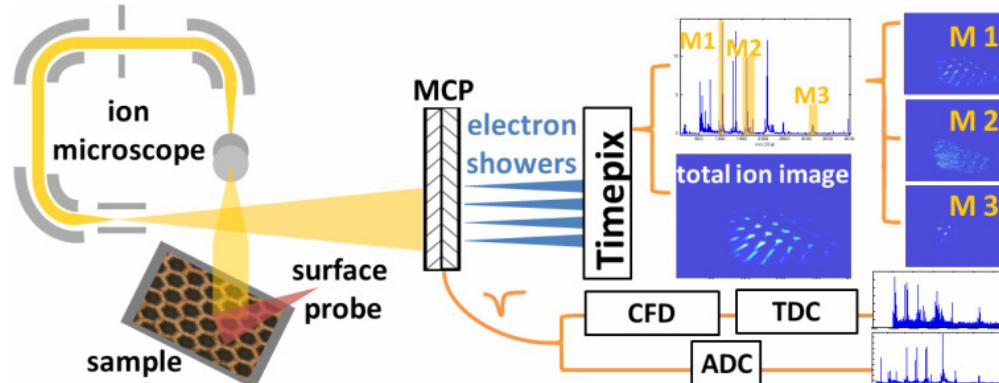


# Active Pixel Detectors for Mass Spectrometry Imaging

Julia H. Jungmann

Donald F. Smith, Andras Kiss, Luke MacAleese, Jan Visser, Ron M. A. Heeren

*FOM-Institute AMOLF & FOM-Institute NIKHEF, Amsterdam, The Netherlands*



Technology and Innovation Group, European Physical Society,  
Workshop 2013, Ravenna, Italy

# Acknowledgements

*Amolf Biomolecular Imaging MS & Nikhef Detector R&D Group*



## **Amolf**

Ronald Buijs, Henk Dekker, Marc Duursma, Gert Eijkel, Frans Giskes, Ron Heeren, Julia Jungmann, Andras Kiss, Ivo Klinkert, Marco Konijnenburg, Luke MacAleese, Henk Neerings, Don Smith, Dirk-Jan Spaanderman, Duncan Veheijde, Marc Vrakking, Sjoerd Wouda

## **Nikhef**

Martin van Beuzekom, Vincent van Beveren, Marten Bosma, Henk Boterenbrood, Bas van der Heiden, Erik Heijne, Joop Roevekamp, Enrico Schioppa, Jan Visser, Jan Visschers

## **Medipix Collaboration**

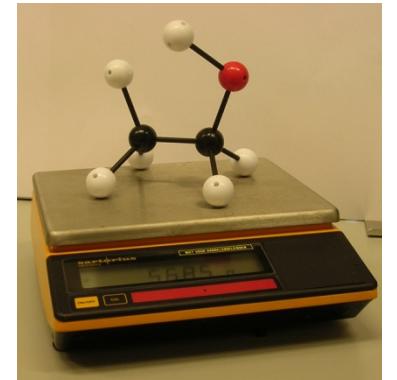
**Funding:** FOM/NWO/STW

# What is Mass Spectrometry Imaging ?

## *Mass spectrometry*

*chemical analyte identification*

- atomic composition of sample molecules (mass= $m$ )
- charge state ( $z$ )



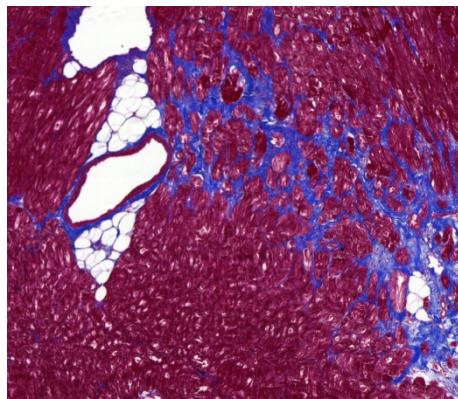
## *Imaging*

- *analyte localization*

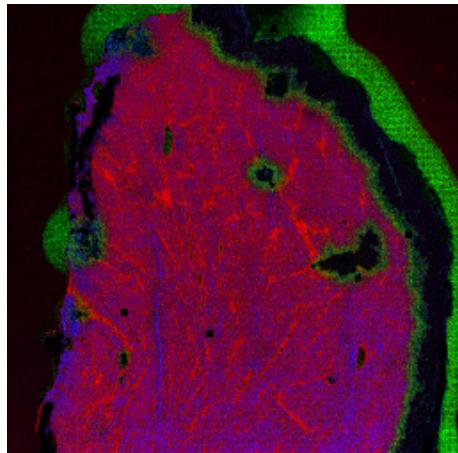
## ***Mass spectrometry + imaging: surface analysis!***

- visualization of the spatial organization
- identification of molecular masses
- accurate (bio)chemical map of sample surface

# Molecular Pathology with MS



Label free imaging



## *MS imaging advantages*

- no labeling required
  - biological molecules are functionally unmodified
- detailed information on
  - molecular identity
  - localization

→ molecular basis to pathology

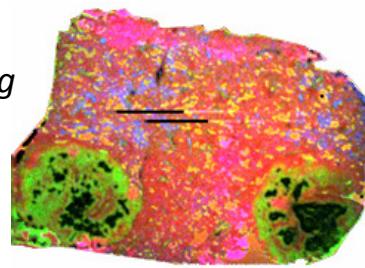
Imaging Mass Spectrometry  
*Mass Spectrometry Reviews* (2007) 26 606-643  
*Chemical Reviews* (2010) 110 3237-3277

# Mass Spectrometry Imaging: Applications

Proteomics  
Lipidomics  
Metabolomics  
Disease studies  
Drug distribution studies  
Forensics

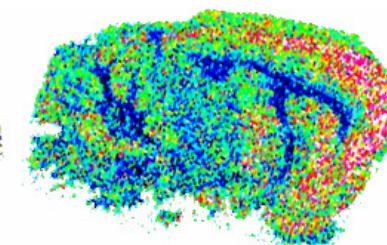
metabolites

*rabbit lung*



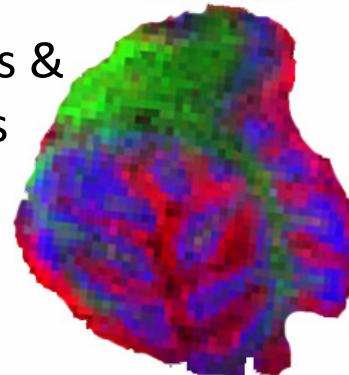
targeted pharmaceuticals

*mouse brain*



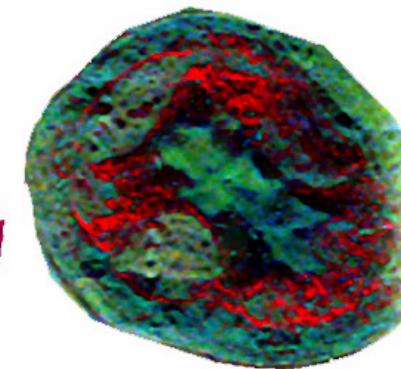
peptides & proteins

*cerebellum (brain)*

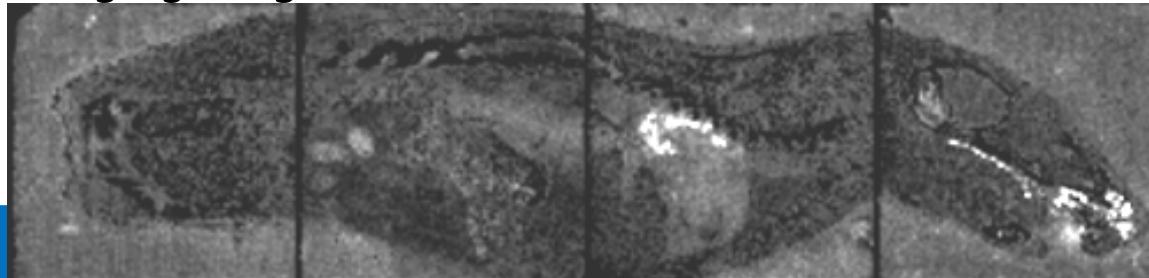


lipids

*breast tumor*

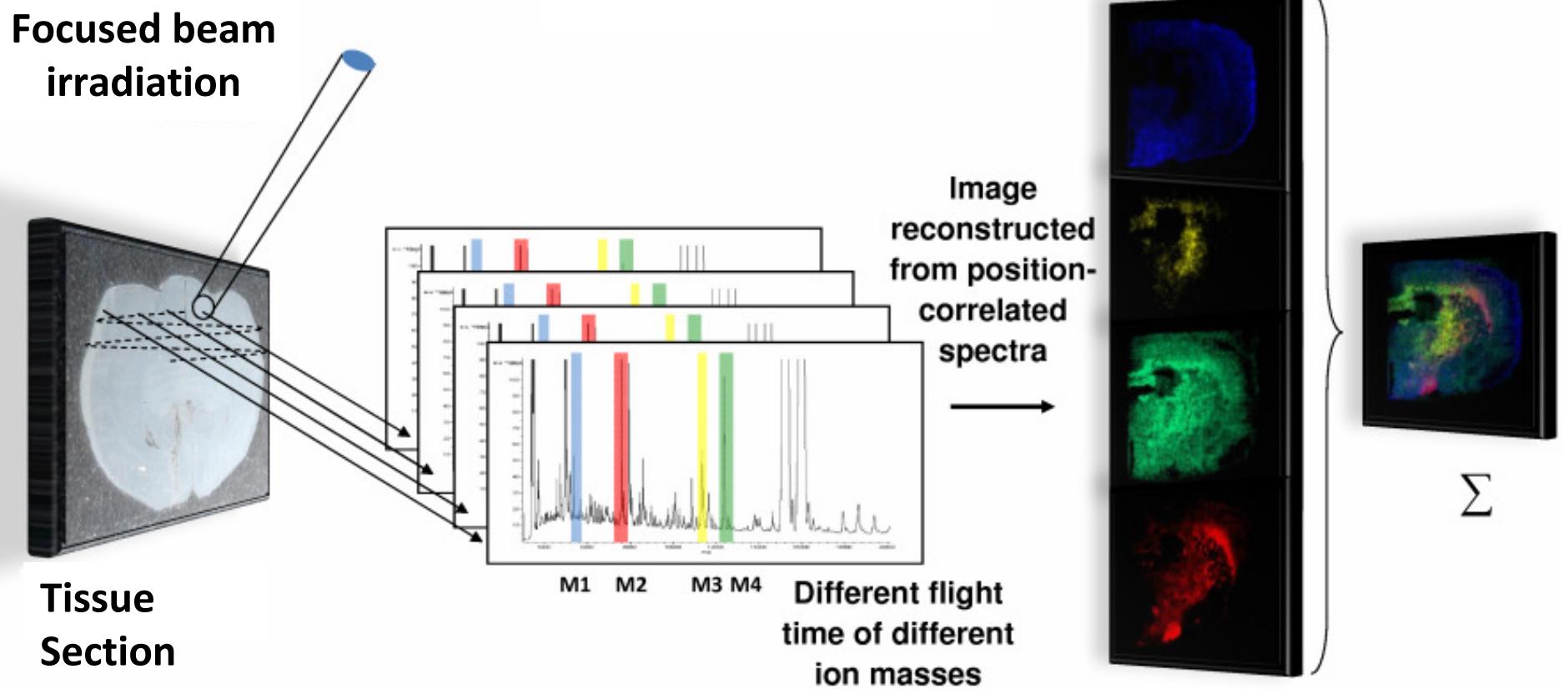


*Imaging drugs and metabolites*

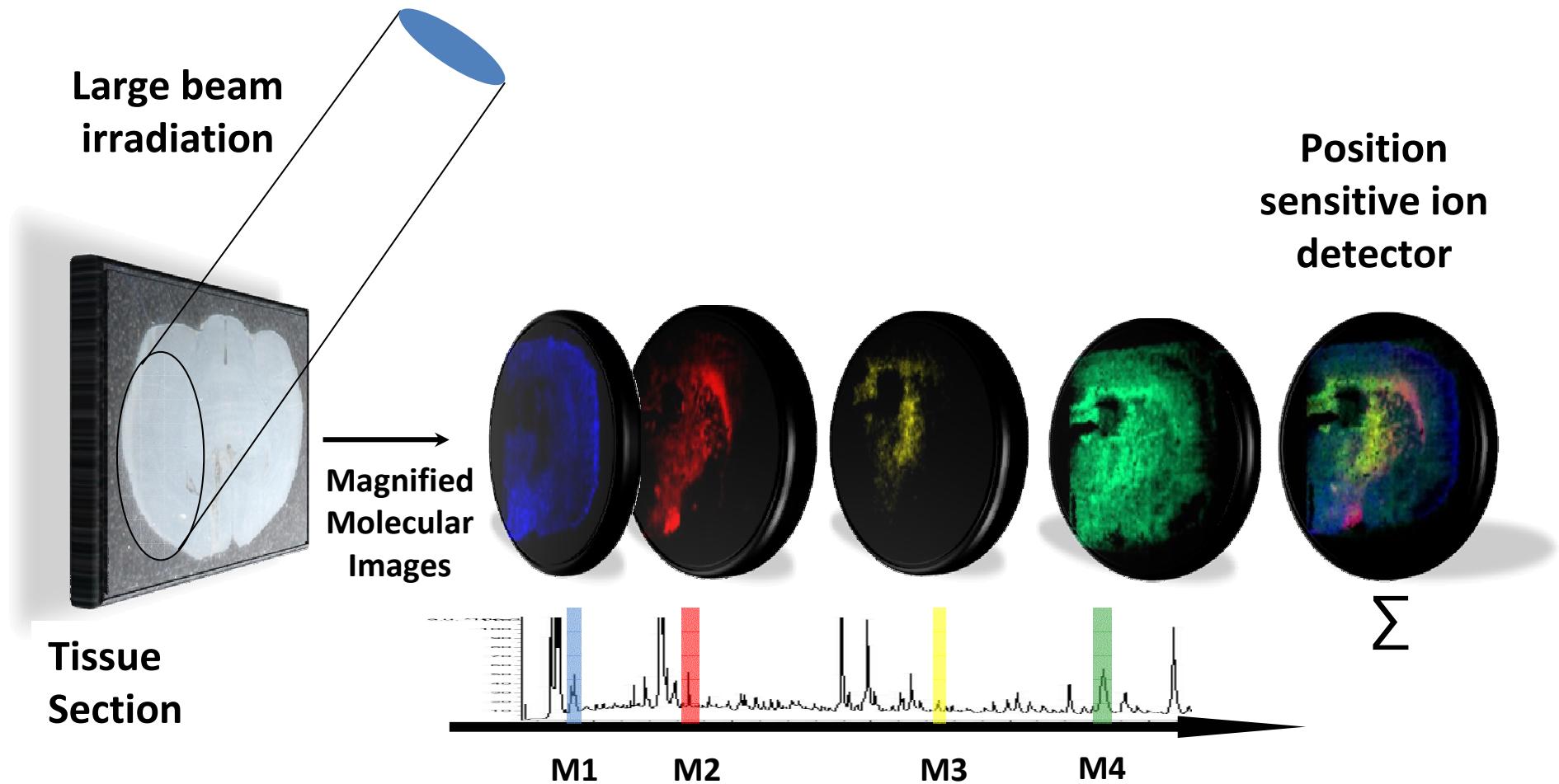


*whole body rat section*

# Microprobe Mode Imaging



# Microscope Mode Imaging



# Microprobe versus Microscope Mode

*Spatial resolution depends on ...*

## Microprobe:

- probe size

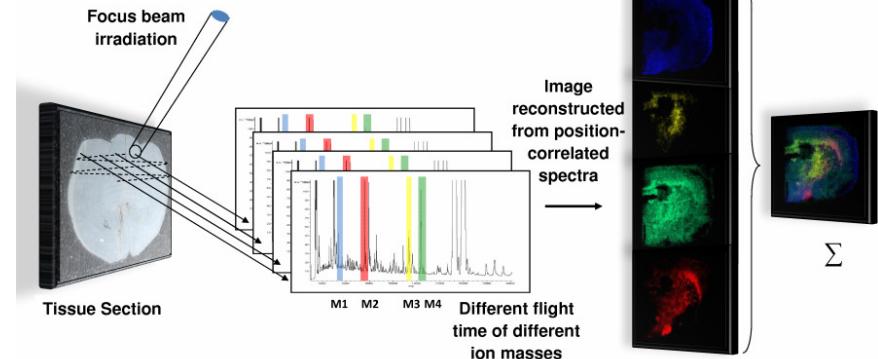
## Microscope:

