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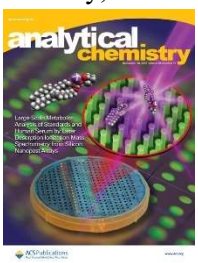
FINAL TECHNICAL REPORT

1. Publications supported in full or in part by the grant

1.1 Published journal articles

1/ Jarod A. Fincher, Andrew R. Korte, Brent Reschke, Nicholas J. Morris, Matthew J. Powell, and Akos Vertes, "Enhanced Sensitivity and Metabolite Coverage with Remote Laser Ablation Electrospray Ionization-Mass Spectrometry Aided by Coaxial Plume and Gas Dynamics," *Analyst*, **2017**, E-pub, ahead of print. http://dx.doi.org/10.1039/C7AN00805H

2/ Hang Li, Pranav Balan, and Akos Vertes, "Molecular Imaging of Growth, Metabolism, and Antibiotic Inhibition in Bacterial Colonies by Laser Ablation Electrospray Ionization Mass Spectrometry," *Angew. Chem. Int. Ed.*, **2016**, *55*, 15035-15039. (Article Featured on Back Cover) http://dx.doi.org/10.1002/anie.201607751



3/ Andrew R. Korte, Sylwia A. Stopka, Nicholas Morris, Trust Razunguzwa, and Akos Vertes, "Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays," *Anal. Chem.*, **2016**, *88*, 8989-8996. (Cover Page Article) http://dx.doi.org/10.1021/acs.analchem.6b01186

4/ Sylwia A. Stopka, Charles Rong, Andrew R. Korte, Sridevi Yadavilli, Javad Nazarian, Trust T. Razunguzwa, Nicholas J. Morris and Akos Vertes, "Molecular Imaging of Biological Samples on Nanophotonic Laser Desorption Ionization Platforms," *Angew. Chem. Int. Ed.*, **2016**, 55, 4482-4486. (Cover Page Article) http://dx.doi.org/10.1002/anie.201511691

5/ Rachelle S. Jacobson, Richard Thurston, Bindesh Shrestha, and Akos Vertes, "In Situ Analysis of Small Populations of Adherent Mammalian Cells Using Laser Ablation Electrospray Ionization Mass Spectrometry in Transmission Geometry," *Anal. Chem.*, **2015**, *87*, 12130-12136. http://dx.doi.org/10.1021/acs.analchem.5b02971

6/ Hang Li, Emmeline Ha, Robert P. Donaldson, Aleksandar M. Jeremic and Akos Vertes, "Rapid Assessment of Human Amylin Aggregation and its Inhibition by Copper(II) Ions by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation," *Anal. Chem.*, **2015**, *87*, 9829-9837. http://dx.doi.org/10.1021/acs.analchem.5b02217

7/ Sylwia A. Stopka, Tarek R. Mansour, Bindesh Shrestha, Eric Marechal, Denis Falconet and Akos Vertes, "Turnover Rates in Microorganisms by LAESI Mass Spectrometry and Pulse-chase Analysis," *Anal. Chim. Acta*, **2016**, *902*, 1-7. (Feature Article) (Cover Page Article) http://dx.doi.org/10.1016/j.aca.2015.08.047

8/ Rohit Shroff, Katharina Schramm, Verena Jeschke, Peter Nemes, Akos Vertes, Jonathan Gershenzon and Ales Svatos, "Quantification of plant surface metabolites by MALDI mass spectrometry imaging: glucosinolates on *Arabidopsis thaliana* leaves," *The Plant Journal*, **2015**, *81*, 961-972. http://dx.doi.org/10.1111/tpj.12760

9/ Sylwia A. Stopka, Bindesh Shrestha, Eric Marechal, Denis Falconet and Akos Vertes, "Metabolic Transformation of Microalgae Due to Light Acclimation and Genetic Modifications Followed by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation," *Analyst*, **2014**, *139*, 5946 - 5954. http://dx.doi.org/10.1039/C4AN01368A

10/ Elizabeth Jaworski, Aarthi Narayanan, Rachel Van Duyne, Shabana Shabbeer-Meyering, Sergey Iordanskiy, Mohammed Saifuddin, Ravi Das, Philippe V. Afonso, Gavin C. Sampey, Myung Chung, Anastas Popratiloff, Bindesh Shrestha, Mohit Sehgal, Pooja Jain, Akos Vertes, Renaud Mahieux, Fatah Kashanchi, "Human T-lymphotropic Virus Type 1-infected Cells Secrete Exosomes That Contain Tax Protein," *J. Biol. Chem.*, **2014**, *289*, 22284-22305. http://dx.doi.org/10.1074/jbc.M114.549659

11/ Bindesh Shrestha and Akos Vertes, "High-Throughput Cell and Tissue Analysis with Enhanced Molecular Coverage by Laser Ablation Electrospray Ionization Mass Spectrometry Using Ion Mobility Separation," *Anal. Chem.*, **2014**, *86*, 4308–4315. http://dx.doi.org/10.1021/ac500007t

12/ Bindesh Shrestha, Robert Javonillo, John R. Burns, Zsolt Pirger and Akos Vertes, "Comparative local analysis of metabolites, lipids and proteins in intact fish tissues by LAESI mass spectrometry," *Analyst*, **2013**, *138*, 3444-3449. http://dx.doi.org/10.1039/c3an00631j

13/ Bennett N. Walker, Cory Antonakos, Scott T. Retterer and Akos Vertes, "Metabolic Differences in Microbial Cell Populations Revealed by Nanophotonic Ionization," *Angew. Chem. Int. Ed.*, **2013**, *52*, 3650-3653. http://dx.doi.org/10.1002/anie.201207348

14/ Anu Vaikkinen, Bindesh Shrestha, Javad Nazarian, Risto K. Kostiainen, Akos Vertes and Tiina J. Kauppila, "Simultaneous Detection of Nonpolar and Polar Compounds by Heat-Assisted Laser Ablation Electrospray Ionization-Mass Spectrometry," *Anal. Chem.*, **2013**, *85*, 177-184. http://dx.doi.org/10.1021/ac302432h

15/ J. A. Stolee, B. Shrestha, G. Mengistu and A. Vertes, "Observation of Subcellular Metabolite Gradients in Single Cells by Laser Ablation Electrospray Ionization Mass Spectrometry," *Angew. Chem. Int. Ed.*, **2012**, *51*, 10386-10389. (Article Featured on Back Cover) http://dx.doi.org/10.1002/anie.201206834

16/ B. N. Walker, J. A. Stolee and A. Vertes, "Nanophotonic Ionization for Ultratrace and Single-Cell Analysis by Mass Spectrometry," *Anal. Chem.*, **2012**, *84*, 7756-7762. http://dx.doi.org/10.1021/ac301238k

17/ J. A. Stolee, B. N. Walker, V. Zorba, R. E. Russo and A. Vertes, "'Laser-nanostructure Interactions for Ion Production," *Phys. Chem. Chem. Phys.*, **2012**, *14*, 8453-8471. (Perspectives Article) (Cover Page Article) http://dx.doi.org/10.1039/C2CP00038E

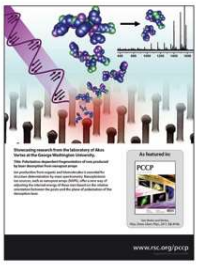
18/ A. Vaikkinen, B. Shrestha, T. J. Kauppila, A. Vertes and R. K. Kostiainen, "Infrared laser ablation atmospheric pressure photoionization mass spectrometry," *Anal. Chem.*, **2012**, *84*, 1630-1636. http://dx.doi.org/10.1021/ac202905y

19/ P. Nemes, H. Huang and A. Vertes, "Internal energy deposition and ion fragmentation in atmospheric-pressure mid-infrared laser ablation electrospray ionization," *Phys. Chem. Chem. Phys.*, **2012**, *14*, 2501-2507. http://dx.doi.org/10.1039/C2CP23411D

20/ G. Parsiegla, B. Shrestha, F. Carriere and A. Vertes, "Direct Analysis of Phycobilisomal Antenna Proteins and Metabolites in Small Cyanobacterial Populations by Laser Ablation Electrospray Ionization Mass Spectrometry," *Anal. Chem.*, **2012**, *84*, 34-38. http://dx.doi.org/10.1021/ac202831w

21/ P. Nemes and A. Vertes, "Ambient Mass Spectrometry for in vivo Local Analysis and in situ Molecular Tissue Imaging," *Trends in Analytical Chemistry (TrAC)*, **2012**, *34*, 22-34. http://dx.doi.org/10.1016/j.trac.2011.11.006

22/ J. A. Stolee and A. Vertes, "Polarization dependent fragmentation of ions produced by laser desorption from nanopost arrays," *Phys. Chem. Chem. Phys.*, **2011**, *13*, 9140-9146. (Article Featured on Back Cover) http://dx.doi.org/10.1039/C0CP02709J



23/ B. Shrestha, J. M. Patt and A. Vertes, "In Situ Cell-by-Cell Imaging and Analysis of Small Cell Populations by Mass Spectrometry," *Anal. Chem.*, **2011**, *83*, 2947-2955. http://dx.doi.org/10.1021/ac102958x

24/ J. A. Stolee, B. N. Walker, Y. Chen and A. Vertes, "Nanophotonic Ion Sources," in: *International Symposium on High Power Laser Ablation 2010*, C. R. Phipps (ed), *AIP Conf. Proc. 1278*, **2010**, 98-110. http://dx.doi.org/10.1063/1.3507188

