PhD in Physics, End-of-Year Seminars, 17th and 18th September 2025

Discriminating superimposed historical inks exploiting a non-invasive and multi-analytical strategy

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Arvedi Laboratory of Non-Invasive Diagnostics, Department of Musicology and Cultural Heritage, University of Pavia

UNIVERSITÀ DI PAVIA
Dipartimento di Fisica

ARVEDI LABORATORY OF NON-INVASIVE DIAGNOSTICS

INDEX OF CONTENTS

Manuscripts: Complex Heritage Artefacts

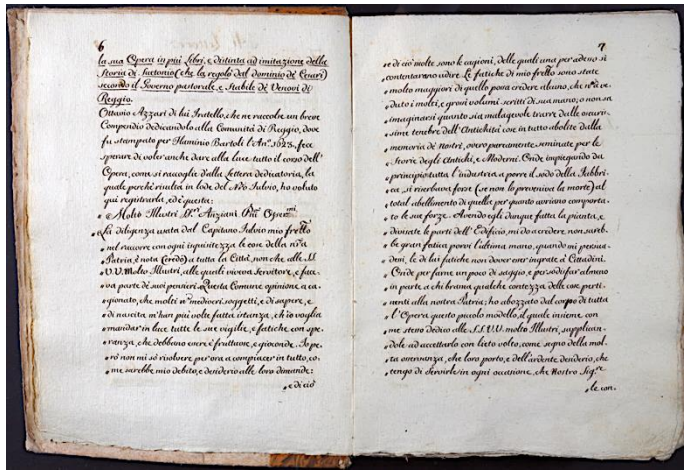
Supports:

- Papyrus
- Parchment
- Paper

Writing materials:

- Inks
- Pigments
- Dyes

Ms.Ald. 211, 14th century, Biblioteca Universitaria di Pavia



Mss-Turri-D-73, 16th century, Biblioteca digitale, Reggio Emilia

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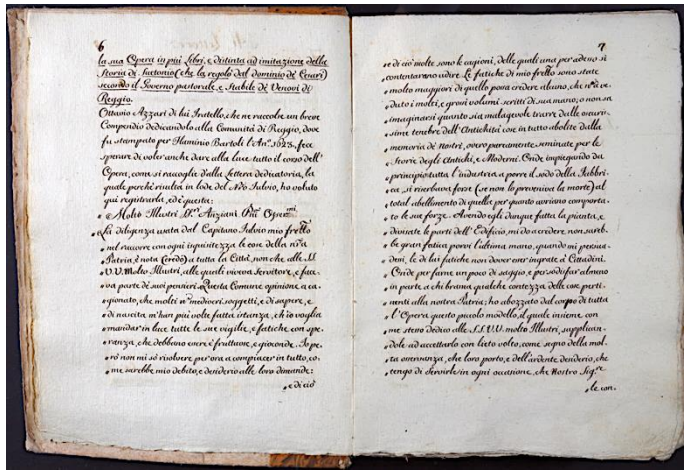

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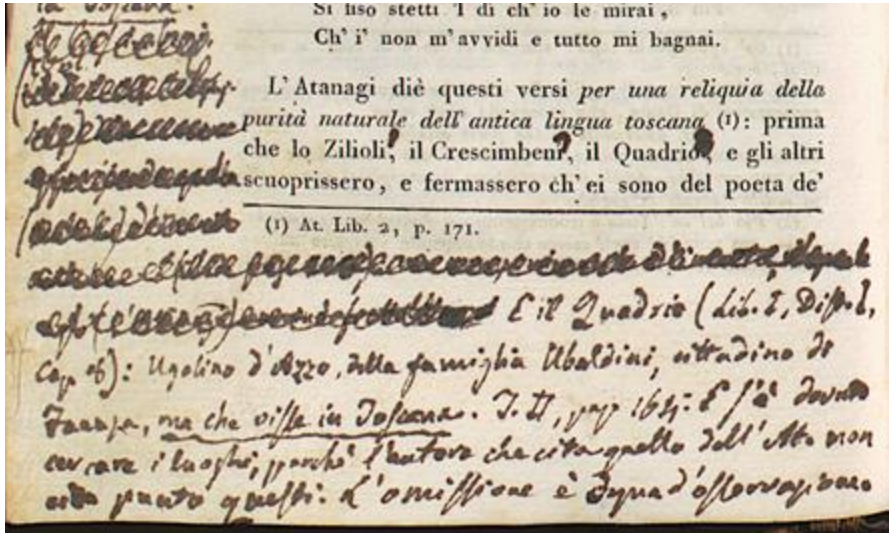
Manipulations of original writings:

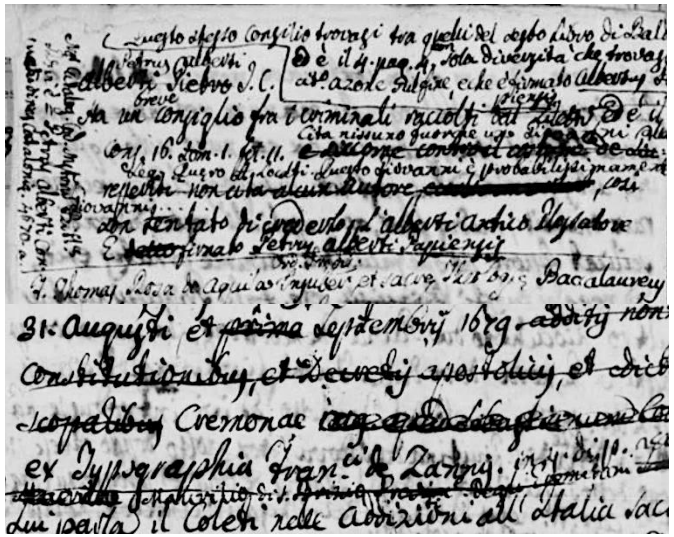
issues arise related to reading and interpreting texts


Si fuso stetti 'l dì ch' io le mirai,
Ch' i' non m'avvidi e tutto mi bagnai.

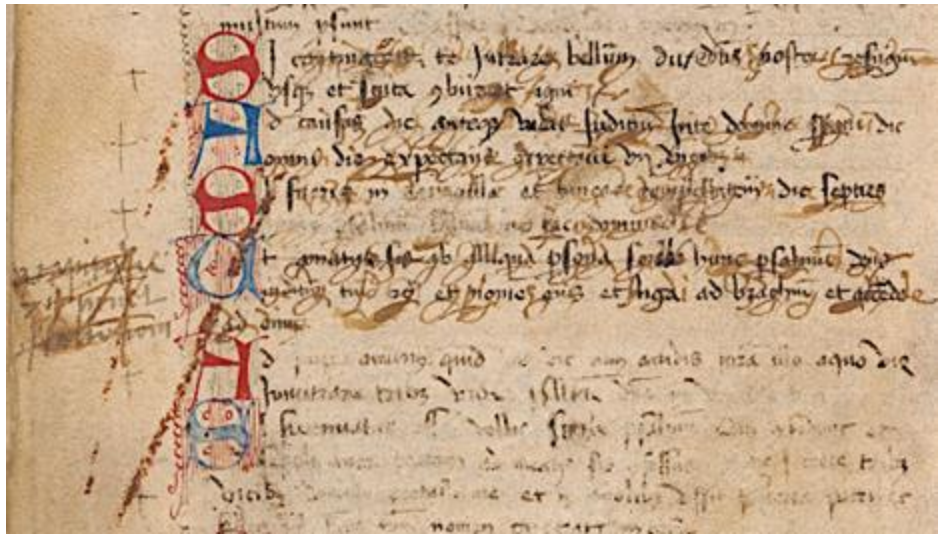
L'Atanagi diè questi versi *per una reliquia della purità naturale dell'antica lingua toscana* (1): prima che lo Zilioli, il Crescimbeni, il Quadrio, e gli altri scuoprissero, e fermassero ch'ei sono del poeta de'

(1) At. Lib. 2, p. 171.

MANZ. 15. 0037-43, 19th century, Biblioteca Nazionale Braidense, Milano



Ms.Tic. 38, 18th-19th century, Biblioteca Universitaria di Pavia



Ms.Ald. 211, 14th century, Biblioteca Universitaria di Pavia

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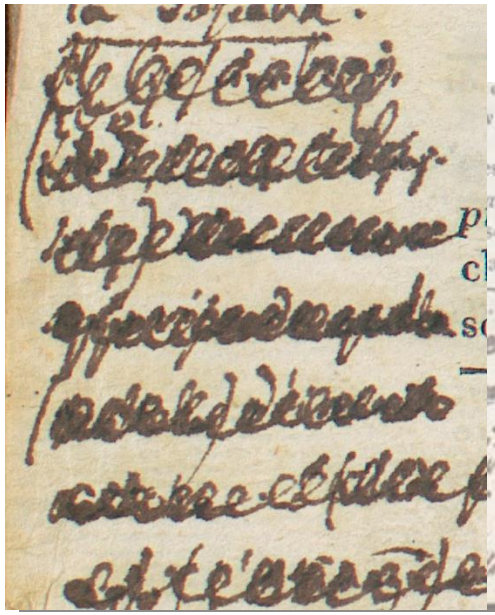

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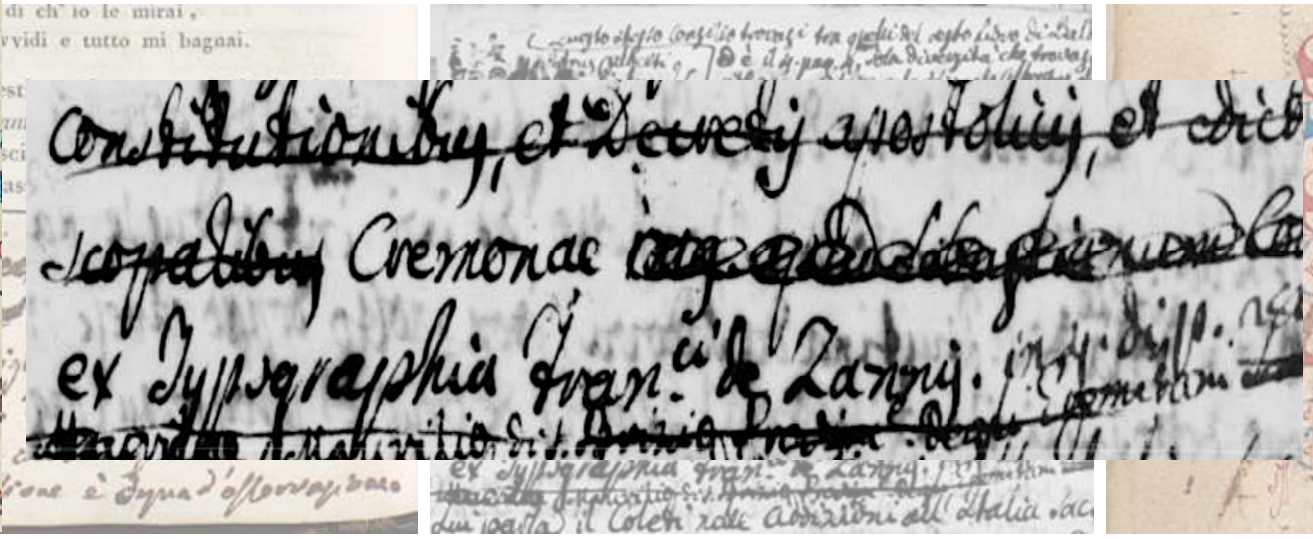

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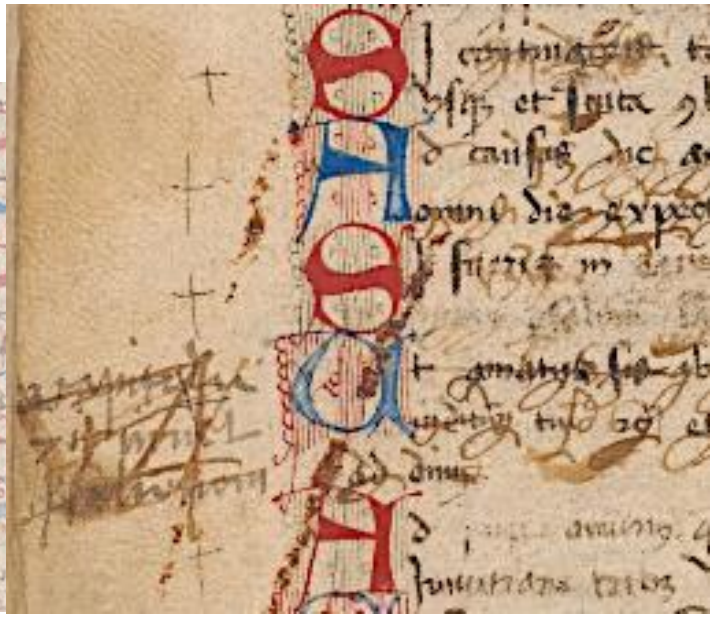
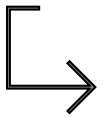


Ms.Ald. 211, 14th century, Biblioteca Universitaria di Pavia

Characterisation and discrimination between inks of different and similar composition and appearance

Improve legibility when the original writing is compromised by overwriting or cross-outs marks



Validation of a non-invasive and multi-analytical methodological approach

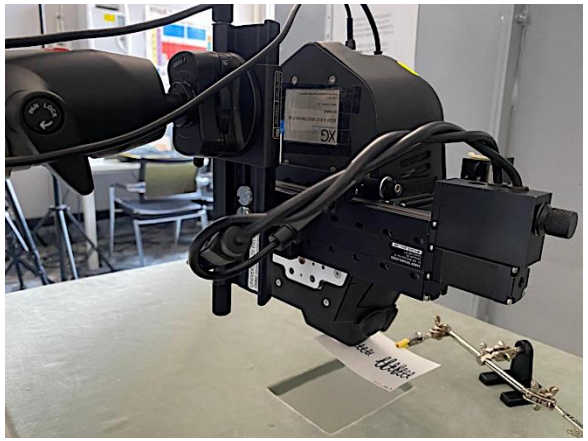
METHODOLOGY

Non-invasive and accessible

X-ray Fluorescence Spectroscopy (XRF)

ELIO - Bruker XGLab s.r.l.

- Single point
- Mapping



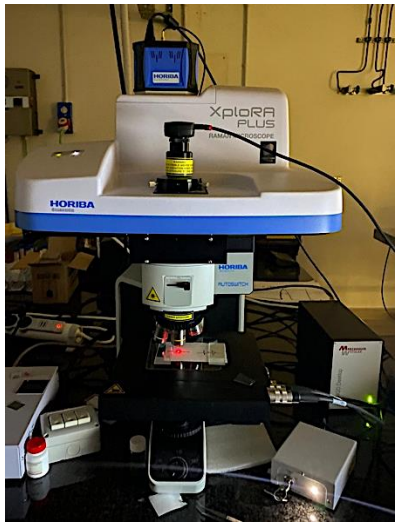
Elemental chemical composition

Micro-Raman Spectroscopy

XploRA PLUS confocal microRaman System Instrument – HORIBA Scientific and Analytical Instruments

Portable Raman spectrometer i-Raman Plus 785S – Metrohm

- Single point
- Mapping



Structural composition

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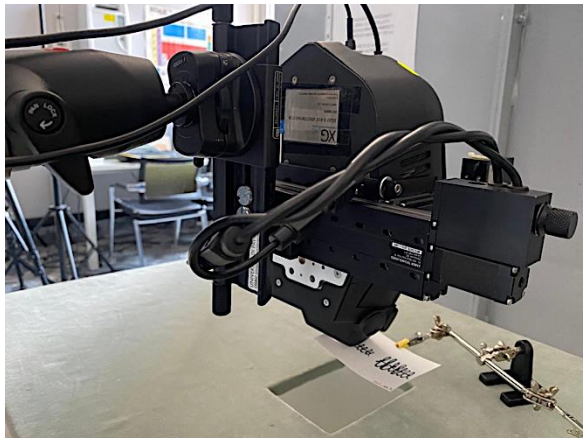

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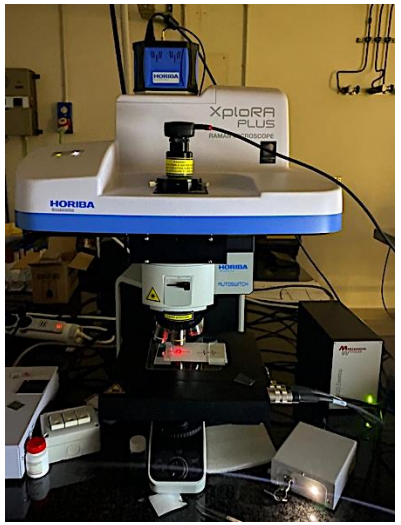
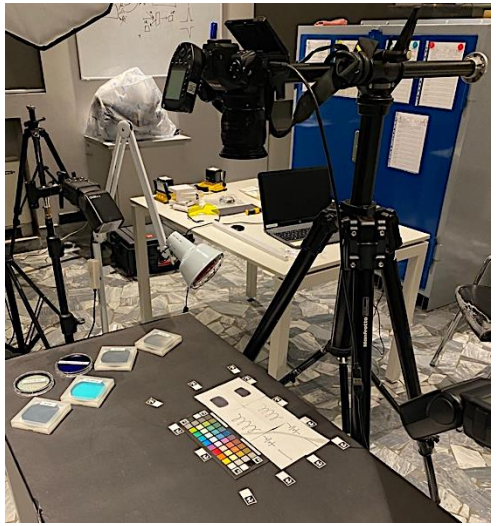


Structural composition

Hypercolorimetric Multispectral Imaging (HMI)

Profilocolore s.r.l.

