Flow Imaging Microscopy for Monitoring Visible Particles

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Abstract

Visible particles (those >150 µm in diameter) pose significant risks to product quality and patient safety. Per USP <1> and <790>, all final containers of a parenteral drug product must be "essentially free" from visible particles. 100% visual inspection is used to satisfy this requirement as it can be performed non-invasively. However, visual inspection is a probabilistic technique as even well-trained human inspectors may not consistently detect all particles in the visible size range in a sample. Recently, researchers have started to explore flow imaging microscopy (FIM), a common subvisible particle (2-100 µm) monitoring technique, as an orthogonal technique for visual inspection to monitor visible and gray zone (100-150 µm) particles present in a sample. In this study, we demonstrate proof-of-concept for how flow imaging microscopy (FIM) can be used to analyze visible particles in biotherapeutic samples.

Materials and Methods

Visible Particle Preparation

- Solutions containing visible particles were prepared as follows:
  - Protein Aggregates: Particles were taken from an expired IVIg sample and diluted 1:100 in PBS
  - Glass shards: A glass syringe was ground into a powder via mortar and pestle, then suspended in PBS

Container Study

- Six vials were washed with isopropanol followed by ultrapure water. Vials were air-dried for 30 minutes after each wash.
- Three "Control" vials were filled with 3 mL of PBS only
- Three "Test" vials were filled with 3 mL of PBS, then spiked with 100 µL of the visible protein aggregate and glass shard solutions
- All containers were inspected for visible particles in a lightbox with gentle swirling before further analysis

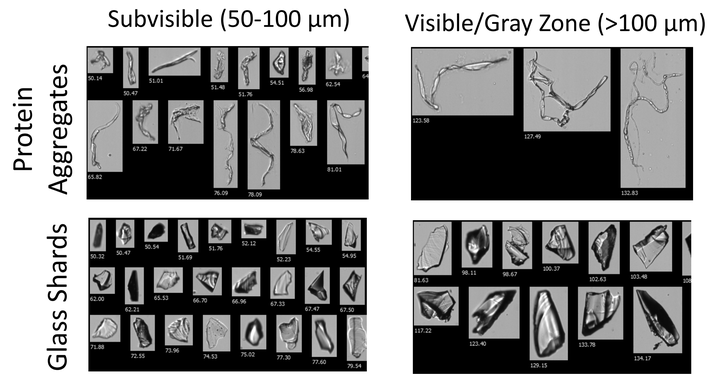
Flow Imaging Microscopy (FIM)

- FIM data was collected using a FlowCam 8100 instrument configured with a 4X objective and FOV300 flow cell.
  - All detected particles >50 µm in size were recorded
- Three 1 mL aliquots were analyzed per sample at 50% efficiency, a 12 dark pixel threshold, and 3 close hole iterations
- Used a simple binary protein-glass classifier to determine the concentration of each particle type in each sample
  - Particles with geodesic aspect ratio <0.25 were called protein aggregates, all others were labeled glass

Visual Inspection Advantages	Flow Imaging Microscopy Advantages
Required for lot release	Quantitative visible data
Non-destructive test	Automated measurements
	Higher sensitivity
	Wider size range
	Reliable particle type info

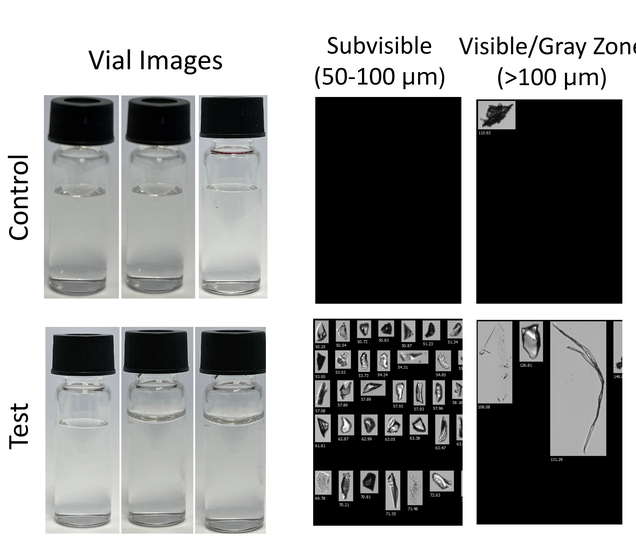
Applications for flow imaging microscopy include:

- Monitoring visible particles during product and process development
- Performing destructive testing and root-cause analysis following batch rejection
- Validating visual inspection processes
- Developing visible particle standards



	Protein	Glass
Protein	86.4%	13.6%
Glass	14.0%	86.0%

Figure 1: (Left) Example images of visible protein aggregate and glass shard particles captured by flow imaging microscopy. (Right) Confusion matrix for a binary protein-glass classifier using a geodesic aspect ratio filter.



Sample	Total Concentration (#/mL)		Particle Type Concentration (#/mL)	
	50-100 µm	>100 µm	Protein	Glass
Control	0	0.2	0.2	0
Test	47.3	1.2	12.5	35.9

Figure 2: (Left) Images of each vial analyzed as part of the container study. (Center) Images of subvisible, gray zone, and visible particles captured by flow imaging microscopy. Note that only one particle was observed in any of the control vials. (Right) Measured particle concentrations in each size range and of each particle type.

Results

Visible particle preparation:

- FIM detected visible, gray zone, and subvisible particles in both visible particle solutions.
- Both particle types exhibited a unique morphology when imaged
  - Glass particles: geometric structures with pronounced edges
  - Protein aggregates: elongated, transparent filaments
- A simple particle property filter classified these particle types with 86% accuracy

Container study:

- No particles were observed in any of the vials during visual inspection.
- Only a single particle was detected in the control vials.
- Test samples contained 1.2 particle / mL in gray zone or visible size range
- Both particle types could be identified in test vials using a simple classifier

Conclusions

- FlowCam analyzes visible and gray zone particles in samples, including those challenging to detect via visual inspection.
- The particle image data FIM provides indicates visible particle type and source.
- FlowCam provides orthogonal visible particle data to support and validate visual inspection processes.