# GreenBeadsIMG4R (SHORT)

## Microscope info:

| Image | | Image4_bead8 | | | |
|---|---|---|---|---|---|
| image's creation | date | 2024-10-17 10:22:18 | | | |
| | method used | from file creation date | | | |
| Actual image depth | | 16 | | | |
| Microscope type | | WideField | | | |
| Objective | NA | 1.4 | | | |
| | im. refractive index | 1.518 | | | |
| Channel(s) | | Wavelengths | | Saturation | sampling (X,Y,Z) |
| | | Ex. (nm) | Em. (nm) | | Nyquist (µm) | Found (µm) | Nyquist/found ratio |
| Channel 0 | | | 440.0 | none | 0.079x0.079x0.236 | 0.063x0.063x0.06 | 0.8, 0.8, 0.3 |
| Bead original coordinates(in pixels) | | 58.0, 390.0 | | | |

## Warnings:

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

## Resolution table:

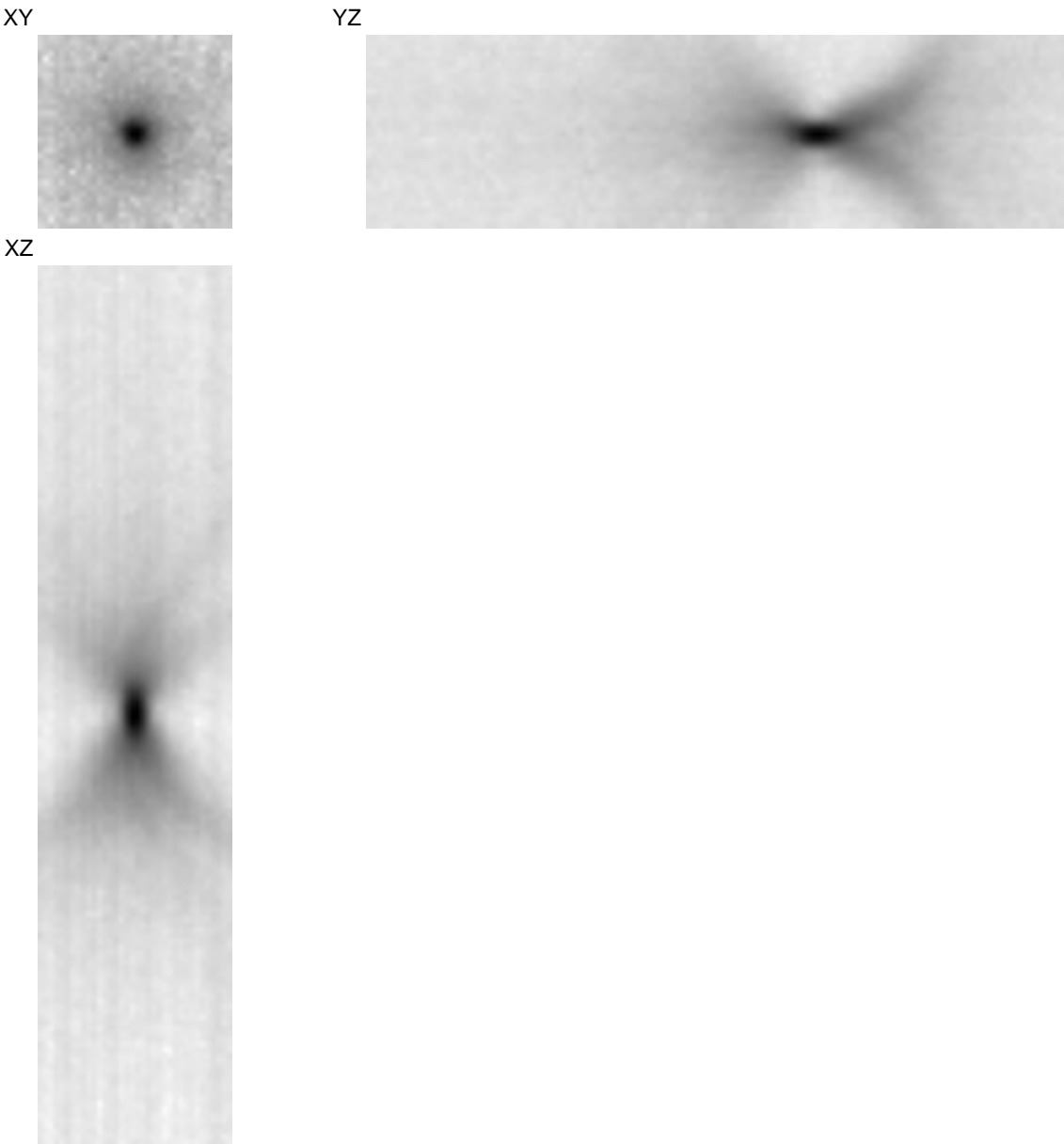| Channel | Sig/Backgnd ratio | Dimension | Measured FWHM (µm) | theory (µm) | Fit Goodness | Mes./theory ratio |
|---|---|---|---|---|---|---|
| Channel 0 (em. 440.0nm) | 6.4 | X | 0.254 | 0.16 | 0.99 | 1.58 |
| | | Y | 0.241 | 0.16 | 0.99 | 1.51 |
| | | Z | 0.502 | 0.603 | 0.99 | 0.83 |

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. 440.0nm) | 0.95 |

Channel #0

XY



YZ



XZ



| Channel 0 (em. 440.0nm) | | | | |
|---|---|---|---|---|
| Sig./Backgnd ratio | LAR | Dimension | FWHM | Fit goodness |
| 6.4 | 0.95 | X | 0.254 | 0.99 |
| | | Y | 0.241 | 0.99 |
| | | Z | 0.502 | 0.99 |

## Analysis parameters

| | | |
|---|---|---|
| **Tool & Operator** | Tool | PSF Profiler (batch) |
| | Versions | MetroloJ_QC v1.3.0, ImageJ v2.14.0/1.54f, Java v1.8.0_322, OS Mac OS X |
| | Operator & date | aaa, October 20, 2024 9:09 AM |
| **data** | result folder | /Users/bumozaza/Desktop/Zeiss WFM/green/Processed/GreenBeadsIMG4R/Image 4/bead8/ |
| | Type of saved data | .pdf, .jpg, .xls |
| | Input data bit depth | 16 |
| **Dimension order** | | XY-(C)Z |
| **Discard saturated samples** | | true |
| **Beads** | Bead detection threshold | Legacy |
| | Center detection method | Centroid |
| | 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 1000.0) |
| | Bead size (µm) | 0.1 |
| | Bead crop Factor | 10.0 |
| | 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 |
|---|---|---|---|---|
| Image 4_bead8 | 2024-10-17 10:22:18 | none | correct | analysed |

Lateral ($res^o_{x,y}$) and axial ($res^o_z$) theoretical resolution values used for widefield microscopes are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy, 2011:

$$res^o_{x,y} = \frac{0.51 * \lambda_{em}}{NA} \qquad res^o_z = \frac{1,77n * \lambda_{em}}{NA^2}$$

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

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

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

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