School of Food Science and Nutrition
FACULTY OF MATHEMATICS AND PHYSICAL SCIENCES
UNIVERSITY OF LEEDS

Working at a Synchrotron

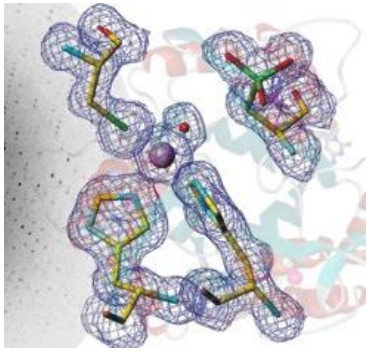
Part 2

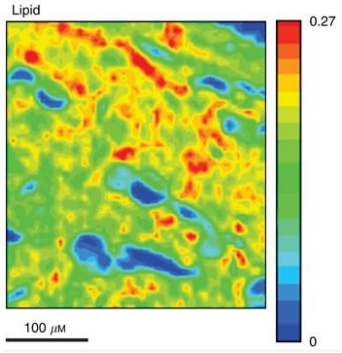
Michael Rappolt

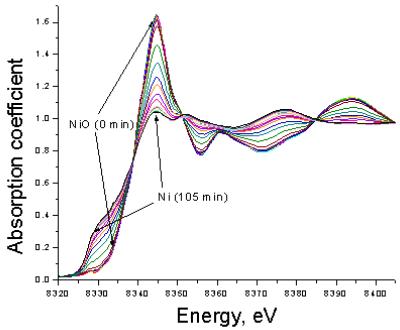
4. BSc Student Lecture: Physics with Introduction to Modern Physics
5th of June 2014, Faculty of Electrical Engineering, University of Ljubljana

Applied Techniques at ELETTRA for Biology and Medicine

- Diffraction: Protein crystallography
- Small Angle X-ray Scattering
- Absorption: XAFS
- Infrared Spectroscopy & Microscopy
- TwinMic
- Imaging: Tomography & Mammography







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Beamlines at ELETTRA: XAFS

*X-ray Absorption Fine Structure

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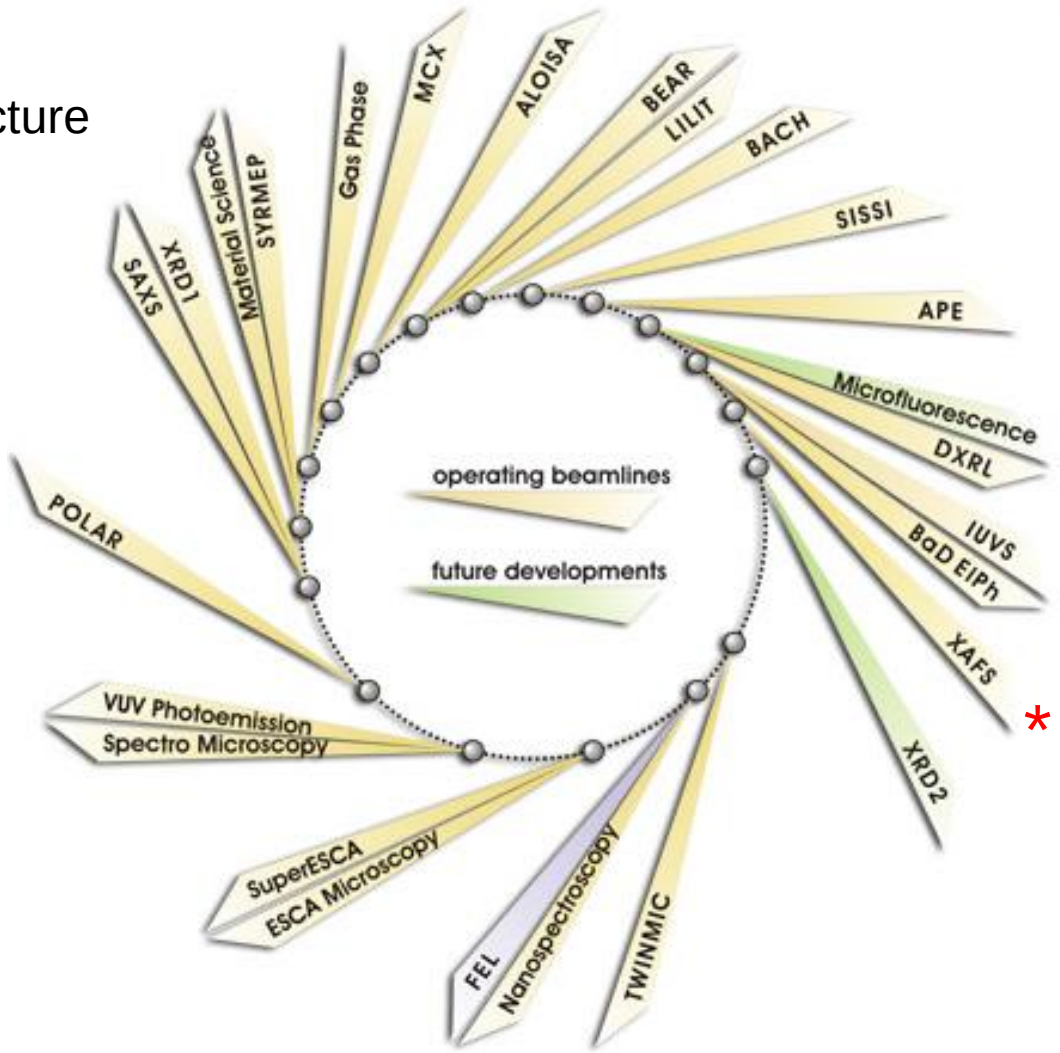

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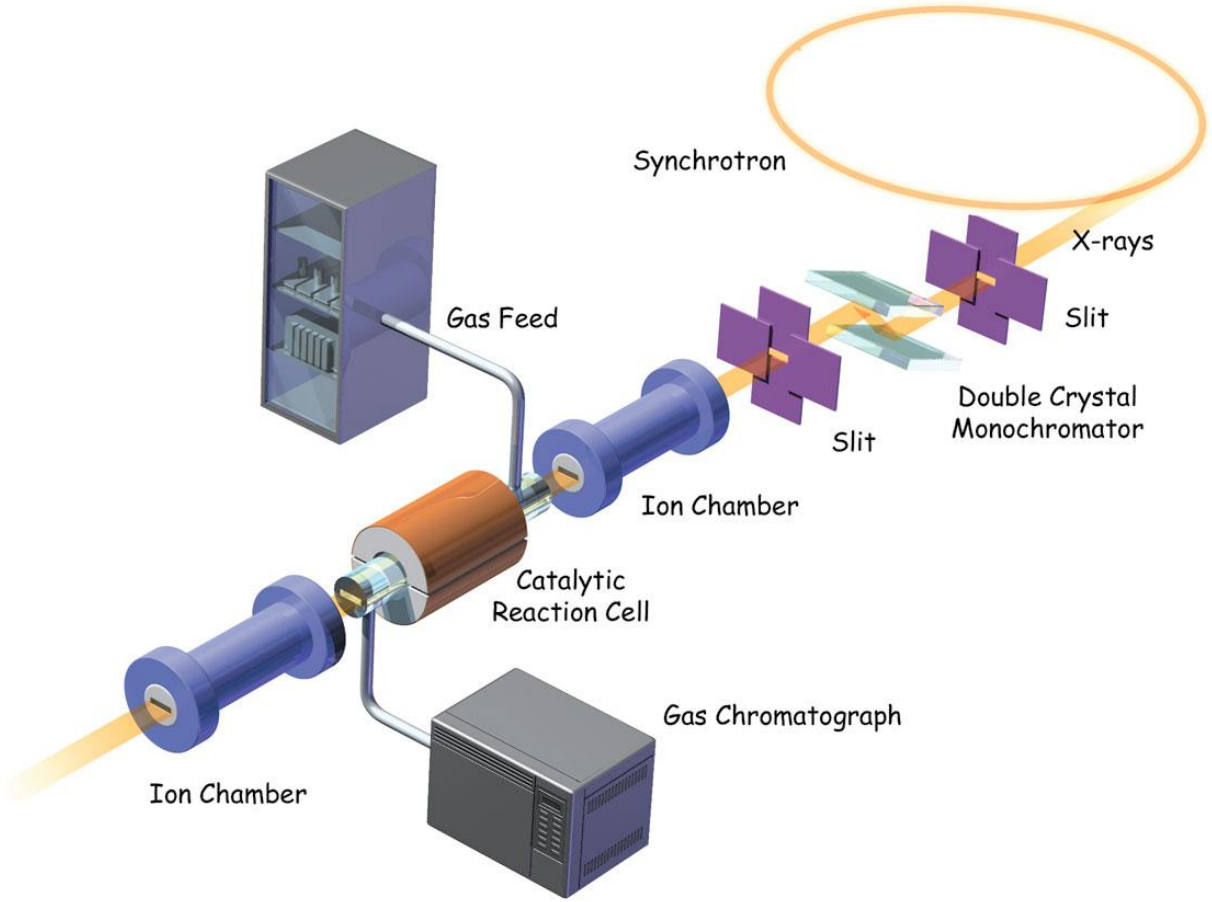
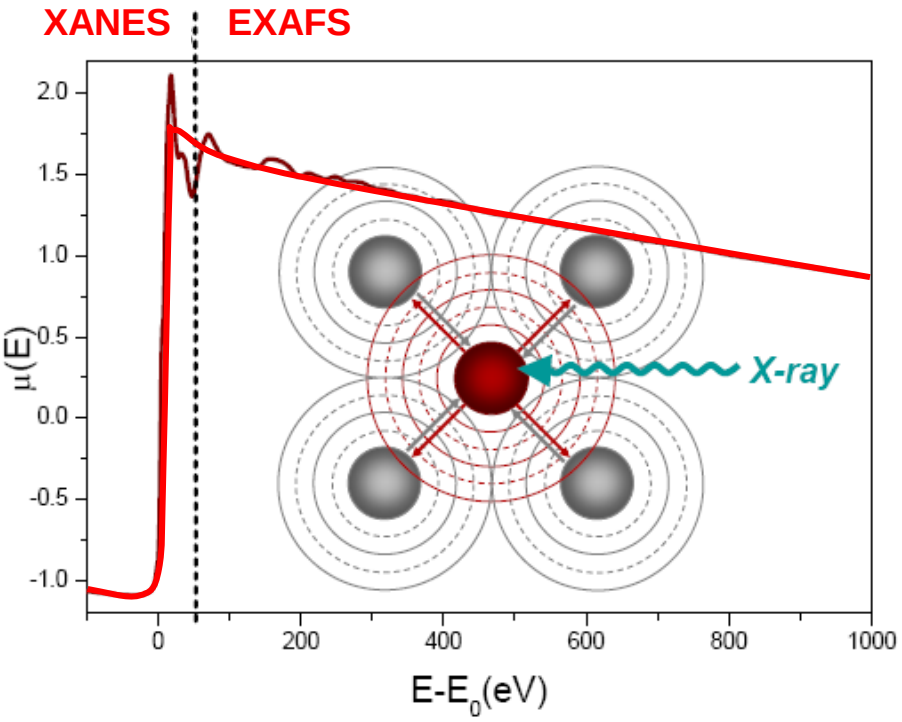
Scheme of a X-ray XAFS Beamline