- ion optics
- detector

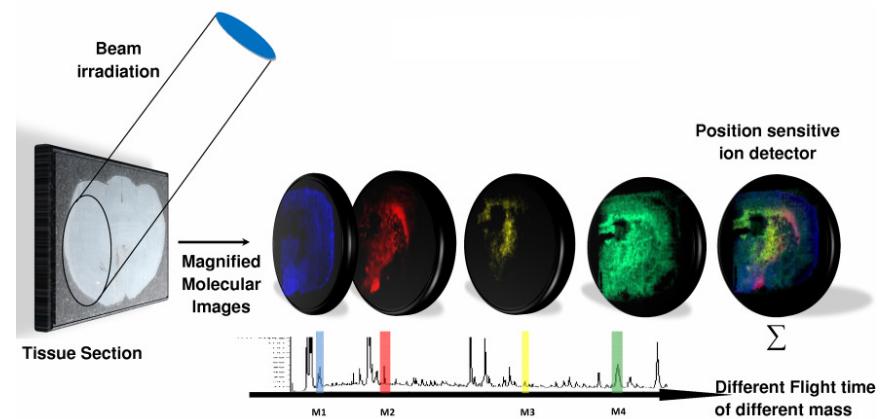
*Throughput depends on ...*

- probe size
- repetition rate

## Microprobe Mode



## Microscope Mode



# Microprobe versus Microscope Mode

*Spatial resolution depends on ...*

**Microprobe:**

- probe size

Probe/pixel size  
Standard: 75-100  $\mu\text{m}$   
Best: 1  $\mu\text{m}$

**Microscope:**

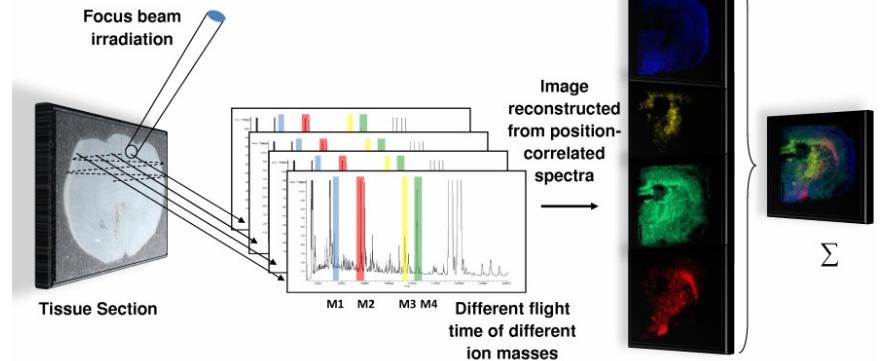
- ion optics
- detector

Probe size: 200-300  $\mu\text{m}$   
Pixel size: <1  $\mu\text{m}$

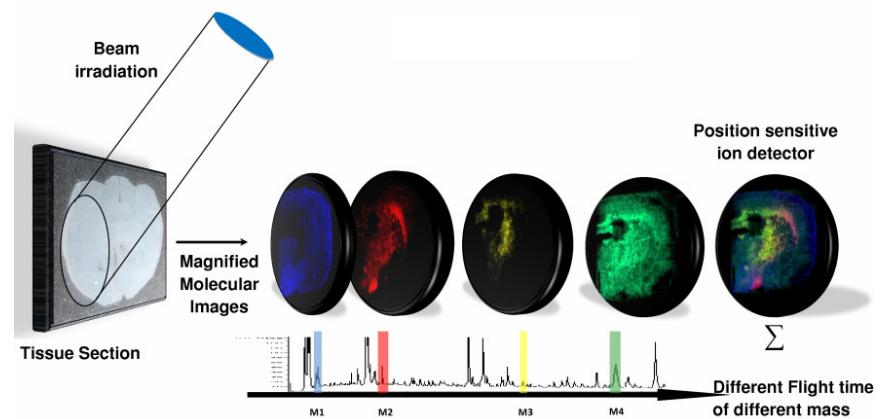
*Throughput depends on ...*

- probe size
- repetition rate

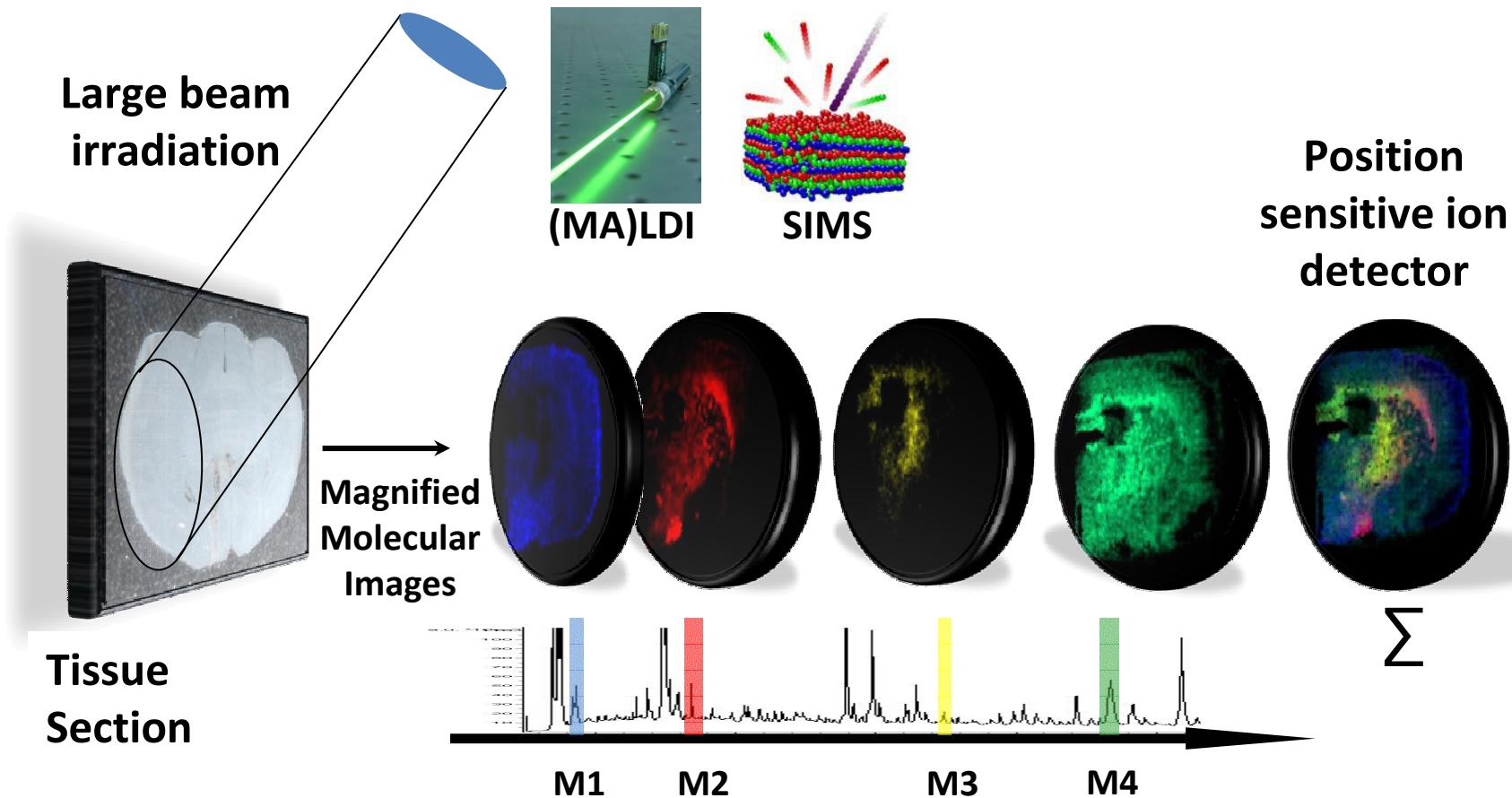
**Microprobe Mode**



**Microscope Mode**



# Microscope Mode Imaging MS



*600 nm MALDI pixel sizes in  
200-300  $\mu\text{m}$  field of view in 1 s.*

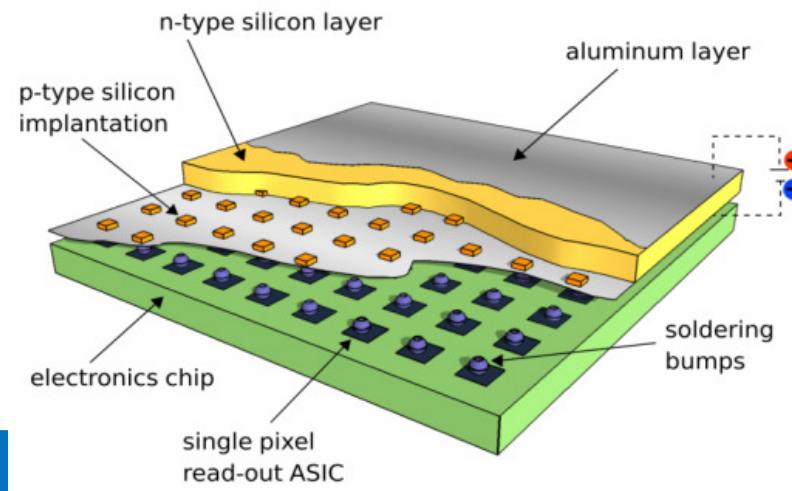
# Active Pixel Detectors

## Mass spectrometry imaging with *active pixel detectors*

- *compact*
- *functionality on the pixel-level*

## Promising features for *fast, sensitive ion imaging*

- time & space information from the same detector
- high spatial resolution imaging
- *direct* imaging acquisition
- highly parallel detection



Jungmann et al., JASMS, 21, 2023-2030, 2010

Jungmann et al., RSI, 81, 103112, 2010

Jungmann et al., Anal. Chem., 83, 7888-7894, 2011

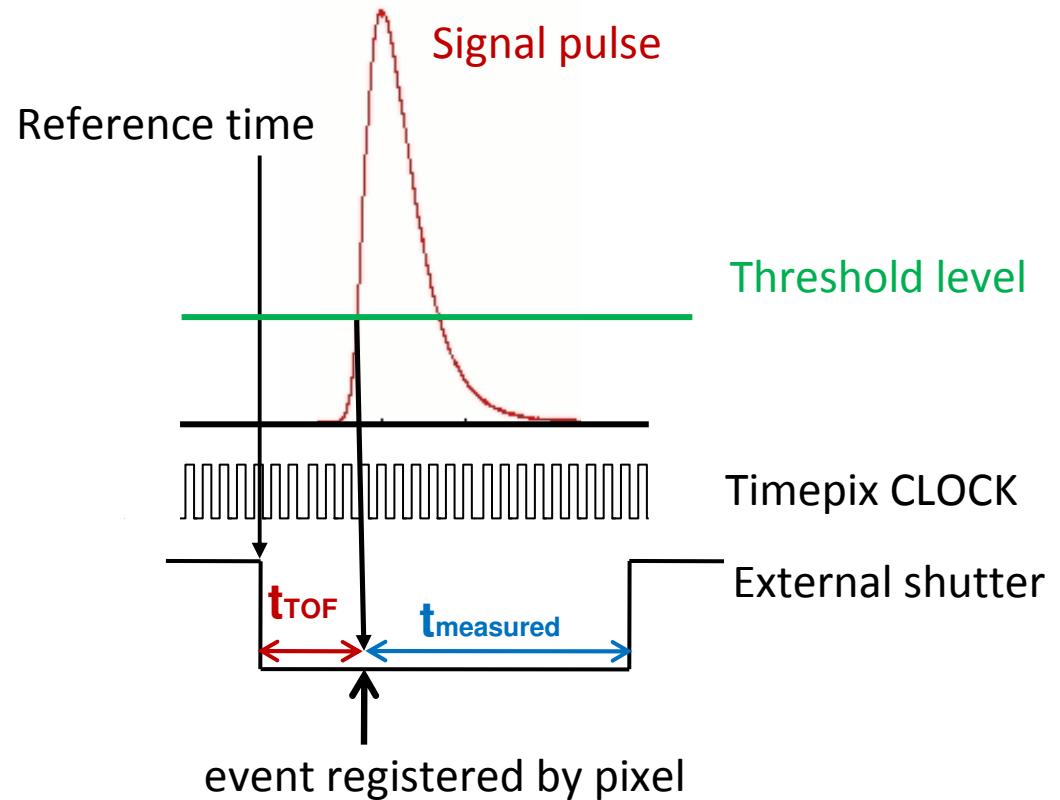
Jungmann et al., JASMS, 23, 1679-1688, 2012

