Method optimization for on-tissue tryptic digestion of formalin-fixed paraffin-embedded human thyroid tissues for MALDI Imaging MS

Nidia Lauzon[1], Ethan Yang[2], Assim Alfadda[3], Pierre Chaurand[2]

[1] Research Institute of McGill University Health Center, Montreal, Quebec, Canada
[2] Department of Chemistry, Université de Montréal, Montreal, Quebec, Canada
[3] Obesity Research Center, King Saud University, Riyadh, Saudi Arabia

Introduction

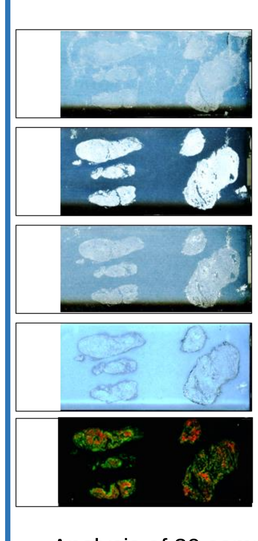
Obesity is associated with numerous diseases including abnormalities of the endocrine system like insulin resistance and dysfunction of the thyroid. Goiter is a manifestation of an increase in the thyroid dysfunction, commonly known to be caused due to iodine deficiency. Obesity is well known to be associated with goiter and an increase with weight leads to an increase in its incidence. We aim to employ Matrix-Assisted Laser Desorption/Ionization (MALDI) Imaging Mass Spectrometry (IMS) to study the changes in the protein distribution directly within the tissue to better understand the relationship between the disease entity and obesity.

Methods

All FFPE tissue sections were sectioned at 5 µm and mounted on ITO conductive slides. Serial sections were also cut and mounted on glass slides for H&E staining. Deposition of trypsin Gold (Promega) and CHCA matrix were performed using the HTX M3 TM-sprayer (HTX Technologies). Profiling and IMS of the tissue sections were performed on a MALDI TOF/TOF Ultraflextreme mass spectrometer equipped with a SmartBeam II Nd:YAG 355 nm laser operating at 2000 Hz, using the medium laser focus setting (Bruker Daltonics). IMS data were acquired using 300 shots per pixel in a mass range of 700-5000 Da. Data analysis was performed with flexAnalysis 3.4 and flexImaging 4.1. Statistical analysis and biomarker discovery were performed using the SCiLS Lab software (2014).

Results

Workflow



FFPE tissue provided by the Obesity Research Center (King Saud University)

Deparaffinization and Antigen Retrieval were automated using the Discovery Ultra instrument (Histopathology Platform)

Tryptic digestion using the TM-Sprayer followed by 4 hrs of incubation at 55°C

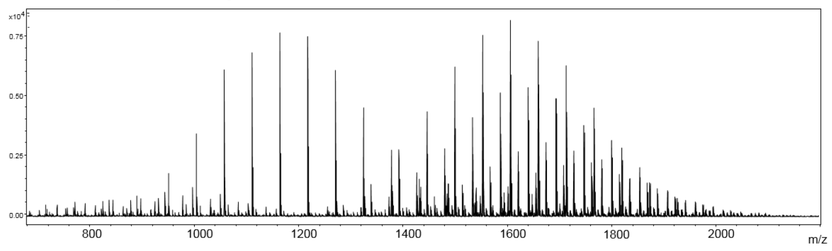
CHCA deposition using the TM-Sprayer

MALDI Imaging

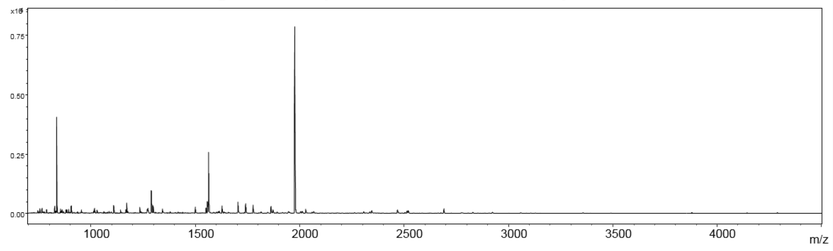
Analysis of 30 samples (15 Lean, 15 Obese) in SCiLS (LDA, ROC Curve plots)

