# Enabling MSI-Guided Laser Capture Microdissection

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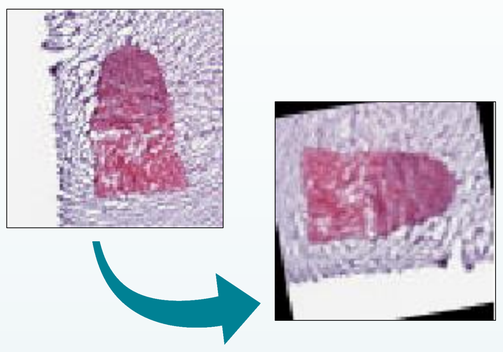
**Introduction:** Coupling MALDI mass spectrometry imaging (MALDI-MSI) with Laser Capture Microdissection (LCM) allows for precise dissection of tissue regions based on molecular features [1]. Automated methods for alignment of the coordinate systems of the MSI and LCM platforms reduces errors associated with manual definition of ROI's and increases throughput, which is a major bottleneck for LCM. Here we present the development of a method to transfer regions of interest from MALDI MSI images to an LCM platform, using consecutive tissue sections mounted on ITO conductive slides for MALDI MSI and on PEN-coated slides for LCM.

**Methods:** The test system consists of a gelatin-embedded pancreatic cancer needle biopsy. 12 µm slices were cut using a cryostat and two consecutive slices were mounted on ITO and PEN slides. The ITO slide was spray-coated with DHB (30mg/mL, MeOH 70%, water 30%, 0.2% TFA) and a MALDI-MSI data acquired with an EP-MALDI source coupled to a Q-Exactive mass spectrometer. The MSI data was imported into MATLAB. The tissue mounted on the PEN slide was stained with hematoxylin and a high resolution optical image acquired using an Aperio Scanscope. The LCM instrument used was an Apotome 2 Axio Observer Z1 microscope equipped with a PALM MicroBeam LCM system (both Zeiss).

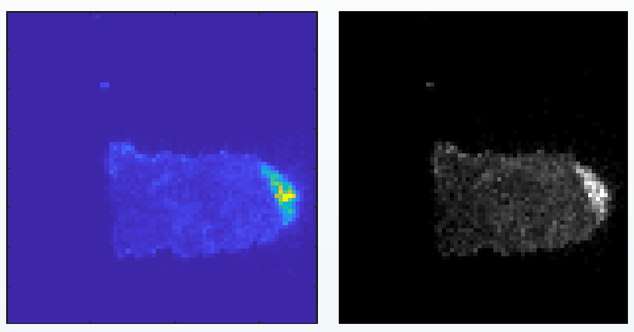
**Results:**

## Digital histology alignment to MALDI image

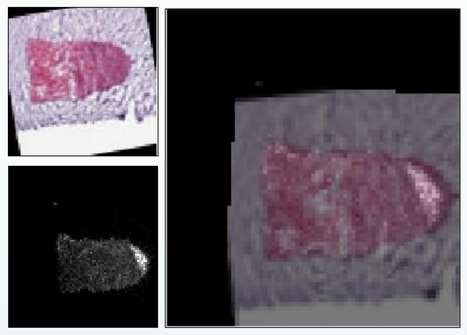
Histological image is acquired and imported into MATLAB. The JPEG image is then cropped to approximately match the MALDI image.



Cropped histological image is resized and rotated to approximately match the MALDI image.

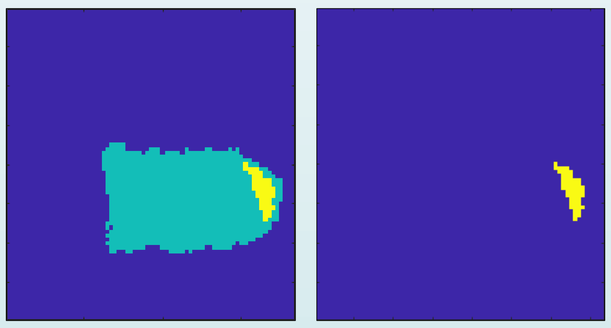


A greyscale MS image of a selected ion is chosen as a fixed image for the alignment.

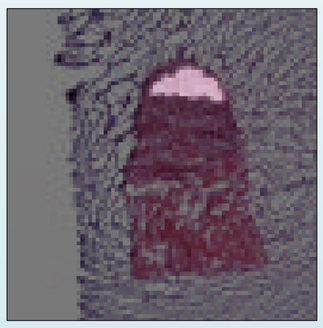


An intensity-based co-registration algorithm [2] is used to align the digital histology to the MALDI image though affine and elastic transformations.

