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CPC Comprehensive Pneumology Center

A New Look at Aerosol Deposition, Dosimetry and Biokinetics of Nanoparticles in the Lung

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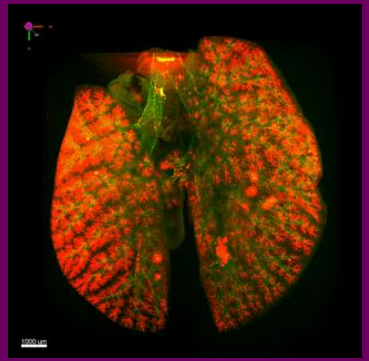
Helmholtz Centrum Munich, Inst. Lung Health and Immunity, Neuherg/Munich Germany



Assoc. Inhalation Toxicology – Webinar Series
6 February, 2023

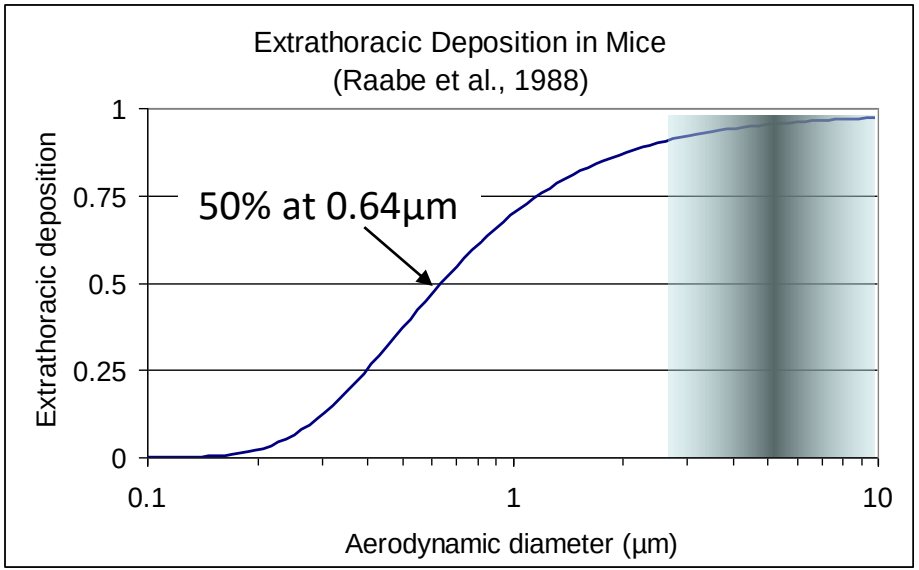
I. Ventilator-assisted aerosol deposition in murine lungs for efficient, dose-controlled drug delivery

II. Multi-modal holistic imaging of the in murine lung with cellular resolution
    I. Artificial-intelligence supported image analysis
    II. Differences between aerosol and bulk liquid application

III. The role of macrophages for nanoparticle clearance from the lung

Problem with nose-only and whole-body inhalation: The NOSE!

Nasal aerosol deposition in mice



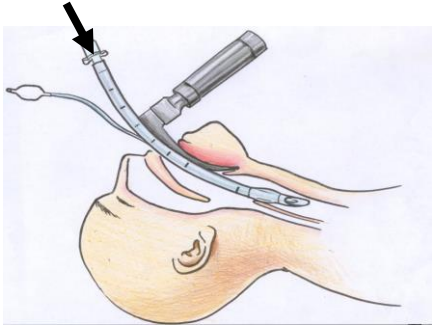
Clinically used nebulizers (MMAD 3 – 5 µm)

⇨ < 0.1% delivered to nose

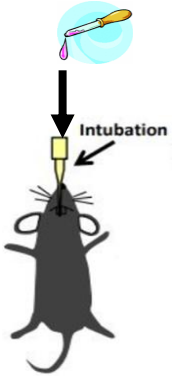
⇨ Low dose rate ---- long exposure times

Pulmonary Drug/Aerosol Delivery Technologies (mice)

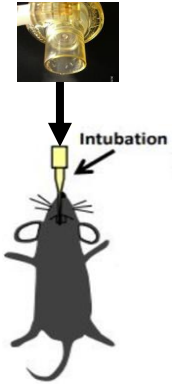
Bulk liquid or aerosol



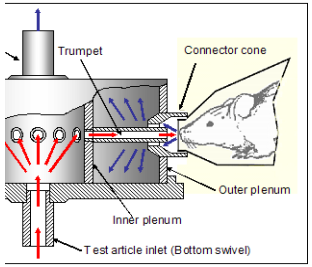
Bulk liquid



Liquid aerosol



Liquid/dry aerosol



Instillation

Inhalation

	Intratracheal	Ventilator-assisted	Nose-only OR whole body
Dose efficiency/delivery period	High (100%)/seconds	Medium (10%)/minutes	Low (<1%)/hours
Technological complexity	Low	Medium	High
Degree of dose control	High	Medium	Low
Degree of clinical relevance	Low	Medium/High	High

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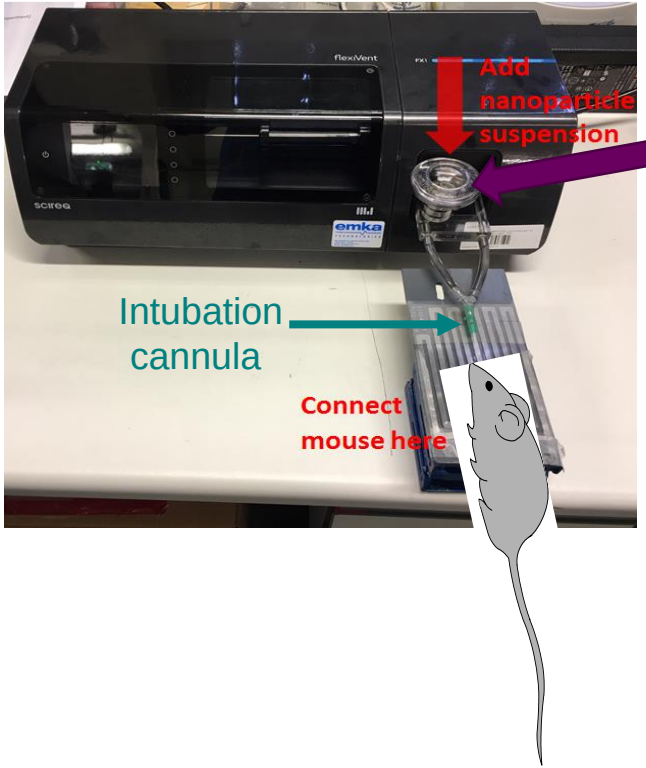
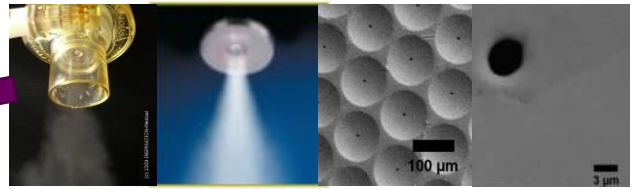
flexiVent: Lung Function Measurement System (for Mice)

Dose-controlled aerosol delivery

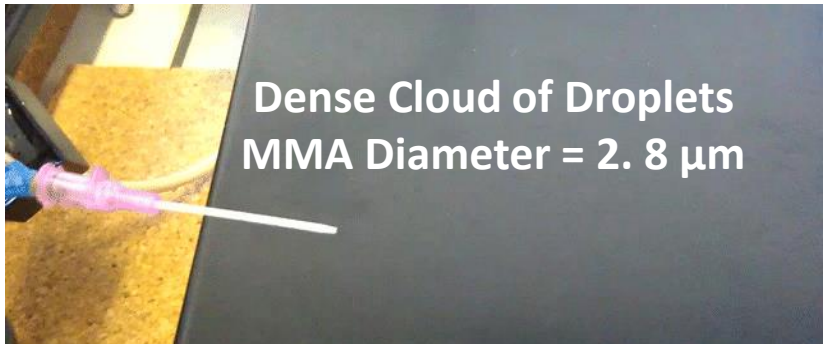


Vibrating Mesh Nebulizer



www.aerogen.com

Aeroneb Lab/Pro
(VMD 3.5 – 6.0 µm)
Vibrating Mesh Nebulizer
(Aeroneb Pro/Lab, Aerogen, Ireland)



Mechanical ventilation of animals

scireq
an emka TECHNOLOGIES company

flexiVent FX
(EMKA/Scireq, France/Canada)

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From Nebulized to Deposited Drug Dose

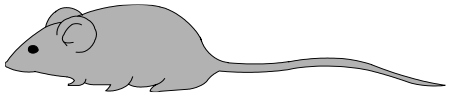
$D_{deposited}$ (from quantitative fluorescence spectroscopy: whole-lung imaging or lung homogenate)

$D_{deposited} = D_{neb} \times f_{inhaled} \times f_{deposited}$

D_{neb} (= $c_{drug} \times 10\ \mu l$)

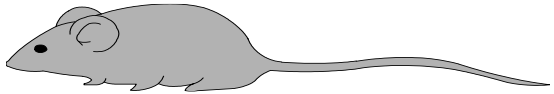
10 µl drug

$D_{delivered/inhaled}$



Gravimetric analysis
(Nebulizer + mount + tubing)

