ICP-MS based methods for the quantitative analysis of nanoparticles in biological samples

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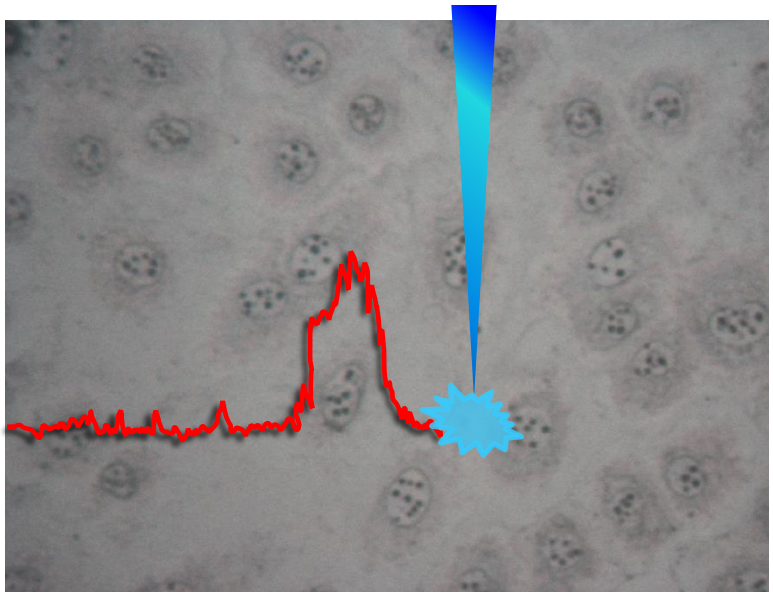
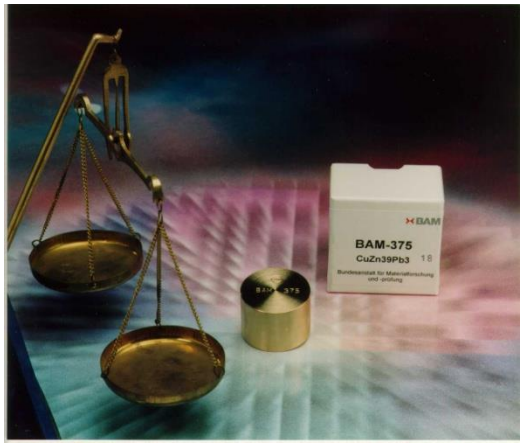


BAM Federal Institute for Materials Research and Testing
Division 1.1: Inorganic Trace Analysis

Outline

- Motivation
- Experimental
- Imaging of metallic nanoparticles (NPs) in single cells
- Elemental imaging of cells
- Conclusions & Outlook



Division: Inorganic Trace Analysis

Inorg. chemical Anal.ysis

- Primary substances
- Elemental trace analysis
- Metallomics
- Isotope analysis




- **Mission:**
- Research and development
- Testing, analysis, approval, and certification
- Consultation, information, and advice

Motivation

- Element content of cells and intracellular distribution

  → understanding of cell physiology

- Cellular uptake of nanoparticles and localisation within cells

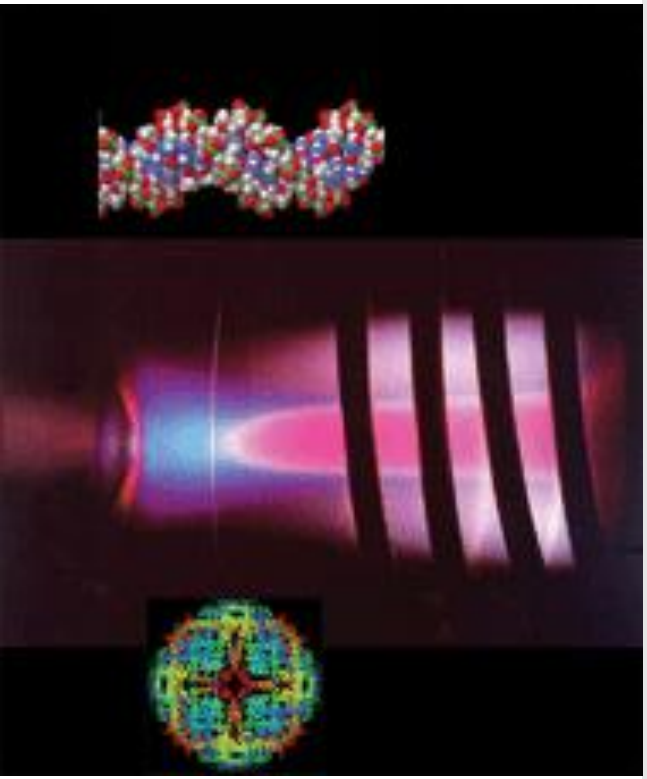
  → medical application and risk assessment of NPs

- But usually average element / nanoparticle content in digested samples with ICP-OES/MS

  → no information about cell heterogeneity and intracellular distribution

→ Determination of naturally occurring elements and nanoparticles (Ag, Au) in single cells
Development of an elemental microscope using imaging by LA-ICP-MS!
Quantification schemes are missing for single cell analysis

Inductively Coupled Plasma Mass Spectrometry – ICP-MS



- Is a mass spectrometric method
- The ion source is a hot plasma (8.000 °C)
- Most elements of the periodic are highly (>50 %) ionized: Multi-element coverage is high!
- The sample (biomolecule) is fully decomposed and atomized; Ionization is independent of matrix!
- **The ICP-MS is nothing else than an atom counter!**
- **Calibration by liquids/standards thus quantification of metals/nanoparticles in biosystems is possible**
- Sensitivity (LOD low pg g^{-1} range, fmol/amol)
- High dynamic range (12 ord. of mag)
- In combination with a **laser ablation** system: analysis of soft materials (tissues, cells)

MOTIVATION (VISION):

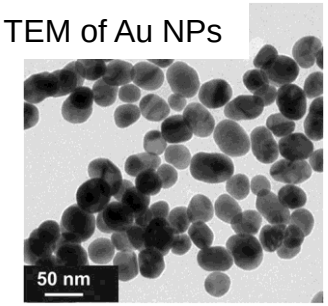
Development of a quantitative elemental microscope with cellular resolution!

