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(DNA Tagged Reagents for Aerosol eXperiments) Barcoded Aerosols Allow for Simultaneous Releases

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(DNA Tagged Reagents for Aerosol eXperiments) Barcoded Aerosols Allow for Simultaneous Releases

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LLNL-PRES-
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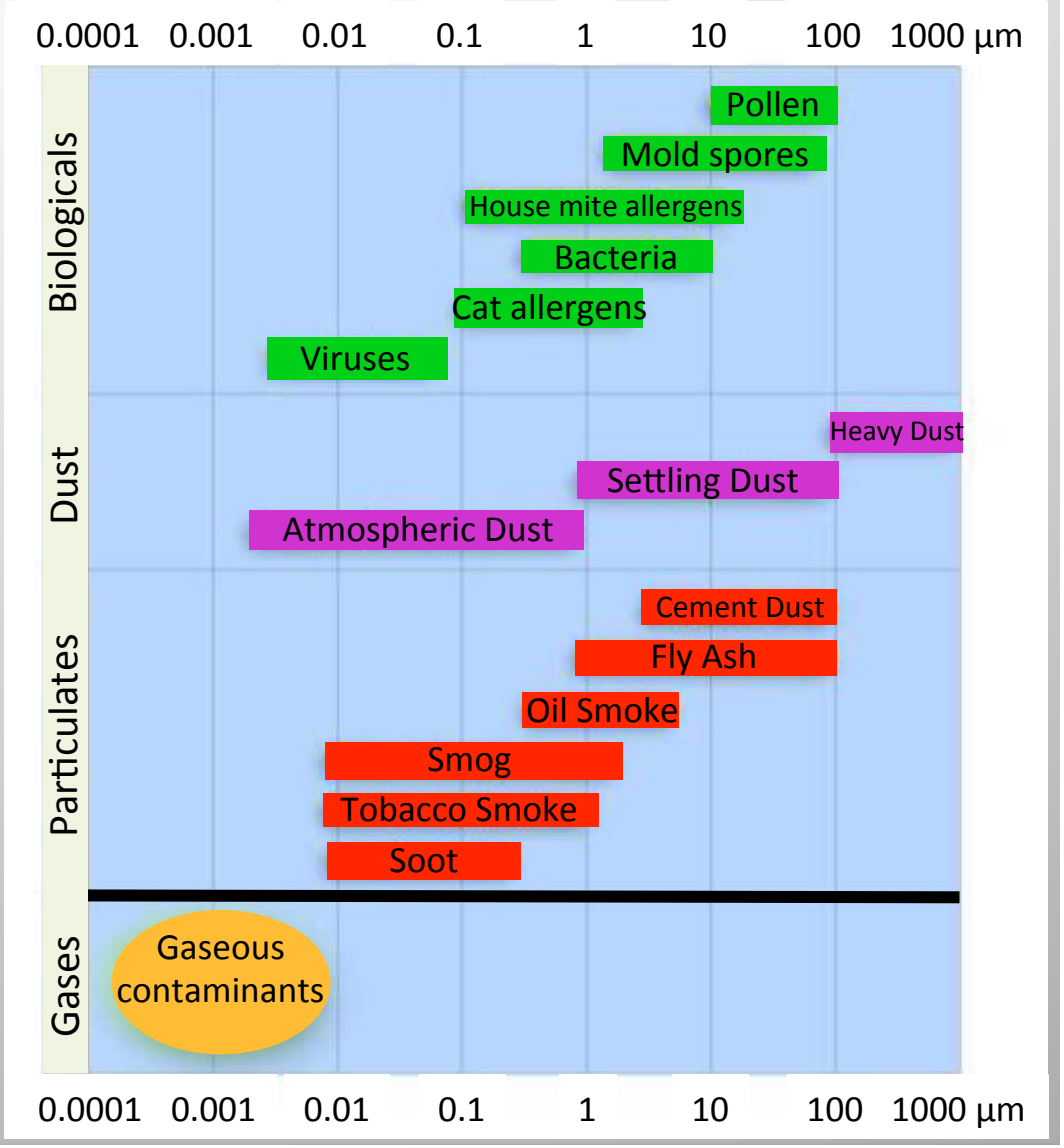
Why Study Aerosols?

- Aerosol: suspension of fine solid particles or liquid droplets in a gas
- Environmental effects
  - Climate
  - Pollution
- Human health effects
  - Allergies
  - Diseases



Hinds, W. C. (1999). *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles* (2nd Ed.). John Wiley and Sons, Inc., New York, NY, pp. 1-14

