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AVA™ Emulation System

User Guide

EP-238 Rev B

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Introduction

The AVA™ Emulation System is our groundbreaking "*Ad Vivo* Architect," bridging the translation gap by merging the throughput of *in vitro* studies with the fidelity of *in vivo* models to create more human-relevant biology at scale. By supporting up to 96 individual Organ-Chip samples ("Emulations") per experiment, AVA delivers high-powered insights that enable faster, more confident decision-making in drug development.

As a 3-in-1 instrument that combines a high-throughput Organ-Chip culture module with a self-contained incubator and an automated microscope, AVA seamlessly creates the precise conditions needed for simultaneous cell culture of up to 96 Emulations in a single experiment. By controlling dynamic media flow, temperature, gas concentration, and relative humidity, AVA creates an Organ-Chip microenvironment that mirrors what cells experience *in vivo*.

This user guide has essential instructions for how to safely and effectively operate AVA. Please ensure all users thoroughly read and understand this guide before operation.

The AVA Emulation System is intended for research use only (RUO).

Manufacturer	Emulate, Inc. 27 Drydock Avenue Boston, MA 02210
Sales & Support	**Sales:** orders@emulatebio.com **Support:** support@emulatebio.com
Intended Use	The AVA Emulation System is intended for Research Use Only (RUO).

Specifications

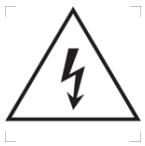
General	• **Model Name:** AVA™ Emulation System • **Weight:** 140 lbs. • **Maximum Power Consumption:** 360 W • **Dimensions (W x H x D):** 30" x 17.5" x 22"
Operating Requirements	• **Instrument Rating:** 24 VDC • **Gas Input Pressure:** 68.9–137.9 kPa (10–20 PSIG) from fixed source • **Gas Input Composition:** 100% CO_2 • **Vacuum Input Pressure:** -70 kPa (-10.2 PSIG)
Laboratory Environment	• **Operating Temperature:** 15–30°C (59–86°F) • **Relative Humidity:** 30–80% (non-condensing) • **Max Altitude:** 2,000 meters (6,562 feet)
Flow	• **Flow Range:** 0 µL/h, or 10–2,000 µL/h +/- 10%
Microscopy	• **Modalities:** Phase Contrast, 3-Channel Fluorescence (DAPI, FITC, Texas Red) • **Objective:** 10X • **Digital Zoom:** 100–200%
AVA Workstation Specifications (minimum)	• **Operating System:** Windows 10 or 11 (64-bit) • **System Storage:** 4 TB (SSD recommended) • **System Memory:** 16 GB • **System Processor:** Intel 8th Gen, Intel Core Ultra • **Network:** 10 / 100 / 1000 BASE-T Ethernet • **Wireless:** 802.11 g/n dual-band wireless • **External Monitor Resolution:** 1920 x 1080

Important Safety Notices

This section includes recommended precautions when handling and using the AVA Emulation System. The information here describes how users can minimize the chance of harming themselves or the module during operation.

Caution Statements

	It is essential that all instructions are followed, and all warnings are understood. Failure to adhere to the instructions or operate the equipment within the stated specifications may cause injury, damage to the instrument, or issues with experiment performance.
	Do not dispose of AVA or any AVA components with household waste. Disposal of AVA and AVA components requires special handling by an appropriate collection facility for recycling and recovery of electrical and electronic equipment.
	AVA is not sterile out of the box. After AVA is unpacked and securely placed on your lab bench, clean thoroughly with 70% pre-moistened ethanol wipes to decrease the risk of contamination.
	Internal heating surfaces are in close proximity to the water pan; please take precaution while refilling the water pan, preparing for a decontamination cycle, or sanitizing the recommended accessible internal surfaces.
	Vent and disconnect all Gas Supply Lines before any planned AVA decontamination, sanitization, or servicing (by Emulate-certified personnel).
	The AVA Emulation System weighs approximately 140 lbs. Attempting to lift or move the system without proper assistance or lifting equipment can result in serious personal injury, equipment damage, and may void the warranty. Please contact Emulate Support if you need to relocate your AVA from the original installation location.
	Ensure AVA is placed on a level, sturdy surface that can support the total weight of AVA as well as other equipment on your bench.
	Never insert fingers or foreign objects into the bays or other moving parts. Injury or instrument damage may result.

	Always follow in-house safety protocols before handling compressed gas.
	Users are responsible for the proper disposal of any single-use components that encounter biological material.
	AVA installation must be completed by Emulate-certified personnel only.
	Service and maintenance for AVA must be performed by Emulate-certified personnel. Never attempt to disassemble or service AVA.
	Please be considerate of the required laboratory environment conditions for AVA. Do not attempt to operate AVA in an environment that exceeds the conditions outlined in this document.
	To power AVA, use only the 24V power supply and cord provided by Emulate. Emulate provides a country-specific power cord. Use of alternative power supplies or cords may cause damage to AVA and void the warranty.

Regulatory Compliance

The AVA Emulation System complies with applicable electrical safety, electromagnetic compatibility (EMC), and environmental regulations for the regions in which it is marketed.

This product is intended for use in a basic electromagnetic environment as defined by IEC 61326-1, such as research laboratories, educational facilities, light industrial settings, and clinical research spaces where electromagnetic disturbances are moderate and controlled.

Certified by TÜV Rheinland (cTUVus mark) to the requirements of IEC 61010-1 and IEC 61010-2-010 for laboratory equipment. For detailed standards and certifications, refer to the Declaration of Conformity available upon request.

This equipment has been tested to the requirements of IEC 61326-1 / EN 61326-1 and the following regional standards:

- EU Directive 2014/30/EU
- FCC Part 15 Subpart B (Class A) – USA
- ICES-003 Issue 7 (Class A) – Canada

This Class A digital apparatus complies with Innovation, Science and Economic Development Canada (ISED) Interference-Causing Equipment Standard ICES-003, Issue 7. Operation of this equipment in a residential environment may cause radio interference, in which case the user may be required to take adequate measures.

Cet appareil numérique de la classe A est conforme à la norme NMB-003 du Canada. L'exploitation de cet appareil dans un environnement résidentiel est susceptible de causer des interférences radio, auquel cas l'utilisateur peut être tenu de prendre des mesures appropriées.

This product complies with the RoHS Directive 2011/65/EU, as amended by Directive (EU) 2015/863, and corresponding regional regulations.

This product contains lead (CAS 7439-92-1) in certain brass components at concentrations greater than 0.1 % w/w. In addition, the AVA Emulation System contains an internal system-on-chip component that contains approximately 0.0010 grams of lead. Lead is included on the European Chemicals Agency (ECHA) Candidate List of Substances of Very High Concern (SVHC). This information is provided in accordance with Article 33 of Regulation (EC) No 1907/2006 (REACH). The use of lead is compliant under RoHS Annex III Exemptions 7(a) and 7(c)-I. As this use is within the scope of the applicable exemptions, it does not affect the overall compliance status of the product.

Additional details, including the full REACH and RoHS Declaration of Conformity, are available upon request.

Limit of Liability

Emulate, Inc. has prepared this guide to the best of their ability; however, no representation or warranty is made regarding the accuracy or completeness of the information provided. All content is subject to change without notice and does not constitute a commitment by Emulate of any of its affiliates. The information, opinions, and strategies described may not be applicable or suitable for all users, and no guarantee is made that the use of this information will achieve any particular outcome.

The software described in this guide is provided under a license or non-disclosure agreement and may be used or copied only in accordance with the terms of that agreement. Copying or reproduction of the software in any form, except as expressly permitted by the applicable agreement, is prohibited.

AVA Site Preparation Checklist

Please refer to the AVA Emulation System Site Preparation Guide for more comprehensive information. Contact Emulate support (support@emulatebio.com) if any items are missing, defective, or you have additional questions.

Inventory Checklist (from Emulate)

Ensure all accessory components are present and undamaged:

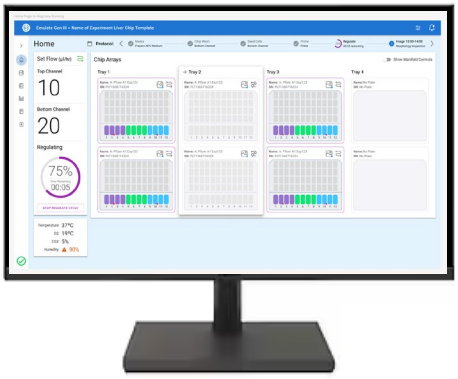
- ☐ AVA™ Emulation System & Power Supply
- ☐ Orb-HM1® & Power Supply (refer to Orb-HM1 User Guide for more information)
- ☐ Orb-AVA Gas Hub Panel
- ☐ Ethernet Cable
- ☐ USB-C-to-Ethernet Cable Adapter

Laboratory Setup

Please ensure the following materials are available and ready to use in your lab:

- ☐ The AVA installation site / lab bench can support the weight and dimensions of AVA and the Orb
- ☐ Three-pronged wall outlet with suitable power is nearby the desired AVA / Orb installation location
- ☐ 100% CO2 gas line can supply 15 PSIG with accessible shut-off
- ☐ CO2 gas outlet has ¼ inch tubing quick connects
- ☐ A vacuum pump capable of delivering -70 kPa
- ☐ Uninterruptible Power Supply (UPS) with battery back-up of at least 900-Watt power capacity (Emulate's internal labs use APC UPS Back-UPS Pro 1500 VA, 900W battery backup). Ensure both AVA and the Orb are connected to the UPS.
- ☐ Workstation PC (laptop or desktop) meets the minimum AVA specifications and is configured by your IT department for use on the scheduled AVA installation day.
- ☐ Accessories for your AVA workstation PC – widescreen monitor, keyboard, mouse, Microsoft Office, and any other items.
- ☐ AVA software is downloaded & installed on your AVA workstation PC

AVA Emulation System Component Overview

AVA Emulation System: A self-contained benchtop Organ-Chip culture module with media flow, incubation, and imaging capabilities operated through UI display screen.	
Chip-Array Consumable: Organ-Chip consumable compatible with AVA. Enables users to create 12 Organ-Chip Emulations per Chip-Array. One AVA can hold up to 8 Chip-Arrays, enabling up to 96 Emulations per run.	
AVA Software & Workstation: The primary interface for AVA experiment planning, instrument control and data management. A dedicated workstation computer must be connected to AVA at all times. *Emulate does not provide a workstation with the AVA Emulation System.* It is recommended to enable internet access on this AVA workstation. Please ensure the workstation supplied for use with AVA aligns with the specifications listed below. Support from your IT team may be required.	

Chip-Array Overview

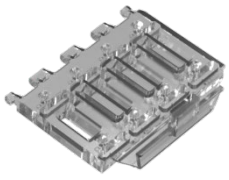
The Chip-Array is an advanced Organ-Chip consumable that integrates 12 fluidically independent, parallel Organ-Chip "Emulations" into a single, high-throughput consumable that is the same footprint of a standard microplate, complying with ANSI/SLAS Microplate Standards. The Chip-Array is made of low-drug-absorbing materials, ensuring precision in biological modeling as well as providing versatility and scale for experimentation.

The Chip-Array is a critical component of the AVA Emulation System, and there are different engagements with Chip-Array in the AVA software that you must familiarize yourself with prior to running any experiments on AVA.

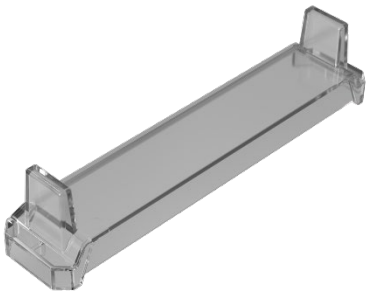
Chip-Array Components & Functions

Chip-Array Components: Each Chip-Array is comprised of several components: the Chip-Array itself (including integrated microfluidic reservoirs), three (3) Pipette Guides, a Pipette Guide Cover, and three (3) Flow Guides.

- Microfluidic Reservoirs: Each Emulation within Chip-Array features independent media reservoirs for the inlets and outlets of both the top and bottom channels. The reservoirs can store up to 1.3 mL of media for each microfluidic channel, enabling automated media flow for up to 3 days when connected to AVA. Media effluent from the outlet reservoirs can be easily collected using a multi-channel pipette or automated liquid handler for downstream analysis.
- Pipette Guide: Enable application of ECM, seeding cells directly into the top and bottom channels.
- Pipette Guide Cover: Minimizes risk of contamination when transferring the Chip-Array(s) between your biosafety cabinet and AVA.
- Flow Guide: Installed after cell seeding steps are complete by removing pipette guides and replacing with flow guides, these components complete the microfluidic circuit between the Emulations on the Chip-Array and the Chip-Array reservoirs, enabling flow operations and keeping your biology viable. Once installed, flow Guides will remain on Chip-Array(s) for the duration of your experiment.



Pipette Guide



Pipette Guide Cover

Flow Guide

Chip-Array Dimensions

Chip-Array dimensions can be found in the below table and diagram:

Specification	Top Channel	Bottom Channel
Dimensions *(width x height)*	1,000 µm x 1,050 µm	1,100 µm x 100 µm
Total Culture Area	12.8 mm^2	18.4 mm^2
Co-culture Surface Area	12.8 mm^2	12.8 mm^2
Volume*	19.7 µL	10.1 µL
Imaging Distance**	172 µm	150 µm
Membrane Thickness	20 µm	
Pore Size	3 µm	

** Volume includes the monoculture region of the channel (if applicable) and the seeding ports.*
*** Imaging distance represents the distance from the bottom of the chip to the membrane surface.*

Chip-Array Specifications & General Applications

Specification	Details
Compatible Cell Types	**The open nature of the Emulate Chip-Array consumable makes it compatible with practically any type of human or animal cell, including:** • Primary cells • Dissociated organoids • Induced pluripotent stem cells (iPSCs) • Immortalized cell lines Note: Due to the rigid nature of the consumable, Chip-Array is not recommended for organ models that require stretch.
Characterization Endpoints	**Image analysis** • Live imaging under flow via AVA: phase contrast and fluorescence microscopy • Fixed imaging: brightfield, phase contrast, and fluorescence microscopy **Omics analysis** • Transcriptomics, proteomics, and metabolomics **Effluent analysis** • Cytokine release, injury markers, barrier function (P_{app}), etc.
Storage Conditions	Ambient temperature (15-25°C)
Shelf Life	1 year from date of manufacture
Sterility	All consumables – including Chip-Arrays, pipette guides, pipette guide covers, and flow guides – are sterilized prior to shipment.

Chip-Array Ordering Info

Product Name	Kit Contents	Catalog Number
Chip-Array Basic Research Kit (8-pack)	**8 sets** of Chip-Arrays to support up to **96 Emulations** on AVA.	**BRK-CA-96**
Chip-Array Basic Research Kit (16-pack)	**16 sets** of Chip-Arrays to support up to **192 Emulations** on AVA.	**BRK-CA-192**

AVA Software Overview

The AVA Emulation System comes pre-installed with AVA Embedded Software, which provides limited control & operation of AVA. The AVA Emulation System requires a dedicated workstation (computer) with **AVA Workstation Software** installed, which is the primary interface for AVA that enables control, operation, experiment design, execution, review, and data storage. The workstation specifications can be found here; AVA software can be installed on any computer meeting these specifications. AVA software can also be used on a stand-alone workstation (installed on a computer with no AVA Emulation System connection) for experiment design and analysis.

There can be only one active connection between AVA software and the AVA Emulation System. While you are running an experiment, do not disconnect the workstation; this can result in experiment disruption and possible data loss. Emulate encourages performing operating system & other workstation updates manually or following a schedule such that active experiments are not disrupted. Automatic OS updates may require, or automatically initiate, a restart of the connected workstation. It is strongly encouraged to disable automatic updates (e.g., Windows Updates) to avoid unintended workstation shut down or reboots during an AVA experiment. While it is unlikely, there is a risk of data loss if the workstation restarts during an experiment.

Network Architecture

The AVA Workstation Software and AVA Embedded Software operate entirely within the local system environment and do not initiate communication with external networks, servers, cloud services, or internet-based resources, **unless allowed by the user.** All data generated by the system is stored locally and may be exported by the user at their discretion. Because the connected computing environment is provided and managed by the customer, responsibility for implementing and maintaining cybersecurity controls—including network security, access controls, and data protection— is the responsibility of the end user.

Data Types

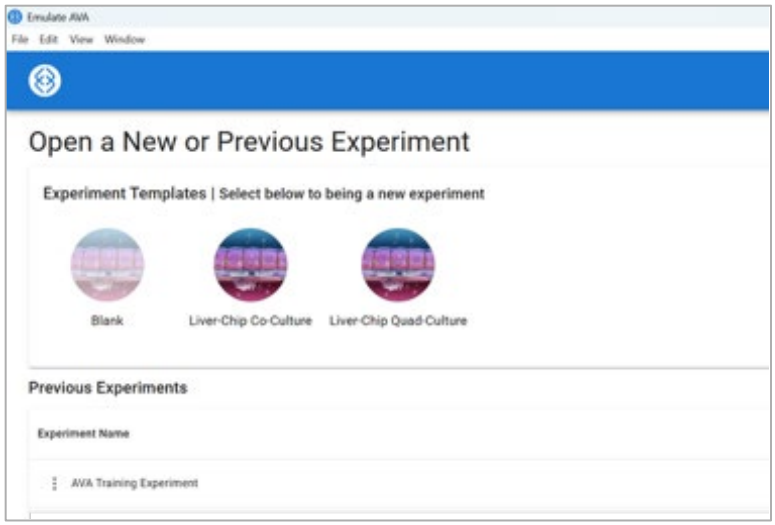
There are several types of data generated by the AVA Emulation System while running an Organ-Chip experiment, such as images (TIFF files), system logs (Excel .xlsx or Text .txt file formats) and other performance data (e.g., environmental sensor values) as Excel (.xlsx) and Text (.txt) formats. After completing an AVA experiment, you can extract the entire experiment data set as a compressed file (.zip).

Data Management

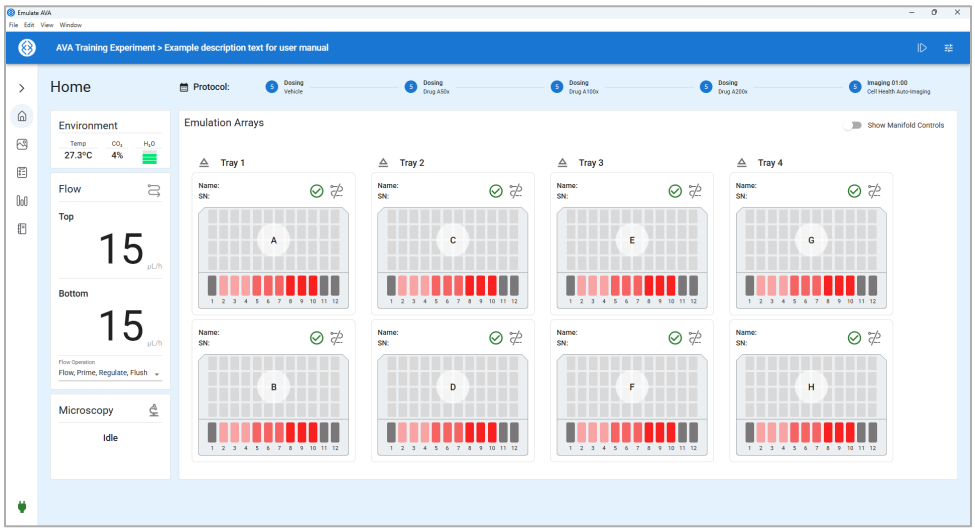
Experiment data and system data is stored on the workstation connected to the AVA Emulation System by default. While an AVA experiment is running, data is stored locally on the AVA internal storage, then automatically transferred to the connected workstation for storage at predefined intervals.

AVA Software Quick Start Guide

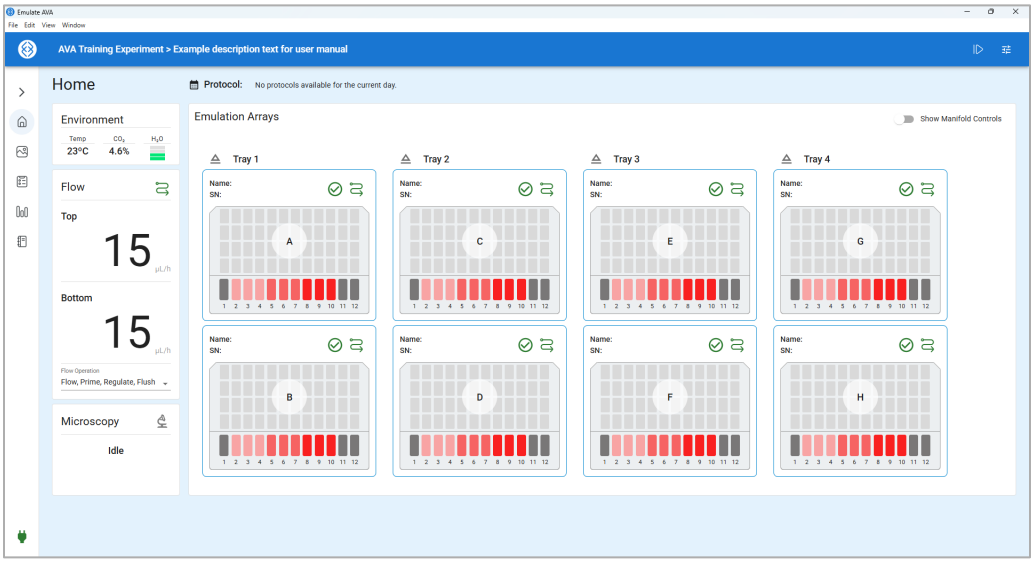
1. **Launch AVA Software:** Double-click the AVA software icon to launch AVA software. If your workstation is connected to an AVA, the software may take 1-2 minutes to open.
2. **Choose / customize an AVA experiment template:** An experiment template contains the necessary information to perform an AVA experiment. Choose from an Emulate-provided template (e.g. "AVA Liver-Chip Array Quad Culture experiment template") or create your own template from the Blank template. You can modify details of your experiment at any point during the experiment.



3. **Start your AVA experiment:** When ready to start your AVA experiment, click **Start Experiment** from to the Conditions view (the first view in experiment design). The AVA Experiment Home view will be displayed:



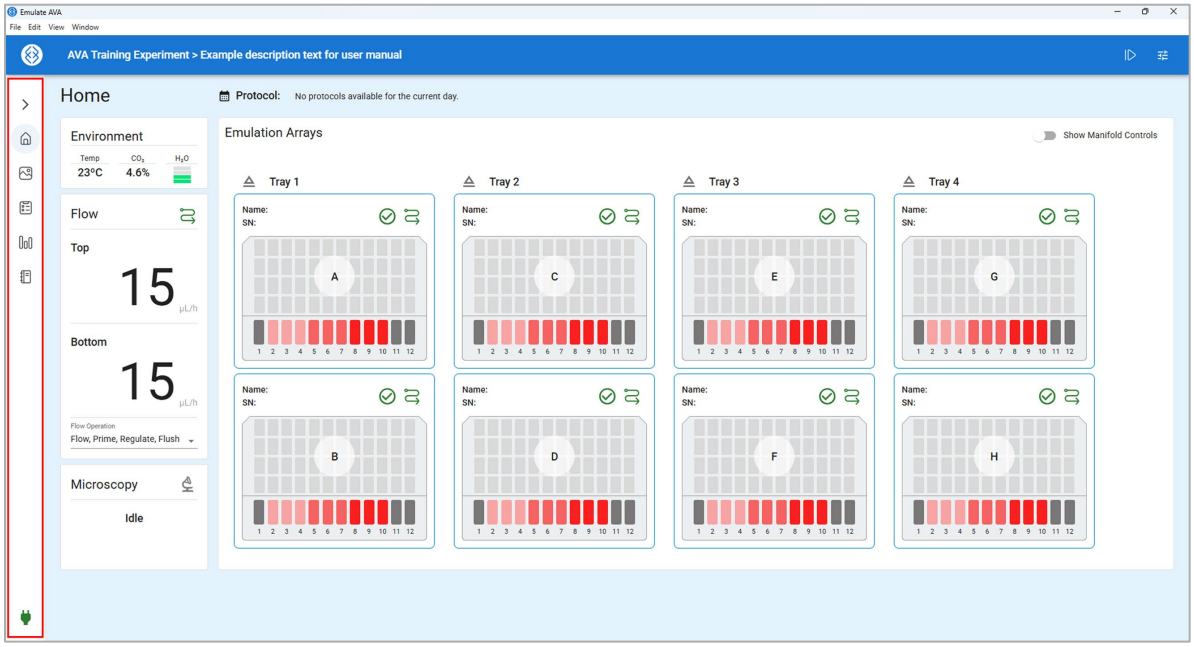
4. **Experiment Runtime & AVA Experiment Home:** The AVA Experiment Home view is the main software view seen during a running experiment. From the AVA Experiment Home view, you can see current environmental conditions (p. 38), view / edit flow settings (p. 39), view Chip-Arrays in the AVA (p. 45), access live imaging or previously generated image sets (p. 51), and more.



Frequently used functions on the AVA Experiment Home include:

- **Environment widget:** The environment widget, located in the upper left corner of the AVA Experiment Home view, displays the current AVA temperature, CO2 concentration, and level of water in the water pan. To review historical data for each of these environmental sensors, click the title (text) of the desired parameter. To edit environment alarm tolerances or disable / enable environment alarms, click on the environment widget itself.
- **Flow widget:** The flow widget, located below the environment widget, displays the current flow status for the top and bottom channels of the Chip-Arrays in the AVA. If flow is actively running, there will be a green serpentine arrow in the upper-right corner of the flow widget. If flow is not actively running, this icon will appear red. The Flow Operation drop down menu below the flow rates provide options to edit currently running flow rates for the top and bottom channels, run specific flow operations like the Regulate Cycle, and more.
- **Chip-Array widgets:** The Chip-Array widgets, featured in the center of the AVA Experiment Home view, display the Chip-Arrays currently in the AVA, the current operation being executed on the Chip-Array(s) (e.g., imaging, flow, etc.), as well as the ability to select a Chip-Array for further info & use cases, such as adding notes or launching live imaging for a specific Emulation.

Navigation Menu: On the left of the AVA Experiment Home view, you will see the navigation menu (red outline, collapsed by default).



In addition to viewing & returning to the AVA Experiment Home view, this navigation menu is used to access:

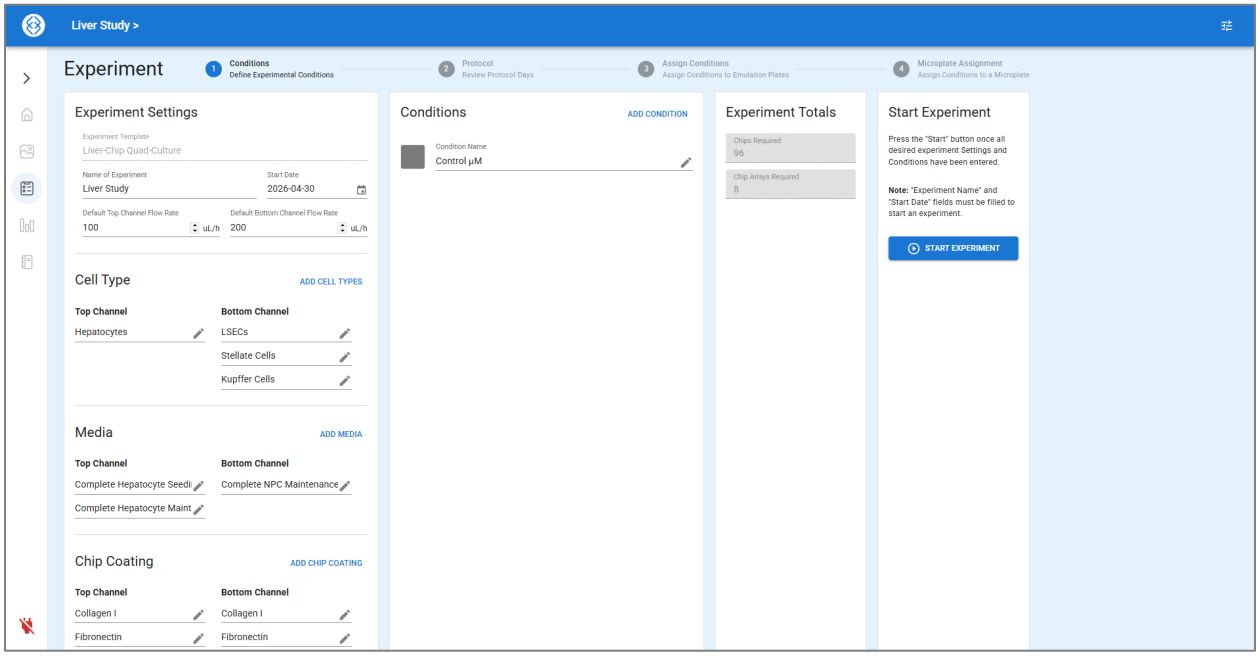
- **Imaging:** Review images, initiate live imaging of Chip-Arrays, or start a morphology scoring session.
- **Experiment Design:** Edit / update information about your AVA experiment.
- **Experiment Data:** Review notes about each Emulation and Chip-Array in your AVA experiment in a tabular format. Data from these tables can be exported.
- **Experiment Logs:** Review information about your AVA experiment, such as environmental condition logs, operational logs, AVA instrument and protocol info in a table format. Data from these tables can be exported.

5. **Experiment Review:** Once an AVA experiment is finished, you can open the experiment to review, edit, export experiment details, files (e.g., images), or other relevant data pertaining to the experiment. To open a completed experiment, from the “select an experiment” dialog, choose a previously completed experiment to display the experiment contents.

