Applications for MALDI-TOF Imaging Present and Future

SimulTOF Systems 261 Cedar Hill Street Marlborough, MA 01752
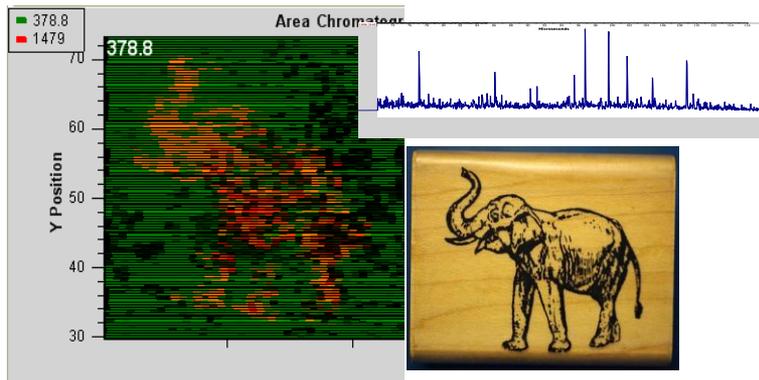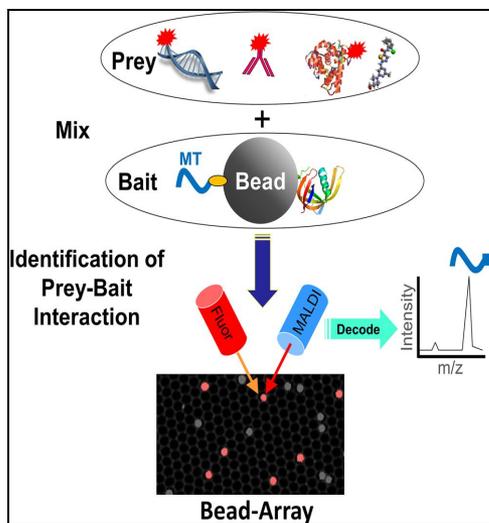
Outline
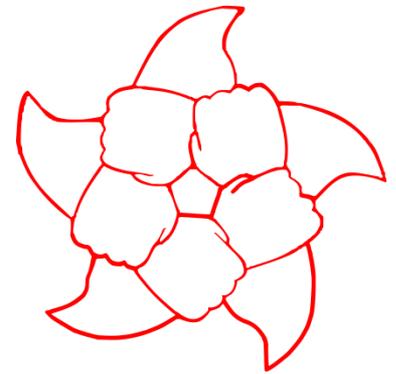
- Protein bead arrays
- Surface Imaging of cells grown on slides
- Biological tissue imaging
- Novel technologies for samples preparations

Protein Arrays

Massively-parallel way to generate information regarding molecular interactions: protein-protein, protein-DNA, protein-drug etc...

- **Affinity Arrays:**
  - Use specific capture molecules to quantify analyte (ELISA type)

- **Reverse Phase Array (Lysate Arrays):**
  - Spot complex sample
  - probe with antibodies

- **Functional Protein Arrays:**
  - Spotted purified protein array
  - Assay protein-protein interaction
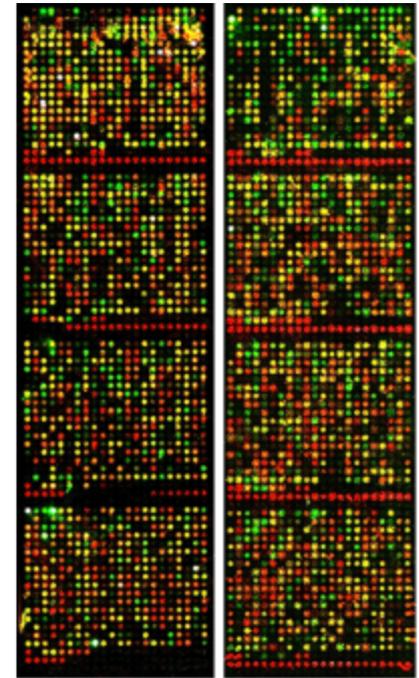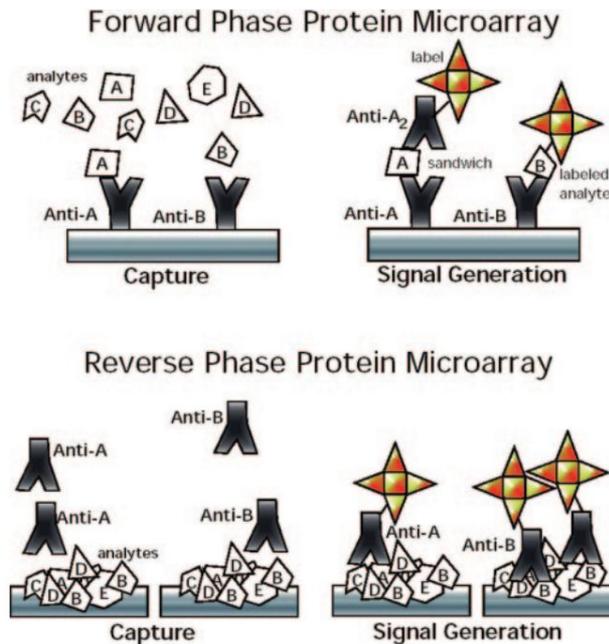
Two microarrays of about 1600 proteins each. The molecules were labelled with fluorophors in order to indicate the percentage of full length proteins.

Detection based on fluorescent signal after secondary interaction

1. Sheehan, KM et al. "*Use of Reverse Phase Protein Microarrays and Reference Standard Development for Molecular Network Analysis of Metastatic Ovarian Carcinoma*" Mol Cell Proteomics.4(4): 346-55 (2005)

Addressing the Weaknesses of Conventional Protein Arrays

- **Inability of Fluorescence to:**
  - Reveal the molecular details of bait and prey interaction
  - Perform label-free detection of small molecule (*e.g.* drug) interaction
  - Facilitate multiplexing (*e.g.* of multiple prey interacting with array)

- **Mechanical or Piezoelectric Protein Printing on Array Surface Results in:**
  - Relatively low array density
  - Poor reproducibility of spot size, shape and uniformity
  - Printing-induced damage to delicate proteins (e.g. drying and/or surface induced denaturation)
  - Non-parallel printing process results in higher costs per array

- **2D Fixed Array Results in:**
  - Poor kinetics for protein binding & interactions
  - Inefficient automation of planar microarray 'chips' (essentially 1 microscope slide per sample)

MALDI-TOF MS
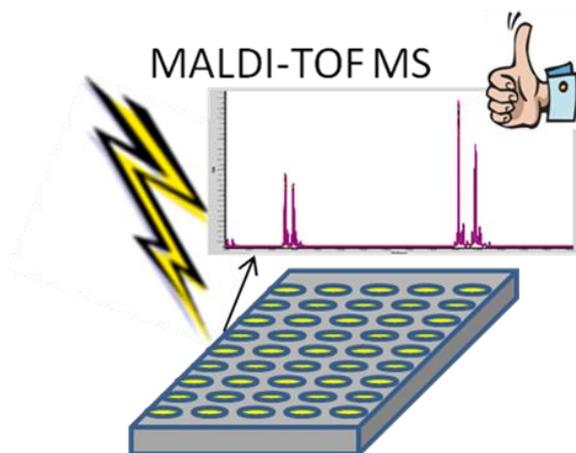
Mass Spectrometric Bead-Array Technology
Overcomes These Limitations

1. Mark J. Lim M J, Liu Z, Braunschweiger K I, Awad A, Rothschild K, "*Correlated matrix-assisted laser desorption/ionization mass spectrometry and fluorescent imaging of photocleavable peptide-coded random bead-arrays*" Rapid Commun. Mass Spectrom. 2014, 28, 49–62
AmberGen, Incorporated, 313 Pleasant Street, Watertown, MA 02472, USA

# AmberGen's Bead-GPS™ Mass Spectrometric Bead-Array Technology

Bead-Array

- **Random bead-array encoded with Photocleavable (PC) Mass-Tags (MT ⌇⬭)**

- **Measuring interaction of prey such as enzymes, DNA, antibodies or small molecules with a bead-library of bait molecules (*e.g.* proteins)**

- **MALDI-MSI decodes the PC-Mass-Tags and in some cases the label-free small molecules**

- **Fluorescence may also be used for quantitative hit detection and correlated with MALDI-MSI**

- **Label-free detection (e.g. drugs)**

Mark J. Lim M J, Liu Z, Braunschweiger K I, Awad A, Rothschild K, "*Correlated matrix-assisted laser desorption/ionization mass spectrometry and fluorescent imaging of photocleavable peptide-coded random bead-arrays*" Rapid Commun. Mass Spectrom. 2014, 28, 49–62.

Workflow

ambergen

Differentially functionalized beads

Complex sample

Incubation / bind

Spread on μwell plate

Fluorescence
For location and quantitation

Add matrix
Analyze
by MALDI-TOF

Bead identification
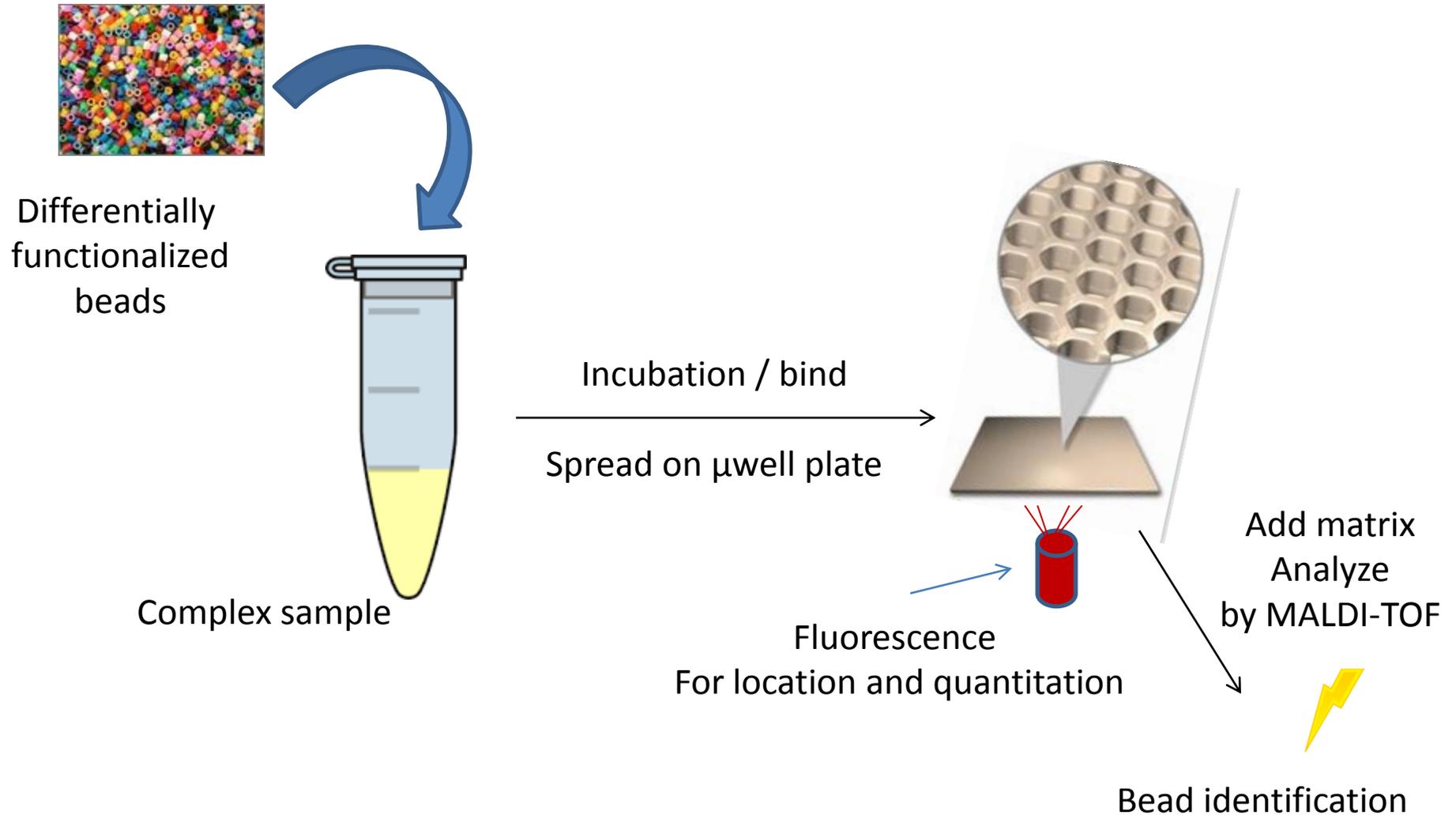
Sample Acquisition

- SimulTOF 200 MALDI-TOF mass spectrometer (SimulTOF Systems, Sudbury, MA)

Capabilities:
- *Max accelerating voltage 20 kV*
- *Max laser pulse frequency 5000 Hz*
- *Max scan speed 10 mm/s*

Acquisition parameters
- Reflector mode using positive-ion polarization
- Acceleration voltage 20 kV
- Mass range 450 – 2000 Dalton
- Focus mass 1,000
- Laser pulse frequency 1000 Hz
- Laser pulse energy 12 µJ
- Scan rate 1 mm/s
-100 µm raster to cover each sample position

