

Semi-Quantitative Mapping of Oncological Therapies with Mass Spectrometry Imaging

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Introduction

Hepatocellular carcinoma (HCC) is one of the challenging cancers to treat and its occurrence is steadily rising in the US.¹ While surgical resection is considered curative, it is not available to patients who have underlying liver problems, such as chronic liver disease. Liver transplantation maybe considered in these cases but recurrence is a possibility and transplant actually occurs for <5% of all HCC cases .

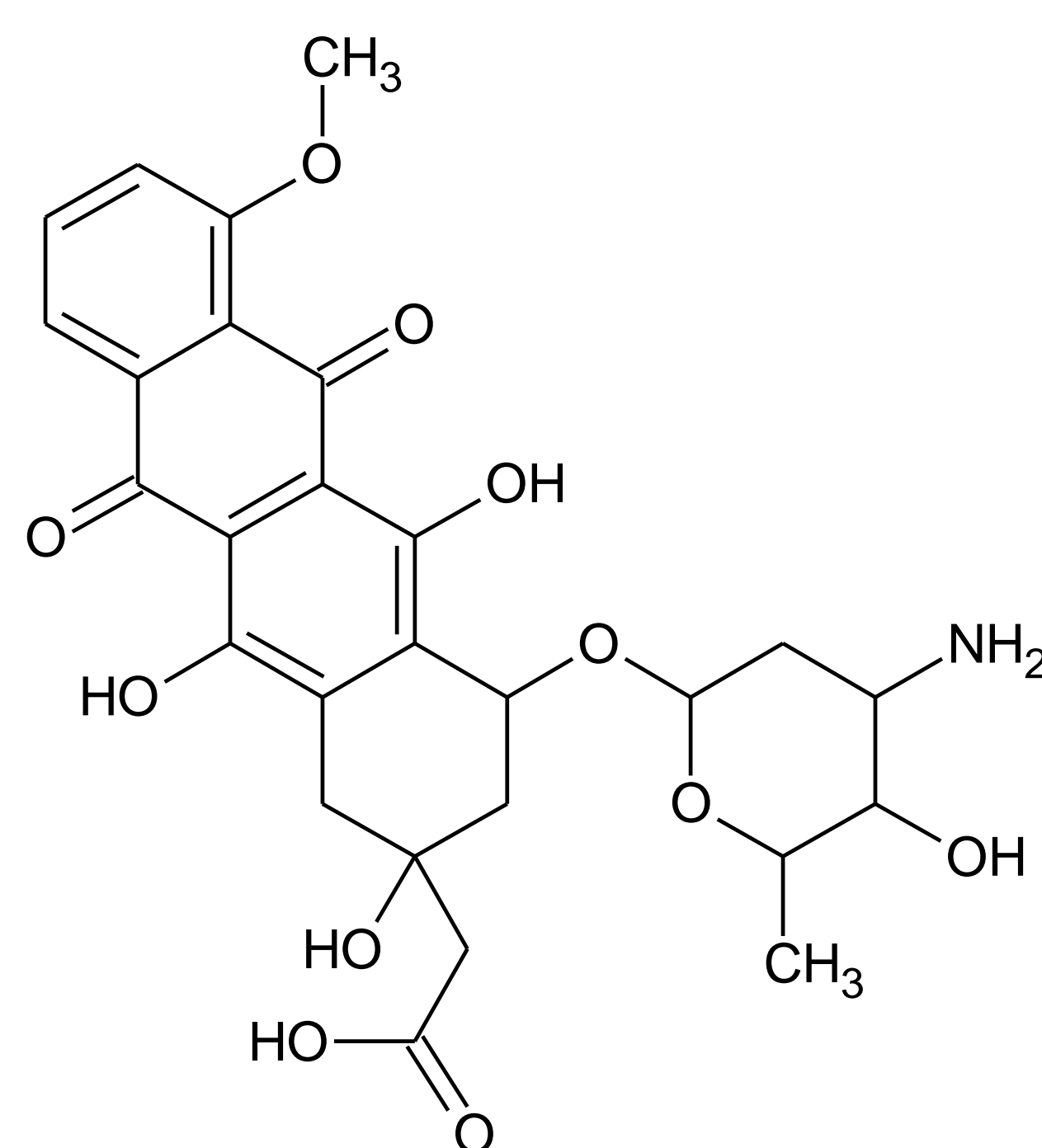


Fig. 1 Doxorubicin DXR (MW 543.525) is a drug derived from a natural product extracted from several wild strains of *Streptomyces*, and used to treat many different types of cancer.