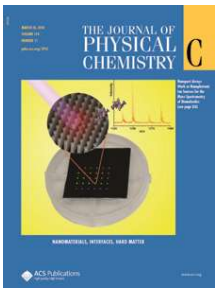
25/ P. Nemes and A. Vertes, "Atmospheric-pressure Molecular Imaging of Biological Tissues and Biofilms by LAESI Mass Spectrometry," *J. Visualized Experiments*, **2010**, *43*, e2097. http://dx.doi.org/10.3791/2097 or http://www.jove.com/index/details.stp?id=2097

26/ B. Shrestha and A. Vertes, "Direct Analysis of Single Cells by Mass Spectrometry at Atmospheric Pressure," *J. Visualized Experiments*, **2010**, *43*, e2144. http://dx.doi.org/10.3791/2144 or http://www.jove.com/index/details.stp?id=2144

27/ C. H. Stephens, B. Shrestha, H. R. Morris, M. E. Bier, P. M. Whitmore and A. Vertes, "Minimally invasive monitoring of cellulose degradation by desorption electrospray ionization and laser ablation electrospray ionization mass spectrometry," *Analyst*, **2010**, *135*, 2434-2444. http://dx.doi.org/10.1039/c0an00155d

28/ B. N. Walker, J. A. Stolee, D. L. Pickel, S. T. Retterer and A. Vertes, "Assessment of Laser-Induced Thermal Load on Silicon Nanostructures Based on Ion Desorption Yields," *Appl. Phys. A*, **2010**, *101*, 539-544. http://dx.doi.org/10.1007/s00339-010-5893-8

29/ B. Shrestha, P. Nemes and A. Vertes, "Ablation and analysis of small cell populations and single cells by consecutive laser pulses," *Appl. Phys. A*, **2010**, *101*, 121-126. http://dx.doi.org/10.1007/s00339-010-5781-2



30/ B. N. Walker, J. A. Stolee, D. L. Pickel, S. T. Retterer and A. Vertes, "Tailored Silicon Nanopost Arrays for Resonant Nanophotonic Ion Production," *J. Phys. Chem. C*, **2010**, *114*, 4835-4840. (Cover Page Article) http://dx.doi.org/10.1021/jp9110103

31/ J. A. Stolee, Y. Chen and A. Vertes, "High-Energy Fragmentation in Nanophotonic Ion Production by Laser-Induced Silicon Microcolumn Arrays," *J. Phys. Chem. C*, **2010**, *114*, 5574-5581. http://dx.doi.org/10.1021/jp906834z

1.2 Book chapter

32/ Peter Nemes and Akos Vertes, "Laser Ablation Electrospray Ionization Mass Spectrometry: Mechanisms, Configurations and Imaging Applications," in: *Ambient Ionization Mass Spectrometry*, New Developments in Mass Spectrometry No. 2, Marek Domin and Robert Cody (eds), The Royal Society of Chemistry, Cambridge, **2015**, pp. 348-371. ISBN-13: 978-1-8497-3926-9 http://dx.doi.org/10.1039/9781782628026-00348

33/ A. Vertes, B. Shrestha, and P. Nemes, "Direct Metabolomics from Tissues and Cells: Laser Ablation Electrospray Ionization for Small Molecule and Lipid Characterization," in: *Methodologies for Metabolomics: Experimental Strategies and Techniques*, Methods in Molecular Biology, N. Lutz, J. V. Sweedler, and R. A. Wevers (eds), Cambridge University Press, **2013**, pp. 140–158. ISBN-13: 978-0-52176-590-9

34/ P. Nemes and A. Vertes, "Laser Ablation Electrospray Ionization for Atmospheric Pressure Molecular Imaging Mass Spectrometry," in: *Mass Spectrometry Imaging: Principles and Protocols*, Methods in Molecular Biology, Vol.: 656, S. S. Rubakhin and J. V. Sweedler (eds), Springer, **2010**, pp. 159-171. http://dx.doi.org/10.1007/978-1-60761-746-4_9

35/ F. Adams and A. Vertes, "Local analysis and imaging by mass spectrometry in nanotechnology," in: *The Encyclopedia of Mass Spectrometry, Vol. 5, Elemental and Isotope Ratio Mass Spectrometry*, D. Beauchemin and D. E. Matthews (eds), Elsevier, **2010**, pp. 486-497. ISBN: 978-0-08-043804-7

2. Patents, licensing and commercialization

2.1 Issued Patents

1/ United States Patent US 9,362,101 B2, Date of Patent: 6/7/2016, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry

2/ United States Patent US 9,000,361 B2, Date of Patent: 04/7/2015, Title: Nanophotonic Production, Modulation and Switching of Ions by Silicon Microcolumn Arrays

3/ United States Patent US 8,901,487 B2, Date of Patent: 12/2/2014, Title: Subcellular Analysis by Laser

Ablation Electrospray Ionization Mass Spectrometry

4/ **United States Patent US 8,829,426 B2,** Date of Patent: 9/9/2014, Title: Plume Collimation for Laser Ablation Electrospray Ionization Mass Spectrometry

5/ **United States Patent US 8,809,774 B2,** Date of Patent: 8/19/2014, Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry, Continuation of United States Patent US 8,487,244 B2

6/ **United States Patent US 8,530,833 B2,** Date of Patent: 09/10/2013, Title: Nanophotonic Production, Modulation and Switching of Ions by Silicon Microcolumn Arrays

7/ **United States Patent US 8,487,246 B2,** Date of Patent: 7/16/2013, Title: Three-Dimensional Molecular Imaging by Infrared Laser Ablation Electrospray Ionization Mass Spectrometry, Continuation-in-part of United States Patent US 8,299,429 B2

8/ **United States Patent US 8,487,244 B2,** Date of Patent: 7/16/2013, Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry, Continuation of United States Patent US 8,067,730 B2

9/ **European Patent EP 2 356 668 B1,** Date of patent: 7/17/2013 Title: Three-Dimensional Molecular Imaging by Infrared Laser Ablation Electrospray Ionization Mass Spectrometry

10/ **United States Patent US 8,110,796 B2,** Date of Patent: 02/07/2012, Title: Nanophotonic Production, Modulation and Switching of Ions by Silicon Microcolumn Arrays

11/ **United States Patent US 8,084,734 B2,** Date of Patent: 12/27/2011, Title: Laser Desorption Ionization and Peptide Sequencing on Laser-Induced Silicon Microcolumn Arrays

12/ **United States Patent US 8,067,730 B2,** Date of Patent: 11/29/2011, Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry

13/ **United States Patent US 7,964,843 B2,** Date of Patent: 6/21/2011, Title: Three-Dimensional Molecular Imaging by Infrared Laser Ablation Electrospray Ionization Mass Spectrometry

14/ **United States Patent US 7,735,146 B2,** Date of Patent: 6/8/2010, Title: Protein Microscope

2.2 Licensing and commercialization

1/ **Commercialization:** On March 9, 2015, in a press release, Protea Biosciences Group, Inc., announced the introduction of a new chip-based technology marketed under the REDIchip™ name (see Figure 7.1). This ultrasensitive analytical tool is based on the silicon nanopost array (NAPA) technology developed with DOE-BES support in my laboratory. The commercialization was facilitated by DARPA funding. https://proteabio.com/media/document/protea_pressRelease_2015.03.09.pdf

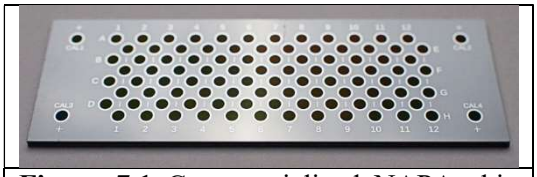


Figure 7.1 Commercialized NAPA chip (Photo: Courtesy of Protea Biosciences, Inc.)

2/ **United States Patent US 8,487,246 B2,** Title: Three-Dimensional Molecular Imaging by Infrared Laser Ablation Electrospray Ionization Mass Spectrometry, Continuation-in-part of United States Patent US 8,299,429 B2, **Licensed** by Protea Biosciences, Inc.

3/ **United States Patent US 8,487,244 B2,** Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry, Continuation of United States Patent US 8,067,730 B2, **Licensed** by Protea Biosciences, Inc.

4/ **Non-provisional patent application US 2016/0203966 A1,** Title: Remote Laser Ablation Electrospray Ionization Mass Spectrometry, **Licensed** by Protea Biosciences, Inc.

5/ **United States Patent US 8,067,730 B2,** Title: Laser Ablation Electrospray Ionization (LAESI) for Atmospheric Pressure, In Vivo and Imaging Mass Spectrometry
Licensed by Protea Biosciences, Inc.

6/ **United States Patent US 7,964,843 B2,** Title: Three-Dimensional Molecular Imaging by Infrared Laser Ablation Electrospray Ionization Mass Spectrometry

Licensed by Protea Biosciences, Inc.

7/ **United States Patent US 7,735,146 B2,** Title: Protein Microscope
Licensed by Protea Biosciences, Inc.

During the 2009-2012 grant period, Protea Biosciences, Inc., built the Alpha and Beta prototypes of the LAESI system. Following the successful testing of these prototypes, they went on to perform the production design for mass spectrometers from Thermo Scientific Inc., and Waters, Co. During the first half of 2012 they rolled out the first commercial product, the LAESI DP-1000 System. This first-generation instrument, coupled with a mass spectrometer, is capable of rapid direct analysis of water containing samples, and 2D and 3D imaging of biomolecules in tissues. The targeted applications include analysis of biofluids, reaction monitoring, drug toxicity studies, and the direct analysis of biological tissues. This first generation instrument does not have single cell capabilities.

