Introducing Halo Labs' Cell Map Reduction Algorithm

A companion Technical Note to App Note 14

Introduction

Automated image particle detection was recently added into USP 788 Method 2 (Membrane Microscopy) as an accepted method for analyzing particulates in manufactured pharmaceuticals.[1] In automated membrane microscopy, particles are detected and differentiated from the membrane background using intensity thresholding to detect, count, and size only the objects that are sufficiently different from the background based on their measured scattered brightfield intensity, measured in Aura systems using backgrounded membrane imaging (BMI). Thresholding and particle acceptance criteria vary significantly between different algorithms, leading to different degrees of detection sensitivity. For example, USP 788 recommends against counting translucent particles and smears, prioritizing opaque particles which are likely insoluble particles and aggregates. Different algorithms can be used to reveal different information about a given sample and as a result, there is no one size fits all detection paradigm. Halo Labs' Aura systems are currently powered by three detection algorithms. In App Note 14, we used the Cell Map Reduction algorithm to accurately detect visible particles without detection fragmentation. Here, we compare it against the Ratio and Threshold and the Ratio and Double Threshold algorithms with respect to the counting linearity of hIgG aggregates as well as the ability to accurately size and count microsphere count particle standards.

Particle Vue Software Detection Algothrims

The following three algorithms were compared using Particle Vue v4.0 and above software:

- **Cell Map Reduction:** This new algorithm analyzes particles in both the visible and subvisible size range. It uses a two-step process where it first finds particles in 5 x 5-pixel cells. If the intensity in that cell is higher than the threshold, then it becomes a candidate for particles and is checked against the corresponding background. Next, if the algorithm determines that particles are present in that cell by comparison to the background image, the cell advances to the next step where candidate cells are grouped for subsequence particle identification and characterization.
- **Ratio and Threshold:** The "original" Halo Labs algorithm, this algorithm first registers (aligns) the membrane image with the measure image to assign co-located pixels. Once this is done, image processing is performed to equalize the image properties before finding the intensity ratio for each co-located pixel. The pixel is counted if the measure image's pixel is 30% darker than the original membrane image and if a neighboring pixel also meets this criterion it is added to the same particle. A particle mask is produced through this approach and is analyzed to produce a particle size distribution.

- **Ratio and Double Threshold:** This algorithm is similar to the Ratio and Threshold algorithm but incorporates one additional step to account for particles that are lighter and more transparent than those detected using the original Ratio and Threshold algorithm. After executing the threshold step described above, this algorithm applies a more sensitive threshold to the previously measured particles by "growing them" on neighboring pixel intensity revealing changes as low as 15% on both dark and bright intensity compared to the membrane.

System Linearity: Human IgG Aggregate Dilution Series

Testing for system linearity on translucent, polydisperse samples is essential when evaluating subvisible analysis accuracy. In Figure 1, we compare how the Particle Vue software algorithms perform when counting the same hIgG samples. Aggregated hIgG was serially diluted before each dilution was loaded into 8 replicate wells with 50 µL of sample per well. While all algorithms produced different counts, with up to a 50% difference in certain cases, they all responded linearly with R^2>0.98 for all algorithms. Additionally, each condition demonstrated low standard deviations during this entire 16-fold serial dilution experiment.

Accurate Sizing and Counting of Polystyrene Microsphere Standards

Microsphere sizing and counting standards are the most widely used particles for particle sizing and counting system calibration and verification. In Table 1, we show how the three different algorithms perform at sizing and counting 10 µm and 25 µm microspheres, both rated as 3k/mL USP particle counting standards.

Average singlet sizes were obtained using the averaging function in a Particle Vue software scatterplot, while counting was performed using the method described in App Note 1. The sizing analysis demonstrates that all three algorithms sized both 10 µm and 25 µm beads appropriately below 7% error. Figures 2a and 2b show that all particle counting was well within the manufacturer count specification of ± 10% for both bead count standard types.

