Assessing Aerosol Exposure from High Speed Cell Sorters

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Introduction

- Cytometry is the measurement of physical and/or chemical characteristics of cells.
- Flow Cytometry is a process in which such measurements are made while the cells pass, single file, through the measuring apparatus in a fluid stream.
- Measure the presence or absence of cell surface receptors, intracellular proteins or nucleic acids, and functional changes
    - Performed rapidly (up to 30,000 cells/sec)
    - Performed quantitatively
- Cell Sorting
    - Isolate cell subsets for subsequent functional or genomic/proteomic analysis

Introduction

- Droplet Sorting
  - Advantages:
    - Speed (30,000 cells/sec)
    - Up to 4 populations simultaneously sorted
    - Sorted cells relatively concentrated
  - Disadvantages
    - Aerosols generated
    - Expensive
    - High speed (high pressure) sorting may damage cells

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How does a droplet cell sorter sort?

- Physical fluidics principle:
  - A stream of fluid is hydrodynamically unstable
    - It will break up into droplets that have a smaller surface area and lower surface tension
    - Droplet formation pattern occurs in an unpredictable manner

Droplet formation in sink

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Introduction

- Droplet generation
  - Droplet pattern can be stabilized
    - Nozzle vibration induces an acoustic wave in stream
    - Droplets form at troughs of waves
    - Breakoff therefore occurs in the same place at a given frequency of vibration

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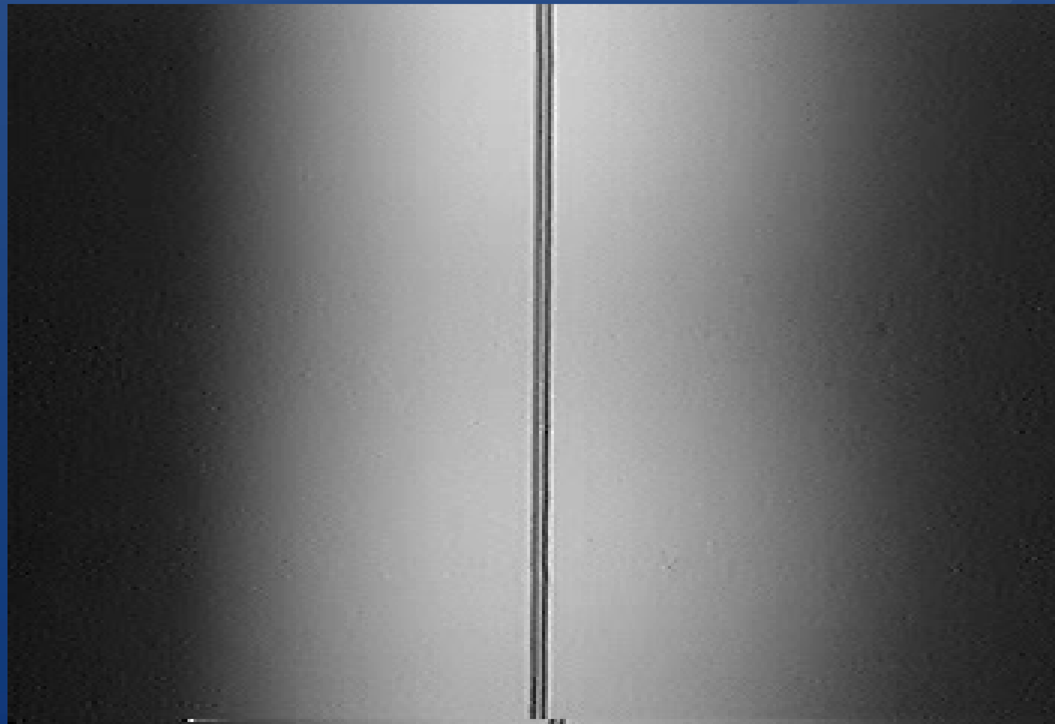
Droplet Controlled by Nozzle Vibration Frequency



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Introduction

- This preliminary study was conducted to ascertain the feasibility of using particle sampling to detect aerosolization created by cell sorting activities.
  - If particle sampling is feasible it could be used to:
    - Quickly assess AMS prior to infectious cell sorting.
    - Identify potential hazardous operating procedures
    - Evaluate cytometer design and modifications

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Introduction

- Currently two methods for measuring aerosol generation are highlighted in the International Society for Analytical Cytology (ISAC) Biosafety Standard for Sorting of Unfixed Cells published in January 2007.
  - T4 bacteriophage method
  - Fluorescent bead method

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- T4 bacteriophage method
  - T4 bacteriophage solution is ran through the flow cytometer.
  - T4 bacteriophage susceptible *Escherichia coli* lawn plates are distributed through out the work area.
  - Plates are incubated for 24 hrs then read for plaques.
  - Number of plaques correlate with the T4 bacteriophage aerosol deposition.

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- Fluorescent bead method
  - Fluorescent beads are suspended in a phosphate buffered saline solution, then ran through the flow cytometer.
  - Microscope slides are places through out the work area.
  - Slides are then examined for aerosolization under a fluorescent microscope.
  - Number of identified fluorescent particles identified correlated to aerosol generation.

