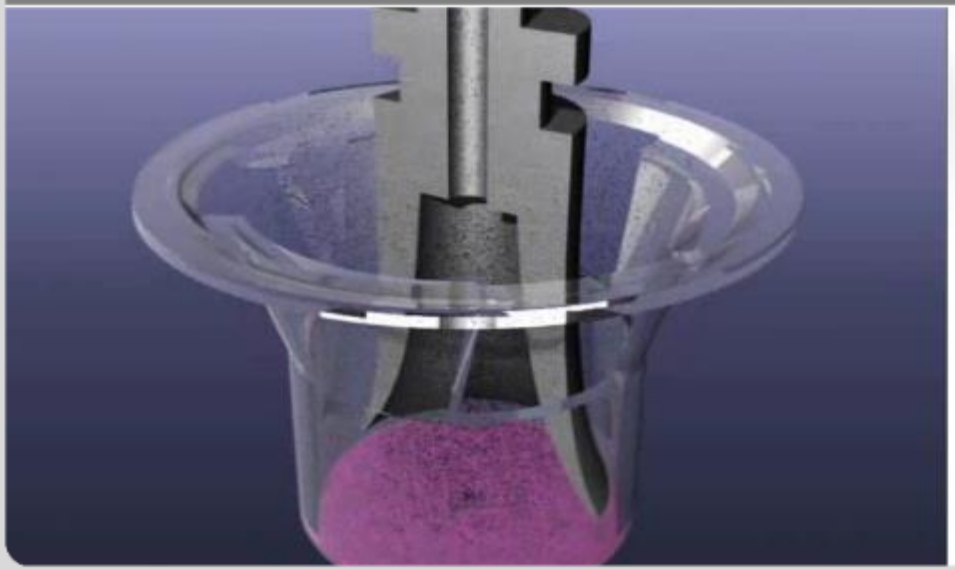
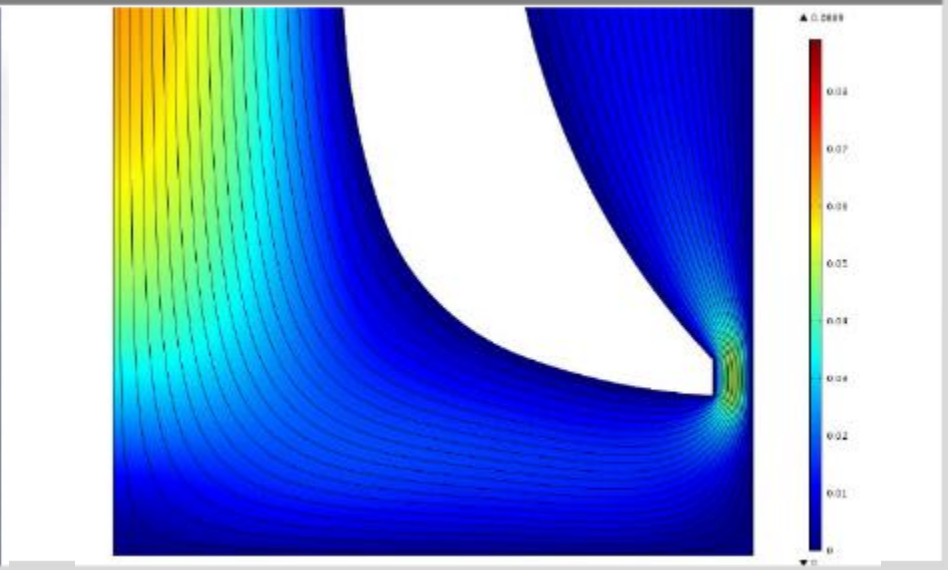
TESTING OF AEROSOLS FOR LUNG TOXICITY BY IN-VITRO STUDIES AT THE AIR-LIQUID INTERFACE FOR UP TO 24 HOURS

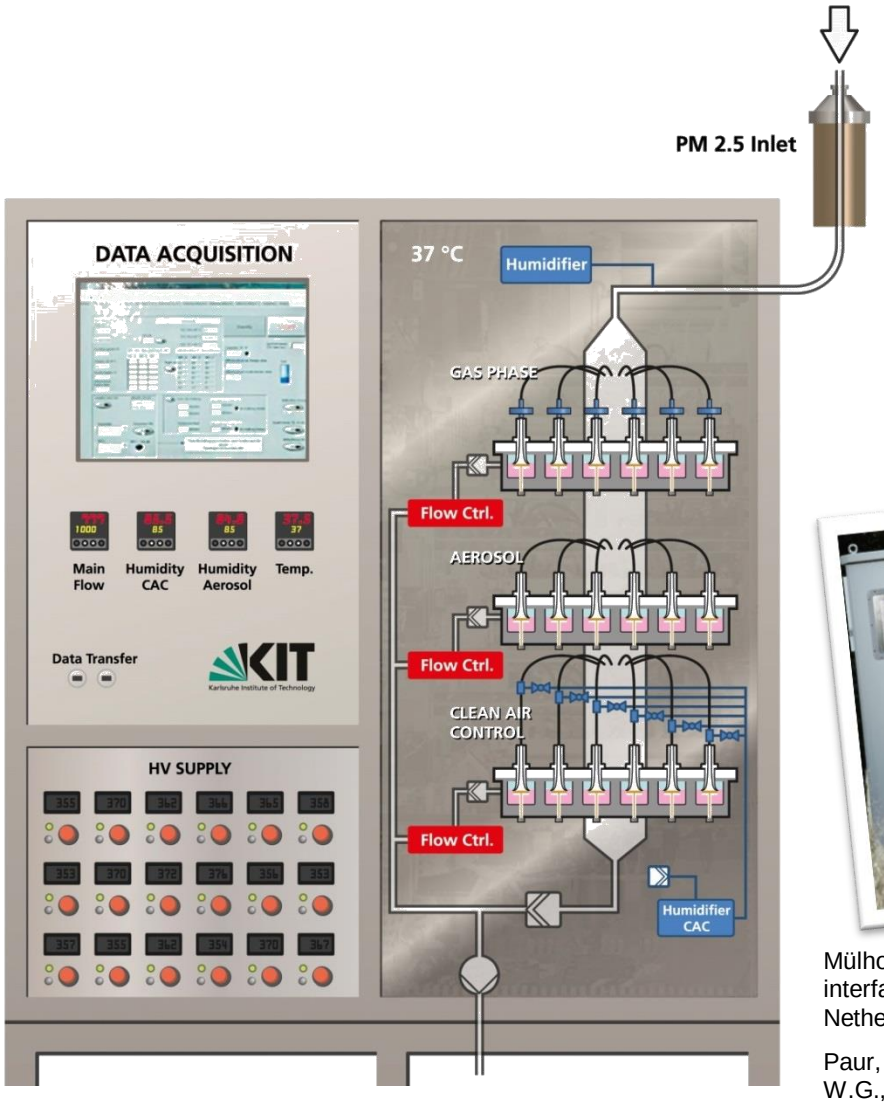
[1]S. Mülhopt, [2]S. Diabaté, [2]M. Dilger,[3]C. Schlager, [3]M. Berger, [3]T. Krebs, [1]H.-R. Paur, [2]C. Weiss, [1]D. Stapf

[1] Institute for Technical Chemistry [2] Institute of Toxicology and Genetics [3] VITROCELL Systems GmbH





The Karlsruhe Exposure System



Mülhopt, S.; Paur, H.R.; Diabate, S.; Krug, H.F. In vitro testing of inhalable fly ash at the air liquid interface. Kim, Y.J. [Hrsg.] *Advanced Environmental Monitoring* Dordrecht : Springer Netherlands, 2007 S.402-14 ISBN 978-1-4020-6363-3