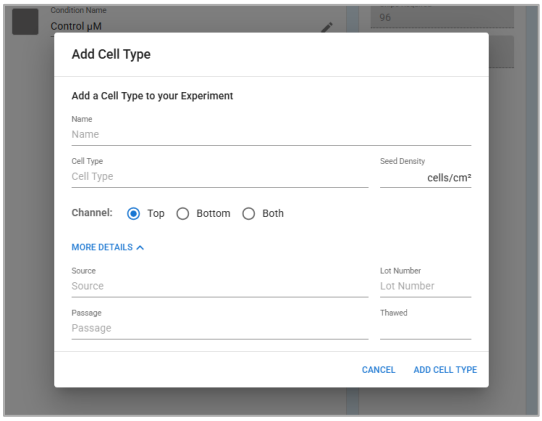
Create an AVA Experiment Template

Define Experimental Conditions on the Conditions View

The **Conditions** view is the first view displayed after opening a template. The Conditions view contains various pieces of information about your experiment, organized into four sections called Cell Type, Media, Chip Coating and Conditions.



A. **Add Cell Types:** Click **Add Cell Types** to display the **Add Cell Type** dialog. Input a **Name** and select a **Channel** for the Cell Type. The information entered for each Cell Type is used throughout the AVA software interface and can be edited at any time, supporting consistency across the experiment workflow.



Other optional information:

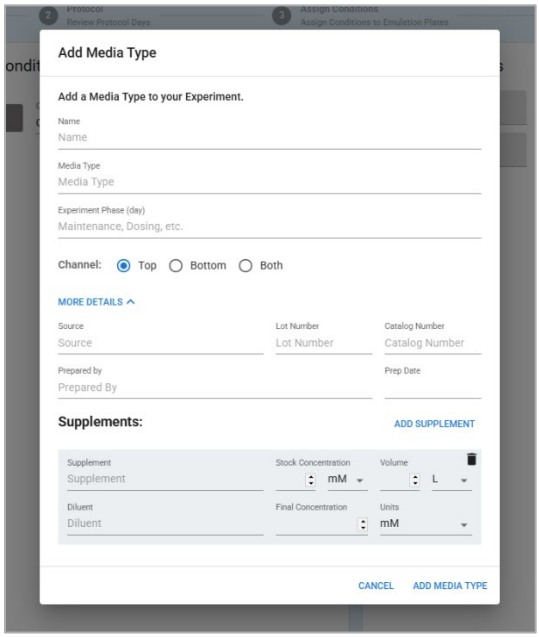
- Cell Type
- Seeding Density
- Expand **More Details** to show additional information for defining cell sources, lot number, passage number / info, and thaw date.

Once finished adding details about the cell entry, click **Add Cell Type**. You will see your new cell type entry appear in the associated channel. If the cell type entry is assigned to both channels, it will appear in both the top and bottom channel.

Other important notes:

- To add another cell type, click **Add Cell Types** and repeat the steps above.
- To edit a cell type entry, click the cell entry you want to edit, and the **Update Cell Type** dialog will appear. Make edits as needed and click **Update Cell Type** to save.
- To delete a cell type entry, click the cell entry you want to delete, and the **Update Cell Type** dialog will appear. Click the Delete button (lower-left corner) and click **Confirm** on the confirmation dialog.

B. **Add Media:** Click **Add Media** to display the **Add Media Type** dialog. Input a **Name** and select a **Channel** for the Media Type. The information entered for each Media Type is used throughout the AVA software interface and can be edited at any time, supporting consistency across the experiment workflow.



Other optional information:

- Media Type
- Experiment Phase (day)
- Click **Supplements** to define independent supplements or a group of supplements used in experiment media composition. For each supplement entry you can define the name, stock concentration and volume, diluent(s) used and final concentration.
- Expand **More Details** to show additional information, for defining media source(s), lot & catalog numbers, who prepared the media and date prepared.

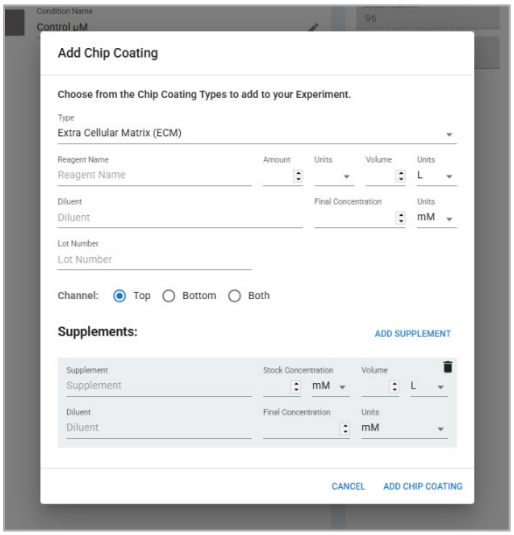
Once finished adding details about the media entry, click **Add Media Type**. You will see your new media type entry appear in the associated channel. If the media type entry is assigned to both channels, it will appear in both the top and bottom channel.

Other important notes:

- To add another media type, click **Add Media** and repeat the steps above.
- To edit a media type entry, click the media entry you want to edit, and the **Update Media Type** dialog will appear. Make edits as needed and click **Update Media Type** to save.
- To delete a media type entry, click the media entry you want to delete, and the **Update Media Type** dialog will appear. Click the Delete button (lower-left corner) and click **Confirm** on the confirmation dialog.

C. **Add Chip Coating:** Click **Add Chip Coating** to display the **Add Chip Coating** dialog. Input a **Type** (drop-down menu), the **Reagent Name** and select a **Channel** for the Chip Coating. The information entered for each Chip Coating is used throughout the AVA software interface and can be edited at any time, supporting consistency across the experiment workflow.
Other optional information:

- Diluent(s) used and final concentration
- Lot Number(s)
- Click **Supplements** to define independent supplements or a group of supplements used in experiment media composition.

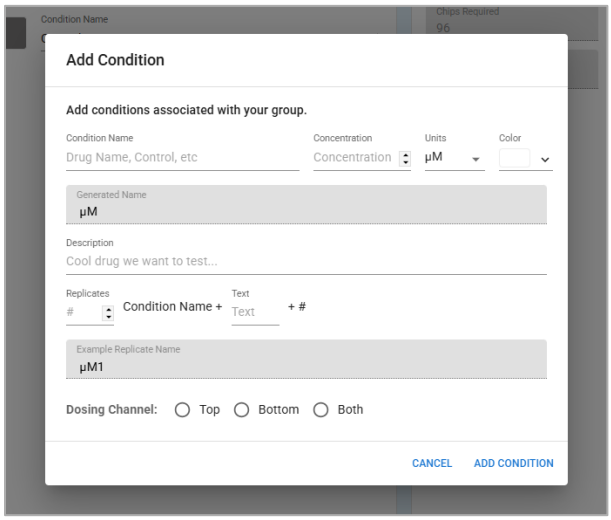


For each supplement entry you can define the name, stock concentration and volume, diluent(s) used and final concentration. Once finished adding details about the chip coating entry, click **Add Chip Coating**. You will see your new chip coating entry appear in the associated channel. If the chip coating entry is assigned to both channels, it will appear in both the top and bottom channel.

Other important notes:

- To add another chip coating, click **Add Chip Coating** and repeat the steps above.
- To edit a chip coating entry, click the chip coating entry you want to edit, and the **Update Chip Coating** dialog will appear. Make edits as needed and click **Update Media Type** to save.
- To delete a chip coating entry, click the chip coating entry you want to delete, and the **Update Chip Coating** dialog will appear. Click the Delete button (lower-left corner) and click **Confirm** on the confirmation dialog.

D. **Add Conditions:** Click **Add Condition** to display the **Add Condition** dialog. Input the **Condition Name**, **Concentration**, **Color**, **Replicates**, **Text** (for replicate assignment incrementation), and select a **Dosing Channel** for the Condition. The information entered for each Condition is used throughout the AVA software interface and can be edited at any time, supporting consistency across the experiment workflow.



Other optional information:

- Condition Description

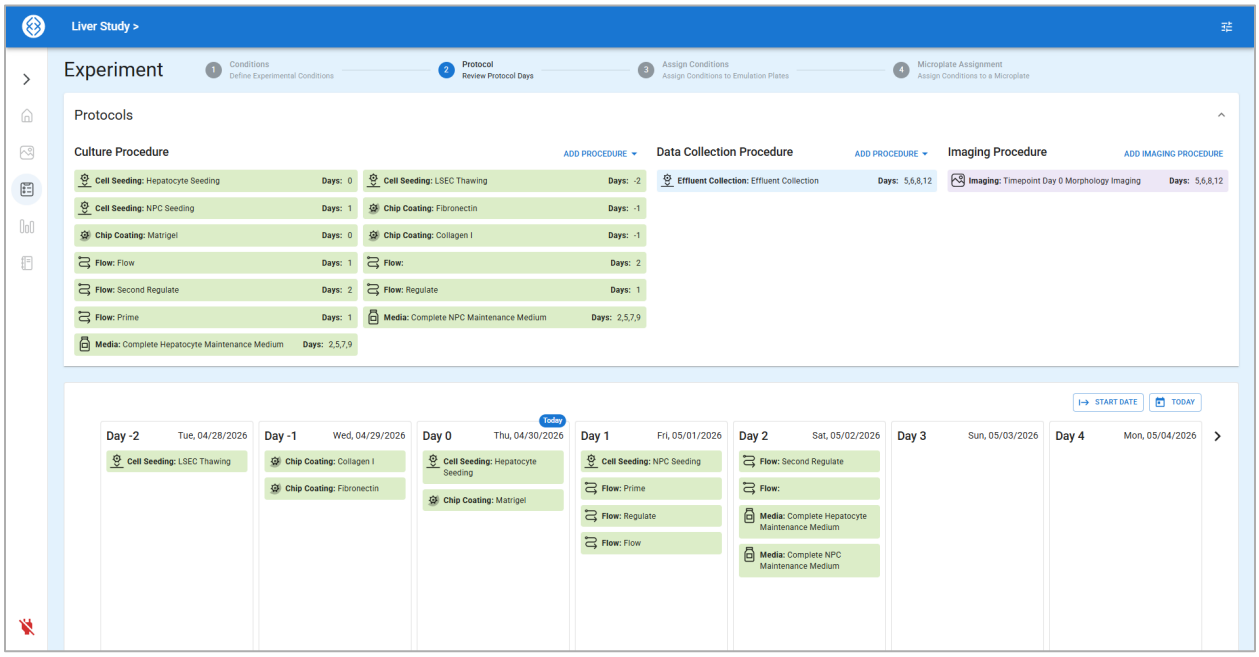
Once finished adding details about a Condition, click **Add Condition**. You will see your new condition entry appear in the Conditions column. The concentration provided in the condition entry will be included in the name automatically.

Other important notes:

- To add another chip coating, click **Add Condition** and repeat the steps above.
- To edit a condition entry, click the condition entry you want to edit, and choose **Edit Condition.** The **Update Condition** dialog will appear. Make edits as needed and click **Update Condition** to save.
- To duplicate a condition entry, click the condition entry you want to duplicate, and choose **Duplicate Condition**. The selected condition will automatically duplicate and appear in the list.
- To delete a condition entry, click the condition entry you want to delete, and choose **Remove Condition**. The selected condition will be automatically deleted.

Review & Customize Experiment Protocol on the Protocol View

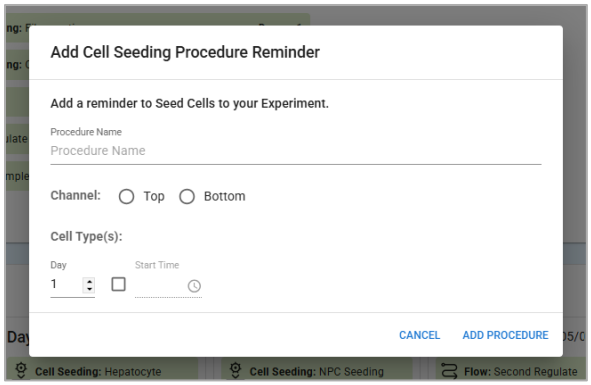
After creating conditions, you will create your experiment protocol on the **Protocol** view. The Protocol view is intended to function as a calendar, with a timeline of procedures that will occur throughout your AVA experiment.



There are different types of procedures / sub-procedures that can be added to the Protocol:

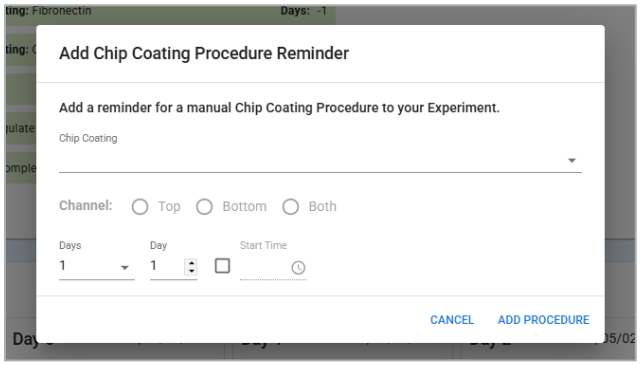
Procedure Type	Sub-Procedure
Culture	• Cell Seeding • Chip Coating • Dose • Flow • Media Replenishment
Data Collection	• Effluent (Sample) Collection
Imaging	• Automated Imaging Procedure

Culture Procedures – Cell Seeding: To add a **Cell Seeding** Culture Procedure reminder, click **Add Procedure** and select **Cell Seeding** from the list.



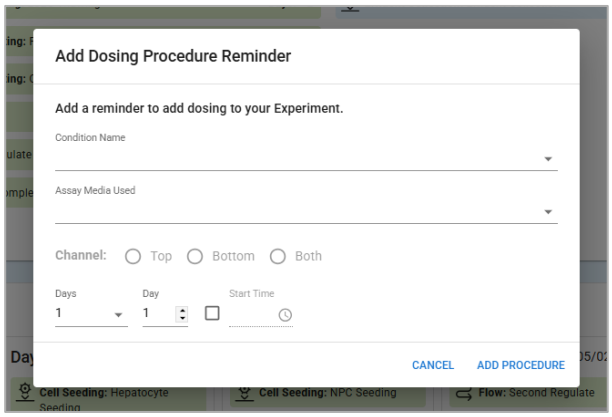
1. Input a **Procedure Name**.
2. Choose the **Channel** (Top or Bottom) to seed. Any **Cell Types** added in the **Conditions** view will appear in their respective channel based on the **Channel** selection; choose the cell types to be seeded.
3. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, add another **Cell Seeding** Procedure and repeat the steps above.
4. Click **Add Procedure** to add the reminder to the desired day(s).

Culture Procedures – Chip Coating: To add a **Chip Coating** Culture Procedure reminder, click **Add Procedure** and select **Chip Coating** from the list.



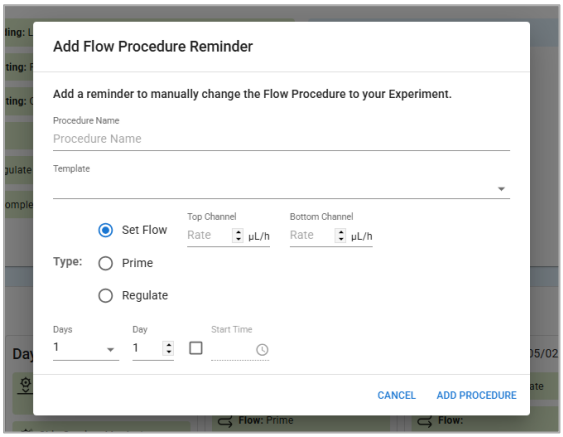
1. Select a **Chip Coating** using the drop-down menu. Any **Chip Coating(s)** added in the **Conditions** view will appear; choose the chip coating that will be used in the reminder.
2. The **Channel** (Top, Bottom, Both) will be automatically assigned (see previous step).
3. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, choose the number of days using the **Days** drop-down menu, then add a reminder time if desired.
4. Click **Add Procedure** to add the reminder to the desired day(s).

Culture Procedures – Dose: To add a **Dosing** Culture Procedure reminder, click **Add Procedure** and select **Dose** from the list.



1. Select a **Condition** using the drop-down menu. Any **Condition(s)** added in the **Conditions** view will appear; choose the condition that will be used in the reminder.
2. Choose **Assay Media** using the drop-down menu. Based on the channel assignment for the Condition, the respective Assay Media will appear in the list (e.g., if you select a Condition that is assigned to both channels, then only assay media assigned to both channels will be displayed in the drop-down menu for Assay Media Used). If you select **Control** as the Condition, the dropdown for Assay Media will be blank.
3. The **Channel** (Top, Bottom, Both) will be automatically assigned (see previous step).
4. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, choose the number of days using the **Days** drop-down menu, then add a reminder time if desired.
5. Click **Add Procedure** to add the reminder to the desired day(s).

Culture Procedures – Flow: To add a **Flow** Culture Procedure reminder, click **Add Procedure** and select **Flow** from the list.



1. Input a **Procedure Name**.

   Please Note: *The* ***Template*** *drop-down menu in the flow procedure dialog is non-functional in this version of AVA software.*

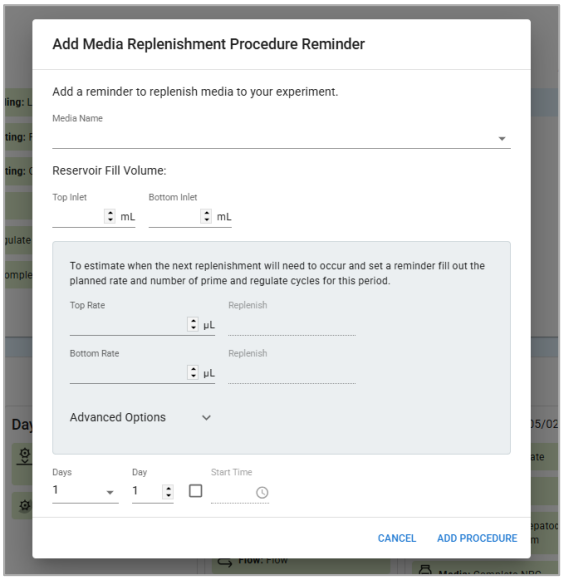
2. Choose a **Flow Type** using the radio buttons – *Set Flow*, *Prime*, and *Regulate*. For the *Set Flow* option, you

can input the top and bottom flow rates to include the values in your procedure reminder.

Please Note: *This function does NOT automatically initiate any flow operation, it is a scheduled reminder.*

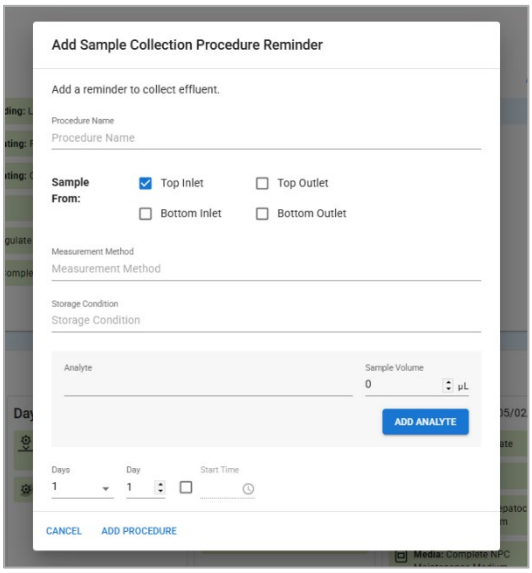
3. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, choose the number of days using the **Days** drop-down menu, then add a reminder time if desired.
4. Click **Add Procedure** to add the reminder to the desired day(s).

Culture Procedures – Media Replenishment:
To add a **Media Replenishment** Culture Procedure reminder, click **Add Procedure** and select **Media Replenishment** from the list.



1. Choose a **Media Name** using the drop-down menu. Any **Media Type** added in the **Conditions** view will appear.
2. Input a **Reservoir Fill Volume** for the **Top Inlet** and **Bottom Inlet**. These values will be used in the calculation of media replenishment timing.
3. Input the expected **Top Rate** (for the Top Channel) and **Bottom Rate** (for the Bottom Channel). Note the approximate time to replenish inlet reservoirs will automatically populate in the **Replenish** field.
4. Expand the **Advanced Options** to also include a *Regulate Cycle* or *Flush Cycle* flow operation in the replenishment timing calculation.
5. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, choose the number of days using the **Days** drop-down menu, then add a reminder time if desired.
6. Click **Add Procedure** to add the reminder to the desired day(s).

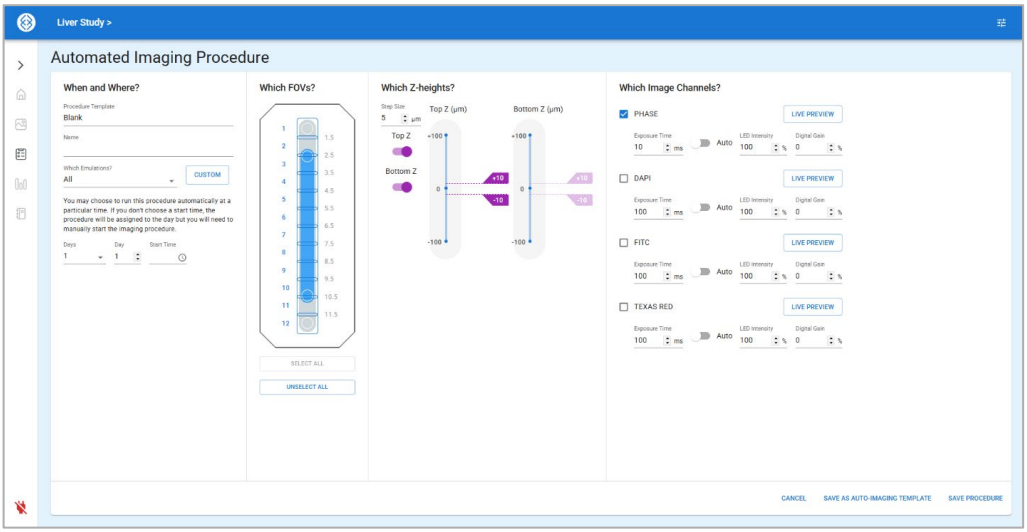
Data Collection Procedures – Effluent (Sample) Collection: To add an **Effluent Collection** Culture Procedure reminder, click **Add Procedure** and select **Effluent Collection** from the list.



1. Input a **Procedure Name**
2. Use the **Sample From** checkboxes to indicate where the effluent collection will come from.
3. Input a **Measurement Method** (e.g., Plate Reader, Immunofluorescence) if desired.
4. Input a **Storage Condition** (e.g., -4°C) if desired.
5. Add analytes to be measured (e.g., ALT) using the **Add Analyte** button, if desired. You can add multiple analytes to a single effluent collection reminder, or you can create an independent effluent collection reminder for each analyte to be measured.
6. Finally, add the **Day** (Experiment Day) and optional **Start Time** to be reminded at a specific time of day. If a **Start Time** is not provided, reminders will appear based on the order they are scheduled (added to the Protocol calendar). If you want to schedule the reminder on multiple experiment days, choose the number of days using the **Days** drop-down menu, then add a reminder time if desired.
7. Click **Add Procedure** to add the reminder to the desired day(s).

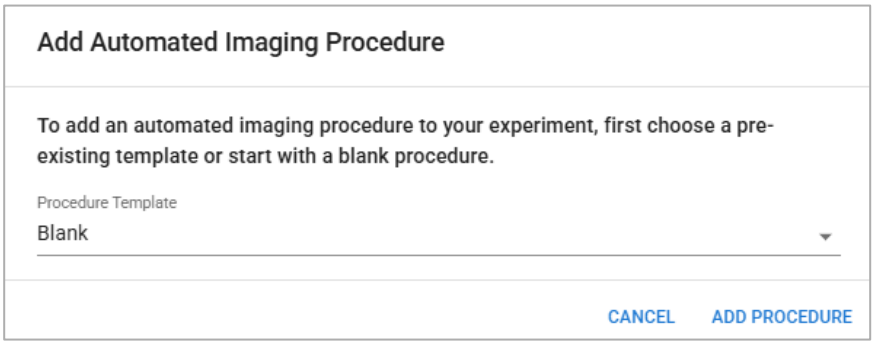
As a reminder, all scheduled **Culture Procedures** and **Data Collection Procedures**, and any information contained within, will be in the form of a reminder (e.g., schedule a media replenishment reminder, or a reminder to coat the bottom channel before cell seeding). There are no automatic instrument / workflow tasks executed when scheduling a **Culture Procedure** or **Data Collection Procedure.**

Imaging Procedure – Automated Imaging Procedures: An automated imaging procedure is a scheduled task for the AVA Emulation System to automatically acquire a set (collection) of images based on the configuration designed by the user.

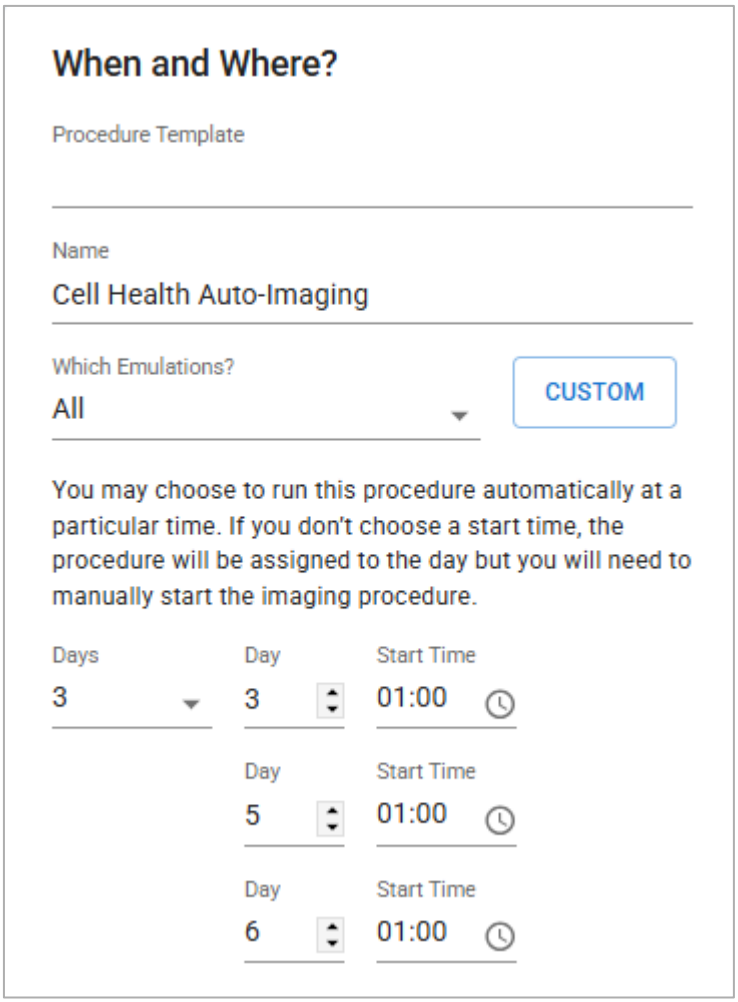


To set up an automated imaging procedure:

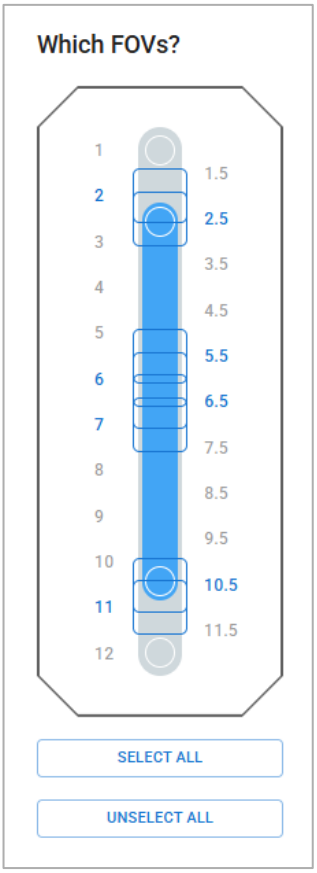
1. Click Add Imaging Procedure.
2. An Add Automated Imaging Procedure dialog box will appear. Use the drop-down to choose the imaging procedure template, either a blank template or an Emulate-provided imaging template containing the recommended imaging procedure steps (also fully customizable). Choose a template and click Add Procedure.



3. The **Procedure Template** field will reflect the name of the procedure template selected in step 2 above.
4. Input a custom name for the procedure in the **Name** field.
5. In the **Which Emulations** drop-down, *All* is selected by default, meaning the imaging procedure parameters seen on this view will be applied to ALL Emulations in your AVA experiment. Click the **Custom** button to display a dialog to select specific Emulations to capture images for.
6. Add the **Day** (Experiment Day) and **Start Time** for the automated imaging procedure. If you want to schedule the imaging procedure to run on multiple experiment days, choose the number of days using the **Days** drop-down menu. The Start Time for the imaging procedures will be the same, but you can customize as needed by checking the box in the desired day row.

