CROSS VALIDATION OF DRUG DISTRIBUTION DETECTED UTILIZING IMAGING MALDI MASS SPECTROMETRY AND QUANTITATIVE AUTORADIOGRAPHY

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OVERVIEW

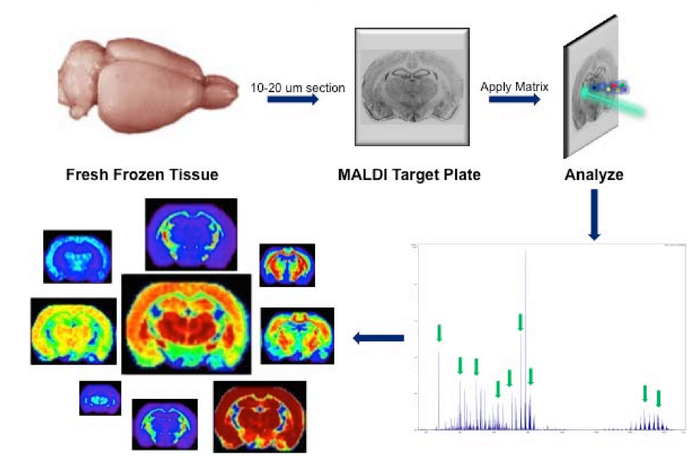
Purpose: To cross-validate label free imaging MALDI mass spectrometry with quantitative autoradiography.

Methods: Genentech Compound ^{14}C-A or Genentech Compound A was dosed at 150 ug/eye in rabbit eye. Eyeball sections were collected at 40 µm for quantitative autoradiography and 15 µm for imaging MALDI MS.

Results: Distribution of Genentech Compound ^{14}C-A via imaging MALDI MS in rabbit eyeball was similar to autoradiography data generated by QPS. Genentech Compound A distribution detected via imaging MALDI MS correlated with Genentech Compound ^{14}C-A distribution.

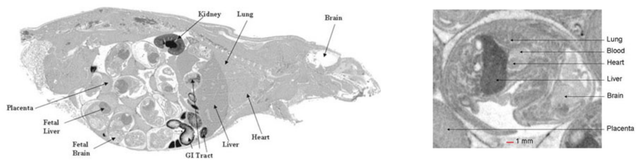
INTRODUCTION

Imaging MALDI MS



An imaging MALDI MS experiment requires a laser to be systematically rastered across an entire tissue section while acquiring a mass spectrum at each ablation spot. The precise x,y coordinates of the recorded mass spectra are then compiled to represent individual "pixels" in an ion image. Specific m/z values can be selected to produce 2D ion density maps representing the location and relative abundance of an analyte within the tissue sample.

Autoradiography



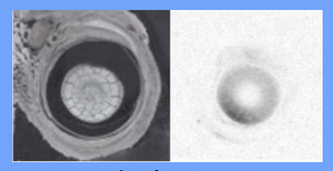
An autoradiograph is an image produced by the radiation emitted from a specimen, such as a section of tissue, that has been treated or injected with a radiolabeled isotope or that has absorbed or ingested such an isotope.

RESULTS

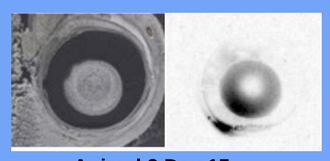
Method Development for Genentech Compound ^{14}C-A Detection via Imaging MALDI MS

Autoradiography

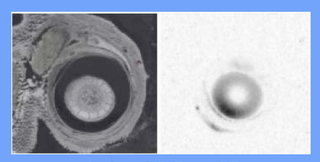
Optical Image



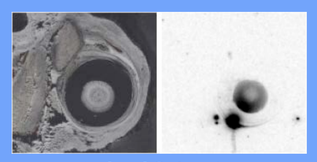
Animal 2 Day 15
Section 6



Animal 2 Day 15
Section 8



Animal 2 Day 15
Section 7

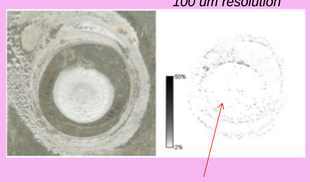


Animal 2 Day 15
Section 9

Imaging MALDI MS

Standard Imaging MALDI Prep

Optical Image | MALDI MS Image *100 um resolution*

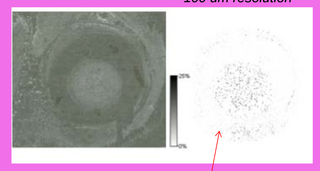


Drug signal missing from lens (possibly due to ion suppression)

Revised Imaging MALDI Prep: Tissue Washed by Dipping

Tissue was washed with 50 mM Ammonium Formate by dipping the slide 3 times for 5 seconds each

Optical Image | MALDI MS Image *100 um resolution*

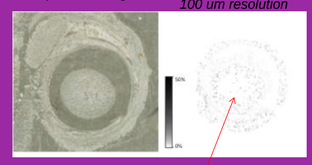


Lens signal was extracted, however hot spot was washed away

Revised Imaging MALDI Prep: Tissue Washed by Spraying

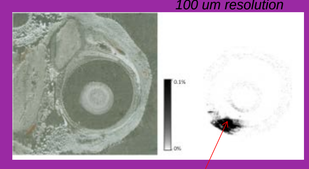
Tissue was washed with 50 mM Ammonium Formate by spraying the slide using an automated sprayer