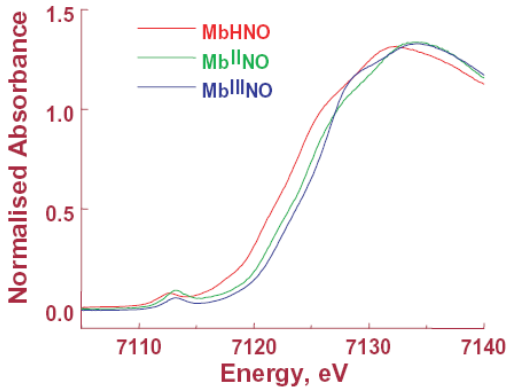


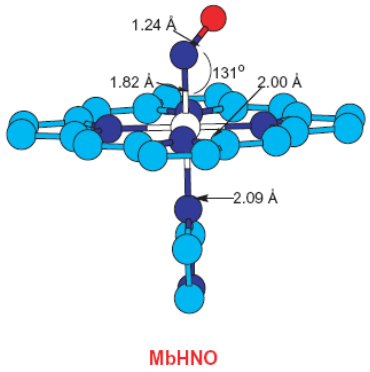
How X-ray Absorption Spectroscopy Works

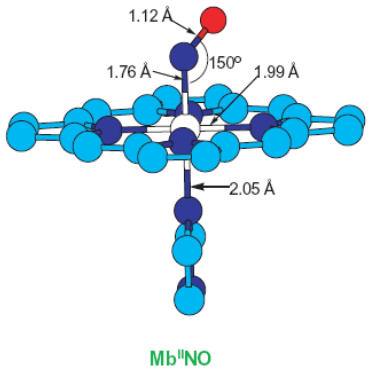


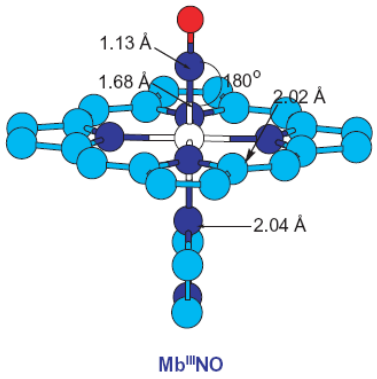
X-ray absorption fine structure is the modulation of the X-ray absorption coefficient (μ) at energies near and above an x-ray absorption edge

XAS Spectroscopy on Heme Proteins









The Fe K-edge XANES of MbHNO, MbIINO and MbIIINO
and the XAFS-derived structures of the active sites of the metalloproteins (Mb = myoglobin).

Rich, A.M., Armstrong, R.S., Ellis, P.J., and Lay, P.A. (1998) *J. Am. Chem. Soc.* **120**, 10827-10836

Immoos, C.E., Sulc, F., Farmer, P.J., Czarnecki, K., Bocian, D.F., Levina, A., Aitken, J.B., Armstrong, R.S., and Lay, P.A. (2005) *J. Am. Chem. Soc.* **127**, 814-815

Beamlines at ELETTRA: SISSI

* Infrared Spectroscopy & Microscopy

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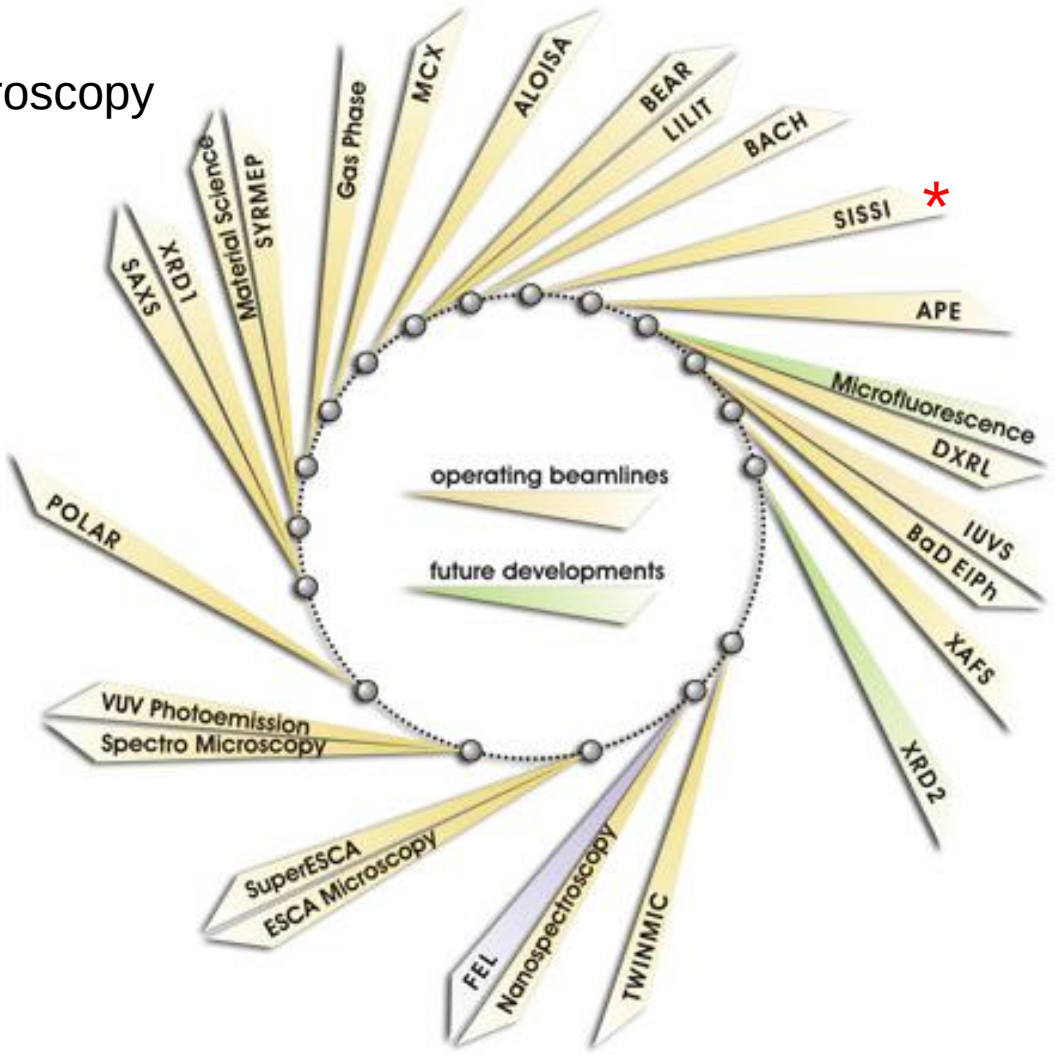
Luca Benevoli



