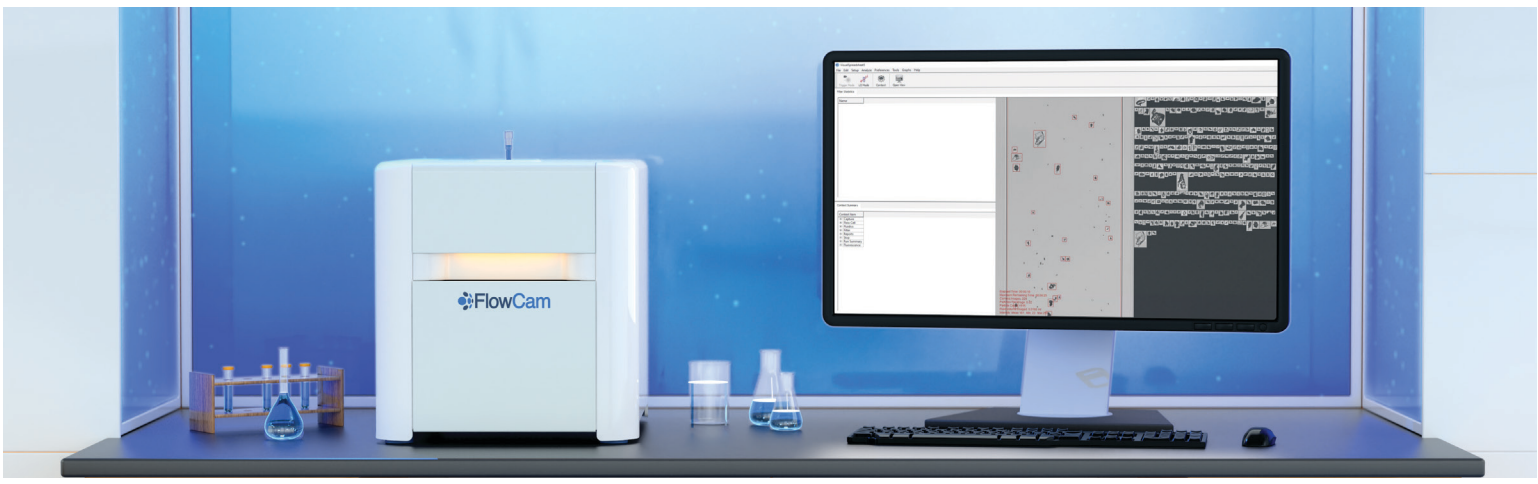
YOKOGAWA

EBOOK

The Ultimate Guide to Flow Imaging Microscopy

FlowCam®

Yokogawa Fluid Imaging Technologies, Inc.



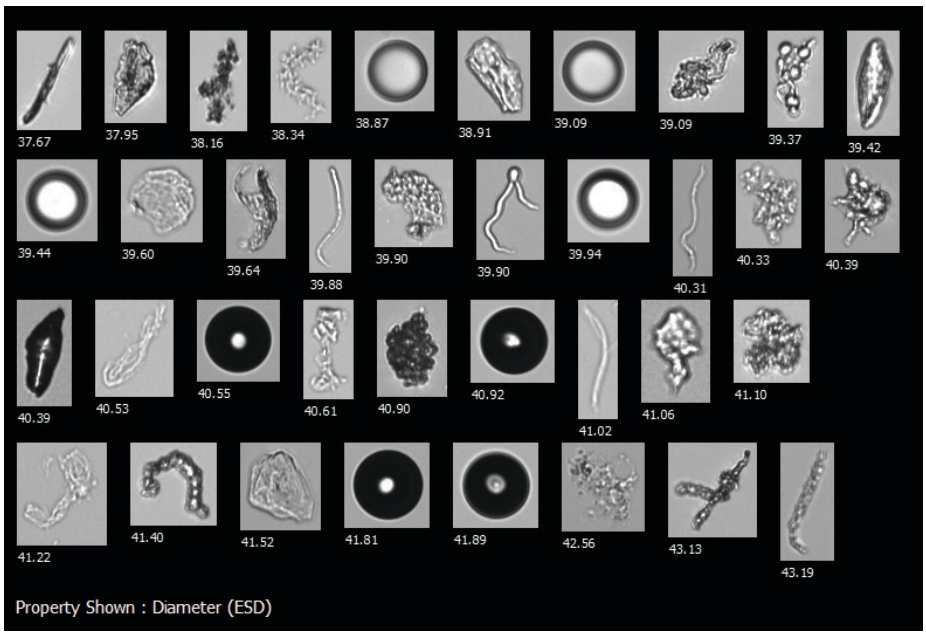
YOKOGAWA FLUID IMAGING TECHNOLOGIES

Flow Imaging Microscopy: Discover a Better Way to Characterize Subvisible Particles

CONTENTS

Overview

In this eBook, we'll briefly review different methods available for analyzing particles, including the advantages and drawbacks of each. Then we'll take a deeper look at flow imaging microscopy (FIM) and how it works. Finally, we'll focus on how FIM is an advantageous technology for many applications in areas including, but not limited to biotherapeutics, aquatic research, food & beverage, printer toner, crop science, microencapsulation processes, and materials characterization.



A VisualSpreadsheet® collage file containing images of protein aggregates, contaminants, silicone oil droplets and other particles.

FlowCam®

CHAPTER ONE

What is Particle Analysis?

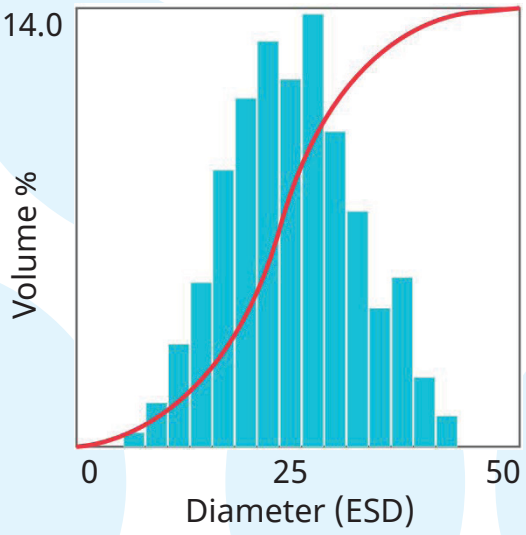
Particle analysis involves taking a sample of a substance and analyzing the individual particles that make up that sample.

Typical measurements of particles of interest include:

- Particle concentration
- Particle size distribution
- Particle count
- Particle shape

For simplicity, results of a particle analysis are typically reported graphically, with particle size plotted against some other variable. Particle size is often stated as equivalent spherical diameter (ESD), which is an estimated diameter based on measurements of the particle assuming the particle is a sphere.

A particle size distribution like that shown on the right is commonly measured and reported during particle analyses. In this graph, the particles are shown by estimated size (ESD). While estimated size distribution is considered valuable data, it only tells part of the story.



While the above graph shows particle size fairly straightforwardly, when shape and/or morphological data is needed, a more in-depth analysis is required to truly characterize a particle.

CHAPTER TWO

A History of Particle Analysis

SECTION 2.1: MICROSCOPY

The introduction of the microscope in the 1600s changed the world for scientists. For the first time, they could observe and record organisms too small to see with the naked eye.

To this day, microscopy remains the most common method for subvisible particle analysis.

ADVANTAGES OF MANUAL MICROSCOPY

The benefit of microscopes is simple: it allows you to study subvisible particles in great detail under a wide range of magnifications.

Microscopes have improved over time, allowing us to look at increasingly smaller particles, even down to the molecular level.

DRAWBACKS OF MANUAL MICROSCOPY

Using manual microscopy for particle analysis is time-consuming. Depending on the sample, it can take hours to prepare the sample, set up the slides, and measure any particles found.

