Recording

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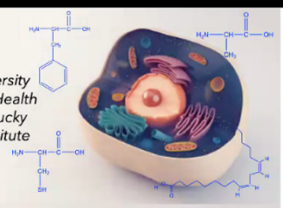
ONLINE WORKSHOP | FEBRUARY 26, 2021

SINGLE CELL METABOLOMICS WORKSHOP

ORGANIZED BY:

Rima Kaddurah-Daouk, *Duke University*
Jennifer Kirwan, *Berlin Institute of Health*
Andrew N. Lane, *University of Kentucky*
Mioara Larion, *National Cancer Institute*



PROGRAM

10:00 **Welcome and Introduction** Rima Kaddurah-Daouk, *Duke University*

Session I Chair: Mioara Larion, *National Cancer Institute*

10:05 **"Single cell metabolomics for biomedical and drug research"**
Thomas Hankemeier & Ahmed Ali, *University of Leiden*

10:35 **"High throughput metabolomics of individual cells in the brain"**
Jonathan Sweedler, *University of Illinois Urbana-Champaign*

11:05 **"Optical methodologies to characterize the metabolic underpinnings of breast cancer "**
Nimmi Ramanujam, *Duke University*

11:35 Break

Session II Chair: Jennifer Kirwan, *Berlin Institute of Health*

11:55 **"Towards super-resolution metabolic imaging using mass spectrometry imaging"**
Ian Gilmore, *National Physical Laboratory, London*

12:25 **"Integrative approaches to study cancer and immune cell metabolism"**
Shawn Davidson, *Princeton University*

General Discussion Chair: Jonathan Sweedler, *University of Illinois Urbana-Champaign*

12:55 Discussants: S. Davidson, I. Gilmore, T Hankemeier, I. Lanekoff, L-I. McCall, N. Ramanujam, J. Sweedler

Sponsored by:

METABOLOMICS SOCIETY

Metabolomics Association of North America

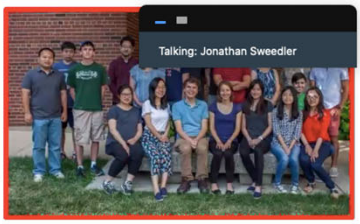
1

Jonathan Sweedler

UIUC

2

Acknowledgements



The Sweedler group
(2014 - 2021)

Collaborators

Rohit Bhargava
Paul Bohn
Charles Lee Cox
Jim Eberwine
Martha Gillette
Rhanor Gillette
Jian Jing
Neil Kelleher
Junhyong Kim
Jean Pierre Mothet
Leonid Moroz
Phil Newmark
Amynah Pradhan
Justin Rhodes
Sandra L. Rodriguez-Zas
Joshua Shrout

Funding from:
The UIUC / NIDA Neuroproteomics Center on Cell to Cell Signaling
NIH, NSF, DOE
American Diabetes Association

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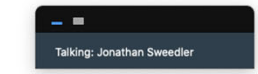
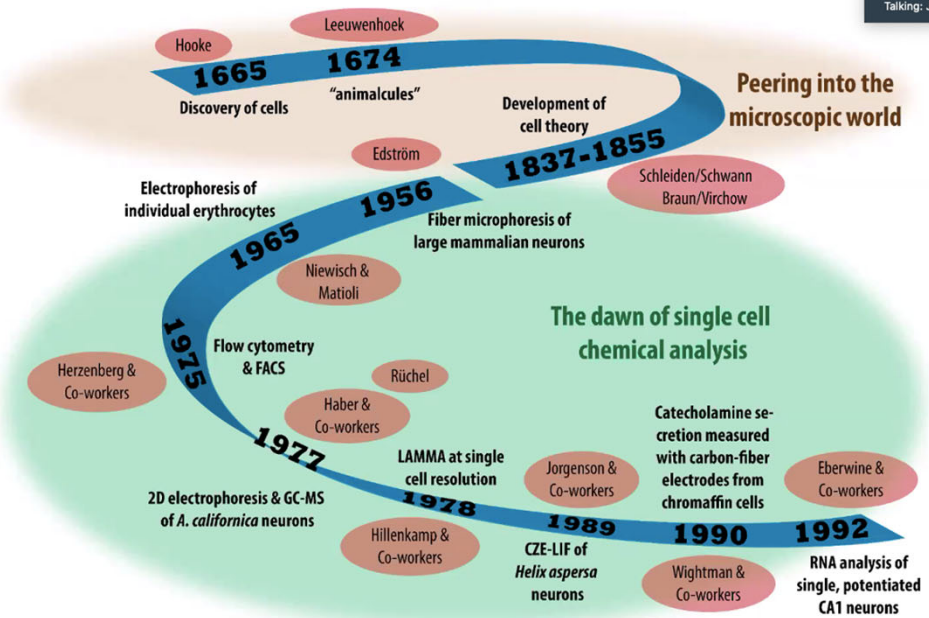
Moving to single cell measurements: a timeline





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Molecular Portrait of a single characterized neu
Single-cell analysis from RNAs to proteins

Talking: Jonathan Sweedler

RNAs

Proteins

Metabolites, Lipids, Signaling molecules

There are about >20,000 distinct lipids, >50,000 metabolites and 200,000 proteoforms reported to be in the brain! Many are not known

While mapping is advanced, the chemical characterization of the brain has lagged behind…

Image from Leonid Moroz, University of Florida

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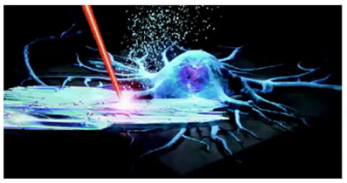
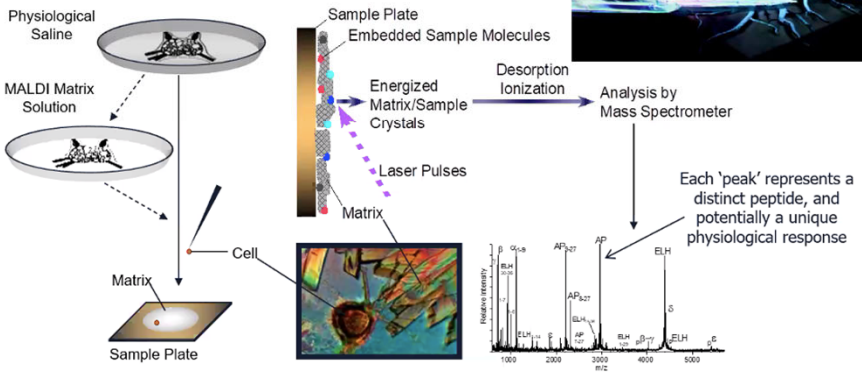
Using mass spectrometry (MS) for peptid
(cytokines, neuropeptides, hormones)

Talking: Jonathan Sweedler





Li et al, Trends Biotechnol. 2000, 18, 151.