Introduction

- Concerns Associated with current methods.
  - T4 bacteriophage method
    - 24 incubation required and extensive prep time required
    - Knowledge of microbiology required
    - Contamination of flow cytometer
  - Fluorescent bead method
    - Individual variability
    - Time consuming
    - Limited particle size
    - Diminished fluorescence

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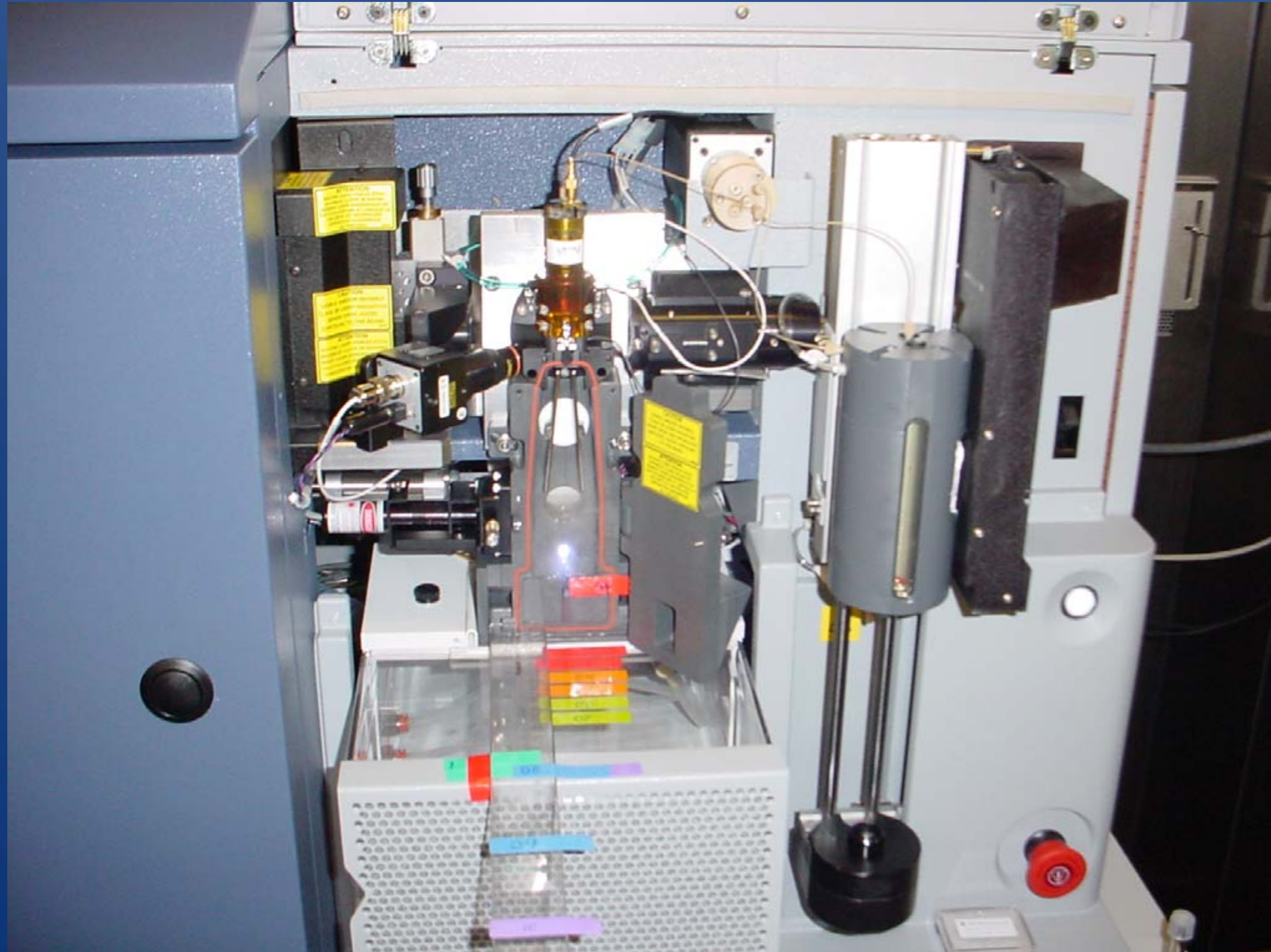
Introduction

- Aerodynamic Particle Sampling
  - Particle sampling not new
  - Historical concerns associated with particle sampling
    - Interference from background particles
    - Inability to differentiate particle size
    - Particle impaction in sampling tube
    - Conservative due to assumption that particles represent biological material

Introduction

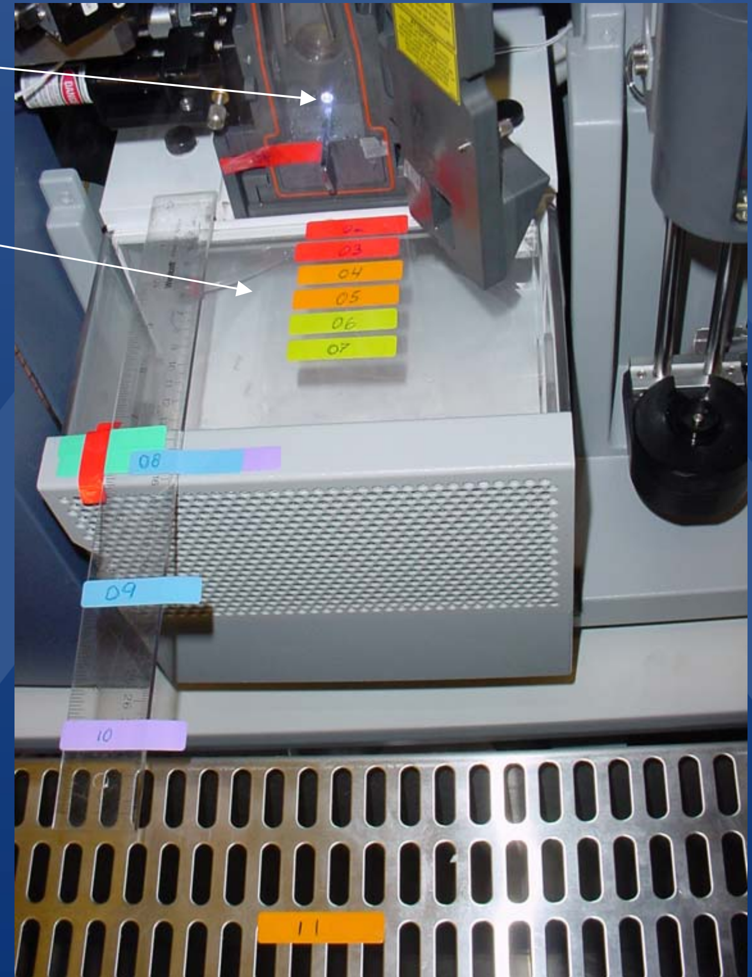
- Aerodynamic Particle Sampling
  - Particles are pulled into instrument and characterized by light scattering technology.
  - Particles are counted and categorized by aerodynamic size.
  - Instrument categorizes sizes between 0.5 microns (μm) and 20 μm.
  - Samples collected and averaged during 20 second intervals.

