

Olympus BX-51 microscope calibration

July 2010

OBJECTIVE	OBJECTIVE	pixel length	um	Calibration pixels/um	Calibration um/pixel
10x objective	10	1245.0	800	1.556	0.643
40x objective NA=0.5	40	933.0	150	6.220	0.161
40x objective NA=1.0	40	930.0	150	6.200	0.161
60x objective NA=0.65	60	934.0	100	9.340	0.107
60x objective NA=1.25	60	934.0	100	9.340	0.107
100x objective	100	1258.0	80	15.725	0.064
Binning = 1		Scale measurement			

Note: Binning on the Jai CVm4+ camera is set to 1.

JAI CVM4+ progressive-scan 24 fps B&W fluorescence CCD

This is the maximum number of pixels - the camera is at maximum resolution

Image size is 1376x1024 pixels [with binning set to 1].

Most users now have camera binning set to 1, you can check easily enough which setting was used by looking at the image size

Note that the 40x and 60x objective has a variable NA adjustment collar but this doesn't affect the calibration value significantly

OBJECTIVE	OBJECTIVE	pixel length	um	Calibration pixels/um	Calibration um/pixel
10x objective	10	621.0	800	0.776	1.288
40x objective NA=0.5	40	462.0	150	3.080	0.325
40x objective NA=1.0	40	465.0	150	3.100	0.323
60x objective NA=0.65	60	468.0	100	4.680	0.214
60x objective NA=1.25	60	467.0	100	4.670	0.214
100x objective	100	627.0	80	7.838	0.128
Binning = 2		Scale measurement			

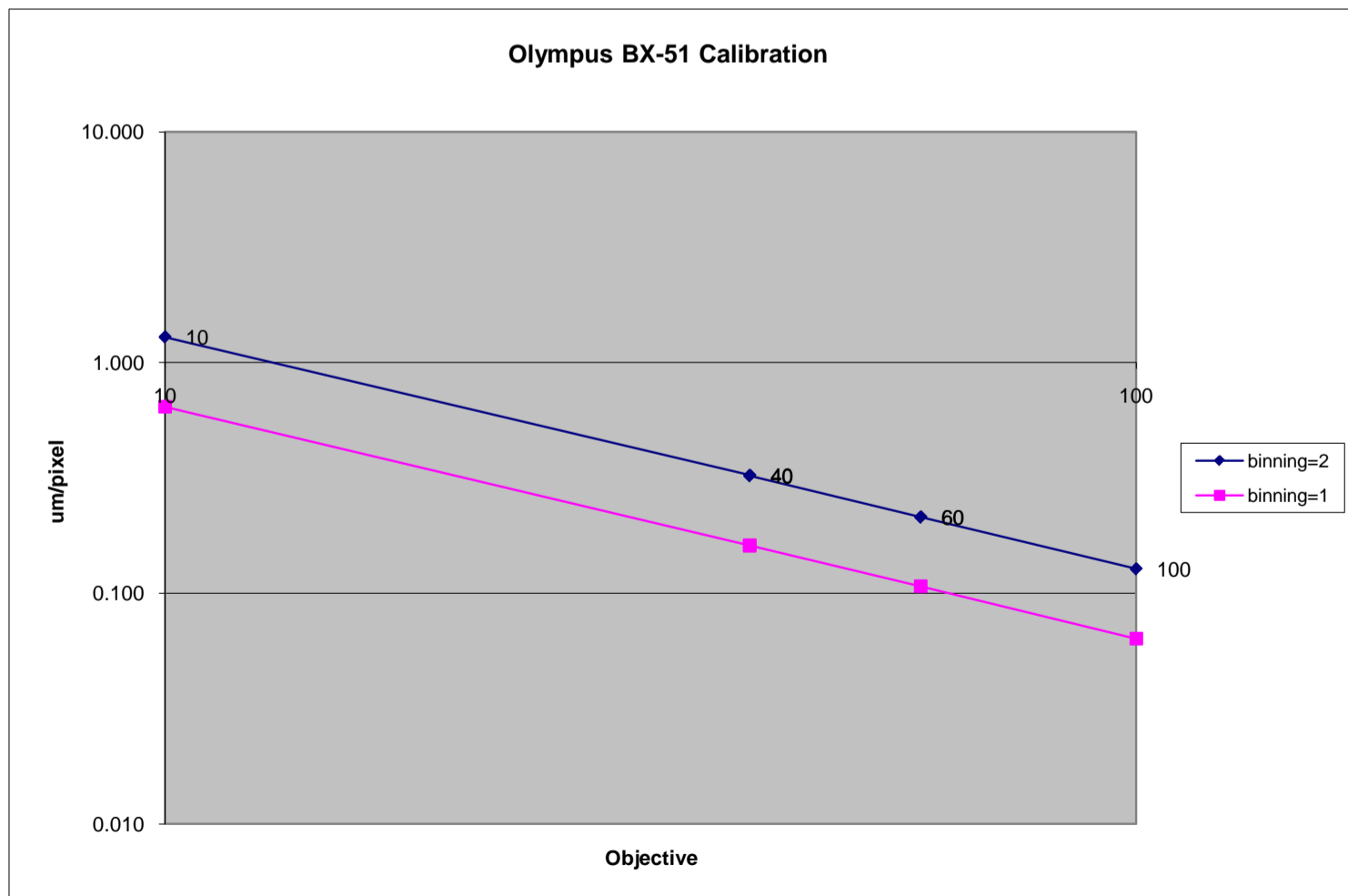
Note: Binning on the Jai CVm4+ camera is set to 2.