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Identification and mapping novel peptides in defined neuronal circuits

Talking: Jonathan Sweedler

Gene expression data (ISH)

Sequence database (direct mRNA translation)

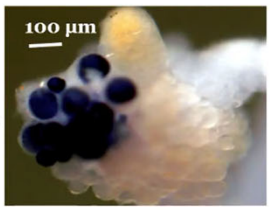
Predicted peptides/PTMs (bioinformatics web tools)

ISH-guided cell isolation

MALDI-TOF MS, MS²

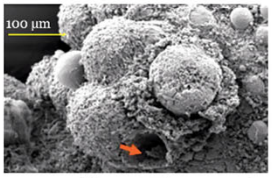
Library of actual peptide gene products found in key neurons in a neural circuit

Romanova, **Neuropsychopharmacology 36**, 2014, 50



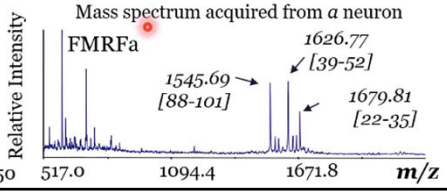
Courtesy L. Moroz

Prohormone mRNA expression in neurons



SEM microphotograph of neurons; the arrow points to where a single neuron was removed for MS

Mass spectrum acquired from *a* neuron



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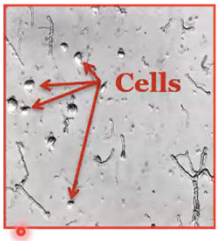
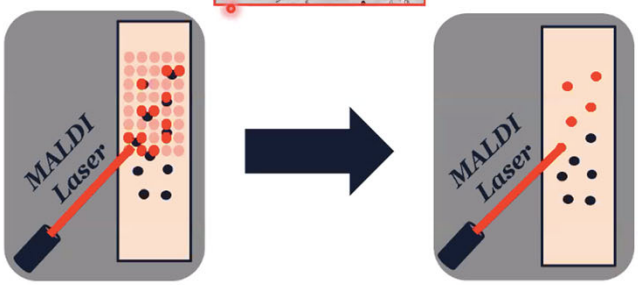
A new approach for high throughput single cell mass spectrometry

Talking: Jonathan Sweedler





Ong. T; Anal Chem. 2015, 87, 7036.

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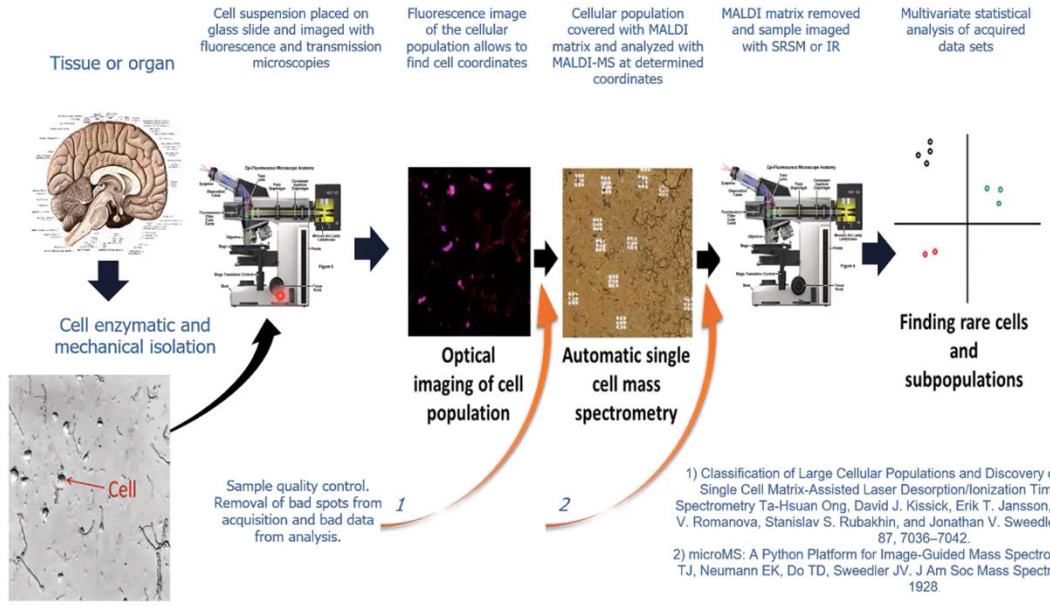
MicroMS guided high throughput Cell Profil

Talking: Jonathan Sweedler



1) Classification of Large Cellular Populations and Discovery of Rare Cells Using Single Cell Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Ta-Hsuan Ong, David J. Kissick, Erik T. Jansson, Troy J. Comi, Elena V. Romanova, Stanislav S. Rubakhin, and Jonathan V. Sweedler* Anal Chem. 2015 87, 7036–7042.

2) microMS: A Python Platform for Image-Guided Mass Spectrometry Profiling. Comi TJ, Neumann EK, Do TD, Sweedler JV. J Am Soc Mass Spectrom. 2017, 28, 1919-1928

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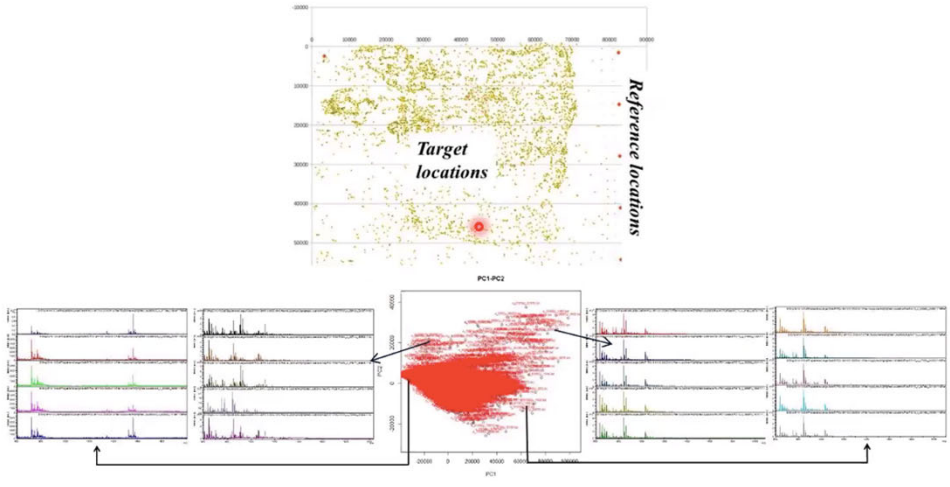
Profiling ~10000 cells… and finding the rare cells via component analysis

Talking:



Ong. T; Anal Chem. 2015, 87, 7036.

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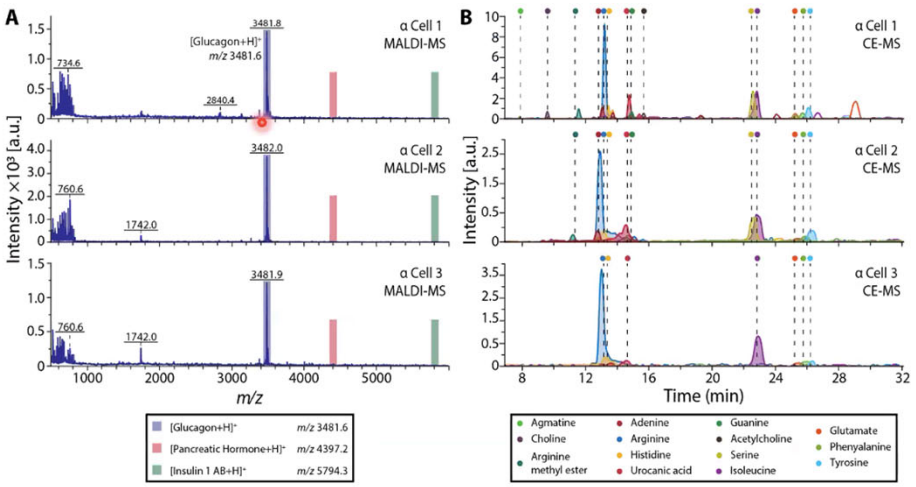
Probing the metabolites via capillary electrophoresis mass spectrometry

Talking:



Comi. T. J., Makurath, M. A., Philip, M. C., Rubakhin, S. S., & Sweedler, J.V. *Analyt. Chem 2017, 89 7765.*

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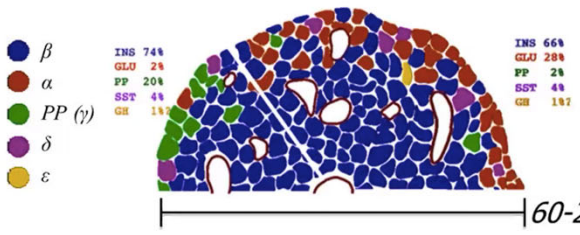
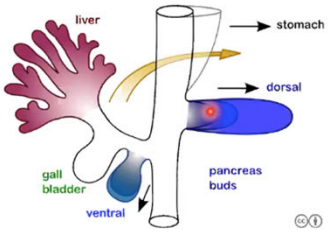
Pancreatic islets of Langerhans are an attractive system for developing single cell analysis