Kiss et al., RSI, accepted

# Space- & Time-Resolved Ion Detection

*Medipix/ Timepix Detector Family*

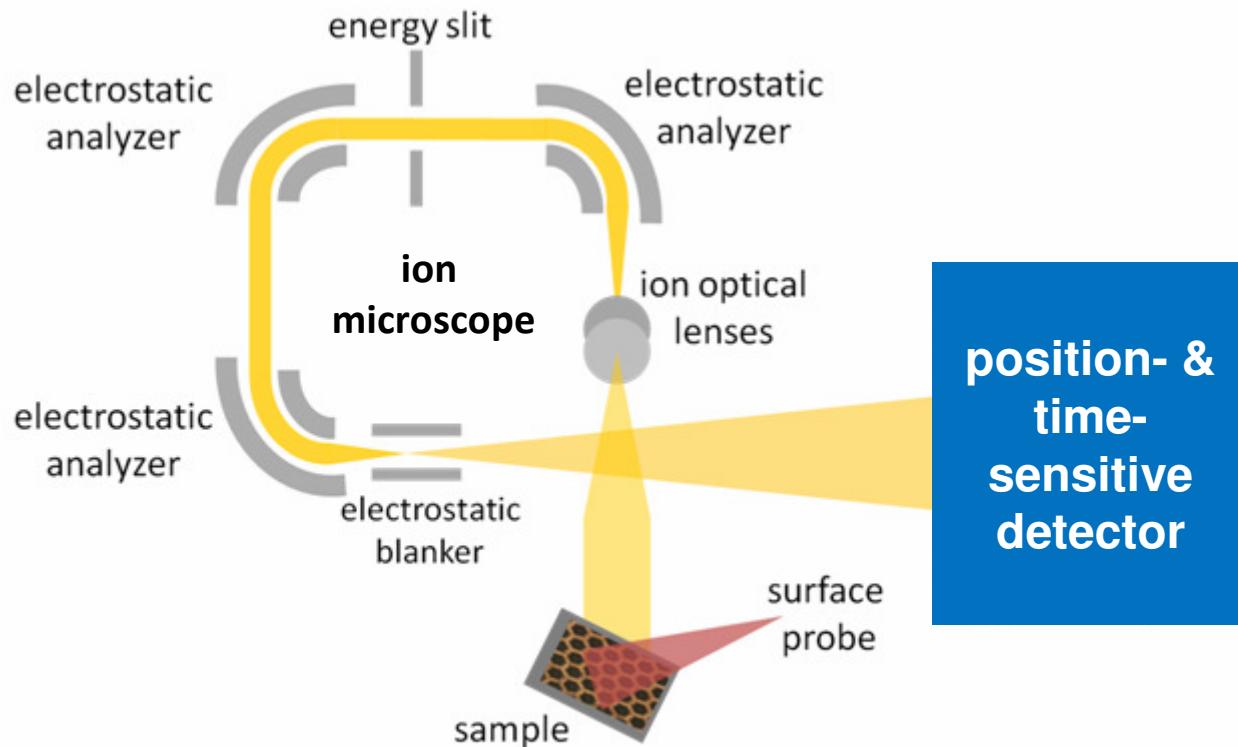
- 512 x 512 pixels  
→ **262 144 individual channels** for *parallel* detection
- Low noise levels
- On the pixel level:  
Time-of-flight measurement  
Time resolution: **10 ns**  
Maximum measurement interval: **118 µs**



$$t_{\text{TOF}} = t_{\text{external shutter}} - t_{\text{measured}}$$

# Overview Experimental Setup

## *Time-of-Flight Ion Microscope Mass Spectrometer*



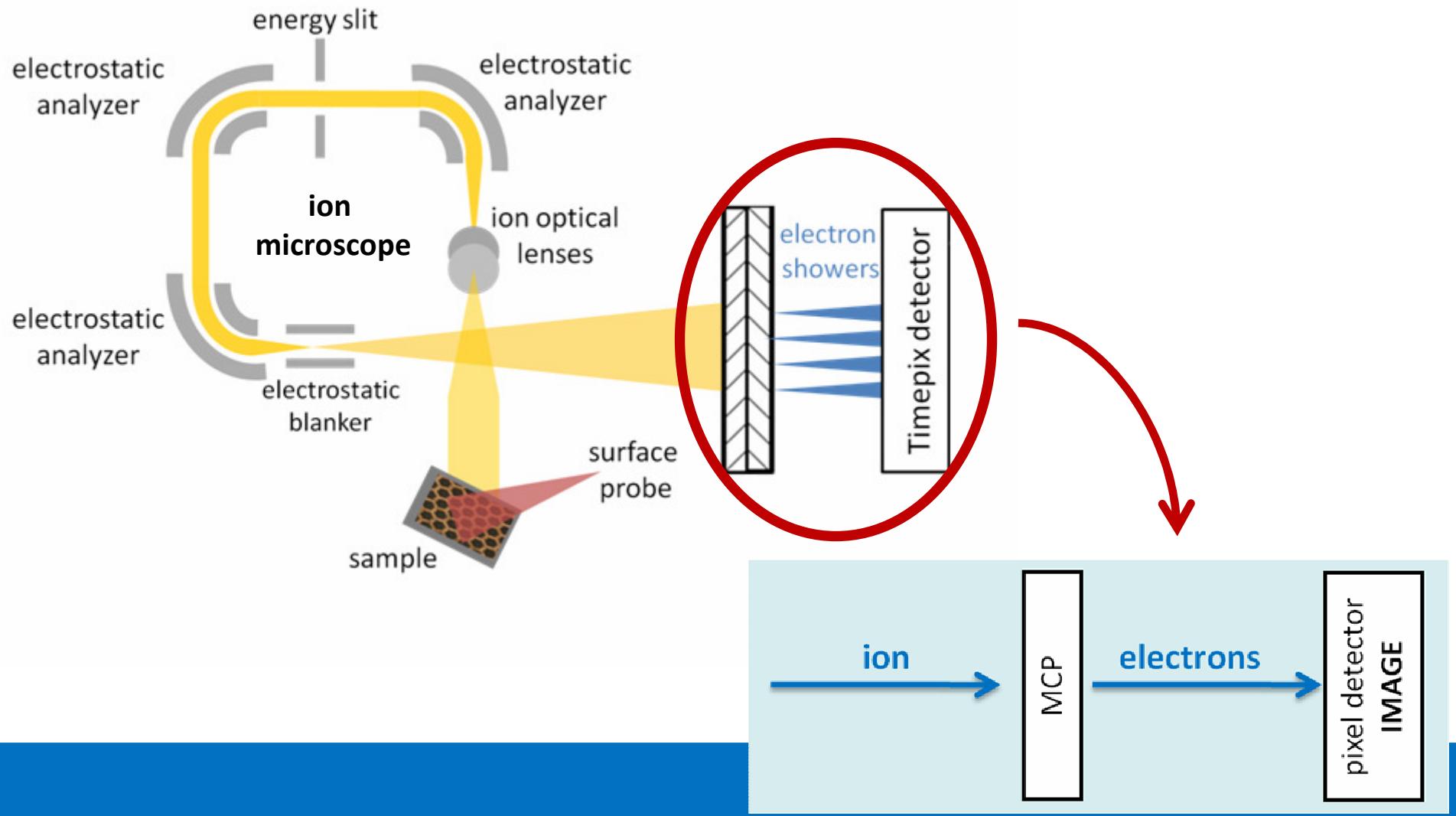
position- &  
time-  
sensitive  
detector

### **Ion microscope**

- Magnifies the ion image  
→ Higher spatial resolution !

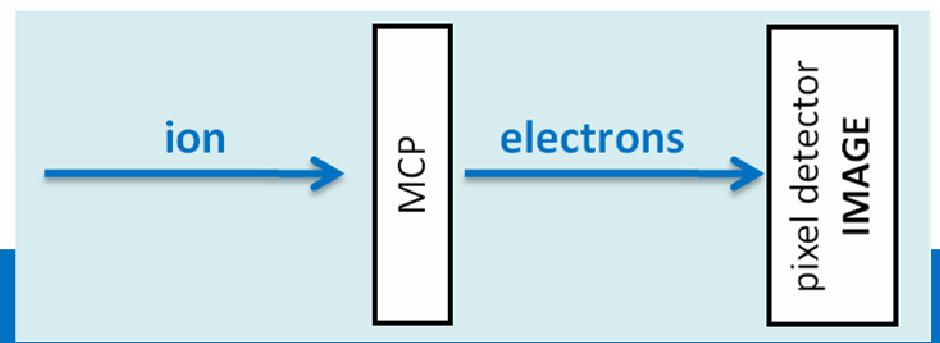
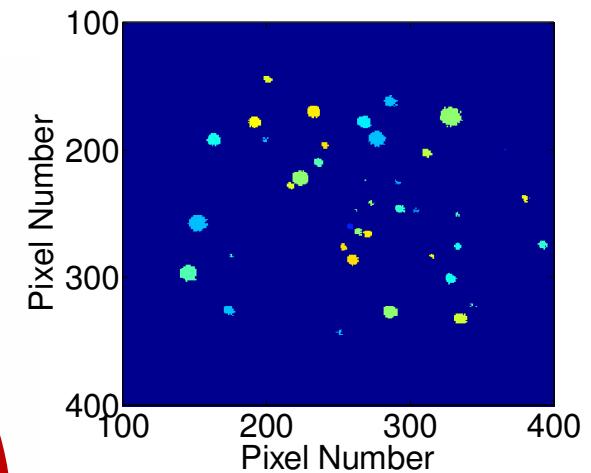
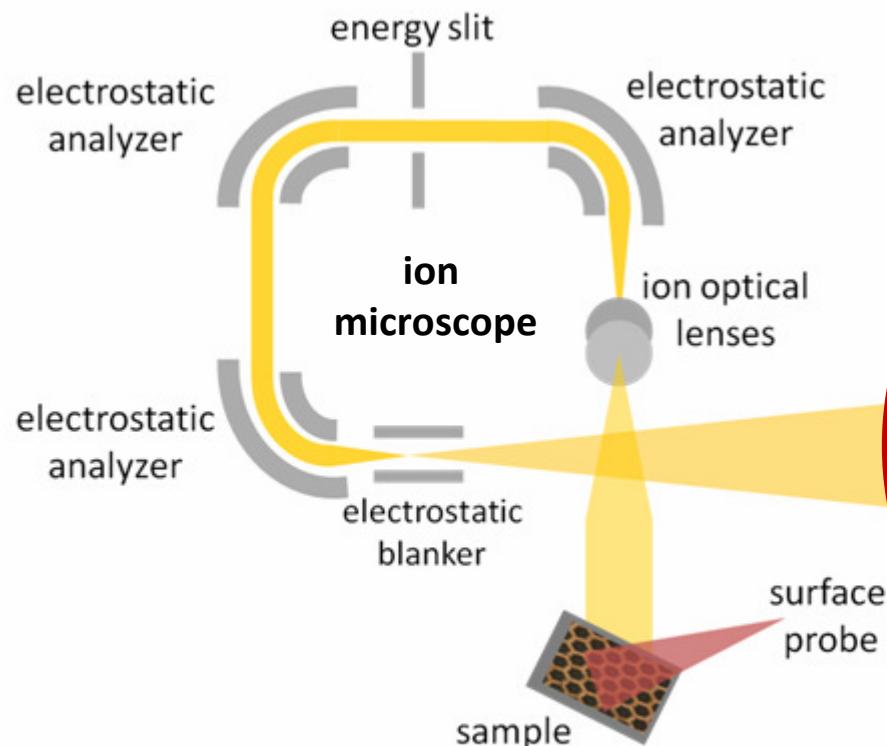
# Overview Experimental Setup

