



Registration40x

Microscope info:

Image		2026-06-15 40xResolution				
image's creation	date	2026-06-17 12:51:43				
	method used	from file creation date				
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	0.163x0.163x0.3	2.2, 2.2, 1.4
Channel 1		450.0	none	0.08x0.08x0.242		2.0, 2.0, 1.2
Channel 2		525.0	none	0.094x0.094x0.282		1.7, 1.7, 1.1
Channel 3		570.0	none	0.102x0.102x0.306		1.6, 1.6, 1.0

Warnings:

(No saturated pixels detected).

The highlighted undersampled channels may alter the result interpretation.

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

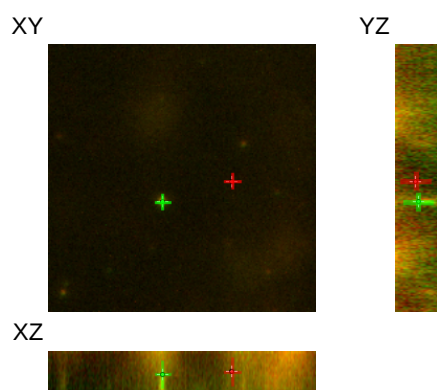
Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		51.61	21.802	21.78
Channel 1	51.61		35.25	35.247
Channel 2	21.802	35.25		0.608
Channel 3	21.78	35.247	0.608	
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)	13.244, 18.281 & 2.702	21.369, 15.925 & 2.327	15.6, 15.6 & 2.026	15.6, 15.6 & 2.402
Bead quality (SB Ratio)	2.5	1.3	2.5	2.8
Title	C1-2026-06-15_40xResolution.tif	C2-2026-06-15_40xResolution.tif	C3-2026-06-15_40xResolution.tif	C4-2026-06-15_40xResolution.tif

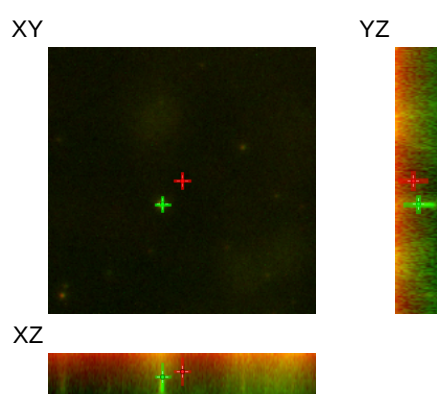
Ratio of the mesured calibrated distance to the reference distance (as calulated using the longest wavelength).
Green: within specifications, red: outside specifications (ie. ratio above 1.0)

Profile view:

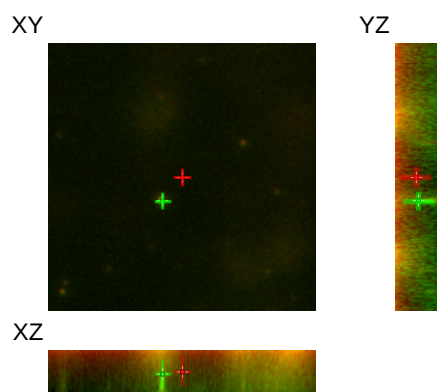
Channel 0 (Em. Wavelength 410.0 nm) vs channel 1 (Em. Wavelength 450.0 nm)



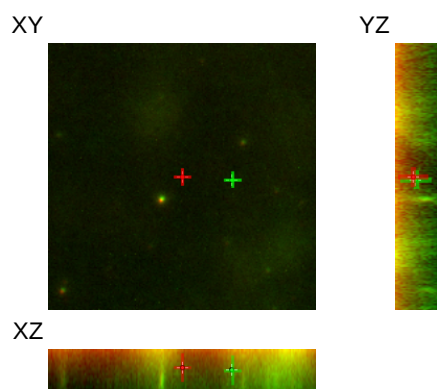
Channel 0 (Em. Wavelength 410.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)



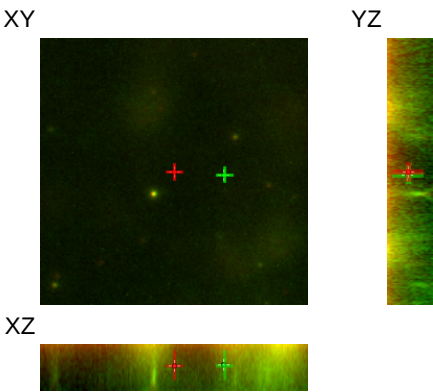
Channel 0 (Em. Wavelength 410.0 nm) vs channel 3 (Em. Wavelength 570.0 nm)



