

Figure 1 shows how a relatively opaque particle can produce a different binary image depending on the threshold value and thus result in inaccurate particle measurements.

When imaging transparent or semi-transparent particles, the particle image pixel may actually be *lighter* than the background pixel, so the simple binarization using dark-only thresholding, as used in most flow imaging microscopy, will not include pixels that are lighter than the background as part of a particle. When dark-only thresholding is used, large, transparent particles are often inaccurately fractionated, or divided, into several smaller particles. This results in inaccurate measurements, and inaccurate count and concentration calculations. Additionally, small, transparent or semi-transparent particles may be missed entirely with improper thresholding.

As a result of fractionation, small particles may be over counted and large particles undercounted. Table 1 illustrates the difference in particle concentration when using dark-only thresholding versus dark and light thresholding.

Proper Optimization for Thresholding Transparent Particles

FlowCam and VisualSpreadsheet software are designed to overcome the issue of thresholding transparent and semi-transparent particles. VisualSpreadsheet allows for thresholding on pixels that are both darker *and* lighter than the background, and supports independent threshold values for each. In addition, a "distance to nearest neighbor" setting enables grouping of nearby pixels that are part of the same particle. Figure 3 illustrates the various thresholding settings available in VisualSpreadsheet. Figure 4 shows the effect of varying the threshold values on a single particle (in this case, a protein aggregate), and the resulting binary image produced by VisualSpreadsheet.

At Fluid Imaging Technologies, we understand these issues and have created a system that is easy to optimize. FlowCam ensures that your measurements, true count and concentration results are accurate.

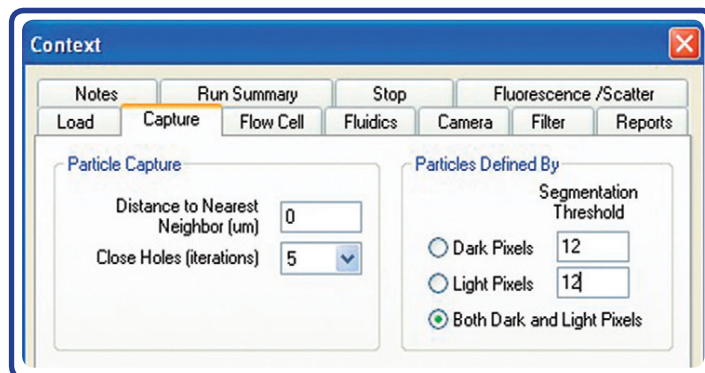


Figure 3: VisualSpreadsheet settings available for adjusting light and dark threshold values, and enabling neighborhood analysis.

Threshold	Total Concentration	Concentration 2-10µm	Concentration 10-25µm	Concentration 25+µm
Dark Only	344,156	186,763	120,694	36,517
Dark + Light + Neighborhood	165,023	42,028	59,469	63,405

Table 1: Concentrations (particles/ml) with varied thresholding

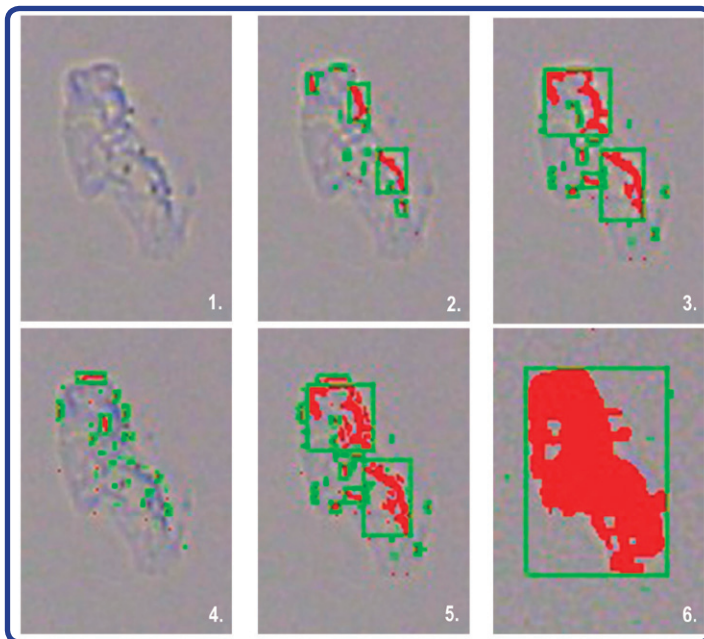


Figure 4: The effect of varying threshold on binarization of protein aggregate image. Red pixels indicate those found to be "particle" based on the threshold (and included in the binary). The green boxes show enclosed "particles" found after thresholding.

Image 1 is the original protein aggregate image. Image #2 shows the result of a threshold of 25 darker than background. Image #3 shows the result of a threshold of 15 darker than background. Image #4 shows the result of a threshold of 15 lighter than background. Image #5 shows the result of thresholding 15 lighter and darker than background. Finally, Image #6 is the same as #5 but with the addition of neighborhood analysis applied. Note the trend from many small particles identified in Image #2 to finally the correct answer of one large particle at Image #6.