Mathematics of signal processing for scientific instrumentation

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Question 1

Describe the concept of Raster Image Correlation Spectroscopy (RICS) and explain how it add new analysis capabilities to the fluorescence fluctuation microscopy toolset (2 pages max). Appended document "Digman05 measuring fast dynamics in solutions and cells.pdf" introduces RICS, but further sources of information can be found via Google Scholar. Evaluated will be clarity, originality, and completeness of your exposition. The inclusion of an explanatory figure is advised.

Resolution of Question 1

Fluorescence fluctuation microscopy (or FFM) analyzes dynamic molecular properties in live cells and provides insight into the subcellular organization of biological functions. Analyzing signal fluctuations from small ensembles (several hundred particles) reveals their concentration, the stoichiometry, the stochastic motion, as well as superimposed signatures of the environment such as spatial confinement and binding events. Thus, fluctuation analysis provides access to dynamic molecular properties that can be used to build physical models of cellular processes.

It posses a powerful arsenal of analysis tools that inestigate the molecular heterogeneity in space and/or time: image correlation spectroscopy (ICS).The family of image correlation spectroscopy methods has grown richer with the appearance of novel variants of previous techniques. All ICS methods analyze the fluctuations of the fluorescence signal to quantify relevant parameters of the processes that cause the fluctuations. One is TICS. It enables the examination of diffusion-related processes by studying the correlation of the fluorescence fluctuations over time for each pixel of an image time series.

Then RICS appears. He was introduced first as a method to study molecular transport in solution and in cells. But now, Raster ICS registers both the temporal and the spatial intensity fluctuations by scanning a laser beam over a sample and exploits the fast pixel-to-pixel and line-to-line sampling to analyze the fluctuations on a faster timescale than TICS. In RICS, the fluorescence fluctuations autocorrelation function is mainly used to determine macroscopic phenomenological parameters such as the diffusion coefficient and the concentration of the molecule of interest. These parameter estimates can be obtained by fitting the model autocorrelation function to the empirical autocorrelation computed from a series of images. So, we can say that RICS is very sensitive to the choice of the parameters. RICS is characterized by scanning parameters such as pixel dwell time and line time, which are not applicable to FCS. The main advantage of RICS over FCS is the spatial correlation introduced by the scanning beam, which can be used to generate diffusion and concentration maps over the sample.

Major benefitis of RICS are:

- it can be done with *commercial laser scanning microscopes* (either one or two photon systems);
- it can be done with *analog detetion, as well as with photon counting* systems, although the characteristic of the detector must be accounted for (time correlations ate very short times due to the analog filter);
- provides an *intrinsic method to separate the immobile fraction*;
- it provides a powerful method to *distinguish diffusion from binding*.

Summing up, the beauty of Raster Image Correlation Spectroscopy or RICS is that it allows us to measure the dynamics from a single image time series.

Like we see in the picture above, raster scan images contain temporal information because pixels are recorded sequentially, and that the spacial correlation in fast diffusion is not zero and in slow diffusion it is.

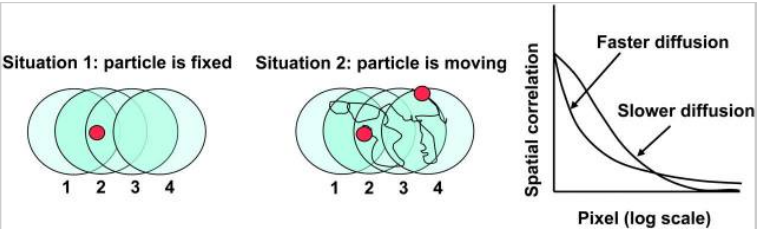

Figure 1: RICS Concept.

Situation 1: if a particle is not moving or slowly moving, a signal will be detected at position 1, 2, and 3 but not at 4 during the scan, because there is a decrease of probability that the laser beam is still in the same position. So, in the first situation, we can say that there is a high correlation for short distance pixels.

Situation 2: if the particle is moving quickly, there is a chance to get some signal even at location 4. So, we can conclude that we can get signal from the same molecule at longer pixel distances.

