Accurate detection of spherical objects in a complex background: supplemental document

1. CONVOLUTION KERNELS FOR ANISOTROPIC PIXELS

While the size of a voxel (3d pixel) along the x- and y-axis in the focal plane is usually the same for a confocal microscope, its size along the z-direction is often larger, as the resolution along the axial direction is not as good as in the focal plane. With the voxel size given by \( v_i, i \in \{x, y, z\} \), the pixel location can be expressed by the coordinates \((x[i], y[j], z[k]) = (iv_x, jv_y, kv_z)\). The boxcar kernel used to calculate the background image is then defined as

\[
k_b(i, j, k) = \begin{cases} 
\frac{1}{N_b} & \sqrt{x^2[i] + y^2[j] + z^2[k]} < R_0 \\
0 & \text{otherwise}
\end{cases}
\]

\[
N_b = \sum_{i,j,k} 1. \quad \sqrt{x^2[i] + y^2[j] + z^2[k]} < R_0
\]

The standard deviation of the Gaussian convolution kernel used for the smoothened image can differ for the axes:

\[
G(i, j, k) = \frac{1}{N} \exp \left( -\frac{1}{2} \left( \frac{x^2[i]}{\sigma_x^2} + \frac{y^2[j]}{\sigma_y^2} + \frac{z^2[k]}{\sigma_z^2} \right) \right)
\]

with

\[
N = \sum_{i,j,k} \exp \left( -\frac{1}{2} \left( \frac{x^2[i]}{\sigma_x^2} + \frac{y^2[j]}{\sigma_y^2} + \frac{z^2[k]}{\sigma_z^2} \right) \right)
\]

2. PIXEL BIASING

The particle coordinates obtained with the Crocker-Grier (CG) method often suffer from pixel biasing [1]. I.e. the determined coordinates have a higher probability to be close to the center of a voxel than between two neighboring voxels. This bias towards integer positions is due to the trend of the CG method to find the position close to the brightest voxel and it is usually strongest for the direction with lowest resolution, usually the z direction along the optical axis of the microscope. The bias is easily detected by plotting the histogram of the fractional part of the coordinates as shown in Fig. S1. The B&∇ method gives coordinates that are less biased, as both brightness and gradient of the smoothed image determine the positions of the peaks in image \( I_2 \) that are used to detect the particles, see the histogram with circles in Fig. S1. In most cases, the pixel bias is removed by applying the fracshift correction.

3. CALCULATION OF THE FILTERED IMAGES \( I_{DC} \) AND \( I_2 \)

For the feature detection using the CG or the B&∇ method, the raw image \( I \) is convolved with a Gaussian filter to reduce the noise in the image. For large images, this is best done using the Fourier transform of the image, \( \hat{I} \), and of the Gaussian kernel given in Eq. 1.

\[
\hat{I}_b = \hat{I} \hat{G}
\]

where the hat (\( \hat{\cdot} \)) represents the Fourier transform. Likewise, the Fourier transform of the background image is obtained using the kernel given in Eq. 2 as

\[
\hat{I}_b = \hat{I} k_b,
\]

The difference of these images gives the image \( I_d \):

\[
I_d = \mathcal{F}^{-1} [I - I_b],
\]
where $F^{-1}$ represents the inverse Fourier transform. Further, all negative values in $I_d$ are changed to zero to have $I_d \geq 0$ everywhere and to obtain isolated peaks for the particles to be detected.

The gradient of the image $I_s$ is used as an input for the B&∇ method. As $I_s = I \otimes G$ is obtained with a convolution, the gradient of the kernel $G$ can be used to obtain $\nabla I_s$:

$$\nabla I_s = F^{-1} \left[ i q_j \hat{G}(q) \hat{I}(q) \right].$$

The kernel $k_c$ (Eq. 3) is then applied in Fourier space to obtain

$$I_g = \sum_{j=1}^{3} F[\partial_j I_s] k_{c,j},$$

$$= \sum_{j=1}^{3} i q_j \hat{G}(q) \hat{I}(q) \hat{k}_{c,j},$$

$$I_g = F^{-1} \left[ \left( \sum_{j=1}^{3} i q_j \hat{k}_{c,j} \right) \hat{G}(q) \hat{I}(q) \right].$$

Finally, all negative values in $I_g$ are changed to zero to have $I_{gc} \geq 0$, and the combined image $I_2$ is obtained by multiplying the clipped images: $I_2 = I_{dc} I_{gc}$.

For the B&Y method, the gradient of the smoothed image and the corresponding spherical
where the equality 
\[ \hat{m} \]
where the calculation is performed for

The signal-to-noise ratio (SNR) used in image analysis is defined as the amplitude
\[ S \]
of the useful signal divided by the standard deviation \( \sigma \) of the noise:

5. SIGNAL-TO-NOISE RATIO

The Hessian matrix \( H(r) \) is calculated using the fast Fourier transform of the smoothened image \( I_s \). As it is a symmetric matrix, the following components are calculated:

\[
H_{xx}(r) = \mathcal{F}^{-1} \left[ -\hat{I}_s(q) q_x^2 \right]
\]

\[
H_{yy}(r) = \mathcal{F}^{-1} \left[ -\hat{I}_s(q) q_y^2 \right]
\]

\[
H_{zz}(r) = \mathcal{F}^{-1} \left[ -\hat{I}_s(q) q_z^2 \right]
\]

\[
H_{xz}(r) = \mathcal{F}^{-1} \left[ -\hat{I}_s(q) q_x q_z \right]
\]

\[
H_{yz}(r) = \mathcal{F}^{-1} \left[ -\hat{I}_s(q) q_y q_z \right]
\]

Here, \( \hat{I}_s(q) \) is the Fourier transform of \( I_s \). As six Fourier back-transformations of the image are required, the calculation of the Hessian matrix is one of the most time consuming parts of our particle-detection code.

5. SIGNAL-TO-NOISE RATIO

The signal-to-noise ratio (SNR) used in image analysis is defined as the amplitude \( S \) of the useful signal divided by the standard deviation \( \sigma \) of the noise:

\[
\text{SNR} = \frac{S}{\sigma}
\]

\( S \) is measured by the standard deviation of the perfect image without noise: \( S^2 = \langle (P - \langle P \rangle)^2 \rangle \).

To estimate the SNR of a microscopy image, we use two images, \( I_1 = P + n_1 \) and \( I_2 = P + n_2 \), taken under the same conditions, which only differ due to the noise \( n_i \). The noise is assumed to have zero mean and to be uncorrelated:

\[
\langle n \rangle = 0
\]

\[
\langle n^2 \rangle = \sigma^2
\]

\[
\langle n_1 n_2 \rangle = 0.
\]
The SNR can then be obtained as

\[ \text{SNR} = \sqrt{\frac{R}{1 - R}} \]

\[ R = \frac{\langle I_1 I_2 - \langle I_1 \rangle \langle I_2 \rangle \rangle}{\sqrt{\langle (I_1 - \langle I_1 \rangle)^2 \rangle \langle (I_2 - \langle I_2 \rangle)^2 \rangle}} \]

REFERENCES

1. J. C. Crocker and E. R. Weeks, “Particle tracking tutorial,” http://www.physics.emory.edu/faculty/weeks/idl/tracking.html.