**Supplemental Information**

Deposition Efficiency and Uniformity of Monodisperse Solid Particle Deposition in the Vitrocell® 24/48 Air-Liquid-Interface in-vitro Exposure System

Michael J. Oldham, Nicolas Castro, Jingjie Zhang, Ali Rostami, Francesco Luccia, Yezdi Pithawalla , Arkadiusz K. Kuczaj, I Gene Gilman, Pasha Kosachevsky, Julia Hoeng, K. Monica Lee

Table of Contents

[Methods - Data Analysis 3](#_Toc18400237)

[Supplemental Figure S1: 6](#_Toc18400244)

[Supplemental Figure S2: 7](#_Toc18400245)

[Supplemental Figure S3: 8](#_Toc18400248)

[Supplemental Figure S4: 9](#_Toc18400250)

[Supplemental Figure S5: 10](#_Toc18400252)

[Supplemental Figure S6: 11](#_Toc18400253)

[Supplemental Figure S7: 12](#_Toc18400255)

[Supplemental Figure S8: 13](#_Toc18400258)

[Supplemental Figure S9: 14](#_Toc18400261)

[Supplemental Figure S10: 15](#_Toc18400264)

[Supplemental Figure S11: 16](#_Toc18400267)

[Supplemental Figure S12: 17](#_Toc18400269)

[Supplemental Figure S13: 18](#_Toc18400271)

[Supplemental Figure S14: 19](#_Toc18400274)

[Supplemental Figure S15: 20](#_Toc18400275)

[Supplemental Figure S16: 21](#_Toc18400276)

[Supplemental Figure S17: 22](#_Toc18400277)

[Supplemental Figure S18: 23](#_Toc18400278)

[Supplemental Figure S19: 24](#_Toc18400279)

[Supplemental Figure S20: 25](#_Toc18400280)

[Supplemental Figure S21: 26](#_Toc18400281)

[Supplemental Figure S22: 27](#_Toc18400282)

[Supplemental Figure S23: 28](#_Toc18400283)

[Supplemental Figure S24: 29](#_Toc18400284)

[Supplemental Figure S25: 30](#_Toc18400285)

[Supplemental Figure S26: 31](#_Toc18400287)

[Supplemental Figure S27: 32](#_Toc18400288)

# 

# Methods - Data Analysis

Detailed equations for particle deposition calculations

Experimentally, this meant that for the first cell culture insert in a channel, the total number of particles that could have entered the first horn, *NH,1*, on the way to the first cell culture insert was the total of the number of particles depositing on the channel exit filter, , plus the number of particles that deposited on all six cell culture inserts (Eq. S1)

|  |  |
| --- | --- |
|  | (Eq. S1) |

Where:

For the second cell culture insert in the channel, the total number of particles (Eq. S2) that could have entered the second horn, *NH2*, on the way towards the second cell culture insert is calculated by subtracting the number of particles that deposited on the first cell culture insert. Subtraction of the number of particles depositing in prior cell culture inserts continued for the number of particles that could have entered subsequent horns to calculate the total number of particles that could have entered each individual horn on the way towards each of the six cell culture inserts in a channel as expressed in (Eq. S2).

|  |  |
| --- | --- |
| … | (Eq. S2) |

Where:

Deposition efficiency, , for each cell culture insert was calculated as

|  |  |
| --- | --- |
|  | (Eq. S3) |

For the one run that included counts of 1.1 and 3.3 µm MMAD particles on the first and last cell culture insert exit filters of a channel, the average of the two filter counts was used to refine the calculation shown in (Eq S1) and (Eq. S2) of what could have entered each horn for deposition efficiency calculations at each cell culture insert in the channel. The total number of particles that could have entered horn number one, *NH1*, in this case was calculated as shown below in (Eq. S4):

|  |  |
| --- | --- |
|  | (Eq. S4) |

Where:

Similar to (Eq. S2), the number of particles depositing in prior cell culture inserts was subtracted, the average value calculated for particles exiting the cell culture insert exit filters, , was also subtracted to calculate what could have entered the horns for subsequent cell culture inserts. For clarity, this calculation is shown below in (Eq. S5) for the second, third and last cell culture inserts.

|  |  |
| --- | --- |
| … | (Eq. S5) |

For this one run that included counts of 1.1 and 3.3 µm MMAD particles on the first and last cell culture insert exit filters values as calculated in (Eq. S5) were used in combination to the total deposited count of particles at each cell culture to estimate the sampling efficiency as calculated in (Eq. S3).

# 

Channel 5

Channel 3

Channel 7

Channel 2

Channel 6

Channel 4

Channel 8

# 

Channel 1

# 

# 

# 

# 

A B C D E F

Columns

Figure S1: Diagram of the Vitrocell® 24/48 ALI in-vitro exposure system (top view) showing the number of 3.3 µm MMAD particles depositing on cell culture inserts in a preliminary run when media or PBS was not placed below the cell culture inserts. The eight channels are from the bottom to top while the columns (A-F) are from left to right with the arrows indicating flow direction.

