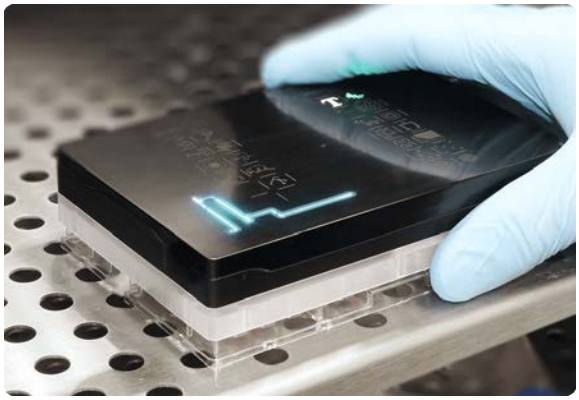
Lucid Scientific

# Resipher

**Installation and User Guide**



This guide walks through setting up the Resipher to get you up and running with real-time continuous monitoring analysis experiments. The Resipher system will enhance your experiments in cellular metabolism by converting standard cell culture plates into smart hand-held readers that sense real-time metabolic changes in response to potential therapeutics. Your data is streamed to researchers in real-time via our collaborative web platform.

# A few useful tips on using Resipher

- **Start simple**
  For your first ever experiment, we recommend you start with a simple experiment, for example, a cell density/seeding experiment. It allows you to get familiar with the Resipher system and confirms the general oxygen consumption behavior of the cells before proceeding to more complex experiments.

- **Let the cells settle**
  It's always a good idea to allow cells to adhere or fall to the bottom of the wells before placing the sensing lids onto the well plates. This prevents cells from accidentally attaching to the sensing probes.

- **100 uL media is the best**
  Resipher experiments work best when there is ~100 uL of media in each well. This ensures the probes stay within liquid for the duration of the experiment and the media has a good oxygen gradient for detection.

- **Outer wells as experimental controls**
  Outer wells (i.e. top and bottom rows) may be used for controls. This reduces the likelihood of evaporation interfering with important data.

- **Evaporation is inevitable**
  PBS can be added to unused wells to reduce media evaporation during experiments.

- **Let the cells do their things**
  During an experiment, it's best to minimize disruptions, for example, opening and closing the incubator. Resipher is a sensitive instrument that responds to small environmental changes.

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# Table of Contents

Resipher magnetically attaches to our disposable lids that rest securely on most standard 96-well plates. It will be referred to as “device” in this document.

The Resipher Base Station stores data from up to 8 connected devices and streams the data in real-time to our secure cloud platform. It will be referred to as the “hub”.

Individually sterilized disposable lids attach between the device and the well plate. Each lid has 32 probes with oxygen sensing material. These will be referred to as “lids”.

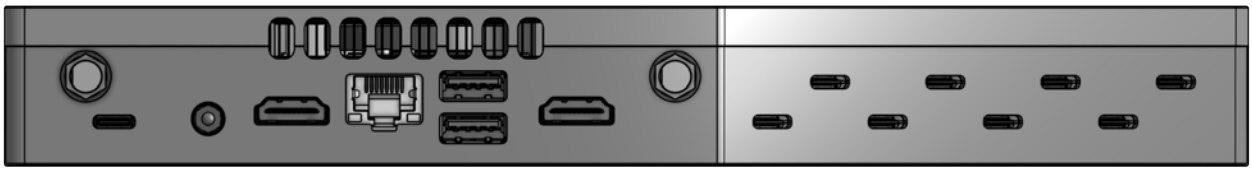
# Lucid Hub

The Hub is an embedded computer, USB-C hub, and network gateway for the control of up to 8 devices. The Hub is also responsible for data storage. All experiment data is stored locally on the Hub's hard drive and streamed securely to our cloud platform. Data can then be viewed and analyzed using our cross-platform web application - Lucid Lab. The Hub is intended to be located on or near a laboratory incubator as 1m is the limit for length of USB-C cable at high data transfer rates. The Hub has strong magnets in each foot that allow you to mount on any side of the incubator.

## Front Panel

- 8 × device port LEDs
- 2 × USB 3.0 ports
- Power button with ring LED
- 3 × vertically-oriented Hub status LEDs

## Back Panel



- 8 × USB-C device ports
- 2 × USB 3.0 ports
- 2 × HDMI output
- Gigabit ethernet
- Power

## Power LED Indicator

The power button ring LED turns solid blue when the device is powered on.

## Device Status LEDs

The row of 8 LEDs indicate the status of each device connected to the USB-C ports on the back.

| State | Description |
|---|---|
| ○ Off | No device connected |
| Yellow | No lid attached |
| Green | Device active, reading |
| Blue | Device tilted |
| Red | Device Error |

## Hub Status LEDs

On the right side of the front panel are 3 vertical LEDs to indicate the Hub's overall status. The LEDs represent status for the internal Hub system, connection to network/internet, and connection to our cloud infrastructure.


| | State | Description |
|---|---|---|
| NETWORK | ○ Off | Cloud disabled |
| | ● Green | Network cloud connected |
| | ● Yellow | Network connected, cloud not connected |
| | ● Red | Network disconnected |
| SOFTWARE | ● Green | Software operating normally |
| | ● Red | Software error |
| SYSTEM | ○ Off | System not running |
| | ● Green | System operating normally |
| | ● Red | System error |

## Connecting the Hub

Place the Hub onto or near the incubator. Connect the provided power cable to plug on the back of the Hub.

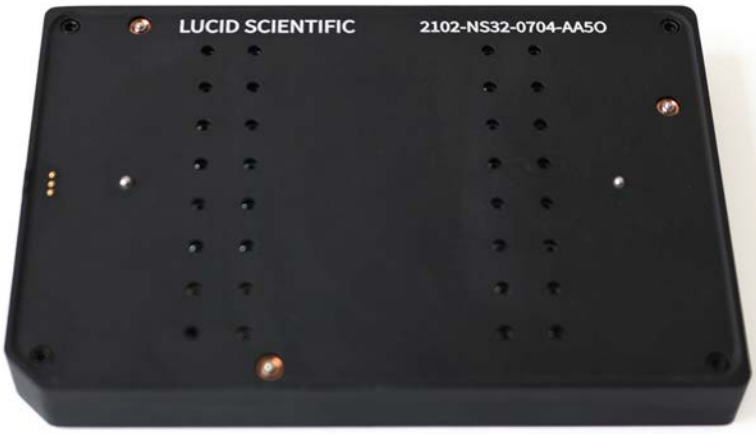
Incubators typically have an opening at the back for cables; feed the included USB-C cable through this for each device you plan on connecting. Connect one end of each cable to a device (inside the incubator) and the other end to one of the USB-C ports on the back of the Hub with the provided USB-C cable.

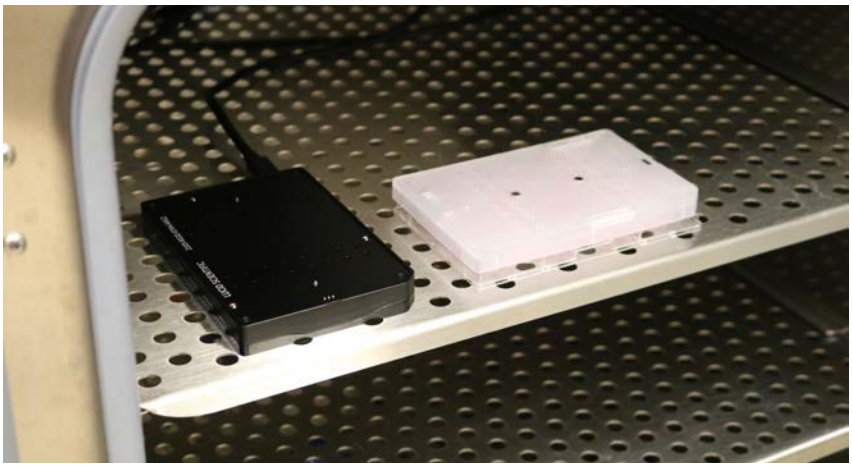
Plug in an ethernet cable to the ethernet port on the Hub. Make sure that internet connection is available from the ethernet outlet. It may be necessary for your IT department to authorize internet access to the Hub using its MAC address (located on the bottom side of the Hub).

