63X_1_2_525 - Batch Summary

Microscope info:

data		1518 analysed images				
images location		/Users/oggsc/Documents/OM/ImageAnalysis/QC/Thunder/PSF/20241015/63X_1_2_525/				
Actual image depth		12				
Microscope type		WideField				
Objective	NA	1.2				
	im. refractive index	1.333				
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	0.109x0.109x0.349	(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)	0.27	0.283	0.898
	FWHM std dev (µm)	0.009	0.009	0.051
	theoretical value (µm)	0.223	0.223	0.86
	number of beads	1159	1132	1195
	mean R2 value	1.0	0.99	0.98
	mean SBR value	0.0		

Measured/theoretical resolution ratios and lateral asymmetry ratios:

Channel	X ratio	Y ratio	Z ratio	Lateral Asymmetry
Channel 0	1.21	1.27	1.04	0.95

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 31, 2024 8:41 AM
data	result folder	/Users/oggsc/Documents/OM/ImageAnalysis/QC/Thunder/PSF/20241015/63X_1_2_525/Processed/63X_1_2_525/
	Type of saved data	.pdf, .jpg, .xls
	Input data bit depth	12
Dimension order		XY-(C)Z
Discard saturated samples		true
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 50.0)
	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
GreenBeads63x.lif - Series005	2024-10-26 09:47:20	correct	859	428	none	valid beads found
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GreenBeads63x.lif - Series004	2024-10-26 09:47:32	correct	981	462	none	valid beads found
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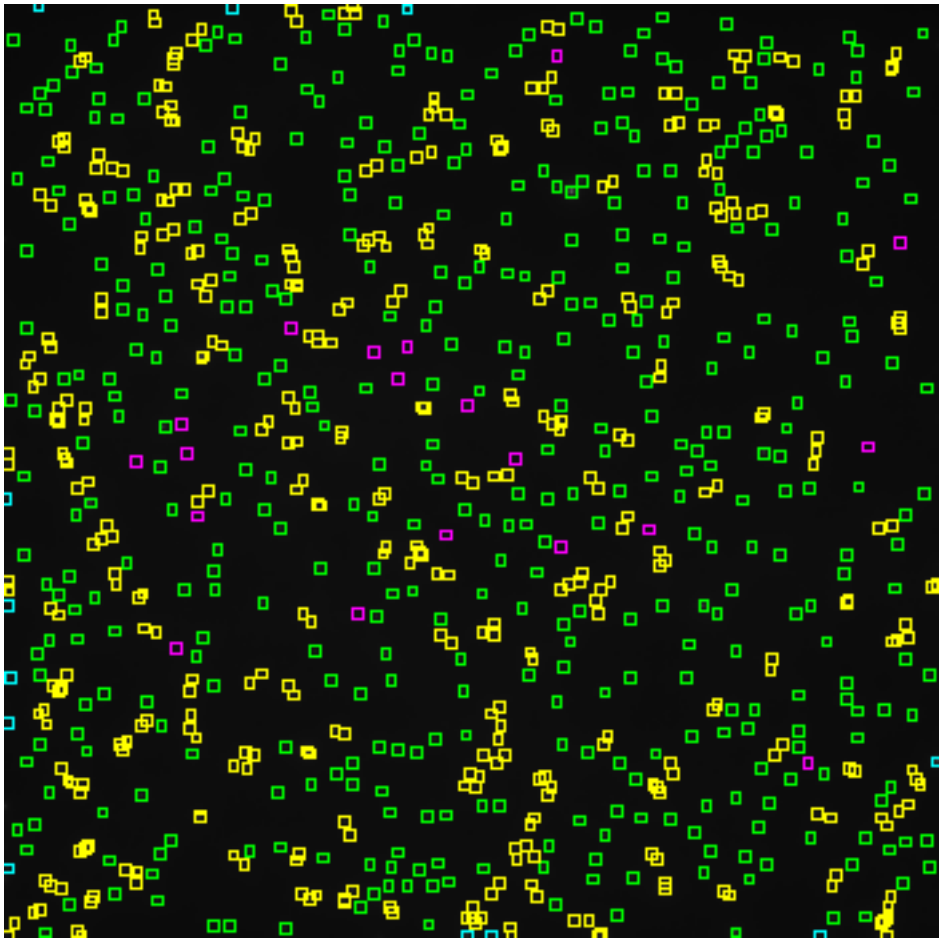
Identified beads

