REFEYN
WEIGHING MOLECULES WITH LIGHT

Refeyn Acquire^{MP} User Manual

Version 2024 R2

Contents

Introduction

Purpose of the software

Acquire^{MP} facilitates taking measurements on Refeyn Mass Photometers. The software also provides features to tune and optimise operating parameters.

Instrument compatibility

This version of Refeyn Acquire^{MP} is compatible with the following Mass Photometer instrument types:

- Refeyn One^{MP}
- Refeyn Two^{MP}
- Refeyn Samux^{MP}
- Refeyn One^{MP} Auto
- Refeyn Two^{MP} Auto
- Refeyn Samux^{MP} Auto
- Refeyn MassFluidix HC add-on for One^{MP} and Two^{MP}
- Refeyn Karitro^{MP}

File compatibility

Measured data and settings stored to file by Acquire^{MP} are generally backwards (but not forwards!) compatible for Acquire^{MP} and for other Refeyn software downstream. This means that files acquired in a particular Acquire^{MP} version can only be opened in Refeyn analysis software (e.g. Discover^{MP}) of the same or later version.

Getting started

Prerequisites

System requirements

The required PC specification for Acquire^{MP} depends on the instrument type it is being used with.

One^{MP} and Two^{MP}

- PC
  - CPU: Intel i7 with 8 cores (e.g. Intel i7-10700)
  - Memory: 16 GB DDR4 RAM
  - Storage: 1TB SSD
- Operating System: Windows 10 Professional
- Display: 1920 x 1080 at 100% zoom

Samux^{MP}, One^{MP} Auto, Two^{MP} Auto and Samux^{MP} Auto

- PC
  - CPU: Intel i9 with 18 cores (e.g. Intel i9-10980XE)
  - Memory: 16 GB DDR4 RAM
  - Storage: 1TB SSD
- Operating System: Windows 10 Professional
- Display: 1920 x 1080 at 100% zoom

Karitro^{MP}

- PC
  - CPU: Intel i7 with 12 cores (e.g. i7-12700T)
  - Memory: 16 GB DDR4 RAM
  - Storage 2TB SSD
- Operating System: Windows 10 Professional
- Display: 1920 x 1080 at 100% zoom

Installing or updating Acquire^{MP}

If installing Acquire^{MP} for the first time, jump to step 4.

1. Ensure that all instances of Acquire^{MP} are closed.
2. Uninstall the old version of Acquire^{MP} by going to Control Panel > Programs > Programs and Features and then selecting Acquire^{MP} to uninstall.
3. Check that the uninstall has been successful by going to C:\Program Files (x86) in the windows file explorer and checking that there is no folder called AcquireMP. If there is, manually delete the folder.
4. Execute the Acquire^{MP} setup executable file (the filename will be of the form: AcquireMP_v2024.1.000_setup.exe). Double clicking on the file will bring up an install wizard.
5. Follow the steps of the installer, ensure that the terms and conditions have been read before accepting.
6. Once the installer has finished, the software is ready to use.

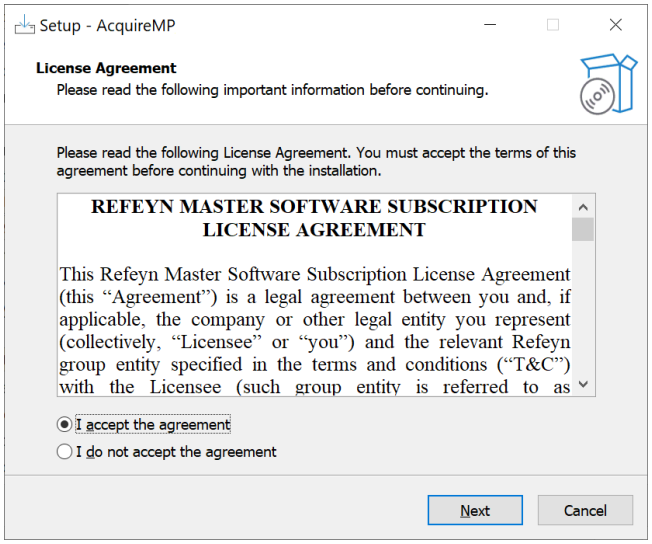


Figure 1: Installation dialog

End User License Agreement (EULA)

When Acquire^{MP} is run for the first time, it will display Refeyn's End User License Agreement. Make sure to read the terms and conditions before accepting. In addition, a choice for Acquire^{MP}'s telemetry mode is shown with the following options:

- Feature preview mode
- Normal mode
- Offline mode

The details of each mode are listed to their right. Accepting will also confirm the current selected telemetry mode, this can be changed in Preferences.

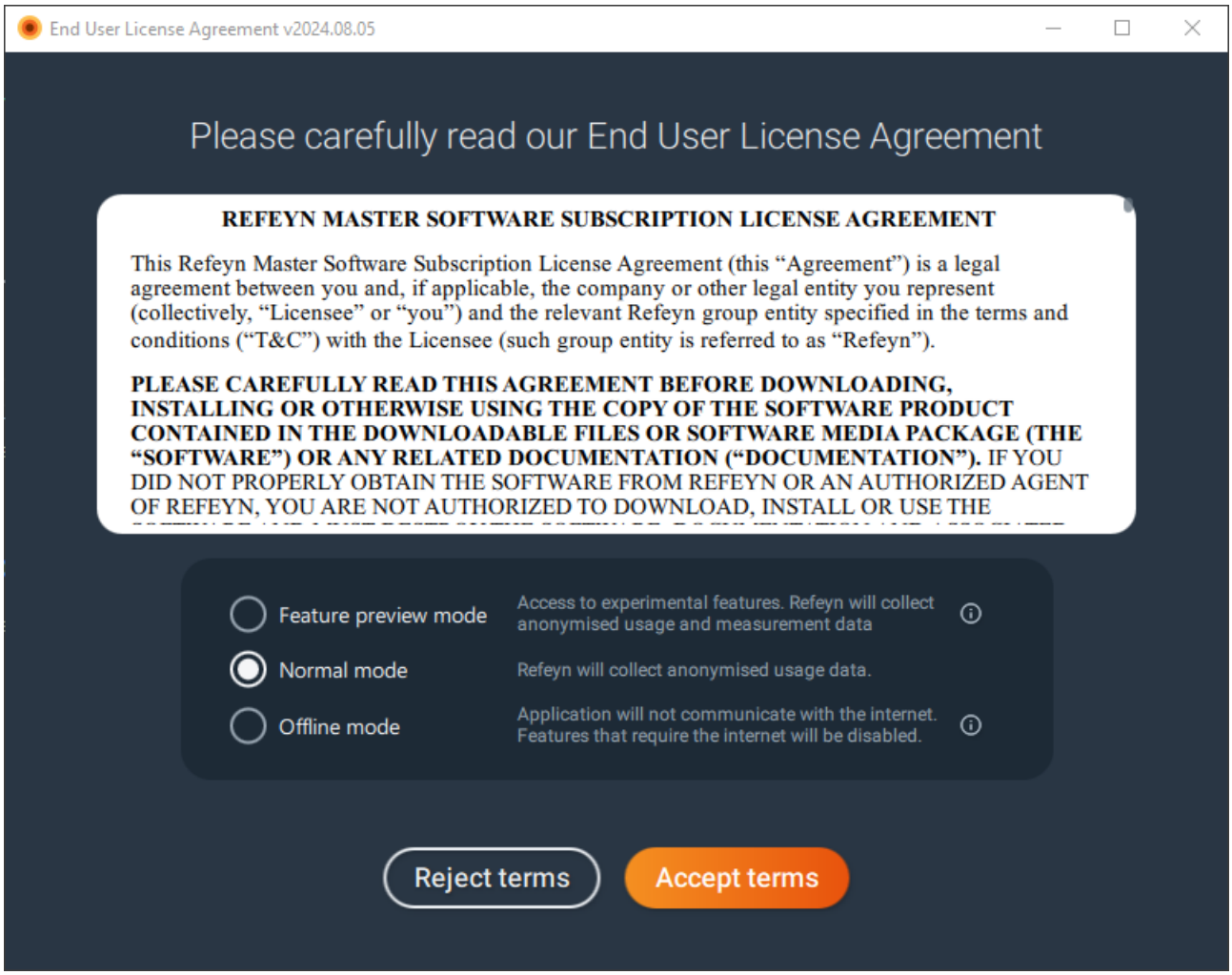


Figure 2: End User License Agreement and mode selection

Activate or deactivate your Acquire^{MP} license

When opening the application for the first time, the program will prompt you to activate the license with the license key. The license key is provided with the software, and appears such as the following example: ABCDE-FGHIJ-KLMNO-PQRST. If the user has yet to obtain a license key, one can be obtained by contacting Refeyn using the contact details.

If the PC on which you are installing Acquire^{MP} has an internet connection, make sure **Use this PC's internet connection** is selected, then simply enter the license key into the box and click **OK**.

If the PC does not have an internet connection or the connection fails, select **Use another PC's internet connection**. The license key code is then to be entered into the box, click **Next** and take note of the *machine code* shown in the dialog. If access is necessary on an additional PC with an internet connection, open the webpage shown in the dialog. A form will be loaded in which the machine code and license key shall be entered. On clicking **Activate**, an .skm activation file will be downloaded. Copy this file onto the desired PC to install Acquire^{MP}. Enter the license key into the Acquire^{MP} dialog and use the **Browse** button to select the .skm activation file you copied over, then click **OK**. Acquire^{MP} will now load.

Deactivating the license

If you wish to move your license for Acquire^{MP} to another PC, you should deactivate the license on the PC you no longer wish to use. To do this, open Acquire^{MP} and go to **Help** > **Deactivate license**. A warning message will be shown. Clicking **Yes** will close Acquire^{MP} and deactivate the license on that PC. You can then activate your license key on another PC. To reactivate a license after deactivating it, simply open Acquire^{MP} again and re-register the license, following the instructions above.

Check drivers and connections

Before starting Acquire^{MP} the required connections between the PC and instrument hardware should be checked.

- Check that the instrument is powered.
- Ensure that the Universal Serial Bus (USB) cable from the instrument is connected to one of the USB 3 ports of the Personal Computer (PC). The USB 3 ports are labelled with the symbol SS.
- Open the Control Panel > Devices and Printers and check that the following USB devices in the instrument are correctly recognised:

USB device	One^{MP}	Two^{MP}/Samux^{MP} (Auto)	One^{MP}/Two^{MP} MassFluidix	Karitro^{MP}
"Digilent USB Device"	X	X	X	X
"Grasshopper3" or "xiC"	X	X	X	X
"uc480 cmos" or "USB 2.0 Camera" or "xiQ"	X	X	X	
"SmarAct MCS2"	X	X	X	X
"USB-3103"	X		X	
"INT-FOEM"			X	
"KaritroMP"				X

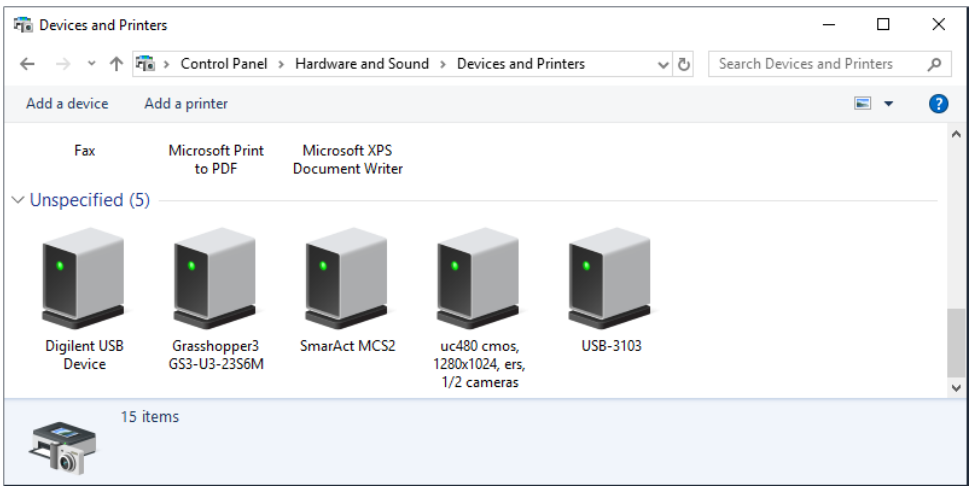


Figure 3: List of USB device names shown in the control panel when the One^{MP} is turned on and correctly connected

If any device is missing:

1) Power-cycle the USB devices by disconnecting and reconnecting the USB cable.
2) Make sure that the correct camera drivers are in use.

Check DAC registry (One^{MP} and One^{MP} Auto only)

The One^{MP}'s Digital-to-analog converter (DAC) card may require its registry to be updated when Acquire^{MP} is run for the first time or if there is a Windows update on the PC. To update the registry, start the program **Instacal** which will then show a prompt to update the registry. Once the update is complete either the PC will need to be restarted or the USB connection of the mass photometer needs to be cycled by removing and plugging in the USB cable to the PC.

Check device status

After starting Acquire^{MP}, the status of each USB device in the Mass Photometer can be checked in **Tools** > **System Status…**. A notification will appear concerning whether any devices have failed to start correctly.

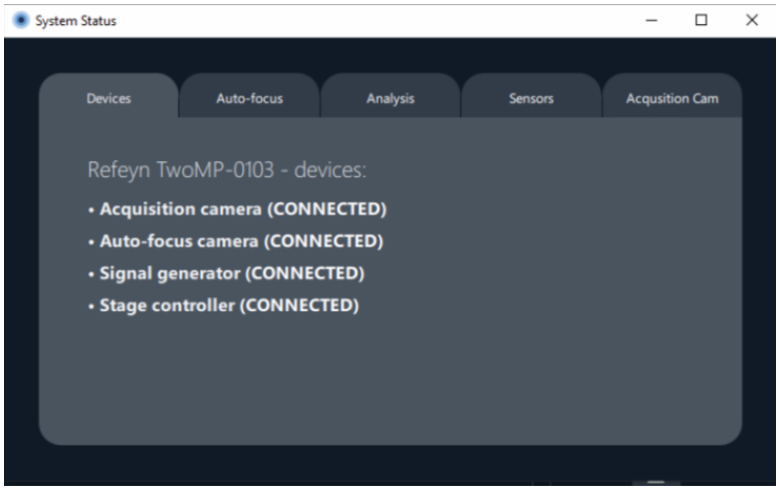


Figure 4: Dialog showing that all devices have started correctly for Two^{MP} and Samux^{MP}. The dialogue for the One^{MP} will also list the DAC

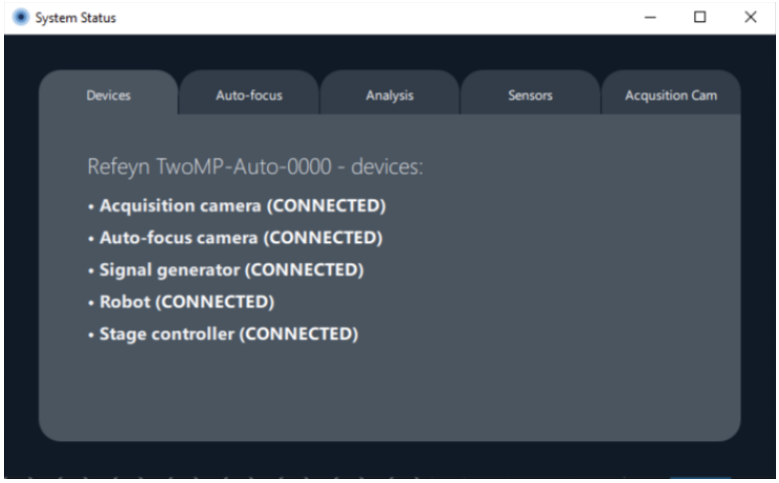


Figure 5: Dialog showing that all devices have started correctly, for Two^{MP} Auto and Samux^{MP} Auto. The dialogue for the One^{MP} Auto will also list the DAC

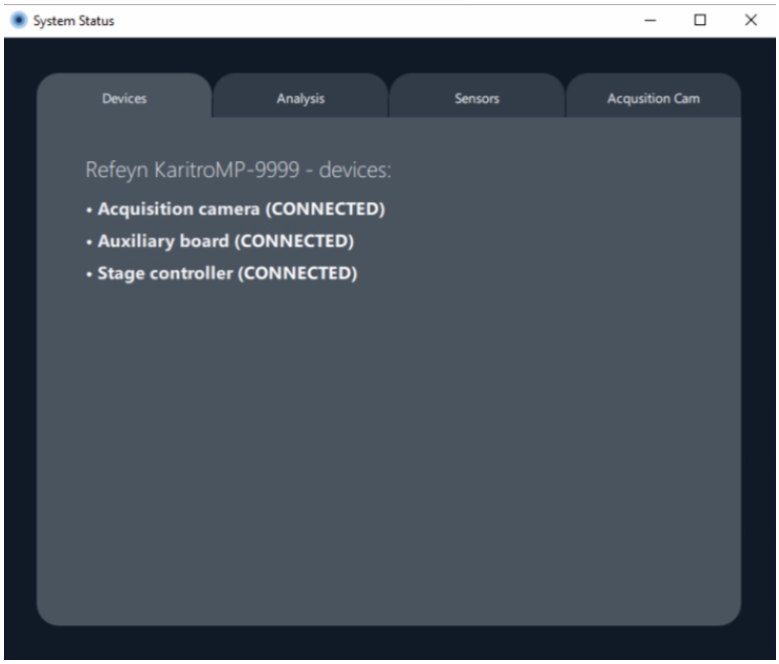


Figure 6: Dialog showing that all devices have started correctly for Karitro^{MP}.

Workflows and functionality

Most of the workflow, user interface, and functionality of Acquire^{MP} are different for each of the different Mass Photometers. Please read the relevant section to learn how to operate your device. For specific details on which functionality is supported for your instrument please refer to the functionality matrix below.

One^{MP} / Two^{MP} / Samux^{MP}

Workflow

1. Define measurement settings.
2. Position your cassette and find focus.
3. Load sample.
4. Record movie and save the directory of the measurement folder.

