



The McCrone Group

The premier microscopy resource, combining the talents and skills of its staff in the areas of materials analysis, instrument sales, and education.

Thank you for joining us. The webinar
Calibrating Your Microscope will begin shortly.

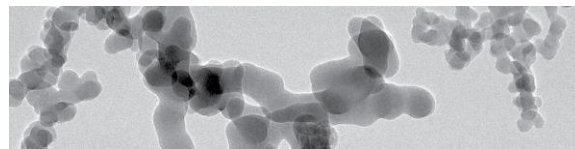
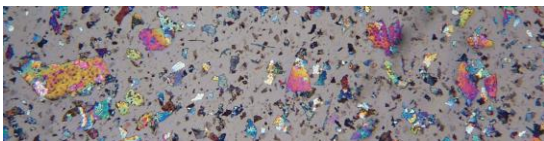
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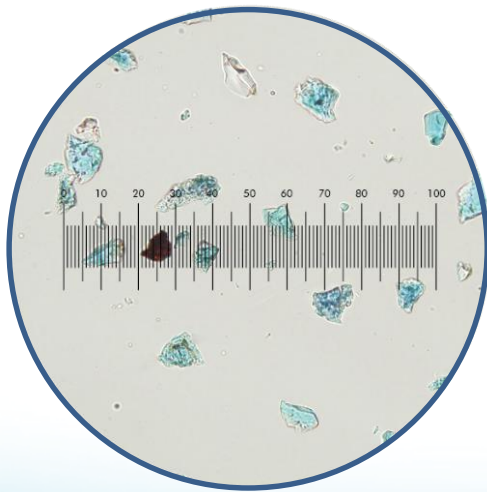
Linkam Thermal Stages • Laboratory Supplies

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Nikon Microscopes and Measuring Equipment



Calibrating Your Microscope

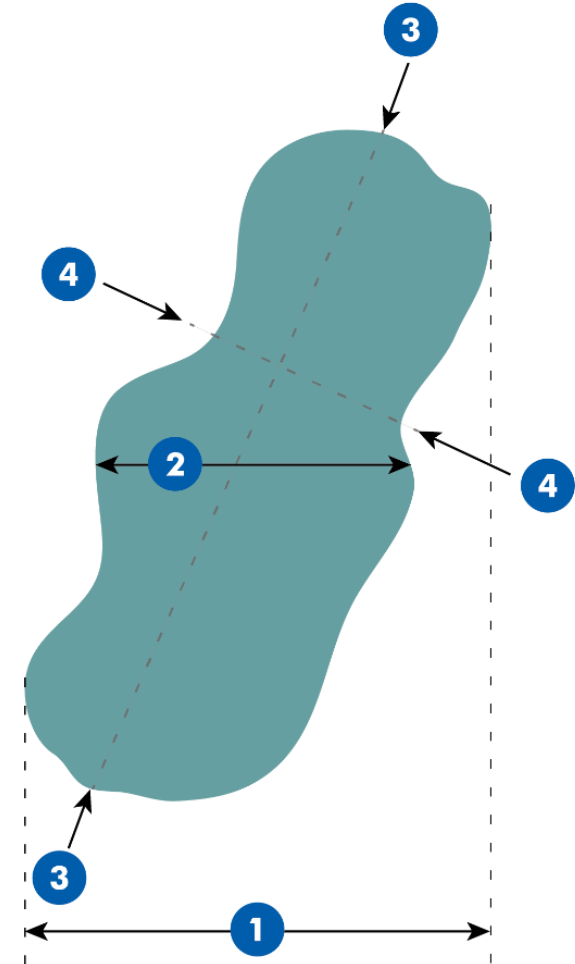


Nicole Groshon
Cleanroom Microscopist

What is Micrometry?

Micrometry is the measuring of linear distance (width, length, etc.) of microscopic samples.

Before we can accurately report particle dimensions, we need to **calibrate our microscope!**



Three Major Components of Calibration

1. Focus your eyepiece reticle to your eyesight.

Benefit: Your eyes may differ in acuity, so focusing your eyepieces separately from one another will prevent squinting, eye strain, tension, and even headaches.

