cytiva

IN Carta software

Protocol design (Mono-nucleated Cells application)

Set up protocol

1. Select the ***Analyze*** tab.
2. In the ***Applications*** 1 tab, select ***Mono-nucleated Cells***.
3. In the ***Protocols*** 2 tab, click the protocol you would like to run and select the ***Modify*** 3 tab. Alternatively, click ***New*** to design a new protocol.
4. Check the ***Target Type*** 5 boxes to activate the correct targets.
5. For each ***Target Type***:
   a. Enter a unique ***Display Name*** 6, if desired.
   b. For organelles, specify the ***Compartment*** 7 in which they should be identified.
   c. Select ***Wavelength*** 8 and a ***Mask Display*** 9 color.
   d. Select ***Segmentation*** 10 method. Table at right provides details on the various methods.
   e. Click the ***Measures*** 11 button and select/deselect measurements for each ***Target Type*** as needed.
   See "Using measurements menu" for more information.
6. For data sets with multiple z slices, it is possible to define a ***Run mode*** 12. It can be ***Multiple Slices, Current Slice*** or ***Best Focus Z.*** In order to specify a custom range of z slices, select ***Multiple Slices*** and click ***Controls*** button 13. Select ***Best Focus Z*** to allow IN Carta automatically determine the slice to be analyzed via image contrast anaysis, for a selected channel. Click ***Controls*** button 13 to specify a channel used for select best focus z-slice.
7. Only one time-point can be analyzed in ***Mono-nucleated Cells*** application. The first point is selected by default, and another time-point selected for analysis in specimen navigation panel. Switch to ***Time-lapse Analysis*** application when analysis of multiple time-points is required.
8. Select the ***Import Protocols*** 4 tab to load previously created analysis protocols or protocols shared by other users.

Optimize segmentation parameters

1. Use the ***Analysis Settings Panel*** to edit available parameters for each ***Target Type***.
   Note: See the table on the next page or click the ***Information*** icon for more details on parameters.
2. Click ***Apply*** to see preliminary segmentation results overlaid on the images.
3. Check segmentation results for each target using any or all of the strategies below:
   - Use the ***Mask***  tool to toggle mask display on/off and to access the ***Target Mask Properties*** menu.
   - Measure object length and area to refine size and area estimates using the ***Sizing***  tool.
   - Apply an ***Area*** or ***Intensity Filter*** as needed.
   - Select the ***Results Review*** tab to display plots of the measured data to refine estimates for algorithm parameters.
4. Update ***Analysis Settings*** as necessary and click ***Apply***. Repeat steps 2 and 3 until segmentation results are satisfactory.
5. Select another well and repeat steps 2 and 3 to verify that ***Analysis Settings*** are appropriate across the plate.
6. Click ***Save*** 14.

Target type	Segmentation method	Description
Nuclei	Fast	• Faster than Robust method, but not as accurate • Use for total intensity assays or when morphology information is not critical
	Robust	• Slower than Fast method, but will provide more accurate nuclear segmentation results
Cell	Fast	• Faster than Robust method, but not as accurate • Use for well-separated, reasonably large cells
	Collar	• Nuclear dilation method • Use when there is no cellular staining, when identifying positive/negative staining, or when precise cell segmentation is not required
	Robust	• Watershed algorithm that very accurately defines cell boundary • Use when morphology or identification of objects within the cell boundary is important
	Soma	• Use with Neurites segmentation • Modified Robust Cell segmentation algorithm to segment soma
Organelle	Fast puncta	• Multi-scale top hat algorithm • Good at identifying puncta of varying size
	Robust puncta	• Good at identifying puncta of varying intensity
	Networks	• Segments network like organelles within the cell (e.g., endoplasmic reticulum, mitochondria, golgi)
	Membrane	• Creates an artificial boundary within the cell. • Use to segment a membrane compartment or to create a new compartment within the cell, for example mitochondria
	Fibers	• Detects filamentous structures including individual and collapsed fibers
	Neurites	• Segments neurite outgrowth and performs its tracing

Using measurements menu

- ***Measures*** are divided into ***Count***, ***Spatial***, ***Intensity***, ***Position***, ***Coordination, Texture*** and ***Colocalization*** categories.
- Each category has some default measurements selected and other available measure can be selected as needed.
- A channel selection is located in the ***Intensity, Texture*** and ***Colocalization*** categories. It allows to perform intensity-relevant measurements on wavelengths other than the one used for segmentation. For example, segment nuclei in the DAPI channel and measure intensity within a nuclear mask in FITC channel.

Run Protocol

1. Click ***Run Protocol*** 15.
2. Click and drag or click ***Select All*** to select wells for analysis.
   Note: Press Ctrl during selection to select non-adjacent wells.
3. Click ***Run***.
   Note: *See* Using batch analysis to run more than one protocol at a time.

