Mass Spectrometry HistoChemistry: A novel spatial single cell multi-omics approach for molecular biomarker discovery from histopathologically documented FFPE samples

Peter Verhaert, Kenneth Verheggen, Nivedita Bhattacharya, Samruddhi Chawan, Marthe Verhaert, Ling Shan, Lin Zhang, Dick Swaab, Wim Develter, Marc Ramael

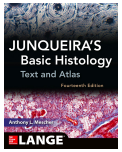
ProteoFormiX, JLABS@BE, Beerse (B), Netherlands Institute for NeuroScience, Amsterdam (NL), Heilig Hart-Z=iekenhuis, Lier (B)

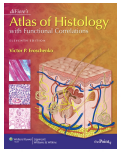
INTRODUCTION

Particular adaptations in MSI sample preparation allow for the specific **top-down** MS detection of ionisable biomolecules from the class of **endogenous peptides** as well as **metabolites**, directly in FFPE tissue sections.
This opens the road to untargeted (discovery mode) analyses of FFPE tissues, an approach which we baptised MSHC (**mass spectrometry histochemistry**), analogous to immunohistochemistry (IHC) a targeted method which employs antibodies to localise specific molecules on FFPE fixed tissue sections.

With a skilled expert team consisting of hardware engineers, software developers, (bio)informaticians, as well as (histo)pathologists and biomedical research scientists, we are continually working on the optimisation of the performance of MSHC.
In terms of sensitivity, robustness, and 'user-friendliness', MSHC appears to have reached the 'technology ready level' sufficient to be applied to the mining of the vast archives of clinically well-documented human FFPE materials piled up in biobanks all over the planet.

THE HUMAN PROTEIN ATLAS





Hence we have initiated the compilation of a MSHC Atlas of the FFPE Human Body (Healthy and Diseased), complementing classical histology atlases.
Obviously, our goal is to integrate this Human FFPE MSHC Atlas with current complementary initiatives, such as the Human Protein Atlas (https://www.proteinatlas.org/), and the Human BioMolecular Atlas Program (https://commonfund.nih.gov/hubmap).

Such *Homo sapiens* FFPE molecular histology atlas will be made accessible for use in pathological applications, including translational disease biomarker discovery.

SAMPLE PREPARATION

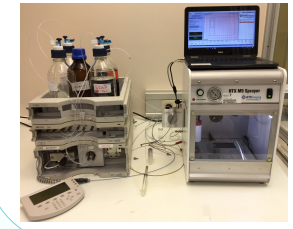
FFPE samples:
- formaldehyde fixation and paraffin embedding of tissues by standard procedures utilized in hospitals and biobanks

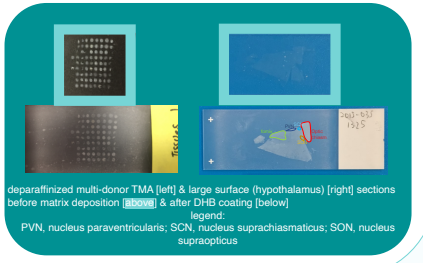
- Microtome sectioning (5μm)
- Sectioned mounted on regular microscope slides

- origin: ***Homo sapiens***
- well documented:
  - hospital biobanks
  - archived research institute collections
  - healthy & diseased
- 2 formats:
  - tissue microarrays (TMA) [multiple donors]
  - large(r) surface [individual specimens]

- model tissues: hypothalamus and its neuropeptide storage and release site, i.e. neurohypophysis / posterior pituitary
- tissues representing diseases, e.g. colon carcinoids

Matrix coating:
- DHB (2,5 dihydroxybenzoic acid) using M5 Sprayer (HTX) after deparaffinization

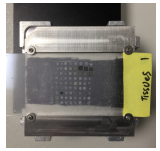




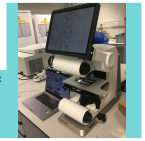
deparaffinized multi-donor TMA [left] & large surface (hypothalamus) [right] sections before matrix deposition [above] & after DHB coating [below]
legend:
PVN, nucleus paraventricularis; SCN, nucleus suprachiasmaticus; SON, nucleus supraopticus

DATA COLLECTION

MSHC Data Acquisition:
- atmospheric pressure MALDI UHR (MassTech) with home modified microscope slide adaptor to accommodate for all commonly used brands of microscope glasses
- connected to LTQ Orbitrap Velos (ThermoFisher Scientific)
- spectra recorded in full profile mode



TMA (left) & brain tissue (above) section [after MSHC analysis in specific hypothalamic nuclei]



ECHO

Orthogonal Imaging Modality:
- H&E staining; IHC
- using Revolve microscope (ECHO) for both brightfield & fluorescence imaging

