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excellence in pure and applied nanoscience

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# Predictive 3D lung models for inhalation toxicology – where are we and where should we go?

**Barbara Rothen-Rutishauser**

Co-Chair BioNanomaterials

Adolphe Merkle Institute

University of Fribourg

Fribourg, Switzerland



# The Adolphe Merkle Institute

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**Polymer Chemistry &  
Materials**  
(Weder)

**Soft Matter Physics**  
(Steiner)

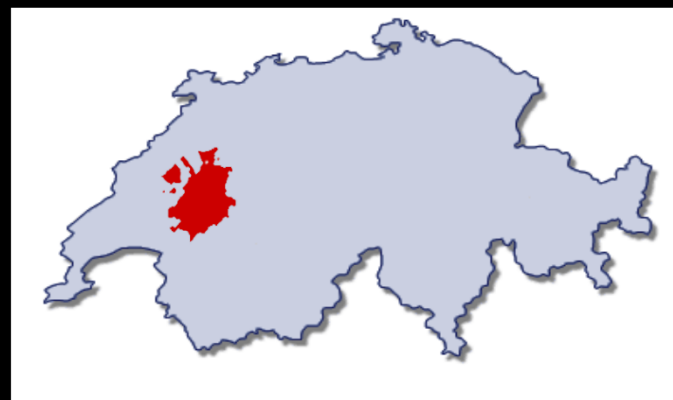
**BioNanomaterials**  
(Fink/Rothen)

**Biophysics**  
(Mayer)

**Assembly**  
(Lattuada)

**Scattering**  
(Balog)

**Macromolecular  
Chemistry**  
(Nico Bruns)





# Inhalation of (nano)particles

## Definition of nanoparticles:

A material with three external dimensions between 1-100 nm

ISO/TS: 27687:2008



<http://www.theguardian.com/uk/2013/jan/27/diesel-engine-fumes-worse-petrol>



<http://www.stern.de/gesundheits/allergie/erkrankungen/>



<http://www.dguv.de/ifa/Fachinfos/Nanopartikel-am-Arbeitsplatz/>



<http://www.spiegel.de/gesundheits/diagnose/>



<http://www.netdoktor.de/Krankheiten/Asthma/Therapie/>

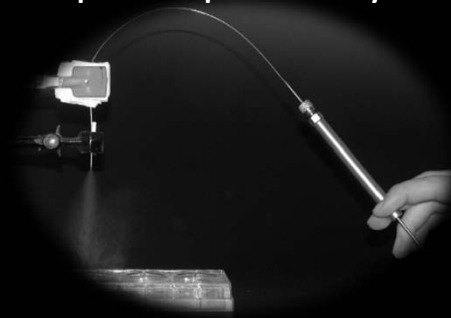


# Hazard assessment of (nano)particles *in vitro*

## 3D Lung models



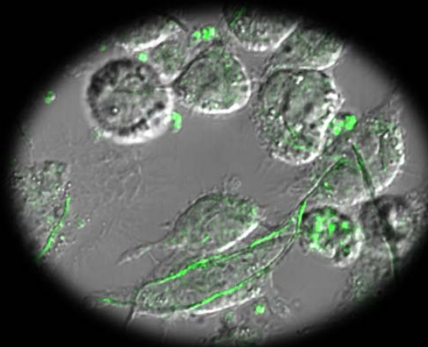
## Air-liquid exposure systems



Definition of realistic:  
"resembling or simulating real life"  
dictionary.com

<http://www.dguv.de/>

## Cell responses

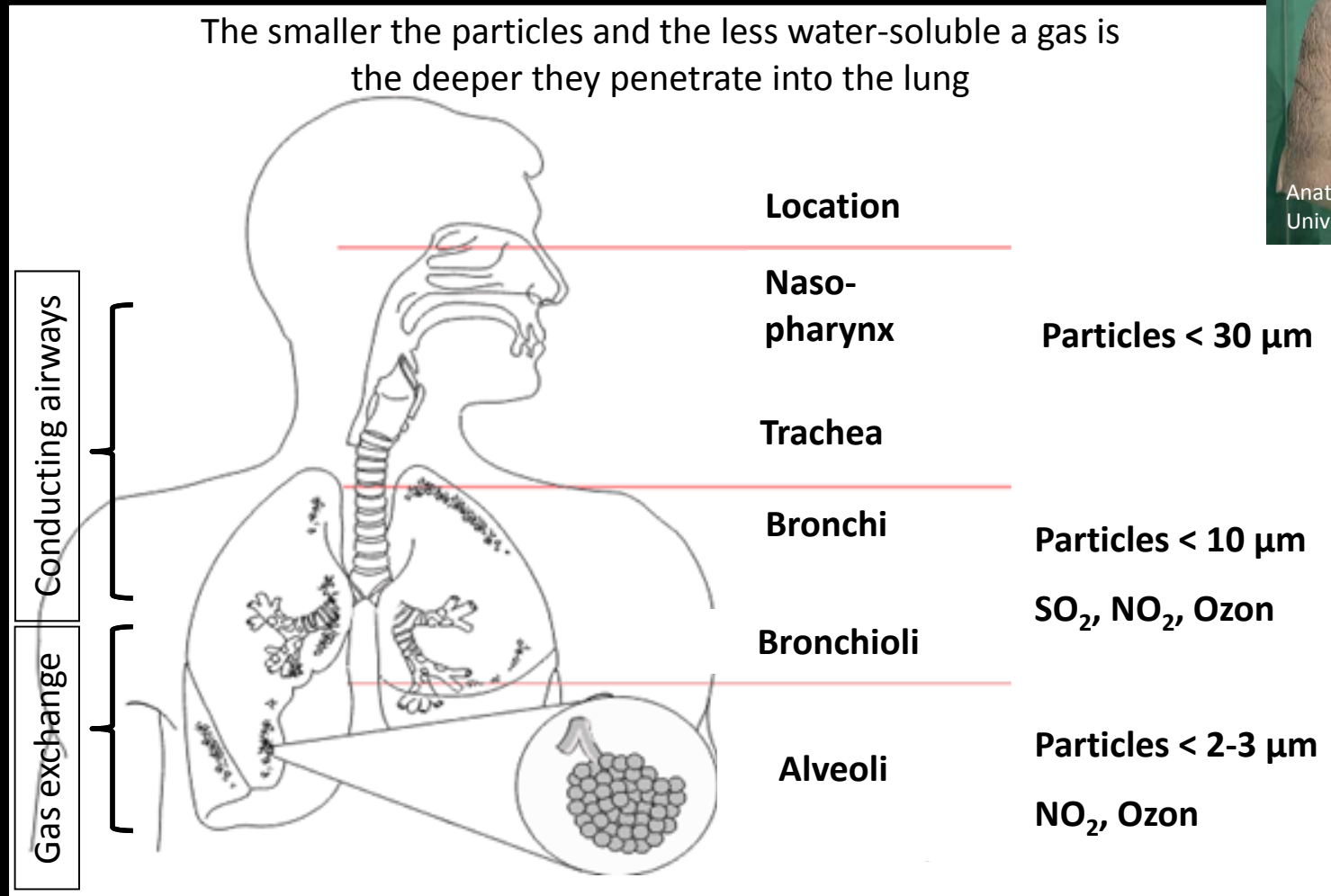
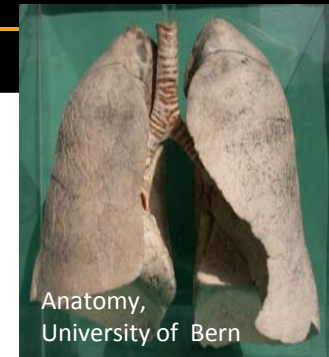


**Prediction of nanomaterials  
toxicity**



# Interaction of inhaled particles with the human lung

The smaller the particles and the less water-soluble a gas is the deeper they penetrate into the lung