*Time-of-Flight Ion Microscope Mass Spectrometer*



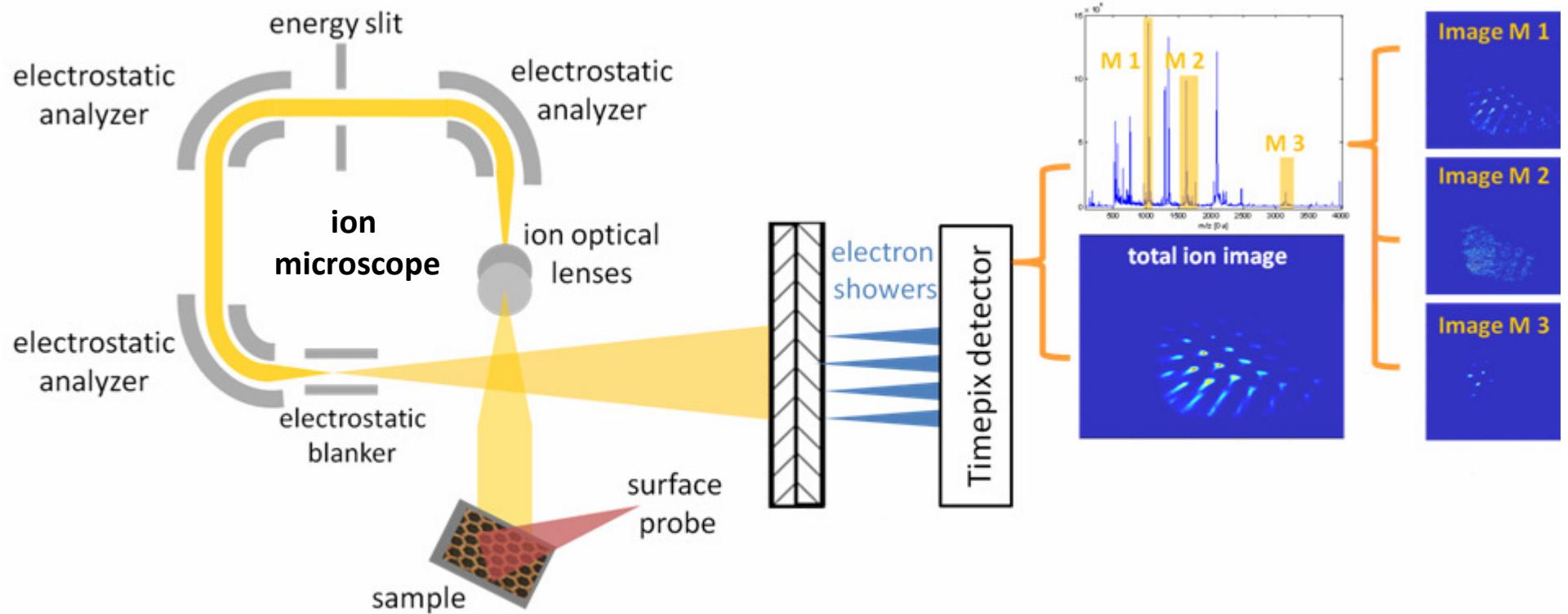
# Overview Experimental Setup

## *Time-of-Flight Ion Microscope Mass Spectrometer*



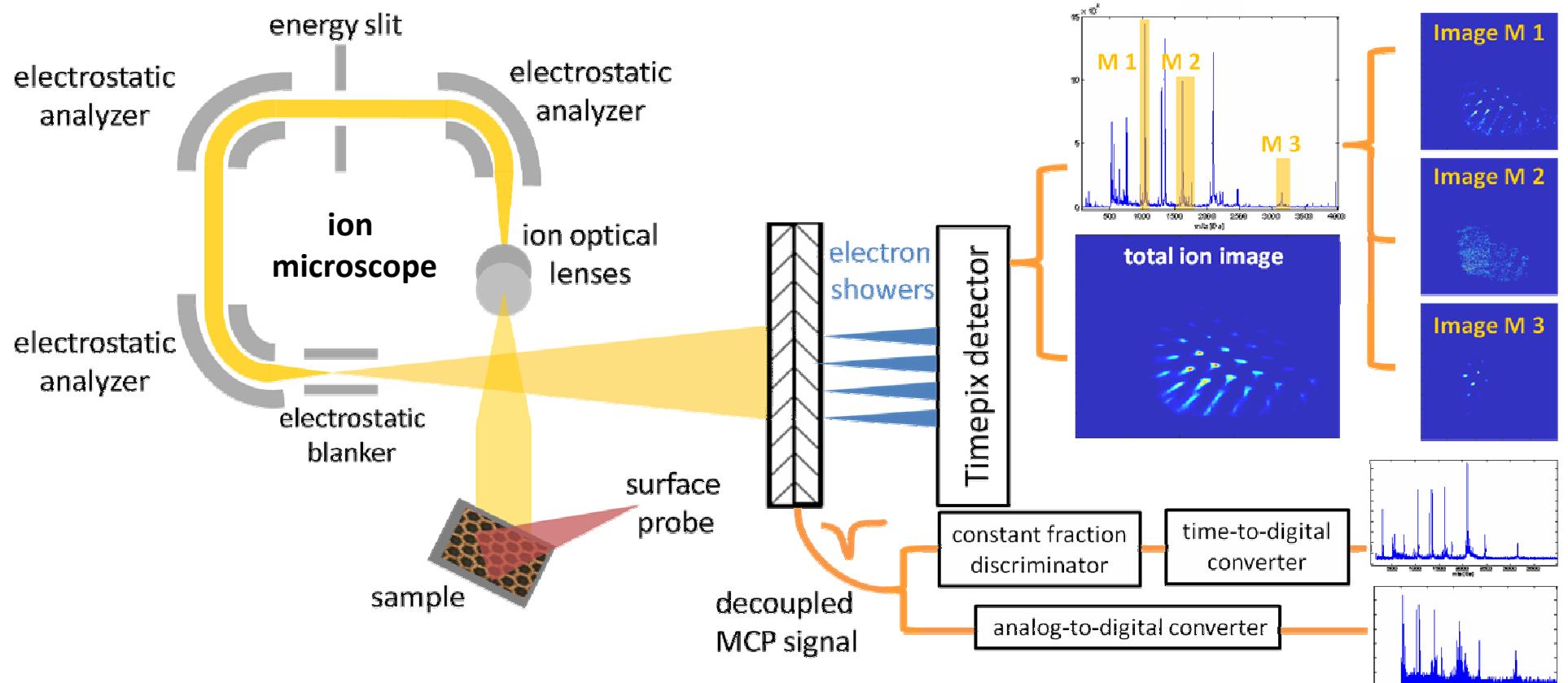
# Overview Experimental Setup

## *Time-of-Flight Ion Microscope Mass Spectrometer*



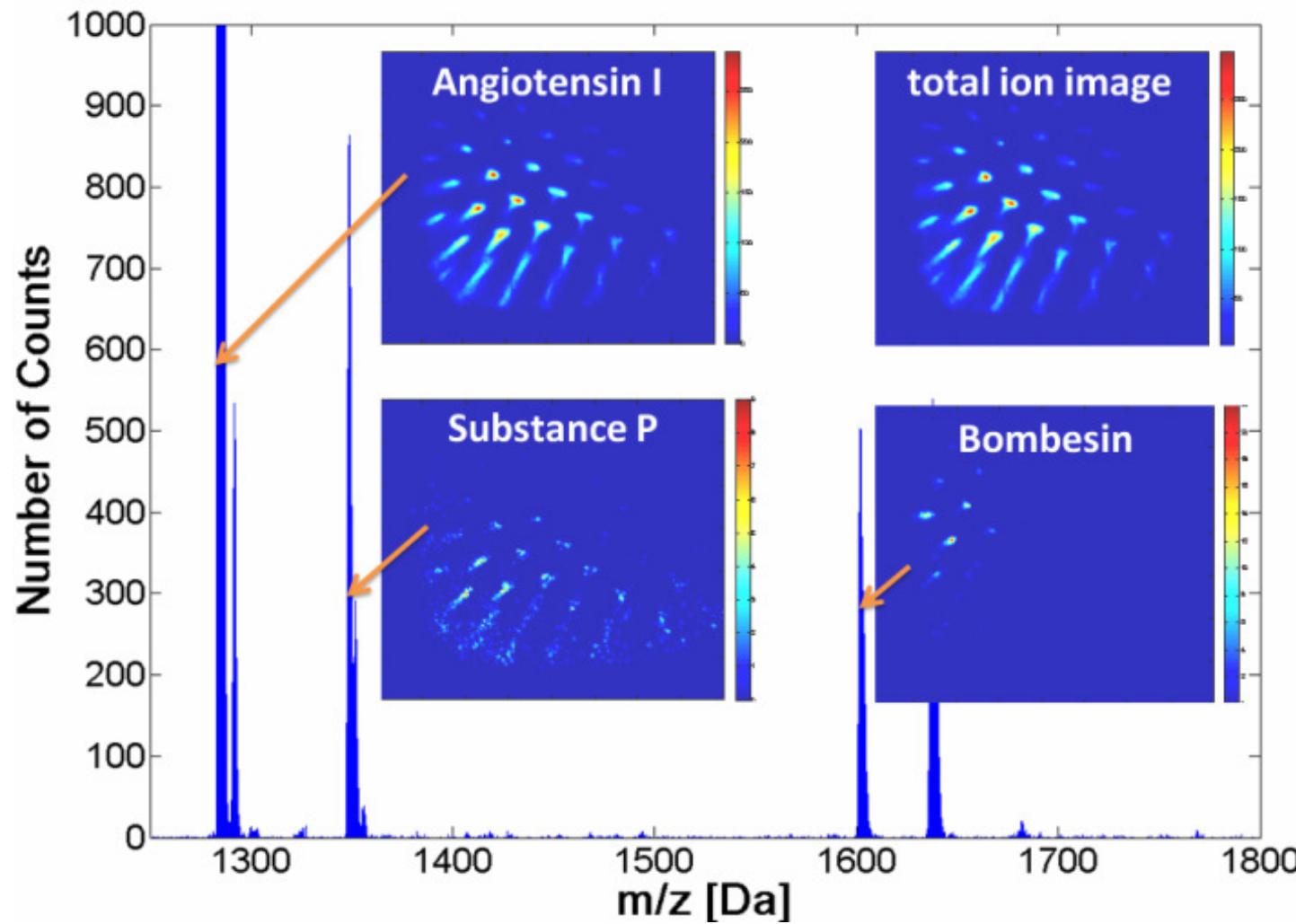
# Overview Experimental Setup

## *Time-of-Flight Ion Microscope Mass Spectrometer*



# MSI Experiment Using the Timepix

*Identification and Localization Possible*

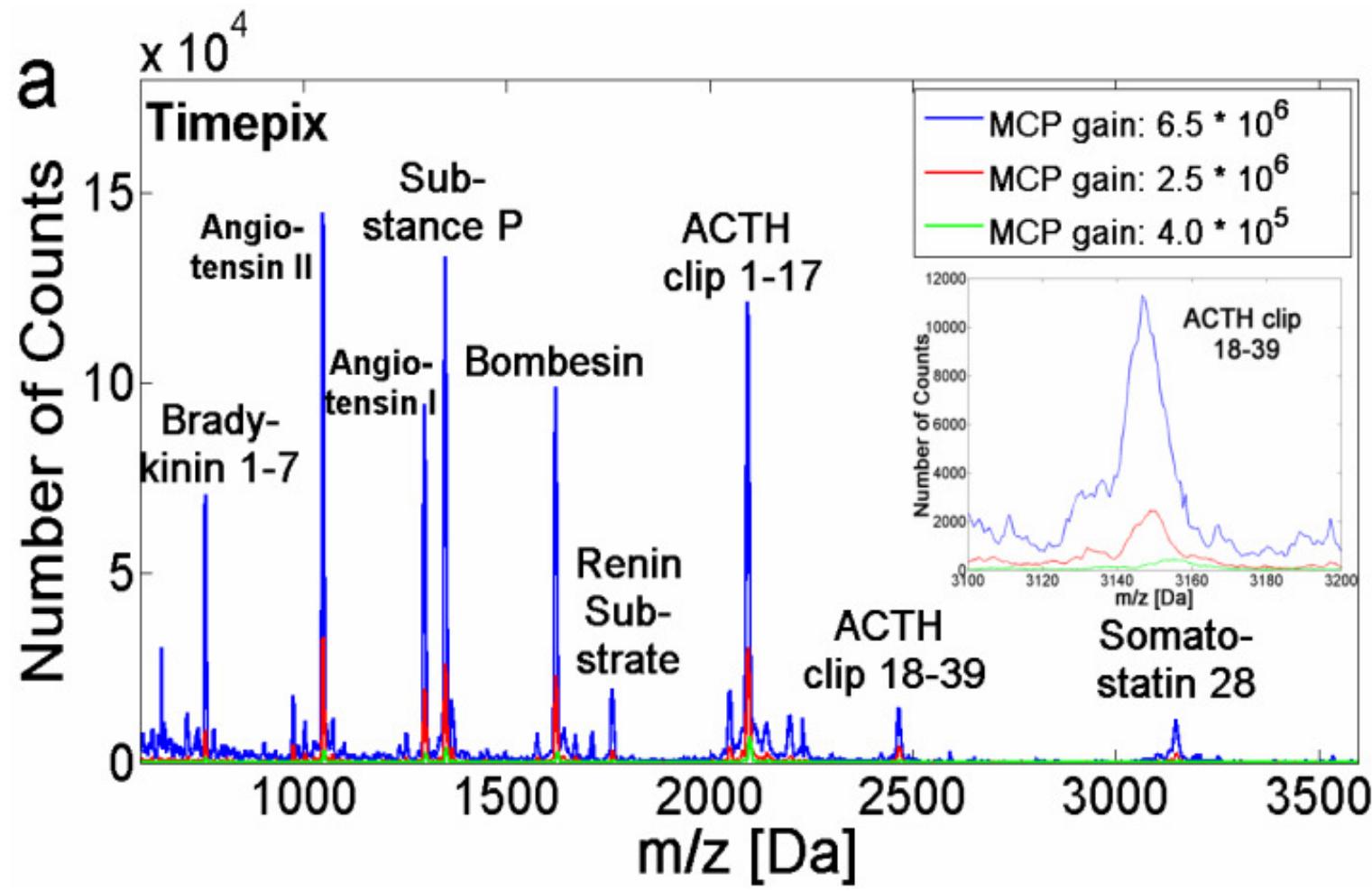


- grid on top peptide standard
- 1000 laser shots
- one single measurement
- ion optical distortion

