FlowCam®

Color vs. Black and White: How to Choose a FlowCam Camera

SUMMARY

While most imaging particle analyzers are only equipped with black and white (monochrome) cameras for image acquisition, FlowCam 8100 instruments offer a choice of either black and white or color cameras. Each configuration offers different performance in common particle analysis tasks like particle sizing and morphology analysis. It is important to select the appropriate setup for a specific application and particle analysis goal. This technical note will describe the strengths and weaknesses of each camera choice and discuss which is better suited for specific applications. The summary of recommendations can be found in Figure 1.

BLACK AND WHITE CAMERA

Advantage: Higher image resolution

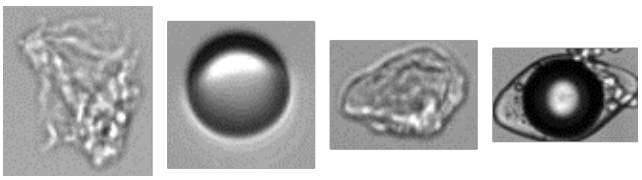
- More accurate particle size measurements
- Helps analyze small particles without distinct coloration

Preferred When:

- Particle sizing accuracy is critical
- Particle identification is of secondary importance
- Particles are colorless (highly transparent or opaque)
- Particle size is near the lower size limit of the instrument

Common Applications:

- Biotherapeutic particle analysis
- Food & beverage characterization
- Materials characterization



COLOR CAMERA

Advantage: Captures particle color

- Better differentiation between particle types
- Helps analyze large, intrinsically colored particles

Preferred When:

- Particle type identification is the focus of the analysis
- Particle size and shape measurement accuracy is less critical
- Particles are colored
- Particles are well above the lower size limit of the instrument

Common Applications:

- Harmful algal bloom monitoring
- Drinking water quality monitoring
- Marine and freshwater phytoplankton research

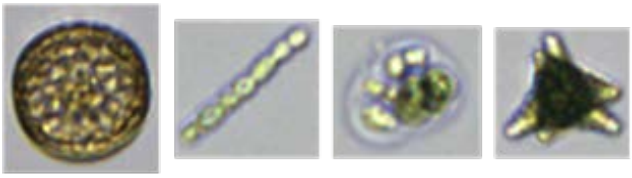


Figure 1. Summary of the benefits of black and white vs. color camera configurations for FlowCam 8100

COLOR VS. BLACK AND WHITE TRADE-OFFS

When choosing a FlowCam camera, the primary trade-off to be considered is spatial versus color resolution. FlowCam units that are configured with a color camera offer lower resolution than an equivalent instrument configured with a black and white camera. While the two configurations use the same number of camera pixels and yield images with the same number of image pixels, the color camera has a lower spatial resolution

because it requires several color-specific camera pixels to measure the color of a single pixel in a camera image.

The combination of color-specific pixels and necessary interpolation reduces the spatial resolution of color cameras relative to a black and white camera containing the same number of pixels on the sensor. In a worst-case scenario, these factors can reduce the resolution of color cameras to 70% of that of an equivalent monochrome camera. Typically, the spatial resolution loss is around 20% or less. While the spatial resolution loss of color cameras may not be significant in standard photography applications, it can be significant when digital cameras are used to perform measurements on instruments like FlowCam.

This lower resolution manifests in part as artifacts in FlowCam images, especially near particle edges. Figure 2 shows images of an edge in both black and white and color to illustrate this effect. While the color transition across the edge is relatively sharp when imaged in grayscale (Figure 2, left), the transition is diffuse and exhibits color artifacts when imaged in color (Figure 2, center). These artifacts transform an approximately one-pixel transition between objects in the black and white image to a three-pixel transition in the color image. As these artifacts are caused by the color-specific camera pixels used in color cameras, they cannot be corrected by casting color FlowCam images to grayscale as the resulting image will still exhibit these artifacts (Figure 2, right).

Color imaging artifacts like those shown in Figure 2 reduce the accuracy of particle size measurements. These artifacts make it difficult to identify where the edges of the particles are in the image and, if misidentified, will cause errors in the reported particle size. Many shape parameters like aspect ratio similarly depend on particle size and may also exhibit errors. As the length of one pixel is approximately 0.6 µm long at 10X magnification, these 1-pixel errors can affect measurement accuracy, especially for particles near the lower size limit for the objective lens in use.

