

Refeyn Karitro^{MP} macro mass photometer

User manual

V2



Table of contents

Warranty	4
1. Safety information	5
1.1. Product identification	5
1.2. Electrical ratings	5
1.3. Embedded laser system ratings	5
1.4. Laser safety information	5
1.5. Environmental operating conditions	6
1.6. Transporting the Karitra ^{MP} macro mass photometer	6
1.7. Servicing and maintenance	7
1.8. Electrical supply to the instrumentation	7
1.9. Intended use	7
2. Site preparation guide	8
2.1. Transport requirements	8
2.2. Shipping crate contents	8
2.3. Room/site requirements	8
3. Getting started	9
3.1. What's in the box?	9
3.2. Switching on the Karitra ^{MP}	9
3.3. Launching the Karitra ^{MP} software	9
4. Typical consumables	10
5. Experimental guidelines	11
5.1. Warming up the instrument	11
5.2. Assembling sample carrier	11
5.3. Adding samples to the sample carrier	13
5.4. Mounting sample carrier	14
5.5. Setting up a measurement in Acquire ^{MP}	15
5.6. Experiment completion and instrument shutdown	15
5.7. Data evaluation	15
6. Troubleshooting guide	16
6.1. Concentration range	16
6.2. Buffer effects	17
6.3. Large unknown particles in the image	17

6.4.	No/few particles binding (bright flashes in image)	18
6.5.	Varying and uneven brightness	19
6.6.	Loss of resolution & grainy data	20
6.7.	Loss of size resolution	21
6.8.	Stage limits error	22
7.	Care and maintenance	22
7.1.	Objective cleaning	22
7.2.	Instrument cleaning	23
7.3.	Lid hinge maintenance	23
8.	Key specifications	23
9.	General information	25
9.1.	Service and support	25
9.2.	Disposal and recycling	25
10.	Disclaimer	26

Warranty

In accordance with Refeyn's Standard Terms & Conditions of Sale ("*Terms & Conditions*"), your Refeyn Karitro^{MP} instrument includes a 12-month parts and labour warranty and has been tested against Refeyn's published specification.

The warranty covers the repair, replacement or other appropriate remedies in accordance with the Terms & Conditions.

It is advised to retain the packaging for the duration of the warranty. This is to ensure full protection of the instrument in the unlikely event of needing to return the instrument, it can be done so safely.

To qualify under the terms of the warranty, the system must be used in accordance with the user manuals and any training provided by Refeyn Ltd.

Failure to do so may invalidate the warranty and no liability is accepted for loss or damage arising from the incorrect use of the Karitro^{MP} system.

Refeyn Ltd reserves the right without prior notice to alter the specification of the Karitro^{MP} system to improve performance and benefits for the user.

For the avoidance of doubt, the Terms & Conditions (or any other terms & conditions agreed between your organisation and Refeyn) shall govern the Warranty. Please refer to these documents for more information.

1. Safety information

1.1. Product identification

Refeyn Karitra^{MP} is manufactured by: Refeyn • Unit 6 • Trade City • Sandy Lane West • Oxford • OX4 6FF • UK

Model number: Karitra^{MP}

Product description: Research grade macro mass photometer

Technical assistance: support@refeyn.com, +44 (0) 1865 203 956

1.2. Electrical ratings

Power supply for Refeyn Karitra^{MP}:

Model No.: VES120PS24

Input Rating: 100-240 VAC • 50/60 Hz • ~2.0A

Output Rating: 24V DC • 5.0A

1.3. Embedded laser system ratings

Refeyn Karitra^{MP} macro mass photometer:

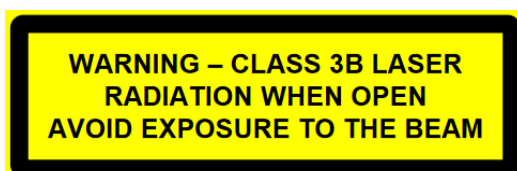
Laser: 450 nm (± 5 nm) • 1.6 W Max • Class 4

1.4. Laser safety information

Caution – Use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.



The Refeyn Karitro^{MP} macro mass photometer is a Class 1 laser product, making it safe to use in an open laboratory environment. The safety interlocking designed into the Karitro^{MP} macro mass photometer eliminates the risk of exposure to the high-power, Class 4 laser system within the instrument. Users should not attempt to remove or tamper with the screws securing the macro mass photometer enclosure or sample lid. If any panel of the instrument is removed then Class 3B laser radiation can be accessible.



Care should be taken when using the instrument. If there is any indication of a fault resulting in the escape of laser radiation from the unit, switch off the unit immediately and contact Refeyn Ltd Technical Support at support@refeyn.com.

1.5. Environmental operating conditions

- Refeyn Karitro^{MP} instruments are intended for indoor, laboratory use only.
- The Karitro^{MP} instrument should be operated on a secure, flat surface in a dedicated area.
- Ensure there is adequate space around the instrument to allow air to circulate and avoid overheating. Cables other than specified for use by Refeyn should be kept tidily away from the instrument.
- For optimum performance, Karitro^{MP} macro mass photometers should be operated in an environment with stable temperature 22°C +/- 2°C (with a temperature variation of less than 1 °C per hour) with relative humidity < 70% at 20°C, at an altitude of < 5000 m above sea level and pollution degree 2.
- As with any analytical instrument, standard precautions must be taken to prevent environmental or cross contamination when collecting/handling samples and loading the coverslip onto the Karitro^{MP} instrument.

