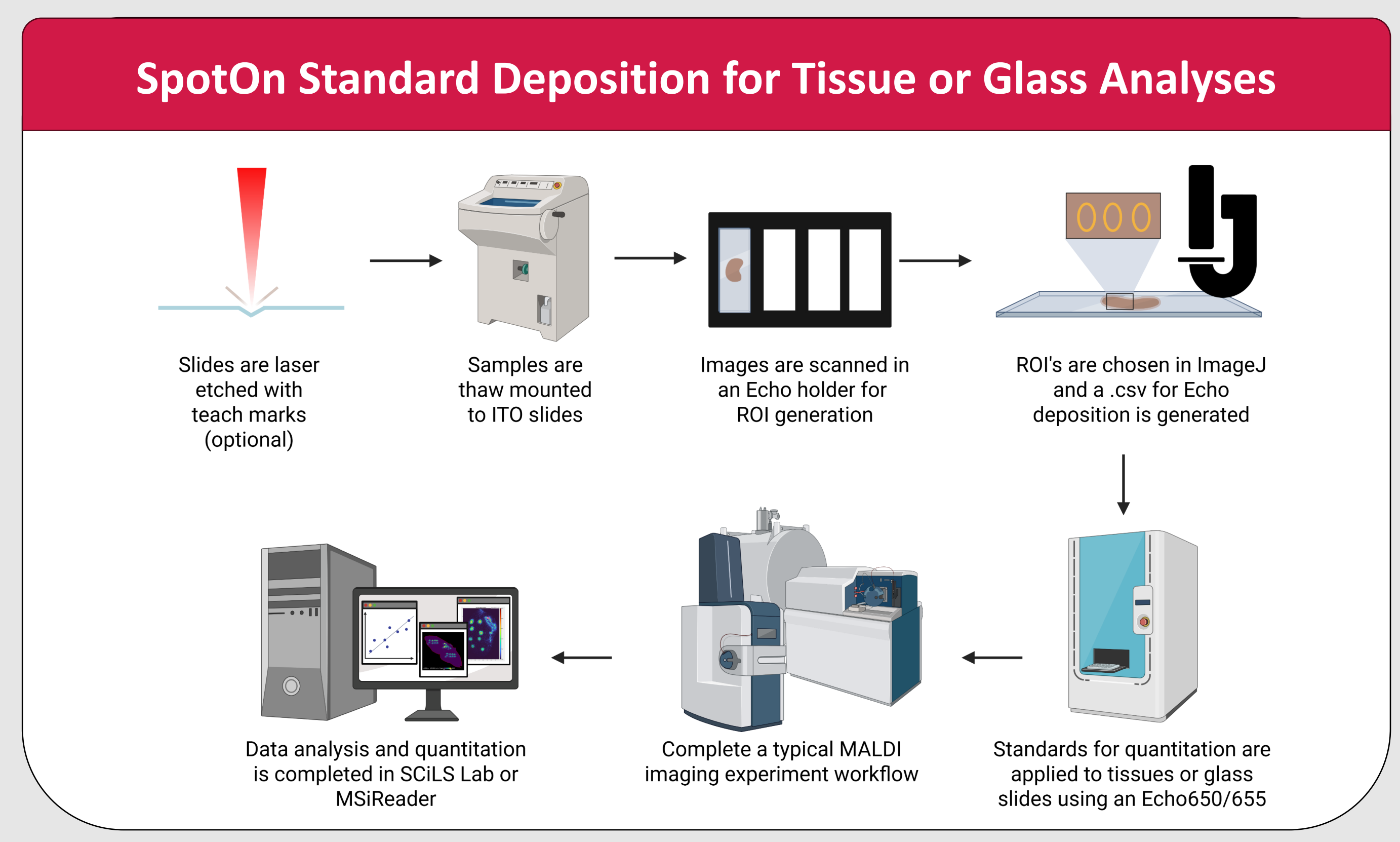


Fig. 3: Workflow for SpotOn application of standards using an Echo acoustic liquid handler for MALDI MSI experiments.

