Recording

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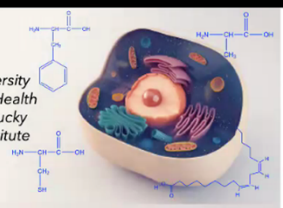
ONLINE WORKSHOP | FEBRUARY 26, 2021

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

Ahmed Ali

University of Leiden

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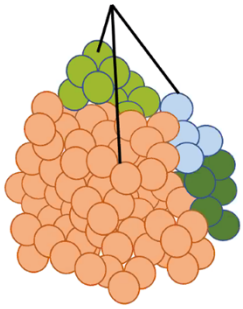
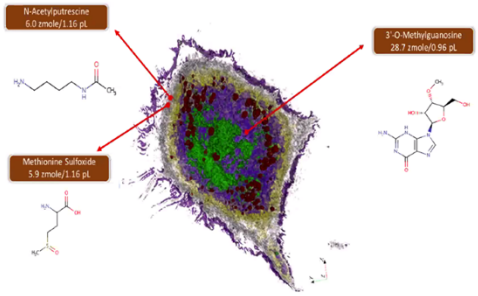
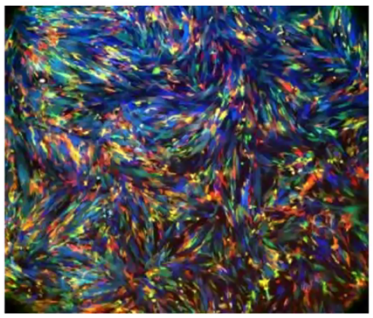
Intro: Why single cell metabolomics?







Rare cell populations | **Intracellular studies** | **Cellular heterogeneity**

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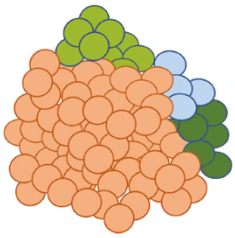
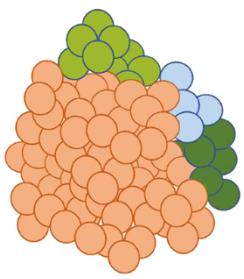
Intro: Rare cell populations



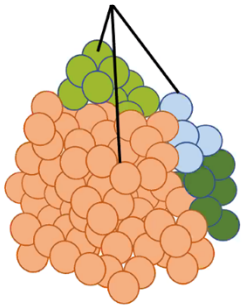
Actual populations

Bulk sampling



Bulk analysis

Single cell sampling



Single cell measurements

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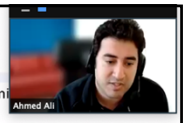
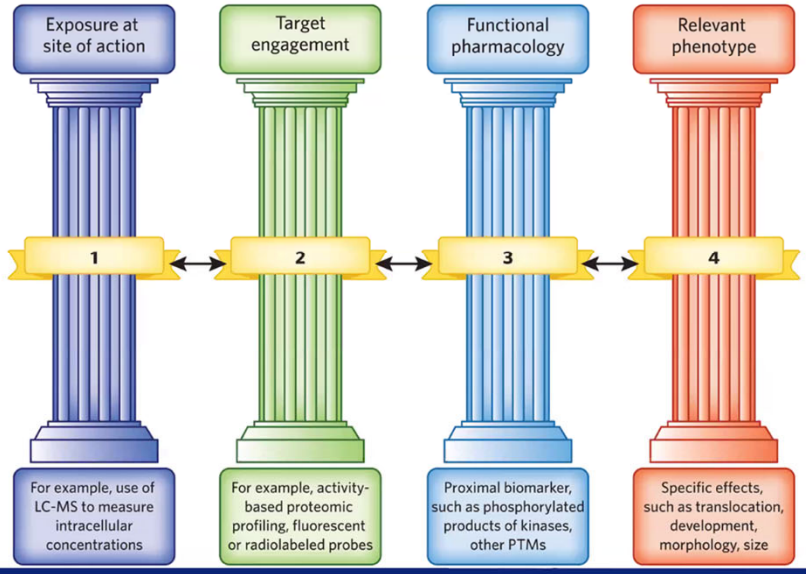
Intro: Intracellular studies



Mark E Bunnage,et al (Nature Chemi

Most drugs fail phase II clinical trials due to missing one or more of these pillars



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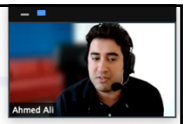
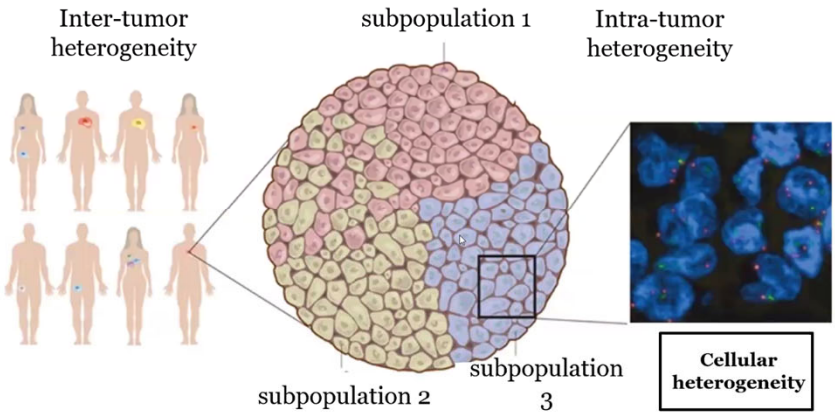
Intro: Cellular Heterogeneity





