# Compound and Inverted Microscope Calibration

(Modified from Standard Methods 10200E)

## 1. SCOPE AND APPLICATION

1.1 This SOP details the procedure for checking the measurement tools (Whipple grid or ocular micrometer) located in the eyepiece of the microscopes used for algal identification. This process is used to ensure that no significant change has occurred to the microscope with respect to magnification. The measurements are used to measure algal specimens during the identification process.

1.2 The measurements performed depend on the overall magnification of the microscope, taking into account the objectives, eyepieces and any additional magnification enhancers.

1.3 Microscopes should be checked/calibrated the first time used or any time parts have been added to or replaced on a microscope. New microscopes should be calibrated before being put into use.

1.4 This method applies to all microscopes used to process samples with LIMS codes beginning with "PRN", "PKD", "DTM", "DTY" and the codes BLOOM-ID and ALGAL-ID.

## 2. SUMMARY OF METHOD

2.1 The total magnification of the microscope is calculated by multiplying the objective magnification by the eyepiece magnification by any additional magnification component on the microscope.

2.2 The Whipple grid or ocular micrometer is viewed through each objective and the width of the entire grid as well as the widths of each increment of the grid are measured using the stage micrometer, recorded in the electronic log, form AB-SCOPE-(version #), and compared to past values. The measurements should not change from year to year unless something is changed on the microscope, such as new objectives or eyepieces. A copy of the measurements on form AB-SCOPE-(version#) are saved as a .pdf file for documentation.

2.3 Calibration checks for analytical measurements, cleanings, and repairs are documented in the Scope_Maint_Logbook in the LIMS Laboratory Logbook module.

## 3. APPARATUS AND EQUIPMENT

3.1 Whipple grid or ocular micrometer (installed in the microscope ocular)

3.2 Stage micrometer (located in the drawer to the left of the Algal Biology room entrance in a small black case the size of a microscope slide)

## 4. PROCEDURE

4.1 Open form AB-SCOPE-(version #).

4.2 Determine the magnification of the microscope for each objective being checked by multiplying the objective magnification strength by the eyepiece magnification strength. If there is any extra magnification component on the microscope, that should be included in the calculation as well.

Note: Both compound and inverted microscopes may have an additional magnification wheel. For example, if the 40X objective is being calibrated, the eyepieces are 15X, and there is an extra 1.5 magnification factor added to the microscope, the magnification strength would be 900X ($40 \times 15 \times 1.5$).


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4.3 Place the stage micrometer on the microscope stage as you would a slide or counting chamber for analysis.

Note: On the inverted microscopes, the stage micrometer must be placed upside down since the objectives are under the stage. Otherwise, you will not be able to see the marks on the stage micrometer when you look through the eyepiece of the microscope. On the compound microscope, the stage micrometer should be placed face up.

4.4 Using the lowest magnification objective, match the line at the left edge of the Whipple grid or ocular micrometer with the zero mark on the stage micrometer scale.

Note: The objective sizes, ocular strengths, and the resulting magnification will vary with the microscope.

4.6 Determine the width of the image of the entire Whipple grid or ocular micrometer as it is projected on the stage micrometer scale. Estimate the width to the nearest 1/1000 millimeter (1 micron) and record the value on form AB-SCOPE-(version #).

Note: The Whipple grid width, in millimeters, is entered on the periphyton and phytoplankton bench sheets for the designated magnification used in counting. It is used in the equation that converts raw plankton counts into densities per volume or area.

4.7 Determine the widths of the 1/10 and 1/100 (or 1/50) divisions in the Whipple grid or ocular micrometer and record on form AB-SCOPE-(version #).

4.8 Repeat steps 4.4 to 4.7 for each objective. Record the values on form AB-SCOPE-(version#).

4.9 If recorded values deviate from expected, a service call is necessary to have someone check the microscope. Enter a note on form AB-SCOPE-(version #).

Note: In general, the ocular micrometers on the compound microscopes are supposed to be approximately 100 µ long at 1000X (10X eyepiece and 100X objective) and each tenth of the grid should be approximately 10 µ long and each one-hundredth of the grid should be 1 µ long.

Note: The width of the Whipple grid depends on the overall magnification of the microscope being used, but in general, the 1/10 squares should be 1/10 of the width of the entire grid. Likewise, the 1/50 squares in the center should be approximately 1/50 of the width of the entire grid.

4.10 Save a copy of form AB-SCOPE-(version #) as a .pdf file with analyst initials and date (format YYYY-MM-DD) to AlgalCommon's Scope Maintenance "AB-SCOPE_saved_pdfs" folder, \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\Log Books\Scope Maintenance\AB-SCOPE_saved_pdfs

Example file name: AB-SCOPE-1.0_MH_2015-06-15

4.11 Document calibration checks for analytical magnification (400x for Zeiss inverted, 1000x for Leica compound) in the Scope_Maint_Logbook in the LIMS Laboratory Logbook module. This is also the location to document microscope cleanings or repairs, changing entry fields as appropriate.

4.10.1 Open the LIMS/Laboratory/Technical/Laboratory Logbook module. Select Scope_Maint_Logbook. For Group ID "Biology", choose radio button "Enter a new log". Highlight the previous calibration check for the microscope calibrated. Click "Enter/Edit". Enter the measurement into the white text box under "Entry Value". Add a comment if needed. Review information, name and date. Save.


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## 5. QUALITY CONTROL AND DOCUMENTATION

5.1 Form AB-SCOPE-(version #), \\fldep1\DEAR\Labs\Lab5\Biology\AlgalCommon\Log Books\Scope Maintenance

5.2 Scope_Maint_Logbook in the LIMS Laboratory Logbook module.

## Appendix of Significant Changes

07/12/2010 Reformatted the layout following SOP LB-001.

07/10/2012 Added code ALGAL-ID.

07/01/2015 Formatted for 508 compliance. Replaced paper logbook with LIMS Laboratory Logbook. Updated links. Created form AB-SCOPE-(version #), an electronic log of microscope measurements.

07/14/2021 Removed once a year and added the first time used in section 1.3.


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