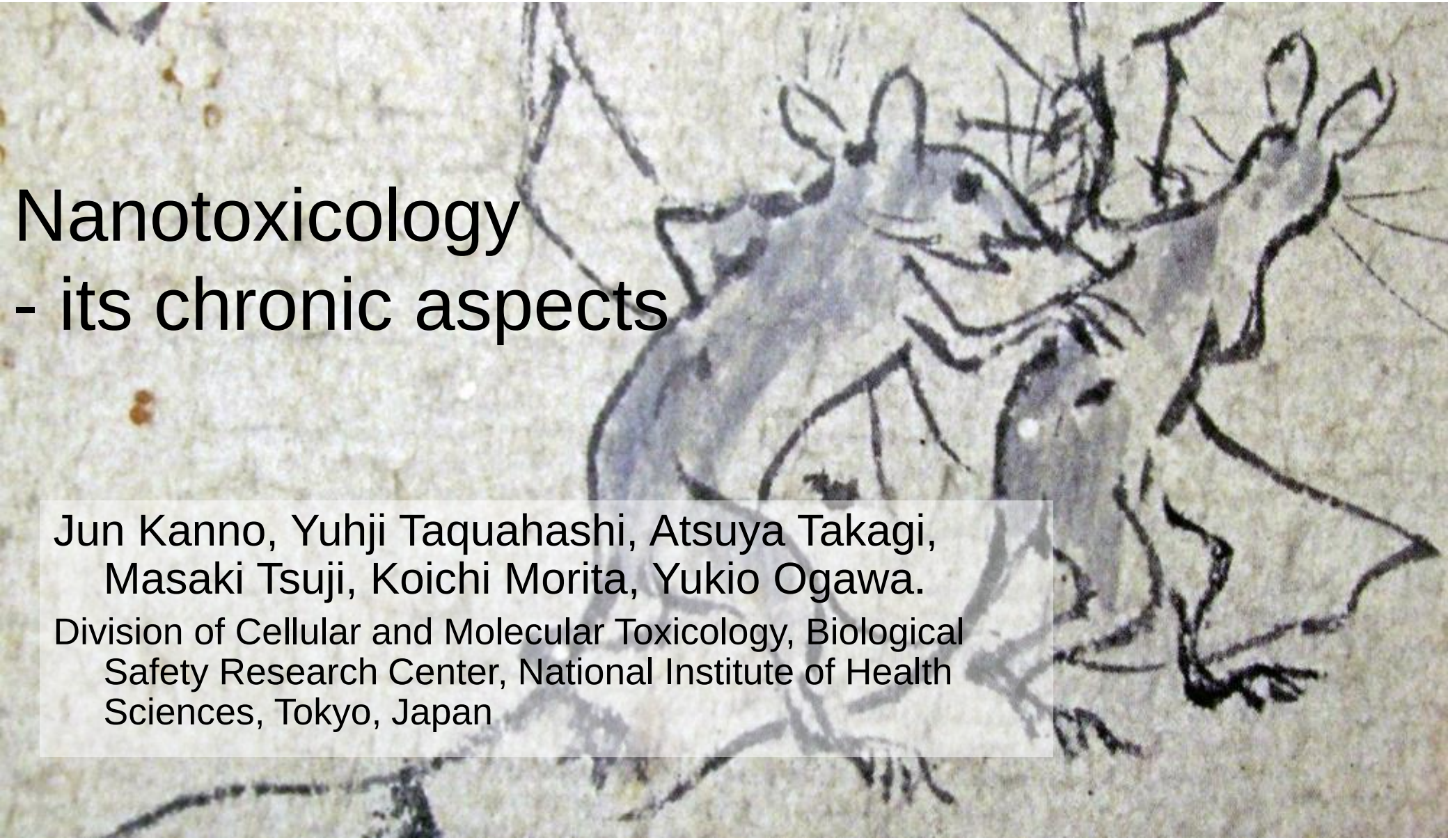
NanOEH6



Nanotoxicology
- its chronic aspects

Jun Kanno, Yuhji Taquahashi, Atsuya Takagi, Masaki Tsuji, Koichi Morita, Yukio Ogawa.

Division of Cellular and Molecular Toxicology, Biological Safety Research Center, National Institute of Health Sciences, Tokyo, Japan

鳥獣人物戯画より

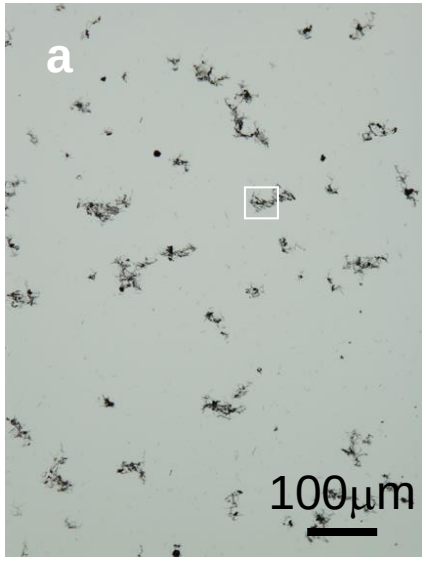
Human Examples of Chronic Particulate Matter Toxicity

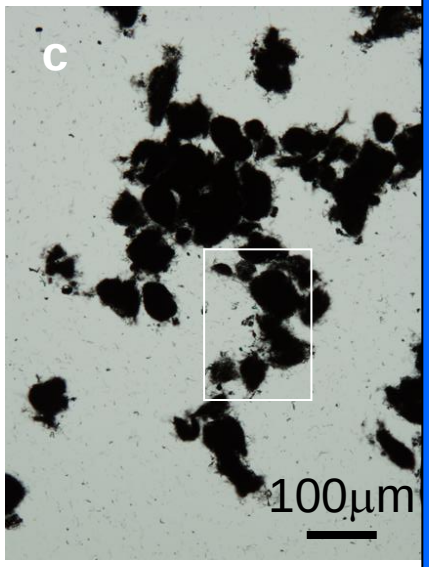
- Asbestos (biopersistent fiber; mesothelioma/ lung carcinoma)
- Thorotrast (3-10 nm-sized biopersistent thorium dioxide particles; reticuloendothelial system (RES) deposition with *in vivo* half life of 22~400 years)
- (Welding fume and cardiovascular diseases)

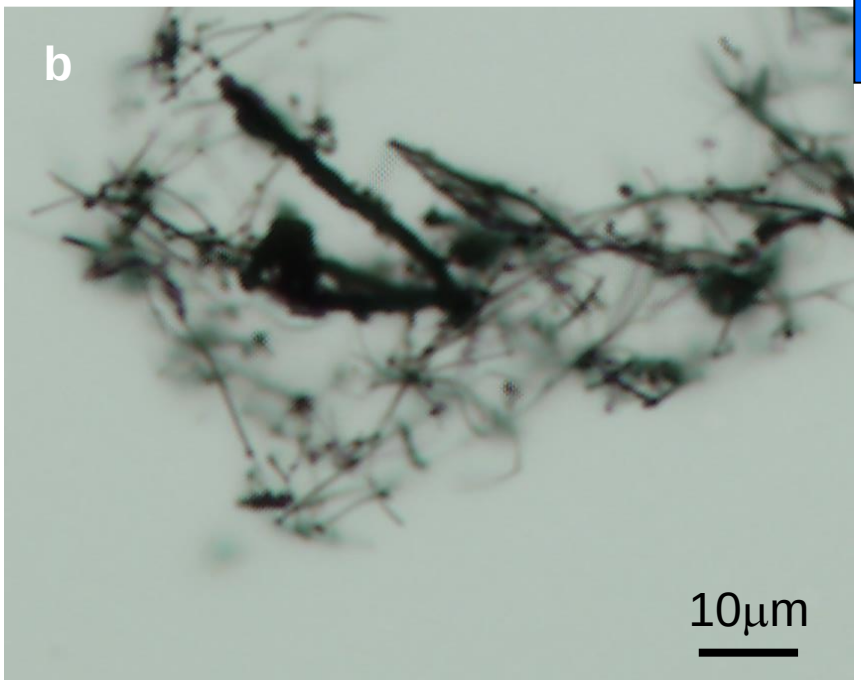
Current status

- Knowledge of asbestos has facilitated the analysis on biological effects of μm-MWCNT.
- Thorotrast foretells entrapment of biopersistent nanoparticles by the reticuloendothelial system for a very long time period.
- Biopersistent nanoparticles may show low acute toxicity, but could show chronic toxicity.
- Study protocols should be set in a case-by-case basis, at least for the moment.

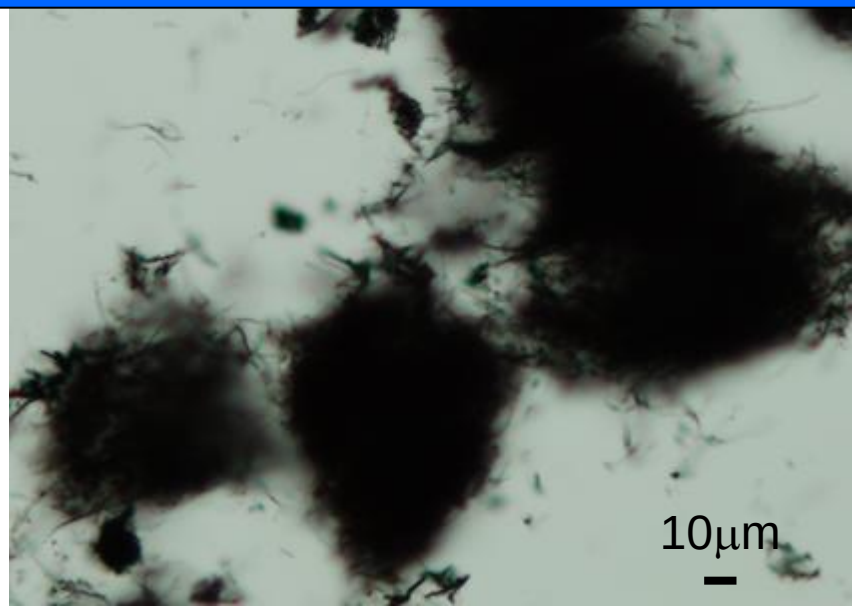
MWCNT (Mitsui)

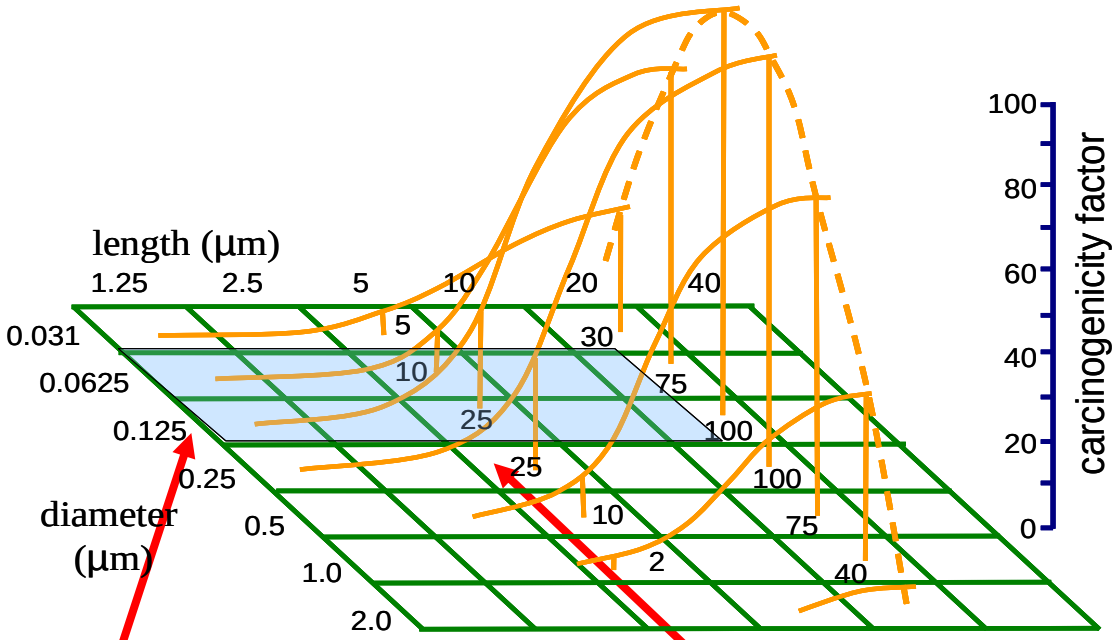






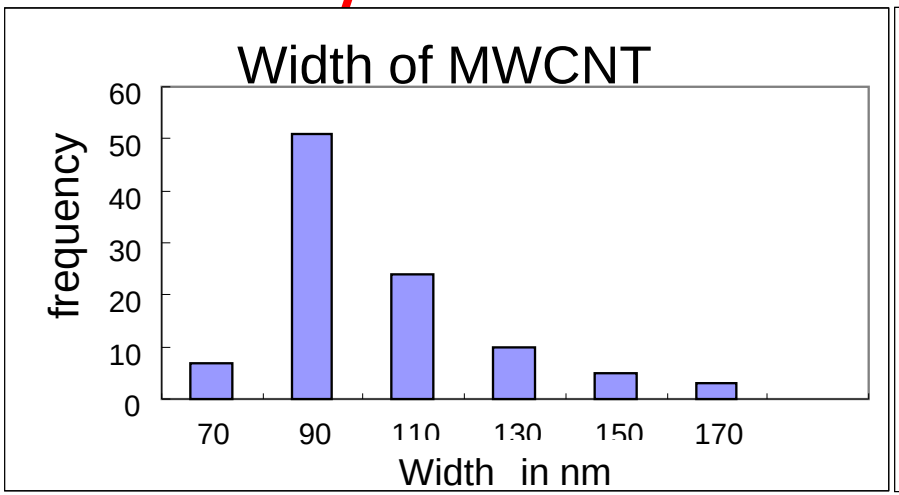
Asbestos (crocidolite)

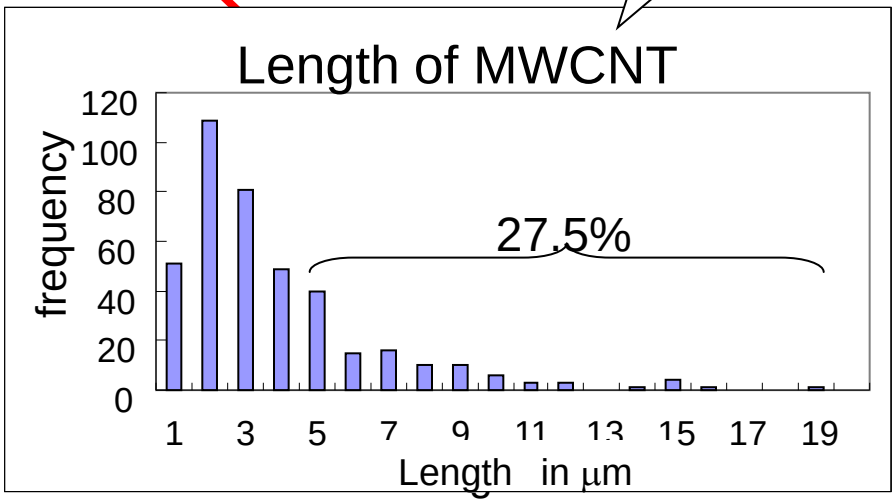




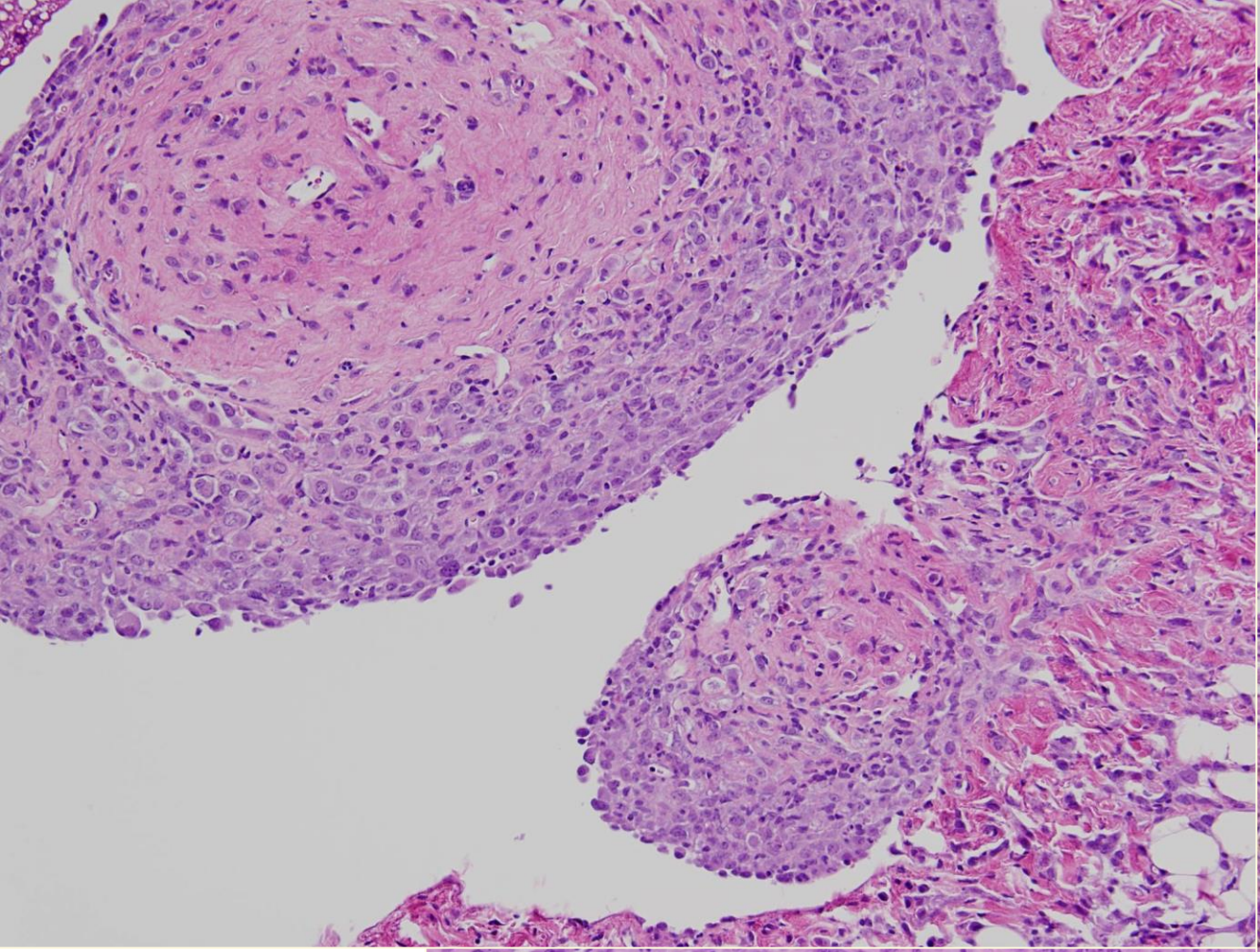
Hypothesis on the carcinogenic potency of a fibre as a function of its size with some data on "carcinogenicity factors". From: Pott (1978).

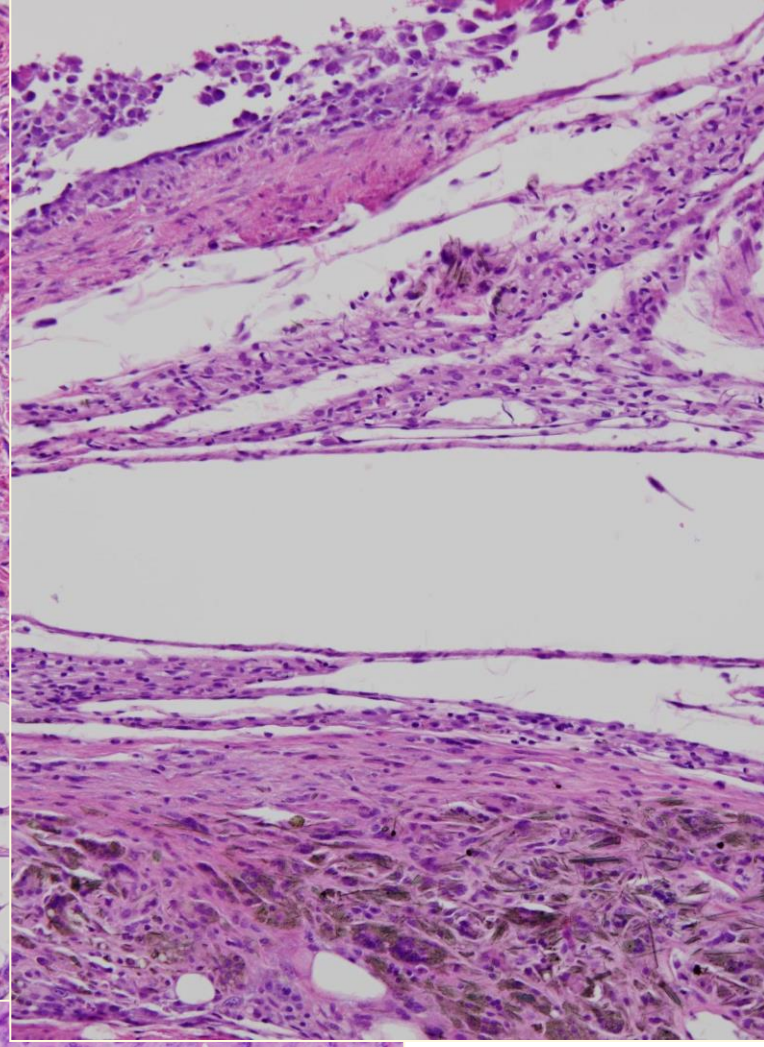
Measured at the Tokyo Metropolitan Institute of Public Health

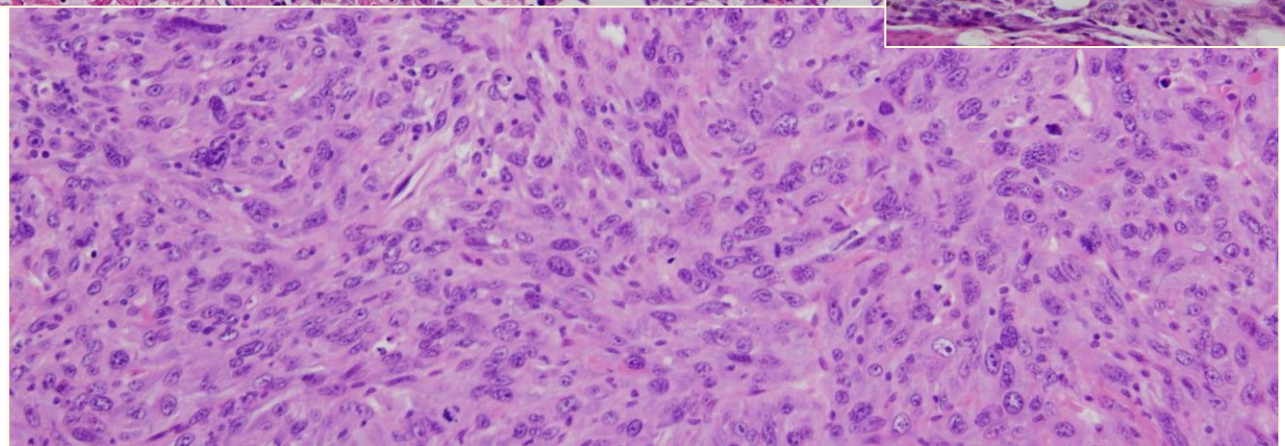




MWCNT：3mg/animal =1.06 X10^9 fiber/mouse ＝ 1.86 X10^8 WHO fiber/mouse)

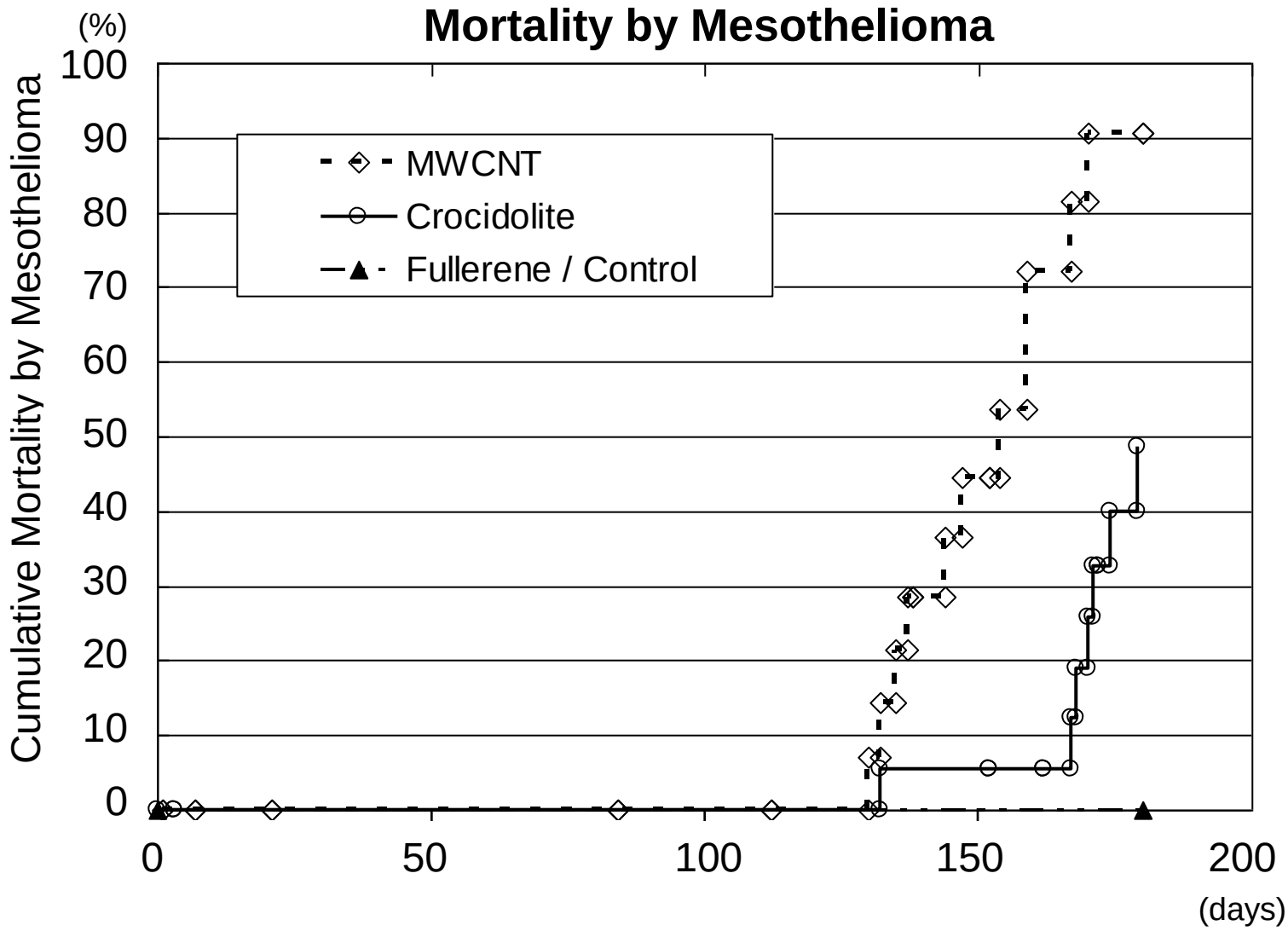






crocidolite

3,000 μg/animal = $1x10^9$ fiber /animal



Takagi et al. 2008

The Journal of Toxicological Sciences (J. Toxicol. Sci.)
Vol.33, No.1, 105-116, 2008

Original Article

Induction of mesothelioma in p53+/– mouse by intraperitoneal application of multi-wall carbon nanotube

Atsuya Takagi[1], Akihiko Hirose[2], Tetsuji Nishimura[3], Nobutaka Fukumori[4], Akio Ogata[4], Norio Ohashi[4], Satoshi Kitajima[1] and Jun Kanno[1]

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(Received November 20, 2007; Accepted December 9, 2007)

NANOTOXICOLOGY

The asbestos analogy revisited

Direct injection of long multiwalled carbon nanotubes into the abdominal cavity of mice produces asbestos-like pathogenic behaviour. What does this finding mean for nanotube safety?