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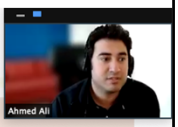
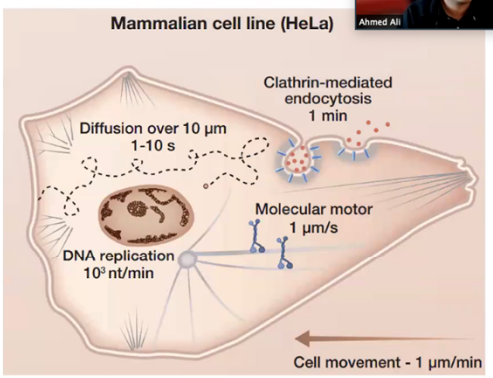
Challenges in single cell metabolomics



- Small sample size (picolitre scale)
- Fast Turnover (Seconds to minutes)
- Complex and diverse compounds (114,100 as of 2019)
- Inability to amplify signals à la PCR



Cellular pool half-life (dominated by)

1 s	**Metabolite** (turnover)	1 min
10 min	**mRNA** (degradation)	10 hr
1 hr	**Protein** (dilution)	1 day

https://www.cell.com/cell/pdf/S0092-8674(16)30208-2.pdf

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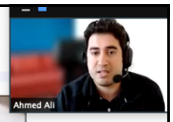
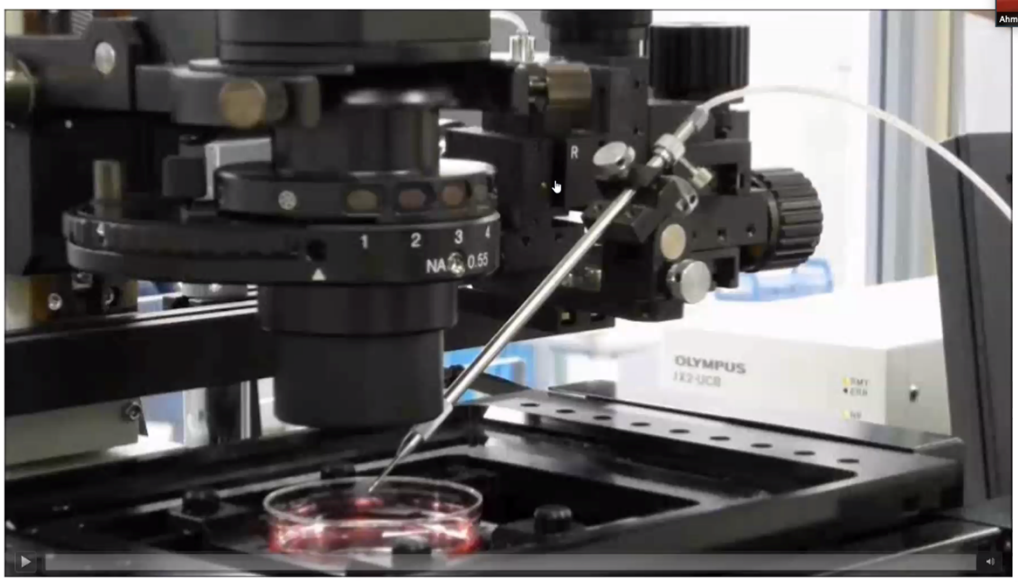
Live Single Cell Mass Spectrometry





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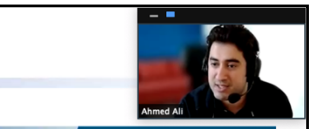
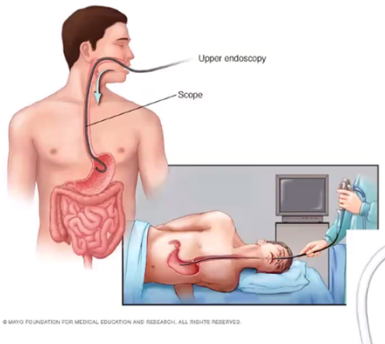
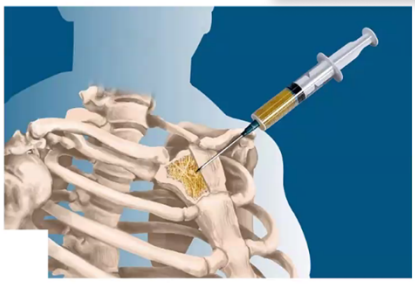
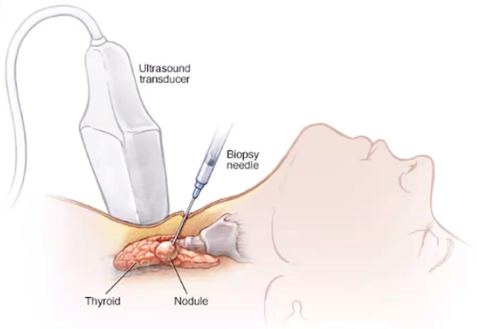
SCM Applications: Cancer Monitoring









https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/biopsy

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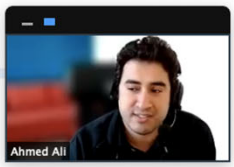
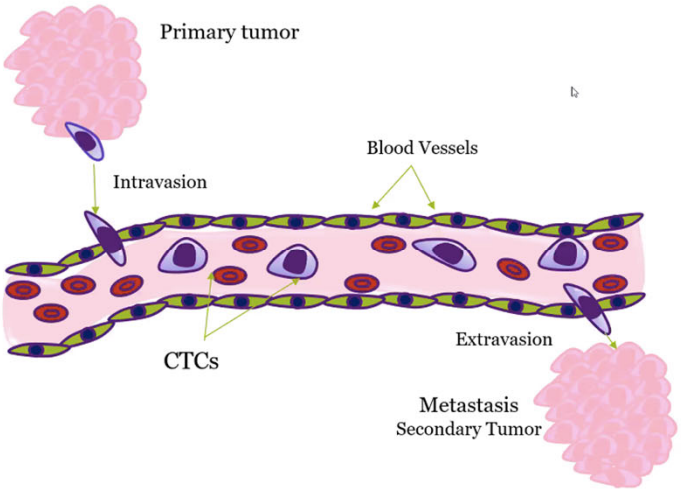
SCM Applications: Liquid Biopsy of CTCs





There are 4-6 **million** cells in 1 milliliter of blood [1]

In cancer patients, **1-6** of these cells are **CTCs** [2]

(1) Dean, L. *Blood and the Cells It Contains*; National Center for Biotechnology Information (US), 2005.
(2)Gold, B.; Cankovic, M.; Furtado, L. V.; Meier, F.; Gocke, C. D. Do Circulating Tumor Cells, Exosomes, and Circulating Tumor Nucleic Acids Have Clinical Utility? A Report of the Association for Molecular Pathology. *J Mol Diagn* **2015**, *17* (3), 209–224. https://doi.org/10.1016/j.jmoldx.2015.02.001.