$f_{delivered/inhaled} = (M_{before} - M_{after}) / M_{neb}$

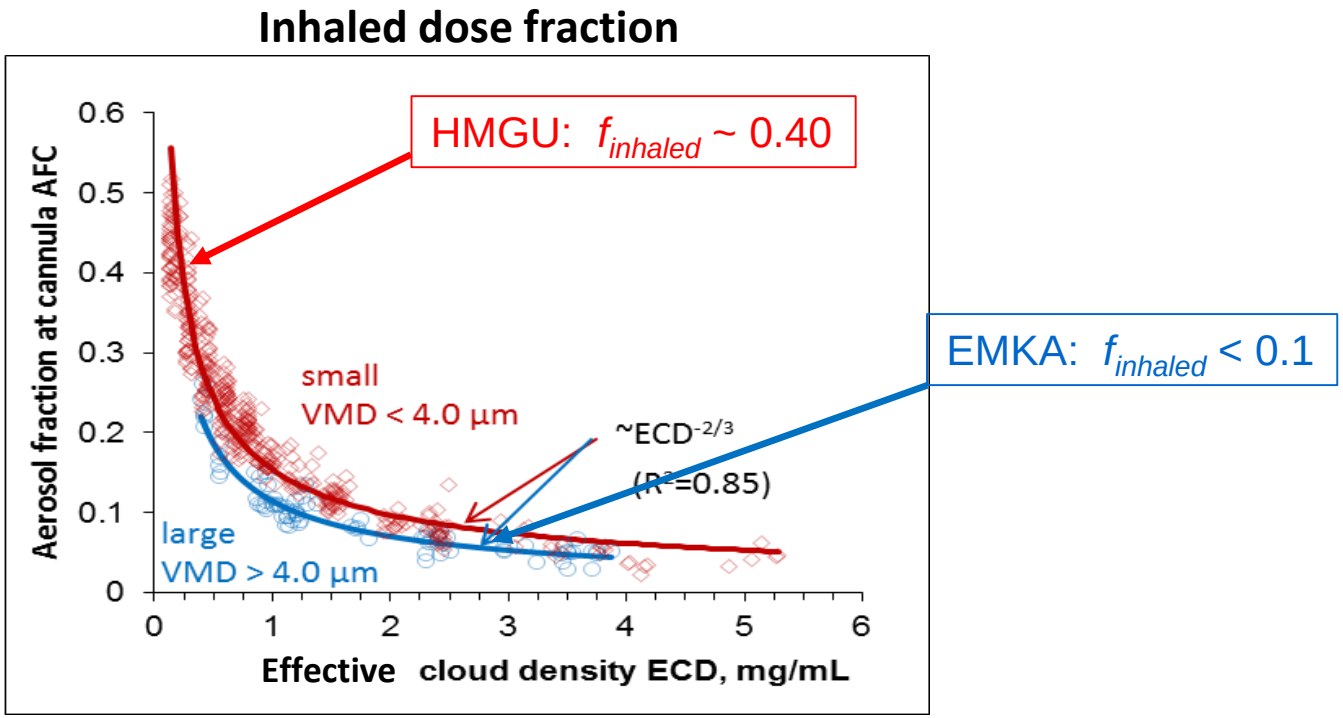


Parameter Matrix for Investigation of Delivered/Inhaled Aerosol Dose

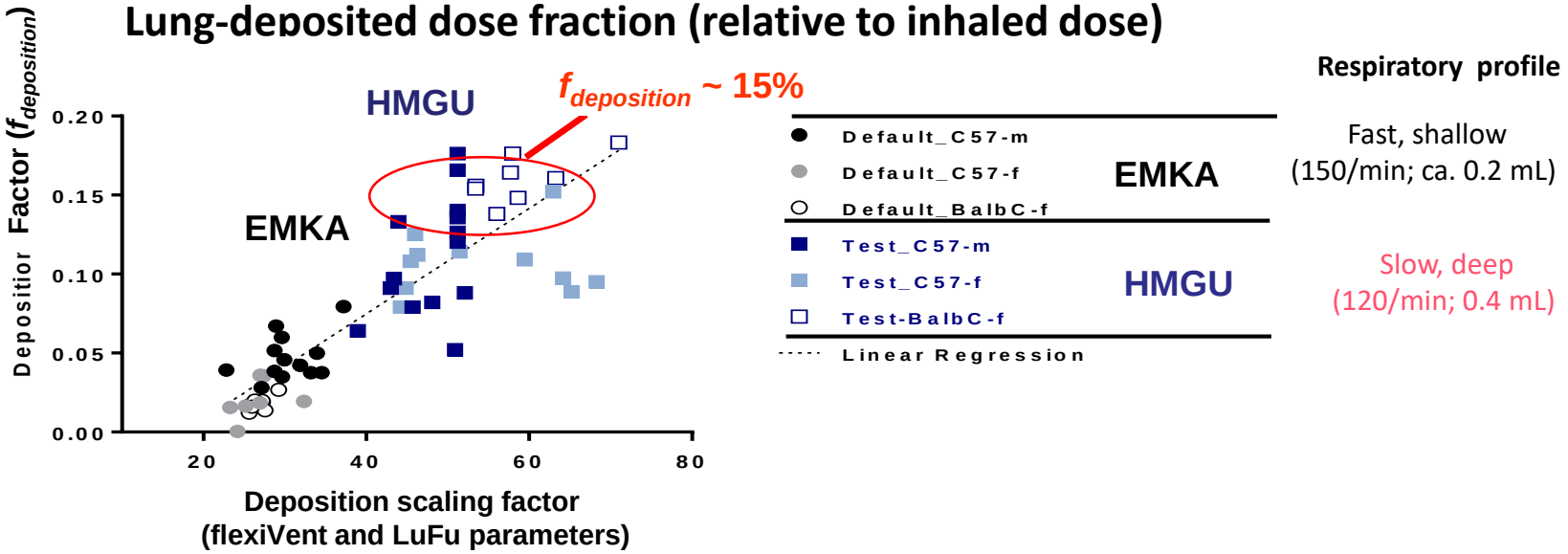
Parameter	Range
Respiratory frequency (breath/min)	120, 180, 240
Tidal volume (µl)	200, 267, 400
Ratio of inhalation/exhalation time (I/E ratio)	2:1, 1:1, 1:2
Nebulizer on-time (duty cycle)	20-333 ms (6-100%)
Nebulizers	3 x Aeroneb Lab (small) 6 x Aeroneb Pro
Droplet mass median diameter (µm)	3.8 – 5.4
Nebulizer aerosol output rate (ml/min)	0.3 – 0.8

More than 60 different settings were tested (n = 3-5)

Inhaled Dose Fraction ($f_{inhaled}$) – Depends on ventilator & nebulizer parameters



Fraction of Inhaled Aerosol Deposited in the Lung ($f_{deposition}$)



⇒ **Up to 15% of inhaled dose can be deposited in the lung**

$f_{inhaled}$ ~ <0.4 (<40%)

⇒ **Up to 6% of invested dose can be deposited in the lung**

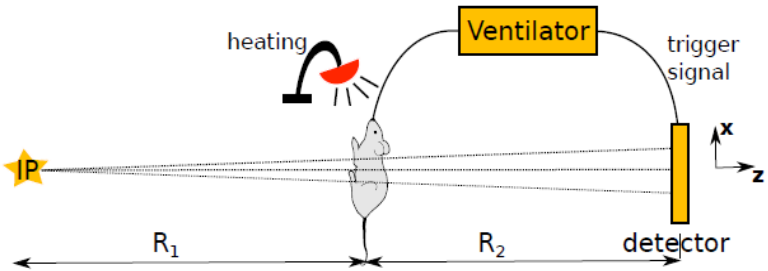
I. *In vivo* imaging of pulmonary delivery of nanoparticles suspensions in murine lungs

II. *Ex vivo* co-mapping of lung morphology and liquid deposition in murine lungs – various routes of application

Propagation-based phase-contrast X-ray imaging of pulmonary delivery – *In vivo*

(Prof. Pfeiffer, Technical Univ. Munich, Germany)

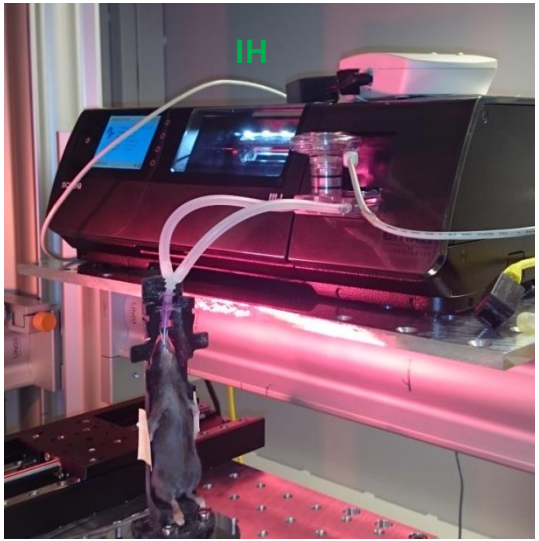
➢ X-ray imaging setup (Munich Compact Light Source)



Allows either an anteroposterior (AP) projection (e.g. of the lungs) or a lateral (Lat) projection (e.g. through the trachea).

➢ Pulmonary delivery setup

Intratracheal Inhalation (ventilator-assisted)



Gradl, et al. Sci. Reports, 8.1: 6788, 2018

Gradl, IEEE Trans. Med. Imaging, 38,2, 649-656, 2019

Gradl et al. Schmid, Morgan, J. Control. Release, 307, 282-291, 2019

doi.org/10.1016/j.jconrel.2019.06.035

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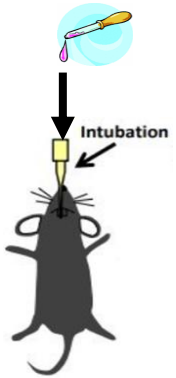
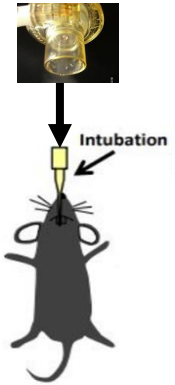
Application/Imaging methods – Contrast agents/dyes

Bulk liquid



Intratracheal Instillation

Liquid aerosol



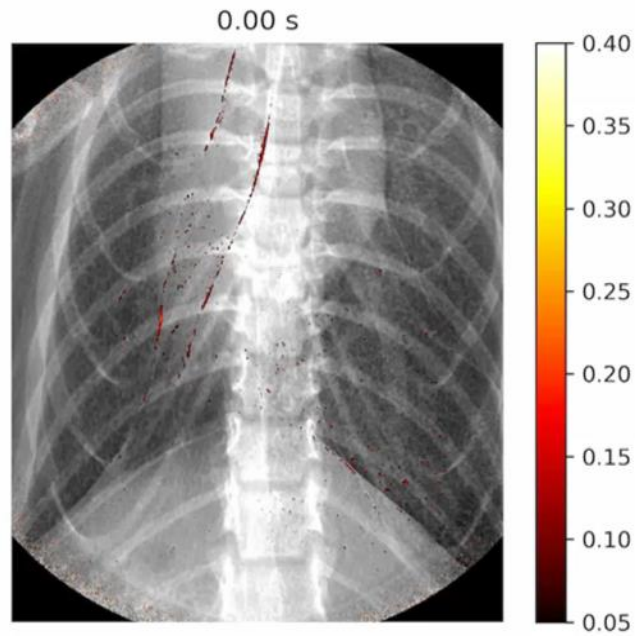
Ventilator-assisted Inhalation

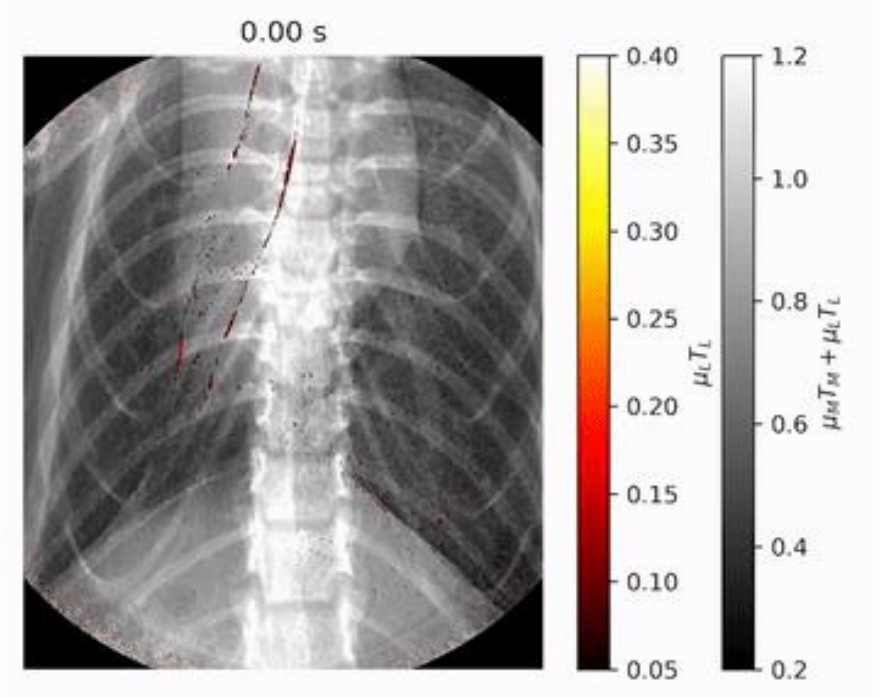
Applied liquid active agents (and mixtures thereof)

Mode	**X-ray/CT imaging**	**Fluorescence microscopy (near-infrared: ~ 700 nm)**
Contrast agents	Iodine (VMD ~ 2 µm)	Polystyrene Particles (VMD ~ 400 nm)
	Iron oxide (VMD ~ 600 nm)	Melamine resin Particles (VMD ~ 450 nm)
	Gold NPs (VMD ~ 20 nm)	
	Quantum dots (VMD ~ 20 nm)	

In vivo Phase-Contrast X-ray Imaging: Intratracheal Instillation

1 image / breath @ 80 breath/min (during 100 ms end-inspiratory breath-hold)





