Co-measuring activity of three caspases with live single-cell imaging

Mauro Silberberg, Agustín A. Corbat, Hernán E. Grecco

maurosilber@df.uba.ar @maurosilber
acorbat@df.uba.ar @aacorbat
hgrecco@df.uba.ar @GreccoHernan

Laboratorio de Electrónica Cuántica, Departamento de Física, FCEyN, UBA
Instituto de Física de Buenos Aires - CONICET

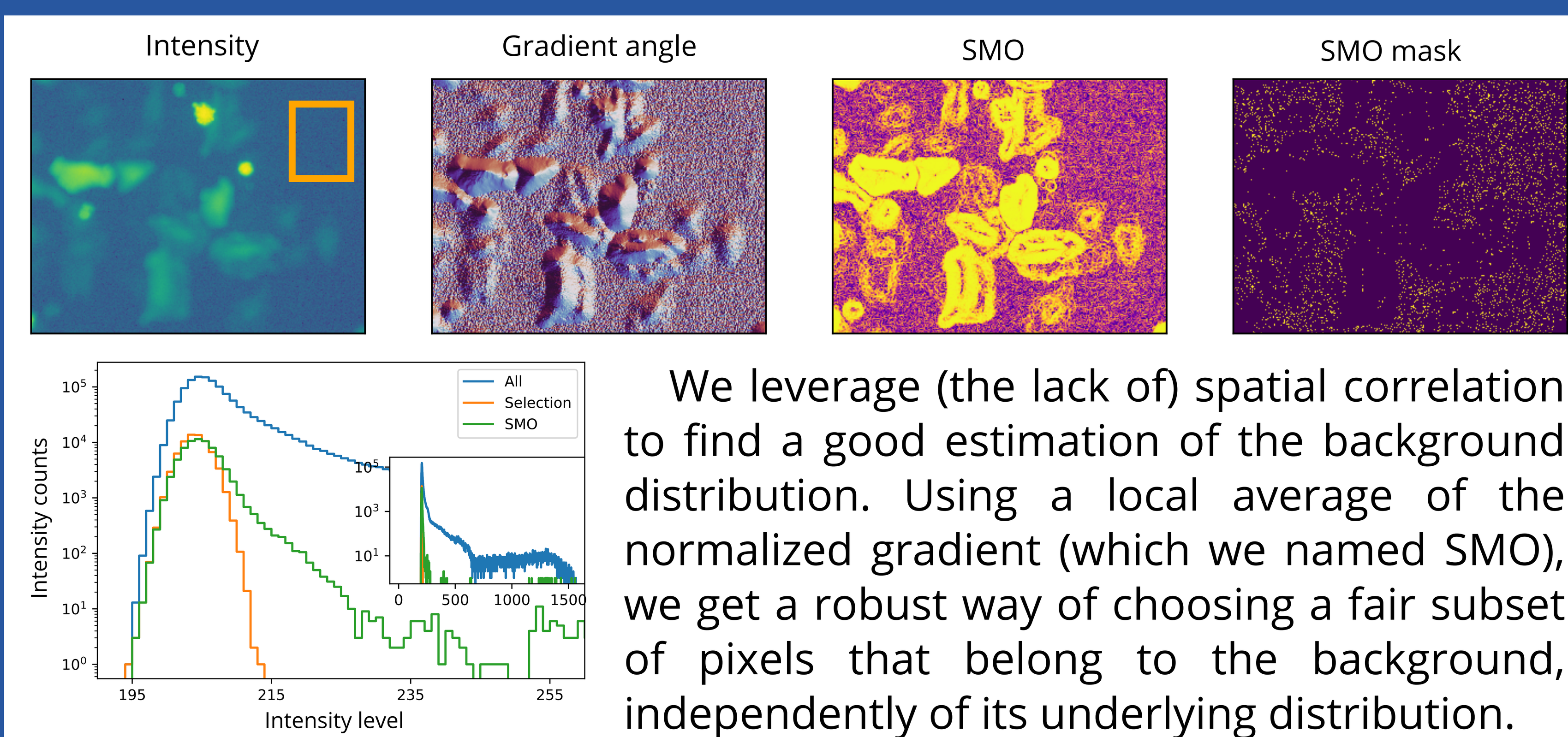
UBA

Understanding signal propagation in biological networks requires simultaneous single-cell measurements of multiple nodes from a large population that samples natural intercellular variability. To co-measure the activation of extrinsic, intrinsic and effector caspases upon apoptotic stimulus, we designed three spectrally separated anisotropy-based FRET biosensors. Automated microscopy was used to monitor a large number of cells over 12 hours. We developed a segmentation algorithm that leverages spatial correlations to identify