For full details of optimal placement of your Karitro^{MP} in your laboratory, see Site Preparation Guidance, below.

1.6. Transporting the Karitro^{MP} macro mass photometer

Refeyn Karitro^{MP} macro mass photometers are intended for static use, in the laboratory position where they have been initially installed. If the system is to be moved to a different location, please contact Refeyn Ltd Technical Support (support@refeyn.com) for advice.

The instrument weighs 25 kg. It is therefore recommended that local policies on manual handling such weights are adhered to, should the unit need to be manually moved.

In all circumstances, the instrument should be lifted by two or more individuals, and anyone with an incapacity to lifting heavy objects should avoid doing so.

As the unit is not intended to be moved, the lifting positions are not indicated ergonomically, however, the unit is robust if lifting from the base.

1.7. Servicing and maintenance

There are no user serviceable parts inside Refeyn Karitro^{MP} macro mass photometers.

These systems must only be installed by trained service personnel from Refeyn Ltd. To avoid any personal risk, users are prohibited from accessing the interior of the Karitro^{MP} system. All external screws are purposely selected to have security features requiring specific tools to unscrew them. This is intentional, and these screws should not be accessed by users or anyone not fully trained by Refeyn Ltd.

1.8. Electrical supply to the instrumentation

Caution: this equipment is not intended for use in residential environments and may not provide adequate protection to radio reception in such environments.

Karitro^{MP} systems must utilize the power supply and cables supplied with each instrument. Under no circumstance should these cables be replaced with similar cables. If in any doubt about the mains cables feeding electricity to the units, contact Refeyn Ltd Technical Support (support@refeyn.com).

Power supply: XP Power VES120PS24

Input: 100 – 240VAC • 50/60 Hz • ~2.0A

Output: 24V DC • 5.0A

Any deviation from the factory-provided electrical connection and power supply parts may affect the safety characteristics of the instrumentation. If there is any doubt, please contact Refeyn Ltd Technical Support (support@refeyn.com).

1.9. Intended use

The Refeyn Karitro^{MP} macro mass photometer is intended for research use only. It is intended to be used for measuring size and contrast distribution of biological particles in solution and is to be operated in a laboratory setting in alignment with the environmental requirements specified in this manual.

2. Site preparation guide

2.1. Transport requirements

The instrument is delivered in a single palletised wooden crate (80 cm x 60 cm x 65 cm, W x D x H) weighing approximately 100 kg. A horizontal clearance of approximately 1 metre is recommended for delivery of the instrument to its intended installation room. A pallet truck will be required to move the crate. The crate should be stored at 10-25°C without major or rapid temperature changes and at lower than 75% humidity prior to unpacking and installation.

2.2. Shipping crate contents

- Refeyn Karitro^{MP} macro mass photometer
- All-in-one instrument computer with mouse and keyboard
- Instrument shipping kit including mains cables, USB cable, microscope objective, immersion oil

2.3. Room/site requirements

The Karitro^{MP} has a footprint of 503 mm x 313 mm x 155 mm (W x D x H) and weighs approximately 25 kg. The instrument must be placed on a heavy, sturdy workbench suitably rated to support the instrument. Internet connectivity is essential for the duration of the installation and occasional availability may be necessary for remote access and updates.

The instrument and PC require two power outlets in total (one for the instrument and one for the PC, 100-250 V, 50/60 Hz). The instrument is fitted with 2 m (6.5 ft) power cables. Power consumption is less than 1 A at 230 V AC. The unit should be positioned to allow easy disconnection of the power supply.

The instrument should not be near air-conditioning units or similar, to minimise exposure to direct airflows.

The supplied workstation and peripherals (keyboard, mouse etc.) shall be placed on a table that is not coupled to the instrument to avoid vibrations caused by operating the computer corrupting measurements (Figure 1). The instrument is vibration sensitive, so it is advised that for the most challenging of experiments the environment should be as free from disturbance as possible; i.e. no excessive vibrations caused by noisy equipment or air currents.

The PC should be configured to save all data during acquisition to a local drive and not to a network drive, for optimal performance. For more details refer to the user manual of Acquire^{MP}.

The installation room should have a stable temperature 22°C +/- 2°C (with a temperature variation of less than 1 °C per hour) with relative humidity < 70% at 21°C and pollution degree 2.

3. Getting started

3.1. What's in the box?

- Karitro^{MP} macro mass photometer
- All-in-one instrument PC
- Mains cables and USB communication cables
- XP 24V power supply

3.2. Switching on the Karitro^{MP}

The Refeyn Karitro^{MP} must be installed and verified by a trained engineer from Refeyn Ltd. Users must receive training in the functionality and use of the instrument before using the instrument.

Ensure the USB cable is connected between the PC and the instrument using a USB3 port on the PC, not a USB2.

Ensure the instrument power switch is in the "OFF" position (denoted "0" on the switch). Ensure the instrument mains cable is securely plugged into XP power supply and that the 4 pin DC Plug supplying 24V to the instrument is plugged into the inlet beneath the on/off switch. Switch on the instrument at the power inlet switch.