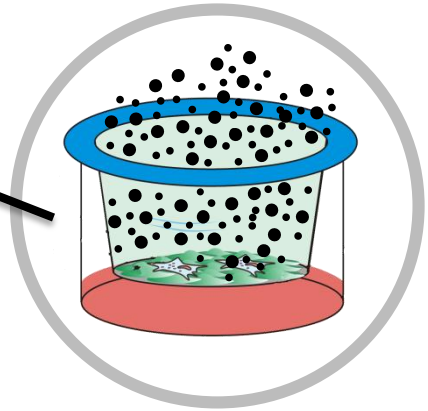
Paur, H.-R., Cassee, F.R., Teeguarden, J., Fissan, H., Diabate, S., Aufderheide, M., Kreyling, W.G., Hänninen, O., Kasper, G., Riediker, M., Rothen-Rutishauser, B., Schmid, O. (2011) Journal of Aerosol Science, 42, 10, 668-692

VITROCELL® automated *in-vitro* exposure station

A lab scale measurement system for the air-liquid interface exposure of human lung cell cultures towards airborne nanoparticles.

- Direct aerosol sampling
- Online dose measurement
- Electrostatic deposition enhancement
- Flow, temperature, and humidification control system
- Data acquisition

- Internal negative control using humidified synthetic air
- Quality assurance by automatic leak test
- Standard exposure protocol is automatically performed by the programmable controller



Mülhopt et al. (2016) *Journal of Aerosol Science, 96*, 18

Patents:
Mülhopt, S.; Paur, H.R.; Wäscher, T.
DE-OS 10 2007 013 938 (2008.09.25)
Mülhopt, S.; Paur, H.R.; Schlager, C.
DE 10 2014 118 846 B4 (2016.06.23)

Aerosols

Manufactured materials

Metals/metal oxides:

Titania

- Aeroxide P25

Silica

- Amorph:
  - Aerosil200
  - Stöber synthesised
- Quartz

Cerium oxide

Silver

Platinum

CNT

Combustion derived aerosols

- **Ship diesel**
  - Diesel fuel
  - Heavy fuel oil
- **Wood combustion**
  - Log wood stoves
  - Pellet boiler
  - Log wood boiler
- **Car emissions**
  - Diesel fuel
  - Driving behaviour
- **Municipal waste incinerator**
- **Nanocomposites of polymer and MNM fillers**

Endpoints

Cell systems

Epithelial cells:
- A549 (alveolar)
- BEAS-2B (bronchial)
- 16HBE14o (bronchial)

Macrophages:
- RAW264.7 (mouse)

2D:
- Co-culture with THP-1 macrophages

3D:
- Triple cultures with THP-1 macrophages and HUVEC endothelial cells

Primary cells:
- MucilAir (Epithelix)

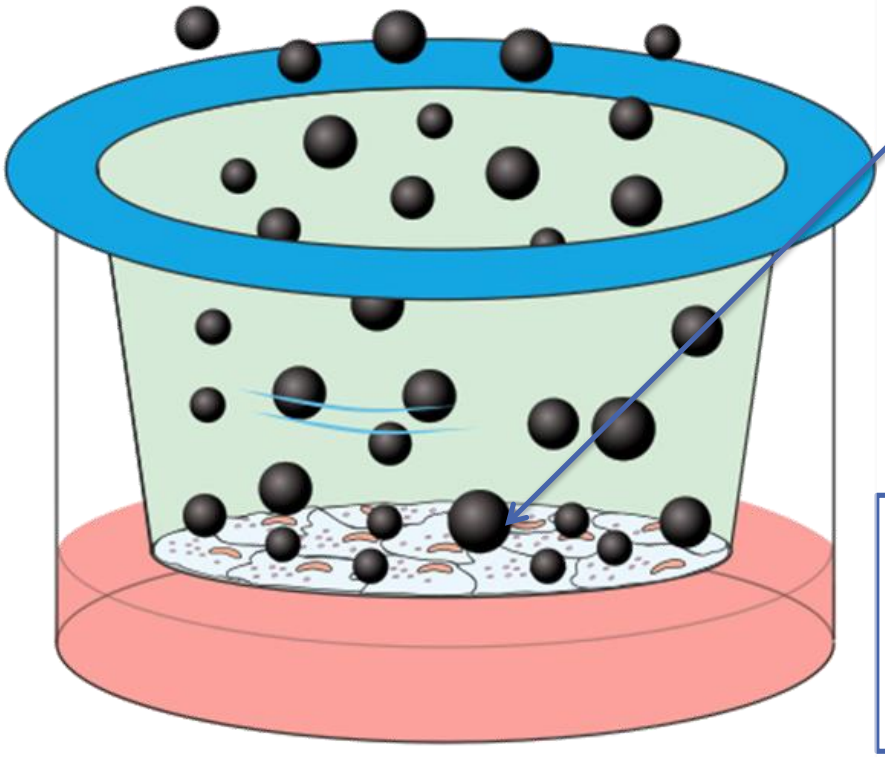
Read out

- **Oxidative stress:**
  - HMOX-1 (WB, RT-qPCR)
  - GSH/GSSG
- **Inflammation**
  - Cytokines (ELISA, RT-qPCR)
- **Cytotoxicity**
  - LDH release, metabolic activity (Alamar Blue, MTS)
- **Genotoxicity**
  - DNA strand breaks (alkaline unwinding, γ-H2Ax expression)
  - high-throughput RT-qPCR with selected gene sets[1]
- **Metabolism of foreign substances**
  - Expression of CYP1A1
- **Genome-wide RNA analysis**
  - RNA-seq (genome-wide analysis)

[1] Fischer et al. Arch. Toxicol. (2016)

Dosimetry at the Air-Liquid Interface

Fluid: gas (air)
Viscosity ~ 1 µPa*s
Density ~ 10^{-3} g/cm³



Airborne particles:
Number concentration c_N [1/cm³]
Mass concentration c_M [µg/cm³]

Particles on cell surface
Number concentration c_N [1/cm²]
Mass concentration c_M [µg/cm²]

