IMAGE CAPTURE METHODS

The imaging technology known as "three-shot color image capture" provides the highest resolution and best tolerances for most color image analysis applications.

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In the last 25 to 30 years, since the inception of personal computers, analysis of digital images has become a commonplace task in both the biological and industrial laboratory. Concurrent with the development of the personal computer has been the development of digital cameras and optical couplers between camera and microscope. As digital camera technology and microscopy have become more refined, the demands for more precise and accurate measurements from the resulting digital images have followed. These measurement demands include not only the physical image parameters, but also the color.

Currently, a system consisting of a high-pixel-resolution CCD (charge coupled device) chip and associated hardware, is the most common method for generating digital images. However, because digital images are inherently monochrome, or black and white, other hardware and software are needed to generate color images.

For CCD cameras, only three ways to create a color image are possible: the Bayer mosaic filter, three CCD chips, and three-shot color sampling. For the most precise and accurate measurements of both spatial and color measurements with the lowest camera cost, the best color generation method is the three-shot color sampling method, because it preserves the native resolution of the CCD chip in the resulting digital image.

This article compares the three-shot and color mosaic methods for color image generation, and shows why the three-shot image is better for image analysis.

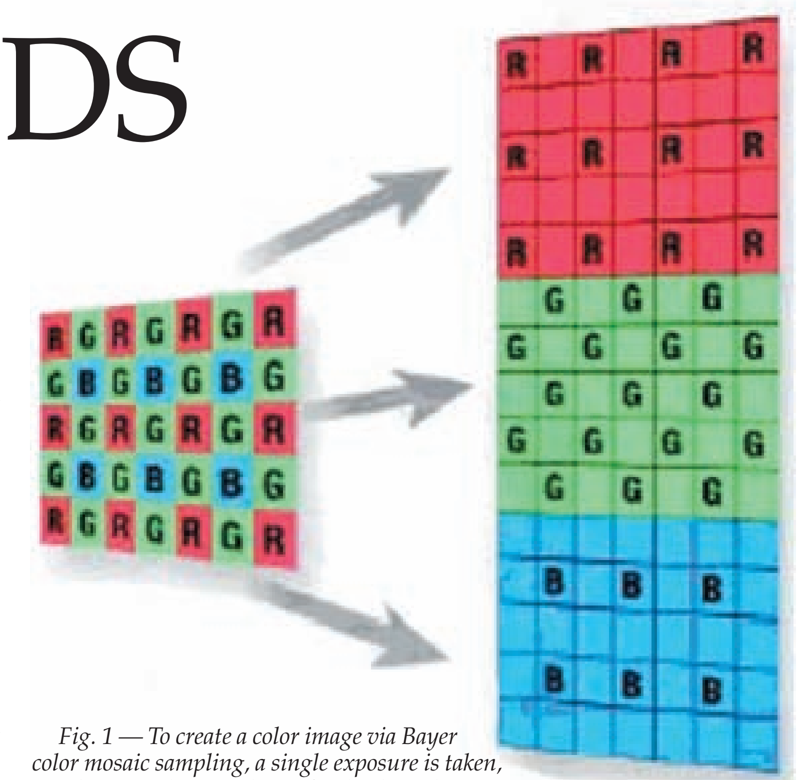

Fig. 1 — To create a color image via Bayer color mosaic sampling, a single exposure is taken, resulting in the sampling of only the red, green, or blue intensity at each pixel location. For each pixel in the resulting image, the two non-sampled colors are then found by interpolation with the adjacent pixels.

Producing a digital color image

The three technologies for producing color digital images are:

- **Three CCD chips:** Merge the three images from three CCD chips. Due to the high cost, three CCD chips are found only in analog cameras today, and will not be discussed further in this article.
- **Bayer mosaic filter:** Glue an RGB (red, green, and blue) Bayer mosaic filter to the front of the CCD and use software interpolation to capture a single-shot color mosaic sampling (i.e., image). In this technology, software interpolates between pixels that detect only pure red, green, and blue to "interpret" the best color for the resulting image pixels. Although this is probably the most common and least expensive method for generating color images, it is not necessarily the best.
- **Three-shot color sampling:** Create three separate images of the whole CCD with red, green, and blue filters, and merge the images. Called three-shot color sampling, this method is based on a movable RGB filter or an LCD (liquid crystal display) filter that changes colors in response to an applied voltage. In the case of the three-shot CCD, each pixel detects red, green, and blue, resulting in an image that more faithfully reproduces the colors in the original subject, and with higher resolving power than the Bayer method.

