MULTI-FORESEE
COST Action CA16101

MULTi-modal imaging of FOREnsic SciEnce Evidence
Tools for Forensic Science

Final Report of 2017

Chair of the Action: Prof. Dr. Simona Francese (UK)
Vice Chair of the Action: Prof. Dr. Massimo Tistarelli (IT)
Working Group 1 Leader: Dr. Manuel Algarra (E) until Q4/2017
Prof. Dr. Martina Marchetti-Deschmann (A) since Q4/2017

Table 1: Members subscribed to WG1 (2018-03-12), including information on methodologies in use and color-coding for industries (grey), academia (yellow) and end-users (green):

Name WG1 Member	email	Institution / Country	Technique/s applied
Simona Francese	s.francese@shu.ac.uk	Sheffield Hallam University (UK)	MALDI MSI
Zdravko Siketic Iva Bogdanovic Radovic	zsiketic@irb.hr iva@irb.hr	Ruder Boskovic Institute, Zagreb (Cro)	MeV-SIMS, imaging
Daniel Vella	daniel.vella@um.edu.mt	University of Malta	FEM with EDS , possibly atomic emission via LIBS
Christophe Champod Andy Bécue	christophe.champod@unil.ch andy.becue@unil.ch	Ecole des Sciences Criminelles (UNIL), (CH)	Polilight PL500 (+ observation filters), LASER (532 + 577nm + obs filters), RUVIS, Hyperspectral imaging (vis and SWIR)
Ivo Šafařík	ivosaf@yahoo.com	Biology Centre, České Budějovice (CZ)	microscopy evaluation
Kristýna Pospíšková	kristyna.pospiskova@seznam.cz	Regional Centre of Advanced Technologies and Materials, Palacký University Olomouc (CZ)	microscopy evaluation
Melanie Bailey	m.bailey@surrey.ac.uk	University of Surrey (UK)	DESI MSI and ToF SIMS imaging
Andrei Tsiatsiuyeu	npc@sudexpertiza.by	Scientific and Practical Centre of the State Forensic Examination Committee (BY)	X-ray Diffractometer, AES with ICP, SEM, Laser (355nm, 532nm, 1064nm)
Manuel Algarra	malgarra@uma.es	University of Malaga (E)	Spectroscopies Techniques 1. XPS; 2. AFM; 3 Raman (Laser (355nm, 532nm, 1064nm)
Matina Marchetti-Deschmann	martina.marchetti-deschmann@tuwien.ac.at	Institute of Chemical Technologies and Analytics, TU Wien (VIenna University of Technology) (A)	(MA)LDI and DESI MSI
Hanna Bednarz Karsten Niehaus	hanna@cebitec.uni-bielefeld.de kniehaus@cebitec.uni-bielefeld.de	Bielefeld University & Centre for Biotechnology (CeBiTec) (D)	MALDI MSI, CLSM, SEM, TEM
Marcel de Puit Jaap van der Weerd	m.de.puit@nfi.minvenj.nl	Netherlands Forensic Institute (NL)	MALDI & LDI ToF, REM

Miklos Veres	veres.miklos@wigner.mta.hu	Wigner Research Institute for Physics of the Hungarian Academy of Sciences, Budapest (HU)	Raman spectroscopy, stimulated Raman spectroscopy, two-photon imaging, FTIR
Ana Cristina Assis	ana.assis@pj.pt	Scientific Police Laboratory of Judiciary Police, Lisbon (PT)	Stereomicroscope, Luminescence & fluorescence reactions, Microspectrophotometry, Micro-FTIR, Micro-Raman
Malgorzata Iwona Szynkowska	miszynk@gmail.com	Lodz University of Technology, Institute of General and Ecological Chemistry (P)	SEM-EDS, LA-ICP-ToF-MS and ToF-SIMS imaging
Alexandra Guedes	aguedes@fc.up.pt	University of Porto (PT)	Raman spectroscopy
Nunzianda Frascione	nunzianda.frascione@kcl.ac.uk	King's College London (UK)	1. Crime Lights 2. IRIS machine (Home Office) (all wavelengths)3. Portable NIR device
Violeta Lazic	violeta.lazic@enea.it	ENEA (I)	REMOTE scanning by: LIF (355 nm), Raman (355 nm), diffusive reflectance (650 nm) and LIBS (1064 nm)
Neil Denison John O'Hara	neil.denison@westyorkshire.pnn.police.uk john.o'hara@westyorkshire.pnn.police.uk	Regional Scienitific Support Services Yorkshire and the Humber (UK)	
Maurice C.G. Aalders	m.c.aalders@amc.uva.nl	University of Amsterdam (NL)	Spectral Imaging (400-1000 nm, 1000-1700 nm), fluorescence spectroscopic imaging
Mimoza Ristova	mima.ristova@gmail.com	Ss Cyril University, Skopje (MK)	XRF, SEM/EDS,
Māra Rēpele	mara.repele@vteb.gov.lv	State Forensic Science Bureau (LV)	MVC, NINcha, VMD
Sony George	sony.george@ntnu.no	NTNU (NO)	Multispectral, Hyperspectral imaging
Thomas Fischer	thomas.fischer@b-tu.de	BTU Cottbus-Senftenberg, D	SEM-EDX, NIR/FTIR microscopy, multispectral imaging
Joanna Vella	joanna.vella@um.edu.mt	University of Malta (MT)	VSC/ ESDA - documents; Crime Light/imaging - fingerprints

Report of WG1's activities of 2017

The following document contains all information regarding WG1's activities, meetings, workshops, and results of the year 2017.
In line with the main objectives of the COST Action, the WG1 started discussions and work on a Round Robin Study (RRS) identifying the benefits of new imaging technologies for forensics giving information beyond state-of-the-art. Demands of end users had to be identified and the contributions of academic participants evaluated.
During the first meeting in Porto in June 2017, WG1 discussed options for the RRS and the actual study design. This meeting was supervised by WG1 leader M. Algarra.
First results of the RRS were presented in Krakow in Oktober 2017, where M. Algarra announced to be no longer able to head the activities of WG1. M. Marchetti-Deschmann was unanimously voted as new WG1 head.

Findings for WG1 during 2017 are summarized as follows:

A. WG1 praticipants:

All participants introduced themselves and it became obvious that this group is extremely heterogeneous. It consists of end users and people from academia, which made the first task - to define a RRS design suited for most of the participants - rather difficult.
However, it was confirmed that the most important imaging technologies were run by members of this WG at the highest level of experience. End-users and academia are involved, yet, industry is at the moment underrepresented and further actions have to be considered (details see table 1).
This survey showed the tremendous opportunities given by this COST action to test novel instrumentation for additional contributions to generate advanced state-of-the-art approaches in Police work.
In a survey undertaken in Q2/2017 it was found that only a few evidences were routinely analyzed by more than one participant (table 2).

Table 2: Evidence analysed by members subscribed to WG1, end users written in green

participant name	Bio-weapons	Body fluids	Chemical weapons	DNA	Drugs	Explosives
S. Francese	YES	YES	YES	NO	YES	YES
A. Guedes	YES	YES	YES	NO	YES	YES
A. Assis	no info	no info	no info	no info	no info	YES
N. Frascione	NO	YES	NO	YES	YES	NO
V. Lazic	Not tested	YES	Not tested	NO	YES	YES
K. Pospiskova	no info	no info	no info	no info	no info	no info
M. Veres	NO	NO	YES	NO	YES	YES
M. I. Szynkowska	NO	YES	NO	NO	YES	no info

participant name	Fibers	Finger marks	Questioned Documents	GSR	Hair	Inks/ toners	Paint
S. Francese	YES	Yes	YES	no info	YES	YES	YES
A. Guedes	YES	Yes	YES	no info	YES	YES	YES
A. Assis	YES	YES	YES	YES	no info	YES	YES
N. Frascione	NO	YES	NO	no info	NO	NO	NO
V. Lazic	Not clear	YES	No	no info	YES	YES	YES
K. Pospiskova	no info	no info	no info	no info	no info	YES	no info
M. Veres	YES	YES	YES	no info	YES	YES	YES
M. I. Szynkowska	YES	YES	YES	no info	YES	YES	YES

B. **End Unser's Review (Interpol):**

Andy Becue and Christophe Champod provided extensive reviews on fingermark analysis (see attached documents):
"Fingermarks and other impressions left by the human body (August 2007- July 2010) "
"Fingermarks and other impressions – a review (August 2010 – June 2013)"
"Fingermarks and other body impressions – a review (July 2013 – July 2016)"

These impressive documentations summarize the most important information needed from end users (e.g. methodologies/biases/performance, fingerprint features, QA, forgery, alteration, ….) for fingermarks and other body part impressions (earmarks, earprints, lips, foot morphology, …). These documents will serve as valuable documentation for further RRS considerations.

C. **RRS experimental design:**

End-users requested that the RRS should be led by them and not by academia, to better communicate the actual needs. However, letting the end-user lead the RRS makes the implementation of other, new technologies difficult. It was argued that "more of the same" will be tested, allowing not for innovations beyond state-of-the-art instrumentation.

Academia showed versatile imaging technologies applicable to forensic evidence, some already implemented in Forensic laboratories, others at the brink to routine analysis.

Following issues were clearly identified, when first steps were undertaken to define an appropriate RRS setting:

- The anticipated RRS has to be flexible to some extent to include as many participants as possible
- New developments will be limited to available national funding, therefore new technologies/concepts are unlikely
- Within the group there was mixed interest for different evidences: fingermarks, blood, fibres, nanoparticles, paint, soil, bacteria, blood, toner, accelerants, explosives, gunshot residues, tapes, were mentioned in particular (table 2).

- Additionally, within in the group many different imaging technologies are available (see table 1), which (a) complicated the decision making process for suited RRS samples, especially in respect to preferred substrates and data generated, and (b) does allow only few comparisons of results, as only few instrumental setting were comparable.

A final decision for RRS was made concordantly at the first WG meeting in Porto in respect to evidences and substrates to meet the demands of most of the participants:

- evidence: fingermarks, ink and blood
- substrate: paper, plastic and cotton

All participants were asked to provide their evidence/substrate preferences (9 possible combinations) within the next two months via a shared document and the group came to an understanding that samples had to be prepared in a controlled surrounding under reproducible conditions. In this matter WG1 decided that,

- S. Francese will prepare fingermarks
- A. Guedes will prepare inks samples
- M. Marchetti-Deschmann will prepare blood samples.

To achieve WG1 milestones in time, samples for each evidence type were prepared at certain time points and shipped to the participants. The participants were asked to document their samples at point of sample receiving (take pictures) and analyse them as soon as possible after receiving the samples documenting the time point of analyses (to take sample aging into account).
The RRS was supervised by M. Marchetti-Deschmann and results of the RRS were presented at the Krakow Meeting (P) in November 2017 (details on samples and results see Annex I).

The overall outcome of this RRS was, that

- time was too short for most of the participants to generate valuable data
- many participants did not follow time-frame given for analyses due to instrument & personnel issues (technical issues, availability), so the sample was sitting in lab undocumented,
- it was not clear for most of the participant what to look for and optimizing method was not possible,
- the depth of analysis was very diverse (fast analysis to get quick answers vs. tedious generation of high datasets to ultimately test the methodology for suitability in Police work),
- and only few results can be compared because so many different techniques were used (only 2x2 participants used same method (SIMS & optical microscopy)

The group came to an unanimously agreement to redo the RRS using evidence of relevance prepared by end users, which will be handed over to the participants in spring 2018 together with a survey asking questions of interest for the police. The RRS will be discussed in detail in Q2/2018 at a WG1 meeting.

Vienna, Tuesday, March 13, 2018

Dr. Martina Marchetti-Deschmann
WG1 Leader

Annex 1

WG1 RRS Result Report

Martina Marchetti-Deschmann (Austria)

Introduction

The main goal of this study was to examine the performance of existing imaging technologies (analytical chemistry instrumentation) on three types of evidences (blood, sweat, ink) prepared on different types of substrate (paper, plastics, cotton). No final goal was defined as participant were asked to present their best data.
Participant were allowed to request a defined number of samples (including samples for test measurements and replicates) in any combination they preferred (max. 7 different samples). All samples were prepared under controlled conditions within the shortest time possible and sent to participants by FedEx and UPS.

The experimental results suggest that most participants were able to gain information from the requested samples. However, data were highly diverse, mostly depending on the aim of the experiments, which were defined by the respective working group. Depending on the time invested more or less information was available.

This RRS demonstrated the capabilities of the many imaging methods available within this COST action but a better concerted RRS has to be carried out to develop a robust protocol and workflow for routine end user's work.

Samples

Substrates: paper (100 or 160 g/cm^2), fabric (white cotton), plastic (PVC)

Evidence:

equine blood (prepared by Martina Marchetti-Deschmann)

prepared as follows:

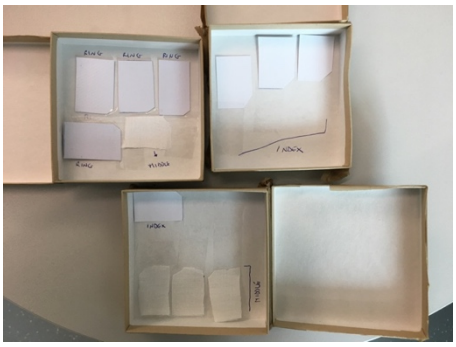
- was shipped on dry-ice from UK (SF) to A (MMD) and immediately stored on -20 °C
- upon arrival (Sept 14th, 2017)
- blood was thawn in a warm water bath (time: 2 hours)
- untreated blood was stored at 4°C (overnight Mon > Tue)
- blood was taken from the fridge and brought to room temperature (30 min)
- 2x 500µL blood were pipetted into an Eppendorf tube (2mL)
- centrifugation for 15 sec on a benchtop centrifuge (low g) to remove particles for 15 sec

- the supernatant was taken for chemical inkjet printing ("printing") on a ChIP-1000 (Shimadzu); before printing, the Piezo head has been washed with iPrOH, ACN, MeOH and water
- for proper printing blood samples had to be diluted:
  500 of water (UHQ) were mixed with 100 µL blood and 400 µL of this solution were put into the printing vessel
- Samples were printed as follows: 80 pL per droplet, 3 droplets per spot resulting in 240 pL / spot; Droplets were deposited in a distance of 250 µm (pitch size) in an area of approx. 5 x 5 mm.
- Beside every printed area, on every surface, 0.5 µL of original blood (no centrifugation or other handling) were deposited at the end of the printing process.
- Samples were handled with Nitril gloves and fixed in paper boxes with adhesive tape.
- All samples were documented (photos were taken).

ChIP parameters (dwell time, waiting time, ….) were optimized for printing (blood droplets). Blood droplets are not uniform and tend to produce satellite droplets. Although the droplets often did not seem to look alike, they always had a volume of 80 pL. Samples were fixed in paper boxes and sent to receiving participant.

Sweat samples (prepared by Simona Francese)

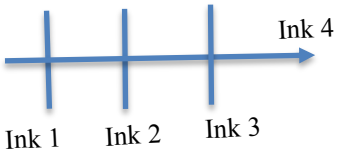
S. Francese turned out to be a "bad" donor, who is sweating only a little bit and leaves only few marks on a given surface. All samples were prepared within 1 day (middle finger on cotton, ring finger on PVC, index finger on paper). Samples were shipped in paper boxes after adding information.



Ungroomed marks were deposited using different fingertips and a fingerprint generator. The following process was used in a standardized manner:
1. Wash hands with soap and warm water. Dry with a clean tissue
2. Wait 15 min while doing computer work (only using keyboard and touching nothing else) to induce sweat
3. Fingertips were rubbed again against each other for 10 seconds and a second mark deposition was made (this should have avoided making a depletion series)
4. Index fingermark were always deposited on paper. Middle fingertip was always used to deposit marks on fabric. Ring fingertips always used for marks on PVC cards. This is also clearly indicated in the box Samples were securely stored in paper boxes, marked and sent to receiving participant.

4 inks (prepared by Alexandra Guedes)

Ink 1- BIC cristal
Ink 2- Pentel SuperB
Ink 3- Staedtler triplus ball
Ink 4- Paper Mate



Ink samples were provided on print paper (100 gr/cm^2) only and drawn as shown. Three parallel strikes from the Inks 1, 2 and 3 were draw and ink 4 across orthogonally with an arrow for future identification of the different inks used in each strike.