While doxorubicin (DXR) use for treatment showed positive effects, its side effects such as cardiomyopathy, bone marrow suppression, hair loss, and typhlitis, are easily triggered when the systemic concentration dose reaches a particular threshold. Thus, novel therapies have been developed to deliver DXR through targeted means instead of administering systemically. Particularly the use of transarterial chemoembolization (TACE) with this drug allows increased loading of DXR via an emulsion while minimizing the systemic exposure.² We developed a method to analyze DXR using mass spectrometry imaging, to gather spatial distribution after it was administered *in vivo*.

Materials

- Waters SYNAPT G2-Si IMS Q-ToF coupled with a MALDI imaging ion source
- HTX M5 Sprayer Matrix applicator
- 2,5-dihydroxybenzoic acid (DHB) from Sigma Aldrich, trifluoroacetic acid from Thermo Fisher
- LCMS grade water & acetonitrile from Fisher Sci.
- Tissue homogenates: Drug-free frozen pig liver (~500mg) mixed with 1mL water and processed using bead homogenizer

Methods

Typical Mass Spectrometry Imaging Workflow

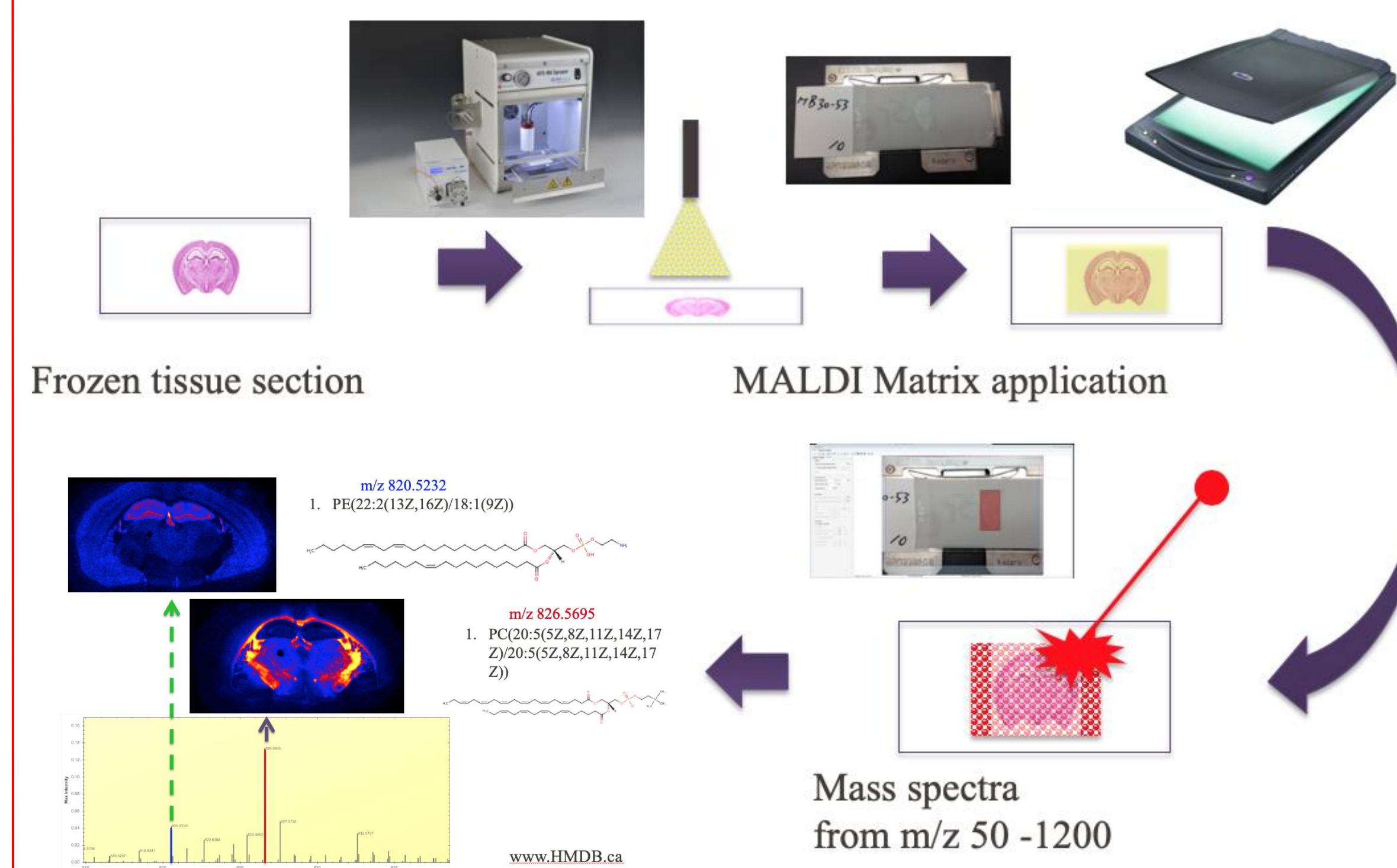


Fig. 2 Typical MSI workflow. Cryosectioned tissue is frozen-mounted on a regular glass slide. After drying under vacuum for 10 minutes, MALDI matrix is applied using the HTX M5 Sprayer matrix applicator. Data was acquired from m/z 50-1200 and was processed using Waters HD Imaging software.

Results and Discussion

Preliminary experiments were done to optimize the MALDI MS parameters to give the best sensitivity for detection of DXR. After establishing positive ion mode is better for DXR analysis, the next step was to determine the signal response of DXR with tissue. A frozen DXR-free liver tissue was cryosectioned and mounted on a regular glass slide. Standard solutions were spotted (1 μ L) on top of the tissue and allowed to dry at room temp under vacuum. DHB was applied as matrix.