Results from MSI of AmberGen Bead-GPS™ Array Scan by SimulTOF Instrument

Matrix peak & calibration standard used for mass calibration

Scan from 5x5 mm area

Even distribution and intensity of mass standard

AmberGen proprietary micro-well plate used in the analysis

Color-coded TIC of 12 peaks from mass range 1500- 1700Da

2D plot of selected signals show their location
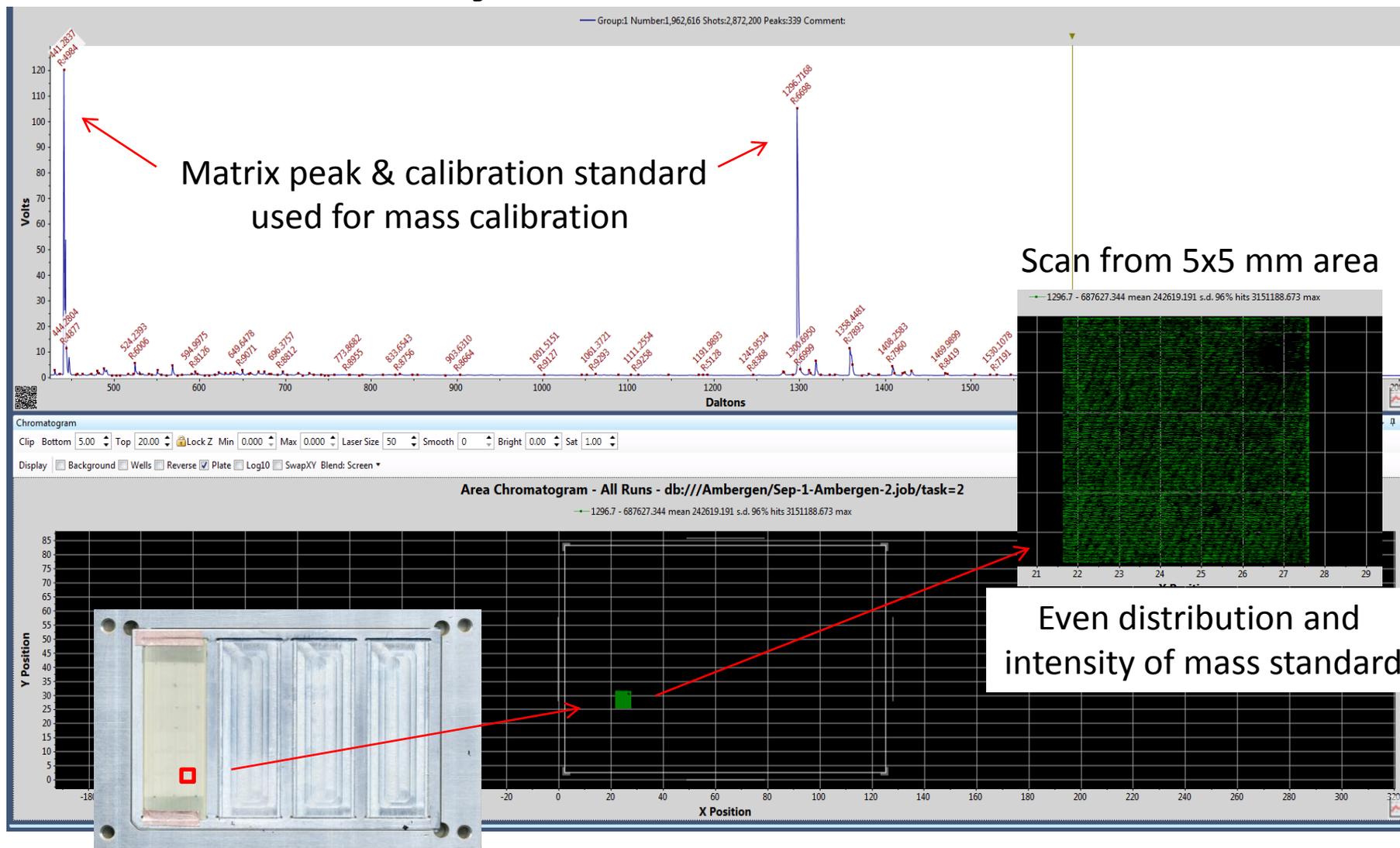
AmberGen Bead-GPS™ using SimulTOF Instrument: MSI of Representative Photocleavable Mass-Tags in 50-Member Library
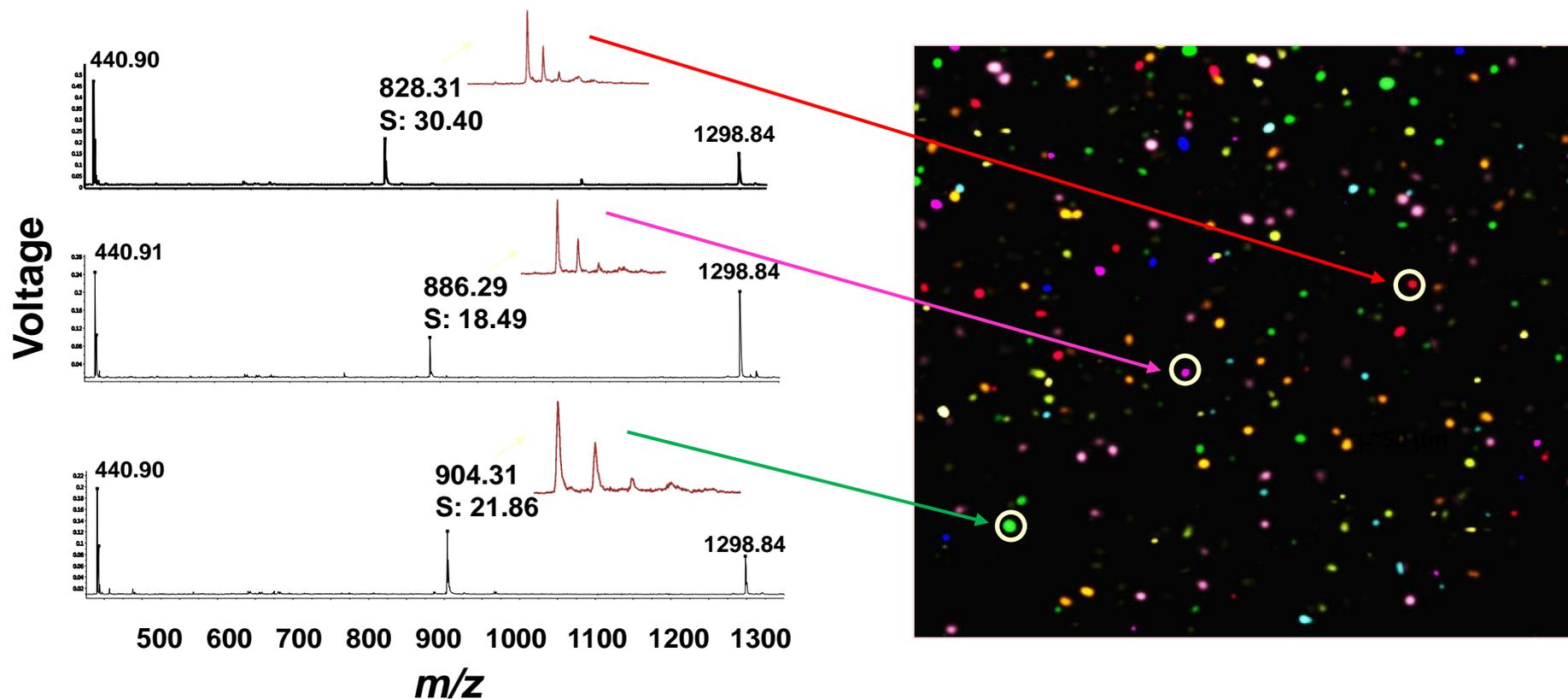
Sample Results:
- SimulTOF software used for PC-Mass-Tag peak detection
- XY coordinates of monoisotopic peak area converted to 2D image of 10 selected PC-Mass-Tags
- 50-member PC-Mass-Tag bead-library also contained 50 recombinant "bait" proteins
- Matrix peak at *m/z* 440.9 & internal standard at *m/z* 1298.8 used for mass calibration

Same sample: Lower mass range

Mass range 900 – 1200 Da

Calibration Standard
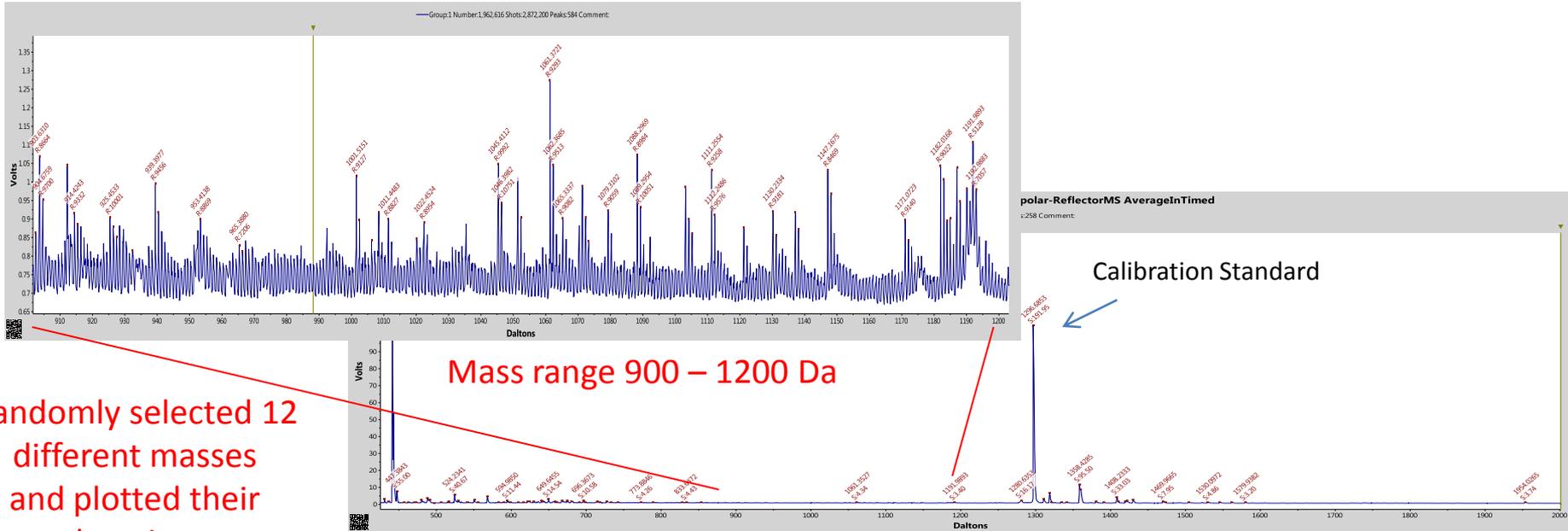
Randomly selected 12 different masses and plotted their location

Application and improvements

- Measuring interaction of prey such as enzymes, DNA, antibodies or small molecules with a bead-library of bait molecules (*e.g.* proteins)

- Huge increase in scaling

- Label free detection

- Molecular details of bait and prey interaction

Surface Imaging of cells grown on slides

Silicone gasket provide isolated compartments for multiple samples or experimental conditions

indium tin oxide coated surface provides electrical conductivity

Growth media added and cell are allowed to incubate

ADEPTRIX
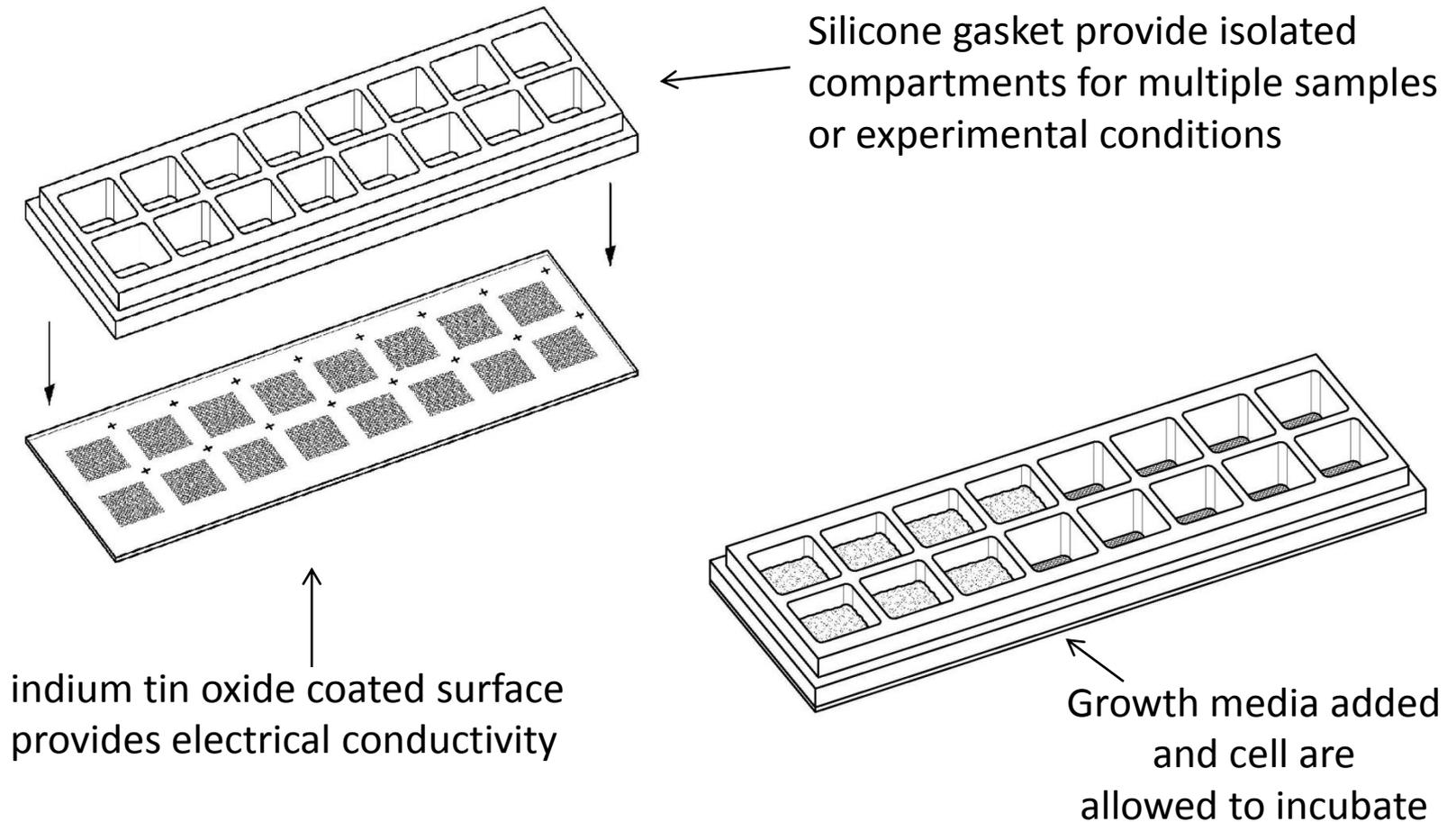
After proper incubation media is discarded and adherent cell remain

Silicone gasket removed

Adherent Cells remain

ADEPTRIX
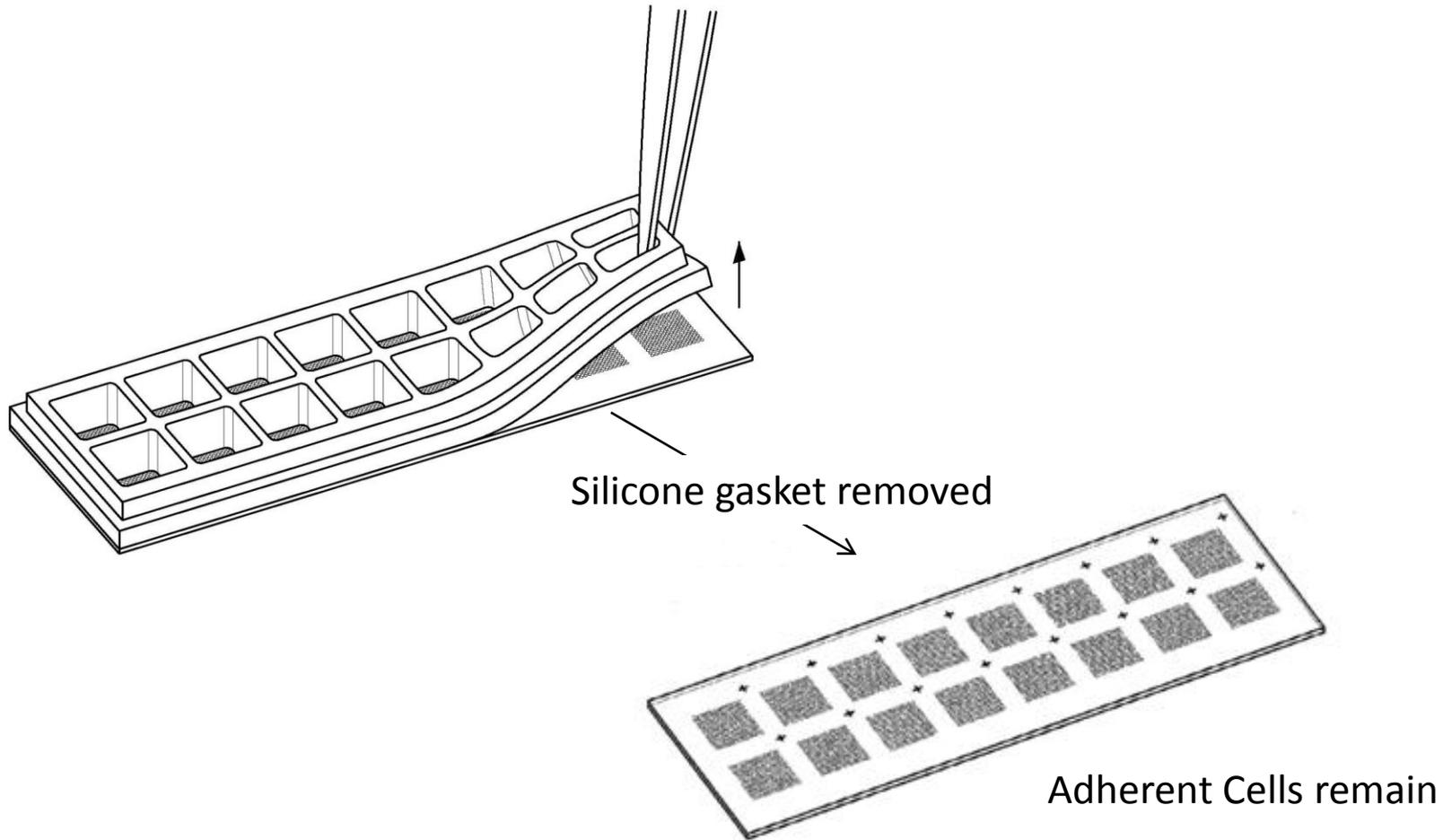
Samples are matrix coated and analyzed directly in the Mass Spectrometer
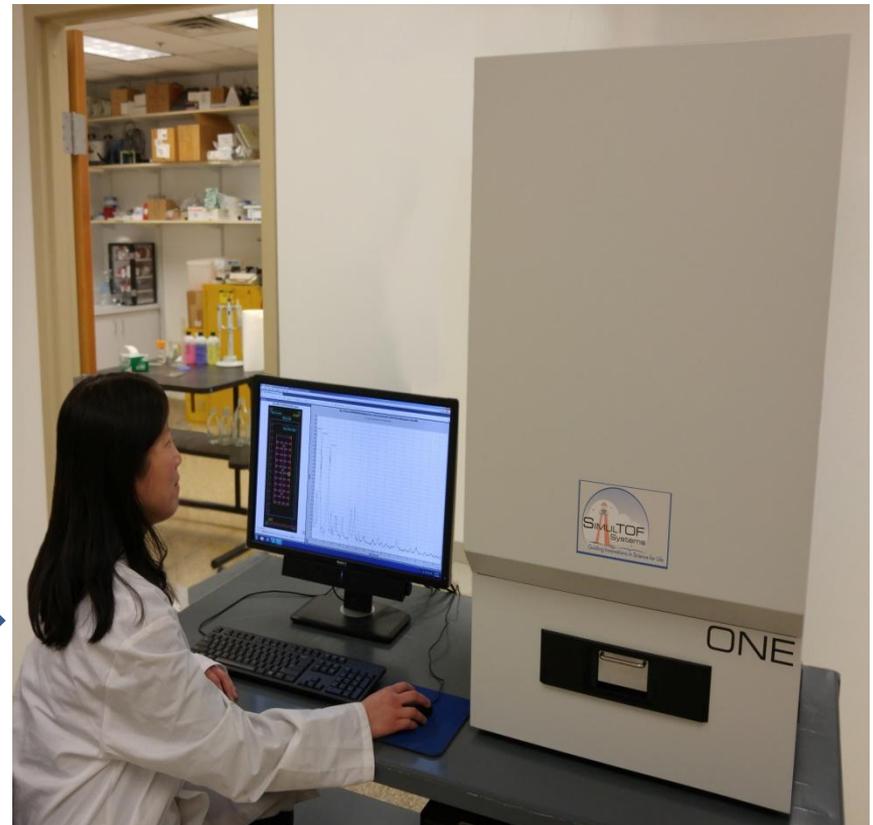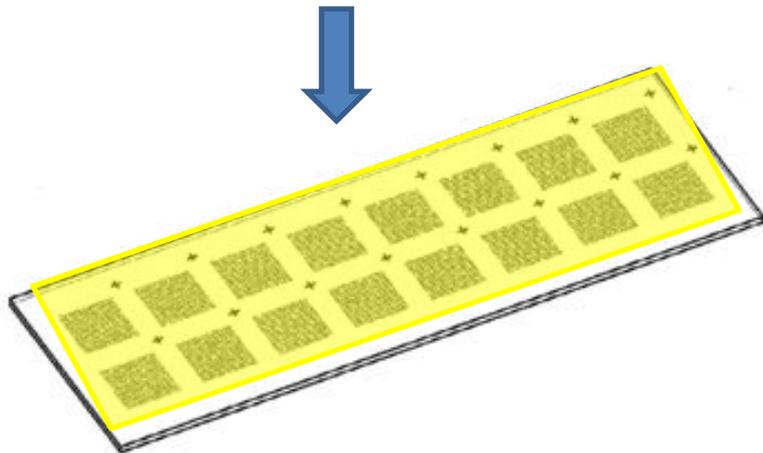
Acquire data as a surface image