Methodology

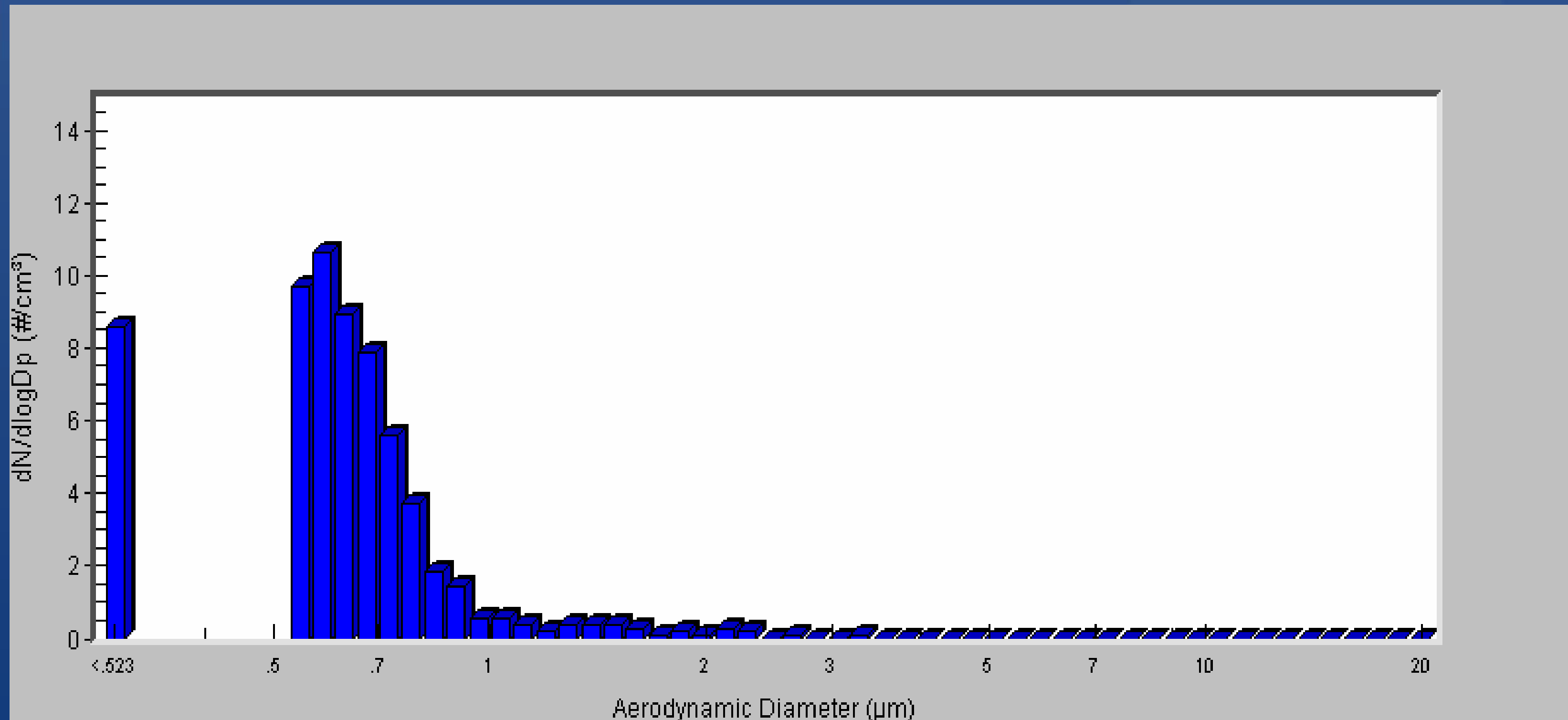


Methodology

- Location 0 – Stream source
- Location 1 – 7 moving away from source in 1.3 cm increments
- Location 8-11 increments increased to 6.1 cm

