

# *In Vitro Toxicity of Complex Aerosols: What are the key drivers?*

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# Air Pollution: A Complex Exposure

- Particulate Matter causes ~6 million premature deaths worldwide
- Combustion is a significant contributor

COOKSTOVES



WILDFIRES



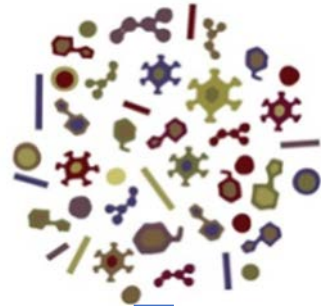
TRASH BURNING



What is the driver of toxicity?

Systematic *In Vitro* data can provide some answers

# How do you measure the toxicity of a particle?



Particles



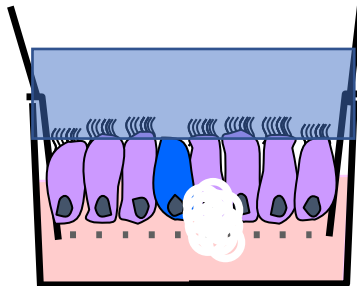
Resuspension Method



Filter



Elute with Solvent



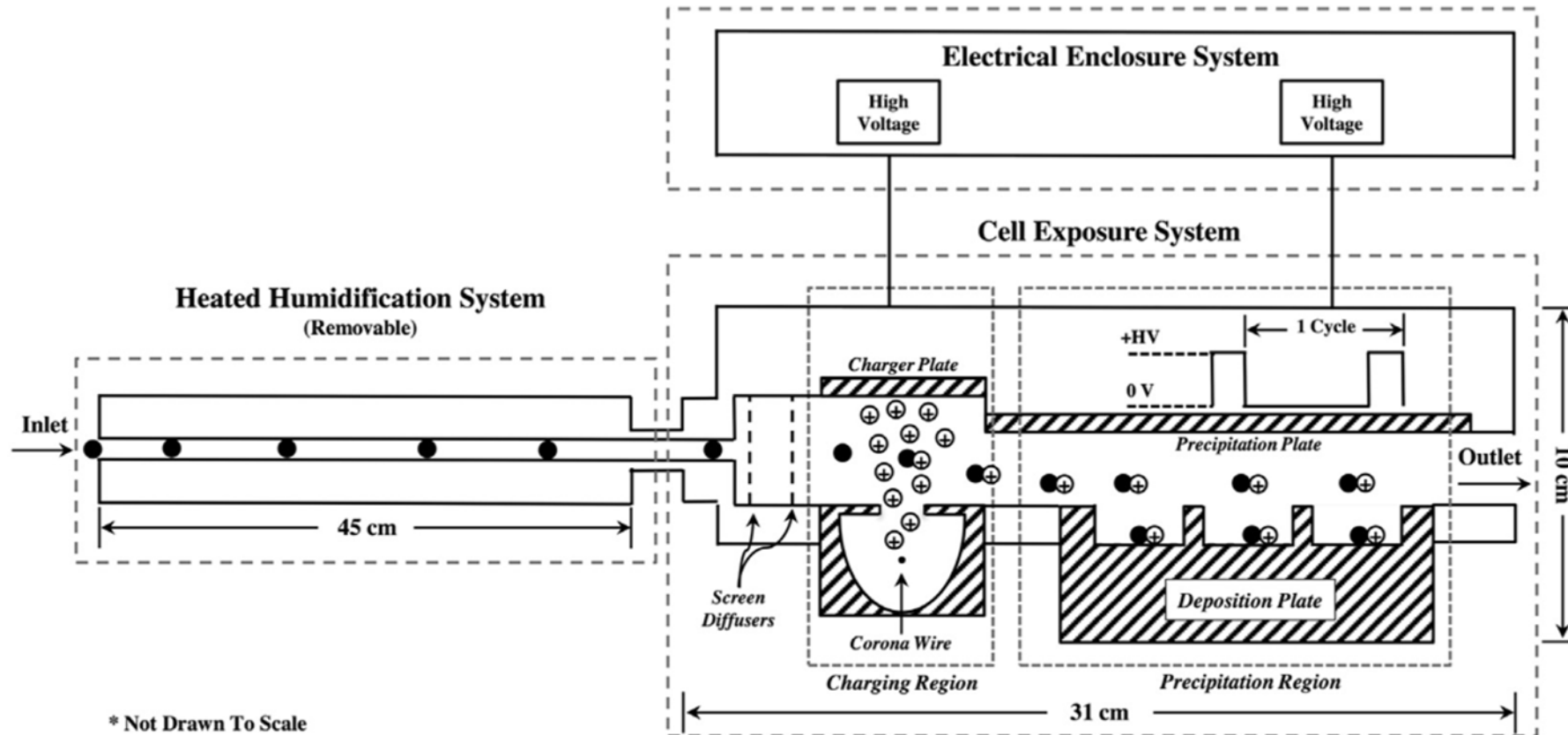
Submerged Cells

# *In Vitro* Technology

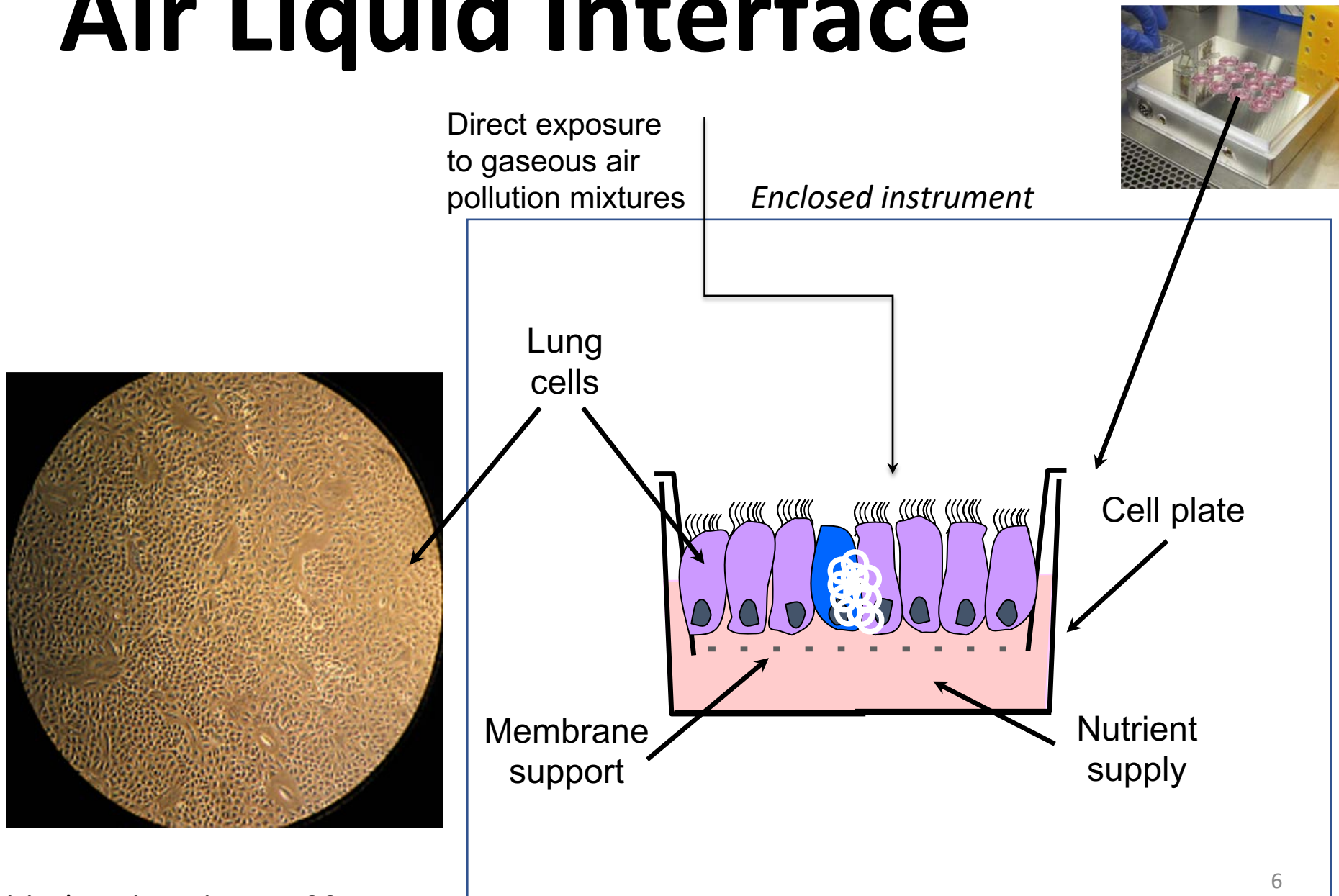
- Invented at UNC
- Electrostatic deposition mimics human lung
- PM composition, size, and interaction with gasses retained