2. Calculate true magnification.

Benefit: Calculating true magnification for each click stop will give perspective to the images you take. Saying an image was taken at click stop 4 doesn't mean much to the viewer, but saying the image was taken at 60x is important.

3. Calibrate your click stops to a certified stage micrometer.

Benefit: Doing so will allow you to measure samples accurately.



At McCrone Associates we check our calibration annually. If the measurements are not within 5% of our original calculations, it is an indication that there might be an issue with the microscope's alignment.

Nikon SMZ1270



Focusing Your Eyepiece Reticle

I. Find a sample to focus on.

Place a sample with fine features, such as typed text in a small font size, on your stage and focus on it.

II. Determine dominant eye and place reticle on that side.

Eyepieces are usually removable and interchangeable with one another. If you have this option on your microscope, the reticle (scale bar) should be placed on the side of the user's dominant eye.

Determining Dominant Eye:

Extend one arm out and hold the thumb of that hand in an upright position. Keeping both eyes open and focused on a distant object, superimpose your thumb on that object. Alternately close one eye at a time. The eye that keeps your thumb directly in front of the object while the other eye is closed is your dominant eye.

III. Focus the dominant eye reticle.

Using your dominant eye only, while keeping the other eye closed, focus the eyepiece crosshairs by rotating the uppermost eye lens of the dominant eye eyepiece until the crosshairs are in focus.

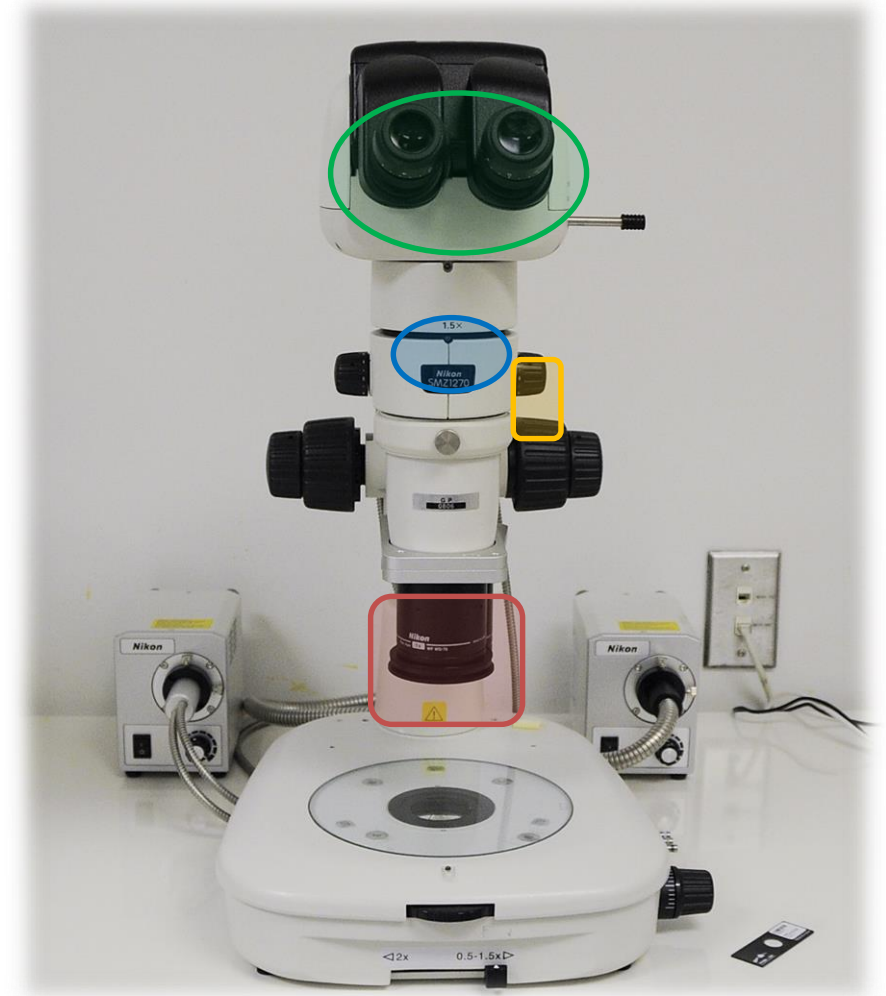
IV. Focus the non-dominant eye eyepiece.

