

# “i c lipid”

## immersion course into mass spectrometry-based lipidomics

by SLING, the Singapore Lipidomics Incubator

### Day 1, Monday

- 09.00-09.45 Opening remarks and introduction of participants  
Discussion of individual participants' aims and objectives (participants).
- 09.45-10.15 Lecture 1: “The emerging field of lipidomics”  
Introduction to lipid classes, biology and biochemistry of lipids, lipidomics as an emerging field
- 10.15-10.40 *Coffee break*
- 10.40-11.00 Lecture 2: “Introduction to lipidomics terminology”  
Overview of common lipids and mass spectrometry terms that will be used throughout the course.
- 11.00-12.00 Lecture 3: “Introduction to lipid extraction”  
Challenges, general liquid-liquid extractions (Bligh & Dyer, Folch), solvents and contaminations, single phase extraction, solid phase extraction (SPE), automation, storage of extracts
- 12.00-14.00 *Lunch*
- 14.00-17.00 Practical Demonstration 1: “Lipid extraction”  
Various extraction modes on three different samples: blood plasma, palm oil, automated bravo extraction.

### Day 2, Tuesday

- 09.00-09.45 Lecture 4: “Liquid chromatography-mass spectrometry (LC-MS) techniques for lipid analysis”  
Thin layer chromatography, normal vs. reversed phase chromatography for LC-MS applications, most common mobile and stationary phases, nanoflow techniques.
- 09.45-10.30 Lecture 5: “Soft ionization methods”  
Basic principles of ESI and MALDI, emerging approaches for surface analysis of lipids
- 10.30-11.00 *Coffee break*
- 11.00-12.00 Lecture 6: “High resolution mass spectrometry of lipids”  
Discussion of how mass accuracy, resolution, and sensitivity relate to analysis of lipids. Overview of instrumentation used for high resolution analysis (Orbitrap, Q-ToF). Ultra-high resolution analysis using FT-ICR MS.
- 12.00-14.00 *Lunch*
- 14.00-17.00 Practical Demonstration 2: “Lipid analysis using Q-ToF and Orbitrap”  
LC-MS analysis of samples extracted on day 1, comparison between mammalian and non-mammalian lipid profiles. TLC.

### Day 3, Wednesday

- 09.00-09.45 Lecture 7: "Tandem mass spectrometry of lipids"  
Collision induced dissociation of lipids, product ion analysis and brief introduction of well characterized fragmentation pathways of membrane lipids
- 09.45-10.30 Lecture 8: "Targeted lipid analysis using triple quadrupole instruments"  
Low-resolution MS instruments (triple quadrupoles, Qtraps), Q1/Q3 scans, product ion scans, precursor ion scans, neutral losses, multiple reaction monitoring (MRM)
- 10.30-11.00 *Coffee break*
- 11.00-12.00 Lecture 9: "Shotgun techniques vs. LC-MS"  
Side by side comparison and discussion of pros and cons (participant exercise)
- 12.00-14.00 *Lunch*
- 14.00-17.00 Practical Demonstration 3: "Lipid analysis using triple quadrupole mass spectrometers"  
Class-specific analysis, structure elucidation (head groups, fatty acyl composition), quantification by MRM and internal standards

### Day 4, Thursday

- 09.00-09.45 Lecture 10: "Quality control measures"  
Technical variation and how to control for it, batch effects, trouble shooting
- 9.45-10.30 Lecture 11: "Emerging bioinformatics resources for mass spectrometry based lipidomics"
- 10.30-11.00 *Coffee break*
- 11.00-12.00 Lecture 12: Participants to be split in 3 groups to work on practical exercises  
(i) Building MRM lists, (ii) Peak annotation, (iii) Workflow for specific biology question.
- 12.00-14.00 *Lunch*
- 14.00-17.00 Practical Demonstration 4: "Raw data handling and analysis"

### Day 5, Friday

- 09:00-09:30 Hot stuff in lipidomics
- 09.30-12.00 Short presentations by participants  
Placed towards the end of the training course this session should serve as an opportunity for feedback and discussion on outstanding issues. Each participant will present approximately 10 min on original aims and achievements during "i c lipid".
- 12.00-14.00 *Lunch*
- 14.00-16.00 Practical Demonstration 5: Sample sets submitted by participants
- 18:00-21:00 ***Farewell dinner***