## Formulas used:

Axial  $(res_z^o)$  theoretical resolution value used for Spinning Disk microscopes is calculated as defined in Toomre and Pawley, Disk-Scanning Confocal Microscopy in Handbook Of Biological Confocal Microscopy 221–238 (Springer US, 2006):

$$res_{z}^{o} = \frac{\lambda_{em}}{n - \sqrt{n^{2} - NA^{2}}}$$

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

Z-axis profiles is fitted using ImageJ Gaussian Curve Fitter and the following formula  $y = a + (b-a) * e^{\frac{-(x-c)^2}{2d^2}}$  (Gaussian fitting).

Measured axial resolution (Full Width at Half Maximum, FWHM) value is derived using FWHM =  $2d\sqrt{2ln(2)}$ 

Compliance with the Shannon-Nyquist criterion uses the formula for widefield Shannon-Nyquist distance calculation:

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

$$\Delta_z = \frac{\lambda_{em}}{2.\,n.\,\left(1 - \cos\left(\alpha\right)\right)}$$