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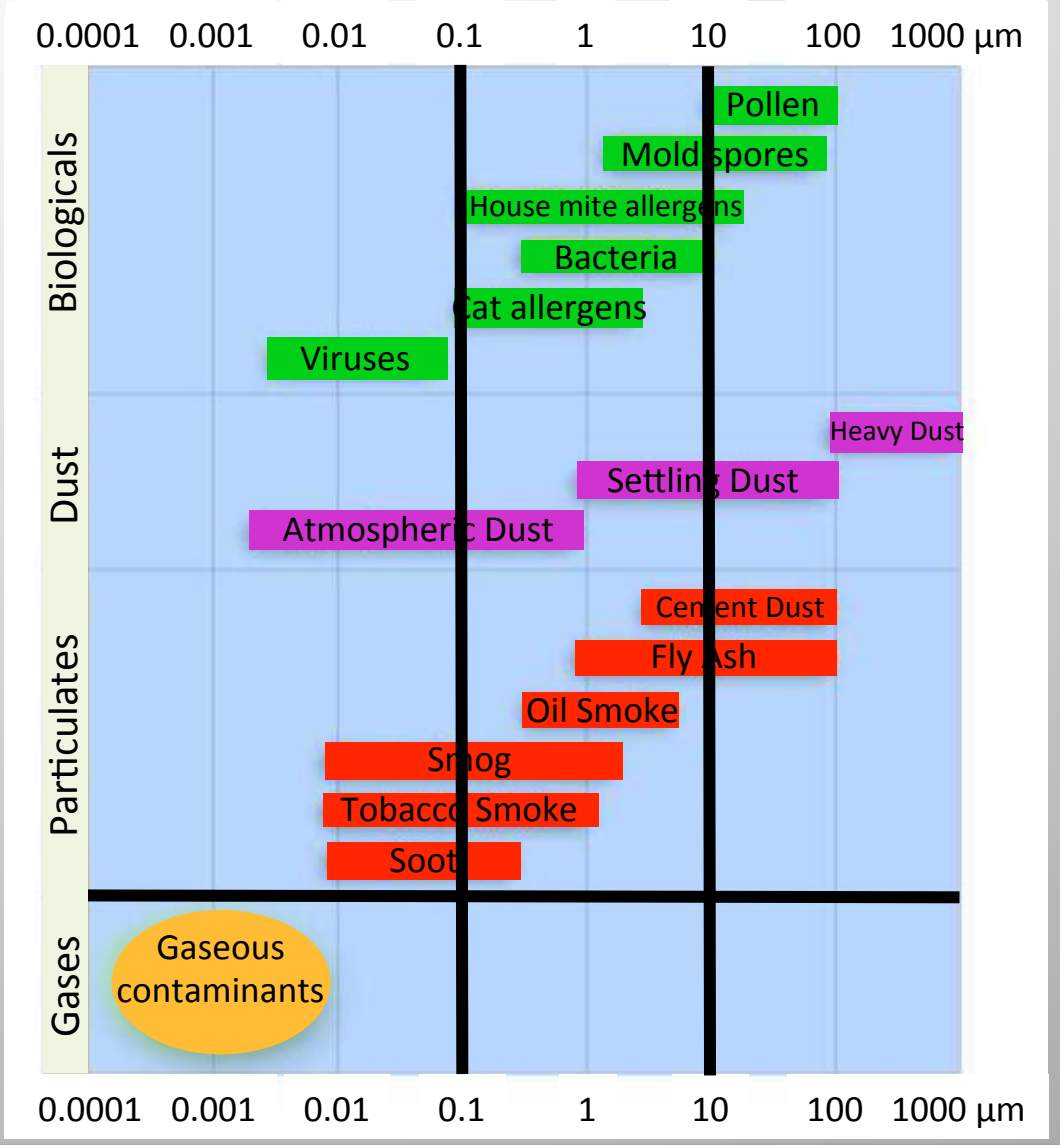
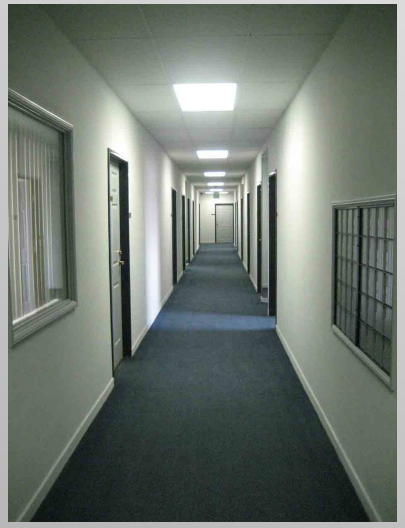
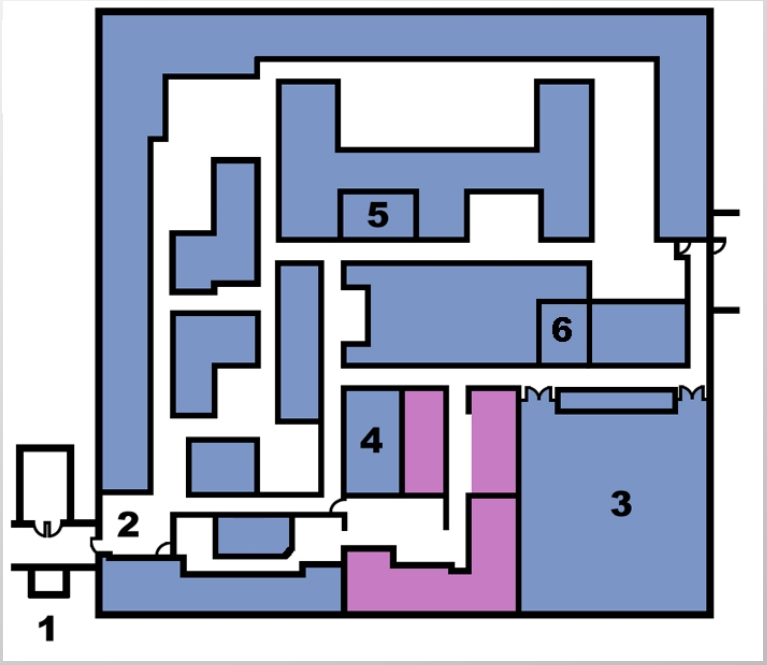


Hinds, W. C. (1999). *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles* (2nd Ed.). John Wiley and Sons, Inc., New York, NY, pp. 1-14

Aerosol Transport Studies

- Track airborne contaminants
- HVAC settings/seasonal effects
- Evacuation routes



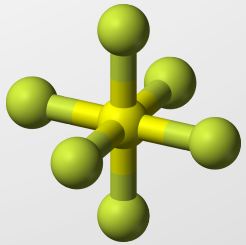


Need experimental feedback for evaluating aerosol dispersion and transport models

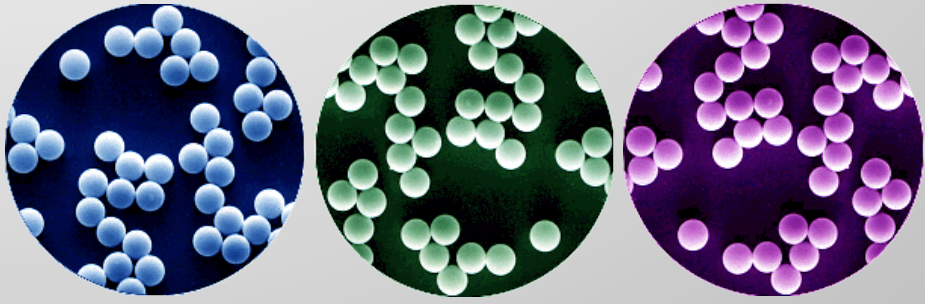
http://cpe.ky.gov/about/directions.htm
http://www.visitingdc.com
http://learnecholocation.blogspot.com

Other Aerosol Transport Study Materials

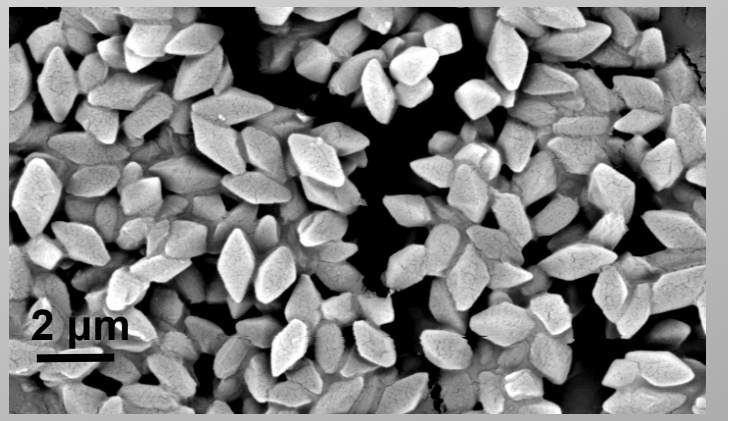
- Sulfur hexafluoride (SF_6)
  - Tracer gas
  - Gas transport ≠ aerosol transport



- Polymer microspheres
  - Fluorescence detection
  - Not safe for human inhalation



