How much and where is it?
Simple questions, challenging tasks:
Mapping the intracellular content of Magnesium and other chemical elements

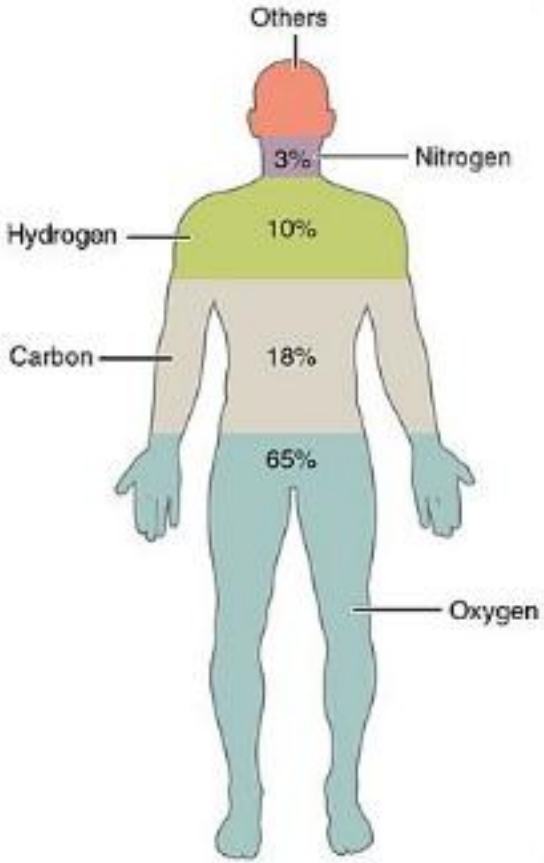
Smolenice, May 11-15, 2014

Back to basics

“How much and where is it?”

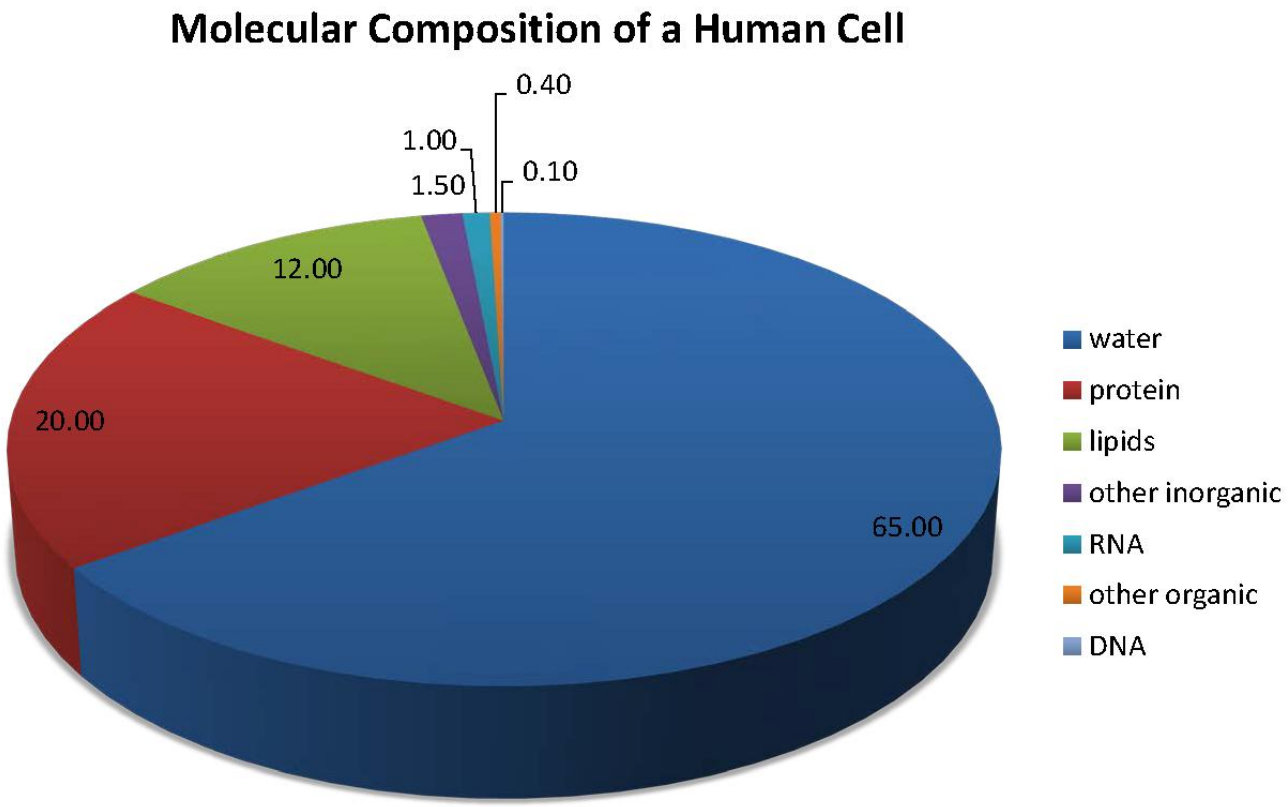
Represents one of the archetypal questions essential in science

Elements of the Human body

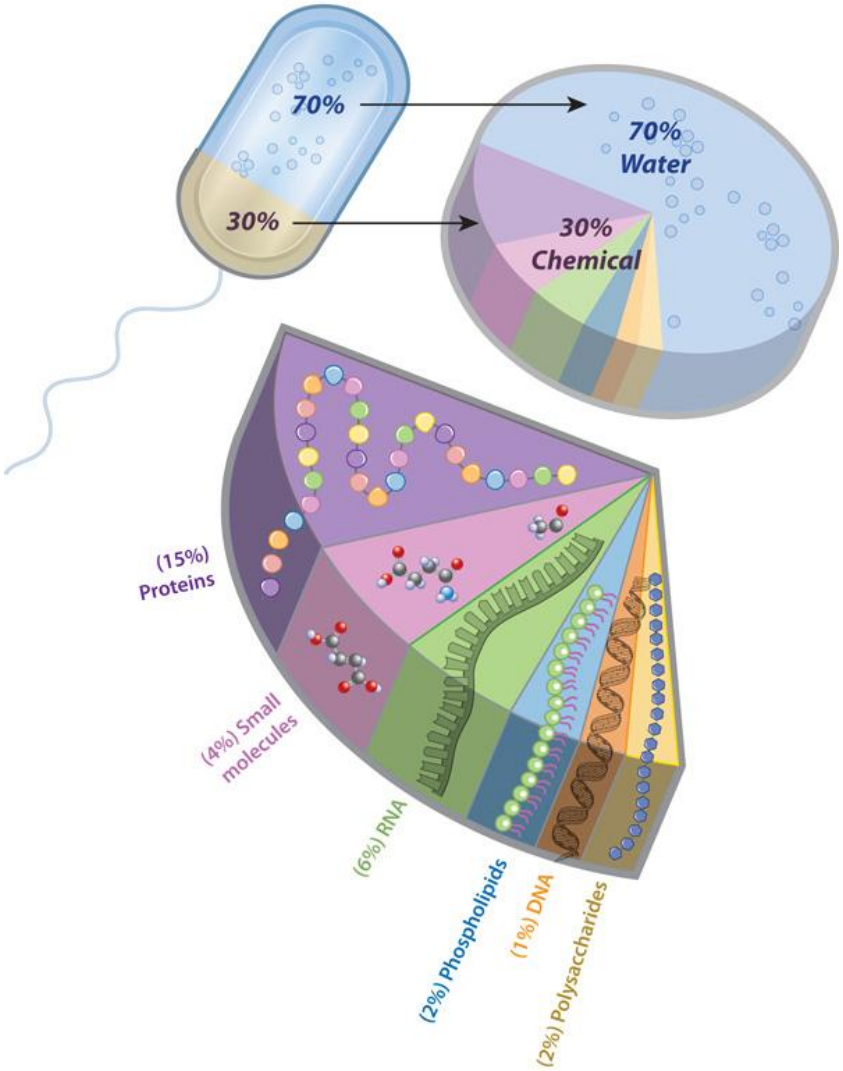


Element	Symbol	Percentage in Body
Oxygen	O	65.0
Carbon	C	18.5
Hydrogen	H	9.5
Nitrogen	N	3.2
Calcium	Ca	1.5
Phosphorus	P	1.0
Potassium	K	0.4
Sulfur	S	0.3
Sodium	Na	0.2
Chlorine	Cl	0.2
Magnesium	Mg	0.1
Trace elements include boron (B), chromium (Cr), cobalt (Co), copper (Cu), fluorine (F), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), selenium (Se), silicon (Si), tin (Sn), vanadium (V), and zinc (Zn).		less than 1.0

Cell composition



Cell composition



Local vs systemic: nuance or disparity

- What is local?
- cells, tissues, organs

- What is systemic?
- body, blood circulation

is there a real distinction?

Magnesium in Brain

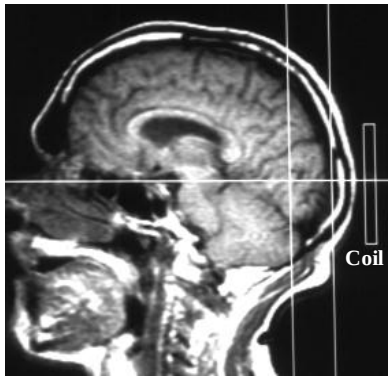
NMR IN BIOMEDICINE, VOL. 9, 24–32 (1996)

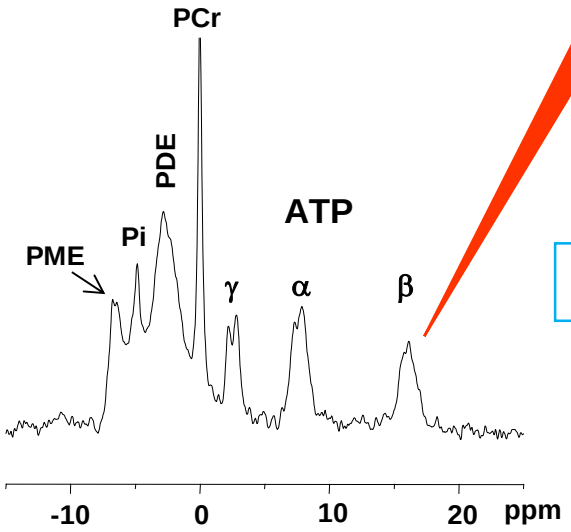
In Vivo Assessment of Free Magnesium Concentration in Human Brain by ^{31}P MRS. A New Calibration Curve Based on a Mathematical Algorithm

S. Iotti,† C. Frassineti,‡ L. Alderighi,§ A. Sabatini,§ A. Vacca,§ and B. Barbiroli†*

† Cattedra di Biochimica Clinica, Dipartimento di Medicina Clinica e Biotecnologia applicata 'D. Campanacci', Università di Bologna, Via Massarenti, 9, 40138 Bologna; ‡ Dipartimento di Scienze Biomediche, Università di Modena; § Dipartimento di Chimica, Università di Firenze, Italy