Once the Hub is powered on and connected to the internet, the power button ring should be blue and the three LED lights to the right of the power button should be green.

## Connecting the device

Put the device into the incubator with the oxygen sensor side facing up  and connect the USB-C coming from the Hub.

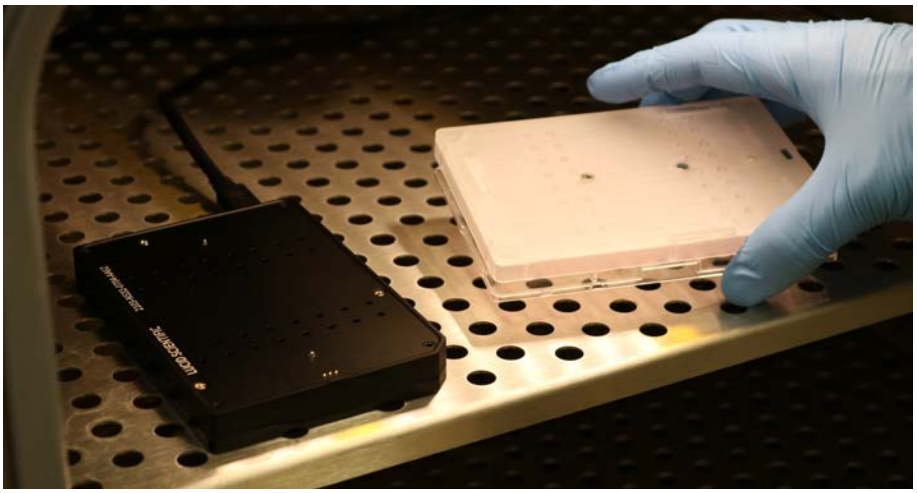


Check the front of the Hub and make sure one of the LEDs on the left is lit up in blue, indicating that a Resipher is connected.

## Place the sensing lid onto the seeded well-plate

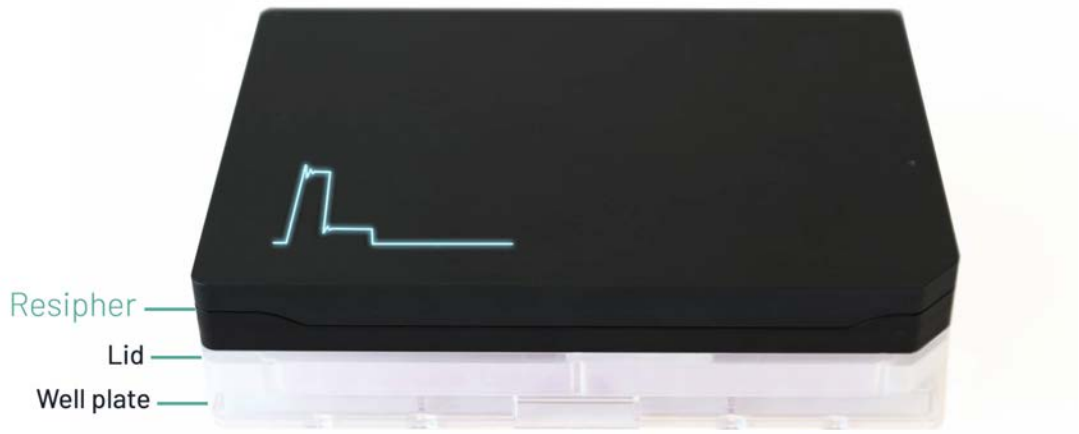
After the cells have settled to the bottom of the wells, place the sensing lid onto the well plate. The sensing lid/well-plate assembly can then be transferred to the incubator.



## Place the Resipher onto the sensing lid/well-plate assembly

Attach the Resipher to the top of the sensing lid. It will snap and fit snugly onto the sensing lid because of magnets embedded in the device and lid. Make sure they are attached properly. There should be no gaps between the sensing lid and the Resipher.

Once they are attached to each other, the indicator LEDs on both the Resipher and the Hub should flash green. The flashing green LED should turn to solid green once the Resipher actuation begins and is stable.



# Lids

The 32x sensing lid

Sensing lids arrive sterilized and calibrated and are compatible with standard 96-well plates including Nunc and Falcon. They can be used immediately after being removed from the packaging, just like pre-sterilized well-plates.

## Important!

Our sensing lids are customized to fit their designed plate styles. They should only be used with their matching plate styles. Please check the label on the lid packaging for its well plate type.

Using mismatching well-plates can damage sensing lids, resulting in failed experiments.

# Compatible 96-well plates

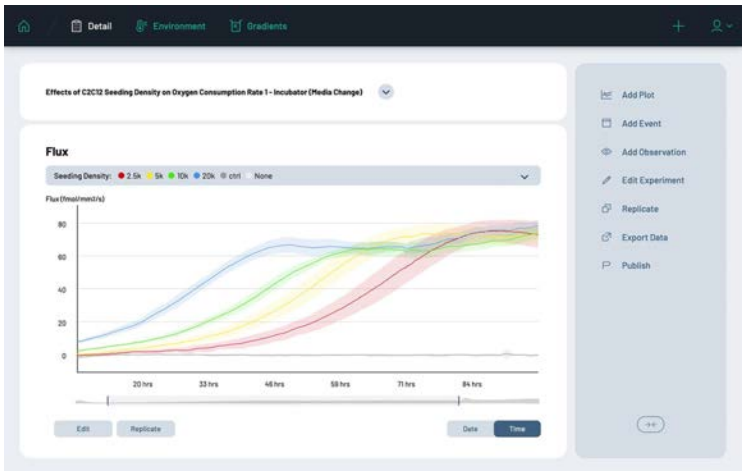
Nunc (Thermo Fisher)

| Cat. No. | Surface | Well Design | Color | Total Vol. | Sterile | Lid | No. Per Pack | No. Per Case |
|---|---|---|---|---|---|---|---|---|
| 167008 | TC-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 1 | 50 |
| 168055 | TC-treated | Flat Bottom | Clear | 400 uL | Yes | No | 1 | 50 |
| 269787 | Non-treated | Flat Bottom | Clear | 400 uL | Yes | No | 1 | 50 |
| 156545 | TC-treated | Flat Bottom | Clear | 400 uL | Yes | No | 10 | 180 |
| 161093 | TC-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 10 | 160 |
| 243656 | Non-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 6 | 108 |
| 260860 | Non-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 10 | 160 |
| 260887 | Non-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 10 | 160 |
| 266120 | Non-treated | Flat Bottom | Clear | 400 uL | Yes | Yes | 25 | 100 |

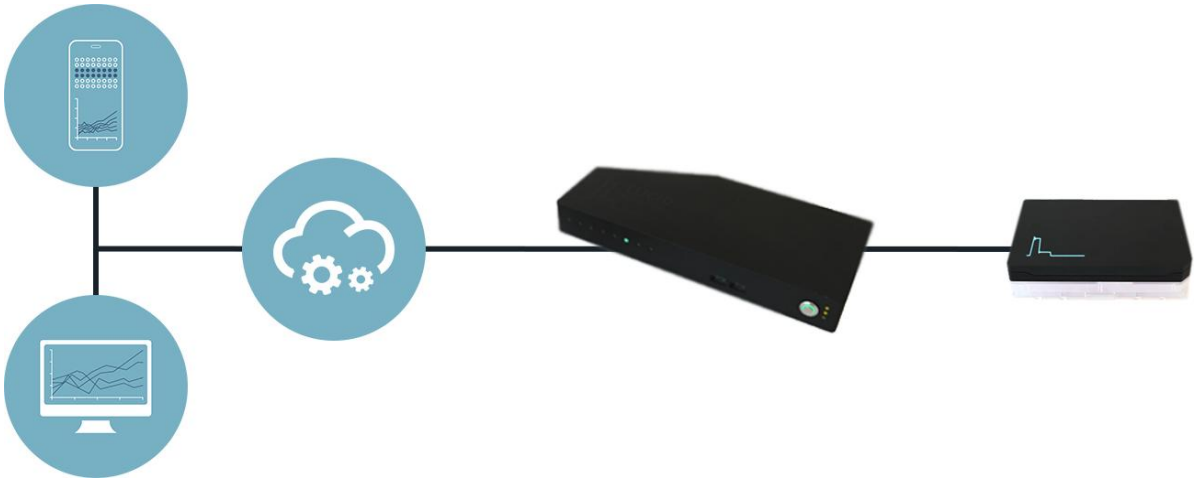
Falcon (Corning)

