MCAM™ User Guide for Automated Workflows

Ramona Optics, Inc.

Updated as of 2022/09/05

The information contained in this document is subject to change without notice.

Disclaimer

The Ramona Optics MCAM™ is a Gigapixel Microscope™ that is provided as a beta-unit for use by the user “AS-IS” and any express or implied warranties, including but not limited to, the implied warranties of merchantability and fitness for a particular purpose are disclaimed.

Licensing

A copy of the license agreement is included along with the software package. If you have not received this agreement please contact Ramona Optics.

All third party licenses can be found within the MCAM™ GUI by selecting “About” in the “Help” menu.

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Technical Specifications

System Ratings

Mains Supply: 100-240 VAC, 50/60Hz, 10A
Indoor use only
Altitudes up to 2000m
Temperatures 15-28C
Maximum relative humidity: 80% up to 31C, decreasing linearly to 50% at 40C
OVERVOLTAGE CATEGORY II (EN 61010-1 2010)
POLLUTION DEGREE II (EN 61010-1 2010)

System Specifications

MCAM™ Dimensions: 483 x 661 x 534mm (width x height x depth)
Mass (weight): 41kg (90lbs)
Electrical outlets: Up to three (3) outlets for the MCAM, Computer, and Monitor Display.

Safety Warnings and Precautions

The MCAM™ system should always be used in accordance with the guidelines of this manual to avoid risk of personal injury and/or damage to the instrument. **If the MCAM™ system is used in a manner not consistent with the manner specified by Ramona Optics, Inc. in this manual, protection provided by the equipment may be impaired.**

Note the following safety warnings:

	The MCAM™ system is heavy (~90 lbs/41 kg). Use caution when lifting. Use your legs and core muscles to lift the equipment, not your back. Keep the load close to your body during the lift. Maintain a firm grip on the load with both hands.
	The MCAM™ system is heavy. For stability, position all system hardware on a stable surface prior to use.
	Prior to moving, cleaning, or performing maintenance on the MCAM™ system, always power off and disconnect power from hardware and disconnect MCAM™ and MCAM™ Workstation.
	The MCAM™ is an electronic system. Do not touch any MCAM™ system hardware with wet hands.
	Ventilation is required for proper function of the MCAM™ system. Make sure to leave a minimum of two inches or 5 cm of space around all sides of the MCAM™ as well as the MCAM™ Workstation.
	MCAM™ system's X, Y, and Z stages can cause pinch hazards for users can move quickly. To avoid pinch hazard, ensure hands are outside MCAM™ imaging chamber before operating the moving MCAM stages using the provided software.
	LEDs on **MCAM™ Reflection Illumination Module** can become very hot after use. Do NOT touch any part of **MCAM™ Reflection Illumination Module** except for **Quick Release Handles** (see **MCAM™ User Manual**).

Avertissements de sécurité et précautions

Le système MCAM™ doit toujours être utilisé conformément aux directives de ce manuel afin d'éviter tout risque de blessure personnelle et/ou de dommage à l'appareil. **Si le système MCAM™ est utilisé d'une manière non conforme aux indications spécifiées par Ramona Optics, Inc. dans ce manuel, la protection offerte par l'équipement peut être compromise.**

Prenez note des avertissements de sécurité suivants :

	Le système MCAM™ est lourd (~90 livres / 41 kg). Faites attention lorsque vous le soulevez. Utilisez vos jambes et les muscles de votre tronc pour soulever l'équipement, pas votre dos. Gardez la charge près de votre corps pendant le soulèvement. Maintenez une prise ferme sur la charge avec vos deux mains.
	Le système MCAM™ est lourd. Pour une meilleure stabilité, placez tout le matériel du système sur une surface stable avant son utilisation.
	Avant de déplacer, nettoyer ou effectuer la maintenance du système MCAM™, éteignez toujours et débranchez l'alimentation du matériel et déconnectez le système MCAM™ et la station de travail MCAM™.
	Le MCAM™ est un système électronique. Ne touchez à aucun composant matériel du système MCAM™ avec vos mains mouillées.
	Une ventilation est nécessaire pour le bon fonctionnement du système MCAM™. Assurez-vous de laisser un espace minimal de 5 cm autour de chaque côté du MCAM™ ainsi que de la station de travail MCAM™.
	Les plateaux X, Y et Z du système MCAM™ peuvent présenter des risques de pincement car ils peuvent se déplacer rapidement. Pour éviter ceci, assurez-vous que vos mains sont à l'extérieur de la chambre d'imagerie MCAM™ avant d'opérer les déplacements des plateaux MCAM à l'aide du logiciel fourni.
	Les LED du **module d'éclairage par réflexion MCAM™** peuvent devenir très chaudes après l'utilisation de l'appareil . Ne touchez à AUCUNE partie du **module d'éclairage par réflexion MCAM™** à l'exception des poignées de dégagement rapide (voir le **manuel d'utilisation MCAM™**).

Sicherheitswarnungen und Vorsichtsmaßnahmen

Das MCAM™-System sollte immer gemäß den Richtlinien dieses Handbuchs verwendet werden, um das Risiko von persönlichen Verletzungen und/oder Schäden am Gerät zu vermeiden. W**ird das MCAM™-System auf eine Art und Weise verwendet, die nicht mit den von Ramona Optics, Inc. in diesem Handbuch angegebenen Verfahren übereinstimmt, kann der durch die Ausrüstung gebotene Schutz beeinträchtigt werden.**

Beachten Sie die folgenden Sicherheitswarnungen:

	Das MCAM™-System ist schwer (Modell A: ~29 lbs/13 kg; Modell B: ~90 lbs/41 kg). Seien Sie vorsichtig beim Heben. Benutzen Sie Ihre Bein- und Rumpfmuskulatur zum Heben der Ausrüstung, nicht Ihren Rücken. Halten Sie die Last während des Hebens nah am Körper. Behalten Sie einen festen Griff an der Last mit beiden Händen.
	Das MCAM™-System ist schwer. Für die Stabilität positionieren Sie die gesamte Systemhardware vor der Verwendung auf einer stabilen Oberfläche.
	Bevor Sie das MCAM™-System bewegen, reinigen oder Wartungsarbeiten daran durchführen, schalten Sie immer zuerst aus und trennen Sie die Stromversorgung von der Hardware und trennen Sie das MCAM™ und die MCAM™-Workstation.
	Das MCAM™ ist ein elektronisches System. Berühren Sie keine Hardware des MCAM™-Systems mit nassen Händen.
	Eine Belüftung ist für die ordnungsgemäße Funktion des MCAM™-Systems erforderlich. Stellen Sie sicher, dass auf allen Seiten des MCAM™ sowie der MCAM™-Workstation mindestens zwei Zoll oder 5 cm Platz gelassen werden.
	Die X-, Y- und Z-Achsen des MCAM™-Systems können sich schnell bewegen und so Quetschgefahren für Benutzer verursachen. Um Quetschgefahren zu vermeiden, stellen Sie sicher, dass Ihre Hände außerhalb der Bildgebungskammer des MCAM™ sind, bevor Sie die beweglichen MCAM-Stufen mit der bereitgestellten Software betreiben.
	Die LEDs am **MCAM™-Reflexionsbeleuchtungsmodul** können nach Gebrauch sehr heiß werden. Berühren Sie KEINEN Teil des **MCAM™-Reflexionsbeleuchtungsmoduls** außer den Schnellverschlussgriffen (siehe **MCAM™-Benutzerhandbuch**).

	Das MCAM™ ist ein elektronisches Gerät. Trennen Sie vor dem Bewegen, Reinigen oder Durchführen von Wartungsarbeiten den Strom.

Equipment Ratings

The MCAM™ system is rated for use in the following conditions:

- Indoor use
- Altitude up to 2000m
- Temperature 15C-28C
- Maximum relative humidity 80% for temperatures up to 31C decreasing linearly to 50% relative humidity at 40C
- MAINS supply voltage fluctuations up to +/-10% of the nominal voltage
- Short duration overvoltages occurring on the MAINS supply
- Only non-conductive pollution (solid, liquid, or gaseous foreign matter that may produce a reduction of dielectric strength or surface resistivity), except that occasionally a temporary conductivity caused by condensation is expected

MCAM™ Features

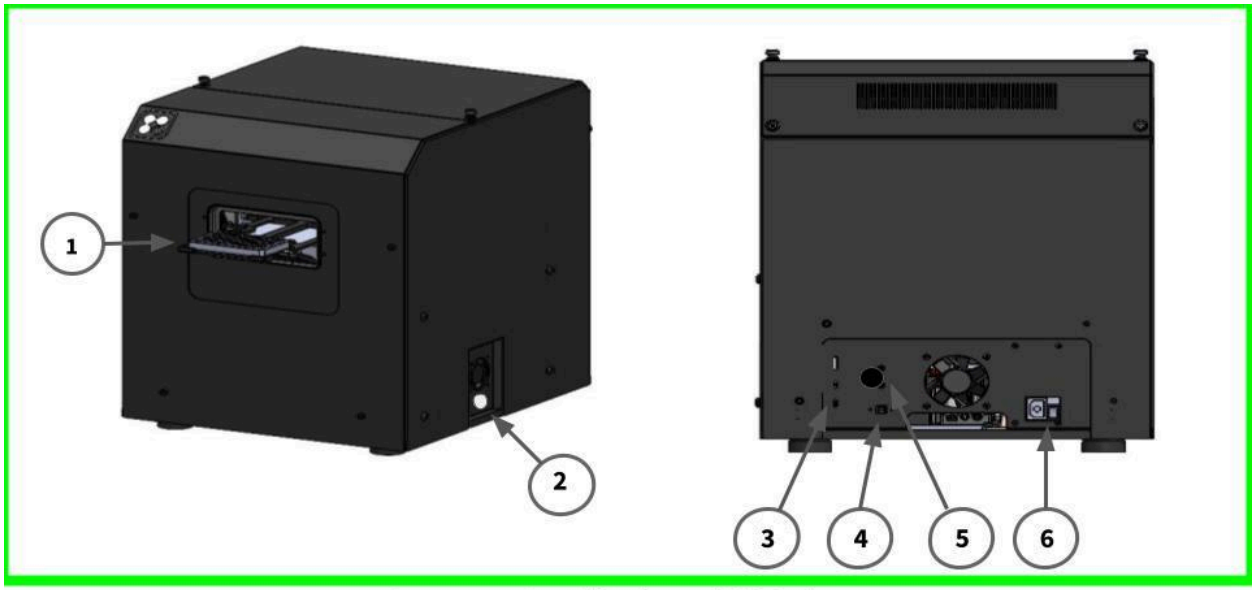


Figure 1:MCAM view with key features highlighted

1. Retractable Stage
2. Power button
3. Thunderbolt port
4. USB B port
5. Manual stage control
6. AC power inlet and switch

Introduction

The intended use of this MCAM™ system is to image 96, 384, 1536 well plates in an automated workcell for cell morphology assessment, contamination detection, organoid debris accumulation, and media quality. In order to use the MCAM™ for imaging within the automated workcell, a protocol must be created within the MCAM GUI and saved in a specific file location that is compatible with GreenButtonGO. Sections 1-3 of this user guide outlines the features of the MCAM™ GUI necessary to optimize imaging parameters for creating a protocol. Section 4 summarizes how to edit the preloaded protocols, create new protocols, and integrate protocols with Green Button Go for automated image acquisition.

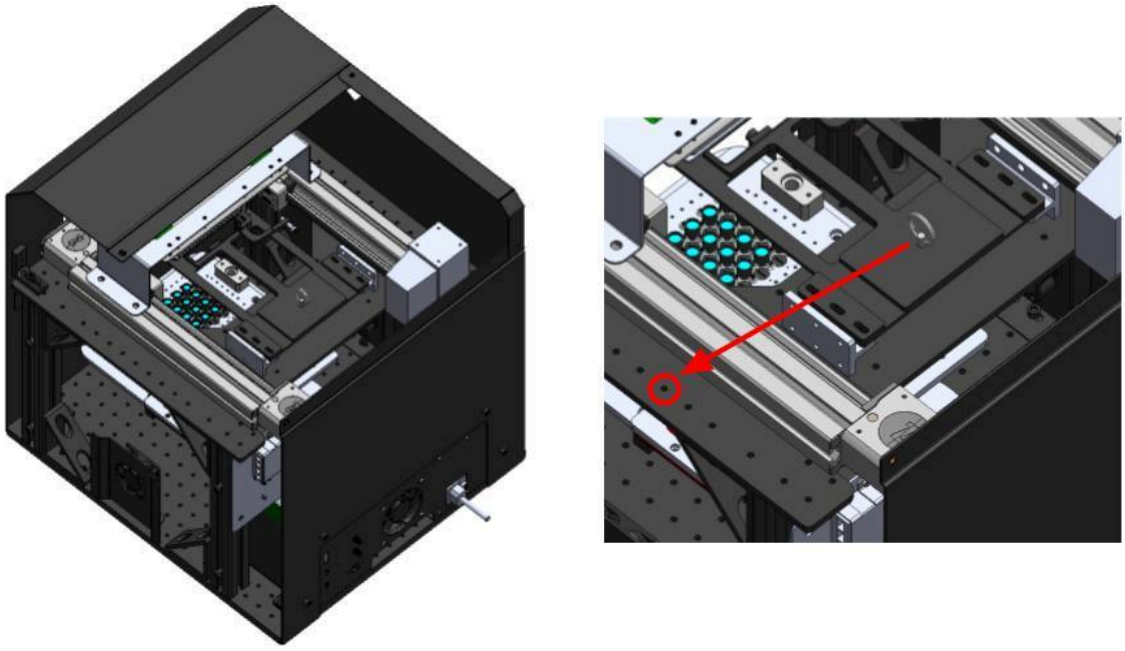
Unpacking the MCAM™

Removing Packing Material and Stage Lock Pin From Inside the MCAM

The MCAM is shipped with protective foam and bubble wrap near the fluorescence illumination modules, as shown in Figure 2. This material needs to be removed before the system can be operated.

