Electronic Supplemental Material

A Versatile Platform for Mass Spectrometry Imaging of Arbitrary Spatial Patterns

Kenneth P. Garrard[1,2,3], Måns Ekelöf[1†], Sitora Khodjaniyazova[1], M. Caleb Bagley[1], and David C. Muddiman[1,3*]

[1]FTMS Laboratory for Human Health Research, Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695, United States
[2]Precision Engineering Consortium, Department of Mechanical and Aerospace Engineering, North Carolina State University, Raleigh, North Carolina 27695, United States
[3]Molecular Education, Technology and Research Innovation Center (METRIC), North Carolina State University, Raleigh, North Carolina 27695, United States

Submitted to: *Journal of The American Society for Mass Spectrometry*
Manuscript Type: Application Note
Supplemental Material: 9 Pages / 7 Figures
Running Title: Imaging arbitrary spatial patterns
Keywords: Mass Spectrometry Imaging, Arbitrary Region of Interest, MALDESI, Vision-System

Author Information

Corresponding Author
*David C. Muddiman, Ph.D.
FTMS Laboratory for Human Health Research
Department of Chemistry
Molecular Education, Technology, and Research Innovation Center (METRIC)
North Carolina State University
Raleigh, North Carolina 27695
Phone: 919.513.0084
Email: dcmuddim@ncsu.edu

ORCID
Kenneth P. Garrard: 0000-0002-0654-4776
Måns Ekelöf
Sitora Khodjaniyazova: 0000-0002-9283-2394
M. Caleb Bagley: 0000-0001-8792-6057
David C. Muddiman: 0000-0003-2216-499X

Present Addresses
[†]EMBL Heidelberg, Meyerhofstraße 1, 69117 Heidelberg, Germany

Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

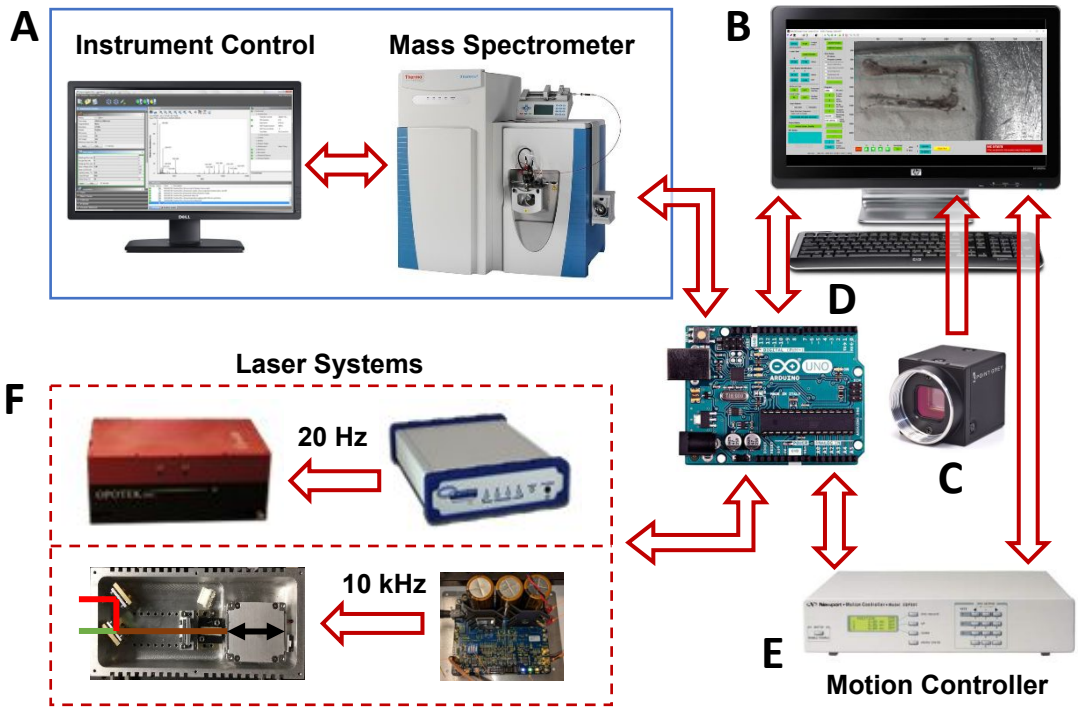

Figure S1. IR-MALDESI System Components.

