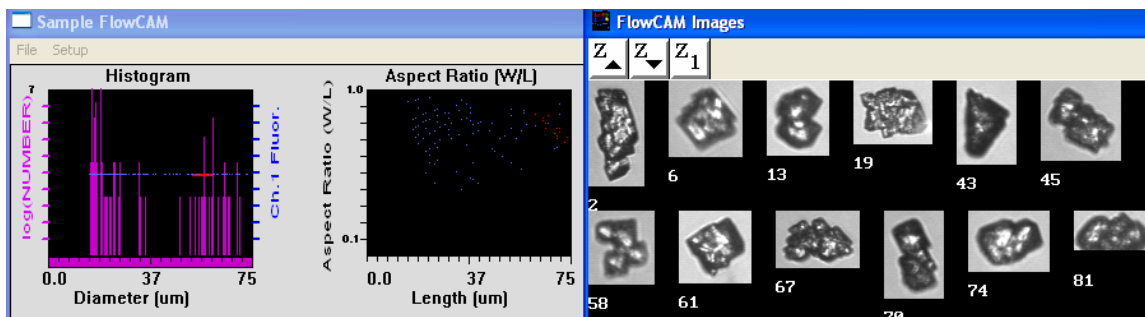


Continuous Imaging Fluid Particle Analysis: A Primer

Lew Brown
Fluid Imaging Technologies
Edgecomb, ME



Introduction: Many scientific endeavors involve the use of particulate matter suspended within a liquid. Typically these endeavors are a part of a “process” which is being studied for reasons of determining cause and effect during a discovery process, and later for monitoring the state of that particular “process” once something is known about the causes and effects. Typical examples include water quality analysis and monitoring, chemical process analysis and monitoring in manufacturing, and pure scientific research. A critical issue in these “processes” is the measurement and analysis of the particulate matter present in the process. In very few cases are the particles under study large enough to be quantified and analyzed by the naked eye. With the advent of the microscope, scientists were now able to study some of these particles in great levels of detail. As technology has advanced over the years, with the introduction of instruments such as the Scanning Electron Microscope (SEM), scientists have been able to look at increasingly smaller particles and even down to the molecular level. This paper will look at the issues associated with fluid particle analysis, discuss some of the historical methods used, and introduce a new technology, the continuous imaging fluid particle analyzer, which offers an automated method for particle analysis which can be used over a broad range of material types/sizes, both in the laboratory and in the field.

Particle counting and analysis: Any discussion of this topic must begin with a basic understanding of exactly what we mean by a “particle”. For the purposes of this paper we will define “particle” as “a minute quantity or fragment, a relatively small portion or amount of something” (Merriam-Webster). Typical measurements of the particles that are of interest to the scientist (or engineer) are particle size distribution (particle count), size, shape, and quantity per unit volume (dilution).

While frequency (particle count per unit volume) seems to be fairly straightforward at first, it can be easily complicated by overlapping particles and “clumping” of particles. This potential problem is easily fixed by diluting the subject matter to separate the particles, and presenting them in a single cell layer, enabling for a straightforward “binary” analysis (particle is either present or it is not). The issue of how we characterize particles presents us with a much more difficult problem, however. For the purpose of analysis, it is usually desirable to quantify particle size as a “single number” which can be plotted against another variable (frequency, dilution, temperature, etc.) on a simple graph. The simplest measure of particle size would be its diameter, but even this is not as straightforward as one would think due to variations in particle shape. In a “perfect world”, all particles would be of the same shape, allowing a single number to be used to quantify size. But in the real world, particles exhibit a variety of shapes, and the problem becomes how to characterize the distribution of particle shapes. A fair amount of research and applied mathematics have been applied to this very problem [1], and is beyond the scope of this paper. The generally accepted industry norm for particle size is to calculate its Equivalent Spherical Diameter (ESD). This permits a single number to quantify the “size” of particles of any shape.

While ESD is a known, established method for characterizing particle size, many applications will also require some quantization of shape to differentiate between particle types which may have an equal ESD within a sample. If it was always possible to obtain uniform samples that contained only one type of particle, this would not be an issue. Real world problems typically consist of analyzing a sample which contains many different particle types in the same fluid. One simple measurement which can be determined relatively easily is the particle's "aspect ratio", which is the ratio of its maximum length to its minimum length. For example, two particles having the same ESD could have an aspect ratio of 1:1 (spherical) or an aspect ratio greater than 1:1 (cylindrical). Since the aspect ratio of two different particle types contained in the sample can differ, this measurement is an easy way of segregating these two particle types even though they may plot with the same ESD.