3.3. Launching the Karitro^{MP} software

Once the Karitro^{MP} has been switched on, open Refeyn Acquire^{MP}. The instrument will initialise and centre the stage. If this is the first time initialising the software then the terms and conditions will be displayed. This must be read and accepted to proceed. For further details on how to use the software see the Acquire^{MP} manual.

4. Typical consumables

For precise macro mass photometry experiments, Refeyn Ltd. recommends the use of Sample Carrier Slides, Sample Well Cassettes and Alignment Assist Tools which have been designed and optimized specifically for mass photometry.

Further information on what the packages include can be found on Refeyn's website (www.refeyn.com/mass-photometry-consumables). For further clarification, send an inquiry to orders@refeyn.com.

Consumables for sample measurement (available from Refeyn through orders@refeyn.com)

- MassGlass® KV Sample Prep Kit – includes cassettes, lids and MassGlass KV slides. Compatible with large viral vector samples.

Calibrants

- SizeFERENCE® Calibrant. Available from Refeyn through orders@refeyn.com

Others

- KaritraMP Alignment Kit - Includes KaritraMP alignment tool, Carbon fiber tip tweezers, and a set of magnets (4). Available from Refeyn through orders@refeyn.com
- Immersion oil, Zeiss Immersol 518 F no. 433802-9010-000
- Lens cleaning tissue. Such as Whatman® lens cleaning tissue, Grade 105, Sigma WHA2105841

5. Experimental guidelines

5.1. Warming up the instrument

Switch on the Karitro^{MP} (Figure 1) and start Acquire^{MP} at least 1 hour before the start of the measurement.



Figure 1 Karitro^{MP} power switch and connections.

5.2. Assembling sample carrier

- 1) The Karitro^{MP} sample carrier consumables have been designed and optimised specifically for mass photometry. These are composed of a sample carrier slide, a cassette, and a plastic lid, and are available from Refeyn Ltd.
- 2) Preparing the sample carrier requires:
 - a. A sample carrier slide
 - b. A sample well cassette
 - c. The alignment tool
 - d. A sample lid
- 3) Using soft-tipped tweezers, take a sample well cassette and place it on the alignment tool in between the designed recess, as shown in Figure 2.

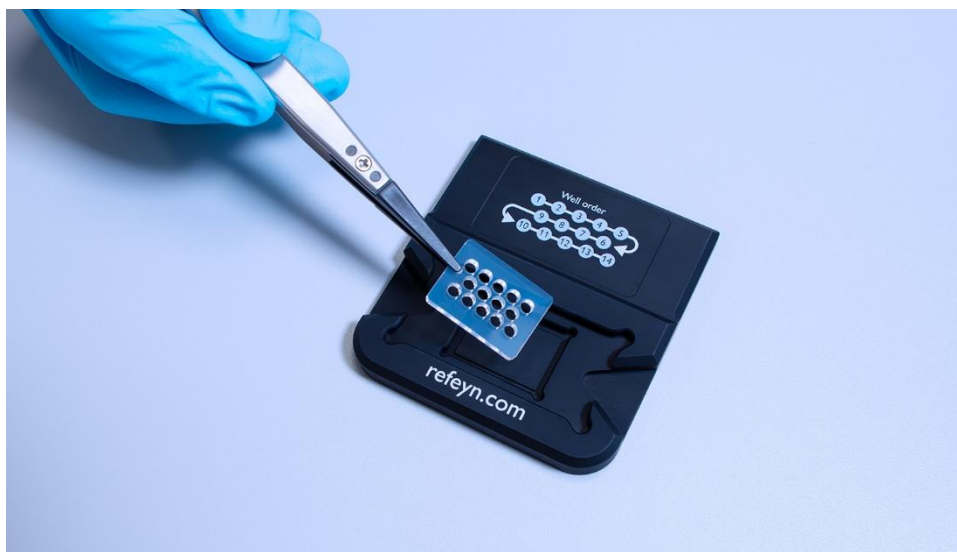


Figure 2: Sample well cassette is placed in the recess of the alignment tool.

- 4) Open the sample carrier slide box and remove a ready-to-use sample carrier slide by taking the larger spacer with a pair of tweezers to lift the sample carrier slide. Using tweezers or powder-free gloves, hold the sample carrier slide and place it on top of the sample well cassette in the alignment tool (Figure 1). Using tweezers, gently press the sample carrier slide to remove the trapped air between the sample well cassette and the sample carrier slide (Figure 3).



Figure 3: Placing a ready-to-use sample carrier slide on the sample well cassette using the alignment tool

- 5) Lift the assembly from the alignment tool, rotate the assembly such that the cassette is facing up and place back into the alignment tool (Figure 4). You are now ready to add samples to your sample carrier.



Figure 4: Remove the assembled sample carrier and rotate so the cassette is facing up. Replace in the alignment tool.

5.3. Adding samples to the sample carrier

- 1) Prepare samples at an appropriate dilution and determine what wells will be used for each sample. Well 1 must contain the SizeFERENCE calibrant. For the appropriate dilution of the SizeFERENCE calibrant, please refer to the SizeFERENCE documentation.
- 2) Add 5 µl of SizeFERENCE or sample to the appropriate wells taking care not to touch the glass surface with the pipette tip. Follow the well order shown in Figure 5.

