

### Formulas used:

Lateral ( $res_{x,y}^o$ ) and axial ( $res_z^o$ ) theoretical resolution values used for Spinning Disk microscopes are calculated as defined in Toomre and Pawley, Disk-Scanning Confocal Microscopy in Handbook Of Biological Confocal Microscopy 221–238 (Springer US, 2006):

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

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

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

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

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 formulas for widefield Shannon-Nyquist distances calculation:

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

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