STAGE MICROMETERS

Introduction

Whenever there is a need to make measurements with an eyepiece graticule, there is also a need to ensure that the microscope is calibrated. The use of a stage micrometer to check the divisions and measurements on the eyepiece reticle is the best way to achieve this calibration. There are a number of factors that can influence the true calibration:-

Errors that may influence the correct measurement of a subject:

- Any inherent inaccuracies of the objective lenses
- Variations in the optical system itself (such as effective tube length)
- Inaccuracies in the reticle scale.

These combined errors need to be taken into account and quantified if measurements taken with the microscope are to be considered accurate within defined limits of uncertainty.

What is a Stage Micrometer?

A Stage Micrometer is simply a microscope slide with a finely divided scale marked on the surface. The scale is of a known true length and is used for calibration of optical systems with eyepiece graticule patterns. This is particularly important when alternating between objectives on one microscope or when using the same graticule in different microscopes.



GRATICULES

What is a Eyepiece Graticule?



A microscope may be equipped with a linear measurement scale fitted into one eyepiece. This eyepiece scale is called a reticle or graticule. The terms reticle or graticule are interchangeable. The reticle can be used to measure any planar dimension in a microscope field, since the ocular can be rotated in any direction and the object of interest can be repositioned with the stage manipulators.

Using the procedure explained below a 'conversion factor' can be derived. This will enable the user to convert the apparent size of a subject as seen through the eyepiece scale, into a real world dimension.

An accurately derived conversion factor will compensate for any of the errors discussed earlier.

To measure an object length, note the number of divisions spanned by the object then multiply by the conversion factor for the magnification used.

The conversion factor is different at each magnification. Therefore, when using a reticle for the first time it is necessary to calibrate the scale by focusing on a second micrometer scale (Stage Micrometer) placed directly on the stage.



Conversion Factor

To get the conversion factor, you are required to calibrate the microscope and its associated reticle with a stage micrometer.

The following is an example procedure:-

• Select your normal or lowest magnification.

• Ensure the eyepiece reticle is in sharp focus.

• Place the stage micrometer on microscope stage. Position and focus so the stage scale is clearly visible.

• Rotate the eyepiece reticle and position the stage in the field of view so that the two scales appear parallel, one positioned above the other.

• Adjust the alignment of the scales so that the zero values correspond.

With the zero values aligned, the stage micrometer scale will either appear longer than the eyepiece scale or alternatively it may appear the same length or shorter than the eyepiece scale.

If the stage scale appears longer than the eyepiece scale

Count the number of stage micrometer divisions that cover the eyepiece graticule s cale.

Calculating the conversion factor

Using a Stage Micrometer with 100 divisions of 0.1mm having a 10mm total length (model S1 or PS1)

Example 1 - Selected objective magnification X4.

Suppose the full length of the reticle scale covered 25 divisions of the stage micrometer. Then the full length of the reticle scale is equivalent to $(25 \times 0.1 \text{mm}) = 2.5 \text{mm} \log 100 \text{mm}$. For an eyepiece reticle with 100 divisions, each division will measure $25 \mu \text{m}$ at the stage for this magnification.

Example 2 - Selected objective magnification X10.

Selecting the X10 objective and repeating the exercise above would show that the reticle scale now covers 10 divisions of the stage scale.

Then the full length of the reticle scale is equivalent to $(10 \times 0.1 \text{ mm}) = 1 \text{ mm}$ long. For an eyepiece reticle with 100 divisions, each division will measure $10 \mu \text{m}$ at the stage for this magnification.

In summary you can apply these conversion factors to state what each division of the eyepiece reticle is measuring for a selected magnification.

X4 1 division = 25μm X10 1 division = 10μm

If the stage scale appears the same length or shorter than the eyepiece scale

In some cases the stage micrometer scale may appear shorter than the eyepiece scale. In that case note how many eyepiece divisions match the full length of the stage micrometer scale.

Calculating the conversion factor

Using a Stage Micrometer with 50 divisions of 0.002mm having a 0.1mm total length (model S12 or PS12).



Example 3 - Selected objective magnification X40.

The full length of the stage scale only spans the first 40 divisions of the reticle scale. Then the first 40 divisions of the reticle scale is 0.1mm long.

So each reticle division is equivalent to $(0.1 \text{ mm} / 40) = 2.5 \mu \text{m}$.

For an eyepiece reticle with 100 divisions the full length of the reticle scale equals 250µm (0.25mm) at the stage for this magnification.