A) RastirX is agnostic with respect to the mass spectrometer, provided there are two "handshake" signals: one to trigger scan acquisition and another indicating that the instrument is ready to acquire a scan. The voltage and polarity of these signals for various instruments is easily accommodated with opto-coupled relays and simple microprocessor (D) code changes. Mass spectrometers interfaced to date include: Thermo Fisher Scientific LQT-FT-ICR Ultra, Q Exactive, Q Exactive Plus, Q Exactive HF-X and Agilent 6560 IM-QTOF.

B) The RastirX user interface computer is any Windows PC with Matlab R2014a or later and the Image Processing and Image Acquisition toolboxes. RAM and HD (or SSD) requirements are modest – 8GB and 250GB, respectively. Three USB ports are needed for communication with the video camera (C), microcontroller (D) and motion control system (E).

C) A video camera with fixed focal length lens. 4K DCI resolution is desirable although any USB camera recognized by the Image Acquisition toolbox will work, as will any webcam.

D) An Arduino Uno microcontroller for synchronization of the laser, stage controller, and mass spectrometer. A very simple custom shield has been built for interfacing TTL I/O pins with the mass spectrometer.

E) A motion controller. Currently a Newport ESP300 is connected to a USB serial port on the user interface PC (B) to send commands and report current position and system status. TTL level signals are sent to and received from the microcontroller (D).

F) A mid-IR laser. Two laser systems are shown: (upper) a 20 Hz pulse rate Opotek Q-switched, tunable laser (2700 - 3100 nm wavelength) along with a Quantum Composers Sapphire 9200 pulse generator for precision triggering, and (lower) a 10 kHz pulse rate JGMA laser (2970 wavelength) with a DM-100 power supply/pulse generator. A menu selection in the RastirX interface is used to indicate which laser is installed.