| Product No. | Surface | Well Design | Color | Total Vol. | Sterile | Lid | No. Per Pack | No. Per Case |
|---|---|---|---|---|---|---|---|---|
| 353072 | TC-treated | Flat Bottom | Clear | 0.37 mL | Yes | Yes | 1 | 50 |
| 351172 | Non-treated | Flat Bottom | Clear | 0.37 mL | Yes | Yes | 1 | 50 |
| 353075 | TC-treated | Flat Bottom | Clear | 0.37 mL | Yes | Yes | 5 | 50 |
| 353916 | TC-treated | Flat Bottom | Clear | 0.37 mL | Yes | Yes | 25 | 100 |
| 353936 | TC-treated | Flat Bottom | Clear | 0.37 mL | Yes | Yes | 14 | 84 |

# **Cloud platform and** Lucid Lab




Experiment data is securely stored both on the Hub and in the cloud. Lucid Lab is our cross-platform web application that allows your team to create and manage experiments, stream and monitor data from devices in real-time, and generate plots that are ready for publishing. Each member of your team can get an account to login and use Lucid Lab with your organization's devices. Team members can collectively monitor and access experiments, monitor device and Hub statuses, receive troubleshooting tips, and even connect with us directly for more advanced support. Lucid Lab is full-featured and accessible on desktop and mobile. Lucid Scientific provides continuous software updates and SSL support.



# Key Terms and Concepts

## OCR/Flux

Oxygen Consumption Rate (OCR) / Extracellular Oxygen Flux is the key measurement provided by Resipher. This measurement is derived from readings of the dissolved oxygen concentration at various depths within the media above the cells. Without any assumptions about the surrounding environment, the system measures the Oxygen Flux (i.e. the amount of oxygen going in or out of a fixed volume). If we assume something below the probe is consuming oxygen (e.g. cells) and that practically all oxygen is being consumed through the media from the air above (e.g. in a plate well), then the OCR and Flux are equivalent measures. Flux/OCR is reported in units of femtomoles per square millimeter per second (fmols/$mm^2$/s).

## $O_2C$

Dissolved Oxygen Concentration ($O_2C$) is what each individual probe measures at a given instant. In a typical 5% CO2 at 37 Celsius, $O_2C$ is around 180 micromolar (um). While the absolute value of $O_2C$ is important in many applications, the relative values of $O_2C$ at different probe heights is what matters for Resipher Flux measurements.

## $O_2C$ Gradient

When cells are consuming oxygen out of the atmosphere through a volume of fluid, a gradient of oxygen concentration forms across that fluid. That is, the oxygen concentration at the top of the fluid is higher towards the top of the fluid and is lower in the fluid closer to the cells. When there is no flux/consumption, there is no gradient (it is “flat”). With increasing flux/consumption the oxygen gradient increases (it becomes “steeper”).

## Probe

The lid contains an array of oxygen probes. The probes consist of an optical fiber with an oxygen-sensitive material at its tip. The probes are moved up and down within the media of the well to measure oxygen at different depths/heights.

## Temperature Dependence

The oxygen-sensitive probes are temperature dependent as the measurements they produce may vary with temperature as well as with oxygen concentration (i.e. they are “cross-sensitive” to temperature). As a result, the flux estimates are temperature dependent as well. Large swings in environmental temperature (e.g. opening an incubator door) induce temporary jumps or dips in reported measurements which are not reflective of the true oxygen flux. Therefore, it is critical to maintain a stable temperature environment (don’t open the incubator door often or else your experimental results will be affected). If possible, keep the incubator door closed throughout the experiment.

# A few tips on seeding cells

## Resipher is best suited for experiments with adherent cells

Unlike traditional oxygen sensors (i.e. Seahorse), Resipher oxygen consumption sensing probes move up and down vertically during experiments. The typical movement range of our sensing probe is 500 µm; the probe moves as close as 500 µm to the bottom of the wells. If your experimental setup requires a different movement range and clearance to the well bottom, please contact Lucid for adjustments. The probe actuation allows Resipher to accurately measure oxygen consumption rates without the need to individually calibrate each probe. Instead of measuring oxygen concentration, Resipher measures the gradient of oxygen content as the probes move vertically within each well. Our software converts the oxygen gradient into oxygen consumption rate and presents the data in an easy-to-read, intuitive fashion. With such dynamic reading technology, we recommend using Resipher with adherent cells that settle and attach to the bottom of the wells. This will ensure a stable environment in each well for oxygen gradient reading. (If oxygen concentration measurements for suspension cells are desired, please contact Lucid Scientific for further discussion before proceeding).

## Columns 3, 4, 9, and 10 are used for the 32x Resipher

For the 32x Resipher, the sensors are located at wells in columns 3, 4, 9, and 10. PBS or media can be added to the rest of the wells to slow down the rate of evaporation.

## Allow cells to settle and adhere to the bottom of the wells after seeding

To avoid cells accidentally attaching to the sensing probes, which may lead to errors in oxygen consumption reading, it is best to allow cells to settle and adhere to the bottom of the wells after seeding. Typically one hour should be sufficient; depending on how adherent the specific cells are. This waiting period can be done by sitting the seeded well plates in an incubator or in a biosafety cabinet at room temperature.

## Clean Resipher before placing inside an incubator or workstation

Before placing the Resipher device into an incubator or workstation, it should be cleaned to reduce the risk of contamination. **Important - Avoid spraying ethanol directly onto the Resipher; it may cause damage to the electronic components.** First, wet a Kimwipe with ethanol, then wipe the Resipher with the wet Kimwipe by lightly wetting the entire surface.

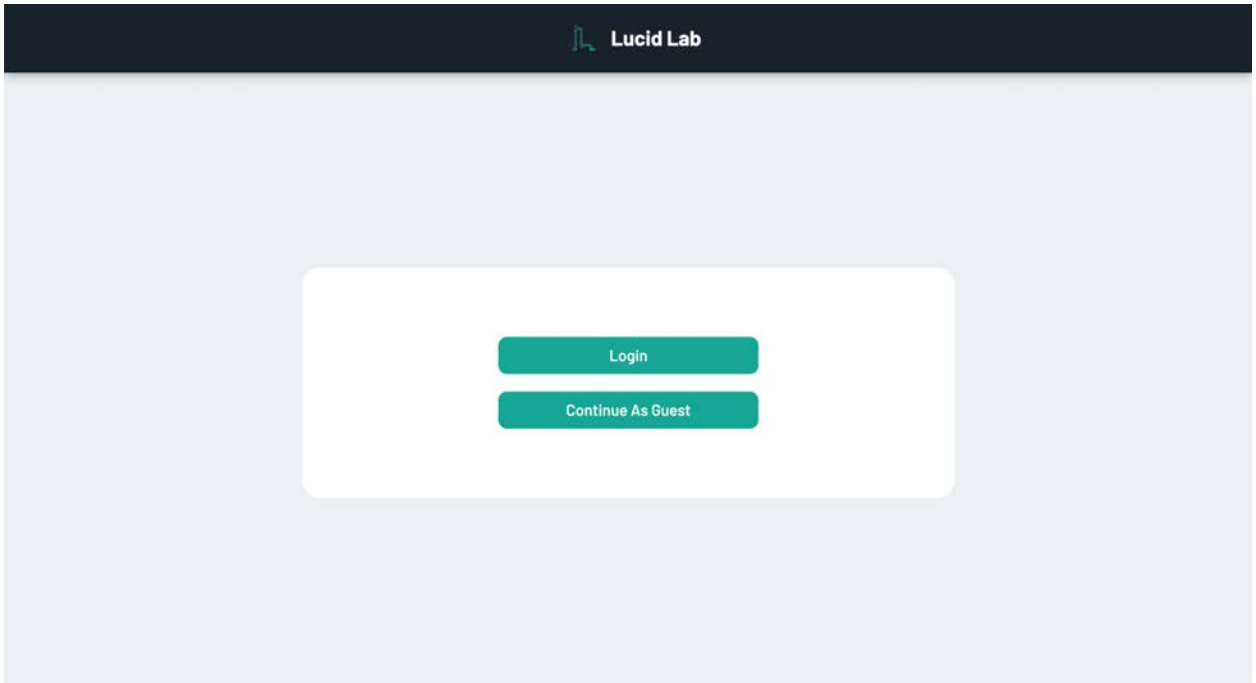
# Lucid Lab – Setting up an experiment

## User Training

Prior to the first experiment, all customers are trained by Lucid on experimental and system setup. Once your Hub is connected to the internet, our customer success team will reach out to you and schedule a training session or provide access to training materials. If your Hub is connected but your training session has not been scheduled, please contact us at info@lucidsci.com.