# Numerous applications

- cell line profiling

- studying cell migration

- cell invasion

- wound healing

- growth conditions

- drug response

ADEPTRIX

Tissue Imaging

## IMS combines molecular specificity with location
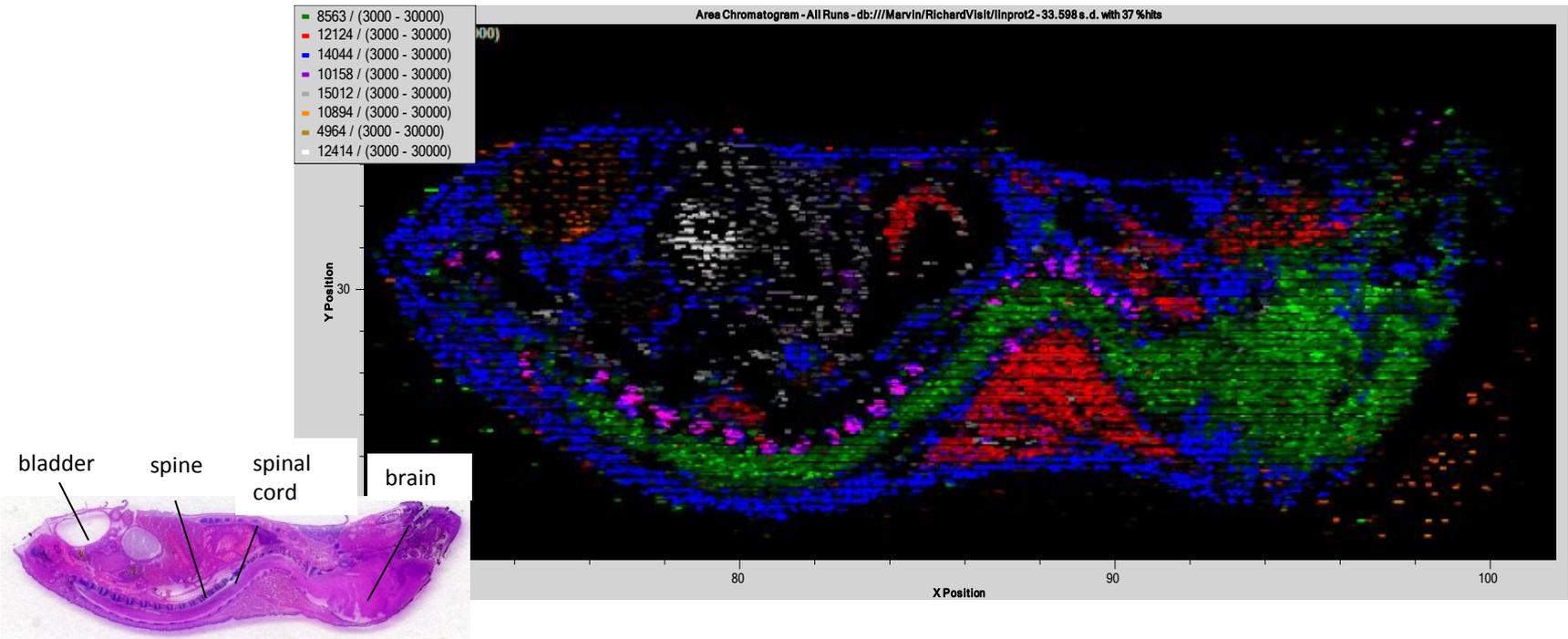
The multiplexed nature of MS analysis allows for the parallel acquisition of many different molecular signals, each which can be reconstructed to give a molecular picture.

Imaging Mass Spectrometry

Sample Preparation

Matrix
SA – proteins/peptides
DHB –proteins/peptides
CHCA- lipids/peptides
DAN– lipids

Application Method
Spraying (manual, robotic)
Sublimation
Spotting (robotic)

Matrix application
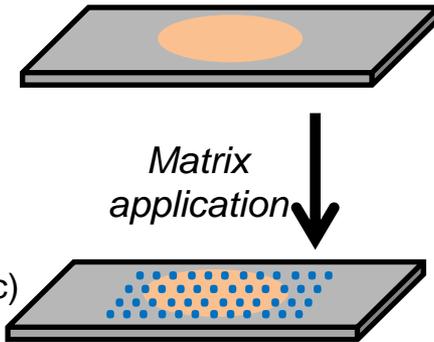
Matrix-Assisted Laser Desorption/Ionization

Laser
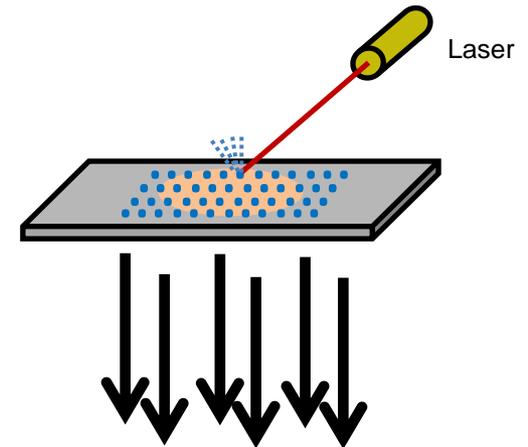
Lipid & Protein Ion Images
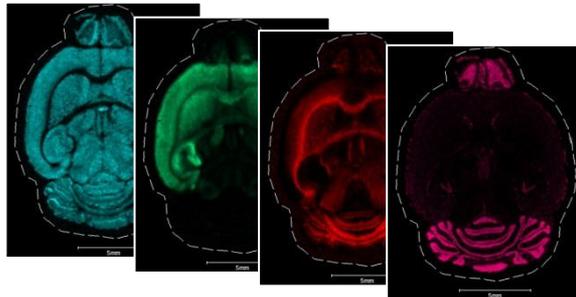
Images for individual m/z values integrated over all pixels

Data Processing
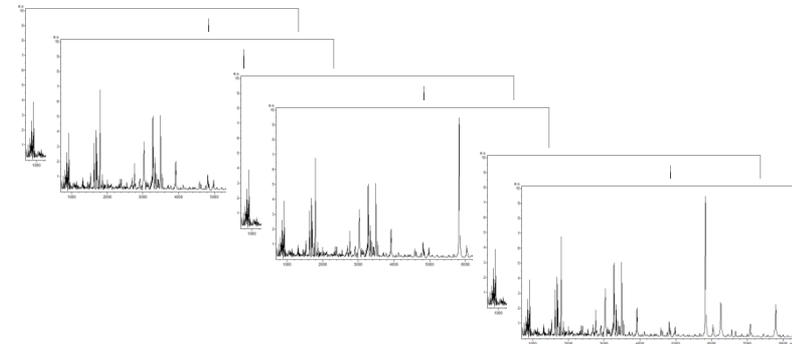
MS spectrum for each x, y coordinate (pixel)

Boone M. Prentice[1], Kevin M. Hayden[2], Marvin L. Vestal[2], Richard M. Caprioli[1]
[1]Mass Spectrometry Research Center, Department of Biochemistry, Vanderbilt University, Nashville, TN 37235
[2]SimulTOF Systems, Marlborough, MA 01752

18

Special IMS Considerations

Low Spatial Resolution (>100 μm) ← → High Spatial Resolution (<10 μm)
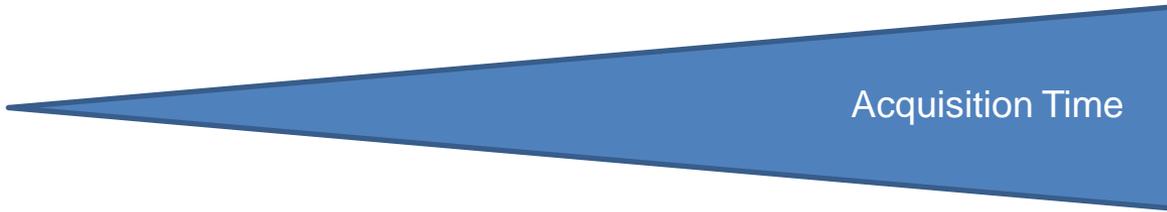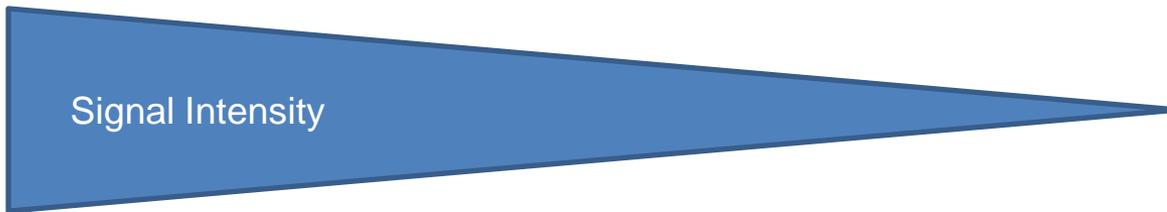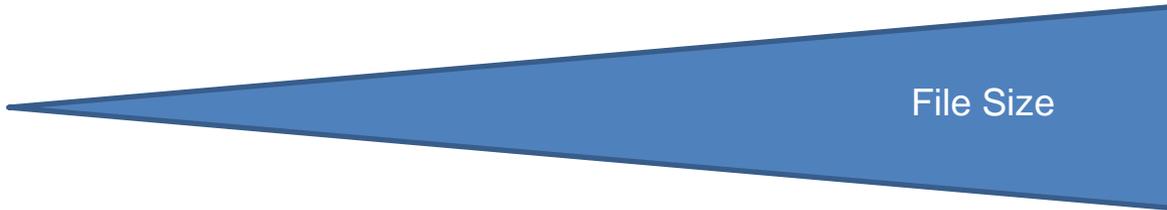
Minutes — Acquisition Time — Hours

1×10^8 — Signal Intensity — 1×10^5

<5 GB — File Size — > 10GB

19

Boone M. Prentice[1], Kevin M. Hayden[2], Marvin L. Vestal[2], Richard M. Caprioli[1]
[1]Mass Spectrometry Research Center, Department of Biochemistry, Vanderbilt University, Nashville, TN 37235
[2]SimulTOF Systems, Marlborough, MA 01752

#Pixels = f(Spatial Resolution, Area)

High-spatial resolution and/or large area IMS experiments can require prohibitive analysis times.
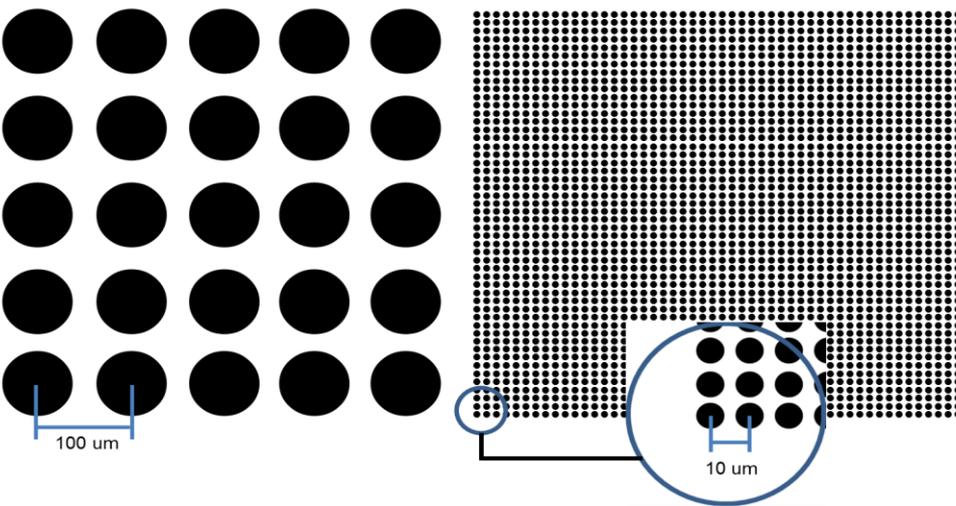
25 pixels

2,500 pixels
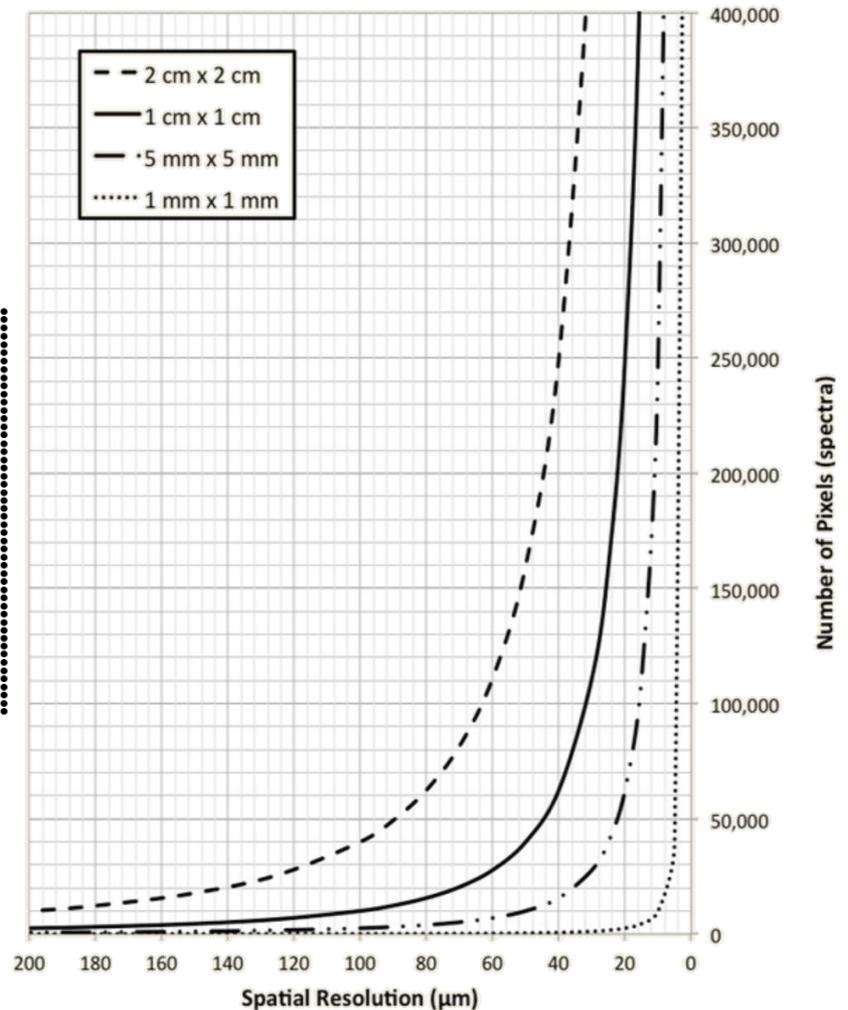
Boone M. Prentice[1], Kevin M. Hayden[2], Marvin L. Vestal[2], Richard M. Caprioli[1]
[1]Mass Spectrometry Research Center, Department of Biochemistry, Vanderbilt University, Nashville, TN 37235
[2]SimulTOF Systems, Marlborough, MA 01752

MALDI Imaging Platforms

Next Generation MALDI TOF
SimulTOF ONE, TWO and THREE

- High mass resolution
- High repetition rate laser (5 kHz Nd:YLF)
- High digitizer acquisition rate (50-100 pixels/sec)
- Continuous laser raster sampling
- MS/MS
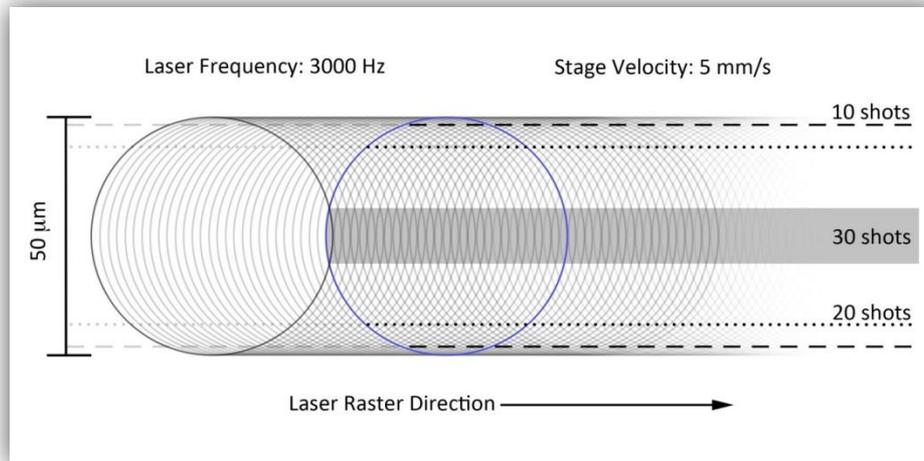
$$Lateral\ Spatial\ Resolution = H.A \left(\frac{v_{stage}}{f_{rep}}\right)$$

J.M Spraggins and R.M. Caprioli, *J. Am. Soc. Mass Spectrom.* **2011**, *22*, 1022-1031.

