Pharmaceutical Compliance Applications of Optical Microscopy

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“Compliance Methods” discusses analytical methods, approaches, and techniques useful to practitioners in compliance and validation. It presents new technology, less common methods, or novel approaches using well-known techniques. We intend this column to be a useful resource for daily work applications.

This installment discusses optical microscopy. Optical microscopy is often a good first step for characterizing pharmaceutical raw materials, investigating production problems, and identifying extraneous matter. Optical microscopy is a well-accepted technique in all sciences, and with training, a wealth of information can be obtained from the microscope. A description of the basic types of optical microscopes, information that can be obtained from optical microscopes, and their applications in pharmaceutical compliance applications are discussed.

Reader comments, questions, and suggestions are requested. Manuscripts or case studies submitted by readers illustrating analytical applications in compliance are also most welcome. Please send your comments and suggestions to column coordinator Paul Pluta at paul.pluta@comcast.net or to coordinating editor Susan Haigney at shaigney@advanstar.com.

KEY POINTS DISCUSSED

The following key points are discussed:

- Optical microscopy provides insight to many compliance applications. Optical microscopes can provide characterization data for many pharmaceutical materials. With training and appropriate standards, the user of an optical microscope can provide valuable information and analysis in compliance activities, such as the identification of many materials including common extraneous and particulate matter.
- Two basic microscopes are best suited for GXP work: Stereomicroscopes and compound microscopes configured with polarizers (polarized light microscopes). Similarities and differences between the two microscope types are explained.
- High quality microscopy data is well accepted, easy to understand, and can be shared with colleagues. Comprehensive training can maximize the data obtained from a microscope and avoid misinterpretation. A picture is worth a thousand words.
COMPLIANCE METHODS

INTRODUCTION
Since 1674 when Anton Van Leeuwenhoek invented the optical microscope, it has been used to study sub-visual particulate. Optical microscopes are a common laboratory tool in many scientific disciplines. Optical microscopy (OM) is accepted by the United States Pharmacopeia (USP) for particle characterization (1) and recognized as the tool of choice for characterizing and identifying particulate matter in medical products (2), parenterals (3), and pharmaceutical production (4,5,6).

While basic training is essential to properly operate an optical microscope, additional technical training will also allow the analyst to maximize quality and types of data that can be obtained from a microscope and avoid misinterpretation. Training is readily available (7, 8) and straightforward. Most microscopes have simple controls and can produce data from properly prepared samples in minutes. Sample preparation is also simple, and in some circumstances, not needed.

One common use of optical microscopy in quality control is to examine liquid dosage forms for undisolved particles of the active pharmaceutical ingredient (API). Another quality control application is to compare particle characteristics of incoming material, for lot-to-lot variation. Another important use is in the characterization of extraneous matter (EM) or particulate matter. Ultimately, OM can identify EM in selected cases or when good reference materials are available.

Microscopic images show a situation with great clarity. They can show details beyond the resolution of the human eye and add optical data not available to the unaided eye. This combination makes optical data easy to understand by production personnel, scientists, managers, and regulators.

This article provides a brief overview of the two basic types of light microscopes, reviews the types of information that can be gained, and shows examples of applications of OM in pharmaceutical compliance.

INSTRUMENTATION
There are two basic types of optical microscopes: Stereomicroscopes and compound microscopes.

Both use visible light to create highly magnified images of a sample. They provide complimentary information and should be used together whenever possible. Both types of microscopes are typically coupled with a high quality digital camera to aid in the documentation of images. Digital microscope cameras are varied, and the technology is changing rapidly.

Stereomicroscopes
The stereomicroscope, also known as the dissecting scope or macroscope, has two distinct images formed in each binocular that are separated by ~14-16°. Each image is generated in separate eyepieces, giving a true three-dimensional perspective. Stereomicroscopes also have the advantage over compound microscopes of forming an image with the same orientation as the sample.

Stereomicroscopes have long focal lengths that can accommodate relatively large samples, such as whole tablets, blister packs, small instrument parts, o-rings, and gaskets. Stereomicroscopes can have fixed magnifications or variable and zoom magnifications. The magnification can range from 2X to approximately 100X. Controls for a stereomicroscope consist of magnification, focus adjustment, and brightness control via an aperture control. If a camera port is available, then a selector to deflect light to an attached camera would also be available.

Lighting for stereomicroscopes can come from several sources. The most accommodating of all sources is a simple gooseneck/fiber optic external source. The brightness and angle of illumination can be easily adjusted by simply moving the fiber light sources closer or further away form the sample. Using low angle illumination from one of these sources can easily accentuate small particles or rough textures. Unlike gooseneck sources, ring lights that are attached to the objective lens can provide uniform illumination across a sample. Stereomicroscopes with illumination in the base can also be beneficial for viewing translucent or transparent samples. Some stereomicroscopes also have interchangeable objective lenses allowing a greater range of magnification.