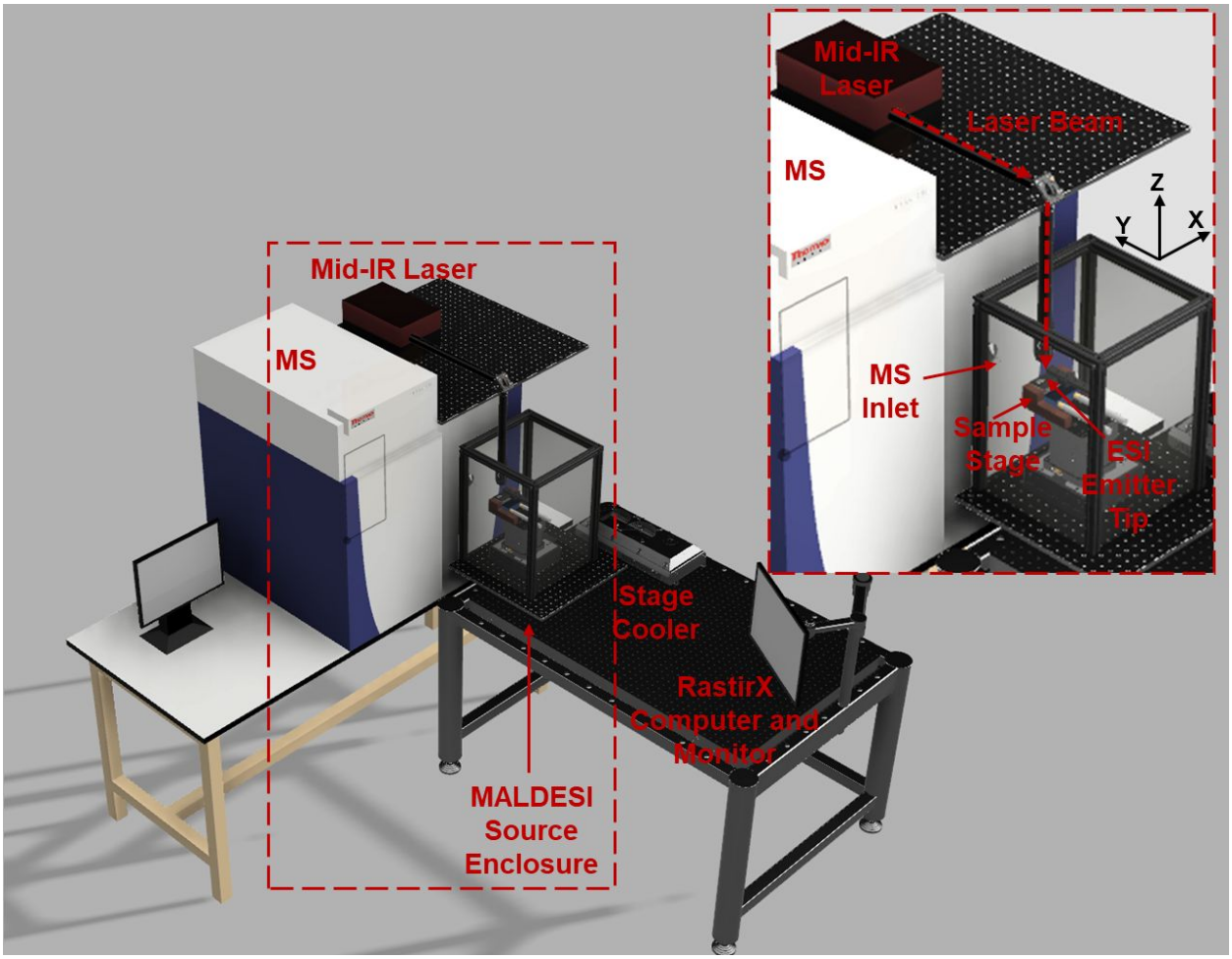

Figure S2. The IR-MALDESI platform. Each of the major components involved in experimental analyses are shown except for certain hardware pieces (i.e. the Newport ESP300, the Arduino Uno microcontroller, the CMOS video camera, and communication cables) to clearly illustrate the major components involved. The inset shows the laser beam path for the exit port on the laser to the sample stage. The camera is not shown but is less than two centimeters in the negative X-direction from the laser beam downtube and is in the same XZ-plane.

Figure S3. The RastirX Graphical User Interface.

A) The RastirX toolbar icons are used to (left-to-right): reset communications with the microcontroller and select a Windows COM port, reset communications with the stage controller and select a Windows COM port, capture the current RastirX user interface screen and save it as a PNG file, close the current command log file and open a new one, initiate the axes homing procedure, toggle between live video and the last image frame acquired, show/hide the pixel calibration line, show/hide the laser spot location tool, show/hide the ROI rectangle, lock/unlock the ROI and its dimensional parameters, launch the ROI Editor, plot the most recently generated ROI and the motion path used to image it, and finally the standard zoom and pan controls for the video image (L).

B) The Pixel Calibration panel shows the length of the calibration line drawn by the user during the setup procedure in camera pixels and its actual length in mm as entered by the user.

C) The Laser Spot panel shows the current position of the laser spot location tool relative to the axes home position.

D) The Scan Region Identification panel defines the area to be imaged. The quantities are updated when the ROI rectangle tool is used to draw or modify the ROI. Its location and size in mm are given along with the spot and line spacing. The number of spots per line and the number of lines are also displayed. Any of the 4 horizontal and vertical dimensional parameters can be changed and the others will be automatically updated. A right-click context menu (not shown) is used to specify which values in the over-constrained parameter set are held constant (i.e., as the user has entered them).

