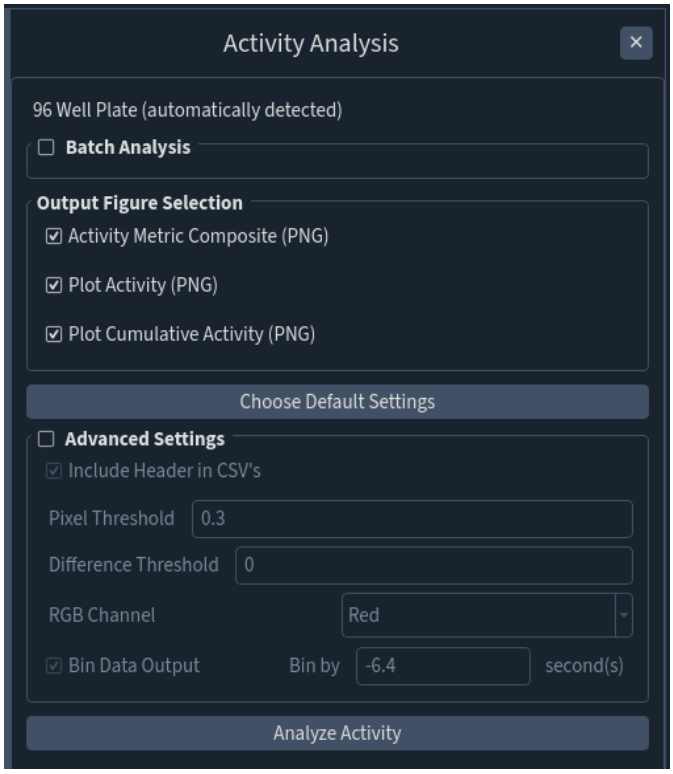
Activity Metric

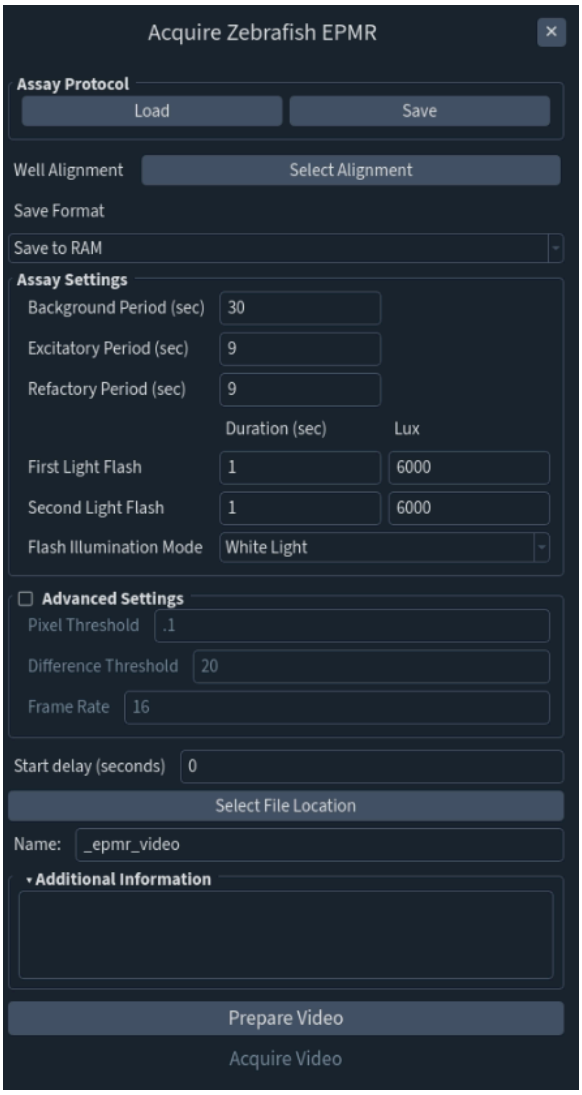
Activity metric analysis provides a rapid method for computing movement in a video. This is accomplished by subtracting each pair of adjacent frames in a video to quantify pixel change. All pixels are treated equally so while total movement is measured over time, we cannot determine “how” something within a frame moves.