THE BENEFITS OF COLOR FOR FLOWCAM MEASUREMENTS

FlowCam 8100 units that are equipped with a color camera configuration trade any potential reduction in sizing accuracy for the ability to distinguish between two or more colored particles. Even a subtle color difference can help users tell different particle types apart when analyzing the particle images. However, some applications benefit more from color images than others. Color information is beneficial when the application involves moderately transparent particles that exhibit pronounced coloration like phytoplankton. Applications that involve highly opaque or transparent particles benefit less from color information. Since FlowCam is a back-lit (brightfield) system, opaque particles will typically be seen as a black silhouette against a white background and exhibit minimal color information. Highly transparent particles may only slightly color the light that passes through them, yielding little color information to aid in particle identification. In these cases, the loss in spatial resolution caused by using the color camera may be counter-productive to particle characterization.

Figure 2. Simulated edge artifacts when imaging a sharp edge using a grayscale camera (left) and a color camera (center). Also shown is the color image of the edge cast to grayscale (right).

HOW TO CHOOSE A FLOWCAM 8100 CAMERA CONFIGURATION

The optimal camera configuration for a specific application primarily depends on the particle characteristics that are most important for that application. Since camera configurations impact both particle size measurements and particle type identification, it is important to ask which is more important for the application—even if both features are relevant. Generally, the color configuration is optimal for applications where identifying different particle types is the focus of the analysis and particle sizing accuracy is not the primary objective of the analysis. Conversely, the black and white configuration is better for applications where particle sizing accuracy are crucial and particle type is of secondary importance. While the relative importance of these two types of data may be similar and even somewhat subjective, deciding which feature is a priority will often determine which camera configuration is optimal.

The other main factors to consider when deciding between grayscale and color camera configurations are particle coloration and particle size relative to the size limitations of the instrument. Applications that involve differentiating colored particles will benefit more strongly from color optics than applications involving particles that lack distinct coloration (e.g., highly opaque or translucent particles). Applications that involve sizing particles that are near the lower size limit of the objective lens would benefit more strongly from a grayscale camera as it avoids the more significant sizing errors color can introduce with small particles. All else being equal, applications that involve particles in the mid-range and near the upper size limit of the instrument and exhibit prominent coloration will be better served by the color camera configuration. Applications that involve opaque or translucent particles that are near the lower size limit of that objective lens are better off with a black and white camera configuration.

COLOR AND GRAYSCALE CAMERAS FOR AQUATIC, BIOPHARMA, AND MATERIALS APPLICATIONS

A prototypical example of where a color FlowCam 8100 configuration would be preferred is in monitoring for harmful algal blooms (HABs). In this application, researchers are interested in using FlowCam to identify toxin-producing species of microalgae in a water sample. Color cameras are almost always used in this application because particle type identification is the focus of the analysis and the particles of interest exhibit intrinsic coloration (e.g., green chlorophyll in phytoplankton) that is useful in distinguishing between plankton genera. Particle size measurement errors are also less pronounced in this application since the large sizes of many microplankton dwarf the sizing errors color optics introduce.

A black and white camera configuration is preferred when particle sizing accuracy is paramount. For example, in biotherapeutic applications the number of particles in different size ranges is an important product quality metric. A black and white camera is the best configuration to monitor the number of particles in various particle size ranges because it minimizes sizing errors. Biopharmaceutical researchers are also often interested in analyzing particles across the entire size range of their FlowCam instrument, often including particles 2-3 µm in diameter for which a one-pixel sizing error can result in a 20-30% change in the measured particle diameter. While many researchers in this domain are also interested in identifying different types of particles, particles encountered in these samples are often highly translucent and primarily exhibit variation in particle shape, not color. Black and white FlowCam units are therefore still the recommended configuration even in particle identification-focused biopharma applications. The black and white camera configuration captures most of the relevant particle morphology information for this analysis while retaining the higher particle sizing accuracy that is often critical in the application.

The optimal optical configuration for some other applications may not be as obvious. For example, users interested in analyzing small but intrinsically colored printer toner particles may be interested in accurately monitoring the circularity (i.e., “roundness”) of these small particles and yet also want to monitor their color. As arguments can be made for both configurations in this application, the “optimal” configuration depends on which particle information is more important for the user—a decision that may be subjective. Typically, black and white FlowCam 8100 units are used for this application since particle circularity is often the main particle feature of interest. However, other users could easily justify using a color configuration for this application if particle coloration was of greater importance to that user.

CONCLUSION

Selecting the appropriate camera type for your FlowCam will help ensure that you obtain the desired particle information for your specific application. If you’d like to discuss color vs black and white camera configurations in more detail, please contact us at info@fluidimaging.com.