nicoya

alto

Operation Hardware Manual

Version 1.1 Revision 2.0
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Table of Contents

1.0 Introduction

1.1 Purpose

The Operating Manual provides information and instructions on safe and recommended usage of Alto.

1.2 Pre-requisites

To ensure Alto is operated safely and according to intended purpose, the following prerequisites must be completed:

- Read the Safety Instructions carefully.
- Install the system according to the instructions in “Getting Started with Alto”.
- Understand the Standard Operating Procedures to perform experiments.
- Be familiar with general laboratory equipment and handling of biological materials.

1.3 Important User Information

The Safety Instructions in this manual must be read by all users prior to installing, using or interacting with the Alto instrument.

The Alto system should not be operated in any other capacity other than as described in this manual. Operating the system outside of the Safety Instructions could expose users to hazards that could lead to personal injury or damage to the instrument.

1.3.1 Intended Use

Alto is a surface plasmon resonance (SPR) system that measures molecular interactions in laboratory-based research. The real-time label-free analysis is intended for research use only and should not be used for diagnostic, clinical or *in vitro* procedures. Based on the number of molecules that can be experimented with, there is both a diverse and broad range of research that can be performed. The data collected is by no means suggestive or instructive, and any decision made based on the resulting data is exclusively the responsibility of the user. Any actions taken based on the resulting data are not considered to be based on Nicoya’s instructions and/or use specifications. A qualified team of application scientists is made available upon purchase of a Nicoya product and consultation should be pursued prior to conducting any experiment where there is doubt on whether it would qualify as “intended use.”

1.4 Regulatory Information

1.4.1 Manufacturer Information

The Alto system is manufactured by Nicoya and the address for the manufacturer is provided below:

Nicoya Lifesciences
29 King St E Unit B
Kitchener, Ontario, N2G 2K4
Canada
Phone: +1 877-673-6777

1.4.2 Power and Temperature

Input Power: 100-240VAC, 50-60Hz, 450W
Operating temperature range is 15-35 degrees Celsius.

1.4.3 Compliance

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with this manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense. Changes or modifications to the instrument which are not expressly approved by Nicoya could void the user's authority to operate the equipment.

- This product meets the applicable Innovation, Science and Economic Development Canada technical specifications.
- This device contains license-exempt transmitters/receivers that comply with Innovation, Science and Economic Development Canada's license-exempt RSS-210. Operation is subject to the following two conditions:
- This device may not cause interference.
- This device must accept any interference, including interference that may cause undesired operation of the device.

L'émetteur/récepteur exempt de licence contenu dans le présent appareil est conforme aux CNR d'Innovation, Sciences et Développement économique Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes:

1. *L'appareil ne doit pas produire de brouillage;*
2. *L'appareil doit accepter tout brouillage radioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.*

2.0 Safety Instructions

2.1 Safety Precautions

The following recommendations are to be followed to ensure safety and proper use of the product.

1. Read and follow all instructions provided.
2. For indoor use only.
3. For research use only.
4. Alto is not waterproof. Do not spill liquids onto the instrument. Clean any fluid spills (following standard lab procedures) in the area around Alto as quickly as possible.
5. Do not operate the system in the presence of strong magnetic fields.
6. Wear gloves and other appropriate personal protective equipment when interacting with the cartridge and operating the system.
7. It is the responsibility of the user to ensure the safety and appropriateness of the chemicals, materials, and fluids being used in the Alto. Utilize all appropriate and recommended safety precautions associated with the materials.
8. The sensor cartridges (cartridges used for samples) are single use and must be disposed after use. Follow all local, state/provincial, and federal regulations for disposal of the cartridges or the instrument if decommissioned.
9. Expected intended use is non-hazardous material only. If a user uses hazardous material or compounds within the cartridge, it is considered as unintended use.
10. Do not remove or disassemble any part of the instrument or the cartridge. The instrument contains no user serviceable components. Do not remove or open the electronics compartment. Contact Nicoya (as outlined in Section 1.4.1) if there is an electrical problem and a service engineer will assist you.
11. Ensure that the instrument is powered down and the power cable is disconnected while servicing any parts in direct proximity of the Alto instrument.
12. It is prohibited to use open fires in the vicinity of this system.
13. Ensure that cartridges are removed and the instrument is in standby mode or shut down when not in use.
14. Do not insert any objects or body parts into the instrument openings (including the front grill, rear ports and the cartridge loading bay).
15. No other objects except for authorized Nicoya manufactured cartridges should be placed in the cartridge loading bay except for the shipping plate.
16. Do not move or relocate the instrument while a test is in progress.
17. The Alto unit must be operated on a levelled surface.

This information is based on our present knowledge. However, this does not constitute a guarantee for any specific product features and shall not establish a legally valid contractual relationship. Users should make independent decisions regarding completeness of the information based on all sources available. Nicoya shall not be
held liable for any damage resulting from handling, contacting, or using this product.

2.2 Disposal and Instrument Decommissioning

The instrument will be disposed of according to local and federal electronics disposal regulations. It is important to ensure that prior to disposal, all cartridges or consumables are removed from the instrument and disposed of separately as per local and/or federal regulations.

3.0 System Details

3.1 Alto Hardware

The Alto instrument is an optical detection system that is integrated with a computer used to perform SPR experiments. The instrument contains optical, thermal, electrical and mechanical modules to enable instrument functionality and detection mechanisms. The fluidic interactions are performed using Digital MicroFluidics (DMF) on a disposable cartridge that is loaded into the instrument for each experiment.

The instrument includes a display touchscreen for accessing the instrument and software (Figure 3.1). The instrument requires a cartridge to perform SPR experiments, which can be installed in the cartridge loading bay of the instrument. The cartridge loading bay is exposed once the cartridge door (Figure 3.1) is opened. The instrument is compatible with WiFi and bluetooth functionality for wirelessly connecting the instrument to additional devices.

The Alto instrument weighs 23 kgs. The dimensions of the instrument are 460mmx350mmx510mm (HxWxD)

Figure 3.1: Alto instrument and components.

3.1.1 Instrument Display

1. The touch screen of the instrument is a capacitive touch screen and is compatible with nitrile gloves. Wet hands or liquid droplets on the display could cause erratic functionality.
2. The touch screen has a resolution of 1920x1080 pixels.
3. The touch screen has an anti-glare coating for user operation and ease. The display brightness is 400 nits(cd/m2).

3.1.2 Instrument Feature Locations

1. The front of the instrument contains a soft power ON/OFF button as well as a USB port (Figure 3.2). The machine vision system is also highlighted in Figure 3.2. The machine vision system in the software will detect any obstacles or issues with the cartridge loading and alert the user about the same in the software.

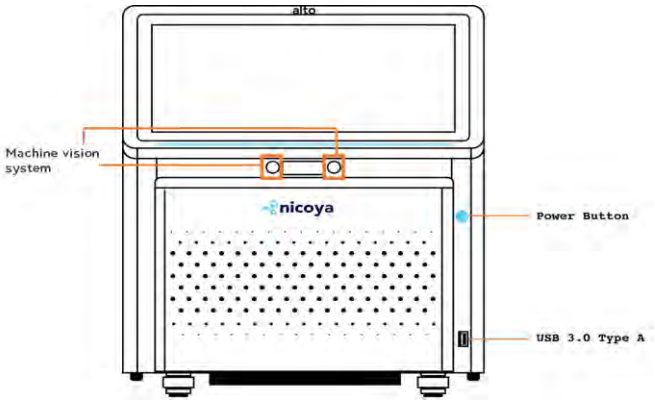

Figure 3.2: Power button, USB ports and machine vision system are located on the front of the instrument.

2. The back of the instrument contains several ports and IOs to support instrument power and connection to accessories as shown in Figure 3.3. The Eco switch should be always set to the off position.

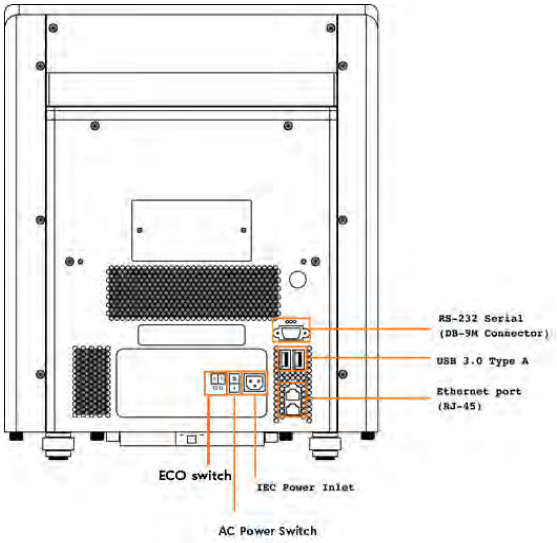

Figure 3.3: Buttons and IOs on the back of the Alto instrument.

3.1.3 Other features

1. The Alto instrument hardware has two speakers, which are located on either side of the Alto instrument (Figure 3.4).

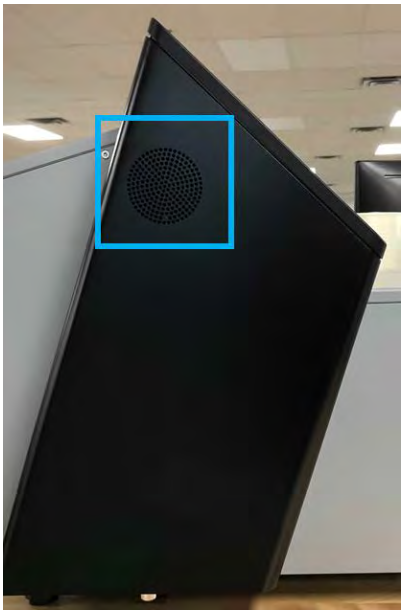

Figure 3.4: Location of the speakers on the Alto instrument.

2. The instrument has a storage drawer for the QC Cartridge and Optics Service Cartridge (OSC)s (Figure 3.5). This drawer contains two compartments, where one holds the Optics Service Cartridge (OSC) and the other compartment holds the QC cartridge along with the OSC wipes that are required regularly in case of need of maintenance. This storage drawer can be easily pulled out by the user.

Figure 3.5: Drawer under the instrument for storing the QC cartridge, OSC cartridge and accessories.

3. The instrument contains a light bar which acts as a status indicator for the state of the instrument (as shown in Figure 3.6). The following colors are used to indicate the different instrument states:

- Startup - Teal
- Initialized/ready to use/run - Blue
- Error - Red (solid)

Figure 3.6: Light bar location on the instrument.

4. The NFC tag present on the cartridge identifies the cartridge type loaded into the bay and assists the user in selecting the correct workflow.

3.2 Alto Cartridge

The Alto cartridge is a core component of the instrument that consists of 16 optical sensors for detection purposes, fluidic functions to execute the SPR experiments and wells for storing samples for the experiment.

3.2.1 Architecture of Alto Cartridge

The Alto cartridge employs the concept of Digital Microfluidics (DMF), which allows for discrete 300 nanoliter sized droplets of sample to be individually controlled by the actuation of a grid of electrodes. The electrodes can be turned on or off in a pre-programmed sequence to dispense, move, merge, mix, and split droplets with a high degree of precision, thereby automating the liquid handling steps. Each cartridge consists of optical sensors for biosensing purposes, DMF electrodes to execute the SPR experiments, and wells for storing and dispensing different reagents.

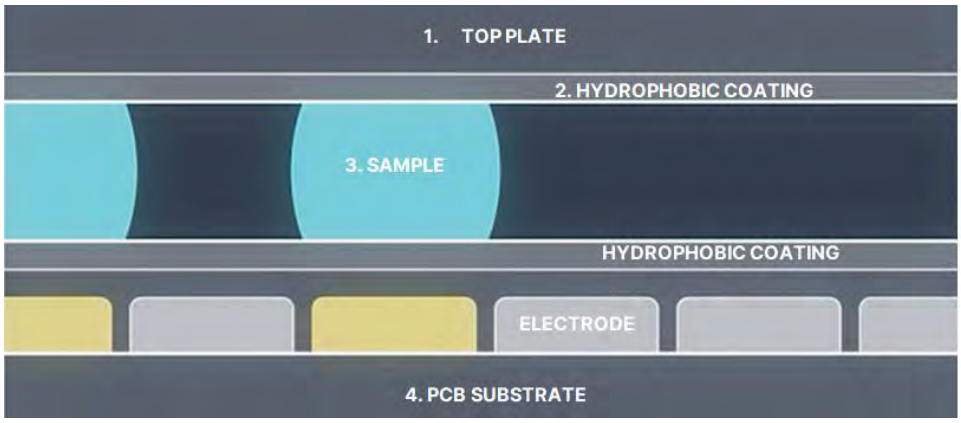

Figure 3.7: Alto cartridge side view.

The Alto cartridge hardware is composed of the following components:

1. **Top plate:** The top plate serves as an interface through which samples are introduced into the cartridge. It includes wells and dispensers. The wells are where the user will load their samples. The dispensers are a combination of electrode and top plate features that dispense multiples of 300 nL droplets from the loaded sample.

2. **Hydrophobic coating:** This is a proprietary coating on the cartridge and is used to facilitate the sample droplet movement.

3. **Sample:** The sample is the blue droplet shown in Figure 3.7. The sample refers to the solution in the wells. This could be running buffer, regeneration buffer, activation solutions, surface cleaning solutions, ligands, or analytes.

4. **PCB substrate:** This is a printed circuit board (PCB) that specifies which electrodes are controlling the droplet movement. The PCB consists of two major parts:
    - Electrodes are located on the top surface of the PCB substrate that can be turned on or off to move droplets onto or off an electrode. The electrodes shaded in yellow in Figure 3.7 are ON and the electrodes shaded in grey are OFF.
    - The contact pads found on the bottom of the PCB substrate connect the control board in the Alto instrument with various electrodes on the PCB.

3.2.2 Alto Cartridge Design

Depending on the cartridge type, the cartridge layout may differ. But overall, the fluidic cartridges will always contain the following areas:

1. **Sensors:** Each cartridge contains 16 sensors as shown in Figure 3.8. The sensors are available with different surface chemistries. The sensors are composed of 100nm gold nanoparticles coated onto the in-cartridge optical fibers used for SPR detection.
2. **Mixing zones:** The mixing zone is where the automated sample dilution will take place.

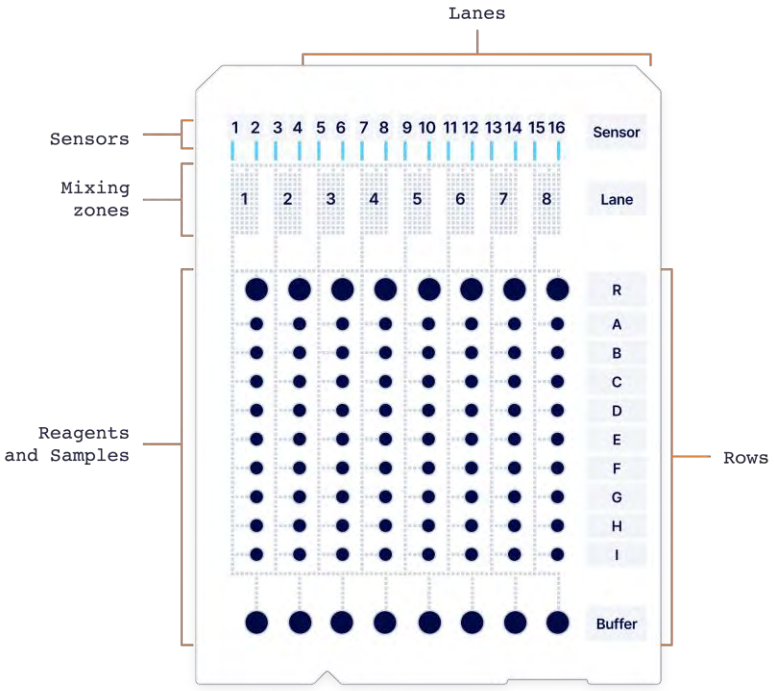

Figure 3.8: Alto cartridge design and zones.

3. **Thermal Zones:** There are two thermal zones on every cartridge; the *reagent thermal zone* and the *sensor thermal zone*:
    - The *reagent thermal zone* encompasses all the wells and controls the temperature for all the wells in the cartridge. The temperature for this zone is set to 15 degrees C or the dewpoint, whichever is higher, to avoid sample degradation.
    - The *sensor thermal zone* controls are around the sensors and are user-controllable between 15 ℃/dewpoint to 50 ℃. The ramp-up time of the temperature change can vary, and this can take up to 25 minutes, depending on the range of temperature change.

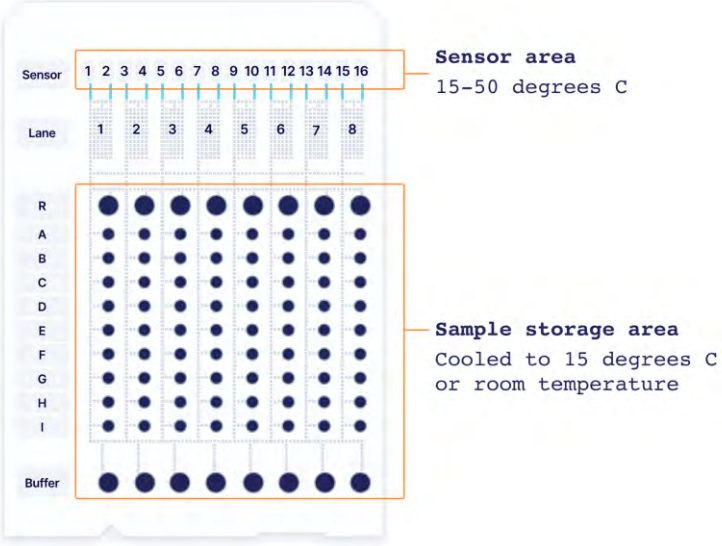

Figure 3.9: Temperature control on Alto cartridges.

4. **Wells:** The different types of wells on every cartridge are defined in Table 3.1 and are shown in Figure 3.10.

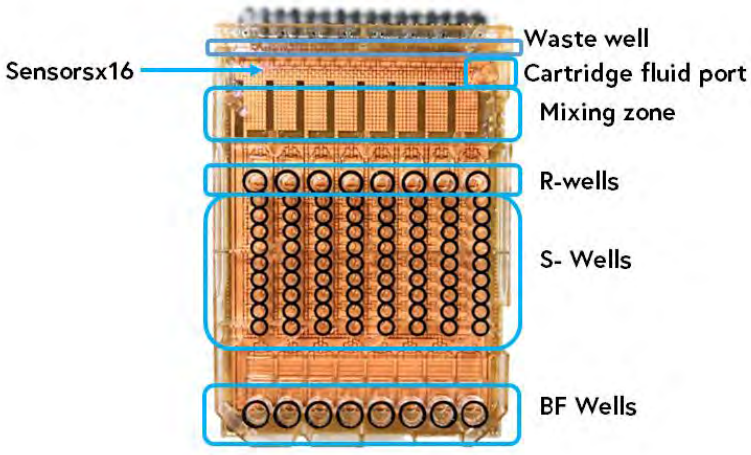

Figure 3.10: Alto Cartridge Top View

Table 3.1: Well types and definition of each well on the cartridge.

Well type	Volume (µL)	Sample to be used	# wells/cartridge
Multifunctional wells (S-wells)	4	Ligand, analyte, activation solutions, blocking solutions	72
Regeneration wells (R-wells)	67	Regeneration solution or surface cleaning	8

		solutions	
Buffer wells (BF-wells)	130	Running buffer	8

3.2.3 Types of Alto Cartridges

1. **Kinetics Cartridge:** The Kinetics Cartridge is recommended for studying the binding kinetics of a ligand to an analyte using 5 different concentrations of analyte. The kinetics cartridge is designed to have 88 wells, where 8 wells will be designated regeneration, 8 wells will be designated for buffer wells and there are 72 wells dedicated to samples depending on the protocol. Surface cleaning, activation, ligand, and analyte solutions along with buffer and regeneration solutions are loaded onto the cartridge to perform ligand immobilization followed by each analyte and regeneration cycle to obtain the necessary binding curves. These will be used to calculate the ka, kd, and KD values after post-processing and analysis of the data.