Step	Function
1	Open the **MCAM Viewer** and click the Open Dataset button. Click into the **dataset** of interest, then click **Open** in the popup to open **dataset** in **MCAM Viewer**
2	**Go to Assays > Activity Analysis** to open the Activity Analysis panel. Well plate configuration (24-, 48-, or 96-well) will be automatically detected by MCAM Viewer.
3	All Activity Analysis Outputs (described in MCAM Viewer Activity Metric

	Analysis) are generated by default. If not all are necessary, **uncheck the box** pertaining to the output you wish not to generate.
4	The features found within Advanced Settings are also activated by default. **Click on Advanced Settings** if there are any changes that need to be made / any features that you want turned off.
5	**Click Analyze Activity** to generate Activity Analysis Outputs

Zebrafish Embryonic Photomotor Response (EPMR) Assay



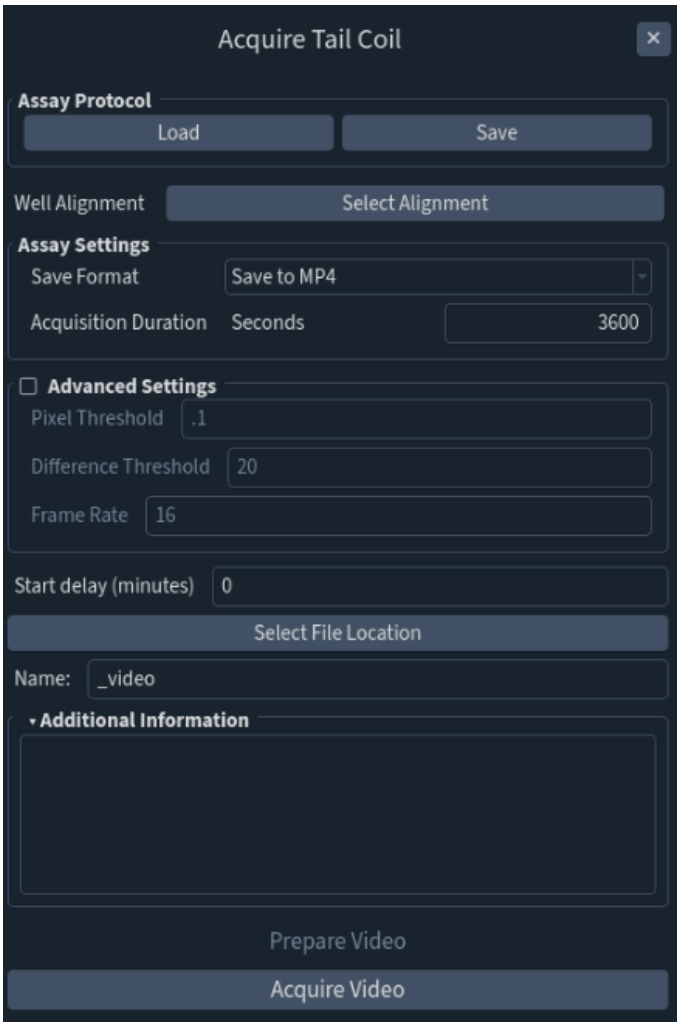
The EPMR assay measures the responses of embryonic zebrafish upon exposure to flashing light stimuli. The MCAM Zebrafish EPMR Assay enables users to set up stimuli and data capture for an EPMR assay experiment.

Step	Action
1	**Go to Assays > Zebrafish > Acquire Zebrafish EPMR** to open the Acquire Zebrafish EPMR Panel. Once the Acquire Zebrafish EPMR Panel opens, image acquisition settings will