Resolving power and correction of CCD imperfections are also very important to the scientist

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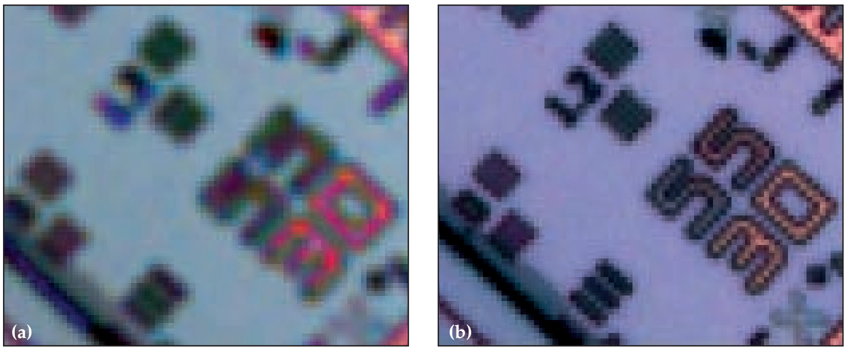

Fig. 2 — Image collected using a single shot sampling (a), compared to the same image collected using a three-shot sampling (b).

when capturing and analyzing images, but in this article we will concentrate our attention on the color capture method.

Diagnostic Instruments, although not the inventor of the three-shot method with an LCD filter, has perfected and patented the integration of a sliding LCD filter into our digital cameras. This LCD slider provides the ability to capture more-sensitive monochrome images as well as the ability to slide the LCD filter into the light path to create highly resolved color images.

Elements of sensor resolution

The pixel is the smallest physical imaging unit on an imaging sensor, and consists of an active light-sensing region (the photodiode) and an area for signal processing. The aperture of the pixel element collects electrons that have been energized and thereby "kicked" into the pixel's potential well by incident light photons that strike the silicon chip during exposure.

The digitization process then assigns a number to each pixel proportional to the intensity of the light to which it was exposed. In a non-color imaging system, the pixel also represents the smallest *resolving* element of the system (excluding any optical system degradation). Note that an electron kicked into the well by a green 550 nm photon looks no different to the digitizer than an electron kicked into the well by a blue 450 nm photon.

In digital RGB color camera systems, the color of the light is determined by sampling light intensity in three broad bands of the visible spectrum: red (~530 to 700 nm), green (~460 to 600 nm) and blue (~400 to 500 nm). The color RGB digital image then consists of an array of data, with each pixel containing RGB values proportional to the red, green, and blue color intensities measured at that specific pixel location.

The next step is to convert this data into an image. As previously stated, the two most common methods for generating color images are the single-shot color mosaic sampling and the three-shot color sampling.

Single-shot color mosaic sampling

This method of color sampling is based on physically attaching a Bayer mask (a filter containing red, green, and blue pixel-sized regions) directly to the CCD so that each pixel is then able to receive only red, green, or blue light from the sample. The filters are most commonly applied in a repeating, four-pixel element called a Bayer Filter Pattern, as seen in Fig. 1.

To create a color image, a single exposure is taken, resulting in the sampling of only the red, green, or blue intensity at each pixel location. For each pixel, the two non-sampled colors are then found by interpolation with the adjacent pixels.

To construct a color RGB image from this sampling method, 66% of the intensity values must be calculated, which means two out of three colors at each pixel. Also note that the 2 x 2 Bayer Filter Pattern is the key resolving element that was used to sample the image (i.e. two green pixels, one red, one blue).

The result is that an image captured by a 2048 x 2048 pixel sensor actually has a spatial resolution of only 1024 x 1024 pixels.

How does this happen?

To explain this result, envision that we create a small ray of red light that falls entirely onto one red pixel with no other light falling on the sensor. What will the image look like? First, the red pixel that the red ray struck will very accurately record its intensity value, and the eight surrounding pixels that received no red light will each register a small value of red light due to interpolation. The resulting image will show red on all nine pixels, when in reality the actual size of the red area was only one pixel.

