

PSF

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)	Nyquist/found ratio
Channel 0		647.0		none	0.062x0.062x0.22	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. (A subresolution bead is used for all channels).

Resolution table:

Channel	Sig/Backgnd ratio	Dimension	Measured FWHM (μm)	theory (μm)	Fit Goodness	Mes./theory ratio
Channel 0 (em. 0.0nm)	<0.1	X	0.817	0.254	0.58	3.22
		Y	1.239	0.254	0.51	4.88
		Z	2.167	0.775	0.7	2.79
Channel 1 (em. 0.0nm)	<0.1	X	0.784	0.221	0.86	3.54
		Y	1.17	0.221	0.88	5.29
		Z	2.35	0.676	0.51	3.48
Channel 2 (em. 0.0nm)	<0.1	X	1.259	0.191	0.95	6.57
		Y	1.068	0.191	0.94	5.58
		Z	2.499	0.585	0.93	4.27
Channel 3 (em. 0.0nm)	<0.1	X	1.174	0.159	0.47	7.39
		Y	1.239	0.159	0.92	7.8
		Z	4.711	0.485	0.44	9.71

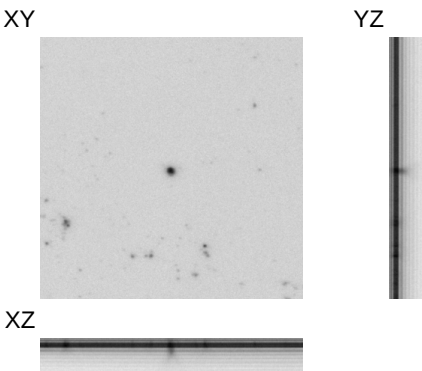
Green: within specifications, red: outside specifications (ie. XY ratios above 1.5 or Z ratio above 2.0)

Lateral asymmetry ratios:

Channel	Ratio
Channel 0 (em. 0.0nm)	0.66
Channel 1 (em. 0.0nm)	0.67
Channel 2 (em. 0.0nm)	0.85
Channel 3 (em. 0.0nm)	0.95

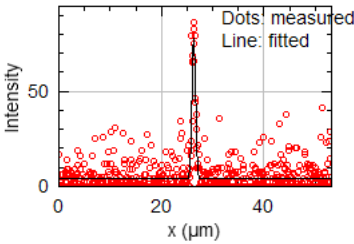
Detailed channel detection info:

Channel #0

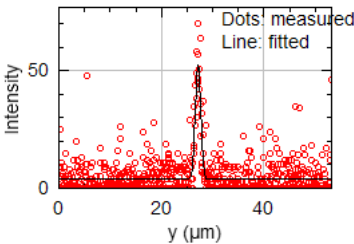


Channel 0 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.66	X	0.817	0.58
		Y	1.239	0.51
		Z	2.167	0.7

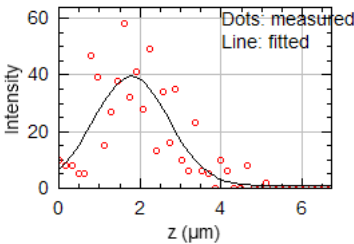
X profile & fitting parameters:
Fit equation $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$
Sum of residuals squared: 33682.8619
Standard deviation: 6.61392
 R^2 : 0.58541
Parameters:
 $a = 3.95439$
 $b = 77.89756$
 $c = 26.30309$
 $d = 0.34692$



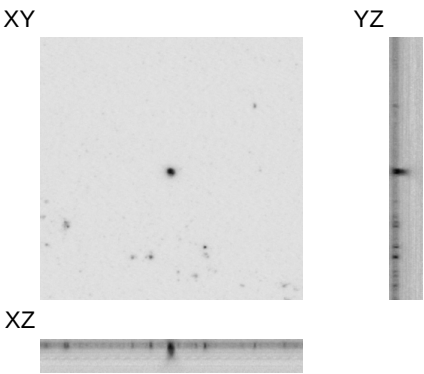
Y profile & fitting parameters:
Fit equation $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$
Sum of residuals squared: 29309.7628
Standard deviation: 6.16965
 R^2 : 0.51188
Parameters:
 $a = 3.93194$
 $b = 52.49991$
 $c = 27.13115$
 $d = 0.52609$



Z profile & fitting parameters:
Fit equation $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$
Sum of residuals squared: 3149.36446
Standard deviation: 8.65938
 R^2 : 0.72529
Parameters:
 $a = 0.87285$
 $b = 39.60420$
 $c = 1.78439$
 $d = 0.92015$

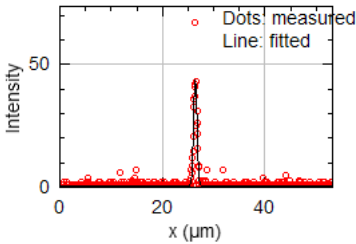


Channel #1

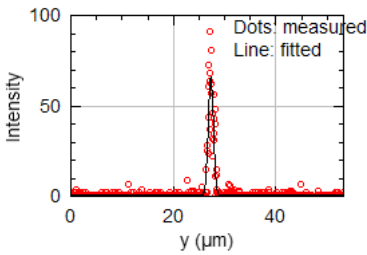


Channel 1 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.67	X	0.784	0.86
		Y	1.17	0.88
		Z	2.35	0.51