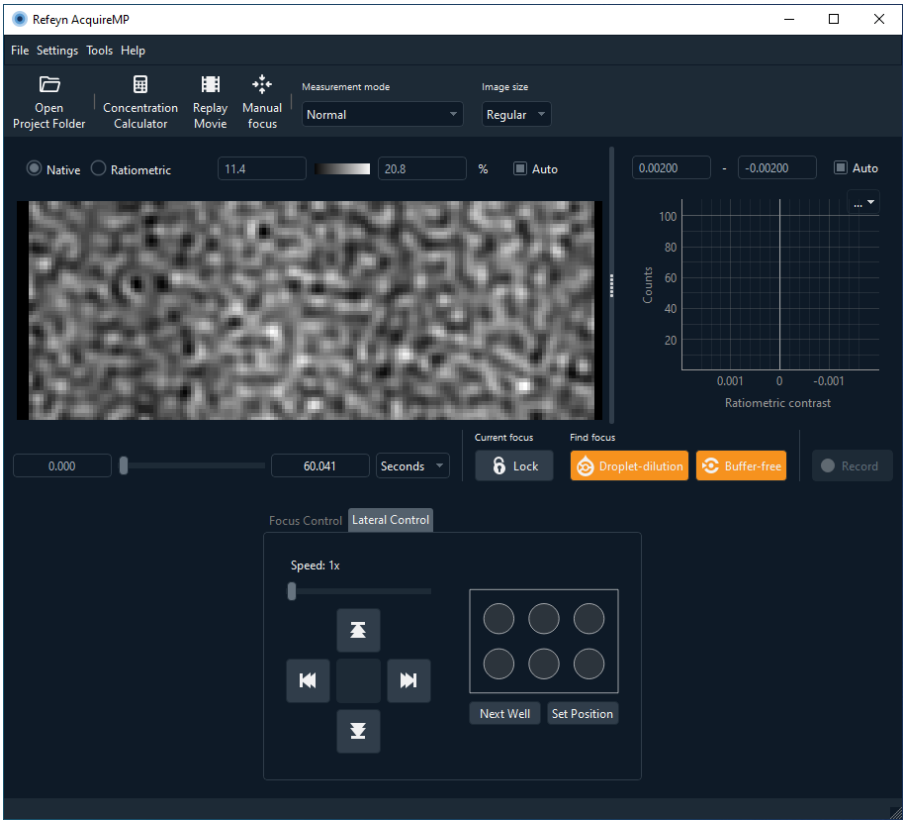


Figure 7: Main window

Main window

Toolbar

The toolbar contains many of the important settings required to optimise the recording as well as useful tools. Details on each of these are provided below.

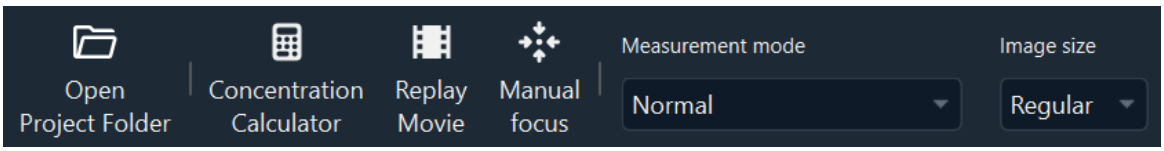


Figure 8: Toolbar. Note: Measurement mode and Image size controls are only visible on the One^{MP} and Two^{MP}

Acquisition view The acquisition camera is the primary camera of the Mass Photometer. It records the images that are used to measure the mass of particles that bind to (or unbind from) the slide. Images from the acquisition camera are displayed in the top half of the Acquire^{MP} main window.

There are two types of acquisition images – *native* and *ratiometric*. Native images contain the native data captured by the acquisition camera. Ratiometric images, on the other hand, are a result of processing multiple native frames in a way that reduces noise and emphasises the changes in native frames across time.

Selecting the **Native** and **Ratiometric** buttons toggles between these views. The pixel brightness limits above the image panel depend on the viewing mode. Native image pixels have a brightness value between 0% and 100%. Selecting **Auto** automatically scales the applied color gradient between the values of the darkest and brightest pixel in each frame. Unchecking the **Auto** box allows users to change the values of the color gradient limits. If a pixel has a value less or greater than the respective minimum or maximum limits then it will be assigned the minimum or maximum color gradient value. In Ratiometric mode a change made to either limit is automatically mirrored in the opposite limit to ensure that both binding and unbinding events are proportional.

Enable Auto in native mode to let the software control the black and white level automatically.

Figure 9: Acquisition movie information panel in Native mode

Data quality scores If the **Manual focus** option is selected, there are five data quality scores on the right side of the interface. They are: Sharpness, Brightness, Saturation, Signal and Motion. These values should remain in the blue regions; if they enter the orange region, this indicates that the data being collected may be unreliable. The exception to this is that the signal monitor should become orange whenever a sample is added due to landing events increasing the signal.

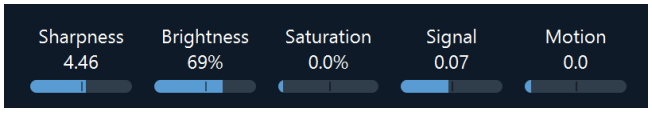
The **Sharpness** value shows how precisely the sample is positioned and held in focus. The minimum recommended sharpness value for the One^{MP} and Two^{MP} is 4%, and 1.6% for Samux^{MP}. Please note that the value can be exaggerated by a dirty glass surface, giving the false impression that the sample is in focus. In this case, the user is advised to move the coverslip to a clean region before starting the recording. Additionally, the sharpness value tends to decrease slightly with image size because the illumination profile is more uniform as the light is distributed over a larger area. During the recording of a movie, sharpness tends to increase as particles bind to the surface. That is expected and no reason for concern. However, a significant drop of the sharpness value usually indicates that the sample moved out of focus, which may impact data quality.

The **Saturation** value shows the percentage of pixels in the native image that are saturated (i.e. overexposed). Ideally, this graph will be a flat line sitting at 0%. A small percentage of saturated pixels is tolerable but the saturation value should not exceed 0.5%.

The **Brightness** value shows how much light the camera has detected in relation to its saturation level. This indicates how efficiently the dynamic range of the camera has been used. A high brightness value (at least 25% for One^{MP}, 50% for Two^{MP}, and 70% for Samux^{MP}) is desirable to minimise shot-noise in the data.

The **Signal** value is indicative of the amount of change in the data. Frames with higher signal usually show particle binding/unbinding events. When recording only buffer, this value should be 0.06% or lower. Please note that the choice of image size influences the signal level. As the image size is increased the same amount of light is distributed over a larger area and therefore per-pixel noise increases. Hence, the signal level for a clean buffer will tend to be higher for larger image sizes.

The **Motion** value gives an indication of the amount of lateral motion detected during the acquisition of a frame. The value is calculated from the frame data by determining the amplitude of the motion signature in the ratiometric frame. Small-scale motions are tolerable, but values exceeding 5% are considered to be considerable motion and may impact data quality significantly.



Autofocus If the Manual focus option is selected, the autofocus indicators will be visible in the bottom-left of the Acquire^{MP} main window. The mass photometer's auto-focus optics produce a circular ring image, shown on the left-hand side of the panel. The radius of this ring is directly related to the vertical (Z) position of the glass slide. Next to the ring image are two plots: the **Focus Position Monitor**, showing the ring radius over time, and the **Radial Profile Monitor**, showing the radial average of the ring image.

Figure 10: Focus position monitor and ring

Acquisition options

Movie length The length of the movie to be recorded can be set by editing the textbox positioned to the right of the movie slider. The value can be defined in seconds or frames, depending on the dropdown option selected next to the textbox. If recording using the Antibody Stability measurement mode, the movie length is fixed at 60s.

Measurement Mode (adjustable on One^{MP} and Two^{MP} only) A particular measurement mode can be chosen depending on the type of measurement being performed. Different measurement modes will activate different analysis tools when the movie is loaded in Discover^{MP}.

- **Normal**: This mode gives the most flexibility and should be used for most measurements.

- **AAV**: This mode is for performing measurements to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).
- **Antibody Stability**: This mode is for performing measurements to calculate the relative abundances of monomers and aggregates in an antibody sample. Use this mode for measurements that are to be analysed with the Antibody Stability module in Streamline^{MP}.
- **Experimental fast detection [EXPERIMENTAL, Two^{MP} only]**: This is an experimental mode intended for use with MassFluidix measurements with highly unstable samples.

Users can choose a measurement mode using the dropdown in the toolbar. By default, Acquire^{MP} will use the **Normal** measurement mode. The Samux^{MP} and Samux^{MP} Auto only operate in **Samux** mode, optimised to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).

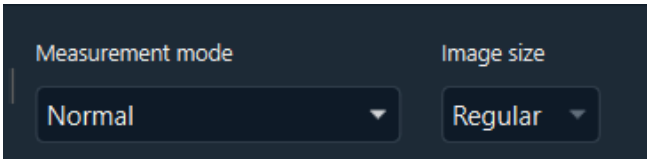


Figure 11: Measurement mode and image size controls

Image Size (Adjustable on One^{MP} and Two^{MP} only) When using the **Normal** measurement mode, the user may choose from three pre-defined image sizes, while the **Antibody Stability**, **AAV** and **Samux** measurement modes have a fixed large image size.

On the One^{MP} the sizes are *Regular*, *Medium*, and *Large*. *Regular* is the default.

On the Two^{MP} the sizes are *Small*, *Regular*, and *Large*. *Regular* is the default.

The **Experimental fast detection** mode is fixed with its own *High framerate* image size.

For guidance on which image size is appropriate for your experiment, see **Image Size** in the **Further Information** section.

Positioning the cassette and focusing

Stage control At the bottom right of the main display window is the lateral control tab, used to move the stage laterally and a focus control tab, containing manual focus functions. For more details on manual focus, see **Manual Focus** in the **Further Information** section.

The lateral movement of the stage is controlled using the arrows and the speed of the movement can be adjusted using the slider.

The stage can also be moved using keyboard shortcuts:

Command	Keyboard Shortcut
Up	Alt + W
Down	Alt + S
Left	Alt + A
Right	Alt + D

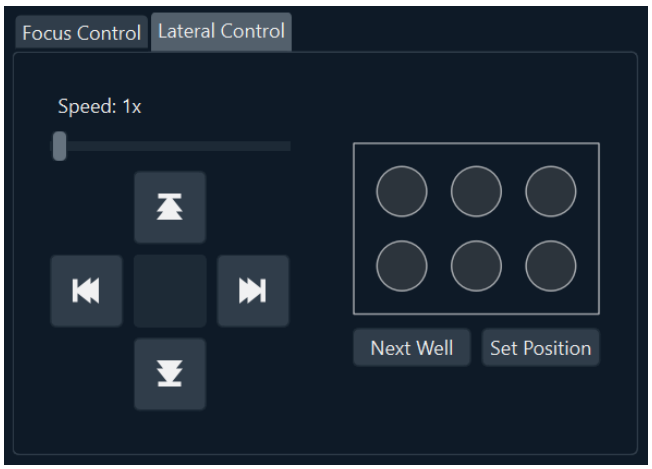


Figure 12: Lateral control tab with stage controls on the left and cassette navigation control on the right

It is recommended to ensure the focus is unlocked before moving the stage laterally.

Cassette navigation First, drop some immersion oil onto the objective, and then place an assembled slide and cassette onto the stage, securing with magnets. In the cassette navigation control, double click on the well you wish to move to and the stage will move accordingly. Then using the arrows, position the well above the objective using the eye safe red laser. For best performance, slightly offset the laser from the centre of the well. Once positioned, click **Set Position**, and you will be able to move to the next well using the **Next well** button. You can also double click on the well you wish to use.

It is recommended to move between wells clockwise when possible to avoid introducing bubbles in the immersion oil.

The cassette navigation tool is only designed to work with pre-cut 6 well cassettes which are available from orders@refeyn.com. For more information, visit https://www.refeyn.com/mass-photometry-consumables.

Find focus Acquire^{MP} has two **Find Focus** features that will automatically focus the system. These are:

- Droplet-Dilution Find Focus
- Buffer-Free Find Focus

Alternatively, a user can decide to use the Manual focus option, the details of which can be found in the **Advanced Features** section of this manual. To focus, the Current Focus **Lock** must be unlocked. If it is locked (indicated by an orange closed padlock in the button), click the **Unlock** button to unlock.

The instrument should be turned on 1 hour before use to allow time for thermal equilibration.

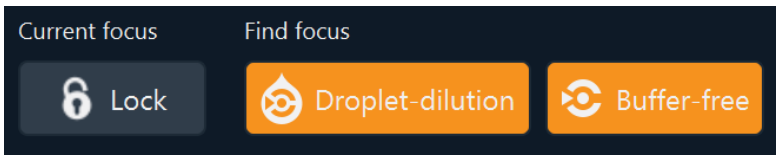


Figure 13: Controls for focus lock, Droplet-dilution find focus and Buffer-free find focus

Droplet-dilution find focus

Droplet-dilution find focus requires access to a particle free buffer which is the same as that used for your sample, but is the preferred method for low mass protein measurements.

To use droplet-dilution find focus, pipette 10–15 µl of particle free buffer into the well, close the lid and click **Droplet Dilution** in the find focus section, and the instrument will find focus.

When run for the first time, Find Focus usually takes up to 20 seconds to complete as it searches across the Mass Photometer's focusing range. On future runs, the previous focus position will be used to speed up the search. For the Find Focus routine to work effectively, the auto-focus and the acquisition cameras need to be set up to give a good image (see Image Calibration).

Verify the quality of the focus as instructed below before opening the lid, adding sample to make the final volume in the well up to 20 µl, closing the lid and clicking **Record**.

Buffer-free find focus

Buffer-free find focus provides a simplified faster measurement process.

To use Buffer-free find focus, ensure the well is centered on the objective, close the lid and click **Buffer-Free**. The system will perform a coarse focus and then ask that sample is added to the well. Pipette 20 µl of sample into the well, and close the lid. If the **Automatically detect if sample is added** checkbox is checked, the system will automatically complete the focussing process, otherwise, click **Sample Added**.

If the **Start recording automatically** checkbox is checked, then the recording will start automatically 6 seconds after the focus is complete. If not, then click the **Record** button to the right of the find focus buttons.

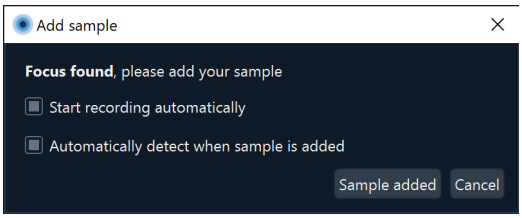


Figure 14: Dialog prompting the user to add sample during Buffer-Free Find Focus

Focus Quality

There are three indicators of focus quality in Acquire^{MP} software. These are:

1. Focus ring
2. Native image
3. Quality scores

The focus ring in the bottom left of the main window should be reasonably round and have reasonably even contrast. If there are gaps, this is generally an indication of bubbles in the immersion oil. To remove these bubbles, unlock the focus, remove the magnets and lift one edge of the cassette assembly, breaking the immersion oil droplet before replacing the slide. Re-secure using the magnets and repeat the focus procedure.

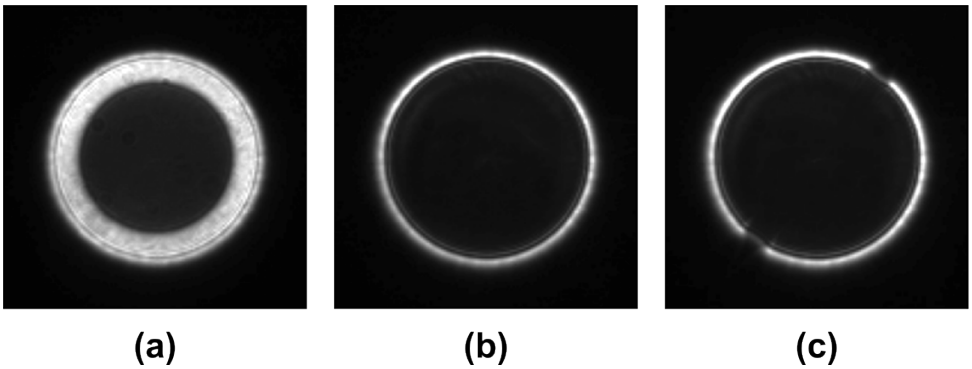


Figure 15: Ring images indicating (a) a good focus with Buffer-free find focus before samples has been added; (b) a good focus following the completion of either find focus method; (c) a bad focus due to a bubble in the immersion oil, suggested by gaps in the ring

The native image in the top left of the main window should show a sharp image of the roughness in the glass. Look around the image for the presence of large particles such as dust which will appear as large light or dark patches as shown below. To do this unlock the focus, move the stage until the particles are outside of the field of view, and repeat the focussing procedure.

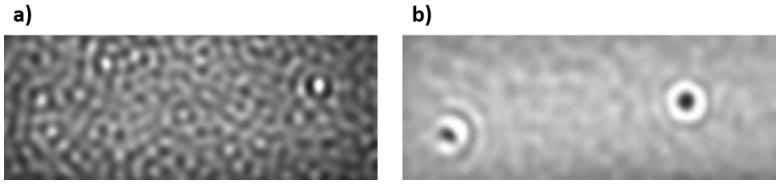


Figure 16: Dirt appears as large a) bright, or b) dark spots in the native image

The quality indicators are positioned in the centre right of the main window. After focus, these should all be blue. Once sample has been added, the signal indicator may move into the orange and this is completely normal.

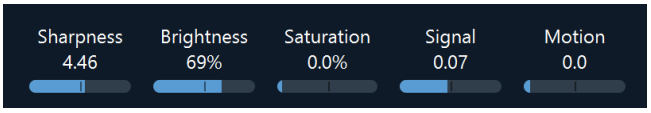


Figure 17: All quality indicators showing blue

Movie recording

Recording and saving Acquire^{MP} organises sample recordings that belong together in projects. To create a new project folder or open an existing one click **Open Project Folder** in toolbar. In the window that opens, create a new folder or select an existing one, and click ok. Alternatively, this can be done following the first recording.

Once recording has begun (see Find Focus), the progress bar in the Acquisition Movie panel shows the recording progress. Acquire[MP] will record up to the number of seconds or frames shown on the right of the progress bar. The movie will stop recording at the selected time or upon pressing the **Record** button again.

When the recording ends, a dialog will appear asking the user whether the recording should be saved, and what filename to save it as. The project for the measurement can also be defined here.

Additional information about the measurement can also be recorded so it can be viewed in Discover[MP]. At the bottom of the dialog is a **Fill Previous Values** button, which fills the values from the previous measurement when clicked. This can be used to complete the fields when multiple recordings of the same sample are to be saved.