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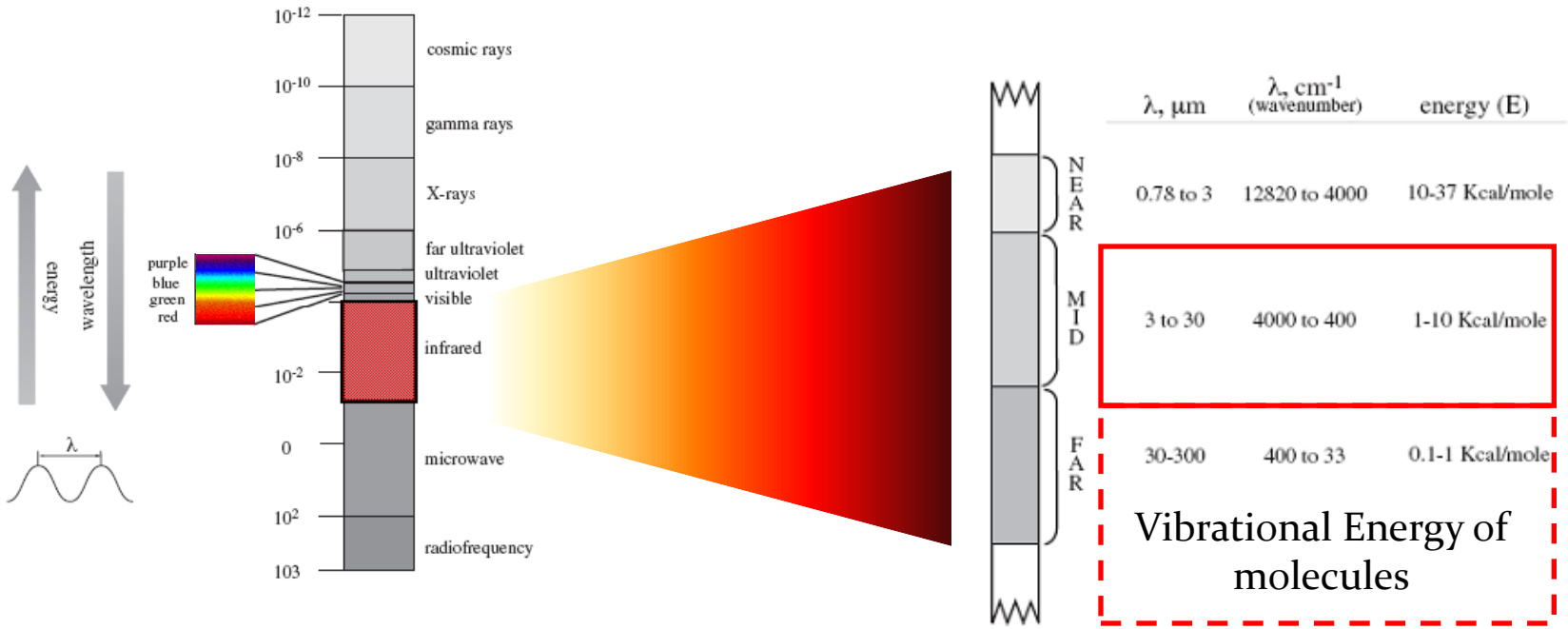
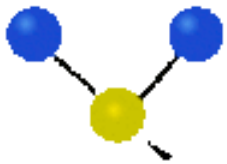
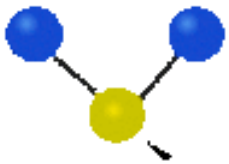
The Infra-Red Region of Light



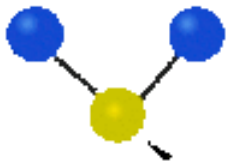
Symmetrical Stretching



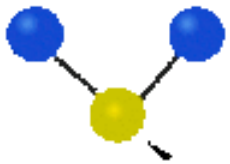
Antisymmetrical Stretching



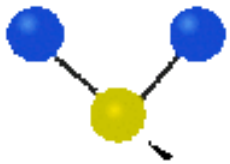
Scissoring



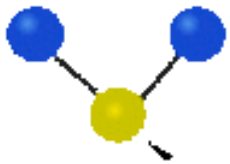
Rocking



Wagging



Twisting



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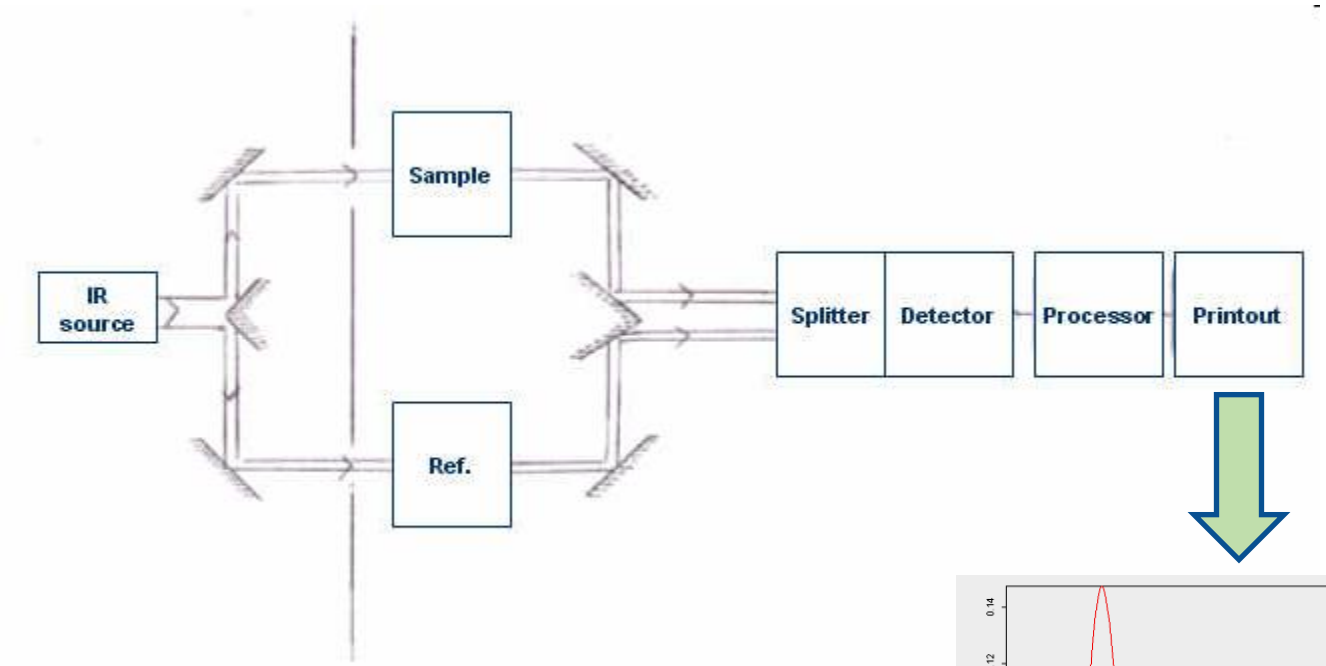
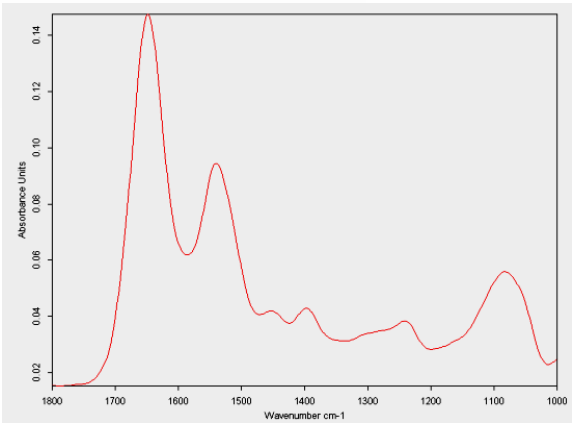
General Set-Up for IR-Spectrocopy





1st Branch *(CNR-INFM)*
Solid State Physics
High Pressures
Time-resolved spectroscopy

2nd Branch *(Elettra)*
Biophysics/Biochemistry
Spectroscopy and Imaging

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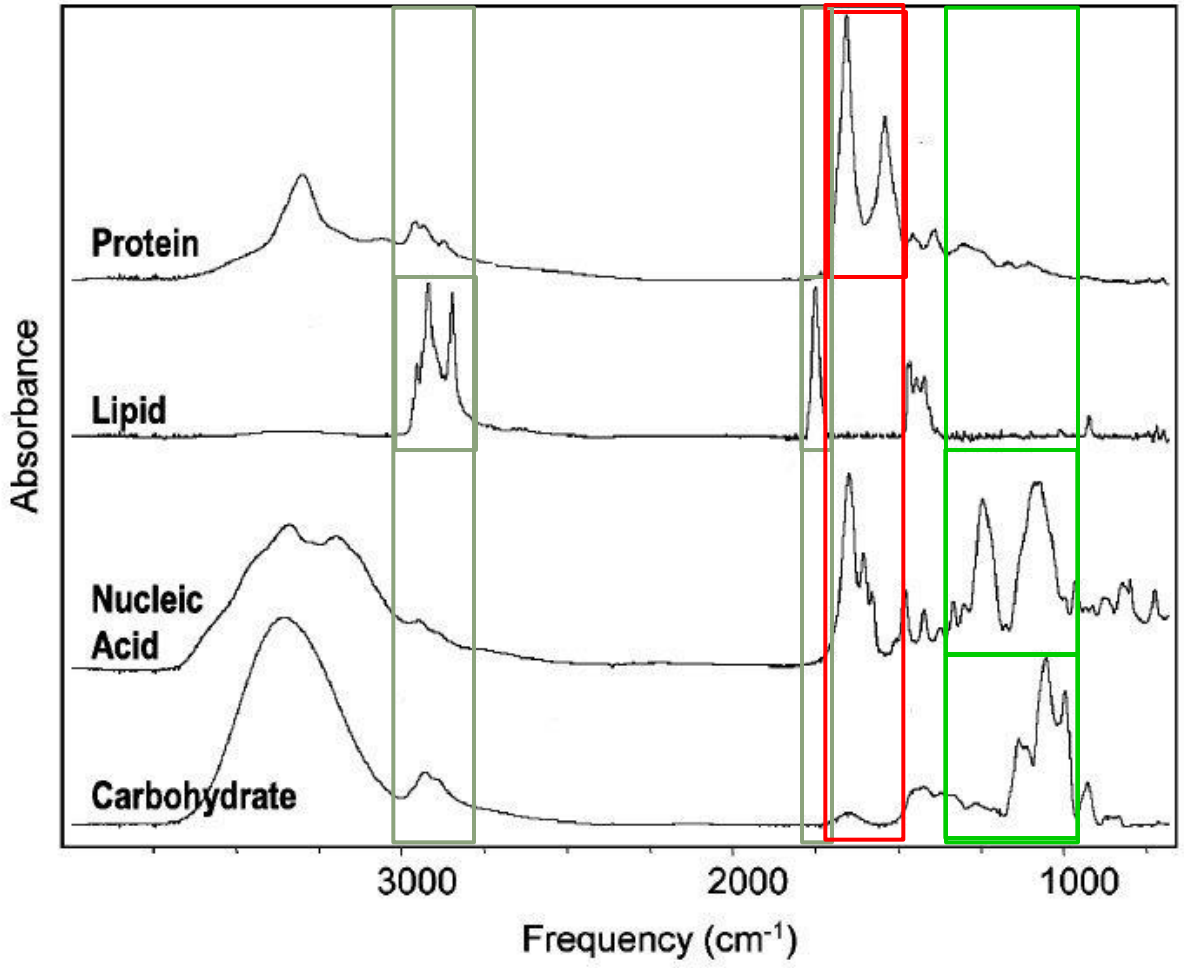
Understanding IR-Bands