Because of the large focal length and the proper orientation of the image from stereomicroscopes,
these instruments lend themselves to sample preparation for other types of microanalysis including compound light microscopy, scanning electron microscopy, and Fourier-transform infrared microscopy. With the right tools, layers can be removed from tablets, extraneous particles can be isolated from solids or solutions, and samples can be cut or sliced to smaller sizes. All this can be done while viewing under higher magnification.

**Compound Light Microscopes**

Compound microscopes create images with a complex collection of lenses, apertures, and filters. Objective lenses are mounted on turret that can be rotated to bring different objective lenses into the optical path, thus changing magnification. Compound microscopes have built-in light sources and condenser lenses to focus the light source. This configuration results in higher resolution and higher magnifications than is obtainable with a stereomicroscope, but at an added cost for the instrument.

Operating a compound light microscope requires greater training and skill than operating a stereomicroscope. To start, most compound microscopes produce inverted images (i.e., image is upside down and reversed right to left). Image brightness, contrast, color temperature of the light source (e.g., yellow, white, or blue tinted light), magnification, and focus are all interconnected in a compound light microscope. Image brightness and contrast can be affected with filters, apertures, and by adjusting the light intensity. Magnification is changed by selecting different objective lens. Focus is changed with coarse and fine focus knobs. Light color temperature is affected by the intensity of the light source and by in-line filters. Having optical color temperature is important because the color of the illuminating light will affect the final color of the sample. Often, changing one condition will affect the other conditions. A well trained analyst can ensure all the lenses and apertures are properly aligned to produce an optimal image.

Samples must be prepared to be thin enough to allow light to travel through them. Most powders can be dispersed in a suspending medium such as mineral oil. Small fibers may be prepared in a similar fashion. Larger samples may need to be sectioned by hand or with a microtome.

While a compound microscope is more expensive and difficult to use, the benefits are well worth the expense and additional training. A compound microscope configured with polarizing filters is referred to as a polarized light microscope (PLM) and is the most practical configuration for most compliance activities. PLMs can determine the optical properties of examined samples such as refractive indices and birefringence. In practical terms, the PLM can characterize crystallinity, particle size and shape of APIs and excipients, differentiate polymorphs, identify most fibers, and aid in the identification of most extraneous matter. PLMs can identify many of the components in a blend and track changes throughout a production process.

While PLMs can provide higher resolution and additional information than a stereomicroscope, the two instruments work best when used together. A stereomicroscope can provide the big picture and a PLM can provide the fine details. For example, a stereomicroscope can show the presence of EM in a tablet, and the PLM can show the details of the EM that can be used to support the identification of EM.

**Digital Microscope Cameras**

The technology for optical microscopes has been around for hundreds of years. However, the technology for digital cameras is relatively new and changing at an amazing rate. State-of-the-art cameras will be replaced by more advanced models in as little as 12-18 months. Digital cameras designed for microscope work can be attached to most microscopes with a camera port. The cameras require a relay or coupling lens that is placed in the light path between the microscope and the camera. This lens will affect the final magnification of the digital image and should be chosen to match the camera image with the field of view seen through the oculars.

Microscope camera resolution, like consumer cameras, is measured in megapixels. The ultimate image resolution is measured by the field of view.
divided by the number of pixels across the field of view. Generally, a three- or four-megapixel format is acceptable for most documentation. Larger formats may be needed for enlargements of samples with fine detail.

Digital cameras attach to a computer through either a USB port or a Firewire (IEEE 1394b) connection, the latter being a faster connection. Software to control the camera and acquire and save images is included with most cameras. The software should be intuitive and allow for rapid and accurate acquisition and saving of digital images. Image formats should include TIFF format, which is an uncompressed format resulting in no loss of image detail. JPEG format is a compressed format and will result in the loss of some of the original image detail. A white balance control is essential to match the color of the digital image with that seen through the oculars.

**MICROSCOPE DATA**
The information obtained from an optical microscope varies by the type of microscope and the level of training by the analyst. The USP defines many optical microscope parameters for particle size measurements, shape descriptors, aggregation states, particle conditions (e.g., fractured, transparency, color), and surface/texture characteristics (1).

Performing simple tests on a microscope slide can greatly expand the amount of information obtained from a sample. Adding drops of water or solvent to a sample can determine the solubility of the sample. Immersing material in a refractive index liquid can identify the refractive index of the material by the Becke Test (10). Performing simple chemical reactions on a sample on a microscope slide can identify the chemical composition of many materials (11). The types of information available from each type of microscope are summarized in the Table.