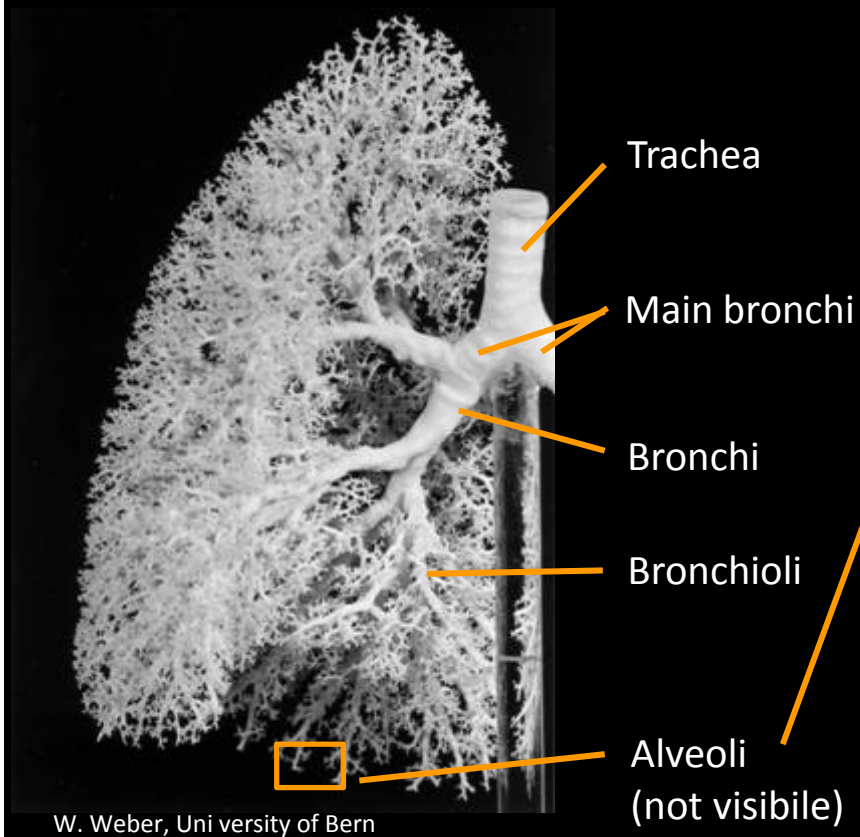
Dr. P. Straehl, BAFU, Abt. Luft-reinhaltung und NIS and Dr. Regula Rapp, Swiss TPH



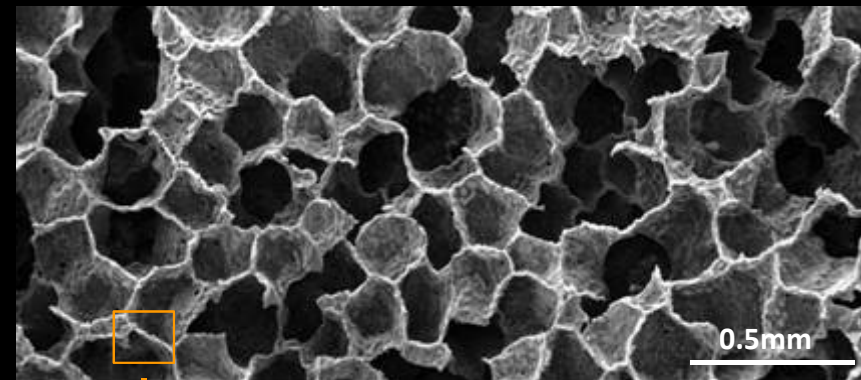


# The human lung structure

## Conducting airways

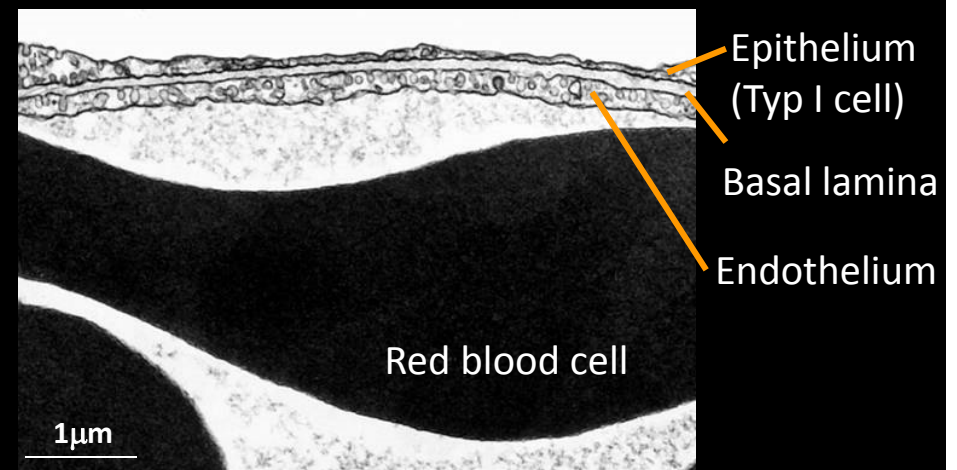


## Gas exchange



Alveolus (Air)