Real case

The examples above give ideal perfect world results. They do not exhibit calibration discrepancies that occur due to any inherent inaccuracies of objectives and the optical system, or the inaccuracies in the eyepiece reticle scale. These errors are more noticeable at higher magnifications using stage micrometers with the finest divisions. The following is such an example using the same Stage Micrometer as example 3 and a X100 Oil immersion objective.

Calculating the conversion factor

Using a Stage Micrometer with 50 divisions of 0.002mm having a 0.1mm total length (model S12 or PS12).

Example 4 - Selected objective magnification X100. The full length of the stage scale only spans the first 95 divisions of the reticle scale. Then the first 95 divisions of the reticle scale is 0.1mm long. So each reticle division is equivalent to (0.1mm / 95) = 1.05µm. For an eyepiece reticle with 100 divisions the full length of the reticle scale equals 105µm (0.105mm) at the stage for this magnification.

Other factors should be taken into consideration before taking this figure of 1.05µm per division as a clear-cut certainty.

Human error can be introduced in taking the measurement, as alignment of the scales can be a little difficult at high magnifications. It is good practice to reduce the effects of human error by taking a number of measurements and averaging the results.

Mathematically calculating that each division = 1.05μ m is OK, but what is the microscopes limit of optical resolution? Assuming a resolution of 0.5μ m or so using oil immersion, how could you report that an object covering 2.5 divisions in the reticle measures exactly 2.625μ m?

Additionally, how can you be sure that the Stage Micrometer is in fact 0.1mm long, with divisions of 2µm. Even if the micrometer is as precise and accurate as the PS12 used above. Just how precise is it, and how do you prove it?

This is of particular importance if the measurements you are taking are critical to your process and required to be part of an ISO quality procedure. This is where a traceable calibration certificate of the Stage Micrometer may be required.

Stage Micrometers & Calibration Certificates

Stage micrometers can be calibrated and provided with a certificate giving the precise details about their accuracy. These calibrated standards provide tractability for the precise calibration and confirmation of accuracy of optical measuring instruments, which is necessary under ISO provisions.



Calibration Standards

Graticules Optics can arrange for the calibration of its Stage Micrometers to be carried out by the most appropriate laboratory to suit the customer requirements - the choice of laboratory is normally dependent on the nature of the calibration and the accuracy required.



a) Calibration by NPL (See Appendix 1)

The National Physical Laboratory carries out measurements at selected points on the scales and grids and issues a certificate of calibration.

b) Calibration by UKAS Accredited Laboratory (See Appendix 2)

A UKAS accredited laboratory carries out measurements at selected points on the scales and grids and issues a calibration certificate.

c) Measurement by Graticules Optics

For applications which do not require a traceable certificate or the accuracy provided by calibrations carried out by NPL or a UKAS accredited laboratory, can provide a Graticules Certificate of Comparison. The scale or grid is compared with NPL calibrated in-house standards and a statement is provided on the accuracy of the item with respect to these standards.

Physical description of Graticules Optics Stage Micrometers

have two distinct Stage Micrometer product ranges, the 'S' and the 'PS' series

'S' series

The 'S' series intended for general everyday routine calibration. The scale is centred on a glass disc mounted in a black anodised aluminium slide 75mm x 24mm x 2mm thick.

'PS' series



If you require traceable calibration to satisfy NIST or ISO, then you need to have a stage micrometer with a unique engraved serial number. In which case you require our 'PS' series Stage Micrometers. These differ from the 'S' series, because the glass disc is mounted in a stainless steel slide with an engraved, unique serial number. Each slide is supplied in a polished wooden presentation/storage box to distinguish it as a traceable standard of high value.



PS Series Stage Micrometers



- ✓ Unique engraved serial number
- ✓ Stainless steel slide mount
- ✓ Supplied in polished wooden box
- ✓ Various scale lengths from 0.1mm to 20mm
- ✓ Negative and positive images for transmitted or reflective light
- ✓ High quality image
- ✓ Available with Internationally traceable certificates of calibration



Recent product introductions have included longer scaled micrometers and micrometers with reflective scales for use with top incident lighting rather than transmitted (bottom) light sources.