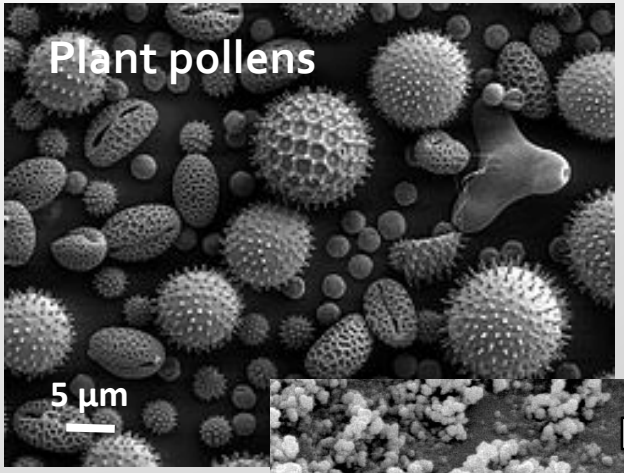
- Spores (e.g. *Bacillus thuringiensis*)
  - Biological pesticide
  - Limited field testing

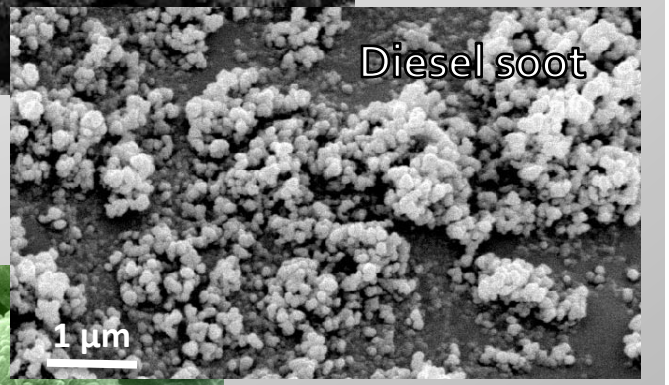


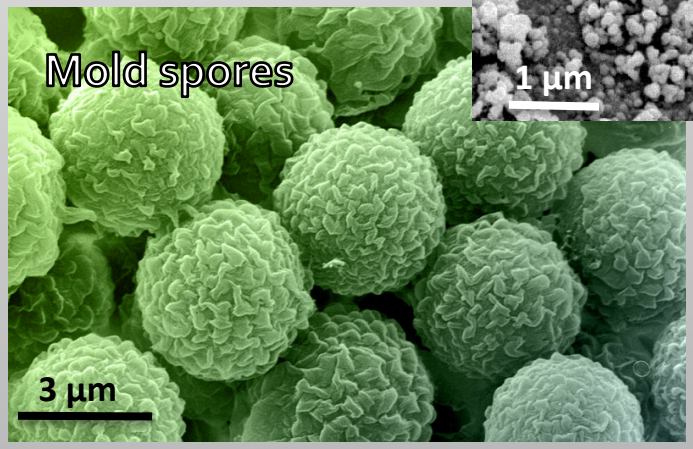
http://en.wikipedia.org/wiki/File:Sulfur-hexafluoride-3D-balls.png
http://www.polymicrospheres.com/
http://www.muski.com/muski_report.htm

Desirable Aerosol Test Particle Characteristics

- Physical properties
  - Size, shape, density
- Safe for human exposure
- Highly sensitive and selective detection
- Cost-effective
- Customizable





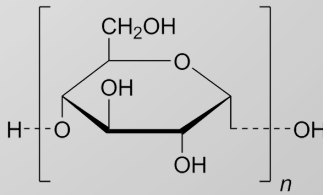


http://en.wikipedia.org/wiki/Pollen
https://courseware.e-education.psu.edu/courses/egee101/L05_petroleum/soot.html
http://science.nationalgeographic.com

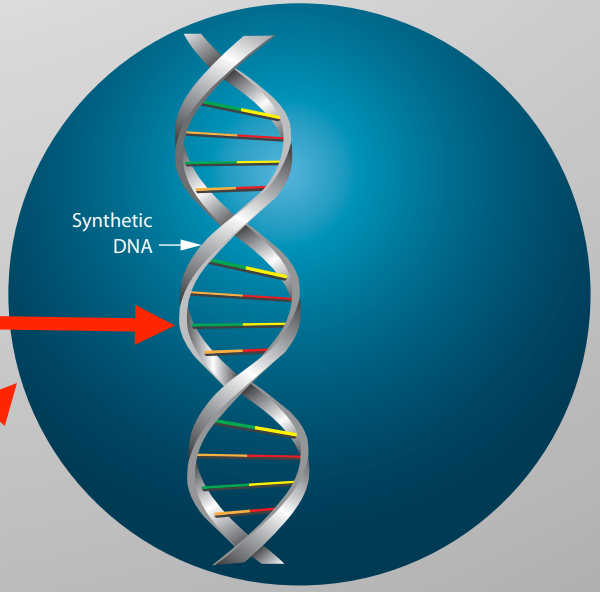
DNATrax particles are composed of inert DNA fragments (barcodes) and sugar

- The simulant is an inert, non-living, non-viable, non-infectious material.
- It consist of two parts:
  1. Non-biological DNA molecules used for identification
  2. Sugar (a food-grade carbohydrate encapsulation material, Maltodextrin)
- 100 DNA base pairs allows for 1.6×10^{60} possible barcodes

- Advantages over spore releases
  - Safe
  - Multiple releases can be conducted in a short time period or simultaneously
  - No clean up needed
  - Low burden for approval
  - size tunable



Maltodextrin Material Safety Datasheet
Inhalation of dust may irritate the nose, throat, and upper respiratory tract. Skin contact is not known to be hazardous to health. Eye contact may cause mild transient irritation. Ingestion of quantities sufficient to produce health effects is not a plausible route of exposure in industrial use



DNA molecule material - Also found in food products containing plant or animal cells

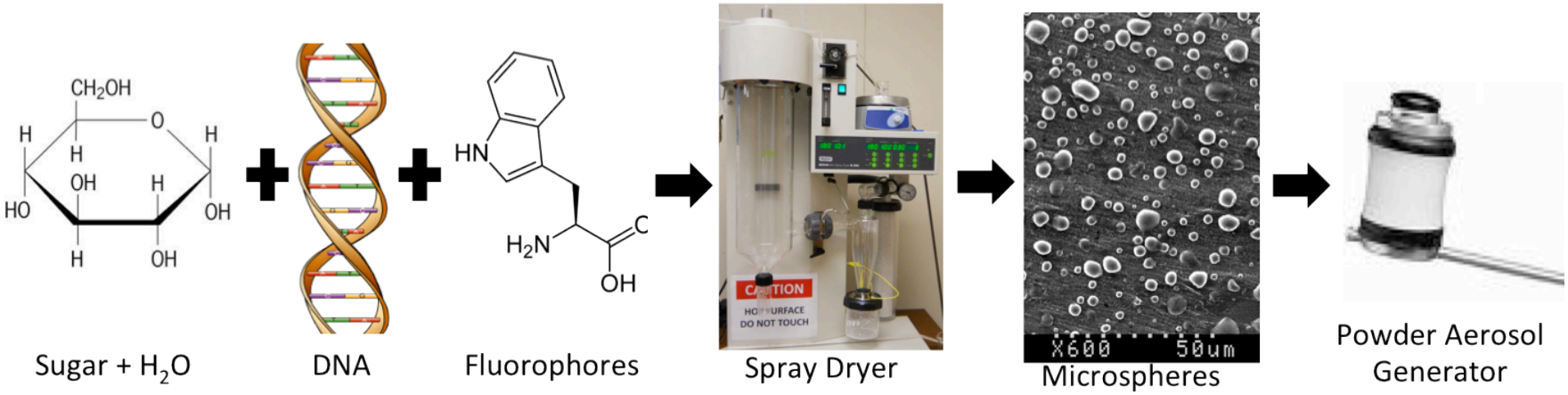
Carbohydrate encapsulation material (Maltodextrin) - Also found in food products such as Splenda® and Equal®