1250-1200
Saturated Acyl chains
1244 : ν_{as} PO^{2-} RNA
1705-1660 : ν C=O RNA
2950-2960 : ν_{as} (CH_3)
1230 : ν_{as} PO^{2-} DNA
1660-1650 : ν C=O DNA
2915-2925 : ν_{as} (CH_2)
1660-1650 : δ C=O DNA
2867-2870 : ν_s (CH_3)
1089 : ν_s PO^{2-} DNA
2845-2855 : ν_s (CH_2) C=O
1084 : ν PO^{2-} RNA
1700-1600: Amide I
80% ν C=O + 10% ν C-N + 10% δ N-H
DNA and RNA ribose
νC-O
1700-1600: Amide II
1700-1750 : ν (C=O)
40% ν C-N + 60% δ N-H
1200-1000
Unsaturated Acyl chains
≥ 3000 : C-H
1600-1500
Ester Band
Complex network of carbohydrate bands

Adapted from: L. M. Miller, G.D. Smith and G. L. Carr, *Journal of Biological Physics*, 29 (2-3), 219-230, **2003**

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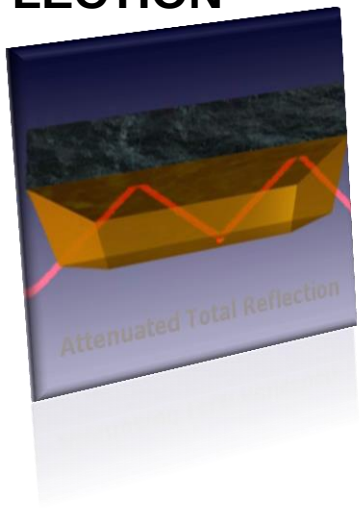
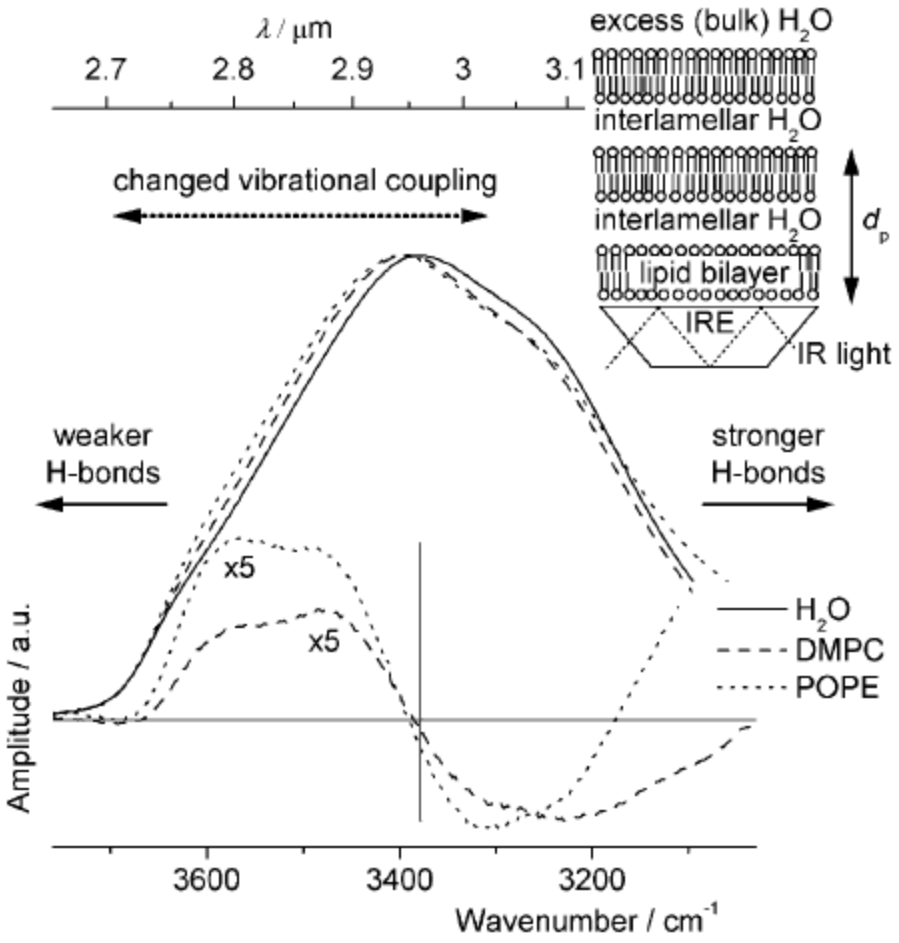
Probing Order of Confined Water: OH-Stretching

ATTENUATED TOTAL REFLECTION



Arsov, Z., Rappolt, M., and Grdadolnik, J. (2009) Weakened Hydrogen Bonds in Water Confined between Lipid Bilayers: The Existence of a Long-Range Attractive Hydration Force. Chem. Phys. Chem. 10, 1438 – 1441.



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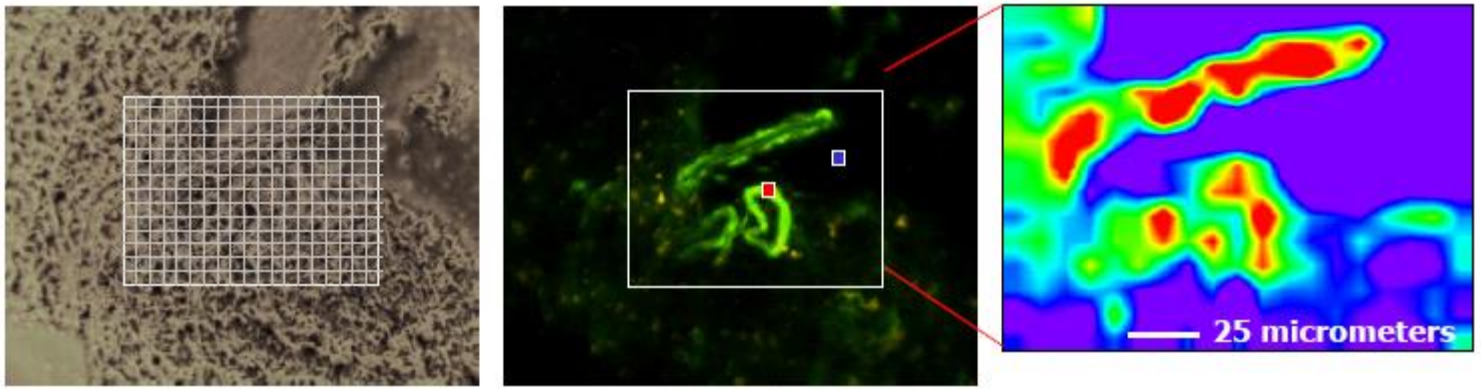
IR-Imaging

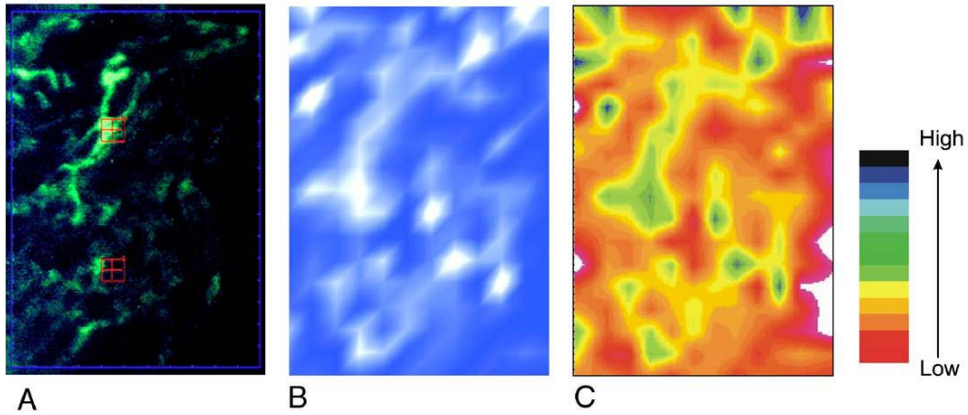
FTIR microspectroscopy provides quite complex chemical information in a simple format (a **chemical image**) that can be easily read also by non-specialist.

