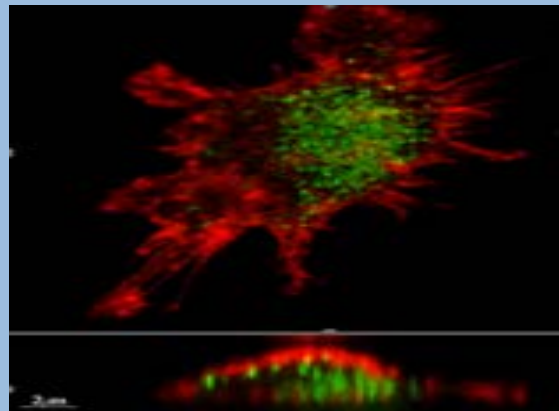
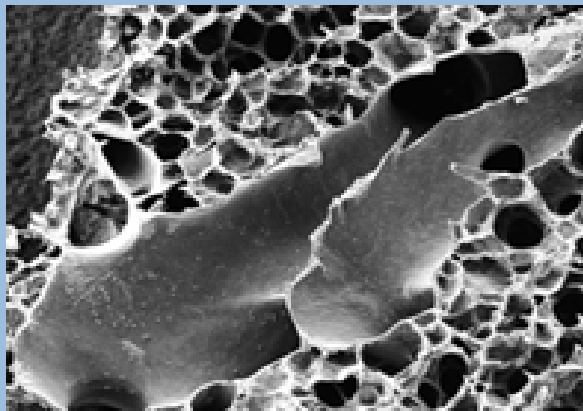


VERT Workshop  
Teheran  
January 13, 2016

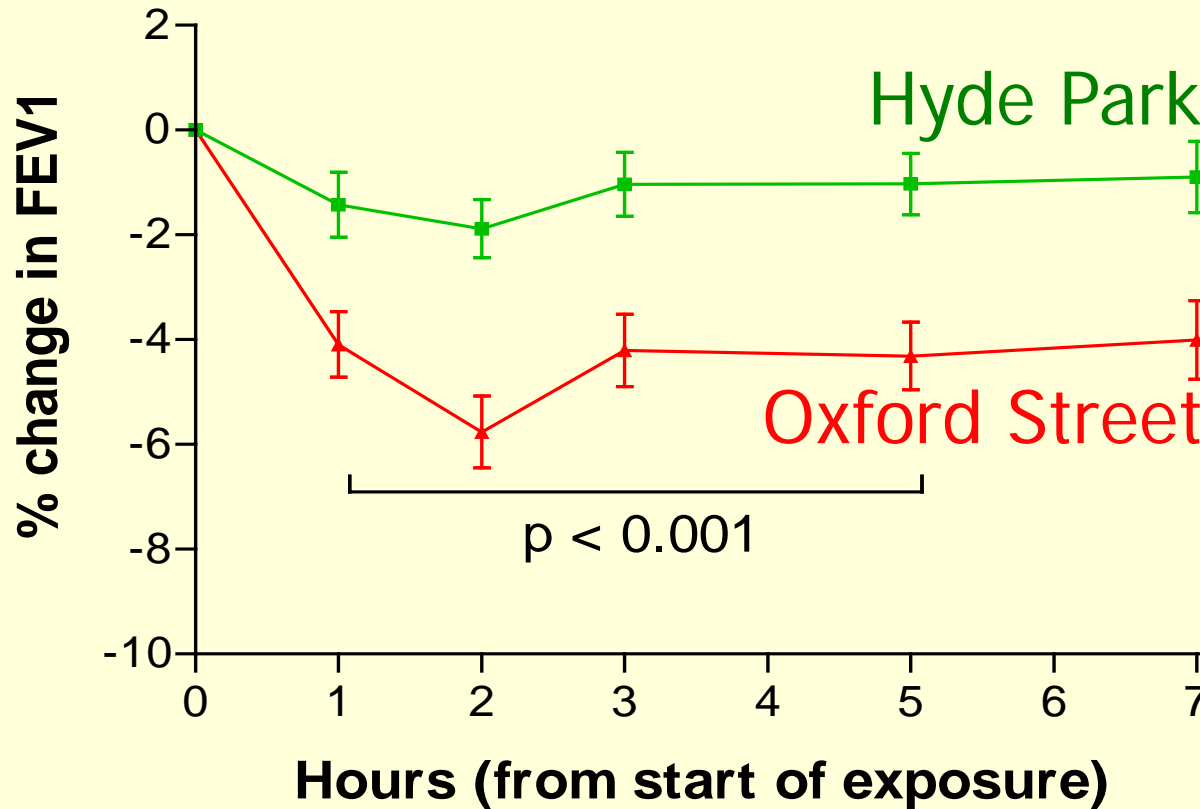
# HEALTH EFFECTS OF COMBUSTION GENERATED PARTICLES

## HOW COMBUSTION GENERATED NANOPARTICLES (UFP) CAN ENTER THE HUMAN ORGANISM – SIZE MATTERS

Peter Gehr  
Prof. em.  
University of Bern  
Bern  
Switzerland



# LUNG FUNCTION OF ASTHMATICS WHILE WALKING ALONG THE DIESEL BUS ROUTE OXFORD STREET, THROUGH HYDE PARK



**WHO (IARC):**

- Diesel exhaust is carcinogenic, June 12, 2012
- Air pollution is carcinogenic, October 17, 2013

Courtesy:

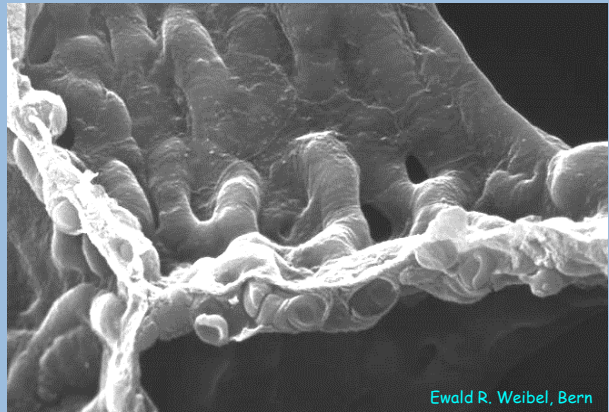
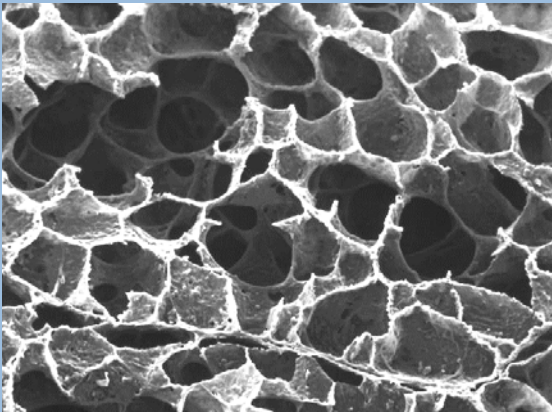
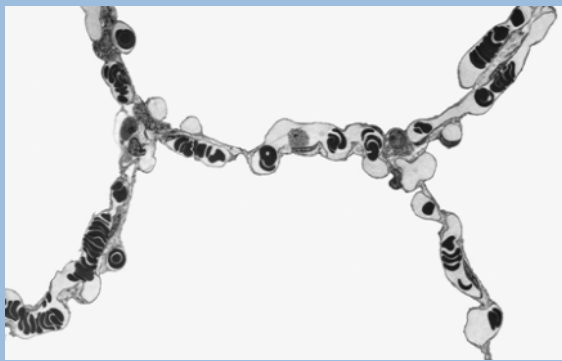
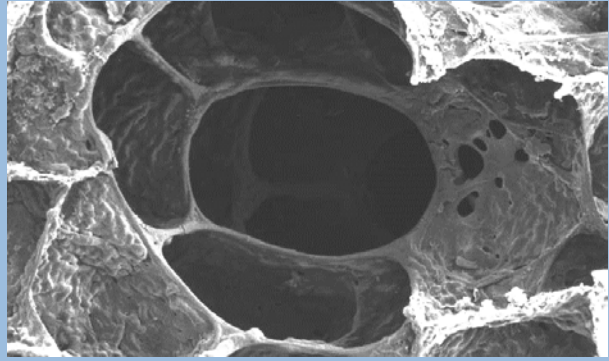
**Nino Künzli**  
Swiss Tropical and  
Public Health Institute  
Basel, Switzerland

# THE LUNG: MAIN PORTAL OF ENTRY FOR NANOPARTICLES

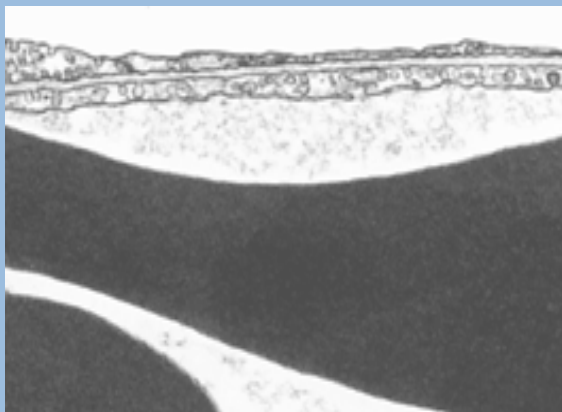
## HUMAN LUNG



Gehr et al., Respir. Physiol., 1978



Ewald R. Weibel, Bern

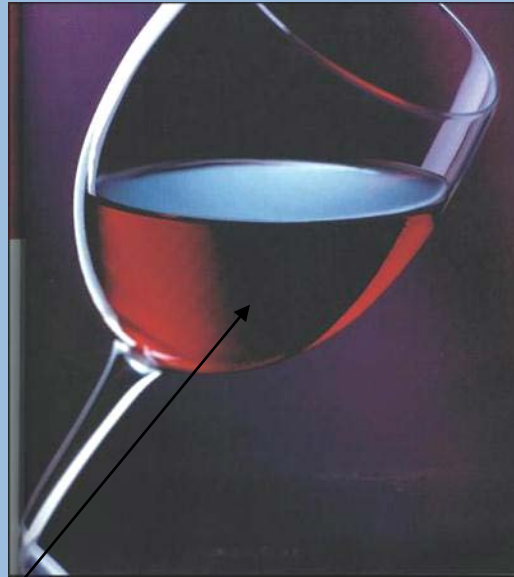


# DID YOU KNOW THIS ABOUT THE HUMAN LUNG?



**Tennis field**

450 Mill. alveoli (M. Ochs, Univ. of Bern)  
with a surface area of **140 m<sup>2</sup>**  
(diameter ¼ mm, gas-exchange region 80-90%)



