



A Powerful Vision

Comparison Illumination

Part 2: Achieving the Best Illumination for Your Comparison Microscope

By Wayne Buttermore, *Leica Product Manager, Forensic Microscopy*

In the last issue of *The Leading Investigator*, we reviewed the many variables that affect color and image intensity while viewing specimens through a comparison microscope. We learned the process for matching color balance and light intensity for the two microscopes from side to side in a systematic, reproducible manner. For Part 2 of this topic, we will discuss several functions to ensure that the image you document through a digital camera will faithfully represent what is seen through the microscope's eyepieces.

To begin with, we know that visually setting up the comparison microscope so that color and intensity match is much easier for the human eye to accomplish, than it is for a camera. Keep in mind that the eye is poor at perceiving small differences in intensity; the brain allows the eye to be a superior imaging device by interpreting color and spatial information in real time, compensating automatically, and ultimately making the eye sensitive enough to match the two images.

One of the challenges of comparison microscopy is that the eye may perceive a perfectly balanced image, but the camera shows disparity from the left to right side. The camera has a much higher sensitivity to small differences in intensity, and those differences are amplified when the image appears on the monitor.

The phenomenon of 'shading error' acknowledges that a microscope's field of view will lack homogeneity in terms of illumination. The problem is best exemplified at very low magnifications when the center of the field of view appears to be brighter than the perimeter of the image. This classic hot spot is caused by the lamp's filament. For a camera, this shading error is observable at all magnifications. With comparison microscopy, this condition may exist on both microscopes, which further complicates the image acquisition process.

How do we deal with shading error?

Using a camera and software system that are specifically designed for microscopy compensate for these normal illumination inconsistencies. One way to alleviate the error is to use the software's **Shading Correction** function. In simple terms, shading correction is a mathematic algorithm that analyzes the value of adjacent pixels, and then equalizes their values across the entire field of view. *Note:* As previously mentioned, the lamp filament is the single most causal element responsible for creating a shading error. Therefore, if the voltage (intensity) is changed, a new shading correction should be applied.

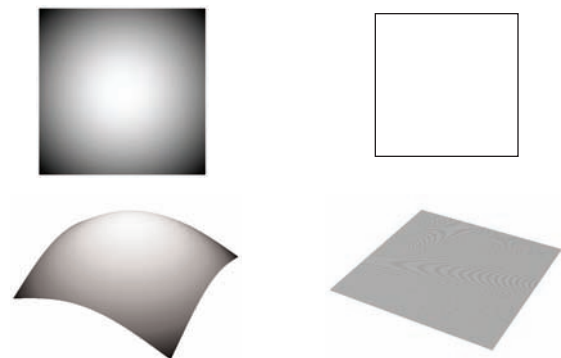


Figure 1. Left image depicts 50% shading, while the right image depicts 0% shading.

Leica LAS software and DFC camera drivers provide the ability to apply and save a shading correction value for both live images and for stored images at each magnification available on

Contents

A Powerful Vision.....	page 1
LED Illumination.....	page 3
Tips and Tricks.....	page 4
Industry News.....	page 5
Glossary.....	page 5

continued on page 2

A Powerful Vision

continued from page 1

your microscope. When you select **Apply to a Live**, you can see the results before the image is captured, thus ensuring that the image is a faithful representation of what is seen through the microscope.

To use the Shading Correction Function:

1. Set the lamp intensity to a comfortable and reproducible level.

If you are using the Leica KL2500 LCD cold light source, reproduce the intensity by setting the LCD readout to 3200K. If this is too bright for the objective, use the middle knob to reduce intensity without changing the voltage to the filament. The detents (A through E) on the adjustment knob provide reproducible set points.



Figure 2. Illumination controls.

2. Make sure the microscope is set up properly for visual examination of the specimen (as discussed in the last issue).
3. Remove the specimen from the optical path so the white light from the illuminator is projected to the objective.
4. In the LAS software, select the **Processing** window; select **Shading Correction**; and then select **Create a Shading Reference**.
5. A new window will open, which allows you to name the **Reference**. It is recommended to use a name that represents the microscope setting.



Figure 3. Leica LAS software window.