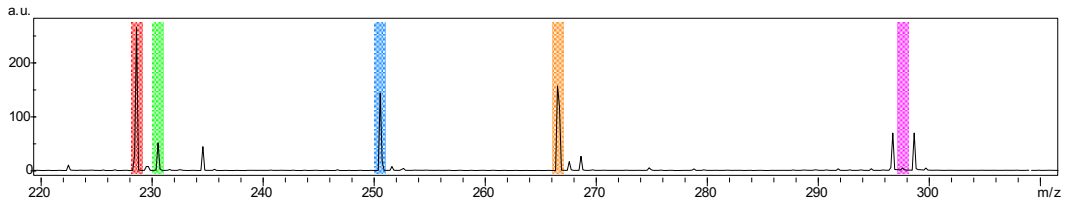
Summarized results of successful imaging experiments

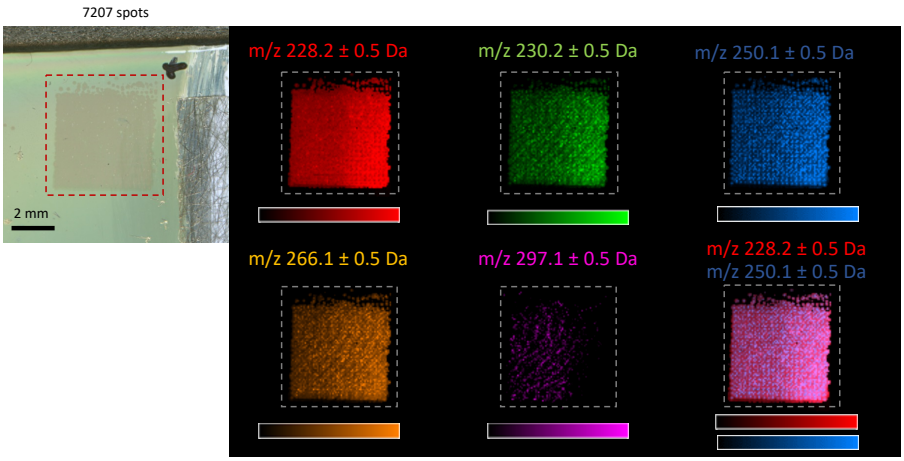
Equine Blood Samples

- Mass Spectrometry Based Imaging:

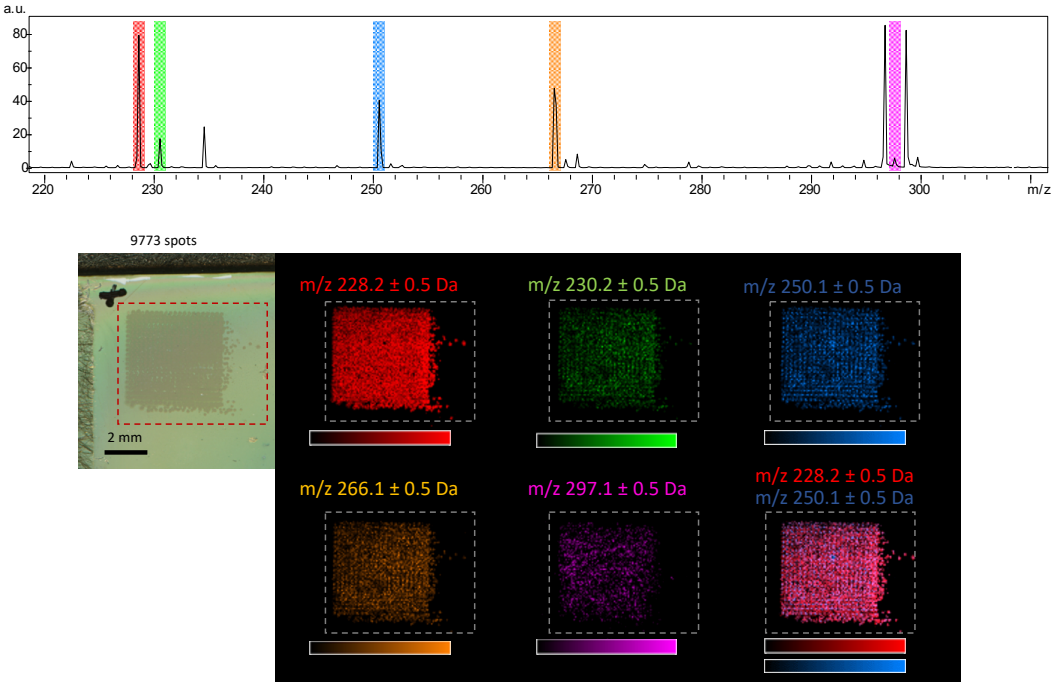
Martina Marchetti-Deschmann: instrumentation: MALDI MSI on an UltrafleXtreme (Bruker), acquisition mass range 100-1000 Da in positive mode, samples were fixed on conductive glass slide (ITO target) with conductive double-sided adhesive tape. Samples were measured by LDI and MALDI after applying CHCA as matrix. To improve ion intensities samples were also covered with a thin film of Ag (sputtering).
Reported limitations: cotton samples were not meaaured because of short current between sample plate and first extraction lens (fibres on surface).

Without putting too much effort into analysis, blood could be measured from plastic after Ag sputtering and matrix deposition (7207 pixels):



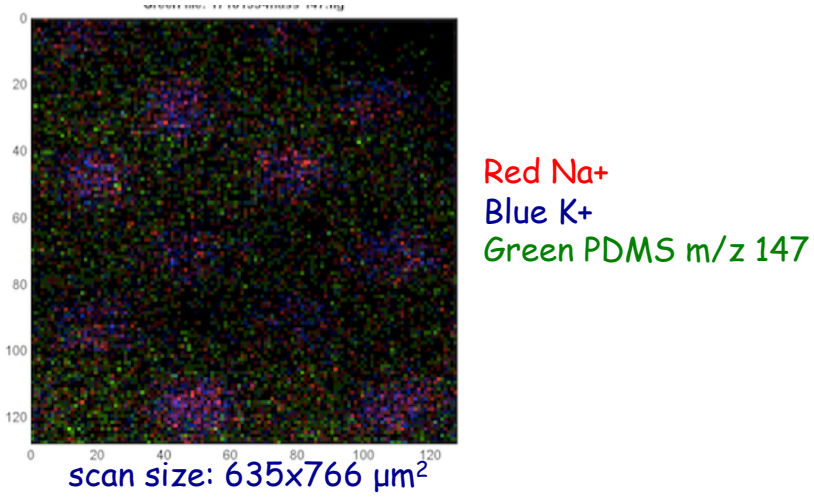


Sample 2 (9773 pixels) clearly showing the possible lateral of MALDI MSI where blood droplet at 250 µm distance could be resolved:



I. Bogdanović Radović: instrumentation: Measurements were performed under vacuum (10^{-6} - 10^{-7} mbar) using the MeV-SIMS setup with a Time-of-Flight (TOF) spectrometer at the Ruđer Bošković Institute (RBI) heavy ion microprobe; -8 MeV Si^{4+} ions; lateral beam resolution 10 x 10 μm^2. The beam was scanned over different areas on a sample for imaging of the intersecting lines. Beam current in the pulsed mode was ~ 0.2 fA.

Samples were mounted on a metal sample holder and blood on plastics was analysed. The printed array could laterally be resolved:



For blood on paper signal intensities were not good enough for imaging but characteristic m/z values were found for blood (not apparent in paper): 30.1, 56.1, 68.2, 70.3, 72.2, 84.2, 86.2

For blood on cotton signal intensities were not good enough for imaging but characteristic m/z values were found for blood (not apparent in cotton): 28.1, 30.1, 44.1, 56.1, 58.1, 59.2, 68.2, 70.3, 72.2

- Spectroscopy Based Imaging:

A. Becue, C. Champod:

Blood droplet was detected, lateral resolution did not allow resolution of small droplets in 250x250 µm rectangular shape.

NB: Best technique in green (easiness, speed), second best in orange

Technique	Parameters	Observations
Naked eye	- Ambiant light	- Obs: 1 drop and 2 squares (all S)
Polilight PL500 (Rofin)	- White light - 415 nm - UV - Luminescence (all λ + orange filter)	- Obs: 1 drop and 2 squares (all S) - Better contrast at 415nm (all S); due to the absorption of blood at this λ - No improvement of marks/contrast in luminescence; no luminescence behaviour (no pics taken) - Imaging is extremely quick (seconds)
LASER (Coherent)	- 532 nm + orange filter - 577 nm + violet filter	- 532nm: 1 drop (all S) and 2 squares (fabric and plastic only) - 577nm: 1 drop (all substrates) - No background luminescence for paper at 577nm (dark)
RUVIS (ArrowHead)	- 254nm	- Obs: 1 drop and 2 squares (plastic)
Hyperspectral imaging (SPECIM)	Vis (400 - 1000 nm) SWIR (1000 – 2500 nm)	- Obs: best contrast obtained at 415nm and 672nm

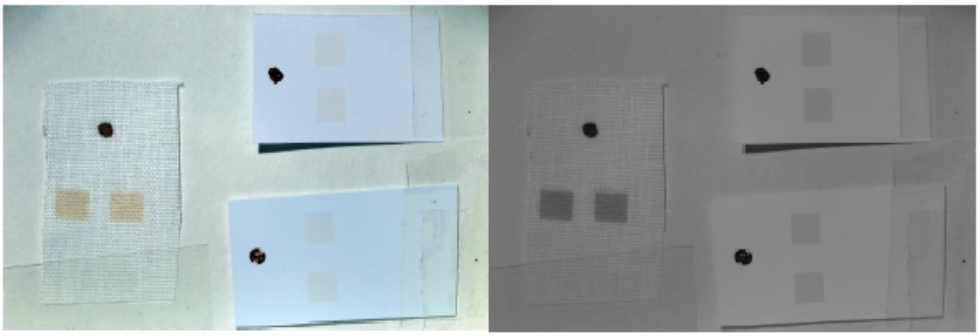


Figure: PL500/white light (left); PL500/415nm + grey scales (right) – no image optimization

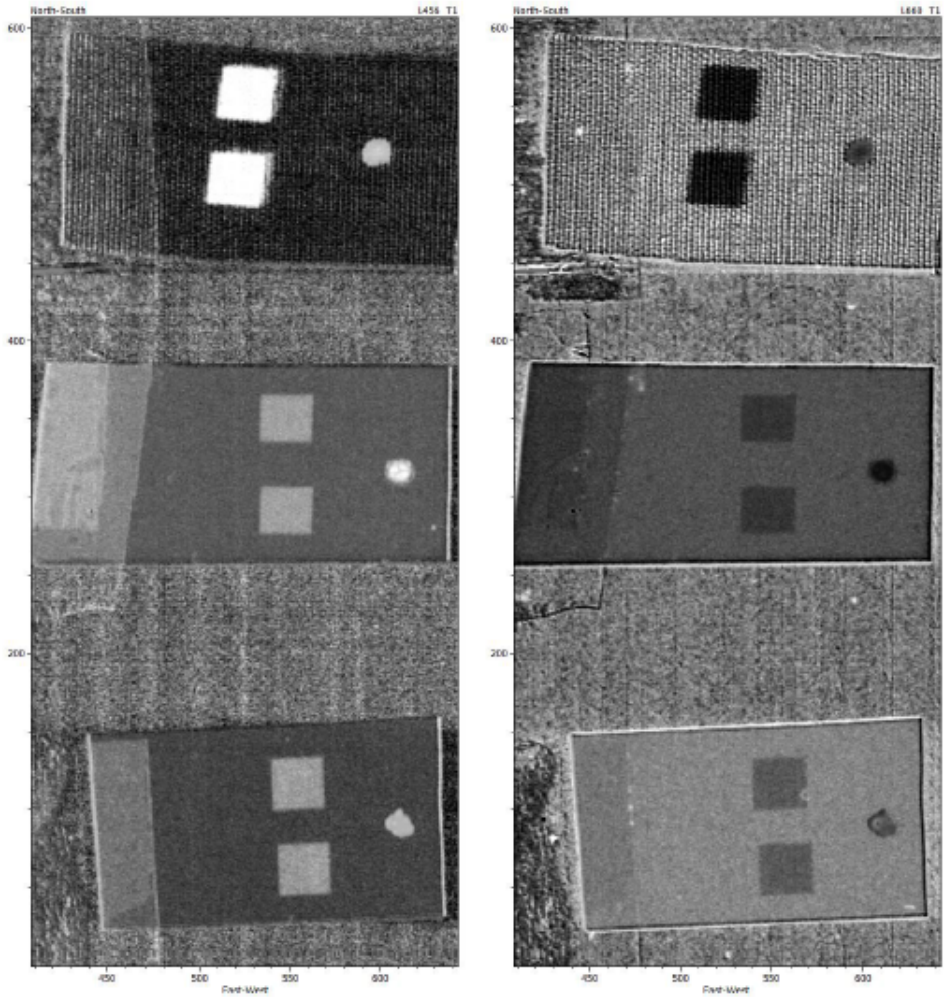
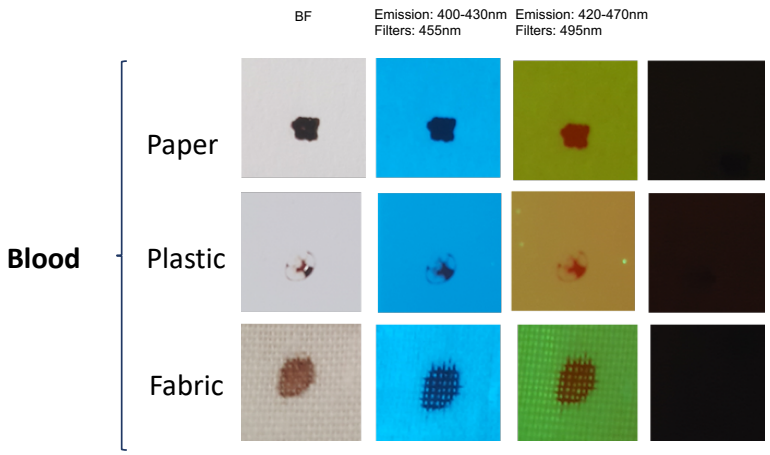


Figure: Hyperspectral imaging performed at 415nm (left) and 672nm (right)

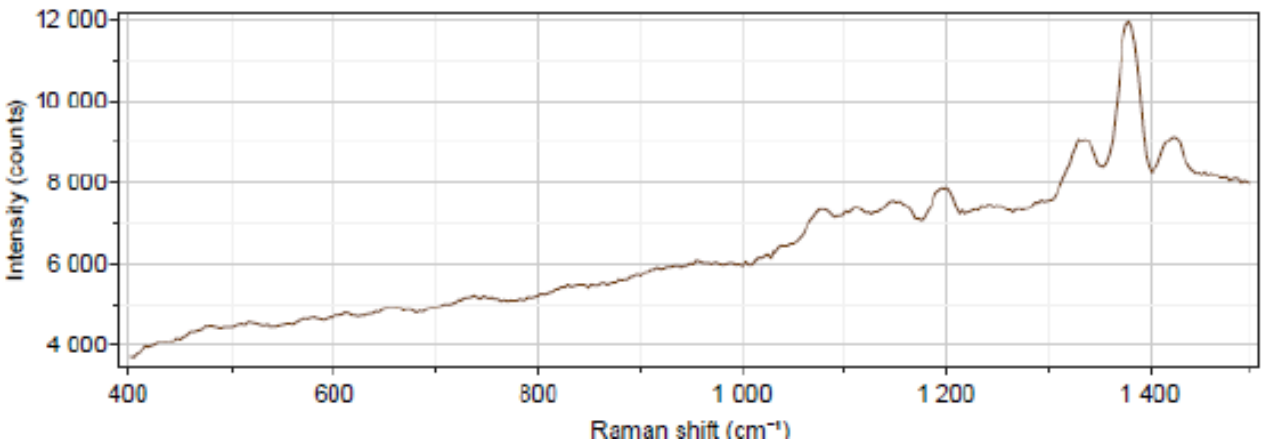
***Nunzianda Frascione*:** instrumentation/methodology: The applied techniques are not been validated yet and therefore could not be used for the purpose of this study. Yet, alternate light sources, IRIS (used at different wavelengths) and a portable NIR device were tested for suitability. All of the

techniques are mainly used for detection purposes only (e.g. location of evidence deposited on surfaces) and would work exploiting either intrinsic properties of the biological evidence (e.g. presence of fluorophores) or fluorescence associated with contaminants. Results were only obtained from blood deposited as droplet on all three substrates (cotton, paper, plastic). The resolution did not give information on smaller lateral resolutions.

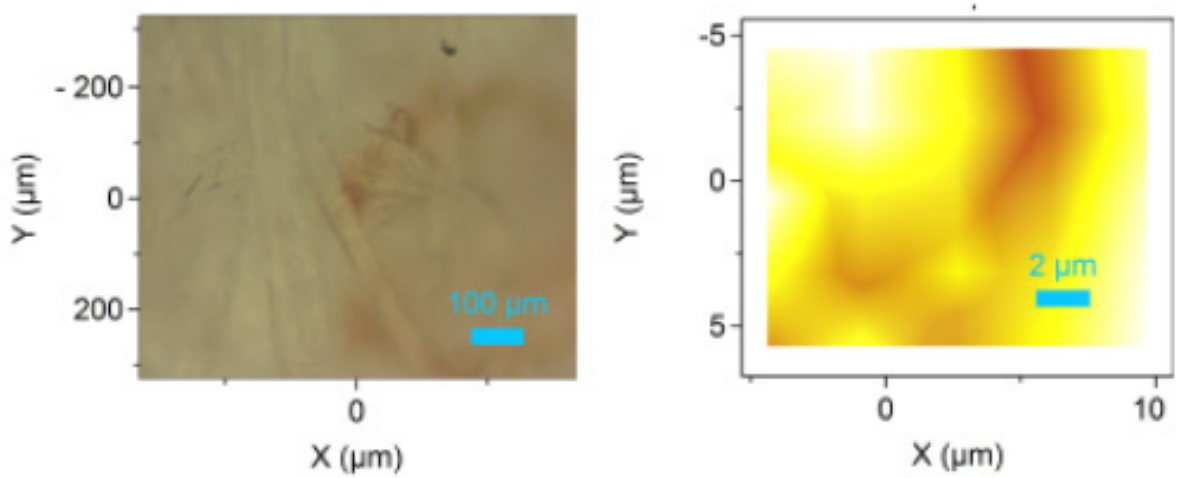


***Alexandra Guedes*:** instrumentation/methodology: Raman spectra of blood were obtained using a Horiba Jobin-Yvon Raman spectrometer XploRATM, equipped with an excitation wavelength of 532 nm from Ar+ laser at a power of 25 mW and with a diffraction gratings with 1200 lines mm-1. An Olympus optical microscope with a Å~100 objective lens was used to focus the laser beam on the sample and collect the scattered radiation. The laser power was reduced 90% with a neutral density filter to avoid thermal decomposition of the samples. Extended scans were performed on the spectral range 400 to 1500 cm-1. The time of acquisition and the number of accumulations varied in order to obtain an optimized spectrum for each analysed point.

