READ THIS FIRST

Morphologi G3

Quick Start Guide

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Part 1: The Morphologi hardware

1. Introduction

This document describes the operation of the Morphologi® G3 particle characterisation system. In simple terms this is an automated microscope and a software package for control, measurement and analysis. The instrument measures the size and shape of a sample of particles, presenting the data according to the user's needs.

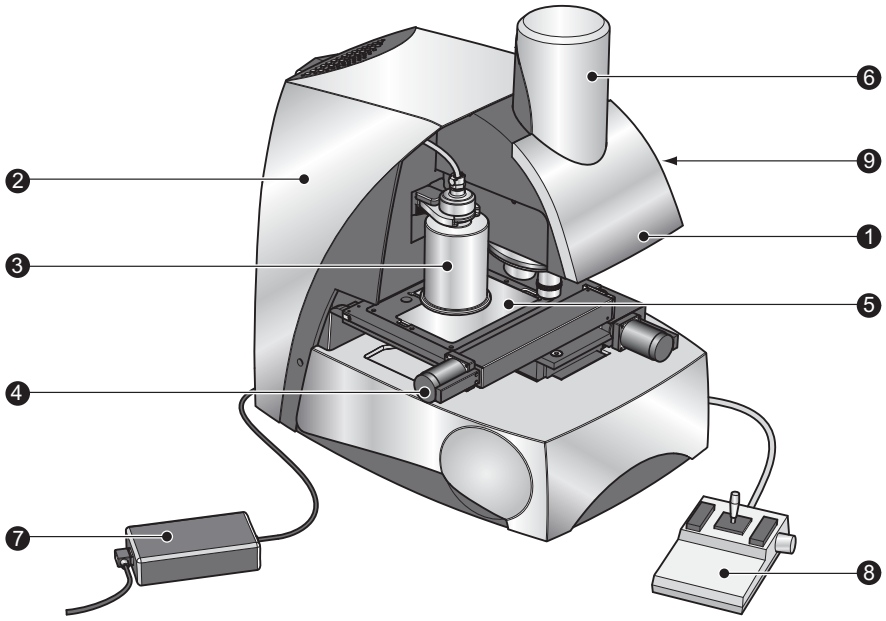
All users must read the companion **Morphologi Essentials Manual** which gives Health and Safety, maintenance, troubleshooting and other vital information.

2. Hardware components

There are two types of system:

- G3S – this has an integral **Sample Dispersion Unit** (SDU) for automatic sample dispersion, but can also be used with manual dispersion onto microscope slides, etc.
- G3 – this has no integral SDU. The user can use a separate sample preparation device or some other sample dispersion unit, or prepare the sample manually.

The components are shown below:



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The components are:

① Optical unit.

② Back panel connections.

③ Integral SDU (G3S only).

④ Precision XY stage.

⑤ Sample plate holder and sample plate.

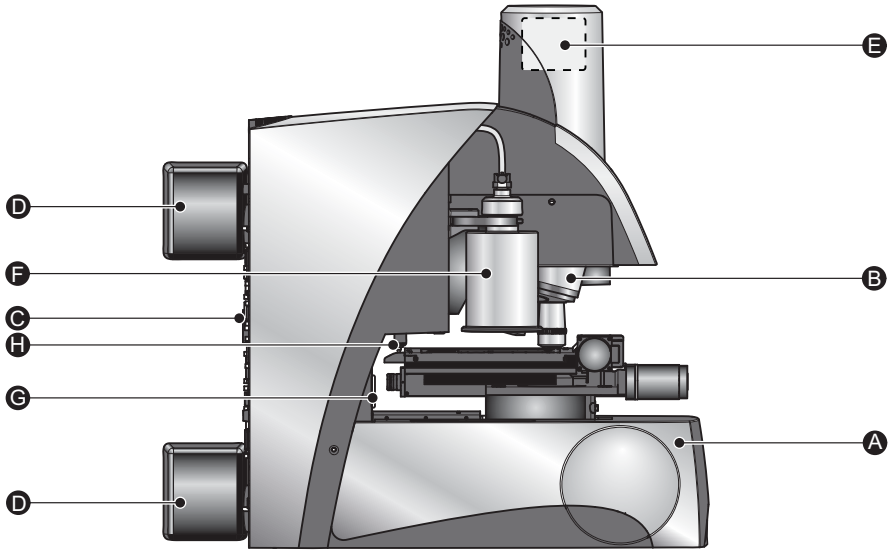
⑥ FireWire™ digital camera (under removable part of cover).