6. Repeat this process for each objective and magnification changer position available on the microscope.

In conclusion, once the intensity is balanced and presented to the camera, you can be confident that the captured image will be representative of the live image seen by the eye through the microscope.



Figure 4. Blank live image before (top), and after (bottom) shading correction is applied.



LED Illumination

Adding LED Illumination to Your Comparison Microscope

by Wayne Buttermore, *Leica Marketing Manager, Forensic Microscopy*

Historically, illumination systems for stereomicroscopes and comparison macroscopes have been based on fiber optic, halogen or fluorescence tube illumination. And now, recent advances in illumination technology allow the utilization of LEDs to create different kinds of illumination that are variable in intensity and have incredibly long life. An LED does not change color throughout its entire lifetime, nor when different levels of voltage are applied, which ensures uniform, consistent color and intensity. And, there is economy of operation – you do not have to replace bulbs every 200 -1000 hours.

LEDs are available in many forms. They can be placed into a single linear array to create a very direct light source; form a ring light; or comprise a single 3W or multi element LED spot illuminator.

Those with microscopes/macrosopes can easily add LED illumination to an existing system. An LED light source can also be configured with a new instrument. Compared to fiber optic systems in particular, LEDs are very affordable. For example, two LED spot illuminators, a controller for both illuminators, and a power supply for a comparison microscope system costs about 1/3 the cost of a comparable fiber optic. An individual LED ring light for a stereomicroscope along with a controller costs about 1/2 the cost of a fiber optic.

Creating optimum illumination for a sample with a ring light is very convenient. One advantage for macroscopy is that a ring light that slips around the macroscope's objectives is very small. For stereomicroscopes, the ring light is larger and is set to the proper diameter, depending on the microscope. Either a 58mm or 66mm interface is appropriate for the Leica S-series or M-series stereomicroscopes. Using the controller and a Visi LED configuration, you can program which of the elements are turned on and create contrast in the sample by rotating or changing the elements that are turned off without moving the sample or the light source.

The average half-life of an LED system is 20,000 hours, meaning that the LED generates half the original light intensity at this point. LEDs are available in a variety of different colors. In fact, many traffic signals now feature LEDs instead of single bulb with a lens cover.

LED is a new technology that provides a great way to illuminate samples. There are many advantages and the technology applies to a wide variety of instrumentation. It is worth checking out.



Figure 1: Spot LED mounted on a comparison microscope



Figure 2: LED ring light mounted on macro objective



Tips and Tricks

How to Quickly Reinitialize Comparison Microscope Stages

Occasionally, the stages on the Leica FS C comparison microscope lose their reference points. This can happen if the instrument is moved or a sudden heavy pressure on the stage occurs; it may be necessary to reinitialize the stage.

How can I tell if the microscope stages have lost their reference points?

During normal operation, if you press the mixed button (MIX) and the full right image button (R) and release them simultaneously, the stages will move to the middle of the Z travel range on both sides. Performing this function is particularly helpful when changing samples, to allow an equal amount of travel on both stages, and it is especially helpful when the new sample height is much larger.

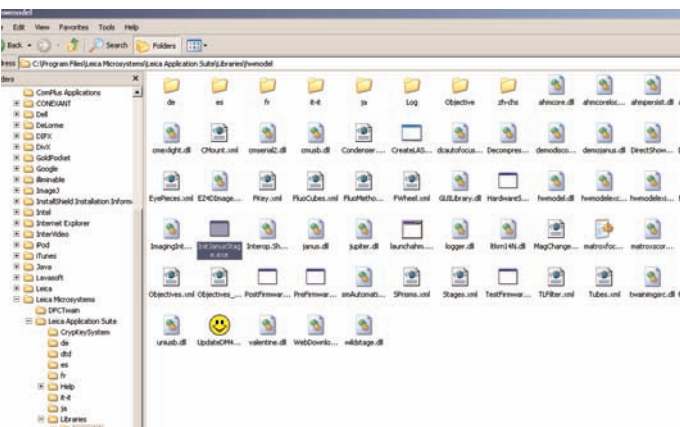
If you press and release the mixed button and the full right image button, but the stages do not move to the middle position, the stages should be re-initialized.

