IRB BARCELONA

INSTITUTE FOR RESEARCH IN BIOMEDICINE

ImageJ
Image Processing and Analysis in Java

PRACTICAL SESSION

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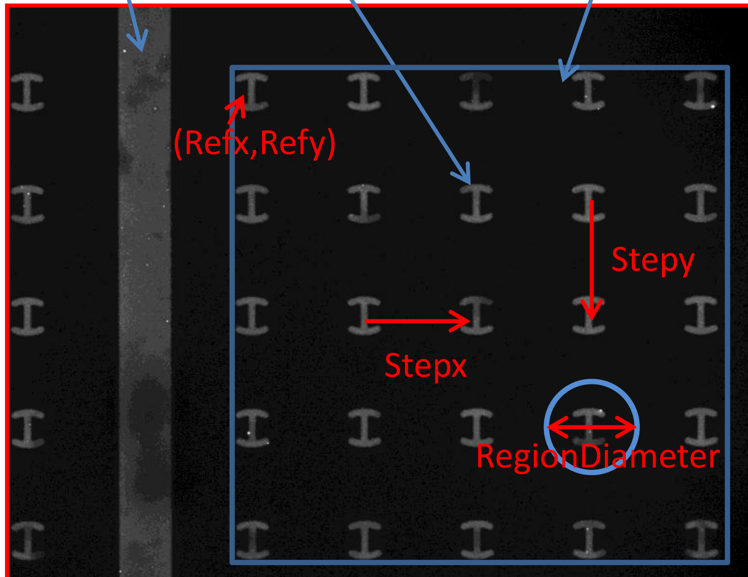
Single nucleus detection (ScanR-Hyperstack dataset)

The control band separating blocks

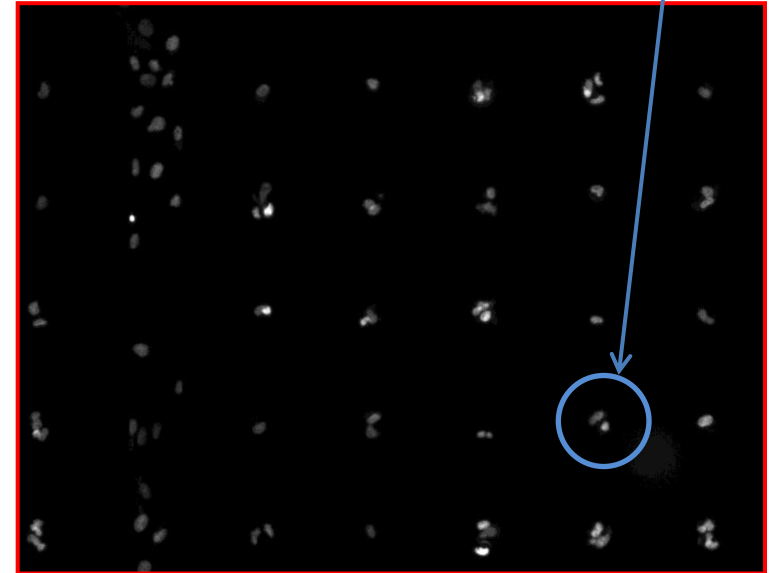
A Cytoo pattern

A region within a block (NumPatternPerLine x NumPatternPerLine)

Analysis region in the nuclei channel



An image from the patterns channel (Alexa 647)



An image from the nuclei channel (DAPI)

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The settings: The images have been taken with a widefield microscope (10x) equipped with a motorized stage. Each image holds at least a 5x5 patterns region from a Cytoo chip block with seeded cells. The acquisition is performed over 3 channels: Cytoo patterns (Alexa 647), nuclei (DAPI) and cytoplasm (GFP). The grid of patterns is assumed regular and the blocks identically centered in the different positions.

The images: They are provided as a 3 channels x 2 frames (positions) hyperstack.

Exercise1: Sequentially generate disks like regions centered on each pattern, call the analysis function and write the number of detected nuclei close to the analysis region (in the nuclei channel).

Exercise2: Copy regions of the cytoplasm channel around the patterns hosting a single cell to a stack (each new region should be copied to a new slice of the stack).

Exercise3: Create a table to store the number of single cells and empty patterns per block region.

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The SingleNucleusDetector Macro

➢ Parameters initialization: required measurements, background/foreground colors, labels for point tool, text properties.

➢ Dialog box: number of patterns per row/column, Analysis regions diameter (RegionDiameter), Minimum area of nuclei, noise threshold.

➢ Image loop: change position (hyperstack frame)

- Calibration (only for the first frame): select the patterns channel (1) and ask the user to spot the centers of 3 reference patterns (up-left, up-right, bottom-left). From these positions deduce the positions of the patterns of the grid (assuming it is regular).

  → Refx and Refy are the coordinates of the up-left pattern.
  → Stepx and Stepy are the x and y distance between patterns.

- *Cells image stack and table Initialization*.

- Pattern loop: wander the pattern center positions

  - Create a disk selection around the pattern center (RegionDiameter).
  - Call the nuclei detection function on this active selection.
  - Write the number of nuclei that have been found for this pattern on the nucleus image.
  - *If a single nucleus has been found copy the bounding box of the analysis region from the cytoplasm channel to the current slice of* StackSingleCell*, add a new slice to this stack (to store the next hit).*
  - *Update single cell and empty pattern counters.*

- *Update a user defined table (single nucleus and empty patterns per block region).*

➢ Remove one slice from the StackSingleCell.