Talking: Jonathan Sweedler



insulin
glucagon
pancreatic peptide
somatostatin
ghrelin
d vessel



- Highly peptidergic
- Known intra- and inter-islet heterogeneity based on peptide content
- Well-studied physiology

We can characterize about 200-800 3-5 micron cells in an individual islet

Suckale, J.; Solimena, M. Front. Biosci **2008, 13, 7156.**

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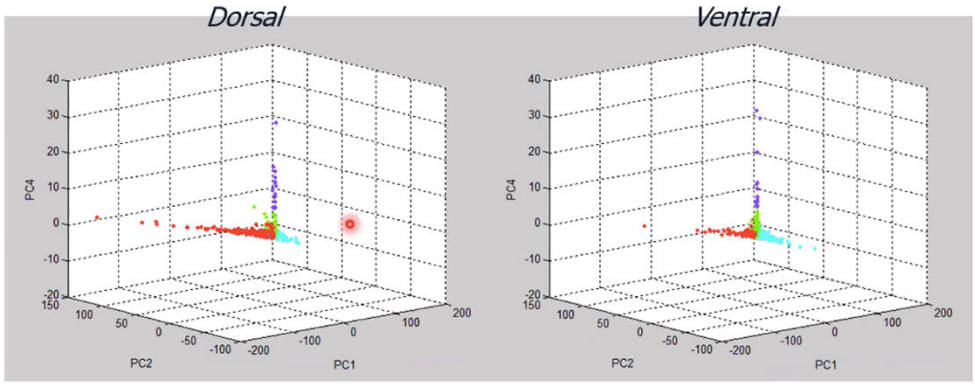
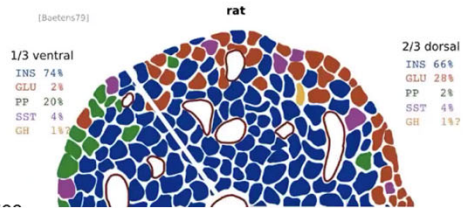
Cell populations between dorsal and ventral lobes agree with previous histological reports

Talking: Jonathan Sweedler



cell-type	Dorsal	Ventral
α	0.43	0.081
β	0.51	0.67
γ	0.045	0.24
δ	0.0090	0.0086
n_{tot}	1768	1738



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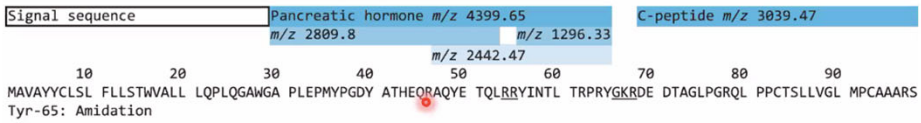
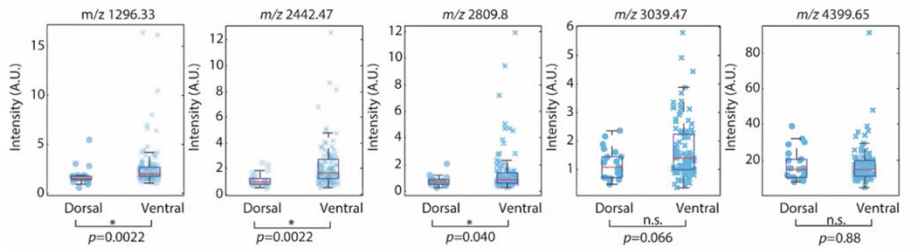
Analysis of single cell spectra: differences between cell types

Talking: Jonathan Sweedler



Significantly different peptide content between subtypes



Jansson et al, ACS Chem Biol. 2016,11, 2588.

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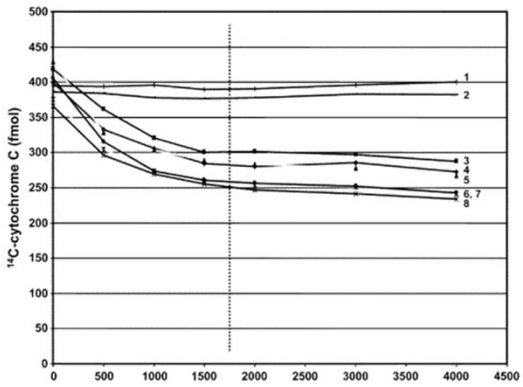
Interlude:
MALDI MS can be "non-destructive"

Talking: Jonathan Sweedler



Amount of [^{14}C]-cytochrome c in deposited spots detected by a phosphor-imaging detector after a series of MALDI mass spectral acquisitions. Six different samples are shown, spots 3–8, to illustrate the consistency of the depletion. The two samples in which no mass spectra were acquired, spots 1 and 2, demonstrate that the depletion is a result of laser desorption/ionization. The vertical dashed line indicates the point in the depletion study that the analyte was no longer detected by MALDI MS.

Anal. Chem., 2002, 74 (24), pp 6200

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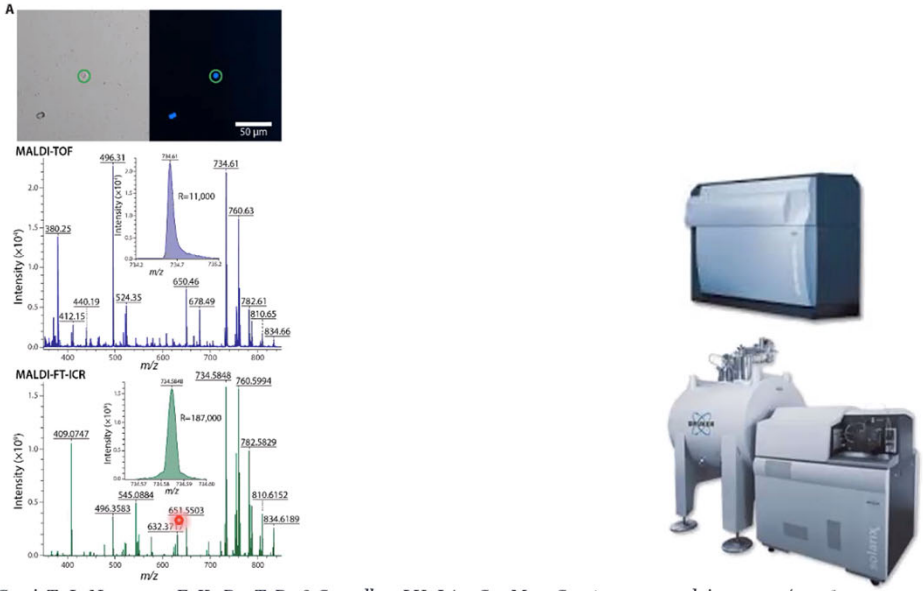
microMS architecture supports facile follow-up analyses

Talking: Jonathan Sweedler



Comi. T. J., Neumann, E. K., Do, T. D., & Sweedler, J.V. J Am Soc Mass Spectrom. 2017. doi: 10.1007/s13361-017-1704-1.