Results

Utilization of SpotOn Plugin

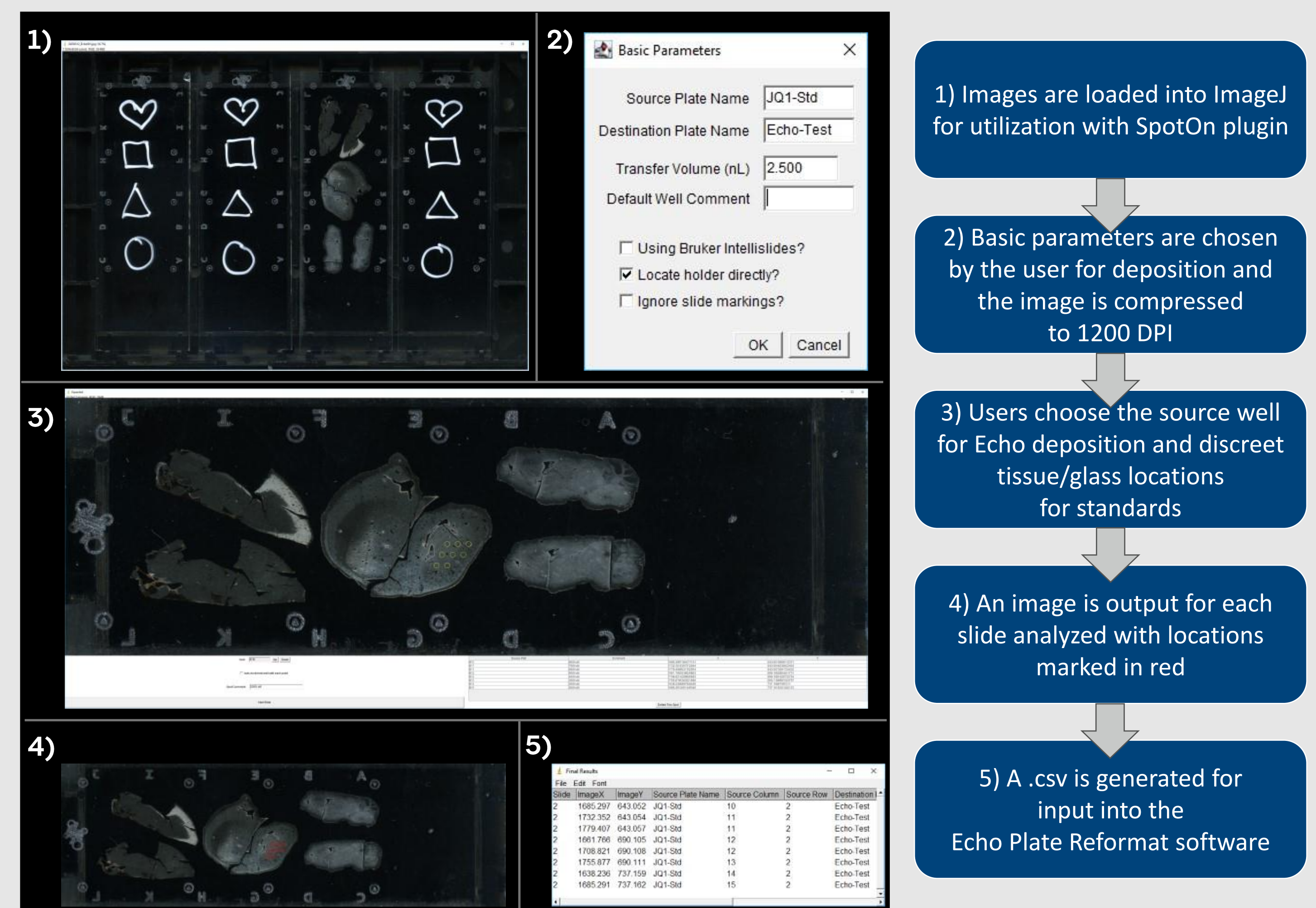


Fig. 4: Walkthrough of SpotOn plugin within ImageJ using liver and brain tissue samples.

