63X_1_4_525 - Batch Summary

Microscope info:

data		253 analysed images				
images location		/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/525/				
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			525.0	all ok	0.094x0.094x0.282	(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)	0.233	0.234	0.573
	FWHM std dev (µm)	0.008	0.008	0.046
	theoretical value (µm)	0.191	0.191	0.72
	number of beads	235	240	241
	mean R2 value	1.0	1.0	0.99
	mean SBR value	13.61		

Measured/theoretical resolution ratios and lateral asymmetry ratios:

Channel	X ratio	Y ratio	Z ratio	Lateral Asymmetry
Channel 0	1.22	1.22	0.8	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 2:36 PM
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Elyra/PSFs/20241014/63X_1_4/525/Processed/63X_1_4_525/
	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 8	2024-10-17 10:22:23	correct	90	38	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
				bead33	none	analysed
				bead34	none	analysed
				bead35	none	analysed
				bead36	none	analysed
				bead37	none	analysed
Image 9	2024-10-17 10:22:24	correct	243	60	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
				bead33	none	analysed
				bead34	none	analysed
				bead35	none	analysed
				bead36	none	analysed
				bead37	none	analysed
				bead38	none	analysed
				bead39	none	analysed
				bead40	none	analysed
				bead41	none	analysed
				bead42	none	analysed
				bead43	none	analysed
				bead44	none	analysed
				bead45	none	analysed
				bead46	none	analysed
				bead47	none	analysed
				bead48	none	analysed
				bead49	none	analysed
				bead50	none	analysed
				bead51	none	analysed
				bead52	none	analysed
				bead53	none	analysed
				bead54	none	analysed
				bead55	none	analysed
				bead56	none	analysed
				bead57	none	analysed
				bead58	none	analysed
				bead59	none	analysed
Image 10	2024-10-17 10:22:15	correct	137	54	Ch.0 saturated	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
					bead33	none	analysed
					bead34	none	analysed
					bead35	none	analysed
					bead36	none	analysed
					bead37	none	analysed
					bead38	none	analysed
					bead39	none	analysed
					bead40	none	analysed
					bead41	none	analysed
					bead42	none	analysed
					bead43	none	analysed
					bead44	none	analysed
					bead45	none	analysed
					bead46	none	analysed
					bead47	none	analysed
					bead48	none	analysed
					bead49	none	analysed
					bead50	none	analysed
					bead51	none	analysed
					bead52	none	analysed
					bead53	none	analysed
Image 7	2024-10-17 10:22:21	correct	81	46	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