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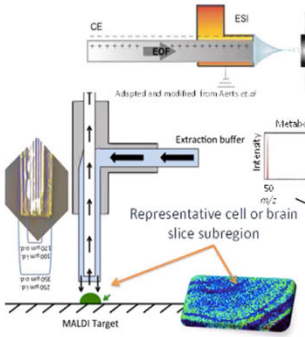
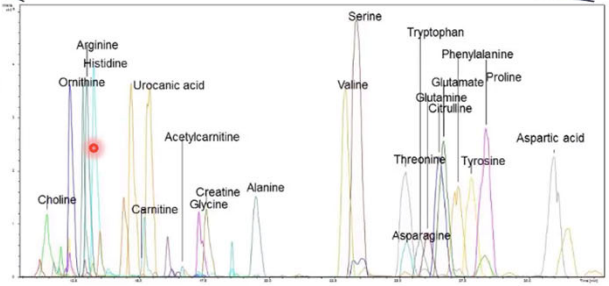
Probing the metabolites via capillary electrophoresis mass spectrometry

Talking: Jonathan Sweedler



Example of CE-MS on a cell after MALDI MS measurement



Comi. T. J., Makurath, M. A., Philip, M. C., Rubakhin, S. S., & Sweedler, J.V. *Analyt. Chem 2017, 89 7765.*

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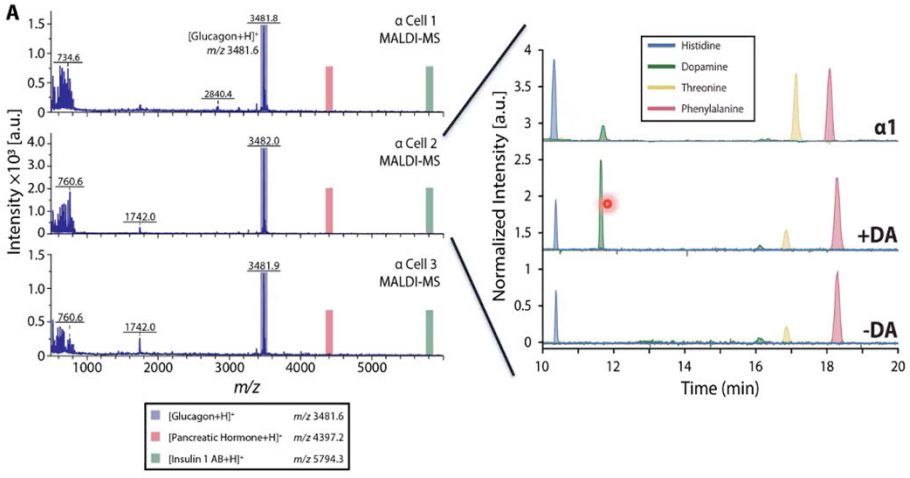
Probing the metabolites via capillary electrophoresis mass spectrometry

Talking:



Comi. T. J., Makurath, M. A., Philip, M. C., Rubakhin, S. S., & Sweedler, J.V. *Analyt. Chem 2017, 89 7765.*

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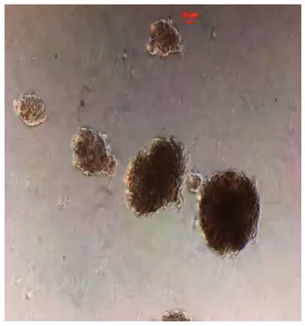
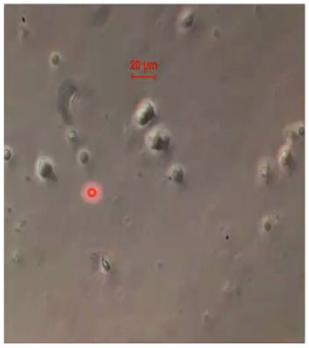
Human islet of Langerhans: Are these structures chemically heterogeneous in a patient and how does this heterogeneity change during the development of diabetes?

Talking: Jonathan Sweedler



Individual islets of varying sizes



Individual cells

Samples are kindly provided by Dr. Chengyang Liu and Dr. Ali Naji, Human Pancreas Analysis Program (HPAP, UPENN)

Sweedler group unpublished

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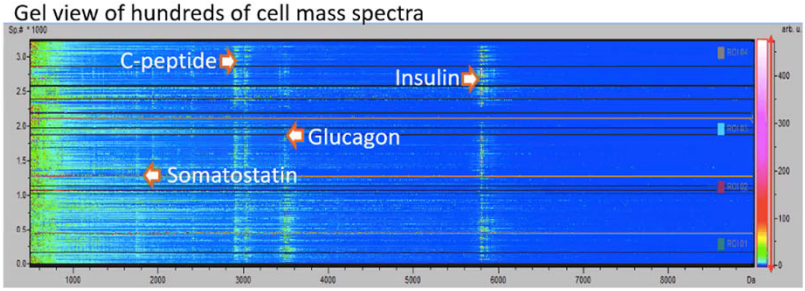
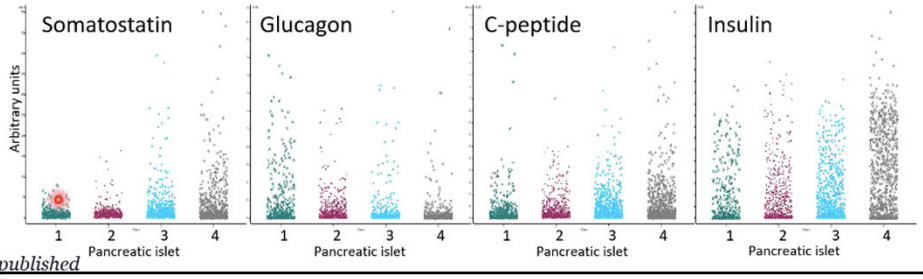
Comparison of chemical profiles of high density populations of four individual pancreatic islets

Talking: Jonathan Sweedler

Gel view of hundreds of cell mass spectra





Sweedler group unpublished

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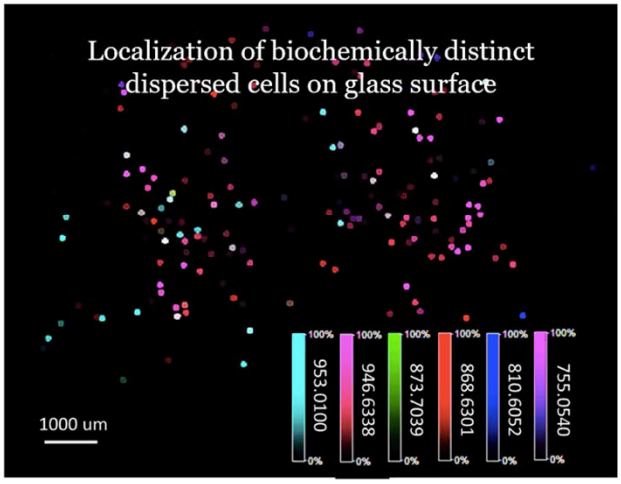
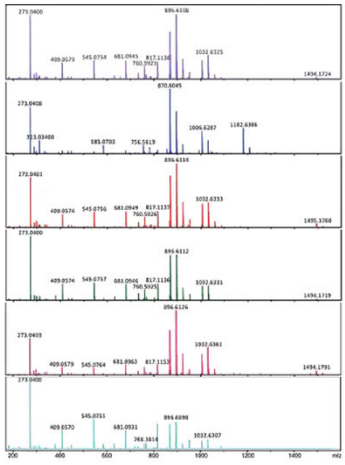
High throughput single cell MALDI MS reveal cellular heterogeneity of the cerebellum



Representative mass spectra



We can see large differences in lipids and other molecules, even from so-called "identical" cells...

Neumann et al, Anal Chem. 2019, 91, 7871.

