A novel Method for Bacterial inactivation Using Engineered Water Nanostructures (EWNS)

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http://hsph.harvard.edu/nano

Background

- Airborne pathogens constitute a major burden of disease
- Bacterial infection through formitesis equally important health risk especially in hospitals and food industry
- Nanotechnology based methods are emerging as intervention technologies for antimicrobial inactivation.
  - Ag nanoparticles (ROS generation via Ag+ ions)
  - TiO_2 nanoparticles (ROS via UV/VIS irradiation)
- Limited only on surfaces – toxic when airborne

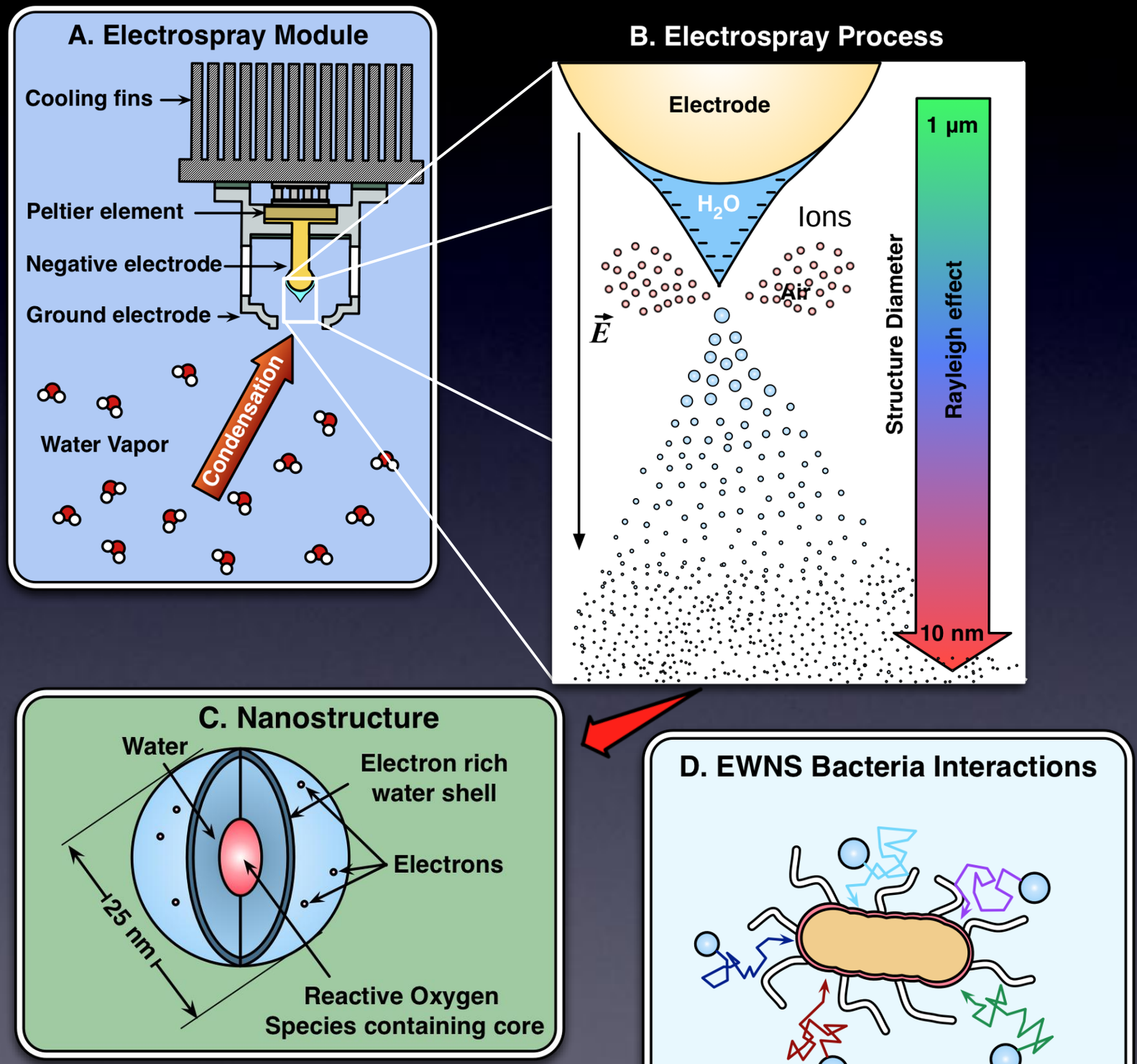
Ideal disinfectant…

- Effective and efficient
- Stay airborne from long enough to inactivate bacteria
- Non – toxic to humans
- Leave no chemical trace – green
- Inexpensive

Potential Solution

Engineered Water NanoStructures (EWNS)

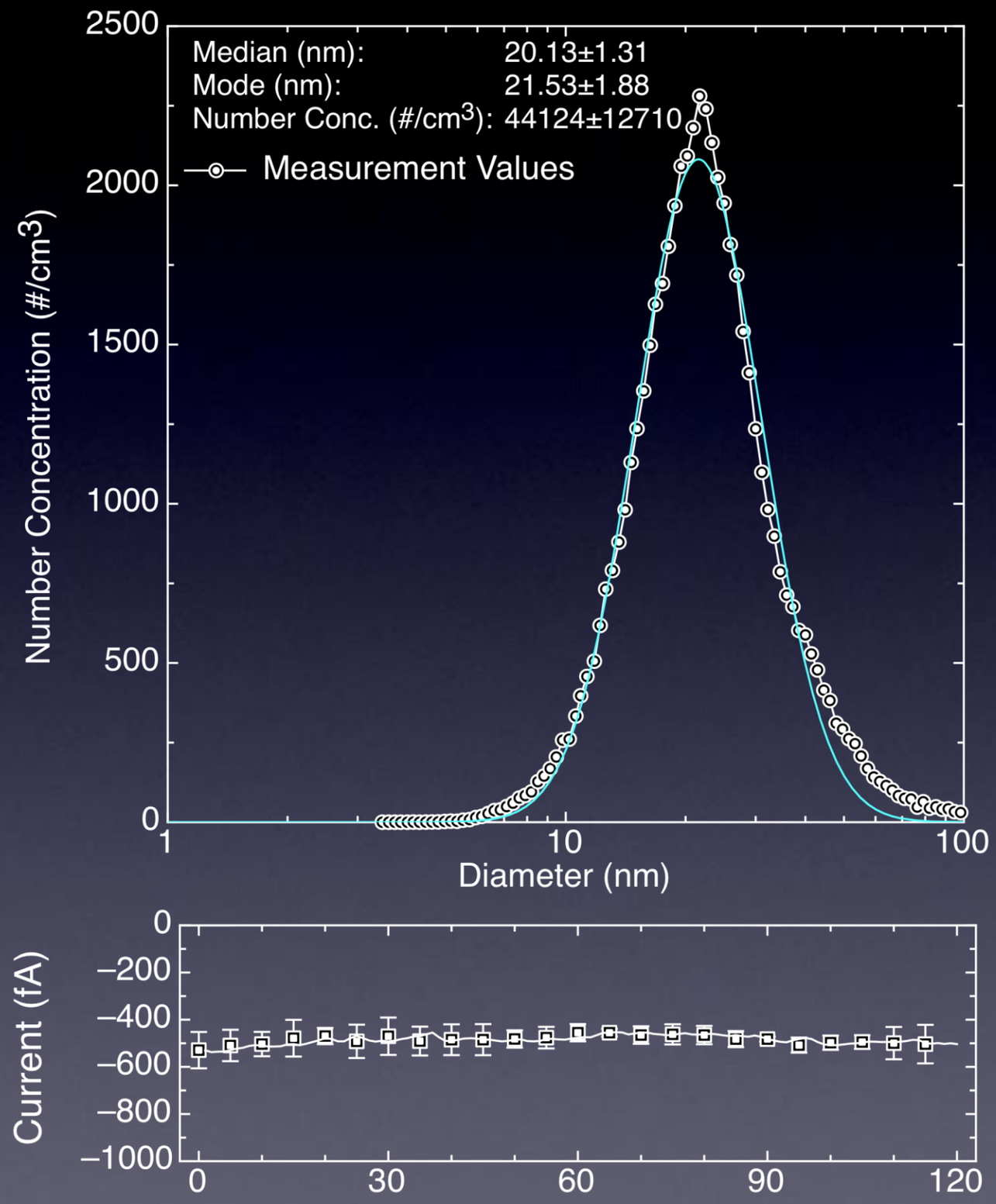
Concept: Engineered Water NanoStructures (EWNS)



Physicochemical Characterization

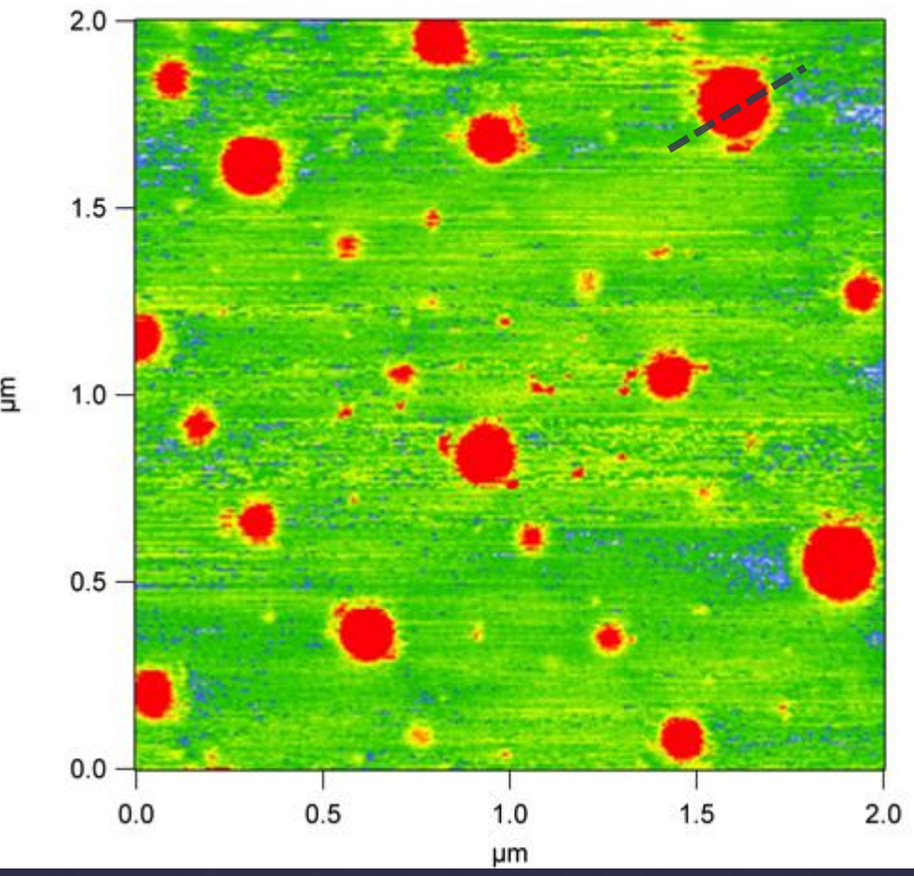
PCM characterization – Surface charge

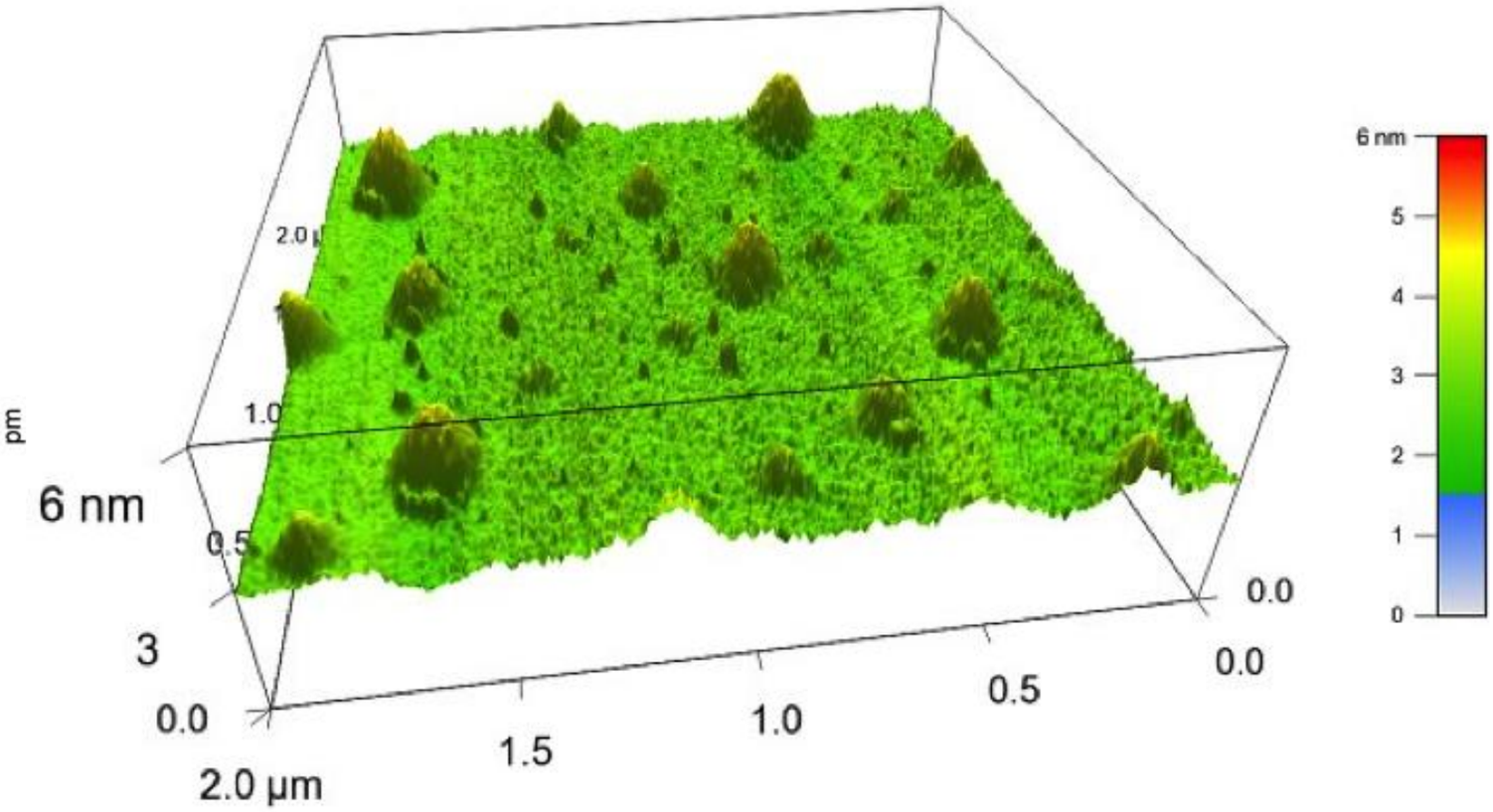
- On average the EWNS have 10 electrons per structure.
- The surface charge increases the surfaces tension that retarding the evaporation.
- The droplets evaporate until the droplet reaches a terminal radius.
- A theoretical model developed by Nielsen that allows to estimate the terminal diameter
- PITFALL: Electric charge can implicate the size measurement as commonly used instruments rely on particle charge neutralization.



