Flow Imaging Microscopes for Particle Characterization of Biopharmaceuticals: 5 Important Considerations

Flow Imaging Microscopes (FIM) provide the in-depth characterization of particles in protein therapeutics as recommended by the FDA. Below are five important features that should be considered and compared when evaluating instrumentation in this arena.

#1 - IMAGE QUALITY

Image quality is extremely important when characterizing, identifying, and differentiating protein aggregates from silicone oil and contaminants in a drug formulation. A high quality image is essential for accurate sizing measurements. Because FIM take measurements directly from the images they collect, the precision and accuracy of the data is directly related to the quality or resolution of the images.

Blurry images make it difficult to distinguish protein aggregates from other contaminants. Additionally, it is challenging to appreciate the morphological differences in aggregated proteins themselves. These differences may be an important indication of bacterial or viral contamination (e.g. aggregates containing round globules versus rods).

Poor image quality can also result in low concentrations or inaccurate characterization because smaller or faint particles (such as those with a low refractive index) are mischaracterized, or missed entirely. This results in increased measurement variation, and a lower statistical confidence.

The ability of a FIM to match patterns and classify particles is also affected by image quality. The true strength of the FIM over other particle analysis techniques is the ability to differentiate particles based upon appearance. The better the overall image quality, the easier it is for the instrument's software to recognize patterns, and in turn classify particles.

Therefore, image quality is paramount to the value of a flow imaging microscopy system. In short, fuzzy images lead to fuzzy measurements, which lead to fuzzy classifications.

#2 - FLEXIBILITY

Not all formulations are alike – particles vary significantly from one formulation to another. Depending on the formulation, these differences may necessitate modifications to capture settings to ensure proper measurement. It is important to choose a system that can be configured to suit the specific formulation you are testing and achieve optimal results. This is especially true if your formulations contain stabilizers, which can affect the refractive index of the matrix.

If a wide range of particles sizes is expected, an instrument may need to support multiple magnifications. A FIM that offers interchangeable 2X, 4X, 10X, and 20X objectives, and can utilize both disposable flow cells and fixed field of view flow cells in a variety of sizes would be preferable.

#3 - INSTRUMENT SENSITIVITY

Many protein aggregates are translucent. This can make them difficult to detect with a particle analyzer, when the refractive index is close to that of the matrix within which they are suspended.

Thresholding is a method of image segmentation used in image processing to discern the boundaries of particles from their background. Having the ability to threshold on either darker pixels (relative to the background), lighter pixels, or both simultaneously can ensure the proper characterization of protein aggregates. This can be especially important when working with high concentration formulations or those with a large amount of stabilizer.

Some systems use simple, dark-only thresholding which may cause transparent aggregate images to be fractionated into smaller particle pieces, or go undetected altogether. This often results in inaccurate measurements, count, or concentration calculations.

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#4 - SAMPLE VOLUME REQUIRED

Protein samples are precious. Drug formulations can be expensive and/or in short supply. Therefore it's important to clearly understand the minimum sample volume required by a particle analyzer so that it can meet your needs and provide accurate results.

It is important to understand how much sample is necessary (if any) to prime the system prior to measurement and analysis. Minimum sample volumes differ from one instrument to another, ranging from 50 µl to 1 mL.

#5 - EASE OF ANALYSIS AND DATA PROCESSING

Once your data is captured using a flow imaging microscope it is important to have an easy way to analyze it. The quality of the software varies significantly from one type of system to another. Choose one that allows you to efficiently interact with your data and extract the information you need quickly and easily.

Some software features you may consider:

- Sorting and filtering particle images based upon criteria you supply, with immediate visual feedback.
- Sophisticated pattern recognition capabilities that immediately find and display all similar-type particles in a heterogeneous sample.
- Creation of user-defined particle-type libraries to instantly enumerate concentrations of specific particle types.
- Satellite software for post-processing data at a remote location or sharing data with others.
- A single system for data acquisition and analysis provides tight integration and eliminates lag time as well as data inconsistencies.
- Ability to export to XLS and other databases provides added flexibility for additional analysis.