- High-resolution image acquisition and calibration system in 34 equispaced bands from 300-1000 nm with 20 nm steps
- Software tools with image-processing functions



Spectral reflectance and colourimetric properties

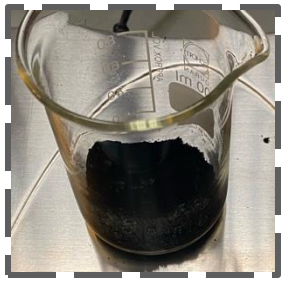
Production of inks and mock-ups

Carbon ink

CI

Giovanni Alcherio, 1411 (Tasseva, J., Analyst, 2017)

- Carbon black
- Gum Arabic
- Water



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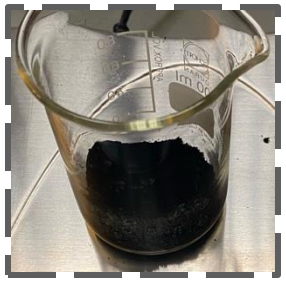

Iron-gall ink

IGI1 – IGI2 – IGI3 – IGI4

Giovanni Battista Palatino, 1545

- Gall-nuts
- Iron(II) Sulphate
- (Copper(II) Sulphate)
- Gum Arabic
- Water

MATERIALS

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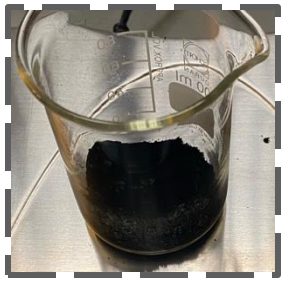

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Logwood ink

LI1 – LI2 – LI3- LI4

Pierre de Ribeaucourt, 1792

- Gall-nuts
- Logwood
- Iron(II) Sulphate
- Copper(II) Sulphate
- Gum Arabic
- Sugar
- Water

MATERIALS

Production of inks and mock-ups

Carbon ink

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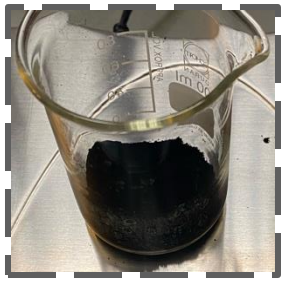
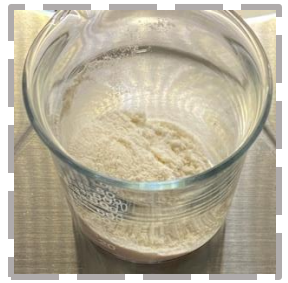


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MATERIALS

Production of inks and mock-ups

- Inks with distinct chemical composition (i.e., carbon ink and iron-gall ink)
- Inks with similar chemical composition (i.e., logwood ink and iron-gall ink)
- Inks with the same raw materials but in different amounts (i.e., iron-gall ink$_{(1)}$ and iron-gall ink$_{(2)}$)

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- Inks with distinct chemical composition (i.e., carbon ink and iron-gall ink)
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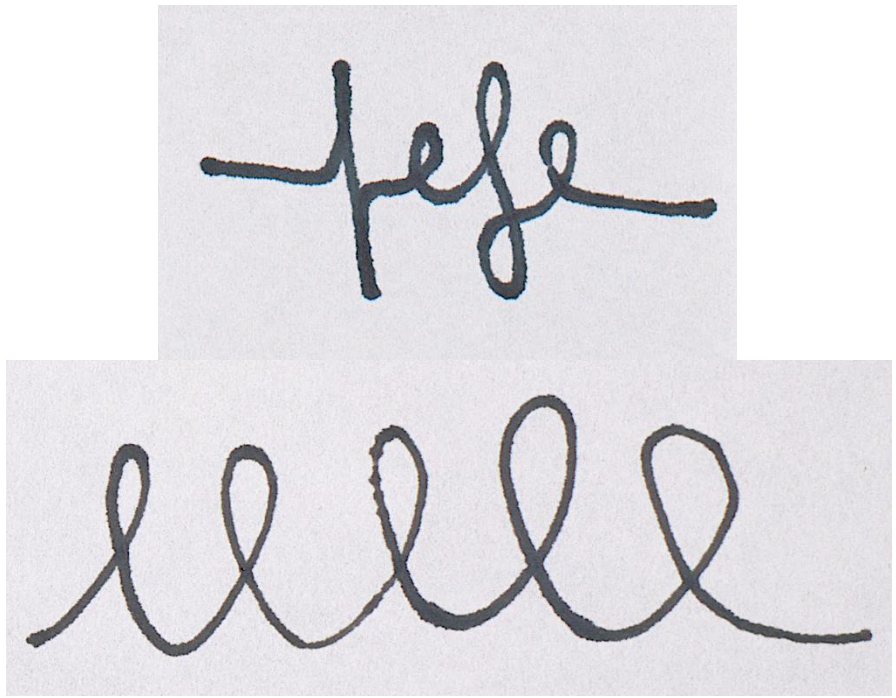
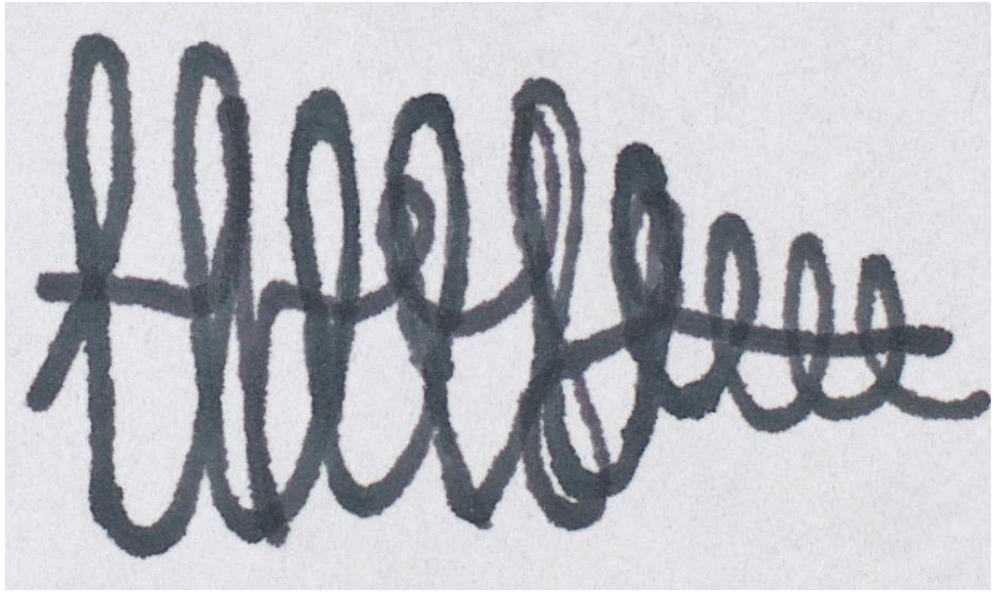


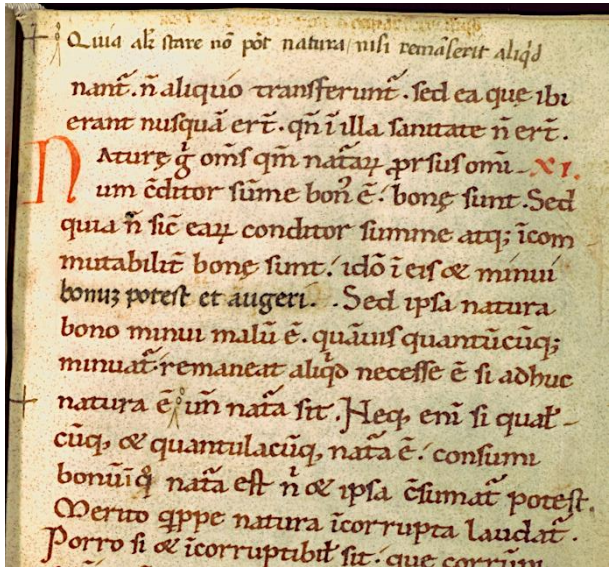


Whatman® paper

Original manuscripts preserved at the *Biblioteca Universitaria di Pavia*

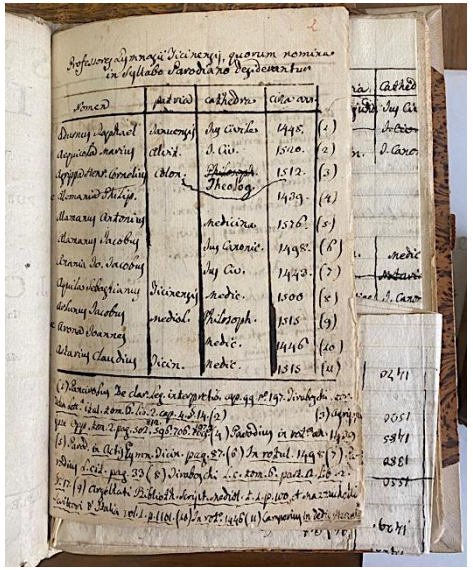
Ms.Ald.52, 11th century

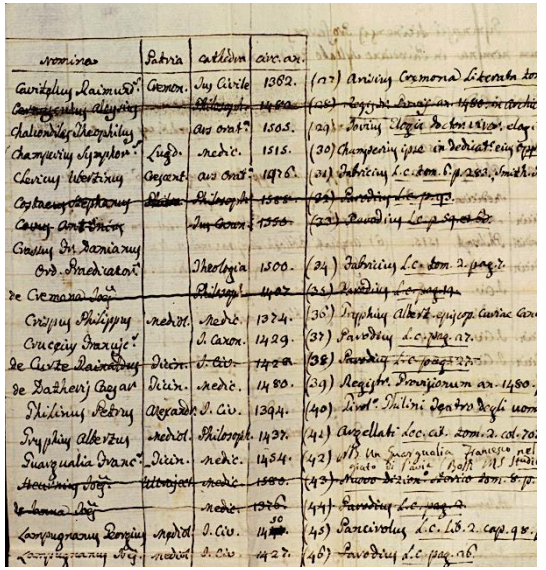
Collection: Manoscritti Aldini



Ms.Tic.67, 18th–19th century

Collection: Manoscritti Ticinesi





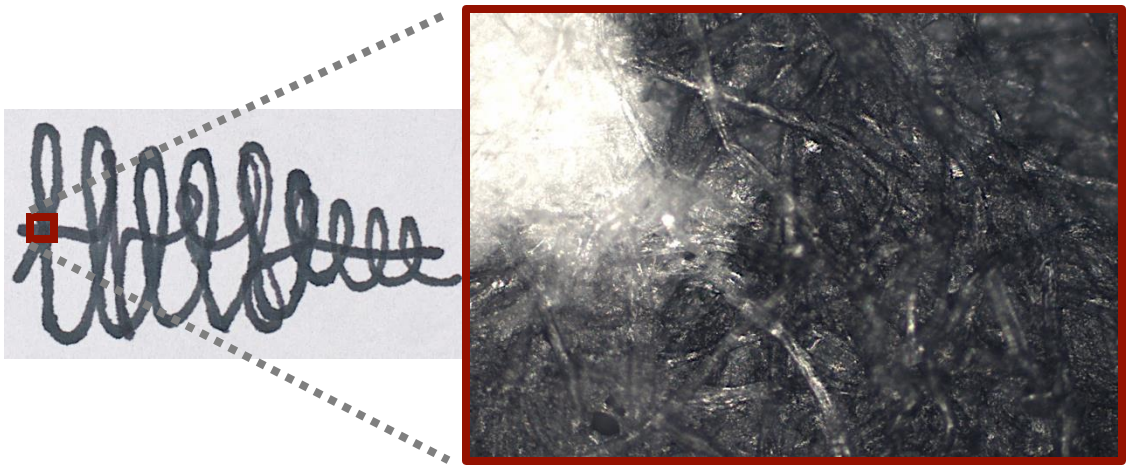
Micro-Raman Spectroscopy

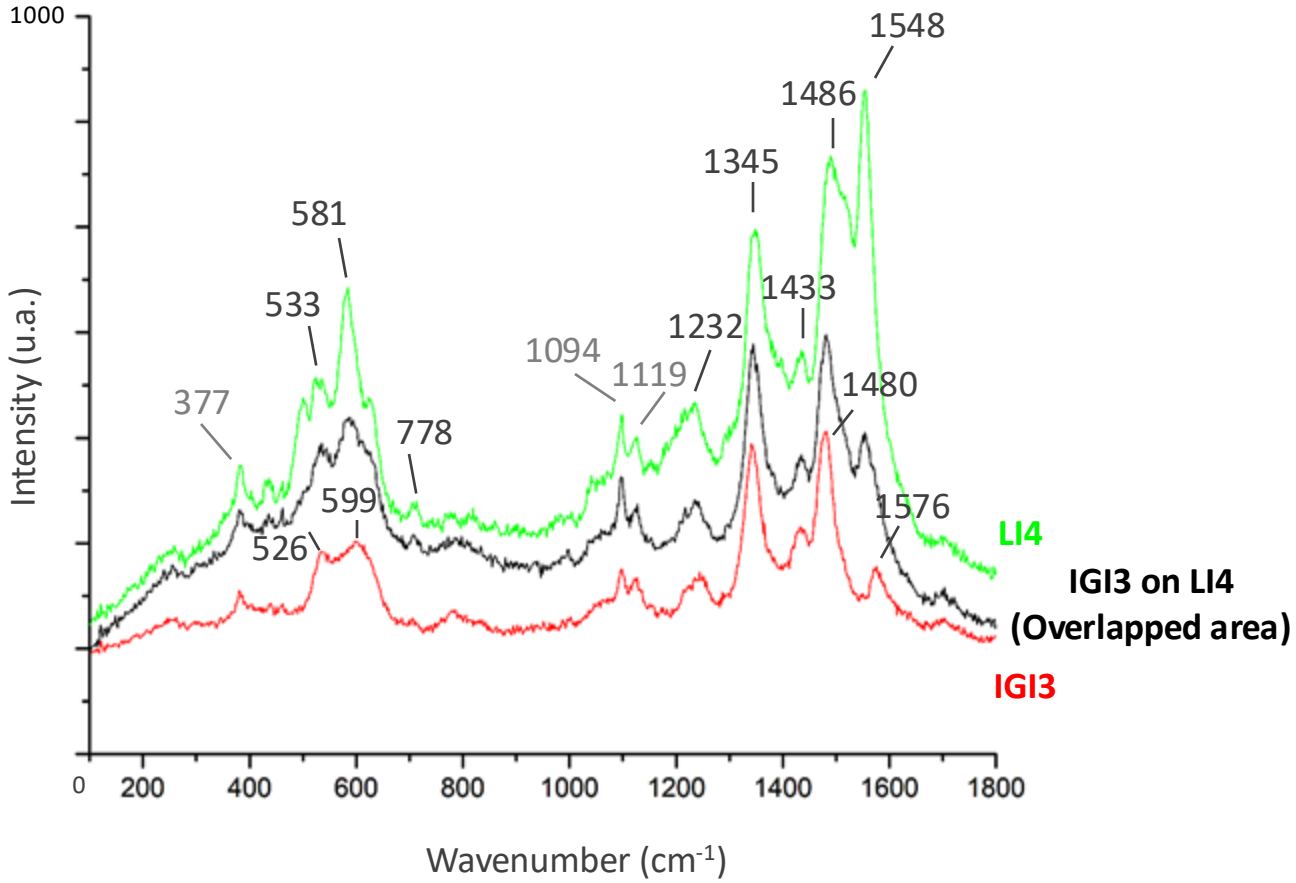
Mapping analysis - Mock-ups

Iron-gall ink(3) on Logwood ink(4)

Bench-top set-up

Laser excitation line: 638 nm line - Objective lens: 10X - Laser power: 1.32×10^4 W/cm^2
Integration time: 15 s - Accumulation: 30
Map size: 214 µm x 203 µm - Spectra acquired: 120





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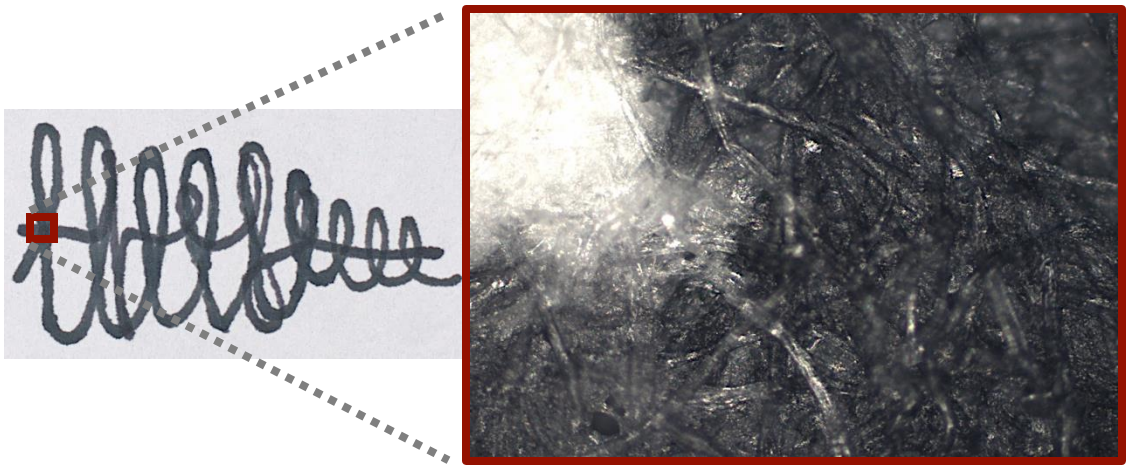
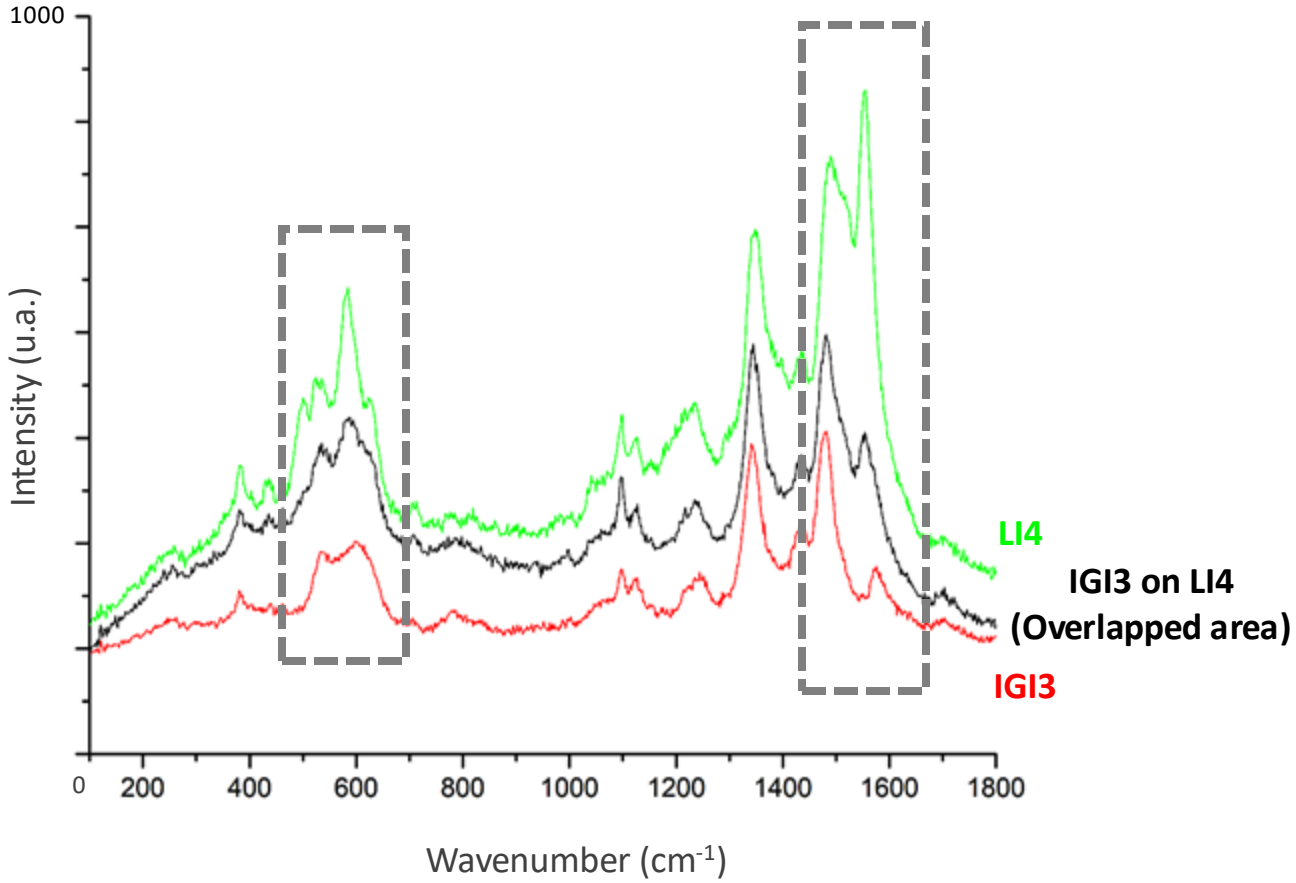


