



## A Powerful Vision

### Digital Imaging – Calibration and Digital Measurement – Part 2

By Rob Kimura

Leica Product Manager, Digital Imaging

Image measurement is the discipline of taking quantitative data from an image, for the purpose of documentation or forensic analysis. In the last issue of *The Leading Investigator*, we discussed the process of manually calibrating a microscope and digital camera for measurement. However, new advancements in automated microscopy, computer software control, and digital cameras offer a faster and more accurate way of calibrating measurements.

Factors to consider when calculating proper magnification for analysis and documentation include eyepiece magnification, camera

adapter, digital camera sensor size, objective magnification, and internal magnification changer (see Figure 1).

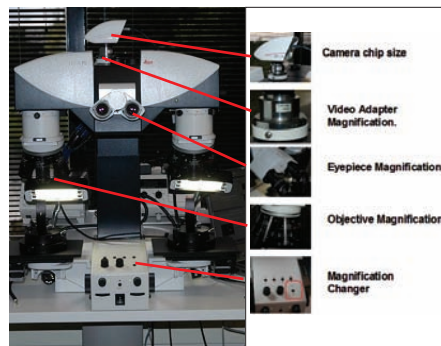


Figure 1: Leica FS C Automated Comparison Microscope Components

Automated comparison microscope systems, such as the one depicted in Figure 1, bridge traditional optical design with electronic

encoding and computer control capability linked to an internal database. This database comprises magnification and numerical aperture information for each objective. As the user changes objectives, the microscope's internal processor can automatically detect the change and determine what type—i.e. oil immersion or mag.—i.e. 10x40x, of objective is now in the optical plane.

Automated microscopes that incorporate an internal magnification chamber can detect which magnifying lens has been inserted into the optical plane. These software-controlled systems often include an automated and a manual control for changing magnifying lenses.

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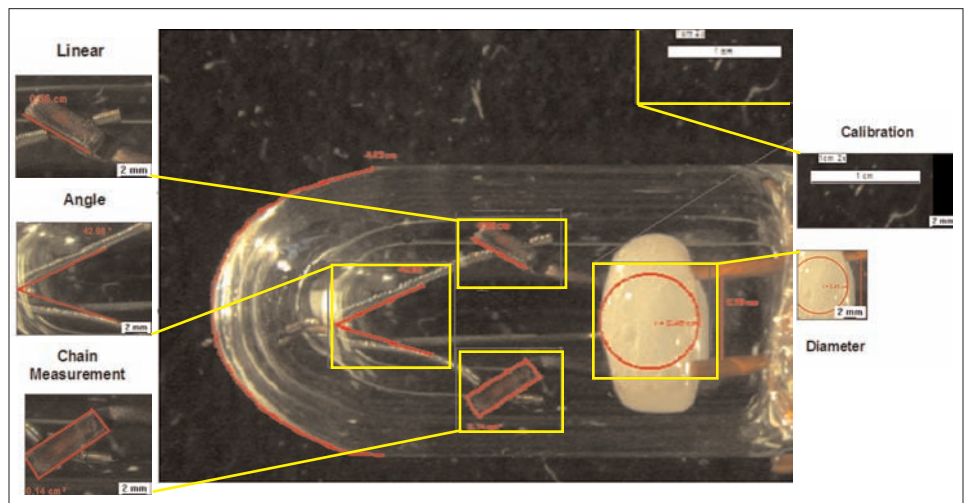


Figure 2: Measurement Example

# A Powerful Vision

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There are many camera choices that integrate with an automated microscope. Some are used with software that can auto-detect chip size and individual pixel size within the camera housing.

Some automated microscope components, such as the camera adapter and eyepieces that are not automated or encoded for automated identification, have fixed magnifications that the user does not normally change. The user can simply input these magnifications within the measurement software, which automatically calculates the correct calibration of the system as it is configured.