Relevant in vitro dose

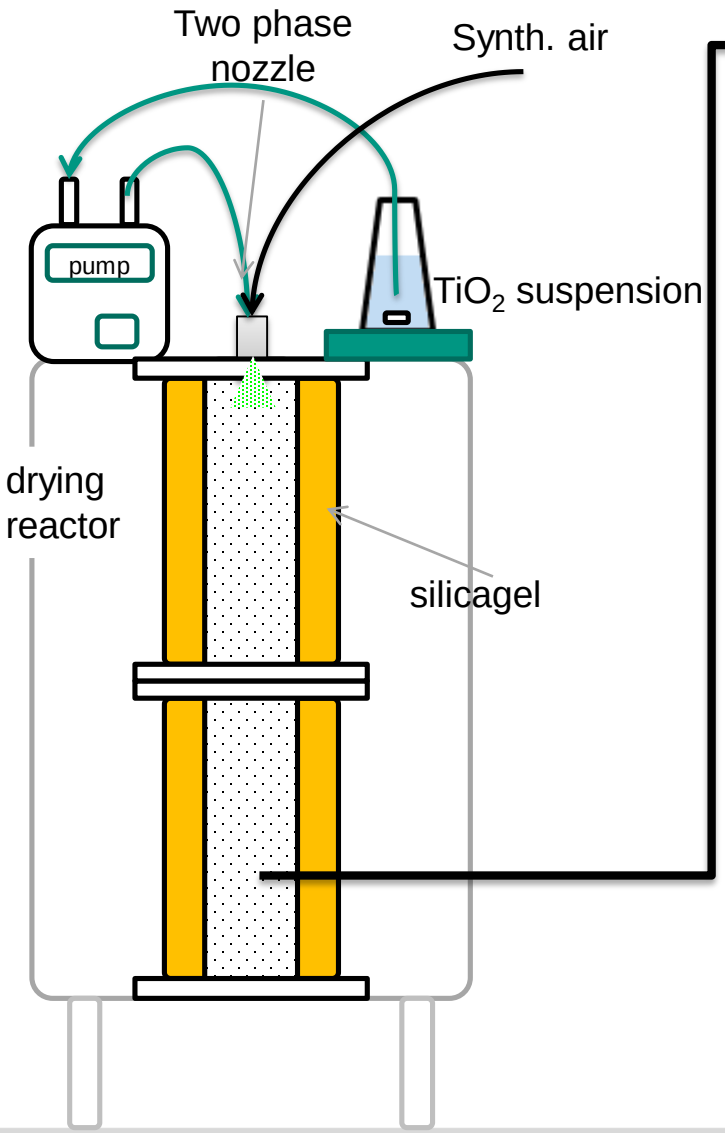
Deposition efficiency

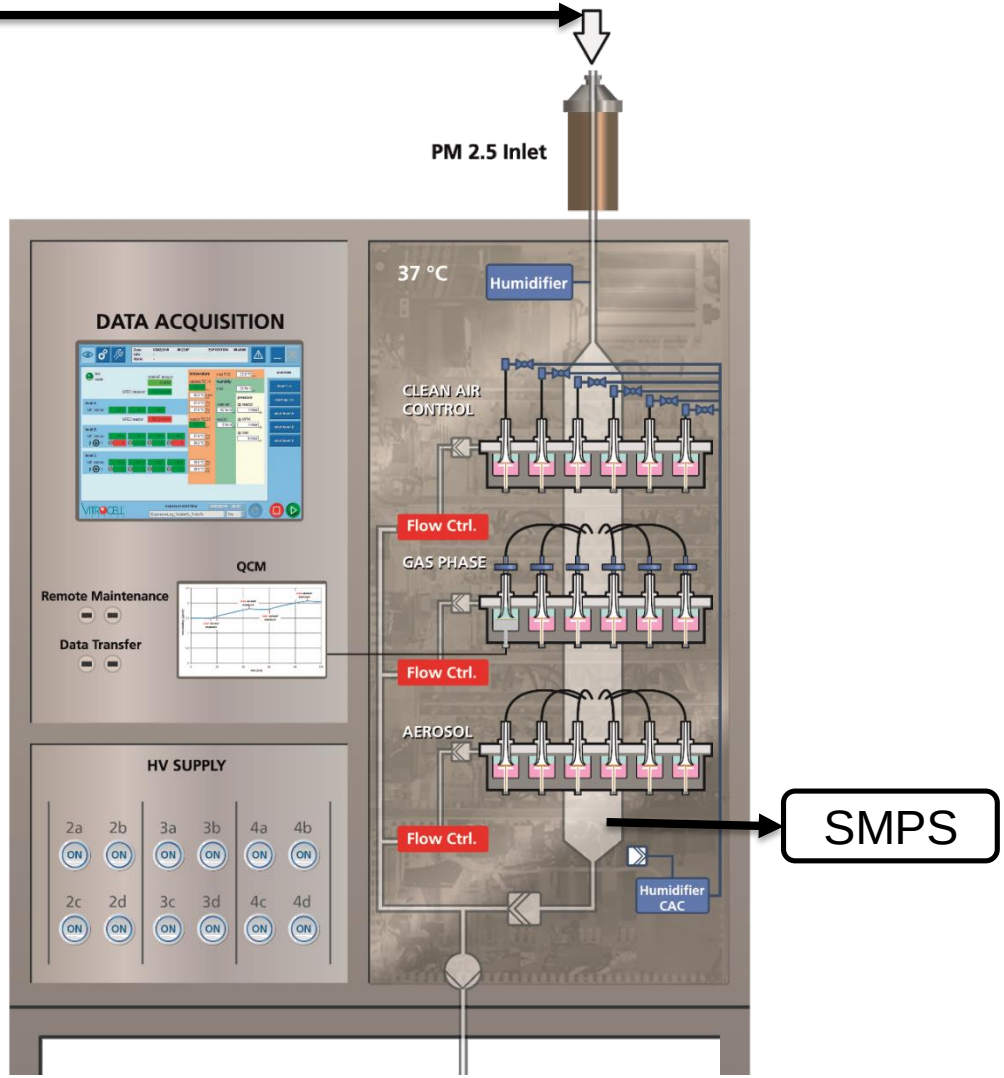
$$W = \frac{deposited\ particle\ mass/number}{exposed\ particle\ mass/number} \quad [\%]$$

Dosimetry methods at the ALI

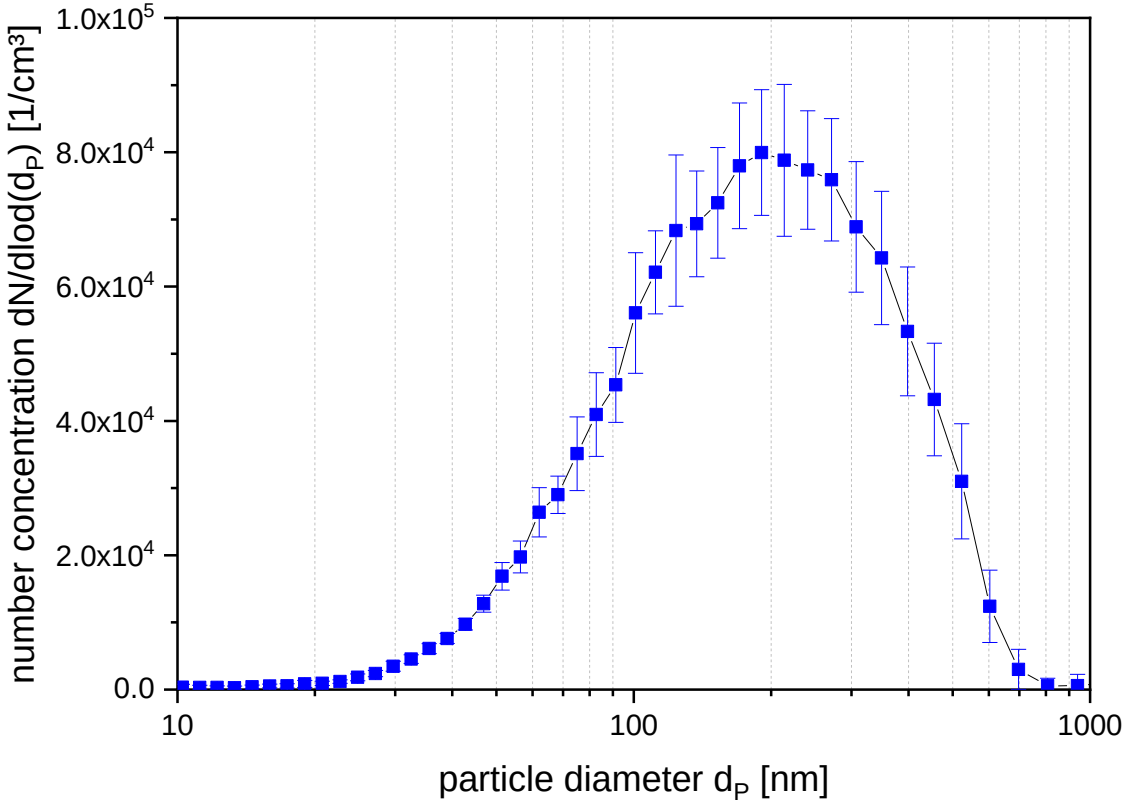
- Fluorescein sodium dosimetry (FNA)
  - spectroscopic measurement of deposited mass
  - c_M [µg/cm²]
- Quartz crystal microbalance
  - Online measurement of mass dose per area
  - c_M [µg/cm²]
    - f(t)
- TEM analysis
  - Image analysis delivers information about particle shape and homogeneity of distribution
  - c_N [µg/cm²]
    - size
    - Shape
- Numerical simulation
  - Deposition efficiencies in dependence of chosen boundary conditions or geometries
  - c_N, c_M
    - $f(d_P)$

