BMG 744
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Mass spectrometry imaging

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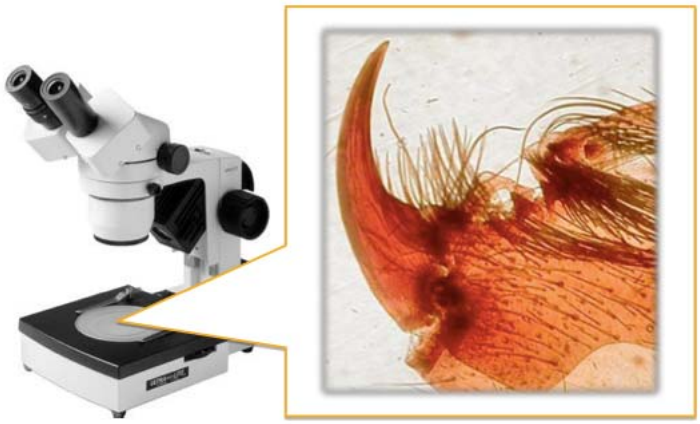
With sincere acknowledgments to David Stella, PhD and Kyle A. Floyd, MS, former students in the Barnes Laboratory (2005-2012), Miranda Collier and Kevin Schey, PhD, Vanderbilt University

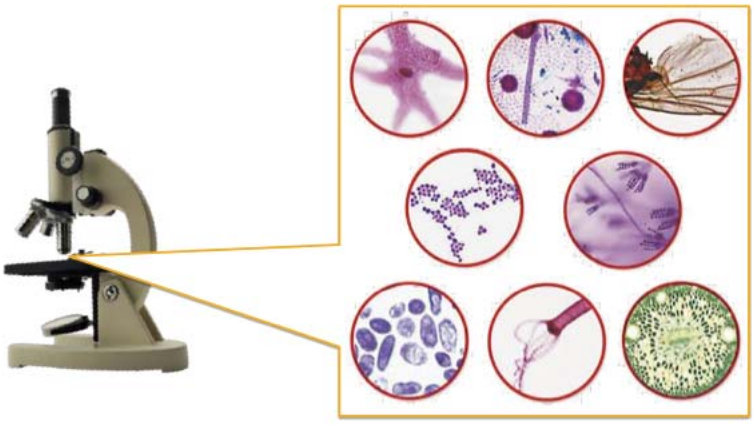
What does MS imaging offer?

- **Can provide information that "grind and find" cannot**
  - **What is in the imaged section?**
  - **Where is it?**
  - **How much of it is there?**
  - **Is it modified?**
- **As we'll see, much as the laser is targeted at the frozen section, it is an *untargeted* assay**

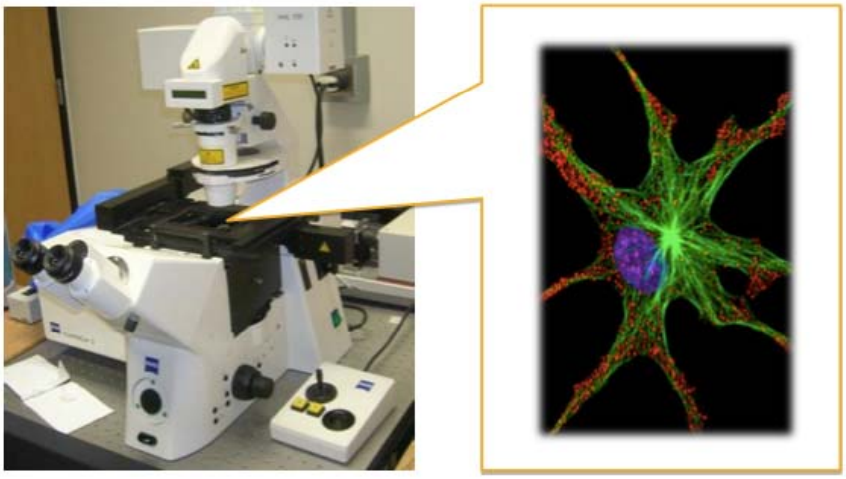
Imaging is widely used in research



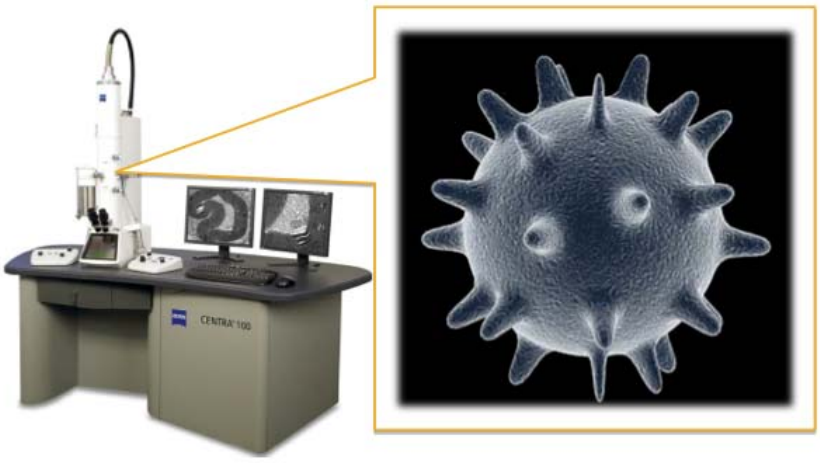
Light microscopy



Confocal Microscopy

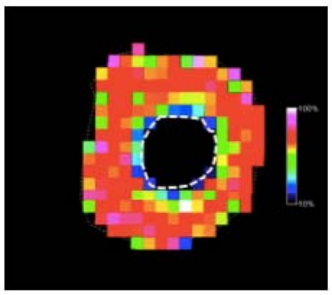


Electron microscopy



Mass spectrometry imaging

MALDI-TOF mass spectrometer



A chemical image

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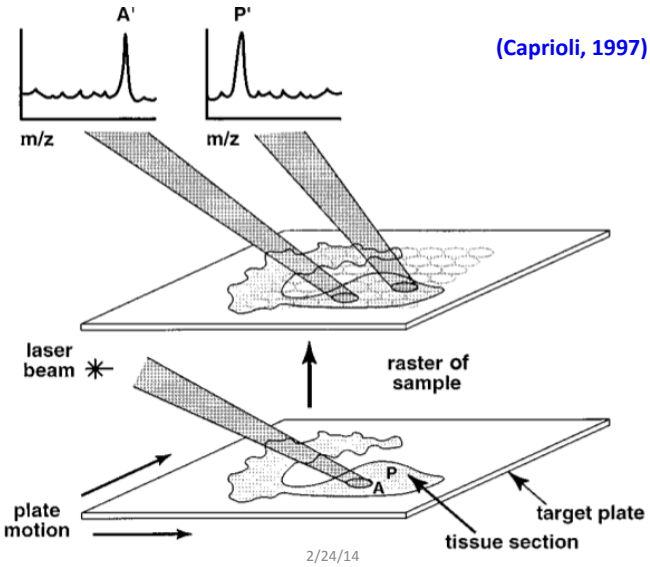
Principle of MALDI imaging



(Caprioli, 1997)

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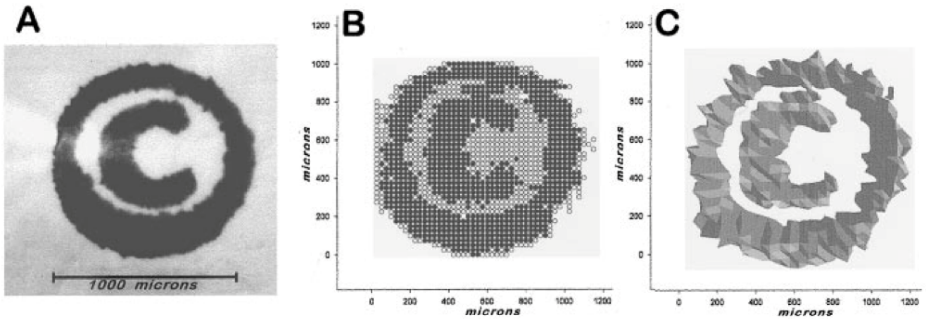
A simple example of MS imaging



Coomassie Blue image

Image based on the detection of the ion for Coomassie Blue

Interpolated image reconstruction

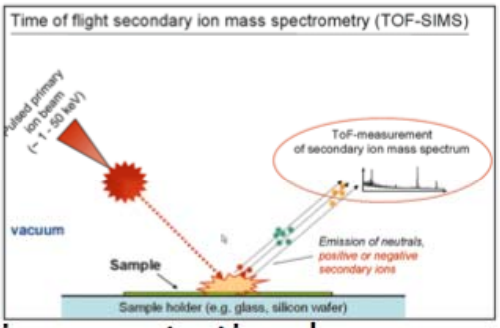
(Caprioli, 1997)

How to get analytes "off" a tissue section

- **Several types of ionization sources are used:**
  - **SIMS** – Secondary Ion MS
  - **DESI** – Desorption electrospray ionization
  - **MALDI**
  - **MALDESI**
  - **LAESI** – Laser Ablation Electrospray Ionization

SIMS

- Used in early studies (elemental analyses)
- Very high resolution (>50 nm)
- Principle of ionization: collated ion beam



