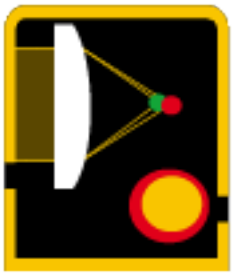


63x_Registration (SHORT)

Microscope info:

Image		2026-05-27_63xResolution				
image's creation	date	2026-05-27 09:43:57				
	method used	from file creation date				
Actual image depth		8				
Microscope type		CLSM				
Pinhole		1.0 AU				
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		640.0		none	0.057x0.057x0.172	0.045x0.045x0.1	0.8, 0.8, 0.6
Channel 1		564.0		none	0.05x0.05x0.151		0.9, 0.9, 0.7
Channel 2		488.0		none	0.044x0.044x0.131		1.0, 1.0, 0.8
Channel 3		405.0		<0.1%	0.036x0.036x0.109		1.2, 1.2, 0.9

Warnings:

Saturation in highlighted channels may affect result.

(All channels sampled following Shannon-Nyquist criterion).
(The bead size is appropriate for this coalignment analysis).

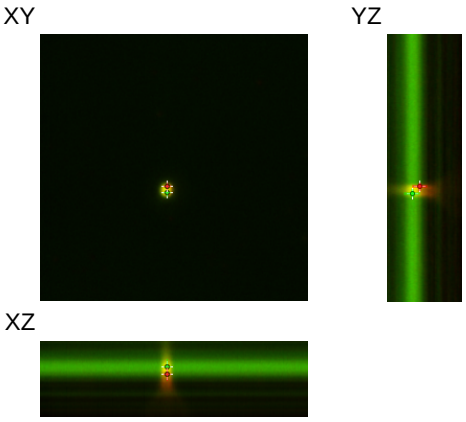
Ratios table:

	Channel 0	Channel 1	Channel 2	Channel 3
Channel 0		2.333	1.146	1.674
Channel 1	2.333		2.683	2.169
Channel 2	1.146	2.683		1.770
Channel 3	1.674	2.169	1.770	
X, Y, Z theoretical resolutions (in µm)	0.233, 0.233 & 0.605	0.205, 0.205 & 0.533	0.178, 0.178 & 0.461	0.148, 0.148 & 0.383
Bead centres'coord.(X,Y & Z in µm)	9.582, 12.033 & 1.9	9.627, 11.543 & 2.5	9.582, 12.167 & 2.5	9.27, 11.9 & 2.4
Bead quality (SB Ratio)	0.0	0.0	0.0	0.0
Title	C1-2026-05-27_63xResolution.czi	C2-2026-05-27_63xResolution.czi	C3-2026-05-27_63xResolution.czi	C4-2026-05-27_63xResolution.czi

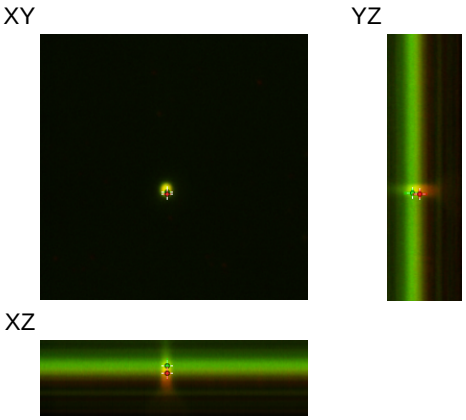
Ratio of the mesured calibrated distance to the reference distance (as calulated using the longest wavelength).
Green: within specifications, red: outside specifications (ie. ratio above 1.0)

Profile view:

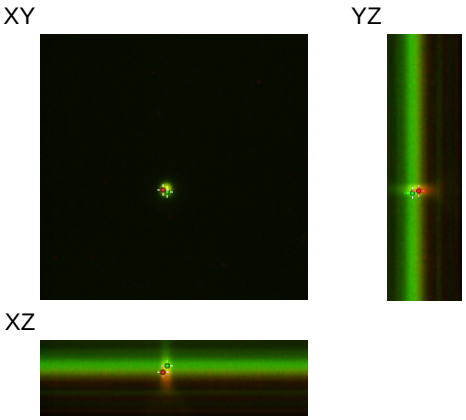
Channel 0 (Em. Wavelength 0.0 nm) vs channel 1 (Em. Wavelength 0.0 nm)



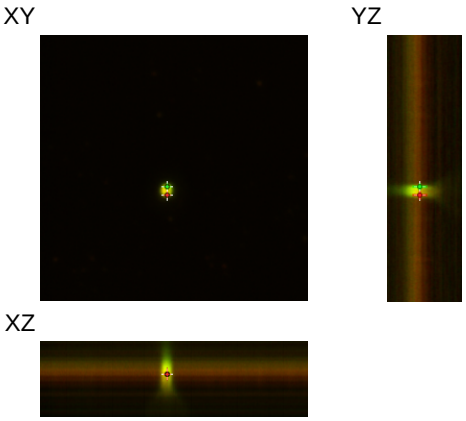
Channel 0 (Em. Wavelength 0.0 nm) vs channel 2 (Em. Wavelength 0.0 nm)



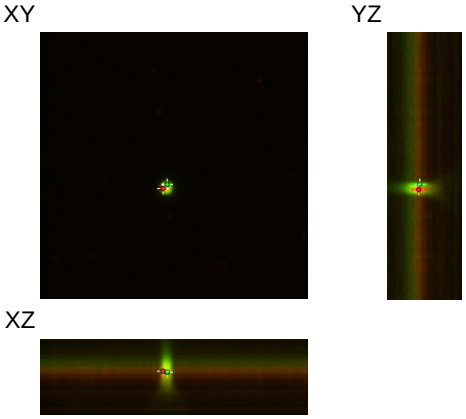
Channel 0 (Em. Wavelength 0.0 nm) vs channel 3 (Em. Wavelength 0.0 nm)



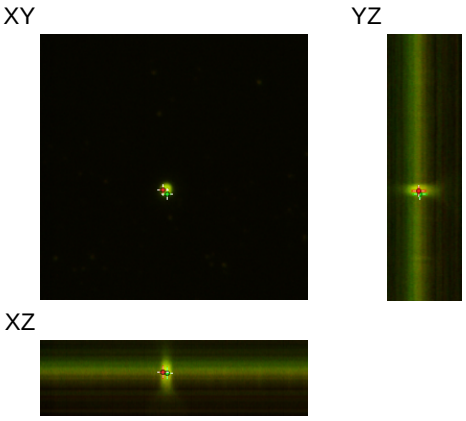
Channel 1 (Em. Wavelength 0.0 nm) vs channel 2 (Em. Wavelength 0.0 nm)



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)



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 23, 2026 11:37 AM
data	result folder	Z:\CTIU\QC\2026-05-27\LSM700\Processed\63x_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 Maximum Intensity
	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
2026-05-27_63xResolution	2026-05-27 09:43:57	Ch.3 saturated	correct	analysed

Formulas used:

The reference resolution distance is calculated as follows:

$$res^o_{\theta,\varphi} = \frac{res^o_x * res^o_y * res^o_z}{\sqrt{(res^o_y * res^o_z * cos\Phi * sin\Theta)^2 + (res^o_x * res^o_z * sin\Phi * sin\Theta)^2 + (res^o_x * res^o_y * cos\Theta)^2}}$$

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

$$\Theta = \arccos \frac{z_B - z_A}{\sqrt{(x_B - x_{A)}{}^2 + (y_B - y_{A)}{}^2 + (z_B - z_{A)}{}^2}}$$

With

$$res^o_{x,y} = \frac{0.51 * \lambda_{ex}}{NA}$$

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

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 ($res^o_{x,y}$) and axial (res^o_z) 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:

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

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

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