Magnesium in Brain

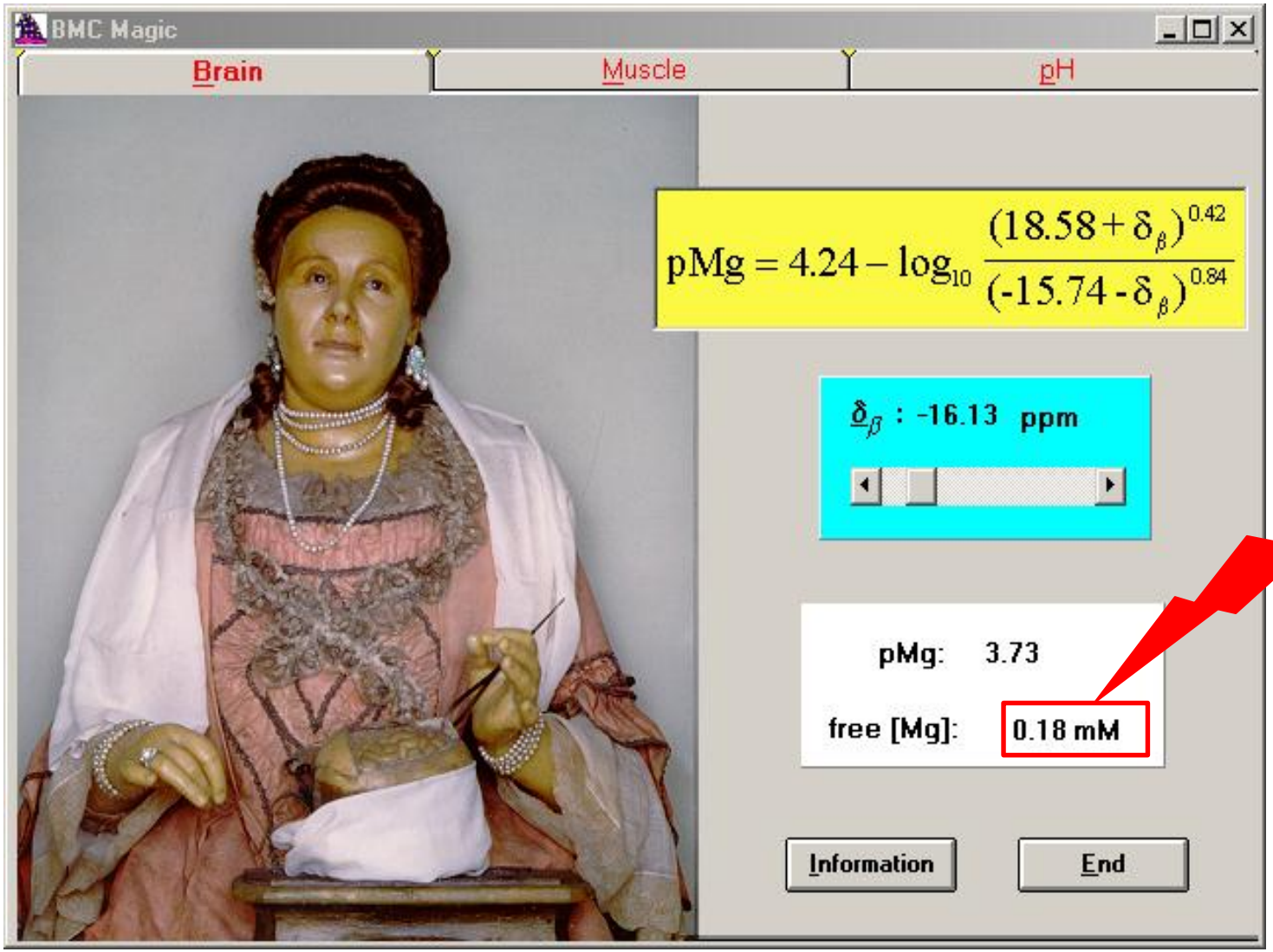




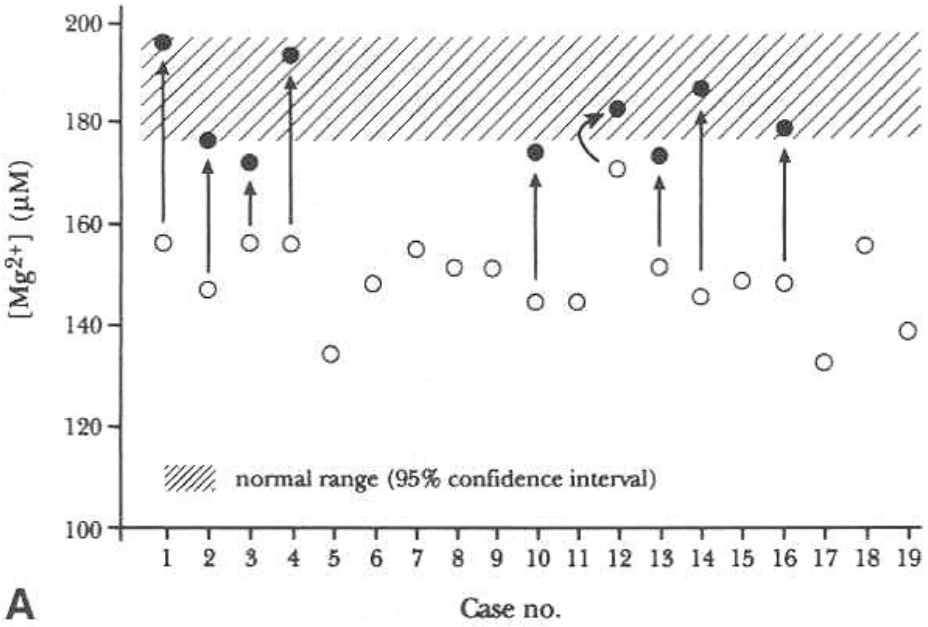
$[Mg^{2+}]$

$MgATP^{2-} \rightleftharpoons Mg^{2+} + ATP^{4-}$

Magnesium in Brain



Magnesium in Mitochondrial Cytopathies



● + CoQ

○ - CoQ

Iotti et al. ***J. Cerebr. Blood Flow Metab.*** **1999; 19:528-532**

ELSEVIER

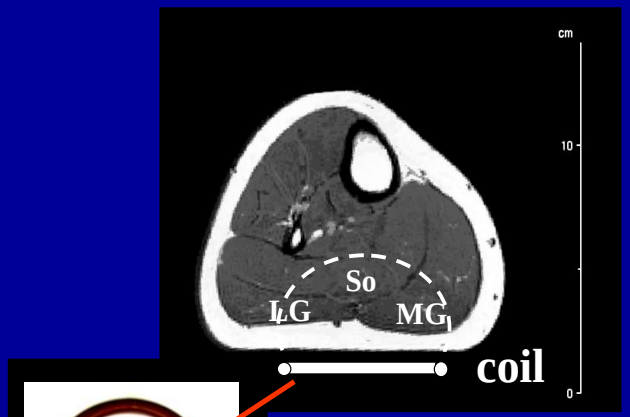
Magnetic Resonance Imaging 18 (2000) 607–614

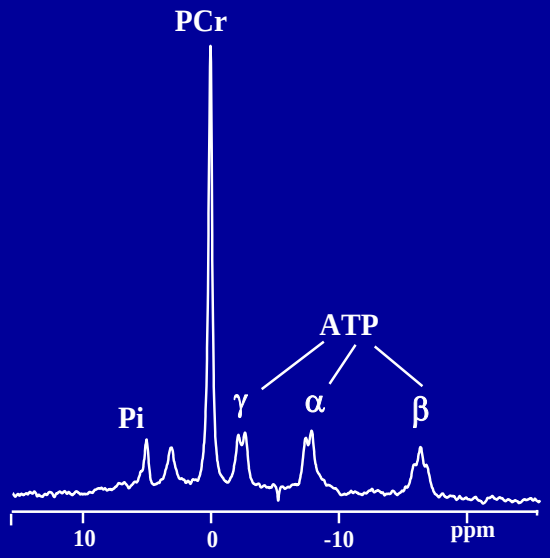
MAGNETIC RESONANCE IMAGING

In vivo ^{31}P-MRS assessment of cytosolic [Mg^{2+}] in the human skeletal muscle in different metabolic conditions

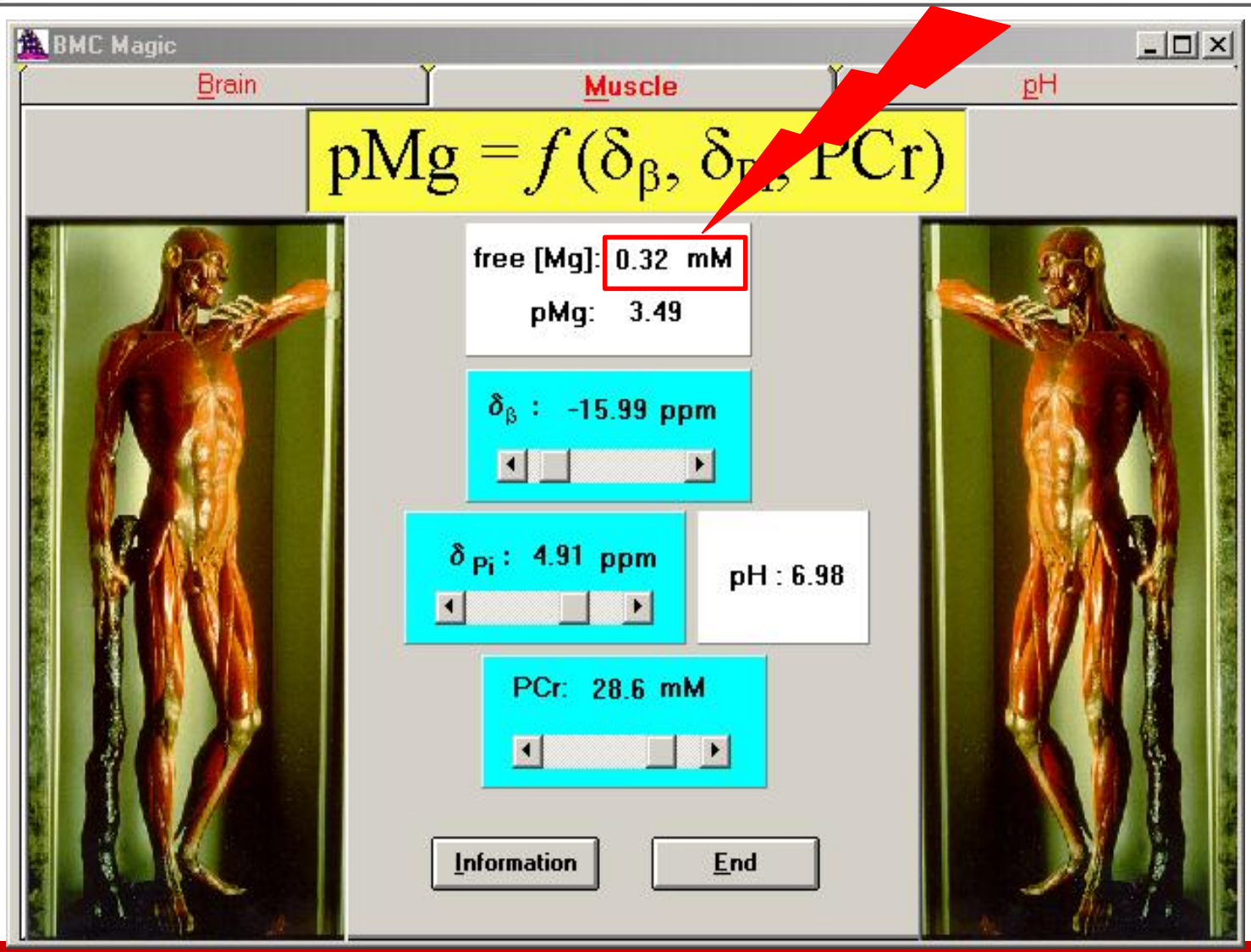
S. Iotti[a,*], C. Frassineti[b], L. Alderighi[c], A. Sabatini[c], A. Vacca[c], B. Barbiroli[a]

Calf muscle ^{31}P-MRS





Magnesium in Muscle



Magnesium Research 2009; **22** (1): 37-43 ORIGINAL ARTICLE

The complex relationship between magnesium and serum parathyroid hormone: a study in patients with chronic intestinal failure

Loris Pironi[2], Emil Malucelli[1], Mariacristina Guidetti[2], Elisabetta Lanzoni[2], Giovanna Farruggia[3], Antonio Daniele Pinna[4], Bruno Barbiroli[1], Stefano Iotti[1,5]

The *opposite* correlations with serum-PTH displayed by muscle [Mg^{2+}] and serum total Mg suggests:

that total circulating Mg and the intracellular free fraction of Mg *are separate pools* undergoing to different regulatory mechanisms

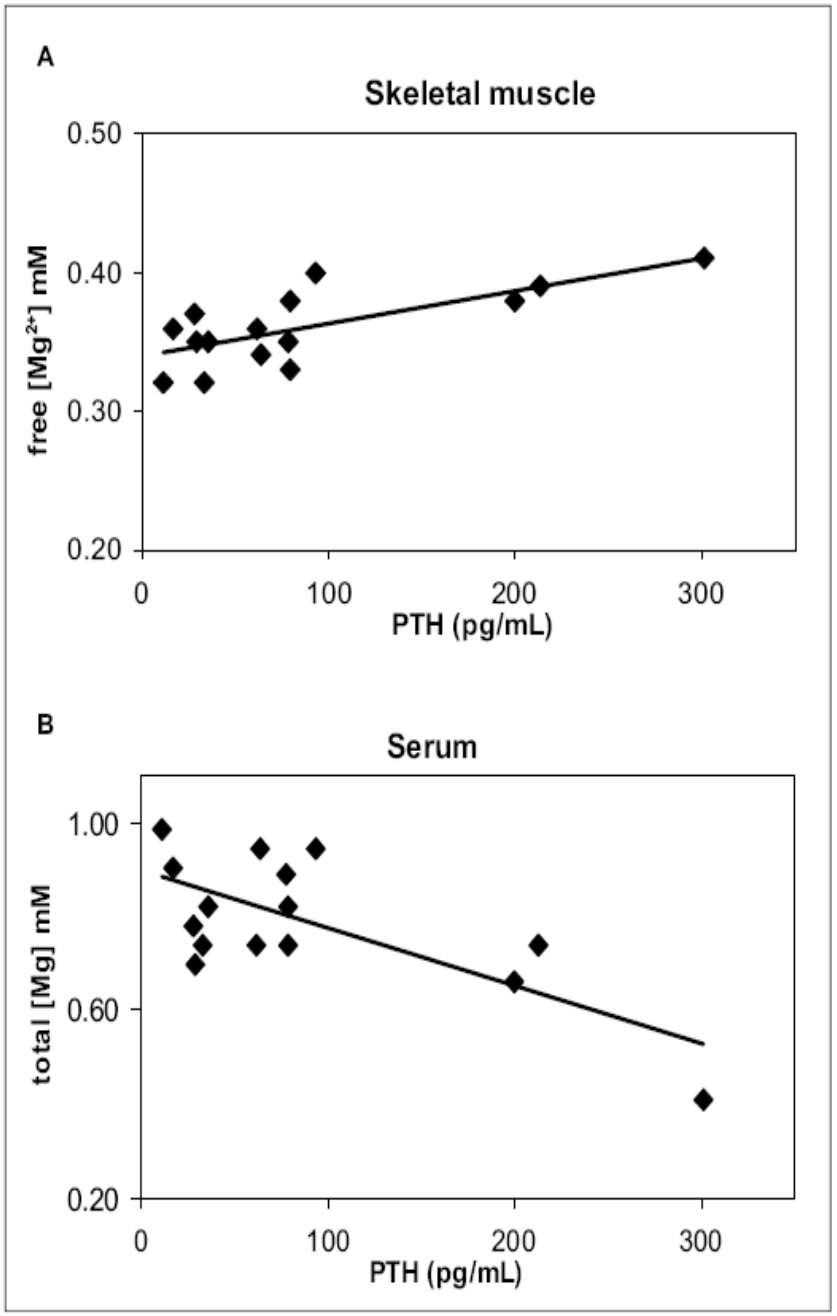
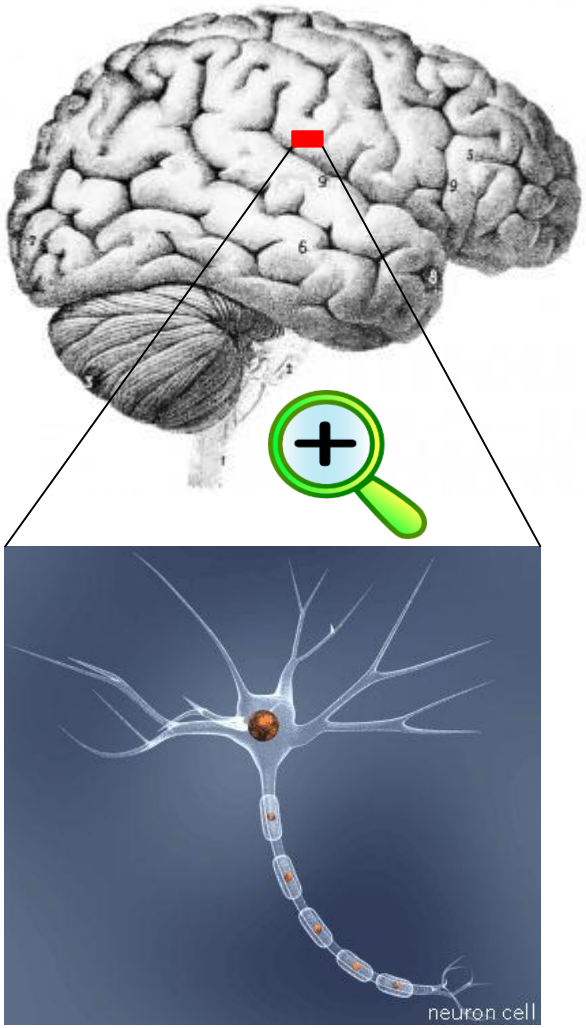
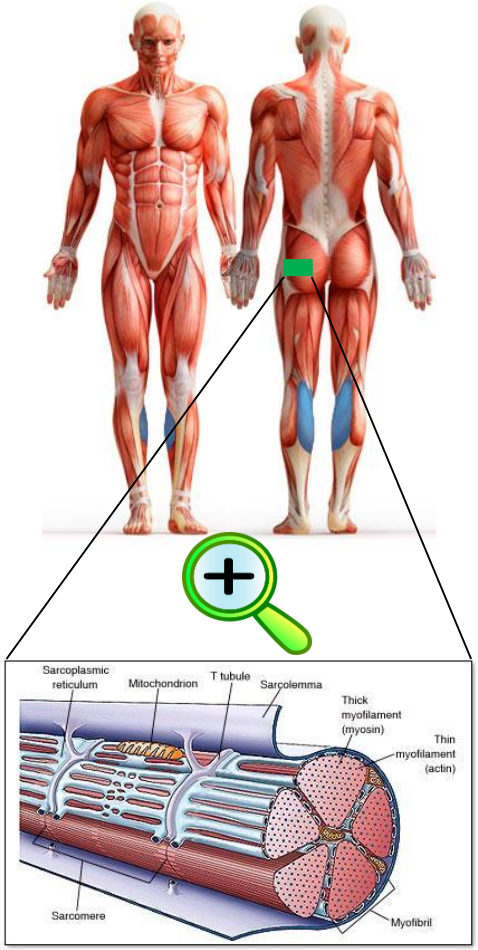