⑦ 40V power supply for control electronics.

⑧ Joystick for moving the XY stage manually.

⑨ Instrument identification label – this gives the Morphologi G3 model and its serial number. To access the label remove the triangular cover. Please quote these numbers when contacting Malvern Instruments.

The main components of the optical unit are shown below:



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The components are:

Ⓐ Main optical body.

Ⓑ Objective nosepiece – houses a set of objectives with different magnifications. Automatic changeover between objectives is controlled by the software.

Ⓒ Back panel connectors.

Ⓓ Lamp houses – the **Essentials Manual** shows how to change their bulbs.

Ⓔ Digital camera.

Ⓕ Integral SDU (G3S only).

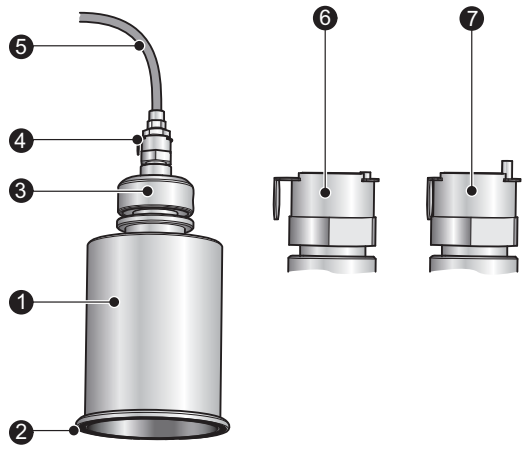
Ⓖ LED displays.

Ⓗ Z limit switch.

Part 2:
Preparing the sample

Correct sample preparation is essential to obtain accurate measurements. The objective is to disperse the sample and distribute it as a monolayer on a glass slide. This section describes sample preparation on systems with the integral SDU.

The sample is placed between circular sheets of aluminium foil between two rings of plastic. This assembly is termed the sample cartridge. After sample is loaded in it, this is placed in the top of a dispersion chamber.



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The parts of the dispersion chamber are:

① Chamber body. The circular sample cartridge is placed in a recess in the top of this.

② Air-tight seal on base – forms a seal against the glass plate.

③ Screw-on cap – holds the sample cartridge in place, keeping the sample air-tight.

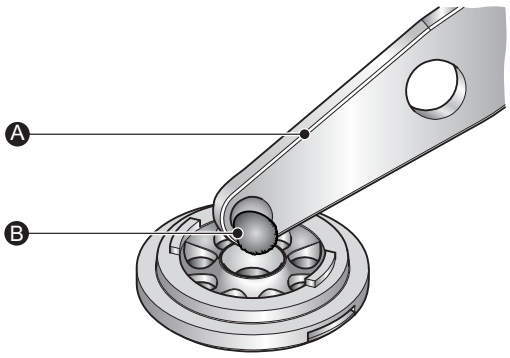
④ Air pipe connector – this is where the air supply fits. If the air pipe / cannot be inserted easily, check that the slider is pushed inward ⑦, not out ⑥.

Loading a G3S sample

To load a sample into the SDU for dispersion:

Place the upper and lower parts of the sample cartridge on the bench.

Use the spoon to scoop up the sample. Load until the hole is just over full then scrape away the excess using a scalpel blade, for example.



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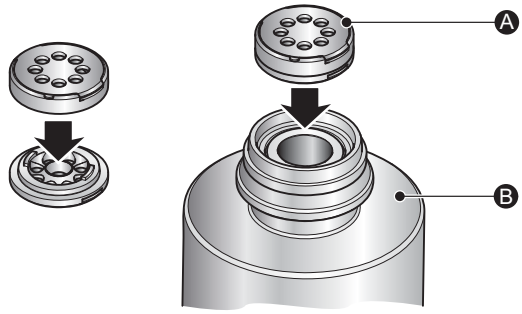
Use the “spoon” Ⓐ to load sample into the centre of the lower sample cartridge Ⓑ. (The lower sample cartridge has a central funnel for the sample, surrounded by 8 air flow holes.)