Other properties can be used to distinguish between particles in a sample. Two properties used in automated analysis systems are "light scatter" and "fluorescence". Light scatter is a function of the optical characteristics of the particle, and varies between different materials and sizes. Light scatter can be measured by directing a laser at the sample and quantifying the amount of light which is either slightly deflected (forward scatter), or is scattered to the side (side scatter) by the sample particles. Fluorescence is a function of the chemical composition of the particle, and can also be used to differentiate particles within a sample. Fluorescence occurs when the particle is illuminated by a narrow wavelength light source, typically a laser. By sorting the light emitted by the particles through narrow band filters, multiple photomultiplier tubes (PMTs) can be used to detect particles with different fluorescence emissions.

In many of these automated systems, adjustment of the electrical gain of the PMT's can also be used to help narrow down the types of particles detected and analyzed. The signals from the PMTs are used as "triggers" to the electronics system which determine when a passing particle in the fluid is sampled and recorded. This greatly reduces the amount of data which needs to be analyzed versus taking continuous samples over some preset time interval. For example, in a sparse sample, measurements are only taken and recorded when a particle of the desired properties is present and not when "empty liquid" is passing through the flow chamber, greatly reducing the amount of data collected for analysis.

Historical methods used in particle analysis: The first use of the microscope to observe and record microscopic life in the 1600's greatly changed the ability of scientists to study objects and phenomena occurring at a level below the limits of the unaided eye. This is certainly true in particle analysis, and the microscope remains even today the most common instrument used for this activity. The major drawback of standard microscopes for particle analysis is the time required, both for sample preparation, and for counting and measuring properties of the particles. Additionally, since this time is required for sample preparation and analysis, the microscope can only be used to observe one static sample at a time. While this is fine for "basic research" in an early discovery phase, it is unacceptable once the requirement is present for looking in detail at a "process". First of all, the second phase of verifying a cause and effect relationship found in the discovery phase requires analyzing a statistically significant quantity of the particles being analyzed. This process is too time consuming for manual methods, and requires that some level of automation be brought to the measurements. Secondly, since this is an analysis of a "process", sampling needs to be accomplished over a time period of the process. Indeed many of these "processes" require continuous analysis over time, an extreme example being the continuous monitoring of particles in a process for purposes of quality control. Surely, other methods are required to achieve these goals.



Scientists have devised various methods and instrumentation for the automation of particle counting and analysis that can go beyond the limitations presented by the standard optical microscope. Some of the

earliest instruments used for automated particle counting are based upon the Coulter principle and are referred to as “Coulter Counters”. Many of these are still in use today as they allow for rapid quantification of particle frequency and size distributions in a heterogeneous sample. The major drawback of these systems is that they are “coulter volume” based for sizing (so can not distinguish particle types), and do not provide any information on the optical properties of the particles [2].

More recently, the “flow cytometer” has become widely used for automated particle analysis. Flow cytometers measure particle light scatter as well as fluorescence. Light scatter introduces the ability to determine some sense of morphology to the particles, and fluorescence aids in distinguishing between particles that either fluoresce or do not fluoresce in relatively narrow wavelengths. Flow cytometers work very well with relatively uniform sample particle sizes typically within the 0.5 to 20 micron range in ESD. However, in order to obtain samples of relatively uniform particle size, pre-processing of the sample from its natural state is usually required. Another major drawback to these systems is that they use a “sheath fluid” apparatus in order to produce laminar flow of the sample through the flow chamber. The sheath fluid apparatus is rather complicated, requires increased set-up time, is limited to particle sizes under 100 microns, and is very expensive to replace.

Another method that has been used in parallel to these instruments being developed is simple imaging of particles in flow. Early studies using this technique made use of photographic film and a “triggered” image capture using either a flash light source or fast shutter with which to “freeze” the particles in flow. As computer technology has evolved, the film has been replaced by digital image capture hardware. With a digital image, many measurements can be made automatically through well understood image analysis techniques originally developed in the remote sensing (reconnaissance and mapping) and medical research fields. Early on, although these measurements could be automated by the computer, they took time (non “real time”) due to the compute intensive nature of digital image processing. This limited the use of these techniques to well funded laboratories that could afford very powerful computers or specialized image processing hardware. The continued exponential growth in speed and reduction of cost of computing hardware has made this type of image analysis available to a much larger group of scientists.