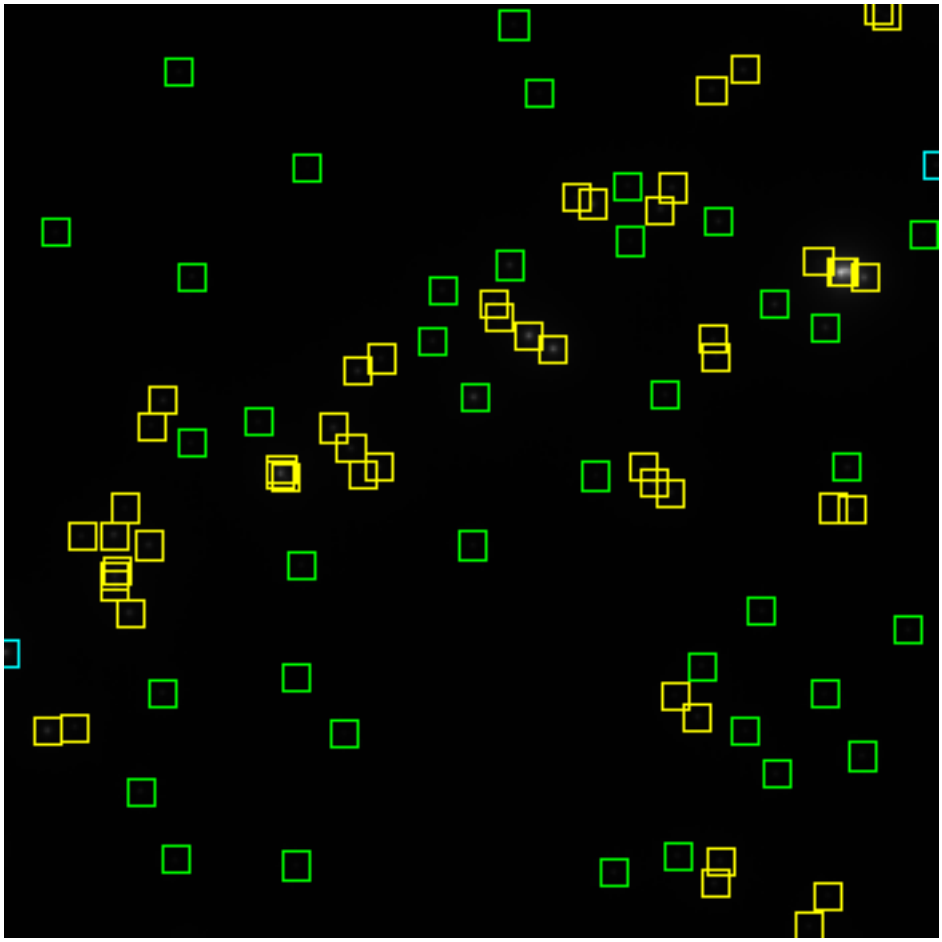
				bead13	none	analysed
				bead14	none	analysed
				bead15	none	analysed
				bead16	none	analysed
				bead17	none	analysed
				bead18	none	analysed
				bead19	none	analysed
				bead20	none	analysed
				bead21	none	analysed
				bead22	none	analysed
				bead23	none	analysed
				bead24	none	analysed
				bead25	none	analysed
				bead26	none	analysed
				bead27	none	analysed
				bead28	none	analysed
				bead29	none	analysed
				bead30	none	analysed
				bead31	none	analysed
				bead32	none	analysed
				bead33	none	analysed
				bead34	none	analysed
				bead35	none	analysed
				bead36	none	analysed
				bead37	none	analysed
				bead38	none	analysed
				bead39	none	analysed
				bead40	none	analysed
				bead41	none	analysed
				bead42	none	analysed
				bead43	none	analysed
				bead44	none	analysed
				bead45	none	analysed
Image 6	2024-10-17 10:22:20	correct	12	10	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
Image 4	2024-10-17 10:22:18	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 5	2024-10-17 10:22:19	correct	19	11	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
Image 1	2024-10-17 10:22:14	correct	1	1	none	valid beads found
				bead0	none	analysed
Image 2	2024-10-17 10:22:16	correct	15	15	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

Identified beads

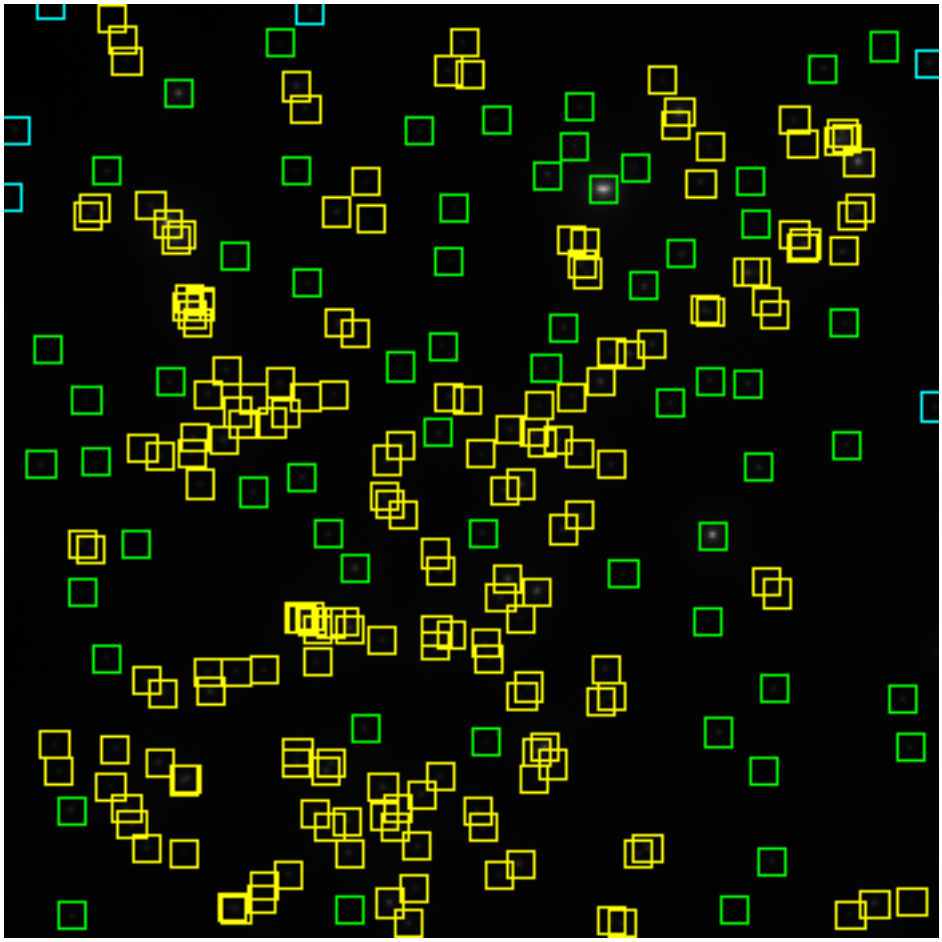
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image8