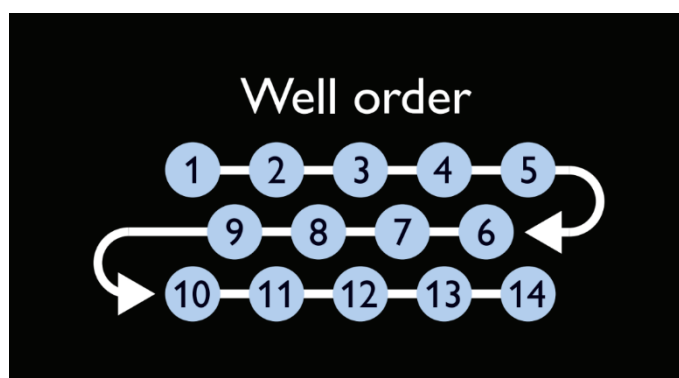


Figure 5: Diagram of the order of the wells in the sample cassette.

- 3) Once SizeFERENCE and samples have been added to all of the wells perform the washing protocol as follows:
 - a. Add 10 µl buffer to all the wells used
 - b. Mix each well thoroughly by pipetting up and down (minimum 3 times) and remove 10 µl
 - c. Add a further 10 µl of buffer to the wells used
- 4) Once all samples have been added to the sample carrier and the washing protocol has been performed place a lid over the sample well cassette.

5.4. Mounting sample carrier

- 1) With a pipette, apply immersion oil on the centre of the objective. For more precision in the amount of oil applied, use reverse pipetting:
 - a. Pour out some oil into a 1.5 mL tube
 - b. Set a pipet to 20 µL and select a tip
 - c. Depress the plunger to the 2nd stop and place the tip in the tube with oil
 - d. Slowly release the plunger to 1st stop; pause until the oil stops moving
 - e. Slowly release the plunger to the upper stop; pause until the oil stops moving
 - f. Move the pipet to Karitro^{MP} objective
 - g. Depress the plunger to the 1st stop to dispense the measured volume of oil on the objective
 - i. Apply oil in a circular motion around and then across the lens
 - h. Discard the tip with unused oil
 - i. If too much oil was added, clean it off (following cleaning instructions detailed in Section 6.1) and place a new drop.
- 2) Carefully mount the sample carrier to the stage.
- 3) Place magnets on the corners of the sample carrier to prevent movement during sample measurement, as shown in Figure 6.



Figure 6: Magnets are placed on the corners of the sample carrier slide to hold it in place.

5.5. Setting up a measurement in Acquire^{MP}

First, close the instrument's lid. Acquire^{MP} will guide you through planning and running of the experiment. For further details please refer to the software manual of the Acquire^{MP} version installed on your system.

5.6. Experiment completion and instrument shutdown

- 1) Once the experiment has been completed, the magnets should be removed, and the sample carrier taken off the instrument. Dispose of the sample carrier in accordance with local procedures.
- 2) Clean the objective lens, following the cleaning procedure outlined in section 6.1.
- 3) If proceeding with further experiments, repeat the steps in sections 4.3 – 4.6 once the objective has been cleaned.
- 4) If no further experiments are to be taken, first close Acquire^{MP}. Then, turn off the instrument using the switch on the rear.

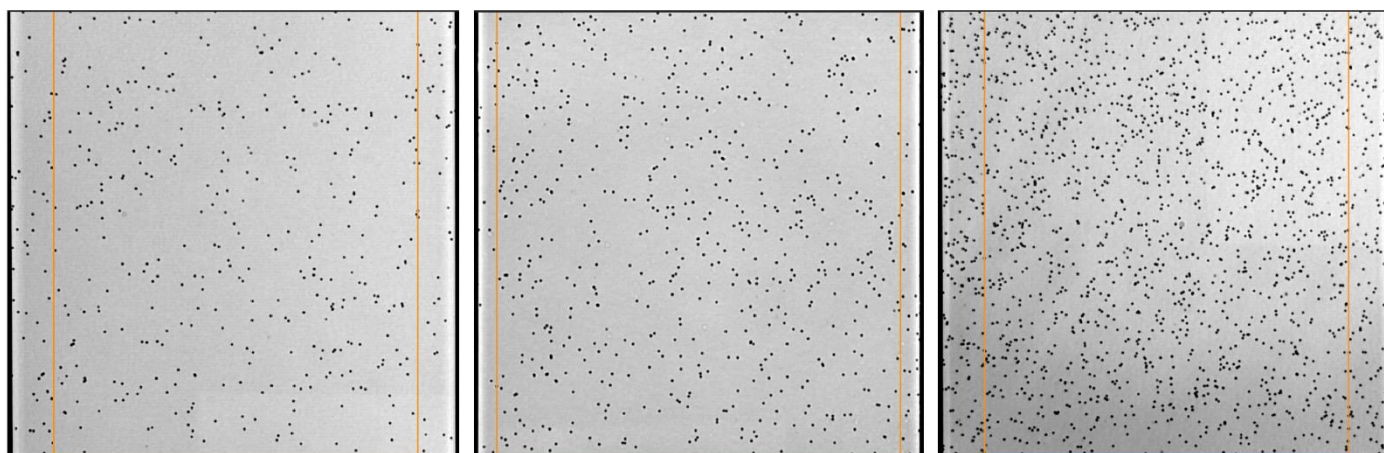
5.7. Data evaluation

Open your measured data (.mpkr or .mpka file) in Discover^{MPK}. For details on how to use Discover^{MPK} refer to the software manual of the specific Discover^{MPK} version that you are using.