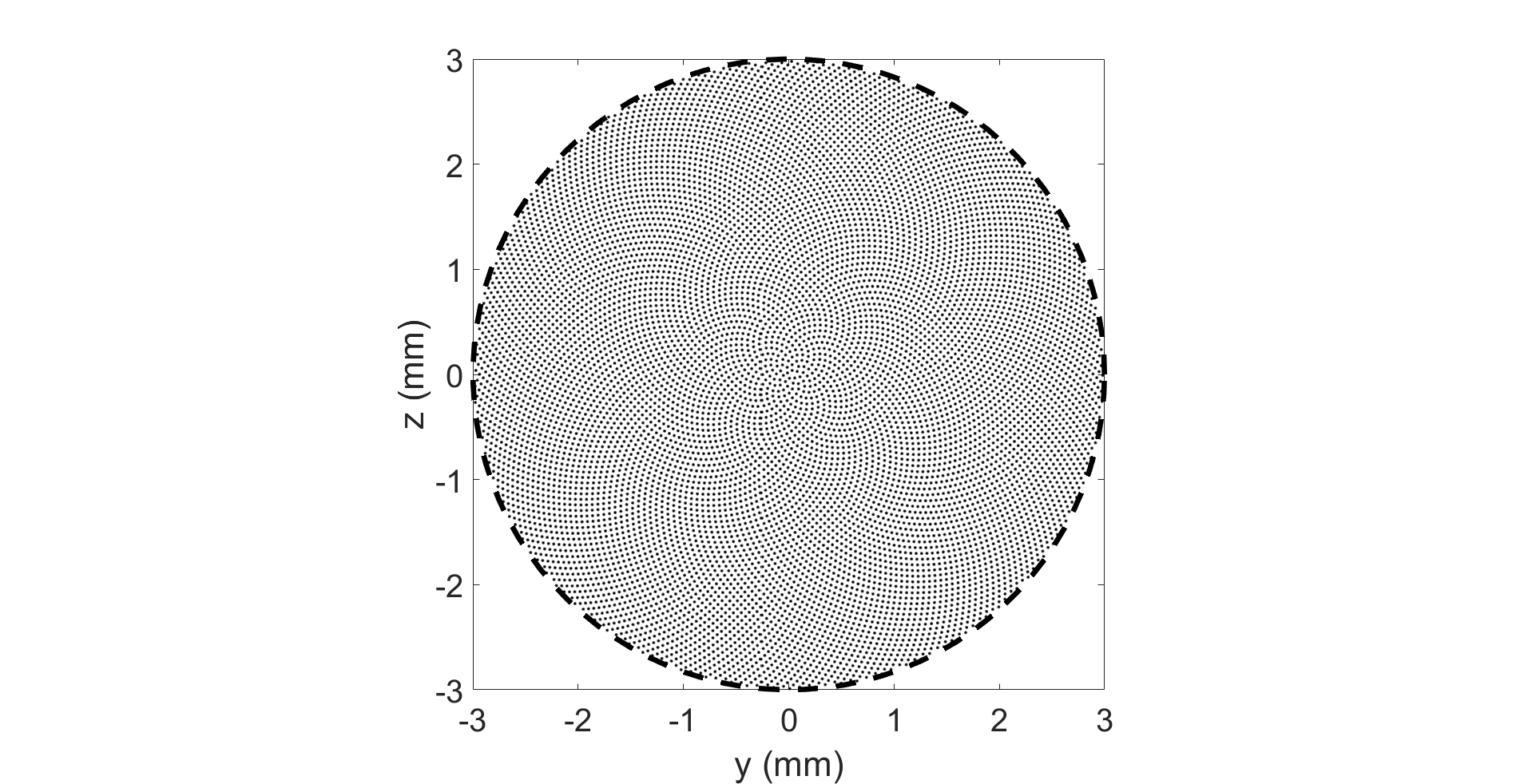


Figure S2: Uniform particle injection starting location at system inlet for Lagrangian simulations.

# Run 1 for 3-3 um MMAD

# 

> 145% of Mean

> 115% to ≤ 145% of Mean

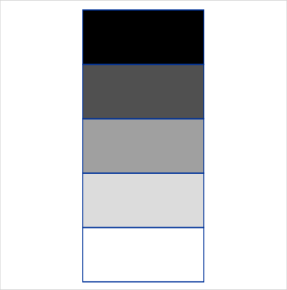
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S3: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #1) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

# Run 2 for 33um MMAD



> 145% of Mean

> 115% to ≤ 145% of Mean

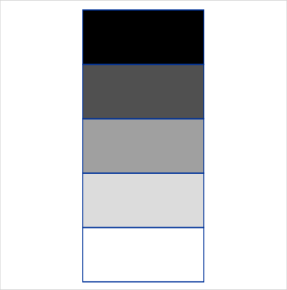
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S4: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #2) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

# Run 3 for 33um MMAD



> 145% of Mean

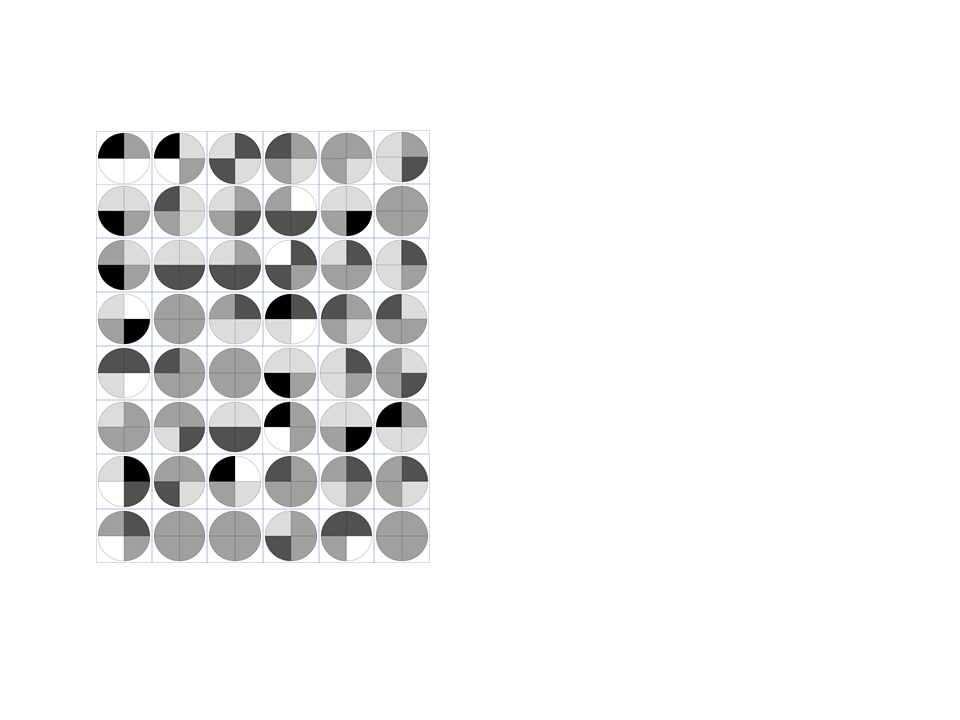
> 115% to ≤ 145% of Mean

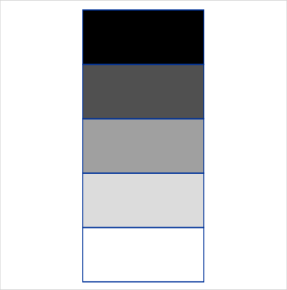
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S5: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #3) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

> 115% to ≤ 145% of Mean

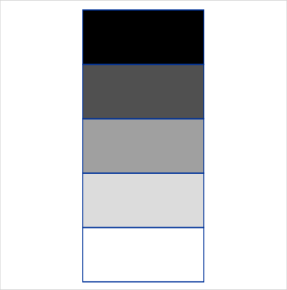
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S6: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #1) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.