# Novel *In Vitro* Technology

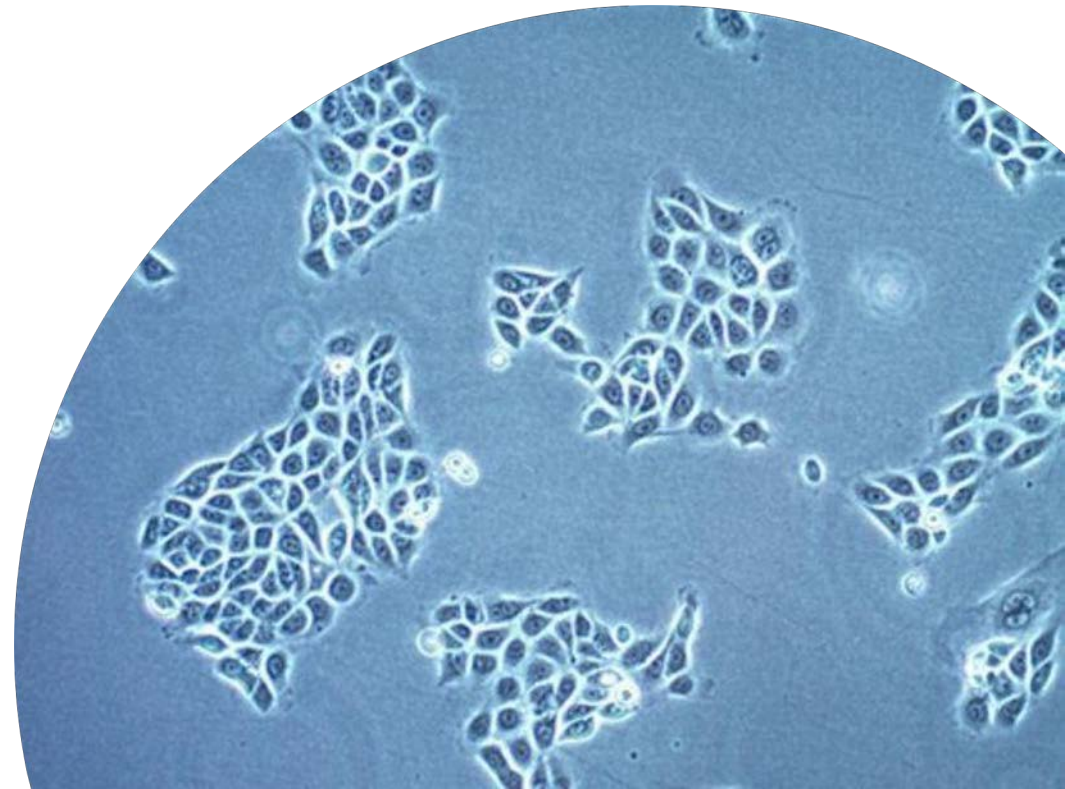


# Air Liquid Interface



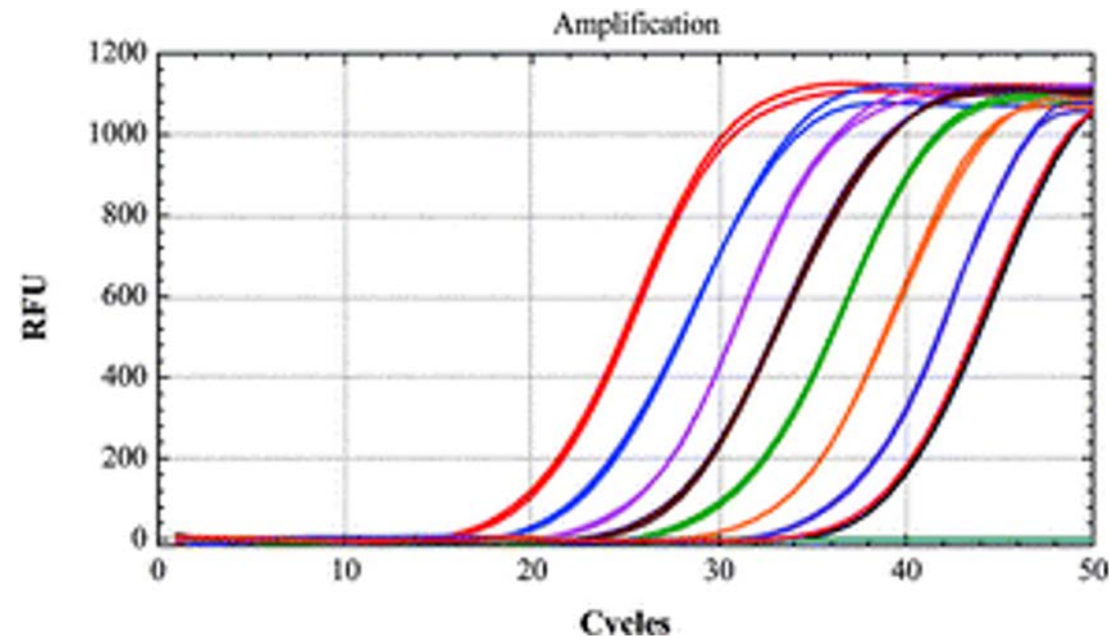
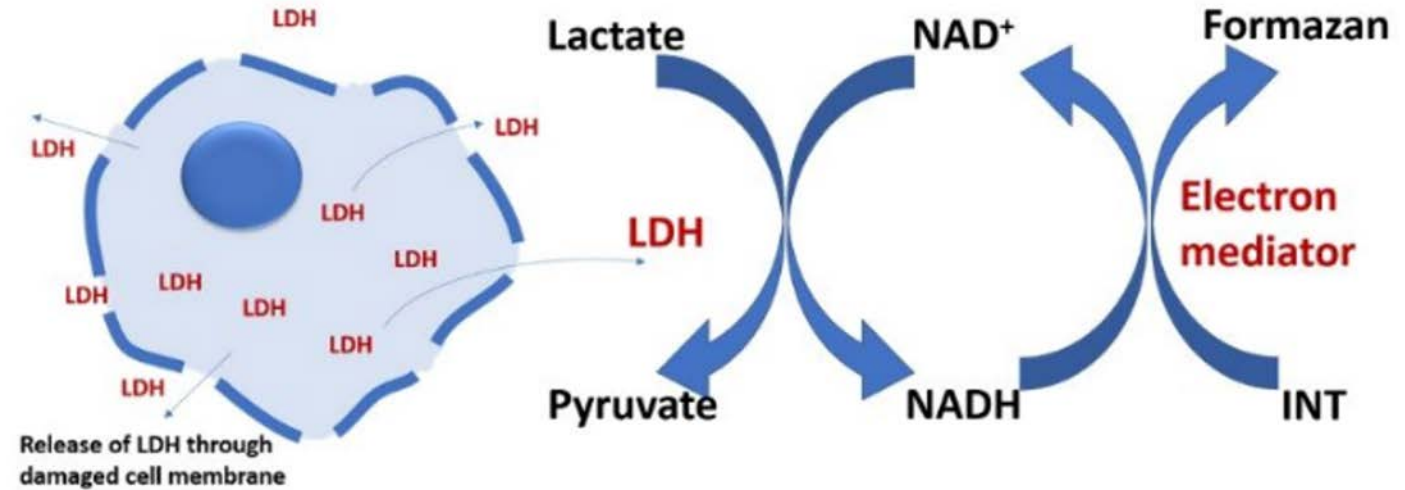
# Lung Epithelial Cells

- Human Bronchial Epithelial Cell Line (16HBE14o, BEAS2BEAS, EpiAirway, primary mice, A549)
- 250,000 cells plated per insert in complete growth medium
- Grown to confluency
- Growth medium replaced with low serum medium two days prior to exposure