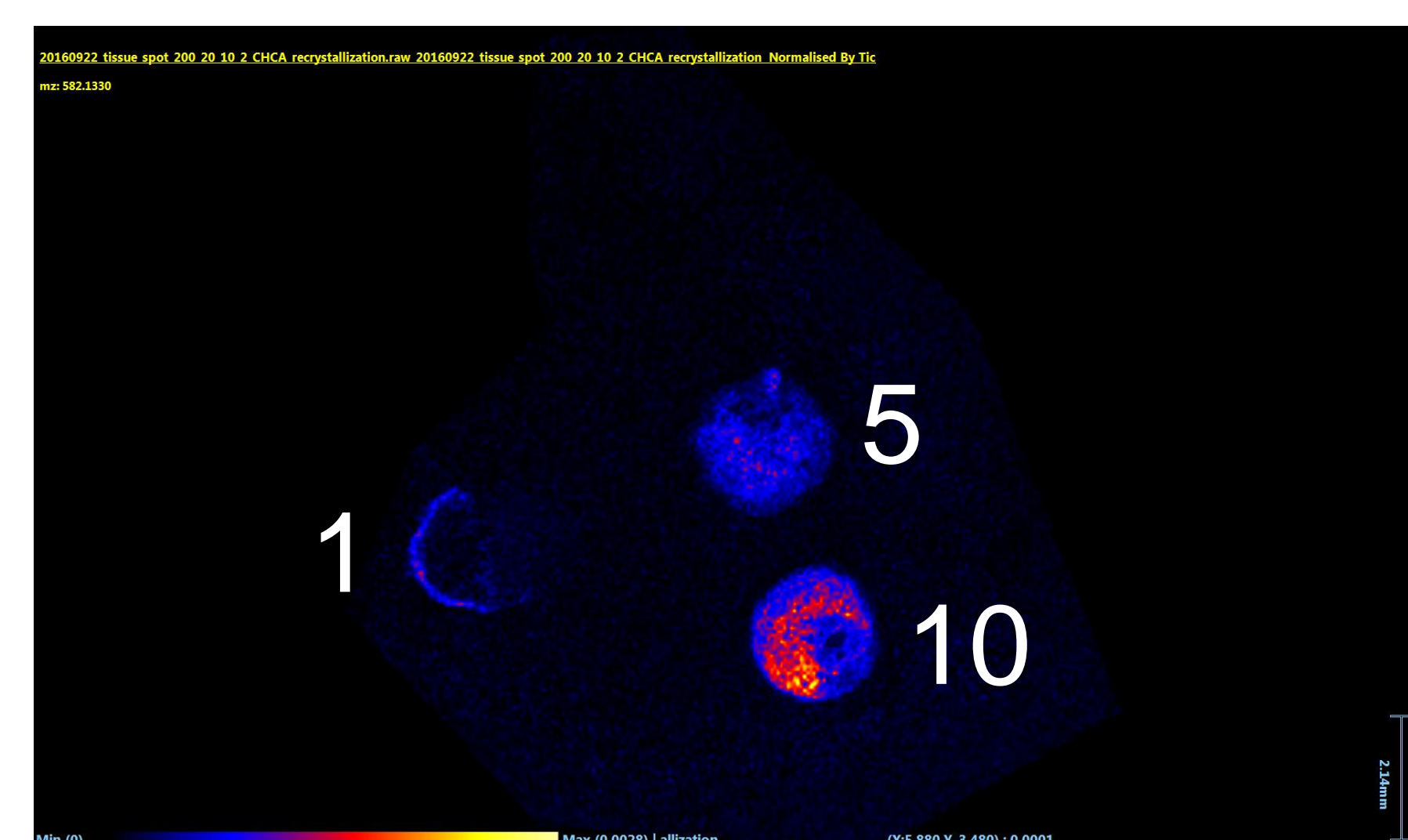


Fig. 3 One microliter spots of DXR solutions applied on top of liver tissue. The solution concentrations were in mg/kg

However, the DXR signal response with this method cannot be reliably compared to DXR when it is administered *in vivo*. The DXR sample droplet is mostly only sitting on top and not thoroughly mixed in the tissue and inhomogeneity in the signal distribution was observed, most likely due to how the droplet dried as shown in Figure 3.

In an attempt to quantify DXR in tissue reliably, a method for constructing a mimetic model for MSI by Barry et al.³ published in Protocol Exchange was followed.

Liver was extracted from a pig and homogenized using a Qiagen TissueLyzer with a 70% homogenate w/v in DI water, and created DXR tissue homogenate solutions (3.9, 7.8, 15.6, 31.3, 62.5 and 125 mg/kg) using serial dilutions. The resulting solutions were pipetted into a tube using a modified 3ml syringe and flash frozen using dry ice and isopropanol. and kept at -80 C until ready to section using Leica cryotome. Sections were cut into 12um thickness and thaw mounted on glass microscope slides (Fig 4A). The resulting products were kept frozen in dry ice/-80 C to prevent numerous freeze thaw cycles.