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e-mail: Agnes_Kane@brown.edu; Robert_Hurt@brown.edu

The possibility that carbon nanotubes would show asbestos-like behaviour in the human body was raised ten years ago with a call for appropriate research[1]. Exposure to asbestos is known to cause mesothelioma — cancer of the lining of the lungs (pleura) and abdominal cavity (peritoneum). The nanotube and asbestos analogy relies on several points of material similarity: small fibre diameter, long length and chemical stability in physiological environments (biopersistence). There are also differences between these two fibrous materials, such as their chemical composition and surface properties, so the validity and usefulness of the nanotube and asbestos analogy have been unclear. Two recent studies provide important new insight into the possibility that carbon nanotubes may indeed induce mesothelioma — a disease that is rare in unexposed populations and is thus a sensitive marker for asbestos exposure.

On page 423 of this issue[2], Ken Donaldson of the MRC/University of Edinburgh and co-workers in the UK and US report that long multiwalled carbon nanotubes (MWNTs) injected directly into the abdominal cavity of mice induce inflammation, formation of nodular lesions called granulomas and early fibrosis or scarring in the mesothelial lining. Shorter nanotubes had much less of an effect, as did carbon black nanoparticles used as a non-fibrous reference material. A seven-day exposure did not induce mesothelioma, but the distribution and severity of these early inflammatory and granulomatous lesions are similar to those induced by long fibres of brown asbestos (amosite), which is known to induce significant toxicity and carcinogenicity in longer-term animal studies.

Another recent study[3] by Jun Kanno of the National Institute of Health Sciences in Japan and colleagues from the Tokyo Metropolitan Institute of Public Health shows that MWNTs, also injected into the abdominal cavity of mice, induce malignant mesotheliomas in p53+/– heterozygous mice — a common genetically engineered mouse model. These mice are a useful laboratory model because they are sensitive to asbestos and can rapidly develop malignant mesothelioma following repeated exposure to asbestos fibres.

Using commercial MWNTs from the same suppliers as Donaldson and co-workers, the Japanese team observed granulomas and fibrosis in the mesothelial lining as well as tumours in 88% of the MWNT-treated mice after 25 weeks, in comparison with 79% in mice injected with crocidolite, a particularly potent form of asbestos. Minimal mesothelial reactions and no mesotheliomas were produced by the same mass dose of (non-fibrous) C_{60} fullerene. The authors conclude that asbestos fibres and MWNTs may have similar carcinogenic potential on the basis of their fibrous geometry, biopersistence and ability to generate tissue-damaging free radicals.

Both of these reports identify key physical properties of carbon nanotubes that may be relevant for potential toxicity

10

NANOTOXICOLOGY

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In the case of carbon nanotubes and other engineered nanoproducts, we are still within a 'window of opportunity' to develop safe material design and manufacturing strategies before commercialization becomes widespread.

Cancer Science
The official journal of the Japanese Cancer Association

Dose-dependent mesothelioma induction by intraperitoneal administration of multi-wall carbon nanotubes in p53 heterozygous mice

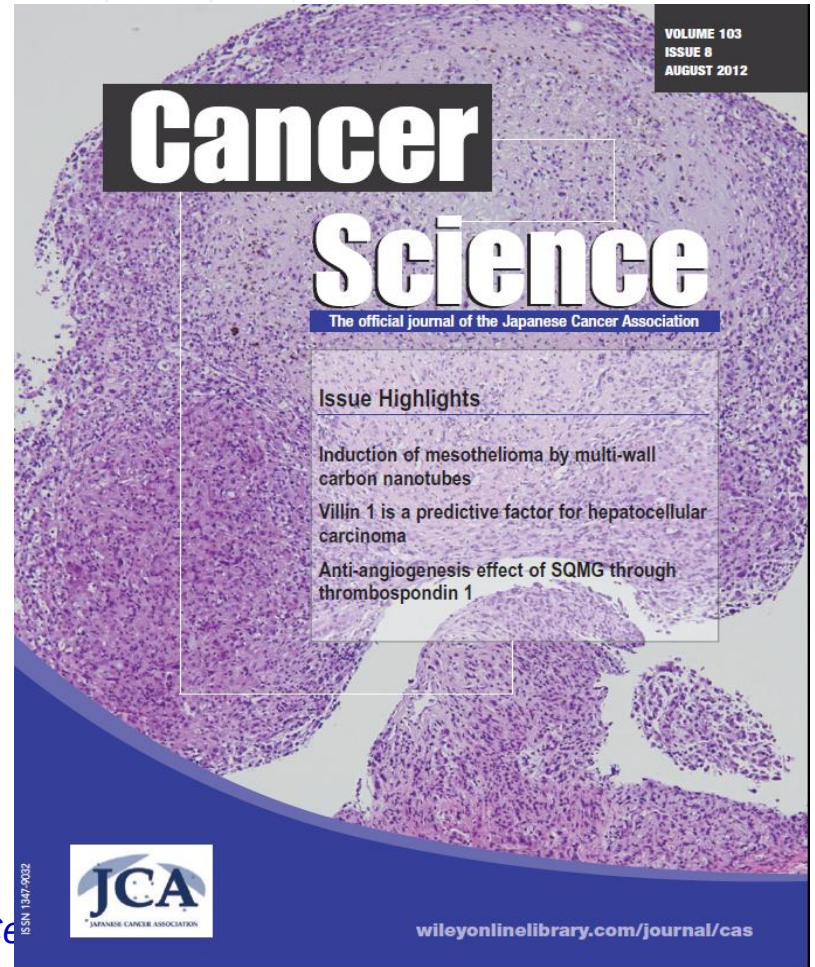
Atsuya Takagi,[1] Akihiko Hirose,[2] Mitsuru Futakuchi,[3] Hiroyuki Tsuda[4] and Jun Kanno[1,5]

[1]Division of Cellular and Molecular Toxicology, [2]Division of Risk Assessment, Biological Safety Research Center, National Institute of Health Sciences, Tokyo; [3]Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences; [4]Nanomaterial Toxicology Project Laboratory, Nagoya City University, Nagoya, Japan

(Received February 21, 2012/Revised March 25, 2012/Accepted April 20, 2012/Accepted manuscript online April 27, 2012/Article first published online June 21, 2012)

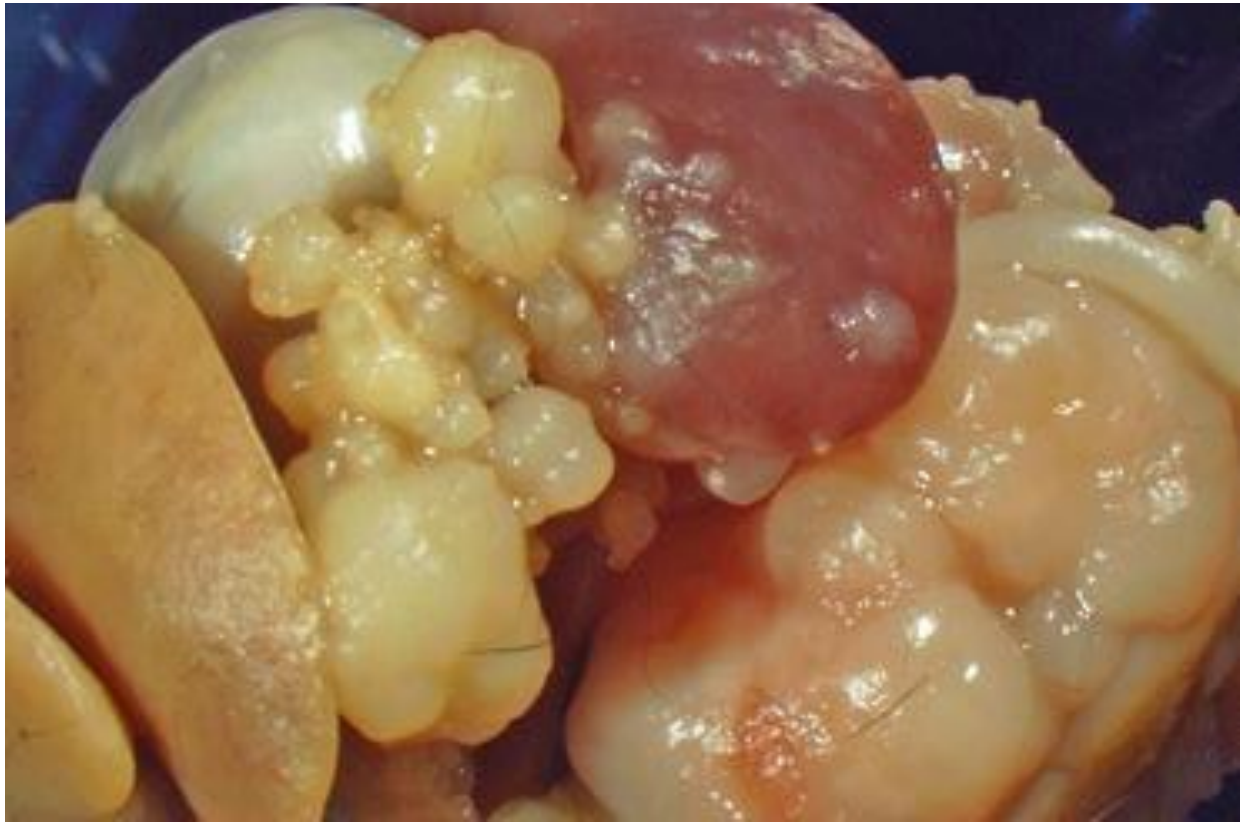
Three doses

300 μg/animal = $1x10^8$ fiber /animal
30 μg/animal = $1x10^7$ fiber /animal
3 μg/animal = $1x10^6$ fiber /animal