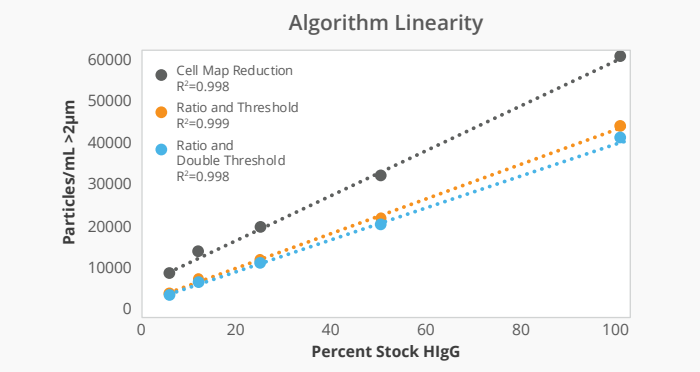

Figure 1: Comparison of the three Particle Vue software algorithms. Aggregated human IgG (stock concentration 0.5 mg/mL) was diluted 1:2, 1:4, 1:8 and 1:16 before each dilution was loaded into 8 replicate wells. Analysis using the three algorithms available with Particle Vue software all demonstrated high linearity and low CV% over a large range of concentrations, with ± 95% CI.

Algorithm Name	Size of 10 µm Bead (10.01 actual)	Size of 25 µm Bead (24.6 actual)
Cell Map Reduction	10.59 µm (st dev = 0.46 µm)	24.72 µm (st dev = 0.90 µm)
Ratio and Threshold	10.19 µm (st dev = 0.31 µm)	24.21 µm (st dev = 0.32 µm)
Ratio and Double Threshold	9.66 µm (st dev = 0.29 µm)	22.94 µm (st dev = 0.46 µm)

Table 1: Accurate Sizing of 10 µm and 25 µm Beads. Data from 24 individual wells (50 µL per well) of 10 µm and 25 µm bead singlets show that the Cell Map Reduction, Ratio and Threshold, and Ratio and Double Threshold algorithms size the beads with errors below 7%.

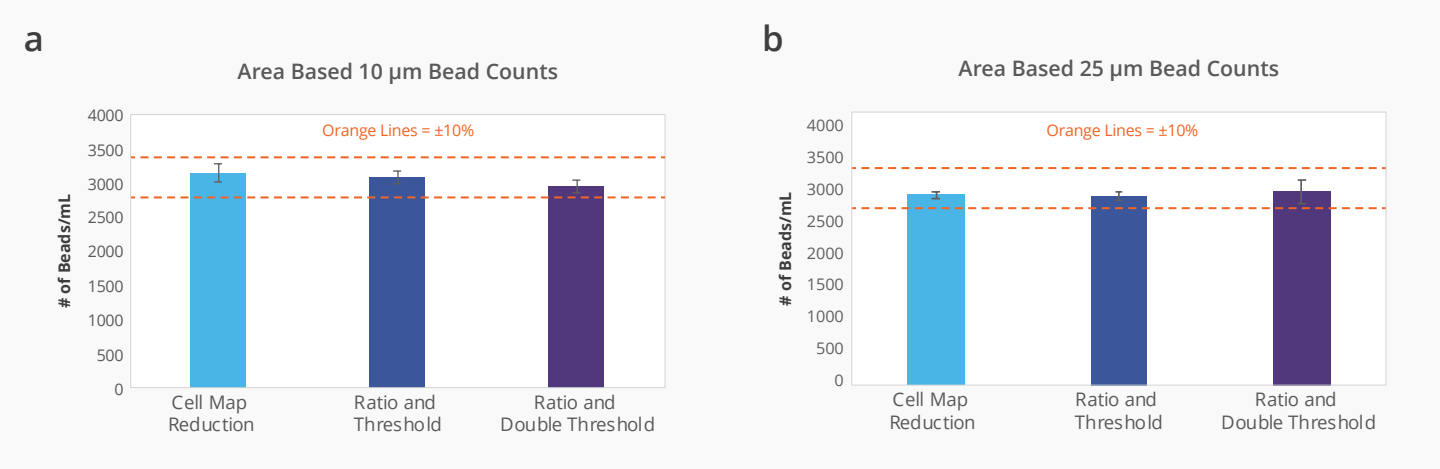

Figure 2: Particle counting data from the same 24 individual wells calculated by taking the total area of all particles divided by the area of a single bead for (a) 10 µm counts standards and (b) 25 µm count standards. Manufacturer stated concentration of beads is 3,000/mL ± 10%. The orange dashed lines represent the specified 10% error specification.

Conclusions

Aura systems utilize BMI to facilitate accurate particle detection, sizing, and counting using different types of algorithms. All three algorithms, including the Cell Map Reduction algorithm, accurately sized and counted biological particles and referenced sizing and counting microsphere particles at low volumes and high-throughput.

References

1. Membrane Microscopy Method for the Determination of Subvisible Particular Matter, *USP <1788.2>*.
2. <788> Particular Matter in Injections, https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/revisionGeneralChapter788.pdf

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