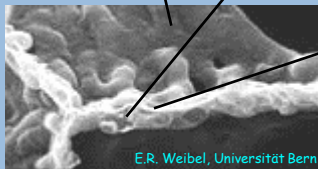
**Red wine glass**

Volume of capillary blood  
involved in gas exchange: **210cm<sup>3</sup>**



**1/50 of the thickness of a  
women's hair**

Thickness of tissue barrier:  
**<1µm**



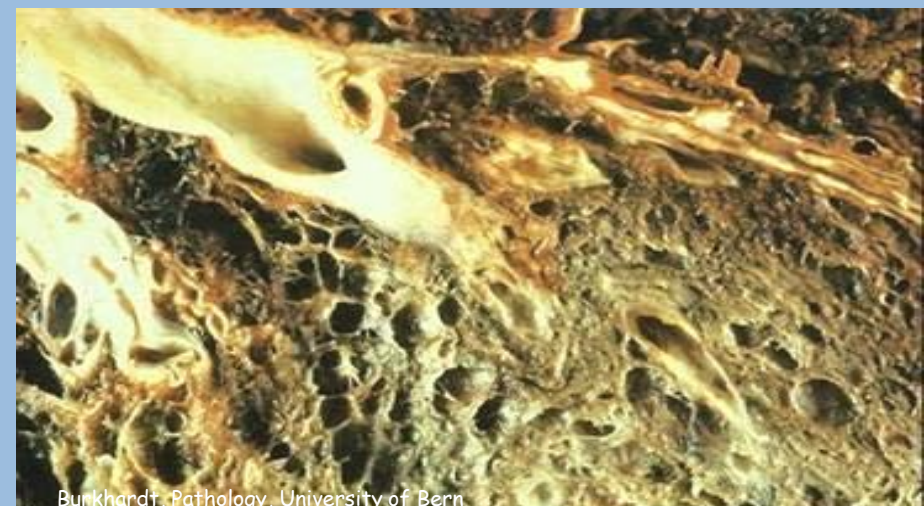
E.R. Weibel, Universität Bern

(B. Rothen-Rutishauser, Universität Bern)

Gehr et al., Respir. Physiol., 1978

# HEALTHY/«CLEAN» LUNG AND POLLUTED LUNG

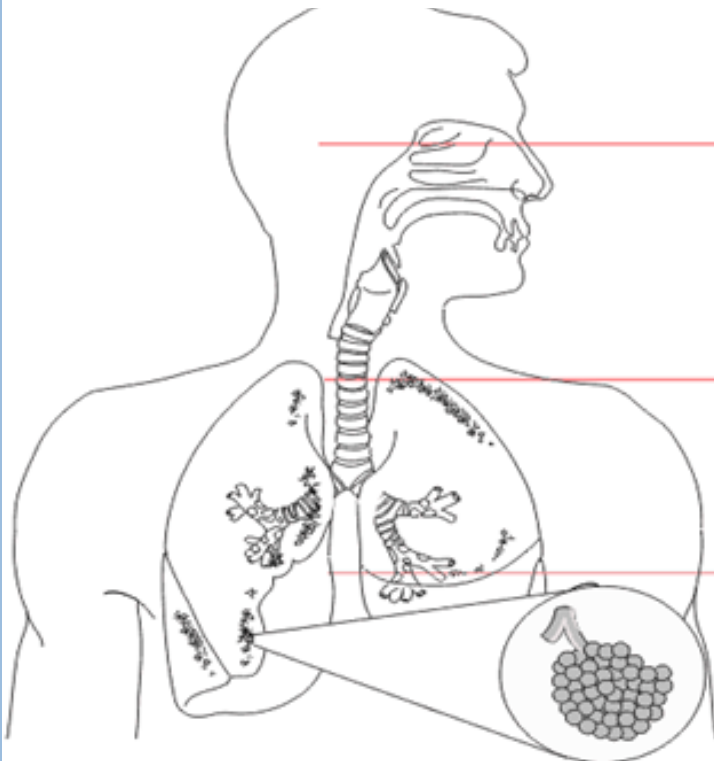
## 1st EFFECT



# PARTICLE DEPOSITION IN THE LUNG

## PENETRATION OF PARTICLES INTO THE LUNG

### Preferential deposition of particles and nanoparticles



| Place         | Particle size                             |
|---------------|---|
| Upper Airways | 5-10 $\mu\text{m}$                        |
| Trachea       | 3-5 $\mu\text{m}$                         |
| Bronchi       | 2-3 $\mu\text{m}$                         |
| Bronchioles   | 1-2 $\mu\text{m}$                         |
| Alveoli       | <1 $\mu\text{m}$<br>(incl. nanoparticles) |

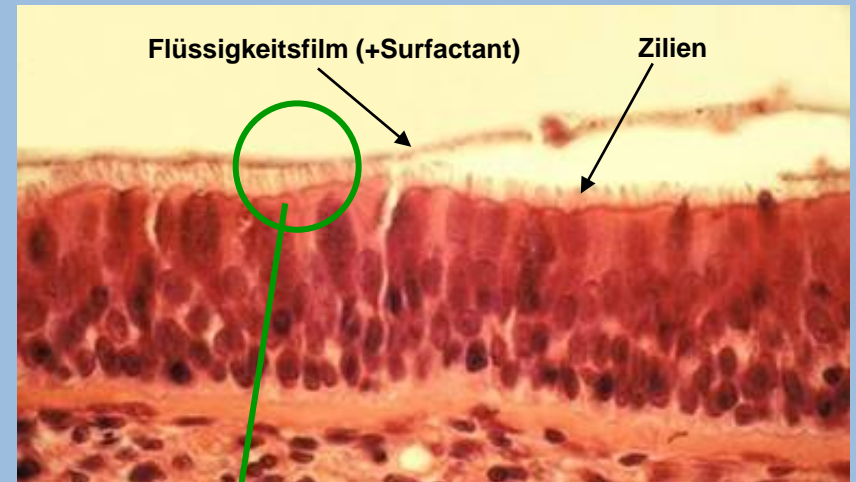
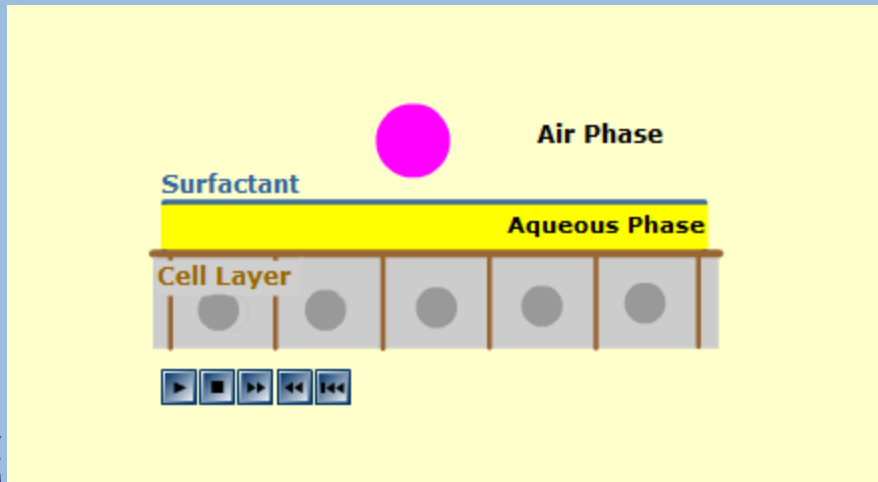
⇒ The smaller the particles the deeper they penetrate into the lung:

(1) There, **nanoparticles** may penetrate into tissue and cells (organelles, nucleus).

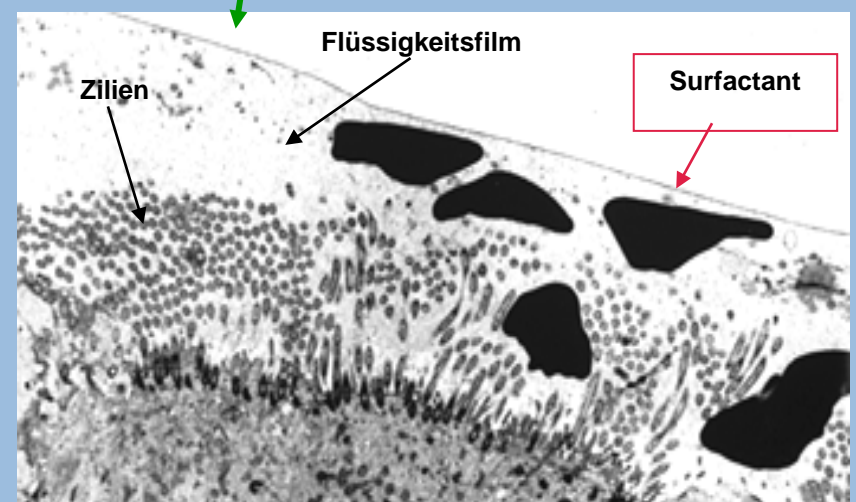
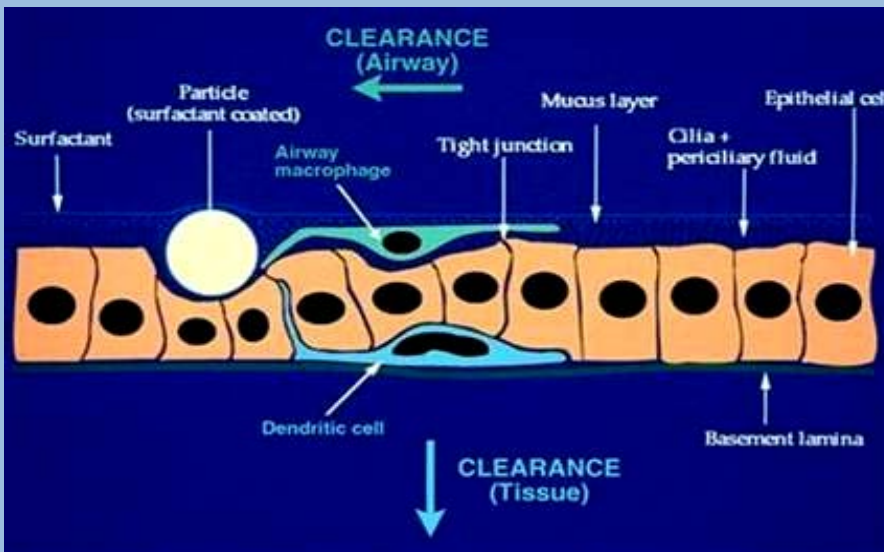
(2) There, **nanoparticles** may translocate into the cappillary blood.

(3) By the blood circulation, **nanoparticles** are transported to other organs.