	automatically adjust to empirically verified optimal values for EPMR assays. However, image acquisition settings can still be modified by the user.
2	**Click Select Alignment** to select a Well Alignment File (see Create a Well Alignment File for more information, and for instructions on creating a Well Alignment File). The well plate configuration documented within the selected Well Alignment File should match the configuration of the well plate being used in the EPMR assay.
3	(Optional) In the Additional Information field, **enter any notes** about the EPMR assay being conducted.
4	To save assay data as an .nc dataset file, **select Save to RAM** under Save Format. To save assay data as an MP4 file, **select Save to MP4** under Save Format.
5	(Optional) Assay Settings open with preset suggested values. However, to customize assay data capture, under Assay Settings, **set durations of**: ● Background Period (sec) - time before First Light Flash ● Excitatory Period (sec) - time between First Light Flash and Second Light Flash ● Refractory Period (sec) - time after Second Light Flash ● First Light Flash (sec) ● Second Light Flash (sec) as per EPMR assay experimental requirements.
6	(Optional) Assay Settings open with preset suggested values. However, to customize assay data capture, under Assay Settings, set intensity values of: ● First Light Flash ● Second Light Flash as per EPMR assay experimental requirements.
7	(Optional) **Enter a value** in the Start Delay field to delay the start of the assay data capture sequence defined in Steps 5 and 6 above.
8	**Click Select File Location** to select a workspace. Always select a folder on the “MCAM_data” drive to store data from MCAM™ acquisitions. The “File Location” will be displayed in the left side panel once defined.
9	**Define a Filename** to name the folder into which all generated files will be saved. A timestamp will be automatically appended to this name.

10	(Optional) At the top of the panel under “Assay Protocol” **click Save** to save this assay protocol for future use. In the future, load previously saved assay data capture parameters by **clicking the Load button** under “Assay Protocol” and select the appropriate EPMR Assay JSON file.
11	If Save to Ram was selected as the Save Format, **click the Prepare Video** button to set up the MCAM system to capture EPMR assay video data.
12	**Click the Acquire Video** button to begin EPMR assay data capture.

Zebrafish Tail Coiling Assay



Step	Action
1	**Go to Assays > Zebrafish > Acquire Zebrafish Tail Coil** to open the **Acquire Tail Coil Panel.** Once the Acquire Tail Coil Panel opens, image acquisition settings will automatically adjust to empirically verified optimal values for Tail Coil assays. However, image acquisition settings can still be modified by the user.
2	**Click Select Alignment** to select a Well Alignment File (see Create a Well Alignment File for more information, and for instructions on creating a Well Alignment File).

	The well plate configuration documented within the selected Well Alignment File should match the configuration of the well plate being used in the Tail Coil assay.
3	(Optional) In the Additional Information field, **enter any notes** about the Tail Coil assay being conducted.
4	To save assay data as an .nc dataset file, **select Save to RAM** under **S**ave Format. To save assay data as an MP4 file, **select Save to MP4** under Save Format. To save assay data as a CSV file, **select Save to CSV** under Save Format. WARNING: This will not save any video data.
5	Assay Settings open with preset suggested values. However, to customize assay data capture, under Assay Settings, **set duration of Acquisition Duration** as per Tail Coil assay experimental requirements.
6	Advanced Settings open with preset suggested values. However, to customize assay data capture, under Advanced Settings, **set values of**: ● Pixel Threshold - threshold to be applied to the absolute difference of pixels between frames relative to the average of the pixels. ● Difference Threshold - threshold to be applied to the absolute difference of pixels between frames. ● Frame Rate - frame rate the assay will acquire video at. as per Tail Coil assay experimental requirements.
7	**Enter a value** in the Start Delay field to delay the start of the assay data capture sequence defined in Steps 5 and 6 above.
8	**Click Select File Location** to select a workspace. Always select a folder on the “MCAM_data” drive to store data from MCAM acquisitions. The “File Location” will be displayed in the left side panel once defined.
9	**Define a Filename** to name the folder into which all generated files will be saved. A timestamp will be automatically appended to this name.
10	(Optional) At the top of the panel under “Assay Protocol” **click Save** to save this assay protocol for future use. In the future, load previously saved assay data capture parameters by clicking the Load button under “Assay Protocol” and select the appropriate Tail Coil Assay JSON file.

11	If Save to Ram was selected as the Save Format, **click the Prepare Video button** to set up the MCAM system to capture Tail Coil assay video data.
12	**Click the Acquire Video** button to begin Tail Coil assay data capture.