In vivo Phase-Contrast X-ray Imaging: Inhalation

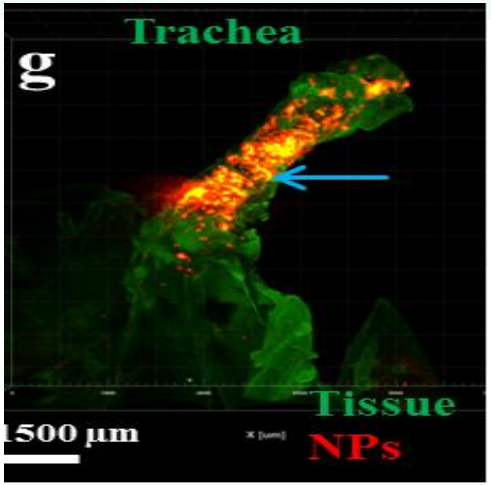
Fractional deposited dose

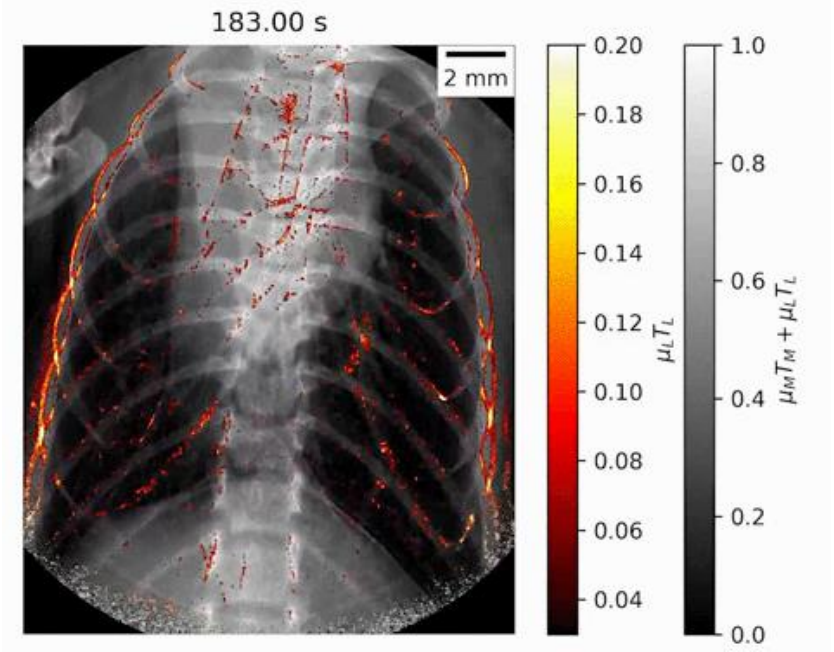
Trachea: 50 – 80% in trachea

Lung: 20 – 50% in lung

Esophagus: <1% in esophagus

(< 3min time for clearance)





I. *In vivo* imaging of pulmonary delivery of nanoparticles suspensions in murine lungs

II. *Ex vivo* co-mapping of lung morphology and liquid deposition in murine lungs – various routes of application

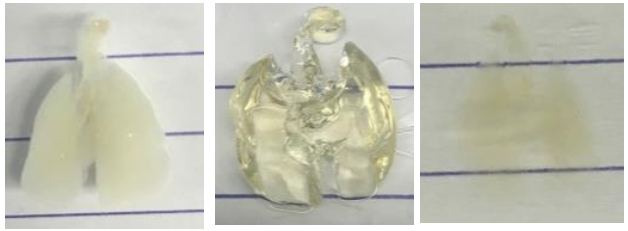
Whole Murine Lung Imaging (Ex vivo)
Tissue Clearing and Light Sheet Fluorescence Microscopy (LSFM)

Tissue Clearing Protocol (3DISCO; ca. 1d)

Ertürk et al., Nat Protocol, 7, 11, 2012

- Dehydration (tetrahydrofuran (THF), 50-100%)
- Lipid removal (dichloromethane (DCM))
- Refractive index matching (BABB: benzyl alcohol (BA)) and benzyl benzoate (BB) or dibenzyl ether (DBE))

Blood removed | Cleared | Cleared & Ref. Index matched



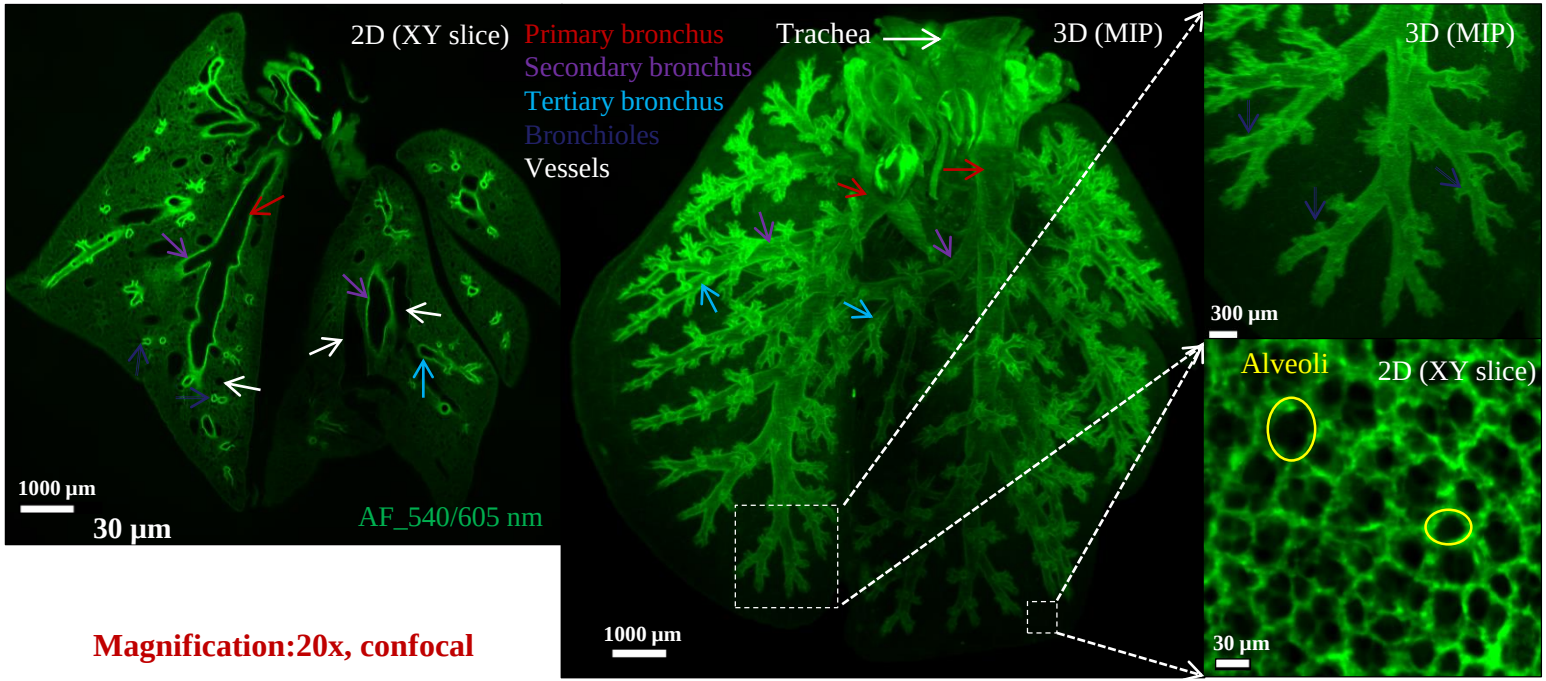
Yang et al. Schmid, ACS Nano, 51, 4, 526-535, 2019

Ultramicroscope II, LaVision Biotec

Thickness of light sheet is about 4 - 10 µm

Whole Lung Morphology – Light Sheet Fluorescence Microscopy (LSFM)

2D & 3D Lung morphology and airway structure generated from autofluorencence images of the tissue (volume function of Imaris) after imaging using LSM

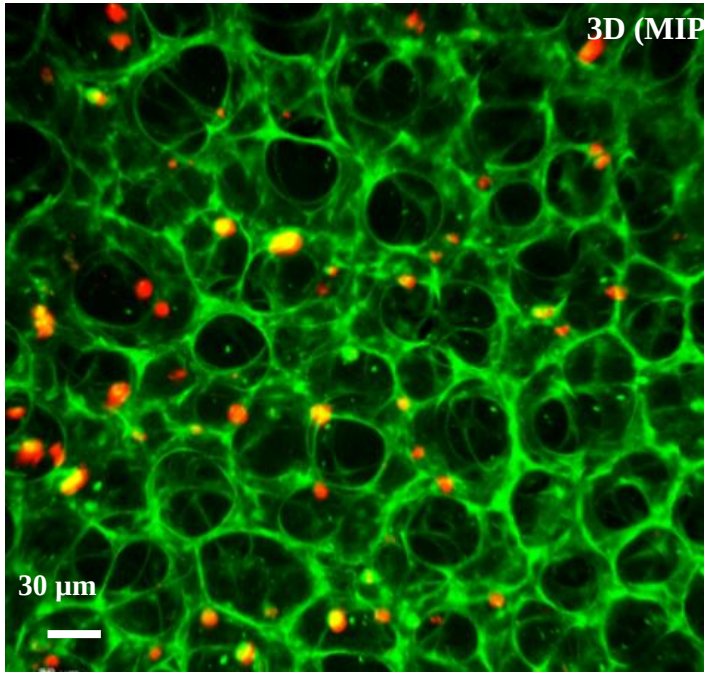


Magnification:20x, confocal

Yang et al. Schmid, ACS Nano, 51, 4, 526-535, 2019

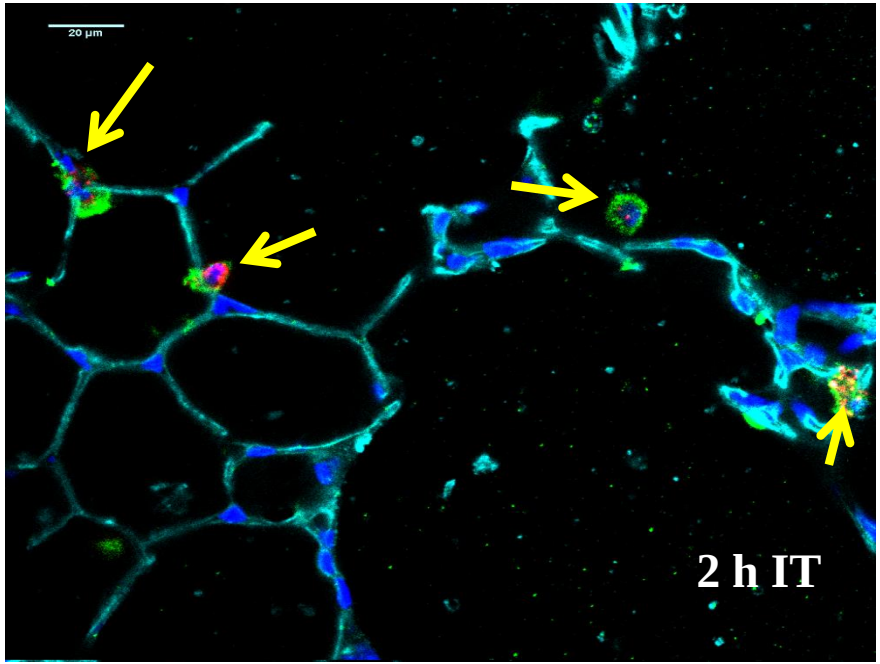
Efficient uptake of nanoparticles by alveolar macrophages

Tissue slice (3D)



Magnification:20x, confocal

Macrophage/epithelial cell uptake ratio of NPs



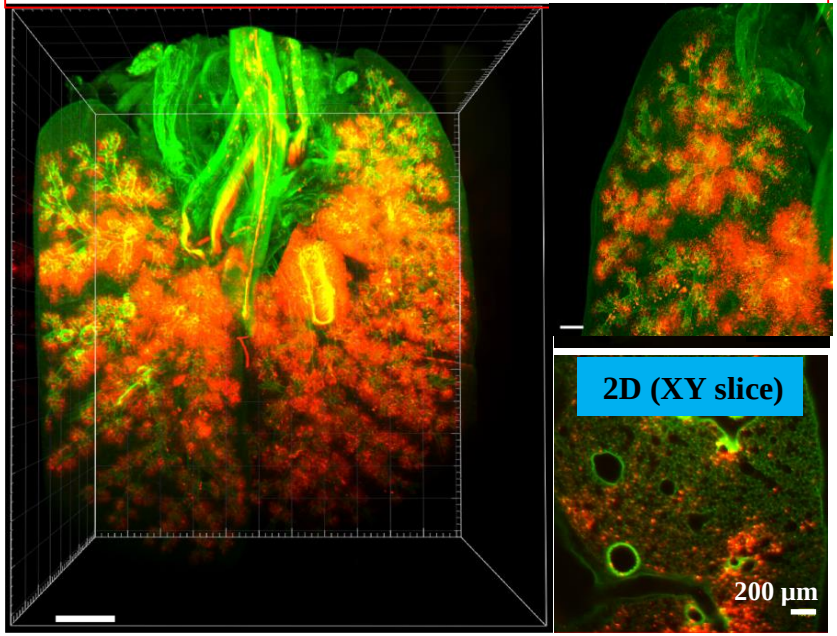
DAPI, Anti F4/80, Podoplanin, NPs

Pulmonary drug distribution: Instillation vs. Inhalation (LSFM)

