FLUID IMAGING TECHNOLOGIES

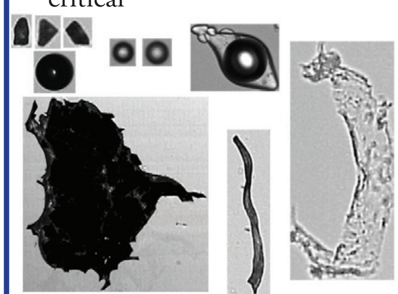
Particle Analysis with Vision®

## FlowCAM® Tech Brief #3

# Color Versus Black & White Cameras

**Summary:** While most imaging particle analyzers only offer gray-scale (black and white) cameras for image acquisition, FlowCAM® offers a choice of either gray-scale *or* color cameras. This Tech Brief will explain the difference between these two camera choices, and discuss the inherent trade-offs that should be considered when choosing which camera to use (summarized in Figure 1).

| Use B&W Camera: | Use Color Camera: |
|---|---|
| •Opaque particles<br>•Transparent particles with no color information<br>•Particles are small relative to calibration factor<br>•Precision in measurements is critical | •Transparent particles with color information that helps characterize them<br>•Particles are large relative to calibration factor<br>•Precision in measurements is less critical |



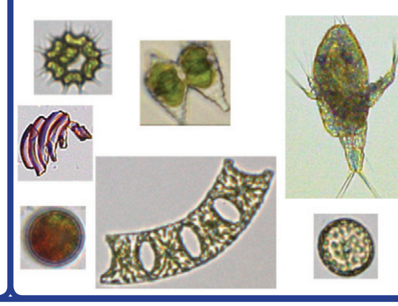


Figure 1: Summary of recommendations.

**Definitions:**

***Gray-Scale (Black and White) Digital Camera*:** In a gray-scale camera, each pixel on the sensor captures information on the quantity of light striking it, or *intensity*, regardless of wavelength. Each pixel in the resultant image is then represented by an 8-bit number where 0=black and 255=white, and the intermediate numbers represent the range of gray between those two extremes.

***Color Digital Camera:*** In a color camera, each pixel is the same as those on a gray-scale camera with the exception that each pixel has a color filter in front of it so that it is measuring the *intensity* of the light striking it only in that color range. The color filters are either red, green or blue, measuring the intensity in the three primary colors.

***Camera Resolution:*** Usually expressed in millions of pixels or *megapixels*, the camera's spatial resolution is found by multiplying the number of horizontal pixels found on the sensor by the number of vertical pixels (e.g. a *1 Megapixel* camera might have a 1,024 x 1,024 pixel sensor array).

***Output Resolution:*** The output resolution is the spatial resolution of the final image created by the sensor in pixels. It is the same as the camera resolution, with the exception that a gray scale camera has only one intensity value per pixel, whereas in a color camera, each output pixel has three intensity values, one each for red, green and blue.

***Bayer Filter Pattern:*** A Bayer filter is the most common color filter array (CFA) pattern used in color cameras. It is the arrangement of color filters in front of the monochrome pixels contained in a color camera (see Figure 2).

***How a Color Image is Formed:*** As described in the definitions, a color camera actually uses a monochrome (gray-scale) sensor with a color filter array (CFA) in front of it. Typically the CFA is the Bayer pattern shown in Figure 2. Figure 3 shows the resulting pattern created by the CFA on the sensor for red, green and blue values. Note that there are actually two times (2X) the number of green sensors versus red and blue ones. The reason for this is because the human eye is most sensitive to green light, so having an emphasis on green yields an image which will be interpreted closest to "true color" by the human eye.

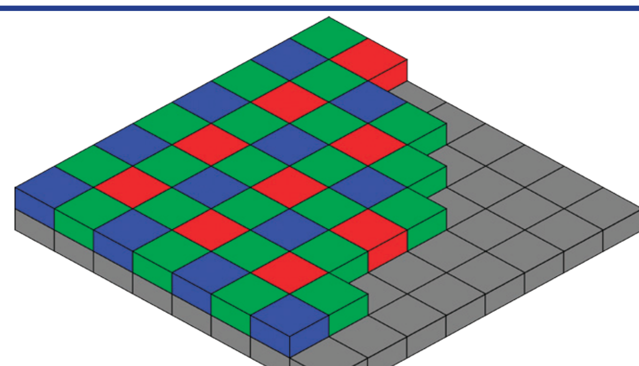


**Figure 2: Bayer color filter array (CFA) arrangement on a monochrome sensor.**
(Image Source: http://en.wikipedia.org/wiki/File:Bayer_pattern_on_sensor.svg)

