BMG 744
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Mass spectrometry imaging

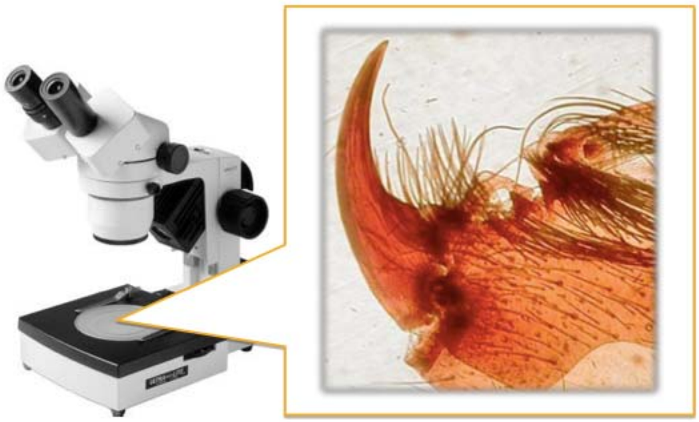
Stephen Barnes, PhD

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

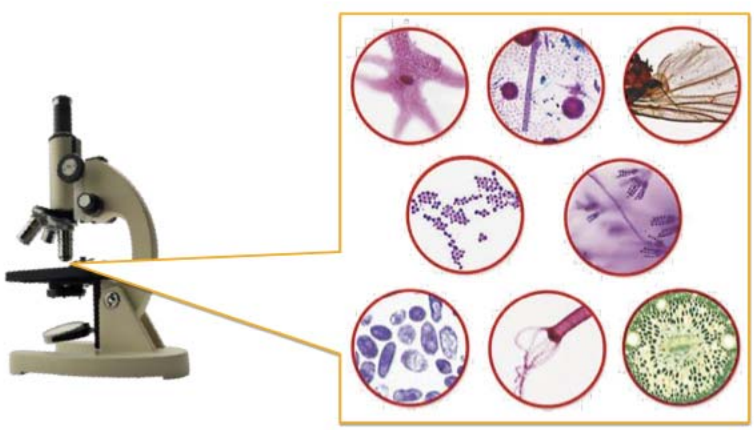
What does MS imaging offer?

- **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**

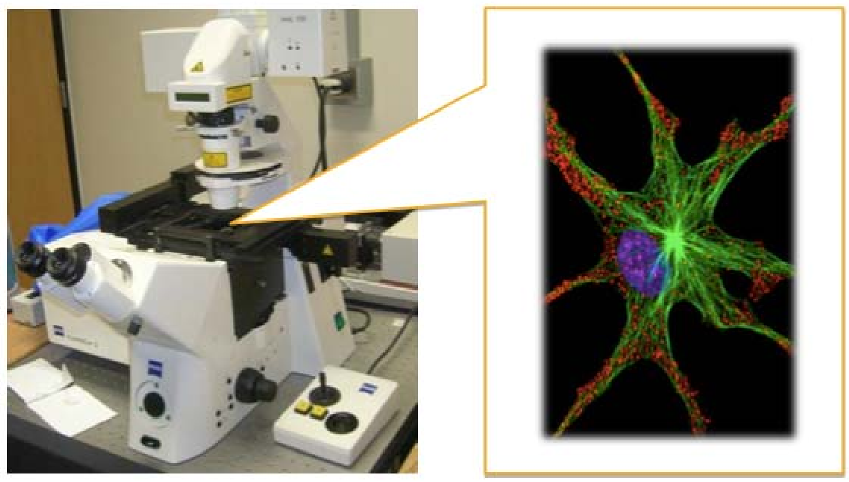
Imaging is widely used in research



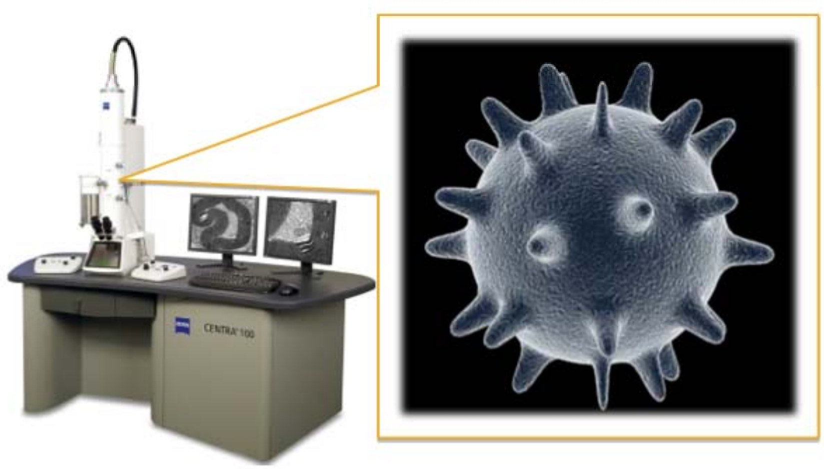
Light microscopy



Confocal Microscopy

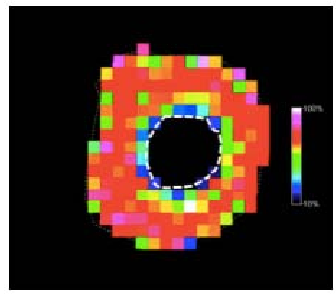


Electron microscopy



Mass spectrometry imaging

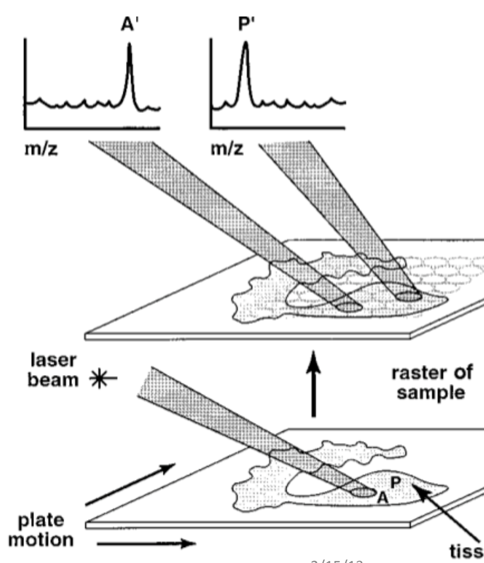
MALDI-TOF mass spectrometer



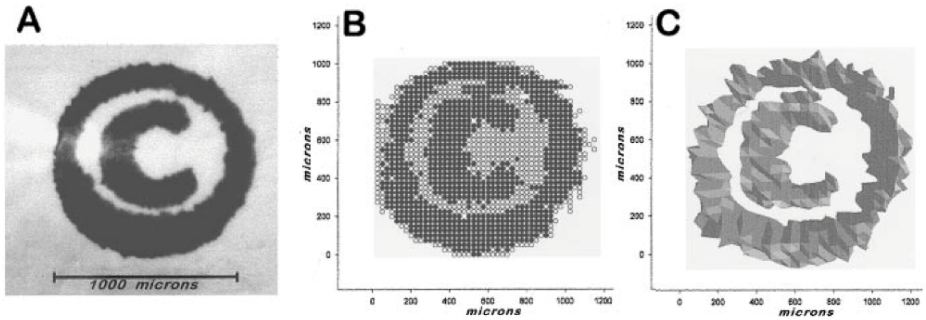
A chemical image

Principle of MALDI imaging

(Caprioli, 1997)



A simple example of MS imaging



Coomasie Blue image

Image based on the detection of the ion for Coomasie Blue

Interpolated image reconstruction

(Caprioli, 1997)