Protea also launched LAESI-based bioanalytical services, including biofluids analysis on 96 and 384 well plate platforms, profiling and monitoring of tissues and cell colonies, tissue imaging in two and three dimensions, and depth profiling.

2.3 Technology awards

On January 1, 2012, the British life sciences magazine The Scientist announced the winners of its "Top 10 Innovations 2011" contest. The LAESI tissue and cell analysis technology, developed at GW with DOE funding, and the corresponding commercial product, LAESI DP-1000, developed by Protea Biosciences, Inc., were among the top ten.

On March 20, 2012, at the International Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy (PITTCON) the LAESI DP-1000 instrument, based on the LAESI technology, received the Editors' Bronze Award.

On June 21, 2012, the R&D 100 Magazine announced that the LAESI DP-1000 instrument, built by Protea Biosciences, Inc., based on the LAESI invention developed in this DOE program, received a 2012 R&D 100 Award. For the 50-th time, these awards are given by an independent panel to the 100 technologically most innovative products of the past year.

On July 17, 2012 The 2012 North American Frost & Sullivan Award for New Product Innovation in Bioanalytics was given for the LAESI DP-1000 system that was built based on the outcomes of this DOE-funded project. The explanation for the award decision stated that the "…pioneering LAESI DP-1000 Direct Ionization System is set to be a key enabling technology for breakthroughs in research, diagnostics and medicine."

3. Results of work accomplished

In order to limit the length of this section, here we only present short excerpts of already published work. Some of the more recent results are presented in detail, as they are not yet available in the form of publications.

3.1 Metabolite analysis by laser desorption ionization mass spectrometry from nanopost arrays

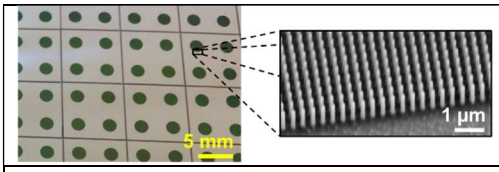


Figure 8.1 Photograph and SEM image of silicon nanopost arrays.

Beyond demonstrating the nanophotonic ionization properties of silicon nanopost arrays (see Figure 8.1) (polarization dependent ion yield and fragmentation), we aimed to show the analytical utility of these platforms for the trace analysis of metabolites and lipids in complex mixtures. To this end, direct analysis of microbial cells (yeast and green algae), serum, and a wide array of standard mixtures were studied in terms of molecular coverage, component identification and quantitative response. Unexpectedly, selective analysis of thin subcellular structures, i.e., lamellipodia, was demonstrated for the first time. Molecular imaging by LDI-MS from NAPA offers a matrix-free approach to determine metabolite and lipid distributions in tissue sections.

3.1.1 Large scale metabolite analysis by LDI-MS from NAPA

Related publications:

Andrew R. Korte, Sylwia A. Stopka, Nicholas Morris, Trust Razunguzwa, and Akos Vertes, "Large-Scale Metabolite Analysis of Standards and Human Serum by Laser Desorption Ionization Mass Spectrometry from Silicon Nanopost Arrays," *Anal. Chem.*, **2016**, *88*, 8989-8996.

Abstract

The unique challenges presented by metabolomics have driven the development of new MS-based techniques for small molecule analysis. We have previously demonstrated silicon nanopost arrays (NAPA) to be an effective substrate for LDI-MS of small molecules. However, the utility of NAPA-LDI-MS for a wide range of metabolite classes had not been investigated. Here we applied NAPA-LDI-MS to the large-scale acquisition of high-resolution mass spectra and tandem mass spectra from a collection of metabolite standards covering a range of compound classes including amino acids, nucleotides, carbohydrates, xenobiotics, lipids, and other classes. In untargeted analysis of metabolite standard mixtures, detection was achieved for 374 compounds and MS/MS spectra were obtained for 287 compounds, without individual optimization of ionization or fragmentation conditions. Metabolite detection was evaluated in the context of 31 metabolic pathways, and NAPA-LDI-MS was found to provide detection for 63% of investigated pathway metabolites (see Figure 8.2). Individual, targeted analysis of the 20 common amino acids provided detection of 100% of the investigated compounds, demonstrating that improved coverage is possible through optimization and targeting of individual analytes or analyte classes. In direct analysis of aqueous and organic extracts from human serum samples, spectral features were assigned to a total of 108 small metabolites and lipids. Glucose and amino acids were quantitated within their natural concentration

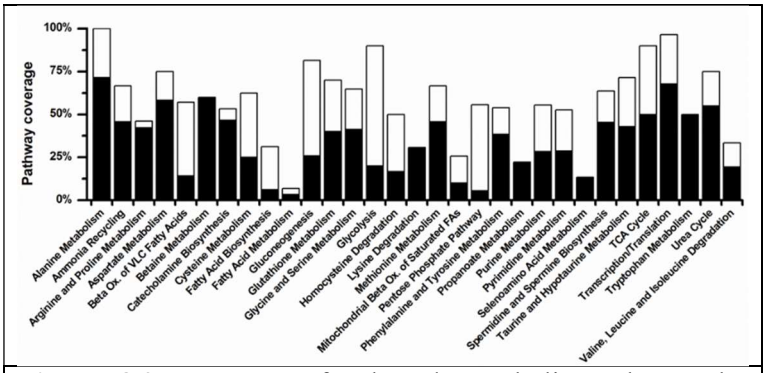


Figure 8.2 Coverage of selected metabolic pathways by NAPA-LDI-MS of metabolite standards under general acquisition conditions.

ranges. The broad coverage demonstrated by this large-scale experiment opens the door for use of NAPA-LDI-MS in numerous metabolite analysis applications.

3.1.2 Metabolic differences in microbial cell populations

Related publications:

Bennett N. Walker, Cory Antonakos, Scott T. Retterer and Akos Vertes, "Metabolic Differences in Microbial Cell Populations Revealed by Nanophotonic Ionization," *Angew. Chem. Int. Ed.*, **2013**, *52*, 3650-3653.

Abstract

Cellular heterogeneity is linked to cell differentiation, the proliferation of cancer and to the development of drug resistance in microbial infections. Due to sensitivity limitations, however, large-scale metabolic analysis at the single cell level is only available for cells significantly larger in volume than *S. cerevisiae* (~30 fL). Here we demonstrate that by a nanophotonic ionization platform and mass spectrometry, over one hundred metabolites, or 18% of the known *S. cerevisiae* metabolome, can be identified in very small cell populations ($n < 100$). Relative quantitation of 4% of the metabolites is achieved at the single cell level. The identified metabolites belong to 63 of the 94 common metabolic pathways with most of them present in amino acid, carbohydrate, nucleotide and lipid biosynthesis and degradation. Following the changes in metabolic states under oxidative stress reveals inter-population differences, i.e., a significant upregulation ($p < 0.002$) of the redox buffer glutathione and the related cysteinylglycine and glutamylcysteine. Downregulation ($p < 4\times10^{-4}$) of amino-deoxychorismate and dihydroneopterin phosphate, used in folate biosynthesis, as well as of oxalureate indicates that the cell redirects resources from cell growth toward fighting oxidative stress. Single cell analyses show that relative standard deviations due to intra-population heterogeneities for the abundance of lysine, methionine, cysteine, and proline, in the unperturbed yeast population are 26%, 30%, 10% and 25%, respectively. Enabling large-scale metabolomic studies of single yeast cells opens the door to following functional changes in evolving heterogeneous microbial populations and the analysis of metabolic noise across a cell population.

3.1.3 Matrix-free molecular imaging by nanophotonic ionization from NAPA

Related publications:

Sylwia A. Stopka, Charles Rong, Andrew R. Korte, Sridevi Yadavilli, Javad Nazarian, Trust T. Razunguzwa, Nicholas J. Morris and Akos Vertes, "Molecular Imaging of Biological Samples on Nanophotonic Laser Desorption Ionization Platforms," *Angew. Chem. Int. Ed.*, **2016**, 55, 4482-4486.

Abstract

Mass spectrometry imaging (MSI) is a comprehensive tool for the analysis of a wide range of biomolecules. The mainstream method for molecular MSI is matrix-assisted laser desorption ionization, however, the presence of a matrix can result in spectral interferences and the suppression of some analyte ions. Here we demonstrate a new matrix-free MSI technique using nanophotonic ionization based on laser desorption ionization (LDI) from a highly uniform silicon nanopost array (NAPA). In mouse brain and kidney tissue sections and in microbial deposits, the

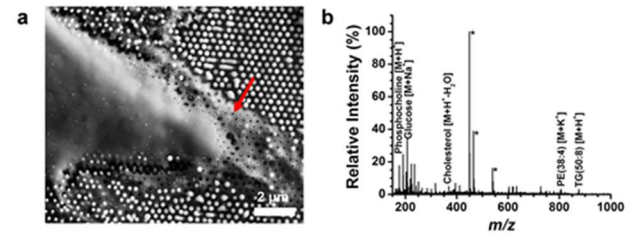


Figure 8.3 (a) SEM image of a HepG2/C3A cell on NAPA, exposed to ~24 mJ/cm^2 laser fluence, with pores (see arrow) in the lamellipodia induced by the irradiated nanoposts. (b) Corresponding mass spectrum indicates metabolite and lipid ions originating selectively from the lamellipodia. Background ions from NAPA are marked by *.

distributions of over 80 putatively annotated molecular species are determined with 40 μm spatial resolution. Furthermore, NAPA-LDI-MS is used to selectively analyze metabolites and lipids from sparsely distributed algal cells and the lamellipodia of hepatocytes (see Figure 8.3). Our results open the door for matrix-free MSI of tissue sections and small cell populations by nanophotonic ionization.