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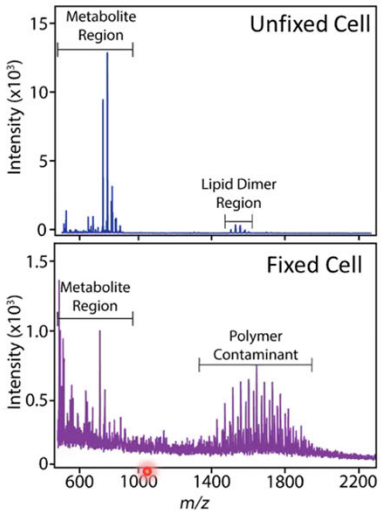
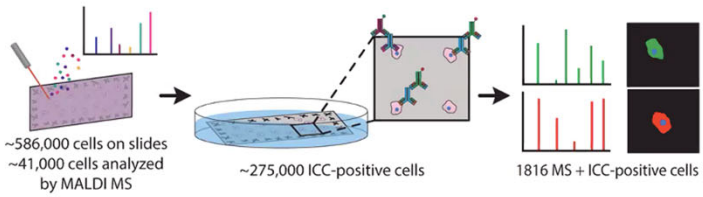
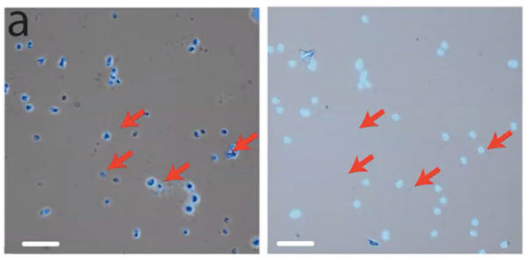
What are the cells?

Adding immunocytochemistry to our MS workflow







Neumann, et al, Angewandte Chemie, 2019, 58, 5910.

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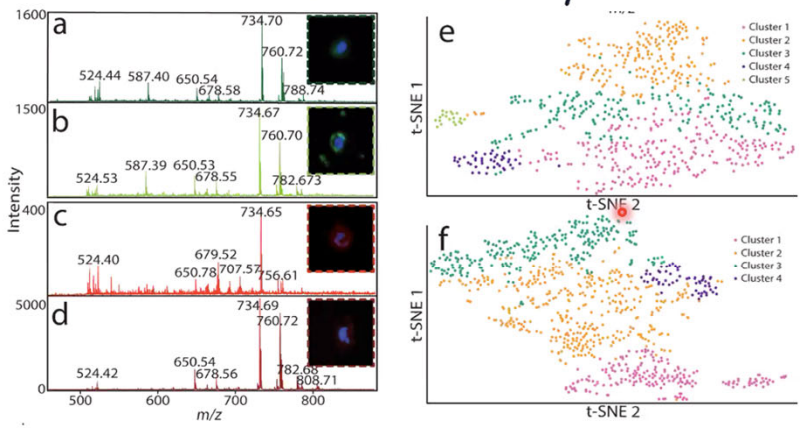
Lipid differences between neurons and astrocytes

Talking: Jonathan Sweedler



(**a and b**) Single cell spectrum for the NF-L-positive cell displayed in the inset.
(**c and d**) GFAP-positive single cell spectra
(e) t-SNE plot of NF-L positive cells colored based on the five clusters determined by HCA.
(f) t-SNE plot of GFAP positive cells colored based on the four clusters determined by HCA.

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Neumann, et al, Angewandte Chemie, 2019, 58, 5910.

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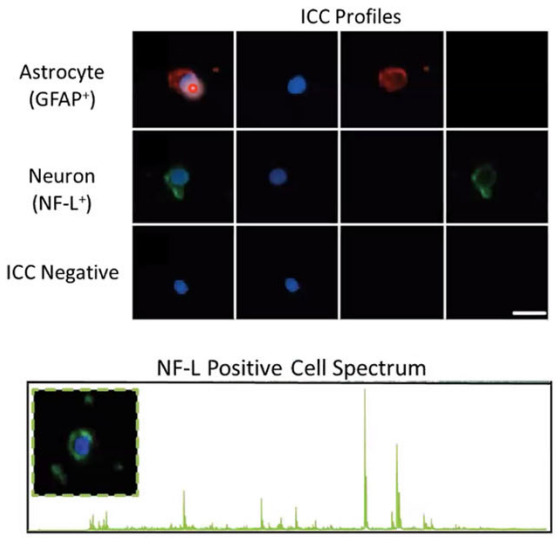
Lipid Signatures of Astrocytes and Neuro

Talking: Jonathan Sweedler



Measured m/z Value	PPM Error	Assignment	Type	Relative Enrichment
837.6607		unknown	GFAP Positive	61%
724.5249	-0.3602	$[PE(O-34:2)+Na]^+$ $[PE(P-34:1)+Na]^+$	GFAP Positive	58%
789.6143	3.3624	$[PE(38:2)+NH_4]^+$	GFAP Positive	39%
752.5564	-0.0810	$[PE(O-36:2)+Na]^+$ $[PE(P-36:1)+Na]^+$	GFAP Positive	28%
768.5880	0.3110	$[PC(O-34:1)+Na]^+$ $[PC(P-34:0)+Na]^+$ $[PE(O-37:1)+Na]^+$ $[PE(P-37:0)+Na]^+$	GFAP Positive	16%
850.6730		unknown	NF-L Positive	60%
731.6060	-0.2064	$[SM(d36:1)+H]^+$	NF-L Positive	30%
732.5538	0.0246	$[PC(32:1)+H]^+$	NF-L Positive	27%
834.6003	-0.5176	$[PC(40:6)+H]^+$	NF-L Positive	26%
784.5828	0.1580	$[PC(34:0)+Na]^+$	NF-L Positive	24%
828.5504	-1.1780	$[PC(38:6)+Na]^+$	NF-L Positive	23%
756.5513	-0.1004	$[PC(32:0)+Na]^+$ $[PE(35:0)+Na]^+$	NF-L Positive	22%
808.5825	-0.2177	$[PC(36:2)+Na]^+$	NF-L Positive	21%
782.5670	-0.0332	$[PC(34:1)+Na]^+$	NF-L Positive	18%
815.6347		unknown	NF-L Positive	16%
762.5044 762.6004	-0.0341 -0.4353	$[PE(36:4)+Na]^+$ $[PC(34:0)+H]^+$	NF-L Positive	11%
772.6218	0.4310	$[PC(O-36:2)+H]^+$ $[PC(P-36:1)+H]^+$	NF-L Positive	06%

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Neumann, et al, Angewandte Chemie, 2019, 58, 5910.

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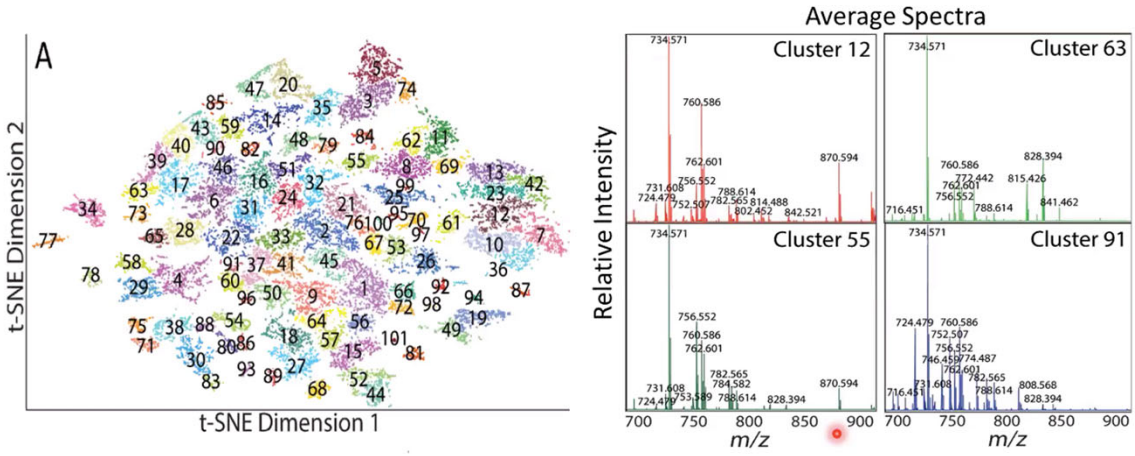
Characterizing the lipids in 30,000 individual cells with MALDI-FT-ICR MS

Talking: Jonathan Sweedler

Individual isolated adult rat cerebellum cells from 6 animals



Separates into 101 Clusters based on Lipid Features

Neumann et al, Anal Chem. 2019, 91, 7871.