E) The Scan Pattern panel is used to select the raster pattern: *one-way* (all scan lines are acquired from left to right) or *meandering* (the first scan line is acquired from left to right, the second from right to left, etc.). In the current version of RastirX, scanning always starts at the upper-left most pixel and proceeds left-right and top-down over the sample.

F) The project name is used to create the files automatically saved by RastirX. The ROI status indicates whether or not the ROI drawn by the user is within the range of travel of the X and Y axes.

G) The MoveTo panel contains two buttons that move the stages to predefined positions LASER (for imaging) and CAMERA (for setup).

H) The Scan Setup panel indicates the status of each required setup item that must be completed prior to imaging.

I) The Program panel contains parameters of the imaging process, e.g., the laser type, number of laser pulses per scan, number of times the image is replicated (for ablation-based 3D imaging), instrument resolving power, and acquisition mode (full handshake or low-latency handshake). The acquisition mode is specific to Thermo Scientific instruments. This panel also displays the number of acquired scans and the number of scans remaining in a run. The LOAD PROGRAM, CANCEL, and RUN buttons are used to send parameters to the microcontroller, reset those parameters, and start acquisition, respectively. The RUN button changes to STOP immediately upon being clicked and can then be used to abort image acquisition.

J) The X and Y stages can be moved manually by entering a distance to move, selecting a speed, and clicking a direction button. The current position of the axes is always displayed.

K) The Single Shot button fires a burst of pulses from the laser on demand. This is primarily used during setup to adjust laser focus and measure laser power with a laser power meter.

L) The video camera image is updated in real time as the stages are moved. ROIs are drawn and edited on top of this image. The displayed units are camera pixels with an aspect ratio of 1:1.

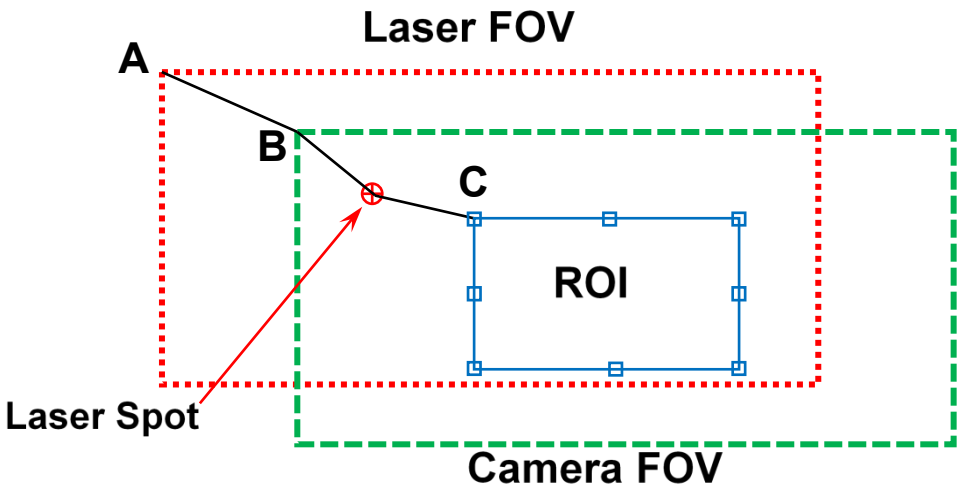

Figure S4. Image calibration.

The camera and laser are not coincident but have overlapping fields of view (FOV). The X and Y offsets from point **A** to point **B** in the figure are found by firing the laser into burn paper (or the sample slide) with the stages at the LASER position and then locating the burn mark with the laser spot tool after moving the stages to CAMERA position. This provides the values needed to translate any point in the camera FOV where the ROI is drawn to the laser FOV where imaging occurs. When the ROI is drawn, the offset from point **B** to **C** is known in camera pixel units. Simple scaling by camera/pixels per mm and translation gives the stage coordinates of the upper left corner of the ROI that will place that point under the laser. The overlapping area of the two FOV rectangles is the sample space that can be imaged with fixed LASER and CAMERA stage positions using this calibration procedure.