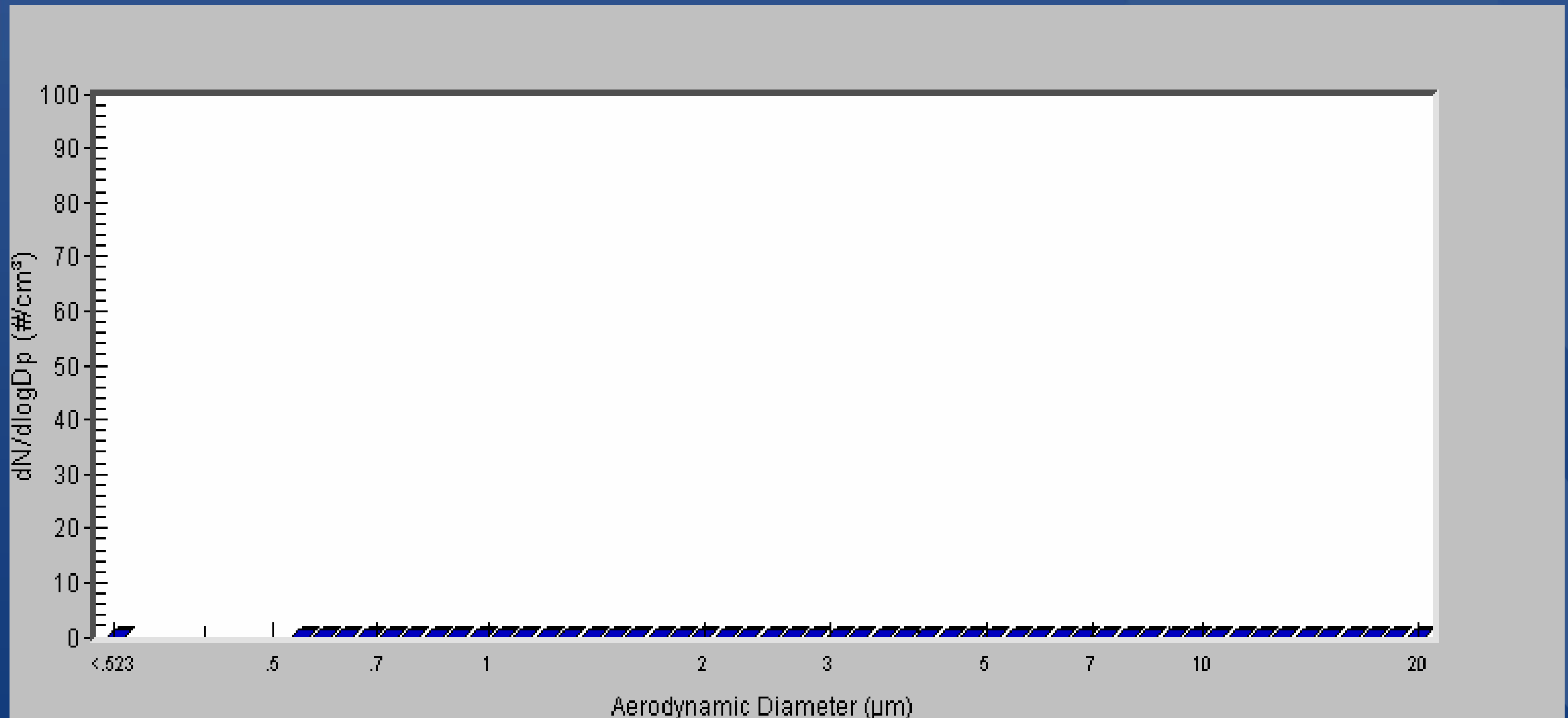


Results



Graph 1: Room No operation

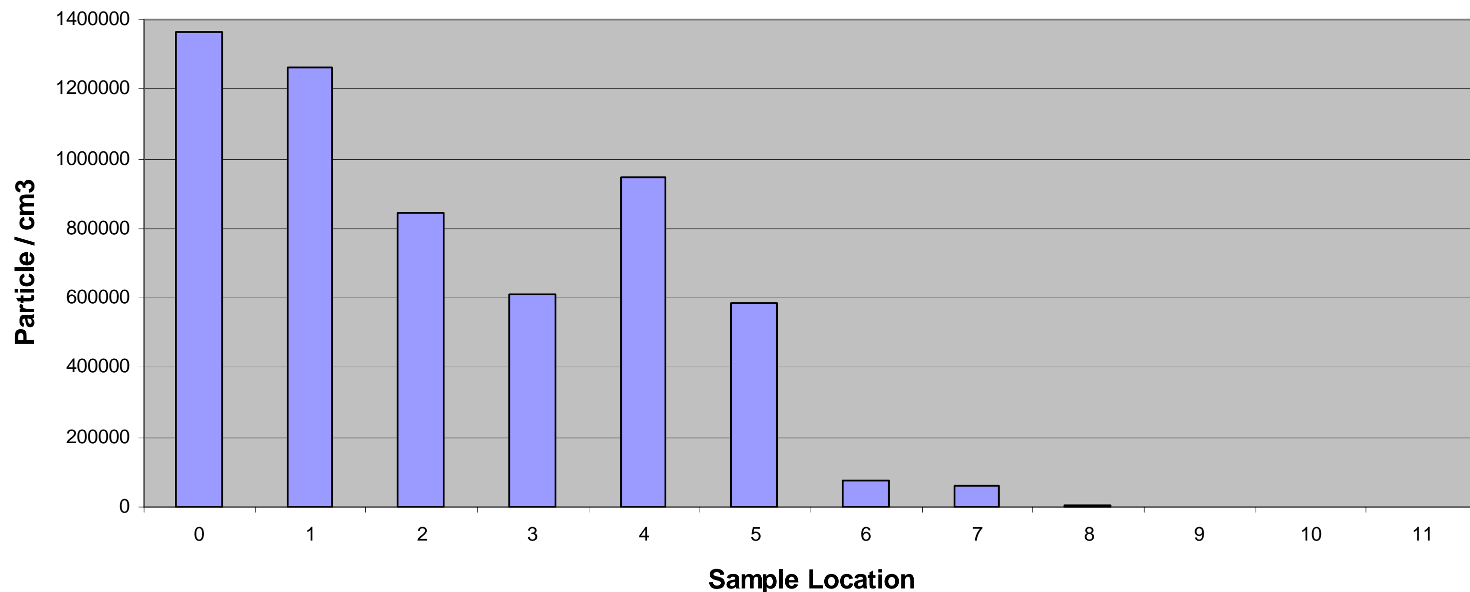
Results



Graph 2: Inside Biological Safety Cabinet No Operation