It's challenging to get results that are statistically significant using manual microscopy. You can only process one small sample at a time, so it's difficult, if not impossible, to know if what you're looking at is representative of the whole.

Human factors must also be considered using microscopy. Tired eyes, interruptions, and time of day can all have an effect on the operator, and therefore the results.

"Comparing indivudual particle shape using a microscope is cumbersome and slow. It's difficult to see more than a handful of particles, and certainly not enough to get a statistically significant sample."

— Ross Clark, Distinguished Research Fellow at CP Kelco, a leading producer of specialty hydrocolliods

SECTION 2.2: VOLUMETRIC PARTICLE ANALYSIS TECHNIQUES

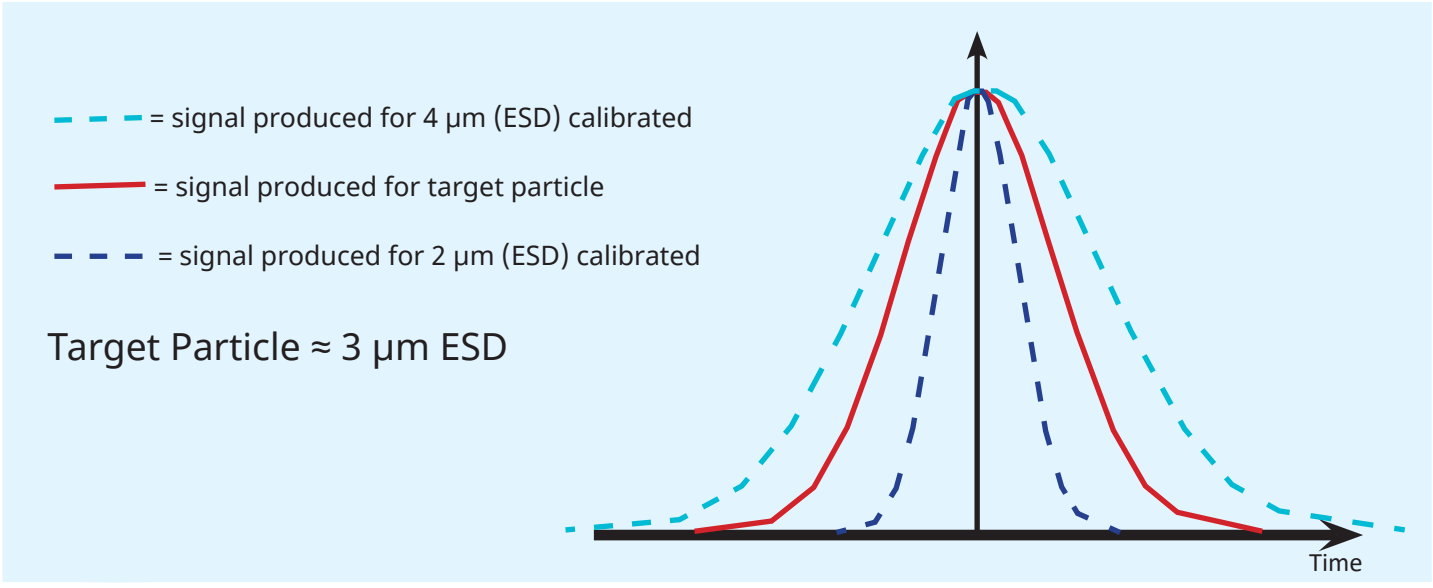
In response to the need for rapid processing of particle data, a variety of volumetric techniques have been developed. Volumetric particle analysis methods include:

- Coulter Counters
- Light Obscuration
- Laser Diffraction
- Light Scattering

These indirect techniques measure a signal that is proportional to the volume of a particle and not the actual physical dimensions of the particle.

The fundamental principle of these indirect techniques is that all particles are assumed to be spherical in shape, and the volume is converted to an equivalent spherical diameter (ESD). In these situations, it is not possible to know the actual shape of the particle, just the size distribution.

The Coulter Counter, shown here counting cells in solution, is an indirect volumetric particle analysis method.



Indirect calculation of particle size based on signal profile, where signal is proportional to volume.

ADVANTAGES OF VOLUMETRIC METHODS

Volumetric methods can rapidly count and size a statistically-significant amount of data—up to tens of thousands of particles per minute.

A particle size distribution that shows particle size versus either frequency or volume is easily created. Detailed particle statistics can be recorded for the entire distribution.

DRAWBACKS OF VOLUMETRIC METHODS

The most significant drawback to volumetric techniques is that they must assume all particles are spheres. These methods are limited to particle counting and size distribution only.

It is common for samples to be heterogeneous, containing a variety of particle types and shapes. Volumetric techniques cannot characterize different particle types in a mixture due to the assumption that all particles are spherical.

SECTION 2.3: FLOW IMAGING MICROSCOPY (FIM)

A flow imaging particle analyzer performs the following three functions all in one instrument:

- Draws a fluid sample through a microscope
- Takes digital images of the magnified particles within the fluid stream
- Characterizes the particles using a variety of measurements

ADVANTAGES OF FLOW IMAGING MICROSCOPY

FIM combines the benefits of manual microscopy with those of volumetric techniques. Microscopic particle measurements are taken from large sample volumes quickly enough to produce statistically significant results. Additionally, multiple measurements are taken for each particle, thereby providing the detailed information often needed for a thorough particle analysis.

The addition of specialized software also provides sophisticated post-processing of data to give you an in-depth analysis of your sample and a better understanding of your data.

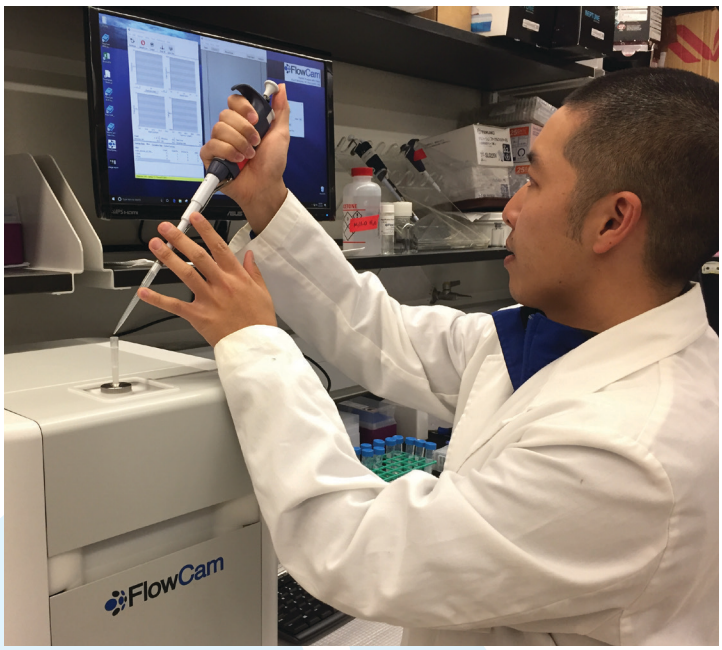
"After using light-obscuration instruments to count particles in a new parenteral product formulation, a project team at GlaxoSmithKline found that the light obscuration method yields a read-out of particles counted in the sample, but is unable to shed light on the nature of the particles. If the particles are not identified, differentiating the actual number of product-related particles can be a difficult task."

-Morrone, Greg J., and Wasfi Al-Azzam. "From Safety Snapshot: An imaging particle analyzer can give researchers a better picture of particles in parenteral formulations." *Drug Discovery & Development*, Volume 14, Issue 5.