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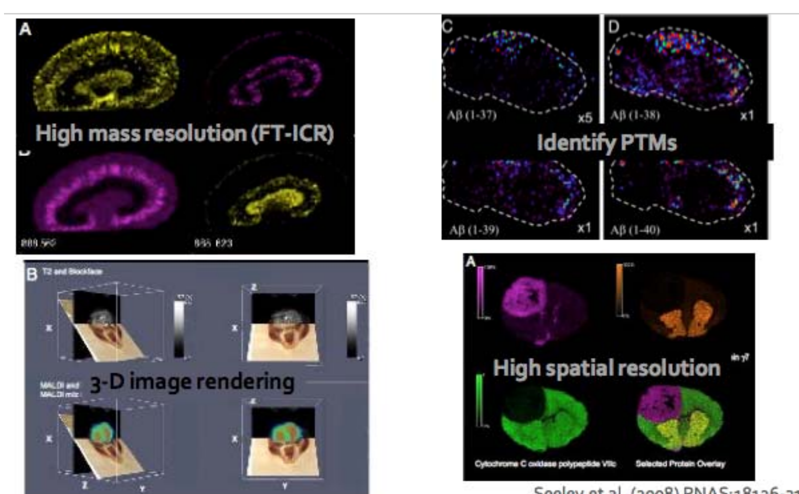
Modern applications of MS imaging



Seeley et al. (2008) PNAS:18126-31.

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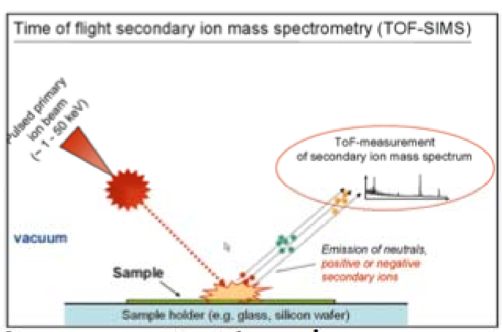
How to get analytes “off” a tissue section

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

SIMS

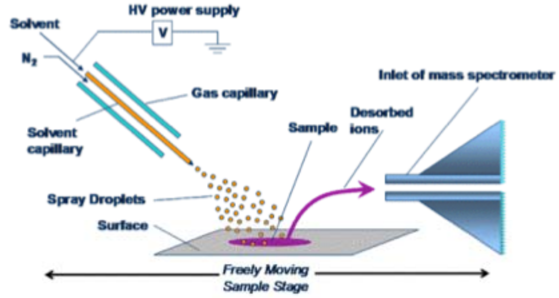
- Used in early studies (elemental analyses)
- Very high resolution (>50 nm)
- Principle of ionization: collated ion beam



- Destructive, penetrating, low mass range

DESI

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



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

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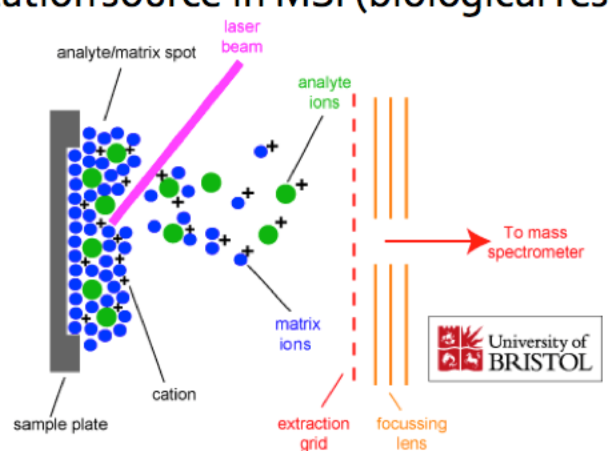
MALDI

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



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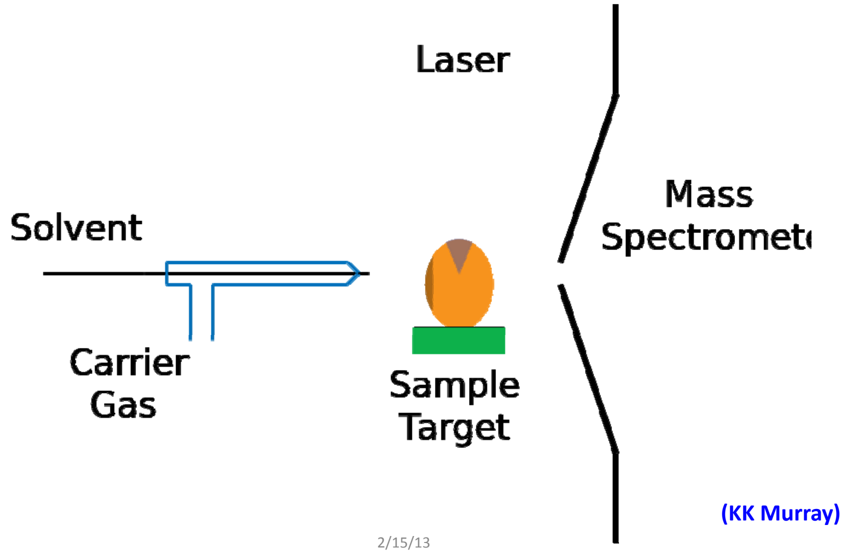
MALDESI



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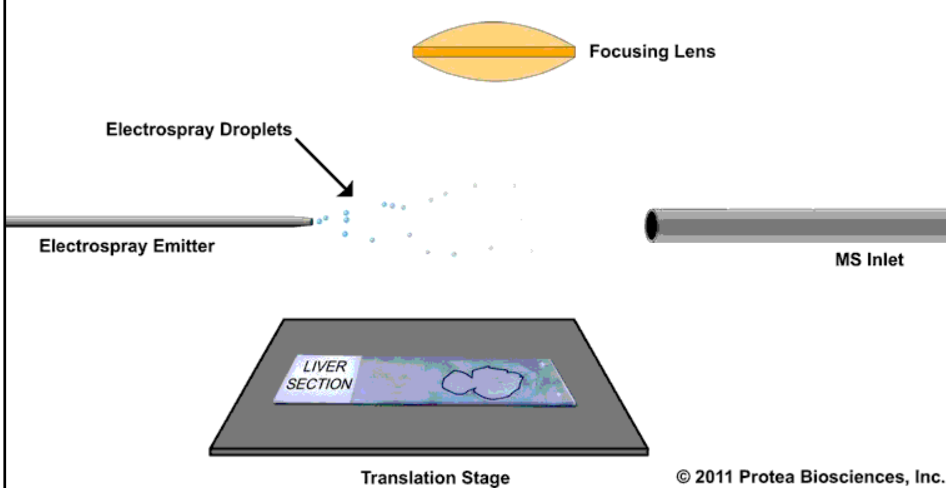
LAESI



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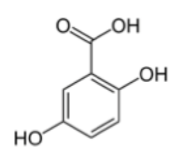
“Profiling” versus “imaging”

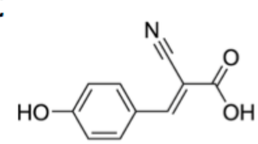
- **Profiling:**
  - Limited, directed information
  - Rapid analysis high throughput
  - Clinical applications and biomarker discovery
- **Imaging:**
  - Extensive, high resolution
  - Time consuming, laborious
  - Useful for investigative research