Metal polyphenol complex



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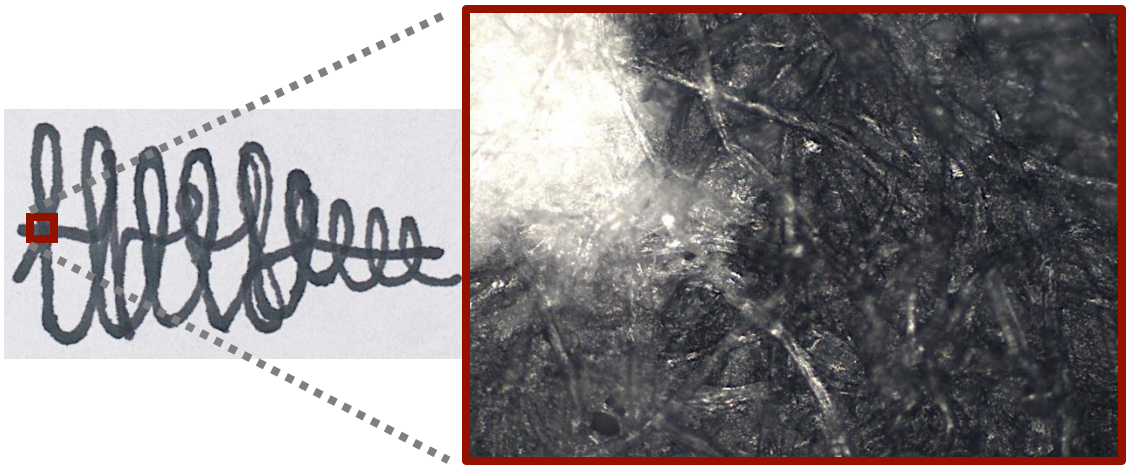

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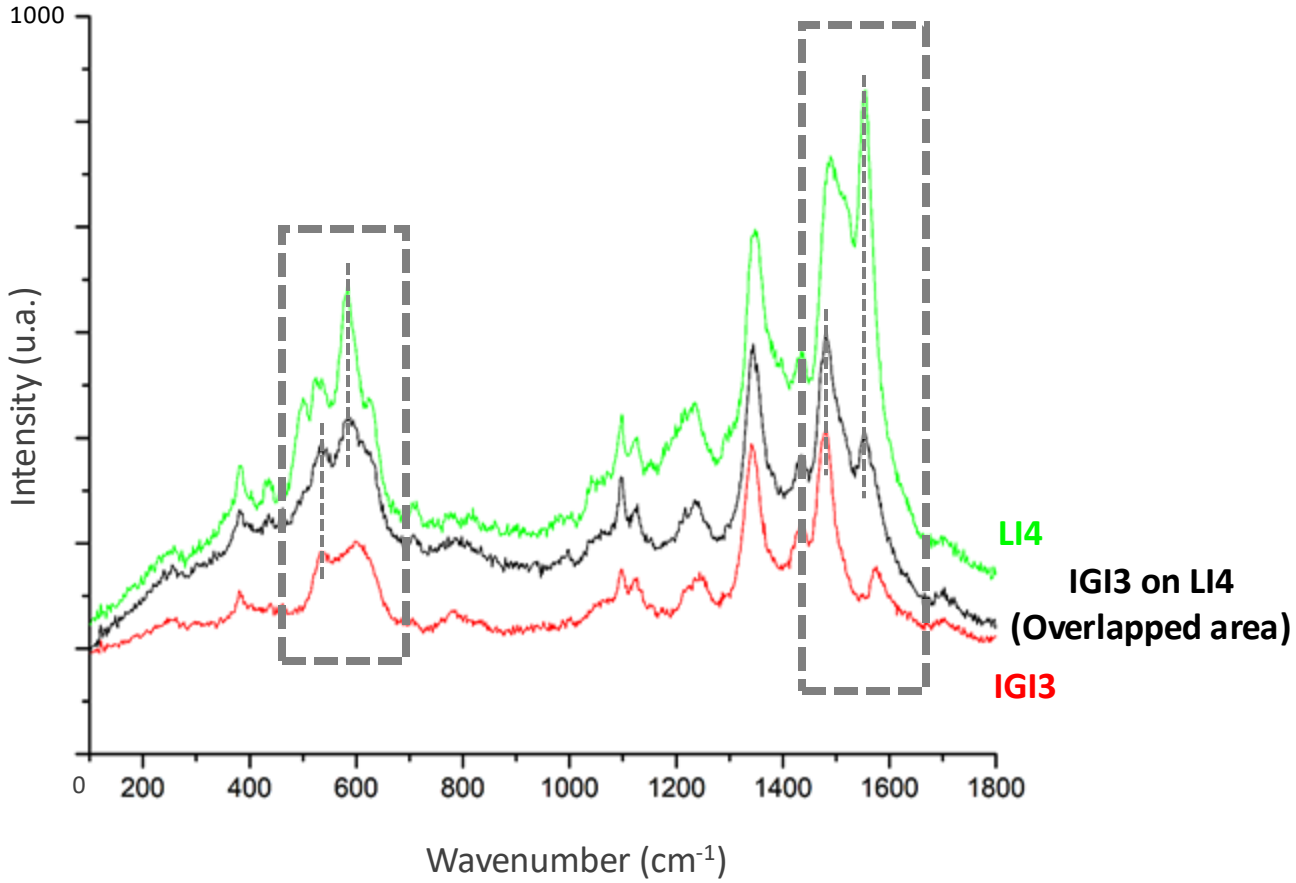


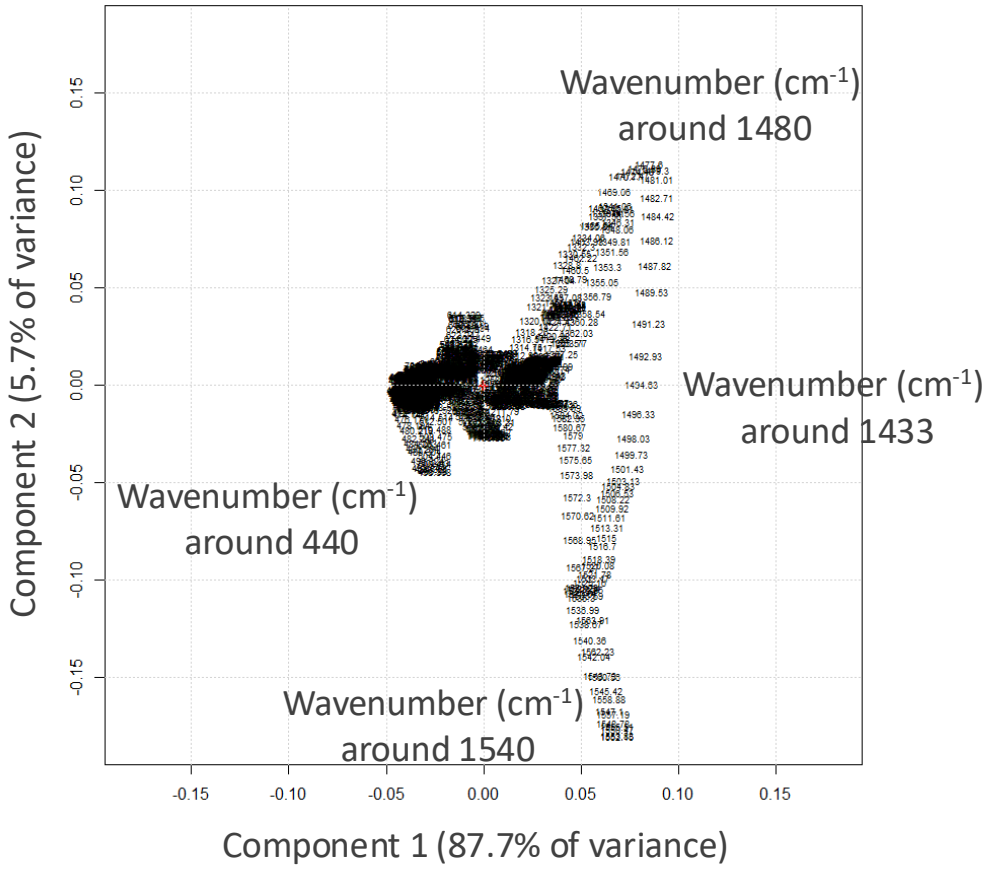
Principal Component Analysis

Mock-ups

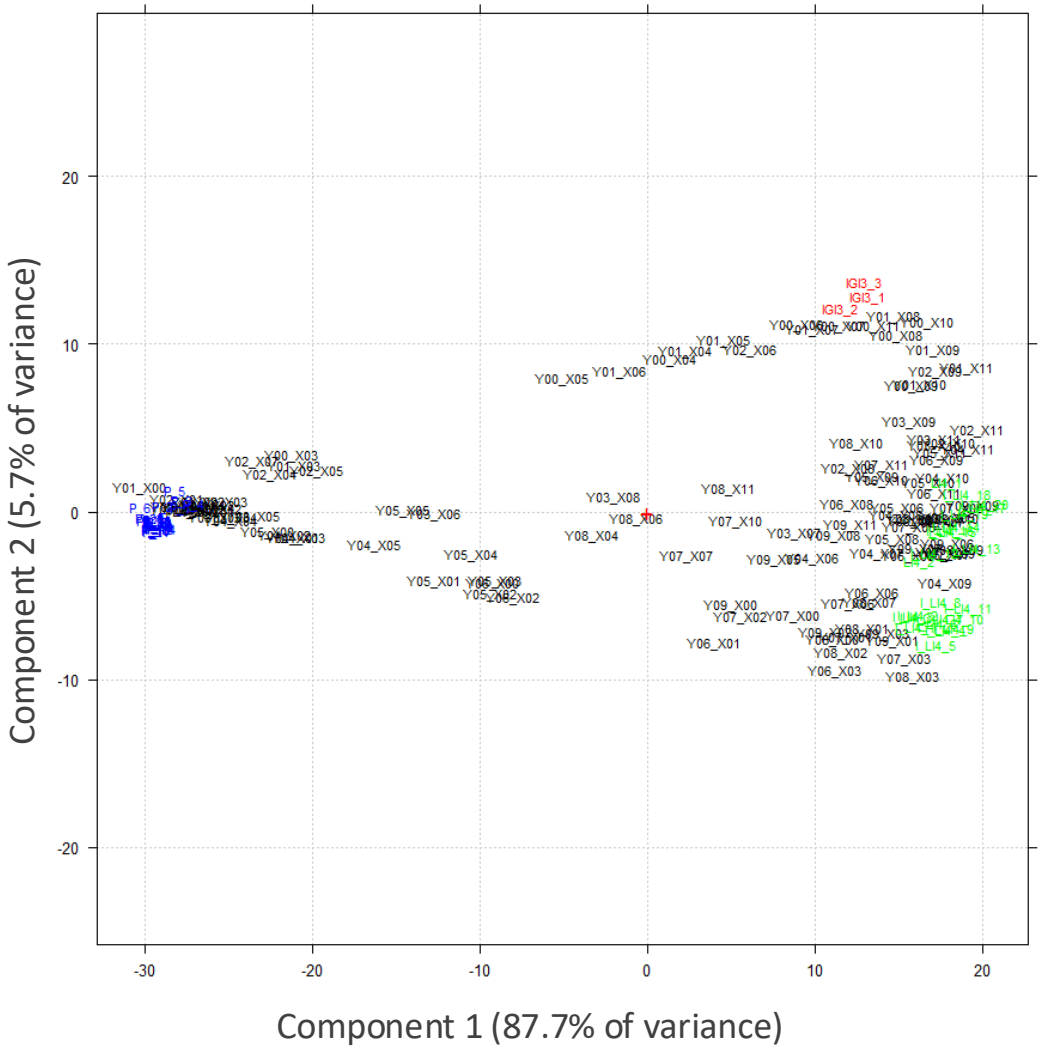
Iron-gall ink$_{(3)}$ on Logwood ink$_{(4)}$

Row pre-processing: Standard Normal Variate (SNV)
Column pre-processing: Centering

Loading plot (93.4% of total variance)



Score plot (93.4% of total variance)



- Paper data - Point analysis
- Logwood ink$_{(4)}$ Data - Point analysis
- Iron-gall ink$_{(3)}$ Data - Point analysis
- Data - Mapping analysis

Principal Component Analysis

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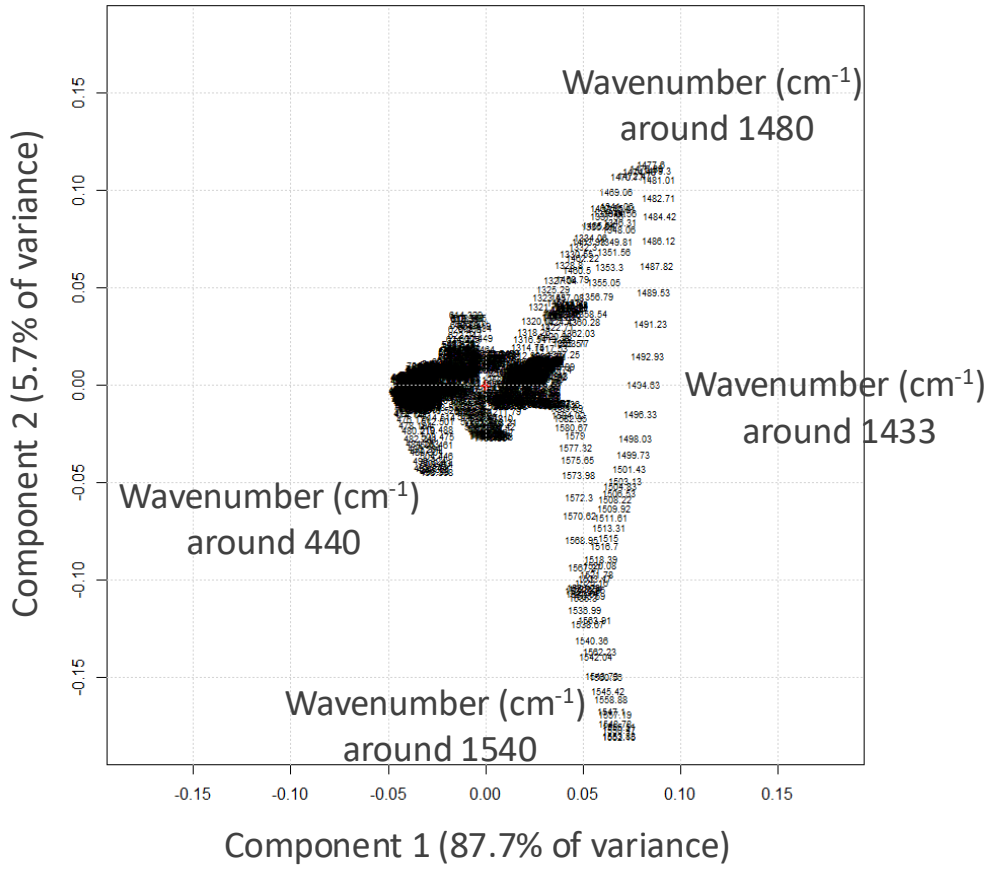

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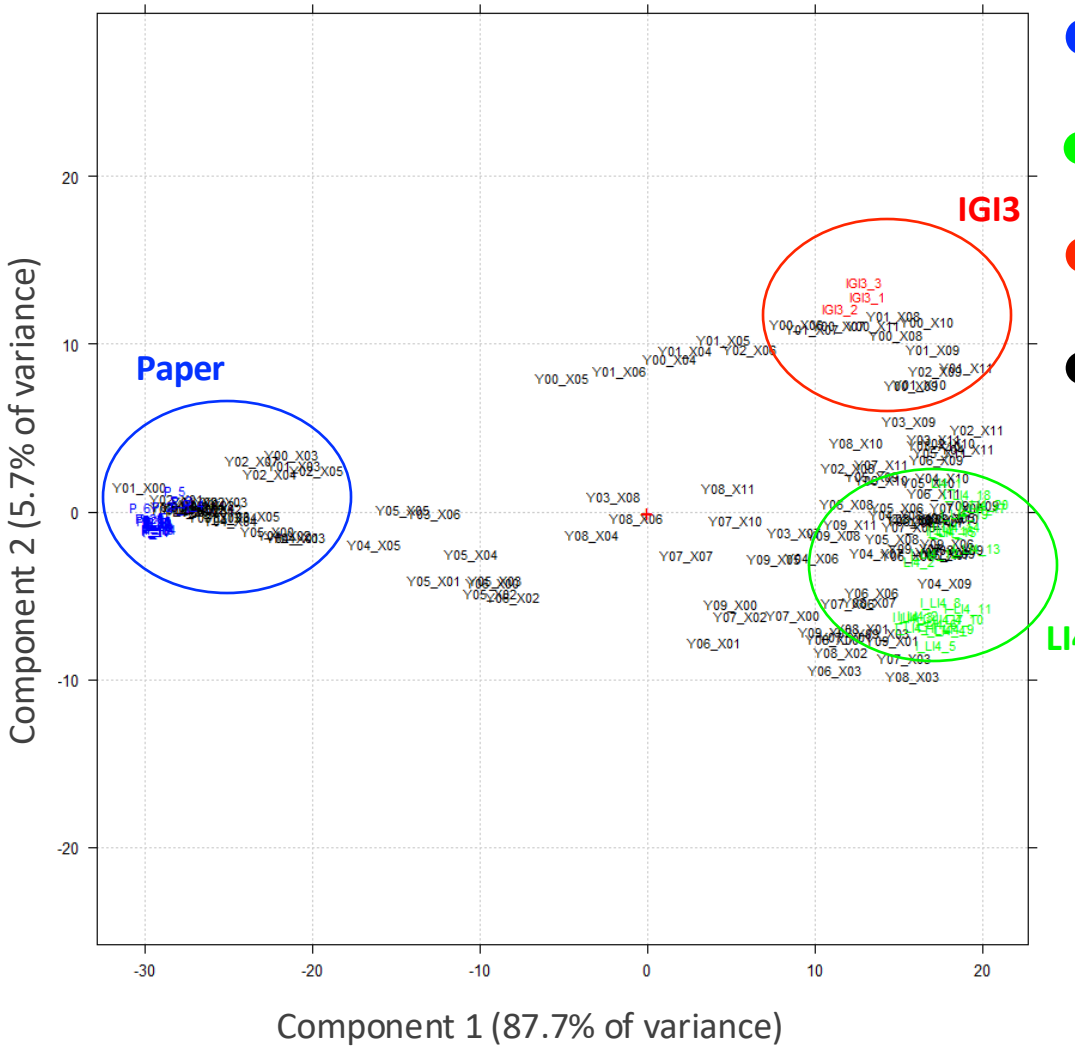

