Identifying Tattoo Pigments in Human Skin Samples with Adverse Reactions based on µXRF and LDI-MS Imaging and Mass Spectral Library Matching

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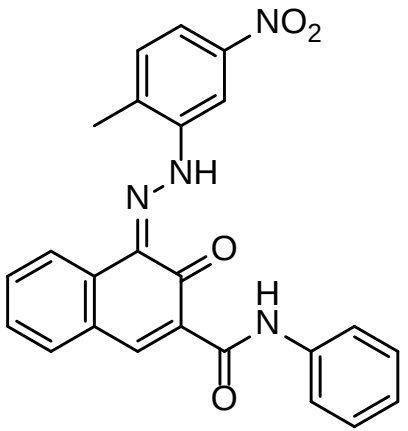
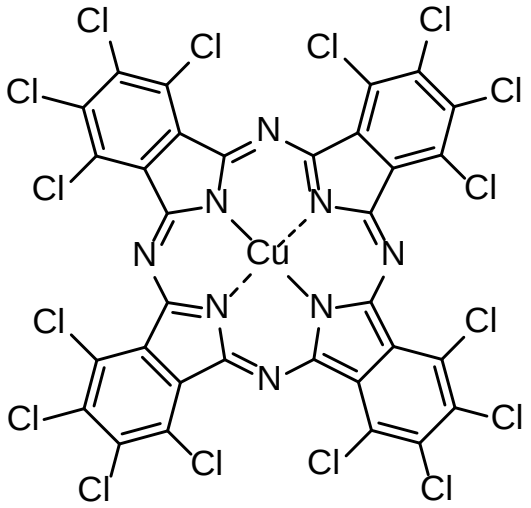
Introduction

- Inks consist of different ingredients (pigments, suspending agents, preservatives,…)
- Insoluble pigments
  - Inorganic (e.g., Carbon Black, TiO_2, Fe_xO_y)
  - Organic (e.g., hydrazone pigments, polycyclic pigments)



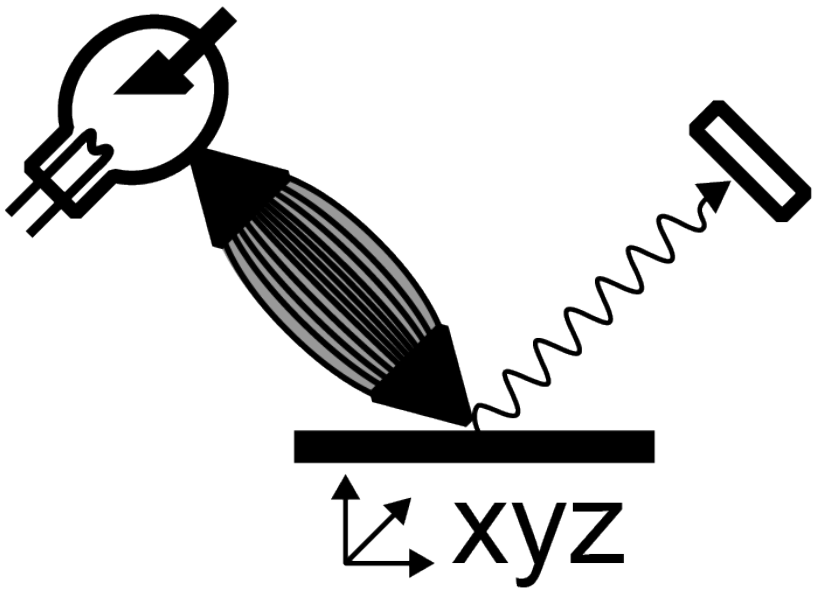
Pigment Red 22
(PR 22)



Pigment Green 7
(PG 7)

- Challenging analysis
- Elemental and molecular (bio)imaging
  - Method without time-consuming sample preparation
  - Detection of inorganic and organic pigments or specific elements of the pigments

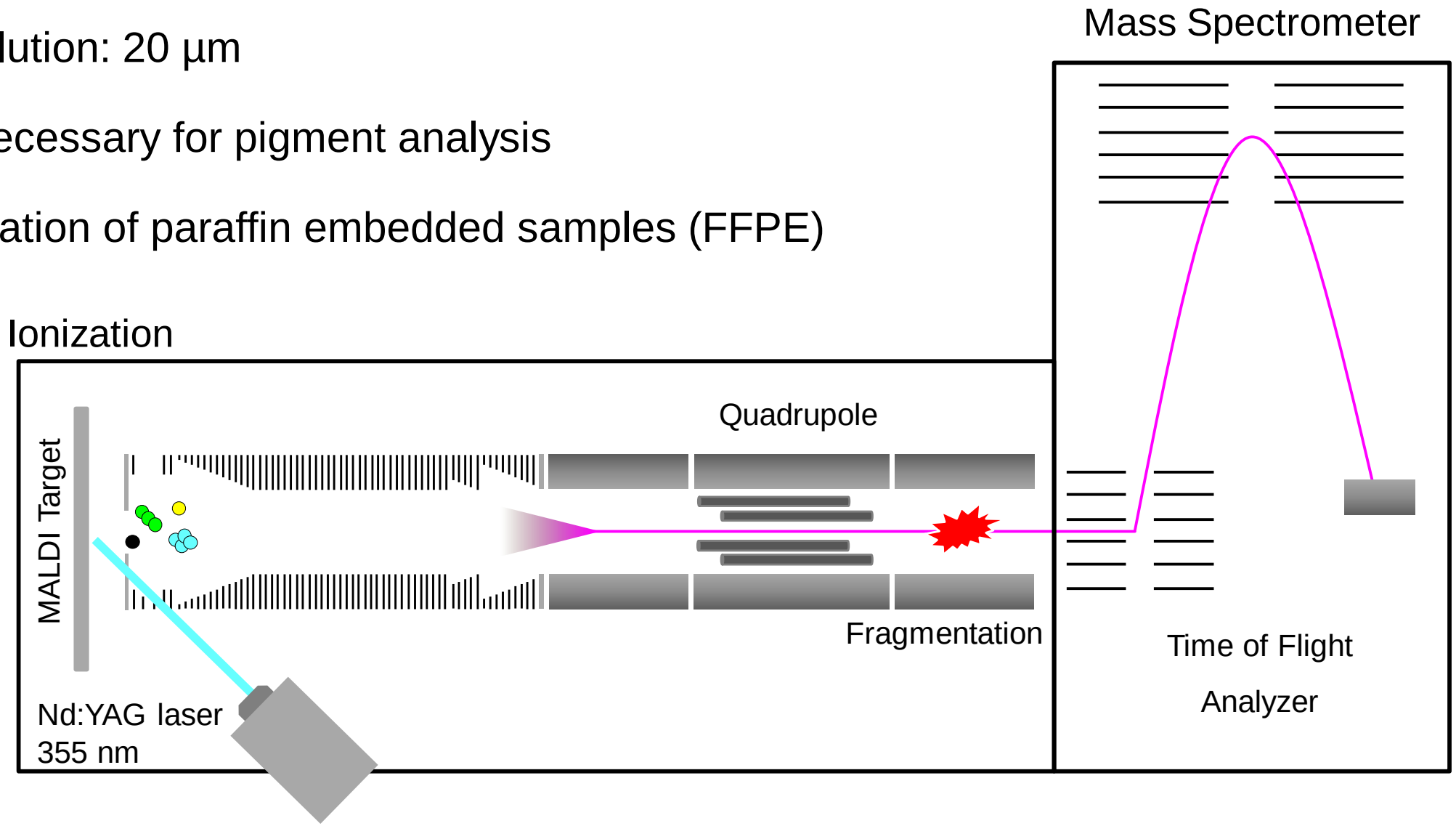
Micro X-Ray Fluorescence (µXRF) – Elemental Imaging



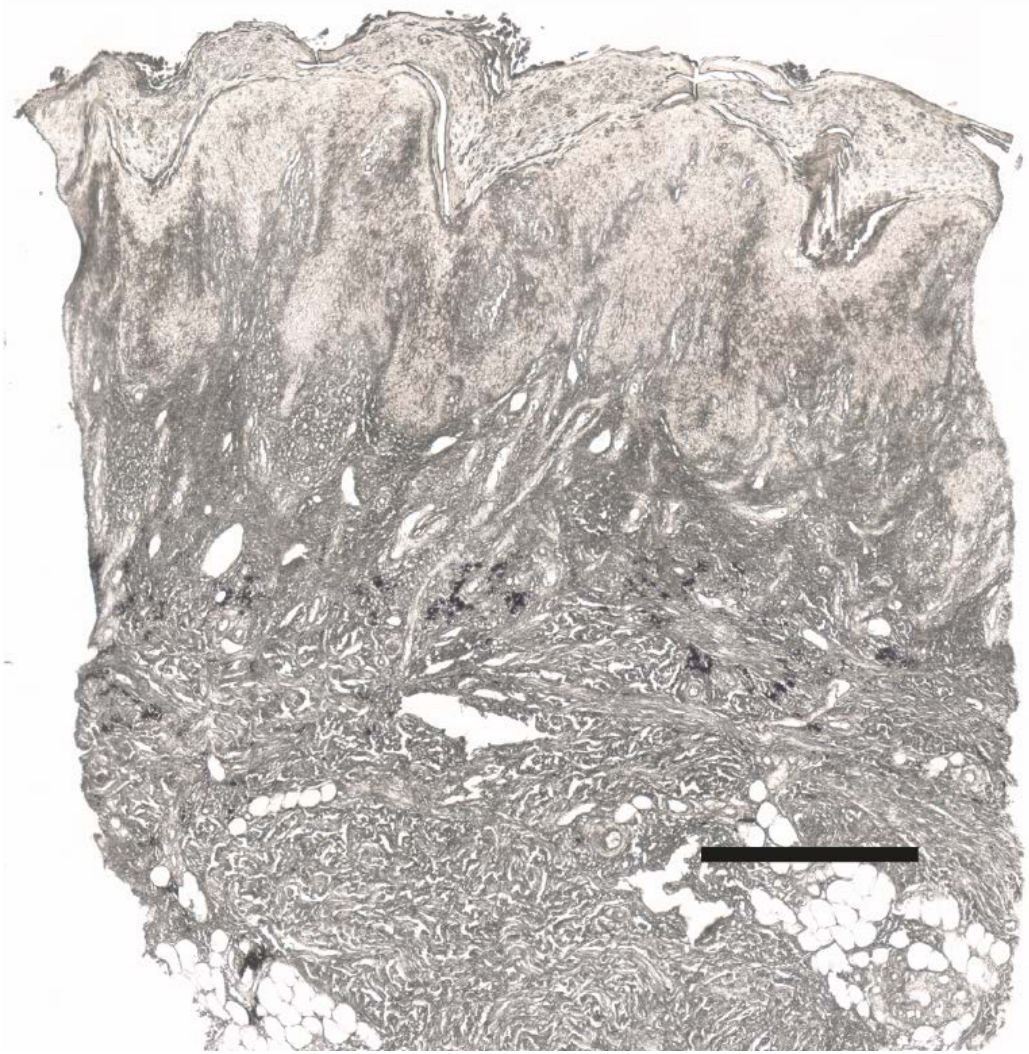
- Fast elemental screening of unknown samples
- Non-destructive technique
- Spatial resolution: 25 µm
- Higher concentrations are necessary

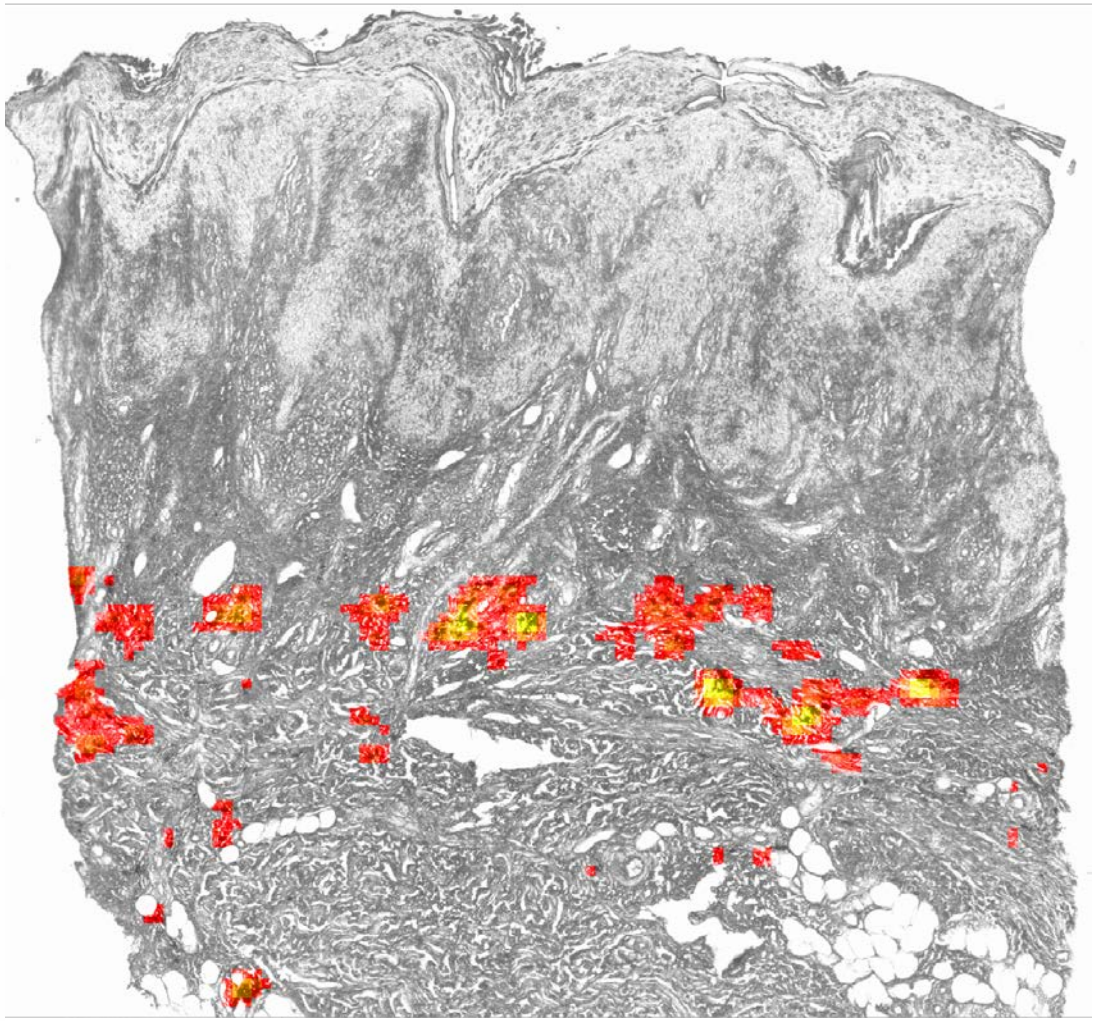
Laser Desorption Ionization-MS Imaging (LDI-MSI) – Molecular Imaging

