

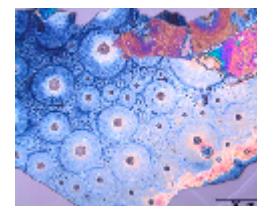
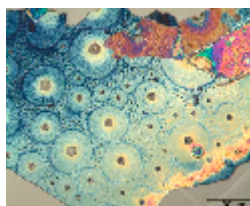
A Digital “Don’t” for Quality Photomicrography
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Product contamination is rare in the highly regulated pharmaceutical industry. However, when contamination does occur, it is a threat to both the consumer and company’s reputation. Pharmaceutical companies need every available resource to fully understand the problem and avoid future mishaps.

To this end, many companies are increasingly turning to digital photomicrography techniques to help document contamination issues. Digital photomicrography is the process of taking digital photographs of a sample and/or its contaminant during inspection using a microscope. This type of imaging is attractive because it visually supports and/or validates the microscopist’s findings and helps visually convey conditions seen with the microscope, which may be difficult to understand in a written report.

However, digital images are easy to alter in attempt to make the contaminant more pronounced or accent certain elements in the sample. While scientists depend on technology to advance their work every day, using photomicrography techniques in this way can be detrimental to end goals. When images are manipulated, important information may be lost or altered from the original sample and microscopists lose the ability to integrate these images with standards from past experiments. Moreover, digitally doctoring images after they have been captured reduces the validity of the scientific data collected during the microanalytical process. Without full confidence in the data, pharmaceutical companies risk missing important information that could help them identify the contamination and ensure the purity of the product.

This article describes, from practical experience, the importance of properly capturing images of microscopic pharmaceutical contamination without using image editing or manipulation software as a secondary process. This work can be accomplished with a quality compound or stereomicroscope with an attached digital camera and skills in sample preparation and microscopy.



Photomicrographs of a glass delamination flake from a pharmaceutical vial at 500X magnification.

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High-quality digital photomicrography is dependent on three factors: the quality of the equipment used (microscope, camera, lighting); precise sample preparation; and, most importantly, the skills of the microscopist.

First, the quality and condition of the microscope plays a key role in capturing quality images. Two types of light microscopes are ideal for the analysis of pharmaceutical contaminants. A low-magnification stereomicroscope can be used for sample preparation and initial visual examination of the sample. A compound polarized light microscope (PLM) provides higher magnifications used for the identification and characterization of particles. Both microscope types must be cleaned and properly aligned. The PLM should preferably be set up to achieve Köhler-type illumination, a method of providing optimum specimen illumination.

(A) This image represents a view through the microscope eyepiece. The microscope is properly aligned, illumination is even, and the camera is properly white-balanced for viewing the sample.

(B) Image captured with the same equipment that is slightly out of focus, has uneven microscope illumination, and an improperly set camera.

Equipment is important in all aspects of microanalytical analysis, especially in photomicrography because capturing images of microscopic contamination necessitates high magnification, high numerical aperture, and high-resolution cameras. Every lens within the microscope and camera apparatus must be particle free. Even minute particles of dust or dirt will blur and obscure the resulting image.

Similarly, proper sample preparation is critical in all forms of microanalyses, especially when photo-documenting a sample. As previously mentioned, a sample that contains contamination may first be examined with a stereomicroscope. If the particle is large enough, it can be removed manually with a tungsten needle and mounted on an appropriate substrate for further analysis.

The cleanliness of the slide, coverglass, instruments, and mounting media are all critical to the outcome of the captured image. Like dust on lens surfaces, foreign debris or air bubbles introduced during sample preparation will ruin or create an eyesore in the digital image taken with the microscope. More importantly, foreign particulate matter introduced during sample preparation could resemble contamination, causing confusion and erroneous conclusions.

Sample preparations performed in a cleanroom helps to ensure that cross-contamination does not occur during the isolation or sample preparation process. Cleanroom classification is based on the number of particles in the air. For example, in a Class 100 (ISO 5) cleanroom environment, there are no more than 100 particles per cubic foot of air $\geq 0.5 \mu\text{m}$. By comparison, a regular laboratory environment has millions of particles in an equal volume of air.

Once the equipment is checked and sample preparation is complete, the last step in procuring high-quality imaging results is applying correct photomicrographic techniques. Microscopists first turn the microscope on and adjust the rheostat to the optimum color

temperature and intensity. The compound microscope needs to be configured for Köhler illumination. Köhler illumination provides the maximum amount of even illumination from inhomogeneous light sources. Additional light sources, such as coaxial illumination or oblique illumination, may be necessary depending on the sample type.

The last and most critical setting is the proper adjustment of the aperture diaphragm. The aperture diaphragm controls the resolving power, the depth of field, and the contrast. Again, proper microscope use is critical, and will dictate the quality of the images captured.

Once a perfect image—as seen looking in the microscope—the camera settings should be adjusted to duplicate that view on the monitor. Most digital cameras used with microscopes are operated via software that controls the camera settings and functionality to capture images. Typically, four camera settings in software may need adjustment prior to capturing an image.

White balance shifts the image sensor to adjust to the color temperature of light through the microscope. Exposure or camera “aperture” setting can be adjusted to control the amount of light reaching the image sensor. Saturation settings help control the color intensity of the image. Contrast adjusts the tones in the image and can be used to match the adjustments made with the aperture diaphragm.

Again, these settings should be adjusted in an effort to match what is seen through the microscope eyepiece with the preview or “live” view in the software just prior to capturing the image.

The main goal when capturing images through the microscope is to achieve an image that accurately represents the view through the eyepieces. Good quality control allows for the detection and photo-documentation of any contamination found within a product. It also builds a better understanding between the microscopist and quality inspectors.

Image editing and manipulation software should never be used to compensate for bad microscopy. Instead, using the right equipment, color-balanced illumination, and good techniques will ensure quality images. Accurate images of the contamination provide a better understanding of the problem and ultimately help the company take the appropriate steps to correct the problem.

About the Author

Kristen Partin teaches courses at the College of Microscopy including Particle Isolation, Manipulation, and Mounting; and Polymers, Paints, and Coatings.

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