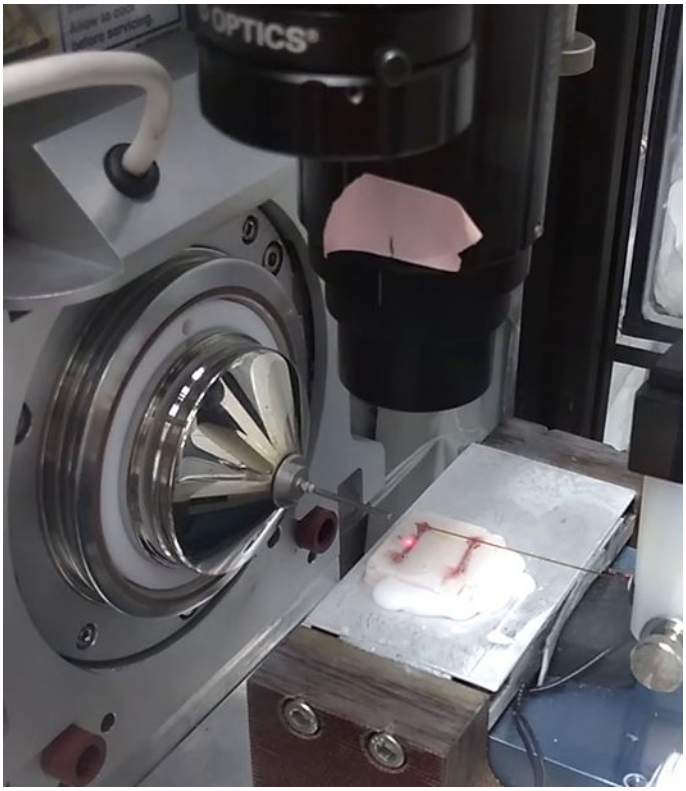

Figure S5. Imaging a mouse femur with the IR-MALDESI and RastirX platforms.

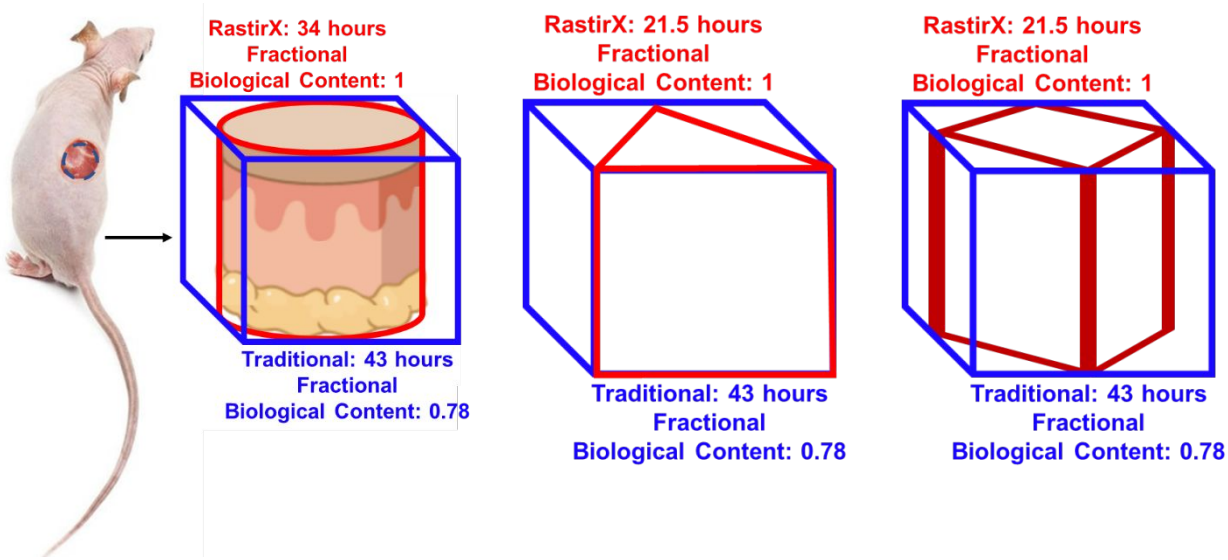

Figure S6. Benefits of RastirX for 3D Imaging.