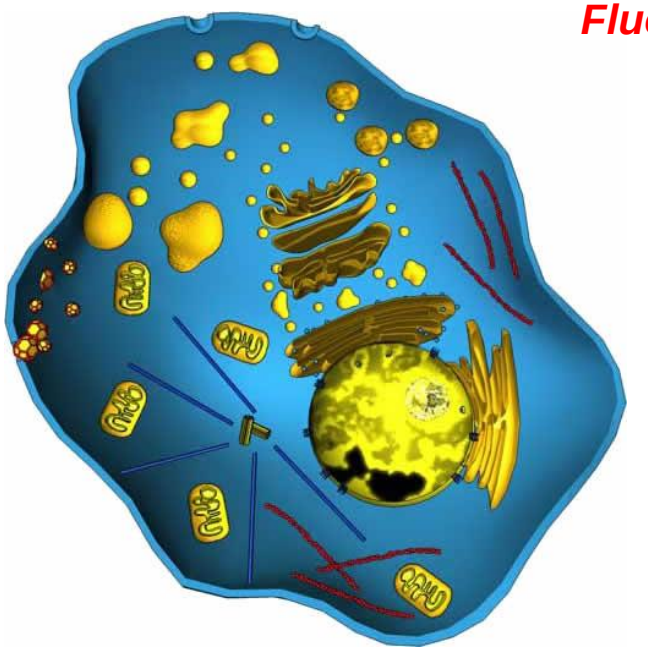
Figure 2. Magnesium correlations with serum PTH in 15 patients. (**A**) muscle free [Mg^{2+}] assessed by ^{31}P MRS; (**B**) serum total Mg assessed by colorimetric assays.

…..moving down

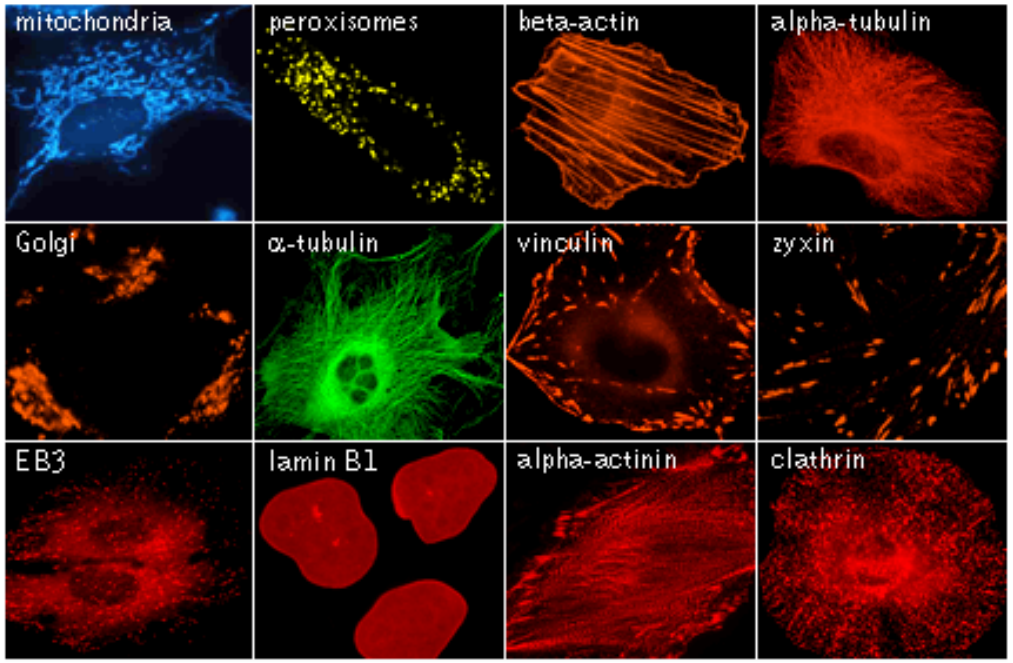

neuron cell


Sarcoplasmic reticulum
Mitochondrion
T tubule
Sarcolemma
Thick myofilament (myosin)
Thin myofilament (actin)
Sarcomere
Myofibril

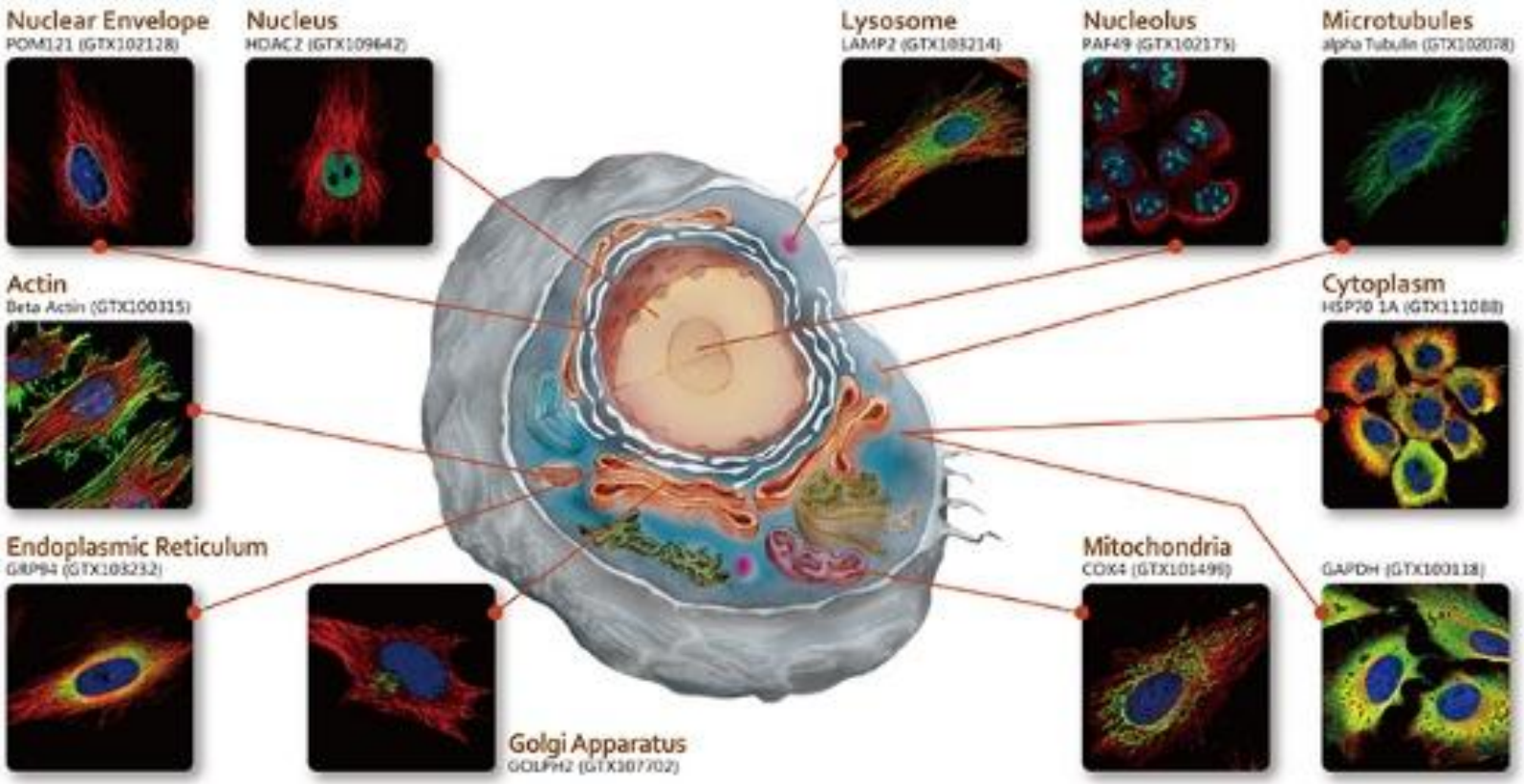
…..and down again



Fluorescently Labelled Subcellular Organelles and Proteins



…..and down again



nanoscience

"A new world is coming. A scientific revolution is under way. A giant revolution on the smallest of scales"

To see this major revolution, you need to **go** deep **down** into the world of the infinitely small

BUT

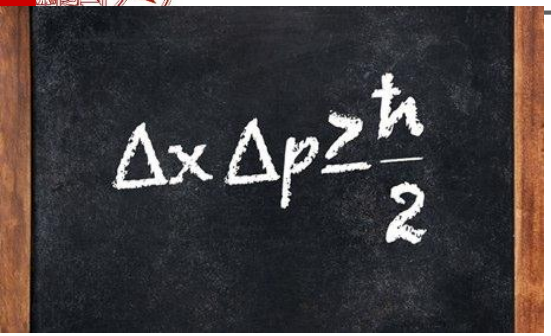
Going deep down to micro (nano) scale world

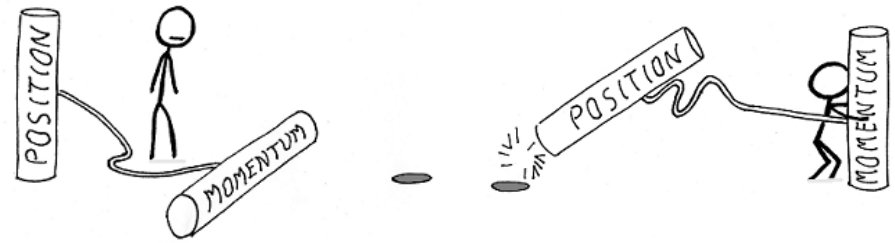
strange things happen

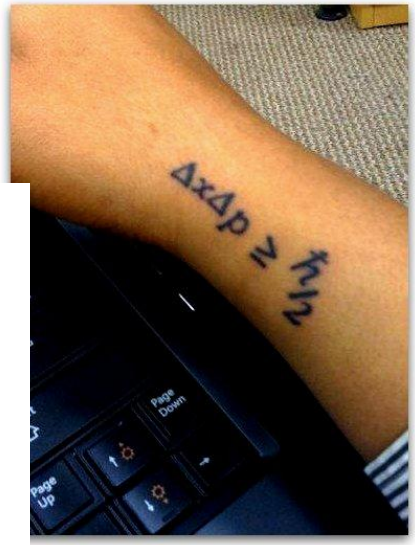

Sprite
Coke
Pepsi
Dr Pepper
V8
Crush
Squirt

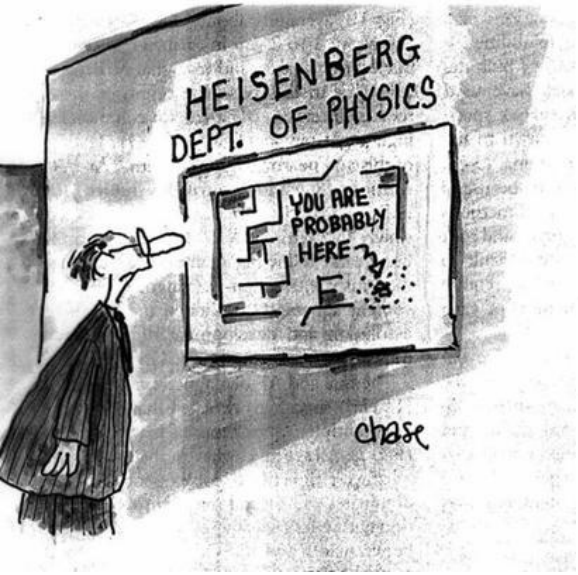
Position and velocity from macroworld to nanoworld