Method Optimization

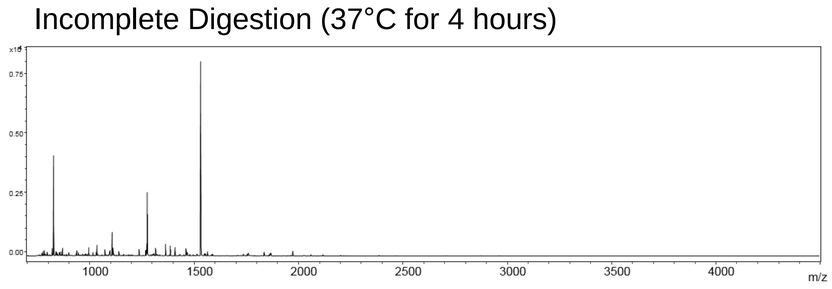
Incomplete Deparaffinization



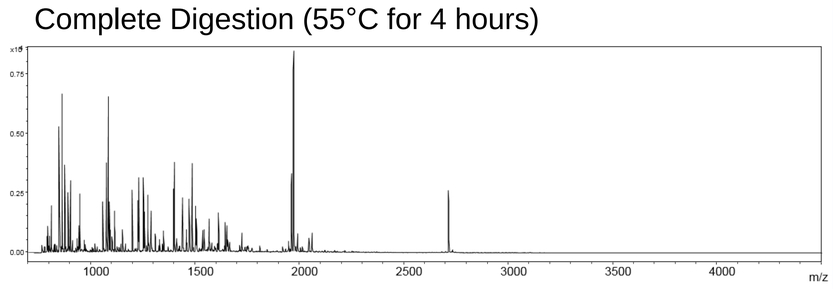
Incomplete Digestion (37°C for 18 hours)



Incomplete Digestion (37°C for 4 hours)



Complete Digestion (55°C for 4 hours)

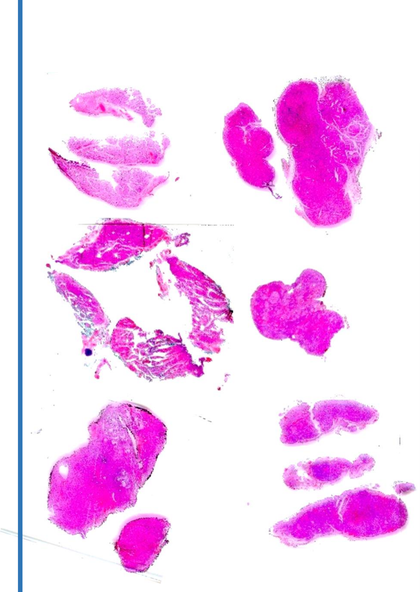


Methods of deposition

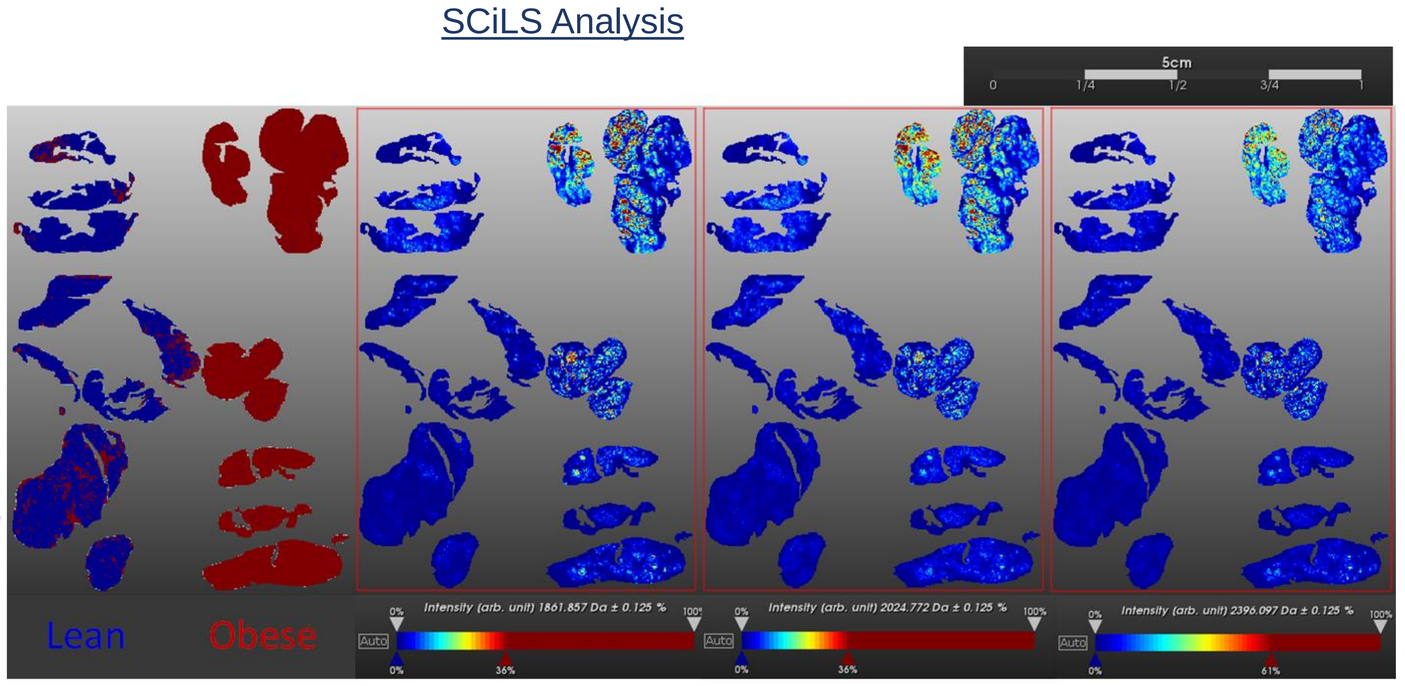
Trypsin
M3 TM-sprayer connected to a syringe pump
83 ng/µL (20 µg in 100 mM NH_4HCO_3)
30°C
10 psi
7.5 µL/min
750 mm/min (VV pattern)
10 passes