Raman analysis of blood reveal a spectrum with a strong band at 1380 cm-1, probably assigned to hemoglobin. Raman spectrum of blood:



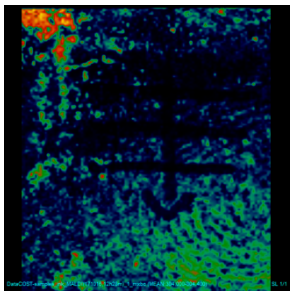
Raman Imaging of blood:



Sweat Samples

- Mass Spectrometry Based Imaging:

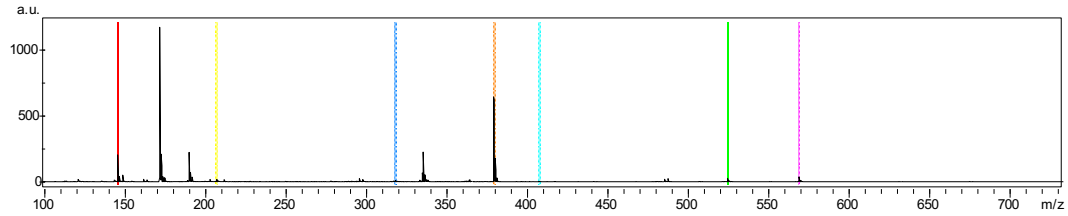
Simona Francese: instrumentation: (MA)LDI MSI, Resolution 150x150 microns, acquisition mass range 100-1000 Da in positive mode, samples were cut out and paper was stuck directly on a MALDI target plate (with a recessed adaptor) and submitted to (matrix assisteted) laser desorption mass spectrometry imaging.
Reported limitations: Instrument issues (data corrupt).

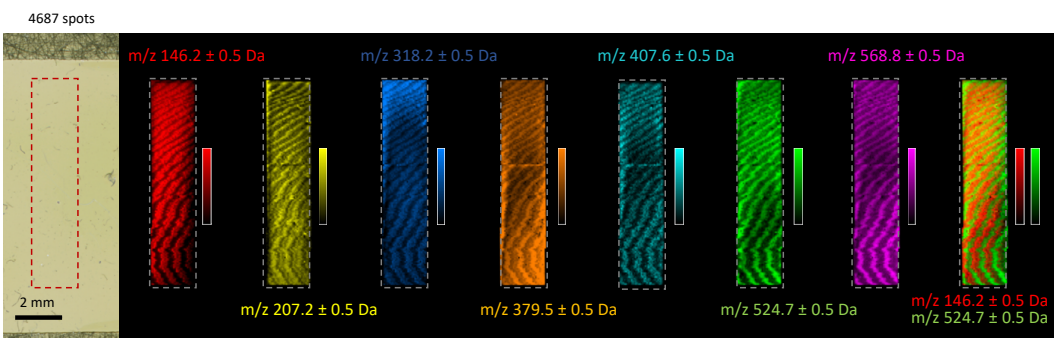


MALDI MSI ion image at m/z 304.2 reveals the presence of a fingerprint (bottom right corner). This signal is unique to the fingerprint and is not detected in the inks.

Martina Marchetti-Deschmann: instrumentation: MALDI MSI on an UltrafleXtreme (Bruker), resolution 50x50 microns, acquisition mass range 100-1000 Da in positive mode, samples were fixed on conductive glass slide (ITO target) with conductive double-sided adhesive tape. Samples were measured by LDI and MALDI after applying CHCA as matrix. To improve ion intensities samples were also covered with a thin film of Ag (sputtering).
Reported limitations: cotton samples were not meaaured because of short current between sample plate and first extraction lens (fibres on surface).

Fingermarls were only detected after Ag sputtering and matrix application on plastic as substrate.



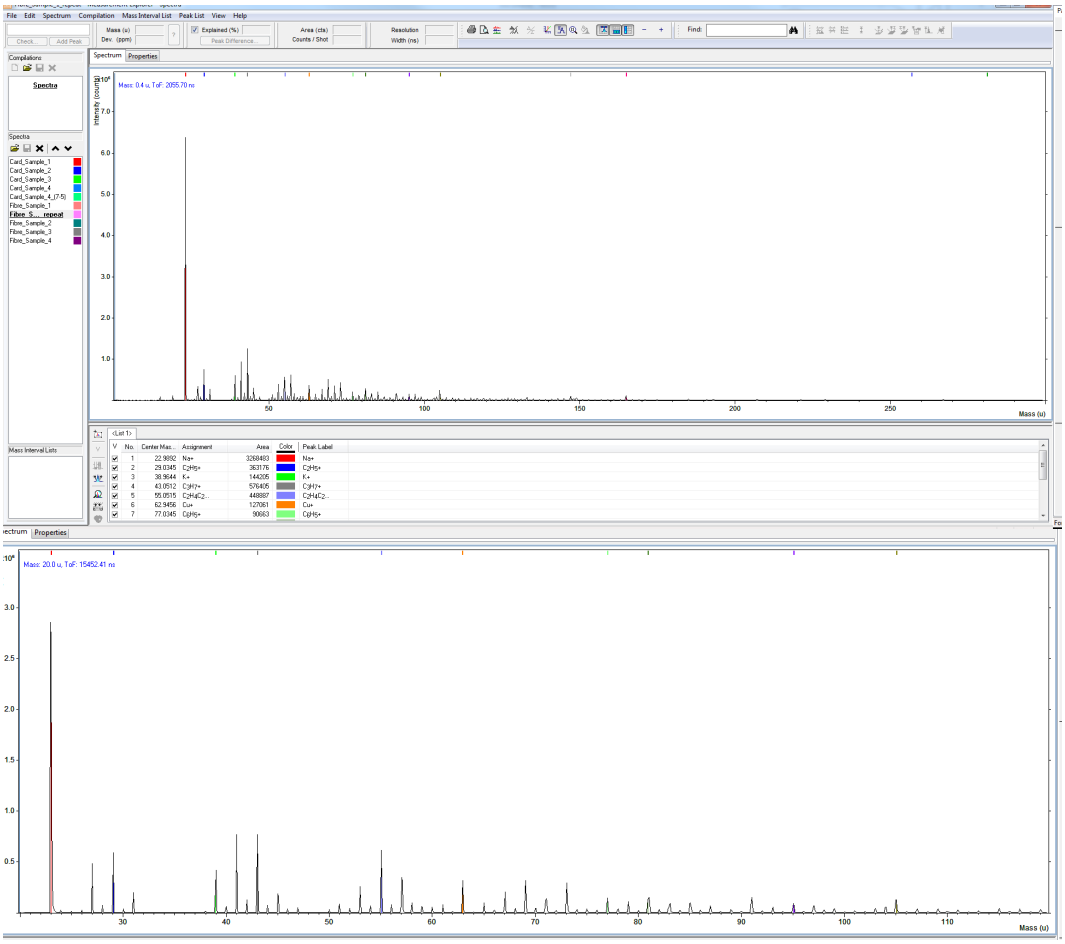


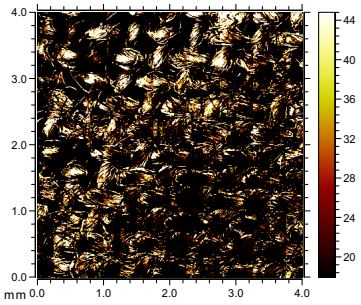
Only a small area was measured due to time constraints. Fingerprint can clearly be deciphered from background ions (overlay m/z 146 & 524).

Melanie Bailey: instrumentation: Ion-TOF 5 SIMS instrument with 7.5 keV Bi^{3+} LMIG primary ion beam
1.00 µA beam current
1 scan
5 shots/pixel
10 frames/patch
0.25 patch size
100 pixel/mm
See scales on images for scan size (mm)
Calibrated CH_3, Na, Al, C_2H_3, Si, C_2H_4, CHO, C_2H_5, K, C_3H_5, C_2H_3O, C_3H_7, C_4H_5, C_3H_3O, C_4H_7, Na_2OH, C_5H_7, C_4H_5O, C_5H_9, SiC_3H_9, C_6H_5, C_6H_{11}, C_7H_7, C_7H_9, C_7H_{11}, C_7H_{13}, $Si_2C_5H_{15}O$, $Si_3C_5H_{15}O_3$, $Si_3C_7H_{21}O_2$.

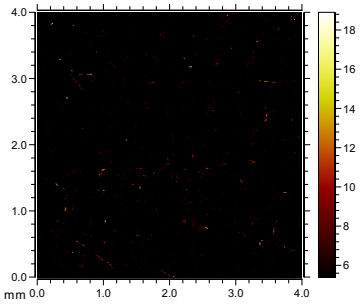
Reported limitations: Due to problems with the ion source, the beam energy was reduced to 7.5 keV. This may have reduced the sensitivity to certain analytes. Data was acquired in .ita format instead of .itm format, which meant that only images that are shown could be provided. Location of the fingerprints on substrate was unknown, which may have led to non-detection of the fingerprint in one of the samples (Card Sample 3).

