Overview

Purpose

- Development of technologies for 3-D imaging mass spectrometry

Methods

- Model system:
  - color coded images of mouse brain slices on printed paper
- Acquisition of two data sets:
  - optical and MS ion images of each model section
- Two registration steps:
  - MS ion images of dyes to corresponding optical images
  - each optical image to the next serial image
- Assembly of 3-D volume of the corpus callosum and the mouse brain
- Incorporation of MS data

Results

- 3-D model of the corpus callosum

Introduction

Medical imaging plays an increasingly important role in many clinical applications. Anatomical, physiological, and functional information can be obtained by computer tomography (CT), positron emission tomography (PET), and magnetic resonance imaging (MRI). However, these imaging techniques, commonly used for the 3-D reconstruction of brain maps, do not enable the study of protein distributions. Imaging MALDI mass spectrometry has been successfully used to obtain the distribution of proteins in thin tissue slices [1-3].

The goal of the present study is to expand this technique by adding a third dimension allowing the 3-D mapping of proteins in specific regions of the mouse brain by imaging serial sections. The 3-D distribution of targeted proteins can be an important tool for the diagnosis, early detection and understanding of disease, such as Parkinson disease, and neuropsychiatric disorders.

Methods

The animal model we have chosen to study is the mouse brain. The methodology required for 3-D imaging mass spectrometry is developed in a first approach with printed images of mouse brain slices on paper to establish stacking parameters.

Figure 1. Nine serial images spanning the full corpus callosum of a male C57Bl/6J mouse brain are downloaded from a brain atlas*. Using PhotoShop™ the downloaded images are converted to a series of model sections by color coding the section periphery and the corpus callosum of each image blue and red, respectively. The colored regions are extracted from the original image and printed at a 1:1 scale on paper. A digital camera is used to record an optical image from each of the model slices before MS ion images of the dyes are acquired using a Voyager DE-STR MALDI TOF mass spectrometer (Applied Biosystems, Framingham, MA) at a spatial resolution of 50 μm. The data acquisition is performed using software developed in-house [4-5].

*http://www.mbl.org/atlas170/atlas170_frame.html

Results

Figure 2 and 3. Each ink color in the printed images of the mouse brain slices on paper produces specific ions from which MS ion images can be constructed. The blue ink used for the brain outline produces two characteristic ion signals at m/z 584 and m/z 1166, and the red ink used for the corpus callosum produces one strong ion signal at m/z 342. This mimics the expression of different proteins in real tissue slices.

Figure 4. The integration of the acquired MS ion images to the optical images requires a registration step to link the images together. This is achieved by placing four black ink dots around the printed images of the mouse brain slices. The four black ink dots are used as landmarks, since they are visible in the optical image and yield unique MS signals below m/z 300. They can be then be identified during the imaging process. To align both images a computer algorithm [6] is used to identify the x,y coordinates of the centers of the dots in both the optical and MS ion images.

Figure 5. The next step in the registration procedure is the alignment of a series of optical images. The external contours of the corpus callosum and the whole mouse brain are extracted and registered to one another, according to their position in the mouse brain, using novel image processing techniques [7].

Figure 6. The final stage is the 3-D reconstruction of the corpus callosum. The surfaces (3-D shape) of both the corpus callosum and the whole mouse brain are constructed from the extracted contours as described in [8]. Subsequently, the MS data of the red dye are extracted out of the registered MS ion images for each individual paper slice. Finally, the 3-D shape of the corpus callosum, the mouse brain and the extracted m/z points are combined and rendered using a commercial renderer [9].

Figure 7. We are currently applying this newly developed methodology to study the 3-D representation of proteins unique to the corpus callosum in mouse brain tissue. Serial sections (20 μm thickness) of a male C57Bl/6J mouse brain are cut in the range of Bregma 2 to -3 and mounted on conductive optically transparent glass slides. Optical images of the 260 slices obtained are recorded and imaging mass spectrometry is performed on selected slices.

Figure 1. Series of optical and MS images of model mouse brain sections.