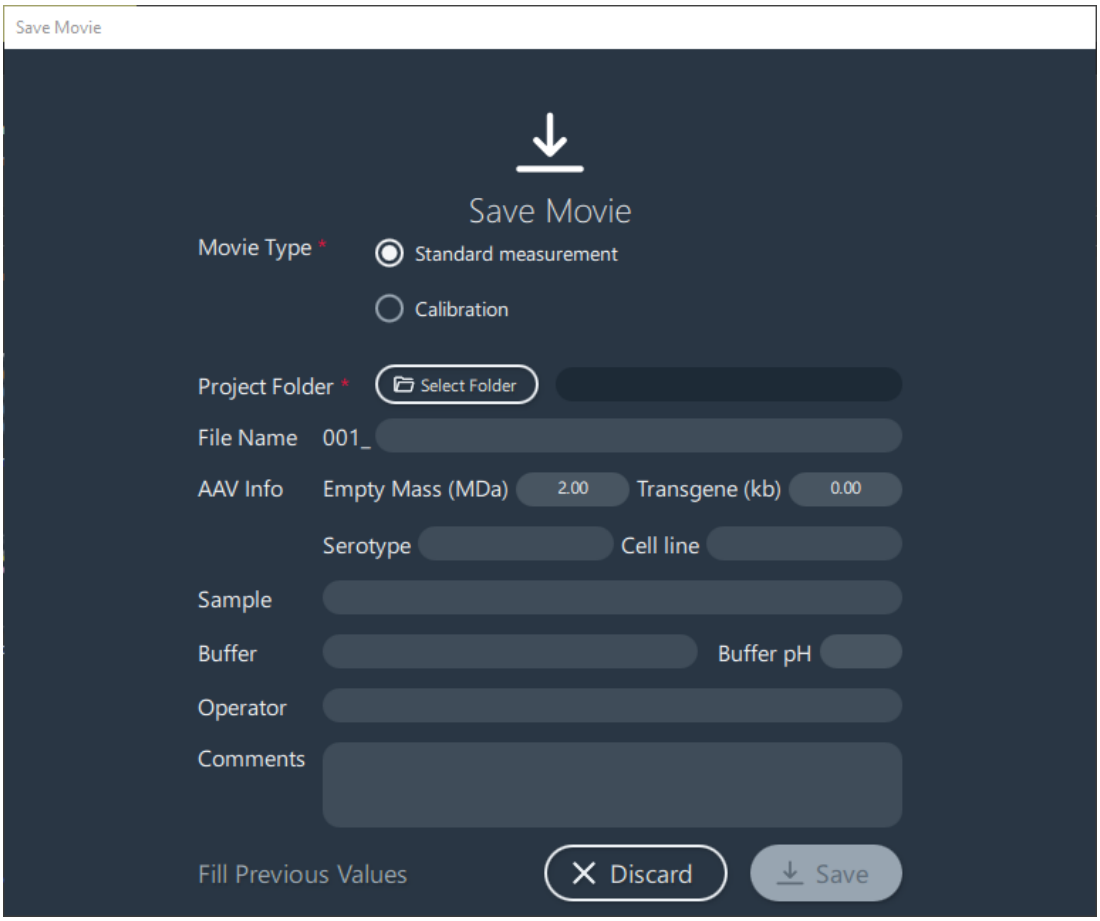


Figure 18: Save movie dialog for Samux[MP]

By default, the saved recordings are compressed to save disk space and will be decompressed when being analysed in Discover[MP]. For details regarding turning file compression on or off, see **Preferences** in the **Instrument Settings** section.

Analysis preview The top right of the Acquire[MP] window displays the **Analysis Preview Panel**.

Unless disabled, this panel will show a preview of the data currently being acquired, in the form of a histogram. By default, the analysis preview histogram shows ratiometric contrast on its X axis.

This can be changed to show mass, bases or base pairs by loading a calibration (.mc) file created in Refeyn Discover[MP]. To do this, right-click on the histogram and select **Load Calibration**. On choosing a calibration file, the histogram will change to show the calibrated units on its X axis. To switch back to ratiometric contrast, right-click on the histogram and select **Clear Calibration**.

It is also possible to easily export the preview histogram as a PNG or SVG image by right-clicking and selecting **Export Histogram**, or copying the image to clipboard via **Copy Histogram**. The live analysis preview can be disabled by selecting the **Disable Analysis Preview** option.

Analysis

Now that the movie recording is complete, the movie can be imported and analysed in Discover^{MP}.

Replay movie

The **Replay Movie** dialog allows movies in the current project folder to be viewed. Movies are selected by clicking on the filename in the panel on the left of the window. Using the radio buttons, the view can be changed between **Native** and **Ratiometric**. The playback timeline can also be changed between seconds and frames using the dropdown in the bottom right.

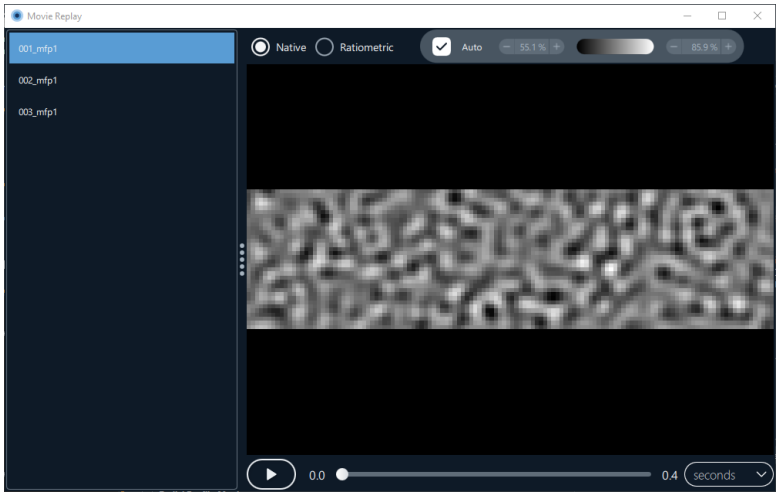


Figure 19: Replay movie window

One^{MP} Auto / Two^{MP} Auto / Samux^{MP} Auto

Workflow

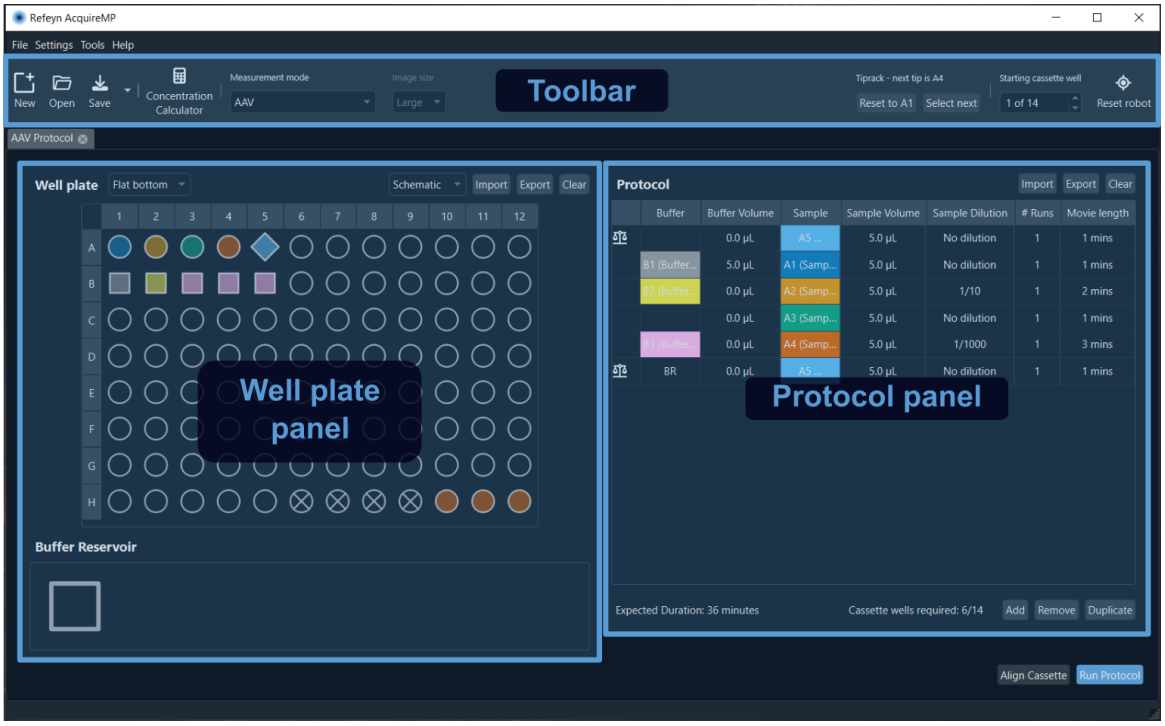
1. Define the well plate design and protocol and set-up the hardware.
2. Define measurement settings.
3. Align the cassette.
4. Run protocol and save the directory of the measurement folder.

A user can choose to add the well plate after defining the protocol to be run by the robot.

Main window

The experiment designer is made up of 3 main elements:

- Toolbar - Located at the top of the window it contains the controls required to open and save experiment files, control the mass photometer and control the robot.
- Well plate panel - This is located on the left of an experiment tab and allows you to define the layout of the well plate for the experiment.
- Protocol panel - This is found on the right of the experiment tab and allows you to define the protocol steps in your experiment.



Toolbar The toolbar contains the tools required to perform a set of measurements. It consists of:

- The **New** button opens a new tab with a blank experiment.
- The **Open** button opens the selected experiment file in a new tab.
- The **Save** button saves the contents of the well plate and protocol table to a pre-existing file if the experiment has previously been saved or ask for a file location to be specified if it has not. The arrow to the right of the **Save** button shows the **Save As…** button which will always prompt for a new file location to be specified. This is a useful way to create a new protocol from an existing one.

- The **Concentration calculator** is a useful tool for calculating required dilutions when the concentration of your stock solution is known. For more information see **Tools & Help**.
- The **Measurement Mode** selector allows the measurement modes to be changed between Normal, AAV and Antibody Stability modes (One^{MP} Auto and Two^{MP} Auto only)
- The **Image Size** selector allows the acquisition image size to be changed (One^{MP} Auto and Two^{MP} Auto in Normal mode only). For guidance on which image size is appropriate for your experiment, see **Image Size** in the **Further Information** section.
- The **Tiprack** section contains the controls related to the pipette tiprack. **Reset to A1** makes the robot start the run at the first tip in the rack and would generally be used when a full tiprack has been inserted. **Select next** allows you to change the starting pipette tip. By default the system will assume the tiprack has not been changed since the last measurement and start the protocol using the next tip position.
- The **Starting cassette well** control facilitates the selection of the first well in the cassette (not the well plate) to be used in the experiment. By default this returns to 1 at the start of each experiment assuming a new slide and cassette have been installed, but if this is not the case, the starting well can be changed here.
- The **Reset Robot** button moves the robot back to its home position.

Figure 20: Toolbar for One^{MP} Auto and Two^{MP} Auto

Start an experiment

To create a new experiment tab, click **New** in the toolbar. This will open up a new tab with a blank well plate panel and protocol panel. Alternatively, you can open a pre-existing experiment file in a new tab by clicking **Open** and selecting the experiment file. Once created, experiments can be saved using the **Save** or **Save As…** buttons in the toolbar.

Populate the well plate panel

The next step is to populate the well plate panel with the samples, buffers and calibrants required for the protocol.

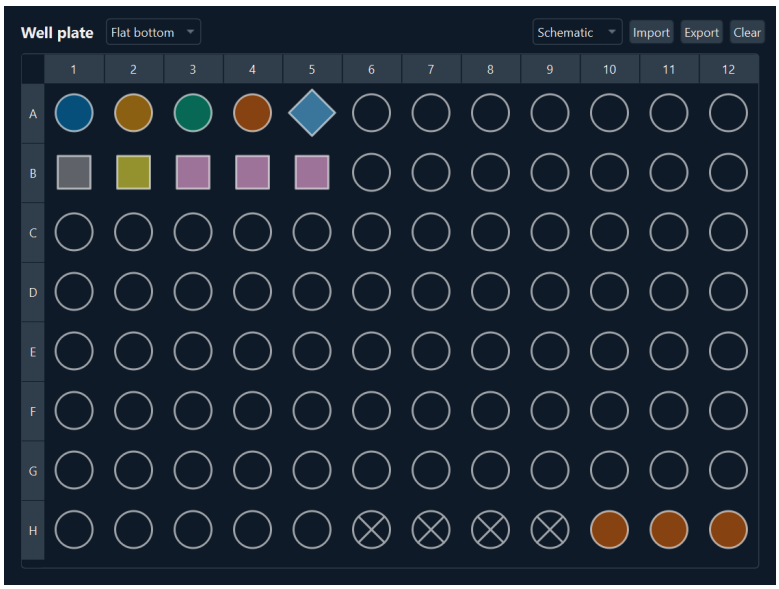


Figure 21: Well plate panel

Add a sample Samples, buffers and calibrants are added to the well plate by double clicking on a well. The **Edit well** dialog can then be populated with the details of the sample.

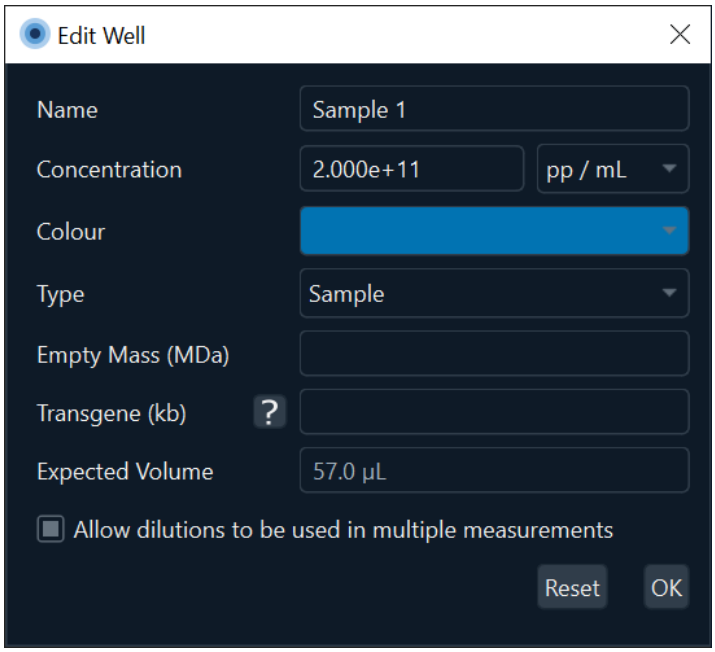


Figure 22: Edit well dialog

The window contains fields to record the **Name** of the material which will be dispensed into that well, as well as its **Concentration** in the desired units. In addition, a colour can also be specified. This colour has no bearing on the experiment, but can be used to help identify different groups of samples and buffers in both the well plate and protocol table.

The material **Type** should also be defined as either **Sample**, **Buffer** or **Calibrant**. This identification will be used by Discover^{MP} when analysing your data. To aid in the identification of these material types, the shape of wells will be changed. Circles are samples, squares are used for buffers and rotated squares for calibrants.

The **Expected Volume** field will be populated automatically, once the protocol table has been completed. This is the minimum amount of sample which must be dispensed into the well for the protocol to run successfully.

Finally, if the **Allow dilutions to be used in multiple measurements** checkbox is selected for the sample well, the robot will perform a single dilution (where requested) and use the same diluted sample for repeats of this measurement defined in the protocol table. If this is not selected, a new dilution will be performed for each individual measurement.

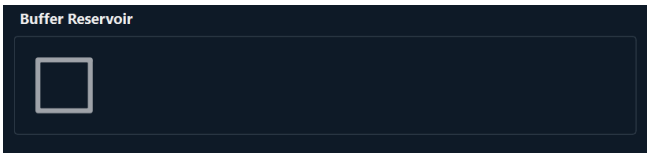
For Samux^{MP} Auto measurements and Two^{MP} Auto measurements in AAV mode, the additional **Empty Mass (MDa)** and **Transgene (kb)** fields are available for Sample wells.

Other well plate functions In addition to those listed above, there are other tools to aid populating the well plate. The well plate type drop-down at the top left allows to specify whether *flat bottom*, *U-bottom*, or *V-bottom* well plate is being used. At the top right of the well plate panel there is the **clear** button which clears the contents of the well plate. By right clicking on a well, you can:

- **Clear** the contents of a well. This can also be done by left clicking on the well and pressing the delete key.
- **Copy** the contents of a well so that you can **Paste** it into a new location. This can also be done by left clicking and dragging a well onto another well.
- **Edit** is another way to access the Edit Well dialogue.
- **Assign well as dirty** changes the colour of the well to mark it as dirty. This is useful if a partially used well plate is being used for an experiment.

All of these functions, other than edit, can be applied to multiple wells by holding the **Ctrl** key and left clicking on multiple wells. Holding **Shift** and left clicking on two wells will select the wells between those two.

Populate the buffer reservoir If your Auto has been equipped with a buffer reservoir, a buffer reservoir panel will appear below the well plate panel.



Double click the buffer reservoir icon to populate the details of the buffer in the reservoir. The name can be specified, as well as a colour. The colour has no bearing on the experiment, but can be used to help identify it in the protocol table.

Populate the protocol table

The next step is to populate the protocol table. To add a new step to the protocol, click the **Add** button below the table. Steps can also be removed and duplicated by clicking on a row and clicking **Remove** or **Duplicate** as appropriate.

Add protocol steps Once a step has been added, a **Sample** to measure must be defined. This is done by clicking and dragging the well which contains the sample you would like to measure onto the sample cell of the appropriate row in the protocol table, or, alternatively, by double clicking the sample cell in the protocol table and selecting the sample well from the well plate panel. If you wish to focus using droplet dilution, or want to dilute the sample as part of the protocol, a **Buffer** must also be specified by assigning the relevant buffer well or the buffer reservoir to the buffer cell. The **Sample Volume**, and if required, a **Buffer Volume** must also be defined. If the buffer volume is set to >0, Droplet-Dilution Find Focus will be used for the measurement. If the buffer volume is set to 0, Buffer-Free Find Focus will be used. Volumes should be set as follows:

14 Well Cassette

Focus Method	Sample Volume	Buffer Volume	Total Volume
Droplet-Dilution Find Focus	5–10 µl	10–15 µl	20 µl
Buffer-Free Find Focus	10–20 µl	0 µl	10–20 µl

24 Well Cassette

Focus Method	Sample Volume	Buffer Volume	Total Volume
Droplet-Dilution Find Focus	5 µl	5 µl	10 µl
Buffer-Free Find Focus	5–10 µl	0 µl	5–10 µl

The next step is to set the **Sample Dilution**. This feature allows samples to be diluted by up to 1/1000. Simply click on the cell and use the drop down menu to select a dilution. To use this feature, make sure a buffer is defined in the protocol step. As dilutions are added, wells will appear with a white cross in the well plate panel. These wells are reserved by the system to use to use when creating dilutions.

When using Droplet-Dilution Find Focus, remember that the sample will be diluted further by the buffer in the cassette well.

Calibration values may change during an experiment, therefore it is recommended to make calibration measurements both as the first and the last runs of an experiment.

The **# Runs** column determines the number of times each step in the protocol is repeated and **Movie Length** can be adjusted to 1, 2 or 3 minutes. Generally speaking, a 1 minute movie is recommended. If you would like all measurements to have a length of 2 or 3 minutes, we recommend changing one measurement and duplicating it.