High Speed MALDI TOF

~345,000 pixels/3hr **Next Generation MALDI TOF**

~21,000 pixels/3hr **MALDI TOF**

~9,800 pixels/3hr **LIT**

~7,700 pixels/3hr **FT-ICR**

~7,200 pixels/3hr **IM Q-TOF**

Legend:
- 2 cm x 2 cm
- 1 cm x 1 cm
- 5 mm x 5 mm
- 1 mm x 1 mm

Number of Pixels (spectra)

Spatial Resolution (µm)

22
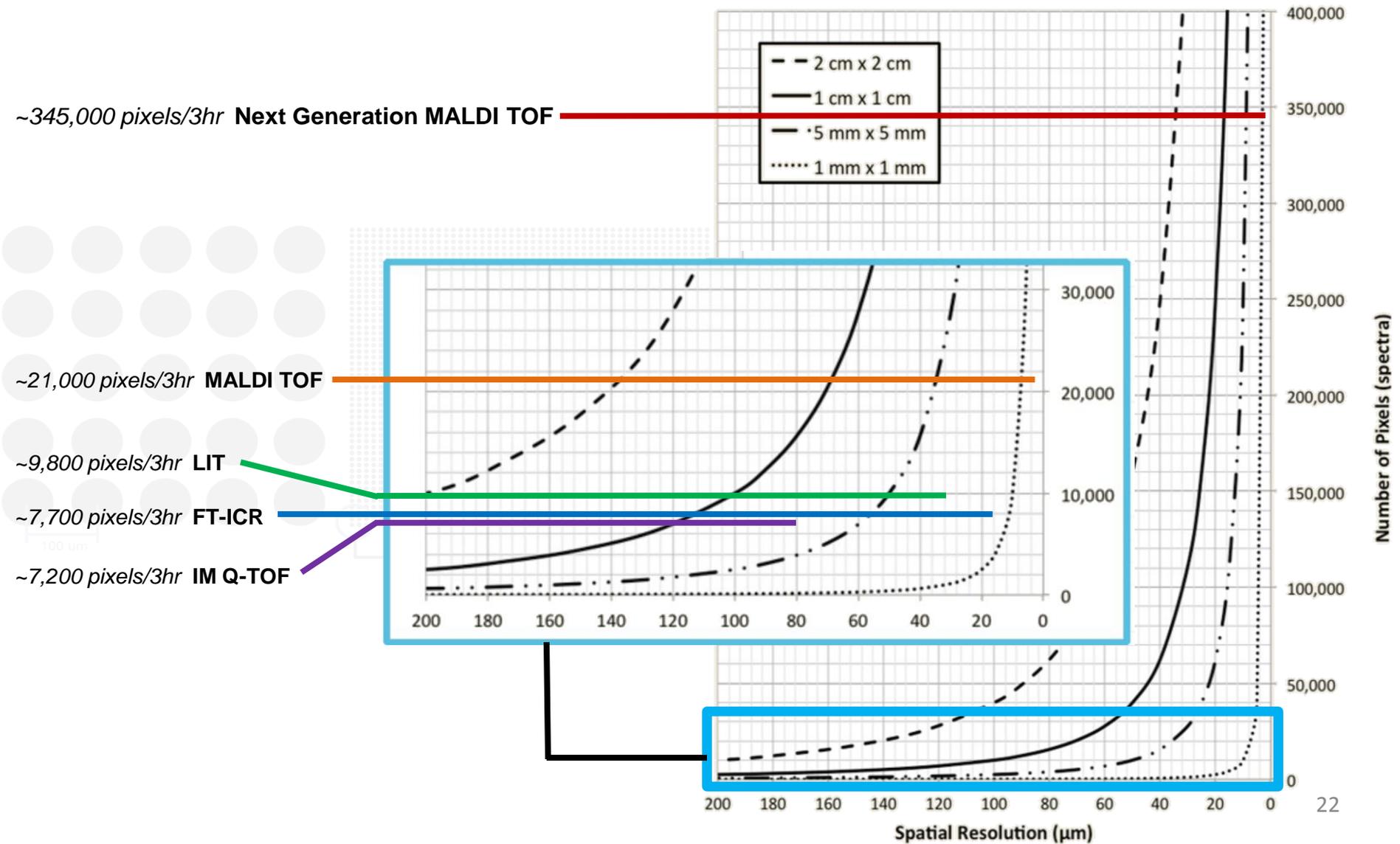
Hemoglobin from whole blood 1:2000 dilution in HCCA matrix

Stainless steel plate not tissue
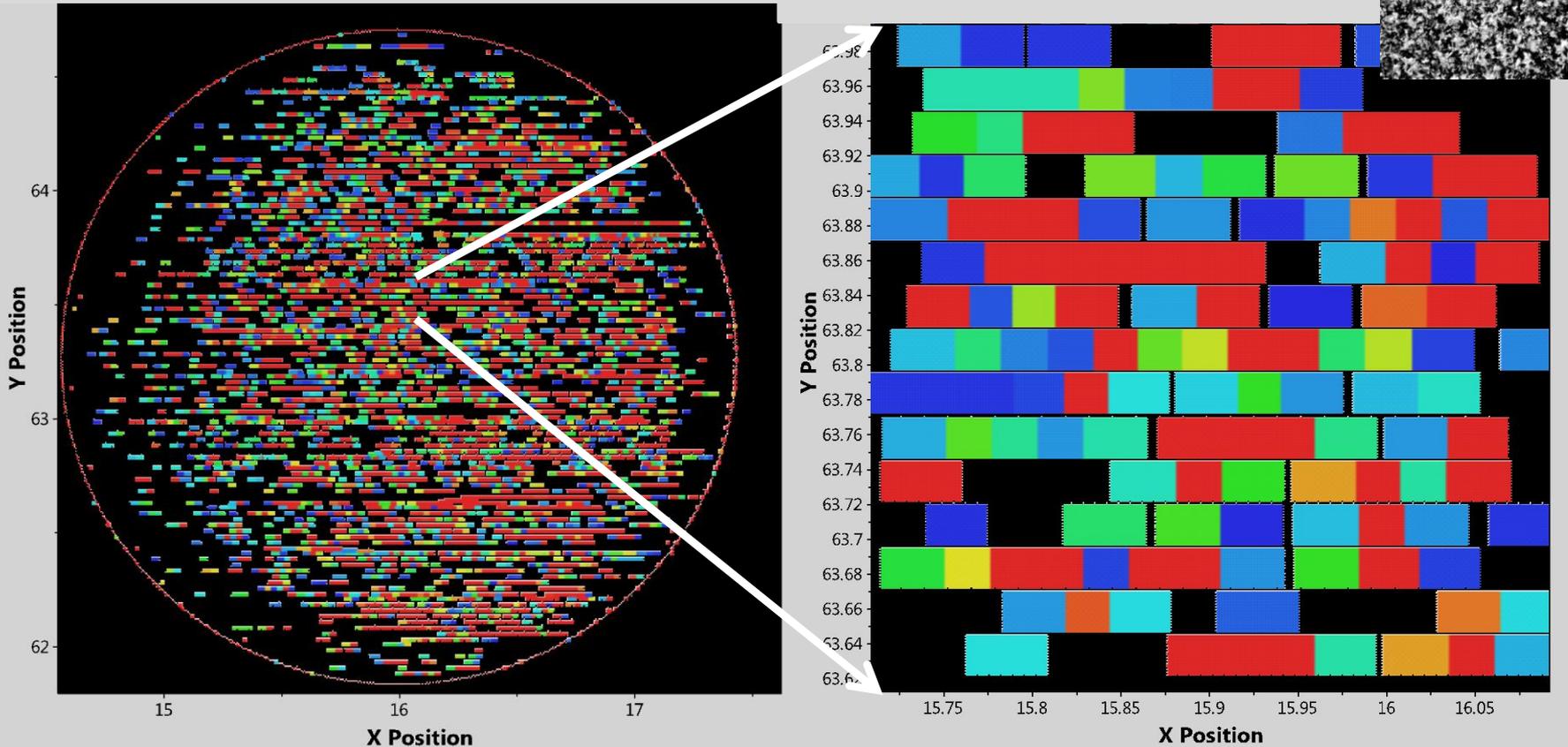
Image of 2.6 mm spot (450,000 laser shots) with 25 μm pixels,
5 kHz laser
25 μm raster @2 mm/s
56 shots/pixel saved
7700 pixels in a 90 s acquisition
~ 90 pixels/s or 972,000 pixels/3 hrs

Blow-up of section of image
Showing 25 μm pixels

Group:1 Number:17D4 Shots:56 Peaks:70

Single
25 µm pixel

Group:1 Number:17D4 Shots:431,872 Peaks:85

Average
Over full
2.6 mm spot
7700 pixels
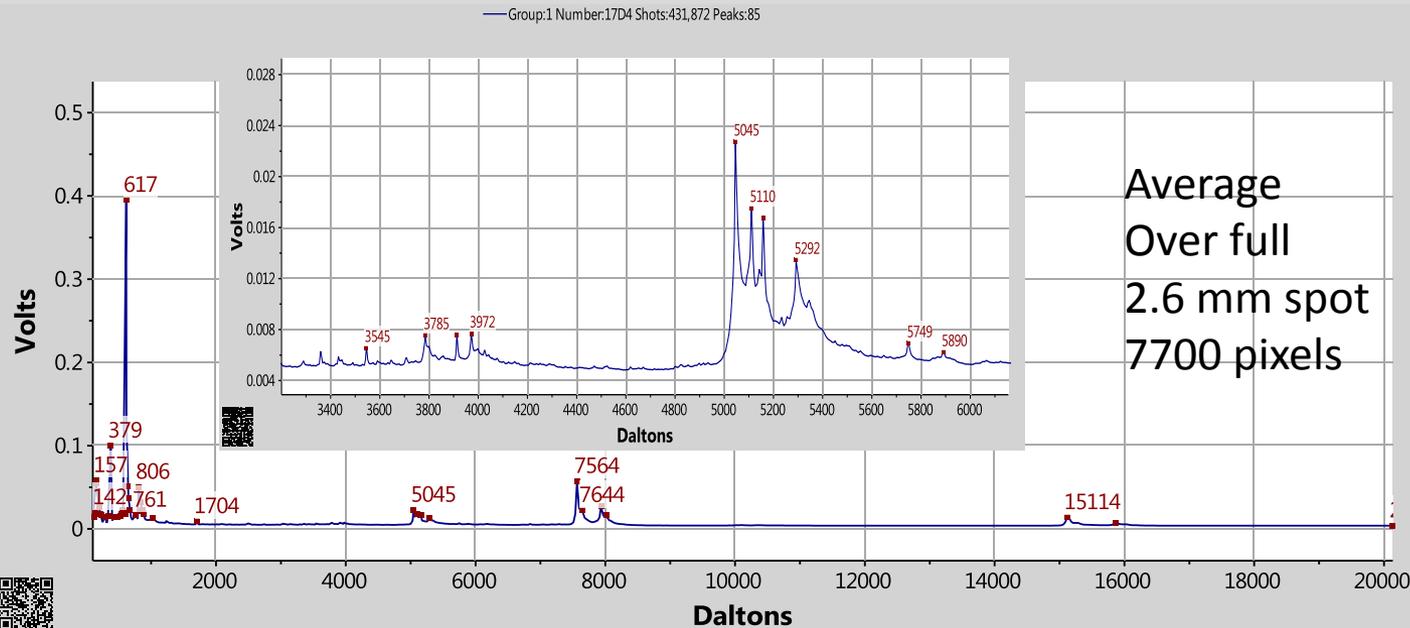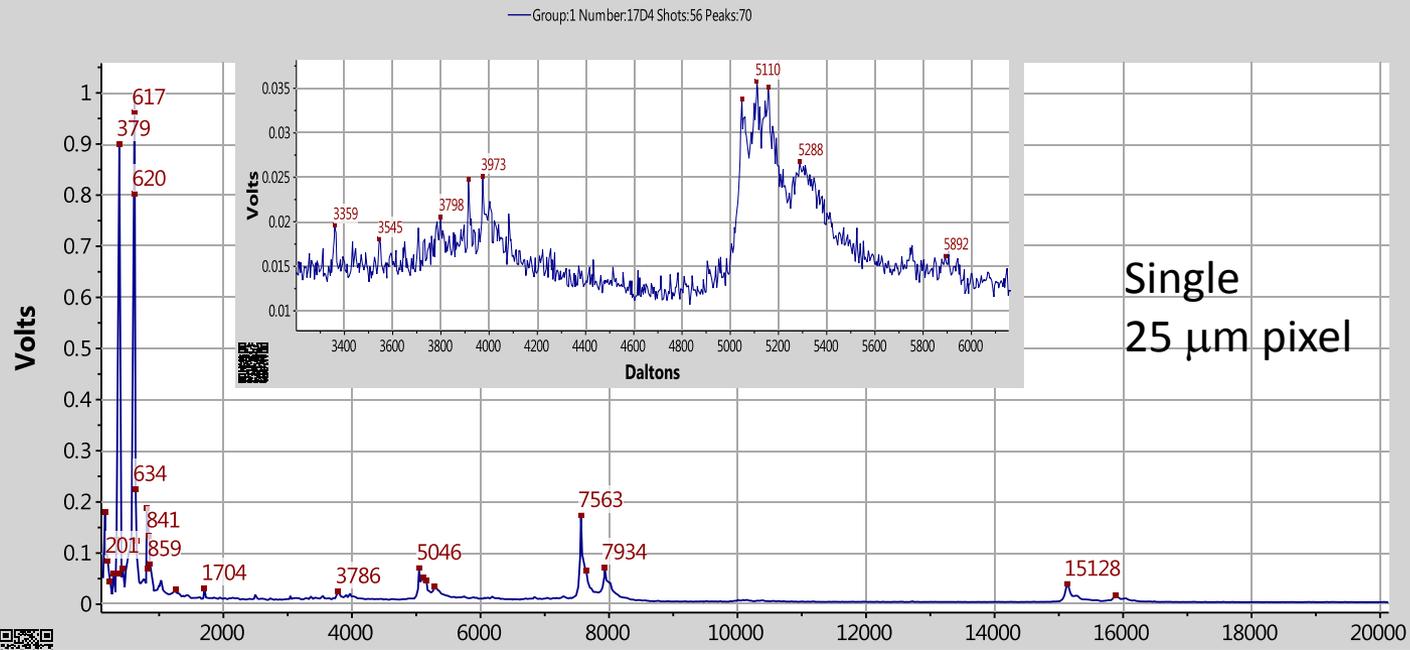
Features of SimulTOF ONE

• 20 kV energy and novel high speed, high mass detector provides *high sensitivity, resolving power and accuracy over broad mass range*

• *Fast sample plate exchange*

• Fully automated and designed for easy-of-use

• *Intuitive software* that requires minimal training

• Up to 100 spectra / second recorded and processed

• New concepts in instrument design provide a system that is *simple, reliable and robust*