Synthetic DNA Barcodes

- Sequences from *Thermotoga maritima*
- ~100 bp oligonucleotides
- Not similar to other microorganisms (no cross reactivity)
- Highly specific quantitative real-time polymerase chain reaction (qRT-PCR) assays

http://www.rcn.montana.edu/library/enlargeimage.aspx?id=37322&size=800

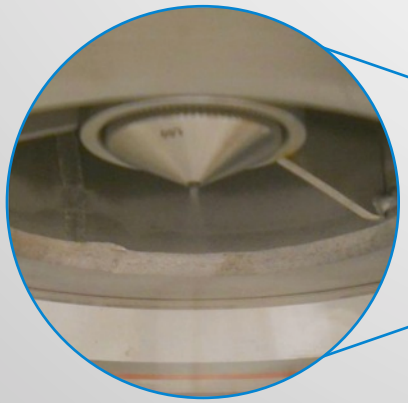
Particle Production



Test particles can be created with unique barcodes to facilitate multiple releases in the same environment

http://www.scq.ubc.ca/decodeing-icelands-dna/

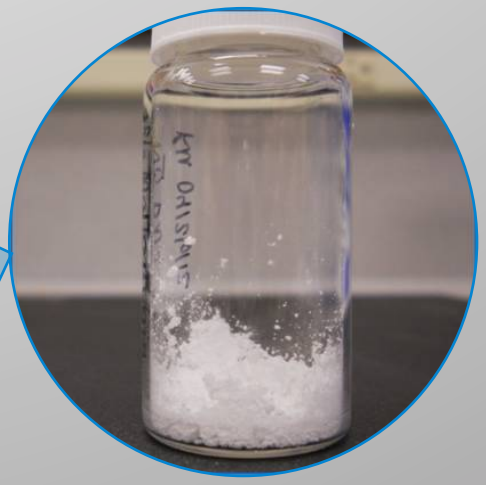
Production-Scale Commercial Spray Dryer



Droplet production using nebulization mechanism

BÜCHI Mini Spray Dryer B-290

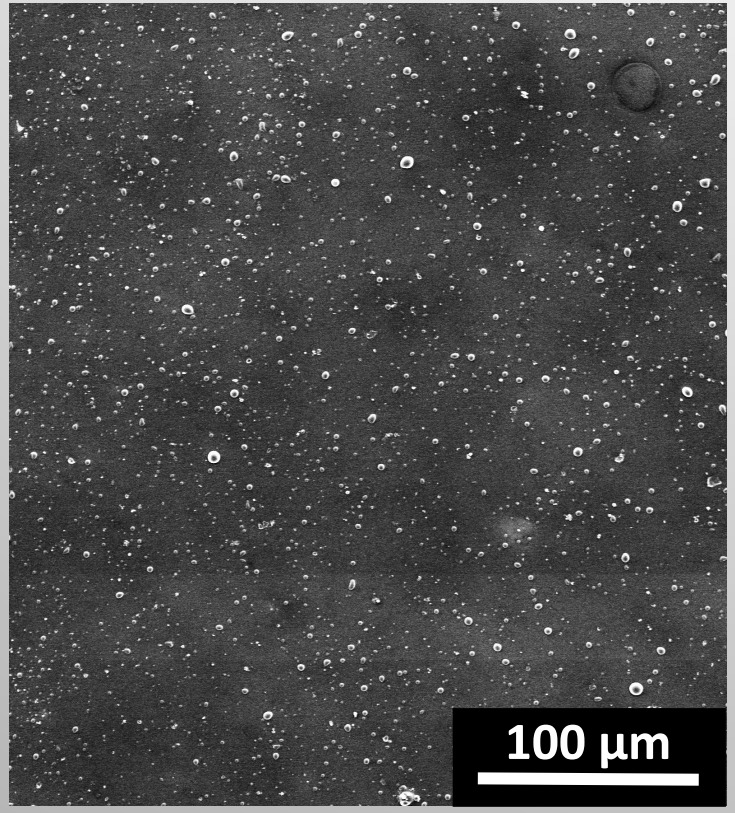
Rapid production of gram quantities of particles