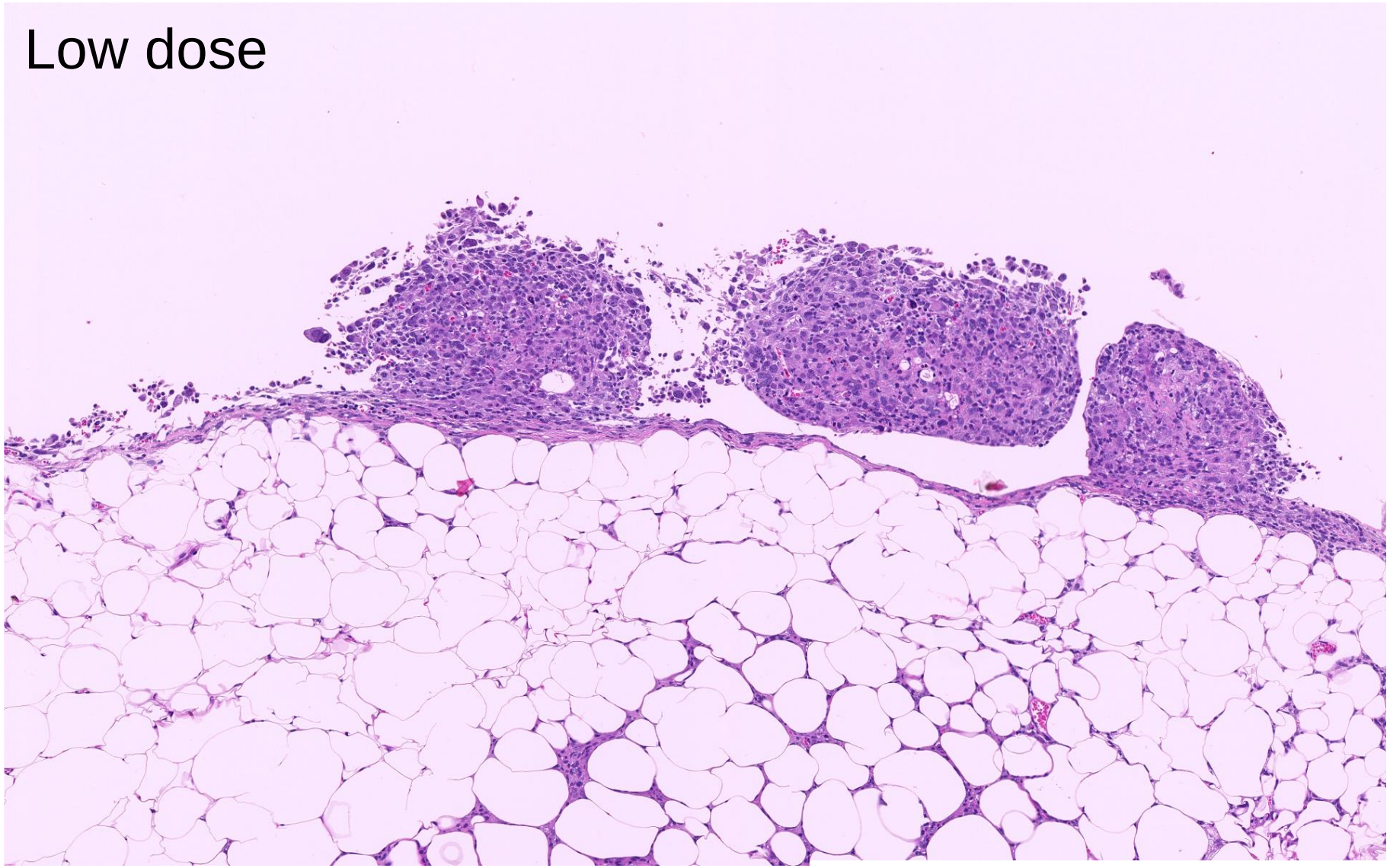
Low dose group #19

3 μg/animal = 1/1,000 of the first study



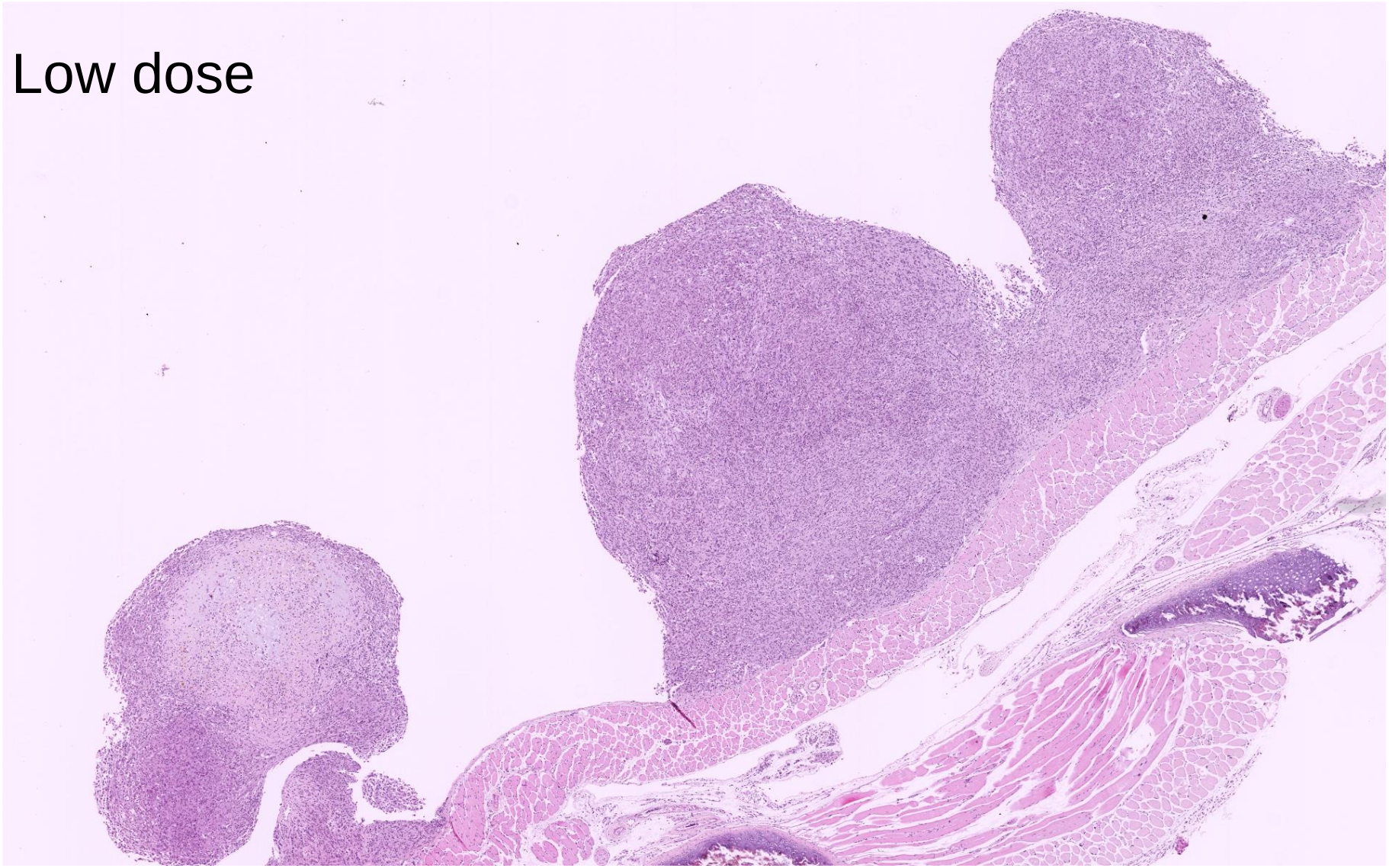
Takagi et al. Cancer Science, 2012

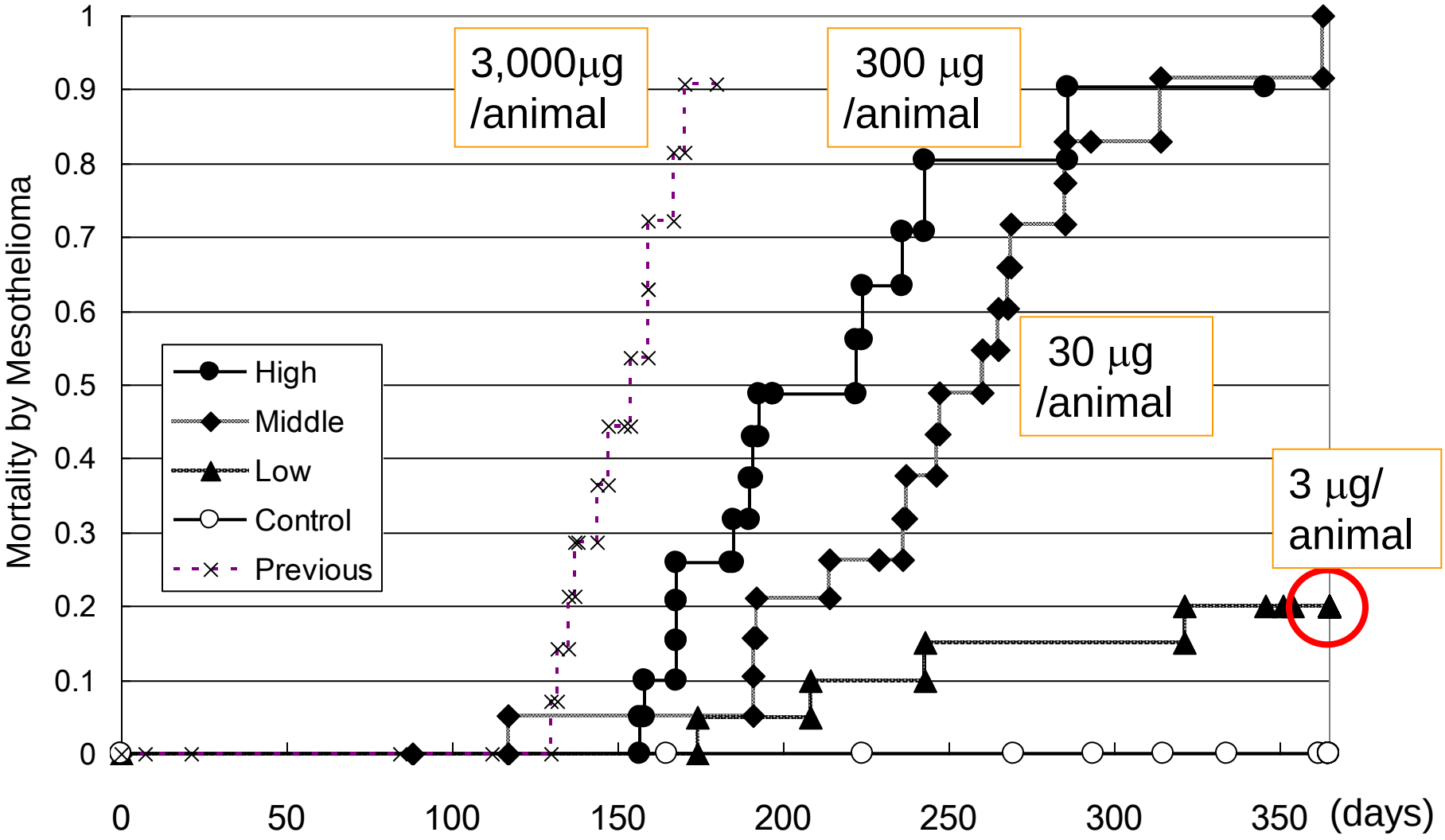
Low dose



Takagi et al. Cancer Science, 2012

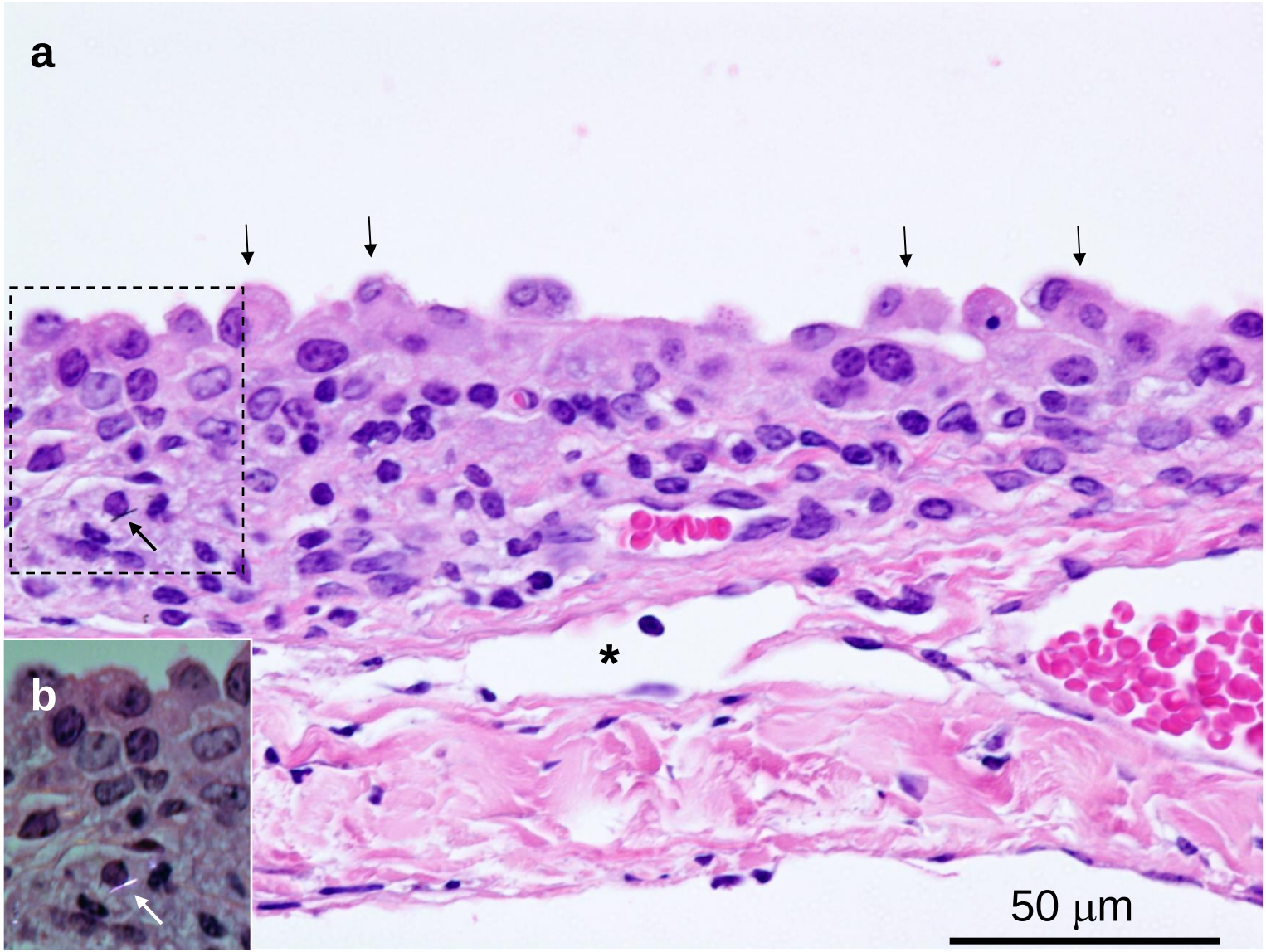
Low dose





Takagi et al. Cancer Science, 2012

Figure 3

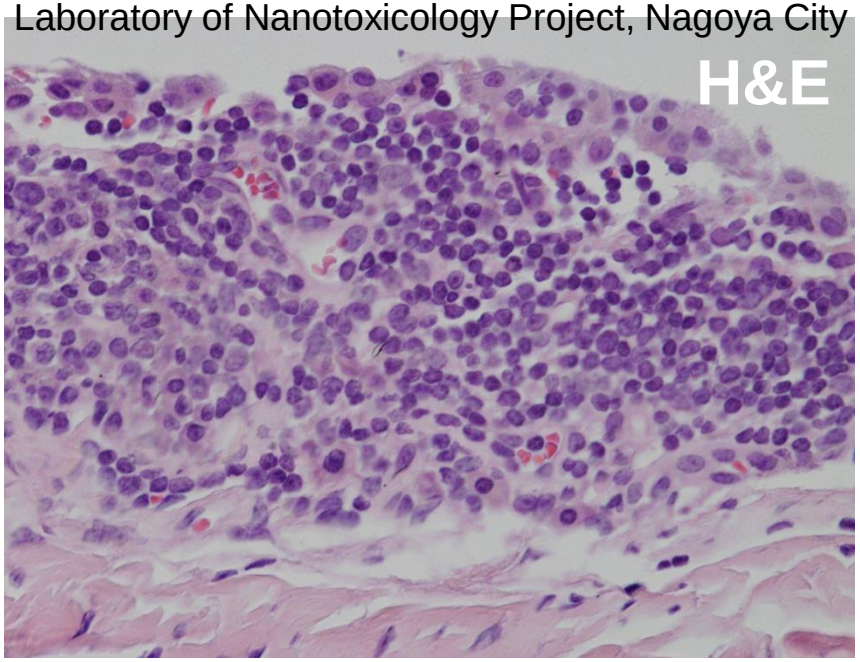


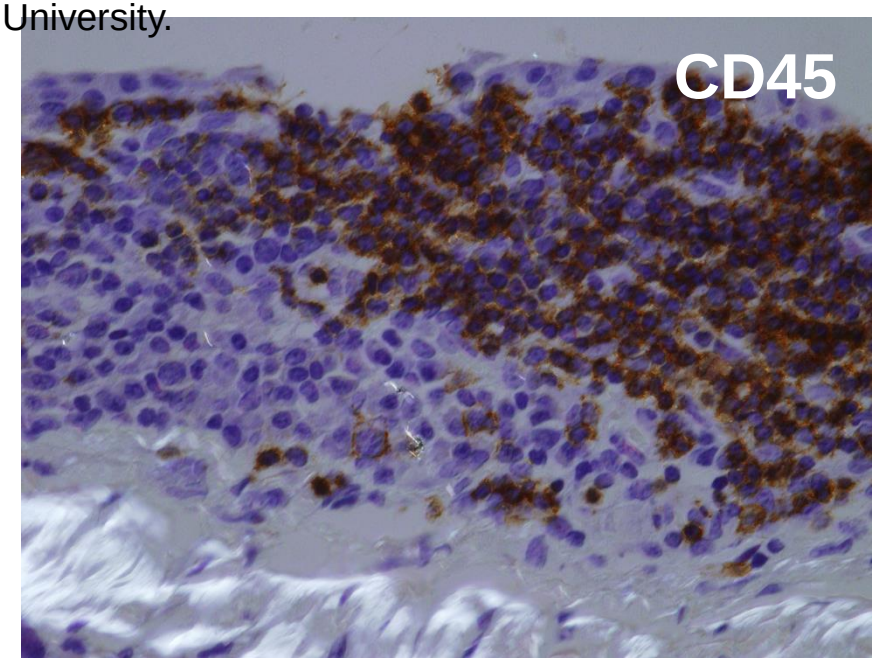
Takagi et al. Cancer Science, 2012

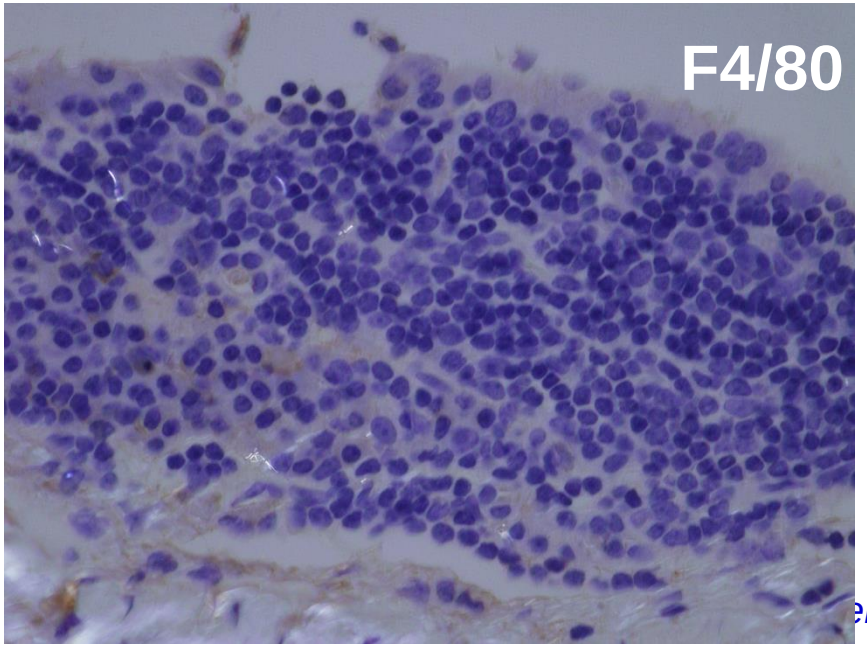
Collaboration: Drs. Mitsuru Futakuchi and Hiroyuki Tsuda

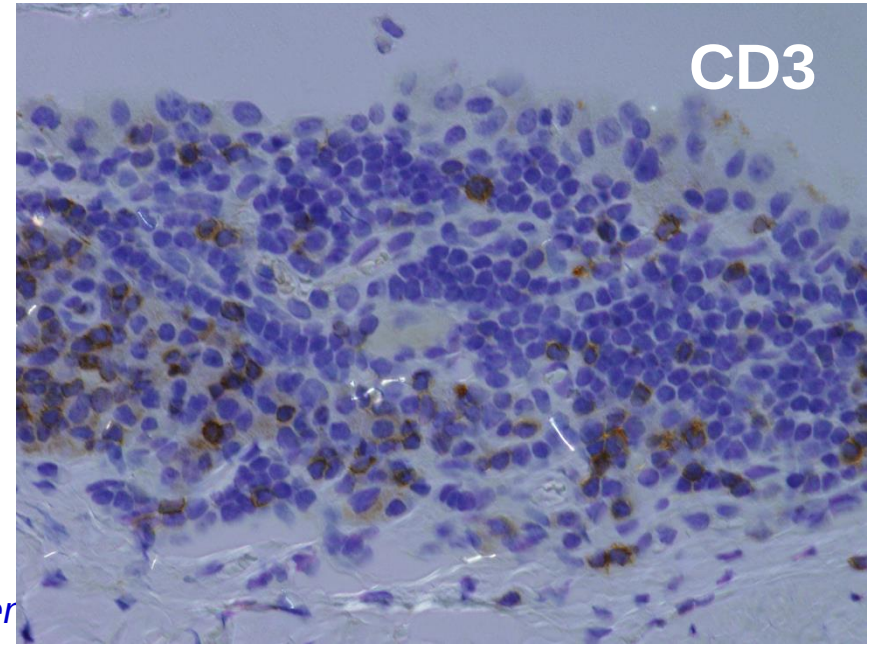
Department of Molecular Toxicology, Nagoya City University Graduate School of Medical Sciences

Laboratory of Nanotoxicology Project, Nagoya City University.









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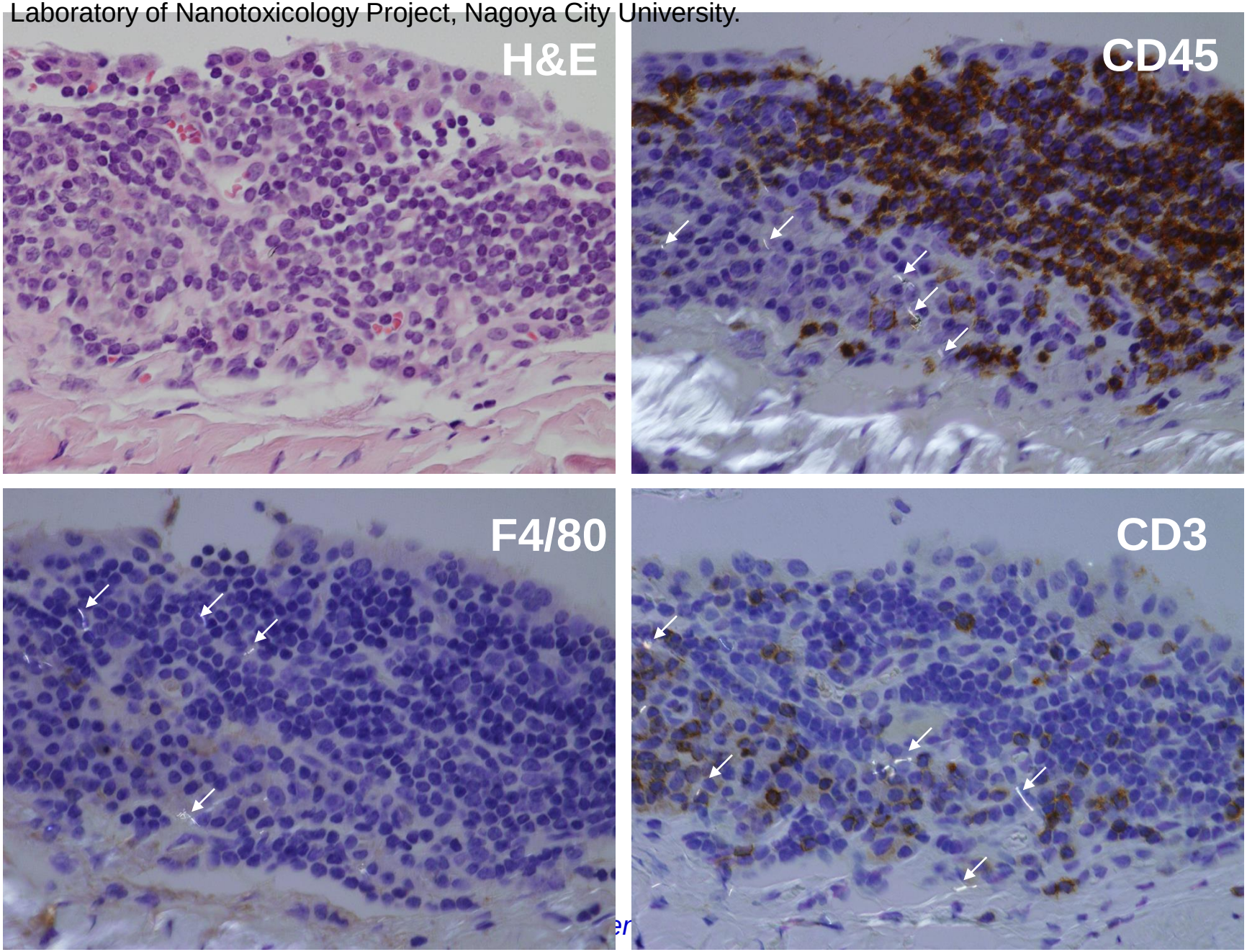
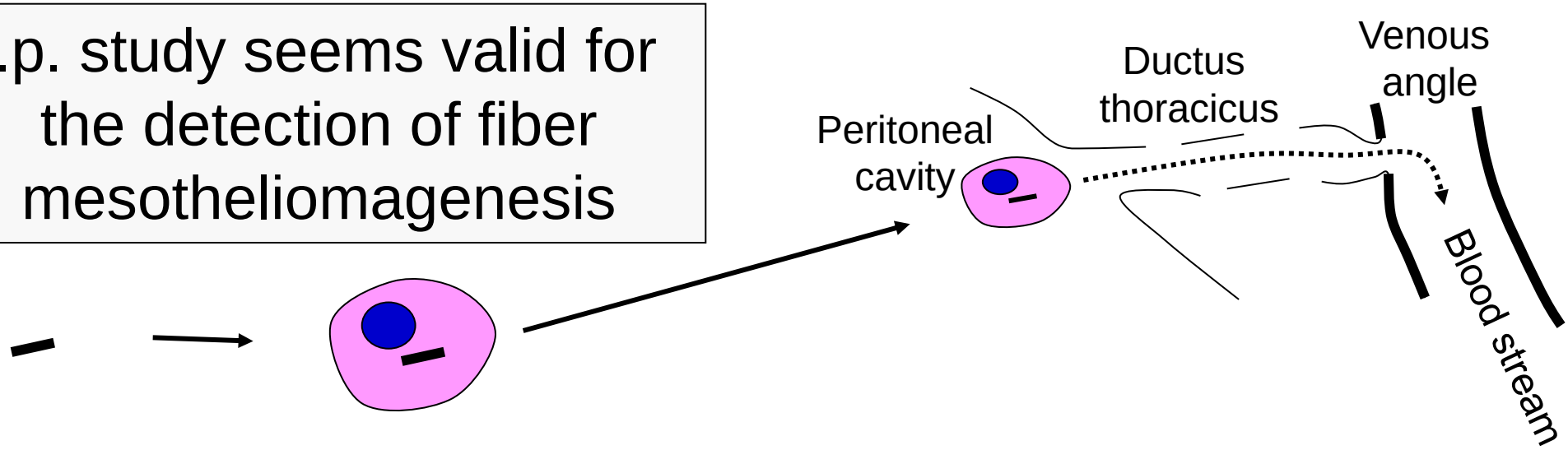
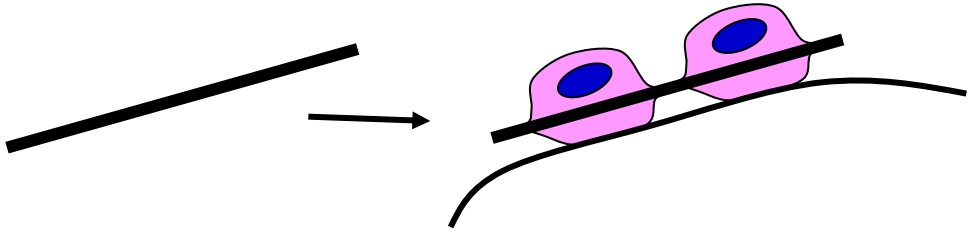


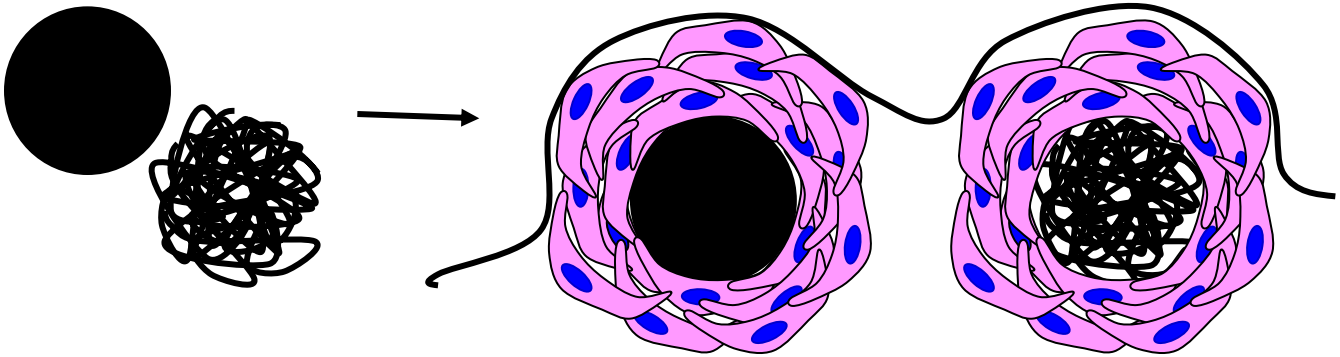
Takagi et al. Cancer Science, 2012

i.p. study seems valid for the detection of fiber mesotheliomagenesis





Mesotheliomagenesis
(frustrated phagocytosis,,,)

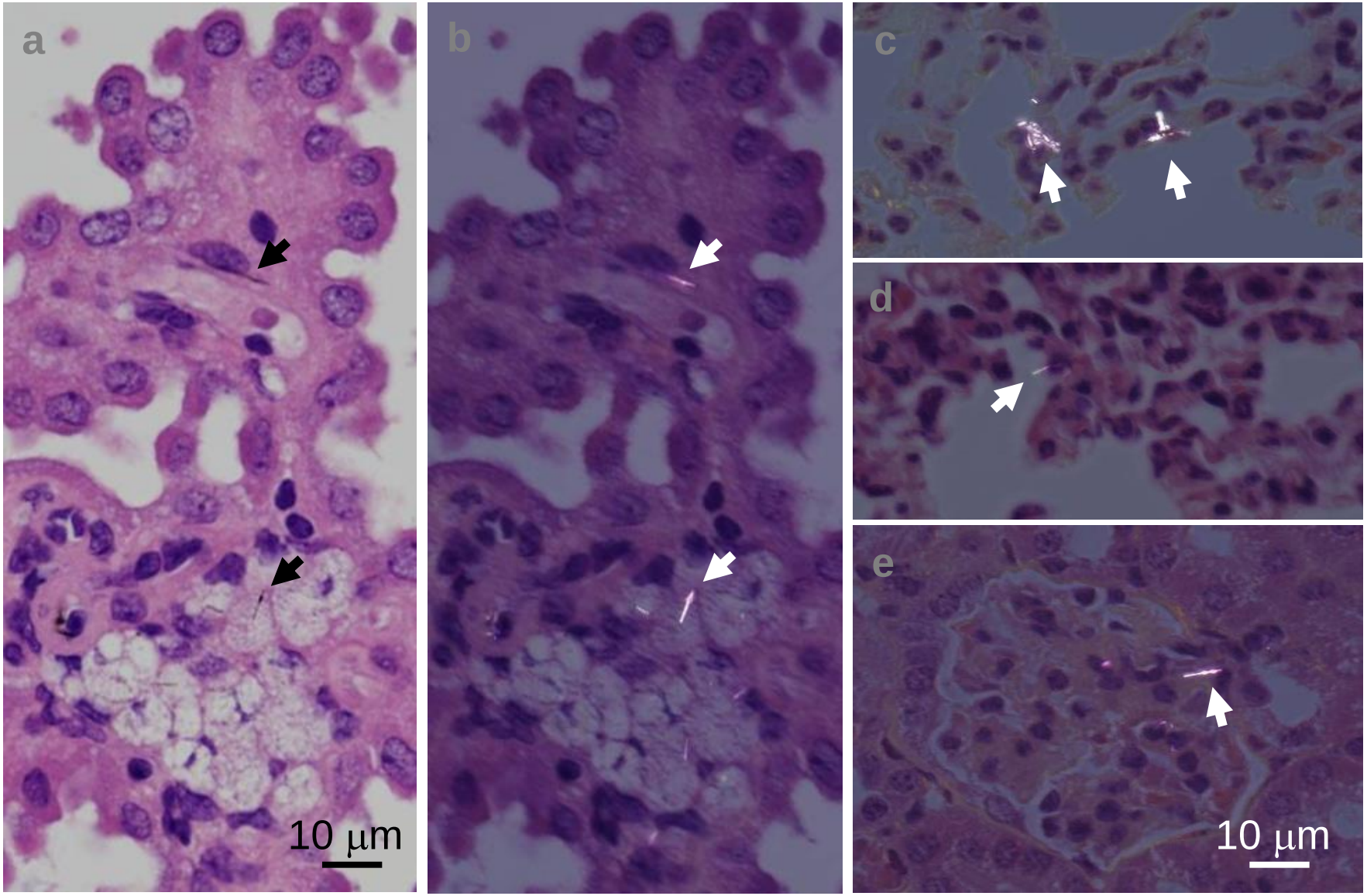


Segregation
(no tumor from this lesion)

Mesotheliomagenesis

- Morphology-based speculation
  - A persistent chronic inflammatory lesion on/ near the surface of mesothelium consisting of fiber-laden macrophage and mononuclear cell accumulation is important
  - Foreign body giant cells, epithelioid cell granuloma, and scar formation are not directly related to mesotheliomagenesis (segregation mechanism).
  - Narrow definition of Frustrated Phagocytosis; persistent single cell phagocytosis that does not lead to giant cell formation, epithelioid cell granuloma formation nor local scar formation.

Unknown (not fully tested yet) is the Chronic Toxicity of Systemic MWCNT Dissemination.



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- Asbestos (biopersistent fiber; mesothelioma/ lung carcinoma)
- Thorotrast (3-10 nm-sized biopersistent thorium dioxide particles; reticuloendothelial system (RES) deposition with in vivo half life of 22~400 years)
- Welding smoke and cardiovascular diseases (heart, brain)

Biodegradation of C_{60} Fullerene Nanowhiskers by Macrophage-like Cells

SHIN-ICHI NUDEJIMA, KUN'ICHI MIYAZAWA
Fullerene Engineering Group, Exploratory Nanotechnology Research Laboratory
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JUNKO OKUDA-SHIMAZAKI, AKIYOSHI TANIGUCHI
Advanced Medical Material Group, Biomaterials Center
National Institute for Materials Science (NIMS)
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JAPAN
NUDEJIMA.Shinichi@nims.go.jp http://www.nims.go.jp/fullerene/index/index.ht

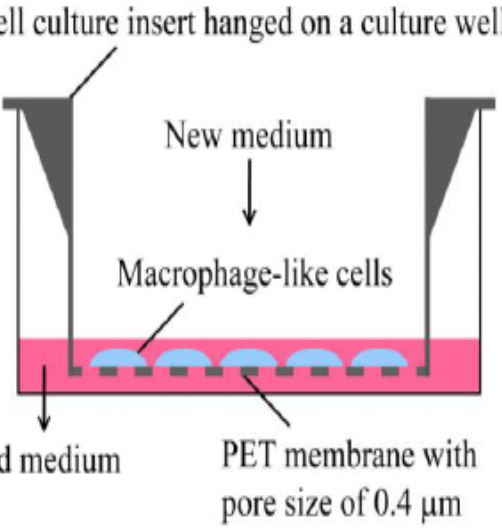
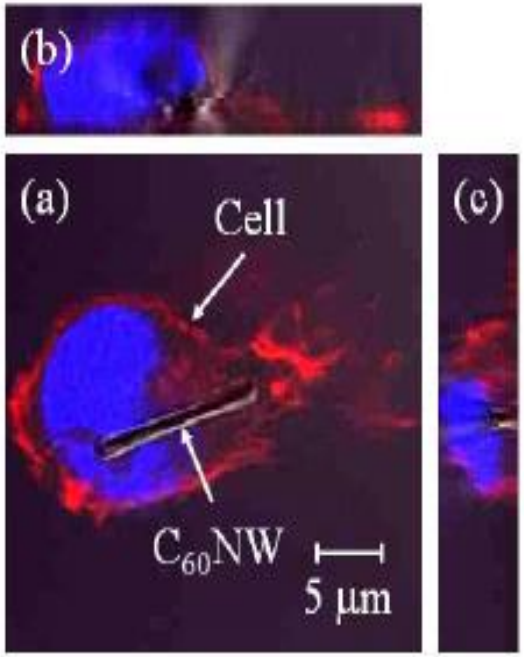


Fig.1. Macrophage-like cells were cultivated on a PET membrane with C_{60}NWs.