Press the two halves of the sample cartridge together, as shown below on the left, then place the sample cartridge Ⓐ in the top of the sample chamber Ⓑ, like this:



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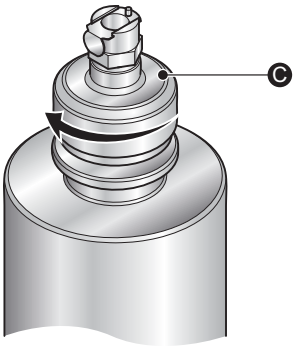
Screw the screw-on cap Ⓒ by hand one turn until it's tight, forming an air-tight seal.



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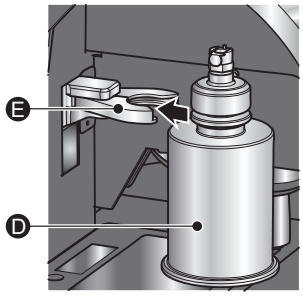
Push the chamber Ⓓ into the clamp Ⓔ on the G3S as shown below:



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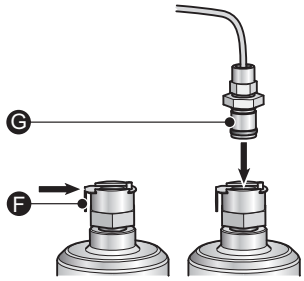
Push the air pipe Ⓖ into the top of the chamber. If it does not fit, check that the slider Ⓕ is pushed in correctly.



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The sample is ready for dispersion.

Making the dispersion

Sample dispersion with the SDU is only possible using the software. There are two ways to work:

- Sample dispersion as part of an SOP sequence. Use the **Sample Dispersion Unit** dialogue to specify injection pressure and time, also settling time.
- Stand alone dispersion or Sample dispersion independent of an SOP – this is useful as a series of sample dispersions can be made in one session and the plates stored ready for use. The equipment can then be cleared away and the dispersed samples measured without the need to stop after each to prepare the next sample. This technique uses the command **Measure-Disperse sample**. The online help for the command gives full details.

This is also useful during method development, to determine the sample quantity needed, the dispersion pressure to use, etc.

Cleaning up

After making a dispersion: For the disposable cartridge, simply through it away, otherwise for the resusable cartridge, remove the sample cartridge and dispose of the used foils, then clean the chamber thoroughly before the next sample is prepared.

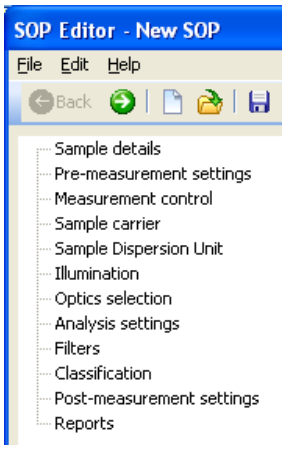
Make sure there is no dirt/grit on the air-tight seal on the base of the chamber.

Part 3: Creating an SOP

This section describes how to create a Standard Operating Procedure (SOP).

Having chosen the correct magnification and checked the dispersion quality, the next step is to create an SOP to make the measurement. This means using the **SOP Editor**, a set of dialogues the user can step through using  and  buttons.

The tree structure shown here appears in the left-hand side of each dialogue. This offers a quick way of moving through the SOP Editor:



To create a new SOP:

1. Select **File-New-SOP** or click the  button to open the SOP Editor.
2. In the **Sample details** dialogue type in the sample name and any notes associated with the measurement. These prompt an operator who runs the SOP.

