Protein or Not? Polysorbate or Not? Advanced Particle Characterization with Fluorescent ID

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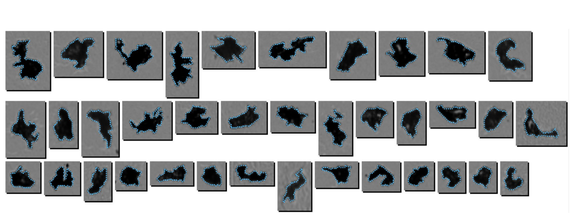
Introduction

Subvisible particle analysis is a key predictor of drug product stability and is a critical quality attribute. Knowing the source of the particles is important in understanding patient impacts and critical to developing mitigation efforts. Until now there has not been a simple, rapid, and easy way to both count and size particles while at the same time, being able to ID their source. Here we introduce Aura™, the first system specifically designed to count, characterize, and ID particles in a rapid and low-volume assay.

Aura systems eliminate the need to use unreliable morphological metrics and cumbersome spectroscopic techniques. It combines membrane microscopy with fluorescence staining. With Aura, both individual particles and entire populations can be identified.

BMI Explained

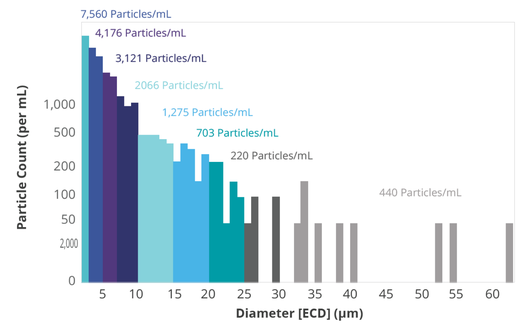
Background Membrane Imaging (BMI) is based on the USP <788> compendial method and has been modernized using automation, image processing, and innovative optics. First a background image of a filter membrane is taken, then samples are vacuumed through the filter and re-imaged. The background and sample images are processed together in order to remove the background texture and clearly identify the particles present in the sample. This step results in particle counts for particles 1 µm – 4 mm ECD as well as shape and size distributions according to USP specifications and is easily validated for both size and counts.



Brightfield Images of subvisible protein aggregates measured with BMI.

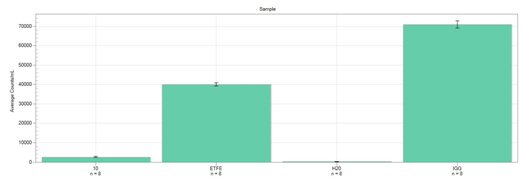
BMI's high refractive index imaging results in high contrast images essential for imaging subvisible particles and is sensitive compared to orthogonal techniques.[1,2]

Detailed to High Level Insights with BMI



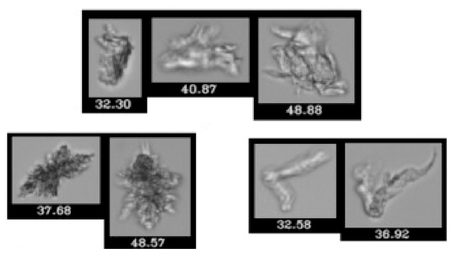
Counts by Condition (Counts/mL)

Sample	Replicates	%CV 2µm..50µm	2µm.. 50µm	Brightfield ≥2µm	≥5µm	≥10µm	≥25µm
10	8	11.85	2672	2675	1815	1445	38
ETFE	8	2.05	40121	40192	17012	6533	996
H20	8	40.32	215	218	22	8	2
IGG	8	2.57	70948	71235	27762	10585	1778



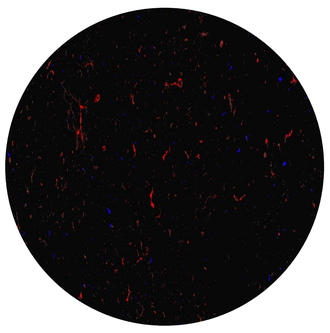
Aura BMI enables the characterization of individual particle size distributions, and its high-throughput format also makes it possible to obtain high level insights at a whole 96-well experiment level. Running replicates and obtaining confidence in your subvisible particle results has never been easier.

Morphology alone does not enable Particle ID



Protein aggregates have varying morphology depending on their nature. They can appear identical to plastic particles as in images above from Japanese Pharmacopeia review[3] (which ones are protein?) or mimic the shape and optical properties of degraded polysorbate components. Morphology, or morphology inspired machine learning are not enough to positively ID these particles.

BMI + Fluorescence Membrane Microscopy

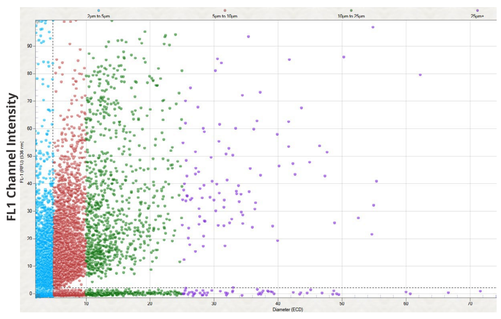


Protein Aggregates: Red Particles
ETFE: Blue Particles. Images acquired with FMM.