• Computer controlled laser fluence

• Self-contained vacuum system

• Single 20A circuit powers a complete system including computer

• No other utilities required
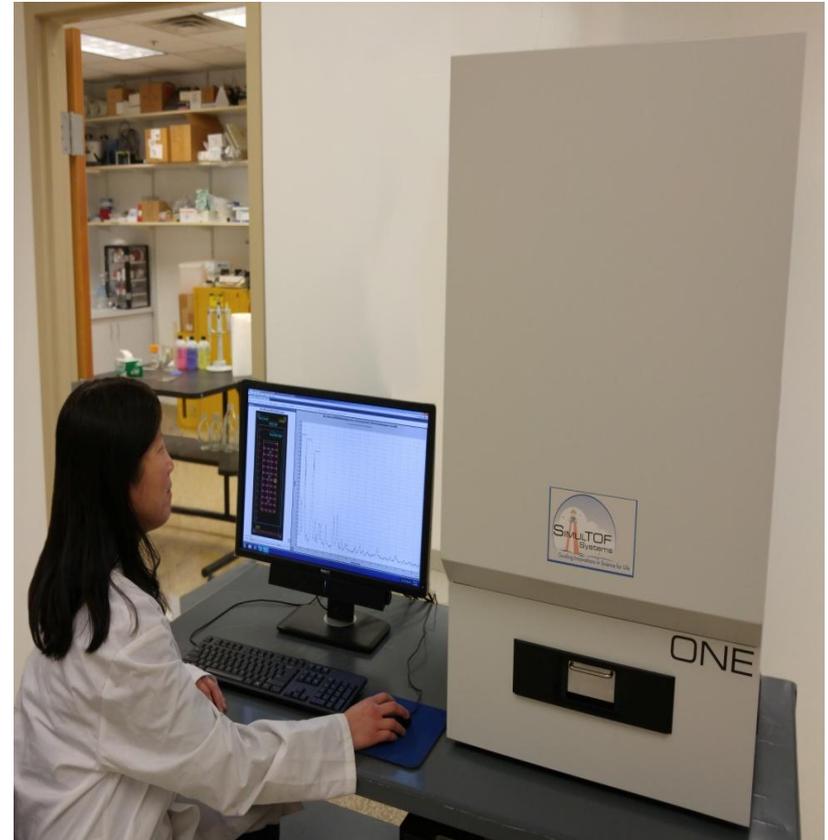
Biological Tissue is a < ideal surface for MALDI-TOF analyses

Tissue itself
-variable in composition
-not flat can distort under vacuum
-non conductive

Sample preparation
-requires thin slices (> 20μm)
-relies of matrix solvent to extract analytes of interest (peptides, proteins)
-do not want matrix solvent to distort sample location
      apposed functions may be limit sensitivity and spatial resolution
-may require washing

Analysis
-Catch all nature is good but can be limiting as well
   you see what ionizes best and what is most abundant

Collimated holes plates filled with polymer

-Developed as a potential interface between SDS-PAGE and MALDI-TOF
-Potentially for *tissue imaging* as well

25μm   35μm

P:200×   7.00 kV   100 μm   METH   18 JUN 107   #0002*

P:1,000×   7.00 kV   10 μm   METH   18 JUN 107   #0003*
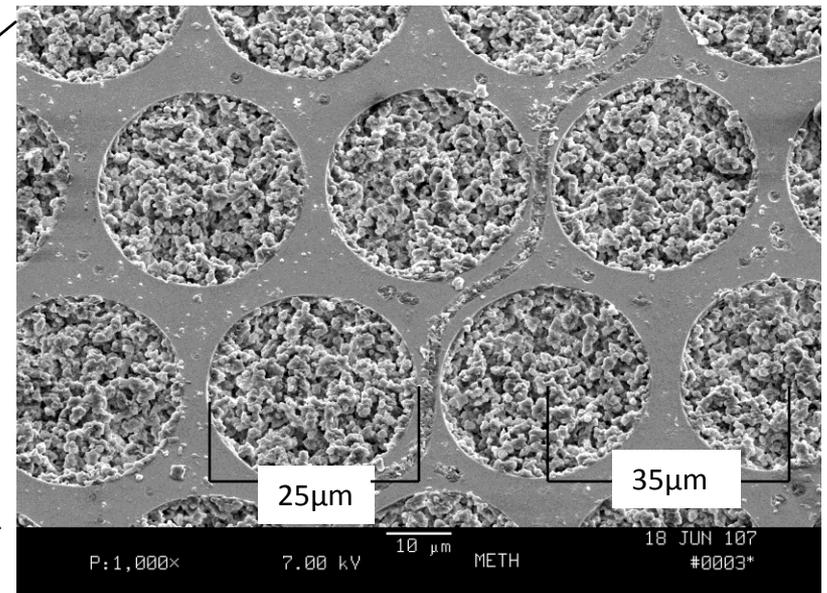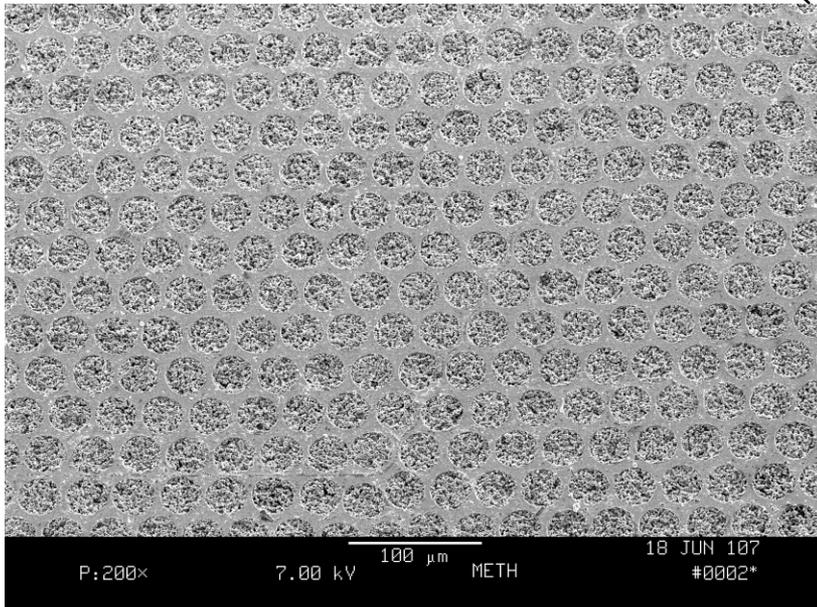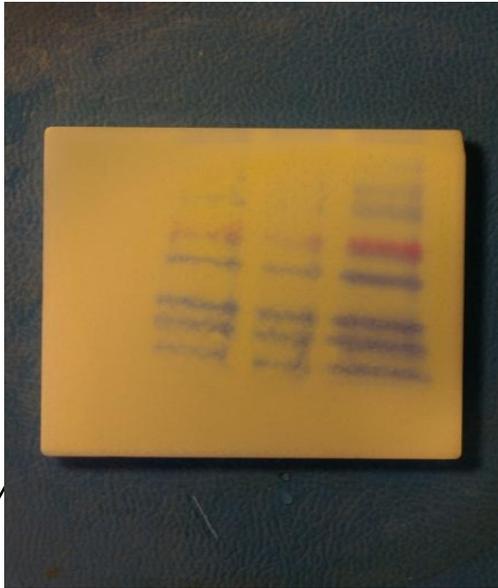
Workflow for Shoot Through CHS Plate for Tissue Imaging

Apply tissue

Extract with appropriate solvent
For as long as you want

Remove tissue

Apply Matrix

-**Lock-in analyte spatially**

-**Decouple analyte extraction and matrix application**

-**Build in specificity for analytes of interest**
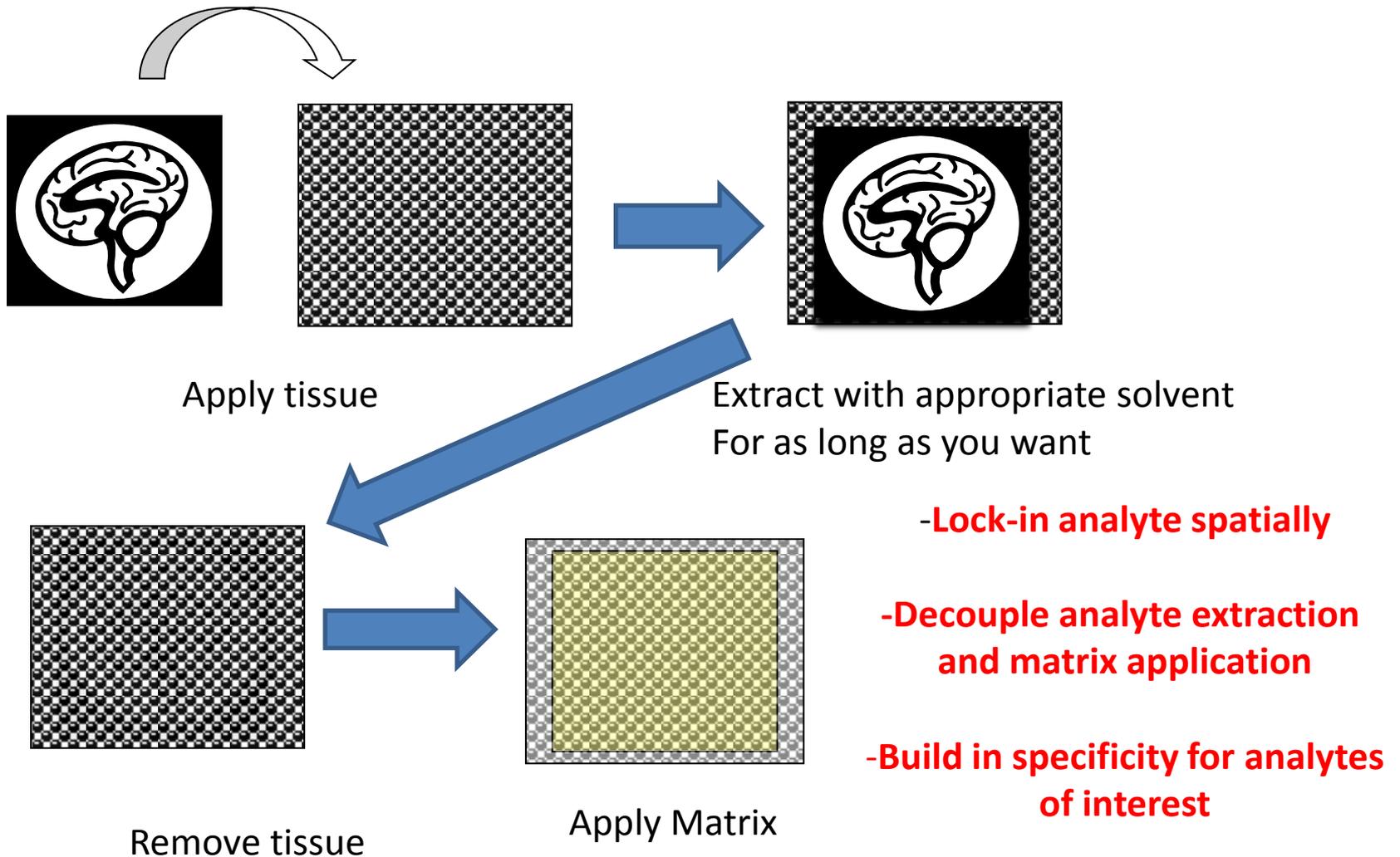
Successfully for the extraction and analysis of exogenous pharmaceutical
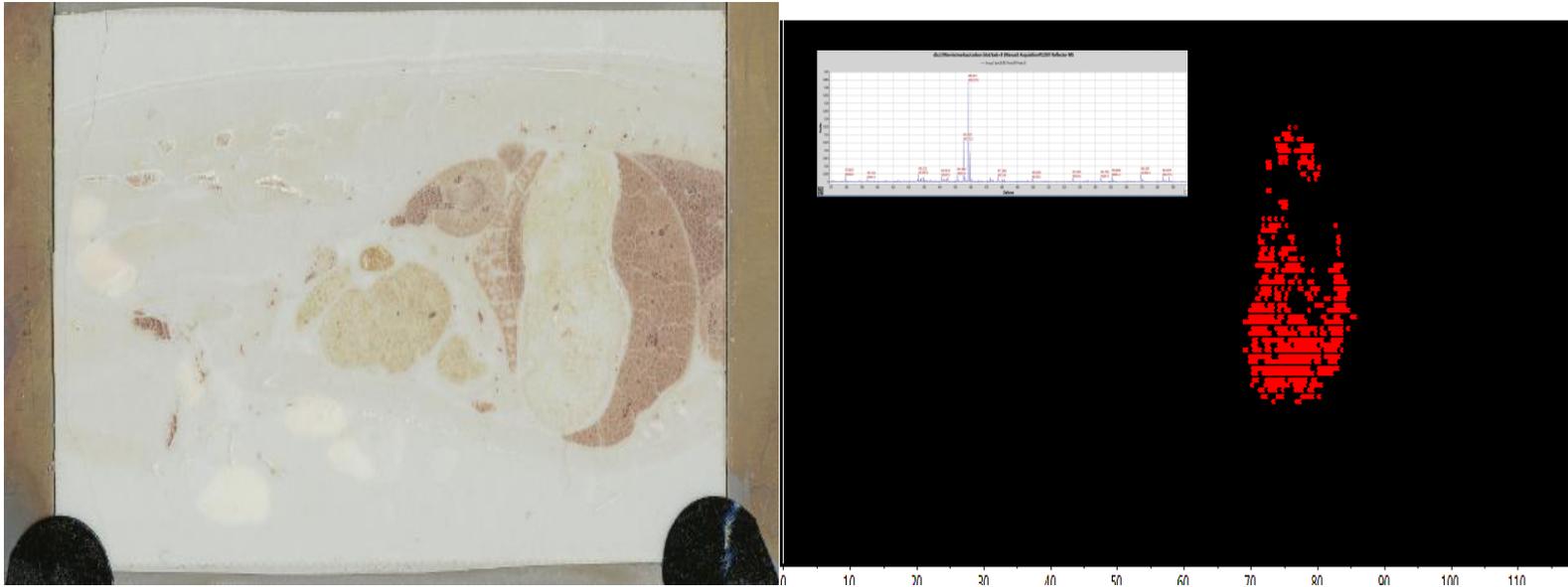
-Apply sample
-Extract with whatever you want for however long you would like
-Remove tissue
-Then apply matrix and analyze

Generated signals of interest form months old samples
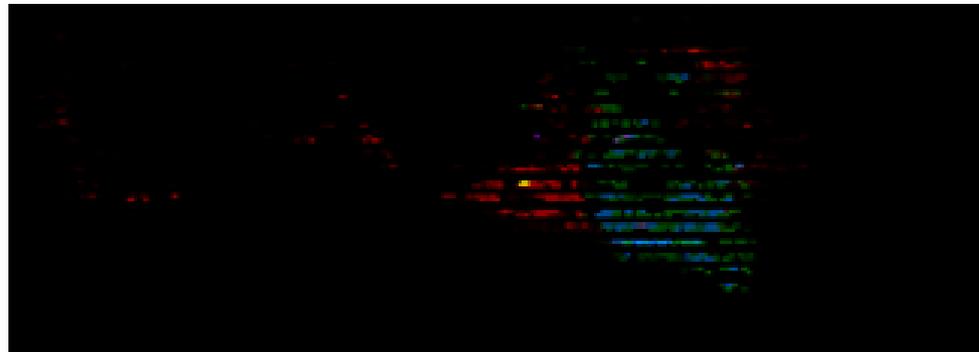
# Can manipulate transport in and out of pores based on solubility

water

Low organic

High organic + Matrix

High organic

Polymer plate

379.09

100

379.09

100
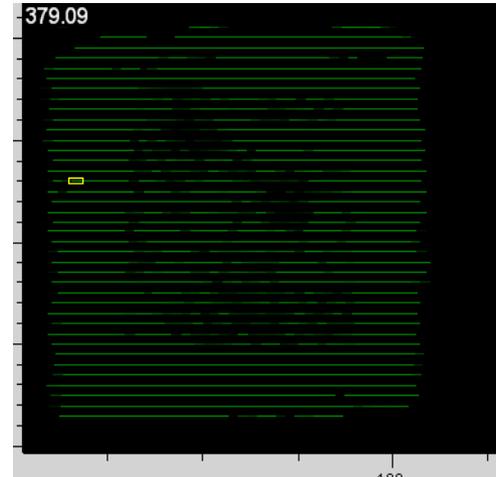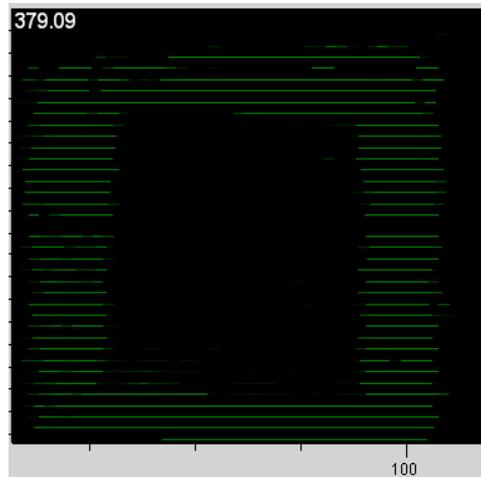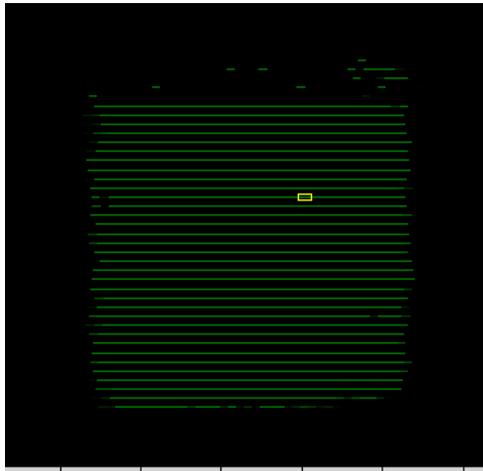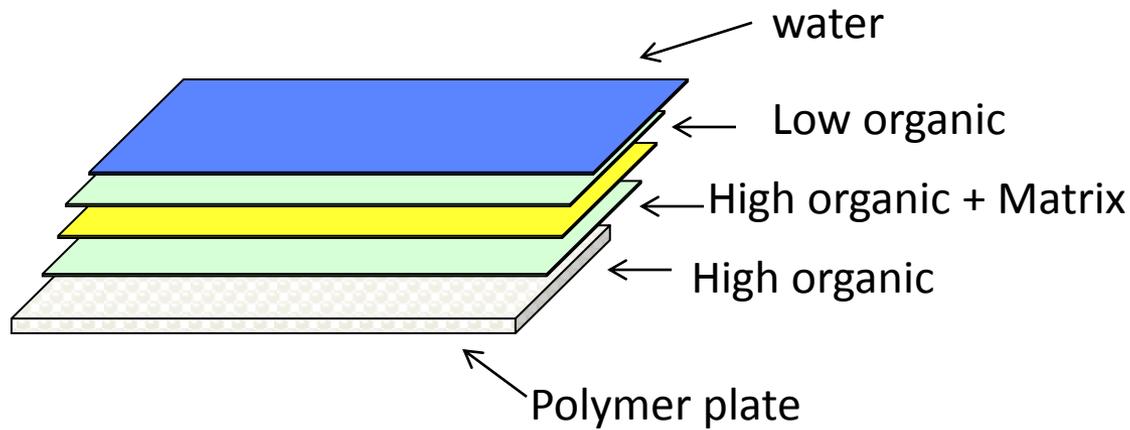
World's Largest Parallel Separation ?