**OPTICAL MICROSCOPY APPLICATIONS IN COMPLIANCE SITUATIONS**
There are numerous applications of optical microscopy in compliance. Four general applications are discussed in the following sections. Figures illustrating these applications are provided.

**Characterization of Raw Materials**
Optical microscopy offers a quick and efficient method to characterize powder materials. Sample prep involves collecting a representative sample, suspending the material in an insoluble medium, and placing the suspension on a microscope slide with a coverslip. Images can be quickly saved from a PLM and compared to library or reference files. Variations in particle shape and size that may affect processing, and the true particle size can be observed (see Figure 1).

**Characterization of Early Development API**
Particle size, shape, crystallinity, and aggregation are important parameters, in all development stages. In this scenario, particles from a representative sample are suspended in a suspending medium and images collected with a PLM. Size measurements can be made by comparing the particle images to a certified stage micrometer collected under the same conditions. Quick evaluations of largest, smallest, and typical particles can be made. It should be noted that due to the small sample size, this method is not statistically significant, but is generally useful for early development of API monitoring (see Figure 2).

**Investigation of Production Issues**
Processing issues can be varied, and their solutions may be complex. No single technique can identify the problem in all situations. Optical microscopy should be considered as a first approach to get a better look at the problem and to determine the next steps in the investigation. Changes in particle habit such as rounding can indicate an overheating problem. Irregular distribution of components in a granulation can indicate an improper mixing problem or an attraction between two components. Changes in crystal habit have shown a conversion of an API to a different polymorph. This was the case for ritonavir where a polymorphic conversion was first detected by PLM (12) (see Figure 3).

**Extraneous Matter Investigation**
Extraneous matter, sometimes referred to particulate matter, generally comes from two sources: Environmental contaminants and process related contaminants.
Environmental sources of EM can include paper, wood, plant fibers, insects, and minerals. Process related EM can include equipment wear particles from metal parts, gaskets, or o-rings lubricants, personal protective equipment, paint coatings, charred product, and portions of product containers.

One of the most popular uses of optical microscopy in compliance applications is the investigation and identification of extraneous or particulate matter. OM is almost always the best tool to start an EM investigation. Simple characteristics of extraneous matter such as color, shape, and opacity can direct an investigation toward environmental sources or toward process related contaminants. Additional analysis by other analytical techniques such as mid-infrared spectroscopy (13) and energy dispersive X-ray spectroscopy can be used to confirm identity of the material. However, complete characterization of the EM optical properties with a PLM can often lead to complete identification without additional testing. This is especially true for fibrous materials. Figure 4 shows examples of paper, cotton, and polyester fibers. Their unique morphology allows for easy recognition and identification. Large libraries are available to compare optical properties of EM with common contaminants (14, 15).

**CONCLUSIONS**

Optical microscopy is a well established technique that can be valuable for many compliance applica-

<table>
<thead>
<tr>
<th>Information</th>
<th>Stereomicroscope</th>
<th>Compound Light Microscope</th>
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<tr>
<td>Shape or habit</td>
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<td>Refractive indices</td>
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<tr>
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**Figure 1:**
Two lots of lactose with different particle characteristics viewed by PLM. The left lot is composed of larger single crystals as shown by the uniform coloration, and the right lot has polycrystalline particles as shown by the non-uniform coloration.
Compliance Methods

Examining materials or problems with an optical microscope can provide insight into many production related activities. Microscopy data of powders can confirm consistency in incoming product. Microscopy data can help elucidate production issues when production processes go out of specification. Optical microscopy is indispensable when investigating sources of extraneous matter.

Optical microscopes are straightforward to operate but do require some additional training to maximize the quality and types of information obtained and understand the wealth of data available from these systems. This training is readily available. Well documented, high-quality optical micrographs are easy to explain and share with colleagues. Reference microscopy data are relatively plentiful from many sources. A picture is often worth a thousand words, and reliable optical microscopy data can quickly explain many GXP compliance issues.

References
1. US Pharmacopeia, USP31-NF26 (official 12/1/08-5/01/09) general chapter <776> Optical Microscopy.
Figure 4:
Examples of paper, cotton, and polyester fibers viewed with a PLM. Paper consists of tapered, tattered fibers, cotton has unique twisted fibers and the pastel colors of the polyester fibers indicate the high birefringence that is characteristic of polyester.


**ARTICLE ACRONYM LISTING**

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>API</td>
<td>Active Pharmaceutical Ingredient</td>
</tr>
<tr>
<td>EM</td>
<td>Extraneous Matter</td>
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<tr>
<td>OM</td>
<td>Optimal Microscopy</td>
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<tr>
<td>PLM</td>
<td>Polarized Light Microscope</td>
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<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
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**ABOUT THE AUTHOR**

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