Characterization of protein aggregates and other particulates in biologics

FlowCam® Application Note #106

FlowCam™
Particle Analysis with Vision
FLUID IMAGING TECHNOLOGIES, INC.

Objective

Particulates in parenteral drug development and production have always been a serious issue. In biopharmaceuticals, including protein therapeutics, the issue is compounded by reported impacts of aggregates and particles on the product's efficacy, safety and immunogenicity. FDA regulations strongly recommend in-depth characterization of the identity and quantity of particles in protein therapeutics. Particle concerns are increasingly common in FDA submissions, and in some cases, particle matters have resulted in drug recalls.

Quantifying particles larger than 10µm and 25µm has been a USP <788> requirement for drug product release since 1975. In protein therapeutics, the addition of "inherent" particles in the form of protein aggregates, alongside the "intrinsic" particles (such as silicone droplets) and "extrinsic" (foreign) particles usually found in parenterals makes particulate characterization more difficult than with small molecule formulations. For this reason, the USP <1787> Informational Chapter states that: "Because multiple potential sources of particles exist, it is important to identify the particles and determine whether they are extrinsic, intrinsic, or inherent."

While conventional microscopy can be used, the time required for sample preparation and the lack of statistical significance caused by small sample sizes are limiting factors. As a result, other automated particle sizing methods such as light obscuration, electrozone sensing and laser diffraction have also been used. The problem with these techniques is that they assume all particles are spherical in shape, recording only an Equivalent Spherical Diameter (ESD) for each particle. As a result, they can not infer information on a particle's identity. In addition, light obscuration, one of the most common techniques used to meet USP requirements, has challenges detecting and properly sizing particles with a low refractive index (such as protein aggregates). As a result of this insensitivity, a batch might pass USP <788> even though it may contain unacceptable levels of protein aggregates which the light obscuration system could not detect.

Method

FlowCam was developed to address existing technology limitations and assist biopharmaceutical scientists to further understand their products, and consequently comply with FDA requirements. The system stores a digital image and over 30 different measurements for each particle. Scientists can therefore characterize particles based on size *and* shape, enabling the automatic differentiation between inherent protein aggregates, intrinsic particles (such as silicone oil droplets) and extrinsic particles (such as fibers, plastic, etc.). Since all images are saved, the results can be visually examined to ensure data accuracy and for troubleshooting. Additionally, FlowCam's VisualSpreadsheet® software has versions designed to meet 21 CFR Part 11 requirements, which is essential in regulated environments. Because of this, FlowCAM is ideal for extended particle characterization during development and release.

The screenshot in Figure 1 shows the results of a FlowCam run (AutoImaging mode) with a polyclonal IgG sample containing silicone droplets. A total particulate load of 315,794 particles/ml was found. Sample images in the right hand window show the diversity of particles found, including both protein aggregates and silicone droplets.

In this particular sample, it is apparent that many of the particles are, in fact, silicone oil droplets. These particles are intrinsic to this formulation which is delivered in a pre-filled syringe containing silicone as a lubricant. VisualSpreadsheet allows for the removal of these silicone droplets and then recalculating particulate load without these intrinsic particles included.

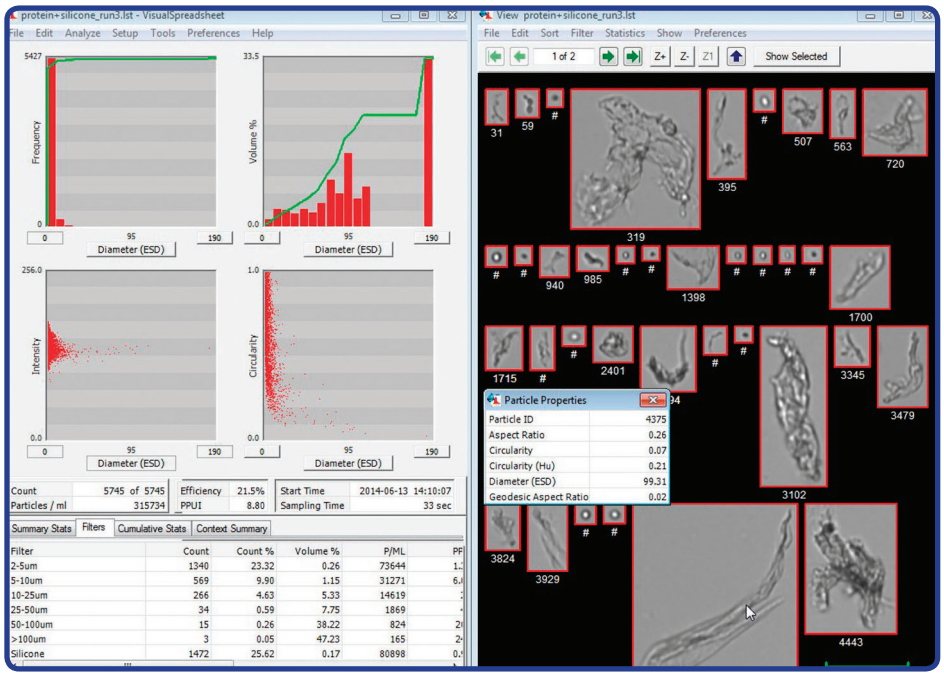

Figure 1: FlowCam results for a protein therapeutic sample.

The last filter shown in the left window of Figure 1 is a silicone filter created in VisualSpreadsheet to isolate the silicone droplets only. Figure 2 (next page) shows the result of selecting this filter and the software displaying the particle images that fit the filter (silicone droplets). Note that the silicone droplets represent 80,898 particles/ml of the original 315,794 particles/ml total found, which is just over 25%.