JAI CVM4+ progressive-scan 24 fps B&W fluorescence CCD

This combines each set of 4 pixels into 1, reducing resolution by a factor of 2 (but increasing sensitivity by a similar amount)

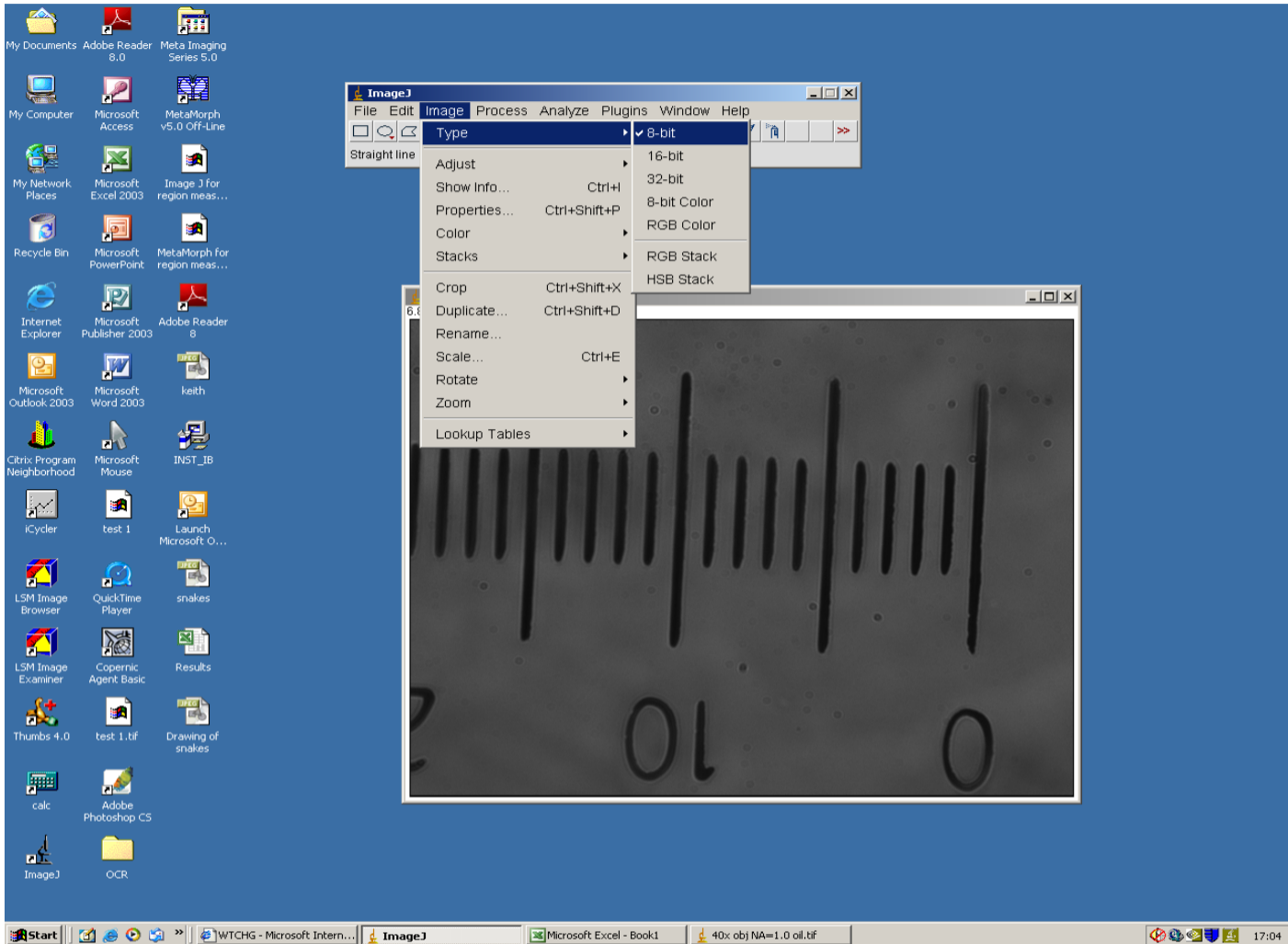
Image size is 688x512 [with binning set to 2]. Most users set binning to 1 with this new camera.

