

Determination of the Fate of Inhaled Nanoparticles

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Problem

Determine the health risks from a large and rapidly growing number of inhaled engineered nanomaterials.

Approaches

1. Survey methods using physical/chemical properties and/or cell culture assays to rapidly examine a large number of engineered nanomaterials.
2. **Detailed in-vivo studies of pathology from exposures to engineered nanomaterials that are in high-level industrial use and/or of particular interest.**

Outline - Toxicologic evaluation of multi-walled carbon nanotube (MWCNT) inhalation exposure.

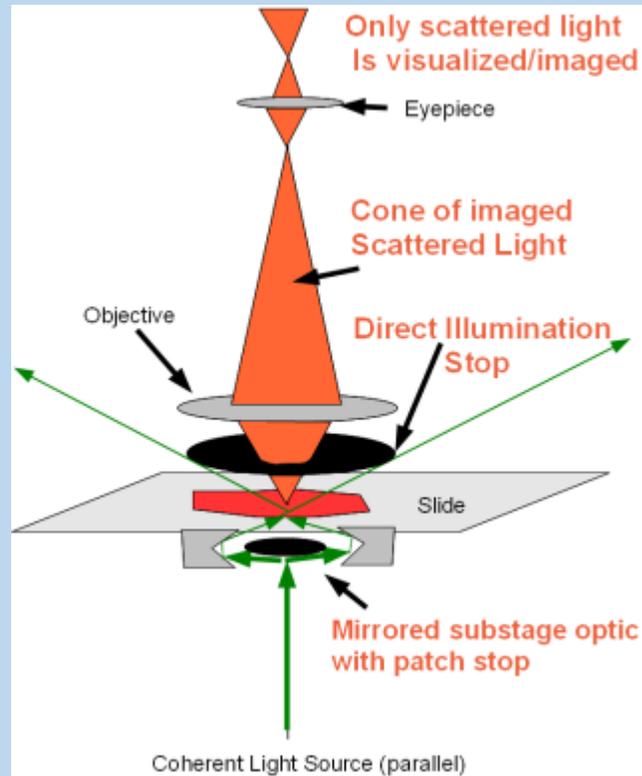
- Enhanced dark-field imaging techniques to detect individual nanoparticles in tissue sections.
- Examine distribution and re-distribution of inhaled MWCNTs in the lungs by methods that enhance detection of single nanoparticles.
- Determine the lung's fibrotic response to MWCNT.
- Test for potential transport outside the lungs and identify potential critical target organs and/or site(s) outside the lungs.

Enhanced dark-field imaging techniques to detect individual nanoparticles in tissue sections.

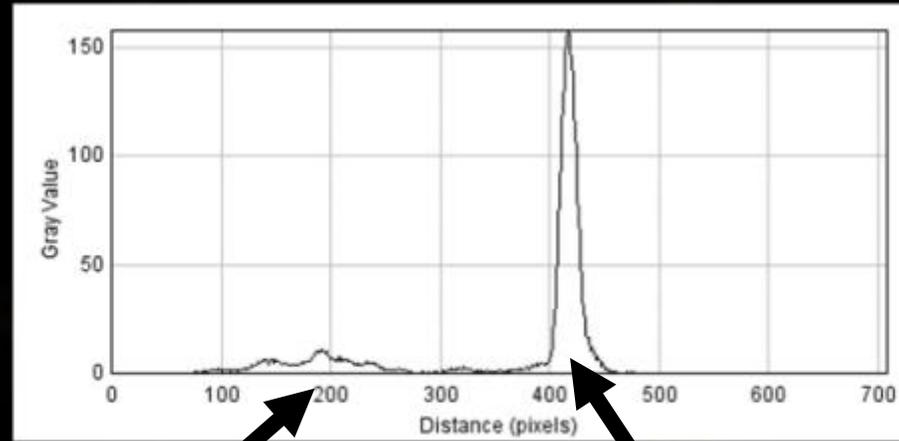
Enhanced Dark-field only images scattered light.

Sub-stage mirrored surface projects direct coherent (parallel) illumination at such an oblique angle that the direct rays passing through the specimen are completely blocked by the ring on the objective lens.

Nanoparticles efficiently scatter light due to high refractive index, dimension(s) that are approximately the same as a wavelength of light and crystal-like structure. Tissue does not.



Enhanced Dark Field image of inhaled TiO₂ nanoparticles deposited in the lungs from a “Nano” spray cleaning product.

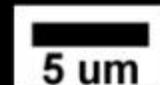


Lung Tissue

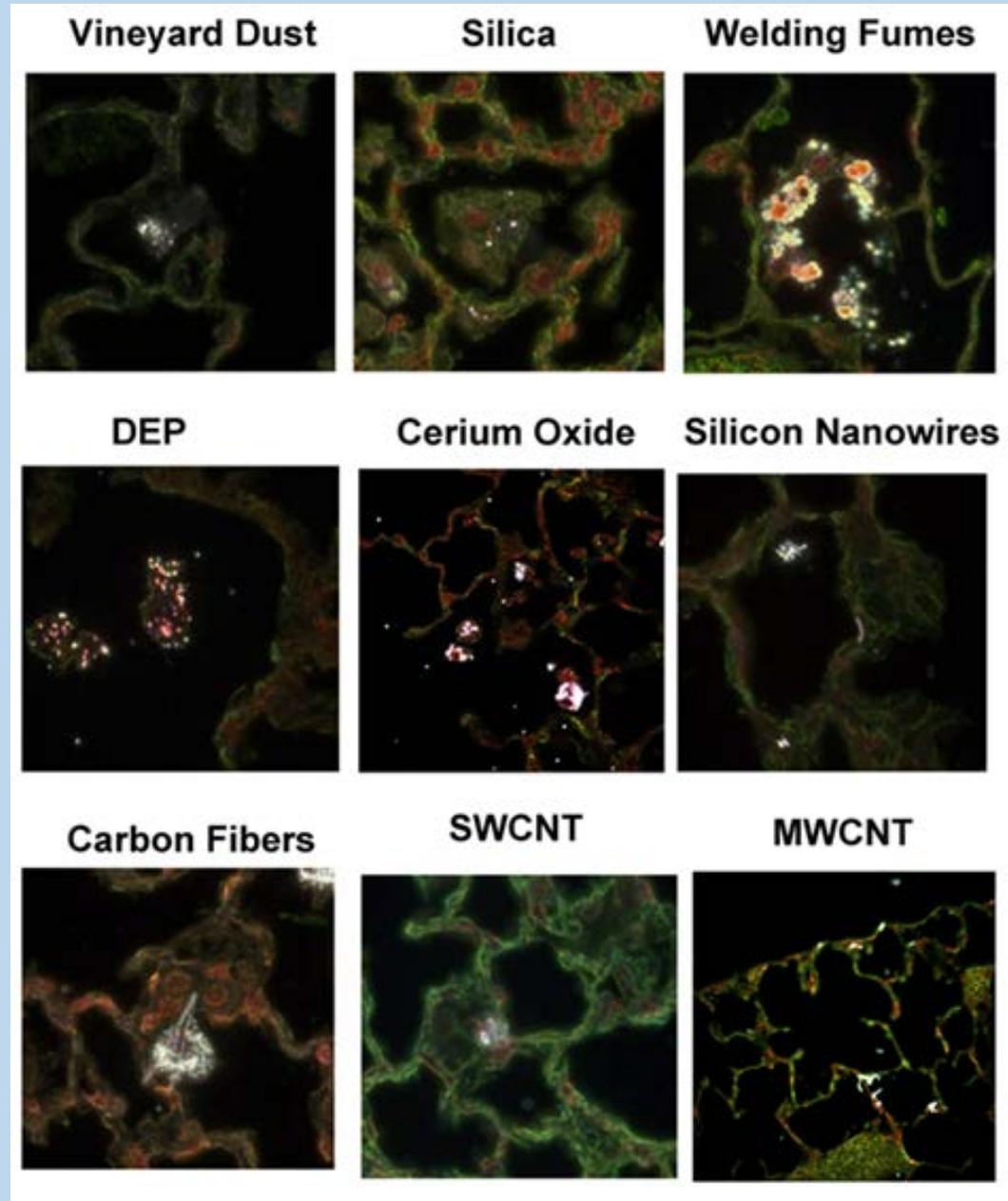
TiO₂ nanoparticles

TiO₂ nanoparticles

Lung Tissue



Enhanced Darkfield Imaging of Particles in the Lungs



Study Details

Mouse inhalation exposure to MWCNT via a whole-body inhalation exposure.