2. **QC Cartridge:** The QC Cartridge is used to run system diagnostics to ensure the functionality of the primary modules of Alto. The QC cartridge will test for the electrical, optical, and thermal functionality. The QC cartridge is used to verify the instrument is functioning normally and diagnose any module failures.

3. **Optics Service Cartridge:** The Optics Service Cartridge (OSC) is a reusable cartridge used to run optics calibration and clean the optics interface of the Alto instrument. Upon completion of optics calibration, the Fiber Cartridge Interface (FCI) obtains a bright reference for the instrument.

4.0 Getting started with Alto

4.1 Initial Equipment Setup Requirements

Prior to removing the instrument from packaging, prepare a space in your laboratory that meets the following requirements for instrument dimensions, power and environmental factors.

Table 4.1: Equipment operational parameters

Parameter	Value
Desk Space Dimensions	60 cm x 50 cm (LxW)
Input Voltage	AC 100-240 V, 50-60 Hz*
Ethernet Connection	Within 10 feet of ethernet port
Heating and Ventilation	15-35 °C, 20-80% Relative humidity non-condensing

**Only use the power cord provided by Nicoya || Surge protected power outlets are recommended.*

4.2 Standard Equipment Supplies

- The Alto Instrument comes equipped with the supplies outlined in Table 4.2 and Table 4.3.

Table 4.2: Alto Instrument Box Contents

Item	Quantity
Alto Instrument	1
6 Foot AC Power Cable	1
10 Foot Ethernet Cable	1
Optics Service Cartridge (OSC)	1
QC Cartridge	1
Shipping Plate (Inside the Instrument)	1
Quick Setup Guide	1

Table 4.3: Alto Accessories Box Contents

Item	Quantity
Tools: 1. Foot Wrench 2. Bubble Level	1 EA
Optics gel	1
Optics Service Cartridge (OSC) Sliders	6
Practice Cartridge	1
Blue Practice Solution	2 x 1.5 mL
Cartridge Fluid	1
Cartridge Fluid Cap Opener	1
Pipette Guide	1
Pipette Guide Hand Screws	2
Table mount kit contains the following: • 2 Table mount brackets • 4 M8 x 60mm socket head cap screws (SHCS) • 4 M8 flat washers • 2 M8 split lock washers • 2 M8 nuts • 1x Allen wrench for SHCS • 1x Open end or combination spanner (for the M8 nut) • 1x HSS Drill bit	N/A

4.3 Unpacking the Alto Instrument

1. Place the instrument box near your prepared benchtop where the instrument will be placed.
2. Open the box lid as shown in Figure 4.1.

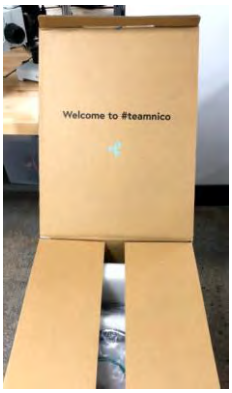

Figure 4.1: Open the box lid for retrieving the instrument.

3. First, remove the accessories from the box. Place the accessory items on a clean, flat surface for later use. Ensure your reserved benchtop space for the instrument is clear.

   Note: The instrument is ~23 kgs, so please seek assistance if necessary.

Figure 4.2: Accessories and foam to remove from the packaging before the user can access the Alto instrument.

4. Remove the uppermost foam piece to expose the instrument.
5. Reach under the long ends of the instrument and find the foam cut-outs that allow your hands to reach underneath. Lift the instrument out of the box and place it on your benchtop.

Figure 4.3: Instrument retrieval from the box.

6. Remove the instrument from the plastic bag. Remove the tape and desiccant from the instrument exterior.

Figure 4.4: Remove the tapes from the Alto once the device is set up on a lab bench.

4.4 Removing the Shipping Plate

1. First, remove the tape and screen protector from the instrument.
2. Push the cartridge door back manually to expose the cartridge loading bay (Figure 4.5).

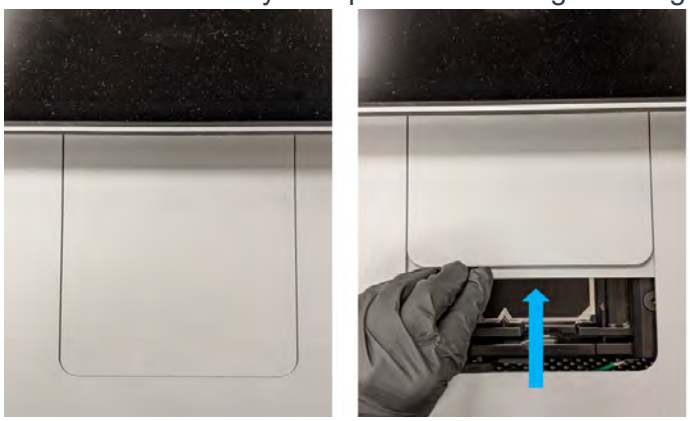

Figure 4.5: Push the cartridge door open manually to expose the cartridge loading bay.

3. The shipping plate will now be visible (Figure 4.6). To remove the shipping plate from the instrument, turn the black screws (highlighted in blue in Figure 4.6) counterclockwise by hand and remove the shipping plate. Store the shipping plate and the screws attached to the plate in the accessories box in case the instrument needs to be transported to a different location.

Figure 4.6: Shipping plate attached to the cartridge loading bay.

4.5.1 Leveling the Instrument

1. To level the instrument, these tools are required:
    a. Bubble level
    b. Foot wrench

2. Place the bubble level inside of the cartridge loading bay as shown in Figure 4.7. If the bubble is centered within the black circle, this means that the instrument is leveled, and no adjustments are needed.

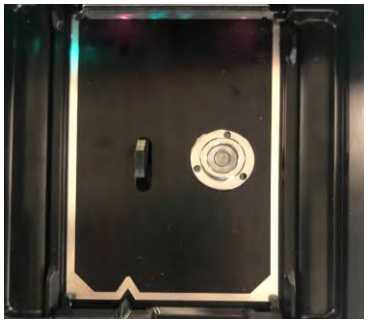

Figure 4.7: Setting up bubble level in the cartridge loading bay.

3. If the bubble is not in the center of the black circle, adjust the feet of the instrument with the foot wrench until the instrument is level (further instructions below).

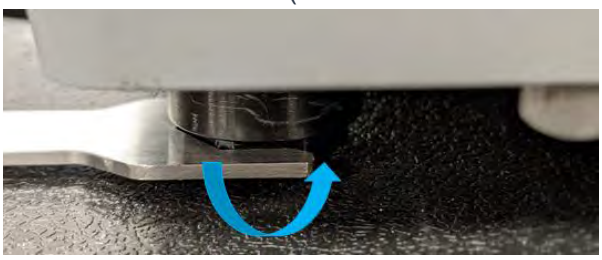

Figure 4.8: Turn the foot counterclockwise using the foot wrench and monitor the bubble level.

4. To level the instrument, turn the foot that is furthest away from the bubble in a clockwise direction (Figure 4.8).
5. Repeat step 4 until the bubble is within the center of the black circle.
6. Once the level process is completed, ensure that all instrument feet are making contact with the table surface and the instrument is not moving.
7. Remove the level from the instrument and place it back in the accessories box.
8. Store the foot wrench in the accessories drawer in the bottom of the unit.
9. Once the instrument is leveled, continue with the table mount bracket attachment process as outlined below.

4.5.2 Optional: Securing Alto to a Lab Bench

This procedure outlines the steps to attach or lock the Alto instrument to a table using mechanical fixture options.

4.5.2.1 Lab Space Requirements

- The provided materials (see below) can accommodate up to 1-3/4" thick bench-tops. If your benchtop is thicker than this, please source longer screws and appropriate accessories.
- In addition to the materials provided (see below), a drill is required. Any drill can be used for this procedure.

4.5.2.2 Materials Provided

To mount Alto onto a bench-top, the following materials are provided in the accessories box:

Foot wrench and bubble level	
HSS (High Speed Steel) drill bit	
2 table mount brackets	
4 M8 x 60mm screws*	
4 M8 flat washers	
2 M8 split lock washers	
2 M8 nuts	
Allen wrench/key, 6mm	

Combination wrench, 13mm	

* Two sets of screws are provided, but only one set is needed for the table mount attachment procedure. It is recommended to use the silver, partially threaded screws since the material is more resistant to rust. If the table being used is not compatible with the partial threading (table is thinner than 1”), use the black, fully threaded screws.

4.5.2.3 Attaching the Table Mount Brackets

1. Rotate the instrument by 90 degrees clockwise to place the unit on its side before starting the feet adjustment. Locate the feet for the instrument, as seen in Figure 4.9. The feet have flats at the very bottom that the foot wrench can interface with.

Figure 4.9: Location of the feet for the instrument.

2. Working with one foot at a time, loosen the foot counterclockwise using the foot wrench until you start to see the screw underneath (Figure 4.10). Unscrew the instrument foot by hand to remove the foot completely and reveal the screw.

Figure 4.10: The process of unscrewing the instrument foot.

3. Put a table mount bracket over the screw, with the larger hole touching the instrument and the smaller hole facing away. Screw the foot back on by rotating it counterclockwise.

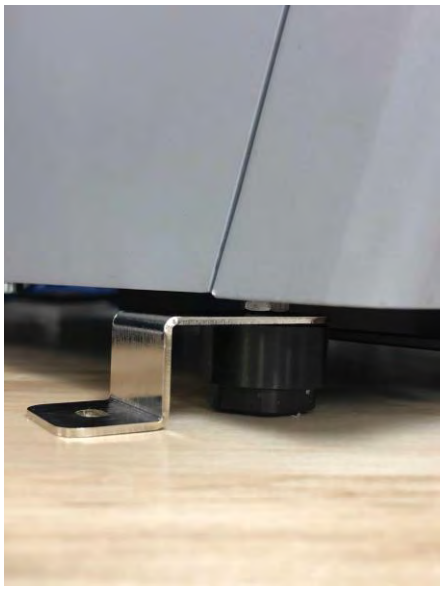

Figure 4.11: Instrument foot with a table mounting bracket installed.

4. Repeat steps 2 and 3 for the instrument foot opposite of the one that has been mounted for the second table mount bracket. For example, if the front-right leg is mounted, proceed with mounting the back left leg.
5. Rotate the instrument by 90 degrees counterclockwise to place the unit upright (back on its feet). Instrument levelling must be performed as outlined in Section 4.5.1 before proceeding with the next steps.

4.5.2.4 Mounting the Brackets to The Lab Bench

1. Using the table mount brackets as guides, mark where the holes will be drilled for each bracket using a pencil or marker.

Figure 4.12: Table mount bracket before being attached to the lab bench.

2. After marking the spot, move the instrument and drill the holes using the provided drill bit.
3. Place the instrument back in its original position and ensure the table mount brackets are directly over the drilled holes.

Figure 4.13: Ensure the table mount bracket is directly over the drilled hole.

4. Place a flat washer on the screw and put the screw through the table mount and lab bench. On the underside of the lab bench, place the second flat washer on the screw, followed by the split-lock washer and the nut, in that order. Rotate the nut counterclockwise until it's finger tight.

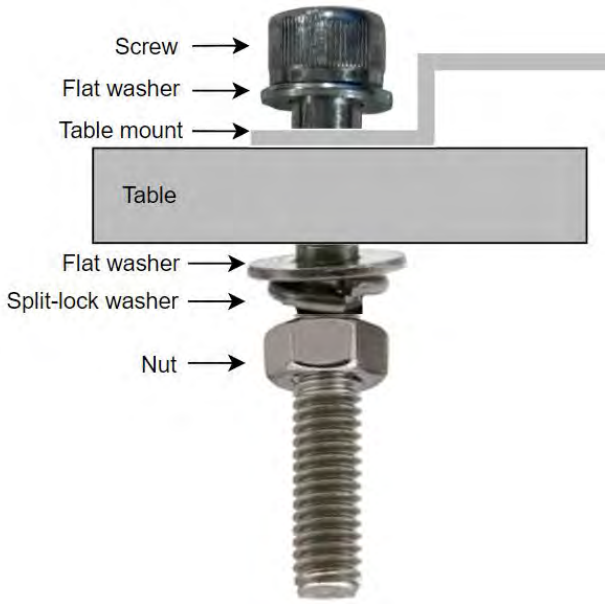

Figure 4.14: Side view for setting up the screw on the table mount.

5. Use the provided combination wrench to tighten the nut onto the screw, counterclockwise. Alternatively, use the provided Allen key to tighten the screw from the top, clockwise.

Figure 4.15: Instructions for screw tightening for the setup.

6. Repeat steps 4-5 for the second table mount bracket attachment.
7. Instrument levelling must be repeated as outlined in Section 4.5.1 to ensure that the instrument is leveled.

4.6 Hardware Setup

4.6.1 Alto Power Connection

1. Turn the I/O AC power switch as shown in Figure 4.16 in the O position (or power off position) before connecting the power cord to the instrument.

Figure 4.16: AC power switch and IEC power inlet location and configuration prior to attaching the power cable.

2. Connect the power cord into the IEC power inlet as highlighted in Figure 4.17.

Figure 4.17: Securing the power cable into the IEC power inlet.

4.6.2 Powering on the Alto

1. Use the power switch on the back of the instrument as shown in Figure 4.19 (left) to power the instrument ON. This button must be toggled on (as shown in Figure 4.18) for the instrument to be powered on. Press the power button in the front of the instrument for 3-5 seconds to turn the instrument on.

Figure 4.18: Instrument is turned on by toggling the AC power switch with the I position depressed.

2. The ECO switch (highlighted in Figure 4.19) should always be in the OFF position.

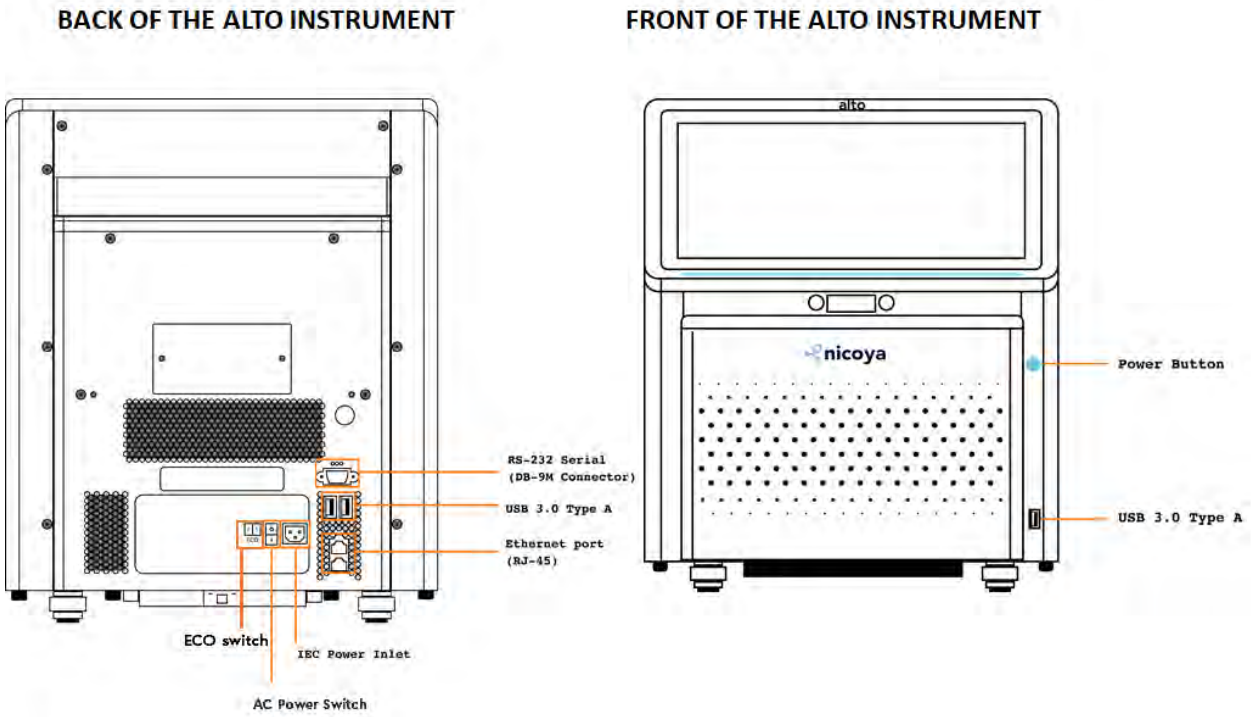

Figure 4.19: Location of the power button (labelled AC Power Switch) at the back (left) and front (right) of the instrument.

3. The instrument will indicate it is successfully powered on by:
    a. A LED light bar underneath the instrument screen will turn on and become teal. Once the LED light bar under the instrument screen turns blue, this indicates that the software has booted on the instrument successfully.
    b. The power button should be illuminated blue as well.
    c. The boot-up screen should read ‘Nicoya’ as the system turns on.
    d. Fans will begin and can be heard starting inside the instrument.
    e. The door will open & close to align the cartridge loading bay.

4.6.3 Accessories

1. There are three USB 3.0 Type A ports available on the instrument. Two USB ports are available on the back of the instrument (Figure 4.20) and one USB port is available on the front of the instrument.

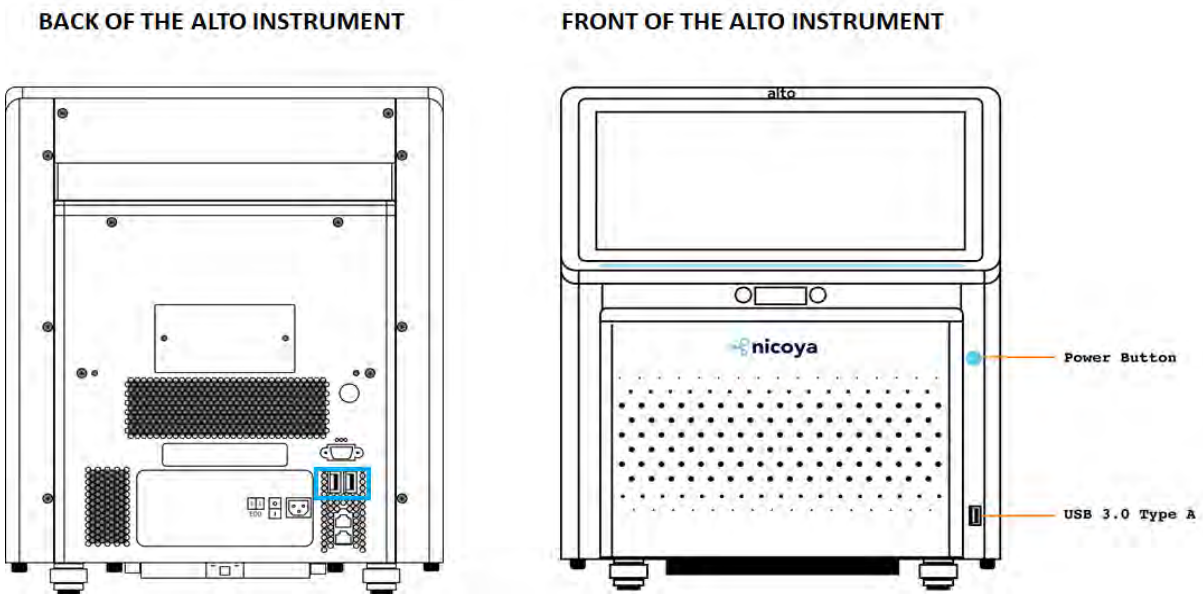

Figure 4.20: Location of two USB ports in the back of the instrument and one USB port in the front of the instrument.

You can use the available USB ports to connect a range of devices, including:

- Liquid Handlers
- Other approved USB devices

2. A RS-232 Serial; DB-9M Connector (Figure 4.21, highlighted in red) is available. Please contact Nicoya for applications that interface through the RS-232 port.
3. The user is provided with two ethernet ports (Figure 4.21, highlighted in blue) for connecting the instrument to the local network and/or the internet. The two ethernet

ports can be used for connecting multiple Alto instruments, if needed. The two Ethernet ports operate as an Ethernet Switch.