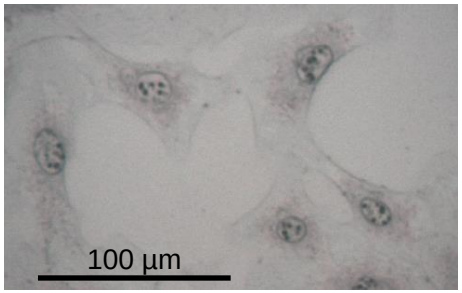
Sample preparation

Ag & Au nanoparticles

- Synthesized by citrate-reduction of $AgNO_3$ or $HAuCl_4$
- Mean diameter Ag NPs 50 ± 15 nm, Au NPs 25 ± 5 nm

TEM of Au NPs





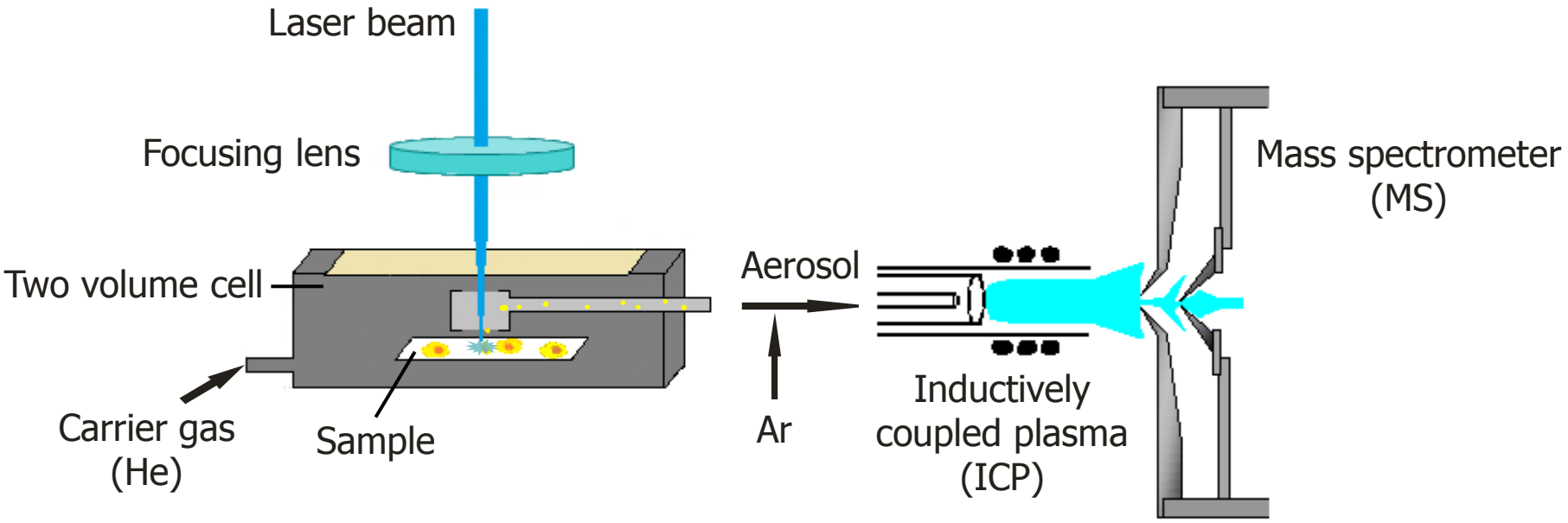
Fibroblast cells (mouse, cell line 3T3):
adherent cells (monolayer) growing on glass slide

Incubation with Ag or Au NPs
in cell culture medium for 3 to 24 h

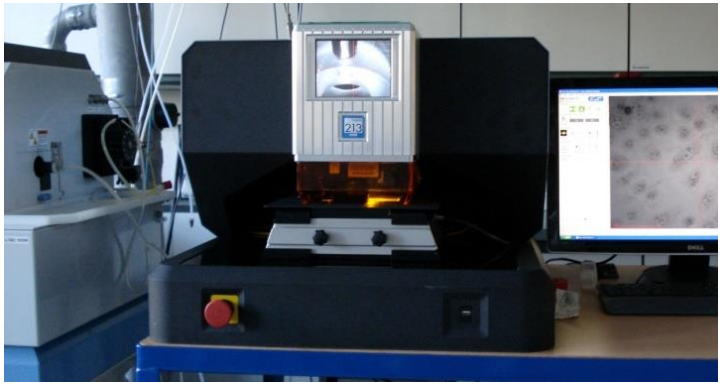
Washing with PBS buffer, fixation with 4 % para-formaldehyde
and drying in graded series of ethanol

Imaging using LA-ICP-MS

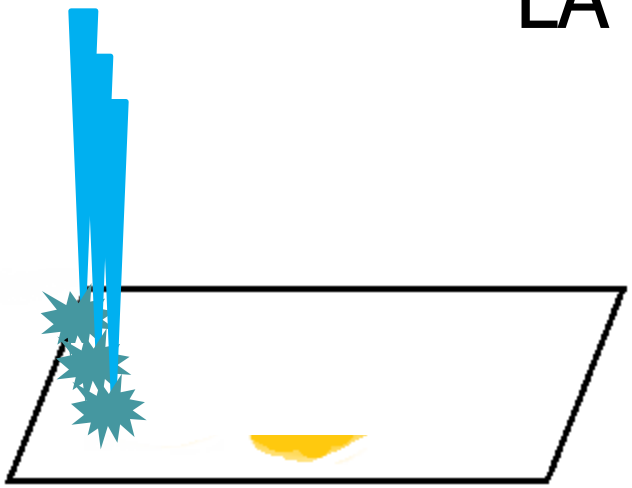
Schematic set-up of LA-ICP-MS

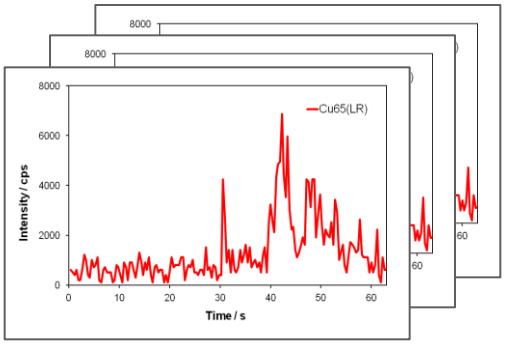


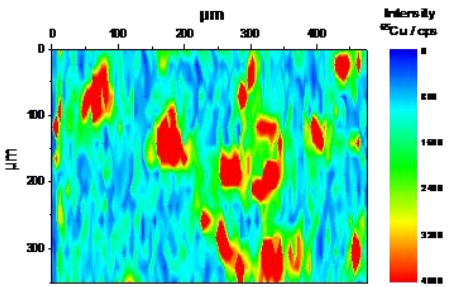
NWR-213 (ESI)
coupled to ICP-SFMS Element XR
(Thermo Fisher Scientific)