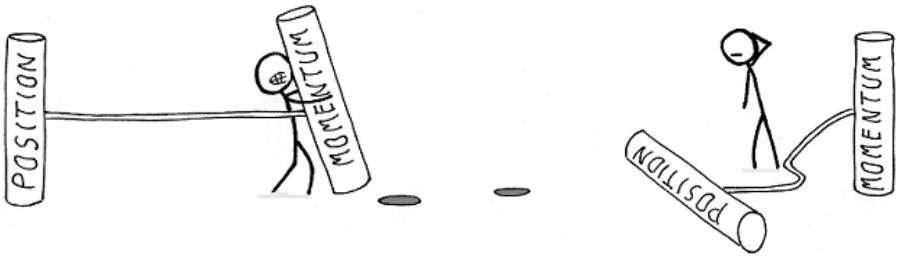
Heisenberg's Uncertainty Principle





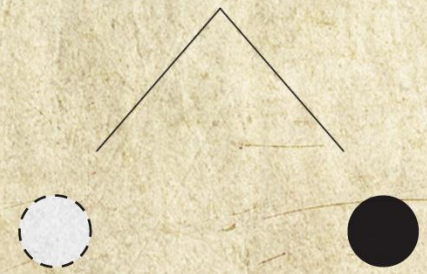






Heisenberg's Uncertainty Principle

you are here

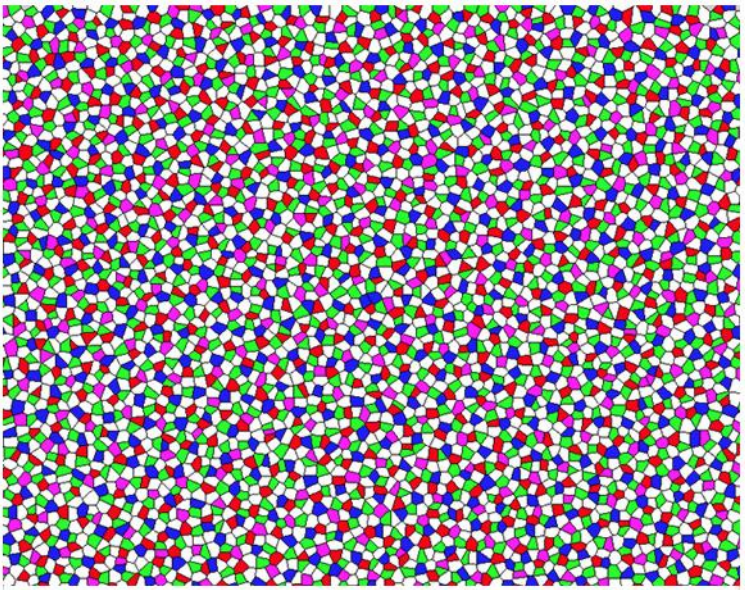


if you're neither here nor there, you aren't really anywhere

Macro and Micro

- Moving from macro to micro
- Different perpectives
- Different informations

Disordered hyperuniformity



PHYSICAL REVIEW E **89**, 022721 (2014)

Avian photoreceptor patterns represent a disordered hyperuniform solution to a multiscale packing problem

These new state of matter have a «hidden order» that allows to behave like crystal at bigger scale and liquid at smaller scale

Spatial distribution of the five different cell types of cone photoreceptors of chicken retina

They are organized into states of disordered hyperuniformity:

a new state of matter that exhibits order over large distances and disorder over small distances

At bigger scale it's like a crystal, but at smaller scale it's liquid-like in that it exhibits similar physical properties in all directions

Mg content: how much and where

- Serum Mg concentration is considered a poor indicator of the body Mg pool representing only 0.3%
- Poor correlation between serum Mg and the tissue content of Mg
- Intracellular Mg depletion can be present despite a normal serum Mg concentration

Content and Position

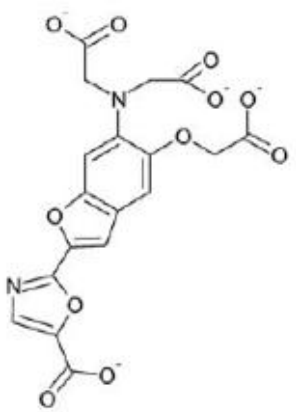
How much and where is it?

Is it possible to measure elemental concentration and spatial distribution at once?

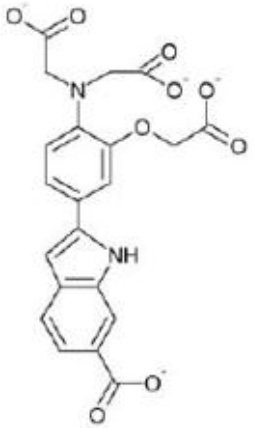
Conceptual limitations?

Technical limitations?

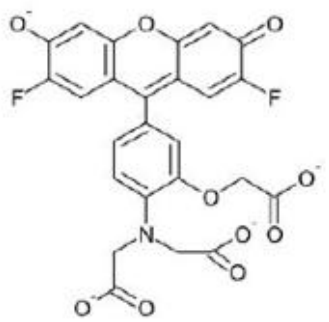
Commercial Magnesium Probes:



mag-fura-2



mag-indo-1



mag-fluo-4

Mg^{2+} fluctuations observed with commercial probes cannot provide enough informations for a full understanding of cellular Mg^{2+} Homeostasis

Limitations:

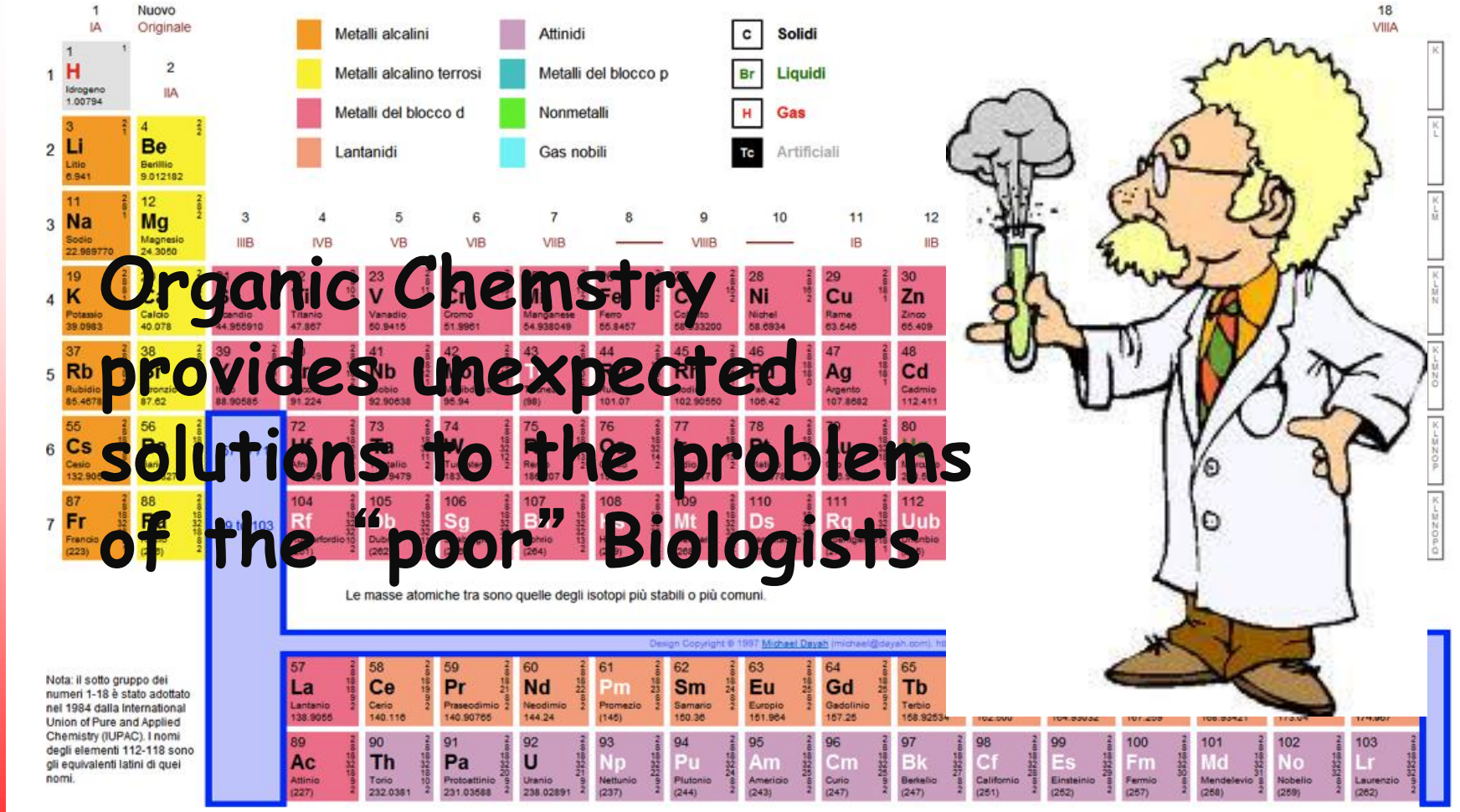
- *Detection of free Mg^{2+} fluctuations*
- *Cross-reactivity with Ca^{2+}*

Main Fluorescent Mg^{2+} Indicators: total or free this is the question!

Indicator	Binding site	Fluorophore	Absorption maximum (nm)		Emission maximum (nm)		Kd (Mg^{2+})	Kd (Ca^{2+})
			Free	Bound	Free	Bound		
Mag-fura-2	APTRA	Furan	369	330	511	491	1.9 mM	25 μM
Mag-indo-1	APTRA	Indole	349	330	480	417	2.7 mM	35 μM
Mag-fluo-4	APTRA	Fluorescein	490	493	None	517	4.7 mM	22 μM
KMG-104	β-Diketone	Fluorescein	502	504	523	523		
DCHQ1	Diaza-18-crown-16	Hydroxyquinoline	363	363	505	505	44 μM	ND
AMg1	APTRA	Naphthalene	365	365	498	498		
CMg1	β-Keto acid	Benzo[*h*]chromene	413	443	556	559	1.3 mM	3.6 mM
Ray and Bharadwaj	NO_2 donor set	Coumarin	488	555	None	602	<1 μM[c]	16.7 μM[c]
Hama *et al.*	Benzo-15-crown-5	Naphthaleneacetamide	258	250	338	338	2.9 μM[c]	1.3 μM[c]
Song *et al.*	Phenanthroimidazole	Calix[4]arene	332	347	409	493	33.6 μM[c]	ND
Ishida *et al.*	Phenanthroline	Porphyrin	496	575	583	631	26.8 mM[c]	ND[d]

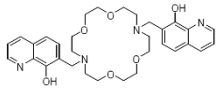
V. Trapani et al., Analyst, 2010, 135, 1855-1866. Review.

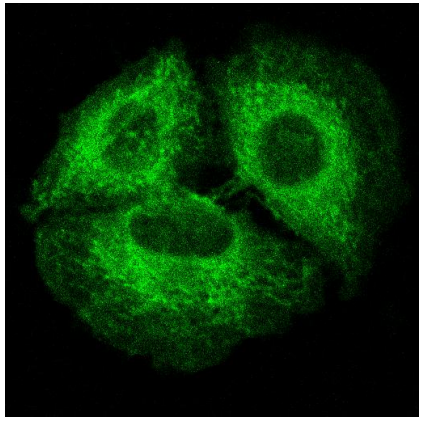
Welcome to the wonderful world of Organic Chemistry!