cells in low signal-to-noise ratio contexts, and uses temporal information to split adjacent cells. Through modelling and simulations, we linked our observable to maximum caspase activity which is a good proxy for caspase activation times. Studying the delay between different caspases allowed us to refine existing models of the apoptotic signalling network.

Cellment: a Python package for segmentation and tracking

Find me at GitHub: https://github.com/maurosilber/cellment

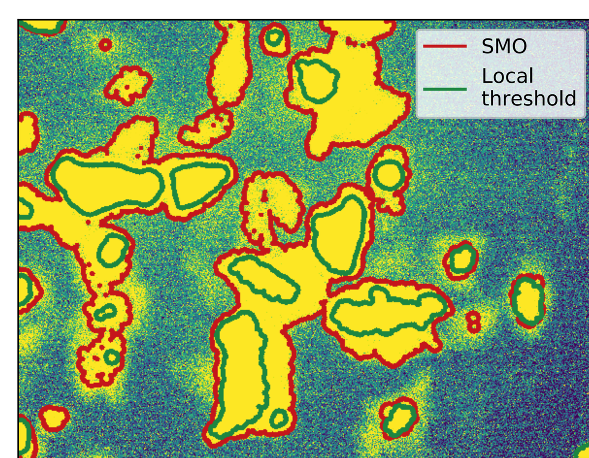
Automatic background estimation with SMO



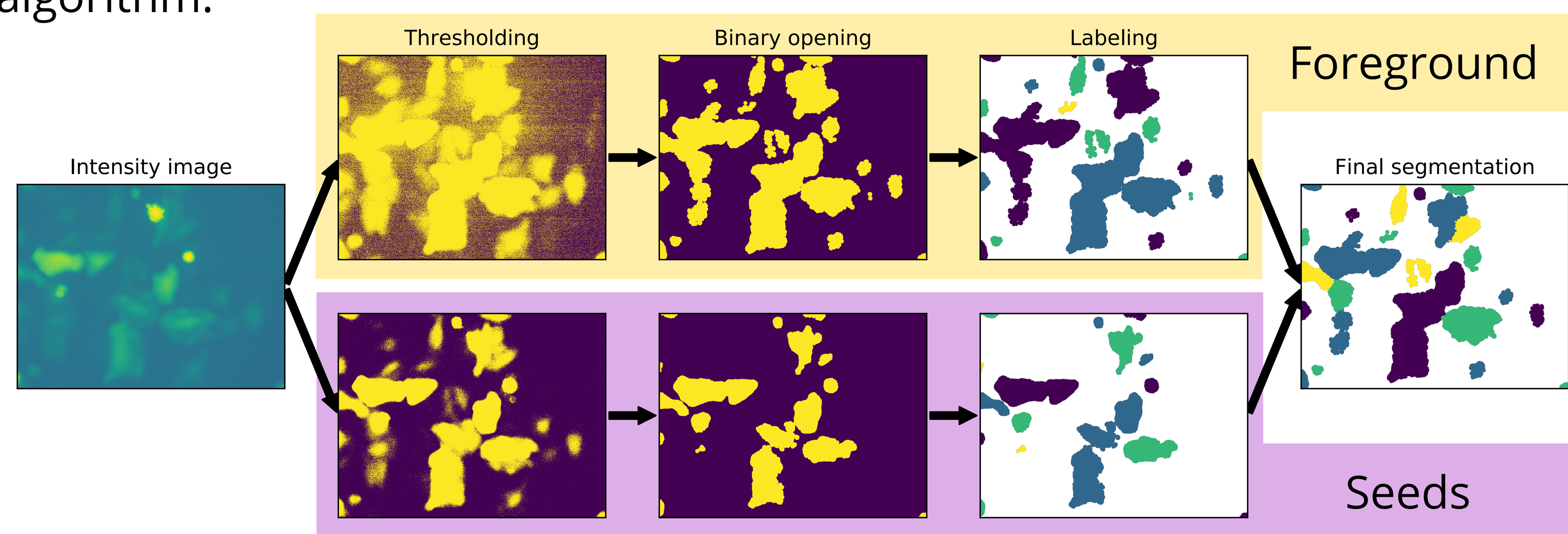
We leverage (the lack of) spatial correlation to find a good estimation of the background distribution. Using a local average of the normalized gradient (which we named SMO), we get a robust way of choosing a fair subset of pixels that belong to the background, independently of its underlying distribution.