# Run 2 for 2-2um MMAD



> 145% of Mean

> 115% to ≤ 145% of Mean

85 – 115% of Mean

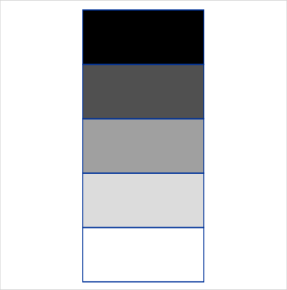
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S7: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #2) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.

# Run 3 for 2-2um MMAD

# 



> 145% of Mean

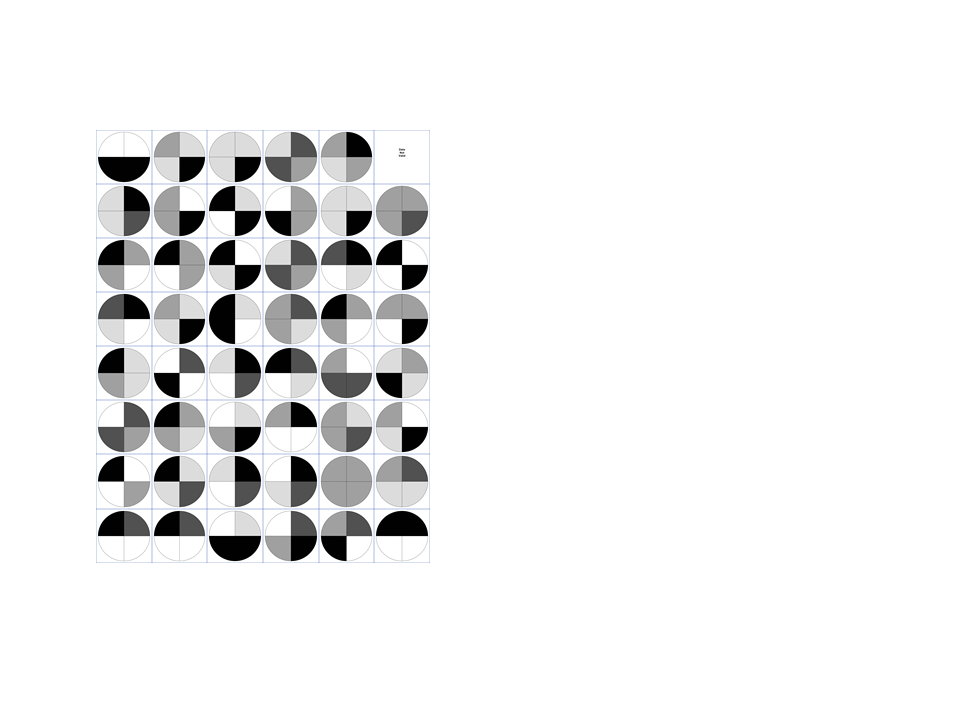
> 115% to ≤ 145% of Mean

85 – 115% of Mean

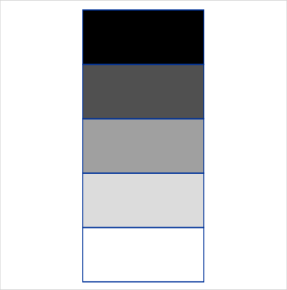
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S8: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #3) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.



# 



> 145% of Mean

> 115% to ≤ 145% of Mean

85 – 115% of Mean

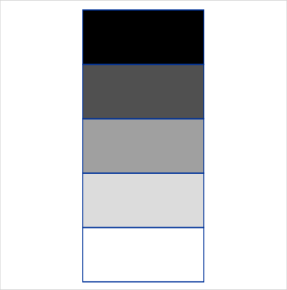
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S9: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #1) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

# Run 2 for 1-1um MMAD

# 



> 145% of Mean

> 115% to ≤ 145% of Mean

85 – 115% of Mean

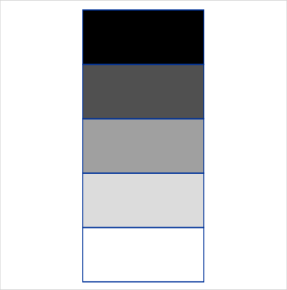
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S10: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #2) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

# Run 3 for 1-1um MMAD

# 



> 145% of Mean

> 115% to ≤ 145% of Mean

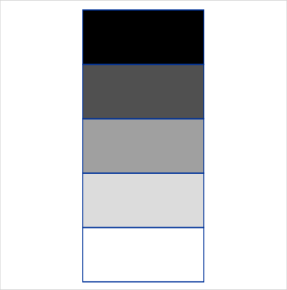
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S11: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #3) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

# Run 1 for 051 um MMAD



> 145% of Mean

> 115% to ≤ 145% of Mean

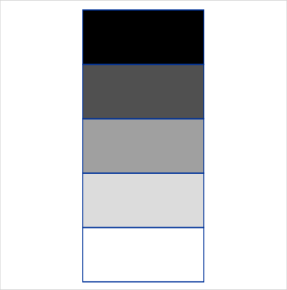
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S12: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #1) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.

# Run 2 for 051um MMAD



> 145% of Mean

> 115% to ≤ 145% of Mean

85 – 115% of Mean

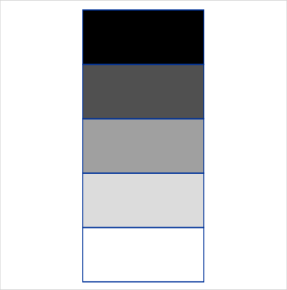
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S13: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #2) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.