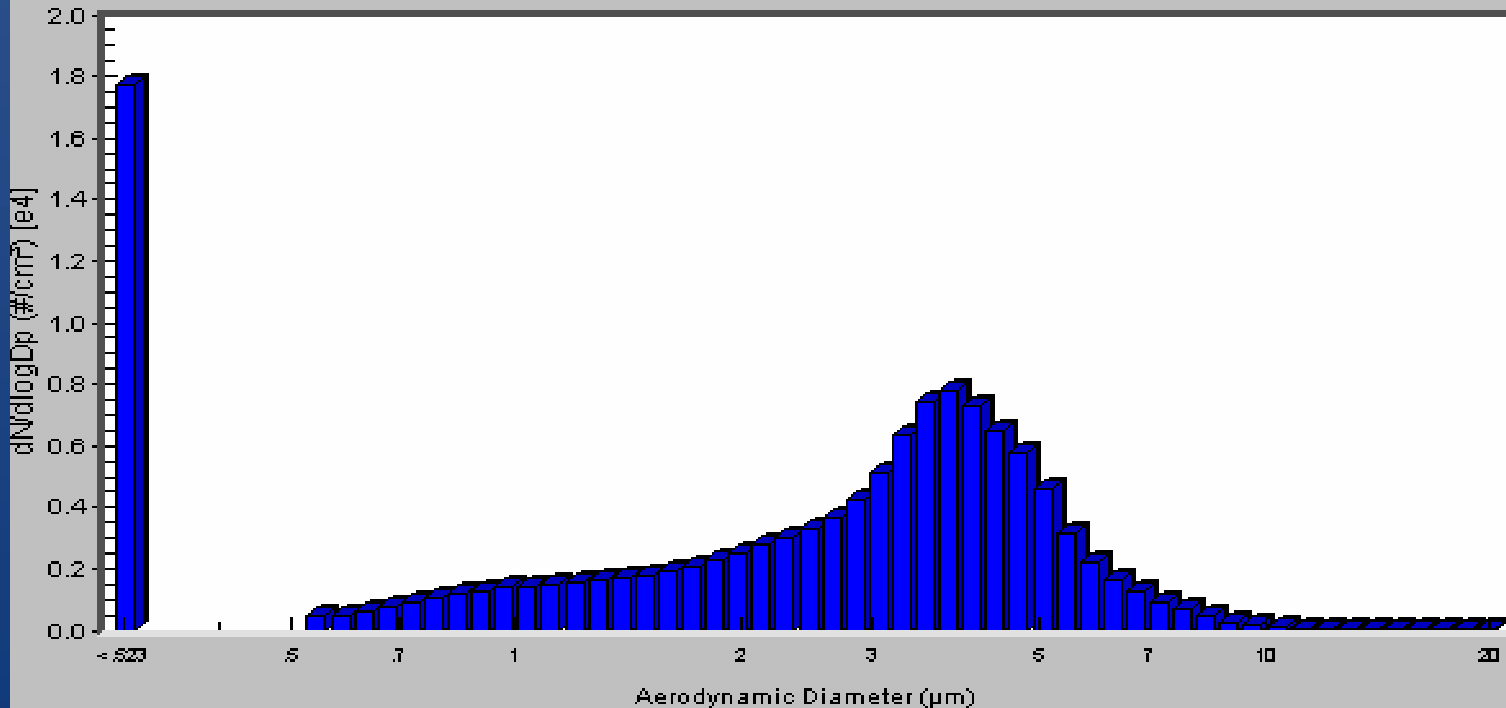
Stream
Waste
Catcher
Aerosol

Results



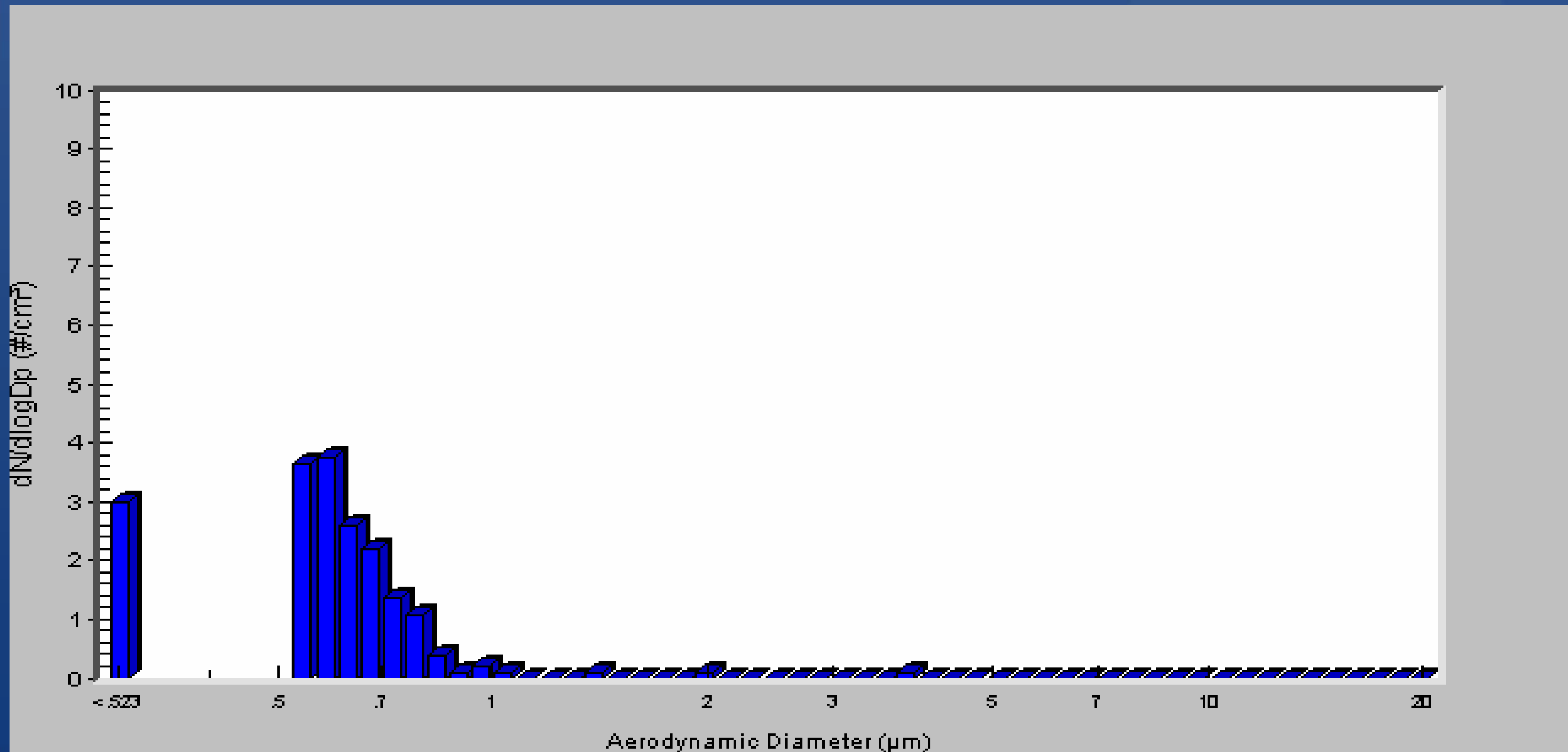
Graph 3: Particle Counts Moving Away from Stream During Replicated Failure

