63X_1_4_585 - Batch Summary

Microscope info:

data	141 analysed images				
images location	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/585/				
Actual image depth	16				
Microscope type	WideField				
Objective	NA	1.4			
	im. refractive index	1.518			
Channel(s)	Wavelengths		unsaturated/total images	sampling (X,Y,Z)	
	Ex. (nm)	Em. (nm)		Nyquist (µm)	correctly sampled/total images
Channel 0		585.0	all ok	0.104x0.104x0.314	(all ok, all ok, all ok)

Warnings:

(no saturation issue detected)

(All images & channels sampled following Shannon-Nyquist criterion)

(A subresolution bead is used for all channels).

Average resolutions values:

		X	Y	Z
Channel 0	average FWHM (µm)	0.266	0.266	0.666
	FWHM std dev (µm)	0.009	0.01	0.023
	theoretical value (µm)	0.213	0.213	0.802
	number of beads	137	136	139
	mean R2 value	0.99	0.99	0.98
	mean SBR value	4.73		

Measured/theoretical resolution ratios and lateral asymmetry ratios:

Channel	X ratio	Y ratio	Z ratio	Lateral Asymmetry
Channel 0	1.25	1.25	0.83	1.0

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

Analysis parameters

Tool & Operator	Tool	Batch PSF Profiler
	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 2:33 PM
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/585/Processed/63X_1_4_585/
	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 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	5.0
	Cropped ROI size in µm	2.31x2.31 (using bead size & background annulus parameters)
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
Measurement rejected	Outliers	true (using IQR)
	R2 ratio below	0.95

Analysis log

image name	creation date	sampling density	identified raw beads	valid beads	saturation	status
Image 6	2024-10-17 10:22:32	correct	43	33	none	valid beads found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
				bead17	none	analysed
				bead18	none	analysed
				bead19	none	analysed
				bead20	none	analysed
				bead21	none	analysed
				bead22	none	analysed
				bead23	none	analysed
				bead24	none	analysed
				bead25	none	analysed
				bead26	none	analysed
				bead27	none	analysed
				bead28	none	analysed
				bead29	none	analysed
				bead30	none	analysed
				bead31	none	analysed
				bead32	none	analysed
Image 4	2024-10-17 10:22:29	correct	30	17	none	valid beads found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
Image 5	2024-10-17	correct	66	30	none	valid beads

	10:22:31					found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
				bead17	none	analysed
				bead18	none	analysed
				bead19	none	analysed
				bead20	none	analysed
				bead21	none	analysed
				bead22	none	analysed
				bead23	none	analysed
				bead24	none	analysed
				bead25	none	analysed
				bead26	none	analysed
				bead27	none	analysed
				bead28	none	analysed
				bead29	none	analysed
Image 1	2024-10-17 10:22:25	correct	23	18	none	valid beads found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
				bead17	none	analysed
Image 2	2024-10-17 10:22:27	correct	39	27	none	valid beads found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed

|						
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
				bead17	none	analysed
				bead18	none	analysed
				bead19	none	analysed
				bead20	none	analysed
				bead21	none	analysed
				bead22	none	analysed
				bead23	none	analysed
				bead24	none	analysed
				bead25	none	analysed
				bead26	none	analysed
Image 3	2024-10-17 10:22:28	correct	27	16	none	valid beads found
				bead0	none	analysed
				bead1	none	analysed
				bead2	none	analysed
				bead3	none	analysed
				bead4	none	analysed
				bead5	none	analysed
				bead6	none	analysed
				bead7	none	analysed
				bead8	none	analysed
				bead9	none	analysed
				bead10	none	analysed
				bead11	none	analysed
				bead12	none	analysed
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed

Identified beads

green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.
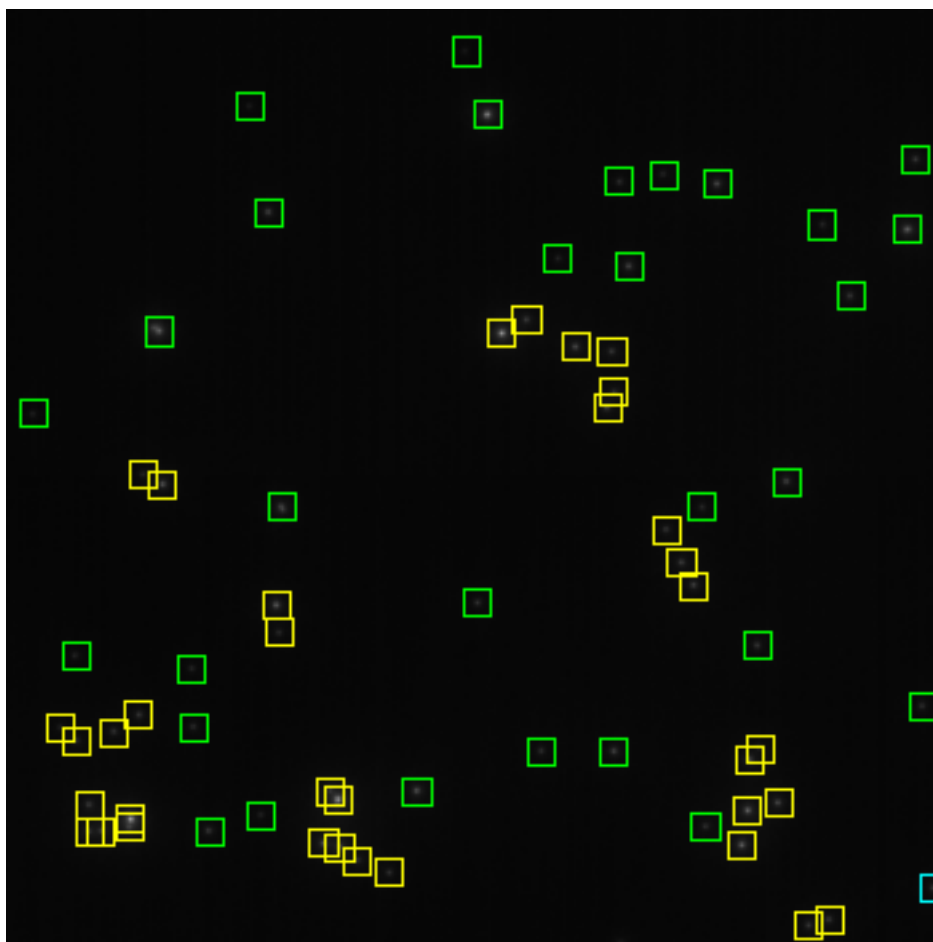
Image6
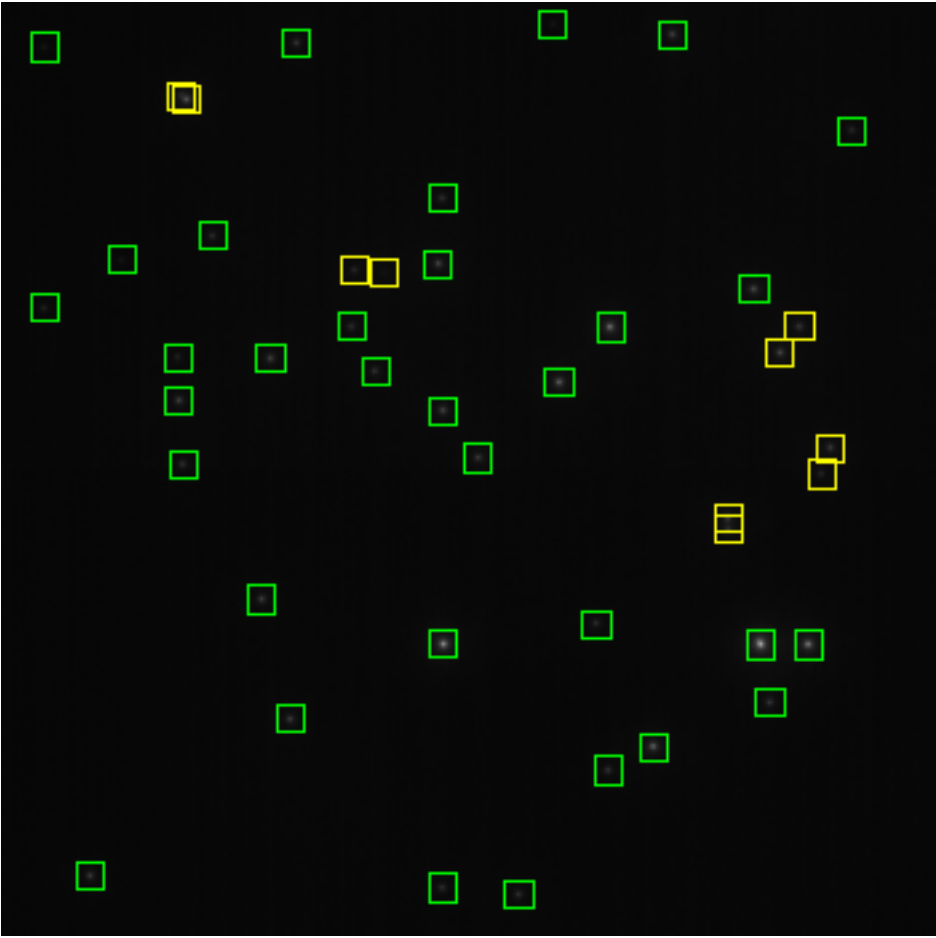
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image4
