FlowCam®

Characterization of Protein Aggregates and Other Particles in Biopharmaceuticals

OVERVIEW

Particulates are ubiquitous in parenteral drug products and remain a concern throughout their development and production[1–3]. These particles must be monitored to satisfy USP particle reporting requirements (e.g. USP <788>). Furthermore, in biopharmaceuticals such as protein therapeutics, these particles have been associated with adverse impacts on the efficacy and safety of the product[4,5]. FDA regulations strongly recommend in-depth characterization of the quantity and types of particles found in biotherapeutics.

Light Obscuration (LO) is the primary compendial technique used to monitor subvisible particles (i.e. particles 2-100 µm in diameter) in parenteral drug products. While LO is effective for counting and sizing opaque particles, it is less effective at analyzing particles in biotherapeutics like aggregates of the active pharmaceutical ingredient (API) which are often highly translucent[6,7]. Another key weakness of LO and other particle analysis techniques is its inability to record particle morphology information—information often related to the types of particles detected by the instrument. Particle morphology information can be used to characterize the types of particles in a sample, detect new and/or unexpected particle types present in a sample, and even detect common artifacts in particle analysis instruments like air bubbles.

One use for particle morphology information is identifying silicone oil droplets in a sample. Silicone oil is commonly used as a syringe lubricant and, during use, the oil layer on the walls of the syringe can become displaced and form additional particles in solution[8,9]. It can be useful to identify these droplets as the oil-water interfaces they create can induce protein aggregation[9,10] and the droplets themselves may contribute to the immunogenicity of the sample[11,12]. Particle analysis techniques that record particle morphology information can be used to differentiate between silicone oil droplets and other common particle types (e.g. API aggregates, glass and metal flakes, cell fragments, bacteria) that may be in a biotherapeutic sample—information that can be informative when making decisions about product quality.

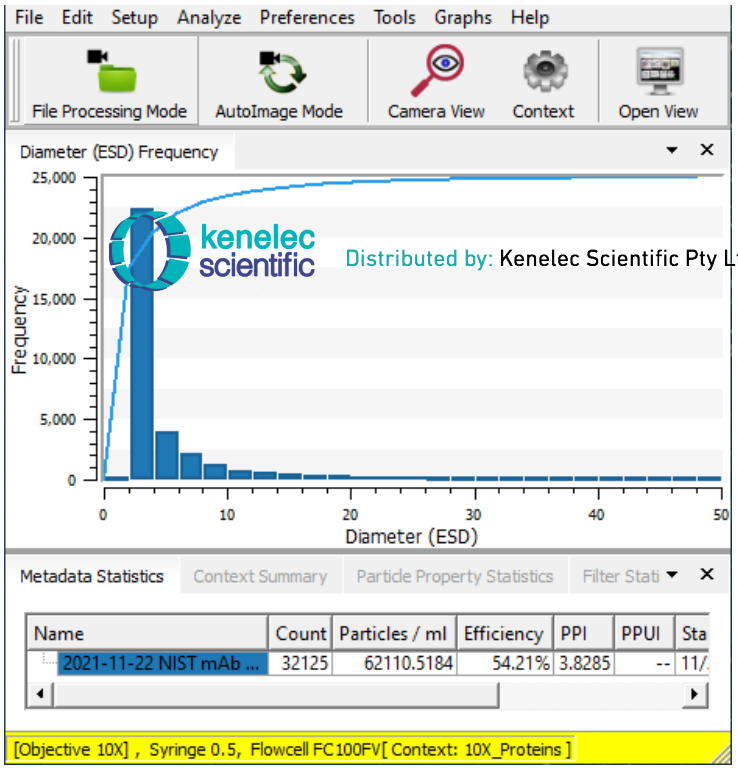
Flow imaging microscopy (FIM) is a USP-recognized orthogonal technique (via USP <1788>) to complement LO and other compendial techniques for particle counting and sizing. FIM instruments like FlowCam capture light microscopy images of particles as they flow through a flow cell. This method of particle detection can count and size particles in a liquid sample. Particle concentrations may also be estimated using the measured particle count and the volume of sample imaged—a quantity that can be calculated using the flow rate of the instrument, the geometry of the flow cell, and the frame rate of the camera. Since a digital image is recorded for each particle, FIM can also be used to capture particle morphology information not available from LO measurements. Scientists can use either the raw image data or the particle measurements recorded by FlowCam's VisualSpreadsheet software to differentiate between particle types (e.g. distinguishing particles from the active ingredient and formulation from contaminant particles)[8,13]. FlowCam LO, a recent advancement in FIM technologies, allows users to perform simultaneous FIM and LO measurements in a single instrumentusing the same sample aliquot, integrating FIM with a compendial particle monitoring technique.