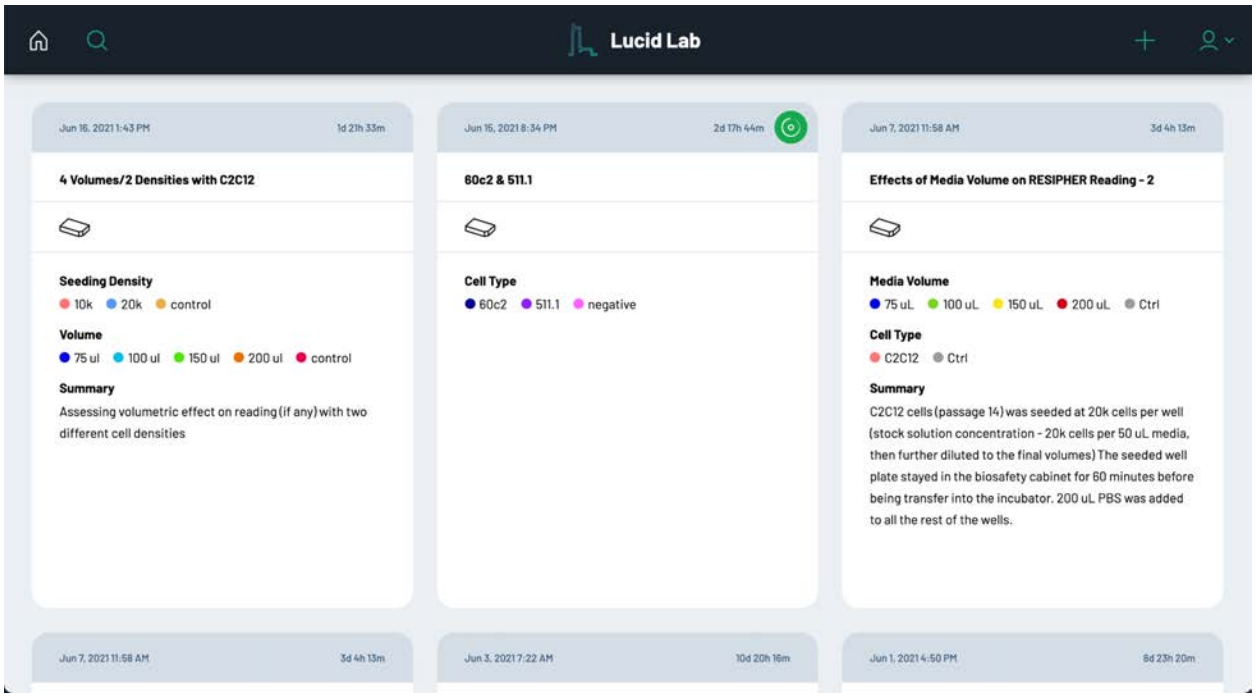
## Login to your account

Make sure the Hub is connected to an active ethernet port with access to the internet. If you have created an account, visit https://lab.lucidsci.com to log onto your user account. If you don't have an account, go ahead and create one. We will confirm your account registration in a timely manner so you can get going with your experiment quickly.