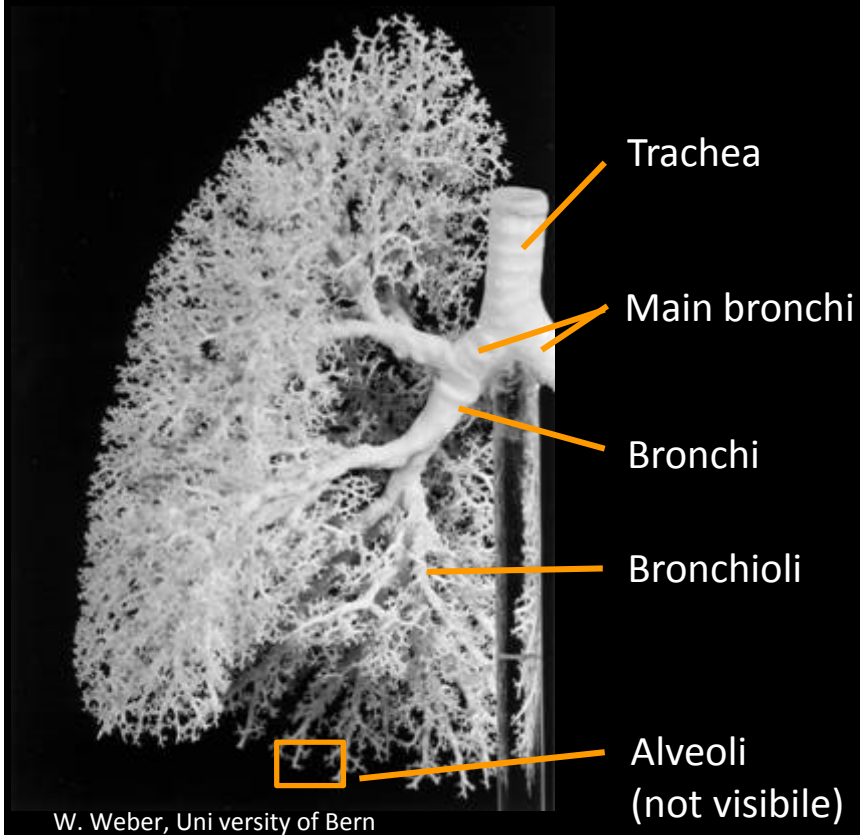
P. Gehr  
University of Bern



Capillary

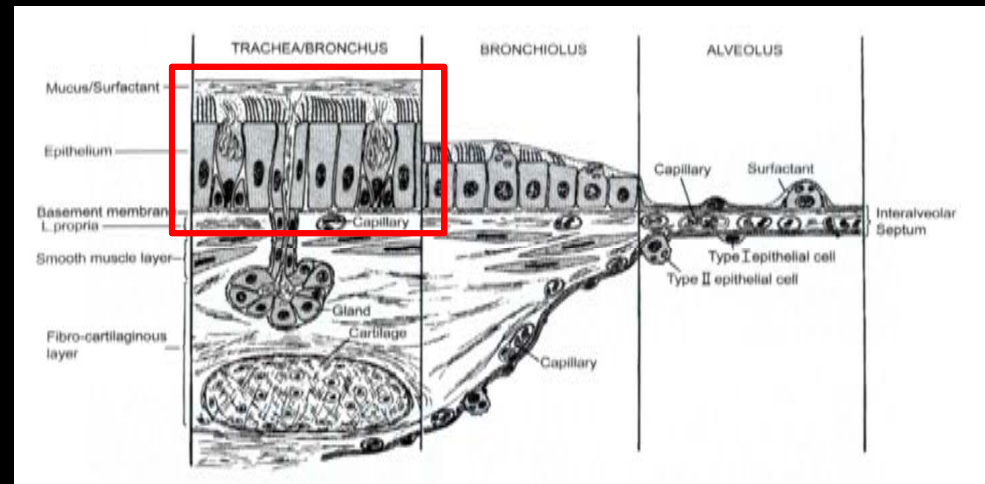


# The human lung: airway – alveolar wall



**Ciliated, columnar/cuboidal  
epithelial cells**

**Thin, outspreaded  
epithelial cells**

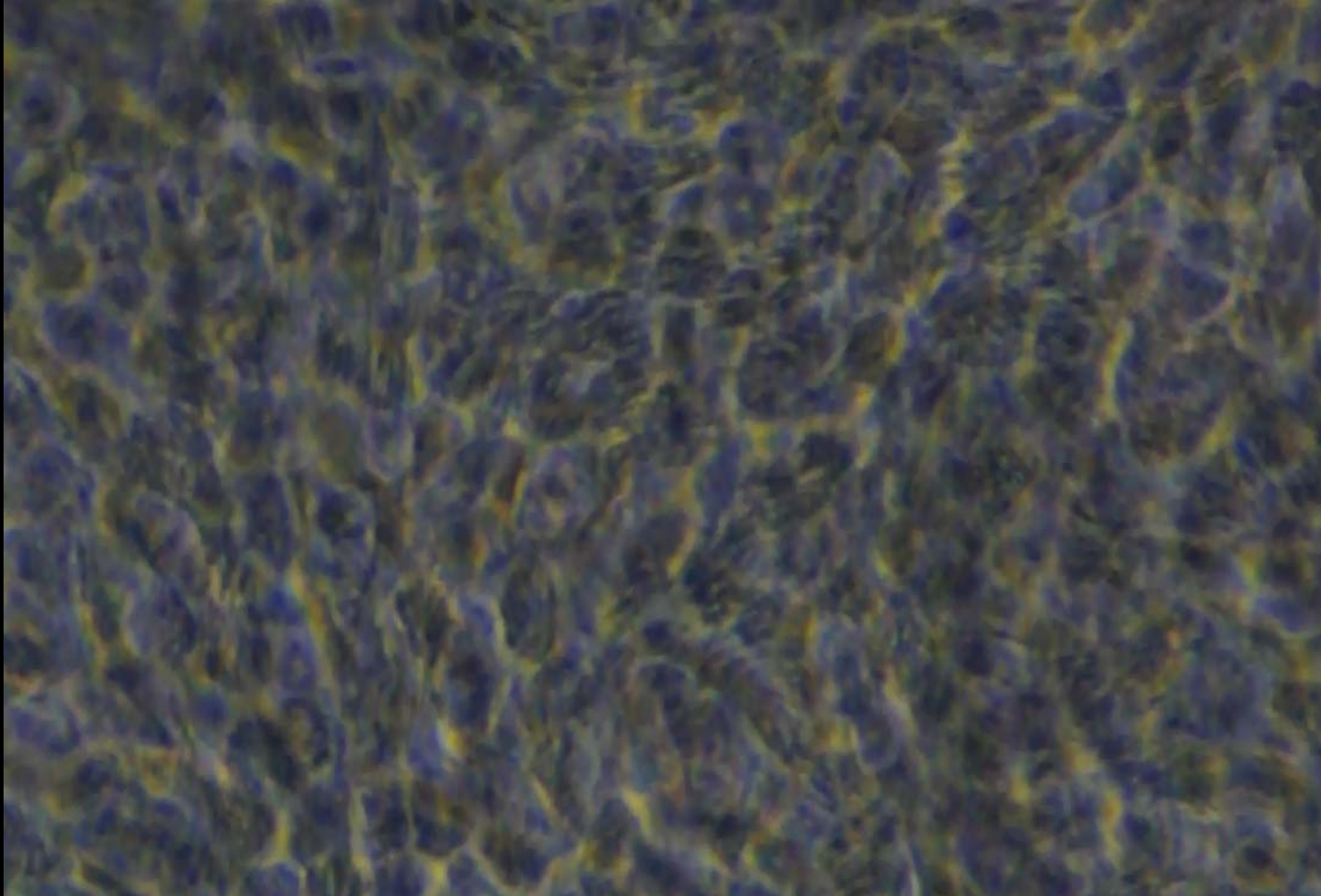


Ochs and Weibel. Functional design of the human lung for gas exchange. In Fishman's Pulmonary Diseases and Disorders, New York, 2008



# Human nasal epithelial cultures

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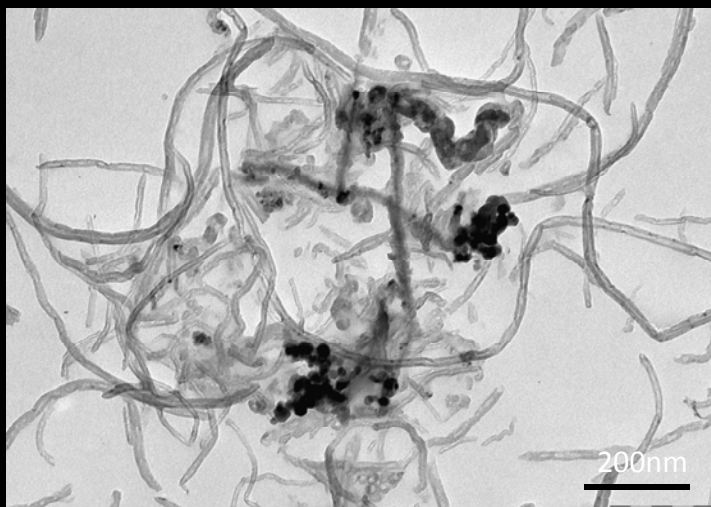
F. Blank, University of Bern





# Air-liquid exposures to nanomaterials

## Multi-walled carbon nanotubes



Wick et al. Tox Letters (2007)

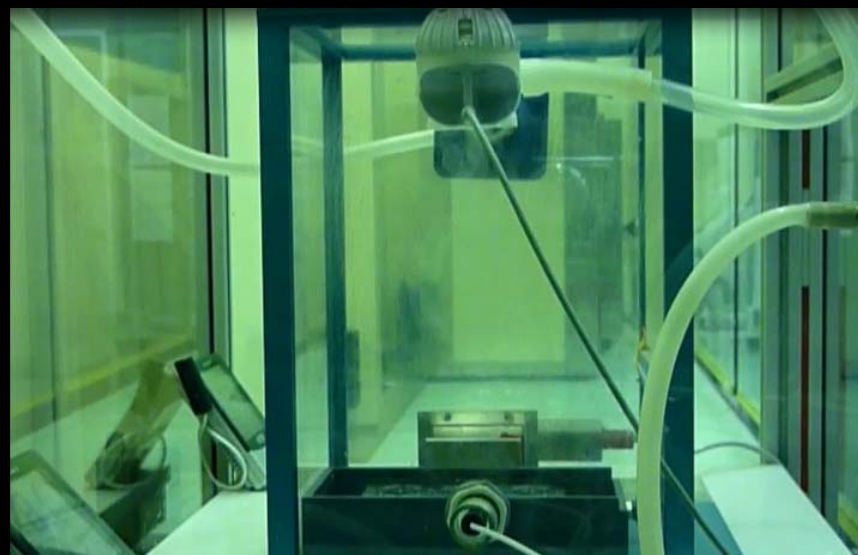


nanotechweb.org



sed-fernseher.eu

## Air-liquid cell exposure system (ALICE)



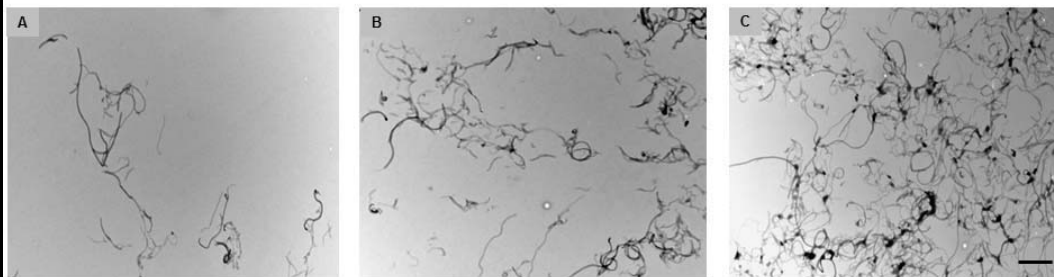
C. Endes

Lenz et al. Part Fibre Tox 2007



# Repeated exposure to carbon nanotube-based aerosols

Multi-walled carbon nanotubes



5 weeks of repeated exposures  
/ 5 days per week  
**10  $\mu\text{g}/\text{cm}^2$**