# PARTICLE DISPLACEMENT (SURFACTANT)



S. Schürch,  
S. Tschanz,  
Univ. Bern

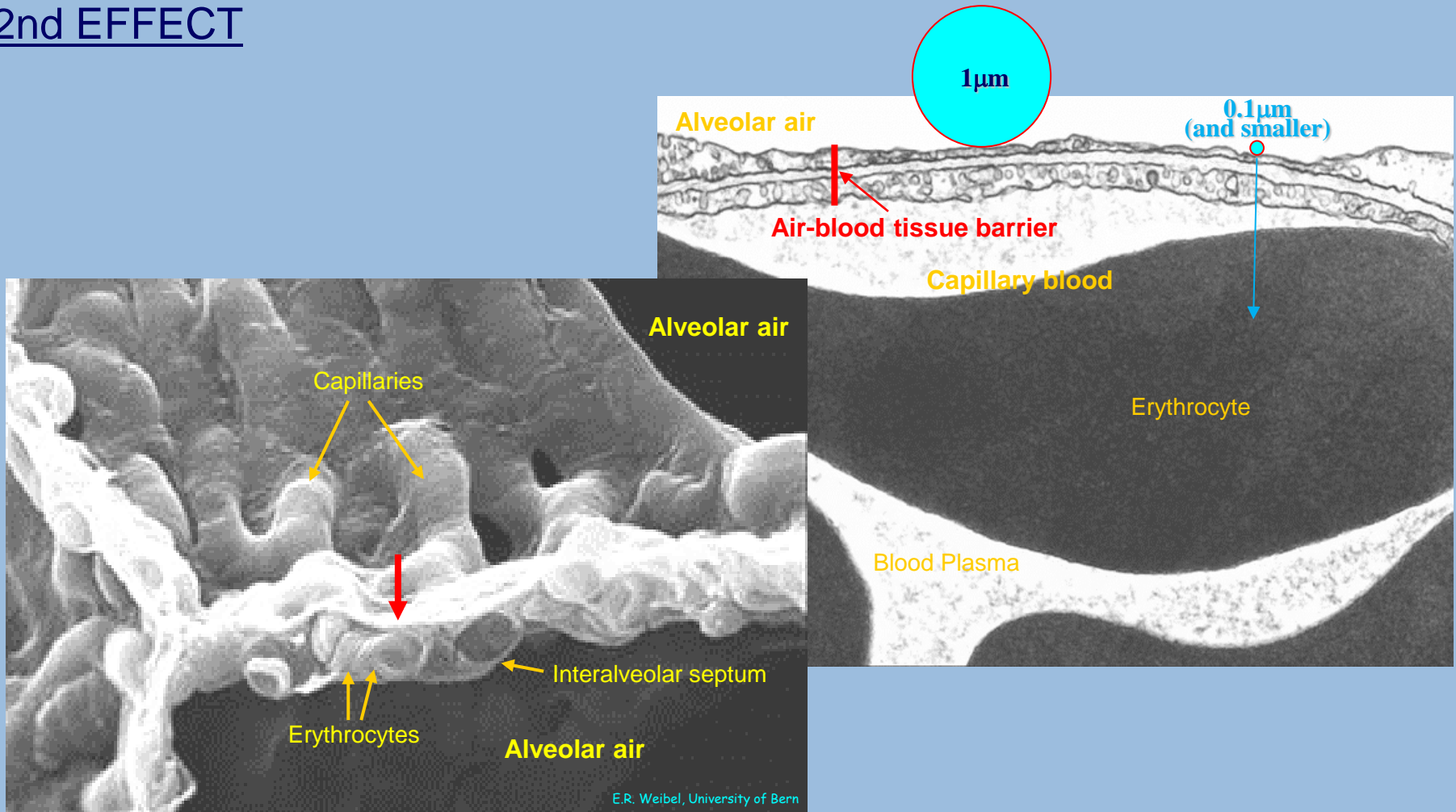


Gehr et al., J. Aerosol Med., 1990  
Schürch et al., Respir. Physiol., 1990  
Gehr et al., J. Aerosol Med., 1996

# TRANSLOCATION OF NANO-PARTICLES FROM AIR INTO BLOOD

(THROUGH AIR-BLOOD TISSUE BARRIER)

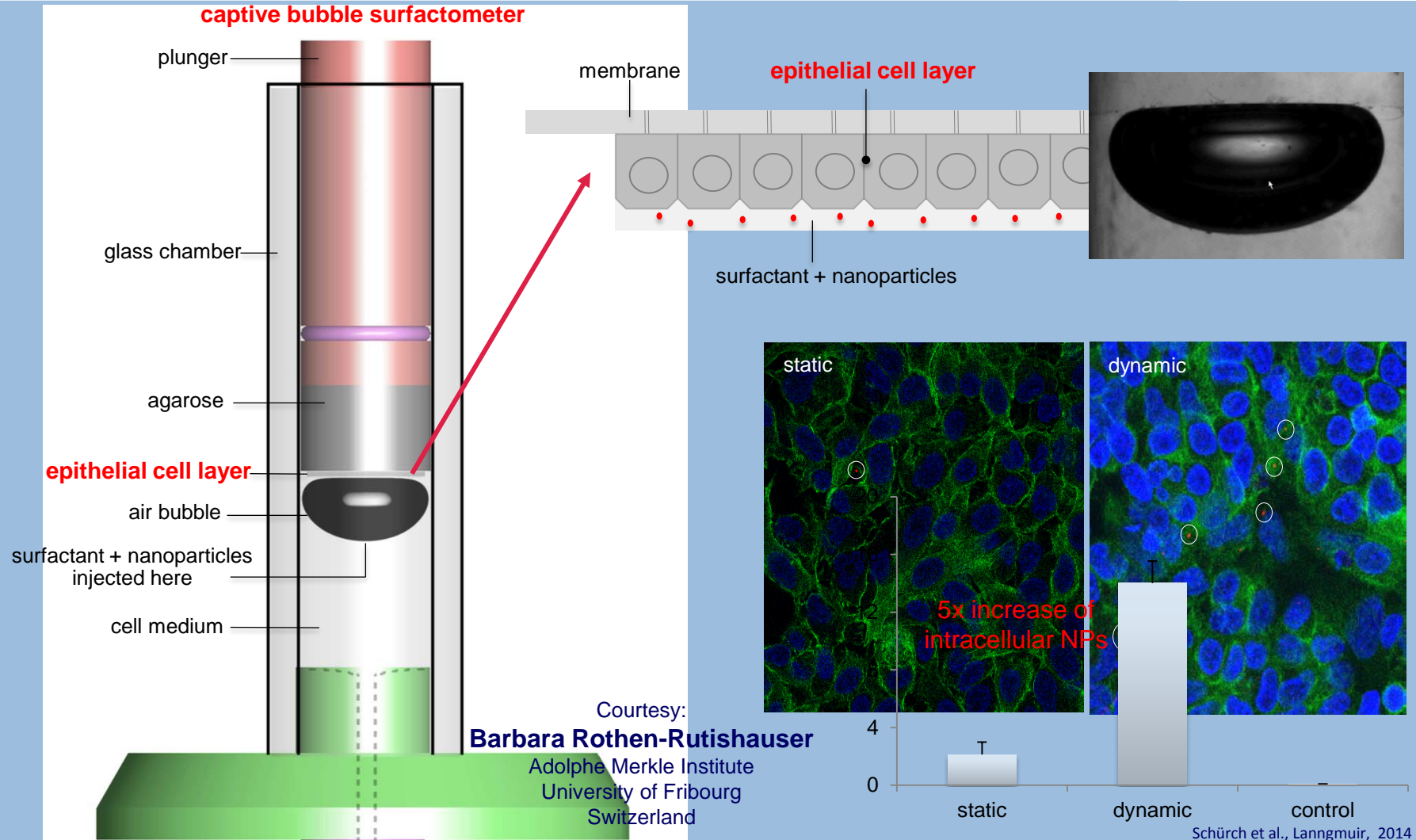
## 2nd EFFECT





# IN VITRO MODEL: BREATHING MOVEMENTS MAY STIMULATE NANOPARTICLE UPTAKE BY CELLS

Courtesy David Schürch, Adolphe Merkle Institute, University of Fribourg

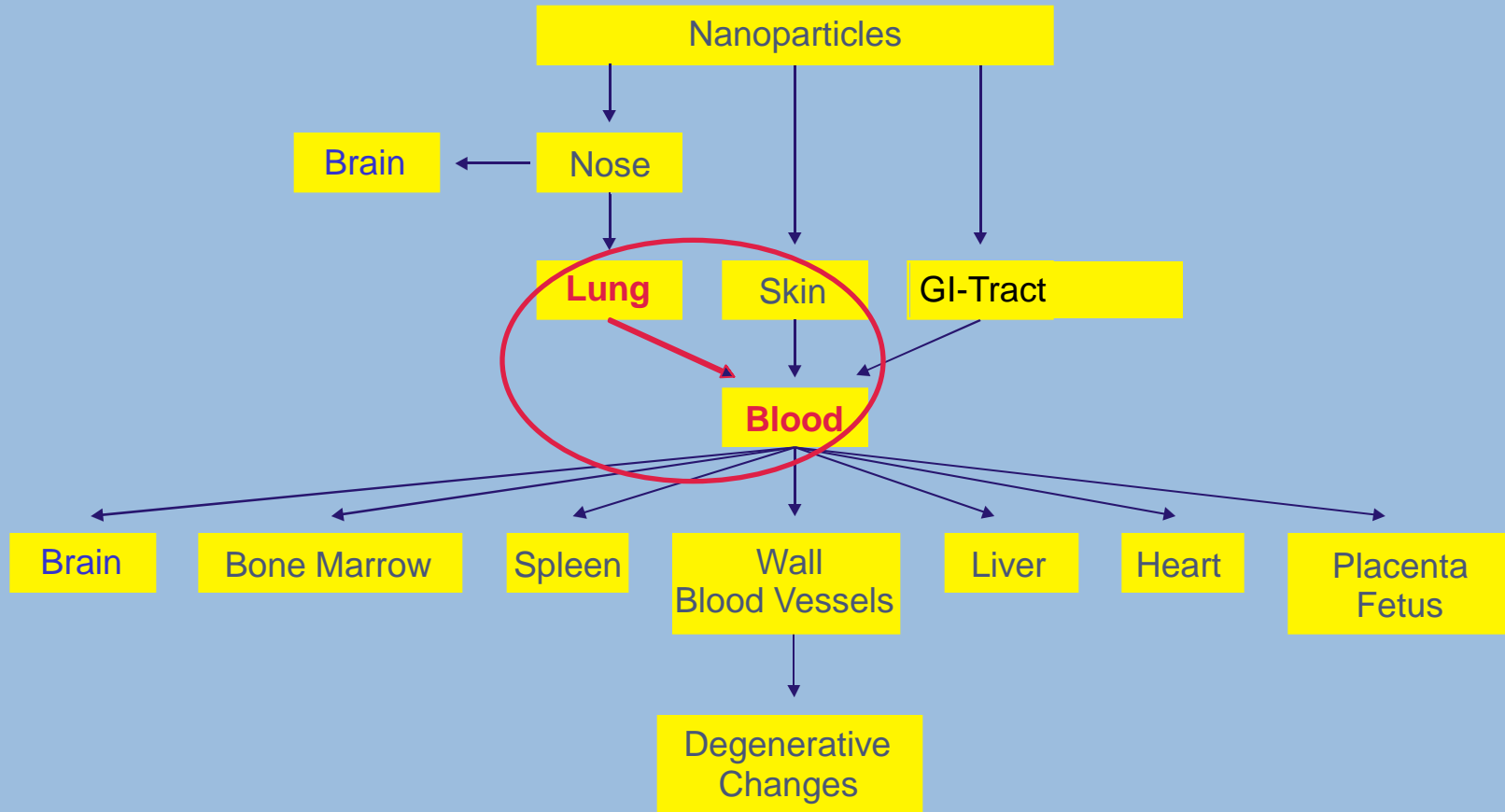


Courtesy:  
**Barbara Rothen-Rutishauser**  
 Adolphe Merkle Institute  
 University of Fribourg  
 Switzerland