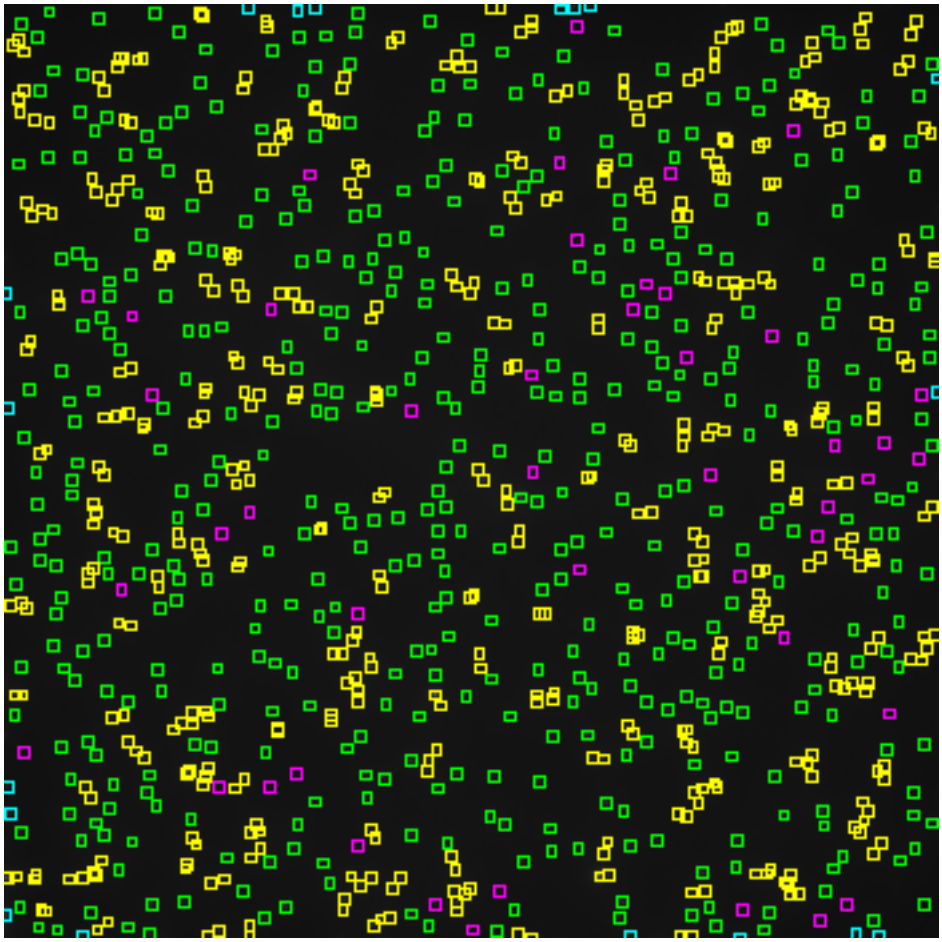
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