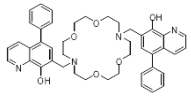
Application in Two-Photon Confocal Microscopy:

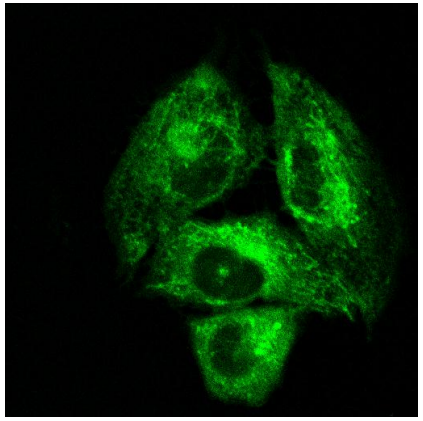
DCHQ1



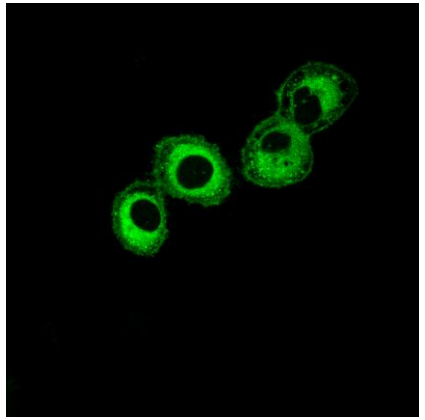


DCHQ5

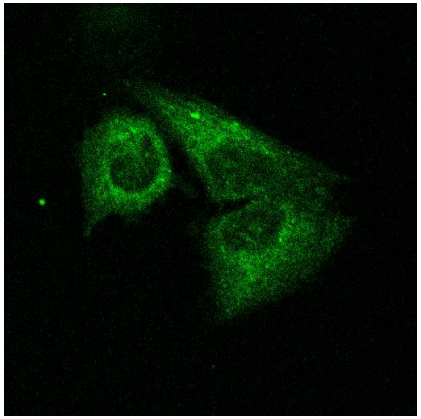


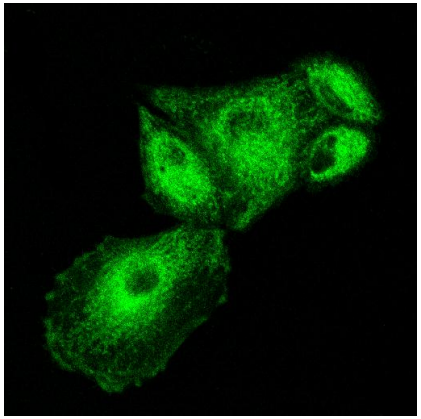


DCHQ11

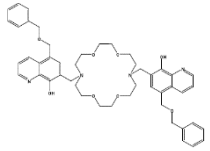


DCHQ12





DCHQ13



DCHQ5

Analyst

PAPER

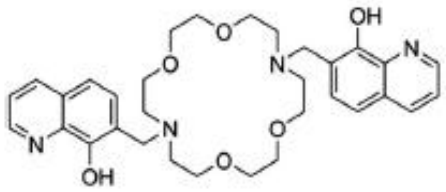
View Article Online
View Journal

A novel fluorescent chemosensor allows the assessment of intracellular total magnesium in small samples†

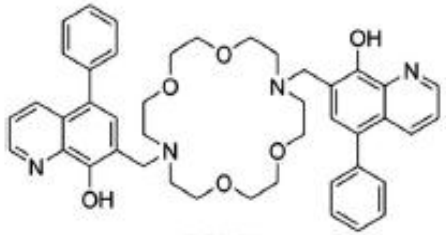
Cite this: DOI: 10.1039/c3an01737k

Azzurra Sargenti,*[a] Giovanna Farruggia,[ab] Emil Malucelli,[a] Concettina Cappadone,[a] Lucia Merolle,[a] Chiara Marraccini,[c] Giulia Andreani,[d] Luca Prodi,[e] Nelsi Zaccheroni,[e] Massimo Sgarzi,[e] Claudio Trombini,[e] Marco Lombardo[e] and Stefano Iotti[ab]

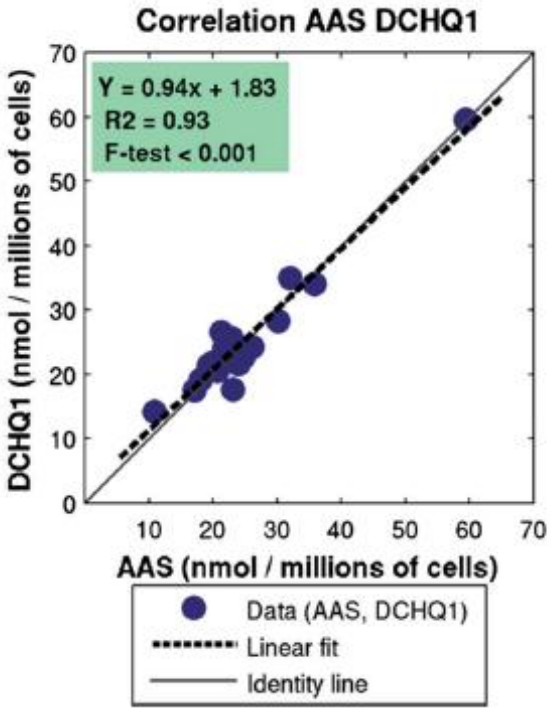
DCHQ5

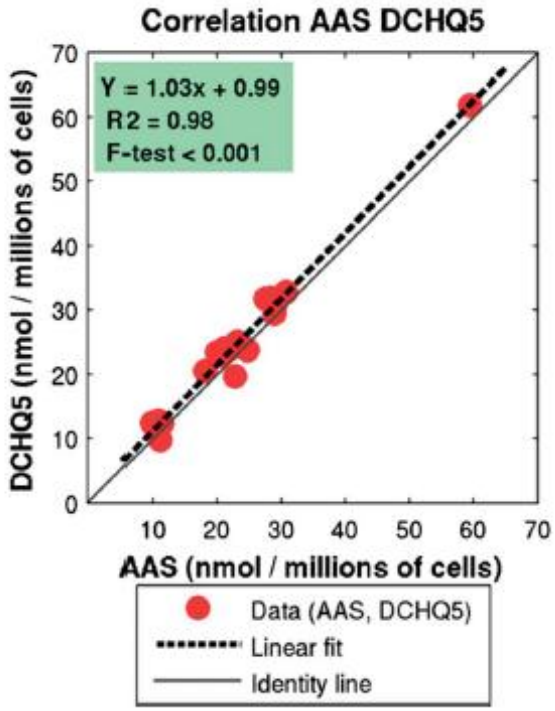


DCHQ1



DCHQ5

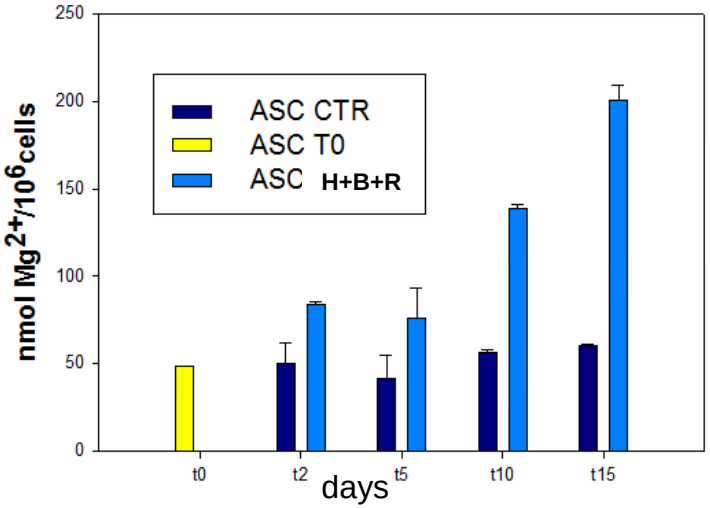




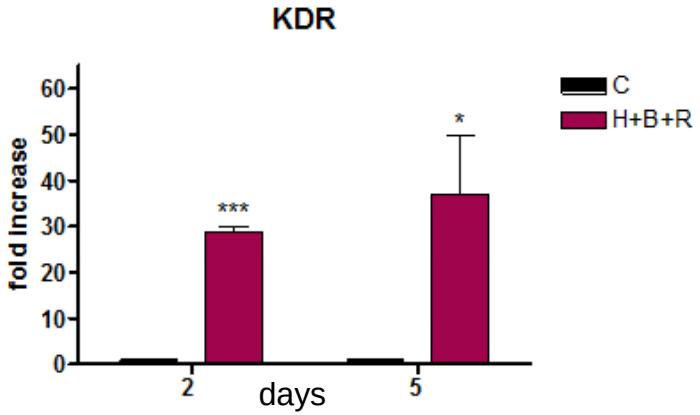
Correlation between the total intracellular Mg assessed by AAS and DCHQ1 or DCHQ5

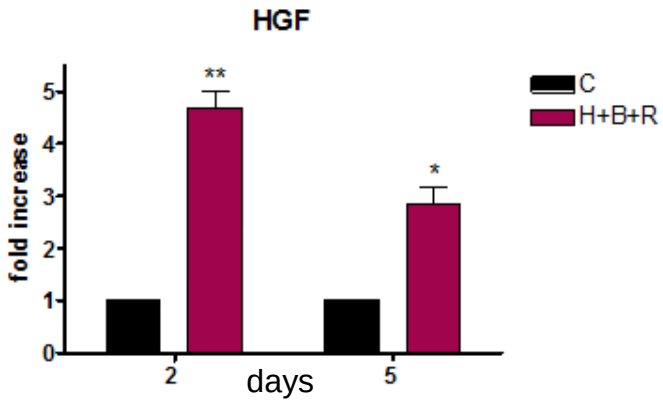
DCHQ5

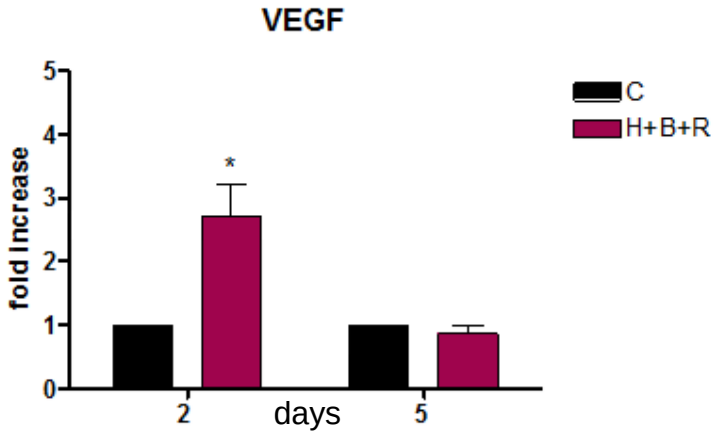
ASC: **Adipose**-derived **Stem Cell**
Hyaluronic + Butyric + Retinoic



VEGF, HGF, KDR:
gene markers of vasculogenic differentiation



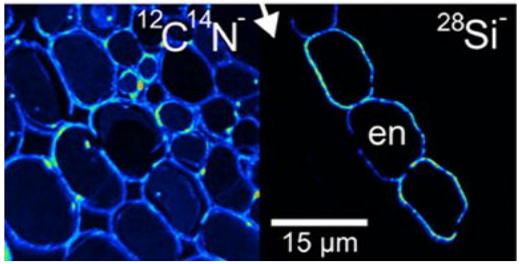




Ion Beam Analysis Techniques

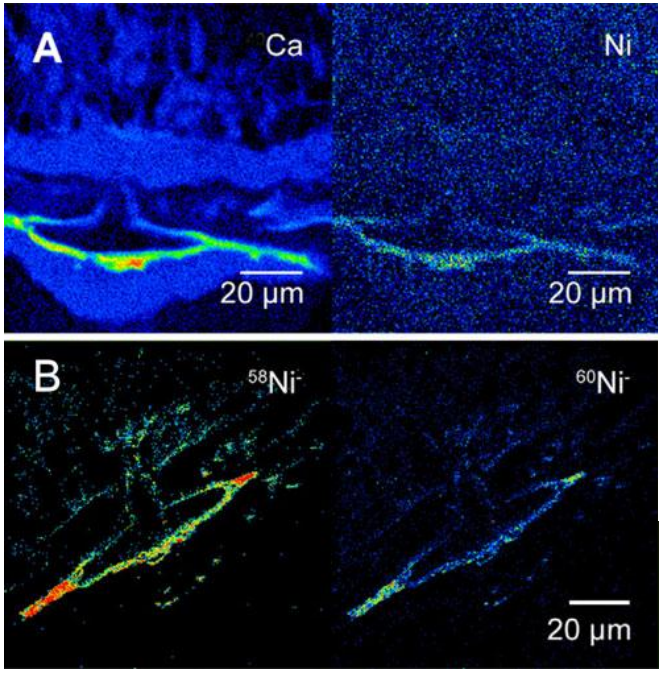
- **Ion Beam Analysis techniques**
- have the capability of *mapping elements in unstained cells* to the microgram per gram (dry weight) sensitivity at higher spatial resolution (nanoscale) than electron microprobe (microscale)

- Different complementary ion beam techniques STIM, PIXE, RBS, NanoSIMS can be applied.
- Scanning Transmission Ion Microscopy
- Particle Induced X-ray Emission
- Rutherford Backscattering Spectrometry
- High-resolution Secondary Ion Mass Spectrometry



- These are destructive techniques and cannot yet be applied to living cells

Quantification or spatial distribution? we have the power to choose!



