



Registration

Microscope info:

Image		2026-06-16_40xResolution				
image's creation	date	unknown				
	method used	original file info & metadata could not be found				
Actual image depth		16				
Microscope type		WideField				
Objective	NA	1.3				
	im. refractive index	1.518				
Channel(s)	Wavelengths		Saturation	sampling (X,Y,Z)		
	Ex. (nm)	Em. (nm)		Nyquist (μm)	Found (μm)	Nyquist/found ratio
Channel 0		405.0	none	0.078x0.078x0.276	0.01x0.01x1.0	0.1, 0.1, 3.6
Channel 1		450.0	none	0.087x0.087x0.306		0.1, 0.1, 3.3
Channel 2		525.0	none	0.101x0.101x0.358		0.1, 0.1, 2.8
Channel 3		570.0	none	0.11x0.11x0.388		0.1, 0.1, 2.6

Warnings:

(No saturated pixels detected).

The highlighted undersampled channels may alter the result interpretation.

(The bead size is appropriate for this coalignment analysis).

Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		1.439	1.553	0.301
Channel 1	1.439		0.118	1.597
Channel 2	1.553	0.118		1.705
Channel 3	0.301	1.597	1.705	
X, Y, Z theoretical resolutions (in μm)	0.159, 0.159 & 0.644	0.177, 0.177 & 0.715	0.206, 0.206 & 0.835	0.224, 0.224 & 0.906
Bead centres' coord.(X,Y & Z in μm)	1.37, 2.104 & 10.5	1.302, 1.859 & 10.5	1.302, 1.839 & 10.5	1.422, 2.115 & 10.5
Bead quality (SB Ratio)	1.6	1.4	1.2	1.6
Title	C1-2026-06-16_40xResolution	C2-2026-06-16_40xResolution	C3-2026-06-16_40xResolution	C4-2026-06-16_40xResolution

Ratio of the mesured calibrated distance to the reference distance (as calulated using the longest wavelength).
The annulus drawn around the bead to compute the background mean intensity is bigger than the image. Increase the cropfactor or decrease the anulus thickness
Green: within specifications, red: outside specifications (ie. ratio above 1.0)

Profile view:

Channel 0 (Em. Wavelength 405.0 nm) vs channel 1 (Em. Wavelength 450.0 nm)

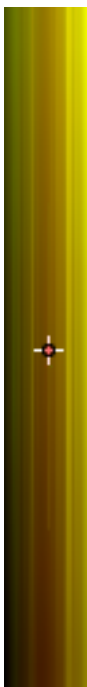
XY



YZ



XZ



Channel 0 (Em. Wavelength 405.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)

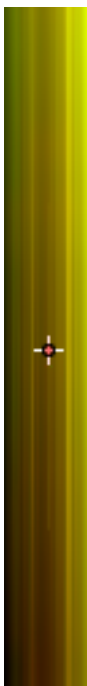
XY



YZ



XZ



Channel 0 (Em. Wavelength 405.0 nm) vs channel 3 (Em. Wavelength 570.0 nm)

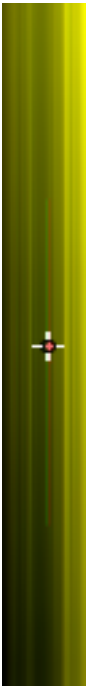
XY



YZ



XZ



Channel 1 (Em. Wavelength 450.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)