- Destructive, penetrating, low mass range

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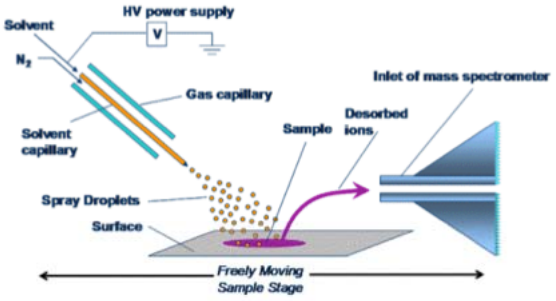
DESI

- Moderate resolution (20 - 300 µm)
- Principle of ionization: Charged solvent spray



- Surface molecules, multiply charged, low - mid mass range

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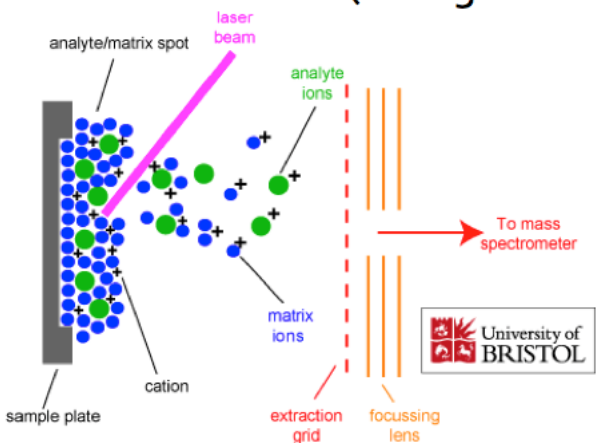
MALDI

- Currently the more commonly used ionization source in MSI (biological research)



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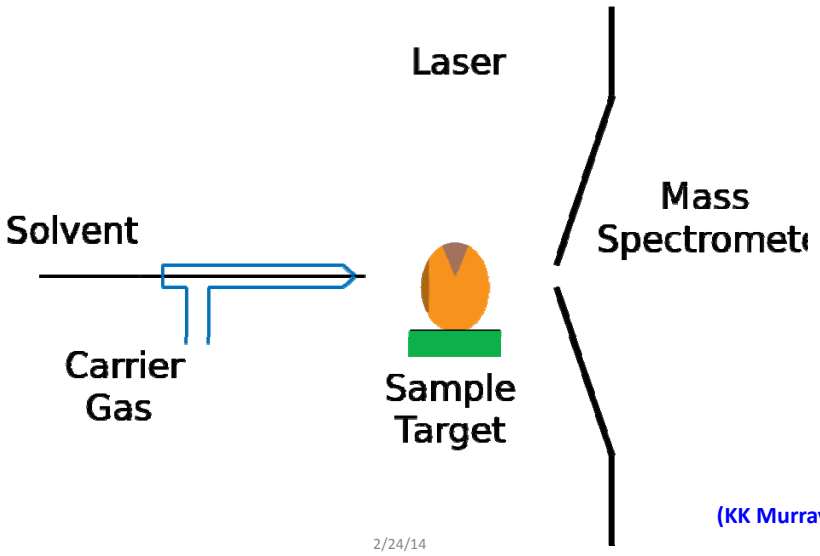
MALDESI



(KK Murray)

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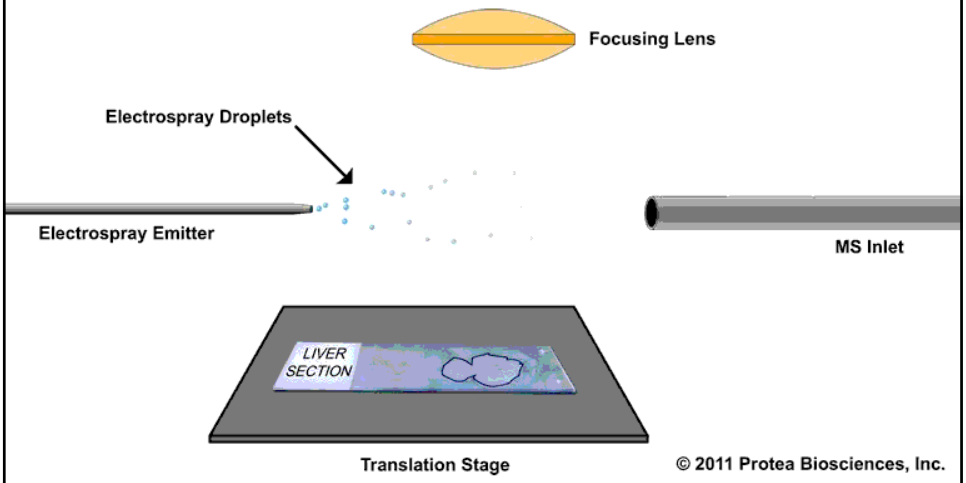
LAESI



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"Profiling" versus "imaging"

- **Profiling:**
  - Limited, directed information
  - Rapid analysis high throughput
  - Clinical applications and biomarker discovery
- **Imaging:**
  - Extensive, high resolution
  - Time consuming, laborious
  - Useful for investigative research

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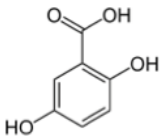
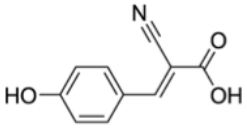
MALDI matrices

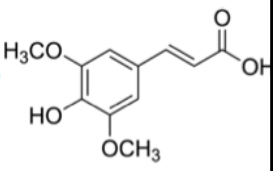
- DHB- 2,5-dihydroxybenzoic acid
  - Commonly used for small molecules



- CHCA- α-Cyano-4-hydroxycinnamic acid
  - Commonly used for peptides and small proteins



- SA- Sinapinic acid
  - Commonly used for peptides and whole proteins (<100 kDa)



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Matrix solvent

- Acetonitrile is the solvent of choice
  - Range of percentage of solvent
    - Depends on application (tissue types)
  - Sometimes the solution is augmented with different additives including detergents
- Acid is also present in the matrix solution
  - Commonly formic acid
  - Promotes ionization
  - Ranges of percentage is also possible (upwards of 10%)

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How to spot the matrix

- Multiple technologies available:
  - Hand-spotting
  - TLC spraying
  - Sublimation
  - Precision mechanical spotting:
    - Acoustic devices.
    - Chemical printers
    - Inkjet printers

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Matrix application I: Manual spotting

✓ Start here!
- Gold standard for signal
- Best extraction
- Biggest crystals
- Best S/N and most signals
- Use for parameter optimization

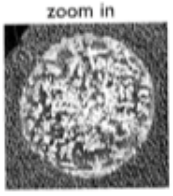
× But don't use for imaging
- Poor resolution
- Limited spot placement accuracy
- Many cell types extracted together

pipette
~250 nL
~1-2 mm diameter

pulled capillary
~100 nL
~800 µm – 1 mm diameter

- Rat brain tissue
- SA
- 20 mg/ml
- 50:50 ACN:H_2O
- 0.25 µl x 2

zoom in



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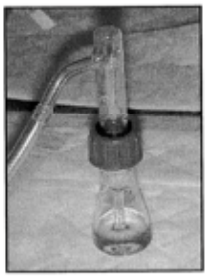
Matrix application III: Manual coating

- TLC reagent sprayer
  - ✓ Commonly used
  - ✓ Variable reservoir sizes (10-25 ml)
  - ✓ Inert
  - × Inconsistent droplets from sprayer to sprayer



WS
#2A/5A

- Artist airbrush
  - ✓ Fine droplets
  - × May corrode

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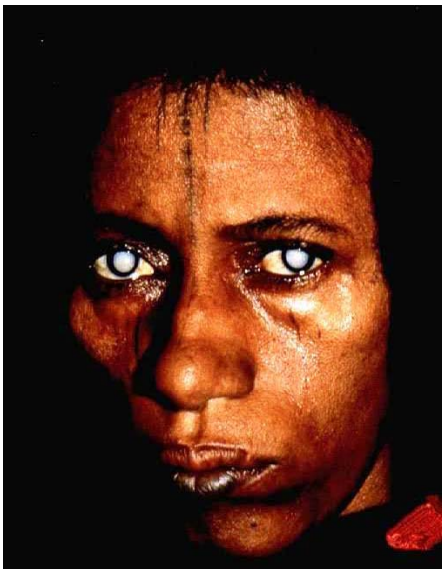
Cataract disease in an Amazonian



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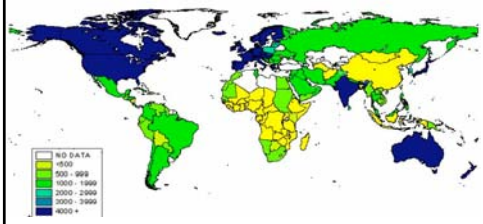
Cataract Disease and Public Health:



- WHO states that age-related cataract is responsible for 48% (~22 million people affected) of blindness in the world.
- Today people are living longer, putting more elderly at risk for cataract disease.
- Currently surgical removal is the only treatment, for which many countries have inadequate resources.
- In 2004 U.S. alone, cataract related medical expenses (i.e., surgical lens replacement) were estimated at $6.8 billion dollars, or ~42% of total vision related costs. (Rein et al. 2006).
- Most common surgery in USA (431,000)

Map from WHO: http://www.who.int/blindness/data_maps/CSR_WORLD_2004.jpg
Picture from WHO: http://www.who.int/blindness/causes/priority/en/index1.html