Optical Image | MALDI MS Image *100 um resolution*



Lens signal was extracted

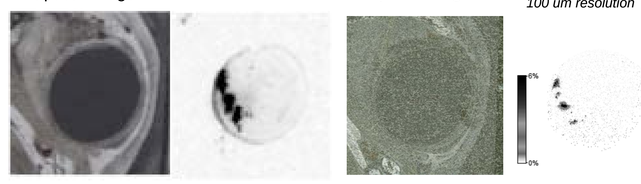
Optical Image | MALDI MS Image *100 um resolution*



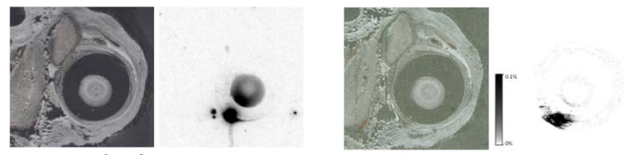
Hot spot was retained

Cross Validation of Genentech Compound ^{14}C-A Distribution

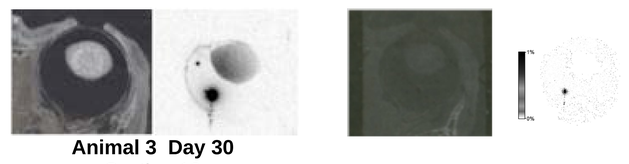
Autoradiography	Imaging MALDI MS
Optical Image	Optical Image / MALDI MS Image *100 um resolution*



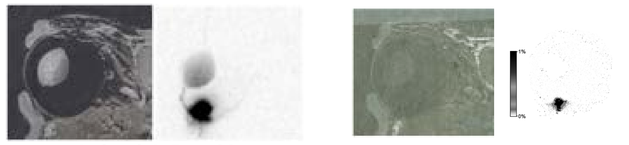
Animal 1 24 hour
Section 13



Animal 2 Day 15
Section 9



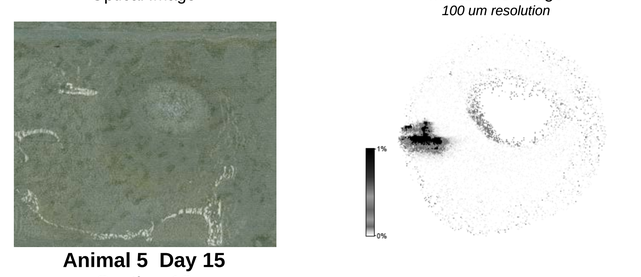
Animal 3 Day 30
Section 12



Animal 4 Day 60
Section 13

Genentech Compound A Imaging MALDI MS Distribution

Optical Image | MALDI MS Image *100 um resolution*



Animal 5 Day 15
Section 13

METHOD

A single dose of Genentech Compound ^{14}C-A or Genentech Compound A at 150 ug/eye was administered to pigmented rabbits via an intravitreous administration. Animals were euthanized at 24 hours, days 15, 30 and 60 post-dose and frozen in a hexane/dry ice bath.

The rabbit head was embedded in 2% carboxymethyl cellulose (CMC), sectioned sagittal at 40 µm thickness for QARL analysis and a serial section was collected at 15 µm for imaging MALDI MS analysis.

Autoradiography

Sections for each rabbit was mounted on a cardboard backing and were exposed to a [^{14}C]- sensitive phosphor imaging plate. Imaging plates and sections were exposed for four days. The imaging plates were scanned using the Typhoon 9410 image acquisition system.

Imaging MALDI MS

Sections were mounted on ITO coated glass slides and mounted using double sided tape. Tissue was washed with 50 mM ammonium formate to increase analyte extraction. A solution of 2,5-Dihydroxbenzoic (DHB) matrix (40 mg/ml, 70/30 Methanol/H_2O plus IS spike) was spray coated onto tissues using an automated sprayer. Imaging MALDI analysis was carried out on the SolariX FTMS. Genentech Compound ^{14}C-A and Genentech Compound A were acquired in positive ion mode utilizing continuous accumulation of selected ions (CASI) with ion detection from *m/z* 150-3000 at 100 µm spatial resolution.

CONCLUSIONS

This study has served to show that autoradiography data can be used to validate imaging MALDI MS data. Although the signal intensity across the two imaging mediums are not 100% identical, the lower sensitivity of the imaging MALDI MS assay can attributed to multiple parameters. 1) The inability to detect drug in the lens is likely due to a matrix effect. 2) The transfer tape used when collecting the tissue sections contributed to signal suppression (via charging), as well as, 3) ion suppression contributions from the embedding media (2% CMC). All in all, combining to an unfavorable imaging MALDI MS outcome; however, all of these parameters are tunable and will be explored further.
Nevertheless, we have demonstrated that appropriate distribution information can be gained from a label free imaging MALDI MS approach as an alternative to the more costly autoradiography approach. Therefore imaging MALDI MS can serve as a useful tool in early assessment of drug distribution in a fast paced drug discovery setting.