ICS2: Image correlation methods for the internal dynamics of cells. ICS and STICS

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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, Rooshin Dalal, Rick Horwitz 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

pixel 43.75 A = 0.0000 k = 0.0000 B = 0.0000

Linear tau
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 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
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
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.
STICS in cells

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).