Selecting the right Scale for your application

Graticule Set Tables

For Transmitted Light:

Objective Magnification	Measured Size Range	Eyepiece Graticule	Stage (object) micrometer
0.5	0.1mm to 10mm	NE5, 5mm in 0.05mm	PS1, 10mm in 0.1mm
0.5	0.01mm to 1mm (10 to 1000microns)	NE28, 1mm in 0.01mm	PS8, 1mm in 0.01mm
1	0.1mm to 10mm	NE1, 10mm in 0.1mm	PS1, 10mm in 0.1mm
1	0.01mm to 1mm (10 to 1000microns)	NE28, 1mm in 0.01mm	PS8, 1mm in 0.01mm
2	0.1mm to 10mm	NE5, 5mm in 0.05mm	PS1, 10mm in 0.1mm
2	0.01mm to 1mm (10 to 1000microns)	NE5, 5mm in 0.05mm	PS8, 1mm in 0.01mm
4	0.01mm to 1mm (10 to 1000microns)	NE5, 5mm in 0.05mm	PS8, 1mm in 0.01mm
10	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS8, 1mm in 0.01mm
10	0.002mm to 0.1mm (2 to 100microns)	NE28, 1mm in 0.01mm	PS12, 0.1mm in 0.002mm
16	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS8, 1mm in 0.01mm
16	0.002mm to 0.1mm (2 to 100microns)	NE5, 5mm in 0.05mm	PS12, 0.1mm in 0.002mm
20	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS8, 1mm in 0.01mm
20	0.002mm to 0.1mm (2 to 100microns)	NE5, 5mm in 0.05mm	PS12, 0.1mm in 0.002mm
40	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS8, 1mm in 0.01mm
40	0.002mm to 0.1mm (2 to 100microns)	NE5, 5mm in 0.05mm	PS12, 0.1mm in 0.002mm
60	0.002mm to 0.1mm (2 to 100microns)	NE1, 10mm in 0.1mm	PS12, 0.1mm in 0.002mm
100	0.002mm to 0.1mm (2 to 100microns)	NE1, 10mm in 0.1mm	PS12, 0.1mm in 0.002mm

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Selecting the right Scale for your application

Graticule Set Tables

For Reflected Light:

Objective Magnification	Measured Size Range	Eyepiece Graticule	Stage (object) micrometer
0.5	0.1mm to 10mm	NE5, 5mm in 0.05mm	PS1REF, 10mm in 0.1mm
0.5	0.01mm to 1mm (10 to 1000microns)	NE28, 1mm in 0.01mm	PS78, 1mm in 0.01mm
1	0.1mm to 10mm	NE1, 10mm in 0.1mm	PS1REF, 10mm in 0.1mm
1	0.01mm to 1mm (10 to 1000microns)	NE28, 1mm in 0.01mm	PS78, 1mm in 0.01mm
2	0.1mm to 10mm	NE5, 5mm in 0.05mm	PS1REF, 10mm in 0.1mm
2	0.01mm to 1mm (10 to 1000microns)	NE5, 5mm in 0.05mm	PS78, 1mm in 0.01mm
4	0.01mm to 1mm (10 to 1000microns)	NE5, 5mm in 0.05mm	PS78, 1mm in 0.01mm
10	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS78, 1mm in 0.01mm
16	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS78, 1mm in 0.01mm
20	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS78, 1mm in 0.01mm
40	0.01mm to 1mm (10 to 1000microns)	NE1, 10mm in 0.1mm	PS78, 1mm in 0.01mm







NATIONAL PHYSICAL LABORATORY

Continuation Sheet

MEASUREMENTS

Intervals on this stage micrometer have been measured using a microscope with a travelling stage. The displacement of the stage was measured by means of a helium-neon laser interferometer, the frequency of the laser having been determined using an iodine-stabilised reference laser.

The slide was placed so that the main inscriptions were uppermost. The scale was viewed using reflected light. The distances between the lines were measured along the longitudinal axis of the scale. The measurements were made at the mid height of the lines from line centre to line centre.

The measurements were taken in a room with temperature maintained to $20 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$. The measurements were corrected to a temperature of $20 \text{ }^{\circ}\text{C}$ using a value of $8.5 \times 10^{-6} \text{ K}^{-1}$ for the coefficient of linear thermal expansion.

RESULTS

Numbering of interval	Length of interval in mm at 20 °C
0 to 10	0.100 1
0 to 20	0.200 0
0 to 30	0.300 0
0 to 40	0.400 0
0 to 50	0.500 0
0 to 60	0.600 0
0 to 70	0.700 0
0 to 80	0.800 0
0 to 90	0.900 0
0 to 100	1.000 0

UNCERTAINTIES

The expanded uncertainty in the interval measurements is 0.000 2 mm.

The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor k = 2, providing a coverage probability of approximately 95 %.

The uncertainty evaluation has been carried out in accordance with UKAS requirements.

NOTE

The results and uncertainties refer to on the day values and make no allowance for subsequent drift.