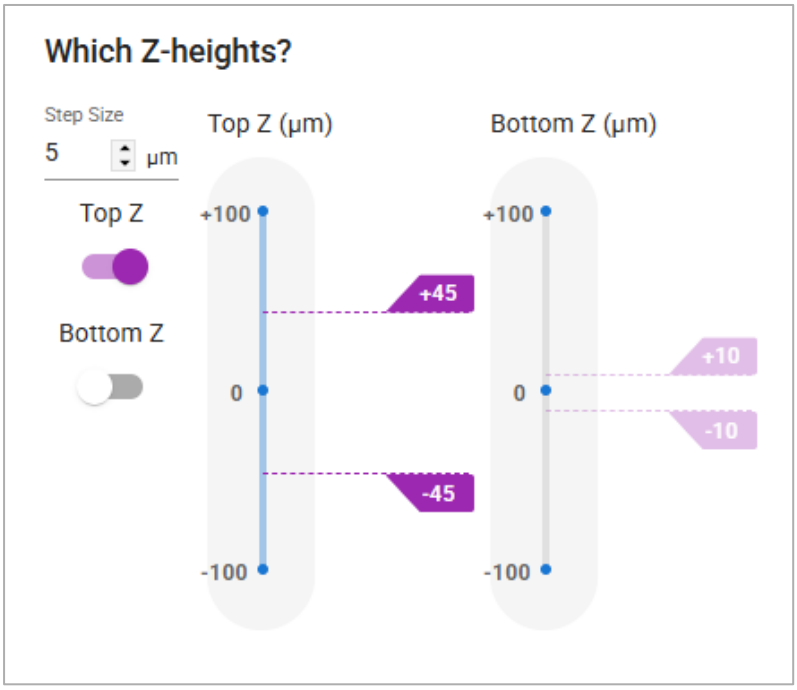


7. Next, choose the fields of view (FOVs) you want to apply the imaging procedure to. A FOV refers to a region, or area, along the chip channels. In AVA software, there are 23 FOVs to choose from with minimal overlap between the whole number options (1-12).
    - When using the **Blank** imaging template, all FOVs are selected by default. Use the **Unselect All** button below the FOV selector to deselect all FOVs, then choose the desired FOVs by clicking the corresponding number on the left or right (e.g., 7)

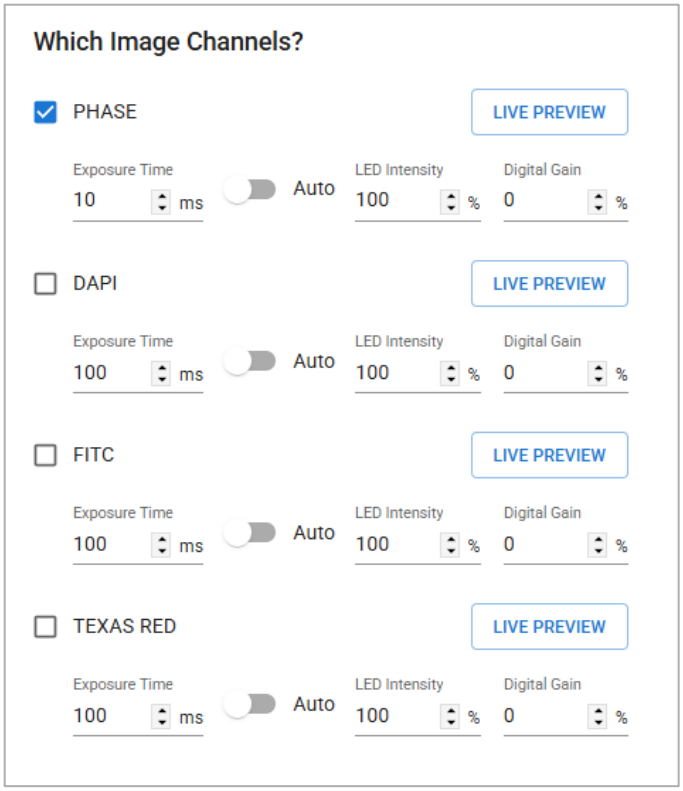


8. Next, choose the **z-slice height(s)** (individual images) to be captured in the top and bottom channels.
    - Adjust the **step size** to the desired incrementation. Setting the **step size** to very small increments (< 5 µm) will capture more images per FOV and there will be less obvious visual distinction between each slice.
    - Use the **purple sliders** to set the **First** and **Last** z-slice in the **Top Z (µm) range**. For reference, the '0' mm value is the approximate location of the membrane that separates the top channel and bottom channel.

      For example, slide the Top Z purple slider to '+15' and slide the Bottom Z purple slider to '0'. With a step size of 5 µm, and the first as last configurations above, this would result in 4 images (z-slices) for the Top Channel at '0', '+5', '+10' and '+15' z-slice heights. Follow this same process for configuring the **Bottom z (µm) range**.

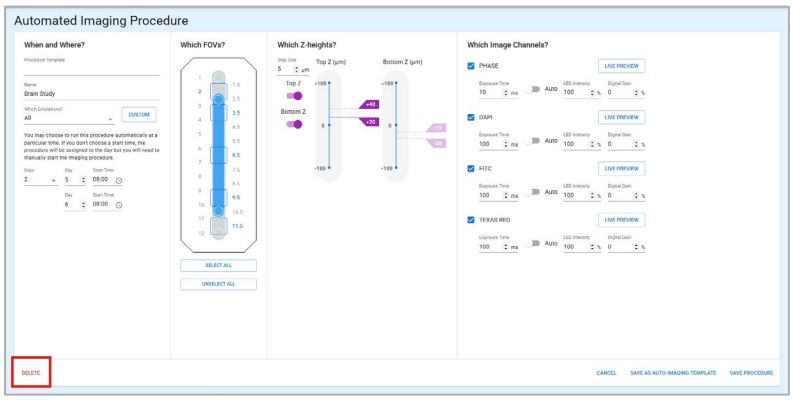


9. Finally, choose the **Imaging Channels**: *Phase Contrast*, *DAPI* (blue), *FITC* (green); and *Texas Red* (red).
    - You will see the associated parameters for each imaging channel, which can be edited from the automated imaging protocol view.
    - Alternatively, you can configure these settings performing a live image preview of the desired imaging channel by clicking **Live Preview**.
    - The adjustments made from this live preview will be applied to the automated imaging protocol and executed on all assigned emulations.



10. When finished with your protocol design, click **Save Procedure**.
    - Click **Save As Auto-Imaging Template** if you intend to reuse a custom auto-imaging template in future experiments.
    - Click **Cancel** to return to the Protocol view.

- To delete a single Imaging Procedure instance, open the specific auto-imaging session from the calendar and click **Delete** in the lower-left corner. To delete an entire auto-imaging series (scheduled across multiple days), open the auto-imaging procedure in the **Protocol** view and click **Delete** in the lower-left corner. This will remove all scheduled instances in the series.

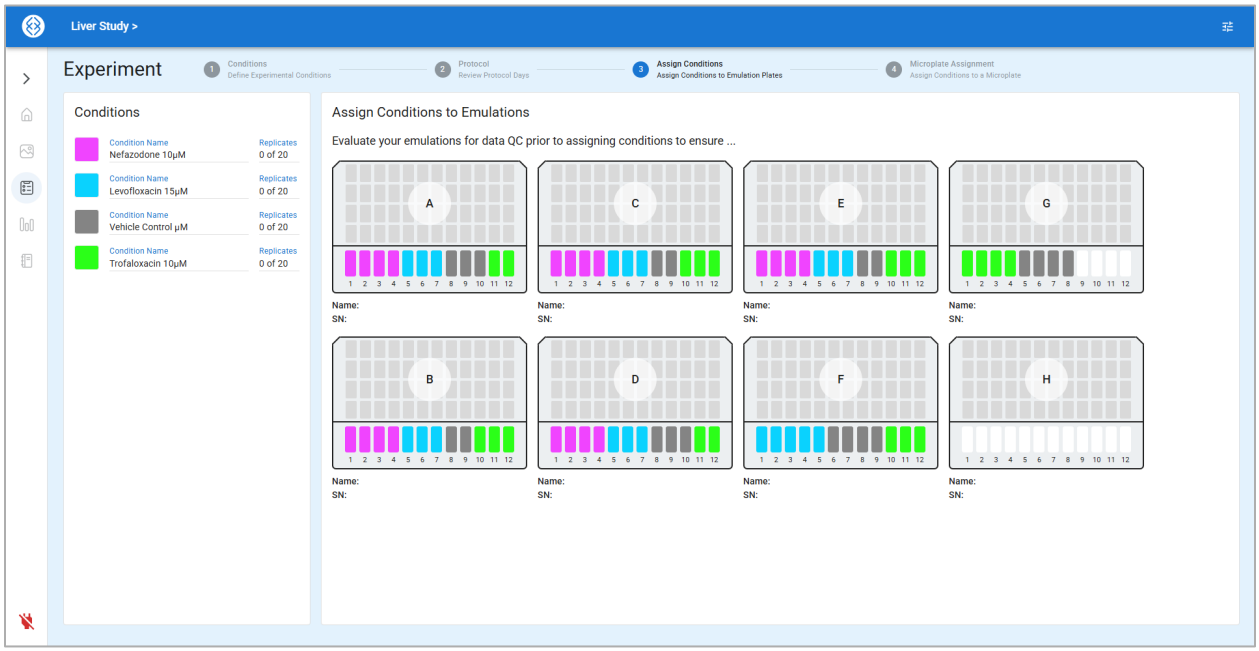


Automated Imaging Best Practices:

- Confirm your automated imaging settings using the live preview feature (next to each imaging channel) to ensure the correct exposure, z-stack ranges for top and bottom channel, etc.
- It is recommended you schedule your automated imaging sessions while the AVA Emulation System is not actively being used (e.g., for scheduled events, like dosing or media replenishment) as certain operations can affect the automated imaging procedure completion time.
- Do not eject trays when running an automated imaging procedure. If you proceed with tray ejection, the automated imaging procedure will be cancelled and must be restarted.
- All Chip-Arrays included in an active experiment must be 'mapped' before any imaging procedure (automated or live) can be executed. This process is vital for accuracy of imaging coordinates and is automatically executed once Chip-Arrays are inserted, a valid QR code is detected, and manifolds have been lowered.
- It is possible to generate a large amount of image data during an AVA experiment. It is recommended to routinely check workstation storage capacity to ensure sufficient storage volume prior to running an experiment or automated imaging procedure.

Assign Experiment Conditions to Chip-Arrays

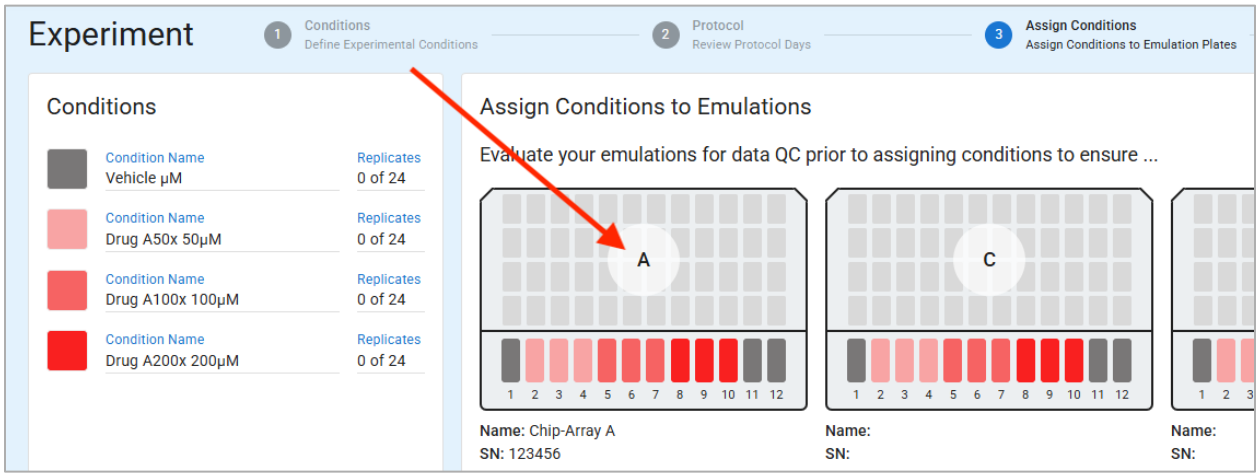
On the Assign Conditions view, the conditions created from the **Condition** view will appear in the left-side list and can be assigned to the Emulations on each Chip-Array in your experiment.



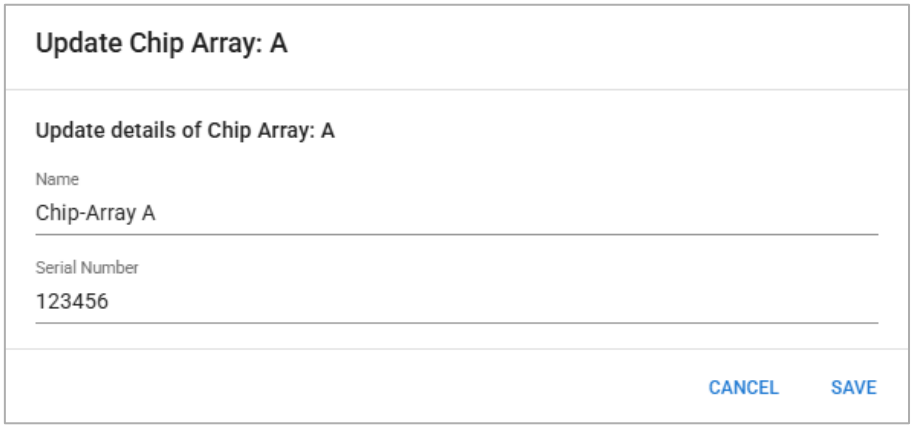
1. Select a Condition in the left Condition list by clicking it. The gray line below the selected condition will turn blue, indicating it is selected & active.
2. Click the individual Emulation (on a Chip-Array) to assign the selected condition.
3. You will see the number of **Replicates** per condition (also assigned on the **Condition** view); this value will decrement as you assign conditions to Emulations. For example, if you have 10 replicates for a given condition, and assign to one Emulation, the **Replicates** field would display “9 of 10”, which means you can assign 9 more Emulations this condition value. The information per condition can be edited by returning to the **Conditions** view (e.g., to add more replicates, or change a typo).
4. It is recommended to assign conditions after you have evaluated all Emulations for cell health. This helps ensure proper distribution of conditions per Emulation & associated cell health.

Add / Update Chip-Array Information

On the **Assign Conditions** view, you can view & update your Chip-Array name by clicking the letter (A, B, C, D, E, F, G, H) on the Chip-Array UI component.

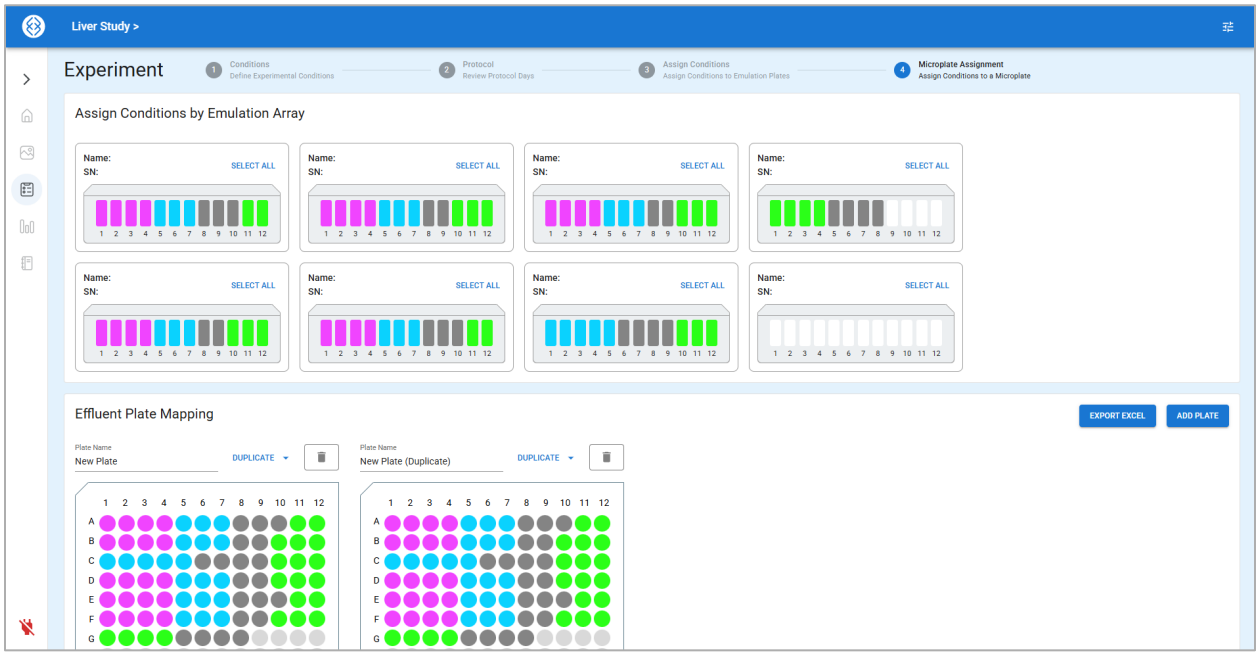


The serial number will be automatically populated from the Chip-Array detection function and is not editable.



Assign Experiment Conditions to Microplate

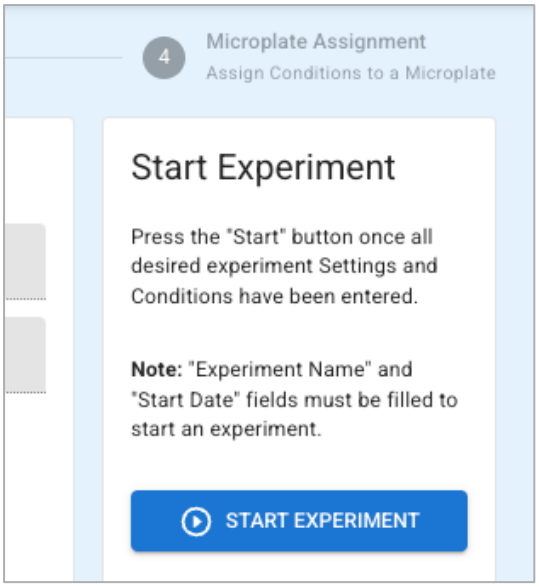
On the **Microplate Assignment** view, Emulations will be displayed as they were assigned from the **Assign Condition** view. This view allows you to assign treatment conditions to a 96-well microplate for sample collection & downstream analysis, minimizing manual data transposition efforts from your AVA experiment design into other analytical platforms & software for downstream analysis (e.g., ELISA) for Emulate workflows.



1. Click **Add Plate** to create a blank, 96-well microplate element in the AVA software.
2. Enter a **Plate Name** in the text field.
3. To assign conditions to the 96-well microplate, use one of the following methods:
   a. Select an individual Emulation condition and click the respective well(s) you want to assign to that condition.
   b. Select multiple Emulation conditions, then click the first well you want to assign this condition to. The AVA software will automatically assign the selected conditions horizontally.
   c. Select **all conditions** on a Chip-Array (easiest option), then click the first well to assign these conditions to (in column 1). You will see the 12 Emulations on the Chip-Array are automatically assigned to the 12 wells on the microplate.
4. You can create a new plate map and repeat this processes as needed, or you can **Duplicate** the plate map and change the plate name.
5. Once you have mapped conditions to the desired number of microplates, export this plate layout to Excel using the **Export Excel** button.

Run an AVA Experiment

When you are ready to begin your AVA experiment, you must click the **Start Experiment** button, found on the Conditions view. This is an important action; any data acquired, information supplied to AVA software, etc. will be tracked and compiled into an “experiment folder” at the completion of your AVA experiment.

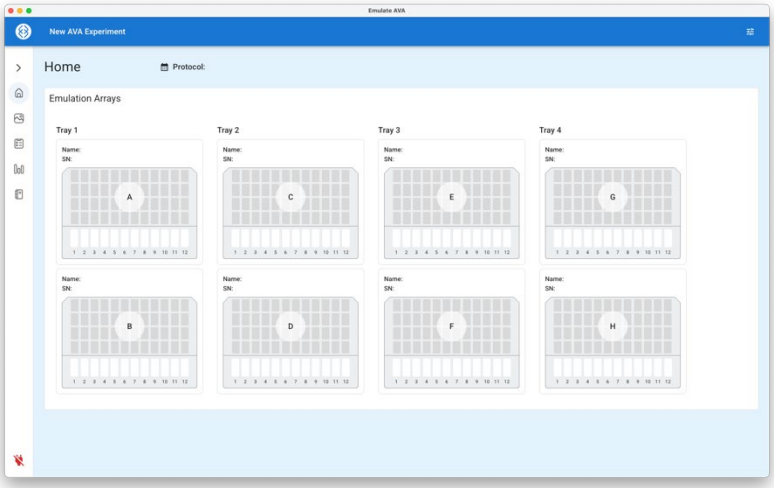


To start an AVA experiment, you must supply *at least* an **experiment name** and **start date**. This allows users to design an experiment very quickly (if needed), however Emulate recommends designing your experiment templates in advance of your start date.

Click **Start Experiment** when ready and address the software confirmation prompt to officially start an AVA experiment. The AVA Experiment Home view will automatically appear once your experiment has started.

AVA Experiment Home

The AVA Experiment Home view is the main experiment runtime software UI where you can see AVA connection status, current environmental conditions, view or edit flow settings, review Chip-Arrays loaded in the AVA, initiate live imaging, and more.



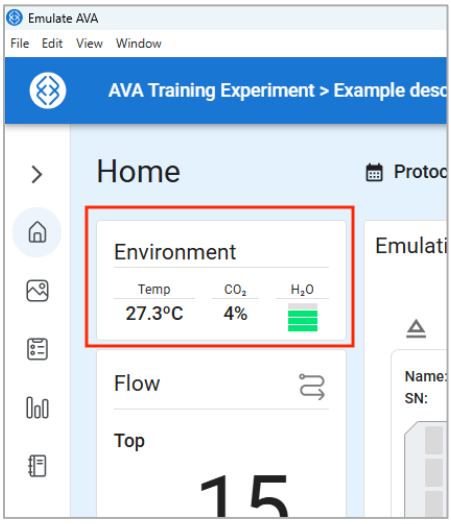
Navigation Menu

On the left of the AVA Experiment Home view, you will see the navigation menu (collapsed by default). This navigation menu is used to access:

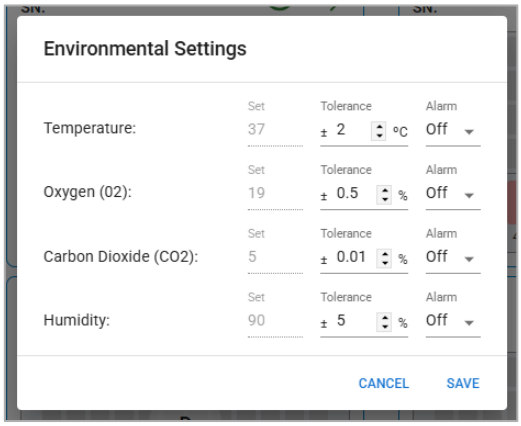
	**AVA Experiment Home:** The primary experiment runtime view where you can see environmental conditions, edit or run flow operations, view Chip-Arrays in the AVA, access live imaging, and more.
	**Imaging:** Review images, initiate live imaging of Chip-Arrays, or start a morphology scoring session.
	**Experiment Design:** Edit / update information about your AVA experiment.
	**Experiment Data:** Review notes about each Emulation and Chip-Array in your AVA experiment in a tabular format. Data from these tables can be exported.
	**Experiment Logs:** Review information about your AVA experiment, such as environmental condition logs, operational logs, AVA instrument and protocol info in a table format. Data from these tables can be exported.

Environment Widget

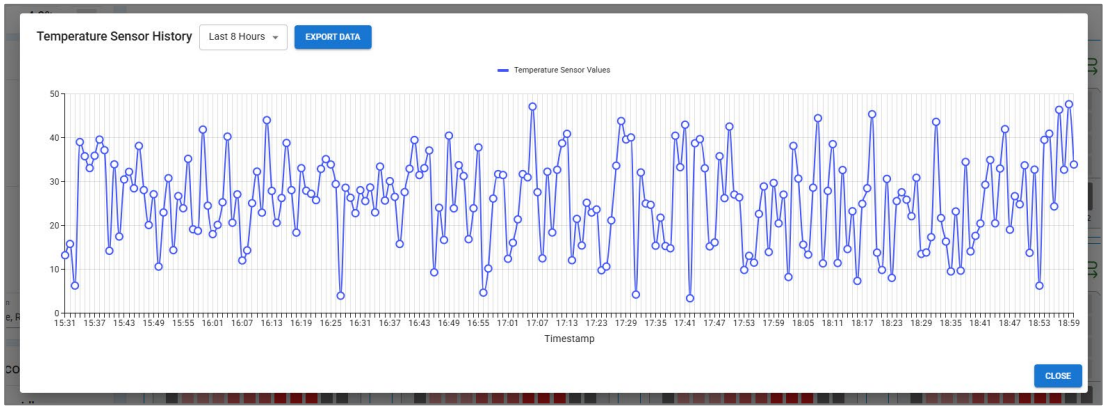
The **Environment** widget, located in the upper left corner of the AVA Experiment Home view, displays the current AVA temperature, carbon dioxide concentration, and water level in the water pan.



- To edit environment alarm tolerances or disable / enable environment alarms, click **Environment**.



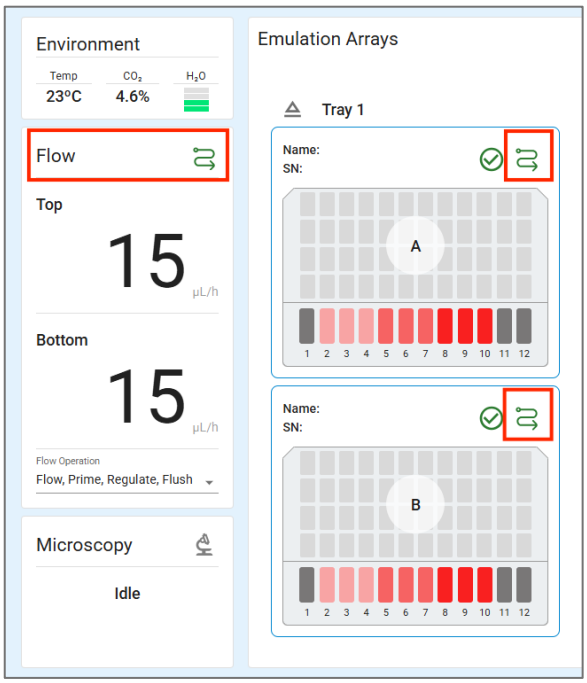
- To review environmental sensor data for temperature, carbon dioxide concentration and water pan level over the last 8 or 24 hours, click **Temp**, **CO_2** or **H_2O** (in the **Environment** widget). If you wish to review the entire history of environmental sensor performance, use the **Export** button (found on the sensor history graph window), choose a file location for the export and click **Save**.



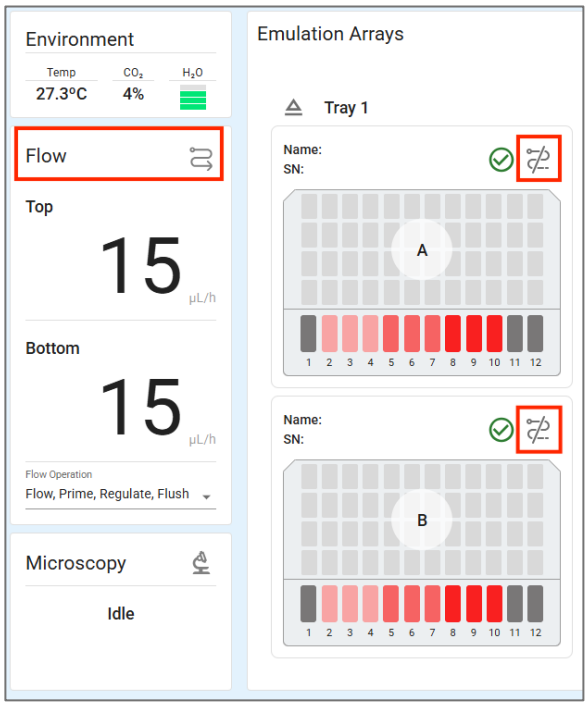
Flow Widget

The **Flow** widget, located below the Environment widget, displays the current flow status for the top and bottom channels of the Chip-Arrays in the AVA.

- If flow is actively running, this widget will display the top and bottom flow rate values and the serpentine arrow in the upper-right corner will be green.



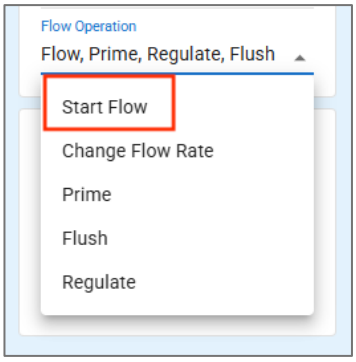
- If flow is *not* running, the serpentine icon will appear gray. If you have specified a default flow rate, they will be displayed for the top / bottom channels. If there was no default flow rate specified, there will be dashes displayed for the top and bottom channel flow rates (not pictured)



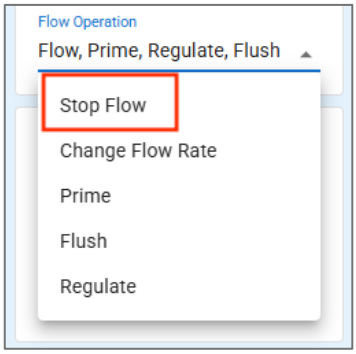
Use the **Flow Operation** drop down menu to start / stop / edit flow rates, or run specific flow operations:

<table>
<tr><th colspan="2">Flow Operations & Descriptions</th></tr>
<tr><td>Start & Stop Flow</td><td>

Start Flow will be displayed if AVA is not running flow. Click Start Flow to start flow in the top and bottom channels. When flow is actively running for a specific tray, there will be a faint blue outline around the tray and the flow (serpentine) icon will display as green.

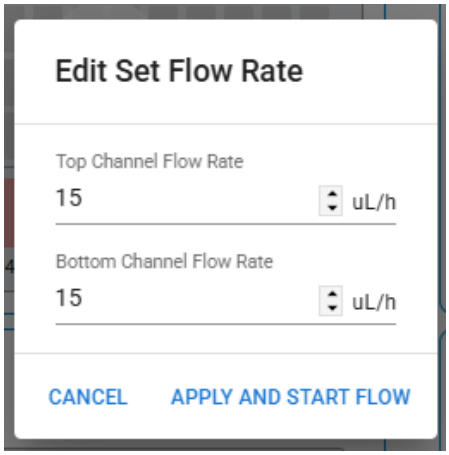


Stop Flow will be displayed if AVA is actively running flow. Click Stop Flow, then click Confirm to stop flow in the top and bottom channels immediately across all Chip-Arrays. When flow is not active for a tray, the flow (serpentine) icon will display as gray with a slash through it, and the tray will have a gray outline.