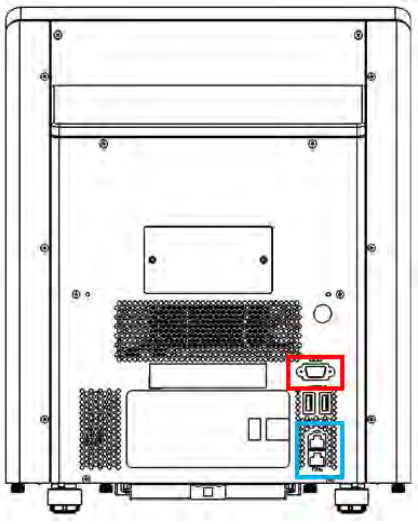

Figure 4.21: Location for one RS-232 Serial; DB-9M Connector (highlighted in red) and two ethernet ports (highlighted in blue) on the Alto.

4.7 Software Setup

The Alto software ecosystem consist of two different software setups that will be referred to throughout this manual:

1. **ACS (Alto Control Software)** - This software will run on the Alto instrument and provides authentication for the users. Once logged in, all methods created by users assigned to the instrument are available (prioritized by user, by default) as well as their workgroup methods to facilitate collaboration.
2. **UP (User Portal)** - This is a web application that is used for designing your experiments, analyzing data, and managing methods on machines.

4.7.1 Setting up a new user account on the User Portal

As a first time Alto user, the customer profile will be set up for users by Nicoya specialists. The customer will provide information for the email addresses for each person who will be accessing the device, as well as indicate their level of access. The level of access for each person can be initially assigned as an administrator or a user with only a limited number of administrators available per instrument.

4.7.1.1 Overview of Nicoya User Portal Dashboard

Once you are logged into the Nicoya User Portal, you will have access to the dashboard. The Nicoya user portal has 4 main functional domains:

1. **Home:** The dashboard will contain information about the users, your Alto unit number, your experiments, and the firmware and software versions as shown in Figure 4.22 and Figure 4.23. The tab contains a few items as outlined in Table 4.4.

Table 4.4: Outlines parameters and definitions for all features available on the Home tab of the Nicoya User Portal.

Parameter	**Definition**	**Example from Figure 4.22**
Type	Connection type either via Wifi or Ethernet	Wifi
Name	Unit number for the Alto	Alto-AC000W018524980
Status	Provide information about the status of the device being online or offline	Offline
User	Provides the name of the device user	Laura Sawyer
Recent experiments section in Figure 4.22		
Experiment	Name of the experiment	Show
Author	Provides the email of the author associated with the	laura0237@gmail.com
Last Updated	Provides the date and time when the experiment method was last updated	2021-04-030 13:10:33
Core Assay	Type of assay used for the experiment design	Direct Kinetics

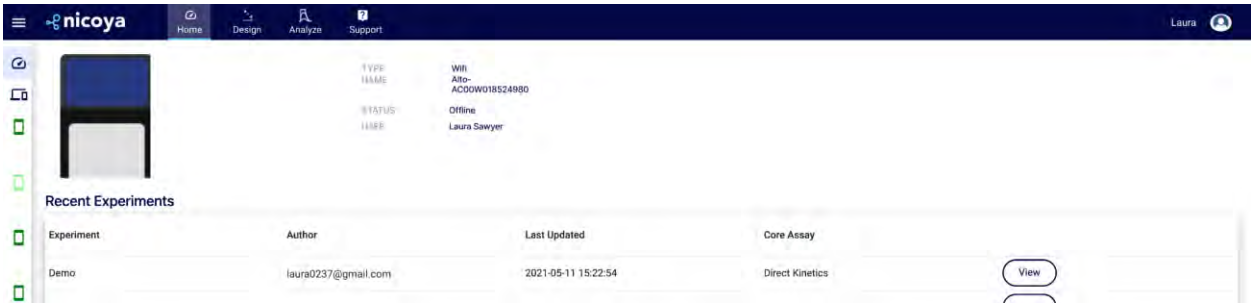

Figure 4.22: Home domain for the Nicoya User Portal dashboard

Figure 4.23: Home domain dashboard for the Nicoya User Portal dashboard showing all devices in the network which are online or offline. The online devices are shown with a green dot next to the device name.

2. **Design:** The design feature will allow for remote assay development and design prior to starting your project on Alto. The detailed description for all the features of this tab will be outlined in Section 4.7.2 of this document.

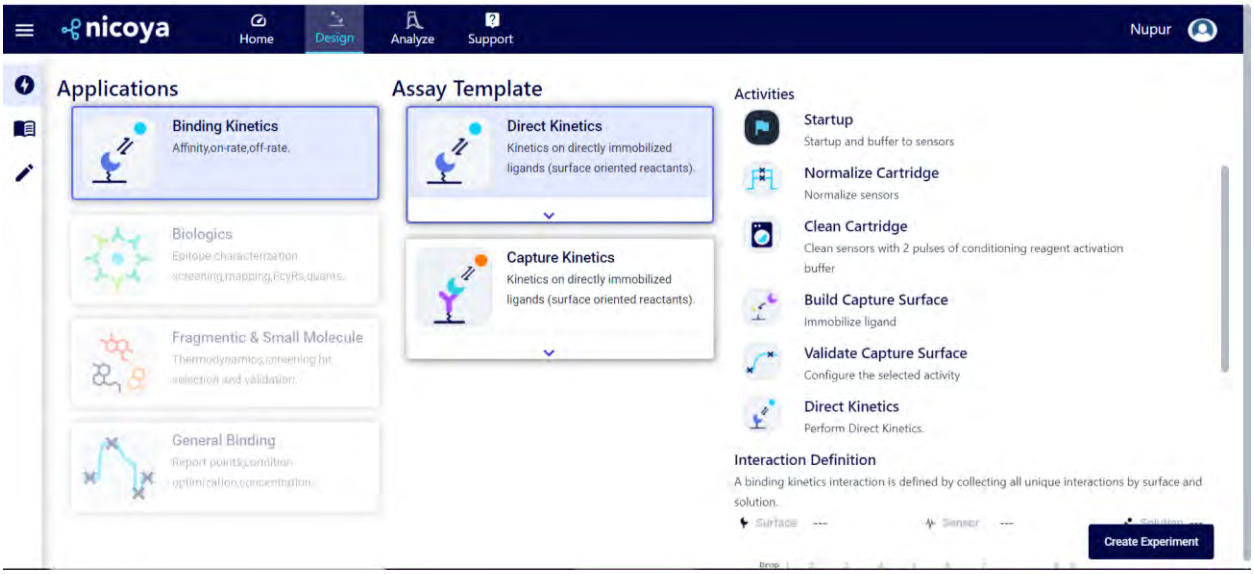

Figure 4.24: Design tab in the Nicoya User Portal outlines the design features that can be used for experimental method design.

3. **Analyzer:** The analyzer feature will allow the user to analyze the data obtained from performing an experiment on the Alto.

Table 4.5: Outlines parameters and definitions for all features available on the “Analyze” tab of the Nicoya User Portal as shown in Figure 4.25.

Parameter	Definition	Example from Figure 4.25
Filter	Option to filter the analyzed experiments	-

Hide Fields	Option to view certain fields in the table versus others	-
Group	Groups the results by experiment, type, and analysis	-
Download	Downloads the table in a CSV format. The "view analysis" button next to each experiment downloads the experiment as a JSON file.	-
Date Completed	Date of analysis completion	-
Operator	E-mail address of the operator that performed the data analysis	bob@gmail.com
Type	Assay type for the experiment as defined by the experiment method	Direct Kinetics
Experiment	Name of the experiment	Quick experiment
Interactions	Number of interactions defined in the experimental method	8
Analysis	Status of the analysis is provided here which could be either complete, in progress or aborted	Aborted

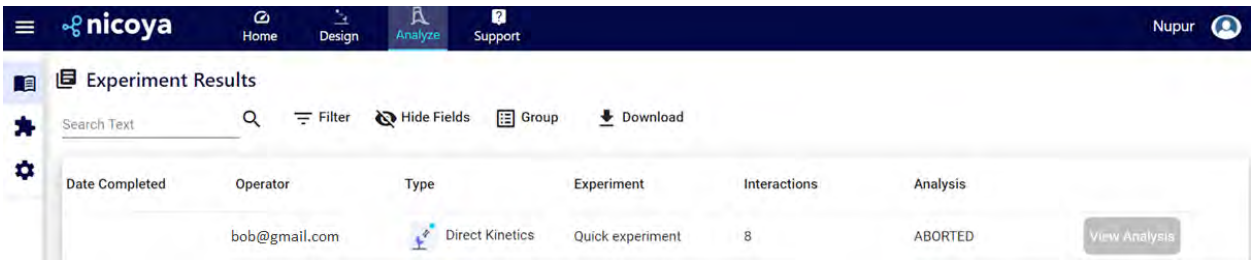

Figure 4.25: Analyze tab on the Nicoya user portal that is used for analyzing the data collected from an experiment with Alto.

4. **Support:** The support table provides contact information for users to get application and technical support.

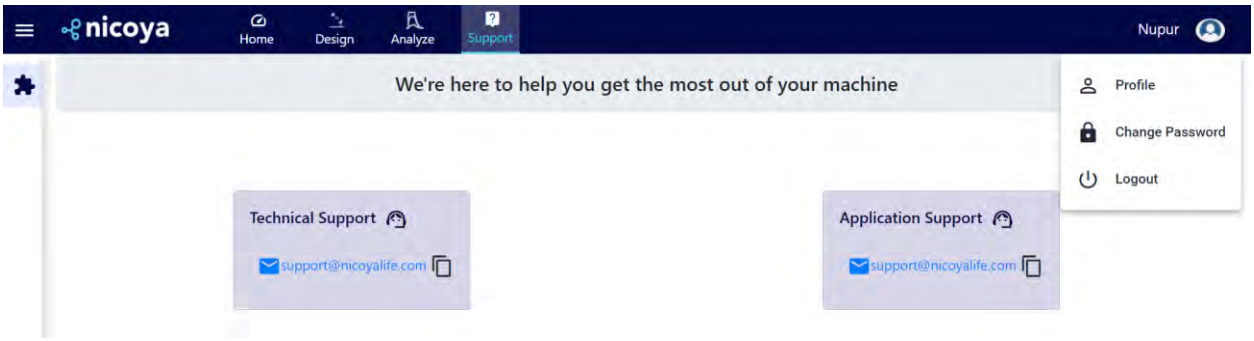

Figure 4.26: Support tab in the Nicoya User Portal provides email addresses for technical and application support related to your experimental design, analysis, or troubleshooting with Alto.

4.7.2 Experiment Design on User Portal

Once the user is logged into the Nicoya User Portal, the user will now be ready to design their experiment. The user will need to choose the “Design” tab from the top of the Nicoya User Portal dashboard to ensure you can enter the space in the Nicoya User Portal to design your experiment on your computer. The user can perform the experiment design remotely and does not need to be present at the Alto device.

The first step is to define the type of interaction the user will be working with under the applications section under the Design tab as shown in Figure 4.27.

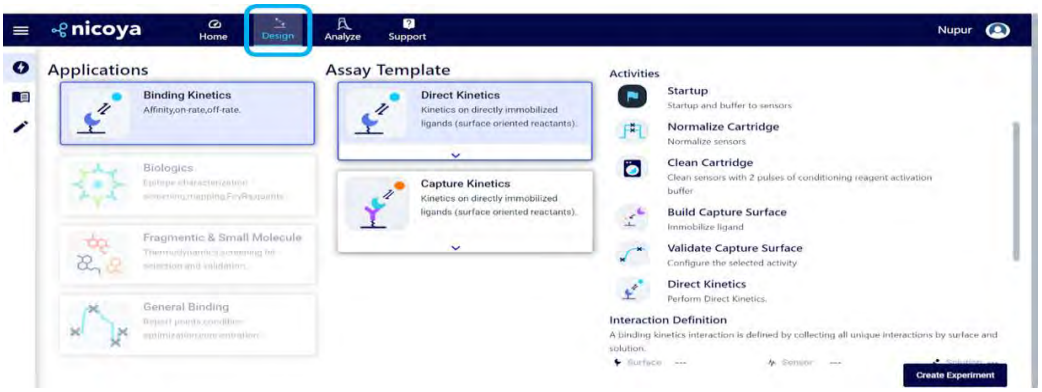

Figure 4.27: Application options and Assay template options with the Alto design software.

The applications section has several options available. Depending on your subscription model, you can choose between the following applications:

1. **Binding Kinetics:** This option should be chosen when the user is looking to perform kinetic experiments to determine the affinity, on and off rate, and KD of the system.
2. **Biologics:** This option should be chosen when the user is looking to perform epitope mapping and characterization experiments along with screening a large set of biomolecules against single or multiple ligands.

3. **Fragment & Small Molecule:** This option should be chosen to perform fragment and small molecule screening-based experiments.
4. **General Binding:** This option should be chosen when the customer is looking to perform a condition optimization experiment like optimizing for buffers, regeneration solutions, and immobilization conditions among others.

At this point, the customer can perform binding kinetics experiments and other features will be introduced with future software releases. Once Binding Kinetics is chosen, the user can select the assay template style. The user has two options - Direct Kinetics or Capture Kinetics. The final cartridge layout and assay template differs for both options. As for choosing which option is ideal for the experiment, the user should consider the following:

1. **Direct Kinetics:** This option should be used to directly attach the ligand to the sensor. The analyte is then introduced to study the binding kinetics for the system.
2. **Capture Kinetics:** This option should be used to couple the capture molecule onto the sensor. Once the capture molecule is coupled to the surface, the ligand is introduced on the surface. This ligand is known to bind to the capture molecule by a certain tag or location on the biomolecule. The user is now able to introduce the analyte for studying the binding kinetics for the system.

Once the assay template is selected, the user can click on “Create Experiment” as shown in Figure 4.28.

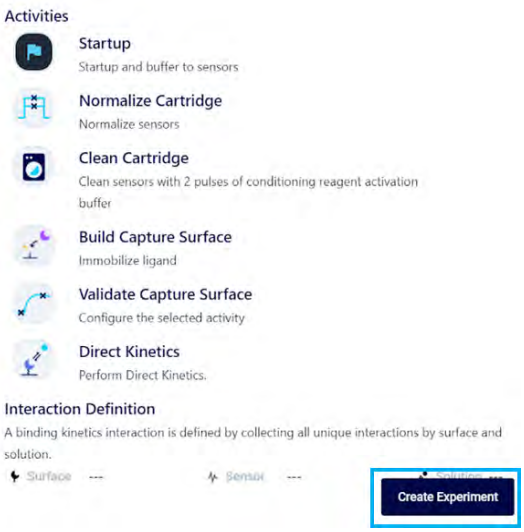

Figure 4.28: Create experiment option

For the new experiment, you will be able to define the following parameters outlined in Figure 4.29.

1. **Project:** The user can choose from a list of existing projects or add a new project.
2. **Experiment name:** The user will enter the name of their experiment.

3. **Experiment description:** The user can provide a short description for the project and the experiment.

Figure 4.29: Define parameters to create a new experiment

Once the parameters are defined, click on “Create Experiment” to define the assay design parameters. The project name will now be defined on the top left and each experimental method will require the user to define parameters in 4 broad categories including Experiment, Method, Samples, and Build.

4.7.2.1 Experiment: Assay Setup

Under the experiment option, the user will be able to view the assay setup parameters on the left along with the cartridge view (As shown in Figure 4.30). The assay setup has quite a few parameters that the user can use to customize for their experiment.

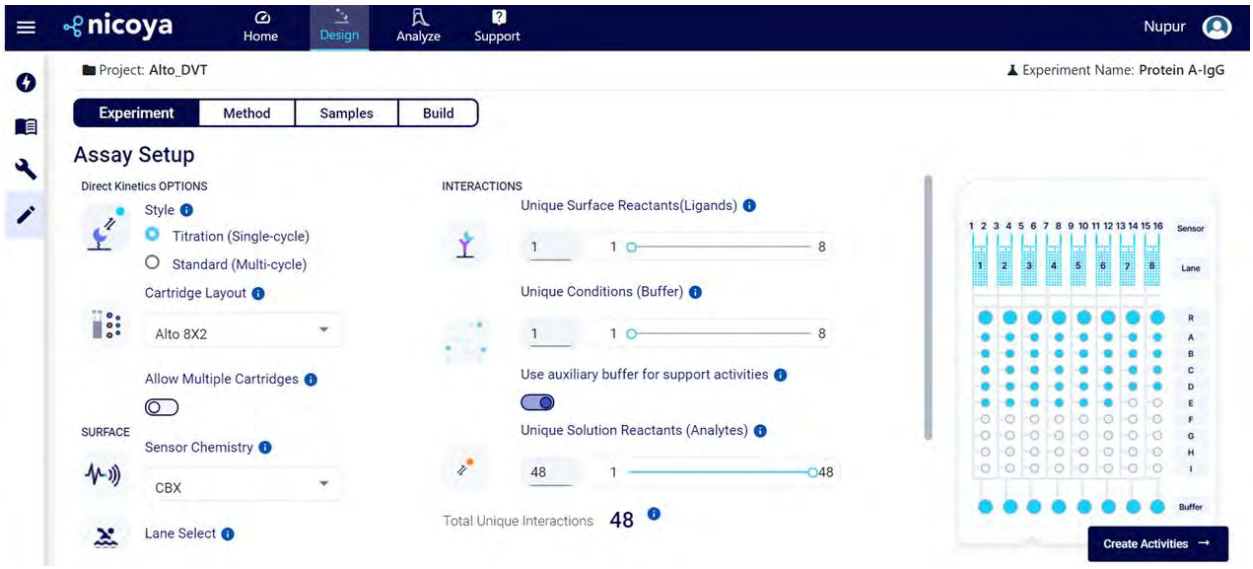

Figure 4.30: Experiment setup by choosing the Assay setup parameters

Direct Kinetics Options

Under the Direct Kinetics Option, the user can define the experiment style and cartridge layout type as shown in Figure 4.31.

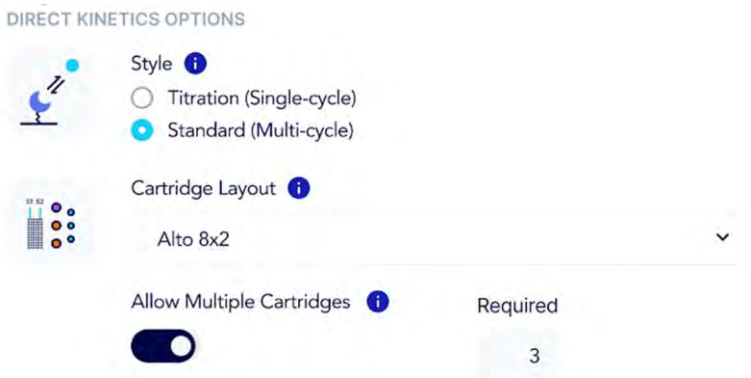

Figure 4.31: Standard option tab parameters

Table 4.6 outlines the parameter definitions as well as default recommendations for a new experiment.

Table 4.6: Parameter definition and default recommendations for Direct Kinetics option for the Assay setup.

Parameter	**Definition**
Titration style	The user can choose between Titration (single-cycle) or standard (multi-cycle) kinetics depending on their experimental needs. - Titration (Single-cycle): SPR analysis method wherein analytes are presented to the immobilized ligand sequentially in order of increasing concentration, with only a single dissociation and regeneration phase after all analyte concentrations have bound to the ligand on the sensor. - Standard (Multi-cycle): SPR analysis method whereby analytes are presented to the immobilized ligand sequentially in order of increasing concentration, with dissociation and regeneration phase after each analyte concentration.
Cartridge layout	The user can choose between different cartridge layouts. The current layout is Alto 8X2. The 8 here refers to the 8 lanes on the cartridge and the 2 indicates the number of sensors per lane.
Allow multiple cartridges	The user can choose to map out the experiment layout for multiple cartridges if the number of ligands or analytes exceeds one single cartridge.

Surface

Under the Surface Option (Figure 4.32), the user can define the sensor chemistry type and choice of lanes used for the experiment as well as cleaning sensors and validating capture surfaces. The Table 4.7 outlines the parameter definitions as well as default recommendations for a new experiment.