First, focus the eyepiece that does not have the scale bar in it. While keeping your dominant eye closed, use the coarse and fine focus knobs to adjust your view until the fine details of the sample you are viewing come into sharp focus.



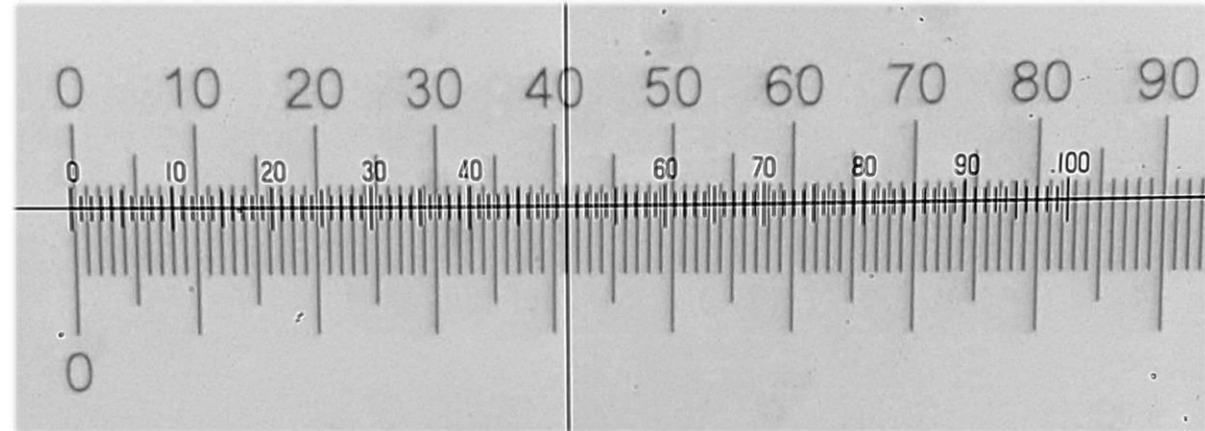
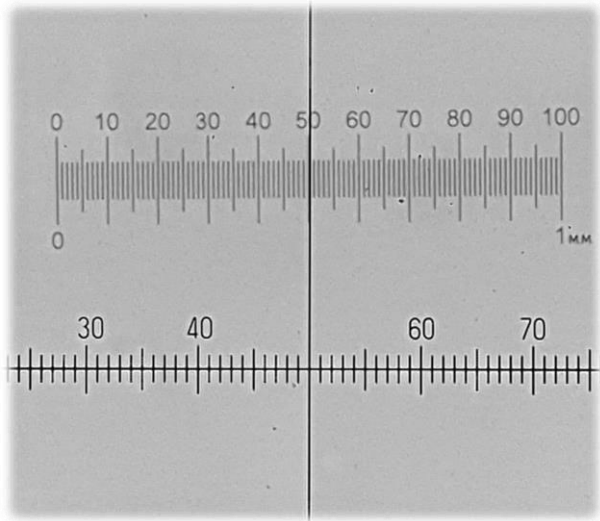
Calculating True Magnification

Eyepiece Magnification	X	Coaxial Illumination Magnification	X	Objective Magnification	X	Knob Magnification	=	True 'Scope Magnification
10	x	1.5	x	1	x	0.63	=	9.5x
10	x	1.5	x	1	x	1	=	15x
10	x	1.5	x	1	x	2	=	30x
10	x	1.5	x	1	x	3	=	45x
10	x	1.5	x	1	x	4	=	60x
10	x	1.5	x	1	x	6	=	90x
10	x	1.5	x	1	x	8	=	120x



Calibrating Your Objectives in 5 Steps

1. Calculate the distance of each stage micrometer division.
2. Line up the micrometer with your eyepiece reticle scale bar.
3. Count divisions and calculate size for that magnification.
4. Increase magnification and repeat calculations for each click stop.
5. Create a sizing chart to keep at your workstation.



