63x_PSF (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). (A subresolution bead is used for all channels).

Resolution table:

Channel	Sig/Background ratio	Dimension	Measured FWHM (µm)	theory (µm)	Fit Goodness	Mes./theory ratio
Channel 0 (em. 0.0nm)	<0.1	X	0.646	0.233	0.7	2.77
		Y	0.795	0.233	0.77	3.41
		Z	2.025	0.605	0.77	3.35
Channel 1 (em. 0.0nm)	<0.1	X	0.563	0.205	0.97	2.74
		Y	0.892	0.205	0.85	4.34
		Z	1.749	0.533	0.87	3.28
Channel 2 (em. 0.0nm)	<0.1	X	0.529	0.178	0.86	2.97
		Y	0.853	0.178	0.83	4.8
		Z	1.373	0.461	0.83	2.98
Channel 3 (em. 0.0nm)	<0.1	Saturated Channel				

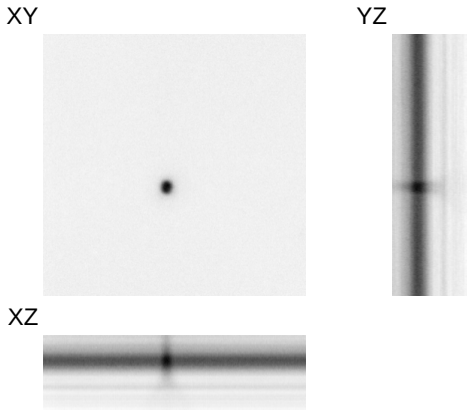
Green: within specifications, red: outside specifications (ie. XY ratios above 1.5 or Z ratio above 2.0)

Lateral asymmetry ratios:

Channel	Ratio
Channel 0 (em. 0.0nm)	0.81
Channel 1 (em. 0.0nm)	0.63
Channel 2 (em. 0.0nm)	0.62
Channel 3 (em. 0.0nm)	Saturated Channel

Detailed channel detection info:

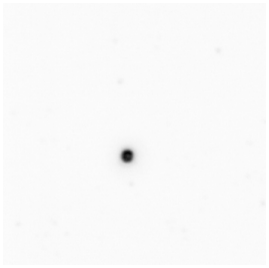
Channel #0



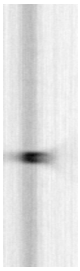
Channel 0 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.81	X	0.646	0.7
		Y	0.795	0.77
		Z	2.025	0.77

Channel #1

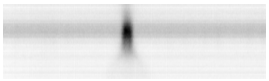
XY



YZ



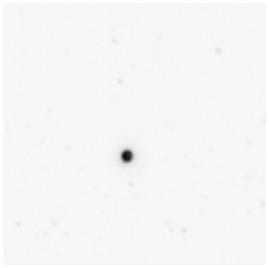
XZ



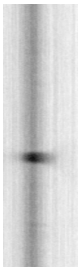
Channel 1 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.63	X	0.563	0.97
		Y	0.892	0.85
		Z	1.749	0.87

Channel #2

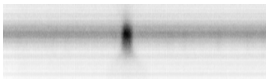
XY



YZ



XZ



Channel 2 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.62	X	0.529	0.86
		Y	0.853	0.83
		Z	1.373	0.83

Analysis parameters

Tool & Operator	Tool	PSF Profiler
	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 11:33 AM
data	result folder	Z:\CTIU\QC\2026-05-27\LSM700\Processed\63x_PSF\
	Type of saved data	.pdf, .jpg, .xls
	Input data bit depth	8
Dimension order		XY-(C)Z
Discard saturated samples		true
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
Square Root PSF Image displayed		true
Tolerance	Applied in this report	true
	X & Y FWHM ratios valid if below	1.5
	Z FWHM ratio valid if below	2.0

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:

Lateral ($res^o_{x,y}$) and axial (res^o_z) theoretical resolution values used for single point scanning confocal microscopes (assuming pinhole and NA values ≥1 & >0.5 respectively) are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy (2011) & Amos, B. et al, Confocal Microscopy in Comprehensive Biophysics 3–23 (Elsevier, 2012):

$$res^o_{x,y} = \frac{0.51*\lambda_{ex}}{NA} \qquad res^o_z = \frac{0.88*\lambda_{ex}}{n-\sqrt{n^2-NA^2}}$$

NA: numerical aperture, λ_{ex}: excitation wavelength, n: refractive index of the lens immersion & mounting media.

Axis profiles are fitted using ImageJ Gaussian Curve Fitter and the following formula $y = a + (b-a) * e^{\frac{-(x-c)^2}{2d^2}}$ (Gaussian fitting).

Measured lateral and axial resolution (Full Width at Half Maximum, FWHM) values are derived using $\mathrm{FWHM} = 2d\sqrt{2ln(2)}$

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

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

$$\Delta_{x,y} = \frac{\lambda_{ex}}{8.NA} \qquad \Delta_z = \frac{\lambda_{ex}}{4.n.(1-cos(\alpha))}$$