To illustrate this point in an even more detrimental manner, consider that if the one pixel-wide red ray were cast onto a blue- or green-masked pixel, the resulting image would show nothing!

Other artifacts also result from this sampling method. Thin white lines and extreme brightness transition edges in images can appear to have color stripes due to sampling and interpolation errors (Fig. 2a).

Three-shot color sampling

The other common method of color sampling is to position a variable color filter (such as

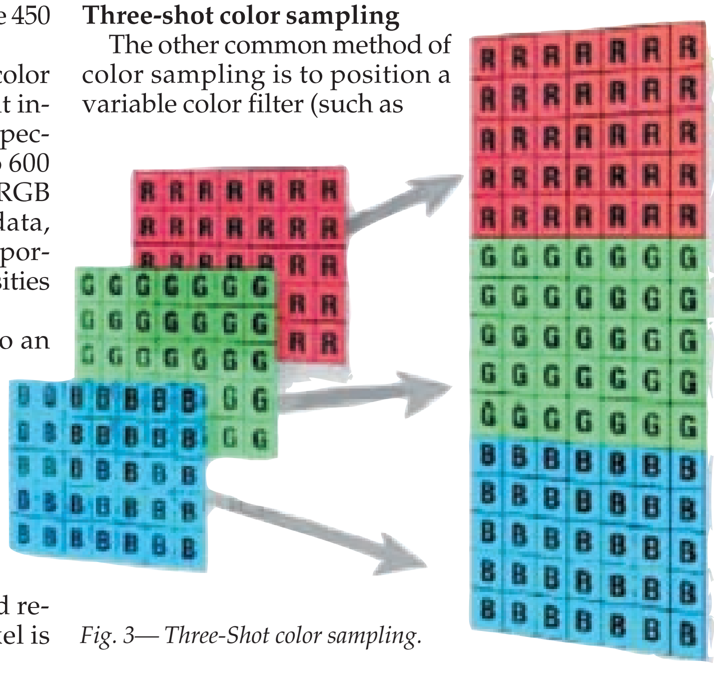

Fig. 3— Three-Shot color sampling.

liquid crystal) in front of the sensor, then sequentially capture a red, green, and blue image. The three images are then combined pixel by pixel to provide an RGB-sampled color at each pixel location (Fig. 3).

Because each color is sampled at each pixel, the resolving element of the system is the pixel, making the stated resolution of the system equal to the resolution of the image sensor. This means that an image captured by a 2048 x 2048 sensor maintains its 2048 x 2048 resolution. Also note that the size of the saved file is no larger than a one-shot file, it just contains data that has been more accurately measured. The results are notably improved, as shown when comparing Fig. 2a and 2b. The colors are more accurate and the edges of high-contrast features are more evident due to the absence of interpolation.

One drawback to this method is that it takes longer to collect the “three-shots,” because each color must be exposed individually. As a result, if the image is changing position with time, the sequential image capture will produce an image with red, green, and blue ghosts of your subject as it moves across the scene. Another possible concern is the time interval for image exposure and capture, as this method takes three times as long. Therefore, if time is an issue with a single shot, it will be more so with the three-shot method.

Match technology to the application

As with any situation, the appropriate solution depends on your needs. If you have moving samples or need high throughput, then single-shot color mosaic cameras would be most appropriate. If your sample is fixed and you have additional time, then you will benefit from the improved resolution and color accuracy of the three-shot color cameras.

Now let’s look at an example demonstrating submicron measurement of features in images captured with the three-shot color sampling method. If a single shot CCD (color mosaic image) had been used instead, the spatial measurements would not have been as precise.

Imaging 200-micron holes

This imaging application from Dynamic Structures and Materials LLC was aimed at evaluating the practical limits and challenges of imaging holes with diameters of 100 to 200 micrometers. They were machined by a proprietary method into an Inconel plate having a thickness of 1/8 in. (3.2 mm), shown in Fig. 4. Of particular concern was the need for images displaying high contrast, high resolution, and good depth of field, so that subsequent measurements of hole diameter and roundness with a tolerance of not greater than 1% deviation could be made. These imaging concerns were best addressed with a three-shot camera, because it provides higher resolution and greater contrast, especially at edges, because it needs no interpolation. The CCD also provided a superior signal-to-noise ratio, due to -32°C (-26°F) ambient cooling.