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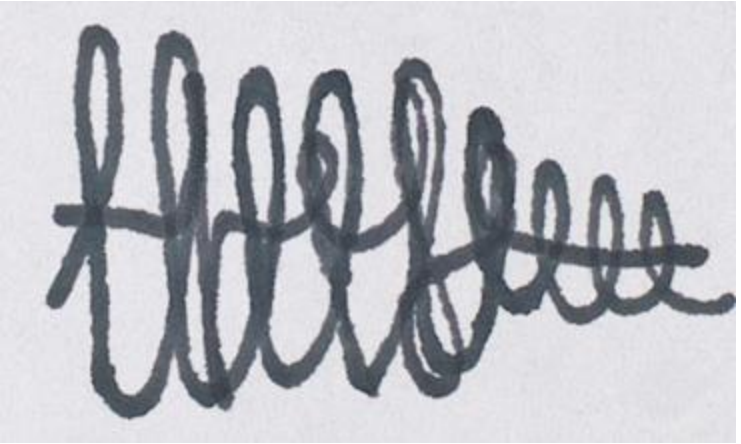
Hypercolorimetric Multispectral Imaging

Mock-ups

Iron-gall ink(3) on Logwood ink(4)

Visible image:



Colorimetry:

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Lab="34.95,-2.16,-6.01"

RGB="87,92,100"
Lab="38.68,-1.12,-6.11"

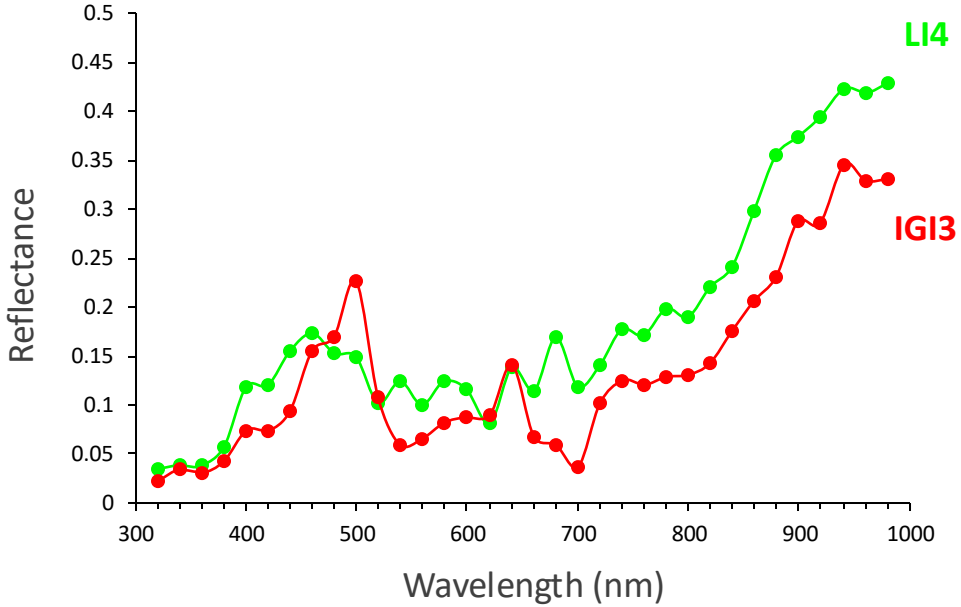


Image of normalised difference indexes:

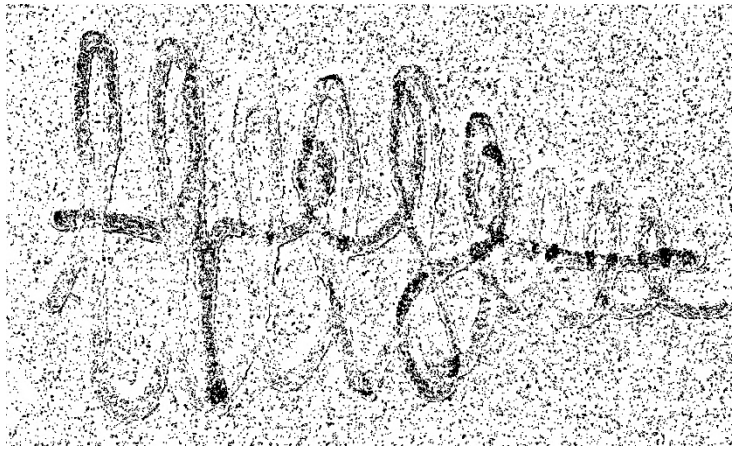
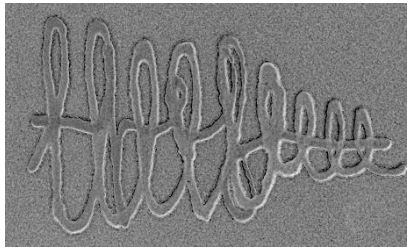


Image of Principal Component Analysis – PCA2:

Image of Principal Component Analysis – PCA3:



Logwood ink$_{(1)}$ on **Logwood ink$_{(4)}$**

80% $FeSO_4*7H_2O$ - 20% $CuSO_4*5H_2O$_70% FeSO4*$7H_2O$ - 30% $CuSO_4*5H_2O$

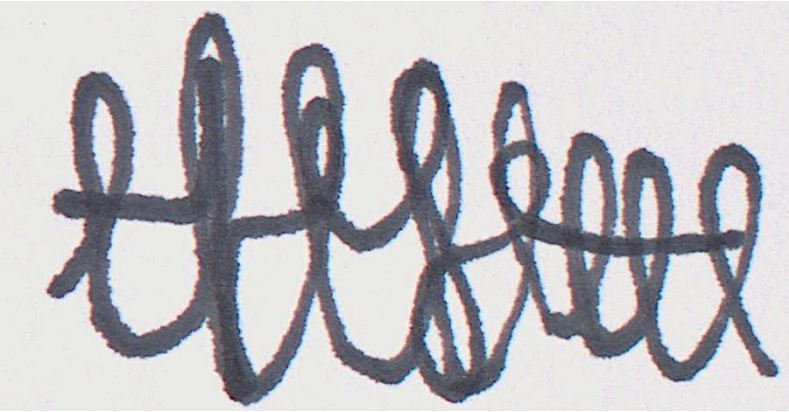
Single point analysis

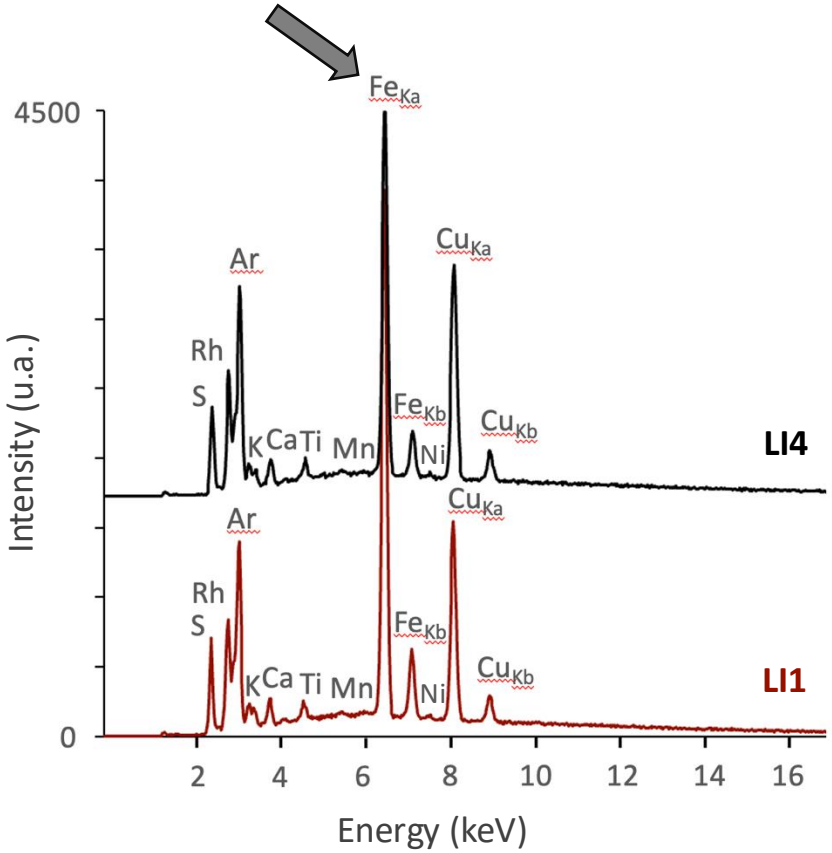
Measurement time: 90 s - Tube voltage: 40 kV - Tube current: 100 µ

Mapping analysis

Tube voltage: 40 kV - Tube current: 100 µA - Map size: 3 cm x 3 cm

Visible image:





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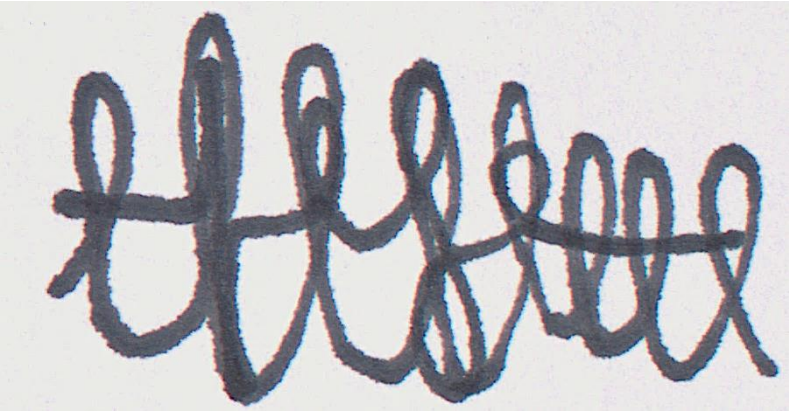
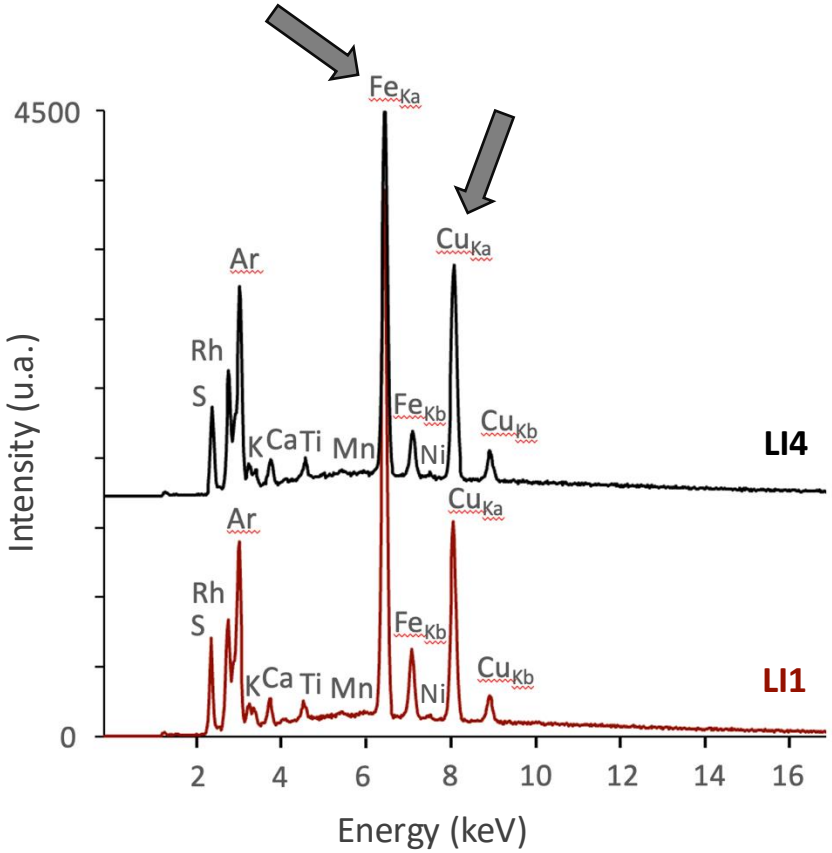
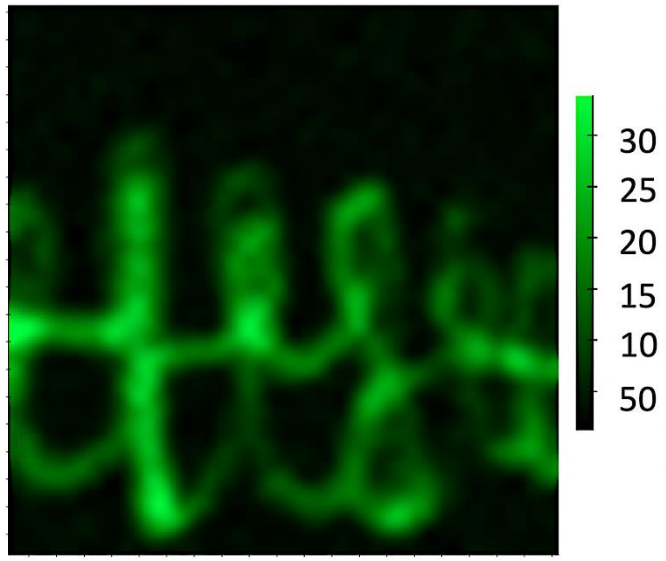
Visible image:





Elemental map Cu_{Ka}:



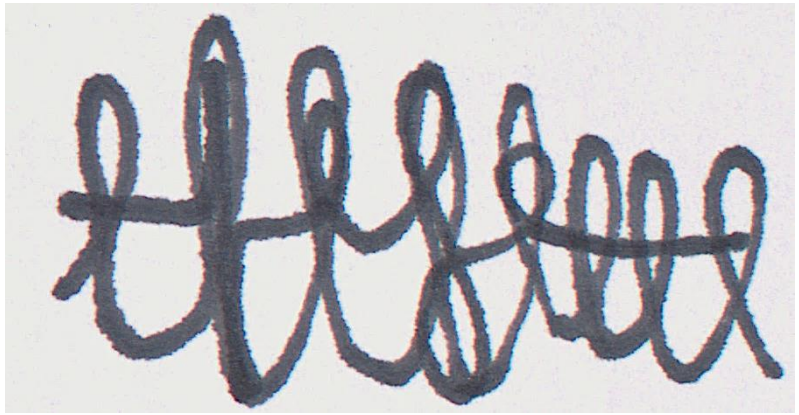
Hypercolorimetric Multispectral Imaging

Mock-ups

Logwood ink$_{(1)}$ on **Logwood ink$_{(4)}$**

80% $FeSO_4*7H_2O$ - 20% $CuSO_4*5H_2O$_70% $FeSO4*7H_2O$ - 30% $CuSO_4*5H_2O$

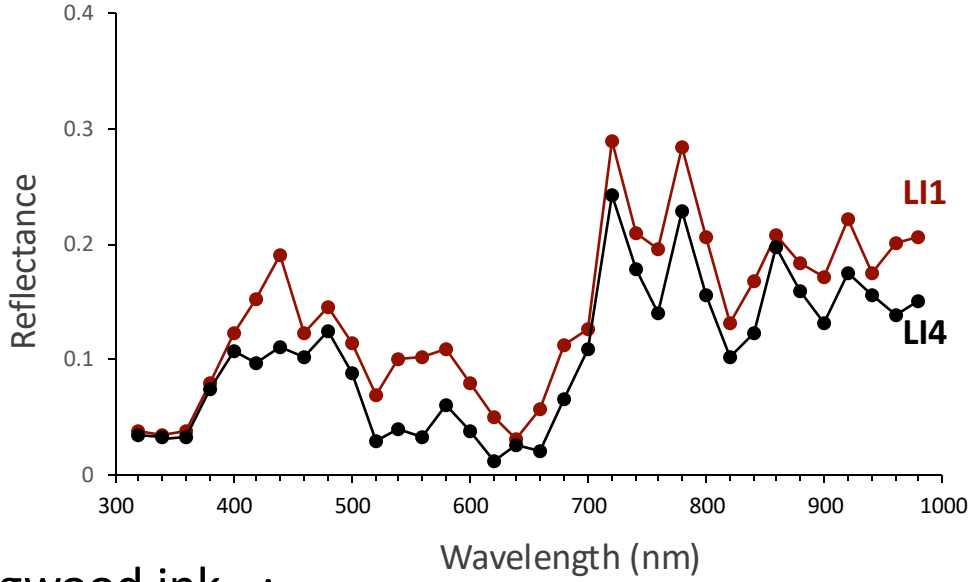
Visible image:



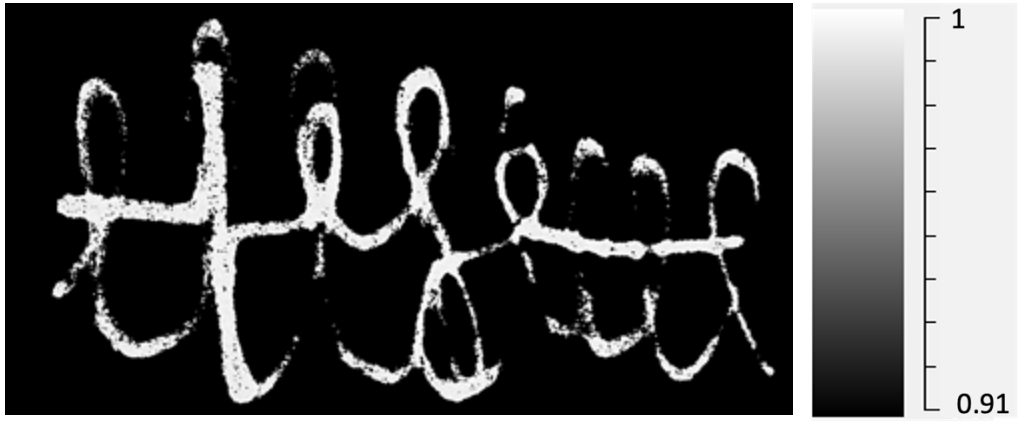
Colorimetry:

RGB="90,92,103"
Lab="39.05,1.04,-7.29"

RGB="88,89,99"
Lab="38.14,1.06,-6.50"



Map of multispectral similarity - Logwood ink$_{(4)}$:



Micro-Raman Spectroscopy
Point analysis - Manuscripts

Ms.Ald.52, 11th century

Collection: Manoscritti Aldini – Biblioteca Universitaria di Pavia

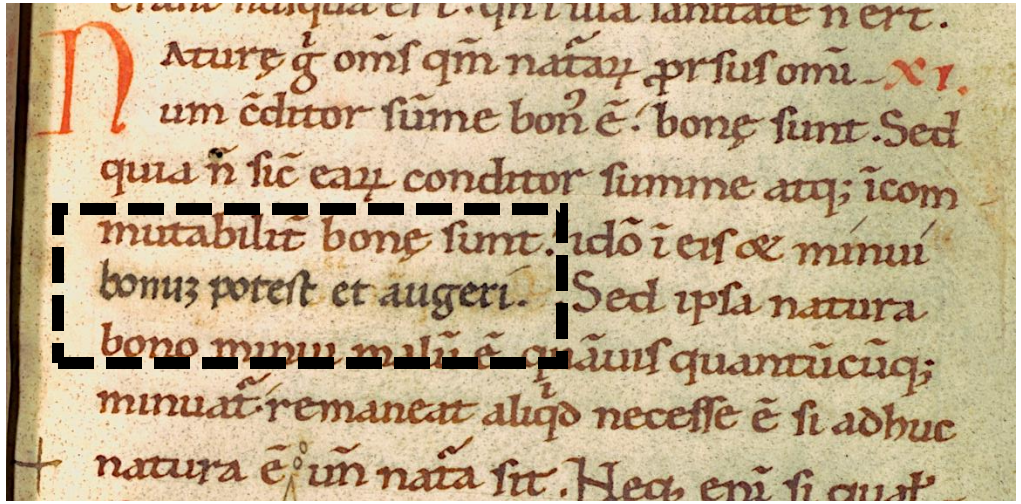
Portable set-up

Laser excitation line: 785 nm line - Laser power: 1.01×10^5 W/cm^2 - Integration time: 5 s - Accumulation: 5

Laser excitation line: 785 nm line - Laser power: 3.03×10^5 W/cm^2 - Integration time: 10 s - Accumulation: 1

Laser excitation line: 785 nm line - Laser power: 2.02×10^5 W/cm^2 - Integration time: 5 s - Accumulation: 3

Visible image



Parchment manuscript

Micro-Raman Spectroscopy
Point analysis - Manuscripts

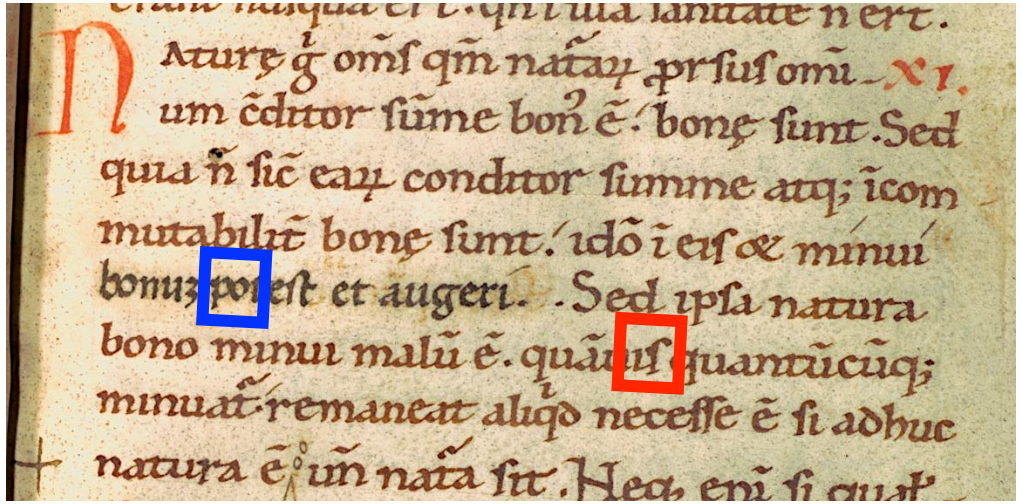
Ms.Ald.52, 11th century

Collection: Manoscritti Aldini – Biblioteca Universitaria di Pavia

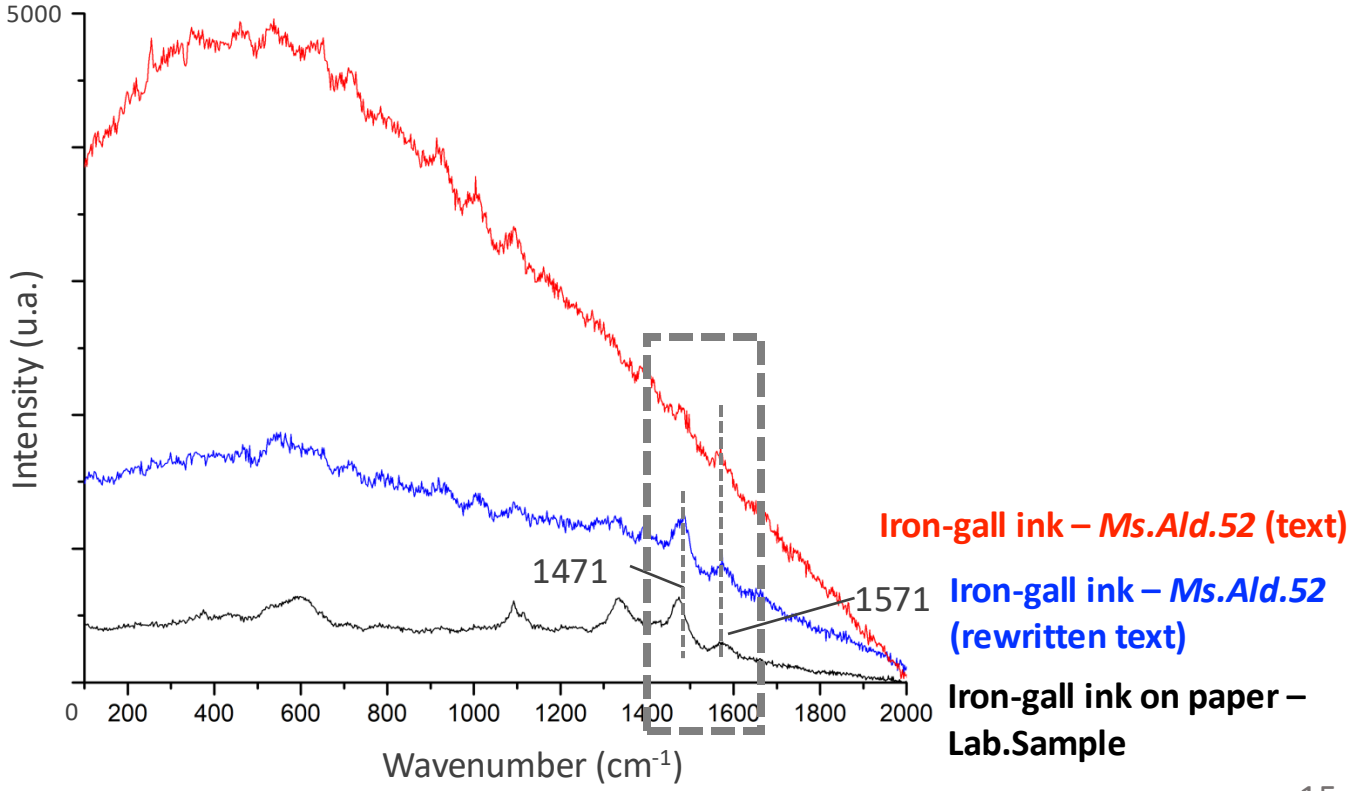
Portable set-up

Laser excitation line: 785 nm line - Laser power: 1.01×10^5 W/cm^2 - Integration time: 5 s - Accumulation: 5
Laser excitation line: 785 nm line - Laser power: 3.03×10^5 W/cm^2 - Integration time: 10 s - Accumulation: 1
Laser excitation line: 785 nm line - Laser power: 2.02×10^5 W/cm^2 - Integration time: 5 s - Accumulation: 3

Visible image



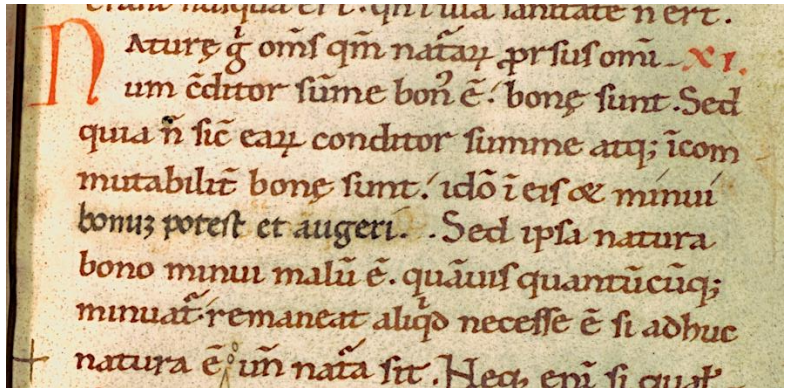
Parchment manuscript



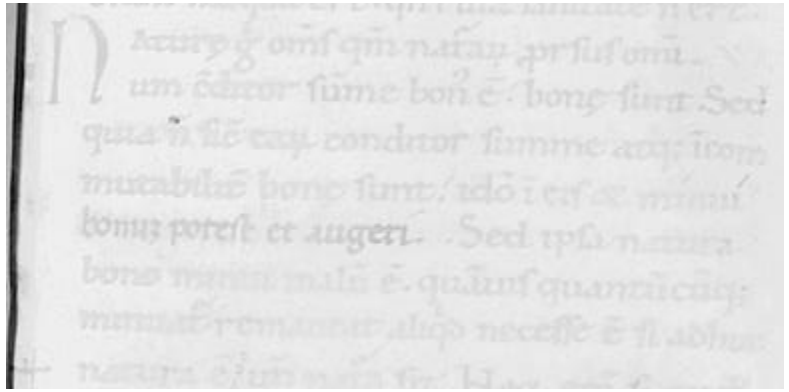
Ms.Ald.52, 11th century

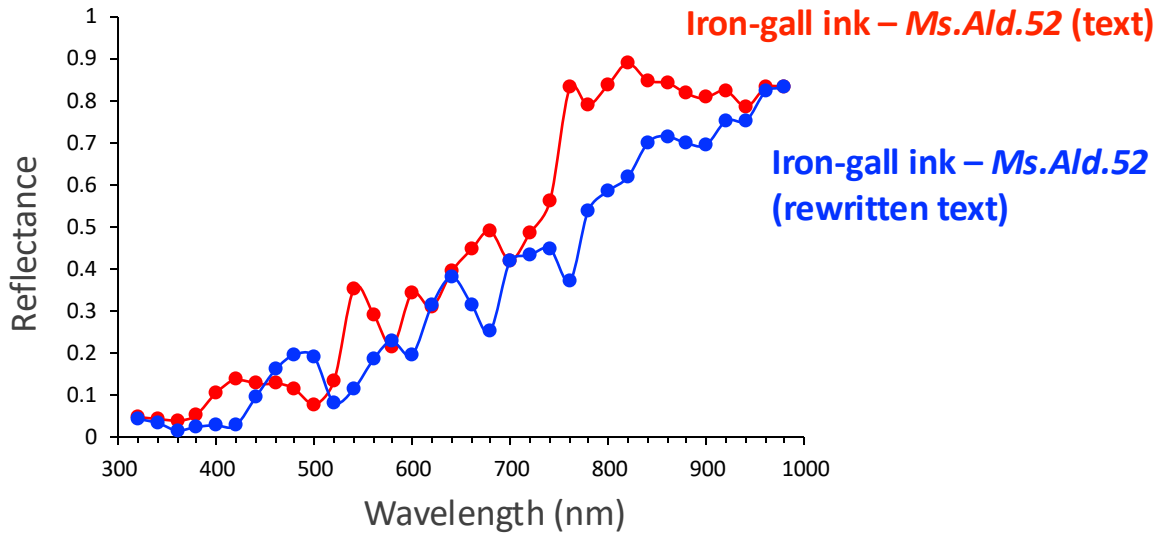
Collection: Manoscritti Aldini – Biblioteca Universitaria di Pavia

Visible image - spectral range 380-780 nm:



Near-infrared image - spectral range 780-1000 nm:





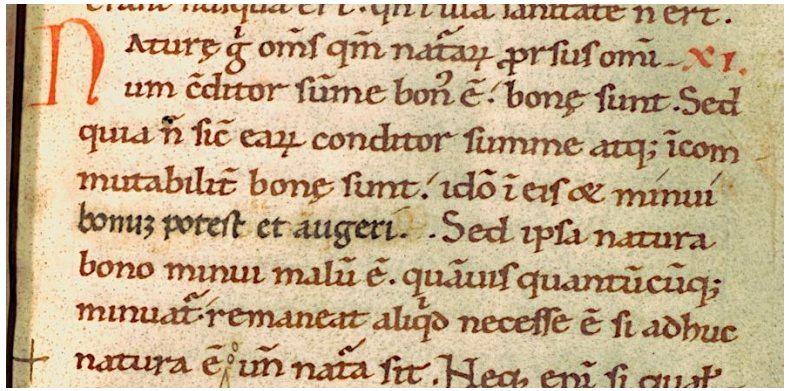
Hypercolorimetric Multispectral Imaging

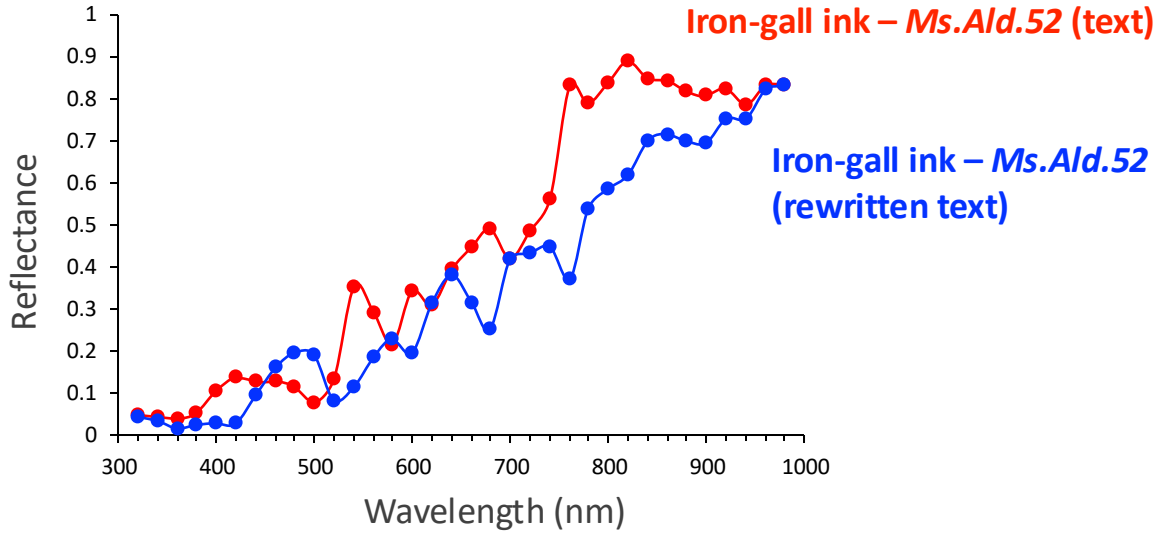
Manuscripts

Ms.Ald.52, 11th century

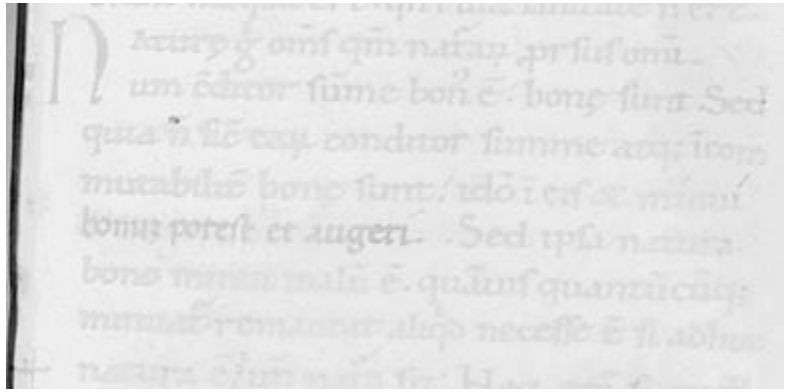
Collection: Manoscritti Aldini – Biblioteca Universitaria di Pavia

Visible image - spectral range 380-780 nm:





Near-infrared image - spectral range 780-1000 nm:



Visible image:

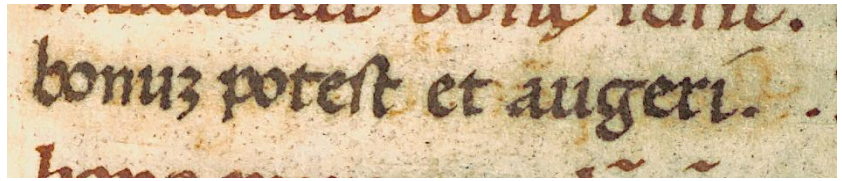
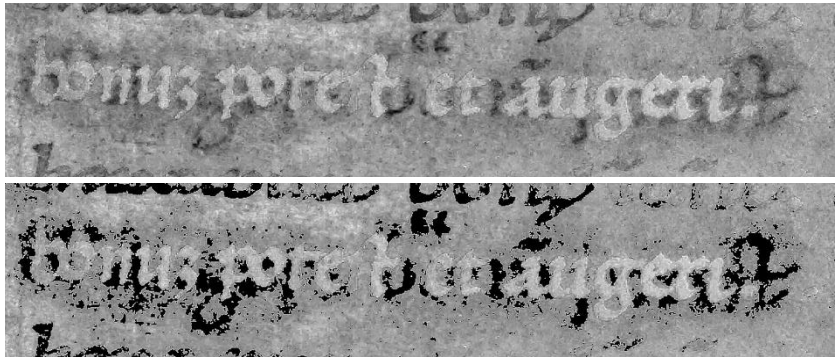
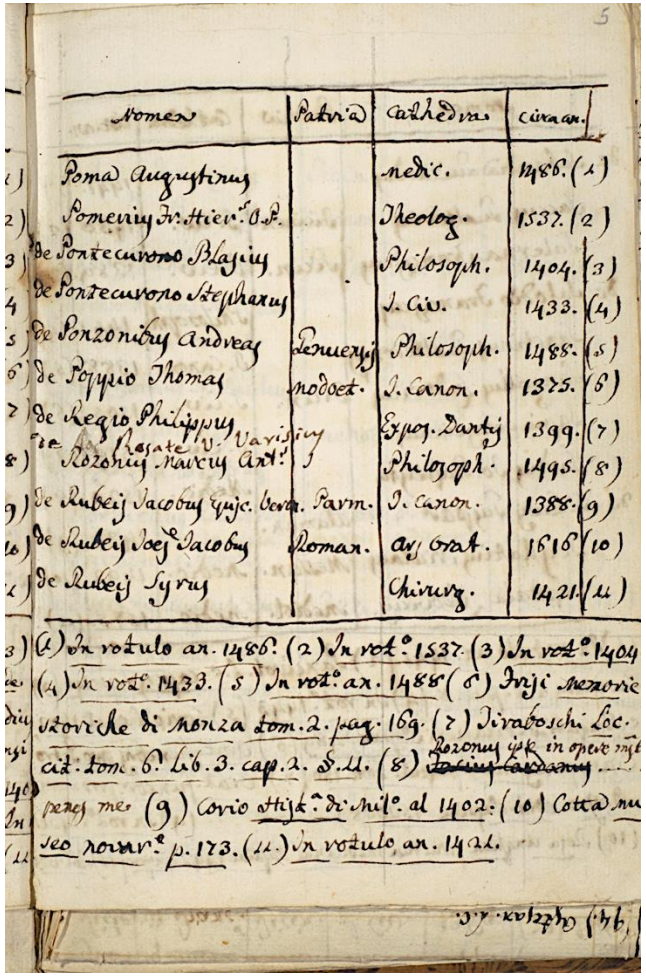


Image of Principal Component Analysis - PCA2:



Ms.Tic.67, 18th–19th century, Autograph section by Siro Comi

Collection: Manoscritti Ticinesi – Biblioteca Universitaria di Pavia



Paper manuscript

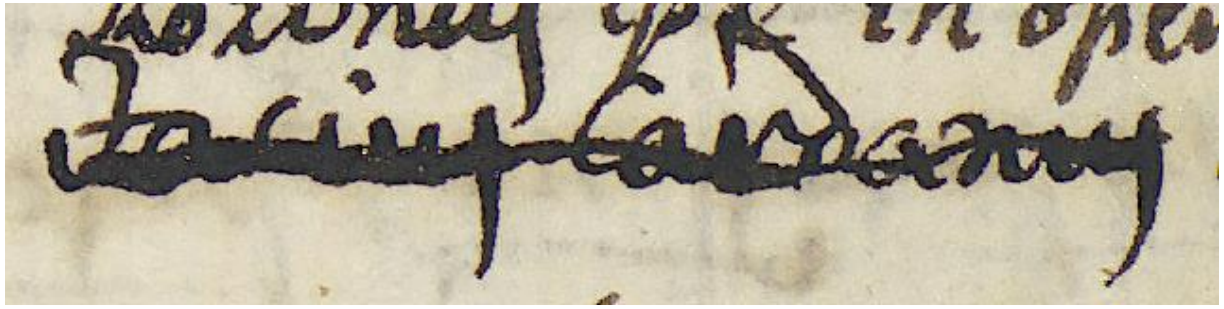
Hypercolorimetric Multispectral Imaging

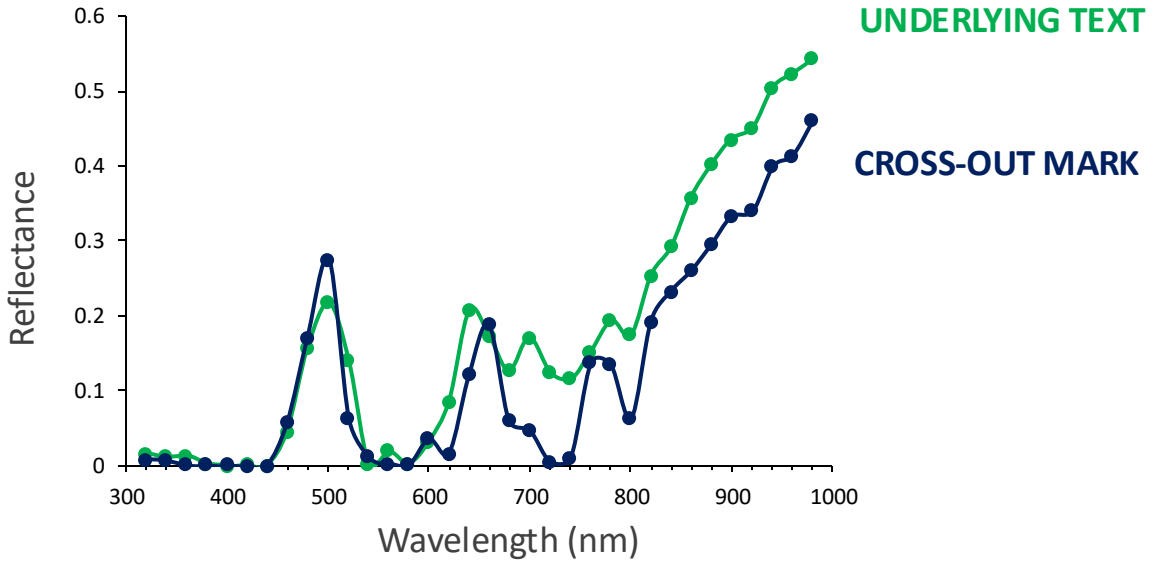
Manuscripts

Ms.Tic.67, 18th-19th century, Autograph section by Siro Comi

Collection: Manoscritti Ticinesi – Biblioteca Universitaria di Pavia

Visible image:





Ms.Tic.67, 18th-19th century, Autograph section by Siro Comi

Collection: Manoscritti Ticinesi – Biblioteca Universitaria di Pavia

Visible image:

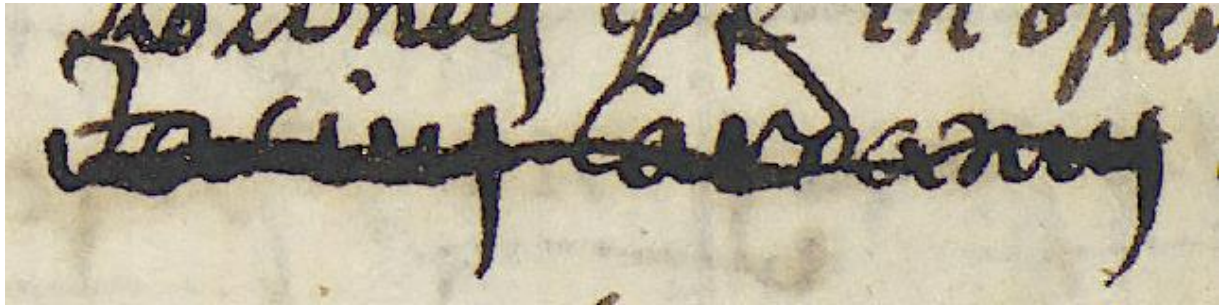
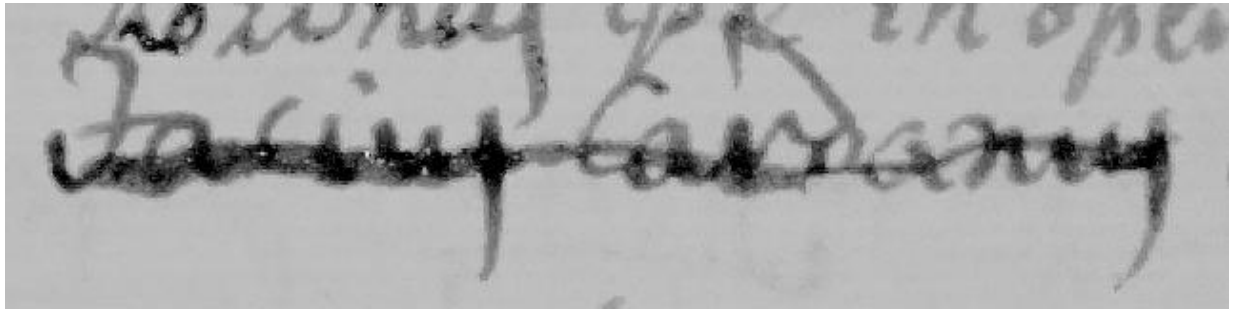
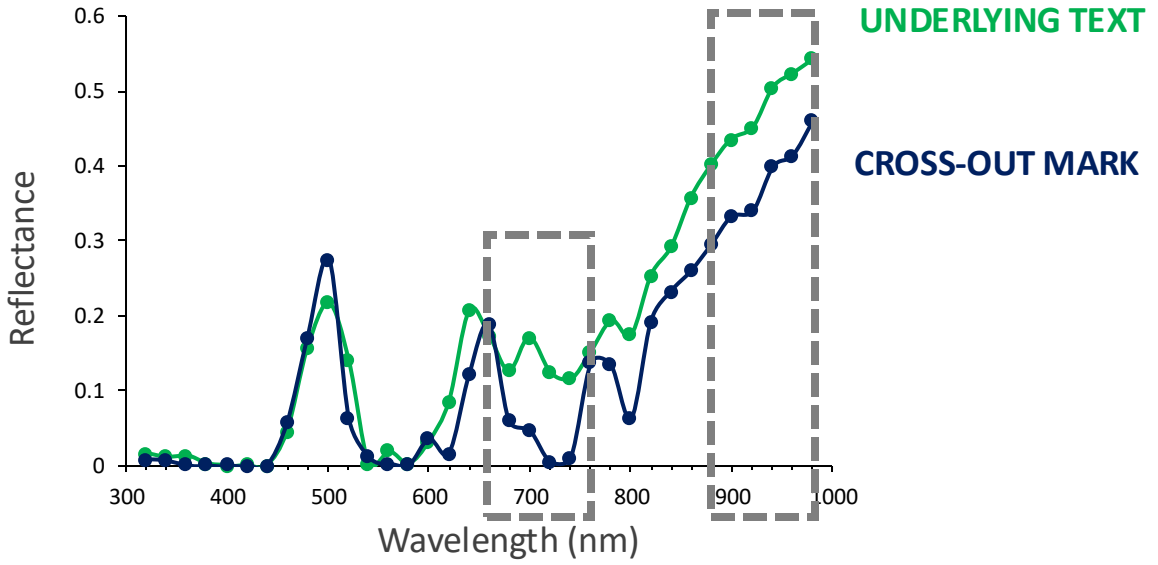


Image of normalised difference indexes:





Hypercolorimetric Multispectral Imaging

Manuscripts

Ms.Tic.67, 18th-19th century, Autograph section by Siro Comi

Collection: Manoscritti Ticinesi – Biblioteca Universitaria di Pavia

Visible image:

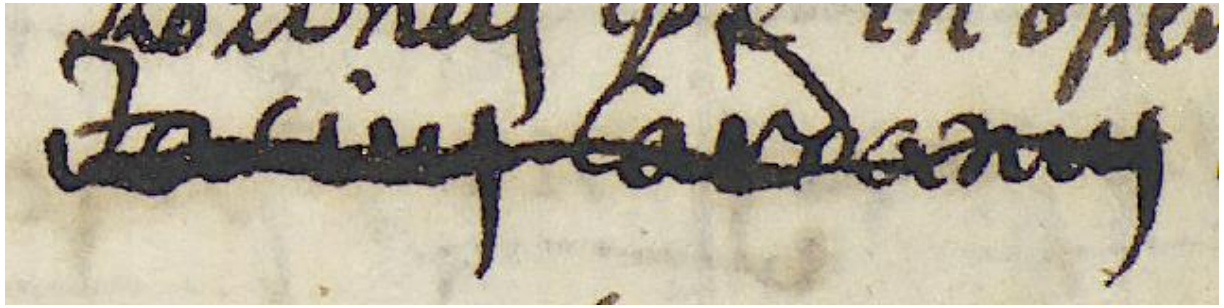
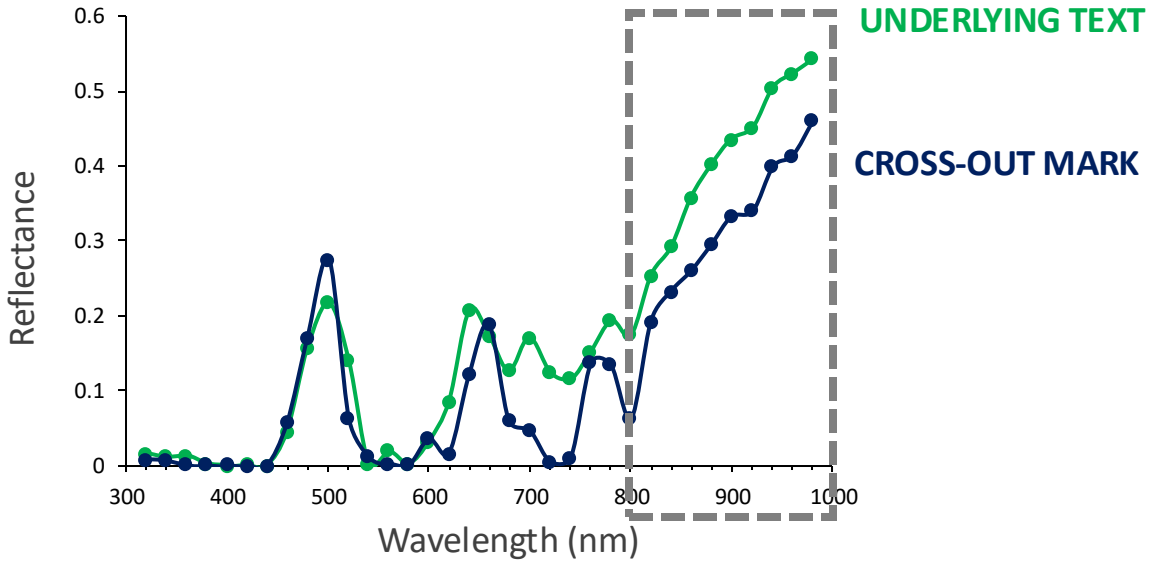


Image of Principal Component Analysis – PCA1:



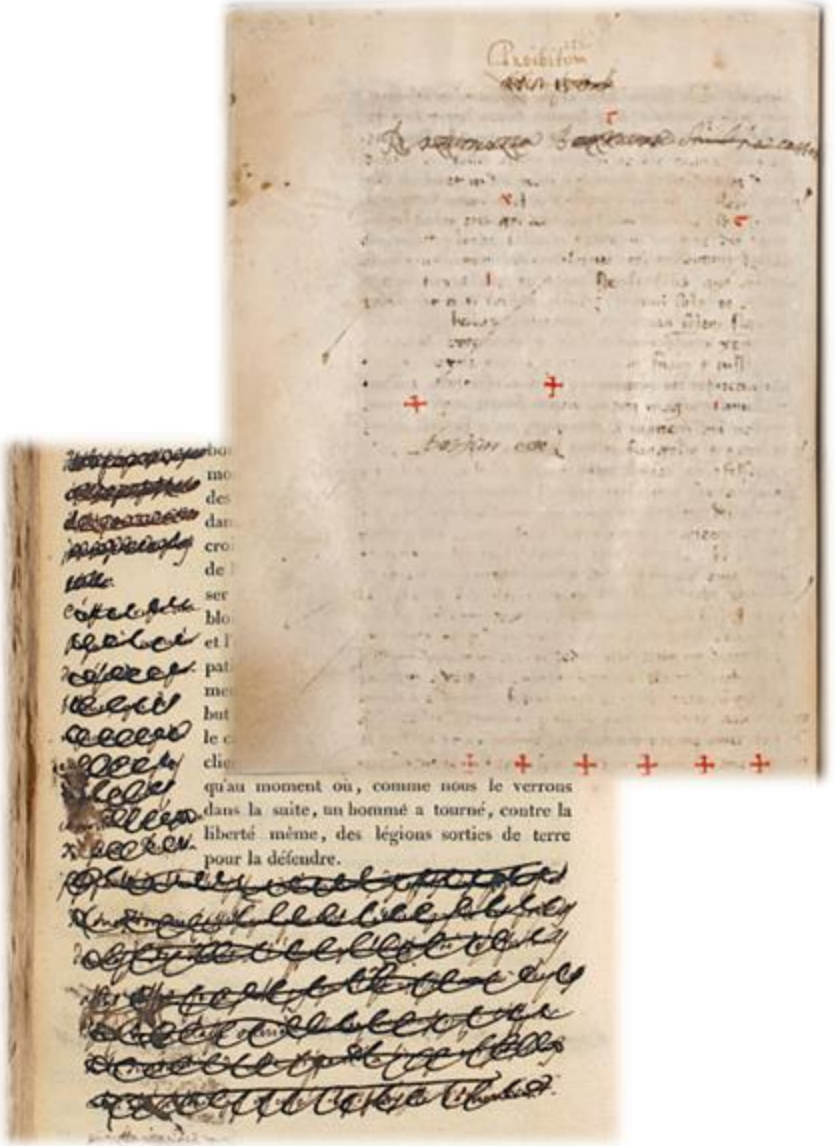
The non-invasive and multi-analytical methodology:

✓ characterisation and discrimination of inks with similar chemical compositions and those used for writing, with the inks superimposed by erasure

✓ enhancing legibility of text overwritten by cross-outs

✓ potential and optimal combined use of different techniques

- Ongoing collaboration with the *Biblioteca Universitaria di Pavia* on case studies of original manuscripts (e.g., Ms.Ald.211, 14th century)



THIRD YEAR OF ACTIVITY

- Ongoing collaboration with the *Biblioteca Universitaria di Pavia* on case studies of original manuscripts (e.g., Ms.Ald.211, 14th century)