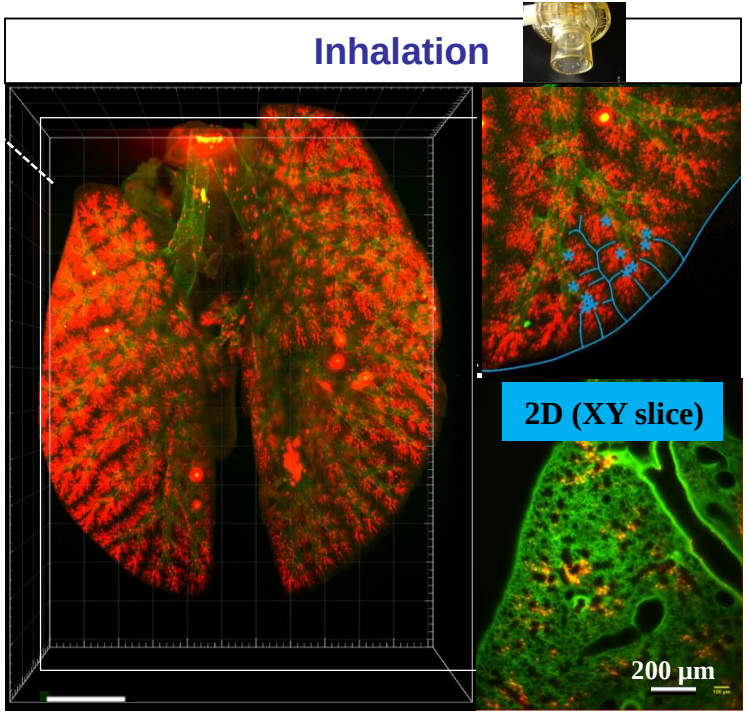
Yang et al. Schmid, ACS Nano, 51, 4, 526-535, 2019

450 nm melamin resin NPs (spherical, fluorescent)

Intratracheal Instillation



Inhalation



Green: lung epithelium

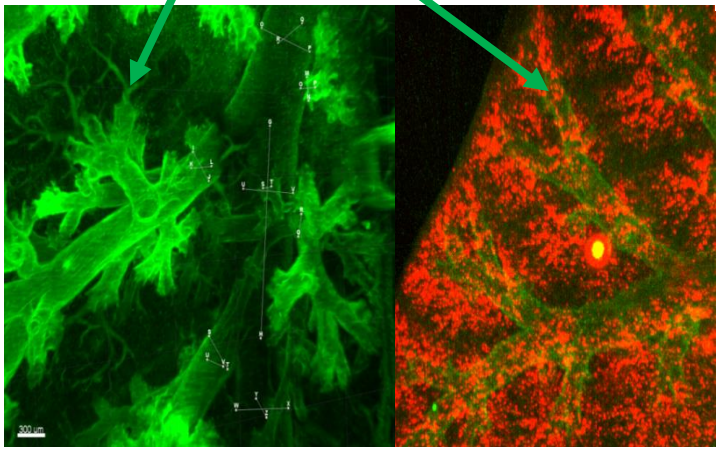
Red: Deposited fluorescent aerosol

⇒ Instil.: Central & patchy deposition

⇒ Inhal.: Uniform deposition throughout lung

„Hot spot" aerosol deposition in Proximal Acinar Region (PAR)

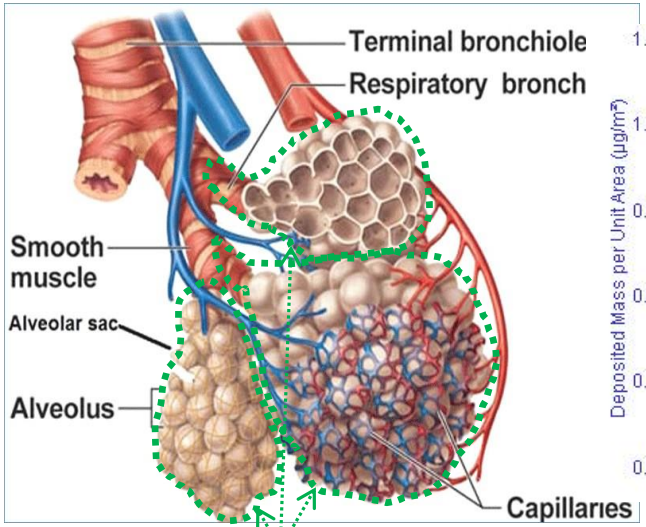
Terminal Bronchioles



Green: Lung epithelium
Red: Deposited fluorescent aerosol

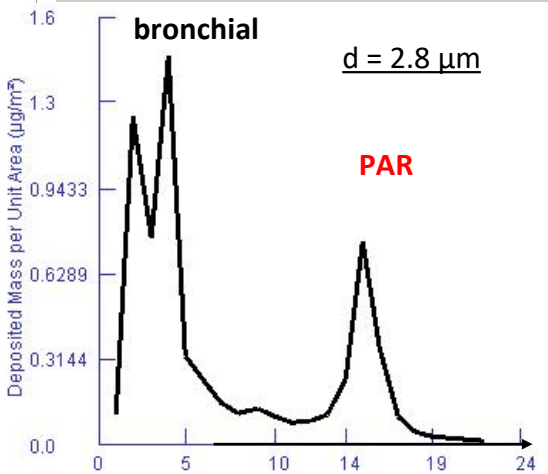
Proximal Acinar Region (PAR)

Respiratory unit of the lung: Acinus



Acini

Comput. lung deposition model (e.g. MPPD)



Lung Generation

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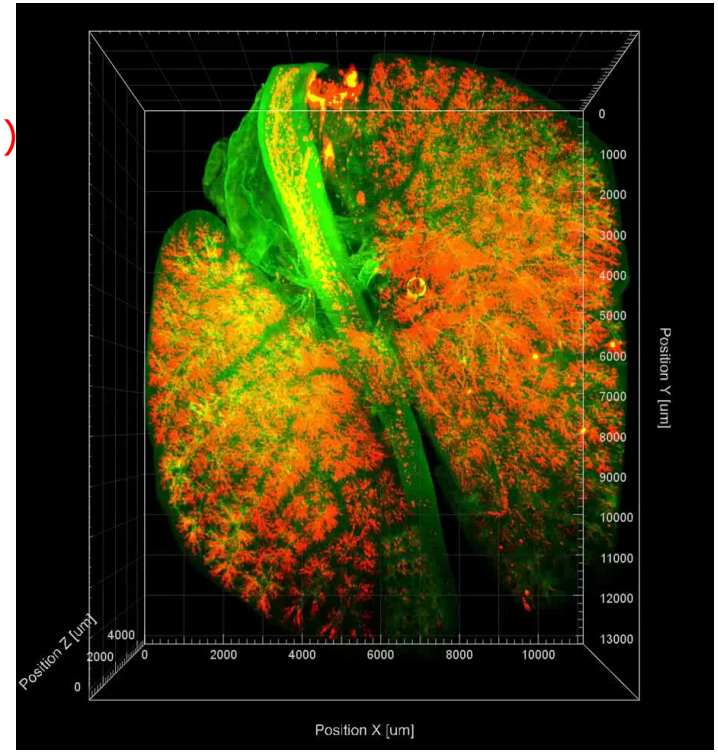
Aerosol Deposition in Murine Lung: 3D LSFM

2.8 µm aerosol depo. in the whole (non-dissected) murine lung (with down to cellular resolution)

Lung epithelium (green)
Deposited Aerosol (red, yellow)



Yang et al. ACS Nano, 13, 1029-41, 2019

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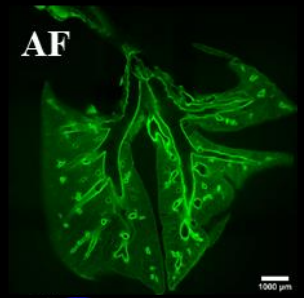
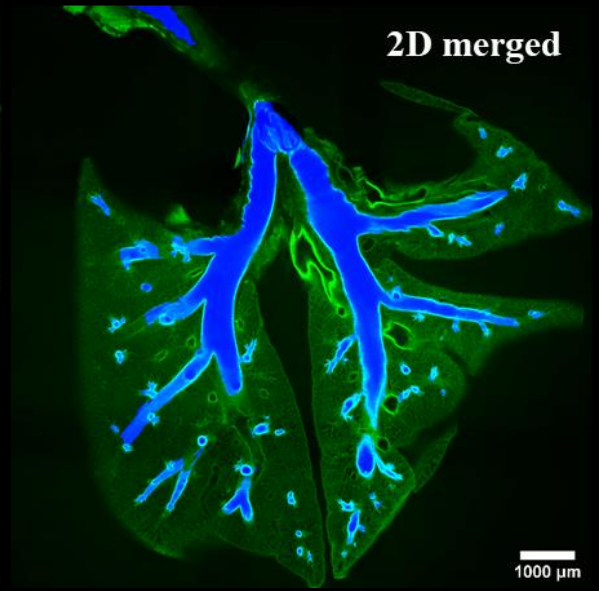
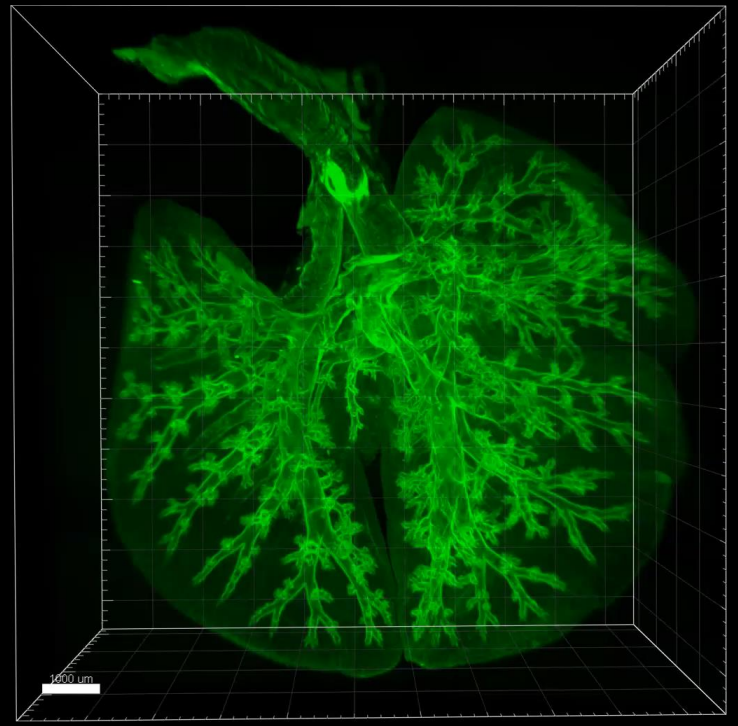
Active learning AI-based analysis for determination of novel regional deposition features

Extraction of ground truth_manual segmentation of whole lung airway tree







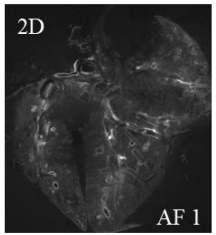
Active learning AI-based analysis for determination of novel regional deposition features

Data-centric active learning AI approach (CNN)

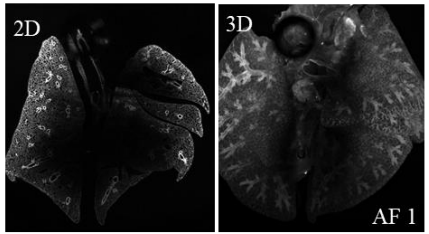
Active Learning: Training nnU-net only on few annotated samples, predication on validation set afterwards, visual inspection of results, corrections of selected cases and adding them to training data, and repeating the process iteratively.