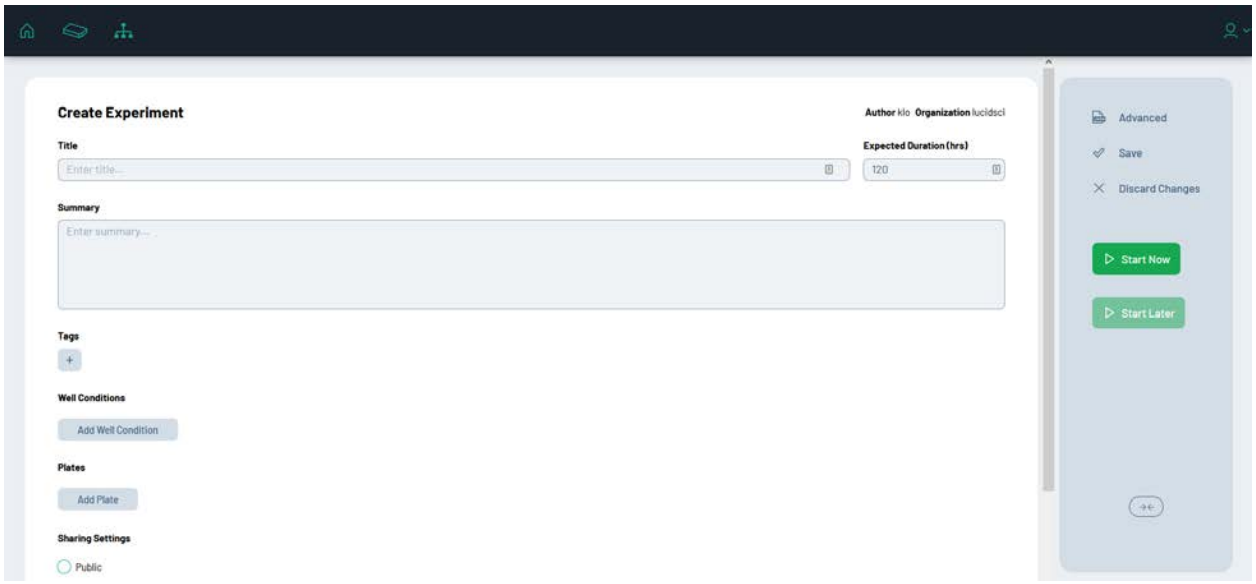
## Create an experiment

Click the “+” button in the top right to create a new experiment



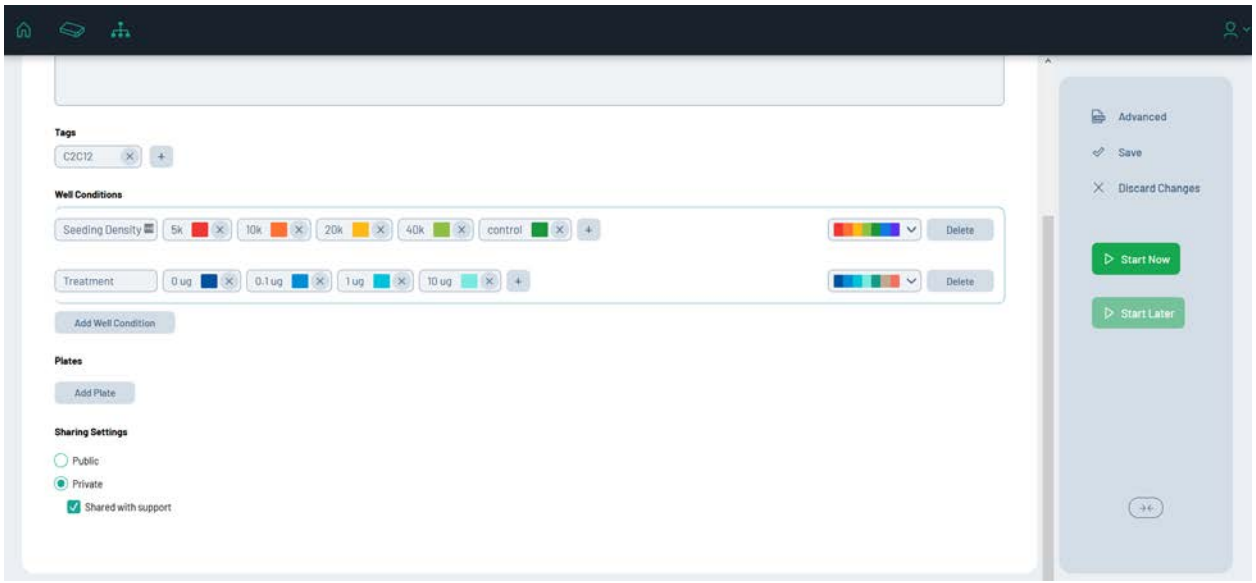
## Enter basic information

Enter a “Title” and indicate the “Expected Duration (hrs)” for your experiment. Note that your experiment does not terminate at the end of the expected duration; the system will simply send you a reminder when the expected duration is reached . You also have an option to further describe your experiment by including detailed information in the “Summary” section. Inserting keywords about your experiment into “Tags” will provide ease of searchability later.



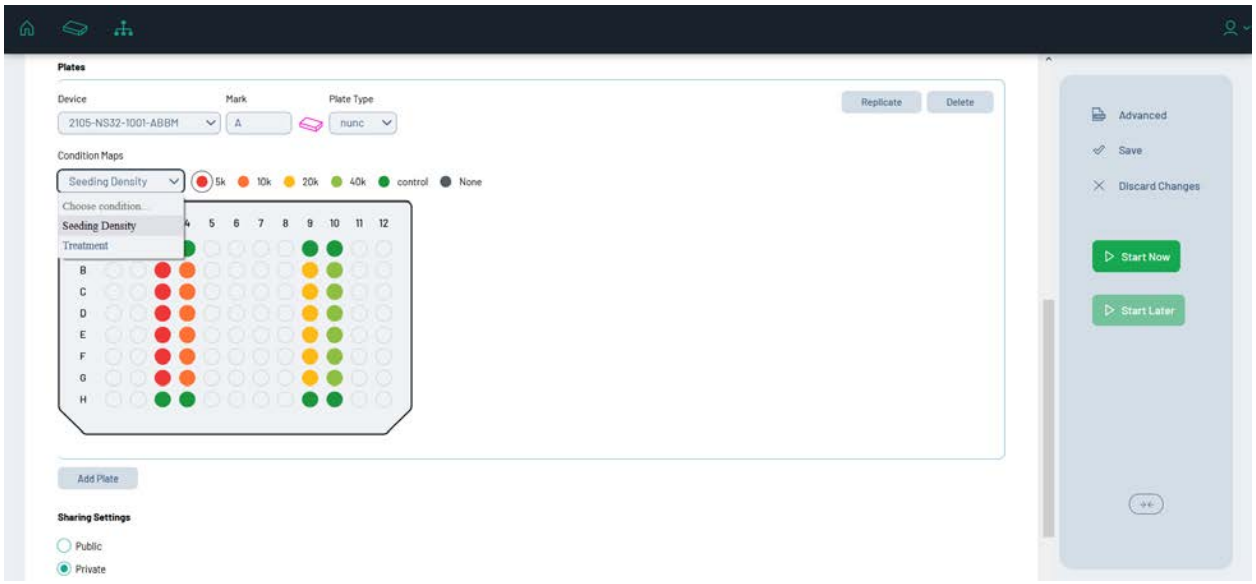
## Create well conditions and values

Click the “Add Well Condition” button, then enter new experimental conditions or select pre-existing ones that appear. Type in experimental values one at a time, press the ENTER key on the keyboard to register each value.



## Configure plates

Once you add values for all “Well Conditions”, scroll down to the “Add Plate” button.
Identify each well by their experimental conditions. Select the correct plate type and device that will be used for the experiment from the drop down menu. The mark indicator can be used when multiple devices are used at the same time to identify plates and devices.



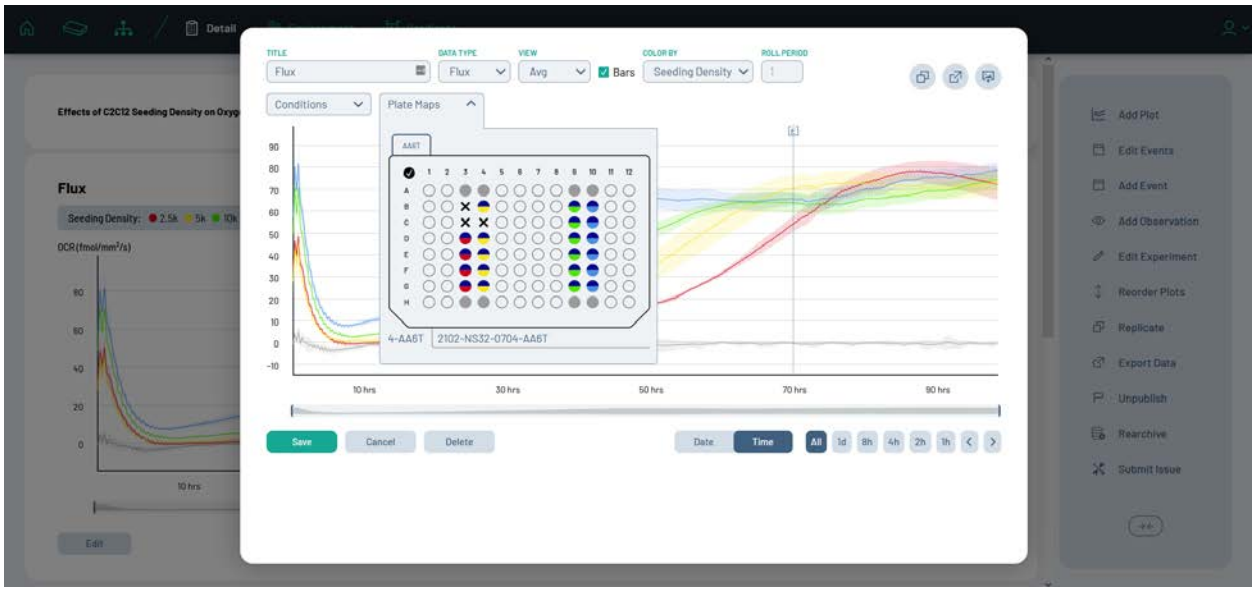

If your experiment is set up and ready to run, click “Start Experiment” to begin. Otherwise, click “Save Changes” to continue editing later. All the finished, running, and draft experiments will appear in the “Home” page.

# Lucid Lab - **Plots**

## Adding a plot

When creating a plot, first add a descriptive title and select a data type (Flux or $O_2C$) from the pulldown. View allows you to choose between raw data from each individual well or the average values of each condition. The conditions drop down allows you to select filter specific conditions to appear in the plot.

The plate map drop down lets you toggle between multiple plates and remove/choose specific wells.

