The brightness and $<N>$ button in the RICS screen

Background

According to Qian and Elson (and the literature on FCS) the average number of molecules $N$ can be calculated from the inverse of the $G(0)$. Since $G(0)$ is the ratio of the variance to the square of the average, this quantity can be calculated for a given time series. The time series we consider is the intensity at any given pixel as a function of time. The SimFCS program calculates the map of the average number of molecules in a pixel $<N>$ using the above idea. To obtain the molecular brightness, we divide the average intensity at one point by the number of molecules $<N>$ at the same point. This is also called the moment analysis method. As a result, we obtain map of $<N>$ and brightness. The units of brightness are related to the pixel dwell time and they are “counts/dwell time/molecule”. Also note that we are calculating $G(0)$ without taking into account the “gamma factor”.

Important notes

The $<N>$ and brightness calculated in this way cannot account for multiple species. This method is substantially different from the PCH method which considers the entire photon counting distribution rather than just the first and second moment as Qian and Elson proposed. However, the moment analysis is very fast and provides a good estimate of the $<N>$ and brightness.

The value of $<N>$ average is obtained from the ratio of the variance to the square of the average, rather that form the “extrapolated $G(0)$” (or $G(1)$) as normally obtained from the autocorrelation curve. Therefore, the two values could be different if there is extra shot noise at channel zero.

The value of $<N>$ obtained from the PCH analysis accounts for the gamma factor. In the way the moment analysis is implemented in SimFCS the gamma factor is ignored.

Tutorial for the use of the $<N>$ and Brightness button

In the RICS screen, read in an image sequence according to the specific file format. Under the tool menu press the $<N>$ and brightness maps button. The moment analysis is applied to the image sequence. Two maps are calculated, one for the $<N>$ and the other for the brightness. An example of the two maps is shown below.
The three images shown are the average intensity, the $<N>$ and the brightness. Note that the brightness map is relatively uniform.

An additional screen is opened automatically that displays in a two dimensional histogram the values of $<N>$ versus brightness. This is done to explore the intensity image for the region where points of large brightness or large $<N>$ values occur. An example of such a correlation histogram is shown below.
In this correlation plot it is clearly shown that the average brightness changes very little while it is the number of molecule that varies from region to region of the cell. By moving the cursor in the correlation histogram screen it is possible to highlight the various parts of the cells where large values of \( <N> \) or large values of brightness occur. It is interesting that there are regions in of the cell where the number of molecules is larger than in others but the brightness is about the same. By exploring the correlation map in the regions of large brightness, as shown in the example below, regions presumably associated with focal adhesions or where large protein aggregates occur are highlighted.
Description of the commands

**Log z-scale.** The correlation histogram is plotted in a log z-scale to better visualize regions of the histogram which are scarcely populated. We recommend using this option.

**Autoscale Histo.** The z-scale of the histogram can be set manually using the fields in the screen to emphasize specific regions of the correlation histogram.

**Color points.** When this flag is on, the intensity image is in color and the selected regions (selected by the cursor position) are drawn in red. When this flag is off, the image is painted in black and white. Sometimes, it is easier to see the red painted colors in a gray background.
Use **white background**. The correlation histogram sometimes is more visible using a white background. This option let you switch from white to black background;

**Radius**. Determine the size of the exploration cursor in fraction of the screen scale. For example, 0.1 means that the diameter of the circle is 10% of the linear size of the correlation plot.

**Image threshold**. Is used the exclude points below the threshold for the calculation of the correlation histogram. The threshold is applied to the average intensity map. For example, points of the background generally are low in brightness. These points can be excluded from the histogram by raising the threshold.

**Autoscale image**. Is used to emphasize regions of the average image. Also, when using the grey scale, using a smaller value for the image max gives a more visible image.

**Smooth**. Is used to sharpen the correlation plot using the famous “Enrico’s’ secret algorithm”.

**Minimum and maximum of x and y axis**. Change these values to zoom in a given region of the correlation histogram.

After changing the values on the screen, the new values take effect when the cursor is moved on the screen and the **recalculate** button is pressed.