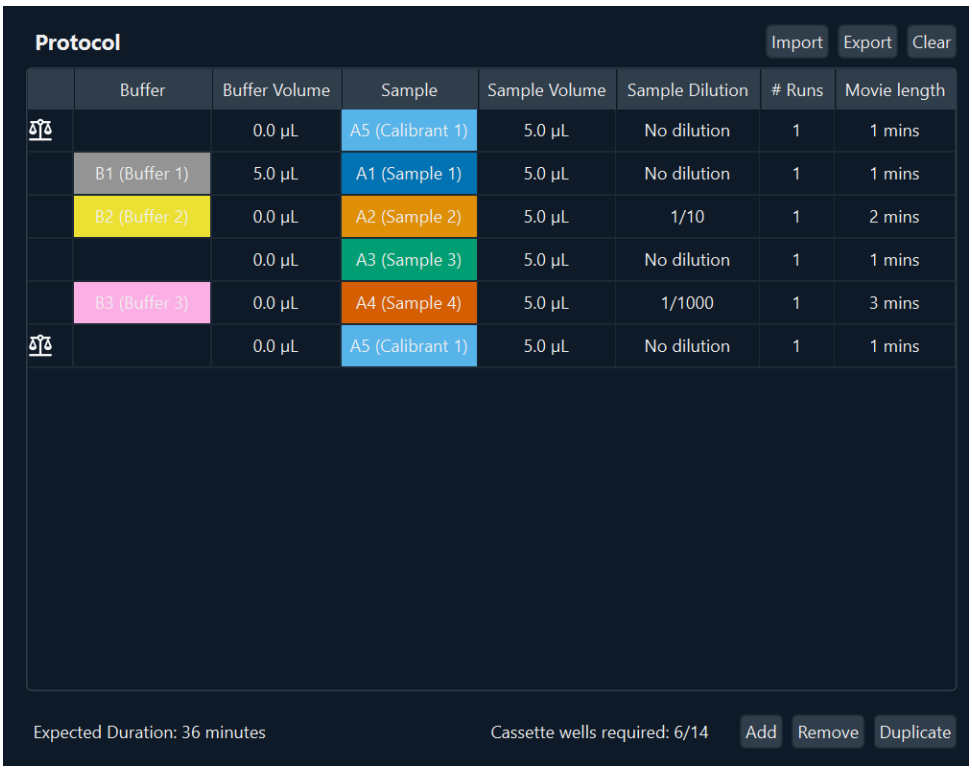


Figure 23: Protocol table

Protocol checks As you set up your protocol, you will be able to see the **Expected Duration** value update. This value is an estimate of how long the protocol will take to run.

Warnings will appear to alert you if the required capacity of a well in the well plate exceeds its physical capacity, or if the number of cassette wells required exceeds the number available.

Saving the Experiment Setup An experiment setup can be saved via the **Save** button in the toolbar which creates an *Automated Mass Photometry Experiment (.ame)* file. This contains the well plate setup and the measurement protocol. The experiment files can then be loaded using the **Open** button.

Exporting protocols and well plate setups can also be done separately via the **Export** buttons at the top of the respective panels, although this is discouraged. If there is a pre-existing protocol table you would like to use, you can use the **Import** command to populate the protocol table from a CSV file. Similarly, a pre-existing well plate setup can be imported from a JSON or a CSV file.

Filling wells

Once the well plate and protocol have been inputted into the software, we recommend using the table view of the well plate to check the minimum volumes of sample or buffer required in each well. Switching the well plate to table view is done by clicking on the dropdown above the well plate design, which can switch between **Table** and **Schematic**.

Filling the buffer reservoir

Filling the buffer reservoir with 5ml of buffer will ensure sufficient buffer is available for all dilutions specified. We recommend filling a clean tube with fresh buffer for each well plate.

Define run settings

Before running a protocol, it is important to check the settings in the toolbar. First, on One^{MP} Auto and Two^{MP} Auto only, the **Measurement Mode** and **Image Size** should be set using the drop down.

The user can choose a particular measurement mode depending on the type of measurement they are performing. Not all measurement modes are available for all Mass Photometry devices. Different measurement modes will activate different analysis tools when loaded in Discover^{MP}.

- **Normal**: This mode gives the most flexibility and should be used for most measurements.
- **AAV**: This mode is for performing measurements to calculate the full/empty ratio of a sample of adeno-associated virus (AAV).
- **Antibody Stability**: This mode is for performing measurements to calculate the relative abundances of monomers and aggregates in an antibody sample. Use this mode for measurements that are to be analysed with the Antibody Stability module in Streamline^{MP}.

For more details on choosing an image size, see the **Image Size** section under **Further Information**.

Next, the starting conditions in the robot should be set:

Tiprack: The software will remember the position of the last tip it picked up in the previous run and will continue from the next position. The software will assume that all subsequent positions on the tiprack contain a tip. When adding a new tiprack, users can reset the software by selecting **Reset to A1**. If you would like to start from a different tip, click **Select next** and a dialog will appear allowing you to select the starting tip by clicking on the starting position and clicking **Select tip** to confirm. The robot will pick up tips column by column from top to bottom. ie. A1»A12, B1»B12…H1»H12

Figure 24: Dialog to select next pipette tip to use

Starting cassette well: For the starting cassette well, the software will normally begin with cassette well 1 (top left position). However, you can input a different starting cassette well if necessary. This can be done by changing the value in the **Starting Cassette Well** control. Note that the well order snakes rather than running only from left to right.

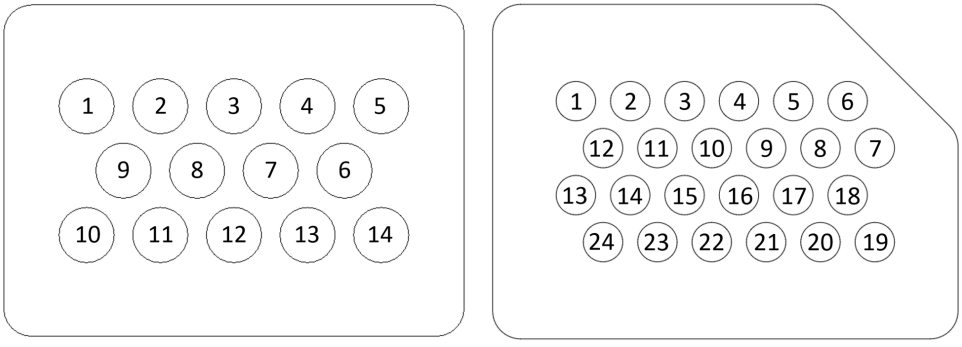


Figure 25: 14 and 24 well cassette layout as seen from above the Mass Photometer stage

Align cassette

Before any run, the cassette must be aligned. This is done after oil has been deposited onto the objective and an assembled clean slide and cassette have been placed onto the stage and secured with magnets. The alignment process will use two wells to map the locations of the wells on the cassette. The easiest way to do this is to click **Align cassette** in the bottom right hand corner. This will automatically run the alignment process.

When assembling the cassette onto a clean slide, make sure to use the aluminium alignment tool provided by Refeyn to ensure the Align Cassette routine works reliably.

In the event that automatic alignment fails, a manual alignment option is available in **Settings > Cassette Settings…** and instructions on how to use this are located in the **Further Information** section of this document.

Run protocol

Once the cassette has been aligned and the well plate, tiprack and waste tip collection box are present you are ready to run the protocol. To do this, click **Run** in the bottom right corner of the tab. This will open a dialog prompting you to select a folder for the data to be saved to.

If there are any errors or warnings, these will be flagged in a pop-up as shown below.

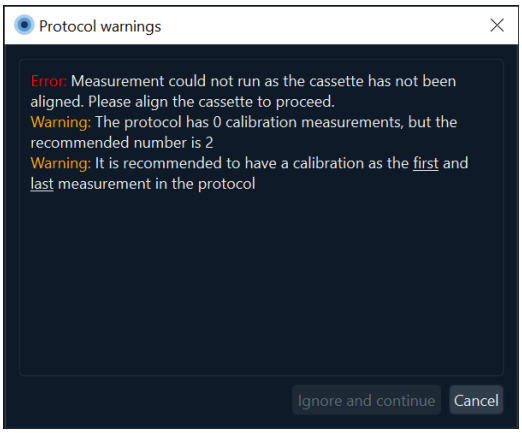


Figure 26: Error and warning example

Finally, a window will appear asking you to confirm that the well plate layout is correct. Once this is confirmed the protocol will begin to run.

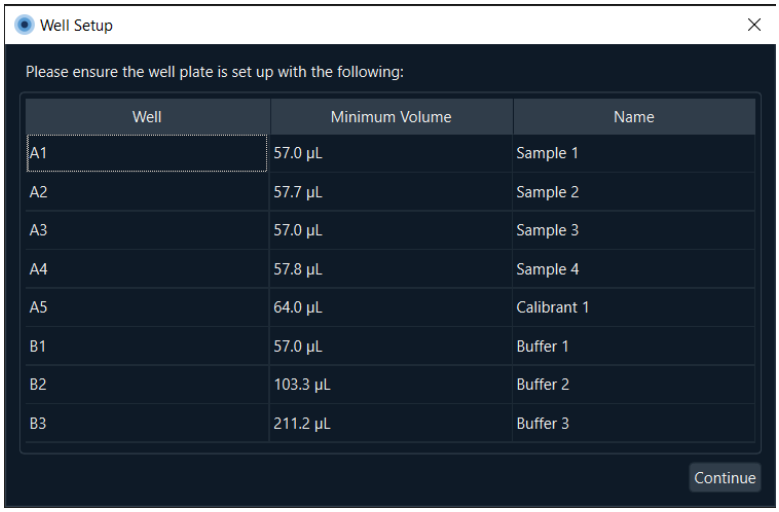


Figure 27: Dialogue confirming the well plate layout

Monitor progress

Once the run has begun, the tab will show the **Progress monitor**, which is made up of 4 sections:

- **Acquisition camera image** - Displays the live ratiometric or native image.
- **Analysis preview** - Gives an indication of the measurement results. This is not the same as the analysed data which provides more detailed information and can be viewed in Discover^{MP}.
- **Progress details** - This provides details on the run progress, highlighting any errors or warnings that may have occurred.
- **Progress bar** - Displays an estimate of progress through the protocol run.

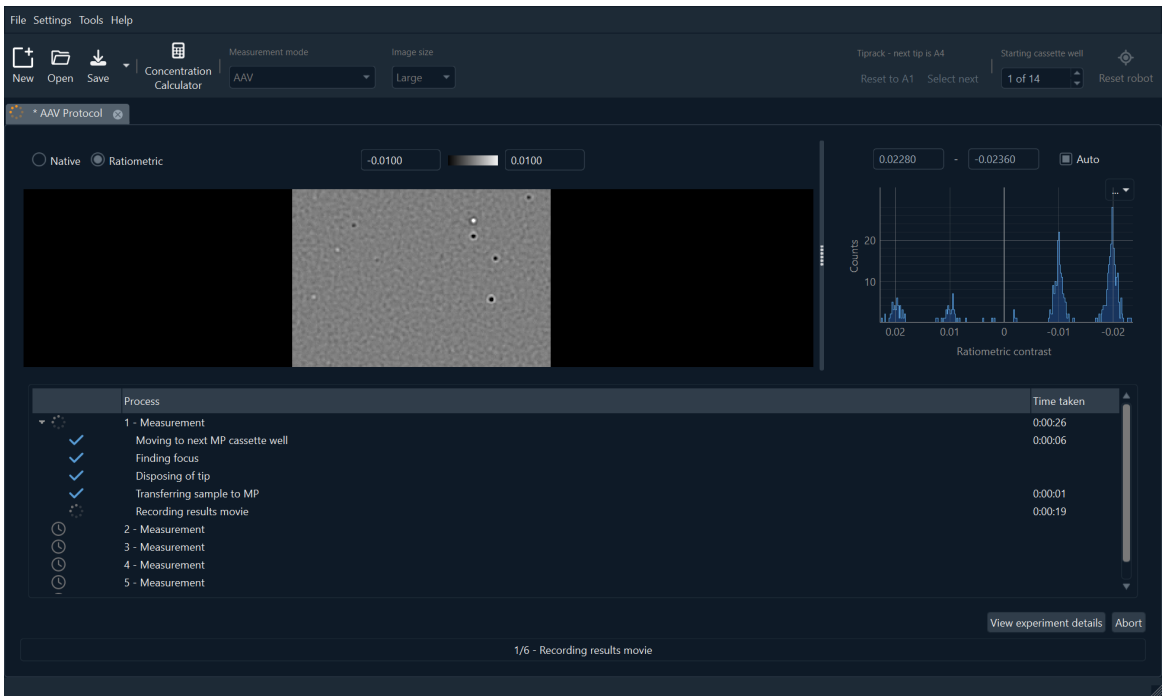


Figure 28: Progress monitor layout

In addition, you can **Abort** the protocol run and **View experiment details** in a pop-up window using the buttons in the bottom right. You can also start creating your next experiment by selecting or opening a new tab. When the protocol completes there is an audible indication.

If the experiment has been halted due to an unexpected error, the robot arm may become stationary in its last operating position. Use the **Reset Robot** button to position the arm of the robot in the resting position.

Analysis

If you chose to analyse whilst the protocol was running then your analysed data can now be visualised in Discover^{MP}. If your data still needs to be analysed, then this must be done in Discover^{MP}.

To return to the experiment design, click **Back to experiment** in the bottom right.

Karitro^{MP}

Workflow

Workflow navigation Just below the menu bar, the workflow navigation panel allows the user to navigate through the different stages of preparation and execution of a Karitro^{MP} measurement.

Figure 29: Acquire^{MP} workflow navigation for Karitro^{MP}

The step-by-step sequence starts with a guide for the set-up of the instrument, followed by screens for the creation and customisation of experiments, and monitoring the execution of these experiments. Each screen is described in more detail in the following sections.

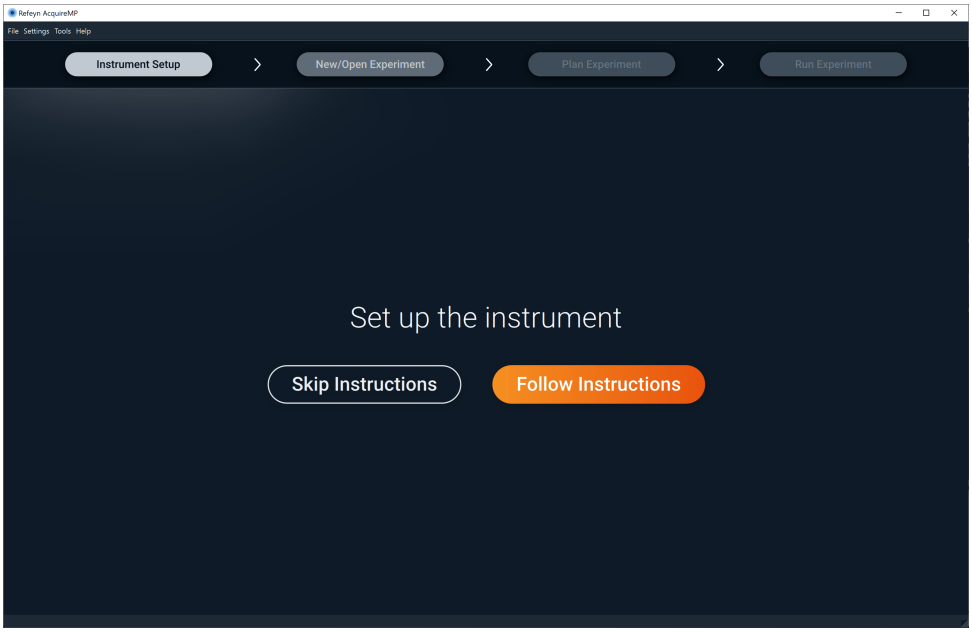


Figure 30: Set Up Instrument screen for Karitro^{MP}

Set Up Instrument On the "Set Up Instrument" screen you are reminded of what needs to be checked or done before any operation of the instrument. While experienced users may skip this screen, inexperienced users may flick through the illustrated steps. The steps include instructions for how to assess cleanliness of the objective, how to ensure that the instrument has warmed up sufficiently, and how to correctly assemble the sample carrier and mount it on the sample stage. Please see the Karitro^{MP} User Manual for further details on those steps.

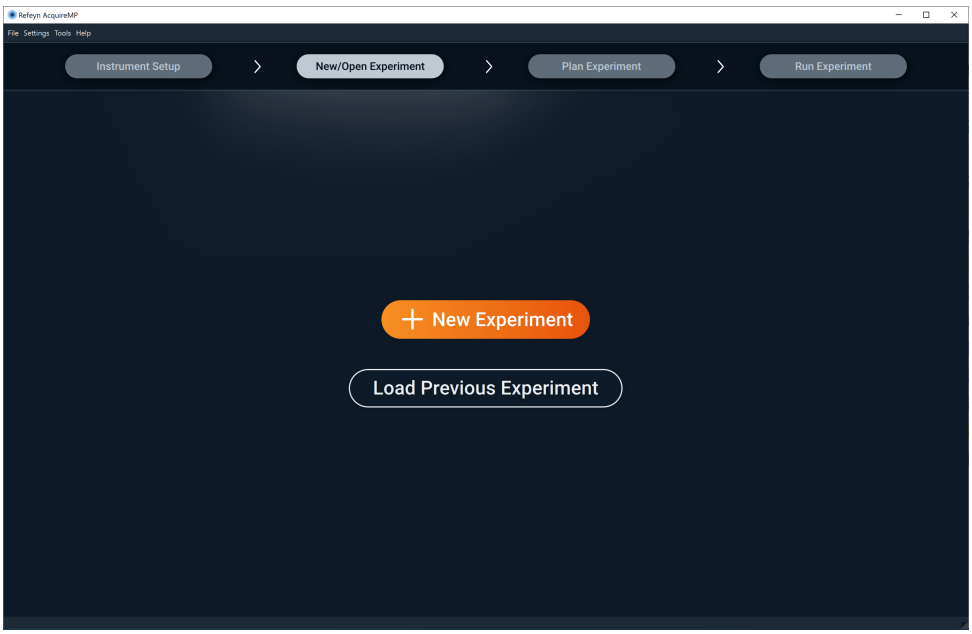


Figure 31: New/Open Experiment screen for Karitro^{MP}

New/Open Experiment Acquire^{MP} uses the term Experiment for the automated measurement and analysis of one Karitro^{MP} well cassette. In the "New/Open Experiment" screen by clicking "New Experiment" you may initialise a new experiment and enter its name and specify the Experiment folder where all related files will be saved. By clicking "Load Previous Experiment" you may instead choose to start from an already existing Experiment plan that you stored previously.