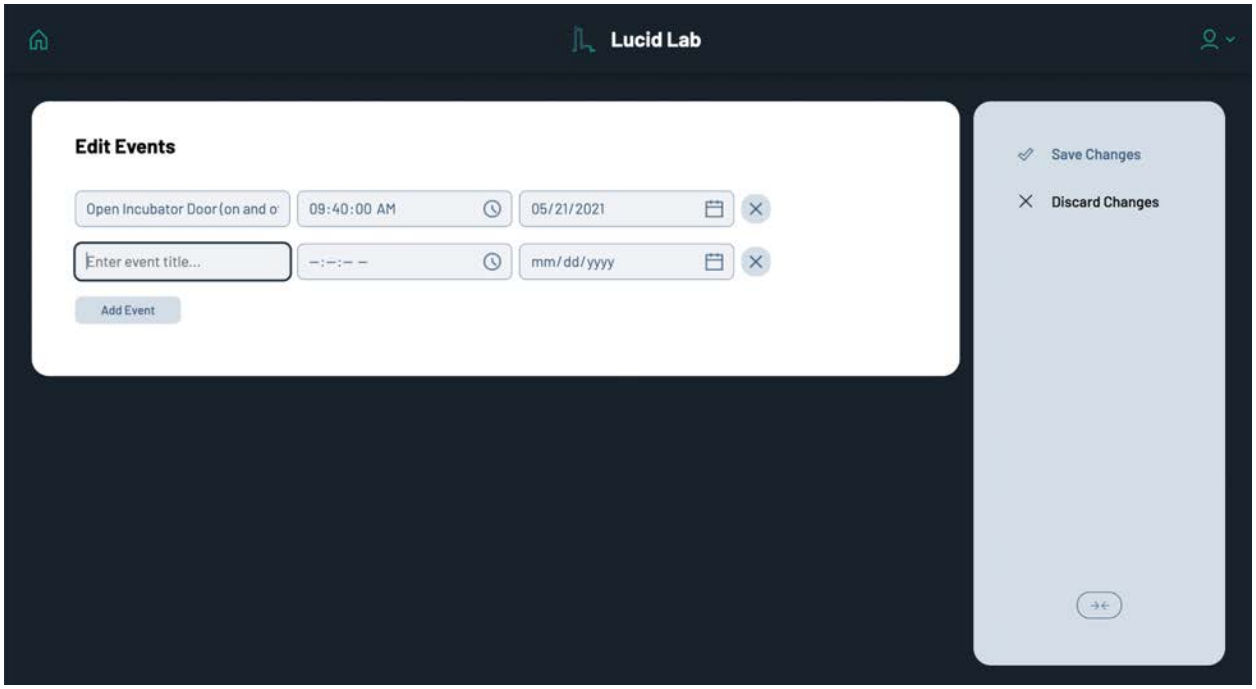


In the top right corner, the three buttons are “Replicate plot”, “Export plot data”, and “Download plot image” in that order. Exporting the plot data will only export what is shown on the plot itself, not all of the data. Exporting complete data will be shown in a later section in the guide.

# Lucid Lab - **Experiments**

## Add Event

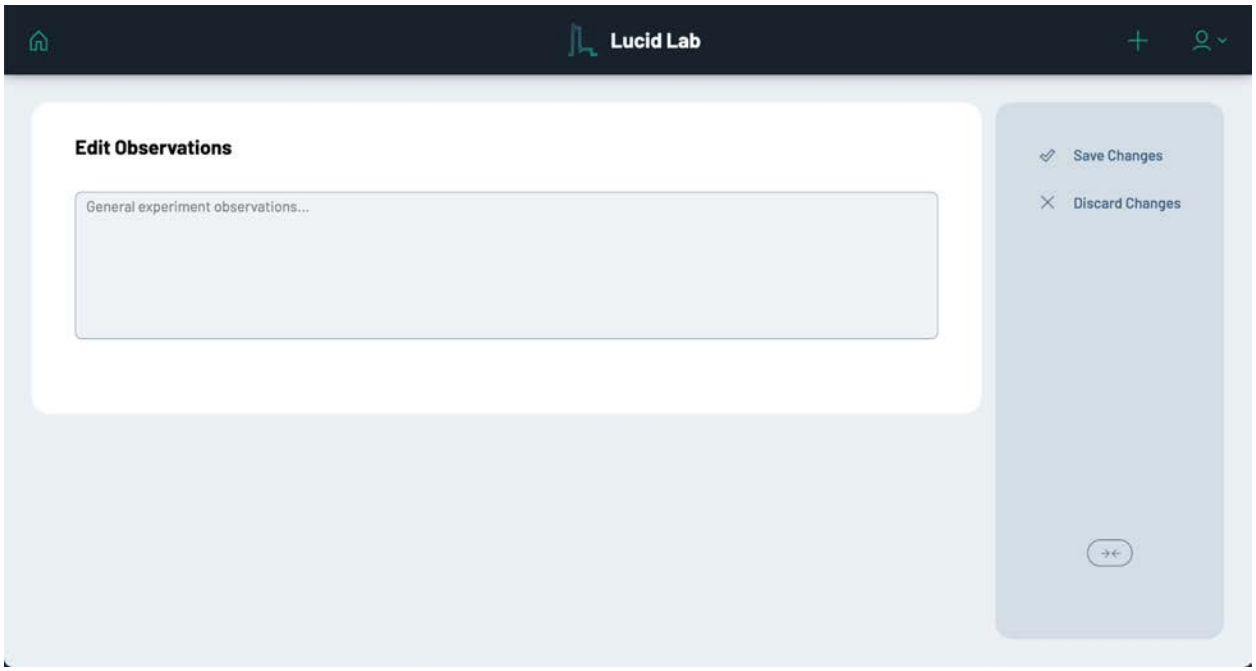
Events can be used to indicate media changes, treatment times, or general changes to cells done at any time during the experiment. When adding an event, type a short description and select a specific time and date. Once finished, be sure to save changes.




An added event will appear on graphs as an “E” icon with a line as seen in the plots.

## Add Observation

Observations are more general notes about an experiment that are not necessarily associated with a specific point in time such as general behaviors, color changes, etc.




## Edit Experiment

Editing an experiment will take you back to the original create experiment page. This allows you to make any changes, i.e. add new conditions.

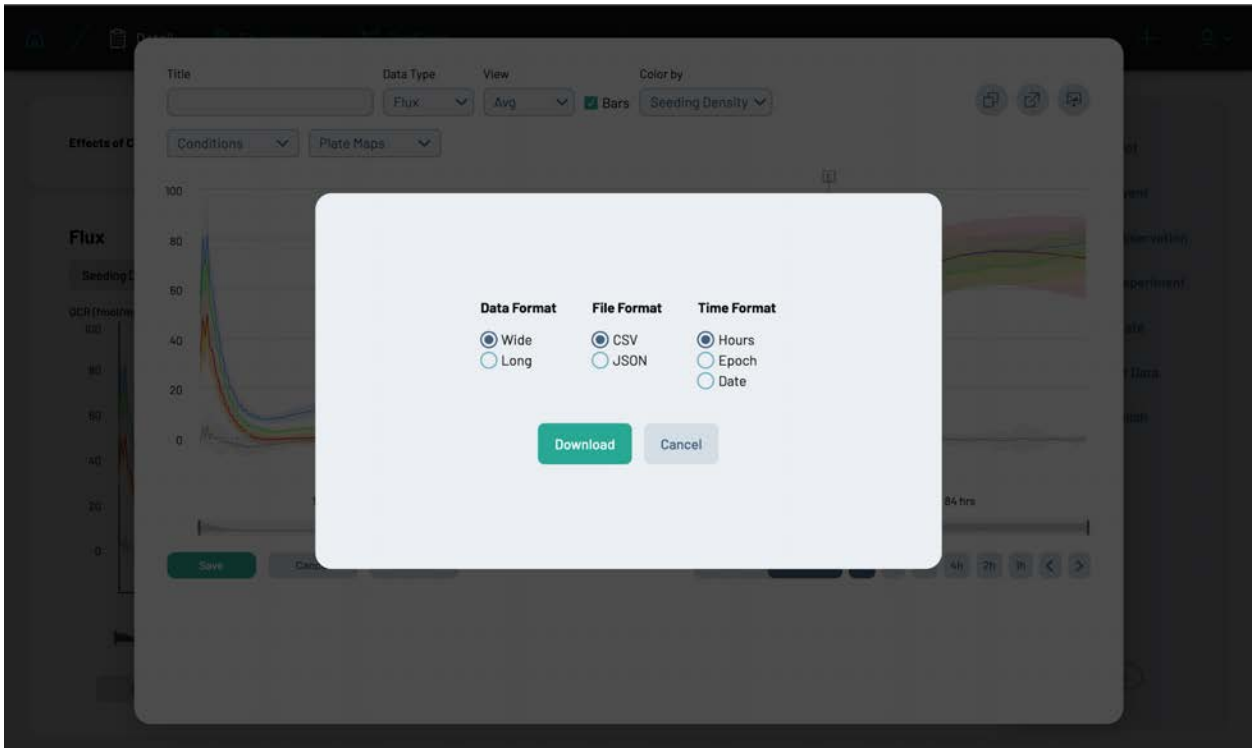
## Replicate Experiment

This copies and creates a new experiment with the exact same experimental setup as the original.