Alzheimer's plaques mapped with SR μ-FTIR



L.M. Miller, P.Dumas et al., Rev. Sci. Instr., 73, 1357-70 (2002)

Liver Fibrosis studies by SR μ-FTIR of collagene



K.Z. Liu et al, BBA ,1758, 960-967 (2006)

Beamlines at ELETTRA: TWINMIC

* X-ray microscopy beamline

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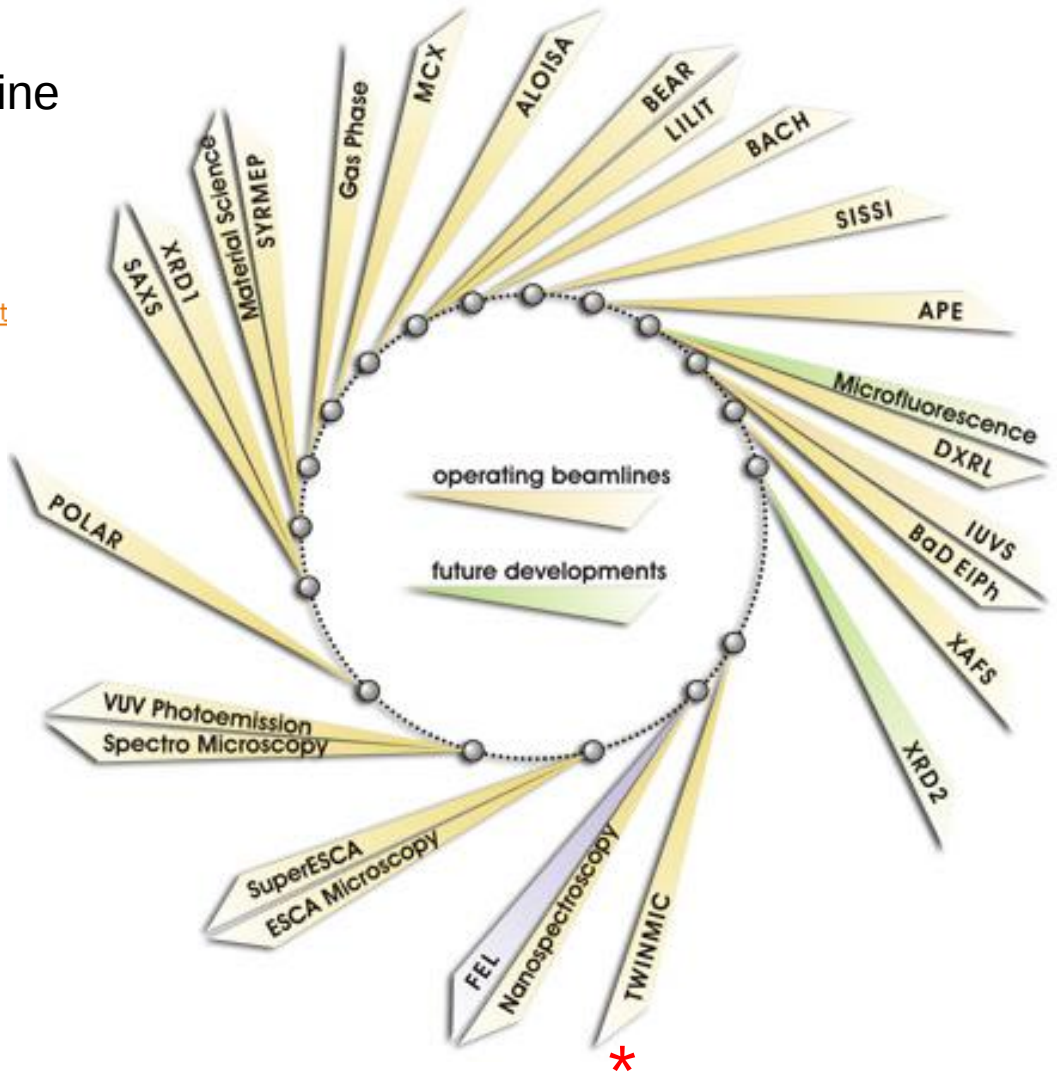

*

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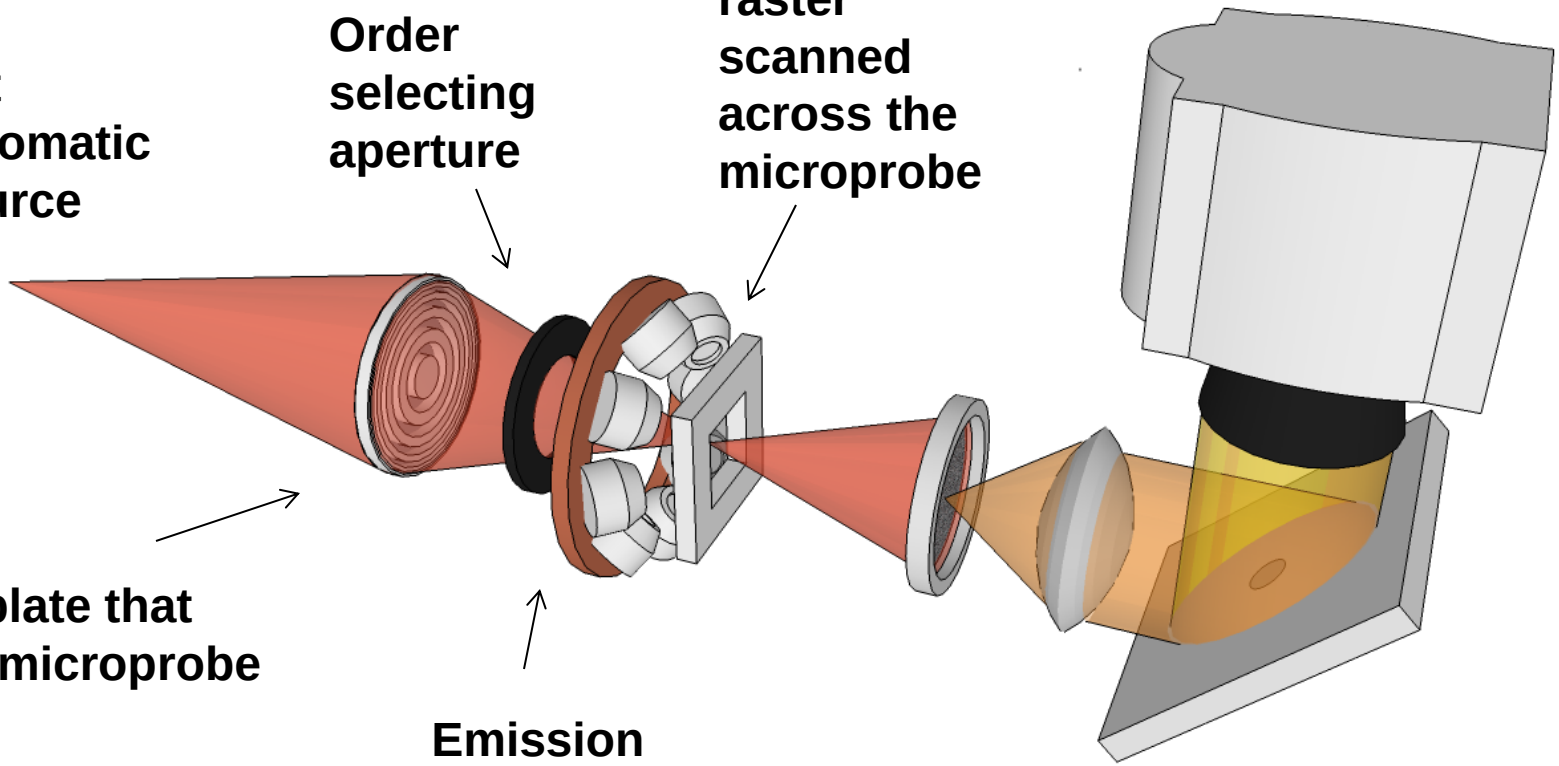
X-ray Microscopy Set-up Combined with Fluorescence

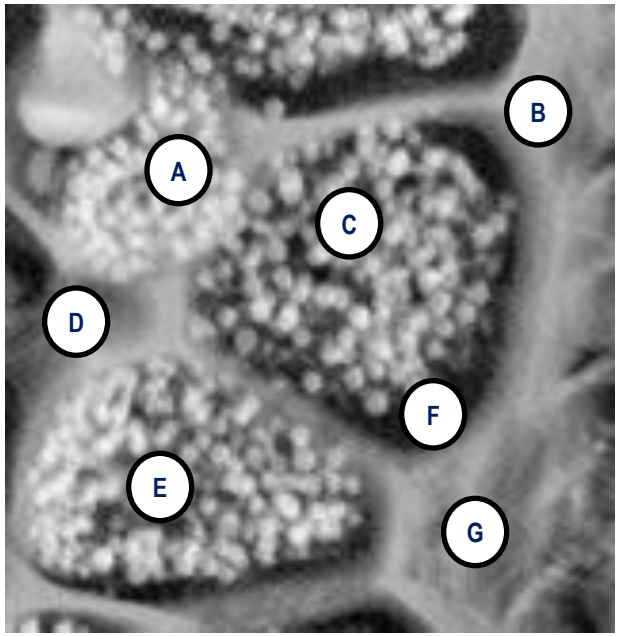


Biogenetics and Food Science: Inside the Wheat

Ivan Kreft,
Fac. of Biotechnology,
University Ljubljana

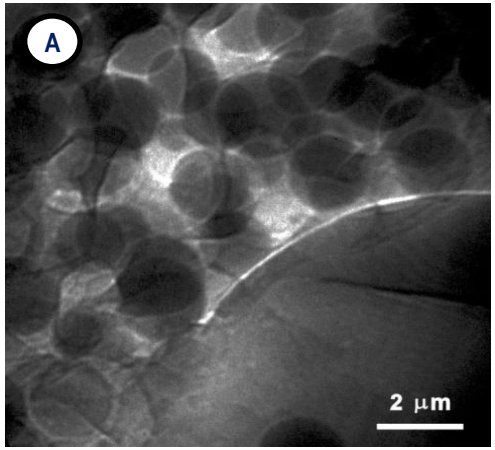
Functionality and toxicity of Zn in wheat and buckwheat analyzed on sub-cellular level