5 mg/m³ MWCNT aerosol for 5 hours/day for 12 days (4 times/week for 3 weeks).

Determine distribution of MWCNT and fibrotic response to MWCNT.

(lungs, lymph nodes, parietal pleura, respiratory musculature and systemic organs)

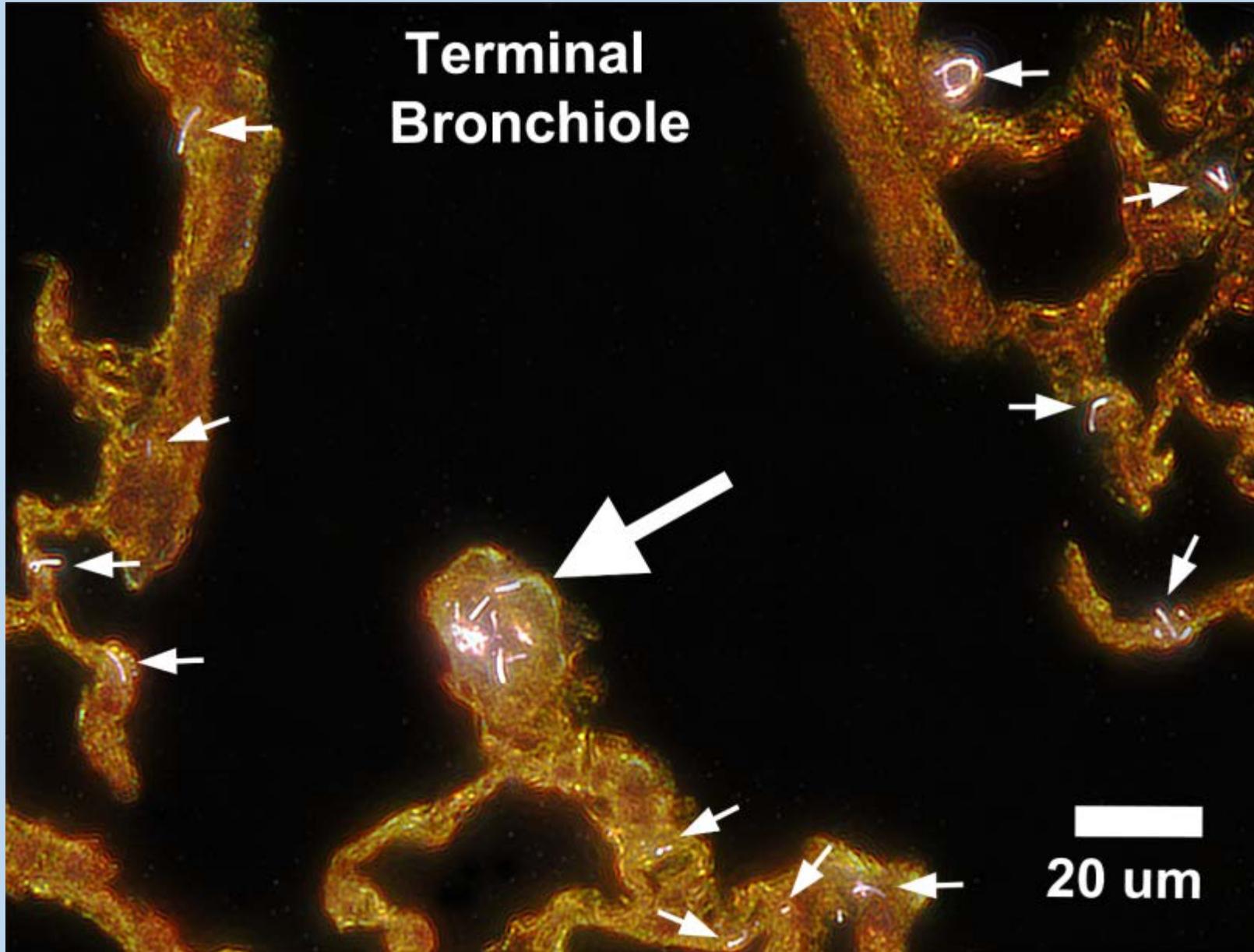
Enhanced darkfield microscopy and morphometric methods were used to detect and count MWCNT in tissue sections.

References:

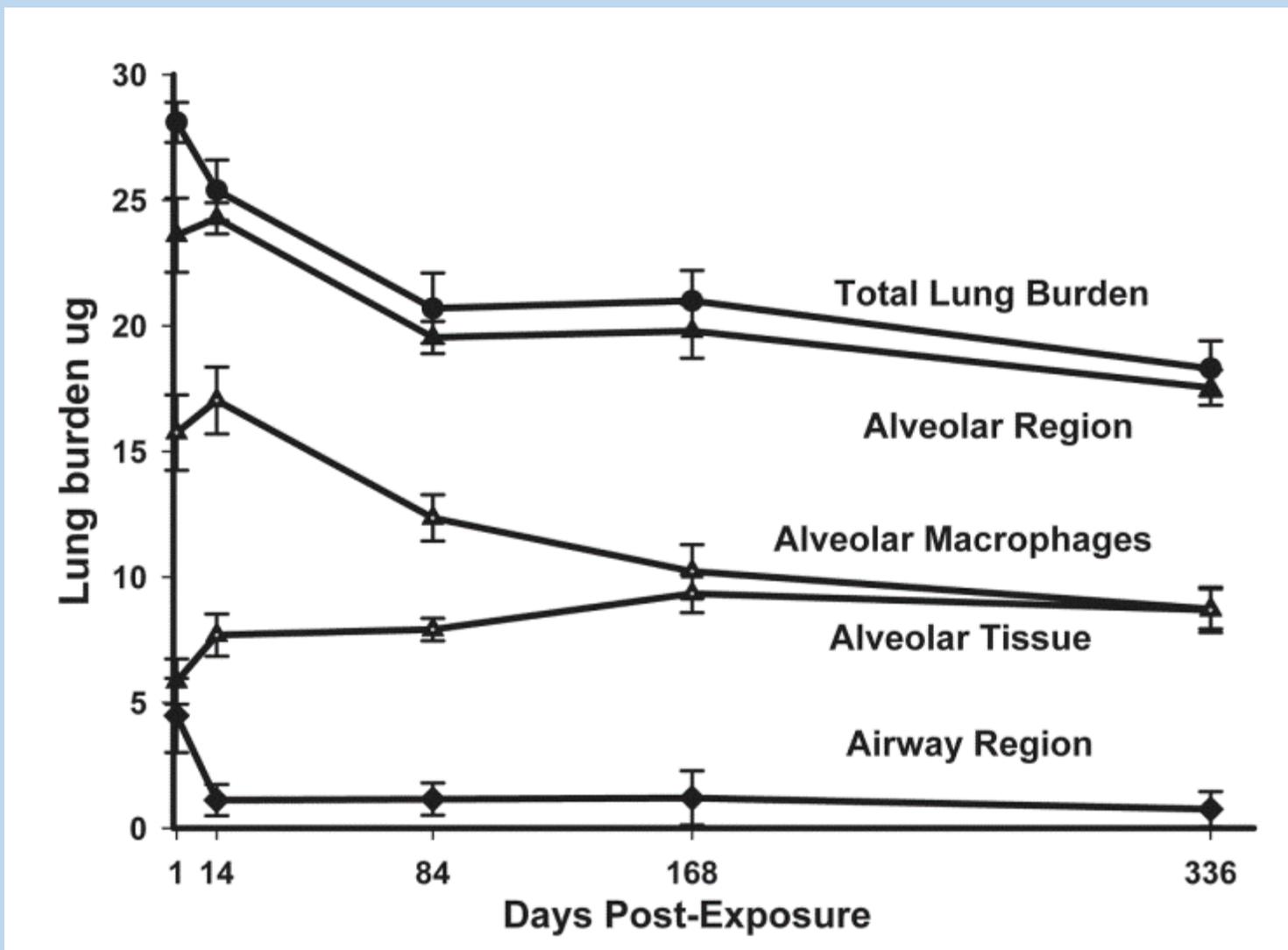
Mercer et al, Part Fibre Toxicol Jul 2013, 10:13.