Jungmann et al., Anal. Chem., 83, 20, 7888–7894, 2011

# Total Ion Spectrum

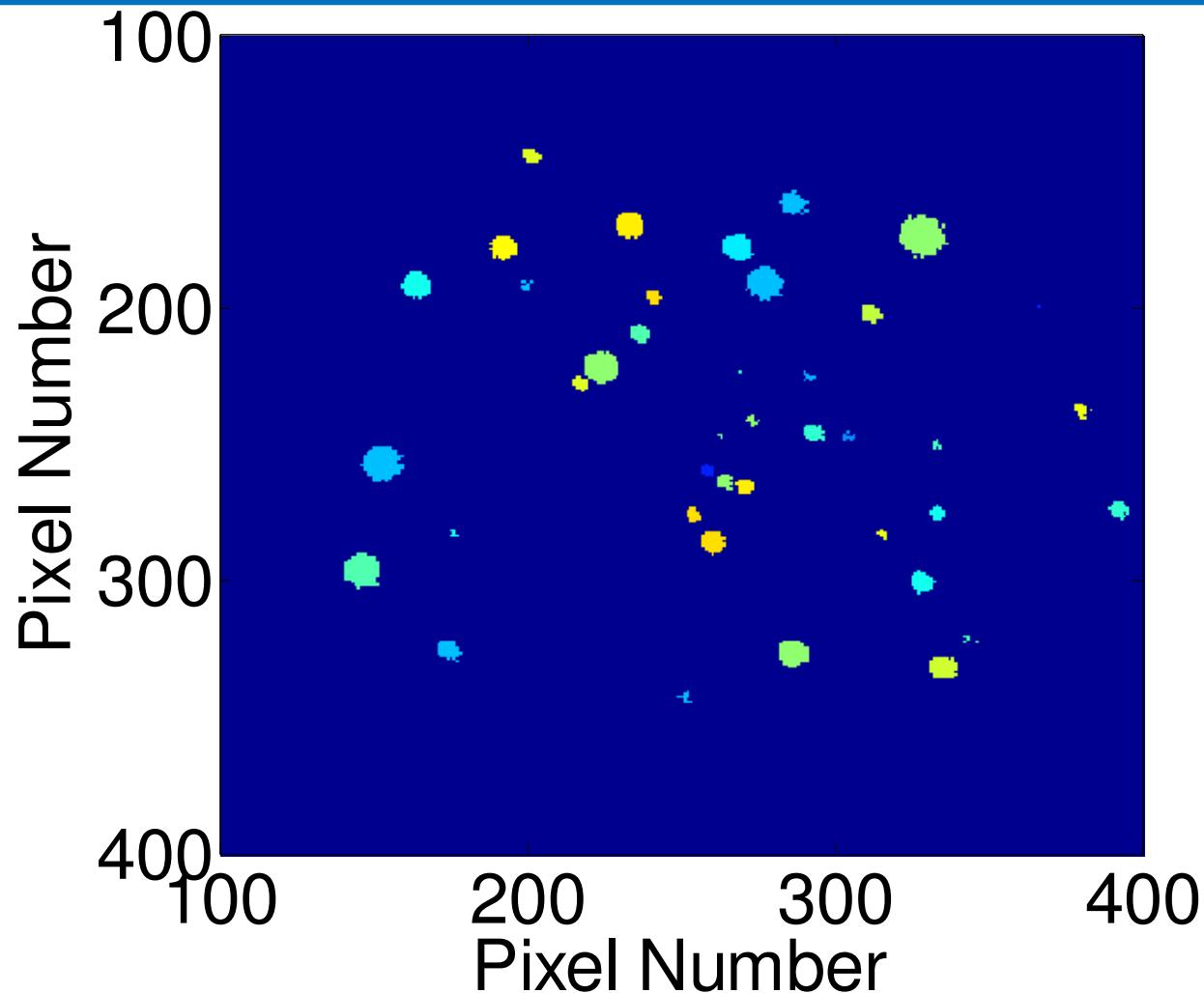
*Function of MCP Gain*



→ mass range in one Timepix measurement window

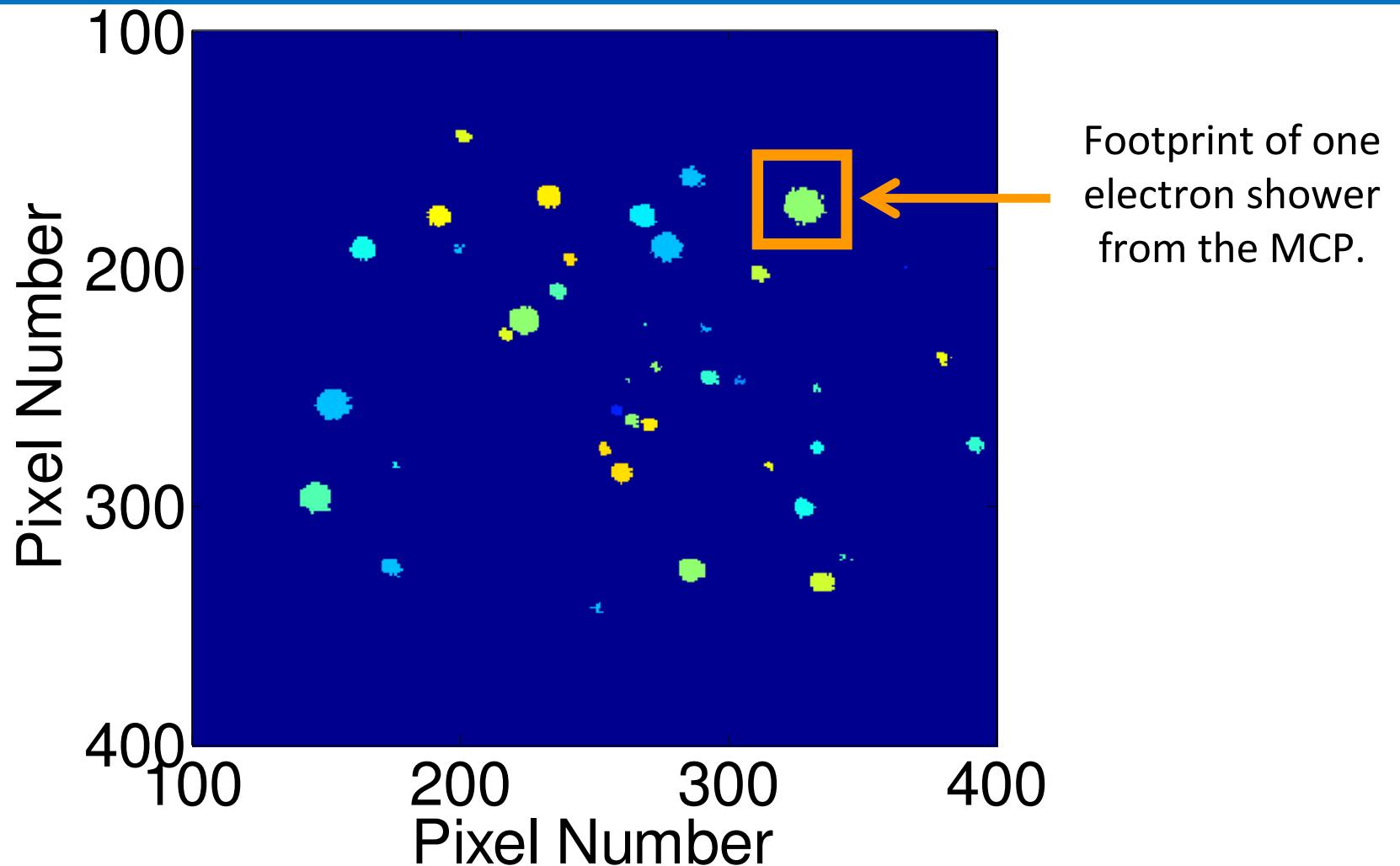
# Ion Detection with the Timepix

*What Does the Data Look Like ?*



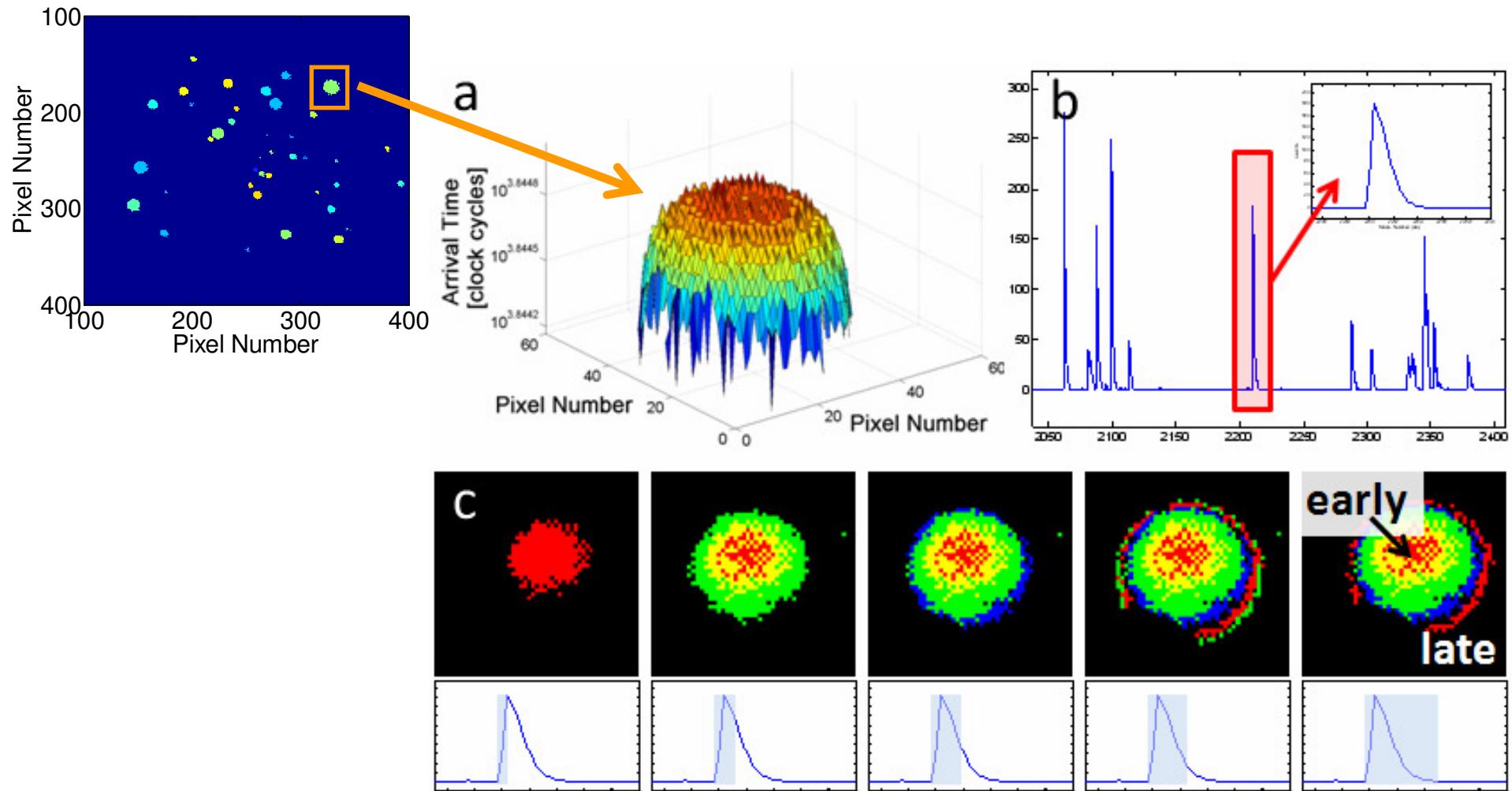
# Ion Detection with the Timepix

*What Does the Data Look Like ?*

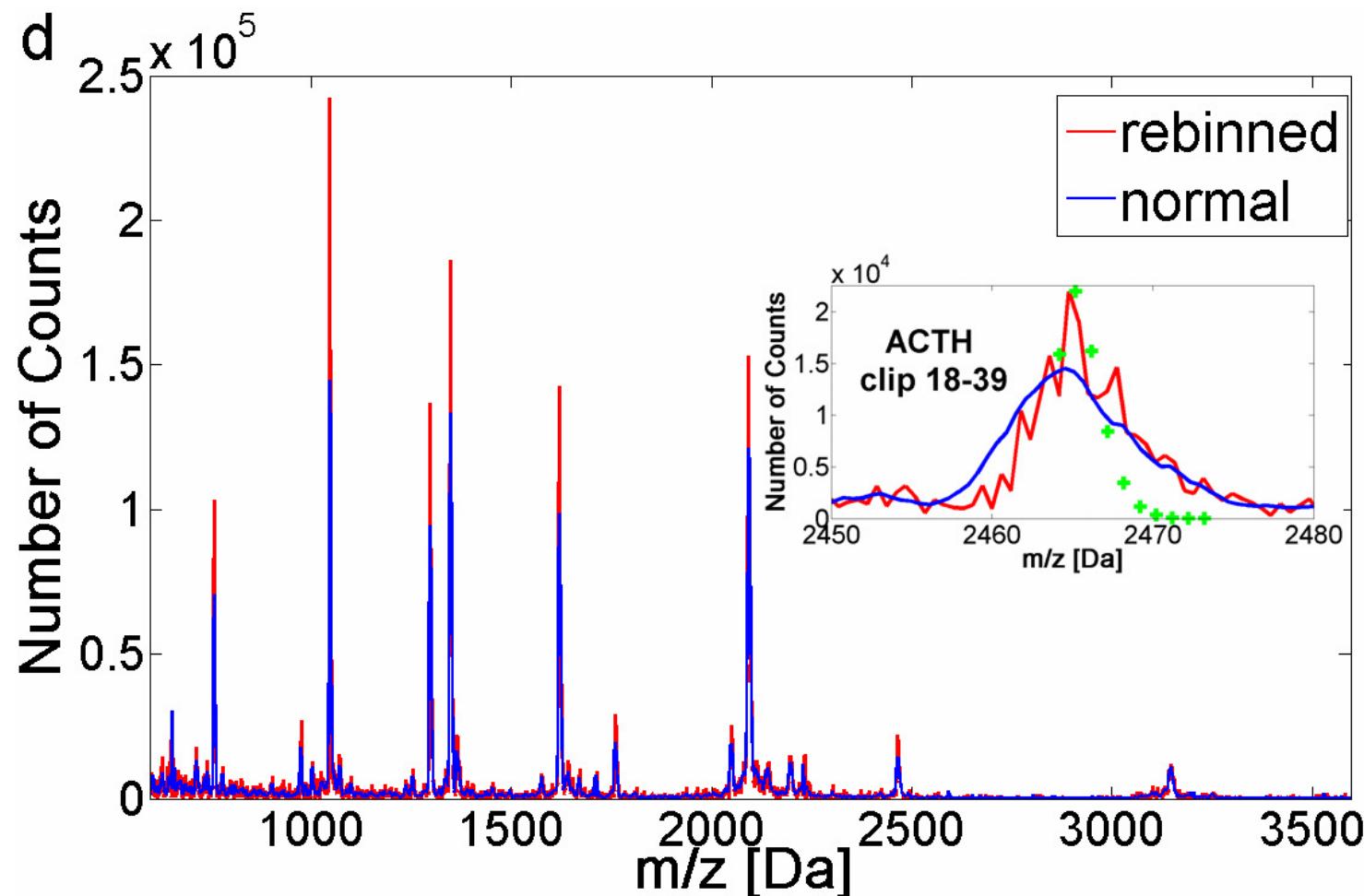


# Ion Detection with the Timepix

*What Does the Data Look Like ?*



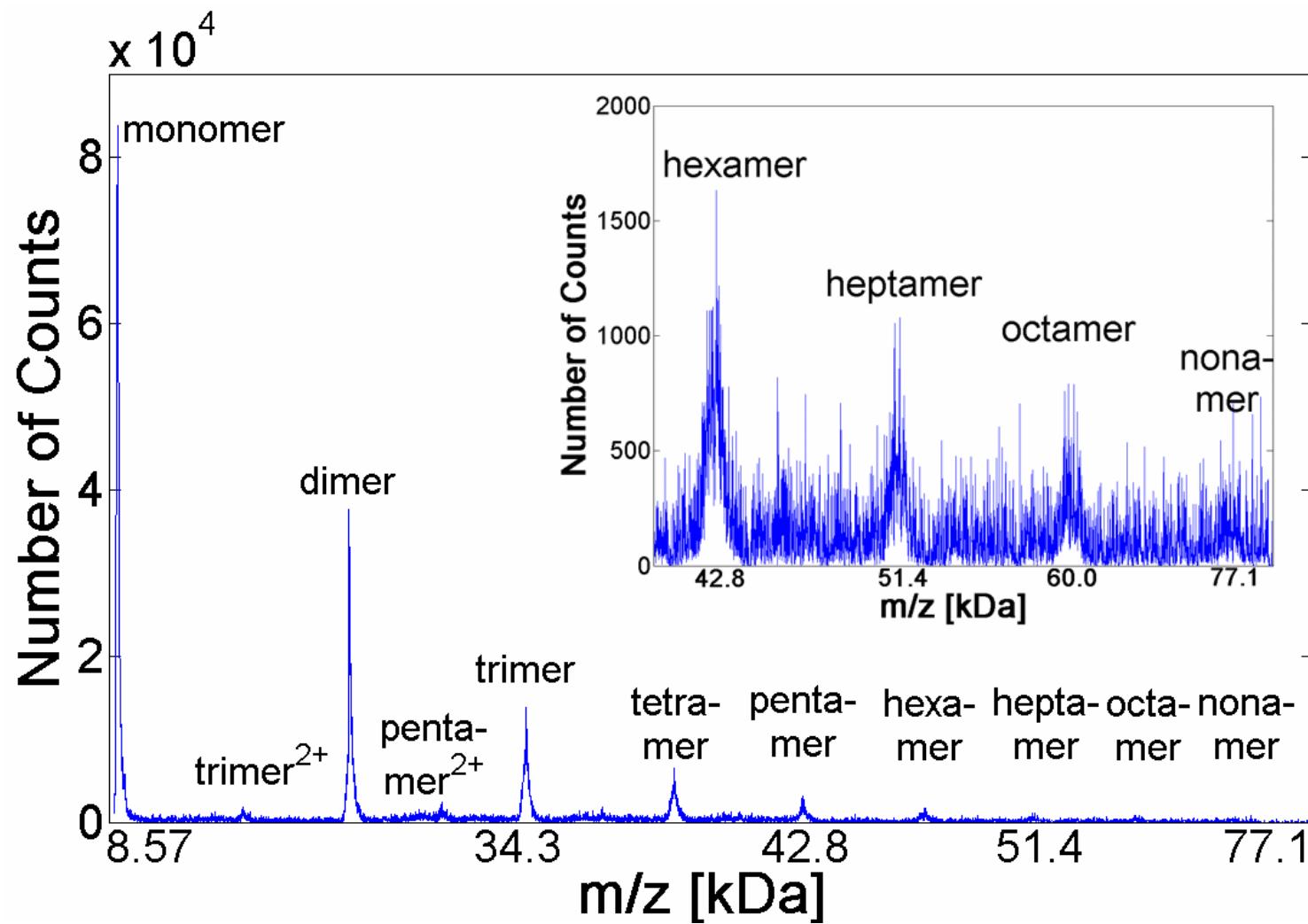
# Improvement of Mass Resolution



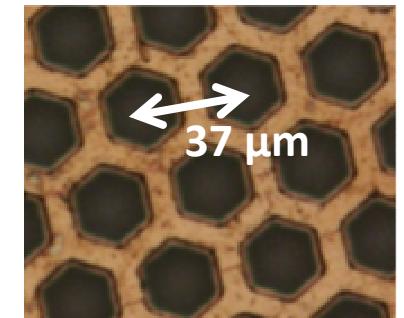
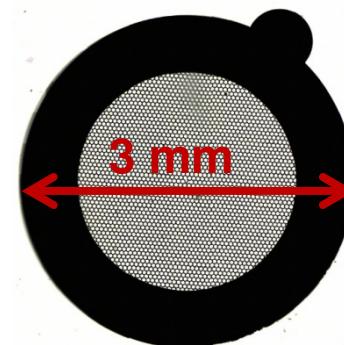
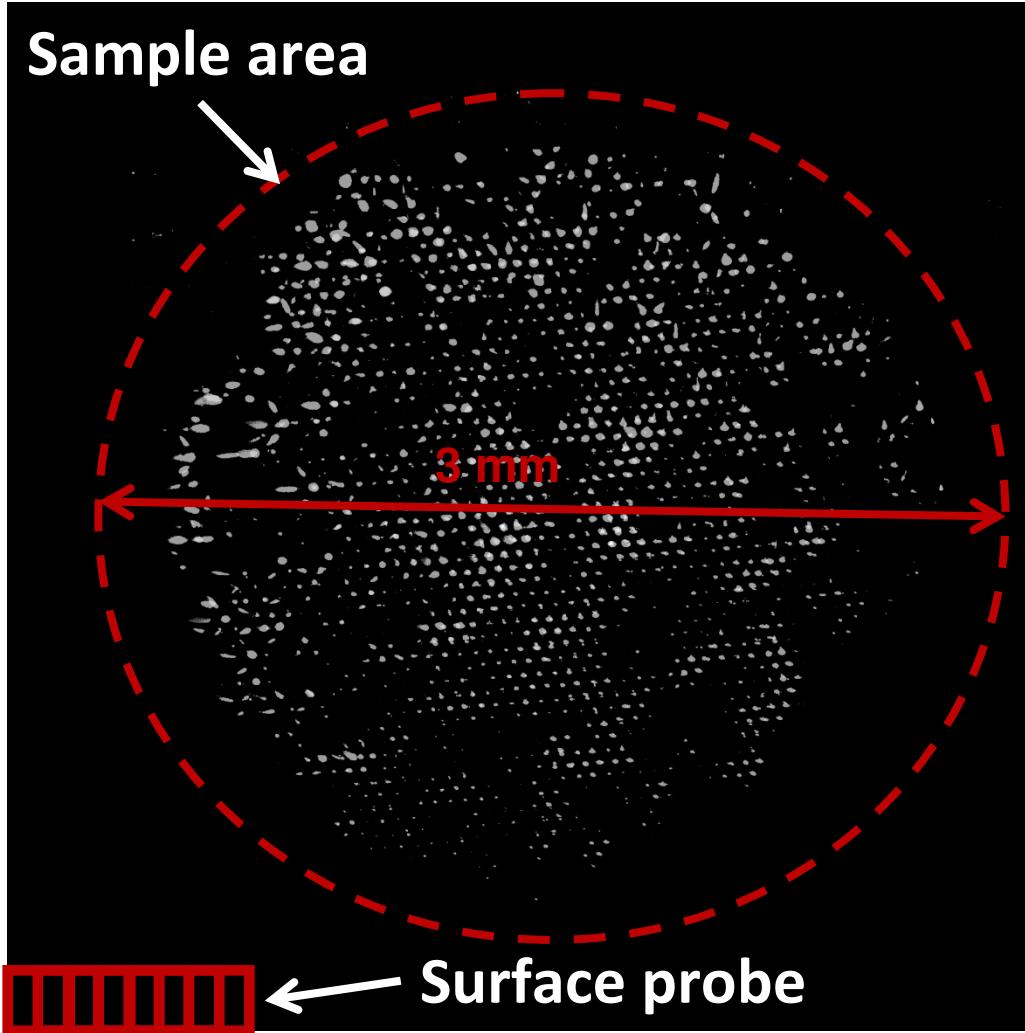
→ Mass resolution only limited by 10 ns pixel clock.