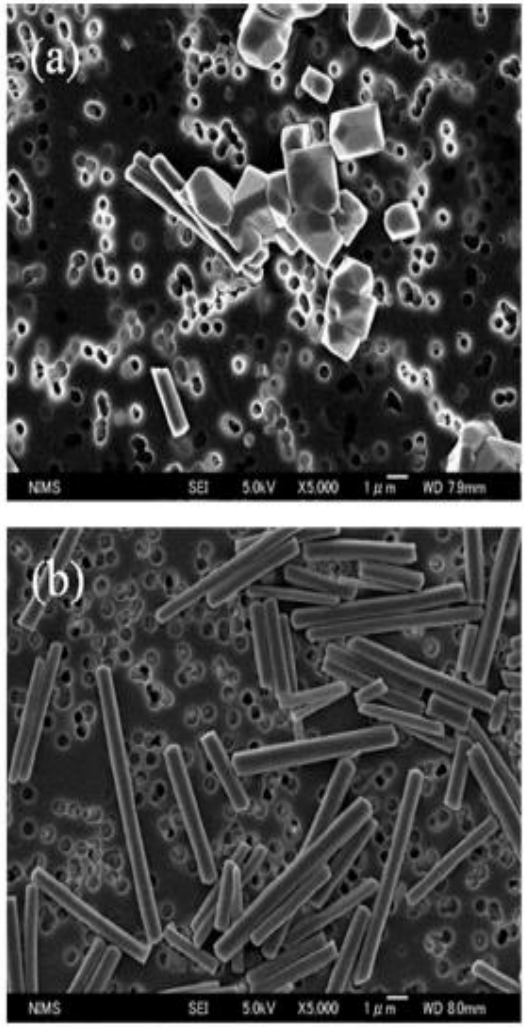
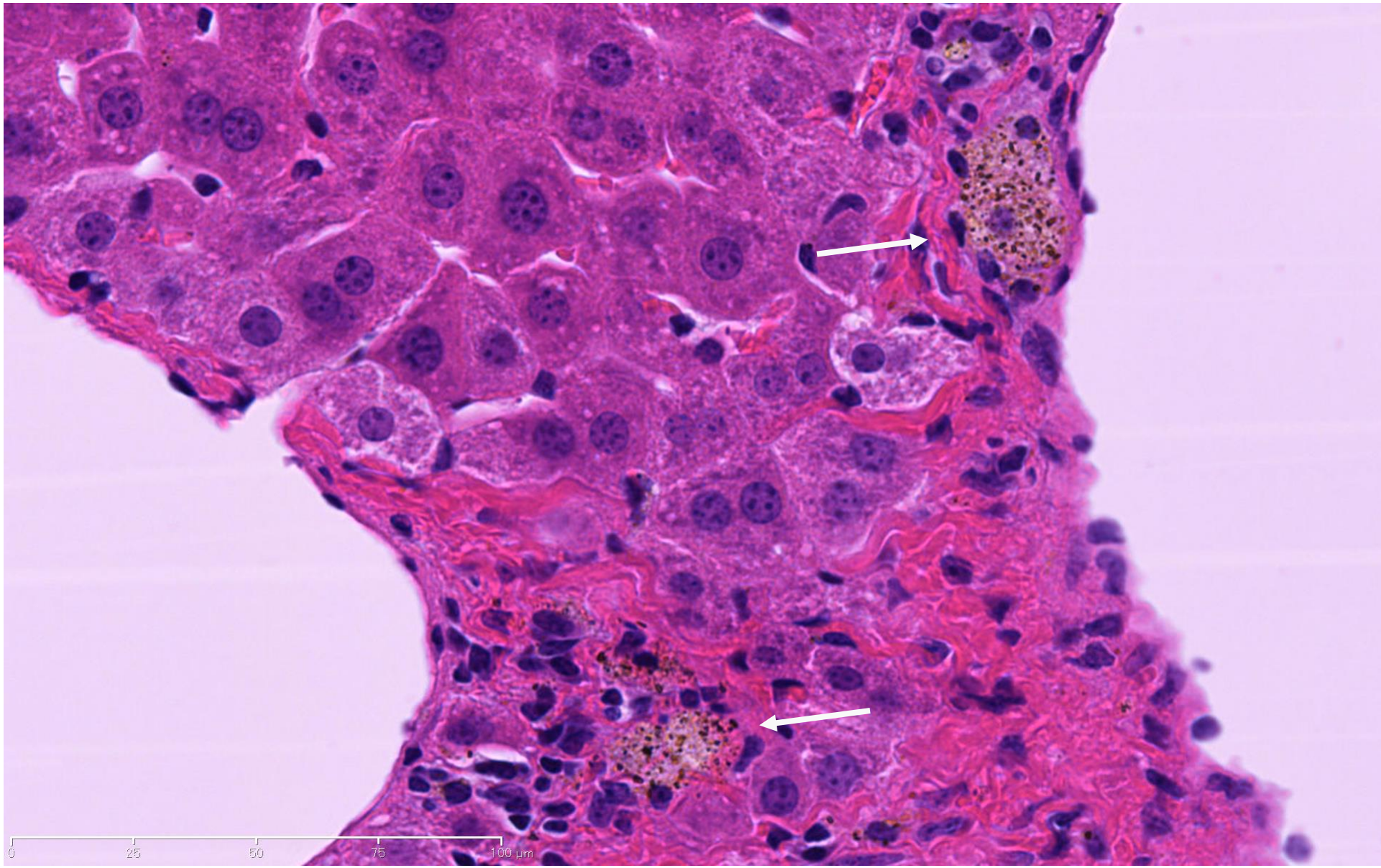
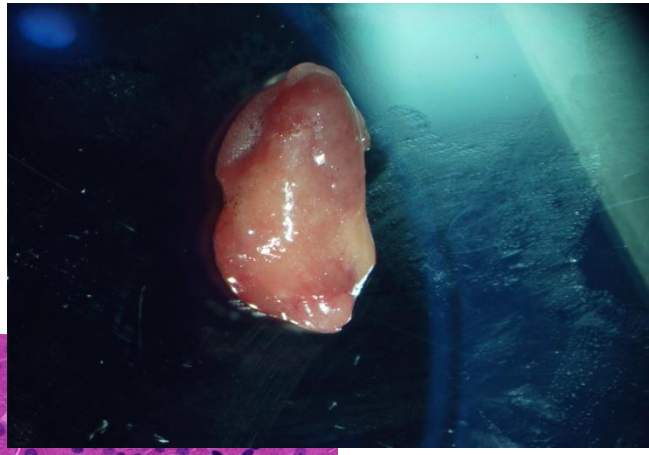


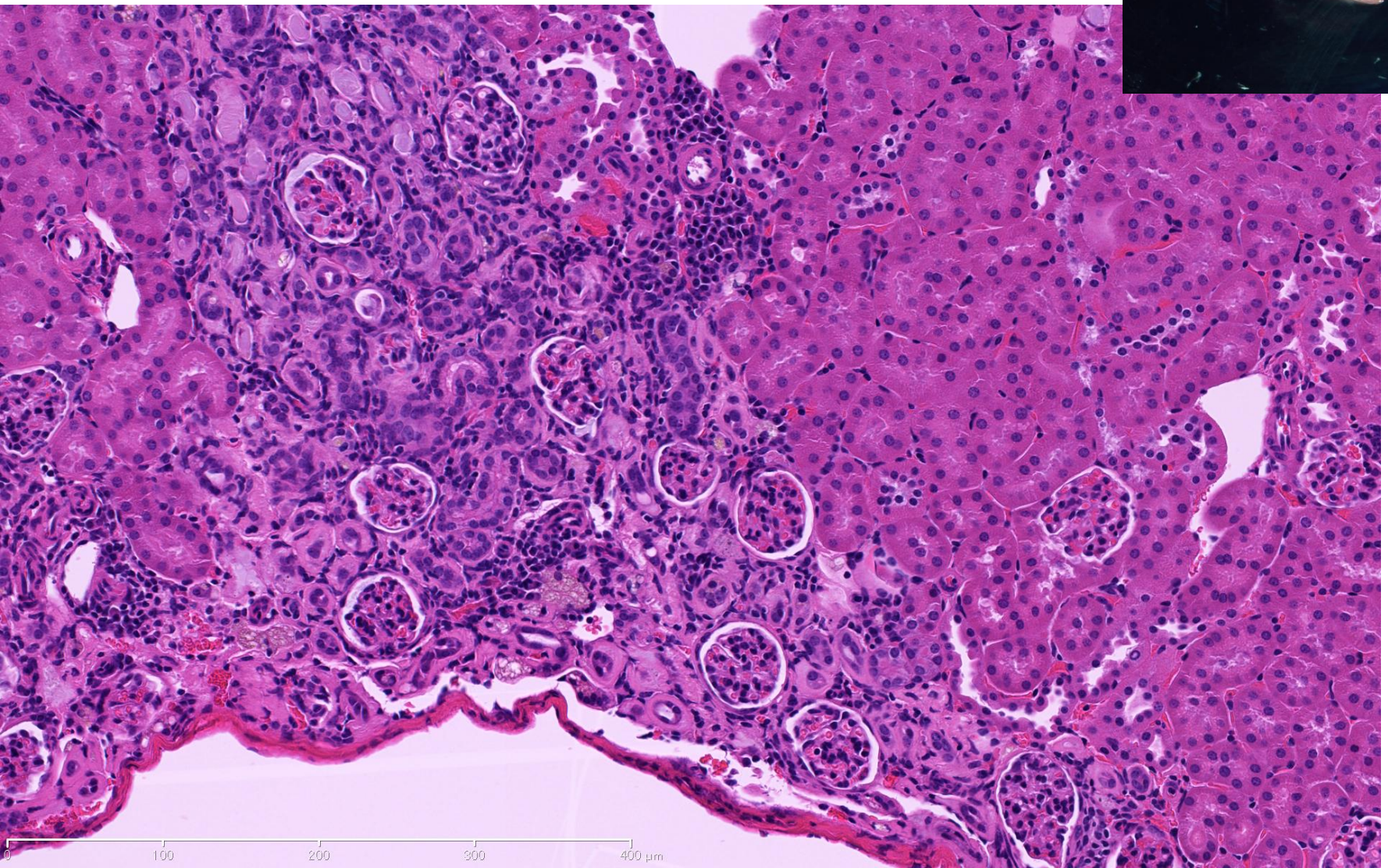
Fig.7. SEM images of the substances on the cell culture insert after the 28 days' exposure of (a) the macrophage-like cells and (b) the culture medium to C_{60}NWs.



Unpublished data

Enhanced renal toxicity:
Renal toxin (methyl cellulose) + C_{60}





Unpublished data

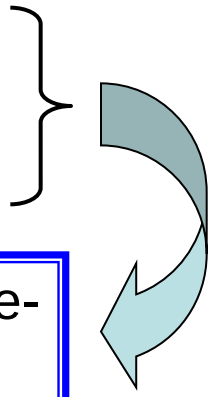
Proposed study direction

1. Known mechanism
   - fiber carcinogenesis - intraperitoneal injection model
   - systemic distribution - i.p. model = i.v. with a filter

---

2. Unknown mechanism
   Animal experiment using human relevant route of exposure*

→ hazard identification
→ mechanism identification (assumption)
→ dose-response data in experimental animals



Assumption of human toxicity and dose-response characteristics

*: inhalation (whole body, intratracheal), dermal, oral

μm-MWCNT
Fullerene whiskers

μm-MWCNT
C_{60}
etc

Propo d study d

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   - systemic distribution - i.p. model

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   Animal experiment using human relevant route*

TiO_2
ZnO
μm-MWCNT
Shorter MWCNT
Other CNT
Nano Metals
Fullerene
whiskers
Etc.

→ hazard identification
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μm-MWCNT
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Propo d study o

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Etc.

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Assumption of human toxicity and dose-response characteristics

*: inhalation (whole body, intratracheal), dermal, oral

Case study: MWCNT (MWNT-7)

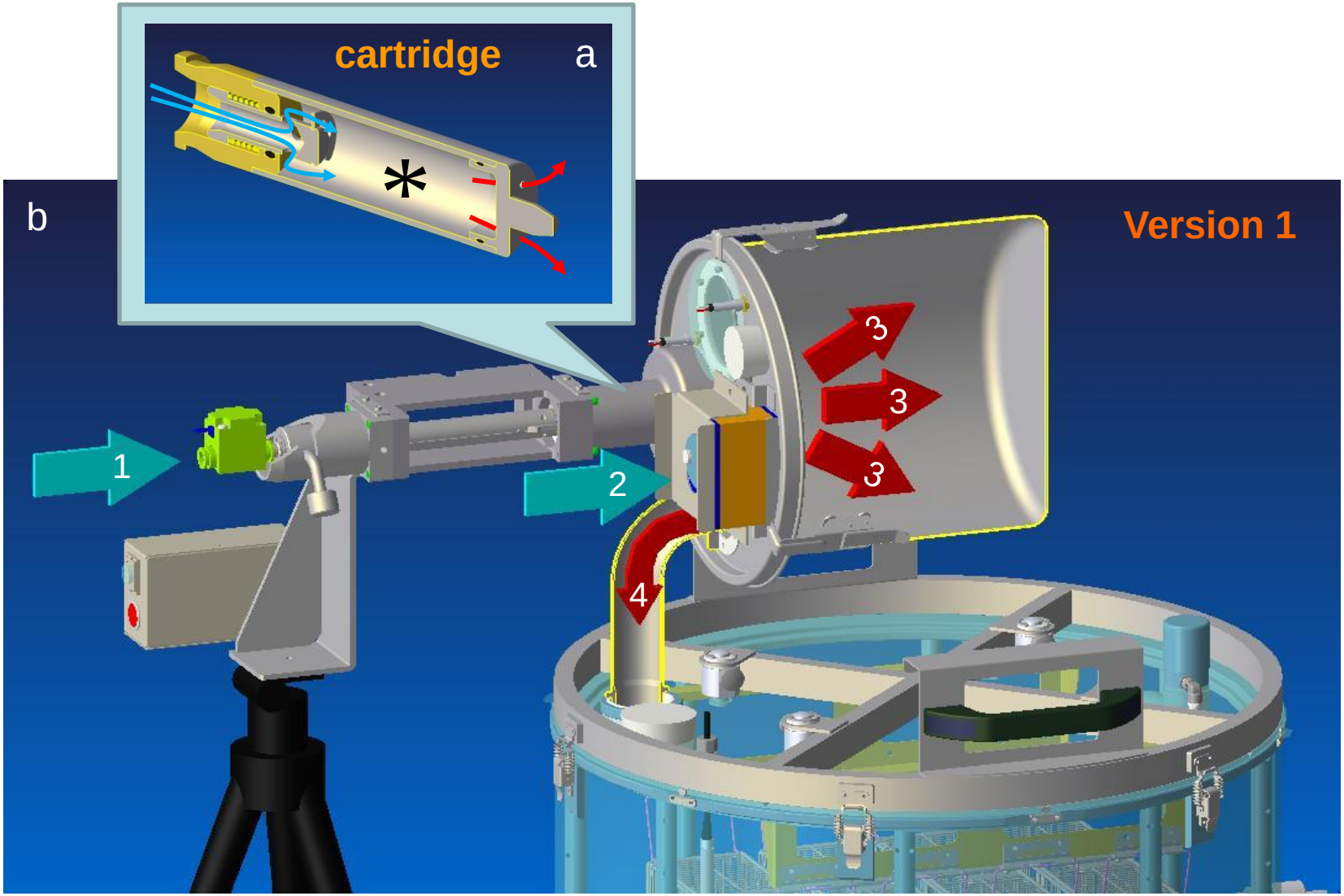
- Human environment allows to inhale dispersed single fibers
  - Low wind in human environment compared to constant rigorous agitation of air in animal chamber
  - Human upper respiratory tract is much longer than rodents'
- Alveolar lesions are important
  - In earlier rodent studies, aggregates/agglomerates induced lesions at proximal segments; masking distal lesions
- Preparing well-dispersed single fiber aerosol
  - Without dispersant
  - No change is size (length and width) of single particles

Taquann method (outline)

Based on two idea of

- Liquid phase dispersion and filtration using volatile dispersant.
- Critical point drying to avoid aggregation by surface tension.

Highly dispersed single MWCNT fibers can be produced in precise aliquots.

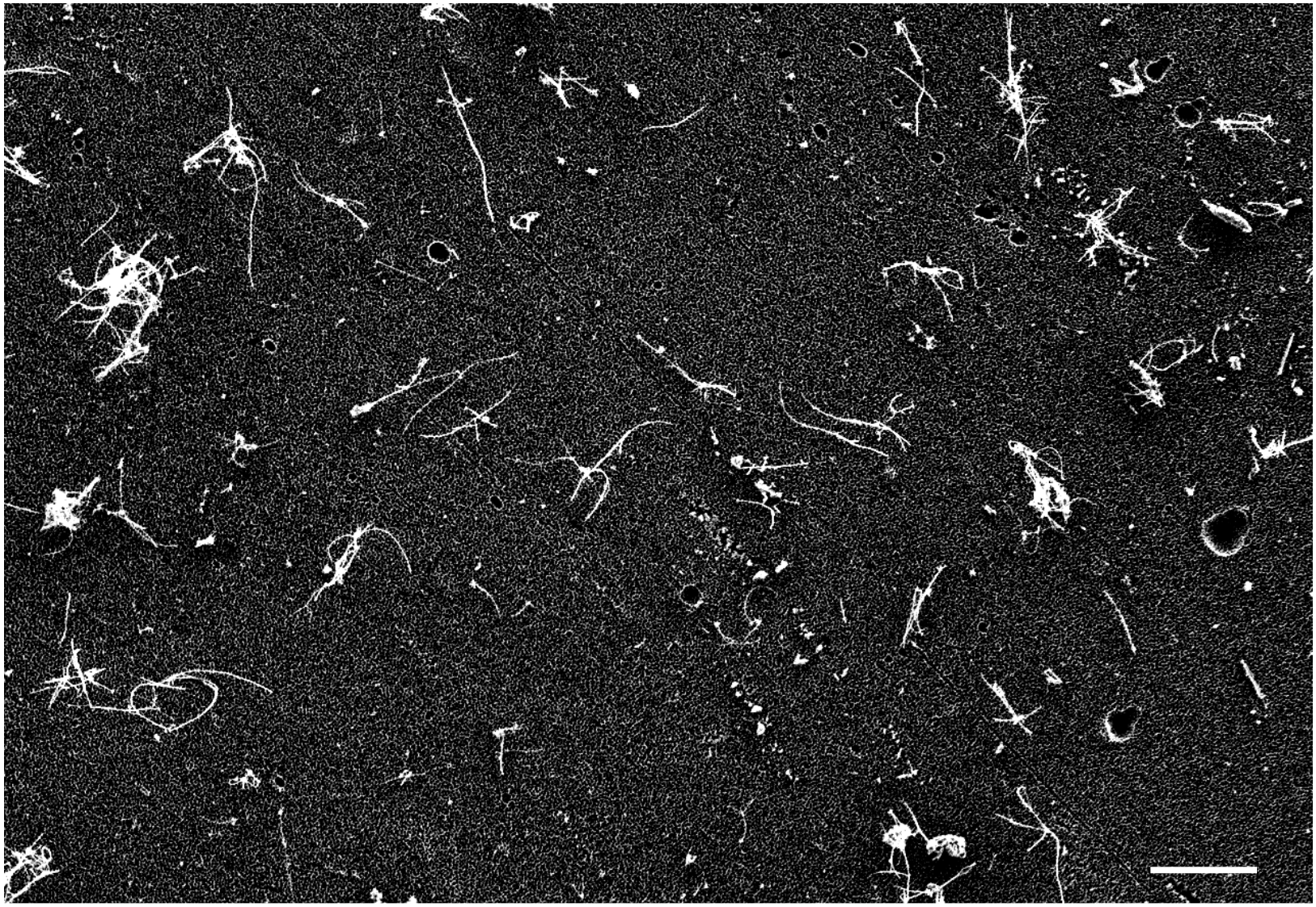


Taquahashi et al., J Tox Sci 38:619-628, 2013


Version 2
2013-10-25

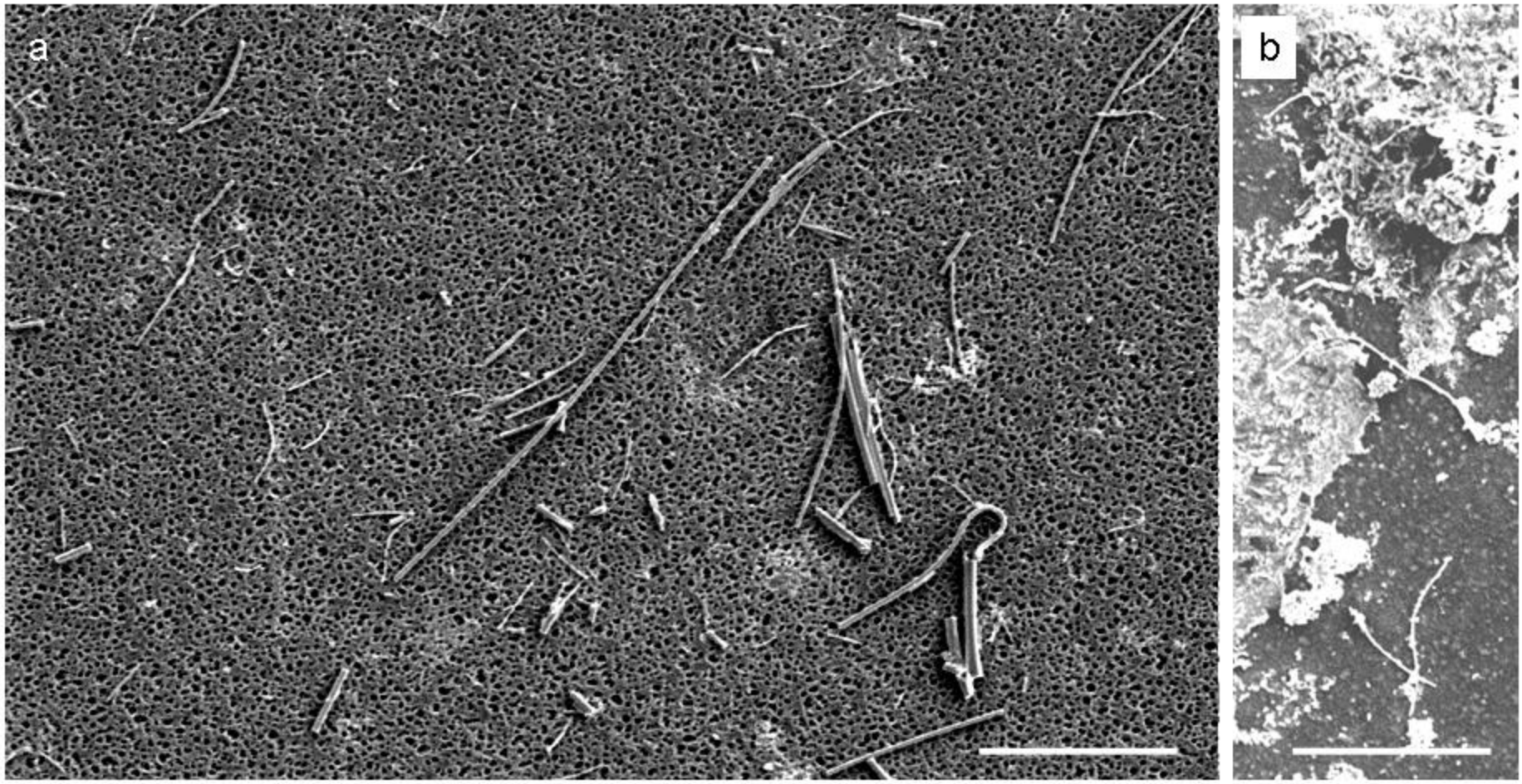
Chamber air sample

Taquahashi et al., J Tox Sci 38:619-628, 2013



5L/min for 3 minutes SEM x1,000 (scale bars are 10 um).