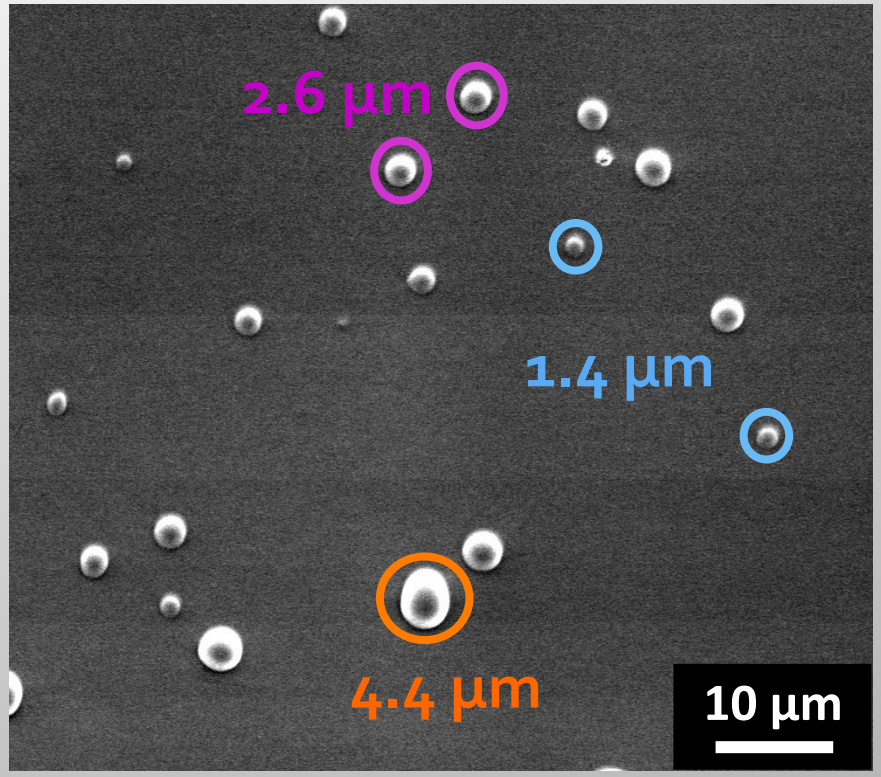


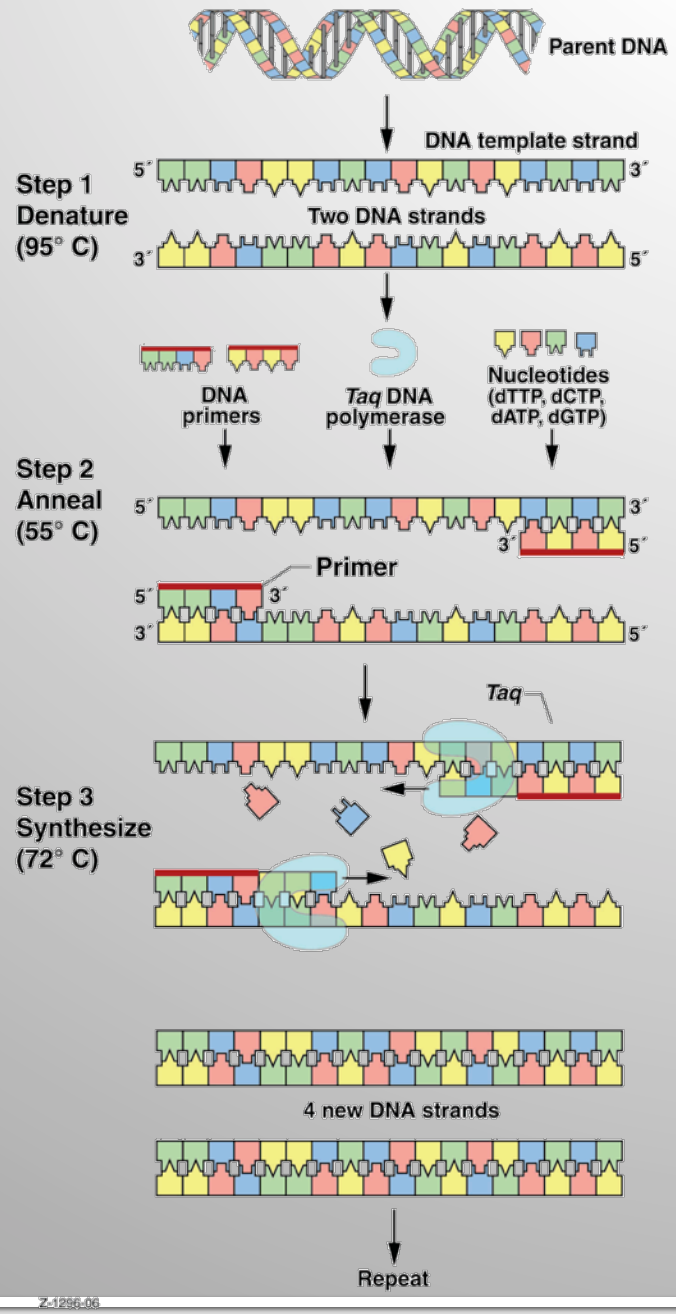
10 min collection time

http://www.buchi.com/Mini_Spray_Dryer_B-290.179.0.html

SEM of Maltodextrin Test Particles



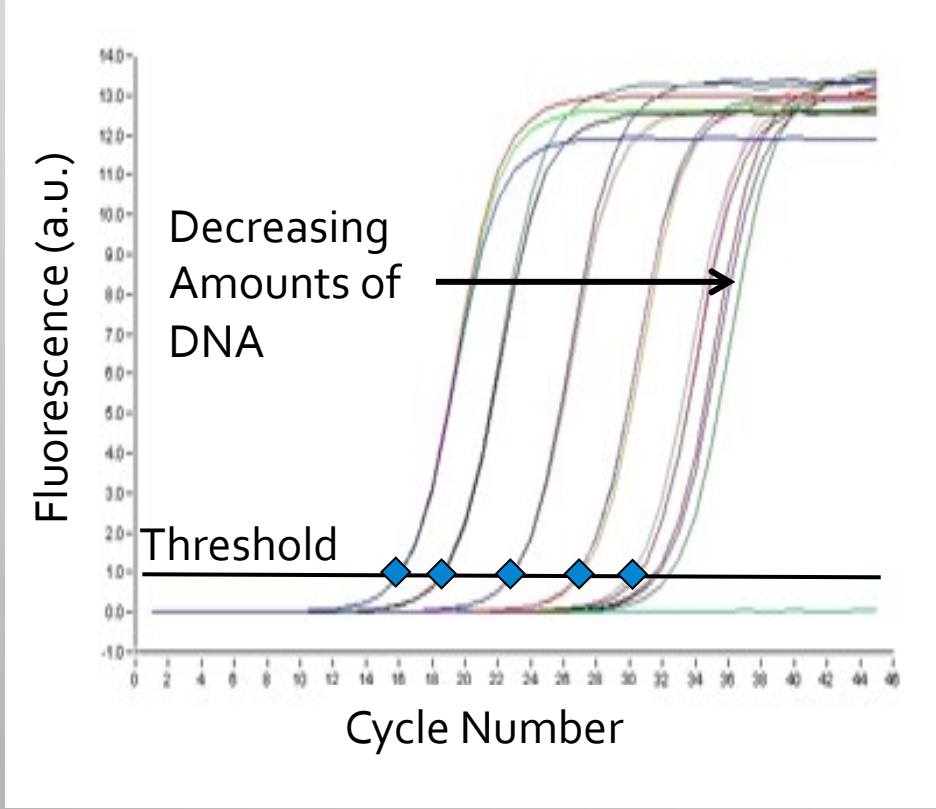


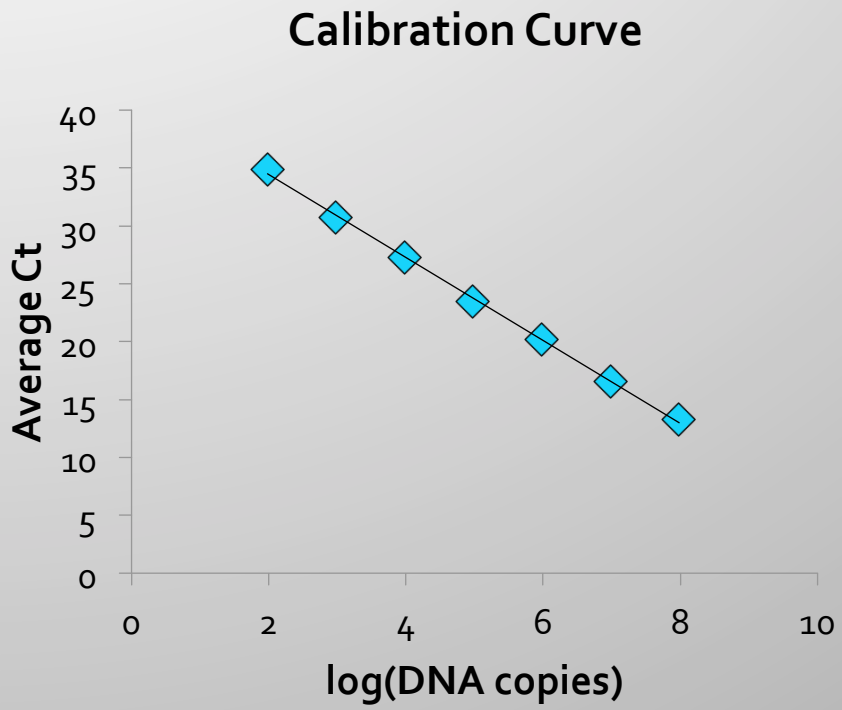


Polymerase Chain Reaction (PCR): Amplification and detection of trace levels of DNA

