

How to change scale bar unit from pixel to calibrated resolution

Step1. find out the calibration resolution first.

Option 1: if you use the entire original converted tiff image (no cropping, no image size change), you can use either Cellsense (below example) or acquisition software FV ASW L100

The screenshot displays the CellSense software interface. The main window shows a grayscale image of a slide with a scale bar at the bottom right indicating 5 mm. The image contains text: "ULTRA PLUS", "S4", "Liver 3078", "Dako", and a QR code labeled "WIMR". A blue square ROI is placed on the image. The software's Properties panel on the left shows the following calibration data:

Property	Value
Name	Image_0120180828.vsi
Path	O:\Bec_CDDE\Folder...
Author	jennifer.chen
Creation Time	28/08/2018 8:23:42 ...
Note	
Company	
Product Version	OLYMPUS VS-ASW 2...
File Size	471.91 MB
Image	
Layer	40x
Frame Count	1
Channel Count	2
Type	16 bit Grayscale
Size (pixel)	30821 x 31845
Size (calibrated)	5 mm x 5.2 mm
Calibration (X)	162.774 nm/pixel
Calibration (Y)	162.774 nm/pixel
Origin (X)	-98.17 mm

The Count and Measure Results panel at the bottom shows a table with the following columns: Object ID, Object Class, Area [m²], Perimeter [m], Mean (Radius) [m], Mean (Gray Intensity Value), Mean (Color Intensity Value), and Shape Factor. The table currently contains three empty rows.

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Option 2: if you only use the cropped portion of the entire original converted tiff image, it is a bit more complex. In this case, it is not too hard because your cropped image comes with a scale bar (50 um in this case). Let's use this as an example to figure out calibrated image resolution.

The screenshot shows the Aperio ImageScope software interface. The main window displays a fluorescence image of a cell with a blue signal. A ruler is visible at the bottom right of the image, labeled "300p". A green box highlights a measurement of "104.1" on the ruler, with a label "50 μm" below it. The software interface includes a menu bar (File, Edit, Image, View, Tools, Window, Help), a toolbar with various icons, and a zoom control panel on the left. The zoom panel shows a slider and buttons for 5%, 10%, 20%, 25%, 50%, 100%, and 71% (highlighted). The status bar at the bottom displays image dimensions and file information: "1360 x 1024 x 3 = 4MB, File = 0MB", coordinates "-68, 0 : 1360 x 1024", "1250, 1021", and "prefetching / trackmap / progressive rendering".

a. click on this ruler icon

b. then draw a line over the original scale bar, you will get the pixel measurement of this scale bar line: 104.1 pixel. The calculation is: $50\mu\text{m}/104.1\text{pixel}=0.48\ \mu\text{m}/\text{pixel}$

300p

104.1

50 μm

1360 x 1024 x 3 = 4MB, File = 0MB

-68, 0 : 1360 x 1024

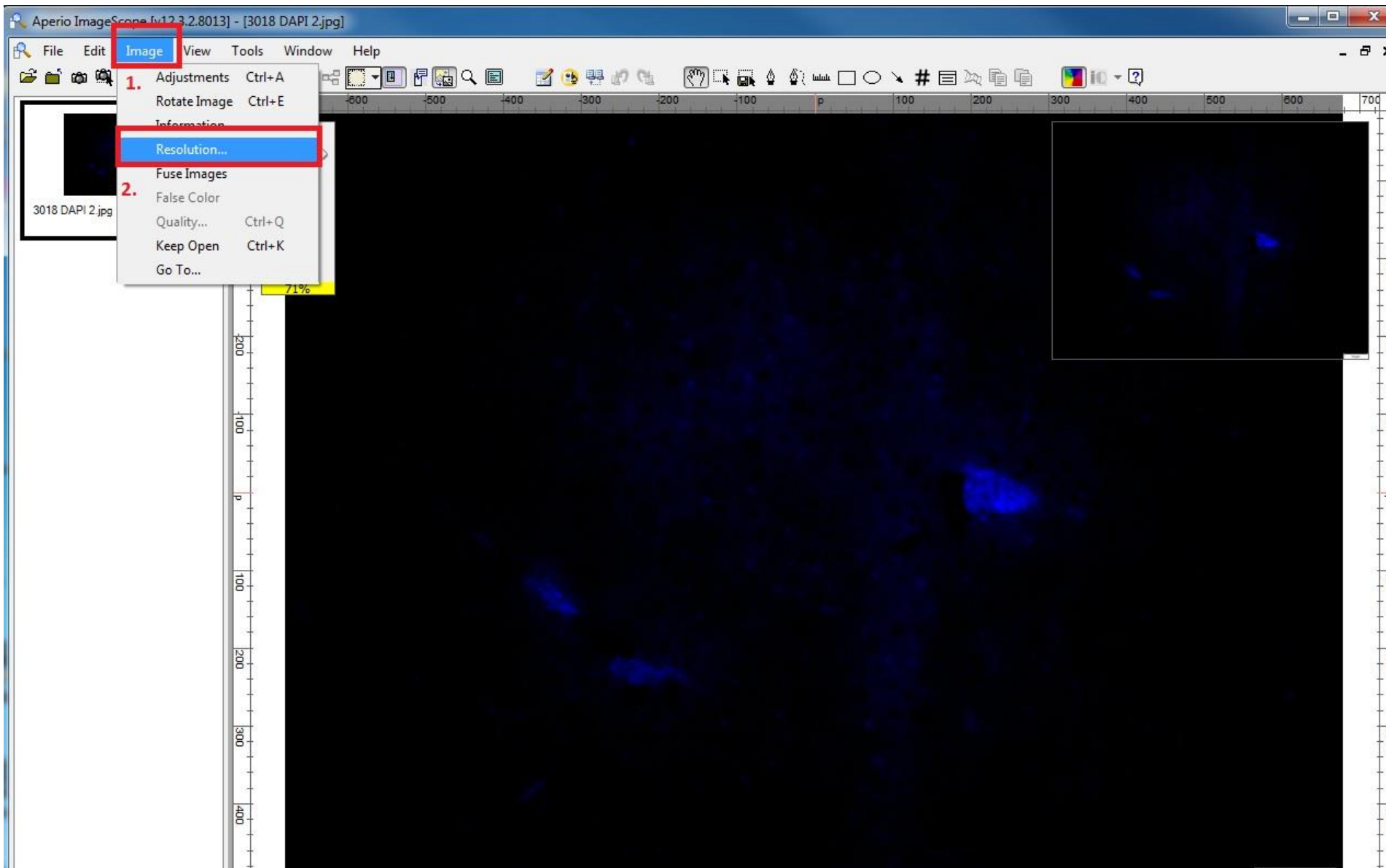
1250, 1021

prefetching / trackmap / progressive rendering

RULER

Step2. open up the target image in ImageScope as usual

Step3. In ImageScope, click "Image" then "resolution"



Step3. You will have a window popping up like this. Now manually type in the calibrated resolution number (0.163 or 0.48 obtained from step1. Note: double check unit! (163 nm/pixel means 0.163 um per pixel)