3.2 Improved molecular coverage and spatial resolution in LAESI-MS

3.2.1 Direct analysis with enhanced molecular coverage by LAESI-MS with ion mobility separation

Related publications:

Bindesh Shrestha and Akos Vertes, "High-Throughput Cell and Tissue Analysis with Enhanced Molecular Coverage by Laser Ablation Electrospray Ionization Mass Spectrometry Using Ion Mobility Separation," *Anal. Chem.*, **2014**, *86*, 4308–4315.

Abstract

Ambient ionization methods, such as laser ablation electrospray ionization (LAESI), facilitate the direct analysis of unperturbed cells and tissues in their native states. However, the lack of a separation step in these ionization techniques results in limited molecular coverage due to interferences, ion suppression effects, and the lack of ability to differentiate between structural isomers and isobaric species. In this contribution, LAESI mass spectrometry (MS) coupled with ion mobility separation (IMS) is utilized for the direct analysis of protein mixtures, megakaryoblast cell pellets, mouse brain sections, and *Arabidopsis thaliana* leaves. We demonstrate that the collision cross sections of ions generated by LAESI are similar to the ones obtained by ESI. In various applications, LAESI-IMS-MS allows for the high-throughput separation and mass spectrometric detection of biomolecules on the millisecond time scale with enhanced molecular coverage. For example, direct analysis of mouse brain tissue without IMS had yielded ~300 ionic species, whereas with IMS over 1 100 different ions were detected. Differentiating between ions of similar mass-to-charge ratios with dissimilar drift times in complex biological samples removes some systematic distortions in isotope distribution patterns and improves the fidelity of molecular identification. Coupling IMS with LAESI-MS also expands the dynamic range by increasing the signal-to-noise ratio due to the separation of isobaric or other interfering ionic species. We have also shown that identification of potential biomarkers by LAESI can be enhanced by using the drift times of individual ions as an additional parameter in supervised orthogonal projections to latent structures discriminant analysis. Comparative analysis of drift time versus mass-to-charge ratio plots was performed for similar tissue samples, e.g., the midvein and the edge of *A. thaliana* leafs, to pinpoint significant metabolic differences (see Figure 8.4).

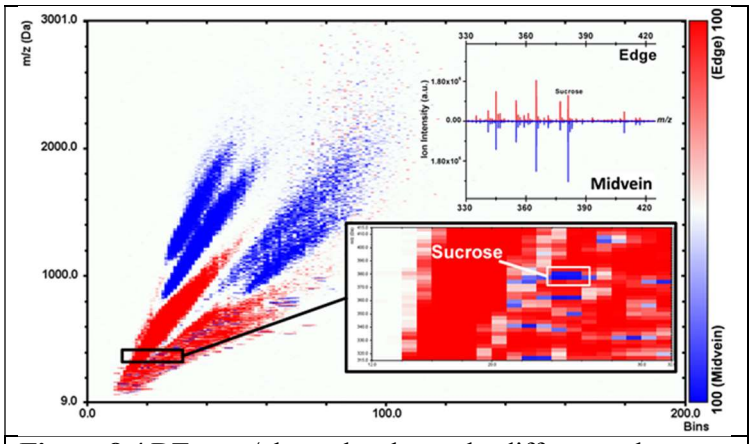


Figure 8.4 DT vs *m/z* heat plot shows the differences between the plots from the edge and midvein of *A. thaliana* leaves. The inset in the bottom right of the panel shows the area zoomed to the vicinity of potassiated sucrose ions. Corresponding mass spectra in the top right of the panel show higher intensity for these ions in the midvein spectrum.

3.2.2 Heat-assisted LAESI for improved ion yields from nonpolar compounds

Related publications:

Anu Vaikkinen, Bindesh Shrestha, Javad Nazarian, Risto K. Kostiainen, Akos Vertes and Tiina J. Kauppila, "Simultaneous Detection of Nonpolar and Polar Compounds by Heat-Assisted Laser Ablation Electrospray Ionization-Mass Spectrometry," *Anal. Chem.*, **2013**, *85*, 177-184.

Abstract

A heat-assisted laser ablation electrospray ionization (HA-LAESI) method for the simultaneous mass spectrometric analysis of nonpolar and polar analytes was developed. The sample was introduced using mid-infrared laser ablation of a water-rich target. The ablated analytes were ionized with an electrospray plume, which was intercepted by a heated nitrogen gas jet that enhanced the ionization of analytes of low polarity. The feasibility of HA-LAESI was tested by analyzing, e.g., naphtho[2,3-*a*]pyrene, cholesterol, tricaprylin, 1,1′,2,2′-tetramyristoyl cardiolipin, bradykinin fragment 1–8, and 1-palmitoyl-2-oleoyl-*sn*-glycerol. HA-LAESI was found better suited for low polarity compounds than conventional LAESI, whereas polar compounds were observed with both techniques. The sensitivity of HA-LAESI for the polar bradykinin fragment 1–8 was slightly lower than observed for LAESI. HA-LAESI showed a linear response for 500 nM to 1.0 mM solutions (n = 11) of verapamil with R^2 = 0.988. HA-LAESI was applied for the direct analysis of tissue samples, e.g., avocado (*Persea americana*) mesocarp and mouse brain tissue sections. Spectra of the avocado showed abundant triglyceride ion peaks, and the results for the mouse brain sections showed cholesterol as the main species. Conventional LAESI shows significantly lower ionization efficiency for these neutral lipids. HA-LAESI can be applied to the analysis of nonpolar and polar analytes, and it extends the capabilities of conventional LAESI to nonpolar and neutral compounds.

3.2.3 Direct analysis of adherent cells by transmission geometry LAESI-MS

Related publications:

Rachelle S. Jacobson, Richard Thurston, Bindesh Shrestha, and Akos Vertes, "In Situ Analysis of Small Populations of Adherent Mammalian Cells Using Laser Ablation Electrospray Ionization Mass Spectrometry in Transmission Geometry," *Anal. Chem.*, **2015**, *87*, 12130-12136.

Abstract

Most cultured cells used for biomedical research are cultured adherently, and the requisite detachment prior to biochemical analysis might induce chemical changes. This is especially crucial if accurate metabolic measurements are desired, given the rapid turnover of metabolites in living organisms. There are only a few methods available for the non-targeted in situ analysis of small adherent cell populations. Here we show that LAESI-MS can be used to analyze adherent cells directly, while still attached to the culture surface. To reduce the size of the analyzed cell population, the spot size constraints of conventional focusing in reflection geometry (rg) LAESI had to be eliminated. By introducing transmission geometry (tg) LAESI and incorporating an objective with a high numerical aperture, spot sizes of 10–20 μm were readily achieved. As few as five adherent cells could be specifically selected for analysis in their culturing environment. The importance of in situ analysis was highlighted by comparing the metabolite composition of adherent versus suspended cells. For example, we observed that cells analyzed adherently yielded higher values for the adenylate energy charge (0.90 ± 0.09 for adherent cells vs 0.09 ± 0.03 for suspended cells). Additionally, due to the smaller focal spot size, tg-LAESI enabled the analysis of ∼20 times smaller cell populations compared to rg-LAESI.

3.3 Transformation, susceptibility, and turnover in microorganisms studied by LAESI-MS

3.3.1 Metabolic transformation and lipid turnover rates in light harvesting microalgae

Sylwia A. Stopka, Bindesh Shrestha, Eric Marechal, Denis Falconet and Akos Vertes, "Metabolic Transformation of Microalgae Due to Light Acclimation and Genetic Modifications Followed by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation," *Analyst*, **2014**, *139*, 5946 - 5954.

Sylwia A. Stopka, Tarek R. Mansour, Bindesh Shrestha, Eric Marechal, Denis Falconet and Akos Vertes, "Turnover Rates in Microorganisms by LAESI Mass Spectrometry and Pulse-chase Analysis," *Anal. Chim. Acta*, **2016**, *902*, 1-7.

Abstract

Metabolic profiling of various microalga species and their genetic variants, grown under varied environmental conditions, has become critical to accelerate the exploration of phytoplankton biodiversity and biology. The accumulation of valuable metabolites, such as glycerolipids, is also sought in microalgae for biotechnological applications ranging from food, feed, medicine, cosmetics to bioenergy and green chemistry. In this report we describe the direct analysis of metabolites and lipids in small cell populations of the green alga *Chlamydomonas reinhardtii*, using laser ablation electrospray ionization (LAESI) mass spectrometry (MS) coupled with ion mobility separation (IMS). These microorganisms are capable of redirecting energy storage pathways from starch to neutral lipids depending on environmental conditions and nutrient availability. Metabolite and lipid productions were monitored in wild type (WT), and genetically modified *C. reinhardtii* strains, *Sta*1 and *Sta*6, with an impaired starch pathway. Lipids, such as triacylglycerols (TAG) and diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS), were monitored over time under altered light conditions. More than 200 ions related to metabolites, *e.g.*, arginine, cysteine, serine, palmitate, chlorophyll *a*, chlorophyll *b*, *etc.*, were detected. The lipid profiles at different light intensities for strains with impaired starch pathway contained 26 glycerolipids, such as DGTS, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), as well as 33 TAG species. Results were obtained over a 72 hour time period under high and low light conditions for the WT species and the two mutants. Our results indicate that LAESI-IMS-MS can be utilized for the rapid analysis of increased TAG production at elevated light intensities. Compared to WT, the *Sta*6 strain showed 2.5 times higher lipid production at 72 hours under high light conditions. The results demonstrate our ability to rapidly observe numerous changes in metabolite and lipid levels in microalgal populations. These capabilities are expected to facilitate the exploration of genetically altered microalgal strains for biofuel production.