GreenBeads63x



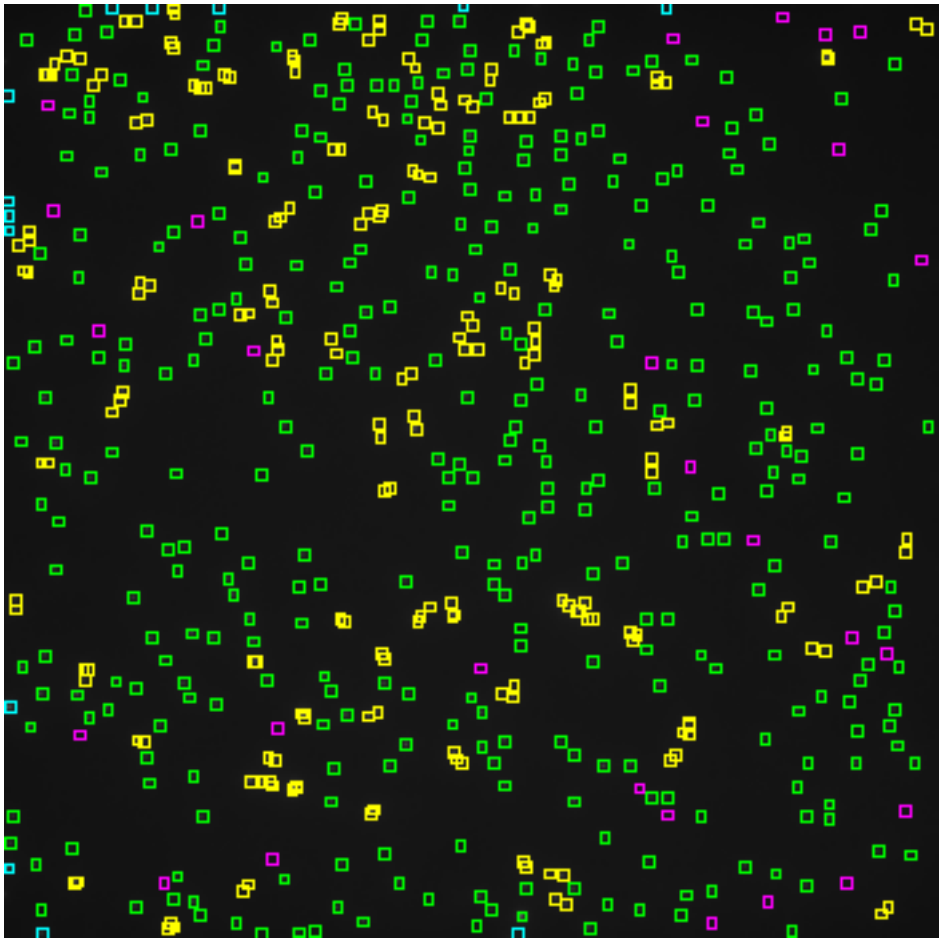
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

GreenBeads63x



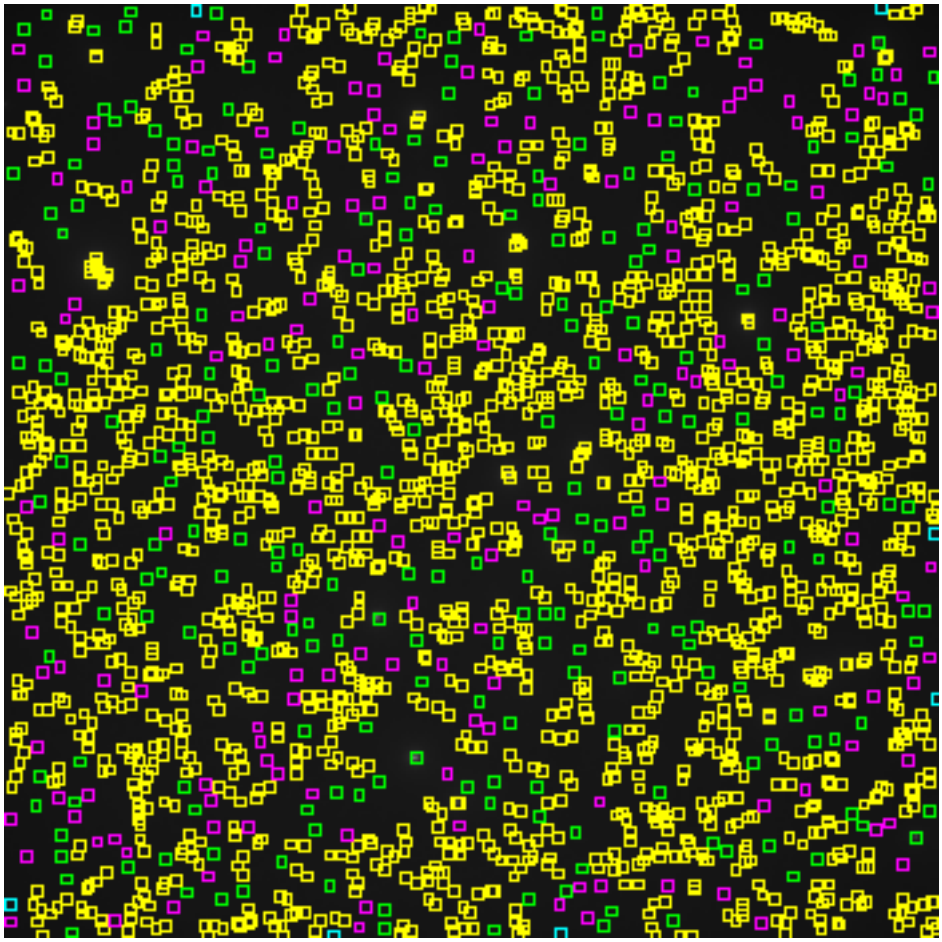
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