## Export Data

Data can be downloaded in multiple formats, including wide/long, CSV/JSON, and hours/epoch/date time. When downloading data, you can also choose which measurements you want downloaded. These include Flux (OCR), raw oxygen concentration, and the environmental data. When downloading two or more data sets, each of them will be downloaded as a separate file within a .zip folder.

- Wide - sensor data points time-aligned
- Long - sensor data points raw time
- CSV - comma delimited for use in Excel, etc.
- JSON - standard syntax with meta-data
- Hours - since experiment start
- Epoch - seconds since January 1, 1970
- Date time - actual date and time in format YYYY-MM-DDTHH:mm:ss.sssZ

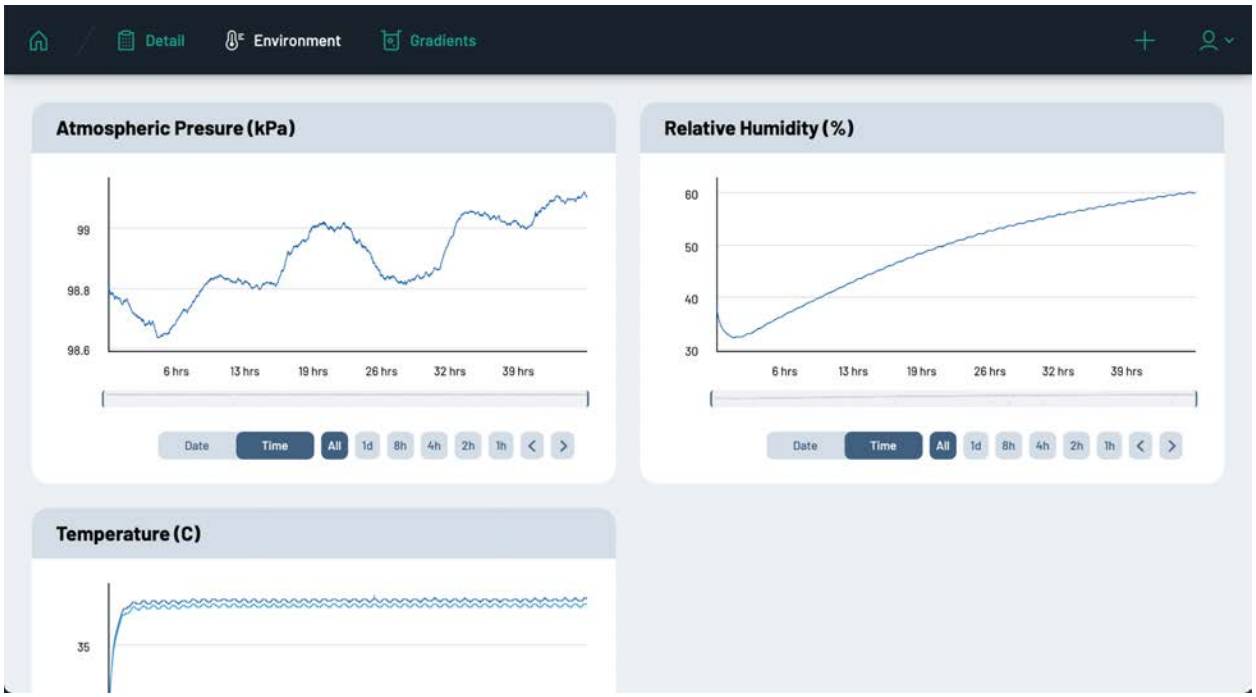


## Publish

Selecting “Publish” will make your experiment and its results visible to every user on the platform, including guests. Doing so allows users to easily collaborate with other scientists.

## Viewing environmental data

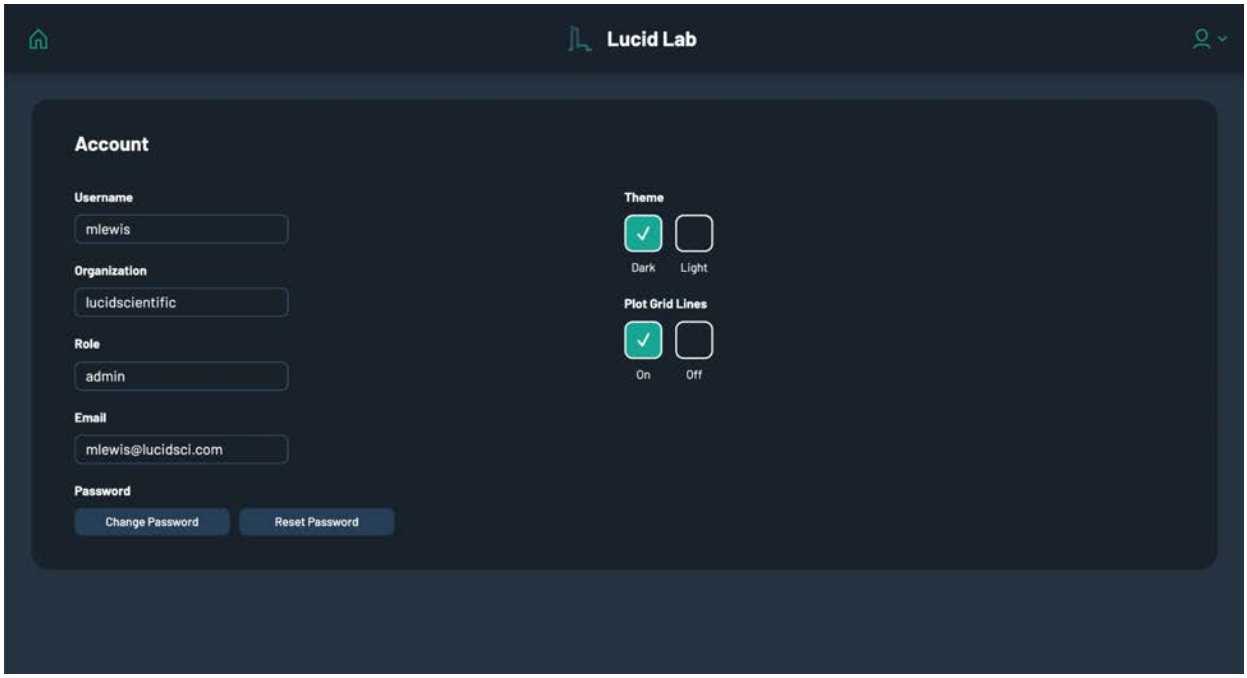
To view environmental data for an experiment, click on the environment tab. This shows you atmospheric pressure, humidity, and temperature data over the entire duration of an experiment.



