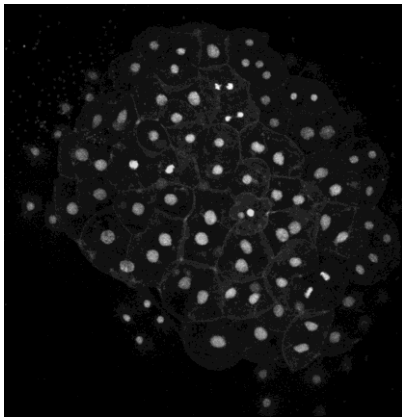
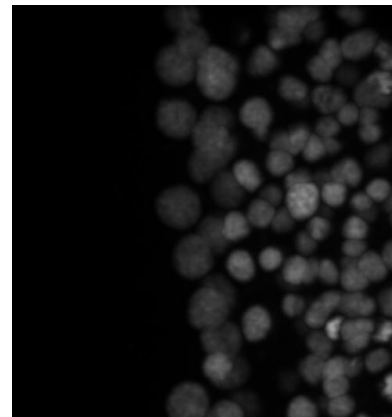


Procesamiento de Imágenes y Bioseñales II - Interpretación de imágenes biológicas y biomédicas en series de tiempo I (Tracking & Flujo Óptico).

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(3D tracking - imagej/FIJI)



(2D OpticalFlow - matlab)

Figure 1. z-Projections from fluorescence Spinning Disk Microcopy of, from left to right: i) annual fish nuclei expressing H2B-RFP at 24 [hpf], animal view (20x, 0.0008 [fps]), ii) cell nuclei expressing H2B-RFP mRNA at 5.3 [hpf], dorsal view (25x, 0.005 [fps]), vegetal pole to the left and animal pole to the right.

The practical session has two main objectives: to quantify cell migration by using tracking and optical flow. To perform tracking, segmentation will be performed followed by distance based tracking. Optical flow will be computed directly from a 2D sequence without segmentation.

1.- 3D Tracking. In this practical work, we will track cell nuclei of annual fish expressin H2B-RFP. Here, we will use FIJI, which is ImageJ variant (also freely distributed) with plugins that allows us to segment, track and analyze biological image sequences.

1. Open the image sequence "track_sequence.tif". For that, go to File->Open and choose the file.
2. For the tracking, we will use the plugin named TrackMate. In order to open the plugin, go to Plugins->Tracking->TrackMate. This will open the tracking window. Figure 2 shows the windows of the tracking procedure in the TrackMate plugin.
3. First, calibration data is necessary. The information, that the stack has, is the original.

