TRAINING UPDATE

Lab Location:	SGAH & WAH	**Date Distributed:**	7/1/2014
Department:	Core Lab	**Due Date:**	7/31/2014
		Implementation:	**8/1/2014**

DESCRIPTION OF PROCEDURE

Name of procedure:

Ocular Micrometer Calibration SGAH.M878, WAH.M875 v0

Description of change(s):

This is a NEW procedure required by CAP.

Calibration must be done initially (Ron performs) and if the ocular or objectives are changed.

Staff must be aware:

- **Do NOT remove the micrometer**
- **Do NOT change objectives on a scope with a micrometer without notifying Ron (or a supervisor)**

This SOP will be implemented on August 1, 2014

Document your compliance with this training update by taking the quiz in the MTS system.

Approved draft for training all sites (version 0)

Non-Technical SOP

Title	**Ocular Micrometer Calibration**
Prepared by	Ron Master Date: 5/21/2014
Owner	Ron Master Date: 5/21/2014

Laboratory Approval		
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

Review:		
Print Name	**Signature**	**Date**

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1. PURPOSE

Size is an important criterion used in identification of parasites. A rough estimation can be obtained by comparing the parasite with the size of human red blood cells. However, if an organism is to be accurately measured, it is advisable to use a calibrated micrometer disc that is placed within the ocular of the microscope. The type most often used is a disc with a line scale divided into 100 ocular micrometer units.

The units on the ocular micrometer are arbitrary and the value of each unit varies with the objective and the microscope. It is necessary to calculate the value with each combination of ocular and objective lenses – low (10x, 20x), high dry (40x), and oil immersion (100x).

The ocular micrometer units are compared with a scale of known dimensions. This may be done by superimposing the image of the unknown ocular scale on the known scale of a stage micrometer (slide with a carefully etched and calibrated scale, divided into 0.01 mm divisions). The ocular micrometer should be recalibrated every time the eyepiece or objective lens is changed.

The ocular micrometer should be calibrated for the microscope in which it is used, and is recalibrated each time the eyepiece or objectives are changed.

2. SCOPE

This procedure applies to all microscopes that are used to identify ova and parasites.

3. RESPONSIBILITY

It is the responsibility of the supervisor or designee to ensure the ocular micrometers on microscopes used for the identification of ova and parasites are calibrated initially and if any change is made to the oculars or objectives.

4. DEFINITIONS

N/A

5. PROCEDURE

5.1 Calibration Frequency

Initially and whenever there is a change in either the eyepieces or the objectives.

5.2 Supplies/Equipment Needed

Stage Micrometer, 0.01 mm divisions (Fisher Scientific 12-561-SM3, Westover Scientific, MP-SM102)

5.3 Calibration Procedure

1. Remove the 10x ocular from the microscope and unscrew the holding ring on the underside of the ocular. Place the micrometer within the ocular so that the engraved side is underneath. Replace the holding ring and insert the ocular into the microscope. Check that the micrometer has been oriented correctly, by looking at the numbers and seeing that they are not reversed. Care must be taken to keep both the micrometer and lens clean and free of dust, which might interfere with observation.
2. Place the stage micrometer on the stage and focus on some portion of the scale.
3. Orient the ocular micrometer to be parallel with the stage micrometer. Adjust the microscope to bring both micrometers into focus.
4. Adjust the stage micrometer so that the '0' line on the ocular micrometer is exactly superimposed onto the line farthest to the left on the stage micrometer.
5. Without moving the stage micrometer, find another point at the extreme right where two other lines are superimposed. This second set of superimposed lines should be as far as possible from the 0 lines, but the distance will vary with objective and microscope.
6. Knowing that each of the divisions of the stage micrometer equals 0.01 mm, determine the total distance (in millimeters) between the two points of superimposition and the number of small ocular units necessary to cover the same distance. For example: Suppose it is found that using the 20x lens, that 98 ocular divisions is equal to 49 stage units or 0.49 mm.
7. Calculate the number of millimeters that is measured by one small ocular unit.
   For example: If 98 ocular units = 0.49 mm
   1 ocular unit = 0.49 / 98 = 0.005 mm
8. Since measurements of the protozoa and other small organisms or structures are usually given in microns rather than in millimeters, the above determination must be converted to microns. One millimeter equals one thousand (1,000) microns, it follows that the millimeter determination multiplied by 1,000 will give the number of microns measured by one ocular unit.
   For example: 0.005 mm x 1,000 = 5 microns
9. Record the calibrations of the ocular micrometer obtained for each of the four objectives. The lines of the stage micrometer will increase in magnification while those of the ocular micrometer will remain the same. With high and oil

magnifications, it will be necessary to center the thinner ocular micrometer line on the broader stage micrometer line for accurate measurement.

10. The size of any object can be quickly determined by measuring it with the ocular micrometer, noting the particular lens combination and referring to the record of the ocular micrometer calibrations.

5.4 Notes:

Do not move ocular lenses from one scope to another.

6. RELATED DOCUMENTS

N/A

7. REFERENCES

Parasitology Laboratory Procedure Manual, Mayo Clinic, Rochester, Minn., 1980, pp 74-75.

8. REVISION HISTORY

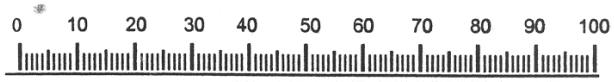
Version	Date	Reason for Revision	Revised By	Approved By

9. ADDENDA AND APPENDICES

Calibration example

Calibration of Ocular Micrometer

1) Make sure that all oculars and lenses are clean and oil-free.
2) Lower stage to accommodate a thick slide.
3) Put the stage micrometer on the scope to be checked.
4) Swing lens to be checked into position and focus carefully until you can see both the ocular and stage micrometer.
5) The ocular micrometer should look like this:

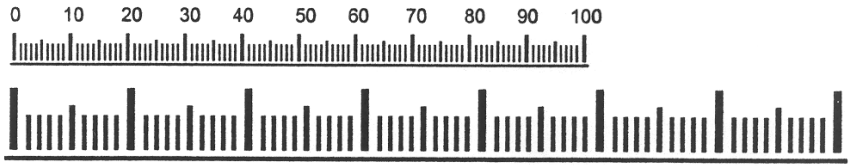


Note that the numbers run from 0-100 and that each division is equal to 1.

6) The slide micrometer will look like this:

Note that is has similar divisions to the ocular micrometer, but there are no numbers indicated, and the value of the entire micrometer is equal to 1. Therefore, each division is equal to 0.01. In addition, the size of the slide micrometer will increase as the magnification of the various lenses increase. (This is about what you could expect to see through the 20x lens.)

7) When calibrating a lens, line the left-most division on the slide micrometer with the zero (0) line on the ocular micrometer, like so:



8) Find where the farthest most right-hand line on the ocular micrometer intersects with a line on the slide micrometer. In this example, the 100 line on the ocular micrometer exactly lines up with the 49th line on the slide micrometer. The values are recorded as follows: Ocular Micrometer = 100; Slide micrometer = 0.49.

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9) To calculate the value of each division on the ocular micrometer in microns, divide the stage micrometer by the ocular micrometer reading and multiply by 1000, such as:

$$\frac{\text{Stage micrometer reading}}{\text{Ocular micrometer reading}} = \text{value in microns} \quad \text{or} \quad 0.49/100 \times 1000 = 4.9$$

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