## Formula:

$$\begin{aligned} & \text{Eyepiece Mag.} \times \text{Objective Mag.} \times \\ & \text{Mag. Changer} \times \text{Camera Adapter Mag.} = \\ & \text{Total Magnification} \end{aligned}$$

## Example:

$$\begin{aligned} & 10x \text{ Eyepieces} \times 4x \text{ Objective} \times \\ & 2x \text{ Mag. Changer} \times .5x \text{ Camera Adapter} = \\ & 40x \text{ Total Magnification} \end{aligned}$$

We know the camera's field of view by the camera chip size and can calculate how many pixels equal a particular distance. From here the user simply selects the unit of measurement he or she would like to calibrate ( $\mu\text{m}$ , mm, inch, etc.). The system is now ready to auto-calibrate acquired images for measurement (see Figure 2).

The benefit of automated calibration is that the user simply selects an acquired image and the correct calibration is set for performing measurements. Then measurements (linear, area, angle, diameter, multi-line) are taken by clicking on the computer screen. As the measurements are completed, final measurement results are displayed.

# How Clean is Clean Enough?

## A Primer on Keeping your Microscope Clean – Part 1

### Determine the Location of Contamination

by Wayne Buttermore

*Leica Marketing Manager, Forensic Microscopy*

No matter how devoted one is to microscope maintenance scheduling through regular service calls, the rigors of daily use, specimen contact, and normal usage will result in occasional contamination of the optical/mechanical components. Contaminants can become apparent when one sees a speck through the microscope's eyepieces. One must first determine the contaminant's location to help decide if it can be cleaned or if a service call is required.

Most contaminants result in a general softening of the microscopic image with a corresponding loss in image quality and contrast. Determining contaminant location is an experiment. First, determine if the problem is a smudge or particle. Then, hypothesize where that type of contamination might be found. Finally, test for the result. The most common sources of contamination are obvious, but it is helpful to review them to find the location of the problem.

- Dust
- Fingerprints
- Makeup (*from the eyes in particular, mascara*)
- Immersion oil
- Biological samples (body fluids from wet preps)
- Mounting media

A microscope system consists of two conjugate planes, one formed with the image and the other formed with the illumination. Often, contamination can be sharply seen and in focus through the microscope along with the image viewed—the contamination is part of the conjugate image plane. On the other hand, if the contaminant is less focused when the specimen is in focus, the contamination is probably located in the illumination plane.

The **Conjugate Image Plane** consists of: field diaphragm, specimen, eyepiece field stop, retina of the eye. The **Conjugate Illumination Plane** consists of: lamp filament, condenser aperture, back focal plane of the objective, and lens of the eye.

### If a speck is seen in the field of view, what steps can be taken to determine where it is?

1. Rotate an eyepiece. Does the speck move with the eyepiece? If so, the contamination location is on the top lens or the eyepiece reticle that is mounted for measurement.
2. Change objectives. Does the speck go away? If magnification is increased, the field of view will get smaller, and the contamination may be located just outside the field of view. This could determine if the objective is the point of contamination.
3. Move the specimen. Does the speck move with the specimen? If so, the slide or specimen may be the source of contamination.
4. Focus the condenser. Does the speck go in and out of focus when the condenser moves up and down? If so, the top lens surface of the condenser is the most likely spot to inspect.

It is important to note that if a service engineer routinely cleans and adjusts the microscope, and its cleanliness is diligently maintained, the exposed surface of a lens system will be the most likely place for accumulation of dust, dirt, and other contamination.

*Next issue:* Learn how to critically inspect lens surfaces. Also, a recommended list of supplies to have on hand to perform simple cleaning jobs will be provided.

# Tips and Tricks

## New Optional LED Light Source for the Leica FS C Comparison Microscope

Contributed by Gary M. Lawrence

*Firearms Toolmark Examiner, Arkansas State Crime Laboratory*

*Thanks for the tip, Gary!*

The LED lighting option described here was obtained with my recently purchased Leica FS C comparison microscope. This lighting option provides a clean, white, and bright illumination and has adjustable intensity for a variety of applications.