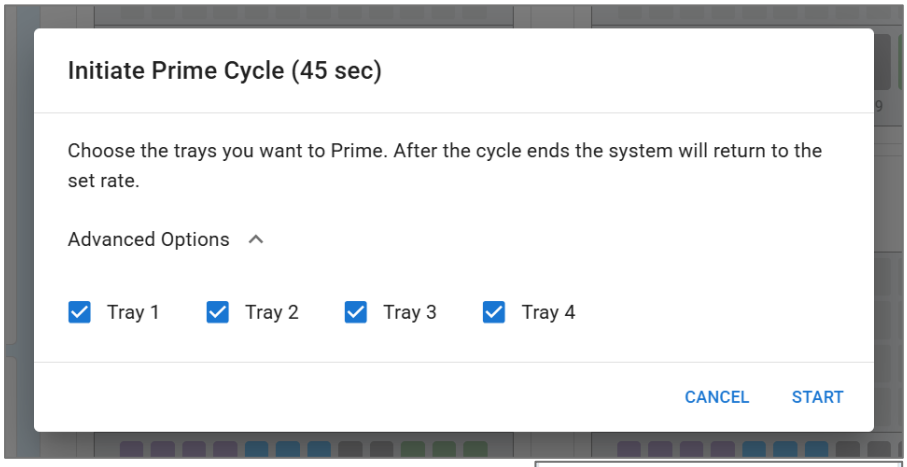
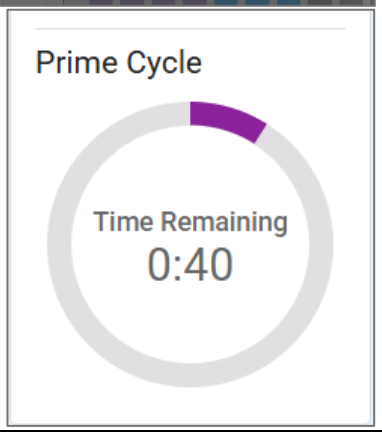


</td></tr>
<tr><td>Change Flow Rate</td><td>

Click Change Flow Rate to display a dialog to edit the top channel and bottom channel flow rates.
To edit flow rates, use the up / down arrows in AVA software, or use a keyboard to input desired rate(s), then click Apply and Start Flow.
Flow rate dynamic range for the top channel and bottom channel is 10 - 2,000 µL / hr. Flow rate must be set in increments of 5 µL / hr.

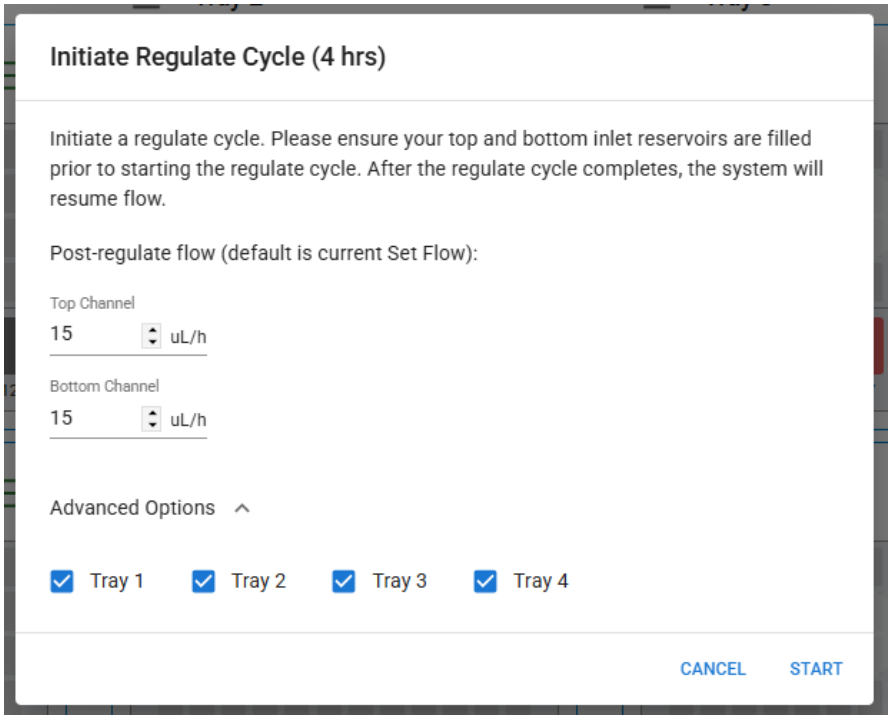


</td></tr>
</table>

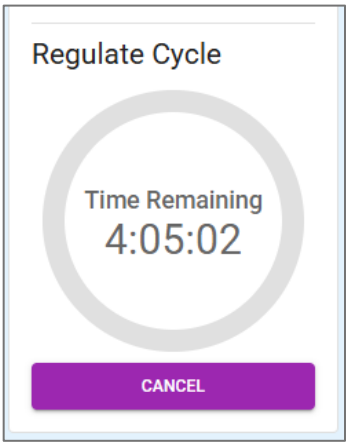
Prime Cycle	• The Prime Cycle perfuses media through the media reservoirs of Chip-Array (but not the Emulation) to ensure no air is trapped within the Chip-Array microfluidic pathways. • To execute a Prime Cycle, select **Prime** and the Prime dialog will appear. When ready, click **Start**. Use the Advanced Options to select specific trays to perform the Prime Cycle, if necessary.  • The Prime Cycle takes only a few minutes to complete for all Chip-Arrays in the AVA. AVA software will display a Prime Cycle timer while the cycle is running. 

Regulate Cycle

- The Regulate Cycle is designed to remove any bubbles that may have been introduced during Chip-Array preparation steps, such as seeding cells.
- To execute a Regulate Cycle, select **Regulate** and the Regulate dialog will appear. It is important to ensure your Top and Bottom Inlet reservoirs are filled to ensure the inlet contents are not fully depleted during the Regulate Cycle. Use the Advanced Options to select specific trays to perform the Regulate Cycle, if necessary. When ready to begin, click **Start**.



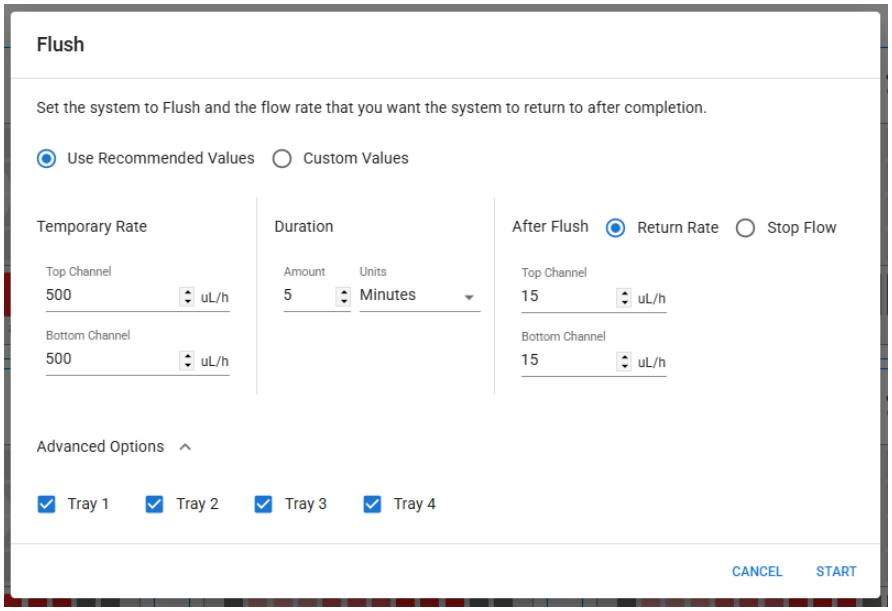
- The Regulate Cycle takes approximately 4 hours to complete for all Chip-Arrays in the AVA. AVA software will display a Regulate Cycle timer while the cycle is running.
- Once the Regulate Cycle finishes, flow will automatically resume in the Top and Bottom Channels at the previously running rate(s); you can adjust the post-Regulate Cycle flow rates on the Regulate dialog if needed.



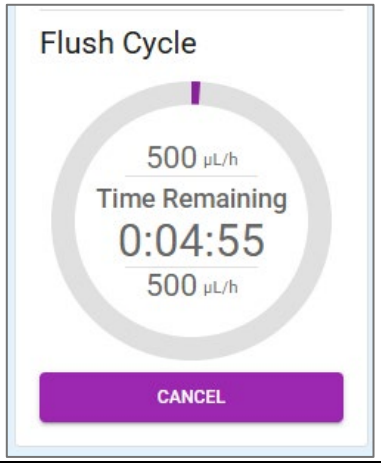
Flush Cycle

- The flush cycle is a transient flow operation used to perfuse fluid through the Chip channels to introduce a drug, compound, treatment, or fresh medium.

- To execute a Flush Cycle, select **Flush** and the Flush dialog will appear:
  - Set a **Temporary Rate** – this setting represents the flow rate that will be executed during your flush cycle for the top and bottom channels.
  - Set a **Duration** – this is the duration of the flush cycle, set in minutes. Do not use the Custom Values feature, it is not operational in AVA software version 1.0.16.
  - Set your **Post-Flush Rate** – this setting represents the flow rate that will be executed immediately after your flush cycle duration has completed.
  - Use the Advanced Options to select specific trays to perform the Flush Cycle, if necessary.

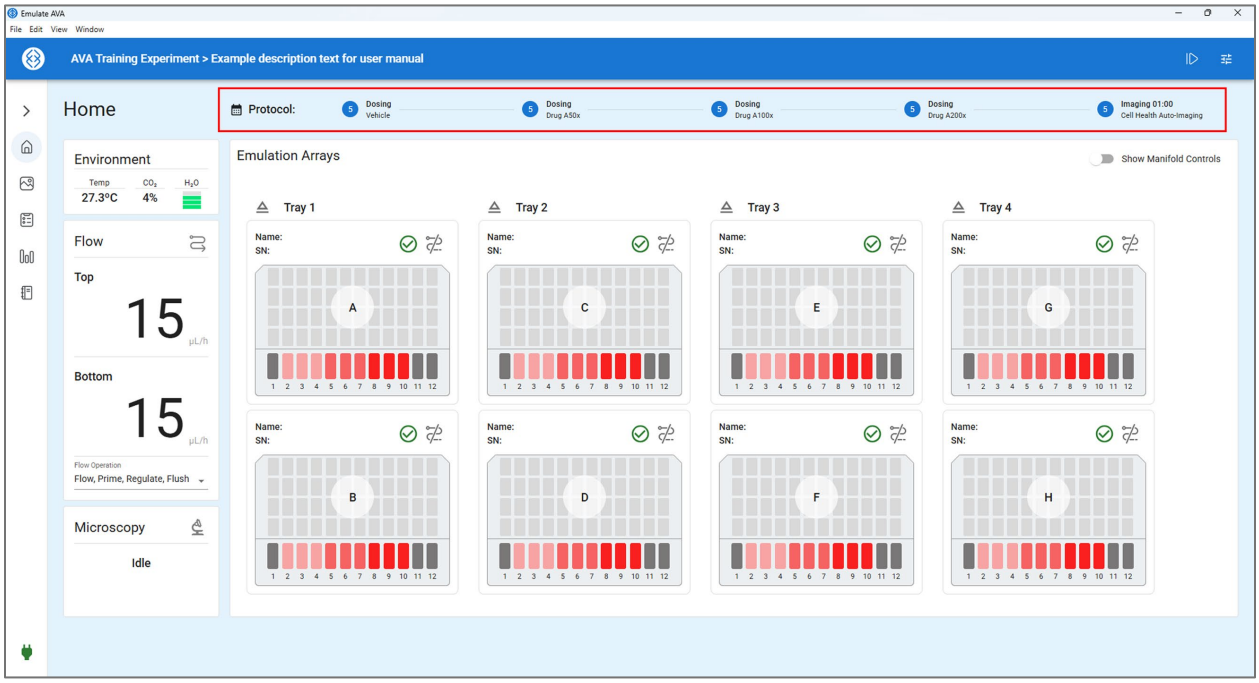


- When ready, click **Start**. AVA software will display a Regulate Cycle timer while the cycle is running.



Protocol Timeline

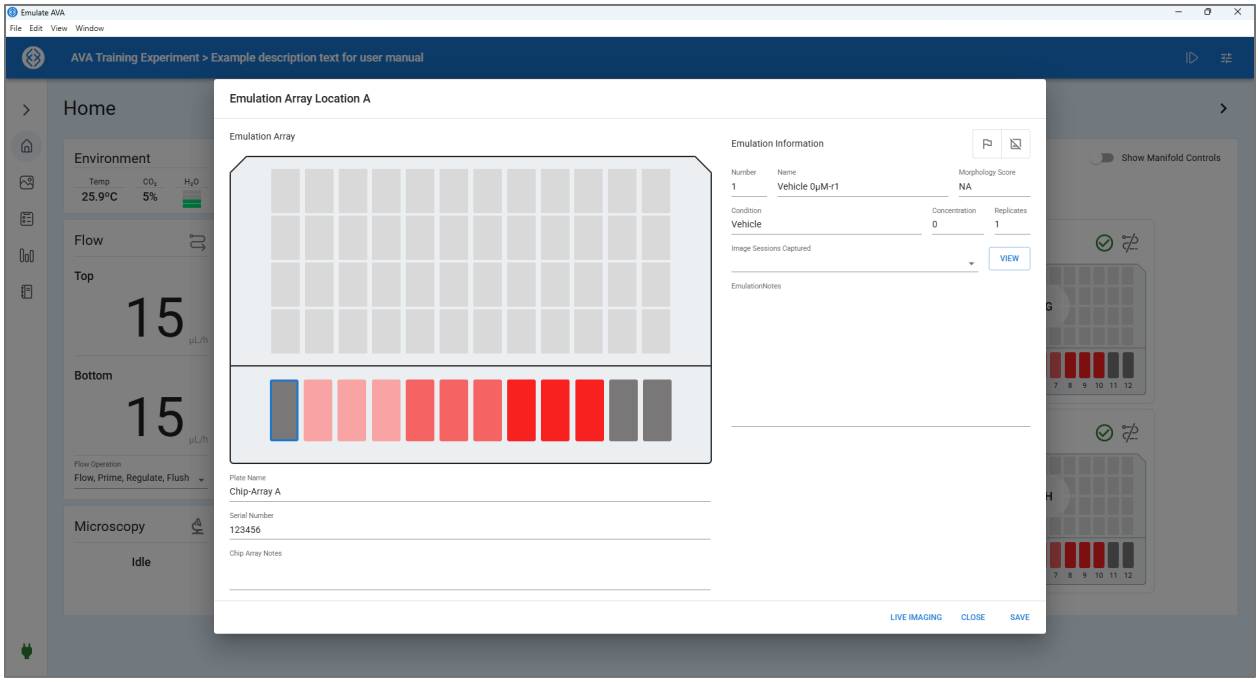
The protocol timeline view, located at the top of the Home view above your Chip-Arrays, displays the current and / or upcoming steps in your protocol. To edit the Protocol, simply open **Experiment Design** (from the navigation menu) and select **Protocol**.



Chip-Array Integration with AVA Software

Component: Chip-Array widget(s)

Chip-Array widgets, featured in the center of the AVA Experiment Home view, display all Chip-Arrays assigned to your experiment, conditions assigned to your Chip-Arrays, the current operation being executed on the Chip-Array(s) (e.g., imaging, flow, etc.), as well as the ability to select a Chip-Array for additional info, live imaging, adding notes, and more. To open the **Chip-Array dialog**, click on a Chip-Array widget on the Home View.



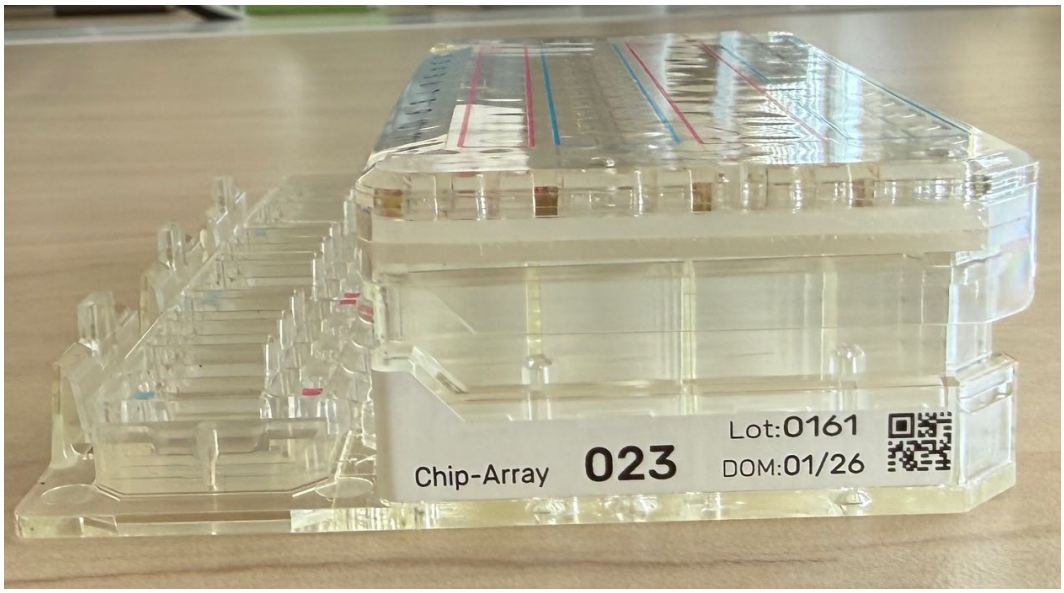
Click an Emulation on the Chip-Array to display specific information about the selected Emulation, including:

a. The **condition name** assigned during experiment design / update, and other condition information such as **concentration** and **replicate number**.
b. A **morphology score**, if assigned (see Morphology Scoring, p.73)
c. **Imaging sessions** captured (if applicable) of images associated with the selected Emulation on the Chip-Array.
d. Add or update **Emulation Notes** (specific to the selected Emulation) or **Chip-Array Notes** (notes pertaining to the selected Chip-Array).

e. **Warnings** or **errors** pertaining to the selected Emulation.
f. **Flag** the selected Emulation to add a small colored dot that will be visible on any UI where the Emulation / Chip-Array are displayed. Use the notes section to describe the purpose of the flag, if useful.
g. **Unassign** the selected Emulation to exclude from upcoming scheduled automated imaging sessions. This will ensure you do not generate unwanted images and consume storage space. Unassigning the Emulation will not unassign condition details or other information associated with that Emulation.
h. Click **Live Imaging** to launch a live imaging session for the selected Emulation. See Live Imaging (p. 51) for more information.
i. Use the **Save** button to save all changes made on the Chip-Array widget view or click **Close** to discard changes.

Function: Chip-Array Detection

Each time AVA trays are inserted, AVA will scan for the presence of Chip-Array(s). When a Chip-Array is detected for the first time, it will be assigned to the actively running experiment with a unique identifier. All operations associated with that Chip-Array will be tracked throughout the duration of the experiment (e.g., ejection / insertion timing), and any information and data assigned to that Chip-Array & Emulations (e.g., notes, morphology scores, images). This information can be reviewed at any time in the Experiment Logs section.

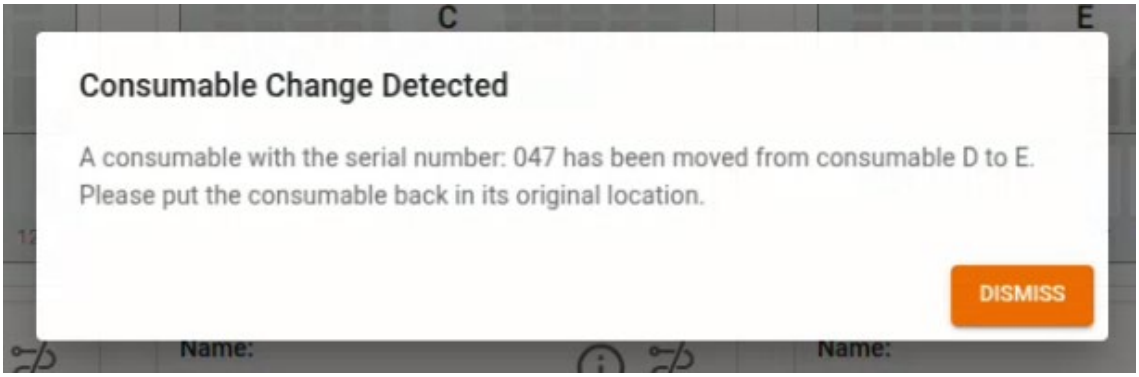


Function: Chip-Array Tracking

Throughout the AVA workflow, it is required to remove Chip-Arrays for various purposes, such as replenishing media and dosing compounds. As these steps are performed, AVA will track activities and operations associated with the Chip-Arrays, such as time of ejection & time of re-insertion, location in AVA, and more.

Please Note: A Chip-Array must always be replaced in the original AVA tray position for the duration of your experiment. During an experiment, when a Chip-Array is introduced to AVA for the first time, the Chip-Array QR code (unique identifier) is paired with the AVA tray position where this Chip-Array is placed. After this process occurs, this Chip-Array must always be replaced in the same tray position for the duration of the experiment.

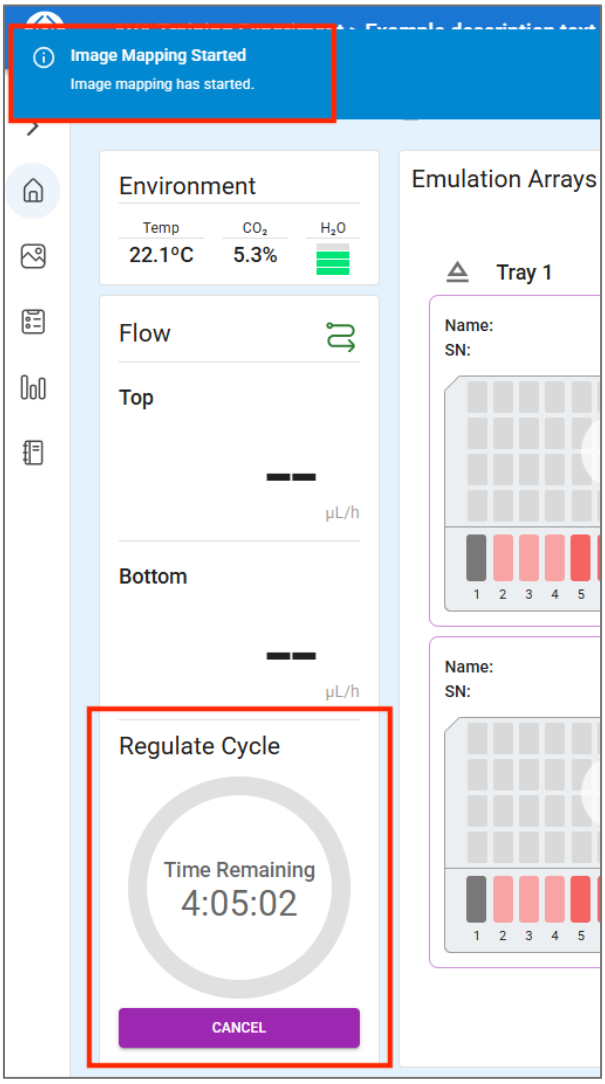
For Example: "Chip-Array -0123" is placed on Tray 1 position A and then Tray 1 is closed, inserting "Chip-Array -0123" into AVA. AVA proceeds to scanning the "Chip-Array -0123" QR code. During this process, "Chip-Array -0123" will be 'assigned' to Tray 1 position A and must always be replaced on Tray 1 for the entirety of the experiment. For example, if "Chip-Array -0123" is replaced on Tray 2, position C, then AVA will display an error that requires this Chip-Array to be ejected and returned to Tray 1 position A.



Function: Chip-Array Mapping

Chip-Array mapping is an automated function that analyzes the properties of each Chip-Array in your experiment to help ensure high-quality images are generated from live imaging and automated imaging sessions.

- Chip-Array mapping is required before any imaging (live and automated) can occur.
- Chip-Array mapping will be performed during the first Regulate Cycle in your experiment and will be applied to all Chip-Arrays in AVA at that time.
- If you ever notice consistently poor focus and / or top or bottom channel misalignment while imaging or reviewing images, you may need to remap the affected Chip-Array(s). Please contact Emulate Support for guidance on the Chip-Array re-mapping process.



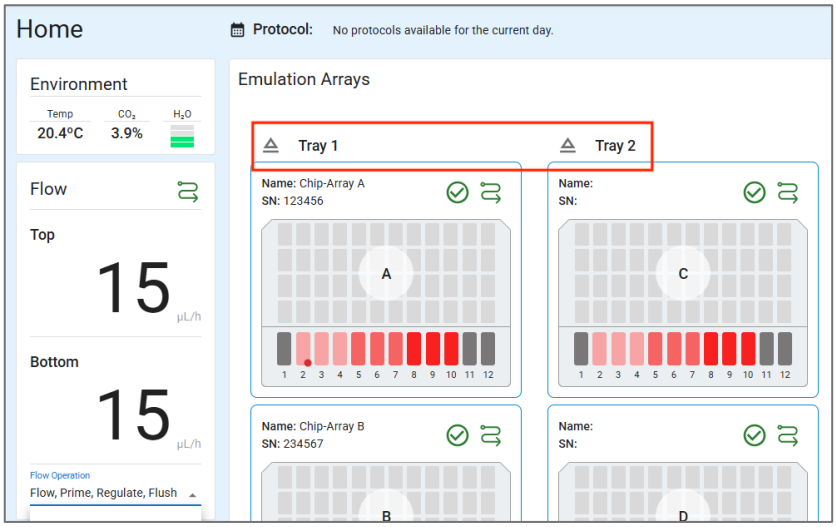
Function: Inserting & Ejecting Chip-Arrays

The AVA Emulation System has a total of 4 trays; each tray can hold 2 Chip-Arrays.

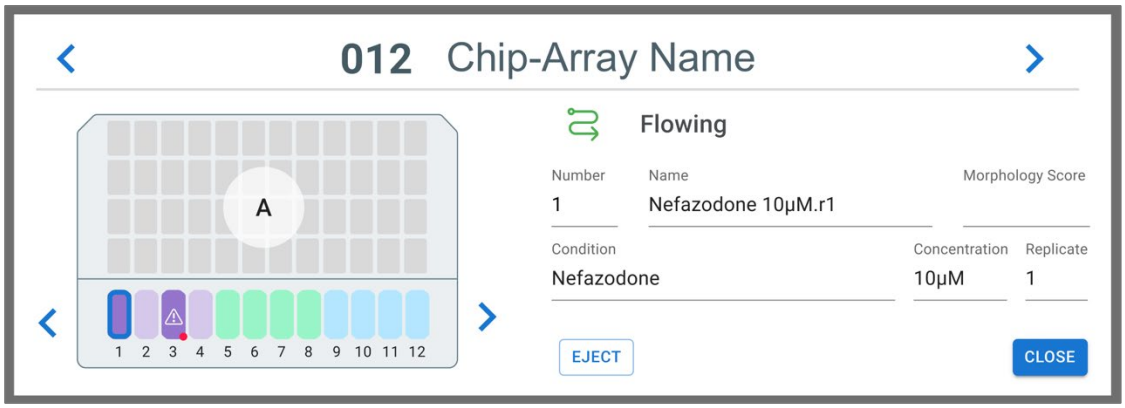
To insert a Chip-Array, you must first choose a tray to eject through one of the following three methods:

- Press the physical tray button corresponding to the desired tray position where the Chip-Array is to be loaded. For example, if you want to insert a Chip-Array in position A, press the illuminated tray control button below tray 1.

- Press the digital eject button on the Home view of AVA Workstation Software (on the connected computer) corresponding to the desired tray position where the Chip-Array is to be loaded.



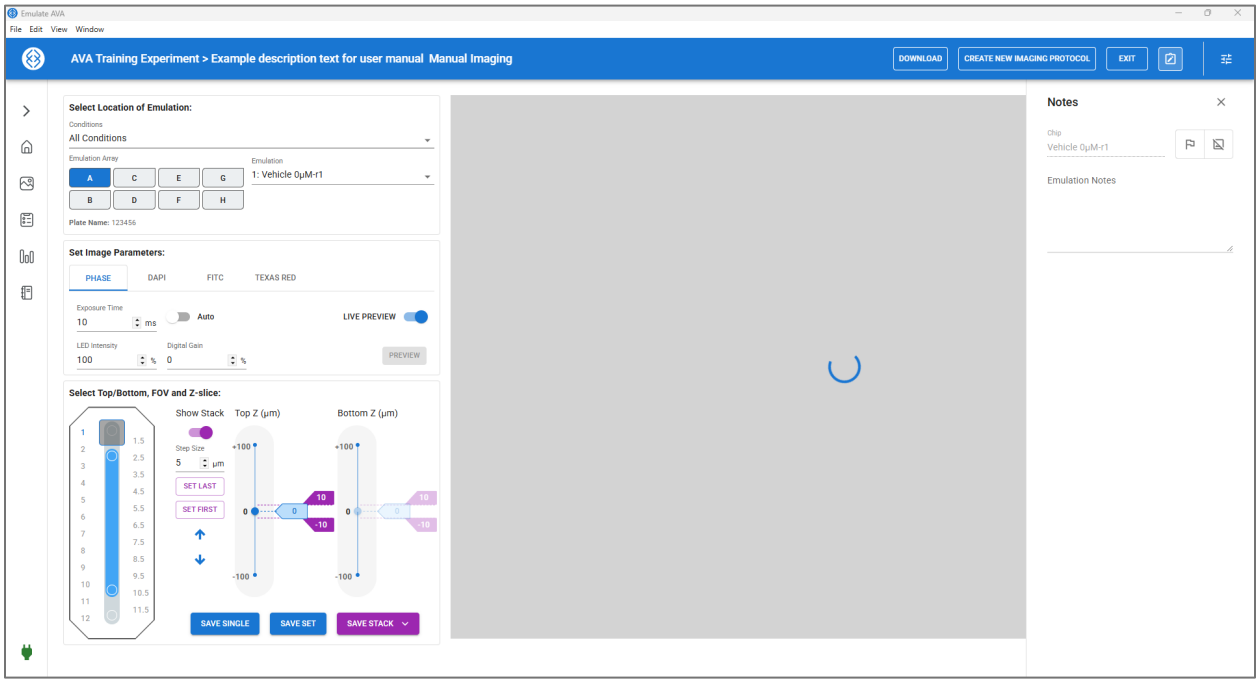
- Press the digital eject button on the AVA Embedded Software (on the AVA built-in screen) corresponding to the desired tray position where the Chip-Array is to be loaded.