Figure 4.32: Surface option tab parameters

Table 4.7: Parameter definitions and default recommendations for Surface options within the Assay setup.

Parameter	Definition	Default recommendation
Sensor chemistry	Users can choose different sensor chemistries. Here, CBX = Carboxyl sensor chemistry, NTA=NTA sensor, etc.	CBX
Lane Select	Users can constrain an assay to selected lanes, in the case of the Kinetics cartridge they can select/deselect lanes 1-8.	Off
Clean Sensors	Option to run an additional cleaning step in sensor activation activity	On
Validate capture surface	Option to select a single concentration of analyte with a known expected response to the ligand captured on the surface used to validate the capture surface.	Off

Interactions

Under the Interactions Option (Figure 4.33), the user can define the number of ligands, analytes, buffers, and unique interactions one would like to perform during the experiment. Table 4.8 outlines the parameter definitions as well as default recommendations for a new experiment.

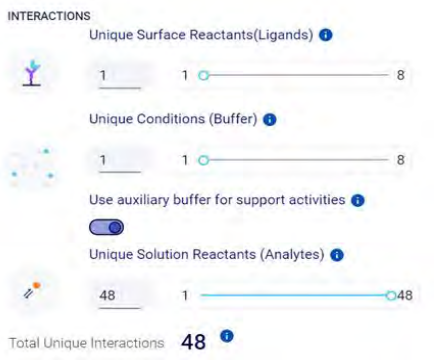

Figure 4.33: Interaction tab options and parameters

Table 4.8: Parameter definitions and default recommendations for defining interactions under the Assay setup.

Parameter	Definition	Default
Unique surface reactants (ligand)	Number of unique ligands for the experiment (1-8)	1
Unique conditions (buffer)	Number of unique buffers for the experiment (1-8)	1
Unique solution reactants (analyte)	Number of unique analytes for the experiment (1-48)	48
Use of auxiliary buffer	This buffer is used for all steps in normalization and ligand immobilization prior to analyte exposure if a buffer switch is desired for kinetic measurements. For example, for a COOH sensor, the user can choose to use PBS-T buffer for normalization and ligand immobilization and PBS-T-BSA buffer for studying the analyte interactions if BSA is needed in the buffer to reduce non-specific binding for the analyte during the experiment.	On
Total unique interactions	Total number of unique ligand-analyte interactions analyzed.	48

Once the experiment parameters are defined, the user can now proceed by clicking on “Create activities” to specify interaction times for each droplet in the experiment.

4.7.2.2 Method: Assay Activities

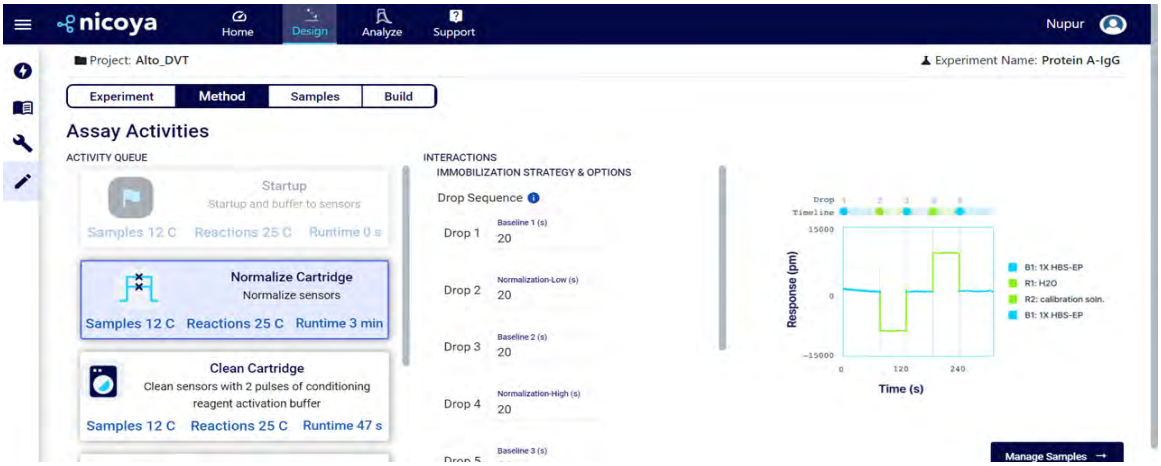

Figure 4.34: Outline for Assay Activities under the “Method” tab

For this step of the experimental setup, the summary of all activities (Figure 4.34) in the queue will define the sample layout and total number of cartridges (and methods). Table 4.9 describes the method parameters and the function of each parameter as well. Once the parameters for contact time are defined for each activity, the user will be able to visualize the time it will take each activity to execute on the core tabs for the activities.

Table 4.9: Method Parameter definitions.

Parameter	Definition
Activity	Activities are the main actions which make up a method. Each is composed of one or more cycles that achieve a specific function in the experiment.
Cycle	A repeat unit of work composed of one or more droplet sequences
Drop sequence	Groups droplets exposed to the sensor in a specific order to accomplish a specific function
Drop	A drop is the sample that the user would like to deliver to the sensor

Normalize Cartridge

Before any samples reach the sensors, the sensors are exposed to two normalization solutions which assess the sensitivity of each sensor. This data is used to correct for any discrepancies in sensitivity across the sensors. Once the user clicks on the “Normalize” tab as shown in Figure 4.35, the user will have access to the drop sequence parameters and will be able to input and change the drop timing, as necessary.

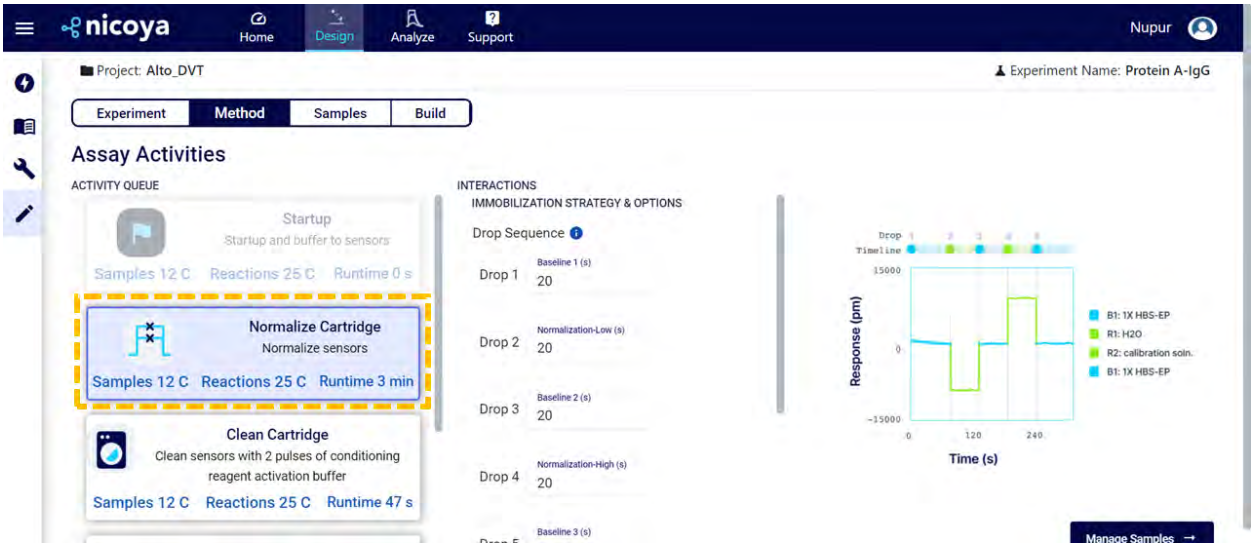

Figure 4.35: Drop sequence variables for normalization of the cartridge.

Table 4.10: Drop sequence variable definition and values for normalize cartridge cycle.

Parameter	Definition	Default sample	Default drop #
Baseline	Buffer droplet to establish a baseline for activity	Auxiliary buffer (An auxiliary buffer is any buffer used prior to the kinetic analysis during the experiment. This buffer should be chosen carefully to ensure that it does not interfere with the ligand or capture molecule coupling to the defined sensor chemistry. For example, for CBX chemistry, the auxiliary buffer should not contain any primary amine	1,3,5

		groups or strong nucleophiles like Tris, BSA or sodium azide for amine coupling as these will compete with the ligand.	
Normalization (Low)	Calibration solution 1 to measure a low response on the sensor to perform a linear calibration	Low RI normalization solution (SKU: ALTO-R-LNORM)	2
Normalization (High)	Calibration solution 2 to measure a high response on the sensor to perform a linear calibration	High RI normalization solution (SKU: ALTO-R-HNORM)	4
Temperature (Sample)	Temperature for sample storage on the cartridge	-	-
Temperature (Reaction)	Temperature for sensors on the cartridge	-	-
Replicates	Number of replicates for the “Normalize Cartridge” cycle	-	-

Clean Cartridge

The second step defines the order of delivering cleaning reagents specific to the sensor chemistry. This step alternates exposure to a cleaning reagent and buffer to rinse the sensor prior to sensor activation or ligand immobilization. The parameters for the clean cartridge are outlined and defined in Table 4.11.

Table 4.11: Drop sequence variable definition and values for clean cartridge cycle.

Parameter	Definition	Default sample	Default drop #
Pulse	Pulses are repeat exposures to the cleaning drop sequence	Dependent on sensor chemistry	1

Baseline	Buffer droplet to establish a baseline for the activity	Auxiliary buffer	2
Cleaning drops	Define the contact time of the cleaning reagent used with the sensor.	Surface cleaning solution for defined sensor type	
Temperature (Sample)	Temperature for sample storage on the cartridge	-	-
Temperature (Reaction)	Temperature for sensors on the cartridge	-	-
Replicates	Number of replicates for the “Clean Cartridge” cycle	-	-

Build Capture Surface

The third step defines the order of building a capture surface or ligand immobilization on a specific sensor chemistry. The parameters for the build capture surface cycle are outlined and defined in Table 4.12.

Table 4.12: Drop Sequence Variables for Ligand Immobilization

Parameter	Definition	Default sample	Default drop #
Baseline	Establish a baseline instrument response (example: this could be a capture molecule or direct ligand immobilization)	Auxiliary buffer	1
Activation	Activation of the sensor with the appropriate activation reagents.	Activation reagents dependent on the sensor chemistry chosen. (For example for CBX chemistry, the activation reagents are EDC-NHS)	2

Immobilization	Ligand immobilization or capture molecule on the sensor	Ligand	3
Buffer Check	Rinse after ligand immobilization or capture molecule immobilization	Auxiliary buffer	4,6
Quench	Blocking solution that deactivates any remaining binding sites	Blocking reagents dependent on the sensor chemistry chosen. (For example, for CBX chemistry, one option is to use 1M Ethanolamine as the blocking solution)	5
Smart Immobilization	Criteria for optimal ligand immobilization	-	-
Clean-up surface post immobilization	Conditioning with regeneration solution of choice prior to analyte injections	-	-
Temperature (Sample)	Temperature for sample storage on the cartridge	-	-
Temperature (Reaction)	Temperature for sensors on the cartridge	-	-
Replicates	Number of replicates for the “Build capture surface” cycle	-	-

Direct Kinetics

The fourth and final step defines the introduction of analyte on the immobilized ligand. Depending on if single cycle kinetics or multi-cycle kinetics options were chosen for the assay setup in Section 4.7.2.1, one may observe differences in drop numbers which would be different

from the options outlined in Table 4.13. Table 4.13 defines the drop sequence options for single cycle kinetics only for the purposes of this manual.

Table 4.13: Drop Sequence Variables for Direct Kinetics for single cycle kinetics option.

Parameter	Definition	Default sample	Default drop #
Baseline	Establish a baseline for each cycle	Running buffer	1,2,10
Analyte	Analyte association or contact time for each concentration will be defined here	Analyte	3-7
Analyte dissociation	Analyte dissociation time will be defined here	Running buffer	8
Regeneration	Regeneration solution contact time to be define here	Regeneration solution defined as per the experiment	9
Temperature (Sample)	Temperature for sample storage on the cartridge	-	-
Temperature (Reaction)	Temperature for sensors on the cartridge	-	-
Replicates	Number of replicates for the direct kinetics cycle	-	-

Once the parameters for each cycle are outlined in the method, the user shall click on “Manage samples” to label all the samples on the cartridge.

4.7.2.3 Sample Identity

The samples tab provides the user the ability to label the different parameters for their samples as shown in Figure 4.36 including the following:

1. Sample name
2. Concentration and concentration units
3. Condition to define the solvent that the reagent or sample is diluted in.
4. Molecular weight in kDa

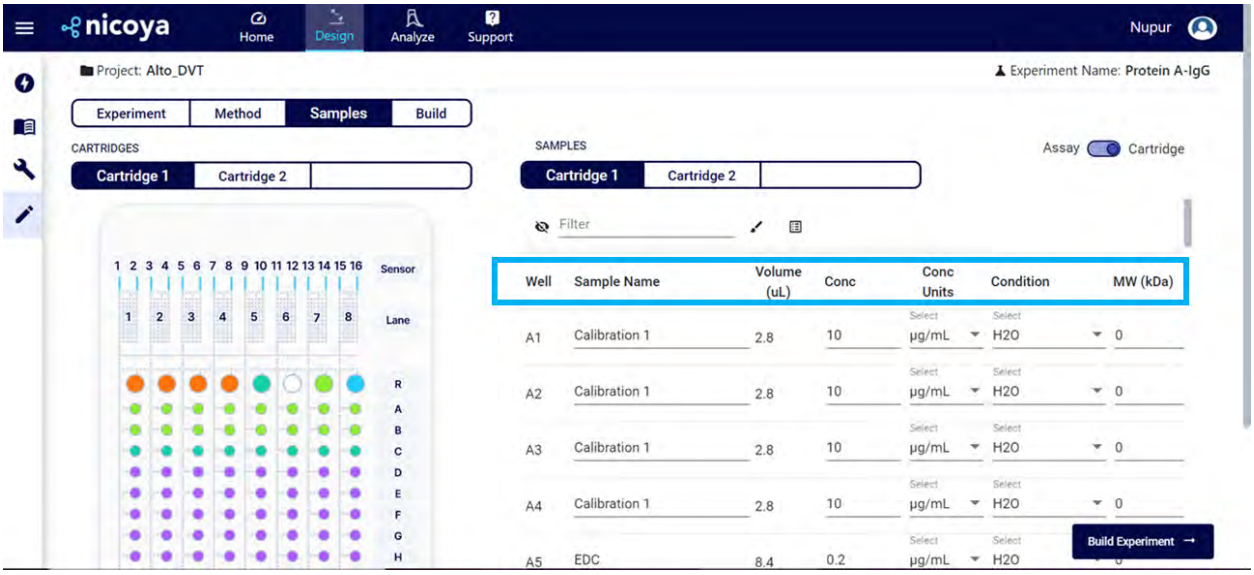

Figure 4.36: Sample labelling under the Assay configuration for the cartridge.

The user can toggle between the Assay configuration and the Cartridge configuration as shown in Figure 4.37 on the top right corner of the software.

Figure 4.37: Toggle option for Assay or Cartridge Configuration

For the cartridge configuration, as shown in Figure 4.38 and Figure 4.39, the user can switch between the different sample types defined on the cartridge between the following categories:

1. **Solution:** All analytes defined on the cartridge wells for all columns (1-8) for rows D-I.
2. **Surface:** Ligands and capture molecules defined on the cartridge wells for all columns (1-8) for row C.
3. **Reagent:** Normalization, activation, and blocking solutions are defined on the cartridge wells A1-A4, B1-B4, A5-A8 and B5-B8 respectively.
4. **Regen:** Regeneration solutions defined in the cartridge wells between columns R1-R4.
5. **Condition:** The solvents that the reagents are diluted in (e.g. PBS-T, NaOAc, H2O etc.) are defined for the cartridge well R7.
6. **Other:** Other buffers or solutions defined on the cartridge wells R5, R6, R8. All running buffer for all columns (1-8) are in the row labelled BF.

Figure 4.38: Solution sample overview in Cartridge configuration.

Figure 4.39: Surface sample overview in Cartridge configuration.

Overall, a default CBX cartridge layout will be as shown in Figure 4.40 using direct kinetics options for the reagent locations.

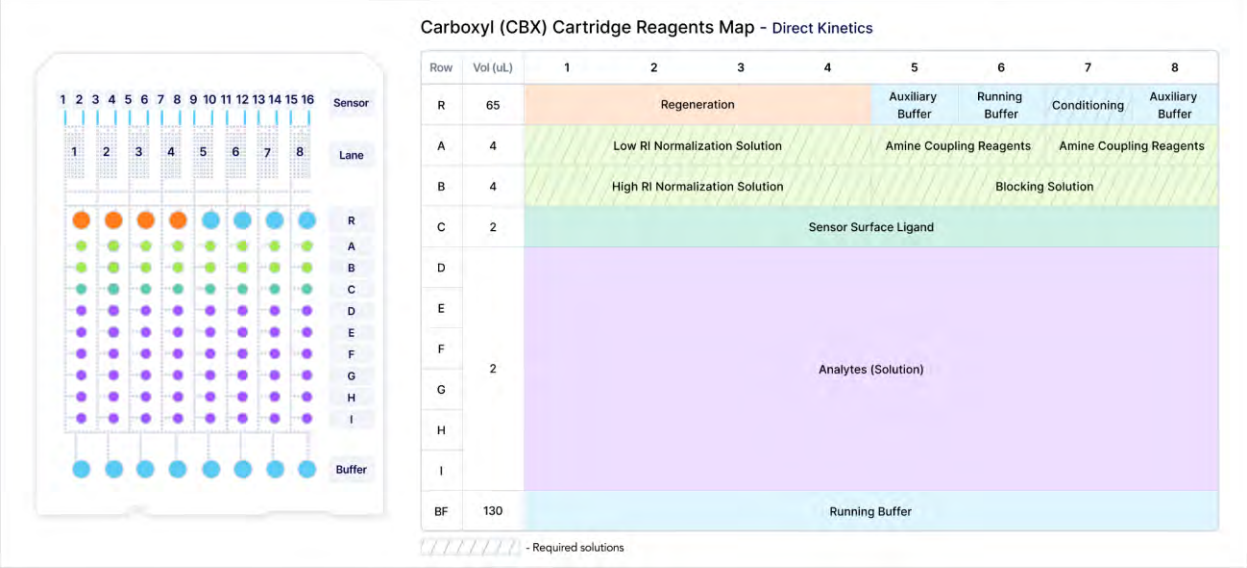

Figure 4.40: CBX cartridge layout for single cycle kinetics experiment method.

Once the samples are labelled and defined, the overall experiment build is ready to be completed and the user can click on “Build experiment” to proceed to the final experiment method step.

4.7.2.4 Build

The build step outlines the estimated runtime for the experiment in hours along with a summary of the number of unique interactions as well as the cartridges used for the test. The user has the option to select a workgroup to share the experimental method with other users or workgroups as shown in Figure 4.41.

Under the timeline section, each cycle number is defined (cycle 1 indicates normalization, cycle 2 indicates cleaning surface, cycle 3 indicates build surface and cycles 4-9 indicate kinetics) for each sensor from 1-16.