XY



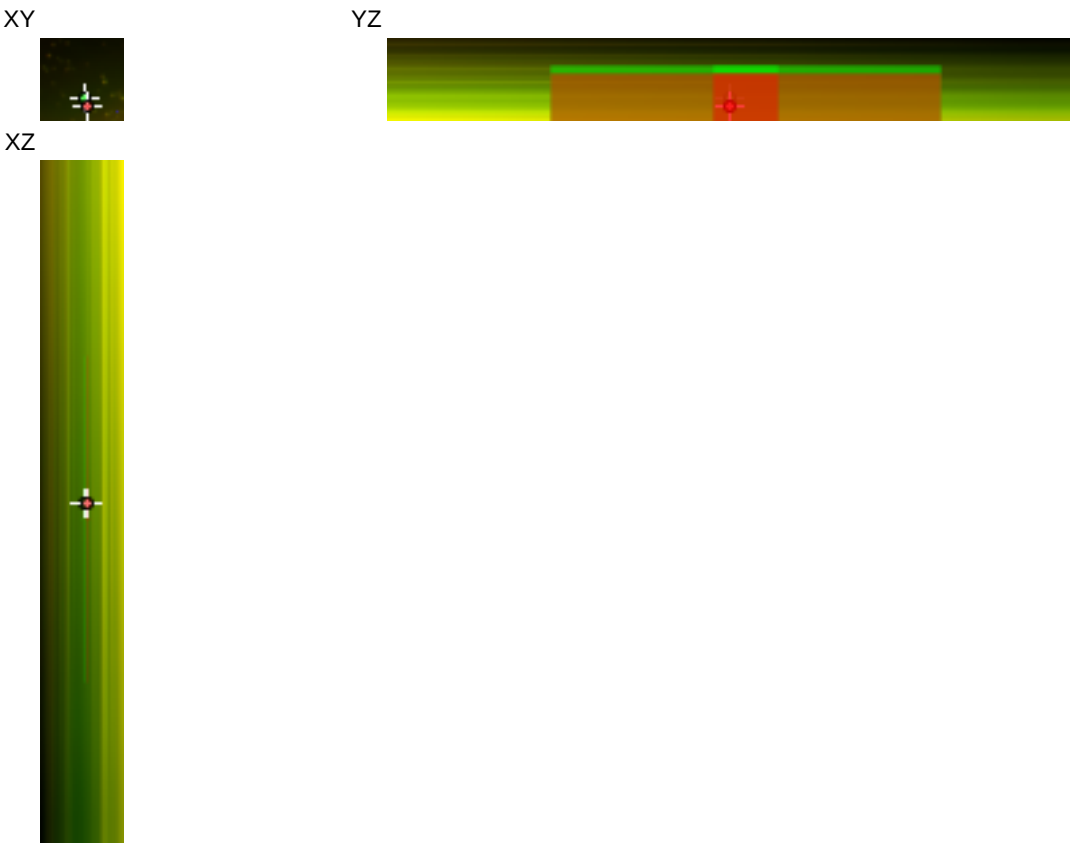
YZ



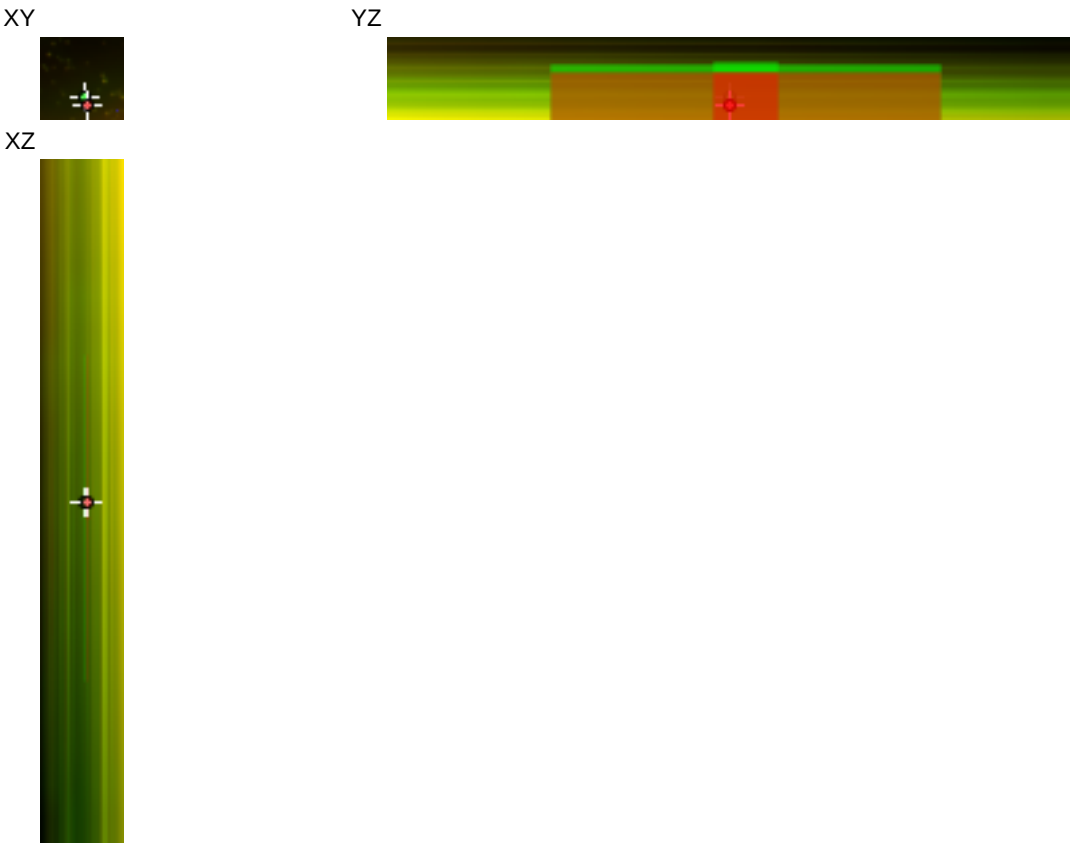
XZ



Channel 1 (Em. Wavelength 450.0 nm) vs channel 3 (Em. Wavelength 570.0 nm)



Channel 2 (Em. Wavelength 525.0 nm) vs channel 3 (Em. Wavelength 570.0 nm)



ISO 21073 co-registration accuracy:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Lateral distance (in μm)		0.254	0.274	0.053
	Axial distance (in μm)		0.0	0.0	0.0
Channel 1	Lateral distance (in μm)	0.254		0.021	0.282
	Axial distance (in μm)	0.0		0.0	0.0
Channel 2	Lateral distance (in μm)	0.274	0.021		0.301
	Axial distance (in μm)	0.0	0.0		0.0
Channel 3	Lateral distance (in μm)	0.053	0.282	0.301	
	Axial distance (in μm)	0.0	0.0	0.0	

Pixel shift table:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	X shift		-6.5	-6.5	5.0
	Y shift		-23.5	-25.5	1.0
	Z shift		0.0	0.0	0.0
Channel 1	X shift	6.5		0.0	11.5
	Y shift	23.5		-2.0	24.5
	Z shift	0.0		0.0	0.0
Channel 2	X shift	6.5	0.0		11.5
	Y shift	25.5	2.0		26.5
	Z shift	0.0	0.0		0.0