- Repeat the process described in the bullet points above to **eject** a Chip-Array.

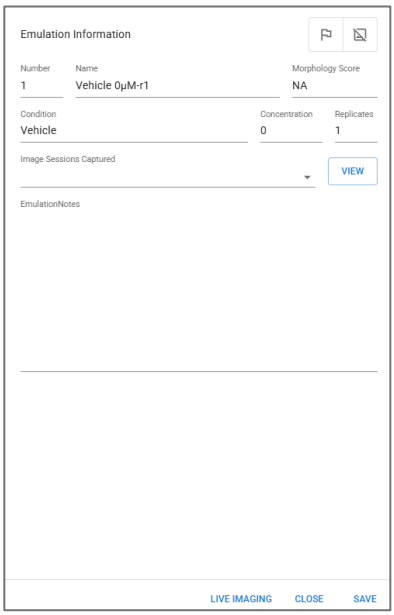
Live Imaging

During an AVA experiment, you can view any Emulation in real-time using the live imaging feature. Below is a screen shot of the Live Imaging UI from AVA software.

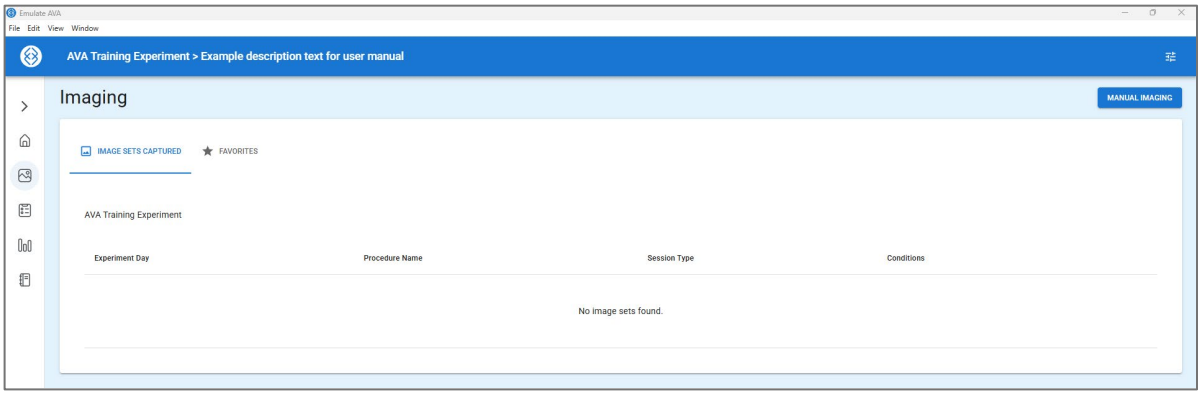


Launch Live Imaging

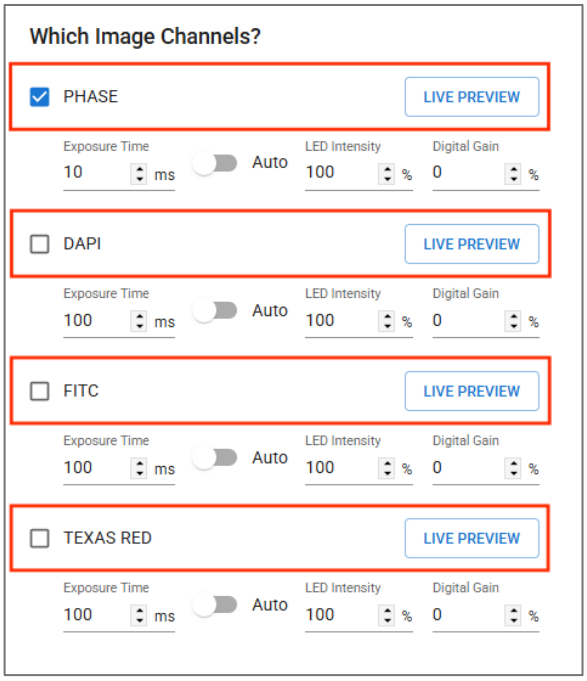
- **From the Home view:** Click a Chip-Array widget to display the Chip-Array widget dialog, then select the desired Emulation, then click **Live Imaging** (lower-right corner). When the Live Imaging view appears, you will see the selected Emulation in the live preview.



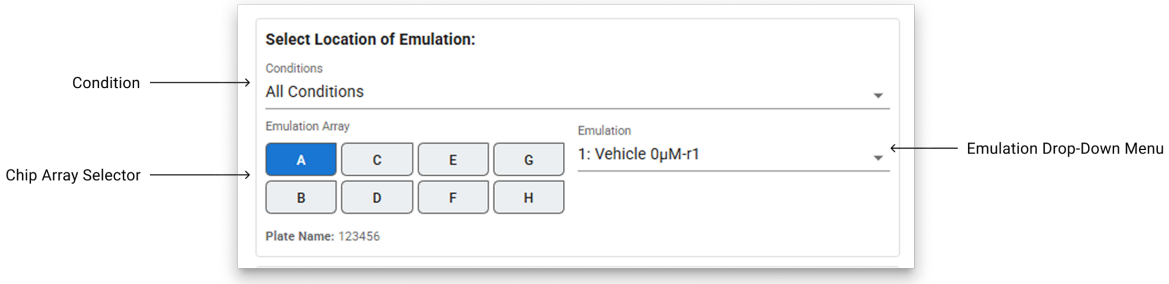
- **From the Imaging Home view:** Using the left navigation menu, click the **Imaging Home** icon to open the Imaging Home view, then click **Manual Imaging** (blue button in upper-right corner of the UI). When the Live Imaging view appears, Emulation 1 on the Chip-Array in position A will be displayed by default.



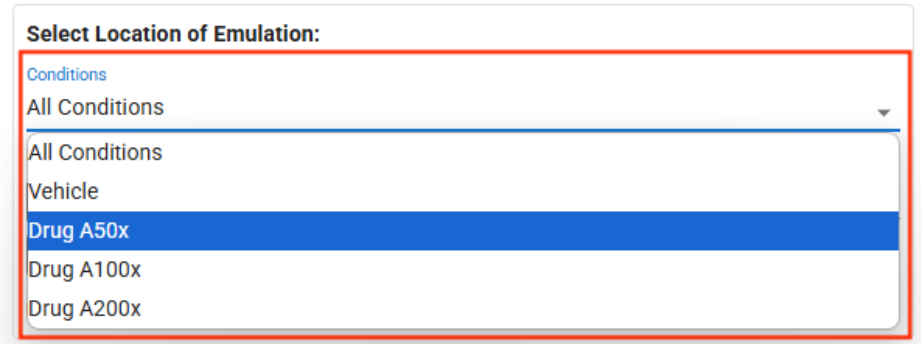
- **From the auto-imaging protocol designer:** Open the Experiment Design > Protocol view, then open an automated imaging procedure or create a new procedure. On this view, you will see four buttons to launch Live Imaging, one associated with each imaging mode available in AVA – phase contrast, DAPI, FITC and Texas Red. When the Live Imaging view appears, Emulation 1 on the Chip-Array in position A will be displayed by default.



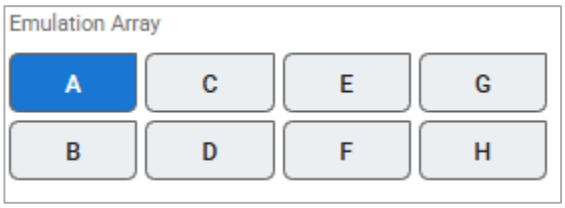
Select Imaging Location



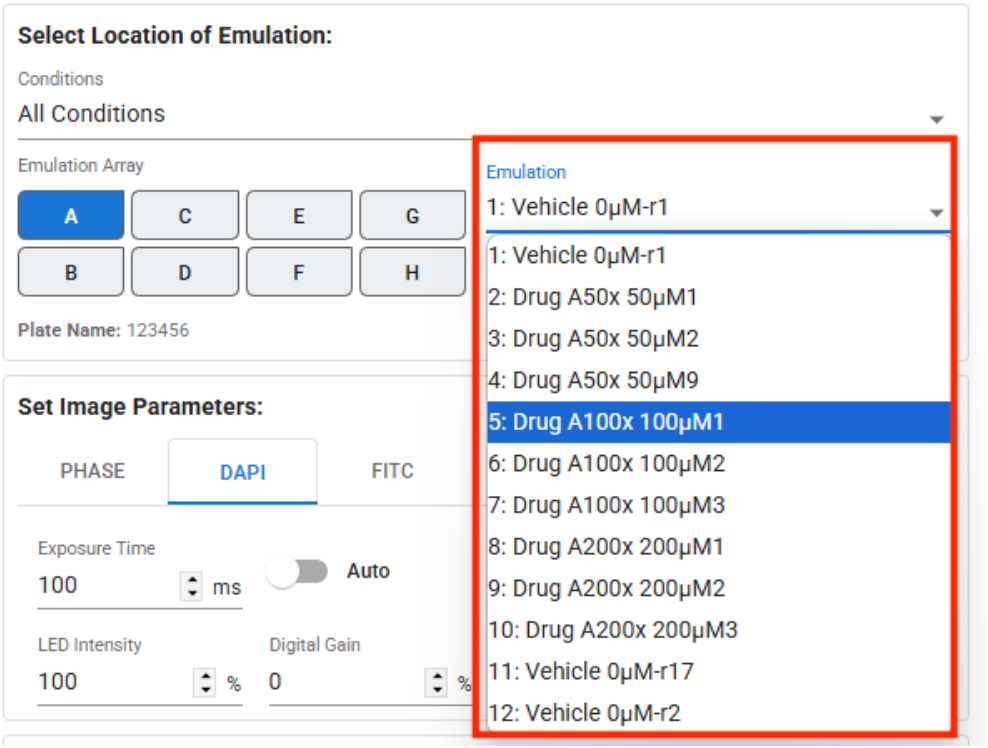
- **By Condition:** Use the **Condition drop-down menu** to filter the selection options in the **Chip-Array selector** & **Emulation selector** drop-down menu based on the Condition selected. Once you make a condition selection, only Chip-Arrays that have the selected condition will be selectable, along with the Emulation drop-down menu. To reset your selection, choose **All Conditions** from the **Conditions** drop-down menu.



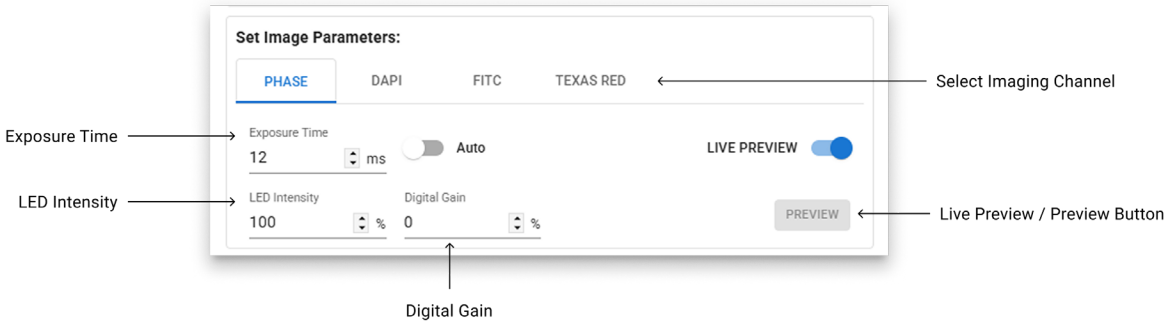
- **By Chip-Array:** The **Chip-Array selector** is used to select a Chip-Array position of the desired Chip-Array for live imaging. If you use the Condition drop-down to choose a specific condition, then only those Chip-Arrays with that condition assigned will be selectable.



- By Emulation: The Emulation drop-down menu enables the selection of the specific Emulation (1-12) for live imaging. If you have assigned specific conditions to your Emulations, then the condition name (including replicate number) will populate this drop-down menu, making it easier to navigate through your experiment conditions and select the correct Chip-Array / Emulation for imaging.

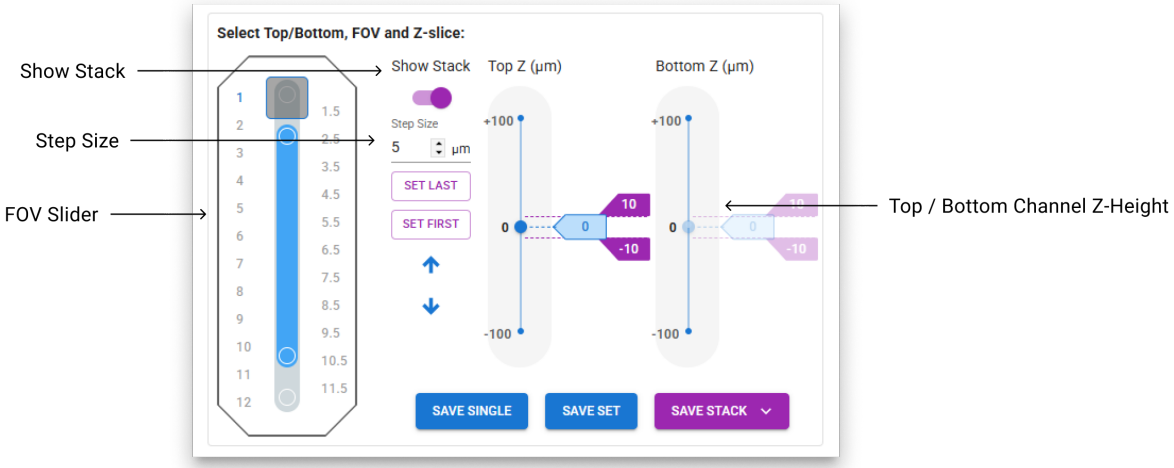


Set Imaging Parameters:



- **Select Imaging Channel:** Click the name (text) of the imaging channel to present the specific parameters for the selected channel. You can tell the channel is selected by the small blue underline below the image channel name. Once you select a channel and adjust the image parameters for that channel (settings below the channel name, see figure). The AVA software will remember those settings as you execute your live imaging session.
- **Exposure Time:** By default, auto exposure is toggled ON. This feature will automatically determine the appropriate exposure time based on the characteristics of the live image. You can disable auto exposure using the **Auto** toggle switch to the right of the exposure time field; doing so allows you to input custom exposure time (in milliseconds) per channel.
- **LED Intensity:** Adjust the LED intensity from 0-100% based on the characteristics you observe while live imaging.
- **Digital Gain:** Adjust the digital gain from 0-100% based on the characteristics you observe while live imaging. Adjusting digital gain amplifies the camera signal to increase image brightness when imaging without affecting exposure time or illumination intensity. This can be useful for visualizing low-intensity signals. Increasing digital gain also amplifies background noise, which may result in a grainier image. For best results, optimize illumination and exposure settings before adjusting this setting.
- **Live Preview / Preview Button:** By default, Live Preview is ON for the phase contrast image channel. For the other 3 imaging channels (DAPI, FITC, Texas Red), Live Preview is OFF. Use the **Preview** button to turn ON live preview as needed.

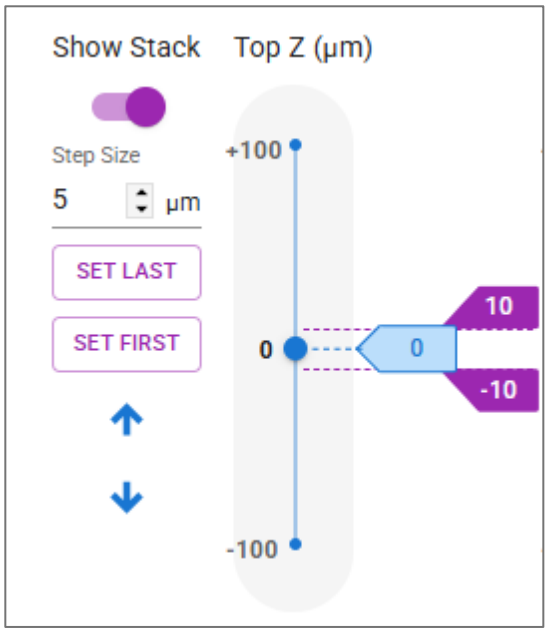
Select FOV, Top/Bottom Channel Z Range(s):



- **FOV Slider:** The **Field of View (FOV) slider** is used to choose a region along the Top / Bottom channels for imaging. During Live Imaging, you can slide the FOV slider up / down to choose the specific region you're interested in imaging. You will see the live preview (if ON) update as you adjust the FOV slider.
- **Step Size:** The **Step Size** field represents the distance between images (Z-slices) for the top channel and bottom channel. You can adjust the step size in microns, which then will affect how the Z-slice selector tool moves. For example, if you have your step size value at '5' µm, then you can slide the blue Z-slice selector arrow / dot in increments of 5. Continue reading for more information on the Z-slice selector.
- **Top / Bottom Channel Z-Height:** Once you select a FOV (region) for imaging, use the Z-slice selector tool(s) to choose the appropriate height(s) for image capture. The Z-slice selector slider – the small horizontal chevron + number / blue dot (per channel) – represent the height of the image will be acquired at. You can slide the Z-slice selector tool up / down to adjust the height of your live image preview. Doing this will adjust the live preview in real-time. These Z-slice sliders are analogous to adjusting the coarse / fine focus knobs on a microscope; the larger your step size, the more dramatic the change in "focus" you will observe when adjusting the Z-slice slider, and vice versa. The "proximity" of the next image is determined based on the step size setting.

- **Show Stack:** The purpose of the Show Stack feature is to allow users to choose the Z-slice ranges to capture in the Top Channel and Bottom Channel. By default, this setting is OFF, meaning any images captured in this configuration will be a single image (by clicking Save Single), or a single image for the top and bottom channel (by clicking Save Both). See saving an image / Z-stack (p. 59) for more information. For example, if you set the top channel Z-slice selector at '+10' and the bottom channel Z-slice selector at '-10', then you will capture two images if you click **Save Set**.

  When **Show Stack is enabled**, you will see two additional brackets / selector tools per channel.

  For the **top channel**, use the z-slice slider labelled **Top Z (µm)**.

  - To **set the upper bracket** – the z-slice image in the stack that is the furthest from '0' – for the **Top Z (µm)**, drag the blue Z-slice selector slider to the desired height; the live preview will represent the current position of the Z-slice selector slider.

    Click **Set First**. You will see the top purple bracket move to the location of the Z-slice selector slider, representing the upper-most (furthest from '0') z-slice to capture in the top channel z-stack.

  - To **set the lower bracket**, drag the blue Z-slice selector slider to the desired height for the last, or "bottom", z-slice image to capture in the z-stack for the top channel and click **Set Last**. You will see the bottom purple bracket move to the location of the Z-slice selector slider, representing the last (closest to '0' compared to the top bracket) z-slice to capture in the top channel z-stack.

    For example, in the **top channel**, if the upper bracket (first Z-slice) is set at +25, the lower bracket (last Z-slice) is set at +5, and the step size is 5 µm, then there would be a total of 5 images (Z-slices) captured in the Top Channel Z-slice set (images at +5, +10, +15, +20, and +25 heights).

For the **bottom channel**, use the Z-slice slider labelled **Bottom Z (µm)**.

- To **set the upper bracket** (the Z-slice image in the stack that is the closest from '0' compared to the bottom bracket), drag the Z-slice selector slider to the desired height; the live preview will represent the current position of the Z-slice selector slider.

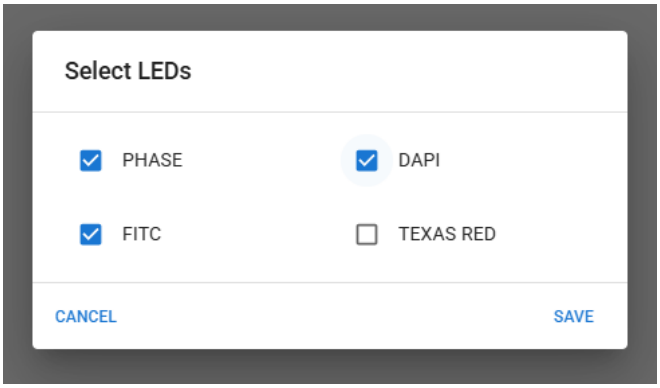
  Click **Set Last**, and you will see the top purple bracket move to the location of the Z-slice selector slider, representing the upper-most (closest from '0' compared to the bottom bracket) Z-slice to capture in the bottom channel Z-stack.

- To **set the lower bracket**, drag the Z-slice selector slider to the desired position / height for the first, or "bottom", Z-slice image to capture in the Z-stack for the bottom channel and click **Set First**. You will see the bottom purple bracket move to the location of the Z-slice selector slider, representing the first (furthest from '0' compared to the top bracket) Z-slice to capture in the bottom channel Z-stack.

Saving an image / Saving a Z-stack: Once you have configured your desired image settings, there are several options for saving an image or set of images.

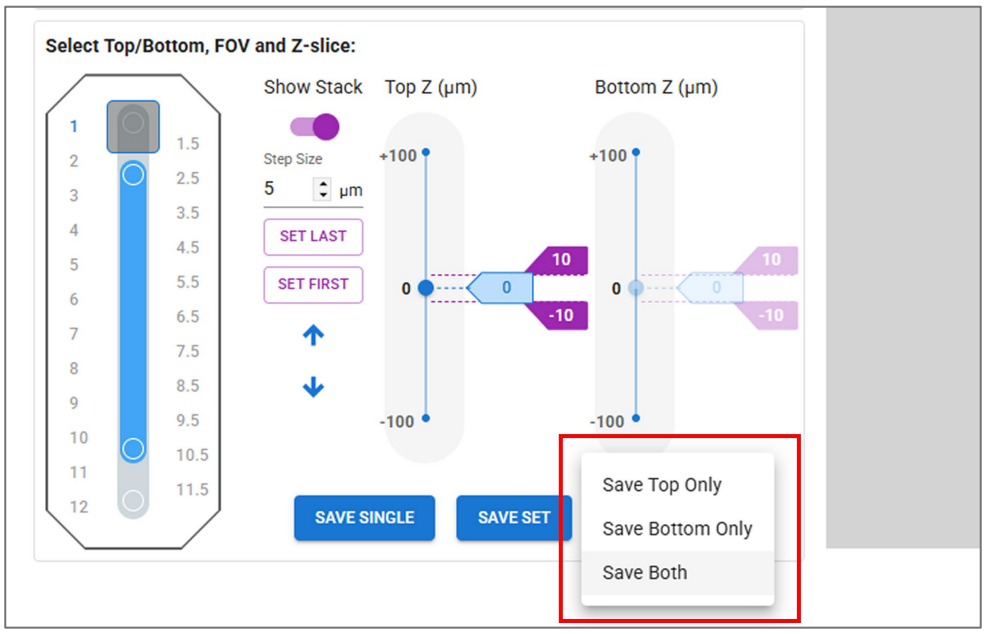
- **Save Single:** This option will save a **single image** (Z-slice) of exactly the region displayed in the live preview of the **currently selected imaging mode**, represented by the blue Z-slice selector slider.

- **Save Set:** This option will save **multiple images** (Z-slices), **pending the selected** (checked) **imaging modes** (e.g. phase contrast, DAPI, FITC, Texas Red) of exactly the region displayed in the live preview of the currently selected imaging mode, represented by the blue Z-slice selector slider.



- **Save Stack:** This option will save **multiple images** (multiple Z-slices, or a Z-stack), pending the configured Z-slice first / last and step size settings for the top / bottom channel.
  - **Save Top Only:** This option will save a Z-stack of only the **top channel**, per the configured Z-slice first / last and step size settings.
  - **Save Bottom Only:** This option will save a Z-stack of only the **bottom channel**, per the configured Z-slice first / last and step size settings.

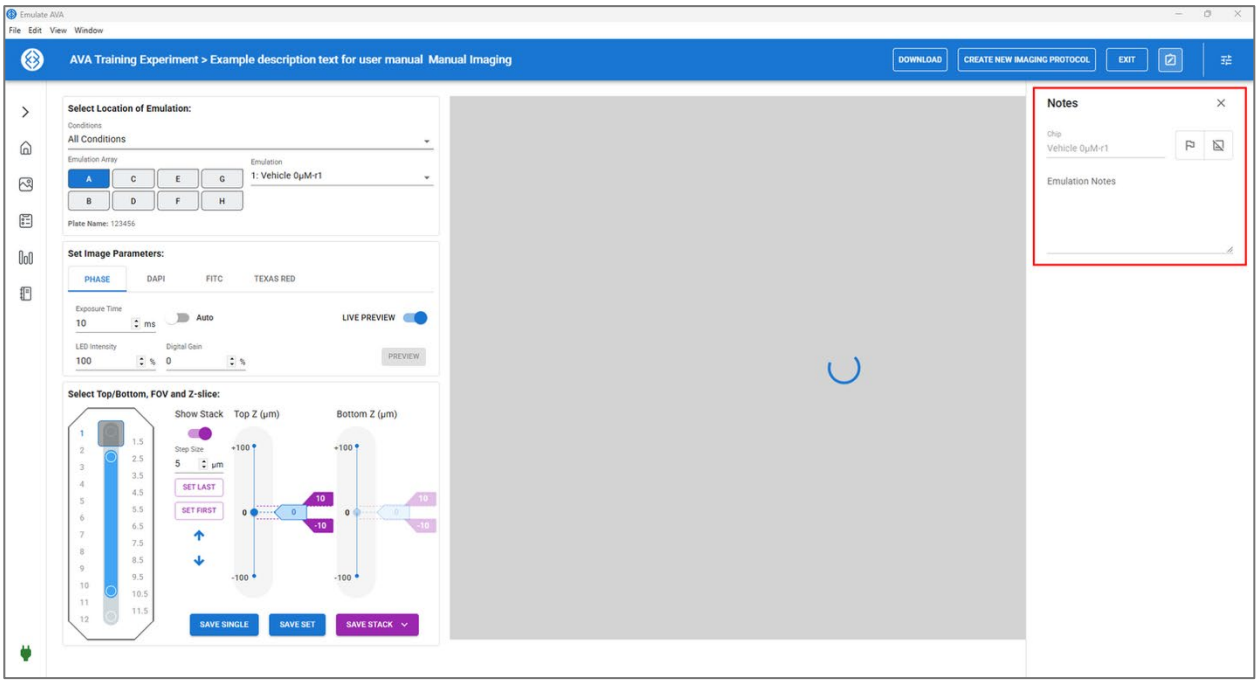
- **Save Both:** This option will save a Z-stack of both the **top and bottom channel**, per the configured Z-slice first / last and step size settings for each channel.



Notes: Click the **Notes** icon in the upper-right corner of the Live Imaging UI (to the right of **Create New Imaging Protocol** button) to expand / collapse the Notes column. In the Notes column you will see the name of the currently selected Emulation for live preview, along with options to:

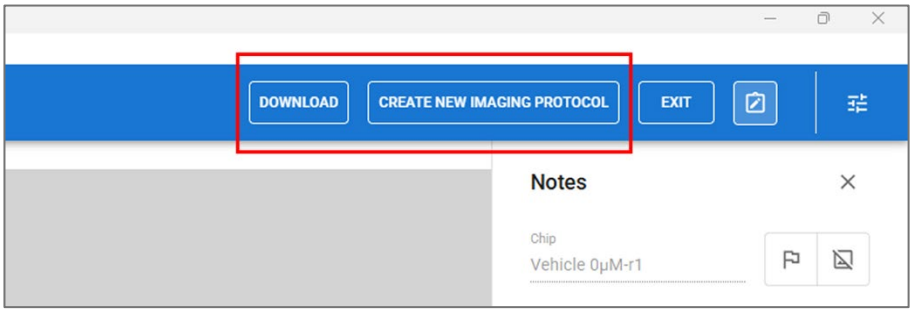
- **Flag the Emulation:** Add a small colored dot to the Emulation that is visible on any software UI that displays the Chip-Array component.
- **Exclude the Emulation**: Exclude the currently selected Emulation from future auto-imaging procedures.

- Add Emulation notes: Add custom user-input notes about the selected image / Emulation, such as flag status, Emulation exclusion, or other important information. Notes added here will be accessible in other areas of the AVA software (e.g., the Home view, Chip-Array Dialog when you select the flagged Emulation).