Analysis on ITO Slides

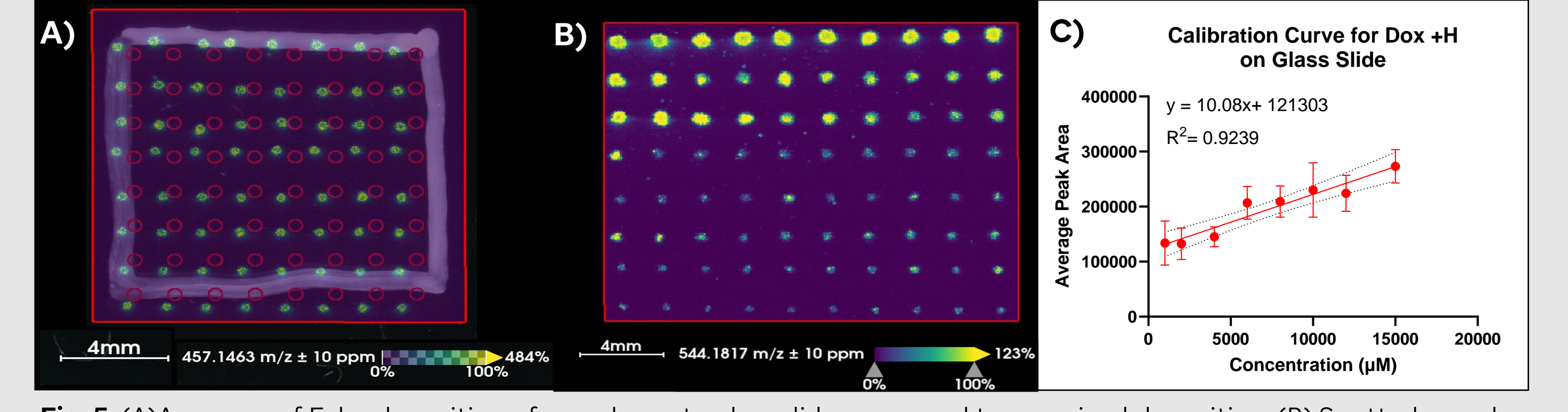


Fig. 5: (A) Accuracy of Echo deposition of samples onto glass slide compared to perceived deposition. (B) Spotted samples (15,000 μM to 1,000 μM) for generation of calibration curve C) for [M+H]⁺ peak for doxorubicin on ITO slides.

MSiReader for Analysis of Doxorubicin

- Calibration curves from 15,000 μM - 1,000 μM were spotted (left) near drug depots (right) within tumors (dosed 50 $\mu\text{g}/\mu\text{L}$ IT). Calibrations were generated using the spatial calibration tool and samples analyzed further by MSi Slicer.

