How to Calibrate a Miscrocope Using a Resolution Target

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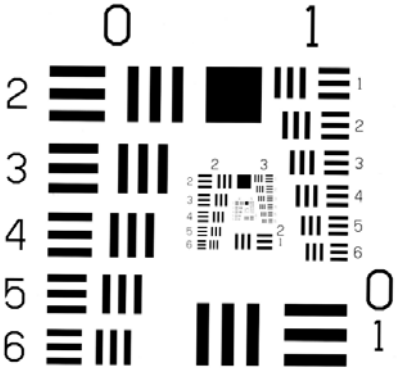
One of the first an easiest ways one can start trying being quantitative when approaching biology problems is to include scale bars in every image that is taken. As we will see throughout the first weeks of the course this will allow us to make all kinds of measurements and estimations not only about numbers and sizes, but also about composition and rates (once we include time in the picture, of course).

In the lab we will use a standard resolution target [1], which can be used for both testing the quality of an optical system or calibrating it. In fig. 1 we present a picture of the resolution target and a table that shows how to read it. The target is separated into groups and each group is divided into different elements. For example, in the lower right corner you have group 0, element 1. Group 0 continues in the upper left corner with element 2 and so on. The table gives the density if lines in lines per millimeter, therefore the periodicity of the lines in each element is 1/density.



Line Pairs per millimeter

Element Number	Group Number							
	0	1	2	3	4	5	6	7
1	1.00	2.00	4.00	8.00	16.00	32.00	64.00	128.00
2	1.12	2.24	4.49	8.98	17.96	35.92	71.84	143.70
3	1.26	2.52	5.04	10.08	20.16	40.32	80.63	161.30
4	1.41	2.83	5.66	11.31	22.63	45.25	90.51	181.00
5	1.59	3.17	6.35	12.70	25.40	50.80	101.60	203.20
6	1.78	3.56	7.13	14.25	28.51	57.02	114.00	228.10
Line pairs/mm = LP					Line width (mm) = 1/(2LP)			
Space width (mm) = 1/(2LP)					Line length = 5(line width)			

Figure 1: Picture of the resolution target and table that shows how to read it [1]

When used for testing the quality of an optical system the idea is that diffraction (light getting diffracted from the feature's edges) and aberrations (mainly due to imperfections in the mirrors and lenses that are part of the system) are the ultimate limitations. The presence of simple periodic features makes it easy to determine when the pattern is starting to be affected by these effects.

This resolution target has a range from 1mm to 4 μm, which is going to be reasonable for most applications in this lab. When using regular objectives one has to make sure that is the focus is not made through the target's glass, the side with the features should be facing the objective in order to reduce possible aberrations. When using an immersion oil objective a large cover slip should be

put in between making sure that no oil gets on the target. Each magnification will have a suitable group to look at.

A good check can be testing the linearity of the microscope: Is there a linear change in magnification when one changes objectives? Additionally, one might one to play with the focus and take different pictures for different focus settings which are close to what appears to be the right one. In that way, when later looking at the pictures with a program like Photoshop, one can have an idea of the sensitivity of the system to small displacements of the focal plane.

References

[1] Newport. Usaf-1951 test targets (www.newport.com).