Anal Bioanal Chem (2012) 402:3263–3273
DOI 10.1007/s00216-011-5484-3

REVIEW

Elemental imaging at the nanoscale: NanoSIMS and complementary techniques for element localisation in plants

Katie L. Moore · Enzo Lombi · Fang-Jie Zhao · Chris R. M. Grovenor

Correlative electron probe microanalysis (A) and NanoSIMS analysis (B) of the same sample of the nickel hyperaccumulator Alyssum lesbiacum

EPMA allowed quantitative analysis, whereas the NanoSIMS provided higher-resolution images

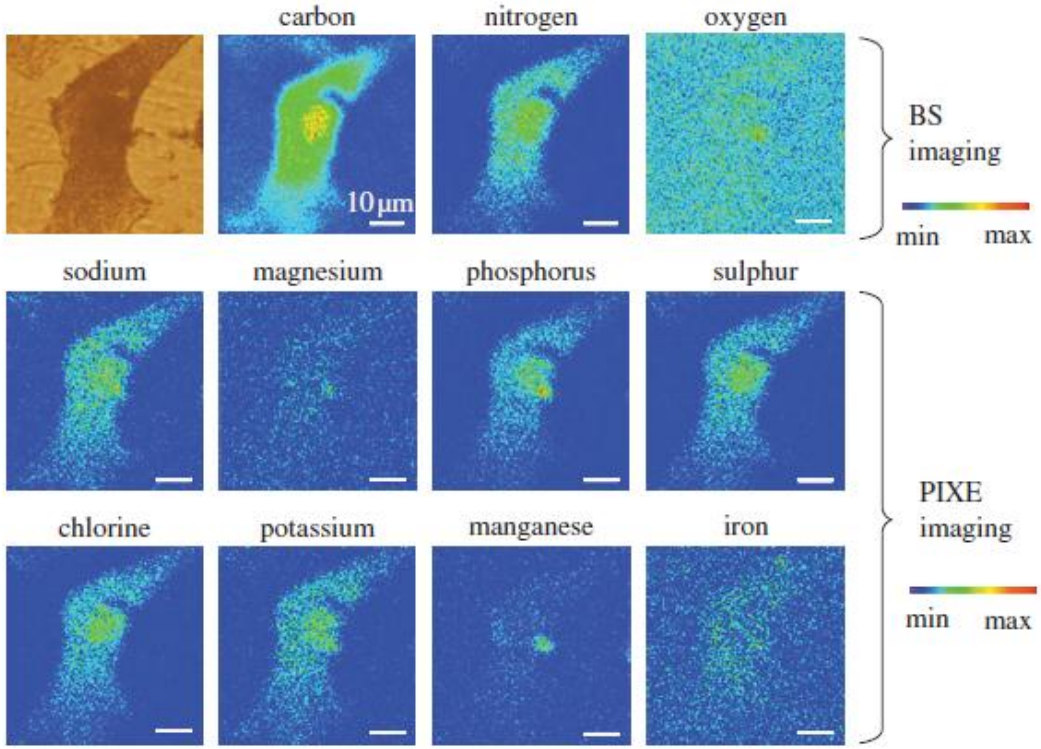
Interface focus

J. R. Soc. Interface (2009) **6**, S649–S658
doi:10.1098/rsif.2009.0166.focus
Published online 15 July 2009

REVIEW

Bio-metals imaging and speciation in cells using proton and synchrotron radiation X-ray microspectroscopy

Richard Ortega*, Guillaume Devès and Asunción Carmona



Multi-element imaging of a single PC12 cell exposed to MnCl2
C, N and O maps were imaged using RBS analysis, elements of higher Z were imaged using PIXE analysis

Quantitation and spatial distribution

Table 1. Typical characteristics of electron, proton and synchrotron X-ray microprobes for trace element imaging, speciation and quantification in single cells (*for ultrathin samples; **for the latest generation of instruments).

X-ray micro-analytical techniques	detection limit	spatial resolution	selectivity	quantification
electron microprobe				
EDS (X-ray energy dispersive spectrometry)	100–1000 $\mu g\ g^{-1}$	0.03*–0.5 μm	multi-elemental	semi-quantitative
EELS (electron energy loss spectroscopy)	1000 $\mu g\ g^{-1}$	atomic**, 0.001 μm	chemical species	
proton microprobe				
PIXE	1–10 $\mu g\ g^{-1}$	0.2**–2 μm	multi-elemental	fully quantitative (combined to BS)
synchrotron microprobe				
XRF	0.1 $\mu g\ g^{-1}$	0.1**–2 μm	multi-elemental	semi-quantitative
XAS	100 $\mu g\ g^{-1}$	1 μm	chemical species	

?

Argonne NATIONAL LABORATORY

Advanced Photon Source

A U.S. Department of Energy, Office of Science,
Office of Basic Energy Sciences national synchrotron x-ray research facility

U.S. DEPARTMENT OF ENERGY

analytical chemistry

Article

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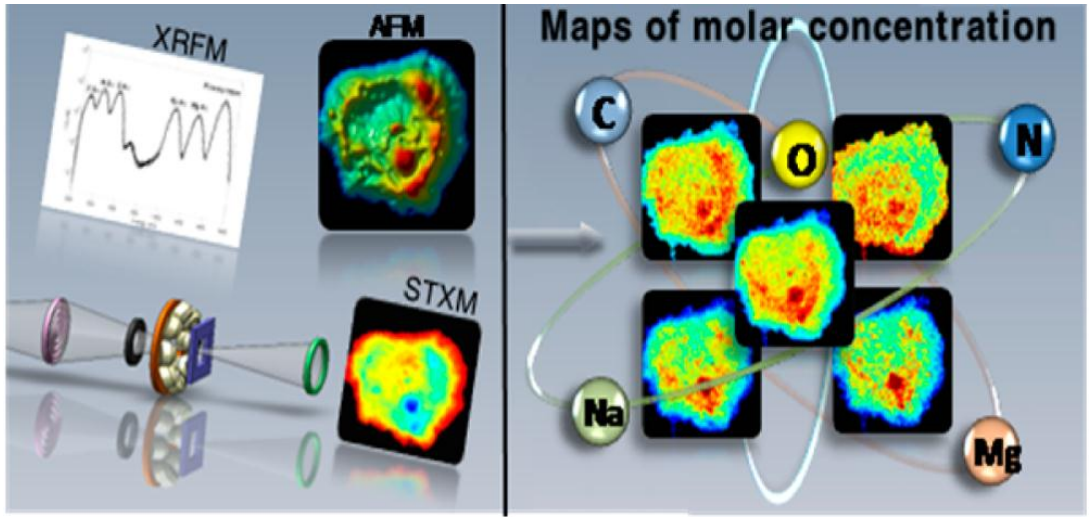
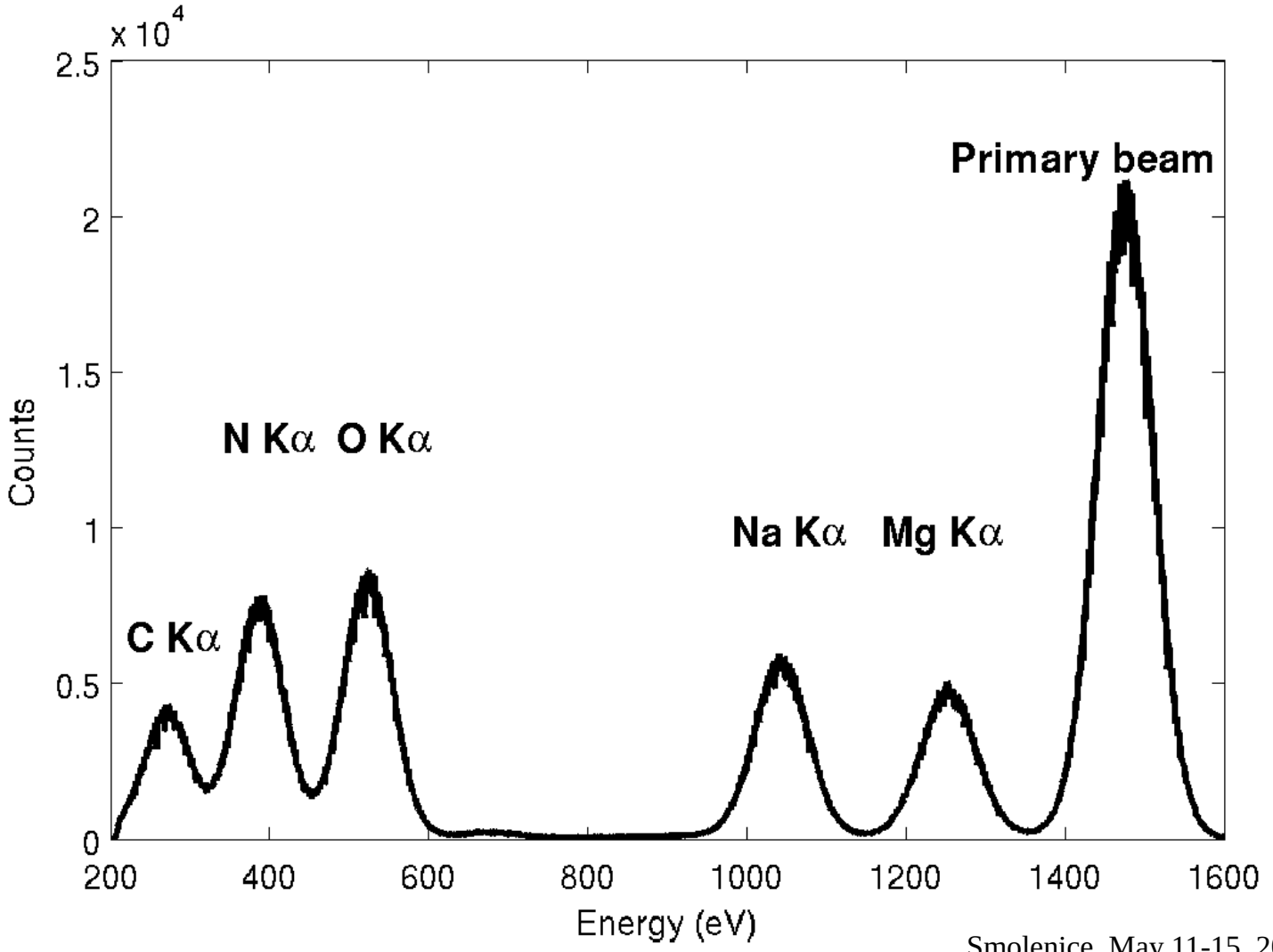
Quantitative Chemical Imaging of the Intracellular Spatial Distribution of Fundamental Elements and Light Metals in Single Cells

Emil Malucelli,[†] Stefano Iotti,[*,†,◆] Alessandra Gianoncelli,[§] Michela Fratini,[∥] Lucia Merolle,[†] Andrea Notargiacomo,[⊥] Chiara Marraccini,[#] Azzurra Sargenti,[†] Concettina Cappadone,[†] Giovanna Farruggia,[†,◆] Inna Bukreeva,[⊥] Marco Lombardo,[‡] Claudio Trombini,[‡] Jeanette A. Maier,[▽] and Stefano Lagomarsino[○,◆]



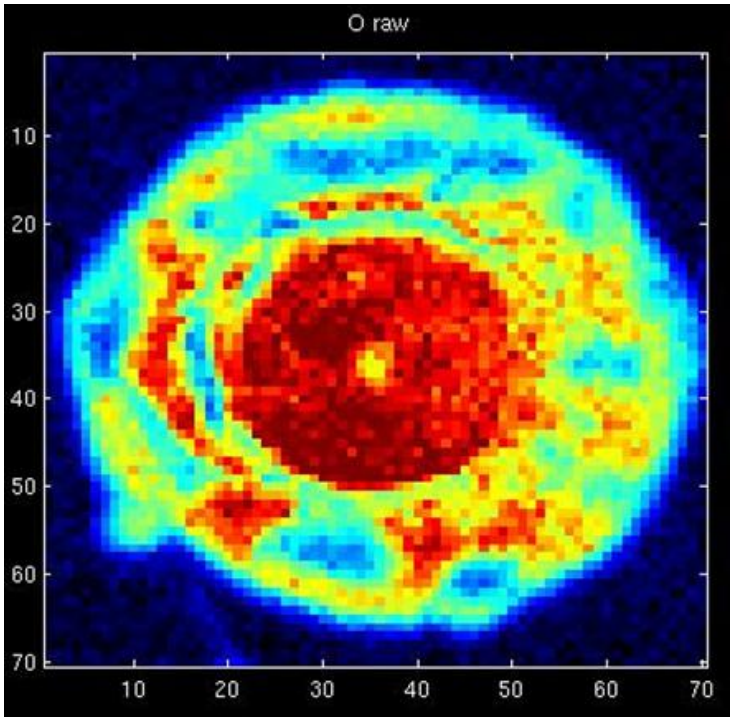
Fluorescence Spectra

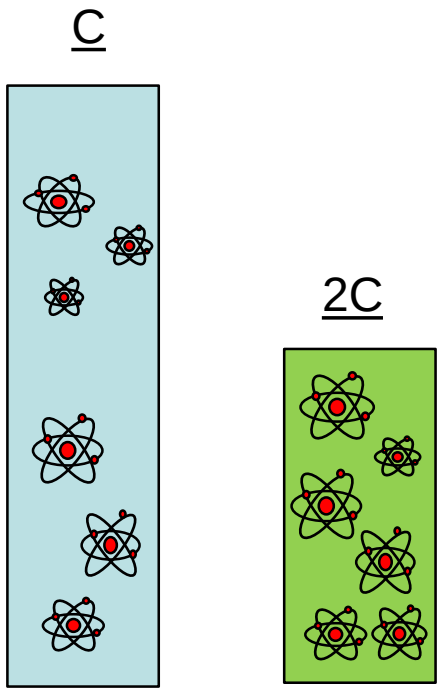


Does the size matters?