LA parameters







Countinuous line scanning → Single line scans → Contour plot

Time scale → µm scale

High intensity: (green)red

Low intensity: blue

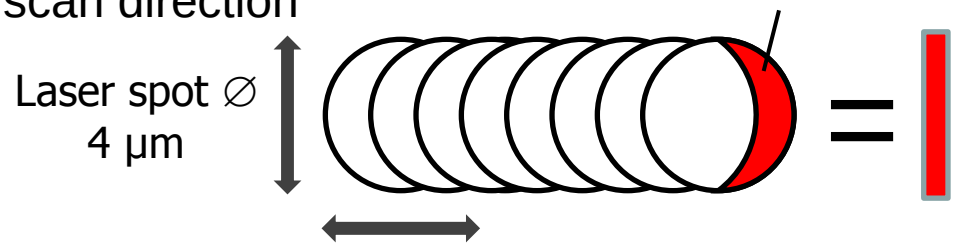
- Complete ablation with one laser shot & minimum background signal
- Minimum influence on neighbouring areas
- Overlap of laser spots

  → Improved spatial resolution in scan direction

Pixel

4 x 0.5 µ

Ablated sample

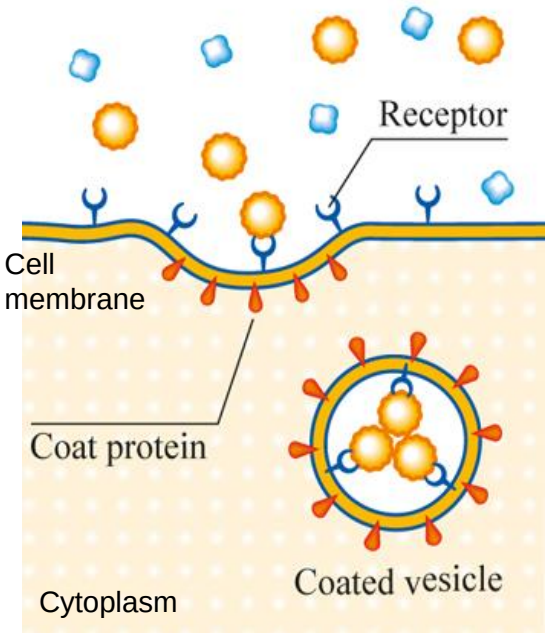


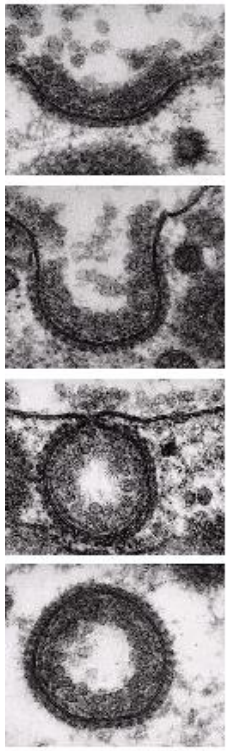
Scan rate 5 µm/s, repetition rate 10 Hz

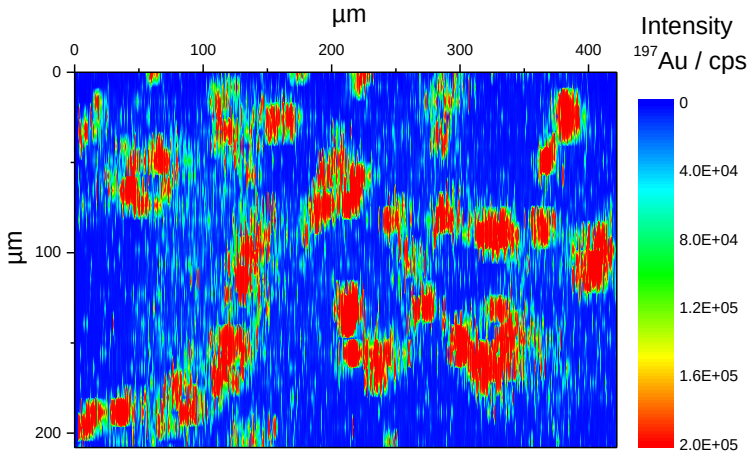
L. Mueller, H. Traub, N. Jakubowski, D. Drescher, J. Kneipp, V. I. Baranov "Trends in Single Cell Analysis by ICP-MS", (2014) ABC 406, 6963-6977, DOI 10.1007/s00216-014-8143-7

Cellular uptake mechanism of NPs

Nanoparticle uptake by endocytosis, localisation in vesicles





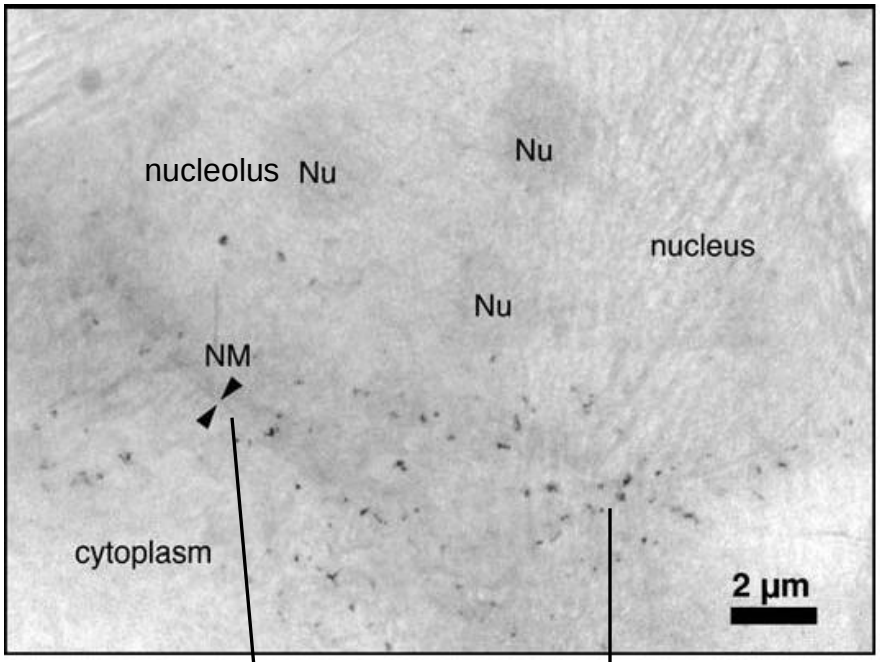


http://en.wikipedia.org/wiki/Endocytosis
http://library.thinkquest.org/C004535/different_cell_types.html

