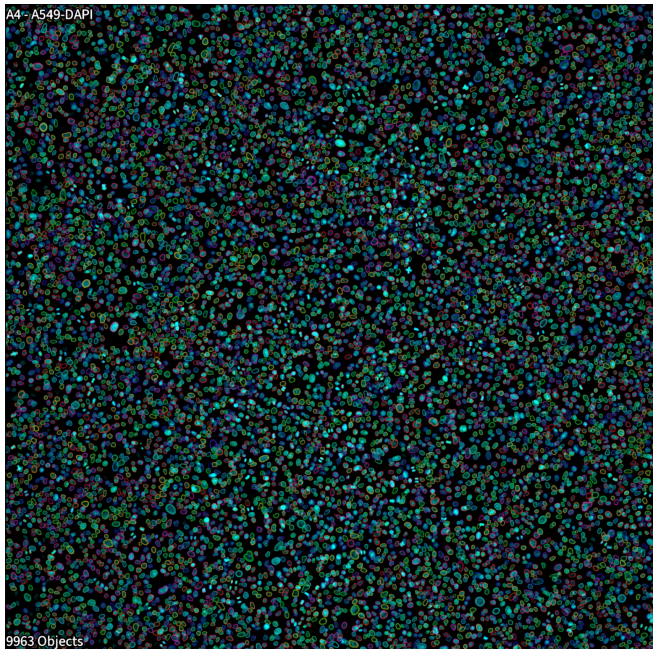
Fluorescent Nuclei Segmentation

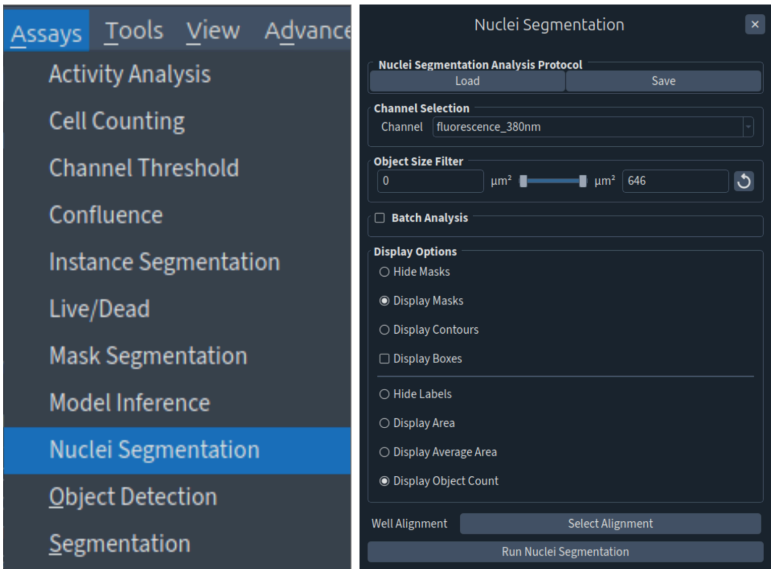
The nuclei segmentation analysis segments and counts individual nuclei in each well of a fluorescence dataset, for example cells stained with DAPI or Hoechst. Unlike the cell counting analysis, which detects bright spots, nuclei segmentation uses a deep-learning model that draws an outline around every nucleus, so touching and clustered nuclei are separated and counted individually. It reports the number of nuclei, the total nuclear area, the average nucleus area for each field of view or well, and the average signal intensity within each nucleus and outputs a csv on a per well basis and a csv with one row per nucleus. This analysis is available on the Vireo and is compatible with 96-, 384- and 1536-well plates.



DAPI stained nuclei detected and segmented within a single well displaying 9,963 objects.

Step	Function
1	**Load the well plate** onto the Vireo.
2	**Capture an XYZC stack at 10x magnification** in a nuclear stain fluorescence channel (for example 380nm for DAPI or Hoechst). See “Nuclear Stain” section for verified dyes and steps for Vireo.
3	**Open the acquired dataset** in the MCAM Viewer software.

4	**Navigate to Assays > Nuclei Segmentation**. A panel will open on the right.
5	For multi-channel datasets, **select the nuclear stain channel** under Channel Selection. The blue (DAPI / Hoechst) channel is selected by default. Brightfield channels are not offered.
6	(Optional) Under Well Alignment, select the alignment file for the well plate in use. Nuclei outside the well boundary are then excluded. If the dataset already contains well plate information this is applied automatically.
7	**Click "Run Nuclei Segmentation"** to run the analysis. A progress bar shows the wells as they are processed; click "Stop Analysis" to cancel.
8	(Optional) Following the analysis, use the Object Size Filter slider to exclude nuclei that are too small (debris) or too large (clumps). The masks, boxes and counts update live. Click "Save Changes" to write the filtered results to disk.
9	Analysis results are displayed on the screen and saved in a folder named "nuclei_segmentation_analysis_results" in the dataset's folder. Three files are generated: "analysis_metadata.nc" containing the analysis results, "nuclei_segmentation_well_summary.csv" summarizing the nucleus count, total area and average area per well, and "nuclei_segmentation_individual_summary.csv" listing the area and signal of every nucleus.



Nuclei segmentation GUI assay panel with channel selection and display options.