24 h exposure experiments with TiO_2 aerosol





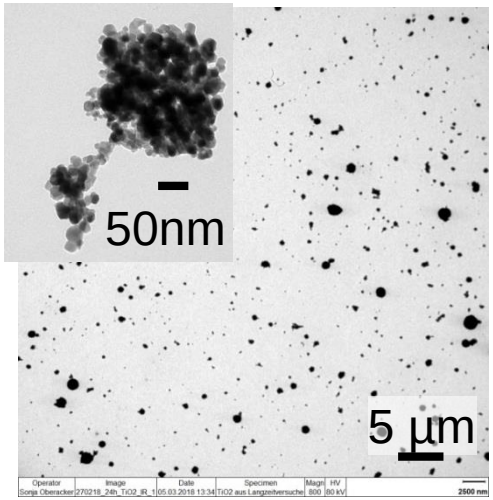
Dosimetry I: Particle number size distribution in the Exposure System determined by SMPS

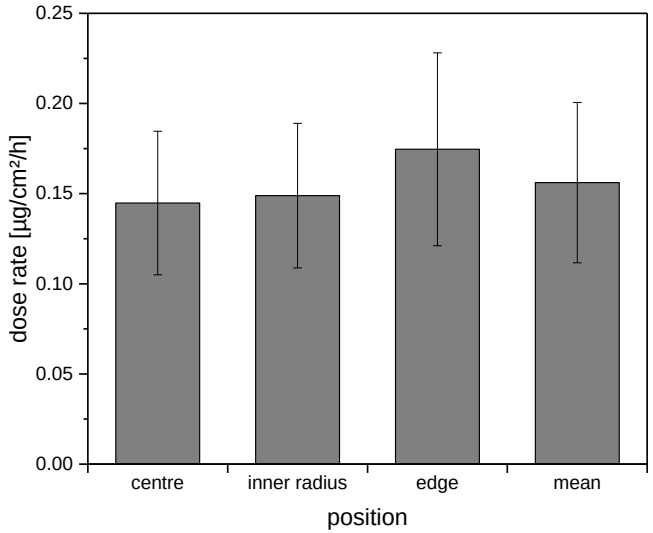


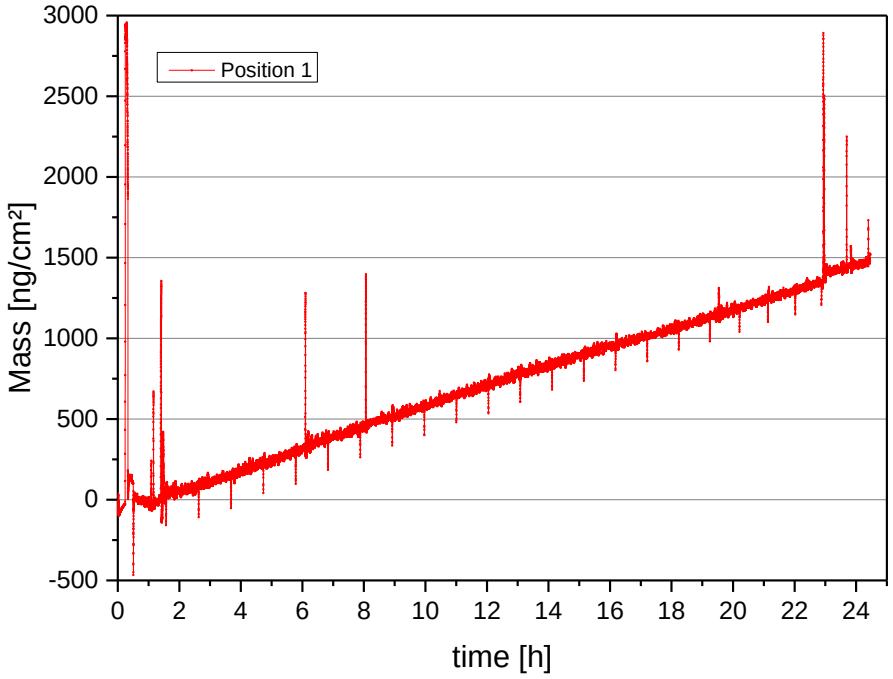
Calculated using the deposition efficiency from fluorescein sodium dosimetry f = 1.5 %: Mass concentration calculated with effective density ρ = 0.638 g/cm³

Modal value x_M	Standard Deviation s_g	Total number conc. c_N	Estimated diff. dose	Estimated dose rate
[nm]	[-/-]	[1/cm³]	[µg/cm²]	[µg/(cm²*h)]
190	2.2	6.0E+04	0.235	0.01