Schürch et al., Lanngmuir, 2014

# TRANSLOCATION I

## WITH BLOOD TO OTHER ORGANS



# TRANSLOCATION II

## AMOUNT OF NANOPARTICLES IN OTHER ORGANS

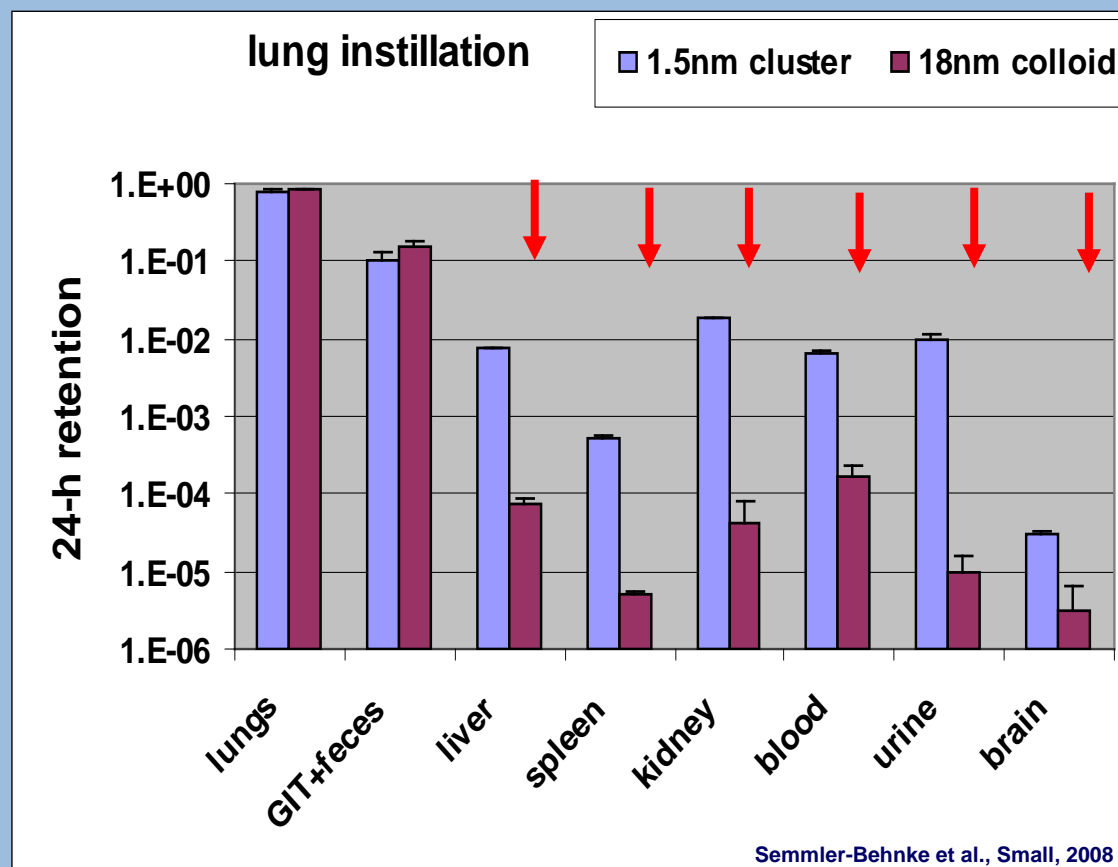
Intratracheal instillation in WKY rats

1-10 µg <sup>198</sup>Au particles in 50 µL saline, negative ionic surface charge

# of particles: 1 · 10<sup>14</sup> (1.4 nm cluster)    2 · 10<sup>11</sup> (18 nm colloid)


G. Schmid, Univ. of Essen, Germany

➤ Mass fractions of gold nanoparticles in different organs after 24 h



Courtesy:  
**W.G. Kreyling**  
(Helmholtz  
Center Munich)

Focus Network  
Nanoparticles and Health

CPC  iLBD  
Institute of  
Lung Biology and Disease

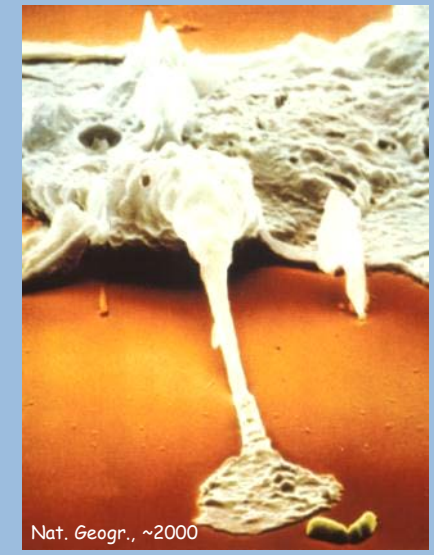
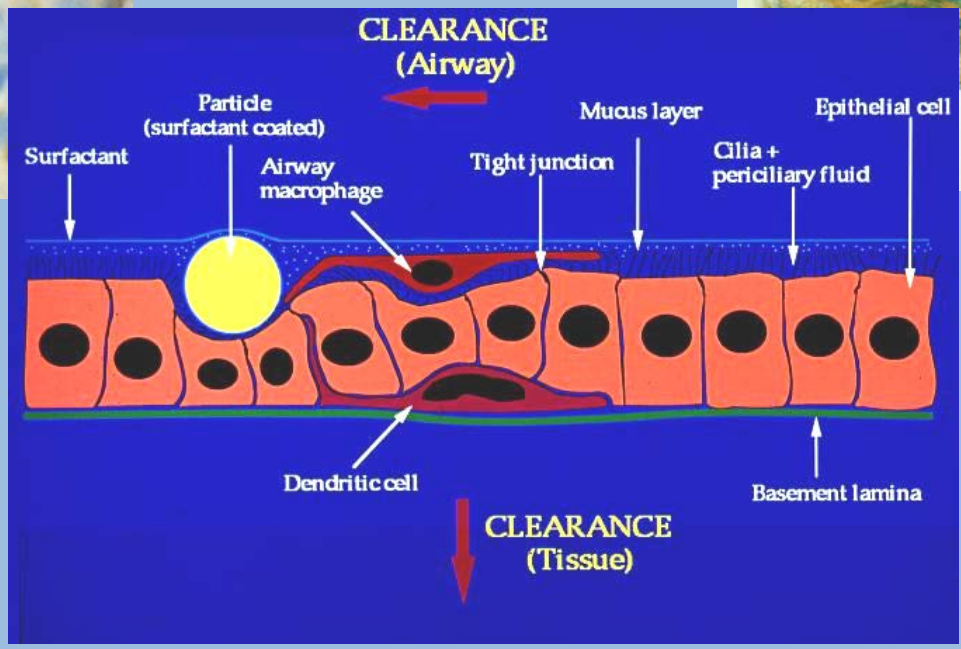
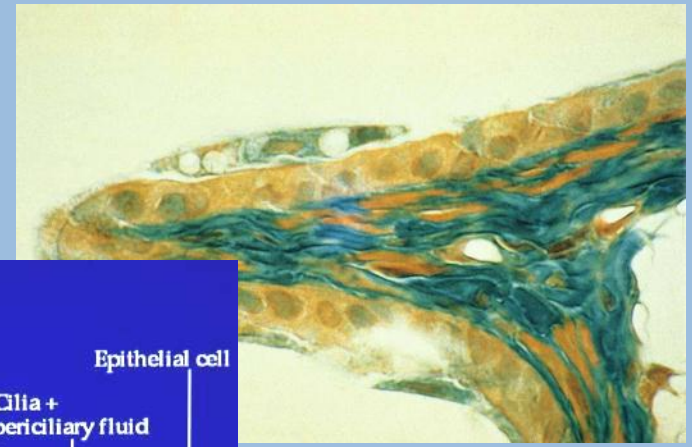
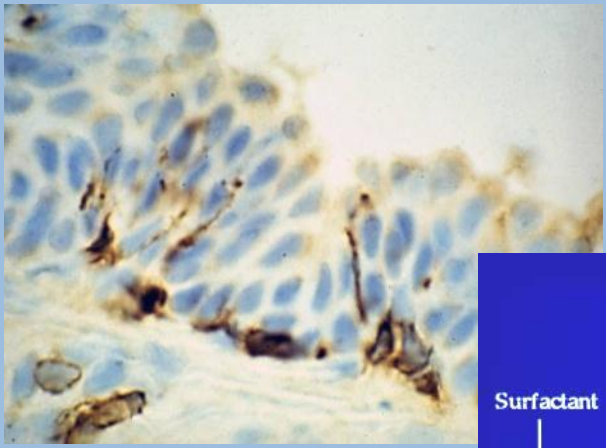
HelmholtzZentrum münchen

# WHAT HAS TO BE CONSIDERED OF NANOPARTICLES FROM COMBUSTION AEROSOLS

- **Size of particles** (nanoparticles)
- **Displacement of nanoparticles** towards epithelial layer (surfactant, surface forces)
- **Distance to capillaries** (translocation)
- **Distance to sensitive cells** (interaction), effect: immune modulation?
- **Interaction with cells** (uptake/penetration, effect: immune modulation, oxidative stress, inflammatory reaction a.o.?)

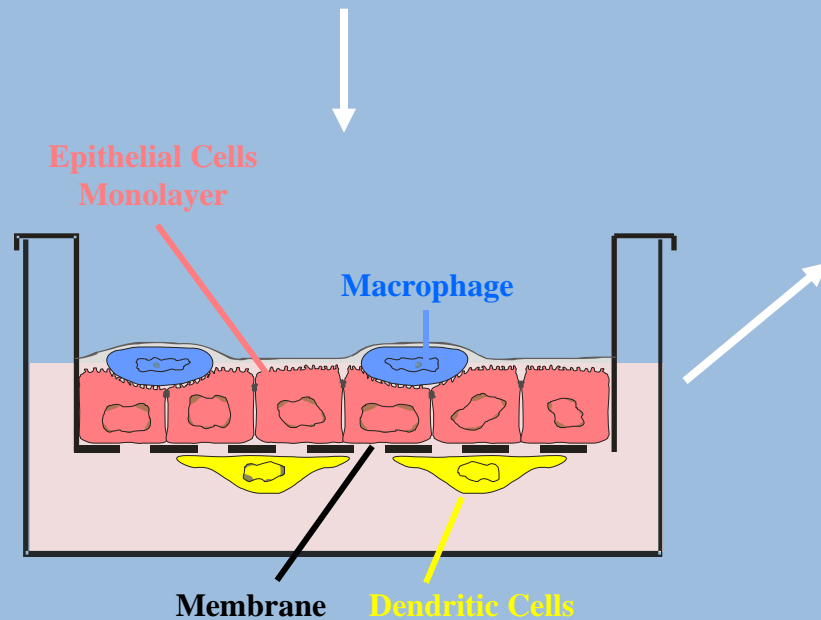
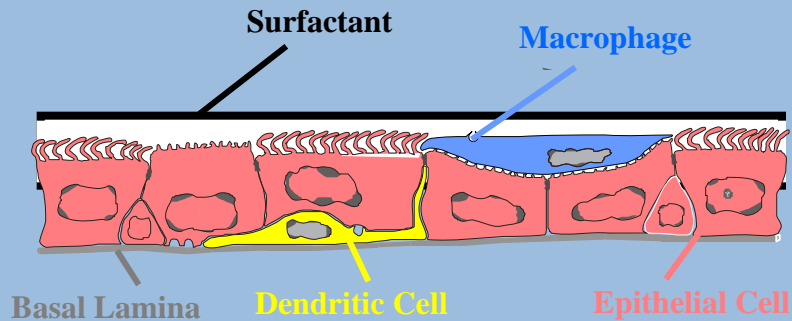
# MAIN ACTORS ARE CELLS

EPITHELIAL CELLS, MACROPHAGES, DENDRITIC CELLS ...