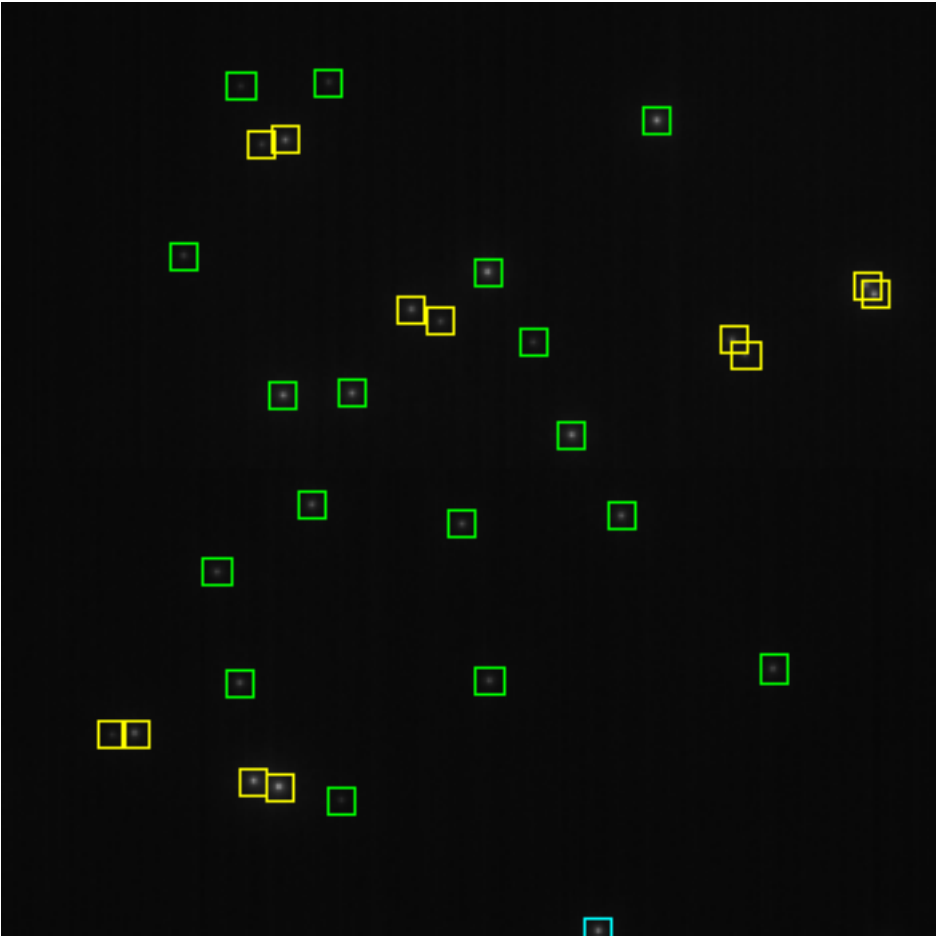
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.
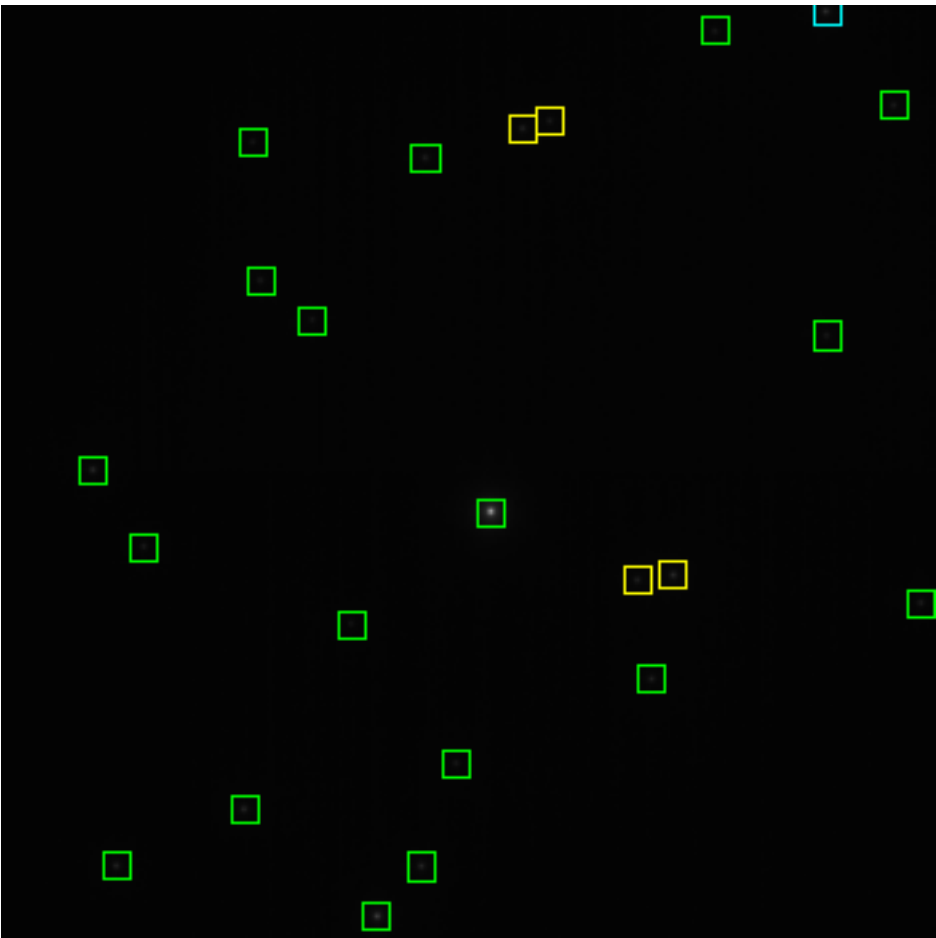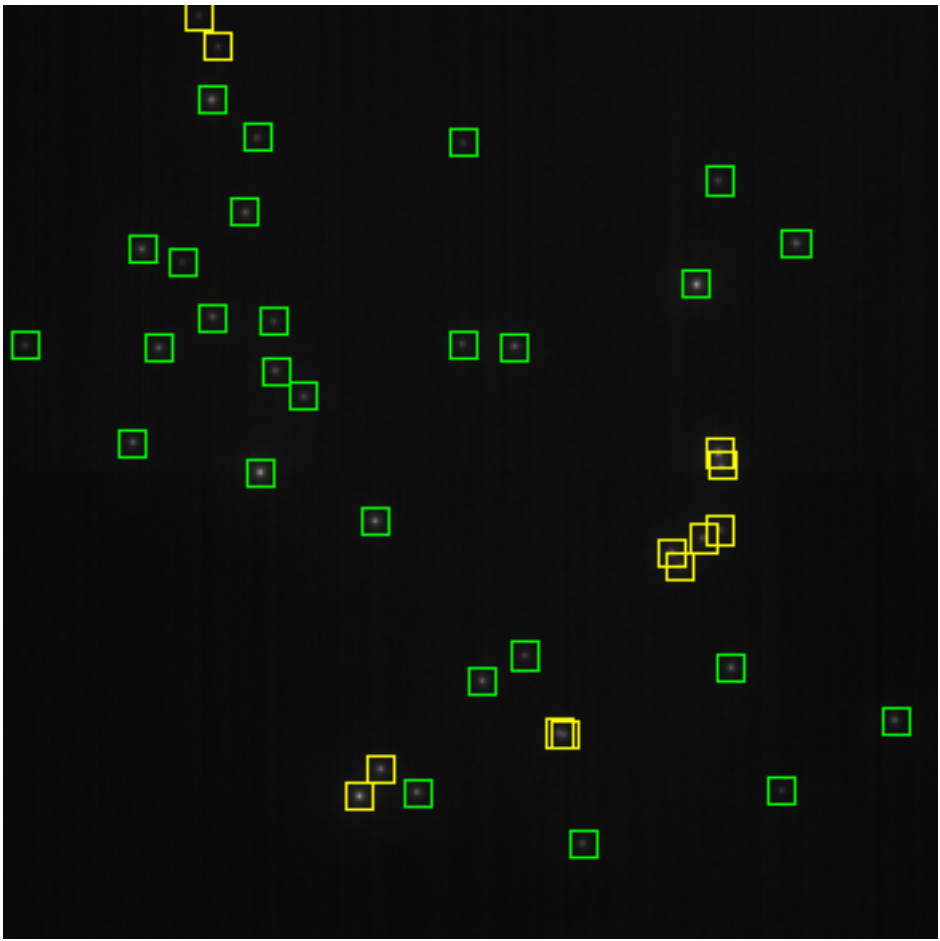