

5	Click Segment Dataset to initiate the analysis and generate Segmented Dataset Outputs.
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Runtime Segmentation Analysis

This workflow runs the Segmentation Analysis at runtime, during image acquisition. Analysis outputs, including object counts, cross-sectional area, circularity, eccentricity, confluence, are computed in parallel to image acquisition, with results ready at the end of the imaging session. This assay 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 > Segmentation. A panel will open on the right.
5	Under "Bounding Box Model Selection", select the appropriate model. Single organoid per well → single organoid focus Single tiny organoid per well → Tiny Single Organoid Focus Multiple organoid per well → multi organoid Leave the default option for "Segmentation Model Selection" as it is.
6	<i>Optional:</i> Select the intended Projection method under "Data Formatting" if the dataset opened contains Tiff stacks.
7	Under "Automated Segmentation Protocol", click "Save" to save the automated segmentation protocol in an intended folder.
8	Click "Segment Dataset".
9	Open MCAM Acquisition GUI and load a new 96- or 384-well plate onto the Vireo. Adjust imaging parameters to appropriate settings. Caution: The biology of the plate needs to be similar to the plate you used to create the automated segmentation protocol described above.

10	Navigate to Assays > Acquire an XYZC stack. A panel will open on the right.
11	Select "minimum" or "laplacian" for Projection.
12	Navigate to Options > Advanced Save Options, click Edit. An "Assay Save Options" window will appear.
13	Click on "Runtime Analysis". Under "Runtime Analysis Protocol", click Load and select the automatic segmentation protocol you saved from step 8. A list of Validation Rules will appear showing correct parameters have been selected. Click Apply.
14	Select the file location to save your images, and provide a name for the folder. Click Acquire Channel Stack.
15	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, bounding boxes, and metrics that can be displayed in MCAM Viewer after navigating to Advanced and selecting the metric of interest (figure below, red box).