- Laser ablation provides cellular resolution
- Sensitivity is sufficient for nano-particle detection in single cells
- Thus „proof of principle“ experiments started

Comparison with X-ray microscopy & TEM

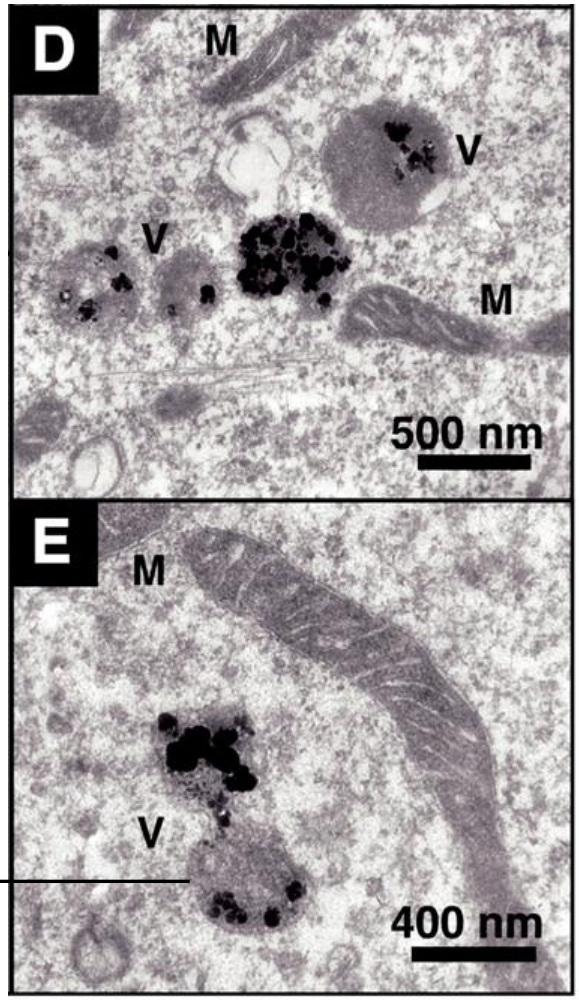
Synchrotron X-ray microscopy



nuclear membrane (NM)

nanoparticles & particle aggegates (black spots)

TEM



Mitochondrion (M)

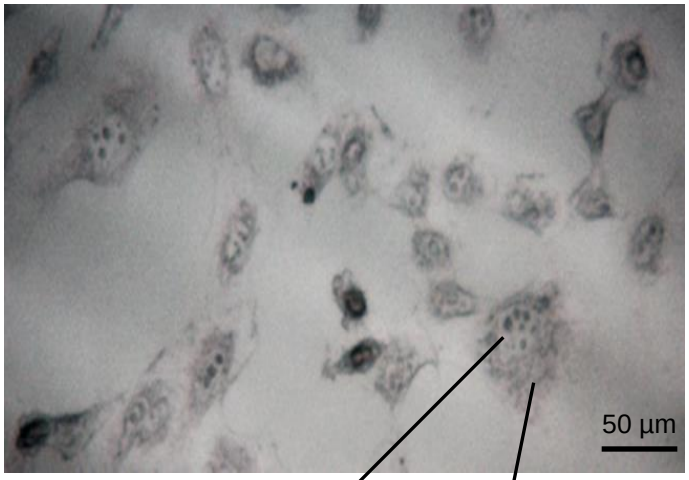
Vesicle (V) with nanoparticles

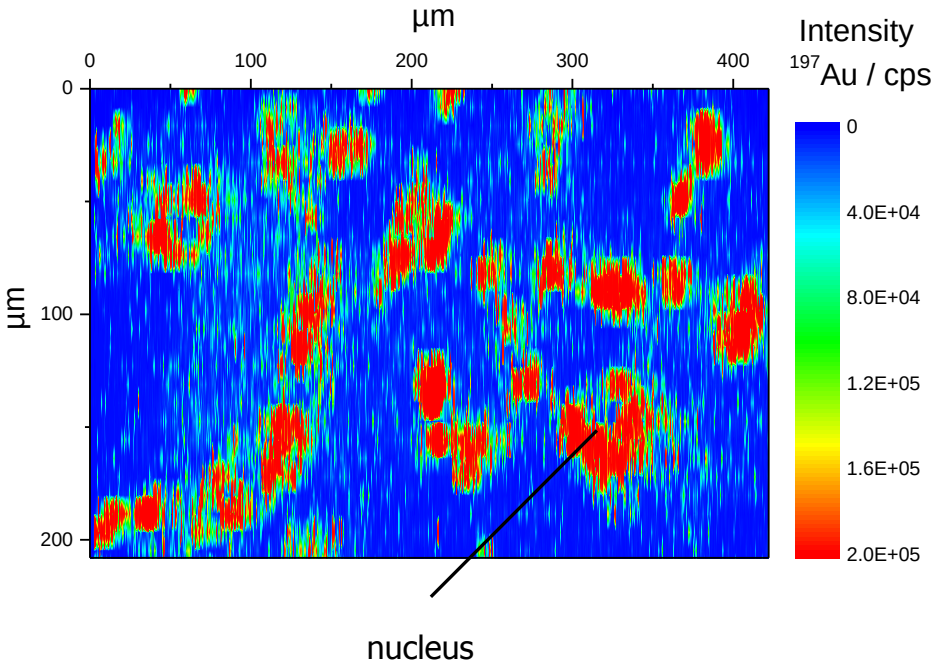
D. Drescher, P. Guttmann, T. Büchner, S. Werner, G. Laube, A. Hornemann, B. Tarek, G. Schneider, J. Kneipp, Nanoscale, 2013, 5, 9193-9198

Uptake of Au NPs by fibroblast cells

- Incubation with Au NPs (100 pM) for 6 h
- Line scan, spot ∅ 8 µm, scan rate 4 µm/s, repetition rate 20 Hz, energy 42 %