Biochemical processes rely on elaborate networks containing thousands of compounds participating in thousands of reactions. Rapid turnover of diverse metabolites and lipids in an organism is an essential part of homeostasis. It affects energy production and storage, two important processes utilized in bioengineering. Conventional approaches to simultaneously quantify a large number of turnover rates in biological systems are currently not feasible. Here we show that pulse-chase analysis followed by LAESI-MS enable the simultaneous and rapid determination of metabolic turnover rates. The incorporation of IMS allowed an additional dimension of analysis, i.e., the detection and identification of isotopologs based on their collision cross sections. We demonstrated these capabilities by determining metabolite, lipid, and peptide turnover in the photosynthetic green algae, *C. reinhardtii*, in the presence of ^{15}N-labeled ammonium chloride as the main nitrogen source. Following the reversal of isotope patterns in the chase phase by LAESI-IMS-MS revealed the turnover rates and half-lives for biochemical species with a wide range of natural concentrations, e.g., chlorophyll metabolites, lipids, and peptides. For example, the half-lives of lyso-DGTS(16:0) and DGTS(18:3/16:0), $t_{1/2}$ = 43.6 ± 4.5 h and 47.6 ± 2.2 h, respectively, provided insight into lipid synthesis and degradation in this organism. Within the same experiment, half-lives for chlorophyll *a*, $t_{1/2}$ = 24.1 ± 2.2 h, and a 2.8 kDa peptide, $t_{1/2}$ = 10.4 ± 3.6 h, were also determined.

3.3.2 Antibiotic susceptibility of microorganisms determined by LAESI-MS

Related publications:

Hang Li, Pranav Balan, and Akos Vertes, "Molecular Imaging of Growth, Metabolism, and Antibiotic Inhibition in Bacterial Colonies by Laser Ablation Electrospray Ionization Mass Spectrometry," *Angew. Chem. Int. Ed.*, **2016**, accepted on September 19, 2016. http://dx.doi.org/10.1002/anie.201607751

Abstract
Metabolism in microbial colonies responds to competing species, rapidly evolving genetic makeup, and sometimes dramatic environmental changes. Conventional characterization of the existing and emerging microbial strains and their interactions with antimicrobial agents, e.g., the Kirby-Bauer susceptibility test, relies on time consuming methods with limited ability to discern the molecular mechanism and the minimum inhibitory concentration. Assessing the metabolic adaptation of microbial colonies requires their non-targeted molecular imaging in a native environment. LAESI is an ambient ionization technique that in combination with MS enables the analysis and imaging of numerous metabolites and lipids. In this contribution, we report on the application of LAESI-MS imaging to gain deeper molecular insight into microbe-antibiotic interactions, and enhance the quantitative nature of antibiotic susceptibility testing while significantly reducing the required incubation time.

3.4 Rapid assessment of noncovalent complex formation by LAESI-IMS-MS

Related publications:
Hang Li, Emmeline Ha, Robert P. Donaldson, Aleksandar M. Jeremic and Akos Vertes, "Rapid Assessment of Human Amylin Aggregation and its Inhibition by Copper(II) Ions by Laser Ablation Electrospray Ionization Mass Spectrometry with Ion Mobility Separation," *Anal. Chem.*, **2015**, *87*, 9829-9837.

Abstract
Native electrospray ionization (ESI) MS is often used to monitor noncovalent complex formation between peptides and ligands. The relatively low throughput of this technique, however, is not compatible with extensive screening. LAESI-MS combined with IMS can analyze complex formation and provide conformation information within a matter of seconds. Islet amyloid polypeptide (IAPP) or amylin, a 37-amino acid residue peptide, is produced in pancreatic beta-cells through proteolytic cleavage of its prohormone. Both amylin and its precursor can aggregate and produce toxic oligomers and fibrils leading to cell death in the pancreas that can eventually contribute to the development of type 2 diabetes mellitus. The inhibitory effect of the copper(II) ion on amylin aggregation has been recently discovered, but details of the interaction remain unknown. Finding other more physiologically tolerated approaches requires large scale screening of potential inhibitors. Here, we demonstrate that LAESI-IMS-MS can reveal the binding stoichiometry, copper oxidation state, and the dissociation constant of human amylin–copper(II) complex. The conformations of hIAPP in the presence of copper(II) ions were also analyzed by IMS, and preferential association between the β-hairpin amylin monomer and the metal ion was found. The copper(II) ion exhibited strong association with the −HSSNN– residues of the amylin. In the absence of copper(II), amylin dimers were detected with collision cross sections consistent with monomers of β-hairpin conformation. When copper(II) was present in the solution, no dimers were detected. Thus, the copper(II) ions disrupt the association pathway to the formation of β-sheet rich amylin fibrils. Using LAESI-IMS-MS for the assessment of amylin–copper(II) interactions demonstrates the utility of this technique for the high-throughput screening of potential inhibitors of amylin oligomerization and fibril formation. More generally, this rapid technique opens the door for high-throughput screening of potential inhibitors of amyloid protein aggregation.

3.5 High-throughput metabolomics of cells and tissues by LAESI-MS

Related publications:

Bindesh Shrestha, Robert Javonillo, John R. Burns, Zsolt Pirger and Akos Vertes, "Comparative local analysis of metabolites, lipids and proteins in intact fish tissues by LAESI mass spectrometry," *Analyst*, **2013**, *138*, 3444-3449.

Rohit Shroff, Katharina Schramm, Verena Jeschke, Peter Nemes, Akos Vertes, Jonathan Gershenzon and Ales Svatos, "Quantification of plant surface metabolites by MALDI mass spectrometry imaging: glucosinolates on *Arabidopsis thaliana* leaves," *The Plant Journal*, **2015**, *81*, 961-972.

Elizabeth Jaworski, Aarthi Narayanan, Rachel Van Duyne, Shabana Shabbeer-Meyering, Sergey Iordanskiy, Mohammed Saifuddin, Ravi Das, Philippe V. Afonso, Gavin C. Sampey, Myung Chung, Anastas Popratiloff, Bindesh Shrestha, Mohit Sehgal, Pooja Jain, Akos Vertes, Renaud Mahieux, Fatah Kashanchi, "Human T-lymphotropic Virus Type 1-infected Cells Secrete Exosomes That Contain Tax Protein," *J. Biol. Chem.*, **2014**, *289*, 22284-22305.

Peter Nemes and Akos Vertes, "Laser Ablation Electrospray Ionization Mass Spectrometry: Mechanisms, Configurations and Imaging Applications," in: *Ambient Ionization Mass Spectrometry*, New Developments in Mass Spectrometry No. 2, Marek Domin and Robert Cody (eds), The Royal Society of Chemistry, Cambridge, **2015**, pp. 348-371. ISBN-13: 978-1-8497-3926-9

Abstract

Direct mass spectrometric analysis of animal tissues is an emerging field enabled by recent developments in ambient ion sources. Label-free *in situ* analysis of metabolites, lipids, and peptides/proteins from intact tissues in whole fish specimens of different gender and age were performed by laser ablation electrospray ionization (LAESI) mass spectrometry (MS). Hypertrophied glandular tissue (gill gland) of adult male *Aphyocharax anisitsi* (bloodfin tetra) was compared with gill tissues in females of the same species. Comparison of a large number of sample-specific ions was aided by a multivariate statistical method based on orthogonal projections to latent structures discriminant analysis. More than 200 different ions were detected in the mass spectra corresponding to primary metabolites, hormones, lipids and peptides/proteins. The gill tissues of the sexually mature males exhibited multiply charged ions in the 6+ to 10+ charge states corresponding to a protein with a molecular weight of 11 380 Da. This protein was present only in the mature male gill glands but absent in the corresponding area of the female and immature male specimens. An additional nine proteins were detected by LAESI-MS in both the male and female gill tissues.

The localization of metabolites on plant surfaces has been problematic because of the limitations of current methodologies. Attempts to localize glucosinolates, the sulfur-rich defense compounds of the order Brassicales, on leaf surfaces have given many contradictory results depending on the method employed. Here we developed MALDI-MS and LAESI-MS protocols to detect surface glucosinolates on *Arabidopsis thaliana* leaves. Quantification was accomplished by spotting glucosinolate standards directly on the leaf surface. The *A. thaliana* leaf surface was found to contain approximately 15 nmol of total glucosinolate per leaf with about 50 pmol mm^{-2} on abaxial (bottom) surfaces and 15–30 times less on adaxial (top) surfaces. Of the major compounds detected, 4-methylsulfinylbutylglucosinolate, indol-3-ylmethylglucosinolate, and 8-methylsulfinyloctylglucosinolate were also major components of the leaf interior, but the second most abundant glucosinolate on the surface, 4-methylthiobutylglucosinolate, was only a trace component of the interior. Distribution on the surface was relatively uniform in contrast to the interior, where glucosinolates were distributed more abundantly in the midrib and periphery than the rest of the leaf. These results were confirmed by two other mass spectrometry-based techniques, laser ablation electrospray ionization and liquid extraction surface analysis. The concentrations of glucosinolates on*A. thaliana* leaf surfaces were found to be sufficient to attract the specialist feeding lepidopterans *Plutella xylostella* and *Pieris rapae* for oviposition. The methods employed here should be easily applied to other plant species and metabolites.