Blood on Cotton 1

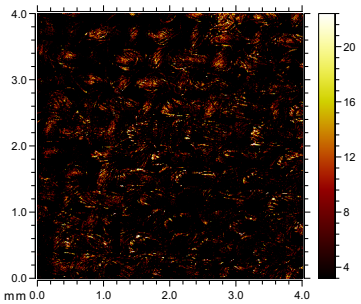




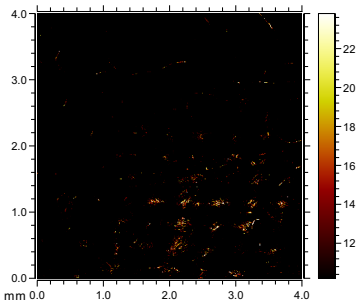
Na+
MC: 45; TC: 2.161e+006



K+
MC: 19; TC: 1.409e+005

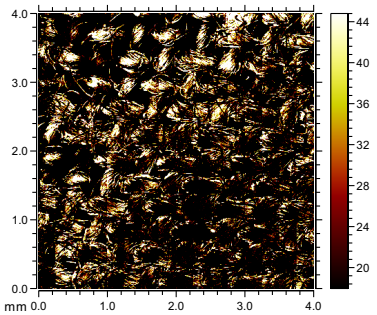


C_2H_5+
MC: 22; TC: 3.381e+005

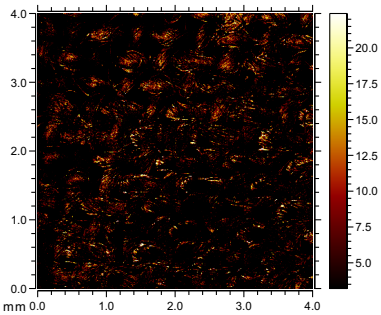


Cu+
MC: 24; TC: 1.291e+005

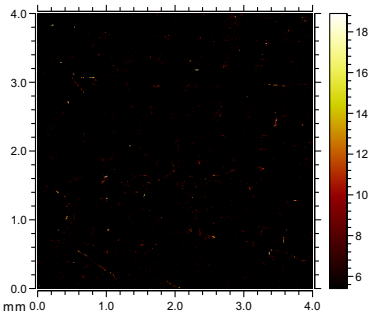
Multi-Foresee



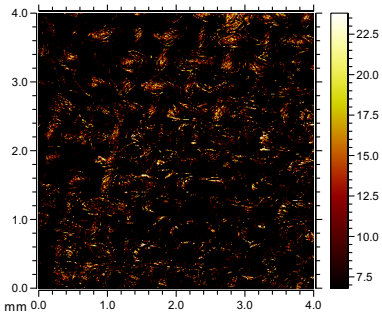
Na+
MC: 45; TC: 2.161e+006



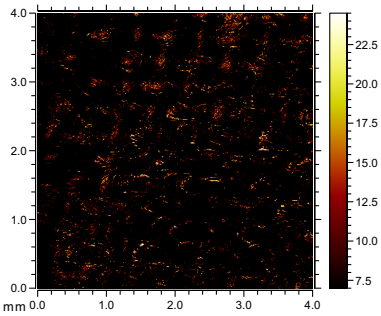
C_2H_5+
MC: 22; TC: 3.381e+005



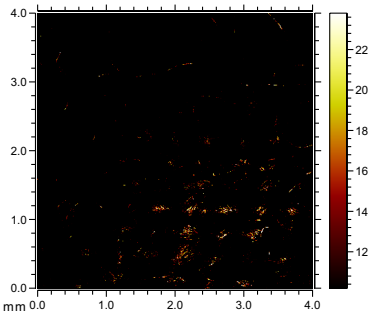
K+
MC: 19; TC: 1.409e+005



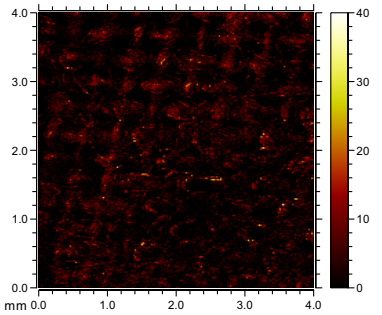
C_3H_7+
MC: 24; TC: 5.119e+005



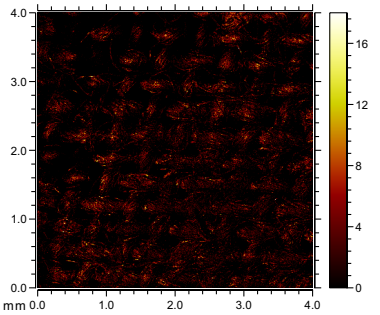
$C_2H_4C_2H_3$+
MC: 25; TC: 4.072e+005



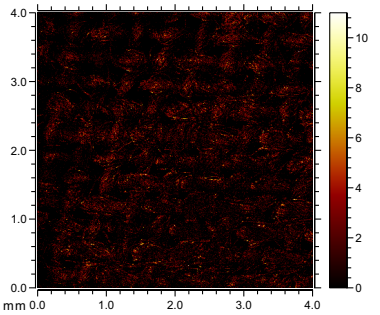
Cu+
MC: 24; TC: 1.291e+005



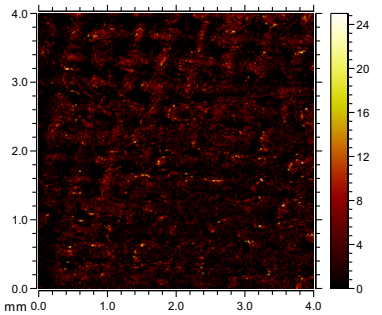
C_6H_5+
MC: 40; TC: 8.790e+004



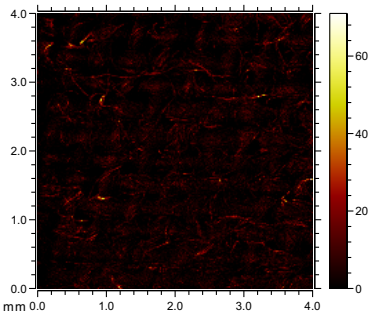
Al_3+
MC: 18; TC: 1.317e+005



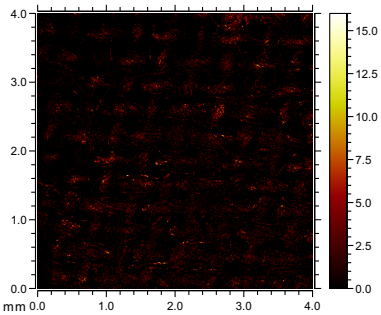
C_7H_{11}+
MC: 11; TC: 8.831e+004



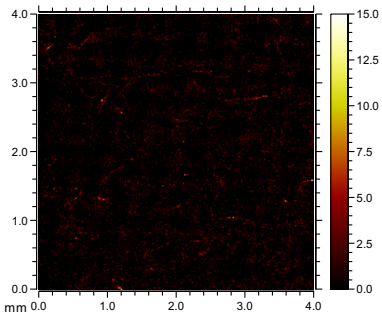
C_8H_9+
MC: 25; TC: 7.280e+004



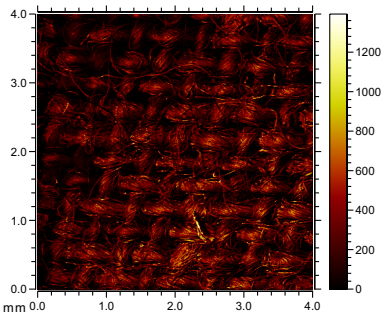
$C_7H_{12}O_2F$+
MC: 71; TC: 1.158e+005



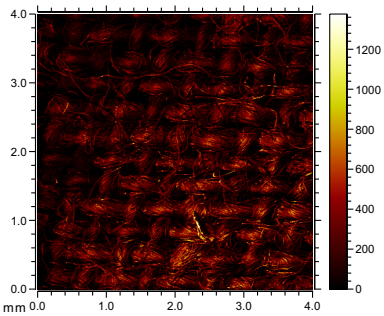
$H_6O_2Al_4F$+
MC: 16; TC: 7.480e+004



$C_{13}H_{15}O_4Na_2$+
MC: 15; TC: 1.351e+004

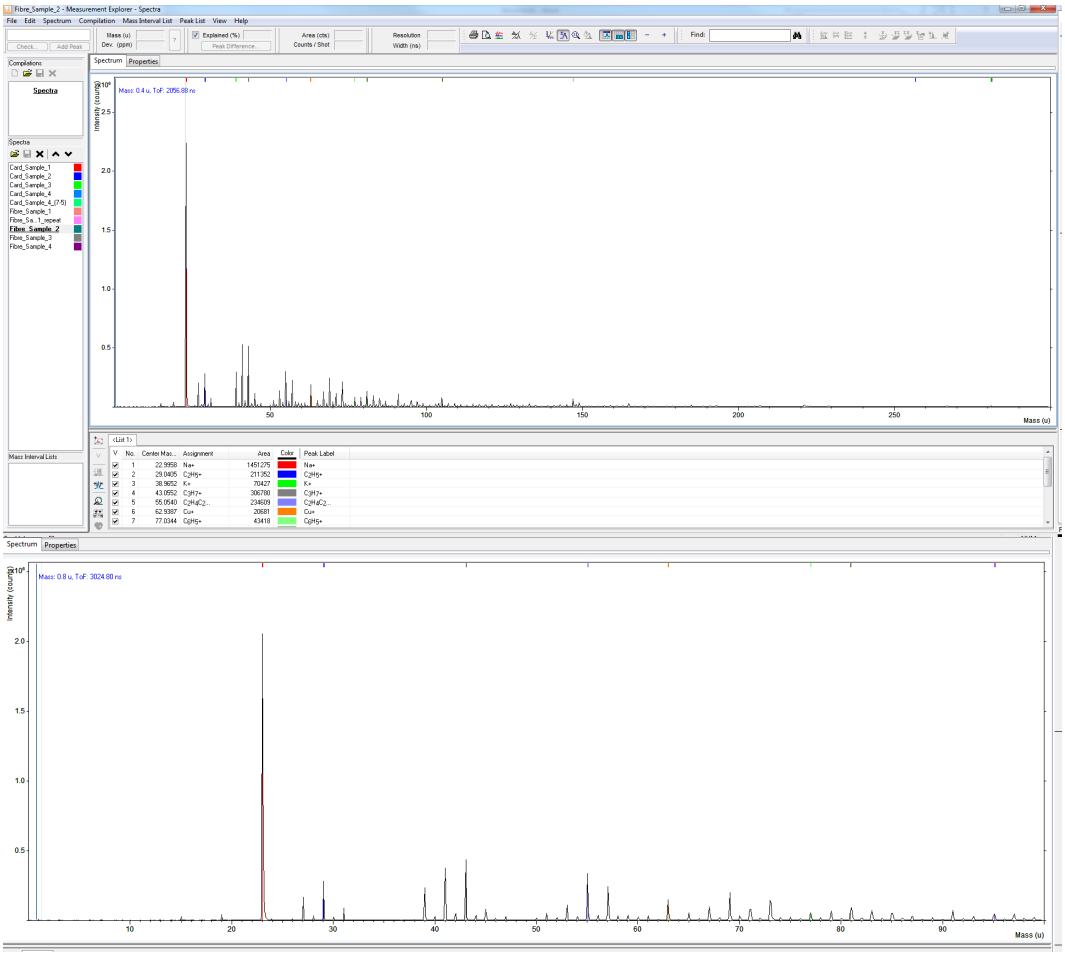


total
MC: 1393; TC: 2.764e+007

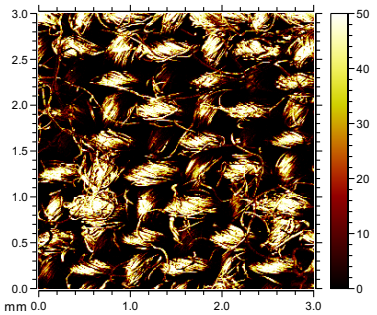


sum of rest
MC: 1376; TC: 2.337e+007

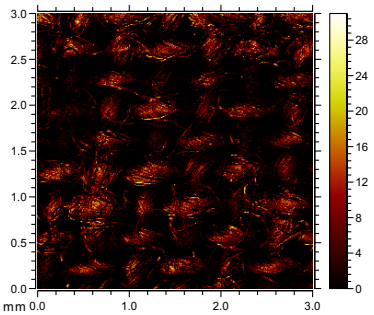
Blood on cotton 2:



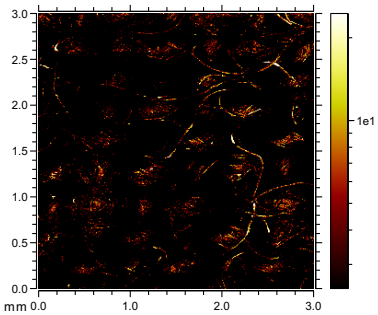
Multi-Foresee



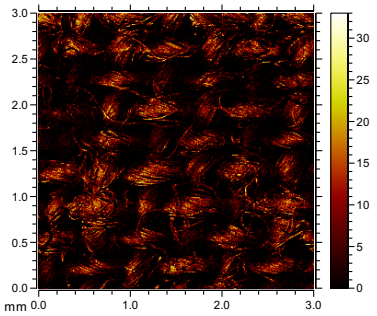
Na+
MC: 50; TC: 1.216e+006



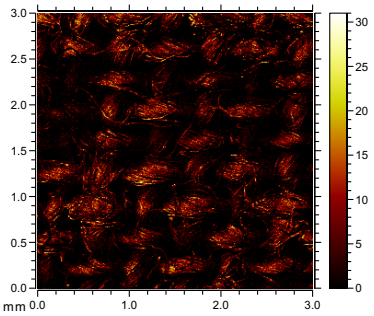
C_2H_5+
MC: 31; TC: 2.025e+005



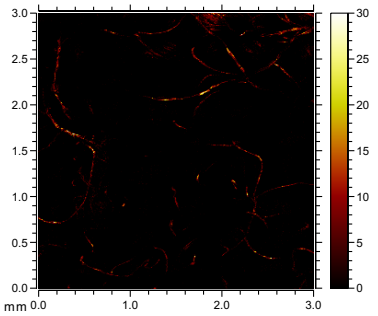
K+
MC: 25; TC: 6.951e+004



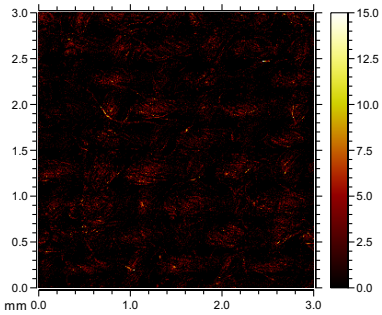
C_3H_7+
MC: 33; TC: 2.860e+005



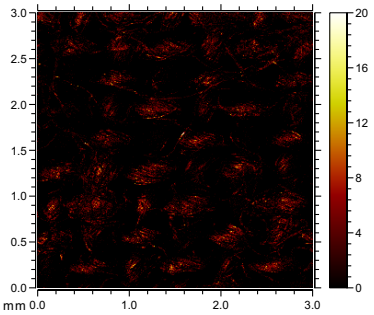
$C_2H_4C_2H_3$+
MC: 31; TC: 2.221e+005



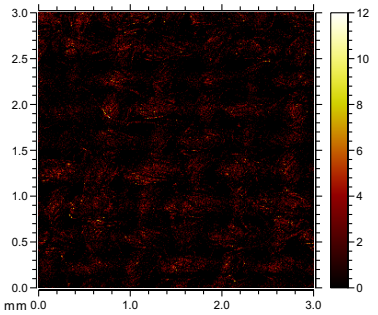
Cu+
MC: 30; TC: 2.063e+004



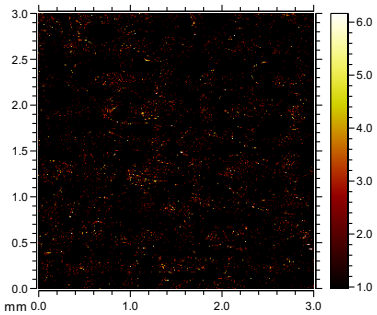
C_6H_5+
MC: 15; TC: 4.278e+004



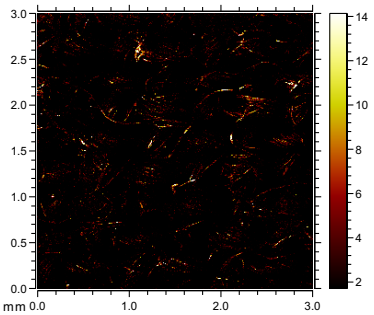
Al_3+
MC: 20; TC: 5.515e+004



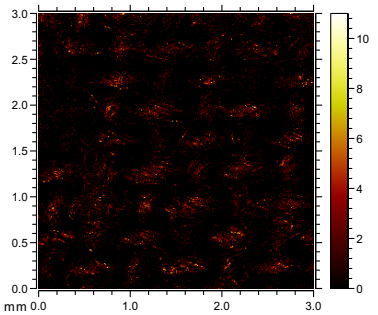
C_7H_{11}+
MC: 12; TC: 4.150e+004



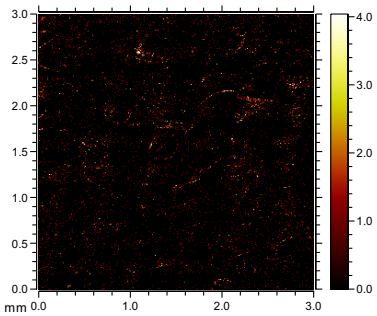
C_8H_9+
MC: 6; TC: 3.075e+004



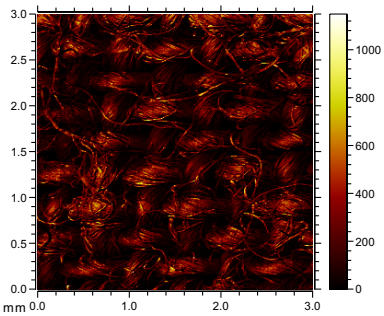
$C_7H_{12}O_2F$+
MC: 14; TC: 6.049e+004



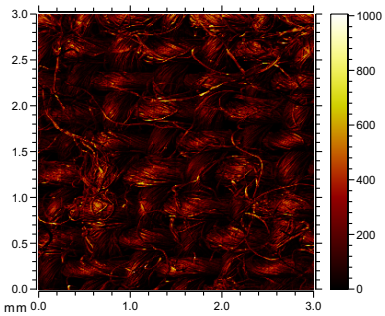
$H_6O_2Al_4F$+
MC: 11; TC: 2.959e+004



$C_{13}H_{15}O_4Na_2$+
MC: 4; TC: 7.286e+003

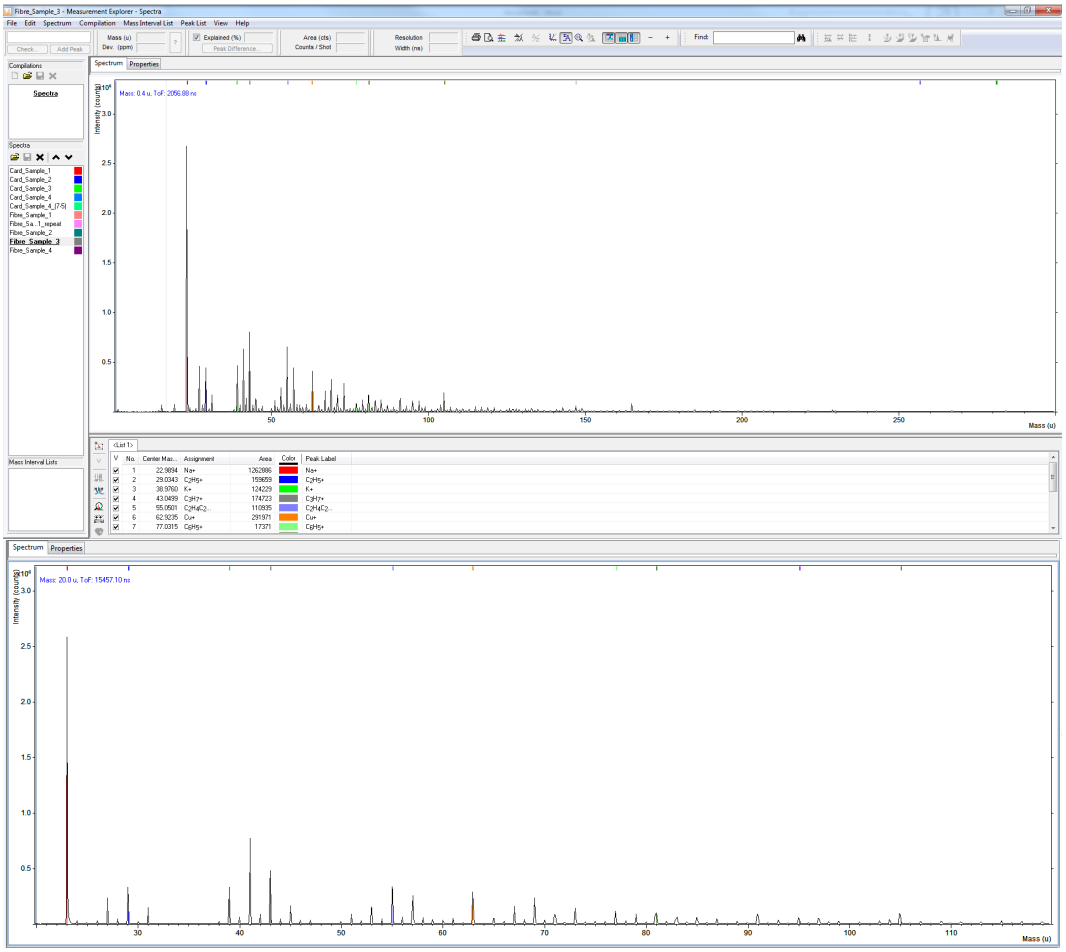


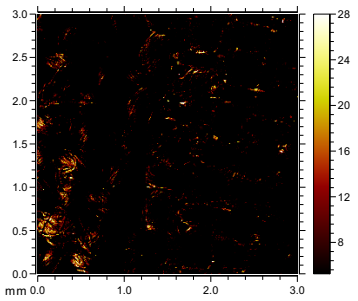
total
MC: 1149; TC: 1.208e+007



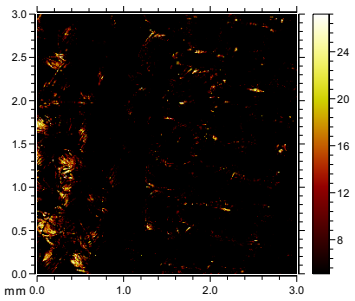
sum of rest
MC: 1006; TC: 9.791e+006

Blood on Cotton 4:

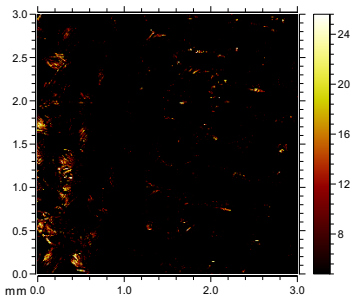




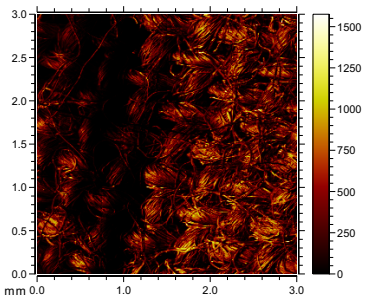
C_2H_5+
MC: 28; TC: 1.394e+005



C_3H_7+
MC: 27; TC: 1.467e+005

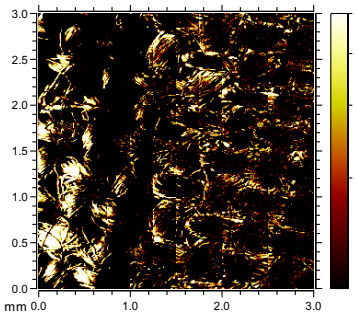


$C_2H_4C_2H_3+$
MC: 26; TC: 9.664e+004

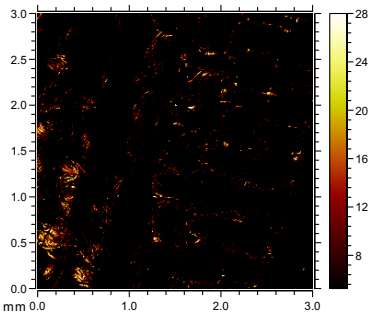


total
MC: 1575; TC: 1.878e+007

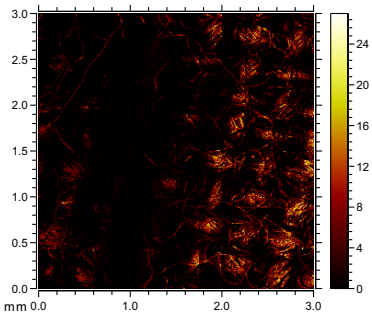
Multi-Foresee



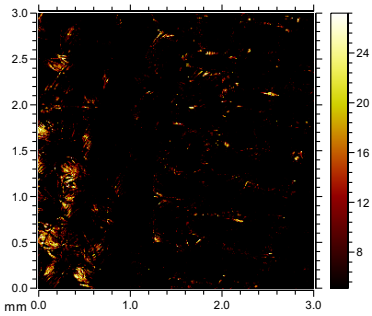
Na+
MC: 50; TC: 7.605e+005



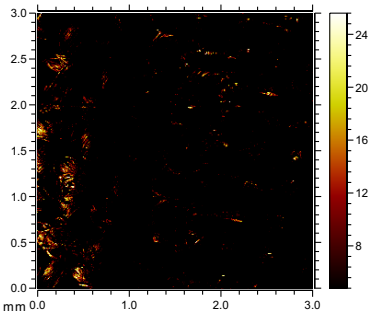
C_2H_5+
MC: 28; TC: 1.394e+005



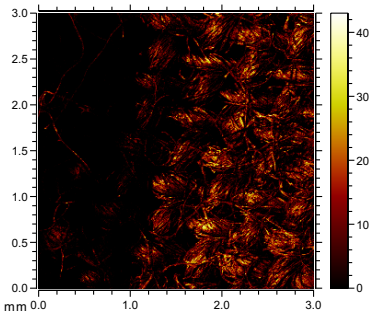
K+
MC: 27; TC: 1.195e+005



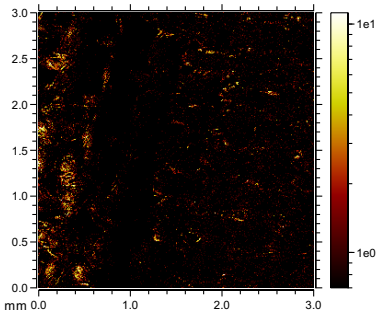
C_3H_7+
MC: 27; TC: 1.467e+005



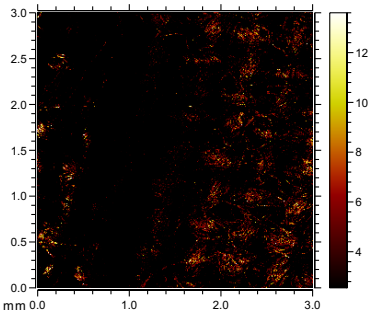
$C_2H_4C_2H_3$+
MC: 26; TC: 9.664e+004



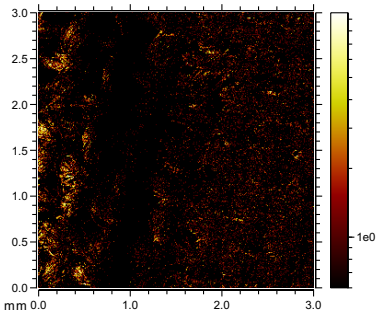
Cu+
MC: 43; TC: 2.819e+005



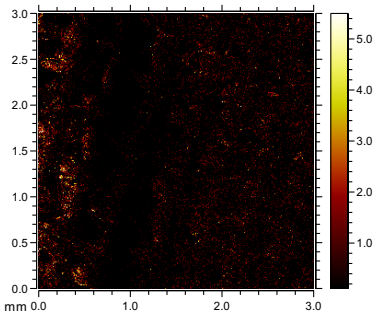
C_6H_5+
MC: 11; TC: 1.680e+004



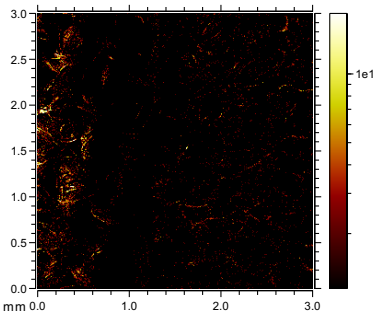
Al_3+
MC: 14; TC: 8.604e+004



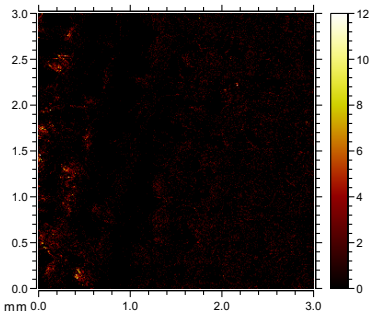
C_7H_{11}+
MC: 10; TC: 2.082e+004



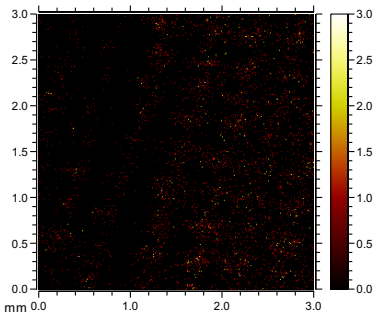
C_8H_9+
MC: 5; TC: 1.579e+004



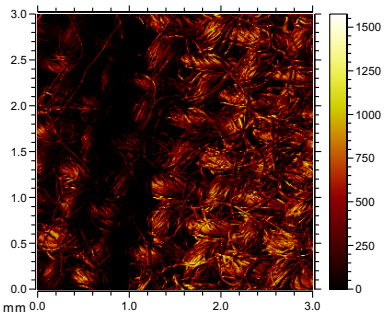
$C_7H_{12}O_2F$+
MC: 18; TC: 2.790e+004



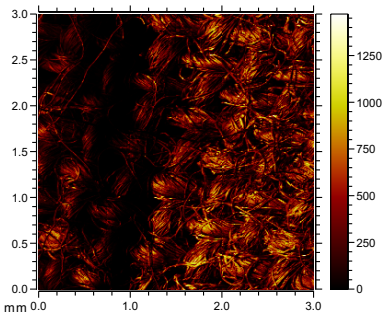
$H_6O_2Al_4F$+
MC: 12; TC: 1.947e+004



Ti_3O_7H+
MC: 3; TC: 4.356e+003

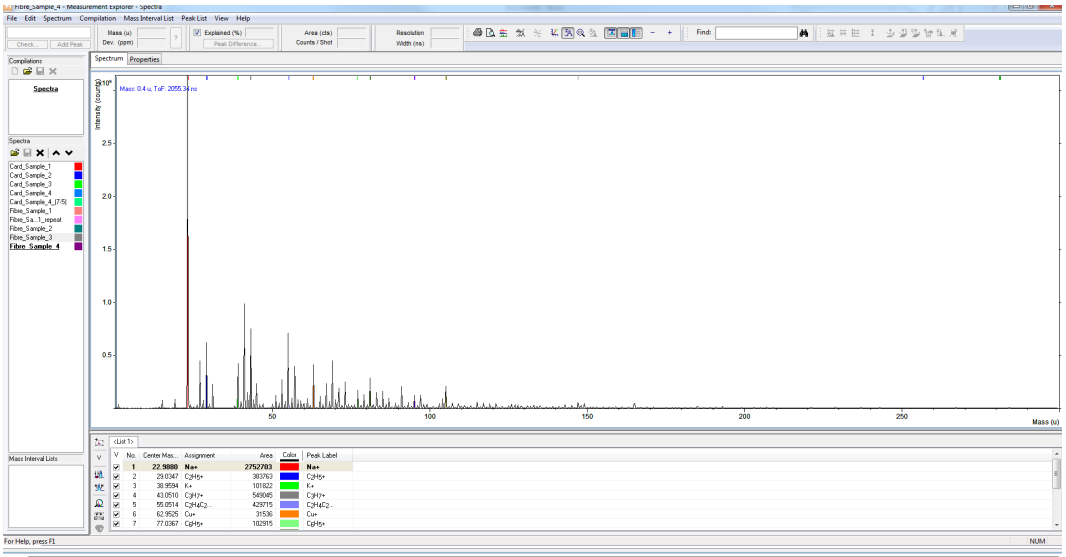


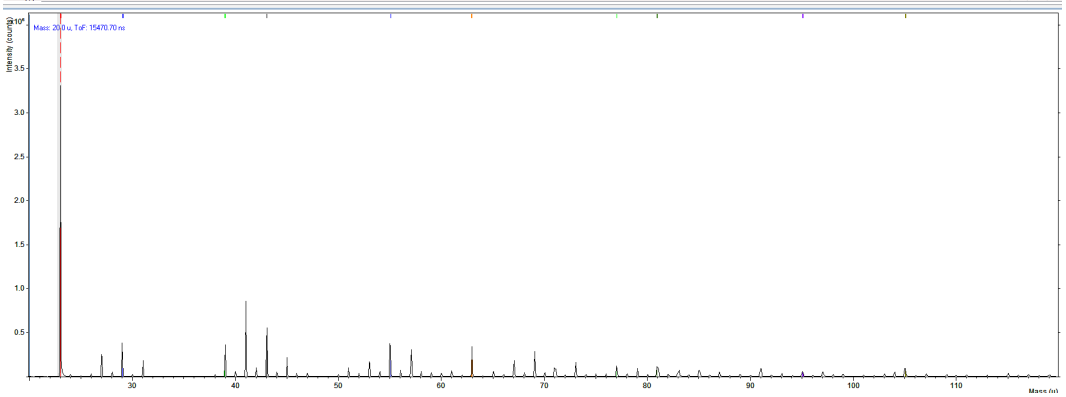
total
MC: 1575; TC: 1.878e+007

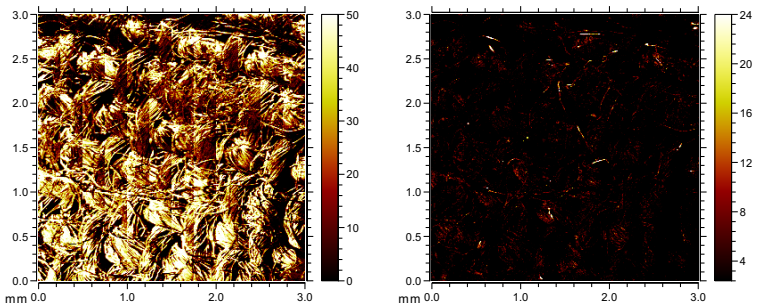


sum of rest
MC: 1472; TC: 1.704e+007

Blood on cotton:

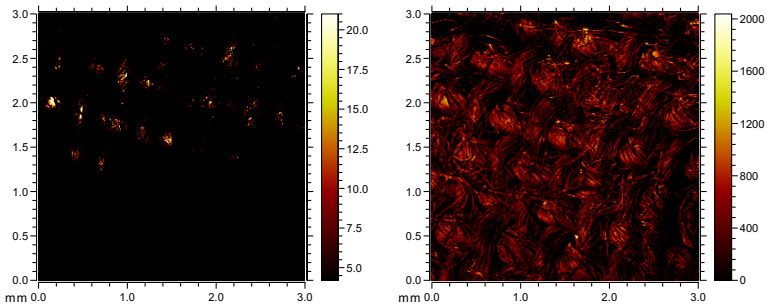






Na+
MC: 50; TC: 1.803e+006

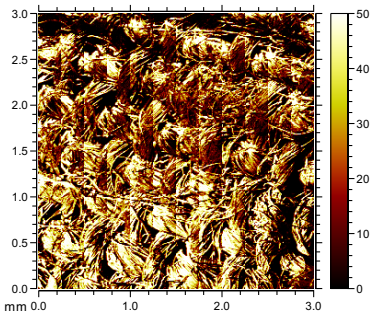
K+
MC: 24; TC: 9.995e+004



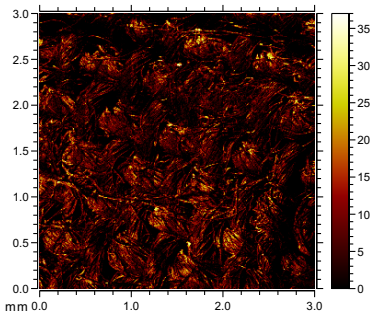
Cu+
MC: 21; TC: 3.142e+004

total
MC: 2038; TC: 1.936e+007

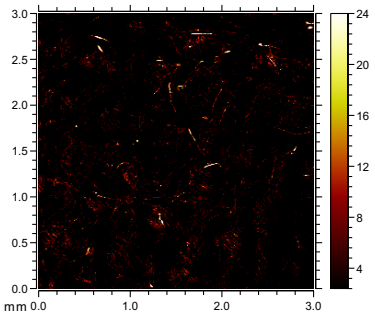
Multi-Foresee



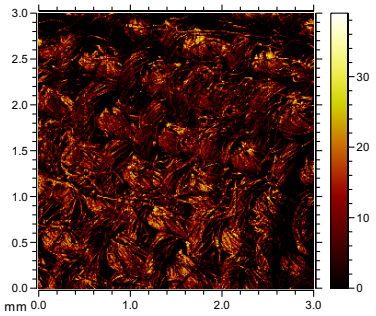
Na+
MC: 50; TC: 1.803e+006



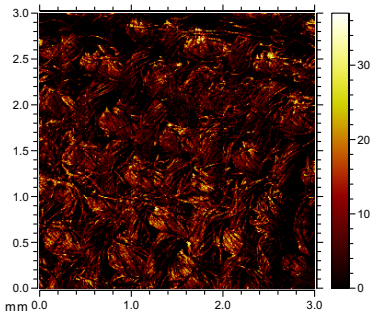
C_2H_5+
MC: 37; TC: 3.524e+005



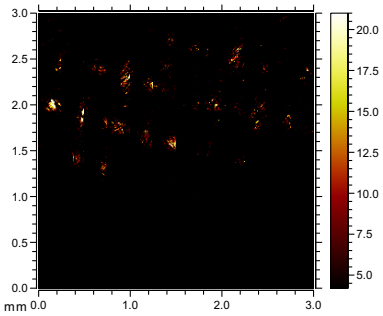
K+
MC: 24; TC: 9.995e+004



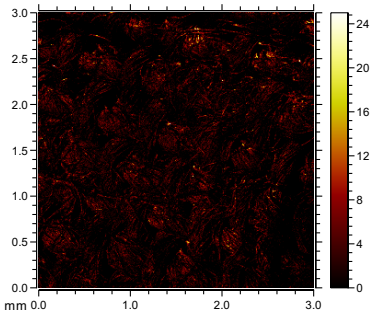
C_3H_7+
MC: 39; TC: 4.776e+005



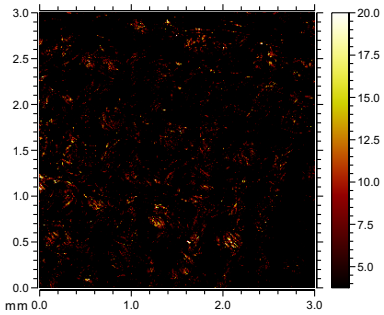
$C_2H_4C_2H_3$+
MC: 37; TC: 3.874e+005



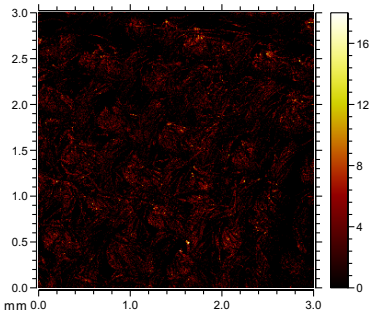
Cu+
MC: 21; TC: 3.142e+004



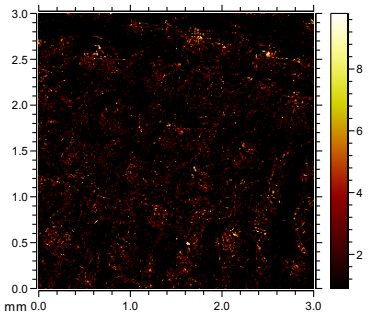
C_6H_5+
MC: 25; TC: 1.009e+005



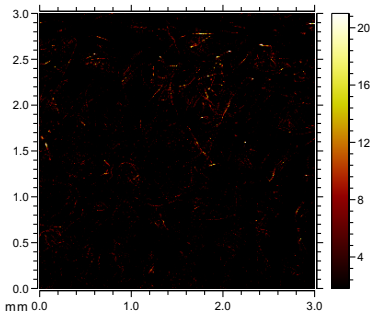
Al_3+
MC: 20; TC: 1.159e+005



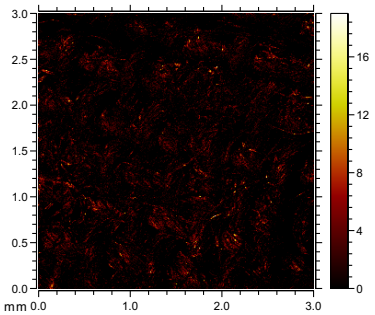
C_7H_{11}+
MC: 18; TC: 7.132e+004



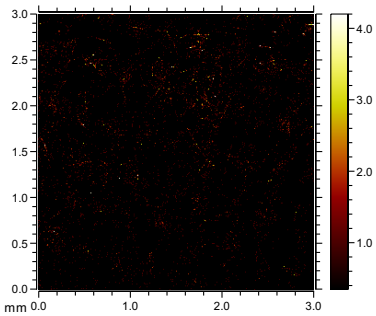
C_8H_9+
MC: 10; TC: 5.840e+004



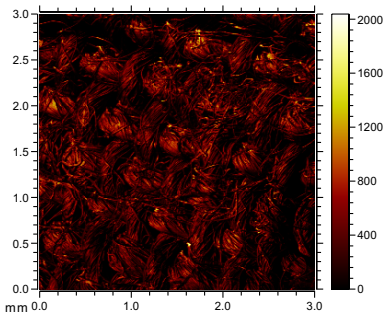
$C_7H_{12}O_2F$+
MC: 21; TC: 5.174e+004



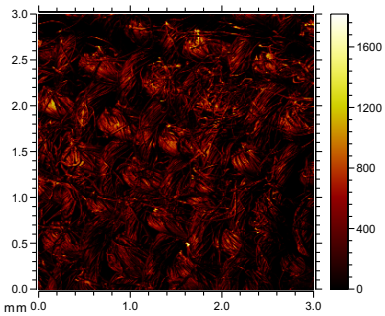
$H_6O_2Al_4F$+
MC: 19; TC: 5.492e+004



$C_{13}H_{15}O_4Na_2$+
MC: 4; TC: 5.064e+003



total
MC: 2038; TC: 1.936e+007



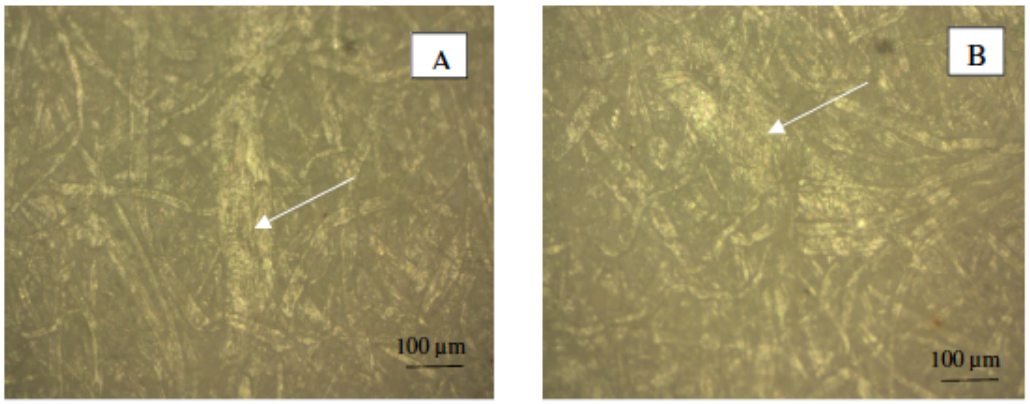
sum of rest
MC: 1817; TC: 1.575e+007

- Spectroscopy Based Imaging:

Alexandra Guedes: instrumentation/methodology: The fingermarks were analysed using a Horiba Jobin-Yvon Raman spectrometer XploRATM, equipped with an excitation wavelength of 532 nm from Ar+ laser at a power of 25 mW and with a diffraction gratings with 1200 lines mm-1. An Olympus optical microscope with a Å~50 objective lens was used to focus the laser beam on the sample and collect the scattered radiation. The laser power was reduced 90% with a neutral density filter to avoid thermal decomposition of the samples. Extended scans were performed on the spectral range 200 to 2000 cm-1. The time of acquisition and the number of accumulations varied in order to obtain an optimized spectrum for each analysed point.