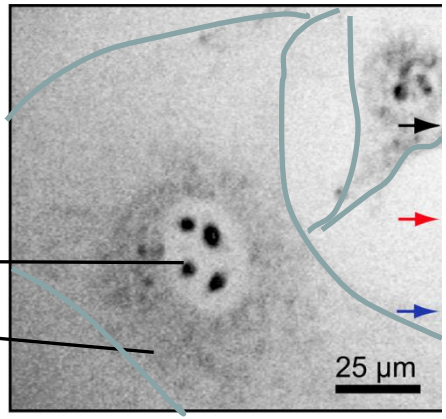
Bright field image before ablation





Imaging of NPs inside a single fibroblast cell

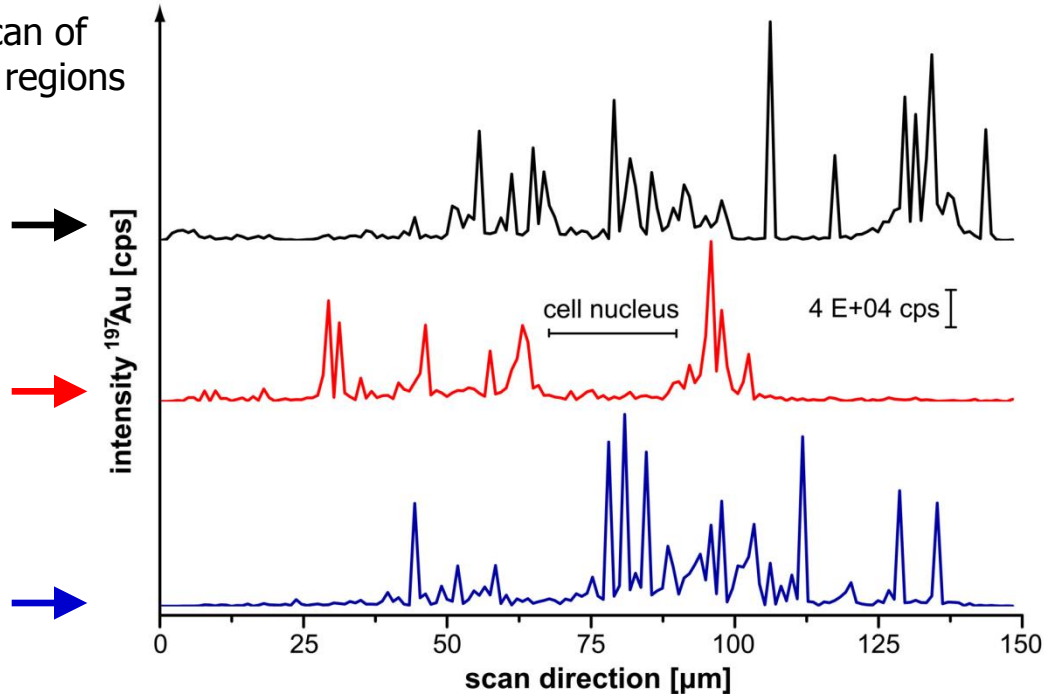
Bright field image



Au
(25 nm ± 5 nm)
100 pm / 3 h

LA-ICP-MS image of the Au intensity distribution (in cps), pixel size 6 x 1 µm

Single line scan of different cell regions



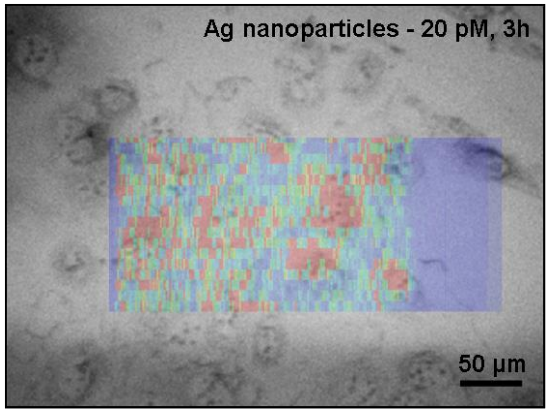
Nanoparticle detected surrounding the nucleus, but our methode integrates over a volume (voxle), thus no depth resolution is availbale

LA parameter:
line scan,
spot ∅ 4 µm,
line distance 6 µm,
scan rate 5 µm/s,
repetition rate 10 Hz,
fluence 0.8 J/cm^2

D. Drescher et al., Anal. Chem., 2012, 84, 9684-9688

Application: Treated cells: Up-take of nanoparticles by single cells

^{109}Ag distribution in fixed 3T3 fibroblast cells superimposed on the corresponding bright field images



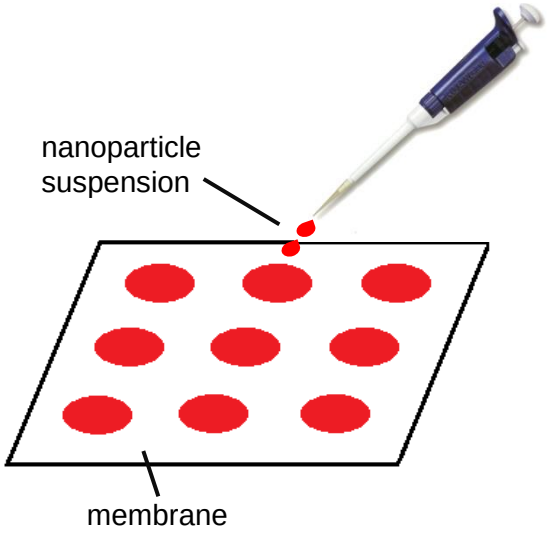
Ag
(50 nm ± 15 nm)

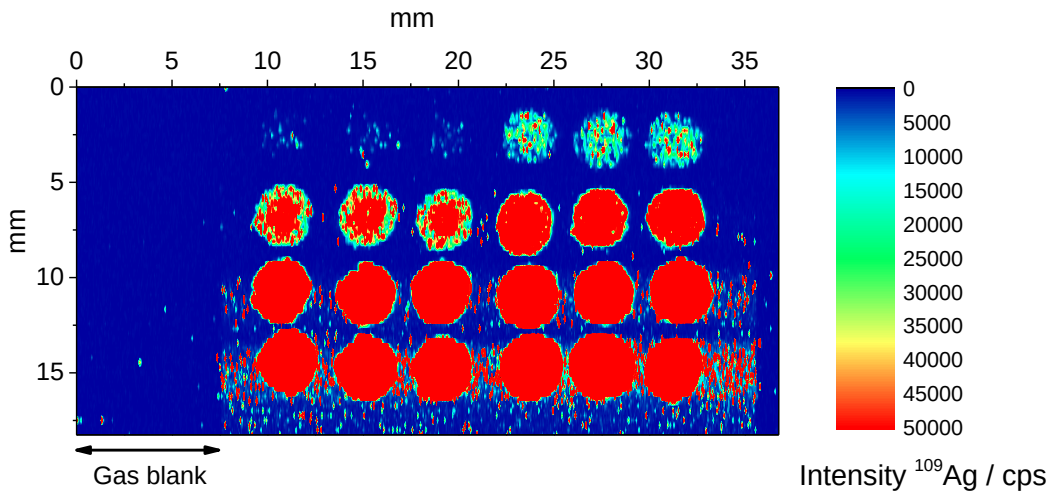
Cells were incubated with Ag nanoparticles in a concentration of 0.2 to 20 pM for 3 or 24 hours.