LAESI combines the microsampling capabilities of mid-IR lasers with the high ion yields of electrosprays to produce sample-specific ions from biomedical and other specimens of high water content. To facilitate fundamental understanding of this technique, four critical aspects of the underlying physical phenomena are discussed. Fast imaging experiments and fluid dynamic calculations indicate that the

sampling process by mid-IR laser ablation is governed by particulate ejection due to the recoil pressure of the expanding laser plume. Spray diagnostic studies show that control over the spraying regimes of the electrospray offers clues to achieve high ion yields. Tailoring the merging of the laser ablation plume with the electrospray offers additional ways to improve sensitivity. Measured internal energies of the ions produced by LAESI and regular electrospray ionization show them to be indistinguishable. Since its inception in 2007, LAESI has been implemented in diverse configurations, including conventional reflection and transmission geometries, heat-assisted LAESI for enhanced detection of apolar molecules, optical fiber-based laser-pulse delivery for improved focusing, and plume collimation for heightened sensitivity. LAESI supports several spatial profiling and molecular imaging modalities for biological tissues. Rastering the surface with the laser beam and collecting spectra point-by-point is the basis of constructing lateral images for ions associated with hundreds of metabolites and lipids. Because ablation by consecutive laser pulses can sample subsurface layers for analysis, depth profiling can also be performed. Combining lateral imaging with depth profiling is the basis of the three-dimensional reconstruction of molecular distributions in tissues. To improve the fidelity of molecular imaging, we introduced and implemented a cell-by-cell imaging strategy that promises to reveal new information on cellular transport and signaling processes in tissues.

3.6 Photonic laser desorption ionization on silicon nanopost arrays

Interactions between pulsed laser radiation and nanostructured materials, with dimensions ranging from 1 nm to 500 nm, can result in enhanced desorption and ionization of organic and biomolecular adsorbates. When the critical dimensions of the nanostructures fall below the characteristic lengths for the involved transport processes, novel regimes of ion production are observed (see Figure 7.1). Systems with dimensions commensurate with the wavelength of the laser radiation are the basis of photonic ion sources with unique properties, including polarization dependent ion yields and fragmentation. The main characteristics of these systems are often governed by altered modes of transport, e.g., ballistic vs. diffusive, energy confinement, plasmon resonances, and local field enhancements. Some structures offer control over the internal energy and the active fragmentation channels for the produced ions. Emerging applications of photonic ion sources in mass spectrometry benefit from ultrahigh sensitivity, a wide dynamic range for detection and quantitation, and a broad coverage of adsorbates ranging from small organic molecules to biopolymers, as well as to highly complex samples like single cells.

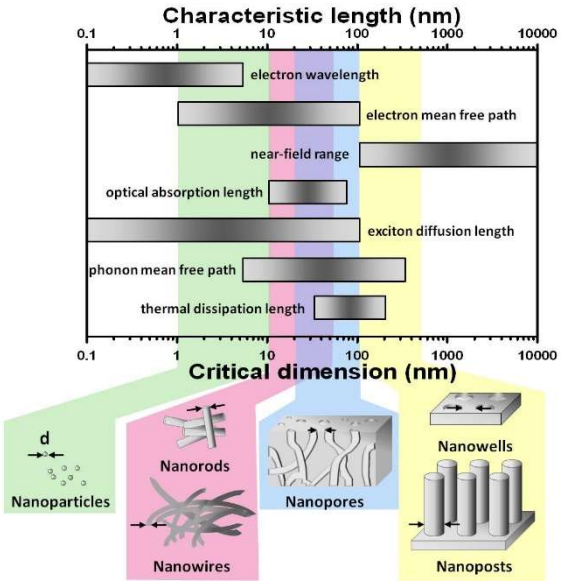


Figure 7.1 Characteristic length ranges for energy and charge carriers that are relevant in the transport properties of nanostructures. Some nanostructures used in laser desorption ionization are depicted and their critical dimensions are indicated with arrows.

3.6.1 Ion production via photonic interactions

Related publications:

J. A. Stolee, B. N. Walker, V. Zorba, R. E. Russo and A. Vertes, "'Laser-nanostructure Interactions for Ion Production," *Phys. Chem. Chem. Phys.*, **2012**, *14*, 8453-8471.

J. A. Stolee, B. N. Walker, Y. Chen and A. Vertes, "Nanophotonic Ion Sources," in: *International Symposium on High Power Laser Ablation 2010*, C. R. Phipps (ed), *AIP Conf. Proc. 1278*, **2010**, 98-110.

B. N. Walker, J. A. Stolee, D. L. Pickel, S. T. Retterer and A. Vertes, "Assessment of Laser-Induced Thermal Load on Silicon Nanostructures Based on Ion Desorption Yields," *Appl. Phys. A*, **2010**, *101*, 539-544.

B. N. Walker, J. A. Stolee, D. L. Pickel, S. T. Retterer and A. Vertes, "Tailored Silicon Nanopost Arrays for Resonant Nanophotonic Ion Production," *J. Phys. Chem. C*, **2010**, *114*, 4835-4840.

Abstract
Nanostructures that have dimensions commensurate with the wavelength of the electromagnetic radiation exhibit near-field effects and, as optical antennas, can couple laser radiation to the local environment. Laser-induced silicon microcolumn arrays behave as nanophotonic ion sources that can be modulated by rotating the plane of light polarization. However, the limited range of surface morphologies available for these substrates makes it difficult to study the underlying mechanism that governs ion production. Here we demonstrate that NAPA can be tailored to exhibit resonant ion production. Ion yields from posts with subwavelength diameter show sharp resonances at high aspect ratios. The resonant enhancement in ion intensities can be modulated by adjusting the periodicity. In addition to strong molecular ion formation, the presence of high energy fragmentation channels is observed. Ion yields from NAPA exhibit dramatic differences for p- and s-polarized laser beams, indicating that energy coupling is similar to antenna arrays. These nanophotonic ion sources can control the degree of ion fragmentation and could eventually be integrated with micromachined mass spectrometers and microfluidic devices.

3.6.2 Internal energy transfer in photonic ionization

Related publications:

J. A. Stolee and A. Vertes, "Polarization dependent fragmentation of ions produced by laser desorption from nanopost arrays," *Phys. Chem. Chem. Phys.*, **2011**, *13*, 9140-9146.

J. A. Stolee, Y. Chen and A. Vertes, "High-Energy Fragmentation in Nanophotonic Ion Production by Laser-Induced Silicon Microcolumn Arrays," *J. Phys. Chem. C*, **2010**, *114*, 5574-5581.

Abstract
Tailored silicon NAPA enable controlled and resonant ion production in laser desorption ionization experiments and have been termed nanophotonic ion sources (Walker et al., *J. Phys. Chem. C*, **2010**, *114*, 4835). As the post dimensions are comparable to or smaller than the laser wavelength, near-field effects and localized electromagnetic fields are present in their vicinity. In this contribution, we explore the desorption and ionization mechanism by studying how surface derivatization affects ion yields and fragmentation. We demonstrate that by increasing the laser fluence on derivatized NAPA with less polar surfaces that have decreased interaction energy between the structured silicon substrate and the adsorbate, the spectrum changes from exhibiting primarily molecular ions to showing a growing variety and abundance of fragments. The polarization angle of the laser beam had been shown to dramatically affect the ion yields of adsorbates. For the first time, we report that by rotating the plane of polarization of the desorption laser, the internal energy of the adsorbate can also be modulated resulting in polarization dependent fragmentation. This polarization effect also resulted in selective fragmentation in vitamin B_{12}. To explore the internal energy of NAPA generated ions, the effect of the post aspect ratios on the laser desorption thresholds and on the internal energy of a preformed ion was studied. Elevated surface temperatures and enhanced near fields in the vicinity of high aspect ratio posts are thought to contribute to desorption and ionization from NAPA. Comparison of the fluence dependence of the internal energies of ions produced from nanoporous silicon and NAPA substrates indicate that surface restructuring or transient melting by the desorption laser is a prerequisite for the former but not for the latter.

3.6.3 Ultratrace and single cell analysis on NAPA

Related publications:

B. N. Walker, J. A. Stolee and A. Vertes, "Nanophotonic Ionization for Ultratrace and Single-Cell Analysis by Mass Spectrometry," *Anal. Chem.*, **2012**, *84*, 7756-7762.