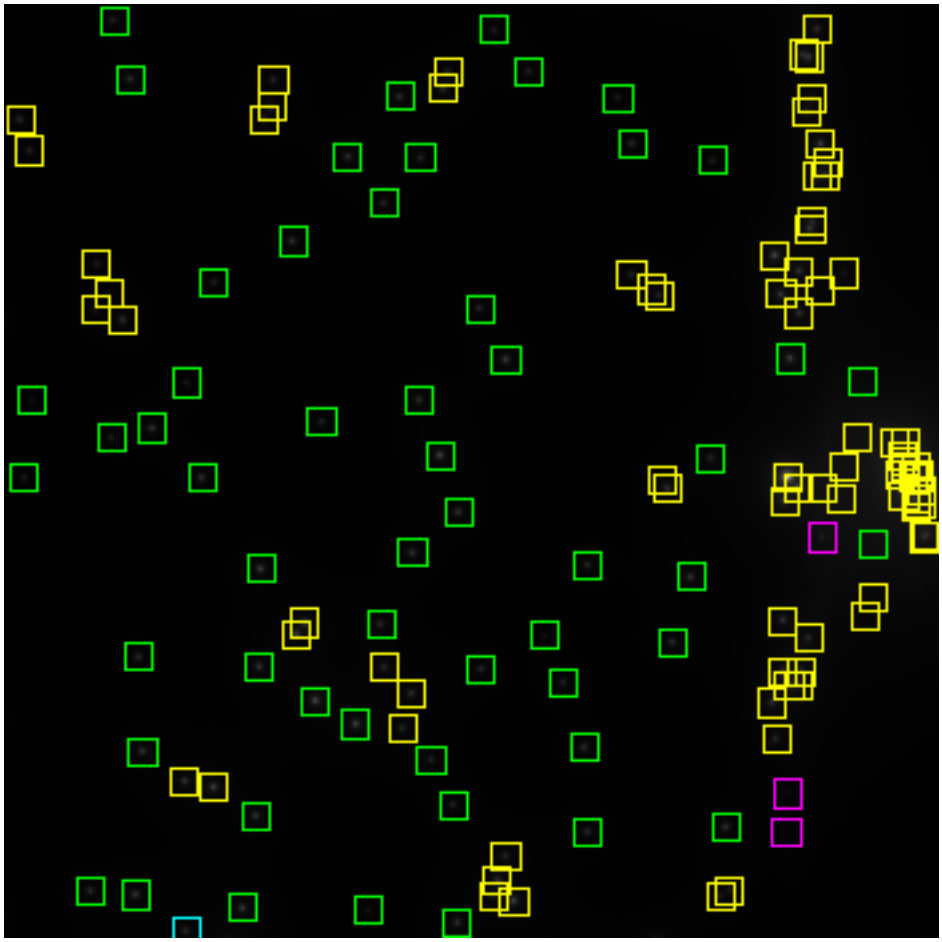
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image9