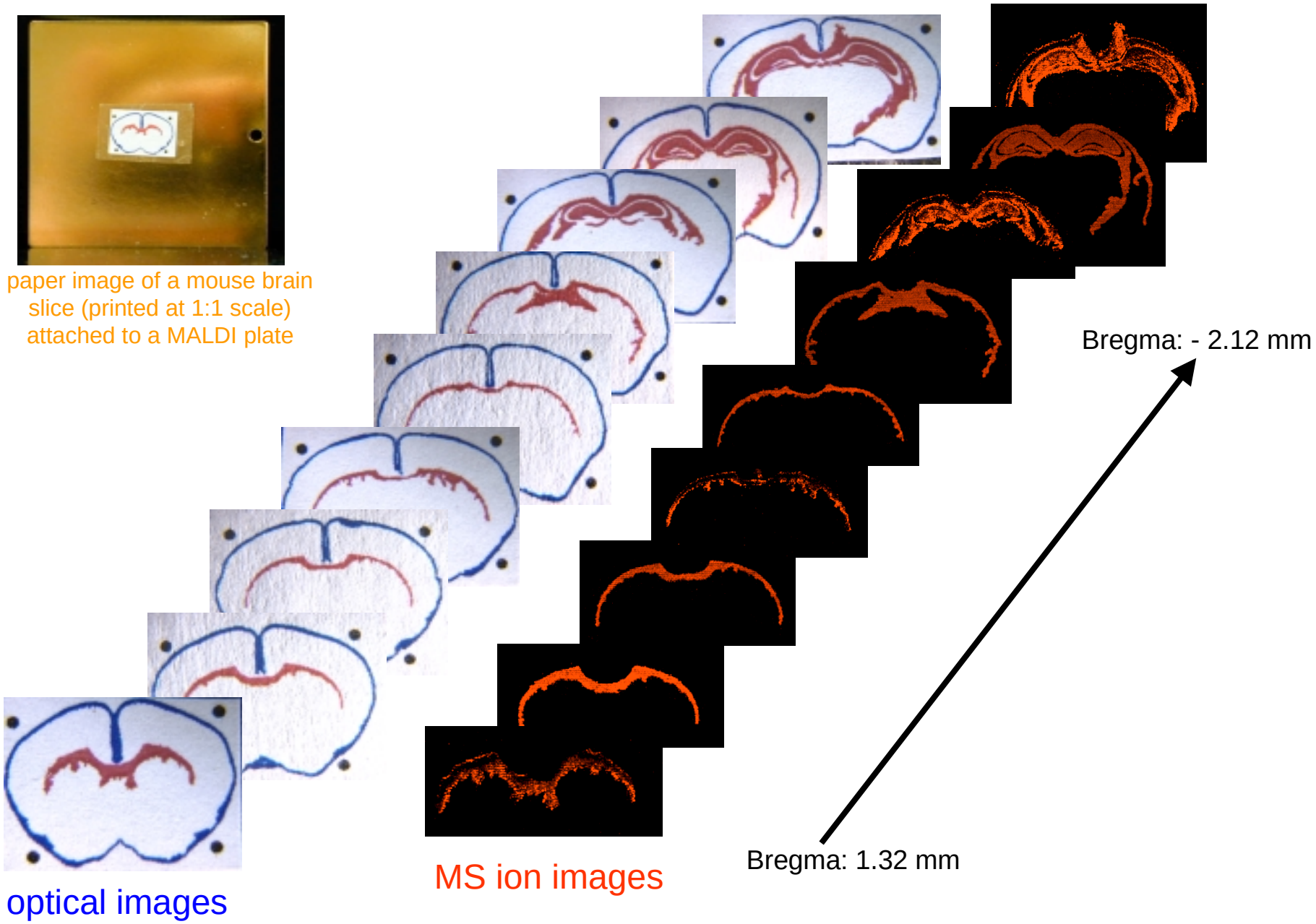


Figure 2. MS analysis of paper slices.

