STAGE MICROMETERS

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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.

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 scale.

Calculating the conversion factor
Using a Stage Micrometer with 100 divisions of 0.1mm having a 10mm total length (Pyser-SGI 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 x 0.1mm) = 2.5mm long.
For an eyepiece reticle with 100 divisions, each division will measure 25µm 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 x 0.1mm) = 1mm long.
For an eyepiece reticle with 100 divisions, each division will measure 10µm 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 (Pyser-SGI model S12 or PS12).

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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 (0.1mm / 40) = 2.5µm.
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 (Pyser-SGI 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 = 1.05µm is OK, but what is the microscopes limit of optical resolution? Assuming a resolution of 0.5µm or so using oil immersion, how could you report that an object covering 2.5 divisions in the reticle measures exactly 2.625µm?

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.

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Calibration Standards

Pyser-SGI Limited Graticules Division 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 Pyser-SGI Graticules Division

For applications which do not require a traceable certificate or the accuracy provided by calibrations carried out by NPL or a UKAS accredited laboratory, Pyser-SGI 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 Pyser-SGI Stage Micrometers

Pyser 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.

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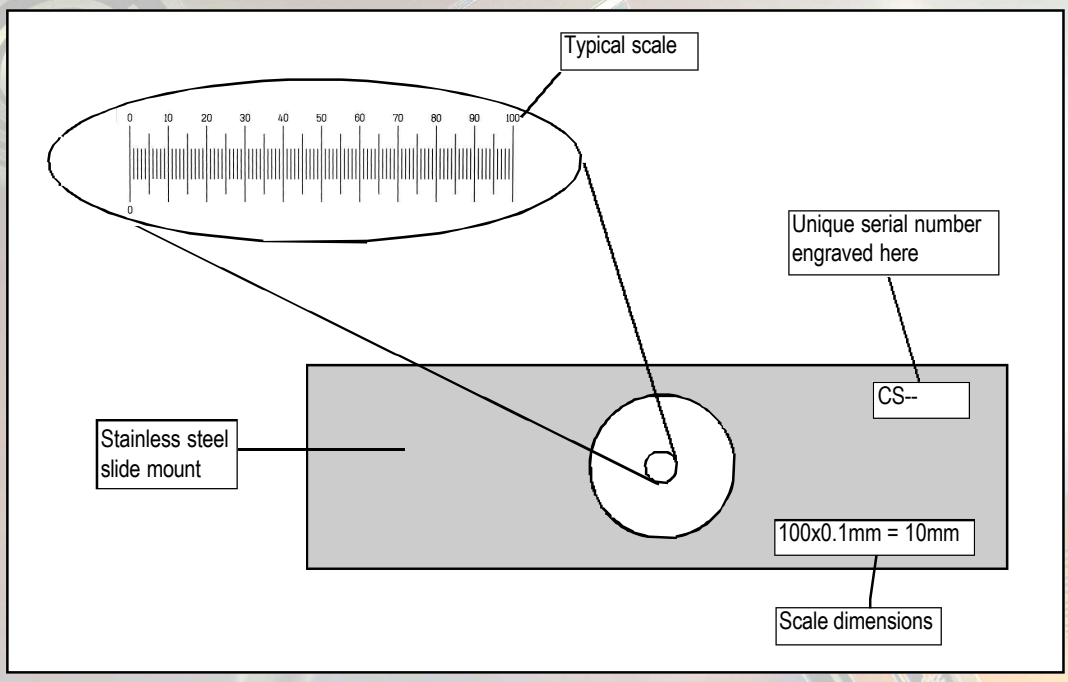
PS Series Stage Micrometers



- ✓ 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.

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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

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APPENDIX 1

NATIONAL PHYSICAL LABORATORY
Teddington Middlesex UK TW11 0LW Switchboard 020 8977 3222

Certificate of Calibration

UKAS CALIBRATION 0478

Stage Micrometer
CS 2205

FOR

Pyser - SGI Ltd
Fircroft Way
Edenbridge
Kent
TN8 6HA

For the attention of I Bennett

DESCRIPTION

A scale formed on a glass disc approximately 15 mm in diameter inset on a metal microscope slide 75 mm x 25 mm x 1 mm. The scale is 1.0 mm long subdivided to 0.01 mm and numbered every tenth line from 0 to 100.

DATE OF CALIBRATION

6 September 2005

IDENTIFICATION

CS 2205
Type PS8 made by Graticules Ltd

Reference: LR0401/05029/ML76/84

Date of issue: 7 September 2005 **Signed:** **(Authorised Signatory)**

Checked by: **Name: Michael B McCarthy** **for Managing Director**

APPENDIX 2

NATIONAL PHYSICAL LABORATORY

Continuation Sheet

MEASUREMENTS

Selected 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 distances between the lines were measured along the longitudinal axis of the scale. The scale was viewed using reflected light, the slide being placed so that the main inscriptions were uppermost.

RESULTS

Numbering of Interval	Length of interval in mm at 20°C
0 to 10	0.1000
0 to 20	0.2000
0 to 30	0.3000
0 to 40	0.4000
0 to 50	0.4999
0 to 60	0.5999
0 to 70	0.6999
0 to 80	0.7999
0 to 90	0.8999
0 to 100	0.9999

UNCERTAINTIES

The expanded uncertainty in the interval measurements is ±0.0005 mm.

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

The uncertainty valuation 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.

Checked by:

CERTIFICATE OF CALIBRATION

DOWDING & MILLS
CALIBRATION
Park Gate Close
Bredbury Park Way
Bredbury
Stockport SK6 2SL
Fax: 0161 406 7979
Telephone: 0161 406 7878
Email: calibration.manchester@dowdingandmills.com

UKAS CALIBRATION
0067

Description of Equipment: A S1 Stage micrometer Scale

Serial N°: SC1197

Customer: Pyser SGI Ltd.
Kent.

Basis of Test: Manufacturer's published accuracy figures.

DATE OF ISSUE
6th October 2005

CERTIFICATE NUMBER
0995L0001

PAGE 1 OF 1

APPROVED SIGNATORY
A.M.BAKER
R.HARGREAVES
I.BOOLEY D.GRESTY

This instrument was examined on the 6th October 2005 at 20±1°C with the following results:

a) The actual distance between the axis of the test graduations over 3 runs as measured using laser interferometer:

Nominal Position (mm)	Mean of measured results (mm)	Variation of measurements (mm)
1.000	1.0003	0.0002
2.000	2.0001	0.0003
3.000	3.0002	0.0001
4.000	4.0002	0.0002
5.000	5.0003	0.0001
6.000	6.0003	0.0003
7.000	7.0002	0.0001
8.000	8.0003	0.0003
9.000	9.0003	0.0001
10.000	10.0005	0.0002

Uncertainty of measurement: ± 0.0005mm

Condition on receipt : Satisfactory
Adjustments made : None
Equipment used : FC 207
Cal Procedure N° : 52
Our Reference N° : 0995L/AH

The reported expanded uncertainty is based on a standard uncertainty multiplied by a coverage factor k=2, providing a level of confidence of approximately 95%. The uncertainty evaluation has been carried out in accordance with UKAS requirements.