Channel 3	X shift	-5.0	-11.5	-11.5	
	Y shift	-1.0	-24.5	-26.5	
	Z shift	0.0	0.0	0.0	
X, Y & Z theoretical resolutions (in pix.)		15.253, 15.253 & 0.644	16.948, 16.948 & 0.715	19.772, 19.772 & 0.835	21.467, 21.467 & 0.906
Centres'coord. in pixels (X, Y & Z)		131.5, 202.0 & 10.5	125.0, 178.5 & 10.5	125.0, 176.5 & 10.5	136.5, 203.0 & 10.5
Title		C1-2026-06-16_40xR esolution	C2-2026-06-16_40xR esolution	C3-2026-06-16_40xR esolution	C4-2026-06-16_40xR esolution

Distances table (uncalibrated):

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		24.382	26.315	5.099
Channel 1	24.382		2.0	27.065
Channel 2	26.315	2.0		28.888
Channel 3	5.099	27.065	28.888	
X, Y & Z resolutions (in pix.)	15.253, 15.253 & 0.644	16.948, 16.948 & 0.715	19.772, 19.772 & 0.835	21.467, 21.467 & 0.906
Centres'coord.(X, Y & Z in pix.)	131.5, 202.0 & 10.5	125.0, 178.5 & 10.5	125.0, 176.5 & 10.5	136.5, 203.0 & 10.5
Title	C1-2026-06-16_40xRe solution	C2-2026-06-16_40xRe solution	C3-2026-06-16_40xRe solution	C4-2026-06-16_40xRe solution