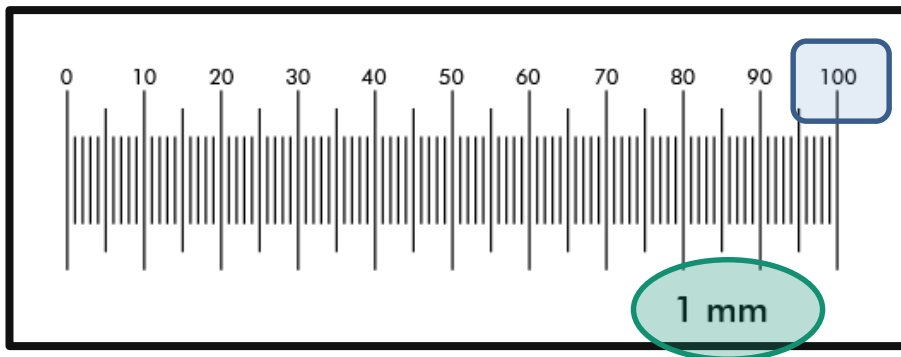
Calibrating Your Objectives (Step 1 of 5)

1. Calculate the distance of each stage micrometer division.

Observe the unit of measure of the certified stage micrometer from the units on the micrometer itself or on the Certificate of Analysis.

Calculate the distance of each division using this formula:

$$\text{Total scale length} / \# \text{ of divisions} = \text{Length of each division}$$



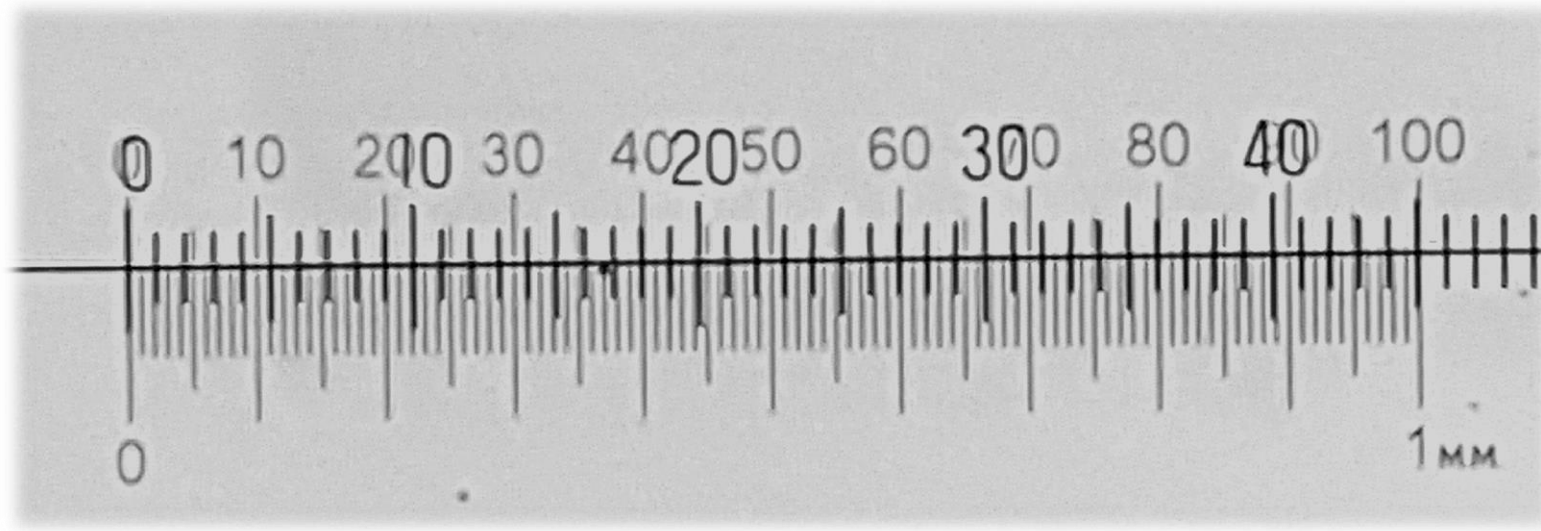
Total scale length **# of Divisions** **Length of each division**