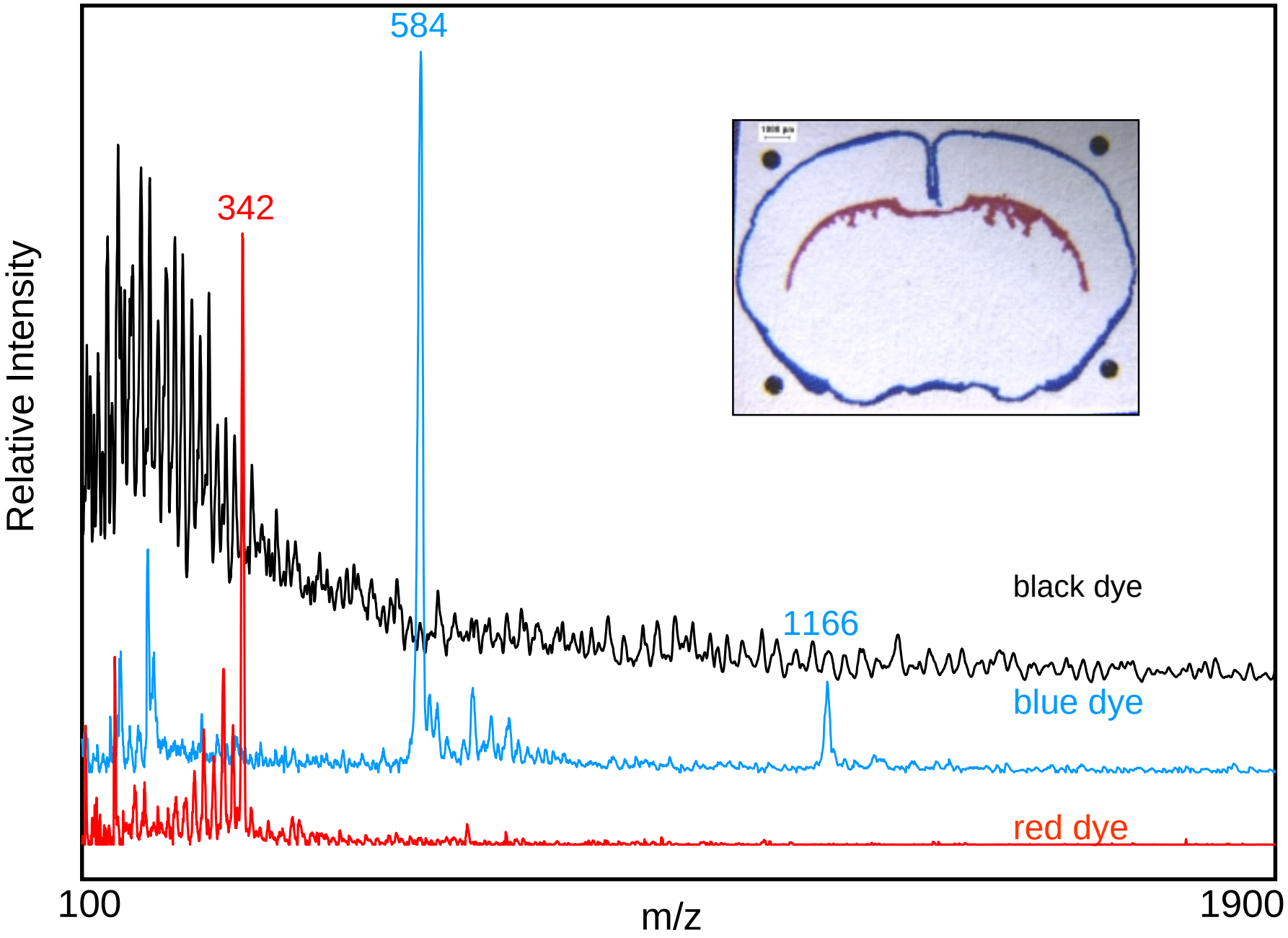
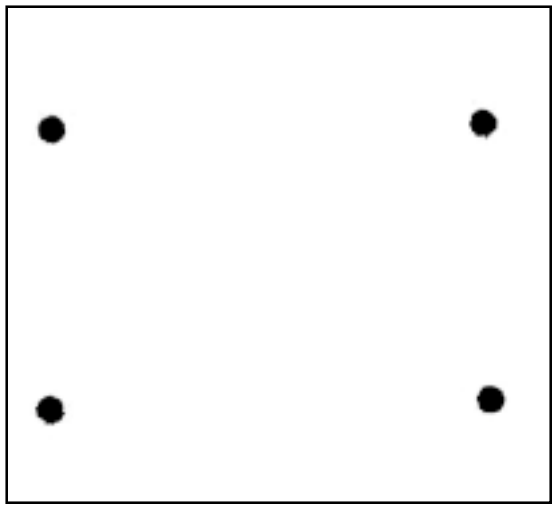


Figure 3. MS Images of Paper Slices.