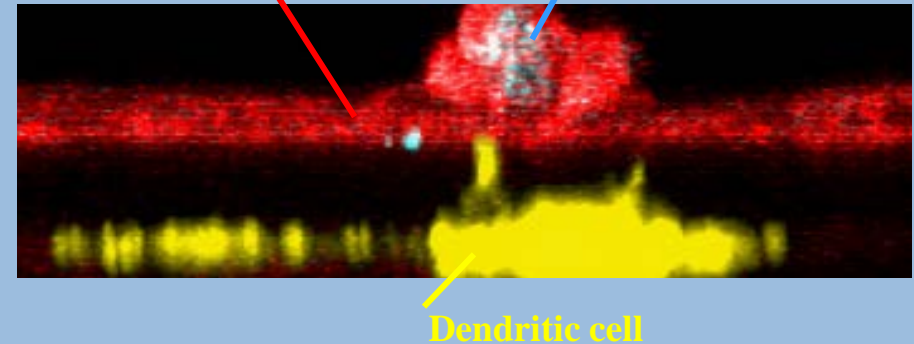


# ... DO THEY COLLABORATE? THE CELL MODEL TO TEST THIS

## THE TRIPLE CELL CO-CULTURE MODEL

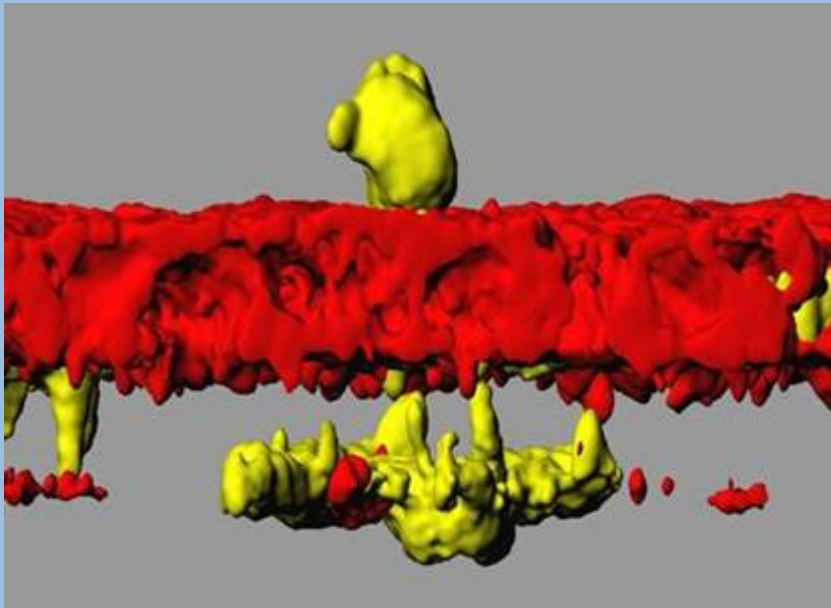


**Epithelial cell monolayer**  
(A549 or 16HBE cells) **Macrophage**

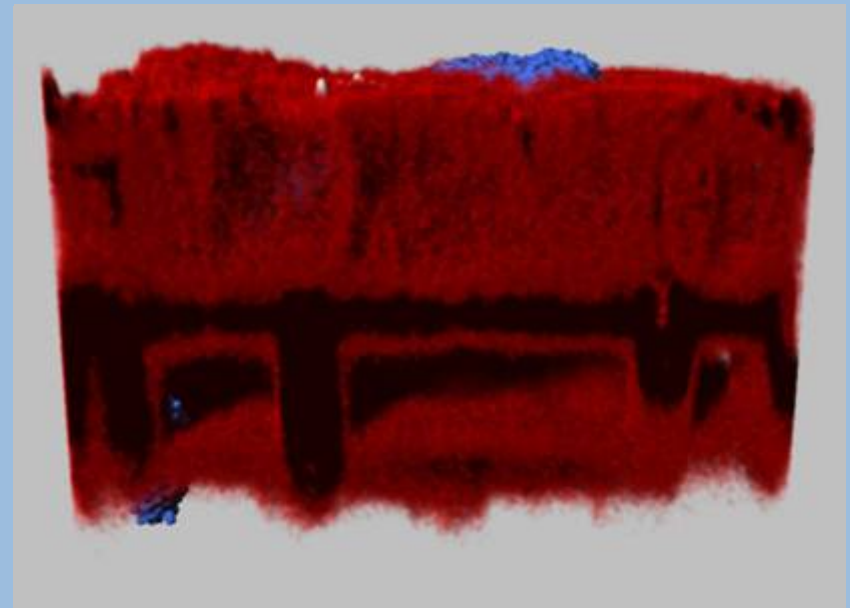


Rothen-Rutishauser et al., *Am. J. Respir Cell Mol. Biol.* 32: 281-899, 2005  
Rothen-Rutishauser et al., *Expert. Opin. Drug Metab. Toxicol.* 4: 1075-1089, 2008

# STRUCTURAL VICINITY OF DENDRITIC CELLS AND MAKROPHAGES (THROUGH THE EPITHELIAL CELL LAYER)



Blank et al., AJRCMB 36: 669-677, 2007



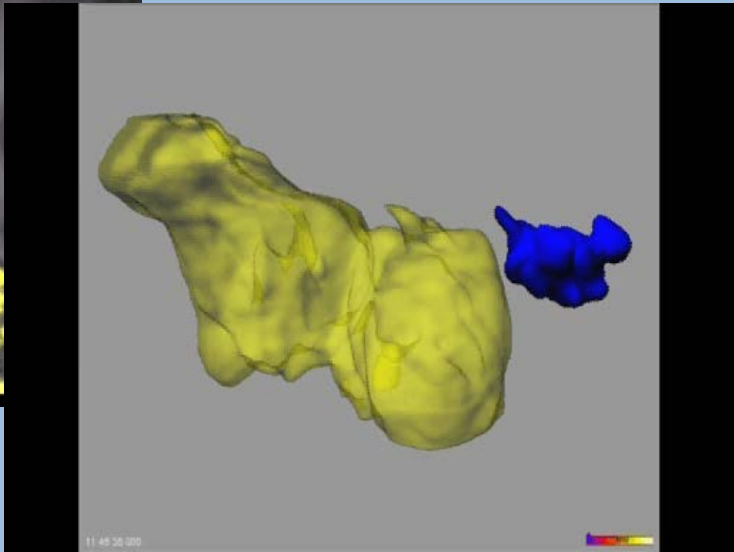
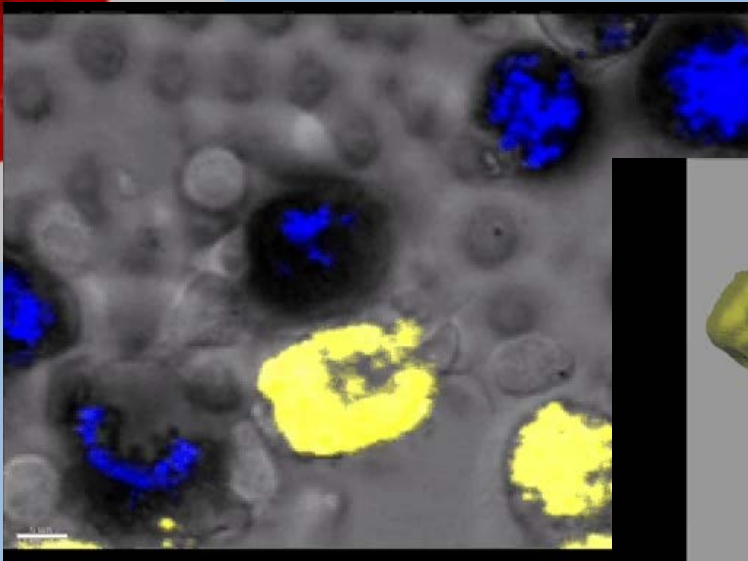
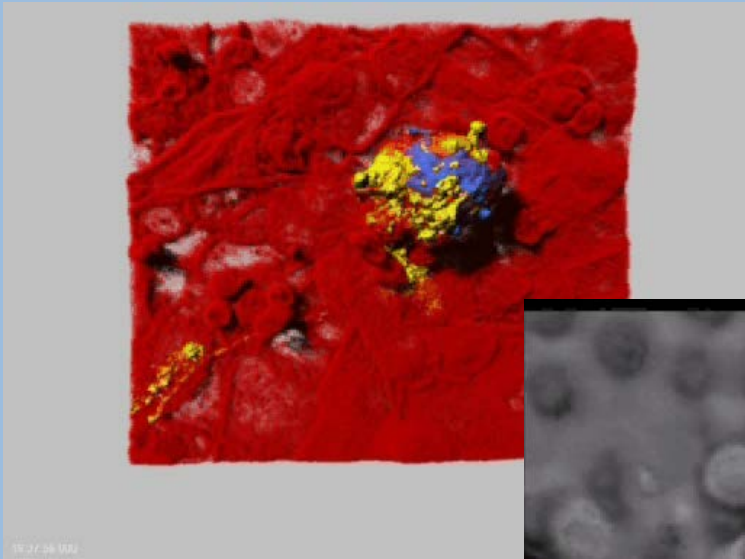
Deconvolution technique  
IMARIS 3D&4D Image Analysis Software  
Bitplane AG, Scientific Software

# CELL-CELL INTERACTIONS

CELLULAR INTERPLAY ->THE CELLS DO  
COLLABORATE!