MALDI matrices

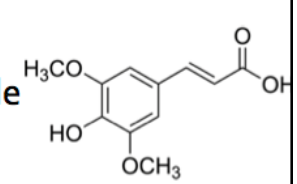
- DHB- 2,5-dihydroxybenzoic acid
  - Commonly used for small molecules



- 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)



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%)

How to spot the matrix

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

Matrix application I: Manual spotting

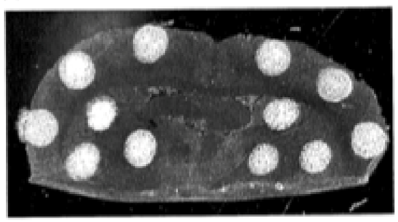
✓ Start here!
- Gold standard for signal
- Best extraction
- Biggest crystals
- Best S/N and most signals
- Use for parameter optimization

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

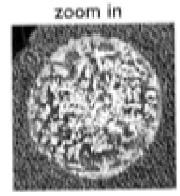
pipette
~250 nL
~1-2 mm diameter

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

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



zoom in



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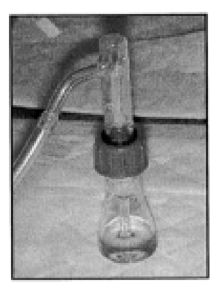
Matrix application III: Manual coating

- 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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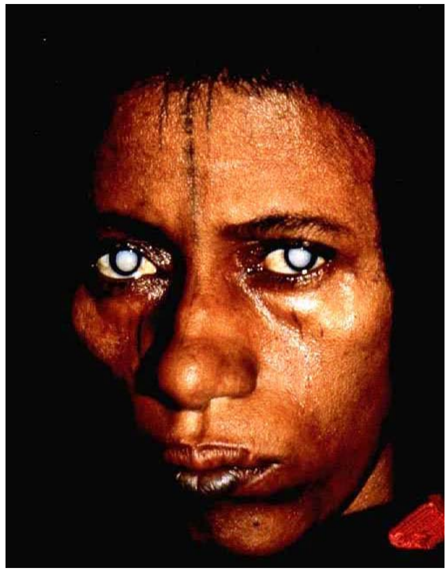
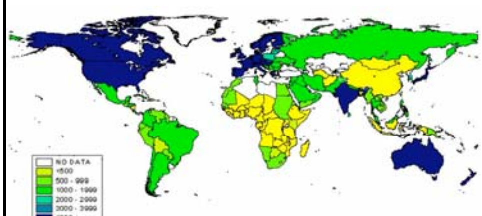
Cataract disease in an Amazonian



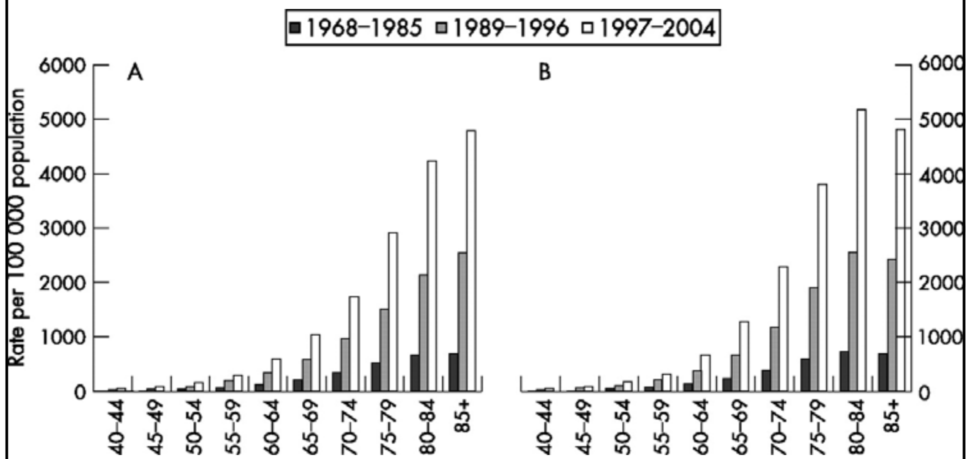
Cataract Disease and Public Health:



- 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

Lens cataract incidence with aging



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

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Lens changes occur in pets, too

Cloudiness is a natural event that occurs with aging and precedes cataract formation. Due to packing of the lens fiber cells.

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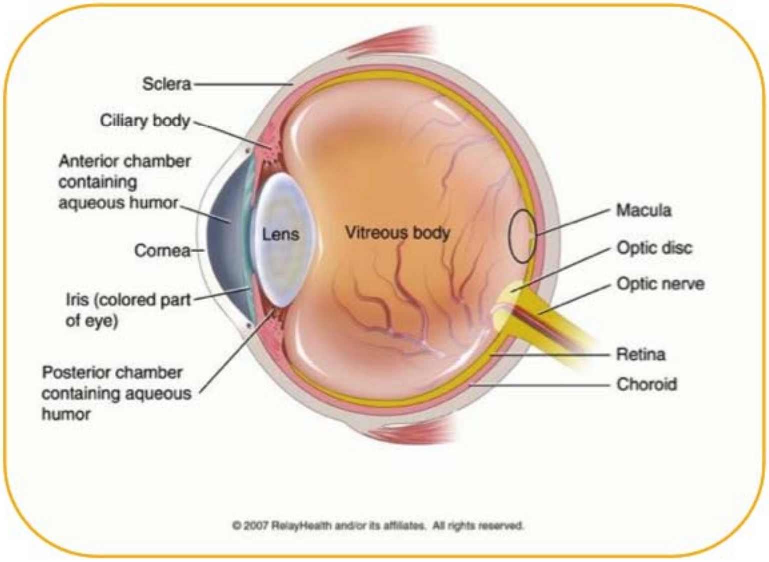
Anatomy of the eye



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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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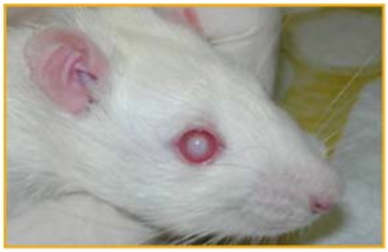
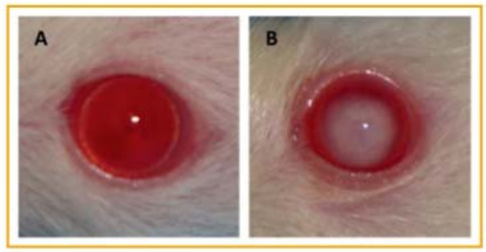
The rat model we use





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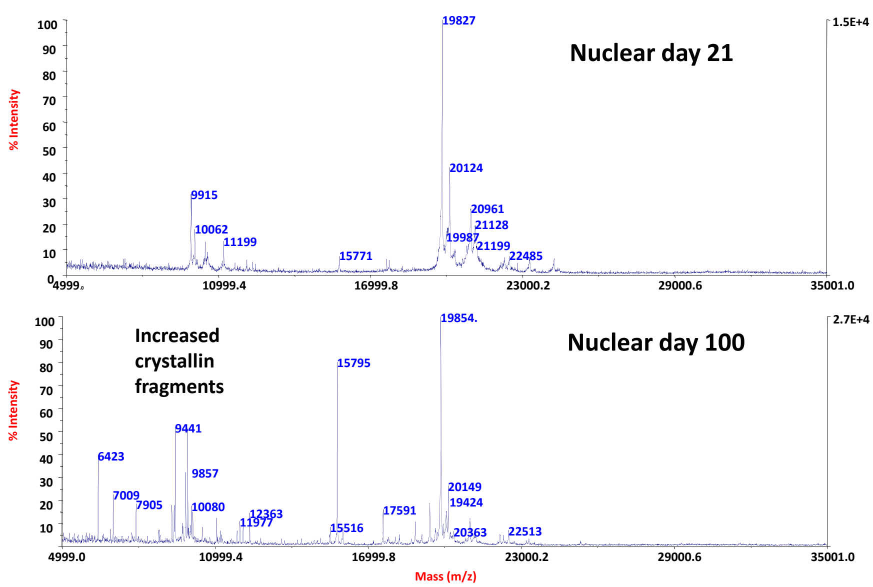
MALDI-TOF MS profiling of nuclear region of SD rat lens