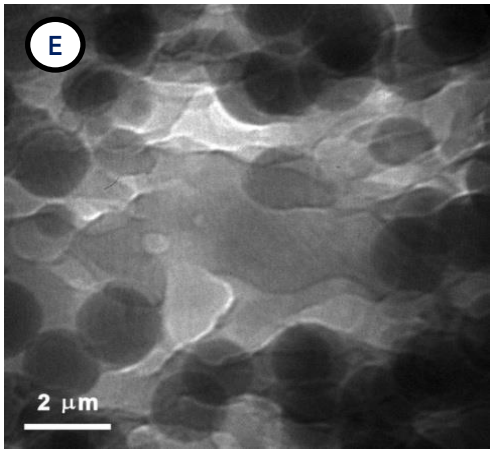
Healthy control wheat



TXM images acquired with a double-frequenced ZP from J. Vila-Comamala (PSI)

TXM, 1s dwell, 740 eV





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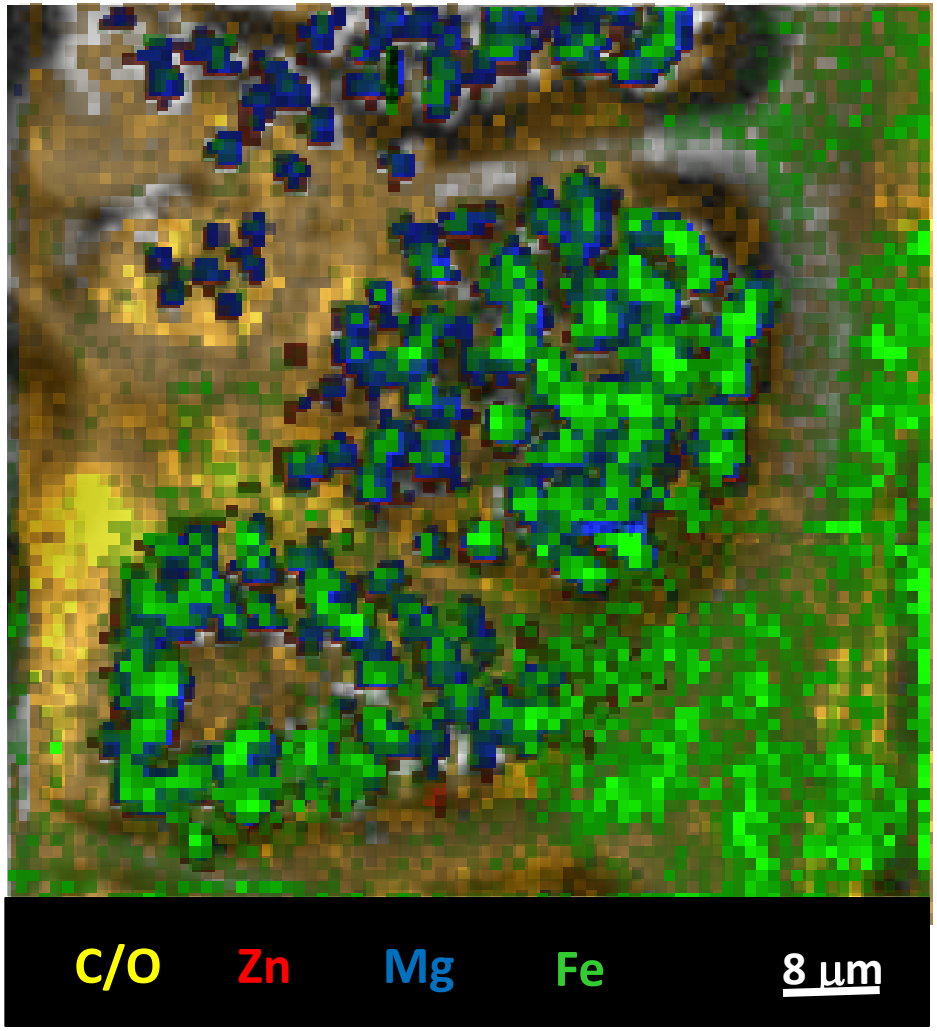
Inside the Wheat: Mic & Fluorescence

Healthy control wheat

E=1686 eV
80 x 80 μm²
80 x 80 px
8 s dwell/ px
1 μm resolution
4 detectors
New STXM optic

Specimen preparation by Paula Pongrac and Katharina Vogel, Uni Ljubljana, Slo



Beamlines at ELETTRA: SYRMEP

* Synchrotron Radiation for Medical Physics

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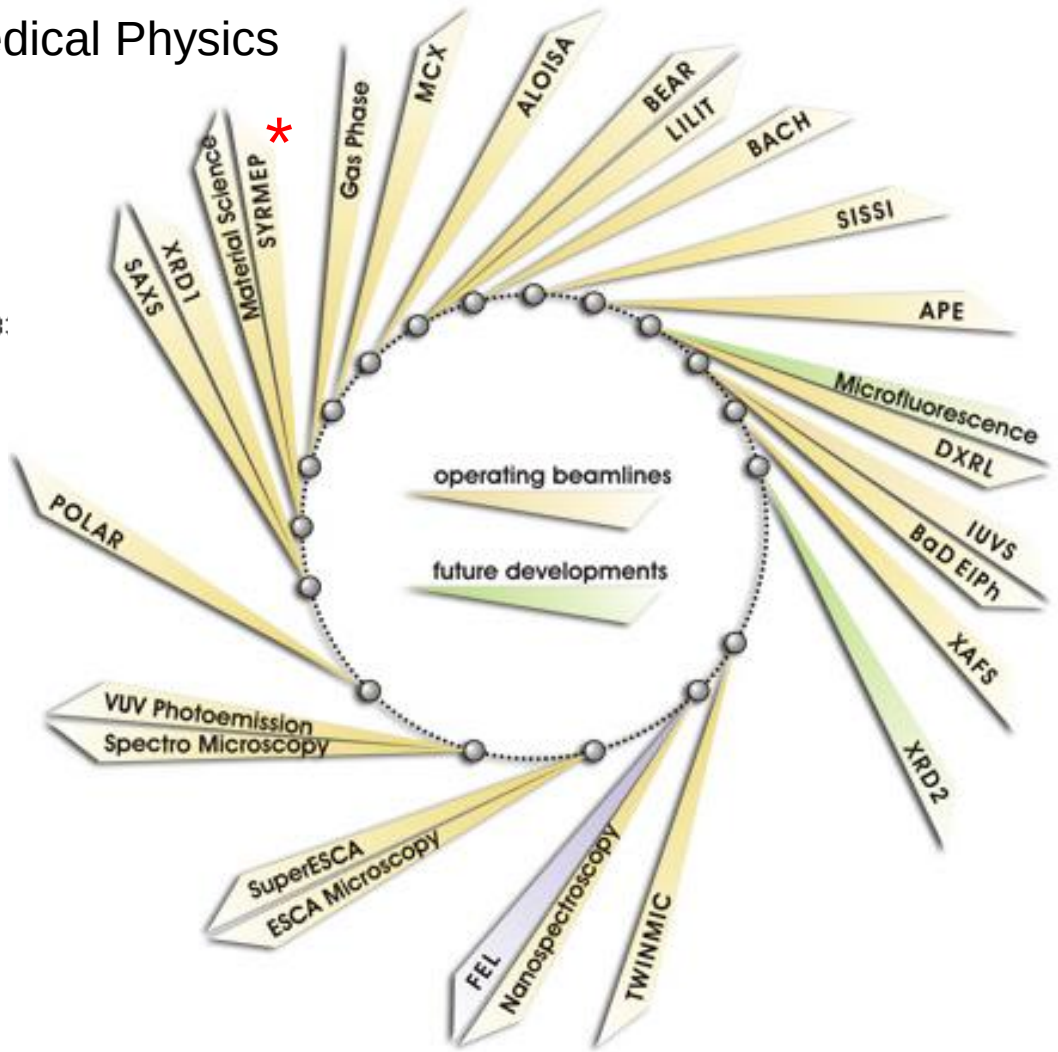