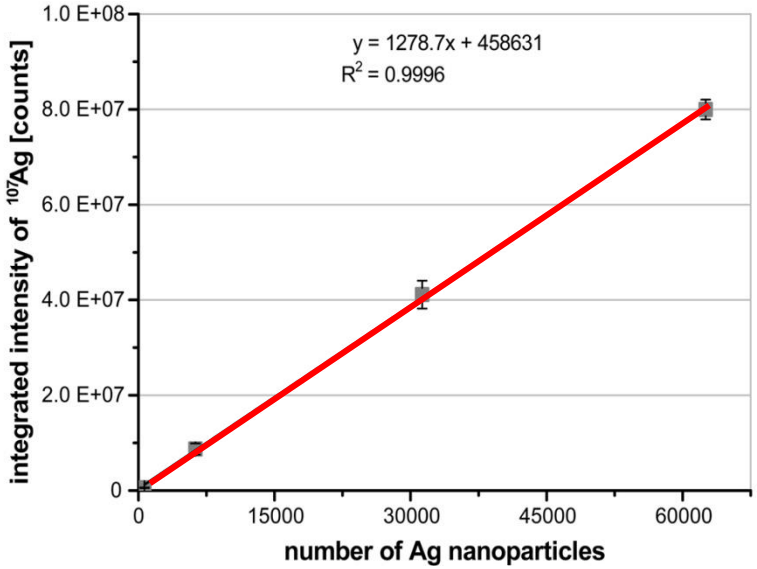
- Laser parameters: Laser spot size **8 µm; rep. rate 10 Hz; scan speed 5 µm/s**

- Up-take of Ag (and Au) nanoparticels by single fibroblast cells is demonstrated
- Agglomerates close to but not inside the nucleus are observed

Calibration with NP suspension on membrane







LA parameter:
line scan, spot ∅ 250 µm,
line distance 250 µm, scan rate 250 µm/s,
repetition rate 20 Hz, fluence 0.8 J/cm^2

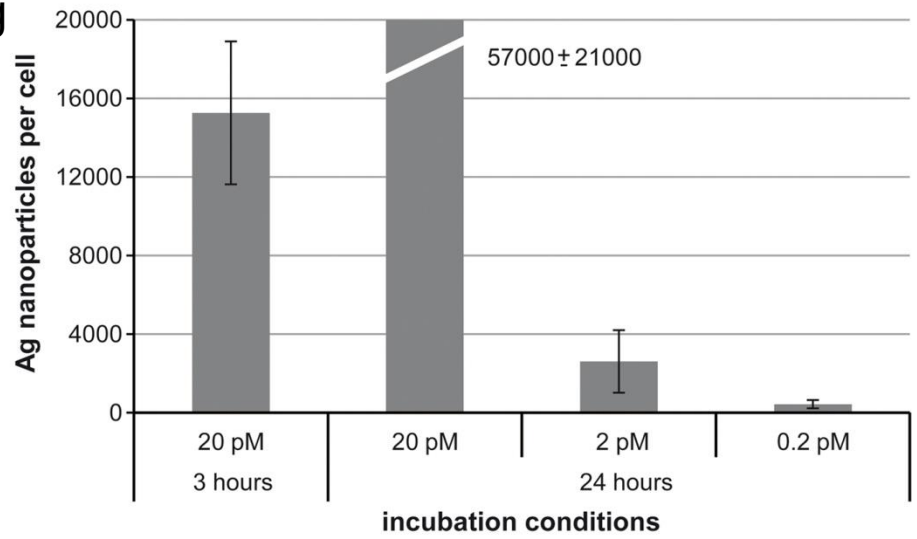
LOD ~ 10 to 50 nanoparticles per spot

Quantification of NPs in single fibroblast cells

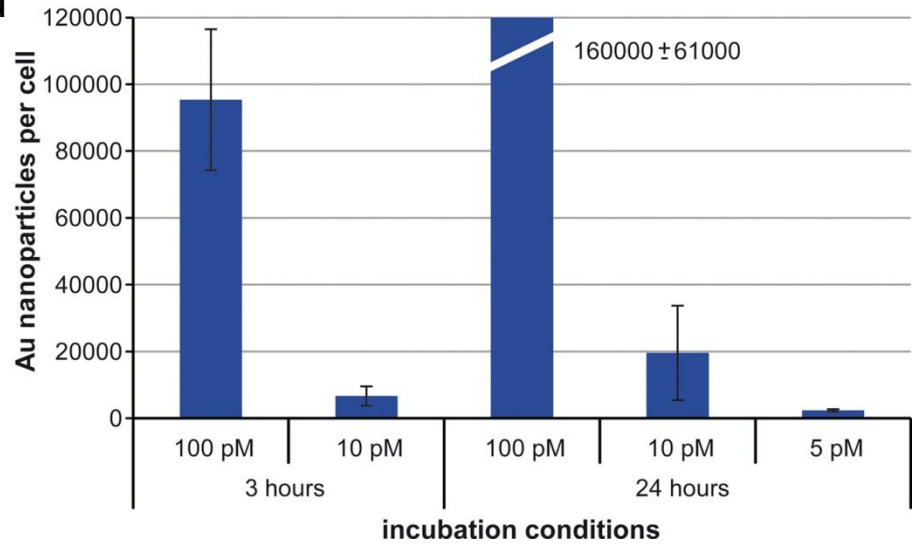
- Incubation with Ag or Au NPs at different concentration levels for 3 or 24 h
- integration over 6 to 10 cells

By use of calibration integrated intensity is converted into number of nanoparticles per cell. The number per cell depends on the concentration in the cell culture medium and on the incubation time