In the RICS approach, we calculate the 2D spatial correlation function (similarly to the ICS method of Petersen and Wiseman):

$$G_{RICS}(\zeta, \Psi) = \frac{< Ix, y)I(x+\zeta, y+\Psi) >}{< Ix, y) >^2} - 1$$

where the variables ζ and Psi represent spatial increments in the x and y directions, respectively. For the autocorrelation function is (for circular scanning):

$$G_{RICS}(\zeta, \pi) = S(\zeta, \pi) \times G(\zeta, \pi)$$

And from where, we can see that the dynamic at a point is independent on the scanning motion (S) of the laser beam.

$$S(\xi, \psi) = \exp\left(\frac{\frac{1}{2}\left[\left(\frac{2\xi\delta r}{w_0}\right)^2\right]}{\left(1 + \frac{4D(\tau_p\xi + \tau_l\psi)}{w_0^2}\right)} \right),$$

$$G(\xi, \psi) = \frac{\gamma}{N}\left(1 + \frac{4D(\tau_p\xi + \tau_l\psi)}{w_0^2}\right)^{-1} \times \left(1 + \frac{4D(\tau_p\xi + \tau_l\psi)}{w_z^2}\right)^{-1/2},$$

Figure 2: Equations taken from the provided article.

The x and y are the horizontal and vertical coordinates of the image or pseudoimage, respectively; δ_r the distance between adjacent points in the line or along the orbit; τ_p and τ_l are the pixel sampling time and time between lines, respectively.

Therefore, we can say that RICS adds new analysis capabilities to the fluorescence fluctuation microscopy toolset because with him we can, by exploiting this time structure, measure dynamic processes such as molecular diffusion in the microseconds to second timescale.

References of Question 1

1. Thomas Weidemann, JonasMucksch, Petra Schwille. Fluorescence fluctuation microscopy: a diversified arsenal of methods to investigate molecular dynamics inside cells.

2. Michelle A. Digman, Parijat Sengupta, Paul W. Wiseman, Claire M. Brown, Alan R. Horwitz, Enrico Gratton. Fluctuation Correlation Spectroscopy with a Laser-Scanning Microscope: Exploiting the Hidden Time Structure.

3. C. M. Brown,R. B. Dalal, B. Hebert, M. A. Digman,A. R. Horwitz, E. Gratton. Raster image correlation spectroscopy (RICS) for measuring fast protein dynamics and concentrations with a commercial laser scanning confocal microscope.

4. Marco Longfils, Nick Smisdom, Marcel Ameloot, Mats Rudemo, Veerle Lemmens, Guillermo Solís Fernández, Magnus Röding, Niklas Lorén, Jelle Hendrix, Aila Särkkä. Raster Image Correlation Spectroscopy Performance Evaluation.

5. https://www.youtube.com/watch?v=zNd1R3jY9VE

6. Michelle A. Digman, Claire M. Brown, Parijat Sengupta, Paul W. Wiseman, Alan R. Horwitz, Enrico Gratton. Measuring Fast Dynamics in Solutions and Cells with a Laser Scanning Microscope

Question 2

Figure 1 shows two experimental autocorrelation curves (red and blue) of two dyes each associated with a different object and one cross correlation curve (green) of the fused object containing both dyes. Please note that the y-axis of the plot represents G+1 as defined in the course notes, i.e., representing the autocorrelation of the fluorescence signal. What is the concentration of fused objects? Assume that measurements have been carried out by a Nikon objective CFI SR Plan Apo IR 60XC WI and $\lambda_{ex,red} \approx \lambda_{ex,blue} \approx 532\,nm$.