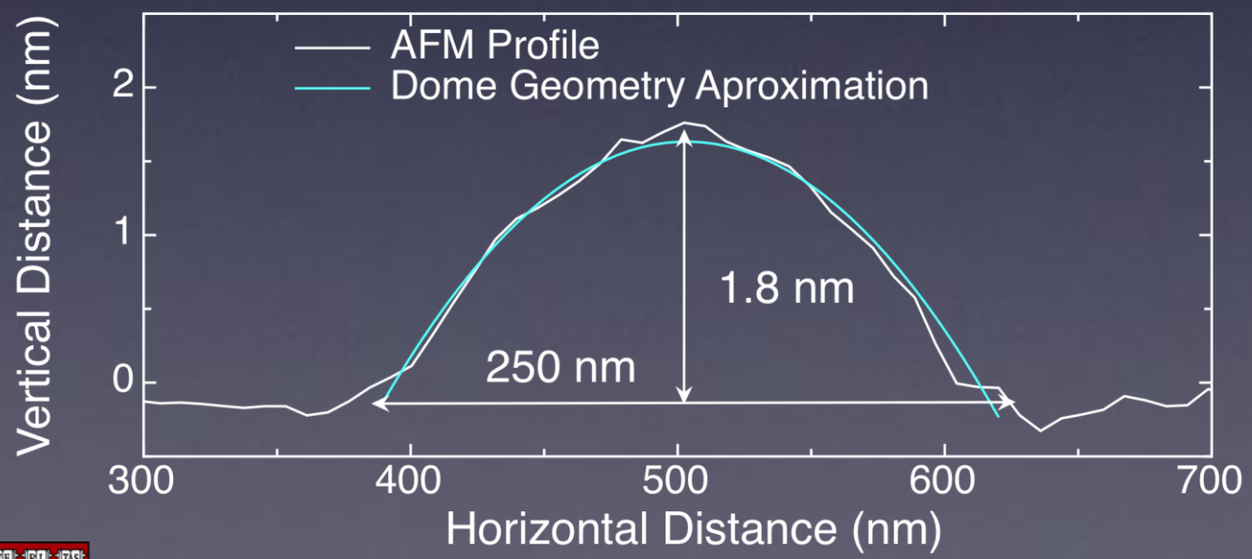
Nielsen J, et al. Atmospheric Chemistry and Physics 11:2031–203

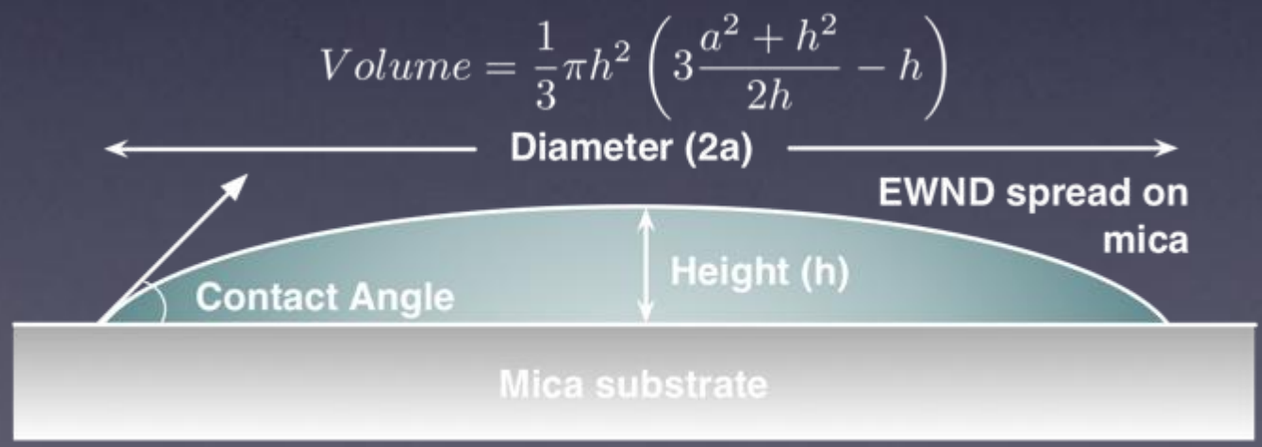
Size: AFM measurement (1/2)



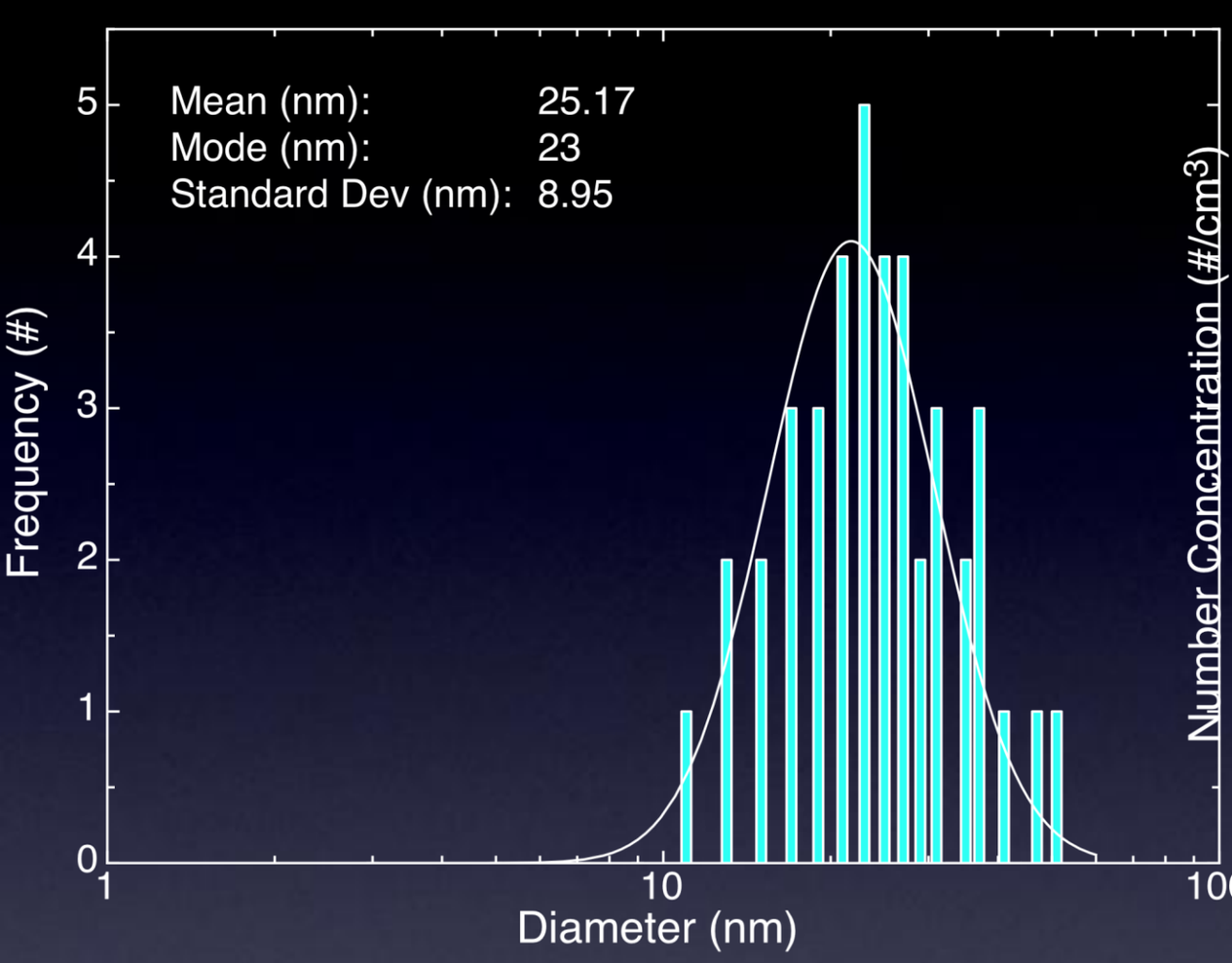


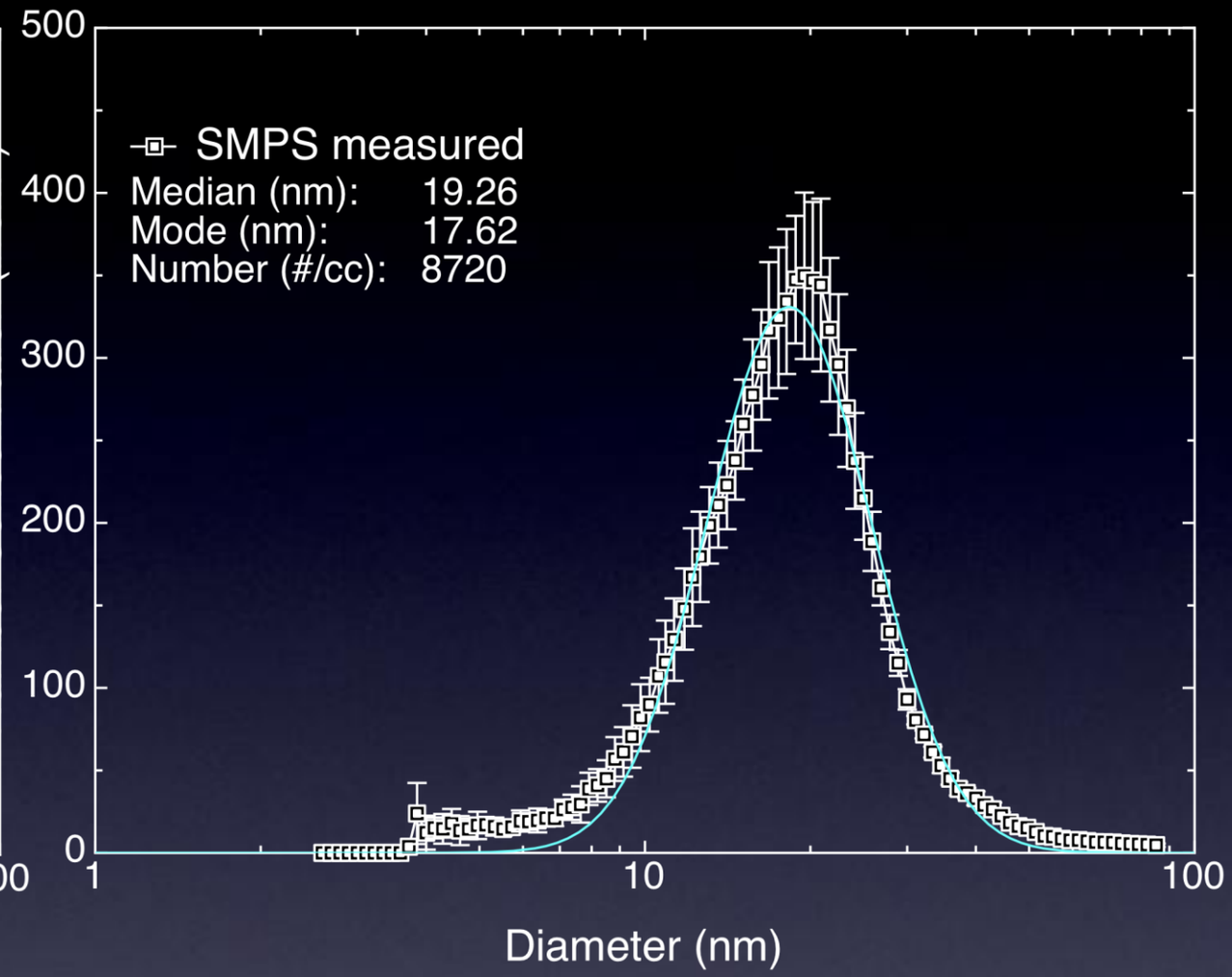
Polydispersity due to the Rayleigh effect





