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
Perform phase correlation in Fourier domain

Combined phase correlation result from all templates

Fit each match to model of DH-PSF

Reconstructed image Blue circle = above threshold

Green plus = validated template match

Experimental Results

<table>
<thead>
<tr>
<th></th>
<th>Human</th>
<th>Single-Peak</th>
<th>Double-Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DH-PSFs found</td>
<td>232</td>
<td>180</td>
<td>197</td>
</tr>
<tr>
<td>Number of matches with human</td>
<td>--</td>
<td>167 (93%)</td>
<td>184 (93%)</td>
</tr>
<tr>
<td>False positives</td>
<td>--</td>
<td>13 (7.2%)</td>
<td>13 (6.6%)</td>
</tr>
<tr>
<td>False negatives</td>
<td>--</td>
<td>65 (28%)</td>
<td>48 (21%)</td>
</tr>
</tbody>
</table>

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.

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