

Analytical Research in the Owens Group

Mass Spectrometry

Matrix Assisted Laser Desorption/Ionization (MALDI)



Owens Research Group

- **Analytical Chemistry- Mass Spectrometry**

- Biological and synthetic polymer analysis using matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry (TOFMS)
- Development of sample preparation techniques for quantitative MALDI analysis
- Development of improved matrix deposition techniques for use in MALDI imaging
- Development of laser spectroscopic techniques for combustion analysis (jointly with David Miller & Nick Cernansky, MEM)
- Development of a combined correlation analysis/genetic algorithm and other chemometric techniques for automating the analysis of mass spectral information

Instrumentation employed: MALDI Time-of-flight mass spectrometers, triple-quadrupole LC-MS, Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer, atomic force microscope, capillary GC-MS, UV-visible absorption, Fourier-transform IR absorption, fluorescence, etc.

What's New and Cool in the World of MALDI Mass Spectrometry on Stratton 4th?

- MALDI Imaging: **Brian Malys**
- Identification of Micro-Organisms by MALDI Mass Spectrometry: **Elsa Gorre**

MALDI TOFMS Imaging

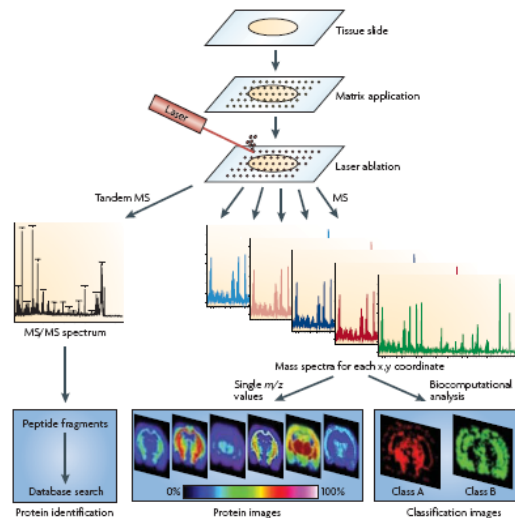
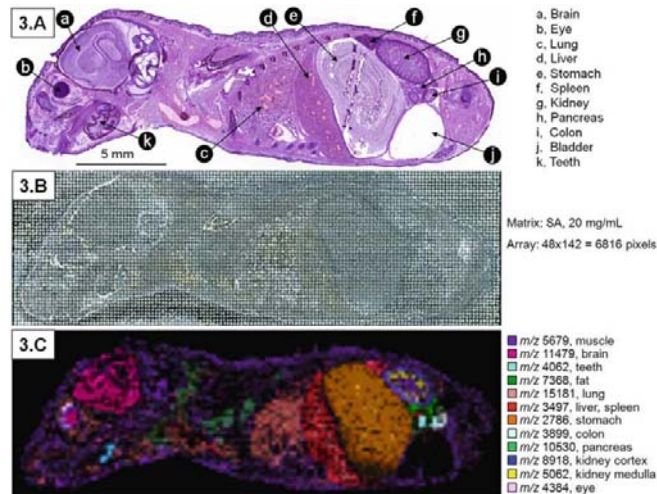


Figure 2 | **Workflow for MALDI IMS analysis.** Schematic outline of a typical workflow for fresh frozen tissue samples. Sample pretreatment steps include cutting and mounting the tissue section on a conductive target. Matrix is applied in an ordered array across the tissue section and mass spectra are generated at each x,y coordinate for protein analysis or tandem MS (MS/MS) spectra for protein identification. Further analytical steps include the visualization of the distribution of a single protein within the tissue (protein image) or statistical analysis to visualize classification images as well as database searching to identify the protein. The scale represents the relative intensity of the protein. IMS, imaging mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; m/z , mass to charge ratio.

Kristina Schwamborn and Richard M. Caprioli, "Molecular imaging by mass spectrometry — looking beyond classical histology", NATURE REVIEWS | CANCER, 2010, 10, 639-46

Whole-Body Tissue Section



MALDI imaging of proteins

Fig. 17.3. Whole-body imaging MS of proteins from a 1-day-old mouse pup. (a) H&E stained section from which numerous organs are clearly visible. (b) Serial section used for imaging MS on which matrix (sinapinic acid) has been automatically deposited in an array manner with a final center-to-center spacing of 200 μm . (c) Overlay of 12 individual organ or tissue-specific protein images, each presented with a different color. See text for details.

