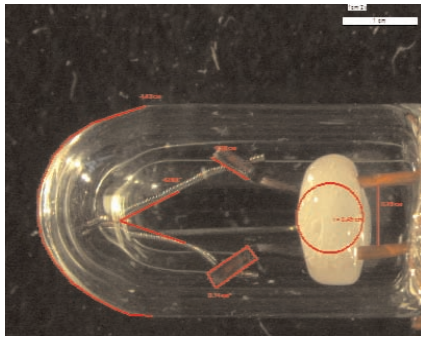




A Powerful Vision



Sample Measurement



Stage Micrometer

Digital Imaging – Calibration and Digital Measurement - Part 1

By Rob Kimura

Leica Product Manager, Digital Imaging

How do I manually calibrate my microscope system?

Image measurement is the discipline of taking quantitative data from an image for the purpose of documentation or forensic analysis.

Image calibration is the most essential process in measurement whether performing manual or automated measuring. In order to accomplish proper measurement, the microscope, camera, and measurement software must be calibrated with a consistent unit of measure and distance. This applies whether a manual microscope or an automated microscope is used. Having the best microscope, the best camera, the best software, and the fastest computer does not insure accurate measurement results. Only the process of correctly calibrating the system will insure accuracy.

In microscopy, there are two common methods of measurement calibration:

- Manual Calibration (most common)
- Automated Calibration (often requires an automated microscope)

Manual calibration requires four assets: A microscope, a camera, measurement software, and a stage micrometer.

To calibrate a manual system, follow these steps:

Place the stage micrometer on the microscope. Acquire a picture of the stage micrometer, write down the objective magnification and magnifica-

tion changer setting (if used) – you will need to remember these numbers.

Open the acquired image in the measurement software and select the calibration mode. (Every measurement software has a manual calibration mode.) The software will prompt for the unit of measure to be calibrated. Then, move the caliper markers over your image. With these caliper markers, measure a known distance from the stage micrometer picture that was just acquired.

As the caliper markers are moved around the screen, the computer tracks the distance between the two markers by pixel counts. The larger the distance, the greater the pixel count. Once the markers are adjusted to correspond to a set distance, an area to type in the distance will appear on the screen.

This process is required in both the X and Y axes. However, many cameras now use square pixels in their chips, so the unit of measure for X should equal the unit of measure for Y. For example, if the Y axis value 353 pixels = 1mm, then the X axis would also be 353 pixels = 1mm. (If unsure whether the camera has square pixels, consult the camera manufacturer.) Once the proper X and Y calibration is established, save the results in a chart. Make sure to indicate the magnification used to acquire calibration when the calibration measurement is saved.

Contents

A Powerful Vision.....	page 1
Tips and Tricks.....	page 2
The Leica LMD Presence....	page 2
Industry News.....	page 3
Glossary.....	page 3

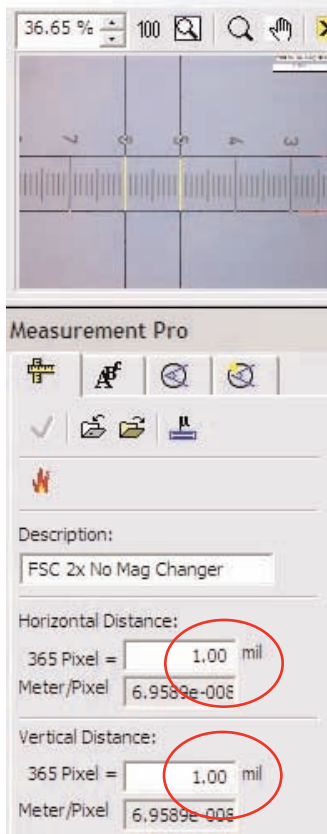
A Powerful Vision

continued from page 1

Follow this process for every magnification scenario that is used to acquire pictures. Once calibrations are completed, measuring can begin.

Some software offers an auto calibration mode to acquire pictures. On a manual microscope, the software will ask the user once they have acquired the image to indicate which calibration magnification to use. Other software requires selection of the calibration magnification in the measurement program. Either way, the process of selecting the right calibration setting is essential. Once you have properly selected the calibration, the areas of interest can be accurately measured.

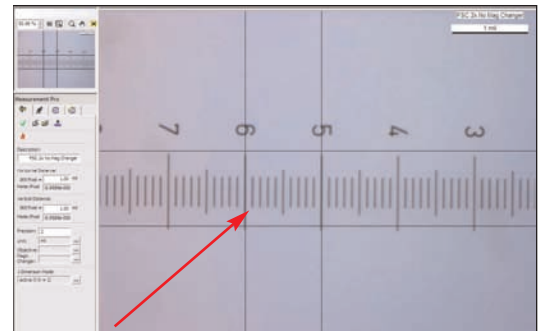
Look for Part 2 in the next issue of *The Leading Investigator*.



