



Acquiring 3D Image Stacks (Nyquist Criterion)

The prerequisite for 3D deconvolution is a Z-stack image. To acquire a Z-stack using AxioVision the optional [Z-Stack](#) module is required. If you want to acquire Z-stacks in several fluorescence channels the [Multichannel Fluorescence](#) module is also required.

For 3D deconvolution the selection of a suitable Z-distance plays an important role. Due to theoretical requirements for deconvolution the distance between the image planes must be at least half the resolution of the optical system in the axial Z-axis. We talk of twofold oversampling of the sample and using this to achieve the so-called Nyquist criterion. The resolution of the optical system in the Z-direction depends on the numerical aperture of the objective used, the refractive index of the immersion fluid and the emission wavelength used.

The following table below shows recommended values for the Z-distance of the images for various conventional objectives. When acquiring the Z-stack these values should not be exceeded, falling below them offers advantages for 3D deconvolution.

	Mag.	N.A.	Immersion	Recommended Z-distance (µm)		
				Dapi	FITC	Rhodamin
1	10	0.3	1.0 (air)	4,650	5,900	6,750
2	20	0.5	1.0 (air)	1,650	2,100	2,400
3	20	0.75	1.0 (air)	0,700	0,950	1,050
4	40	0.75	1.0 (air)	0,700	0,950	1,050
5	40	1.3	1.51 (oil)	0,350	0,450	0,500
6	40	1.2	1.33 (H ₂ O)	0,350	0,450	0,550
7	63	1.4	1.51 (oil)	0,300	0,400	0,450
8	63	1.2	1.33 (H ₂ O)	0,350	0,450	0,550
9	100	1.3	1.51 (oil)	0,350	0,450	0,500
10	100	1.4	1.51 (oil)	0,300	0,400	0,450

Furthermore it is important to acquire enough image planes above and below the structure of interest, because in these planes color values are located which belong to the structure of interest. These color values are relocated in the deconvolution process. Note: This is not necessary for the Nearest Neighbor method.