- Amplify a specific target fragment of DNA
- Every cycle theoretically doubles the number of fragments
- 1 copy becomes 1 billion copies after 30 cycles
- Fluorescence increases as the number of copies increases

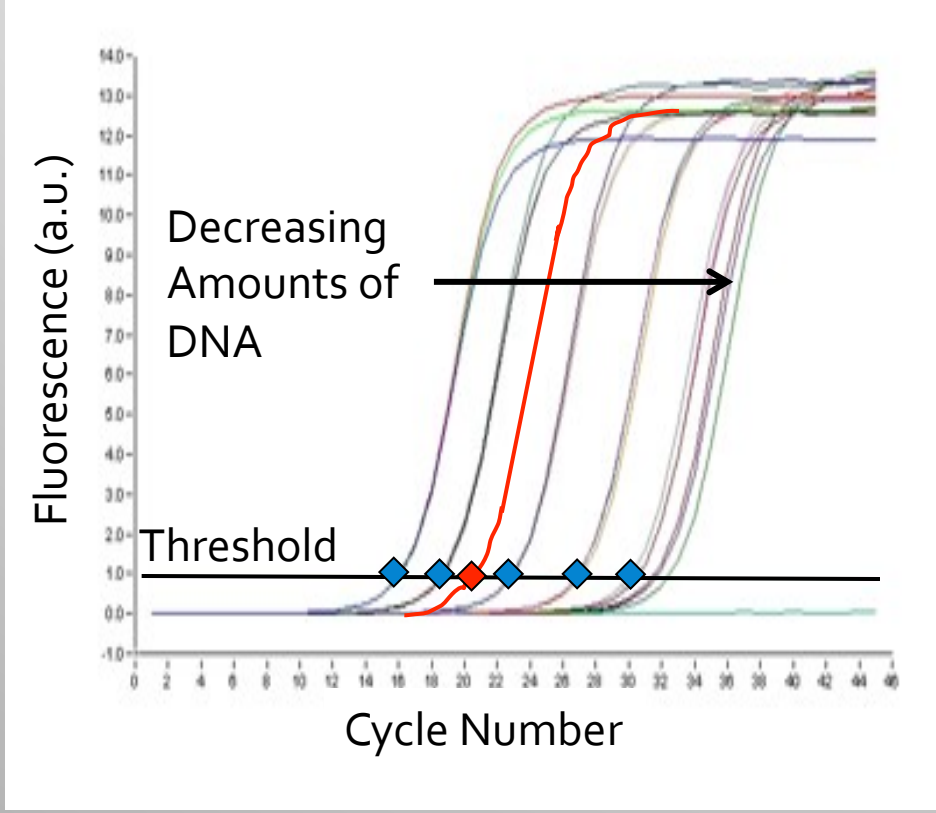
PCR allows quantification of DNA in a sample

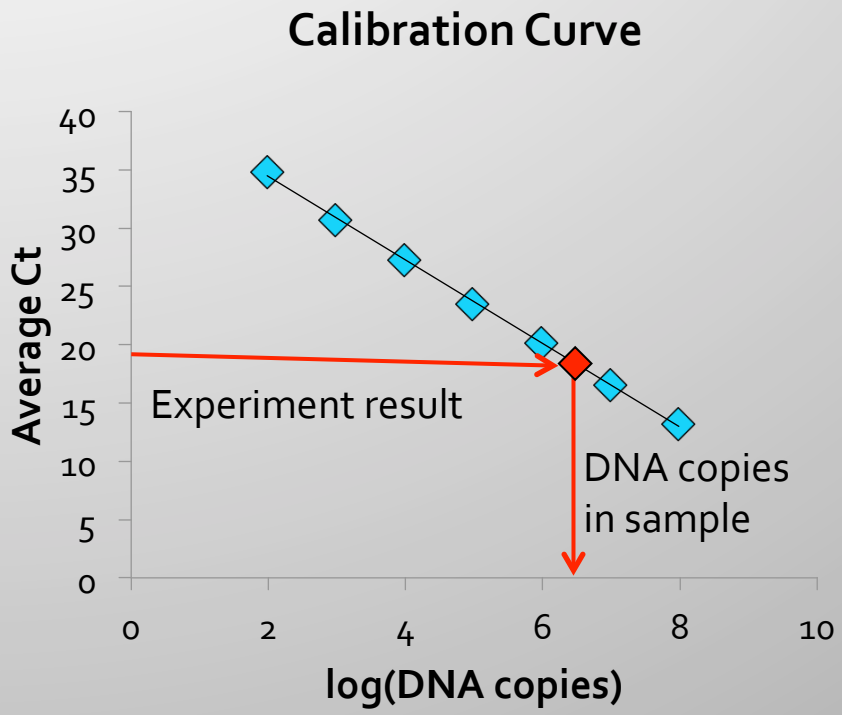




$$C = C_0 E^n$$

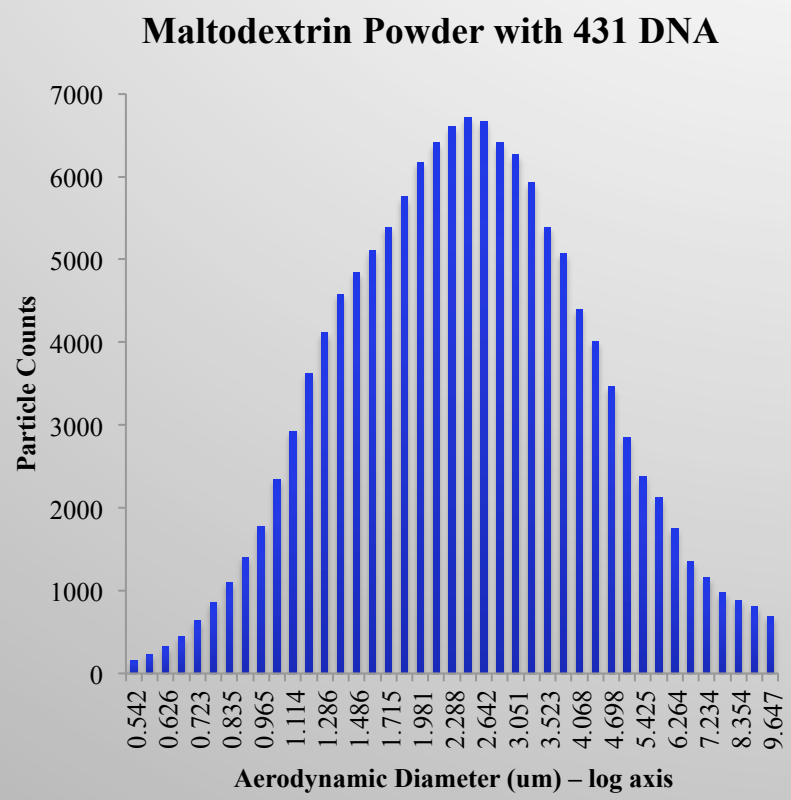
PCR allows quantification of DNA in a sample