Mercer et al, Part Fibre Toxicol Aug 2013,10:38.

Initial MWCNT distribution in the lungs



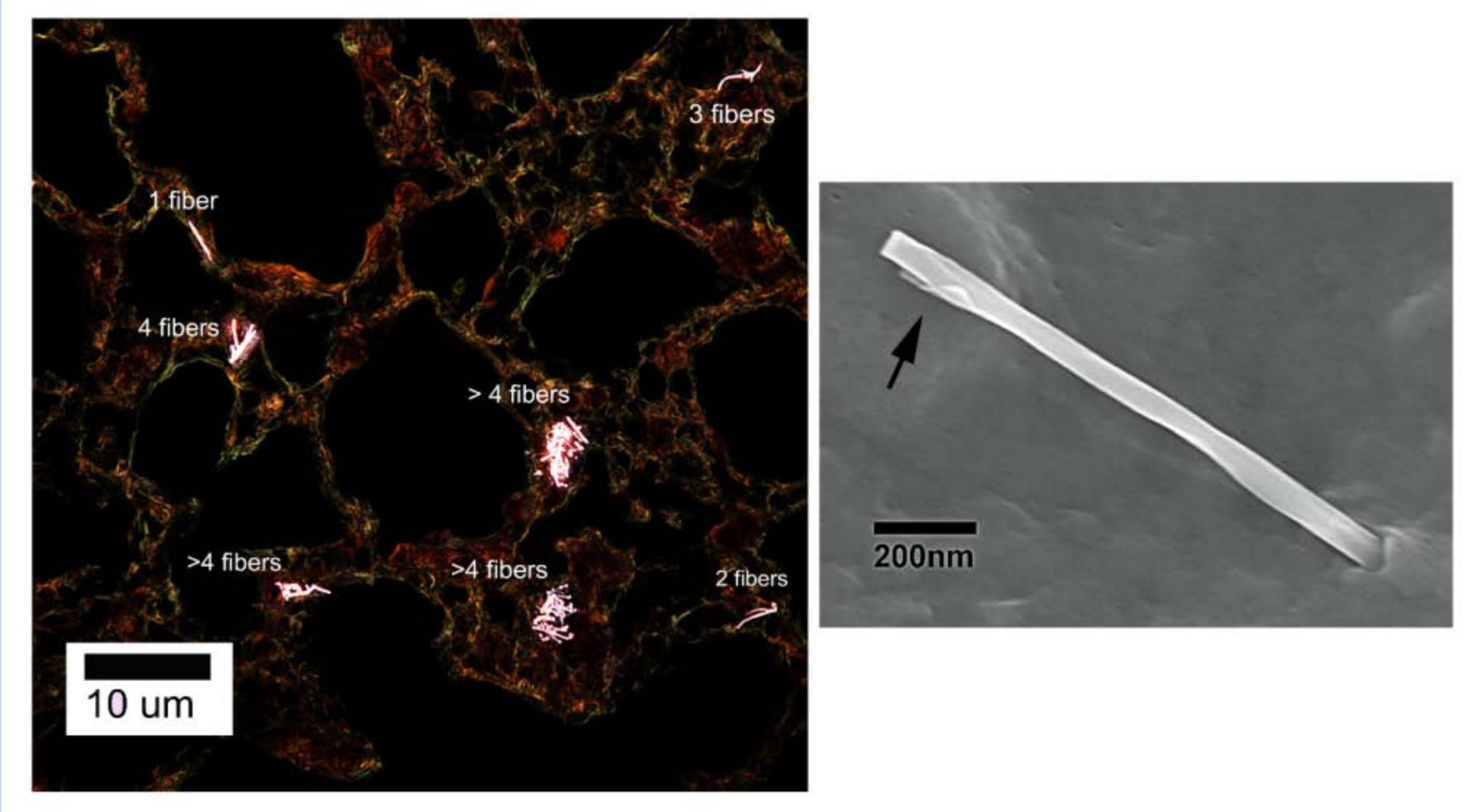
Slow clearance of MWCNT burden from the alveolar region of lungs



Alveolar Region contains majority of Total Lung Burden.

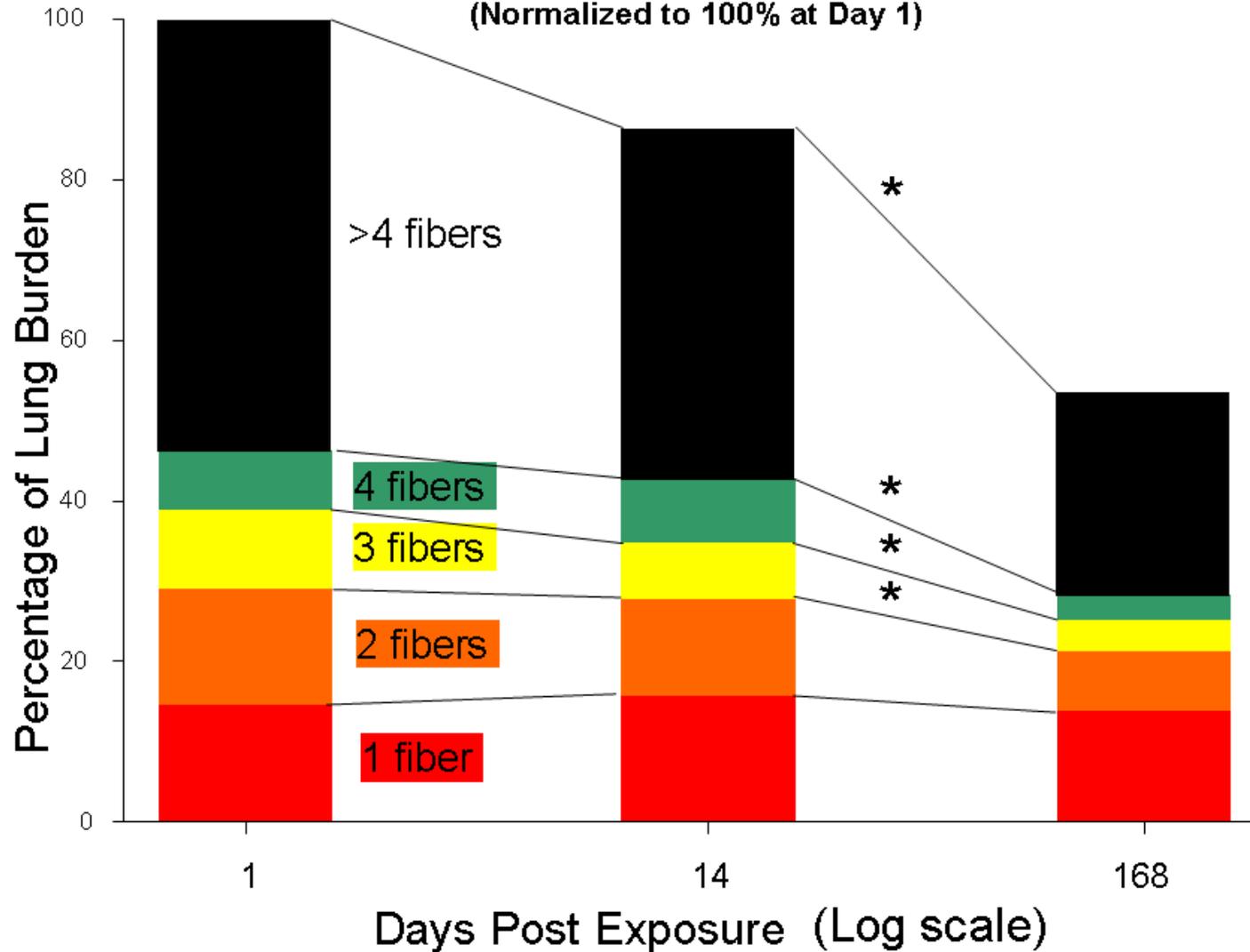
Burden of MWCNT in Alveolar Tissue increases after exposure?