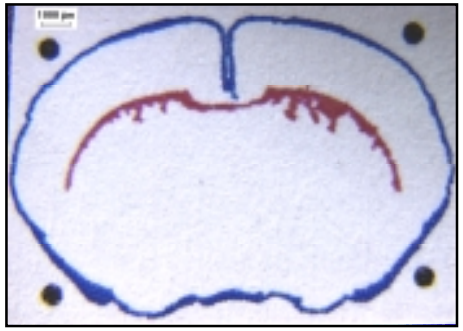
black dye



m/z 10-300

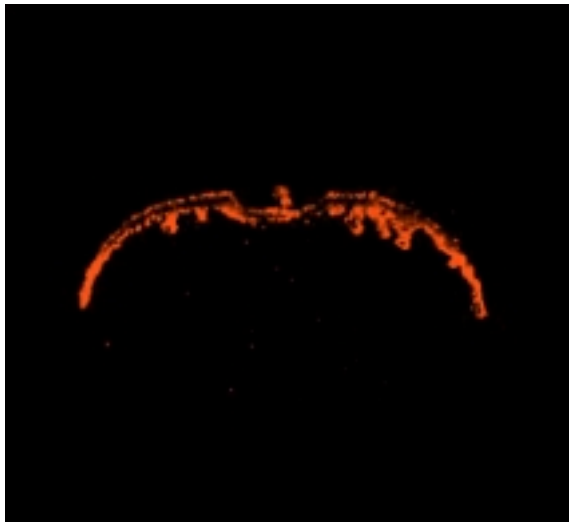
blue dye

m/z 584



Optical image

red dye



m/z 342

Figure 4. Registration of optical image to corresponding MS image.

