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| Microscope Calibration & Measurement with an Ocular Micrometer |
| **Purpose** | This procedure provides instructions for MICROSCOPE CALIBRATION & MEASUREMENT.Size is an important criterion in the identification process of many parasites. Measurements are made with an ocular micrometer. An ocular micrometer consists as a disc inserted into the ocular of the microscope. The divisions on the ocular micrometer are arbitrary and unit less until calibrated to a specific microscope. The unit values will also vary with different objectives and microscopes. It is necessary to establish these values with each combination of lenses: low, high dry and oil immersion. A stage micrometer is used to determine the unit values on the ocular micrometer. Calibration establishes the number of micrometers (μm) in one (1) ocular unit (U). |
| **Policy Statements** | This procedure applies to Microbiologists/Virologists that measure objects with a microscope. |
| **Materials** |  |
|  | **Equipment** |
|  | * Binocular microscope with multiple objectives
* Ocular micrometer disk
* Stage micrometer
	+ Scale: 0.1 mm and 0.01 mm divisions
* Immersion oil
* Lens Paper
* Lens cleaner
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| **Special Safety Precautions** | Microbiologists/virologists are subject to occupational risks associated with specimen handling. Refer to the safety policies**:**1. [*Biohazard Containment*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20201%20%20%20Biohazard%20Containment%20R.doc)
2. [*Safety in the Microbiology/Virology Laboratory*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20202%20Safety%20in%20the%20Microbiology%20Lab%20Policy%20R.docx)
3. [*Biohazardous Spills*](file:///G%3A%5CLAB%5CMicro%20Procedure%20Manuals%5CMC%20200%20%20%20%20Safety%5CMC%20204%20Biohazardous%20Spills%20R.docx)
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| **Quality Control** | * Recalibration is required anytime new objectives or oculars are put into use.
* Document calibration results for the Microscope desktop reference.
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| **Calibration** | To function as a scale for measurement (length and width), the ocular micrometer is calibrated to the microscope using a stage micrometer. Calibration of the ocular micrometer is accomplished by comparing the unknown units on the ocular micrometer with the known scale of the stage micrometer by superimposing their images (see Fig 1.) |
| **Procedure** | 1. Remove the 10X ocular from the microscope and insert the ocular micrometer objective.
2. Place the stage micrometer on the stage.
3. Focus on some portion of the etched scale on the stage micrometer.
4. Identify the large (0.1 mm) and small (0.01 mm) unit divisions on the scale. Make sure you understand the divisions on the scale before proceeding. Seek help and/or clarification from a coworker if needed.
5. Superimpose the images of the ocular micrometer onto the stage micrometer by aligning together there ZERO “0” lines.
6. Without moving the stage micrometer, find another point where there are two additional lines exactly superimposed. This distance will vary with each objective and microscope (see Fig 1.) The second set of lines should be as far from the ZERO “0” lines as possible.

 Fig. 11. Count the number of ocular divisions between the ZERO “0” lines and the point where the second set of lines superimpose. Next, on the stage micrometer, count the number of 0.1 mm divisions between the ZERO “0” lines and the second set of superimposed lines.
2. Calculate the portion of a millimeter that is measured by a single ocular unit.
3. Calculate the calibration factors as follows. Examples:

1. To determine the size of any microscopic object: measure the object with the ocular micrometer to get the number of **ocular units (U)**. Note the objective used for the measurement and multiply the noted **ocular units (U)** by the objectives calibration factor. In general, as the magnification increases, the calibration factor decreases.
2. Example:

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| **Objective** | **Calibration Factor** | **Ocular Units of Object** | Size of Object |
| 10X | 8.0 μm | 3.8 U | 3.8 U x (8.0 μm/U) = 30.4 μm |
| 40X | 2.0 μm | 15 U | 15 U x (2.0 μm/U) = 30 μm |
| 100X | 0.8 μm | 38 U | 38 U x (0.8 m/U) = 30.4 μm |

1. For each objective magnification, there will be a factor generated (1 ocular unit = certain number of micrometers).
2. The calibration factors should be posted either on the base of the microscope or nearby for easy reference.
3. Comparison of the measurements obtained with measurements from reference books and manuals should assist in the organism identification.
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| **Limitations** | 1. After a scope has been calibrated, the oculars and objectives cannot be interchanged with another microscope. New calibration factors would need to be established if this were the case.
2. Each microscope used to measure organisms must be calibrated as a unit. The original oculars and objectives that were used to calibrate the microscope must also be used when measuring an organism.
3. The objective containing the ocular may be stored until needed. This single ocular can be inserted when measurements are taken. However, this particular ocular containing the ocular disk must have also been used as the ocular during microscope calibration.
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| **Method Performance Specifications** | 1. The calibration factors will only be as good as the initial visual comparison of the ocular ZERO “0” and stage micrometer ZERO “0” lines.
2. As a rule of thumb, the high dry objective (40X) calibration factor should be approximately 2.5 times more then the calibration factor for the oil immersion objective (100X). The low power objective (10X) calibration factor should be approximately 10 times the oil immersion objective (100X) calibration factor.
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| **References** | 1. Ash, Lawrence R. and Orihel, Thomas C. 1987. *Parasites: A Guide to Laboratory Procedures and Identification*. ASCP Press. Chicago
2. Garcia, L. S. 2001. *Diagnostic Medical Parasitology*, 4th ed., P 723. ASM Press, Washington, D.C.
3. Garcia, Lynne, Editor in Chief. 2010. *Clinical Microbiology Procedures Handbook*, 3rd ed., ASM Press, Washington D.C.
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| **Training Plan/ Competency Assessment** | **Training Plan** | **Initial Competency Assessment** |
| 1. Employee must read the procedure
2. Employee will observe trainer performing the procedure.
3. Employee will demonstrate the ability to perform procedure, record results and document corrective action after instruction by the trainer.
 | 1. Direct observation.
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| **Historical Record** |  |  |  |  |
|  | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1.0 | Becky Carlson | 09/01/1993 | Initial Version |
| 1.1 | Eddy Morrow | 06/27/2007 | Modified whole document to include disk and stage micrometer images, calculation examples, procedure notes, and limitations. |
| 1.2 | Tina Gronquist | 08/11/2014 | Updated into online format. |
|  | 2 | Becky Carlson | 4/4/2015 | Re-numbered from MC 809 |  |  |
| 2 | Susan DeMeyere | 8/8/2017 | Changed to docx file.  |
| **Archived by:** |  | **Archived Date:** |  |