Dosimetry II: Image evaluation and QCM



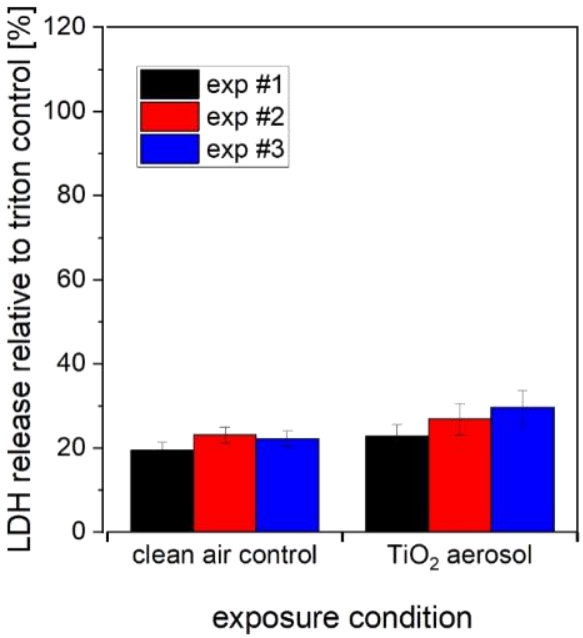


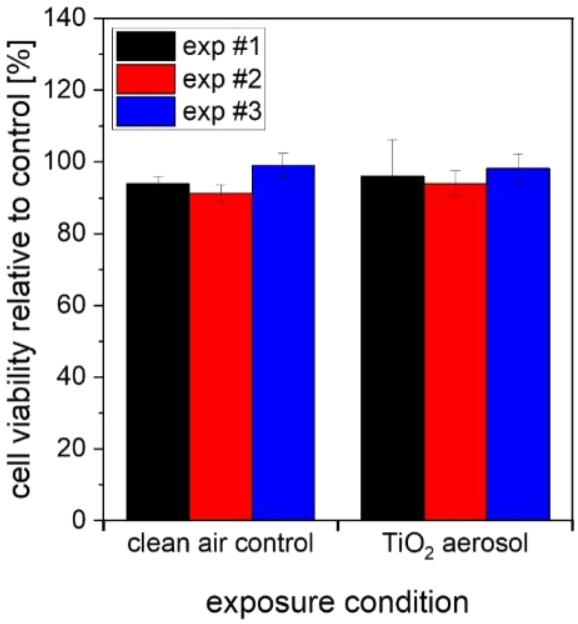


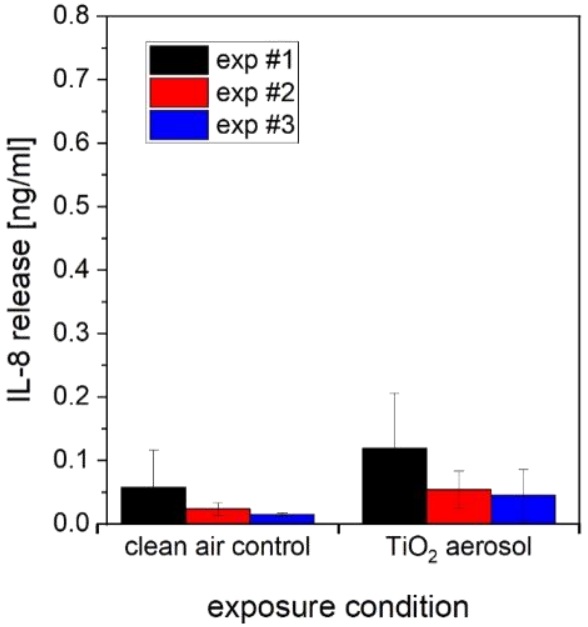
- QCM measurement

Dose rate = 0.062 µg/(cm²*h)

Cell viability (Alamar Blue assay) and cytotoxicity (LDH assay) of TiO_2 exposed A549 cells