Other Functions:

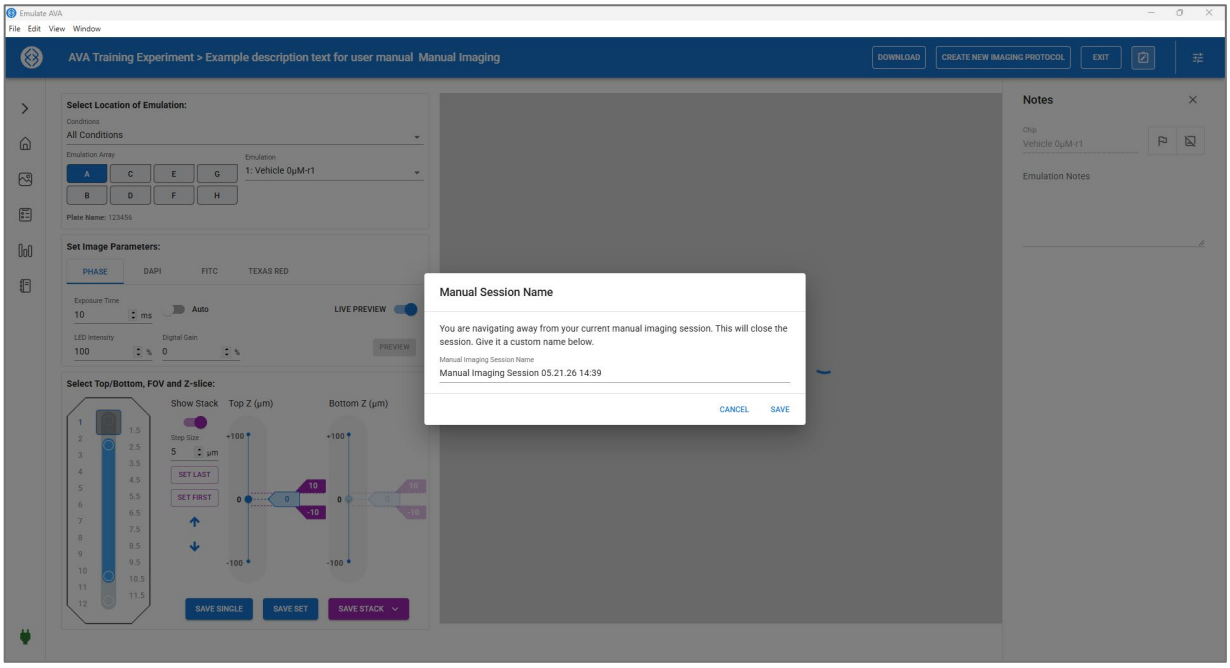
- Download Image: This will automatically save an image of the current live preview.
- Create New Imaging Protocol: This will exit your manual imaging session and launch the auto-imaging procedure designer view. Once this view loads, the parameters from your live imaging session will automatically populate the auto-imaging protocol designer settings. You can then customize the auto-imaging routine. For example, if there were observations for a specific Emulation, you could create a custom automated imaging procedure of this specific region (or regions) and schedule at the desired interval.



Exiting Live Imaging:

Whenever a Live Imaging session is initiated, it must be exited before other operations can be performed in AVA software. To do this, use the **Exit** button in the upper-right corner of the Live Imaging UI. The software will prompt you to enter a name for the live imaging session; a default name will be provided.

Click **Save** to exit the live imaging session or click **Cancel** to return to live imaging. If a live imaging session has been idle for longer than 5 minutes, the software will display a prompt requesting user input. If this prompt is not addressed after a few minutes, the live imaging session will exit automatically.



The purpose of exiting / auto-exiting a live imaging session is to ensure scheduled AVA operations, such as auto-imaging, are not delayed or interfered with due to a live imaging session. Additionally, exiting a live imaging session also helps to organize & manage images captured during that live imaging session; when you return to the Image Home to review images, you will see a specific live imaging session that you can select and review all images captured during that session.

Review AVA Experiment Results

The types of data that can be reviewed in the AVA software are images, tables containing data from AVA instrument operation, environmental sensor data, and user-input data (e.g., morphology scores, notes, etc.).

At any time during your AVA experiment, or after the experiment is complete, you can review data that has been acquired automatically (e.g., images) or through user intervention (e.g., notes, live imaging, etc.).

It is also possible to extract these data for additional processing & analysis using third-party software applications, like Microsoft Excel, ImageJ, etc. Continue reading to learn how to review data using the AVA software as well as how to extract data for analysis using your preferred tool(s).

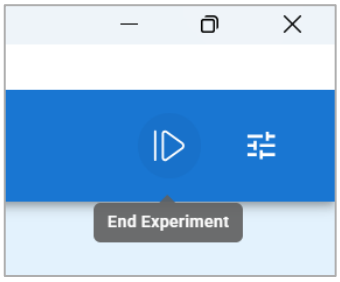
This section of the user manual will explain how to perform the following experiment review functions:

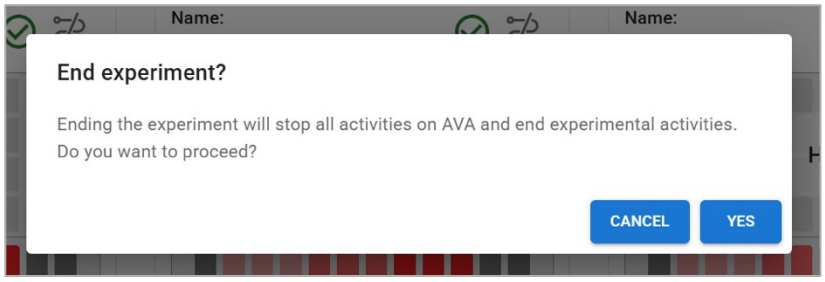
- Ending Your AVA Experiment
- Imaging Home & Image Review
- Morphology Scoring
- Experiment Data
- Experiment Logs

Ending Your AVA Experiment

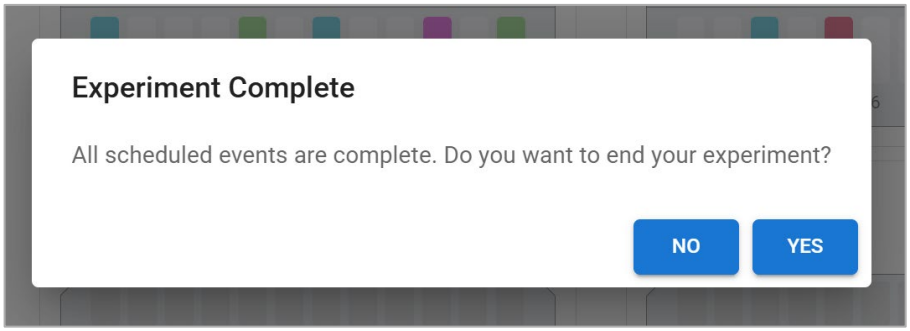
When you have completed the final step in your AVA experiment, you must “end” the experiment using AVA Workstation software. There are two ways to end your AVA experiment:

- **Press the End Experiment button:** The **End Experiment** button is located in the upper-right corner of the AVA Experiment Home view, next to System Settings. Press **End Experiment** to display a confirmation dialog; press **Yes** to end your experiment or **Cancel** to return to the AVA Experiment Home view.





- **Automatic End Experiment dialog:** After the final scheduled protocol event has been marked ‘complete’ (e.g., effluent collection procedure), AVA will display a dialog asking if you are ready to end your experiment. Press **Yes** to end your experiment or **No** to return to the AVA Experiment Home view. You can continue your experiment as long as required by your protocol. Use the End Experiment button (described above) when ready to end the experiment.



- After ending your experiment, you will return to the **Choose an Experiment** view, where you can open the recently ended experiment for review. Please note that after ending an experiment, the AVA Experiment Home view will be disabled when the experiment is opened for review.

Imaging Home:

- **Imaging Home** is where you can find all images associated with an experiment, organized by the type of imaging session (live or automated); think of an *imaging session* as a "folder" of images acquired during a live or scheduled automated imaging session.

  Each row displayed on the Imaging Home view represents an imaging session. Use the column labels to help sort / filter your imaging sessions. To open and review images from a session, click the desired imaging session (row).

- From the **Imaging Home** view, you can **Download** a complete set of images captured in an imaging session, initiate **Morphology Scoring** (e.g. for Liver-Chip Array Quad Culture Experiments), review your **Favorited** images from the experiment, and open a set of images to perform **Image Review**.

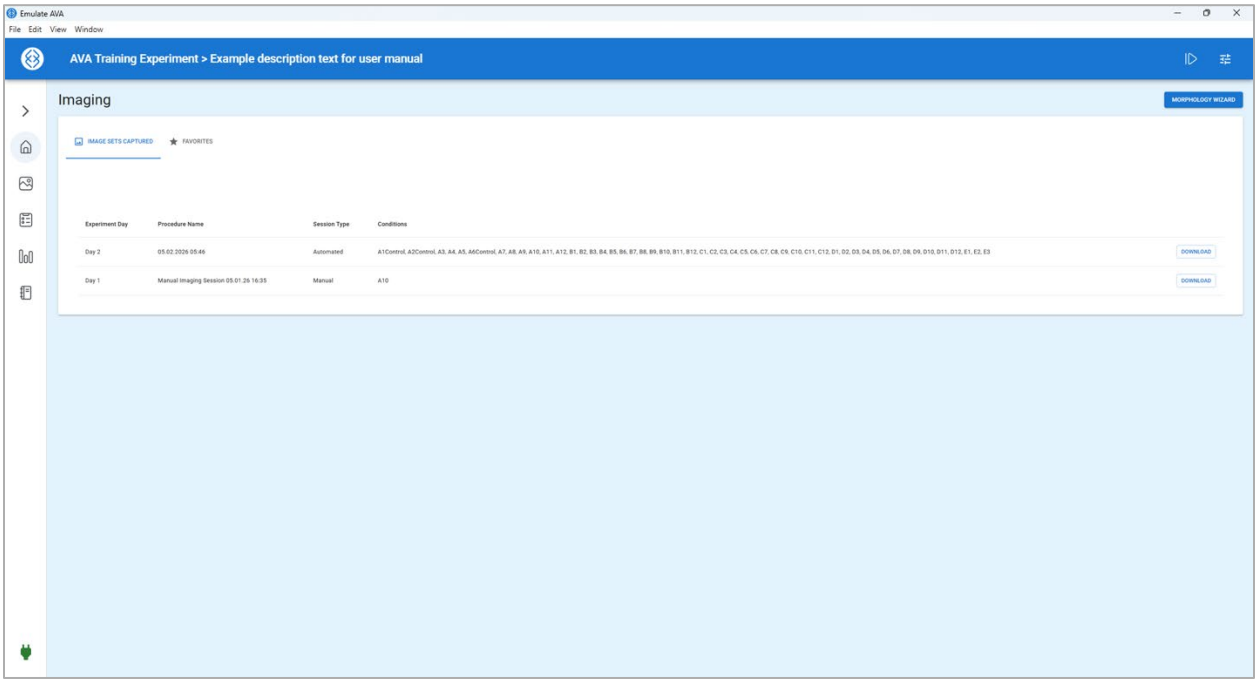
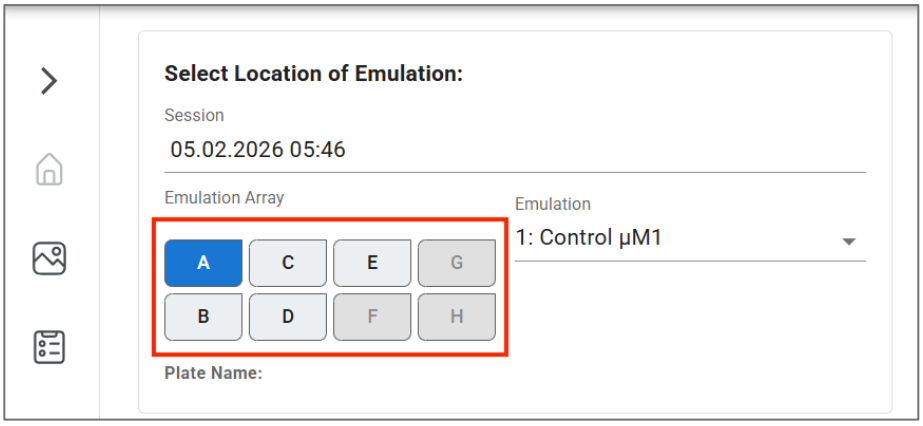


Image Review:

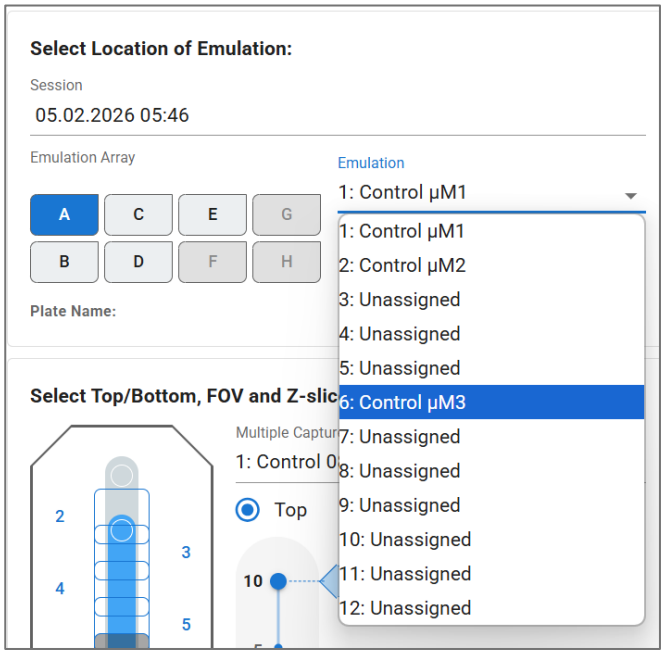
Reviewing images from both live and automated imaging sessions is a routine workflow on AVA. To open an image set for review, simply click on a row (e.g. a live or automated image session) available on the **Image Home**, or click the **Favorites** tab and open an individual image for review.

Image Review Tools: Select Chip-Array and Emulation

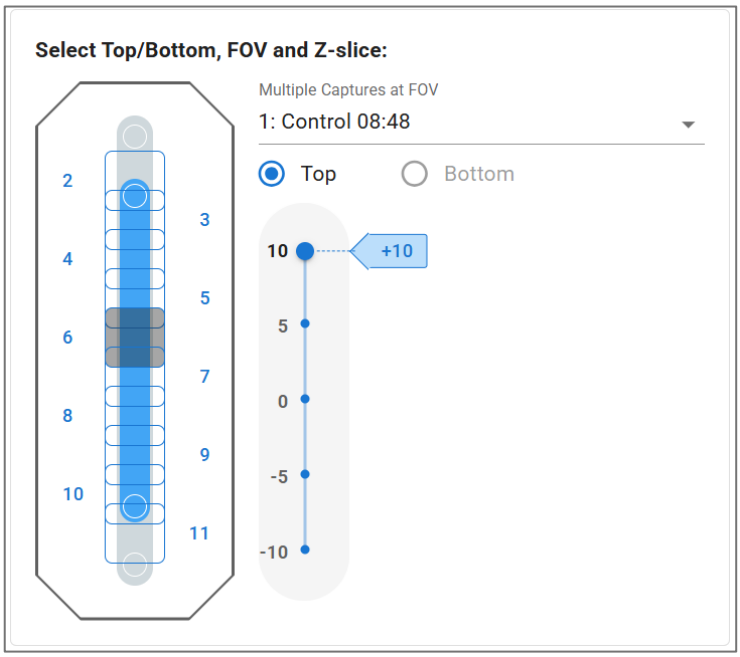
- **Chip-Array Selector:** The Chip-Array selector is used to select a Chip-Array containing images for image review. If you use the Condition drop-down to choose a specific condition, then only those Chip-Arrays with that condition assigned will be selectable.



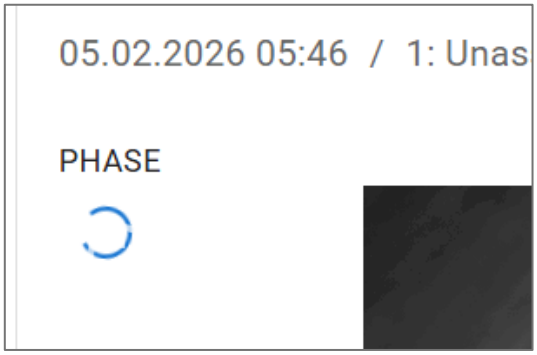
- **Emulation Drop-Down Menu:** The Emulation drop-down menu enables the selection of a specific Emulation (1-12) on the selected Chip-Array for image review. If you have assigned specific conditions to your Emulations, then the condition name (including replicate number) will populate this drop-down menu, making it easier to navigate through your experiment conditions and select the correct Chip-Array / Emulation to review images from.



- **Select FOV, Channel and Z-Slice(s):** The FOV and Z-slice selection tools below represent the regions on the Emulation channel where images were acquired during a live or automated imaging session. For example, in the image below, the FOV labeled '6' is the currently selected FOV for image review, denoted by the dark gray background. To the right is the Z-slice selector component, which shows the number of Z-slices captured for each FOV. In the example below, the Z-slice selection component indicates there were 5 Z-slices captured for each FOV, and currently the Z-slice at position '+10' is selected for display in image review.

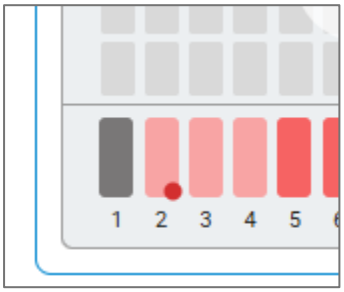


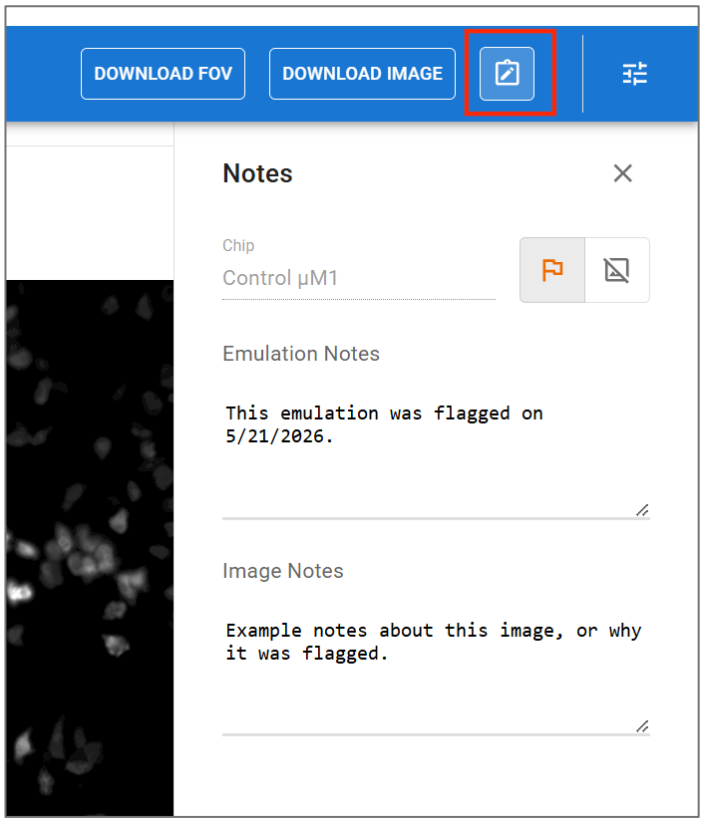
- **Choose Image Channels:** Use the checkboxes to select the imaging channels to view images from. AVA software will display images from the selected channels based on the FOV / Z-slice selection. As you zoom, pan, etc., each image displayed will be adjusted based on your selections.



When you select a new FOV or Z-slice to view you will see a small blue spinning wheel below the channel name, indicating the image is being loaded into the view.

- **Notes**: Click the **Notes** icon in the upper-right corner of the Live Imaging UI (to the right of **Create New Imaging Protocol** button) to expand / collapse the Notes column. In the Notes column you will see the name of the currently selected Emulation for live preview, along with options to:
    - **Flag the Emulation:** Adds a small, red-colored dot to an Emulation on a Chip-Array. The flag is visible throughout the AVA software wherever you see a Chip-Array component.

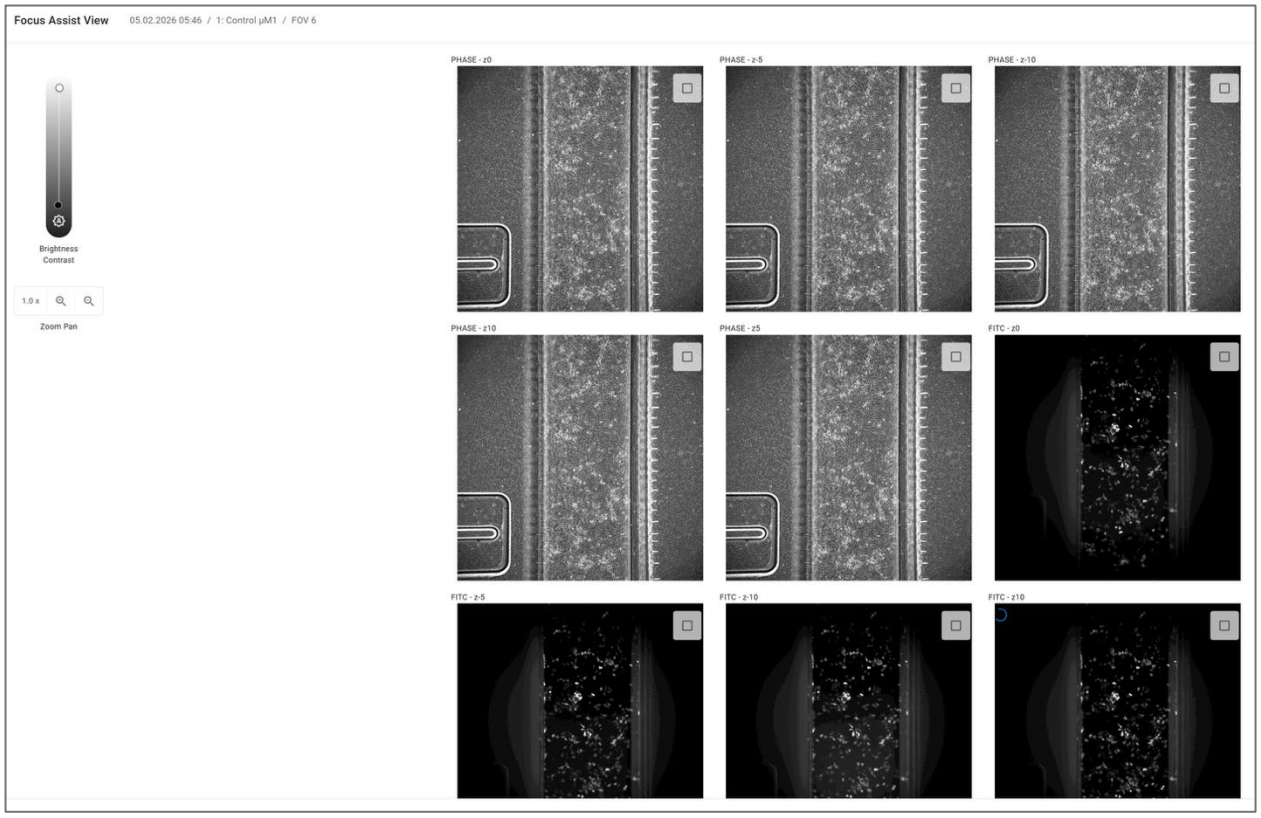




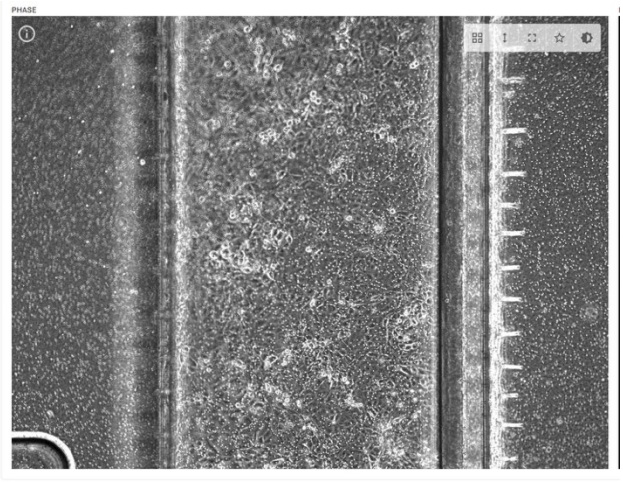
    - **Exclude the Emulation**: The button to the right of the flag button is the exclude feature. Excluding an Emulation will tell the AVA software to exclude the selected Emulation from future auto-imaging procedures.
    - **Add Emulation notes**: Add custom user-input notes about the selected Emulation, such as reasoning for flagging or exclusion, or other important information. Notes added here will be accessible in other areas of the AVA software, such as from the Experiment Home view > Chip-Array widget.
    - **Add Image Notes**: Add custom user-input notes about the selected image. Image notes will be accessible solely through the image review UI.

- **Focus Assist:** The 4-square grid icon, located in the upper-right corner of an image during image review, launches the **Focus Assist** feature.

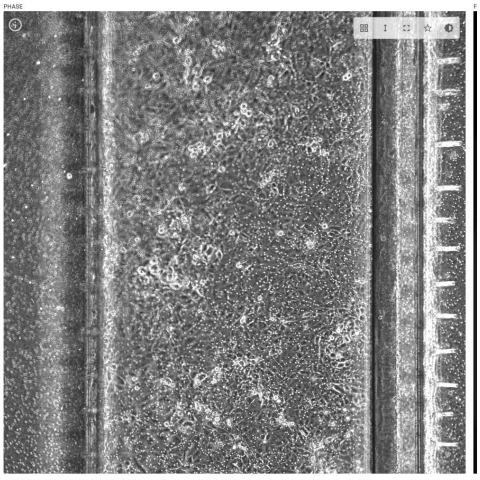
Focus Assist displays all Z-slices acquired for the selected FOV. The purpose of this feature is to allow you to see all Z-slices side-by-side to more easily determine the most representative image for a Z-stack for the selected FOV. In the event you are looking at images from multiple imaging channels (example below; user initiated focus assist while viewing a phase contrast image and FITC image), all Z-slices for the images currently displayed will be populated in focus assist. Use the checkbox in the upper right corner of each Z-slice image to select the desired, representative thumbnail image for the selected FOV. Next time you open Image Review for this Emulation & FOV, the selected image will be displayed by default.



- **Fill Container:** Click the **Fill Container** feature to zoom in on the channel of the currently displayed Z-slice, excluding the edges the FOV that do not have cells.

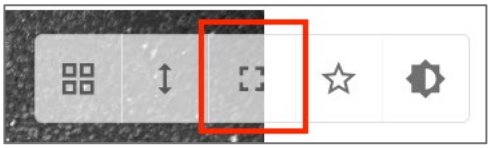


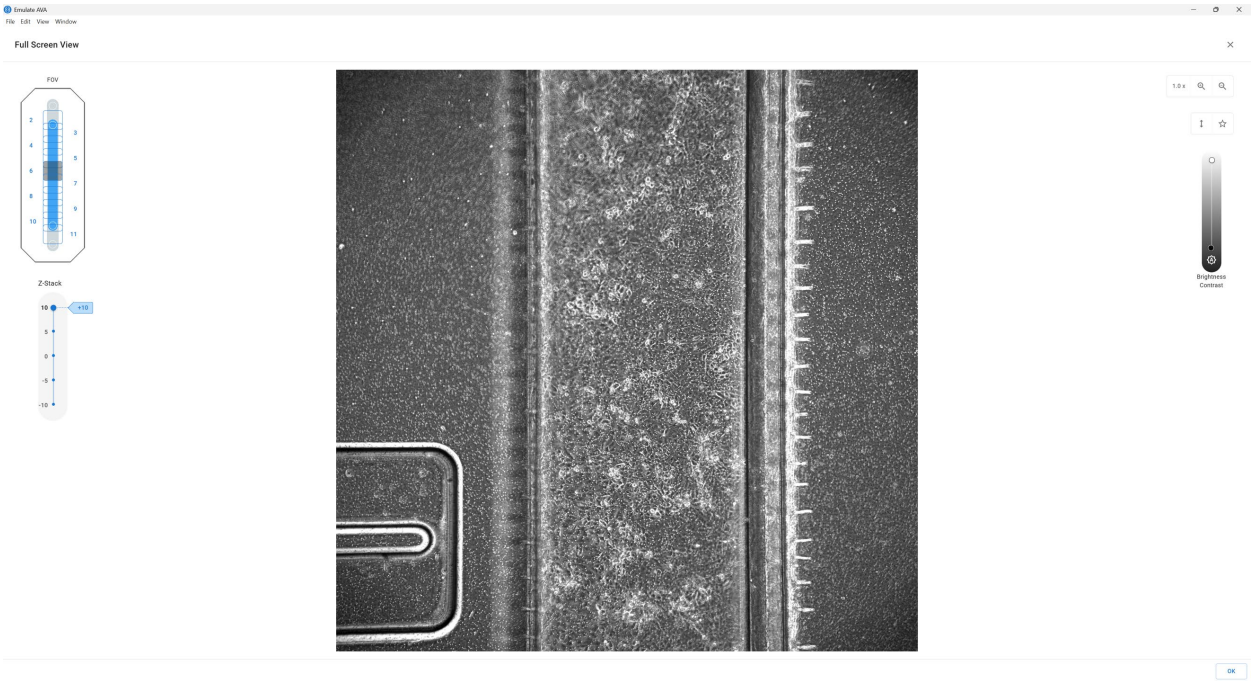
Fill Container OFF



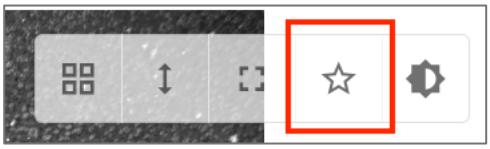
Fill Container ON

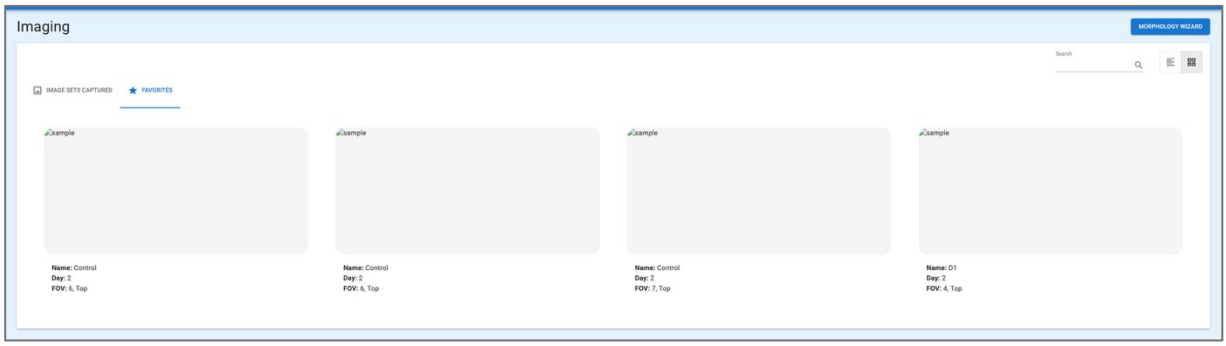
- **Full Screen:** Click the Full Screen button to display a full-screen view of the selected Z-slice. Image review controls are accessible from this view, allowing you to choose additional FOVs and Z-slices for review while in full screen mode.