Abstract

Recent mechanistic studies have indicated that at subwavelength post diameters and selected aspect ratios nanopost arrays (NAPA) exhibit ion yield resonances (Walker, B. N.; Stolee, J. A.; Pickel, D. L.; Retterer, S. T.; Vertes, A. *J. Phys. Chem. C* **2010**, *114*, 4835–4840). In this contribution we explore the analytical utility of these optimized structures as matrix-free platforms for laser desorption ionization mass spectrometry (LDI-MS). Using NAPA, we show that high ionization efficiencies enable the detection of ultratrace amounts of analytes (e.g., ~800 zmol of verapamil) with a dynamic range spanning up to four orders of magnitude. Due to the clean nanofabrication process and the lack of matrix material, minimal background interferences are present in the low mass range. We demonstrate that LDI from NAPA ionizes a broad class of small molecules including pharmaceuticals, natural products, metabolites and explosives. Quantitation of resveratrol in red wine samples shows that the analysis of targeted analytes in complex mixtures is feasible with minimal sample preparation using NAPA-based LDI. We also describe how multiple metabolite species can be directly detected in single yeast cells deposited on the NAPA chip. Twenty four metabolites, or 4% of the yeast metabolome, were identified in the single cell spectra.

3.6.4 Cellular heterogeneity revealed by nanophotonic ionization

Related publications:

B. N. Walker, C. Antonakos, S. T. Retterer and A. Vertes, "Metabolic Heterogeneity in Microbial Cell Populations Revealed by Nanophotonic Ionization," *Angew. Chem. Int. Ed.*, **2012**, submitted.

Abstract

Cellular heterogeneity is linked to cell differentiation, the proliferation of cancer and to the development of drug resistance in microbial infections. Due to sensitivity limitations, however, large-scale metabolic analysis at the single cell level is only available for cells significantly larger in volume than *S. cerevisiae* (~30 fL). Here we demonstrate that by a nanophotonic ionization platform and mass spectrometry, over one hundred metabolites, or 18% of the known *S. cerevisiae* metabolome, can be identified in very small cell populations ($n < 100$). Relative quantitation of 4% of the metabolites is achieved at the single cell level. The identified metabolites belong to 63 of the 94 common metabolic pathways with most of them present in amino acid, carbohydrate, nucleotide and lipid biosynthesis and degradation. Following the changes in metabolic states under oxidative stress reveals inter-population differences, i.e., a significant upregulation ($p < 0.002$) of the redox buffer glutathione and the related cysteinylglycine and glutamylcysteine. Downregulation ($p < 4\times10^{-4}$) of amino-deoxychorismate and dihydroneopterin phosphate, used in folate biosynthesis, as well as of oxalureate indicates that the cell redirects resources from cell growth toward fighting oxidative stress. Single cell analyses show that relative standard deviations due to intra-population heterogeneities for the abundance of lysine, methionine, cysteine, and proline, in the unperturbed yeast population are 26%, 30%, 10% and 25%, respectively. Enabling large-scale metabolomic studies of single yeast cells opens the door to following functional changes in evolving heterogeneous microbial populations and the analysis of metabolic noise across a cell population.

3.7 Measuring and adjusting internal energy in LAESI

3.7.1 Internal energy deposition and ion fragmentation

Related publications:

P. Nemes, H. Huang and A. Vertes, "Internal energy deposition and ion fragmentation in atmospheric-pressure mid-infrared laser ablation electrospray ionization," *Phys. Chem. Chem. Phys.*, **2012**, *14*, 2501-2507.

Abstract

Mid-infrared laser ablation of water-rich targets at the maximum of the 2.94 μm absorption band is a two-step process initiated by phase explosion followed by recoil pressure induced material ejection. Particulates and/or droplets ejected by this high temperature high pressure process can be ionized for mass spectrometry by charged droplets from an electrospray. In order to gauge the internal energy introduced in the LAESI process, we apply the survival yield method and compare the results with ESI and MALDI. The results indicate that LAESI yields ions with internal energies indistinguishable from those produced by ESI. This finding is consistent with the recoil pressure induced ejection of low micrometer droplets that does not significantly change the internal energy of solute molecules.

3.7.2 Heat-assisted LAESI for improved ion yields from nonpolar compounds

Related publications:

A. Vaikkinen, B. Shrestha, J. Nazarian, R. Kostiainen, A. Vertes and T. J. Kauppila, "Simultaneous Detection of Nonpolar and Polar Compounds by Heat-Assisted Laser Ablation Electrospray Ionization Mass Spectrometry," *Anal. Chem*., **2012**, submitted.

Abstract

A heat-assisted LAESI (HA-LAESI) method for the simultaneous mass spectrometric analysis of nonpolar and polar analytes was developed. The sample was introduced using mid-infrared laser ablation of a water-rich target. The ablated analytes were ionized with an electrospray plume, which was intercepted by a heated nitrogen gas jet that enhanced the ionization of analytes of low polarity. The feasibility of HA-LAESI was tested by analyzing, e.g., naphtho[2,3-*a*]pyrene, cholesterol, tricaprylin, 1,1',2,2'-tetramyristoyl cardiolipin, bradykinin fragment 1-8, and 1-palmitoyl-2-oleoyl-*sn*-glycerol. HA-LAESI was found better suited for low polarity compounds than conventional LAESI, whereas polar compounds were observed with both techniques. The sensitivity of HA-LAESI for the polar bradykinin fragment 1-8 was slightly lower than what was observed for LAESI. HA-LAESI showed linear response for 500 nM – 1.0 mM solutions (n = 11) of verapamil with $R^2 = 0.988$. HA-LAESI was applied for the direct analysis of tissue samples, e.g., avocado (*Persea americana*) mesocarp and mouse brain tissue sections. Spectra of the avocado showed abundant triglyceride ion peaks, and the results for the mouse brain sections showed cholesterol as the main species. Conventional LAESI shows significantly lower ionization efficiency for these neutral lipids. HA-LAESI can be applied to the analysis of nonpolar and polar analytes, and it extends the capabilities of conventional LAESI to nonpolar and neutral compounds.

3.8 Direct analysis and imaging by LAESI mass spectrometry

3.8.1 Energy harvesting bacteria and biofilms

G. Parsiegla, B. Shrestha, F. Carriere and A. Vertes, "Direct Analysis of Phycobilisomal Antenna Proteins and Metabolites in Small Cyanobacterial Populations by Laser Ablation Electrospray Ionization Mass Spectrometry," *Anal. Chem.*, **2012**, *84*, 34-38.

P. Nemes and A. Vertes, "Atmospheric-pressure Molecular Imaging of Biological Tissues and Biofilms by LAESI Mass Spectrometry," *J. Visualized Experiments*, **2010**, *43*, e2097.

Abstract

Due to their significance in energy and environmental and natural product research, as well as their large genetic diversity, rapid in situ analysis of cyanobacteria is of increasing interest. Metabolic profiles and the composition of energy harvesting antenna protein complexes are needed to understand how environmental factors affect the functioning of these microorganisms. Here, we show that LAESI mass spectrometry enables the direct analysis of phycobilisomal antenna proteins and report on numerous metabolites from intact cyanobacteria. Small populations ($n < 616 \pm 76$) of vegetative *Anabaena* sp. PCC7120 cyanobacterial cells are analyzed by LAESI mass spectrometry. The spectra reveal the ratio of phycocyanin (C-PC) and allophycocyanin (APC) in the antenna complex, the subunit composition of the phycobiliproteins, and the tentative identity of over 30 metabolites and lipids. Metabolites are tentatively identified by accurate mass measurements, isotope distribution patterns, and literature searches. The rapid simultaneous analysis of abundant proteins and diverse metabolites enables the evaluation of the environmental response and metabolic adaptation of cyanobacteria and other microorganisms.

3.8.2 Following cellulose degradation by LAESI

Related publications:

C. H. Stephens, B. Shrestha, H. R. Morris, M. E. Bier, P. M. Whitmore and A. Vertes, "Minimally invasive monitoring of cellulose degradation by desorption electrospray ionization and laser ablation electrospray ionization mass spectrometry," *Analyst*, **2010**, *135*, 2434-2444.

Abstract

Minimally invasive desorption electrospray ionization-mass spectrometry (DESI-MS) and laser ablation electrospray ionization-MS (LAESI-MS) were used to look for soluble cellulose degradation products produced by accelerated aging in unsized cotton paper. Soluble extracts from papers aged 144 to 26 856 hours were first analyzed in solution using traditional electrospray ionization-MS (ESI-MS). Results were compared to those from direct analysis of condensed phase degradation products extracted from the absorbent paper substrate using DESI-MS and LAESI-MS. ESI-MS results showed evidence of oligosaccharide degradation products ranging from cellobiose to cellononaose; using DESI-MS and LAESI-MS, products from cellobiose to cellodecaose and glucose to cellooctaose, respectively, were observed. As degradation proceeded, increased quantities of both low and high molecular weight oligosaccharides were observed. The analytical approaches developed in the control study were applied for the detection of degradation products in two naturally-aged books dating from the 19th century, both made from cotton and linen. Oligosaccharides ranging from glucose to cellopentaose were observed.

3.8.3 Tissue imaging of metabolites by LAESI

Related publications:

A. Vertes, B. Shrestha, and P. Nemes, "Direct Metabolomics from Tissues and Cells: Laser Ablation Electrospray Ionization for Small Molecule and Lipid Characterization," in: *Methodologies for Metabolomics: Experimental Strategies and Techniques*, Methods in Molecular Biology, N. Lutz, J. V. Sweedler, and R. A. Wevers (eds), Cambridge University Press, **2013**, pp. 140–158. ISBN-13: 978-0-52176-590-9

P. Nemes and A. Vertes, "Ambient Mass Spectrometry for in vivo Local Analysis and in situ Molecular Tissue Imaging," *Trends in Analytical Chemistry (TrAC)*, **2012**, *34*, 22-34.