Step 1: Remove the top Panel first, then the side panel. The top panel locks all panels in place, so after it is removed, any other panel can be removed for maintenance.

Step 2: Remove Stage Pin from stage and place in hole at side of MCAM as shown



Step 3: Remove Packing material from the system, save for repacking as it is anti-static packaging.

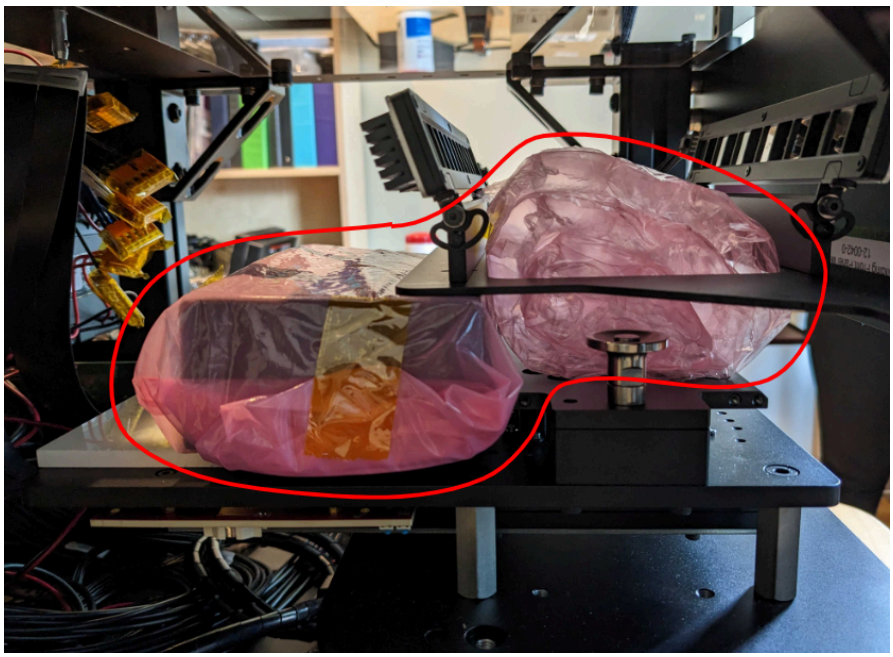


Figure 2. Protective packing material which must be removed

Enabling the System Power Supply

The MCAM is shipped with the system power switch disconnected from the system power supply. After the MCAM is unpacked and the protective foam and bubble wrap have been removed from inside the MCAM, the power switch cable (shown on Figure X0) should be connected.

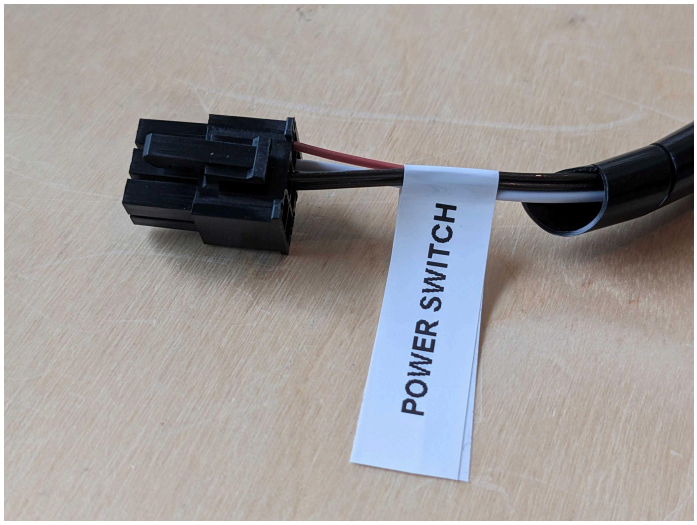


Figure X0: Power supply enable key

The power supply is located at the rear of the MCAM and can be reached once the left side panel is disassembled, as shown in Figure X1. The power switch cable can be found near the power supply.

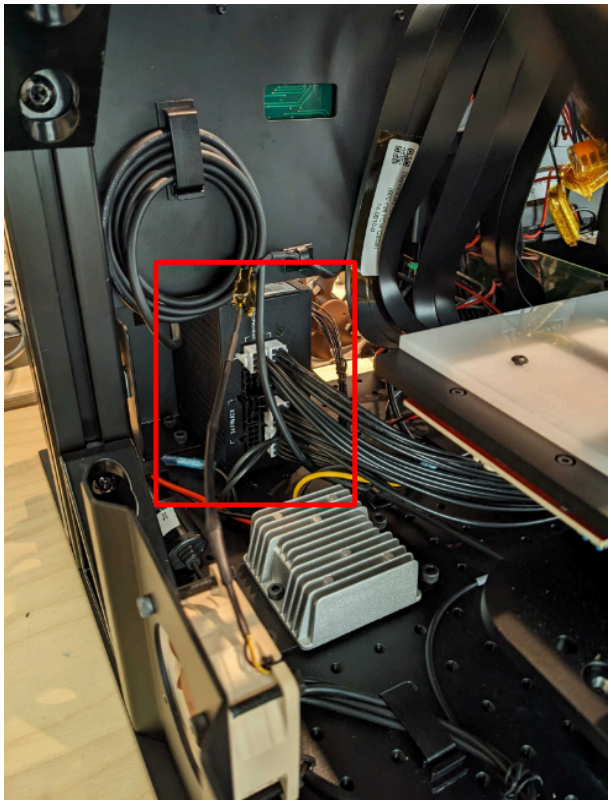


Figure X1: System power supply

Figure X2 shows the port on the power supply into which the power switch cable must be connected. The cable should click into place with the locking clasp engaged.

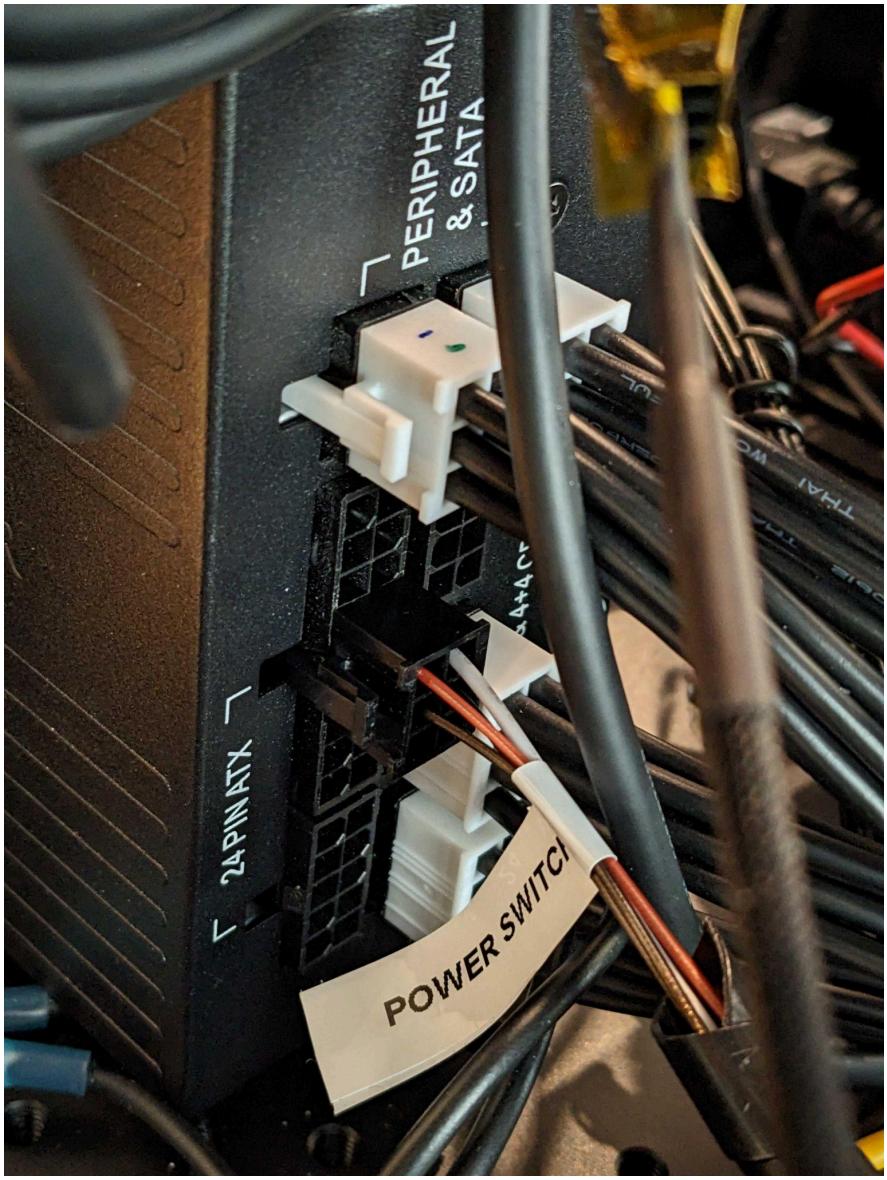


Figure X2: Power switch cable properly connected to the power supply

MCAM™ Accessories

Monitors

Recommended monitor options for the MCAM™:

- Dell 27 Monitor - P2722H - Full HD 1080p, IPS Technology, 8 ms Response Time
  Can be purchased from Amazon:
  https://www.amazon.com/Dell-27-Monitor-Technology-Response/dp/B096W49KBL

- Dell 24 Monitor - P2422H - Full HD 1080p, IPS Technology, ComfortView Plus Technology
  Can be purchased from Amazon:
  https://www.amazon.com/Dell-24-Monitor-Technology-Comfortview/dp/B096T36G8L

1. Graphical User Interface (GUI)

1.1 Opening and Closing the MCAM™

After installing the acquisition software, and turning on the MCAM™, the Graphical User Interface (GUI) can be started by clicking on the Ramona Optics application launcher on the sidebar. The MCAM viewer will automatically start when the GUI opens. If the GUI has already been opened and the MCAM viewer has been turned off, it can be started using the “Open MCAM” button in the middle of the display (Figure 2). By default, the MCAM™ will attempt to connect to each of two components:

- The transmission illumination board
- The stage motor

If any of these components are unavailable, the user can choose to disconnect them by opening the “Advanced” menu on the toolbar, clicking “Advanced Settings”, navigating to the “subcomponents” tab, and deselecting the subcomponents . This will automatically disable the ability to change the settings for these components.

The MCAM™ can be closed using the “Exit” button from the File menu, or by exiting the GUI with the red “X” button in the upper right corner. Either method will stop all ongoing operations and safely close the MCAM™.

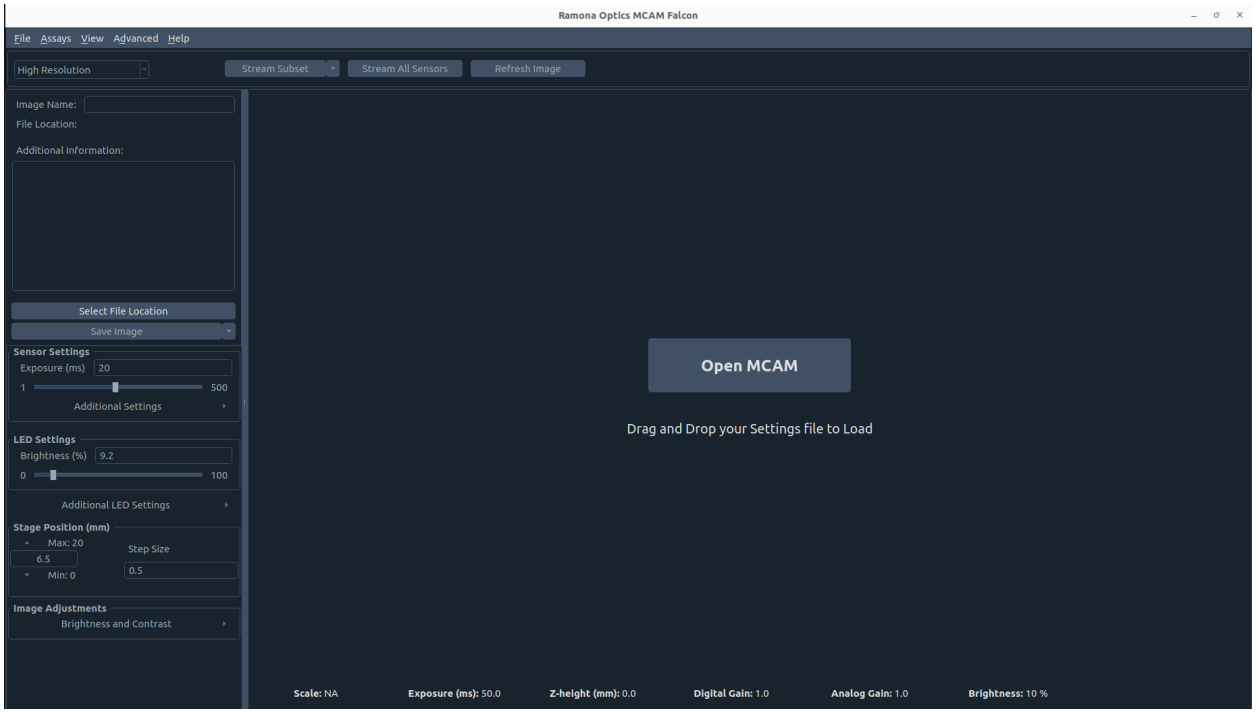


Figure 2: GUI start screen, with the “File” menu open and highlighted.

1.2 GUI Navigation

The user can zoom and pan in the GUI to view any section of the streamed or acquired images. To zoom, either scroll up and down on the mouse wheel, or move the mouse while holding the right click mouse button. To pan, move the mouse in any direction while holding down the left click button.