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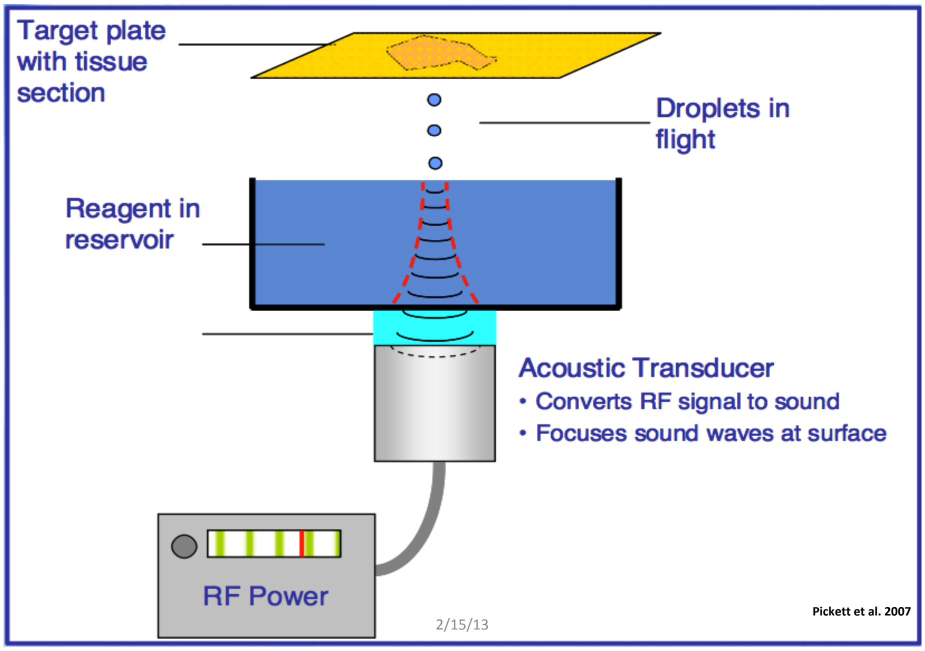
Acoustic spotting onto frozen lens section



Pickett et al. 2007

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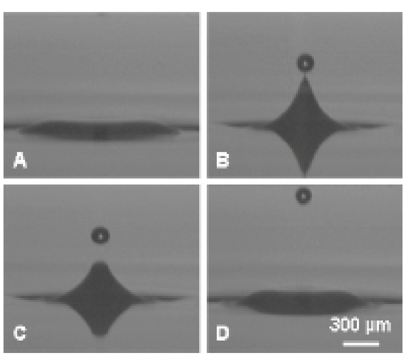
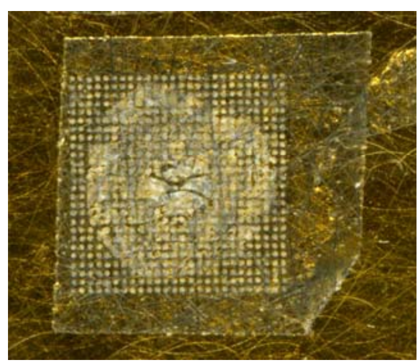
Spotting to the frozen rat lens



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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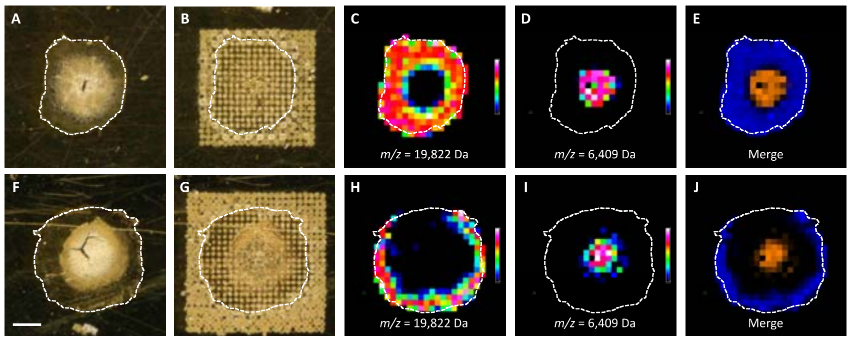
Lens Cataract: imaging reveals a geographic distribution of protein forms



What are the proteins that imaging is detecting?

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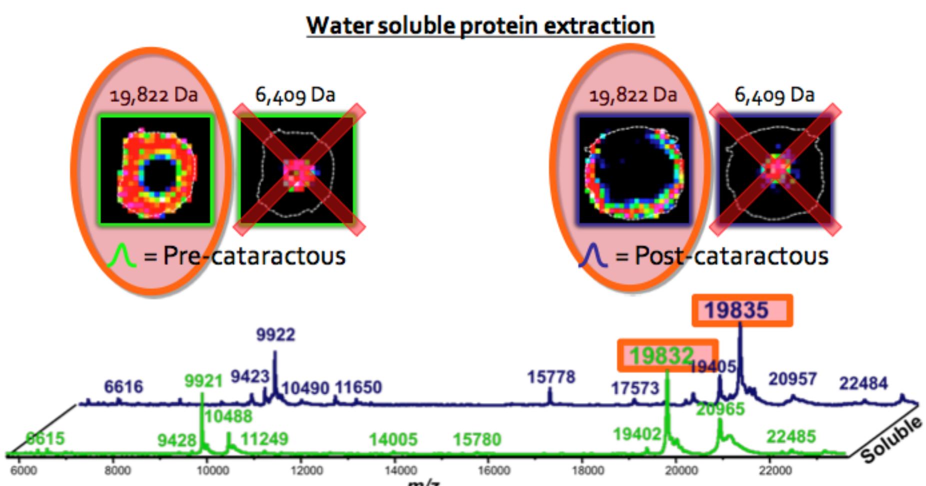
Stella et al., 2010