GreenBeads63x



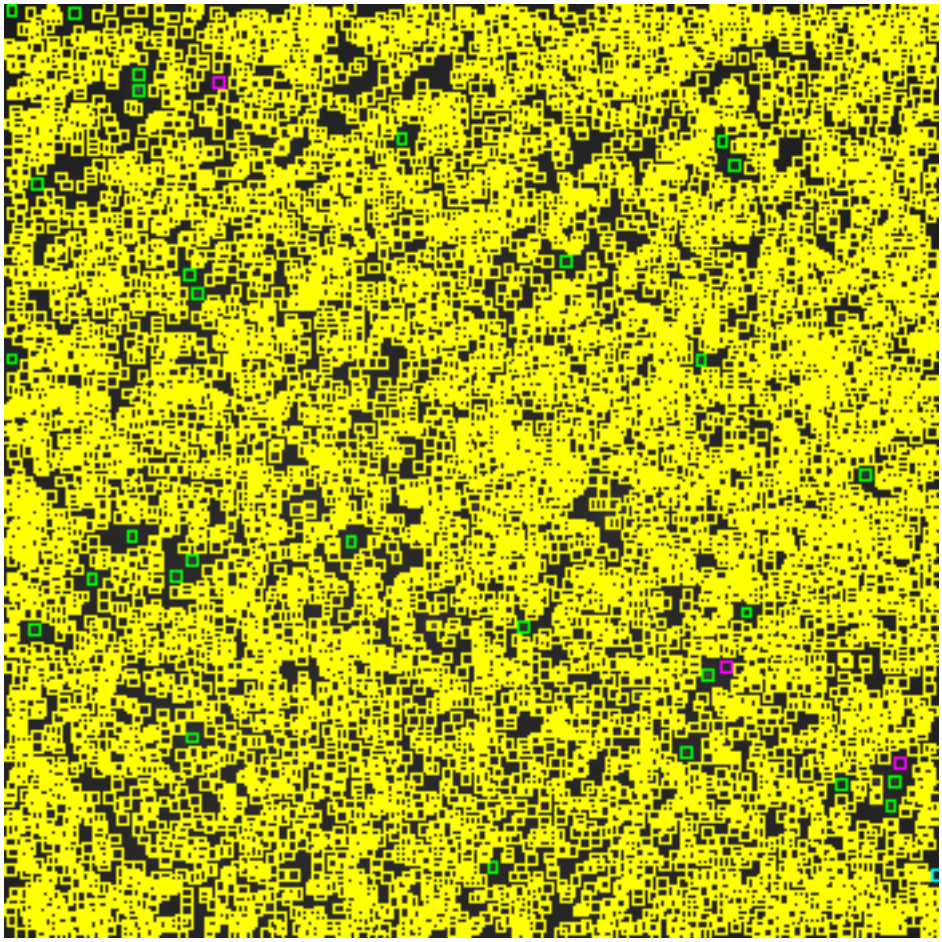
green: valid bead, yellow: too close to another bead, magenta: too close to stack's top or bottom, cyan: too close to the image's edges.

GreenBeads63x



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.

GreenBeads63x



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:

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

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