# Run 3 for051um MMAD

# 



> 145% of Mean

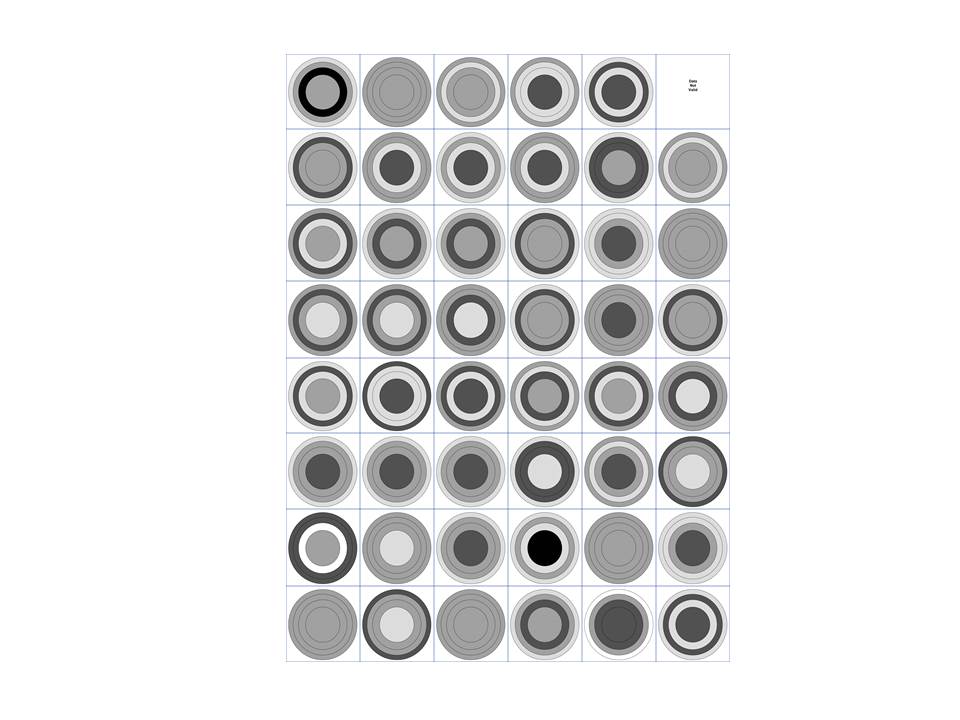
> 115% to ≤ 145% of Mean

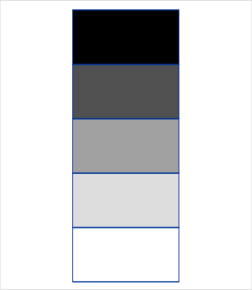
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S14: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #3) in four equal area quadrants expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

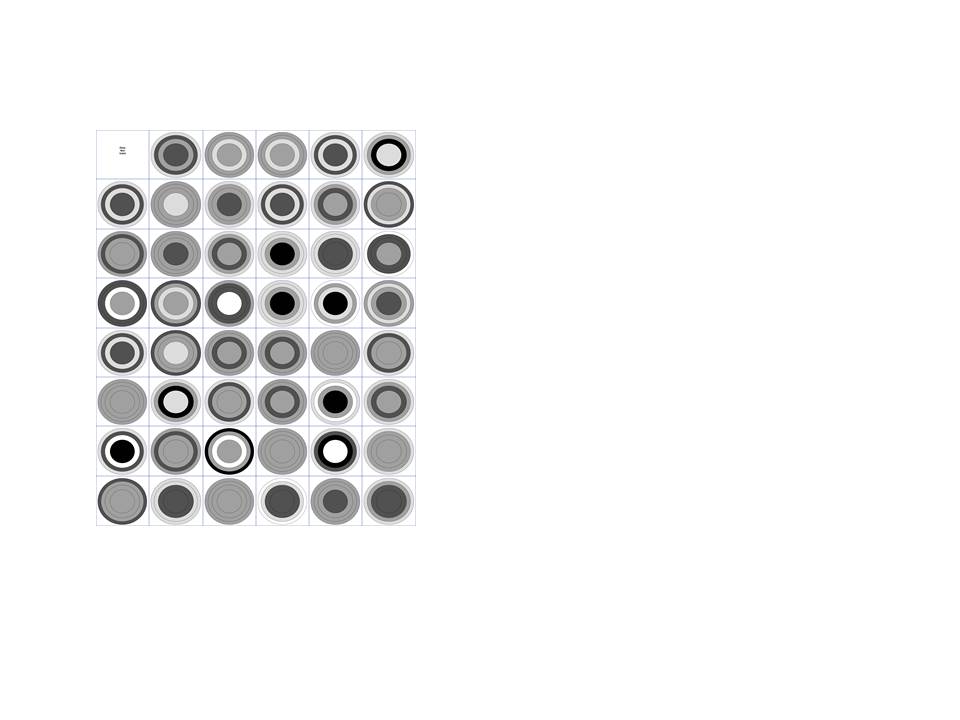
> 115% to ≤ 145% of Mean

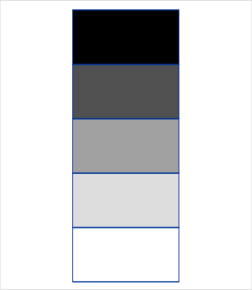
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S15: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #1) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

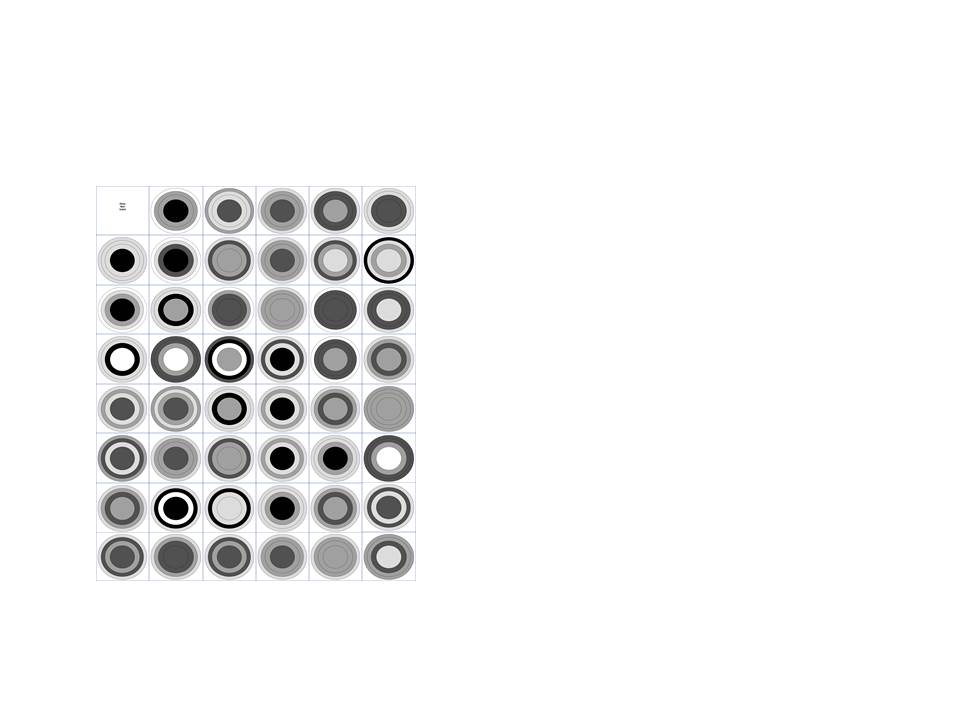
> 115% to ≤ 145% of Mean

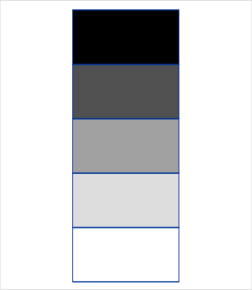
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S16: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #2) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