=> Occupational lifetime exposure



<http://www.dguv.de/ifa/Fachinfos/Nanopartikel-am-Arbeitsplatz/Schutzmaßnahmen/index-2.jsp>

**Full working lifetime** exposure to  $1 \mu\text{g}/\text{m}^3$   
aerosol concentration of CNT ranged from  
**12.4 to 46.5  $\mu\text{g}/\text{cm}^2$**  alveolar mass retention  
Gangwal et al. Env Health Persp 2011

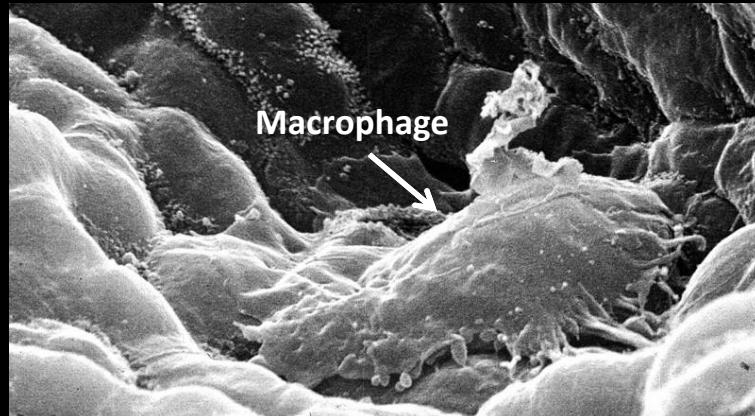
Chortarea et al. Nanotoxicology 2015

Nanotoxicology: RSC Toxicology Award Seminar, 19<sup>th</sup> of April 2016, London



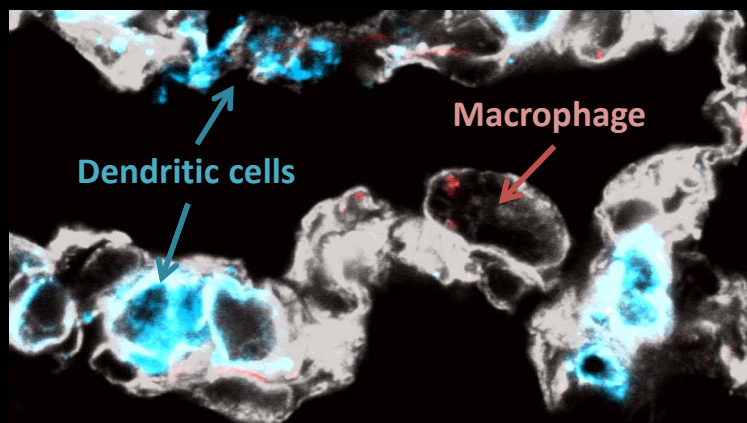
# The human lung: airway – alveolar wall

## *In vivo* (human lung)



P. Gehr, University of Bern

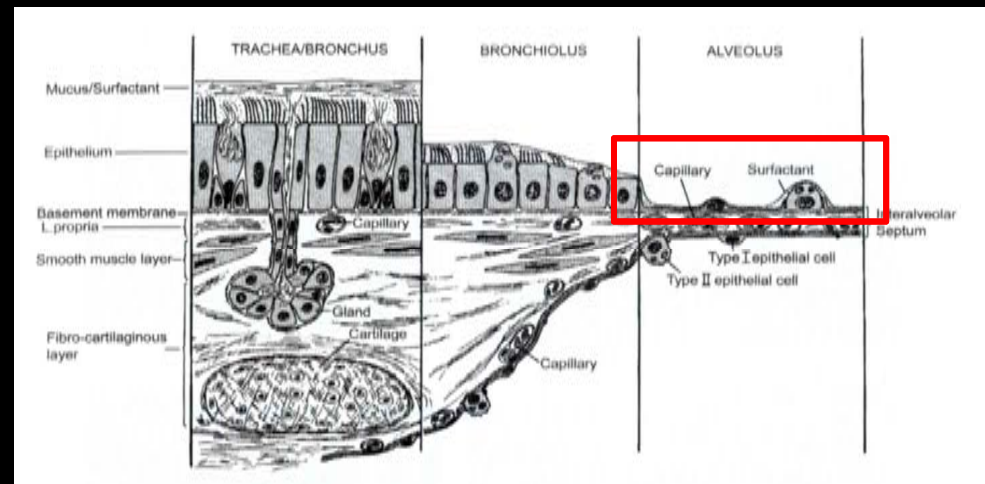
## *In vivo* (mouse lung)



F. Blank, University of Bern

**Ciliated, columnar/cuboidal  
epithelial cells**

**Thin, outspread  
epithelial cells**

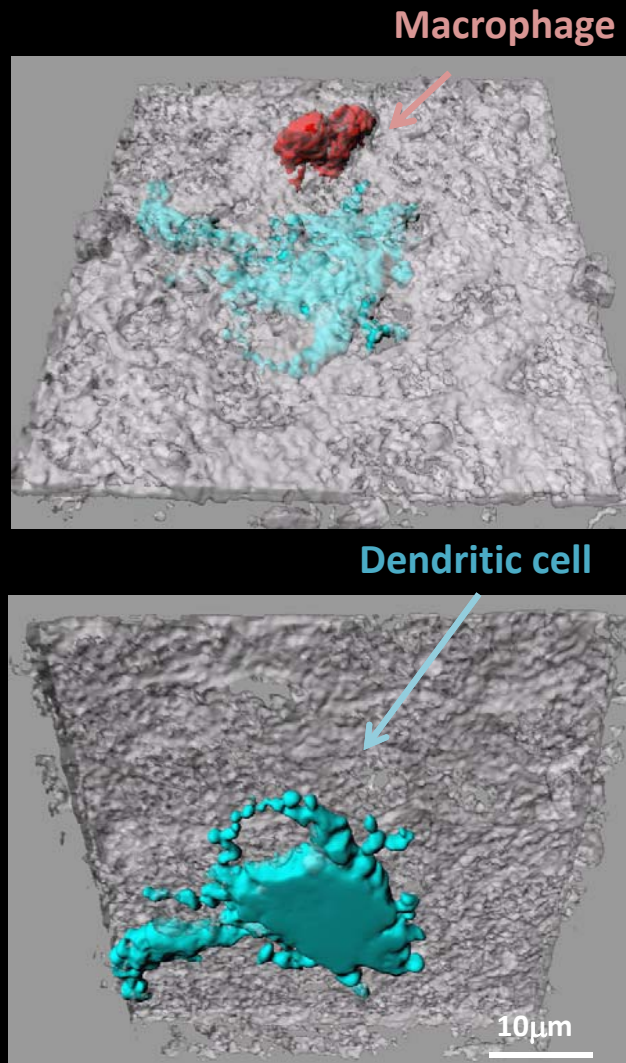


Ochs and Weibel. Functional design of the human lung for gas exchange. In Fishman's Pulmonary Diseases and Disorders, New York, 2008