Regulatory agencies are now asking for drug developers to characterize particulate matter in biologics, with a new emphasis on particles in the 2-10µm size range in addition to the classical >10µm and >25µm reporting used in the past. With biologics, it is particularly important to actually *characterize* the particles (intrinsic, extrinsic and inherent) as opposed to just *counting* the total number of particles. This characterization is only possible using imaging techniques because of their ability to measure shape and other parameters which can be used to distinguish the particle *type.*

An experiment was conducted to study the efficacy of different software filtering algorithms on properly identifying silicone droplets[1]. In order to measure how effective each filter is, the data set was first hand-classified visually, and a particle type (protein, silicone, etc.) was assigned to each particle record. The human eye-brain system is much more adept at *inferring* content based on subtle cues than is possible algorithmically. So after each particle was assigned a "type (known)" visually, each filter's results ("type classified as") could be compared to determine filter accuracy.

The filters used are as follows:

- Aspect ratio (width/length): historically has been a de facto standard method of distinguishing silicone droplets[2], and was thus used as a baseline.
- Hu Circularity: produces better results for circularity than other measures when looking at particles with boundary defects, i.e. when the perimeter of the circle is not perfect[3].
- Statistical filter: uses statistical pattern recognition to find similar particles to ones chosen as examples by the user.
- Custom value filter: using the three particle parameters for known silicone droplets that have the smallest Coefficient of Variability (CV).

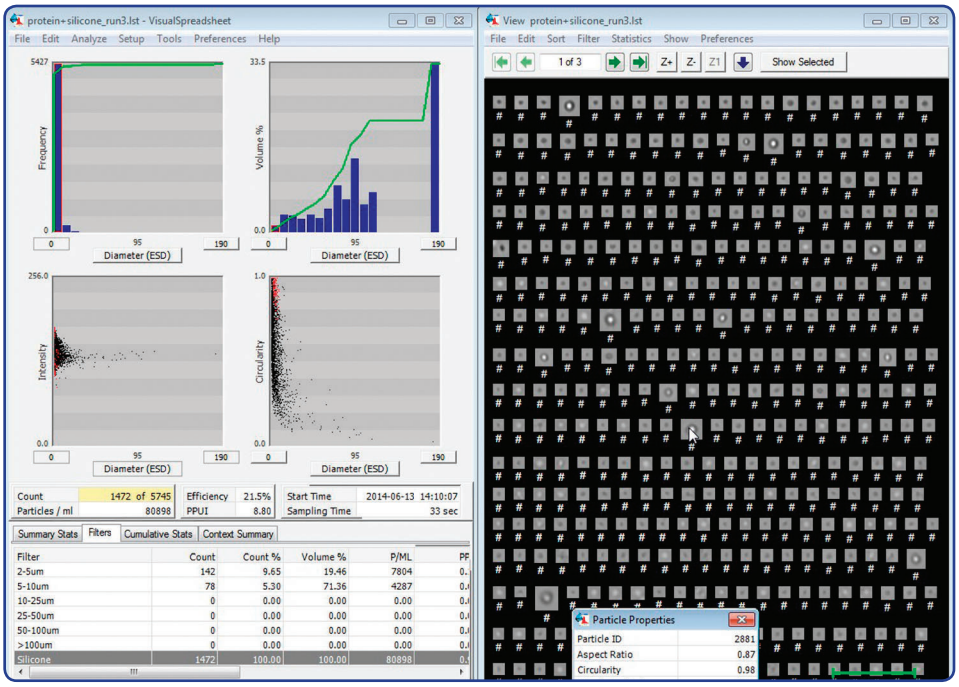

Figure 2: FlowCam results for same protein therapeutic sample, showing the number of silicone droplets found by the filter and the corresponding particle images (right).

Filter	% Increase Overall vs. Baseline (Aspect Ratio filter)
Aspect Ratio ≥ 0.85	N/A (baseline)
Circularity (Hu) ≥ 0.95	42%
Statistical Filter	73%
Customized Value Filter	130%

Summary Results:

The results clearly show improved accuracy for silicone characterization in the sample by using filters containing more discriminatory particle properties (Hu Circularity), as well as more powerful statistical based methods.

Conclusions:

Several conclusions can be drawn from this example/experiment:

- FlowCam offers a powerful method for characterization of particulates in biologics, enabling separation into distinct particle types (extrinsic, intrinsic, inherent).
- The ability to see each particle image is critical to understanding filter efficacy.
- The availability of a larger variety of particle properties aids in better filter discrimination.
- Advanced measurements (e.g. Hu Circularity), and advanced techniques (e.g. statistical filtering) can significantly increase filter accuracy.

Dynamic imaging particle analysis using FlowCam is a superior method for characterizing particulate content in biologic formulations.

References:

1. *Brown L, Bernt W. (2014), A Comparison of Methods for Quantifying Silicone Droplets in Biologics Using Dynamic Imaging Particle Analysis. Proceedings of 2014 Workshop on Protein Aggregation and Immunogenicity, Breckenridge CO, July 2014*
2. *Sharma, D.K., Oma, P., & Krishnan, S, (2009). Silicone Microdroplets in Protein Formulations-Detection and Enumeration. Pharmaceutical Technology, 33 (4), 74-79.*
3. *Žunic, J., Hirota, J., & Rosin, P.L. (2008). A Hu moment invariant as a shape circularity measure. Pattern Recognition, 43 (1), 47-57.*