Developing 3-D Imaging Mass Spectrometry

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Imaging MALDI mass spectrometry has been successfully used to obtain the distribution of proteins in thin tissue slices [1]. 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.

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. Nine serial images spanning the full corpus callosum of a male C57Bl/6J mouse brain are downloaded from a brain atlas (http://www.mbl.org/atlas170/atlas170_frame.html). 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 [2-3].

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, as shown in Figure 1. 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. 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. Therefore, they can be identified during the imaging process. To align both images a computer algorithm [4] is used to identify the x,y coordinates of the centers of the dots in both the optical and MS ion images. 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 [5]. 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 [6]. 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 [7]. The resulting 3-D model is presented in Figure 2.

This study shows the successful development of stacking parameter tools for 3-D imaging mass spectrometry. The next stage is the construction 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.

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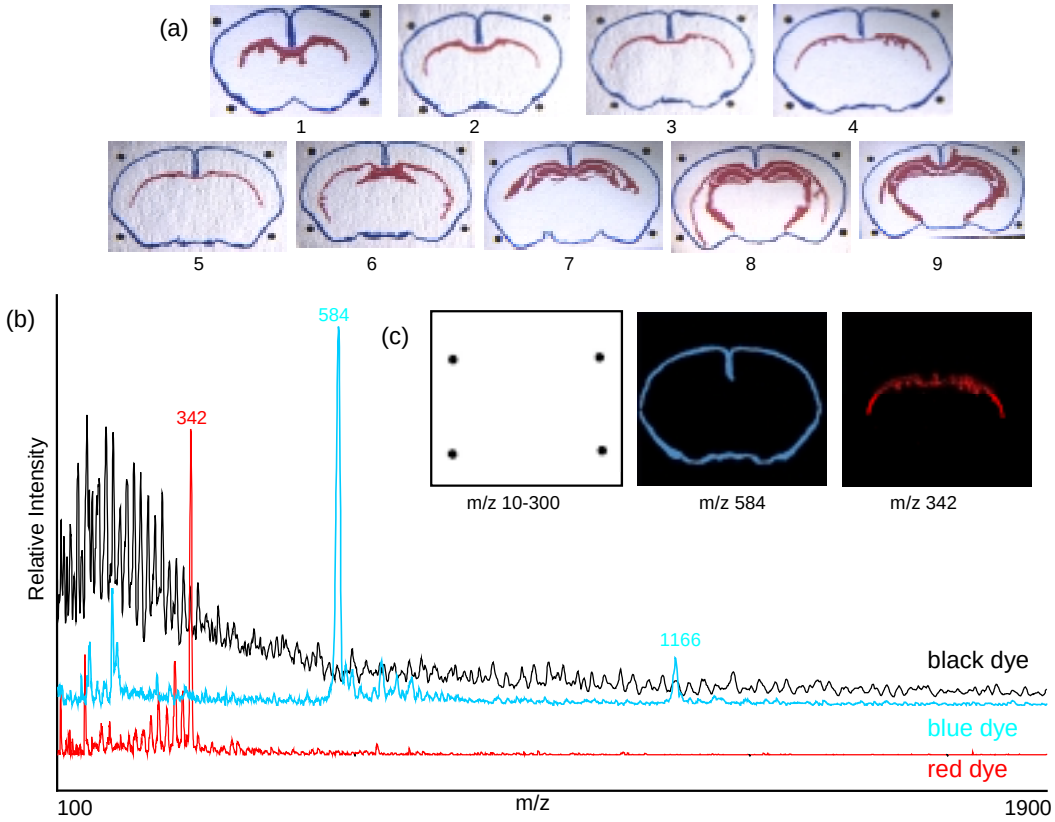

Figure 1. MS analysis of model sections. (a) optical images of the printed mouse brain slices, (b) mass spectra of the different dyes, and (c) constructed MS images of the dyes (slice 4).

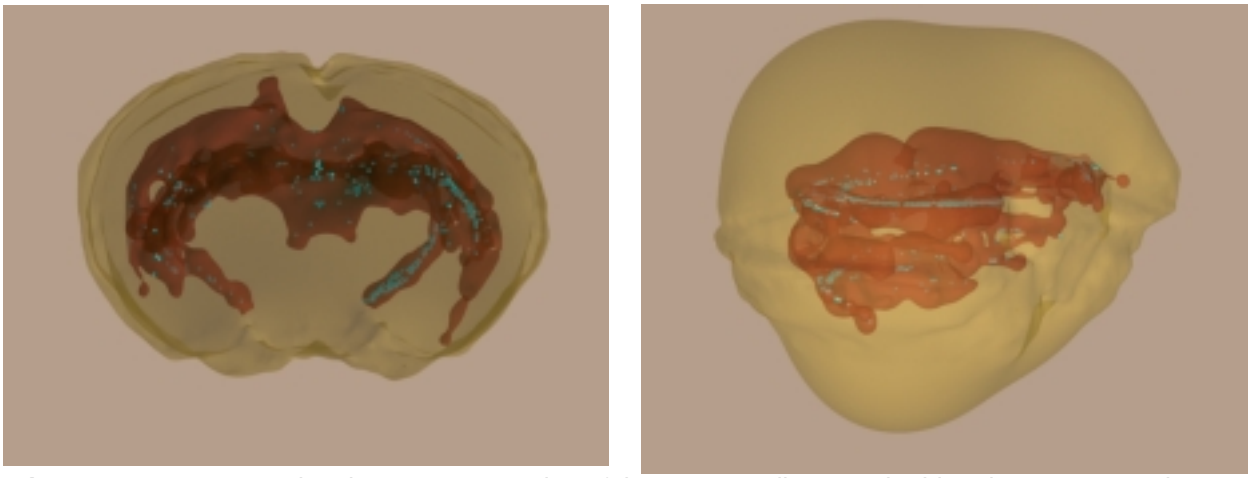

Figure 2. Computer-assisted 3-D reconstruction of the corpus callosum. The blue dots represent the MS data obtained from the blue dye.