

Abstract

Subvisible particles are ubiquitous in biotherapeutics and have been associated with adverse reactions and other changes in product quality. Flow imaging microscopy (FIM) is a commonly used analytical technique to monitor subvisible particles in these therapies. This method involves capturing brightfield microscopy images of particles present in a flowing liquid sample to determine particle concentration, size, and morphology. These measurements allow researchers to quantify the subvisible particle content and help determine the source(s) of these particles. This poster provides an overview of applications for monitoring subvisible particles in protein therapies and other biologics using flow imaging microscopy.

Materials and Methods

Subvisible Particle Imaging

- Samples containing typical inherent and intrinsic subvisible particle types found in drug products were analyzed using a FlowCam 8100 instrument at 10X magnification

Accelerated Stability Study

- 1 mg/mL BSA formulations in PBS were prepared with and without 0.1% (v/v) polysorbate 80 (PS80)
- Samples were stressed for 4 hours via either shaking (plate rocker, max settings) or heating at 60°C
- Analyzed subvisible particle content using FlowCam LO, a combined flow imaging microscopy-light obscuration (LO) instrument

Protein-Silicone Oil Classification

- Protein Aggregate Generation: 1 mg/mL IVIg formulation in PBS was shaken via plate rocker for 4 hours
- Silicone Oil Droplet Generation: A 5% (v/v) silicone oil suspension was emulsified via blending
- Samples were analyzed via FlowCam 8100
- All particles larger than 3 μm were analyzed via VisualAI software, an integrated, convolutional neural network-driven protein aggregate-silicone oil droplet classifier.

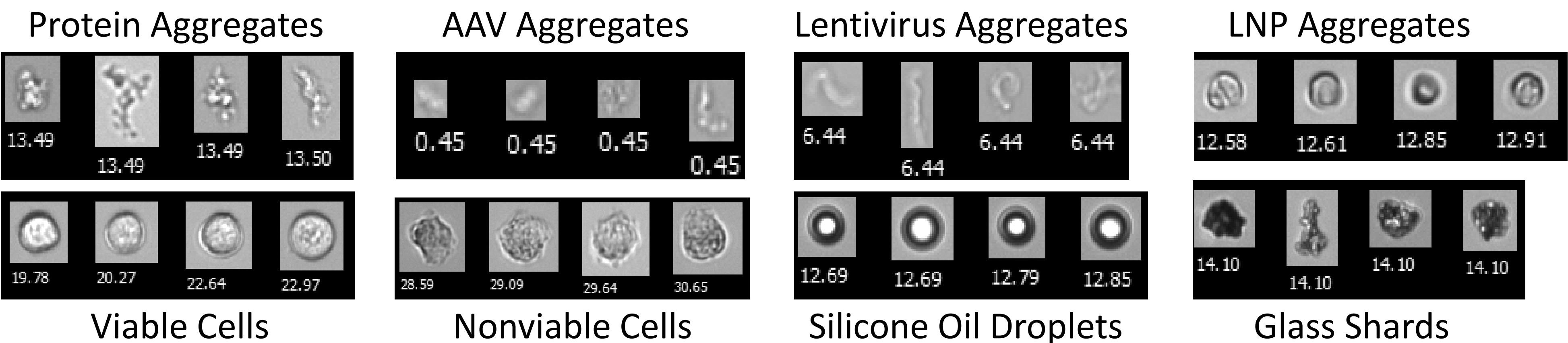


Figure 1: Example particle images of common particle types found in biopharmaceutical drug products