At the top left of the window (Figure 3, #1) the current workspace is displayed. This is the location where all files will be saved. If a workspace has not yet been selected a message will be displayed suggesting to select a workspace.

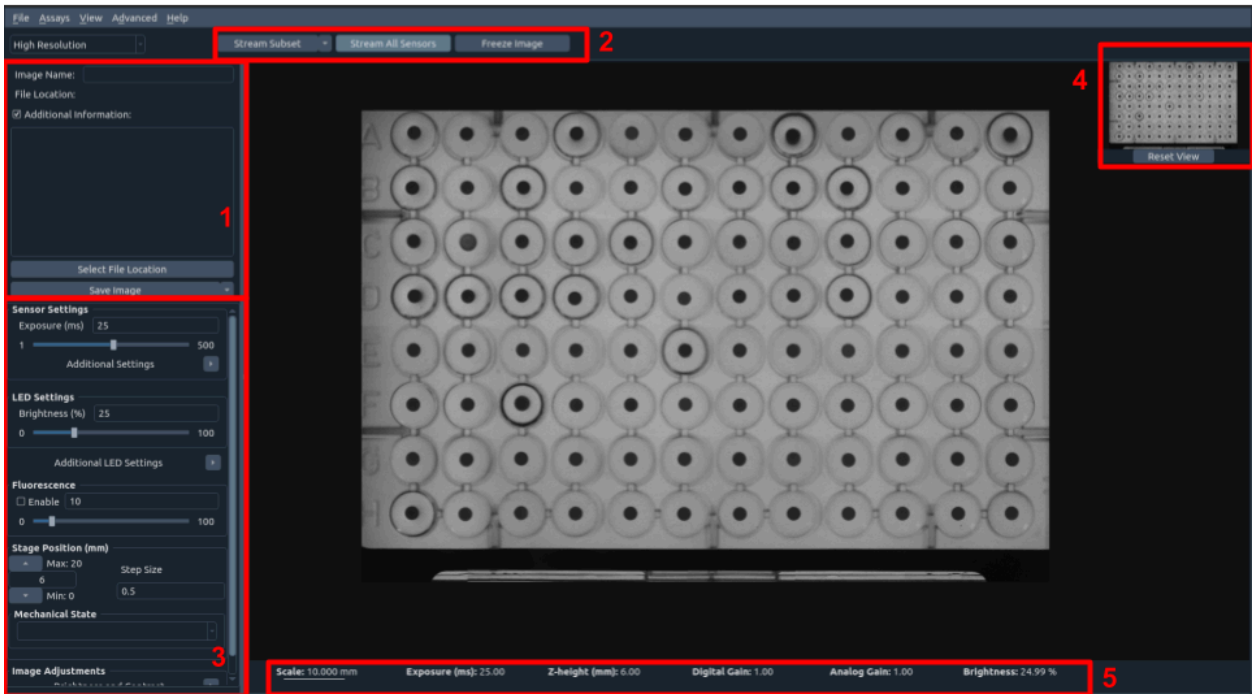


Figure 3: Falcon GUI layout with 1) Selected Workspace, 2) Image Acquisition Mode Buttons, 3) Image Acquisition Settings Panel, 4) Picture-in-picture and 5) Key Image Specifications highlighted.

At the top of the GUI the Image Acquisition Mode toolbar allows the user to control all 24 cameras at once using the “Stream All Sensors” button or in subsets with the “Stream Subset” function (Figure 3, #2). The user can also freeze the image in order to pause the sensor streaming at key points in time to allow for further examination of the frame.

The image acquisition settings panel is on the left side of the viewing window (Figure 3, #3). This panel is used for adjusting the sensor and LED settings, fluorescence control, and stage positioning. Additional details for each subsection is described in section 2.

While panning, the picture-in-picture (Figure 3, #4) in the right-hand corner of the screen shows what portion of the full image is currently displayed.

Figure 4: Key Image Specifications

Some key image parameters are displayed at the bottom of the viewing window (Figure 3, #5 and Figure 4) including a scale bar providing an approximate scale. Note that the scale bar assumes a constant width for each pixel, and may not be accurate across the entire sample if 3D features are

present. Additionally on the right side, RGB color values of the LEDs are displayed which have been preselected using calibration data.

2. MCAM™ Image Acquisition Settings

2.1 Settings Panel

Most of the MCAM™ image acquisition settings can be adjusted using the Settings Panel on the left side of the GUI (Figure 3, #3). The panel can be hidden to make more room on the screen by clicking on the white line border between the settings panel and the image, and sliding to the left. The GUI allows the user to set the system parameters within a range of values acceptable for most common experimental setups. More customized settings can be set in the Advanced submenu.

Most settings values can be adjusted either by using the sliders or by directly typing a value into the text box. When using the text box, the accepted values will still be limited to the maximum and minimum values shown on the respective slider. In addition, the setting displayed in the text box may adjust to a value slightly higher or lower than the value entered. This is because for certain settings (particularly digital and analog gain), the MCAM™ can only accept distinct values. The GUI will automatically round the entered setting to the nearest acceptable value, and send that parameter to the MCAM™ system.

2.2 Loading/Saving Settings

On first use, the GUI will load a set of predetermined default settings which were chosen to produce generally high quality images. Afterwards, the GUI will automatically load the settings from its last use. Because each unique imaging environment and sample may have different optimized settings, the settings for individual experiments can be saved and loaded using the “File” menu in the toolbar. The default settings can also be re-loaded using the “Restore Default Settings” button in the same menu.

2.3 Image Acquisition Settings

The sensor exposure, gain, and LED parameters can all be set from the setting panel on the left side of the GUI, as well as contrast levels of the displayed image. These settings are further explained below.

2.3.1 Exposure:

This setting controls the amount of time that the camera sensor acquires light for each frame. A longer exposure will yield a brighter image with more motion artifacts while shortening the exposure will darken the image with fewer motion artifacts. Additionally this setting will affect the framerate when using streaming acquisition modes with longer exposures decreasing potential framerate. In order to maximize framerate, consider keeping the exposure as low as possible and balance lighting with the brightness setting.

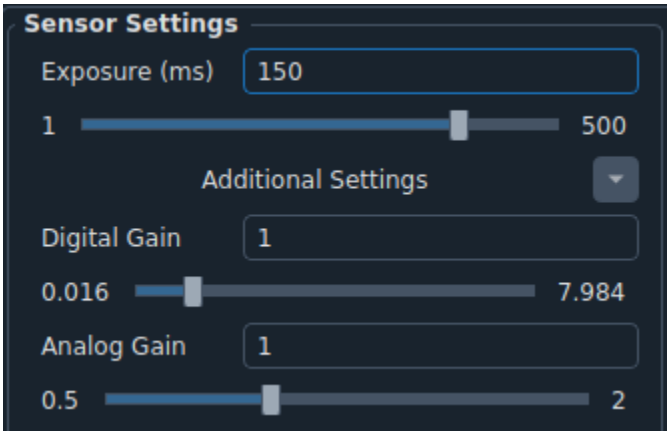


Figure 5: Camera sensor settings.

2.3.2 Digital and Analog Gain:

Gain settings will magnify the signal produced by the image sensors. Analog gain increases the sensitivity of the sensors using hardware while digital gain increases the signal using software to multiply the signal once it has been converted to a digital signal. Increasing gain values will increase image intensity but will also increase noise proportionally. Keeping gain values as low as possible will reduce inherent signal noise.

2.3.3 Brightness:

Located under “LED Settings”, this will control the overall brightness of the LEDs used to illuminate the sample.

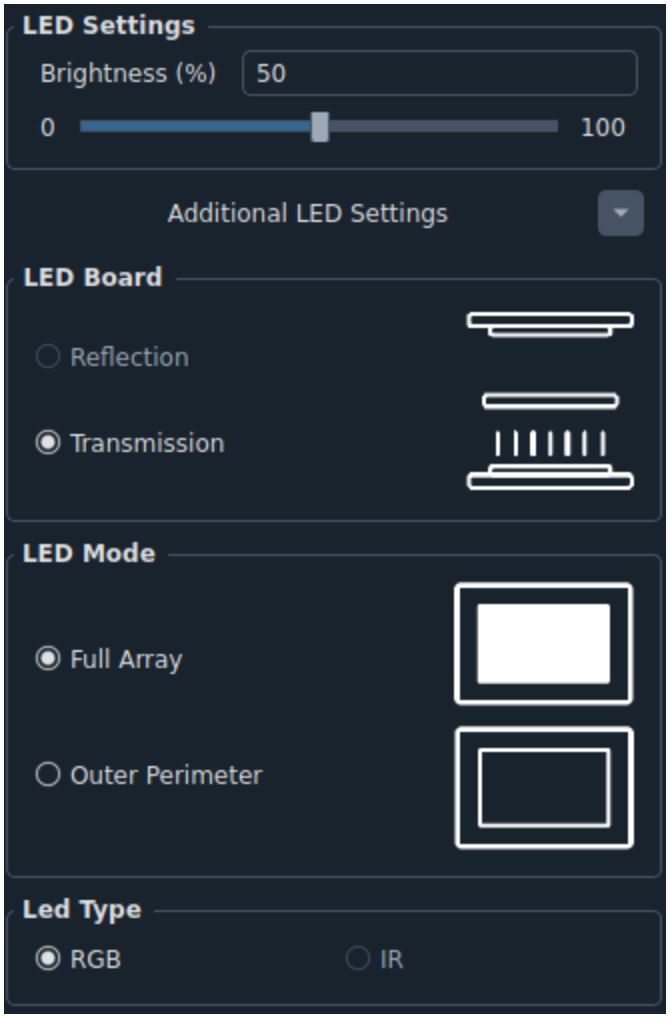


Figure 6: GUI LED board settings

2.3.4 LED Board Selection:

The transmission LED board is the standard light source for use with the MCAM™ Kestrel when imaging semi-transparent samples and is installed at the top of the unit. Selecting this light source, the stage will be lit from above and pass through the sample before reaching the camera sensors. The reflection LED board is not available in this version of the MCAM..

2.3.5 LED Board Mode:

Currently, the LED board can be set to one of two modes, shown in Figure 6. “Full Array” will light up every LED on the board, while “Outer Perimeter” will light up a single row of LEDs on the outside of the chosen board. These settings apply to the LED board.

2.3.6 Z-Stage Height Settings:

The location of the Z-stage can be changed using either the up and down arrows or by manually entering a location (given in millimeters from the highest point where the stage can be positioned) as shown in Figure 7. Step size controls the increment by which the arrows will adjust the height. Adjusting the Z-stage location will move the stage and sample in and out of focus.

Figure 7: Z-stage settings.

2.3.7 Mechanical State:

To eject and retract the plate stage, select the mechanical state option in the left side bar. A dropdown menu will appear with the option to select “Acquisition” “Sample Loading'' and “Sample Unloading” (Figure 8). “Sample Loading” and “Sample Unloading” will cause the stage to eject from the MCAM™ while “Acquisition” will retract the stage into the device. In the case of a mechanical failure, a manual stage control is located in the back left of the device (Figure 1.5).

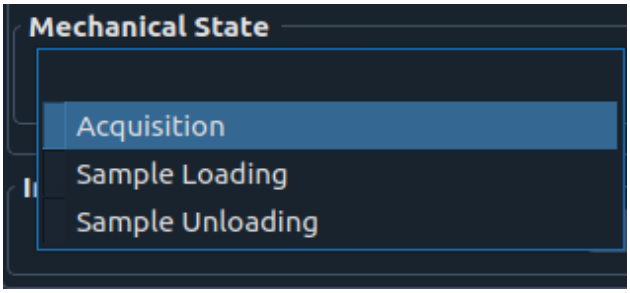


Figure 8: Ejecting and retracting the plate stage

2.3.8 Gamma:

Adjusting this value will control the displayed image’s sensitivity to bright and dark tones and is an effective method of controlling the overall image contrast as perceived by humans. Reducing Gamma will decrease shadows and darker tones yielding a brighter image (Figure 9, 10).

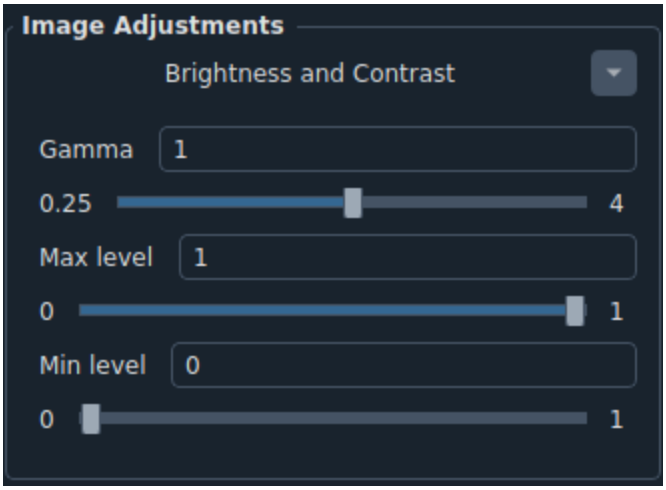


Figure 9: GUI contrast settings.

2.3.9 Max and Min Levels:

The “Max level” and “Min Level” sliders are used to set the contrast of the image as displayed in the GUI (Figure 9). All pixels in the raw image data with values equal or greater to the selected “Max Level” will be displayed using the highest pixel value, while any data pixels with values below the “Min Level” will show up as black. The remaining middle range of pixels will be displayed using the full range of display pixel values. These settings are only for immediate display, and do not impact the data being gathered or saved. Figure 10 shows the impact on the displayed image of changing the Max and Min levels.