The CCD-microscope combination consisted of a two-megapixel SPOT RT camera attached to an Olympus BH2 reflected/transmitted light microscope. To achieve the most accurate measurements, the image contrast and resolution were compared by a variety of techniques: differential interference contrast, brightfield, and darkfield.

Results showed that although the overall contrast of the hole was highest when struck simul-

Fig. 4 — Inconel plate with seven machined holes is shown with a low magnification overview. The image was collected using simultaneous transmitted and reflected illumination.

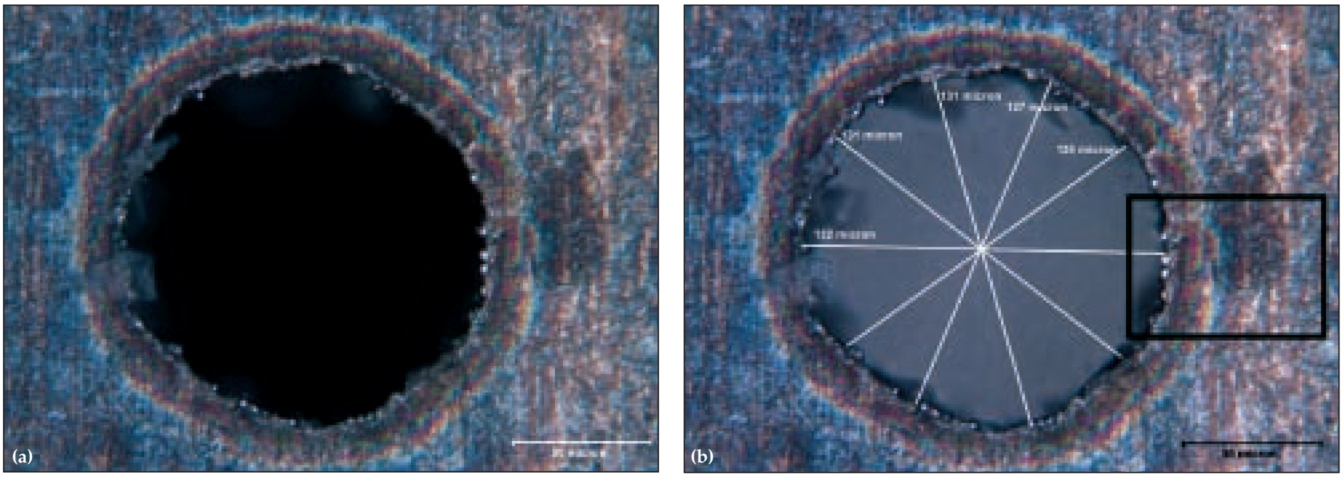

Fig. 5 — Hole in Inconel plate has the same fields of view, but is collected at 400X magnification. The image of reflected light only is shown at (a), and the image of both reflected and transmitted light are shown at (b). Several diameter measurements have been added. The metal debris attached along the perimeter has greater contrast in (a). This debris has a detrimental effect with regards to segmentation of the hole. The outlined area in (b) is shown in Fig. 7.

Summary of metrologic qualities of the holes measured in Fig. 5

Image	Area, µm^2	Perimeter	Major ellipse axis	Minor ellipse axis	Roundness	Maximum diameter
Figure 5A	12477	463.4	128.7	123.8	1.37	128.7
Figure 5B	12466	441.5	128.6	123.7	1.25	132.4

(Spot 4.5; Image Pro Plus 5.0.)