Michelle L. Reyzer, Pierre Chaurand, Peggi M. Angel, Richard M. Caprioli, "Direct Molecular Analysis of Whole-Body Animal Tissue Sections by MALDI Imaging Mass Spectrometry", in S.S. Rubakhin, J.V. Sweedler (eds.), *Mass Spectrometry Imaging*, Methods in Molecular Biology 656, DOI 10.1007/978-1-60761-746-4_17, Springer Science Business Media, LLC 2010

MS versus Histology

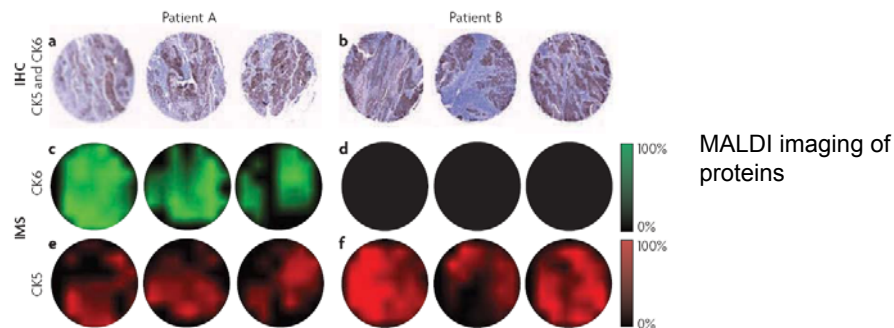
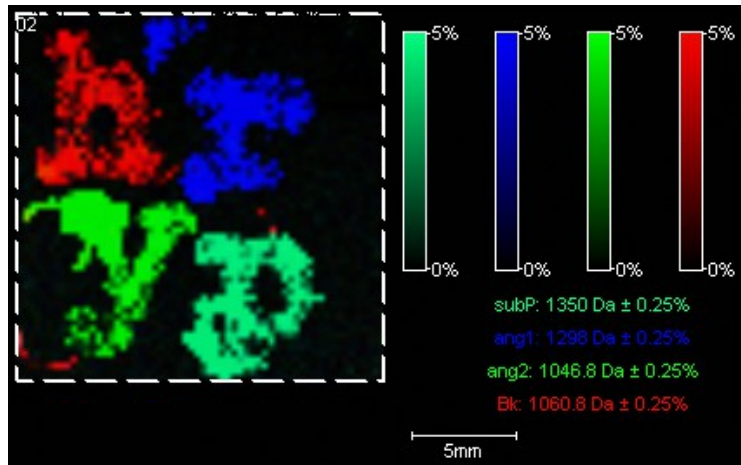


Figure 3 | **Comparison of IHC and MALDI IMS results.** Immunohistochemical (IHC) staining with an antibody against cytokeratin 5 (CK5) and CK6 of triplicate lung cancer biopsy samples from two different patients (patient A or patient B) showing positive staining in all biopsy samples (parts a and b). The corresponding peptide image of a 1407.7 Da fragment of CK6 (green; parts c and d) reveals this peptide to be present only in one patient, whereas the 1410.7 Da fragment of CK5 (red; parts e and f) is present in all biopsy samples. The scale represents the relative intensity of the peptide. IMS, imaging mass spectrometry; MALDI, matrix-assisted laser desorption/ionization. Image courtesy of M.R. Groseclose, Vanderbilt University, USA.

Kristina Schwamborn and Richard M. Caprioli, "Molecular imaging by mass spectrometry — looking beyond classical histology", *NATURE REVIEWS | CANCER*, 2010, 10, 639-46

Peptide Imaging by MALDI



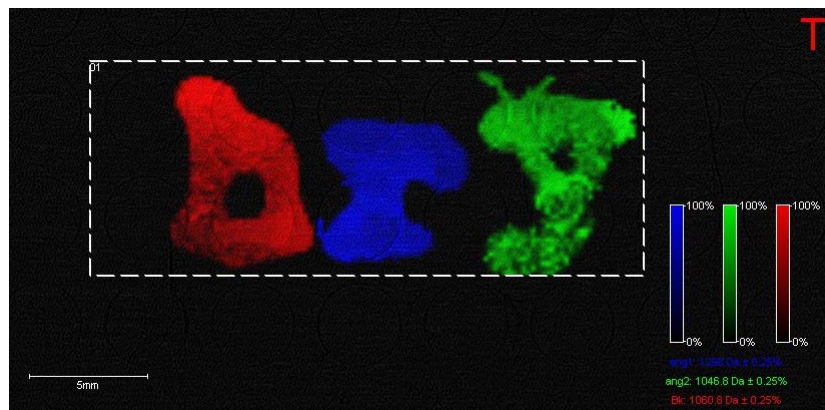
Analytes stamped from solutions: bradykinin as "h" shown in red, angiotensin I as "r" shown as blue, angiotensin II as "y" shown as bright green and substance P as "p" as pale green.