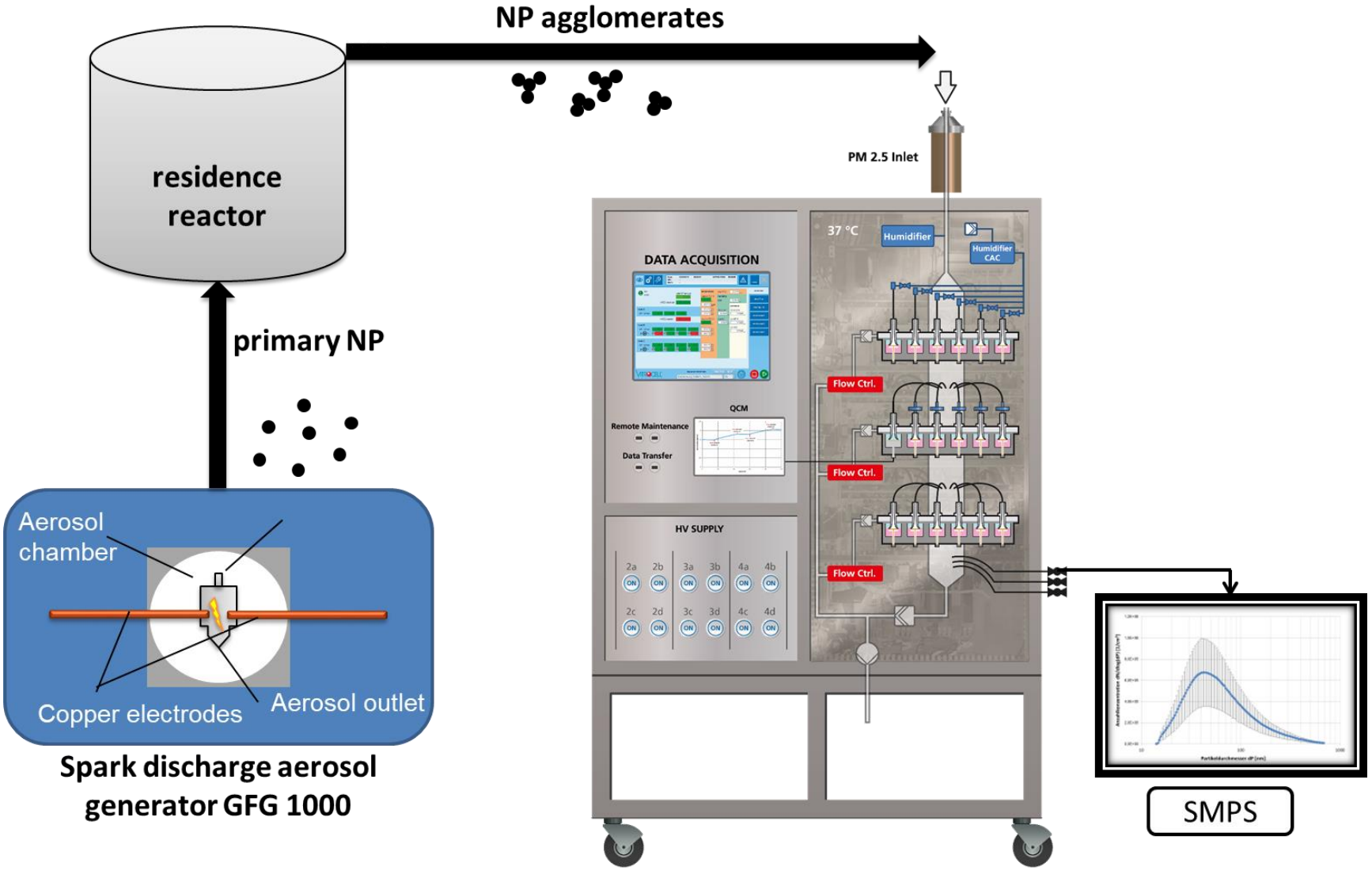




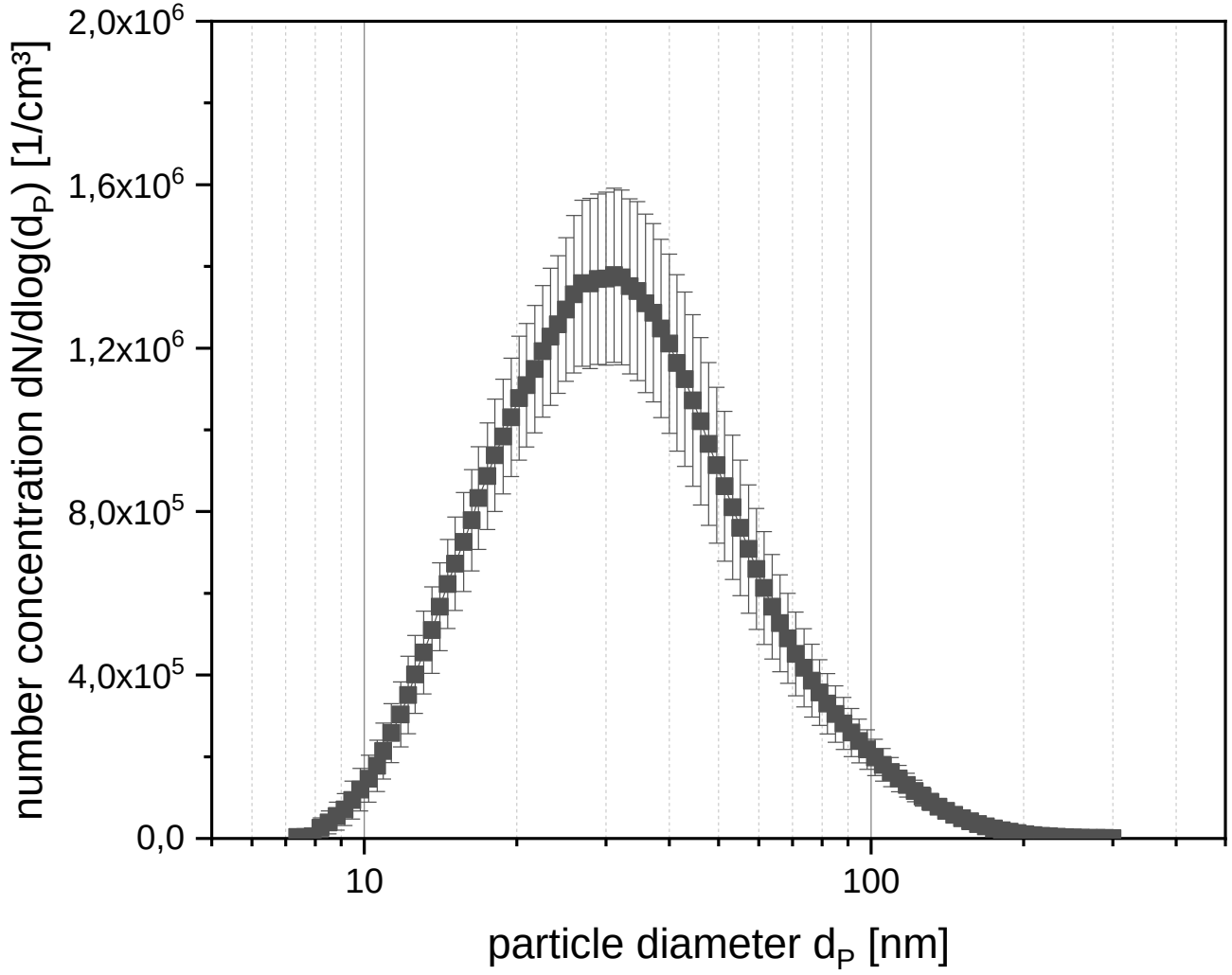


- After 24 hours exposure of A549 towards TiO_2 no viability loss is observed.
- After 24 hours exposure towards TiO_2 no significant cytotoxicity or inflammatory response can be observed.

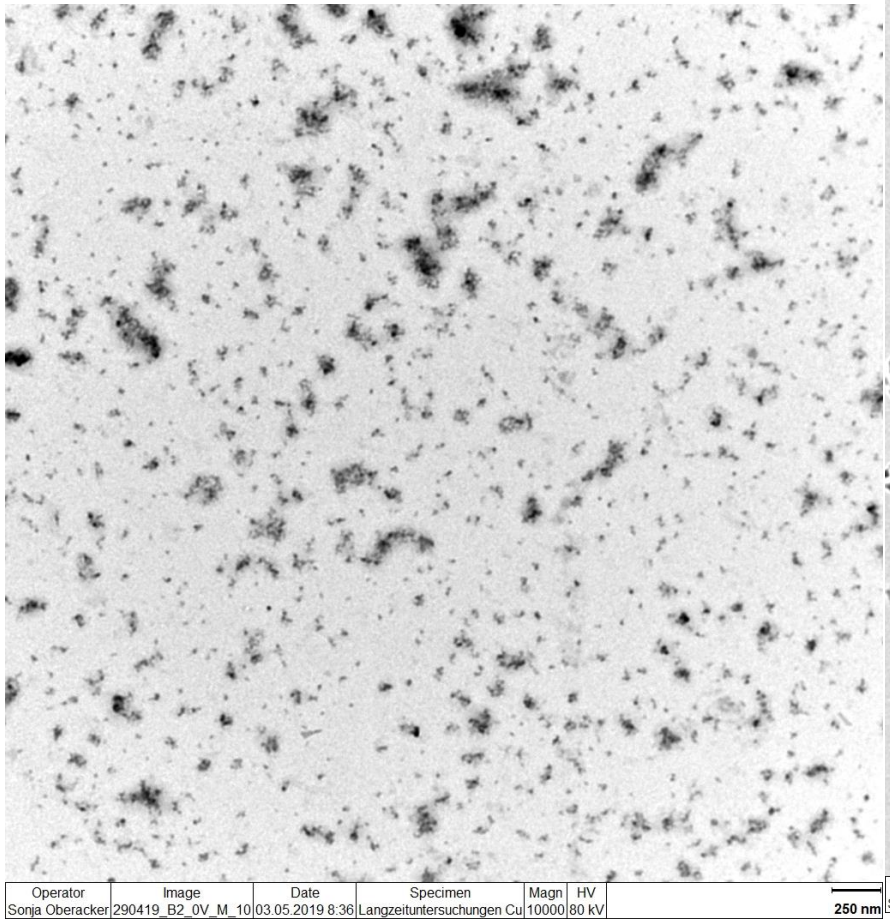
Experimental set up

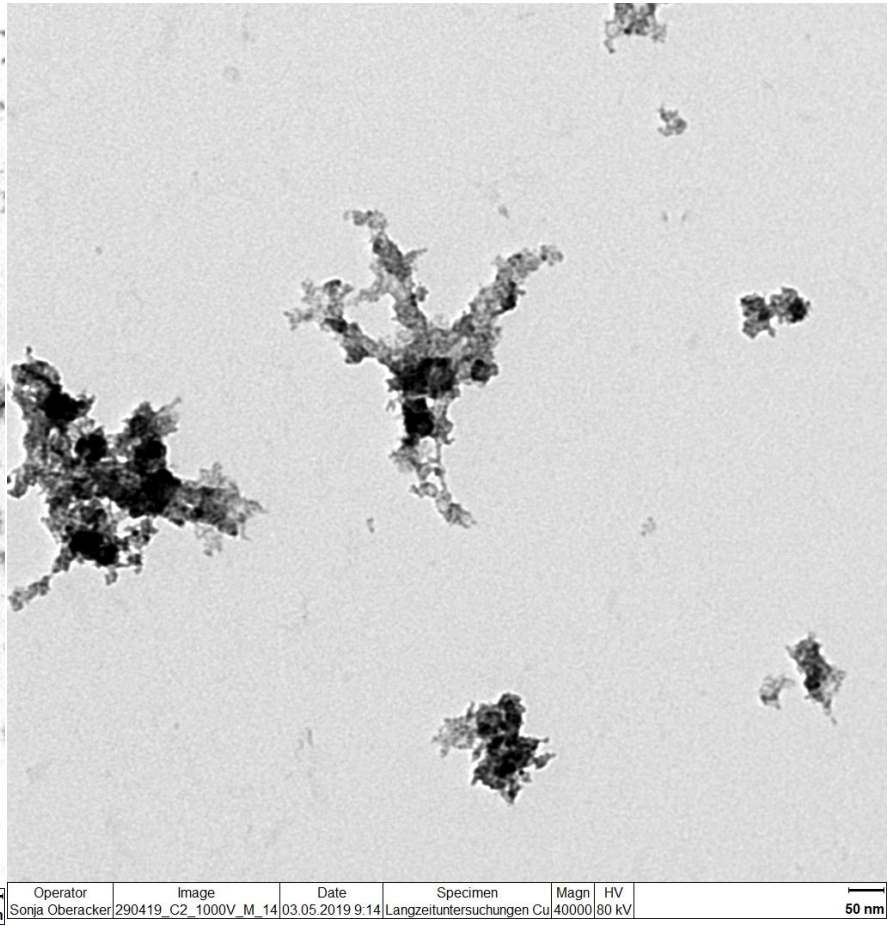


Particle number size distribution of CuO in the Exposure System determined by SMPS