From the lysed lung



Taquahashi et al., J Tox Sci 38:619-628, 2013

On-going study: Taquann-Direct Injection System whole body MWCNT inhalation study (C57BL/6 p53 +/- male)

Group/ Exposure*	Conc.			Sampling				
	mg/m^3		Animal no.	0D	13W	26W	39W	52W
Control 0 μg/cartridge	0	Pathology	48	3	7	7	8	8
		Burden		3	3	3	3	3
Taquann L 250 μg/cartridge	1	Pathology	48	3	7	7	8	8
		Burden		3	3	3	3	3
Taquann H 500 μg/cartridge	2	Pathology	48	3	7	7	8	8
		Burden		3	3	3	3	3

*: 2hr exposure per week for 5 weeks (total 10 hr)

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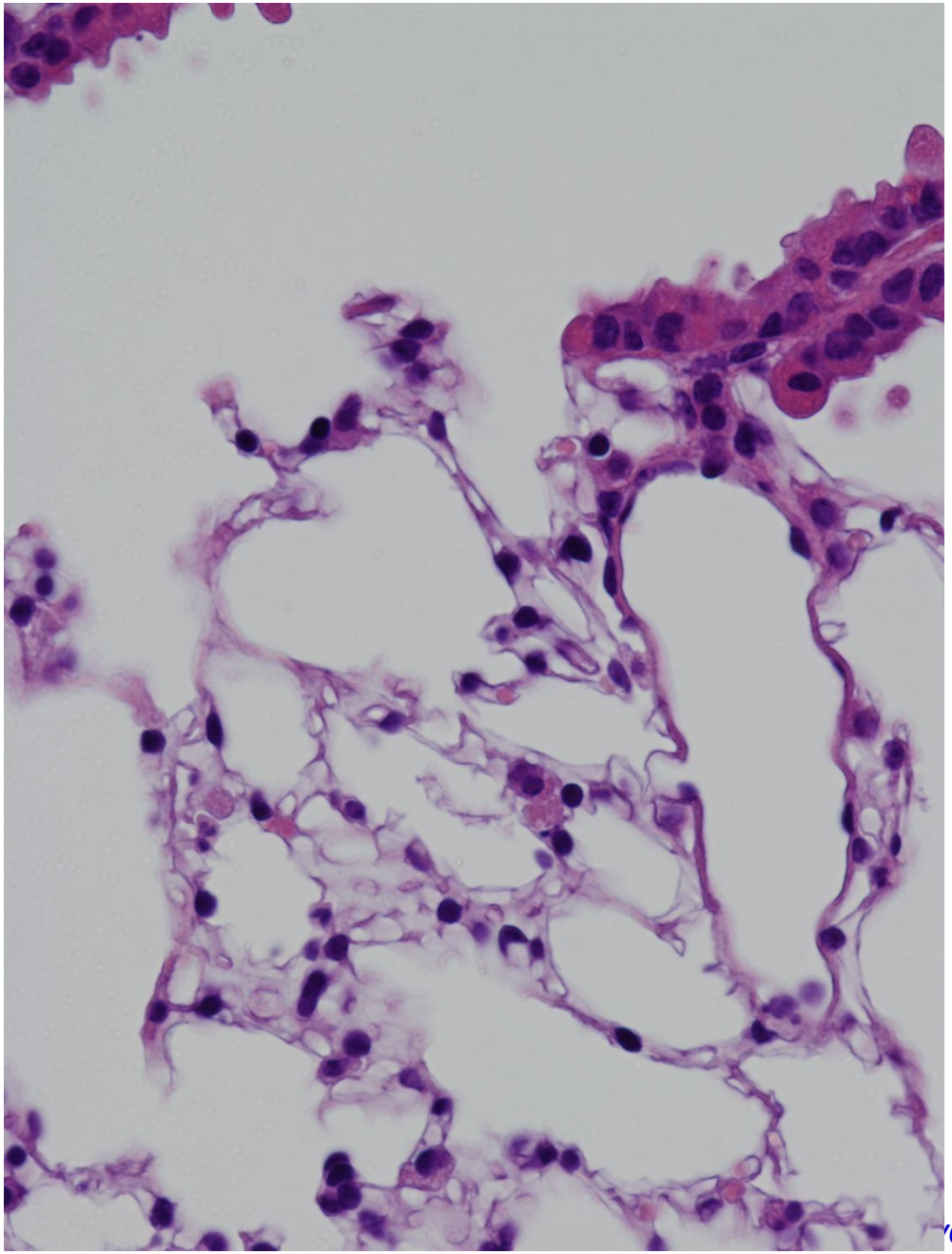
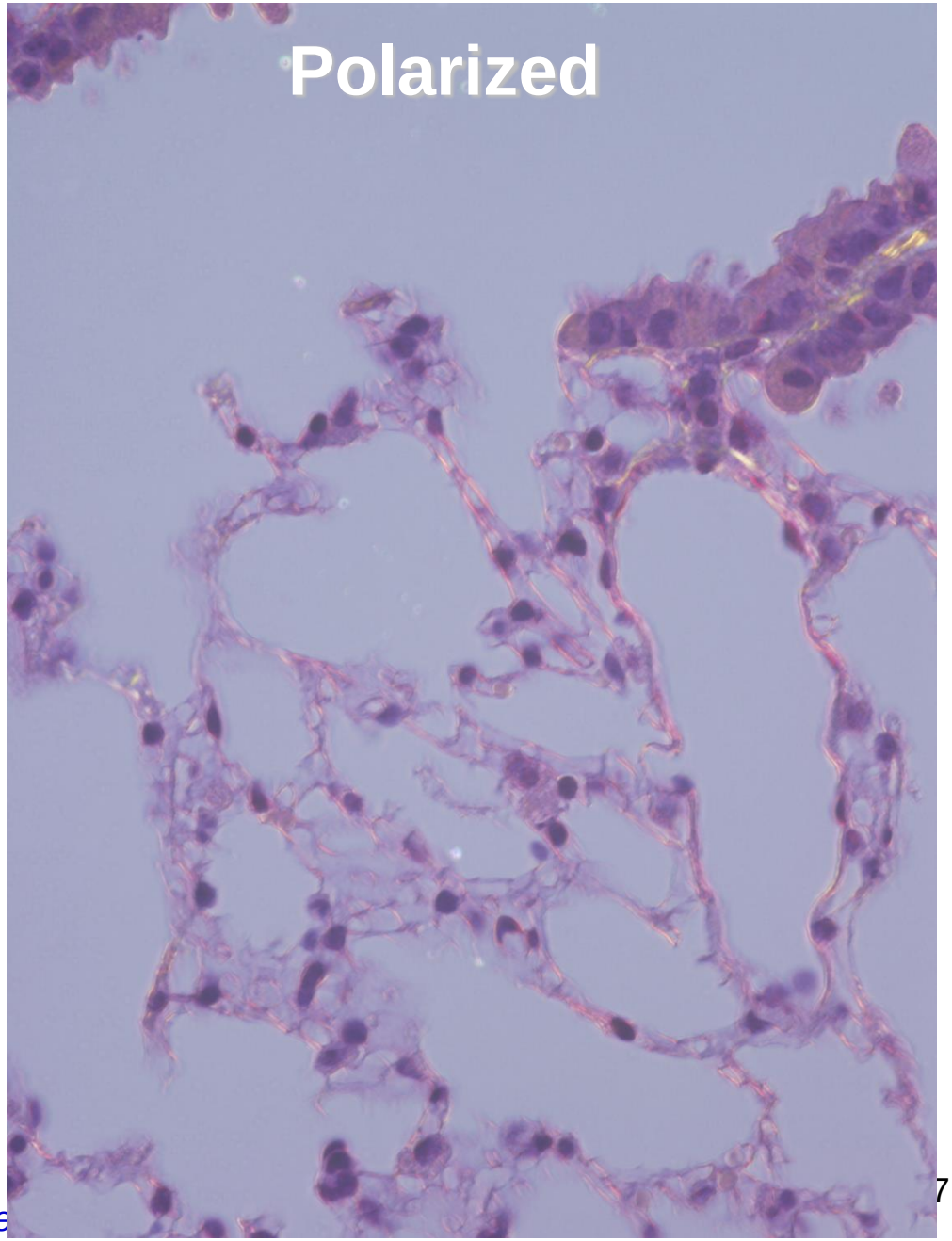
		Burden		3	3	3	3	3

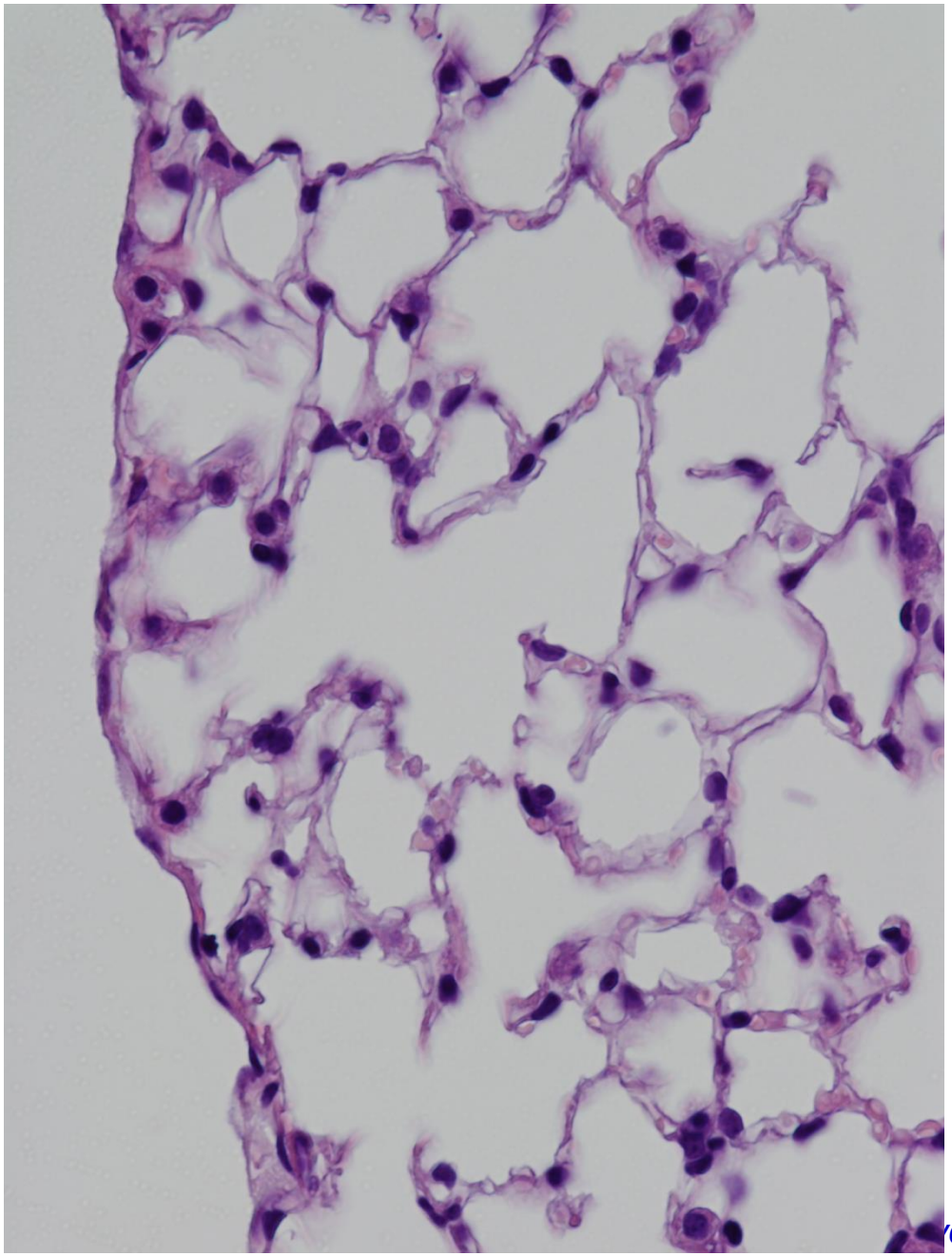
*: 2hr exposure per week for 5 weeks (total 10 hr)