LIMITATIONS OF FLOW IMAGING MICROSCOPY

The ability of an imaging system to resolve particle details is essential for accurate measurement. The optical system and the sensor of the instrument affect its ability to size and characterize subvisible particles.

Because of this, it is important to select the correct objective lens on these types of instruments specifically for the sample you are analyzing to ensure accurate results for the particles being studied. Flow Imaging Microscopy is currently capable of counting and characterizing particles in the 300 nm - 5 mm range.



Dr. Cheng Her uses FlowCam at the University of Colorado Center for Pharmaceutical Biotechnology with Dr. John Carpenter.

CHAPTER THREE

A Closer Look at Flow Imaging Microscopy

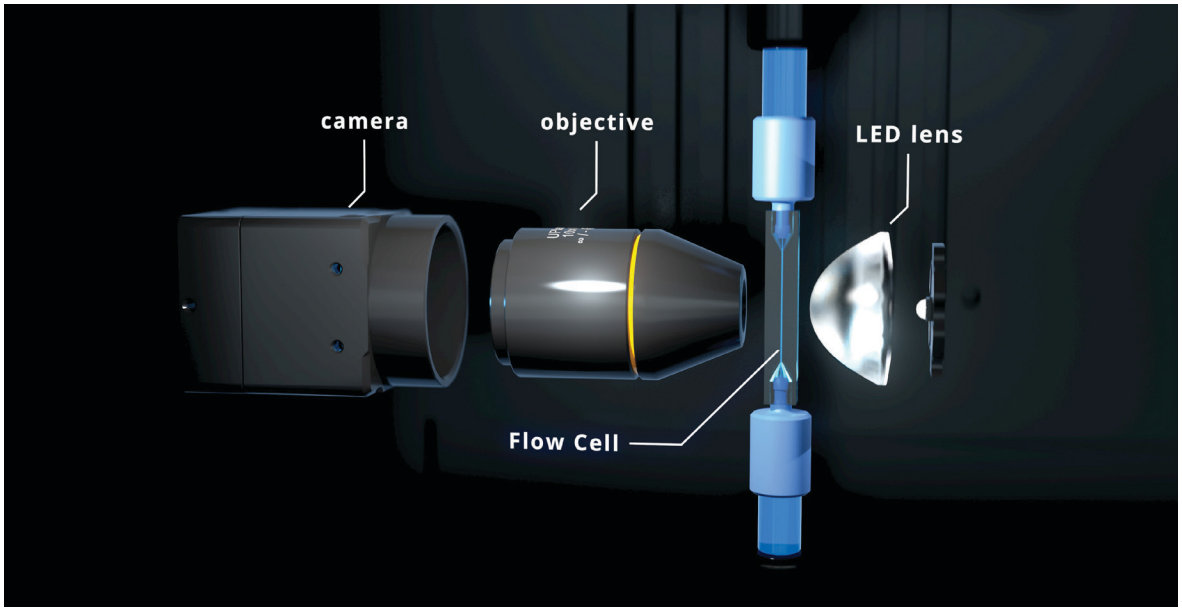
SECTION 3.1: HOW FLOW IMAGING MICROSCOPY WORKS

Flow imaging microscopy uses digital images to measure the size and shape of each particle in a sample. Essentially, the operator in classical microscopy is replaced by a computer that extracts the information from the images.

The sample containing the particles streams through the flow cell past the microscope optics. Thousands of particle images are captured per second.

To capture sharp images of moving particles, they are “frozen” in space using a strobed LED illumination source combined synchronously with a very short shutter speed.

As each frame of the camera’s field of view is captured, the software extracts the particle images from the background in real time and stores them.



SECTION 3.2: DIRECT PARTICLE MEASUREMENTS

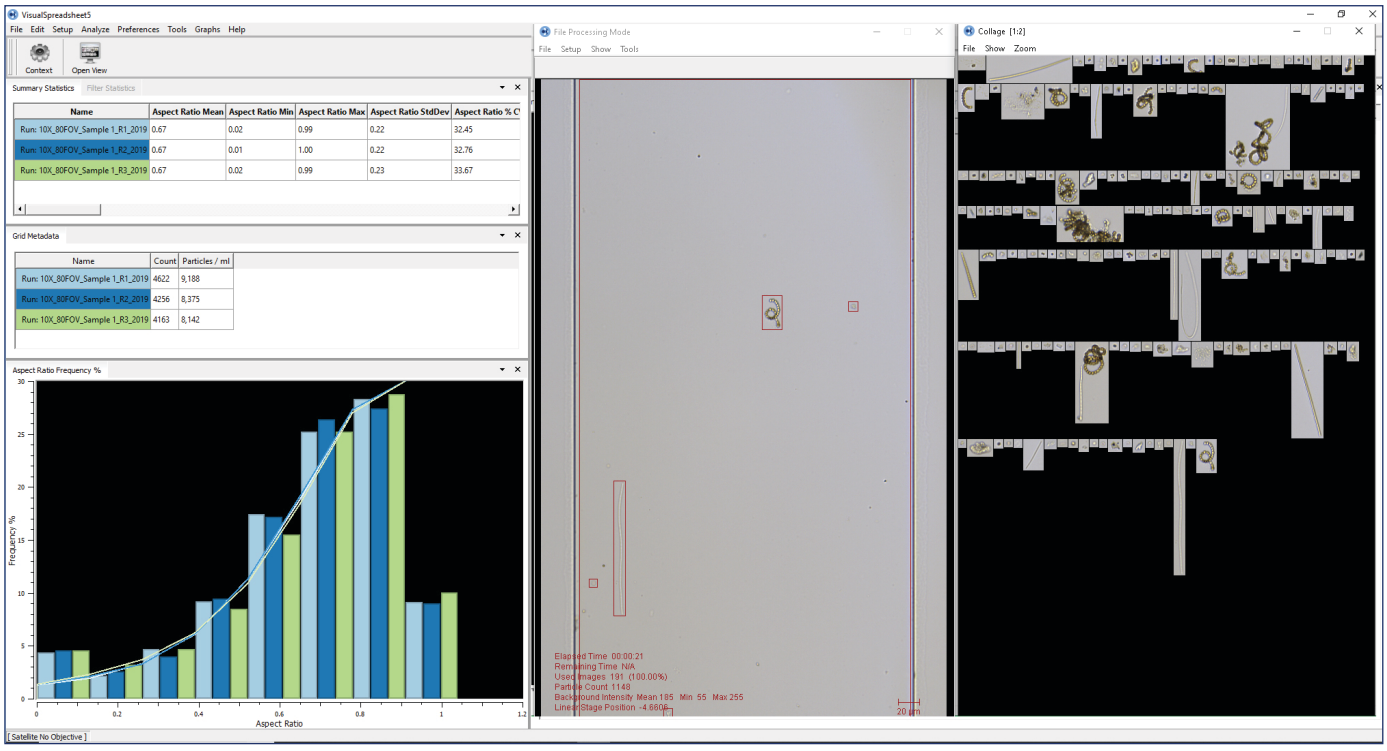
In an imaging-based system, particle measurements are made directly from the image of the particle. Since the system's optics are fixed and the magnification is known, distance measurements on the image can be directly converted to real distance measurements on the object.

No generalizations are made about a particle's shape. The user can also view the image to ensure that the data is being properly interpreted.

> ***"Image quality is extremely important when characterizing protein aggregates. We need to be able to differentiate them from silicone oil and other contaminants in drug formulations early in the process. FlowCam allows us to do this quickly and easily."***
>
> -Dr. Jeff Schwegman, Founder and CEO
> AB BioTechnologies, Bloomington, IN

Common measurements include:

- Equivalent spherical diameter (ESD)
- Length, width, and aspect ratio
- Area and volume
- Circularity and elongation
- Edge gradient
- Intensity, average intensity, and sigma intensity
- Transparency
- and more (40+ morphological characteristics)



FlowCam screen during image capture. The middle window is the full field-of-view of camera on the flow cell. Red boxes indicate particles found. The right window is the 'collage window' of particle images that are saved and stored. The main window (left side) shows particle measurements summary graph and statistics that populate at the end of the sample run. The main window can be customized to show desired properties and display preferences.

