

## OrangeBeadsReport2

## Microscope info:

Image		Image5_bead21							
image's	date	2024-10-17 10:22:31							
creation	method used	from file creation date							
Actual image depth 16									
Microsco	Microscope type		WideField						
	NA	1.4	1.4						
Objective	im. refractive index	1.518							
Channel(s)		Wavel	engths		sampling (X,Y,Z)				
		Ex. (nm)	Em. (nm)	Saturation	Nyquist (µm)	Found (µm)	Nyquist/found ratio		
Channel 0			590.0	none	0.105x0.105x0. 317	0.063x0.063x0. 06	0.6, 0.6, 0.2		
Bead original pix	1117.0,	295.0				-			

#### Warnings:

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

#### Resolution table:

Channel	Sig/Backgn d ratio	Dimension	Measured FWHM (µm)	theory (µm)	Fit Goodness	Mes./theory ratio
Channel 0 (em. 590.0nm)	3.6	X	0.033	0.215	0.09	0.15
		Υ	0.839	0.215	0.06	3.91
		Z	Center detection failed			

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

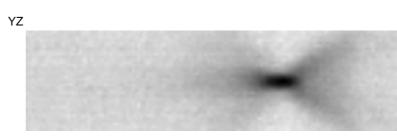
#### Lateral asymmetry ratios:

Channel	Ratio
Channel 0 (em. 590.0nm)	0.04

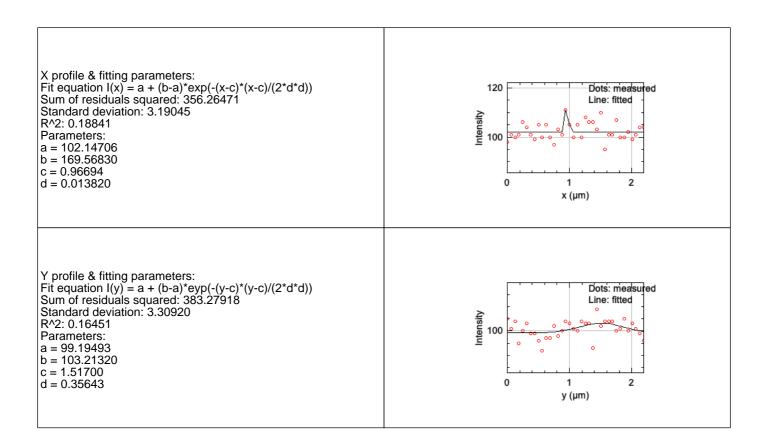
## Detailed channel detection info:

## Channel #0





Channel 0 (em. 590.0nm)					
Sig./Backgnd ratio	LAR	Dimension	FWHM	Fit goodness	
		X	0.033	0.09	
3.6	0.04	Υ	0.839	0.06	
		Z	Center detection failed		



### Analysis parameters

	Tool	PSF Profiler (batch)			
Tool & Operator	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 10:52 AM			
data	result folder	/Users/bumozaza/Desktop/Zeiss WFM/orange/Processed/OrangeBeadsReport2/Image 5/bead21/			
data	Type of saved data	.pdf, .jpg, .xls			
	Input data bit depth	16			
Dim	ension order	XY-(C)Z			
Discard s	aturated samples	true			
	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			
Beads	Background annulus distance to bead edges in µm	0.5			
Deads	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			
	Applied in this report	true			
Tolerance	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 5_bead21	2024-10-17 10:22:31	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:

$$res_{x,y}^o = \frac{0.51*\lambda_{em}}{NA}$$
  $res_z^o = \frac{1,77n*\lambda_{em}}{NA^2}$ 

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

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

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

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