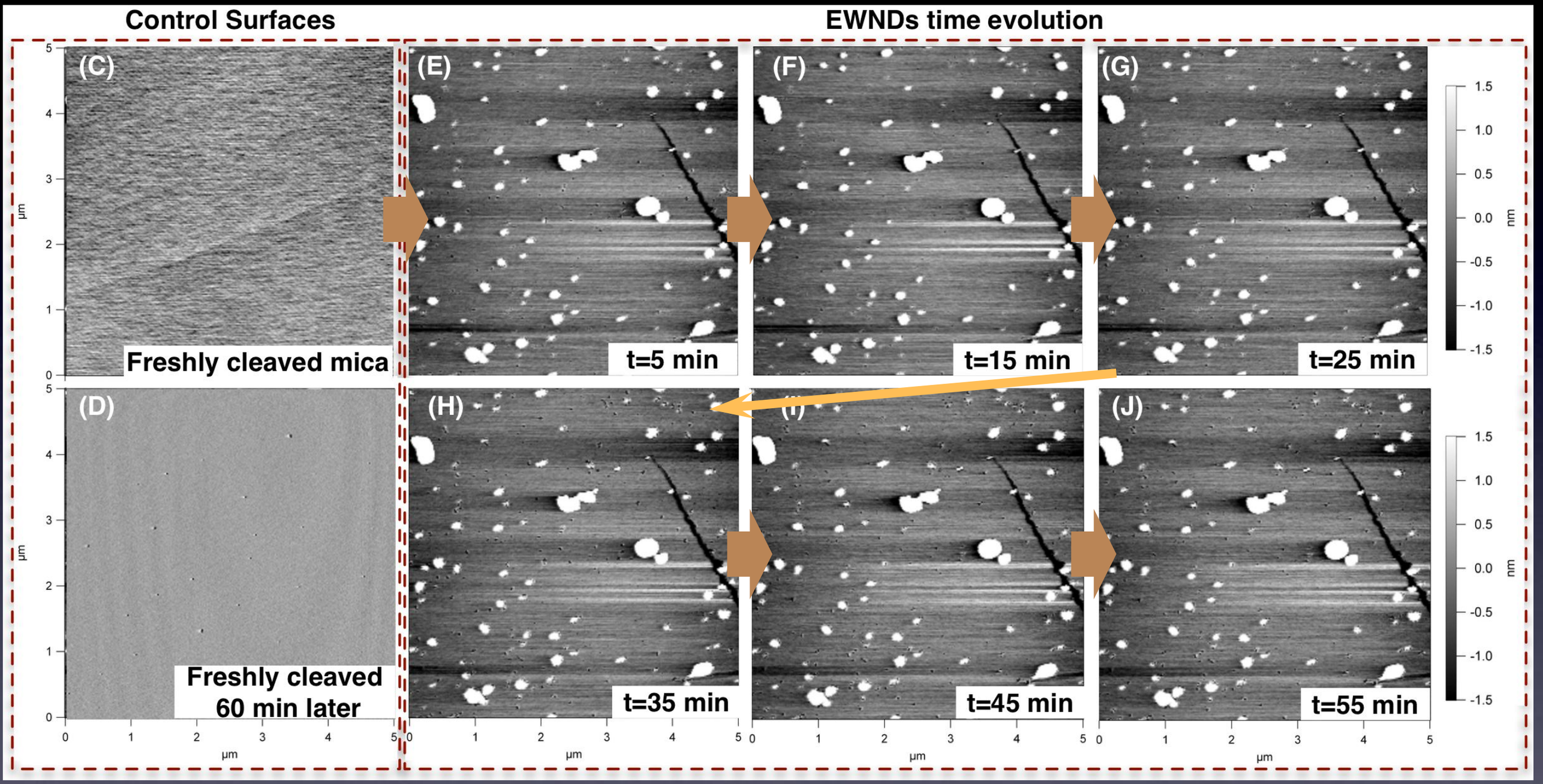
Size: Size distribution (2/2)





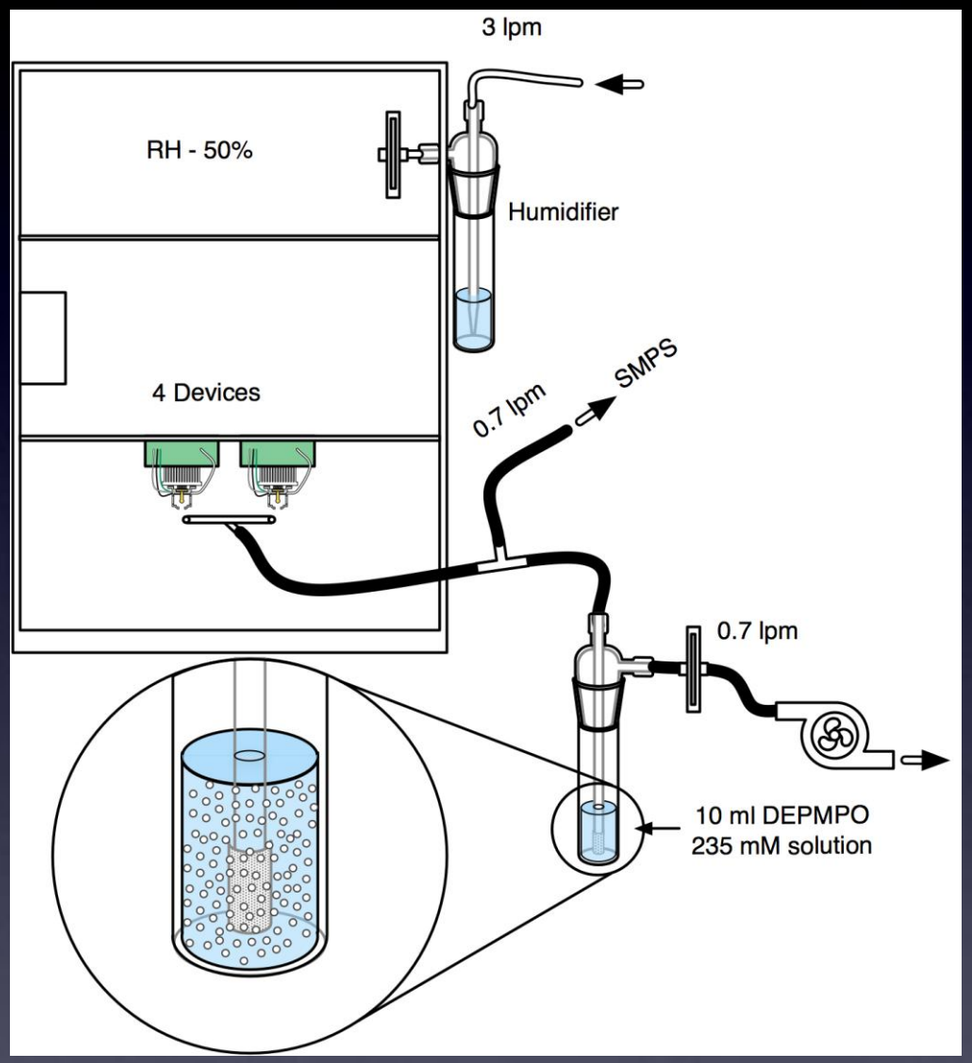
- The mean EWNS diameter is 25 nm (AFM measurement)
- SMPS, charge adjusted mean diameter was found 19 nm

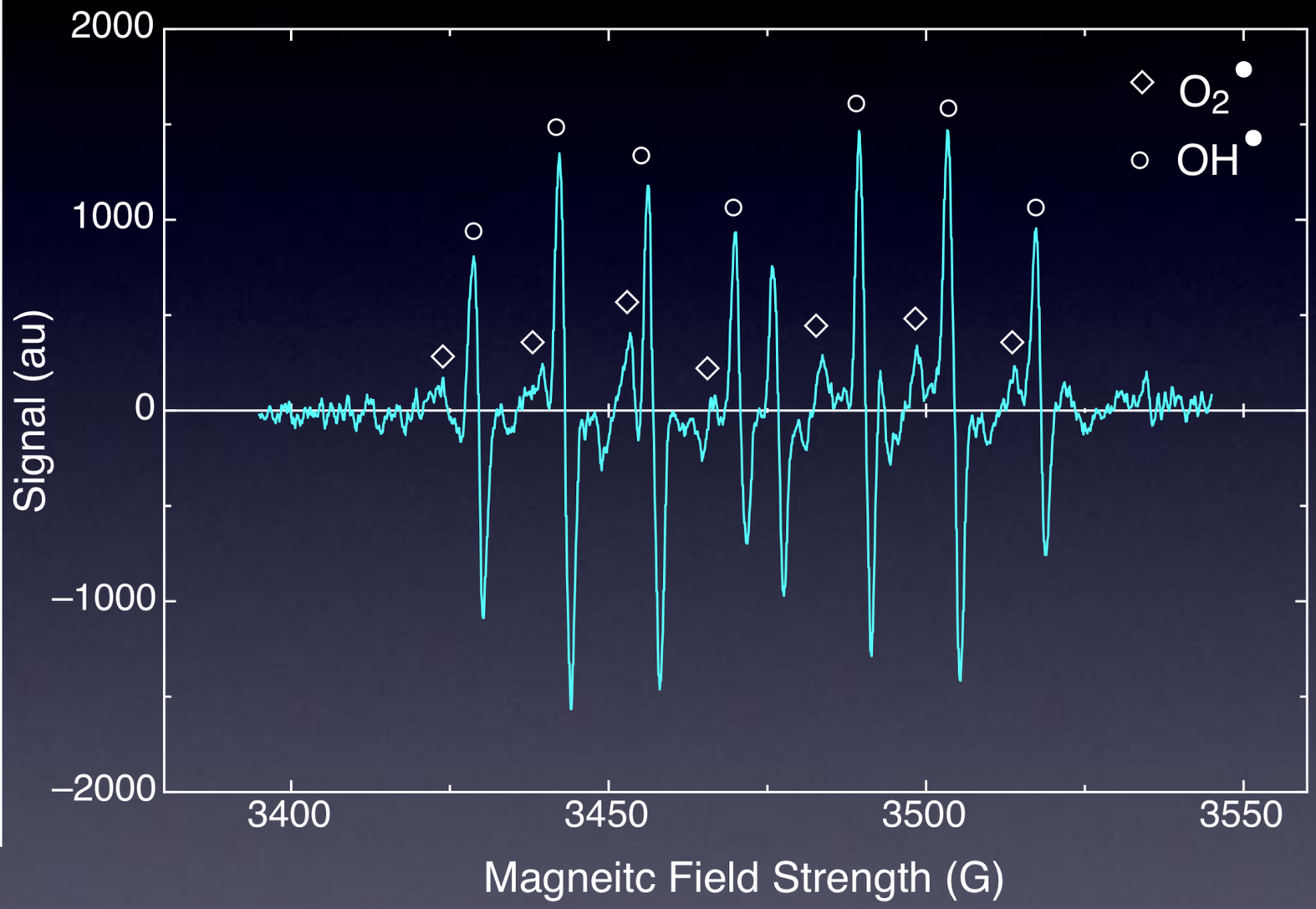
Lifetime (1/1)



The EWNDs are very stable and there is no noticeable evaporation for almost an hour.

PCM Characterization – ROS detection using ESR





The EWNS are loaded with both $O_2^{\bullet}$ and $OH^{\bullet}$.

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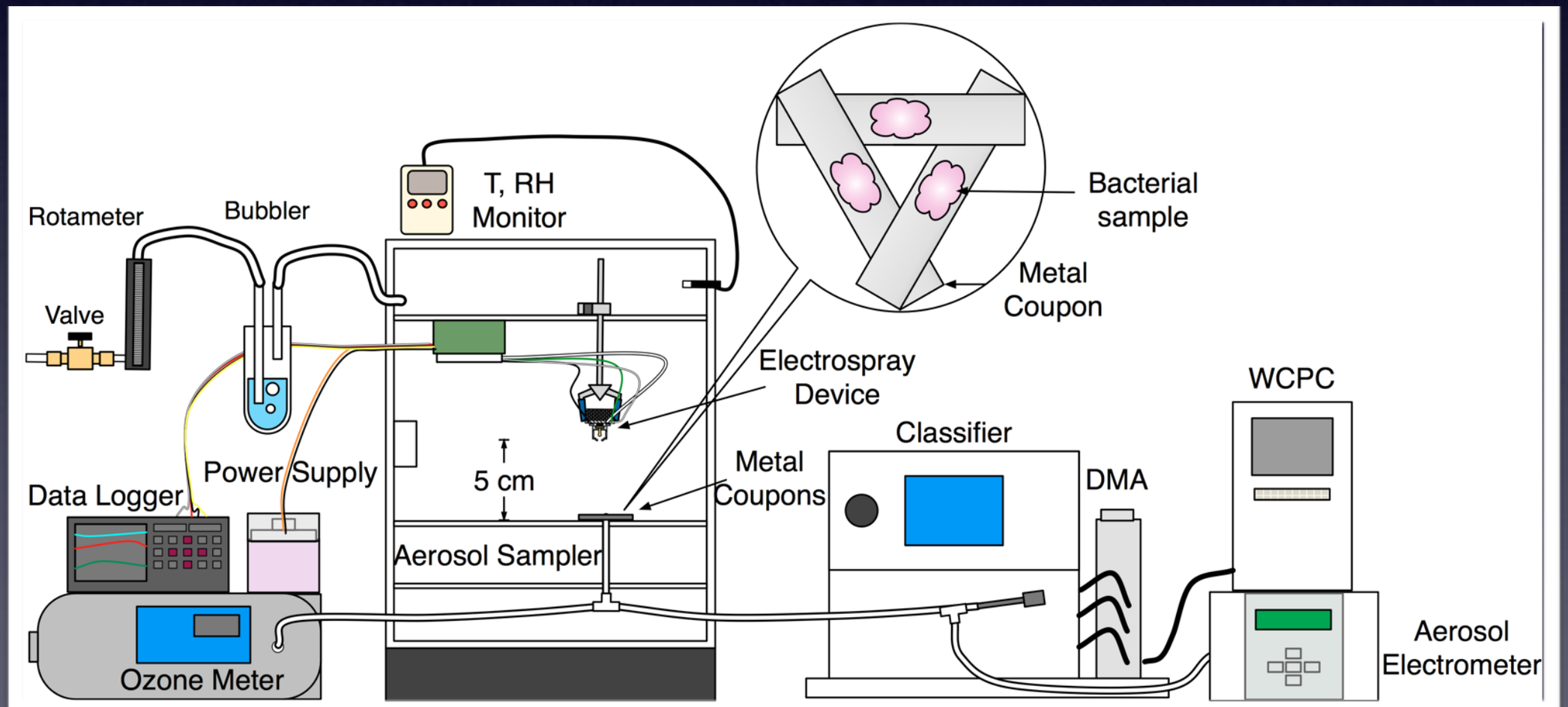
Antimicrobial properties Applications