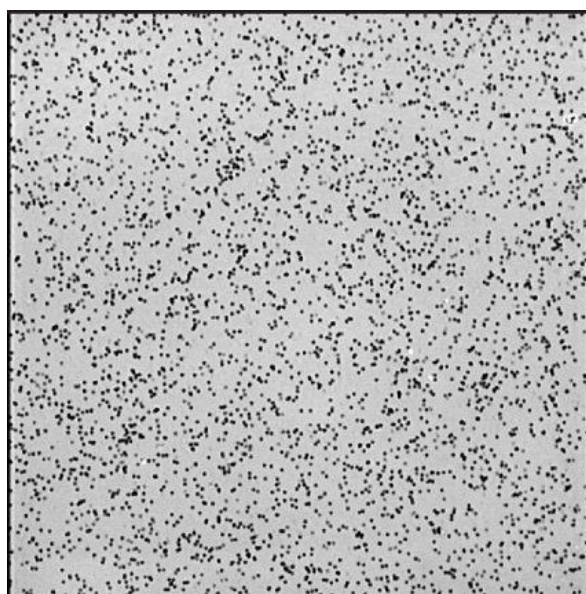
6. Troubleshooting guide

6.1. Concentration range

A range of acceptable surface coverages are shown in the images below, from relatively sparse (left) to relatively dense (right). A density much higher than that on the right can prevent the accurate identification of individual particles resulting in a loss of resolution.

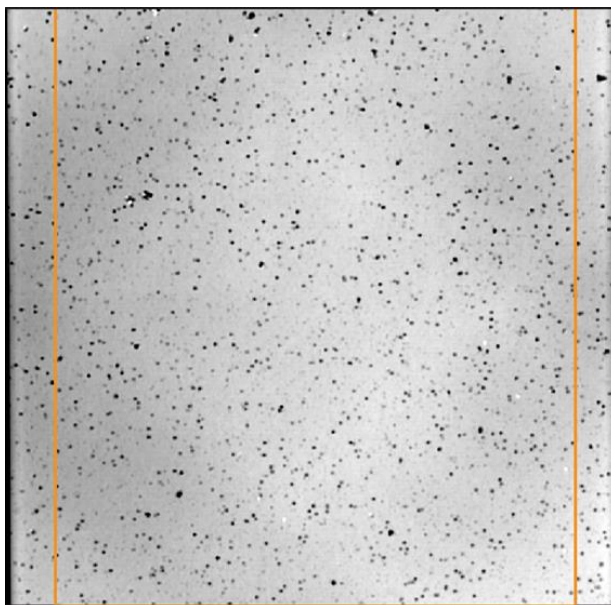


An example of surface coverage too dense to give reliable results is shown below:



In cases where the sample is overloaded in this manner it can affect the distribution observed as the overlapping events prevent the accurate measurement of the particles. Note: In heterogenous samples this can be harder to identify as

smaller, lower contrast particles can be harder to observe in the image. An example of this can be seen below with many smaller particles being less easily observed amongst the higher contrast particles.



6.2. Buffer effects

Some buffer components can have effect on the binding of particles to the glass surface, for example, Polysorbate-80 can have effects on the binding of Adenovirus at the levels found in common storage buffers. To check for effects of a buffer component, it is recommended to measure the same sample with a series of decreasing concentrations of such buffer component. In the absence of any buffer component effects, the ratio of events between populations should remain consistent across different dilutions. If significant changes in ratios are observed, then it is recommended to dilute the buffer component of interest further until such changes are no longer observed or, if possible, prepare the sample without these components and then follow the guidance on concentration range in 5.1 to find an appropriate concentration range.

6.3. Large unknown particles in the image

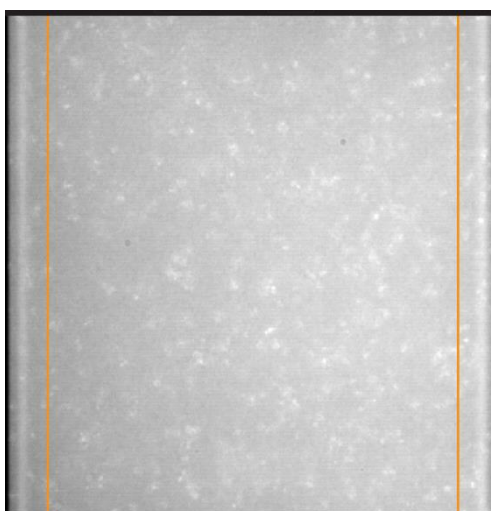
Sometimes particles can be observed in the image that are far larger than 150nm. These can present themselves in a number of ways with a selection example shown below. These are not able to be characterised effectively by Acquire^{MP} due to their size and shape but may result in artefacts being observed in the results. Particles can be from a number of sources including dirt within the buffer, dirt on the surface of the sample carrier or aggregates within the sample. To determine whether the particles are originating from the buffer, surface or sample, it is recommended to measure the buffer alone in order to characterise the background that may be present in other measurements. If the particles are only observed in sample wells, then this suggests that these events are originating from the sample and may be a reflection of a real population of larger particles being present within the sample. If the particles are present only in buffer wells, the buffer can be filtered to remove

any particles present or fresh buffer can be prepared if the buffer has expired. If the particles are still present, then it may be a result of dirt on the sample carrier. The risk of this occurring can be minimised by avoiding contact with the surface when preparing and loading the sample carrier and storing the sample carrier slides appropriately with the lid closed when not in use.



