



**63X\_1\_2\_525**

Microscope info:

Image		GreenBeads63x.lif-Series005_bead263				
image's creation	date	2024-10-26 09:47:20				
	method used	from file creation date				
Actual image depth		12				
Microscope type		WideField				
Objective	NA	1.2				
	im. refractive index	1.333				
Channel(s)		Wavelengths		Saturation	sampling (X,Y,Z)	
		Ex. (nm)	Em. (nm)		Nyquist (μm)	Nyquist/found ratio
Channel 0			525.0	none	0.109x0.109x0.349	0.103x0.103x0.099
Bead original coordinates(in pixels)		1482.0, 962.0				

Warnings:

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

Resolution table:

Channel	Sig/Backgnd ratio	Dimension	Measured FWHM (μm)	theory (μm)	Fit Goodness	Mes./theory ratio
Channel 0 (em. 525.0nm)	1.7	X	0.269	0.223	1.0	1.21
		Y	0.278	0.223	1.0	1.24
		Z	0.815	0.86	0.98	0.95

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

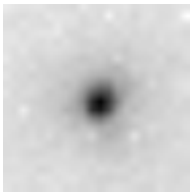
Lateral asymmetry ratios:

Channel	Ratio
Channel 0 (em. 525.0nm)	0.97

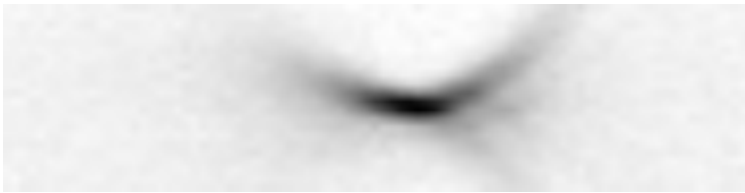
Detailed channel detection info:

Channel #0

XY



YZ

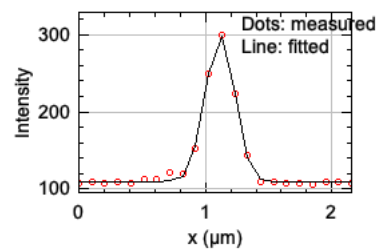


XZ

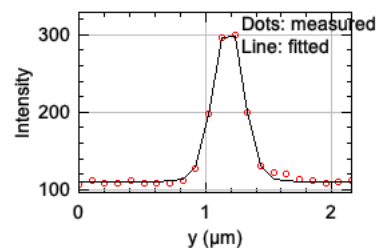


Channel 0 (em. 525.0nm)				
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness
1.7	0.97	X	0.269	1.0
		Y	0.278	1.0
		Z	0.815	0.98

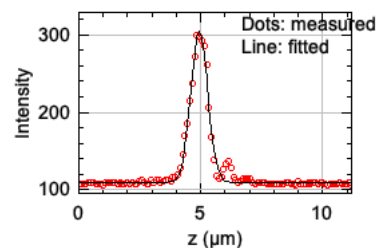
X profile & fitting parameters:  
Fit equation  $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$   
Sum of residuals squared: 239.67699  
Standard deviation: 3.37834  
R<sup>2</sup>: 0.99591  
Parameters:  
a = 109.69319  
b = 299.62313  
c = 1.11665  
d = 0.11419



Y profile & fitting parameters:  
Fit equation  $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$   
Sum of residuals squared: 257.41656  
Standard deviation: 3.50113  
R<sup>2</sup>: 0.99633  
Parameters:  
a = 110.92616  
b = 316.03143  
c = 1.18182  
d = 0.11789



Z profile & fitting parameters:  
Fit equation  $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$   
Sum of residuals squared: 3917.38936  
Standard deviation: 5.88788  
R<sup>2</sup>: 0.98165  
Parameters:  
a = 109.91731  
b = 304.62888  
c = 4.93738  
d = 0.34629



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 31, 2024 8:15 AM
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Thunder/PSF/20241015/63X_1_2_525//Processed/63X_1_2_525/GreenBeads63x.tif - Series005/bead263/
	Type of saved data	.pdf, .jpg, .xls
	Input data bit depth	12
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	true
	Background annulus thickness in $\mu\text{m}$	0.5
	Background annulus distance to bead edges in $\mu\text{m}$	0.5
	Multiple beads in image	true
	Bead identification method	Using Find Maxima (prominence of 50.0)
	Bead size ( $\mu\text{m}$ )	0.1
	Bead crop Factor	5.0
	Cropped ROI size in $\mu\text{m}$	2.31x2.31 (using bead size & background annulus parameters)
	Bead rejection distance to top/bottom	2.0 $\mu\text{m}$
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
GreenBeads63x.tif - Series005_bead263	2024-10-26 09:47:20	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:

$$res_{x,y}^o = \frac{0.51 \cdot \lambda_{em}}{NA} \quad res_z^o = \frac{1.77 n \cdot \lambda_{em}}{NA^2}$$

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

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

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

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