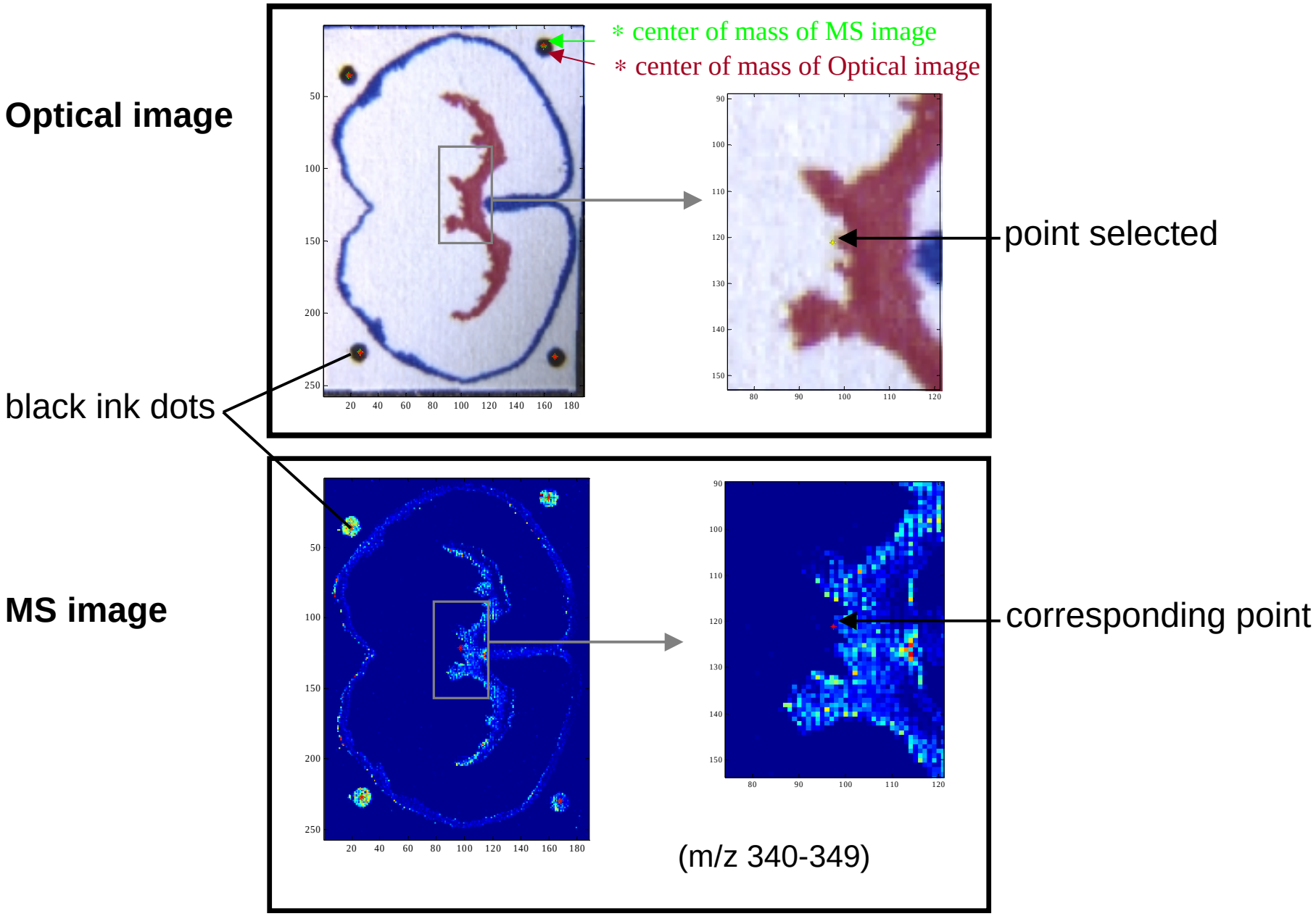


Figure 5. Extraction of the contours of (a) the corpus callosum, (b) the whole mouse brain, and (c) overlay of the the edges of the corpus callosum of all nine slices.

