



Registration

Microscope info:

Image		2026-06-12_40xResolution				
image's creation	date	2026-06-12 11:29:01				
	method used	from Metadata				
Actual image depth		8				
Microscope type		CLSM				
Pinhole		1.0 AU				
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	647.0		none	0.062x0.062x0.22	0.069x0.069x0.16	1.1, 1.1, 0.7
Channel 1	564.0		none	0.054x0.054x0.192		1.3, 1.3, 0.8
Channel 2	488.0		none	0.047x0.047x0.166		1.5, 1.5, 1.0
Channel 3	405.0		none	0.039x0.039x0.138		1.8, 1.8, 1.2

Warnings:

(No saturated pixels detected).

The highlighted undersampled channels may alter the result interpretation.

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

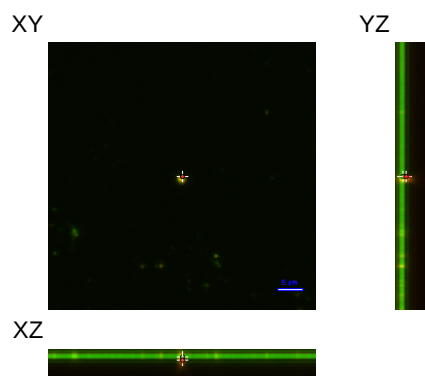
Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		0.928	3.209	0.928
Channel 1	0.928		3.024	0.000
Channel 2	3.209	3.024		3.024
Channel 3	0.928	0.000	3.024	
X, Y, Z theoretical resolutions (in μm)	0.254, 0.254 & 0.775	0.221, 0.221 & 0.676	0.191, 0.191 & 0.585	0.159, 0.159 & 0.485
Bead centres' coord.(X,Y & Z in μm)	26.621, 26.621 & 1.52	26.621, 26.621 & 2.24	26.517, 27.38 & 2.36	26.621, 26.621 & 2.24
Bead quality (SB Ratio)	0.0	0.0	0.0	0.0
Title	C1-2026-06-12_40xResolution.czi	C2-2026-06-12_40xResolution.czi	C3-2026-06-12_40xResolution.czi	C4-2026-06-12_40xResolution.czi

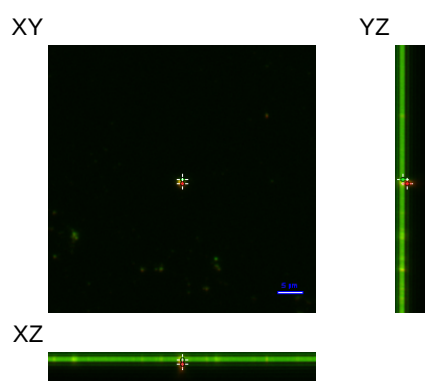
Ratio of the mesured calibrated distance to the reference distance (as calulated using the longest wavelength).
Green: within specifications, red: outside specifications (ie. ratio above 1.0)

Profile view:

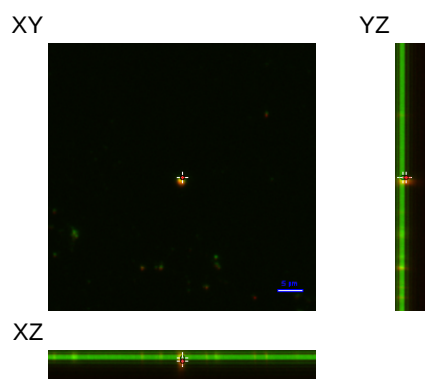
Channel 0 (Em. Wavelength 0.0 nm) vs channel 1 (Em. Wavelength 0.0 nm)



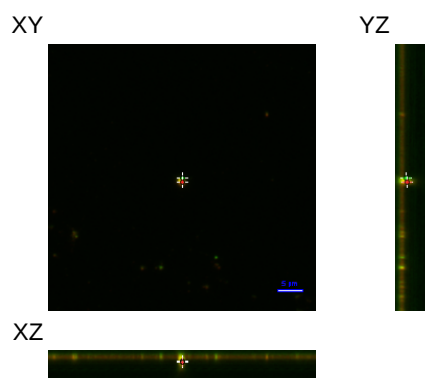
Channel 0 (Em. Wavelength 0.0 nm) vs channel 2 (Em. Wavelength 0.0 nm)



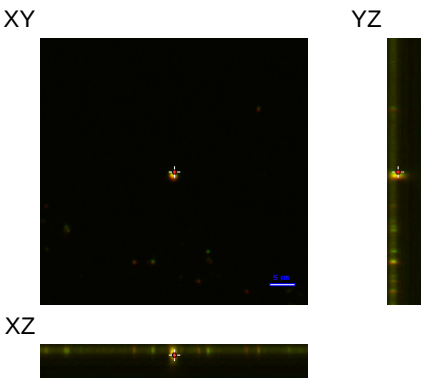
Channel 0 (Em. Wavelength 0.0 nm) vs channel 3 (Em. Wavelength 0.0 nm)



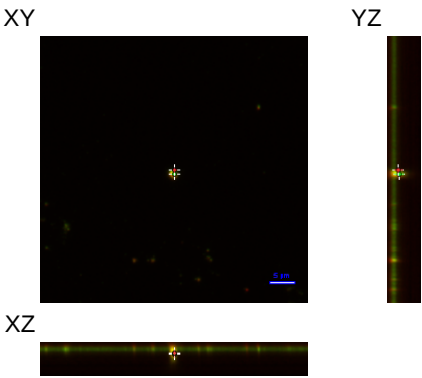
Channel 1 (Em. Wavelength 0.0 nm) vs channel 2 (Em. Wavelength 0.0 nm)