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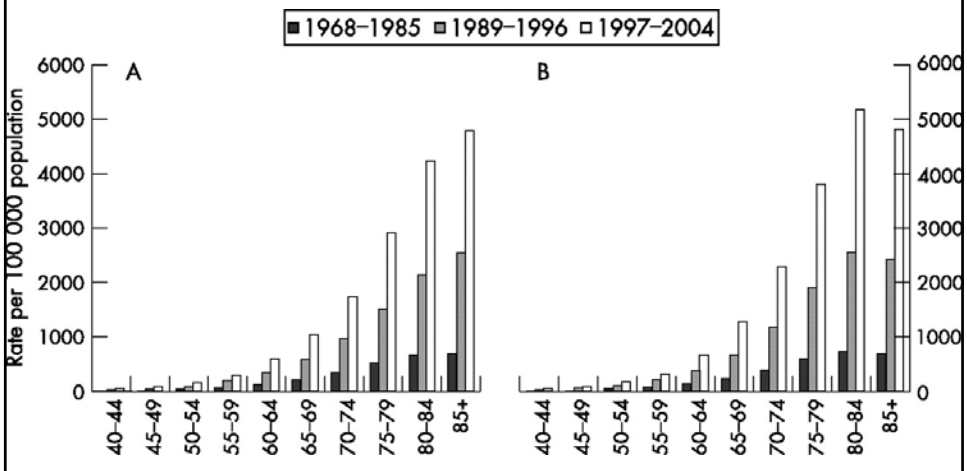
Lens cataract incidence with aging



Keenan et al., Br J Ophthalmol 2007;91:901-904

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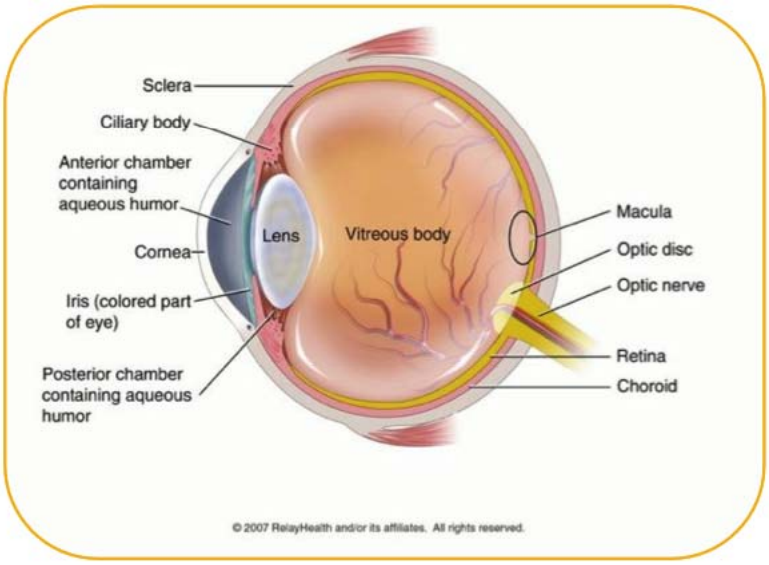
Anatomy of the eye



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Issues that relate to the lens

- Understand more about the protein localization in the ocular lens.
  - Interesting lens facts:
    - From "womb to tomb"
    - No protein turnover
    - Limited translated proteome
      - Expanded PTM proteome though!
    - Predominantly alpha crystallin proteins
      - small heat-shock proteins

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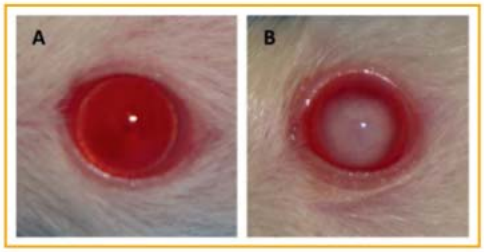
The rat model we use



- ICR/f rat (Ihara/Inherited Cataract Rat, strain-f)
  - Model of age-related disease.
  - Spontaneously develops cataracts by 10 weeks of age.
    - Possible result of early oxidative insult.
    - Compare 21-day vs. 100-day

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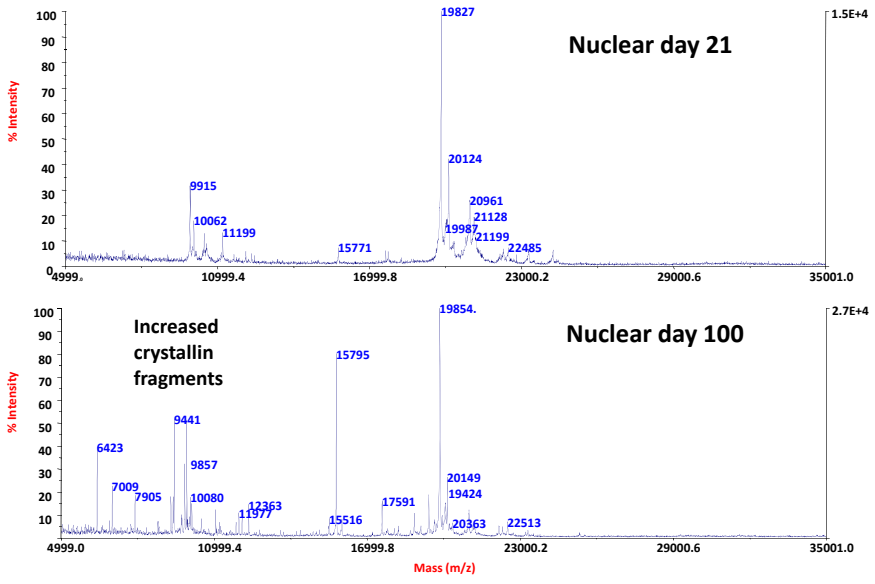
MALDI-TOF MS profiling of nuclear region of SD rat lens



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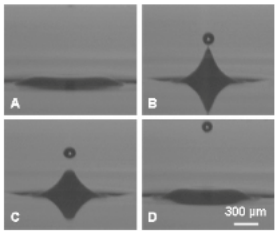
Acoustic spotting of a lens tissue slice



Aerni et al. 2006

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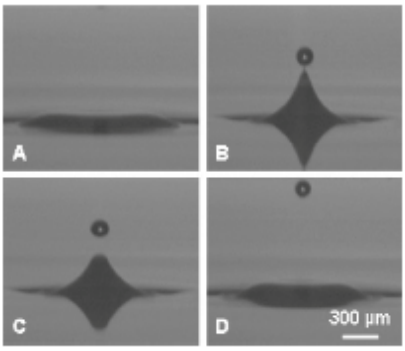
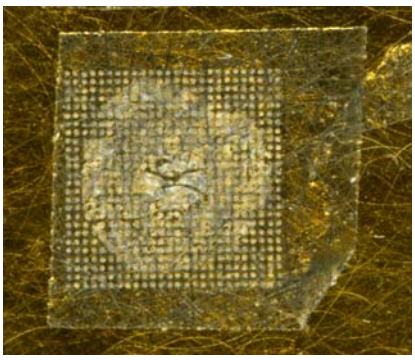
Spotting to the frozen rat lens



Aerni et al. 2006



- **Our Deposition Method**:
  - Overlay squares of 400 µm, for a final array resolution of 200 µm.
  - 40 cycles of 1 drop per spot per cycle; 170 pL total volume per spot.
  - **Matrix:** 50% Acetonitrile, 45% Water, 5% Formic Acid, 15mg/mL Sinapinic Acid, with/without 0.1% Triton-X.

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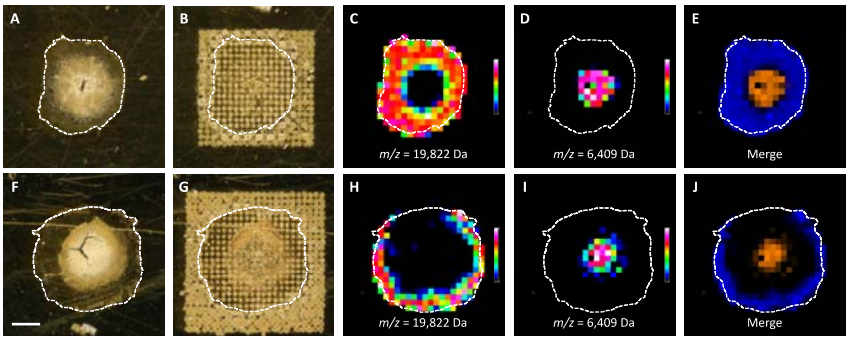
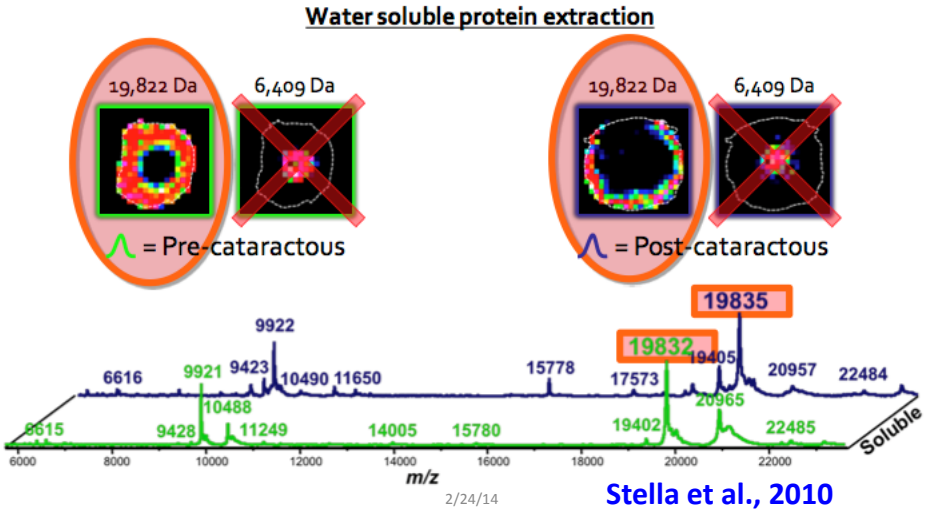
Lens Cataract: imaging reveals a geographic distribution of protein forms



What are the proteins that imaging is detecting?