# Biological Impact

- **Cytotoxicity**
  - Release of Lactate dehydrogenase (LDH)
- **Gene Expression**
  - Inflammation markers
    - IL-6 & IL-8
  - Oxidative stress markers
    - HMOX1 & COX-2





# Exposure Experiments

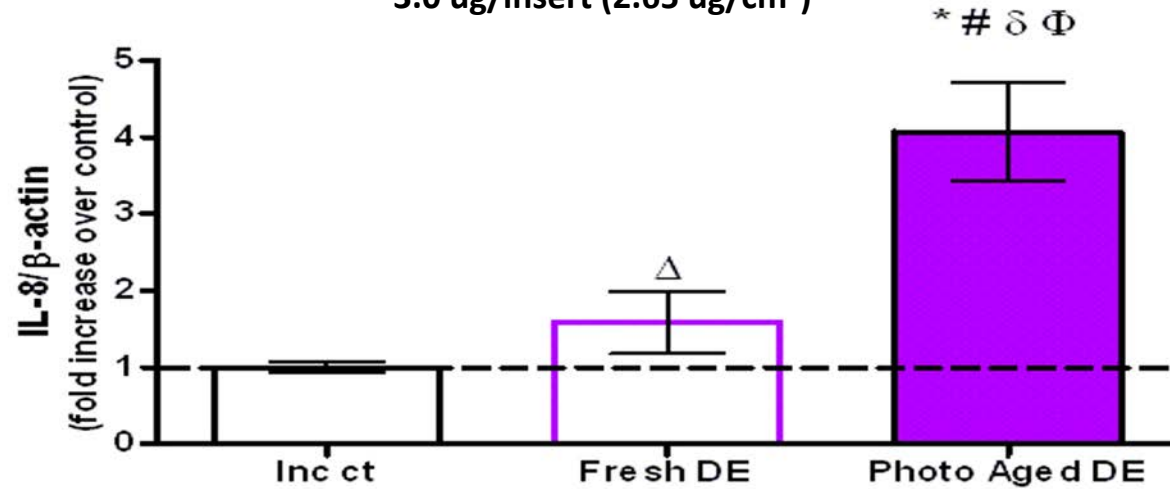
- Diesel emissions
- Photochemically “aged” and fresh
- Comparison with resuspension
- Wide array of genes



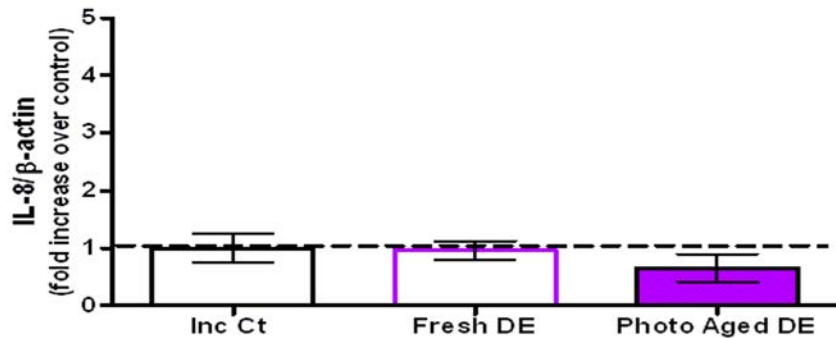
Lichtveld, Environ. Sci. Technol. 2012, 46, 9062–9070

# Genomic Responses

~ 3.0 ug/insert (2.65 ug/cm<sup>2</sup>)

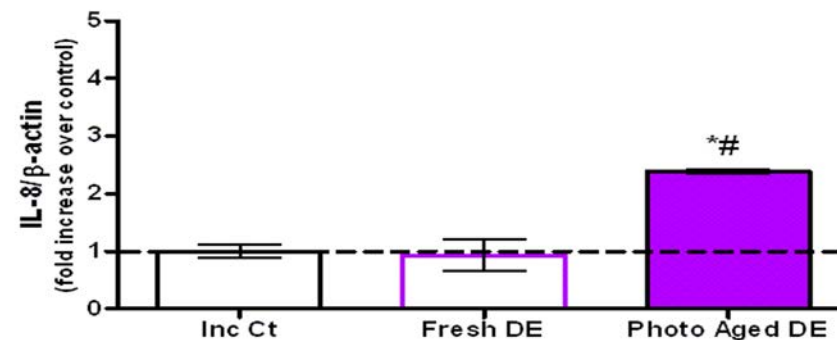


~ 3.0 ug/insert (2.65 ug/cm<sup>2</sup>)