Channel 1 (Em. Wavelength 0.0 nm) vs channel 3 (Em. Wavelength 0.0 nm)



Channel 2 (Em. Wavelength 0.0 nm) vs channel 3 (Em. Wavelength 0.0 nm)



ISO 21073 co-registration accuracy:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Lateral distance (in μm)		0.0	0.767	0.0
	Axial distance (in μm)		0.72	0.84	0.72
Channel 1	Lateral distance (in μm)	0.0		0.767	0.0
	Axial distance (in μm)	0.72		0.12	0.0
Channel 2	Lateral distance (in μm)	0.767	0.767		0.767
	Axial distance (in μm)	0.84	0.12		0.12
Channel 3	Lateral distance (in μm)	0.0	0.0	0.767	
	Axial distance (in μm)	0.72	0.0	0.12	

Pixel shift table:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	X shift		0.0	-1.5	0.0
	Y shift		0.0	11.0	0.0
	Z shift		4.5	5.25	4.5
Channel 1	X shift	0.0		-1.5	0.0
	Y shift	0.0		11.0	0.0
	Z shift	-4.5		0.75	0.0
Channel 2	X shift	1.5	1.5		1.5
	Y shift	-11.0	-11.0		-11.0
	Z shift	-5.25	-0.75		-0.75
Channel 3	X shift	0.0	0.0	-1.5	
	Y shift	0.0	0.0	11.0	
	Z shift	-4.5	0.0	0.75	
X, Y & Z theoretical resolutions (in pix.)		3.676, 3.676 & 4.847	3.204, 3.204 & 4.225	2.772, 2.772 & 3.656	2.301, 2.301 & 3.034
Centres'coord. in pixels (X, Y & Z)		385.5, 385.5 & 9.5	385.5, 385.5 & 14.0	384.0, 396.5 & 14.8	385.5, 385.5 & 14.0
Title		C1-2026-06-12_40xResolution.czi	C2-2026-06-12_40xResolution.czi	C3-2026-06-12_40xResolution.czi	C4-2026-06-12_40xResolution.czi

Distances table (uncalibrated):

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		4.5	12.281	4.5
Channel 1	4.5		11.127	0.0
Channel 2	12.281	11.127		11.127
Channel 3	4.5	0.0	11.127	
X, Y & Z resolutions (in pix.)	3.676, 3.676 & 4.847	3.204, 3.204 & 4.225	2.772, 2.772 & 3.656	2.301, 2.301 & 3.034
Centres'coord.(X, Y & Z in pix.)	385.5, 385.5 & 9.5	385.5, 385.5 & 14.0	384.0, 396.5 & 14.8	385.5, 385.5 & 14.0
Title	C1-2026-06-12_40xResolution.czi	C2-2026-06-12_40xResolution.czi	C3-2026-06-12_40xResolution.czi	C4-2026-06-12_40xResolution.czi

Distances table (calibrated):

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Measured distance in μm)		0.72	1.137	0.72
	Reference distance in μm)		0.775	0.354	0.775
Channel 1	Measured distance in μm)	0.72		0.776	0.0
	Reference distance in μm)	0.775		0.257	0.775
Channel 2	Measured distance in μm)	1.137	0.776		0.776
	Reference distance in μm)	0.354	0.257		0.257
Channel 3	Measured distance in μm)	0.72	0.0	0.776	
	Reference distance in μm)	0.775	0.775	0.257	
X, Y & Z resolutions (in μm)		0.254, 0.254 & 0.775	0.221, 0.221 & 0.676	0.191, 0.191 & 0.585	0.159, 0.159 & 0.485
Centres' coord.(X, Y & Z, in μm)		26.6, 26.6 & 1.5	26.6, 26.6 & 2.2	26.5, 27.4 & 2.4	26.6, 26.6 & 2.2
Title		C1-2026-06-12_40xR esolution.czi	C2-2026-06-12_40xR esolution.czi	C3-2026-06-12_40xR esolution.czi	C4-2026-06-12_40xR esolution.czi

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 22, 2026 3:16 PM
data	result folder	Z:\CTIU\QC\2026-06-12\LSM800\Processed\Registration\
	Type of saved data	.pdf, .jpg, .xls
	Input data bit depth	8
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
2026-06-12_40xResolution	2026-06-12 11:29:01	none	Ch.3 undersampled	analysed

Formulas used:

The reference resolution distance is calculated as follows:

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

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

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

With

$$res_{x,y}^o = \frac{0.51 * \lambda_{ex}}{NA}$$

$$res_z^o = \frac{0.88 * \lambda_{ex}}{n - \sqrt{n^2 - NA^2}}$$

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 Spinning Disk microscopes are calculated as defined in Toomre and Pawley, Disk-Scanning Confocal Microscopy in Handbook Of Biological Confocal Microscopy 221–238 (Springer US, 2006).

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

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

$$\Delta_{x,y} = \frac{\lambda_{ex}}{8 * NA}$$

$$\Delta_z = \frac{\lambda_{ex}}{4 * n * (1 - \cos(\alpha))}$$