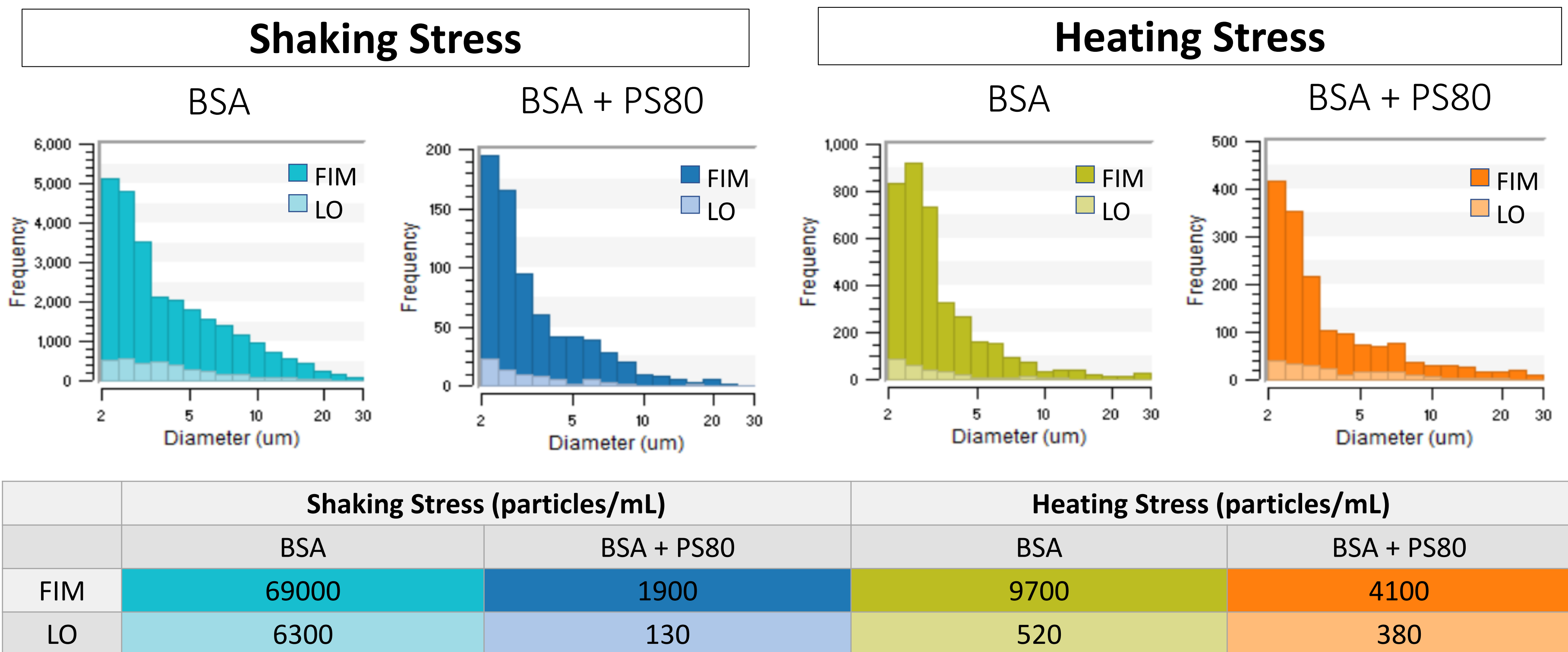


Figure 2: FlowCam LO histograms for BSA with and without PS80 exposed to shaking and heating stress. Particle concentrations for each experimental condition are shown below the histograms.

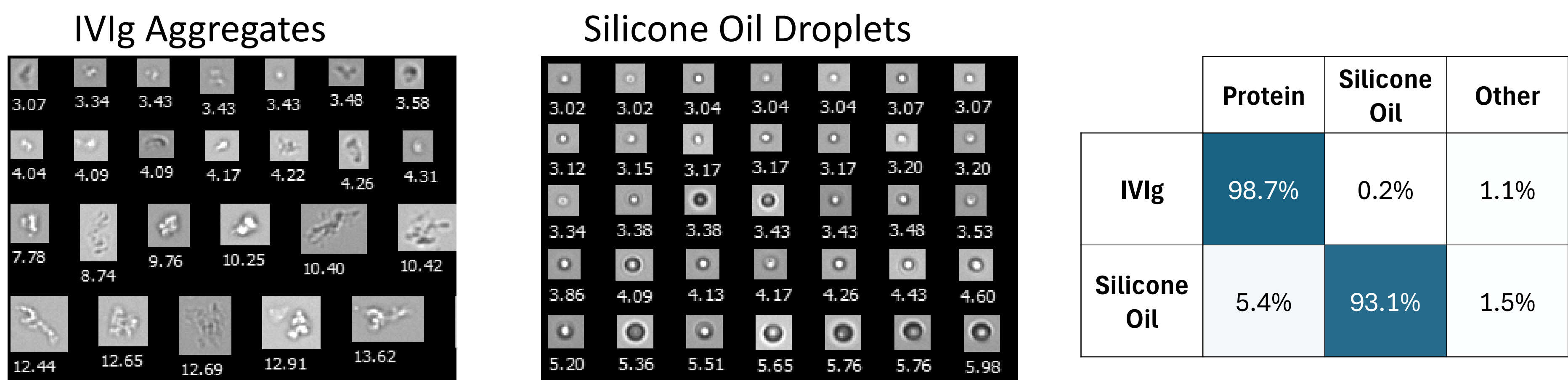


Figure 3: VisualAI classification performance on protein aggregates and silicone oil droplets. (Left) Randomly selected images of IVIg aggregates and silicone oil droplets. Values below each image are a particle size in μm. (Right) Confusion matrix showing the classification accuracy on images of each particle type.

Results

- Subvisible Particle Imaging (Figure 1):**
 - A variety of common particle types were analyzed with FlowCam, many of which exhibited a distinct morphology
- Stability Study (Figure 2):**
 - In the absence of PS80, shaking stress generated higher concentrations and larger sizes of subvisible particles than heating stress.
 - Adding PS80 reduced particle concentrations and sizes generated by both stresses, but had a much greater impact on shaking stress. This is the expected effect of adding surfactant.
 - Light obscuration (LO) measurements show similar trends as FIM data, but with lower measured particle concentrations and sizes.
 - This is expected behavior: LO is less sensitive to protein aggregates than FIM.
- Protein-Silicone Oil Classification (Figure 3):**
 - VisualAI classified both protein aggregates and silicone droplets with over 90% accuracy.

Conclusions

- Flow imaging microscopy with FlowCam provide useful subvisible particle concentration, size, and morphology data for biotherapeutic development. Applications include:
 - Identifying and analyzing relevant particle types in protein, cell, and gene therapies.
 - Designing formulations and drug products to minimize particle formation.
 - Monitoring protein aggregate and silicone oil droplet content in protein therapies