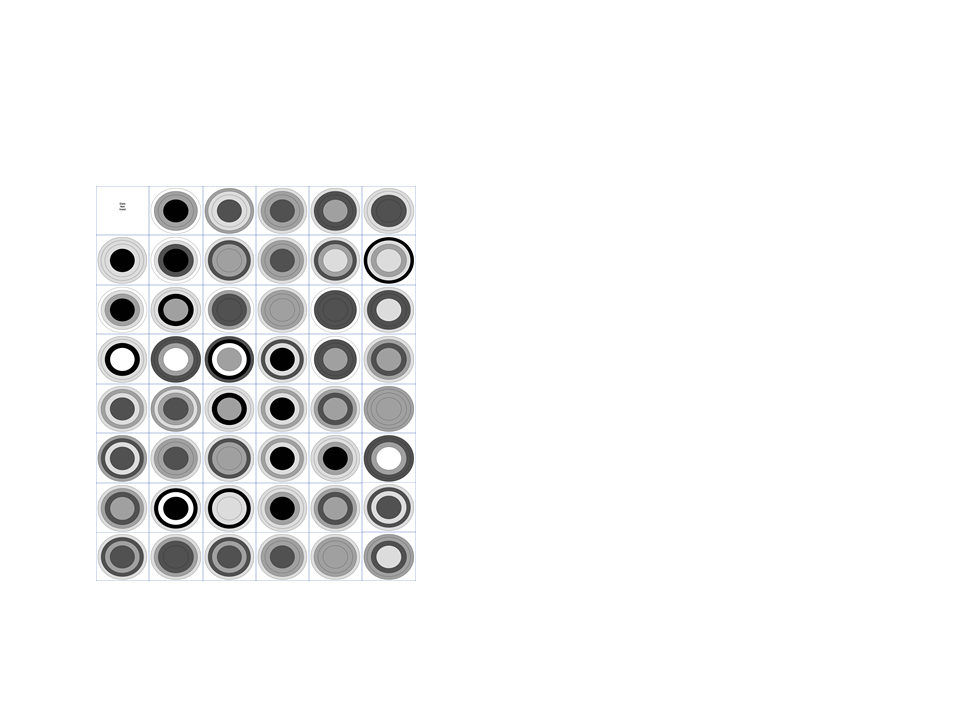
> 115% to ≤ 145% of Mean

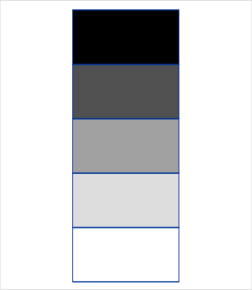
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S17: Particle deposition uniformity across cell culture inserts for 3.3µm MMAD particles (Run #3) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

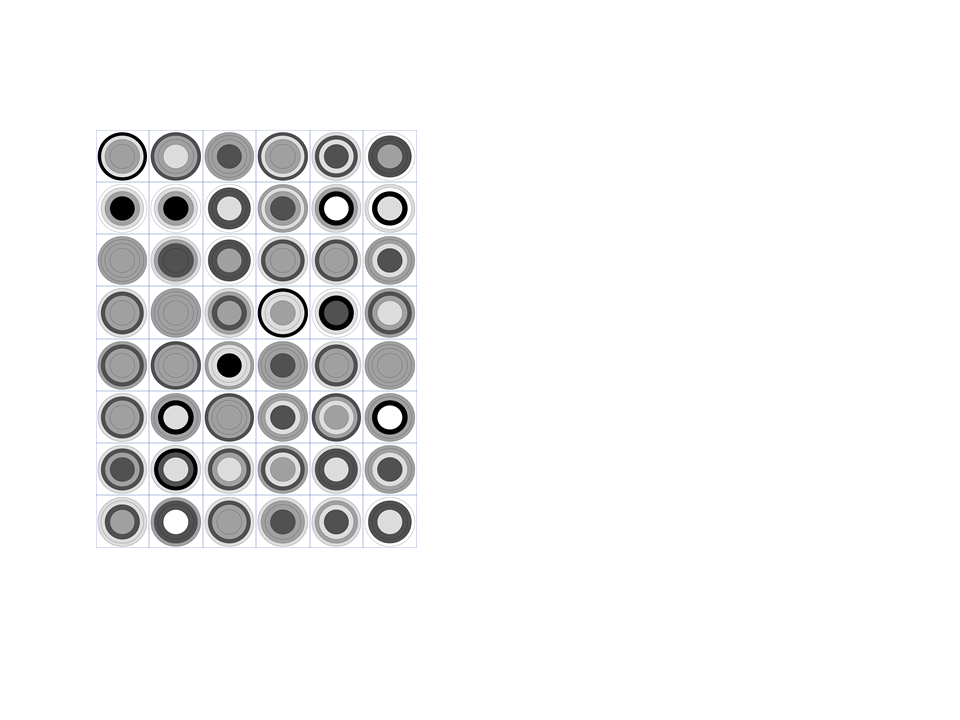
> 115% to ≤ 145% of Mean

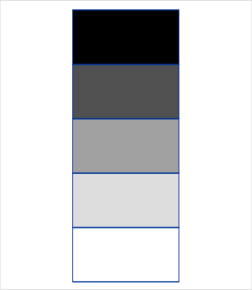
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S18: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #1) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