# Accessible Mass Range on Our Ion Microscope

*Ubiquitin to 78 kDa*

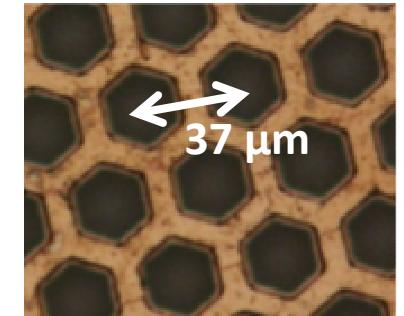
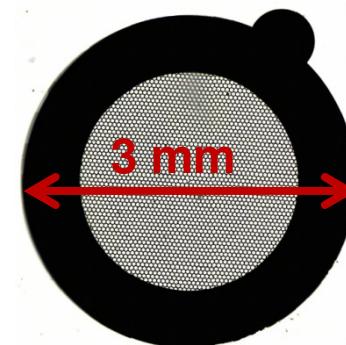
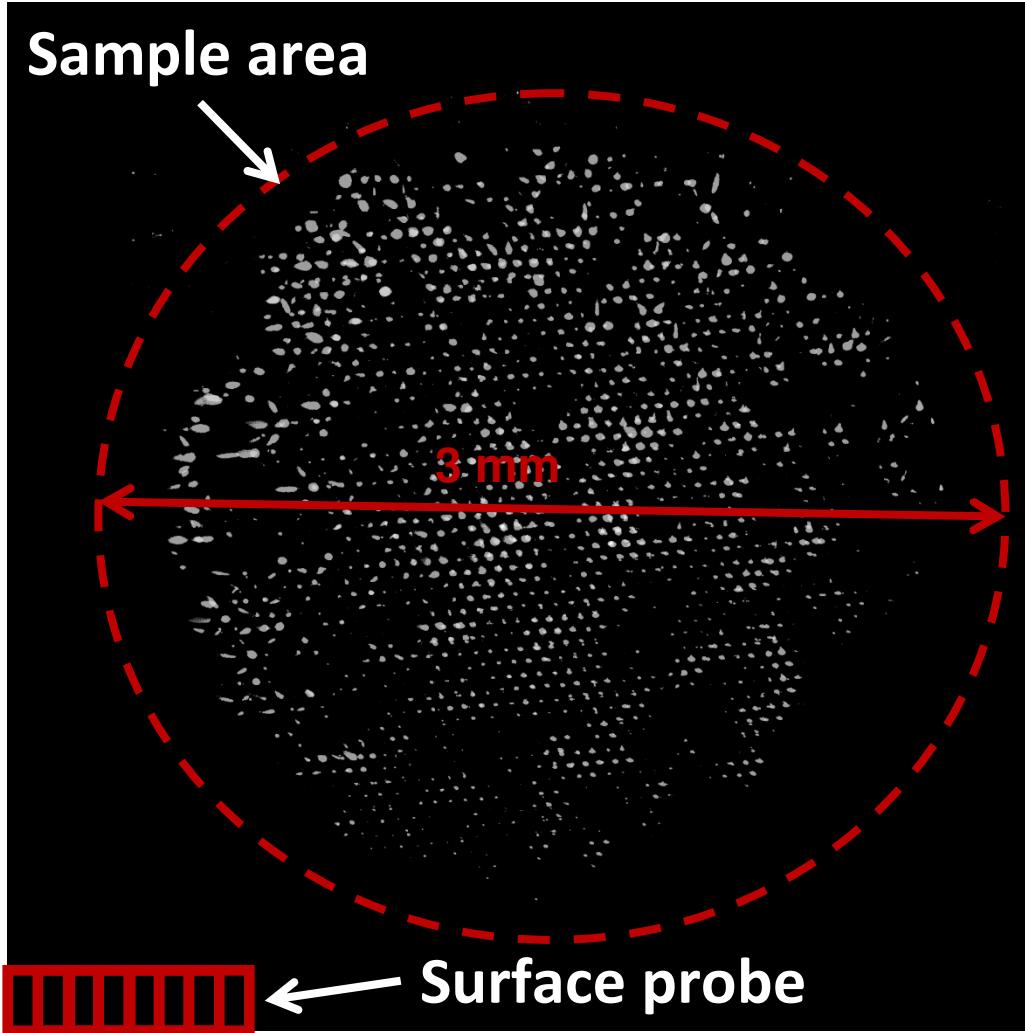


# Large-Area MSI Established on Standards



- Grid on top peptide standard
  - mosaic acquisition
  - 100 laser shots per position
  - laser spot size:  $\phi = 200 \mu\text{m}$
  - step size:  $100 \mu\text{m}$
- Overlap between successive images !

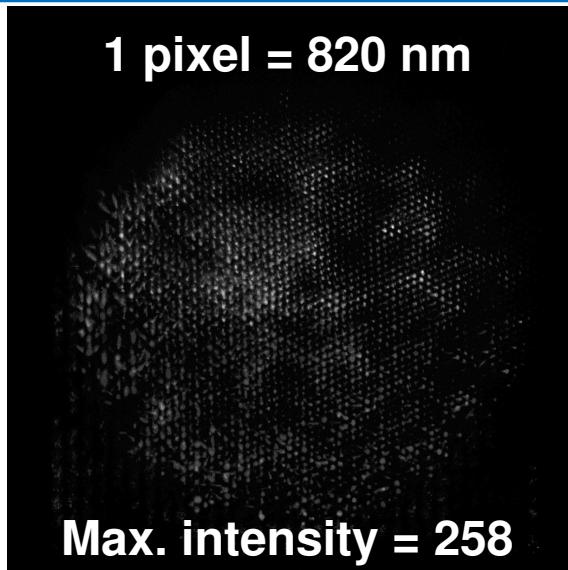
# Large-Area MSI Established on Standards



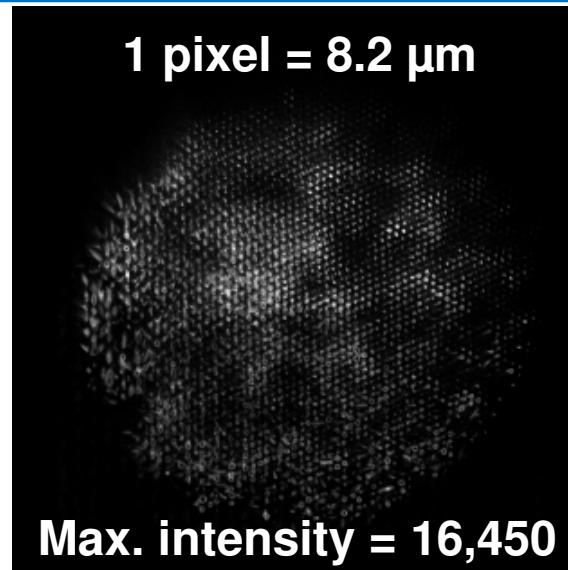
- Grid on top peptide standard
  - mosaic acquisition
  - 100 laser shots per position
  - laser spot size:  $\phi = 200 \mu\text{m}$
  - step size:  $100 \mu\text{m}$
- Overlap between successive images !