## Cluster of interest alignment with digital histology



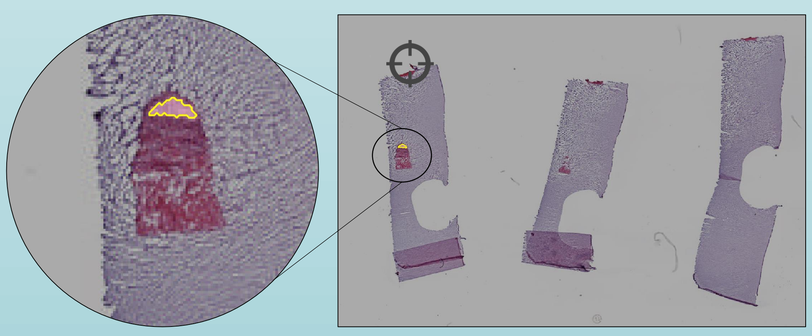
K-means cluster analysis is performed on the MSI data and a cluster selected as an image mask.



The affine and elastic transformations found previously are used to project the cluster mask to the histological image.

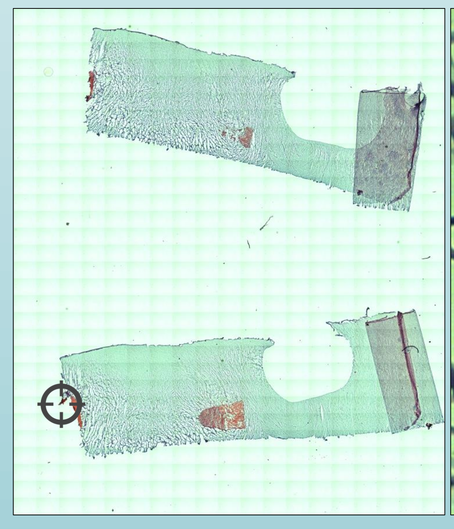
The cluster mask is layered on top of the cropped area histological image, filling the remaining pixels with zeroes. The image is then rescaled to the original dimensions.

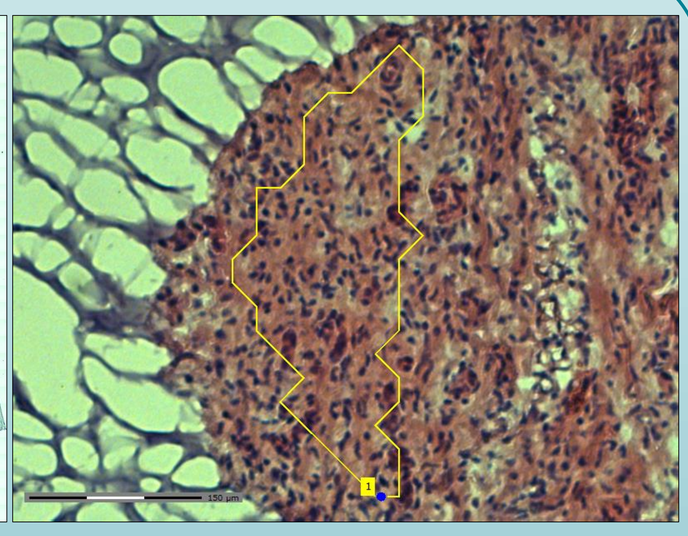
## Digital histology to LCM conversion



Holes in the cluster mask are filled and the coordinates of the border are extracted using a segmentation algorithm. A filter on the maximum number of allowed vertices can be applied.

The coordinates of reference points in the histological image are located in the LCM PALM RoboSoftware coordinate system. The coordinates are used to define the coordinate transformation, and thus to convert the coordinates of the vertices of the ROI into PalmRobo coordinates. Vertices of the selected region are written in a text file in the PALM RoboSoftware Element format.






**Conclusions:** The presented method enables rapid transfer of coordinates from a MALDI image to an LCM instrument, increasing throughput and reducing errors due to freehand cutting. The method is applicable to consecutive tissue sections, and ROI's can be defined either by MSI or via histopathological specification.

**References:** 1.Dilillo et al., Mass Spectrometry Imaging, Laser Capture Microdissection, and LC-MS/MS of the Same Tissue Section, *J. Proteome Res.* 2017, 16, 8, 2993-3001; 2.Image Registration App, by Brett Shoelson, July 2019.

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