6.4. No/few particles binding (bright flashes in image)

In some cases, it can be difficult to observe particles as they are not binding to the surface. This is often observed as bright flashes moving across the image with an example of this shown below.

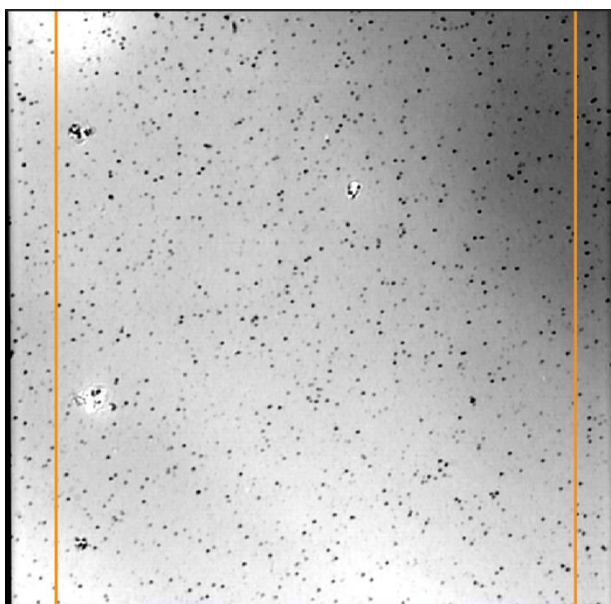


In these cases, it may be that the buffer being used is not conducive to particle binding. This could be occurring for several reasons but has been observed previously under the following conditions.

- Low ionic strength buffer: In cases where the buffer has a low ionic strength it has been observed that some samples have a reduced affinity for the surface. If possible, modifying the buffer to increase the ionic strength may help in this case.
- Tween-80: Tween-80, a component of the adenovirus storage buffer A195, has been observed to reduce the ability of adenovirus samples to bind to the surface at higher levels. A dilution of a sample stored in A195 buffer by 100-fold (0.0002% Tween-80) in PBS restores binding to the surface with results consistent with PBS alone.

6.5. Varying and uneven brightness

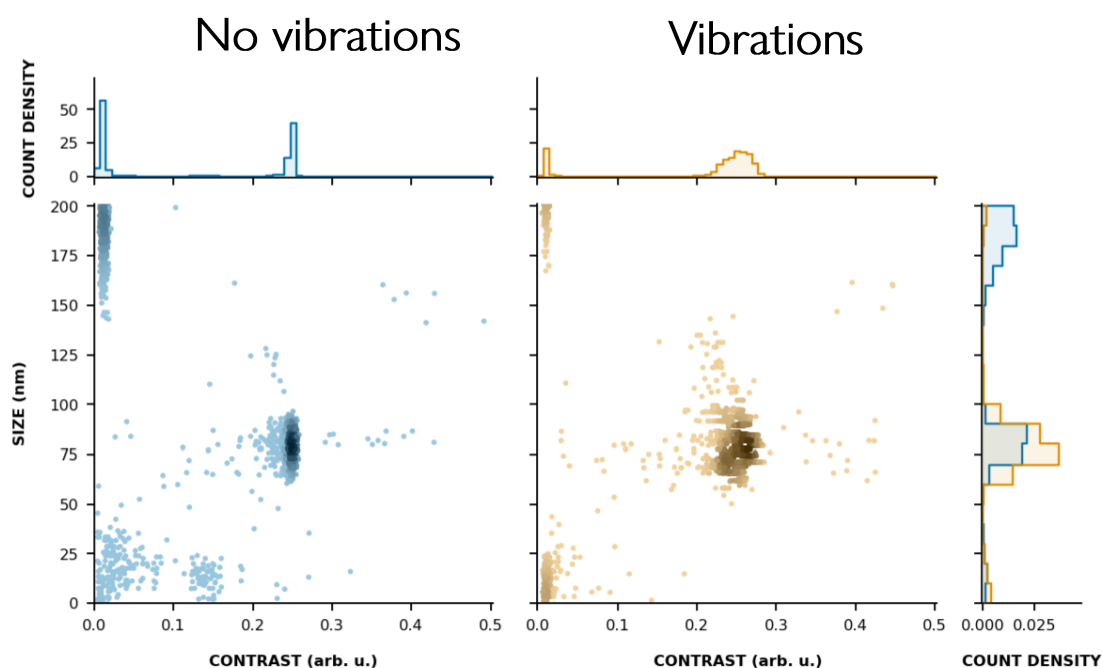
In some cases, large differences in brightness across a field of view may be observed, which can vary from well to well and/or sweep to sweep. This may present itself in the manner observed below with dark and bright patches observed rather than the standard, uniform, brightness as seen in unaffected experiments. This can cause shifts in contrast between sweeps and result in artefacts.