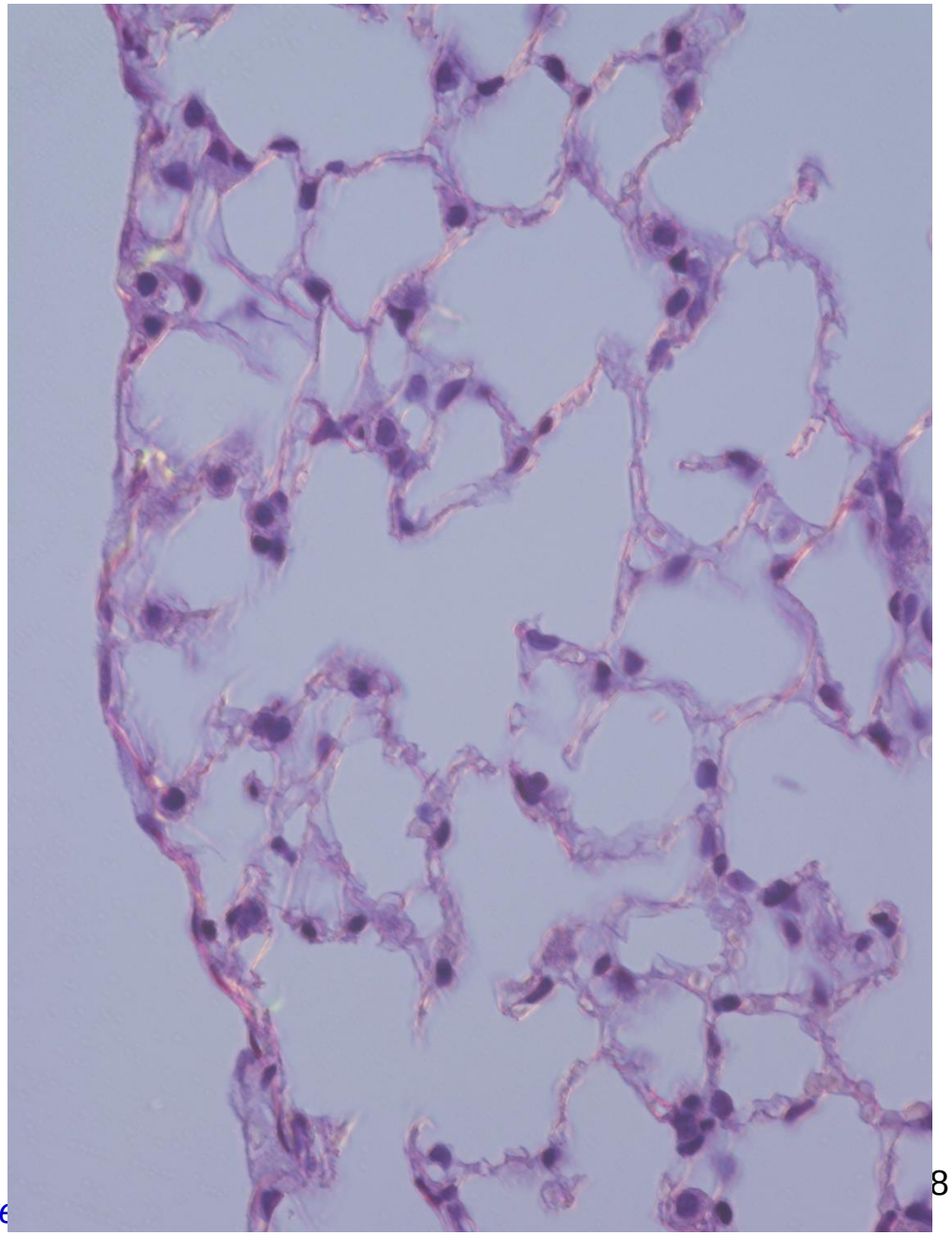
Control



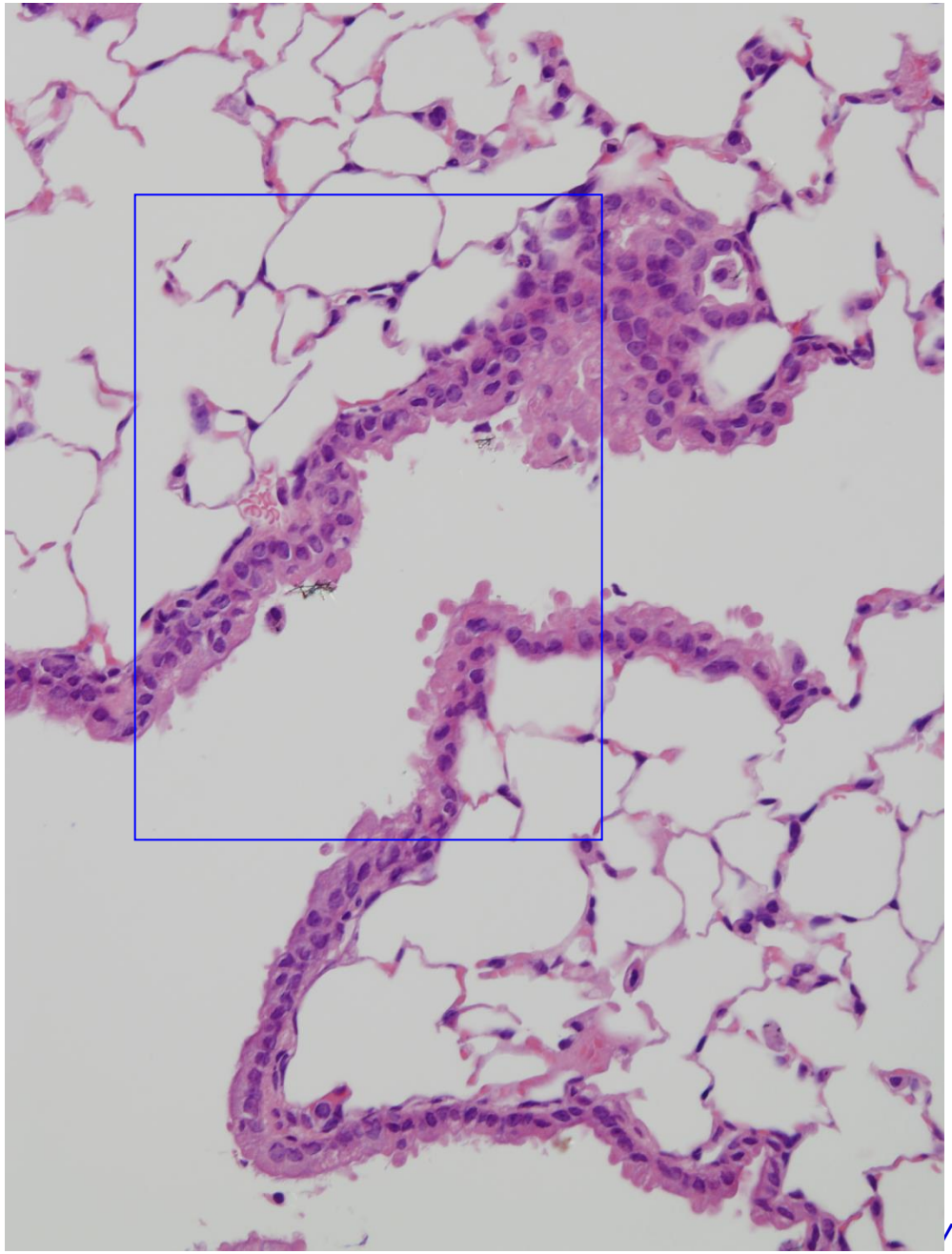


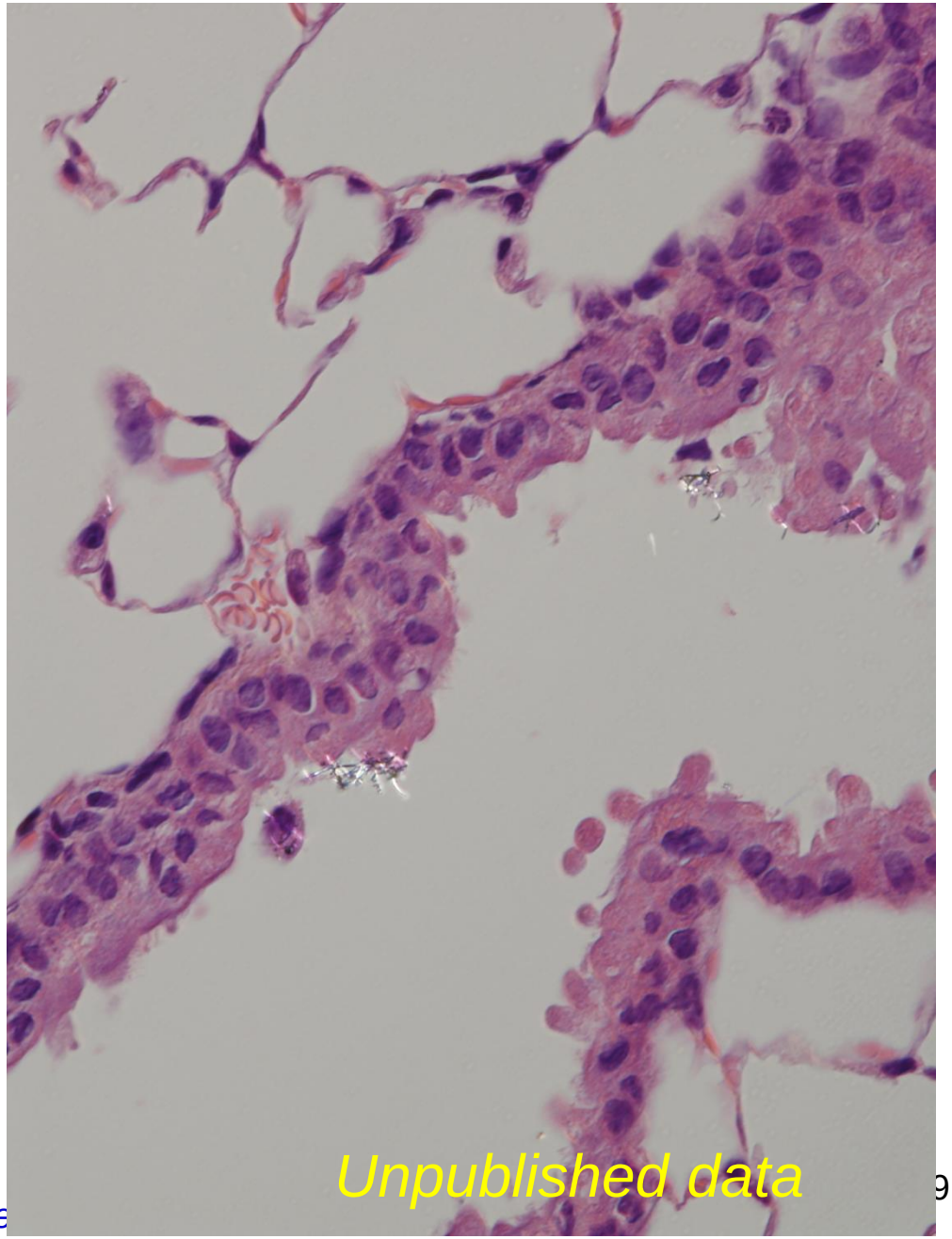
Control





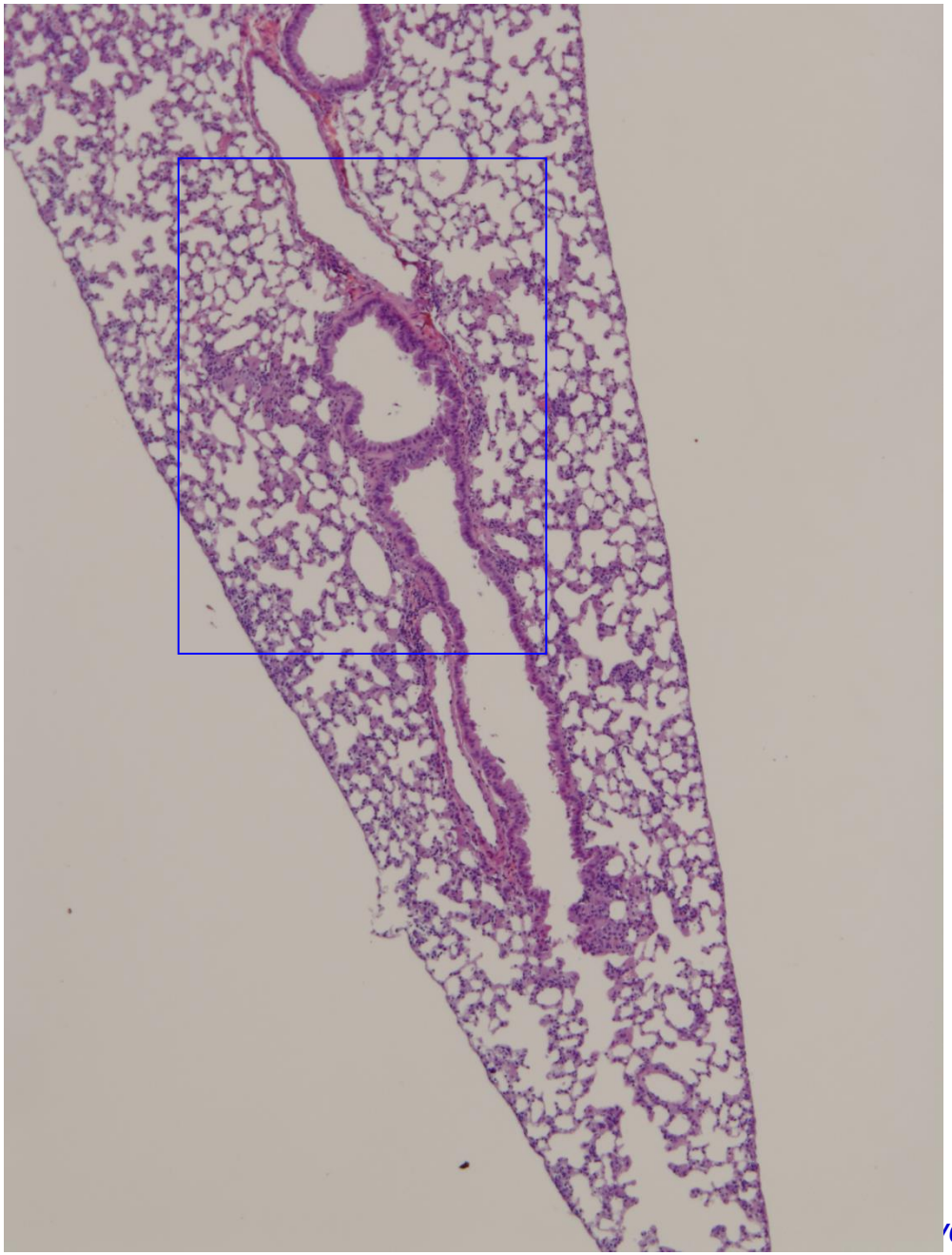
High Day 0

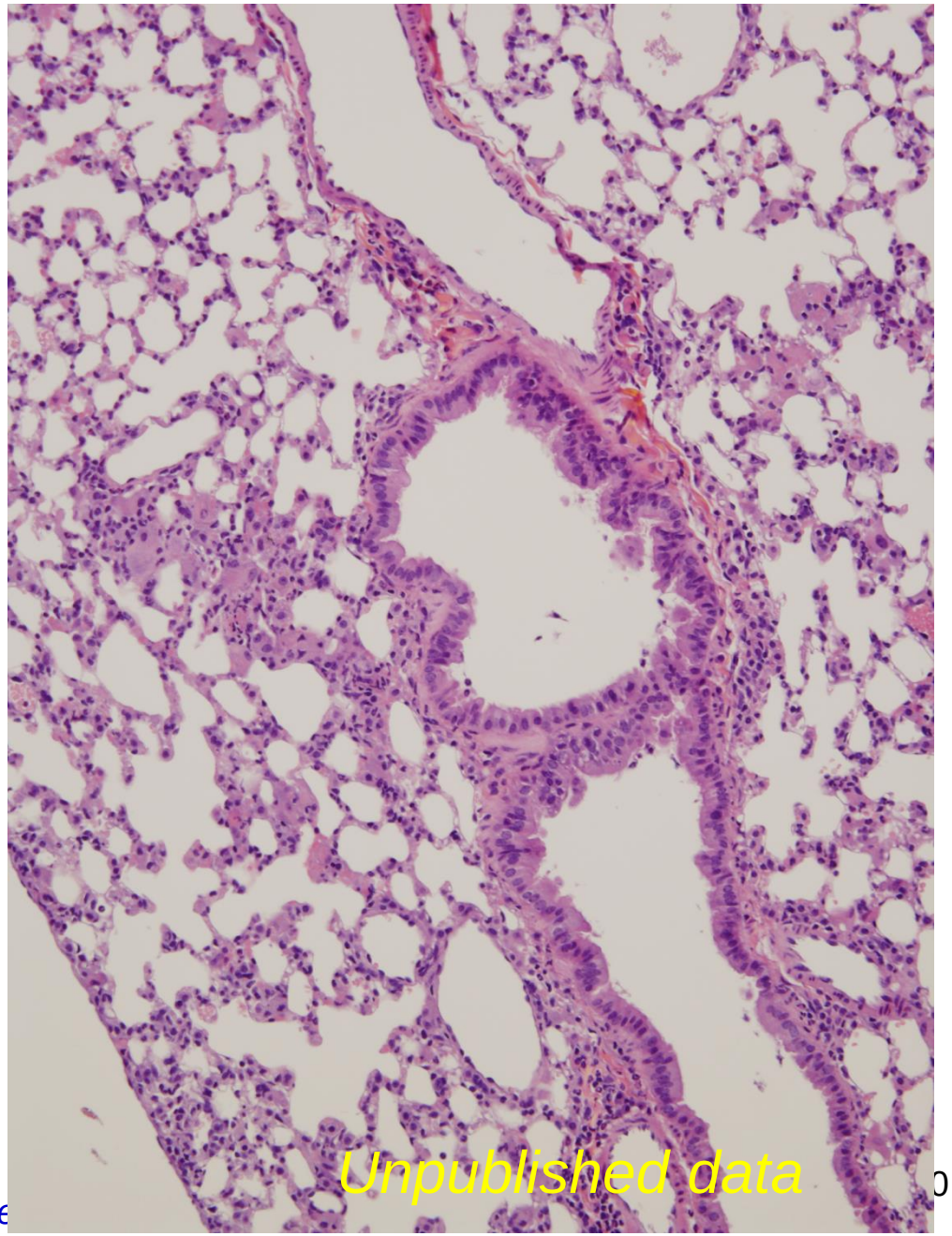




NANO-AP_13W_High_162

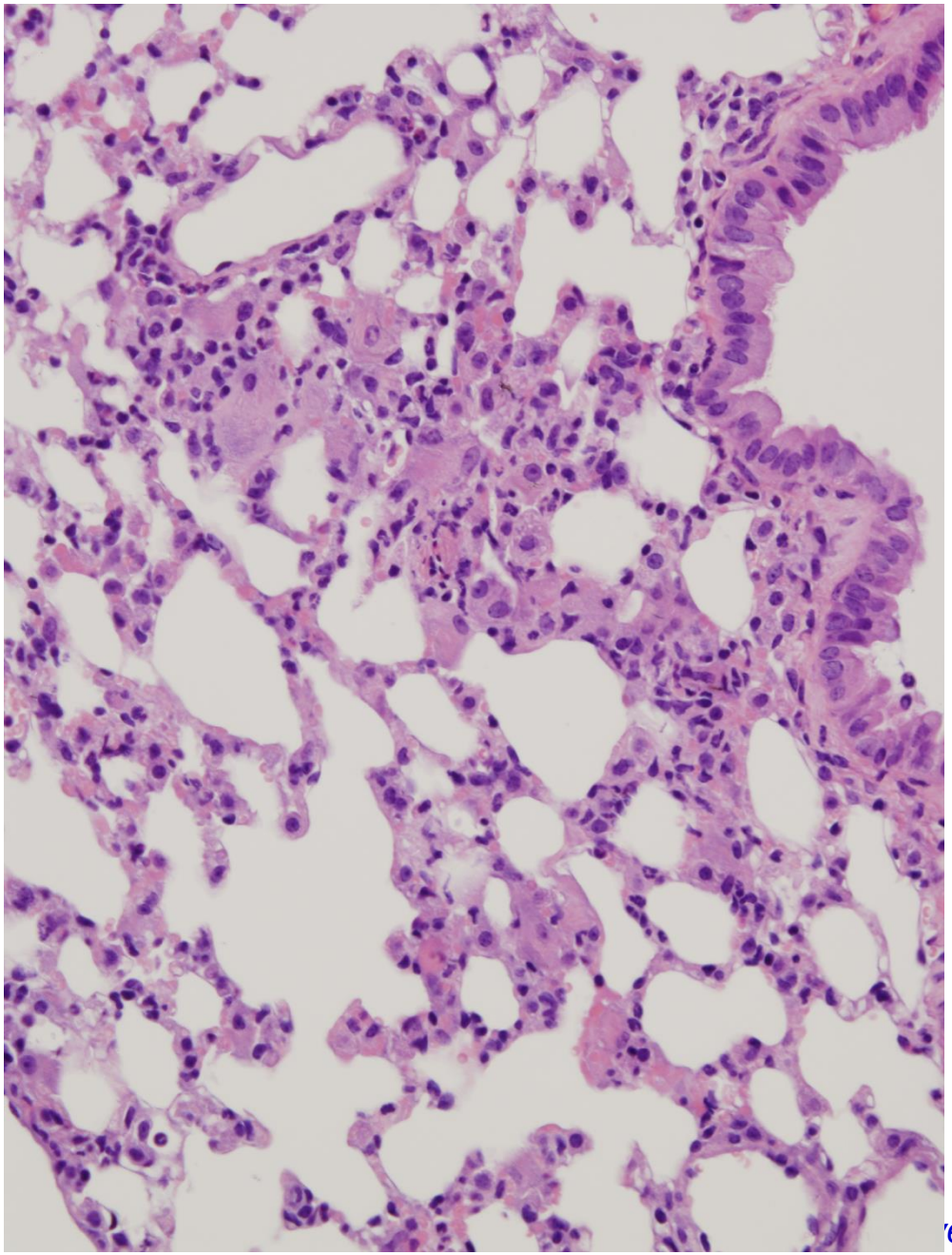
High 13W

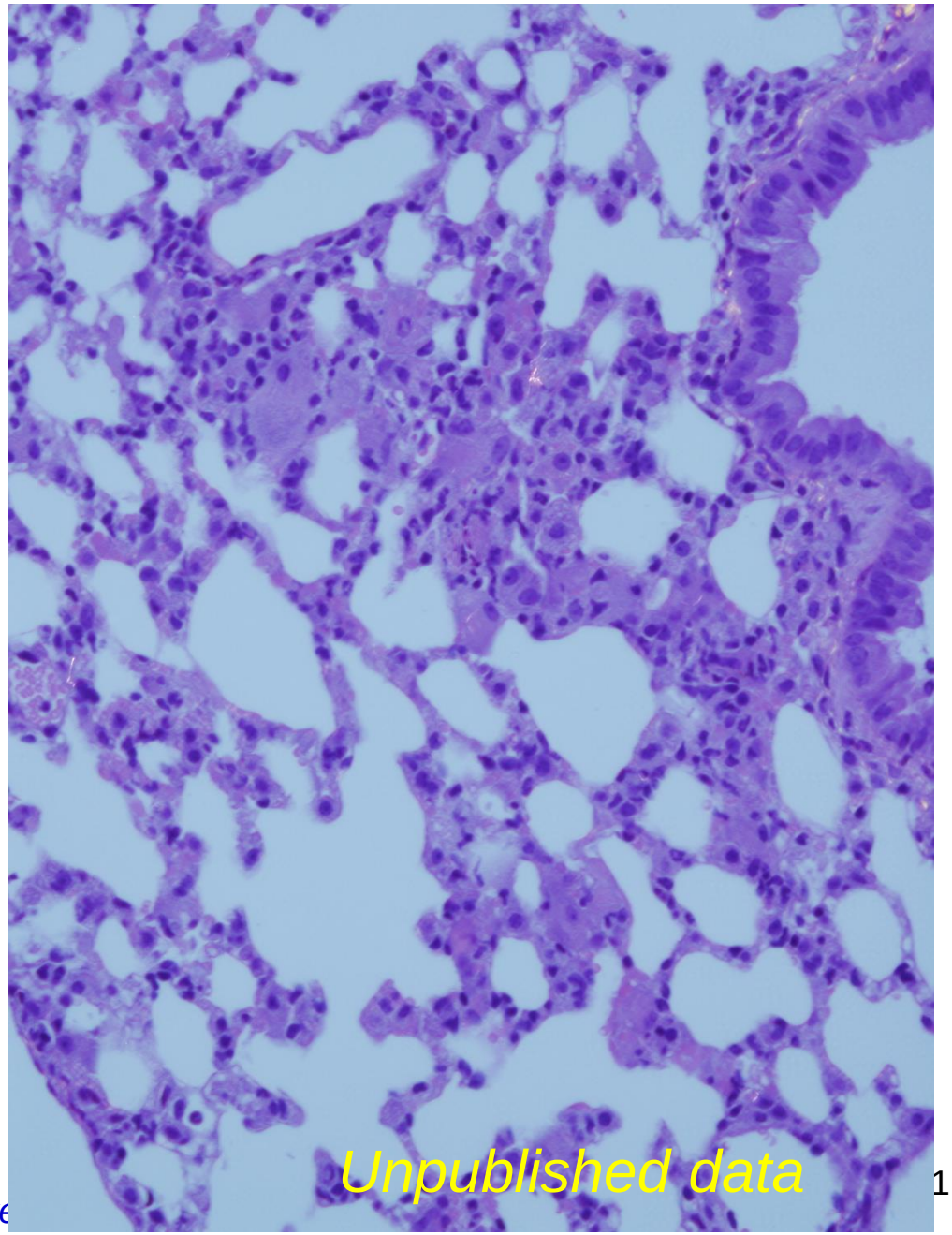




Unpublished data

High 13W





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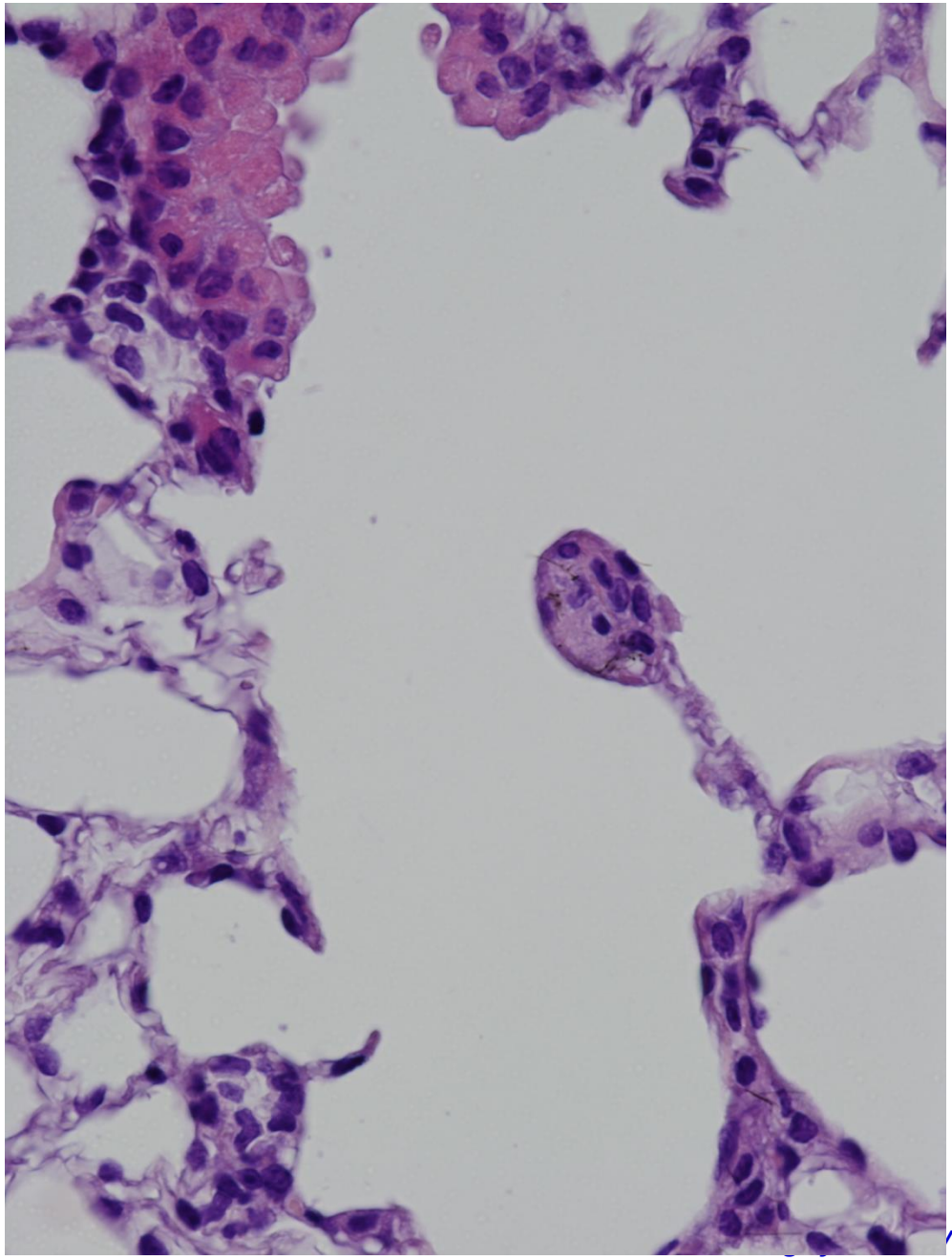
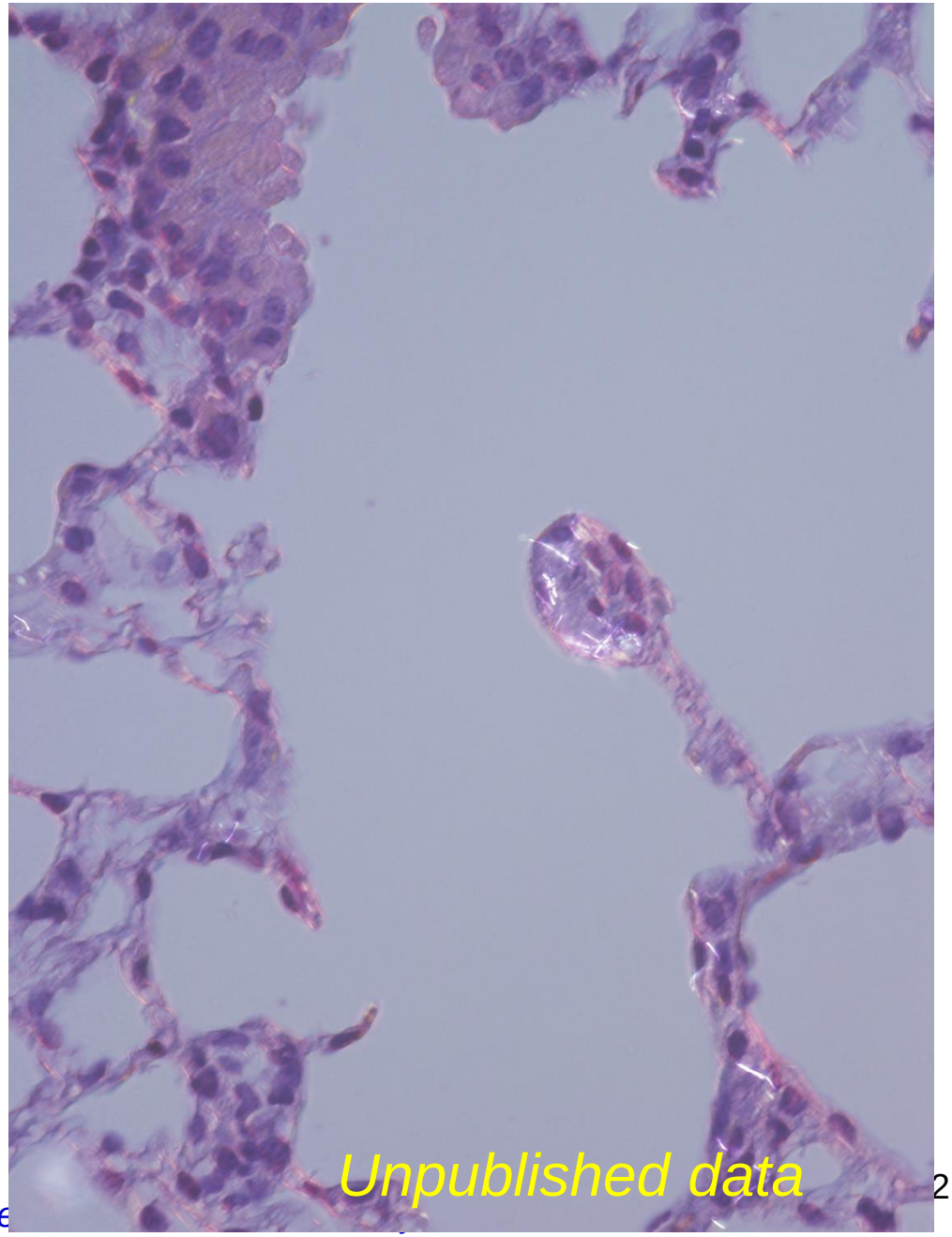
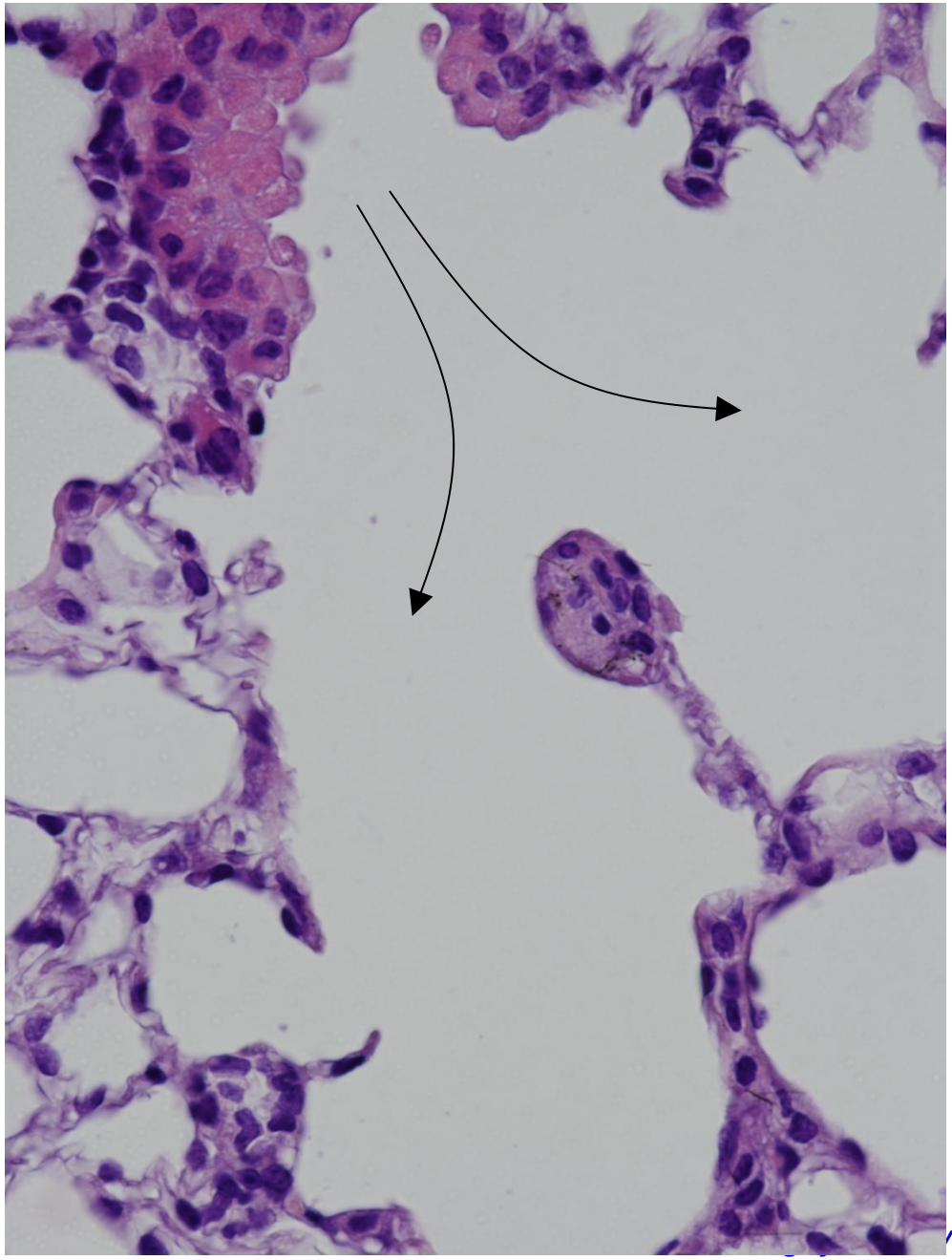


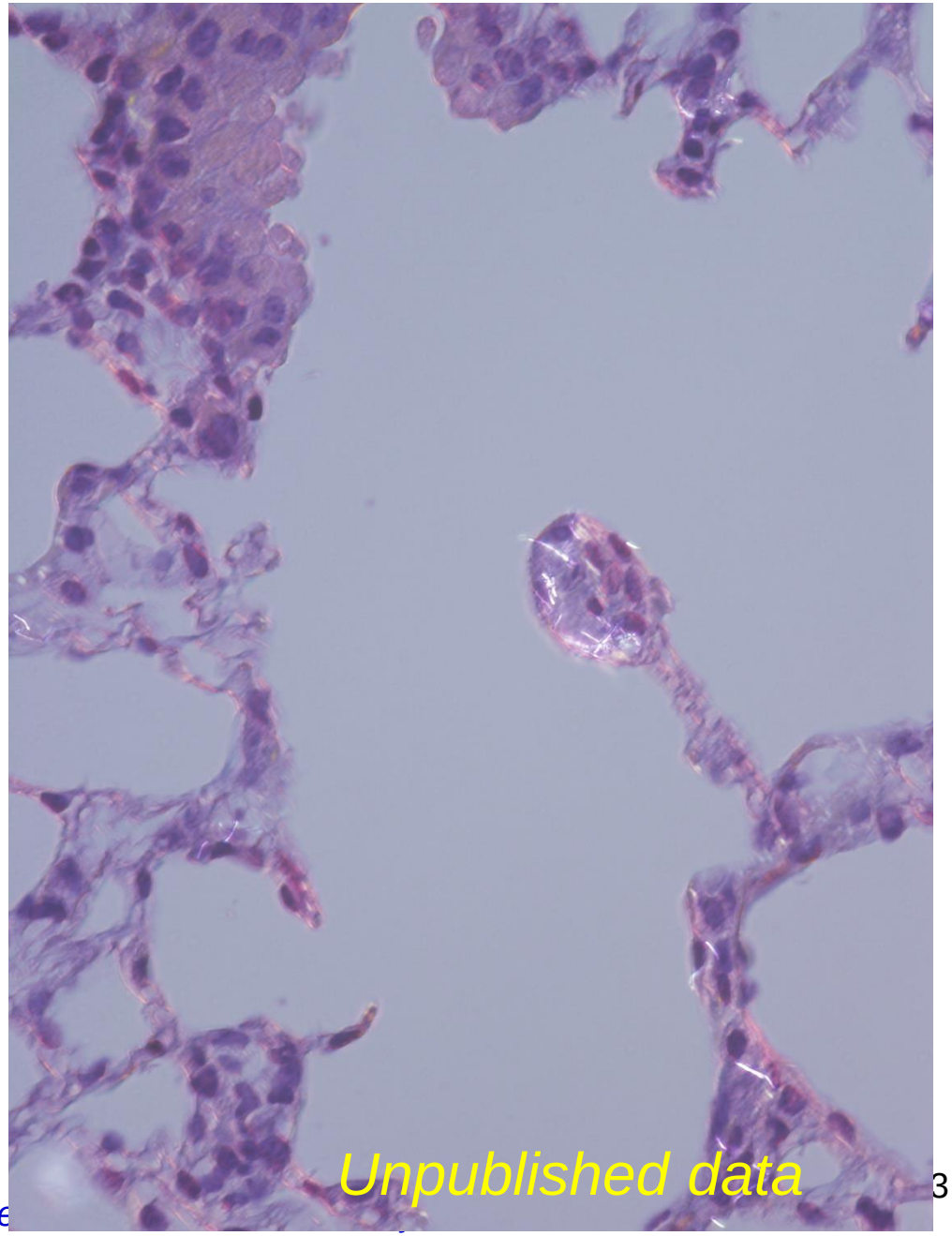


Unpublished data

NANO-AP_13W_High_179_513

High 13W





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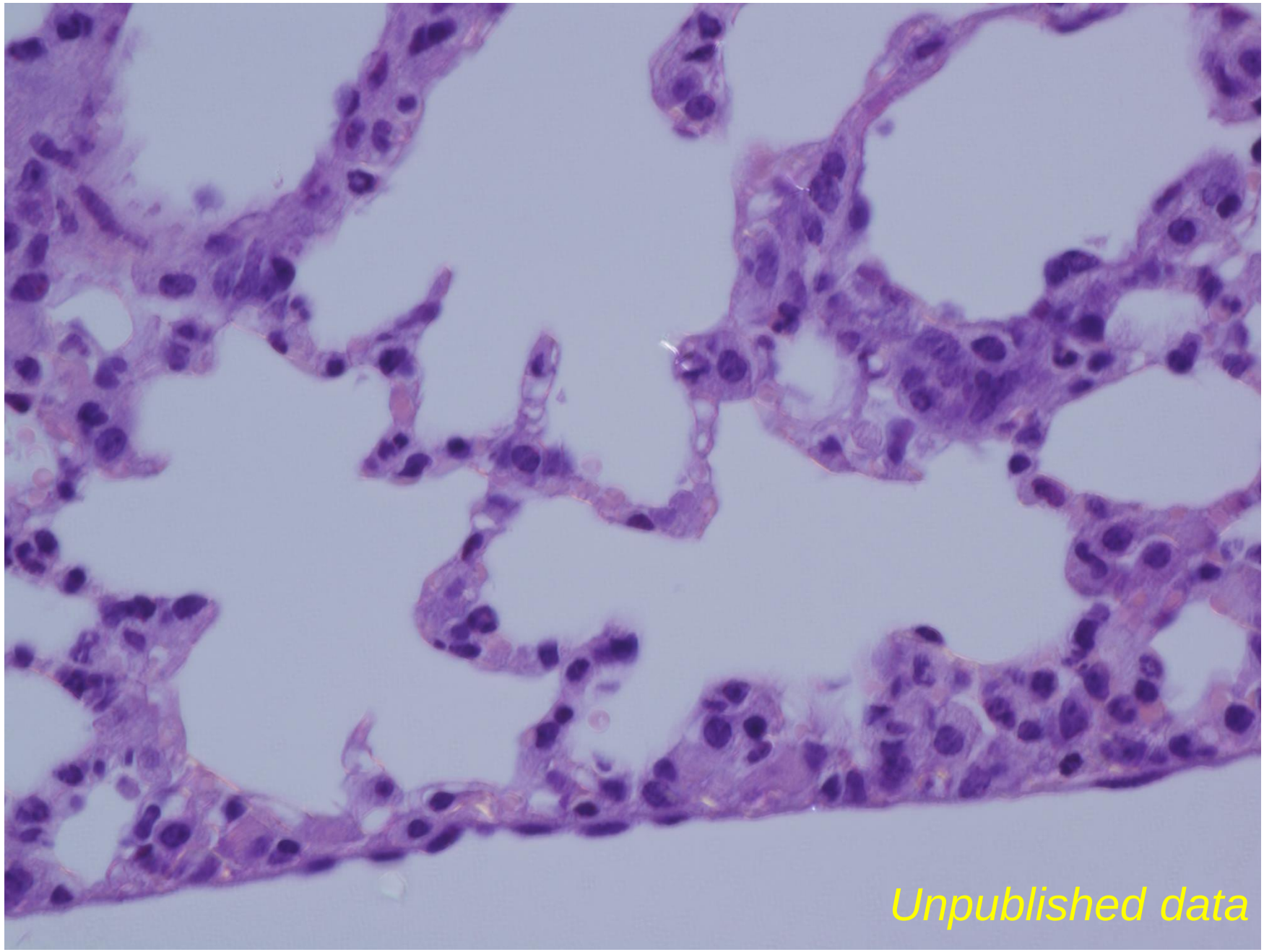