A new technique, continuous imaging particle analysis: A new instrument for fluid particle analysis has been developed by Fluid Imaging Technologies Inc. (Edgecomb, Maine) called the FlowCAM® (Flow Cytometer And Microscope). This instrument combines the capabilities of a flow cytometer with a digital imaging microscope. The basic architecture is shown in Figure 1. Like a flow cytometer, the basic architecture can be divided into three interrelated subsystems: the fluid system which delivers the fluid under study into the flow chamber; the optical system containing light sources, detectors, and other optics; and the electronic system which controls the overall apparatus and does the actual processing of the images and data gathered by the various detectors.

The FlowCAM® can be configured in a variety of different ways depending upon the needs of the scientist. In its simplest configuration, continuous imaging, the instrument uses only the digital imaging subsystem to continuously image the sample as it flows through the flow chamber. Illumination of the sample is accomplished by the electronics triggering a flash LED light source and the frame grabber at preset intervals. The computer then processes and stores the images to disk. In order to save storage space, the entire image field is not stored. Instead, a sub-image is made

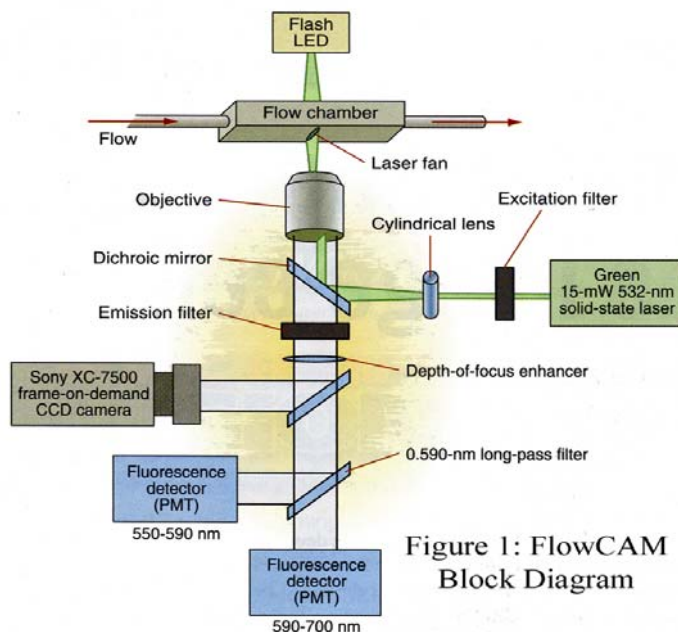


Figure 1: FlowCAM Block Diagram

by creating a “bounding box” around each particle in the image field and storing only the particle images on disk. As each particle image is acquired, data about the particle such as length, width, ESD, area, and aspect ratio is calculated and stored indexed to the image itself.

In other configurations of the FlowCAM, the instrument can use either a fluorescence or forward light scatter voltage from a PMT as a “triggering” mechanism to collect data and images as the sample flows through the flow chamber. Essentially, this amounts to a flow cytometer with selected image capture based upon parameters (such as size, fluorescence, or scatter) input by the scientist. As in continuous imaging mode, sub-images of particles are collected with each trigger generated by the PMT, and associated data for each particle (size, aspect ratio, fluorescence strength, etc.) is stored indexed to the particle image. The data collected for each image also contains the location of the particle within the imaging field of view, which enables eliminating redundant data which might be collected if a particle were to become attached to the flow cell.

Regardless of the configuration, data from the sample flow can be gathered continuously over time with the only limitations being the speed of the processor, and the overall limit for data storage imposed by the disk drive(s). This means that the instrument can not only be used in the laboratory for analyzing discrete samples, but can also be used *in-situ* to continuously monitor a process.

Once data is collected from a sample or process, it can then be presented to the scientist for review in two different modes: image collage or interactive scattergram. In image collage mode, the scientist can review collage images containing multiple particles in order to visually classify particle types and morphology [2]. In interactive scattergram mode, the data is presented in a similar fashion as it would be on a flow cytometer. A two-dimensional plot of particles is displayed on a graph containing two variables. The simplest plot could be particle ESD versus fluorescence, with each “dot” on the plot representing a single particle. Other plots could be generated such as particle size versus aspect ratio (this plot would easily show and distinguish between two different types of particles contained in the sample). Once the scattergram is displayed, the user can then “interact” with it by defining a box around a particular group of particles on the scattergram. This will then bring up for display an image collage containing all those particles within the box (Figure 2). This

is extremely useful in isolating for display images that only contain a particular type of particle within a heterogeneous sample.