Results



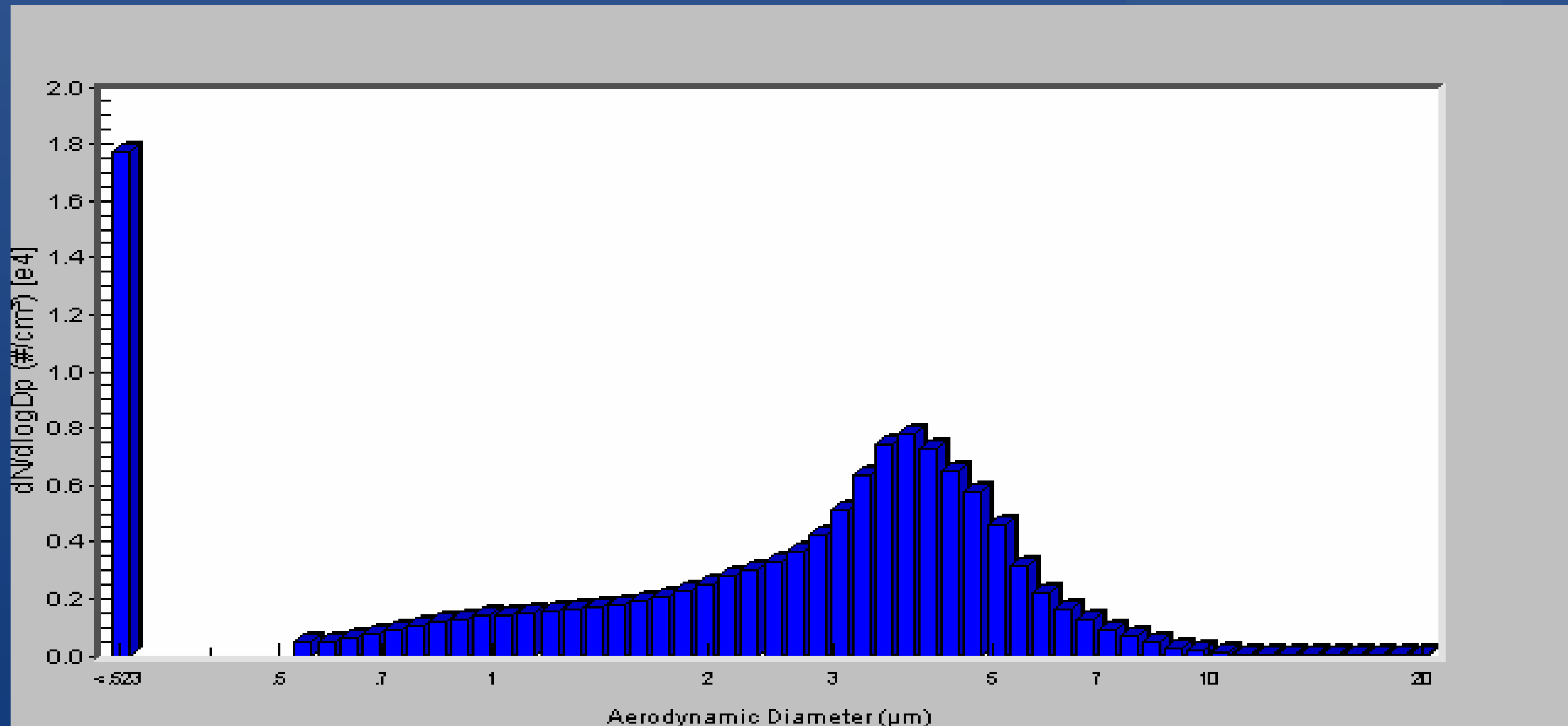
Graph 4: Particles @ Location 0 During Replicated Failure

