



63X_1_2_525

Microscope info:

| | | | | | | |
|--------------------------------------|----------------------|-------------------------------------|----------|------------|-------------------|---------------------|
| Image | | GreenBeads63x.lif-Series003_bead322 | | | | |
| 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) | | 1128.0, 741.0 | | | | |

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) | <0.1 | X | 0.134 | 0.223 | 0.3 | 0.6 |
| | | Y | 0.174 | 0.223 | 0.33 | 0.78 |
| | | Z | 0.164 | 0.86 | 0.07 | 0.19 |

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

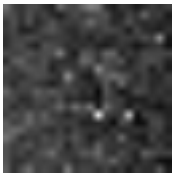
Lateral asymmetry ratios:

| Channel | Ratio |
|-------------------------|-------|
| Channel 0 (em. 525.0nm) | 0.77 |

Detailed channel detection info:

Channel #0

XY



YZ

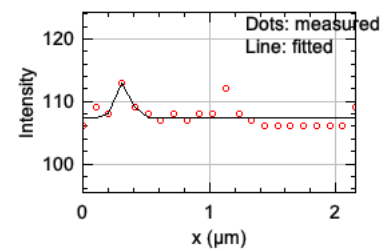


XZ

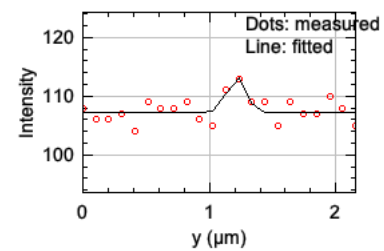


| Channel 0 (em. 525.0nm) | | | | |
|-------------------------|------|-----------|-------|--------------|
| Sig./Backgnd ratio | LAR | Dimension | FWHM | Fit goodness |
| 0.0 | 0.77 | X | 0.134 | 0.3 |
| | | Y | 0.174 | 0.33 |
| | | Z | 0.164 | 0.07 |

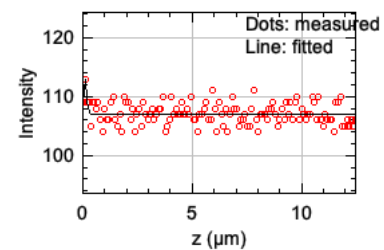
X profile & fitting parameters:
Fit equation $I(x) = a + (b-a) \exp(-(x-c)/(2*d*d))$
Sum of residuals squared: 44.06856
Standard deviation: 1.44862
R^2: 0.42599
Parameters:
a = 107.31134
b = 113.15034
c = 0.32133
d = 0.056972



Y profile & fitting parameters:
Fit equation $I(y) = a + (b-a) \exp(-(y-c)/(2*d*d))$
Sum of residuals squared: 53.89041
Standard deviation: 1.60194
R^2: 0.45440
Parameters:
a = 107.16515
b = 113.44820
c = 1.21315
d = 0.074064



Z profile & fitting parameters:
Fit equation $I(z) = a + (b-a) \exp(-(z-c)/(2*d*d))$
Sum of residuals squared: 374.28016
Standard deviation: 1.72351
R^2: 0.10185
Parameters:
a = 107.05337
b = 112.89882
c = 0.10095
d = 0.069655



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 8:25 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/bead322/ |
| | 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 μm | 0.5 |
| | Background annulus distance to bead edges in μm | 0.5 |
| | 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 |
| 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_bead322 | 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, 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 $FWHM = 2d\sqrt{2\ln(2)}$

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

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

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