Formulas used:

The reference distance is calculated as follows:

$$r_{ref} = \frac{res_x^o * res_y^o * res_z^o}{\sqrt{(res_y^o * res_z^o * cos\Phi * sin\Theta)^2 + (res_x^o * res_z^o * sin\Phi * sin\Theta)^2 + (res_x^o * res_y^o * cos\Theta)^2}}$$

$$\Phi = \arccos \frac{x_B - x_A}{\sqrt{(x_B - x_{A)}{}^2 + (y_B - y_{A)}{}^2}}$$

$$\Theta = \arccos \frac{z_B - z_A}{\sqrt{(x_B - x_{A)}{}^2 + (y_B - y_{A)}{}^2 + (z_B - z_{A)}{}^2}}$$

$NA \leq 0.7 \quad res_{x,y}^o = \frac{0.377*\lambda_{ex}}{NA} \quad res_z^o = \frac{0.626*\lambda_{ex}}{n-\sqrt{n^2-NA^2}}$

$NA > 0.7 \quad res_{x,y}^o = \frac{0.383*\lambda_{ex}}{NA^{0.91}} \quad res_z^o = \frac{0.626*\lambda_{ex}}{n-\sqrt{n^2-NA^2}}$

x_A, y_A, z_A and x_B, y_B, z_B are the bead coordinates in channel A and B respectively, NA: numerical aperture, λ_{ex}: excitation wavelength, n: refractive index of the lens immersion & mounting media.

Lateral ($res_{x,y}^o$) and axial (res_z^o) theoretical resolution values used for single point scanning multiphoton excitation microscopes are calculated as defined in Zipfel, W.R., Willams, R.M. & Webb, W.W. Nonlinear magic: multiphoton microscopy in the biosciences, Nature Biotechnology 21, 1369–1377 (2003).

Compliance with the Shannon-Nyquist criterion for k photon excitation uses the following formulas for Shannon-Nyquist distances calculation (and a fixed k value of 2):

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

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