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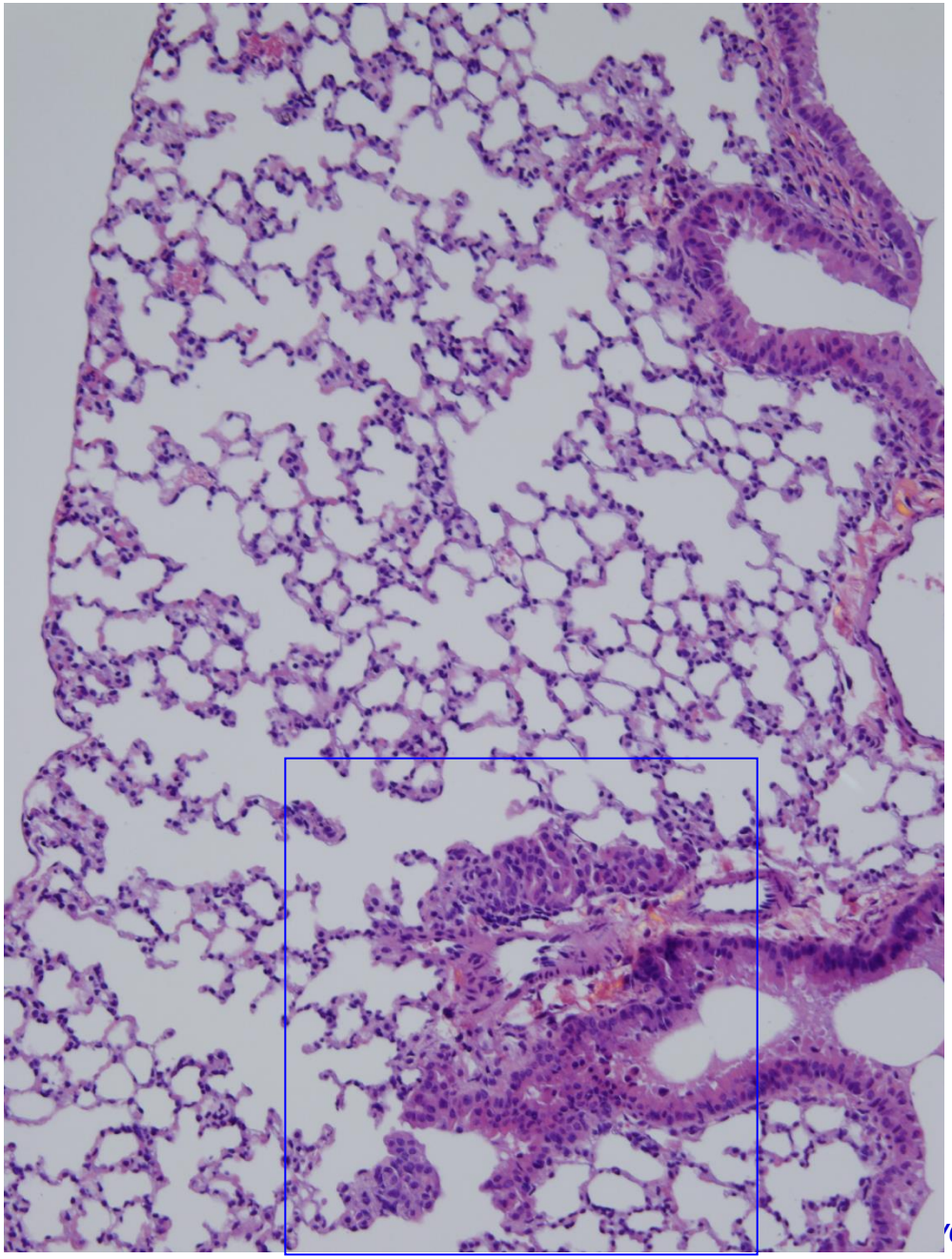
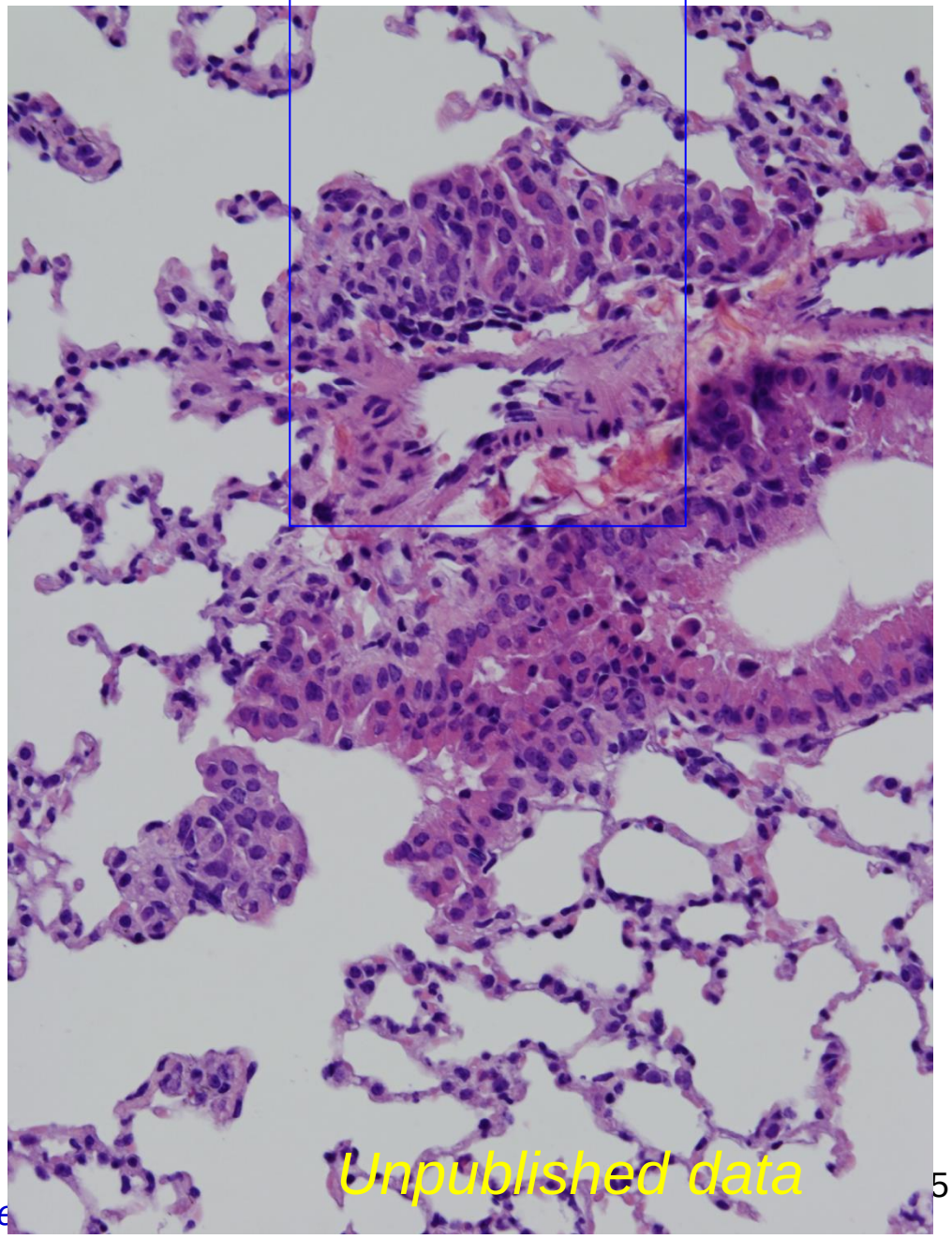
High 13W





Unpublished data

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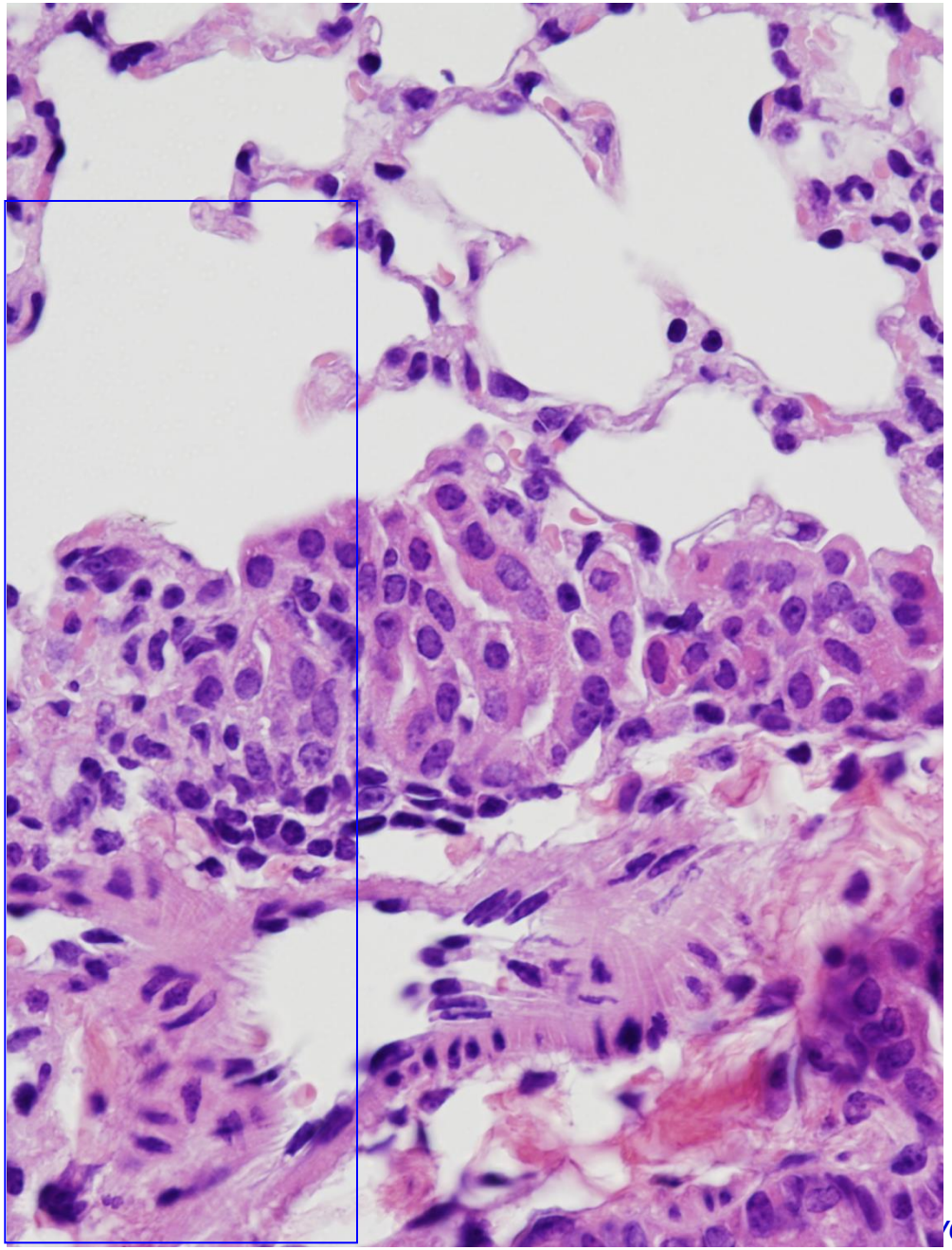
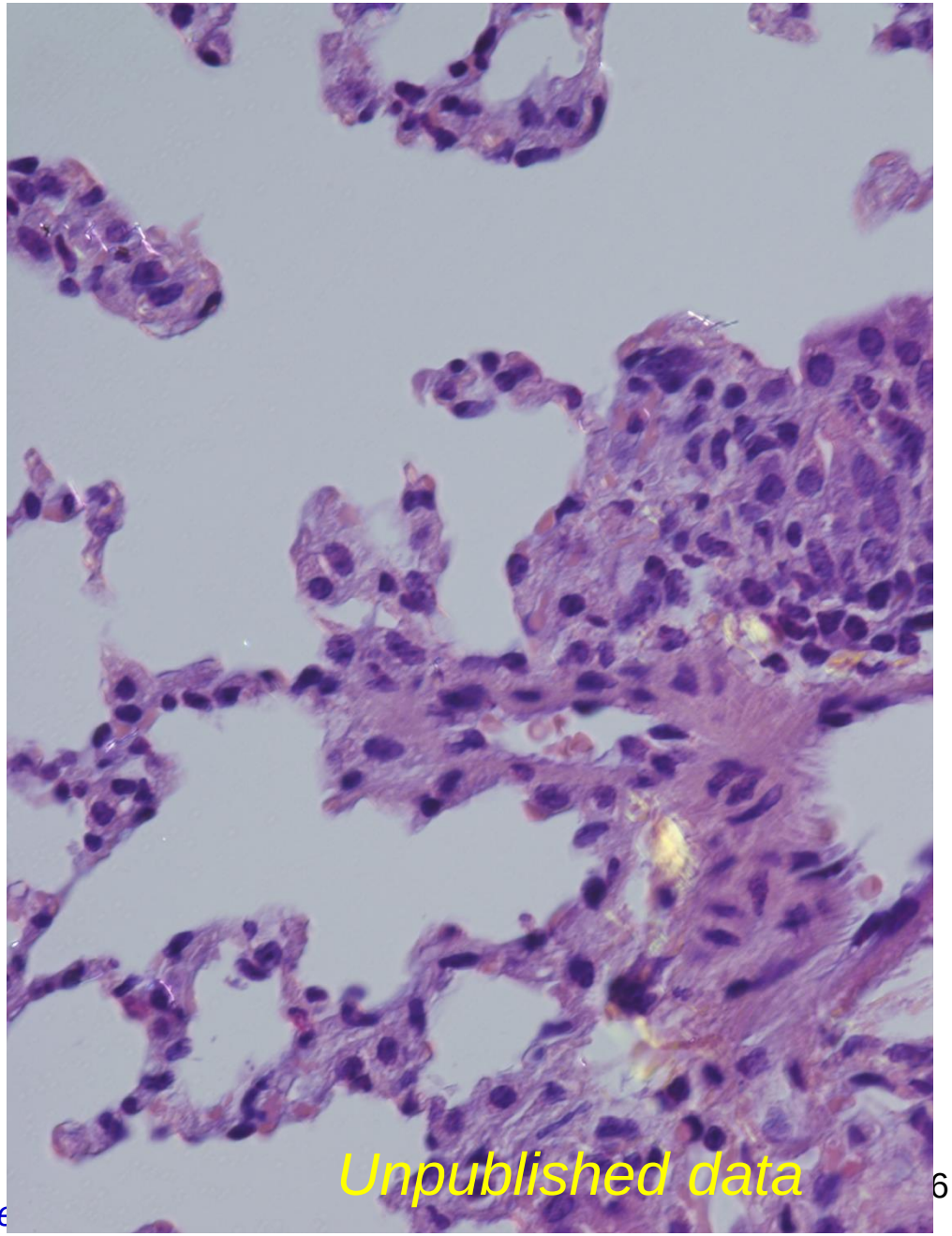
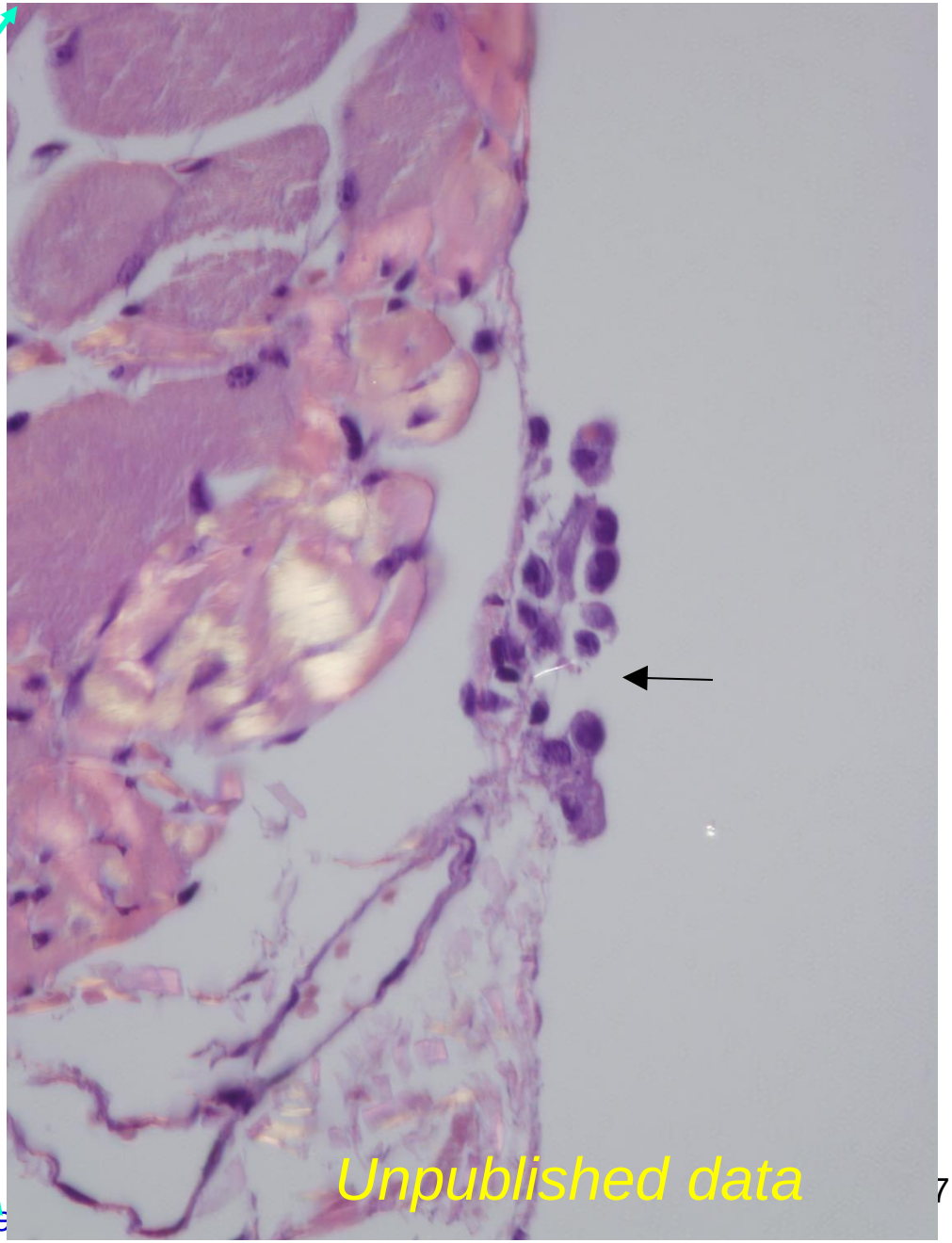
High 13W