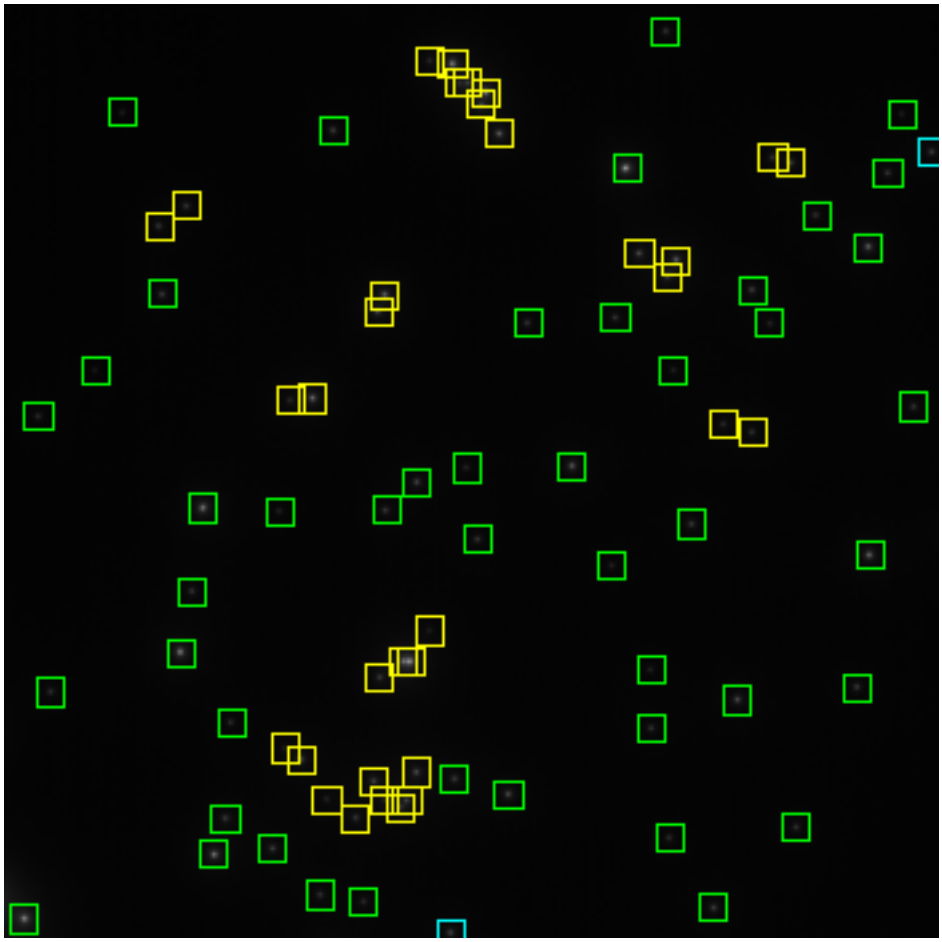
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image10



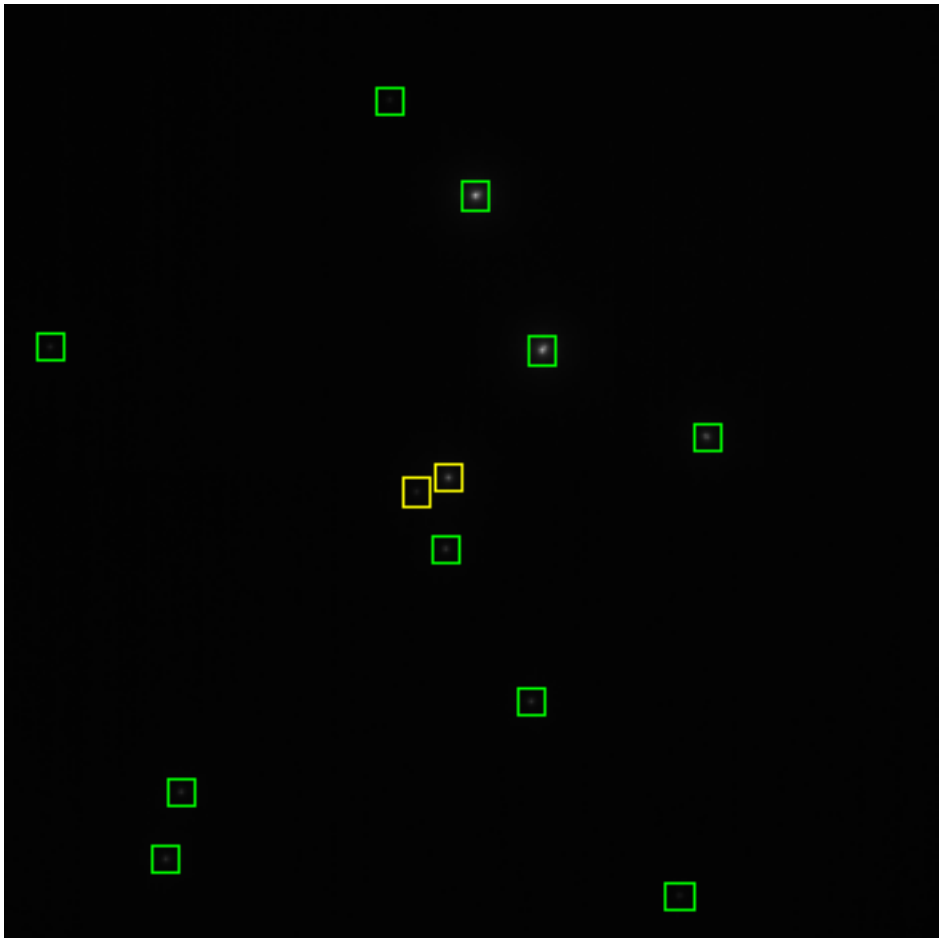
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image7



