

### Formulas used:

Lateral ( $res_{x,y}^o$ ) and axial ( $res_z^o$ ) theoretical resolution values used for single point scanning confocal microscopes (assuming pinhole and NA values  $\geq 1$  &  $>0.5$  respectively) are calculated as defined in Wilhelm, S. Confocal Laser Scanning Microscopy (2011) & Amos, B. et al, Confocal Microscopy in Comprehensive Biophysics 3–23 (Elsevier, 2012):

$$res_{x,y}^o = \frac{0.51 * \lambda_{ex}}{NA} \quad res_z^o = \frac{0.88 * \lambda_{ex}}{n - \sqrt{n^2 - NA^2}}$$

NA: numerical aperture,  $\lambda_{ex}$ : excitation wavelength, n: refractive index of the lens immersion & mounting media.

Axis profiles are fitted using ImageJ Gaussian Curve Fitter and the following formula  $y = a +$

$(b - a) * e^{\frac{-(x-c)^2}{2d^2}}$  (Gaussian fitting).

Measured lateral and axial resolution (Full Width at Half Maximum, FWHM) values are derived using  $FWHM = 2d\sqrt{2\ln(2)}$

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

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

$$\Delta_{x,y} = \frac{\lambda_{ex}}{8.NA} \quad \Delta_z = \frac{\lambda_{ex}}{4.n.(1-\cos(\alpha))}$$