



63X\_1\_2\_525

Microscope info:

|                                      |                      |                                     |          |            |                   |                     |
|--------------------------------------|----------------------|-------------------------------------|----------|------------|-------------------|---------------------|
| Image                                |                      | GreenBeads63x.lif-Series003_bead114 |          |            |                   |                     |
| image's creation                     | date                 | 2024-10-26 09:47:42                 |          |            |                   |                     |
|                                      | 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) |                      | 188.0, 1037.0                       |          |            |                   |                     |

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         | 0.279              | 0.223       | 1.0          | 1.25              |
|                         |                   | Y         | 0.274              | 0.223       | 0.99         | 1.23              |
|                         |                   | Z         | 0.939              | 0.86        | 0.99         | 1.09              |

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

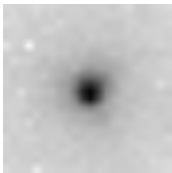
Lateral asymmetry ratios:

| Channel                 | Ratio |
|-------------------------|-------|
| Channel 0 (em. 525.0nm) | 0.98  |

Detailed channel detection info:

Channel #0

XY



YZ

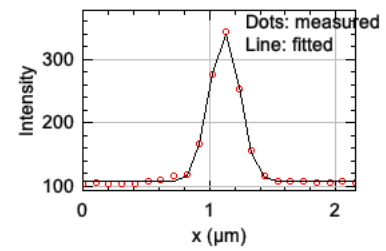


XZ

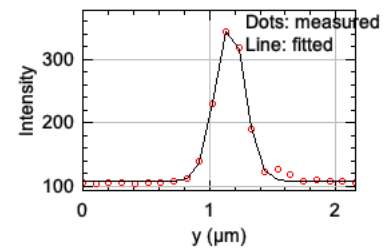


| Channel 0 (em. 525.0nm) |      |           |       |              |
|-------------------------|------|-----------|-------|--------------|
| Sig./Backgnd ratio      | LAR  | Dimension | FWHM  | Fit goodness |
| 1.7                     | 0.98 | X         | 0.279 | 1.0          |
|                         |      | Y         | 0.274 | 0.99         |
|                         |      | Z         | 0.939 | 0.99         |

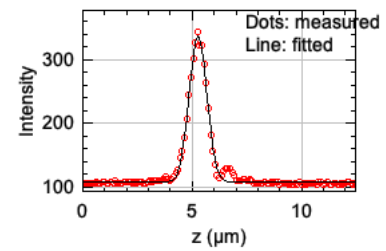
X profile & fitting parameters:  
Fit equation  $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$   
Sum of residuals squared: 134.36712  
Standard deviation: 2.52951  
R<sup>2</sup>: 0.99852  
Parameters:  
a = 107.58583  
b = 340.99825  
c = 1.11981  
d = 0.11859



Y profile & fitting parameters:  
Fit equation  $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$   
Sum of residuals squared: 454.75929  
Standard deviation: 4.65352  
R<sup>2</sup>: 0.99542  
Parameters:  
a = 109.10286  
b = 354.63369  
c = 1.16345  
d = 0.11618



Z profile & fitting parameters:  
Fit equation  $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$   
Sum of residuals squared: 3141.37058  
Standard deviation: 4.99315  
R<sup>2</sup>: 0.99074  
Parameters:  
a = 107.48846  
b = 337.64267  
c = 5.26206  
d = 0.39886



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 8:22 AM                                                                                                              |
| data                            | result folder                                              | /Users/oggsc/Documents/OM/ImageAnalysis/QC/Thunder/PSF/20241015/63X_1_2_525//Processed/63X_1_2_525/GreenBeads63x.tif - Series003/bead114/ |
|                                 | 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 - Series003_bead114 | 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:

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

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:

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

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