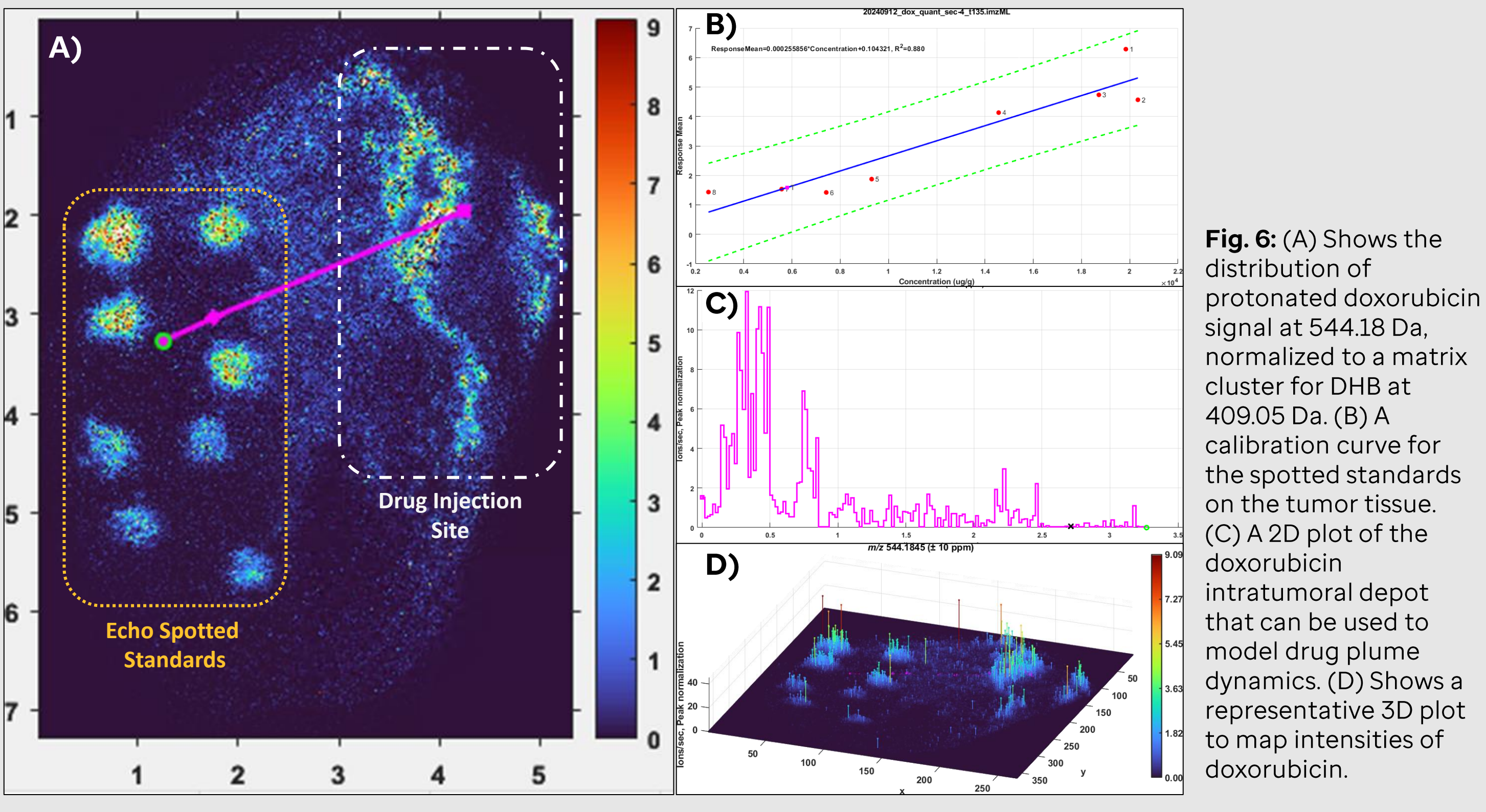