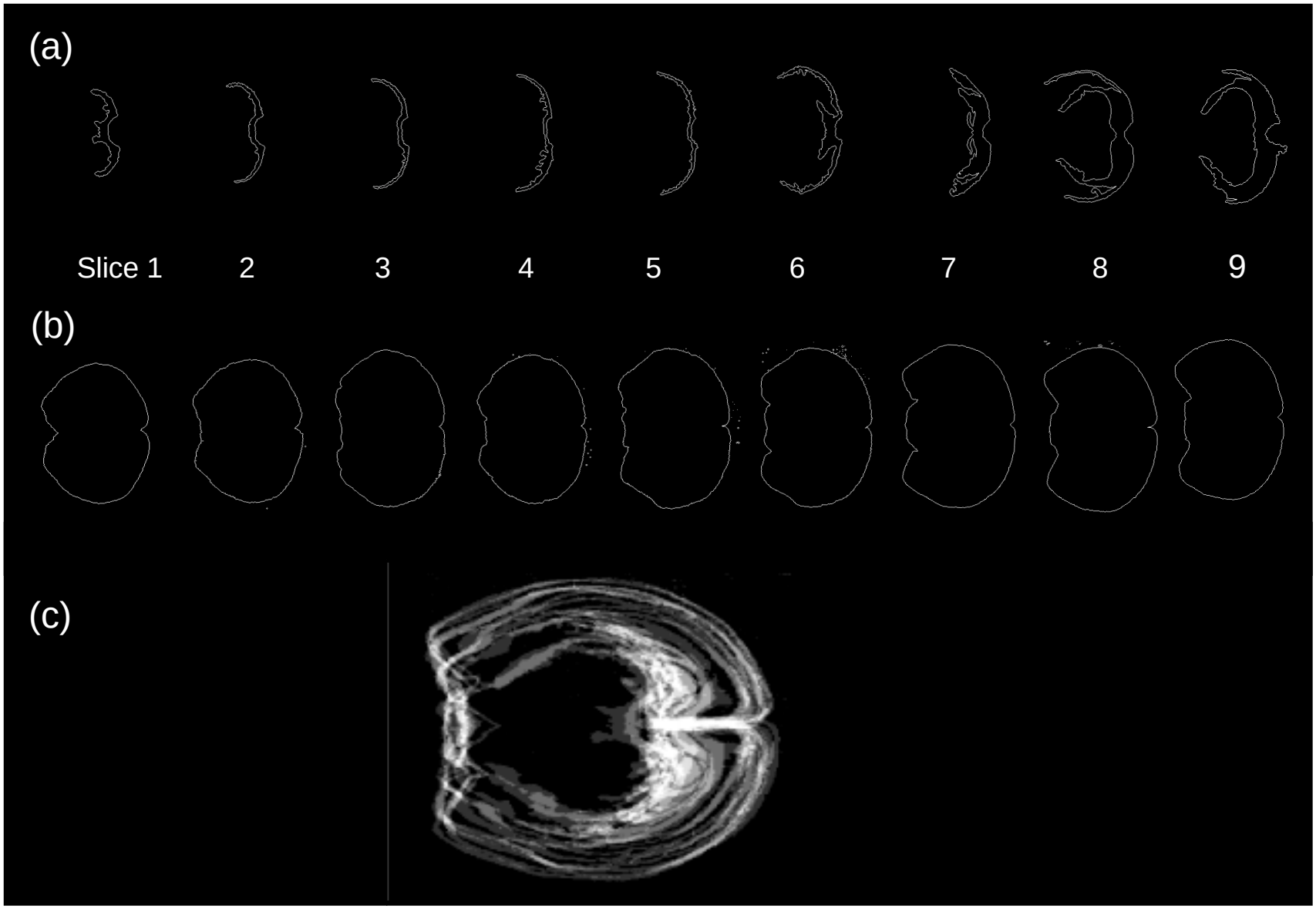
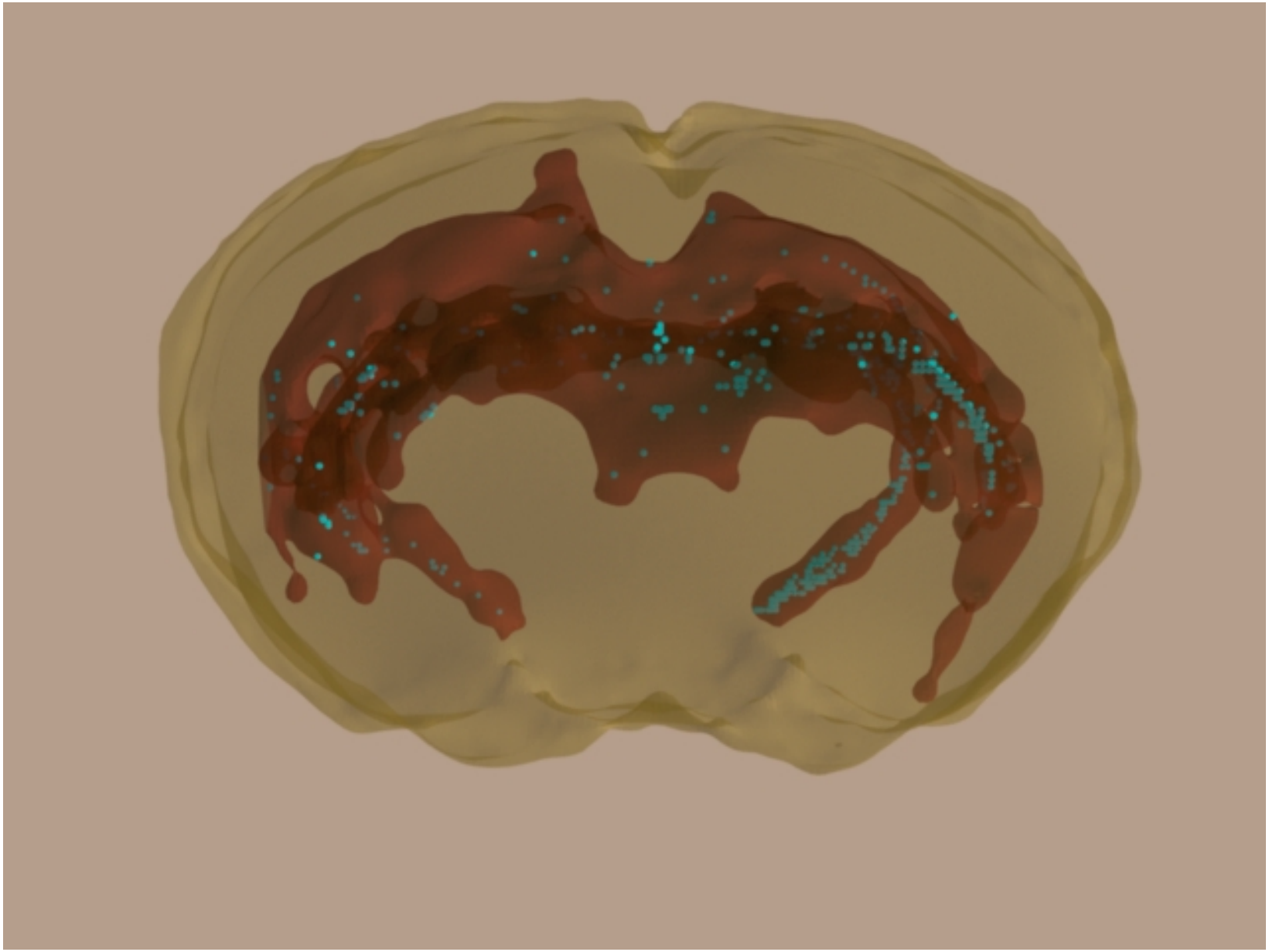


Figure 6. Computer-assisted 3-D reconstruction of the corpus callosum.