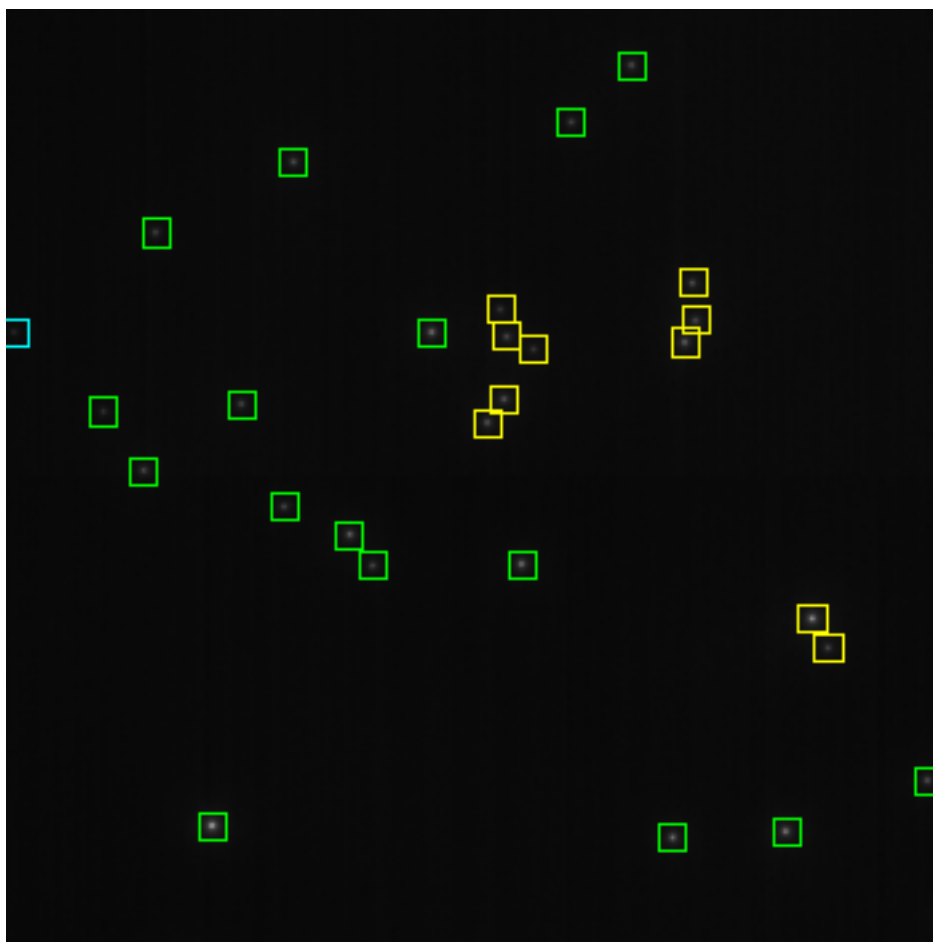
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image1

green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

<u>Formulas used:</u>

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

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

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

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