Assessing the effect of background counts on the determination of the correct radius of a cylinder using the MT method

The MT method uses two different algorithms for the determination of the radius and shape of a cellular protrusion. One algorithm is intended to measure the average radius and the second is for the measurement of the local radius.

The first algorithm is used in real time to track the average radius of the protrusion. It is based on the calculation of the nth-harmonic where n is the harmonic chosen for the measurement of the radius. Generally, n is 8 or 16 or 32. As the beam orbits along a structure, the following pattern is observed.

For this analysis we use a cylinder of 400 nm radius. The analysis is done using the 8th harmonics, the radius of the orbit is 12 pixels=500nm, the PSF is 200nm (xy) and 600 nm(z). The modulation is 10%. The DC background count was set at DC=1 unit when measured well outside the cylinder.

The recovered value according to the table is reported below as a function of the intensity of the fluorescence of the cylinder.
With the constant modulation method, the recovered radius is very much on target even for DC values of 1.5 with a background of 1.0!

The conclusion is that working at the appropriate radius is crucial for the proper recovery of the cylinder size. When the proper value is chosen, the background has very little effect. By choosing a smaller radius, i.e., being closer to the cylinder, reduces the effect of the background.

From the carpet point of view, the recorded modulation is shown below for the worst case (DC=1.57 and DC background=1).

![Modulation Graph](image)

As you can see, the modulation is still very well visible.

The question is now about the real data. Two things could happen

1. The cylinder boundary is fuzzy,

2. The protein is not uniform on the cylinder

I will try simulating filled cylinders just to be sure that this will just give a different apparent radius, but not a different S/N

Now we explore the second method to measure the radius, which is based on measuring the modulation due to each one of the oscillations in the direction perpendicular to the cylinder axis (in this case we have 8 oscillations). In the worst case, when the DC was 1.57 and the DC of the background was 1, we obtain a relatively uniform image of the radius as shown below.
The average is about 410nm and the STD is about 30 nm (this is per orbit, not average over time). The conclusion is that both methods, even in the case of large background give approximately the same values.