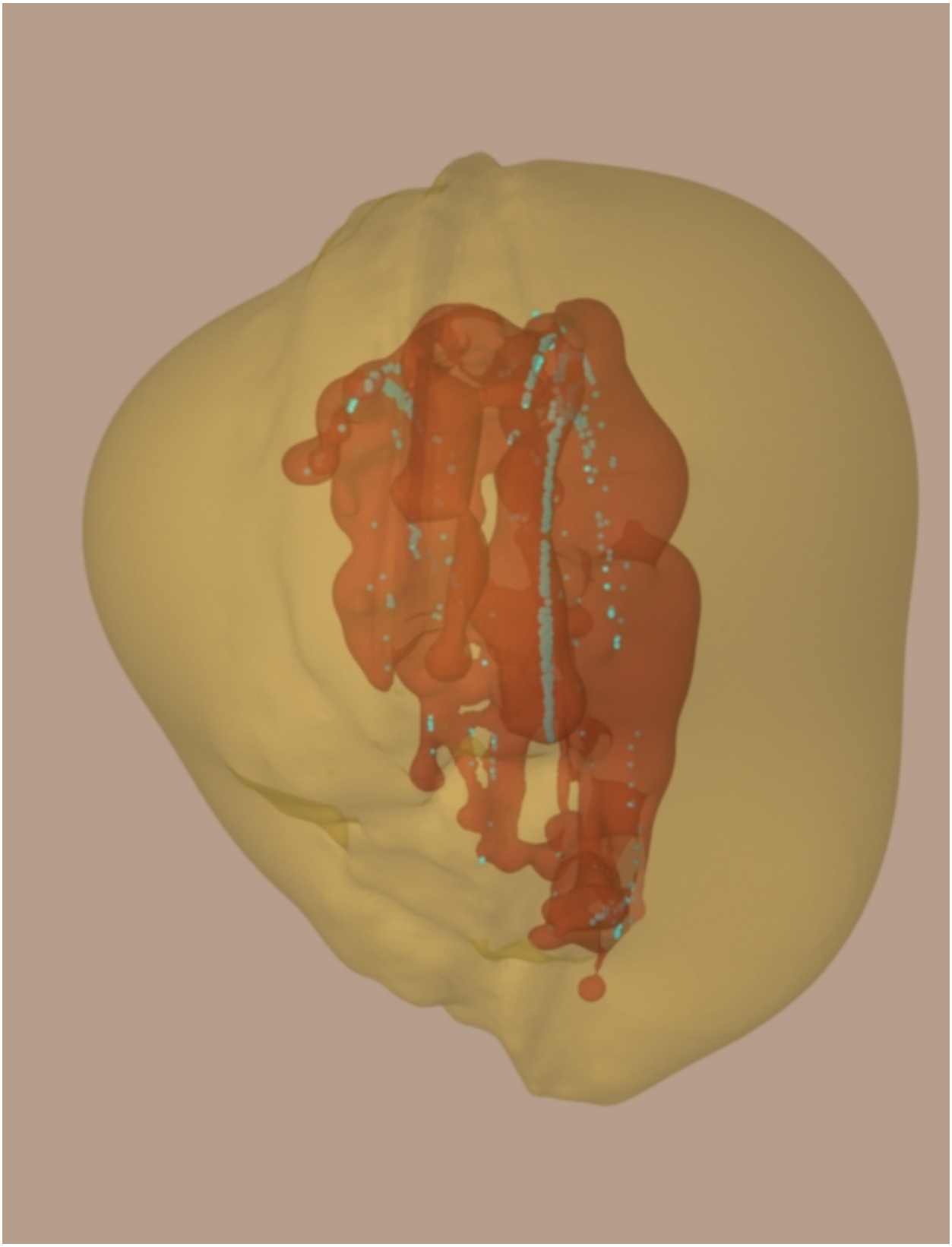
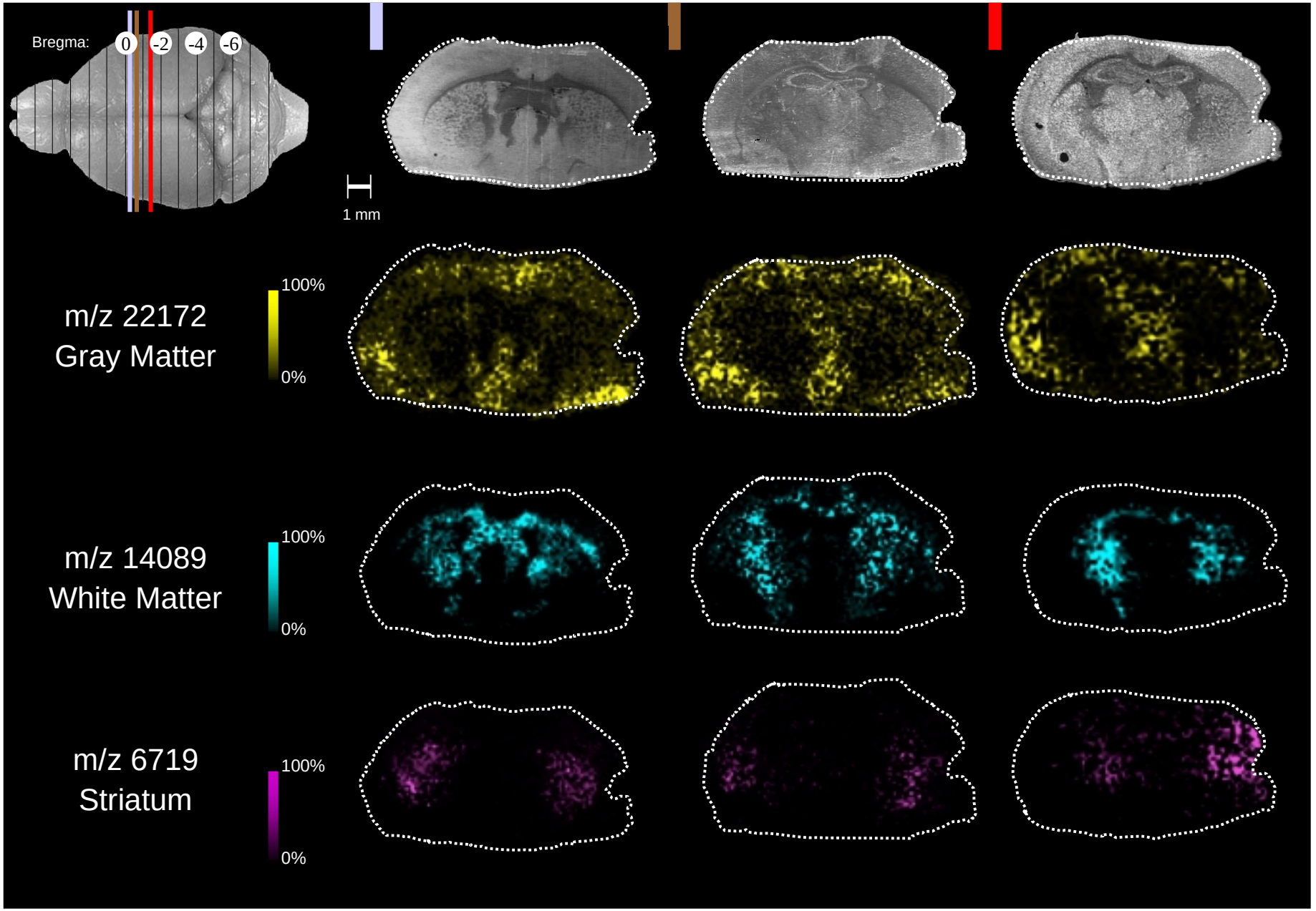


Figure 7. MS images of serial mouse brain sections.



Conclusions

The stacking parameters essential for 3-D imaging mass spectrometry were successfully developed with printed images of mouse brain slices on paper. The next stage is the integration of 3-D warping parameters using real mouse brain tissue in order to advance this technique for the 3-D mapping of marker proteins in the mouse brain.

References

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[9] Maya, http://www.aliaswavefront.com

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