Formulas used:

The reference distance is calculated as follows:

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

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

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

$res_{x,y}^o = \frac{0.51*\lambda_{em}}{NA}$ $res_z^o = \frac{\lambda_{em}}{n-\sqrt{n^2-NA^2}}$

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_{em}: emission 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 widefield microscopes are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy, 2011.

Compliance with the Shannon-Nyquist criterion uses the following formulas for Shannon-Nyquist distances calculation:

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

$\Delta_{x,y} = \frac{\lambda_{em}}{4.NA}$ $\Delta_z = \frac{\lambda_{em}}{2.n.(1-cos(\alpha))}$