- Molecular information (pigments)
- Spatial resolution: 20 µm
- No matrix necessary for pigment analysis
- Deparaffinization of paraffin embedded samples (FFPE)



LDI-MS Imaging of Tattooed Skin





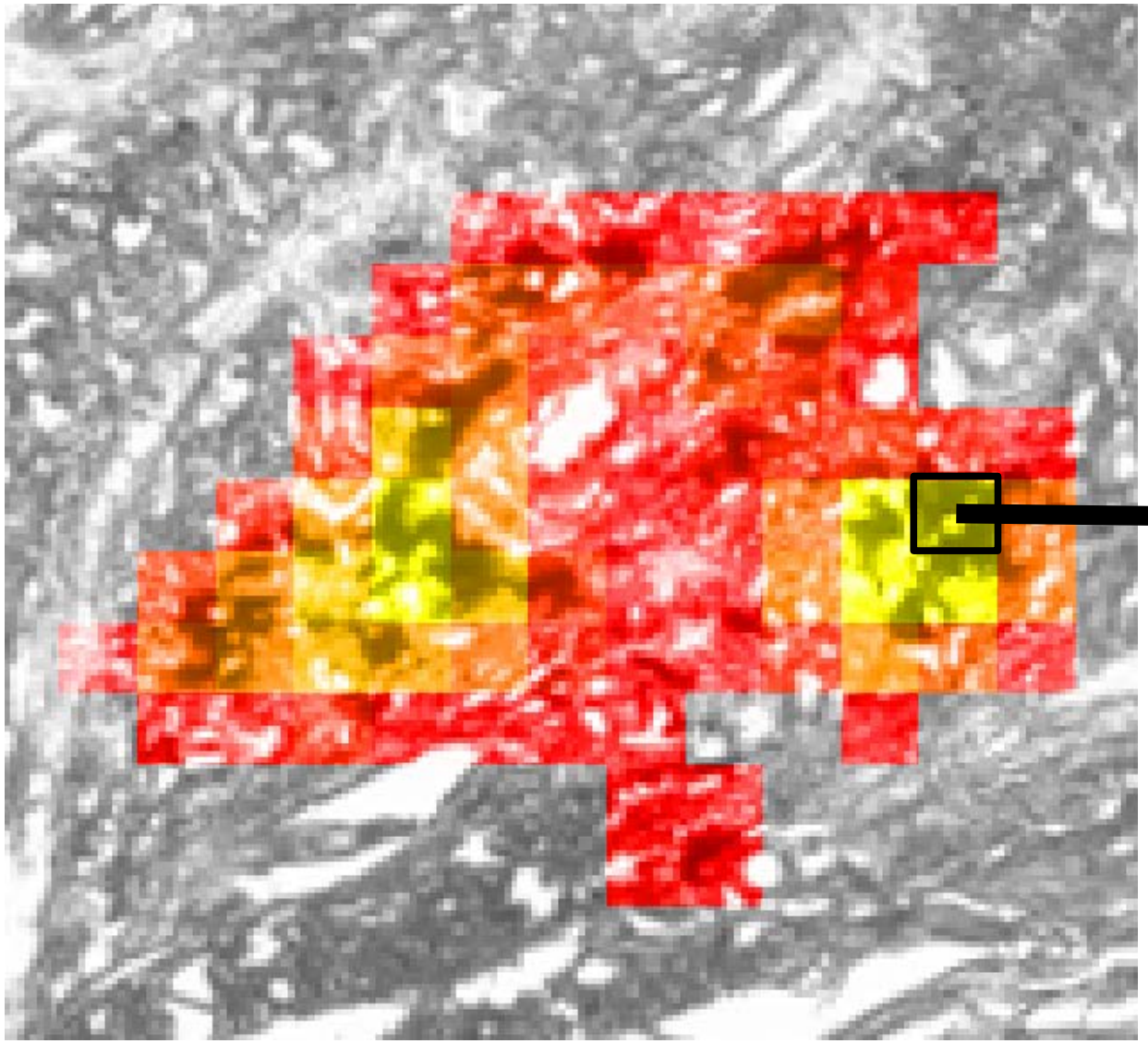
Epidermis

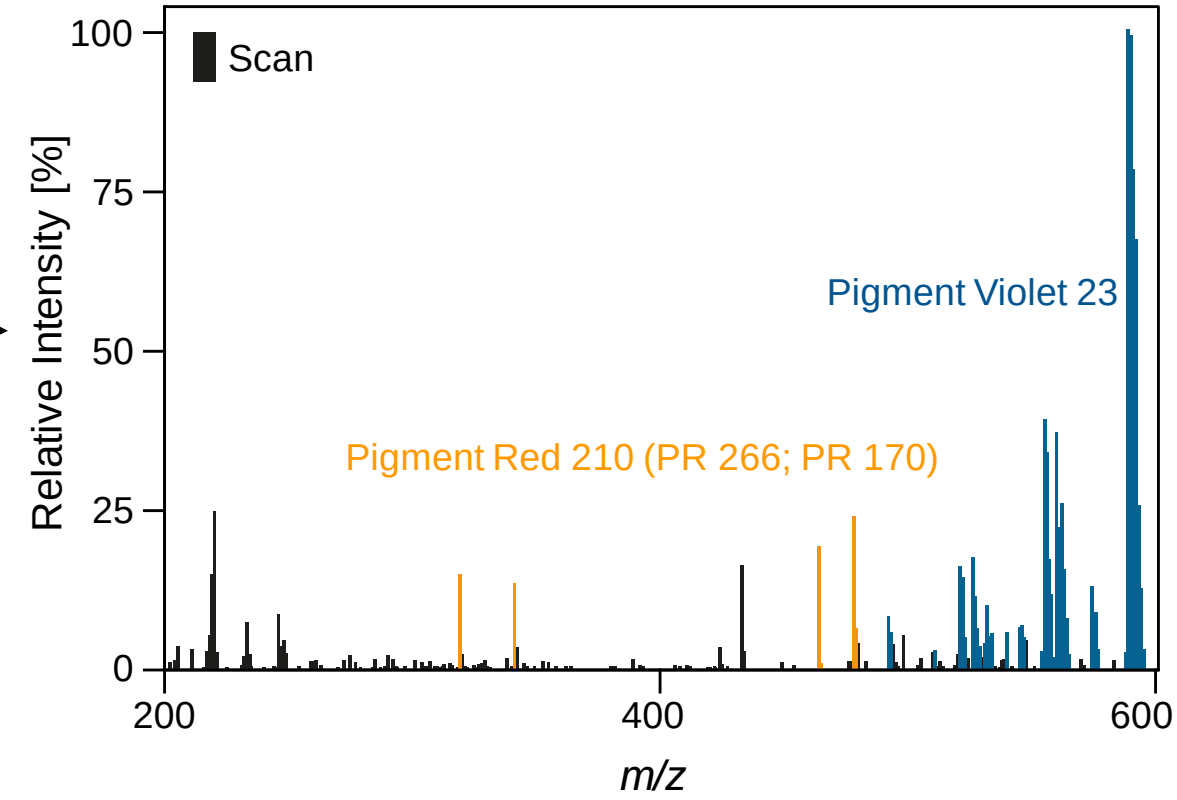
Dermis

Deposition of Tattoo Pigments in the Dermis

Min Intensity Max

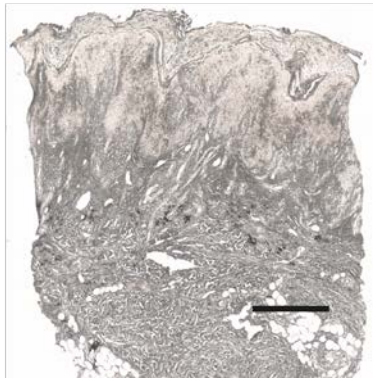
LDI-MS Imaging of Tattooed Skin





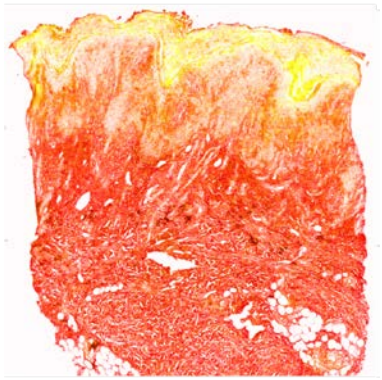
Workflow

Microscopy



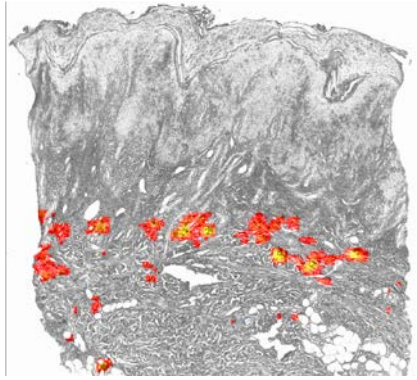
histology

µXRF



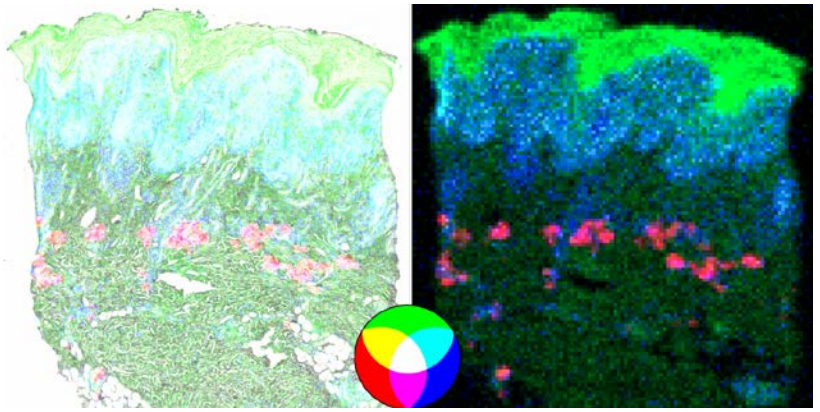
elemental

LDI-MS



molecular

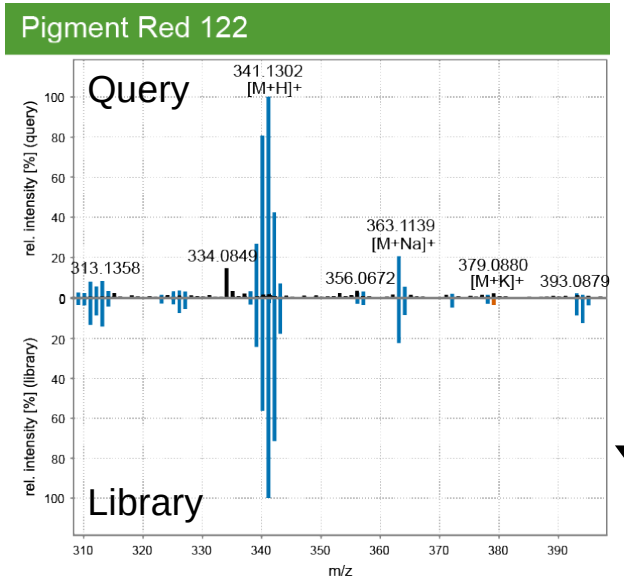
Overlay



Colocalization of pigment specific agglomerates

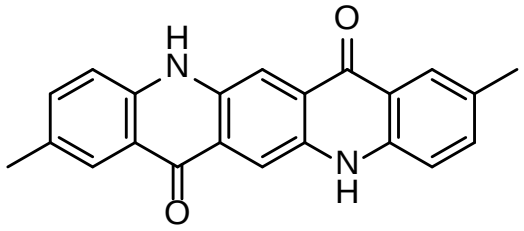
Pigment search

MZmine



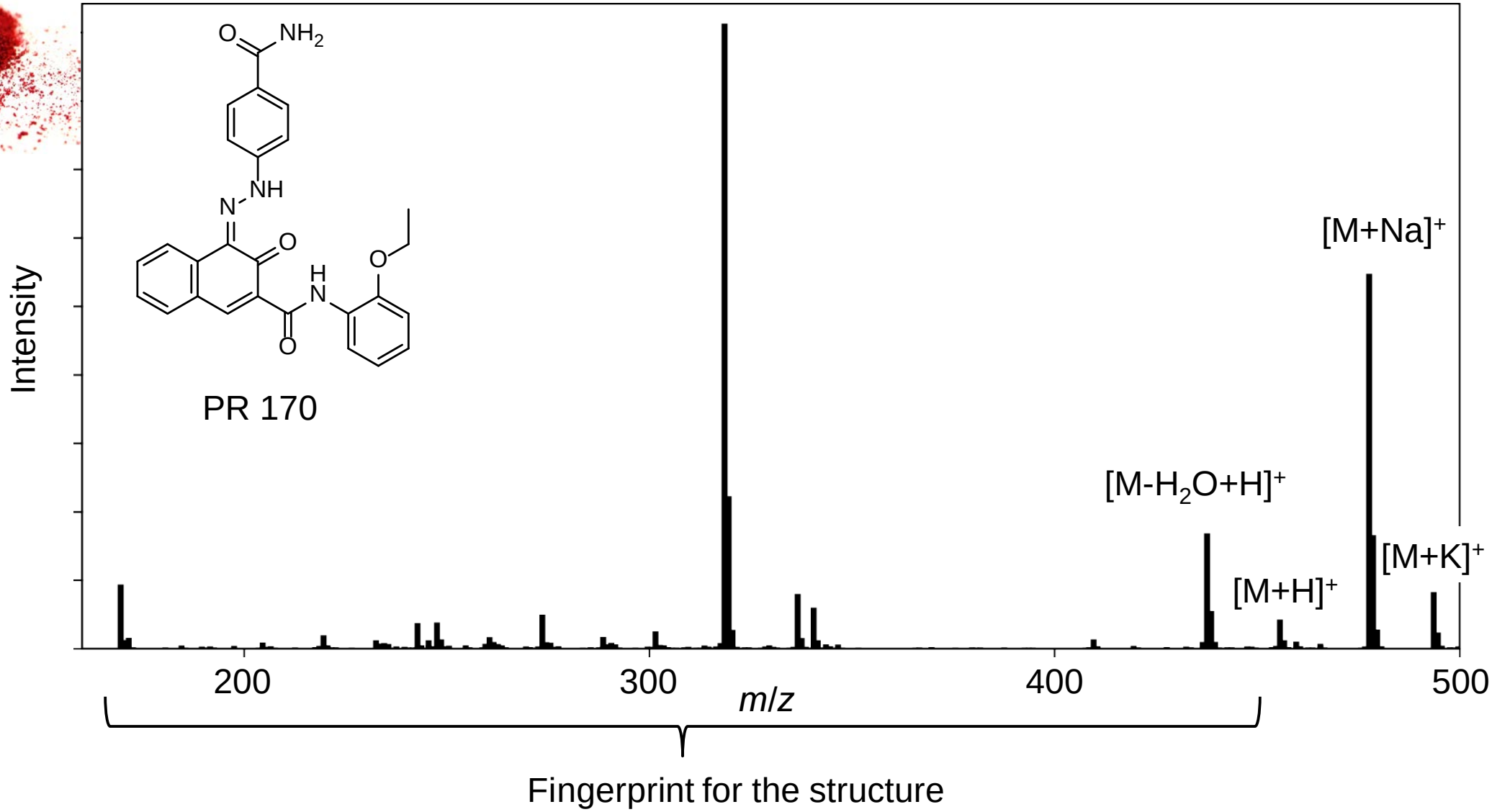
Verification

- Localization
- Specific elements by µXRF



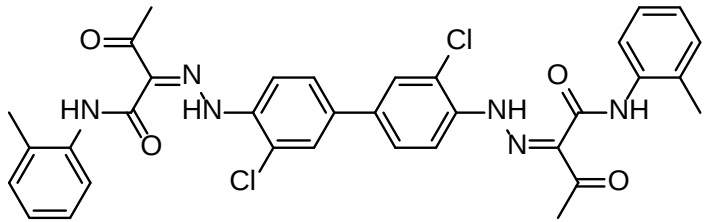
Reference pigment for mass spectral library creation