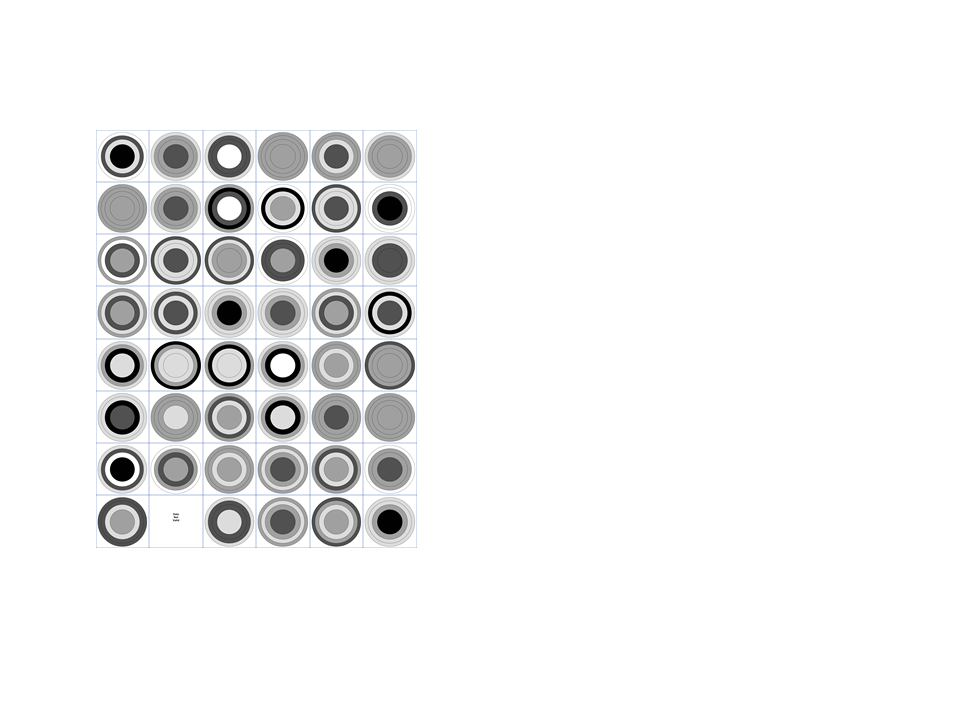
> 115% to ≤ 145% of Mean

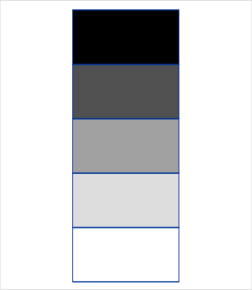
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S19: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #2) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.





> 145% of Mean

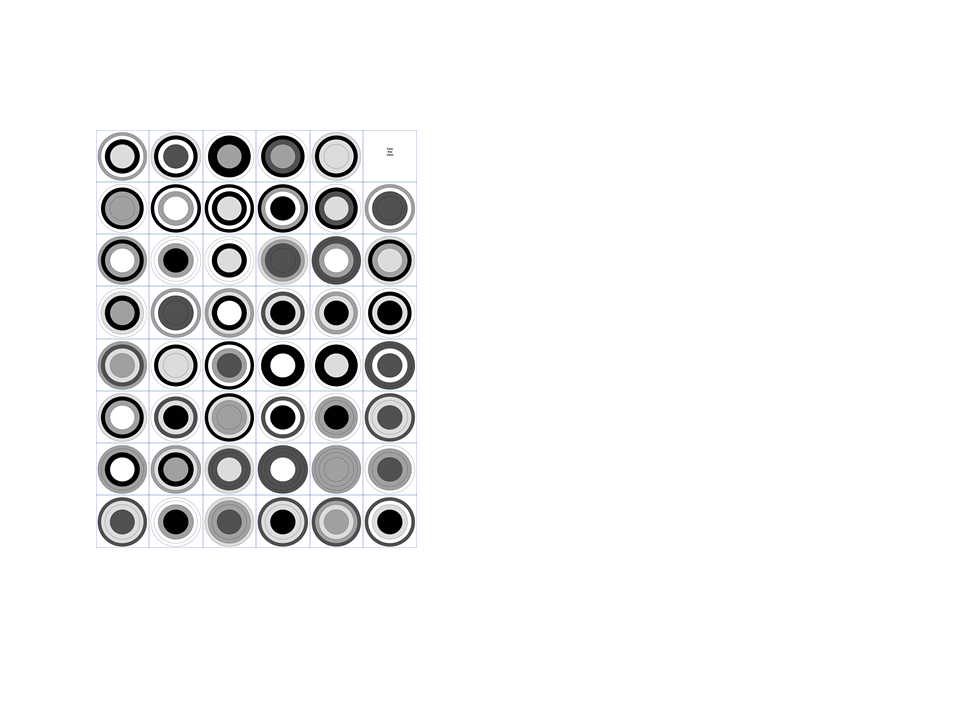
> 115% to ≤ 145% of Mean

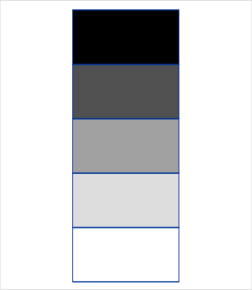
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S20: Particle deposition uniformity across cell culture inserts for 2.2µm MMAD particles (Run #3) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

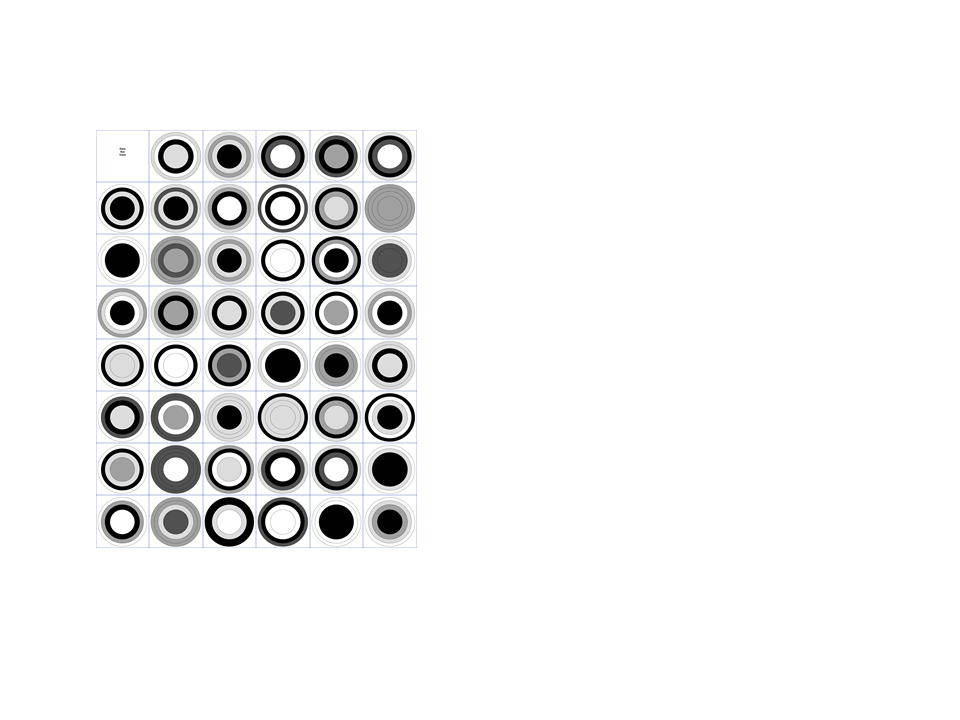
> 115% to ≤ 145% of Mean

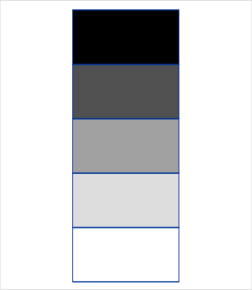
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S21: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #1) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