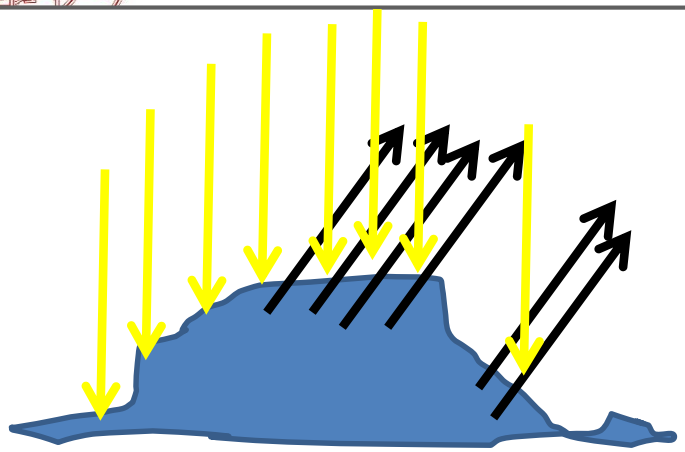
$$dR_i = w_i S \rho dz \times \frac{\Omega}{4\pi} \varepsilon(E_i) \omega_i P_i \tau_i(E_0) I_0(E_0) \times e^{-[\mu_m(E_0) cosec(\alpha) + \mu_m(E_i) cosec(\beta)]\rho z}$$

This equation gives the mass fraction ***not the concentration***





ELEMENTAL MAP



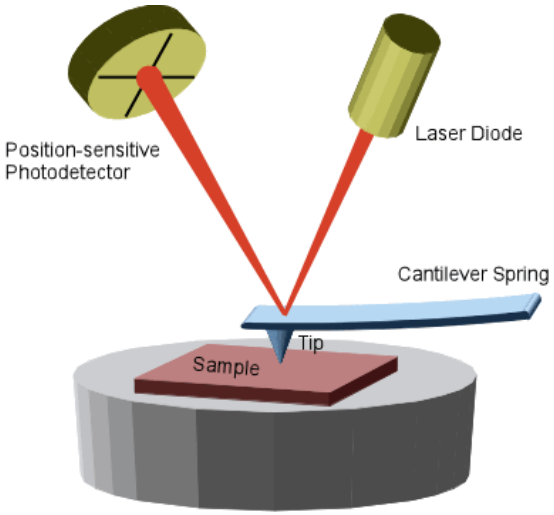
We need to know the morphology (i.e.volume)

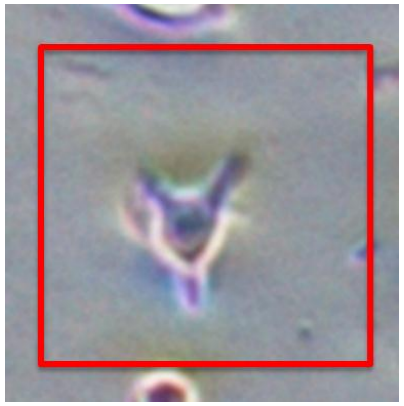
Fluorescence → **composition**

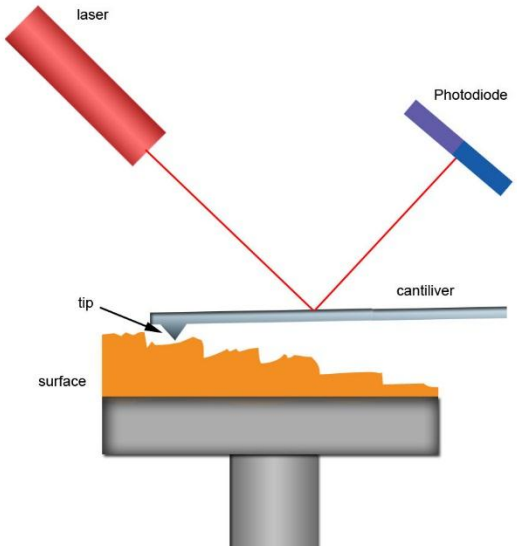
2D Trasmission + **AFM** → **Morphology**

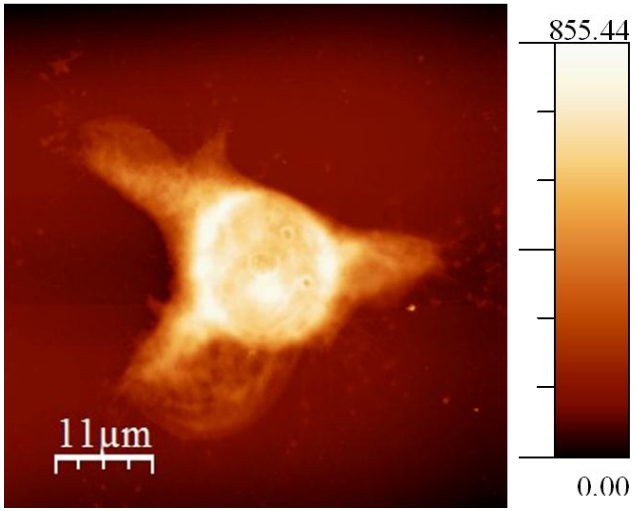
Quantitative maps: structure and composition