Aqueous extract of the lens



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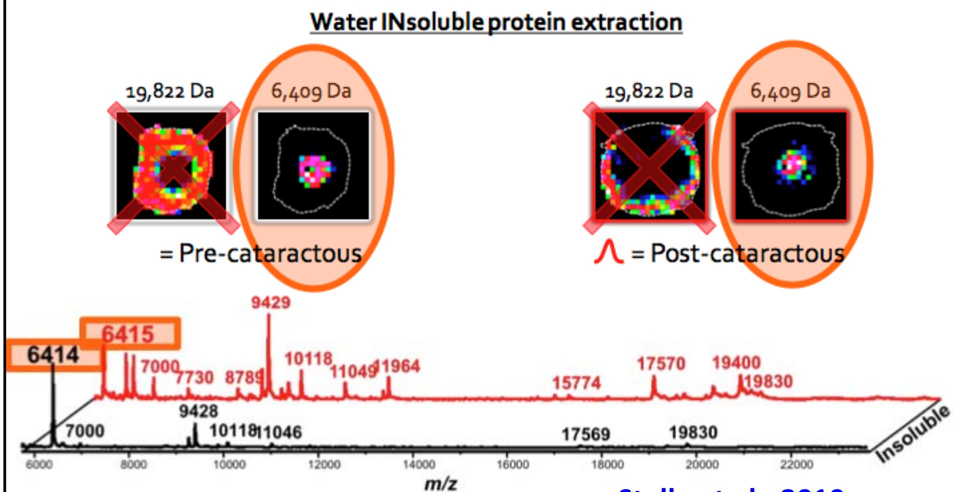
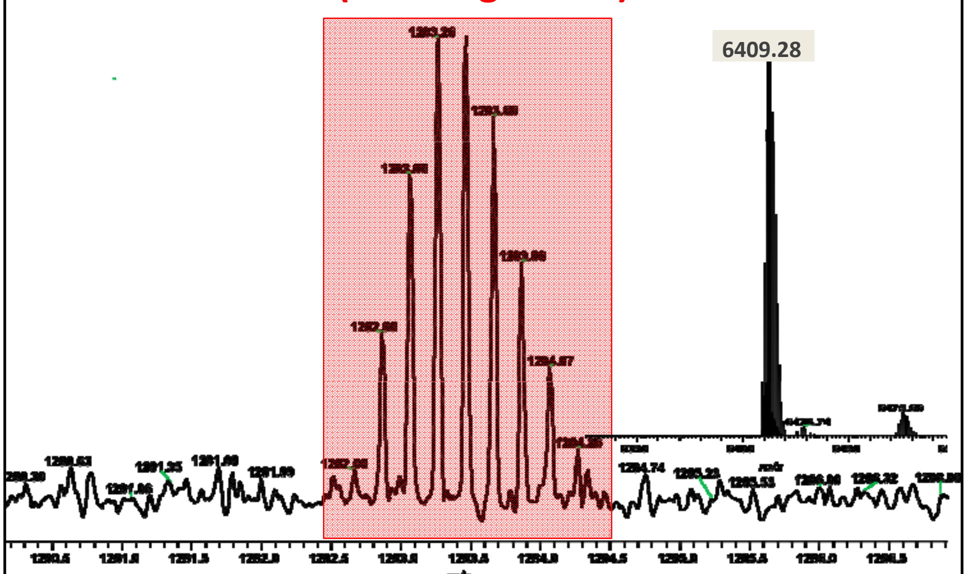
Stella et al., 2010

Water-insoluble/urea soluble



Stella et al., 2010

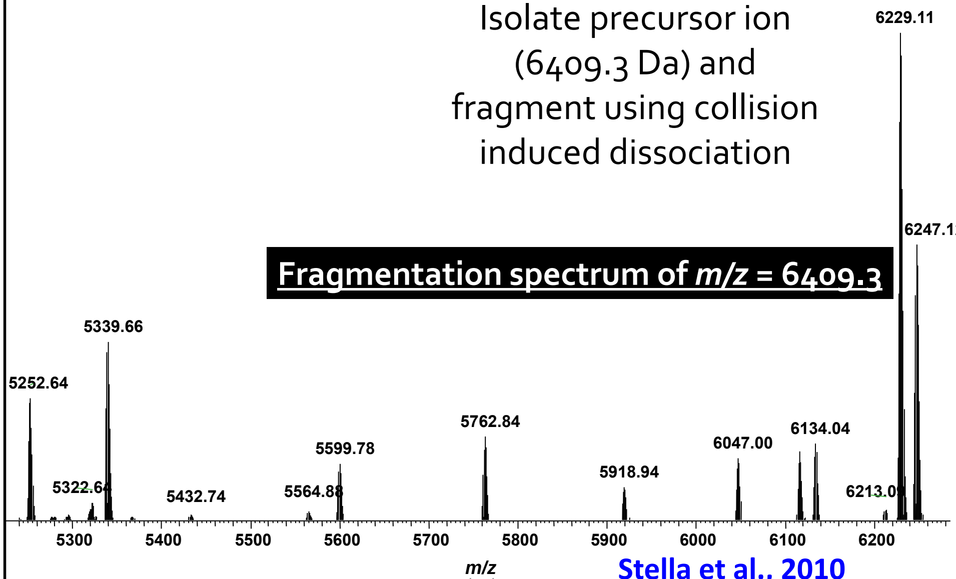
Top-down identification of *m/z*=1283.3 (5+ charge state)



Stella et al., 2010

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

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



Stella et al., 2010

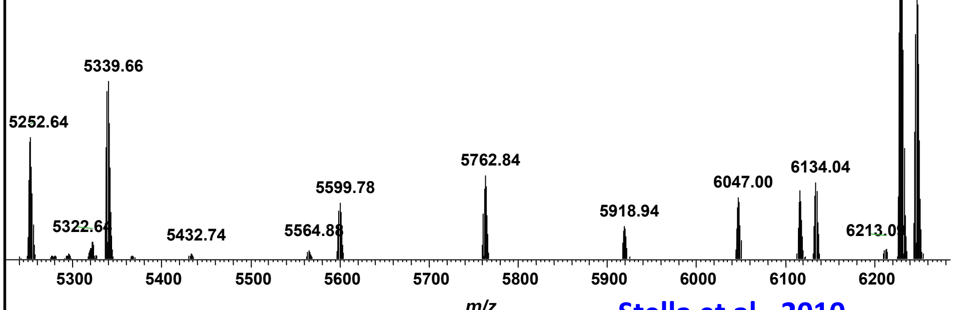
Top-down ID of (M+H$^+$)= *6,409.28 Da*

αA-crystallin

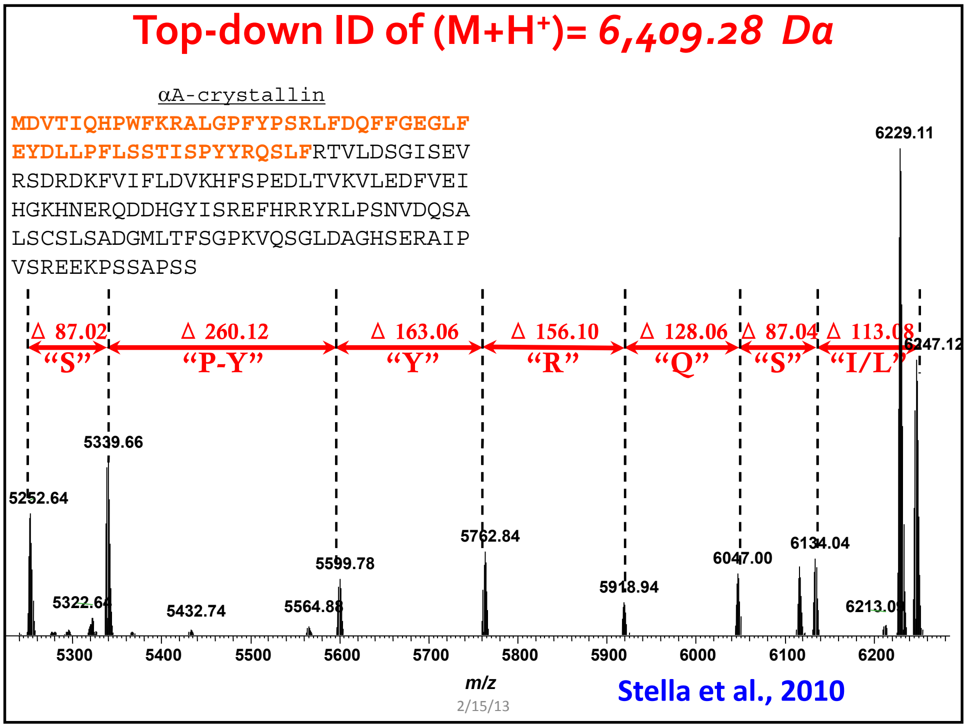
MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLF
EYDLLPFLSSTISPYYRQSLFRTVLDSGISEV
RSDRDKFVIFLDVKHFSPEDLTVKVLEDFVEI
HGKHNERQDDHGYISREFHRRYRLPSNVDQSA
LSCSLSADGMLTFSGPKVQSGLDAGHSERAIP
VSREEKPSSAPSS