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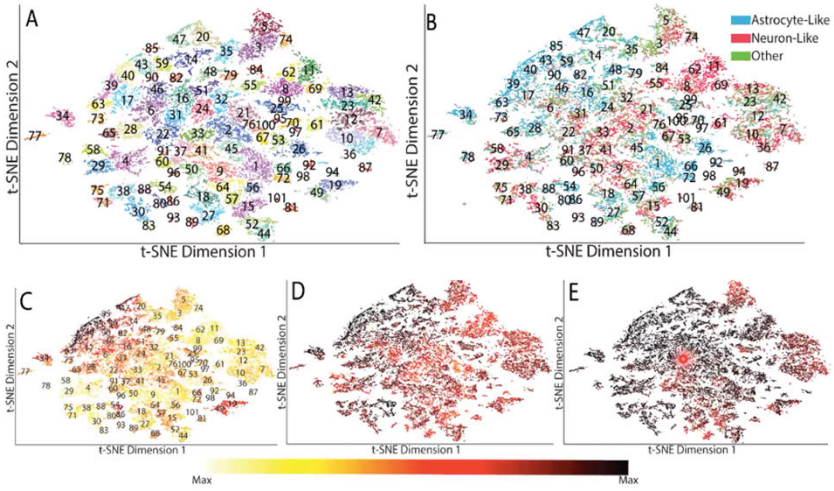
Characterizing the lipids in 30,000 cells with single cell MALDI-FT-ICR MS

Talking: Jonathan Sweedler



t-SNE recolored by relative amount of **(C)** phosphatidylcholine, **(D)** sphingomyelin and **(E)** phosphatidylglycerol lipid species to visualize the localizations of lipid classes.

Neumann et al. Anal Chem. 2019, 91, 7871.

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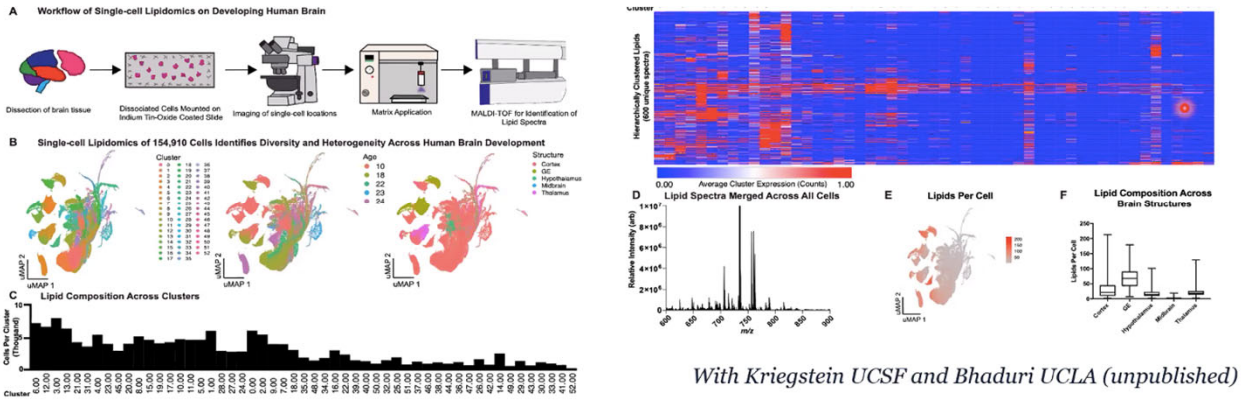
Creating an atlas of single cell li… profiles of the developing human brain

Talking: Jonathan Sweedler



With Kriegstein UCSF and Bhaduri UCLA (unpublished)

Single-cell lipidomics of the cortex, ganglionic eminences (GE), hypothalamus, midbrain, thalamus of the developing human brain between gestational weeks (GW) 10 – 23 with more than 150,000 cells assayed; 53 unique lipidomic clusters (bottom left) visualized by uniform manifold approximation and projection (UMAP) were identified via Louvain clustering. Some of these clusters were enriched in lipids at specific ages while other clusters were intermixed across stages or regions. On average, there were 2923 cells per cluster ranging from 184 to 9153. The lower right shows summed mass spectra from all cells, demonstrating how bulk analysis is insufficient for assaying lipid diversity, the UMAP recolored by number of cells, and the distribution of lipids per cell for each sampled brain structure.

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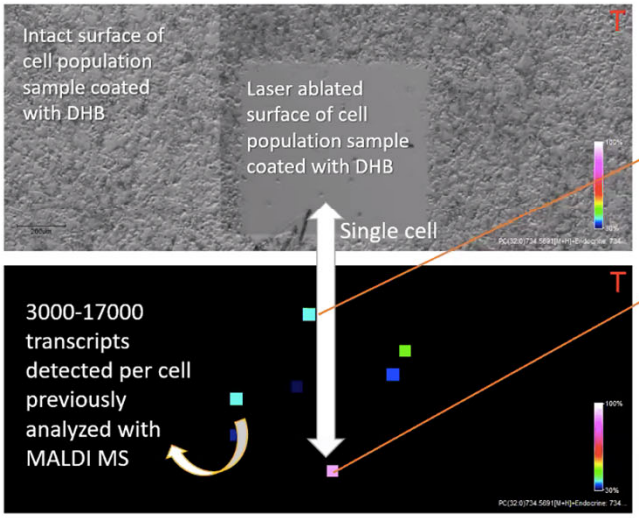
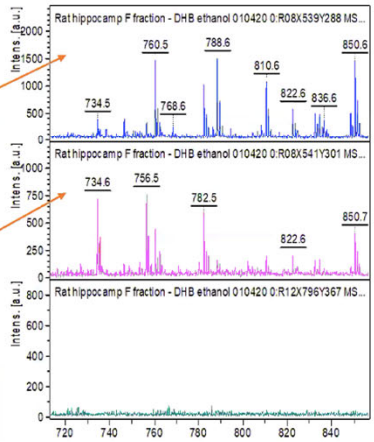
Hyphenation of single cell mass spectrometry and trans…

Talking: Jonathan Sweedler

Ra…hippocampal cell population analyzed using single cell MALDI MS. Individual cells are collected and sent to the Eberwine laboratory for transcriptomics



Examples of mass spectra obtained from individual cells



Lipids found in hippocampal cell extracts

Lipid	exact mass
• PC(32:0)	734.5694
• PC(32:0)+Na	756.5514
• PC(34:1)	760.5851
• PC(32:0)+K	772.5253
• PC(36:1)	788.6164
• PC(34:1)+K	798.5410
• PC(34:0)+K	800.5566
• PC(38:6)	844.5253

DHB MALDI matrix is water soluble and can be removed with PBS wash. However, it's requires higher laser beam intensity - 60%.

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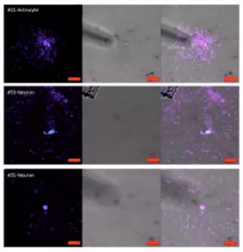
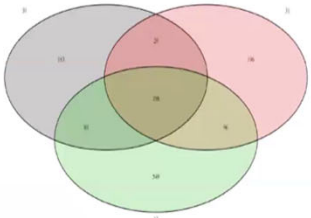
The greatest information from a single cell ever!

Talking:

With Jim Eberwine U Penn

Isolate individual cells, perform MS, and then send to Eberwine's group for transcriptomics. single cell transcriptomics after MS!





