

63X_1_4_525

Microscope info:

Image		Image8_bead2						
image's date 2024-10-17 10:22:23								
creation	method used	from file creation date						
Actual image depth		16						
Microsco	ope type	WideField						
	NA	1.4	1.4					
Objective	im. refractive index	1.518						
		Wavel	engths		sampling (X,Y,Z)			
Chan	nel(s)	Ex. (nm)	Em. (nm)	Saturation	Nyquist (µm)	Found (µm)	Nyquist/found ratio	
Channel 0			525.0	none	0.094x0.094x0. 282	0.063x0.063x0. 06	0.7, 0.7, 0.2	
Bead original pix	1124.0,	442.0						

Warnings:

(No saturated pixels detected). (All channels sampled following Shannon-Nyquist criterion). (A subresolution bead is used for all channels).

Resolution table:

Channel	Sig/Backgn d ratio	Dimension	Measured FWHM (µm)	theory (µm)	Fit Goodness	Mes./theory ratio
Channel 0 (em. 525.0nm)		X	0.236	0.191	1.0	1.23
		Υ	0.252	0.191	1.0	1.32
		Z	0.547	0.72	0.99	0.76

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. 525.0nm)	0.94

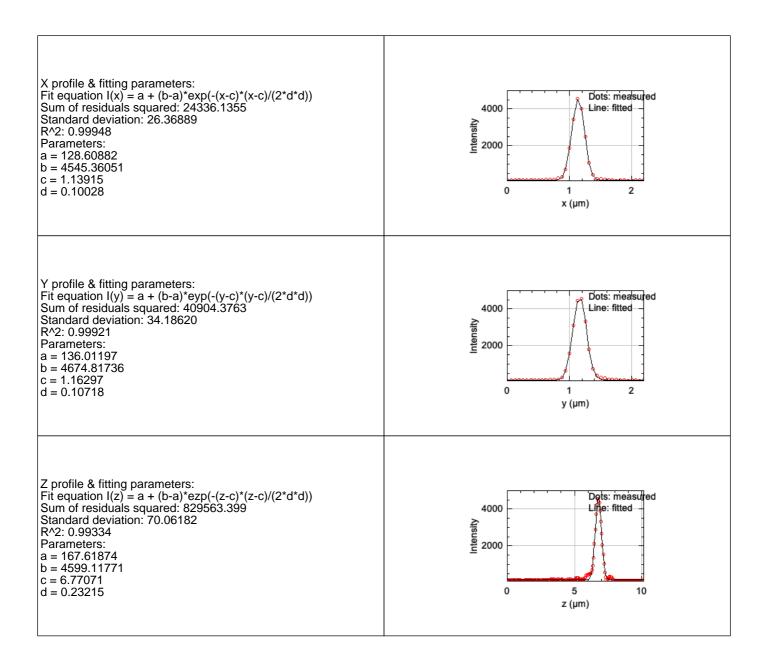
Detailed channel detection info:

Channel #0





Channel 0 (em. 525.0nm)					
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness	
		X	0.236	1.0	
16.0	0.94	Υ	0.252	1.0	
		Z	0.547	0.99	



Analysis parameters

	Tool	PSF Profiler (batch)				
Tool & Operator	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 2:34 PM				
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/525//Processed/63X_1_4_525/Image8/bead2/				
data	Type of saved data	.pdf, .jpg, .xls				
	Input data bit depth	16				
Dim	ension order	XY-(C)Z				
Discard s	aturated samples	false				
	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				
Beads	Background annulus distance to bead edges in µm	0.5				
Deads	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				
	Applied in this report	true				
Tolerance	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 8_bead2	2024-10-17 10:22:23	none	correct	analysed

Formulas used:

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:

$$res_{x,y}^o = \frac{0.51*\lambda_{em}}{NA}$$
 $res_z^o = \frac{1,77n*\lambda_{em}}{NA^2}$

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

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

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

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