Determining number of MWCNT fibers per MWCNT structure to assess redistribution in the lungs.



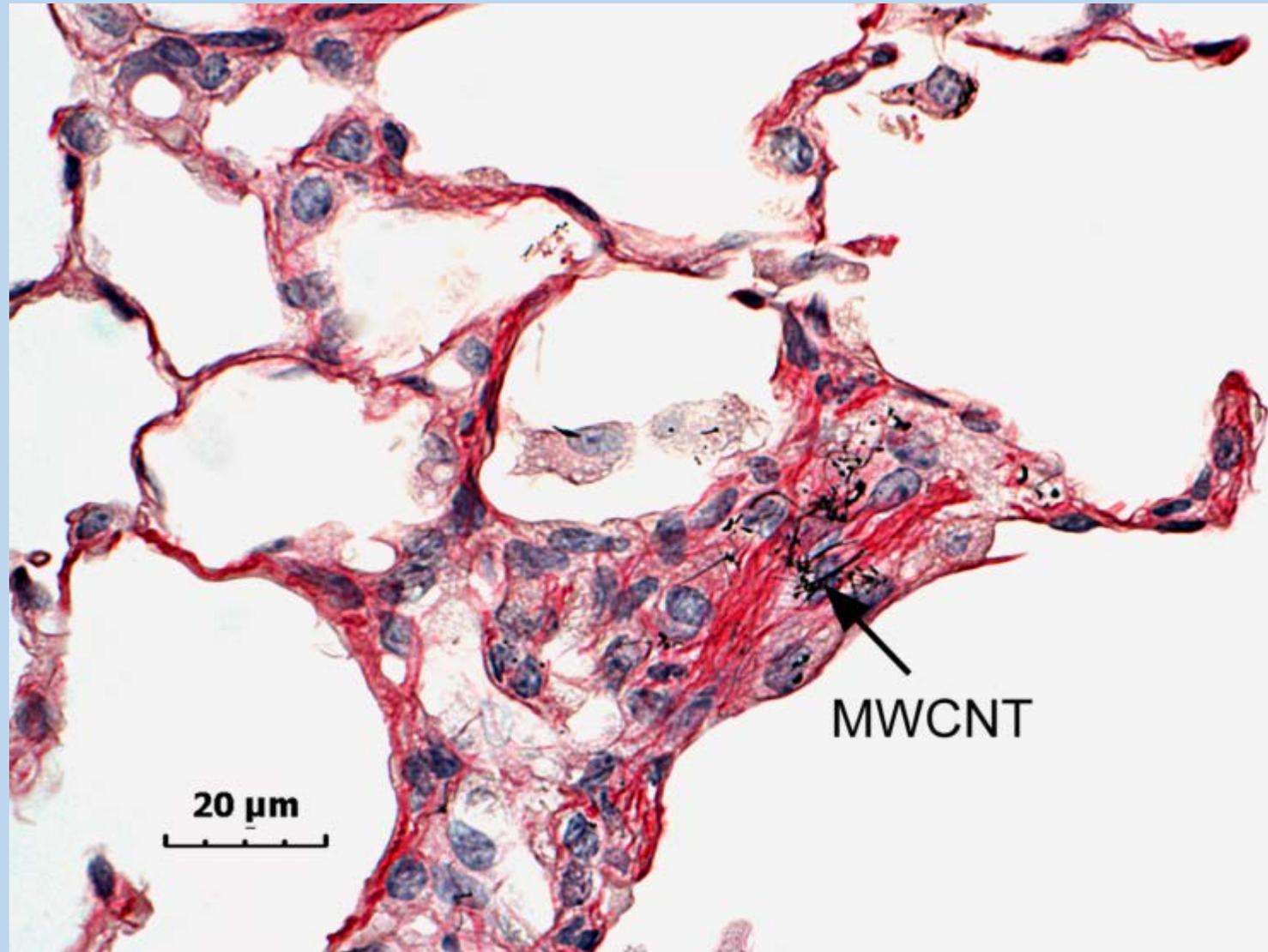
Percentage of Total Lung Burden

(Normalized to 100% at Day 1)

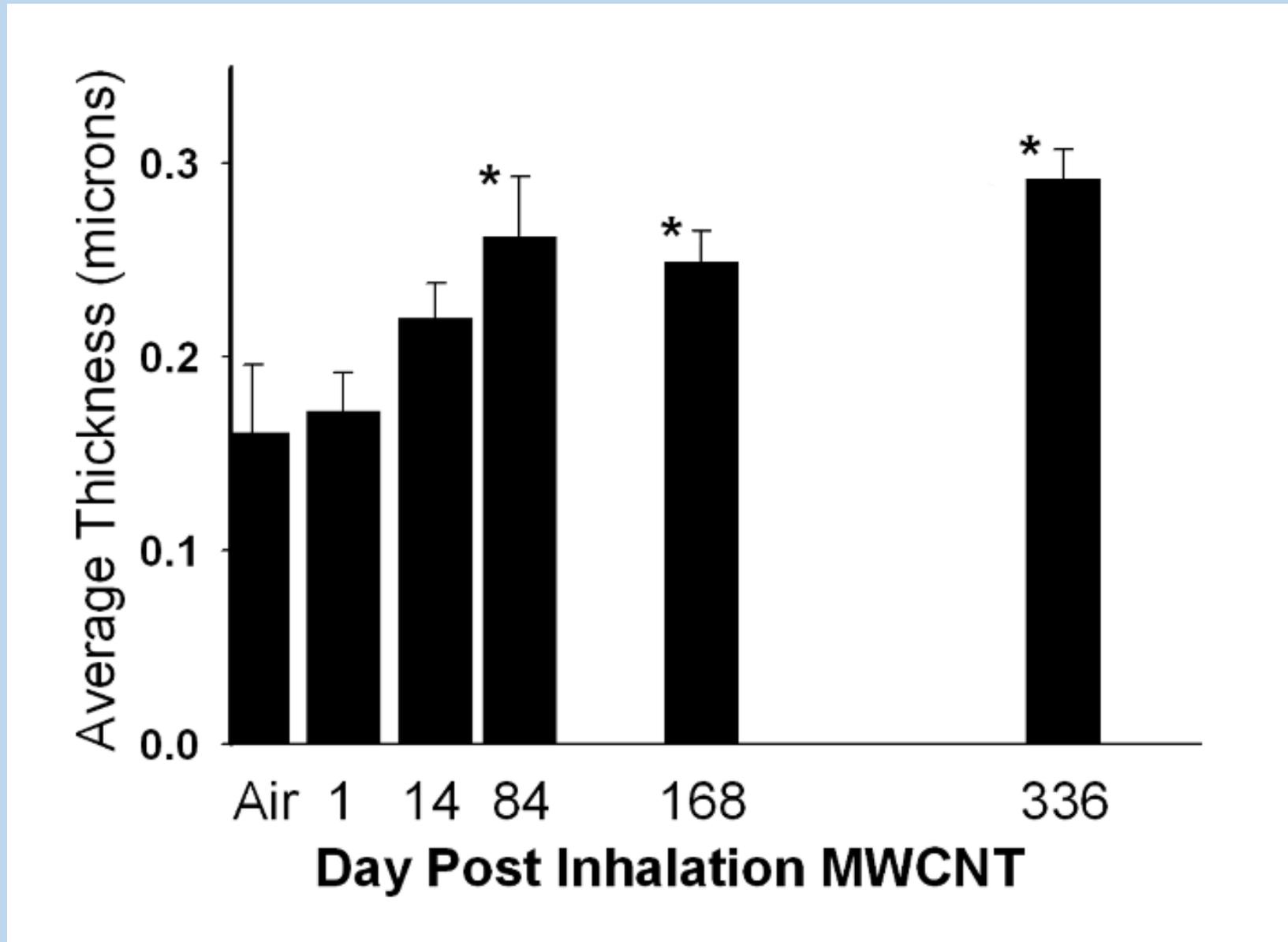


MWCNT structures containing > 4 fibers (typically loaded macrophages) decline significantly after exposure.