# Microscope versus Microprobe Imaging

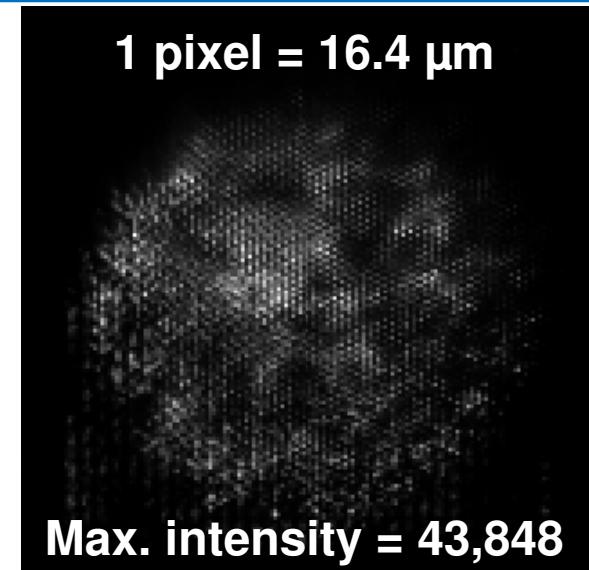
1 pixel = 820 nm



1 pixel = 8.2 μm



1 pixel = 16.4 μm

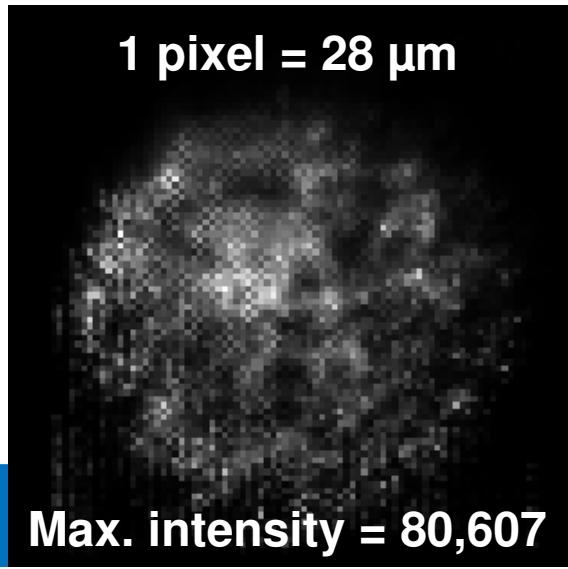


Max. intensity = 258

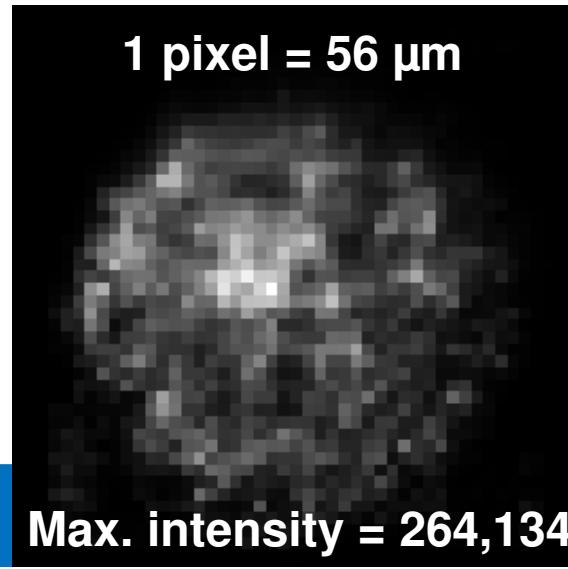
Max. intensity = 16,450

Max. intensity = 43,848

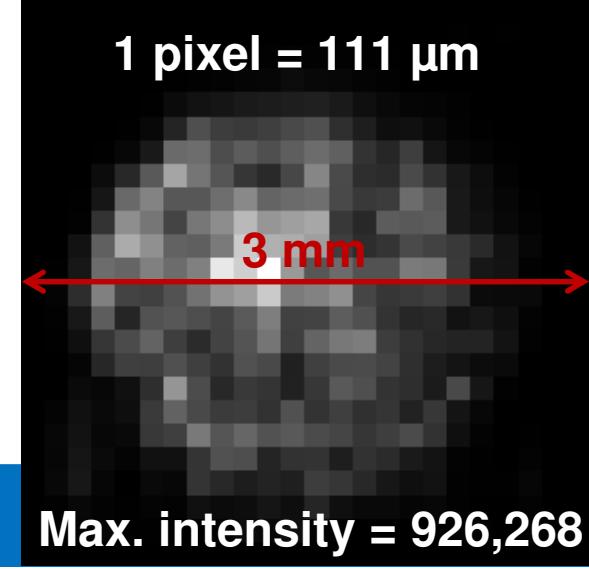
1 pixel = 28 μm



1 pixel = 56 μm



1 pixel = 111 μm



Max. intensity = 80,607

Max. intensity = 264,134

Max. intensity = 926,268

# Microscope versus Microprobe Imaging

1 pixel = 820 nm

1 pixel = 8.2  $\mu$ m

1 pixel = 16.4  $\mu$ m

At a spatial resolution of 1  $\mu$ m:

Max.

1

43,848

$\mu$ m

Microscope mode (10 Hz): < 3 hours

Microprobe mode (1 kHz): 10 days

Max. intensity = 80,607

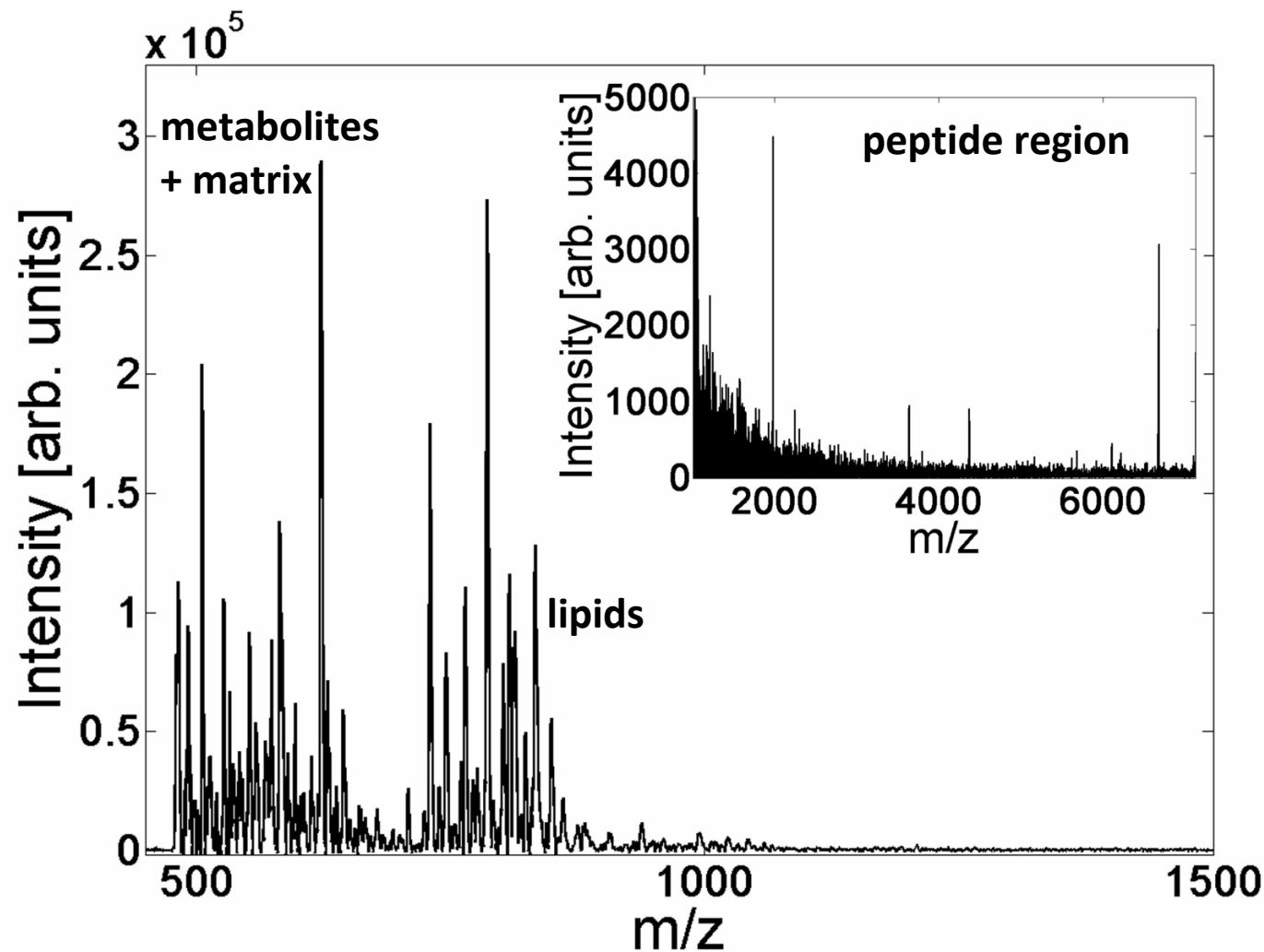
Max. intensity = 264,134

Max. intensity = 926,268

# Experiments on Mouse Testis

*Mass Spectrum*

MALDI

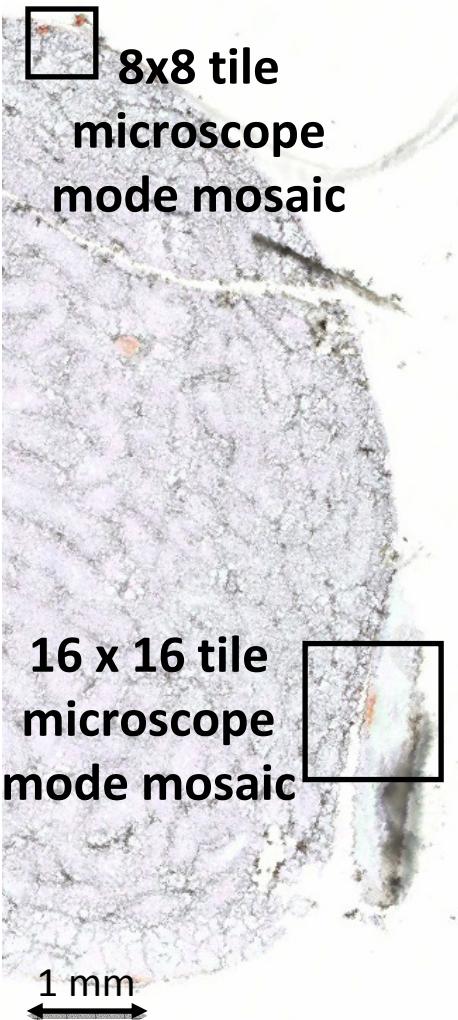


Biological samples: *Physiological* concentrations!

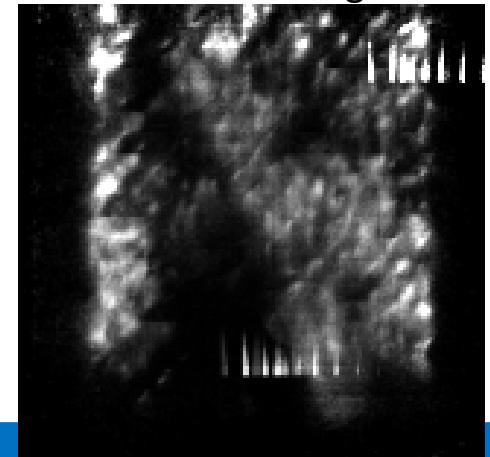
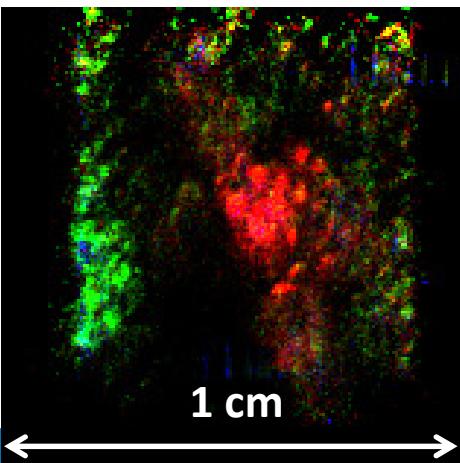
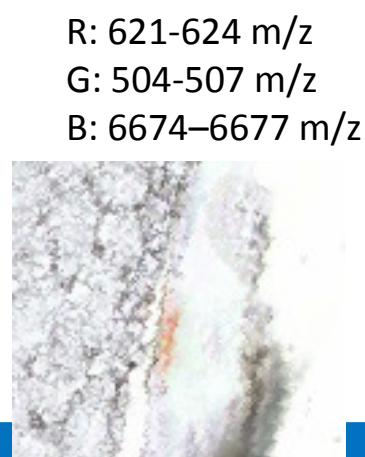
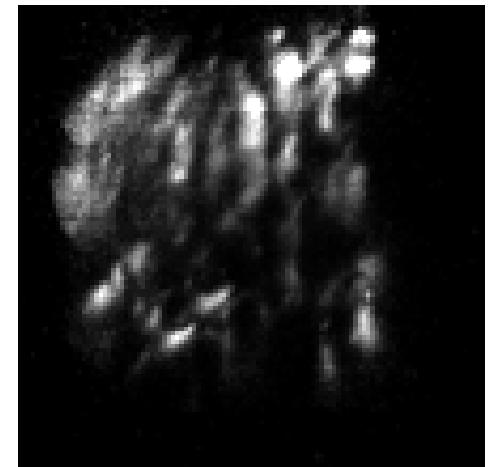
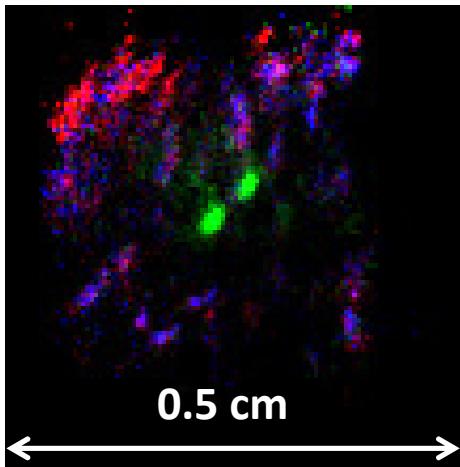
1 bin = 1 Da.

# Experiments on Mouse Testis

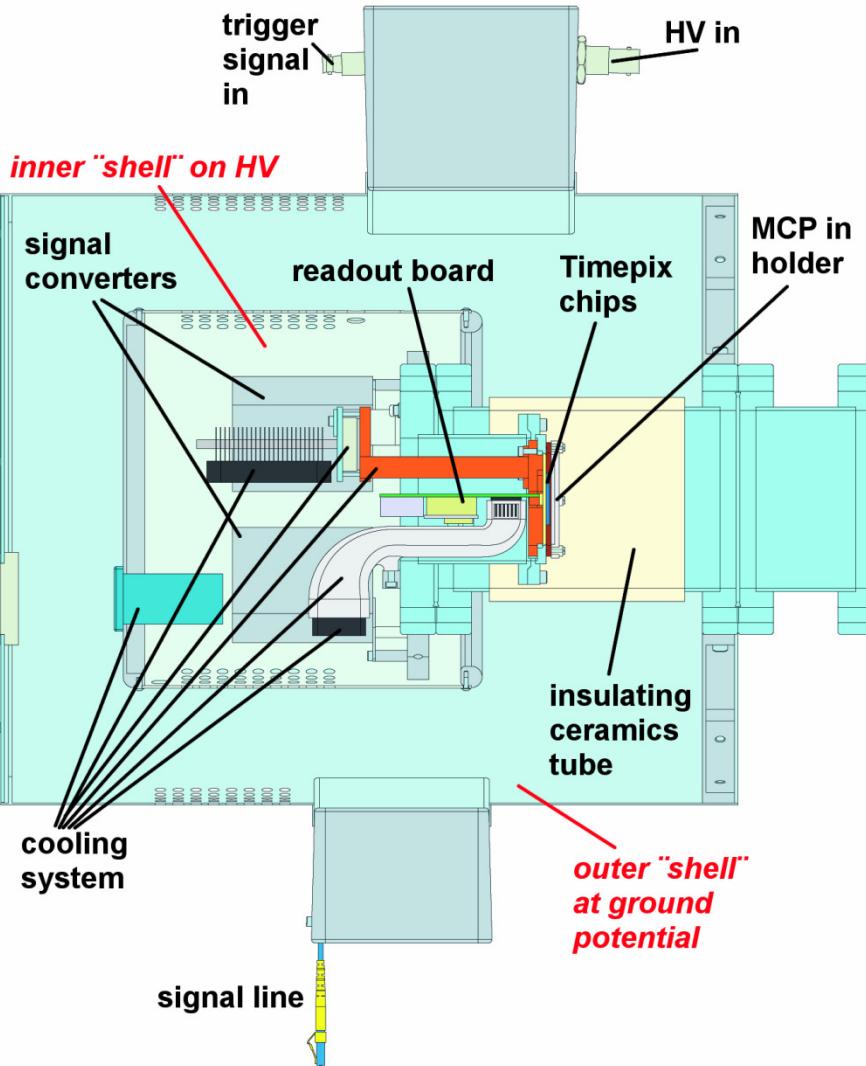
*MS Images*



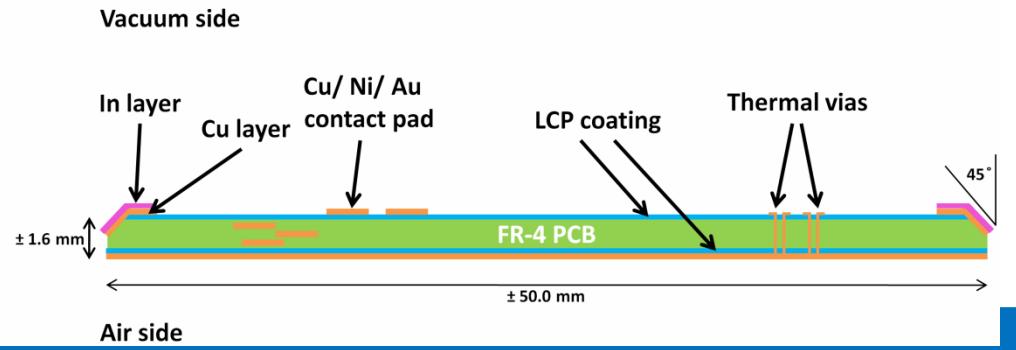
R: 621-624 m/z  
G: 595-599 m/z  
B: 491-495 m/z