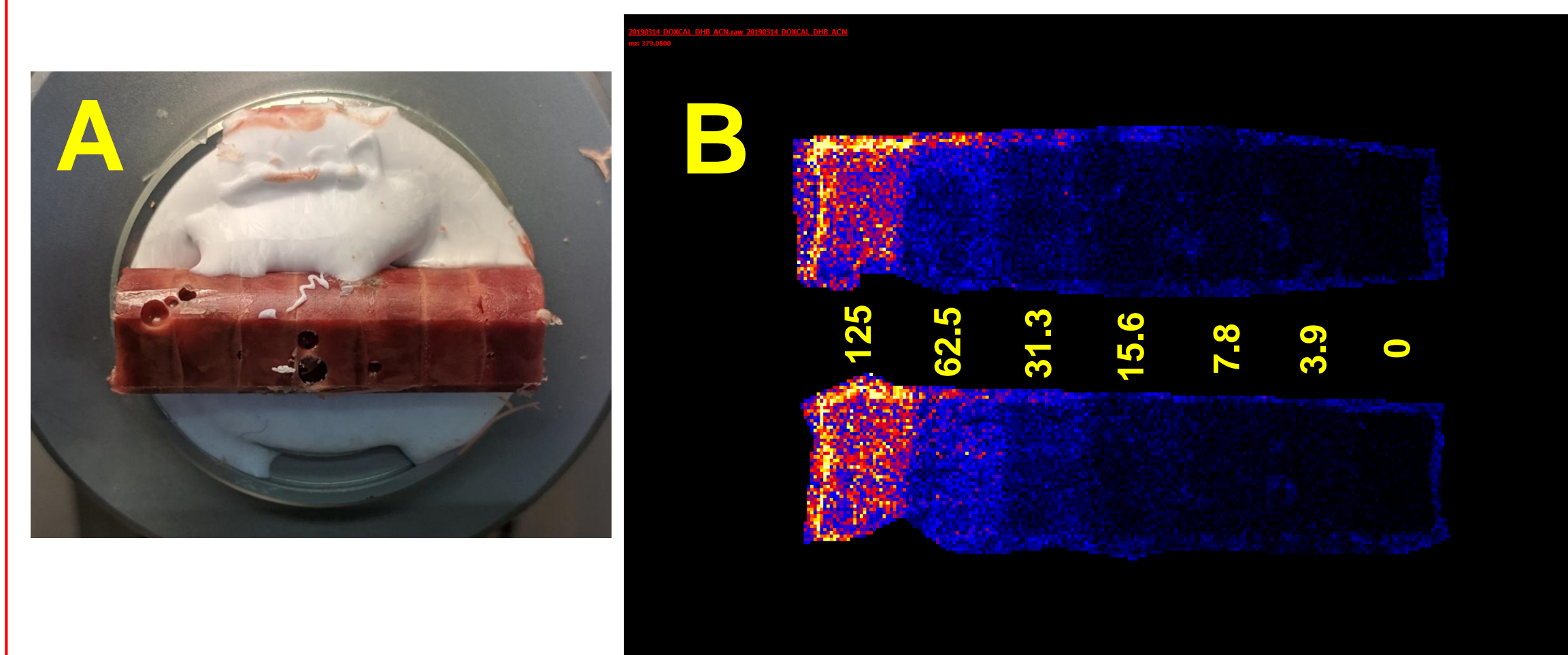


Fig. 4 A) Frozen DXR standards in tissue homogenate mimetic model mounted on the cryotome stage. B) MSI of 2 adjacent sections from the mimetic model placed on 1 slide. The numbers represent concentrations in mg/kg.