CHCA
M3 TM-sprayer connected to an isocratic LC pump
5 mg/mL (50% ACN, 0.1% TFA)
65°C
10 psi
0.1 mL/min
1200 mm/min (VV pattern)
12 passes

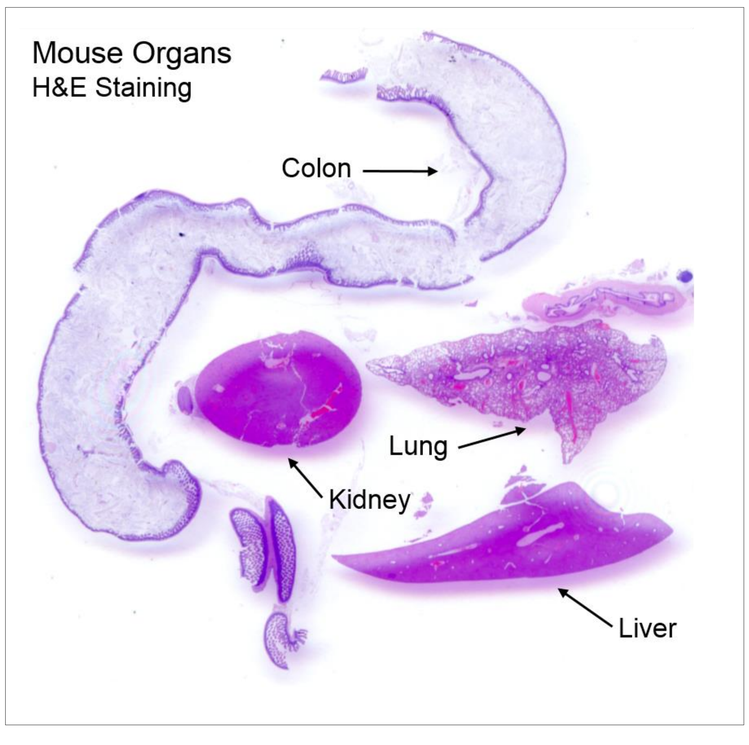
SCiLS Analysis

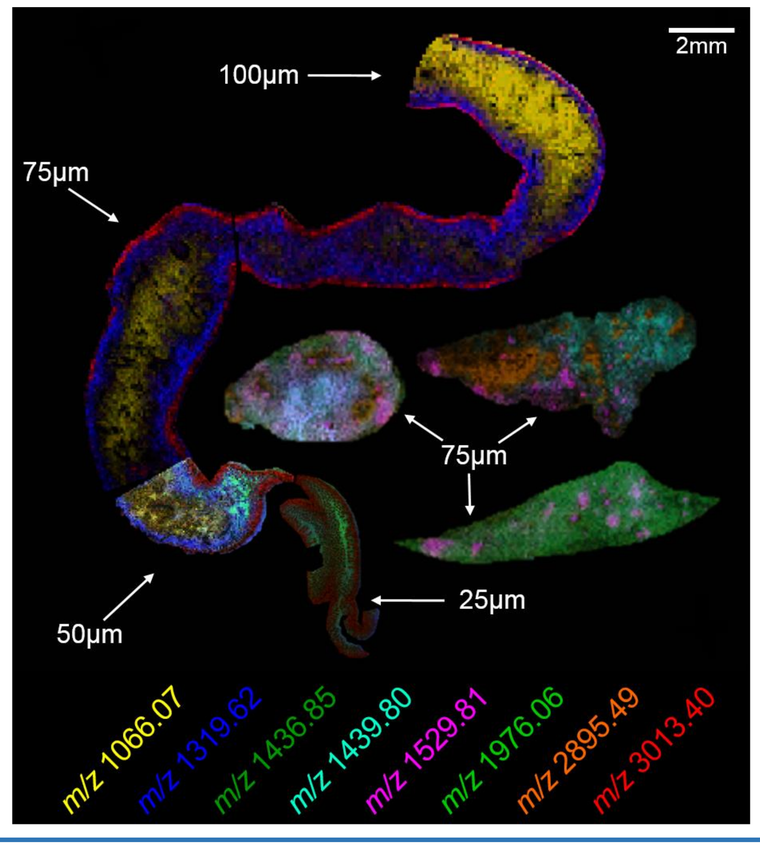


H&E Staining



High-Resolution Imaging





Conclusion

Our work demonstrates the potential of using this protocol for on-tissue tryptic digestion of FFPE human thyroid. By raising the temperature at 55°C during the incubation process, we were able to yield the best enzymatic digestion in order to study the changes in the protein distribution within the thyroid tissue. A shorter incubation time also allows to perform the complete protocol in one workday (8hrs). We also verified that this protocol can be used on other FFPE tissue types. In all cases, high digestion efficacy was achieved and high-resolution MALDI IMS datasets were generated.

Acknowledgements

The authors would like to acknowledge financial support from King Saud University and Fazila Chouiali from the RI-MUHC histopathology platform for her time and effort in optimizing the on-tissue FFPE deparaffinization/antigen retrieval protocol.