



Registration

Microscope info:

Image		Composite				
image's creation	date	unknown				
	method used	original file info & metadata could not be found				
Actual image depth		16				
Microscope type		WideField				
Objective	NA	0.3				
	im. refractive index	1.0				
Channel(s)	Wavelengths		Saturation	sampling (X,Y,Z)		
	Ex. (nm)	Em. (nm)		Nyquist (μm)	Found (μm)	Nyquist/found ratio
Channel 0		405.0	none	0.338x0.338x4.396	0.01x0.01x1.0	0.0, 0.0, 0.2
Channel 1		450.0	none	0.375x0.375x4.885		0.0, 0.0, 0.2
Channel 2		525.0	none	0.438x0.438x5.699		0.0, 0.0, 0.2
Channel 3		570.0	none	0.475x0.475x6.187		0.0, 0.0, 0.2

Warnings:

(No saturated pixels detected).

(All channels sampled following Shannon-Nyquist criterion).

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

Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		1.229	1.229	1.811
Channel 1	1.229		0.000	3.021
Channel 2	1.229	0.000		3.021
Channel 3	1.811	3.021	3.021	
X, Y, Z theoretical resolutions (in μm)	0.689, 0.689 & 7.965	0.765, 0.765 & 8.85	0.893, 0.893 & 10.325	0.969, 0.969 & 11.21
Bead centres' coord. (X, Y & Z in μm)	3.682, 1.979 & 20.0	2.771, 2.208 & 20.0	2.771, 2.208 & 20.0	5.068, 1.953 & 20.0
Bead quality (SB Ratio)	100.6	223.8	71.3	45.4
Title	C1-Composite	C2-Composite	C3-Composite	C4-Composite

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)

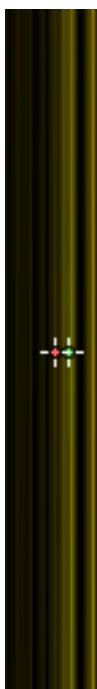
XY



YZ



XZ



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

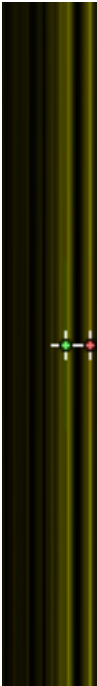
XY



YZ



XZ



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

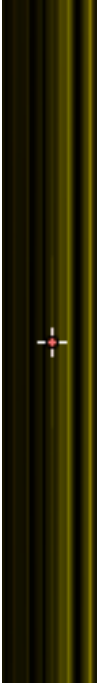
XY



YZ



XZ



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

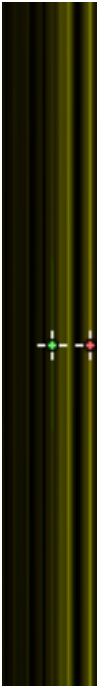
XY



YZ



XZ



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

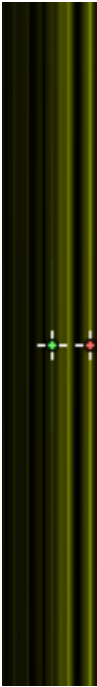
XY



YZ



XZ



ISO 21073 co-registration accuracy:

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Lateral distance (in μm)		0.94	0.94	1.386
	Axial distance (in μm)		0.0	0.0	0.0
Channel 1	Lateral distance (in μm)	0.94		0.0	2.311
	Axial distance (in μm)	0.0		0.0	0.0
Channel 2	Lateral distance (in μm)	0.94	0.0		2.311
	Axial distance (in μm)	0.0	0.0		0.0
Channel 3	Lateral distance (in μm)	1.386	2.311	2.311	
	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		-87.5	-87.5	133.0
	Y shift		22.0	22.0	-2.5
	Z shift		0.0	0.0	0.0
Channel 1	X shift	87.5		0.0	220.5
	Y shift	-22.0		0.0	-24.5
	Z shift	0.0		0.0	0.0
Channel 2	X shift	87.5	0.0		220.5
	Y shift	-22.0	0.0		-24.5
	Z shift	0.0	0.0		0.0
Channel 3	X shift	-133.0	-220.5	-220.5	
	Y shift	2.5	24.5	24.5	
	Z shift	0.0	0.0	0.0	
X, Y & Z theoretical resolutions (in pix.)		66.096, 66.096 & 7.965	73.44, 73.44 & 8.85	85.68, 85.68 & 10.325	93.024, 93.024 & 11.21
Centres' coord. in pixels (X, Y & Z)		353.5, 190.0 & 20.0	266.0, 212.0 & 20.0	266.0, 212.0 & 20.0	486.5, 187.5 & 20.0
Title		C1-Composite	C2-Composite	C3-Composite	C4-Composite

Distances table (uncalibrated):

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		90.223	90.223	133.023
Channel 1	90.223		0.0	221.857
Channel 2	90.223	0.0		221.857
Channel 3	133.023	221.857	221.857	
X, Y & Z resolutions (in pix.)	66.096, 66.096 & 7.965	73.44, 73.44 & 8.85	85.68, 85.68 & 10.325	93.024, 93.024 & 11.21
Centres' coord. (X, Y & Z in pix.)	353.5, 190.0 & 20.0	266.0, 212.0 & 20.0	266.0, 212.0 & 20.0	486.5, 187.5 & 20.0
Title	C1-Composite	C2-Composite	C3-Composite	C4-Composite

Distances table (calibrated):

		Channel 0	Channel 1	Channel 2	Channel 3
Channel 0	Measured distance in μm)		0.94	0.94	1.386
	Reference distance in μm)		0.765	0.765	0.765
Channel 1	Measured distance in μm)	0.94		0.0	2.311
	Reference distance in μm)	0.765		8.85	0.765
Channel 2	Measured distance in μm)	0.94	0.0		2.311
	Reference distance in μm)	0.765	8.85		0.765
Channel 3	Measured distance in μm)	1.386	2.311	2.311	
	Reference distance in μm)	0.765	0.765	0.765	
X, Y & Z resolutions (in μm)		0.689, 0.689 & 7.965	0.765, 0.765 & 8.85	0.893, 0.893 & 10.325	0.969, 0.969 & 11.21
Centres' coord.(X, Y & Z, in μm)		3.7, 2.0 & 20.0	2.8, 2.2 & 20.0	2.8, 2.2 & 20.0	5.1, 2.0 & 20.0
Title		C1-Composite	C2-Composite	C3-Composite	C4-Composite

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 9:54 AM
data	result folder	Z:\CTIU\QC\2026-06-16\Evident\Processed\Registration\10x\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
Composite	unknown	none	correct	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:

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

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