In long experiments, it might be crucial to estimate the background from the image itself, instead of via an independent experiment, as we observed an increase in background level with time.

Dual-threshold segmentation for well-defined edges

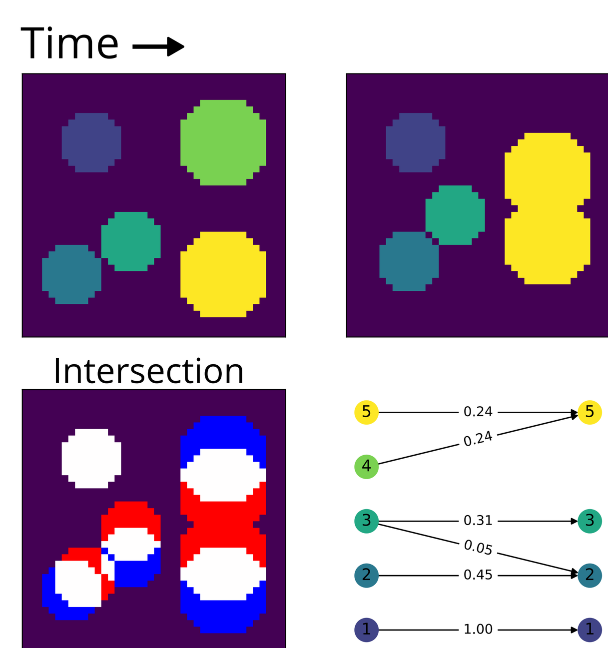


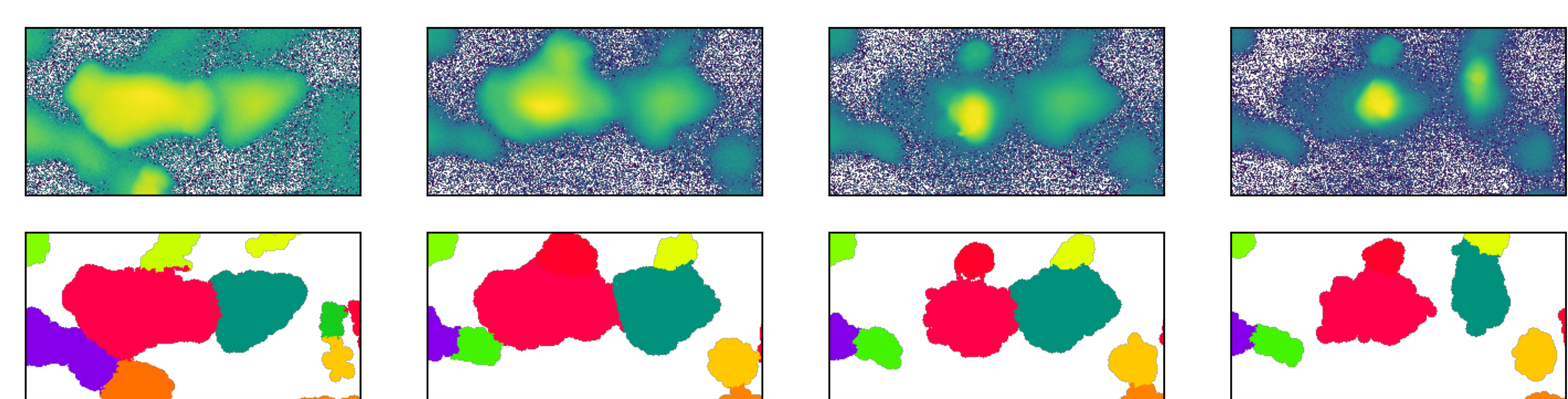
Having a good estimate of the background distribution, it's simple to select a threshold value to perform an intensity-based segmentation. But, while thresholding at the median gives a better estimate of the cell boundary than other methods, it fails to split neighbouring cells. To solve this, we use two thresholds. First, a foreground segmentation is done by thresholding at the median