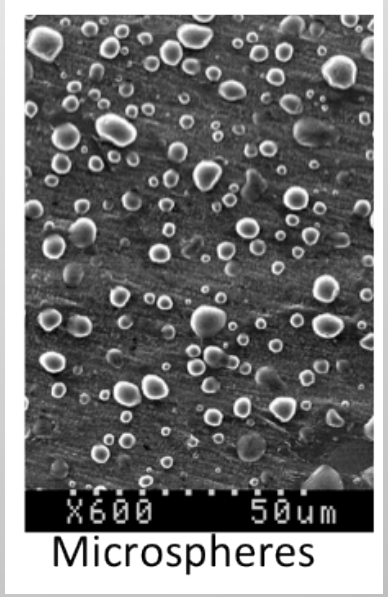


5 copies of the DNA barcode per 2 µm particle

Tunable Maltodextrin Simulant



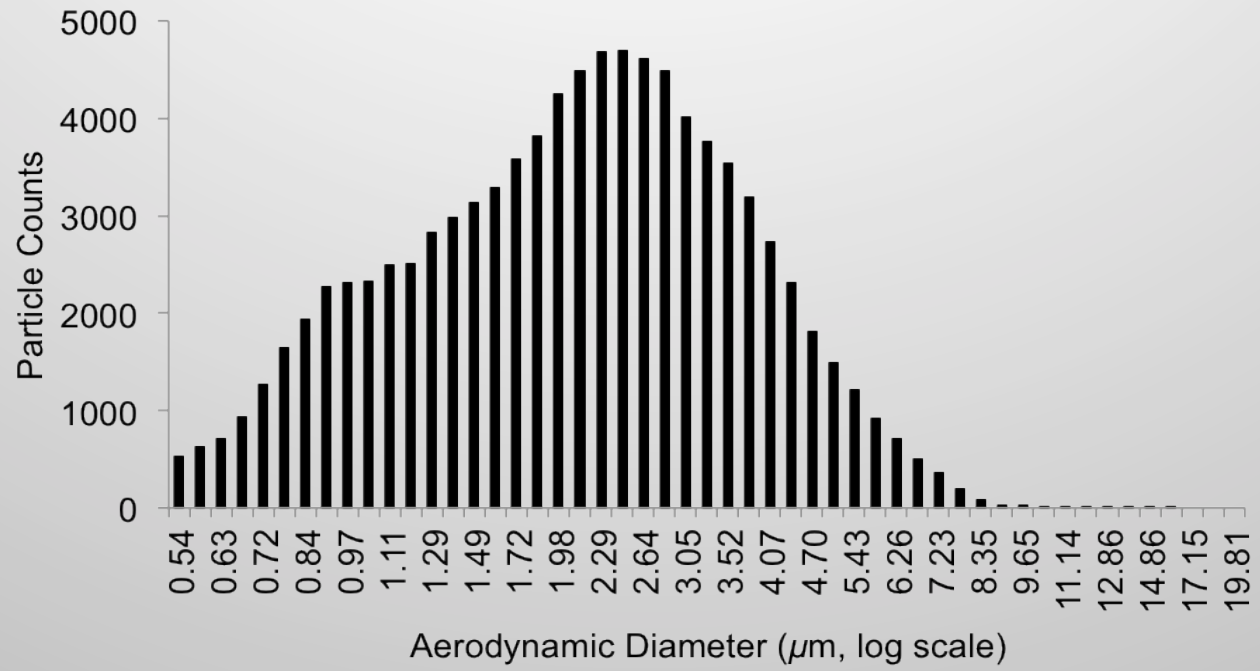
- Particles are size tunable
  - Smaller or larger mean diameter distributions are possible
  - Material is fully characterized, including size and number of barcodes per particle
  - Gram to kilogram quantities available



Spray-dried maltodextrin microspheres exhibit a log-normal distribution of aerodynamic diameters, as measured by Aerodynamic Particle Sizer (APS).

Geometric mean aerodynamic diameter = 2.5 μm

Quantifying DNA Barcodes in Particles



14.4 DNA barcodes per particle on average

How many barcodes in each particle size bin?

