VisualAI™ for Sample-Agnostic Flow Imaging Microscopy Particle Analysis

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FlowCam® Yokogawa Fluid Imaging Technologies, Inc.

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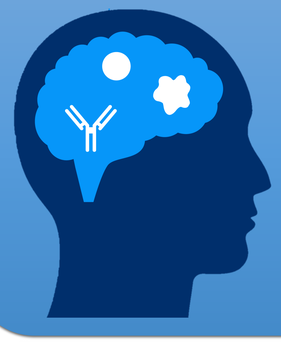
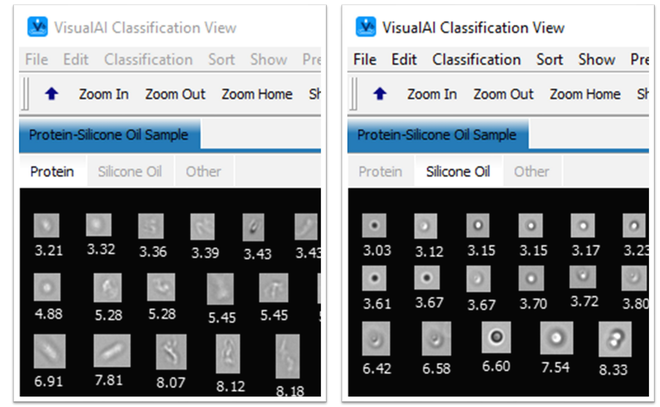


Abstract

- AI approaches are effective for identifying particles in flow imaging microscopy (FIM) data
- AI-based image analysis tools rely on large image libraries captured on the FIM instrument(s) and protein formulations to be analyzed. These data requirements can make AI tools difficult to develop
- VisualAI is a **pre-trained AI utility** that classifies images of protein aggregate and silicone oil droplet particles larger than 3 µm in **any protein formulation captured on any FlowCam 8100/FlowCam LO**
- We demonstrate VisualAI's performance on classifying FIM images of protein aggregates and silicone oil droplets as well as those from mixed samples



Materials and Methods

- **Protein aggregate prep:**
  - Prepared ~1 mg/mL solutions of BSA, ovalbumin, IVIg, or NIST mAb in PBS
  - Stressed 5 mL aliquots of each protein formulation via:
    - Freeze-thaw: 4 cycles x (freeze @ -20°C, thaw @ 25°C)
    - Shake: Plate rocker at max speed for 4 hours
- **Silicone oil emulsion prep:**
  - Blended 5% silicone oil in PBS mixtures for 20 s
- **Mixture preparation:**
  - Prepared 1.5 mL samples containing 25%, 50%, and 75% BSA aggregates and the remainder silicone oil droplets
- **Flow Imaging Microscopy (FIM)**
  - Analyzed each protein sample + one emulsion on a different FlowCam 8100
  - Varied flow rates and focus settings to simulate use cases

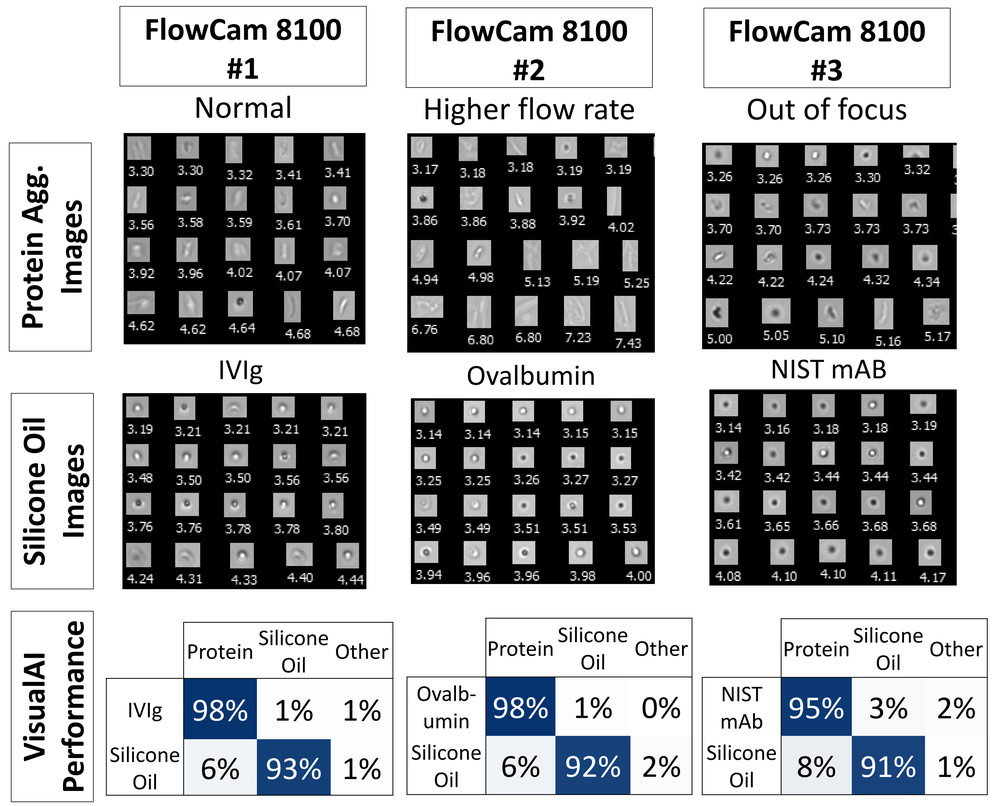


Figure 1: Sample FIM images of protein aggregates and silicone oil droplets captured on three FlowCam 8100 units. The aggregated protein is denoted below the images. Also shown is VisualAI's classification performance (bottom row) as a confusion matrix.

Results

- Pure Sample Performance (Figure 1):
  - **>90% classification accuracy** on particles >3 µm in size **regardless of the protein and instrument used**
  - Similar accuracy on an out-of-focus unit
- Mixture Performance (Figure 2):
  - Reported protein aggregate content is **consistent and linear** with actual content

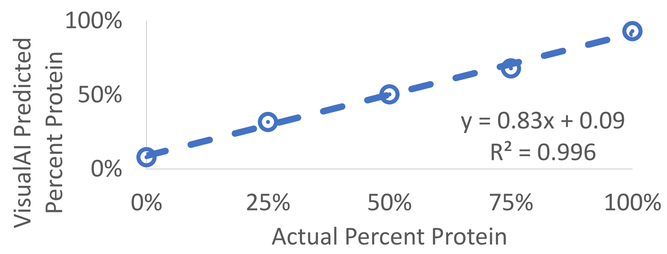


Figure 2: VisualAI-predicted protein concentrations in mixed samples

Classification Approach		VisualAI	Prev. AI-Based Methods	Property-Based Methods
		Use pre-trained AI tools to classify images	Develop and apply AI tools to classify images	Use particle properties to classify images
Performance	Analysis Time	Minutes	Minutes	Hours
	Accuracy	High	**Very High**	Low
Robustness	Instrument	High	Low	Moderate
	Sample	High	Moderate	Moderate
Ease-of-use	Images Needed	None	10,000s	100s
	Software Integration	Yes	No	**Yes**

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