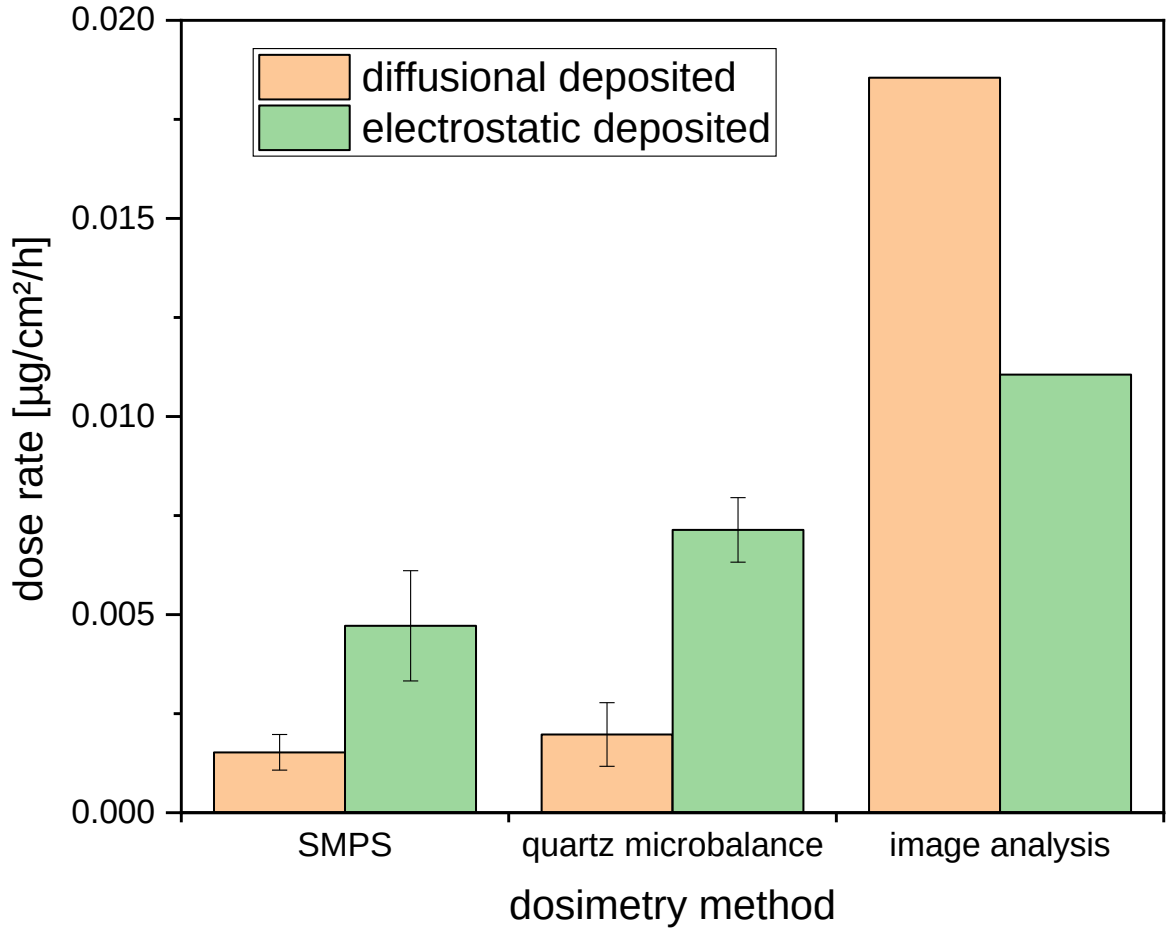
TEM images of Cu NP



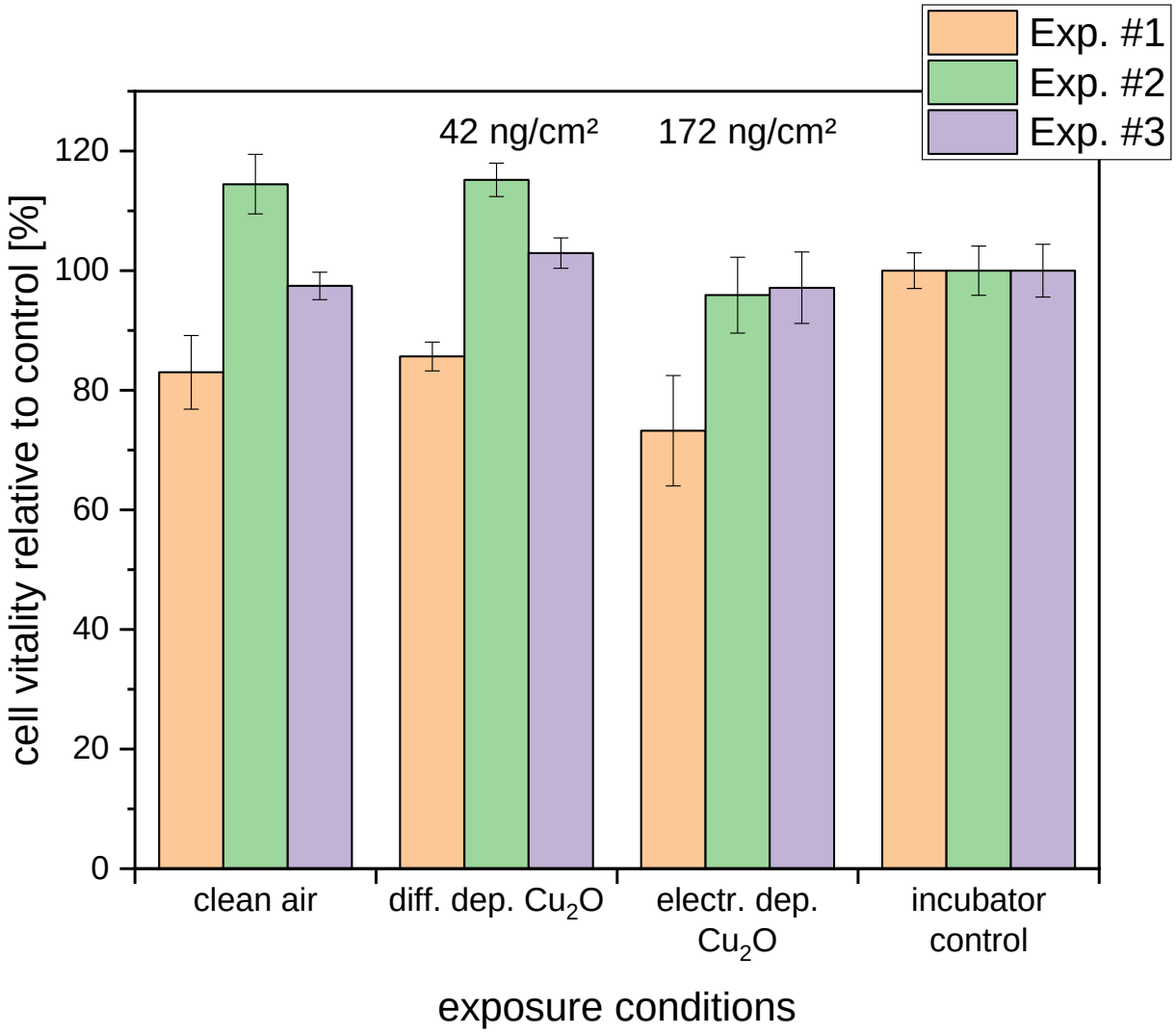


- Effective density according Charvet et al. (2014), J. Nanopart Res: $\rho_{eff} = 15.528 * d_P^{-0.826}$, with $d_P = 30$ nm $\rho_{eff} = 0.9354$ g/cm³

