Advances in Flow Imaging Microscopy Techniques for Submicron Particle Analysis

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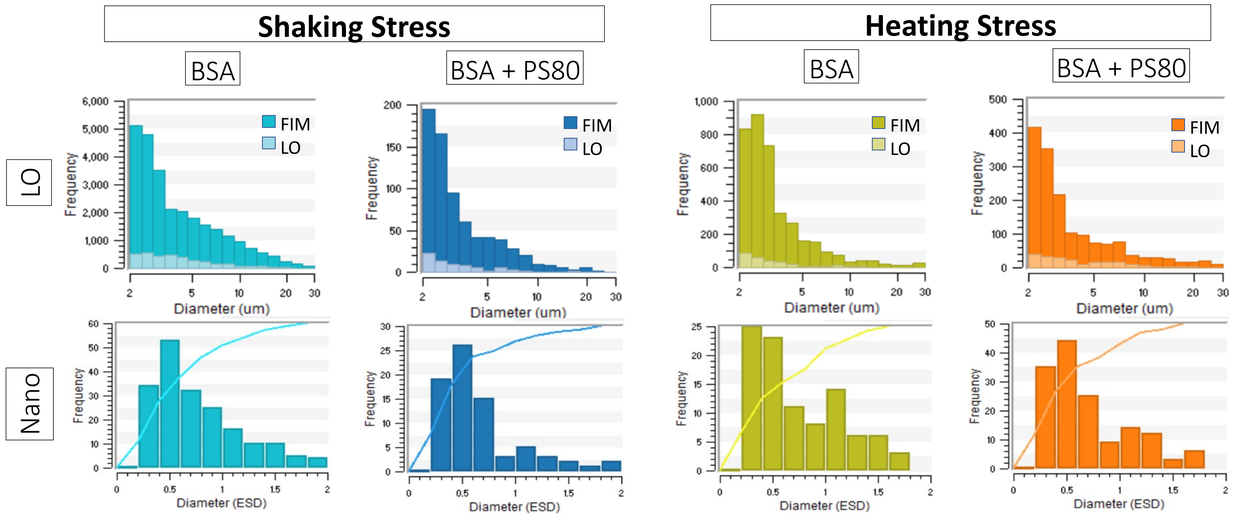
Abstract

Flow imaging microscopy (FIM) is increasingly used to monitor the number, size distribution, and types of particles in biotherapeutic samples (e.g., protein and other API aggregates, cells, and container-derived particles like silicone oil droplets). Recent innovations in FIM technologies combined with complementary measurement techniques like dynamic light scattering (DLS) can comprehensively characterize particle aggregation in biotherapeutics over a wide size range. This study assessed the size distribution of nanoparticle, submicron, and subvisible protein aggregates formed under different stress conditions. FlowCam LO, which combines FIM and light obscuration in a single instrument, was used to measure subvisible particles and FlowCam Nano was used to image submicron particles.

Materials and Methods

- Formulations were prepared in PBS containing 1 mg/mL BSA 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
- Protein aggregate and particle content were analyzed via:
  - FlowCam LO: FIM + LO, 2-70 µm subvisible particles
  - FlowCam Nano: Submicron FIM, 0.3-2 µm submicron particles
  - DynaPro NanoStar: DLS, 0.5-2000 nm nanoparticles

Scan this QR code to learn more about this study, FlowCam LO, and FlowCam Nano



	Shaking Stress (particles/mL)		Heating Stress (particles/mL)	
	BSA	BSA + PS80	BSA	BSA + PS80
FIM	69000	1900	9700	4100
LO	6300	130	520	380
Nano	36000	14000	18000	28000

Figure 1: FlowCam LO and FlowCam Nano histograms for BSA with and without PS80 exposed to shaking and heating stress. Shaking stress (blue plots) generated higher concentrations and larger sizes of subvisible and submicron particles than heating stress (green and orange plots) in BSA w/o PS80. Adding PS80 reduced particle concentration and size of subvisible and submicron particles generated by shaking stress, with a smaller effect on particles generated by heating stress. Particle concentrations for each instrument and experimental condition shown below histograms.

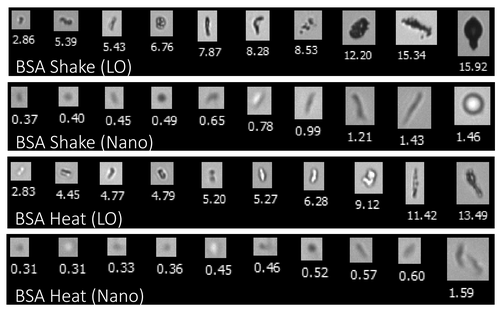


Figure 2: FlowCam particle images from stressed BSA samples.

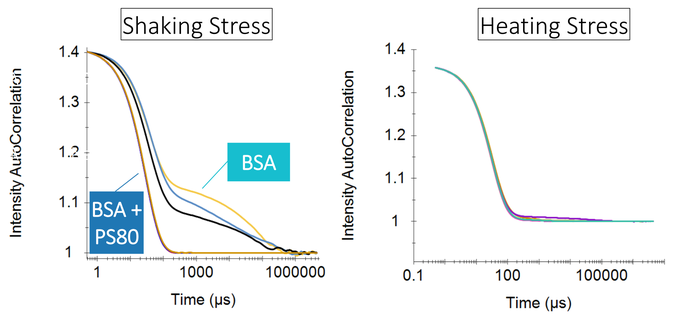


Figure 3: Autocorrelation plots for BSA aggregates generated by (left) shaking stress and (right) heating stress with and without PS80. Three replicates are shown per sample.

Results

- FIM (Figure 1):
  - In BSA without PS80, shaking stress generated higher concentrations and larger sizes of subvisible and submicron particles than heating stress
  - PS80 reduced subvisible and submicron particle concentration and size in the sample exposed to shaking stress, with a smaller effect on those generated through heating stress
- LO (Figure 1):
  - LO results show similar trends to FIM, with fewer particles measured due to non-detection and/or undersizing of transparent particles
- DLS (Figure 3):
  - Measurements show a reduction in particle size for shaken BSA samples with the addition of PS80
  - Heat-stressed sample measurements indicate less aggregation and minimal PS80 effect

Conclusions

Particle content in samples consistent with the suspected mechanism of aggregation:

- Heating: Monomer unfolding + aggregation in bulk → smaller aggregates, minor PS80 effect
- Shaking: Protein film formation + destruction at interfaces → larger aggregates, significant PS80 effect
- Combining FIM, LO and DLS data provides a comprehensive picture of protein aggregation in the subvisible, submicron, and nanoparticle size range