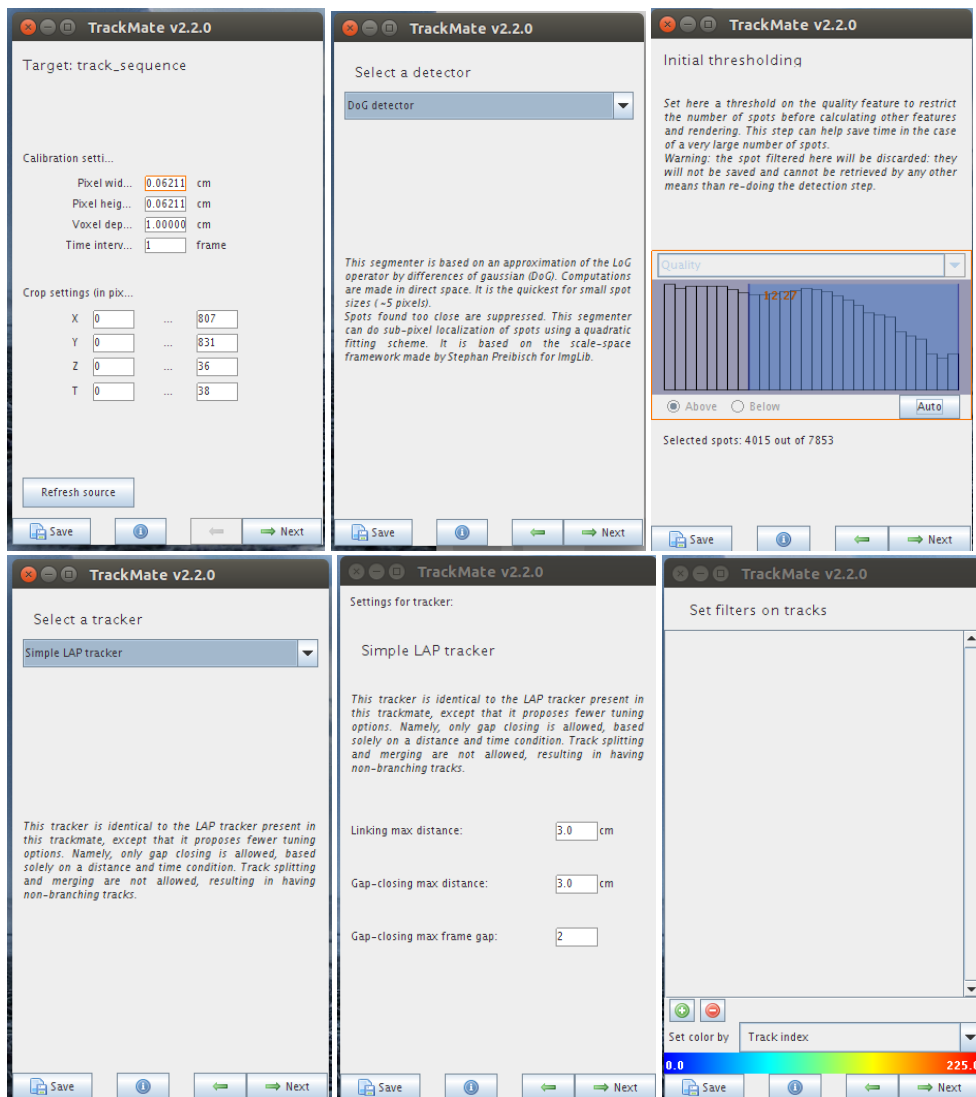


Figure 2. Steps for detection and tracking (from left to bottom and top to bottom).

4. Press “Next” to go to the following steps. After calibration, it is necessary to select the detection algorithm. There are 4 options to detect nuclei, select DoG detector.
5. Press “Next” in order to fill detector parameters. In this case, we have to provide “Estimated blob Diameter” and detection threshold. Select proper values for those parameters and preview results. You can navigate through z-slices and time using the scrolls in the image window. Press next to detect nuclei in the complete sequence (this can take some minutes).
6. After the nuclei detection output results will appear. Press “Next” and a filter window will appear. Here you have to select a detection threshold in order to filter detection. There is the option manual and “Auto”, select one. Press “Next” and the program will ask for the visualization way: 2D Hyperstack or 3D Viewer. We recommend 2D due to 3D may not work.
7. Press “Next” and we can add filter for the detected nuclei, as for example, size, diameter, etc.

8. Press "Next" 3 options will appear for tracking. Test all algorithms and choose one. Every algorithm has parameters that should be provided. Choose the more suitable values for that in each case.
9. Press "Next" and the tracking results will be shown, drawing the trajectories in the stack. Now we have visualization options, this help us to draw the trajectories with different colors. By default the colors are select by ID. In the next window, it is possible to filter trajectories by different parameters, for example, mean speed, maximum speed, track displacement. Also, you have the button named "Analysis", where you can observe the detection and track results. These tools you can use to answer the questions.

After finishing the previous procedure, please answer the following questions:

P1: Which parameters did you selected for the detection algorithm?, Why? Which parameters did you choose for the tracking algorithm? Why?, which tracking algorithm provides the best results?

P2: Given the parameters selected by you for tracking, which was the highest mean speed?, Which was the highest instant speed?

P3: Which was the largest track displacement? Which was the mean track displacement?

2.- Optical Flow. In this example, we want to identify groups of cells with different movement behavior. To this aim we will use the Optical Flow method (OF). The OF delivers a vector of movement for each pixel that you will find already computed in "C:\RSI\School2014\lightsheet_epulgar_of\hs_ms\". Using matlab we will take the vector fields to: visualize, and to identify populations in a directional chart.

- a) In the OF folder, you will find a vector field (two files) for each time. For instance "of_scales_3_param_1_x_0_1.bin" is the "x" component of movement between time 0 to time 1.
- b) Open Matlab and locate the "plotOF.m" script, you can select the time to plot (input parameter "timeToPlot"). To execute click on the green "play" button. An additional script "plotOFRose.m" plots angular histograms.

P4: About the OF results: is the resulting vector field uniform?, can you identify groups of cell movement? identify the main advantage/drawback of this method compared to tracking.