1479

1749 1st analysis
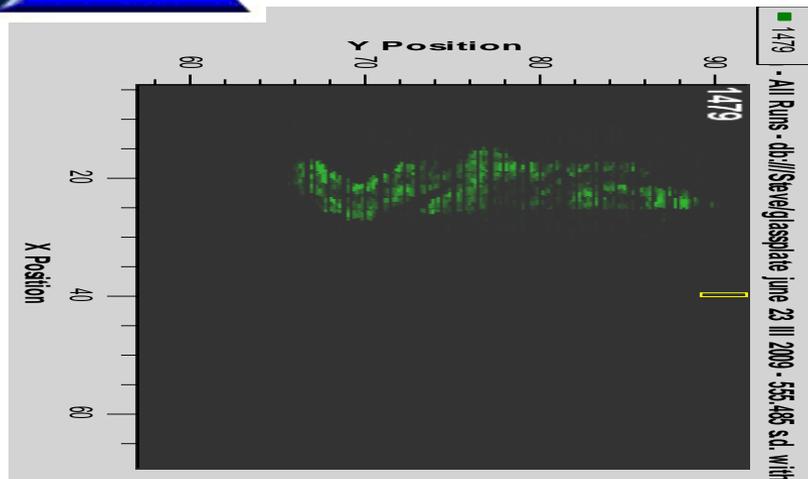
~200,000 parallel columns
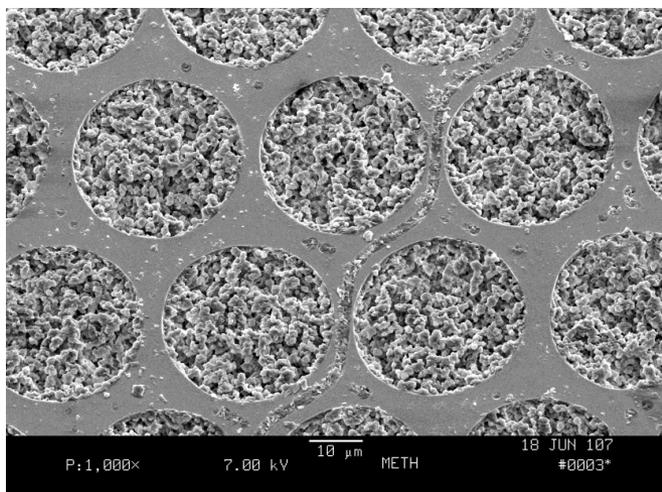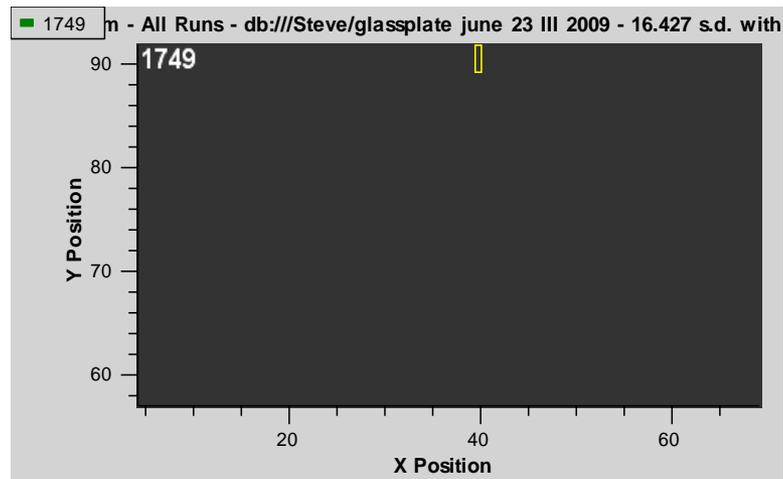
1749 2nd analysis
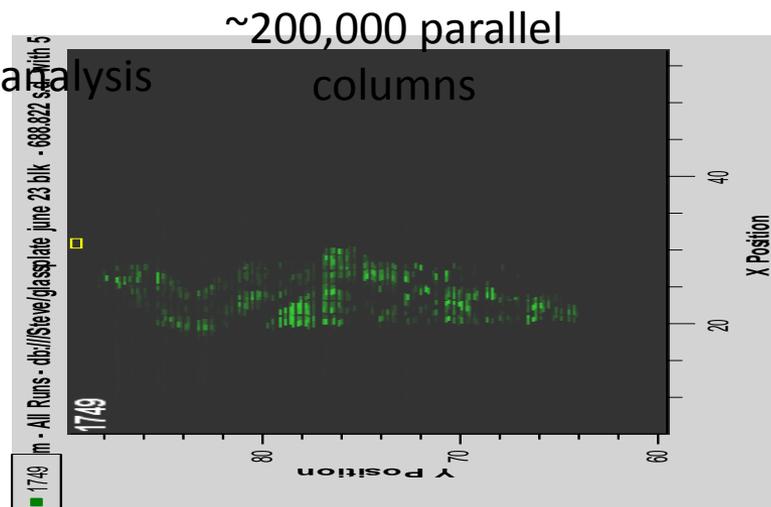
Can induce protein digestion with an immobilized enzyme membrane

Peptide Capture

Immobilized
Enzyme Mem.

Sample blots

Group:1 Spot:59 Shots:200   db:///Steve/Oct 2009/4pro 19 Oct#179 PSD-MS

927.5049
925.5187
1034.4808
1088.6400
1149.5125
1283.7030
1422.9483
1439.7964
1479.7950
1537.7969
1567.7402
1639.9632
1661.9034
1772.7072

Volts
0.02
0.01
0.00

Daltons
1000   1500
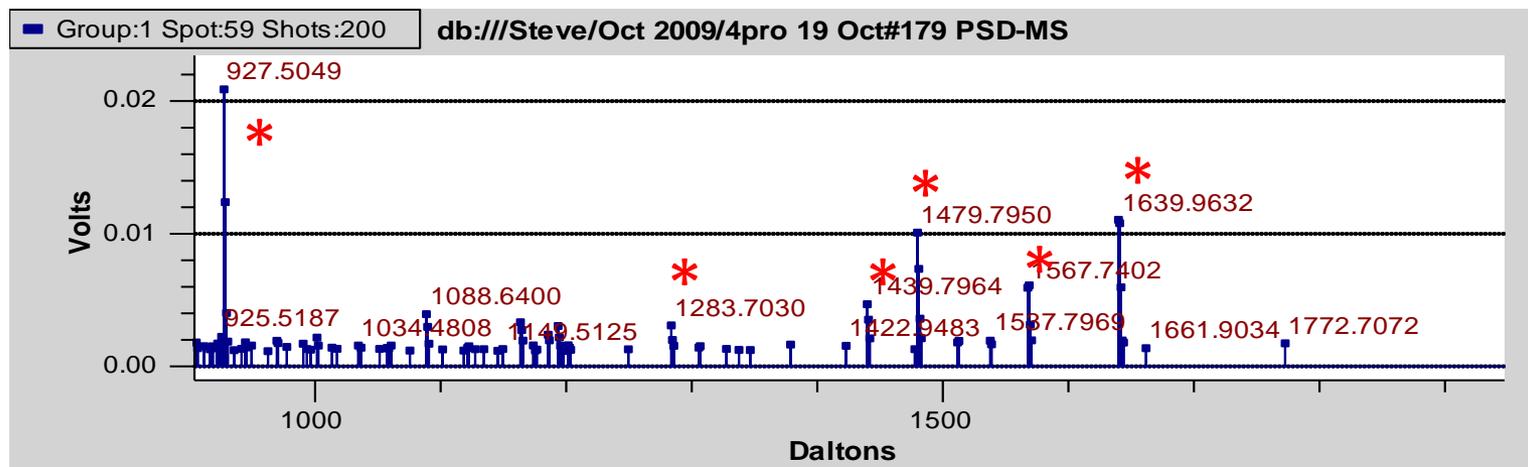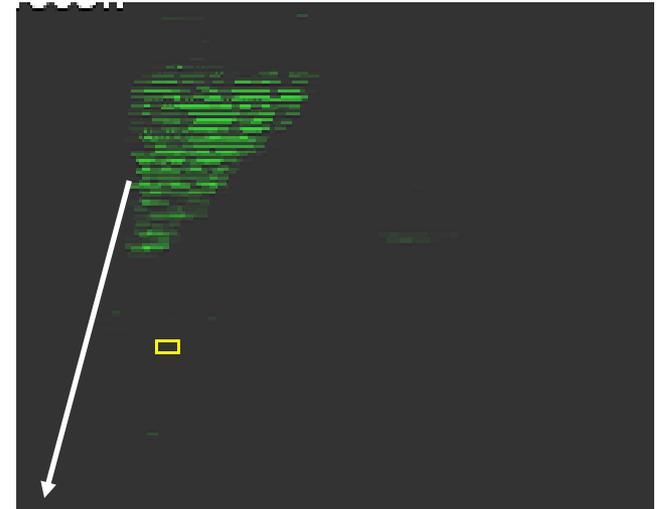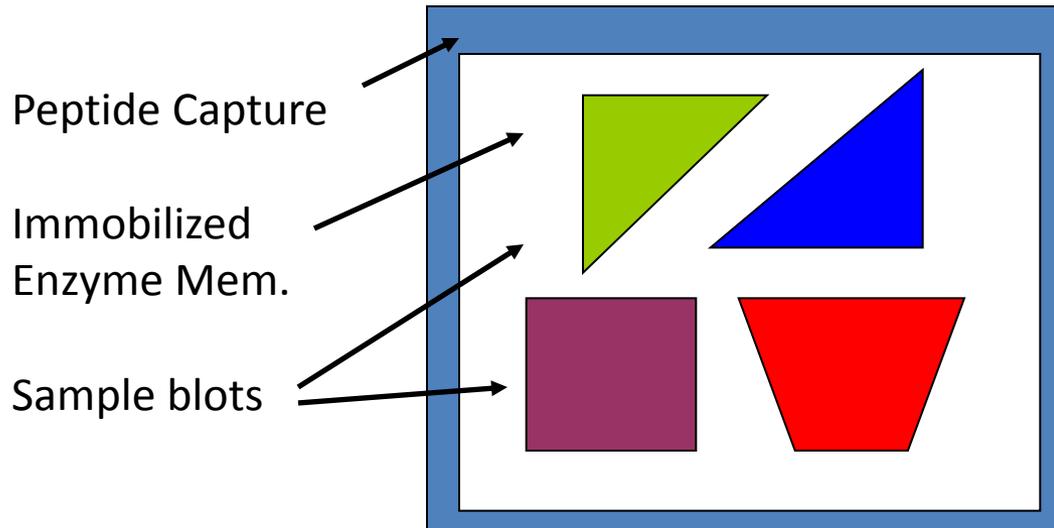
Even Sample Application / Digestion and Matrix Elution

Overlay of Matrix peak

379 Da

BSA MYO

CYTO-C

LYZO
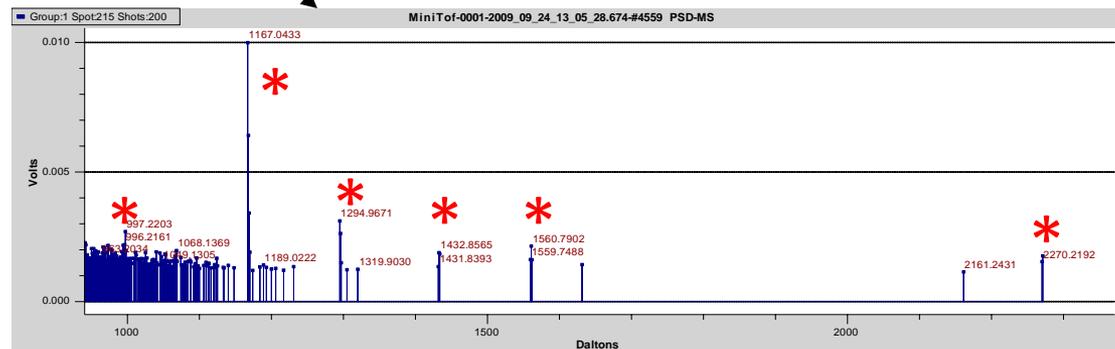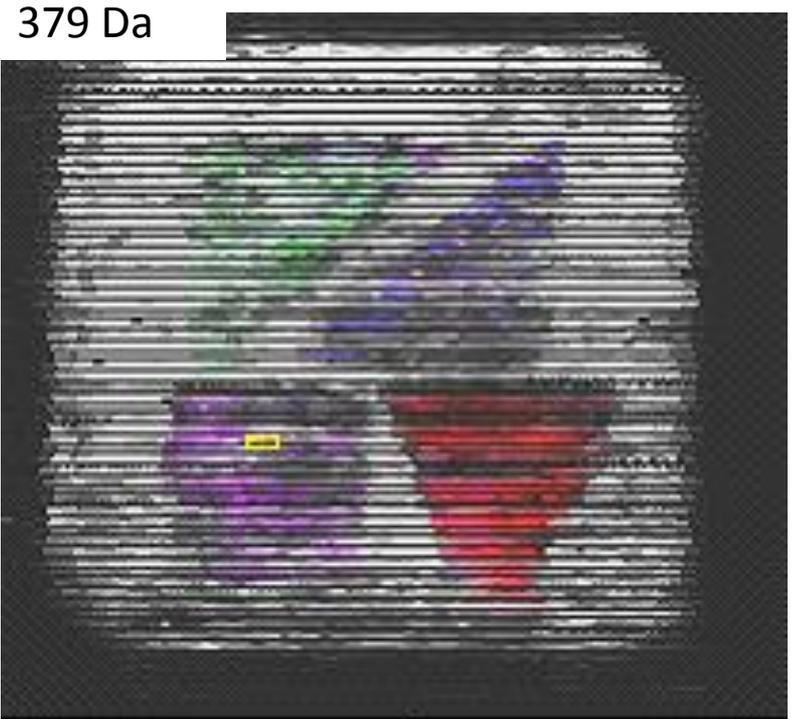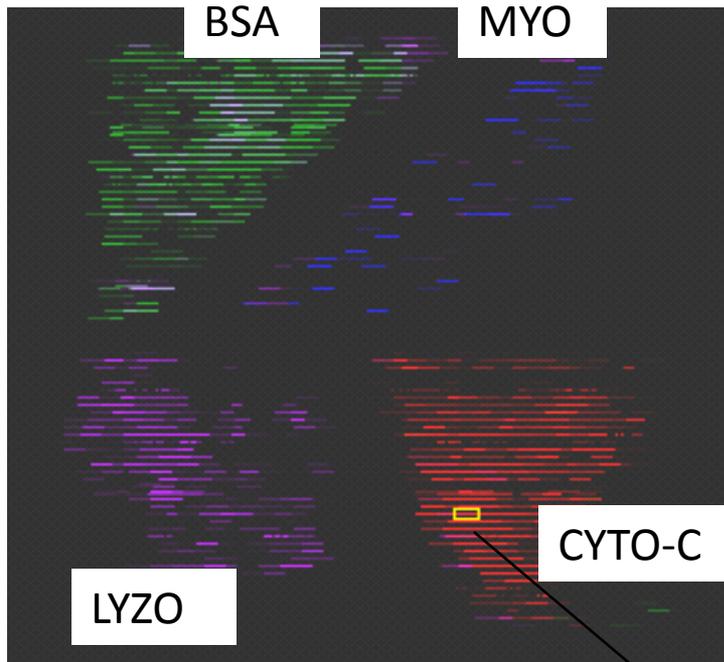
Vitreous Carbon plate for Tissue Imaging
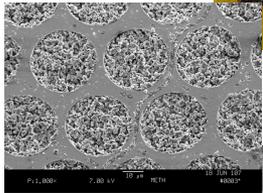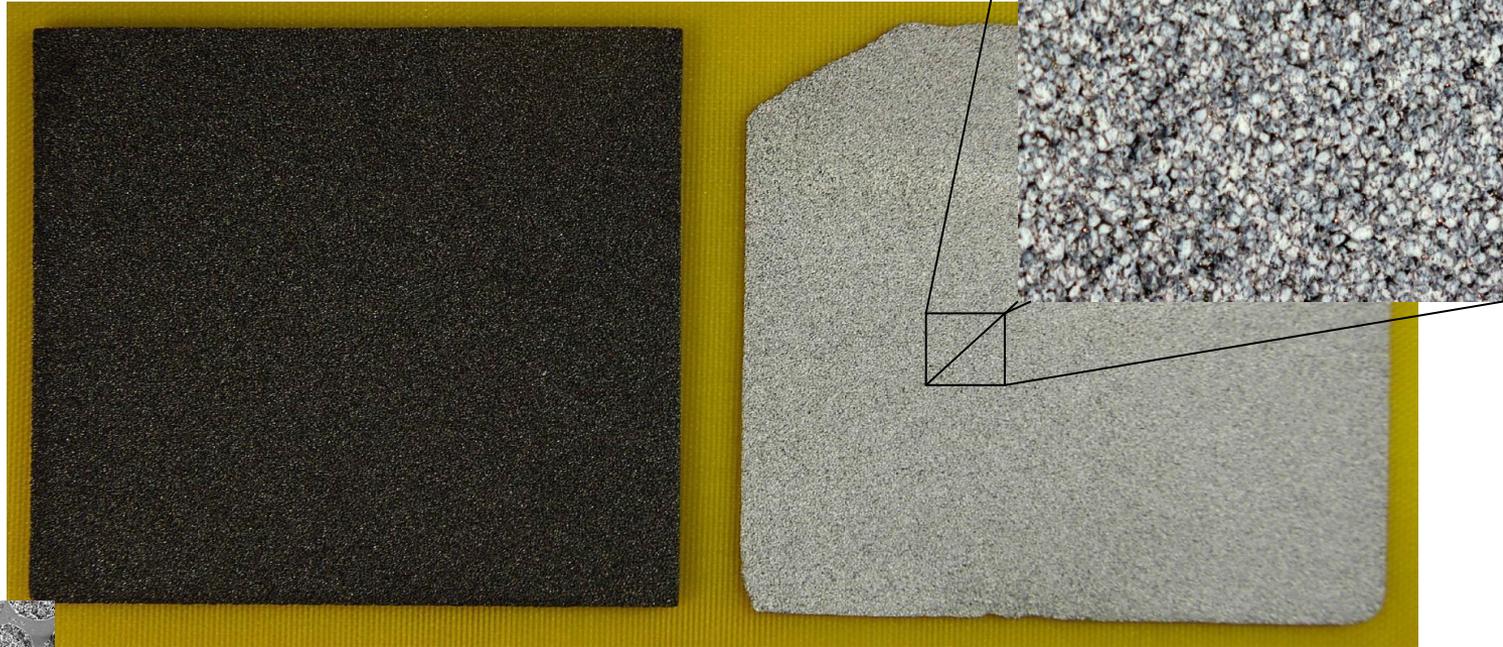
Porous Vitreous Carbon substrate