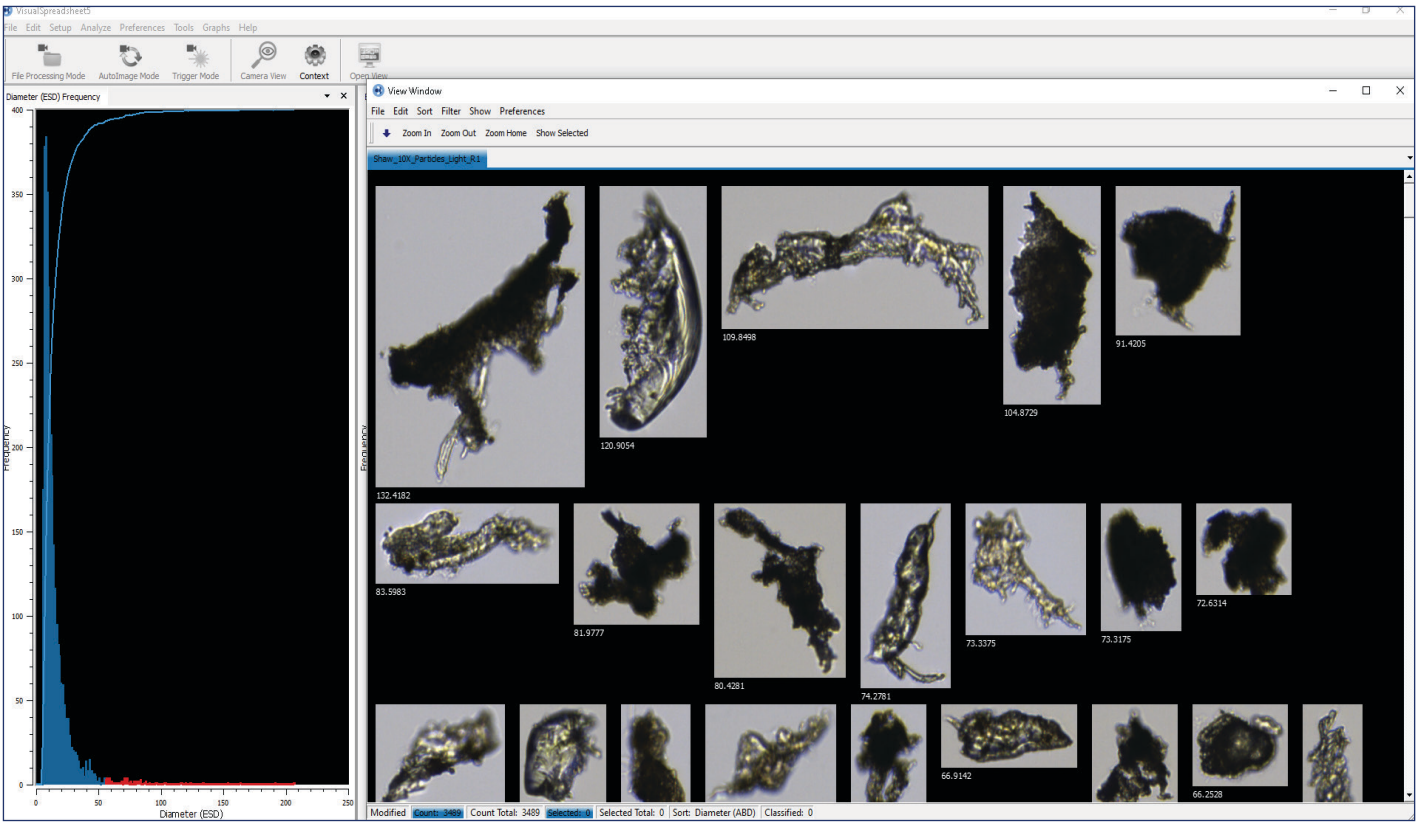
SECTION 3.3: DATA PROCESSING - SORTING AND FILTERING MEASUREMENTS

SORTING

The FlowCam system includes VisualSpreadsheet® software for both acquisition and analysis of data. VisualSpreadsheet provides the ability to sort and filter your data based on any of the measurements (or combination of measurements) acquired for the particles. The results are displayed as particle images as well as in a tabular format, and can also be exported to Excel.

The user can interact with the auto-generated scattergram to quickly select particles of interest from any of the configurable graphs.

> ***"You can get more information from FlowCam than from any other type of instrument. Going to FlowCam with a particle problem is just the best feeling in the world because it turns data into useful information that you can use to solve a real problem."***
>
> -Dan Berdovich, Owner
> Micromeasurement Laboratories, Inc.



VisualSpreadsheet interactive scattergram: only the largest particles have been selected from the histogram in the left window (red). The right window displays those particles, revealing that they are contaminants, not intrinsic particles.

FILTERING

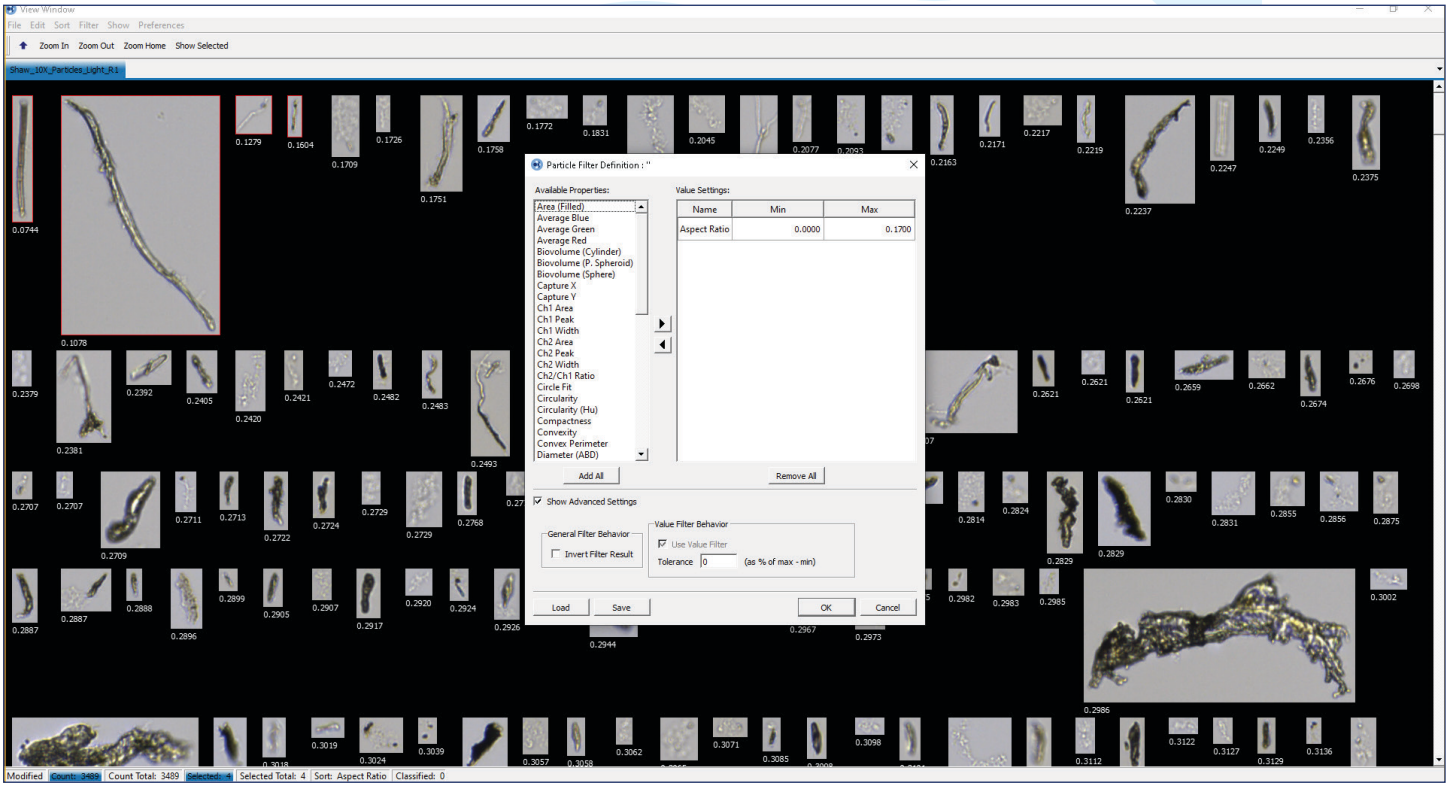
You can also build filters based on particle properties with VisualSpreadsheet to automatically isolate particles of a particular type.