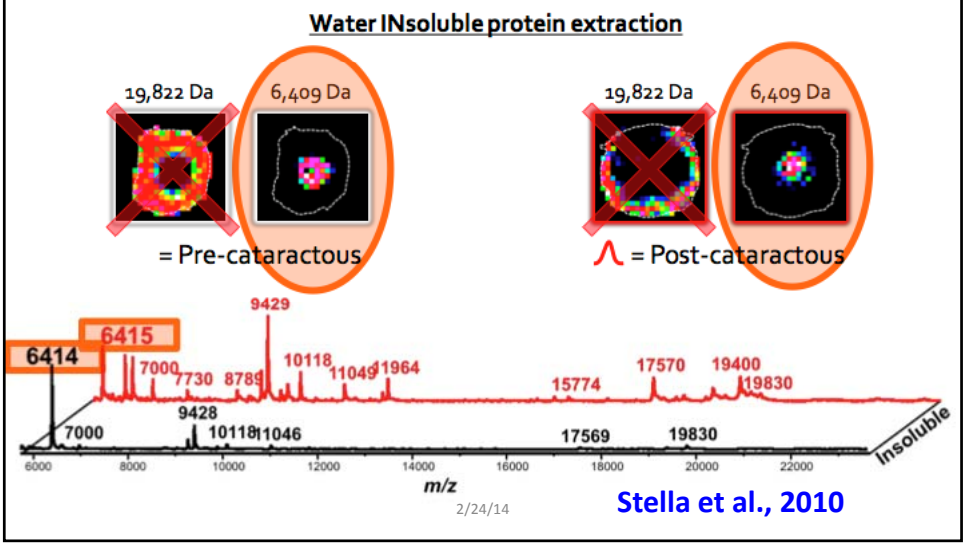
Stella et al., 2010

Aqueous extract of the lens



Stella et al., 2010

Water-insoluble/urea soluble



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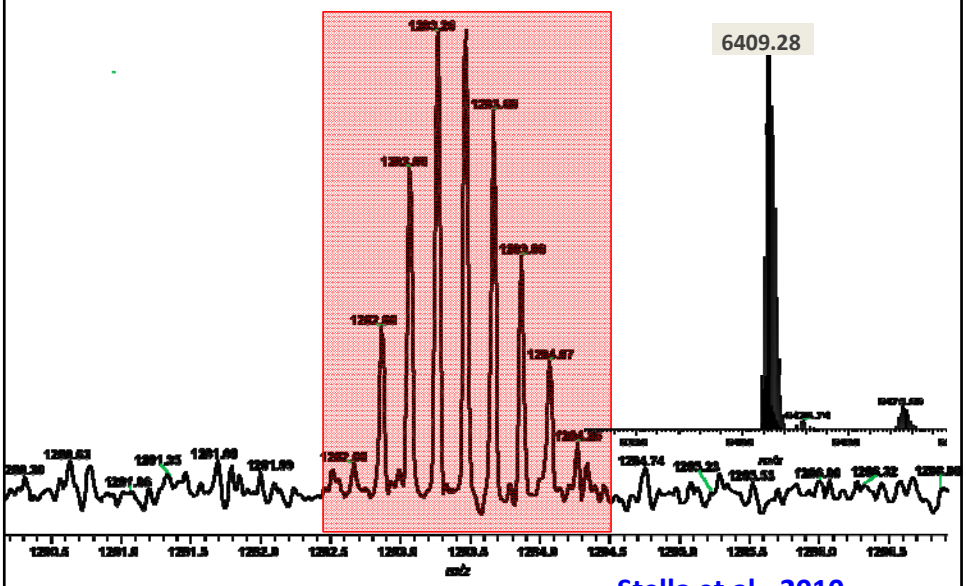
Stella et al., 2010

Top-down identification of *m/z*=1283.3 (5+ charge state)



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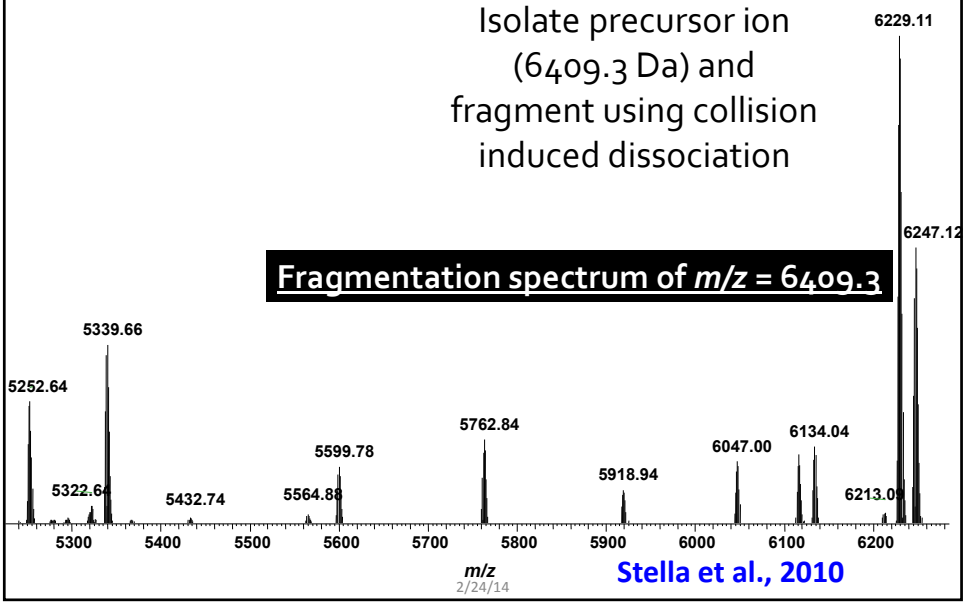
Stella et al., 2010

Top-down identification of *m/z*=6409.3

Isolate precursor ion (6409.3 Da) and fragment using collision induced dissociation



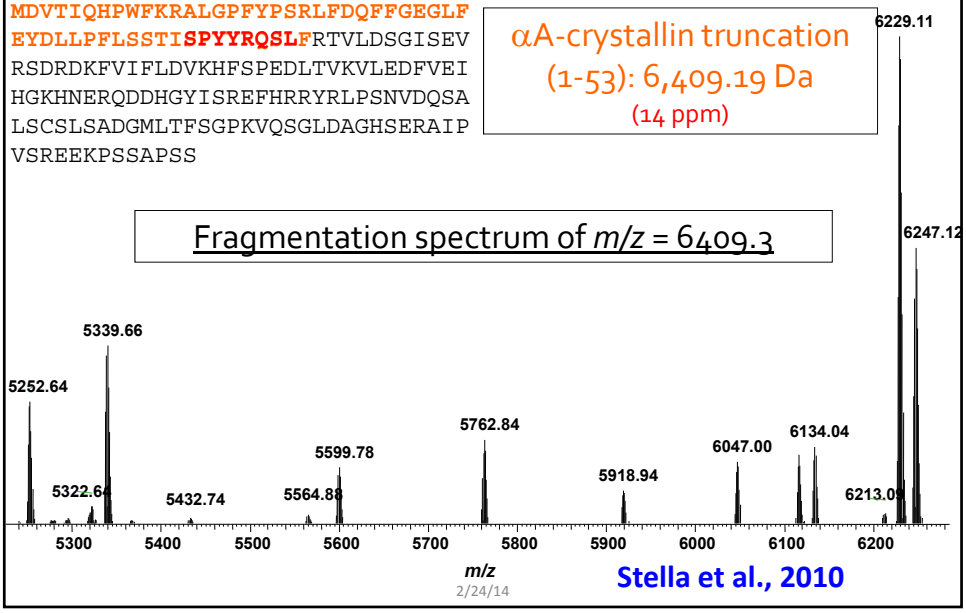
Top-down ID of (M+H⁺)= *6,409.28 Da*

αA-crystallin

MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLF
EYDLLPFLSSTI**SPYYRQSLF**RTVLDSGISEV
RSDRDKFVIFLDVKHFSPEDLTVKVLEDFVEI
HGKHNERQDDHGYISREFHRRYRLPSNVDQSA
LSCSLSADGMLTFSGPKVQSGLDAGHSERAIP
VSREEKPSSAPSS

αA-crystallin truncation (1-53): 6,409.19 Da (14 ppm)