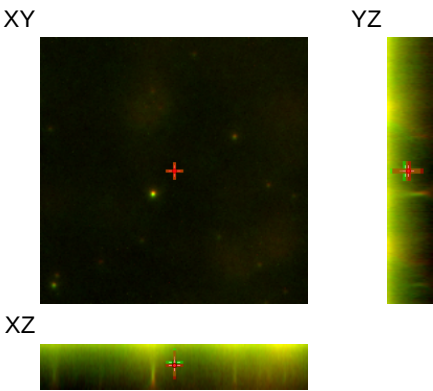
Channel 1 (Em. Wavelength 450.0 nm) vs channel 2 (Em. Wavelength 525.0 nm)



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)		8.46	3.569	3.569
	Axial distance (in μm)		0.375	0.675	0.3
Channel 1	Lateral distance (in μm)	8.46		5.778	5.778
	Axial distance (in μm)	0.375		0.3	0.075
Channel 2	Lateral distance (in μm)	3.569	5.778		0.0
	Axial distance (in μm)	0.675	0.3		0.375
Channel 3	Lateral distance (in μm)	3.569	5.778	0.0	
	Axial distance (in μm)	0.3	0.075	0.375	

Pixel shift table:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	X shift		50.0	14.5	14.5
	Y shift		-14.5	-16.5	-16.5
	Z shift		-1.25	-2.25	-1.0
Channel 1	X shift	-50.0		-35.5	-35.5
	Y shift	14.5		-2.0	-2.0
	Z shift	1.25		-1.0	0.25
Channel 2	X shift	-14.5	35.5		0.0
	Y shift	16.5	2.0		0.0
	Z shift	2.25	1.0		1.25
Channel 3	X shift	-14.5	35.5	0.0	
	Y shift	16.5	2.0	0.0	
	Z shift	1.0	-0.25	-1.25	
X, Y & Z theoretical resolutions (in pix.)		0.919, 0.919 & 1.872	1.009, 1.009 & 2.055	1.177, 1.177 & 2.397	1.278, 1.278 & 2.603
Centres'coord. in pixels (X, Y & Z)		81.5, 112.5 & 9.0	131.5, 98.0 & 7.8	96.0, 96.0 & 6.8	96.0, 96.0 & 8.0
Title		C1-2026-06-15_40xResolution.tif	C2-2026-06-15_40xResolution.tif	C3-2026-06-15_40xResolution.tif	C4-2026-06-15_40xResolution.tif

Distances table (uncalibrated):

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		52.075	22.081	21.989
Channel 1	52.075		35.57	35.557
Channel 2	22.081	35.57		1.25
Channel 3	21.989	35.557	1.25	
X, Y & Z resolutions (in pix.)	0.919, 0.919 & 1.872	1.009, 1.009 & 2.055	1.177, 1.177 & 2.397	1.278, 1.278 & 2.603
Centres'coord.(X, Y & Z in pix.)	81.5, 112.5 & 9.0	131.5, 98.0 & 7.8	96.0, 96.0 & 6.8	96.0, 96.0 & 8.0
Title	C1-2026-06-15_40xResolution.tif	C2-2026-06-15_40xResolution.tif	C3-2026-06-15_40xResolution.tif	C4-2026-06-15_40xResolution.tif

Distances table (calibrated):

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Measured distance in μm)		8.468	3.633	3.582
	Reference distance in μm)		0.164	0.167	0.164
Channel 1	Measured distance in μm)	8.468		5.786	5.778
	Reference distance in μm)	0.164		0.164	0.164
Channel 2	Measured distance in μm)	3.633	5.786		0.375
	Reference distance in μm)	0.167	0.164		0.617
Channel 3	Measured distance in μm)	3.582	5.778	0.375	
	Reference distance in μm)	0.164	0.164	0.617	
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)		13.2, 18.3 & 2.7	21.4, 15.9 & 2.3	15.6, 15.6 & 2.0	15.6, 15.6 & 2.4
Title		C1-2026-06-15_40xResolution.tif	C2-2026-06-15_40xResolution.tif	C3-2026-06-15_40xResolution.tif	C4-2026-06-15_40xResolution.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\Registration40x\
	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_40xResolution	2026-06-17 12:51:43	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:

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

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