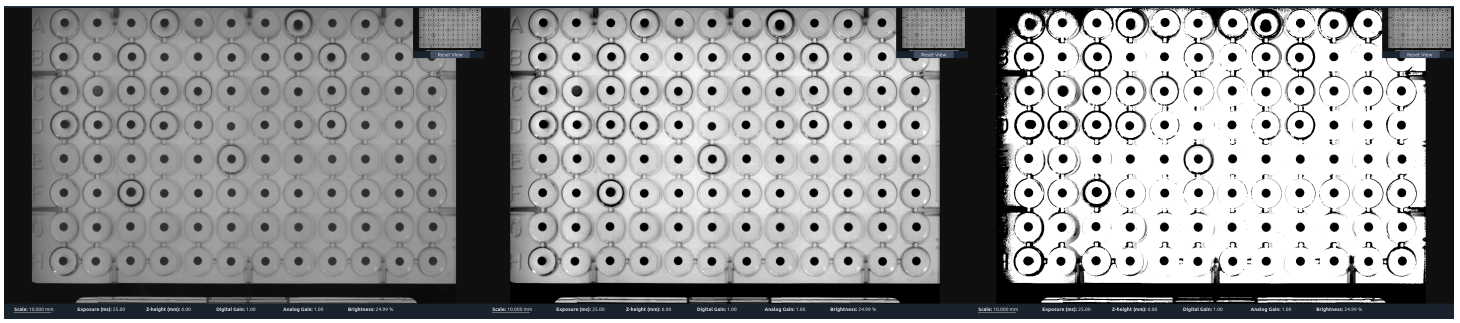


Figure 10: Images with different Max and Min levels: 0 and 1 (left), 0.7 and 0.2 (center), 0.5 and 0.5 (right).

3. Stream and Acquire Images

Buttons for streaming images are located at the top left of the GUI (Figure 11). The GUI can display images streamed from all 24 cameras or images streamed by a subset of selected cameras. If the user would like to freeze an image to capture specific instances, the “Freeze Image” is available.

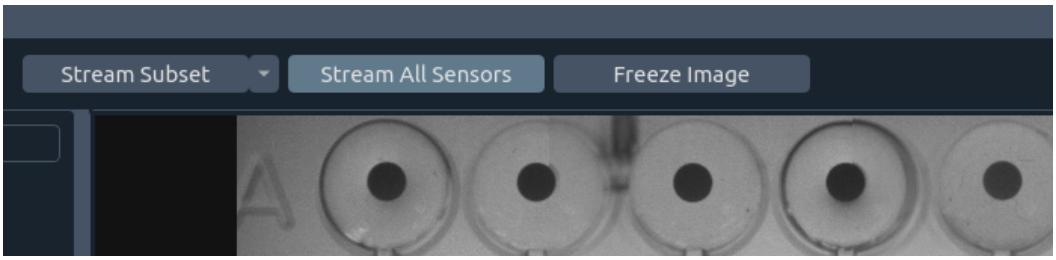


Figure 11: Buttons for streaming sensors

The GUI can stream from any one of the cameras (see Figure 11) or groups of cameras in the array, in full resolution mode. When streaming in single camera mode, the user can double click on any part of the image to stream from the respective camera capturing that area. All cameras not currently streaming will appear darker (Figure 12). Streaming can be stopped at any time by pressing “Freeze Image”, which will pause the streaming.

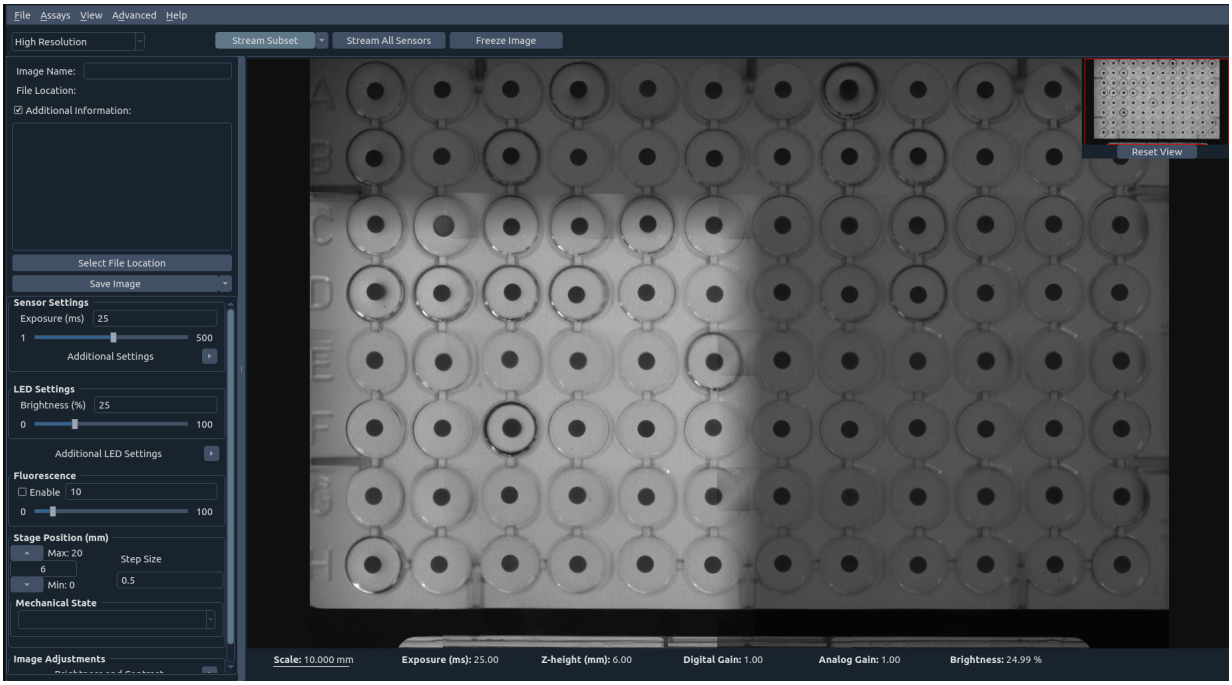


Figure 12: Streaming from a subset of cameras

3.1 Save and Export Images

3.1.1 Save Image via toolbar

MCAM™ data can be exported or saved from the GUI in several ways. First, high resolution images can be exported using the “Save Image” button in the left panel (Figure 13). First, assign the image a specific name in the “Image Name” section. Next, click the “Select File Location” to assign a directory for the image to be saved in. Lastly, select “Save Image” to capture a screenshot of the section of the plate that is in view. This will save the full resolution MCAM™ data in a single “ .nc” file which can be opened for analysis in the Ramona Optics custom MCAM™ Image Viewer or in MathWorks software. “Open Workspace” will open the directory in which the most recently saved images were placed. “.nc” files use the netcdf4 data format. More information on the implementation and usage of this file structure can be found here: https://unidata.github.io/netcdf4-python/. Notes can be entered in the “Additional Information” field which are saved as metadata in the image file and displayed in the MCAM™ Viewer with the image.

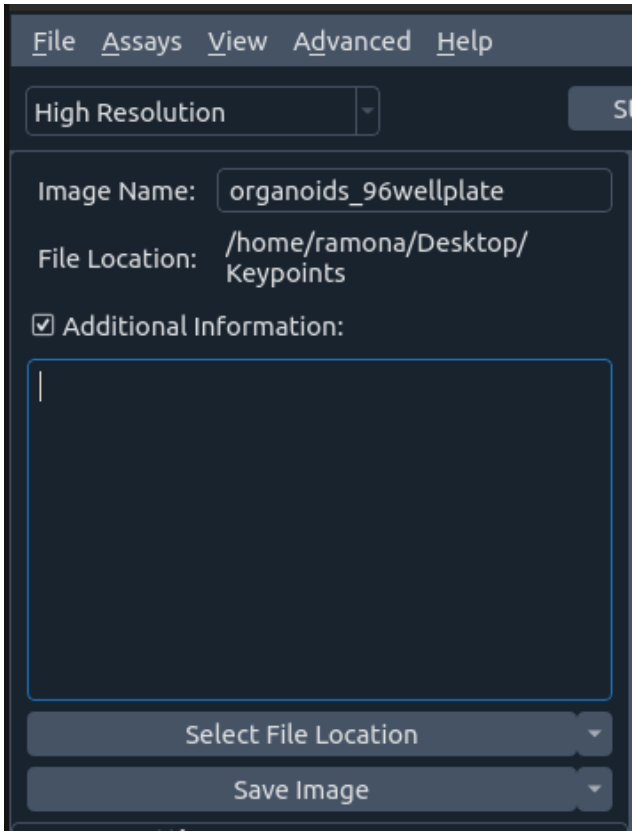


Figure 13: Save settings.

Saved images are given a filename according to the following rules:

1. The user defines the prefix of this filename by entering in the “Image Name” field (Figure 13).
2. The suffix of this filename is generated as a timestamp with the following format YYYYMMDD_HHMMSS_mmm where:
    - YYYY is the year when the image is saved, e.g. 2020.
    - MM is the month when the image is saved, e.g. 08.
    - DD is the day when the image is saved, e.g. 06.
    - HH is the hour (in 24 hour format) when the image is saved, e.g. 15.
    - MM contains the minute when the image is saved, e.g. 43.
    - SS contains the seconds when the image is saved, e.g. 52.
    - mmm contains the milliseconds when the image is saved, e.g. 549.
    - The final timestamp will appear as: 20200806_154352_549

3.1.2. Screenshot function

Screenshots of the currently displayed images can be saved using “Capture Screenshot” in the “File” menu of the toolbar. This screenshot will include the main canvas, the settings toolbar, and the picture-in-picture and the main settings (Figure 14).

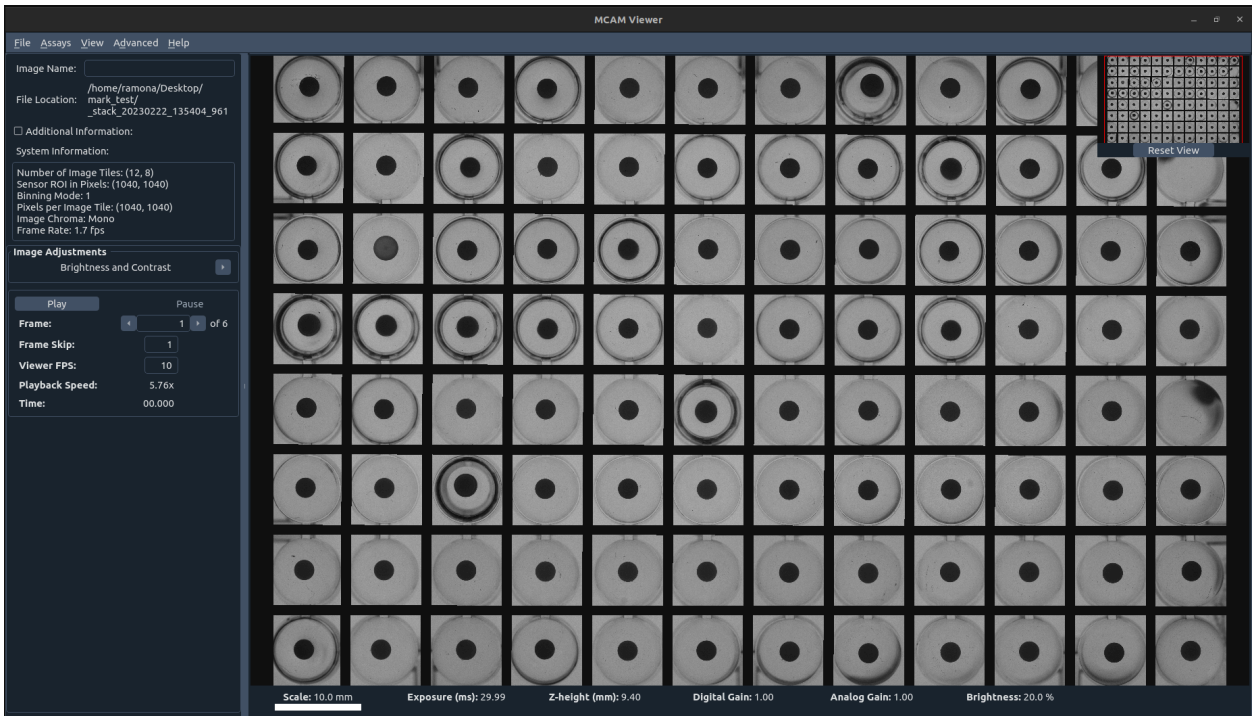


Figure 14: Screenshot of GUI Images

Note: If a filename is entered in the “Image name” field and the workspace is changed, the filename will be deleted and the user will need to enter a new filename.

3.1.3 Acquiring individual well images

If the user requires high resolution images of each well in individual tif files, navigate to the “Assays” menu and select “Acquire and Save Image Assay” (Figure 15). In the “Save Mode” dropdown, select “Export Images as Tif”. Next, the user will need to define the well plate configuration which is largely defined by the number of wells in the plate being imaged. Multiple Well Plate Configurations are preloaded as part of the software and include 6, 24, 48, 96, and 384 well plates. Once a well plate configuration file is selected, the wells in the image will be outlined in blue. Ensure the blue outlines are aligned correctly before acquiring the image using the “Take Image” button.