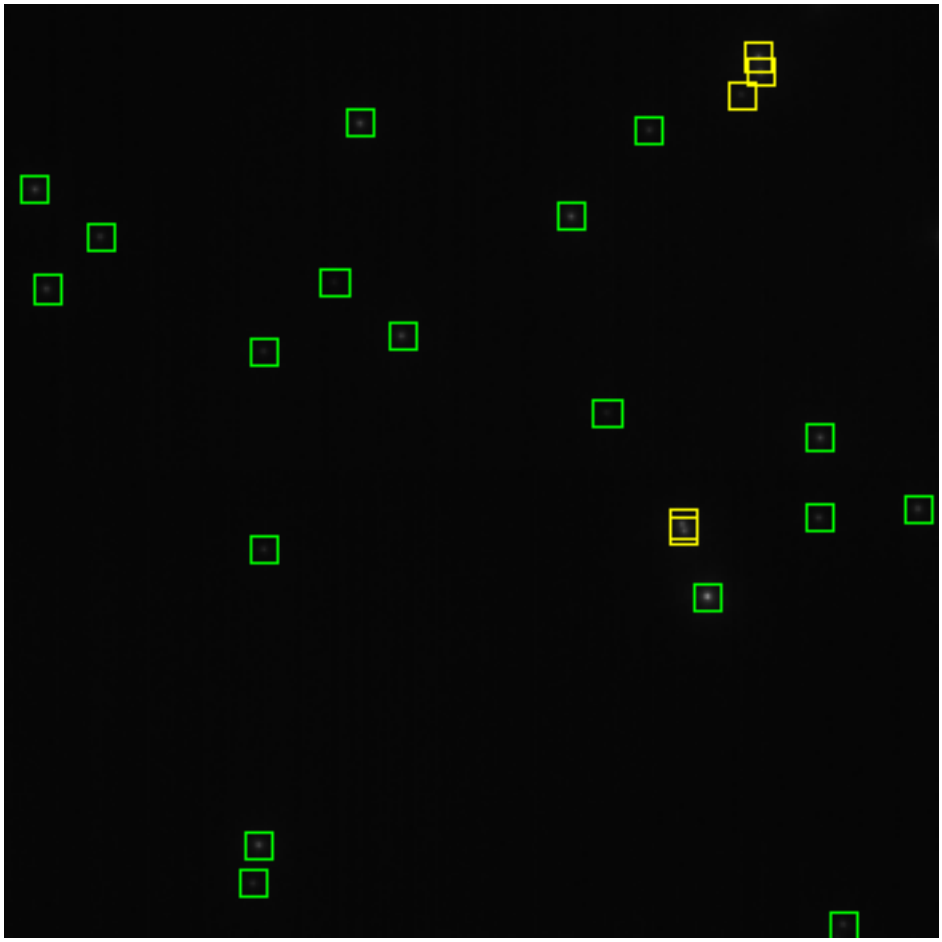
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image6