Filters can be created, saved, and applied to future runs, or in post-processing mode of past runs.

Value filters can be used to isolate particles within a specified range of any particle property. Statistical filters can be used to identify images similar to a population of user-selected images.

"Data is one thing, but having instantaneous information is another...
FlowCam is an integral part of our screening process and enables us to quickly get the answers we need to drive the formulation development"

-Dr Jeff Schwegman, Founder and CEO
AB BioTechnologies, Bloomington, Indiana



A VisualSpreadsheet value filter was used to isolate "long and skinny" particles, limiting the display to only particles with an aspect ratio (width/length) from 0 to 0.25.

CHAPTER FOUR

Flow Imaging Microscopy Applications Overview

SECTION 4.1: Detection of Protein Aggregates and Contaminants in Parenteral Drug Formulations

SECTION 4.2: Identification of Aquatic Microorganisms

SECTION 4.3: Crop and Soil Sciences

SECTION 4.4: Food and Beverage Characterization

SECTION 4.5: Printer Toner Quality Assurance

SECTION 4.6: Microencapsulation Process Analysis

SECTION 4.7: Outlier Characterization - Column Packing Material Quality Control

SECTION 4.8: Additive Manufacturing - Characterizing Metal Powders

SECTION 4.9: Particle Differentiation in a Heterogeneous Sample - Wash Water

SECTION 4.1: DETECTION OF PROTEIN AGGREGATES AND CONTAMINANTS IN PARENTERAL DRUG FORMULATIONS

Particulates in parenteral drug development have always been a serious concern. In biopharmaceuticals the issue is compounded by reported impacts of aggregates and contaminant particles on the product's efficacy, safety, and immunogenicity. FDA regulations strongly recommend in-depth characterization of particles in protein therapeutics and other biologics in addition to size and quantity measurements.

FlowCam is an excellent tool for characterizing subvisible particles in these biopharmaceutical drugs. The instrument allows users to determine the number, size, and types of particles present in these therapies (Figure 1). This information can be used not only to assess the total particle load of a sample, but also to monitor particle population(s) of interest (e.g. inherent vs. intrinsic particles in a formulation) and to detect and identify any anomalous particles.

As a label-free method, FlowCam can be used to analyze many biotherapeutics and their aggregates. These include proteins, cells, adeno-associated viruses, and other APIs. Many drug delivery platforms and their aggregates such as hydrogel spheres (Figure 2) can also be analyzed with FlowCam. FlowCam can also be used to look for instrinsic and extrinsic particles common between these formulations such as silicone oil droplets and glass flakes.

Unlike other technologies, FlowCam can analyze a wide size range of particles. Particles between 300 nm and 2 µm are visible via FlowCam Nano (Figure 3), while those between 2 µm and 1 mm are visible via FlowCam 8000 and FlowCam LO. Ifyour particles are detectable by brightfield light microscopy, FlowCam can detect them.

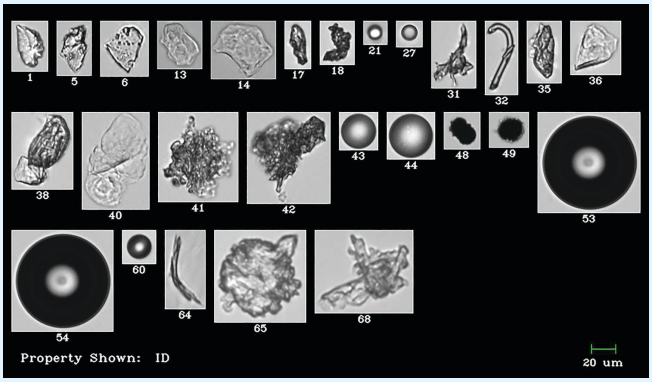


Figure 1. A VisualSpreadsheet collage file containing images of protein aggregates, glass shards, silicone oil droplets and various other intrinsic, extrinsic and inherent particles

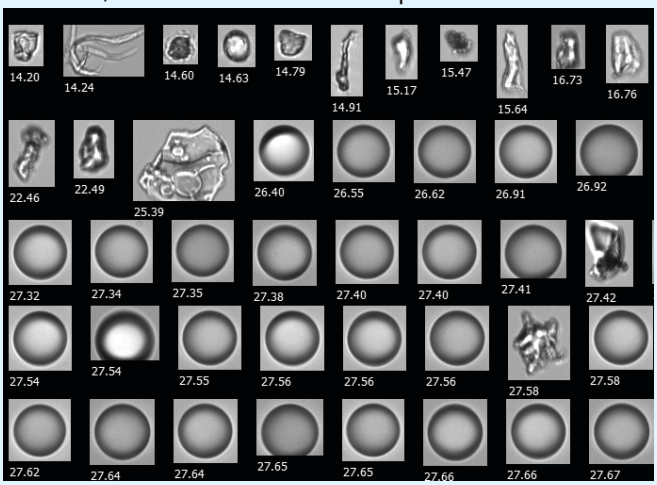


Figure 2. In this sample it is easy to tell the difference between the microspheres used for drug delivery and the contaminant particles

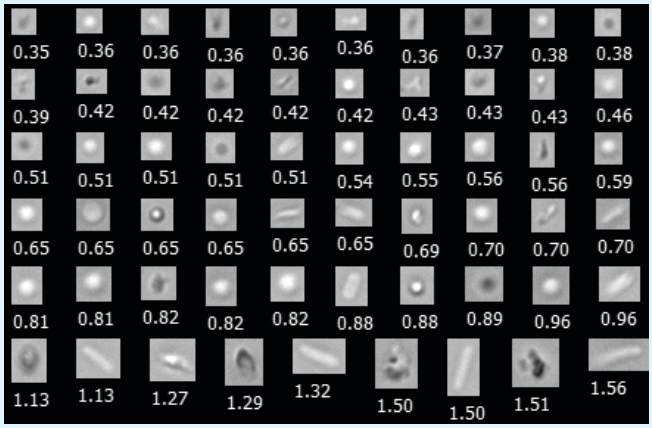


Figure 3. A biopharmaceutical sample imaged by FlowCam Nano (particle diameter shown in µm)

SECTION 4.2: IDENTIFICATION OF AQUATIC MICROORGANISMS

FlowCam was developed by biological oceanographers to study marine phytoplankton in the late 1990s. Today, use of FlowCam in the aquatic sciences includes marine and freshwater research, drinking water monitoring, algae cultivation, harmful algal bloom monitoring, and more.