This can be a result of air bubbles within the immersion oil. The best way to prevent this is to be careful when placing the sample carrier slide on the objective. Slowly lowering the slide onto the objective and avoiding large vertical stage movements can reduce the likelihood of trapping bubbles.

6.6. Loss of resolution & grainy data

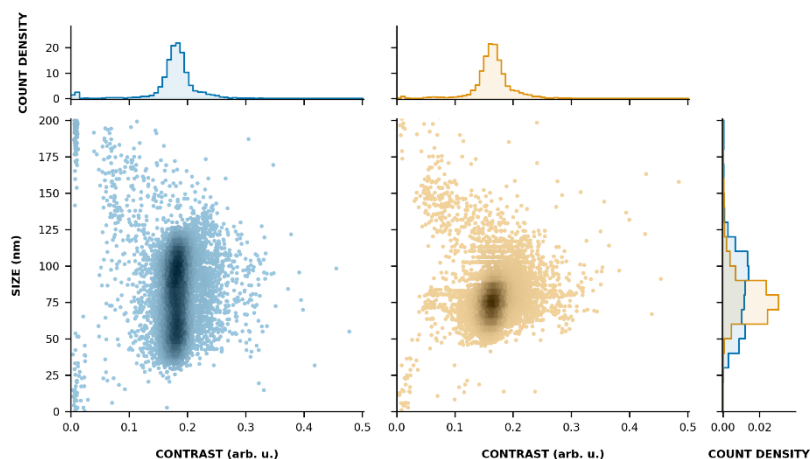
In some cases, a loss of resolution and the data becoming more granular with lines may be observed in the data. An example of this can be seen below.



This may be due to vibrations that may also be observed in the movies in particularly severe cases. Depending on the source, this may affect some or all the sweeps collected. It may be necessary to move the instrument to an alternative bench away from the source of vibrations.

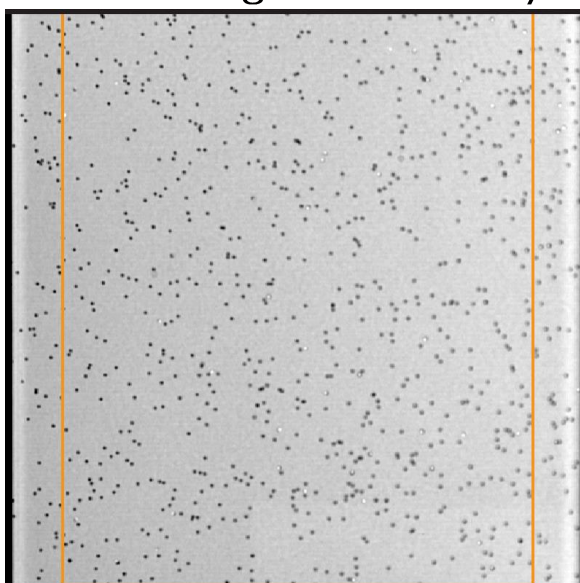
6.7. Loss of size resolution

In some cases, you may observe a spread in the size dimension that is likely to affect most, if not all, wells in a cassette. An example of this can be observed below where the same sample was measured with and without the issue. In the movies, this is observed as shown below with particles of the same size coming into focus at different times across the field of view.

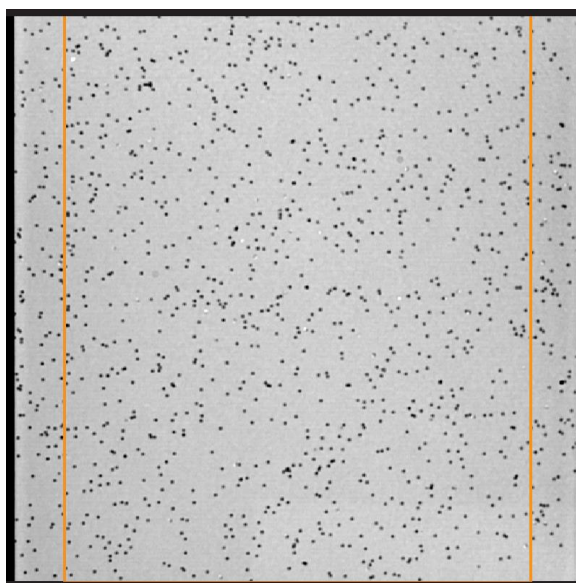


This is observed in situations where part of the sample carrier is raised, likely because of oil, dirt or other residue between the sample carrier and the stage. If oil is seen between the sample carrier and the stage, remove the sample carrier. Clean the stage and objective gently with a lens tissue as described in sections 6.1 and 6.2. Prepare and load a new cassette, then place on the instrument using a conservative amount of immersion oil. If the problem persists between sample carriers it may be necessary to clean the stage. The process for this can be seen in section 6.2.