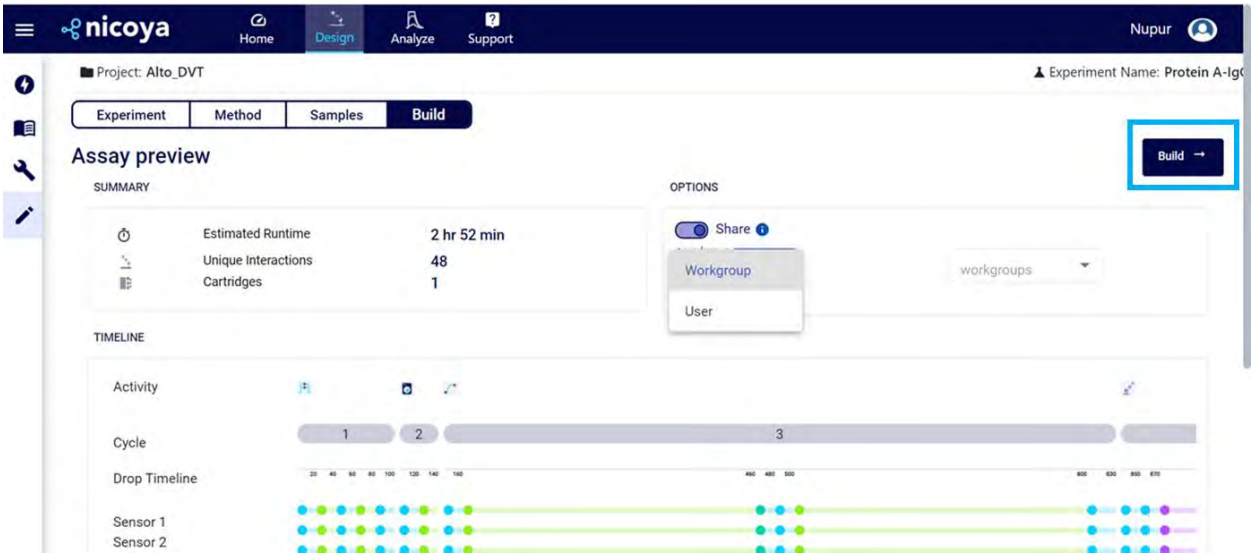

Figure 4.41: Build experiment method overview.

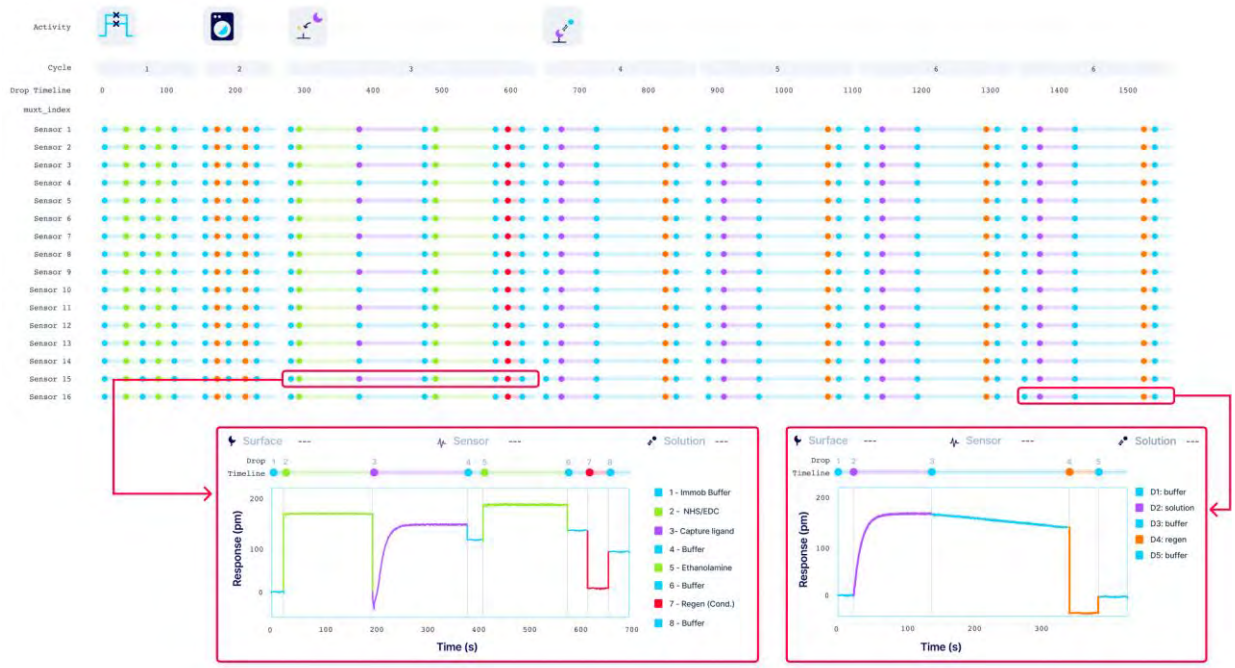

Figure 4.42: Zoomed in view for a specific sensor and drop sequence selection.

Once the user has designed the experiment and clicked on “Build” located at the top right corner as shown in Figure 4.42, it will automatically be saved to the online server. Once the user logs into the Alto Control Software on the Alto device, the user will automatically be able to review the full setup on the Alto instrument.

5.0 Standard Operating Procedures

5.1 Starting the Instrument

1. Power on the instrument using the procedures outlined in Section 4.6.1 of this manual.
2. Once the instrument is powered on, wait for the instrument to startup.
3. Once the instrument is started, the Alto control software will startup automatically.

5.2 Setup an Internet Connection

To connect your instrument to the internet, you can either use an ethernet cable or WiFi:

5.2.1 Option 1: Setup an Ethernet Cable Connection

1. To connect your instrument to the internet, you will need to use an ethernet cable. Attach an ethernet cable in the port highlighted in Figure 5.1. Connect one end of the ethernet cable to the Alto instrument and the other end to your router or wall connection to complete the setup.

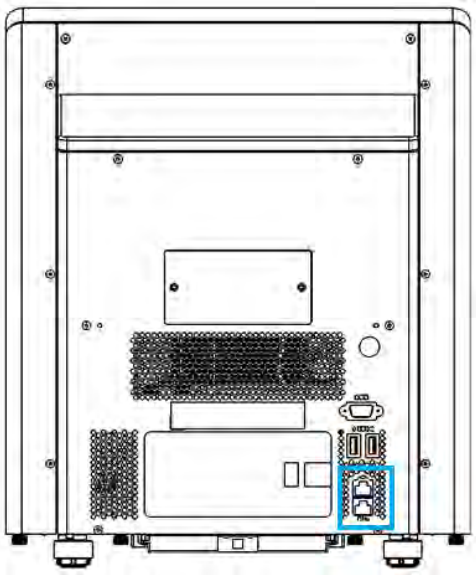

Figure 5.1: Location of Ethernet ports on the back of the Alto instrument.

5.2.2 Option 2: Connect to a Wireless Network

It is important to note that the ethernet connection will ensure a stable internet connection compared to using WiFi option as this may be unstable.

1. Please choose a wireless network to set up your Alto-16 instrument in the Alto Control Software.
2. Once the password is entered and the WiFi connection is established, you will be able to proceed to use the Alto-16 Control Software.

5.3 Alto Control Software Login

1. Once the Alto instrument is turned on, the main login view with user selection is displayed. This allows the user to select themselves from the list of users as shown in Figure 5.2.

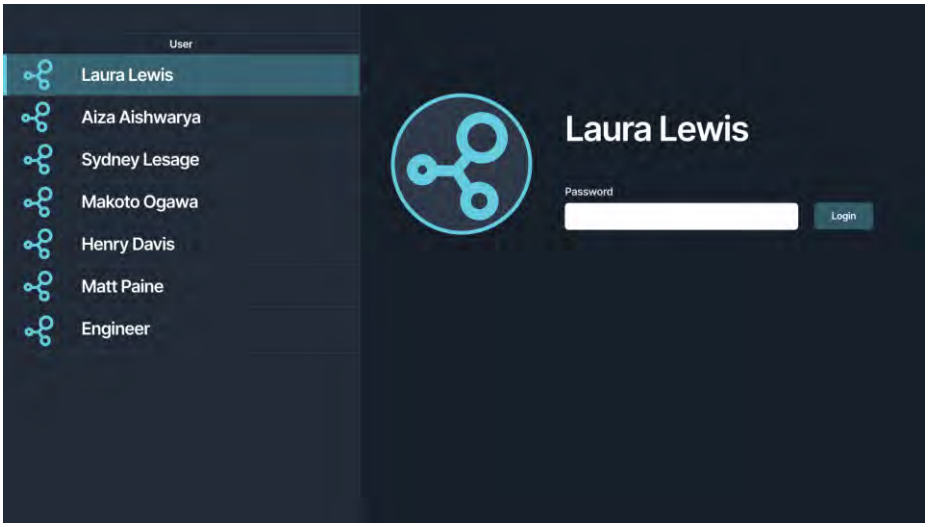

Figure 5.2: User login selection

2. Once you select a user from the user list, the login screen will give you the option to enter your password and login. The default selection is the last or current user on the Alto system.
3. Once you are logged in, you will be taken to a new experiment page.

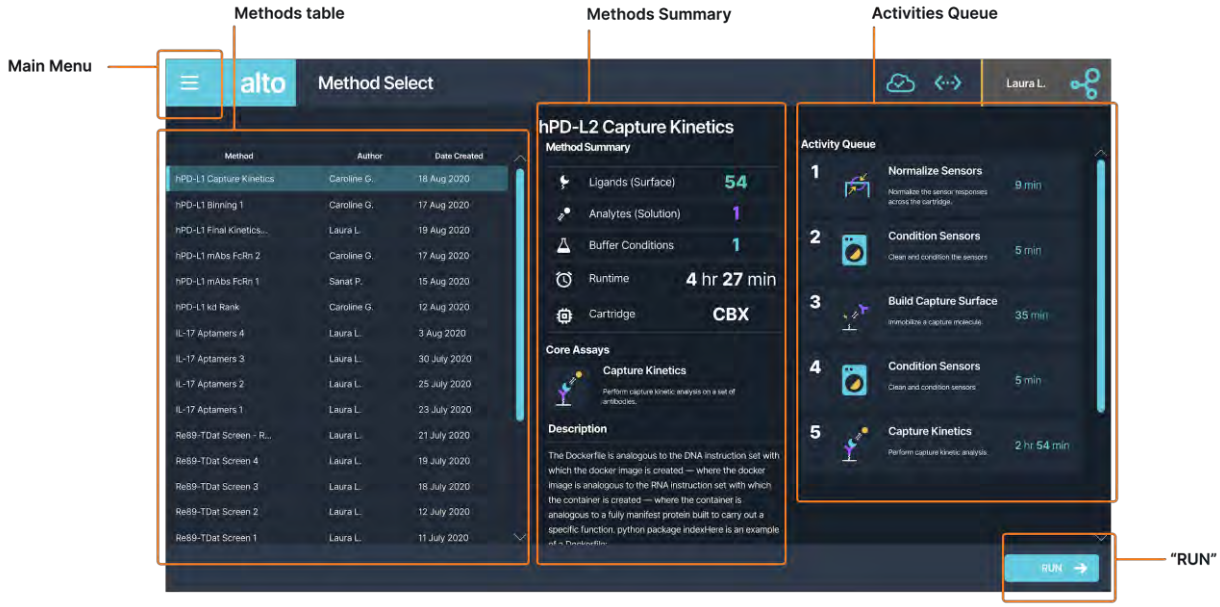

Figure 5.3: Features available on the Method Select screen for the Alto device.

4. All the features available on the Method Select screen are outlined in Table 5.1.

Table 5.1: Method Select screen parameters and definition.

Parameter	Definition
Main menu	The main menu button provides access to unit information, servicing options along with user switching options. These include Software updates, Diagnostics, Optics Calibration, About the instrument, Logout, and Power off options. The main menu options will only be accessible from the Method Select screen and will appear inactive for the other Alto Control software screens.
Methods table	The methods table provides a full list of experiments designed using the user portal with the names, authors, and dates for each experimental method.
Methods summary	The methods summary provides an overview of the selected experiment under the methods table section for the number of ligands, analytes, cartridges, buffers, core assay type, and overall experiment run time.
Activities Queue	The activity queue defines every activity that will be performed for a certain experiment.
Run	The run button will guide the user through the process of loading the cartridge and samples to start the experiment on the Alto device.

5. A system diagnostics test is recommended if this is the first time the instrument is being operated after installation. The diagnostics test consists of working with the Optics Service Cartridge (full process overview is outlined in Section 5.4) and QC cartridge (full process overview is outlined in Section 5.5) to service the instrument prior to performing experiments. Depending on the prerequisites for servicing via each cartridge type, if the system diagnostics test is not needed, the user can skip to Section 5.6 of this manual to continue with experimental setup.

5.4 Optical Calibration with Optics Service Cartridge

The Optics Service Cartridge (OSC) is a reusable cartridge used to run optics calibration and clean the optics interface of the Alto instrument. Upon completion of optics calibration, the Fiber Cartridge Interface (FCI) obtains a bright reference for the instrument. The cartridge has a

lifetime of 5 years when used as intended. 6 OSC Sliders are provided with the instrument that are easily replaceable by the user.

5.4.1 When to use the OSC

The cartridge should be used in the following scenarios:

1. When the instrument is first received or installed, the user should run optics calibration prior to using the QC cartridge for the diagnostics test.
2. Once a month, if not recommended otherwise.

5.4.2 OSC Replacement parts

1. Once the cartridge has been used 5 times, the OSC Slider will need to be replaced. This procedure can be performed outside the Alto instrument as outlined in the Optics Service Wipe installation section of this protocol below.
2. If there is any suspicion of dust on the optical mating face of the cartridge, the OSC Slider should be replaced.

Figure 5.4: Optical mating surface in the Alto instrument from the cartridge view.

Conditions to Avoid

Be careful not to drop the cartridge or collect dust on the optics service surface as this can compromise cartridge functionality and prevent reuse. The customer should keep a log of the usage of the cartridge. The cartridge wipes will need to be replaced every 5 uses to ensure usability.

5.4.3 Materials needed for Optical Calibration

- Optics Service cartridge
- 80% Isopropanol or 80% Ethanol
- OSC Slider
- Lint free wipes
- Gloves

5.4.4 Optics Service Wipe Installation

The Optics Service Cartridge has an Optics Service Wipe installed on it to clean the optical mating face of the instrument. The OSC will be delivered with the first Optics Service Wipe installed in the cartridge. When the OSC needs to be replaced after 5 uses, assemble the cartridge as defined below:

5.4.4.1 OSC Wipe Installation

1. Remove the OSC from the packaging and place on a clean surface or kim wipe.

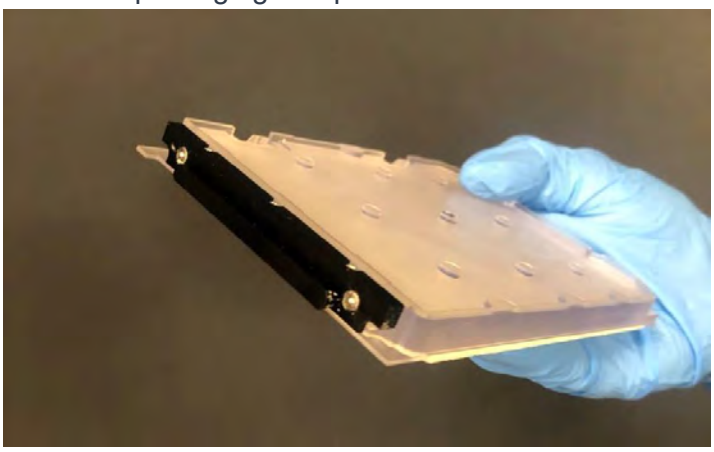

Figure 5.5: OSC side view without the OSC Slider.

2. Remove the OSC sliders from the packaging. Place the 5 Optical Sliders in the accessories box and take one of the Optical Sliders for assembly onto the OSC.
3. Slide the OSC Slider onto the cartridge (Figure 5.6). The OSC Slider will come preloaded with lint free wipes. Install the OSC slider onto the OSC as shown in Figure 5.6. The OSC slider will automatically stop moving once it reaches the end of the cartridge opposite of where the user started the installation.

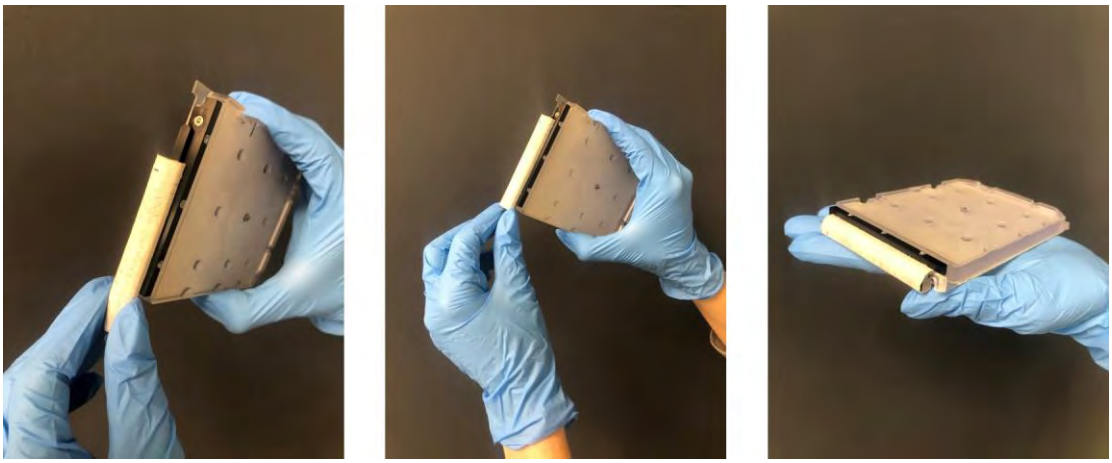

Figure 5.6: Installing the OSC Slider onto the OSC.

5.4.4.2 Installing the OSC into the Alto

4. Once the OSC slider is loaded, place the cartridge on a lint free wipe as you progress to the Optics Calibration Protocol in the Alto Control Software.
5. Spray the surface with 80% IPA on the entirety of the wipe. Wait for 1 minute to ensure that the wipe is damp but not wet prior to inserting into the instrument.

5.4.5 Optics Calibration Protocol

1. Click on the main menu button of the Alto Control Software to open the menu and select “Optics Calibration” (Figure 5.7).

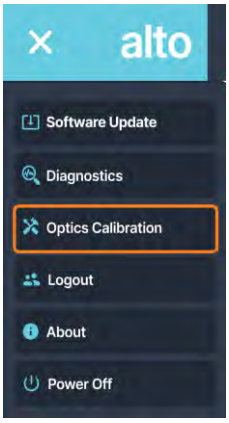

Figure 5.7: Optical calibration selection on the Alto Control Software menu.

2. The software will prompt the user to load the OSC into the instrument. Load the OSC into the cartridge loading bay, with the Optical Slider face pointing towards the instrument at a 15-degree angle (Figure 5.8).

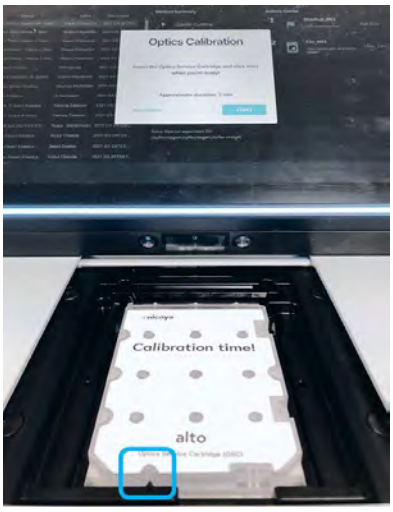

Figure 5.8: Loading the OSC into the instrument. It is important to note that the notch highlighted in the blue box must align with the bottom of the cartridge loading bay.

3. Once the cartridge is placed in the loading bay, the cartridge will be detected and the “Start” button will become clickable for the Optics Calibration Procedure as shown in Figure 5.9. Click the start button to start the calibration procedure. This process will take about 2 minutes.