Dataset challenges

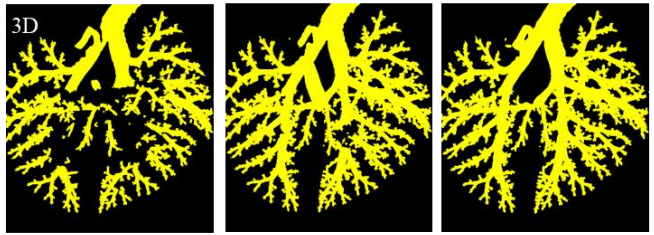
Iteration 1 (3 training cases) | **Iteration 3 (14 training cases)** | **Iteration 6 (21 training cases)**

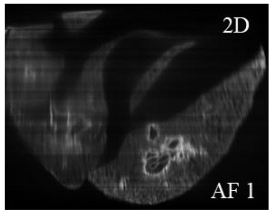


Massive imaging artifacts: fake lung structure, obscure airway,…

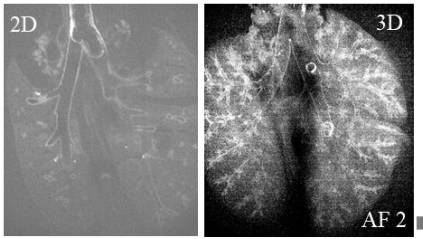


Out of focus center, poor center illumination due to blood residual

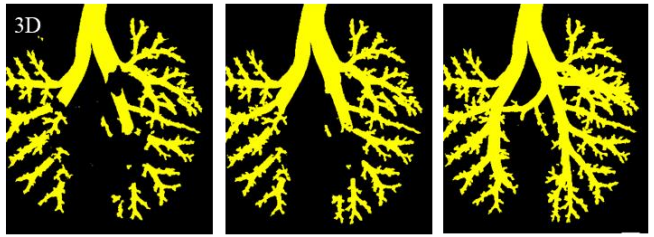




Inconsistent slice illumination



Heterogenous imaging wavelength, very weak AF, highly variable signal to noise ratio



Method Improvement: Adapt Data Augmentation to specific challenges: Gaussian Blur, Gaussian Noise, local blurring transform, local contrast transform, local brightness transform, local sharpness transform, custom slice illumination transform, blank rectangle transform

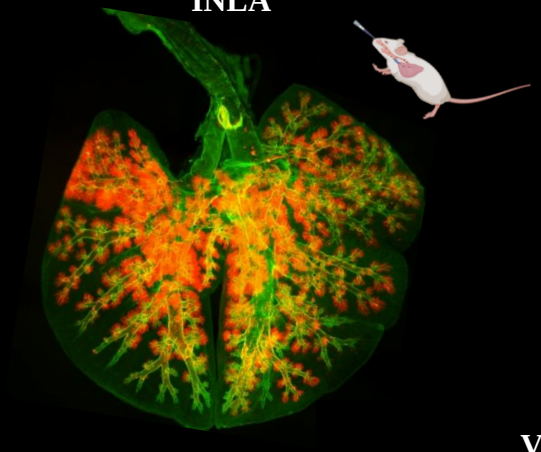
Collaboration with

Dr. Fabian Isensee and Team (DKFZ)

Dr. Marie Piraud and Team (Helmholtz AI)

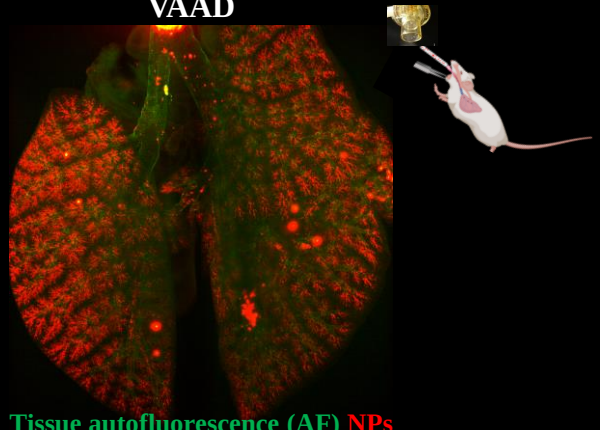
Distinct pulmonary deposition profiles for multiple delivery routes revealed by tissue-cleared LSFM

Intranasl liquid aspiration
INLA

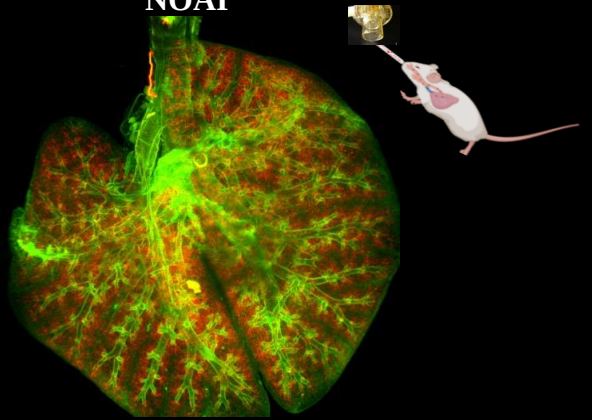


Intratracheal liquid instillation
ITLI

Ventilator-assisted aerosol delivery
VAAD



Nose-only aerosol inhalation
NOAI



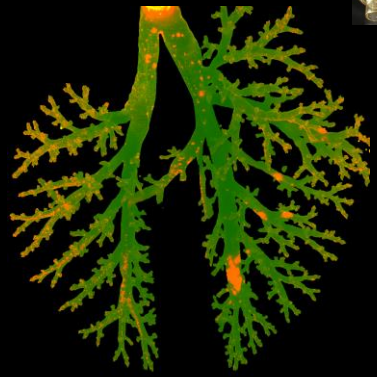
Tissue autofluorescence (AF) NPs

Distinct bronchial deposition profiles for multiple delivery routes

Intranasl liquid aspiration
INLA

Intratracheal liquid instillation
ITLI

Ventilator-assisted aerosol delivery
VAAD

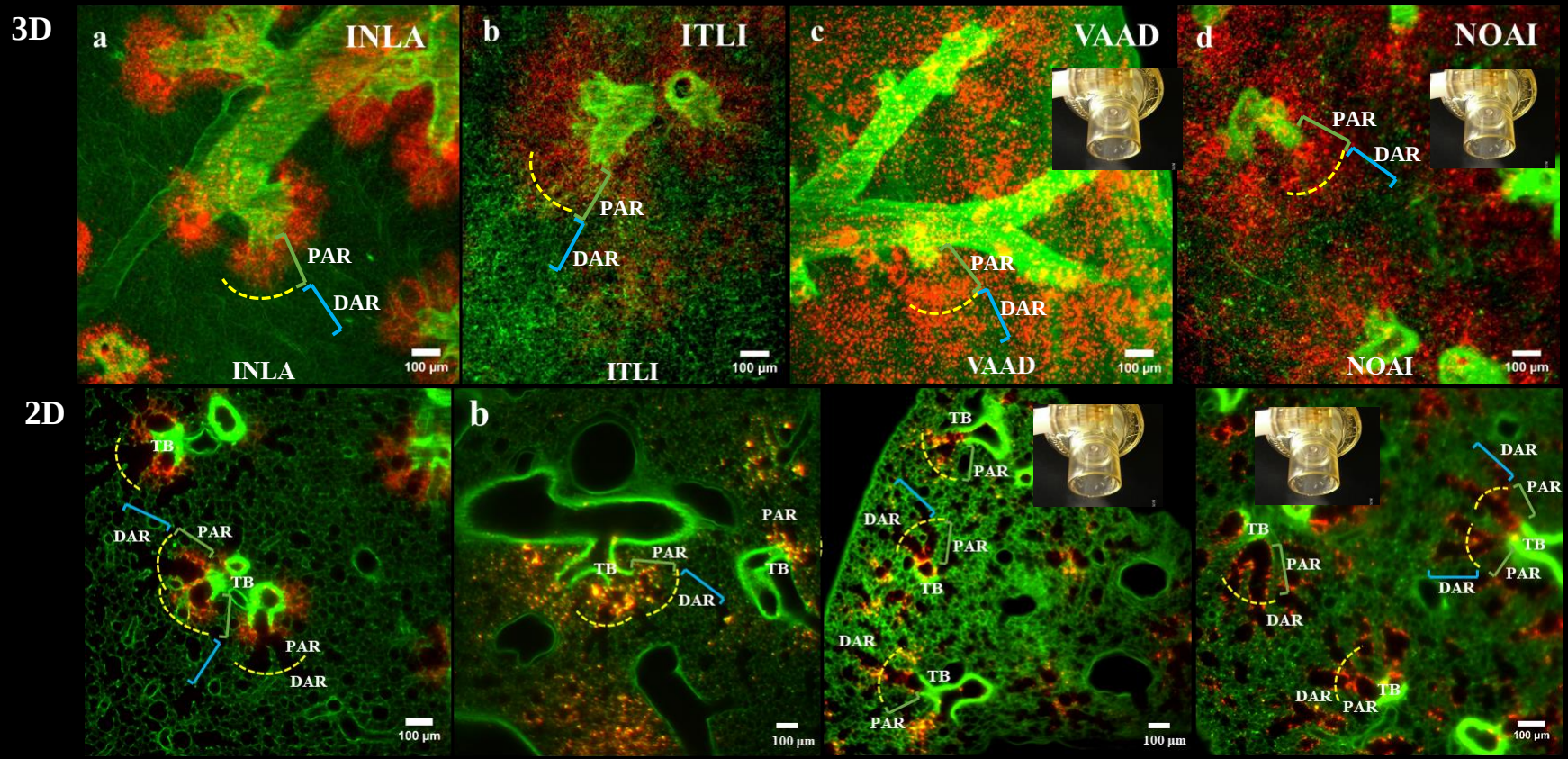


Nose-only aerosol inhalation
NOAI

Tissue autofluorescence (AF) NPs

Acinar deposition feature for multiple delivery routes at cellular resolution

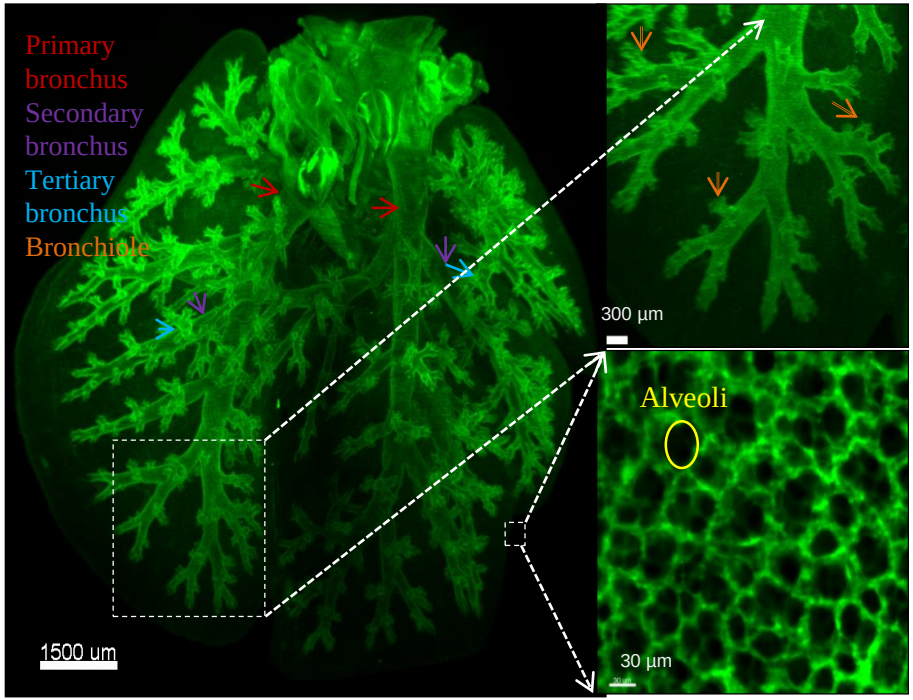
Proximal or Distal acinar regions (PAR or DAR) TB: terminal bronchioles