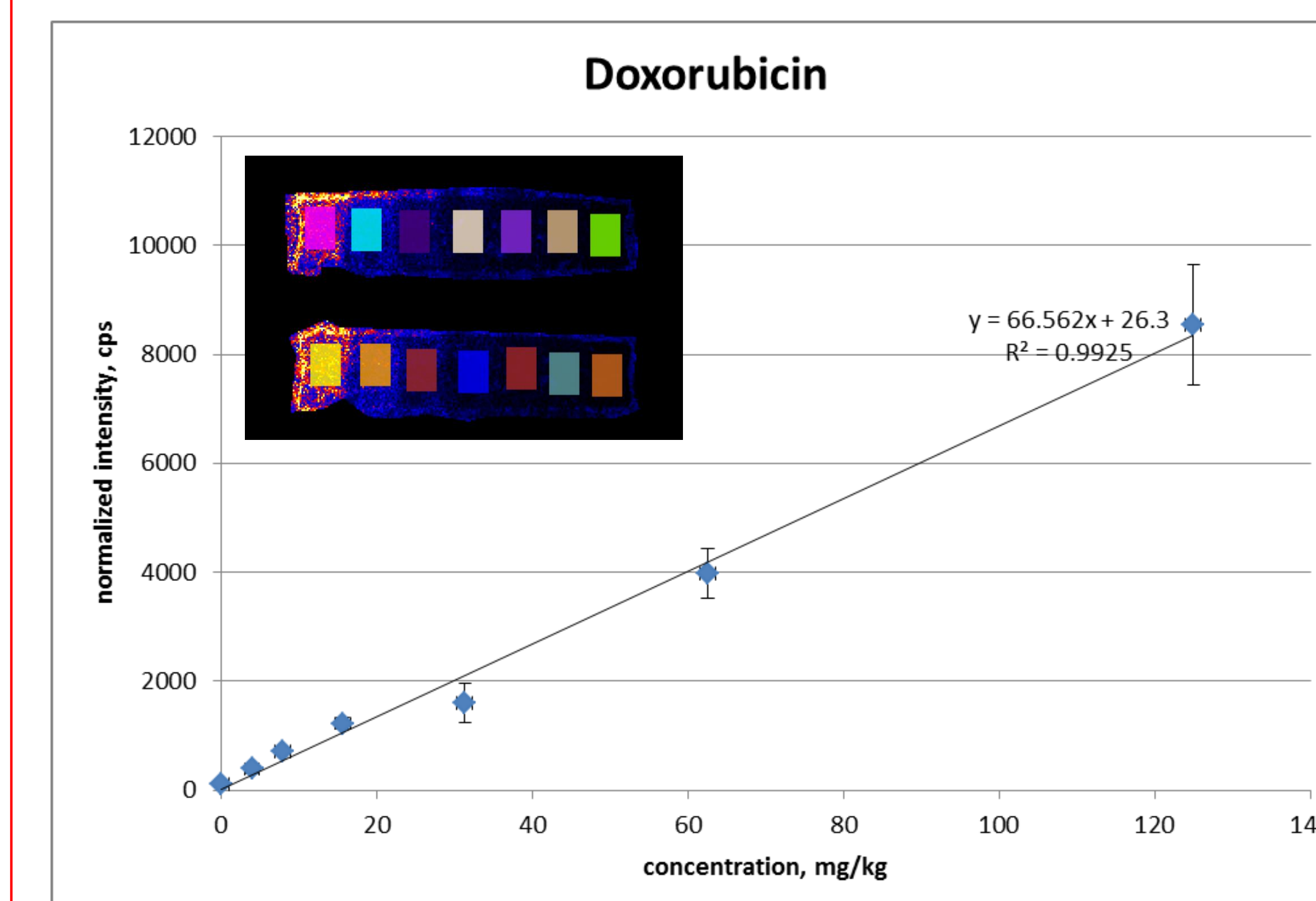


Fig. 5 DXR signal response plotted on the graph. The signal intensities were extracted from the respective layers (location as depicted on the inset). The numbers represent concentrations in mg/kg.

Signal intensities were extracted using HDI software (Waters Corp.) and imported to Progenesis Q1 (Waters Corp.) for normalization and processing. The calibration curve generated has a correlation $R^2 > 0.99$. The m/z chosen for DXR was an ion fragment at m/z 379.08.

A procedure of TACE on a pig is presented below. A catheter was inserted into the pig liver (Figure 6A) and an emulsion of DXR in lipiodol was administered, while using xray and CT for image guidance and confirmation(Figure 6B). The liver was explanted and analyzed for DXR using MSI (Figure 6C).