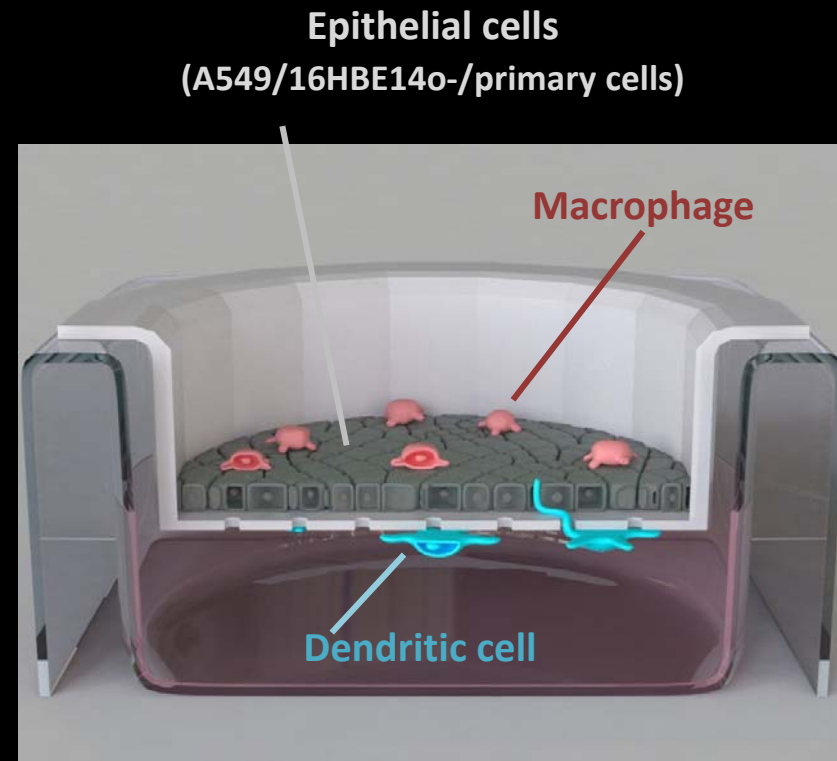




# Triple cell co-culture of lung epithelial tissue



Rothen-Rutishauser et al. Swiss Med Wkly 2010



Rothen-Rutishauser et al. Am J Respir Cell Mol Biol 2005;  
Blank et al. Am J Respir Cell Mol Biol 2007  
Rothen-Rutishauser et al. Review, Exp Opin Drug Metab Toxicol 2008  
Lehmann et al. Eur J Pharm Biopharm 2010

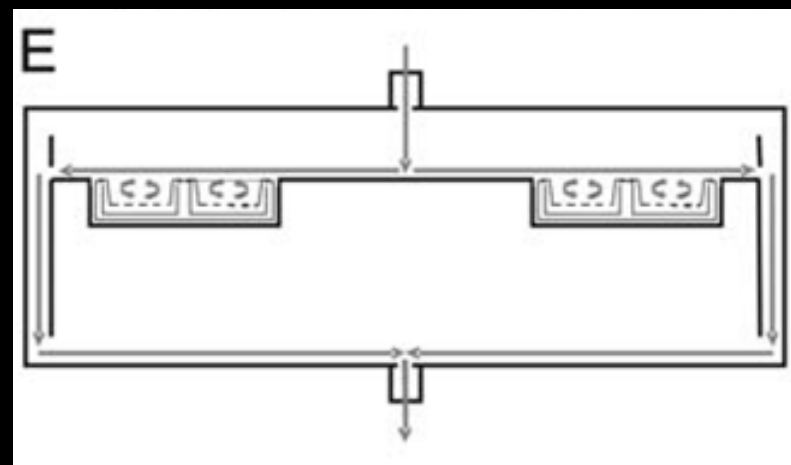
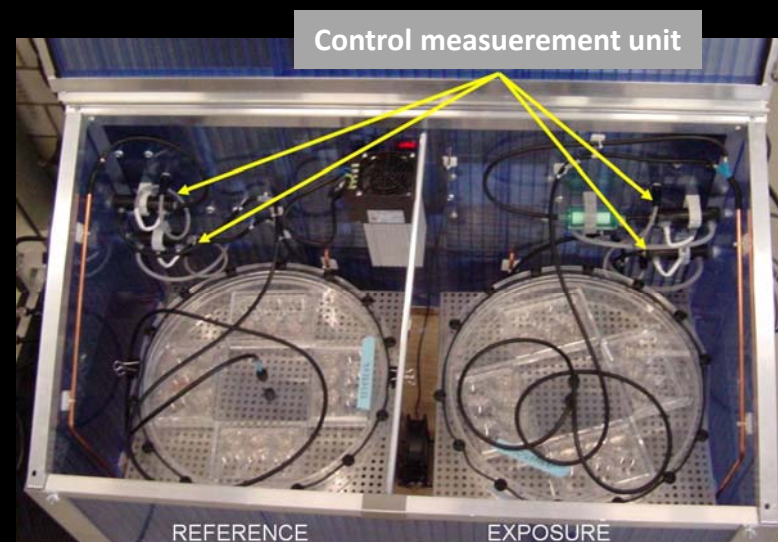




# Exhaust emission exposure system



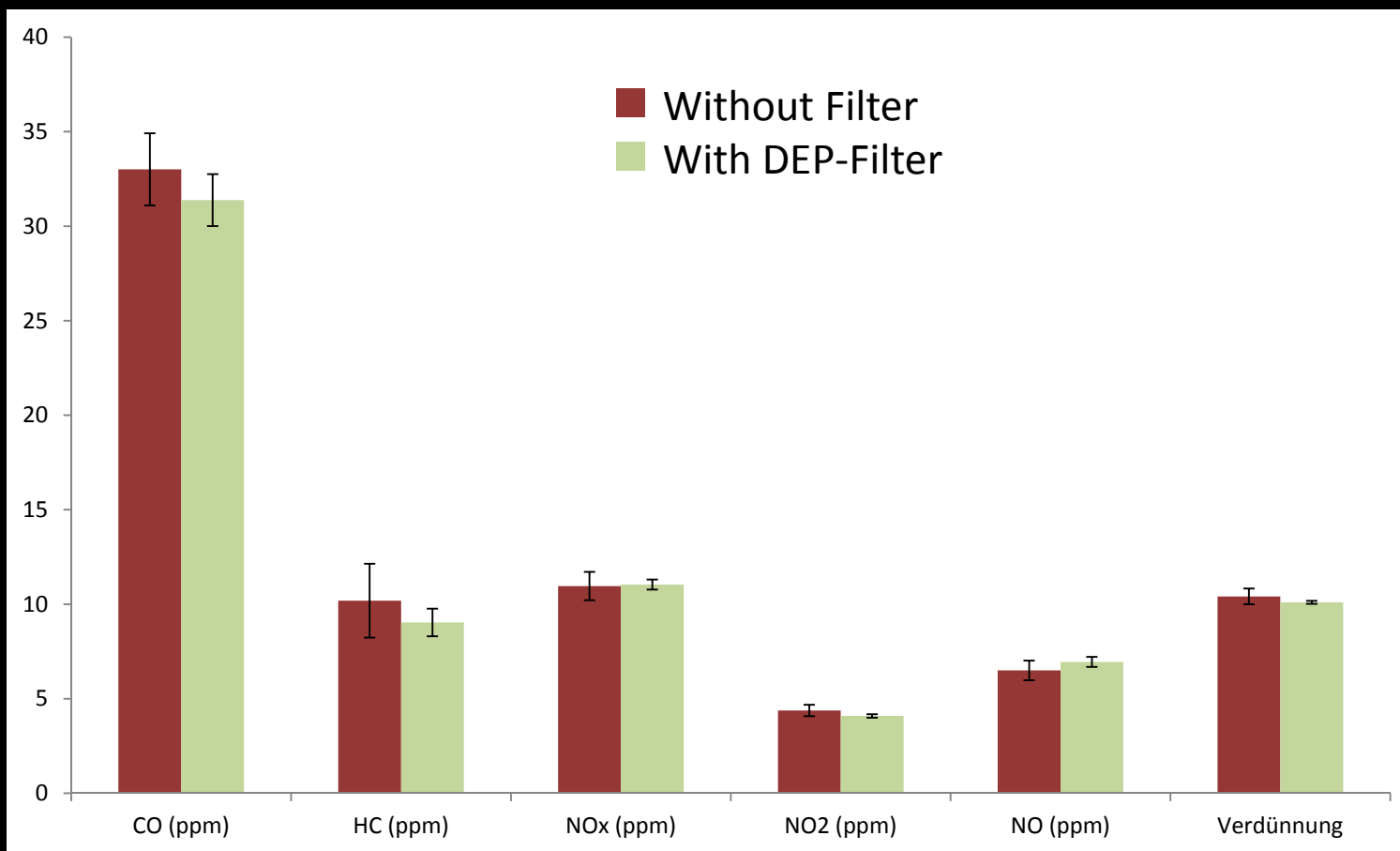
Müller et al. Environ Sci Technol 2009  
Steiner et al. Tox Letters 2012