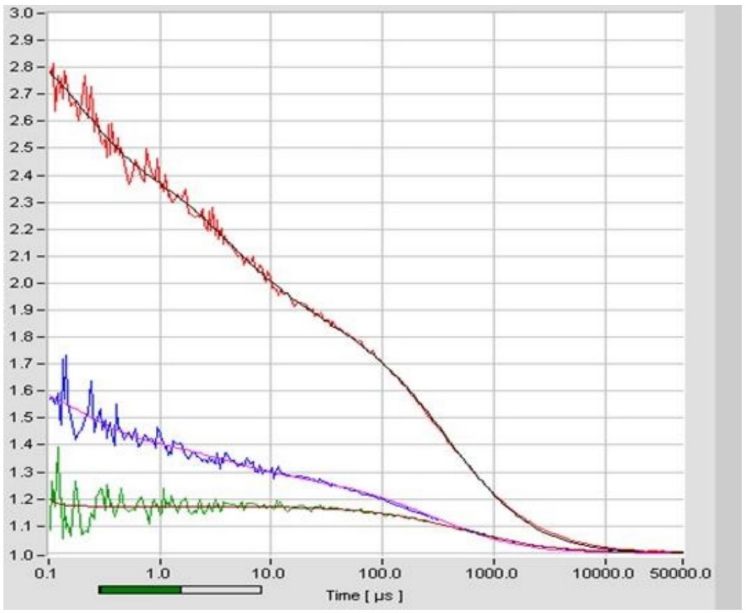

Figure 3: Autocorrelation (blue/red) and crosscorrelation (green) curves of an FCCS experiment.

Resolution of Question 2

From the statement of this second exercise, we know that figure 1 shows 2 experimental autocorrelation curves of 2 dyes each associated with different object (the red and blue) and one cross correlation curve of the fused object containing both dyes.

What we want to know is the concentration of fused objects. So, for that, we have to applied the following equation:

$$< C_{R,B} >= \frac{G_x(0)}{G_R(0) \cdot G_B(0) \cdot V_{eff}}$$

where:

- $G_R(0)$ and $G_B(0)$ is the autocorrelation of red and blue curves, respectively, in $t = 0\mu s$;
- $G_x(0)$ is the crosscorrelation of the green curve;
- V_{eff} is the effective volume element, given by:

$$V_{eff} = \pi^{3/2} \cdot W_r \cdot W_z$$

.

From the graph, it is removed that:

- $G_x(0) = 1.2$;
- $G_R(0) = 2.79 - 1 = 1.79$;
- $G_B(0) = 1.59 - 1 = 0.59$.

It remains to know the value of V_{eff}. But, for that, we need to know first the values of W_r and W_z, which are the spatial intensity volume distribution of the fluorescence emission for the red and blue curves. So, we will have:

$$W_r = \frac{0.61 \cdot \lambda}{NA}$$
$$W_z = \frac{2 \cdot n \cdot \lambda}{NA^2}$$

As we are assuming that the measures have been carried out by a Nikon objective CFI SR Plan Apo IR 60XC and $\lambda_{ex,red} \approx \lambda_{ex,blue} \approx 532\,nm$, replacing on top:

$$W_r = \frac{0.61 \cdot \lambda}{NA} \Leftrightarrow W_r = \frac{0.61 \cdot 532 \times 10^{-9}}{1.27} \Leftrightarrow W_r = 2.56 \times 10^{-7} m$$

$$W_z = \frac{2 \cdot n \cdot \lambda}{NA^2} \Leftrightarrow W_z = \frac{2 \cdot 1.33 \cdot 532 \times 10^{-9}}{1.27^2} W_r = 8.77 \times 10^{-7} m$$

Therefore,

$$V_{eff} = \pi^{3/2} \cdot W_r \cdot W_z \Leftrightarrow \pi^{3/2} \cdot 2.56 \times 10^{-7} \cdot 8.77 \times 10^{-7} \Leftrightarrow V_{eff} = 1.25 \times 10^{-12} m^3$$

And substituting in the concentration equation we will have:

$$< C_{R,B} >= \frac{G_x(0)}{G_R(0) \cdot G_B(0) \cdot V_{eff}} \Leftrightarrow < C_{R,B} >= \frac{1.2}{1.79 \cdot 0.59 \cdot 1.25 \times 10^{-12}} \Leftrightarrow < C_{R,B} >= 9.09 \times 10^{11} m^{-1}$$

References of Question 2

1. http://www.fcsxpert.com/classroom/theory/what-is-confocal-volume.htmloptics
2. https://www.janelia.org/sites/default/files/Support20Teams/Light20Microscopy/Nikon20Objectives.pdf
3. slides provided from classes