Time savings were calculated based on a square ROI area of 25 mm^2, with a laser XY spatial resolution of 50 µm (100 shots × 100 lines), an MS scan rate of 1.6/second, and 25 layers analyzed in the Z dimension. Fractional biological content is a ratio of time spent on meaningful sample divided by total time spent collecting data. The size of the acquired data file is also reduced by the same ratio.

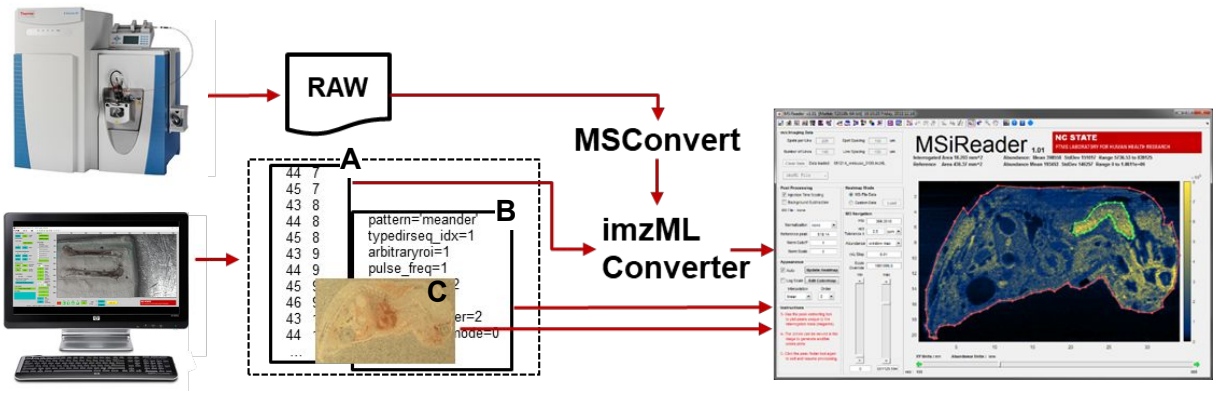

Figure S7. Integration with MSiReader.

Workflow through MSConvert [1] and the imzMLConverter [2] to MSiReader is shown. RastirX creates two files, (**A**) ROI location and (**B**) scan parameters, that are needed to convert RAW data to imzML while preserving the spatial location and spacing of each scan. The ROI location file identifies the X and Y coordinates (in integer units) of each scan. The imzMLConverter uses this file to create an imzML [3] data set that can be read into MSiReader. The parameter file records the spot and line spacing and scan pattern (one-way or meander) along with all other RastirX selections and options (e.g., pulses per scan). The third file (**C**) is an optical image captured automatically by RastirX prior to image acquisition. This optical image can be inherently co-registered (i.e., overlaid) with all molecular images created by MSiReader for this data set.

References

1. Kessner, D., Chambers, M., Burke, R., Agus, D., Mallick, P. ProteoWizard: open source software for rapid proteomics tools development. *Bioinformatics*. **24**, 2534-2536 (2008).

2. Race, A.M., Styles, I.B., Bunch, J. Inclusive sharing of mass spectrometry imaging data requires a converter for all. *J. Proteomics*. **75**, 5111-5112 (2012).

3. Schramm, T., Hester, A., Klinkert, I., Both, J.P., Heeren, R.M., Brunelle, A., Laprevote, O., Desbenoit, N., Robbe, M.F., Stöckli, M., Spengler, B., Römpp, A. imzML--a common data format for the flexible exchange and processing of mass spectrometry imaging data. *J. Proteomics*. **75**, 5106-5110 (2012).