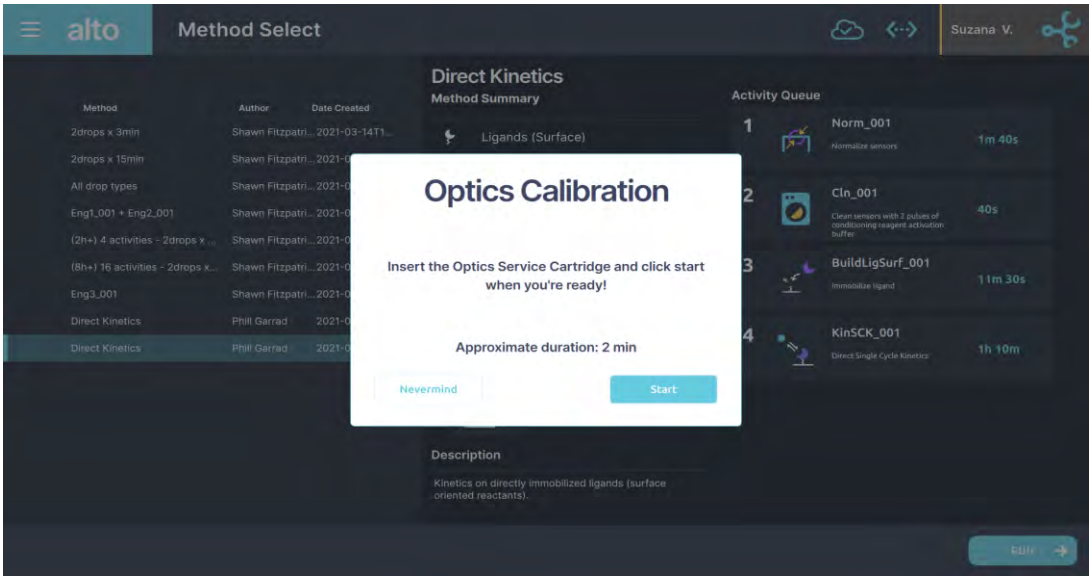

Figure 5.9: Once the OSC is placed in the loading bay, click “Start” to begin the calibration procedure.

4. Once the optics calibration is complete, select “Done”.
5. The cartridge loading bay will be automatically exposed to remove the cartridge from the instrument. The bright and dark references will be taken at this point.

   **If there is an error reported instead of a successful calibration, select “Try Again” or “Exit”. If the error is observed after 3 continuous attempts, please contact your Customer Success Scientist for next steps.

6. Once the OSC is removed from the instrument, place it in the plastic bag provided by Nicoya. Store the cartridge in the marked drawer under the instrument (Figure 5.10).

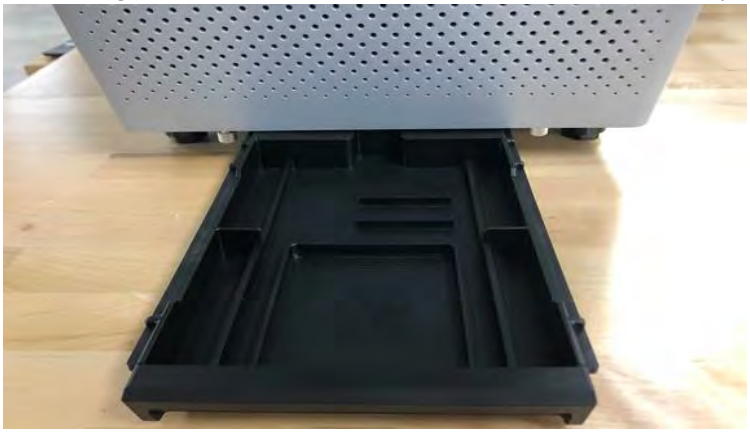

Figure 5.10: Location of the drawer located underneath the instrument.

5.4.6 Disassembling the OSC Slider

1. To disassemble the OSC Slider so that it can be replaced, push the slider off as shown in Figure 5.11.

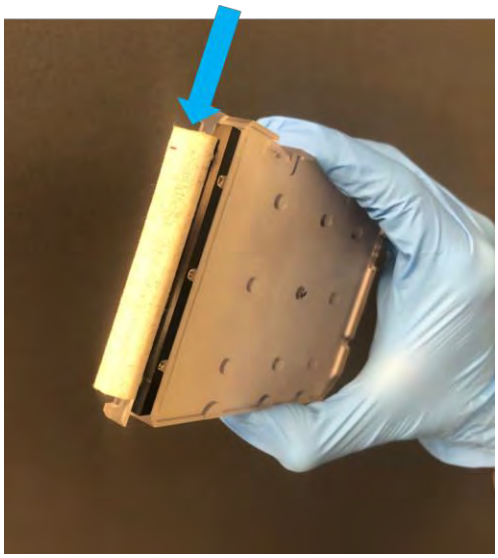

Figure 5.11: Push on the OSC Slider to unlock the slider and remove it from the OSC.

5.5 Diagnostic Test with the QC Cartridge

The QC cartridge is used to run system diagnostics on the instrument and ensure functionality of the primary modules of Alto. The QC cartridge will test for the electrical, optical, and thermal connectivity.

5.5.1 When to use the QC Cartridge

The QC Cartridge is required to be used during instrument installation and is recommended in the following scenarios:

1. This is the first time the instrument is installed, and operational qualification is performed after the optical calibration is complete.
2. The instrument has not been used for an extended period(2 weeks or more)
3. After restarting the instrument from a critical error. If the instrument detects a critical error the lightbar will turn red and the user will be prompted to restart the unit. After the unit is initialized, it is recommended that the user runs a diagnostic test. System errors may be detected by the instrument and will be indicated by the LED light bar turning red.
4. The instrument is relocated from one space to another.
5. The instrument encounters a power loss or unexpected electrical shutdown.
6. If a full system diagnostic is preferred by the user prior to running experiments to check for instrument functionality.

The QC Cartridge is reusable and stored within the drawer provided in the instrument. The shelf life for the QC cartridge is 5 years if used as intended and when there is no damage to the cartridge. If the cartridge is dropped on the floor, this can lead to cartridge damage and you may need a replacement for any future use. Please contact your Customer Success Scientist in case of damage to the cartridge for next steps.

5.5.2 Adding Optics Gel to the QC Cartridge

Optics Gel must be added to the QC Cartridge Ferrule Fiber Assembly (FFA) prior to running system diagnostics.

5.5.2.1 Materials Needed

- QC cartridge
- Gloves
- Optics Gel (3mL syringe barrel and plunger)
- Luer lock needle tip
- 80% IPA or Ethanol
- Lint-free wipe

5.5.2.2 Gel Application Instructions

1. Screw the luer lock needle tip onto the lure end of the syringe barrel.
2. Remove the plug from the other end of the barrel and insert the plunger.

Figure 5.12: Assembly of the optics gel syringe.

3. Wipe the outer edge of the black Ferrule Fiber Assembly (FFA) with 80% IPA or Ethanol using a lint-free wipe (Figure 5.13).

Figure 5.13: Wipe the outer edge of the black ferrules with a lint free wipe soaked in 80% IPA.

4. Hold the cartridge in your hand such that the FFA is visible and easily accessible for adding the optics gel (Figure 5.14). It is recommended to place the green tip into the black ferrule at an angle of 45 degrees to the axis of the black ferrule.
5. Dispense the gel into the black ferrule until some gel squeezes out from the side of the ferrule.
6. Proceed to fill the rest of the ferrules with the gel.

Figure 5.14: Optical gel application for the QC cartridge.

7. Repeat steps 4-6 for each of the 16 ferrules using caution not to touch the gel after it is applied to the fibers. ****DO NOT touch the gel once it is applied. This could result in the gel being removed from the ferrules.**
   **If the gel is removed, repeat steps 3-6, making sure to wipe-off any excess gel that is visible around the ferrules before reapplying. Do not clean the inside of the ferrule.

8. If the user would like to reuse the needle, wipe the needle tip with lint-free wipe to prevent build-up of gel. Place the optics gel back into a cool, dry place for storage or in the accessories box. Dock the cartridge inside of the instrument and proceed with your experiment.
   Note: It is better to have more gel than not enough gel. While too much gel over-time can cause a build-up on the Fiber Cartridge Interface (FCI), it can be easily cleaned with an Optics Service Cartridge (OSC).

5.5.3 Diagnostics Test Protocol

1. Click on the main menu button of the Alto Control Software to open the menu and select “Diagnostics” as shown in Figure 5.15.

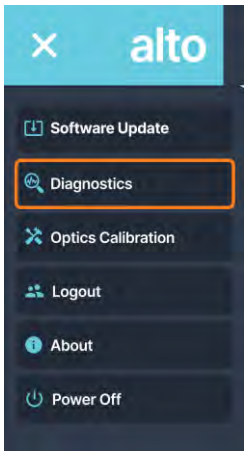

Figure 5.15: Select the “Diagnostics” option in the menu to proceed with the testing.

2. Install the QC cartridge in the instrument with the notch aligned and facing outward as shown in Figure 5.16.

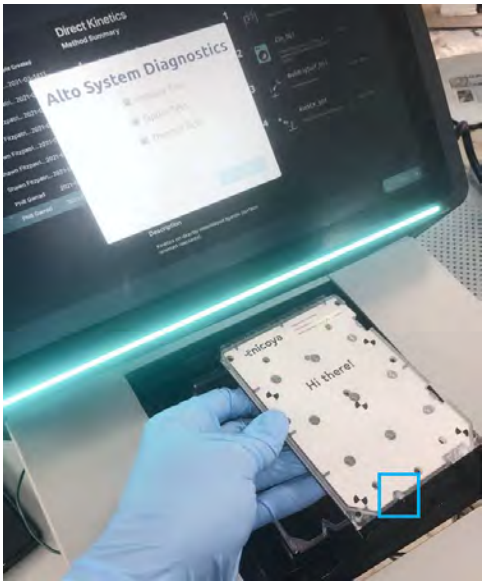

Figure 5.16: Alignment of the cartridge notch for loading the cartridge into the cartridge loading bay for Alto.

3. Once the cartridge is loaded, select from the three test options available:
    a. **Voltage test:** This test should be performed to check the performance of the electrodes to ensure fluidic protocols via DMF can be completed. This test can be performed if irregular data acquisition is observed while running an experiment using the Alto control software.

    b. **Optics test:** This test should be performed to check the optics pipeline and ensure all channels are operating properly. This test can also be performed if poor data quality is consistently observed over multiple experiments.

c. **Thermal test:** This test should be performed to ensure that the thermal module is operating to control both the reagent and sensor zones of the instrument. This test should be performed if there is suspected damage to the thermal module.

It is recommended that all the test options are selected for the Installation and/or Operational Qualification for auditing purposes. After installation, this cartridge is only required to be employed under one of the other examples in the 'When to use the QC Cartridge' section above.

4. Once the test options have been selected, click "Start" to proceed with the test. The full diagnostic test takes approximately 30 minutes. During this test, the user will be unable to operate the instrument for any other experiments.

Figure 5.17: Option selection for one or multiple tests for diagnostic purposes

5. Once the test is complete and successful, the screen in Figure 5.18 is shown. The green checkmarks in front of each selected test indicate that the instrument is good to proceed with the kinetics cartridge for the performance qualification test (if this is the first time using the instrument) or with the cartridge selected for your experiment.

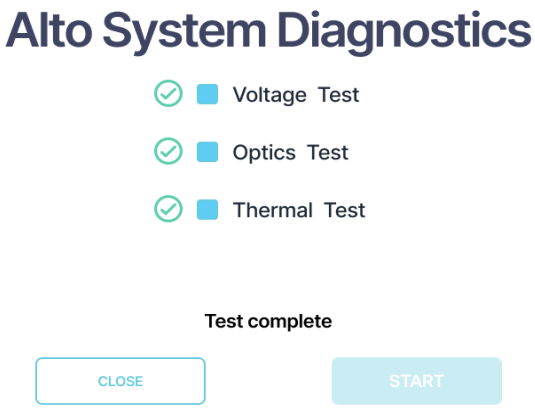

Figure 5.18: Success criteria for Alto system diagnostics.

If the test is unsuccessful or any of the selected tests have failed, a “red cross” next to the test type will be shown as in Figure 5.19. It is recommended to perform the test again before you contact your Customer Success Scientist for next steps.

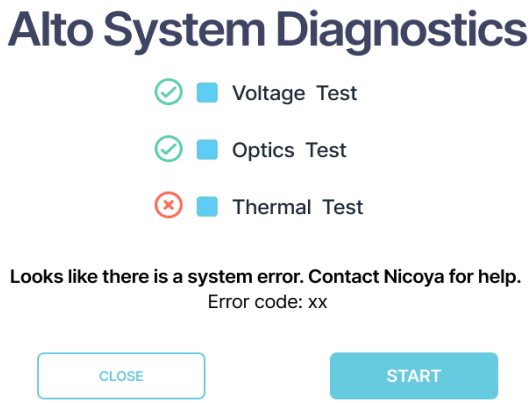

Figure 5.19: Failure criteria for Alto system diagnostics.

6. Once the process is complete, the cartridge will be ejected automatically. The user can select, click on “Close” to return to the previous view (Method Select screen) . Remove the QC Cartridge by lifting the cartridge out from the instrument.
7. The QC Cartridge can now be placed in the plastic bag provided and stored in the drawer built into the base of the unit.

5.6 Experimental Setup

The user will need to design and set up the experiments on the User Portal first including any edits to the experimental methods before starting the experiment on the Alto. Please refer to Section 4.7.2 for further information about how to set up your experiments on the User Portal.

5.6.1 Required Materials

To prepare for your experiments, the following materials will be required:

- Alto instrument with corresponding power and USB cables
- Kinetics cartridge
- Lint-free wipes
- Running buffer, filtered (0.2 µm filter)
- Samples
- 0.1% Tween 20 added to all samples

- Cartridge fluid
- Calibration solutions (provided by Nicoya)
- Relevant personal protective equipment

5.6.2 Loading Your Experiment

1. To start your experiment, navigate to the dashboard of the Alto Control Software. Once the Method Select screen is loaded, you can now choose the method named “Alto PQ Experiment” (Figure 5.20) and click on the “RUN” button at the bottom right corner of the software screen. Table 5.2 outlines all the parameters you can see for your experiment once the method is selected.

Figure 5.20: Selecting the “Experiment” method on the Method Select screen. This experiment is designed to immobilize the Protein A as the ligand and IgG as the analyte on a COOH sensor.

Table 5.2: Parameter descriptions for the Method select screen in Figure 5.20.

Parameter	Description
Method	A user-designed experiment workflow with all available parameters & variables
Author	Name of the creator of the method
Date created	Date when the method was created
Method summary	A summary of all experimental parameters
Ligand	Biomolecule coupling to the sensor
Analyte	Biomolecule free flowing in solution
Buffer condition	Buffer compositions for the experiment

Runtime	Total time for the experiment run
Cartridge	Cartridge type
Core Assay	Type of assay selected for the experiment
Description	Any additional information about your experiment

5.6.3 Unpacking the Cartridge

1. The cartridges are provided in sealed mylar bags (Figure 5.21). You can identify the type of cartridge with the Product name, SKU, Expiry, and storage conditions information provided on the label.

Figure 5.21: Cartridge packaged in the mylar bag and should be cut on the dotted line in the figure.

2. Once you have identified the cartridge to use, wear gloves to remove the cartridge from the packaging using the edges.
3. To unpack the cartridge, use scissors to open the cartridge from either end of the packaging. Make sure to store the packaging materials in a safe place as these will be used for disposal of the cartridge after.
   Note: Be extremely careful to not drop the cartridge on the floor or hit the cartridge on a surface. This can lead to disassembly and cartridge failure and render the cartridge ineffective for use. This could also result in damage to the Alto instrument.

5.6.4 Loading the Cartridge

1. The software setup screen will now instruct the user to load the cartridge (Figure 5.22).

Figure 5.22: Cartridge docking screen.

2. To load the cartridge into the instrument, pick up the cartridge using the edges as shown in Figure 5.23.

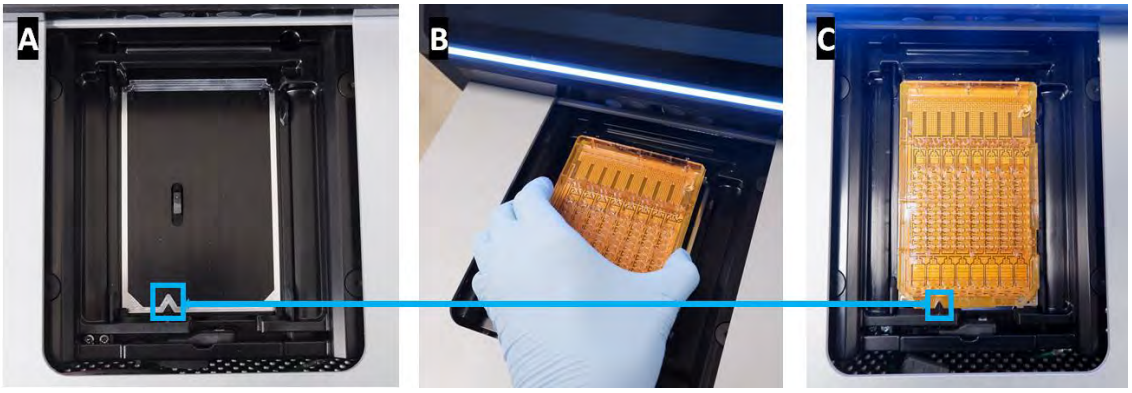

Figure 5.23: (A) Exposed view of the cartridge loading bay with the notch highlighted in the blue; (B) Loading the cartridge into the cartridge loading bay; (C) Cartridge is loaded and the notch aligns with the cartridge as highlighted in the blue box.

3. The cartridge loading orientation is important to ensure that the cartridge is sitting properly inside the instrument. To ensure that this is done correctly, the cartridge must sit flat and should not be at an angle. The notch in the cartridge ensures that it is oriented correctly in the cartridge bay. The cartridge will be detected inside the instrument due to the presence switch located within the cartridge loading bay (Figure 5.24).

Figure 5.24: Location of the presence switch in the Alto instrument.

4. Once the cartridge has been loaded, select "Ready to fill" to move onto the next step of inserting cartridge fluid.

5.6.5 Loading the Cartridge Fluid

The cartridge fluid is a silicone oil solution that seals the samples in the instrument and helps transport the droplets across the cartridge. Each cartridge packaging will contain one cartridge with one cartridge fluid syringe.

1. Locate the cartridge fluid syringe in the cartridge box. Remove the cartridge fluid syringe from the cartridge box. Store the cartridge fluid syringes at room temperature when not in use. To unpack the cartridge fluid syringe, use the notches on the packaging to remove the syringe (Figure 5.25)

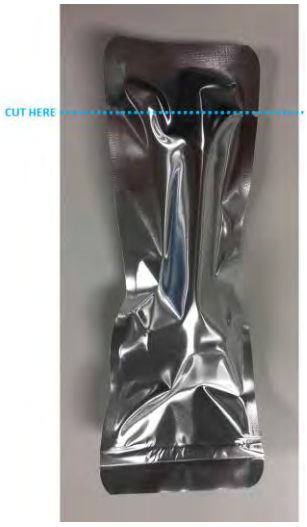

Figure 5.25: Unpacking the cartridge fluid.

2. The software screen (Figure 5.26) will direct you to load the cartridge fluid into the cartridge.

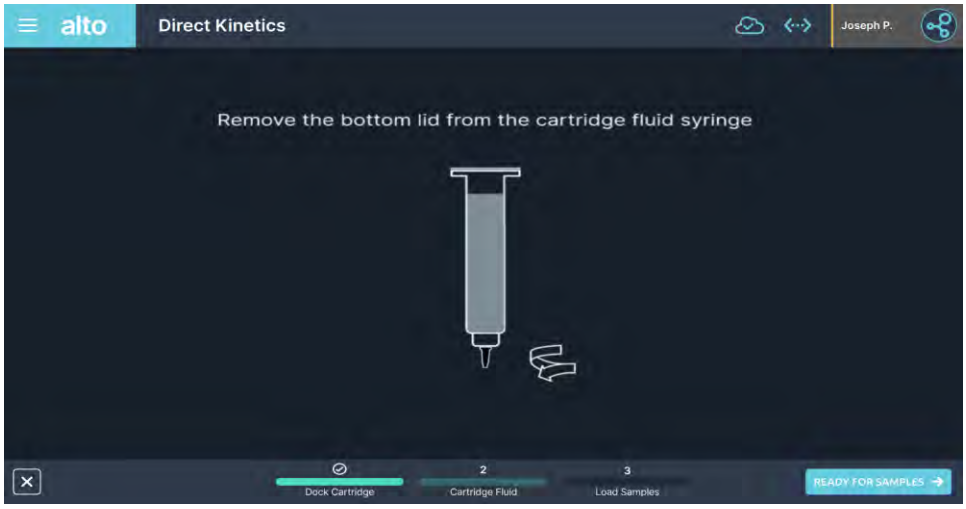

Figure 5.26: Loading the cartridge fluid into the cartridge.