Surface Inactivation

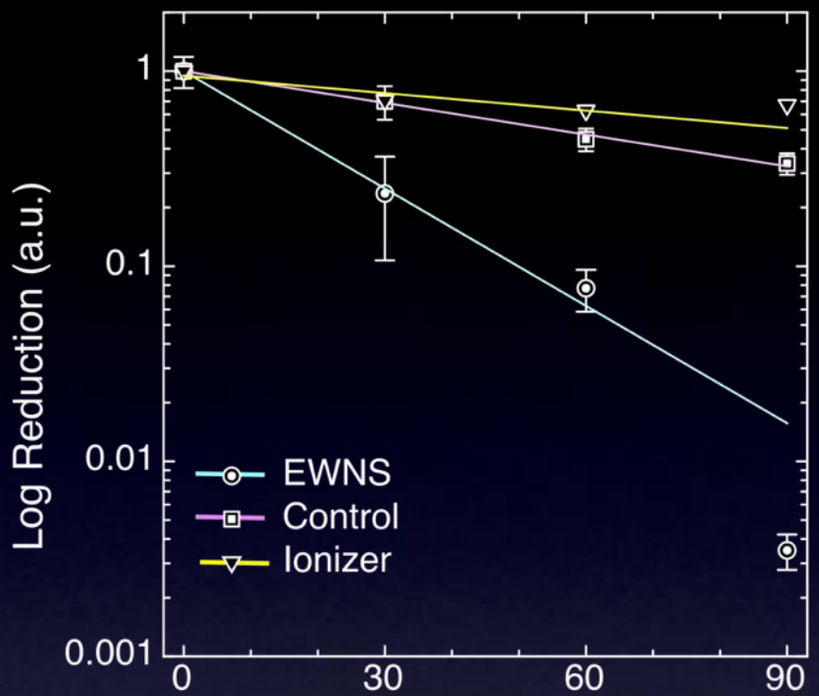
Surface bacteria inactivation - Methods

- Three type of bacteria (gram negative, gram positive, mycobacterium & spores)
- Surface: Stainless steel
- Controls: Air, ions
- Dose at 9000 #/cc (trhee time points, 30 min, 60 min, 90 min)

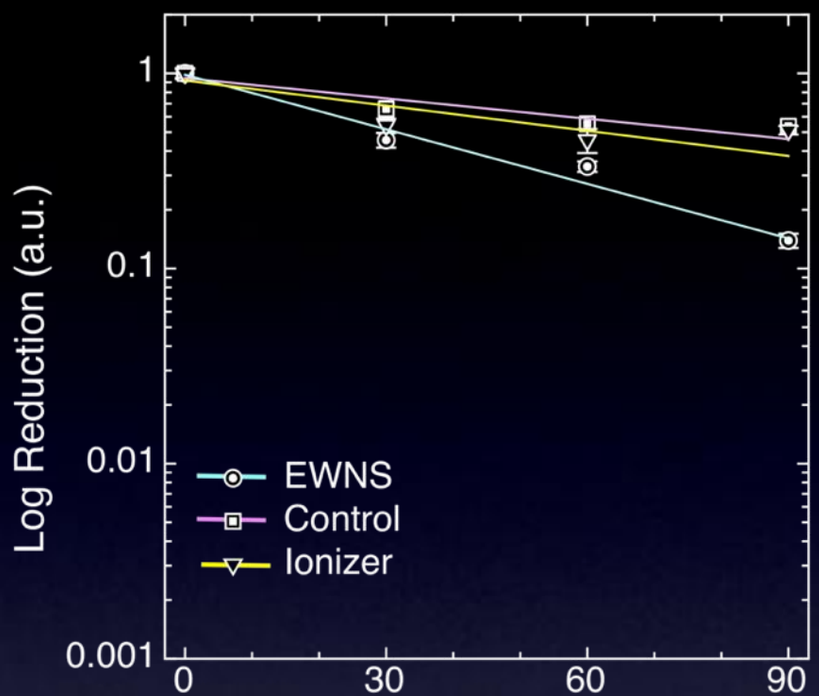


Pyrgiotakis G et al. J Nanopart Res. doi: 10.1007/s11051-012-1027

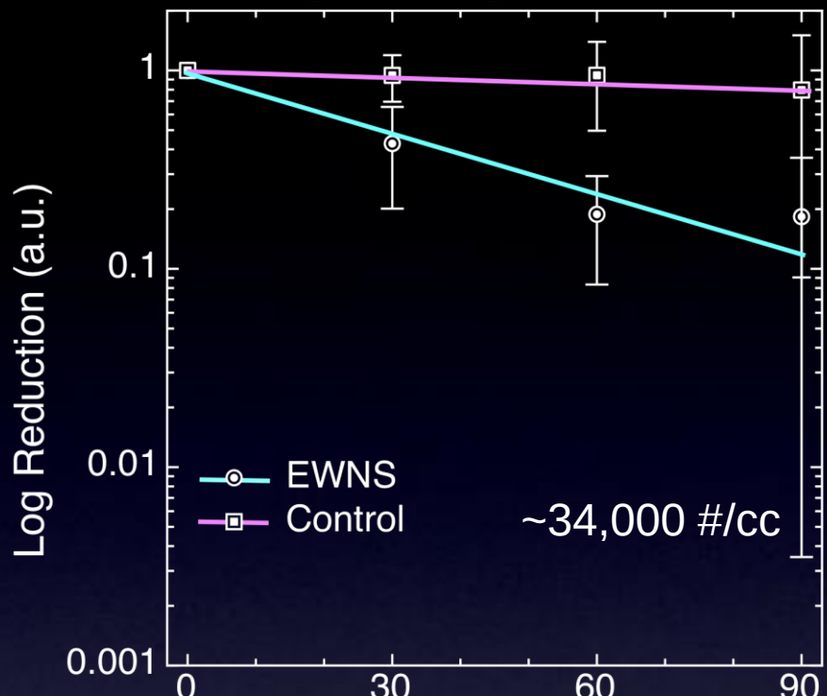
Surface inactivation – Results (1/2)



Serratia Marcescen

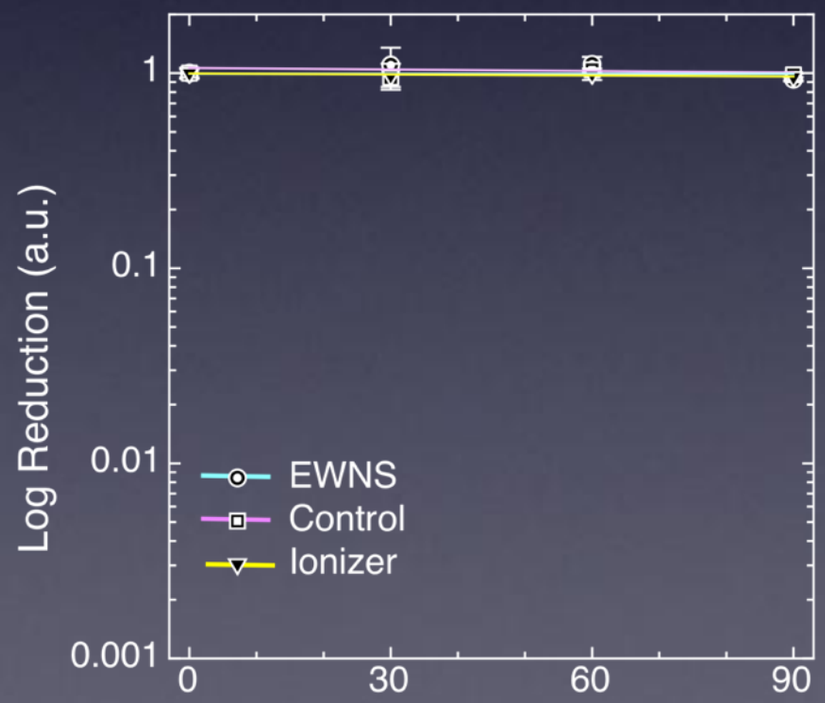


Staphylococus Aureus



Mycob. parafortuitum

- The structures were effective in inactivating the bacteria with the gram negative to be more susceptible than the gram positive.
- Mycobacterium has one log reduction (at higher dose)
- Spores, the ultimate survivors, under the same conditions were not effective at all.



Bacillus Subtillis

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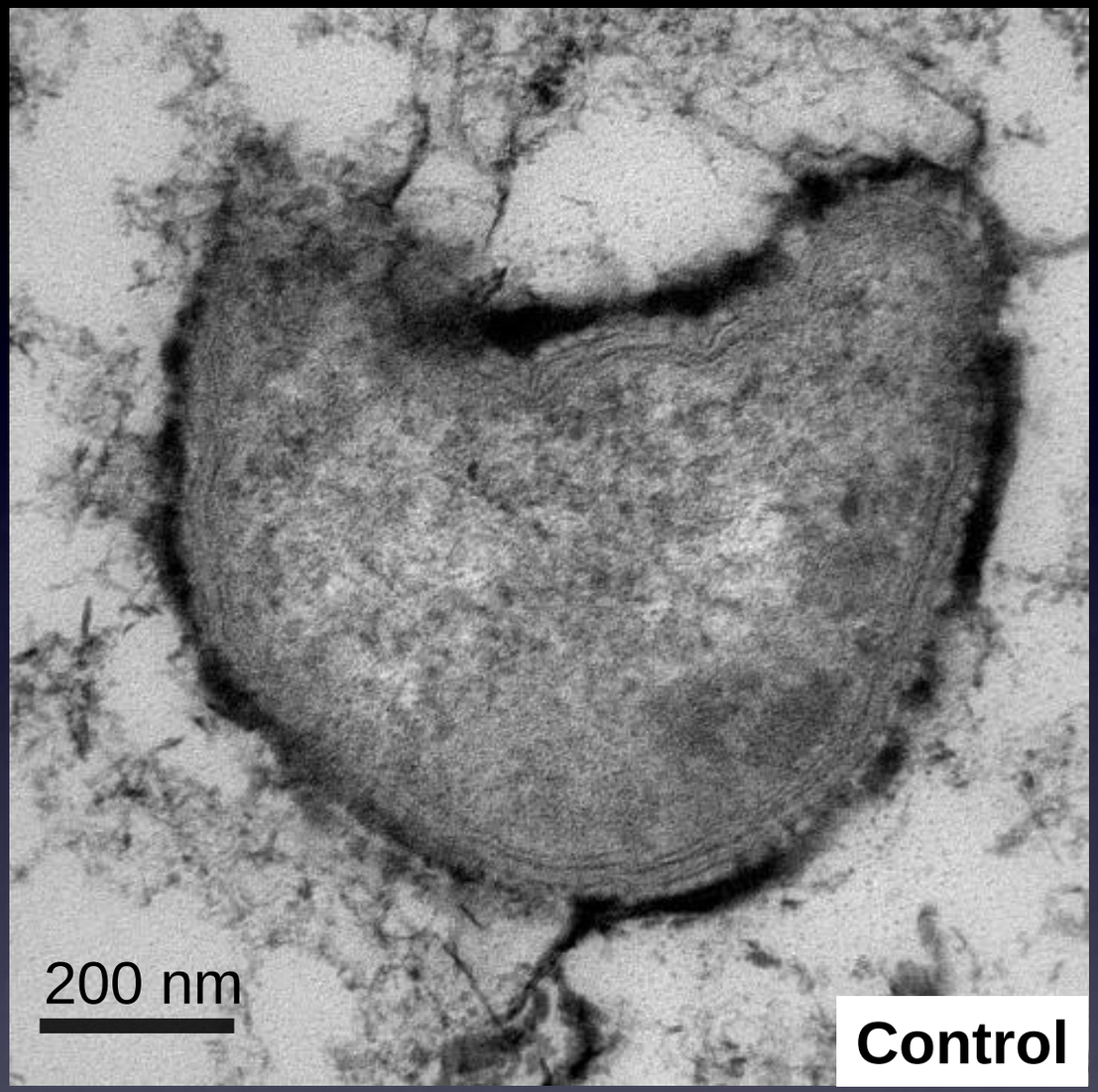
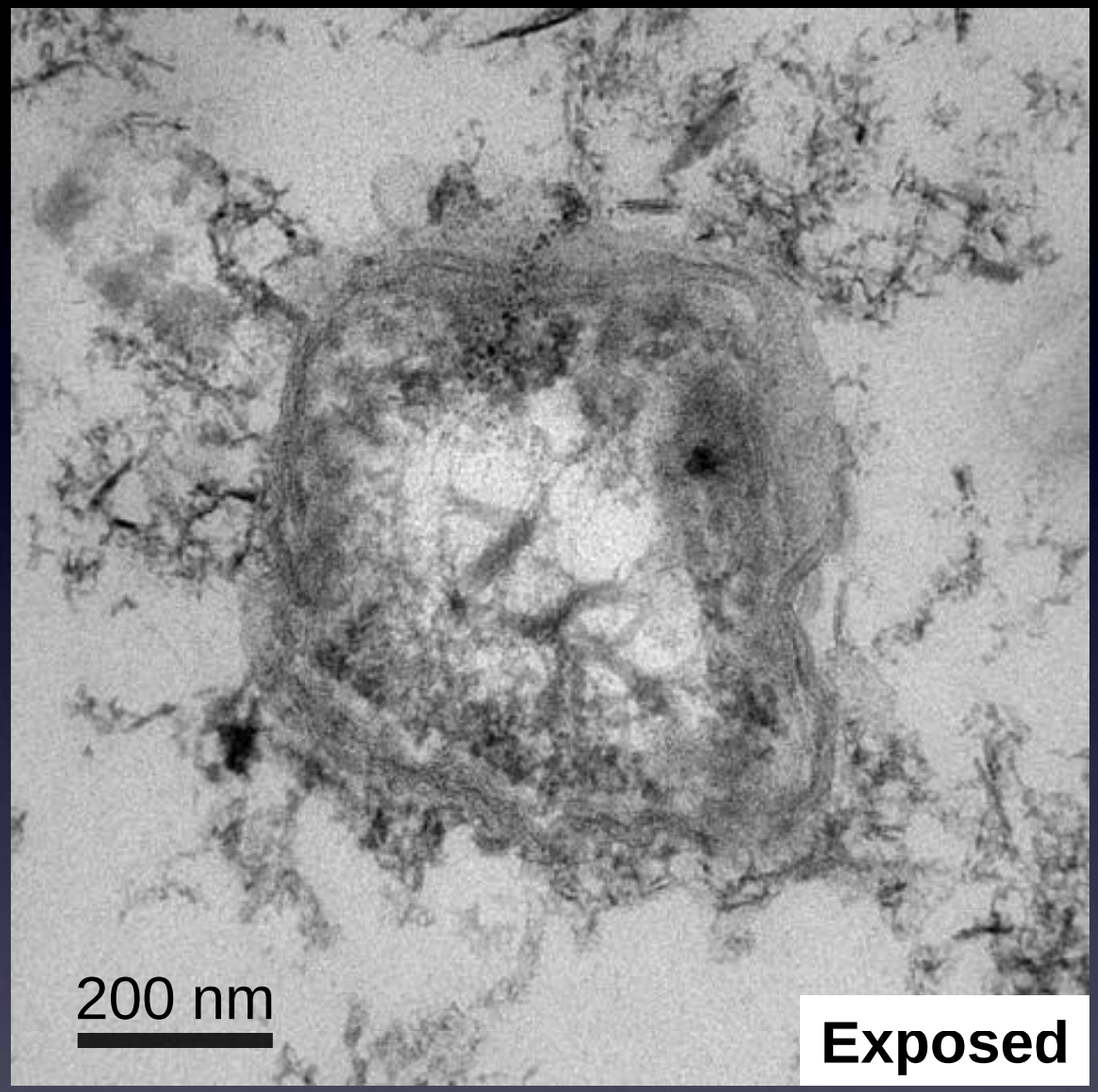
Surface inactivation – Results (2/2)