Example: 1000 μm / 100 divisions = 10 μm /division

Calibrating Your Objectives (Step 2 of 5)

2. Line up the micrometer with your eyepiece reticle scale.

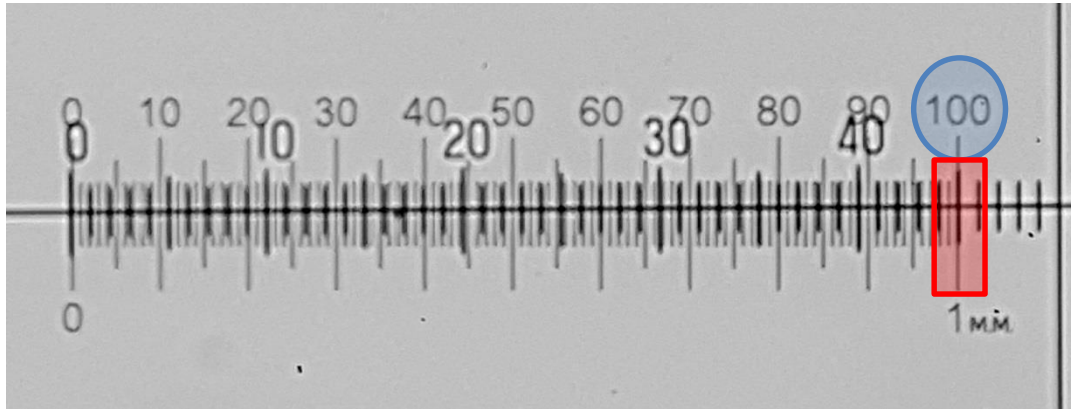
Place the stage micrometer on the stage of the microscope and bring the scale into focus. Position the stage micrometer so that the zero of the stage micrometer overlaps with the zero on the eyepiece reticle scale.



Calibrating Your Objectives (Step 3 of 5)

3. Count divisions and calculate size of a single division for that magnification.

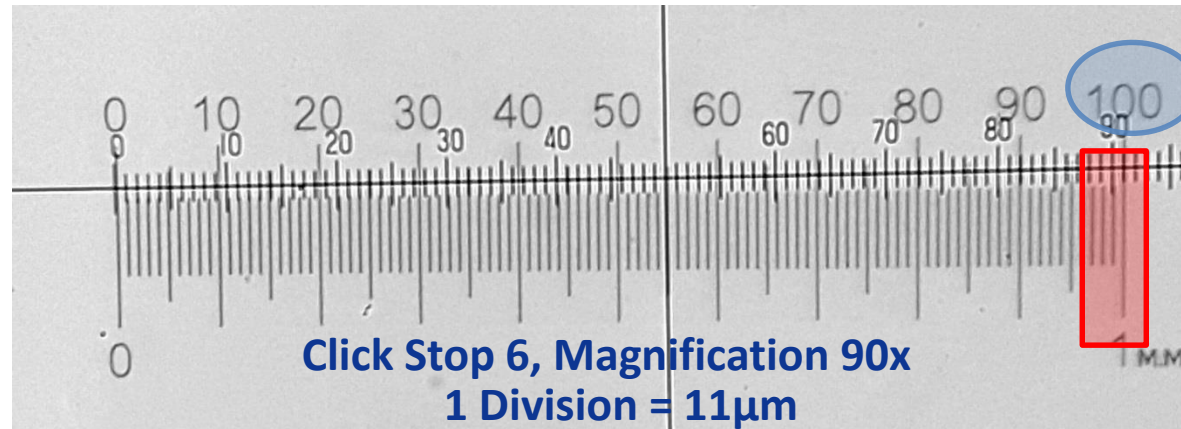
Count the number of stage micrometer divisions that match up with the largest visible number of eyepiece reticle divisions and enter in the following equation:



No. of Stage Micrometer Divisions	÷	No. of Eye- Piece Reticle Divisions	x	µm per Stage Micrometer Division	=	One Eyepiece Reticle Division (µm)
↓		↓		↓		↓
100 Stage Micrometer Divisions	÷	45 Reticle Divisions	x	10 µm per Stage Micrometer Division	=	One Eyepiece Reticle Division is 22 µm at 45x

Calibrating Your Objectives (Step 3 -Practice)

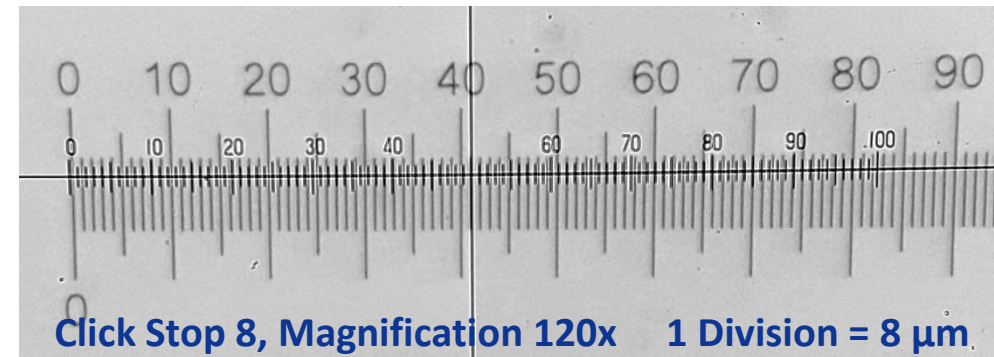
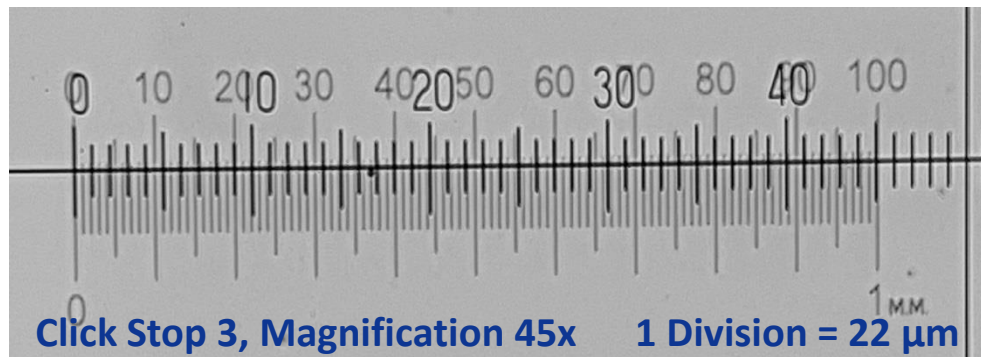
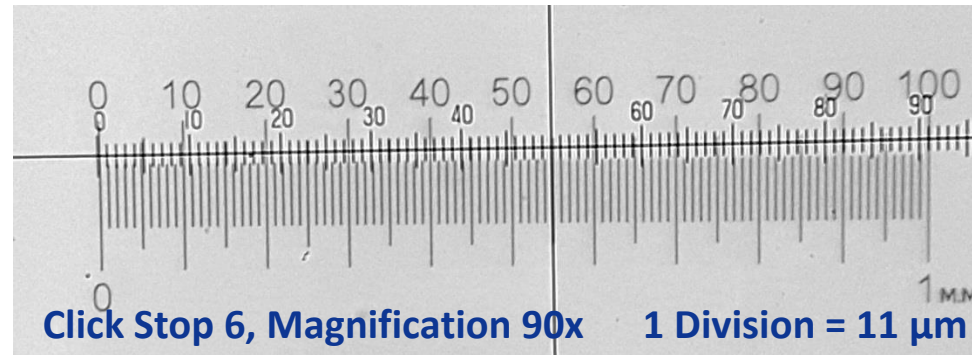
No. of Stage Micrometer Divisions	÷	No. of Eye- Piece Reticle Divisions	×	µm per Stage Micrometer Division	=	One Eyepiece Reticle Division (µm)
↓		↓		↓		↓
100 Stage Micrometer Divisions	÷	91 Reticle Divisions	×	10 µm per Stage Micrometer Division	=	One Eyepiece Reticle Division is 11 µm at 90x



