

# Automated, Robust Recognition and Extraction of the Double-helix Point Spread Function in Fluorescence Microscope Images

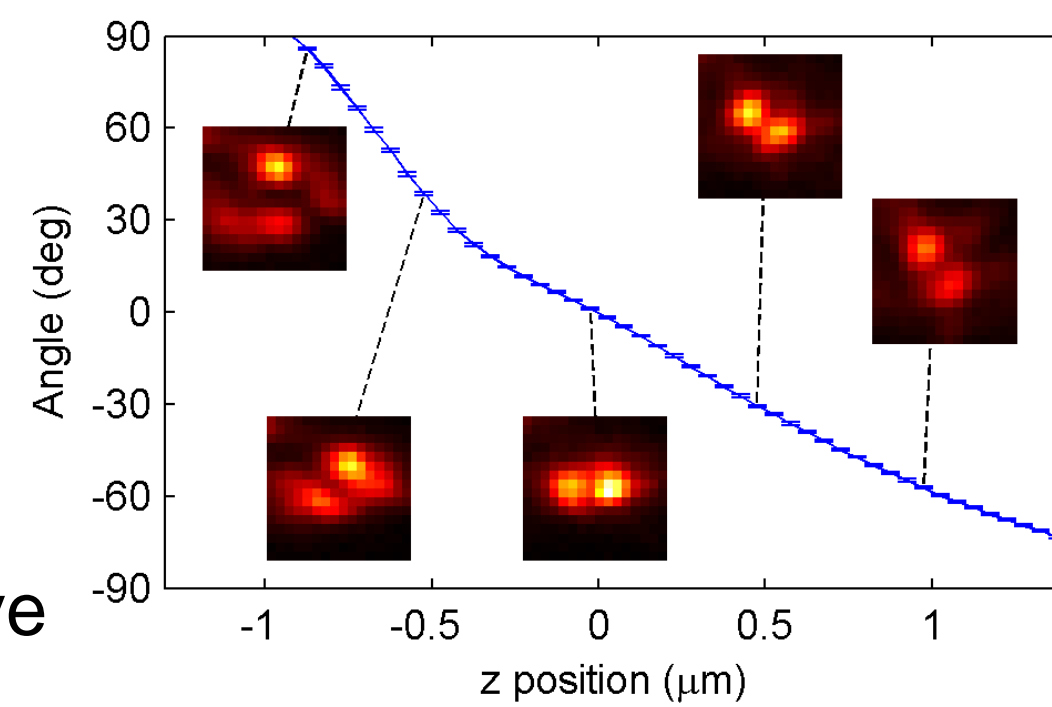
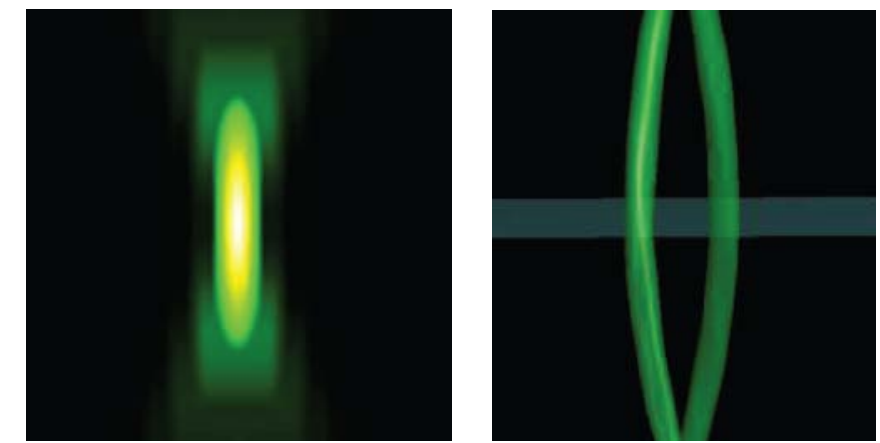
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## Background