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

Fragmentation spectrum of *m/z* = 6409.3



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	11,956.08	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§
Full length → Crystallin, alpha A	19,822.89	1-173§

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

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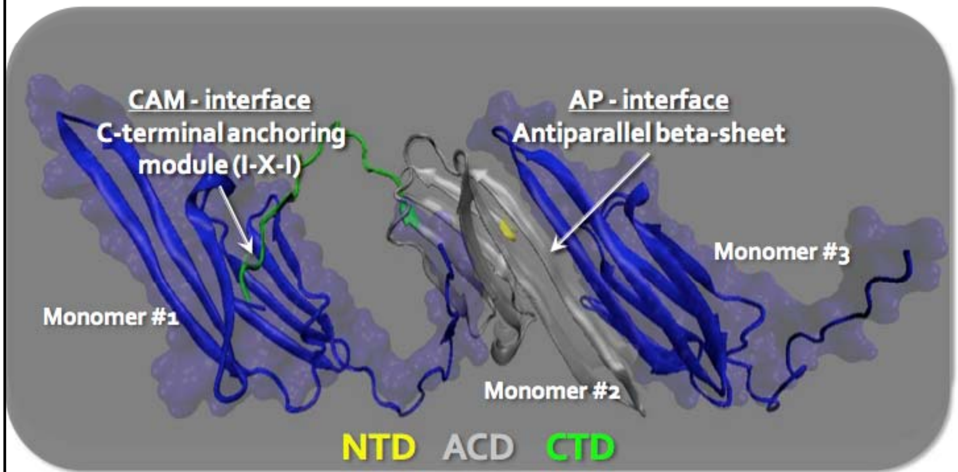
Stella et al. (2010) Invest Opth Vis Sci:5153 - 61.

Structure of αA-crystallin



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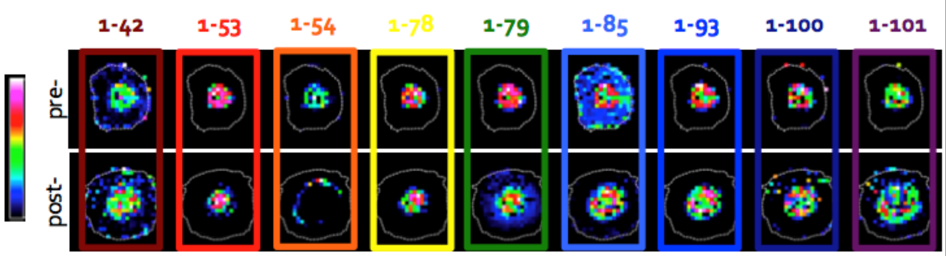
Distribution of truncated αA-crystallins

1. Nuclear localization



MDVTIQHPWFKRALGPFYPSRLFDQFFGEGLFEYDLLPFLSSTI
SPYYRQSLFRTVLDSGISEVRSDRDKFVIFLDVKHFSPEDLTVK
VLEDFVEIHGKHNERQDDHGYISREFHRRYRLPSNVDQSALSC
SLSADGMLTFSGPKVQSGLDAGHSERAIPVSREEKPSSAPSS

NTD ACD CTD

Stella et al., 2010

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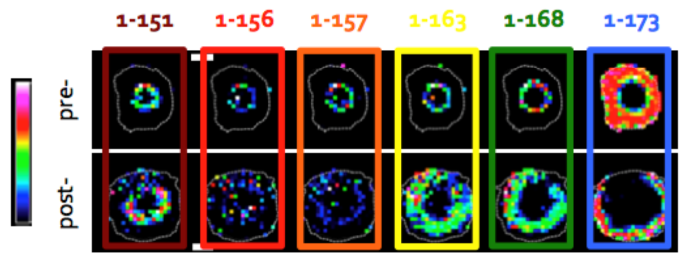
Distribution of truncated αA-crystallins

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

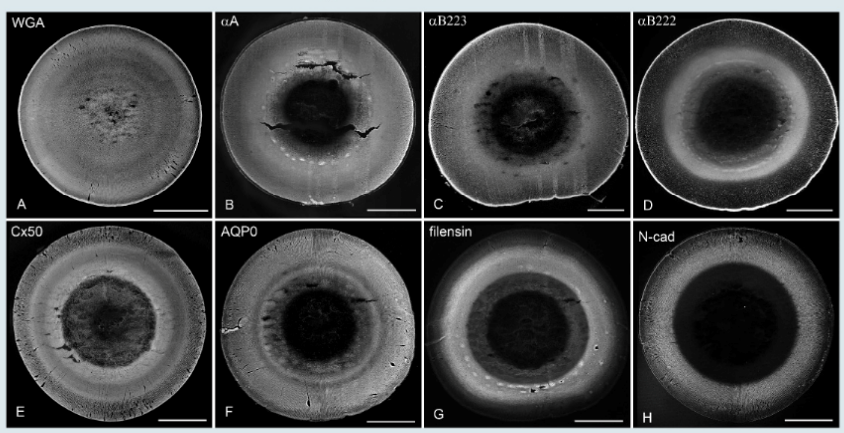

Figure 3(A-H): Composite of mouse lens sections labelled with wheat germ agglutinin (WGA) or antibodies to lens proteins. A) WGA (Invitrogen W32466); B) αA221 (Enzo ADI-SPA 221); C) αB223 (Enzo ADI-SPA 223); D) αB222 (Enzo ADI-SPA 222); E) connexin 50 (Cx50 from Dr. Thomas White); F) Aquaporin0 (AQP0; 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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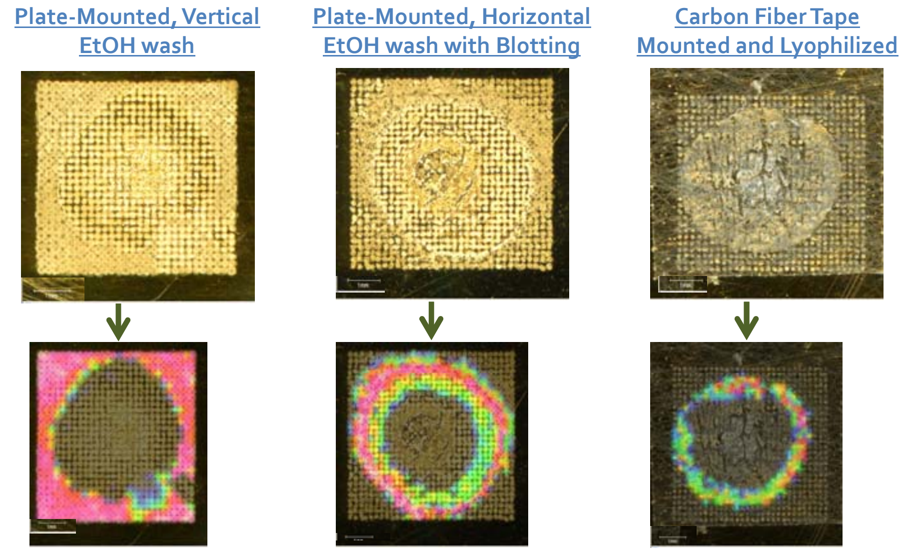
Clark et al., ARVO, 2011