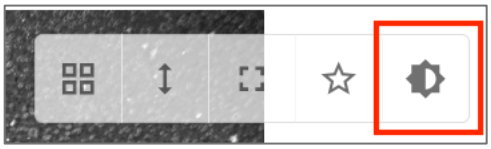


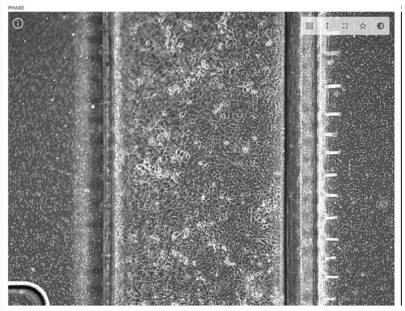
- **Favorites:** During image review, you can favorite images using the star icon, found in the upper-right corner of the displayed image. These favorited images will be grouped together on the **Favorites** tab, found on the Image Home view.



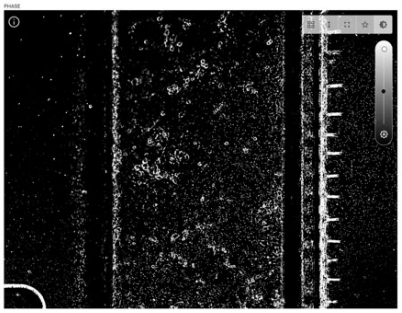


- **Histogram:** Use the Histogram feature to adjust the brightness of the acquired image. The image will be adjusted based on the darkest & brightest regions detected in the image.

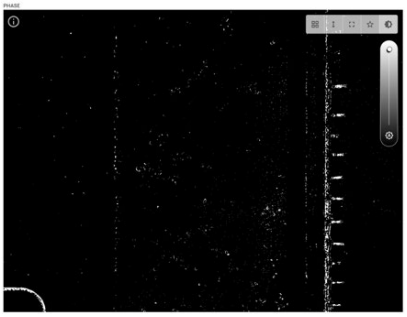




Histogram OFF



Histogram Middle Adjustment



Histogram Fully Dark

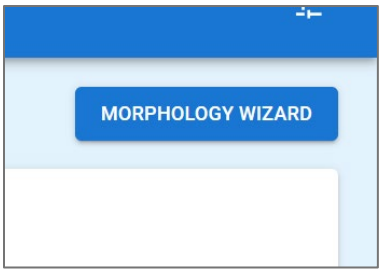
- **Download FOV:** This function is located in the upper-right corner of image review and will download all Z-slices (images) from all image channels for the selected FOV. Follow the software prompts to complete the download.

- **Download Image:** This function is located in the upper-right corner of image review and will download the currently displayed Z-slice (image) only. Follow the software prompts to complete the download.

Morphology Scoring:

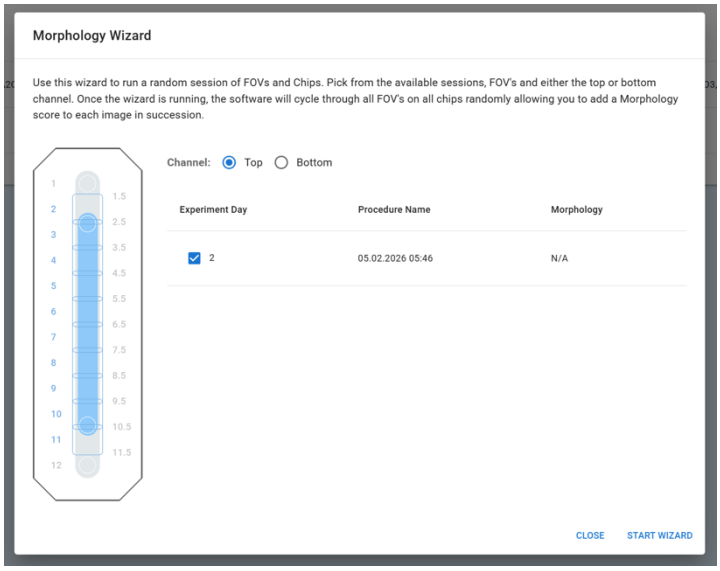
This feature enables users to assign a morphology score (0-4 and “N/A”) to an Emulation in order to represent the health of the overall Emulation. A morphology score represents the average of the individually assigned morphology scores per FOV, per top and bottom channel, per Emulation. During a morphology scoring session, you can select one or more automated image sessions to score at a time; images from these sessions will be randomized by channel to mitigate bias. No information about the specific Emulation, FOV, channel, condition, etc. will be presented to the user during a morphology scoring session.

To start a morphology scoring session:



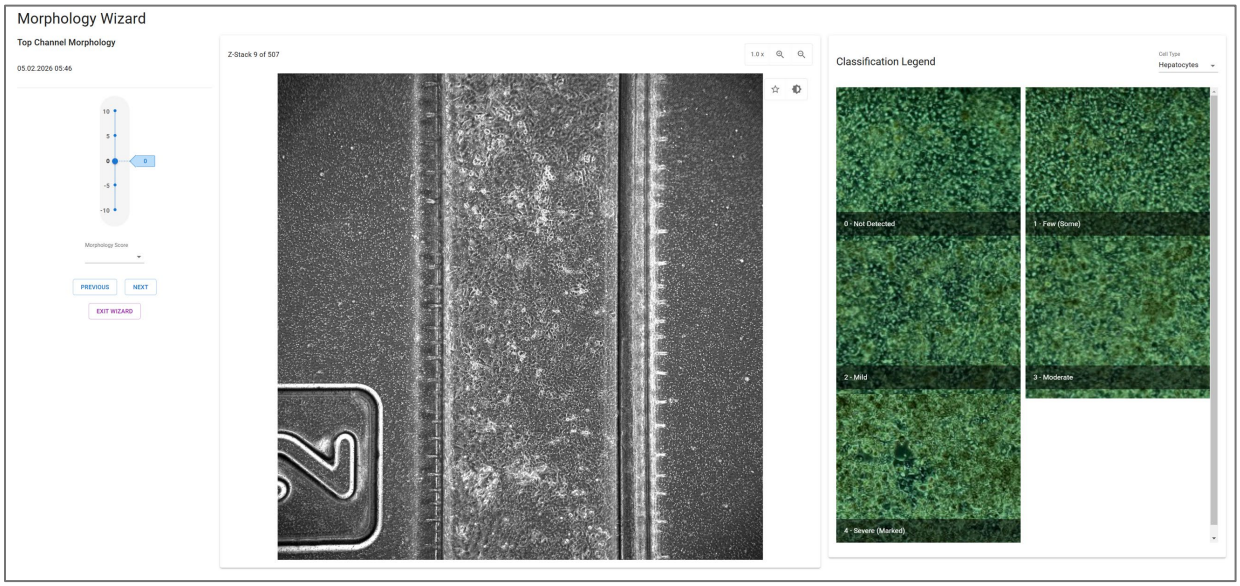
1. Open the Imaging Home view (from left-navigation menu).
2. Click **Morphology Wizard** to display the wizard dialog. If this button is deactivated, then your experiment does not contain any images that were generated from an automated imaging procedure.
3. Select a **Channel** (Top or Bottom); the available image sets will be displayed in the table below.
4. Click the checkbox next to the desired image sessions to include in your scoring session.

   After choosing image sessions, the interactive Emulation graphic will update to indicate the available FOVs for the morphology scoring session.



5. Select FOVs on the Emulation to include / excludeimages in your scoring session.
6. Click **Start Wizard** to launch the scoring session.

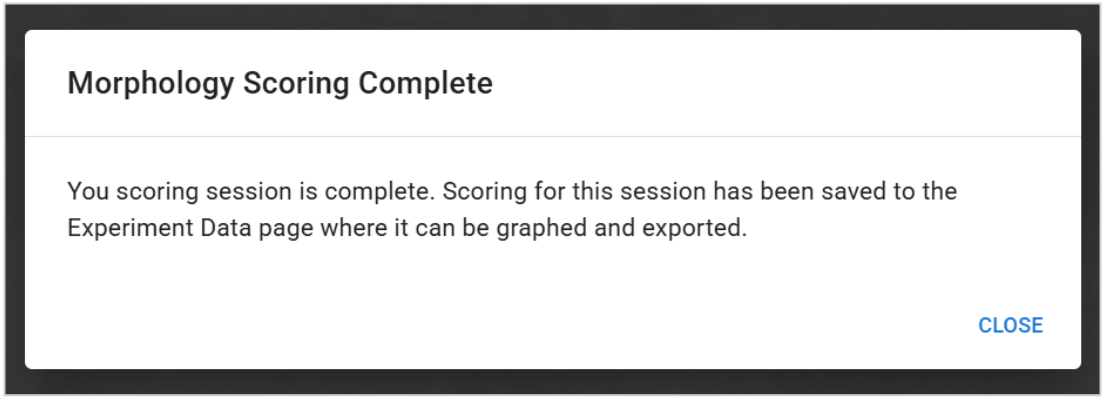
The left side of the Morphology Wizard displays the selected channel (top or bottom), imaging sessions included in the wizard, and controls for scoring:



- **Z-slice slider:** Use the Z-slice slider to adjust the image height / Z-slice ("focus") for the currently selected FOV and channel. If there is only one Z-slice for the displayed FOV, then the slider will be fixed at the height position for the displayed image. If there are multiple Z-slices for the FOV displayed, they will be represented on this slider and available for selection & display.
- **Morphology Score Assignment:** Use the Morphology Score drop-down menu to assign a value of 0-4 (in 0.5 increments), or 'N/A', to the currently displayed image.
- **Previous / Next FOV:** Use the **Previous** and **Next** buttons to navigate through the selected image set(s) for the selected channel. Remember the images from the selected set(s) are randomized. Images / FOVs that have been scored previously will be included in the randomization algorithm, but the previously assigned score will be maintained.
- **Classification Legend:** On the right side of the Morphology Wizard view, you will see the **Classification Legend**. This is intended to provide general morphology scoring guidance for the Top and Bottom channels. Use the **Cell Type** drop-down menu to display the legend for other cell types in the Liver Quad Culture Model.
- **Other Controls:**
  - **Favorite:** Click the small star in the upper-right corner of the displayed image to favorite the image. All favorited images will be available for review on the **Favorites** tab on the Imaging Home view.
  - **Brightness:** Click the half-filled circle icon next to the Favorites icon to display the digital brightness slider. Manually adjust the slider by dragging the circle up or down, or click the {A} to toggle auto-brightness.

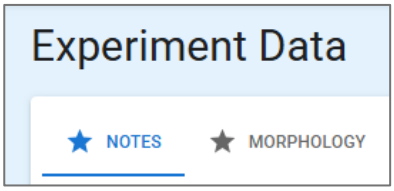
  - **Zoom / Pan:** Use the zoom drop-down to zoom in or out of the displayed image. When zoomed in, the Pan hand will appear, allowing you to click and drag to navigate the zoomed-in image.

- **Pause Scoring Session / Exit Wizard:** It is possible to exit and return to the same Morphology Scoring session (same set of images) later. Use the **Exit Wizard** button to exit the wizard. When you want to initiate the scoring session again, simply follow steps 1-5 above.
- **Completed Scoring Session / Exit Wizard:** When you have scored the final FOV in the set of images in the scoring session, the Morphology Scoring Complete dialog will appear. Click **Close** to close the scoring session and return to the Imaging Home view or click **Go To Scores** to go to **Experiment** Data / Morphology table view.



Experiment Data:

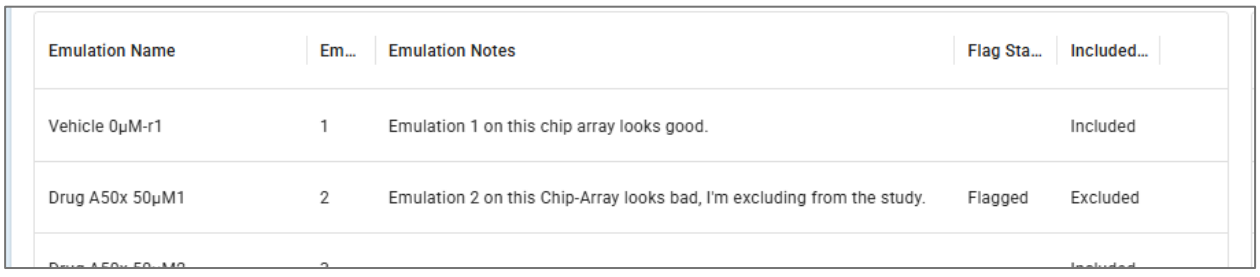
The **Experiment Data** view organizes all user-input data into two tabs called **Notes** (e.g., general notes, morphology scores, and more) and **Morphology,** which offers more tools for viewing & interacting with morphology score data.



The **Notes** tab view organizes data by Chip-Array, as individual tables. Each Chip-Array table contains the following information:

- **Plate Name (A-H):** This corresponds to each Chip-Array's tray position in AVA (A – H) as well as the user-defined Chip-Array name (if provided).
- **Chip-Array Serial Number:** This information is automatically imported from the Chip-Array detection process after AVA reads the unique QR code of the Chip-Array.
- **Chip-Array Notes:** This column contains user-provided notes for the Chip-Array, which are accessible when clicking a Chip-Array widget from the AVA Experiment Home view.

Data in each Chip-Array table represents the 12 Emulations per Chip-Array. Each row represents the Emulations on the Chip-Array, and the column headers represent the data associated with each Emulation.

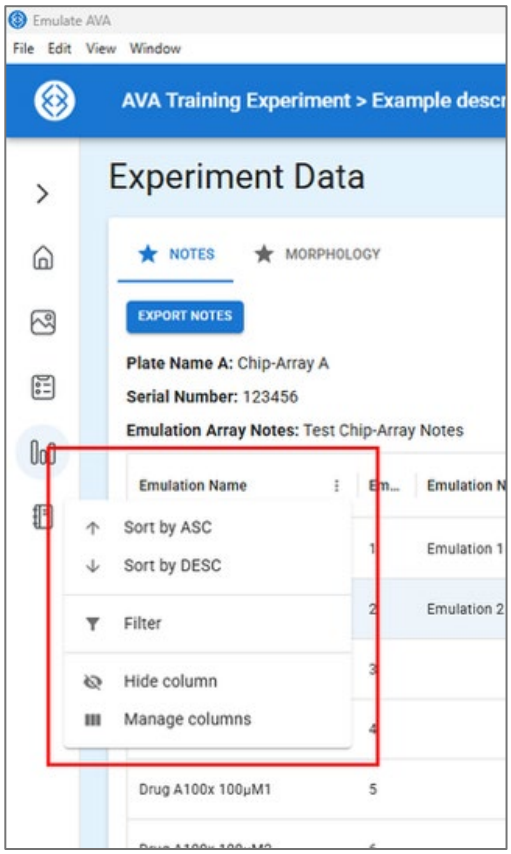


Emulation Name	Em...	Emulation Notes	Flag Sta...	Included...
Vehicle 0µM-r1	1	Emulation 1 on this chip array looks good.		Included
Drug A50x 50µM1	2	Emulation 2 on this Chip-Array looks bad, I'm excluding from the study.	Flagged	Excluded

- **Emulation Name:** The name automatically generated during Condition definition and assignment.
- **Emulation Number:** The Emulation number on the Chip-Array (1-12)

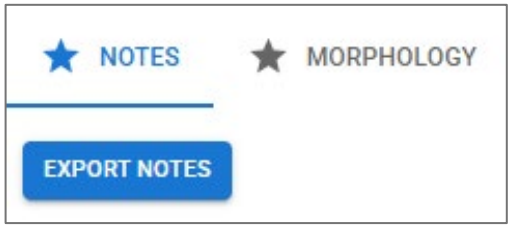
- **Morphology Score:** Calculated value representing the average of the individual FOV morphology scores as input by the user. See Morphology Scoring (p. 73) for more information.
- **Emulation Notes:** User-provided notes.
- **Flag Status:** Indicates whether the Emulation was flagged or not flagged by the user. If this value is empty in the table it represents “unflagged”.
- **Included / Excluded Status:** Indicates whether the Emulation is included or excluded from the study.

You can sort, filter and manage data in each table by clicking the small 3-dot menu next to the desired column header. Edits will be applied to the table (Chip-Array) only, not all tables (Chip-Arrays) in your experiment.

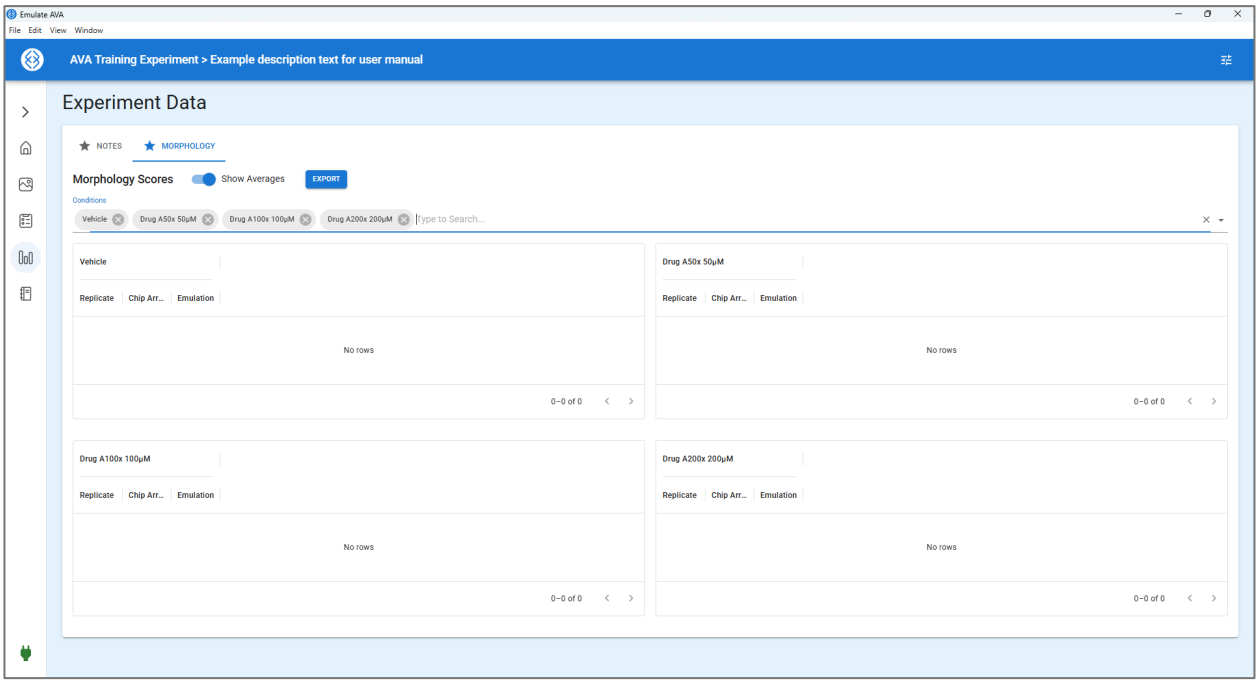


- **Sort by ASC:** Sort the table from smallest to largest based on the column values.
- **Sort by DESC:** Sort the table from largest to smallest based on the column values.
- **Unsort:** Resets the table configuration back to the default.
- **Filter:** Input specific values to filter the data table by.
- **Hide Column:** Hides a specific column.
- **Manage Columns:** Choose which columns to hide/unhide for the selected table.

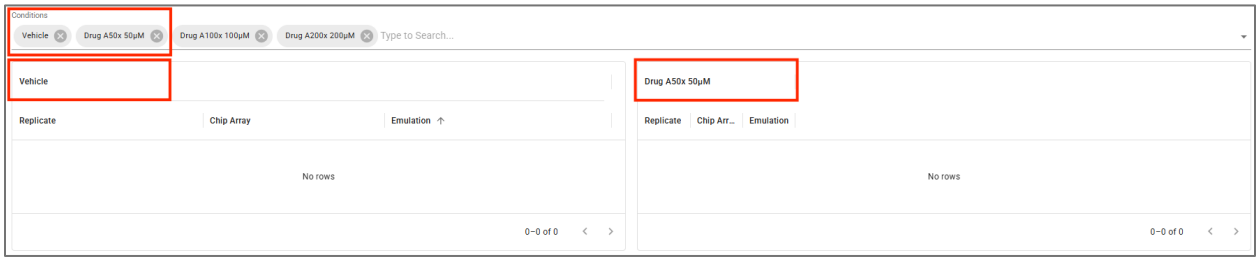
You can **export** all data from the Notes view to an Excel file by clicking the Export Notes button. Use the system dialog to choose a save location and file name.



Morphology: The **Morphology** tab is used to the morphology scores supplied by the user during their morphology scoring session(s) for all experiment conditions.



- Data tables correspond to individual experiment conditions (e.g., Control) that were assigned to Emulations / Chip-Arrays in your experiment.

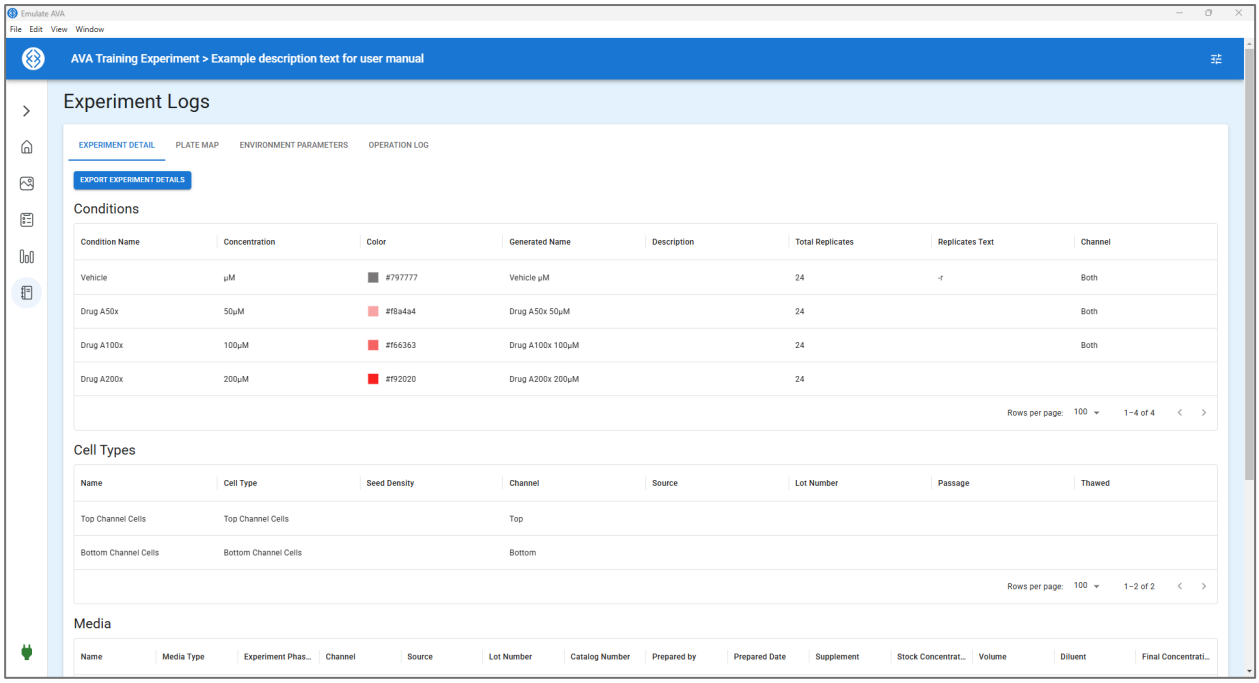


- Each table presents replicate chips assigned to that condition in rows (not pictured).
- You can adjust the data displayed in the morphology score tables to show the average, or independent values for top and bottom channels.
- You can export data to Excel for additional analysis using the Export button in the upper-right corner of this view. Use the system dialog to choose a save location and file name.

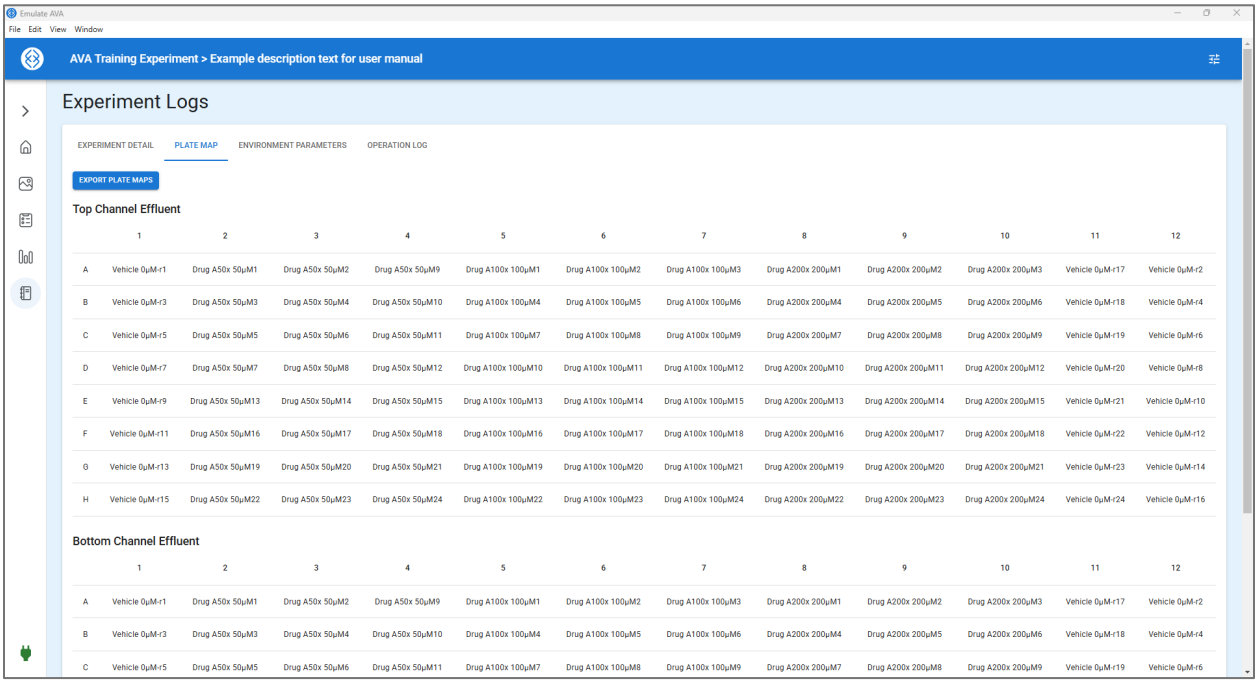
Experiment Logs:

The **Experiment Logs** view organizes user-input data (e.g., experiment design, plate maps, etc.) and AVA-generated data (e.g., operation dates and times) by tab into exportable data tables. Use the built-in tools to sort, search, filter and organize the data tables based on your needs.

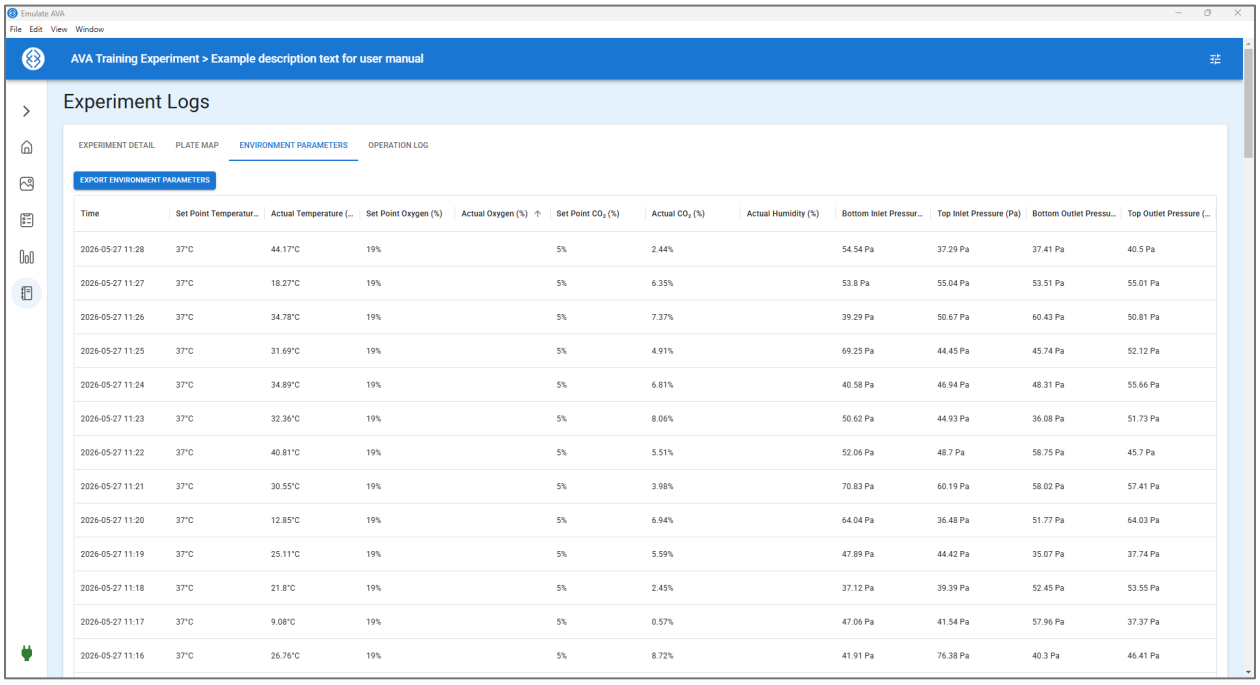
- **Experiment Detail:** This tab displays all data input on the Conditions tab (Experiment Design) into separate tables by Condition, Cell Type, Media and Chip Coating. You will see the data within each table organized by the parent category and by row, representing the individual entries per parent category. For example, if you created two Chip Coatings in your experiment, then you will see two rows in the Chip Coating table, one for each chip coating value generated. You can export data from this view to an Excel file by clicking the **Export Experiment Details** button above the tables. Use the system dialog to choose a save location and file name.