Filled with Polystyrene monolith
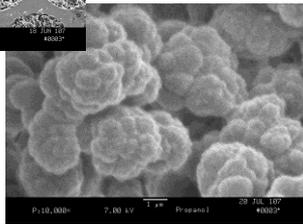
propanol
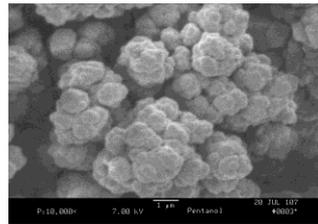
pentanol
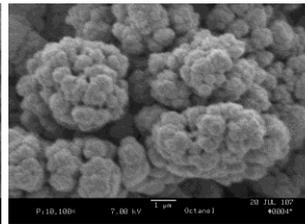
octanol
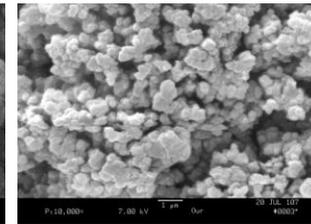
in-house polymer

Control over
Pores size
Surface chemistry

We have not abandoned the Molecular Scanner

Protein migration direction

Individual Proteins

PAGE Gel

Capture/digestion Membrane
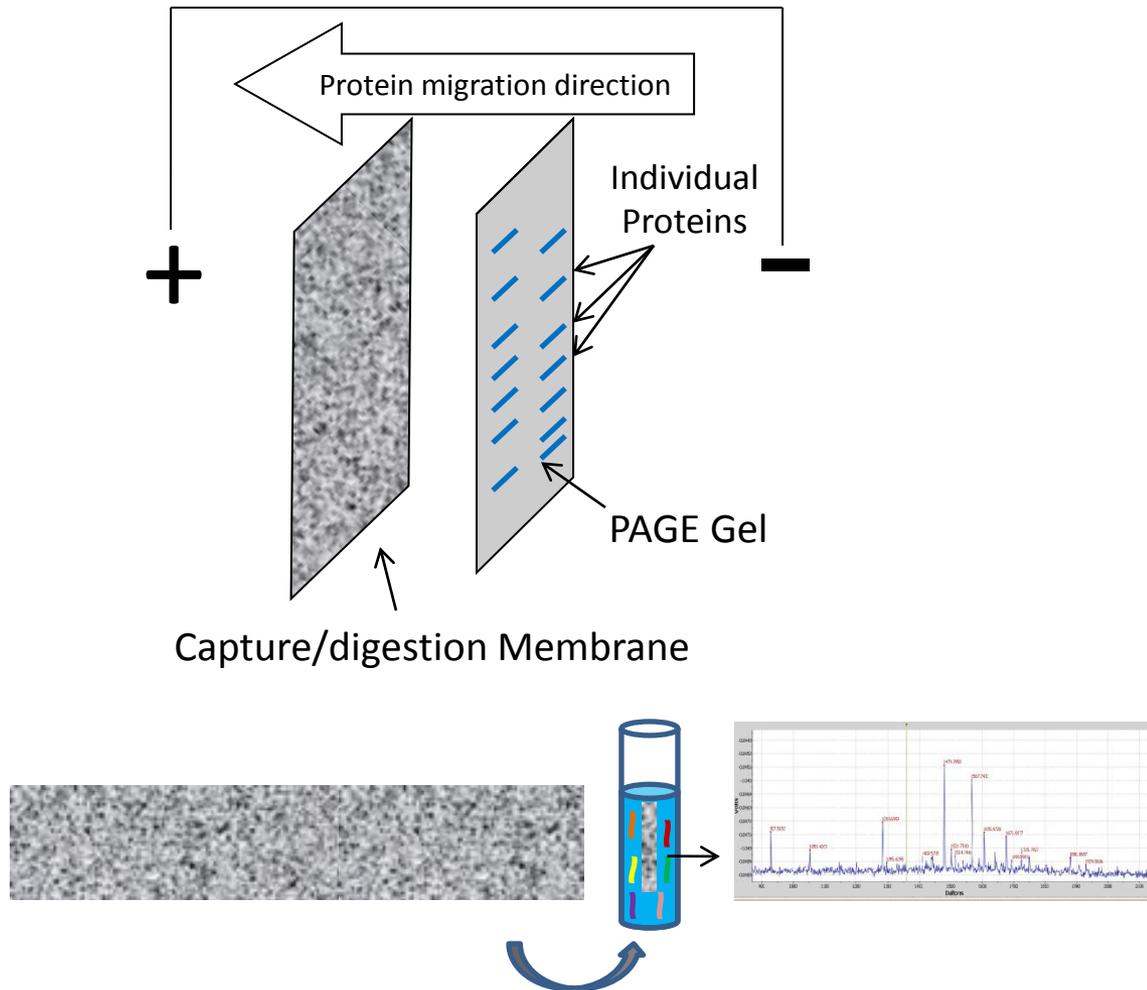
Dual purpose membrane
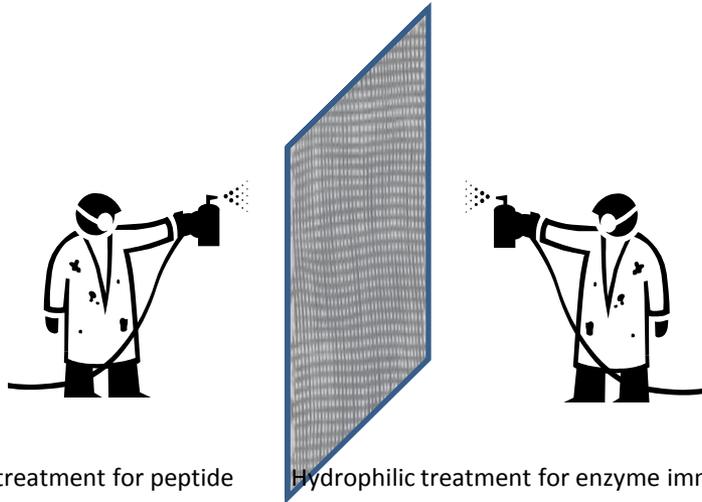
Hydrophobic treatment for peptide capture

Hydrophilic treatment for enzyme immobilization
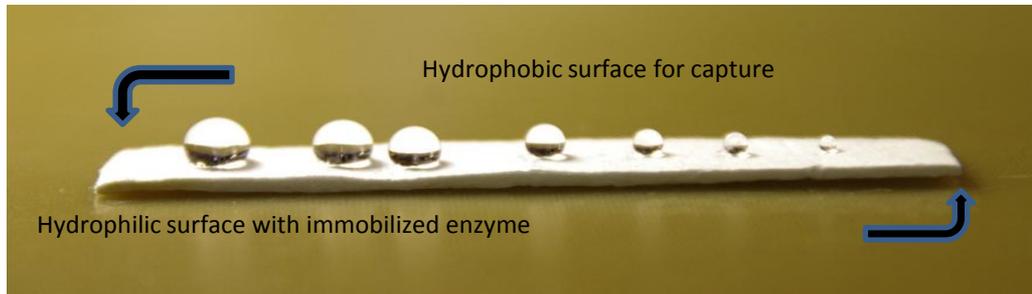
Hydrophobic surface for capture

Hydrophilic surface with immobilized enzyme
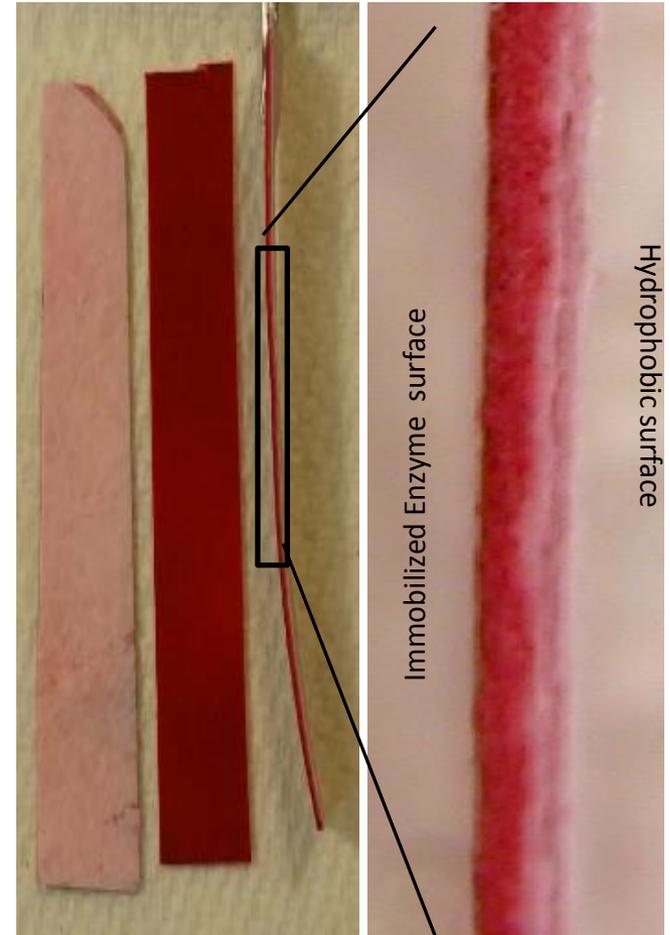
Immobilized Enzyme surface