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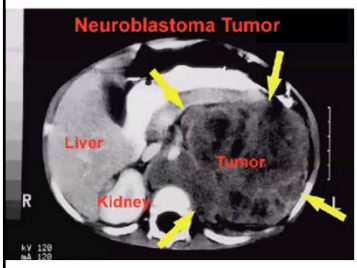
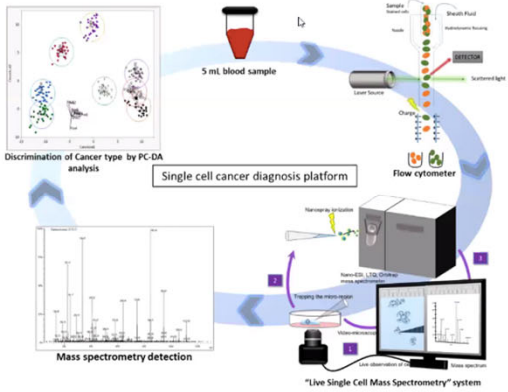
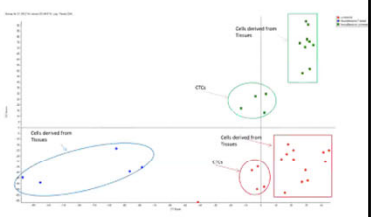
SCM Applications: Neuroblastoma (1st Iteration)

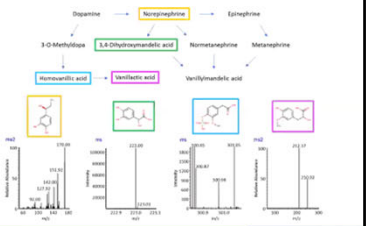


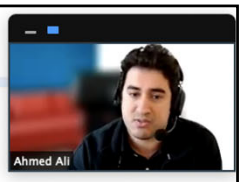
rof. Eiso Hiyama M.D

Prof. Tsutomu Masujima









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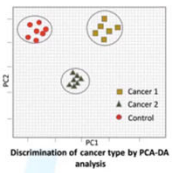
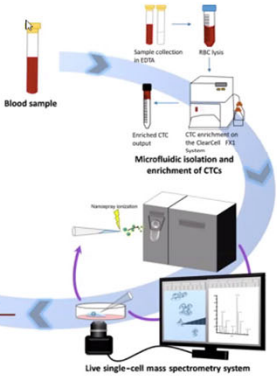
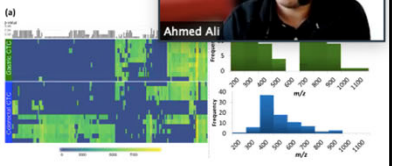
SCM Applications: Gastric and Colorectal cancer (2nd It

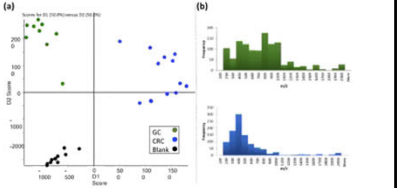
Colorectal cancer

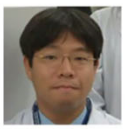
Gastric cancer











Prof. Kazufumi Honda M.D

Yasmine Abouleila PhD

国立研究開発法人
国立がん研究センター
National Cancer Center Japan

NUS

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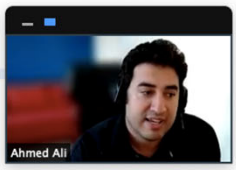
SCM Applications: Larger scale studies (3rd Iteration)



Cancer type	Cell type	Isolation method	Number of cells
Neuroblastoma	CTCs	FACS	64
Neuroblastoma	CTCs	IsoFlux	14
Neuroblastoma	Tissues	FACS	45
Nephroblastoma	CTCs	FACS	10
Nephroblastoma	Tissues	FACS	37
Pancreatoblastoma	CTCs	FACS	56
Hepatoblastoma	CTCs	FACS	12
Hepatoblastoma	Tissues	FACS	14
Bile duct	CTCs	FACS	2
Lymphocytes	B and T cells	FACS	16

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(Live) Single-Cell Mass Spectrometry Shortcomings

- Qualitati
- Exhaustiveness
- Low Throughput
- Robustness
- Data analysis

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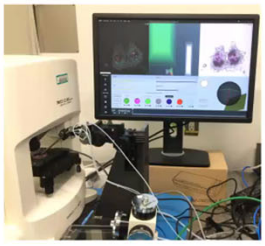
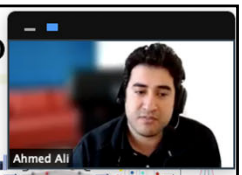
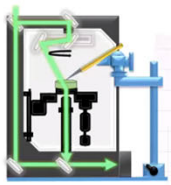
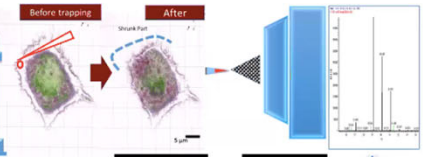
Qualitative: 3D Holography and tomography integratio





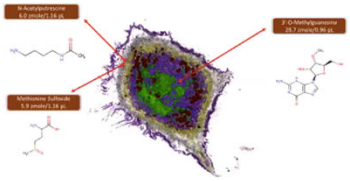




TECHNOLOGY FEATURE

METABOLOMICS: SMALL MOLECULES, SINGLE CELLS

Sensitive mass spectrometry and innovative cell-sampling techniques allow researchers to profile metabolites in single cells, but the field is still in its infancy.



