silicone droplets represent 80,898 particles/ml of the original 315,794 particles/ml total found, which is just over 25%.

Regulatory agencies are now asking for drug developers to characterize particulate matter in biologics, with a new emphasis on particles in the 2-10µm size range in addition to the classical >10µm and >25µm reporting used in the past. With biologics, it is particularly important to actually *characterize* the particles (intrinsic, extrinsic and inherent) as opposed to just *counting* the total number of particles. This characterization is only possible using imaging techniques because of their ability to measure shape and other parameters which can be used to distinguish the particle *type*.

An experiment was conducted to study the efficacy of different software filtering algorithms on properly identifying silicone droplets[1]. In order to measure how effective each filter is, the data set was first hand-classified visually, and a particle type (protein, silicone, etc.) was assigned to each particle record. The human eye-brain system is much more adept at *inferring* content based on subtle cues than is possible algorithmically. So after each particle was assigned a "type (known)" visually, each filter's results ("type classified as") could be compared to determine filter accuracy.

The filters used are as follows:

- Aspect ratio (width/length): historically has been a de facto standard method of distinguishing silicone droplets[2], and was thus used as a baseline.
- Hu Circularity: produces better results for circularity than other measures when looking at particles with boundary defects, i.e. when the perimeter of the circle is not perfect[3].
- Statistical filter: uses statistical pattern recognition to find similar particles to ones chosen as examples by the user.
- Custom value filter: using the three particle parameters for known silicone droplets that have the smallest Coefficient of Variability (CV).

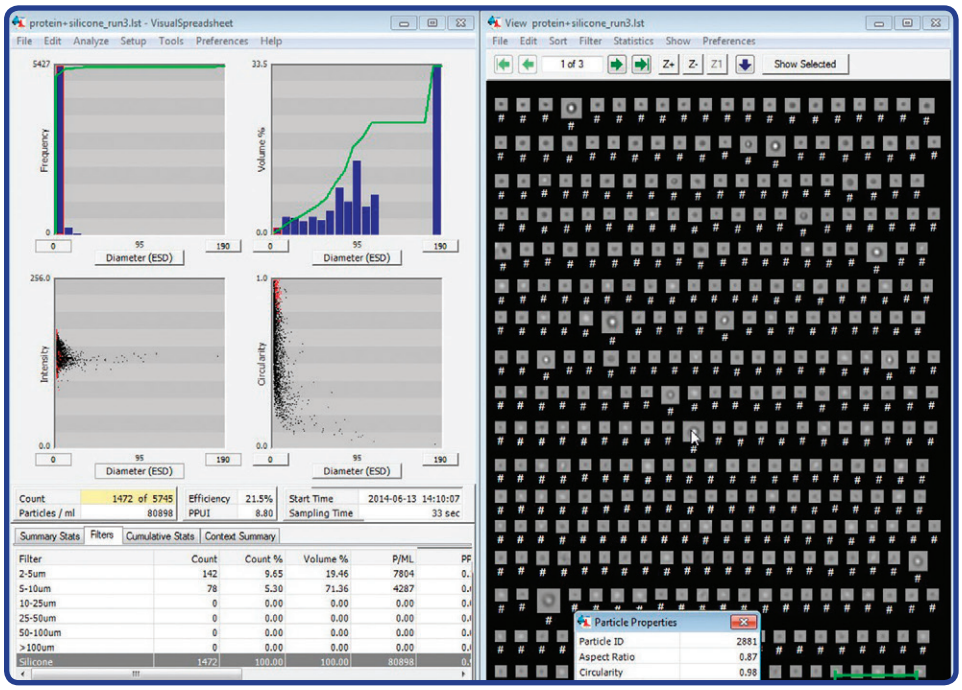

Figure 2: FlowCDPI results for same protein therapeutic sample, showing the number of silicone droplets found by the filter and the corresponding particle images (right).

Summary Results:

Filter	% Increase Overall vs. Baseline (Aspect Ratio filter)
Aspect Ratio ≥ 0.85	N/A (baseline)
Circularity (Hu) ≥ 0.95	42%
Statistical Filter	73%
Customized Value Filter	130%

The results clearly show improved accuracy for silicone characterization in the sample by using filters containing more discriminatory particle properties (Hu Circularity), as well as more powerful statistical based methods.

Conclusions:

Several conclusions can be drawn from this example/experiment:

- FlowCBN offers a powerful method for characterization of particulatesin biologics, enabling separation into distinct particle types (extrinsic, intrinsic, inherent).
- The ability to see each particle image is critical to understanding filter efficacy.
- The availability of a larger variety of particle properties aids in better filter discrimination.
- Advanced measurements (e.g. Hu Circularity), and advanced techniques (e.g. statistical filtering) can significantly increase filter accuracy.

Dynamic Imaging Particle Analysis (DIPA) using FlowCBN is a superior method for characterizing particulate content in biologic formulations.

References:

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2. Sharma, D.K., Oma, P., & Krishnan, S, (2009). Silicone Microdroplets in Protein Formulations-Detection and Enumeration. *Pharmaceutical Technology*, 33 (4), 74-79.
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