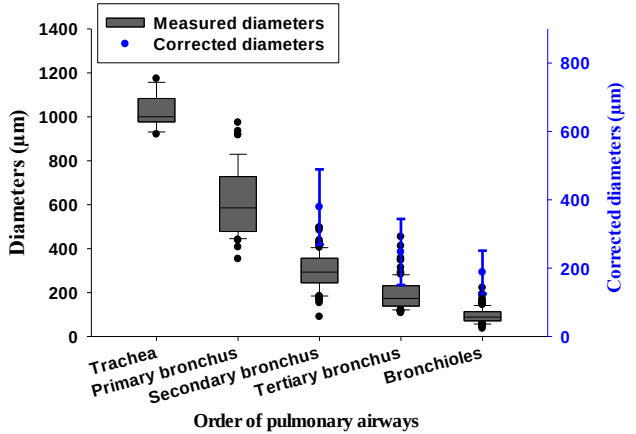
Aerosol deliveries (VAAD and NOAI) allow for deeper lung (distal acini) and more uniform transportation than INLA and ITLI

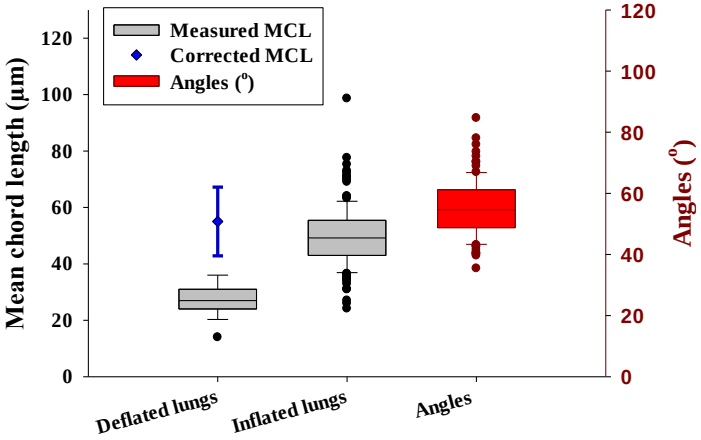
Quantitative Lung Morphometry

Morphology of murine lung – autofluorescence image

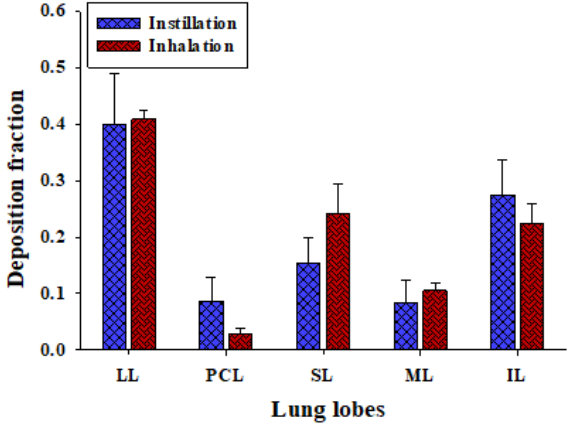


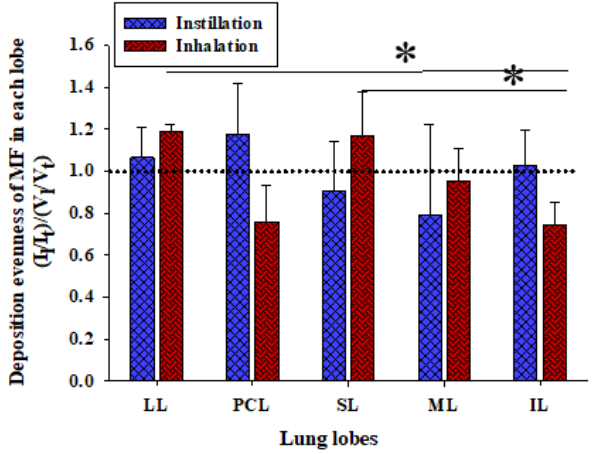
Yang et al. Schmid, ACS Nano, 51, 4, 526-535, 2019

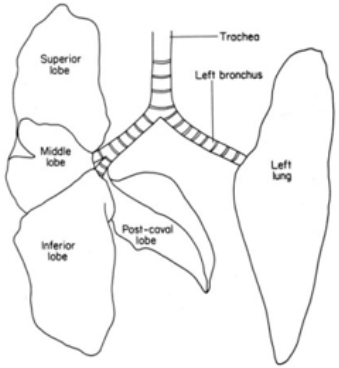




➢ Lobe-specific particle deposition: Intratr. Instillation & VA Aerosol Inhalation







$$N = \frac{Il/It}{Vl/Vt}$$

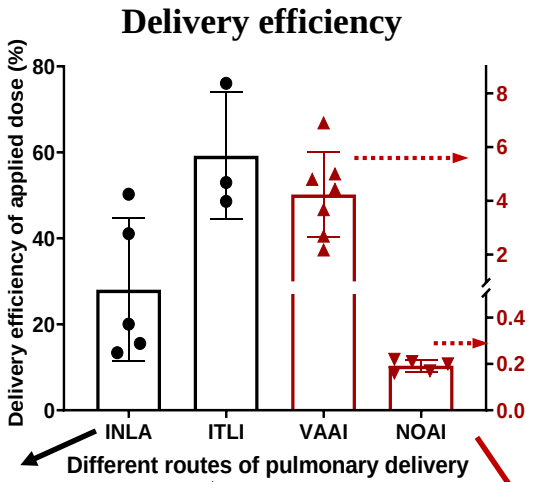
Yang et al. Schmid, ACS Nano, 51, 4, 526-535, 2019

HELMHOLTZ MUNICH

Whole lung dosimetry – four routes of pulmonary application

Dosimetry

Spectrofluorometry in homgenized lungs



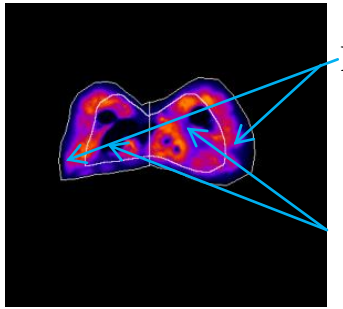
Intranasal liquid aspiration (IN**LA**)

Intratracheal liquid instillation (IT**LI**)

Ventilator-assisted aerosol inhalation (VA**AI**)

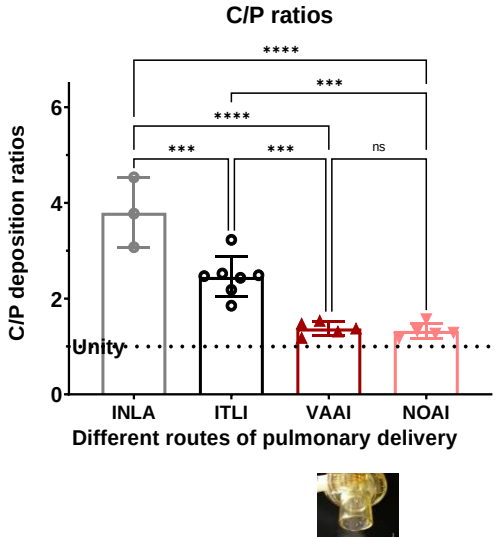
Nose-only aerosol inhalation (NO**AI**)

AI-based analysis of bronchial/acinar deposition – four routes of application

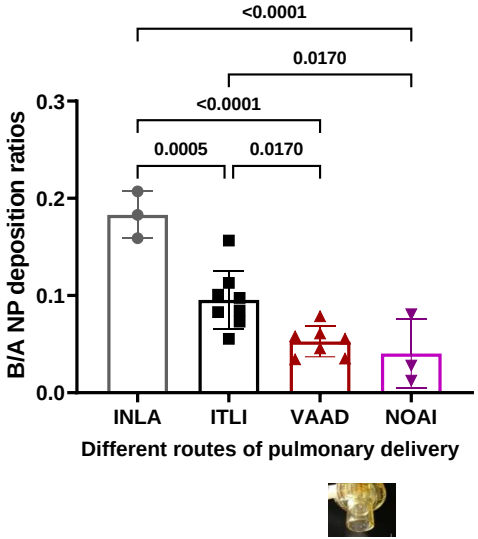


$$C/P = \frac{\text{central / periphery(Intensity)}}{\text{central / periphery (Area)}}$$

Central/Peripheral (C/P) deposition ratio



Bronchial/acinar (B/A) deposition ratio

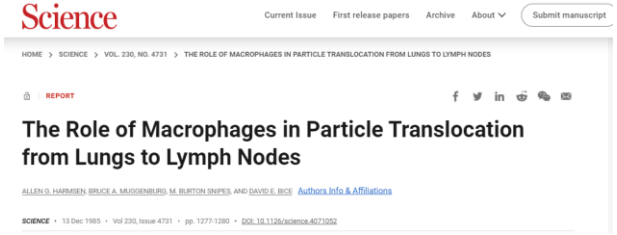


➡ C/P deposition ratio is highly predictive for bronchial/acinar ratio

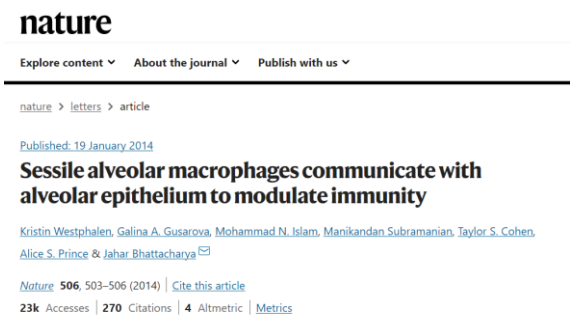
HELMHOLTZ MUNICH

I. Understanding the role of macrophages in particle clearance from the lung

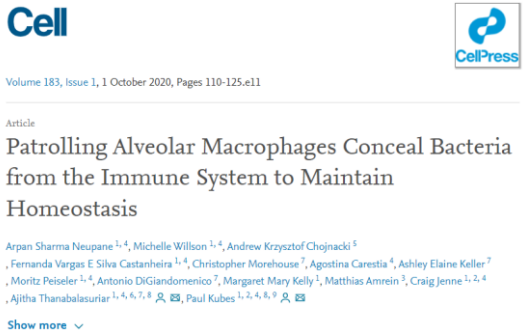
Previous knowledge on the migration of lung (alveolar) macrophages in literature



- Pulmonary alveolar macrophages laden with microparticles migrated to the tracheobronchial lymph nodes (year of 1985)



- Stimulated by LPS or bacterial infection, the **AMs remained sessile and attached to the alveoli**,they established intercommunication through synchronized Ca2+ waves in ex vivo perfused lung (year of 2014)



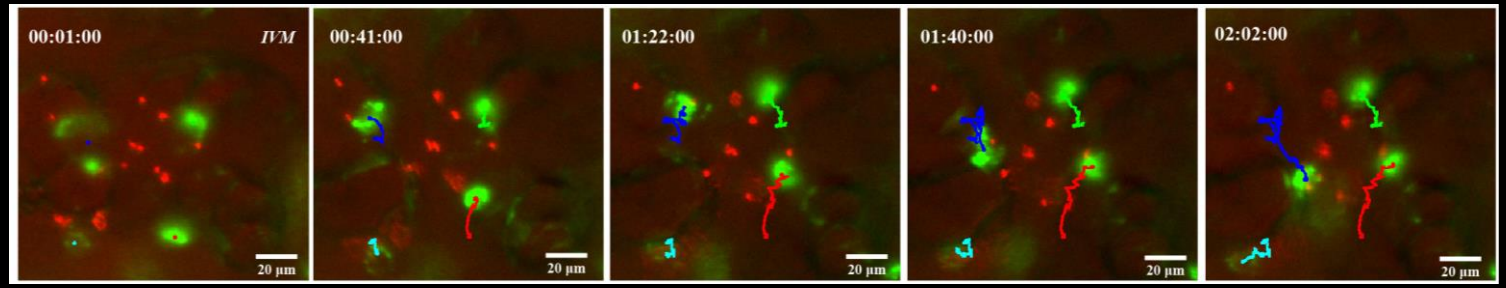
Alveolar macrophages show directed movement toward inhaled bacteria (year of 2020)

