ToF-SIMS for Biological Research – Sample Preparation Techniques

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Polytechnical institute:
- Owned by the swedish government
- Ca 830 employees, ca 550 in Borås
- 8 technical divisions, including
  - Measurement technology
  - Energy technology
  - Construction and Mechanics
  - Fire technology
TOF-SIMS instrument at SP

- TOF-SIMS IV, purchased December 1999
- Bi$_n$ LMIG source
- C$_{60}$ source
- Heating and cooling (LN$_2$) in loadlock and main chamber
TOF-SIMS group at SP

Ca 30 % contract work for industry
  – Failure analysis, materials characterization, production problems, quality control, ...
  – Medical device and pharmaceutical industry, manufacturing industry, electronics, etc.

Ca 70 % research projects
  – Collaboration with academic research groups
  – Collaboration with industry
  – Mainly bioscience (also combustion, polymers, coatings, metrology, ...)

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Examples of research projects (ToF-SIMS group)

• **ToF-SIMS imaging of biological samples**
  – lipid model systems, cells and tissue
  – Improve lateral resolution, 3D analysis and identification of biomolecules

• **Geochemistry**
  – Detection and localisation of organic molecules (microorganisms) in geobiological samples
  – Detection of biomarkers in fluid inclusions

• **Marine antifouling**
  – Formulation, characterization and evaluation of new coatings

• **Biomaterals**
  – Surface modification for optimizing clinical function
  – Characterization of implant surface and implant/tissue interface
Outline

1. Introduction to ToF-SIMS of biological samples
2. Sample preparation strategies and techniques
   • Freezing /drying
   • Surface preparation
3. Examples
   • Tissues
   • Cells
Current methods used for biological samples

FE-SEM

TEM

Fluorescence

Mass spectrometry

Histology

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Advantages of TOF-SIMS

- **Identification** of biomolecules by mass spectrometry \((m/z < \sim 5000)\)
- **Mapping** of biomolecules in biological samples \(< 1 \mu m\)
- **Chemical characterisation** of small structures

Without labelling and staining!
ToF-SIMS analysis of biological samples

Opportunities in biomedical research:

- Basic knowledge about the chemical composition of specific structures in cells and tissue
- Disease-induced (or other stress-related) changes in local chemical composition
- Localization of pharmaceuticals and mapping of drug-induced chemical changes
ToF-SIMS analysis of biological samples

Challenges:

- Analysis in vacuum! → Freezing, drying?
- Sample specificity! → How to expose the relevant structures on the sample surface?

Sample preparation!

- Chemical complexity of biological samples?
- Quantification, identification, image interpretation?

Data interpretation!

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Preparation strategies – the vacuum problem

Biological structures (e.g. cell membranes) depend on water
→ Analysis requires dried or frozen sample

Air drying will cause chemical rearrangements
→ Freeze drying necessary

Normal freezing gives rise to crystallization, which may damage the structures and cause chemical redistribution
Amorphous ice produced by
→ Plunge freezing (> $10^4$-$10^5$ K/s), or
→ High-pressure freezing

Slow freeze drying at low temperatures (water recrystallization occurs at around -80 - -90°C)
Plunge freezing

Sample ”plunged” into liquid nitrogen cooled ethane or propane at -185 C

Prevents boiling at sample surface, which otherwise limits the heat transfer

Used by us for preparation of lipid bilayer systems and cell samples
Preparation strategies – surface preparation

Cryomicrotoming

Frozen tissue → Cutting of sections of frozen tissue → Section placed on substrate → Analysis after freeze drying

Freeze fracturing

Sample sandwiched between two substrates → Plunge freezing → Separation of the two substrates → Analysis in frozen hydrated state or after freeze drying

Ion sputtering ($C_{60}^+$)

Freeze dried or frozen hydrated sample → Material removal by ion sputtering → Analysis of sputtered sample
Tissue examples

1. Mouse brain tissue

2. Adipose tissue from patients with chronic kidney disease (CKD)
Tissue preparation

Plunge freezing or high-pressure freezing often difficult/impossible
→ Crystallization may be accepted

Cryosectioning at -15 - -20 C provides flat tissue surfaces without smearing
→ typically 15 µm thick
→ successive sections can be used for ”3D analysis” or for complementary analysis (histology, SEM, …)
→ attachment of sections on substrate by ”finger-thawing” (or pressed into indium substrate)
→ risk for contamination (OCT,…)

Freeze drying

Analysis at room temperature or below
Mouse brain tissue, negative TOF-SIMS spectrum

Detected lipids:

