



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

Microscope info:

|                                      |                      |                                     |          |            |                   |                     |
|--------------------------------------|----------------------|-------------------------------------|----------|------------|-------------------|---------------------|
| Image                                |                      | GreenBeads63x.lif-Series005_bead269 |          |            |                   |                     |
| 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) |                      | 1504.0, 215.0                       |          |            |                   |                     |

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) | 1.8               | X         | 0.256              | 0.223       | 0.99         | 1.15              |
|                         |                   | Y         | 0.29               | 0.223       | 0.99         | 1.3               |
|                         |                   | Z         | 0.883              | 0.86        | 0.98         | 1.03              |

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

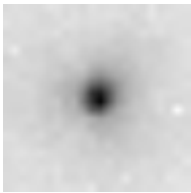
Lateral asymmetry ratios:

| Channel                 | Ratio |
|-------------------------|-------|
| Channel 0 (em. 525.0nm) | 0.88  |

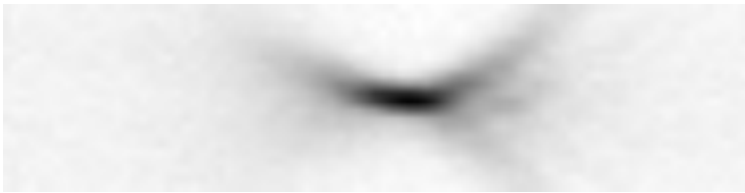
Detailed channel detection info:

Channel #0

XY



YZ

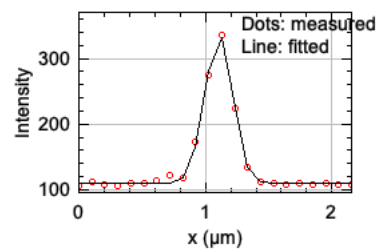


XZ

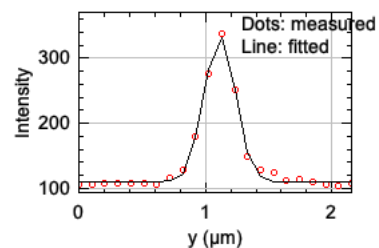


| Channel 0 (em. 525.0nm) |      |           |       |              |
|-------------------------|------|-----------|-------|--------------|
| Sig./Backgnd ratio      | LAR  | Dimension | FWHM  | Fit goodness |
| 1.8                     | 0.88 | X         | 0.256 | 0.99         |
|                         |      | Y         | 0.29  | 0.99         |
|                         |      | Z         | 0.883 | 0.98         |

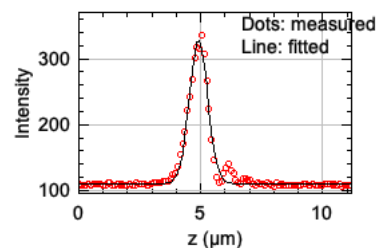
X profile & fitting parameters:  
Fit equation  $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$   
Sum of residuals squared: 388.37121  
Standard deviation: 4.30045  
R<sup>2</sup>: 0.99515  
Parameters:  
a = 109.58511  
b = 335.59959  
c = 1.10667  
d = 0.10889



Y profile & fitting parameters:  
Fit equation  $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$   
Sum of residuals squared: 641.49936  
Standard deviation: 5.52699  
R<sup>2</sup>: 0.99253  
Parameters:  
a = 110.15614  
b = 333.06876  
c = 1.11481  
d = 0.12316



Z profile & fitting parameters:  
Fit equation  $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$   
Sum of residuals squared: 6169.36692  
Standard deviation: 7.38892  
R<sup>2</sup>: 0.97851  
Parameters:  
a = 110.46768  
b = 328.21228  
c = 4.90676  
d = 0.37478



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/bead269/ |
|                                 | 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_bead269 | 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:

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

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