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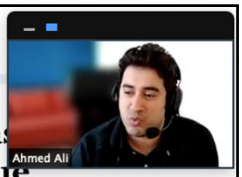
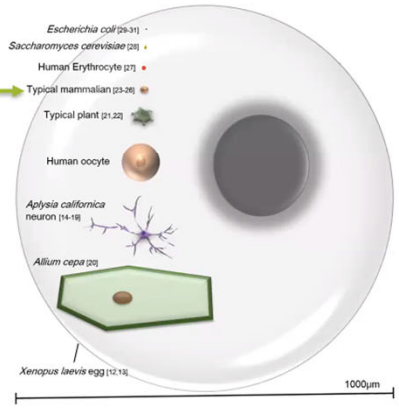
Exhaustiveness



Small sample volume

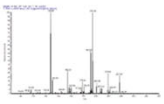
Volume is in picolitres



Destructive meas technique

- Electron Ionization
- Chemical Ionization
- Fast Atom Bombardment
- Matrix Assisted Laser Desorption Ionization
- ElectroSpray Ionization
- Nano Spray Ionization

- Quadrupole
- Ion Trap
- Time Of Flight
- Fourier Transform



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Exhaustiveness

Pharmacokinetics

What does the **cell** do to the drug ?

Quantify
- Drug
- Metabolite

PK-PD

Correlate
Conc. vs Effect

Pharmacodynamics

What does the **drug** do to the cell ?

Predict
- Screen for drug effect
- Predict the effect

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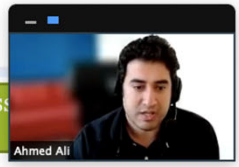
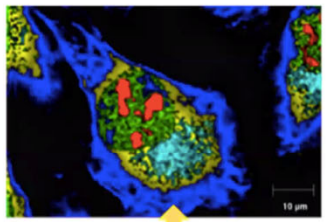
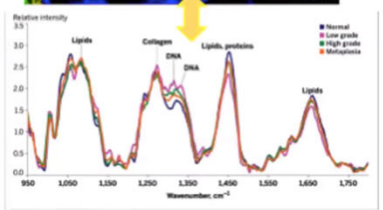
Solution: Integrate with non-invasive technique

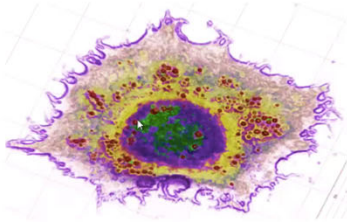


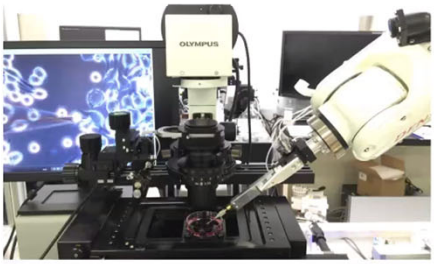
Raman spectroscopy

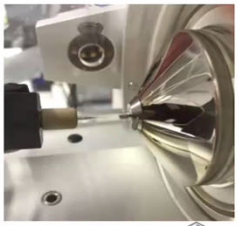
Mass

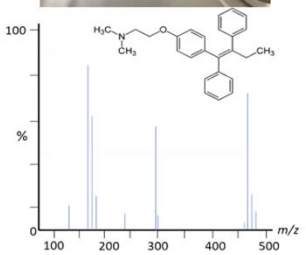












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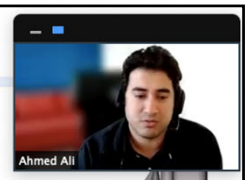
Experimental design

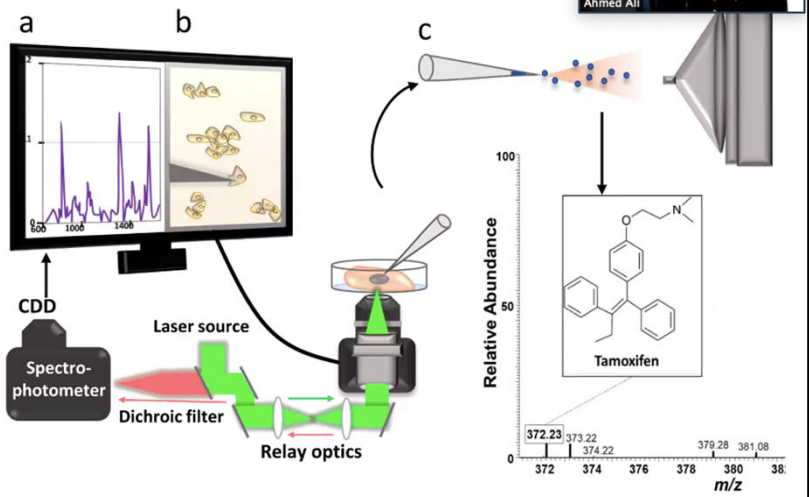


Cells: HepG2 cells

Drug: Tamoxifen (10 µM)

Incubation Time: 24 hours

Groups: 2 (Treated and Untreated cells (control))