Optimization of spotting methods



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Slide adapted and modified from Kyle Floyd

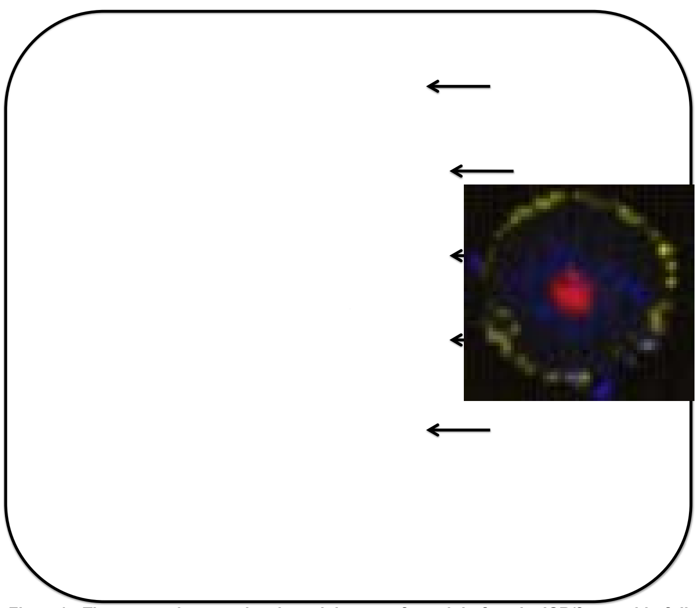

Figure1: Tissue sections and selected images for adult female ICR/f rat with full cataracts. These five sections were taken from the same lens, with four sections taken between each imaged section, and saved for future proteomic analysis.

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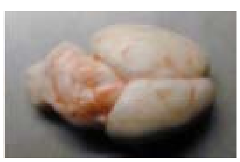
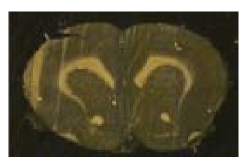
Small molecule imaging

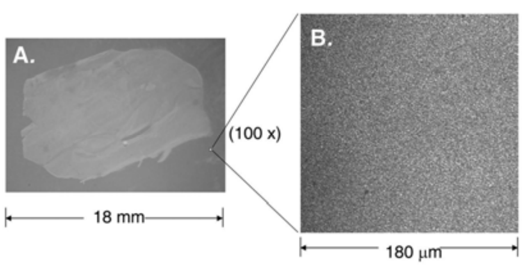
Imaging of lipids in tissue



Fresh frozen tissue

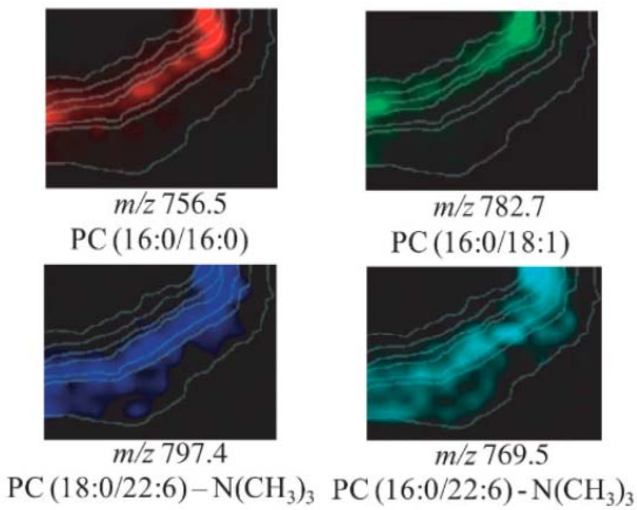


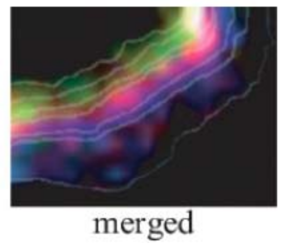
Frozen tissue sectioning
Cut and transfer 10-20 micron section to conductive glass slide



DHB (MALDI matrix) is transferred by sublimation.
This produces a very uniform coating (as shown in B) and high resolution images

Differential lipid distribution in the retina





MALDI-QIT-TOF
Hayasaka et al. 2008
8 μm sections sprayed with matrix (in MeOH) using an airbrush

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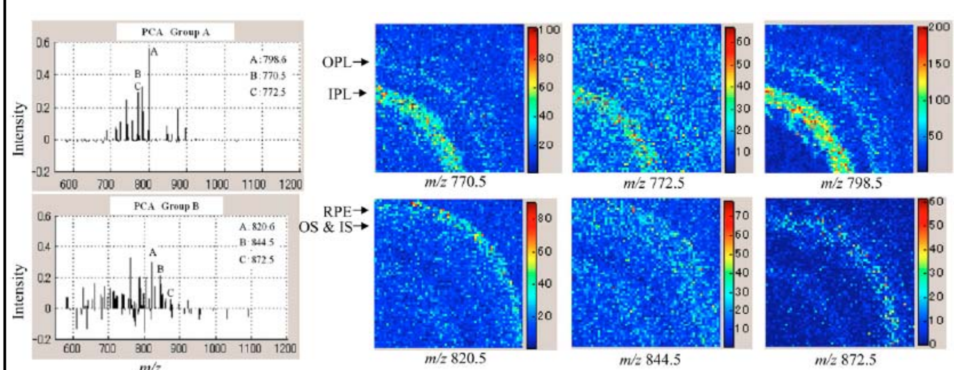
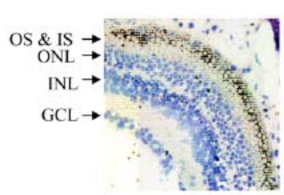
Using a higher resolution approach





The same Japanese group used an "evaporation" method to apply the matrix – see next slide

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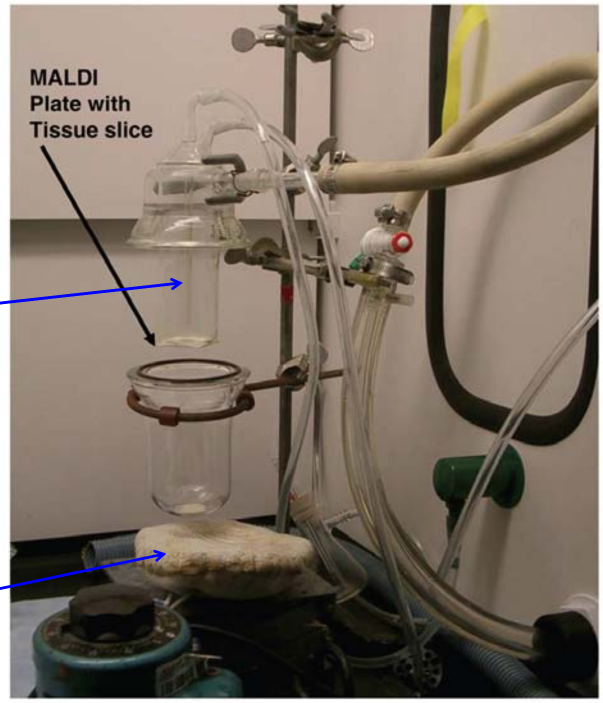
Sublimation method for applying MALDI matrix to the tissue slice