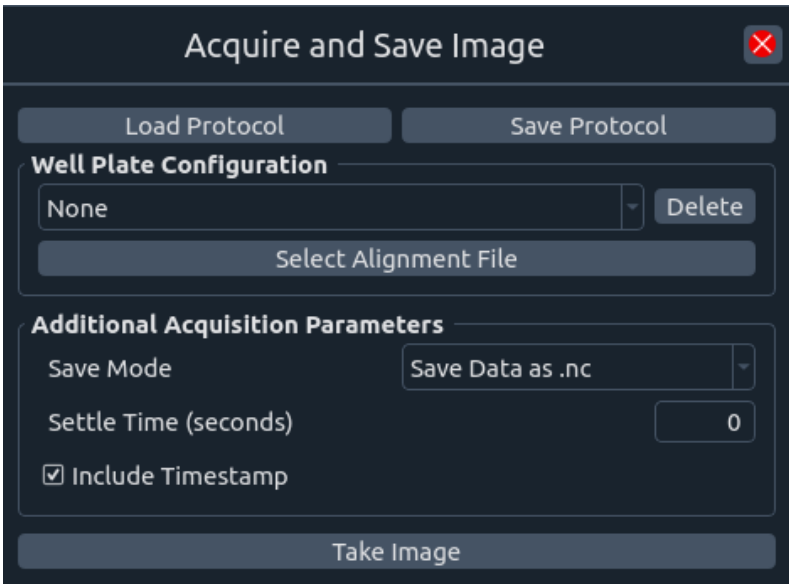


Figure 15: Acquire and Save Image prompt

3.1.4 Defining New Well Plate Configuration

If a new well plate configuration needs to be defined, the user will need to create a new Well Alignment file by navigating to “Assays'' and selecting “Well Alignment”(Figure 16). Next, enter the number of columns and rows present on the current plate, then select “Detect Well Alignment” and the software will generate red outlines for each well across the plate. Generally, the red well outlines will not be aligned completely across the plate. The user will need to manually align the wells in the four corners of the plate by double clicking the dot in the center of the well and dragging the outline into the proper

orientation. Once the wells in the four corners of the plate have been aligned correctly, select “Update Well Alignment” to automatically align the rest of the wells. After confirming the alignment is correct, select “Save Well Alignment” and name the new well alignment file to correspond to the new plate type.

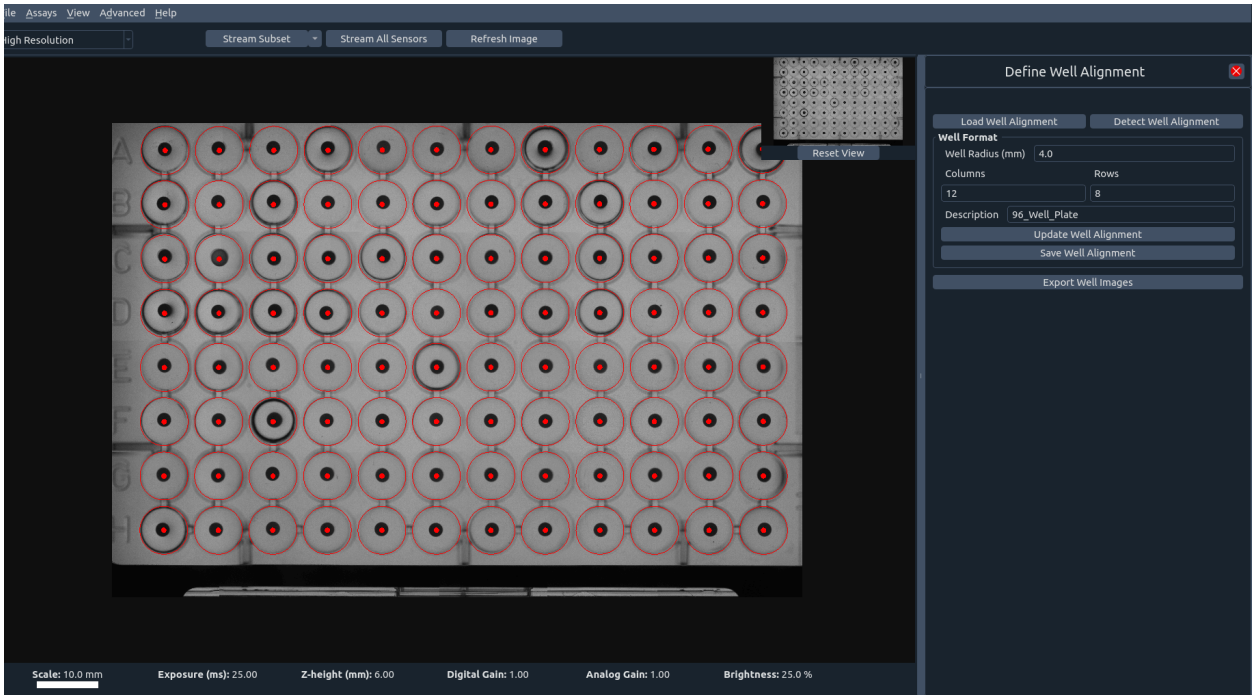


Figure 16: Defining Well Alignment

3.2 Acquire Video or Z-Stack

To acquire a video, select “Assays” from the top toolbar menu and select “Acquire Video”. An “Acquire Video” prompt will appear on the right side of the screen (Figure 17). Click “Select File Location” to designate the location the video file will be saved. The “Additional Information” box is available for any notes the user would like to include about the video. The user also has the option to create a video of the full array to capture the entire plate or a specific subset of wells within the plate. When acquiring videos, the user can adjust the frames per second and acquisition duration in the “Frame Rate Settings” section of the Acquire Video prompt. The MCAM GUI will automatically calculate the total number of frames and estimate the size of the video file and available memory of the system.

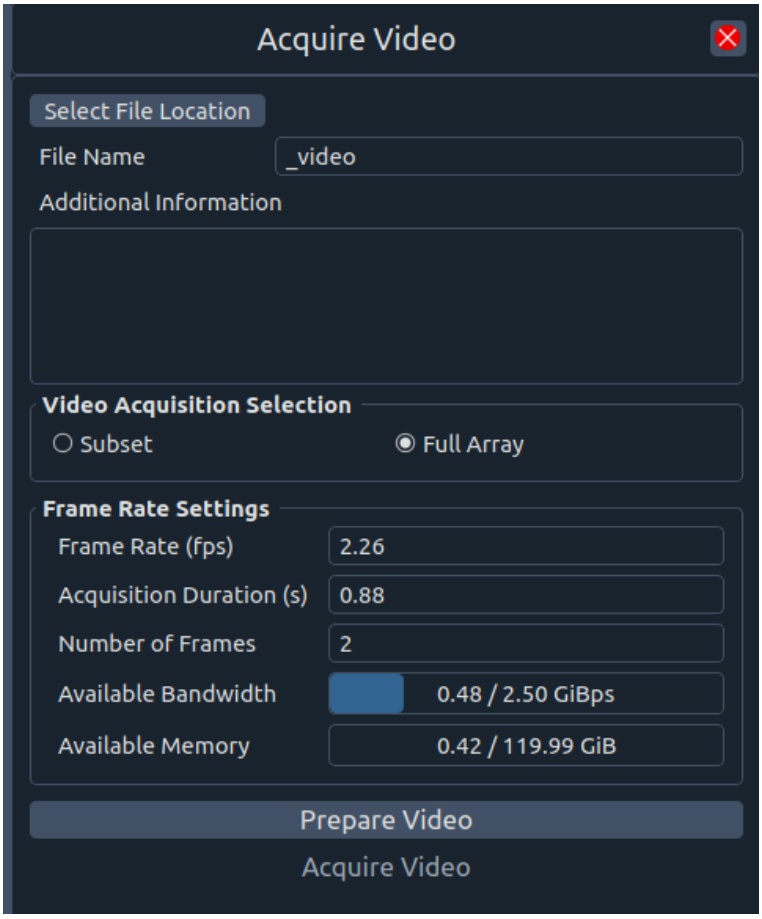


Figure 17: Acquire Video Prompt in Assays menu

To acquire a Z stack, select “Assays” from the top toolbar menu and select “Acquire Z Stack”. An “Acquire Z Stack” prompt will appear on the right side of the screen (Figure 18). If a Z-stack protocol has already been defined and saved select “Load Protocol”. To create a new protocol, select the correct “Well Plate Configuration” file that corresponds to the imaging plate. To set the range of the Z stack, adjust the start height (bottom of the stack) and the end height (top of the stack) in the “Z stage” section. The mid height will be automatically calculated based on the start and end height. The step size indicates the range between each stack that is collected. The number of steps will be automatically calculated based on the start height , end height, and step size supplied by the user. In the “Save Settings” section of the prompt, select the file location and input a folder name based on the naming convention suggested in 3.1. To save the Z-stack protocol for future use, click the “Save Protocol” button in the top right of the prompt.

Figure 18: Acquire Stack Prompt in Assays menu

3.2 MCAM compatibility with Green Button Go automated scheduler

The MCAM-B is compatible with automated workcells and has been integrated into Biosero's automated laboratory scheduling system, Green Button Go. An example workflow in the Green Button Go interface is outlined below (Figure 19). For support with Green Button Go, please contact Biosero.

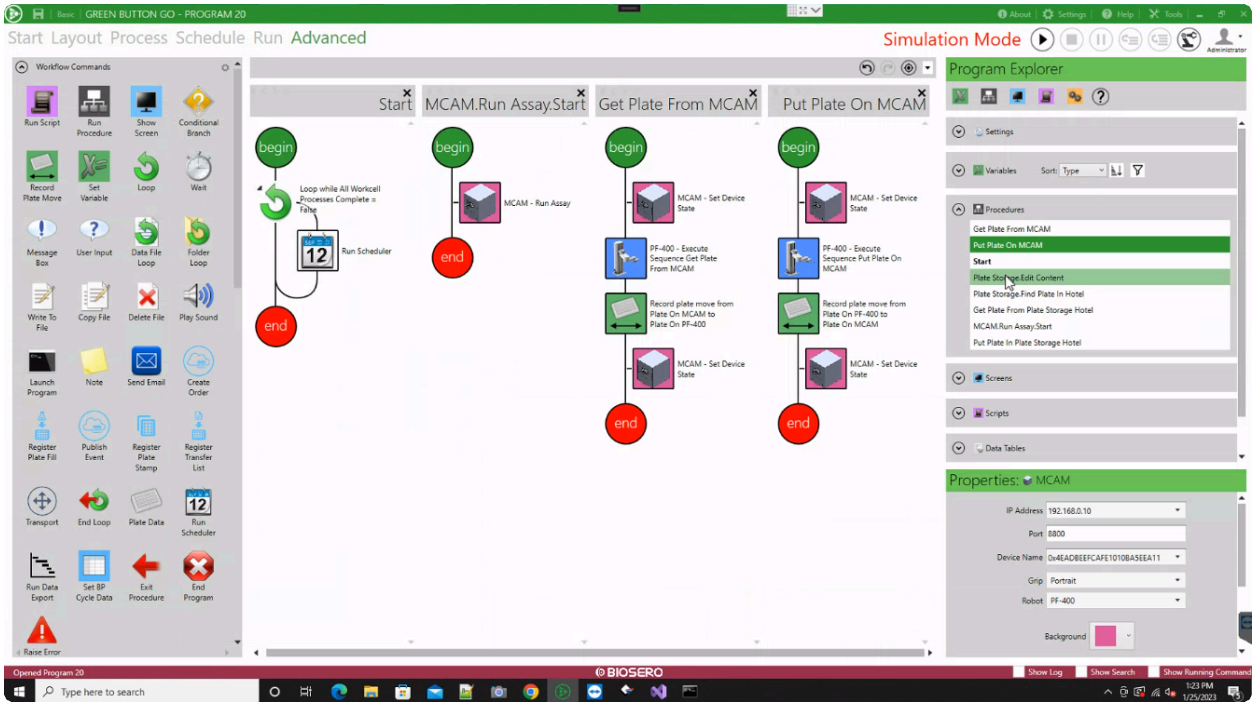


Figure 19. Green Button Go integration with MCAM

3.3 Existing protocols in the MCAM GUI

Below is a summary of the potential plate types that will be used in the assays as well as their physical dimensions and suggested imaging parameters. An assay protocol for each plate type in Table 1 will be preloaded on the MCAM GUI as a starting point. To load an existing protocol select “assays” in the toolbar and click “Acquire Z Stack”. Open “Load Protocols” at the top of the prompt, navigate to the Desktop and open the folder labeled “Protocols.” In that folder, an assay for each plate type in Table 1 will be available along with the corresponding “Well Alignment” files. Alternatively, the user can drag and drop the protocol .json files into the GUI to load the desired protocol. It is suggested before running any of the preset acquisition protocols in the workcell to adjust the imaging settings based on the user’s input material (see section 2). To save any changes made to an existing protocol, simply click “Save Protocol” after optimizing the imaging parameters.

Table 1: Suggested imaging parameters based on plate type

Potential Assay Plates							Suggested Imaging Parameters		
Company	**Catalog Number**	**Wells**	**Shape**	**Walls**	**Material**	**Well dimensions mm (height, top, bottom)**	**Z-stack depth (mm)**	**Number of slices**	**Step size (mm)**
Greiner	655180	96	F	Clear	Polystyrene	10.9 x 6.96 x 6.58	2	5	0.5
Corning	7007	96	U	Clear	Polystyrene	11.3 x 6.86 x 1.80	3	7	.5
Greiner	651161	96	V	Clear	Polystyrene	10.03 x 6.18 x 1.80	3	7	.5
Greiner	655090	96	F	Black	Polystyrene	10.9 x 6.96 x 6.58	2	5	0.5
Corning	4520	96	U	Black	Polystyrene	12.54 x 6.86 x 1.85	2	5	0.5
Corning	4516	384	U	Black	Polystyrene	12.54 x 3.63 x 1.80	2	5	0.5
Perkin Elmer	6057300	384	F	Black	Cyclic Olefin	12.7 x 3.26 x 3.26	1.5	4	0.5
Greiner	657160	6	F	Clear	Polystyrene	16.5 x 35.58 x 35.58	3	7	0.5
Greiner	662160	24	F	Clear	Polystyrene	16.5 x 16.28 x 15.7	2	5	0.5
Greiner	677180	48	F	Clear	Polystyrene	16.5 x 11.95 x 11.37	1	20	0.05

Note - Bottom dimension of flat well plates is the width of the flat surface. Bottom dimension of U and V bottom plate is the depth of curvature

3.3 Quick Guide to creating a new protocol

If a new plate type is being integrated into the workcell, the user will need to create a new protocol. It is recommended to have organoids plated in the new plate type during new protocol optimization to capture the imaging parameters appropriately. To create a new protocol follow the instructions below.