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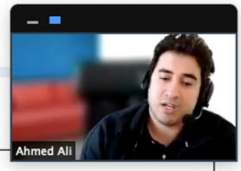
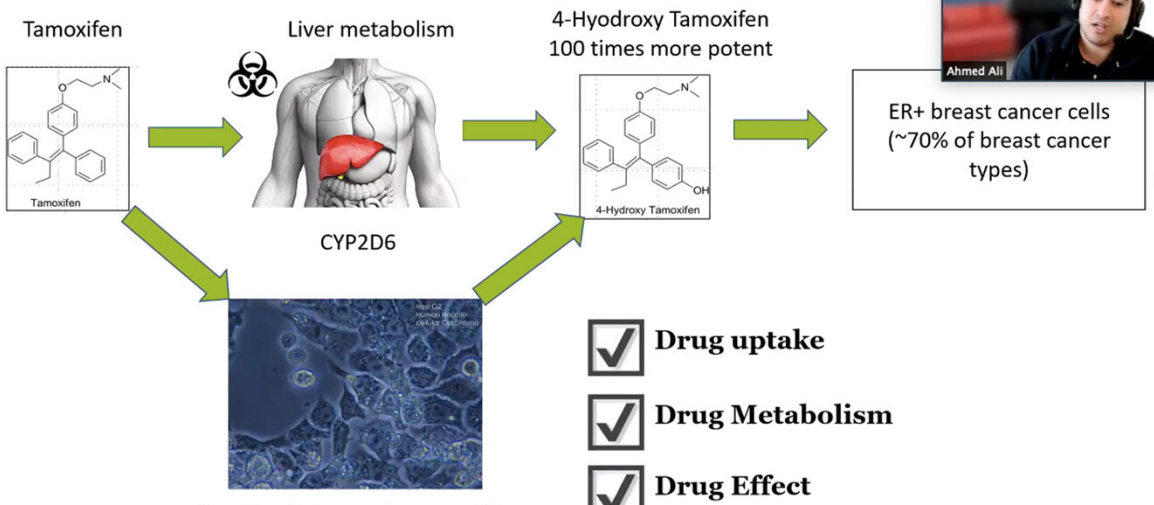
Targeted Drug





https://www.webmd.com/cancer/ss/slideshow-liver-cancer-gui

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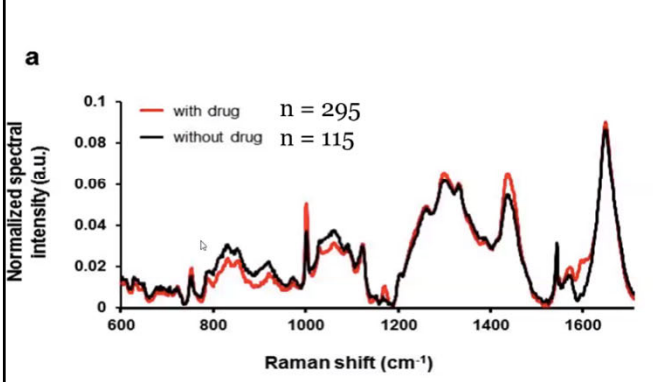
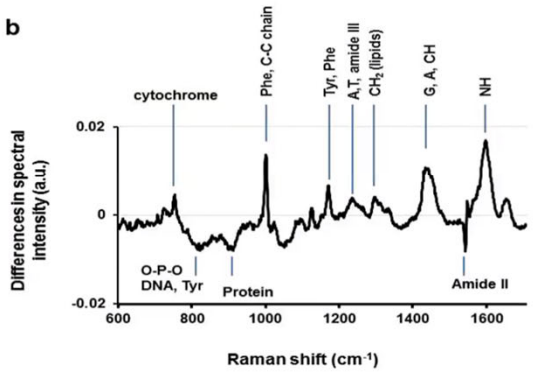
Raman: Profile of treated vs control cells

Talking: Ahmed Ali

Composite spectra of treated & control cells



Treated cells spectra subtracted from control cells spectra



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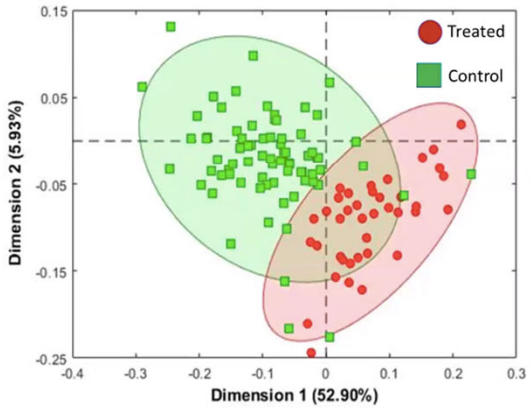
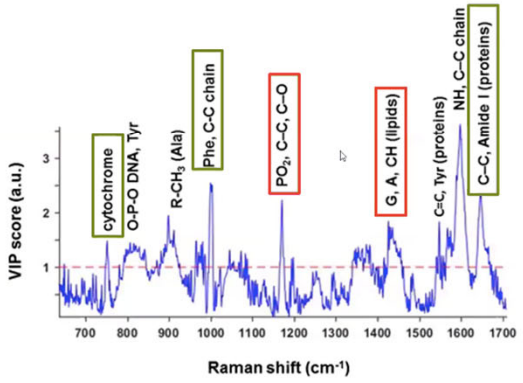
Raman: Predictive model of drug effects