Certain FlowCam models combine the fluorescence detection capabilities of a flow cytometer with the imaging capabilities of a microscope. The system enables the user to identify and enumerate phytoplankton and zooplankton, and save an image of each particle and/or organism.

FlowCam users build libraries to semi-automate the identification process with the help of FlowCam example libraries. FlowCam models with fluorescence capabilities can further distinguish organisms containing chlorophyll - including green algae and diatoms - from detritus and cyanobacteria (Figure 1).

Using FlowCam Cyano, drinking water utilities and monitoring agencies can quickly detect, identify, and quantify cyanobacteria (the primary producers of cyanotoxins and taste and odor compounds), allowing them to make informed testing and treatment decisions.

In the marine environment, FlowCam is used by researchers, educators, and aquaculturists to study primary production and to detect and monitor harmful algal blooms that can produce toxins that cause fish kills and shellfish closures (Figure 2).

Figure 1. Use FlowCam Cyano to separate diatoms and green algae (top row) from cyanobacteria (bottom row).

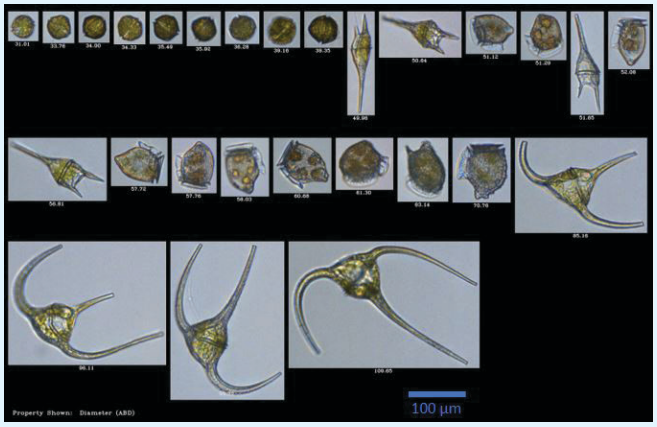


Figure 2. FlowCam can capture marine dinoflagellates like these from the Gulf of Maine.

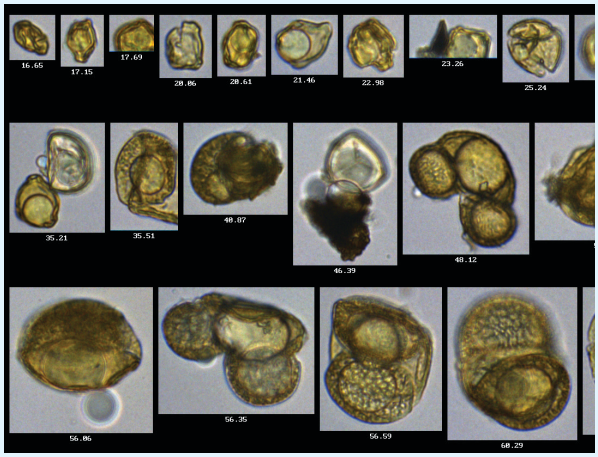
Figure 3. FlowCam can capture zooplankton like the Daphnia and copepods shown here

For more information, visit the Aquatic Applications page at fluidimaging.com

SECTION 4.3: CROP AND SOIL SCIENCES

FlowCam applications in Agronomy include:

- Monitoring the microencapsulation process of fertilizer particles
- Determining presence of and monitoring health and growth of soil microbes, mites, forest litter invertebrates and nematodes
- Determining seed viability and observing naturally occuring defects in plant development
- Analyzing pollen particles and pollen shell capsules



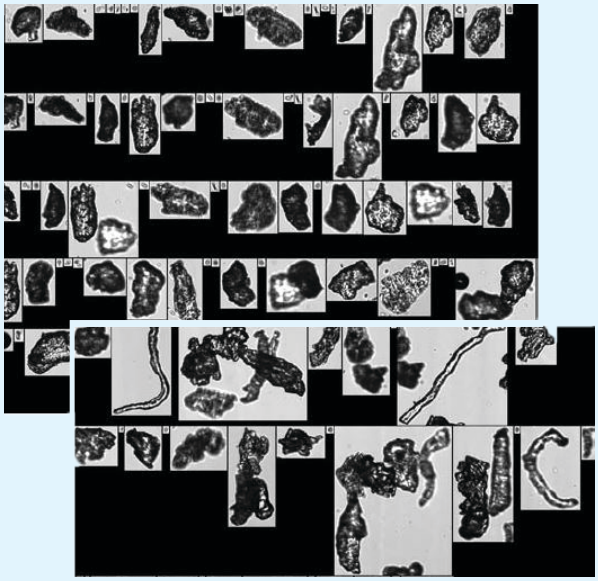
Pollen particles imaged by FlowCam

SECTION 4.4: FOOD AND BEVERAGE CHARACTERIZATION

Ingredients are critical in all facets of the food and beverage industry. FlowCam allows the user to isolate different particle types from a heterogeneous mixture in order to ensure the contents and detect process flaws early.

One example is hydrocolloids. Xanthan gum, guar gum, pectin, and other products are used to impart thickening, stabilizing, texturizing, and other properties to foods, beverages, and personal care products. Careful formulation, production, and packing are required to meet dispersability, hydration rate, powder flow, and other key performance characteristics. All of these are affected by individual particle size and shape.

Similarly, agglomerated particles and blends such as pectin blended with sugar demand compatible particle sizes to ensure they remain locked together. If they become separated, required characteristics aren't delivered.



Xanthan gum and cellulose gum particles imaged by FlowCam

"The worst part of a particle size or shape issue is that it probably never occurs to the processor to check gum particle size or shape or that of any other ingredient. Instead, the recipe or the machinery are blamed."

-Ross Clark, Distinguished Research Fellow
CP Kelco

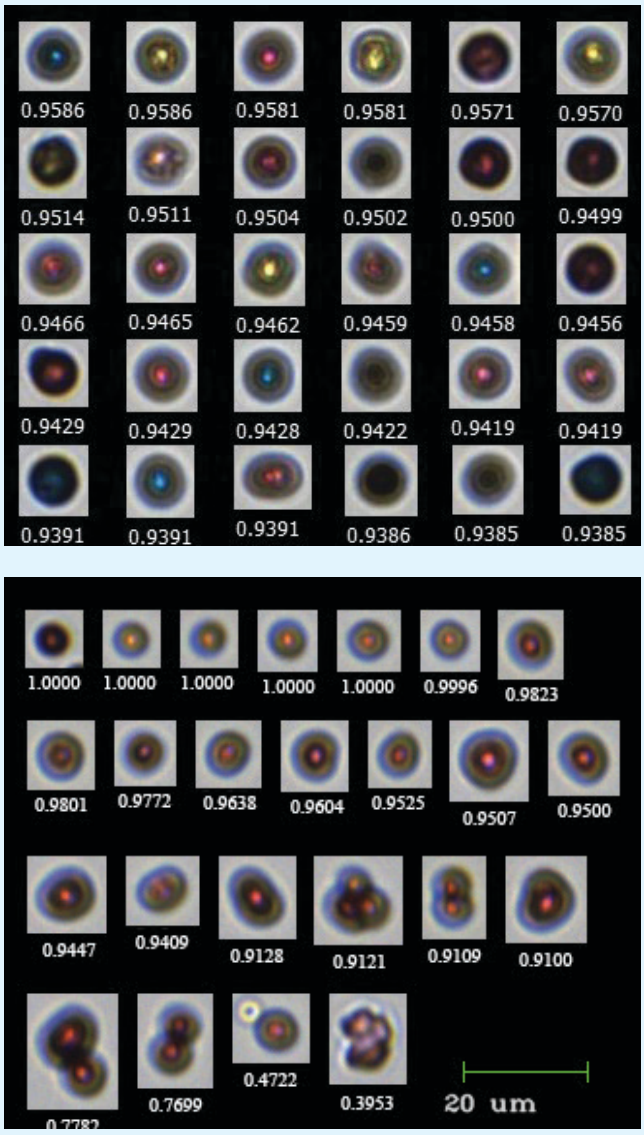
SECTION 4.5: PRINTER TONER QUALITY ASSURANCE

The size and shape of printer toner particles can considerably impact the image resolution and efficiency of a printer. The consistency of these particles also influences the distribution of charge the particles hold and, as a consequence, can affect overall image quality.