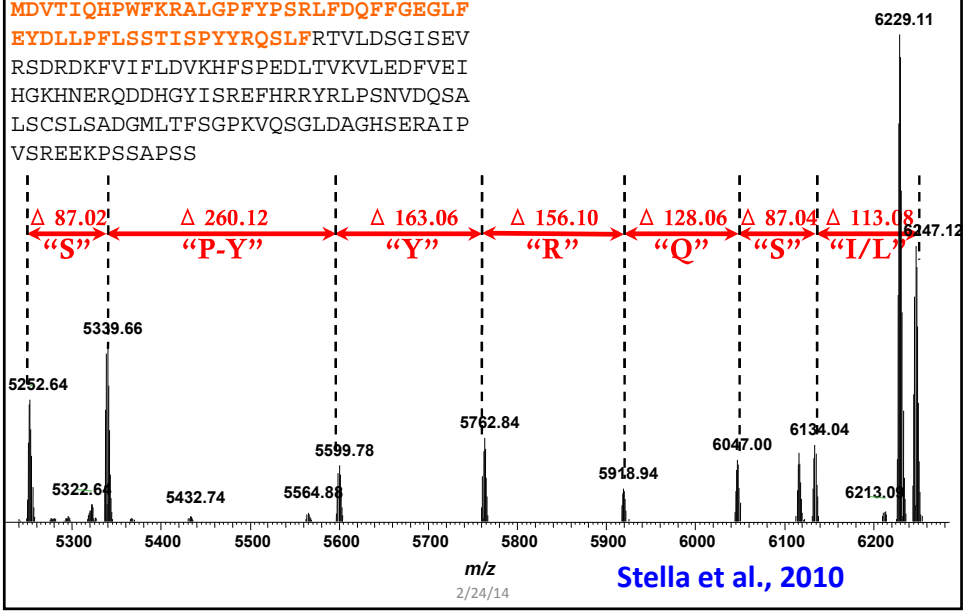
Fragmentation spectrum of *m/z* = 6409.3



Top-down ID of (M+H⁺)= *6,409.28 Da*

αA-crystallin

MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLF
EYDLLPFLSSTISPYYRQSLFRTVLDSGISEV
RSDRDKFVIFLDVKHFSPEDLTVKVLEDFVEI
HGKHNERQDDHGYISREFHRRYRLPSNVDQSA
LSCSLSADGMLTFSGPKVQSGLDAGHSERAIP
VSREEKPSSAPSS



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Stella et al., 2010

Top-Down Protein Assignment

Protein ID	Predicted Mass (Da)*	Residues
Crystallin, alpha A	5,053.50	1-42
Crystallin, alpha A	6,409.19	1-53
Crystallin, alpha A	6,565.29	1-54
Crystallin, alpha A	9,284.76	1-78
Crystallin, alpha A	9,421.80	1-79
Crystallin, alpha A	10,110.11	1-85
Crystallin, alpha A	11,041.61	1-93
Crystallin, alpha A	11,842.04	1-100
Crystallin, alpha A	11,956.06	1-101
Crystallin, alpha A	17,562.77	1-151
Crystallin, alpha A	18,043.96	1-156
Crystallin, alpha A	18,200.06	1-157
Crystallin, alpha A	18,823.44	1-163
Crystallin, alpha A	19,383.70	1-168
Crystallin, alpha A	19,822.89	1-173

Full length →

* = N-terminal acetylation included (+42.01 Da)

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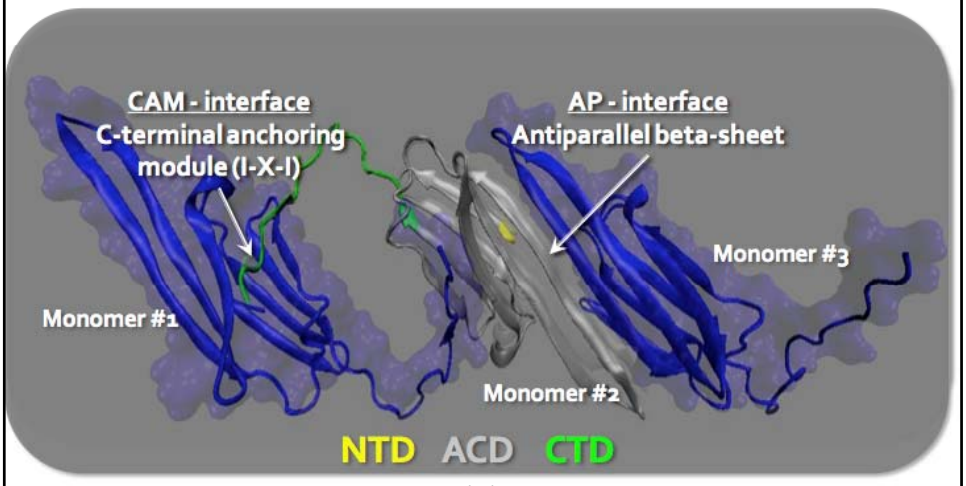
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

Structure of αA-crystallin



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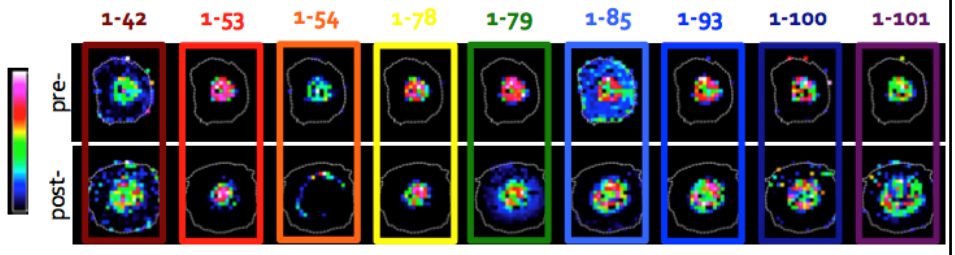
Distribution of truncated αA-crystallins

1. Nuclear localization



MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLFEYDLLPFLSSTI
SPYYRQSLFRTVLDSGISEVRSDRDKFVIFLDVKHFSPEDLTVK
VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

NTD ACD CTD

Stella et al., 2010

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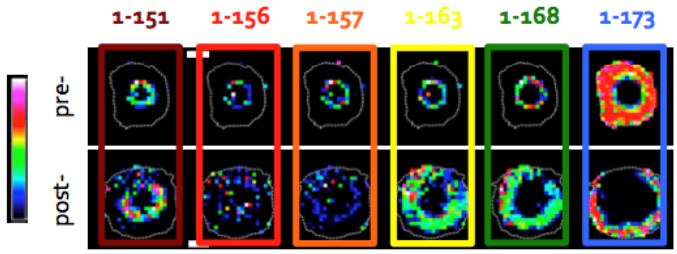
Distribution of truncated αA-crystallins

2. Cortical/Nuclear-ring localization



MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLFEYDLLPFLSSTI
SPYYRQSLFRTVLDSGISEVRSDRDKFVIFLDVKHFSPEDLTVK
VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

NTD ACD CTD

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Stella et al., 2010

Histochemical imaging of concentric shells in lens

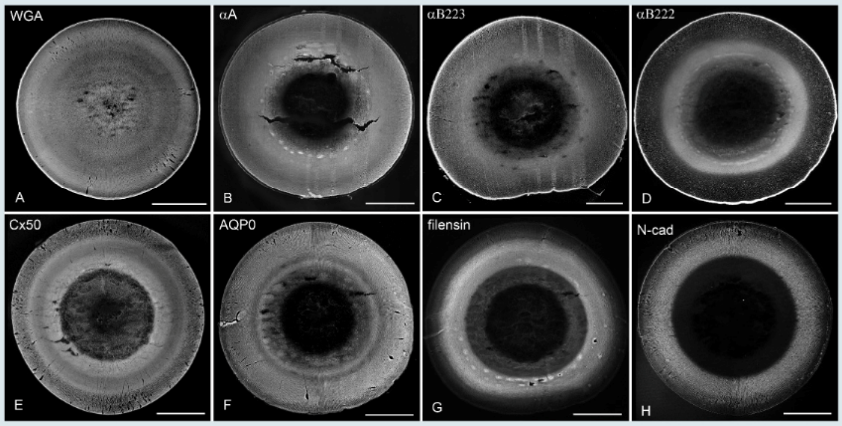

Figure 3(A-H): Composite of mouse lens sections labelled with wheat germ agglutinin (WGA) or antibodies to lens proteins. A) WGA (Invitrogen W32466); B) αA221 (Enzo ADI-SPA 221); C) αB223 (Enzo ADI-SPA 223); D) αB222 (Enzo ADI-SPA 222); E) connexin 50 (Cx50 from Dr. Thomas White); F) Aquaporin0 (AQP0; ADI-AQP02); G) filensin (Dr. Roy Quinlan 3241); H) N-cadherin (Ncad; BD 610920). Note the distinct patterns of shells for each label and the abrupt change from one shell to another. Magnification bars=500μ

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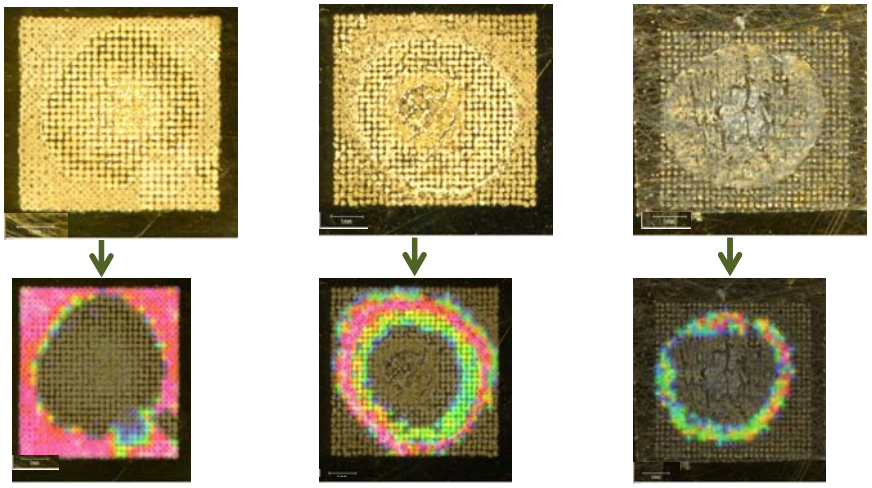
Clark et al., ARVO, 2011