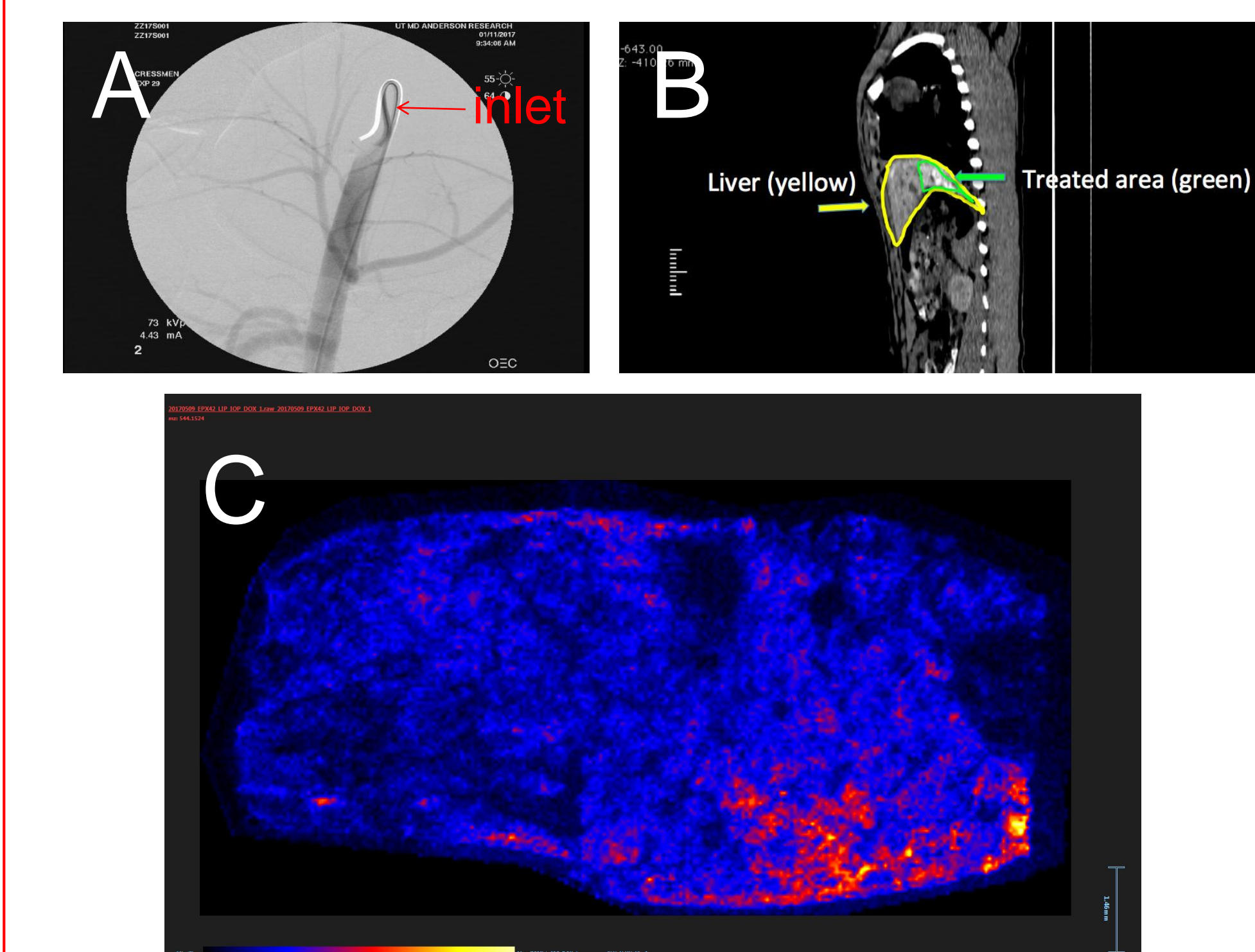


Fig. 6 A) An arteriogram of the pig showing the catheter placement in the vascular anatomy. Treatment and local drug delivery is applied in this way using a microcatheter through the base catheter and selectively advancing it into a branch vessel. B) A sagittal CT image of a pig 24h after the procedure to track the treatment. Denser white area corresponds to treated territory. C) MSI of DXR in liver

Although DXR was detected in this sample, this MSI analysis was performed as a separate experiment. To directly correlate the signal intensities between the samples, it is recommended that the mimetic model and sample are analyzed on the same slide.

Summary and Conclusion

Doxorubicin can be characterized by MALDI MSI to determine its spatial distribution in tissue, and using the mimetic model protocol, semi quantification can be achieved. For the next step of this project, the sample and mimetic model would be run on the same slide.

Selected References

- 1) Petrick, J.L., Kelly, S.P., Altekruse, S.F., McGlynn, K.A. and Rosenberg, P.S., Future of Hepatocellular Carcinoma Incidence in the United States Forecast Through 2030, *J Clin Oncol.* 2016; 34:15, 1787-1794.
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