



63X\_1\_4\_610

Microscope info:

|                                      |                      |                         |          |            |                   |                     |
|--------------------------------------|----------------------|-------------------------|----------|------------|-------------------|---------------------|
| Image                                |                      | Image3_bead2            |          |            |                   |                     |
| image's creation                     | date                 | 2024-10-17 10:22:36     |          |            |                   |                     |
|                                      | 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)      | Nyquist/found ratio |
| Channel 0                            |                      |                         | 610.0    | none       | 0.109x0.109x0.328 | 0.063x0.063x0.06    |
| Bead original coordinates(in pixels) |                      | 929.0, 219.0            |          |            |                   |                     |

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. 610.0nm) | 10.4              | X         | 0.273              | 0.222       | 1.0          | 1.23              |
|                         |                   | Y         | 0.267              | 0.222       | 1.0          | 1.2               |
|                         |                   | Z         | 0.67               | 0.836       | 0.98         | 0.8               |

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

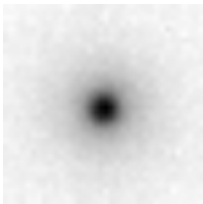
Lateral asymmetry ratios:

| Channel                 | Ratio |
|-------------------------|-------|
| Channel 0 (em. 610.0nm) | 0.98  |

Detailed channel detection info:

Channel #0

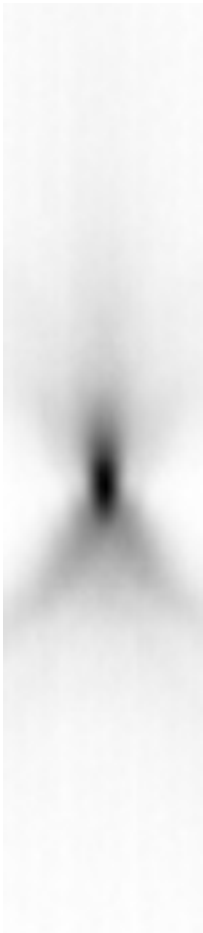
XY



YZ

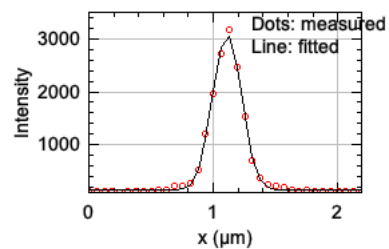


XZ

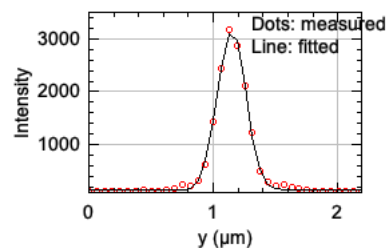


| Channel 0 (em. 610.0nm) |      |           |       |              |
|-------------------------|------|-----------|-------|--------------|
| Sig./Backgnd ratio      | LAR  | Dimension | FWHM  | Fit goodness |
| 10.4                    | 0.98 | X         | 0.273 | 1.0          |
|                         |      | Y         | 0.267 | 1.0          |
|                         |      | Z         | 0.67  | 0.98         |

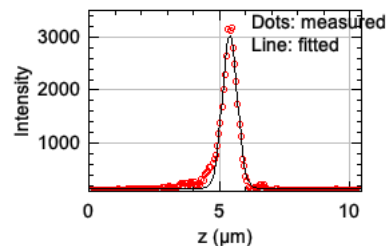
X profile & fitting parameters:  
Fit equation  $I(x) = a + (b-a) \cdot \exp(-(x-c)/(2 \cdot d))$   
Sum of residuals squared: 86361.6675  
Standard deviation: 49.67370  
R^2: 0.99629  
Parameters:  
a = 133.26039  
b = 3073.40354  
c = 1.11129  
d = 0.11599



Y profile & fitting parameters:  
Fit equation  $I(y) = a + (b-a) \cdot \exp(-(y-c)/(2 \cdot d))$   
Sum of residuals squared: 75897.5289  
Standard deviation: 46.56716  
R^2: 0.99686  
Parameters:  
a = 134.13760  
b = 3159.83229  
c = 1.14826  
d = 0.11321



Z profile & fitting parameters:  
Fit equation  $I(z) = a + (b-a) \cdot \exp(-(z-c)/(2 \cdot d))$   
Sum of residuals squared: 1113111.11  
Standard deviation: 79.98244  
R^2: 0.98263  
Parameters:  
a = 147.15336  
b = 3025.99790  
c = 5.39666  
d = 0.28457



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 25, 2024 2:38 PM                                                                                    |
| data                            | result folder                                              | /Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/610//Processed/63X_1_4_610/Image3/bead2/ |
|                                 | Type of saved data                                         | .pdf, .jpg, .xls                                                                                                |
|                                 | Input data bit depth                                       | 16                                                                                                              |
| Dimension order                 |                                                            | XY-(C)Z                                                                                                         |
| Discard saturated samples       |                                                            | false                                                                                                           |
| 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 1000.0)                                                                        |
|                                 | 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   |
|---------------|---------------------|------------|------------------|----------|
| Image 3_bead2 | 2024-10-17 10:22:36 | 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:

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

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