In this case study we show how flow imaging microscopy and FlowCam can help analyze particles in biotherapeutic samples. A simulated protein formulation consisting of silicone oil microdroplets spiked in a protein formulation was prepared and analyzed with a FlowCam 8100 FIM instrument. FIM was able to effectively capture images of the translucent particles in this sample—information that was used to determine an accurate particle concentration for this sample. These images were also analyzed with simple particle morphology analysis tools within VisualSpreadsheet to identify the number of silicone oil particles included in this sample.

METHODS

A NIST monoclonal antibody (mAb) sample spiked with silicone oil droplets was prepared and analyzed using FlowCam. NIST mAb aggregates were generated by filling a 15 mL conical tube with 2 mL of 1 mg/mL NIST mAb formulation in phosphate-buffered saline (PBS) and vigorously shaking by hand. Silicone oil emulsion was prepared by preparing a 5% v/v solution of silicone oil in PBS and blending the samples with a lab blender for 20 seconds. 0.2 mL of this solution was spiked into the NIST mAb solution. 1 mL of this sample was analyzed using a FlowCam 8100 instrument equipped with a grayscale camera, a 10x objective, and an 80 µm FOV flow cell.

Figure 1 shows the FlowCam output for this NIST mAb sample in VisualSpreadsheet, the software used to operate the instrument and perform data analysis. This window contains information about the particles in this sample including the particle concentration and size distribution. As shown in this window, the NIST mAb sample contained approximately 62,000 particles/mL with a highly asymmetric particle size distribution. While this type of information is available using LO, FlowCam also returns images of each particle that are rich in particle morphology information. Sample FlowCam images for this sample are shown in Figure 2.



As shown in the images, the sample exhibited a highly heterogeneous particle population – a variety of particle morphologies ranging from dark spheres to light thin rods. This morphology information is not accessible by LO alone. Additionally, many of the lighter, more transparent particles in this sample may not be detectable at all via LO due to their relatively poor contrast against the background fluid of the sample. FlowCam LO, a FlowCam model that can perform simultaneous FIM and LO analysis of samples, can be used to confirm the limited sensitivity of LO towards these translucent particles.

One particle population of note in this sample is made up of the dark, circular particles with a bright outer ring and often a bright center. These particles are the silicone oil microdroplets that were spiked into the sample during preparation. FlowCam images allow the user to differentiate between these microdroplets, protein aggregates, and other particle types present in a sample—useful information when trying to analyze the particle content of formulations containing multiple particle types. This particle type information is not available from other common particle analyses like LO and even other techniques for analyzing particle morphology. For example, silicone oil detection is not possible from backgrounded membrane imaging as these droplets can pass through the membrane used to remove fluid from samples prior to imaging.

Figure 1. VisualSpreadsheet window showing particle population for the NIST mAb-silicone oil emulsion mixture. The top histogram shows the size distribution of particles at different concentrations. The bottom table shows some overall particle statistics for the sample such as the total particle count and concentration.