Mass Spectral Library Creation – LDI-MS^1(+)

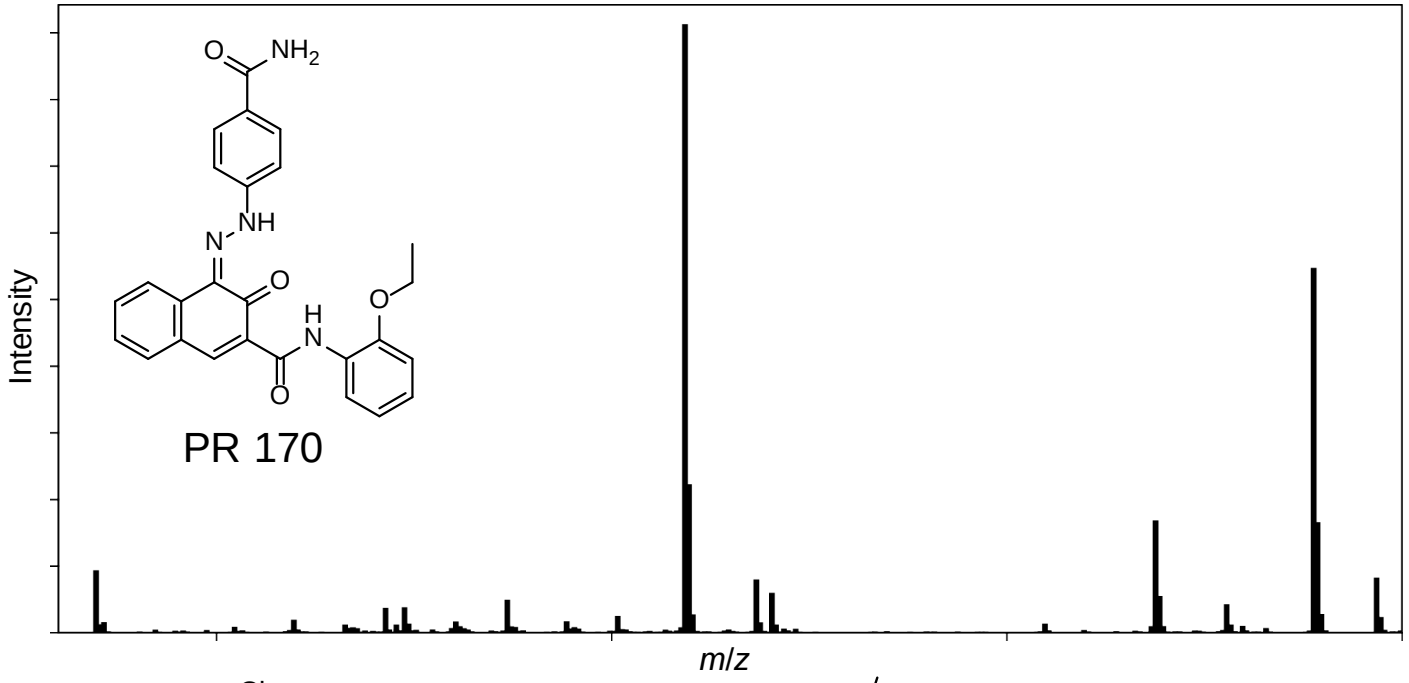


Fingerprint for the structure

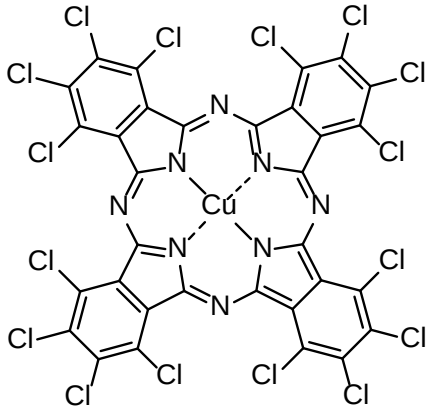
Mass Spectral Library Creation – LDI-MS^1(+)



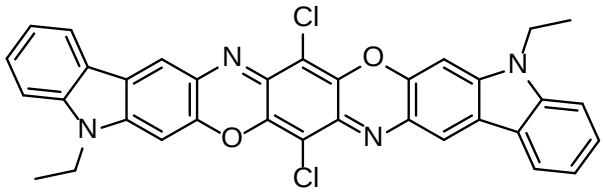
PY 14



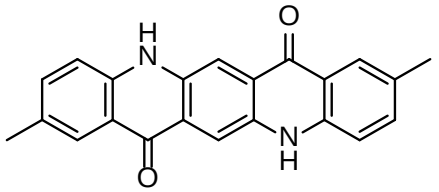
PR 170



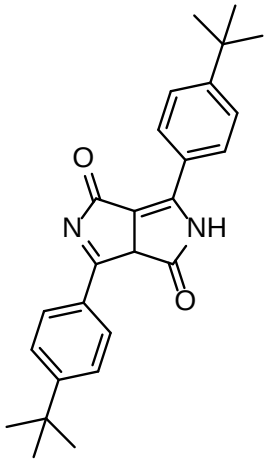
PG 7



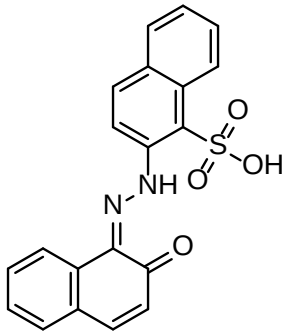
PV 23



PR 122

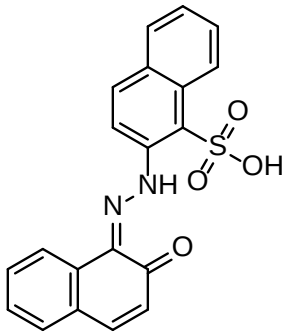


PO 73



PR 49

Mass Spectral Library Creation – LDI-MS^1(+) and LDI-MS^1(-)



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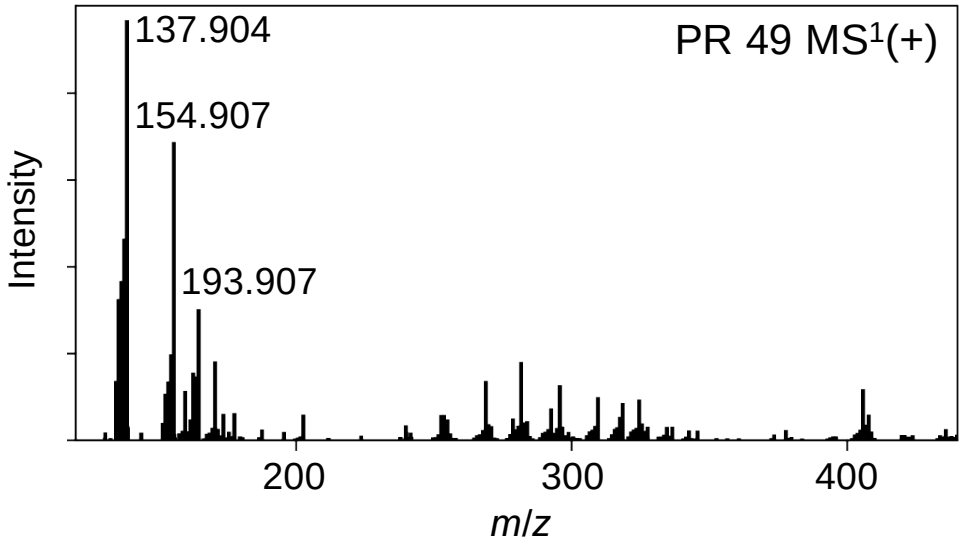
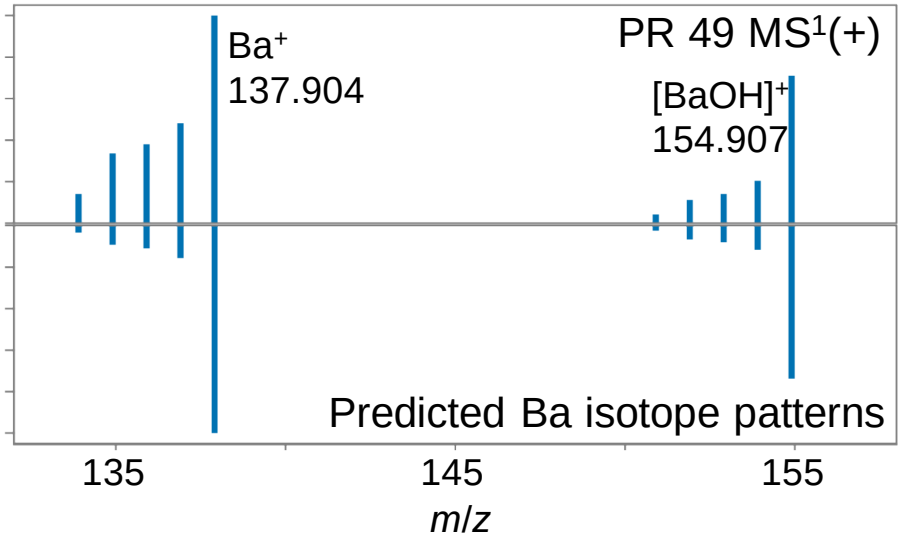
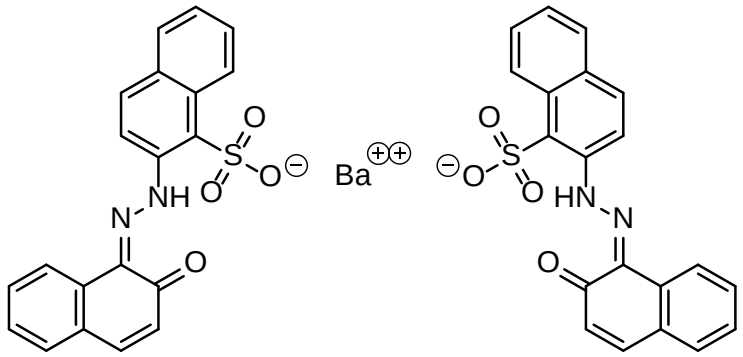
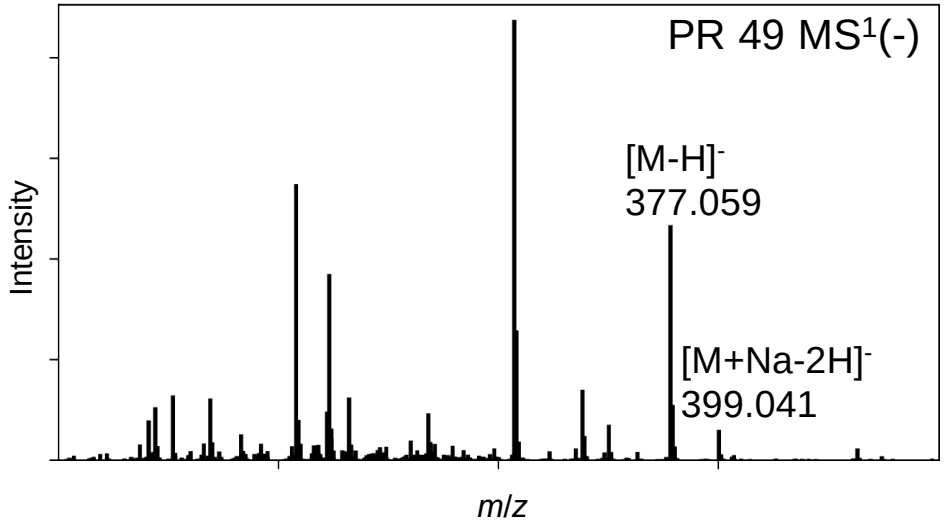