The optical images of the fingermarks performed on paper were not very conclusive regarding the marks identification. However once it was referred that the marks were in the centre of the paper sample, Raman analyses were performed in the areas were the paper fibbers showed a higher reflectance (Fig. 2A and 2B). However, the fluorescence from the paper substrate prevented the detection of fingermark signals and therefore no Raman spectrum was obtained.

Optical images of fingermarks on paper. The arrow indicates the selected analysed areas, A and B:



A. Becue, C. Champod:

NB: Best technique in green (easiness, speed), second best in orange

Technique	Parameters	Observations
Naked eye	- Ambiant light	- Obs: no fingermark visible (all S)
Polilight PL500 (Rofin)	- White light - UV - Luminescence (all λ + orange filter)	- Obs: no fingermark visible (all S) - This is quite surprizing for plastic, but plausible if very faint mark
LASER (Coherent)	- 532 nm + orange filter - 577 nm + violet filter	- Obs: no fingermark visible (all S) - No auto-luminescence (paper)
RUVIS (ArrowHead)	- 254nm	- Obs: no fingermark visible (all S) - The absence of result on plastic is pointing towards a very faint mark
Hyperspectral imaging	Not applied	N/A
Cyanoacrylate fuming + RUVIS	NB: cyanoacrylate fuming has been applied due to the absence of results with optical means	- Obs: fingermark visible, few ridge details + dotty results > very faint secretion residue, close to sweat only

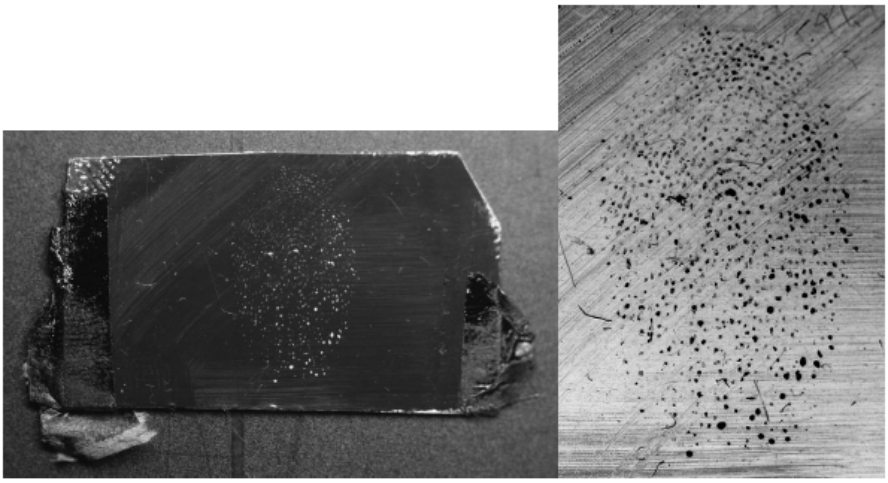
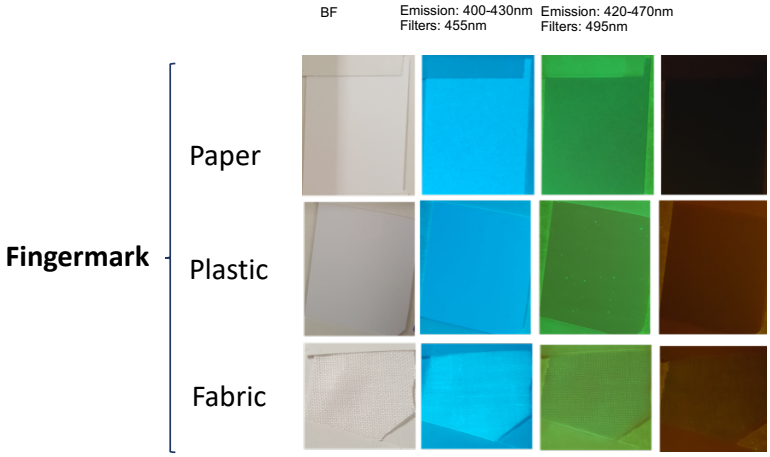


Figure: RUVIS observation of the fingermark left on plastic, after cyanoacrylate fuming

***Nunzianda Frascione*:** The applied techniques have not been validated yet and therefore can not be used for the purpose of this study. Yet, alternate light sources, IRIS (used at different wavelengths) and a portable NIR device were tested for suitability. All of the techniques are mainly used for detection purposes only (e.g. location of evidence deposited on surfaces) and would work exploiting either intrinsic properties of the biological evidence (e.g. presence of fluorophores) or fluorescence associated with contaminants. No fingermarks were detected.

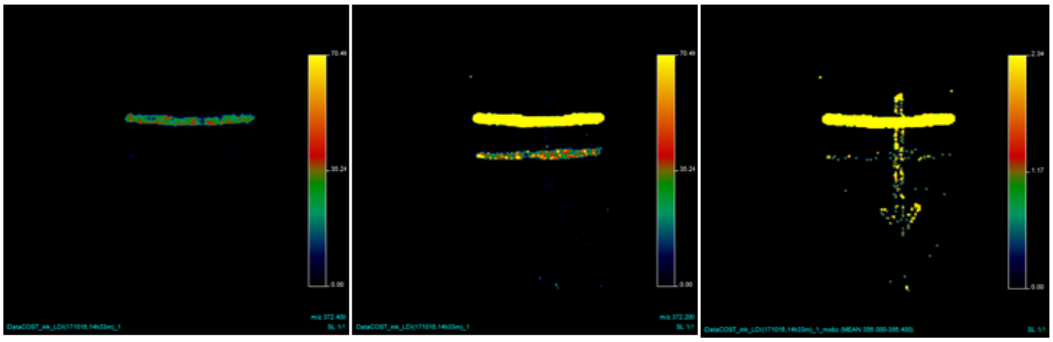


Ink Samples

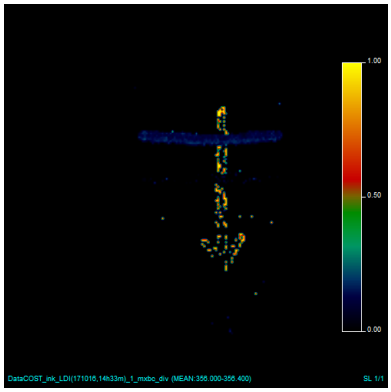
- Mass Spectrometry Based Imaging:

Simona Francese: instrumentation: (MA)LDI MSI, Resolution 150x150 microns, acquisition mass range 100-1000 Da in positive mode, samples were cut out and paper was stuck directly on a MALDI target plate (with a recessed adaptor) and submitted to Laser desorption mass spectrometry imaging

In **LDI** it was possible to detect only 3 of the 4 inks used. Total ion current shows the 4 inks with the third bottom horizontal strike being very weak in intensity and a unique image could not be obtained. In one case (top horizontal ink strike) a few unique signals were detected for that specific ink (left panel LDI image of m/z 372.4). Some ion signals are in common to the top two horizontal ink strikes (middle panel LDI image of m/z 372.2) and some signals are in common between the top two horizontal ink strikes and the inked arrow (left panel LDI image of m/z 356.2). The LDI images are not normalised though the first 2 were generated using the same brightness and contrast
The intensity of the signals in common is different for the three inks. Whilst it could be argued that it is a differential pressure to have determined this different intensity, another possibility is that those particular components are truly present in different amounts and could be representing ink discriminants.



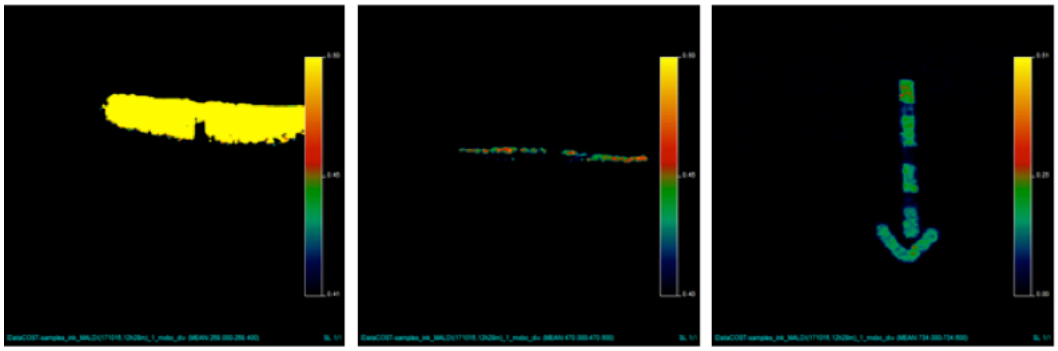
Normalised LDI MS images of: Left panel m/z 372.4. Middle panel m/z 372.2. Right panel m/z 356.2.



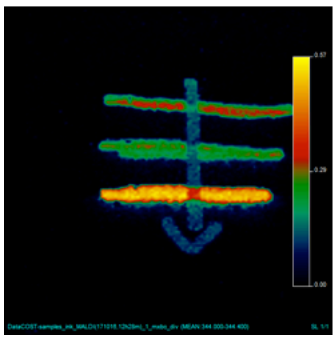
LDI image of the signal at m/z 356.2 normalised against the total ion current. It is not possible to comment on the order of deposition unless a method is implemented of the kind described by Bright NJ et al. Anal Chem. 2012 May 1;84(9):4083-7

The use of matrix for **MALDI MSI analysis** yielded many more ion signals as expected. It was possible to differentiate at least 3/4 inks due to unique components as shown in Fig 4 (m/z 268.2,

470.3 and 734.3 in left, middle and right panel in the figure respectively). The signal at m/z 734.3 clearly showing the "arrow ink" this time, is absent in the LDI MSI analysis.
As expected there are also signals that are in common with the 4 inks as Figure 5 shows. The signal at m/z 344.2 seems to be present in greater abundance in the bottom horizontal ink strike. This could be a genuine occurrence or the result of differential pressures when depositing the ink on paper.



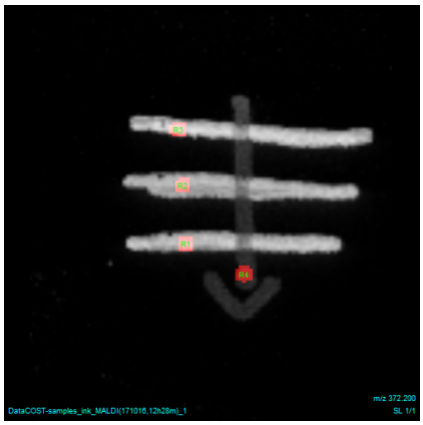
Normalised MALDI images of the ion signal at m/z 268.2, 470.3 and 734.3 in left, middle and right panel in the figure respectively.



MALDI MS image of the ion signal at m/z 344.2. This component is present in all of the 4 inks though the intensity of the signal varies greatly.

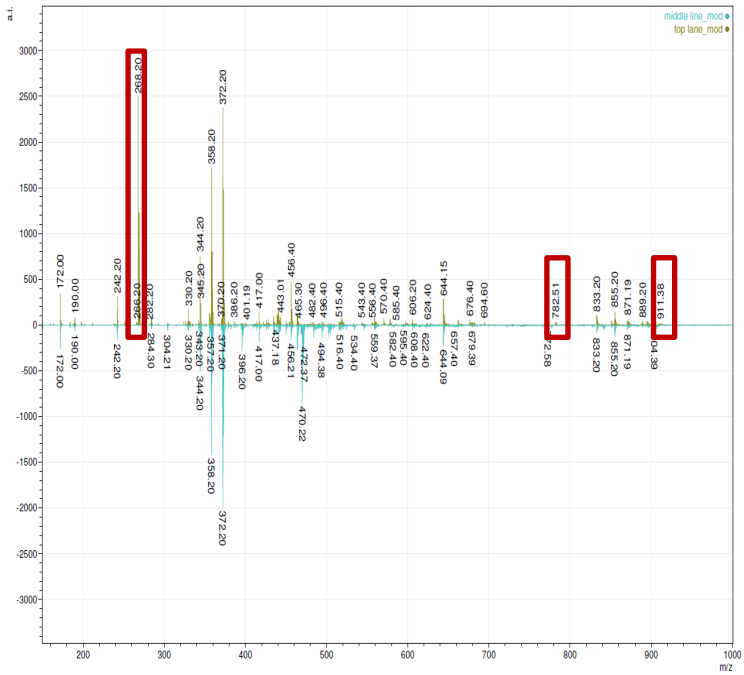
To perform some sort of discriminant analysis, Region of interests can be drawn and the overall spectrum exported to observe difference in the composition of the different inks and therefore clearly differentiate them.

Differently from LDI, MALDI MSI could also detect the presence of a fingerprint (though the ridge detail is not useful) which was for sure not left by the SHU team as we handled the sample with gloves at all times



Circular regions of interest of the same size drawn over each ink line to extract spectral pattern for comparison between different inks (ROI) as a means to discriminate the different inks themselves

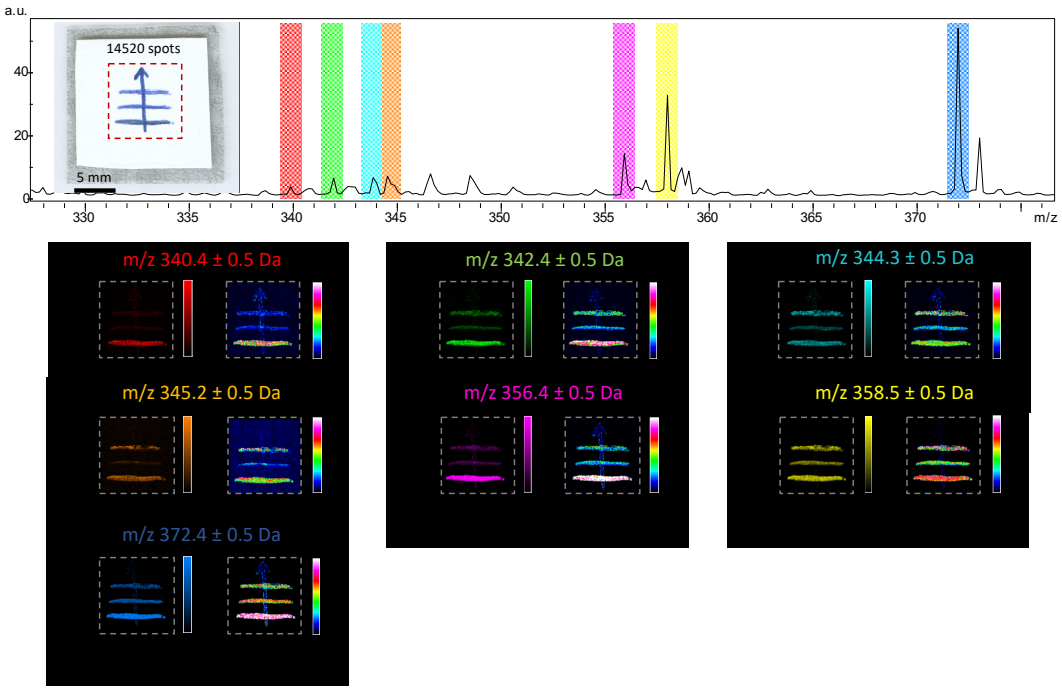
Comparison of MALDI MS average spectra of R4 (top panel, arrow ink strike) and R1 (bottom panel, bottom horizontal ink strike). Unique signals could be detected only present in the arrow ink



Comparison of MALDI MS average spectra of R2 (top panel, middle ink strike) and R3 (bottom panel, top horizontal ink strike). Unique signals could be detected only in the middle ink lane as well as in the top ink lane (such as for the latter a m/z 470.2).