Re-suspension

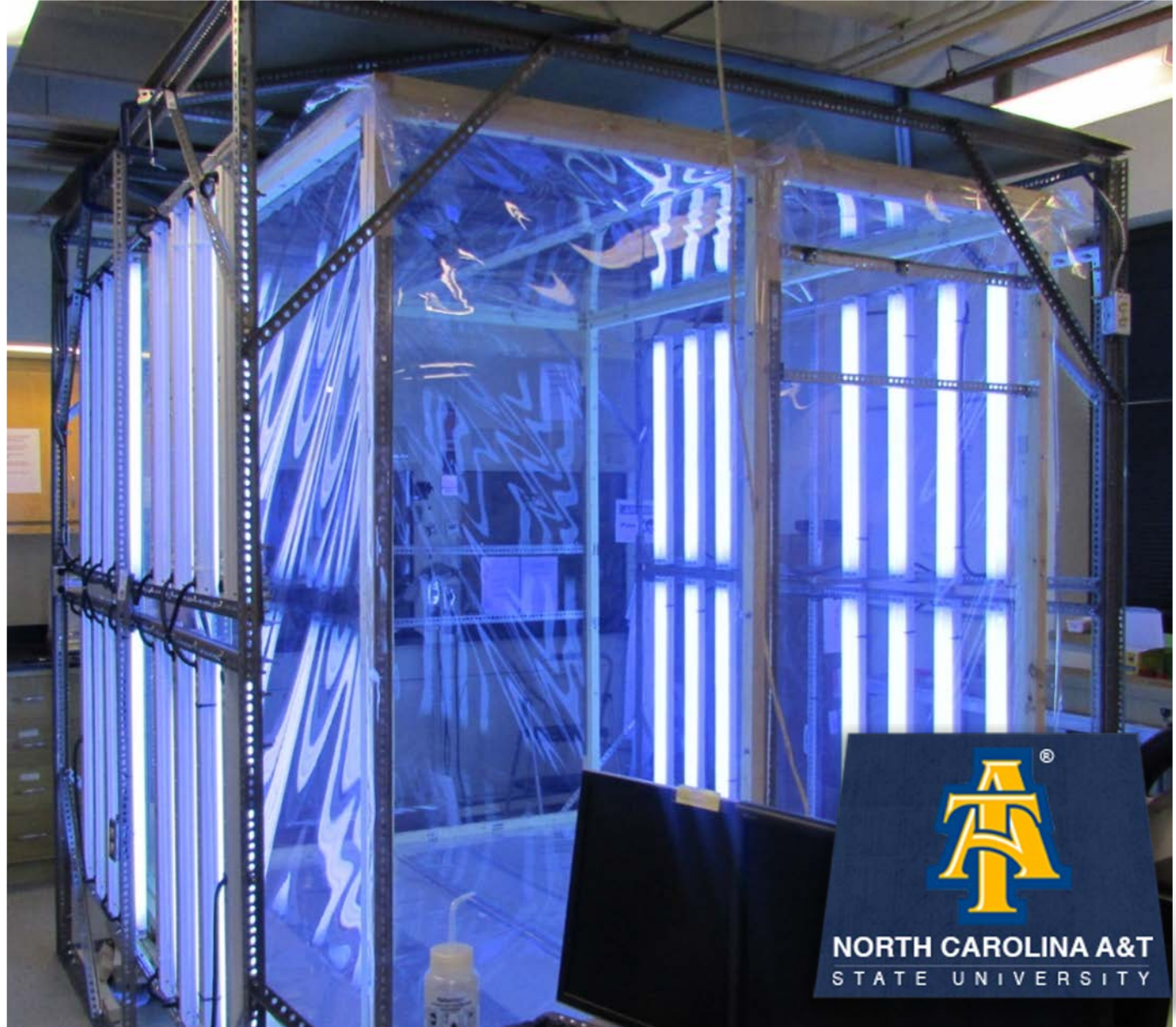
~ 48.0 ug/insert (42.4 ug/cm<sup>2</sup>)