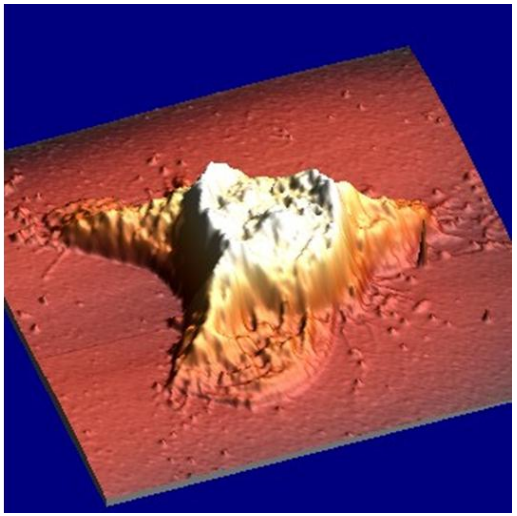
Atomic Force Microscopy



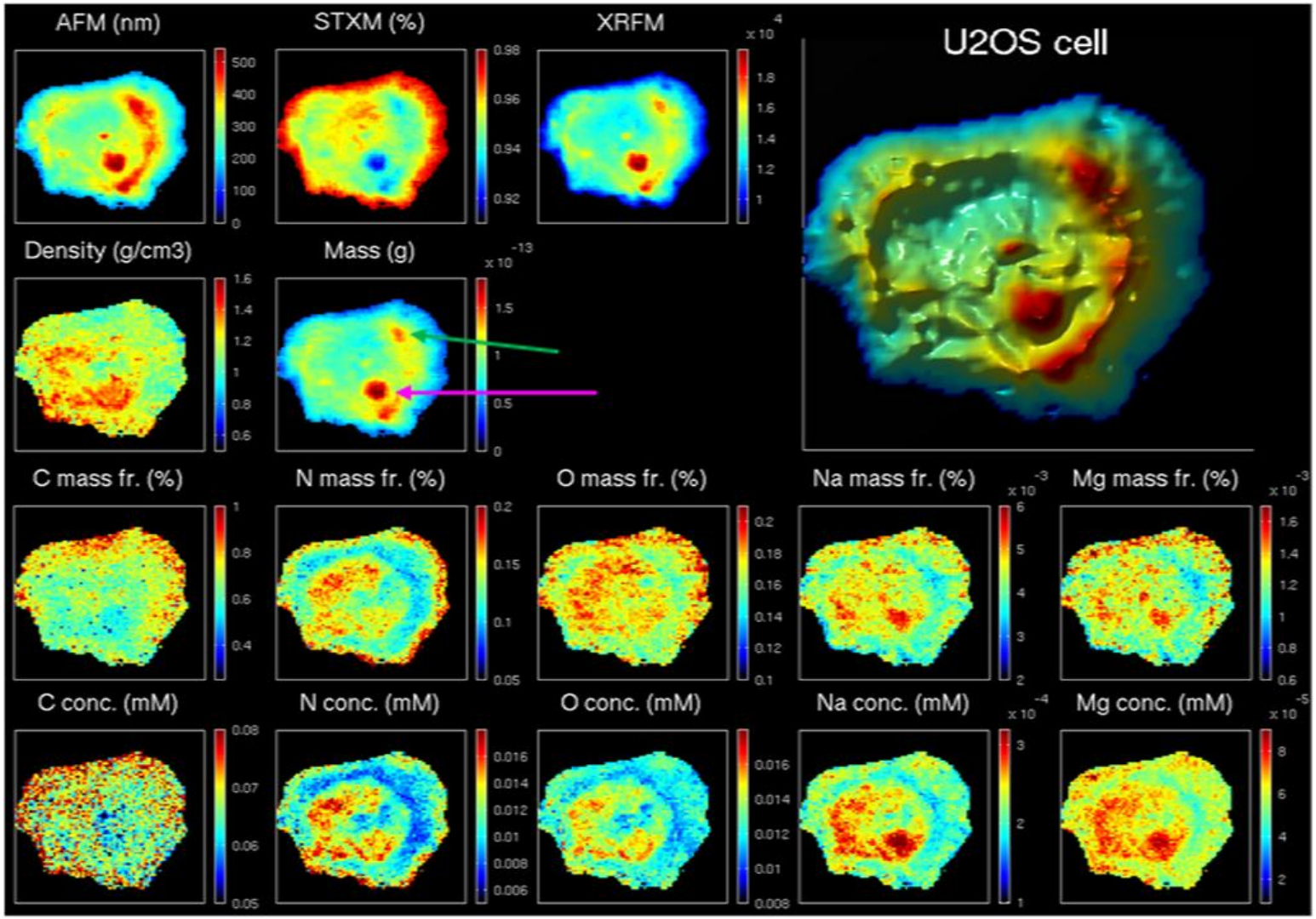




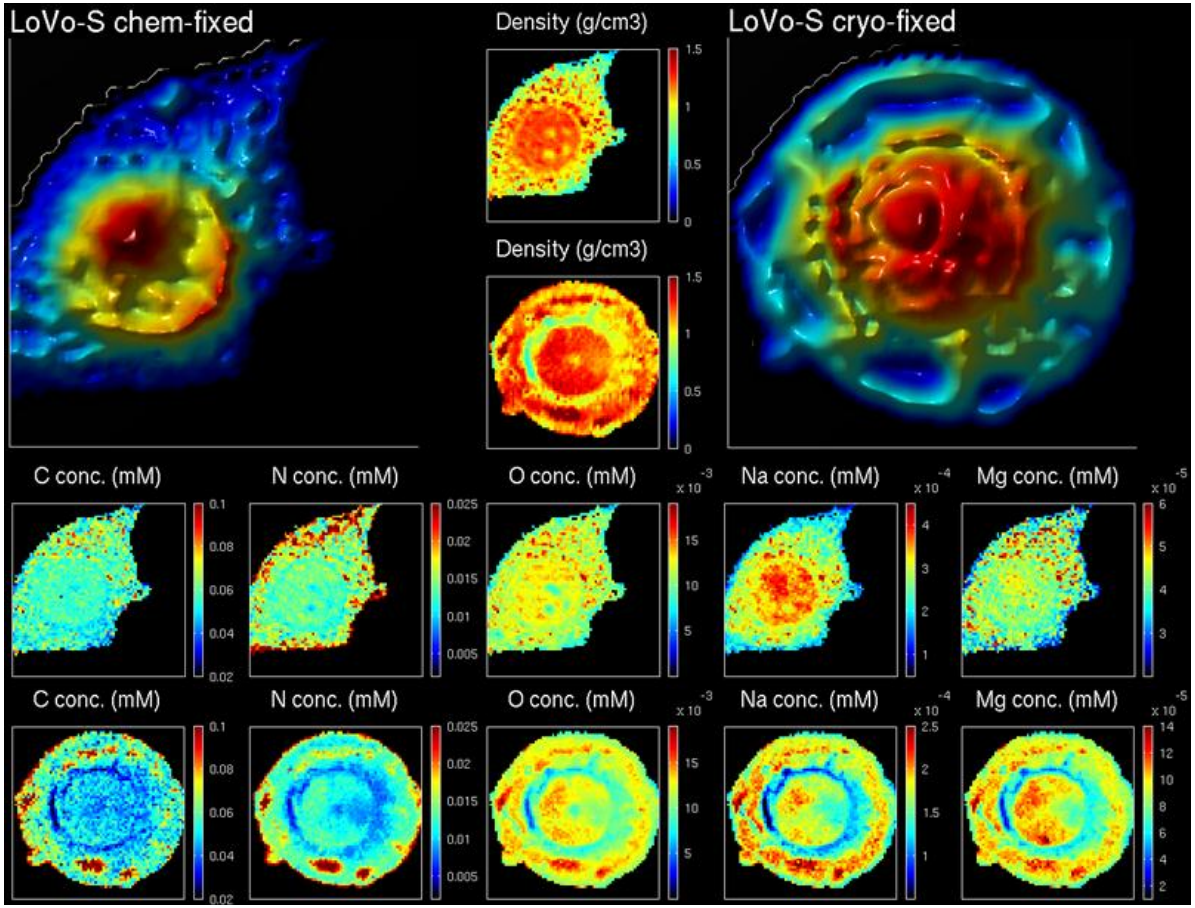




U2OS – Osteosarcoma cells



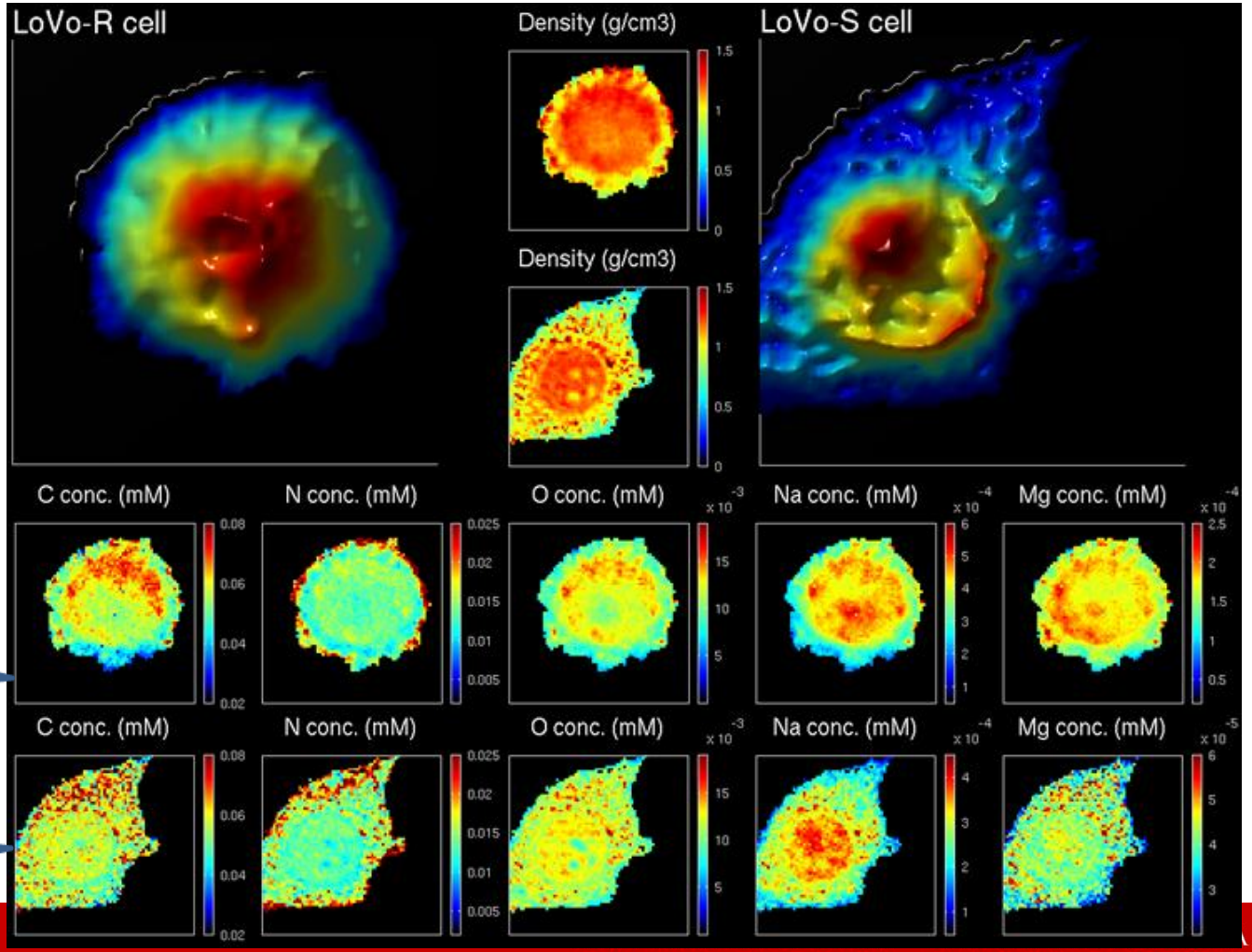
Cell fixation methods



cryofixed cells show a peri-nuclear ring of low density corresponding to endoplasmic reticulum not evident in chemically fixed cells

Effect of doxorubicine resistance on elemental distribution

LoVo cells: human colon carcinoma



LoVo-R

LoVo-S

Single cell analysis: is it a true improvement?

"Isolated material particles are abstractions, their properties being definable and observable only through their interactions with other systems"
Bohr 1934 "Atomic Theory and the description of Nature"

".....on the basis of strictly empirical investigations, that the sheer reversal of our prior analytic dissection of the universe by putting the pieces together again, whether in reality or just in our minds, can yield no complete explanation of the behavior of even the most elementary living system"
Paul Weiss 1973 "The Science of Life"

"When scientists reduce a whole complex system such as cells, genes or even elementary particles to the fondamental elements trying to explain their functions merely in term of the elemental components, they lose the possibility to understand the self-organizing activity of the whole system, hence the peculiar property of the complex systems such as the biological ones"

Filippo Conti in " Moving from the reductionistic to olistic approach in the study of biological systems"

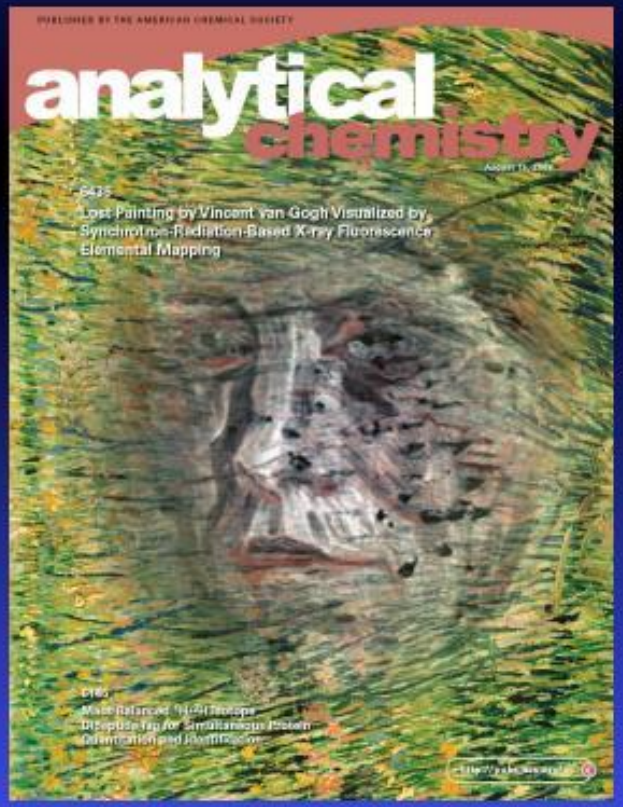
Science and Beauty

The French physicist, mathematician, historian and philosopher of science
Pierre Duhem : Beauty Is a Fundamental Value of Science

Anal. Chem. **2008**, *80*, 6436–6442

Visualization of a Lost Painting by Vincent van Gogh Using Synchrotron Radiation Based X-ray Fluorescence Elemental Mapping

Joris Dik,*,† Koen Janssens,‡ Geert Van Der Snickt,‡ Luuk van der Loeff,§ Karen Rickers,∥ and Marine Cotte⊥,⊗



Vincent van Gogh
Patch of Grass
Paris, 1887

Kröller-Müller Museum
Otterlo, the Netherlands

Patch of grass

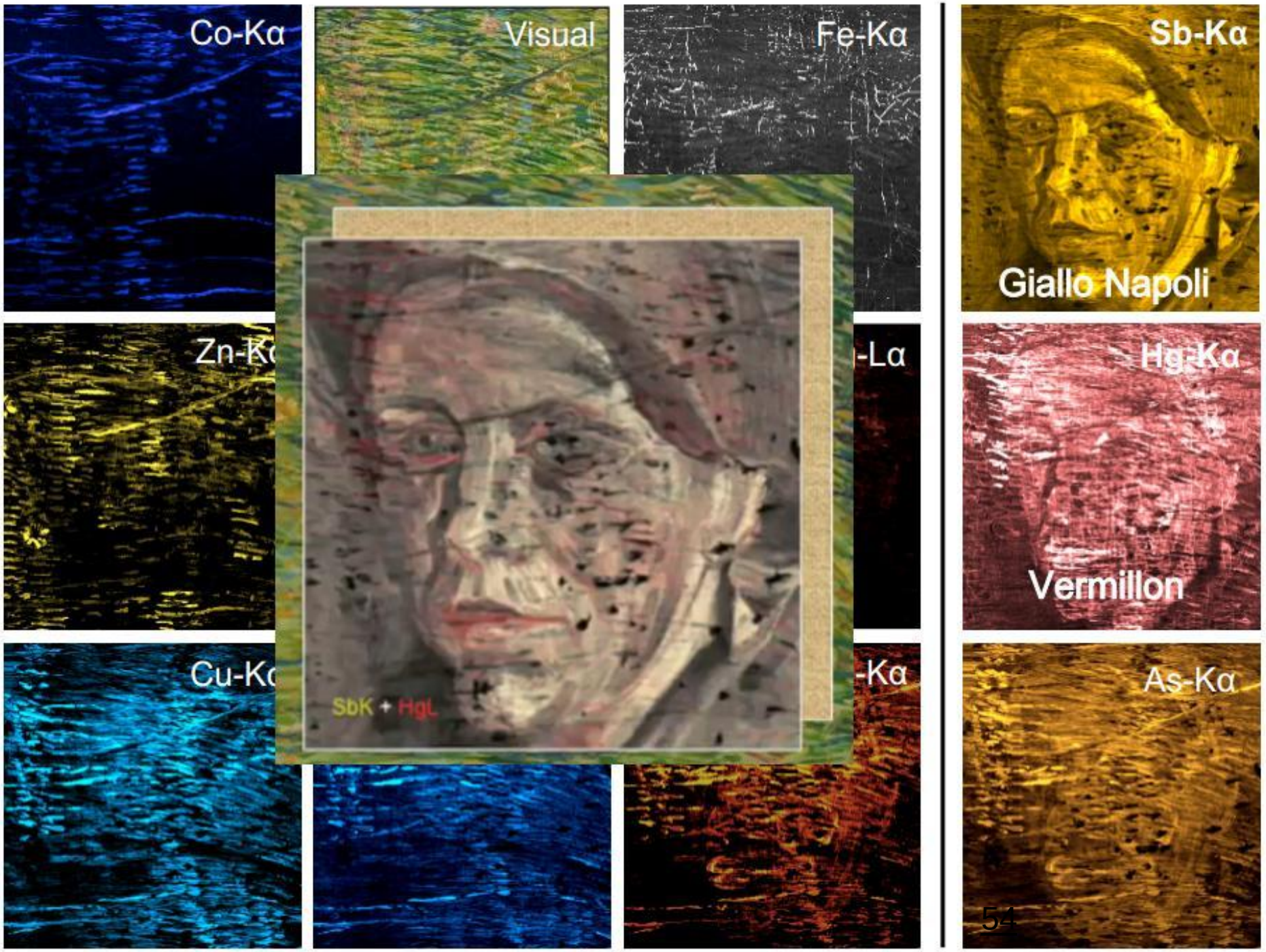
X-ray radiography

Portrait of a woman (1884/85)?

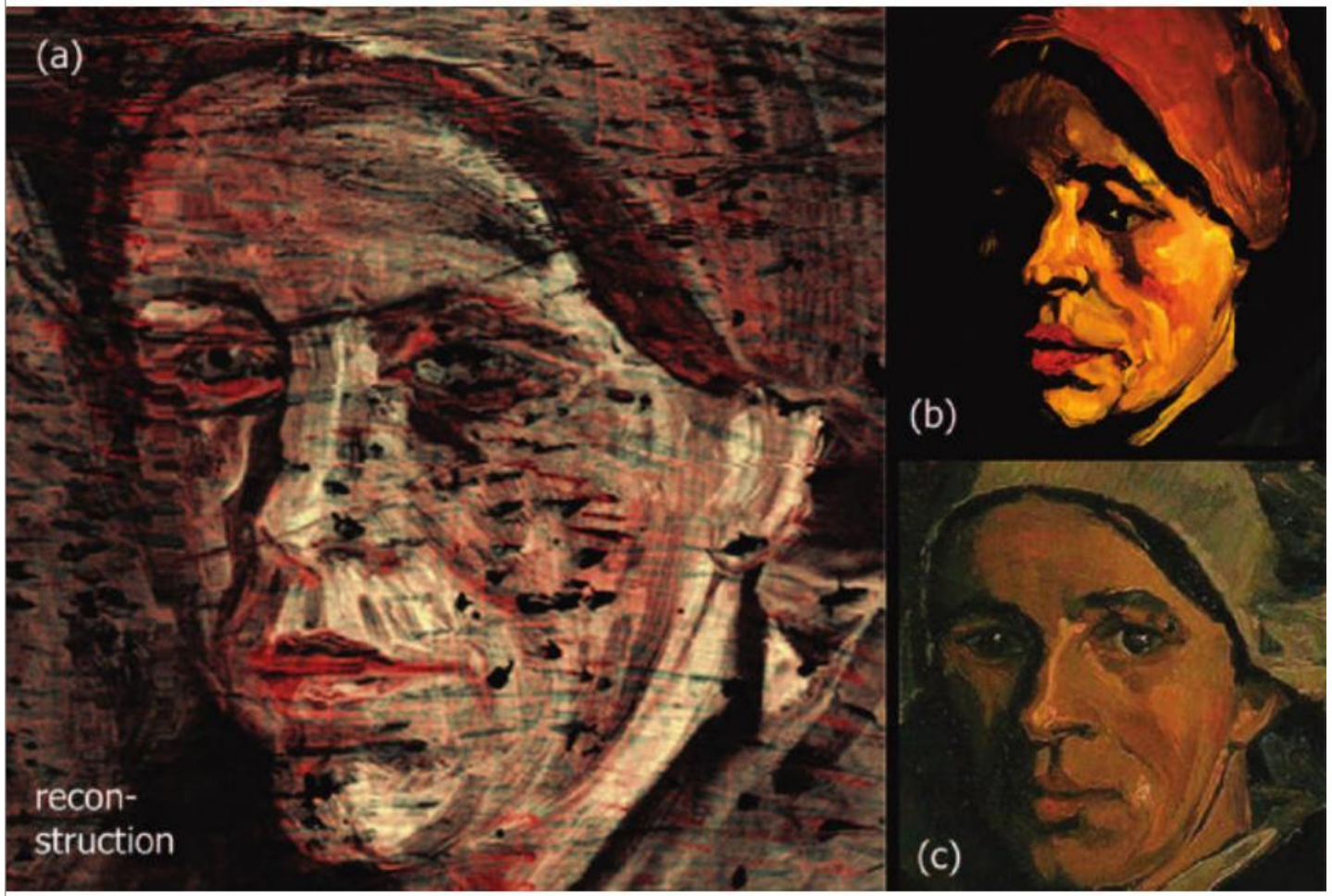
Conventional X-ray radiography has the following limitations:

- it is sensitive only to the heavy metals
- It sees all the painting layers

SR-XRF, to map the distribution of the different elements


Co-Kα
Visual
Fe-Kα
Sb-Kα
Giallo Napoli
Zn-Kα
-Lα
Hg-Kα
Vermillon
SbK + HgL
Cu-Kα
-Kα
As-Kα

WHEN SCIENCE MEETS ART



- Concettina Cappadone
- Emil Malucelli
- Giovanna Farruggia
- Azzurra Sargenti
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