



Registration63x

Microscope info:

Image		2026-06-15_63xResolution				
image's creation	date					
	method used					
Actual image depth		16				
Microscope type		WideField				
Objective	NA	1.4				
	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		410.0	none	0.073x0.073x0.22	0.103x0.103x0.201	1.4, 1.4, 0.9
Channel 1		450.0	none	0.08x0.08x0.242		1.3, 1.3, 0.8
Channel 2		525.0	none	0.094x0.094x0.282		1.1, 1.1, 0.7
Channel 3		570.0	none	0.102x0.102x0.306		1.0, 1.0, 0.7

Warnings:

(No saturated pixels detected).

The highlighted undersampled channels may alter the result interpretation.

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

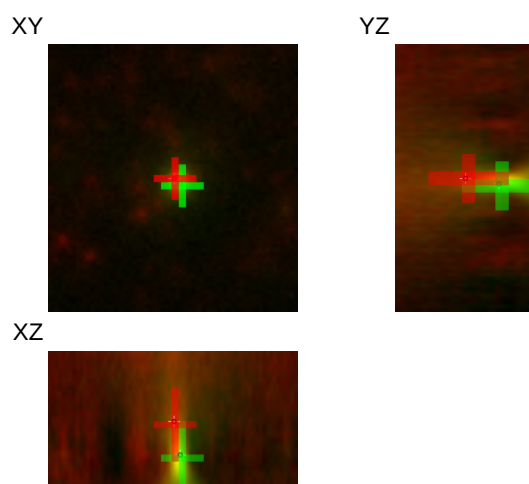
Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		2.267	2.061	2.397
Channel 1	2.267		0.944	1.147
Channel 2	2.061	0.944		0.652
Channel 3	2.397	1.147	0.652	
X, Y, Z theoretical resolutions (in μm)	0.149, 0.149 & 0.562	0.164, 0.164 & 0.617	0.191, 0.191 & 0.72	0.208, 0.208 & 0.781
Bead centres' coord.(X,Y & Z in μm)	3.869, 4.075 & 3.122	3.663, 3.921 & 2.115	3.663, 4.075 & 2.115	3.714, 4.075 & 1.762
Bead quality (SB Ratio)	4.0	1.2	4.9	5.3
Title	C1-2026-06-15_63xResolution.tif	C2-2026-06-15_63xResolution.tif	C3-2026-06-15_63xResolution.tif	C4-2026-06-15_63xResolution.tif

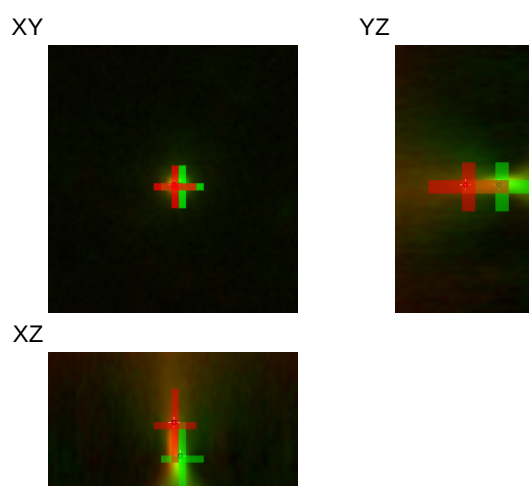
Ratio of the mesured calibrated distance to the reference distance (as calulated using the longest wavelength).
Green: within specifications, red: outside specifications (ie. ratio above 1.0)

Profile view:

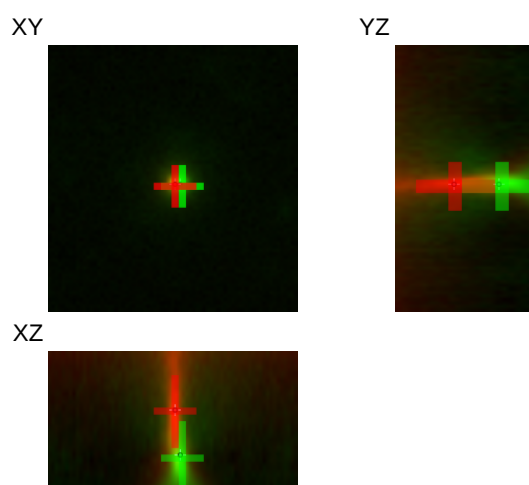
Channel 0 (Em. Wavelength 410.0 nm) vs channel 1 (Em. Wavelength 450.0 nm)



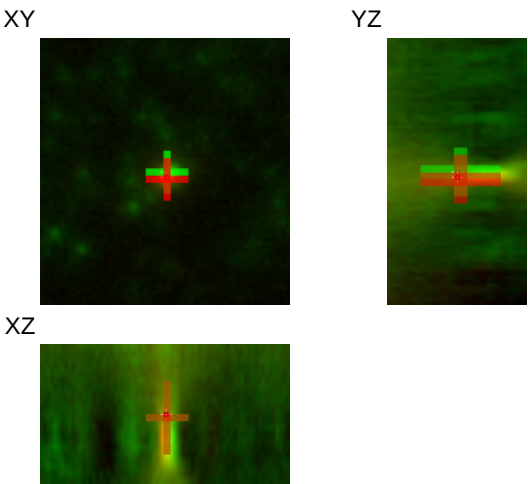
Channel 0 (Em. Wavelength 410.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)