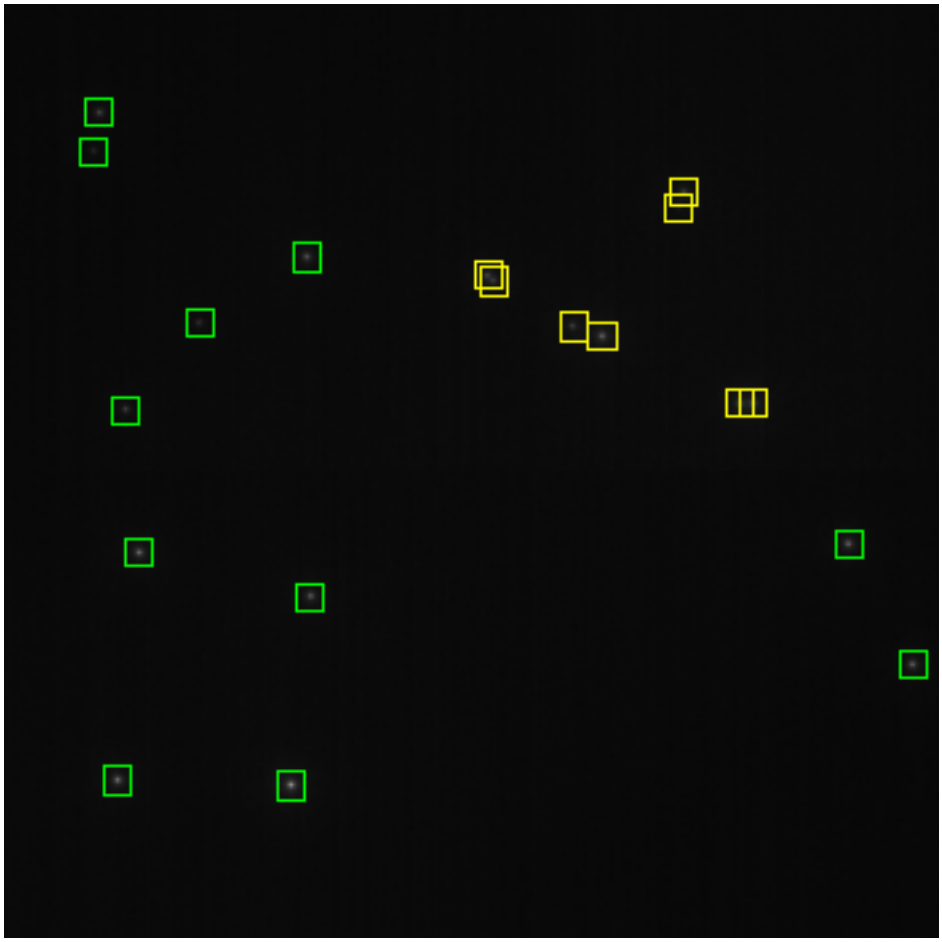
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image4



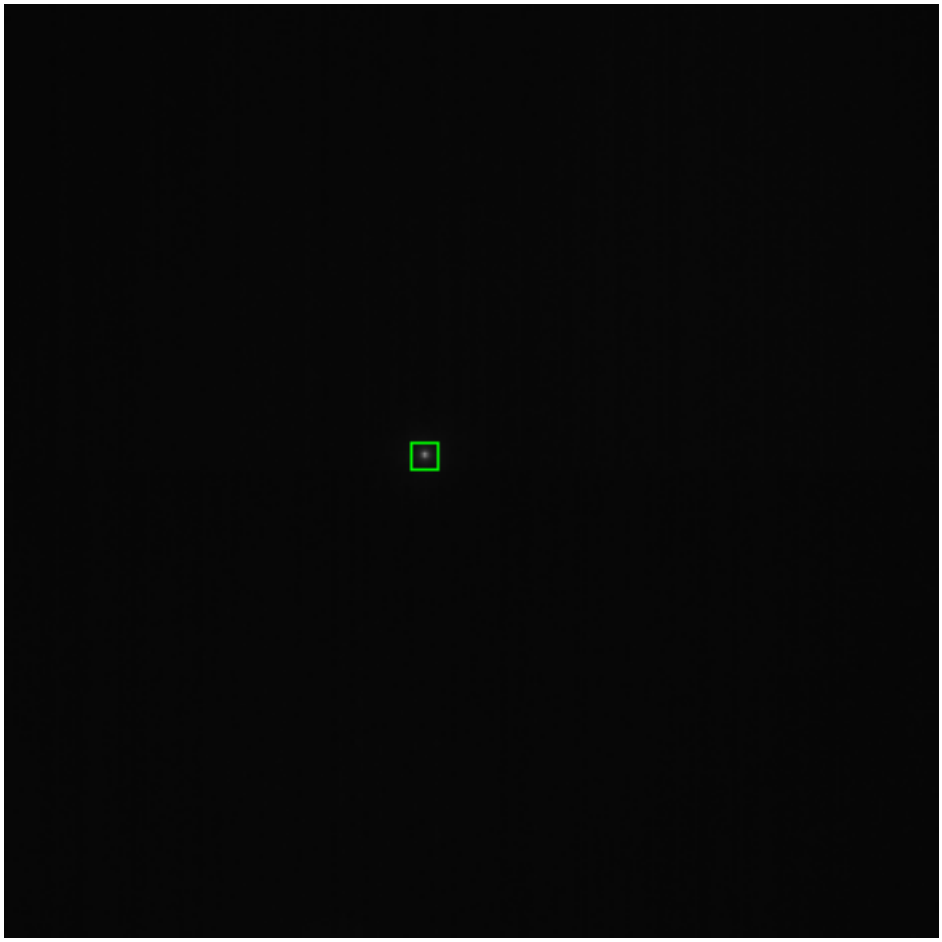
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