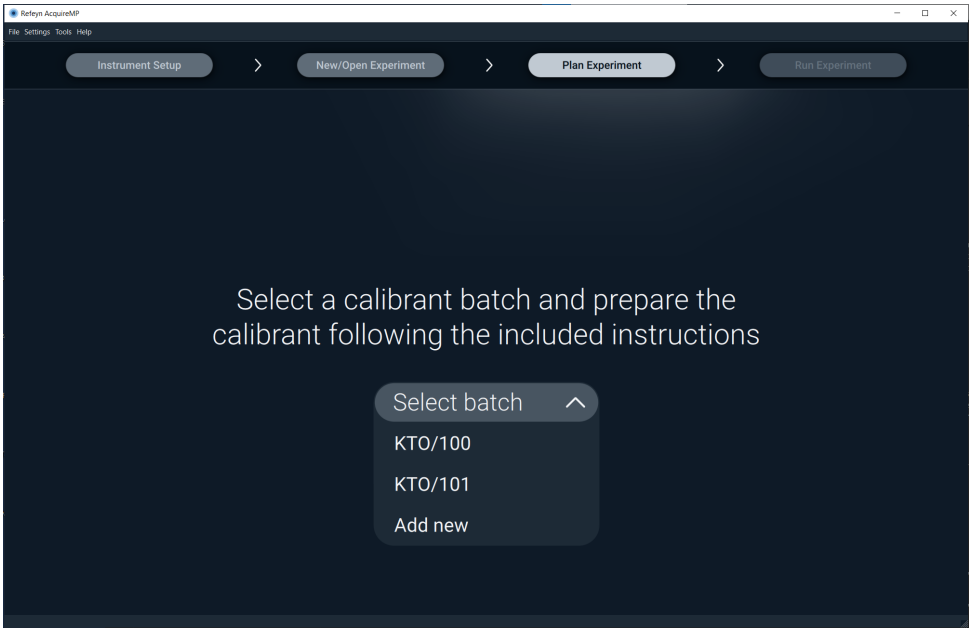


Figure 32: Calibrant batch selection screen for Karitro^{MP}

Plan Experiment After initialising an Experiment, if you have not previously set a calibrant batch as default, you will be prompted to select the calibrant batch you will be using. Acquire^{MP} currently includes the definitions for two SizeFerence calibrant batches, KTO/100 and KTO/101. Definitions for future batches may be imported by selecting the "Add new" option at which point you will be prompted to select a batch file.

After selecting a calibrant batch, you will be prompted to confirm your choice and you will be asked whether you want to save the selected calibrant as the default. When a calibrant has been saved as the default this will remain set for three months. During this time the batch selection screen will be skipped after initialising an Experiment. You may choose to change the selected calibrant batch by using the "Change" button in the calibrant well row on the "Plan Experiment" screen.

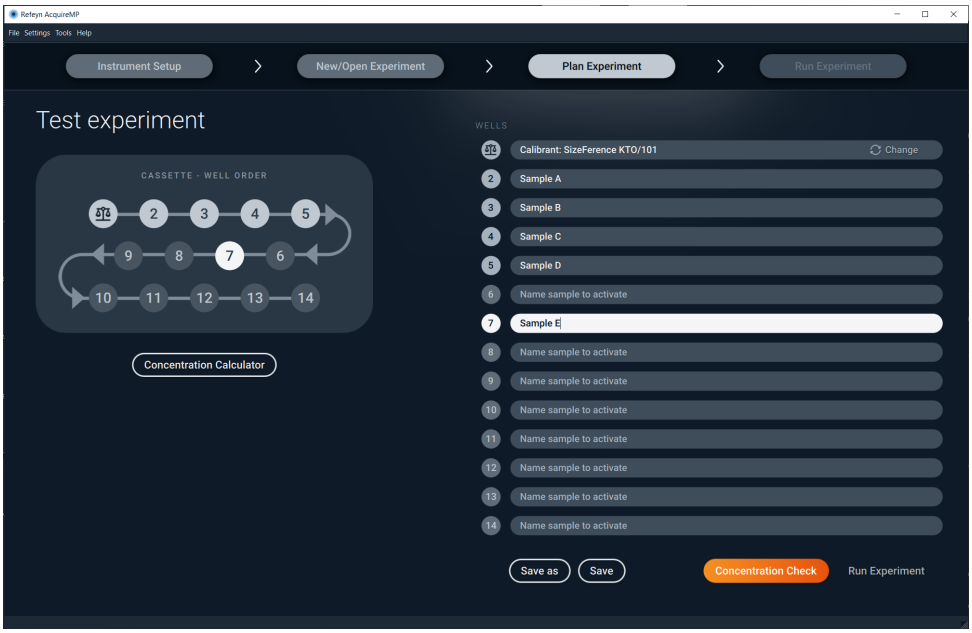


Figure 33: Plan Experiment screen for Karitro^{MP}

At this point the "Plan Experiment" screen is shown where you define and name the cassette wells that contain samples. On the left of the screen a chart of the well cassette is displayed. As illustrated by arrows, the order in which the wells are measured form the letter "Z" on the cassette. Note that in every Experiment the first well of the cassette contains the standard Refeyn SizeFerence calibrant at the recommended concentration.

The plan of the Experiment can be saved to a .kex file to be reloaded at a later time in the "New/Open Experiment" screen. Saving to the Experiment folder can be triggered by clicking "Save" and is automatically done when the execution of the Experiment is started. The .kex file can also be saved to a location of your choice by clicking "Save As".

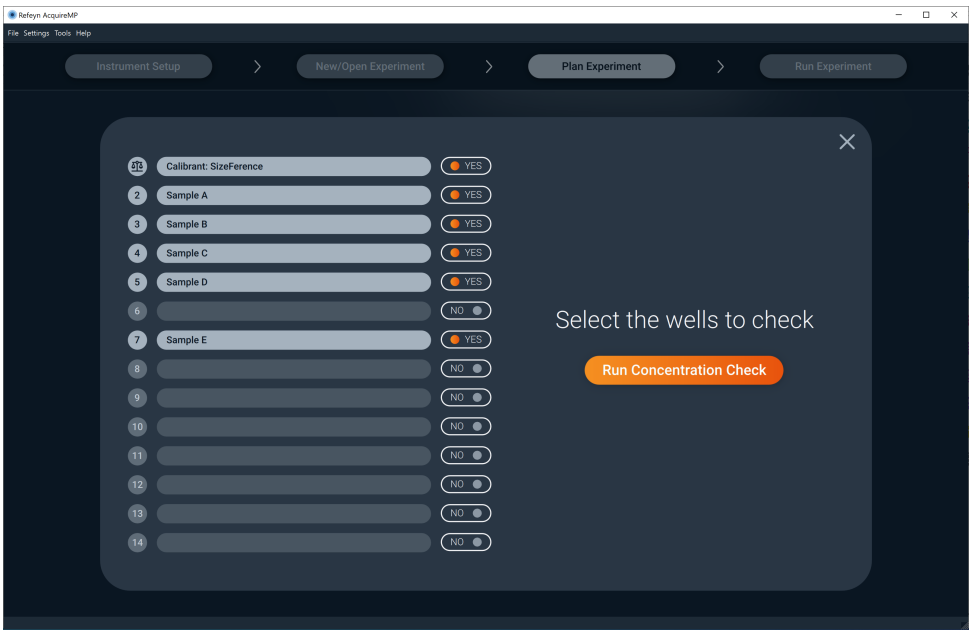


Figure 34: Screen to select the wells for running a concentration check on a Karitro^{MP}

If you are unsure about whether or not the particle concentration on the glass surface is within the suitable range for a measurement you may press "Concentration Check", select the relevant sample wells, and launch the test routine. When finished it will show images that you can inspect to estimate whether the concentration of your sample is adequate or need to be adjusted. For guidance on how to judge whether the particle concentration is suitable for a measurement refer to the examples in Figure 35 and Figure 36 as well as the respective section in the Karitro^{MP} User Manual.

- Too low concentration of particles leads to poor statistics of the measured distribution. This means suboptimal conditions to measure, locate, and detect small populations of particles in the size/contrast distribution and low definition of the shape of the distribution.
- Too high concentration of particles may lead lower than optimal size and contrast resolution and to decreased detectability of (especially low-contrast) particles.

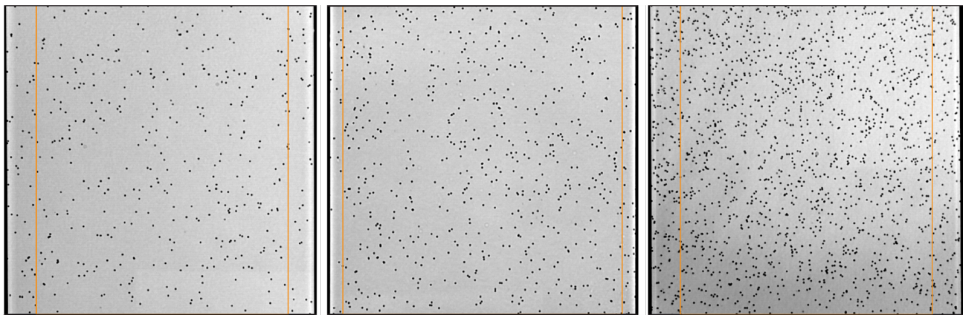


Figure 35: Examples for recommended particle concentrations on the glass surface for Karitro^{MP} measurements

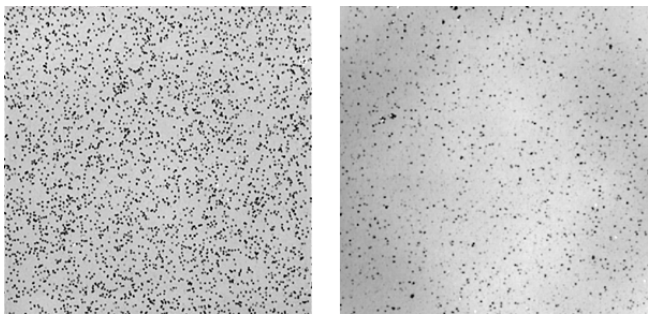


Figure 36: Examples for too high particle concentrations on the glass surface for Karitro^{MP} measurements

When all is ready press the “Run Experiment” button to start the automated measurement sequence.

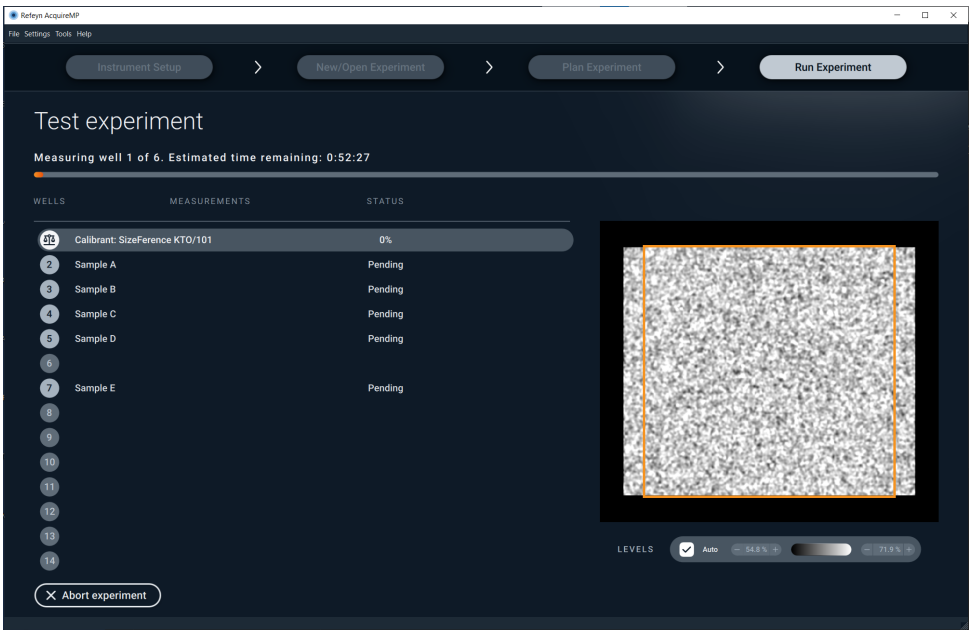


Figure 37: Plan Experiment screen for Karitro^{MP}

Run Experiment After an Experiment has started its progress can be monitored in the “Run Experiment” screen. The Experiment goes through the following sequence of procedures:

1. Cassette alignment: The software detects the center position of the first well. Using the known geometry of the well cassette the software is now able to move any well to the location above the objective where it can be measured.
2. Calibration: The calibration measurement is carried out from the first well. The calibration is needed to convert raw particle data to particle size in nanometers and standardised particle contrast. Note that the calibration measurement requires twice as much data and therefore takes more time than measurements from regular sample wells.
3. Finally, after the calibration measurement is completed, measurements from the sample wells are recorded as specified in the Experiment plan.

Every well measurement starts with finding focus and then continues with recording focus sweeps at 15 (for the calibration well 30) different locations within the respective well. The current status of each measurement is shown in the Run Experiment table and errors are displayed if any occur.

If the calibration measurement fails, the Experiment needs to be repeated with a new cassette. Calibration measurements fail very rarely and are usually the consequence of damage or debris on the glass surface or incorrect concentration of the calibrant.

The Experiment of an entire cassette (i.e. calibrant & 13 samples) takes about 90 min. The estimated remaining time of the Experiment is displayed in the progress bar. As the Experiment procedure is fully automated the user may step away from the instrument and return to it after completion.

All data are analysed concurrently with the acquisition of the data. Data are stored in the Experiment folder that you specified to both a large .mpkr file, which includes the results (i.e. particle sizes and contrasts) and the raw movie data, and a small .mpka file, which only contains the results without the movie data. Discover^{MP}K can open both .mpkr and .mpka files but replay of movies is only possible for measurements loaded from .mpkr files. While it is more convenient to move and work with the small .mpka files. Refeyn recommends to keep a copy of your .mpkr files for your future reference and for any potential future reevaluation of your results.

MassFluidix

Acquire^{MP} includes a semi-automated workflow for the Refeyn MassFluidix HC system (only available for Two^{MP} instruments in manual mode). This section contains information about the software workflow steps. For more information about the MassFluidix hardware setup and physical troubleshooting as well as instructions for the use of the software *OxyGEN*, please refer to the MassFluidix User Manual.

Accessing the MassFluidix workflow

To access the MassFluidix workflow, go to **Tools** > **MassFluidix**. This brings up a dialog dedicated to operating MassFluidix measurements and cleaning routines. While the main Acquire^{MP} window is still visible in the background, it cannot be accessed until the MassFluidix window is closed.

Note that the Acquire^{MP} MassFluidix dialog should not be run at the same time as the software *OxyGEN*.

Navigating the workflow stages

The MassFluidix dialog presents the MassFluidix workflow as a sequence of stages (instructions, controls, displays, and menus). The stages, in the order they are typically presented, are as follows:

- Initialisation
- Hardware check
- Beginning of day clean
- Routine selection
  - **Measurement**
  - Inter-measurement clean
  - Full system clean
  - Buffer flush

with the **Measurement** stage itself consisting of

- Setup
  - Select sample holder
  - Priming the sample line
  - Measurement setup
- Alignment
- Start flow
- Buffer measurement
- Sample measurement
  - Start sample flow
  - Sample detection
  - Sample dilution adjustment
  - Sample measurement recording
- Cleaning

In general, navigating between these stages is done by using the buttons at the bottom of the MassFluidix workflow window. Proceeding to the next stage is usually done via the **Continue** button; going back to the previous stage if possible is usually done via the **Back** button, while to stop the workflow, a button titled **Abort** must be used. Stopping the workflow also stops any of the ongoing fluid flow.

Acquire^{MP} is equipped with a safety feature that stops the workflow and the fluid flow if the input flow rate does not match that of the output. This response is not instant, so if a leak is detected by the user, using the **Abort** button to stop the flow is a safer option.

The following sections explain the stages in detail.

Initialisation

Upon opening the MassFluidix workflow dialog the **Initialisation** stage is presented. Here a basic device check is performed. If either the MassFluidix device is not connected to the PC, or the compressor is not powered on and set to position P1, it will not be possible to continue with the workflow.

If you run into a different problem that prevents you from accessing the next stages, there might be a problem with your MassFluidix device.

Hardware Check

Following the Initialisation stage, a sequence of steps with illustrations that instruct the user to check specific components of the hardware setup is presented. The images can be expanded by clicking the button at their top right. For convenience, these steps can be skipped using the **Skip** button.

Note that this step is *not* intended to instruct users how to set up the system. For that, please refer to the **MassFluidix HC User Manual**.

Beginning of Day Clean

Following the Hardware Check, the user can choose to do a Beginning of Day Clean. Similar to the Inter-measurement Clean, it will clean the sample line from the M-switch to the chip and prime the sample line with the intended buffer from line 1. It uses ports 1 (buffer), 2 (NaOH) and 3 (water) as well as the buffer line.

Both the sample and buffer lines should be placed inside their respective waste containers.

WARNING: the system should be monitored by the user during the entire process.

Routine Selection

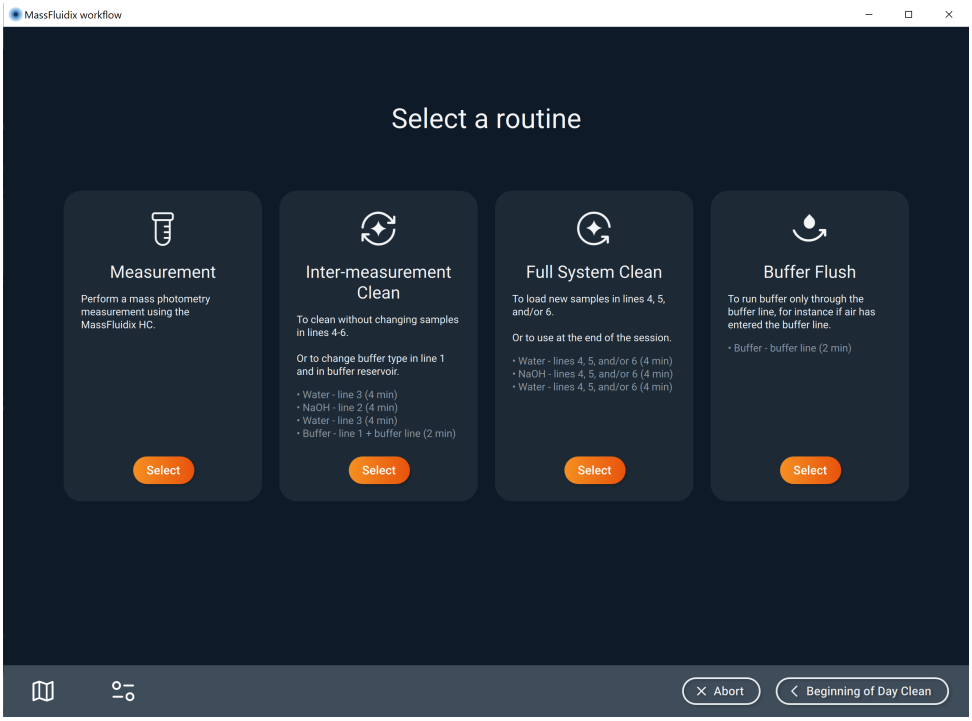


Figure 38: MassFluidix Routine Selection menu

After the Beginning of Day Clean, a selection of MassFluidix routines is available:

- Measurement
- Inter-measurement Clean
- Full System Clean
- Buffer Flush

The **Measurement** routine takes the user through the recommended MassFluidix measurement workflow, starting with the **Setup**. The other options related to system cleaning are described in the Inter-measurement Clean, Full System Clean, and Buffer Flush sections.