Fibrotic response to inhalation exposure of MWCNT

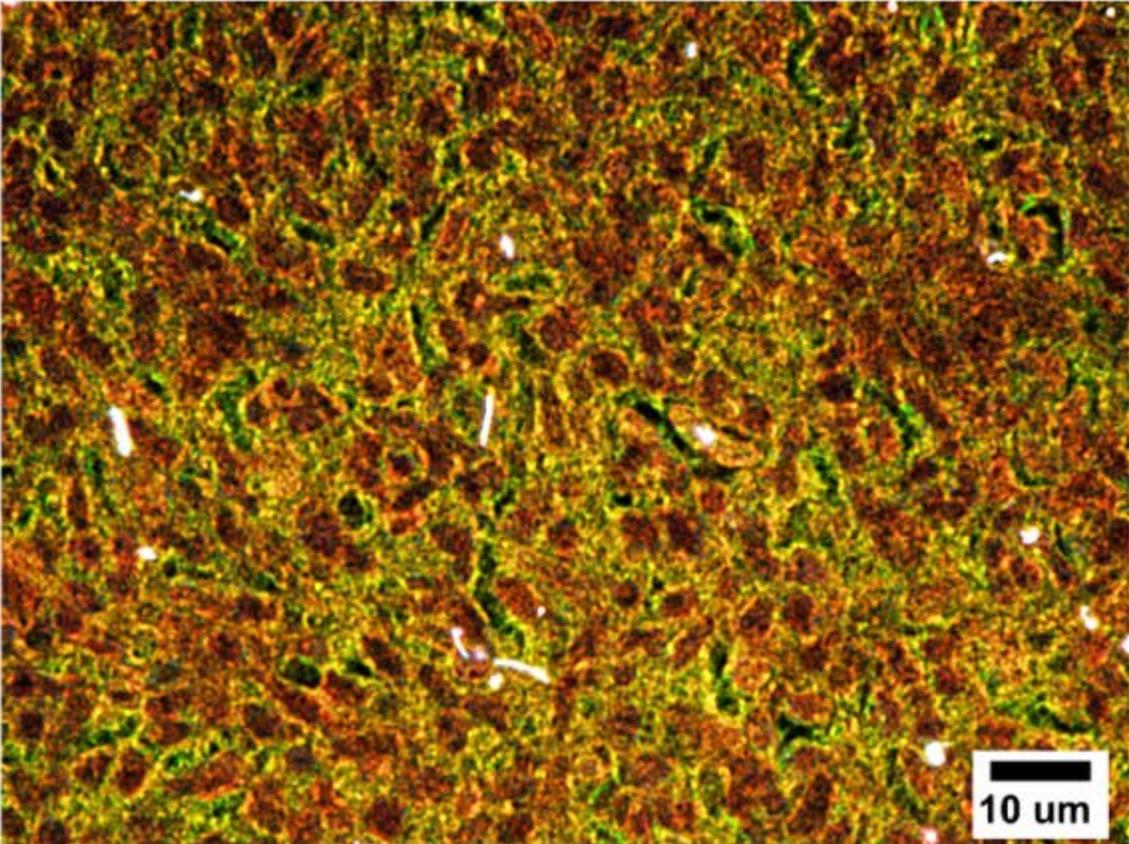


Average size (thickness) of fibrillar collagen significantly increases after MWCNT inhalation.

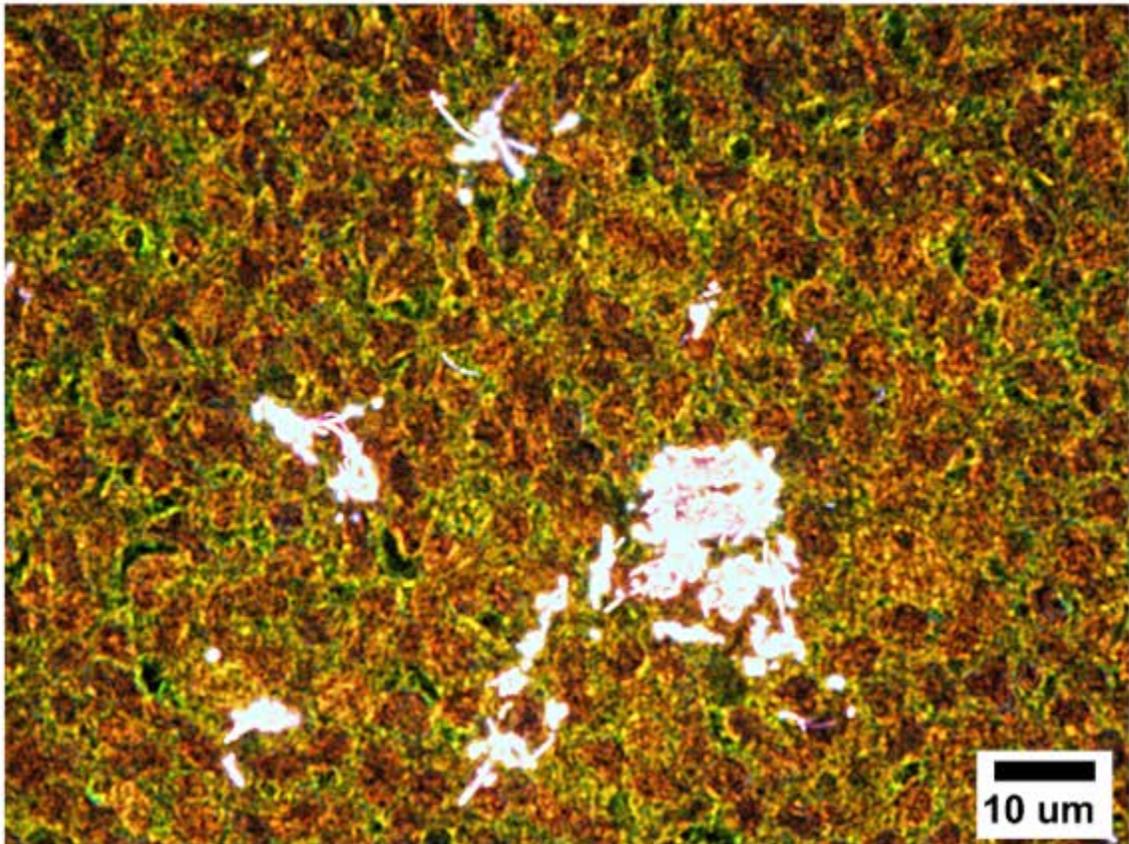


Enhanced dark-field microscope images of MWCNT accumulation in tracheobronchial lymph nodes
(1 and 336 days after 12 day inhalation exposure)