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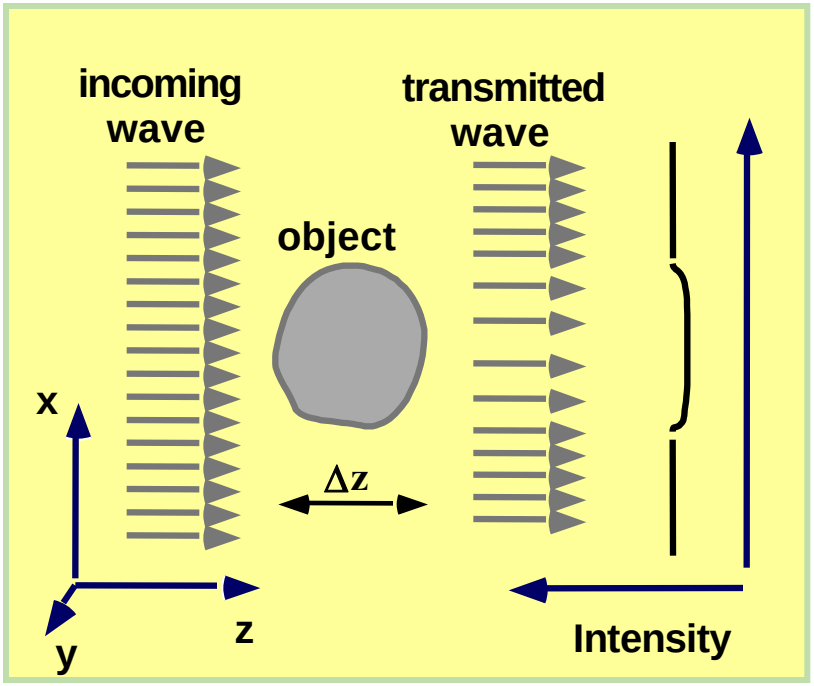
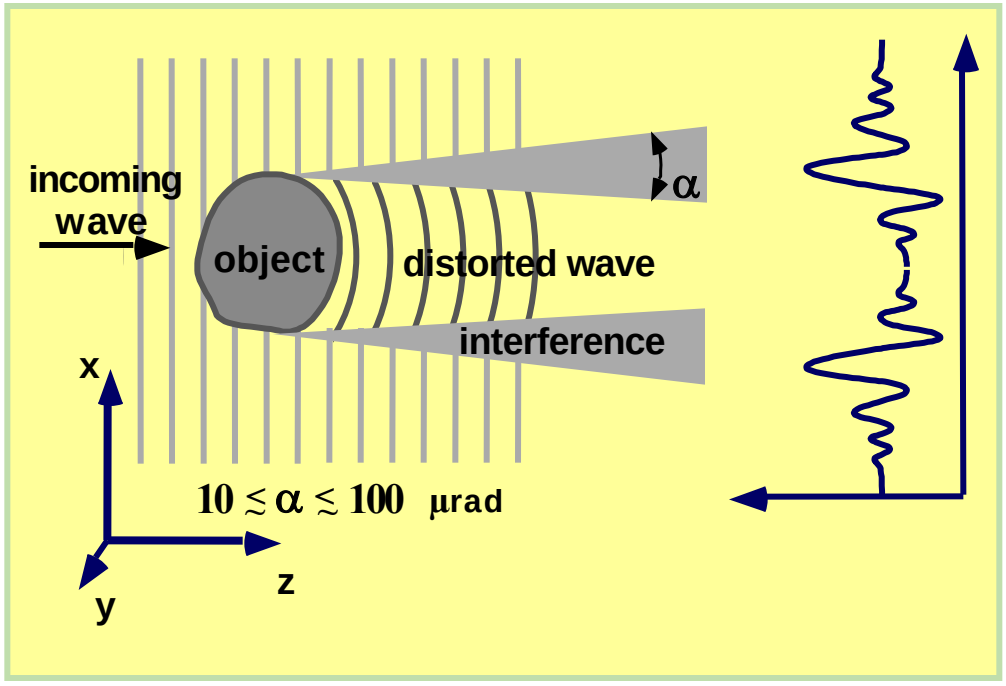
Conventional *vs.* Phase Contrast Imaging



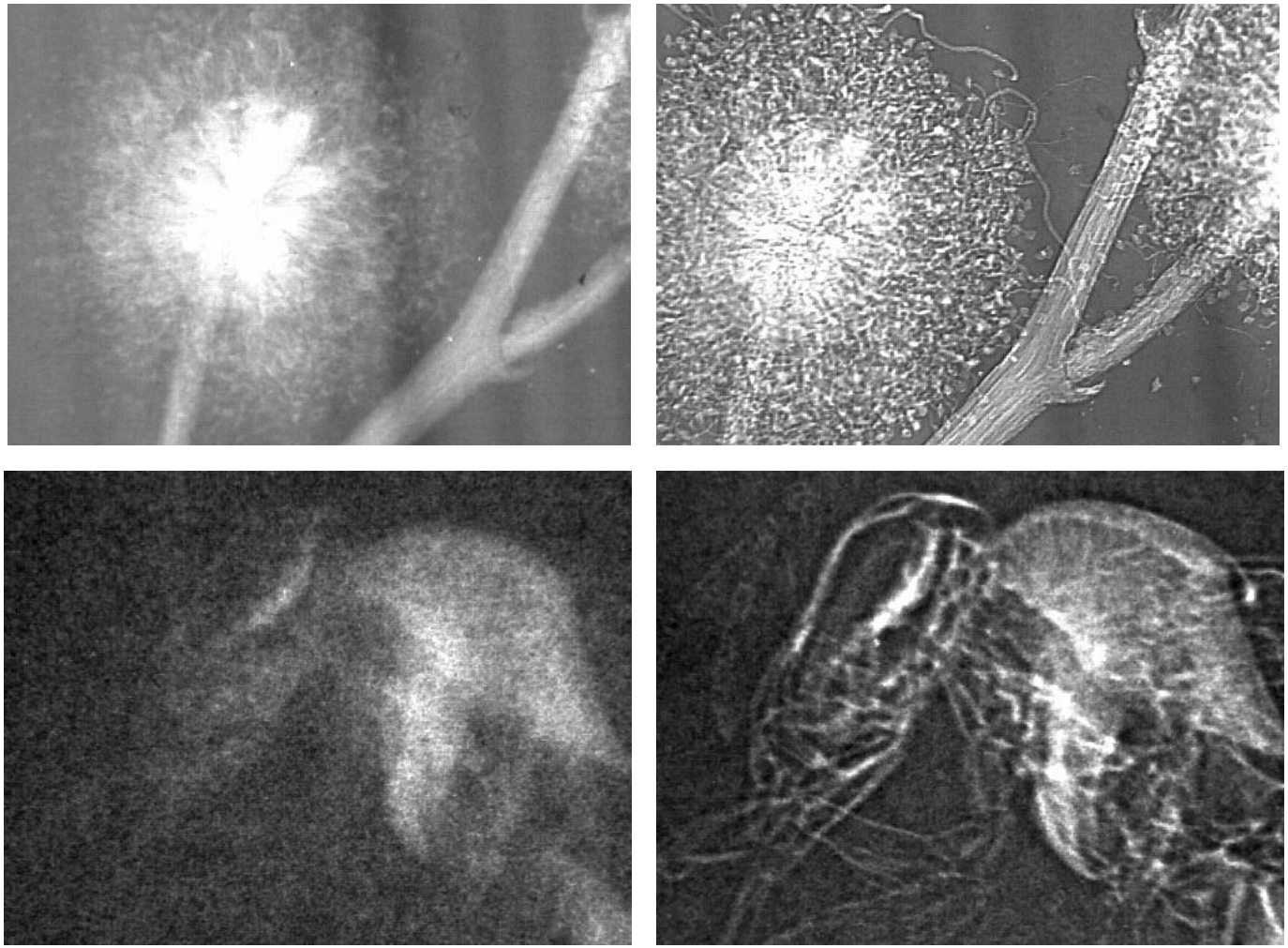
Conventional Radiology



Phase Contrast

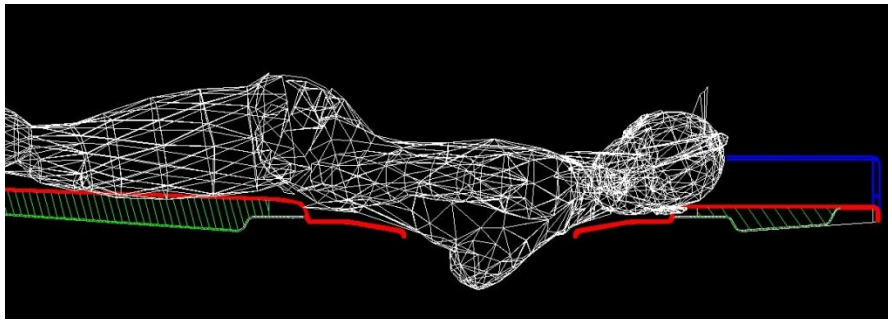
***Phase sensitive* techniques** are based on the observation of the *phase shifts* produced by the object on the incoming wave. Contrast arises from interference among parts of the wave front differently deviated (or phase shifted) by the sample. Edge enhancement effects.