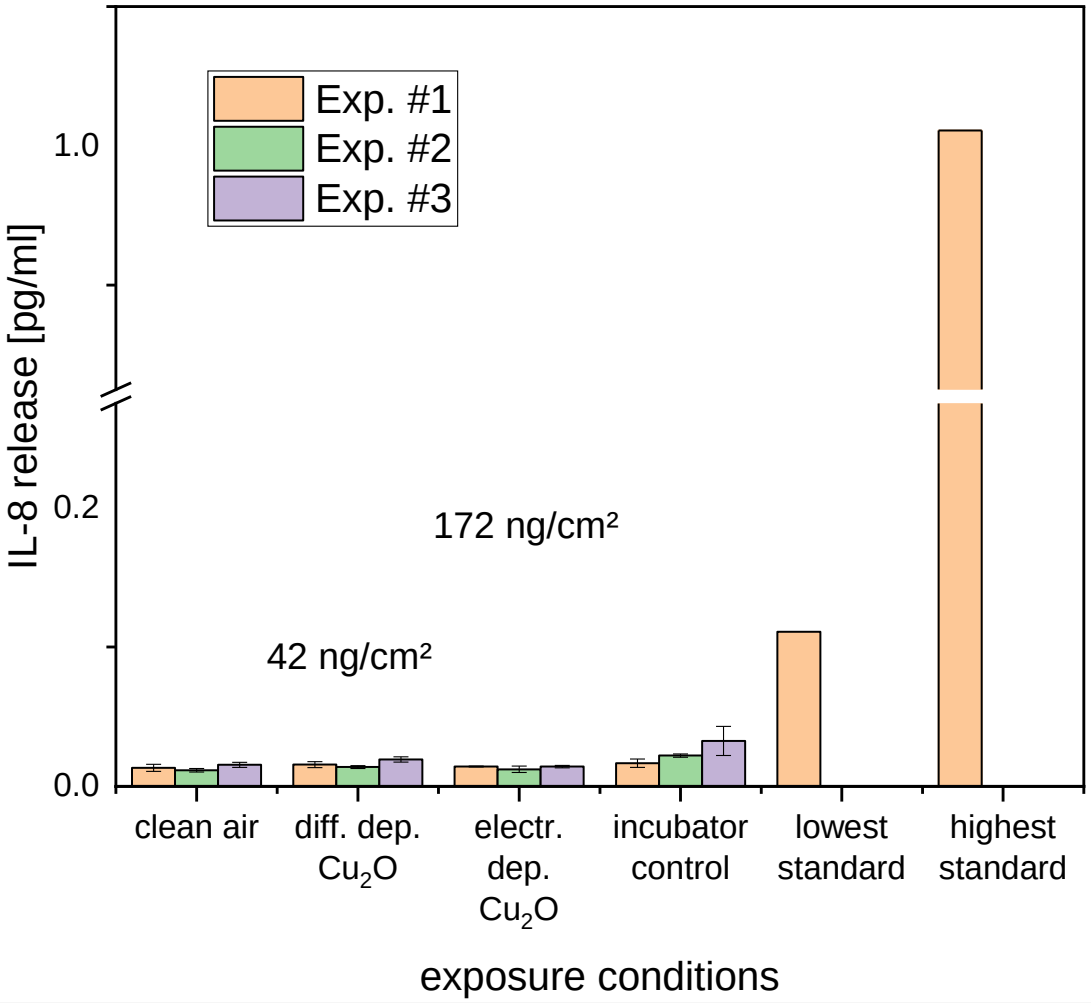
Copper particle doses: comparison of methods



AlamarBlue assay

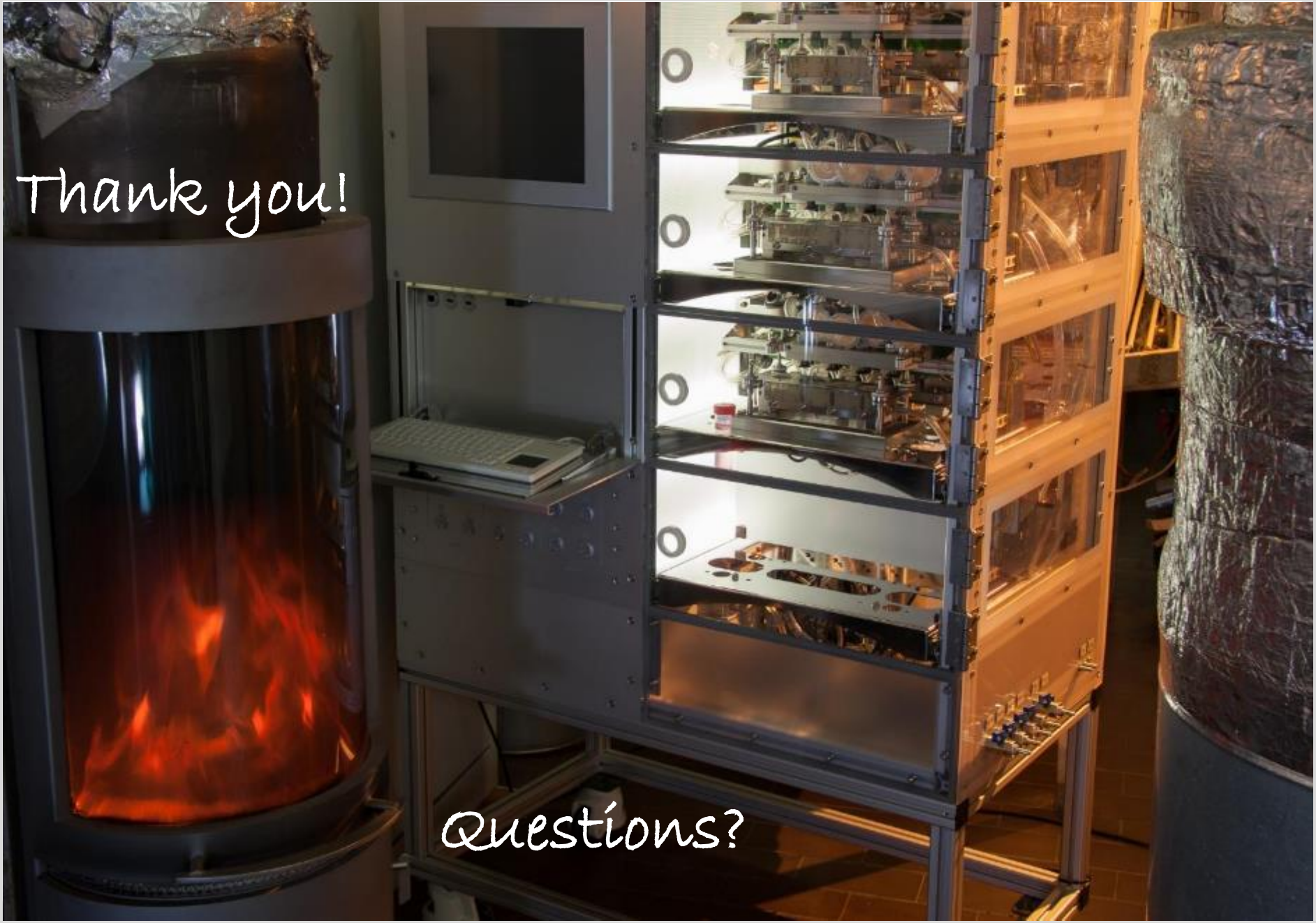


Inflammatory response



Summary

- Long term exposures towards clean air are performed without viability loss or cytotoxic effects.
- After 24 hours exposure of A549 towards TiO_2 no viability loss, significant cytotoxicity or inflammatory response can be observed.
- After 24 hours exposure of A549 towards Cu_2O no viability loss is observed.
- After 24 hours exposure towards Cu_2O no significant cytotoxicity or inflammatory response can be observed.
- Dose rate is a parameter for biological responses.


Thank you!
Questions?