$u^b$

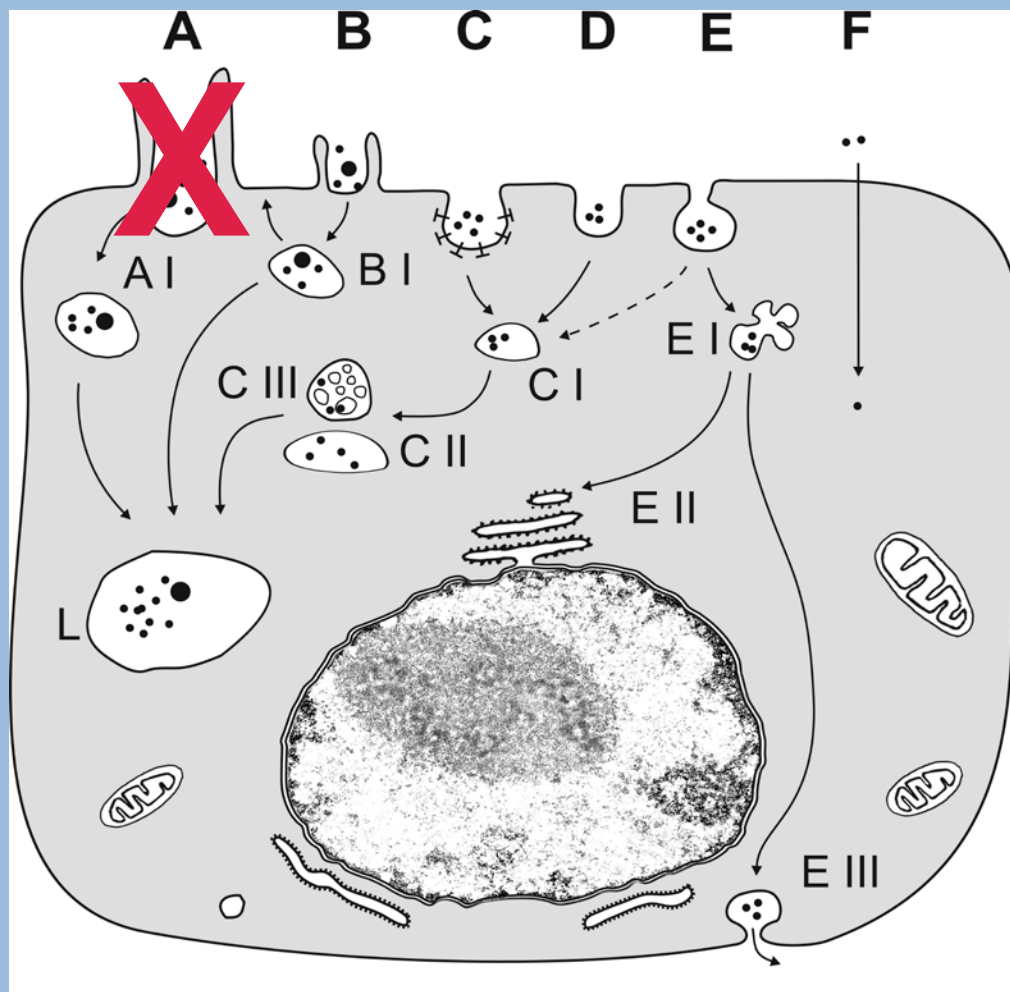
b  
UNIVERSITÄT  
BERN



Blank et al., Am. J. Respir. Cell Molec. Biol., 2007



# A BURNING QUESTION: HOW DO NANOPARTICLES ENTER CELLS?

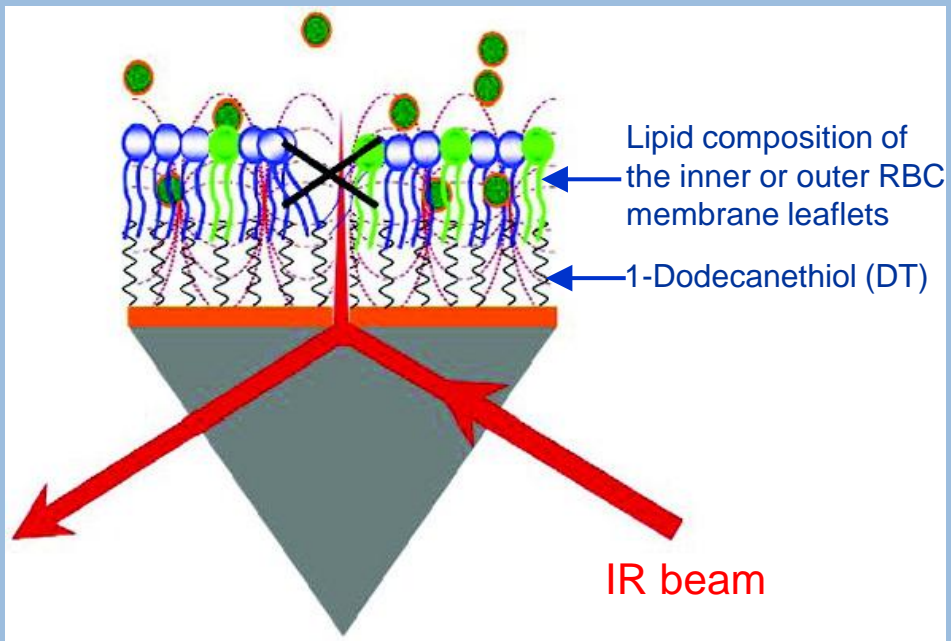


- (A: Phagocytosis)
- B: Macropinocytosis
- C: Clathrin-mediated endocytosis
- D: Clathrin and caveolae independent endocytic pathways
- E: Caveolae-mediated endocytosis**
- F: Adhesive interaction (entering):** *interaction of nanoparticles with cell membrane, effect on fluidity, nanoparticles may slip into cell between phospholipid molecules (→ U. Nienhaus, KIT)*

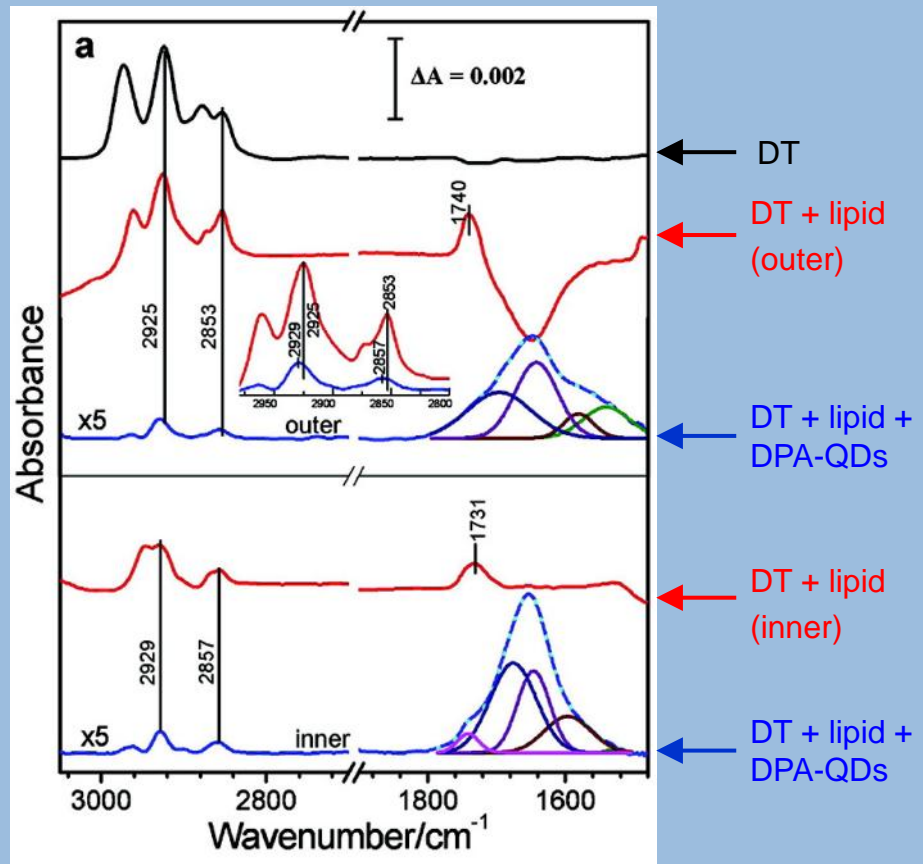
Brandenberger et al., Small, 2010

# ... AND AN ANSWER:

## ELECTROCHEMISTRY AND SURFACE-ENHANCED INFRARED ABSORPTION SPECTROSCOPY ON MODEL MEMBRANES (DAP-QDs)



Electrochemistry: voltammograms indicate that lipid layers do not conduct current upon DPA-QD exposure → no holes formed!



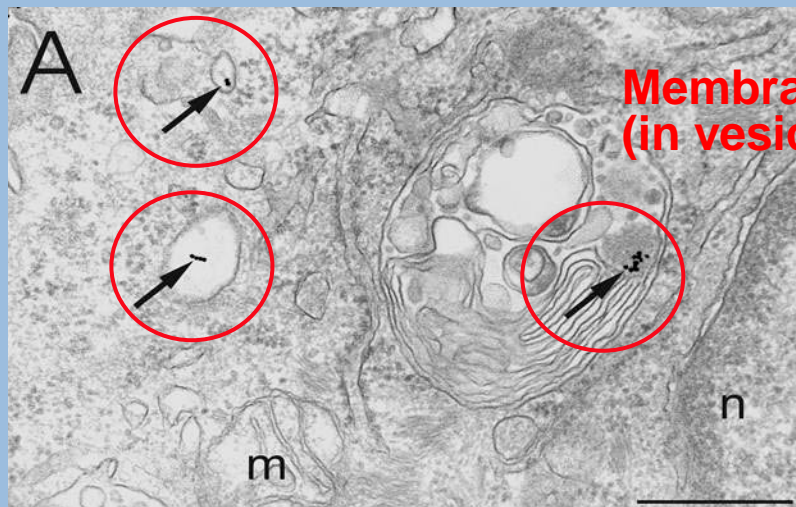
SEIRAS: Membrane flexibility is enhanced in the presence of DPA-QDs

Courtesy:  
G.U. Nienhaus,  
Institute of Applied Physics, KIT

Wang et al., *ACS Nano* 6 (2012) 1251-1259

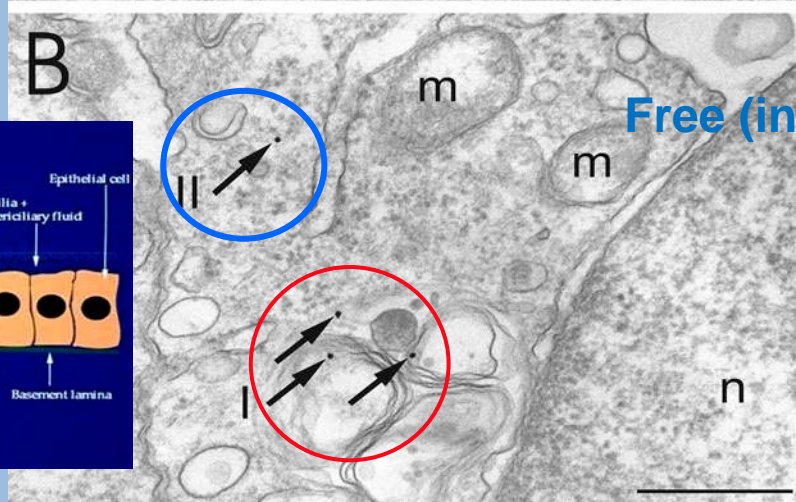
(Rothen-Rutishauser et al., *Environ. Sci. Technol.*, 2006)  
(Rothen-Rutishauser et al., *In Donaldson and Borm, Taylor&Francis*, 2007)

# NANOPARTICLES IN CELLS



Membrane bound  
(in vesicles/lysosomes)

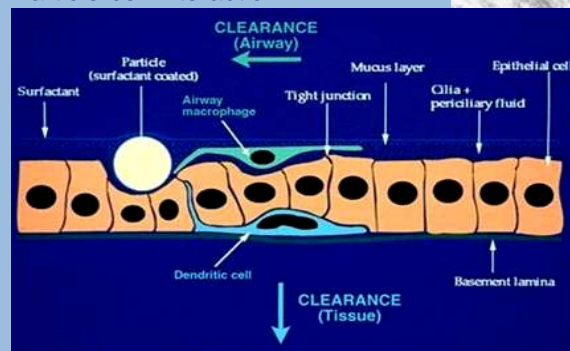
Plain Au nanoparticles



Free (in cytosol)

PEG coated Au nanoparticles  
→ more nanoparticles in cytosol

Particle-cell interaction



Brandenberger et al., Small 2010

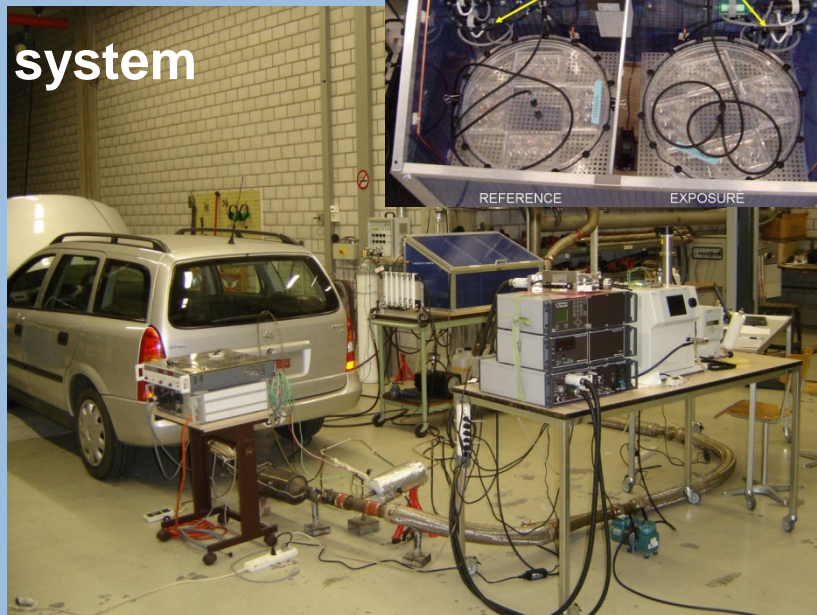
# WHAT WE SHOULD CONSIDER

## WHEN WORKING WITH NANOPARTICLES (UFP)

- **risk = f(hazard, exposure<sub>time</sub>)** for a given size
- **effect = f(dose, time<sub>after exposure</sub>)** for a given size
- **Interaction of nanoparticles with biological systems is primarily a function of size (*size matters*):**  
peneatration, translocation, effect/reaction
- **Important are furthermore:**  
material, corona, agglomeration, **time<sub>after exposure</sub>** etc.

