**Stochastic Antigen Distribution Induced Fluctuation of Immunofluorescence:**

**Basic assumptions:**

1. **Suppose a cell/nucleus that contains N proteins is divided into *m* segments, the protein number () in each segments obeys multinormial distribution:**

**where**

**.**

**For this distribution, we have**

1. **Unless otherwise specified, protein distribution inside the cell/nucleus is considered to be uniform, which implies . In this case, we use to represent .**
2. **In all following derivation, we use the limit , so that each segment is infinitesimally small, and therefore . We can replace discrete with a function , where**

**Mean and Variance of image pixels/voxels (uniform ditribution case):**

1. **Suppose a 3D point spread function (PSF) , which is the detector (either a PMT or a single pixel of CCD)’s typical response (ignore the optical shot noise) to a single protein particle at position (x,y,z), then we have:**

**where is the intensity value of a typical pixel/voxel, , which is the integration over the whole space. Obviously the slope of vs is**

1. **Suppose the PSF has a gaussian form on all three dimensions, ie**

**Then**

**Usually , so**

1. **To compare with the single particle analysis results, just integrate over the middle xy plane**

**Therefore**

1. **For thin samples, where , the PSF on z dimension is replaced by its maximal value, and therefore**
2. **Unfortunately these results are not directly applicable because we do not know the real value of mean and variance.**

**Estimated Mean and Variance of image pixels/voxels (uniform distribution case):**

1. **Under the same assumption as the last section, the measured mean and variance of the image pixels/voxels are:**

**Since is localized, the magnitude of decays rapidly with the distance between and , therefore**

**where is a constant related to the interpixel/voxel distance as well as the actually geometry of data points (when is large enough, approaches , and becomes insensitive to the geometry). Combining the last two formulas, we get**

**For big (say ), the terms in the bracket is roughly 1, and we therefore get the same result as the last section.**

1. **If we bin pixels/voxels together, this is equivalent to redefine the “bin” spread function (BSF) as the summation of the PSF (rename as ) of binned pixel/voxels:**

**The result is therefore**

**where is the number of binned pixels/voxels. As we discussed before, if is big enough, , so**

**which degrades to a multinormial formula (particularly for , it becomes binormial) with an extra coefficient .**

1. **For an epi-fluorescence microscope equipped with an high end CCD camera (with the fill factor > 90%), the BSF for large area binning ( >> 1) is close to a step function that covers the binned area, therefore can be simply replaced by , and we return to a classical binormial distribution.**

**The effect of optical shot noise (uniform ditribution case):**

1. **Optical shot noise is the fluctuation of photon emission/counting, which is due to the stochastic nature of the emission/counting process. This process obeys Poisson distribution**

**where is the number of fluorescent particles, is the average number of photon emission/counting per fluorescent particle, and is the total number of emitted/counted photon.**

1. **The variance and mean of the photon emission/counting of multinormially distributed fluorophore particles are**
2. **To include optical shot noise into our model, we redefine I as the total number of fluorescent particles of a pixel/voxel. The previous formulas will therefore be rederived considering the stochastic photon emission/counting process:**

**To conclude, the shot noise gives the estimated mean intensity an extra factor , and gives the estimated intensity variance an extra factor , so the resulting slope will have an extra factor of . Here is the noise term that we would like to avoid, because by considering the photon emission/counting process, the calculation of the single protein spot intensity will also give an extra factor , which corresponds to the term of the slope. In general, higher excitation intensity, longer exposure time, and higher detector efficiency all makes bigger. If is not much bigger than 1 (which is true for my confocal), the shot noise slope can be measured (we can image the same area multiple times, calculate variance and mean for each pixels, and then fit the result to a linear function) and subtracted from the result.**