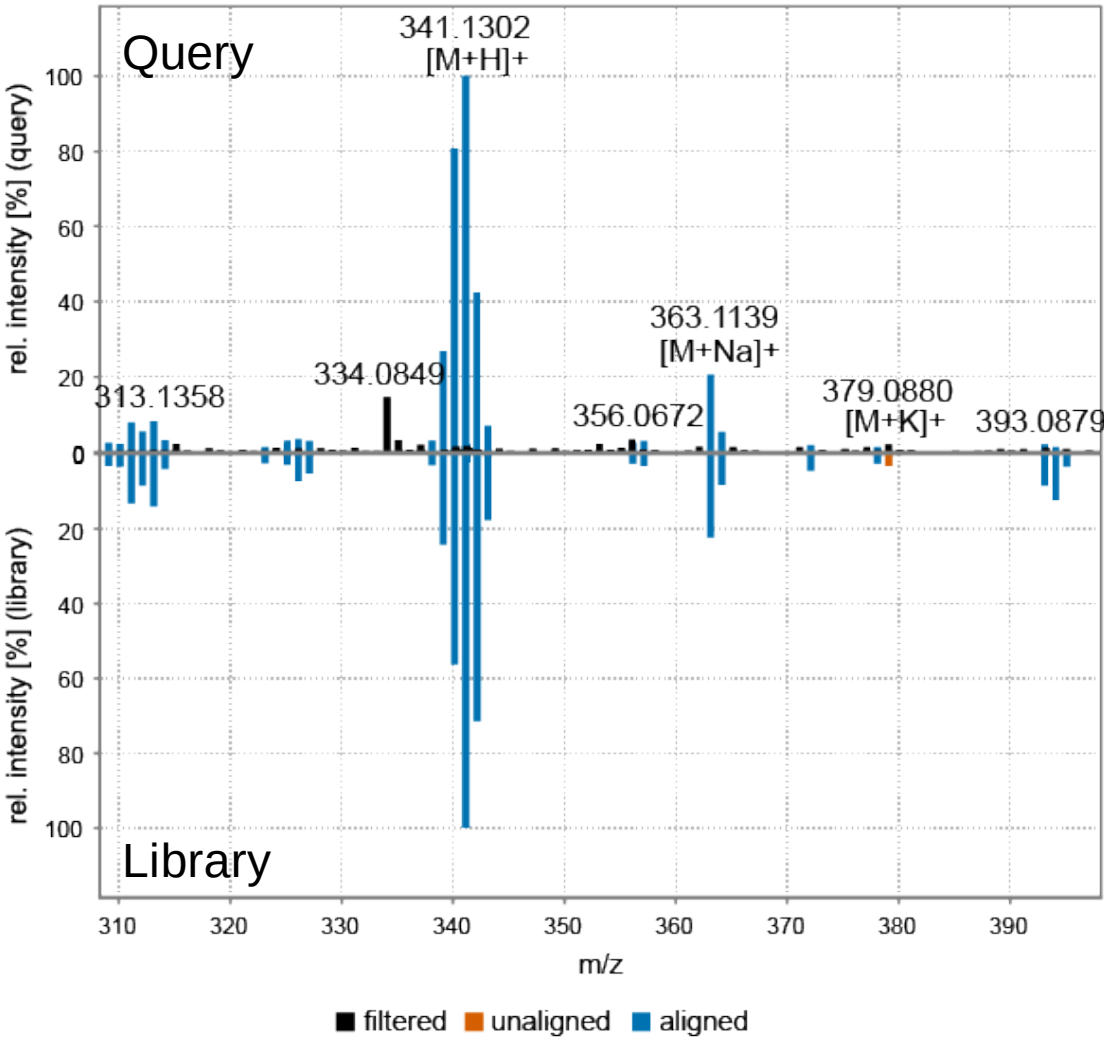


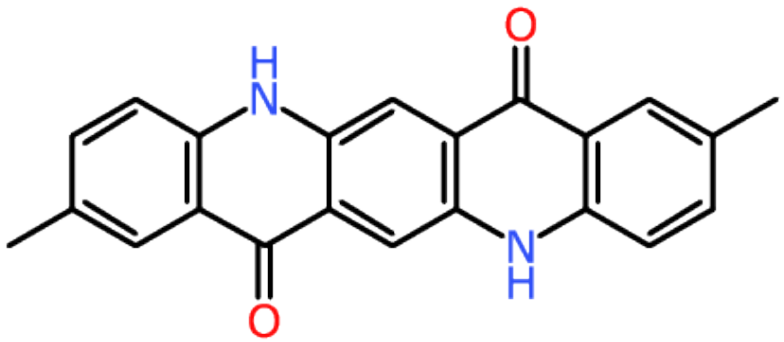




Library Match Window

Pigment Red 122 | 42/48 97% | 0.919





ALL
PDF
EMF
EPS
SVG

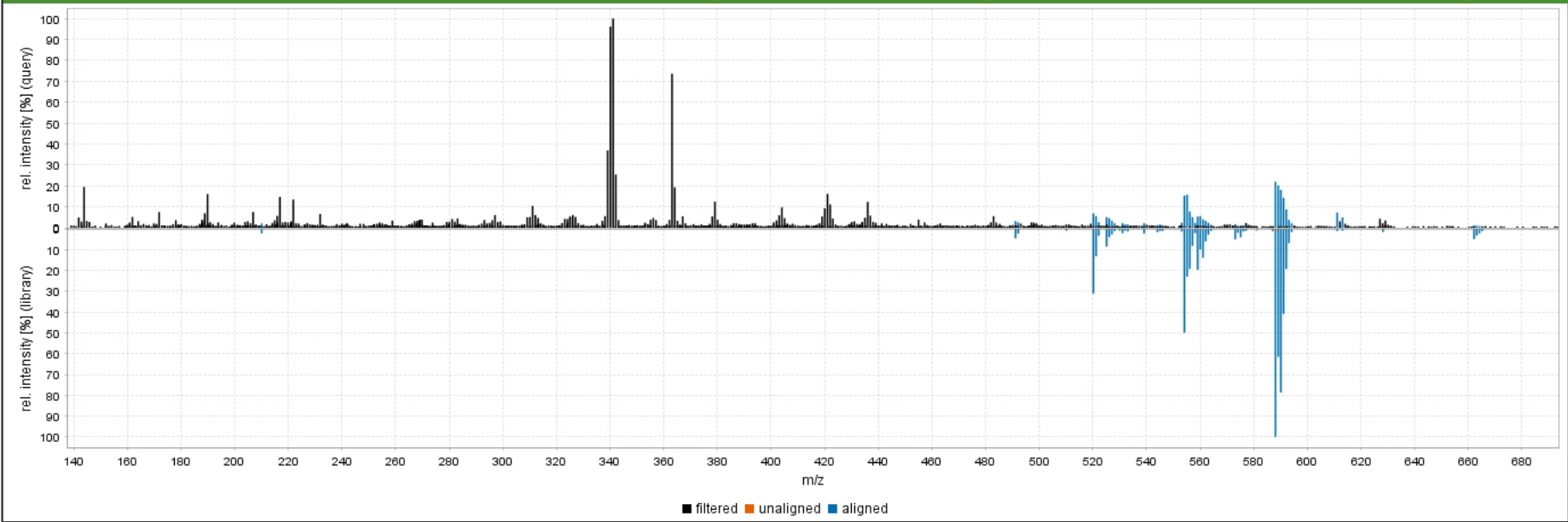
MZmine 2

Matched 23 compounds for feature list row1186

Windows | Setup dialog | Toggle sorting: MATCH SCORE | Couple y-zoom | Set chart font

Pigment Violet 23

51/53
100%
0.920



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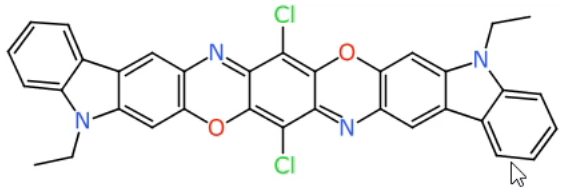


Compound information

Name: Pigment Violet 23

Formula: C34H22Cl2N4O2

Exact mass: 588.112

Ion mode: Positive

InChI:
InChI=1S/C34H22Cl2N4O2/c1-3-39-23-11-7-5-9-17(23)19-13-21-27(15-25(19)39)41-33-29(35)32-34(30(36)31(33)37-21)42-28-16-26-20(14-22(28)38-32)18-10-6-8-12-24(18)40(26)4-2/h5-16H,3-4H2,1-2H3

InChI key:
CGLVZFOCZLHKOH-UHFFFAOYSA-N

SMILES:

Instrument information

Instrument type: qTof

Instrument: timsTOF fleX

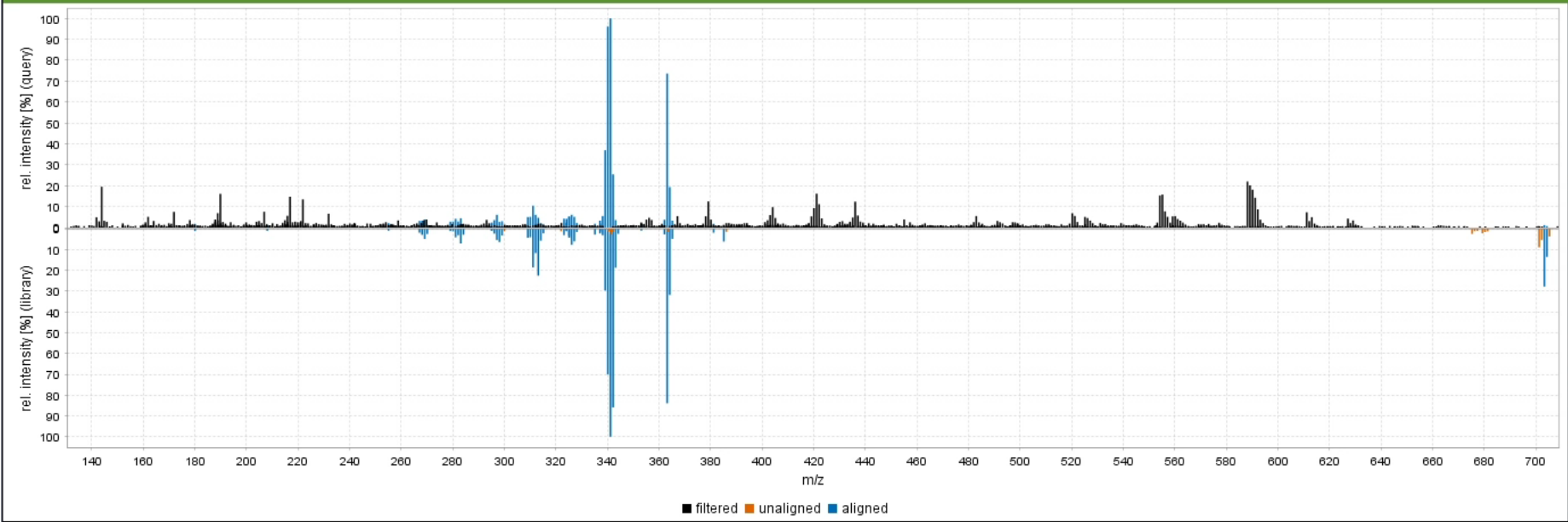
Ion source: LDI

Acquisition: Crude

Software: mzmine2

Pigment Red 122

53/90
90%
0.893



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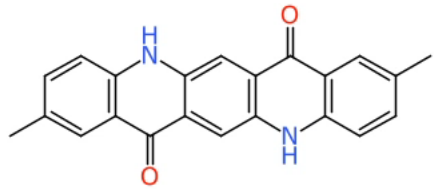


Compound information

Name: Pigment Red 122

Formula: C22H16N2O2

Exact mass: 340.1212

Ion mode: Positive

InChI:
InChI=1S/C22H16N2O2/c1-11-3-5-17-13(7-11)21(25)15-9-20-16(10-19(15)23-17)22(26)14-8-12(2)4-6-18(14)24-20/h3-10H,1-2H3,(H,23,25)(H,24,26)