# EFFECTS OF DIESEL EXHAUST ON BIOLOGICAL SYSTEMS

## Exposure system



## Exhaust system

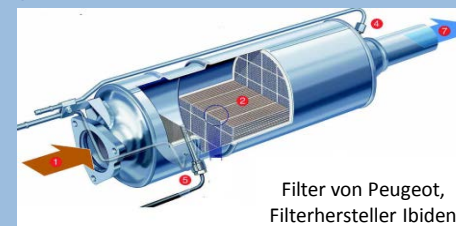
- Opel Astra X20DTL, 35 km/h
- Fuel: low sulfur diesel (>10mg/kg, Greenergy SA)
- Lube oil (V10.237, Motorex)
- Exhaust dilution 1:10

⇒ Without particle filter

⇒ With a silicon carbide diesel particle filter

Müller et al. Environ Sci Technol 2009;  
Steiner et al. Tox Letters 2012 in press

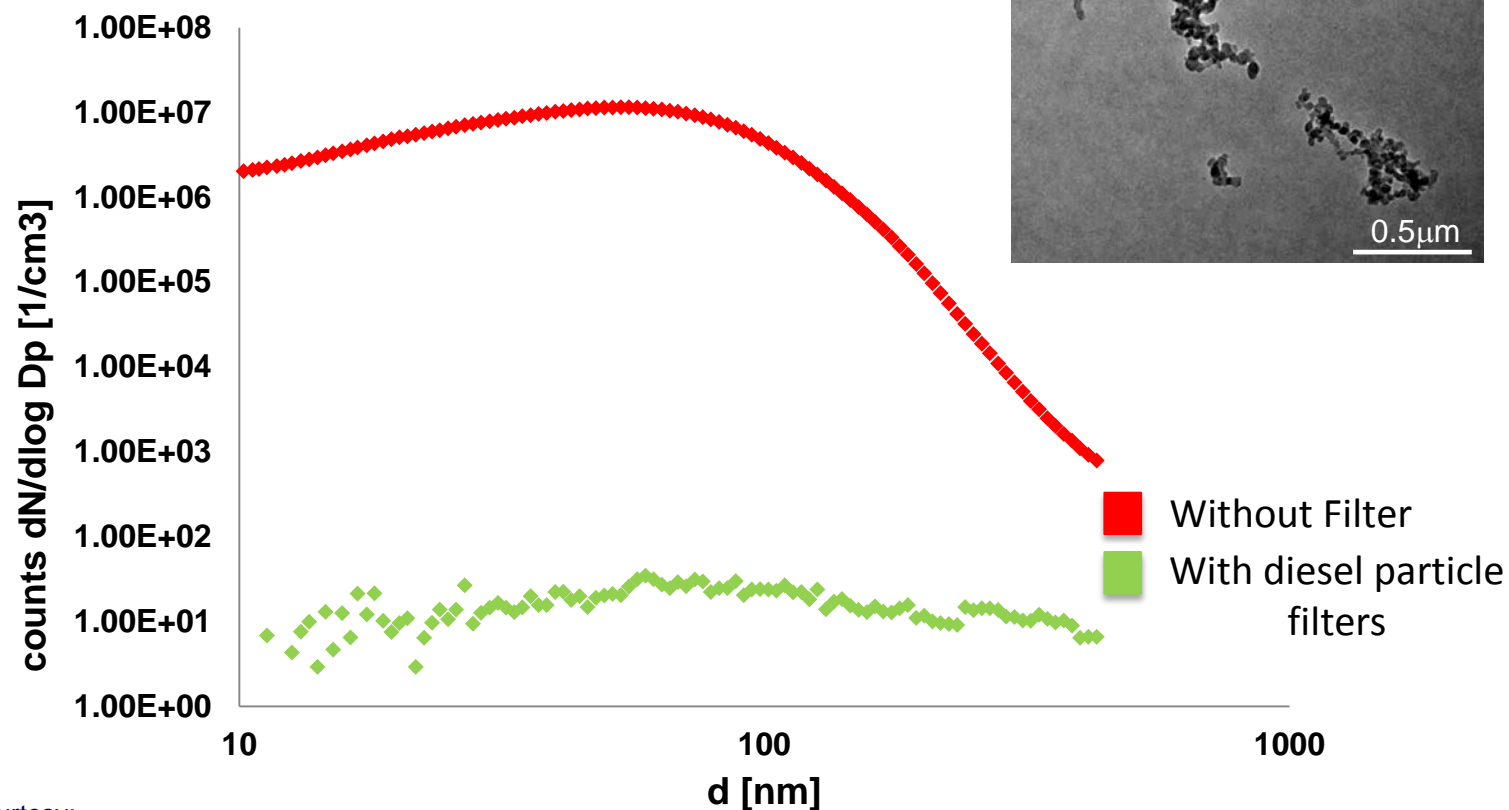
Courtesy:  
**Barbara Rothen-Rutishauser**  
Adolphe Merkle Institute  
University of Fribourg  
Switzerland



Filter von Peugeot,  
Filterhersteller Ibiden

# DIESEL EXHAUST I

## PARTICLE SIZE DISTRIBUTION



Courtesy:

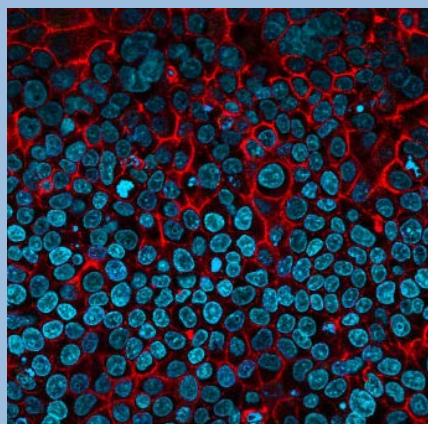
**Barbara Rothen-Rutishauser**

Adolphe Merkle Institute  
University of Fribourg  
Switzerland

Steiner et al., Atmos. Environ., 2013

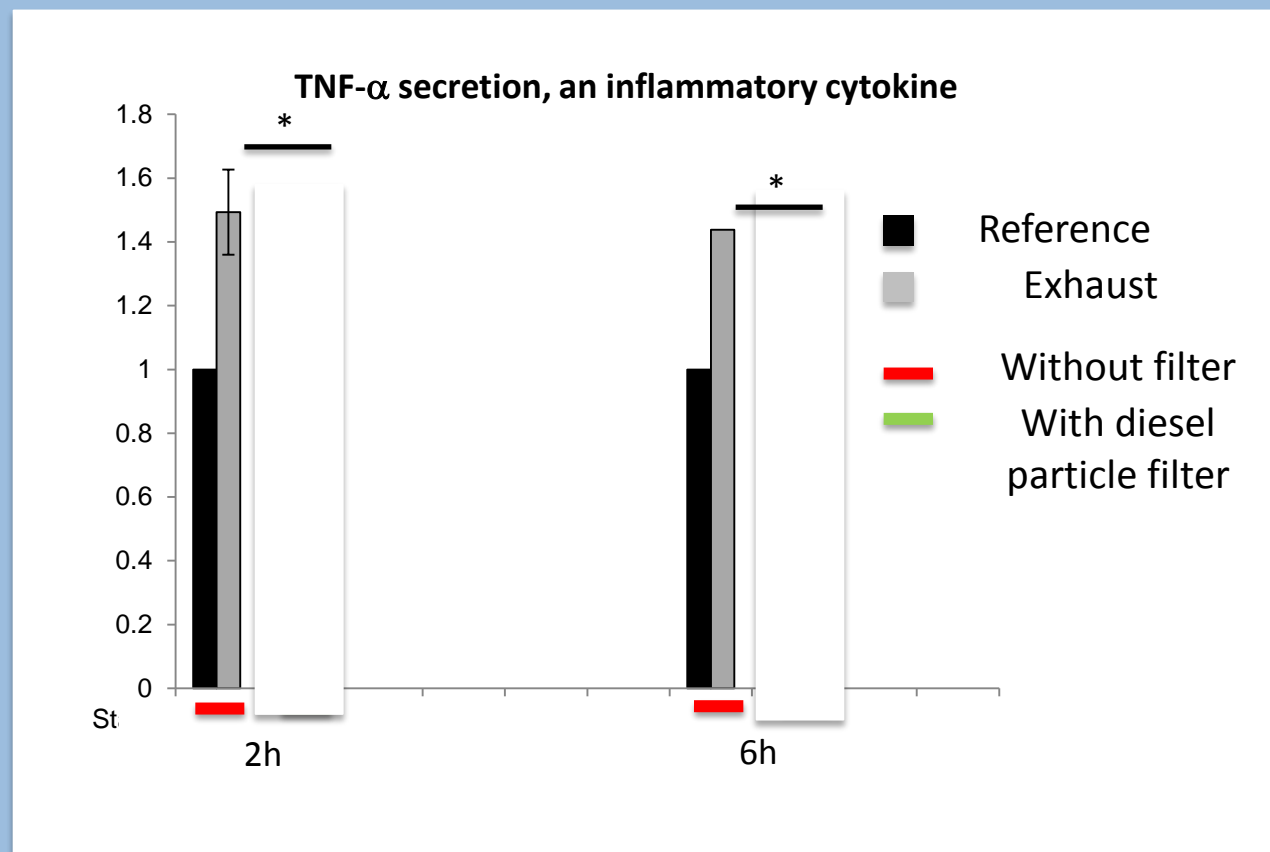
# DIESEL EXHAUST II

## INFLAMMATORY REACTION OF CELLS



Confocal light micrograph  
(blue: nuclei, red: actin)

Courtesy:  
**Barbara Rothen-Rutishauser**  
Adolphe Merkle Institute  
University of Fribourg  
Switzerland



Steiner et al., Atmos. Environ., 2013

# WHAT SHOULD BE CONSIDERED

**SIZE MATTERS! UFP CAN TRANSLOCATE INTO BLOOD IN LUNGS!**

- Diesel exhaust, air pollution were declared carcinogenic (many UFP)
- Distance to source of air pollution (e.g. traffic) is crucial
- Filters contribute substantially to reducing adverse health effects from diesel exhaust particles (>99% removed from exhaust)
- UFP (ultrafine particles) enter cells and tissue very easily
- UFP can translocate into blood in the lungs, translocation to secondary organs -> lung is main portal of entry for UFP
- Effects on lungs:
  - Reduced pulmonary function in adults (asthmatics) (1<sup>st</sup> slide)
  - Reduced development and function of lungs in neonates (not shown)
- *Speculations (Translocation through internal tissue barriers) e.g.:*
  - *Blood-brain-barrier (e.g. Alzheimer's disease?)*
  - *Blood testis barrier (Development/maturation of sperms?)*
  - *Blood thymus barrier (Development of T-lymphocytes?)*