# The High Voltage Setup



- Timepix-based detection system
- UHV compatible
- 2 versions: simple & HV
- HV: positive/negative ion mode

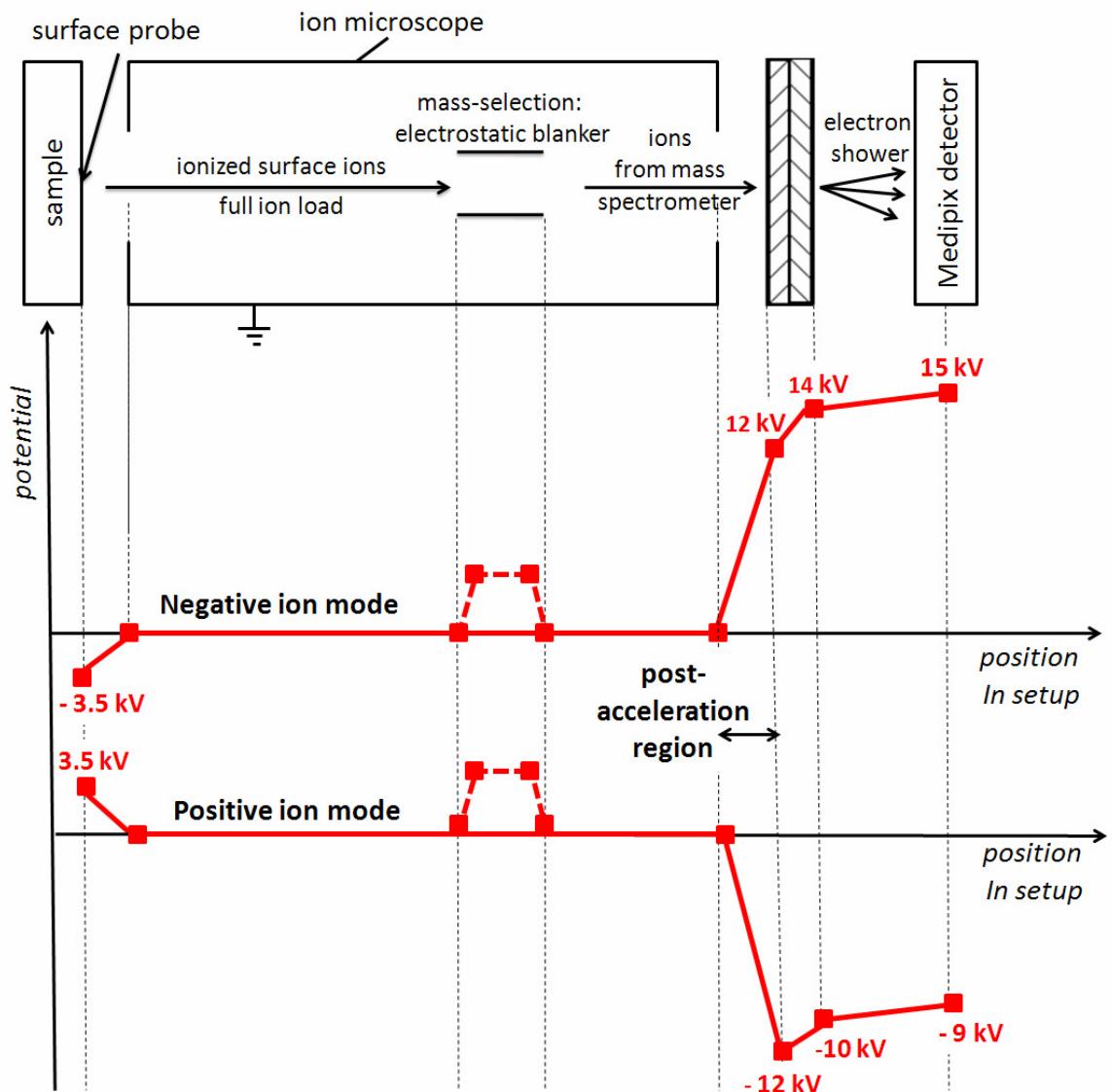


# High Voltage Project: Detect Large Biomolecules

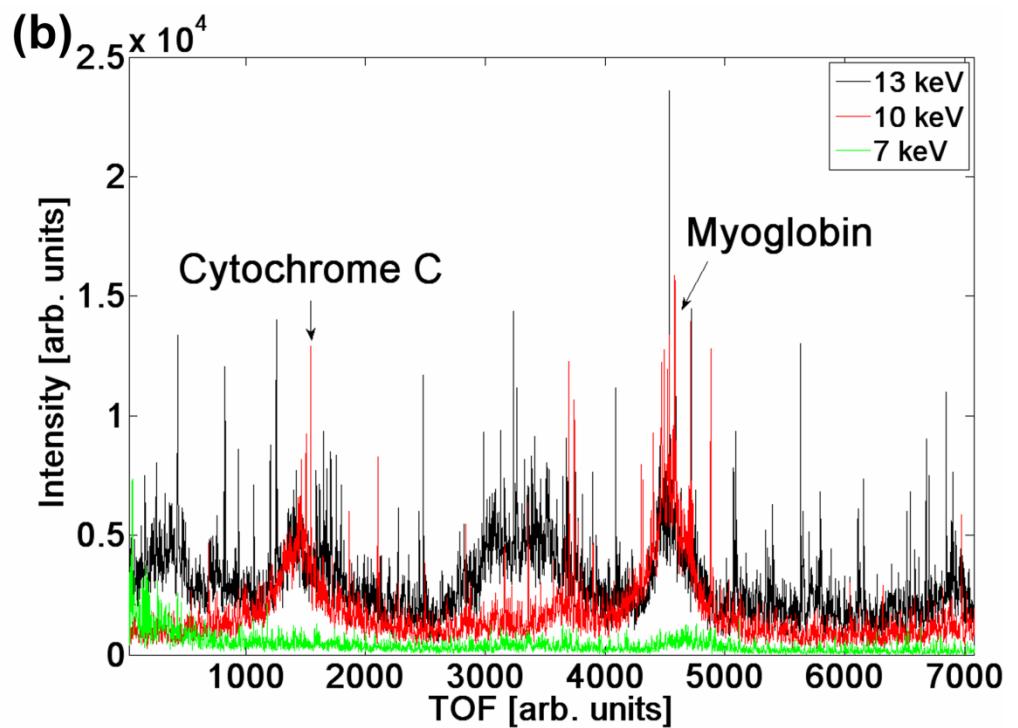
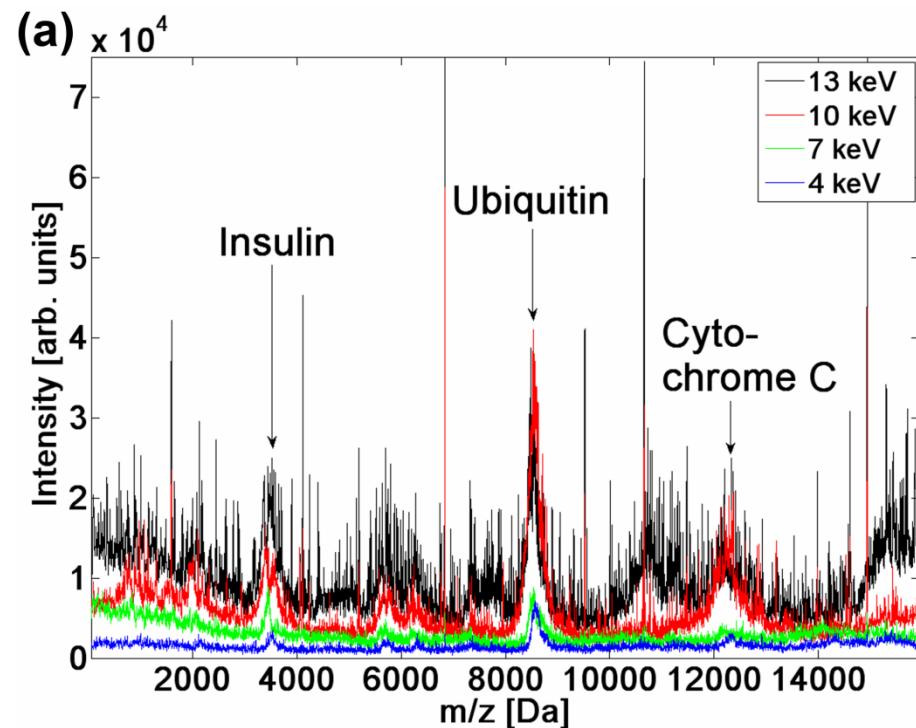
Previously:  
Timepix at ground  
potential

Now:  
- *positive* and *negative*  
ion mode  
- Timepix at HV

→ Larger acceleration  
voltage!

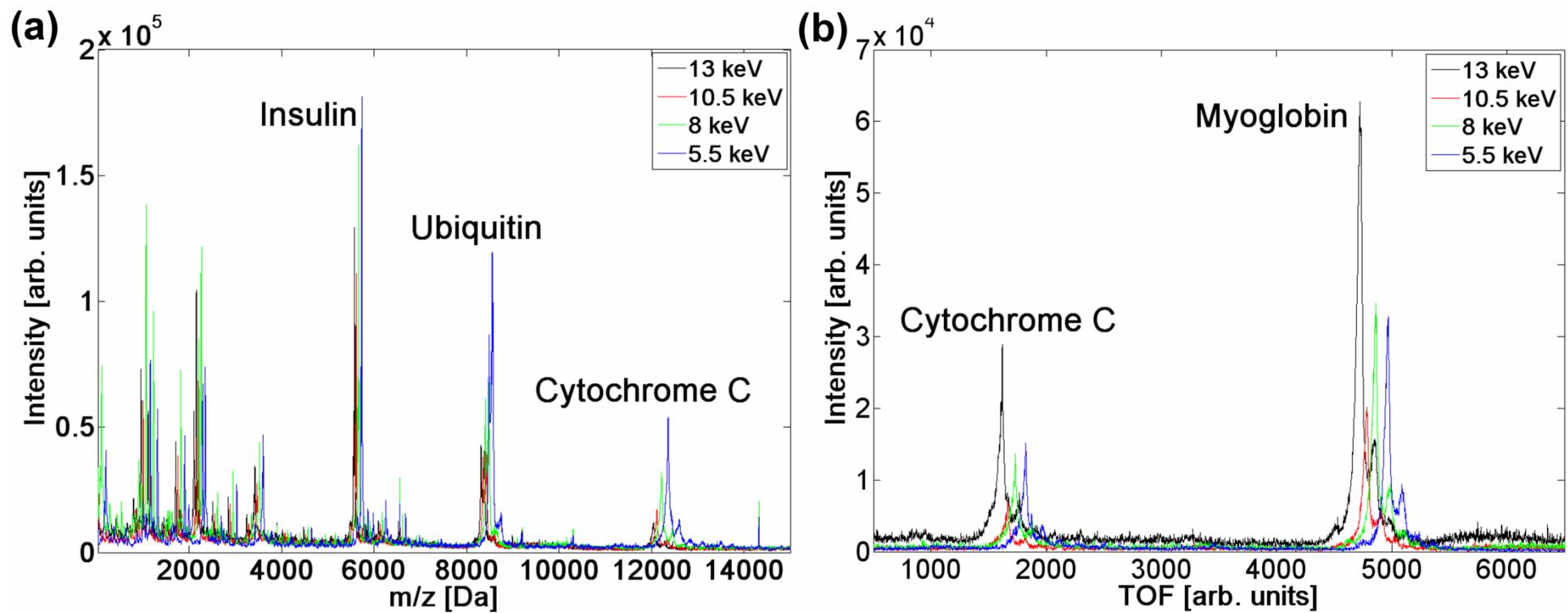


# First Results: Negative Ion Mode



Protein calibration standard

# First Results: Positive Ion Mode



Protein calibration standard

# What Can the Timepix Do for Us?



- *Space- and time-resolved* imaging using the Timepix
- Highly parallel detection: *high dynamic range*
- *Large-area imaging* works well on biologically relevant samples
- Analytes at *physiological concentrations* can be detected
- Tested for data handling, *spatial resolution*, dynamic range, mass resolution and mass range, ...
- *Wish list:* Chip with 100 ps time bins, 1 ms maximum measurement interval, multi-hit capabilities on pixel level, higher readout rate, high fluence tolerance



Thank you!

50μm

[www.amolf.nl/medipix](http://www.amolf.nl/medipix)

# Contact/Information

## **Heeren MSI Facility @ Amolf:**

Prof. Dr. Ron M. A. Heeren

FOM-Institute AMOLF

Science Park 104

1098 XG Amsterdam, The Netherlands

[heeren@amolf.nl](mailto:heeren@amolf.nl) · [www.amolf.nl/bims](http://www.amolf.nl/bims)

## **Contact Speaker:**

Paul Scherrer Institute

Swiss Light Source Detector Group

5232 Villigen PSI, Switzerland

[julia.jungmann@psi.ch](mailto:julia.jungmann@psi.ch)

50 μm