InChI key:
TXWSZJSDZKWQAU-UHFFFAOYSA-N

SMILES:
CC1=CC2=C(C=C1)NC3=CC4=C(C=C3C

Instrument information

Instrument type: qTof

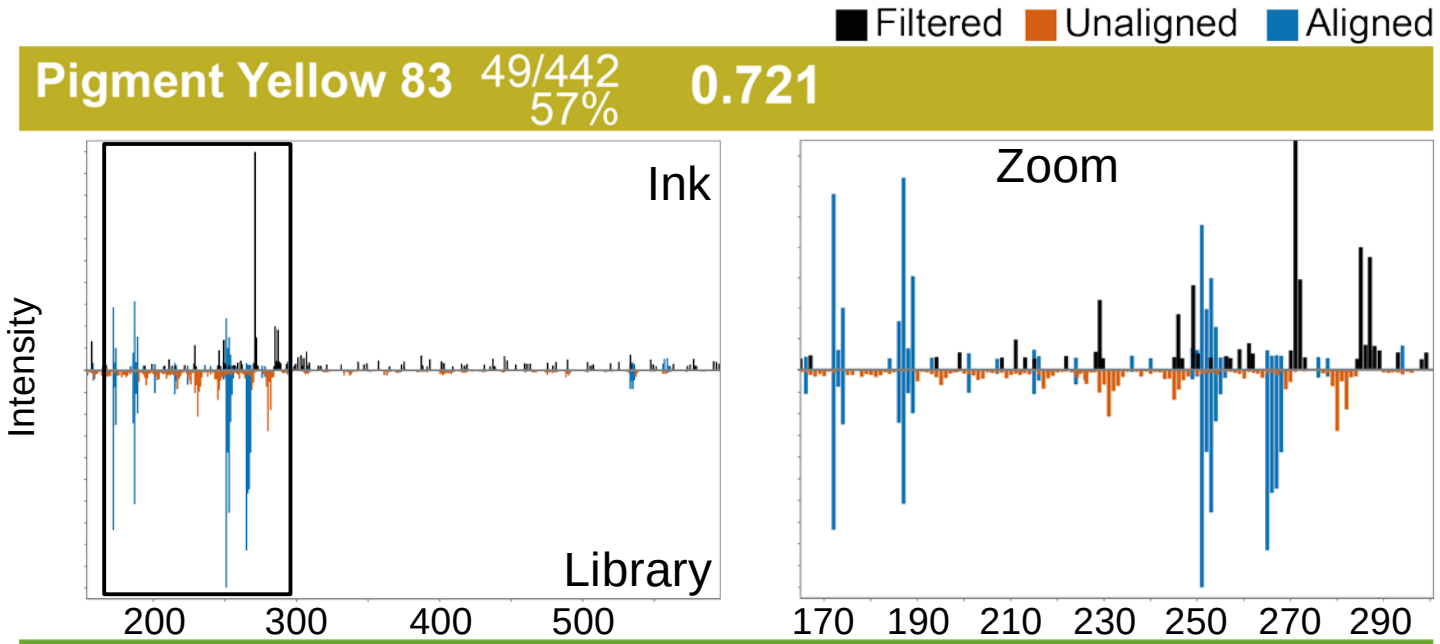
Instrument: timsTOF fleX

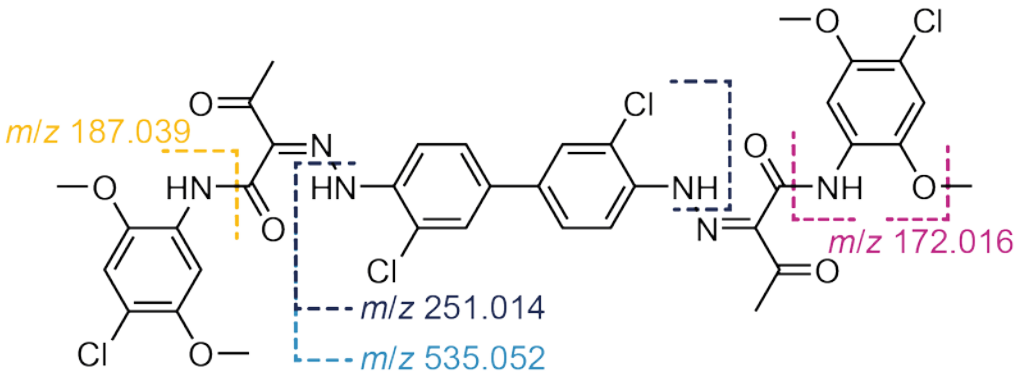
Ion source: LDI

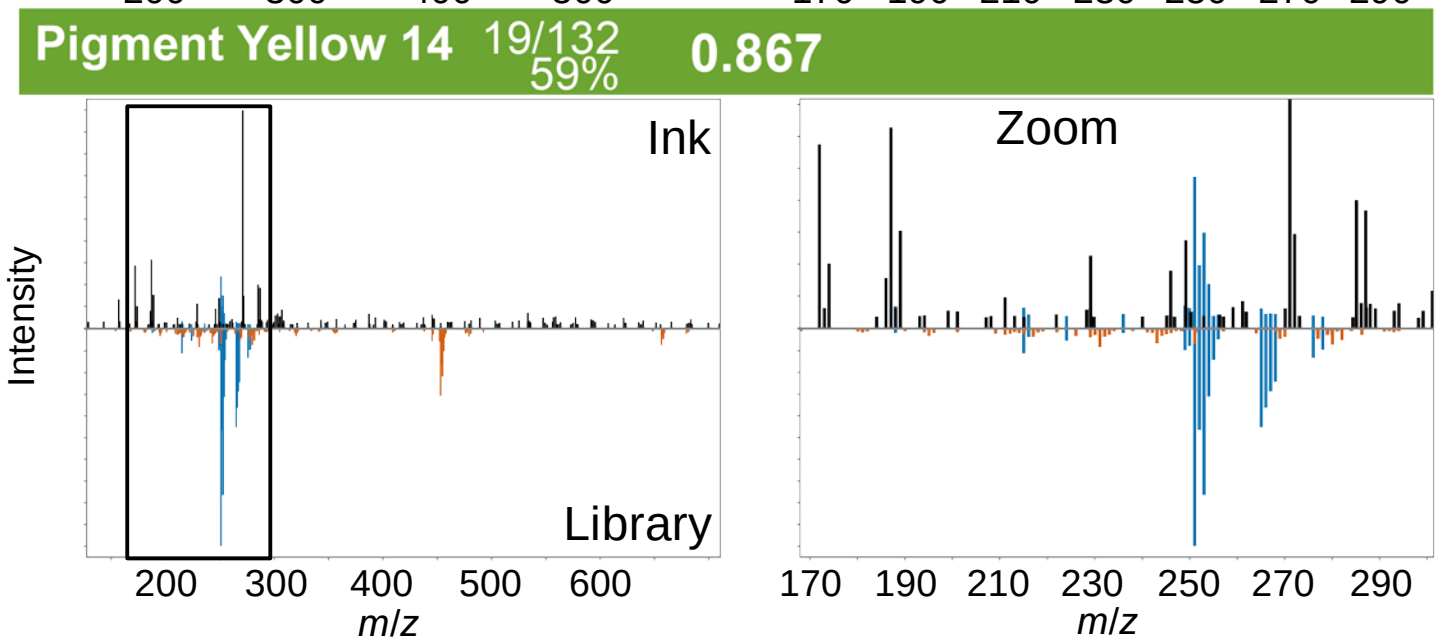
Acquisition: Crude

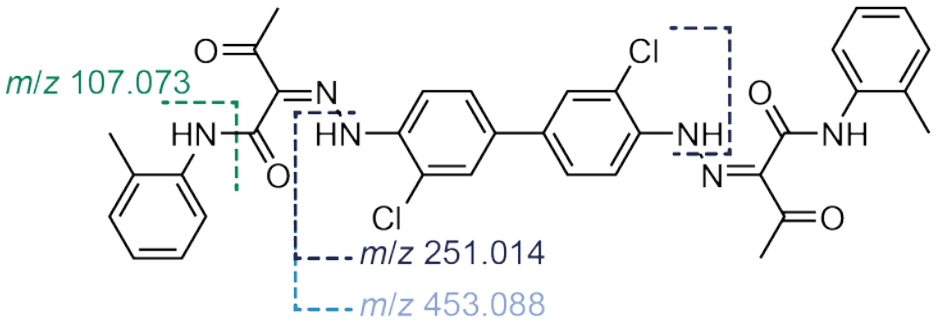
Software: mzmine2

Matching Results of Structurally Related Pigments

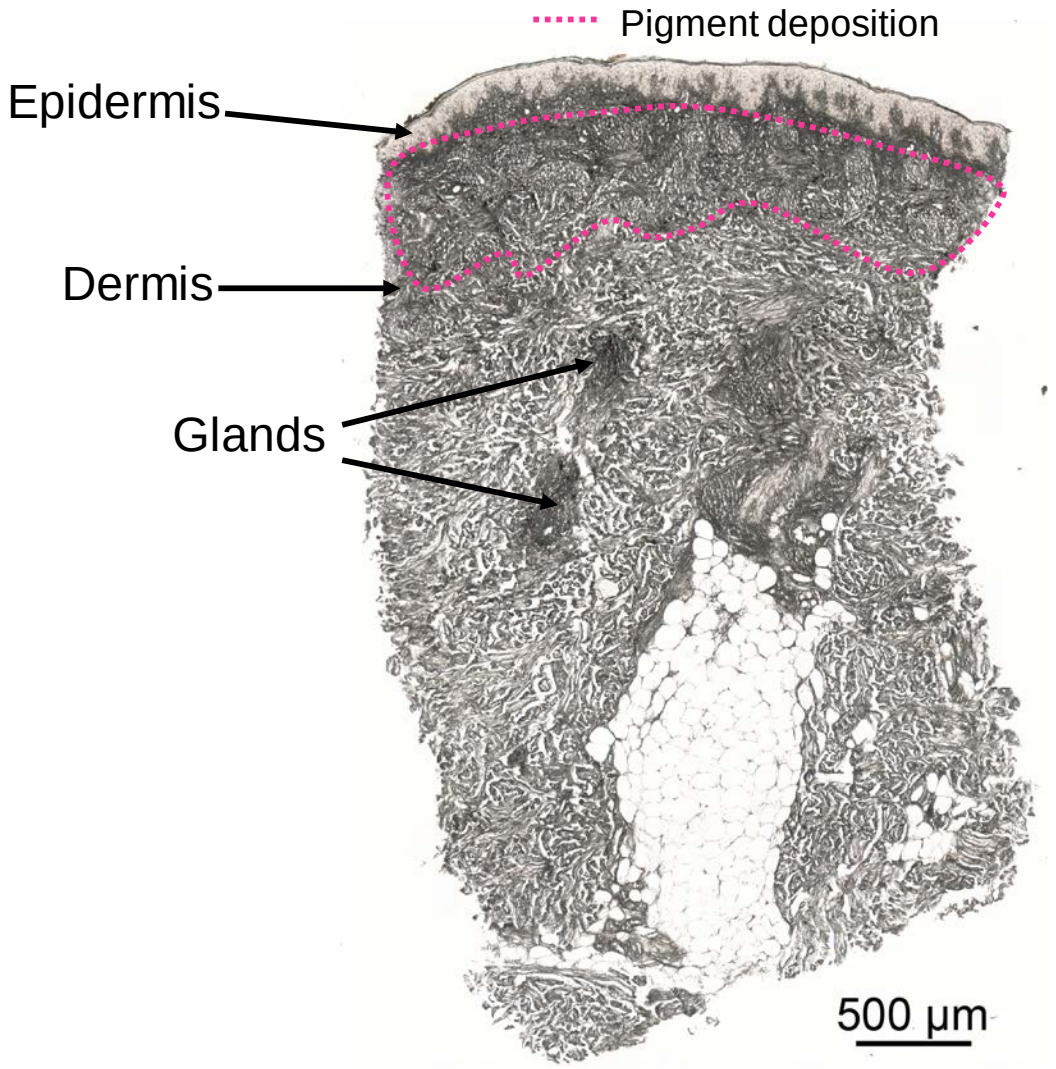


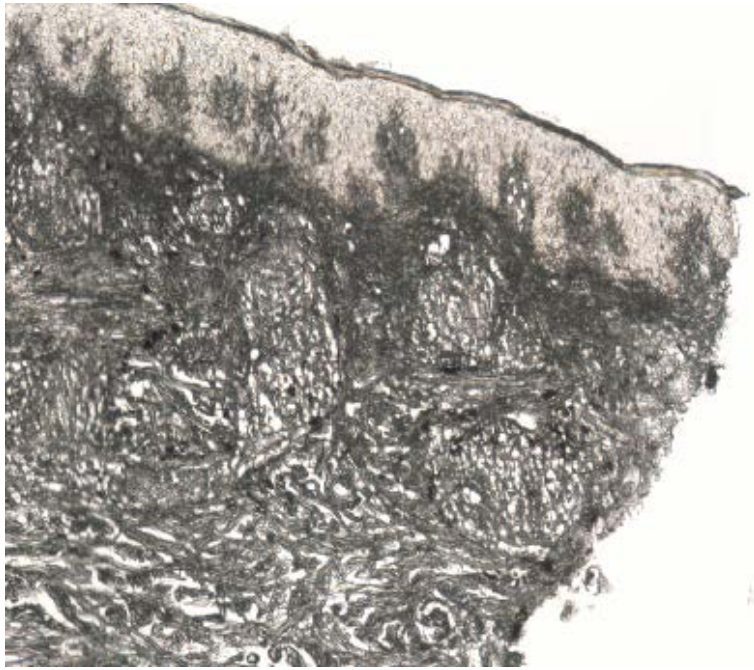




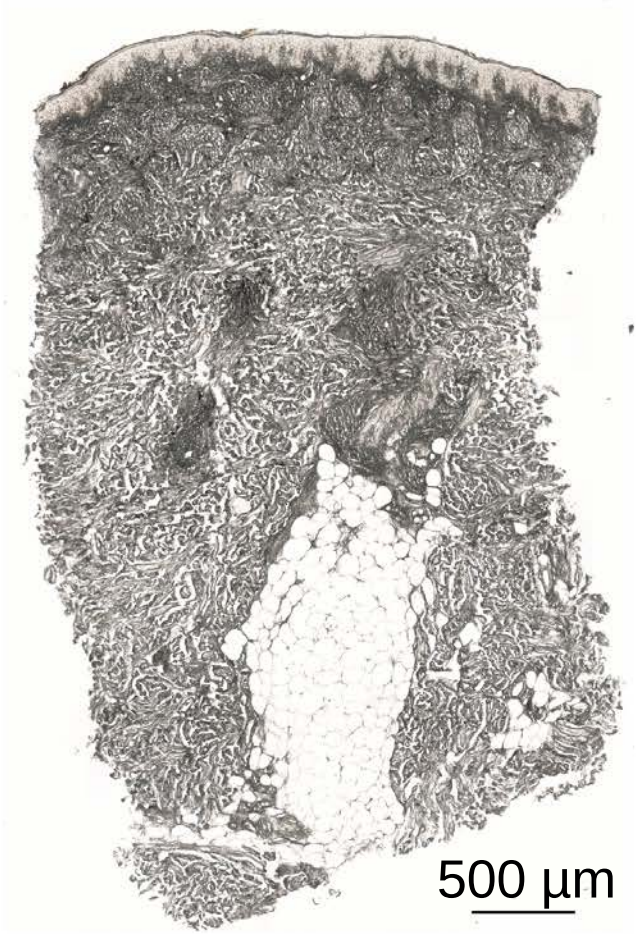


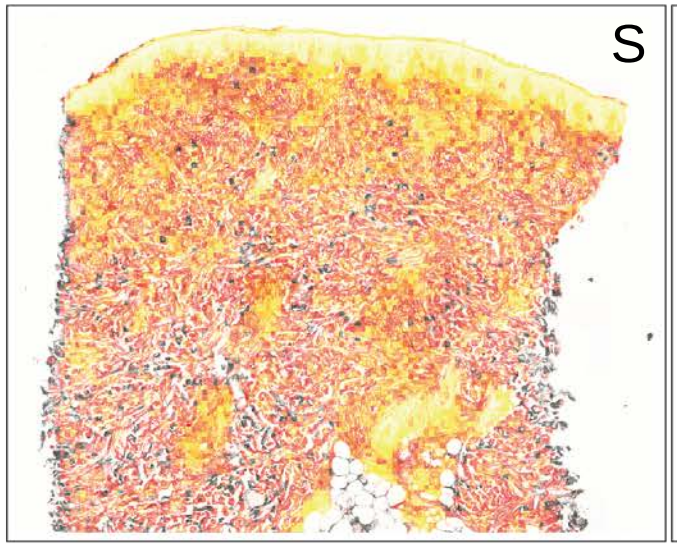
Tattooed Skin Samples

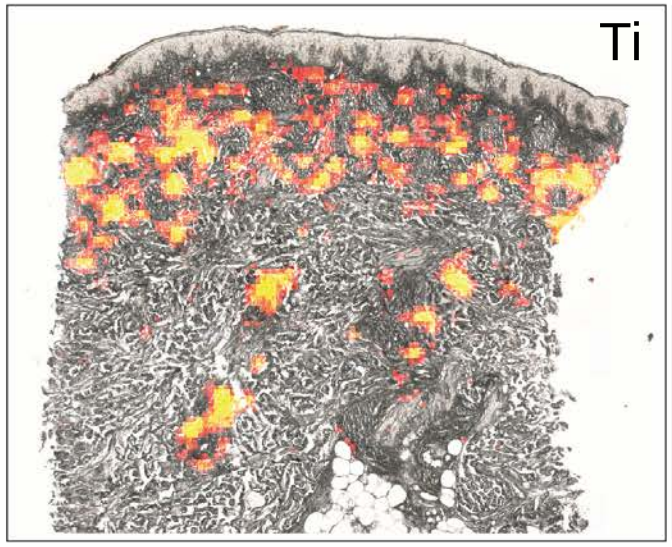


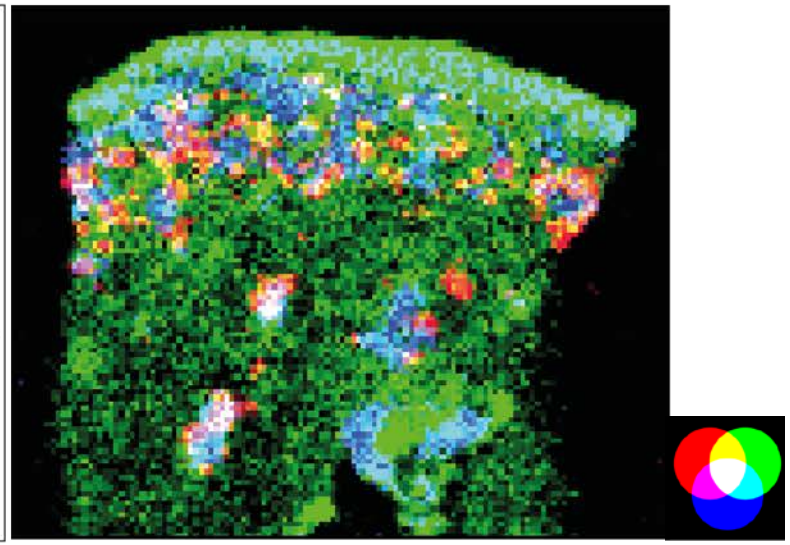


Elemental Bioimaging of a Tattooed Skin Sample

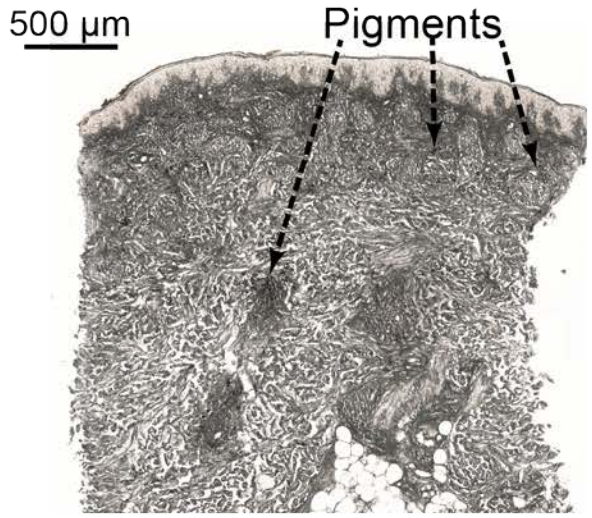




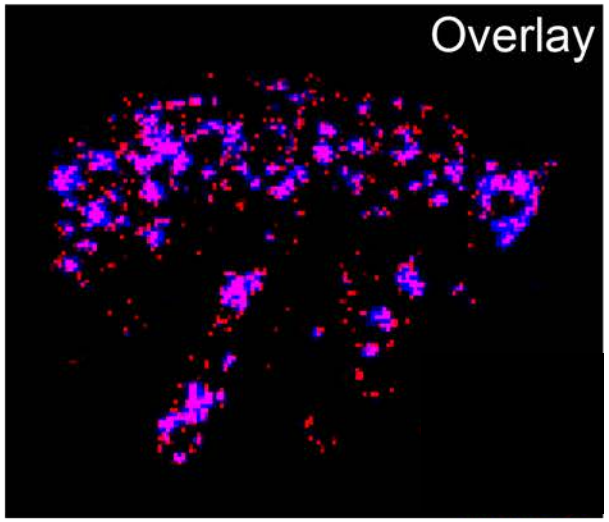




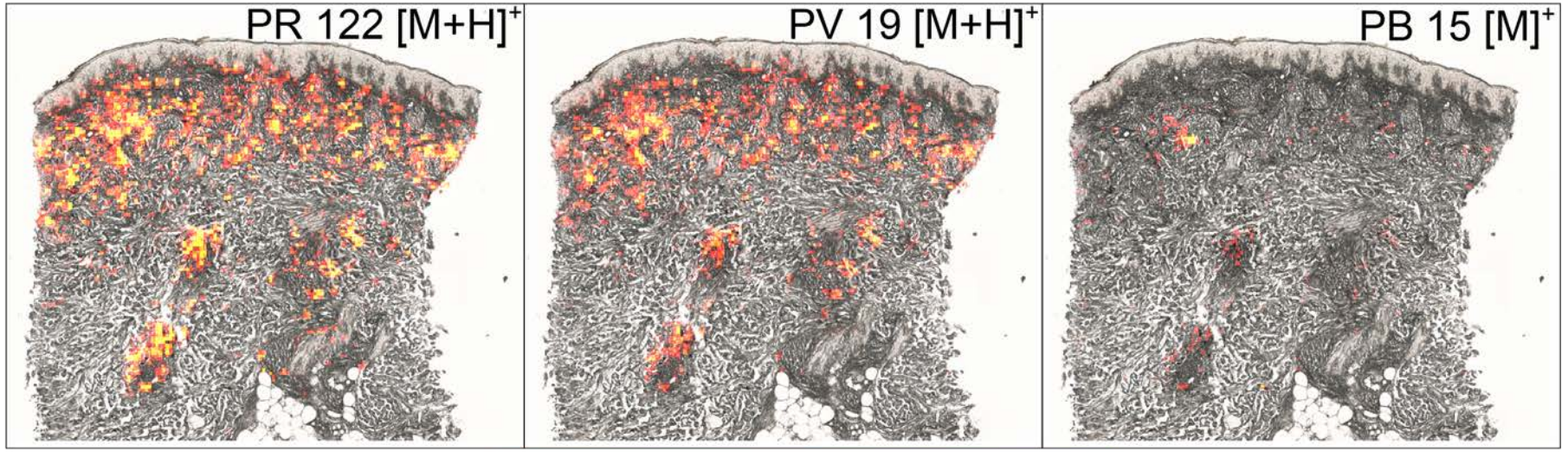
Combination of Elemental and Molecular Bioimaging