How to re-initialize the stages:

Leica's documented way to update stage initialization is through the Leica LAS software. The firmware update routine is found in the OPTION top level menu. However, there is an undocumented shortcut that allows the initialization to occur without opening Leica LAS and/or the firmware's software.

The shortcut:

This shortcut allows direct access to stage initialization.



caption

Open **Windows Explorer** or **My Computer** window

1. Select the **c:** drive
2. Select the folder to the following path: **C:\Program Files\Leica Microsystems\Leica Application Suite\Libraries\hwmodel**
3. Left click once on the file name (**InitJanusStage.exe**), right click,

and select **Create a Shortcut**, which will create a new icon in the directory you are in. Click on **Shortcut**, and drag the icon to your desktop.

Whenever you want to initialize the stages, simply double click the shortcut on your desktop and the initialization routine will begin. Each stage will travel to the minimum and maximum limits while the tracking bar is displayed on the screen. Once the initialization is complete (about 1 minute), close the window on your desktop.



Industry News

The annual meeting of the **MAAFS (Mid-Atlantic Association of Forensic Scientists)** will be held at the Hyatt Regency Washington on Capitol Hill in Washington DC, May 23-25, 2007. The program includes Chemically Prepared Toners (CPTs), The John Walker Spy Case, and much more.

More information: www.maafs.org

The **38th annual AFTE (Association of Firearm and Toolmark Examiners)** Training Seminar will take place at the Hyatt Regency, San Francisco, on May 27-June 1, 2007. AFTE welcomes everyone to a fantastic training conference. Your host committee comprises not only the San Francisco Police Department – Firearms and Toolmark Unit, but also firearms examiners from agencies all over Northern California such as Contra Costa County Sheriff's Office, Santa Clara County, Fresno DOJ, Sacramento DOJ, BATFE-Walnut Creek, Sacramento County, and the Oakland PD.

More information: www.afte.org

The **INTER/MICRO** Conference will be held July 9-13, 2007. Each year the McCrone Research Institute sponsors the weeklong conference in Chicago, offering presentations on all aspects of microscopy, methods, and materials.

More information: www.mcri.org

The 2007 **ASQDE** Meeting will be held August 11-16, 2007 at the Boulder, CO Millennium Harvest House. The theme for the meeting is Document Examination - In Theory and in Practice.

More information: www.asqde.org

The NIJ (National Institute of Justice) and the FBI Laboratory Division invite you to the **Trace Evidence Symposium** at the Sheraton Sand Key in Clearwater Beach, FL on August 13-17, 2007. Attend educational workshops, listen to plenary sessions and case presentations, and learn more about the field. Who should attend: Trace evidence examiners, prosecutors, defense attorneys, violent crime investigators, and others.

More information: www.ojp.usdoj.gov

The 2007 Annual Meeting of the **Southern Association of Forensic Scientists** will be held September 9-14, 2007 in Atlanta. A full program is planned including Advanced Structure Elucidation (DI), LC Tandem MS Use in Post-mortem Cases, and Statistics in DNA Analysis.

More information: www.southernforensic.org



Glossary

Light Emitting Diode: A light-emitting diode (LED) is a semiconductor device that emits incoherent narrow-spectrum light when electrically biased in the forward direction. This effect is a form of electroluminescence. The color of the emitted light depends on the chemical composition of the semiconducting material used, and can be near-ultraviolet, visible or infrared.

Shading Correction: In electronic imaging, shading correction is a mathematical algorithm used to compensate for inhomogeneous illumination of a specimen field created by the light source. The end result is a homogeneously illuminated microscope field of view.

LED Half-Life: Half life is the means to measure the effective use time of an LED. It is the length of time, usually expressed in hours, that an LED degrades to 50% of its original maximum intensity (usually 20,000 to 25,000 hours).



Editorial Staff

Editor-in-Chief:	Molly Lundberg
Managing Editors:	Pam Jandura, Wayne Buttermore
Graphic Design:	M.N. Kennedy

Note: We are interested in your comments and thoughts about the newsletter. Please feel free to email your comments to molly.lundberg@leica-microsystems.com.