Distances table (calibrated):

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Measured distance in μm)		0.254	0.274	0.053
	Reference distance in μm)		0.177	0.177	0.177
Channel 1	Measured distance in μm)	0.254		0.021	0.282
	Reference distance in μm)	0.177		0.177	0.177
Channel 2	Measured distance in μm)	0.274	0.021		0.301
	Reference distance in μm)	0.177	0.177		0.177
Channel 3	Measured distance in μm)	0.053	0.282	0.301	
	Reference distance in μm)	0.177	0.177	0.177	
X, Y & Z resolutions (in μm)		0.159, 0.159 & 0.644	0.177, 0.177 & 0.715	0.206, 0.206 & 0.835	0.224, 0.224 & 0.906
Centres' coord.(X, Y & Z, in μm)		1.4, 2.1 & 10.5	1.3, 1.9 & 10.5	1.3, 1.8 & 10.5	1.4, 2.1 & 10.5
Title		C1-2026-06-16_40xR esolution	C2-2026-06-16_40xR esolution	C3-2026-06-16_40xR esolution	C4-2026-06-16_40xR esolution

reference distance is calculated using the longest wavelength

Analysis parameters

Tool & Operator	Tool	Co-registration
	Versions	MetroloJ QC v1.3.1.2, ImageJ v2.16.0/1.54p, Java v1.8.0_452, OS Windows 11
	Operator & date	Hanna, July 2, 2026 10:00 AM
data	result folder	Z:\CTIU\QC\2026-06-16\Evident\Processed\Registration\40x\Processed\Registration\
	Type of saved data	.pdf, .jpg, .xls
	Input data bit depth	16
Dimension order		XY-(C)Z
Discard saturated samples		false
Beads	Bead detection threshold	Legacy
	Center detection method	Legacy Fit Ellipses
	Discard bead if more than one particle are thresholded	false
	Background annulus thickness in μm	1.0
	Background annulus distance to bead edges in μm	0.5
	Multiple beads in image	false
Tolerance	Applied in this report	true
	Ratio valid if below	1.0

Analysis log

image name	creation date	saturation	sampling density	status
2026-06-16_40xResolution	unknown	none	Ch.0,1,2,3 undersampled	analysed

Formulas used:

The reference distance is calculated as follows:

$$r_{ref} = \frac{res_x^o * res_y^o * res_z^o}{\sqrt{(res_y^o * res_z^o * \cos\Phi * \sin\theta)^2 + (res_x^o * res_z^o * \sin\Phi * \sin\theta)^2 + (res_x^o * res_y^o * \cos\theta)^2}}$$

$$\Phi = \arccos \frac{x_B - x_A}{\sqrt{(x_B - x_A)^2 + (y_B - y_A)^2}}$$

$$\theta = \arccos \frac{z_B - z_A}{\sqrt{(x_B - x_A)^2 + (y_B - y_A)^2 + (z_B - z_A)^2}}$$

$$res_{x,y}^o = \frac{0.51 * \lambda_{em}}{NA} \quad res_z^o = \frac{\lambda_{em}}{n - \sqrt{n^2 - NA^2}}$$

x_A, y_A, z_A and x_B, y_B, z_B are the bead coordinates in channel A and B respectively, NA: numerical aperture, λ_{em} : emission wavelength, n: refractive index of the lens immersion & mounting media.

Lateral ($res_{x,y}^o$) and axial (res_z^o) theoretical resolution values used for widefield microscopes are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy, 2011.

Compliance with the Shannon-Nyquist criterion uses the following formulas for Shannon-Nyquist distances calculation:

$$\alpha = \arcsin\left(\frac{NA}{n}\right)$$

$$\Delta_{x,y} = \frac{\lambda_{em}}{4.NA} \quad \Delta_z = \frac{\lambda_{em}}{2.n.(1 - \cos(\alpha))}$$