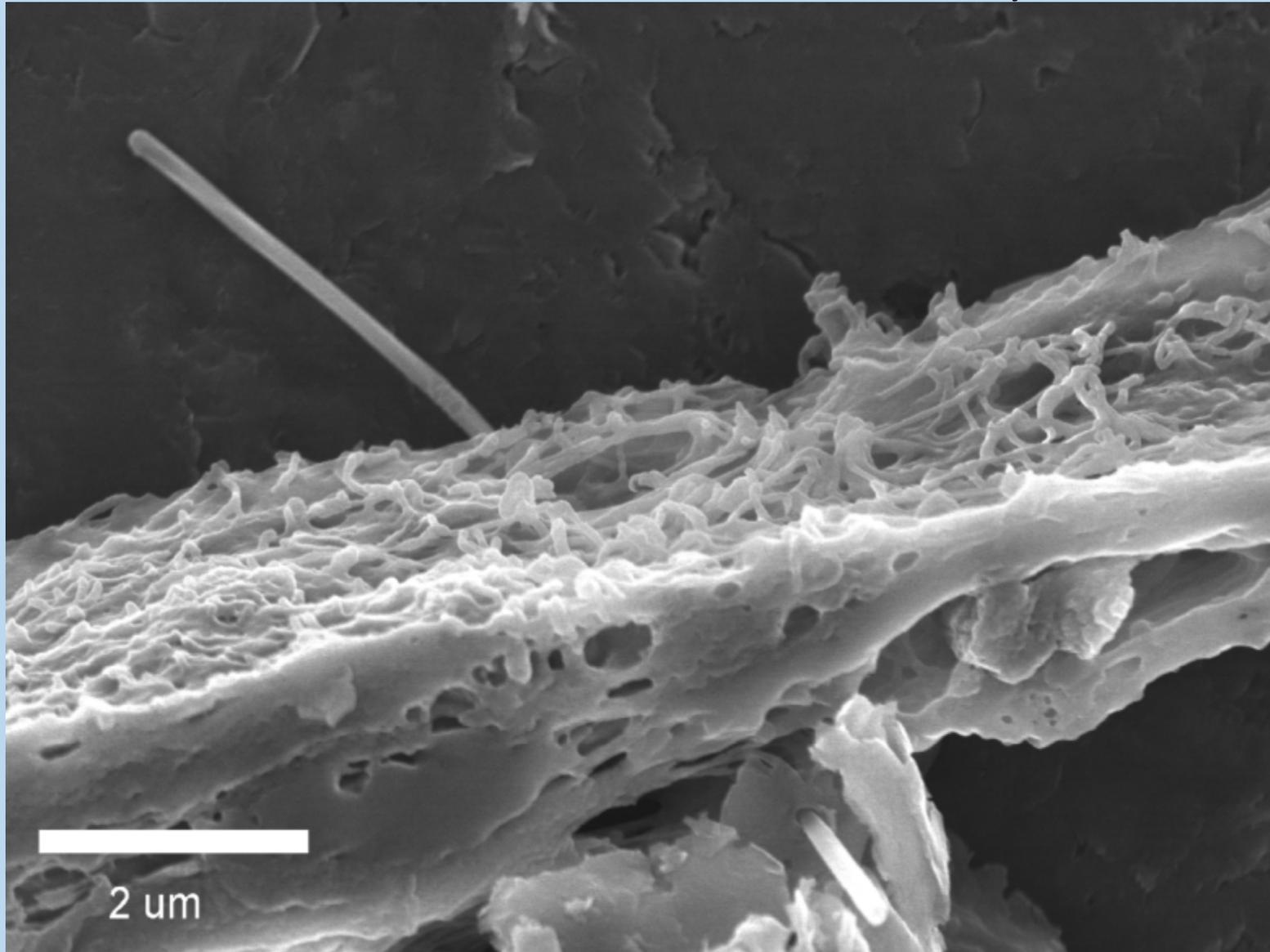
1 Day



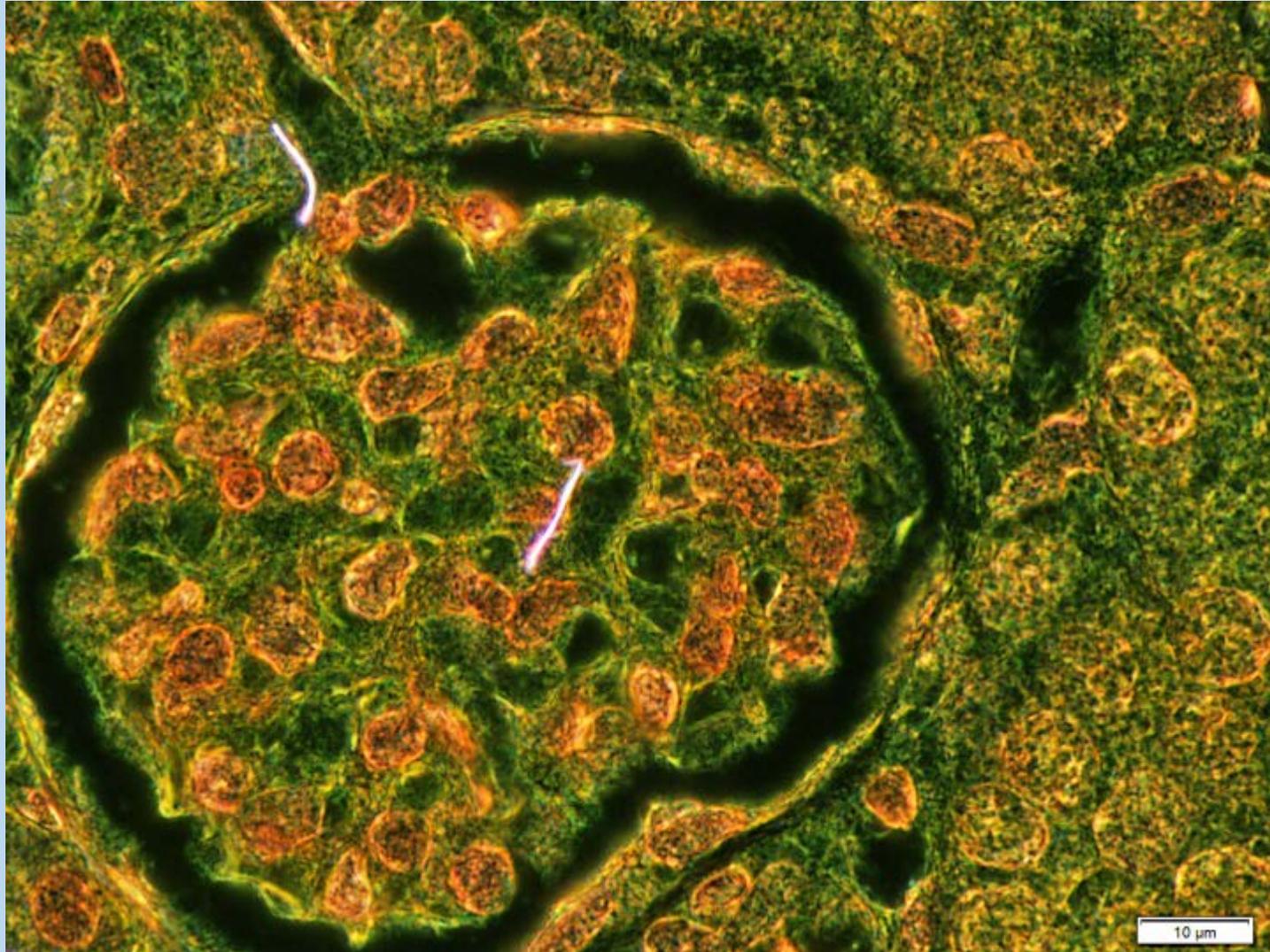
336 Days



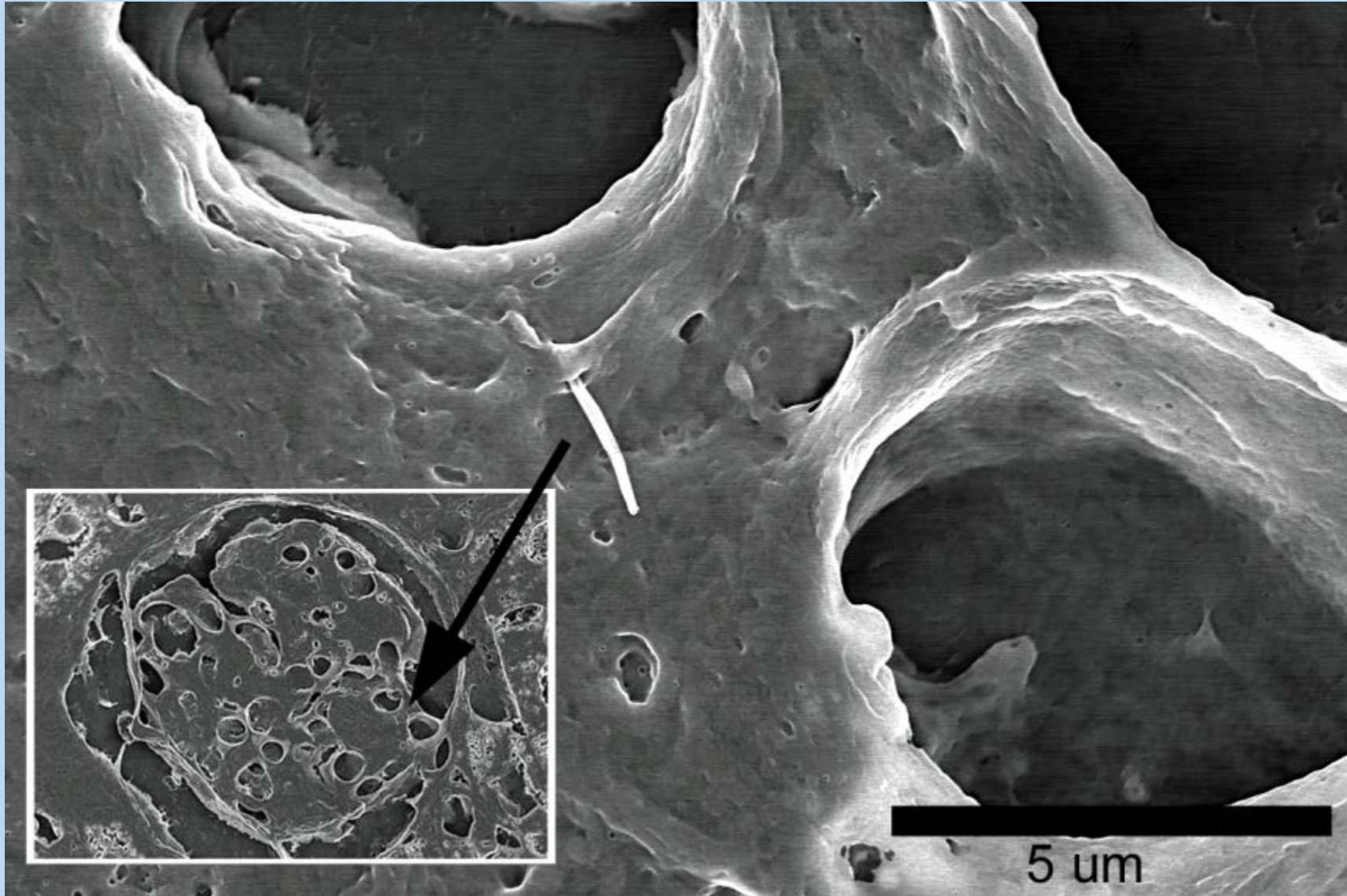