Müller et al. Environ Sci Technol 2009  
Müller et al. Tox Env Chem 2012



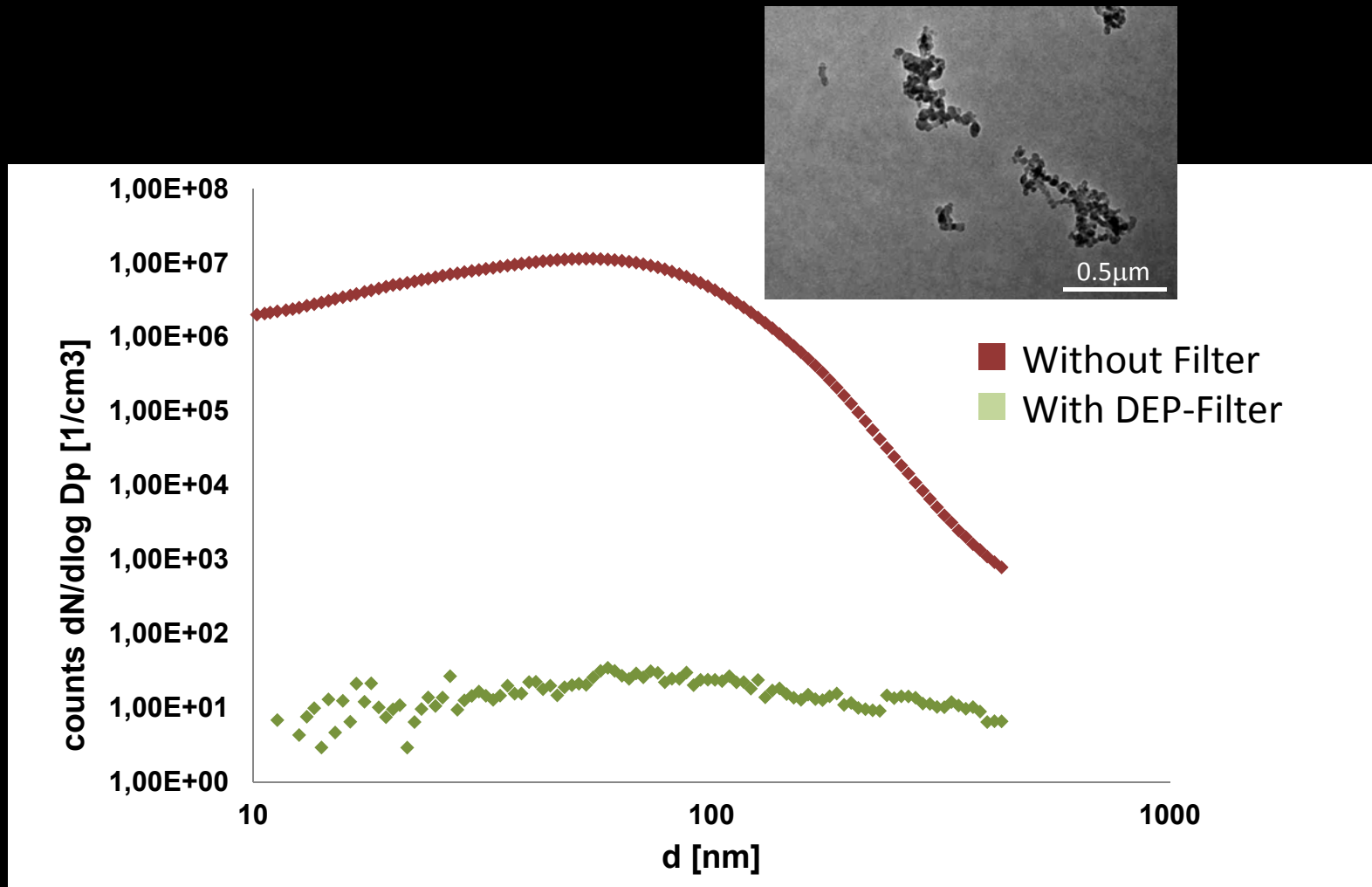
# Exhaust characterisation



Steiner et al. 2013 Atmos Environ



# Particle size distribution



Steiner et al. 2013 Atmos Environ



# Exhaust emission exposure system

Marker	Diesel no filter	GDI 1 no filter	GDI 1 uncoat. GPF	GDI 2 no filter	GDI 2 uncoat. GPF	GDI 2 coated GPF
Particle number (#/cm <sup>3</sup> )	~10 <sup>6</sup>	~10 <sup>5</sup>	~10 <sup>2</sup>	~10 <sup>5</sup>	~10 <sup>4</sup>	~10 <sup>4</sup>
CO (mg/km); extra high part!	~10	~1500	~1500	~750	~750	~750
NOx (mg/km); extra high part!	~1500	~20	~20	~20	~20	~20
Cytotoxicity (LDH)	↑	-	-	-	-	-
Morphologie (Microscopy)	-	-	-	-	-	-
Oxidative stress	↑↑	↑	↑↑	-	-	-
Pro-inflammation	↑↑	-	↑↑	-	-	-
AhR-upregulation	↑	↑↑	-	-	-	-

Stable and reproducible system to study the effect of **exhaust emissions** *in vitro*  
⇒ Differentiation between gaseous compounds and particles

Removal of the particles alone is **not sufficient for reduction of exhaust toxicity**





# The air-blood tissue barrier *in vitro* to study NP translocation

Arch Toxicol (2015) 89:1469–1495  
DOI 10.1007/s00204-015-1518-5



REVIEW ARTICLE

## Progress and future of *in vitro* models to study translocation of nanoparticles

Hedwig M. Braakhuis<sup>1,2</sup> · Samantha K. Kloet<sup>3</sup> · Sanja Kezic<sup>4</sup> · Frieke Kuper<sup>5</sup> ·  
Margriet V. D. Z. Park<sup>2</sup> · Susann Bellmann<sup>5</sup> · Meike van der Zande<sup>6</sup> · Séverine Le Gac<sup>7</sup> ·  
Petra Krystek<sup>8</sup> · Ruud J. B. Peters<sup>6</sup> · Ivonne M. C. M. Rietjens<sup>3</sup> · Hans Bouwmeester<sup>6</sup>

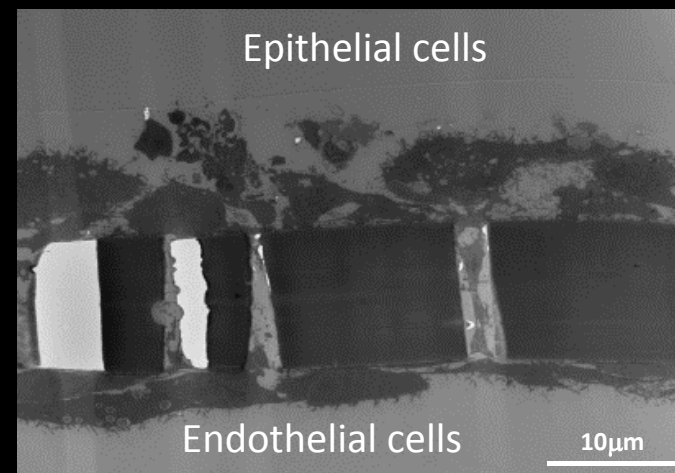
Human type II alveolar epithelial cells / pulmonary  
microvascular endothelial cells