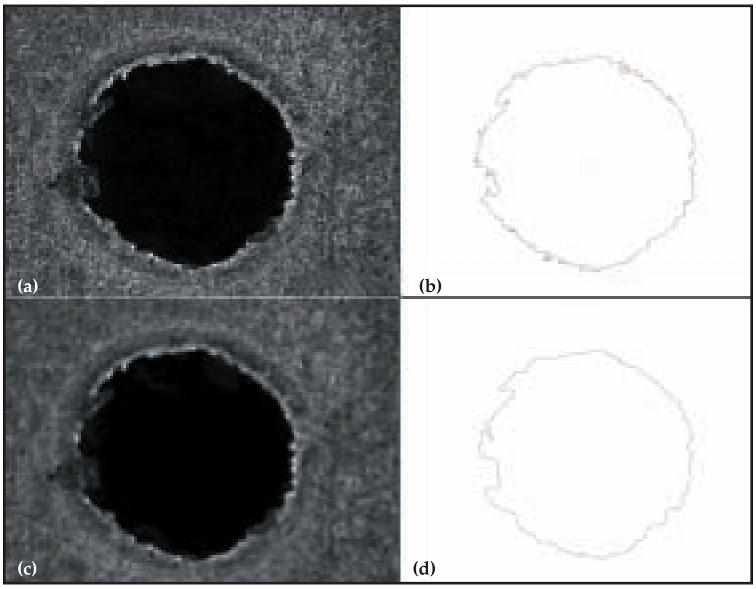

Fig. 6 — Same image as Fig. 5a is shown after an 'edge detection' filter in (a) and 'Gaussian blur' in (c). An outline of these areas is measured in (b) and (d). Note that the perimeter of the area analyzed in (d) is much smoother than in (b). (see Table).

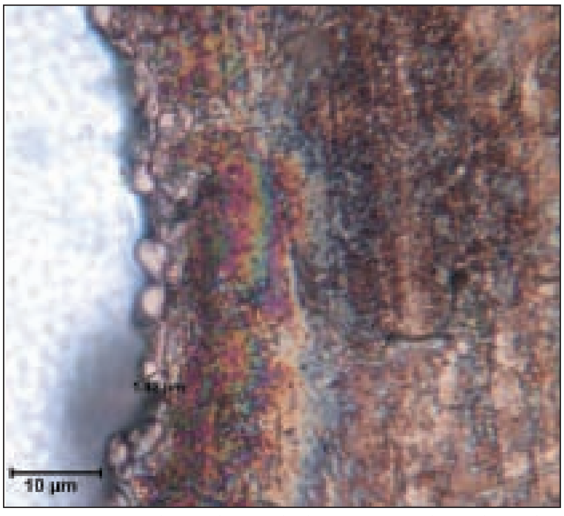

Fig. 7 — High-resolution light micrograph made with a 100X, 0.9 N.A. Plan Apo objective. See corresponding area outlined in Fig. 5.

taneously with both reflected and transmitted light, the contrast at the hole's perimeter was highest when only reflected light was used, as shown in Fig. 5a and 5b. This difference was caused by less light scattering at the hole's perimeter, which caused an unacceptable level of either erosion or dilation during the image processing steps. Manual linear measurements of the hole's diameter, made using the SPOT Advanced software, provided a good means to qualitatively visualize the roundness (Fig. 5b).

For the more detailed measurements of the hole by segmentation (such as thresholding), it was necessary to compare the results from the same image subjected to different image-processing steps. This was necessary because some of the perimeter associated with the jagged edges of the hole was actually caused by the presence of adhering machined debris.

Figure 6 shows results for two differently processed images, one by edge-detect filter and the other by Gaussian blur. This shows how the results from image analysis (including hole-diameter, perimeter, roundness) may vary depending on the image processing method (Table 1). More advanced measurements from Image-Pro Plus 5.0 show that the measurements of area and the major and minor ellipse axes are within the required tolerance.

However, the roundness, perimeter, and maximum diameter have greater than 1% variability. In this application, the CCD and microscope optics provided the necessary resolution, but the adhering metal blocked its ability to locate the exact edge of the hole by segmentation. Figure 7 demonstrates the high resolution attainable for an optimally prepared region of the hole. The grain labeled in this figure is only 1.4 micrometers wide.

Relevant considerations

When selecting a digital camera for life science or industrial research, it is important to consider its color capture method, as it allows the user to obtain images with the most accurate spatial measurements and best color accuracy. Although brief, the metal-plate example nicely illustrates many of the relevant characteristics involved in making extremely accurate measurements from digital images. These include, but are not limited to, the type of sampling (3-Shot vs. 1-Shot), CCD resolution, microscope optics, microscope contrast technique and illumination, sample preparation, and image processing.

Although it was not a task in the present example, the three-shot method provides superior results for quantitative analysis of color or shade. Areas of application include analysis of thin-film interference, forensic comparisons, automatic analysis of grain orientation, particle size analysis, and reflectivity measurements. ◆