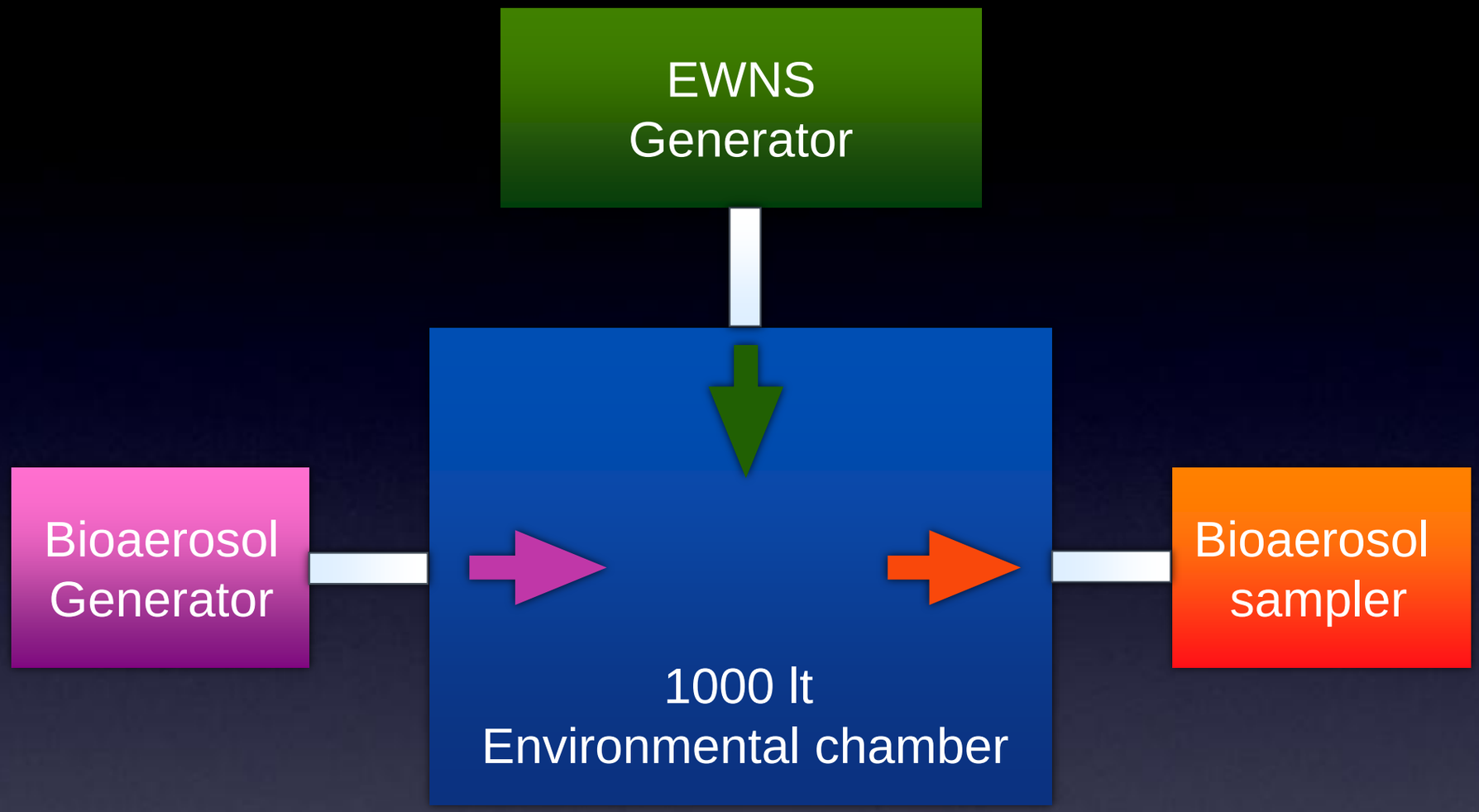


- After 90 minute exposure (TEM pic on the right)
- Destruction of the cell-wall
- The dead bacteria lost its shape and have developed a large vacuole

Antimicrobial properties Applications

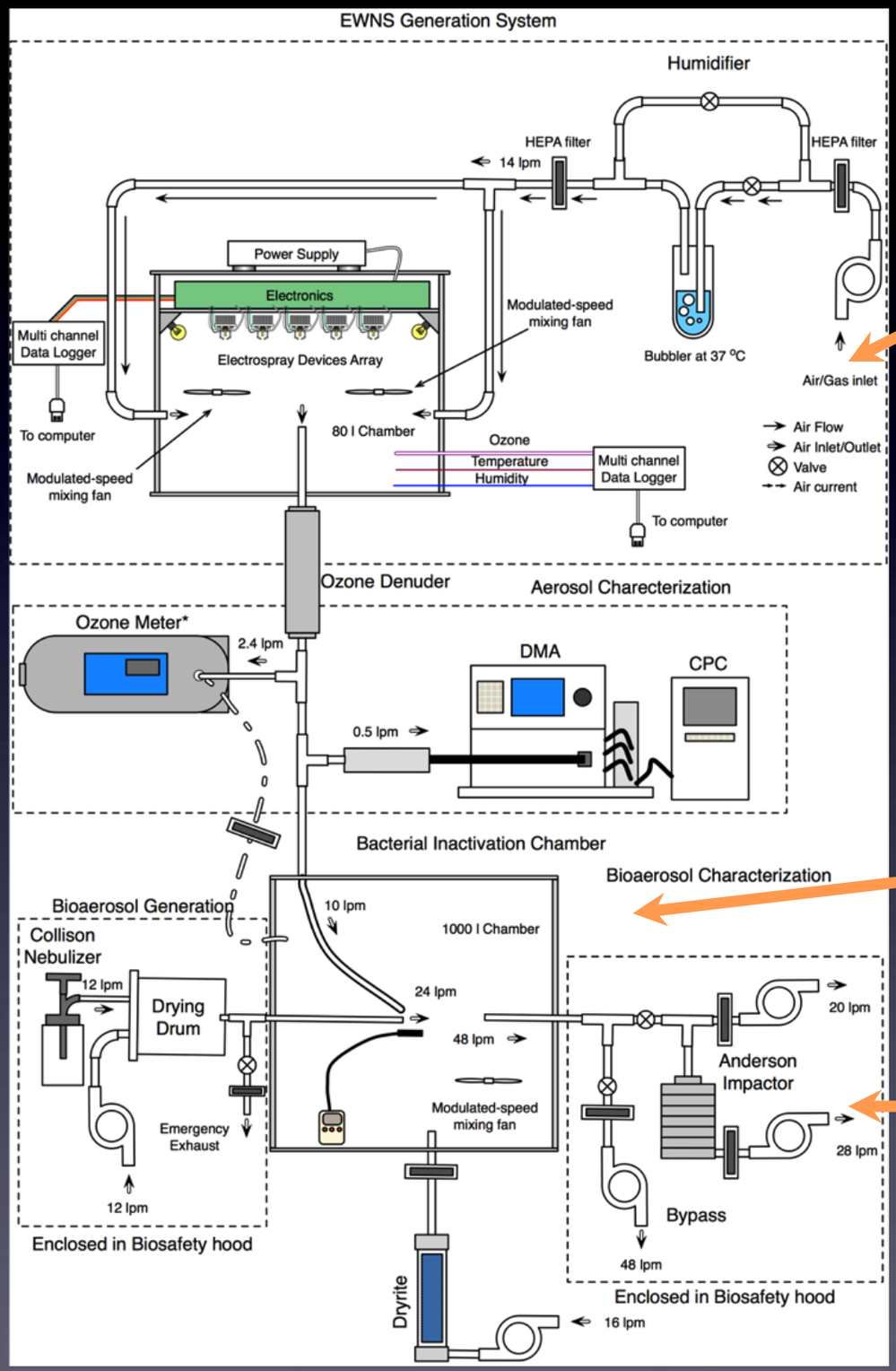
Air Inactivation

Air Disinfection - Methods



- Generate a bioaerosol of known concentration and mix it with a EWNS aerosol of controlled number of EWNS.
- Monitor the concentration of the bioaerosol as a function of time.
- Two exchange rate scenarios 2.9 ACH and 1.7 ACH