1. Open the MCAM™ GUI as described in section 1.
2. Load the new plate type containing organoids by navigating to the Mechanical State dropdown and select “load acquisition” to eject the stage.
3. Once the plate is positioned securely in the stage, select “Acquisition” in the Mechanical State dropdown.
4. Using the wells containing organoids, adjust the Sensor Settings, LED settings, and any additional Image Adjustments according to section 2 of the manual.

5. If a “Well Alignment” file hasn’t been created for this particular plate type, select “Assays” and “Well Alignment”.
    a. Input the correct number of columns and rows in the “Well Format” section.
    b. Select “Detect Well Alignment” to generate red outlines for each well across the plate.
    c. If the red outlines are not aligned correctly, click and drag the outlines into the proper alignment for the 4 corner wells of the plate and select “Update Well Alignment” when complete. (see 3.1.4 for how to)
    d. If all the red outlines are correctly aligned, “Save Well Alignment” and continue to step 6.
6. Navigate to the “Acquire Z stack” prompt under the “Assays”menu in the toolbar. In the “Z Stage” section, determine the start and end height of the stack as outlined in section 3.2. Determine the desired step size based on experimental design.
    a. Note: Increasing the number of stacks in the acquisition will impact the speed of acquisition. This will need to be optimized based on the desired speed of acquisition.
7. In the “Options” section the user can select either .nc (MCAM Viewer only) or .Tif files as well as setting timing delays if necessary.
8. In the “Savings Settings” select a file location and folder name for the Z Stack images to save in.
9. To save the protocol for future use click “Save Protocol” in the top right of the prompt.
10. The user can now proceed with imaging by selecting “Acquire Z Stack” at the bottom.

4. Image Acquisition Optimization

4.1 Calibration Graphs

The GUI contains interactive graphs to assist with calibrating the system. These can be opened by selecting “Calibration Graphs” under “View” in the toolbar. Both graphs will display data for whichever cameras were most recently being streamed or acquired from. If the full camera array field of view was recently acquired, the graphs will display data for the array. If the subset function was being used, the graphs would only reflect the data from the cameras selected in the subset.

If the graphs are open, they will by default update for each new frame. If this causes the GUI to slow down to an unacceptable level, this can be disabled by deselecting “Update graphs on every frame” under “Advanced”→“Graphing” in the toolbar. If automatic update is deselected, the color

histogram can be updated by acquiring a new full resolution image or by clicking “Update Histogram”, and the focus graph will be updated whenever the stage height changes or a new camera(s) is streamed from.

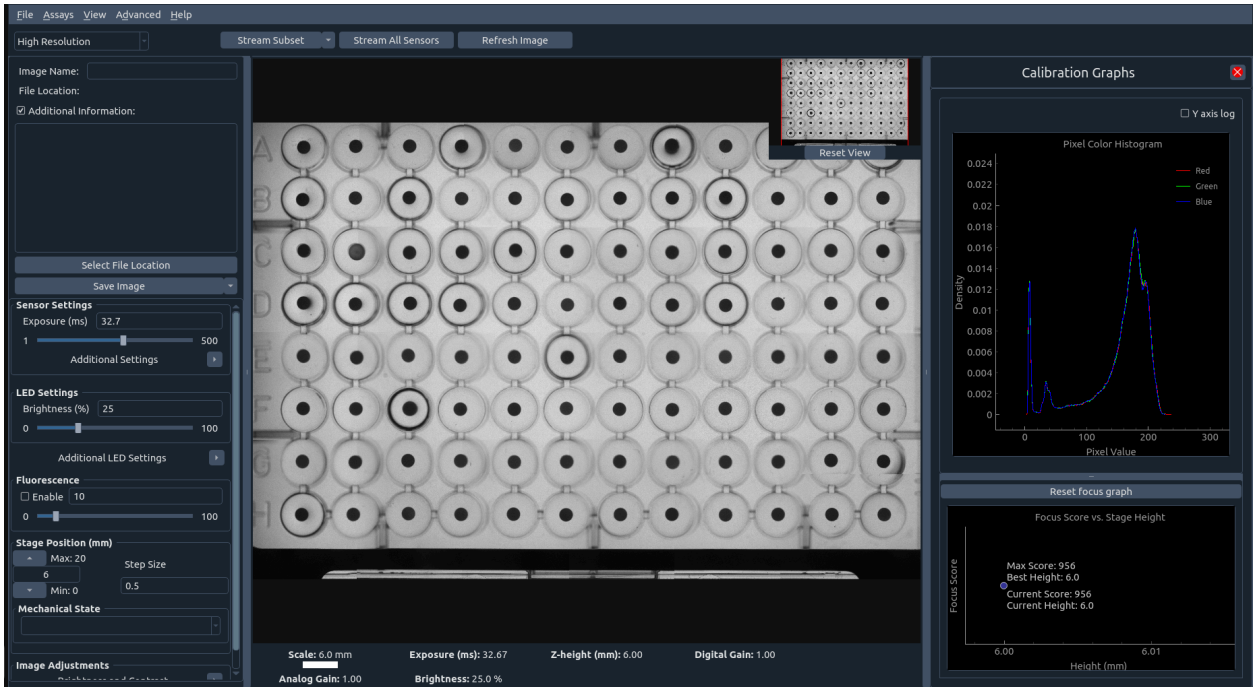


Figure 20: GUI with color histogram and focus graph open.

4.2 Color Histogram

The color histogram displays what percentage of the pixels take on a given value between 0 and 255. This can help the user find the ideal LED and exposure/gain settings to achieve the desired white balance in the acquired images. By default, the histogram displays on a linear scale, but this can be switched to logarithmic by right clicking on any of the plotted lines and selecting “Plot Options” -> “Transform” and selecting “Log X” or “Log Y”. The histogram will always display the color values for the raw data, and will not be affected by changing the displayed contrast with the Max and Min Level sliders.

4.3 Focus Graph

The focus graph (Figure 20, bottom right) displays the calculated focus score for a given camera(s) at different stage heights. The graph is intended to help the user find the stage height which best focuses the current sample. The graph will update as the arrow buttons are used to move the MCAM™

stage up and down. The highest point of the resulting curve is the most focused. Because each camera or set of cameras will have a slightly different focus graph curve, the graph resets each time the streaming mode or camera being streamed from is changed. The focus score is purely qualitative, and should only be used to compare images of the same sample taken under identical lighting and imaging conditions. Altering the exposure, gain, or other system parameters will likely change the focus score and make the graph difficult to interpret. If the system parameters or environment changes, the user should press the “Reset focus graph” button to ensure the data is accurate.

4.4 Advanced Settings

Additional settings can be found in the “Advanced” menu and are described below (Figure 21).

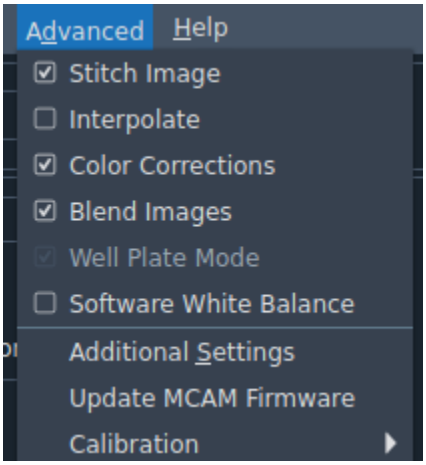


Figure 21: Advanced Menu

4.4.1 Image Stitching

The individual images can be displayed in the GUI as stitched or unstitched. The full image is stitched using a calibration file specific to the individual MCAM™ setup (see System Calibration), which the GUI automatically loads and uses to place the images in the GUI canvas. If the image is un-stitched, the central square from each imaging sensor will be displayed with a small border between them (Figure 22). Whether the image is stitched or unstitched makes no impact on the GUI performance.

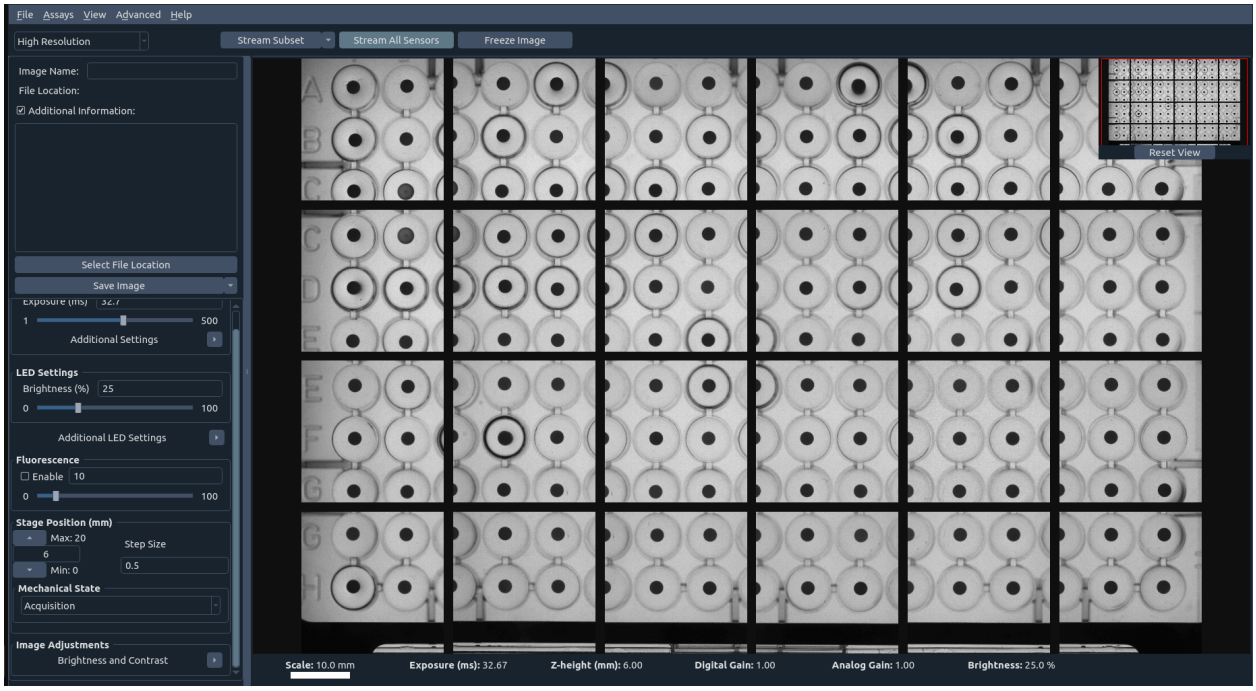


Figure 22: GUI with unstitched image

4.4.2 Select Components

By checking or unchecking respective boxes, the user can activate or deactivate components connected to the MCAM™.

4.4.3 Sensor Corrections

Checking this box applies sensor corrections calculated during system calibration. The default state for this setting is active.

4.4.4 Additional Settings

The additional settings window allows the user to set the minimum and maximum values that the GUI will accept for the exposure and gain settings (Figure 23).

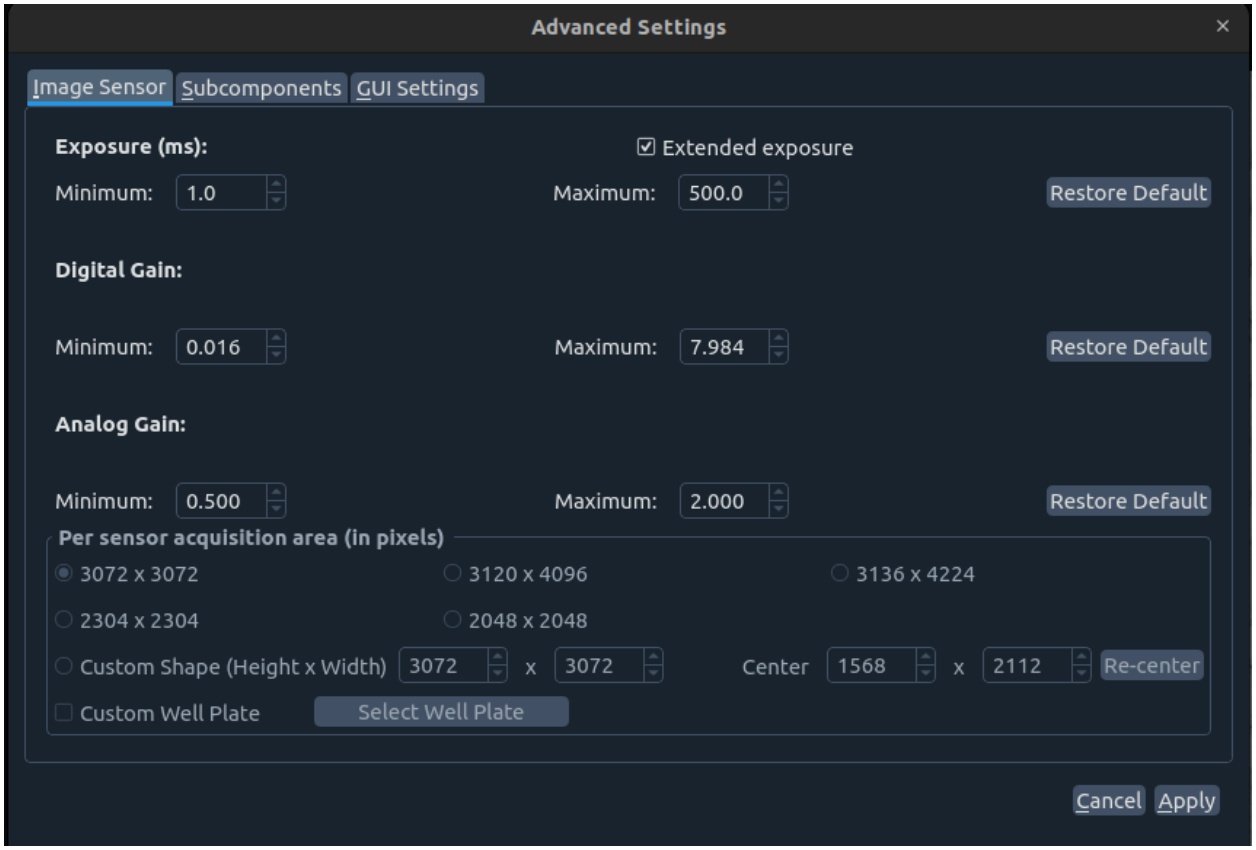


Figure 23: Additional Settings Window.