Ag

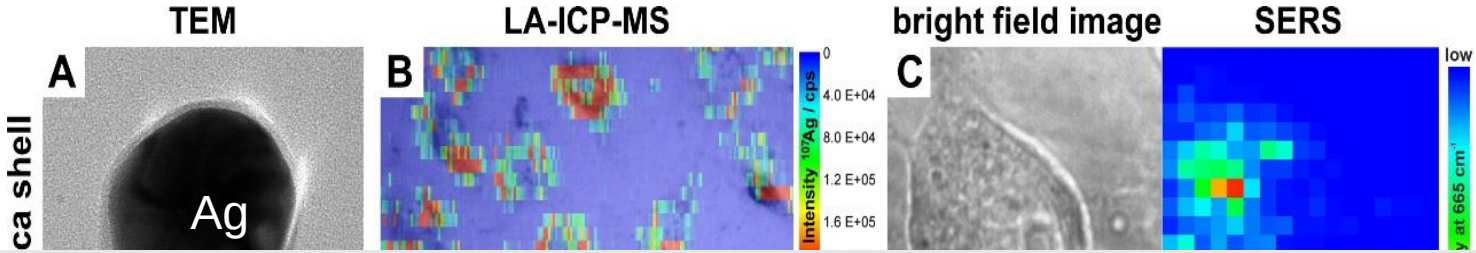


Au



D. Drescher, C. Giesen, H. Traub, U. Panne, J. Kneipp, N. Jakubowski, Anal. Chem., 2012, 84, 9684-9688

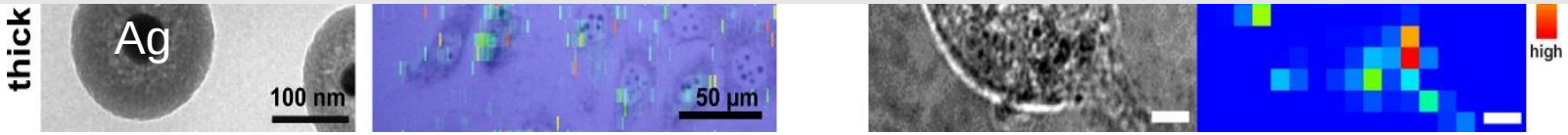
Combination of ICP-MS and Surface Enhanced Raman Spectroscopy (SERS)



665 cm^{-1} (ν(C-S) of cysteine)

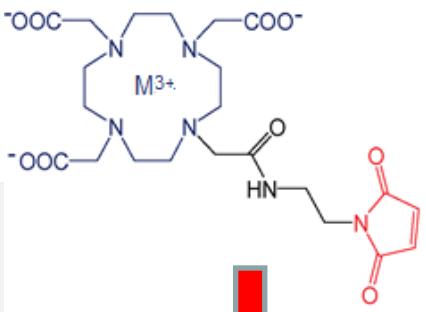
D. Drescher , I. Zeise, H. Traub, P. Guttmann, S. Seifert, T. Büchner, N. Jakubowski, G. Schneider, and J. Kneipp, "In situ Characterization of SiO_2 Nanoparticle Biointeractions Using BrightSilica" (2014) Adv. Funct. Mat., 24, 3765-3775.

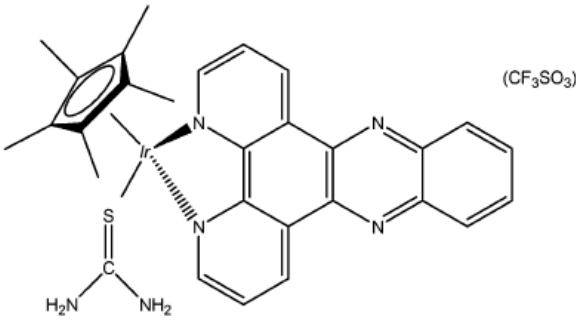
L. Mueller, H. Traub, N. Jakubowski, D. Drescher, J. Kneipp, V. I. Baranov "Trends in Single Cell Analysis by ICP-MS", (2014) ABC 406, 6963-6977, DOI 10.1007/s00216-014-8143-7



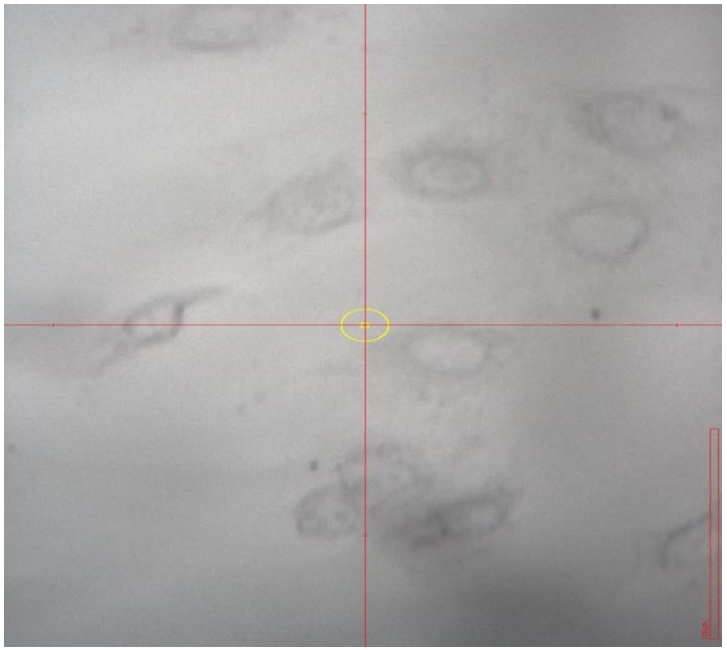
Combination of LA-ICP-MS micro-mapping with Raman micro-spectroscopy provides chemical information from both, the nanoparticle and the biological system. Thus the distribution and quantity of nanoparticles in single cells can be measured to understand their intracellular processing and cytotoxic behaviour.