Talking: Ahmed Ali

PLS-DA model of treated and untreated cells



Biomarkers for tamoxifen activity



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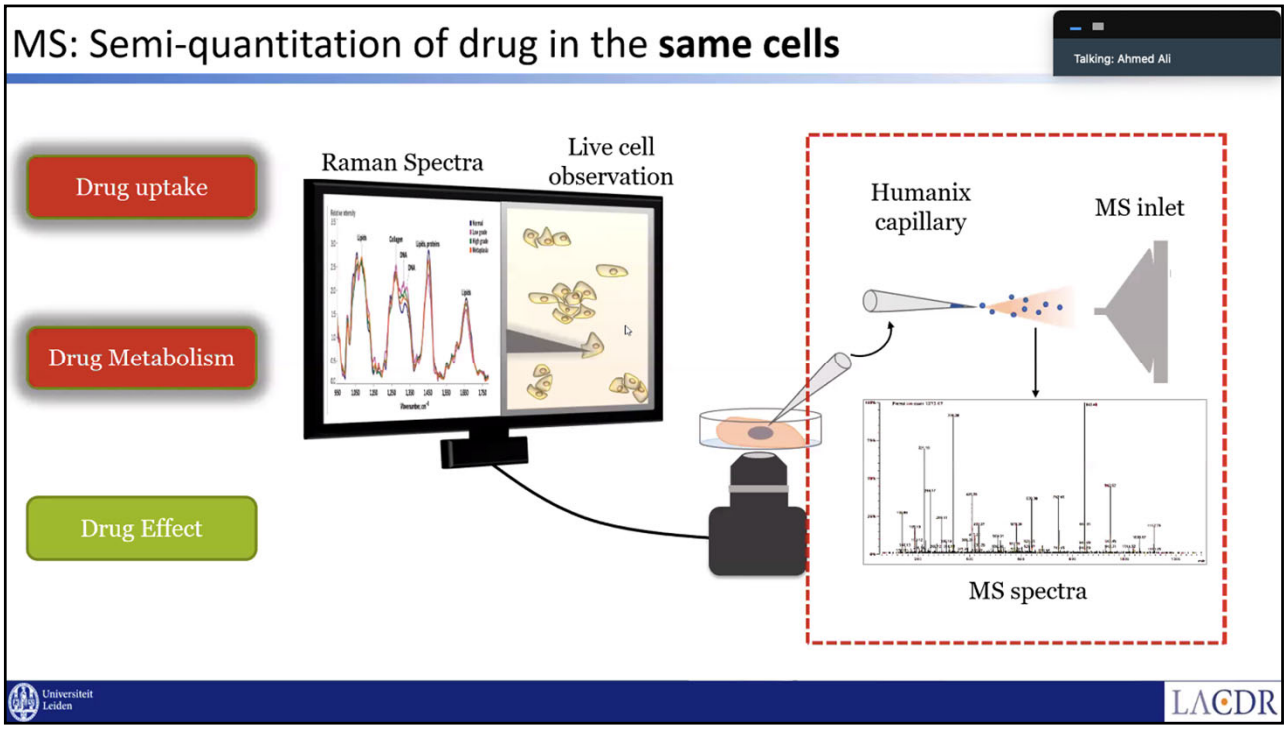
MS: Semi-quantitation of drug in the **same cells**
Talking: Ahmed Ali
Drug uptake
Drug Metabolism
Drug Effect
Raman Spectra
Live cell observation
Humanix capillary
MS inlet
MS spectra
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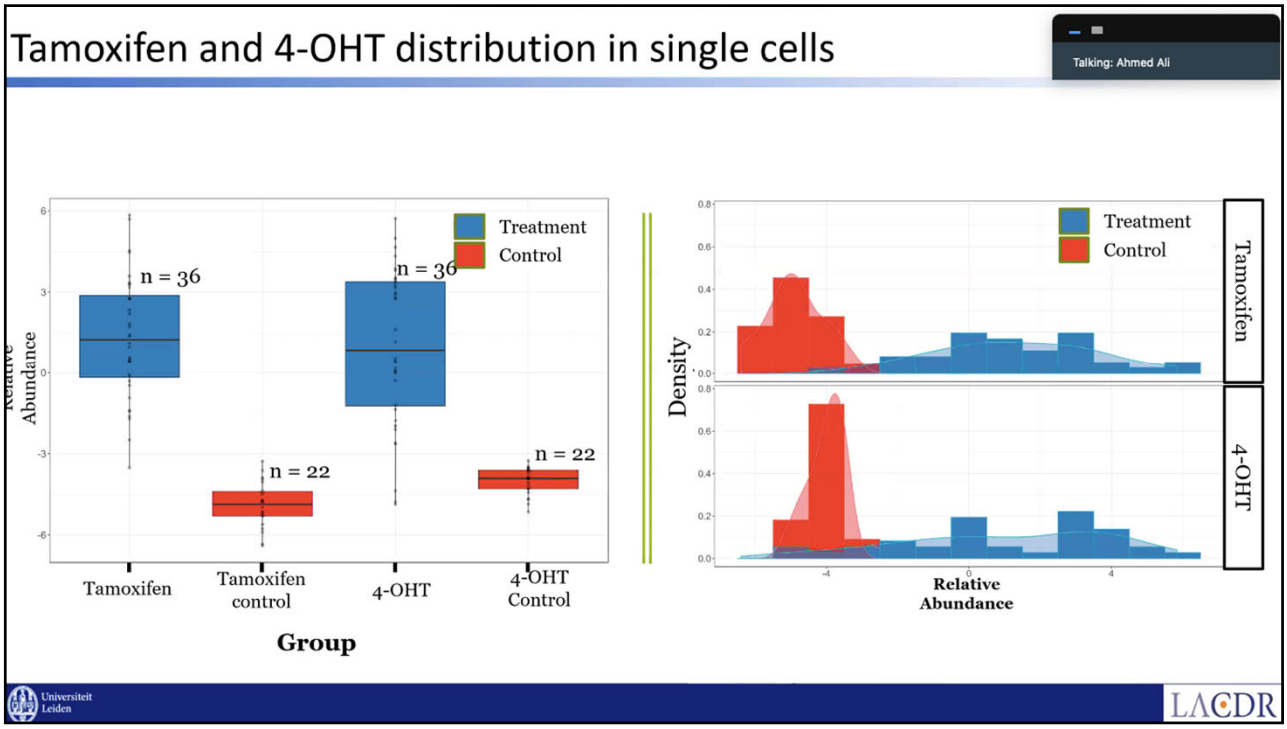
Tamoxifen and 4-OHT distribution in single cells
Talking: Ahmed Ali
Treatment
Control
n = 36
n = 22
n = 36
n = 22
Tamoxifen
Tamoxifen control
4-OHT
4-OHT Control
Group
Density
Relative Abundance
Tamoxifen
4-OHT
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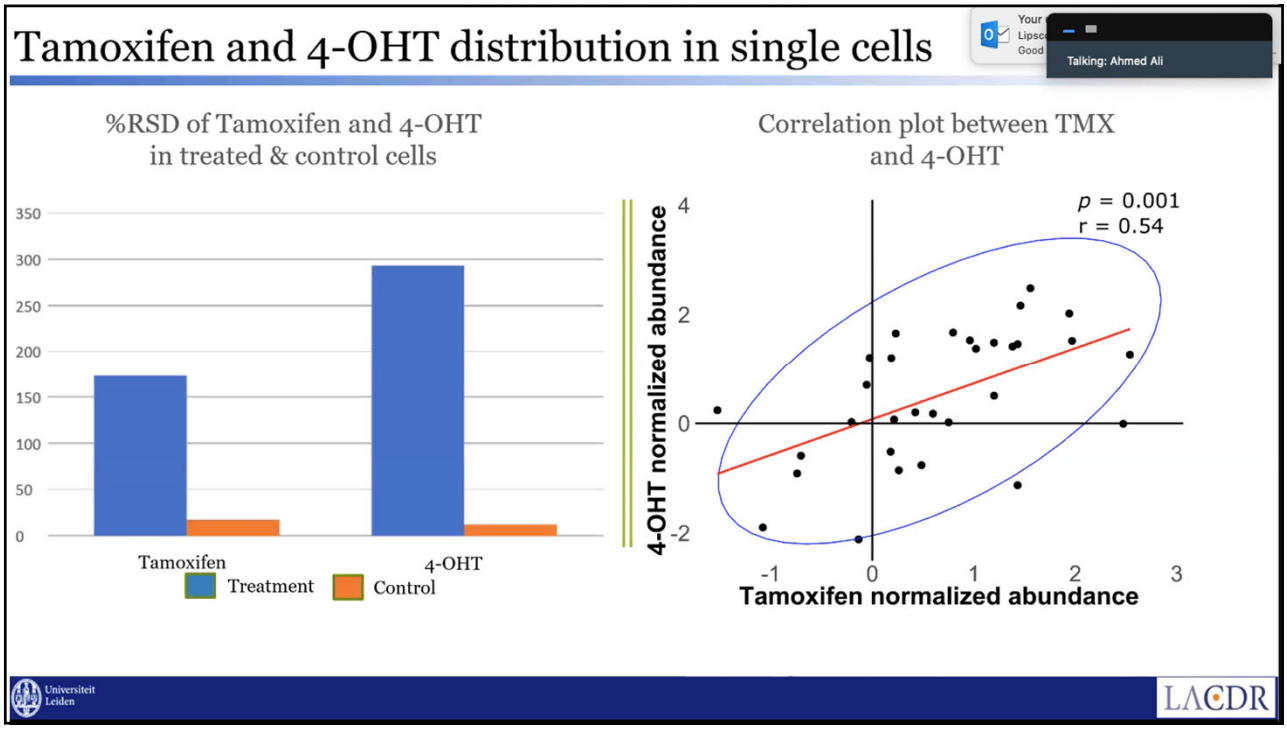
Tamoxifen and 4-OHT distribution in single cells
Talking: Ahmed Ali
%RSD of Tamoxifen and 4-OHT in treated & control cells