5. Viewing Data Acquired by the MCAM

The images acquired by the MCAM can be viewed in a variety of ways:

1. **Per-micro camera images**: Data can be exported on a per-micro camera level allowing them to be viewed and analyzed in isolation. A metadata file includes information about the MCAM system that was used to acquire the images in a machine readable format.
2. **Stitched image of the entire FOV**: Data can be exported using the GUI into a single stitched image. This is most appropriate for publication quality images. Unfortunately, many image viewers struggle to load images more than 100 Megapixels in size, limiting the use of this image format.

NC of the original data: The standard NC files that are used throughout the MCAM software retain the original data. This data can be loaded into other applications such as Python and Matlab, but requires users to apply many of the corrections we apply in the MCAM Viewer application. For more information about the dataset, please refer to our documentation found online:

https://docs.ramonaoptics.com/python_metadata.html

5.1 MCAM™ Viewer

The MCAM™ Viewer software allows for viewing images that have been previously acquired and saved using the MCAM™ GUI.

The MCAM™ viewer software is provided as a distributable executable (see MCAM™ Viewer Installation, section 5.2) and can be run using Windows 10 or Linux systems. Compatible file types include ".nc" files which use the netcdf4 data format as well as ".jpg" images. Note: ".jpg" images generated by exporting screenshots from the GUI may also have an associated ".json" file containing relevant metadata which can also open in the viewer.

In the MCAM Viewer, image contrast can be adjusted using the Gamma, Minimum Level and Maximum Level settings in the bottom left corner. Metadata that has been saved with image files is displayed on the left side of the viewer window. Additionally, functionality similar to the MCAM™ GUI is available including the focus graph, color histogram, sensor corrections, stitching, and raw versus rgb display (see section 4 more information).

5.2 MCAM™ Viewer Installer

5.2.1 Windows:

https://drive.google.com/file/d/1S8Mlv0NT-9vwTrm7t2wXOEFrh8jllfWw/view
Before starting with the installation of the software, it is recommended to ensure that any old versions of the software are uninstalled. You may uninstall old versions by Opening the Windows start menu, and searching for "Apps & Features" then selecting "Uninstall MCAM Viewer".

5.2.2 Linux:

https://drive.google.com/file/d/14vqRB6t8UYrO8KlxkQ8juXyVRVMB3Q8-/view
All required software has been installed on the MCAM workstation prior to delivery.

5.2.3 Software Updates:

Contact us at help@ramonaoptics.com if you think your software may be out of date.

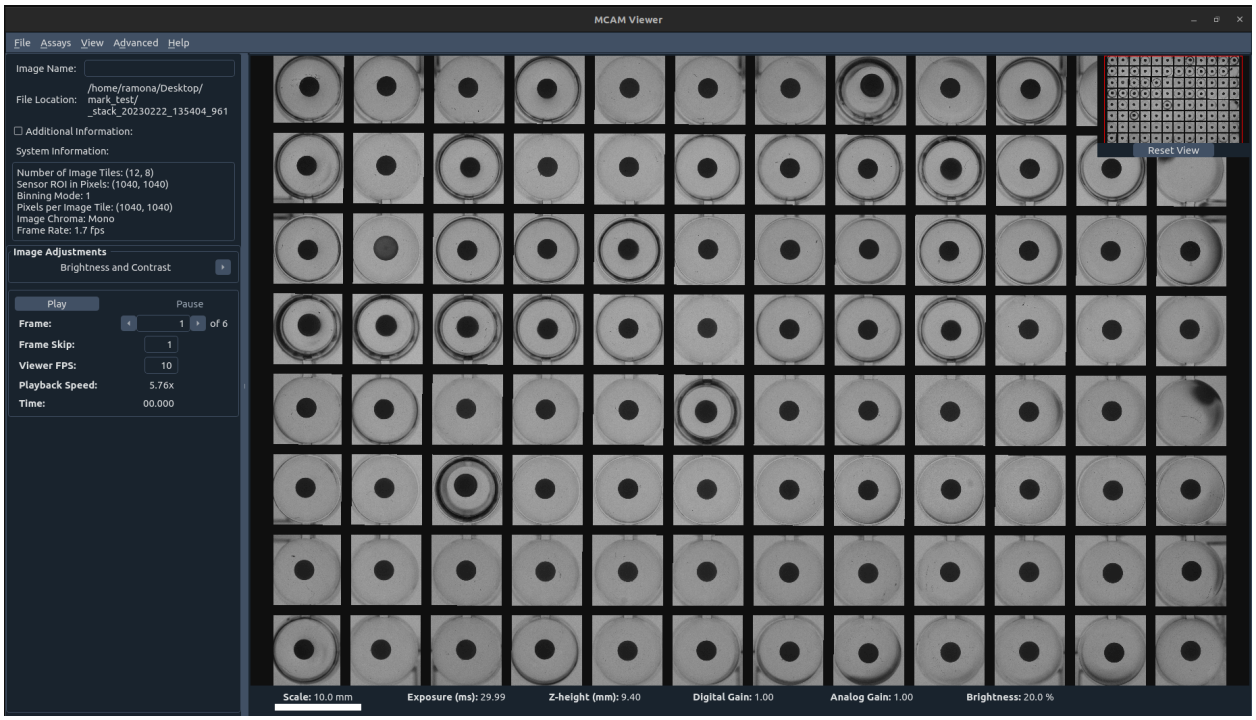
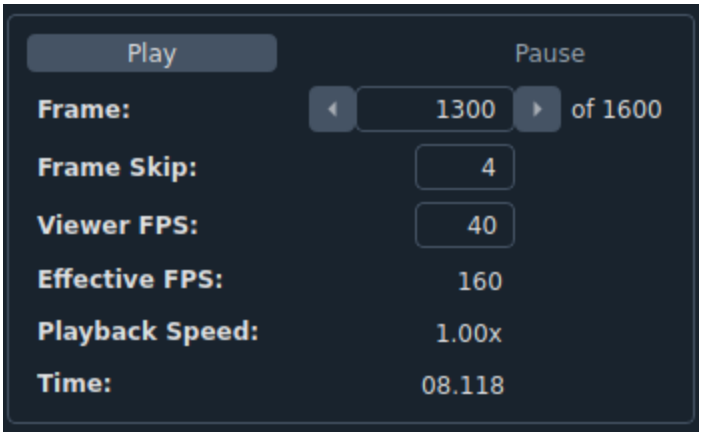


Figure 24: MCAM™ Viewer Window.

5.3 Video Playback

The MCAM™ Viewer allows for video playback with multiple settings affecting playback speed.
Frame Skip allows the user to specify an interval of frames per the player will skip between frames.
Viewer FPS allows the user to request a playback FPS. This is dependent on the amount of data in each frame.
Combining these options allows for a user to reach a higher effective FPS than otherwise possible.



5.4 Stitching Images Through MCAM™ Viewer

These instruction can be supplemented with these slides

1) With your desired single frame dataset loaded into the MCAM™ Viewer navigate to File>Export Image (as stitched).
2) The stitching window will pop up with your image in the viewer.
3) Select the output resolution of your stitched image. The default “Native Resolution” will create a stitched image at the resolution of the raw data included in the field of view (FOV). The maximum size is listed in parentheses.
4) Once resolution is selected, frame the viewer to the FOV you would like your stitched image to cover. You can navigate this viewer the same as in the MCAM™ Viewer and MCAM™ Falcon.
5) To quickly fit the FOV to the full composite press the “Reset View” button.
6) Once the preview window is set, press the “Stitch” button. This will bring up a File Navigator that will allow you to select the filename of the stitched image.
7) Stitching process can be monitored in the textbox at the bottom of the stitching window.

6. Introduction to the MCAM Web API

The MCAM provides a web API for seamless integration into automated workflows. At its core, the MCAM web API tries to approach a REST API while enabling control over the MCAM’s mechanical capabilities.

6.1 System Architecture

The MCAM server computer, that runs the web service for the MCAM acquisition control and image analysis, listens on port 8800 for incoming requests. The system is assumed to be connected to the local network via ethernet. The MCAM and the MCAM server are assumed to always be connected to one other. The controlling scheduling system (e.g. Green Button Go) is assumed to be installed on a different computer, and able to communicate with the MCAM server on the same secure network.

Network Connection

The MCAM is assumed to be connected to the local network via ethernet. At minimum, a 1 Gbps network connection is required, and a 2.5 Gbps connection is recommended. Depending on the demands of the application, a 10 Gbps can be accommodated on the MCAM server.

Security Model

The MCAM assumes that it is placed in a secured facility. The server is configured to listen to any requests that arrive at its IP address. The server can be configured to require certain credentials described below, but these are only advised to prevent accidental requests, and not malicious requests to the MCAM server.

HTTP Basic Authentication

To enable HTTP Basic Authentication, the server must be started with the environment variable `RAMONAOPTICS_WEBMCAM_AUTHENTICATION` set to “httpbasic”. The username and password can be set from the `RAMONAOPTICS_WEBMCAM_USERNAME` `RAMONAOPTICS_WEBMCAM_PASSWORD` environment variables.

Shared File Server Installation

Upon installation, the Ramona Optics will work with the client to mount the shared file server so that it appears as a local folder on the MCAM server. We briefly describe a few known share file configurations that are compatible with Ramona Optics’ MCAM server.

6.2 API Function Calls

There are 8 basic API endpoints:

1. `GET /v1/status`

a. The response contains an entry with information pertaining to the MCAM server application. The main information returned in this endpoint is the version of the server application described in `owl_version`
    i. owl_version: The version of the MCAM server application.

2. `GET /v1/mcam/search`
    a. This call is generally issued once upon instrument startup.
    b. It returns a list of available serial numbers that may be available to clients to open.
3. `GET /v1/mcam/{serial_number}`
    a. Return status information fo the MCAM serial number specified in the request
4. `POST /v1/mcam/{serial_number}`
    a. The MCAM server application claims control over the MCAM instrument.
    b. The MCAM will automatically enter a retracted state when it is open.
    c. This endpoint can take 10-30 seconds to startup and ensures that the motion subsystem of the MCAM is ready to take commands.
    d. This command may fail for the following reasons:
        i. The MCAM is powered off while the MCAM server is powered on
        ii. Another application is connected to the MCAM (e.g. the native application).
5. `DELETE /v1/mcam/{serial_number}`
    a. The MCAM may enter a retracted state when the communication connection is closed.
    b. This call is generally called if the user wishes to use the MCAM instrument with the native graphical application for troubleshooting.
6. `GET /v1/mcam/{serial_number}/state/search`
    a. Provides the list of available states for a particular MCAM.
7. `GET /v1/mcam/{serial_number}/state`
8. `POST /v1/mcam/{serial_number}/state`
    a. Requests that the MCAM enters a particular state.
    b. `"acquisition"` state:
        i. In this state the sample holder (if present) is retracted and the MCAM is ready to acquire images.
        ii. The MCAM will automatically enter this state when the `/run_assay` endpoint is called.
    c. `"loading"` state:
        i. The sample holder is extended into the defined loading position.
    d. `"unloading"` state:
        i. The sample holder is extended into the defined unloading position.
9. `GET /v1/mcam/{serial_number}/assay/search`

    a. Returns a list of strings, each an identifier of the different types of the available assays (or experiments) that can be run on the MCAM.
10. `GET /v1/mcam/{serial_number}/assay/{assay_name}/configuration`
    a. Return a list of strings, each containing a valid configuration of the different available assay configurations.
11. `GET /v1/mcam/{serial_number}/assay/{assay_name}`
    a. Returns the assay schema.
12. `POST /v1/mcam/{serial_number}/assay/{assay_name}`
    a. This is the main function to call to acquire and process data. It is described in detail in subsequent sections.

6.3 API Function Workflow

The API is documented in detail in the staging website. The openapi.json can be downloaded from the staging website. Most requests are HTTP Post requests and take in their parameters through a json request body.

The general workflow can be summarized by:

1. Only a single client application is assumed to be communicating with the MCAM at once.
    a. Initially, no locking mechanism exists to avoid conflicts between multiple clients.
2. The client application (installed a different computer) checks the `GET /v1/mcam/search` endpoint.
    a. If the returned value of serial_numbers is an empty list, then the webserver is not connected to the MCAM instrument (this can occur if a user was trying to access the MCAM with the native application).
    b. If the returned value of the serial_numbers contains one or more strings, it indicates the serial number of the MCAM connected by the server.
3. The client application initiates a connection with the MCAM of its choice through the `POST /v1/mcam/{serial_number}` endpoint
4. The client application then probes:
    a. `GET /v1/mcam/{serial_number}/state/search`
        i. To get a list of the available mechanical states of the particular MCAM.
    b. `GET /v1/mcam/{serial_number}/assay/search`
        i. To get a list of the available assays for the MCAM
5. The client application sets the state of the MCAM to load the sample by issuing a post request to `POST /v1/mcam/{serial_number}/state` with the desired state.