MassTech

Spectroswiss

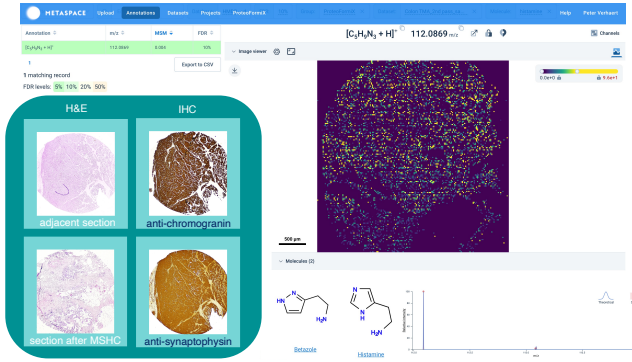
MSHC Data Processing:
- Home-built software (ProteoFormiX' imzML Heatmap Generator) for quick image or m/z based MSHC data browsing [after conversion of *.raw and *.xml data files to *.imzml and *.ibd]
- Mozaic MSI software (SpectroSwiss) for performant data processing and presentation [directly from (multi-Gb) *.raw and *.xml MSHC files]
- MetaSpace annotation for metabolite type analytes present in (biochemical) databases, such as HMDB and CheBI
- NeXtProt and UniProt for annotation of human known and novel peptides

METASPACE
The platform for metabolite annotation of imaging mass spectrometry data

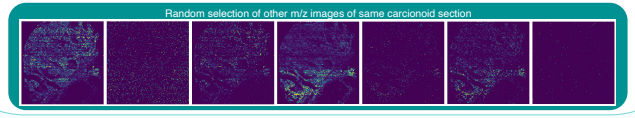
neXtprot UniProt

hmdb ChEBI

RESULTS [1]: Colon carcinoid metabolomics

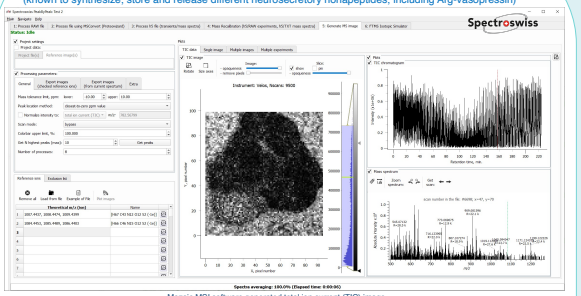


Random selection of other m/z images of same carcinoid section



RESULTS [2]: Brain (neuro)peptidomics

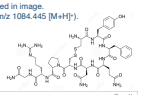
Model tissue: hypophysis including posterior pituitary
(known to synthesize, store and release different neurosecretory nonapeptides, including Arg-vasopressin)

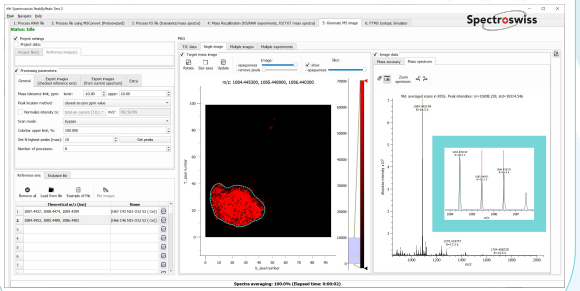


Mozaic MSI software generated total ion current (TIC) image.
Right panel shows TIC chromatogram (top) and mass spectrum (bottom) of specific scan selected in image.
Vertical green line shows position of neuropeptide ion of interest (Arg-vasopressin; a cyclic nonapeptide; m/z 1084.445 [M+H]$^+$).

$C_{46}H_{65}N_{15}O_{12}S_2$

[Cys1-Cys6] Cys - Tyr - Phe - Gln - Asn - Cys - Pro - Arg - Gly - NH_2





Target data analysis: Cyclic nonapeptide **Vasopressin** (monoisotopic mass m/z 1084.445) is iimaged (Mozaic MSI software). Right panel shows averaged mass spectrum of region of interest (ROI), with an isotopic envelope of the compound of interest shown in inset. Isotopic envelope structure suggests overlap of the A+2 isotopologue with monoisotopic peak of other compound, most probably vasopressin with opened cystine ring (reduced disulfide bridge).

CONCLUSIONS / FUTURE PERSPECTIVES

- MSHC of human FFPE tissues allows routine imaging at 15 μm lateral resolution (current pixel size limit is 10 μm) of metabolites as well as endogenous peptides with high mass accuracy (<1-2 ppm).
- Multi-Gbyte data from sections of (neuro)peptide peptide synthesizing pituitary and hypothalamic 'nuclei' demonstrate that MSHC qualifies as another single cell 'omics' technology.
- Large MSHC data sets from human carcinoids show unique *disease specific* metabolite distributions, indicating that MSHC based patient stratification is already feasible today.

- We warmly welcome interested collaborators to our *Homo sapiens* FFPE Tissue Atlas project.

ACKNOWLEDGEMENTS

eit Health
imec
istart
Johnson&Johnson INNOVATION | JLABS
BlueHealth Innovation Center supported by Microsoft
FLANDERS INVESTMENT & TRADE
AGENTSCHAP INNOVEREN & ONDERNEMEN
Vlaanderen is ondernemen