Optimization of spotting methods

Plate-Mounted, Vertical EtOH wash	Plate-Mounted, Horizontal EtOH wash with Blotting	Carbon Fiber Tape Mounted and Lyophilized



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Slide adapted and modified from Kyle Floyd

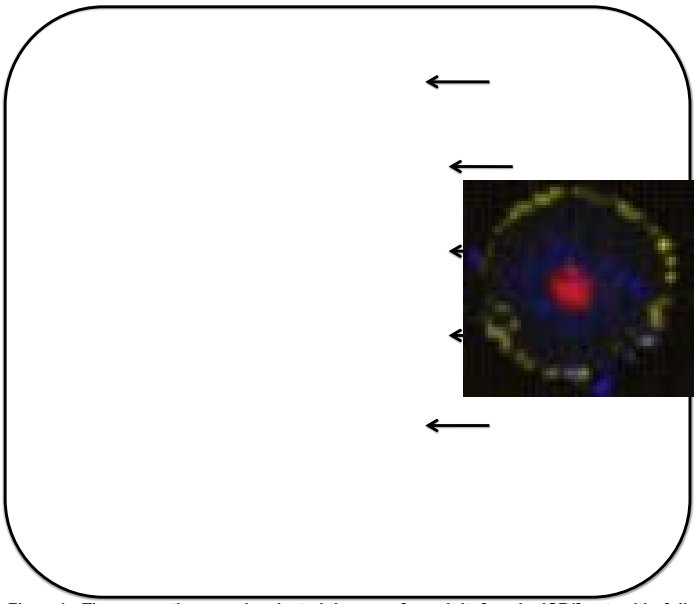

Figure1: Tissue sections and selected images for adult female ICR/f rat with full cataracts. These five sections were taken from the same lens, with four sections taken between each imaged section, and saved for future proteomic analysis.

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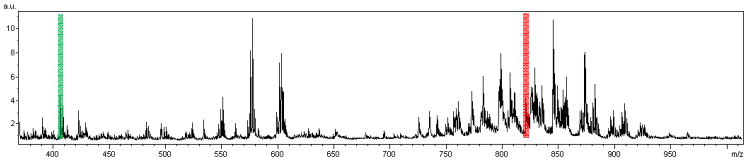
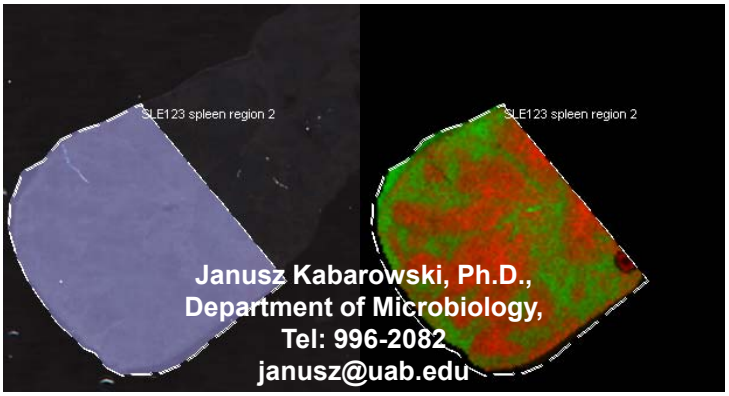
Small molecule imaging

MALDI-IMS for spatial characterization of biomolecules.





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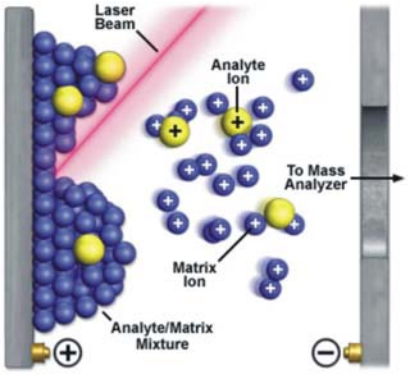
Matrix-Assisted Laser Desorption/Ionization (MALDI):

Matrix molecules absorb laser light, enter an excited state, and collide with sample molecules, facilitating charge transfer to create ions.



MALDI-TOF instrument

Mass Spectrometric Imaging for biomedical tissue analysis
Kamila Chughtai and Ron M.A. Heeren
Chem Rev. Vol.110(5): pp3237–3277, 2010.

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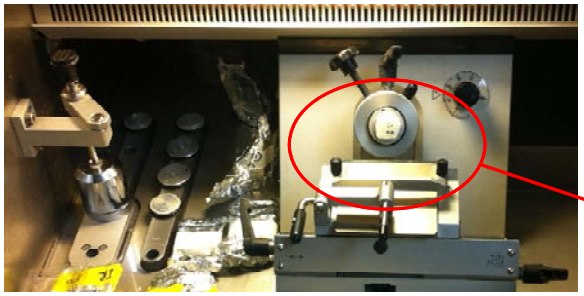
Cryosection preparation onto ITO slides and scanning digital image for "teaching" FlexControl software on MALDI-TOF instrument.



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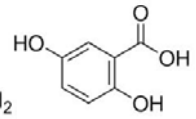
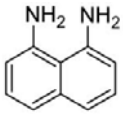
Vacuum sublimation is used to apply an even microscopically thin uniform layer of matrix compound onto tissue section without the need for solvents.

Sublimation: *the transition of a substance from solid to gas phase without an intermediate liquid phase*.

MALDI matrices for lipid imaging:

DHB: 2,5-dihydrobenzoic acid  +ve mode

1,5-diaminonapthalene  -ve mode

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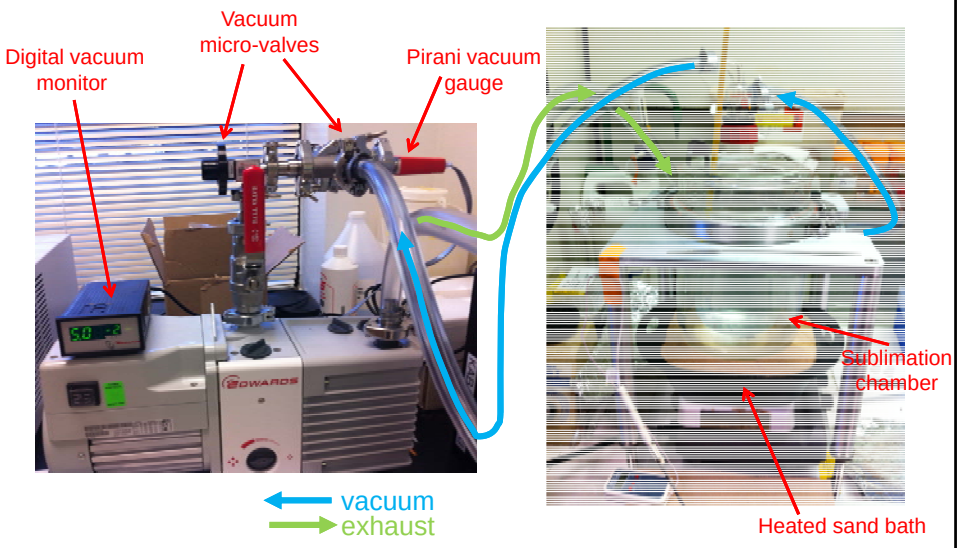
How do we do it ?

Vacuum sublimation apparatus.



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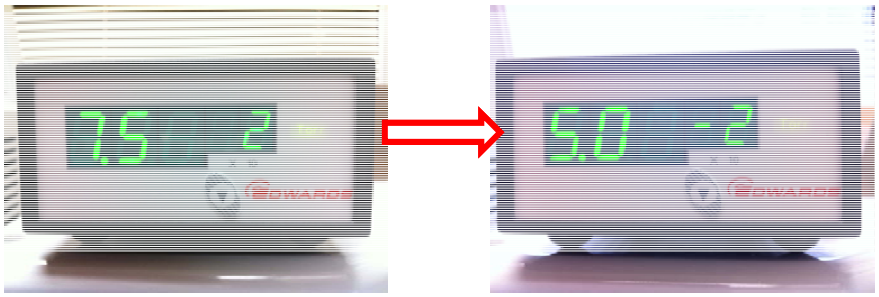
Vacuum at 0.05 Torr in sublimation chamber (atmospheric pressure is 750 Torr).