Calibrating Your Objectives (Step 4 of 5)

4. Increase magnification and repeat calculations for each click stop.

Every time you increase magnification, you will need to realign the zeros from both scales and count the divisions. Continue this process until you have calculated the size of one division at each magnification.



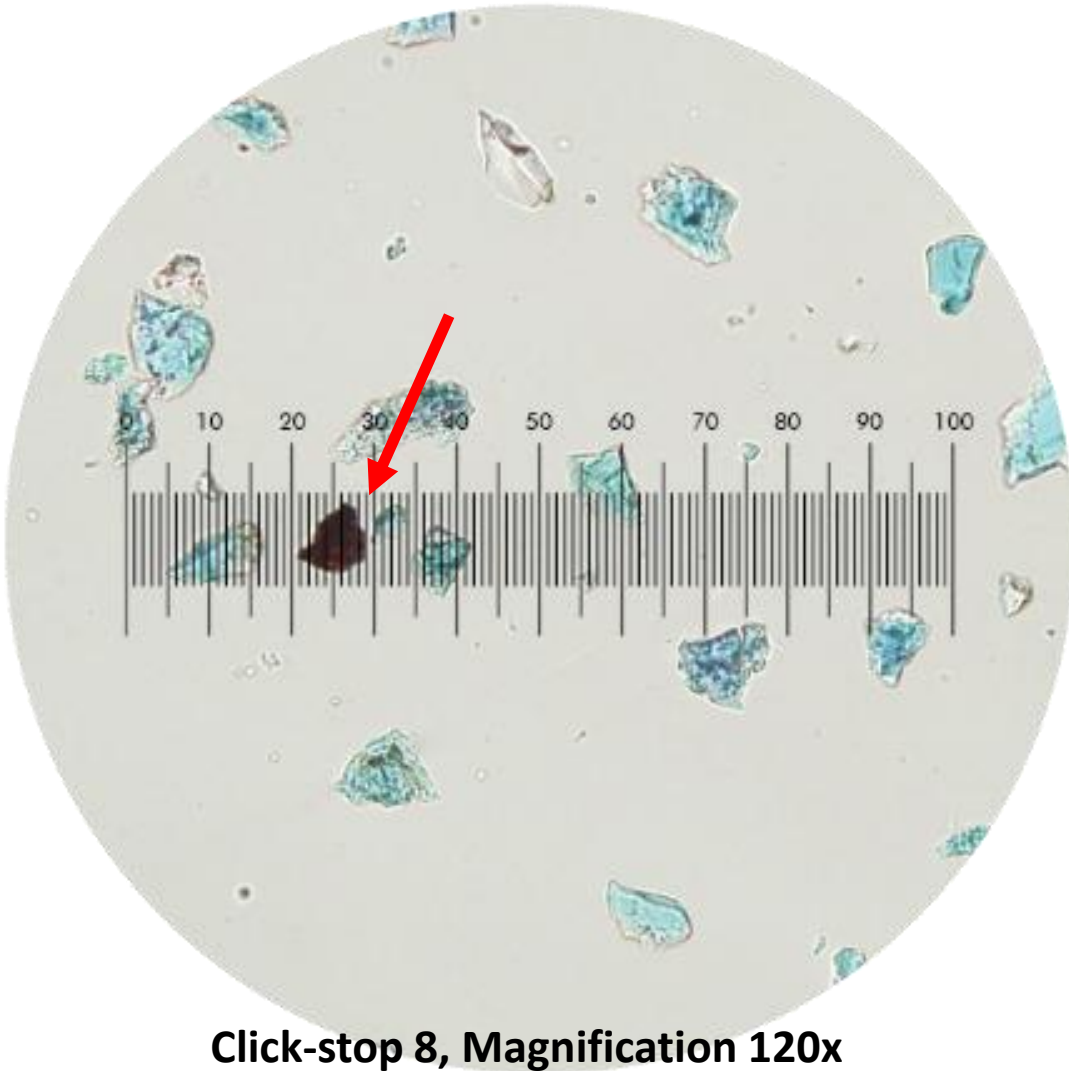
Calibrating Your Objectives (Step 5 of 5)