Air Disinfection - Methods



EWNS generator system

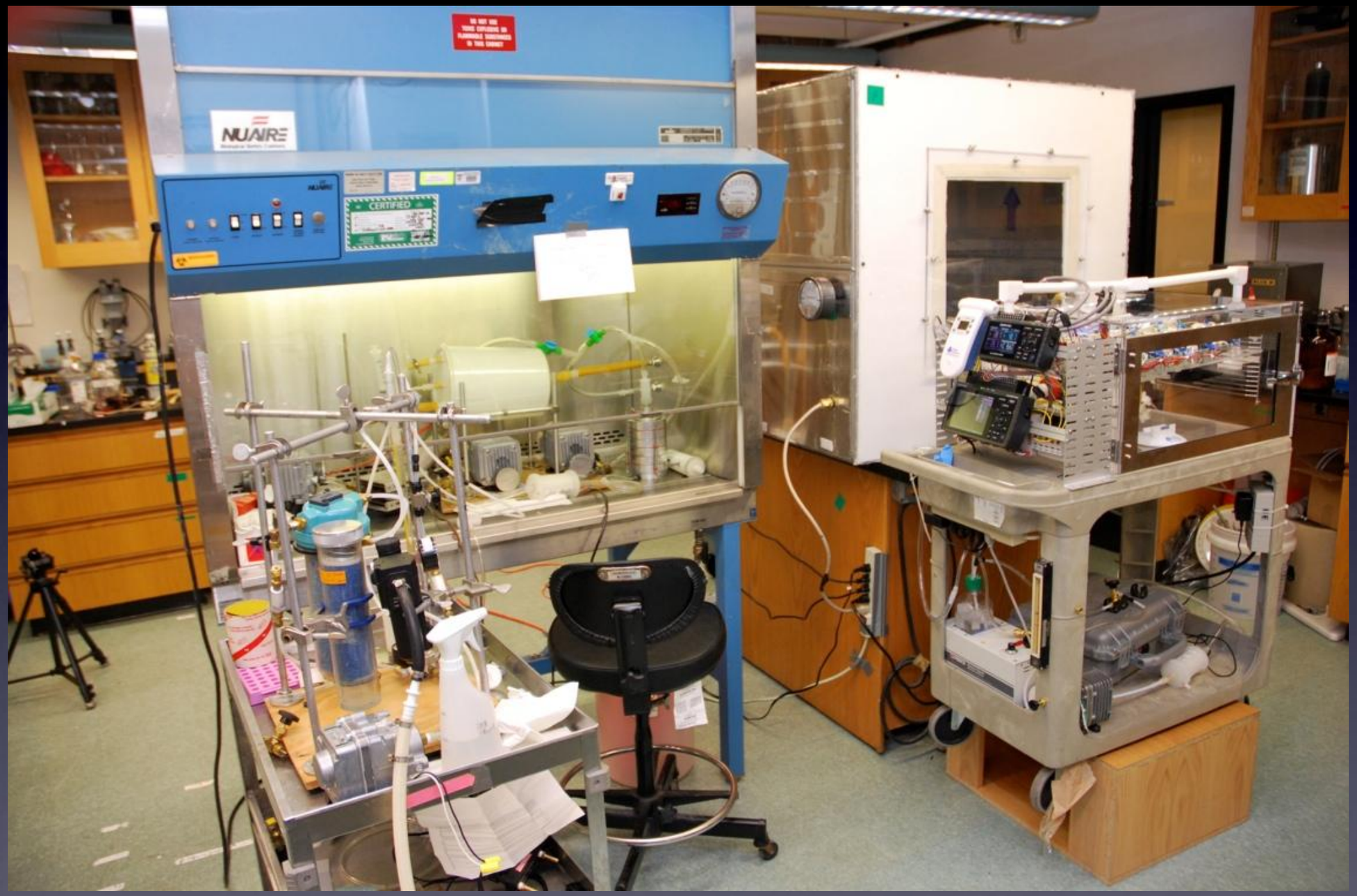
EWNS aerosol characterization system

Environmental chamber

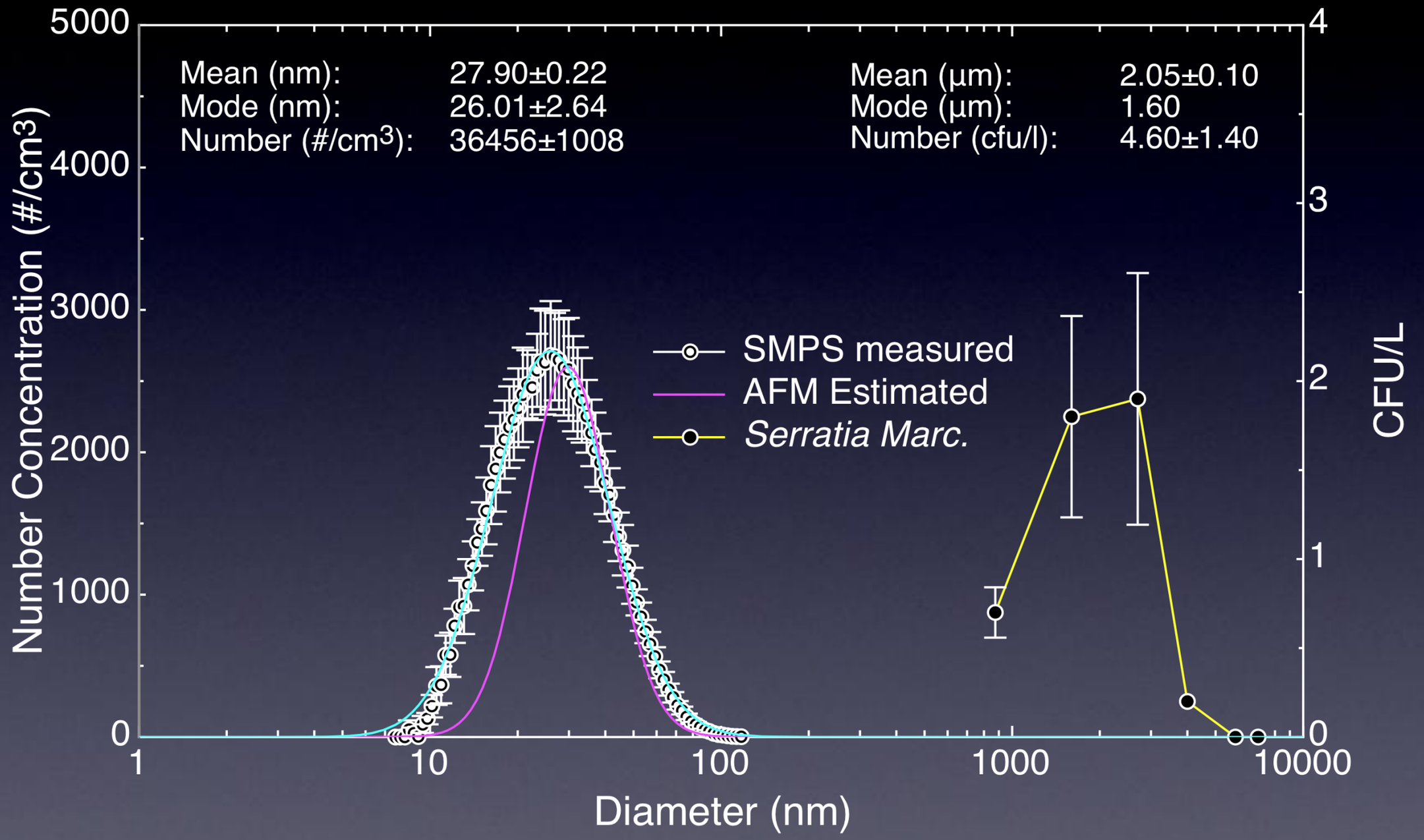
Bioaerosol generation system

Bioaerosol sampling system

Air Disinfection - Methods



Air Disinfection – Results (1/4)



2.9 ACH: R_b=EWNS/Bacteria=7.9 10^6 EWNS/cfu

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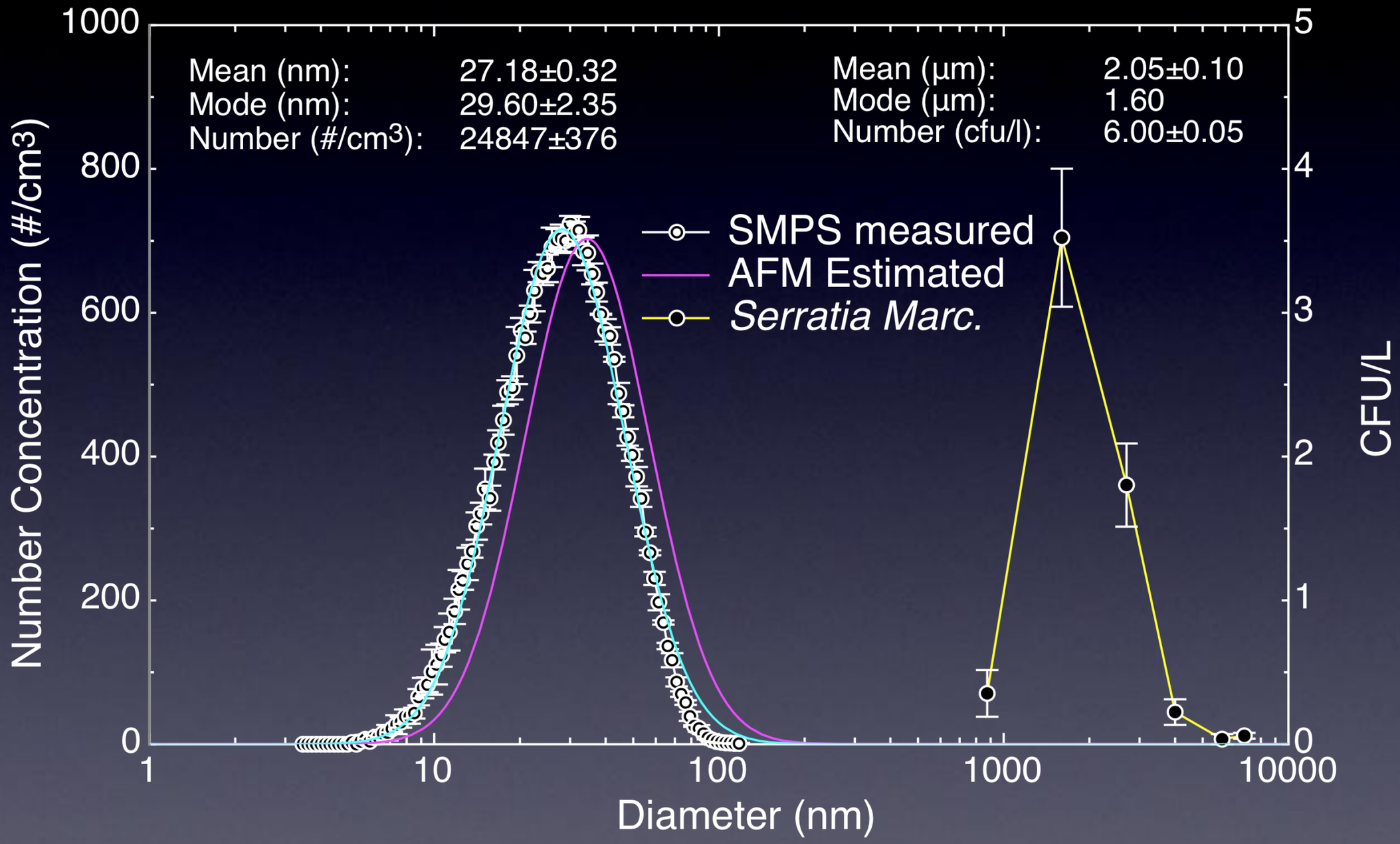
Air Disinfection – Results (2/4)



1.7 ACH: R_b=3.3 10^6 EWNS/cfu

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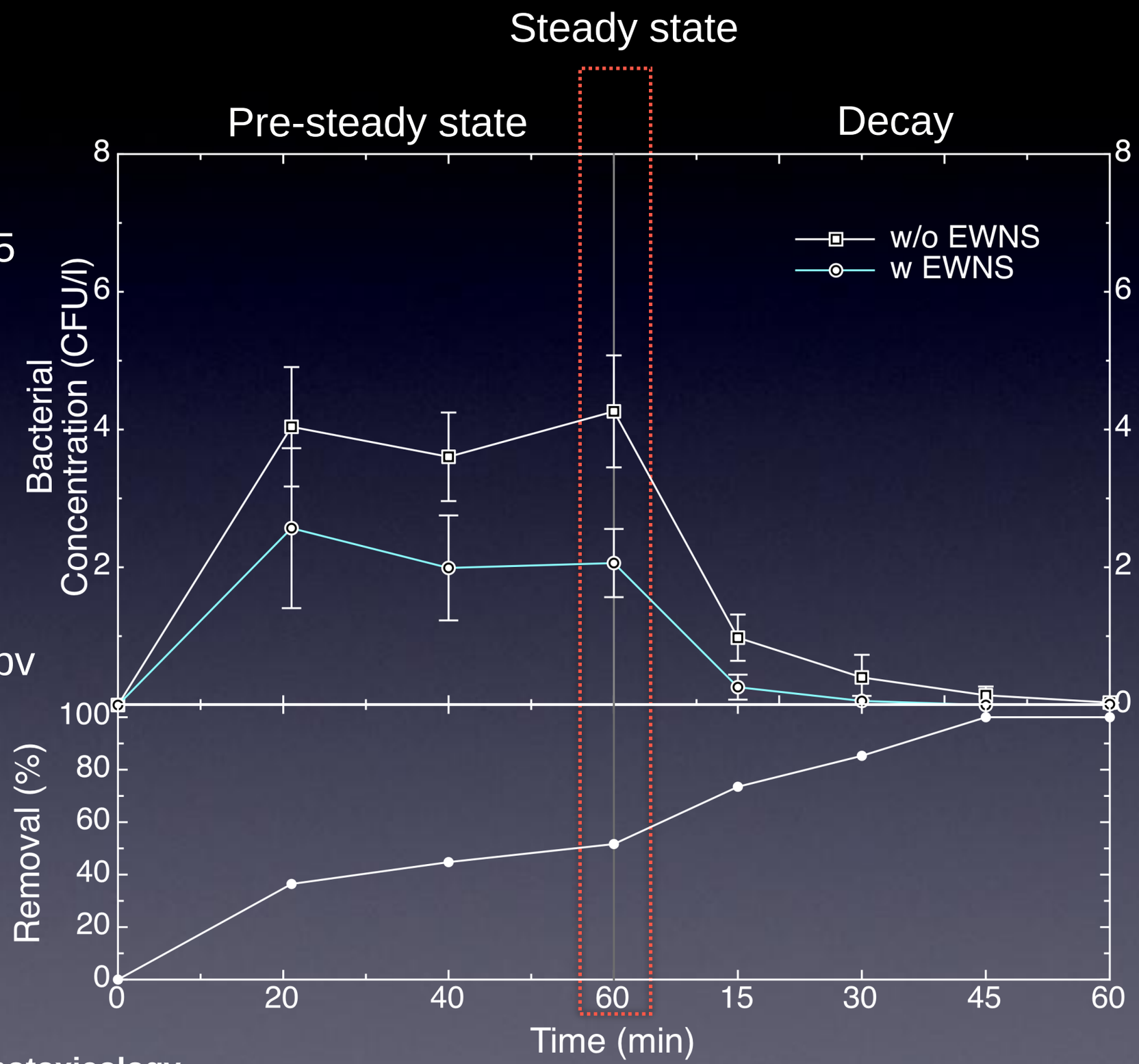
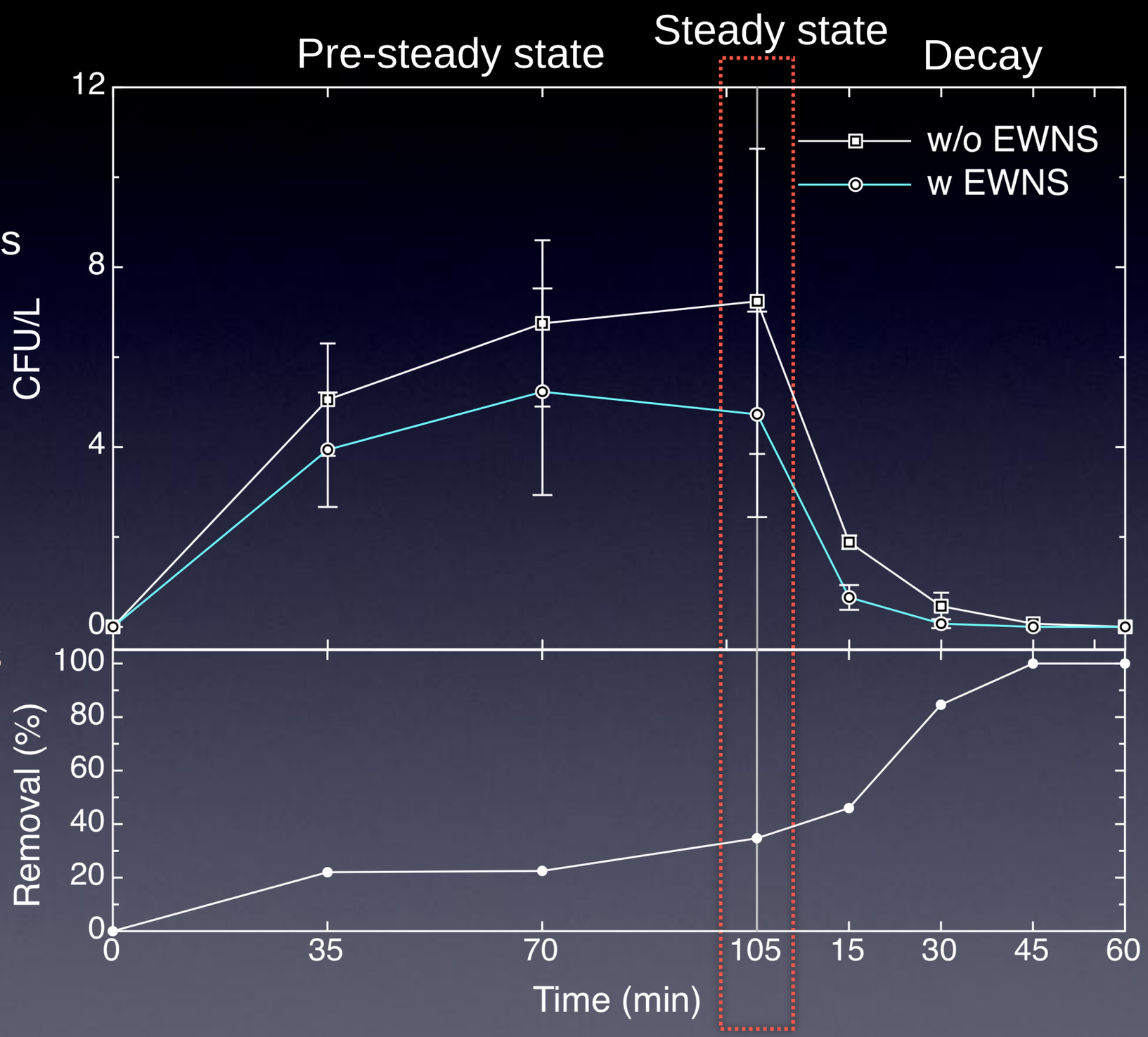
Air Disinfection – Results (3/4)

- Enviromental chamber conditions
  - R_b=8.7 10^6 EWNS/cfu
  - 3.0 ACH, res. Time= 19.5 mins
  - RH: 40%
  - T: 25 ^oC
  - Ozone 110.25±11.78 ppbv
- The EWNS can hinder the growth of bacteria (50% less CFU with the EWNS)
- Can completely remove the bacteria in ~30 mins.