µXRF Results



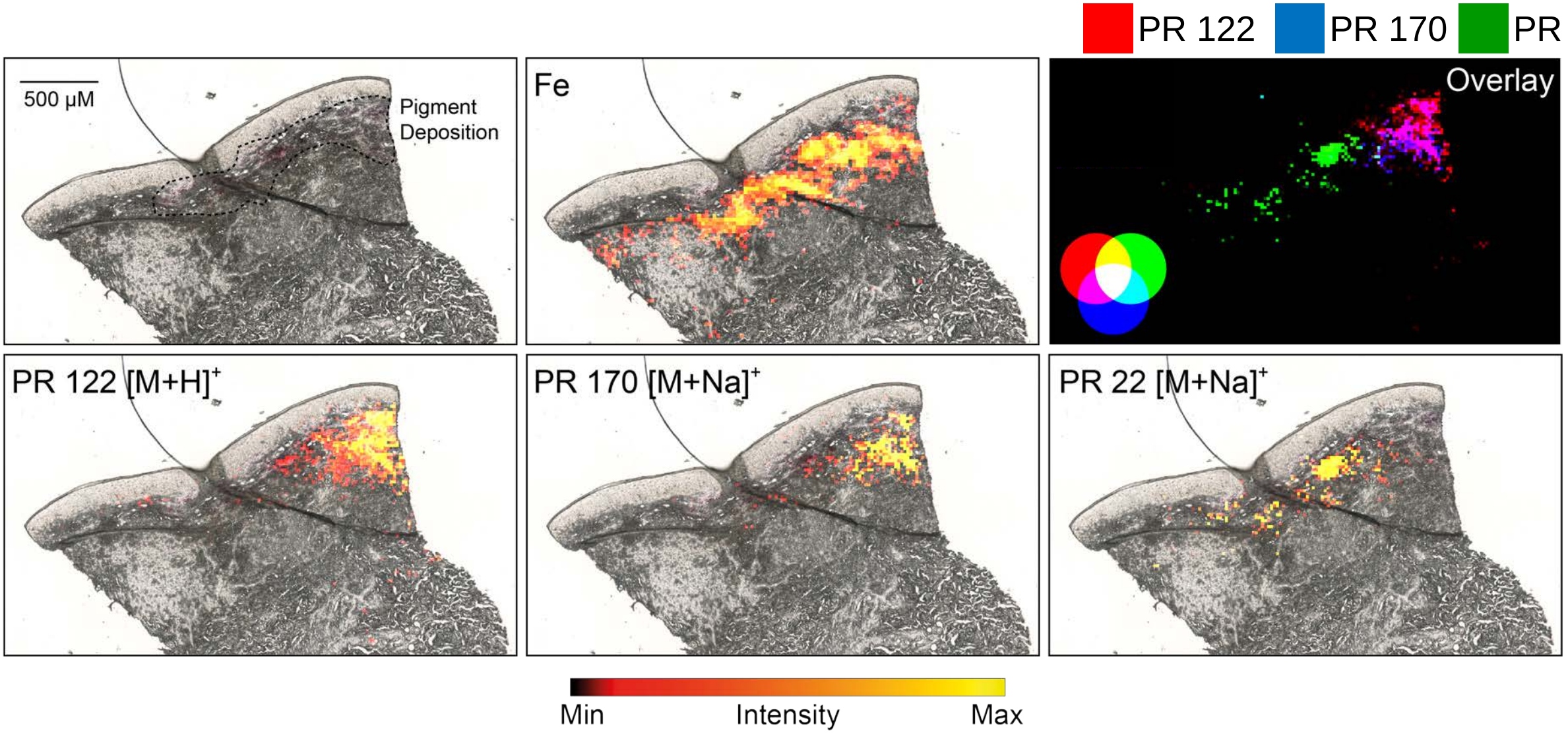
LDI-MS Results



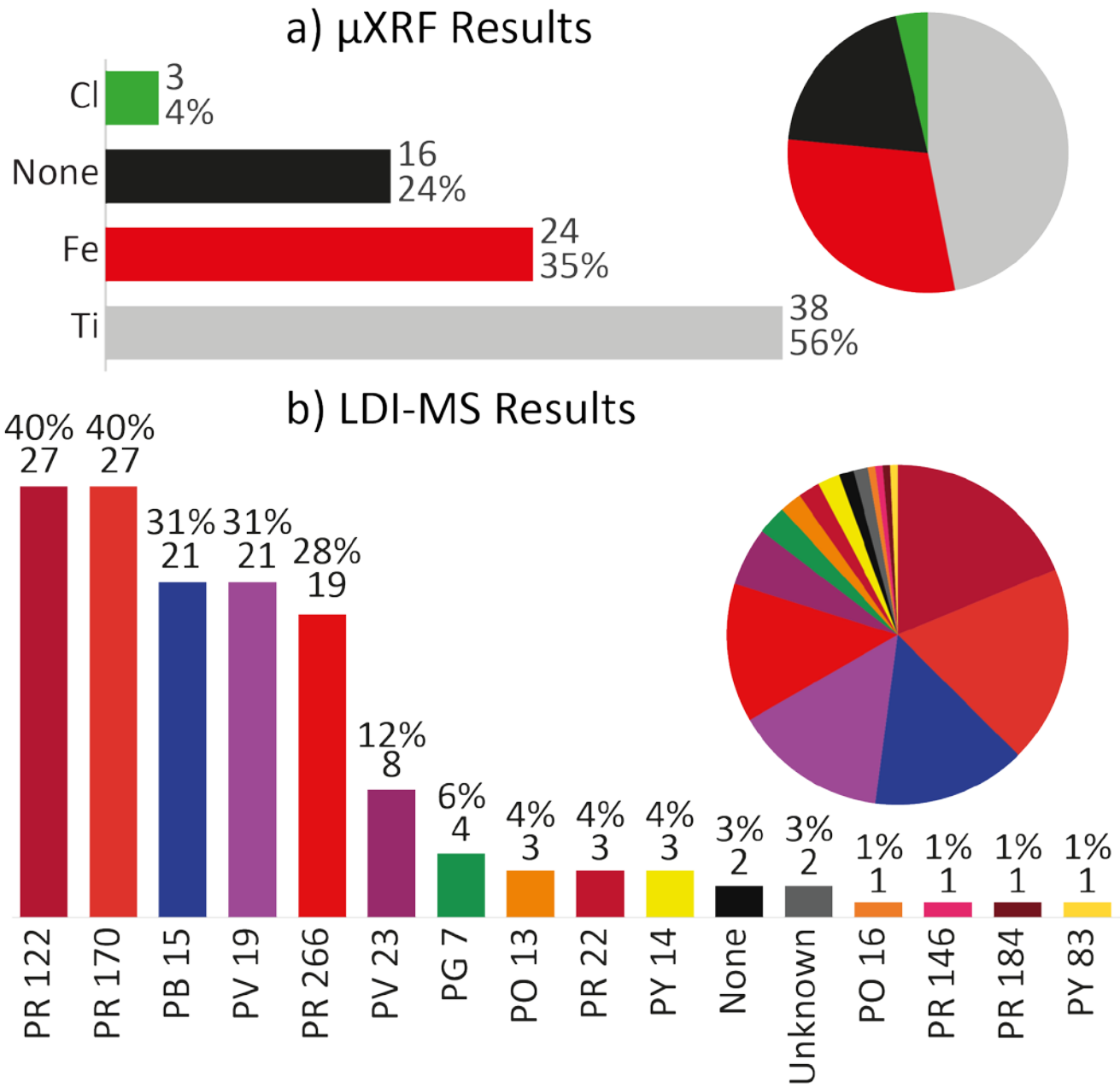
Ti PR 122 Mixed

Min Intensity Max

Combination of Elemental and Molecular Bioimaging for Another Sample



Analysis Results of 68 Skin Samples and Their Co-occurrence



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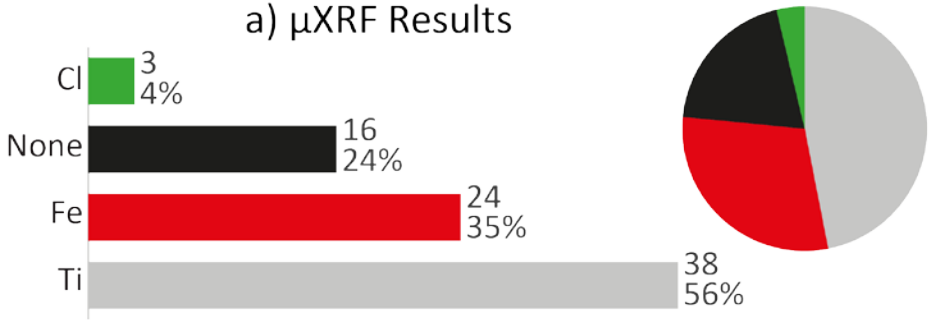
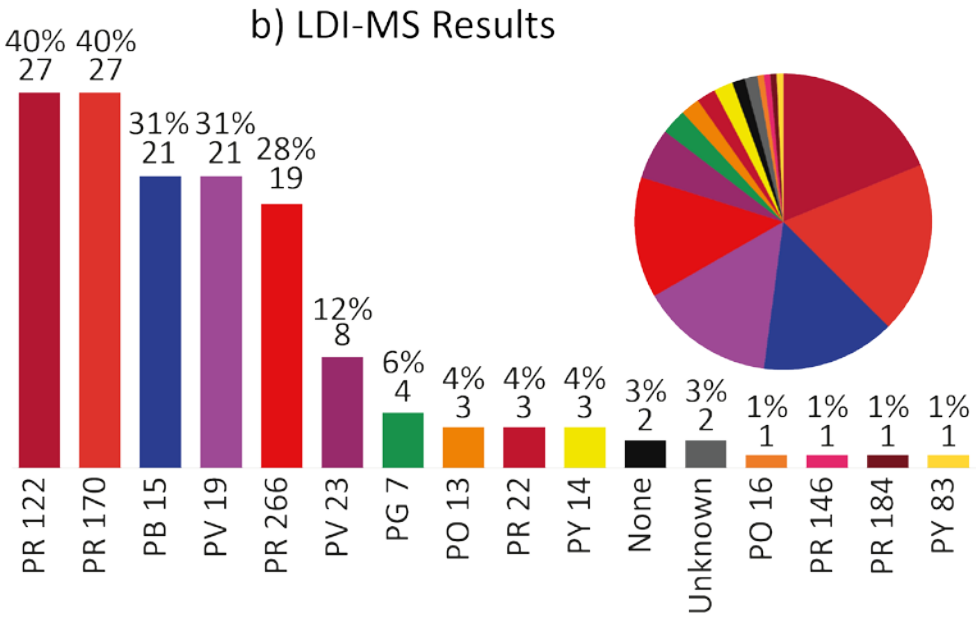
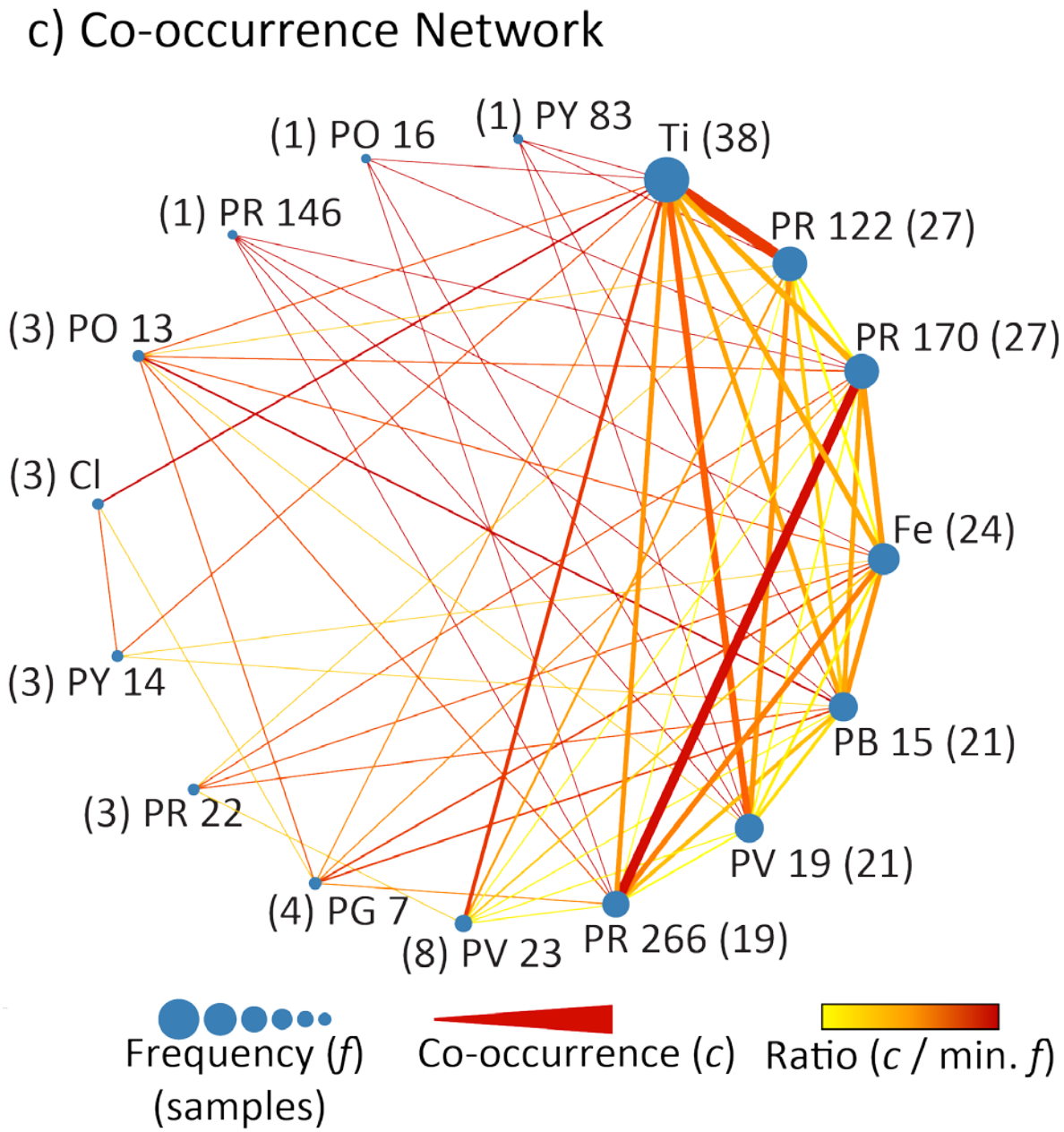
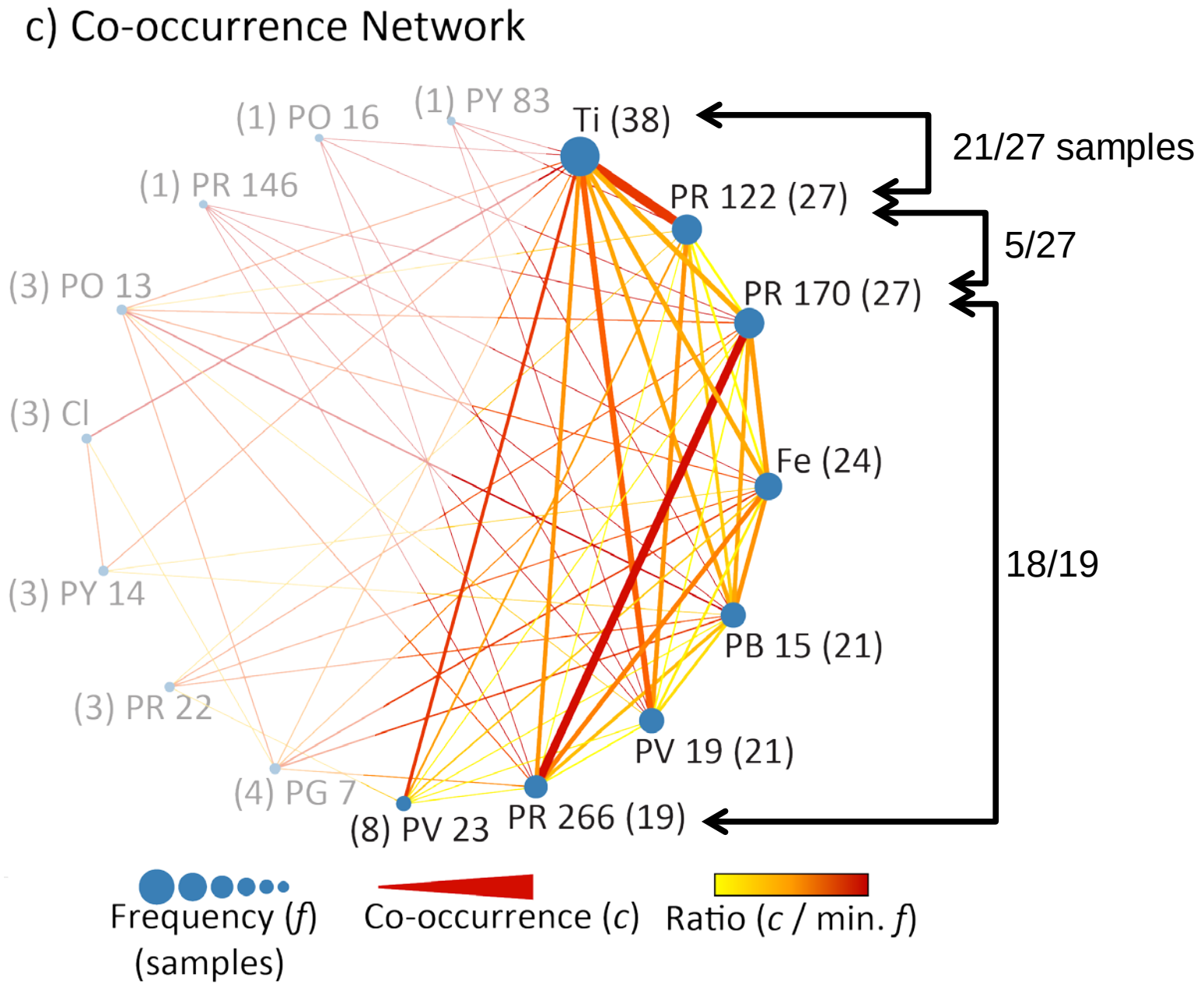


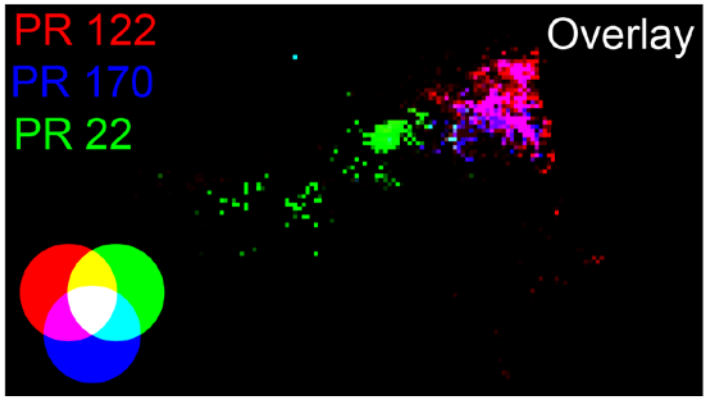




Analysis Results of 68 Skin Samples and Their Co-occurrence

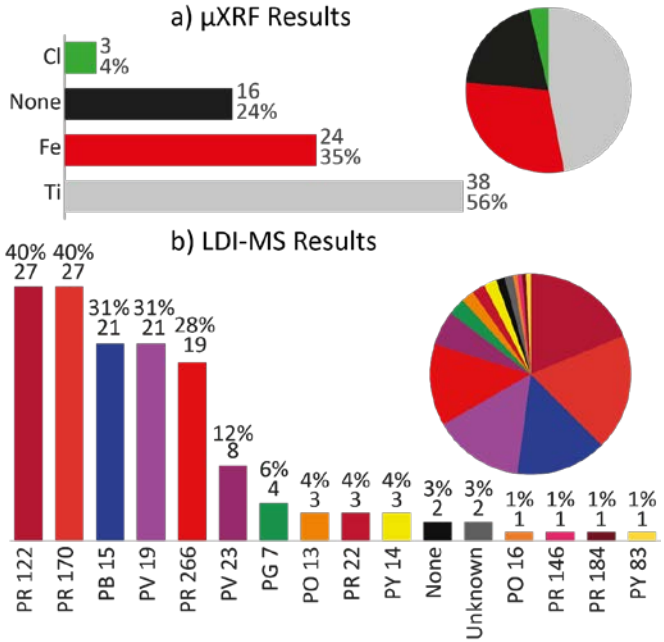


Summary and Conclusion



Complementary elemental and molecular bioimaging of skin samples:

- **µXRF**: Fast screening of **elements** (e.g., Fe, Ti,…)
- **LDI-MS**: Detection of **organic** pigments and **Carbon Black**