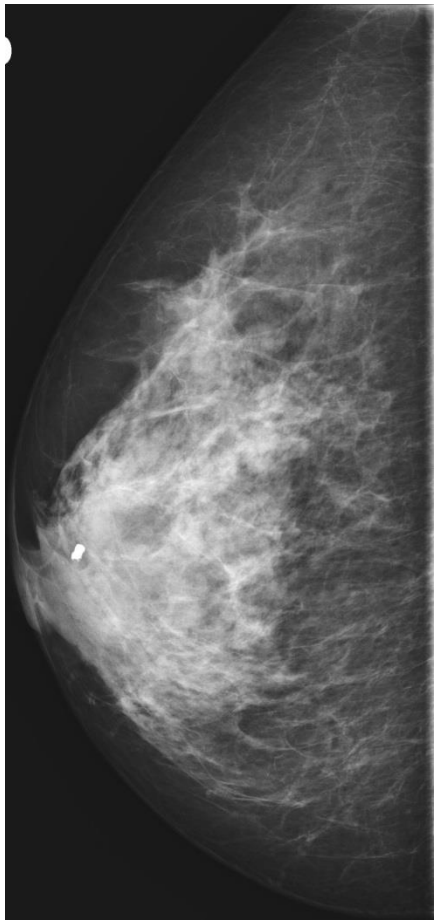
Examples: Conventional *vs.* Phase Contrast Imaging



Conventional radiology **Phase Contrast**

Mammography: Lower Dosage & Higher Resolution





Conventional Unit

@ the Synchrotron

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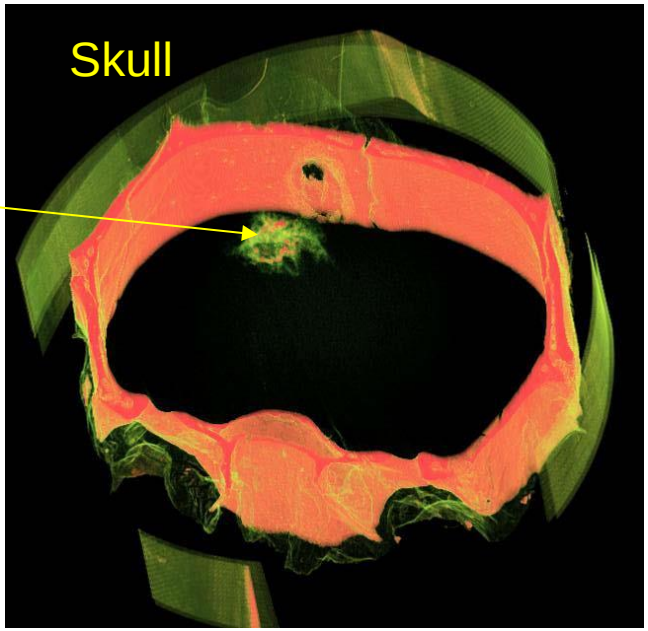
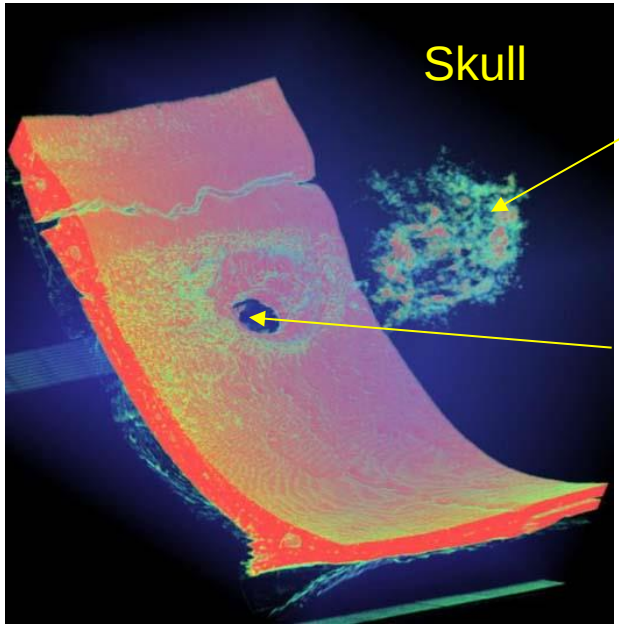
The Mouse... Found in the Experimental Hall

The Mouse... Used for Tomography

volume highlighted by the Au-loaded cells

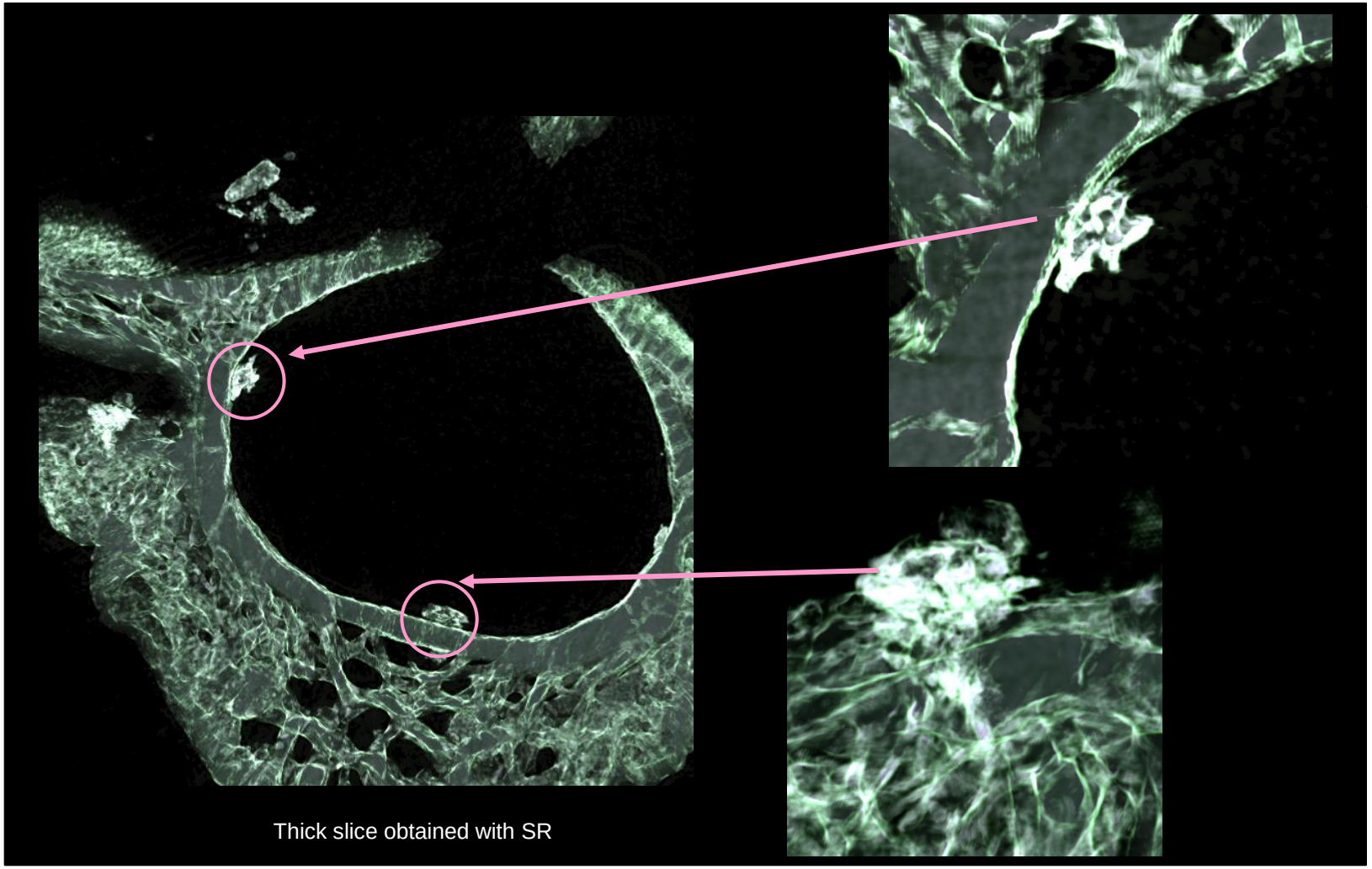




volume highlighted by the Au-loaded cells

hole performed in the skull to inject the cells

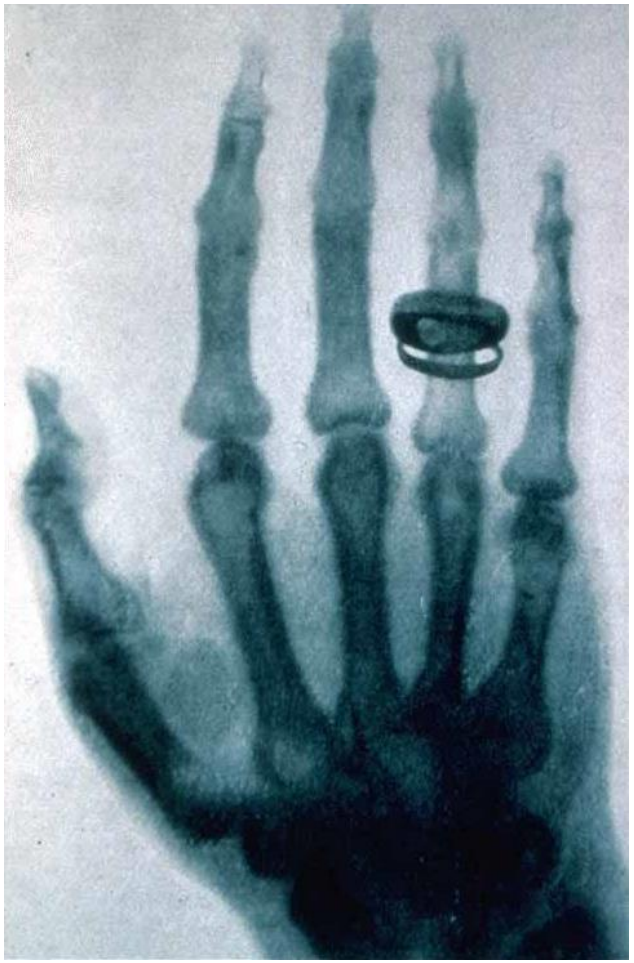
The Mouse... Used for Tomography (II)



Metastasis spread in the spine

Thank you!

My special thanks go to:



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Austrian Academy of Sciences, IBN, Graz, Austria
**working at the Austrian SAXS Beamline Trieste*

Veronika Kralj-Iglic and Ales Iglic
Universtity of Ljubljana, Slovenia

Sigrid Bernstorff (SAXS, Luca Olivi (XAFS), Lisa Vaccari (SISSI), Burkhard Kaulich (TwinMic) and Ralf Menk (Syrmep)
ELETTRA, Sincrotrone Trieste, Italy

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