Setting Calibration

Excel			
1	Vector	cm	0.69
2	Angle	°	42.98
3	Chain	cm	4.43
4	Vector	cm	0.75
5	Circle	cm	0.45

Data input into Microsoft Excel®



Calibration Marker

Tips and Tricks

A Simple, Inexpensive Way to Create Improved Illumination Support

Contributed by Terry Weaver, Arizona DPS, Flagstaff
Thanks for the tip, Terry!



I found a unique way to enhance adjustability of the graduated illumination support arms featured on the new Leica FS C comparison microscope. The degree of adjustability is often dependent on the sample being viewed and/or the type of illuminator used.

A simple and inexpensive solution: Use the mounting posts from the cold light bar as an intermediate holder. When the oblique fiber optic illuminator is installed, this set-up allows the illuminator to not only be moved from side to side, but vertical adjustment of the light guide can be accomplished as well.

The Leica LMD Presence

By Andy Lee
Leica LMD Application Specialist



Hong Kong Harbor

In August, at the IAFS (International Association for Forensic Sciences) 2005 Exhibition and Meeting held in Hong Kong, invited symposium speakers Christine Sanders of the Rosalind Franklin University (North Chicago, IL) and

Andy Lee of Leica Microsystems, presented information about the use of the Leica Laser Microdissection System for rapid sperm separation in forensic science.

In September, at the 16th Promega International Symposium on Human Identification held in Grapevine, Texas. Invited speaker Kelli Langly of the North Louisiana Criminalistic Laboratory (Shreveport) presented ongoing forensic research validation data using the Leica Laser Microdissection System for sperm separation.

In October, at the ASCLD (American Society for Crime Laboratory

continued on page 3

Directors) 2005 Meeting in Phoenix, AZ, Andy Lee presented a technical paper regarding the use of the Leica Laser Microdissection System for sperm separation and other possible forensic applications. The interest level remains very high within the forensic laboratory community for technological uses of laser dissection beyond sperm separation. These include the specific separation of epithelial transfer evidence and parental DNA analysis of minute amounts of fetal material.

Television popular culture has also embraced Leica's Laser Microdissection technology through an appearance on last season's CSI:NY. During November or December 2005, the Leica AS LMD will have an encore appearance on a new episode of CSI:NY!

Industry News

Leica Microsystems hosted a full day Digital Imaging seminar at the Illinois State Police Forensic Science Center in Chicago on September 21, 2005. Lectures were presented on illumination techniques, camera technology, software control, measurement, annotations, and Image Pro Express. Later, the groups rotated through five workshops on illuminations method, camera control, Image Pro Express software, LAS (Leica Application Suite) software, and Leica IM (Image Manager) archiving tools.

"Blueprint for Success: Leadership by Design," was the theme of the American Society of Crime Laboratory Directors (ASCLD) Annual Symposium on October 23-28, 2005. The ASCLD Annual Symposium stimulates, promotes, and develops excellence in forensic science management. The meeting was held in Phoenix, Arizona.

More information: www.asclcd.org

The 31st Annual Northeastern Association of Forensic Scientists (NEAFS) Meeting will be held at the Hyatt Regency in Newport, RI on November 8-13, 2005. The preliminary schedule and registration form are available online. More information: www.neafs.org

The National Academy of Sciences will present a symposium on Forensic Science November 16-18, 2005 at the National Academy of Sciences Building in Washington, DC. The program covers a variety of topics including the past, present, and future of forensic science. More information: www.nas.edu

The AAFS 58th Annual Meeting will be held February 20-25, 2006 at the Washington State Convention & Trade Center in Seattle. The theme is "Mass Disasters: Natural and Man Made". More information: www.aafs.org

AAFS, Florida Gulf Coast University (FGCU), and Court Television Network, LLC (Court TV) will present the 9th Forensic Science Educational Conference on May 5-7, 2006. The three-day conference provides instruction to 100 science educators on the use of scientific methods in the investigation of crime.

More information: www.aafs.org

The 4th European Academy of Forensic Science Conference (EAFS) will be held in Helsinki, Finland on June 13-16, 2006. The EAFS 2006 program will include presentations on recent developments in forensic science, which will assist senior scientists and managers. Workshops will target scientists at an earlier stage in their career and staff who have changed fields. The conference takes place at Finlandia Hall, the leading conference and concert center in Helsinki. More information: www.enfsi.org

Glossary

Stage Micrometer: A special ruled scale (often on a glass slide) used for calibration of magnification, and calibration of an eyepiece reticule. Stage micrometers are configured for transmitted or reflected light and may be in either English or Metric units. Outside agencies certify the calibration of stage micrometers to NIST or ISO standards.

Pixels: A pixel (a combination of the words picture and element) is the smallest area of an image that can be assigned an intensity and color value. Software used to display images will often provide a zoom function to allow the captured image pixel to be displayed at various sizes. A calibration is required to know the area a pixel represents at the sample.

Calibration: A process used to determine the relationship between distances measured at the sample and distances measured in an image, either when viewed in the eyepiece or an image captured by camera.



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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.