Left of image in focus only

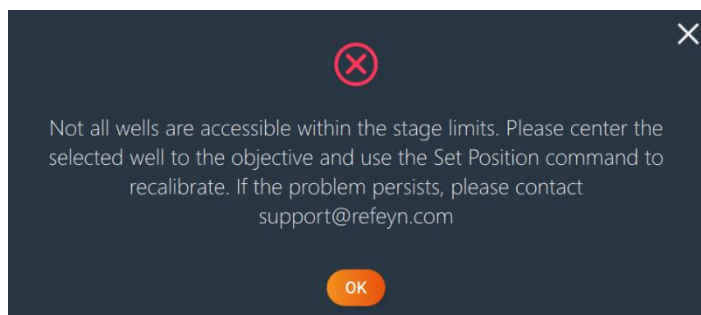


All events in focus

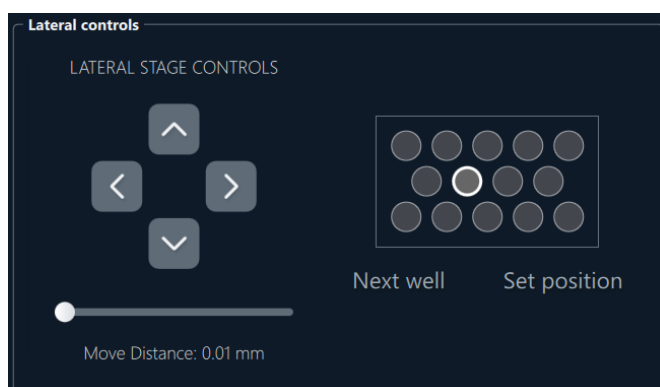


6.8. Stage limits error

When performing cassette alignment during a measurement protocol, if the stages have become desynchronized with Acquire^{MP}, it is possible for the following error to occur:



If this occurs, follow the guidance in the error message to resynchronize the sensor position. First, navigate to File -> Preferences, select the Advanced Mode option, and save the Preferences settings. Then, navigate to Tools -> Switch to Manual Control, use the Lateral Stage Controls to center a well above the objective, select the corresponding well and press Set Position.



This should resolve the problem; verify by returning to Auto Control and running the protocol. If the error persists, shut down Acquire^{MP}, power cycle the Karitro^{MP}, and relaunch Acquire^{MP} before re-attempting to run the protocol. If the error persists, contact support@refeyn.com.

7. Care and maintenance

7.1. Objective cleaning

When measurements are completed, remove the sample carrier slide from the stage. Dispose of the slide, cassette and lid as per local regulations.

Close Acquire^{MP}, then power off the instrument using the switch on the rear.

The objective must then be cleaned following the steps below:

- Fold a lens cleaning tissue and lightly wet with isopropanol.
- Gently wipe the oil off the objective and allow to dry.
- Repeat with as many lens tissues as required.

For more detail, watch [this](#) YouTube video (from 1:50 onwards)

7.2. Instrument cleaning

Unless otherwise required by local procedures, there is no need to clean the Karitro^{MP} more than once a week.

To disinfect the instrument, dampen a sheet of absorbent tissue with Chemgene or a suitable alternative and wipe all exterior surfaces of the instrument. The absorbent tissue must be moist enough to ensure the cleaning agent is in contact with the exterior surfaces of the instrument. Use further sheets of tissue as required. The instrument should subsequently be cleaned with water.

To clean the instrument, dampen a sheet of absorbent tissue with purified water and wipe all exterior surfaces of the instrument. The absorbent tissue must be moist enough to ensure water is in contact with the exterior surfaces of the instrument. Use further sheets of tissue as required.

If the area around the objective requires cleaning, use a fresh sheet of tissue dampened with isopropanol to gently wipe the area, **taking care to NOT touch the objective lens.**

7.3. Lid hinge maintenance

It is recommended to adjust the torque of the lid hinge occasionally so as to provide some resistance when opening and closing. An ideal torque will allow the hinge to remain open when left at a 45-degree angle.

As an extra note in regards to the operation of the lid, make sure to always open and close the lid slowly and carefully to avoid causing any disturbances in the sample area.

8. Key specifications

Refeyn Karitro^{MP} macro mass photometer specifications*

- Size range 40-150 nm (diameter)
- Contrast resolution <5% (SD/mean, as measured for AdV particles)

- Contrast precision $\pm 2\%$ (SD/mean)
- Size resolution < 30 nm (FWHM, as measured for virus-like particles and silica nanoparticle standards)
- Size precision < 6 nm (SD, measured at 120 nm)
- Optimal concentration $10^8 - 10^9$ particles/mL
- Wavelength 450 nm
- Field of view $39 \times 47 \mu\text{m}^2$
- Pixel size 86 nm

*All specifications subject to meeting the installation requirements

9. General information

9.1. Service and support

Refeyn offers service and technical support for Karitro^{MP} systems:

Refeyn Ltd, Unit 9, Trade City Sandy Lane West Oxford, OX4 6FF, UK

Email: support@refeyn.com

9.2. Disposal and recycling



Before disposing of this equipment, you must:

- Check with the appropriate local organization to obtain advice on local rules and regulations about disposal and recycling.
- Contact Refeyn. before disposal begins.

10. Disclaimer

This user guide is proprietary to Refeyn, and no part of this manual may be reproduced, copied or duplicated without the express written permission of Refeyn.

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