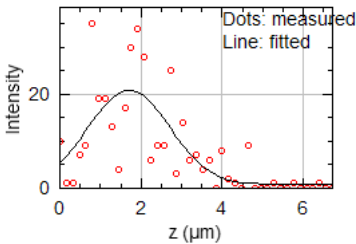
X profile & fitting parameters:
Fit equation $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$
Sum of residuals squared: 2436.39253
Standard deviation: 1.77880
 R^2 : 0.86324
Parameters:
a = 0.65961
b = 43.56044
c = 26.39607
d = 0.33292



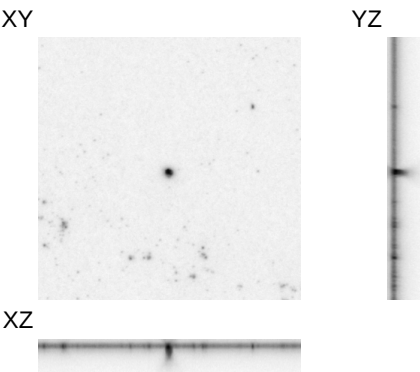
Y profile & fitting parameters:
Fit equation $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$
Sum of residuals squared: 7238.15623
Standard deviation: 3.06598
 R^2 : 0.87877
Parameters:
a = 0.64029
b = 65.87679
c = 27.28839
d = 0.49676



Z profile & fitting parameters:
Fit equation $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$
Sum of residuals squared: 1828.74186
Standard deviation: 6.59860
 R^2 : 0.55811
Parameters:
a = 0.84418
b = 20.94403
c = 1.70600
d = 0.99777



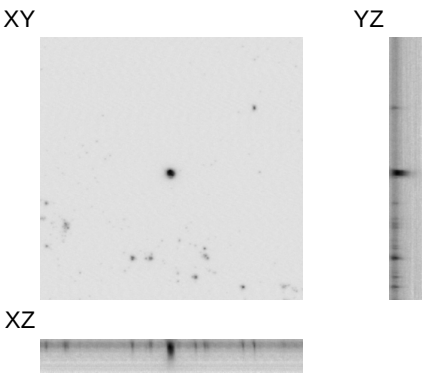
Channel #2



Channel 2 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.85	X	1.259	0.95
		Y	1.068	0.94
		Z	2.499	0.93

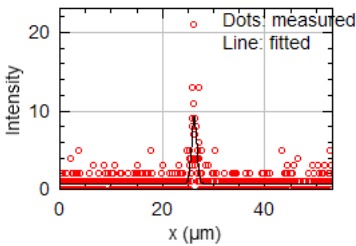
X profile & fitting parameters: Fit equation $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$ Sum of residuals squared: 1244.06573 Standard deviation: 1.27109 R^2: 0.94967 Parameters: a = 0.064541 b = 42.18824 c = 26.43754 d = 0.53448	
Y profile & fitting parameters: Fit equation $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$ Sum of residuals squared: 1361.57921 Standard deviation: 1.32977 R^2: 0.94062 Parameters: a = 0.13659 b = 43.83573 c = 27.26732 d = 0.45371	
Z profile & fitting parameters: Fit equation $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$ Sum of residuals squared: 639.30367 Standard deviation: 3.90148 R^2: 0.93852 Parameters: a = -0.55352 b = 41.52249 c = 2.20678 d = 1.06142	

Channel #3

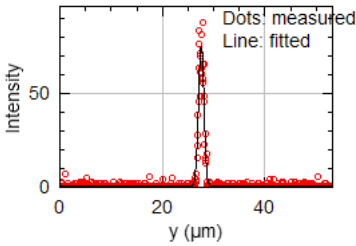


Channel 3 (em. 0.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
0.0	0.95	X	1.174	0.47
		Y	1.239	0.92
		Z	4.711	0.44

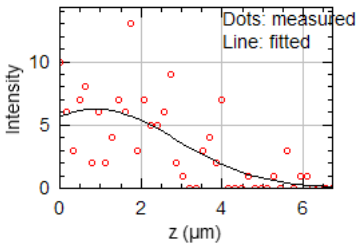
X profile & fitting parameters:
Fit equation $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$
Sum of residuals squared: 995.06252
Standard deviation: 1.13679
 R^2 : 0.47347
Parameters:
a = 0.71870
b = 9.22427
c = 26.18792
d = 0.49842



Y profile & fitting parameters:
Fit equation $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$
Sum of residuals squared: 6016.57595
Standard deviation: 2.79531
 R^2 : 0.92392
Parameters:
a = 0.66627
b = 75.52960
c = 27.55673
d = 0.52634



Z profile & fitting parameters:
Fit equation $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$
Sum of residuals squared: 236.73124
Standard deviation: 2.37412
 R^2 : 0.49542
Parameters:
a = 0.033292
b = 6.25931
c = 0.88941
d = 2.00058



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, June 22, 2026 3:13 PM
data	result folder	Z:\CTIU\QC\2026-06-12\LSM800\Processed\PSF\
	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
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
2026-06-12_40xResolution	2026-06-12 11:29:01	none	Ch.3 undersampled	analysed

Formulas used:

Lateral ($res_{x,y}^o$) and axial (res_z^o) 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):

$$res_{x,y}^o = \frac{0.51 * \lambda_{ex}}{NA} \quad res_z^o = \frac{0.88 * \lambda_{ex}}{n - \sqrt{n^2 - NA^2}}$$

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 $FWHM = 2d\sqrt{2\ln(2)}$

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

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

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