   To prompt the operator to add documentation before or after making a measurement, select the relevant check box(es) under Additional options. We recommend selecting the first check box at least. This adds a Documentation tab to the Pre-measurement dialogue, asking for details from the operator. When the dialogue is complete click the button.
3. In the **Pre-measurement settings** dialogue, type in any instructions operators need to follow at the SOP start (for example, on how to prepare the sample) and select the **Show these instructions...** check box. These instructions will pop up on the screen when an operator runs the SOP.
4. In the Measurement control dialogue specify one of the following:
   - **Fixed number of slides/plates** – if the sample preparation method requires that the sample is distributed over four plates, for example, specifying 4 here should ensure that the operator includes all four slides in the analysis. Specify whether to combine or separate the results from these slides.
   - **Minimum number of particles** – if the measurement requires a number of particles, for example to achieve statistical significance, set this here. If merging multiple optics, after the minimum number is reached measurement will continue until all optics finish. This is to prevent bias towards the first optic(s) used.

   During the analysis the software prompts for further samples until the specified requirement is met.
5. In the **Sample carrier** dialogue, under **Carrier** select the slide plate to use. We recommend using tilt compensation for all measurements except quick test measurements using a 2.5X objective, or using a 5X objective in just a small scan area.
6. In the **Sample Dispersion Unit** dialogue, check the **Use SDU** box if using the integrated SDU. Increase the **Settling time** for smaller particles.

Part 4: Making a measurement

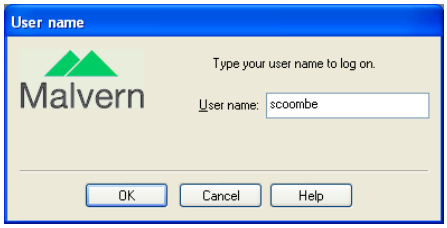
Introduction

These instructions show how to make a measurement. Work through the steps in the order given here.

1. Turning on the instrument and starting the software

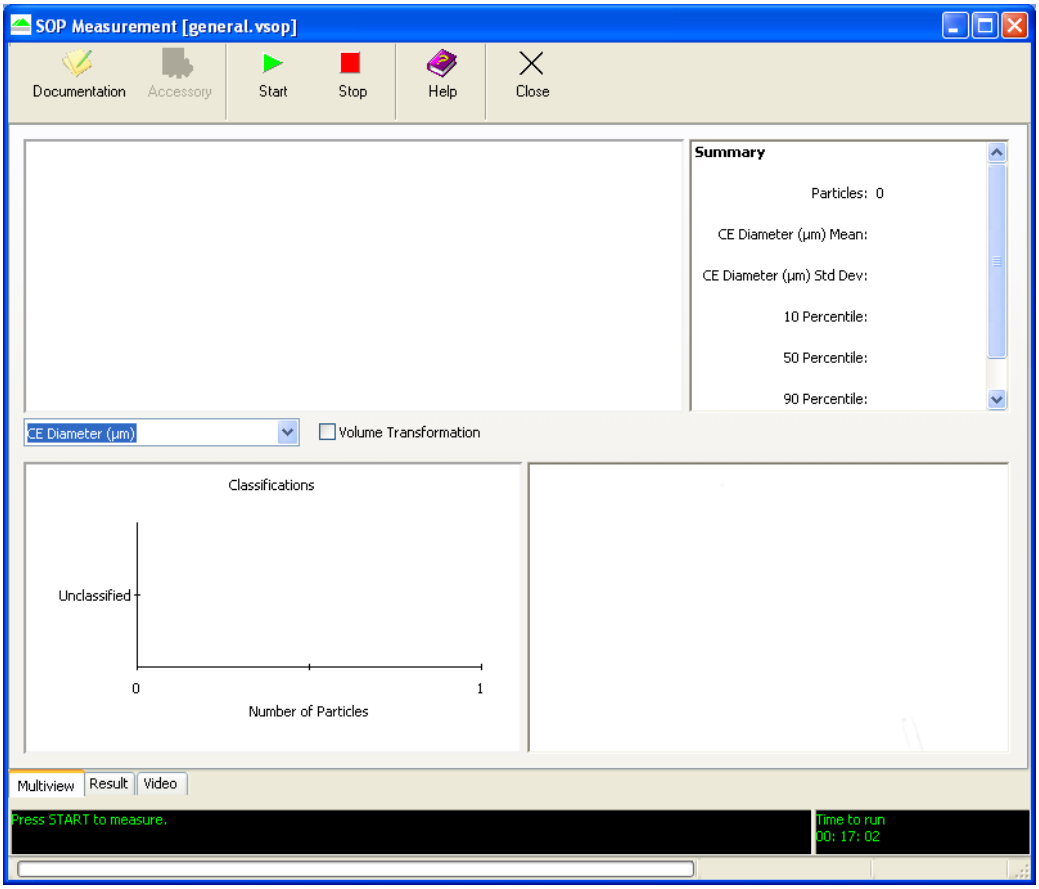
1. Turn on the instrument by pressing the power switch on the back of the instrument.
2. Start the software by double-clicking the 