Results



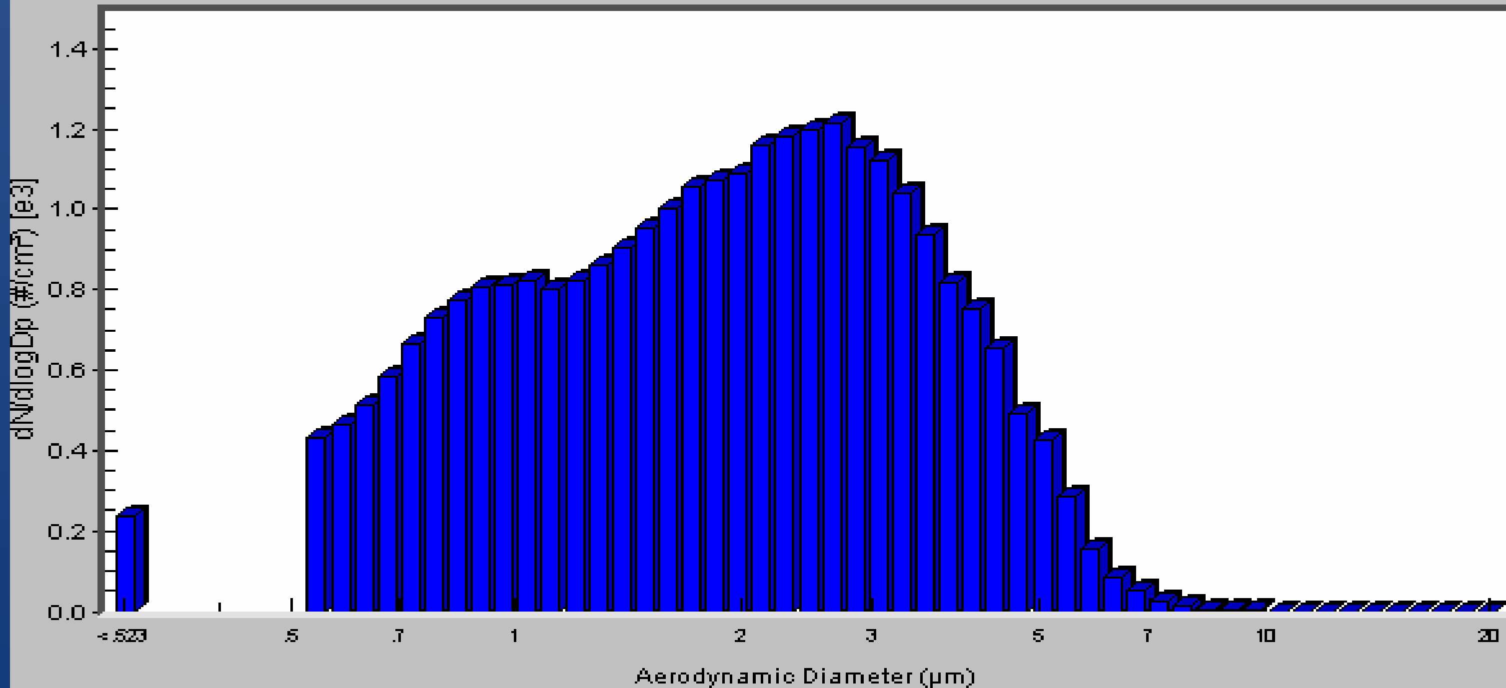
Graph 5: Particles @ Location 11 During Replicated Failure

Results



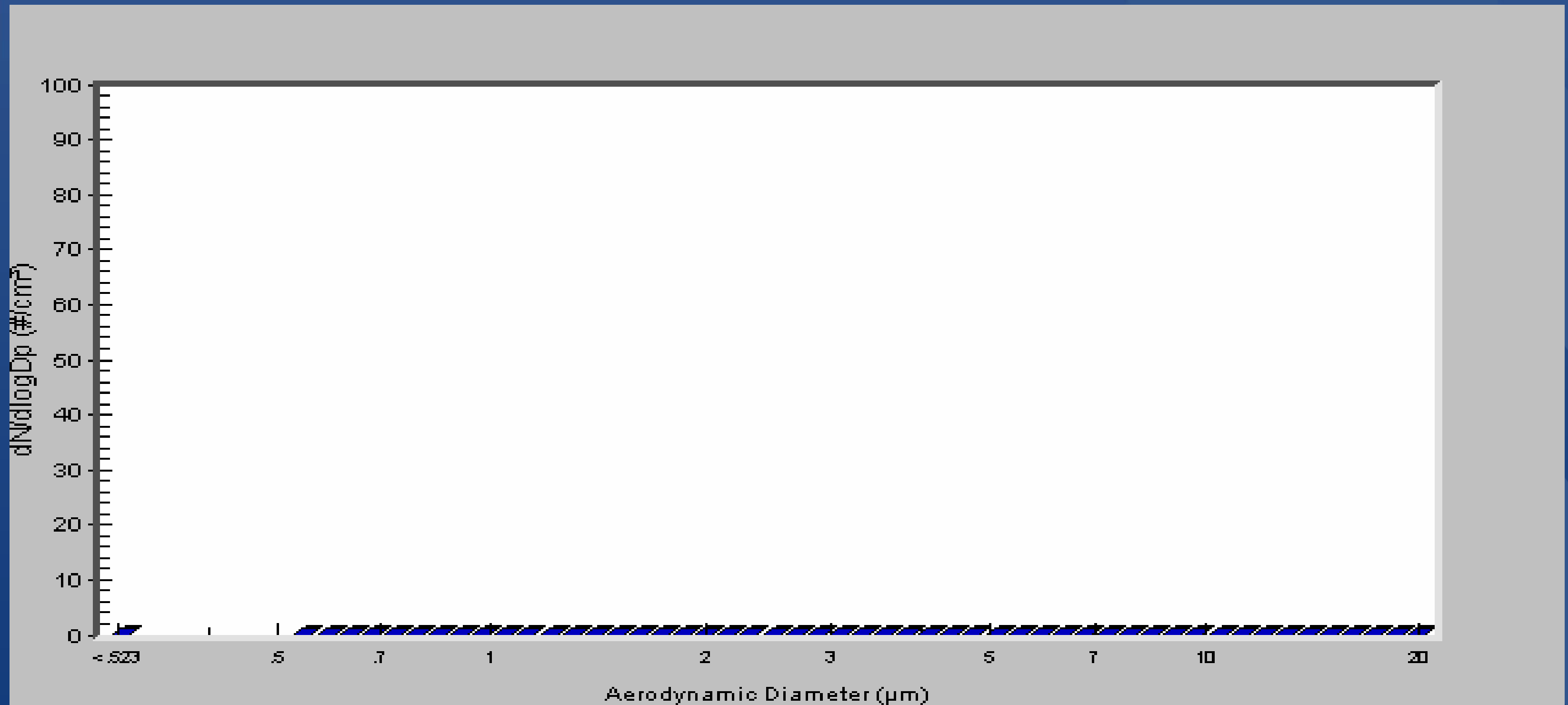
Graph 6: Particles @ Location 0 During Replicated Failure @ 70 psi