Air Disinfection – Results (4/4)

- Enviromental chamber conditions
  - R_b=3.3 10^6 EWNS/cfu
  - 1.7 ACH, res. Time= 35 mins
  - RH: 47%
  - T: 25 ^oC
  - Ozone 148.54±15.56 ppbv
- The EWNS can hinder the growth of bacteria (~40% less CFU with the EWNS)
- Can completely remove the bacteria in ~ 30 mins and faster than scenario 1.



Toxicological Characterization

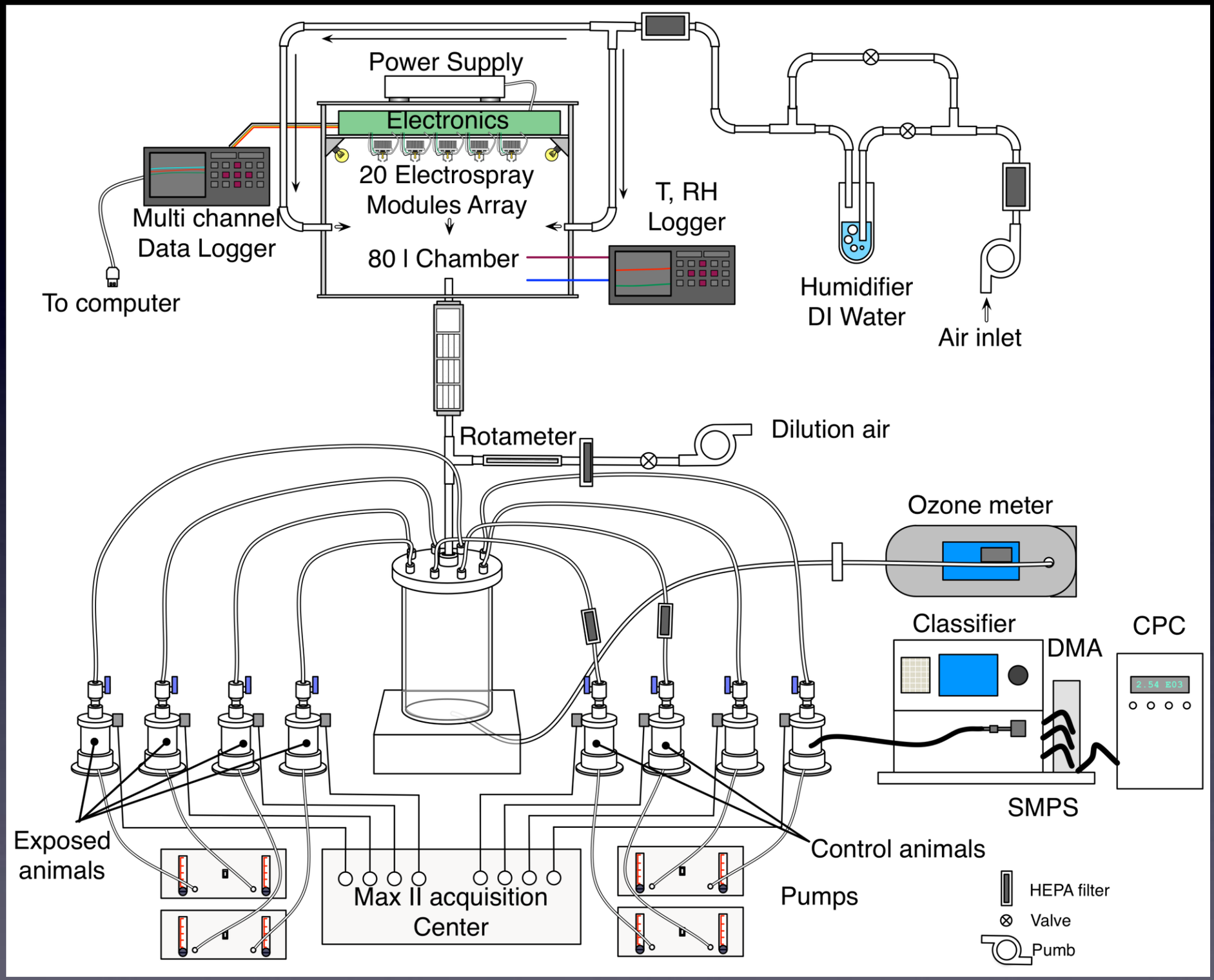
using a rodent model

Toxicological characterization - Methods

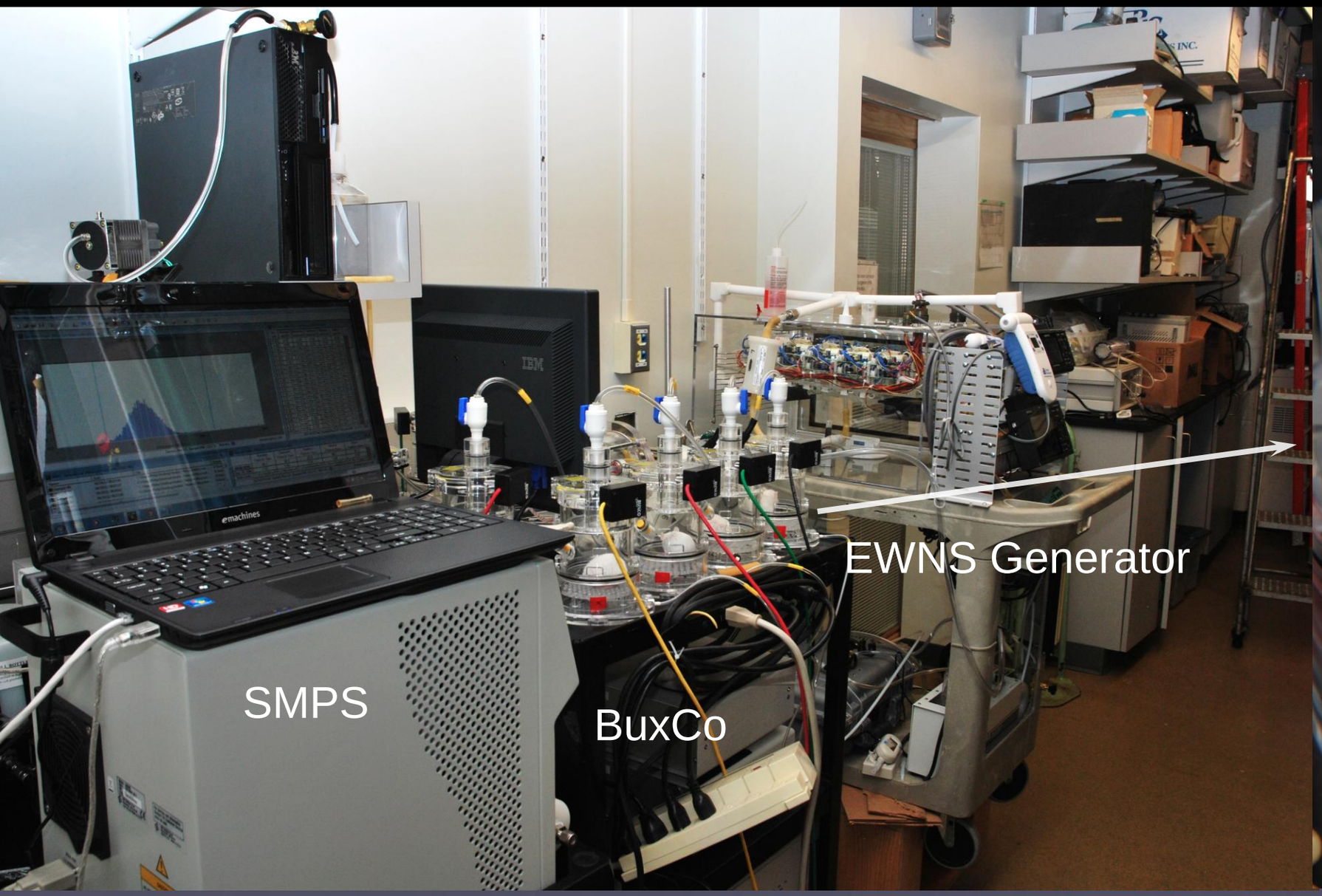
Study design

- Exposure system - Full body inhalation chambers
- 3 Groups of 6 mice (2 control, 4 exposed) for each dose
- 3 doses: 10,000 (surface inactivation), 40,000 p/cm^3 (air inactivation) and 60,000 p/cm^3
- 24 hours endpoint
- Bronchoalveolar lavage (BAL) and nasal lavage (NL) will be performed (obligatory nose breathers).
- Look for inflammation biomarkers:
  - Lactate dehydrogenase (LDH) – BAL/NL
  - Myeloperoxidase (MPO) – BAL
  - Albumin – BAL
  - Neutrophils – BAL
- Breathing patterns