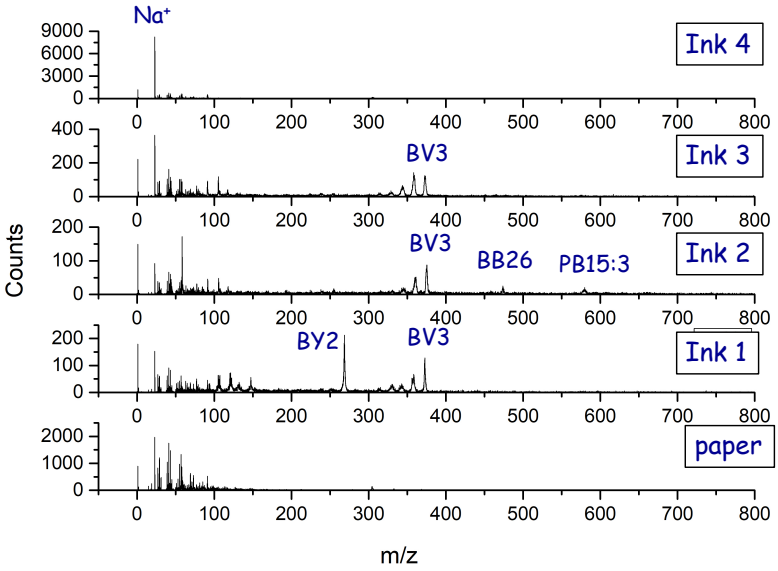
Martina Marchetti-Deschmann: instrumentation: MALDI MSI on an UltrafleXtreme (Bruker), resolution 50x50 microns, acquisition mass range 100-1000 Da in positive mode, samples were fixed on conductive glass slide (ITO target) with conductive double-sided adhesive tape. Samples were measured by LDI and MALDI after applying CHCA as matrix. To improve ion intensities samples were also covered with a thin film of Ag (sputtering).
Reported limitations: cotton samples were not meaaured because of short current between sample plate and first extraction lens (fibres on surface).

Ink could be measured from paper and plastic. Using paper as substrate ink were distinguishable after Ag sputtering. Inks could be distinguished, but no effort was put into distinguishing which ink was drawn first although hints are already seen in a way that ink4 (arrow) was drawn last. Region of interest will be defined in the future where line crossings will be investigated.



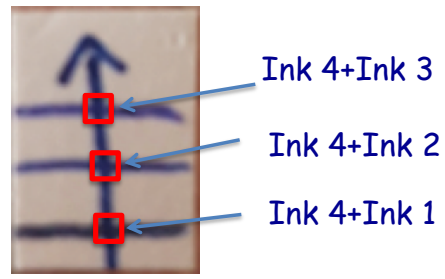
I. Bogdanović Radović: instrumentation: Measurements were performed under vacuum (10^{-6} - 10^{-7} mbar) using the MeV-SIMS setup with a Time-of-Flight (TOF) spectrometer at the Ruđer Bošković Institute (RBI) heavy ion microprobe; -8 MeV Si^{4+} ions; lateral beam resolution 10 x 10 μm^2. The beam was scanned over different areas on a sample for imaging of the intersecting lines. Beam current in the pulsed mode was ~ 0.2 fA.

Samples were mounted on a metal sample holder and ink on paper was analysed:

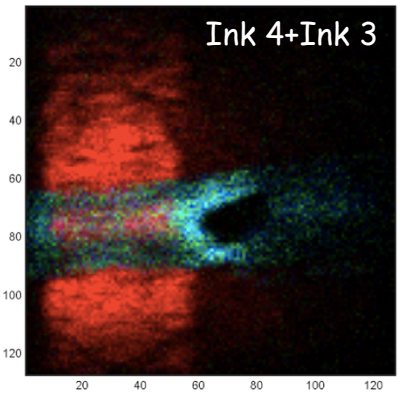


Comparison of SIMS MS spectra of ink 1, 2, 3 & 4.

Detailed study of regions of interest give information about ink layers:

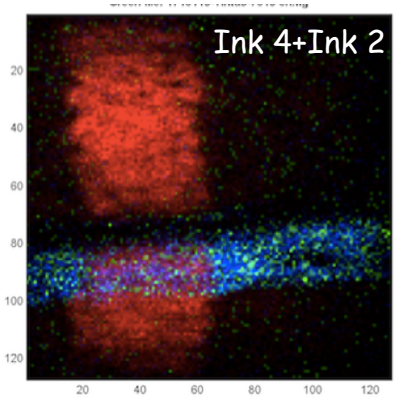


scan size: 635x766 µm²



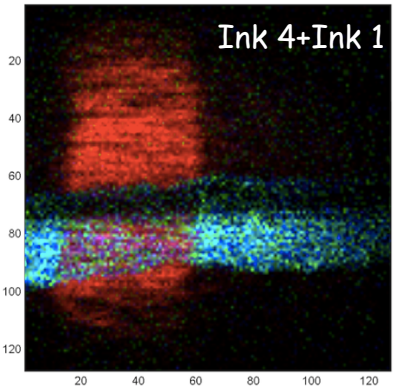
Ink 4 R (Na+)
Ink 3 B,G (BV3)

Ink 3 is above Ink 4



Ink 4 R (Na+)
Ink 2 B (BV3), G(BB26)

Ink 2 is above Ink 4



Ink 4 R(Na+)
Ink 1 B(BY2), G (BV3)

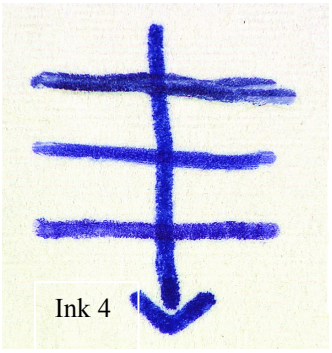
Ink 1 is above Ink 4

- Spectroscopy Based Imaging:

Ana Cristina de Almeida Assis: Instrumentation: VSC 8000 Video Spectral Comparator from Foster&Freeman, High resolution 5 MP digital camera with SRI (Super Resolution Imaging) at 19 MP.

Spectrophotometer from J&M Tidas coupled to a Zeiss Microscope with a camera with 1280x960 (1.2 MP). Spectra acquisition with an image magnification of 200x with a flexible adjustable measurement diaphragm.

VSC 8000 Video Spectral Comparator:



Ink 1

Ink 2

Ink 3

Image of all inks obtained with natural light magnified 27x.

These inks are distinguishable by this observation. With this method it was possible to identify the type of inks: 1) 2) 3) ballpoint pen ink, 4) liquid ink (gel pen). Usually this is the first observation when ink analysis is performed. The accuracy of the information obtained by this method depends on the forensic expert experience to this kind of trace evidence.

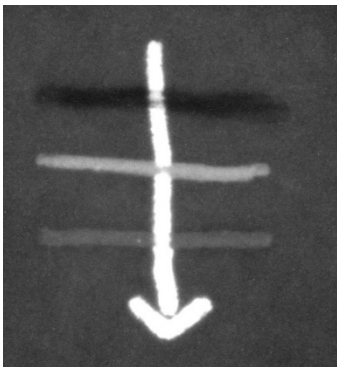
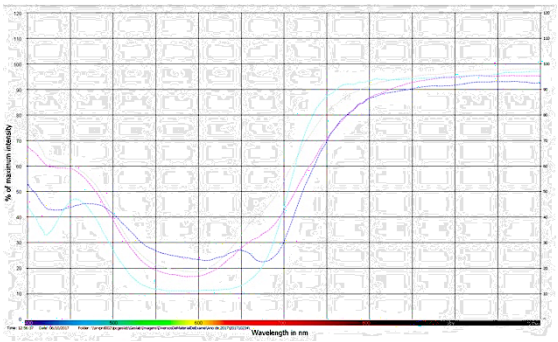


Image of all inks obtained with IR florescence light. The wavelengths of the excitation light are 605-730 nm and a camera filter of 850 nm has been selected.
In the resulting image, a greater difference of intensity in the examined inks is directly related to the brightness of pixels shown.

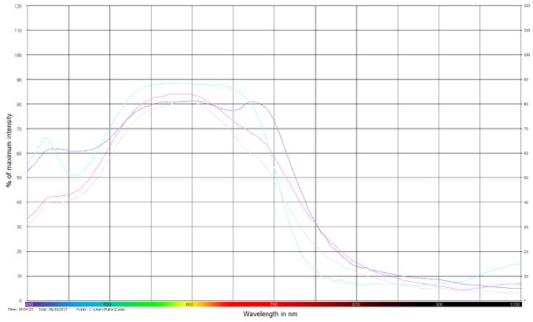
The absorption/florescence reactions of the inks are different from each other. This technique provides an obvious and objective forensic information concerning their differentiation.
One limitation of this kind of analysis is that is only possible when the inks are in the same piece of paper. This method is always used in inks differentiation when the inks are manuscript in the same paper. A second limitation is that not all the inks have different absorption/fluorescence reactions.
The analyse time of this procedure is fast and produce indisputable forensic intelligence.
In a real case, when these results are obtained, no more techniques are used.

Multi-modal imaging

The paper was used as white reference and for a more reliable comparison, three spectra from each ink were taken. The average of the inks spectra was used to compare them.



Reflectance spectra of the inks:
1) dark blue, 2) pink, 3) green and 4) light blue.



Absorption spectra of the inks:
1) dark blue, 2) pink, 3) green and 4) light blue.

The inks 2 and 3 show similar spectra while 1 and 4 have very distinct spectra. The differences between inks 2 and 3 are not conclusive with this technique.

Micro spectrophotometer

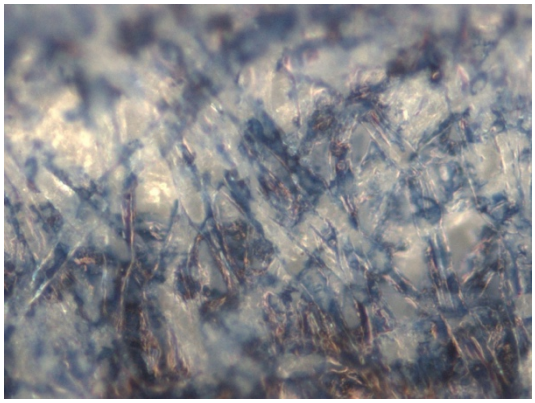


Image of ink 1 magnified 200x.

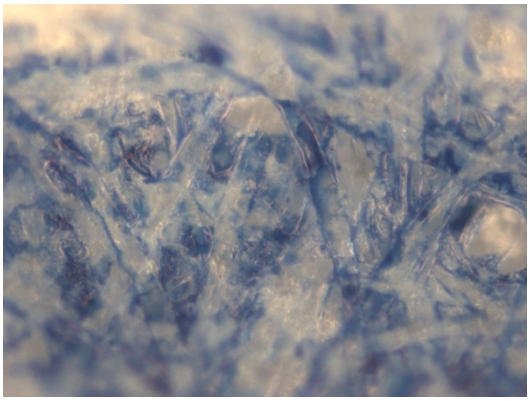


Image of ink 2 magnified 200x.

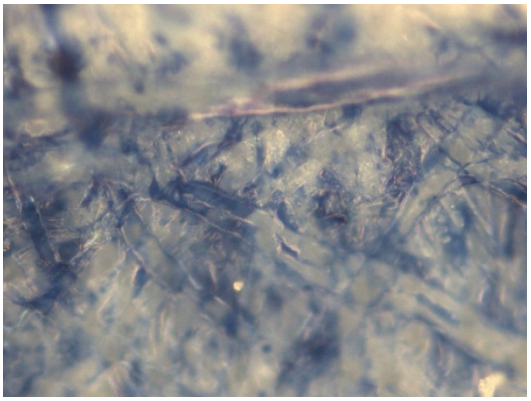


Image of ink 3 magnified 200x.

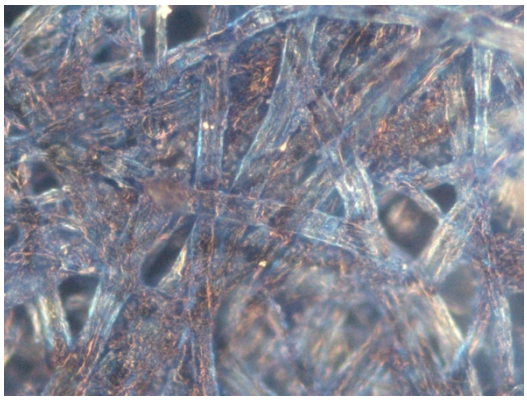
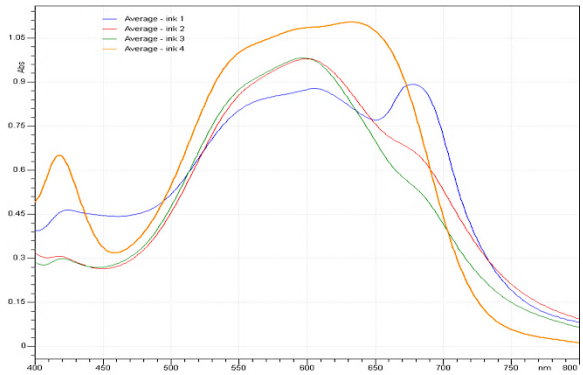


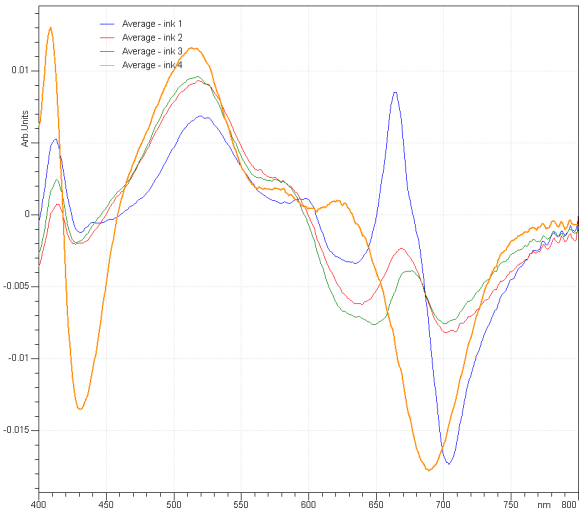
Image of ink 4 magnified 200x.

Multi-modal Imaging

For acquisition of the absorption spectra a TIDAS MSP-800 microspectrophotometer, consisting of a microscope (Zeiss®, Axiotech 100) coupled to a spectrophotometer (J&M Tidas®), was used. Inks were analysed in the visible region between 400 and 800 nm in the reflectance mode. This mode of obtaining the spectra, besides being non-destructive, allows to have a sample with no previous treatment for analysis. The paper in analysis was then fixed to a microscope base slide, and placed on the stage of the microscope with the microspectrophotometer instrument. The paper was used as white reference, and for a more reliable comparison, five spectra of each ink were taken (in different places of the ink). The average of the inks spectra was used to compare them. The measurements were obtained using a diaphragm to select each area, under the following conditions: for the microscope [Diaphragm dimensions (220.0 × 127.0 m), image resolution (1280x960), objective with 20× magnification and light intensity of the microscope 10 (maximum)] and for the spectrophotometer [Interpolation (YES), Step (1 nm), Representation (Absorbance AU), Scan type (Single Scan), Accumulations (3), Bunching (1 Pixel)].



Absorption spectra of the inks.

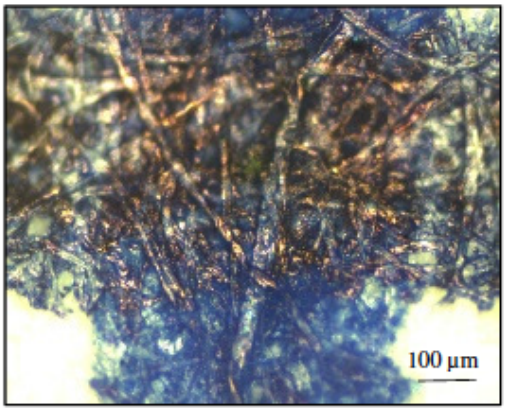


First derivative of the inks. The graphics shows that ink 1 and 4 are different from each other and different from the rest. The inks 2 and 3 have similar spectra, however the first derivative indicates a difference in the minimum values around 640 and 650 nm.

Conclusions: To distinguish these inks the best methodology was the video spectral comparator which provides an irrefutable imaging forensic information with the use of IR fluorescence light. These imaging and multi-imaging techniques have the advantage to be non-destructive.

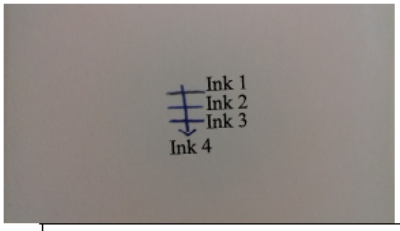
***Ana Cristina de Almeida Assis*:** Instrumentation: Raman spectra of ink were obtained using a Horiba Jobin-Yvon Raman spectrometer XploRATM, equipped with an excitation wavelength of 532 nm from Ar+ laser at a power of 25 mW and with a diffraction gratings with 1200 lines mm^{-1}. An Olympus optical microscope with a Å~100 objective lens was used to focus the laser beam on the sample and collect the scattered radiation. The laser power was reduced 90% with a neutral density filter to avoid thermal decomposition of the samples. Extended scans were performed on the spectral range 200 to 1700 cm-1. The time of acquisition and the number of accumulations varied in order to obtain an optimized spectrum for each analysed point.