3. To remove the bottom lid from the cartridge fluid syringe the customer can use the CF cap opener which can be found in the accessories kit. Figure 5.27 shows the step-by-step instructions to undo the bottom lid of the cartridge fluid syringe using the CF cap opener.

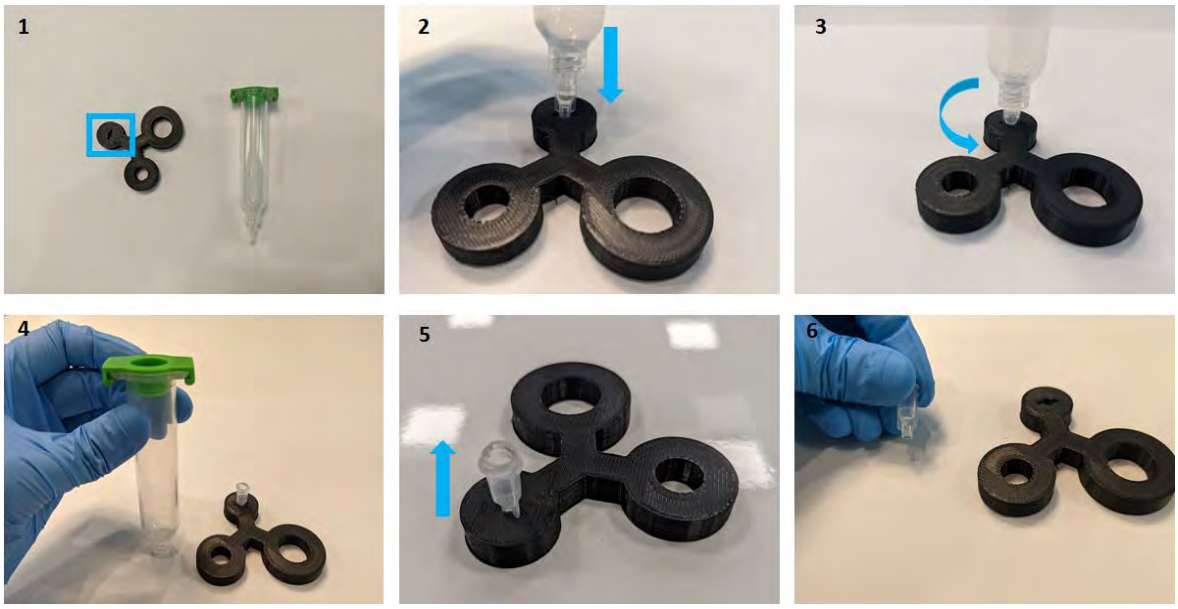

Figure 5.27: Unscrew the cartridge fluid cap by turning anti-clockwise prior to installing on the cartridge.

4. Once the cap is removed, load the cartridge fluid syringe in the fluid port as shown in the software prompt in Figure 5.28.

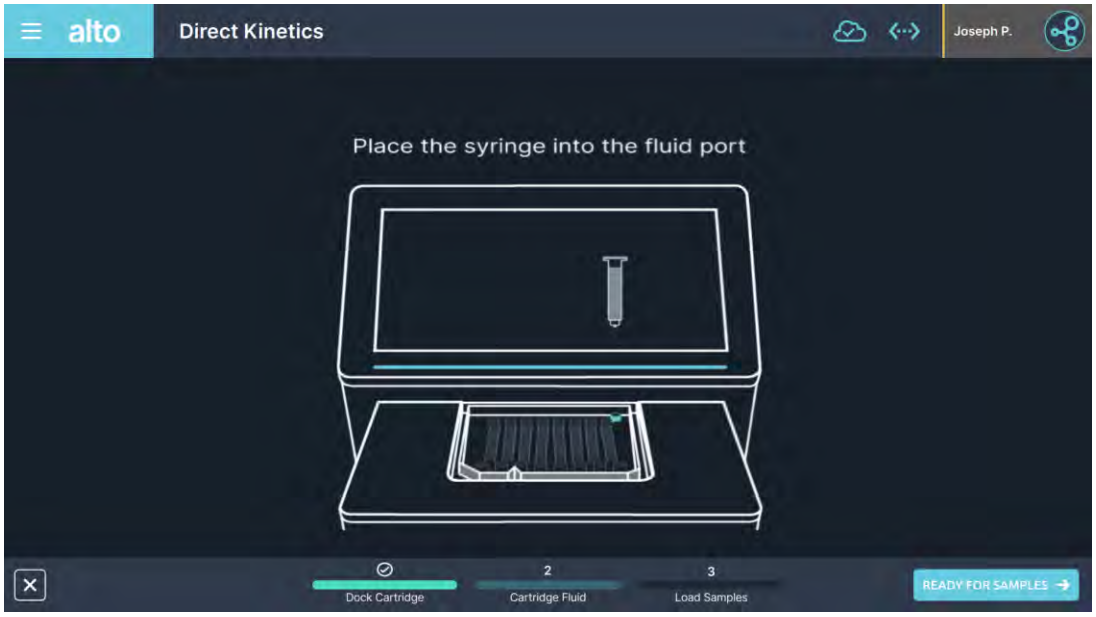

Figure 5.28: Cartridge fluid syringe loading into the fluid port on the cartridge.

5. Lower the cartridge fluid syringe onto the cartridge fluid port on the cartridge (Figure 5.29 (A) and (B)). The cartridge fluid syringe luer tip should fit snug on the cartridge fluid well. To ensure that the cartridge fluid syringe is connected properly, the user should not have to hold the cartridge fluid syringe to sit in a vertical position (as shown in Figure 5.29 (B)). Once the syringe is secured on the cartridge, remove the top cap of the syringe (as shown in Figure 5.29 (C)) and the cartridge fluid will start loading into the cartridge. Wait for 1 minute for all the cartridge fluid to load into the cartridge and the cartridge fluid syringe is empty (Figure 5.29 (C)).
   Important: Do not twist or turn the syringe for loading.

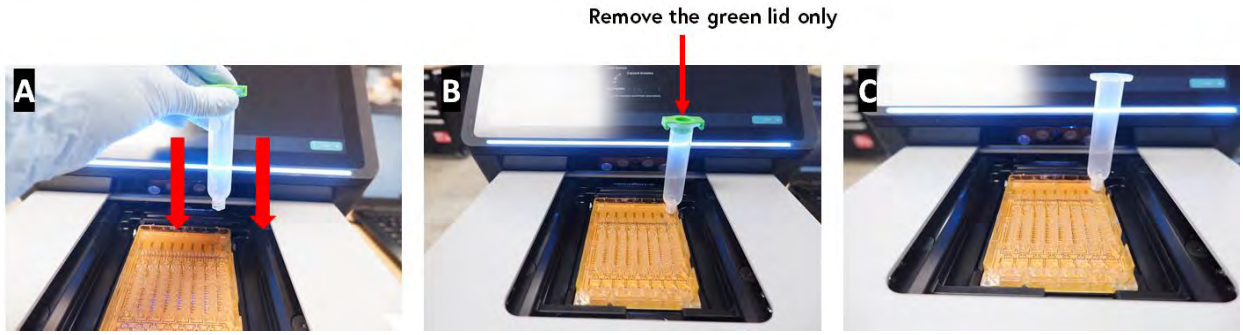

Figure 5.29: (A) Lower the cartridge fluid syringe onto the cartridge fluid port (B) Remove the top cap from the cartridge fluid syringe to start loading the cartridge fluid into the cartridge. (C) Please ensure that the cartridge fluid syringe is empty before proceeding to the next step.

6. Once the syringe is empty (Figure 5.30), hold the cartridge with one hand on the top and lift the syringe slowly and carefully from the cartridge to avoid any spills or lifting the cartridge off the cartridge loading bay.
   Note: Avoid hitting the cartridge fluid syringe at any other spots on the cartridge except for the cartridge port.

Figure 5.30: Remove the empty cartridge fluid syringe from the cartridge.

7. The user can now click on “Ready for samples” in the software for sample loading into the cartridge.

5.6.6 Loading Samples

1. You will now be directed to load your samples into the cartridge. Before loading your samples, review your sample location and information on the software screen. To do this, click on the interactive cartridge to ensure that the sample details are precise (Figure 5.31). You can select the different wells using the touch screen. For example, if you can click on the R1-R4 well (little green well on the cartridge in Figure 5.31 that is highlighted in blue). Wells on the cartridge may be selected, and corresponding samples for selected wells will be highlighted in the table. Individual wells and rows may be selected by the user as well. The scroll bar of the sample table can be used to see all sample details.

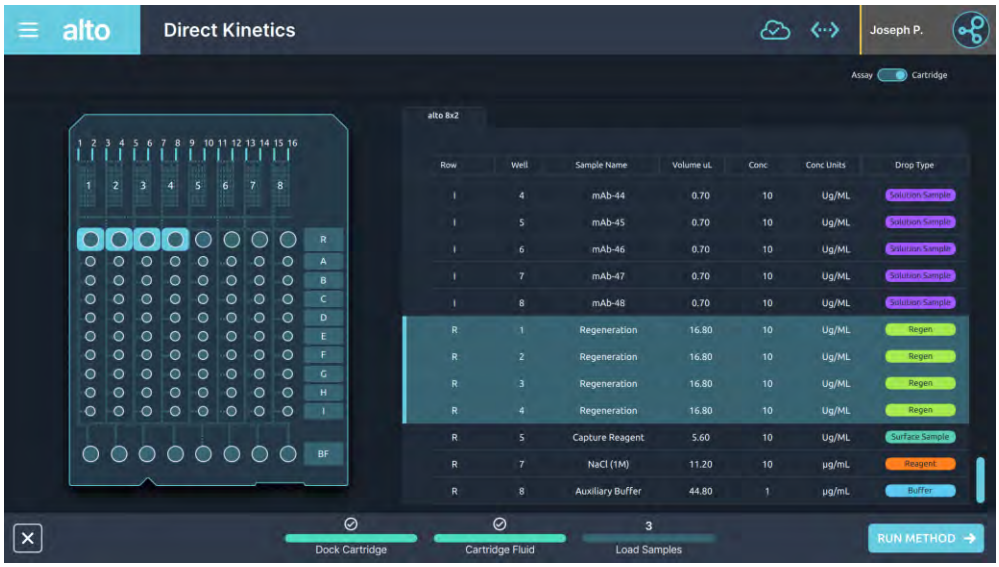

Figure 5.31: The table will highlight samples being used in the wells and/or rows that are selected on the cartridge diagram.

2. To load the samples, it is recommended to start from the top of the cartridge and go down one row at a time. At this point, the user will prepare to fill the samples referencing each well on the screen.
3. The user can tap on the wells on the screen one-by-one as they fill them to help guide them through this process. If the user chooses not to tap the wells, the software will prompt them before running that they might have missed some wells. They may ignore this prompt and continue to start their experiment.

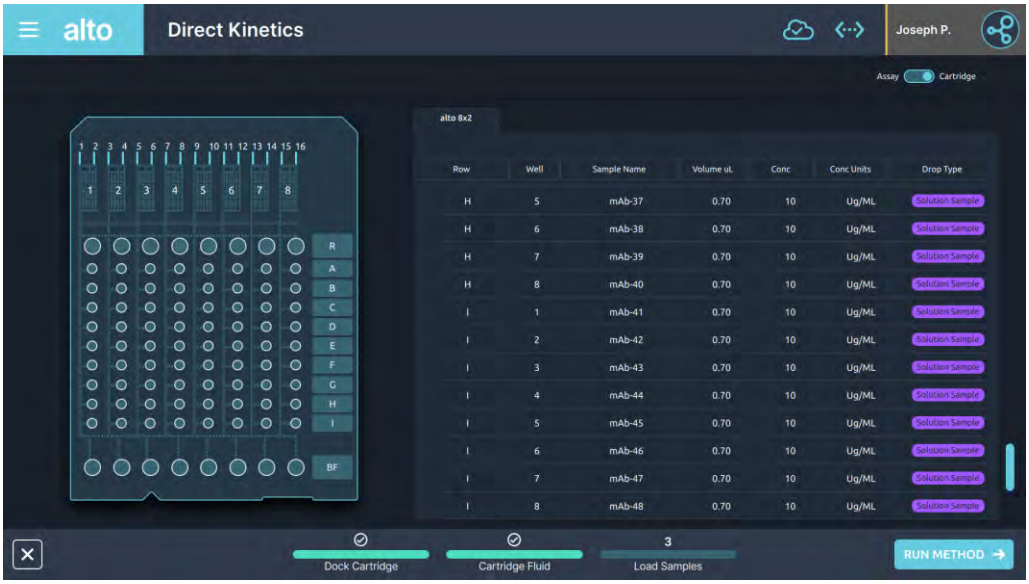

Figure 5.32: Sample overview for the experiment method.

4. To pipette the samples, please find the appropriate pipettes for the following sample types:
    - For row R (regeneration) wells, we recommend using a 10-100 µL pipette.
    - For rows A-I (sample) wells, we recommend using a 1-10 µL pipette.
    - For row BF (buffer) wells, we recommend using a 20-200 µL pipette.

5.6.6.1 Reverse pipetting for samples

To load the sample, it is extremely important to use the “Reverse pipetting technique” for loading the samples into the wells.

- Set the pipette to the required volume.
- Press to full stop (second stopping point) and submerge into solution for transfer.
- Release slowly (example: fully fill the tip) – fast release can aspirate solution into the pipette and damage the pipette.
- Remove any droplets from the exterior of the tip.
- The pipette tip should be immersed in the cartridge fluid but not touch the bottom plate (Figure 5.33). Once the pipette is immersed in the cartridge fluid, dispense the sample into the well and go to the first stop. ***Do not press down to the second stop.***

Figure 5.33: Lower the pipette tip into the cartridge fluid without touching the bottom of the cartridge or electrode.

- Remove the pipette tip carefully from the cartridge.
- Discard the pipette tip and load a new pipette tip for the next sample. It is important to change the pipette tips to avoid cross contamination of samples and oil carryover in between samples.

5.6.6.2 Using the pipette guide

1. To load the samples into the cartridge, the user has the option to use the pipette guide. The pipette guide and the hand screws are provided in the accessories kit as shown in Figure 5.34.

Figure 5.34: Pipette guide hand screws and pipette guide assembly.

2. To load the pipette guide into the instrument, align the pipette guide as shown in Figure 5.35 and align the holes on the pipette guide with the holes on the cartridge loading bay as shown in Figure 5.35.

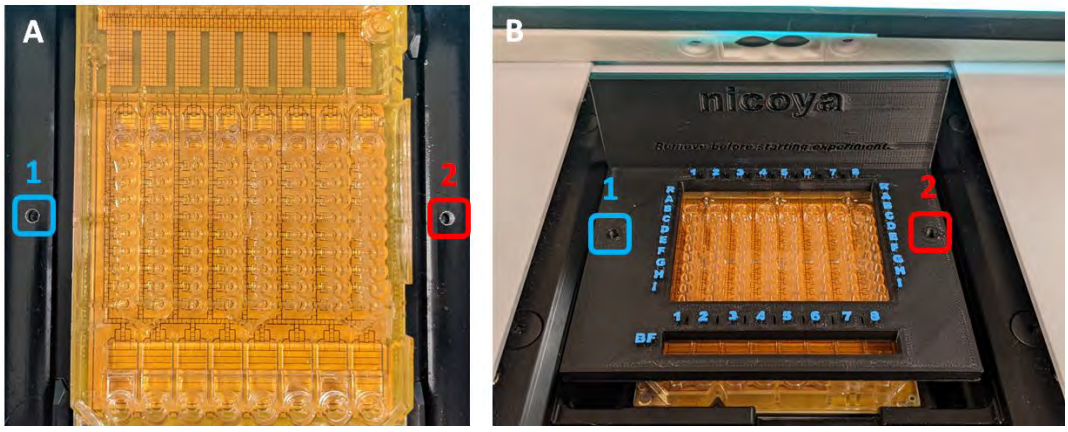

Figure 5.35: (A) Holes 1 and 2 on the cartridge loading bay (B) The pipette guide is placed onto the cartridge loading bay with the holes for the pipette guide (1) and (2) aligning with the holes for the cartridge loading bay labelled (1) and (2) in Figure 5.35(A)

3. Once the pipette guide is in position, insert the screws into the holes and turn clockwise to tighten as shown in Figure 5.36 to secure the pipette guide to the cartridge loading bay. The user will now be ready to load their samples.

Figure 5.36: Securing the pipette guide onto the cartridge loading bay with the hand screws.

4. Once the sample is loaded in the pipette, lower the pipette tip into the appropriate cartridge well (Figure 5.37).

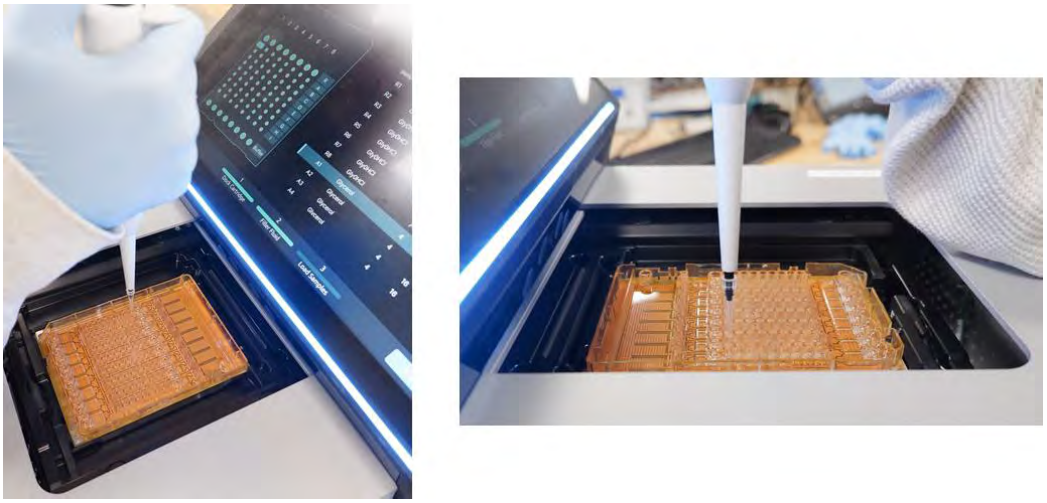

Figure 5.37: Loading samples into the cartridge.

Once all the samples are loaded into the cartridge, unscrew the pipette guide and remove the pipette guide from the loading bay before clicking on “Run method” on the software. **Note:** It is extremely important to remove the pipette guide to avoid damage to the instrument.

5.7 Running the Experiment

1. To start the test, click on the “RUN METHOD” button at the bottom right corner of the software. A warning will appear (Figure 5.38) to confirm if the user does not select all the wells that need to be filled with samples.

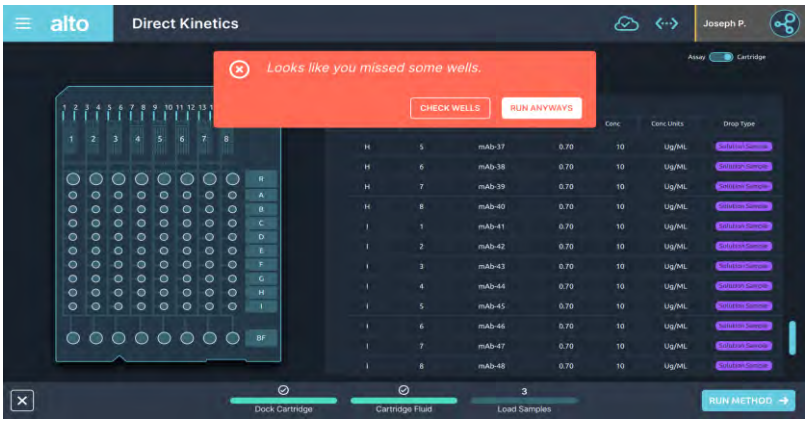

Figure 5.38. Warning screen to confirm if the user does not select all the wells that needed to be filled with the samples.

2. When the warning appears, the user will now have 2 options:
    - Run anyways - User is confident they have filled all appropriate wells and wants to start their run
    - Check wells - Users may have missed selecting a well during their sample load (assuming they were using the cartridge diagram as a guide) and choose to go back to double check.
3. The software will now ask you if you are ready to run the experiment. Click on “LET’S GO!” (Figure 5.39).