Hydrophobic surface

Excellent digestion reproducibility

927.50 Da
R ~ 35,000

1567.75 Da
R ~ 30,000
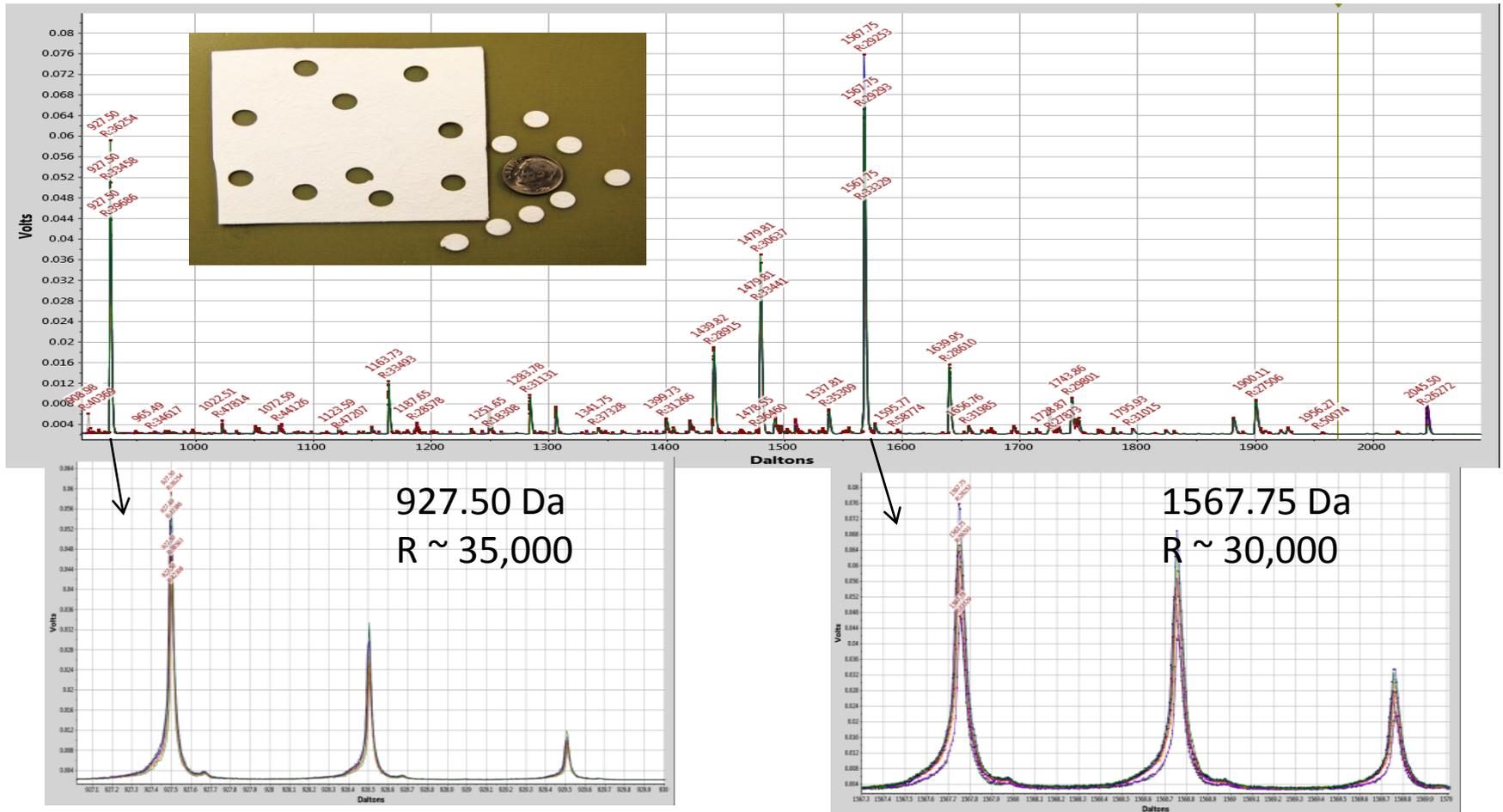
Fractions eluted and analyzed by PMF for protein ID

34 fractions
eluted and analyzed
by MALDI-TOF MS
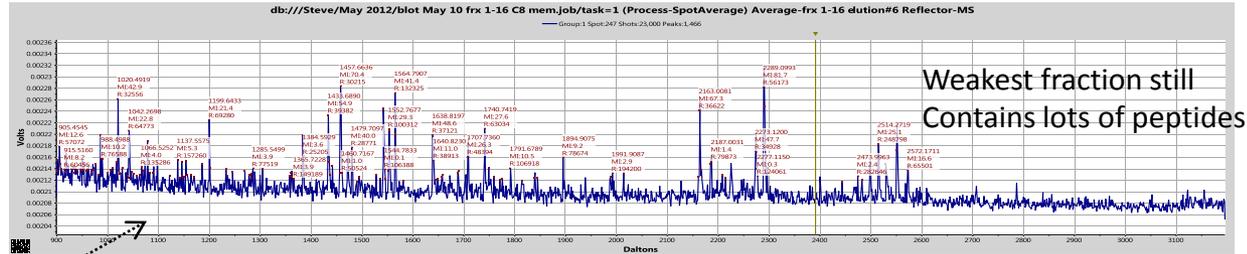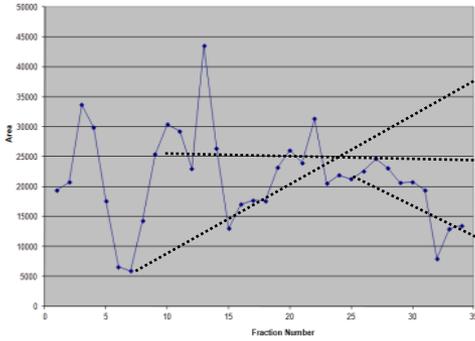
Weakest fraction still
Contains lots of peptides
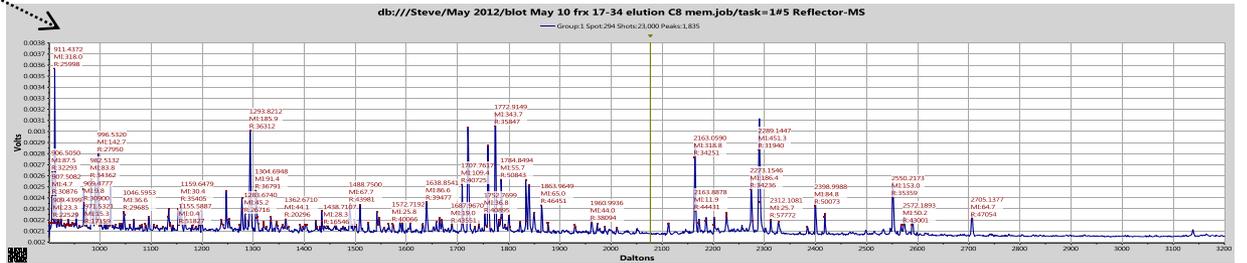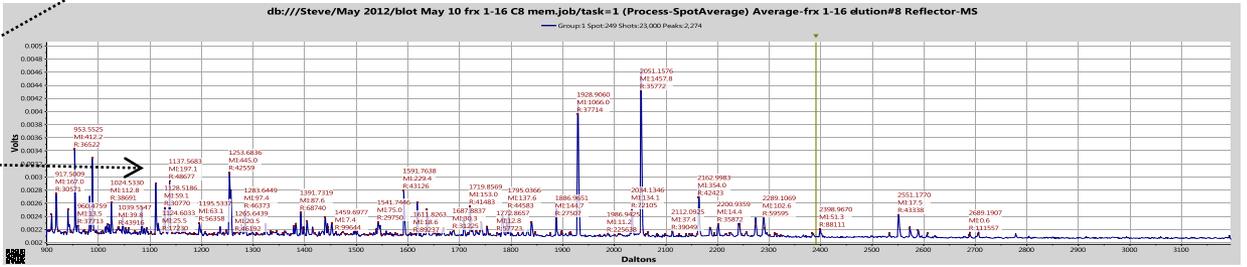
*Different fractions contain peptides originating
from the proteins in that band --good for PMF--*

# Resolution of the separation is maintained

Avg. 6 peptides per ID
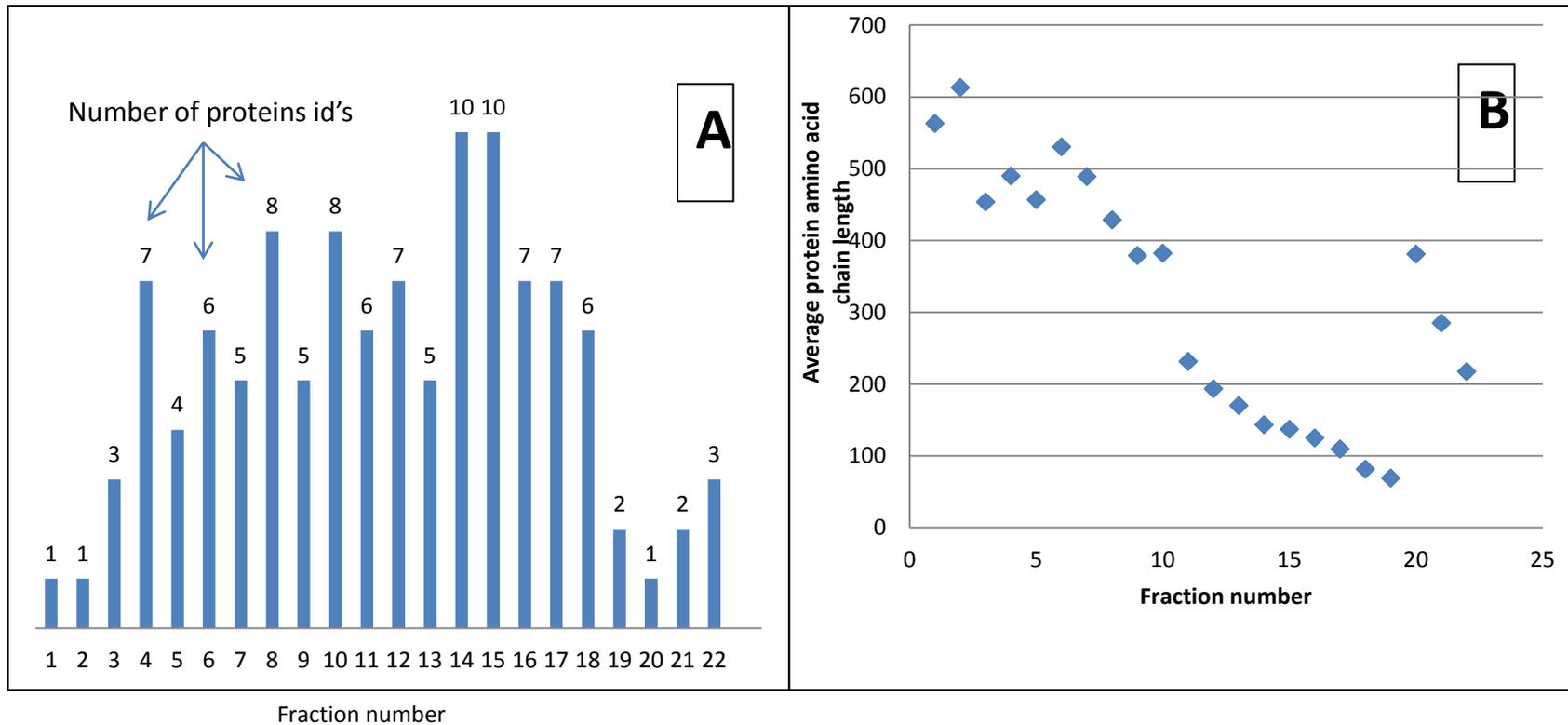
Figure 9: A)Bar-graph showing the number of proteins identified in each fraction. B) Plot of the average molecular weight for proteins in each fraction plotted as a function of fraction number.

Currently we use modified 384 well plate. We have machined the plate to Create strips composed of 2 adjacent Rows
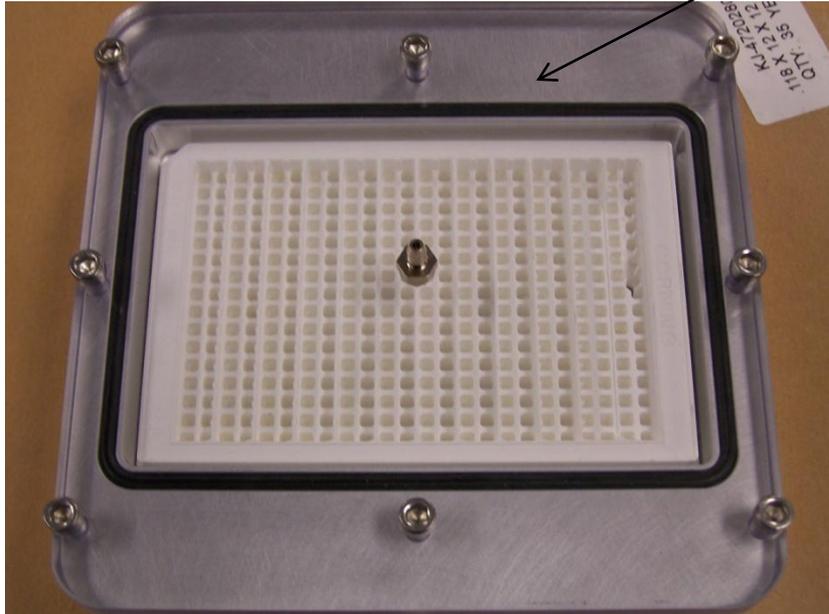
Would like to have a plate with such strips but would like the compartments to be in the form of tapered slots.

Top view

Side view

8 mm

2 mm

10mm

1mm
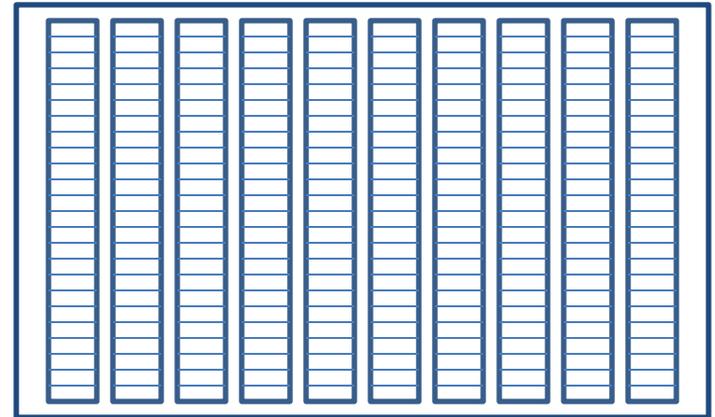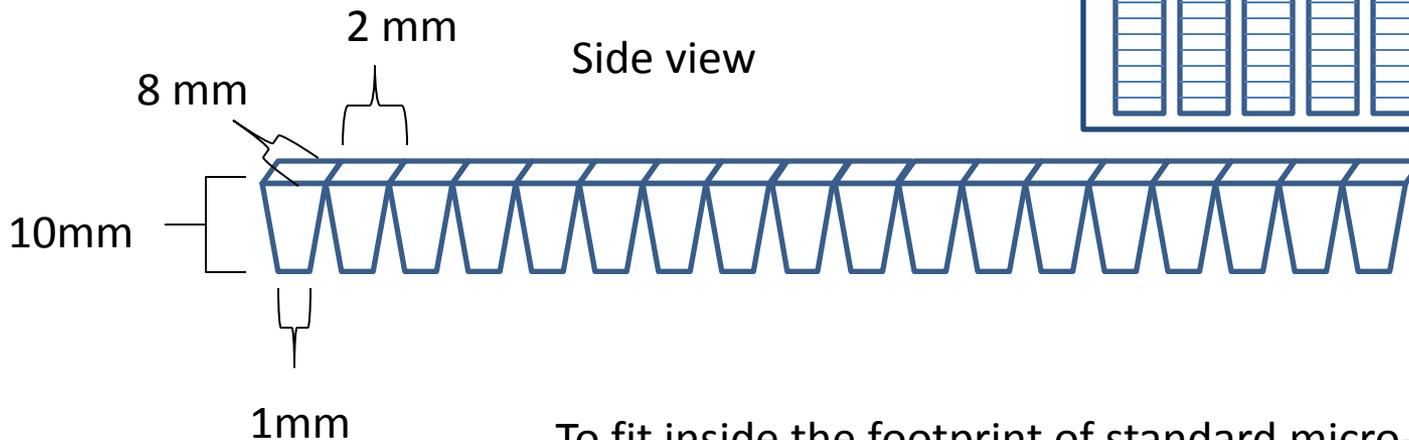
To fit inside the footprint of standard micro-titer plate
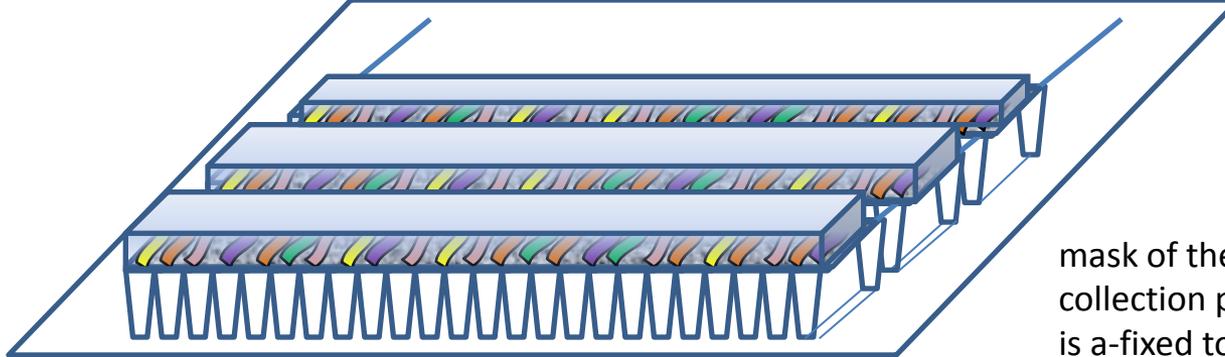
mask of the same size and dimension as collection plate but with open bottomed wells is a-fixed to MALDI target
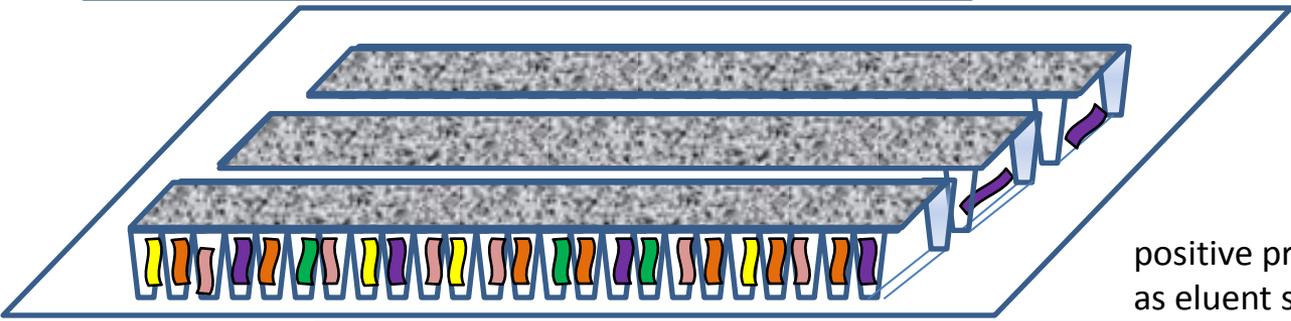
positive pressure elution using MALDI matrix as eluent solution
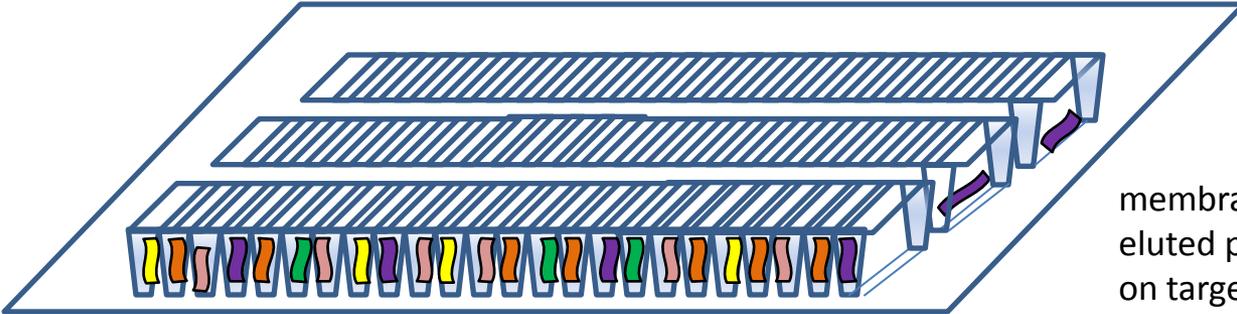
membrane removed matrix solution containing eluted peptides allowed to dry and concentrate on target surface

mask removed and sample analyzed in MALDI mass spectrometer

Enjoy the rest of the show!

Pay us a Visit!

SimulTOF Systems
261 Cedar Hill St, Suite 100
Marlborough, MA 01752