Combining BMI with Fluorescence Membrane Microscopy (FMM) allows rapid and specific identification of particles. After particles are counted with BMI, a fluorescent dye is added to the membrane to tag particles of interest. Highly specific and rapid stains such as Thioflavin T (ThT) can be used to stain and positively identify subvisible protein particles. The workflow can be adjusted to allow for solution or membrane-phase staining. Membrane-phase staining after the particles have already been counted using BMI does not result in false positive counts since only particles that were first counted with BMI are evaluated.

Easy + Fast Particle ID

Aura software (Particle Vue software v3.1) automatically detects fluorescence from stained particles and displays them in relation to unstained particles for easy particle discrimination. As seen in scatterplot below displaying the average fluorescent intensity of every particle measured, using ThT protein particles display strong fluorescence and are easily discriminated from the non-fluorescent ETFE plastic particles.



Thresholds for High-Throughput Particle ID

Particle Vue software allows users to set manual thresholds or create logical expressions to filter data and to specifically count particles of interest. From this large dataset of particles, sub-populations of particles can be separated and quantified by their ID. In this example, a six-sigma threshold was set to find fluorescent particles that are six standard deviations above background fluorescence.

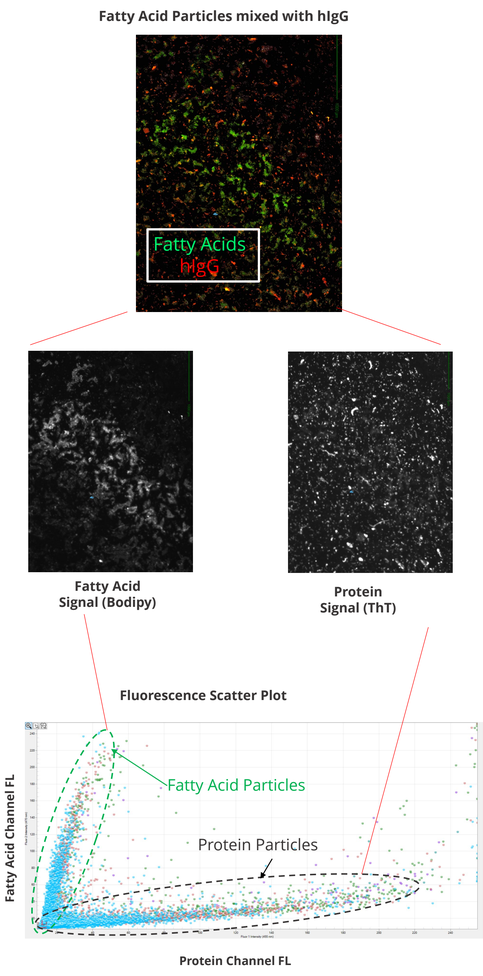
Sample	Replicates	ECD > 5um (/ml)	ECD >5um & FL > Bck + 6σ (/ml)	% >5um above Threshold
ETFE	24	15857	244	**1.5%**
IgG	24	43494	43396	**99.8%**
IgG + ETFE	24	66343	58223	**87.8%**

Summary table of protein vs. non-protein particles.

As shown above, applying a threshold yields particle counts separated by ID. This information is essential in understanding the source of the particles as well as developing mitigation plans.

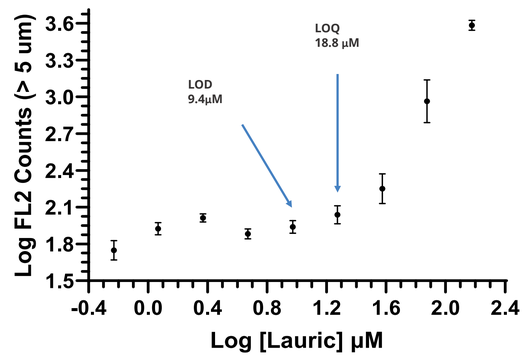
High-Throughput Polysorbate Particle ID

Polysorbate degradation can result in particulate formation. It is frequent and can occur in long term sample storage due to oxidation.[4] Since the morphology of polysorbate particles is similar to protein aggregates, their characterization and identification has been elusive, until now.



High-throughput identification and differentiation of subvisible polysorbate and protein particles. Samples were imaged and counted using BMI, and FMM was conducted using ThT for protein ID and Bodipy D3821 for degraded polysorbate ID.

The Aura FMM polysorbate assay is sensitive, specific, and enables visual confirmation of the presence of subvisible polysorbate degradation components mixed with protein.



LOD and LOQ of polysorbate particle detection in the presence of protein.

References

[1] Helbig *et al. J Pharm Sci*. 109 (1) Jan 1, 2020

[2] Vargas *et al*. *Intl. J Pharm*. 2020 Mar 30;578:119072.

[3] Kiyoshi *et al*. *J Pharm Sci*. 108 (2), 2019

[4] Kishore *et al*. *Pharma Res*. 2011 May;28(5):1194-210