Setup

In the **Setup** stage, the user must specify which sample holder will be used for the measurement, then prime the sample line with the intended buffer from the selected sample holder. They will then select which chip channel to use, what the sample dilution should be, as well as measurement movie length, measurement mode, and image size.

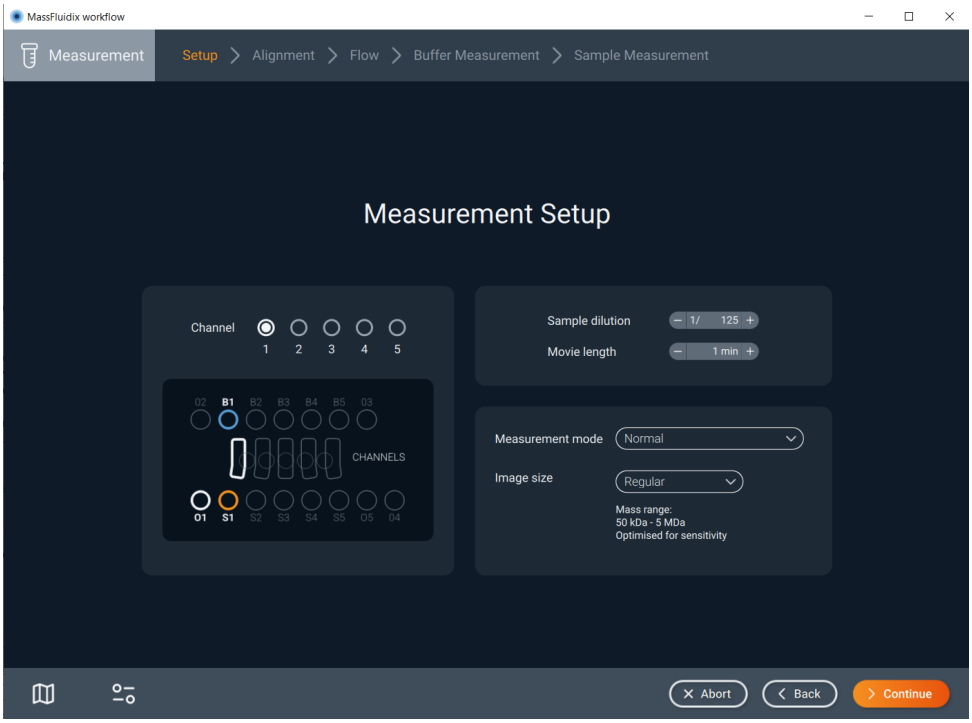


Figure 39: Sample Setup stage

After entering the relevant information, continuing brings the user to instructions on setting up the microfluidic chip. Once the user has placed the chip on the photometer, secured it with clamps and pressed **Continue**, the photometer stages will automatically move to the saved first alignment circle position. It will then bring the user to the **Alignment** stage.

Alignment

In the **Alignment** stage, the user is asked to verify that the laser is in the alignment circle; if not, they must move it themselves with the alignment tools. Then the lid must be closed and the automatic channel alignment can be started. If this is not successful the user can retry or abort, otherwise they are brought to the **Start Flow** stage.

Start Flow

At this stage, it is important to open the lid and ensure that the tubes are properly connected to the chip before pressing **Start Buffer Flow**. When the flow starts, the user should monitor it and ensure that there are no leaks. This should be done visually by observing the chip, and it is also recommended to use the Graphs.

If a leak is detected, the **Stop flows** button should be pressed. This gives an opportunity to fix the setup and try this step again. If the buffer flowrate isn't achieved automatically, the buffer flow is paused and the step can be retried by pressing the **Retry** button.

Once the buffer flowrate has been reached, the user is asked to check for leaks, then close the lid before continuing to the **Buffer Measurement** stage.

Buffer Measurement

In the **Buffer Measurement** stage, the user can record a movie of the buffer as a control by clicking the button **Find focus and record**. Focus will be automatically found before the recording, unless it has been found previously. The user can also move the photometer stages with the lateral stage controls to find a suitable spot for a recording if the glass shows imperfections or scratches (while being cautious to not leave the measurement channel).

After a movie is recorded, the **Save Movie** dialog will pop up. Here the user can fill in information about the sample and buffer, and choose where to save the movie.

Once the user has finished recording buffer movies they can proceed with the sample measurement. If the system is not in focus, the software will first find focus before proceeding.

WARNING: Proceeding to the **Sample Measurement** stage starts the sample flow. This means it will not be possible to come back to previous steps, and a cleaning will be required afterwards.

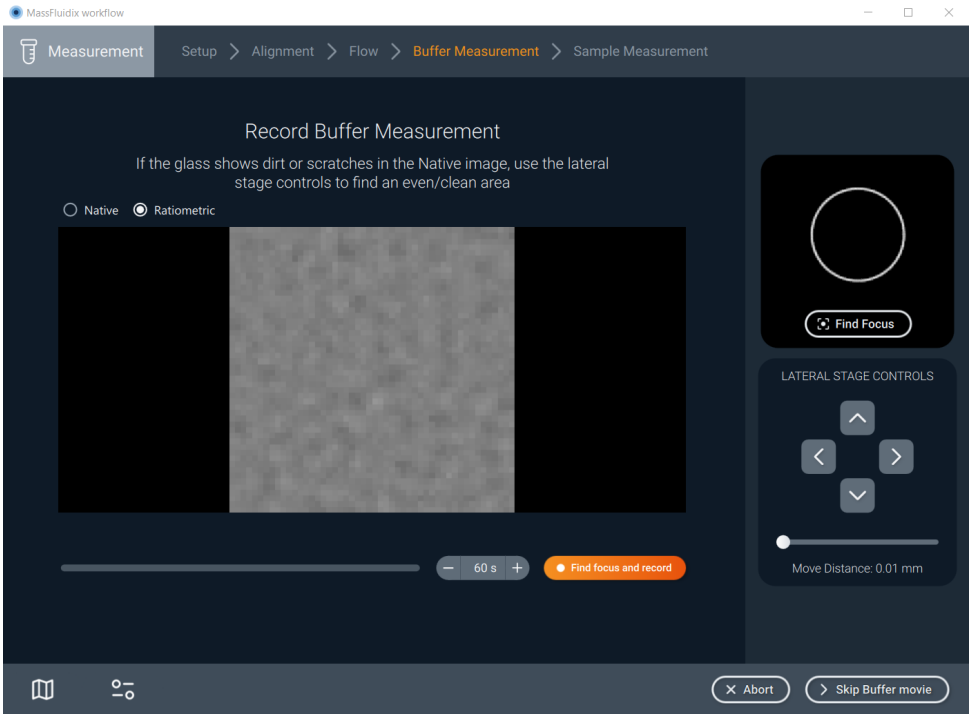


Figure 40: Buffer Measurement page

Sample Measurement

As soon as this stage is entered, the system starts the sample flow from the sample position (4, 5, or 6) that was selected in the **Sample Setup** step. The user is prompted to observe the live ratiometric video and verify when the sample has reached the observation window (the moment when a significant number of binding events can be observed) by clicking the **Sample detected** button. Depending on the length of the tubes, this might take 2-3 minutes.

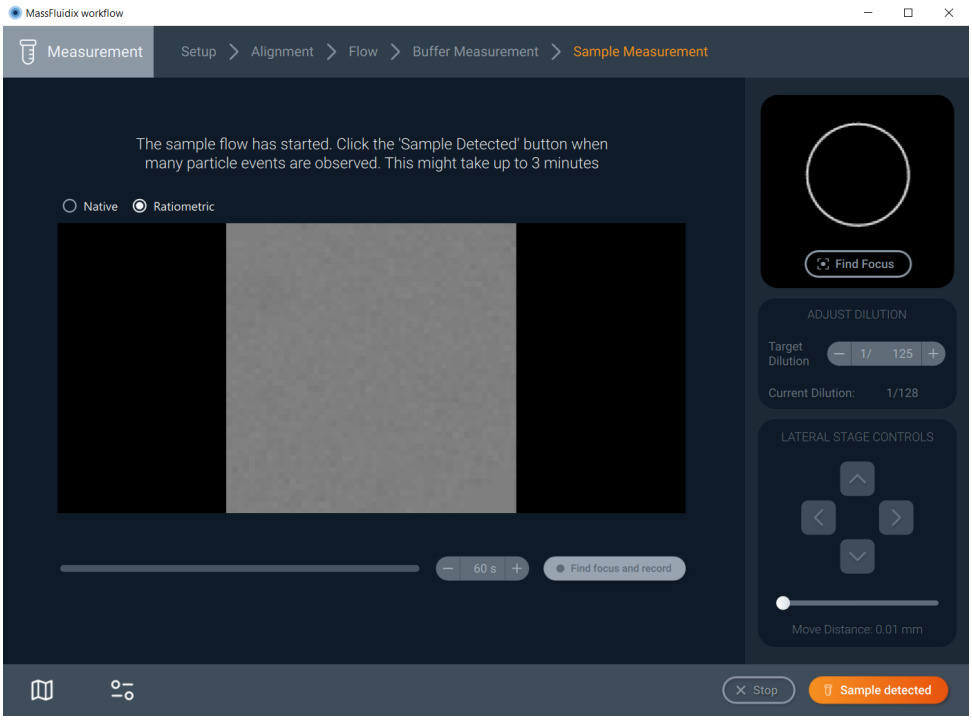


Figure 41: Waiting for sample to reach the chip

Once the user has confirmed that the sample has been detected, the system attempts to reach the sample dilution chosen in the **Setup** stage, and then opens the **Sample Measurement** page. Similar to the **Buffer Measurement** page, it gives controls for the user to perform a measurement as well as an additional tool for adjusting the dilution if too many or too few counts are observed at the initial dilution. Note that, if the user does not confirm sample detection after 4 minutes, the dialog will go to the **Sample Measurement** page automatically, but a warning will appear. If no events appear after 4 minutes the workflow should be aborted to troubleshoot the system.

Once the user has finished recording movies, they can proceed with the cleaning by clicking the **Cleaning** button after a recording, which will present the **Cleaning Menu**. Note that aborting at this point will also present the **Cleaning Menu**.

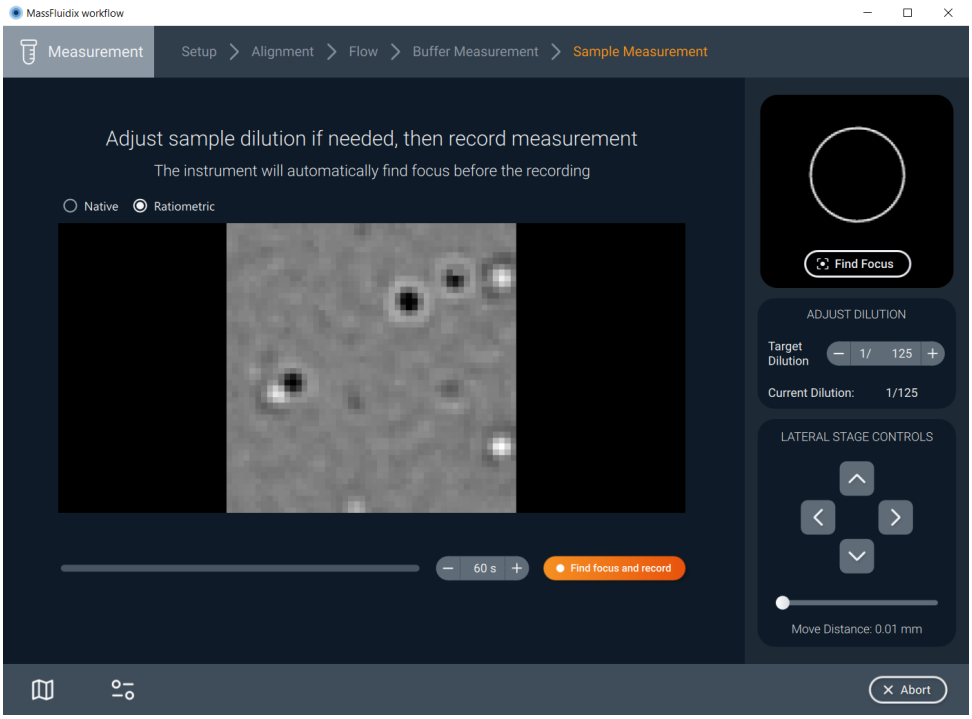


Figure 42: Sample Measurement page

Cleaning Menu

After sample has started flowing, it is necessary to clean the line at the end of a measurement to avoid contaminating future measurements. In the **Cleaning Menu** it is possible to choose between Inter-measurement Clean and Full System Clean.

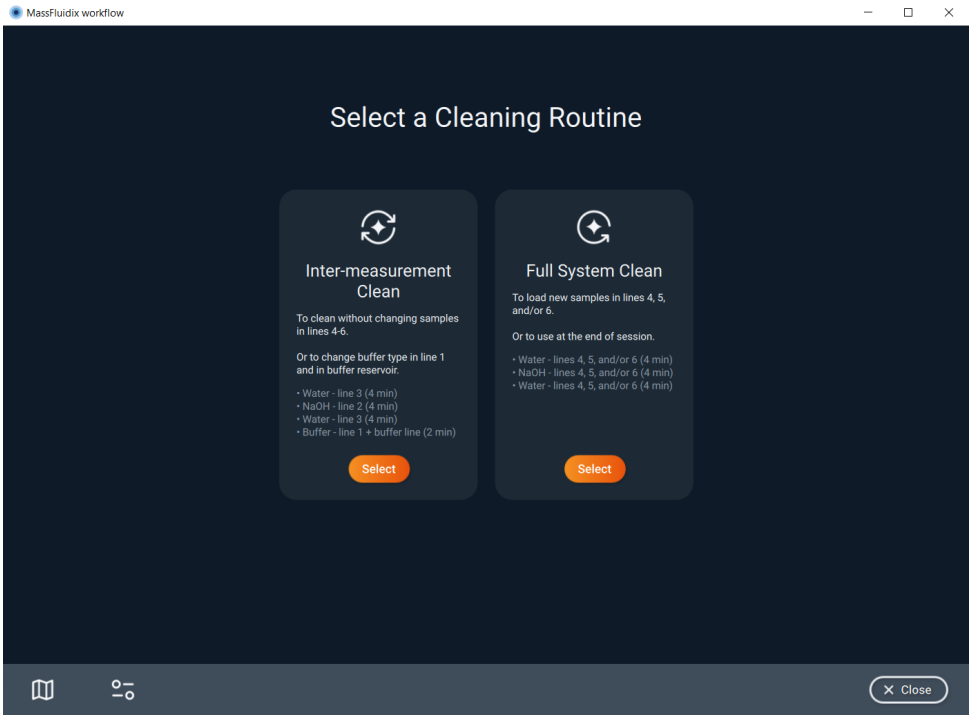


Figure 43: Cleaning Menu

Full System Clean

The **Full System Clean** routine is designed to clean the entirety of the sample line, from the sample port (4, 5 or 6) to the end of the sample tubing. This routine ought to be run when one or more sample channels are about to be used with a different sample. It is also recommended to run it after the last measurement of the day as well as before using a channel that hasn't been used in a while.

The user can select which sample channels (4, 5, and 6) they wish to clean, and the system then performs the cleaning procedure through the chosen channels, prompting the user to swap out 1.5 mL tubes with the necessary contents (water and NaOH) when required.

WARNING: the system should be monitored by the user during the entire process.

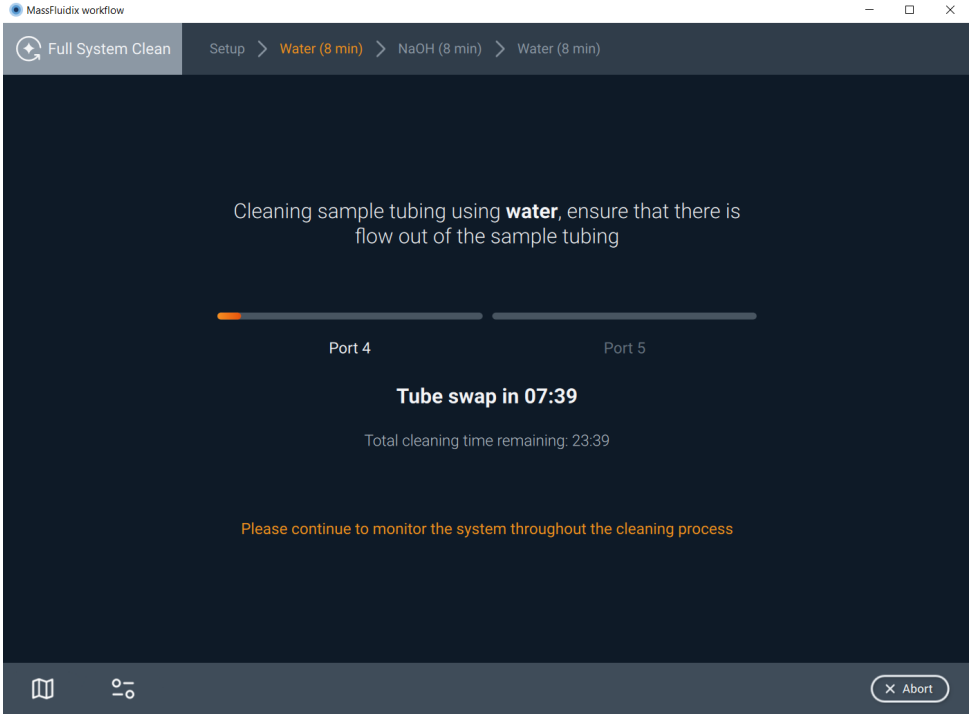


Figure 44: MassFluidix workflow in the middle of the System Clean routine

Inter-measurement Clean

The **Inter-measurement Clean** routine cleans the sample line only from the M-switch to the chip. It can be performed between measurements instead of the **Full System Clean** if the samples in ports 4, 5 and 6 are not being changed. It will ensure that the used sample remaining in this part of the tubing doesn't contaminate the next measurement. This routine uses ports 1, 2, and 3 to run buffer, NaOH, and water, respectively, as well as the buffer line.

WARNING: the system should be monitored by the user during the entire process.