Image characterization using FIM can help to determine the size, shape, circularity and material uniformity of printer toner particles during and after production.

FIM is crucial to the ability to measure particle circularity, one of the principal properties relevant to quality control analysis in the manufacturing of printer toner. Other high-volume particle analysis techniques are able to determine particle size, but since they assume that all particles are spheres, they do not allow for particle shape analysis.

Using VisualSpreadsheet, software filters can be created to look for toner aggregates, by filtering for low circular fit and circularity properties. VisualSpreadsheet also gives the user the ability to see not only the whole particle size distribution (PSD) but also the ability to analyze specific ranges and outliers separately.



Color printer toner particles imaged by FlowCam. Circularity value is shown beneath each image. Particles are shown in order of decreasing circularity, where 1 is a perfect circle.

SECTION 4.6: MICROENCAPSULATION PROCESS ANALYSIS

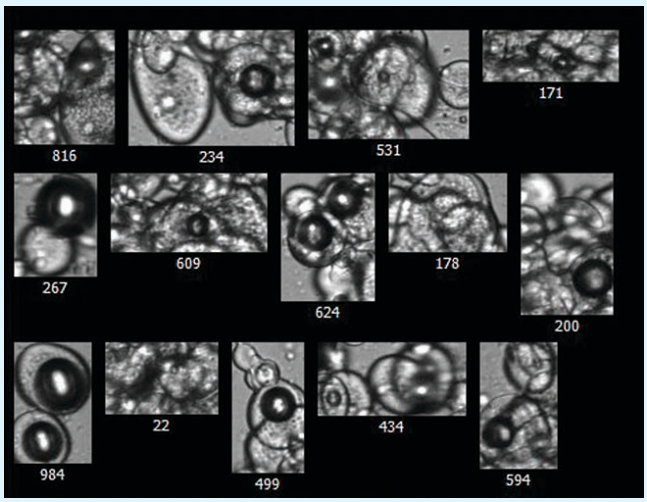
Microencapsulation is a process by which small amounts of a substance (an active ingredient) are packaged inside a second substance to shield the active ingredient from the surrounding environment. The process is used extensively for delivering particles in a wide range of applications, from pharmaceuticals to foods to detergents.

Flow Imaging Microscopy provides unique insight into the microencapsulation process. While studying the effects of temperature, concentration, pH or other variables that affect the process, you can monitor capsule formation in real time, optimizing your process and ensuring quality.

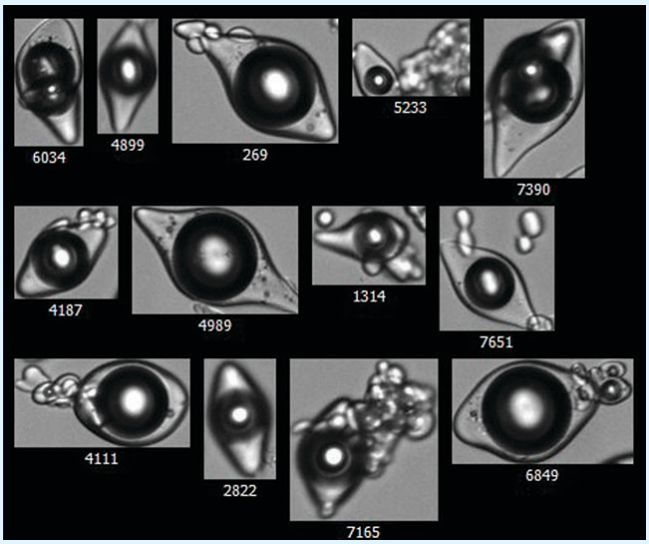
In this example, FlowCam was used to monitor coacervate formation in a test vat as the sample cooled under constant agitation.

Samples were collected and analyzed every 15-30 minutes. Visual examination of particle images and statistical pattern recognition analysis confirmed that at t_0+ 39 minutes the most clean coacervates were formed. After this point, the gelatin began to attach itself to the capsule walls, causing agglomeration and eventually disintegration of the coacervates.

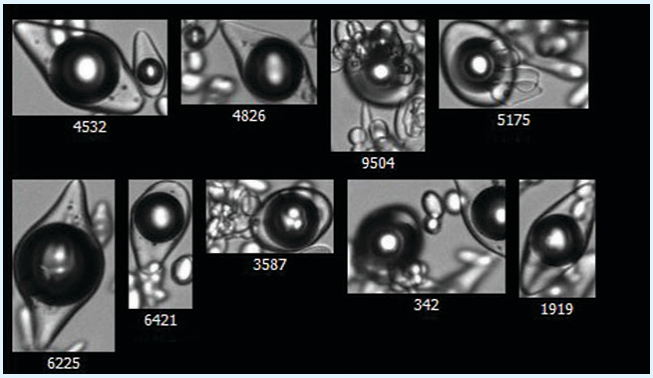
FlowCam yields tremendous insight into the process of coacervate formation, and can be an indispensable tool for microencapsulation research & development and quality control applications.



Sample analyzed at 9 minutes. Dark circles are active ingredients.



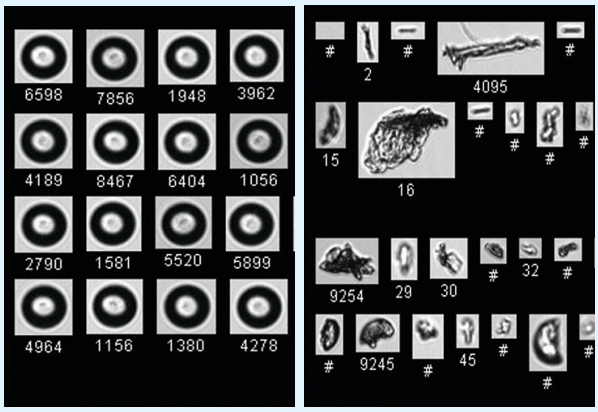
Sample analyzed at 39 minutes. Coacervates are fully formed.



Sample analyzed at 58 minutes. Coacervates still visible, agglomeration beginning to occur.

SECTION 4.7: OUTLIER CHARACTERIZATION - COLUMN PACKING MATERIAL QUALITY CONTROL

In column chromatography the elution rate of different components in a mobile phase is dependent on the size and shape of particles in the stationary phase. Elution rate precision is greatly improved when a stationary phase is made up of uniform particles. FlowCam provides critical size and shape information which allows for tighter column density control, and in turn, better control of column performance. FlowCam can help trace damaged (non-spherical) particles that are often present in different lots of column packing material.



Round, acceptable particles vs. irregular, unacceptable particles

SECTION 4.8: ADDITIVE MANUFACTURING - CHARACTERIZING METAL POWDERS

Metal Additive Manufacturing (also known as 3-D printing) requires carefully engineered compound metal powders in order to make high-quality end products. ISO/ASTM provides specifications for certain characteristics in order to meet quality standards. These include particle size distribution, morphology, contamination, and requirements for used metallic powders.