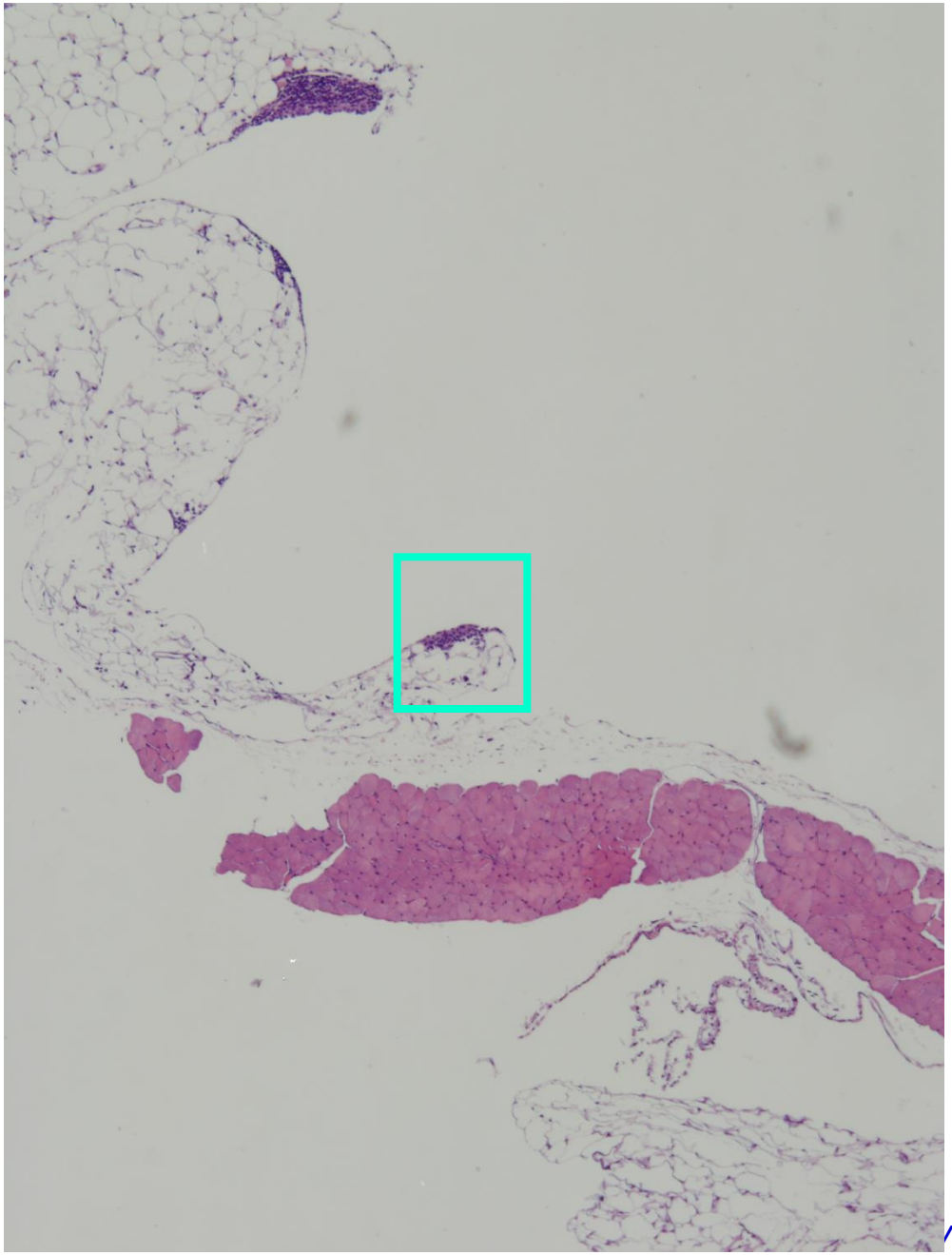


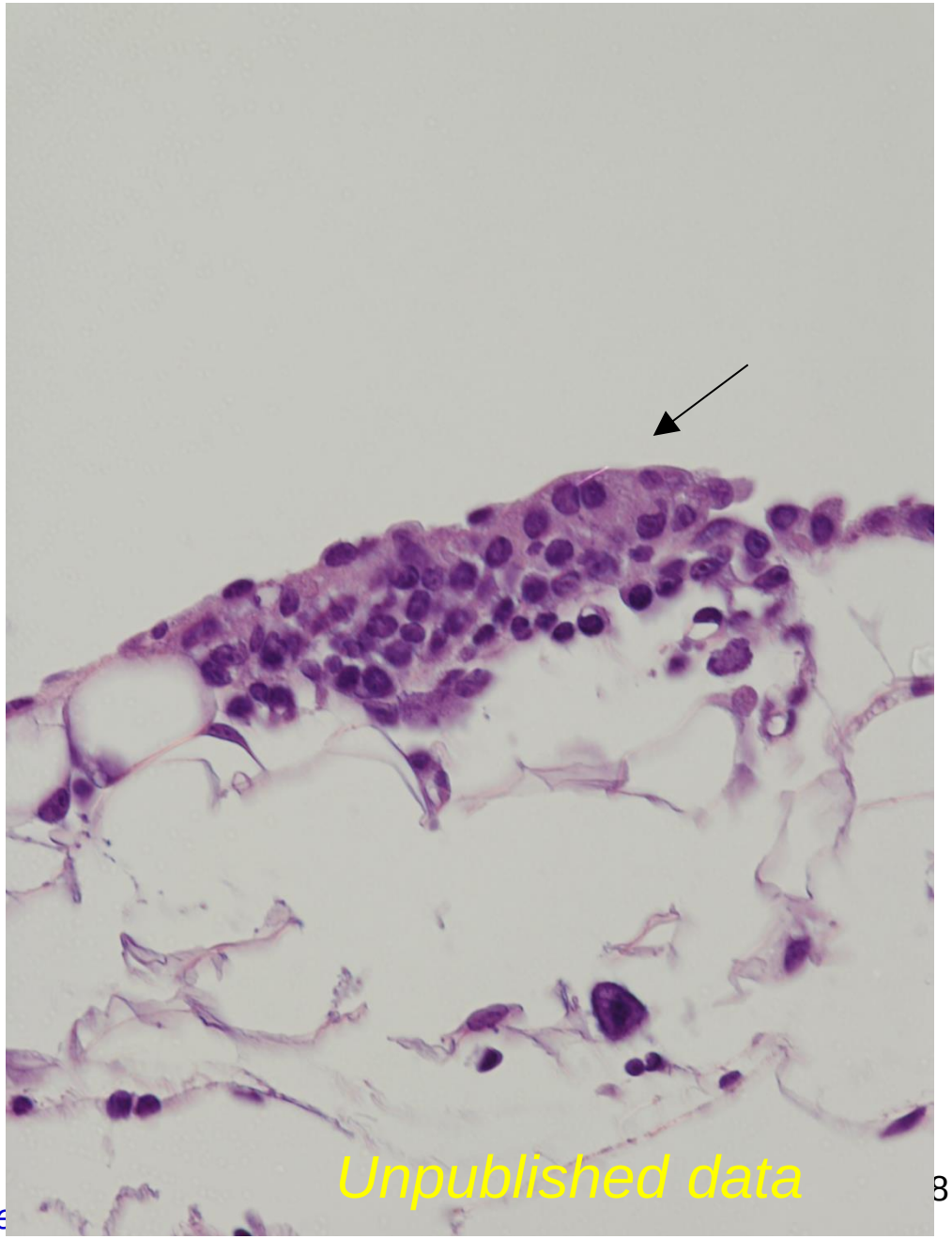
Unpublished data

Low 13W



Low 13W





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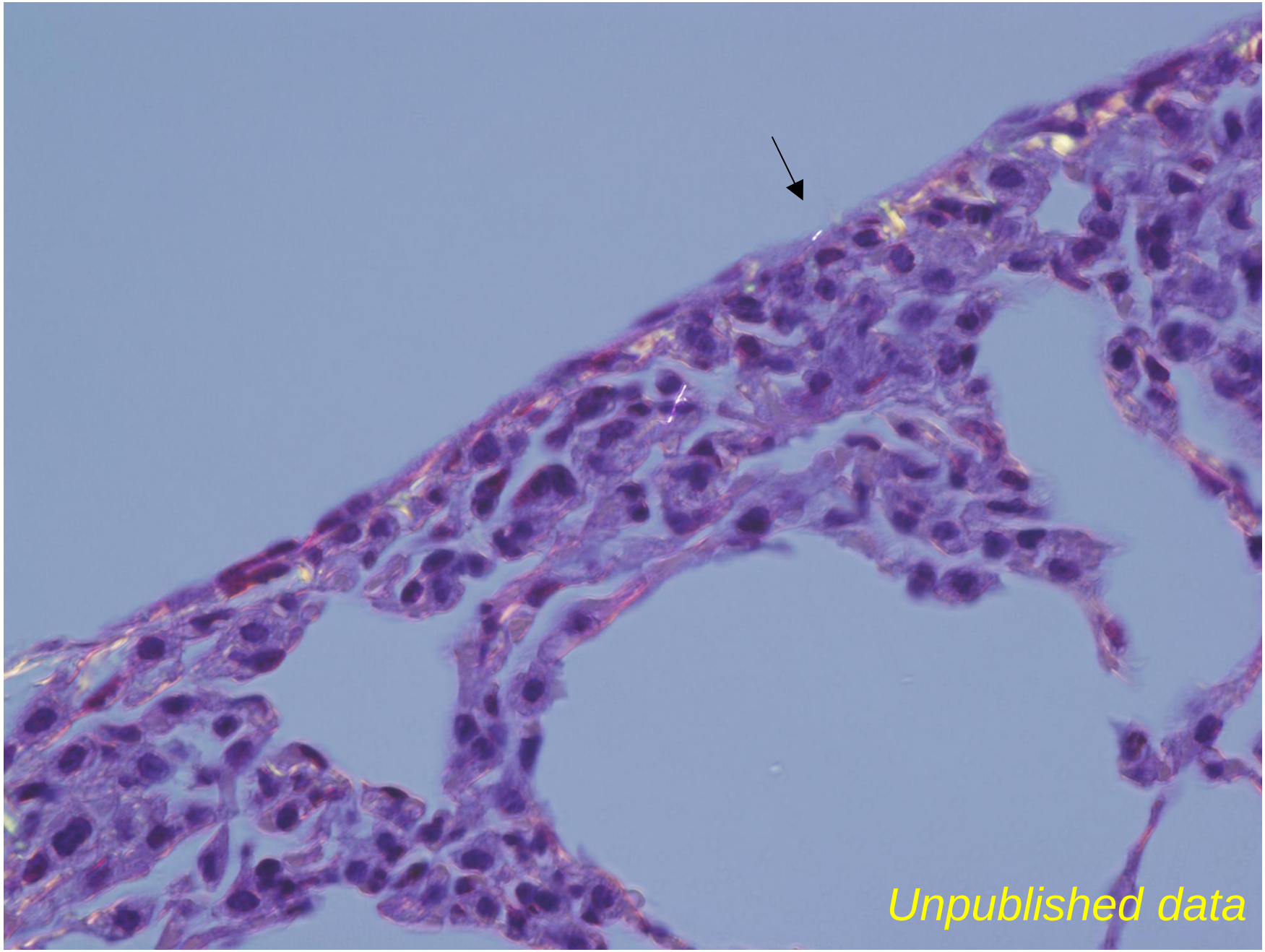


Nanos to be tested

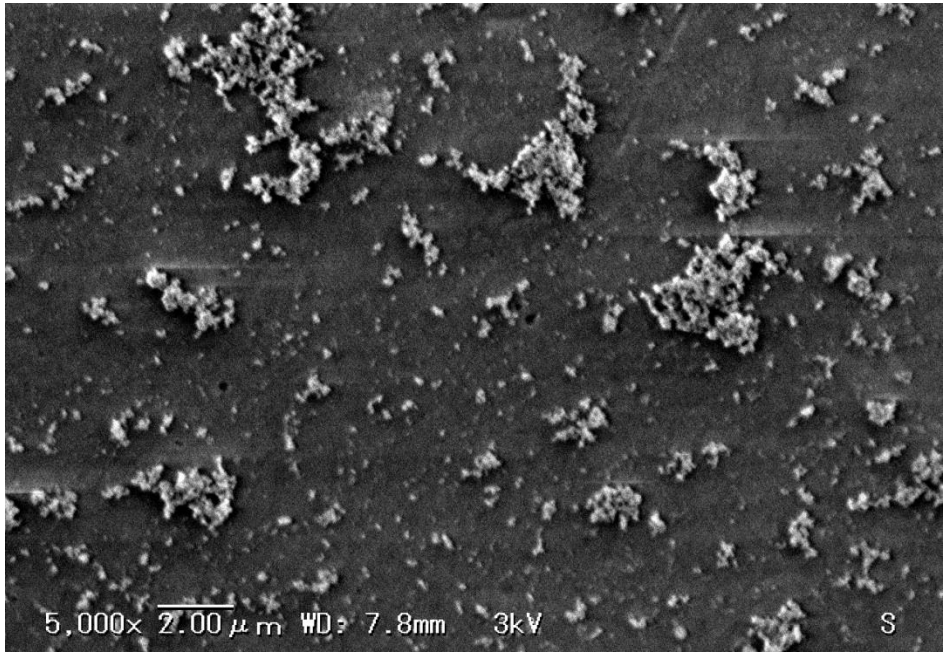
Application of Taquann System

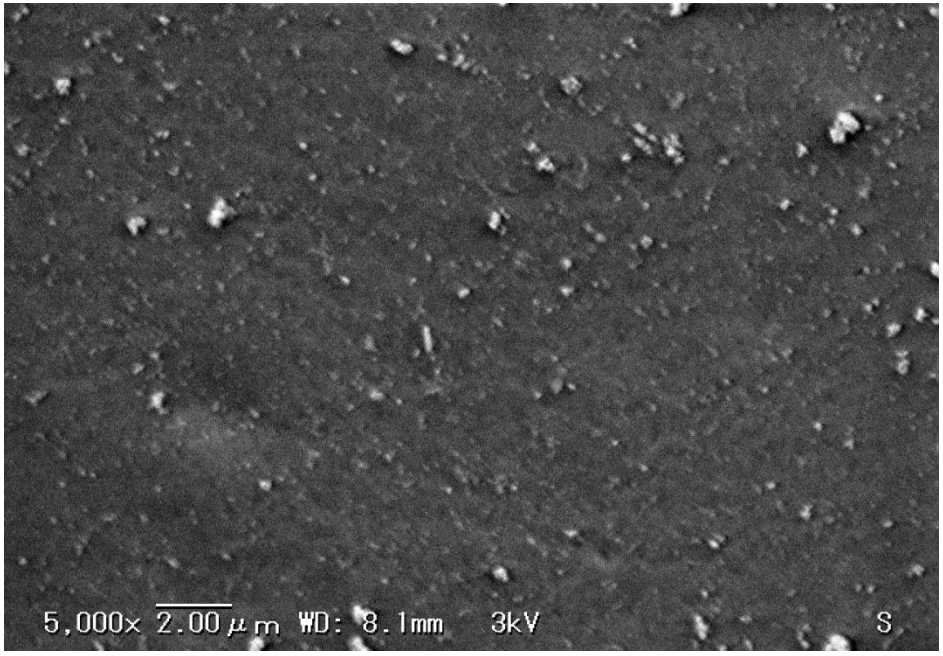
- CNTs
- TiO_2
- ZnO
- C_{60}
- Ag, An, Zn
- Graphen
- Nano-Cellulose
- others

- NM with Specific Affinity
  - DNA/RNA
  - Protein
    - = enzyme inhibition
    - = antibody-like
    - = amyloid/ prion-like

  Aptamer-conjugated

Taquann trial: TiO2 (primary particle diameter; 35 nm)



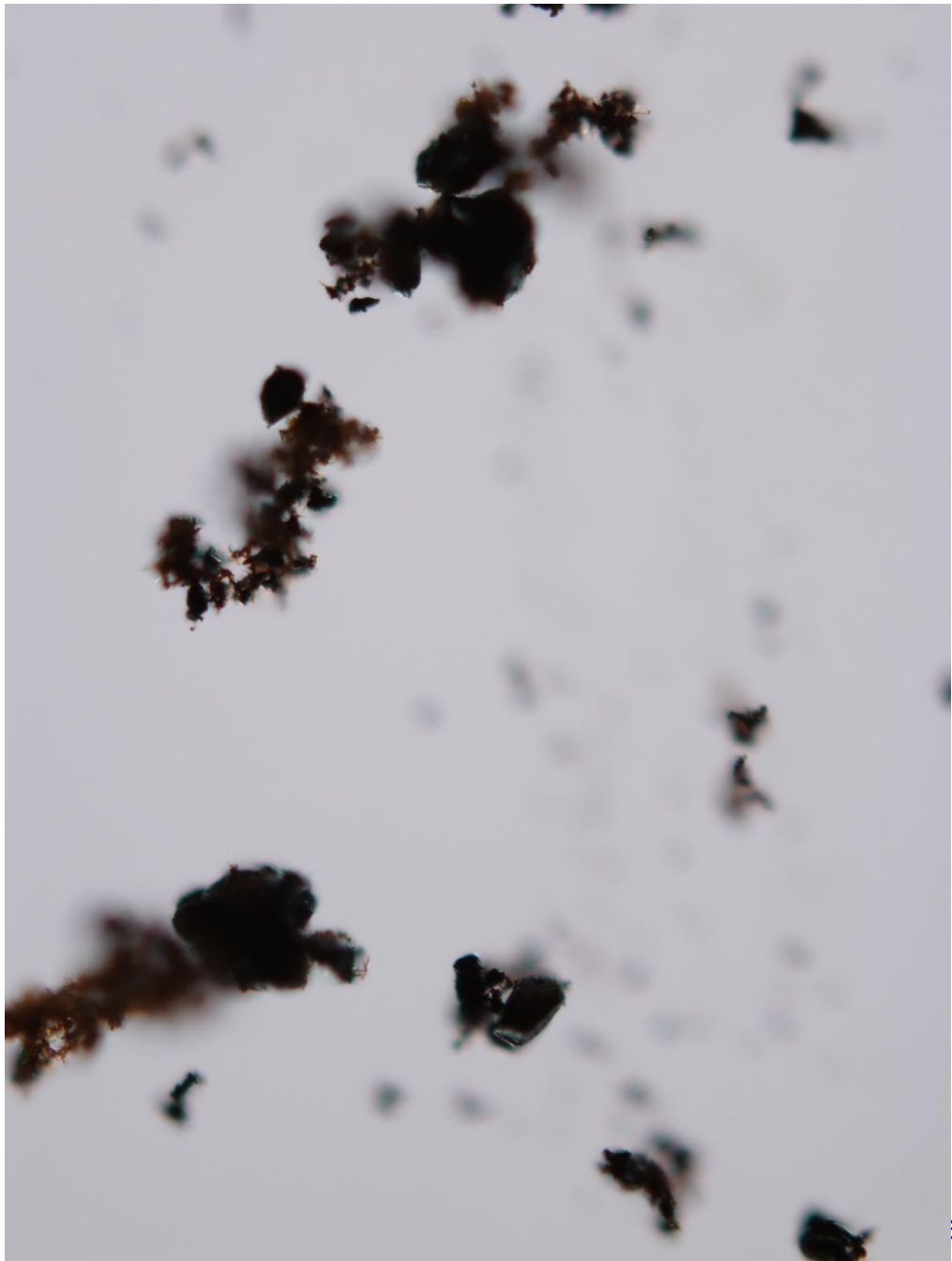


Bulk

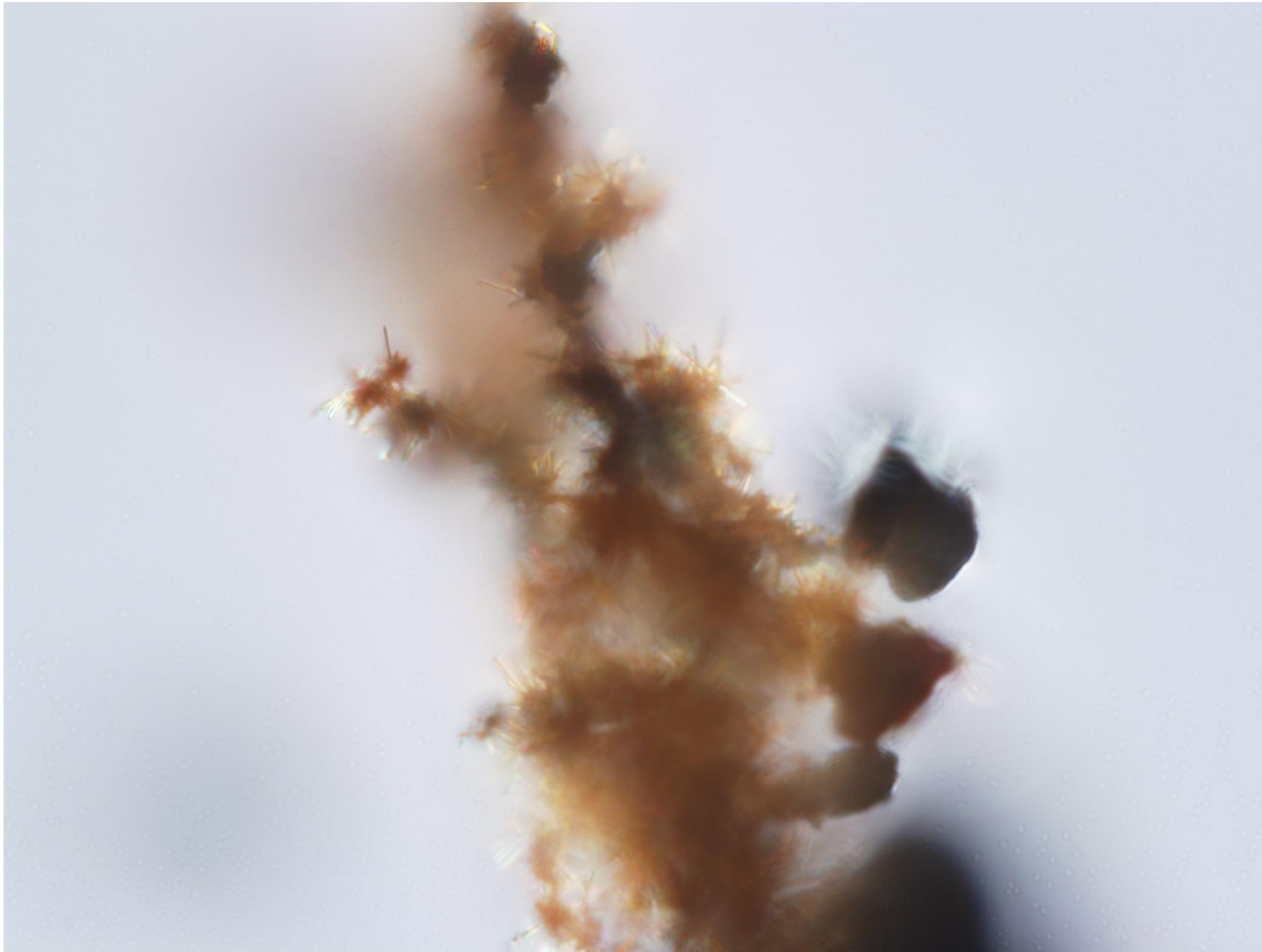
Suspension in water 5,000x

Taquann 5,000x

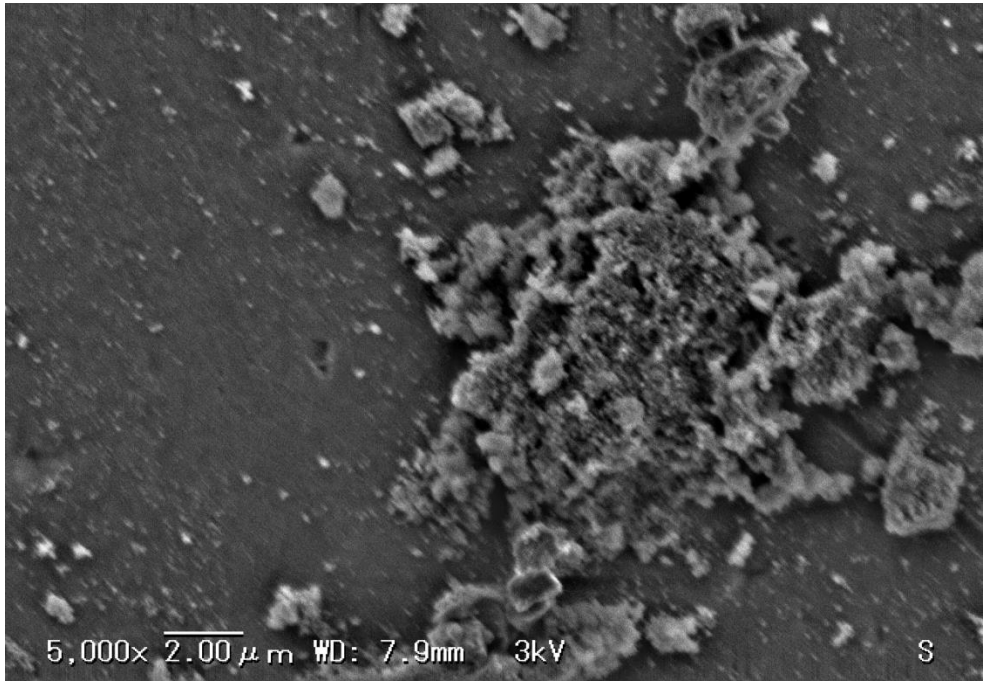
Taquann trial: C60



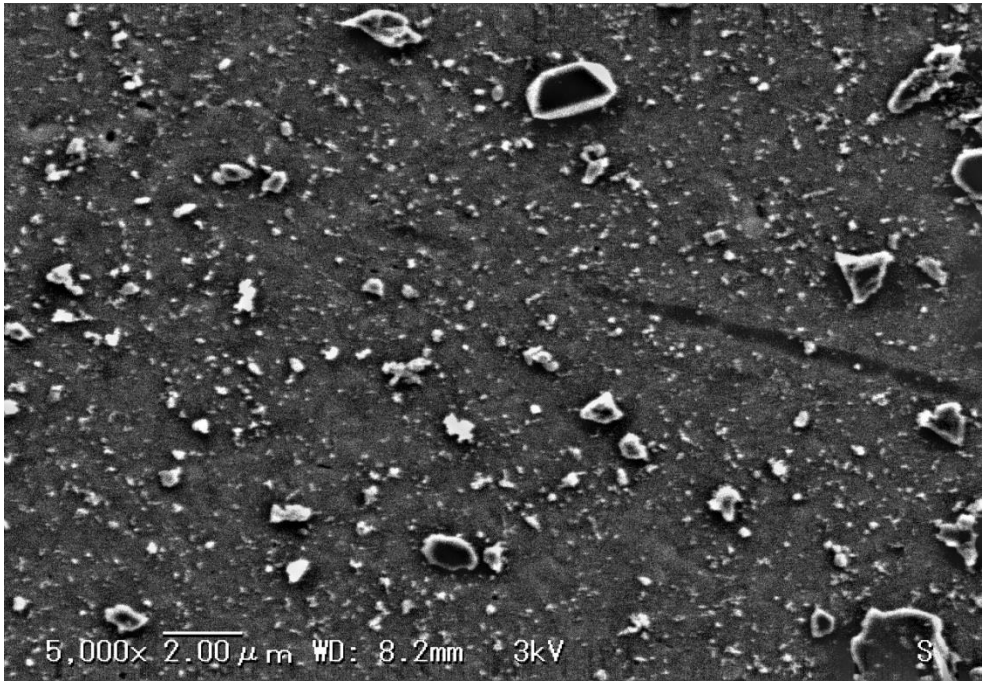
54
c60 Taquann 3A24 9 x40



C60



Bulk 5,000x



Taquann 5,000x

Summary

- For the monitoring of general pulmonary toxicity (acute and chronic), a small scale inhalation system together with more universal dust generation system is developed.
- This Taquann method generates well-dispersed MWCNT without aggregate/agglomerates and without dispersants.
- The size of the single particle is no affected
- Taquann + Cartridge-direct injection system keeps operator and room clean.
- Pilot study showed single fibers reaching alveolar spaced without proximal lesions.
- Can be used for other nanoparticles as long as insoluble to tert-butyl alcohol

---

- Improved second version is under testing.

μm-MWCNT
Fullerene whiskers

μm-MWCNT
C_{60}
etc

Curre[illegible] study di[illegible]

1. Known mechanism
   - fiber carcinogenesis - intraperitoneal injection model
   - systemic distribution - i.p. model

---

2. Unknown mechanism
   Animal experiment using human relevant route*

TiO_2
ZnO
μm-MWCNT
Shorter MWCNT
Other CNT
Nano Metals
Fullerene
whiskers
Etc.

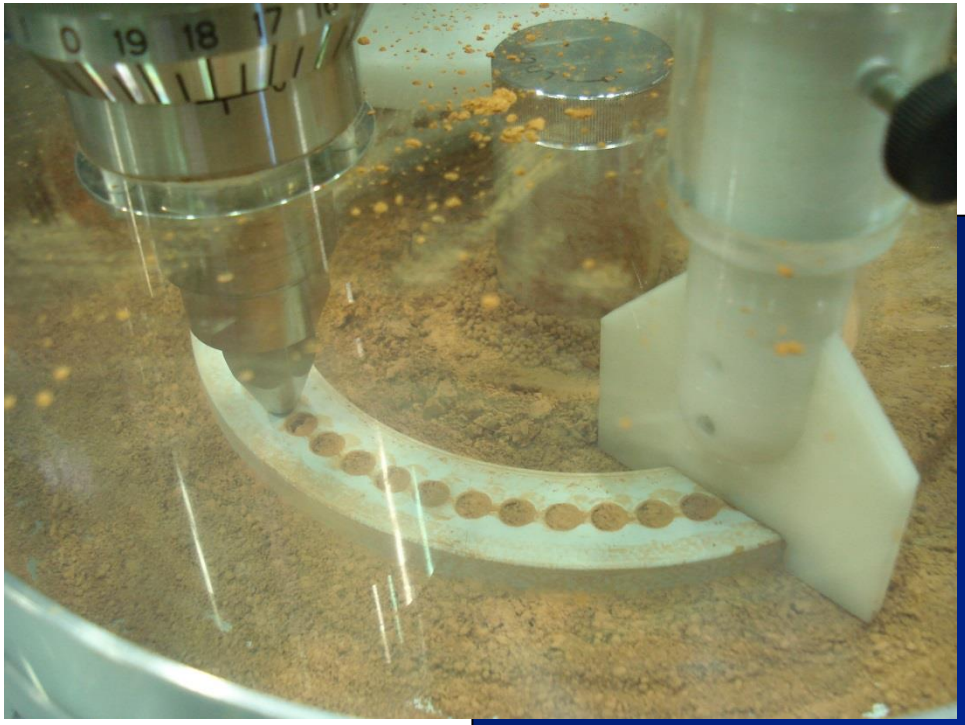
→ hazard identification
→ mechanism identification (assumption)
→ dose-response data in experimental animals

Assumption of human toxicity and dose-response characteristics

*: inhalation (whole body, intratracheal), dermal, oral

Conclusion

- For the monitoring of general inhalation toxicity, both acute and chronic, an universal dispersion method is introduced; Taquann method.
- A small scale whole body inhalation system (Taquann-direct injeciton system) was made; the equipment is relatively cheap and ready for varieties of nanomaterial samples.
- The authors hope that these two methods/systems will facilitate inhalation toxicity studies for monitoring the unpredictable toxicity induced via whole body inhalation.



END