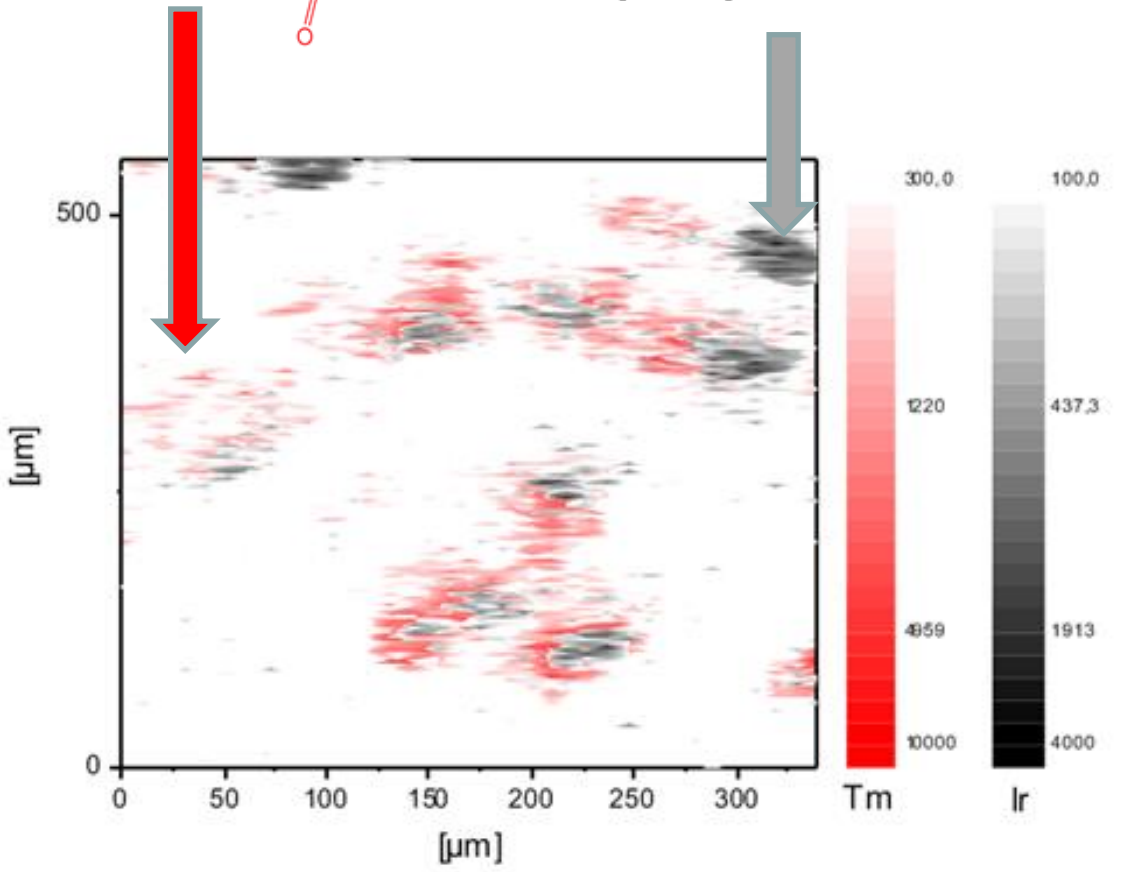
Staining of cells by mDOTA(Tm-red) and intercalator (Ir-black)





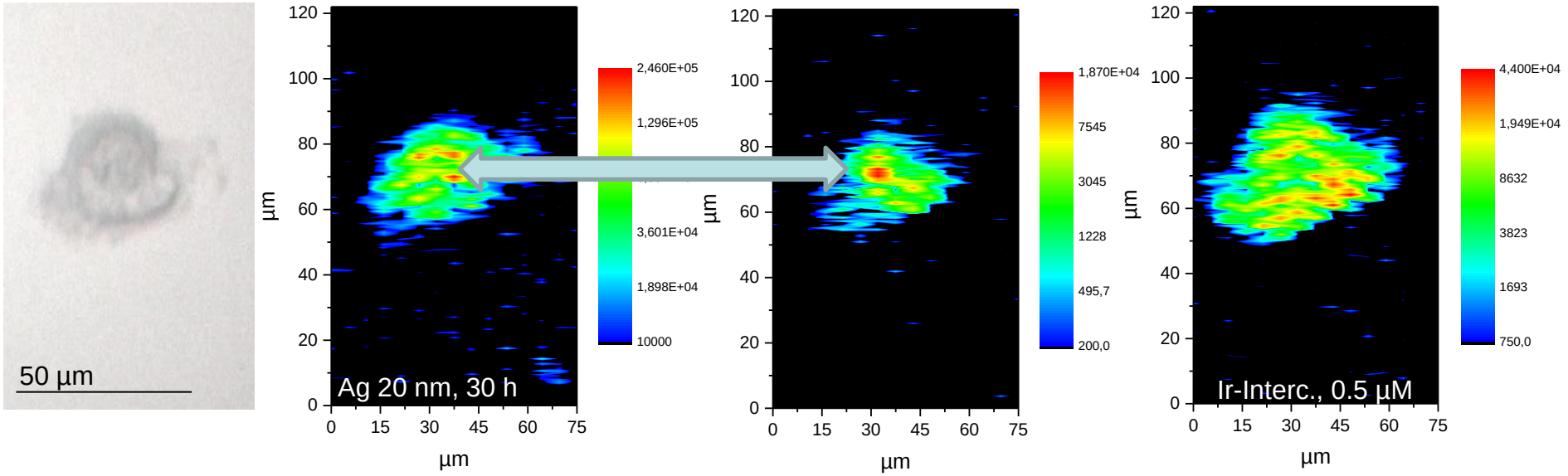
Total amount per cell:
Intercalator: 81 fg (±16 fg)
mDOTA: 400 fg (±16 fg)





Single cell analysis: simultaneous detection of nanoparticles, proteins and nucleus

A549 Q-nano, Ag 20 nm NP 30 h incubation, Ir-Intercalator 0,5 µM, mDOTA(Tb) 10 µM, 6 µm laser spot size, 5 Hz repetition
(Herrmann_LA_Zellen_050215_07)



- Again, Ag agglomerates are enriched around the nucleus
- mDOTA intensity is a measure for protein concentration and will be used as internal standard

Conclusions & Outlook

- **LA-ICP-MS is a new analytical tool to localize and quantify metals (Cu, Zn), Ag- and Au-nanoparticles in single cells**
- **Combination of multimodal spectroscopies can provide quantitative and chemical information**
- **Development of a quantitative elemental microscope looks feasible**

Outlook

- Development of multimodal spectroscopies
- Validation of the quantification strategy using ICP-MS after digestion of cell suspension
- Application to other metallic nanoparticle and cell systems
- Combination with immuno-assays to measure protein expression during nanoparticle uptake (DFG Project!).
- New LA system $\rightarrow$ Impovement of spatial resolution

….and partners

- Janina Kneipp, Daniela Drescher, Tina Büchner – HUB

- Jutta Tentschert, Harald Jungnickel,
- Andrea Haase

Risiken erkennen – Gesundheit schützen