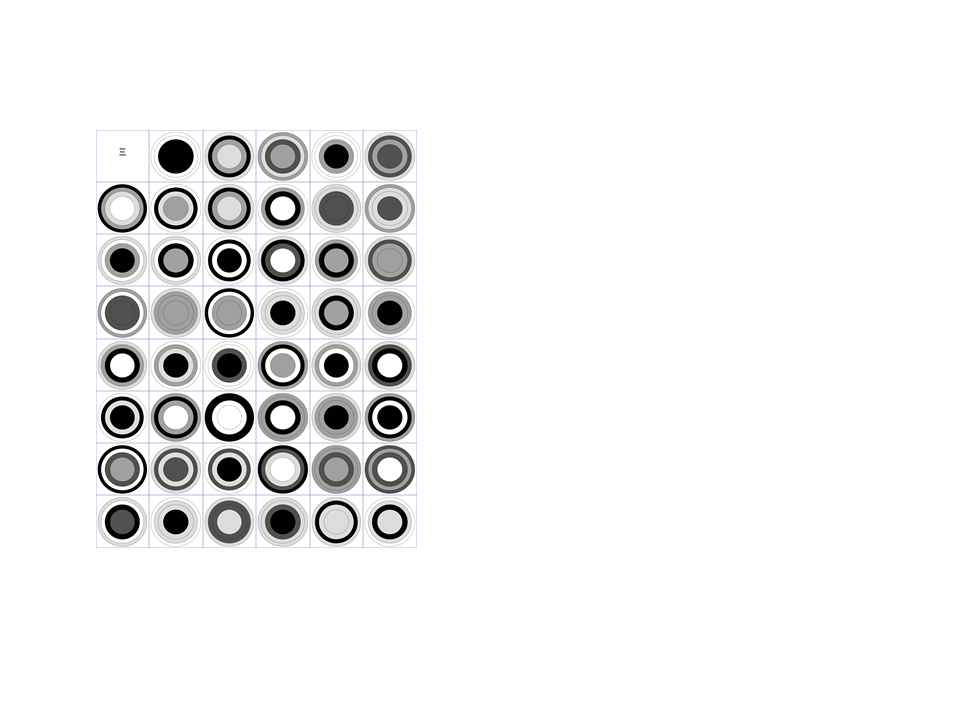
> 115% to ≤ 145% of Mean

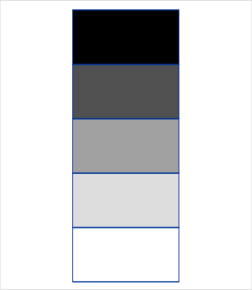
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S22: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #2) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

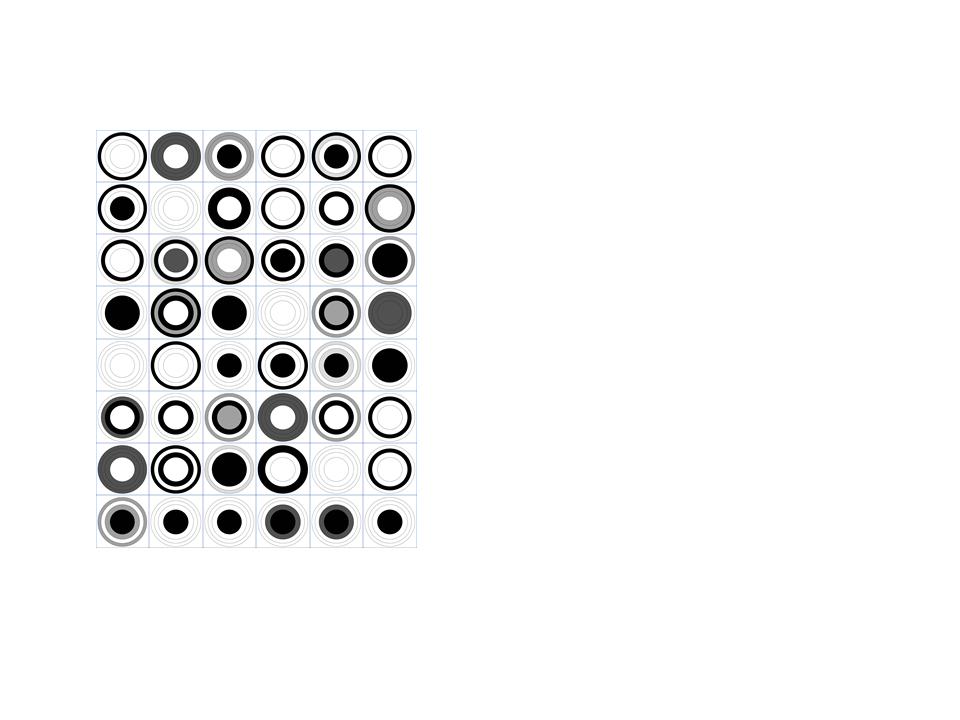
> 115% to ≤ 145% of Mean

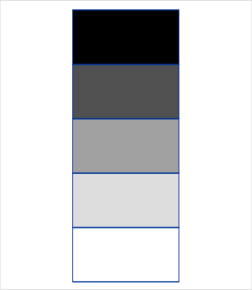
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S23: Particle deposition uniformity across cell culture inserts for 1.1µm MMAD particles (Run #3) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.