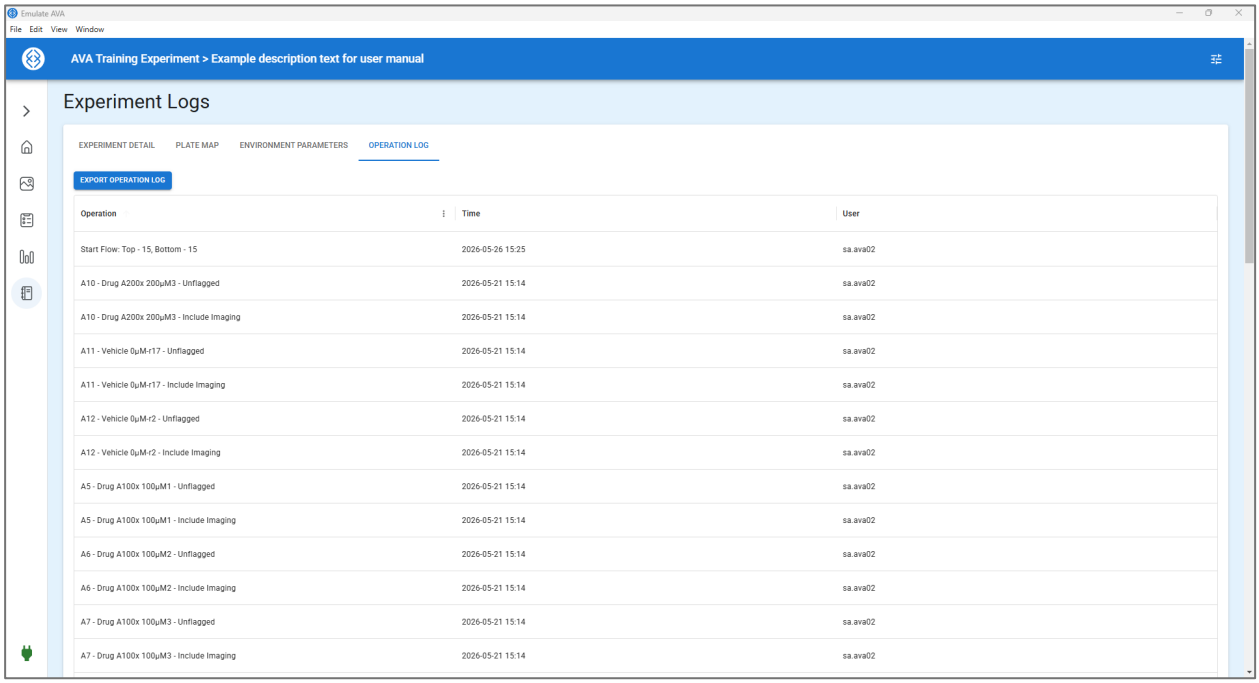
- **Plate Map:** This tab displays the generated condition name (including replicate number) that have been assigned to a microplate map (on the Experiment Design view). You can adjust the condition assignment displayed on this tab by visiting the Microplate Assignment view and making the necessary changes, then returning to this tab. You can export the plate map(s) to an Excel file by clicking the **Export Plate Maps** button above the table. Use the system dialog to choose a save location and file name.



- **Environment Parameters:** This tab displays the environmental parameter sensor log data for temperature, oxygen, carbon dioxide, and humidity acquired during an experiment, from the date and time that the experiment was started on AVA to when the user confirmed the end of the experiment. Export environmental data to an Excel file using the **Export Environment Parameters** button above the data table. Use the system dialog to choose a save location and file name.

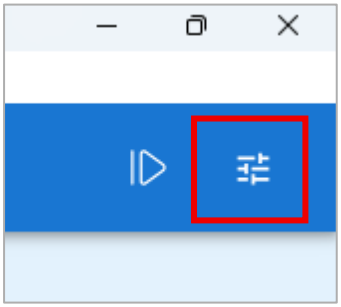


- **Operation Log:** This tab displays all AVA operations executed during an experiment; from the date and time that the experiment was started on AVA to when the user confirmed the end of the experiment. Export the operation log data to an Excel file using the **Export Operation Log** button above the data table. Use the system dialog to choose a save location and file name.



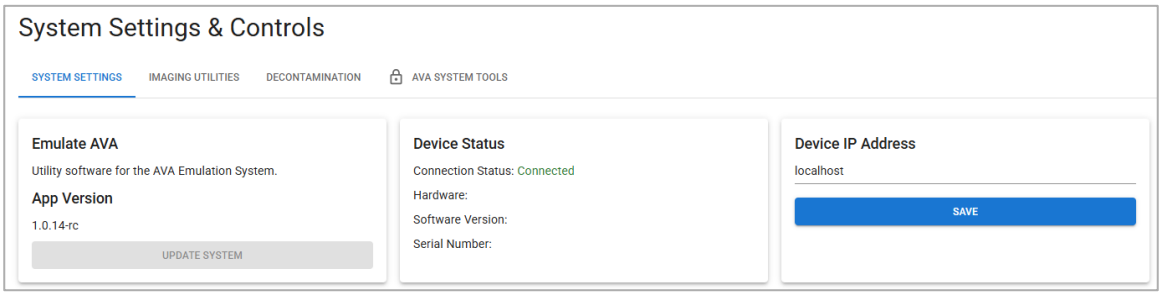
System Settings

AVA System Settings is found in the blue ribbon at the top of the AVA software UI. Click the System Settings icon to open the System Settings view.

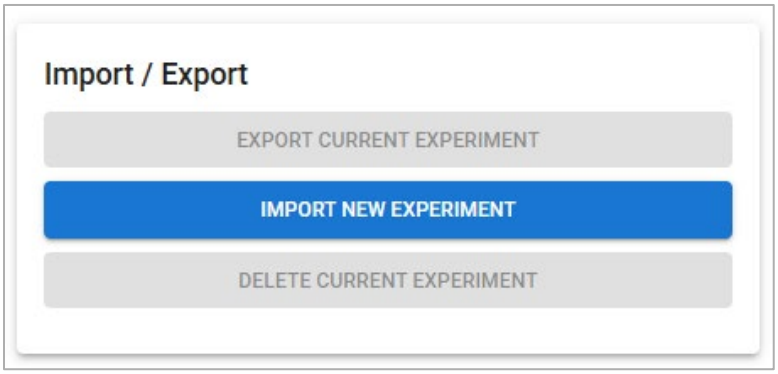


System Settings:

- View Software Version: Displays the current AVA software version.
- Device (AVA) Status: Displays the current instrument/software connection status, hardware version, software version and AVA serial number. This information cannot be edited directly.
- Device (AVA) IP Address: Displays the current IP address of the AVA. Do not edit unless instructed by Emulate-trained personnel.



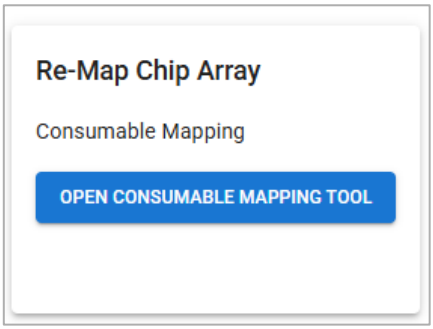
- Import / Export / Delete Experiments:
  - **Import Experiment:** The **Import Experiment** function allows users to upload & view results from a previously performed AVA experiment. This is valuable when you want to view data "offline" (not using the computer connected to AVA) such as a computer in your office or while at home. Click the **Import New Experiment** button, locate the experiment file, click **Open** and wait for the import process to complete.



  - **Export Experiment:** The **Export Experiment** function allows users to move experiments from the AVA workstation computer to another computer for storage / archival, experiment review and analysis, or other purposes. Click the Export Current Experiment button to display a system dialog to select a save location for the experiment. Choose a location, click **Save**, and wait for the export process to complete.
  - **Delete Experiment:** The **Delete Experiment** function allows users to delete experiments that have been archived in a permanent (or semi-permanent location), such as a shared network drive, to free up storage space on the AVA and the AVA workstation computer. To delete an experiment, click **Delete Experiment** and choose the experiment you wish to delete from the list. You will be prompted to type "DELETE" to confirm the experiment deletion. Please note, once you delete an experiment using this function, all data associated with this experiment will be deleted (e.g., images). *This action cannot be undone.*
  - **Power Options – Shut Down:** Press this button to shutdown AVA. Follow the on-screen prompts to complete the shutdown safely.

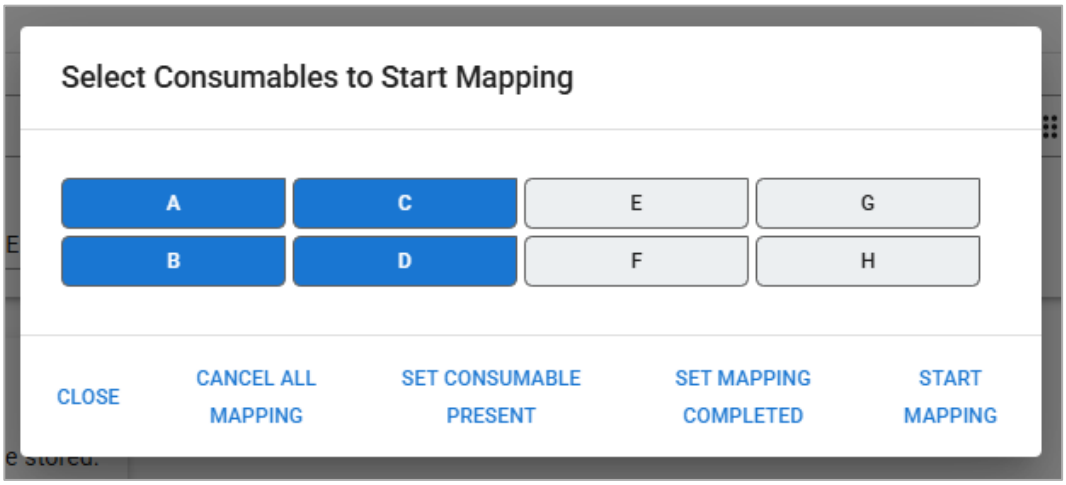
- Imaging Utilities:
  - Image Naming: You can customize the AVA-generated image file names using the Image Naming utility. You will see the default naming arrangement; use the 6-dot component within each name parameter to delete or move the position of the name parameter. You can also add custom text and separators (underscore or dash) as naming parameters. You will see a name preview that reflects the changes made to the individual image naming building blocks above.

  - Consumable Mapping Tool: This function allows users to manage Chip-Array detection and mapping states to ensure proper imaging operation and high-quality images are generated by AVA. Chip-Array mapping is an automated process AVA executes to ensure high-quality images are generated during live and automated imaging sessions.



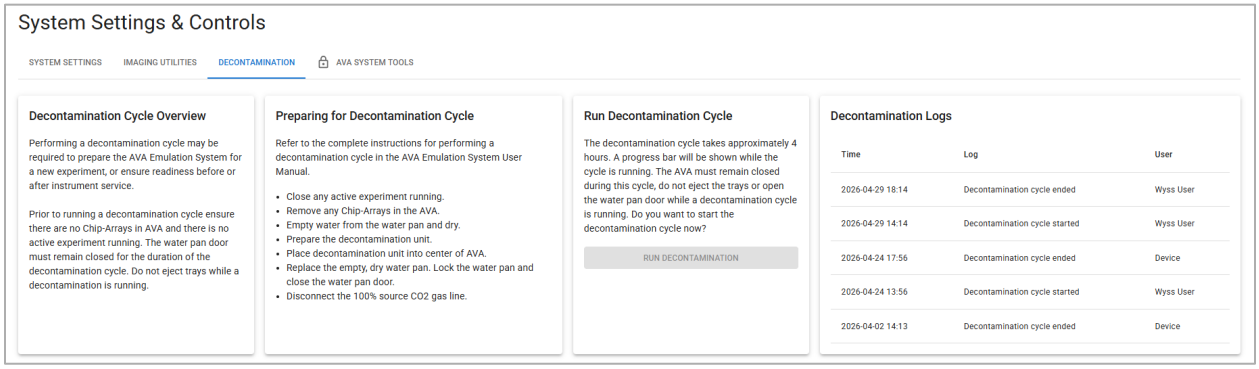
If image quality is poor (e.g., focus is routinely not achieved, poor channel alignment, etc.), it may be necessary to remap a Chip-Array. If you notice poor image quality or issues with Chip-Array detection, please contact Emulate Support and they will direct you how to resolve the situation using the Consumable Mapping Tool.

- Click Open Consumable Mapping Tool



- Click the Chip-Array positions to select Chip-Arrays for remapping or presence detection.
- Set Consumable Present – Use this function if you insert a Chip-Array, but AVA does not show this Chip-Array as 'present' on the Experiment Home view.
- Set Mapping Completed – Use this function if you do not see a green checkmark on a Chip-Array on the Experiment Home view, but you are able to acquire good images from that Chip-Array.
- Start Mapping – Use this function if you do not see a green checkmark on a Chip-Array on the Experiment Home view and you are unable to acquire good images of that Chip-Array. This process will take approximately 30-40 minutes per Chip-Array selected.

- **Decontamination:** At certain times, or as directed by Emulate Support, it may be necessary to decontaminate AVA. The Decontamination tab displays high-level instructions for performing the decontamination cycle, log files from previous decontamination cycle runs, and the button to initiate the decontamination cycle ("Run Decontamination"). Follow the on-screen prompts and the instructions outlined in the section called Decontaminating your AVA Emulation System in this document.



- **AVA System Tools:** This tab is intended for use by trained Emulate Field Service Engineers and should not be accessed or modified by anyone other than Emulate-trained personnel. Modifying the settings on this tab can have serious consequences on AVA functionality and may void the warranty.

Post-Experiment Procedures

Cleaning AVA

Use 70% pre-moistened ethanol wipes for cleaning surfaces and follow aseptic protocols. Cleanable surfaces of the AVA instrument are:

- Exterior housing
- Trays for Chip-Array
- Tray-loading bays
- Water pan bay

Decontaminating Your AVA Emulation System

Emulate recommends using MycoFog™ for decontaminating AVA when necessary, such as after a service visit, preventative maintenance, or suspected contamination event. Prior to starting the decontamination cycle:

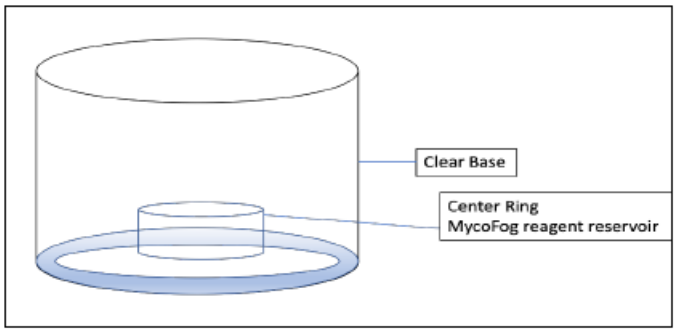
- Confirm the environmental temperature within AVA is 37˚C using the environment widget on the AVA display or in AVA Workstation software.
- Fully charge your MycoFog device (approximately 8 hours).
- Ensure there is no active experiment; it is not possible to perform a decontamination cycle while an experiment is running.
- Please Note: Emulate is not responsible for the performance of the MycoFog device, the following instructions are included in this document for convenience. You should refer to the manufacturer's instructions for the most up to date protocol.

To execute a decontamination cycle, please follow the steps below:

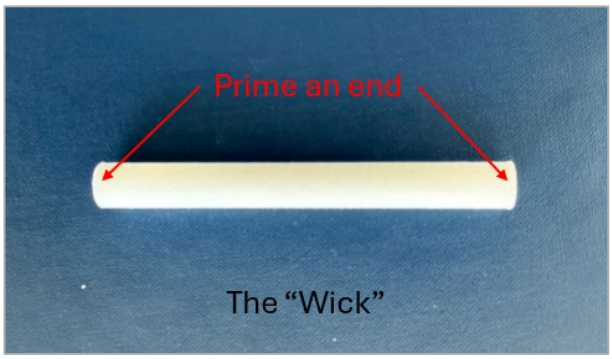
1. Ensure that AVA is powered ON and has reached 37°C.
2. Ensure the MycoFog device is fully charged. The charge indicator light will be ON while charging, and OFF when charging has completed. Use the USB cable provided with the MycoFog device for charging.
3. Remove one reagent bottle and wick from the MycoFog box.

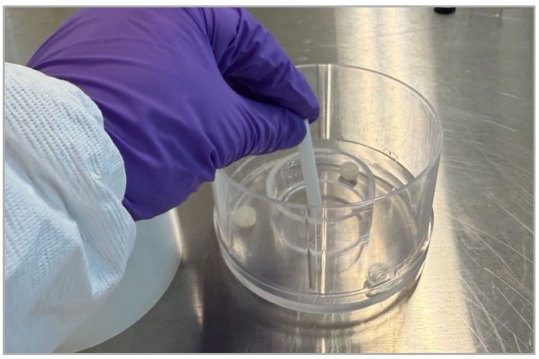
4. Open the MycoFog device by twisting the upper cylinder counterclockwise and lifting it off the clear base lower cylinder. The wick holder is attached inside the upper cylinder; pull it out to remove it.

5. Pour the contents of the reagent bottle into the MycoFog reagent reservoir, located in the center ring of the lower cylinder.

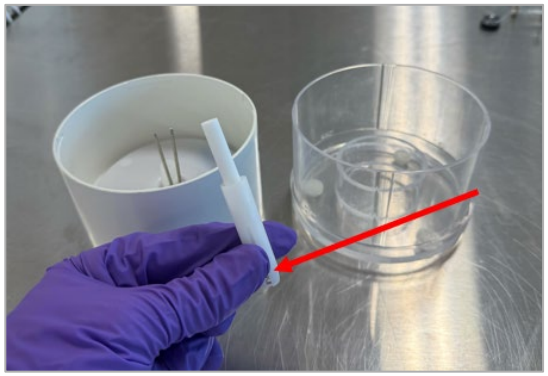


6. Prime the wick by placing one end into the MycoFog reagent reservoir (as pictured below) for one minute.





7. After priming the wick for one minute, place the primed (wetted) end of the wick into the wick holder. The primed (wetted) end of the wick should be placed in the wick holder first, as indicated by the red arrow in the image below.



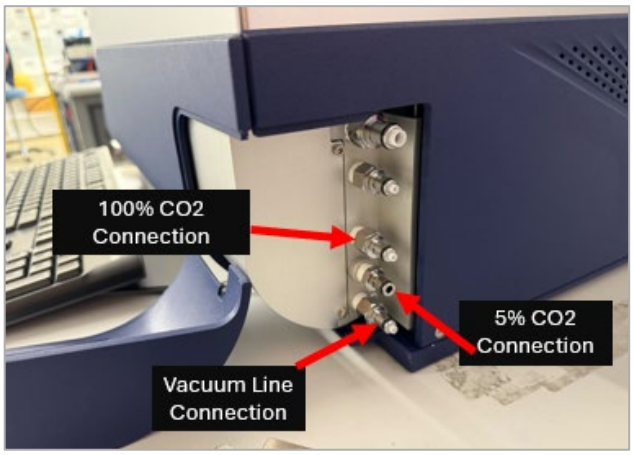
8. Reinstall the wick holder to the MycoFog upper shell by pushing it into place in the upper cylinder.

9. Replace the MycoFog upper shell onto the clear base lower cylinder, then rotate clockwise to lock in position.

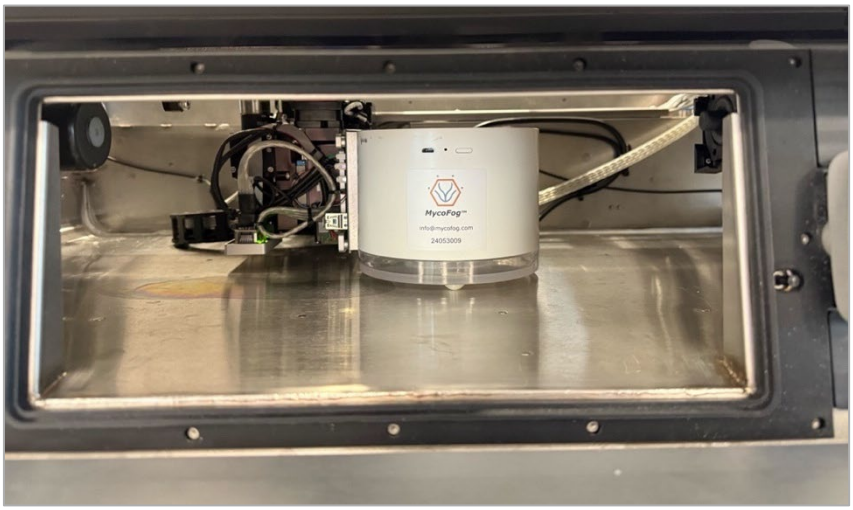
   Do not tip or rotate the clear base lower cylinder, this may cause reagent to spill.

10. Once the MycoFog device is reassembled, wait an additional 5 minutes for the wick priming to finish.

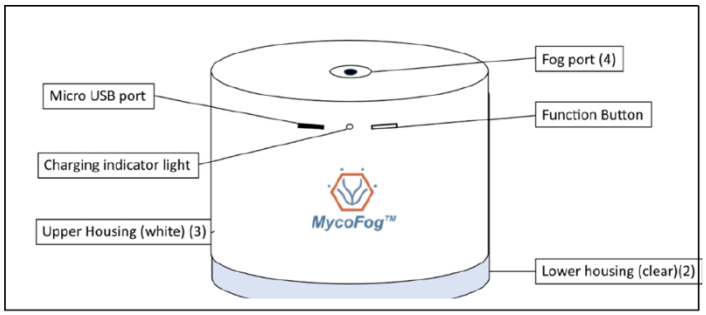
11. Prepare AVA for MycoFog decontamination by:
    a. Disconnecting the 5% CO_2 line from the back panel on AVA (pictured right).
    b. Removing the water pan (front of AVA) and empty the contents. Spray with isopropyl alcohol and wipe down until dry.



12. After 5 minutes has elapsed, wick priming is complete. Ensure the water pan and 100% CO_2 line are disconnected from the AVA. Place the MycoFog device in the center of AVA, through the water pan door. See image below for reference.



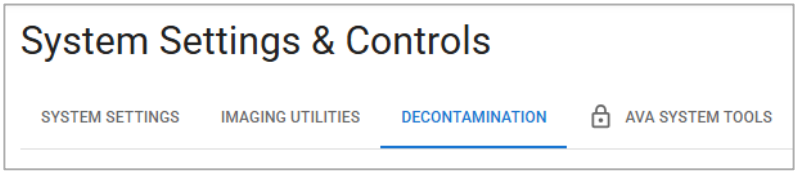
13. Press the **Function** button once. This button is located on the front of the MycoFog device next to the charging indicator light (see diagram below).

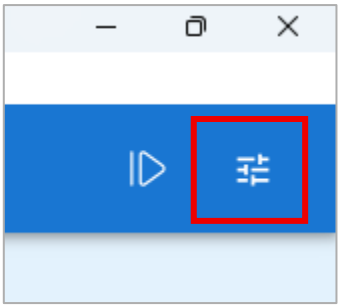


IMPORTANT! Once the **Function** button is pressed, the MycoFog™ device will begin the decontamination process. It is imperative that you perform **Step 14** and **Step 15** *immediately* after pressing the **Function** button.

14. Reinsert the water pan into the AVA instrument, secure in place using the two hand-rotatable clamps, then close the water pan door.

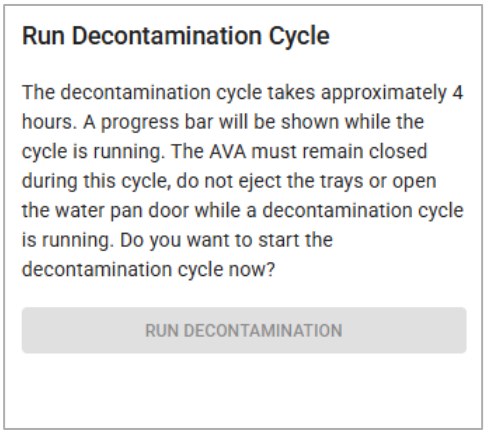
15. Initiate the Decontamination cycle in the AVA software:
    a. Click the **System Settings** icon (found in the upper-right corner in the blue ribbon).
    b. Click the **Decontamination** tab.



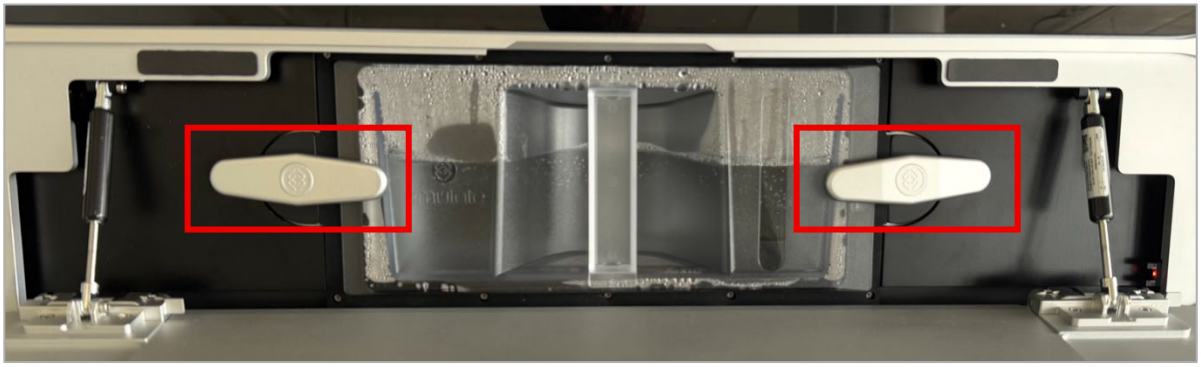


    c. Press **Run Decontamination** and follow the on-screen prompts.

Please Note: *A decontamination cycle cannot be performed while an experiment is actively running.*



16. The decontamination cycle takes approximately 4.5 hours; during this time AVA cannot be used. Do not open the water pan door. Tray doors will be locked as well. There will be an on-screen timer that displays the remaining time for the cycle to complete.
17. Once the decontamination cycle is complete, remove the MycoFog device. Refill the humidity cartridge with lab grade water and reinstall it into the AVA instrument by securing in place using the two hand-rotatable clamps, then close the water pan door.



18. AVA is now decontaminated and ready for use.

Other helpful links:

- Info about MycoFog Incubator Decontamination (link)
- 37°C Protocol Validation for MycoFog (link)
- MycoFog Website (link)

Advanced Features

Consumable Location Tracking and Chip-Array Plate Mapping

- The AVA Emulation System incorporates logic to track the identity and location of specific Emulations via consumable location tracking routines.
- Consumable IDs and tray positions are logged with associated experimental metadata, supporting downstream data traceability and audit requirements.
- The UI offers a visual mapping interface to verify Emulation and tray locations, including occupancy and status.
- Individual Emulation outlets from the Chip-Array can be mapped to individual wells on a collection plate (e.g., 96-well plate).
- System logs maintain a consumable usage history to support QC, reuse prevention, and traceability workflows.

Remote Experiment Monitoring

- The system captures real-time operational metrics including pressures, set flow rates, manifold status, image acquisitions, and environmental conditions.
- Alerts and warnings are displayed in the user interface and recorded in a persistent log for support traceability.
- Remote access to the system is possible via a secure external workstation interface. This allows for passive monitoring, log extraction, and basic parameter control.
- Real-time performance data helps maintain throughput consistency and allows early detection of system drift.

Automation Enablement

- The Chip-Array consumable features an SBS plate design for compatibility with standard off-the-shelf liquid handlers, robotic arms, high content imagers, and plate-readers.
- All pipetting workflows on the Chip-Array can be performed using a multichannel pipette. Refer to the respective Organ-Chip protocols for details.
- The height of the region containing the Emulations within the Chip-Array consumable meets the clearance specifications of 14.35 ± 0.76 mm per ANSI standards.
- The reservoir height of the Chip-Array consumable is 21.6 mm without the reservoir lid. Chip-Arrays can be placed under high content imagers without the lid for post-experiment imaging purposes.

Troubleshooting and Support

Common System Errors and Fixes

Flow inconsistencies:

- Review protocol to ensure that the media equilibration steps have been correctly performed.
- Ensure Chip-Array flow guides are properly connected and aligned across each Emulation by paying close attention to an audible snap.
- Ensure that the Prime and Regulate steps have completed successfully.
- Inspect the impacted Emulations under live imaging for bubbles. Run a Flush Cycle to displace any bubbles that may be causing blockages.
    - If a bubble is still present, then detach the flow guide for the specific Emulation, reattach the pipette guide, and manually flush the bubble out with a pipette.
    - After this, it is highly recommended to detach the pipette guide, re-prime the Chip-Array, reattach the flow guide, and rerun Regulate.

Environmental control issues:

- The AVA instrument is programmed to maintain temperature and CO_2 concentration with tolerances around the set points. If the AVA instrument is outside of these tolerances, users will be notified via an alert on the external workstation UI.
- Review the live operational logs to assess trends or fluctuations in environmental readings.
- If issues persist, contact Emulate support.

Issues running Automated Imaging:

- The AVA software allows users to schedule “Automated Imaging” sessions, where the AVA microscope will image specific FOVs on specific Emulations designated by the user at a certain date and time. If the AVA fails to capture these images, attempt to open a “Live Imaging” session and manually capture images to determine if this is a microscope failure. If “Live Imaging” works, then this means the microscope is functional, and the failed “Automated Imaging” session is likely related to the software. Contact Emulate support for further troubleshooting.

Software crashes and recovery steps:

- Perform a soft reset via the UI.
- Perform a hard reboot using the AVA power switch.
- Download the most recent logs and contact Emulate support with the log file ID.