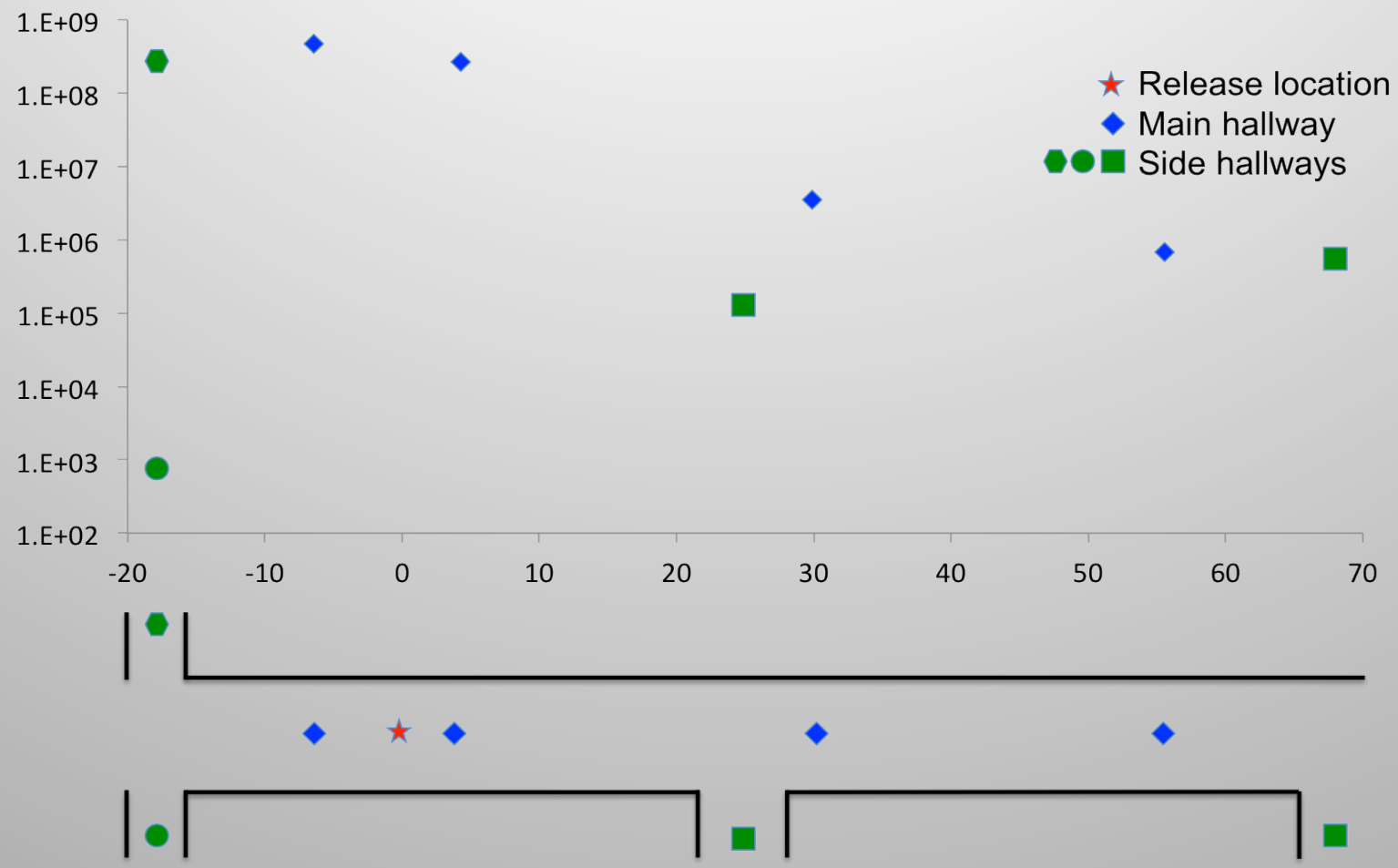
Quantifying DNA Barcodes in Particles

$$\frac{5.2 \times 10^{6} \text{ DNA barcodes}}{3.6 \times 10^{5} \text{ particles}} = \textbf{14.4 DNA barcodes per particle on average}$$

Aerosol Release

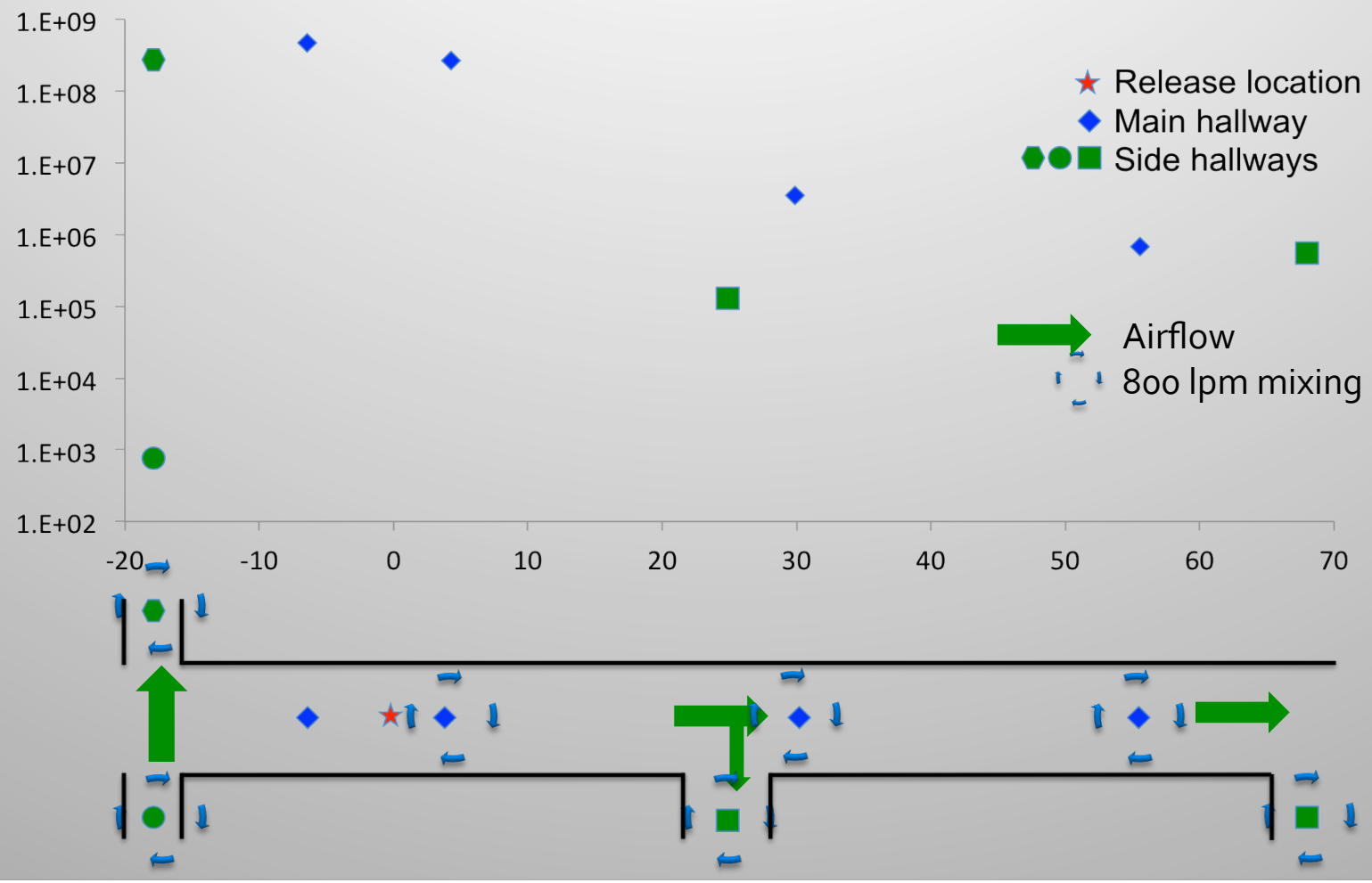
DNATrax detected on filters from 1 g release

- 1 gram of simulant material was released.
- Dry filter units (DFU) collected particles for ~30 minutes (each DFU collects two filters)



DNATrax detected on filters from 1 g release

- Filters were collected and analyzed by PCR
- Airflow patterns can be determined by particle location



Conclusions and summary

- DNATrax is a safe aerosol test particle
  - FDA-approved food additives
  - Advantageous properties
  - Water soluble
  - Biodegradable

- Other applications
  - Food safety
  - Model validation
  - Penetration testing with size dependence
  - Building certification

Team

- LLNL personnel in the Field and Lab
  - George Farquar
  - Elizabeth Wheeler
  - Ruth Udey
  - Sally Hall
  - Beth Vitalis
  - Christine Hara
  - Cindy Thomas
  - Roald Leif
  - Holly Franz
  - Brian Baker
  - Maxim Shusteff
  - Cindy Kaeser
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