350
300
250
200
150
100
50
0
Tamoxifen
4-OHT
Treatment
Control
Correlation plot between TMX and 4-OHT
4-OHT normalized abundance
4
2
0
-2
p = 0.001
r = 0.54
-1
0
1
2
3
Tamoxifen normalized abundance
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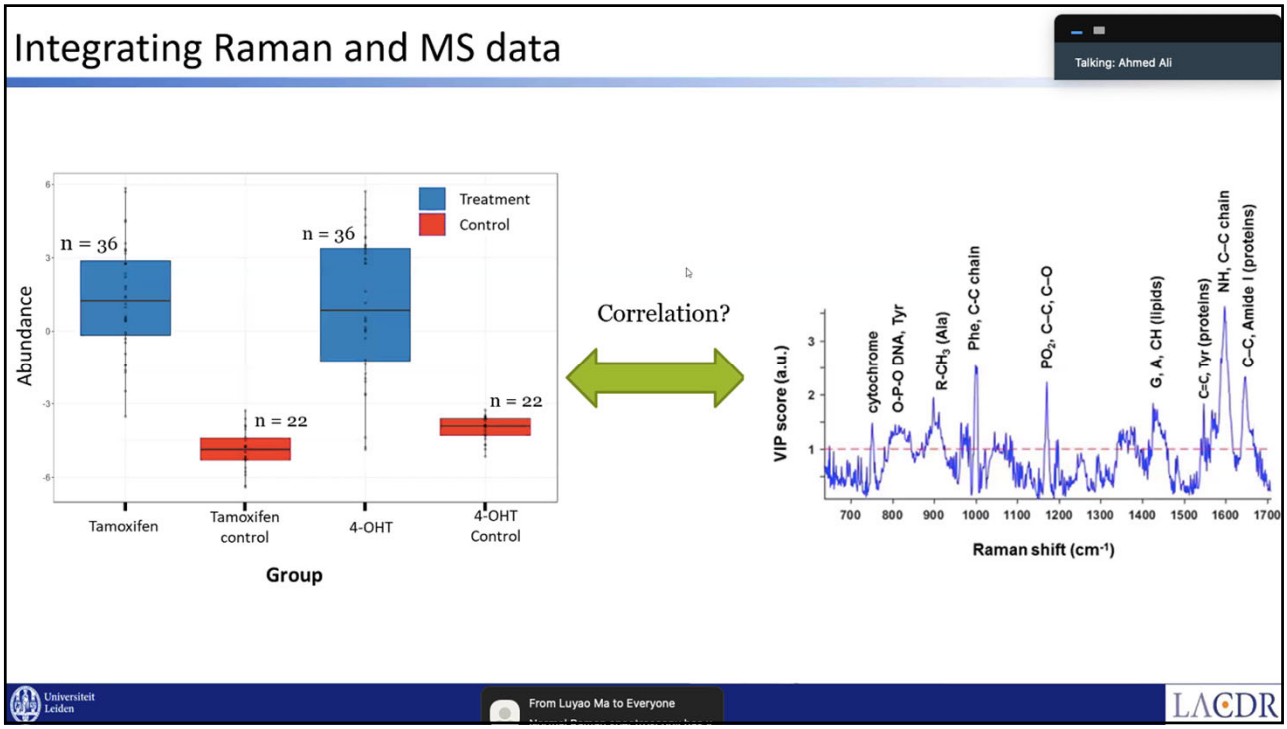
Integrating Raman and MS data
Talking: Ahmed Ali
Treatment
Control
n = 36
n = 36
n = 22
n = 22
Abundance
Tamoxifen
Tamoxifen control
4-OHT
4-OHT Control
Group
Correlation?
VIP score (a.u.)
cytochrome
O-P-O DNA, Tyr
R-CH3 (Ala)
Phe, C-C chain
PO2, C–C, C–O
G, A, CH (lipids)
C=C, Tyr (proteins)
NH, C–C chain
C–C, Amide I (proteins)
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
Raman shift (cm-1)
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From Luyao Ma to Everyone
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Live Single-Cell mass spectrometry shortcomings

