

Runtime Single Point Segmentation Analysis

This workflow runs the Single Point Segmentation live during XYZC stack acquisitions, where the analysis segments objects of interest at runtime, during image acquisition, when there is one object of interest at the center of each well. Analysis outputs, including cross-sectional area, circularity, eccentricity, confluence, are computed in parallel to image acquisition, with the analysis ready at the end of an imaging session. This analysis currently only applies in the brightfield channel.

Step	Function
1	Open MCAM Acquisition GUI and load a 96-well or 384-well plate onto the Vireo.
2	Navigate to Assays > Acquire an XYZC stack, and acquire images at 4x or 10x magnifications with brightfield illumination.
3	Open the acquired dataset in the MCAM Viewer software.
4	Navigate to Assays > Single Point Segmentation. A panel will open on the right.
5	Under "Single Point Segmentation Protocol", click "Save" to save the automated segmentation protocol in an intended folder.
6	Click "Segment Dataset".
7	Open MCAM Acquisition GUI and load a new 96- or 384-well plate onto the Vireo.
8	Navigate to Assays > Acquire an XYZC stack. A panel will open on the right.
9	Select "minimum" or "laplacian" for Projection.
10	Navigate to Options > Advanced Save Options, click Edit. An "Assay Save Options" window will appear.
11	Click on "Runtime Analysis". Under "Runtime Analysis Protocol", click Load and select the single_point_segmentation_protocol.json you saved from step 6. A list of Validation Rules will appear showing correct parameters have been selected. Click Apply.
12	Select the file location to save your images, and provide a name for the folder. Click Acquire Channel Stack.
13	CSV outputs summarizing area, circularity, eccentricity, region intensity, object count, and confluence are saved in the dataset's folder. The metadata.nc file

	now includes the segmentation masks and metrics that can be displayed in MCAM Viewer after navigating to Advanced and selecting the metric of interest (figure below, red box).
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