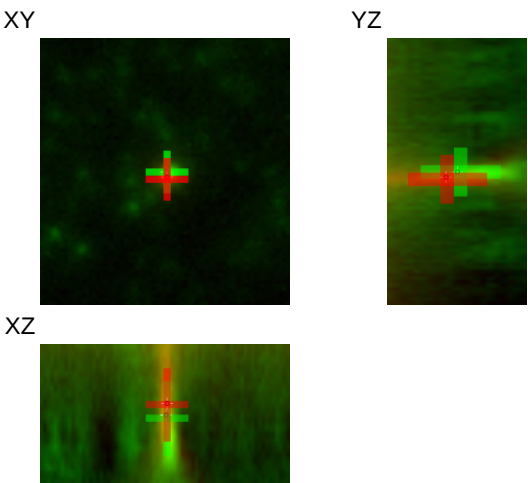
Channel 0 (Em. Wavelength 410.0 nm) vs channel 3 (Em. Wavelength 570.0 nm)



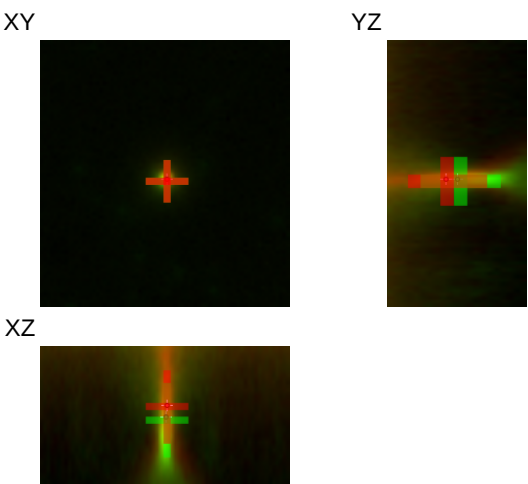
Channel 1 (Em. Wavelength 450.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)



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)		0.258	0.206	0.155
	Axial distance (in μm)		1.007	1.007	1.359
Channel 1	Lateral distance (in μm)	0.258		0.155	0.163
	Axial distance (in μm)	1.007		0.0	0.352
Channel 2	Lateral distance (in μm)	0.206	0.155		0.052
	Axial distance (in μm)	1.007	0.0		0.352
Channel 3	Lateral distance (in μm)	0.155	0.163	0.052	
	Axial distance (in μm)	1.359	0.352	0.352	

Pixel shift table:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	X shift		-2.0	-2.0	-1.5
	Y shift		-1.5	0.0	0.0
	Z shift		-5.0	-5.0	-6.75
Channel 1	X shift	2.0		0.0	0.5
	Y shift	1.5		1.5	1.5
	Z shift	5.0		0.0	-1.75
Channel 2	X shift	2.0	0.0		0.5
	Y shift	0.0	-1.5		0.0
	Z shift	5.0	0.0		-1.75
Channel 3	X shift	1.5	-0.5	-0.5	
	Y shift	0.0	-1.5	0.0	
	Z shift	6.75	1.75	1.75	
X, Y & Z theoretical resolutions (in pix.)		1.448, 1.448 & 2.791	1.589, 1.589 & 3.063	1.854, 1.854 & 3.573	2.013, 2.013 & 3.88
Centres'coord. in pixels (X, Y & Z)		37.5, 39.5 & 15.5	35.5, 38.0 & 10.5	35.5, 39.5 & 10.5	36.0, 39.5 & 8.8
Title		C1-2026-06-15_63xResolution.tif	C2-2026-06-15_63xResolution.tif	C3-2026-06-15_63xResolution.tif	C4-2026-06-15_63xResolution.tif

Distances table (uncalibrated):

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		5.59	5.385	6.915
Channel 1	5.59		1.5	2.358
Channel 2	5.385	1.5		1.82
Channel 3	6.915	2.358	1.82	
X, Y & Z resolutions (in pix.)	1.448, 1.448 & 2.791	1.589, 1.589 & 3.063	1.854, 1.854 & 3.573	2.013, 2.013 & 3.88
Centres'coord.(X, Y & Z in pix.)	37.5, 39.5 & 15.5	35.5, 38.0 & 10.5	35.5, 39.5 & 10.5	36.0, 39.5 & 8.8
Title	C1-2026-06-15_63xResolution.tif	C2-2026-06-15_63xResolution.tif	C3-2026-06-15_63xResolution.tif	C4-2026-06-15_63xResolution.tif

Distances table (calibrated):

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Measured distance in μm)		1.04	1.028	1.368
	Reference distance in μm)		0.458	0.499	0.571
Channel 1	Measured distance in μm)	1.04		0.155	0.388
	Reference distance in μm)	0.458		0.164	0.338
Channel 2	Measured distance in μm)	1.028	0.155		0.356
	Reference distance in μm)	0.499	0.164		0.546
Channel 3	Measured distance in μm)	1.368	0.388	0.356	
	Reference distance in μm)	0.571	0.338	0.546	
X, Y & Z resolutions (in μm)		0.149, 0.149 & 0.562	0.164, 0.164 & 0.617	0.191, 0.191 & 0.72	0.208, 0.208 & 0.781
Centres' coord.(X, Y & Z, in μm)		3.9, 4.1 & 3.1	3.7, 3.9 & 2.1	3.7, 4.1 & 2.1	3.7, 4.1 & 1.8
Title		C1-2026-06-15_63xResolution.tif	C2-2026-06-15_63xResolution.tif	C3-2026-06-15_63xResolution.tif	C4-2026-06-15_63xResolution.tif

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, June 17, 2026 1:00 PM
data	result folder	Z:\CTIU\QC\2026-06-15\Thunder\Processed\Registration63x\
	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
2026-06-15_63xResolution		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:

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

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