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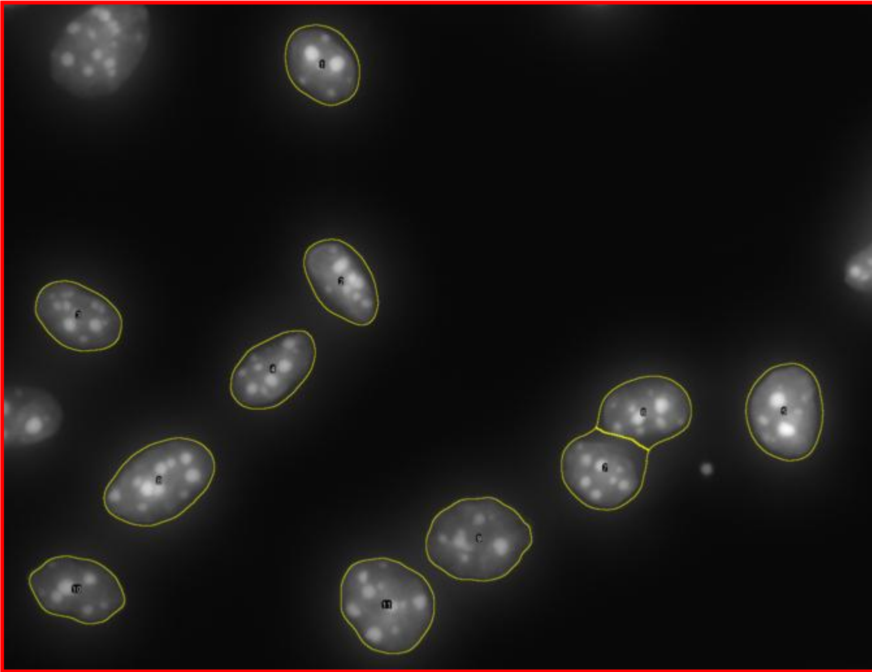
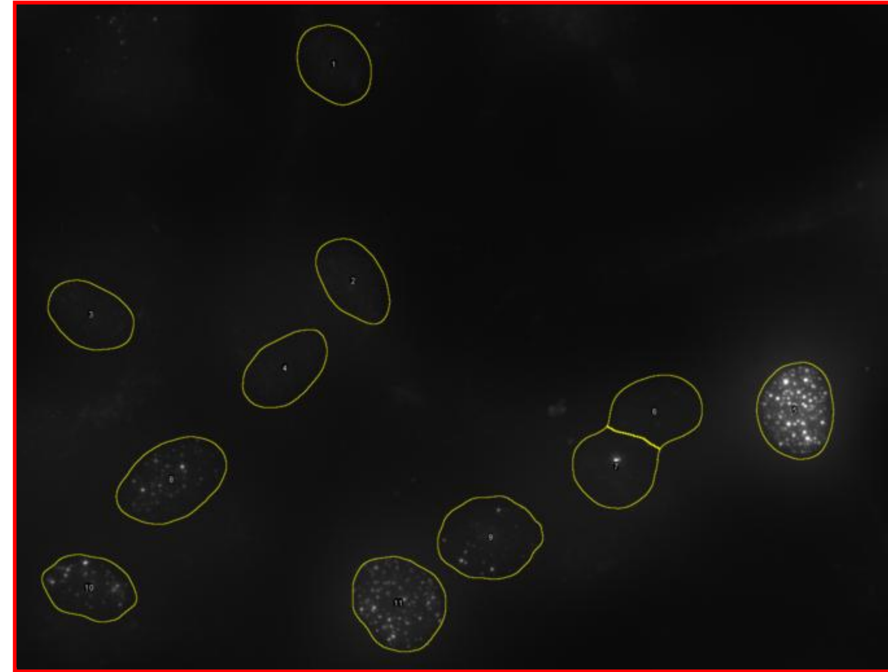
DNA Foci Detection (ScanR-zproj dataset)



An image from the nucleus channel (DAPI)



An image from the foci channel (GFP)

The settings: The images have been taken with a widefield microscope (100x) equipped with a motorized stage in 2 channels (nuclei and DNA damage foci).

The images: They are provided as a 2 channels x 1 frame (single position) hyperstack.

The parameters: The minimum area of a nucleus: MinNucArea (pixels), the minimum intensity level to consider a nucleus as foci positive: MinInt (the maximum intensity in the foci channel for each nucleus is considered), the analysis radius used for the foci (around twice the largest spot radius): AnalysisRad, the detection threshold for the foci (minimum intensity increase compared to surrounding): AnalysisThr, the minimum area of the foci (pixels): MinFociArea.

DNA Foci Detection Macro

The results map

Frame	Nucleus index	Max Intensity	Detected Foci
1	1	226	0
1	2	179	0
1	3	220	0
1	4	215	0
1	5	3120	75
1	6	234	0
1	7	3550	1
1	8	2803	8
1	9	2296	7
1	10	3044	11
1	11	2477	37

The results table

The macro generates a map where the foci channel (green), the detected nuclei (white) and the detected foci (red) are overlaid. A table is also generated to report the number of foci per nucleus and the maximum intensity detected in the foci channel: the index of the nuclei of the map match those of the table.

Exercise: Modify the macro so that the average intensity of the foci is also reported in the table.
Note: the average should be performed only over the detected foci, not inside each nucleus!