- Ongoing processing of data from diagnostic campaigns on original manuscripts (e.g., manuscripts by Alessandro Manzoni, 19th century; 13th century reused polyphonic parchments)

THIRD YEAR OF ACTIVITY

- Ongoing collaboration with the *Biblioteca Universitaria di Pavia* on case studies of original manuscripts (e.g., Ms.Ald.211, 14th century)

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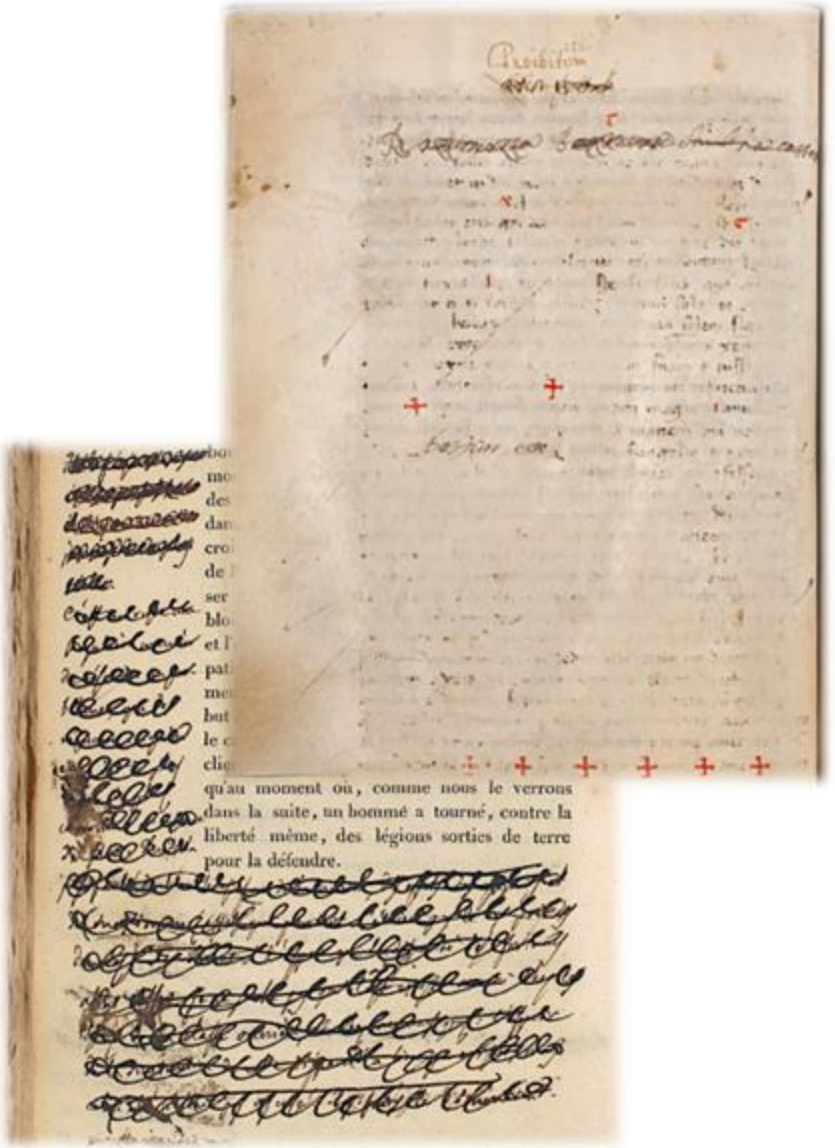
- Implementation of other analytical techniques in collaboration with *Optics Department of the Institute of Physics of the Czech Academy of Sciences in Prague* (e.g., Micro X-Ray Fluorescence spectrometry; Terahertz Imaging; Surface-Enhanced Raman Scattering; Spatially Offset Raman Spectroscopy)



Thank you for your attention!

Dr. Chiara Delledonne (chiara.delledonne@unipv.it)

Department of Physics, University of Pavia

Arvedi Laboratory of Non-Invasive Diagnostics, Department of Musicology and Cultural Heritage, University of Pavia

UNIVERSITÀ DI PAVIA
Dipartimento di Fisica

ARVEDI LABORATORY OF NON-INVASIVE DIAGNOSTICS

MATERIALS

Production of inks and mock-ups

Carbon ink

Giovanni Alcherio, 1411
(Tasseva, J., Analyst, 2017)

Carbon black - 0,6 g
Gum Arabic - 0,7 g
Water - 9 ml

Iron-gall ink

Giovanni Battista Palatino, 1545

Gall-nuts - 15,4 g
Iron (II) Sulphate - 10,2 g
Gum Arabic - 5,1 g
Water - 150 ml

Logwood ink

Ribeaucourt, 1792

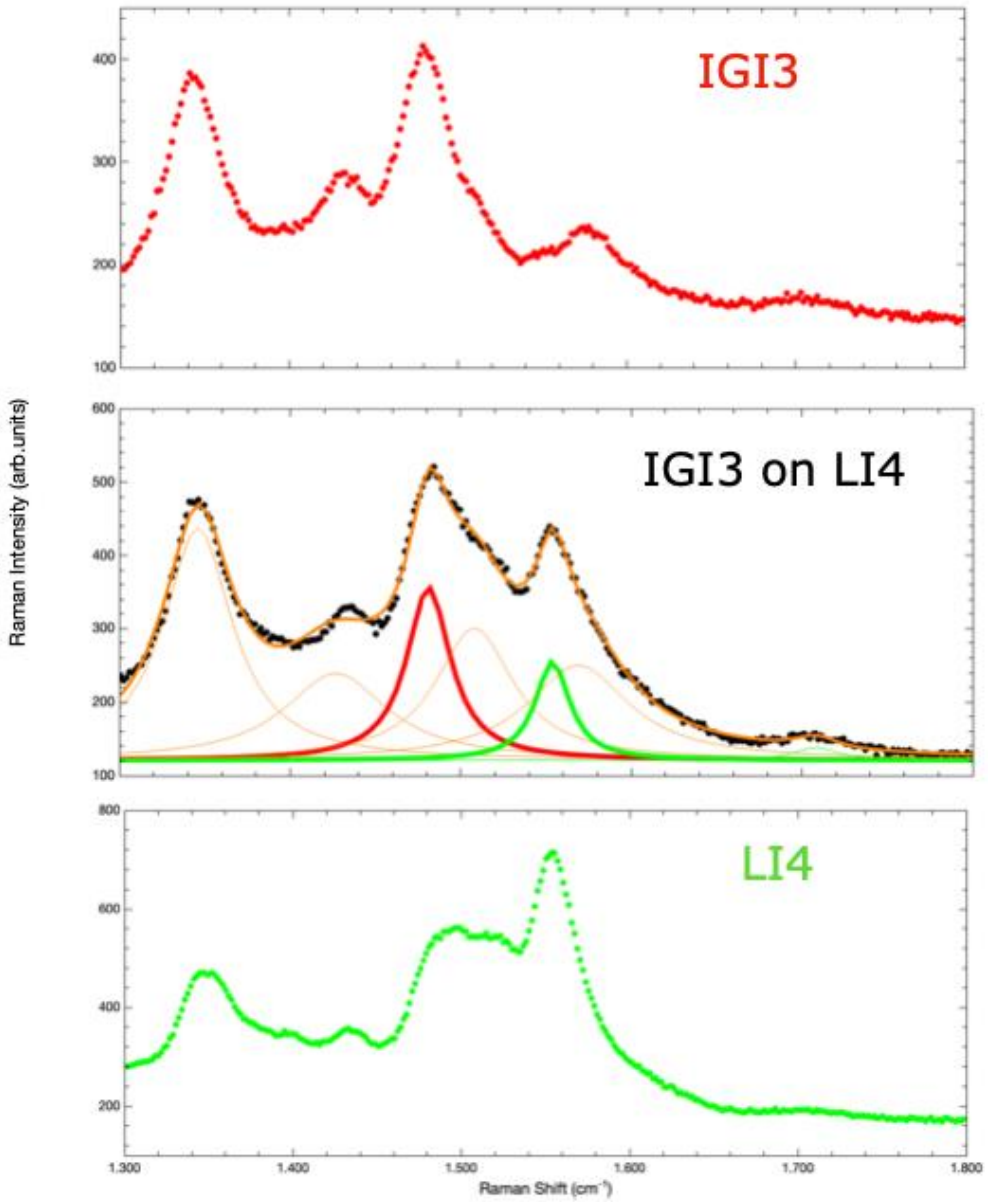
Gall-nuts - 12,2 g
Logwood - 6,1 g
Iron (II) Sulphate - 6,1 g
Copper(II) Sulphate - 1,5 g
Gum Arabic - 4,6 g
Sugar - 1,5 g
Water - 293,7 ml

RESULTS

Micro-Raman Spectroscopy

Mock-ups

Iron-gall ink$_{(3)}$ on
Logwood ink$_{(4)}$



Bench-top set-up
Mapping analysis

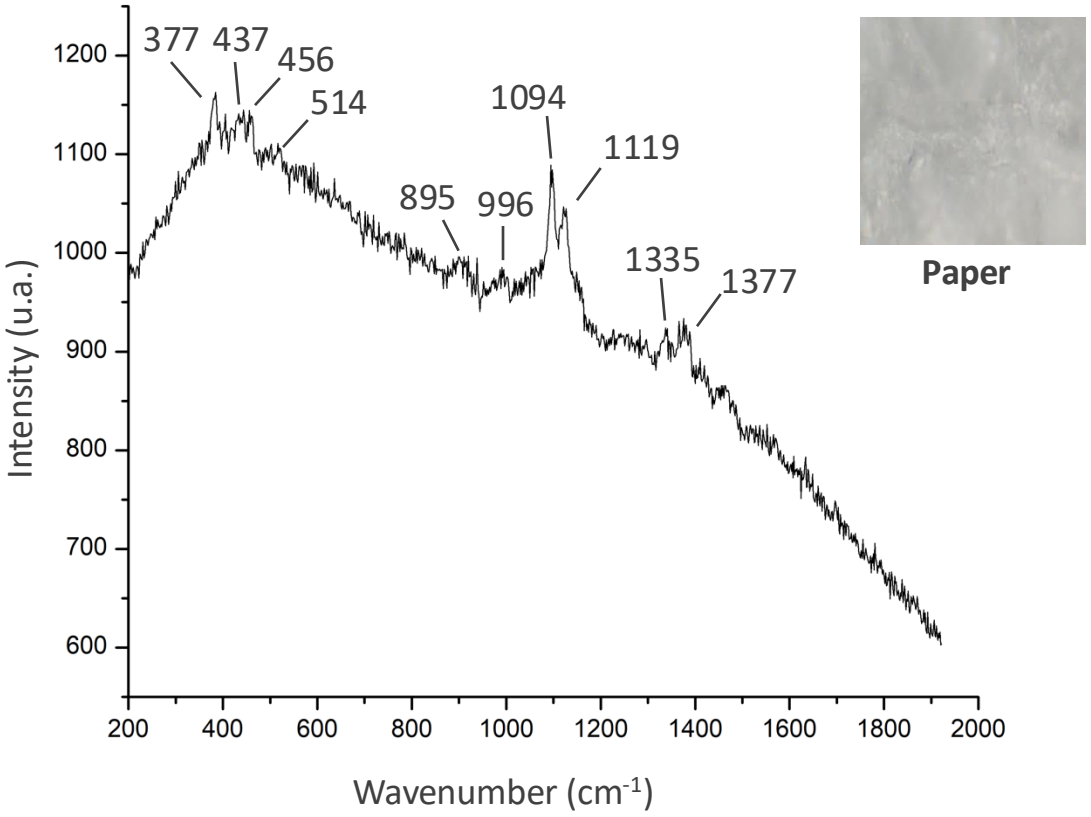
Laser excitation line: 638 nm line
Objective lens: 10X
Laser power: 1.32×10^4 W/cm^2
Integration time: 15 s
Accumulation: 30
Map size: 214 µm x 203 µm,
Step = 23,8 µm Y e 22,7 µm X
Spectra acquired: 120

Micro-Raman Spectroscopy

Mock-ups

Bench-top set-up - Point analysis

Laser excitation line: 638 nm line - Objective lens: 50X long working distance - Laser power: 5.23×10^4 W/cm^2 - Integration time: 10 s - Accumulation: 10



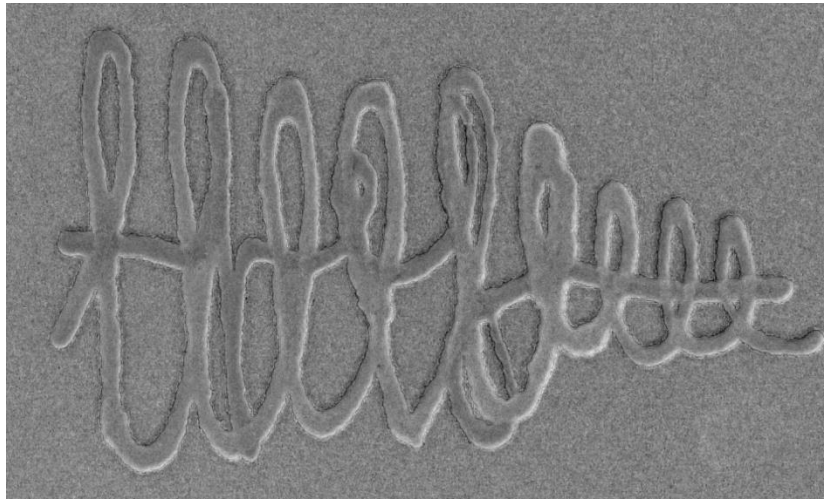
Hypercolorimetric Multispectral Imaging

Mock-ups

Iron-gall ink$_{(3)}$ on Logwood ink$_{(4)}$

Image of Principal Component Analysis – PCA2:

Image of Principal Component Analysis – PCA3:

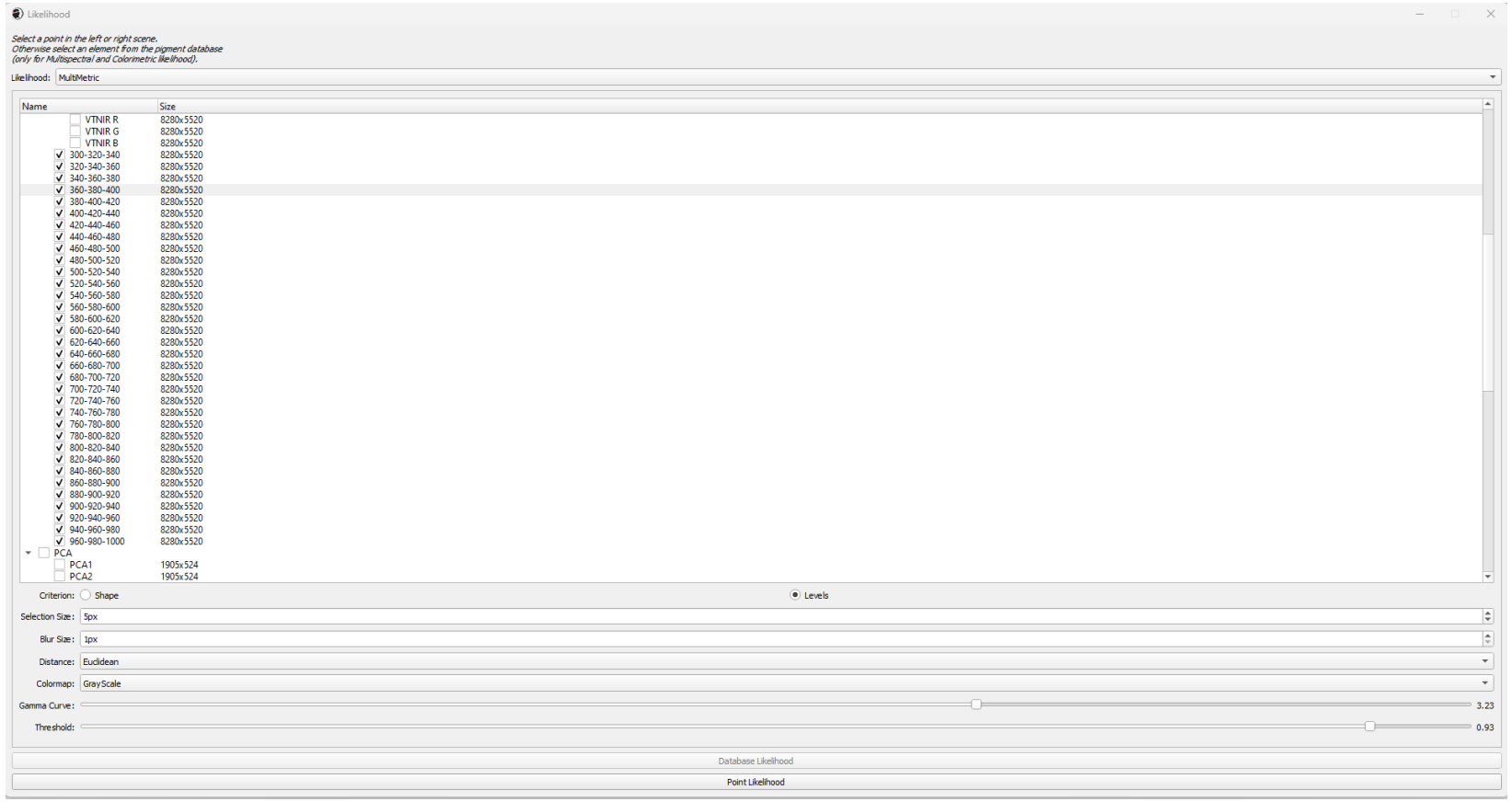


Canali in Ingresso	300-320-340, 320-340-360, 340-360-380, 360-380-400, 380-400-420, 400-420-440, 420-440-460, 440-460-480, 460-480-500, 480-500-520, 500-520-540, 520-540-560, 540-560-580, 560-580-600, 580-600-620, 600-620-640, 620-640-660, 640-660-680, 660-680-700, 680-700-720, 700-720-740, 720-740-760, 740-760-780, 760-780-800, 780-800-820, 800-820-840, 820-840-860, 840-860-880, 860-880-900, 880-900-920, 900-920-940, 920-940-960, 940-960-980, 960-980-1000
Coefficienti PCA	1.9746, 0.0113, 0.0055, 0.0021, 0.0017, 0.0015, 0.0010, 0.0008, 0.0006, 0.0004, 0.0003, 0.0002, 0.0002, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0001, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000