background and then applying morphological operators. Repeating this for a higher percentile (eg. 90%) of background, we find seeds to split neighboring cells via the watershed algorithm.



Tracking as feedback for segmentation

We developed a graph-based tracking algorithm where nodes correspond to objects at a given time and edges encode overlap at adjacent times. If a node has more than one incoming edge, we can infer from relative overlap areas either to which node it corresponds, or if they are merged cells that need to be splitted. In the latter case, we use intersections as seeds for the watershed algorithm.





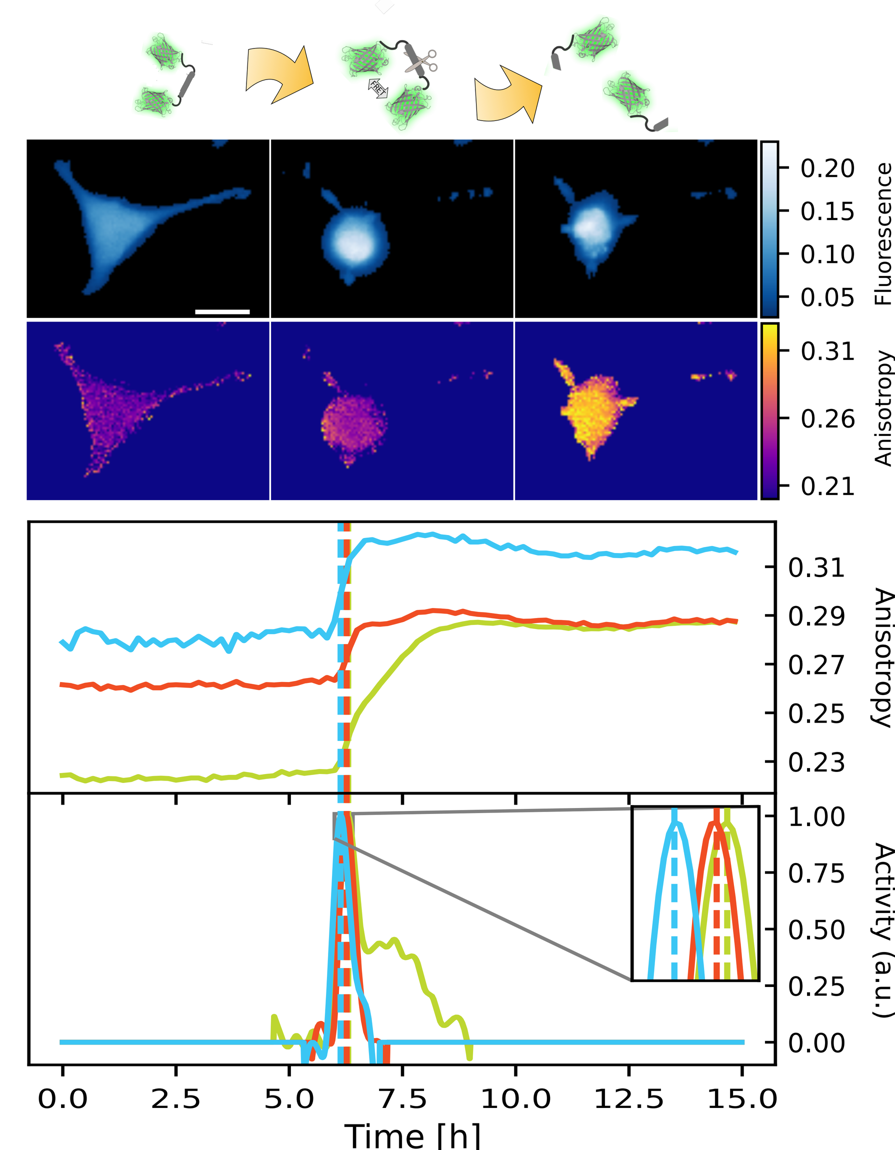
Simultaneous monitoring of three caspases activity