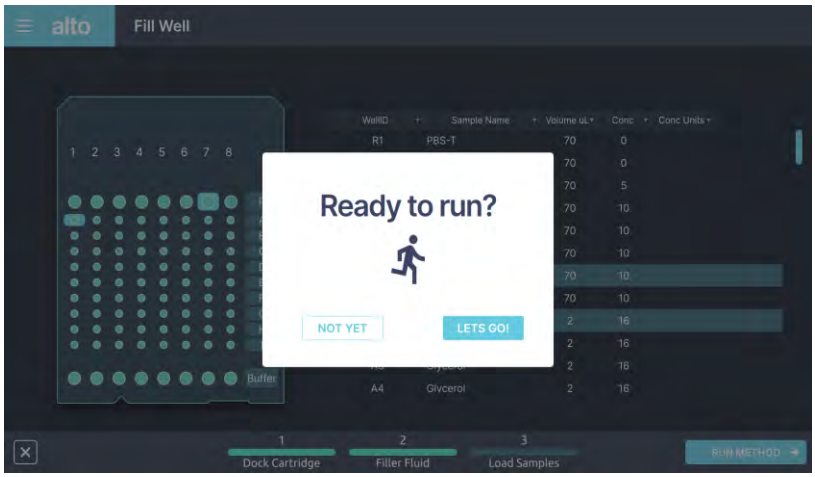

Figure 5.39: Initiate cartridge and experiment method loading by clicking on “LET’S GO!”

4. Once the cartridge is loaded, and the temperature is set, the experiment will begin.
5. The user will be able to see the data from their experiment appear on the screen in real time with response on the y axis in pm and time on the x axis in seconds.
6. A detailed description of the features available on the software screen are outlined in Table 5.3.

Table 5.3: Features provided in the software to visualize the data as also shown in Figure 5.40.

Group	Term	Definition
Context	Activity	Activities are the main actions which make up a method. Each is composed of one or more cycles that achieve a specific function in the experiment. These are defined in more detail in 5.7.1 “Visualizing an Activity”.
	Cycle	A repeat unit of work composed of one or more droplet sequences
Chart grouping	Lane	The lane here refers to groupings of sensors (for example - 1 lane consists of 2 sensors on Alto 8x2 cartridge layout). The software will have one graph per lane, showing all sensor responses in that lane (For example - for Alto 8x2 cartridge this will be 8 graphs with 2 sensorgrams per graph)
	Cartridge	The software will have 1 graph with all sensor responses streaming in the one graph simultaneously. (For example - for Alto 8x2 cartridges, this is one plot with 16 sensorgrams)
	Sensor	The software will provide individual sensorgrams for each sensor on the cartridge for this setting.
	Response - Raw	The data from each individual sensor channel.
	Response-Referenced	The corrected data where the reference channel is subtracted from the active channel response.
Thermal	Reagent temperature	Temperature of samples & reagents stored on cartridge that are not currently being analyzed (includes mixing area)
	Sensor temperature	Temperature of samples/reagents at sensor surface currently being analyzed

5.7.1 Visualizing by Activities

All activities for the running experiment will be listed in a queue. An activity may be selected from its data. Each activity will be listed in a list format (Figure 5.40).

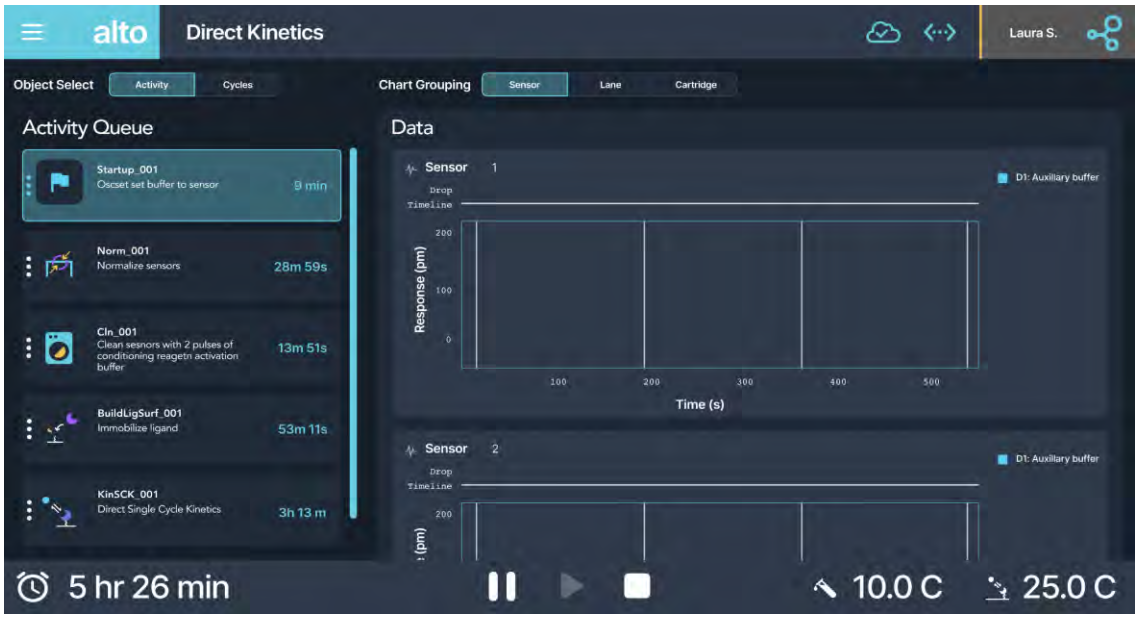

Figure 5.40: List of activities available for this experiment method.

The different activities that are available include:

1. **Normalize Sensors:** In this step, the sensors are exposed to two normalization solutions which assess the sensitivity of each sensor. This data is used to correct for any discrepancies in sensitivity across the sensors.
2. **Condition Sensors:** Clean and condition the sensors across the cartridge with the appropriate cleaning (dependent on sensor chemistry and can vary).
3. **Build Capture Surface:** Surface activation with sensor appropriate activation solutions and immobilization of the ligand or capture molecule on the sensor surface.
4. **Kinetics:** Introducing your analytes to perform a kinetic analysis.

5.7.2 Visualizing by Cycles

1. The user is able to see the queue for all cycles for the experiment by selecting the "Cycles" tab from the Object Select toggle on the top left of the screen.The user will be able to see each cycle of the activity along with the time required to execute each step as well (Figure 5.41).

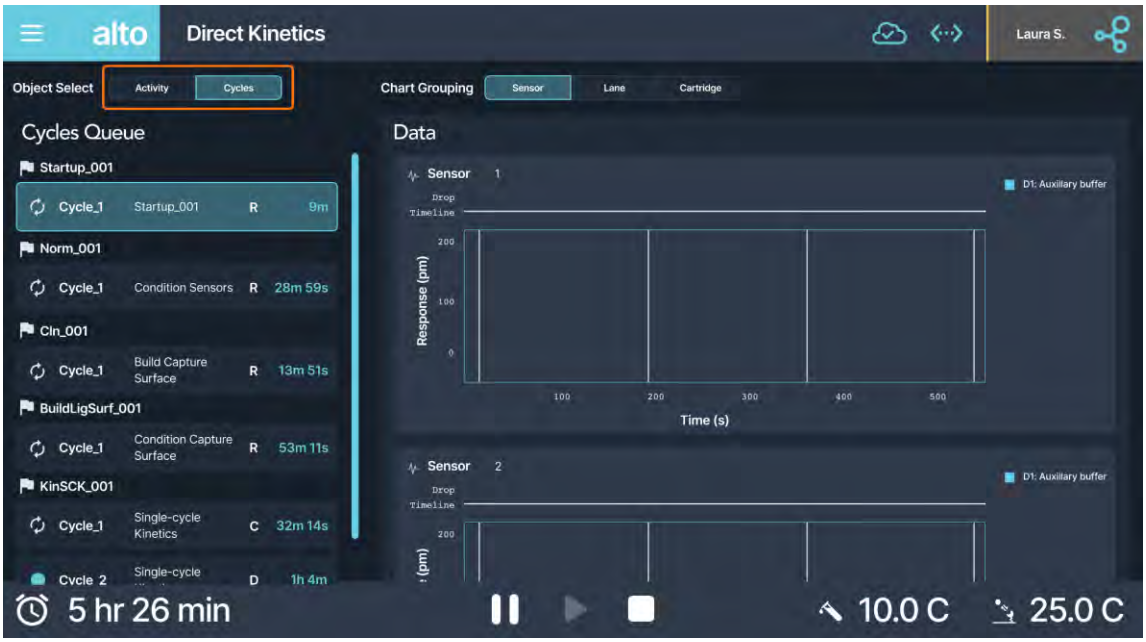

Figure 5.41: Running experiment showing cycle queue.

5.7.2.1 Experiment Pause

1. Experiments can be paused using the "Pause" button located in the bottom navigation bar as highlighted in Figure 5.42.

Figure 5.42: Experiment Pause Button on the Alto Control Software

2. A confirmation popup will appear to either "Pause" or "Cancel". Click on “Cancel” if the user is ready to pause the experiment as shown in Figure 5.43. Click “Cancel” to return to the running experiment.

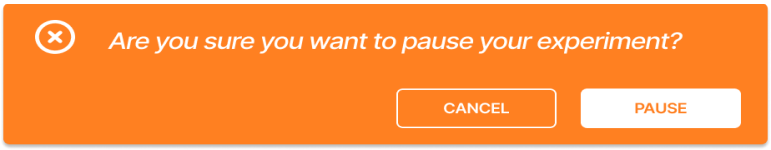

Figure 5.43: Experiment pause user confirmation prompt in the Alto Control Software.

5.7.2.2 Experiment Abort

1. Experiments can be stopped (aborted) through the "Stop" button located on the bottom navigation bar as highlighted in Figure 5.44.

Figure 5.44: Experiment Abort Button on the Alto Control Software

2. A confirmation popup will appear to "Stop and Eject" the current run or return to the running experiment ("nevermind") as shown in Figure 5.43.

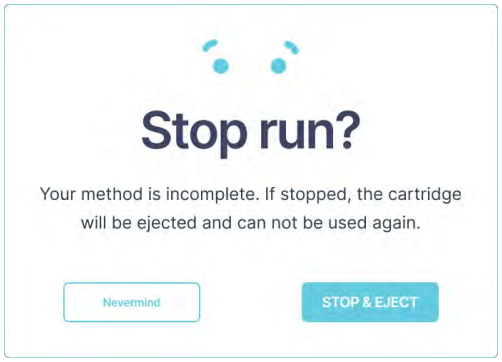

Figure 5.43: Experiment stop user confirmation prompt in the Alto Control Software.

3. Stopping and ejecting the running experiment will result in the cartridge being ejected with the software returning to method selection.

5.7.3 Cartridge Removal and Disposal

1. Once the experiment is complete, the cartridge is ready to be disposed of and the user has two options:
    - In the Alto software, click on “Eject Cartridge” to open the Alto door to expose the cartridge (Figure 5.44). The eject cartridge option allows the user to remain on the chart screen to look at their data. After they can select go to methods when done.
    - Go to methods and the cartridge will eject while the software will return them back to the method screen automatically after the cartridge has been ejected.

Figure 5.44: Eject cartridge or return to the method select screen once the experiment is complete.

2. Once the door is open, wear gloves and remove the cartridge from the instrument (Figure 5.45).

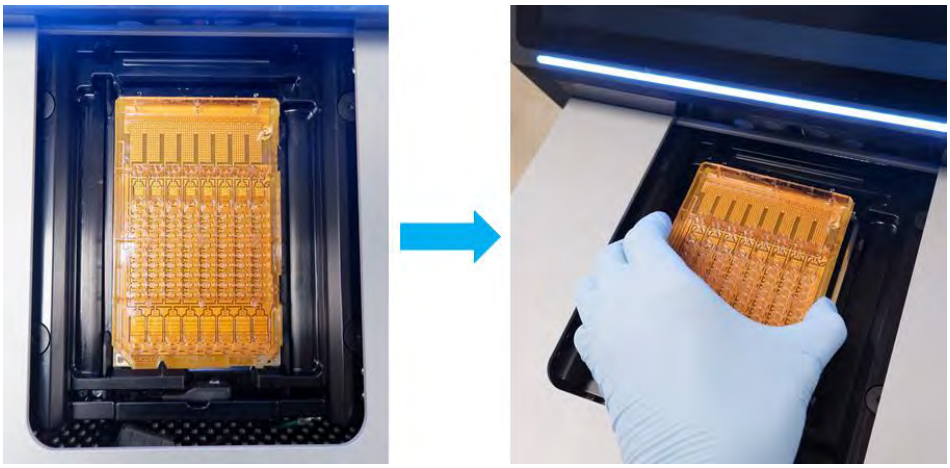

Figure 5.45: Remove the cartridge from the cartridge loading bay.

3. Place the cartridge on a clean and flat surface. It is recommended to put the waste cartridge in the original packaging prior to disposal. Please follow the local regulations for cartridge disposal.

5.8 Instrument Processes

At the end of an Alto experiment, the user can either start a new experiment, check the instrument's status, logout, or power off the instrument.

5.8.1 Instrument Shutdown

If the instrument will not be used for an extensive period (beyond 7 days), shutdown the instrument.

1. To place the instrument in shutdown mode, ensure that the cartridge is removed from the instrument.
2. Click on the “Main Menu” button as shown in Figure 5.46 and select the Power off button.
3. Select "Power off" on the confirmation popup and turn off the power supply switch on the back of the instrument.

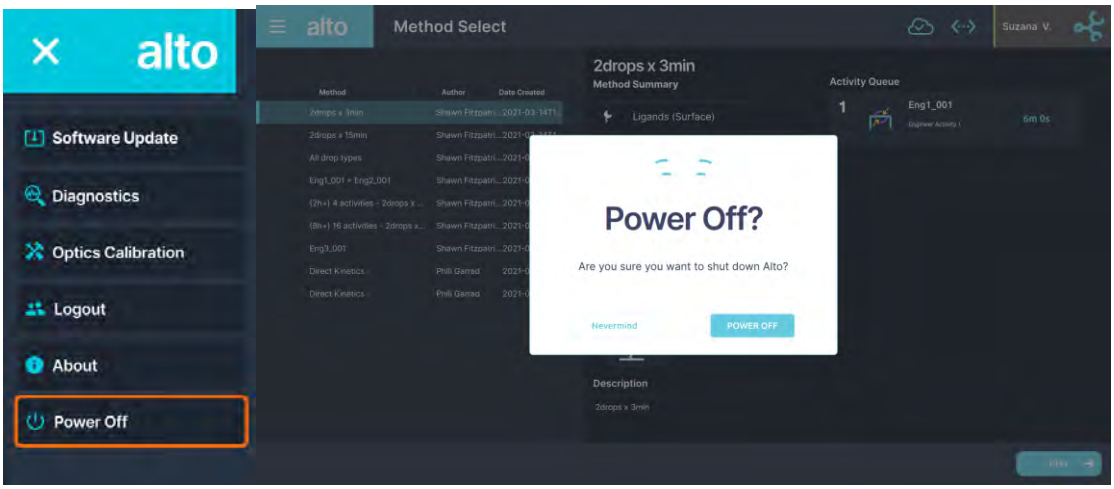

Figure 5.46: Power off software selection screen.

5.8.2 Instrument User Logout

To log out of the device, go to the main navigation menu and select "Logout" and confirm in the popup as shown in Figure 5.47. This will ensure that the user is logged out of the Alto device.

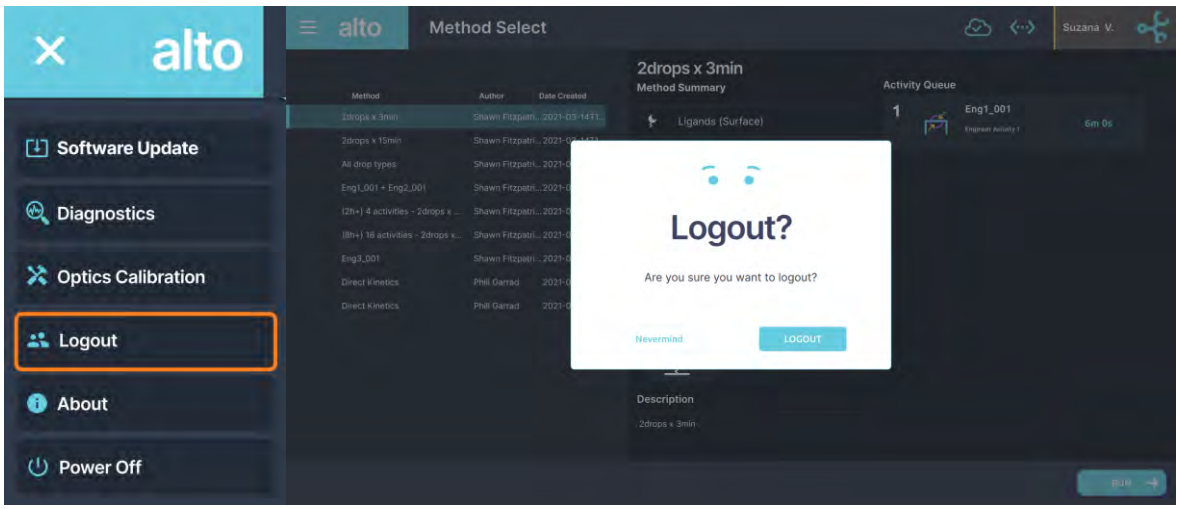

Figure 5.47: User Logout software selection screen.

5.8.3 About and Contact Support

The user can check on the instrument software version, model number, serial number, and IP address by accessing the “About” function on the Alto Control Software. To perform this operation, click on the “Main menu” and select the “About” option as shown in Figure 5.48. This will provide the user with information about the Alto device such as software updates, model and serial number, IP address and support information.

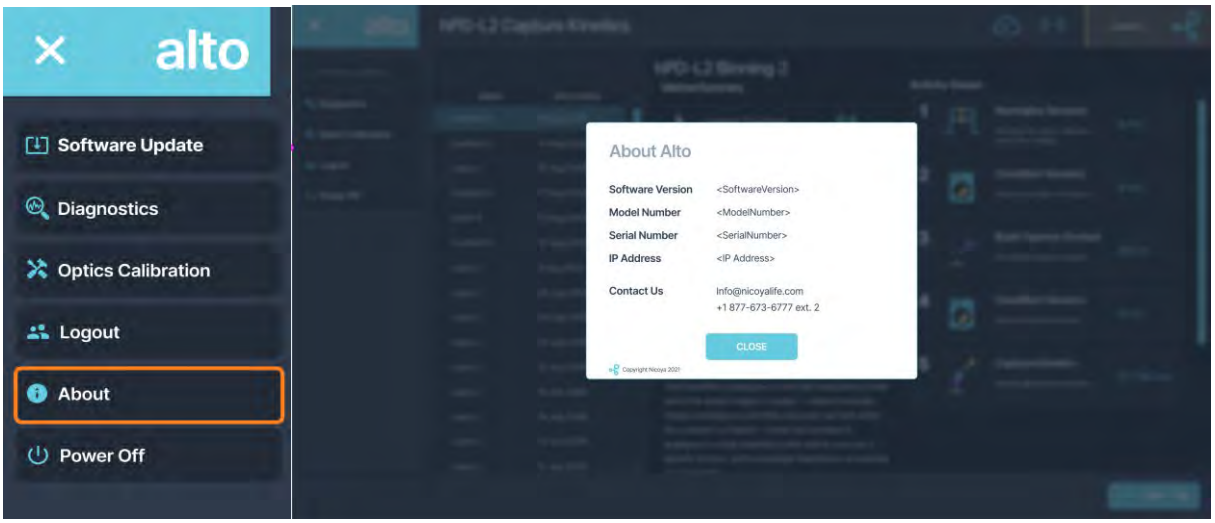

Figure 5.48: Instrument information software selection screen.

5.9 Data Output Files

1. Once the experiment has finished, the data will be automatically uploaded to the Nicoya User Portal for analysis.