Penetration of Visceral Pleura by MWCNT



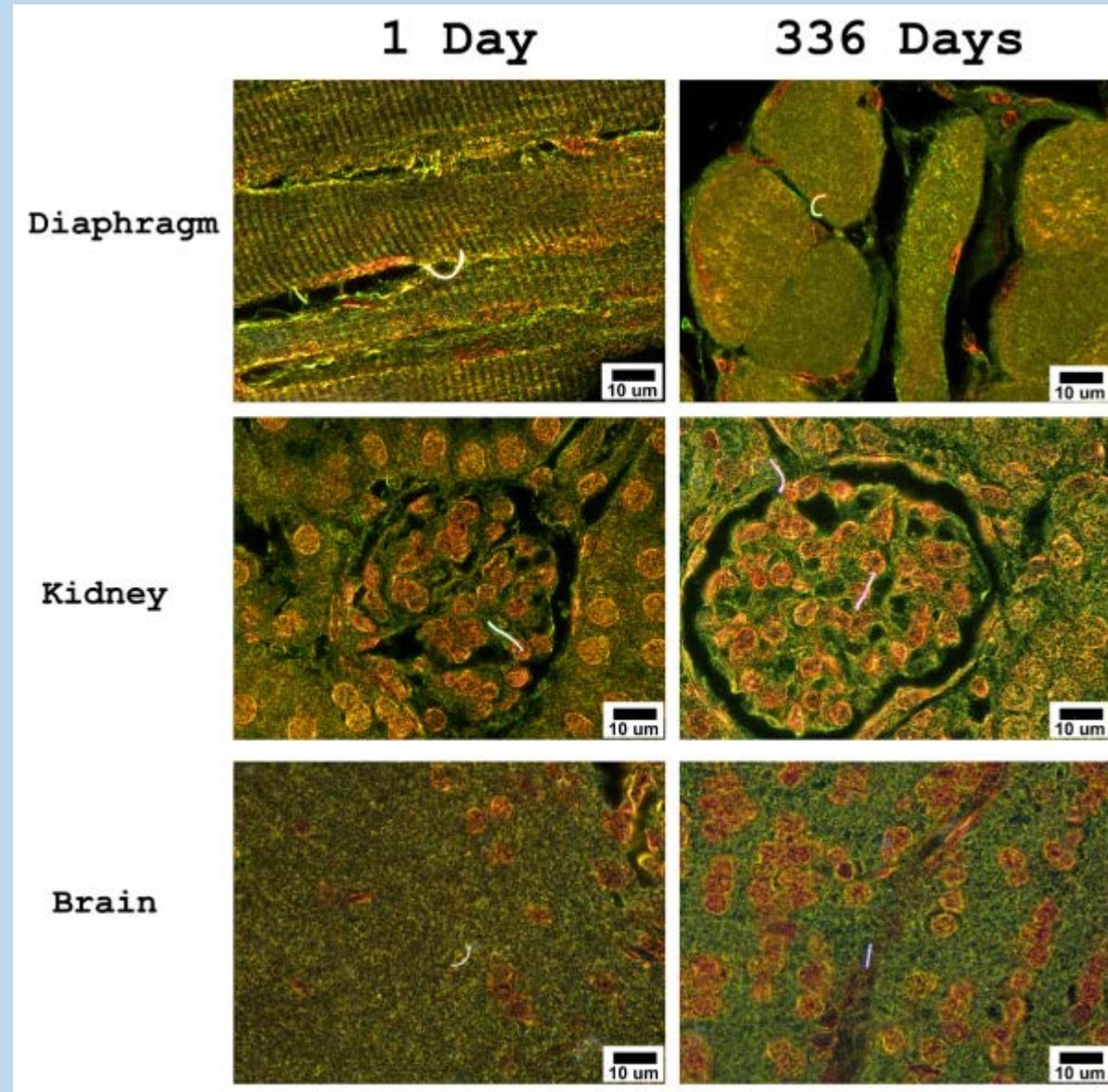
Singlet MWCNT in Kidney 336 days after 12 day inhalation exposure