Toxicological characterization - Methods



Toxicological characterization - Methods



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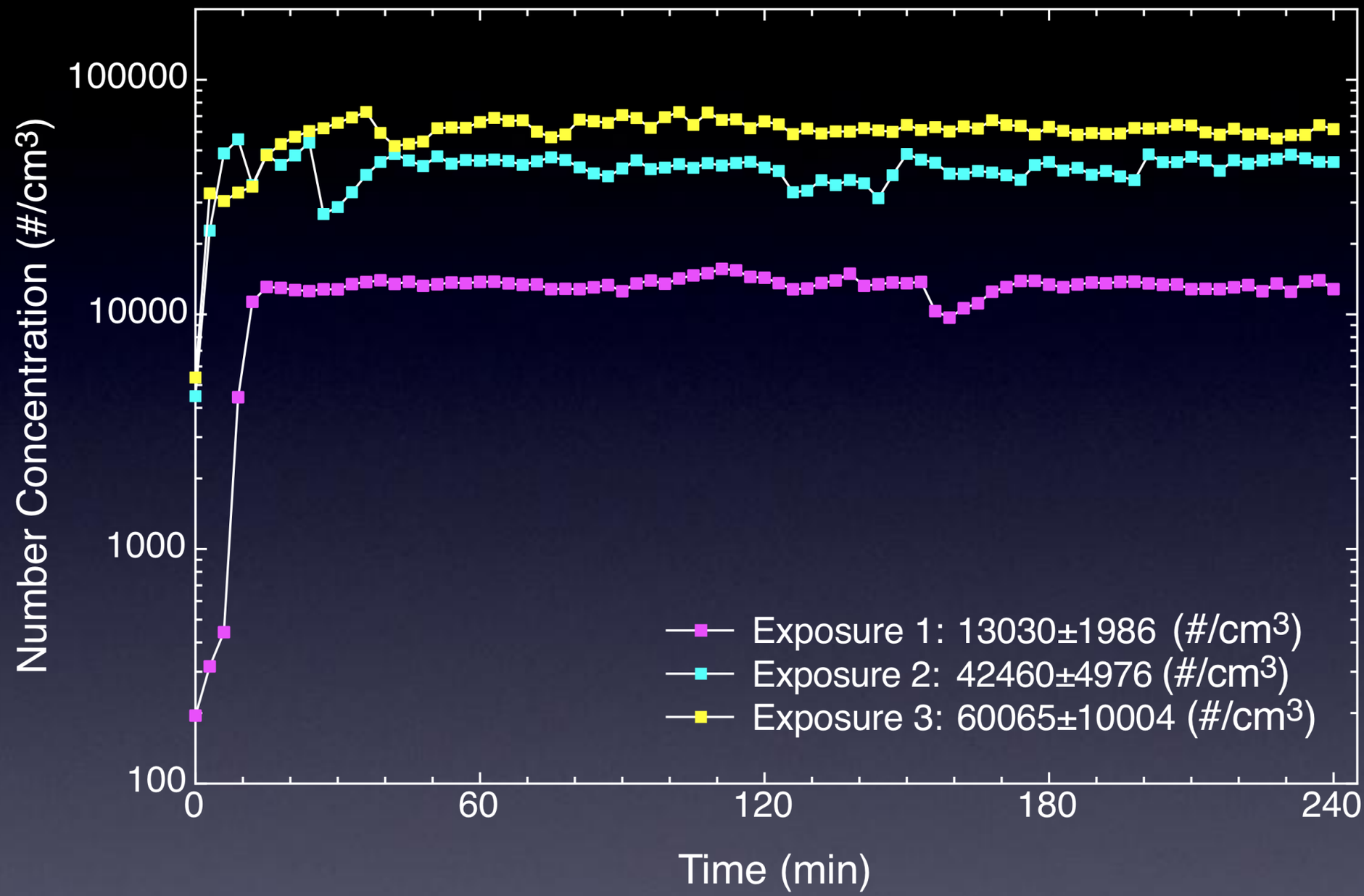
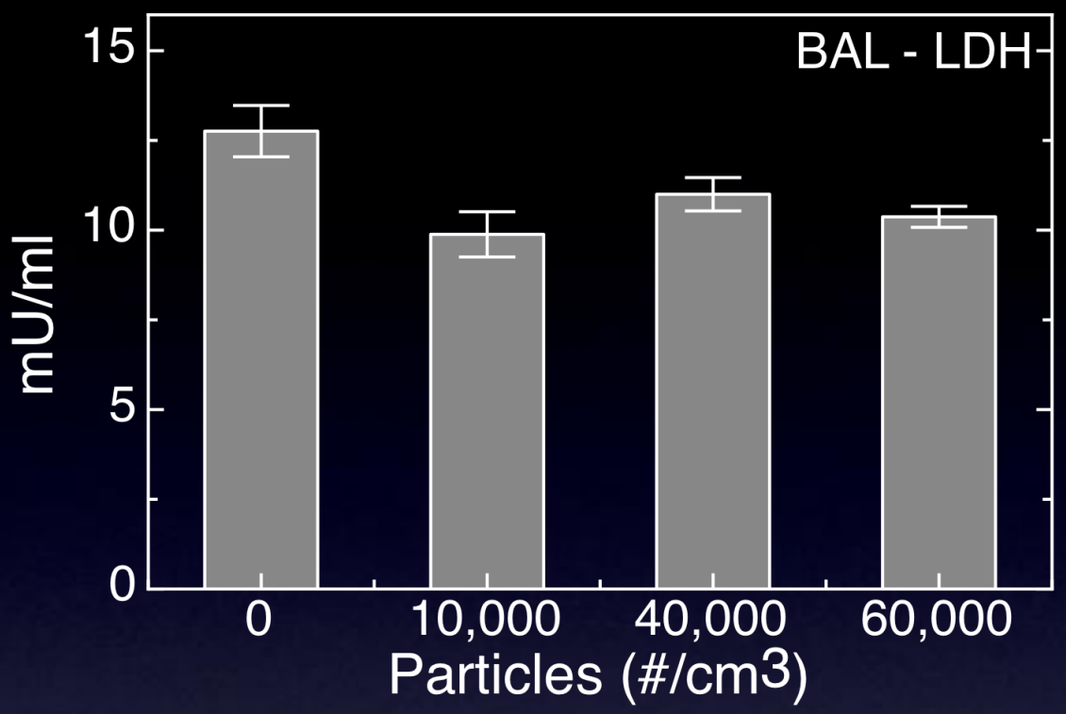
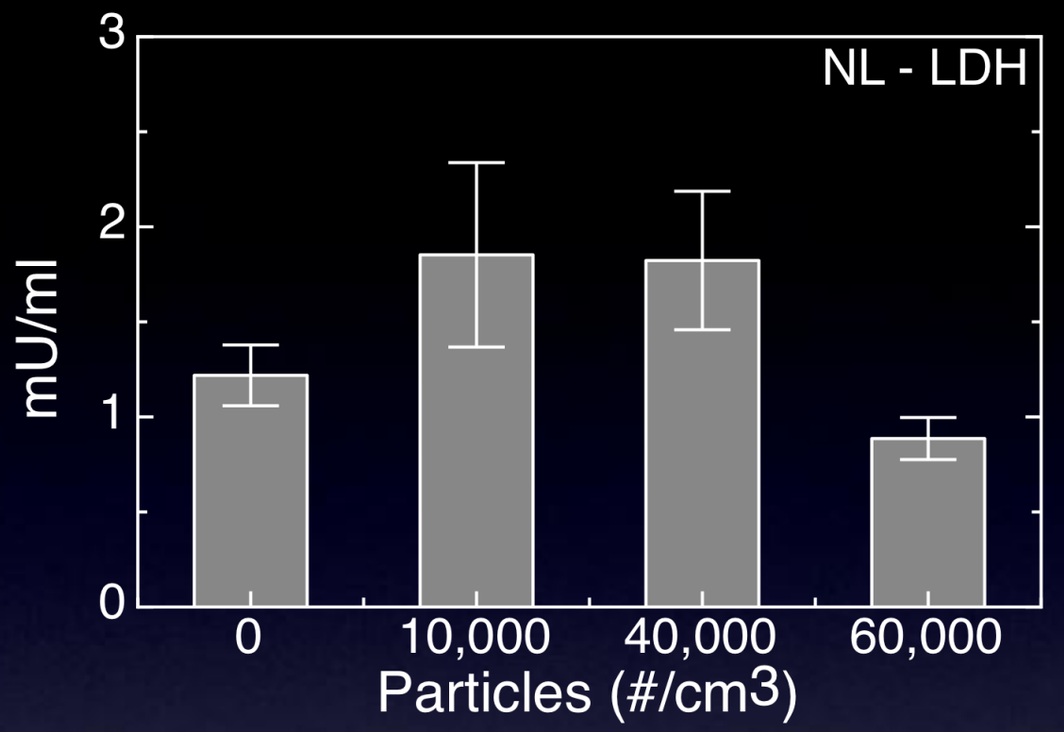
Toxicological characterization – Results (1/3)

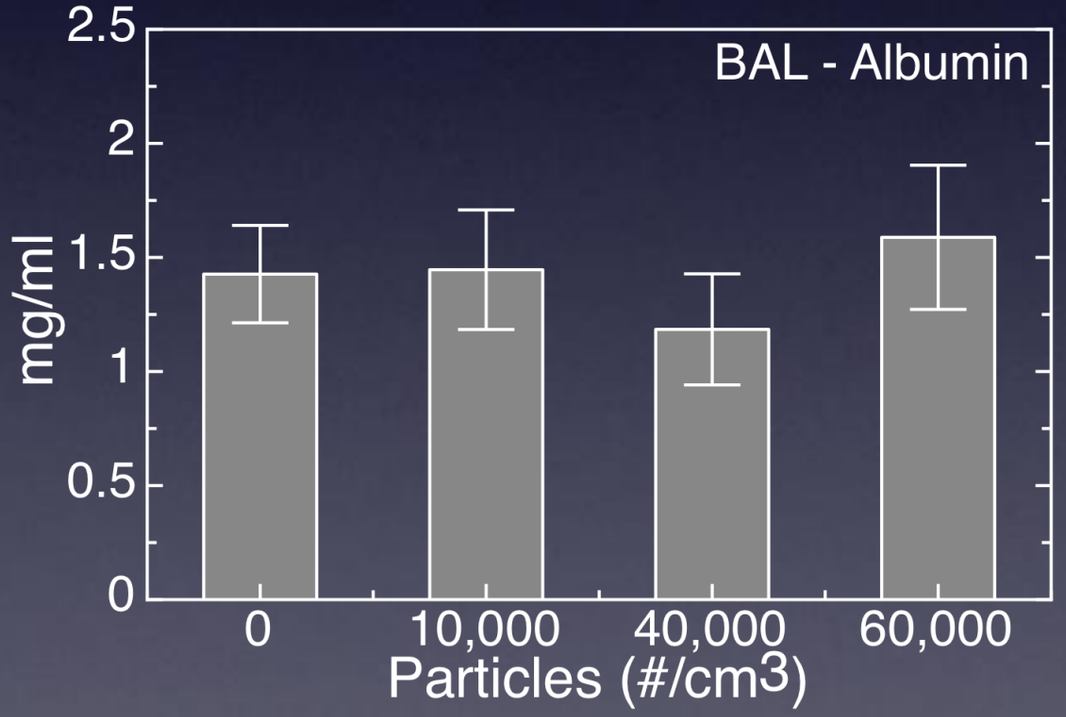


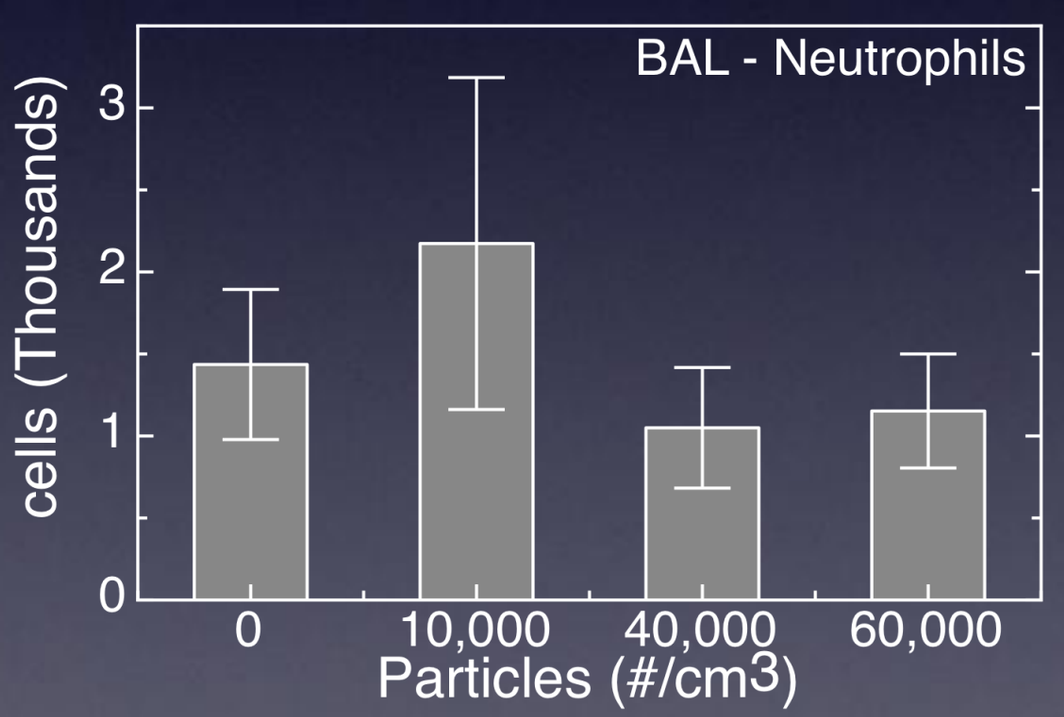
Very steady particle flow over a long periods of time. ~13,000 ,42,000 and 60,000 #/cc with 10, 25 and 35 ppbv of ozone respectively.

Toxicological characterization – Results (2/3)



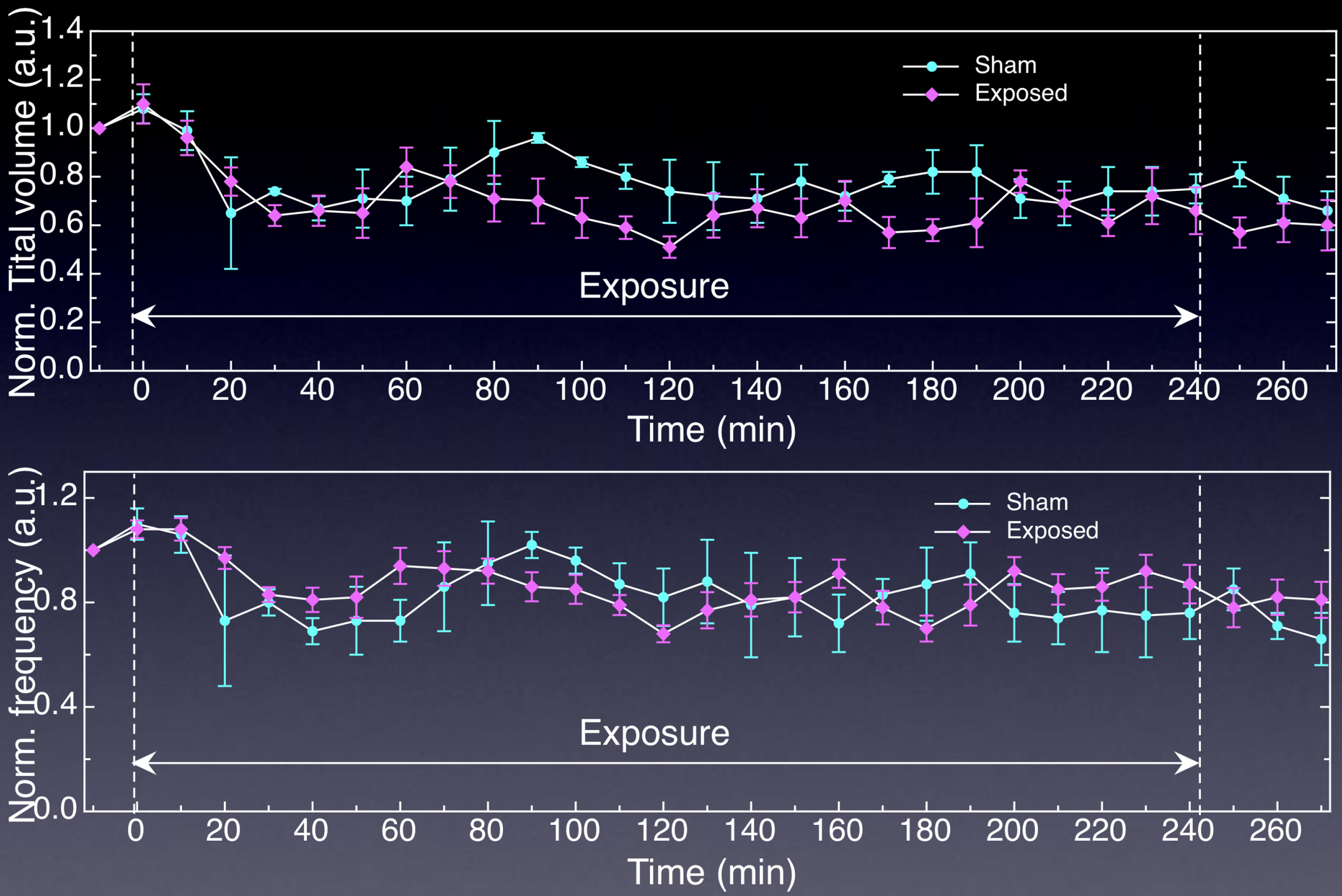






No statistically significant inflammation responses.

Toxicological characterization – Results (3/3)



No significant change in the breating pattern

Summary

- The Sustainable, green technology, chemical free using water with a great potential to revolutionize the pathogen disinfectin
- Unique PCM properties:
  - 10 ectrons per structure
  - loaded with $OH^{\bullet}$ and $O_2^{\bullet}$ radicals.
- Effective in air and on surfaces
- The toxicological evaluation showed that there is no effect at the concentrations that are effective for the surface and air inactivation.

Future Work

- Explore other applications:
  - Open wound – wound healing
  - Fresh produce disinfection
  - Air disinfection – airborne transmitted diseases (TB), flu

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Trust Science Innovation

Loaning an electrometer

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