Note that the 40x and 60x objective has a variable NA adjustment collar but this doesn't affect the calibration value significantly

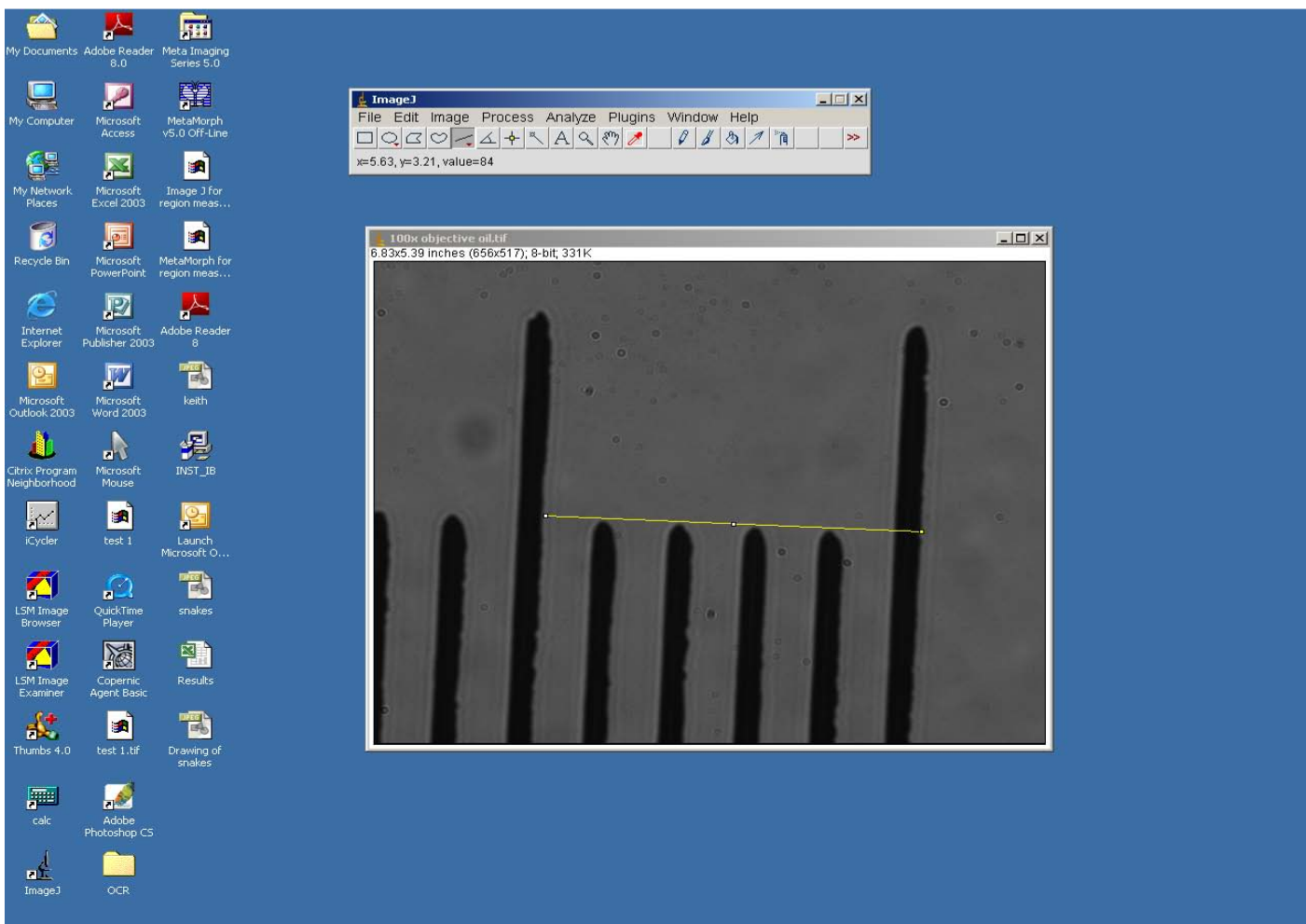


Calibrated using ImageJ

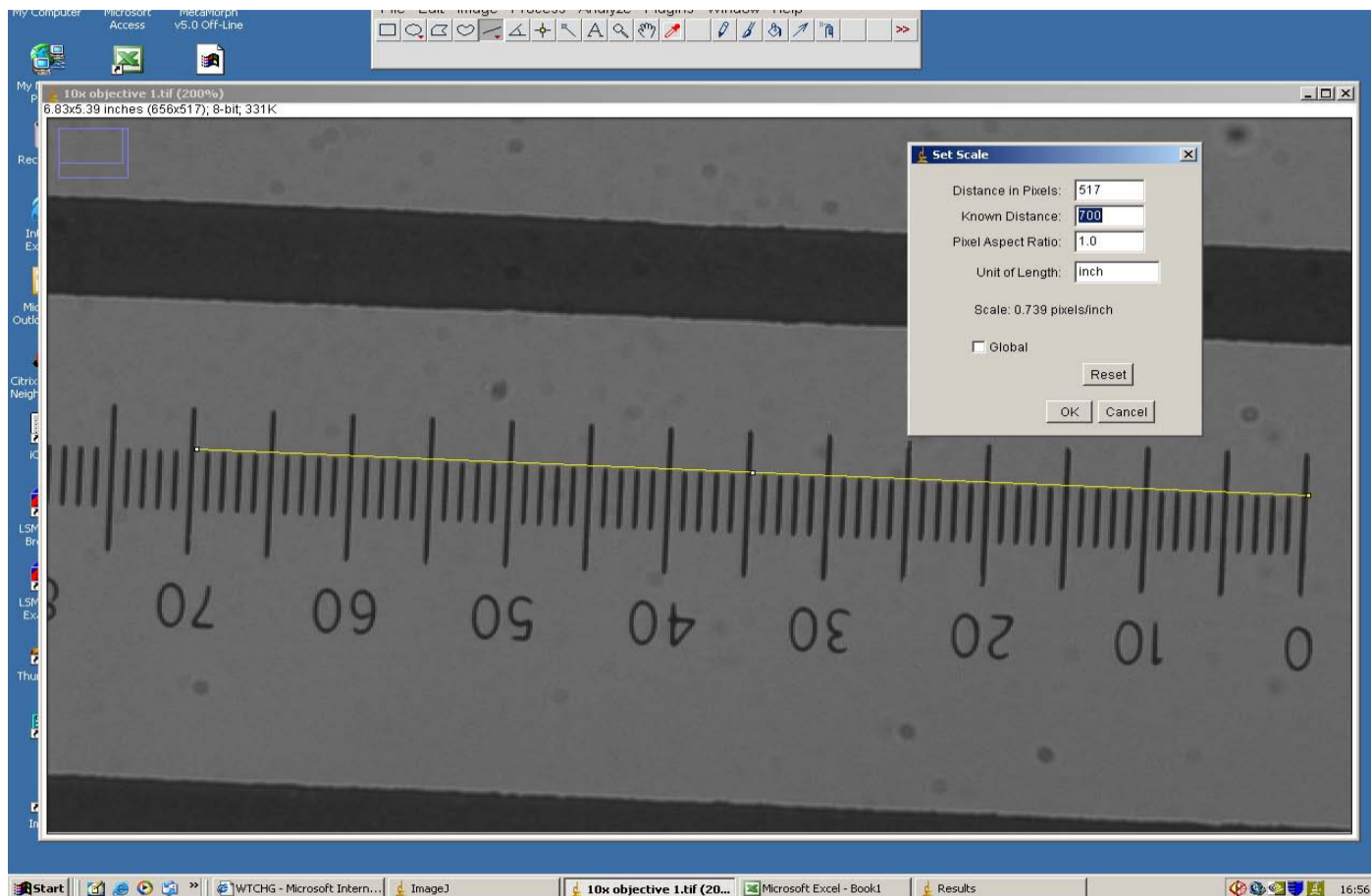
Use image, type, 8-bit (e.g. converted to 256 grey levels)
Change image type from 36-bit RGB to 8-bit for set scale



Use 'straight line' selection - the button is 'pushed in' [greyed out], right click for other options
Draw a line with the mouse (left click down)



Use Analyze, Set scale to obtain the calibration
The scale length measured is 700 um (the scale is 1mm long and is graduated from 0 to 100 in units of ten)



You can use Analyze, Set Scale to input both values using the calibration data in this pdf file
Select OK and the image will be calibrated for ImageJ area/length measurements in um etc..

See our *Microscopy Web Site* links for the freeware image analysis software **ImageJ**

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