750 Torr (atmospheric pressure)

0.05 Torr

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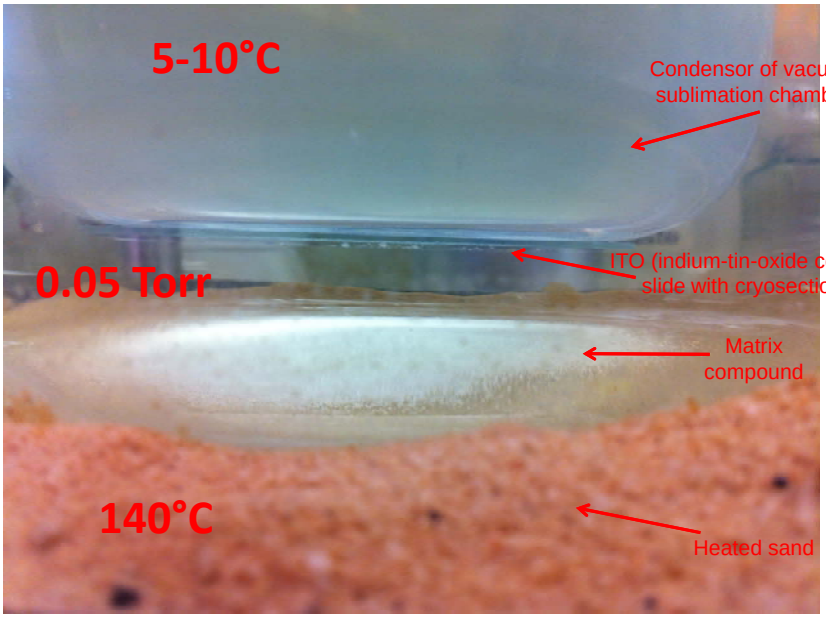
Vacuum sublimation.



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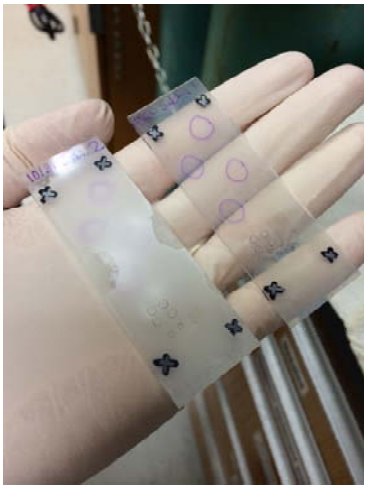
Slides post-sublimation.

Deposition of the matrix compound is at the molecular level because gaseous molecules recrystallize at the relatively cold surface of the tissue section attached to the cold condenser.

The uniformity of matrix deposition onto the slide attached to the cold condenser surface reflects the random Brownian motion of the released gaseous matrix molecules.



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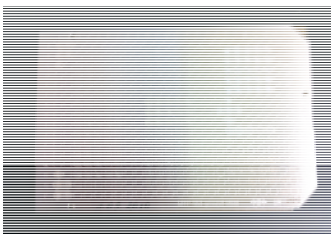
Adaptation of MALDI plate for imaging cryosections on slides.

Conventional MALDI plate



Adapted MALDI plate for cryosections

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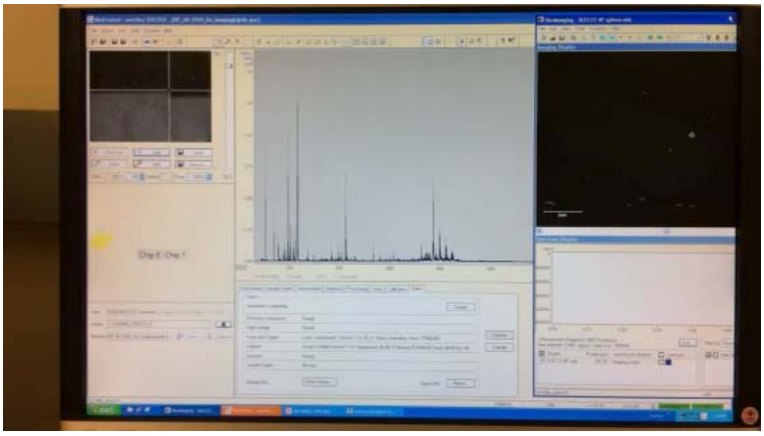
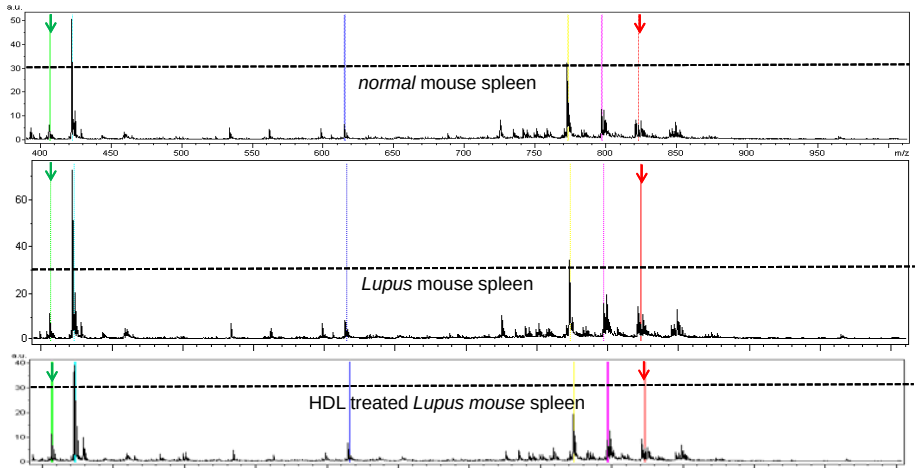
MALDI-IMS in motion.



Some examples of MALDI-IMS

MALDI-IMS on mouse spleen.



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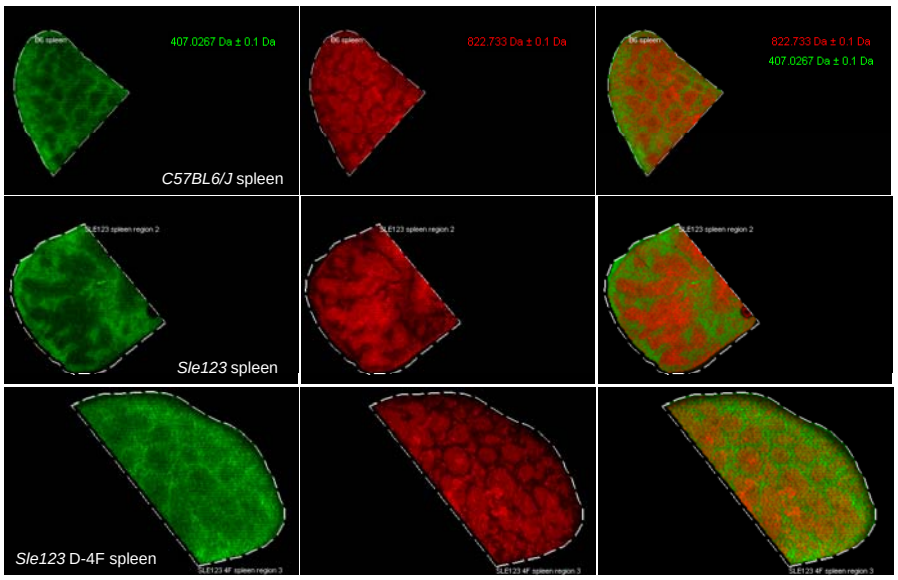
MALDI-IMS on mouse spleen.