P. Nemes and A. Vertes, "Laser Ablation Electrospray Ionization for Atmospheric Pressure Molecular Imaging Mass Spectrometry," in: *Mass Spectrometry Imaging: Principles and Protocols*, Methods in Molecular Biology, Vol.: 656, S. S. Rubakhin and J. V. Sweedler (eds), Springer, **2010**, pp. 159-171.

F. Adams and A. Vertes, "Local analysis and imaging by mass spectrometry in nanotechnology," in: *The Encyclopedia of Mass Spectrometry, Vol. 5, Elemental and Isotope Ratio Mass Spectrometry*, D. Beauchemin and D. E. Matthews (eds), Elsevier, **2010**, pp. 486-497.

Abstract

LAESI MS offers a means to achieve local analysis with spatial resolution approaching 50 μm. Under appropriate experimental conditions (e.g., temperature and humidity), the structural and chemical integrity of biological tissues and cells is essentially retained around the ablated spot. Assuming proportionality between the LAESI MS signal and the metabolite concentration in the tissues, spatial variations in the chemical composition of these systems can be interrogated. The ion signal measured across the tissues allows the reconstruction of the corresponding molecular image. To perform LAESI mass spectrometry imaging, the sample is mounted onto a sample holder. Using two computer-controlled independent translation stages (X and Y directions), the sample is positioned at the focal point of the ablating mid-infrared light. The generated ions are simultaneously mass-analyzed, and the data are stored for each X-Y coordinate of the interrogated area. The molecular image of the sample for a particular species is reconstructed by representing the intensity of the related ion signal on a false color scale and correlating it with the coordinates for every pixel of the interrogated area. Most plant tissues are structurally different from tissues found in animals. In plants, the waxy cuticle and the rigid cell walls act as natural barriers for unwanted water loss in a LAESI imaging experiment. In contrast, when animal tissues are dissected, evaporative water loss is relatively rapid. Because water serves as the energy coupling medium in LAESI experiments, animal tissue sections are generally kept cold or frozen during analyses. A Peltier-cooling stage, equipped with a heat sink and a fan, can maintain the appropriate conditions for several hours and ensure that the water content of tissues does not appreciably change during the LAESI MSI time frame.

Using high mass-resolution time-of-flight MS in conjunction with LAESI, it becomes feasible to deconvolute the spatial distribution of metabolites that possess an identical nominal mass. For example, the ion images of GABA and choline, which differ only by 38 mDa in their monoisotopic mass, have been found to populate tissue sections in a vastly different manner. Similar distributions can be obtained for any detected m/z.

The massive data sets generated in LAESI imaging can be evaluated via Pearson co-localization maps. The calculated Pearson co-localization map highlights the areas where two selected ions are found together. Co-localization of metabolites can be exploited in the discovery of metabolic pathways active in certain tissue regions.

3.9 Laser ablation atmospheric pressure photoionization

Related publications:

A. Vaikkinen, B. Shrestha, T. J. Kauppila, A. Vertes and R. K. Kostiainen, "Infrared laser ablation atmospheric pressure photoionization mass spectrometry," *Anal. Chem.*, **2012**, *84*, 1630-1636.

Abstract

In this paper we introduce laser ablation atmospheric pressure photoionization (LAAPPI), a novel atmospheric pressure ion source for mass spectrometry. In LAAPPI the analytes are ablated from water-rich solid samples or from aqueous solutions with an infrared (IR) laser running at 2.94 μm wavelength. Approximately 12 mm above the sample surface, the ablation plume is intercepted with an orthogonal hot solvent (e.g., toluene or anisole) jet, which is generated by a heated nebulizer microchip and directed toward the mass spectrometer inlet. The ablated analytes are desolvated and ionized in the gas-phase by atmospheric pressure photoionization using a 10 eV vacuum ultraviolet krypton discharge lamp. The effect of operational parameters and spray solvent on the performance of LAAPPI is studied. In its current version, LAAPPI offers ~300 μm lateral resolution. In addition to polar compounds, LAAPPI efficiently ionizes neutral and nonpolar compounds. The bioanalytical application of the method is demonstrated by the direct LAAPPI analysis of rat brain tissue sections and sour orange (*Citrus aurantium*) leaves.

3.10 Analysis of single plant cells and their subcellular compartments

Related publications:

B. Shrestha, J. M. Patt and A. Vertes, "In Situ Cell-by-Cell Imaging and Analysis of Small Cell Populations by Mass Spectrometry," *Anal. Chem.*, **2011**, *83*, 2947-2955.

B. Shrestha and A. Vertes, "Direct Analysis of Single Cells by Mass Spectrometry at Atmospheric Pressure," *J. Visualized Experiments*, **2010**, *43*, e2144.

B. Shrestha, P. Nemes and A. Vertes, "Ablation and analysis of small cell populations and single cells by consecutive laser pulses," *Appl. Phys. A*, **2010**, *101*, 121-126.

B. Shrestha and A. Vertes, "Relative Quantitation in Single Cell Metabolomics by Laser Ablation Electrospray Mass Spectrometry," in: *Plant Metabolism: Methods and Protocols*, Methods in Molecular Biology, G. Sriram (ed), pp. 31-39. ISBN-13: 978-1-62703-660-3

J. A. Stolee, B. Shrestha, G. Mengistu and A. Vertes, "Observation of Subcellular Metabolite Gradients in Single Cells by Laser Ablation Electrospray Ionization Mass Spectrometry," *Angew. Chem. Int. Ed.*, **2012**, *51*, 10386-10389. http://dx.doi.org/10.1002/anie.201206834

Abstract

Molecular imaging by mass spectrometry (MS) is emerging as a tool to determine the distribution of proteins, lipids, and metabolites in tissues. The existing imaging methods, however, mostly rely on predefined rectangular grids for sampling that ignore the natural cellular organization of the tissue. Here we demonstrate that laser ablation electrospray ionization (LAESI) MS can be utilized for in situ cell-by-cell imaging of plant tissues. The cell-by-cell molecular image of the metabolite cyanidin, the ion responsible for purple pigmentation in onion (*Allium cepa*) epidermal cells, correlated well with the color of cells in the tissue. Chemical imaging using single-cells as voxels reflects the spatial distribution of biochemical differences within a tissue without the distortion stemming from sampling multiple cells within the laser focal spot. Microsampling by laser ablation also has the benefit of enabling the analysis of very small cell populations for biochemical heterogeneity. For example, with a ~30 μm ablation spot we were able to analyze 3-4 achlorophyllous cells within an oil gland on a sour orange (*Citrus aurantium*) leaf. To explore cell-to-cell variations within and between tissues, multivariate statistical analysis on LAESI-MS data from epidermal cells of an *A. cepa* bulb and a *C. aurantium* leaf and from human buccal epithelial cell populations was performed using the method of orthogonal projections to latent structures discriminant analysis (OPLS-DA). The OPLS-DA analysis of mass spectra, containing over 300 peaks each, provided guidance in identifying a small number of metabolites most responsible for the variance between the cell populations. These metabolites can be viewed as promising candidates for biomarkers that, however, require further verification.

In recent years an increasing number of methods have been exploited for the proteomic and metabolomic analysis of single cells and have provided new insight into cellular subtypes. Local analysis on a subcellular level, however, requires new approaches. Heterogeneity of metabolite distributions within a cell is attributed to functional organization, compartmentalization into organelles, macromolecular crowding, and metabolite channeling as a result of the colocalization of enzymes. This heterogeneity results in metabolite gradients within a cell and compartmentalization of metabolites in particular organelles. The intracellular production, reaction, and redistribution of metabolites do not always follow the kinetics established in vitro at low concentrations. Subcellular trafficking between compartments often relies on active transport facilitated by transporter proteins. For example, secondary metabolites can accumulate in the vacuole by the help of ABC transporters. Determining the subcellular distributions of metabolites is challenging because of their high diffusion rates and rapid turnover.

Most techniques for the subcellular analysis of eukaryotic cells rely on the isolation of organelles by nonaqueous fractionation and require extensive sample preparation prior to chemical analysis. By using tagging or labeling techniques, the distribution of some preselected metabolites can be followed by fluorescence resonance energy transfer. More recently, cell-membrane lipid distributions have been analyzed by secondary ion mass spectrometry (SIMS) and selected metabolite levels have been determined

in the cytoplasm, cytosolic lipid droplets, vacuole, granule, and nucleus by nano-electrospray ionization mass spectrometry. There are, however, few label-free multispecies methods that capture the spatial localization of diverse metabolites within a cell.

Femtosecond laser pulses have been used for disrupting and dissecting subcellular organelles, such as mitochondria and nuclei, in living mammalian cells. This nanosurgery technique, however, is typically performed without opening the cell, therefore the resulting ablation products are not available for analysis. In laser ablation electrospray ionization (LAESI) of biological samples, a mid-infrared laser generates a plume in the surrounding environment by bursting the cells open. The ejected material is ionized by an electrospray and analyzed by a mass spectrometer. The ablation and analysis of metabolites in single cells has been achieved by delivering the mid-IR laser pulses with an etched optical fiber for LAESI analysis. Herein, we report the in situ chemical analysis of metabolites localized in subcellular compartments by the combination of microdissection and LAESI–MS. We demonstrate the direct multispecies molecular analysis of subcellular compartments by this ambient ionization method. Large metabolite gradients between the cytoplasm and nucleus of *Allium cepa* epidermal cells are observed using this novel technique.