Hermanns et al. Cell Tissue Res 2009

Co/Triple cell co-culture models composed of human  
epithelial cells / macrophages / mast cells / endothelial cells

Alfaro-Moreno et al. Eur Respir J 2008

Klein et al. Part Fiber Toxicol 2013



Jud et al. Biores Open Access 2015



## ....where to go from here

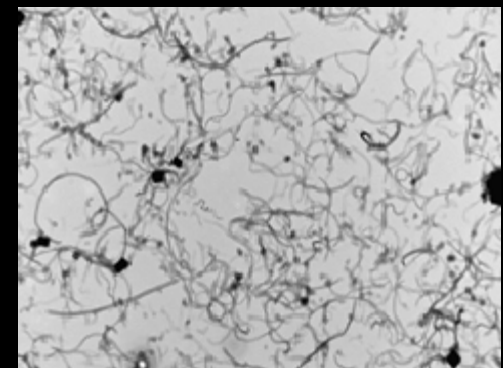
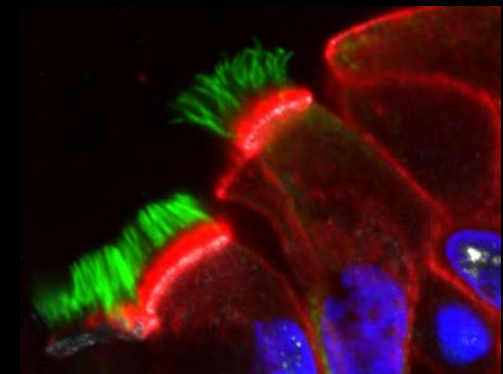
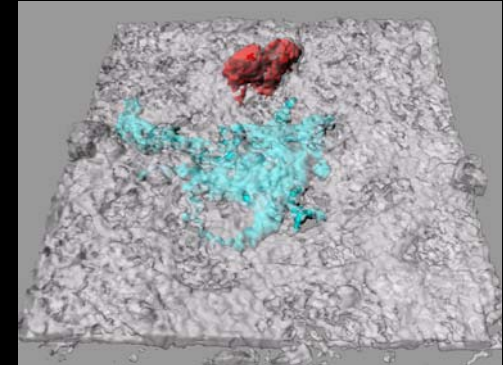
### 3D lung models

- Represent more physiologically relevant situations
- Structural-functional characterisation
- There is not one optimal model yet for all questions

**Air-liquid exposures** mimicking realistic inhalations of nanomaterials (dose)

More complex models including

- **breathing** patterns
- (blood) **flow**





....that is all nice

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But is it relevant?



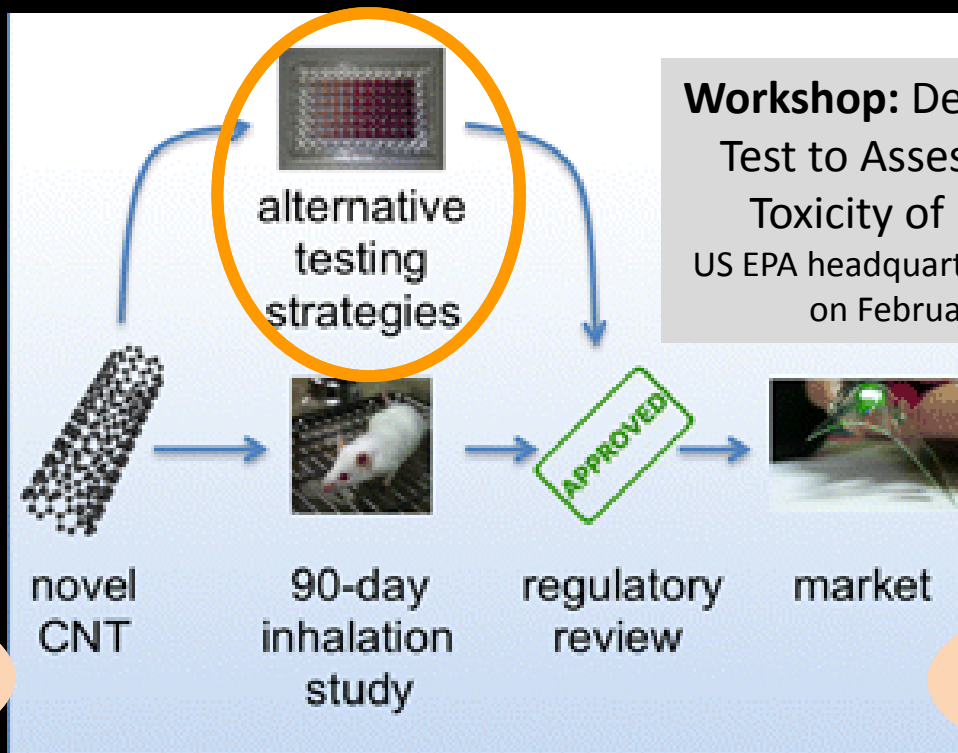
# Testing of new chemicals and nanomaterials

Researchers



Industry

Policy /  
Organisations



Regulatory  
authorities

Godwin et al. ACS Nano (2015)





# MWCNTs can induce pulmonary fibrosis

Vietti et al. *Particle and Fibre Toxicology* 2013, **10**:52  
http://www.particleandfibretoxicology.com/content/10/1/52



## RESEARCH

## Open Access

### Towards predicting the lung fibrogenic activity of nanomaterials: experimental validation of an in vitro fibroblast proliferation assay

Giulia Vietti<sup>1\*</sup>, Saloua Ibouadaten<sup>1</sup>, Mihaly Palmay-Pallag<sup>1</sup>, Yousof Yakoub<sup>1</sup>, Christian Bailly<sup>2</sup>, Ivana Fenoglio<sup>3</sup>, Etienne Marbaix<sup>4</sup>, Dominique Lison<sup>1</sup> and Sybille van den Brule<sup>1</sup>

Mercer et al. *Particle and Fibre Toxicology* 2011, **8**:21  
http://www.particleandfibretoxicology.com/content/8/1/21

