# Optical Sectioning Properties of Revolution DSD

**Sectioning and Signal to Noise ratio**

Spinning at 6000 rpm, the disk produces a dynamic SIP which is projected into the specimen focal plane where it is in sharp focus. At increasing defocus (dependent on the objective properties, imaging wavelength and specimen scattering) the SIP loses contrast and eventually becomes impossible to differentiate from the general wide-field or out-of-focus fluorescence. The manner in which the confocal signal, C changes with focus position, z is known as the axial response function, C(z) . A convenient way to describe the axial response function is by its full-width-half-maximum or FWHM as illustrated in Figure 1 below.

The theoretical FWHM ARF data for the Revolution(R) DSD are listed in the table. This will help to explain how we estimate signal to noise ratio and also how we can trade signal for optical sectioning and choose the DSD SIP best suited to particular types of specimen.

Increasing FWHM delivers more signal when the axial (z) dimension of the feature of interest is greater than the FWHM. Think of the FWHM as equivalent to the optical section thickness and the area under the ARF as the confocal signal, 2C. The chosen DSD confocal FWHM results in the fractional confocal signal, FCS (2C/WF) that we collect in the transmitted and reflected signals of the DSD.

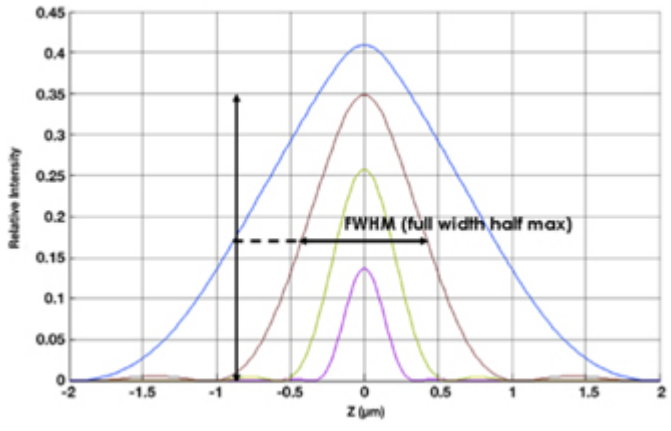


Figure 1: Shows the theoretical axial response function (ARF) of the DSD system with different SIP pitches from 40-320 µm and showing the full width half maximum or FWHM. All data computed for 60X/1.4 oil immersion lens.

T = 0.5 WF + C; R = 0.5 WF – C; [Equation 1]

2C = T – R; WF = T + R; [Equation 2]

If we use the notation from Equations 1 and 2, we can compute the SNR as follows:

Signal = 2C - the confocal signal;

Noise ˜ v(RN2 + WF) where RN is the detector read noise and WF is the square of the WF signal shot noise, vWF. Note that noise adds in quadrature.

SNR ˜ 2C/v(RN2 + WF); [Equation 3]

In a CCD detector of high quality, like the Clara the read noise is around 4.5 electrons rms, while the shot noise (the noise due to the Poisson statistics of the WF signal) is typically in the range 10 to 180 (v100 - v30,000). Hence the DSD is a shot noise limited system, or its SNR is limited primarily by the WF signal statistics and not the camera read noise. However, the camera read noise will have an impact in low signal conditions.

Depending on the specimen and the goals of the study, we can trade signal to noise for axial resolution, magnification or specimen exposure time and illumination level. This series of trade-offs becomes most complex for live cell specimens, where specimen health, photo-bleaching and toxicity can be critically important. Photo-bleaching leads to loss of signal over time and toxicity in live specimens.

In this respect the DSD compares well with laser scanning confocal since photo-bleaching is a nonlinear effect in which high illumination power has the greatest effect. Figure 2 shows measured Mito-Tracker photo-bleaching with living MDCK cells imaged by the DSD.

Table 1 shows the FWHM of the DSD axial response for a range of objectives and DSD’s two disk patterns with pitch 160 and 320 µm.Because DSD must detect confocal signal in the presence of the much larger wide-field signal, its signal-to-noise ratio is constrained by the wide-field shot noise. This means that signal must be traded with optical sectioning. Very thin optical sections result in poor signal-to-noise ratio; hence DSD SIP design is intended to optimize the trade-off (Equation 3). With this in mind, we have formulated some general guide lines for DSD imaging.

Use a lower power objective (with highest NA available) and select “high sectioning” for larger specimens such as embryos.

Use a high power objective and select “high signal” for live cell specimens.

With living specimens, use the minimum exposure and/or source intensity possible to deliver the minimum required SNR for your analysis and measurements.Many factors must be considered when choosing an instrument for scientific research. DSD-based systems will not replace point scanners or laser spinning disk in all conditions, but they do represent a viable and cost-effective alternative in many applications ranging from analysis of fixed tissue to live-cell imaging. DSD can be tuned to a broad range of fluorophores, operate at high and low magnification, and acquire high-resolution images at frame rates in the order of 1 to 10 Hz. At 2x2 binning and with regions of interest this frame rate can exceed 25 Hz, with a limit of 100Hz defined by the disk rotation speed.