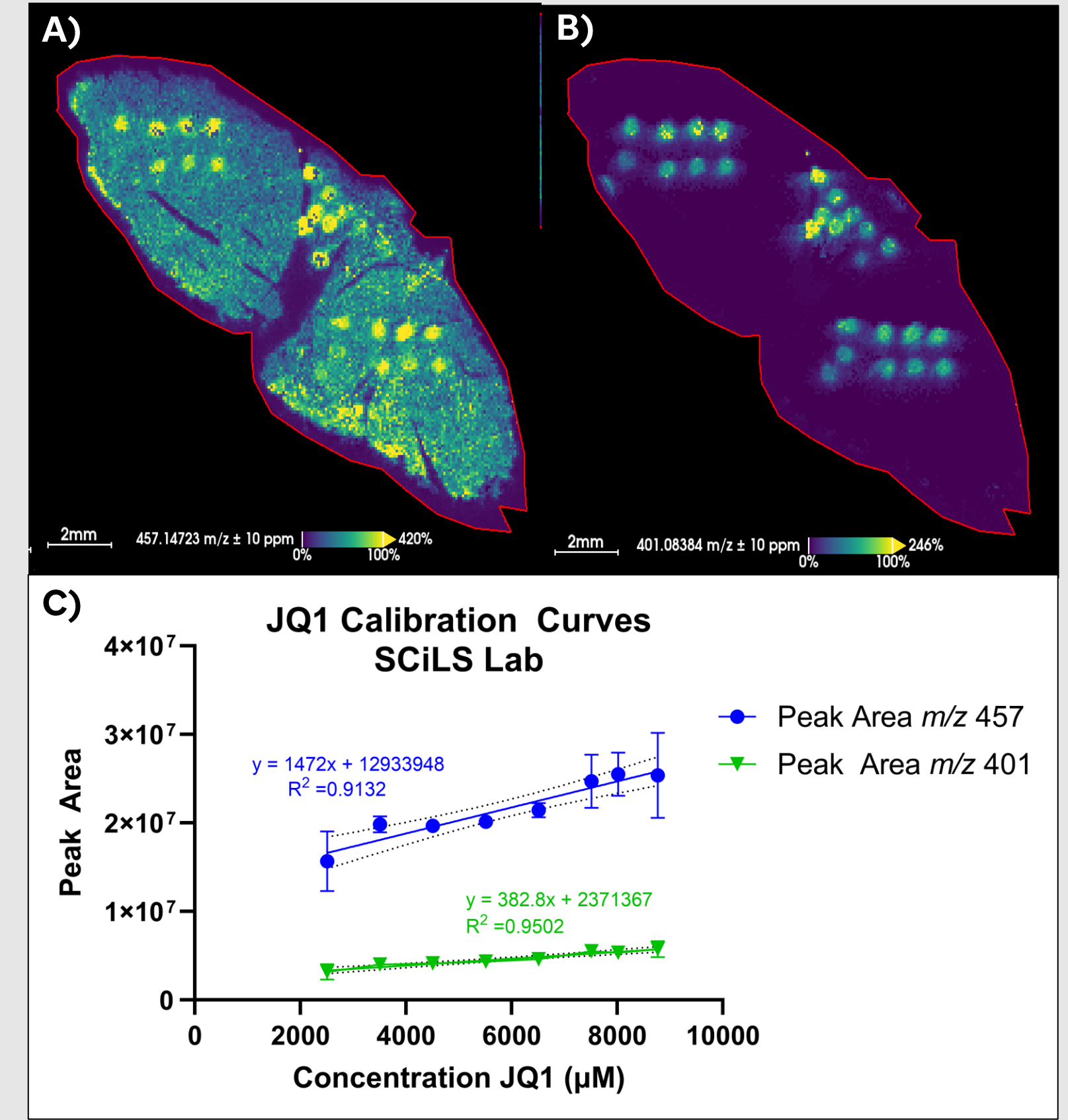


