63X_1_4_585

Microscope info:

Image		Image6_bead13					
image's creation	date	2024-10-17 10:22:32					
	method used	from file creation date					
Actual image depth		16					
Microscope type		WideField					
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			585.0	none	0.104x0.104x0.314	0.063x0.063x0.06	0.6, 0.6, 0.2
Bead original coordinates(in pixels)		395.0, 982.0					

Warnings:

(No saturated pixels detected). (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. 585.0nm)	4.6	X	0.278	0.213	0.99	1.3
		Y	0.264	0.213	1.0	1.24
		Z	0.658	0.802	0.98	0.82

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

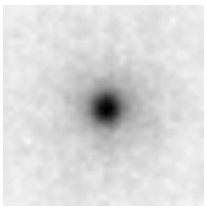
Lateral asymmetry ratios:

Channel	Ratio
Channel 0 (em. 585.0nm)	0.95

Detailed channel detection info:

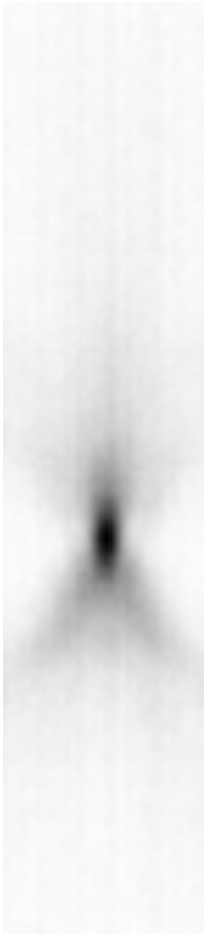
Channel #0

XY

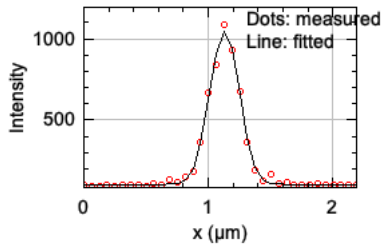
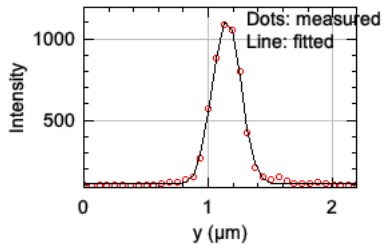
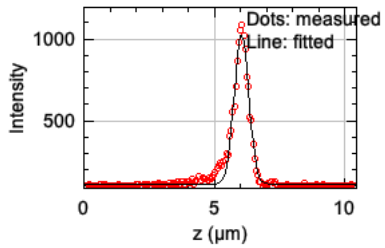


YZ

XZ



Channel 0 (em. 585.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
4.6	0.95	X	0.278	0.99
		Y	0.264	1.0
		Z	0.658	0.98

X profile & fitting parameters: Fit equation I(x) = a + (b-a)*exp(-(x-c)*(x-c)/(2*d*d)) Sum of residuals squared: 14263.6567 Standard deviation: 20.18745 R^2: 0.99413 Parameters: a = 105.82358 b = 1048.15688 c = 1.13090 d = 0.11806	
Y profile & fitting parameters: Fit equation I(y) = a + (b-a)*eyp(-(y-c)*(y-c)/(2*d*d)) Sum of residuals squared: 8062.63841 Standard deviation: 15.17765 R^2: 0.99702 Parameters: a = 108.15952 b = 1124.01353 c = 1.14791 d = 0.11212	
Z profile & fitting parameters: Fit equation I(z) = a + (b-a)*ezp(-(z-c)*(z-c)/(2*d*d)) Sum of residuals squared: 132847.312 Standard deviation: 27.63133 R^2: 0.97906 Parameters: a = 113.36809 b = 1024.88018 c = 6.03499 d = 0.27950	

Analysis parameters

Tool & Operator	Tool	PSF Profiler (batch)
	Versions	MetroloJ_QC v1.3.1.1, ImageJ v2.14.0/1.54f, Java v1.8.0_322, OS Mac OS X
	Operator & date	SO, October 25, 2024 2:32 PM
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/585//Processed/63X_1_4_585/Image 6/bead13/
	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 Maximum Intensity
	Discard bead if more than one particle are thresholded	true
	Background annulus thickness in µm	0.5
	Background annulus distance to bead edges in µm	0.5
	Multiple beads in image	true
	Bead identification method	Using Find Maxima (prominence of 1000.0)
	Bead size (µm)	0.1
	Bead crop Factor	5.0
	Cropped ROI size in µm	2.31x2.31 (using bead size & background annulus parameters)
	Bead rejection distance to top/bottom	2.0 µm
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
Image 6_bead13	2024-10-17 10:22:32	none	correct	analysed

Formulas used:

Lateral ($res^o_{x,y}$) and axial (res^o_z) theoretical resolution values used for widefield microscopes are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy, 2011:

$$res^o_{x,y} = \frac{0.51*\lambda_{em}}{NA} \qquad res^o_z = \frac{1{,}77n*\lambda_{em}}{NA^2}$$

NA: numerical aperture, λ_{em}: emission 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:

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

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