Genes expressed in all three cells

Ace	Ttfh1	Srrm1
Acsm1	Ttpn3	Srrm2
Adck2	Kiff3a	Sub4
Angptl6	Klhdc10	Sstr5
Ankrd13a	Krt10	Taok3
Ap4m1	LOC287004	Tarbp2
Apobp2	LOC317471	Tas1r2
Arrdc2	LOC365985	Tcea2
Asic3	LOC498575	Tenm3
Atic	LOC102723236	Tox38
B3galt2	Lca1m	Tmem2
B3gnt3	Map1a	Tmem41a
Bap4	Map1s	Tmem59l
Bcor	Map4k3	Tnrc18
Bid	Map10	Ttyc3
C5ar2	Mapk13	Tspan33
Cacna1h	Mast3	Txnrd3
Ccdc59	Max	U2af1
Cuic124	Mdga1	Ube2e3
Cdc25a	Megf8	Uba2z
Cdc42bpb	Mesp2	Ubr4
Cdc42bpg	Mpvep	Unc45a
Cdc42ep4	Mpv17l2	Usp45
Cdr2	Mrlap1	Vsig10
Cehpa	Nfasc	Wdr24
Chrnb2	Nimt	Wipi2
Chst11	Nomo1	Wnk4
Cnfn	Nus1	Wnt11
Crtc1	Ocal1	Wsb2
Ctu1	Pak2	Ybx3
Dact3	Paor5	Zc3h7b
Ddi2	Pdk3	Zc3h18
Ddx27	Pgls	Zdhhc14
Dnaaf2	Phospho1	Zfp706
Dnajc6	Pitrl1	Zfpm2
Dvl2 Ell	Plk3r2	
Enrp2	Ptcd3	
F10	Ptec	
Fat1	Ptdrn2	
Fgd6	Pthlr	
Fgf2	Psd	
Fib3	Ptpn1	
Fmc1	Ptpn12	
Fndc1	Pvrl2	
Fnta	RGD1309388	
Foxj2	RT1.CE6	
Foxm4	RT1.N3	
Frmd6	Rrgn1	
Galnt2	Rps7bp	
Gan	Rgs17	
Gbx1	Rpusd4	
Gfod2	Scaf1	
Gfra4	Scube1	
Glb1l2	Sec23b	
Gpr180	Sfxn4p	
Grk5	Slc1a7	
H2afb3	Slc2a3	
Hef1	Slc5a3	
Hspa1a	Slc13a3	
Htatsf1	Slc27a1	
Ier5l	Slc35f5	
Ifitr	Slitrk4	
Inpp4a	Smurf1	
Ipsec3	Sox4	

With Eberwine, UPenn

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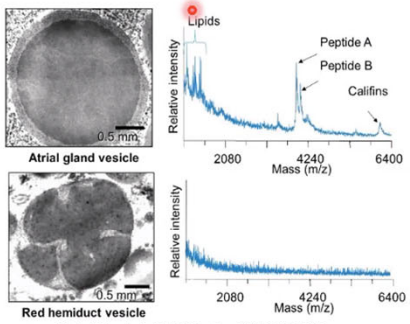
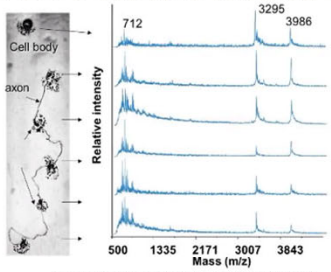
Subcellular profiling of neurons!

MALDI MS of hormones in a large dense core vesicle, lipids and neuropeptides in an axon, and vitamin E in a damaged cultured cell

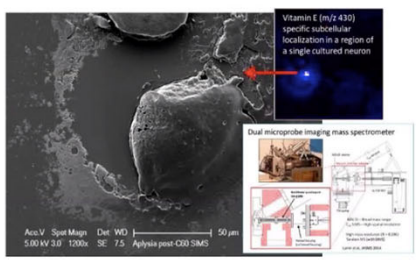
Talking: Jonathan Sweedler



Rubakhin et al, Nat Biotechnol. 2000 18, 172



Rubakhin et al, Analyt. Chem. 2003 75, 5374



Lanni et al, J Am Soc Mass Spectrom. 2014 25, 1897

Each type of separation, mass analyzer and ionization source provides distinct advantages in terms of spatial resolution, detectability and observed molecular class

The major components in subcellular domains are measureable

Can we make these measurements high throughput?

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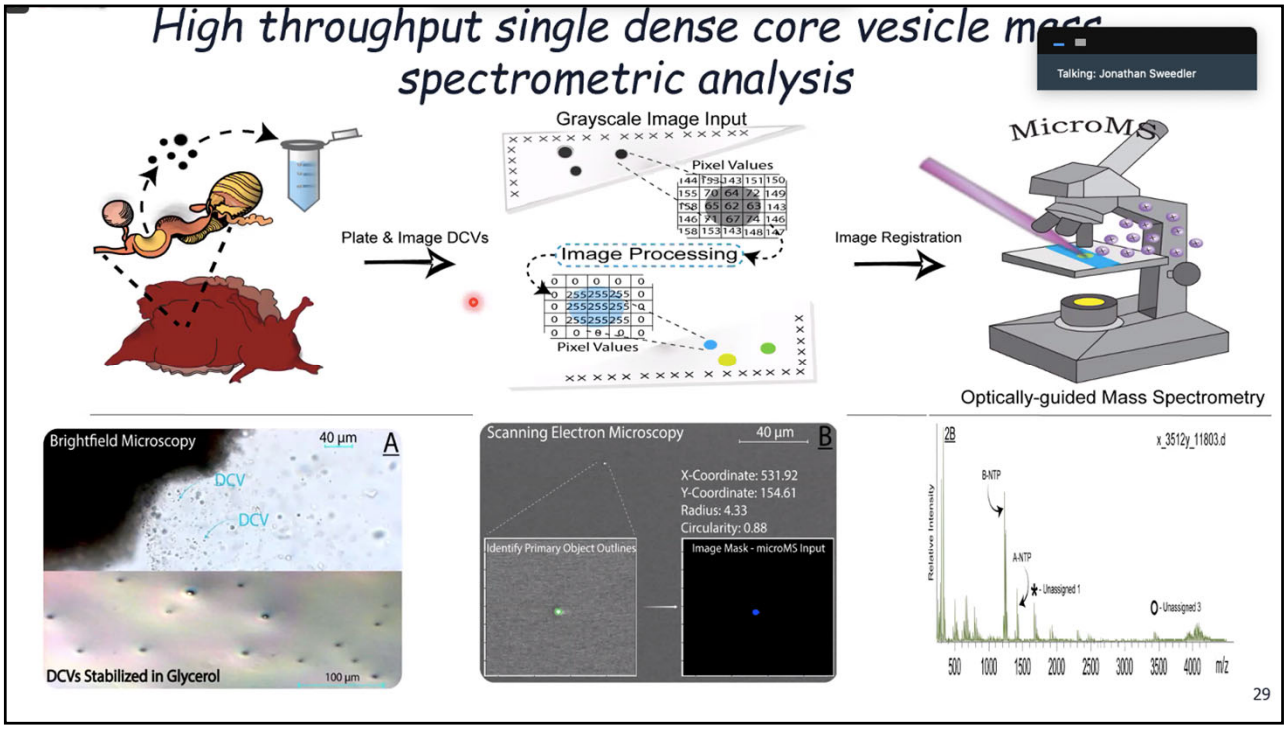
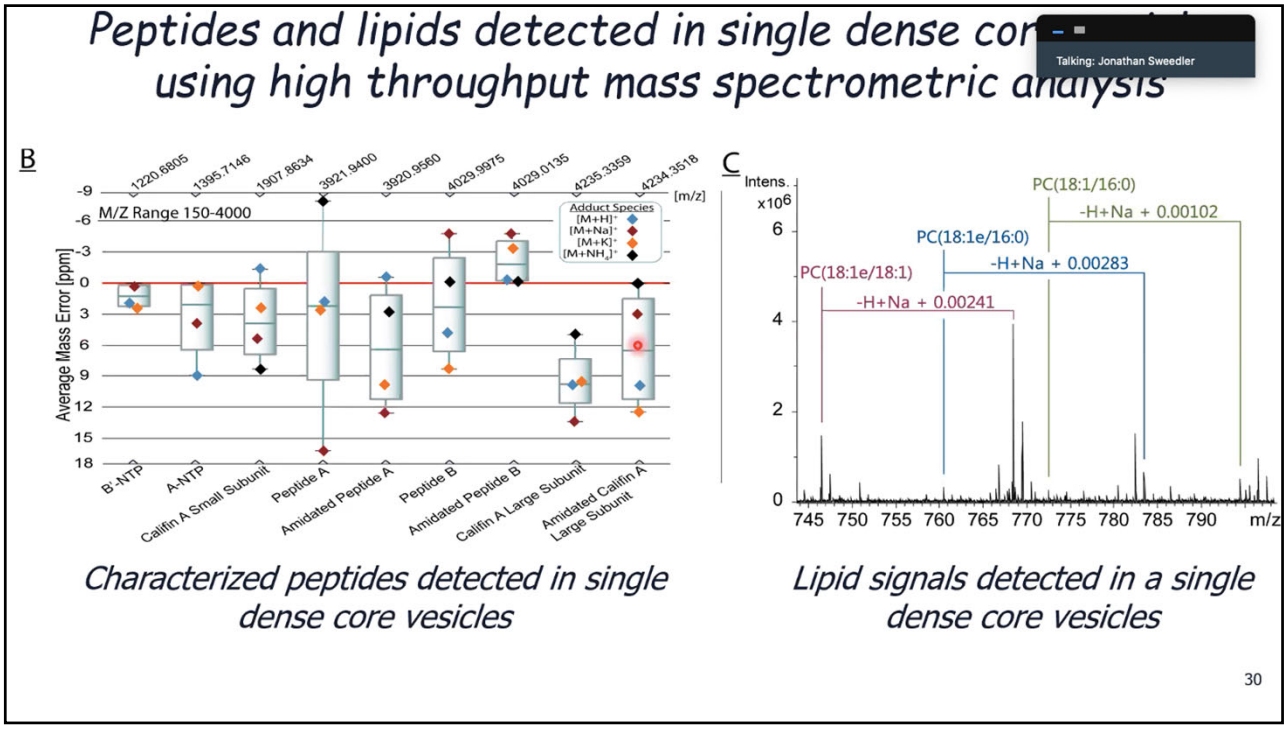