Via homoFRET measurements of anisotropy, we simultaneously monitor three enzymatic activities in a single cell. Our sensors consist of pairs of two spectrally similar fluorophores joined by linker motifs, which are specific to a certain caspase. The pair shows a lower anisotropy than the fluorophore by itself. Hence, when the enzymes start acting and the sensor is cleaved, an increase in anisotropy is produced.

As the cleavage is unidirectional, our readout is proportional to the integral of the caspase activity. Therefore, the derivative of the anisotropy curve is related to the instantaneous activity.

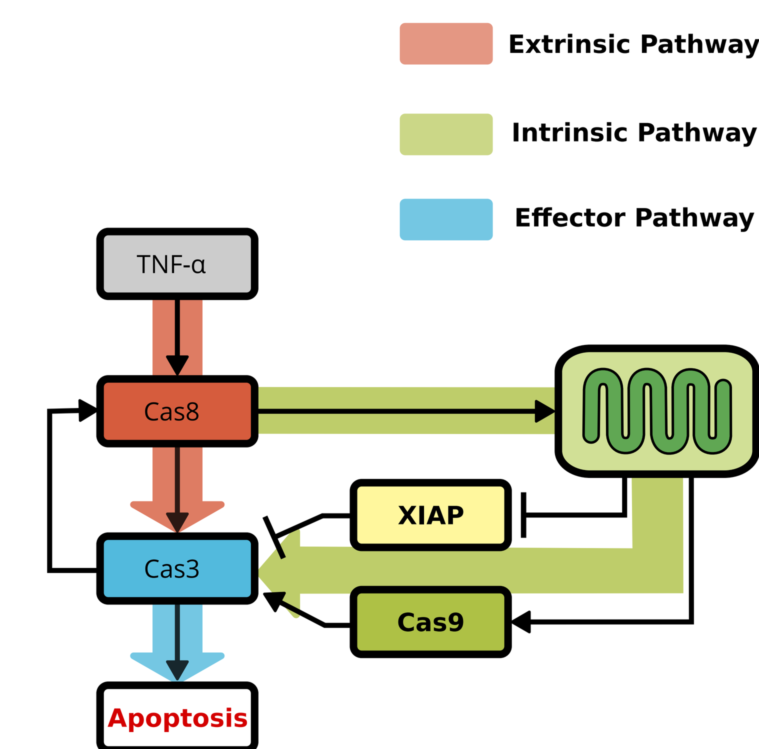
Through simulations and control experiments, we found the time of maximum activity to be a robust proxy for caspase activation time.

As the onset of apoptosis can occur anytime throughout the experiment, we studied time differences between activation of caspases.