6. The client application issues a call to the `POST /v1/mcam/{serial_number}/assay/{assay_name}` endpoint as described below
    a. After the call to run_assay, the MCAM may be in an arbitrary state. Typically, it will be left in the “acquisition” state, though this can change between assays and the particular assay configuration.
7. The client application sets the state of the MCAM to unload the sample by issuing a call to the `POST /v1/mcam/{serial_number}/state` with the particular state of interest.
8. Repeat steps 5-7 as desired.
9. Once the connection is no longer needed, one can call the endpoint `DELETE /v1/mcam/{serial_number}` to release the resources associated with the MCAM from the server.

The assay endpoint

We focus our attention on the `POST /v1/mcam/{serial_number}/assay/{assay_name}` endpoint.

The endpoint call specifies both the MCAM serial number that will complete the operation, and the assay name that will be executed. The body of the request contains 4 keys:

- save_location: a string containing the location of the output files.
    - Required
    - This path is relative to the mcam server itself
- metadata: a dictionary containing metadata to be added to the acquired dataset.
    - Optional
    - This dictionary can contain multiple key value pairs. All keys must be strings. The values may be strings, floating point numbers, or integers.
- configuration: A string containing preconfigured settings for the particular assay.
    - Optional
    - If not provided the default parameters for the given assay will be used.
        - The default parameters are almost surely not the ones you want to use.
- parameters: A dictionary containing key value pairs to overwrite in the assay parameters specified in the assay_filename.
    - The default key value pairs are those provided by the settings file defined in assay_filename.

For example, they may be specified as json application data. As a cURL request, these are written as

```
curl -X 'POST' \

'http://mcam_ip_address.local:8800/v1/mcam/0x4EADBEEFCAFE1010BA5EDA11
/assay/acquire_and_save \
  -H 'accept: application/json' \
  -H 'Content-Type: application/json' \
  -d '{
  "save_location": "/shared_folder/data/my_file_location",
  "metadata": {
     "barcode": "A329812",
     "operator_name": "John Doe"
  },
  "parameters": {}
}'
```

6.4 Environment Variables used by the MCAM Server

The MCAM server can be configured via the following environment variables to adjust the server settings:

Environment Variable	Usage	Allowed values	Example
RAMONAOPTICS_WEBMCAM_AUTHENTICATION	Specifying the authentication method.	httpbasic or empty (no authentication required)	`httpbasic`
RAMONAOPTICS_WEBMCAM_USERNAME	Username when httpbasic authentication is used.	Any string, spaces are discouraged.	`ramona`
RAMONAOPTICS_WEBMCAM_PASSWORD	Password when httpbasic authentication is used.	Any string, spaces are discouraged.	`gigapixel`
RAMONAOPTICS_WEBMCAM_EXTRA_AVAILABLE_SERIAL_NUMBERS	Specifying additional serial numbers that “appear available” when testing the MCAM.	A list of comma separated strings.	`RAMONAOPTICS_WEBMCAM_EXTRA_AVAILABLE_SERIAL_NUMBERS=0x4EADBEEFCAFE1010BA5EDA11`
RAMONAOPTICS_WEBMCAM_STARTUP_SERIAL_NUMBER	Serial number to which the WebMCAM Server connects to upon startup.	A single serial number (string)	`RAMONAOPTICS_WEBMCAM_STARTUP_SERIAL_NUMBER=0x4EADBEEFCAFE1010BA5EDA11`
RAMONAOPTICS_WEBMCAM_PORT	Network port on which the server lists.	A number specifying the port on which the server listens	`RAMONAOPTICS_WEBMCAM_PORT=8800`
RAMONAOPTICS_WEBMCAM_SSL_KEYFILE	SSL keyfile used for https communication.	A valid local path.	`RAMONAOPTICS_WEBMCAM_SSL_KEYFILE=/home/ramona/cert/webmcamdemo.ramonalabs.com+3-key.pem`
RAMONAOPTICS_WEBMCAM_SSL_CERTFILE	SSL certfile used for https communication.	A valid local path.	`RAMONAOPTICS_WEBMCAM_SSL_CERTFILE=/home/ramona/cert/webmcamdemo.ramonalabs.com+3.pem`

7. MCAM Server Setup

7.1 Remote Access Via SSH

Ensure that the MCAM and MCAM server are powered on, and connected to the network.

a. The MCAM computer hosts an SSH server.
b. If no SSH key is setup on the machine, a local login can be used to install the

```
cat id_rsa.pub >> ~./ssh/authorized_keys
```

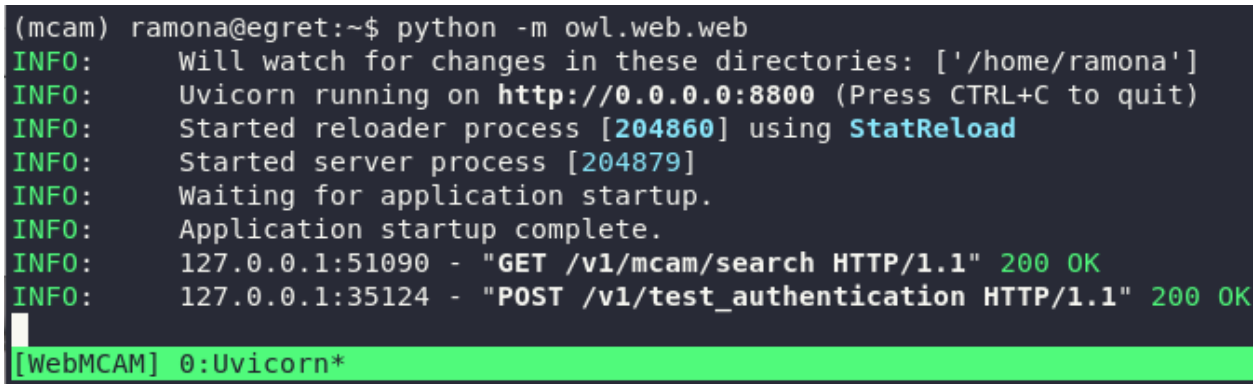
If working behind a router or firewall, you will have to set up the correct firewall rules and port forwarding rules to the connection through.

7.2 Troubleshooting Firewall Issues

The MCAM computer will attempt to launch the web server in a tmux session on bootup. However, access from another computer may be blocked due to network security. To verify that the MCAM server is correctly started, use a terminal on the MCAM server to connect to the tmux session. You can connect to the tmux session through the command:

tmux a

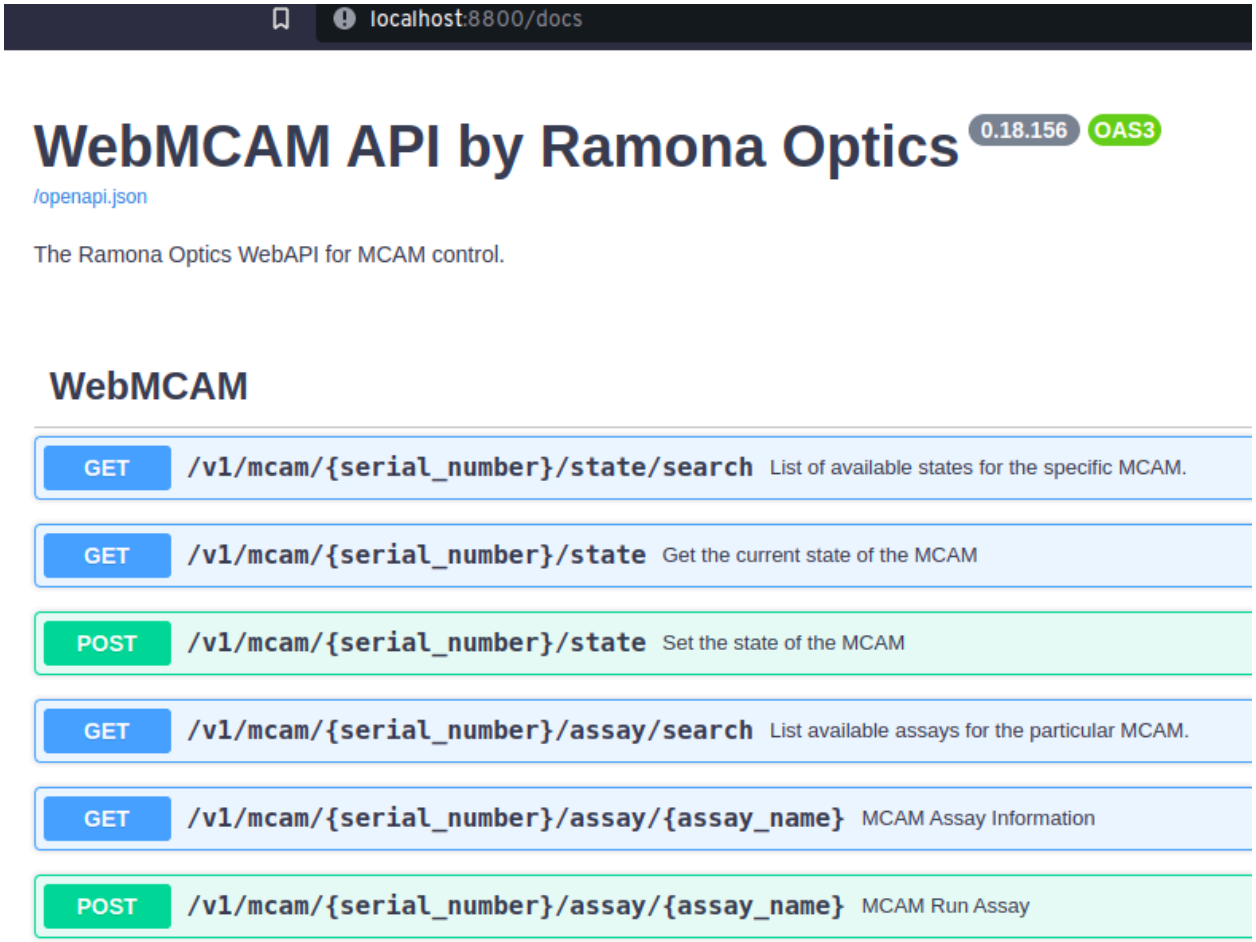
and you will have access to the running web server.



From the MCAM computer, you can check that the server is running by connecting to the website through the installed web browser (Firefox or Google Chrome):

https://localhost:8800/docs

Verify that the screen looks similar to the above image of the correctly running web server.



Verify you can connect to the documentation page in a web browser by navigating to the ip address and the port shown in the tmux session.

Once you have verified that the server is correctly configured on the MCAM computer, you should disconnect from the tmux session:

Ctrl+b, then d

To list the IP addresses of the MCAM computer, you can use the command

ip a

The IP addresses will be listed next to network each interface. The IP address will often start with 10.X.X.X where each X represents a number between 0 and 255 (inclusively). Once the IP

address of the MCAM server has been identified, you can attempt to connect to the MCAM server on your workstation through the address:

https://10.X.X.X:8800/docs

If you are unable to connect to the WebMCAM webpage, a temporary solution to get around a problematic firewall is to use a WiFi hotspot on the MCAM computer and connect to it directly. This should allow direct communication without any firewall interference. If you are resorting to this solution, you should contact your network administrator to ensure that a long term solution can be found to connect to the MCAM server through a wired ethernet connection.

7.3 Windows SAMBA Setup

If a Windows SAMBA is required Ramona Optics will install the required cifs utilities on the MCAM server. Users may work with the Ramona Optics engineering team to ensure that the shared drive configuration is correctly added so that the shared drives are permanently mounted.

If a Windows SAMBA is required Ramona Optics will install the required cifs utilities on the MCAM server. Users may work with the Ramona Optics engineering team to ensure that the shared drive configuration is correctly added so that the shared drives are permanently mounted. A sample configuration may resemble:

```
sudo apt install cifs-utils
# Create the credentials file
echo username=myusername  > /home/ramona/.smbcredentials
echo password=mypassword >> /home/ramona/.smbcredentials
# echo domain=domain     >> /home/ramona/.smbcredentials
# Add the line to /etc/fstab
//localhost/sambashare  /shared  cifs
credentials=/home/ramona/.smbcredentials,file_mode=0755,dir_mode=0755,user=ramona,group=ramona,nobootwait
0       0
sudo mount -a
# Ensure that the drive is visible
sudo reboot
# Ensure that the drive is still visible
```

7.4 Staging Website

https://webmcamdemo.ramonalabs.com:8800/docs/

Gaining access to the virtual MCAM shell

If you need to gain access to a virtual MCAM bash shell, please contact Ramona Optics by email and provide them your public key for SSH at help@ramonaoptics.com . We will provide you information about the login IP and the port.

7.5 Customization of Assays

The MCAM API allows for a large amount of customization based on the required experiments. All experimental parameters are exposed to the end user based on key-value pairs stored in a json dictionary. Many of the parameters are described in the endpoint `GET /v1/mcam/{serial_number}/assay/{assay_name}` . Please contact Ramona Optics for more information about assay parameters.

7.6 Known Limitations

1. Long running assay may cause hangups with blocking http requests.
    a. Currently assays are expected to run between 1-second and 60-seconds.
    b. However, longer assays may run up to 300-600 seconds (5 minutes) which may cause TCP requests to drop.
2. Multiple client requests can conflict.
    a. Currently there is no locking mechanism stopping multiple clients from issuing calls.
    b. All API calls are handled sequentially meaning that once an assay is started, it cannot be polled.
3. Assay parameters cannot be easily validated.
    - An endpoint that can validate the choice of parameters for a given assay without executing it.
    - In future versions of the MCAM server, we plan to create validation endpoints that simply validate, without executing the assay.
4. The software of the MCAM Server cannot be updated through a web API call.
5. Mounting network attached storage cannot be accomplished through a web API call.