Image correlation methods for the internal dynamics of cells. ICS and STICS

Enrico Gratton

Laboratory for Fluorescence Dynamics

LFD workshop
Fluctuation correlation spectroscopy applied to images

**ICS**
The size and number of membrane aggregates (Originally developed by Niels Petersen and later by Paul Wiseman)

**tICS**
The time autocorrelation at one pixel (Paul Wiseman and Niels Petersen)

**STICS**
Diffusion and velocity in 2-D (Ben Herbert and Paul Wiseman)

**kICS**
Distinguish between diffusion and binding on the basis of spatial correlations (Paul Wiseman)

**RICS**
Fast diffusion in solution and cells (Michelle Digman and Enrico Gratton)

**N&B**
Number and brightness of aggregates in solutions and in cells (Michelle Digman and Enrico Gratton)
Introduction to the math of ICS

Principle of the method

Spatial correlation
Temporal correlation
Spatio-temporal cross-correlation
Number fluctuations
Binding fluctuations
The spatial correlation method

This is the basis of the mathematical operation for the spatial correlation operation in 2D
Displace the frame by $\Delta X$ and multiply the intensity at each pixel of the original frame with the intensity of the pixels in the frame displaced by $\Delta x$.

Sum all the values of these products and divide by the average intensity squared.

Assign this value to the position $(1,0)$ of a new image called the correlation image.

Repeat for several delays in the x direction and in the y direction. You end up with the correlation image. Which is half the size of the original image. We then past 4 parts to display the correlation image as a symmetric image.
The larger are the particles, the larger is the spatial correlation.
The effect of the number of particles

The less particles we have, the larger is the correlation, because the average is less with fewer particles, while the product of the intensity goes with the square of the particle intensity.
Two particle sizes
General results of ICS

Image correlation spectroscopy gives the size of aggregates and the number of aggregates.

The aggregates must be large (larger than the PSF) to measure their average size.

The beauty of ICS is that it is an average method. You don’t need to count the particles or measure their size individually.

The limit of ICS

The sample must be immobile during the measurement, which is generally true for one single image.

It is intrinsically a 2-D technique. The membrane must be flat and all the particles must be at the same focus.

The field must be flat, no deformation of the image at the borders.

It is a purely static method.
How to do ICS??

The ICS algorithm is available under ImageJ and in the RICS page for the SimFCS software.

Others image software packages offer similar routines to automatically count particles and determine the image distribution.

What happens if we collect many successive frames and the particles move slowly from one frame to the other?

If we autocorrelate the time series along one pixel, this is the tICS method!!

At each pixel, if there is a particle a any time, at a later time the particle will not be there if it is diffusing away!

Of course, this method will work if particles move very slowly, in the time scale of the frame time.
Introduction to spatio-temporal correlations: STICS

![Image showing a graph with linear tau on the x-axis and pixel values on the y-axis, indicating some data points and a graph with green lines showing variability over time.]

(pixel 43.75 A = 0.0000 k = 0.0000 B = 0.0000)
The principle of STICS

If we spatially cross-correlate each successive image, the shape of the correlation function should not change, although the position of the particles could change.

To detect the particle motion, we correlate one frame with a frame after some time delay.

This is the STICS technique!!
Basic explanation of the tICS method

Pick one pixel, plot the intensity as a function of time. Calculate the time autocorrelation function from this time series.

It is also possible to calculate cross-correlation between the time sequence at any two pixels.

The time resolution of this method depends on the time between frames.

For confocal microscopy, the frame time is in the second range, but for fast cameras and line scan, the time between two successive reading of the intensity could be in the ms range.
Basic explanation of the STICS principle

Particles diffusing

Particles drifting
Effect of diffusion

Due to diffusion, the STICS correlation will broaden
What happens if the particles move at constant velocity?
Effect of drifting velocity

STICS of slowing drifting particles in the negative y direction
Is the STICS method limited to slow diffusing and drifting particles?

Until now, STICS works only for slow diffusing or slow drifting particles. There are ways to overcome this limitations: fast cameras and line STICS!

With fast cameras, the frame rate can be as fast as 1 ms and in the line mode, this is also the typical time for a line.

A diffusion coefficient of 1 $\mu$m$^2$/s corresponds to a transit time of about 15-20 ms depending on the waist of the PSF. The STICS analysis should be able to pick up diffusion processes as fast as $D=5\mu$m$^2$/s using these approaches.

Examples of data acquired with the Cascade Camera at 10 ms per frame.
How can we visualize the STICS results as binding on a fixed adhesion?

Assume that we have an adhesion in which binding of a fluorescent protein to random sites can occur.

Along the time axis the fluctuations in intensity correspond to the on-off statistics. However, time correlations at one pixel is not telling us if the particle is moving away from the pixel or is coming on and off. Cross-correlation along two lines will give a “sharp correlation only if the “same” site remain on or off. As we cross-correlate two lines far away in time, the cross-correlation should disappear.
At a time delay of 1 (=10 ms) all the correlation planes are identical.

At a frame delay of 10 (=100 ms) we don’t see a relaxation process.

At a frame delay of 100 (=1000 ms) we definitively see a relaxation process which appears as a decrease of the amplitude of the correlation function.

This process is due to binding-unbinding since diffusion is not occurring in this sample (TIRF image).