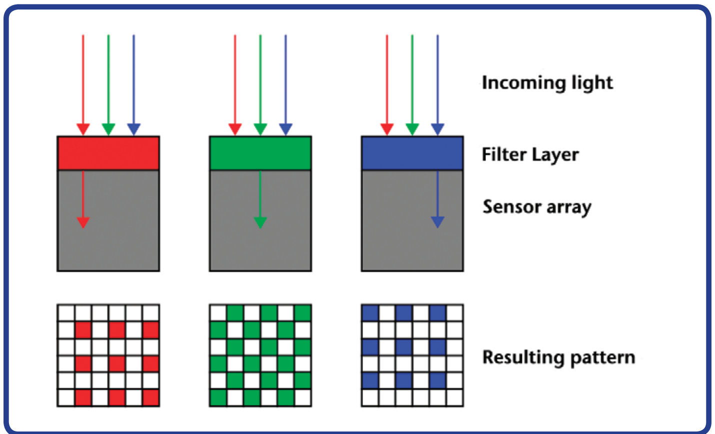



**Figure 3: Cross-Section of Bayer color filter array (CFA) arrangement and resulting pattern on monochrome sensor.**

(Image Source: http://en.wikipedia.org/wiki/File:Bayer_pattern_on_sensor_profile.svg)

If we were to take the direct output of the sensor ***as is***, the output image would have far less resolution than the camera image. For example, a 1 Megapixel camera image would produce a 0.5 Megapixel green image, and 0.25 Megapixels each for the red and blue images. In order to ***reconstruct*** an output image of equal resolution to the original camera resolution, a process called ***demosaicing*** is used. Essentially, this process ***interpolates*** the two color values for each pixel that are missing by looking at neighboring pixel values for that color. There are many different interpolation algorithms, and each camera manufacturer uses their own. As a simple example, Figure 4 shows what is called ***bilinear*** interpolation, whereby for each pixel in the camera image, the neighboring nearest 8 pixels are considered.

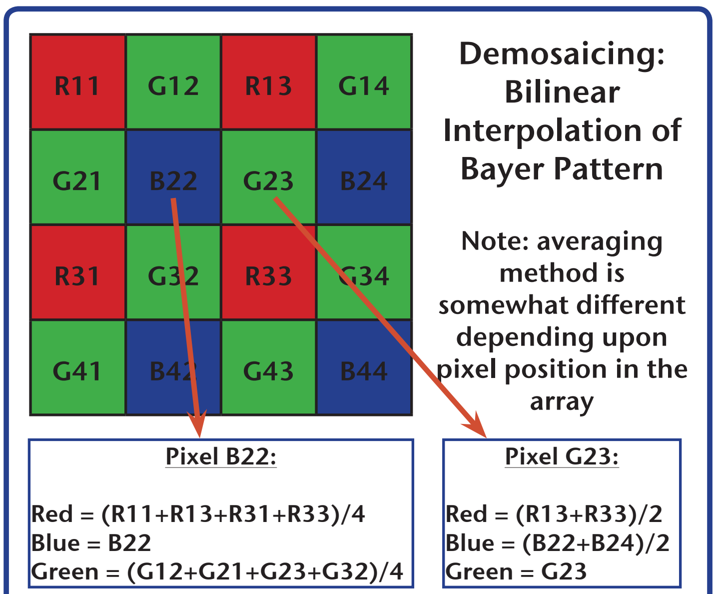



**Figure 4: *Demosaicing* of Bayer color filter array (CFA) showing resulting RGB pixel values created using bilinear interpolation.**

**What This Means for FlowCAM Camera Choices:** Since color sensors are merely gray-scale sensors with a CFA overlay, there is really no such thing as a "color" camera. As shown above, the CFA creates a color image by sampling the three primary colors (red, green and blue) separately in ***physically different*** locations, and then ***making up*** color values at other locations via interpolation. This means that a color camera has inherently lower resolution than a gray-scale camera will. Using demosaicing, the output image is brought up to the original camera resolution with RGB values at each pixel. So, in reality, the difference in resolution is not as great as it initially appears. Where it will show the most will be on edges, where color ***aliasing*** will occur, as simulated in Figure 5.

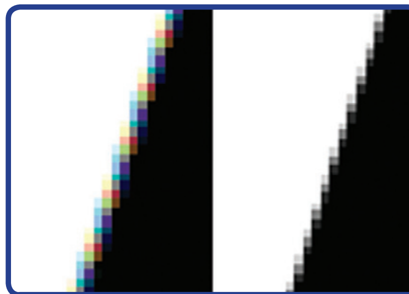


**Figure 5: Simulation of edge artifacts caused by color interpolation, left image is color camera, right image is gray-scale camera.**

**Color versus Black & White Camera Trade-offs:** As detailed above, when choosing which camera to use in your FlowCAM, the ***primary*** trade-off to be considered is ***spatial*** versus ***color*** resolution. The monochrome camera will give you higher ***spatial*** resolution. This is particularly important when looking at objects which are relatively small compared to the calibration factor at the magnification being used, where *every pixel counts* in determining size and shape measurements. An example of this would be 8µm-10µm size particles when using the 10x magnification, where calibration is around 0.6µm/pixel.

When making this decision, it is always important to keep in mind the end goal of imaging particle analysis, which is to *separate* and *characterize* different particle types in a heterogeneous mixture. This is done by *filtering* the particles using either the value or statistical filtering capabilities of VisualSpreadsheet®. If the ***color*** information is particularly useful for an application, such as in identifying plankton, then a color camera may be appropriate. Keep in mind that FlowCAM is a back-lit (brightfield) system whereby opaque particles will only be seen as a black *silhouette* against a white background, so there is no benefit to color in these situations. Even when the particles are transparent, if there is no *strong* color component to distinguish them from other particles, the loss in spatial resolution caused by using the color camera may be counter-productive to particle characterization.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