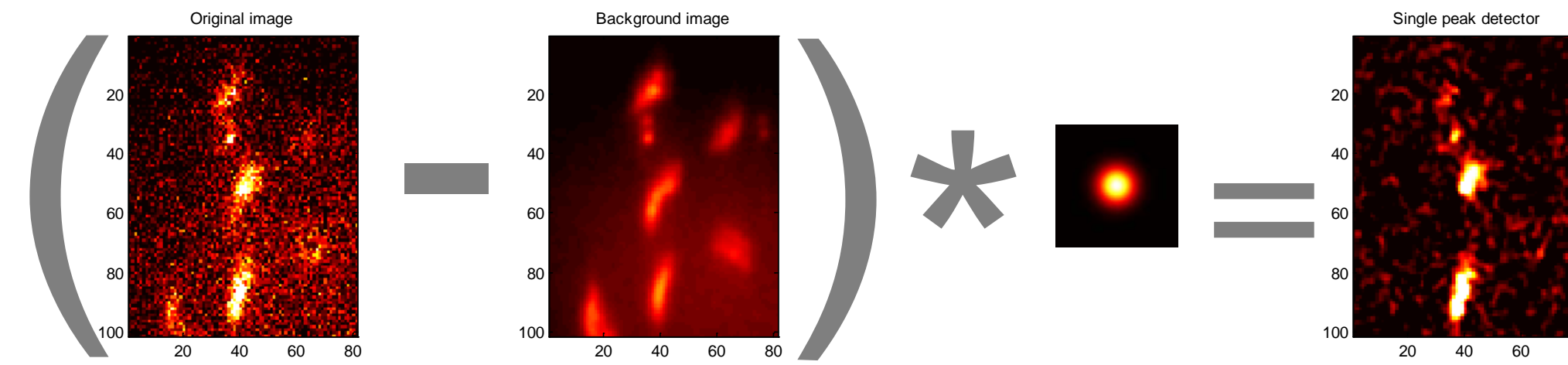


Diffraction-limited microscopy cannot adequately resolve the location of the fluorescently labeled DNA in these cells. We combine superlocalization with a double-helix point spread function (DH-PSF) to localize the DNA molecules in three dimensions.

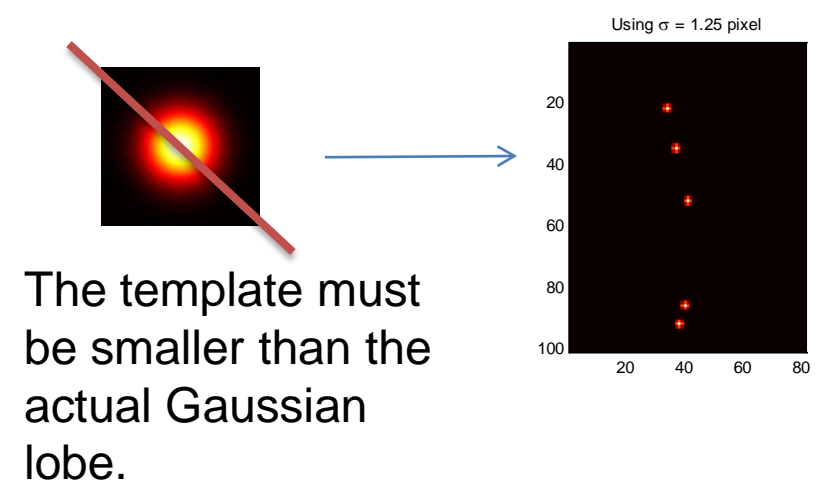


The double-helix point spread function is much more sensitive to variation in depth (z).