HELMHOLTZ MUNICH

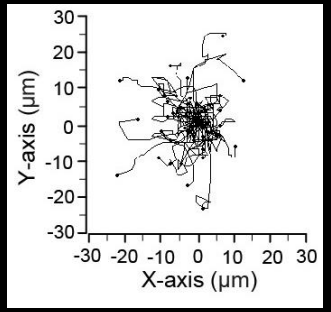
Alveolar macrophages (AMs) show directed movement toward inhaled particles

Intravital microscopy indicates the active migration of AMs to and particles

PKH labelled AMs

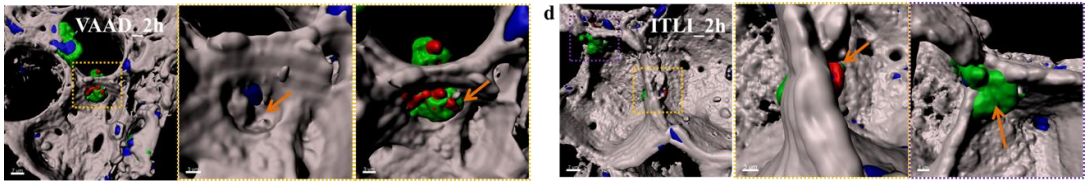


Prof. Dr. Markus Rehberg
and Qiongliang Liu

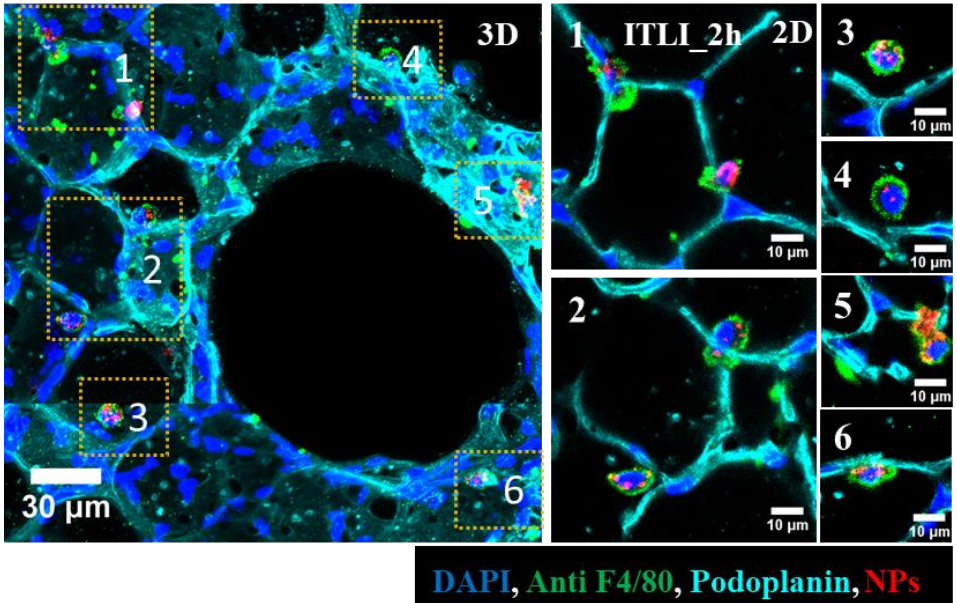


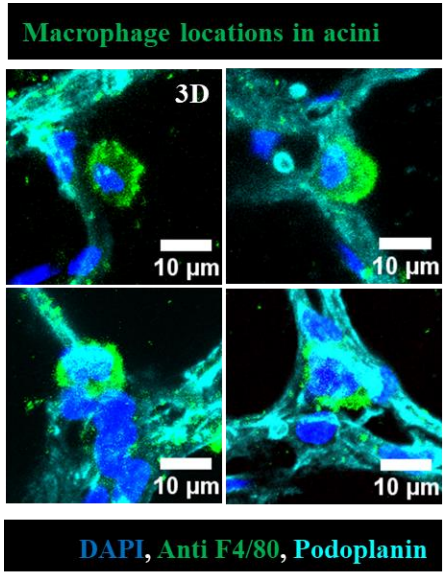
Particle uptake and relocation mediated by alveolar macrophages (in *ex vivo* precision cut lung slices)

➡ Visualization of AM „squeezing“ through pore of Kohn

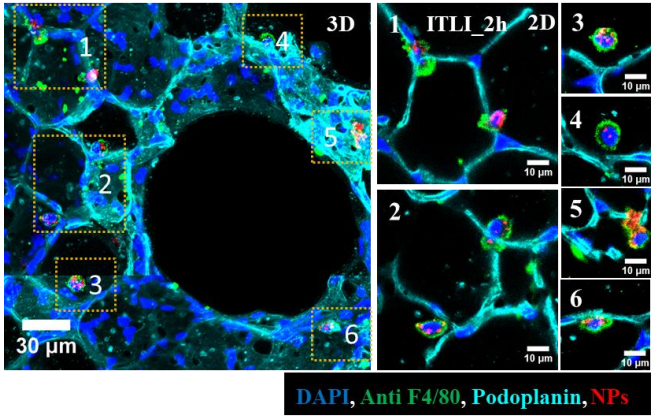


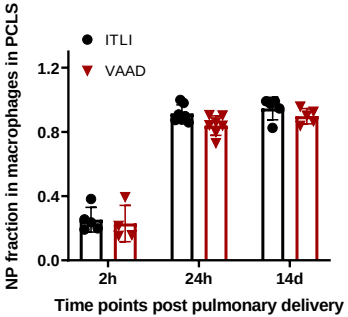
➡ Precisce location of al AMs

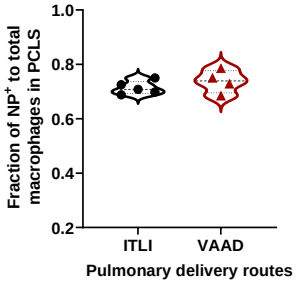


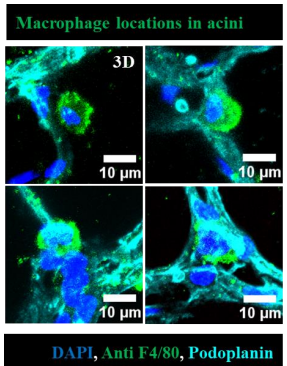


Particle uptake of and relocation by alveolar macrophages (AMs) in *ex vivo* precision cut lung slices

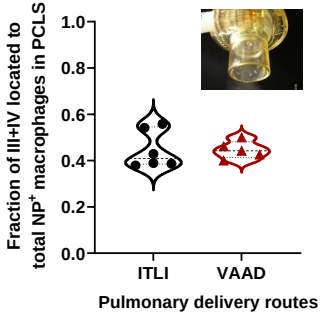






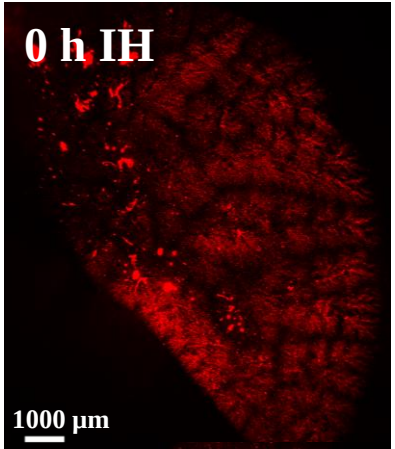


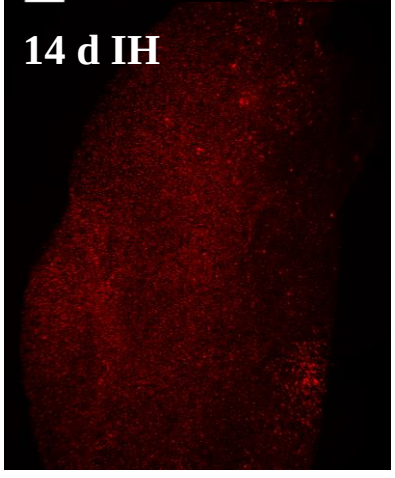
Fraction of interstitial NP⁺ AMs

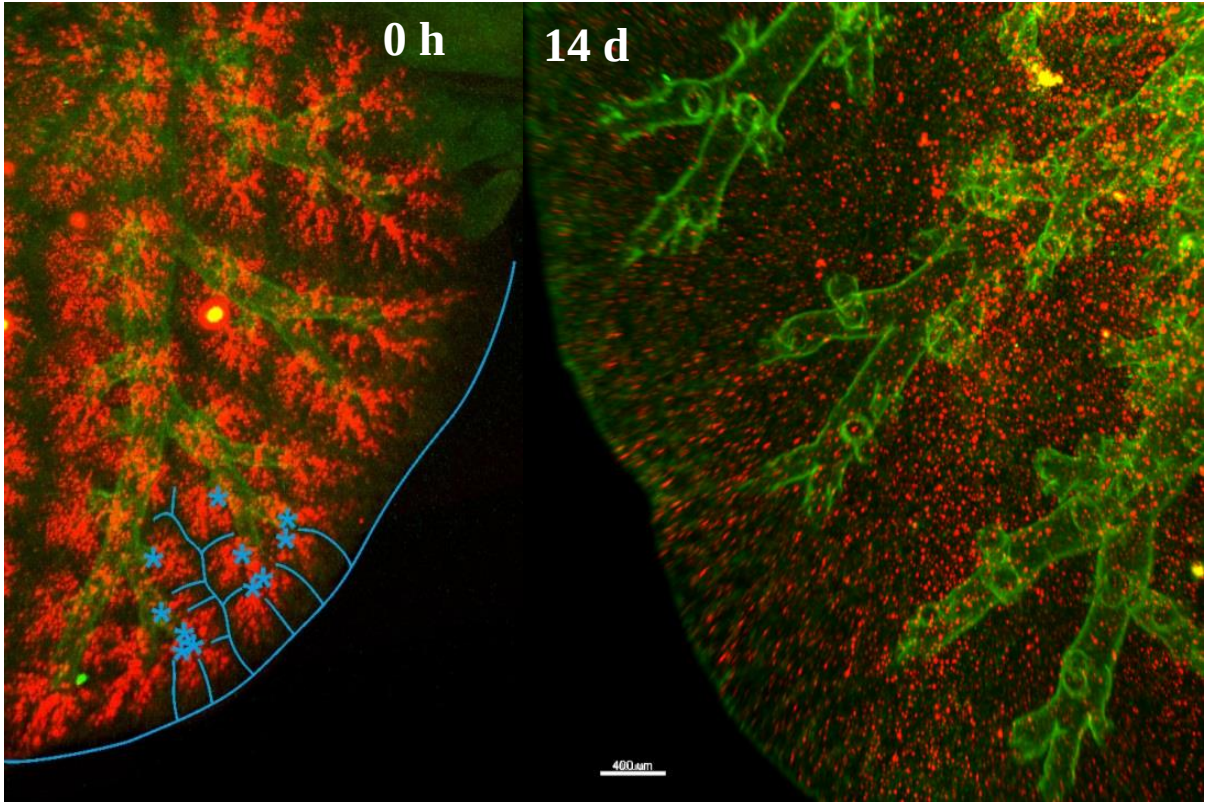


- **Deposited particles are rapidly and effectively taken up by AMs**

Redistribution of nanoparticles







Macrophages play a critical role in particle relocation

Summary

I. Deposition features of bulk liquid delivery vs. aerosol inhalation
   I. central/bronchial vs. peripherial/acinar deposition
   II. Patchy vs. uniform deposition,
      I. yet, hot spot deposition in proximal acinar region
      II. reaches deeper into acinar region (than bulk liquid)