> 145% of Mean

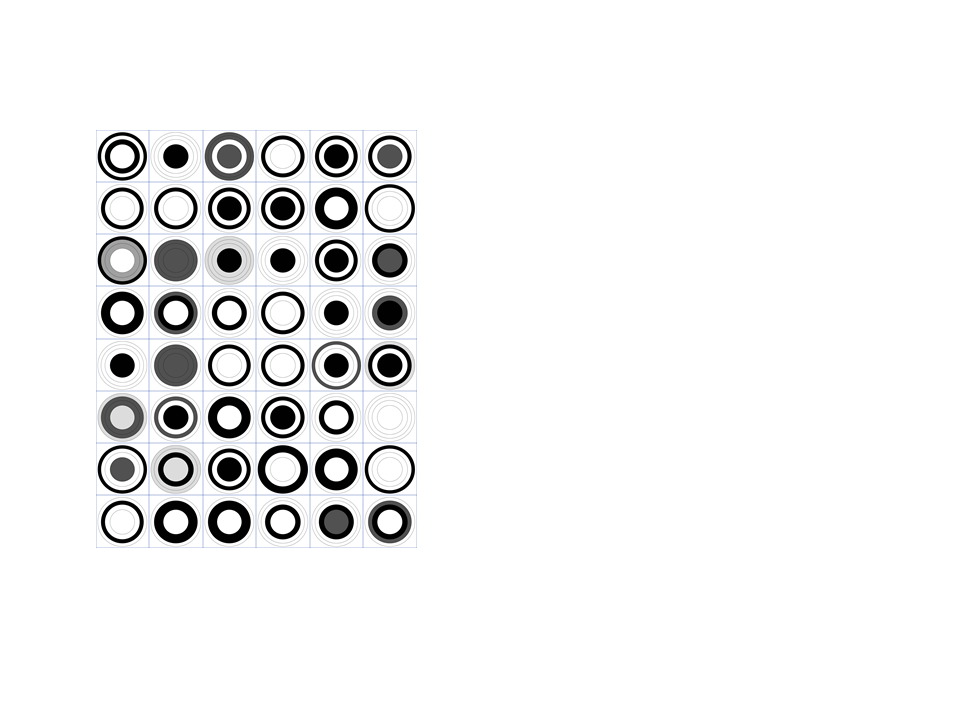
> 115% to ≤ 145% of Mean

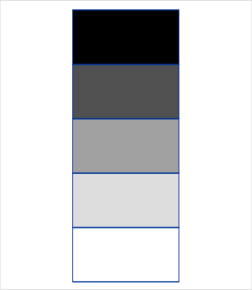
85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S24: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #1) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.





> 145% of Mean

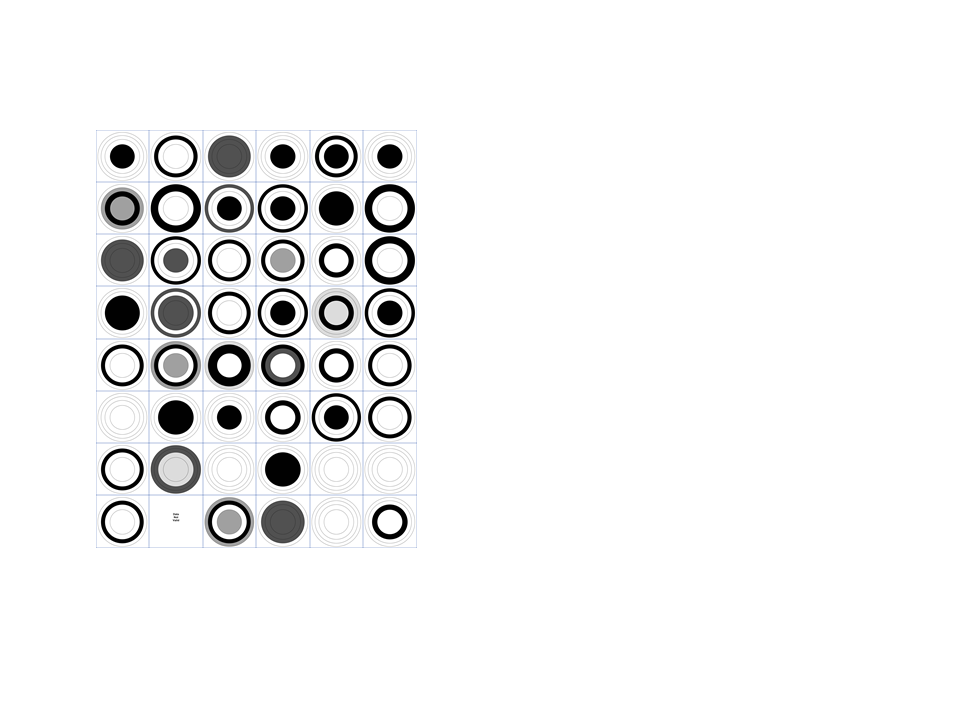
> 115% to ≤ 145% of Mean

85 – 115% of Mean

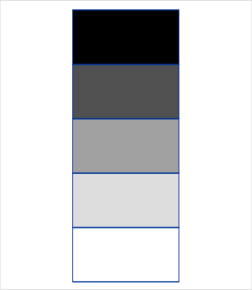
55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S25: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #2) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right.



# 



> 145% of Mean

> 115% to ≤ 145% of Mean

85 – 115% of Mean

55% to ≤ 85% of Mean

≤ 55% of Mean

Figure S26: Particle deposition uniformity across cell culture inserts for 0.51µm MMAD particles (Run #3) in a circle and 3 rings with equal area expressed as percent of mean. The eight channels are from the bottom to top with airflow moving from left to right while the columns (A-F) are from left to right. “N/A” designates sample not made available.

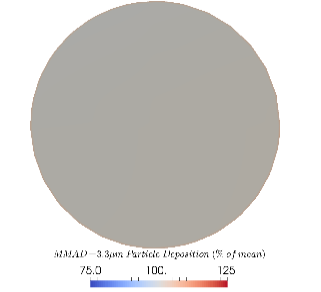
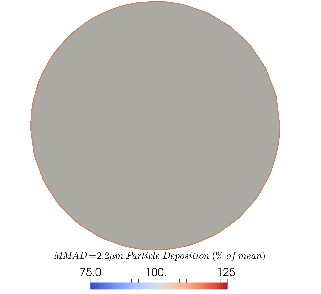
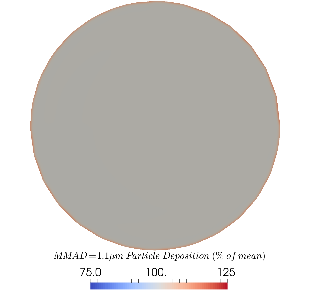
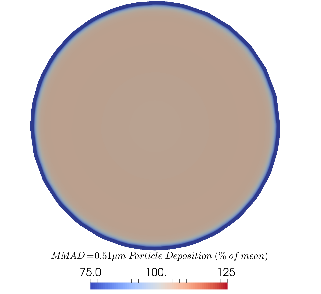


Figure S27. Eulerian CFD predicted particle deposition uniformity expressed as percent of mean, for different particle diameters from left to right (first = 0.51, second = 1.1, third = 2.2 and fourth = 3.3µm MMAD) for the fourth cell culture insert in a row.