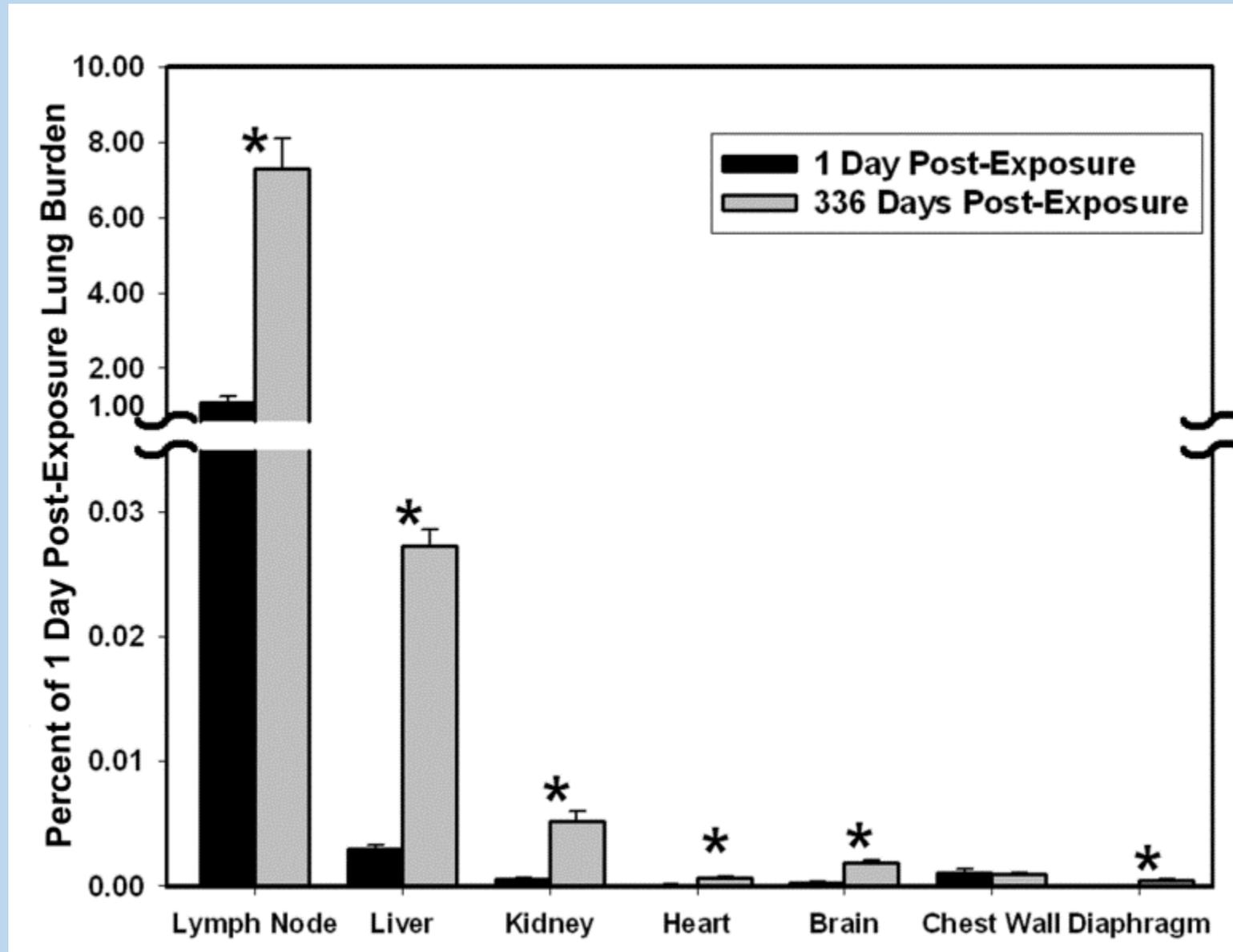
FESEM images of singlet MWCNT in Kidney 336 days after 12 day inhalation exposure



MWCNT in extra-pulmonary organs after inhalation exposure



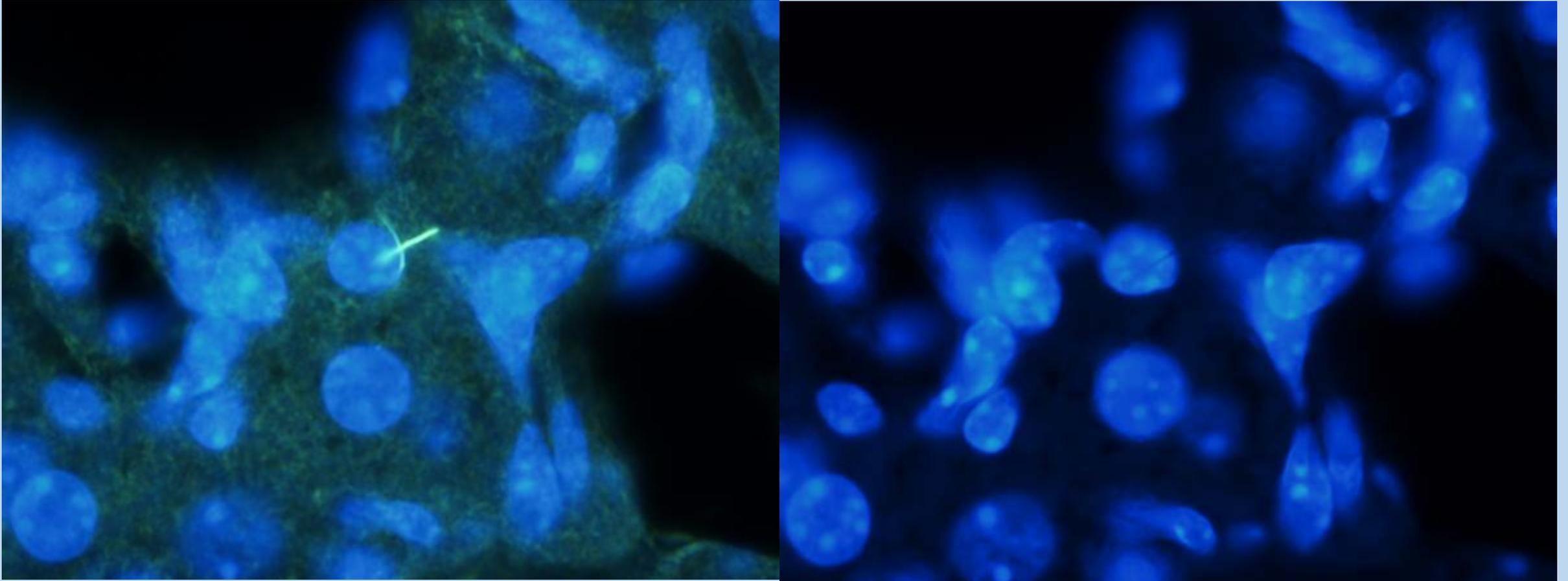
Accumulation of MWCNT in lymph nodes and extra-pulmonary organs after exposure.



Conclusions

- Inhaled MWCNTs distribute primarily to the alveolar region and have a slow clearance from the alveolar region.
- MWCNTs deposited in the lungs disaggregate into smaller structures (triplets, doublets, singlets). These smaller structures are primarily found in the alveolar interstitium.
- MWCNT produce a chronic, progressive fibrotic response in the alveolar interstitium.
- Chronically, MWCNT become concentrated in the lymphatics draining the lungs and accumulate as singlet fibers in systemic organs.

Measurement of liver nuclear burden following MWCNT inhalation.



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A number of approaches can be employed to assess the health risks from inhaled engineered nanomaterials. Our research has focused on identifying potential critical target site(s) by using newly developed microscopic imaging techniques to detect individual nanoparticles in the lungs and follow the transport of nanoparticles that are potentially transported to other organs. This presentation will discuss our use of this method in the study of inhaled multi-walled carbon nanotubes (MWCNT) in the mouse. The mouse inhalation was a whole-body inhalation exposure with 5 mg/m³ MWCNT aerosol for 5 hours/day for 12 days (4 times/week for 3 weeks). The distribution of MWCNT was determined in the lungs, tracheobronchial lymph nodes draining the lungs, the parietal pleura, respiratory musculature and systemic organs at 1 day, 14 days, 168 days and 336 days after the 12 day exposure period. Enhanced darkfield microscopy and morphometric methods were used to detect and count MWCNT in tissue sections. Field emission scanning electron microscopy (FESEM) was also used to examine details of MWCNT structure in the various tissues. Tracheobronchial lymph nodes were found to contain 1.08 and 7.34 percent of the initial lung burden at 1 day and 336 days post-exposure, respectively. Although agglomerates account for approximately 54% of the burden in the lungs, only singlet MWCNT were observed in the diaphragm, chest wall, liver, kidney, heart and brain. On average, there were 15,371 and 109,885 fibers per gram in liver, kidney, heart and brain at 1 day and 336 days post-exposure, respectively. The burden of singlet MWCNT in the lymph nodes, diaphragm, chest wall and extrapulmonary organs at 336 days post-exposure was significantly higher than at 1 day post-exposure. Results demonstrate that inhaled MWCNT, which deposit in the lungs, are transported to the parietal pleura, the respiratory musculature and the systemic organs in a singlet form and chronically accumulate in systemic organs following exposure.