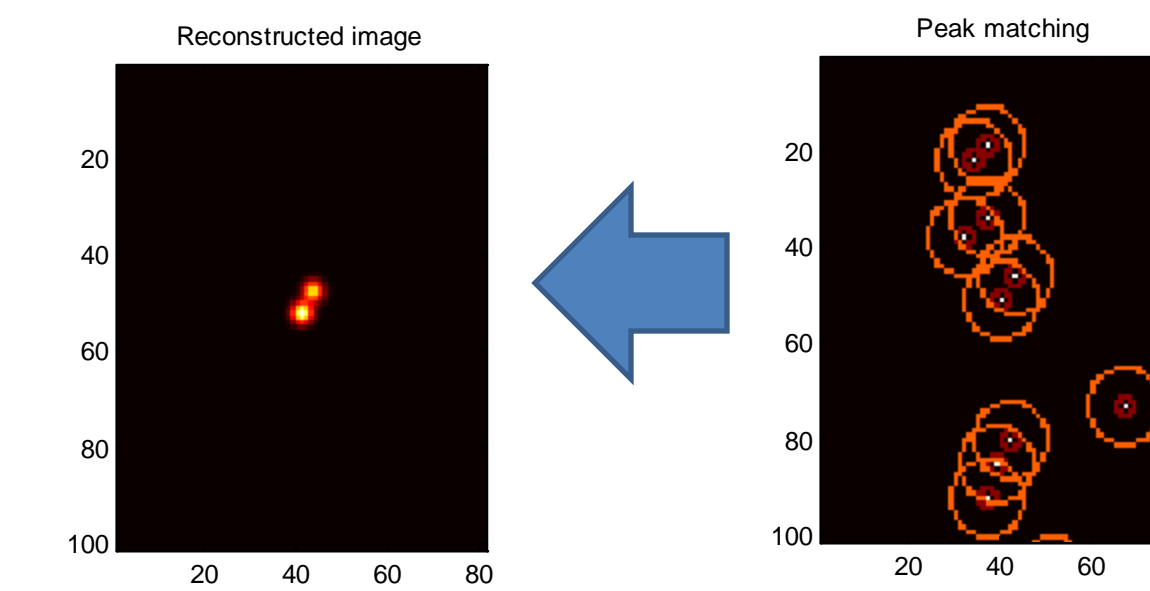
## Single-Peak Detection



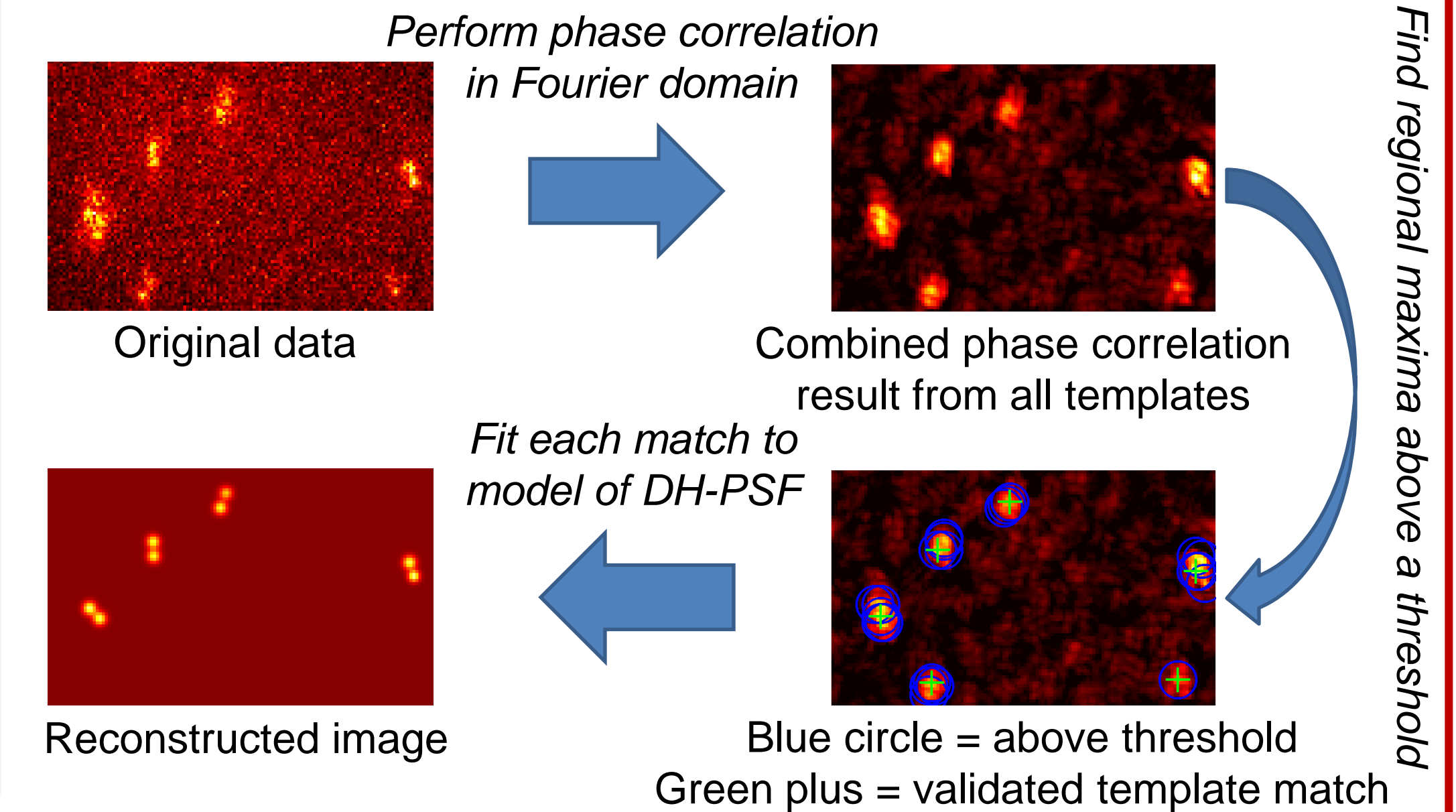
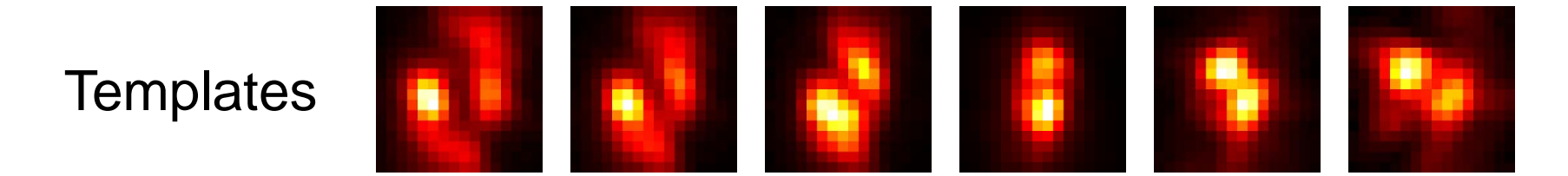
The single peak detection algorithm works by template matching, followed by peak matching.



The template must be smaller than the actual Gaussian lobe.