5. Create a sizing chart for your work station.

Instead of making calculations every time you measure a particle, create a cheat sheet to expedite your observation process.

Click stops and Magnification								
Divisions		0.63 (9.45x)	1 (15x)	2 (30x)	3 (45x)	4 (60x)	6 (90x)	8 (120x)
	1	105	67	33	22	17	11	8
	2	210	134	66	44	34	22	16
	3	315	201	99	66	51	33	24
	4	420	268	132	88	68	44	32
	5	525	335	165	110	85	55	40
	6	630	402	198	132	102	66	48
	7	735	469	231	154	119	77	56
	8	840	536	264	176	136	88	64
	9	945	603	297	198	153	99	72
	10	1050	670	330	220	170	110	80

Measuring a Particle



Click stops and Magnification

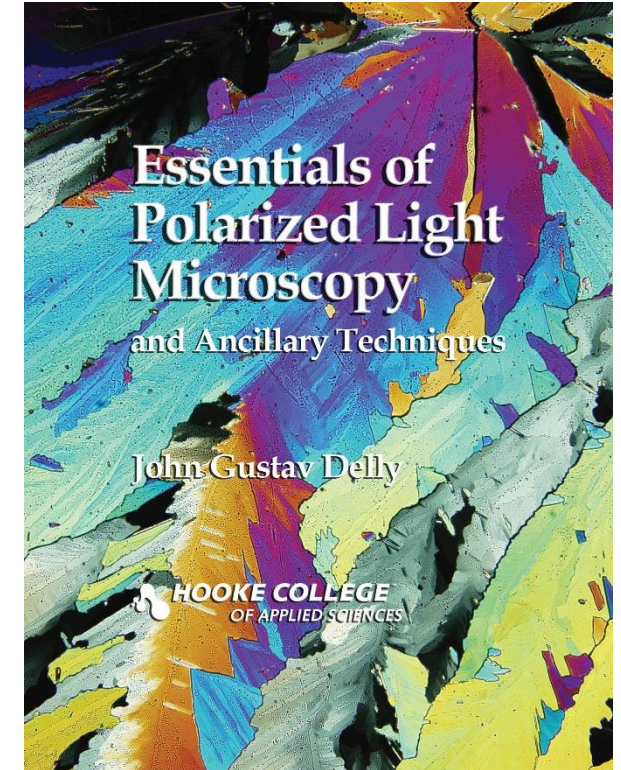
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9	945	603	297	198	153	99	72
10	1050	670	330	220	170	110	80

Divisions

References & Acknowledgements

Delly, John Gustav. “Micrometry and the Calibration of the Eyepiece Micrometer.” *Essentials of Polarized Light Microscopy and Ancillary Techniques*. Westmont, IL: The McCrone Group, Inc., 2017. 51-66.

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Thank you for joining us.

Nicole Groshon

Cleanroom Microscopist

ngroshon@mccrone.com • www.linkedin.com/in/ngroshon • (630) 887-7100