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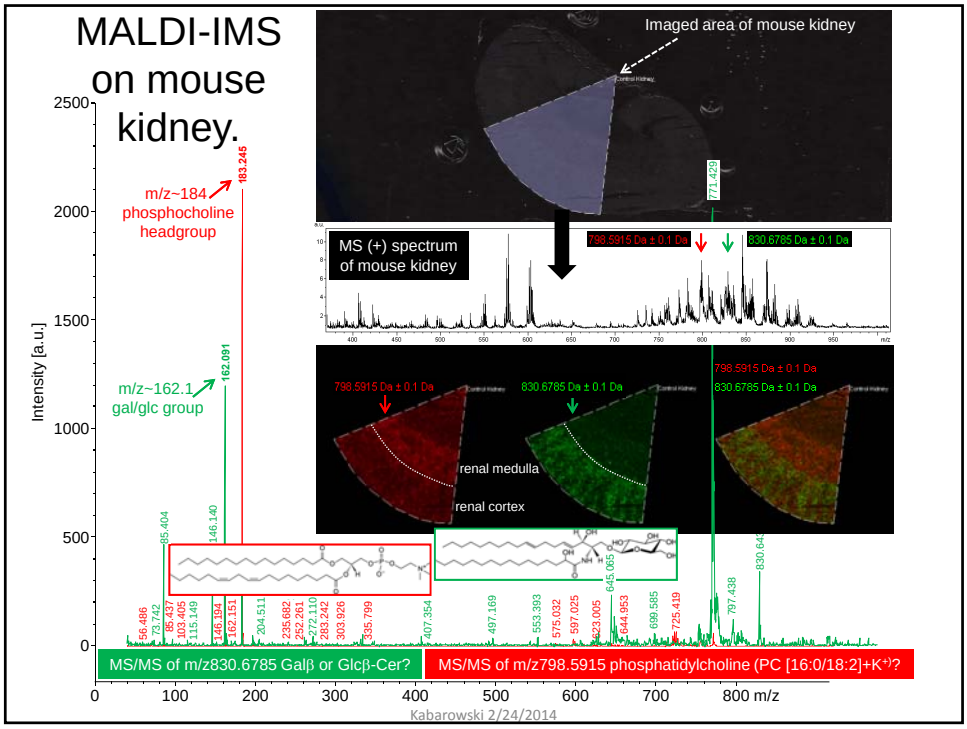
MALDI-IMS on mouse kidney.
Imaged area of mouse kidney
MS (+) spectrum of mouse kidney
798.5915 Da ± 0.1 Da
830.6785 Da ± 0.1 Da
m/z~184 phosphocholine headgroup
m/z~162.1 gal/glc group
renal medulla
renal cortex
Intensity [a.u.]
MS/MS of m/z830.6785 Galβ or Glcβ-Cer?
MS/MS of m/z798.5915 phosphatidylcholine (PC [16:0/18:2]+K+)?
800 m/z
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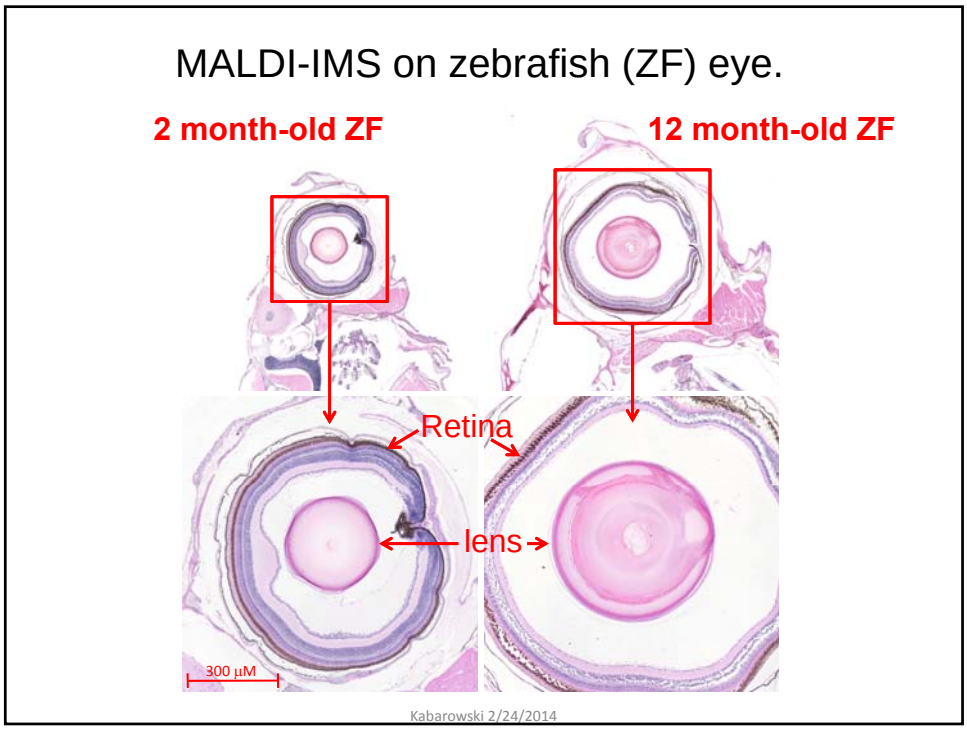
MALDI-IMS on zebrafish (ZF) eye.
2 month-old ZF
12 month-old ZF
Retina
lens
300 μM
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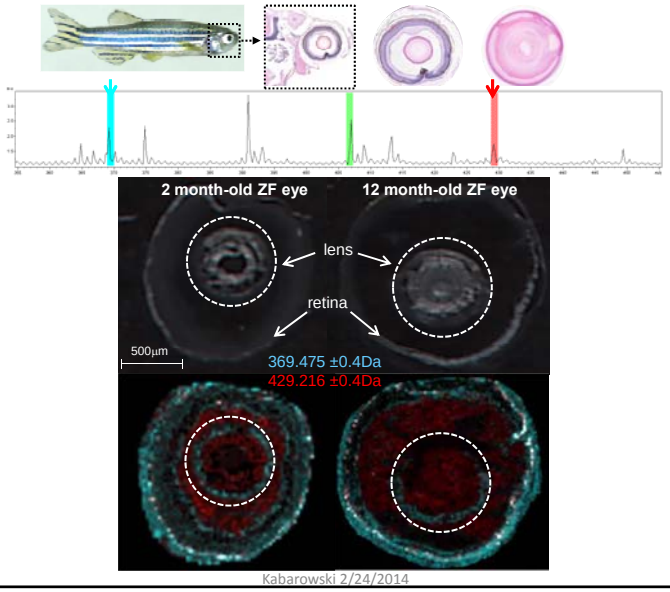
MALDI-IMS to identify age-related changes in ZF eye lens lipid distribution.



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Suggested reading:

Mass Spectrometric Imaging for biomedical tissue analysis.

Kamila Chughtai and Ron M.A. Heeren.

Chem Rev. Vol.110(5): pp3237–3277, 2010.

FOM-Institute for Atomic and Molecular Physics, Science Park 104, 1098 XG Amsterdam, The Netherlands

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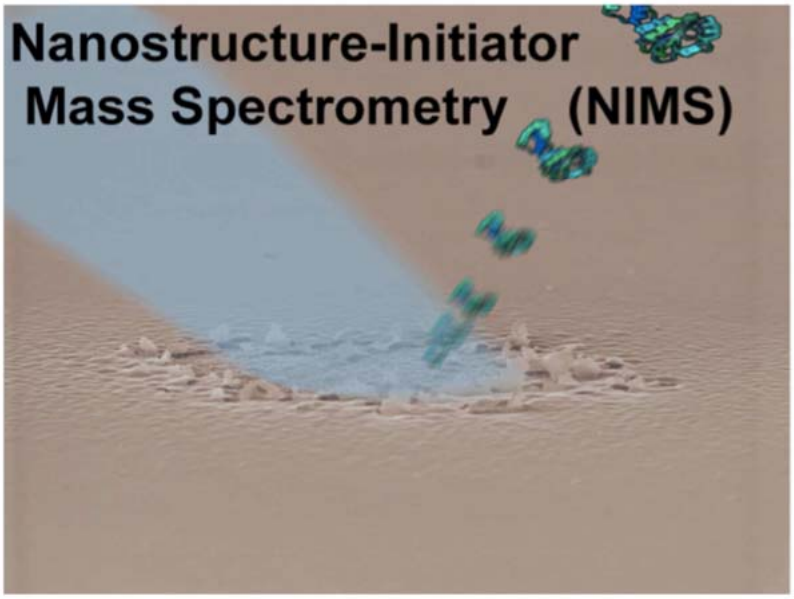
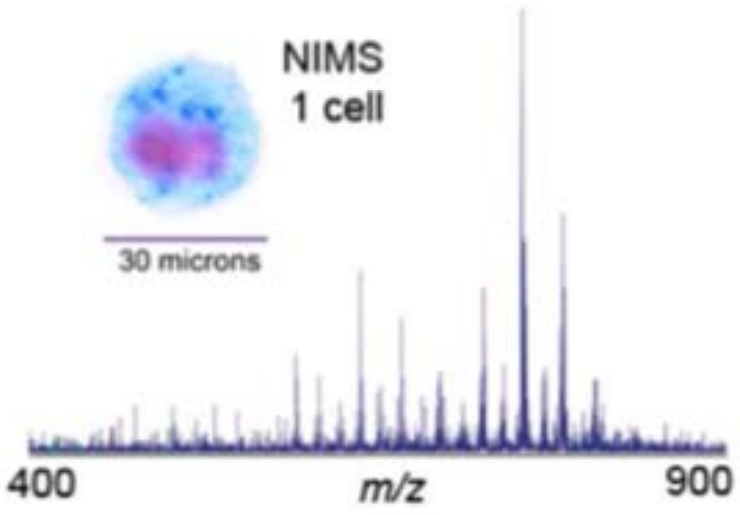


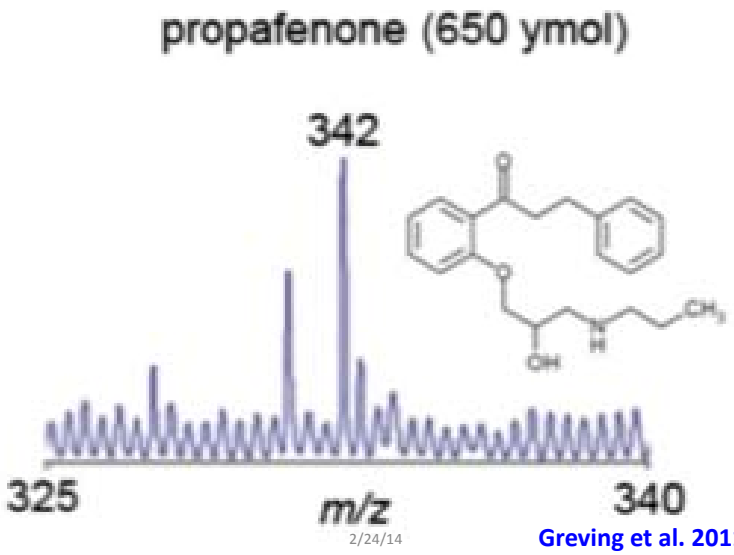
2/24/14 Greving et al. 2011

Spectrum from a single cell



2/24/14 Greving et al. 2011

Extreme sensitivity



2/24/14

Greving et al. 2011

Mass Spectrometry Imaging Workshop
April 22-25, 2014
Vanderbilt University

https://www.msrc.mc.vanderbilt.edu/aims2014

Registration: **$750 before Mar 15**
$950 after Mar 15, up to Mar 31

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