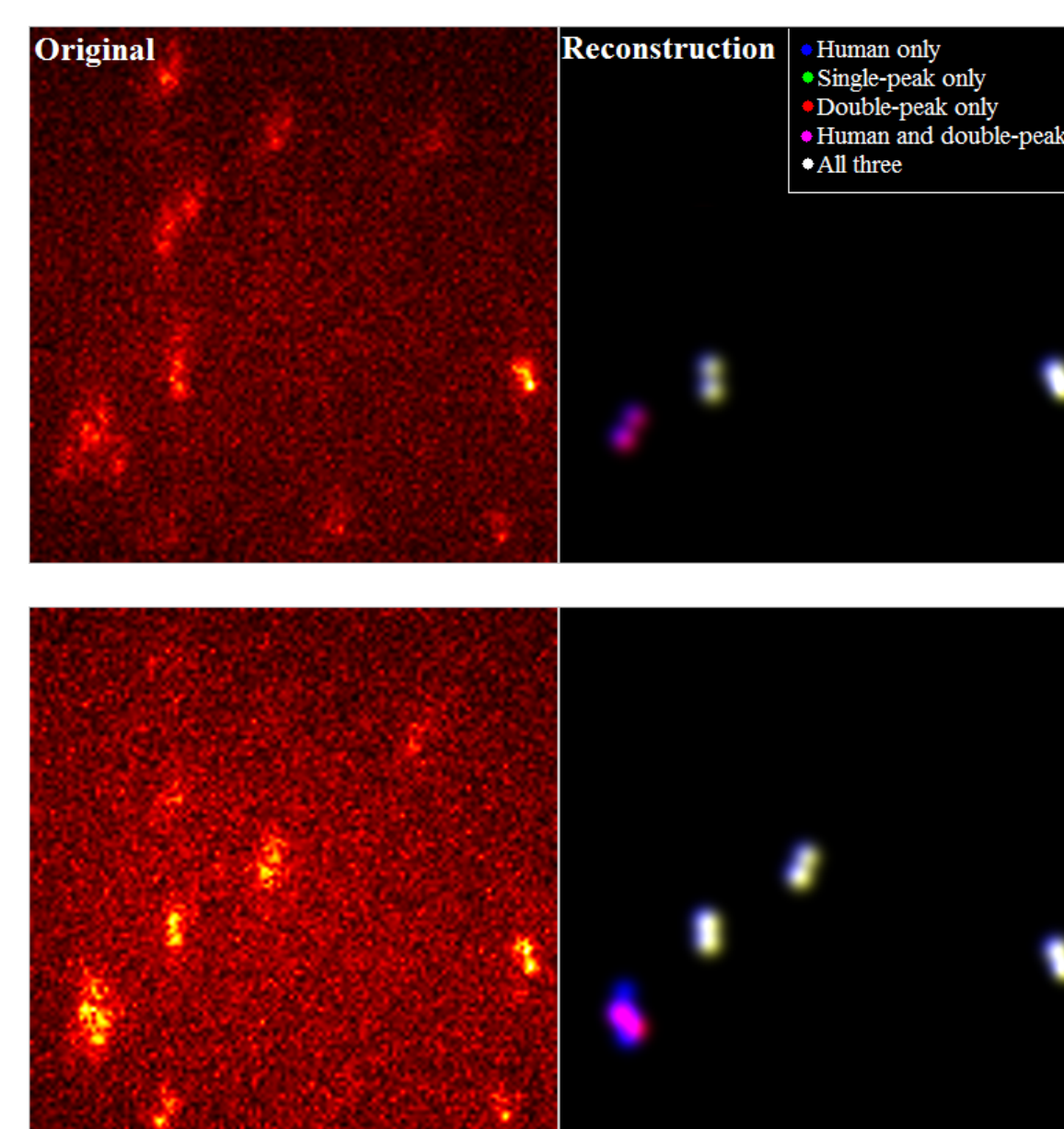


## Double-Peak Detection



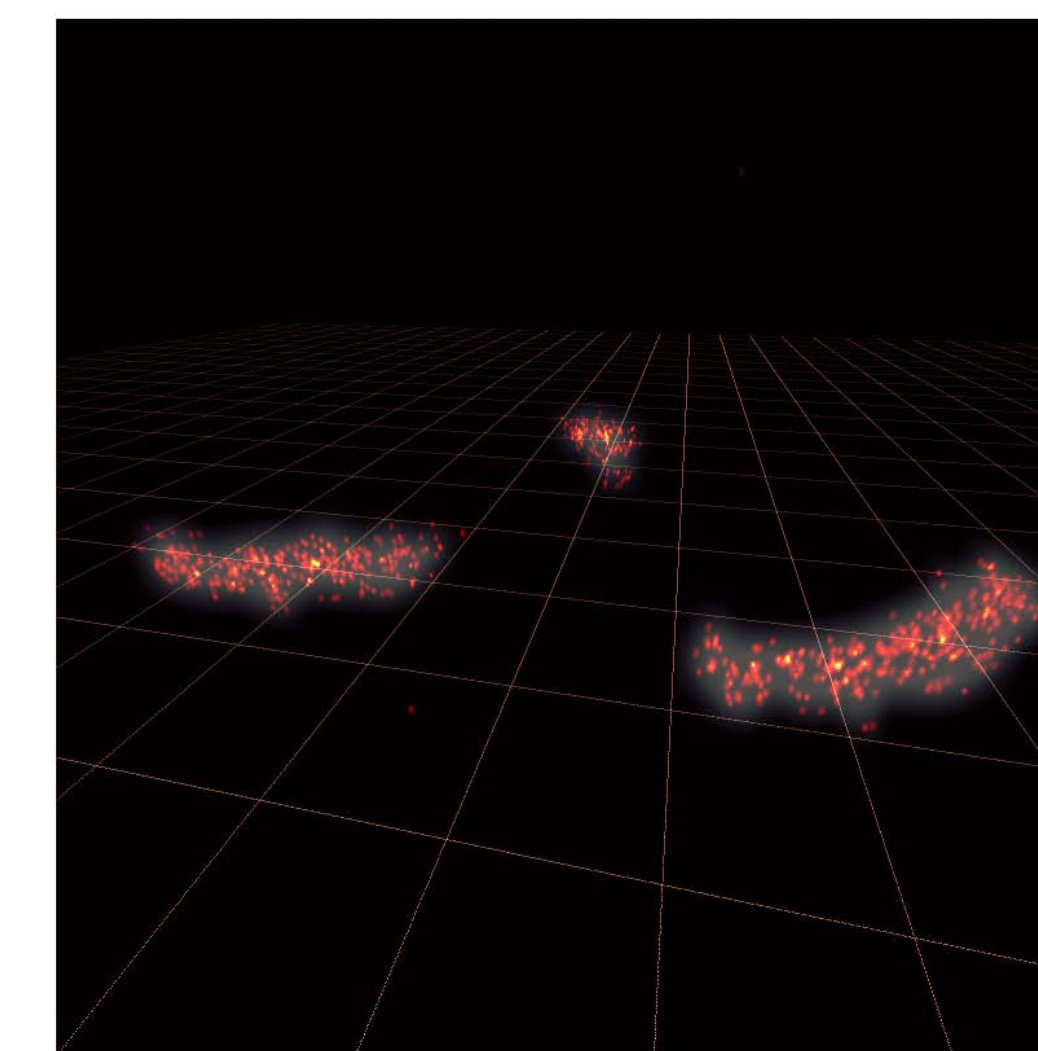
## Experimental Results

	Human	Single-Peak	Double-Peak
Total DH-PSFs found	232	180	197
Number of matches with human	--	167 (93%)	184 (93%)
False positives	--	13 (7.2%)	13 (6.6%)
False negatives	--	65 (28%)	48 (21%)



By using more information, the double-peak algorithm is able to outperform the single-peak algorithm compared to a human observer on a test set of some of the hardest cases.