Talking: Ahmed Ali

- Qualitative only
- Exhaustiveness
- **Low Throughput**
- Robustness
- Data analysis

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Live Single-Cell mass spectrometry shortcomings

Talking: Ahmed Ali

- Qualitative only
- Exhaustiveness
- **Low Throughput**
- Robustness
- Data analysis

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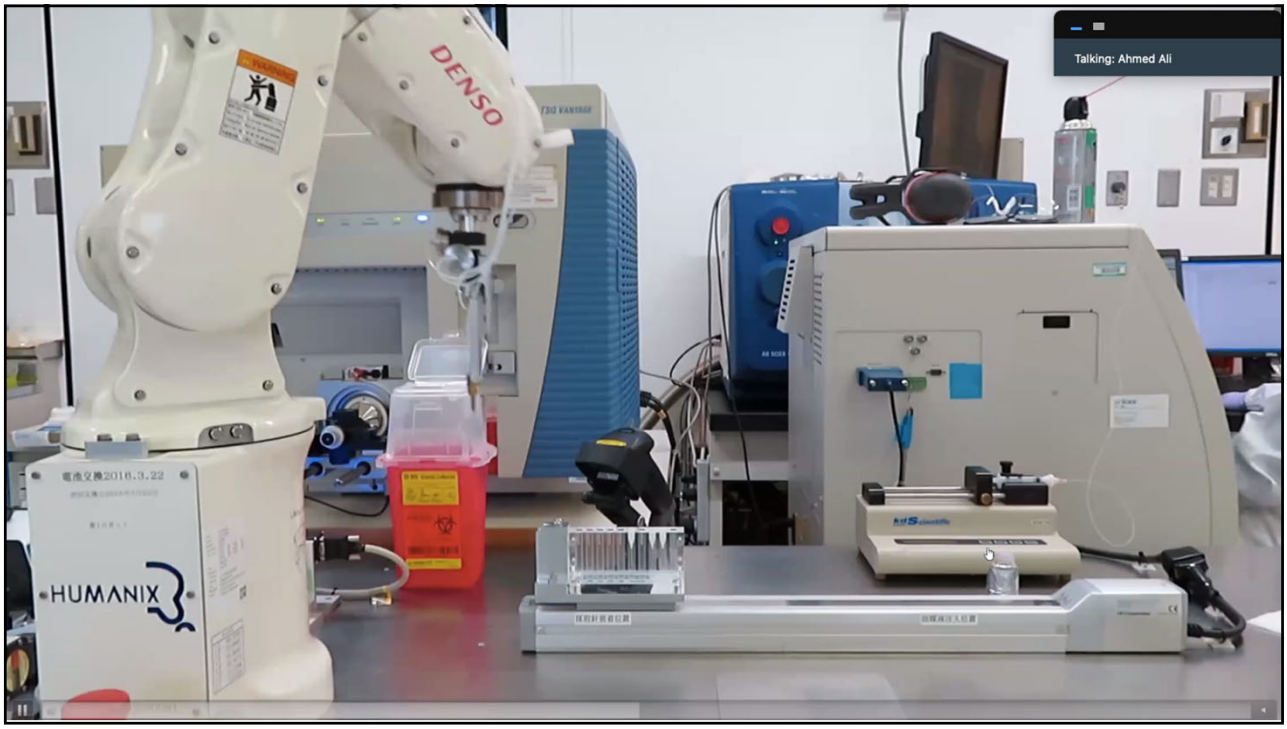

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Data Analysis: Unified Data analysis pipeline for LSC-MS in R

Talking: Ahmed Ali

- Convert raw thermo files
- Data processing
- Unsupervised filtering
- Normalization
- Unsupervised analysis
- Supervised analysis
- Fancy statistics

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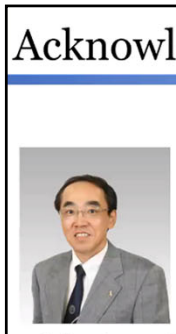
Acknowledgments


Prof. Eiso Hiyama


Dr. Arno Germond


Dr. Yasmine Abouleila


Prof. Tsutomu Masujima

BDR

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