Re-suspension

# Exposure Experiments

- Biomass Burning
- White Pine, Smoldering
- Fresh and Aged
- Ultrafine exposures



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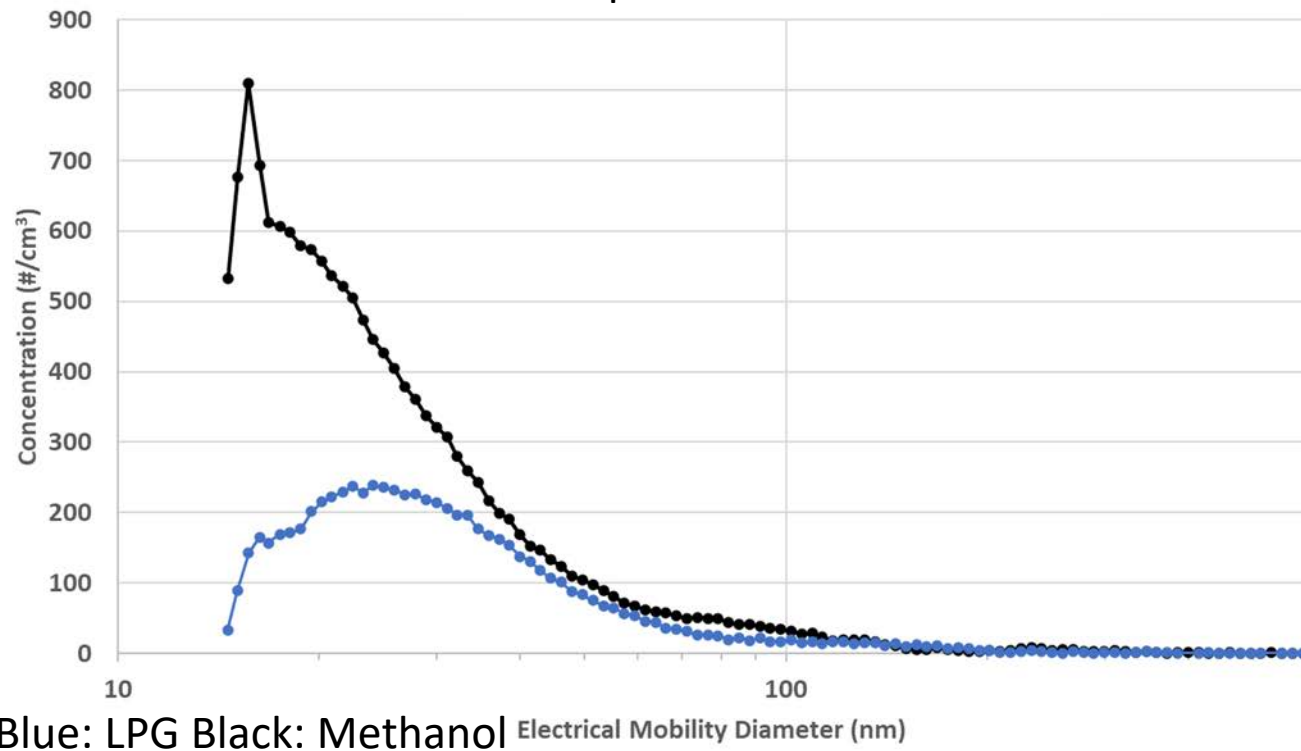
# Preliminary Biomarker Results Ultrafine PM

- Cytotoxicity
  - Significant difference at immediate and 4-hr post exposure
  - More release from cells then at immediate collection.
- Genomic Responses (vs incubator)
  - Significant increase in all genes for cells exposed to ultrafines at immediate collection
  - Similar response as aged exposures
  - After 4 hours:
    - IL-6 and IL-8 had a significant decrease in expression
    - HMOX1 was significantly increased in expression
    - No significant difference in expression of COX-2