Hypercolorimetric Multispectral Imaging
Mock-ups

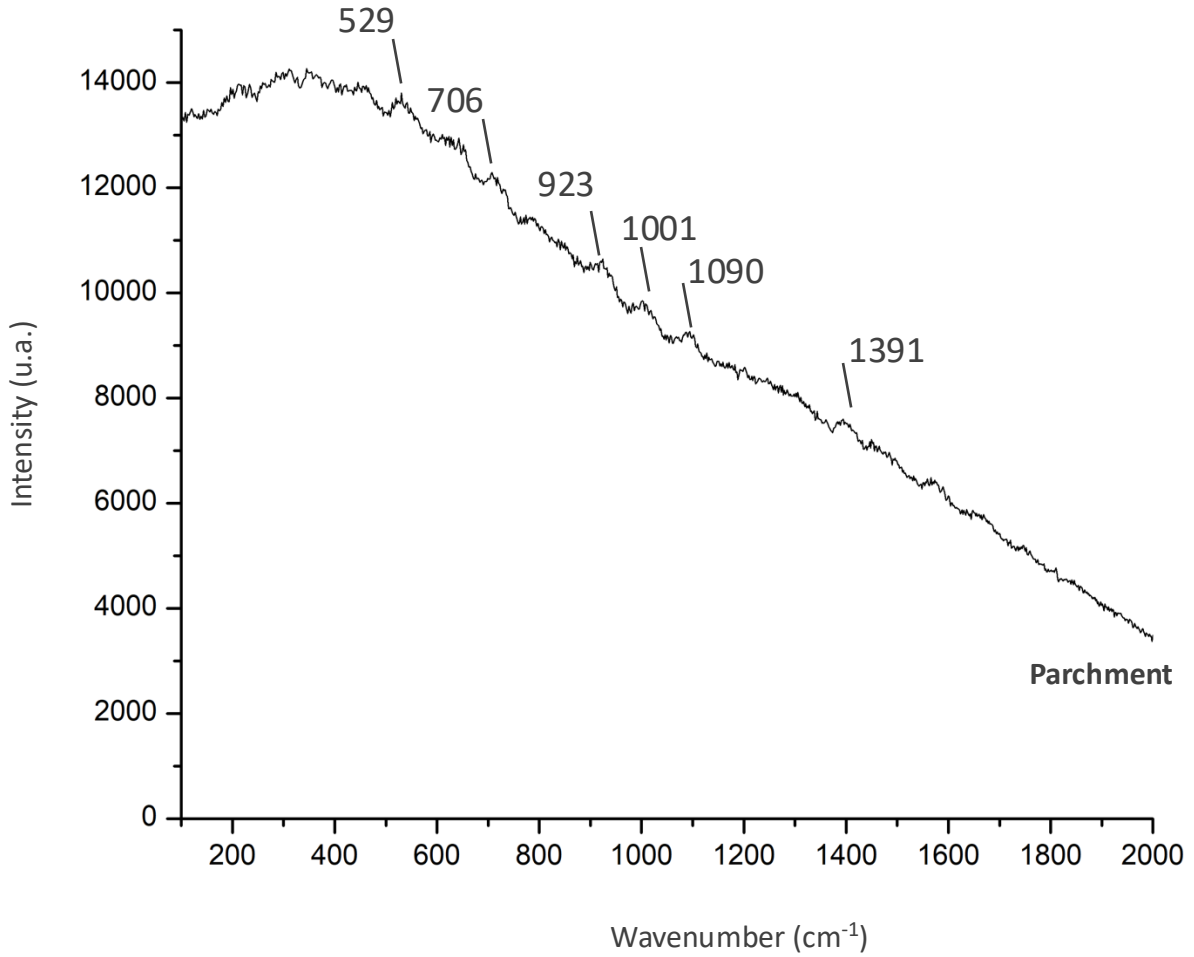
Logwood ink$_{(1)}$ on Logwood ink$_{(4)}$

Map of multispectral similarity - Logwood ink (4):



RESULTS

Ms.Ald.52, 11th century
Collection: Manoscritti Aldini – University Library of Pavia

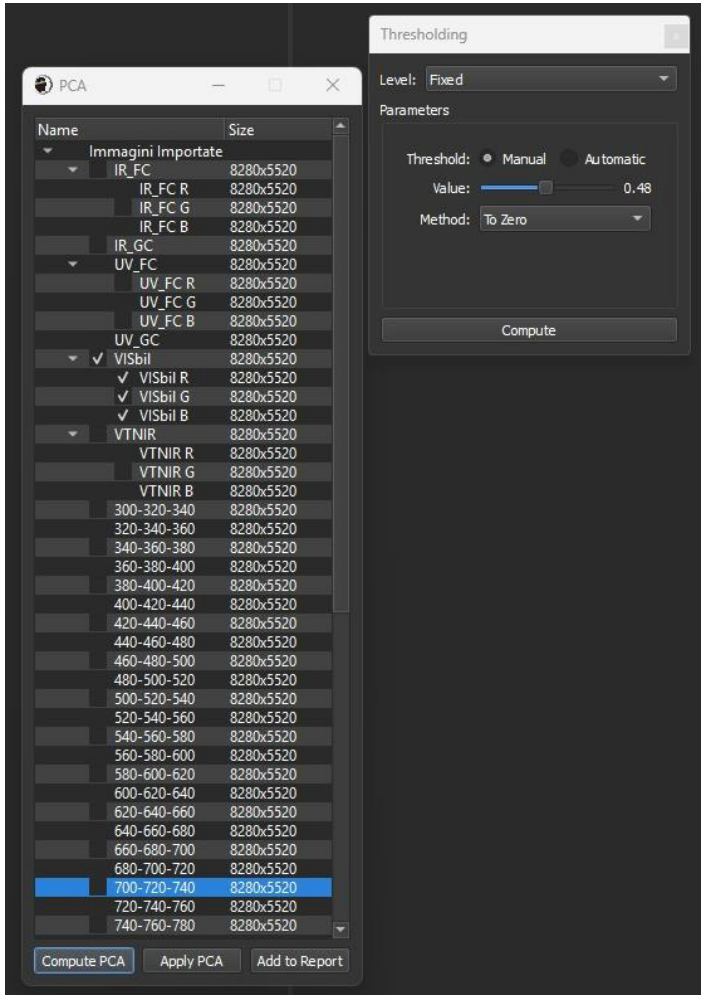


Portable set-up:
Laser excitation line: 785 nm line -
Laser power: 4.04×10^5 W/cm^2 -
Integration time: 10 s -
Accumulation: 1

Ms.Ald.52, 11th century
Collection: Manoscritti Aldini – University Library of Pavia

Image of Principal Component Analysis – PCA2:

Canali in Ingresso	VISbil R, VISbil G, VISbil B
Coefficienti PCA	0.0797, 0.0012, 0.0004



Colorimetry:

RGB="96,84,68"
Lab="36.99,5.19,13.22"

RGB="137,96,74"
Lab="46.70,20.76,23.87"

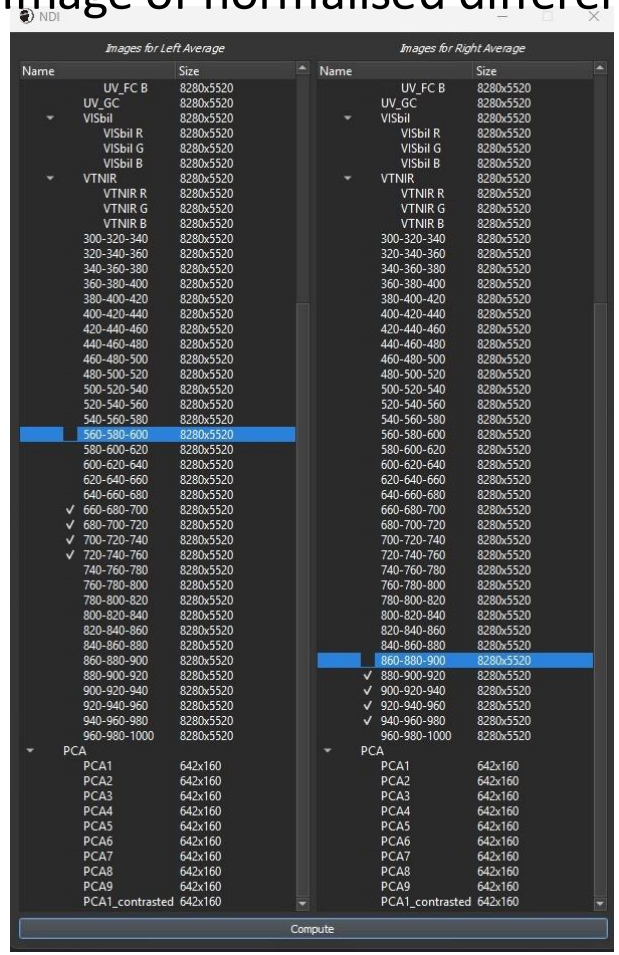
Hypercolorimetric Multispectral Imaging

Manuscript

Ms.Tic.67, 18th-19th century, Autograph section by Siro Comi

Collection: Manoscritti Ticinesi – Biblioteca Universitaria di Pavia

Image of normalised difference indexes:



Colorimetry:

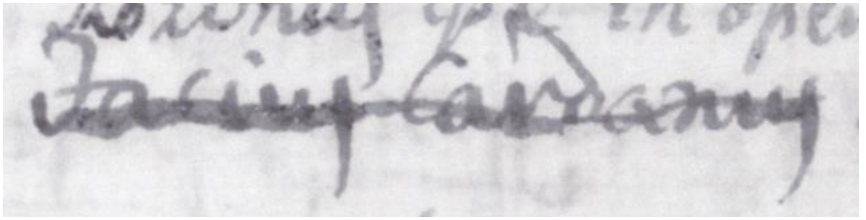
RGB="64,62,62"
Lab="25.76,1.29,0.66"

RGB="69,65,62"
Lab="27.44,1.59,3.26"

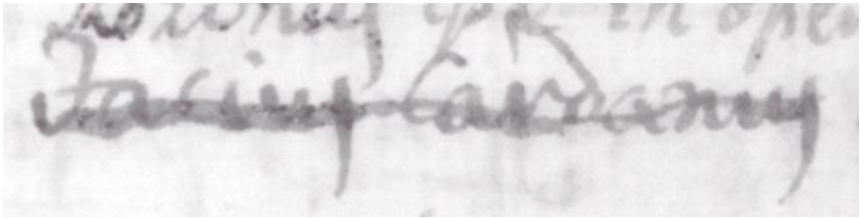
Image of Principal Component Analysis – PCA1:

Canali in Ingresso	PFCL IR1 R, PFCL IR1 G, PFCL IR1 B, PFCL IR2 R, PFCL IR2 G, PFCL IR2 B, PFCL IR3 R, PFCL IR3 G, PFCL IR3 B
Coefficienti PCA	0.1026, 0.0005, 0.0001, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000, 0.0000

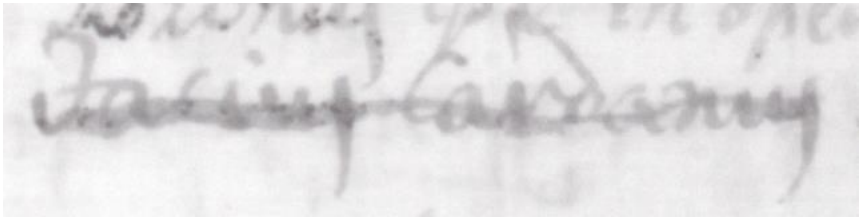
IR1-760-1000 nm



IR2-850-1000 nm



IR3-930-1000 nm



MICRO-RAMAN – SETTINGS

XploRA PLUS – HORIBA Scientific and Analytical Instruments
Software: LabSpec6 - HORIBA Scientific and Analytical Instruments

i-Raman Plus 785S – Metrohm
Software: BWSpec® - B&W Tek, Metrohm

MICRO-RAMAN – SETTINGS

Bench-top instrumentation: XploRA PLUS confocal microRaman System Instrument -

Olympus Microscope BX41 and Raman module

Laser excitation lines – Power:

- 532 nm - 100 mW
- 638 nm - 100 mW
- 785 nm - 300 mW

Portable instrumentation: Portable Raman spectrometer i-Raman Plus -
BAC151C compact Raman microscope compatible with B&W Tek portable Raman system

Laser excitation lines – Power:

- 785 nm - 300 mW

Objective lens:

- 5X
- 10X
- 50X

HMI – SETTINGS

Software:

- CapturePick® for the image acquisition
- SpectraPick ®for the image calibration processing
- PickViewer® for the image post-processing, provides image processing tools

Several kinds of analyses are possible with PickViewer, such as:
add and integrate any other imaging data (fluorescence, X-ray, thermal etc.); multichannel images viewer; any pixel colorimetry and spectral reflectance read-out; mapping by colour, spectra, arbitrary channels; principal components analysis; contrast enhancement through digital imaging processing algorithms; neural network based clustering; colour and spectral signature database; two ways mapping by database entry; any channel to RGB false colours visualization; channels math, indexes and normalised contrast.

HMI – SETTINGS

The calibration procedure follows three steps:

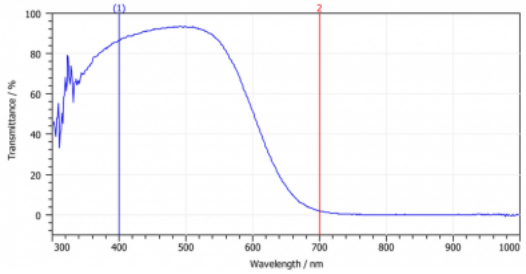
1) white patches analysis to achieve correct white balance and even exposure across all the subject;
2) running an Artificial Intelligence based optimizer that finds the mathematical function to translate camera digital values of the colour-checker into radiometric and colorimetric measurements;
3) applying the calibration to the acquired images and producing 34 monochromatic images, containing the spectral reflectance at 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, 640, 660, 680, 700, 720, 740, 760, 780, 800, 820, 840, 860, 880, 900, 920, 940, 960, 980 nm.
   The achieved precision across the whole 36 megapixel image is better than 95% on the spectral reflectance images and colour error less than CIE2000 $\Delta E = 2$ for the colour image.

HMI – SETTINGS

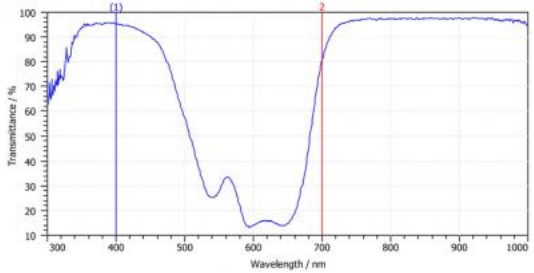
The radiometric references consist in a number of white patches (to sample any uneven lighting) surrounding the subject and of a 36 patches colour-checker.
These references are built using colour's samples out of the NCS – Natural Colour Systemic®© catalog and their spectral reflectances are accurately measured in Profilocolore lab in the range of 220 to 1050 nm at 0.7 nm accuracy (Instrument System Spectroradiometer CAS 140 CT and dark room).

Optical filters:

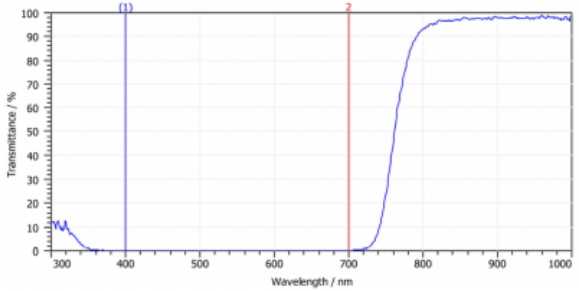
PFCL-A_77x2



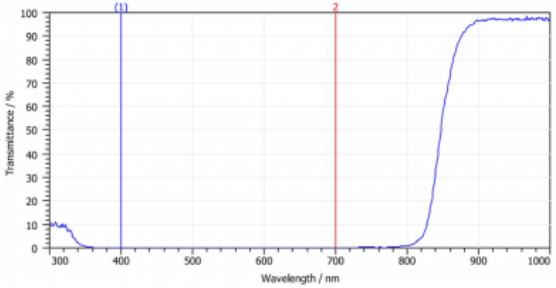
PFCL-B_77x2



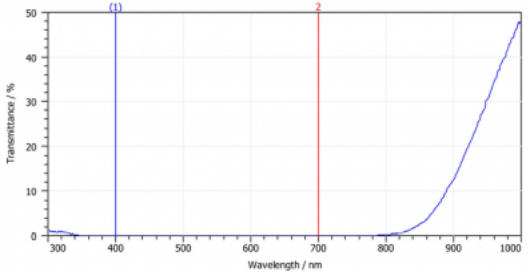
PFCL-IR1-760_77x2



PFCL-IR2-850_77x2



PFCL-IR3-930_77x2



HMI – SETTINGS

Hypercolorimetric Multispectral Imaging (HMI), Profilocolore srl (Roma, Italia):
the system consists of a modified camera, a set of optical filters with different spectral transmittances, a checkerboard target for colour calibration and white balance of the images, white markers for balancing the exposure, and sophisticated software for image acquisition, calibration and processing.

Nikon Z8 mirrorless 45.7 megapixel digital camera (Minato, Tokyo, Japan), modified under a Nikon-Profilocolore project, to reach the full spectral range of sensitivity of silicon sensors from about 300 nm to 1000 nm.
AF-S 50mm 1:1.4 G Nikkor lens (Minato, Tokyo, Japan) mounted with FTZ adapter.

Lighting was obtained by Two Godoz TT600 xenon flashes (T = 5600 K) (Shenzhen, Guangdong, China) controlled by the Godox X2T-N transmitter and used after removing their front plastic lenses, thus allowing also the UV wavelength to be emitted.
Philips infrared incandescent lamps (PAR38 IR 175W E27 240V Red 1CT/12).
Visible images of fluorescence induced by ultraviolet light are acquired using Madatec CR230B-HP UV LED lamps (365 nm UV source with a power of about 3 W) (Pessano con Bornago, Italy).

XRF – SETTINGS

Portable XRF spectrometer ELIO by Bruker XGLab s.r.l. (Milan, Italy)
Low-power X-ray tube with Rh anode and 1.2 mm of analytical spot.

Working conditions

- Single point method, 90 s, 40 kV, 100 µA - 2048 acquisition channels
  Net area counts of the peak (Kα) normalized to net area counts of the peak of Rh-Lα.
- Mapping method - 40 kV, 100 µA - 2048 acquisition channels
  Movement setup: Rows = 40, Columns = 40, Spectrum time = 1 s, Rows distance = 750 µm, Columns distance = 750 µm,
  Area = 3 cm x 3 cm

Software ELIO 1.60.57 by Bruker XGLab s.r.l. (Milan, Italy)