- Phosphatidylcholine (PC)
- Cholesterol
- Sphingomyelin
- Sulfatides
- Phosphatidylinositol (PI)
- Vitamin E

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TOF-SIMS images from mouse brain tissue

7 x 7 mm²

Palmitate, $C_{16}H_{31}O_2^-$
Cholesterol, $(M-H)^-$
ST 24:0/1+h24:0/1, $(M-H)^-$
Sphingomyelin
PI 38:4, $(M-H)^-$
Palm+chol+sulf
Sagittal section, caudate putamen

- Colocalization of cholesterol and sulfatide
- Complementary localization with PC
- Spots in N-containing ion images shows cell nuclei
Temperature-controlled measurements

Lipid migration: Cholesterol migrates to surface at T> ~0C
ToF-SIMS analysis of adipose tissue from kidney patients and controls

- Subcutaneous fat tissue
- 7 kidney patients and 6 controls
- Biopsies frozen, cryosectioned (15 µm thickness), placed on glass and stored at -80 C
- Freeze dried immediately before TOF-SIMS analysis
Adipose tissue from CKD patients, positive TOF-SIMS spectrum

**Triglycerides (TAG):**
- Main ingredient in animal fat
- Energy storage in adipose tissue

**Detected lipids:**
- TAG, Diacyl glycerol (DAG) and Fatty acids (FA)
- Phosphatidylcholine
Adipose tissue from CKD patients, positive TOF-SIMS spectrum
Principal component analysis (PCA)

Identifies correlations and systematic variations in large data sets

- Each individual has a characteristic pattern/distribution of glycerol lipids
- Compared to the controls, patients tend to have higher relative signal from unsaturated DAGs
Positive TOF-SIMS images of adipose tissue

Field of view: 500 × 500 µm²

PHOSPHOCHOLINE

Sample "A"

Sample "B"

- Phosphocholine and DAG complementary localized
- Different spatial distributions for saturated and unsaturated DAGs
TOF-SIMS analysis of cells

Aim:
- Chemical composition of subcellular structures
- Mapping of (inhomogeneous) spatial distribution of lipids on cell membrane
Cell examples

1. Surface-adhering hTERT cells (fibroblasts)

2. Chemical imprinting of PMLN cells (leukocytes)
TOF-SIMS analysis of cells

Sample preparation:

1. hTERT (fibroblasts) on SiO₂ substrate
2. Removal of salt by rinsing in NH₄HCOO
3. Drying: plunge freeze + freeze drying
4. TOF-SIMS analysis

Or, alternatively

1. Fixation in glutaraldehyde
2. Rinsing in deionized water
3. Drying: plunge freeze + freeze drying
4. TOF-SIMS analysis
TOF-SIMS analysis of hTERT cells

Video image of cell sample
ToF-SIMS of hTERT cells

199 x 199 µm²

Phosphatidylcholine fragments

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ToF-SIMS of hTERT cells

199 x 199 µm²

- K inside cell, Na outside
- Some K leakage out from cell
- Phosphatidylcholine not redistributed
hTERT, profile

125x125 µm, m/z 86

Profile:
signal intensity along line in image

- Sharpness of cell edge: ca 1.4 µm
- Lipids in filopodia detectable
3D analysis of cells

How to expose cell interior for TOF-SIMS analysis?

- Ion etching with $C_{60}^+$ ions (Breitenstein et al)
- Freeze fracturing (Winograd et al)
- Cryosectioning (Arlinghaus et al)
- Chemical imprinting
- Removal of cell membrane by Triton-X detergent
Cell imprinting:

- Transfer sample molecules to substrate surface with retained lateral distribution

- Imaging TOF-SIMS of chemical imprint

Advantages:
- Optimised analysis conditions
- Access to intracellular regions
TOF-SIMS images of cell imprint

phosphocholine$^+$
184 u

(Ag-cholesterol)$^+$
493-496 u

Ag$_3^+$
323 u

CH$_4$N$^+$
30 u

(Ag-cholesterol$_2$)$^+$
879-882 u

Total ion image
TOF-SIMS images of cell imprint
Concluding remarks

• Chemical analysis of biological samples with subcellular resolution possible with ToF-SIMS
  – Mass range 0 - ~2000 Dalton (lipids, peptides, pharmaceuticals, …)

• Sample preparation critical for obtaining relevant information
  – Different methods should be applied based on the requested information

• Collaboration with biomedical research groups important
  – Formulation of relevant biological questions

• SI Ontario now very well equipped for ToF-SIMS analysis of biological samples
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and

Good luck!