

63X\_1\_2\_525

### Microscope info:

Image		GreenBeads63x.lif-Series003_bead146					
image's	date	2024-10-26 09:47:42					
creation	method used	from file	from file creation date				
Actual ima	12						
Microscope type		WideField					
	NA	1.2	1.2				
Objective	im. refractive index	1.333					
		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	0.109x0.109x0. 349	0.103x0.103x0. 099	0.9, 0.9, 0.3
Bead original pix	1510.0,	866.0					

# 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
		Х	0.272	0.223	1.0	1.22
Channel 0 (em. 525.0nm)	n. 1.7	Υ	0.271	0.223	0.99	1.21
		Z	0.943	0.86	0.99	1.1

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)	1.0

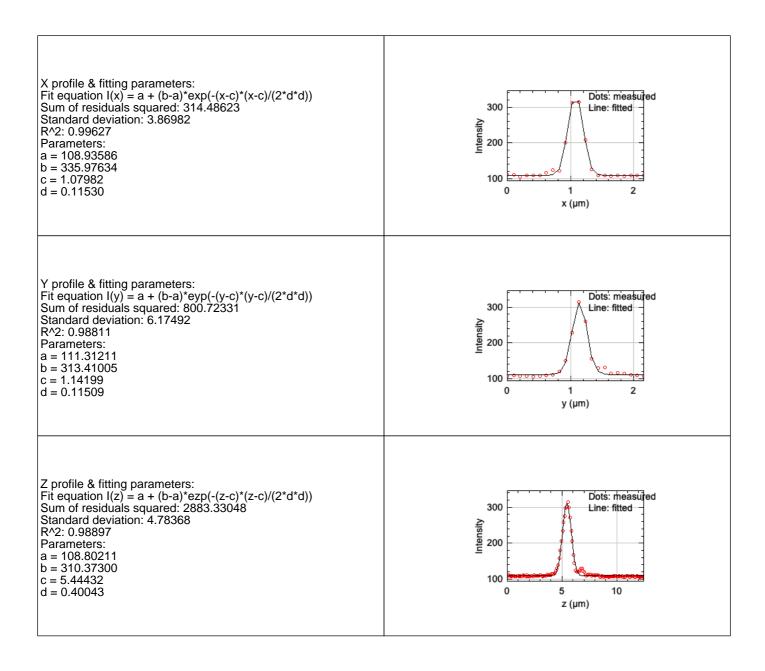
# Detailed channel detection info:

# Channel #0





Channel 0 (em. 525.0nm)					
Sig./Backgnd ratio	LAR	Dimension FWHM		Fit goodness	
		X	0.272	1.0	
1.7	1.0	Υ	0.271	0.99	
		Z	0.943	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 31, 2024 8:23 AM		
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Thunder/ PSF/20241015/63X_1_2_525//Processed/63X_1_2_525/Gr eenBeads63x.lif - Series003/bead146/		
data	Type of saved data	.pdf, .jpg, .xls		
	Input data bit depth	12		
Dim	ension order	XY-(C)Z		
Discard s	aturated samples	true		
	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 50.0)		
	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
GreenBeads63x.lif - Series003_bead146	2024-10-26 09:47:42	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,  $\lambda_{em}$ : 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:

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

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