Buffer Flush

The **Buffer Flush** routine should be performed when swapping to a different type of buffer or when the buffer in the buffer channel has become stale. It flushes the buffer channel with the solution from the buffer reservoir.

WARNING: the system should be monitored by the user during the entire process.

Graphs

On the left of the bottom bar there are two buttons that are used to display the system diagram and the flow and pressure graphs. They are not required to run a measurement or cleaning routine, but they can help monitor the system to detect leaks or issues. Please refer to the **MassFluidix User Manual** for more information on how to detect them.

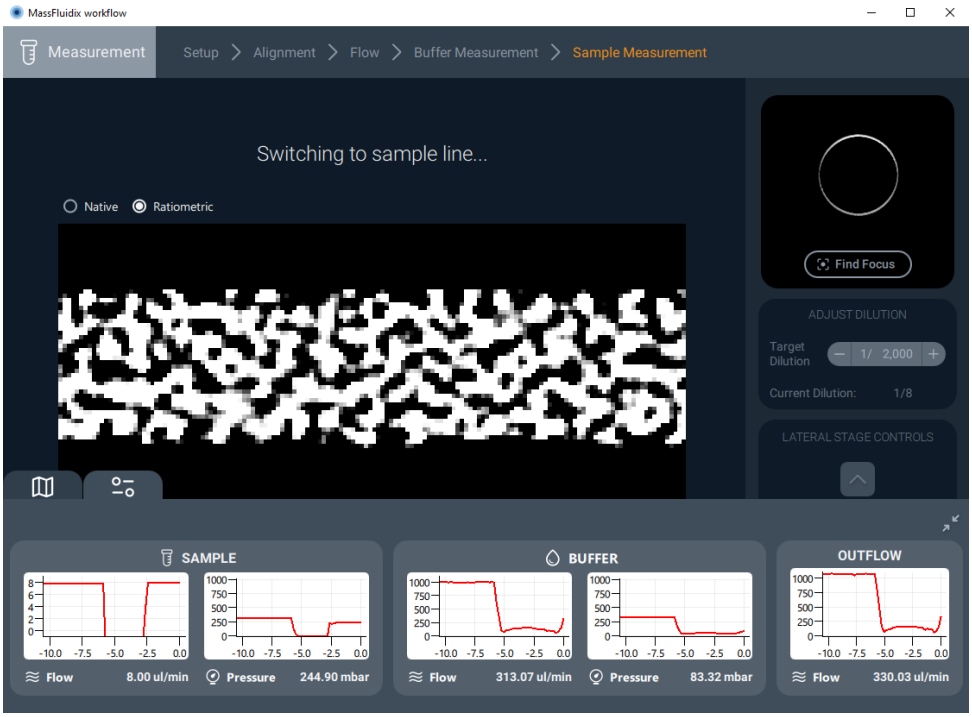


Figure 45: Pressure and sample graphs open

Tips & Tools

Concentration calculator

The concentration calculator allows users to calculate the volumes of reagent and solvent needed to dilute a solution to a particular concentration. It also allows users to convert between mass concentration and molar concentration given a molecular weight. The concentration calculator can be opened from the **Tools** menu.

To calculate the volumes of reagent and solvent needed for a dilution, enter the current and target concentration of the solution into the corresponding fields in the dialog. The dialog will display the dilution factor and, once a target volume has been entered, the required reagent and solvent volumes. If the calculation requires a molecular weight, due to conversions between mass and molar concentrations, a warning message will appear prompting the user to complete the molecular weight field.

To convert between mass and molar concentrations, enter the concentration into the current concentration field, adjusting the units as required. Then enter the molecular weight, and the converted concentration will be shown below the current concentration field, in the units specified by the dropdown to the right.

The dialog will show a warning if the dilution is too large (indicating that the units may be incorrect), or if the target concentration is too low to be detected by the Mass Photometer.

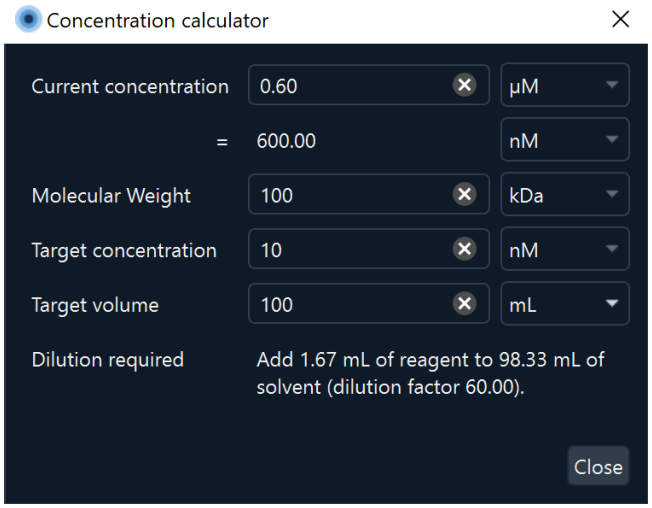


Figure 46: Concentration calculator

Tips

Upon startup of Acquire^{MP}, a dialog will appear with tips on how to perform a normal mass photometry measurement. These tips are intended to guide new users though the measurement process and are not a replacement for proper training. The dialog can be disabled by unchecking **Show tips at startup** in the bottom-left corner. The dialog can be re-enabled by opening the dialog from the **Help** menu, and checking the box again.

The dialog presents each step of the measurement process as a separate page. The user can advance to the next step using the **Next** button, or by selecting a step using the list on the left. The main Acquire^{MP} interface remains active while the dialog is open, so users can perform each step with the tips dialog open. Some steps include links to external resources, such as video tutorials.

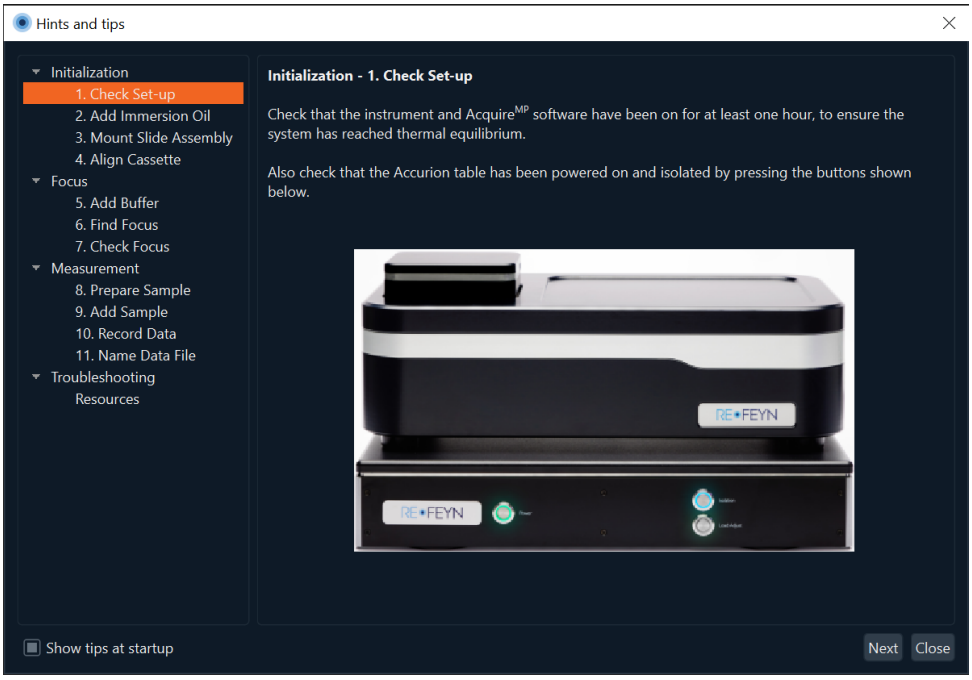


Figure 47: Startup tips dialog

Further Information

Functionality matrix

Depending on the device different functionalities are available in Acquire^{MP}. Key Acquire^{MP} features and their availability/options for each instrument type are listed in the table below.

	One^{MP} & Two^{MP}	One^{MP} Auto & Two^{MP} Auto	Samux^{MP}	Samux^{MP} Auto	Karitro^{MP}
Normal measurement mode	X	X			
AAV measurement mode	X	X			
Antibody Stability measurement mode	X	X			
Samux measurement mode			X	X	
Image Size	Multiple options	Multiple options	Fixed	Fixed	Fixed
Movie Length	Custom	1, 2 or 3min	Custom	1, 2 or 3min	Fixed
Droplet-Dilution Find Focus	X	X	X	X	N/A
Buffer-Free Find Focus	X	X	X	X	N/A
Manual Focus	X		X		
Manual Stage Control	X		X		

	One^{MP} & Two^{MP}	One^{MP} Auto & Two^{MP} Auto	Samux^{MP}	Samux^{MP} Auto	Karitro^{MP}
Automatic Cassette Alignment		X		X	X
Analysis Preview	X	X	X	X	
Analysis During Recording	X	X	X	X	X
Auto Robot Control		X		X	
Advanced Settings	X	X			X
Concentration Calculator	X	X	X	X	X
Hints and Tips	X	X	X	X	

Image Size

Increasing the image size means that more events can be detected in a single frame which results in improved statistics. However, changing to a larger image size lowers the mass sensitivity. This is due to the same amount of light being distributed over a larger area, decreasing the per-pixel intensity and signal-to-noise ratio. The increased noise is reflected by a grainier ratiometric image and a higher signal score when measuring clean buffer. Increasing the image size also increases the file sizes and processing times.

Ultimately, the optimal image size is completely sample dependent, but the limit of detection using the *large* field of view on the One^{MP} and Two^{MP} is 100 kDa and 70 kDa respectively. Ensure that the smallest population in a sample is significantly larger than this before moving to the *large* image size.

Note that the small image size is included for legacy reasons on the Two^{MP} as there is no detection improvement between the *small* and *regular*; hence, we recommend *regular* is used for low mass samples.

Note that the detection area does not exactly match the total area of the acquisition image because a border mask is applied during analysis to exclude peaks partly outside the image.

Instrument type	Image size	Binned pixels	Dimensions of imaged area	Detection area
One^{MP} (Auto)	Regular	128 × 34	10.8 µm × 2.9 µm	18.0 µm^2
One^{MP} (Auto)	Medium	128 × 81	10.8 µm × 6.8 µm	56.4 µm^2
One^{MP} (Auto)	Large	128 × 128	10.8 µm × 10.8 µm	95.0 µm^2
Two^{MP} (Auto)	Small	150 × 38	10.9 µm × 2.7 µm	29.8 µm^2
Two^{MP} (Auto)	Regular	150 × 59	10.9 µm × 4.3 µm	46.3 µm^2
Two^{MP} (Auto)	Large	233 × 166	16.9 µm × 12.0 µm	202.4 µm^2
Samux^{MP} (Auto)	Samux^{MP}	233 × 166	16.9 µm × 12.0 µm	202.4 µm^2
Karitro^{MP}	Large	750 × 550	64.2 µm × 47.1 µm	3018.8 µm^2

Manual Focus

Find focus

The user also has the option to find the focus manually. This feature is only available when using the One^{MP}, Two^{MP} or Samux^{MP}. To perform a manual find focus, a droplet of buffer must be placed in the measurement well as would be done for a Droplet-Dilution Find Focus. The user must then use the coarse focus controls to reach a point where the roughness of the glass surface can be seen in the acquisition image and simultaneously a bright ring is visible in the auto-focus image. Once the ring is apparent, the fine focus controls can be used to bring the sharpness of the acquisition image to an acceptable level.

Figure 48: Mass photometer close to focus; both the auto-focus ring and glass roughness are visible

The data quality scores above the focus control panel displays the values for sharpness, saturation, signal and motion which are considered acceptable when the Mass Photometer is in focus. The sharpness needs to be brought into the acceptable range using the Fine Focus controls. The optimal focus is achieved when the sharpness is greatest. Clicking on the **Sharpness** indicator displays a plot showing its value over time. Figure 49 shows an example of how the sharpness changes as the slide moves in the Z direction; it shows a noticeable maximum sharpness peak.

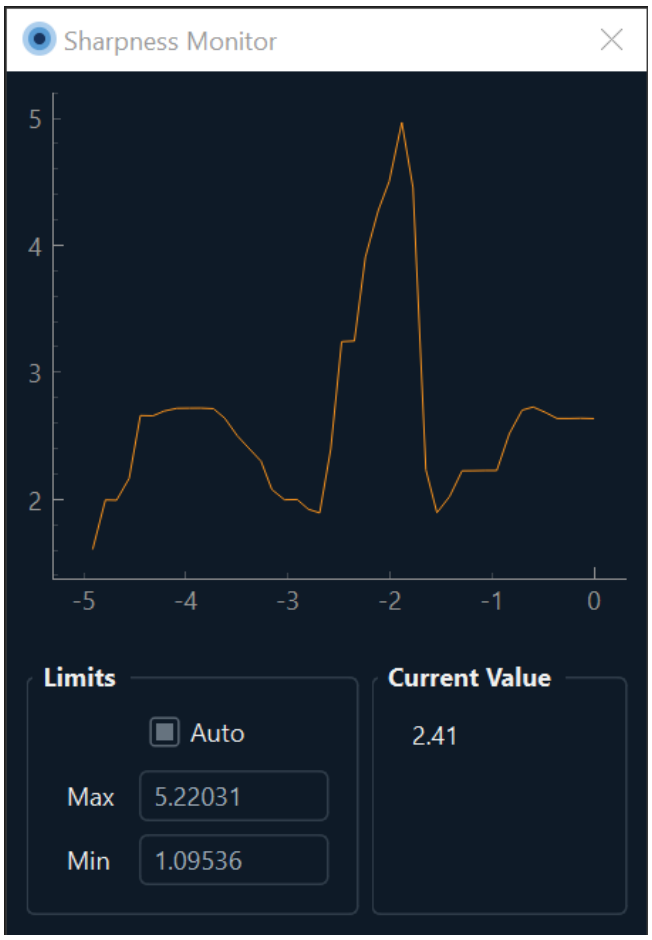


Figure 49: Finding the maximum sharpness value

When the instrument is in optimal focus the acquisition camera image in native mode will clearly display the glass surface (see Figure 50).

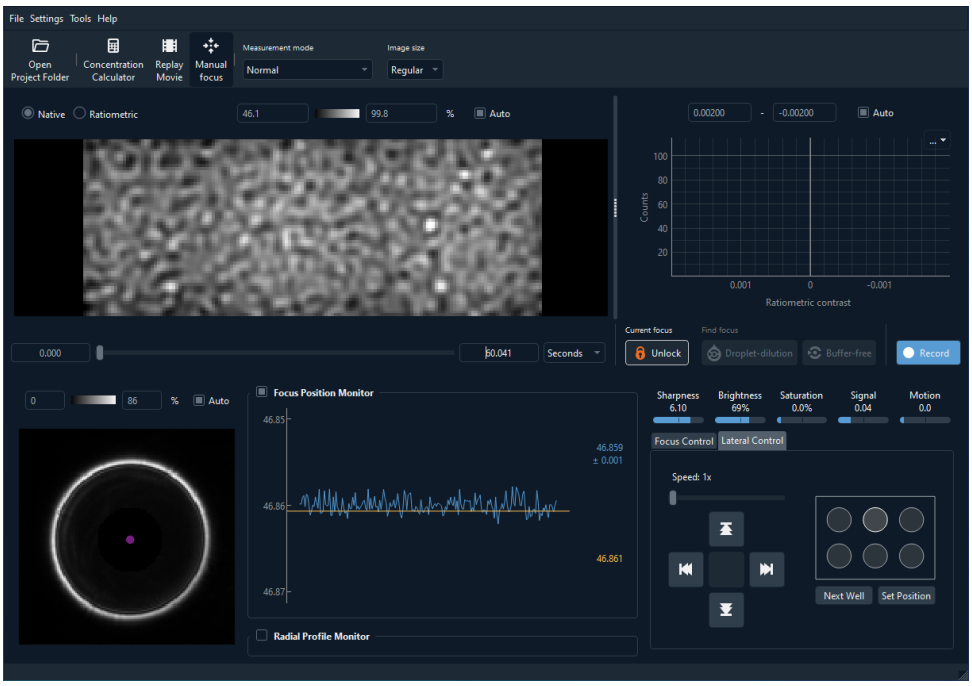


Figure 50: Acquire^{MP} with the mass photometer in optimal focus

Data quality scores can give a good indication if focus has been found:

- If the Brightness value is too low, there is not enough illumination across the Acquisition image and the exposure time should be increased.
- The Saturation can be adjusted by modifying the exposure time of the acquisition image. The ideal is to have the exposure time as high as possible while keeping saturation at 0%. This is optimised using the **Calibrate Acquisition Image** tool which is accessed by clicking **Settings > Acquisition…**.
- The Signal parameter should be as low as possible in order to observe landing events of small proteins. If the signal is too high (slider turns orange), the recommendation is to move to a cleaner area of the well or to replace the slide.
- The Motion value should be minimised by isolating the Mass Photometer from external sources of vibration, making sure that it is mounted horizontally and using the provided magnets to secure the slide on the sample carrier.

In certain cases, drift is observed shortly after the position of the sample carrier is changed. For best results, it is recommended to wait until the position of the sample carrier has reached an equilibrium before recording is commenced.

Stabilise focus

Once the position of the optimal focus has been achieved and equilibrated (equilibration time is about one second), selecting the **Set Focus** button saves the auto-focus ring radius as a reference point for achieving a focused image.

The target ring radius is shown as a orange horizontal line in the **Focus Position Monitor** graph. The measured radius of the auto-focus ring is shown updating in real-time as a blue line.

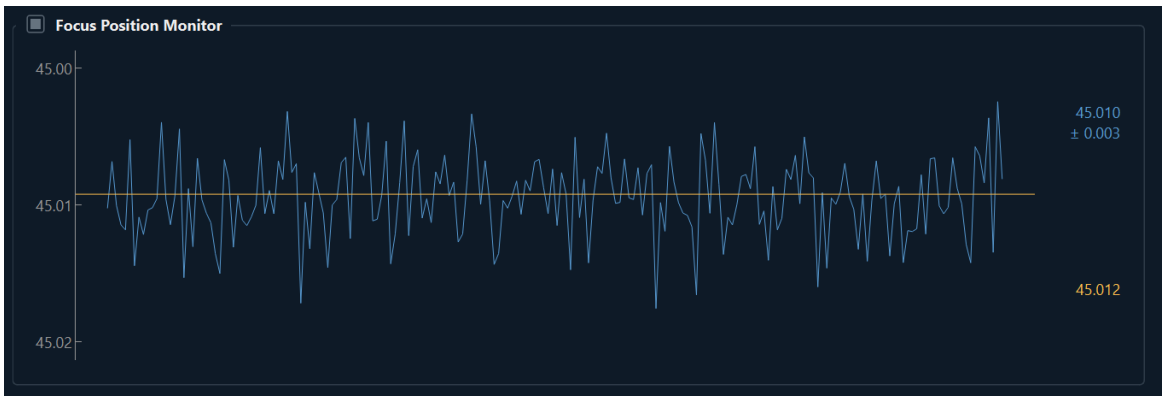


Figure 51: Focus position monitor when a target radius has been set

The current focus **Lock** button becomes enabled once a target radius has been set. On selecting Lock Focus, the Acquire^{MP} application enters Focus Stabilising mode; the sample stage is moved in the focusing plane to maintain a constant auto-focus ring diameter. In this mode, the Mass Photometer dynamically counteracts any drift in the focal position of the sample. The current focus **Lock** button displays a red unlock icon while in Focus Stabilising mode.