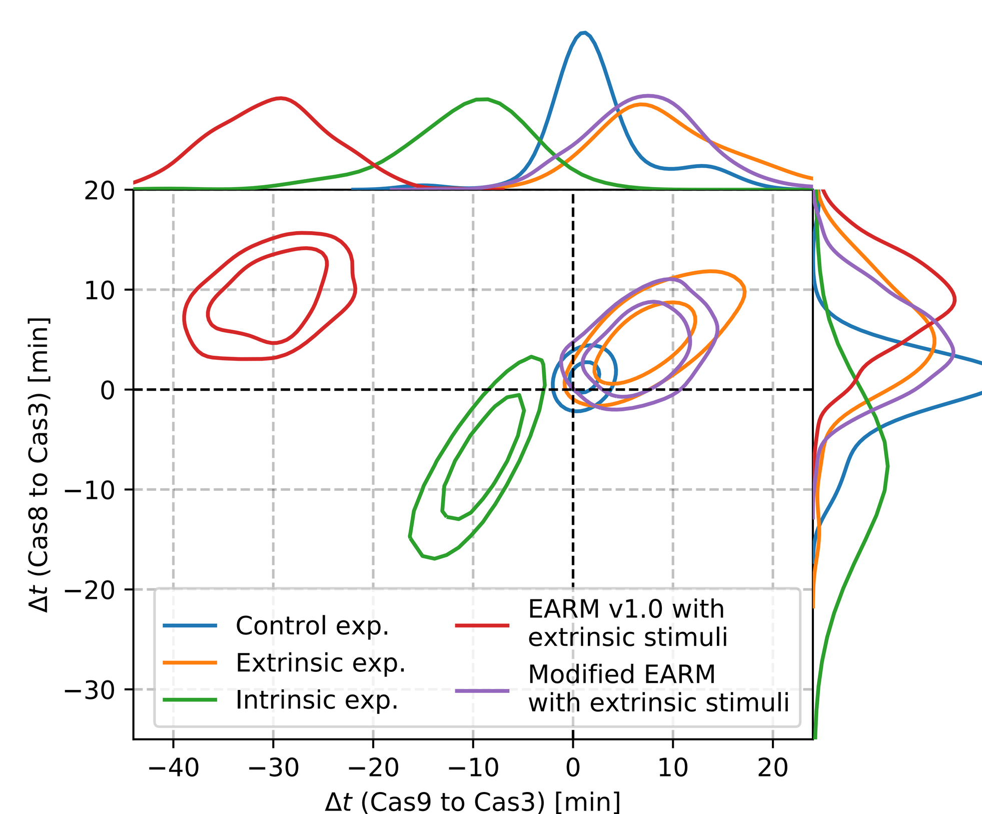


Correlation in activation times deliver insight into apoptotic network modelling

The apoptotic signalling cascade can be divided into extrinsic, intrinsic and effector pathways. The apoptotic network can be portrayed by describing the activation dynamics of its key nodes which are the proteases known as caspases. While all caspases are activated whether apoptosis is initiated via an extrinsic or instrinsic stimuli, correlating activation times revealed a different network behavior in each case.



In experiments stimulating the extrinsic pathway, a delay of both caspases 8 and 9 with respect to caspase 3 was observed. While the Extrinsic Apoptosis Reaction Model (EARM) [1] correctly predicted one, it showed an inverse timing for the other. Modifying some parameters, we were able to reproduce the observed timing.



Instead, stimulating the intrinsic pathway, we observed a reversed timing with respect to extrinsic stimulus, where caspase 3 is delayed.

Currently, we are investigating possible modifications to EARM to explain both behaviours within the same model.

References

[1] Albeck, John G., et al. "Modeling a snap-action, variable-delay switch controlling extrinsic cell death." PLoS biology 6.12 (2008).

[2] Corbat, Agustin A., et al. "Co-imaging extrinsic, intrinsic and effector caspase activity by fluorescence anisotropy microscopy." Redox biology 19 (2018): 210-217.

Poster PDF