Brian Malys

CHCA matrix applied by ESD

300 um raster size

Peptide Imaging by MALDI



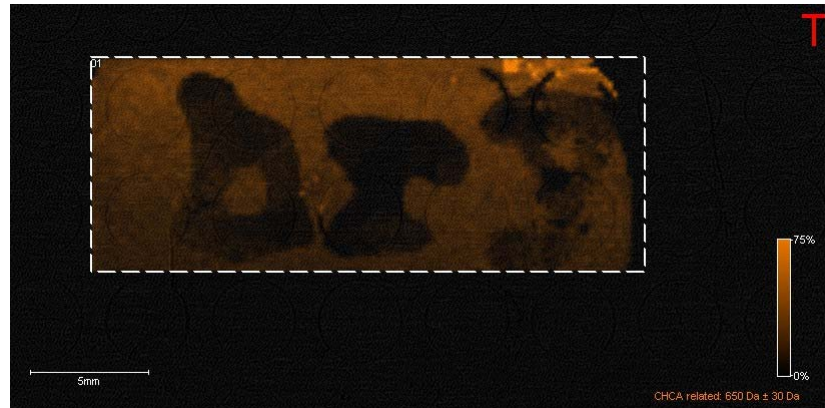
Analytes stamped from solutions, bradykinin as "h", angiotensin I as "r" and angiotensin II as "y".

Brian Malys

CHCA matrix applied by ESD

200 um raster size

Peptide Imaging by MALDI



Analytes stamped from solutions, bradykinin as "h", angiotensin I as "r" and angiotensin II as "y". Normalized intensity of peaks related to CHCA clusters shown in orange.

Brian Malys

CHCA matrix applied by ESD

200 μ m raster size

Bacterial Identification

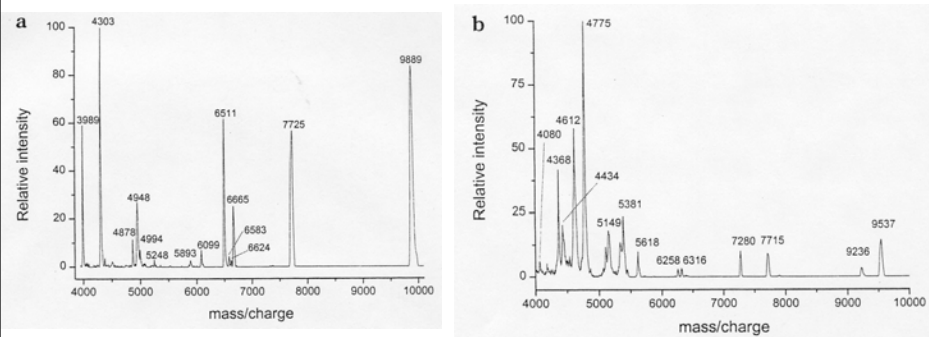


Figure 2. Positive ion MALDI spectra from (a) *B. subtilis* (8 h growth time), matrix SA; (b) *E. coli* (32 h growth time), matrix CHCA (see Experimental Section for more details).

C. Fenselau et al., Anal. Chem., 71(14), 2732, 1999.

Identification of Micro-Organisms

Bruker BioTyper

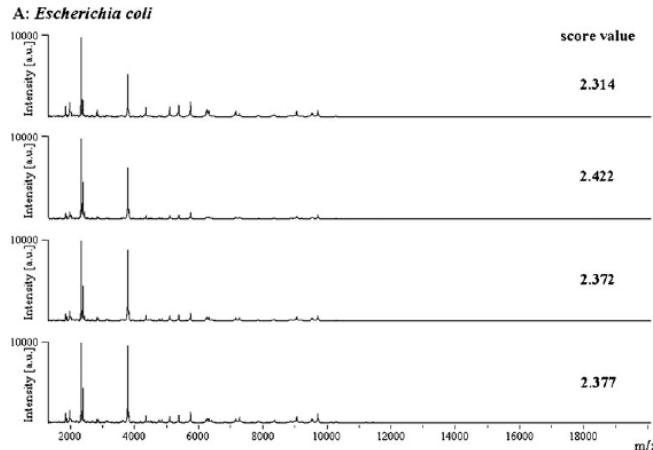


Fig. 1 Colony-to-colony differences of the MALDI-TOF MS based identification of clinical bacterial isolates. Clinical isolates of the Gram-negative rods *Escherichia coli* (A) and *Pseudomonas aeruginosa* (B) obtained from four different colonies were analyzed by the rapid microorganism identification method combining MALDI-TOF MS and MALDI BioTyper. Colony-to-colony differences are minimal.

