Error estimate of z-coordinate in 3D Single Molecule Localization

Tung-Han Hsieh

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The relation between z-coordinate of the molecules and the width in x, y direction is through the calibration curve (the defocusing curve):

$$w_{x,y}(z) = w_0 \mathrm{Re}\left(\sqrt{1 + \left(\frac{z - c_{x,y}}{d_{x,y}}\right)^2 + A_{x,y}\left(\frac{z - c_{x,y}}{d_{x,y}}\right)^3 + B_{x,y}\left(\frac{z - c_{x,y}}{d_{x,y}}\right)^4}\right) \tag{1}$$

where w_0, $A_{x,y}$, $B_{x,y}$, $c_{x,y}$, and $d_{x,y}$ are calibration parameters determined from experiments. In general these parameters are complex numbers (except the parameter w_0). The z-coordinate of the molecules can be obtained via solving z in Eq(1) with respect to the fitted width w_x and w_y from experimental measurements.

There are two ways to determine the z coordinate. The first one is to solve z_x and z_y for the input of w_x and w_y, and then take the average of z_x and z_y. The second one is to solve w_x/w_y for z. We describe the error estimate of z through the input of w_x, w_y and their errorbars δw_x, δw_y for both methods.

1 Method 1: average of z_x and z_y

For convenience, we first consider that all calibration parameters are real numbers. We define the variable $u_{x,y}$ as:

$$u(z) = \left(\frac{z - c}{d}\right)^2 + A\left(\frac{z - c}{d}\right)^3 + B\left(\frac{z - c}{d}\right)^4 \tag{2}$$

Here we have suppressed the labels x and y for simplicity, which will be put back when necessary. Then Eq(1) can be rewritten as

$$w(z) = w_0\sqrt{1 + u(z)} \tag{3}$$

Suppose that there is no errorbars in the calibration parameters. The only errorbar is δw, which comes from the fitting for $w(z)$. Then the relation between δw and δz is

$$\delta w = \frac{w_0^2}{2|w(z)|}\delta u \tag{4}$$

where

$$\delta u = \sqrt{\left[2\Big(\frac{z-c}{d}\Big)\right]^2 + \left[3A\Big(\frac{z-c}{d}\Big)^2\right]^2 + \left[4B\Big(\frac{z-c}{d}\Big)^3\right]^2}\,\delta z \tag{5}$$

Putting Eq(4) and Eq(5) together one can solve δz from δw:

$$\delta z = \frac{2|w(z)|}{w_0^2}\delta w \left\{\left[2\Big(\frac{z-c}{d}\Big)\right]^2 + \left[3A\Big(\frac{z-c}{d}\Big)^2\right]^2 + \left[4B\Big(\frac{z-c}{d}\Big)^3\right]^2\right\}^{-1/2} \tag{6}$$

Therefore, for the input of calibration parameters, $w_{x,y}(z)$, and $\delta w_{x,y}$, we can obtain $\delta z_{x,y}$. Finally, errorbar δz_a of the average z_x and z_y is

$$\delta z_a = \frac{1}{2}\sqrt{(\delta z_x)^2 + (\delta z_y)^2} \tag{7}$$

Now for in general the parameters A, B, c, and d may be complex numbers, we modify Eq(6) as

$$\delta z = \frac{2|w(z)|}{w_0^2}\delta w \left\{\left|2\Big(\frac{z-c}{d}\Big)\right|^2 + \left|3A\Big(\frac{z-c}{d}\Big)^2\right|^2 + \left|4B\Big(\frac{z-c}{d}\Big)^3\right|^2\right\}^{-1/2} \tag{8}$$

That is, take the absolute square of the three terms inside the overall square root, so that the resulting $\delta z_{x,y}$ is real and positive quantity. Then Eq(7) can be used to obtain the final errorbar of z coordinate.

2 Method 2: solving for $w_x(z)/w_y(z)$

For $v(z) = w_x(z)/w_y(z)$, its errorbar δv is

$$\delta v = v\sqrt{\left(\frac{dw_x}{w_x}\right)^2 + \left(\frac{dw_y}{w_y}\right)^2} \tag{9}$$

Here we understood that v, w_x, and w_y are all functions of z. On the other hand, $v(z)$ can also be written in the defocusing relation (suppose that all calibration parameters are real for this moment):

$$v(z) = \frac{\sqrt{1+u_x(z)}}{\sqrt{1+u_y(z)}} \tag{10}$$

where $u_x(z)$ and $u_y(z)$ are defined in Eq(2). Thus we can obtain the errorbar of v as

$$\delta v = \frac{1}{2} v \sqrt{\left(\frac{\delta u_x}{1+u_x}\right)^2 + \left(\frac{\delta u_y}{1+u_y}\right)^2} \tag{11}$$

where δu_x and δu_y are defined as Eq(5). Therefore

$$\begin{aligned}
\left(\frac{\delta v}{v}\right)^2 &= \frac{(\delta z)^2}{4}\left\{\left|\frac{1}{1+u_x}\right|^2 \left[4\left|\frac{z-c_x}{d_x}\right|^2 + 9|A_x|^2\left|\frac{z-c_x}{d_x}\right|^4 + 16|B_x|^2\left|\frac{z-c_x}{d_x}\right|^6\right]\right. \\
&\quad \left. + \left|\frac{1}{1+u_y}\right|^2 \left[4\left|\frac{z-c_y}{d_y}\right|^2 + 9|A_y|^2\left|\frac{z-c_y}{d_y}\right|^4 + 16|B_y|^2\left|\frac{z-c_y}{d_y}\right|^6\right]\right\} \\
&= \left(\frac{dw_x}{w_x}\right)^2 + \left(\frac{dw_y}{w_y}\right)^2
\end{aligned} \tag{12}$$

So the estimated errorbar of z coordinate δz can be calculated.