II. Ventilator-assisted (VAAI) vs. nose-only (NOAI) aerosol inhalation
   I. 10-30-fold higher dose efficiency (similar to clincial dose rate)
   II. No nasal deposition **- clincally more relevant**

III. Exact mapping of macrophages throughout lung
   I. Particle uptake
   II. Localization & migration within lung

Perspectives

Need to investigate the relevance of aerosols as compared to bulk liquid application in preclincial studies

I. Partico-/pharmacokinetics

II. Pharmaco-/toxicodynamics

III. Improved substance-induced animal models?

- Elastase - emphysema
- Bleomycin - fibrosis
- ……

Acknowledgement

Dr. Lin Yang

Institute of lung health and immunity (LHI/CPC), DZL, HMGU
Schmid lab
Dr. Pramod Kumar,
Dr. Ali Farnoud,
Dr. Ali Doryab,
Anna Fuchs,
Penghang Chen
Andreas Schröppel
Dr. Otmar Schmid

Stoeger lab
Qiongliang Liu,
David Kutschke,
Dr. Carola Voss
Prof. Dr. Markus Rehberg,
Dr. Tobias Stoeger,

Burgstaller lab
Dr. Arunima Sengupta,
Dr. Gerald Burgstaller

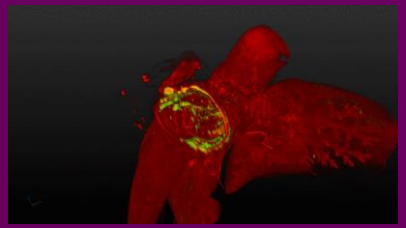
Helmholtz core facility
Dr. Annette Feuchtinger

Helmholtz Imaging platform,
German Cancer Research Center (DKFZ)
Darya Trofimova,
Sebastian Ziegler,
Dr. Fabian. Isensee,
Dr. Paul Jäger
Helmholtz AI, HMGU
Ruolin Shen,
Dr. Marie, Piraud

CPC/LHI, HMGU
Schiller lab

Dr. Herbert Schiller

Dr. Ali Önder Yildirim
CPC organization and secretary team
The CPC-M bioArchive team



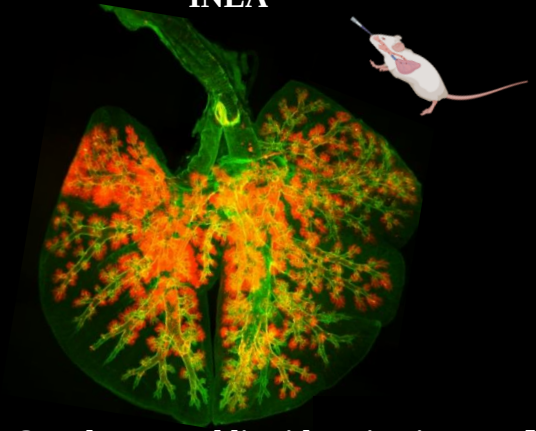
Thank you

Questions

Comments?

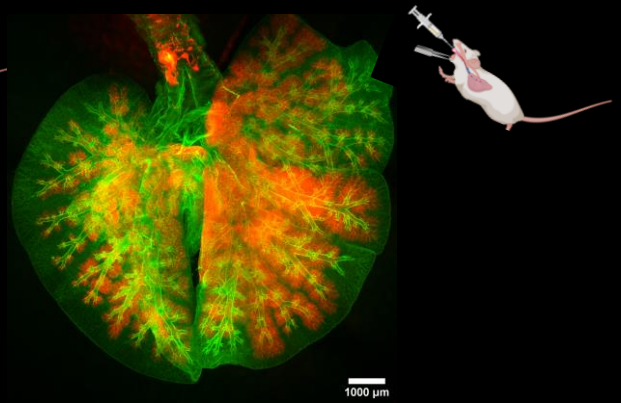
Distinct pulmonary deposition profiles for multiple delivery routes revealed by tissue-cleared LSFM

Intranasl liquid aspiration
INLA

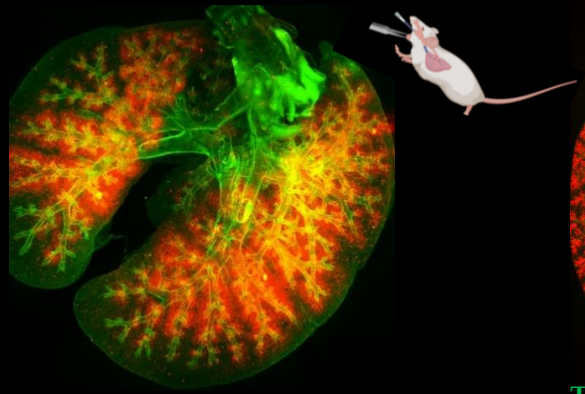


Intratracheal liquid instillation
ITLI

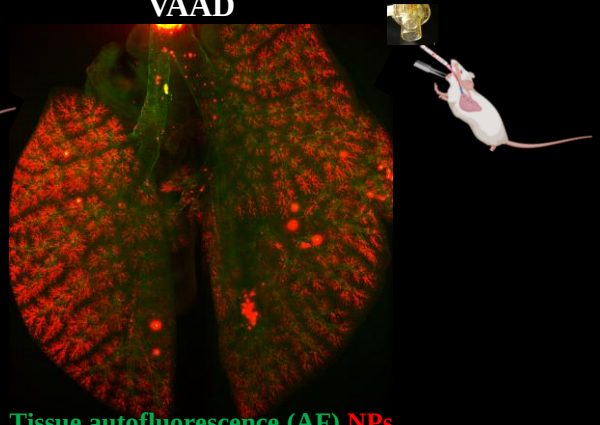
Microsprayer aerosol delivery
MSD



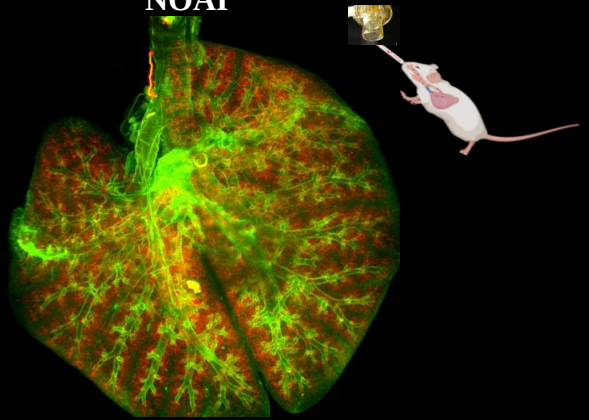
Oropharyngeal liquid aspiration
ORIA



Ventilator-assisted aerosol delivery
VAAD



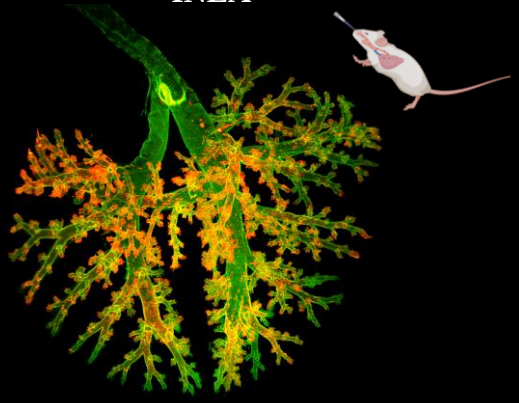
Nose-only aerosol inhalation
NOAI



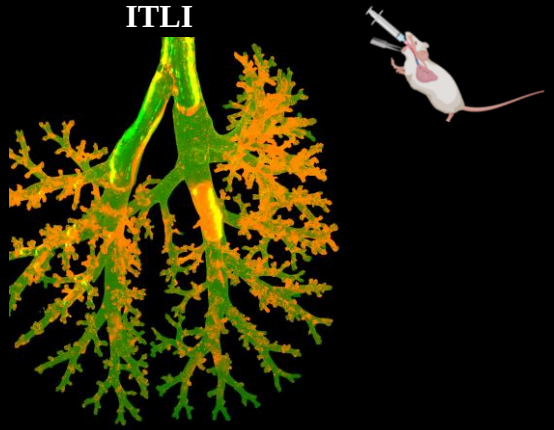
Tissue autofluorescence (AF) NPs

Distinct pulmonary deposition profiles for multiple delivery routes revealed by tissue-cleared LSFM

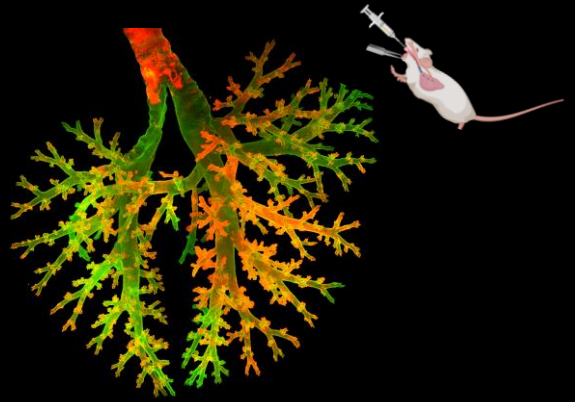
Intranasl liquid aspiration
INLA



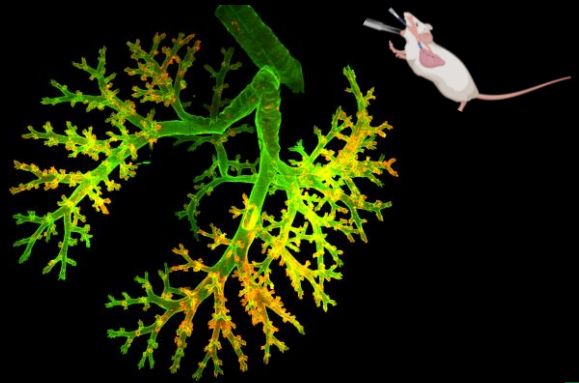
Intratracheal liquid instillation
ITLI



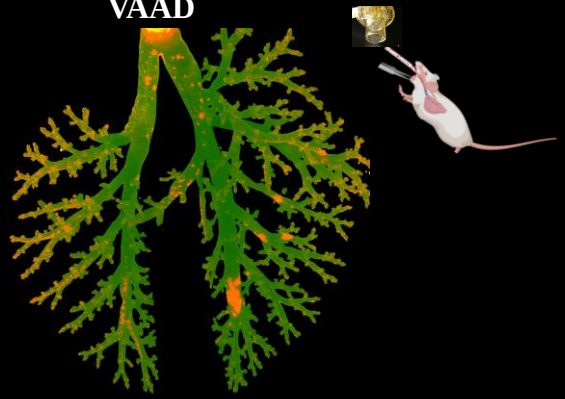
Microsprayer aerosol delivery
MSD



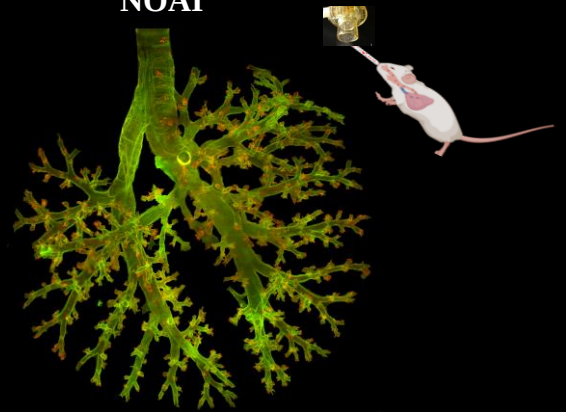
Oropharyngeal liquid aspiration
ORIA



Ventilator-assisted aerosol delivery
VAAD



Nose-only aerosol inhalation
NOAI



Tissue autofluorescence (AF) NPs