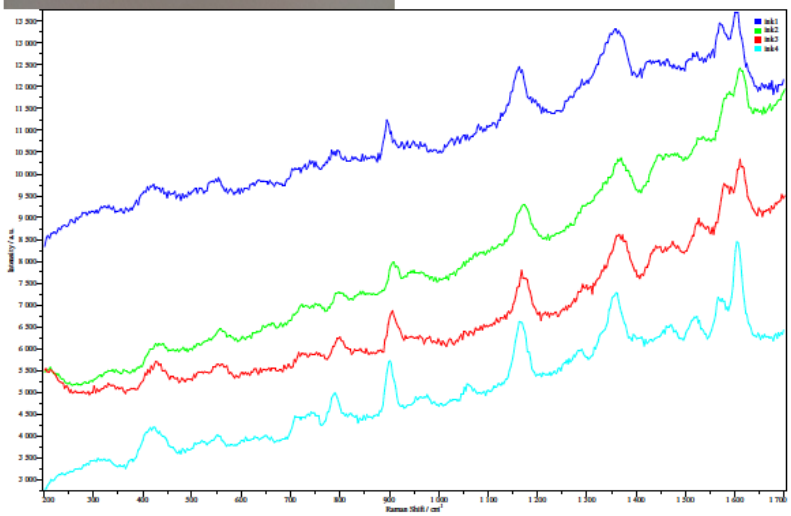
Due to the magnification of the optical images of the inks on paper it was not possible to take optical images of all the inks together:



Optical image of two crossed inks on paper

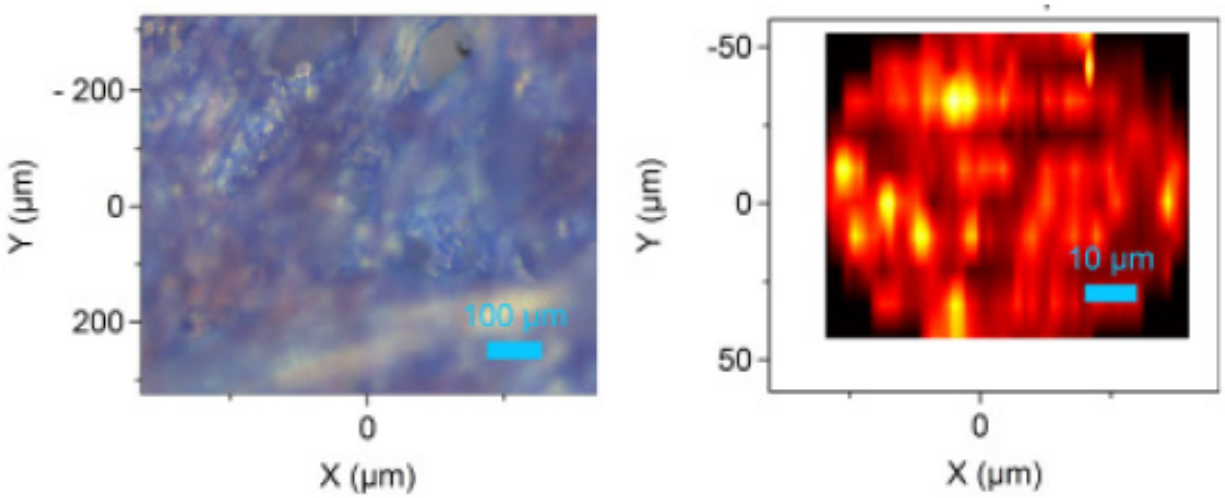
Raman analysis on the four ink strikes reveal Raman spectrum corresponding to distinct inks with similar components:





Raman spectra of the different analysed inks

The optical image of one of the strikes (Ink 4) and a mapping from the area were obtained. The mapping shows distinct intensities of the Raman signal of ink (red and yellow):



Raman Imaging of ink 4

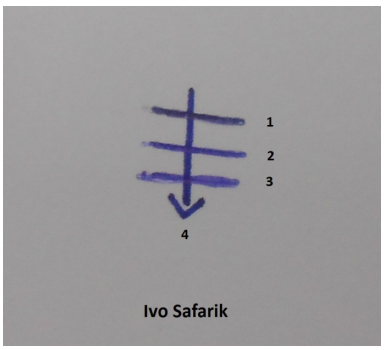
Ivo Safarik: Instrumentation/methodology: Microscopy images of ink lines were taken using an optical microscopy (Olympus); from one ink line 10 independent microscopy images were taken. Exactly the same conditions (magnification, illumination etc.) were used during the microscopy in order to take images of all four ink lines tested (altogether 40 microscopy images taken). Together with ink images also images of stage micrometer were taken. Using XnView software the 200 x 200 µm images were cropped from the original images for the subsequent evaluation.

Every 200 x 200 µm image was analysed using ImageJ software. Using Color Histogram each image was analysed in order to obtain RGB values (in the range between 0 – 255; both means and mode values were collected). The arithmetic mean values were calculated from 10 sets of values for any ink sample analyzed.

Every 200 x 200 µm image was also analyzed using Color Inspector 3D plugin (ImageJ software). Using RGB color space and Median Cut display mode, the number of colors was reduced to one. The RGB values were taken for each image. The arithmetic mean and median values were calculated from 10 sets of values for any ink sample analyzed.

In order to analyze the similarity of the ink colors, "Color tools for webmasters" (http://www.colortools.net/) was used. Using the subroutine „Colors Mixer Tool" the individual RGB values were converted into hexadecimal scale. Comparison of two colors was performed using the „Color Matcher Tool" subroutine; the results are given in percentage.

Histogram analysis was performed on the microscopy images of four tested inks (Fig. 1). Each ink was analyzed on 10 independent images using Color Histogram as described above. The RGB values obtained are presented in Table 1.



Ink written document used for the analysis

Table 1. RGB values obtained by the analysis of microscopy images using Color Histogram subroutine in ImageJ software

	Ink 1	Ink 2	Ink 3	Ink 4
Red mean (arithmetic mean from 10 images)	88.4	85.6	138.0	39.6
Red mode (arithmetic mean from 10 images)	49.8	27.9	142.9	16.5
Green mean (arithmetic mean from 10 images)	121.0	127.4	151.7	95.4
Green mode (arithmetic mean from 10 images)	91.9	101.6	156.5	69.2
Blue mean (arithmetic mean from 10 images)	166.1	207.5	200.0	181.1
Blue mode (arithmetic mean from 10 images)	172.2	207	196.2	180.6

Additional analysis was performed using Color Inspector 3D plugin (ImageJ software) as described above. The RGB values obtained are presented in Table 2.

Table 2. RGB values obtained by the analysis of microscopy images using Color Inspector 3D plugin (ImageJ software; RGB color space and Median Cut display mode, the number of colors reduced to one).

	Ink 1	Ink 2	Ink 3	Ink 4
Red (arithmetic mean from 10 images)	89.8	81.5	134	35.6
Red (median from 10 images)	83	79	133	35.5
Green (arithmetic mean from 10 images)	116.9	123.3	147.9	91.7
Green (median from 10 images)	113	122	147.5	94

Blue (arithmetic mean from 10 images)	162.1	203.5	196.1	177.1
Blue (median from 10 images)	163	203.5	196.5	179.5

Using "Color tools for webmasters" the similarity of the ink colors was tested; the results are given in percentage (Table 3).

Table 3. Similarity of the tested ink expressed in percentage (numbers in black are based on the average RGB mean values from Color Histogram; the numbers in red are based on average RGB values using Color Inspector 3D plugin, RGB color space, Median Cut display mode, the number of colors equal to one.

	Ink 1	Ink 2	Ink 3	Ink 4
Ink 1		94	85	88
		93	86	88
Ink 2	94		89	87
	93		89	87
Ink 3	85	89		78
	86	89		77
Ink 4	88	87	78	
	88	87	77	

In order to test the reliability of the procedure, randomly selected images of one ink (two groups of five images) were analyzed in the same way as the tested ink samples. It was observed that the same ink in two parallel samples exhibited 98-99 % similarity.

Conclusion: It was observed that the tested ink written document was written with 4 different blue inks. Based on the color similarity test we can conclude that Ink 1 and Ink 2 were the most similar ones (but definitely different ones), while the Ink 3 and Ink 4 exhibited the lowest similarity. Without other experiments we can just suggest that the similarity less than 95 % can indicate substantial difference in the ink type, while at values higher than 95 % other tests should be performed. Appropriate freeware can be used successfully for inks comparison.

***Kristýna Pospíšková*:** Instrumentation/methodology: Microscopy images of ink lines were taken using an optical microscopy (Microscope Olympus IX 70); from one ink line 10 independent microscopy images were taken. Exactly the same conditions (magnification, illumination, etc.) were used during the microscopy in order to take images of all four ink lines tested (altogether 40

microscopy images taken). Together with ink images also images of stage micrometer were taken. Using XnView software the 350 x 350 µm images were cropped from the original images for the subsequent evaluation.

Every 350 x 350 µm image was analysed using ImageJ software. Using Color Histogram each image was analysed in order to obtain RGB values (in the range between 0 – 255; both means and mode values were collected). The arithmetic mean values were calculated from 10 sets of values for any ink sample analyzed.

Every 350 x 350 µm image was also analyzed using Color Inspector 3D plugin (ImageJ software). Using RGB color space and Median Cut display mode, the number of colors was reduced to one. The RGB values were taken for each image. The arithmetic mean and median values were calculated from 10 sets of values for any ink sample analyzed.

In order to analyze the similarity of the ink colors, "Color tools for webmasters" (http://www.colortools.net/) was used. Using the subroutine „Colors Mixer Tool" the individual RGB values were converted into hexadecimal scale. Comparison of two colors was performed using the „Color Matcher Tool" subroutine; the results are given in percentage.

Histogram analysis was performed on the microscopy images of four tested inks (Fig. 1). Each ink was analyzed on 10 independent images using Color Histogram as described above. The RGB values obtained are presented in Table 1.

Fig. 1. Ink written document used for the analysis.

Table 1. RGB values obtained by the analysis of microscopy images using **Color Histogram** subroutine in ImageJ software.

	Ink 1	***Ink 2***	***Ink 3***	***Ink 4***
Red mean (arithmetic mean from 10 images)	**96,5**	**82,3**	**41,9**	**10,5**

Red mode (arithmetic mean from 10 images)	94,2	79,9	36,3	1,8
Green mean (arithmetic mean from 10 images)	**111,4**	**121,2**	**59,8**	**37,1**
Green mode (arithmetic mean from 10 images)	110,9	120,6	54,1	25,5
Blue mean (arithmetic mean from 10 images)	**136,2**	**180,2**	**149,5**	**111,8**
Blue mode (arithmetic mean from 10 images)	136,3	183,5	148,7	108,4

Additional analysis was performed using Color Inspector 3D plugin (ImageJ software) as described above. The RGB values obtained are presented in Table 2.

Table 2. RGB values obtained by the analysis of microscopy images using **Color Inspector 3D** plugin (ImageJ software; RGB color space and Median Cut display mode, the number of colors reduced to one).

	Ink 1	***Ink 2***	***Ink 3***	***Ink 4***
Red (arithmetic mean from 10 images)	**92,7**	**78,3**	**37,9**	**7,4**
Red (median from 10 images)	95,5	82,5	35,0	5,0
Green (arithmetic mean from 10 images)	**107,4**	**117,3**	**55,8**	**33,0**
Green (median from 10 images)	111,5	119,5	53,5	33,5
Blue (arithmetic mean from 10 images)	**132,3**	**176,2**	**145,5**	**107,8**
Blue (median from 10 images)	135,5	178,0	143,0	108,5

Using “Color tools for webmasters“ the similarity of the ink colors was tested; the results (from arithmetic mean values) are given in percentage (Table 3).

Table 3. Similarity of the tested inks expressed in percentage (numbers in black are based on the average RGB mean values from **Color Histogram**; the numbers in red are based on average RGB values using **Color Inspector 3D** plugin, RGB color space, Median Cut display mode, the number of colors equal to one).

	Ink 1	*Ink 2*	*Ink 3*	*Ink 4*
Ink 1		91	84	76
		91	84	76
Ink 2	91		83	71
	91		83	71
Ink 3	84	83		88
	84	83		88
Ink 4	76	71	88	
	76	71	88	

In order to test the reliability of the procedure, randomly selected images of one ink (two groups of five images) were analyzed in the same way as the tested ink samples. It was observed that the same ink in two parallel samples exhibited 98-99 % similarity (Table 4).

Table 4. Examination of the reliability of the procedure based on the similarity testing within one ink sample expressed in percentage (numbers in black are based on the average RGB mean values from **Color Histogram**; the numbers in red are based on average RGB values using **Color Inspector 3D** plugin, RGB color space, Median Cut display mode, the number of colors equal to one).

	Ink 1	*Ink 2*	*Ink 3*	*Ink 4*
Similarity within one ink sample (%)	99	99	98	98
	99	98	98	99

Conclusion: It was observed that the tested ink written document was written with 4 different blue inks. Based on the color similarity test we can conclude that Ink 1 and Ink 2 were the most similar ones (but definitely different ones, similarity only 91 %), while the Ink 2 and Ink 4 exhibited the lowest similarity (71 %). Without other experiments we can just suggest that the similarity less than 95 % can indicate substantial difference in the ink type, while at values higher than 95 % other tests should be performed. Appropriate freeware can be used successfully for inks comparison.

A. Becue, C. Champod:

Technique	Parameters	Observations
Naked eye	- Ambiant light	- Obs: slight color differences between the strokes
Polilight PL500 (Rofin)	- White light - UV - Luminescence (all λ + orange filter)	- Obs: slight color differences between the strokes (WL)
LASER (Coherent)	- 532 nm + orange filter - 577 nm + violet filter	- Obs: no differentiation between inks - No luminescence behaviour
RUVIS (ArrowHead)	Not applied (RUVIS not adapted to paper)	N/A
Docucenter NIRVIS (Projectina)	- exc. DOCU Filter - em. 760nm	- Obs: differentiation of the 4 strokes (if the pressure is identical -> 4 inks) - Imaging is extremely quick (seconds)
Hyperspectral imaging (SPECIM)	Vis (400 - 1000 nm) SWIR (1000 – 2500 nm)	- Obs: differentiation of the 4 strokes - Operations required (SNV + Smoothing + 2nd deriv.)

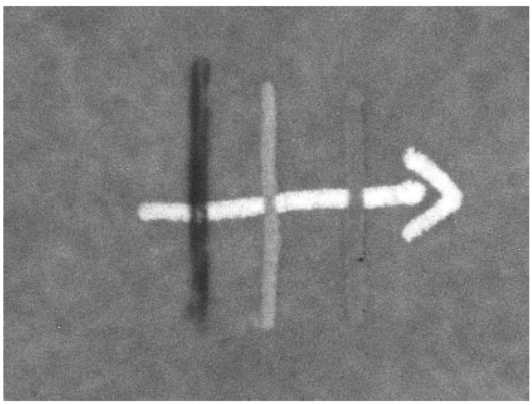


Figure: Docucenter NIRVIS (Projectina)

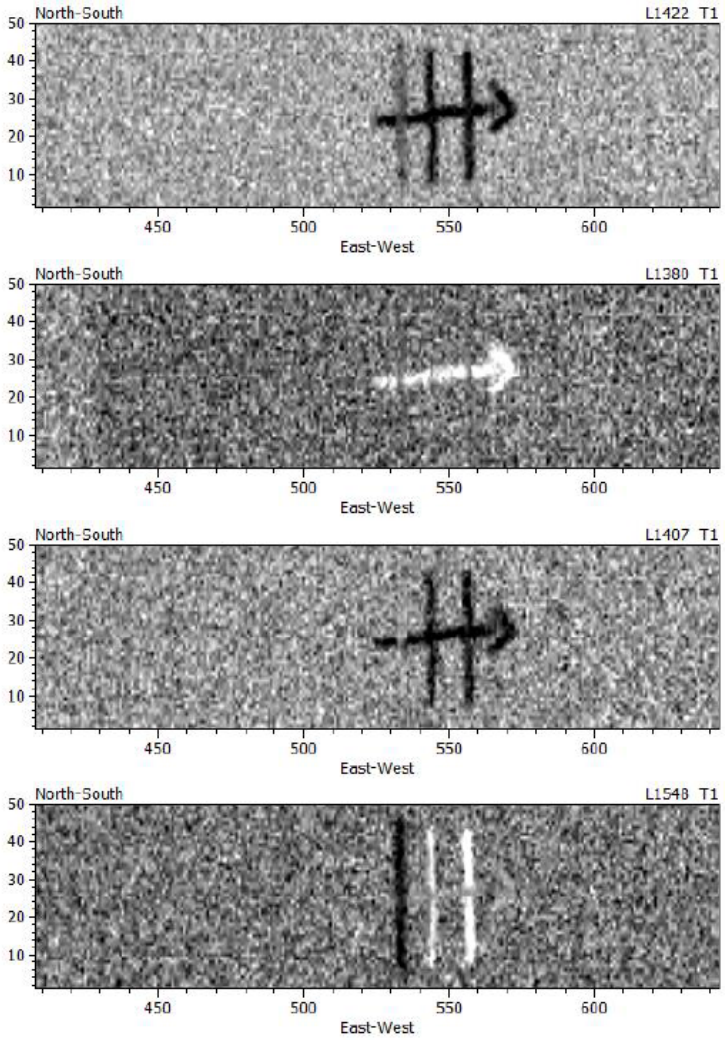


Figure: Hyperspectral imaging (various wavelengths)

***Nunzianda Frascione*:** The applied techniques are not been validated yet and therefore could not be used for the purpose of this study. Yet, alternate light sources, IRIS (used at different wavelengths) and a portable NIR device were tested for suitability. All of the techniques are mainly used for detection purposes only (e.g. location of evidence deposited on surfaces) and would work exploiting either intrinsic properties of the biological evidence (e.g. presence of fluorophores) or fluorescence associated with contaminants. Ink could not be differentiated.