Reference: 2019100460/1

Checked by: Up

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Certificate of Calibration

Date of Issue 18 March 2020

SUPPLEMENT TO CALIBRATION CERTIFICATE NUMBER 3257470001

Certificate No. 3257470001S

Page 1 of 3

Issued by Trescal Ltd Park Gate Close, Bredbury Park Way Bredbury, Stockport SK6 2SL, UK Tel: +44 (0)161 4067878



APPROVED SIGNATORY David Gresty (038) (Signed electronically)

Customer:

Graticules Optics Limited, 17-19 Morley Road Tonbridge, Kent, TN9 1RN

Location of calibration:

Trescal Ltd, Park Gate Close, Bredbury Park Way Bredbury, Stockport, SK6 2SL, UK

Equipment Details:

Description:	Stage Micrometer		
Manufacturer:	Graticules Optics Ltd		
Type No:	S8	Date of Receipt:	05 March 2020
Range:	1mm x 0.01 mm	Order No:	2704
Serial No:	SC3757	Our Reference:	00568116
Calibrated By:	Afonso Achas	Date of Calibration:	07 March 2020

Calibration Summary

This certificate is issued in accordance with the laboratory accreditation requirements of the United Kingdom Accreditation Service. It provides traceability of measurement to the SI system of units and/or to units of measurement realised at the National Physical Laboratory or other recognised national metrology institutes. This certificate may not be reproduced other than in full, except with the prior written approval of the issuing laboratory.

Status on Receipt: In Tolerance

The equipment conformed to the stated specification at the points measured, due allowance having been made for the uncertainty of the measurement.

Status on Despatch: In Tolerance

The equipment conformed to the stated specification at the points measured, due allowance having been made for the uncertainty of the measurement.

Action(s) Taken: Full Calibration

Ambient Conditions: Temperature: 20 ±2 °C

07 March 2022 Date of next calibration:

s quoted refer to the measured values only, with no account rtainty multiplied by a coverage factor k=2 providing a con In Calibration". results given within this certificate only relate to the item calibrated. The uncertainty limits ain its calibration. The reported expanded uncertainty is based upon a standard uncer tainty evaluation has been derived from EA-402 M-2013 "Evaluation of the Uncertainty of Massurement being taken of the instruments ability to rfidence level of approximately 95%. The

EMS 00004-ISS30-Feb2020





Calibration Equipment Used

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Asset No	Due Date	Asset No	Due Date
FC309	31/05/2020	FC481	30/04/2020

Key to results annotations, where applicable

The measurements marked thus have been determined to be outside the specification, due allowance having been made for the uncertainty of the measurement.
The measurements marked thus are within the specification by a margin less than the measurement uncertainty; this indicates that conformance is more probable than non-conformance.
The measurements marked thus are outside the specification by a margin less than the measurement uncertainty; this indicates that non-conformance is more probable than conformance.
The measurement uncertainty is significant in relation to the unit specification and it is therefore not possible to demonstrate conformance unambiguously.
The measurements marked thus do not lie within the scope of the Laboratory's prevailing UKAS accreditation but are reported herein for completeness.



Certificate of Calibration	Certificate Number
Issued By TRESCAL Ltd	3257470001S
UKAS Accredited Calibration Laboratory 0013	Page 3 of 3

Basis of Test: Accuracy requirements of manufacturers published accuracy figures (0.0015 mm)

This scale was measured using a laser interferometer with the following results:-

a) The actual distance between the axis of the graduations over three runs

Nominal Position (mm)	Measured Result (mm)		Mean Result	Variation of Measurements	
(11111)	Run 1	Run 2	Run 3	(mm)	(mm)
0.100	0.1000	0.1001	0.1000	0.1000	0.0001
0.200	0.2000	0.2001	0.2000	0.2000	0.0001
0.300	0.2999	0.3000	0.3000	0.3000	0.0001
0.400	0.4000	0.4000	0.3999	0.4000	0.0001
0.500	0.5000	0.4999	0.4999	0.4999	0.0001
0.600	0.6001	0.6000	0.6000	0.6000	0.0001
0.700	0.7001	0.7000	0.7000	0.7000	0.0001
0.800	0.8001	0.8000	0.7999	0.8000	0.0002
0.900	0.9001	0.9001	0.9000	0.9001	0.0001
1.000	1.0000	1.0000	0.9999	1.0000	0.0001

* Denotes outside tolerance

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Uncertainty of Measurement	$\pm 0.0005 \text{ mm}$

Calibration Procedure	QCD/CALP/52
Our Reference:	AA325747

--End of Results--