LED light unit



Control box

The LED light unit, when mounted on the articulating arm and rotating mount, provides a variety of lighting directions. The control box features an on/off switch along with a rotating dial for lighting intensity. Even at the lowest setting, the LED light unit provides a soft but clean white light.

The nineteen (19) LEDs provide an even distribution of non-focused light, which results in an even illumination of the samples on the stage. The LED light unit does not get hot and it is easily manipulated into position. I would encourage any examiner with a Leica FS C microscope to think about adding this option to their current inventory of accessories.



19 LEDs

# Industry News

The AAFS 58th Annual Meeting was held February 20-25, 2006 at the Washington State Convention & Trade Center in Seattle, Washington. It was great to see so many of you there!

More information: [www.aafs.org](http://www.aafs.org)

AAFS, Florida Gulf Coast University (FGCU), and Court TV® will present the 9th Forensic Science Educational Conference on the FGCU campus on May 5-7, 2006. The three-day conference provides instruction to middle and high school science educators on the scientific method in crime investigation.

More information: [www.aafs.org](http://www.aafs.org)

AAFS President-Elect James G. Young, MD, and Eileen Young will lead a delegation of AAFS members on a ten-day tour of northern Europe, June 9-18, 2006. The tour will coincide with the 4th European Academy of Forensic Science Meeting in Helsinki, June 13-16, 2006.

More information: [www.aafs.org](http://www.aafs.org)

The 4th European Academy of Forensic Science Conference will be held at Finlandia Hall in Helsinki, Finland on June 13-16, 2006. The EAFS 2006 program will serve two groups. Presentations on recent developments in forensic science will assist senior scientists and managers. Workshops will target scientists at an earlier stage in their career and staff who are new to the field.

More information: [www.enfsi.org](http://www.enfsi.org)

AAFS, Indiana University-Purdue, and Court TV® will present the 10th Forensic Science Educational Conference on the university's Indianapolis campus on June 22-24, 2006. The three-day conference provides instruction to secondary school science educators on the scientific method in crime investigation.

More information: [www.aafs.org](http://www.aafs.org)

The 37th Annual AFTE Training Seminar will take place at the Springfield Convention Center, Springfield, MA, June 26-30, 2006. The host committee invites you to participate in this seminar and encourages early registration. More information: [www.afte.org](http://www.afte.org)

The ASQDE (American Society of Questioned Document Examiners) Conference, "Complex Examinations: Meeting the Challenges," will be held August 19-24, 2006 at the Portland, Oregon DoubleTree Hotel. Workshops on Signatures (A. Frank Hicks and Howard C. Rile, Jr.), and Difficult Handwriting Problems (Lloyd Cunningham) are under development. Workshops on Motor Control and Complexity Theory (Dr. Bryan Found), and Photocopiers (Dr. Reiner Eschbach, Xerox) are currently planned. Abstracts are due May 1, 2006 (papers due July 1, 2006). More information: [www.asqde.org](http://www.asqde.org)

# Glossary

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**LED (Light Emitting Diode):** A display and lighting technology used in almost every electronic product on the market, from a tiny on/off light to digital readouts, flashlights, traffic lights, and perimeter lighting. LEDs are also used as the light source in multimode fibers, optical mice, and laser-class printers.

**Conjugate Image Plane:** In normal observation mode (using the eyepieces), the conjugate set of object or field planes can be simultaneously viewed when the specimen is in focus. This observation mode is referred to as the orthoscopic mode, and the image is known as the orthoscopic image.

**Conjugate Illumination Plane:** The other conjugate set of aperture or diffraction planes requires the ability to focus on the rear aperture of the objective. This can be accomplished using an eyepiece telescope in place of an ocular, or a built-in Bertrand lens on microscopes that are so equipped. This observation mode is termed the conoscopic, aperture or diffraction mode, and the image observed at the objective's rear aperture is known as the conoscopic image.



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*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](mailto:molly.lundberg@leica-microsystems.com).*