Using batch analysis

1. Click ***Batch*** 
2. Select ***New Batch***.
3. Enter a name for the ***Batch Container*** and click ***Ok***.
4. With the ***Batch Container*** selected in the ***Batch Containers panel***, select multiple data sets from the ***Acquisition Experiments panel*** and select one or multiple analysis protocol. Click ***Add Selected.***
5. Click ***Submit for Processing***. Results are saved to each image stack location.
6. Click the ***Monitoring*** icon to see the status of the batch analysis.

Parameter	Targets			Explanation	Tips
	Nuclei	Cells	Organelles		
Area filter	✓	✓	✓	A post segmentation step to remove large or small objects from final data	• Use to remove objects with an area outside the range selected (i.e., small segmented background noise or large artifacts)
Background suppression			✓	Advanced parameter for neurite segmentation to correct for low contrast	• Default value is a good starting point and should be changed only when changing sensitivity and width parameters don't improve segmentation • Higher values reduce noise in the segmentation mask
Cell area		✓		Average cell area (µm²)	• Use size tools to establish appropriate value • 2 × the nuclear area is a good estimate for monolayer cells • After initial segmentation use ***Results Review*** plot to refine
Collar radius		✓		Distance (µm) to be dilated from the edges of the nuclear mask	• Use to generate an artificial cell boundary when there is not a sufficient cell marker
Connected component segmentation			✓	Advanced parameter for neurite segmantation, which triggers the use of an algorithm to segment broken neurites	• Turn on by default and turned off only when changing senstivity and width parameters dont improve segmentation
Diameter	✓			Typical target diameter (µm)	• Use size tools to establish appropriate value. • After initial segmentation, use ***Results Review*** plot to refine
Enhance Image			✓	Advanced parameter for neurite target to improve image contrast prior to segmentation	• Turned off by default and should be turned on only when changing sensitivity and width parameters don't improve segmentation
Intensity filter	✓	✓	✓	A post segmentation step to remove bright or dim objects from final data	• Use to exclude objects with a mean intensity outside the selected range
Min. length			✓	Approximate length (µm²) of the shortest fiber to be segmented	• Larger values will detect longer continuous objects while smaller values will detect small fragments or break up incorrectly joint objects • This value is not a filter so the algorithm may still identify objects smaller than the specified length
Minimum target area	✓			Approximate area (µm²) of smallest nucleus (Used for optimization of other algorithm functions. This does not mean that smaller nuclei cannot be segmented)	• Use size tools to establish appropriate value • Default value is a good starting point • After initial segmentation, use ***Results Review*** plot to refine
Minimum/maximum target area or target diameter			✓	Approximate area (µm²) or diameter (µm) of the smallest and largest organelles to be segmented	• These values adjust the steps of the algorithm to favor objects of a specified size
Minimum/Maximum width			✓	Approximate thickness (µm) of the smallest and largest fibers and neurites to be segmented	• To segment individual fibers, use very small min value since actin filament width is typically close to the resolution limit • Increase Min/Max width for lower magnification images when segmenting individual fibers/neurites isn't possible
Noise removal	✓	✓	✓	Noise removal filter to improve segmentation results for samples with few cells and/or low contrast	• Use if background is high, illumination is uneven, or if there is debris in the field-of-view (FOV)
Ratio		✓		Controls the size of soma required for downstream neurite segmentation	• Similar values result in larger soma • Decrease sensitivity first if soma is over-segmented. If soma mask still expands beyond cell borders then increase ratio value
Scales			✓	Number of distinct sizes of objects to be identified	• Increasing the number of scales allows for detection of a larger range of organelle sizes • When scales equal **1:** Only the minimum area is considered **2:** Both minimum and maximum values are used **3 or more:** Values between minimum and maximum are used
Sensitivity	✓	✓	✓	Determines whether pixels are assigned to objects or background based on intensity relative to local background	• Lower contrast images typically require higher sensitivity • If object borders are not well defined, adjust sensitivity • If too many small false positives are found in background, use the area filter to remove
Sensitivity threshold			✓	Noise suppression filter	• Increase if too many organelles are identified with ***Sensitivity*** slider at lowest value • Decrease if too few organelles are identified with ***Sensitivity*** slider at maximum value • Increase or decrease by increments of 0.25
Thickness (membrane)			✓	Distance (µm) to erode cell boundary to create membrane compartment	• After membrane is defined, it can be used as a compartment for subsequent organelles
Thickness (network)			✓	Thickness (µm) of individual structures of the network	• Default value is a good starting point • Increase to identify thicker structures, decrease to identify thinner structures
Tubeness			✓	Advanced parameter for neurite target to obtain continuous segmentation mask	• Default value is a good starting point and should be changed only when changing sensitivity and width parameters don't improve segmentation • Higher values result in smoother segmentation masks

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