High throughput single dense core vesicle mass spectrometric analysis
Talking: Jonathan Sweedler
Grayscale Image Input
Pixel Values
Plate & Image DCVs
Image Processing
Pixel Values
Image Registration
MicroMS
Optically-guided Mass Spectrometry
Brightfield Microscopy
40 µm
A
DCV
DCV
DCVs Stabilized in Glycerol
100 µm
Scanning Electron Microscopy
40 µm
B
X-Coordinate: 531.92
Y-Coordinate: 154.61
Radius: 4.33
Circularity: 0.88
Identify Primary Object Outlines
Image Mask - microMS Input
2B
x_3512y_11803.d
B-NTP
A-NTP
Unassigned 1
Unassigned 3
Relative Intensity
500 1000 1500 2000 2500 3000 3500 4000 m/z
29

31


Peptides and lipids detected in single dense core vesicles using high throughput mass spectrometric analysis
Talking: Jonathan Sweedler
B
1220.6805
1395.7146
1907.8634
3921.9400
3920.9560
4029.9975
4029.0135
4235.3359
4234.3518
[m/z]
M/Z Range 150-4000
Adduct Species
[M+H]+
[M+Na]+
[M+K]+
[M+NH4]+
Average Mass Error [ppm]
-9
-6
-3
0
3
6
9
12
15
18
B-NTP
A-NTP
Calfin A Small Subunit
Peptide A
Amidated Peptide A
Peptide B
Amidated Peptide B
Calfin A Large Subunit
Amidated Calfin A Large Subunit
C
Intens.
x10^6
6
4
2
0
PC(18:1/16:0)
-H+Na + 0.00102
PC(18:1e/16:0)
-H+Na + 0.00283
PC(18:1e/18:1)
-H+Na + 0.00241
745 750 755 760 765 770 775 780 785 790 m/z
Characterized peptides detected in single dense core vesicles
Lipid signals detected in a single dense core vesicles
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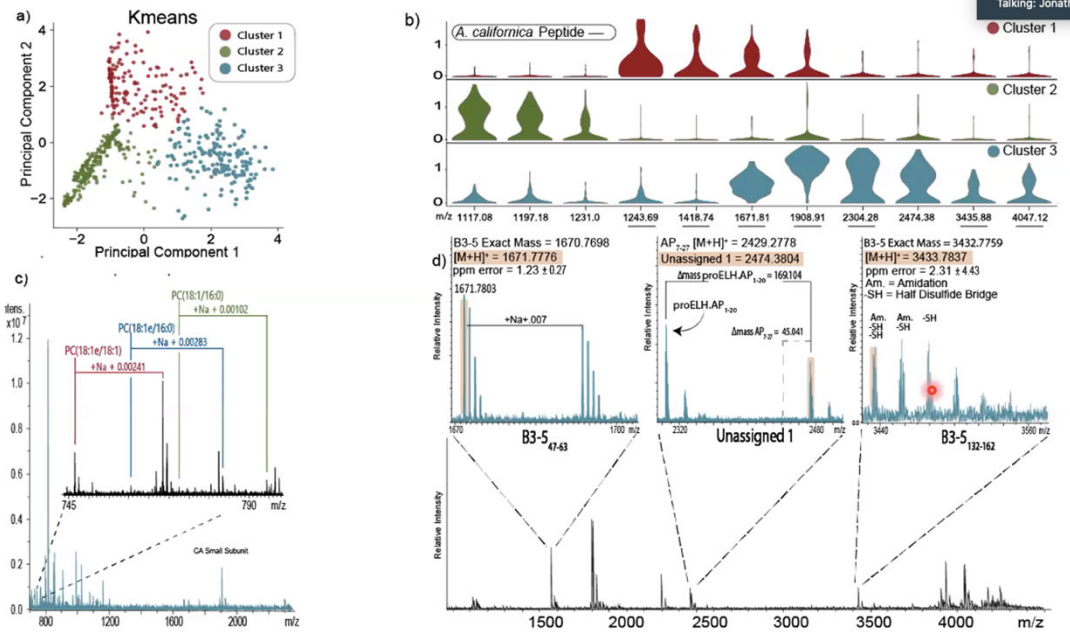
Three different DCV populations based on chem



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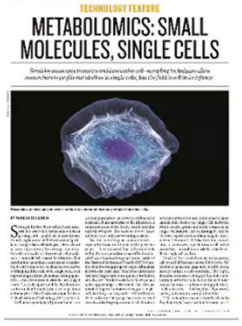
Conclusions

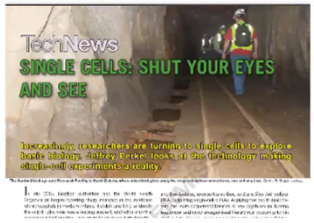
Cellular heterogeneity is high, even in a well-defined cell types.

In tissues such as endocrine structures, the CNS and the ENS, the spatial and chemical complexity requires approaches with single cell chemical resolution.

In every organism studied, *unexpected* and novel transmitters and peptides are still being found

A particular challenge is the dynamic range of the brain, from length, time and chemical complexity…





Reviews and overviews

- Exploring the Fundamental Structures of Life: Non-targeted, Chemical Analysis of Single Cells and Subcellular Structures, Neumann et al, **Angew Chem Int Ed Engl.** 2019, 58, 9348.
- Categorizing Cells on the Basis of their Chemical Profiles: Progress in Single-Cell Mass Spectrometry, Comi et al, **J Am Chem Soc. 139**, 2017, 3920.
- Single-cell analysis at the threshold, Chen et al, **Nature Biotechnology 34**, 2016, 1111.
- Single cells: shut your eyes and see, J. Perkel, **Biotechniques 61**, 2016, 165.
- Metabolomics: Small molecules, single cells, M. Fessenden, **Nature 540**, 2016, 153

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