Sampling metallic powder using FlowCam can provide all of this information, and determine if the particles are of the required aspect ratio (round), if contamination is present, or if used powders are suitable for reuse. The suitability of the particles has the ability to affect bulk powder performance during manufacturing as well as the final qualities of the printed material.

Round, acceptable metal particles

Irregular metal particles that could cause problems in the printing process

[Read more: Farzadfar, S.A. et. al (2020) doi: 10.1016/j.powtec.2020.07.092]

SECTION 4.9: PARTICLE DIFFERENTIATION IN A HETEROGENEOUS SAMPLE - WASH WATER

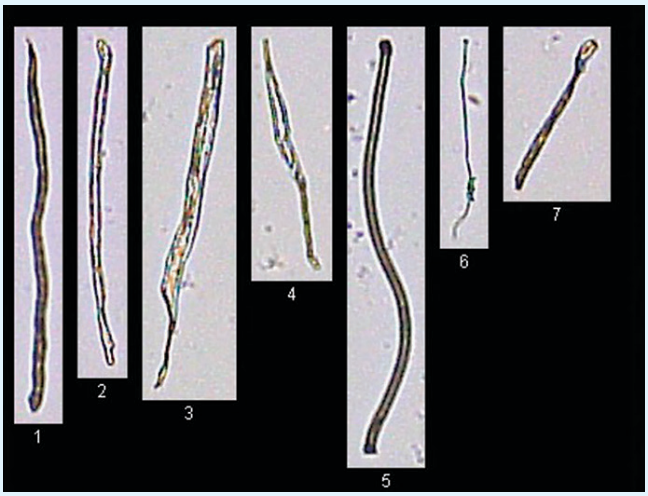
FlowCam excels at analyzing heterogeneous samples, where multiple particle types are present. In these situations, imaging particle analysis and pattern recognition techniques can provide an automated method for characterizing the types and quantities of particles present.

In this example, a wash water sample from a manufacturing process for electronic devices was analyzed. These devices are washed to remove traces of fibers, metals and plastics from the manufacturing process. It is important that the wash water contains less than a certain number of each of these particle types, as too many particles could cause failures. Additionally, the types and quantity of particles present in the wash water serves as an indicator for any problems arising in the production process.

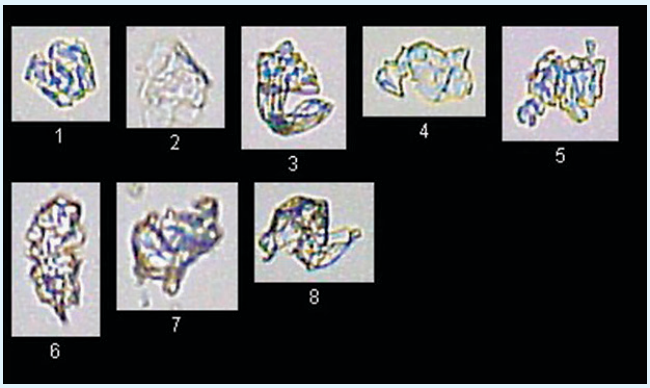
Analysis of this sample revealed a diversity of particle types: long, skinny fiber particles, semi-transparent metal shavings, and more opaque plastic particles.

A library for each particle type was built based on particle characteristics, and each subsequent run was automatically filtered into the different particle types. The corresponding volume percent, particles/mL, and PPM was simultaneously calculated.

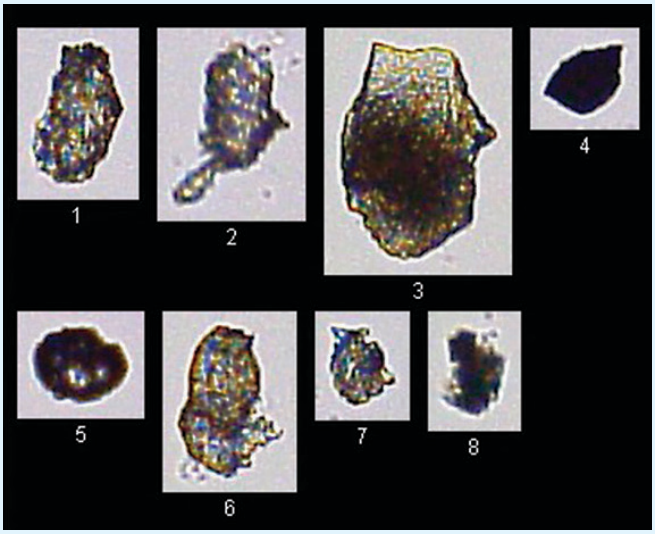
FlowCam allows the user to quickly determine if there is an issue with their manufacturing process in real time and make the necessary adjustments.



Fibers



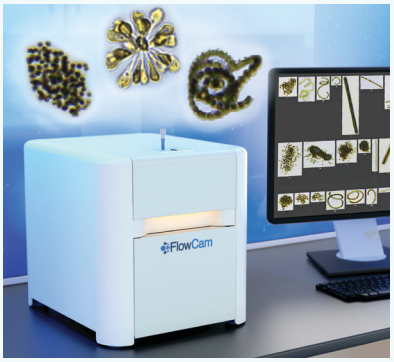
Metals



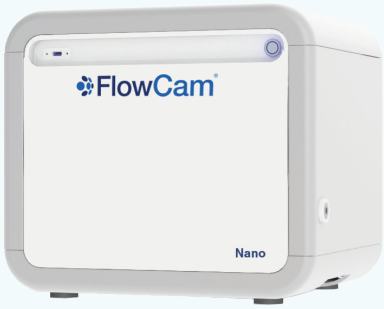
Plastics

Covering the Full Spectrum of Flow Imaging Technology

FlowCam 8000 Series
Particles 2 µm to 1 mm
multiple objectives

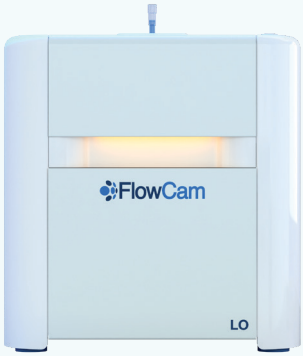


FlowCam Cyano
Distinguish between cyanobacteria and other algae

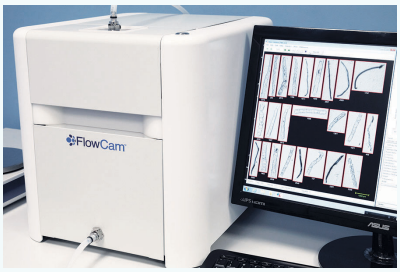


FlowCam Nano
Particles 300 nm to 2 µm

FlowCam 5000
Particles 2 µm to 300 µm
single objective



FlowCam LO
Obtain Flow Imaging and Light Obscuration data in one instrument



FlowCam Macro
Particles 300 µm to 5 mm

ALH for FlowCam™
Integrates with 8000 series and FlowCam LO for high-throughput processing

VisualSpreadsheet Software
FlowCam's image analysis software

Don't see your specific application?

Have additional questions?

Wondering if the FlowCam will work for you?

contact@fluidimaging.com

ABOUT YOKOGAWA FLUID IMAGING TECHNOLOGIES

Yokogawa Fluid Imaging Technologies, Inc., manufactures industry-leading particle analysis instrumentation based on digital imaging technology. Our flagship product, FlowCam, is the first automated particle analysis instrument to use digital imaging for measuring size and shape of microscopic particles in a fluid medium.

With applications in marine & freshwater research, biopharmaceutical research & development, municipal water, industrial manufacturing, and many other markets, Yokogawa Fluid Imaging Technologies leads the way in imaging particle analysis.

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