Fig. 6: (A) Shows the distribution of protonated doxorubicin signal at 544.18 Da, normalized to a matrix cluster for DHB at 409.05 Da. (B) A calibration curve for the spotted standards on the tumor tissue. (C) A 2D plot of the doxorubicin intratumoral depot that can be used to model drug plume dynamics. (D) Shows a representative 3D plot to map intensities of doxorubicin.

Results

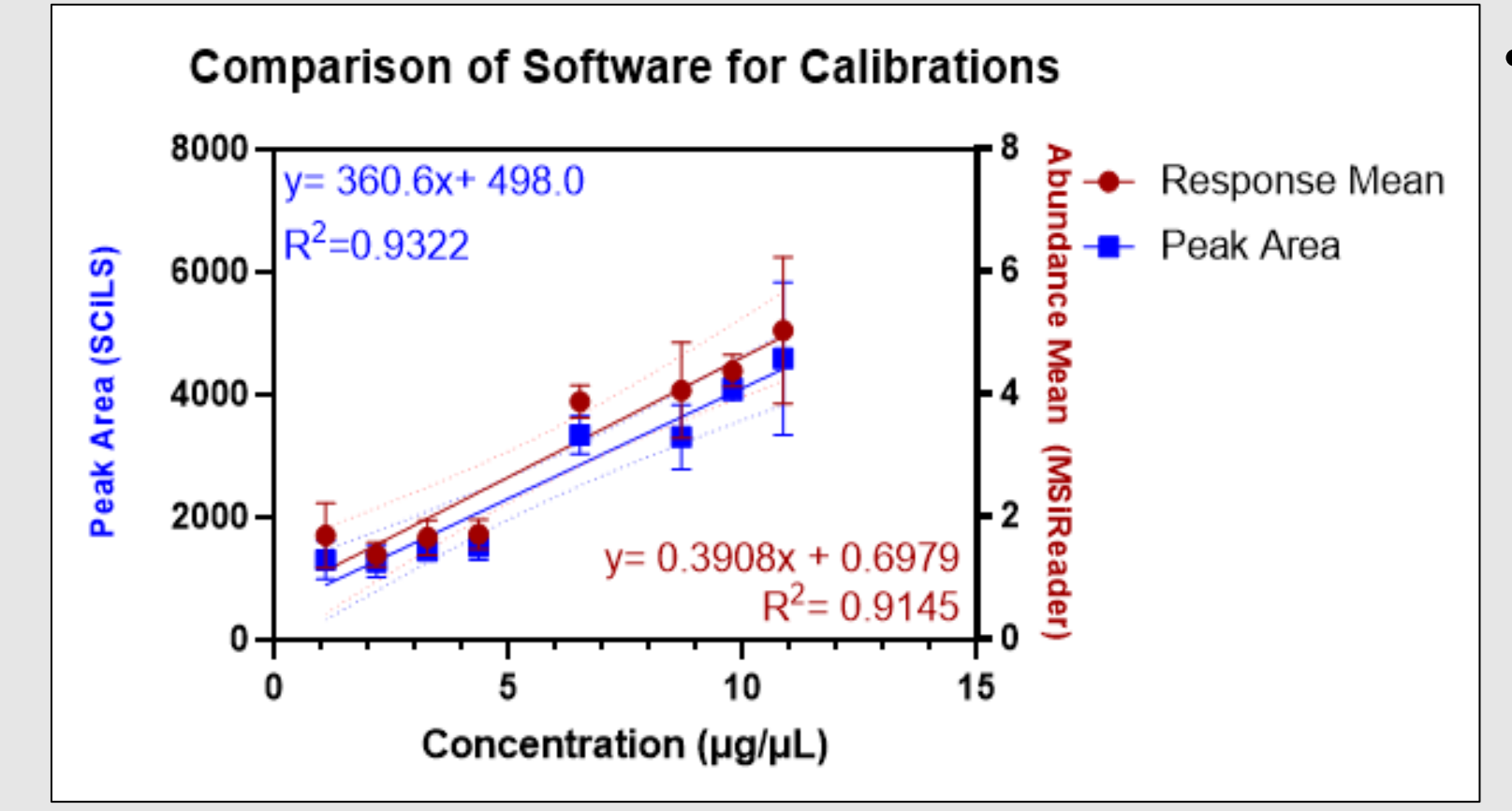
SCiLS Lab for Analysis of JQ1



- Three calibration curves were spotted on liver tissue dosed *i.v.* with JQ1 (10 mg/kg) ranging from 8,000 μM to 2,000 μM
- In-source fragmentation products were only observed for spotted standards and used to generate ROI's for standards
- Normalized peak areas for 457 and 401 Da were utilized to generate calibration curves for JQ1 standards

Fig. 7: Results from a dosed liver spotted with JQ1. Images correspond to m/z for JQ1, 457.14 Da (A) and the in-source fragmentation product of JQ1, 401.08 Da (B) both normalized to a deuterated internal standard of JQ1 at 459.16 Da. (C) Shows the workup of calibration curves for both analytes.

Software Comparison of Calibrations for Doxorubicin



- While each software reports in different units, a comparison of values shows that either software is viable for creating linear outputs when using the same tissues and normalizations.

Fig. 8: Comparison of calibration curves generated from Echo deposition of doxorubicin standards onto tumor tissues within SCiLS Lab (blue) and MSiReader (Red), with data normalized to matrix cluster of DHB.

Conclusions & Future Directions

- A robust software plugin for use with ImageJ has been developed to enable the discreet deposition of standards onto tissues and glass slides.
- Some variations in deposition locations between perceived and actual positions can be attributed to issues caused by the refractive index of the glass when attempting to co-register.
- Calibration curves generated across glass, single tissues, and multi-tissue studies show high reliability and goodness of fit scores.
- Software from MSiReader and SCiLS lab are comparable for analyses and have different features and tools beneficial for deeper understanding of datasets.
- We aim to use this tool in tandem with MSI to probe drugs in the clinical pipeline to more fully interrogate PK/PD of novel therapeutics.

References

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Acknowledgements

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