- FFPE or frozen skin thin sections
- Simple sample preparation



Annotation results:

- Pigments in 68 of 68 skin samples
- Mixture of up to 7 pigments
- Total of 140 pigment annotations with 14 unique structures
- Ti, Fe, PR 122, PR 170, PB 15, PV 19, PR 266, and PV 23

Acknowledgements

WWU, Münster
- Karst Lab & Hayen Lab

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- Dr. Sebastiaan van der Bent
- Dr. Thomas Rustemeyer

IVDK, Göttingen:
- Dr. Steffen Schubert

Identifying Tattoo Pigments in Human Skin Samples with Adverse Reactions based on µXRF and LDI-MS Imaging and Mass Spectral Library Matching

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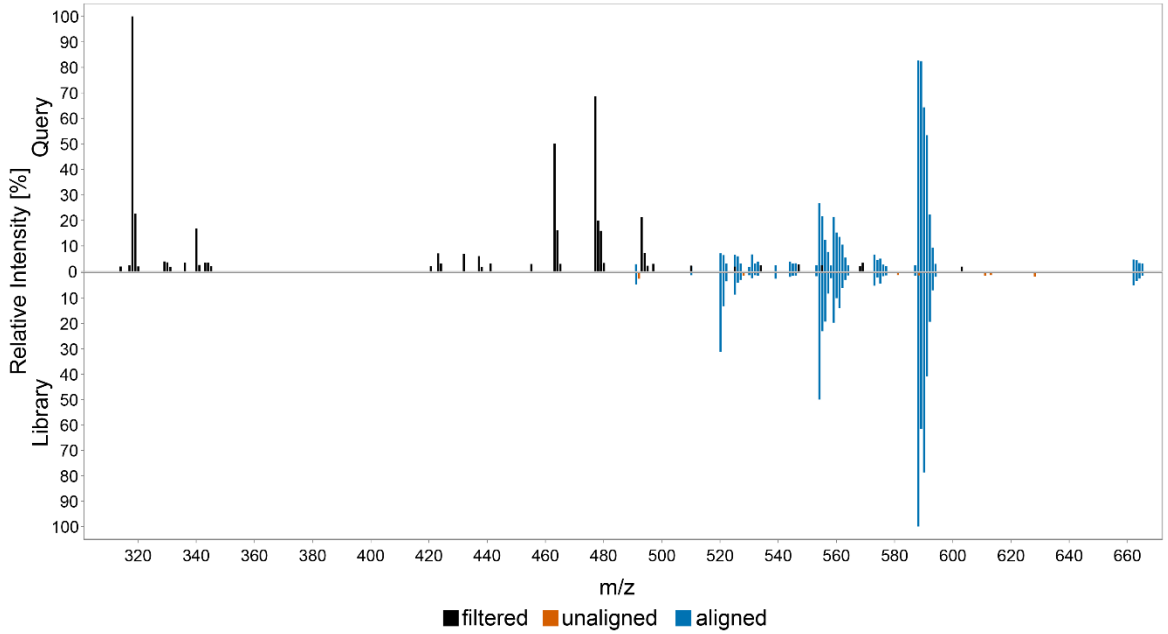
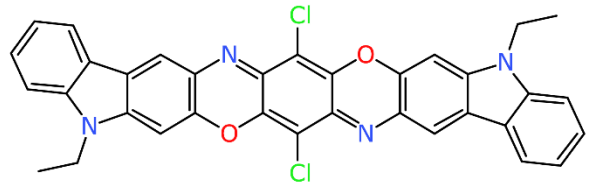
[2]Tattoo Clinic (Tattoo poli), Department of Dermatology, Alrijne Hospital, Leiden, The Netherlands

