Generalized Flow Imaging Microscopy Capture Settings to Streamline Quality Assessments of Injectable Biologics

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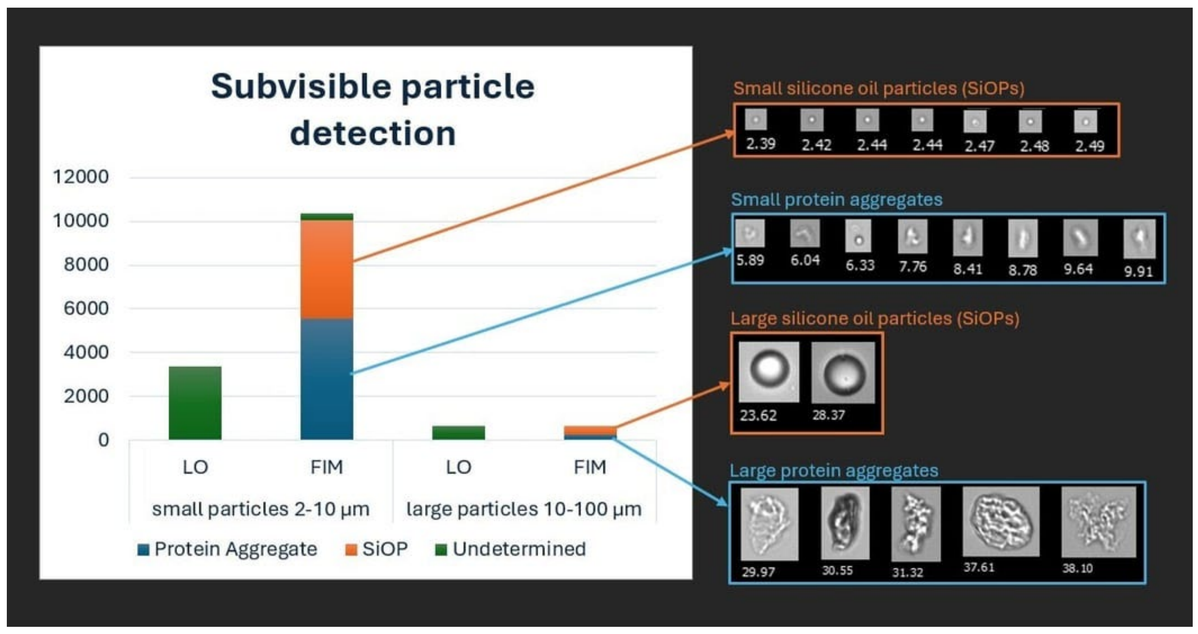
Introduction

Flow Imaging Microscopy (FIM) with FlowCam is an information-rich orthogonal technique for quantifying and characterizing subvisible particulates in parenteral biologics formulations, per USP <1788>. FlowCam software is uniquely powerful in that it allows the user to optimize image capture settings to account for differences in the refractive index of particles of different origin. This flexibility helps overcome challenges often encountered when characterizing particles found in injectable biologics. The FIM general settings developed in this study allow accurate size, concentration and morphological measurements of common particle types encountered in protein therapy development and manufacturing and aim to aid the standardization of FIM as an analytical technique.

Background

Image-Based Subvisible Particle Detection

The ability to visualize subvisible particles (SVPs) between 1 µm and 100 µm within parenteral drug formulations is a key benefit of FIM, compared with non-imaging technologies like Light Obscuration (LO). FIM provides high-sensitivity detection of small translucent particles and the ability to differentiate larger aggregated proteins from silicone oil particles (SiOPs), a key benefit when assessing safety and efficacy. The optical properties, for example, refractive index, of different particle types can pose challenges to the consistent definition of relevant pixels in an image. In this study, we used FlowCam to identify general image capture settings that optimize FIM measurements of a broad range of SVP types.



FIM with FlowCam Image Capture Settings: Definitions and Values

Image Capture Parameter	Value Description	General Settings
Particles Defined By	Determines whether pixel values lower or higher than the background, are used to define the object.	Dark pixels only
Dark Pixels Threshold (Intensity Units)	Threshold decrease in pixel intensity below background for a pixel to be included in the segmentation.	12
Light Pixels Threshold (Intensity Units)	Threshold increase in pixel intensity above background for a pixel to be included in the segmentation.	n/a
Close Holes (Iterations), (CHI)	Number of erosion-dilation cycles used to include internal pixels not originally selected by intensity thresholding.	3
Distance to Nearest neighbor,(DNN) (µm)	Minimum distance that two particles must be separated by to be identified as separate particles.	0

Using “Dark Pixels only” helps eliminate “haloing” artifacts that can appear when Light Pixels are used.

A low Dark Pixel threshold value improves sensitivity for low-contrast regions of particles.

CHI ensures gaps are filled within the segmentation.

Low/disabled DNN avoids capturing fragmented images of individual particles and thus improves counting.

Results

Various values for image capture parameters were assessed using a combination of qualitative and quantitative optimization strategies to identify general settings that improved segmentation of both opaque and translucent SVP standards. Improved segmentation provides more accurate measurements of SVPs typically found in protein biologics formulations.