Results



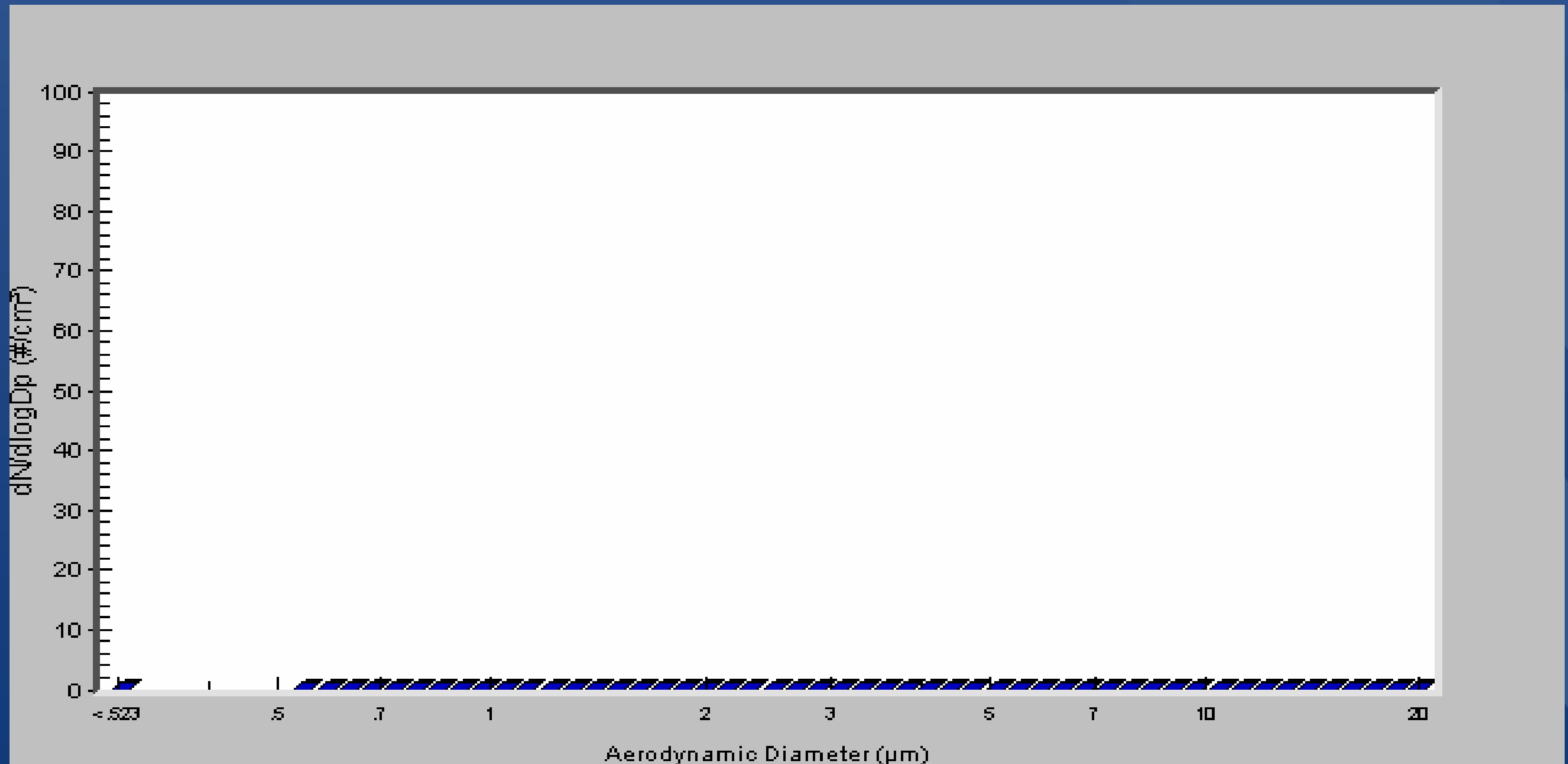
Graph 7: Particles @ Location 0 During Replicated Failure @ 20 psi

Results



Graph 8: Measurements w/o Stream Disruption

Results



Graph 9: Measurements w/ Door Closed

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Discussion

- When background particles are eliminated aerodynamic particle sampling is capable of assessing potential inhalation exposure from cell sorting.
  - Further studies will be conducted utilizing a particle sampler which has capabilities of detecting particles tagged with a fluorescent compound.
  - An ultraviolet (UV) laser will be used to identify particles that contain a UV-excitable dye (Clear Blue), which has been dissolved in the fluidics system of the cell sorter.
  - This will allow counting and sizing of aerosols unambiguously identified as being generated by the cell sorter versus those in the ambient air.

Discussion

- Goals of Future Studies
  - Evaluate current methods for test AMS
  - Develop safe operating procedures in the event of stream disruption
    - Primary Barriers
  - Assess equipment modifications for potential aerosolization hazards
  - Provide accurate quantification of potential aerosolization hazards to be used by safety specialist in conducted risk assessments.
    - Personal Protective Equipment

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Discussion

- Problems associated with using microbial exposure to determine risk of developing disease.
  - Particles generated may or may not carry infectious agent
    - Fixed samples as an alternative
  - Individual susceptibility
    - Immunizations
  - Virulence of infectious particles

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Acknowledgments

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