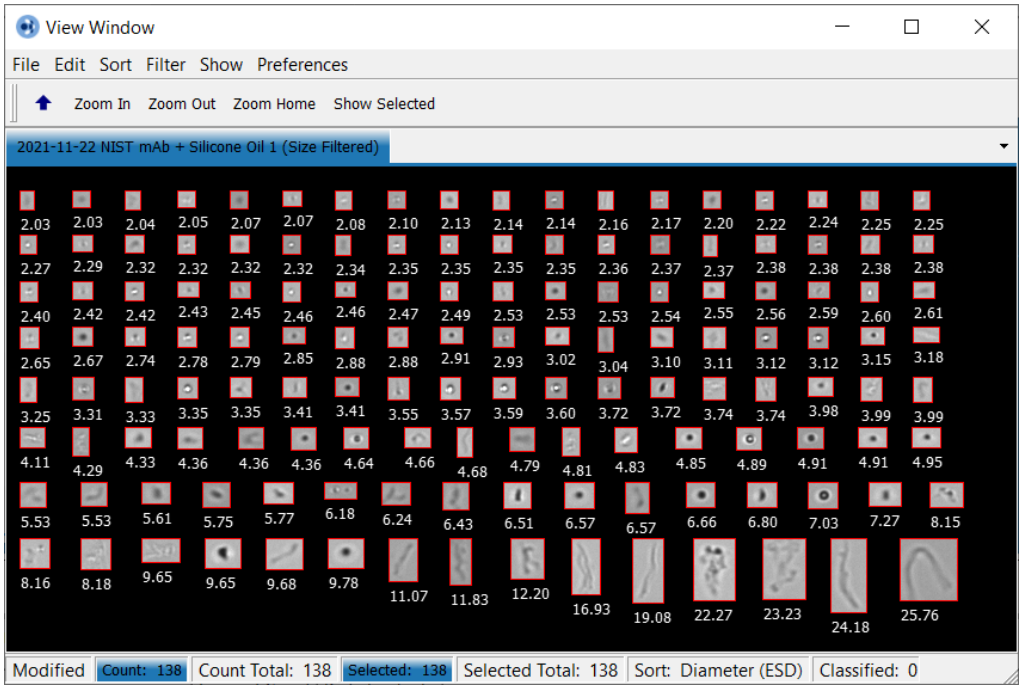


Figure 2. VisualSpreadsheet window showing sample particle images for the NIST mAb-silicone oil emulsion mixture. Values below each image are the diameter (equivalent sphereical diameter) for the particle in µm.

DATA ANALYSIS

VisualSpreadsheet can be used to automatically differentiate between the distinct protein aggregate and silicone oil droplet populations in this sample. During data collection, the raw images of each particle are used to compute several particle properties such as particle diameter, aspect ratio, and intensity (i.e. color). Different particle types will often exhibit different values for these particle properties. VisualSpreadsheet can exploit these property differences between particle types to develop particle filters and classifiers to automatically select and/or sort particles from a sample containing multiple particle types.

A simple silicone oil filter was generated using this FlowCam dataset as a demonstration of the particle analysis tools within VisualSpreadsheet. The software's "Like Selected Particles (Statistical)" feature was used to create this filter. "Like Selected Particles (Statistical)" constructs a particle filter using a set of user-selected FlowCam images in a dataset and applies that filter to the entire dataset to find images of similar particles. 50 images of silicone oil droplets from this sample were used to prepare this filter using this feature.

Figure 3 shows some of the images the filter identified as silicone oil droplets in this sample, nearly all of which exhibit the concentric ring structure characteristic of this particle type. Based on this filter, the NIST mAb sample contained approximately 4,700 silicone oil droplets / mL. These tools within VisualSpreadsheet allow users to rapidly develop simple filters to identify particle populations of interest within a mixed sample—even with just the images from the mixed sample. These simple filters could be further improved by collecting images of individual particle types and developing filters based on these images.

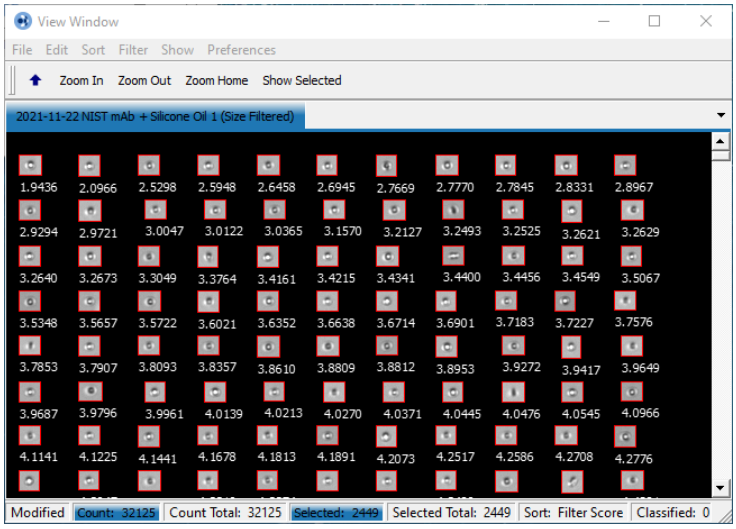


Figure 3. VisualSpreadsheet window showing particle images identified as silicone oil via use of a statistical filter. The value below each image is a score that indicates how silicone oil-like the image is. Lower values of this filter indicate images that most closely match the properties of the originally-selected images.

CONCLUSIONS

The results shown here demonstrate how FlowCam can be used to analyze the number and type of particles in a typical biotherapeutic sample—even samples containing translucent particles. The particle images returned by FlowCam can be used to identify and count the different particle types in a sample such as silicone oil microdroplets—information that is not available from many other particle analyzers. FlowCam allows the user to get a more complete picture of the particle contents of their biotherapeutics which can be used to help ensure the quality and efficacy of that formulation.

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