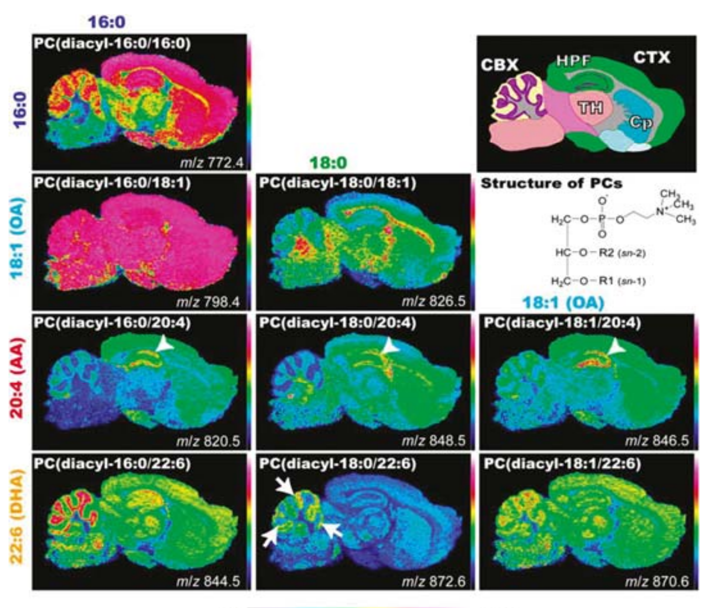
MALDI plate is upside down – ice is placed inside the glass "finger" to condense the DHB

A cold trap (not shown) is needed after the heated chamber to protect the vacuum pump

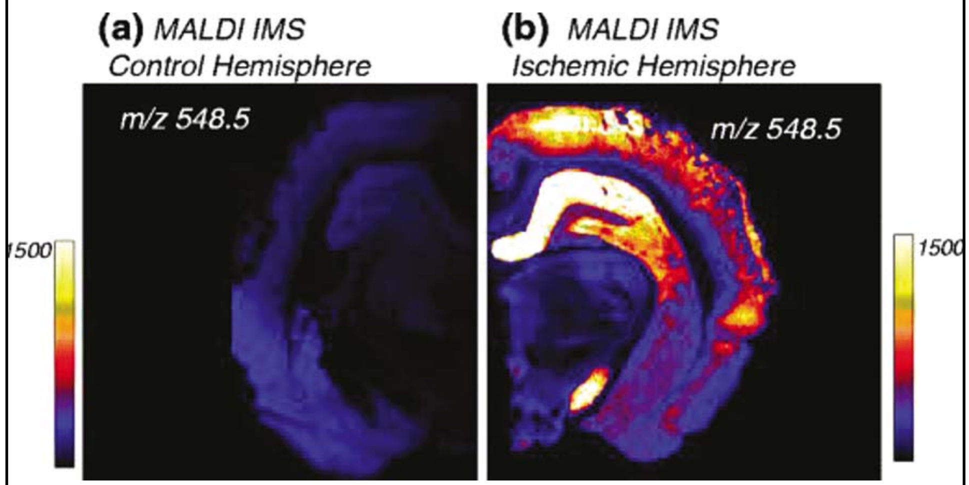
Sand bath to heat the DHB



PC species distribution in the brain



Ceramides and ischemia by IMS



The ion was determined to be Cer 18:0/18:1 (-H_2O)

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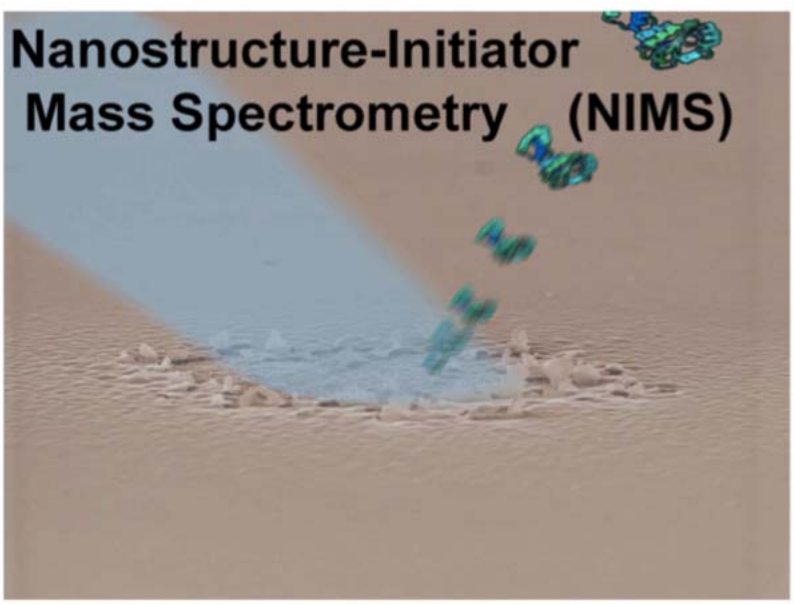

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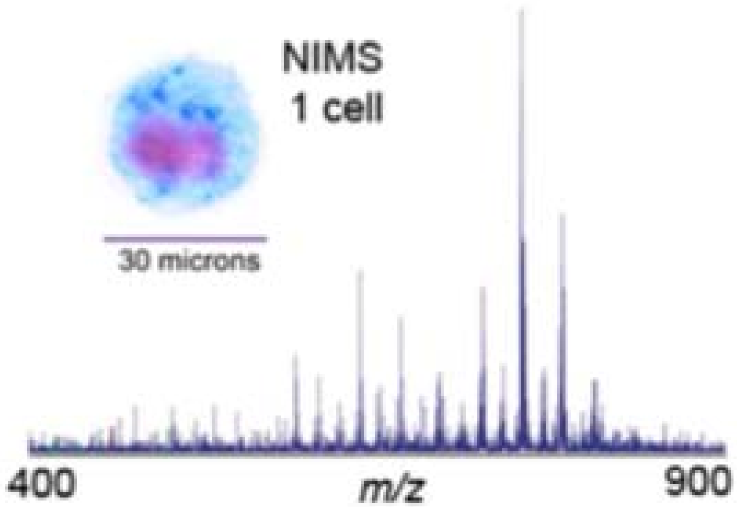
Greving et al. 2011

Spectrum from a single cell



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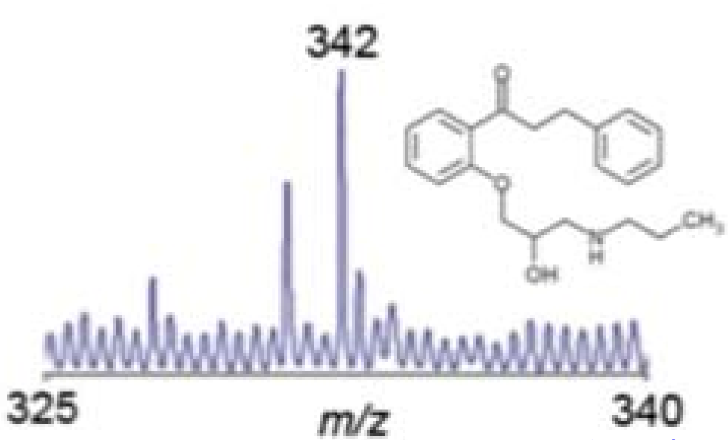
Greving et al. 2011

Extreme sensitivity

propafenone (650 ymol)



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Greving et al. 2011

Mass Spectrometry Imaging Workshop
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Registration: **$750 before Mar 15**
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