There are a number of characteristics that make the FlowCAM unique versus other instrumentation used for particle

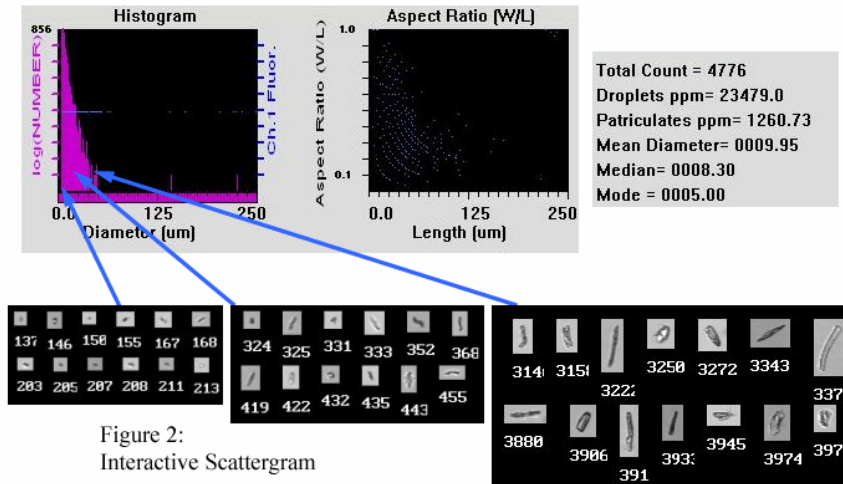


Figure 2:
Interactive Scattergram

analysis. Firstly, unlike traditional flow cytometers, the FlowCAM does not use sheath fluid hardware. This greatly decreases set up and maintenance time for its use, and allows for a wide range of particle sizes to be studied, between 1 micron and 3 millimeters using different sized flow cells. Secondly, the FlowCAM uses a proprietary, patented optical element with the objective lens in order to increase the system’s depth of focus. Since imaging depth of field decreases with increasing objective lens power, this added optical element greatly increases the clarity of imaging the instrument is capable of at higher magnifications. Additionally, the enhanced depth of field allows it to be used at higher flow rates (up to 10 milliliters/sec) than other instruments. As noted above, the FlowCAM also can be used for continuous analysis of a fluid flow, rather than only discrete samples typical of other methods. Finally, since imaging is an integral part

of the FlowCAM, measurements such as length/width (not just ESD), area and aspect ratio are easily calculated on the fly, and the images are available for both visual and computational post-processing.

The FlowCAM has been extensively tested, and its performance validated for a number of parameters against traditional manual methods [2]. These tests have validated the contribution of the depth of field enhancing optics to accuracy in particle sizing, the accuracy of particle sizing and counting, the ability to accurately distinguish between particle types based upon size distribution, and the ability to continuously monitor a sample stream and detect changes in the sample's content.

Some typical examples of FlowCAM applications: The FlowCAM was originally developed for oceanographic studies of microplankton and phytoplankton [2], which encompasses a fairly broad range of particle sizes (20 to 200 microns). These studies also had a requirement for continuous monitoring, either of a stationary location in a body of water or of a continuous sample being collected on board a moving ship. However, because of the instrument's flexibility outlined above, it can and has been used on a wide variety of other particle analysis applications such as on fluid toners, oil-in water (petrochemical), salt particles, and coatings analyses. Indeed, many new applications for the technology are being experimented with as of this writing.

Conclusions: A new analytical instrument, the FlowCAM, has been developed that has opened up new applications for scientific studies involving automated particle analysis. The key benefits of the technology used in FlowCAM are the ability to analyze continuous flow, the ability to analyze particle shape, and, most importantly, the fact that digital images of particles are acquired and saved for further analysis in post-processing. Because of the flexibility of the FlowCAM's architecture, it can be applied to a wide variety of applications of particle analysis, with potential future growth both in the instrument's capabilities and the types of problems it can address. For this reason, the instrument is truly an "enabling technology" to the scientist, researcher, product developer, QC and manufacturing personnel, etc. concerned with particle analysis.



References:

- 1.) Rawle, Alan: The importance of particle sizing to the coatings industry, Part 1: Particle size measurement. *Advances in Colour Science and Technology*, Vol 5 No 1 (Jan 2002)
- 2.) Sieracki, Christian K., Sieracki, Michael E., Yentsch, Charles S.: An imaging-in-flow system for automated analysis of marine microplankton. *Marine Ecology Progress Series* (Jul 1998)

Lew Brown is the Manager of Marketing & Sales for Fluid Imaging Technologies. He may be contacted at (207) 882-1100 or lew@fluidimaging.com.