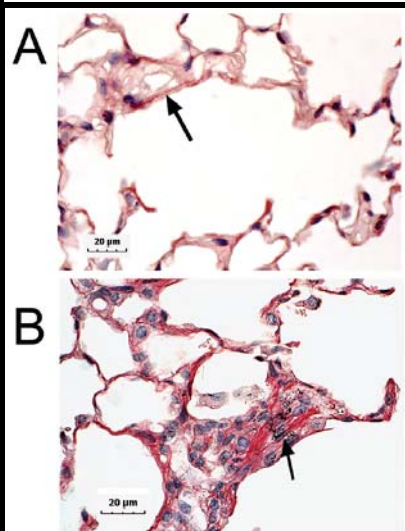


## RESEARCH

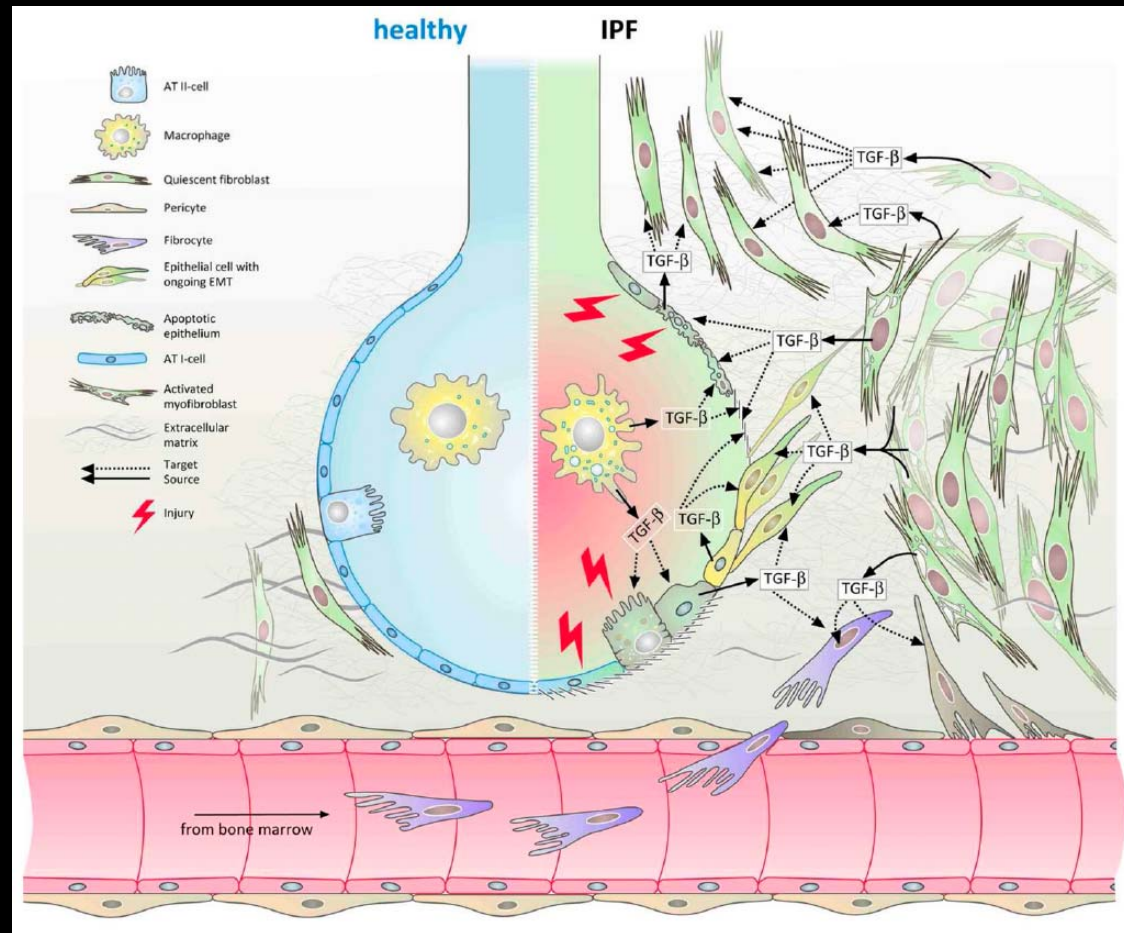
## Open Access

### Pulmonary fibrotic response to aspiration of multi-walled carbon nanotubes

Robert R Mercer<sup>1,2\*</sup>, Ann F Hubbs<sup>1</sup>, James F Scabilloni<sup>1</sup>, Liying Wang<sup>1</sup>, Lori A Battelli<sup>1</sup>, Sherri Friend<sup>1</sup>, Vincent Castranova<sup>1</sup> and Dale W Porter<sup>1,2</sup>



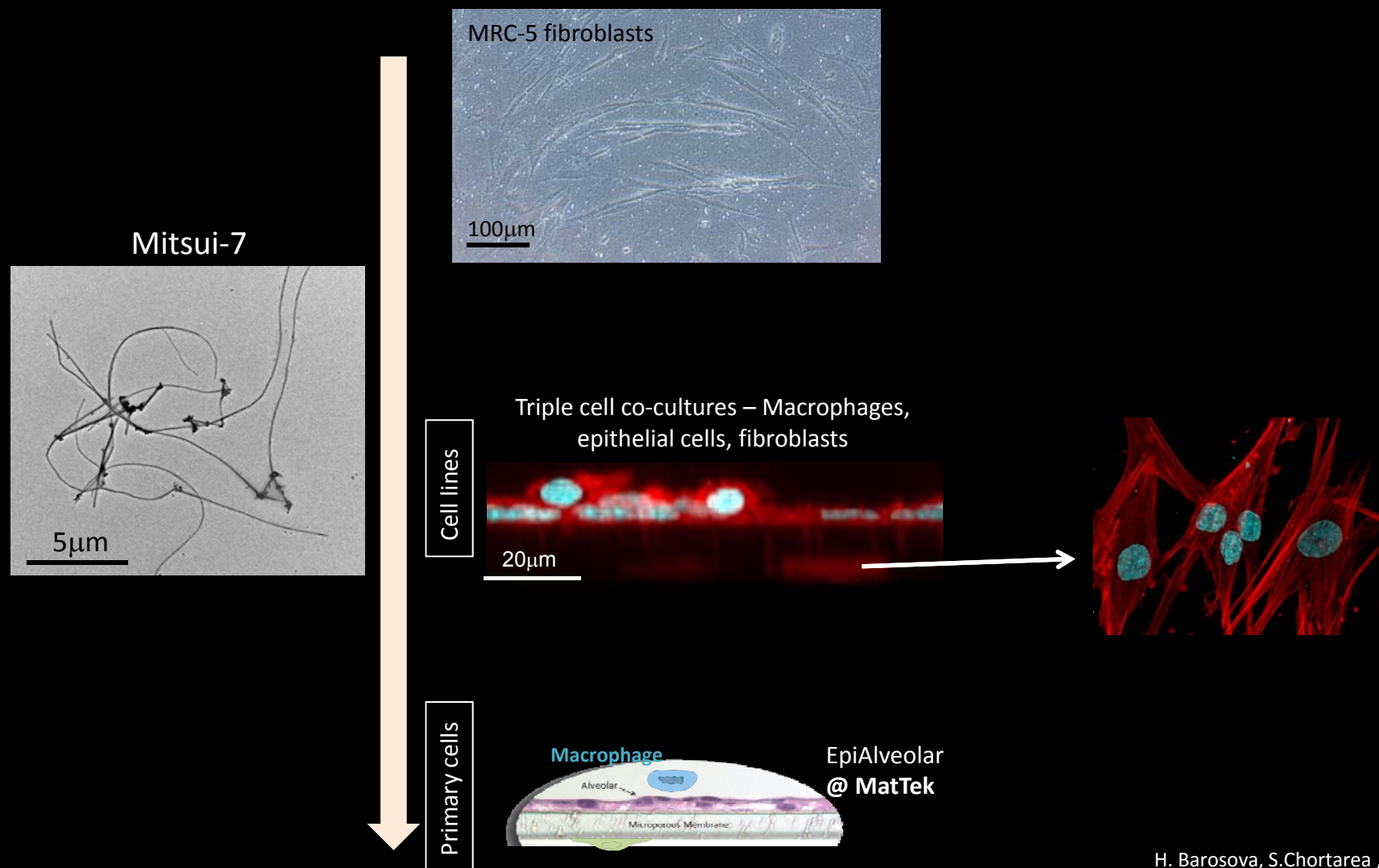
**Figure 5** Light micrograph of fibrotic response to interstitial MWCNTs. Comparison of Sirius red stained collagen fibers in PBS treated (SA) and MWCNT treated lungs (SB, 80 ug dose at 56 days post-aspiration). Figure 5A illustrates the typical long and thin



Fernandez and Eickelberg, *Proc Am Thorac Soc* 2012



# Study design



H. Barosova, S.Chortarea , F. Zerimariam

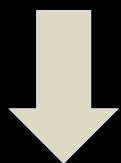


# 3D lung models as *in vitro* alternatives

VISION



[www.lushprize.org](http://www.lushprize.org)



- Versatile, simple as possible and **reproducible**
- Realistic in simulating the **human organ** of interest
- Improved prediction by **3D models**
- Able to **reproduce** findings of *in vivo* studies

Standardized protocols

Lung parenchyma

Co-cultures

(pro-)fibrotic response

Replacement of the 90-day sub-chronic inhalation assay in rodents





# Acknowledgments

## BioNanomaterials group



### AMI members

### Adolphe Merkle Foundation

### University of Fribourg

### Collaboration partners:

- Dr. Fabian Blank, University of Bern
- Prof. J. Czerwinski, Biel
- Prof. Vicki Stone, Heriot-Watt University, Edinburgh
- MatTek Corporation
- Dr. Amy Clippinger and Dr. Monita Sharma, PISC



Ch. Bisig



S. Chortarea



H. Barosova

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ZUR FÖRDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG

BIO-INSPIRED  
MATERIALS  
NATIONAL CENTER OF COMPETENCE  
IN RESEARCH

Opportunities and Risks of Nanomaterials  
National Research Programme NRP 64