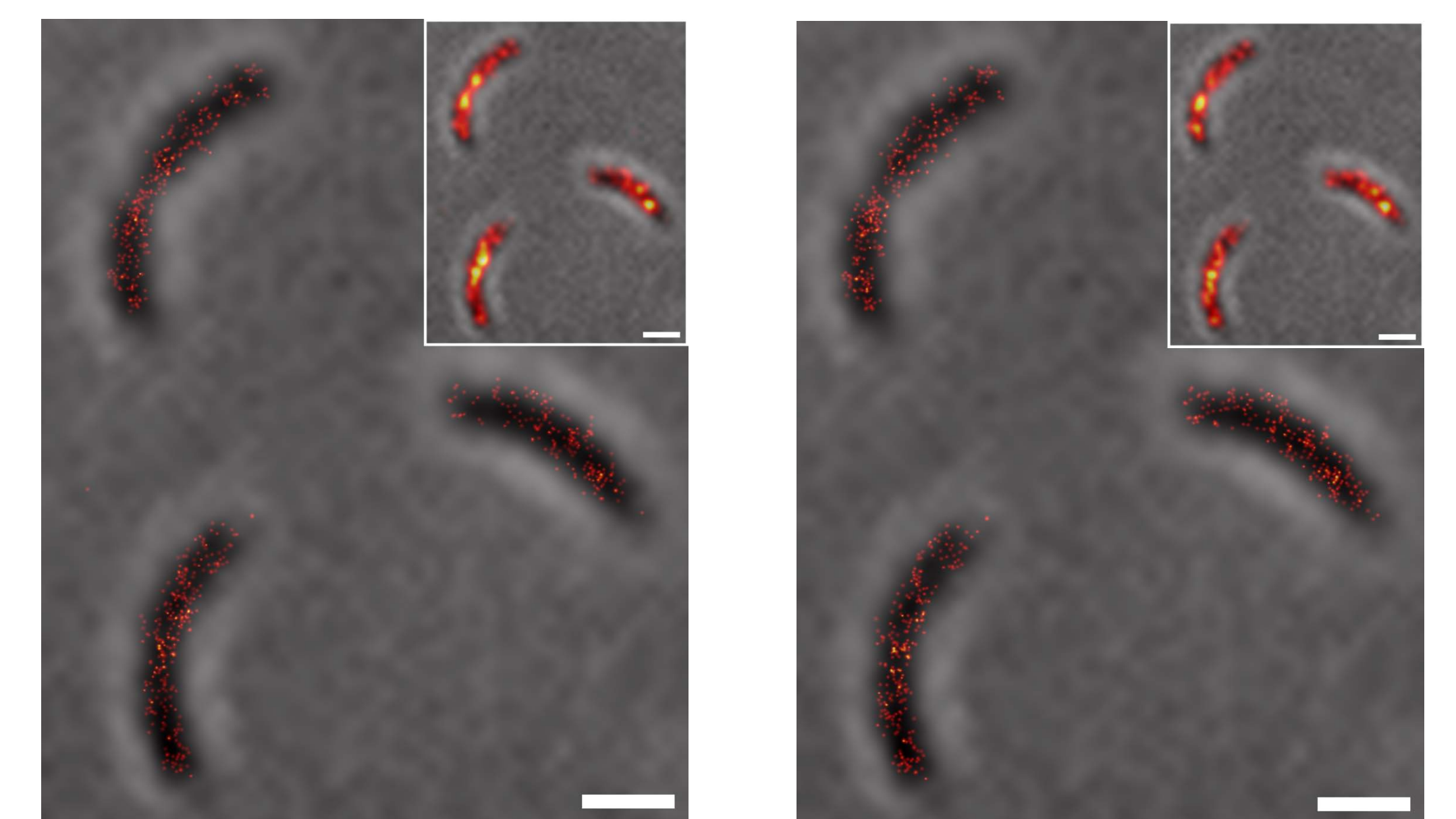
### Perspective view



Automated extraction of the points enables the fast, practical visualization of the distribution of DNA throughout the cell.

### Superresolution images of DNA locations within bacteria

2D view: fluorescence (red) overlaid with white light (gray)



Double-peak detection

Single-peak detection

Insets show diffraction-limited versions of the superresolution images, scalebar = 1 μm