# Exposure Experiments

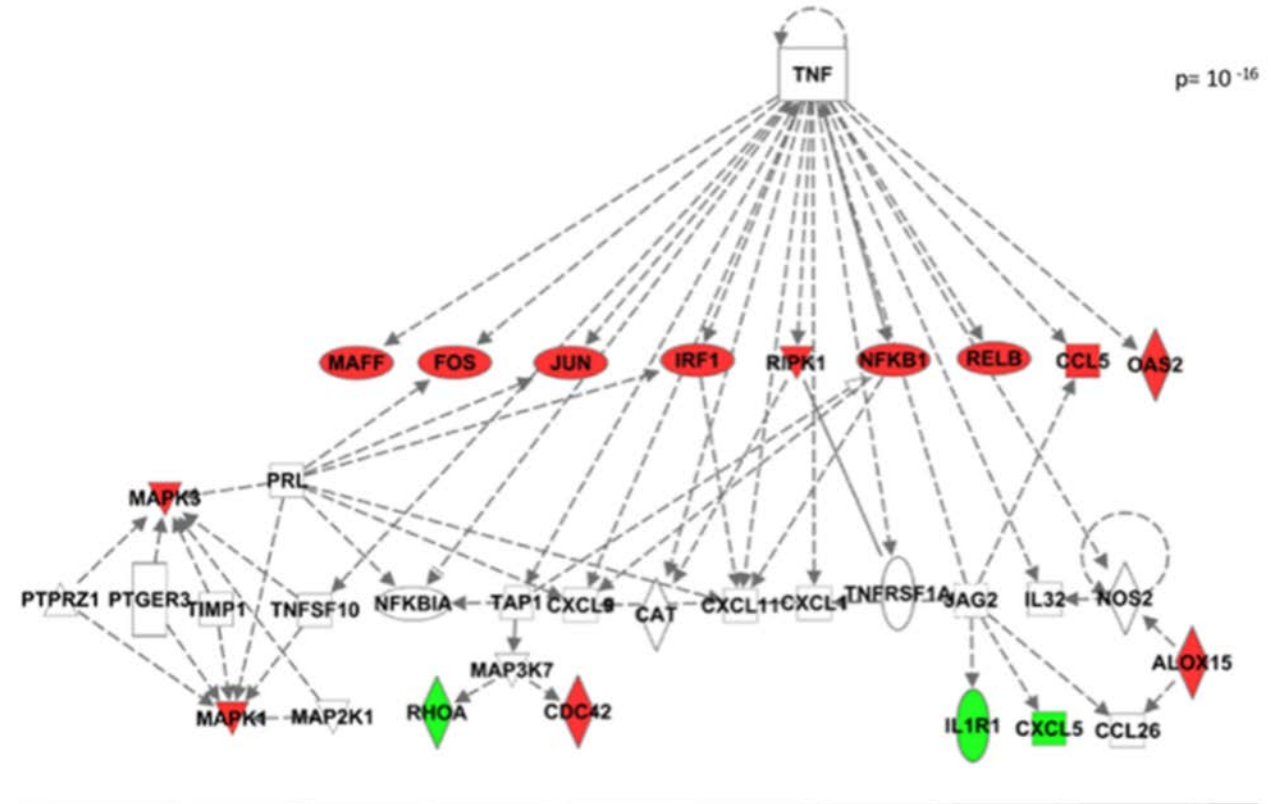
## LPG and MeOH Cookstoves Fresh, Ultrafines

Mean particle size distribution



# Exposure Experiments

## Ambient Conditions (BEE-TEX campaign)



- Clusters of chemicals identified, correlated with gene expression levels
- Benzene was a prominent pollutant among clusters
- Relevant pathway signaling of three genes (FN1, TNF, and TP53) that have been shown to be associated with lung cancer.

# Impacts

- **Chemistry and Size Matters**
  - Demonstrate oxidized particles are more toxic
  - Ultrafine cytotoxicity and similar genomic responses to aged aerosols
  - Influence interventions and regulations based on size and mass
- **Toxicity estimation**
  - More realistic analog of human inhalation will change the way *in vitro* studies are conducted in the field and lab
  - Successful establishment of methods and use of novel instrumentation in field
  - Used to identify hot spots of toxicity, confirm laboratory findings of drivers of toxicity



Questions?

<http://vizuete.web.unc.edu>