19.11.2021

Pigment Violet 23

46/53
98%
0.867





Compound information

Name: Pigment Violet 23

Formula: C34H22Cl2N4O2

Exact mass: 588.112

Ion mode: Positive

InChI:
InChI=1S/C34H22Cl2N4O2/c1-3-39-23-11-7-5-9-17(23)19-13-21-27(15-25(19)39)41-33-29(35)32-34(30(36)31(33)37-21)42-28-16-26-20(14-22(28)38-32)18-10-6-8-12-24(18)40(26)4-2/h5-16H,3-4H2,1-2H3

InChI key:
CGLVZFOCZLHKOH-UHFFFAOYSA-N

SMILES:
CCN1C2=CC=CC=C2C3=CC4=C(C=C31)

Instrument information

Instrument type: qTof

Instrument: timsTOF fleX

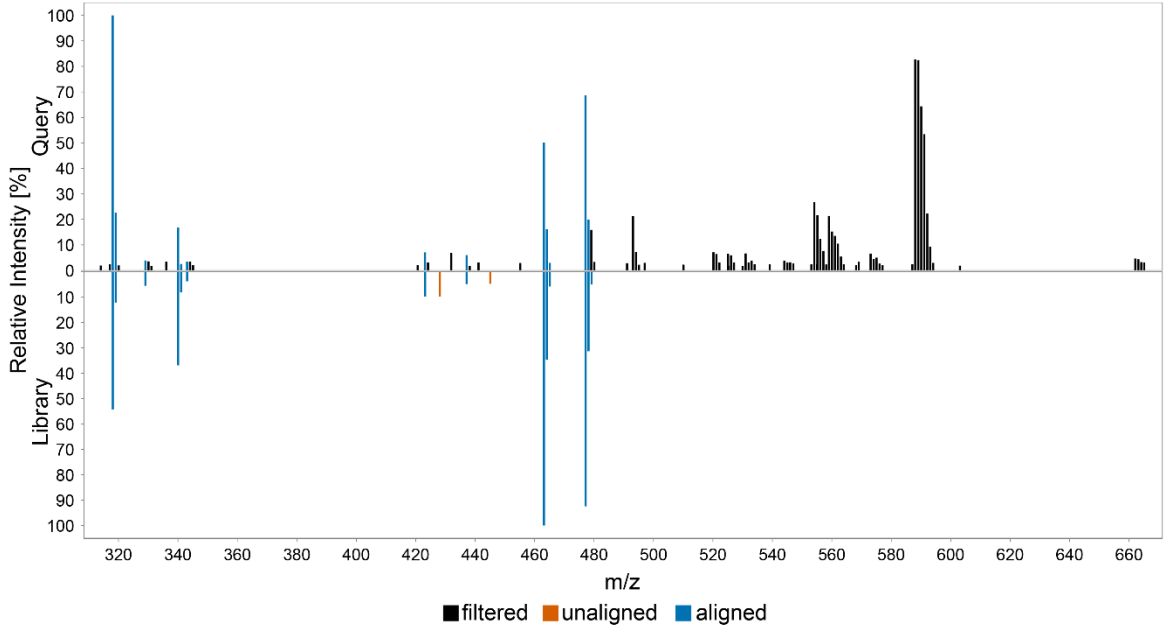
Ion source: LDI

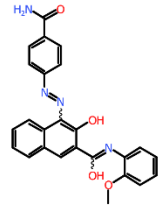
Acquisition: Crude

Software: mzmine2

Pigment Red 210: Pigment Red 266; Pigment Red 170

21/24
96%
0.829





Compound information

Name: Pigment Red 210; Pigment Red 266; Pigment Red 170

Formula: C25H20N4O4

Exact mass: 440.1484

Ion mode: Positive

InChI:
InChI=1S/C25H20N4O4/c1-33-21-9-5-4-8-20(21)27-25(32)19-14-16-6-2-3-7-18(16)22(23(19)30)29-28-17-12-10-15(11-13-17)24(26)31/h2-14,30H,1H3,(H2,26,31)(H,27,32)

InChI key:
HULNYTPFPARJMG-UHFFFAOYSA-N

SMILES:
COC1=CC=CC=C1NC(=O)C2=CC3=CC=

Instrument information

Instrument type: ToF

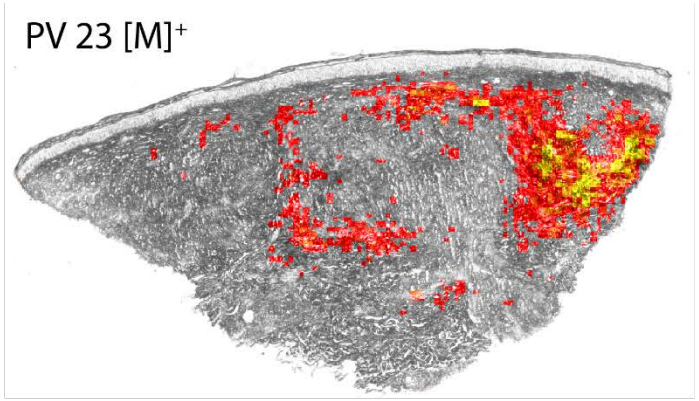
Instrument: timsTOF fleX

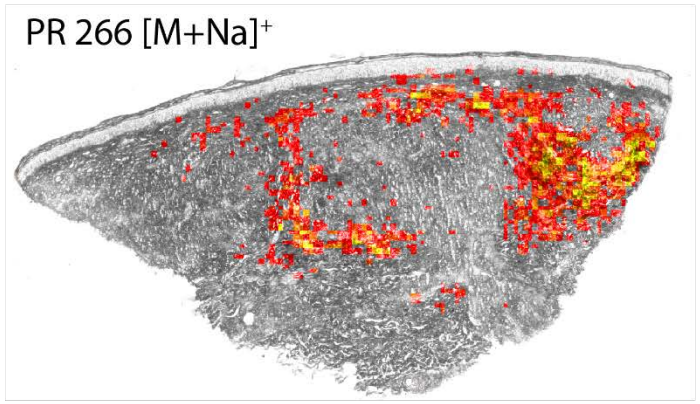
Ion source: LDI

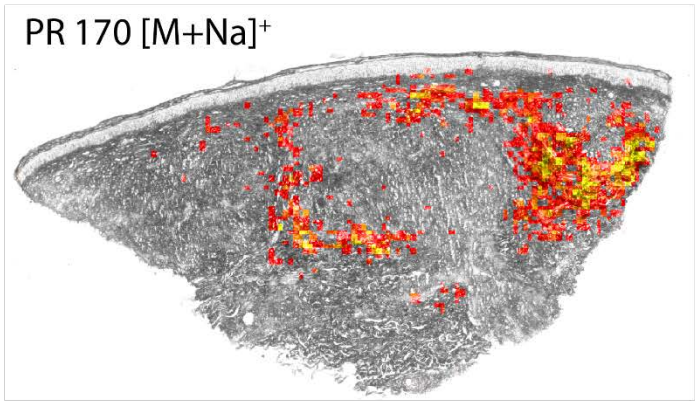
Acquisition: Crude

Software: mzmine2

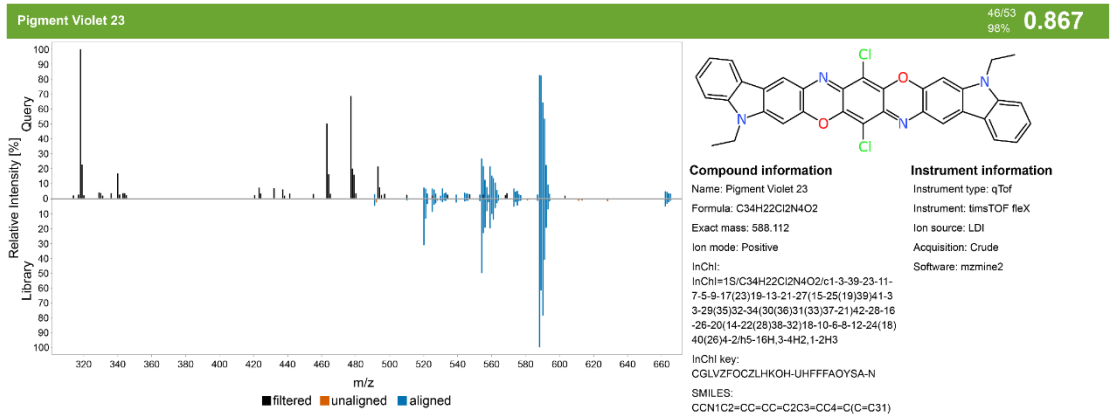
Molecular Bioimaging after Library Matching

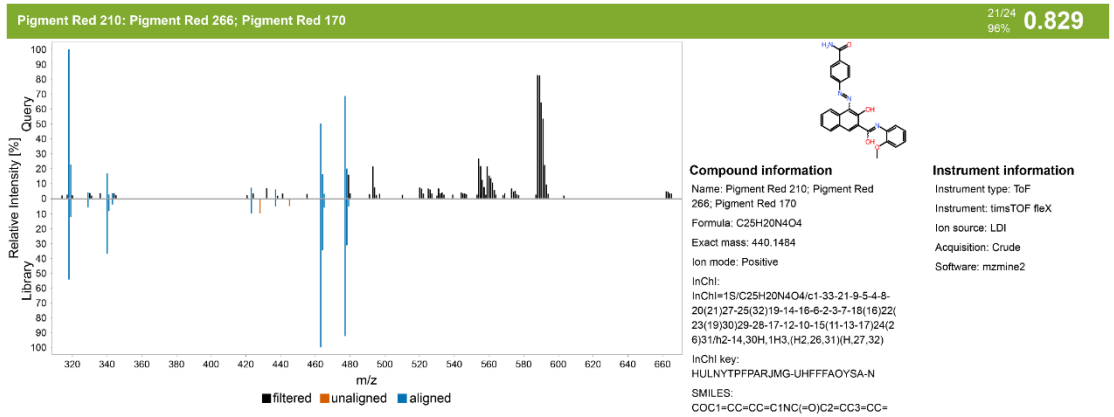
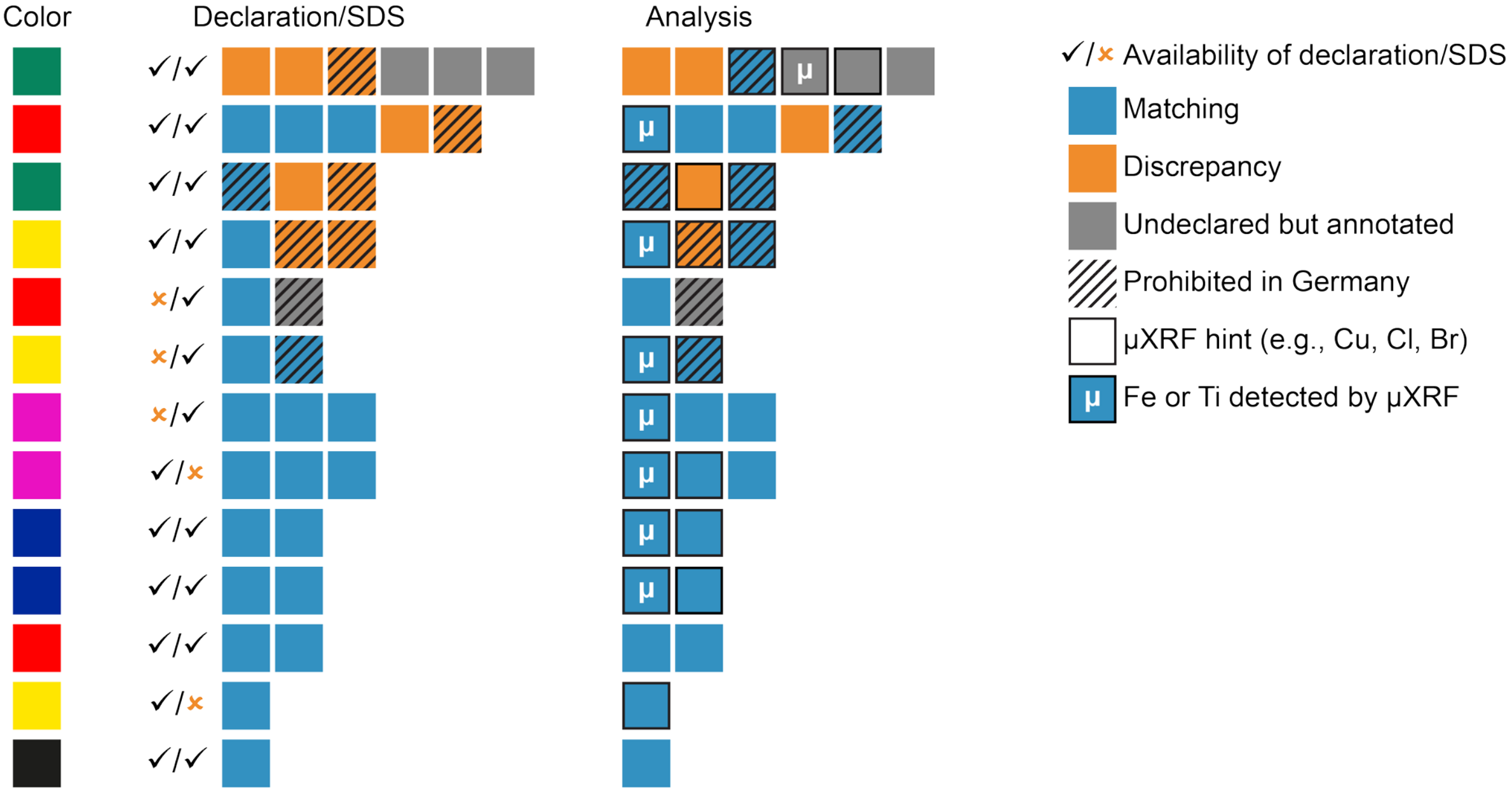






Min Intensity Max





Elemental and Molecular Analysis of 13 Tattoo Inks