Kazuyuki Sogawa, Masaharu Watanabe, Kenichi Sato, Syunsuke Segawa, Chisato Ishii, Akiko Miyabe, Syota Murata, Tomoko Saito, Fumio Nomura, "Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms", *Anal Bioanal Chem*, 2011, DOI 10.1007/s00216-011-4877-7.

Identification of Micro-Organisms

Bruker BioTyper

Conventional method	MALDI BioTyper method	16S rRNA method
<i>Aeromonas hydrophila</i> group	<i>Aeromonas caviae</i> <i>Aeromonas hydrophila</i>	<i>Aeromonas caviae</i> <i>Aeromonas hydrophila</i>
<i>Bordetella parapertussis</i>	<i>Bordetella bronchiseptica</i>	<i>Bordetella bronchiseptica</i> or <i>Bordetella parapertussis</i>
<i>Enterobacter cloacae</i>	<i>Enterobacter asburiae</i> <i>Enterobacter ludwigii</i>	<i>Enterobacter cloacae</i> <i>Enterobacter cloacae</i>
<i>Enterococcus casseliflavus</i>	<i>Enterococcus phoenicolicola</i>	<i>Enterococcus casseliflavus</i>
<i>Haemophilus parahaemolyticus</i>	<i>Haemophilus parainfluenzae</i>	<i>Haemophilus parainfluenzae</i>
<i>Haemophilus parainfluenzae</i>	<i>Haemophilus parahaemolyticus</i>	<i>Haemophilus parahaemolyticus</i>
<i>Lactobacillus acidophilus</i>	<i>Lactobacillus gasseri</i>	<i>Lactobacillus gasseri</i>
<i>Stenotrophomonas maltophilia</i>	<i>Pseudomonas geniculata</i> <i>Pseudomonas hibiscicola</i>	<i>Stenotrophomonas maltophilia</i> <i>Stenotrophomonas maltophilia</i>
<i>Streptococcus bovis</i>	<i>Streptococcus pasteurianus</i>	<i>Streptococcus pasteurianus</i>
<i>Vibrio fluvialis</i>	<i>Vibrio furnissii</i>	<i>Vibrio furnissii</i>

Table 2 Comparison of the results obtained by use of the conventional and MALDI-TOF MS-based methods and the 16S rRNA method

Kazuyuki Sogawa, Masaharu Watanabe, Kenichi Sato, Syunsuke Segawa, Chisato Ishii, Akiko Miyabe, Syota Murata, Tomoko Saito, Fumio Nomura, "Use of the MALDI BioTyper system with MALDI-TOF mass spectrometry for rapid identification of microorganisms", *Anal Bioanal Chem*, 2011, DOI 10.1007/s00216-011-4877-7.

Owens Research Group

- Example Undergraduate Research Projects (last 5 years)
 - Forensic Investigation of Arson Accelerants (Gasoline) via GC/MS (2013-14, Joseph Trusello)
 - Investigation of the Solution and Solid-Phase Fluorescence Behavior of MALDI Matrices, and the Effect of Solvent on MALDI Signal Intensity (2012-13, Francisco Guevara- Bruker Daltonics)
 - GC/MS Analysis of Diacetal in Beer (2011-12, Marisa Egan- Victory Brewing)
 - Analysis of Peptide Trypsin Digestion Fragments via Triple Quadrupole LC/MS (2011-12, Craig Gunnett)
 - MALDI and ESI-MS Investigations of Peptides (2012, Lucas de Lima Ferreira)

Owens Research Group

- Example Undergraduate Research Projects (last 5 years)
 - Investigation of the Kinetics of Trypsin Digestion of Peptides by MALDI TOFMS (2010, Collin Murphy and Sean Devenny, Francisco Guevara)
 - Determination of the Off-Flavor Components of Beer by Solid Phase Extraction GC/MS (2009-10, Alex Bilinski, jointly with Dr. Phil Handel, Food and Culinary Sciences)
 - Development of Thick-Film Gelatin Based Substrates for Optical Holography (2008-09, Adria Wilson- Duke Univ, in collaboration with Dr. Richard Billmers, RL Associates)

Let's Do Some Analytical Chemistry Together!

- Christina Emeigh
- Elsa Gorre
- Brian Malys
- Michelle Piotrowski

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<http://www.chemistry.drexel.edu/people/owens/index.htm>