Image5



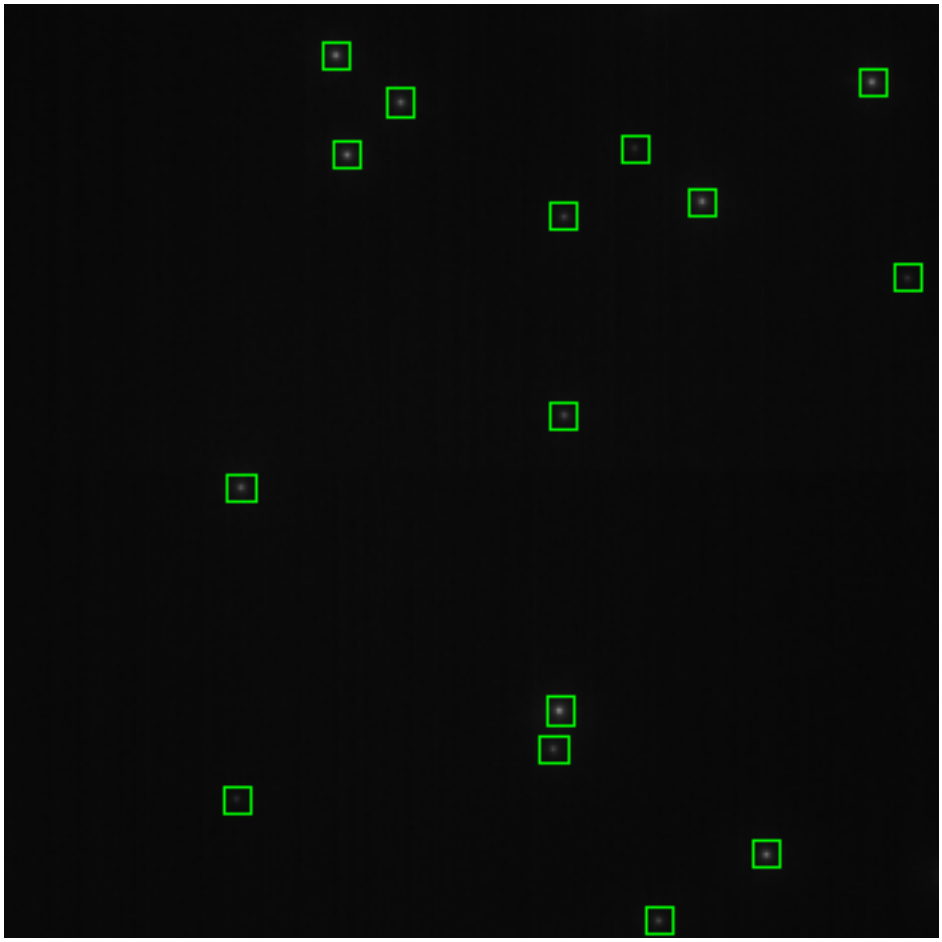
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

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.

Image2



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.

Formulas used:

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

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

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

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