# TRAFFIC RELATED PM FROM HIGHWAY 405 CAUSE ATHEROSCLEROSIS IN MICE

Araujo et al, Circul Res 2008

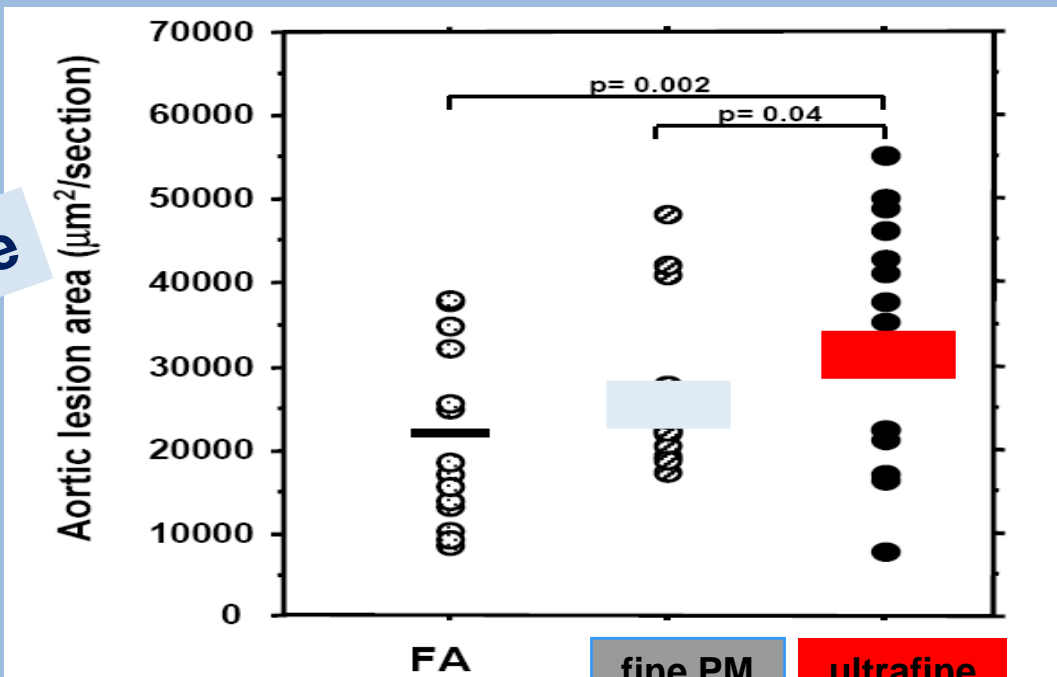
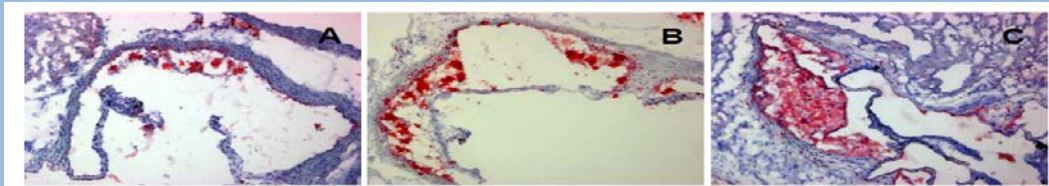
## Exposure:

40 days

5h / day

3 days / week

Toxicology example



Picture from  
**Nino Künzli, MD, PhD; MPH**  
Professor and Deputy Director  
Swiss Tropical and Public Health Institute, Basel  
Dean, Swiss School of Public Health, Zurich  
Switzerland

fine PM (PM2.5)  
ultrafine PM (PM0.1)

# UFP SUMMARY

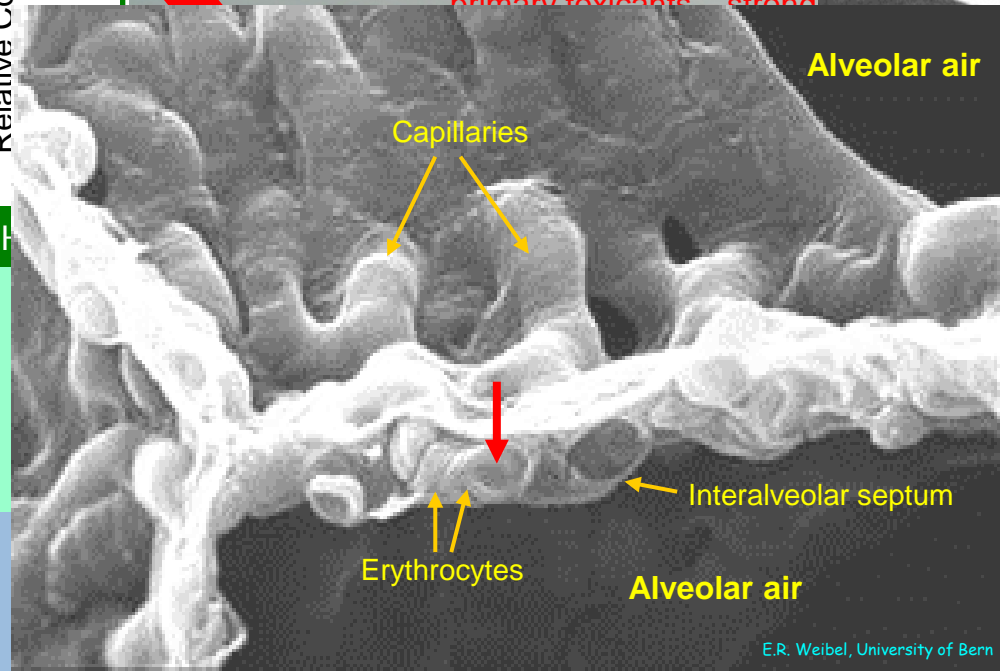
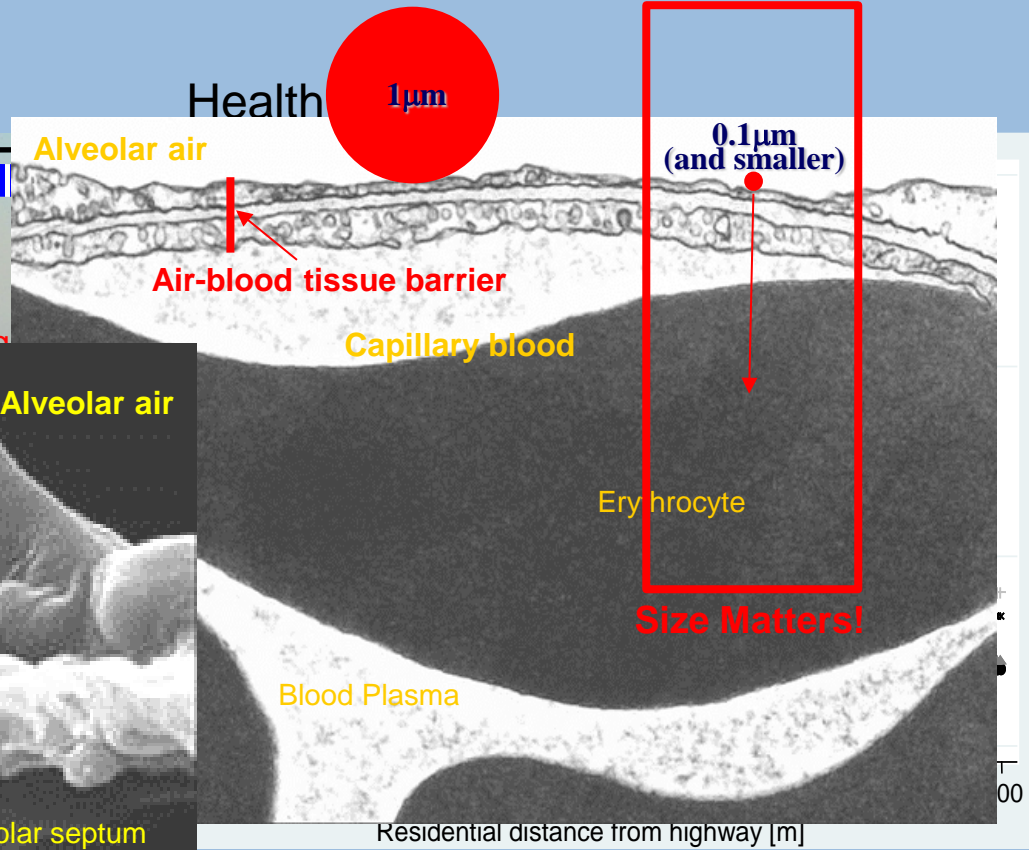
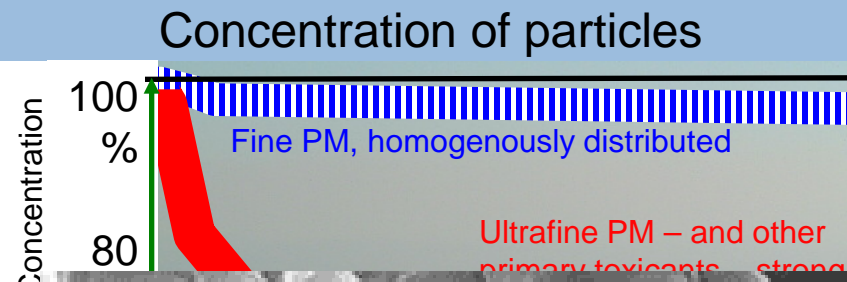
COMBUSTION AEROSOLS HAVE EFFECTS ON  
LUNG FUNCTION/HEALTH (PARTICLE-LUNG INTERACTION)

*u<sup>b</sup>*

b  
UNIVERSITÄT  
BERN

- Deposition on internal surfaces of the lungs
- Displacement of particles towards epithelial layer by surfactant at air-aqueous phase interface (surface forces)
- Interaction with pulmonary cells (epithelial, defence system): cellular interplay, intracellular trafficking
- Translocation through air-blood tissue barrier into capillary blood
- Tanslocation to secondary organs by blood circulation
- **Particle size matters!**

# CONCENTRATION OF PARTICLES AND HEALTH – DISTANCE FROM BUSY ROAD



E.R. Weibel, University of Bern

...res from  
...o Künzli  
... Tropical and Public Health Institute  
... Basel, Switzerland

# ACKNOWLEDGEMENTS



## University of Bern

Barbara Rothen-Rutishauser

*Today: Universities of Fribourg/Bern*

Martin Clift

*Today: University of Fribourg*

Fabian Blank

Christina Brandenberger

*Today: University of Michigan*

Loretta Müller

*Today: University of North Carolina*

Andrea Lehmann

*Today: RMS Foundation R. Mathys*

Michael Gasser

*Today: Fed. Dpt. Home Affairs*

David Raemy

*Today: Insel Hospital Univ. of Bern*

Marc Wehrli

Oliver Baum

Sandra Frank

Andrea Stokes

Barbara Tschirren

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Christian Mühlfeld

*Today: Medizinische Hochschule Hannover, Germany*

## University of Calgary, Canada

Samuel Schürch

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ETH: Zurich

EMPA: St.Gallen

IST: Lausanne

Helmholtz Zentrum: München

Universität Ulm: Ulm

Universität Marburg: Marburg

Heriot-Watt University: Edinburgh

**Nino Künzli, MD, PhD; MPH**  
Professor and Deputy Director  
Swiss Tropical and Public Health  
Institute, Basel, Switzerland

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