While the focus is locked, manual control of the sample stage is disabled and the auto-focus Settings and Calibration may not be updated. It is also not possible to load a previously saved settings file.

To re-enable these functions, exit Focus Stabilising mode by clicking the **Unlock** button.

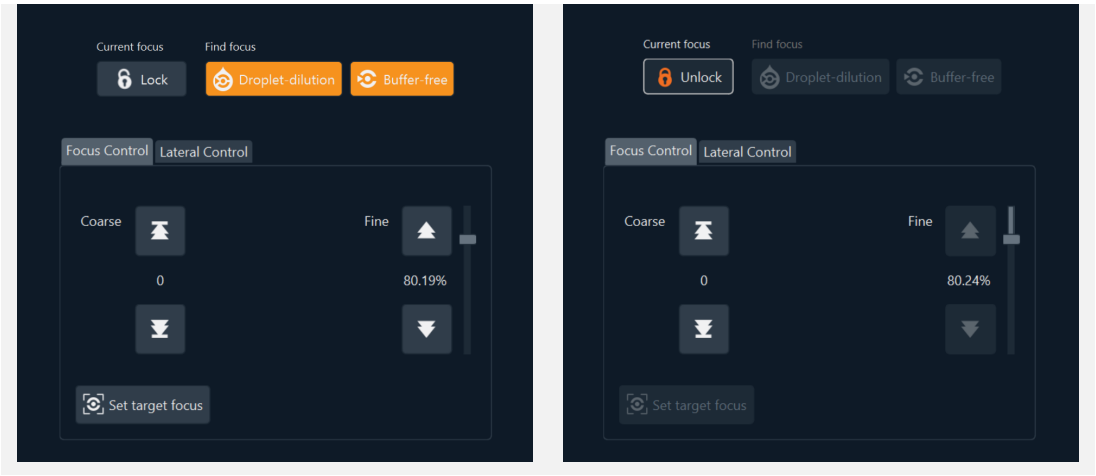


Figure 52: Lock Focus button enabled (left) and locked (right)

Manual Cassette Alignment

To perform a manual cassette alignment on a One^{MP} Auto, Two^{MP} Auto or Samux^{MP} Auto go to **Settings > Cassette Settings…** which will open the cassette settings dialog.

To perform a manual alignment:

1. Click **Go to first calibration well**.
2. Use the arrows to move the stage so that the highlighted well centered on the objective.
3. Click **Validate Position**.
4. Click **Go to second calibration well**.

5. Use the arrows to move the stage so that the highlighted well centered on the objective.
6. Click **Validate Position**.

The calibration is then saved.

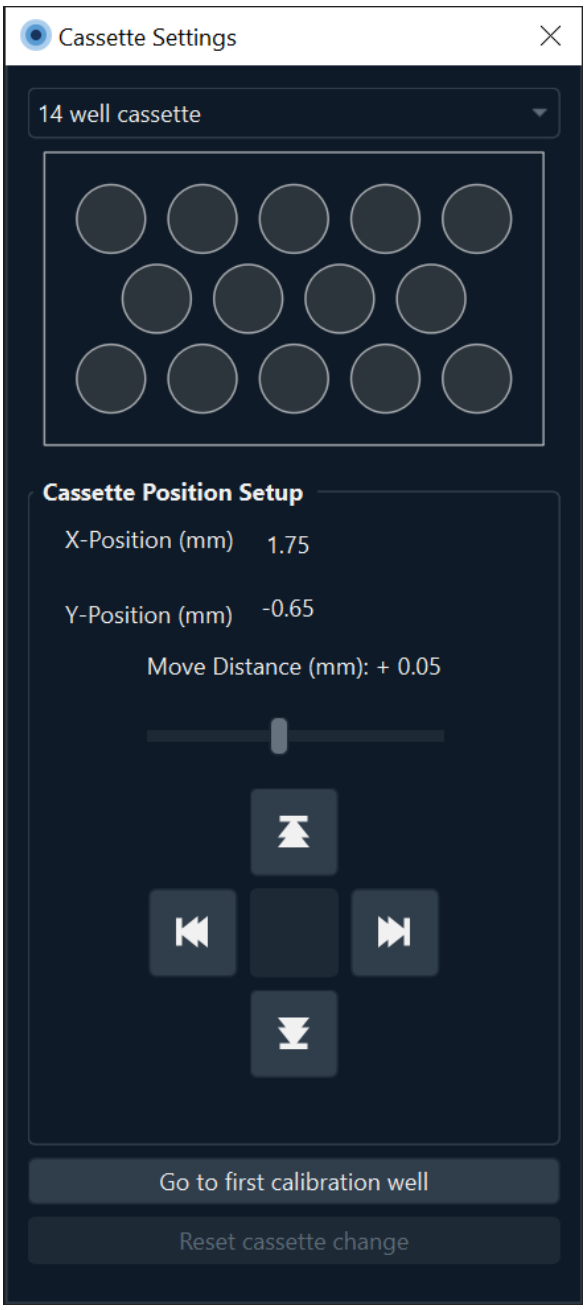


Figure 53: Cassette Settings window with tools for manual cassette alignment and for One^{MP} and Two^{MP} Auto, changing the cassette type

For the Two^{MP} Auto there is the option to change cassette type using the dropdown menu at the top of the window. If the type is changed, a manual alignment is required to save the change and therefore, it is important to set up a slide with the new cassette type on the stage first.

Instrument Settings

Preferences

Acquire^{MP} has a small set of preferences that the user can interact with via **File > Preferences**.

Telemetry mode

In the Preferences dialog, the current telemetry mode can be updated.

- **Feature preview mode** - Allows access to experimental features. Measurement data, and basic data about the user's use of the software will be anonymously reported to Refeyn, including error reports.
- **Normal mode** - Basic data about the user's use of the software will be anonymously reported to Refeyn, including error reports.
- **Offline mode** - Acquire^{MP} will not communicate with the internet, or allow any feature that requires the internet.

Other settings

By default, saved acquisition movies are compressed to reduce memory usage. When loaded in Discover^{MP} the movies are decompressed before being analysed. This compression can be turned on or off by checking or unchecking the **Compress movies** option in the preferences dialog.

By default, Acquire^{MP} performs movie analysis during the recording of the movie and thus produces result (.mpr) files. This feature can be disabled by unticking the **Save analysed movies** option, although note that this will prevent the software from flagging recordings that exhibit signs of suboptimal quality via "High activity". With analysis during recording being disabled, Acquire^{MP} will produce unanalysed movie files (.mp), for which the analysis will have to be done in Discover^{MP}.

With **Show antibody warning** checked, when the user is prompted to select a measurement mode and they select Antibody Stability, a dialog will be shown to explain the Antibody Stability mode and how measurements taken in this mode can work with Refeyn's Antibody Stabilty analysis module - a separate application.

To show advanced settings (not available for Samux^{MP} and Samux^{MP} Auto) the user can press the **Advanced mode** button. This will bring up a message detailing the risks that can occur if the advanced settings are changed. The user will be questioned whether they would like to continue. To hide advanced settings, the user shall press **Leave advanced mode**. This option is not saved across sessions and by default will be turned off each time the software is started. Changes to the advanced settings can severely damage data acquisitions and analysis and should only be used when advised by Refeyn internal teams.

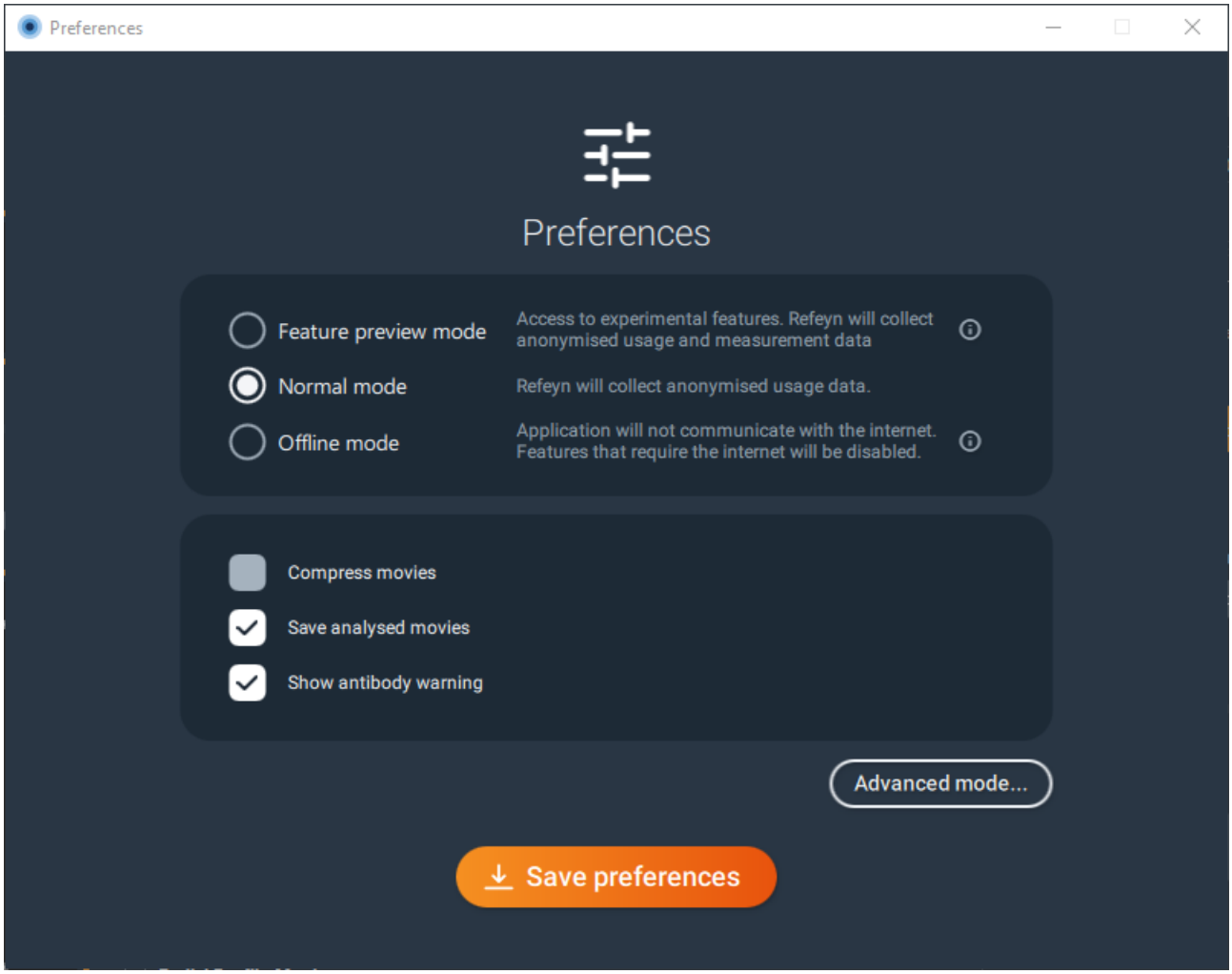


Figure 54: Preferences dialog

Save and load device settings

Acquire^{MP} handles projects and settings separately as this allows movies within the same project to have different instrument settings.

On startup, Acquire^{MP} remembers the most recently saved system settings. If Acquire^{MP} is being run for the first time, it will load the factory settings, or if there are none, the default settings. Pressing **Save Settings** updates the system settings, which will be loaded next time Acquire^{MP} is run. Pressing **Settings** > **Reset Settings** reverts the settings to the default. Acquire^{MP} can also save settings to a file to be reloaded later. This is done by going to **Settings** > **Save Settings As** and then choosing the location to save the current settings. **Settings** > **Load Settings** reloads a previously saved settings file. Settings files will affect all different settings defined under the Settings menu.

After running Acquire^{MP} for the first time, settings changes should not be required for most data acquisition experiments.

Troubleshooting

Image Calibration

This procedure should not be performed as part of a regular protocol and should only be used when either:

- The glass surface is not brightly or uniformly illuminated.
- The autofocus ring is off centre, for One^{MP}, Two^{MP}, and Samux^{MP} instruments.

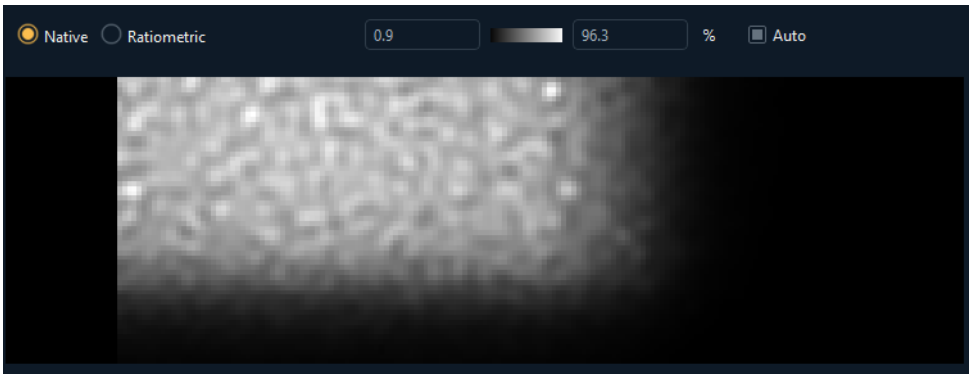


Figure 55: Partial illumination in the acquisition image which requires the acquisition image to be calibrated

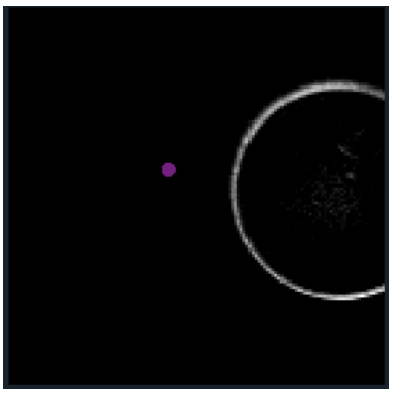


Figure 56: Off-centre auto-focus ring which requires the acquisition image to be calibrated

To calibrate the acquisition image, open the image calibration dialog found in **Settings > Calibrate**. Follow the steps listed in the dialog and click **Run Image Calibration**. This process takes approximately two minutes, and will optimise the settings for each image size simultaneously, as well as centre the auto-focus ring for One^{MP}, Two^{MP}, and Samux^{MP} instruments.

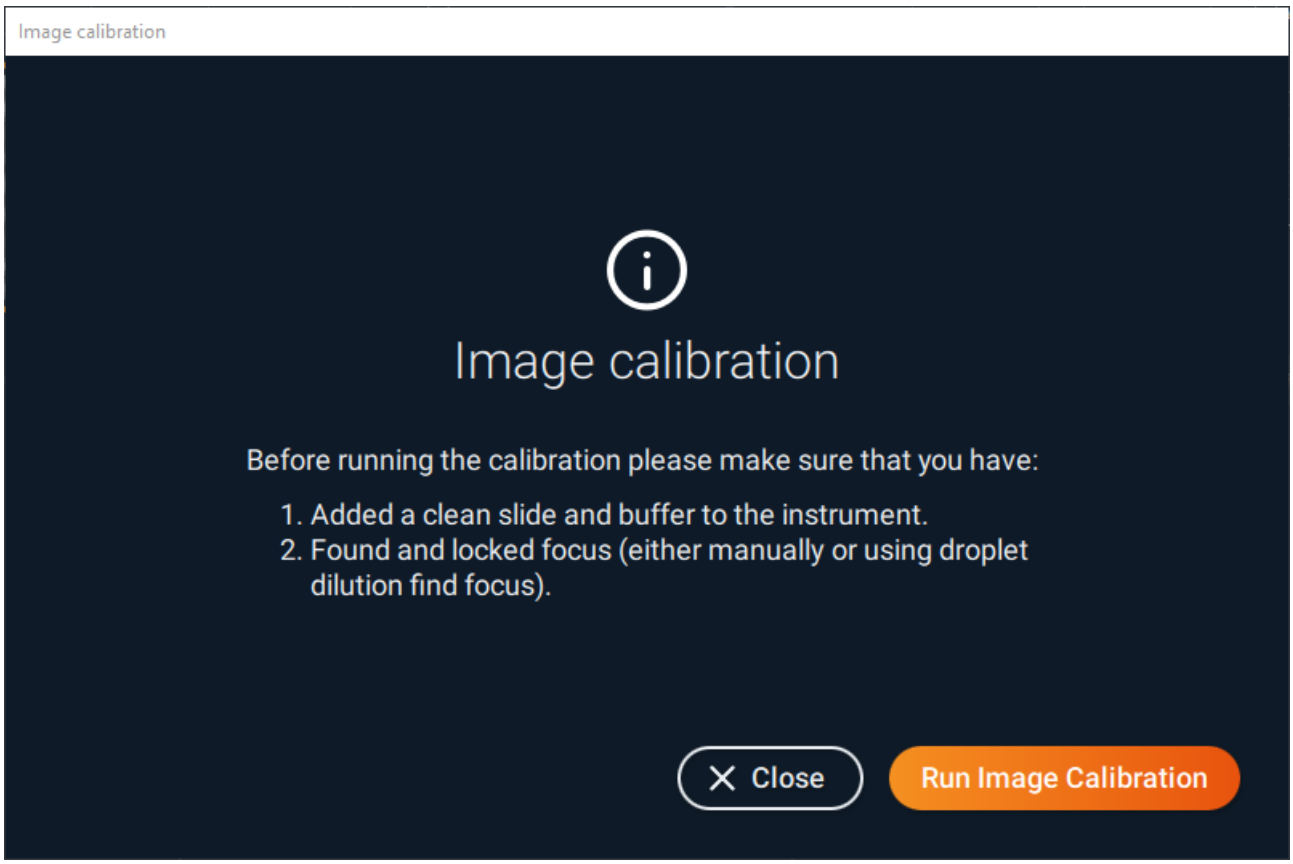


Figure 57: Image Calibration dialog

Figure 50 shows an example of when the instrument is the correct state to be calibrated as the auto-focus ring is clearly visible.

General information

Refeyn Ltd service and support

Refeyn Ltd offer service and technical support for our Mass Photometry systems, and the Refeyn software applications.

Applications and service support

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