General Settings are effective across SVP refractive indices

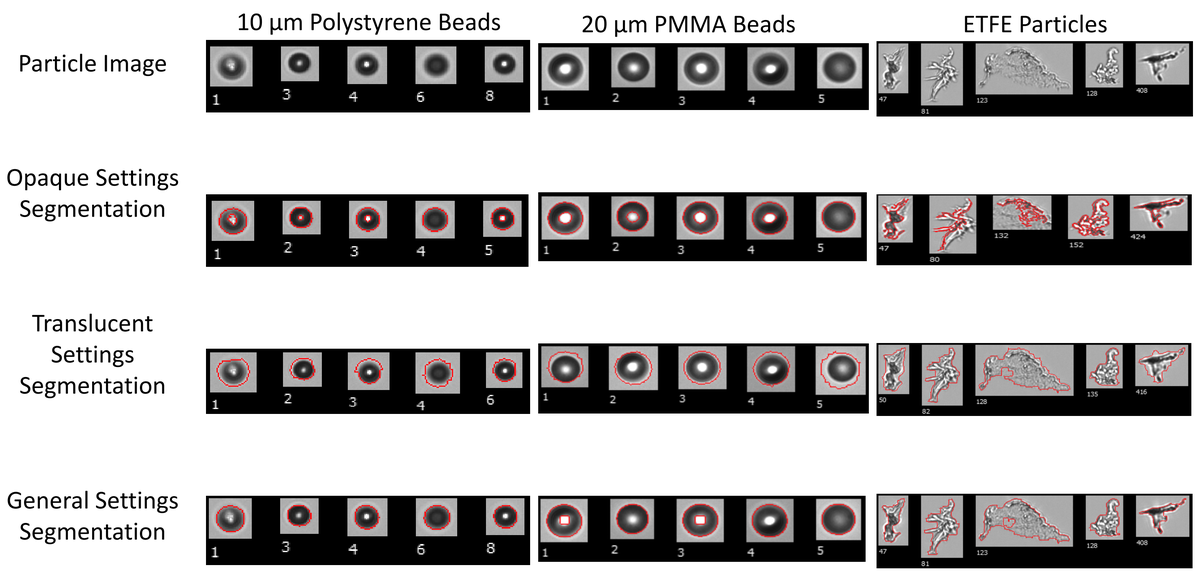


Figure 1. Comparison of segmentation settings effects on spherical and non-spherical SVPs with differing refractive indices. Red lines illustrate object segmentation boundaries defined by Opaque, Translucent, and General settings developed for SVPs of differing refractive indices, respectively. General settings were qualitatively most effective at edge tracing object boundaries across SVP particle types - in agreement with visual inspection of high-refractive index polymer spheres and lower-refractive index particles like ETFE, whose optical and morphological properties are similar to those of protein aggregates.
Image ID numbers are shown below each particle image.

Effective Detection of Common SVPs Found in Protein Therapy Development and Manufacturing

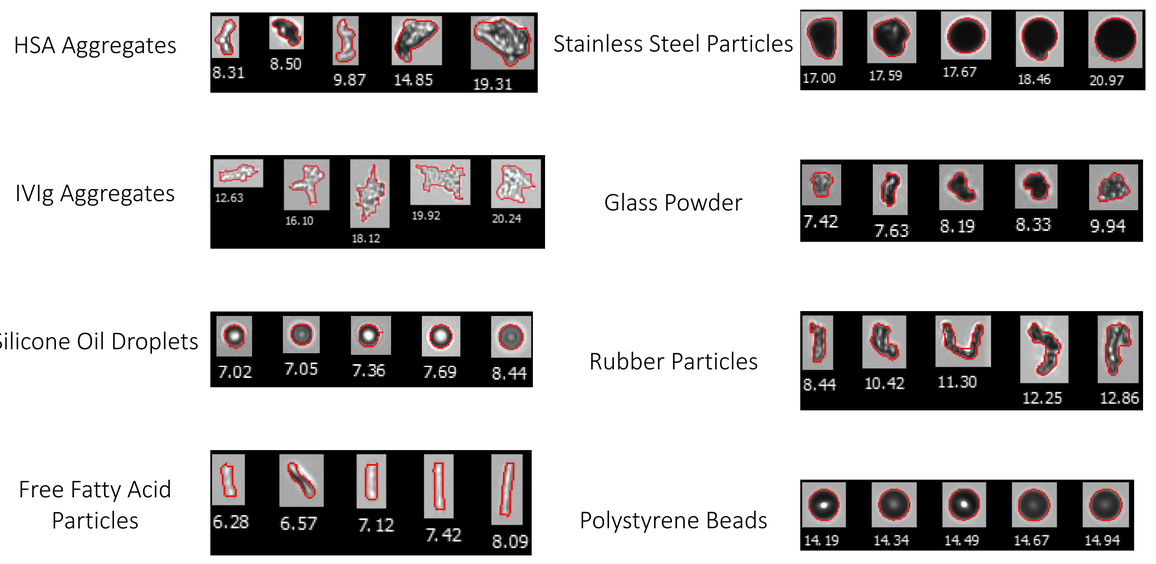


Figure 2. General segmentation settings proved to be effective in defining particles derived from many different sources relevant to drug product manufacturing and stability.

Conclusions

The improved detection and characterization of SVPs using the General capture settings described in this study, demonstrate the value of using FIM as a standard technique for analysis of particulate matter in biologics formulations, therefore overcoming some of the limitations of current compendial techniques such as LO measurements.

Questions about flow imaging microscopy?

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