# Lucid Lab - **Account**

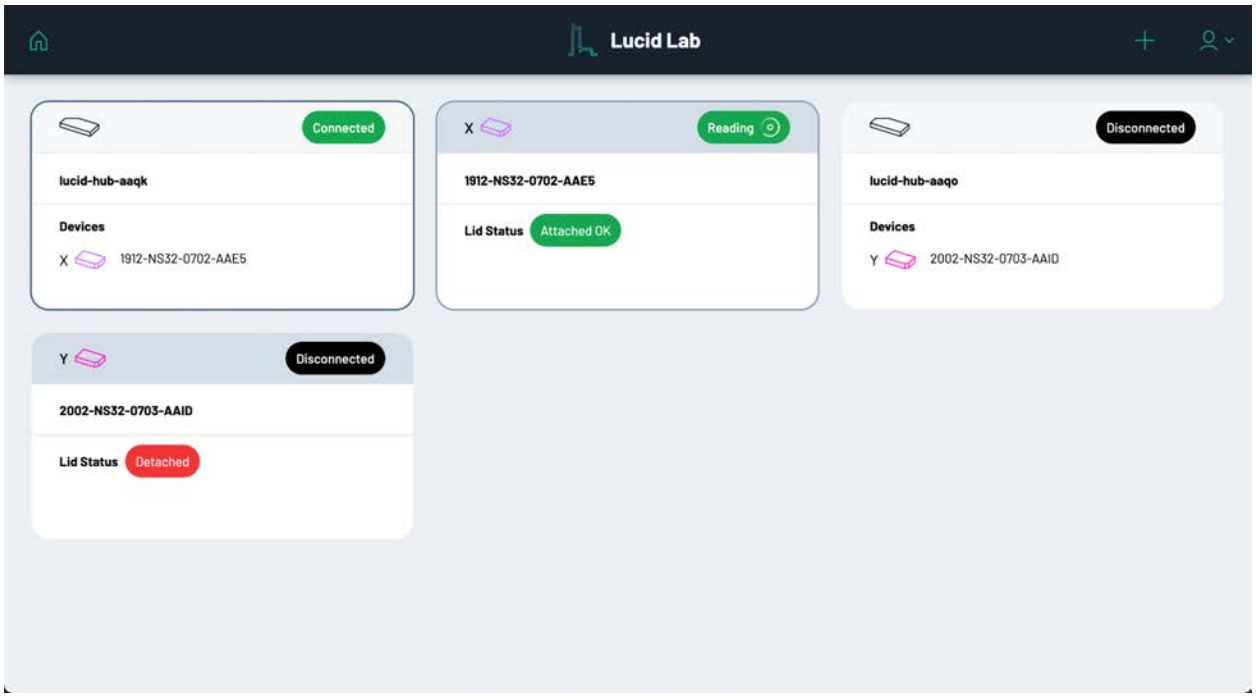
## Changing/resetting passwords and account info

In the top right corner of the app, click on the person icon to see a drop down menu. By clicking your username, a new page will open and show your username, organization, role, and registered email address. You can also change and reset your password from here and toggle between light & dark themes.



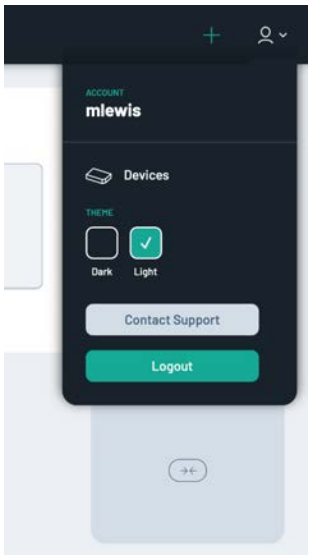
# Lucid Lab - **Devices**

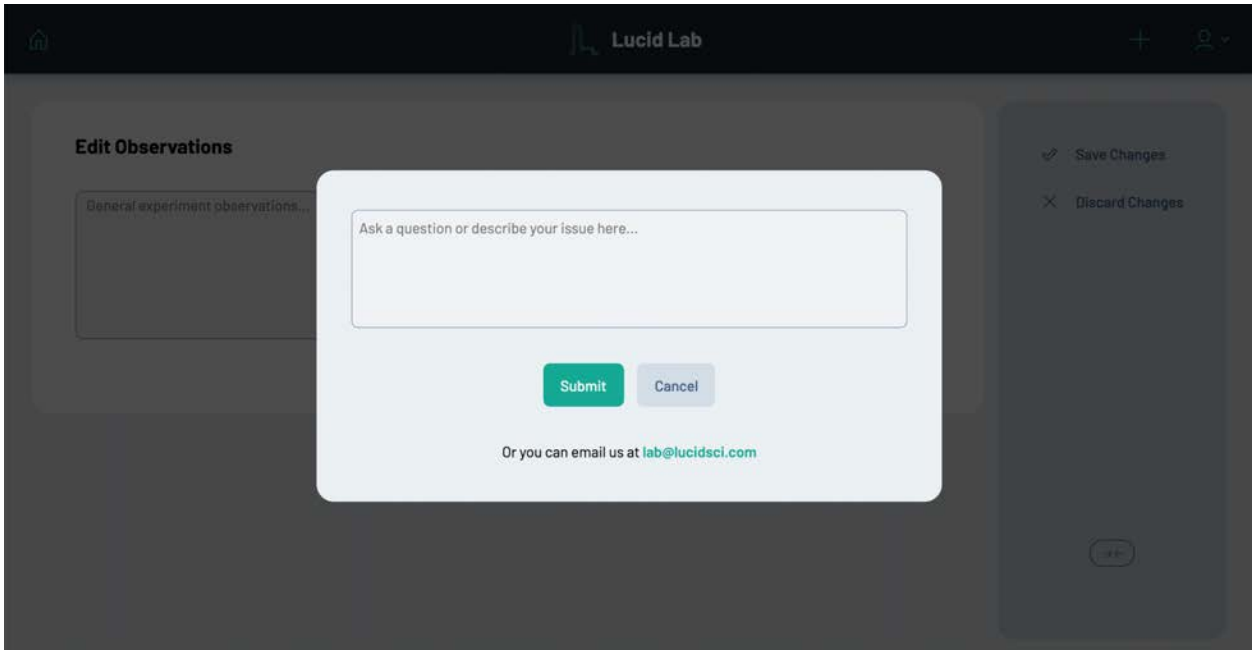
The second option in the account drop down menu will show you all devices that have been or are connected to your Hub currently. It will also show the status of the devices, including whether or not they are connected or running.



# Lucid Lab **– Support**

The last few options allow you to toggle between light/dark themes, contact support, and logout from your account. Should you have any questions, do not hesitate to put your questions into the support box and we will answer you promptly.



# Certifications

## Federal Communications Commission (FCC) Interference Statement

The device complies with Part 15 of FCC rules. Operation is subject to the following two conditions:

- This device may not cause harmful interference.
- This device must accept any interference received, including interference that may cause undesired operations.

This device has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This device generates, uses, and can radiate radio frequency energy, and if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation.

## Open Source Software

The Hub and device contain third-party software including free/open-source licensed software. To request for more information (such as obtaining access to this software), please contact us at info@lucidsci.com.

## Data Restrictions

This system is for research use only. While it is designed and implemented to securely store and access data, users are instructed not to enter sensitive information such as PII/PHI. The Hub and web app are not certified for HIPAA or GLP/GMP. The system is intended for research purposes only and should not be used for clinical or diagnostic purposes.

# IT Support FAQ

**Q:** Why does the Hub require WAN/Internet access?
**A:** Primarily for remote system performance monitoring, data analysis support, and software updates. The internet connection also enables researchers to monitor their experiment data remotely.

**Q:** How do I connect the Hub?
**A:** Via ethernet to a DHCP-enabled network.

**Q:** What OS does the Hub run?
**A:** Ubuntu 18.04 LTS

**Q:** Is this a network-level hub or switch?
**A:** No, it is a USB hub and embedded computer - not a network-level hub.

**Q:** Does the Hub require inbound firewall access?
**A:** No, all WAN connections are outbound TCP connections initiated by the Hub.

**Q:** What domains does the Hub connect to?
**A:** *.lucidsci.com and *.amazonaws.com

**Q:** Can you provide the specific subdomains?
**A:** Yes, please contact us for an up-to-date list of the specific subdomains.

**Q:** What outbound ports are the connections made with?
**A:** 443 (https) and 22 (ssh) and 8883 (mqtt)

**Q:** Where is the data stored?
**A:** The data is stored both locally on the Hub's hard drive and on AWS S3.

**Q:** Is the cloud data transmitted and stored securely?
**A:** All data is sent to and retrieved from AWS using TLS/SSL. The data is stored encrypted at rest using AWS S3.

**Q:** Does the Hub support WiFi?
**A:** Yes, but a wired connection is preferred.

**Q:** Is there a default password?
**A:** No, each Hub has a unique factory generated password.

**Q:** Can I install network monitoring software agents on the Hub?
**A:** Yes, we support the installation of linux-supported network monitoring agents such as CrowdStrike Falcon.

**Q:** Is there support for advanced network configuration options such as assigning a static IP address?
**A:** Yes, by connecting a keyboard and an HDMI monitor to the Hub and pressing ctl+alt+F2 you can access the network connection editor. This UI supports advanced configurations such as manual IPv4 configurations.