icon.
3. If this dialogue appears, it will show your Windows login name. Click **OK**.
4. Leave the instrument **on for five minutes before making a measurement** to let ite stabilise.

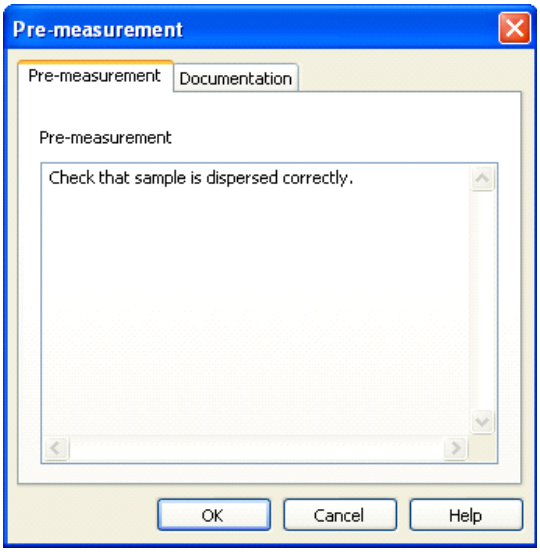


2. Running the SOP measurement

1. Check that the microscope symbol in the **Status bar** is not crossed out.
2. Use **File-New-Measurement** to open a measurement file for the results. (If this step is omitted the result is saved to the current measurement file.)
3. Select **Measure-SOP** or click the  button. In the **Open** dialogue select the newly created SOP and click **Open**.
4. The **Measurement Manager** appears, running a short initialisation routine: When the **Start** button turns green and the **Status bar** says “Press START to measure” click the **Start** button.

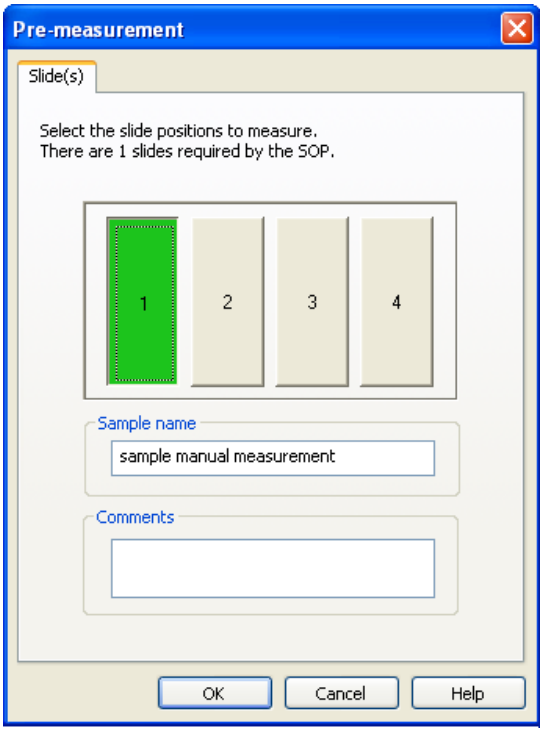


5. If the SOP has any sample details and pre-measurement instructions, these are displayed by this **Pre-measurement** dialogue:



The **Pre-measurement** tab describes actions to perform. Follow these instructions. If the SOP needs sample information or other comments from the operator, type these in on the **Documentation** tab.

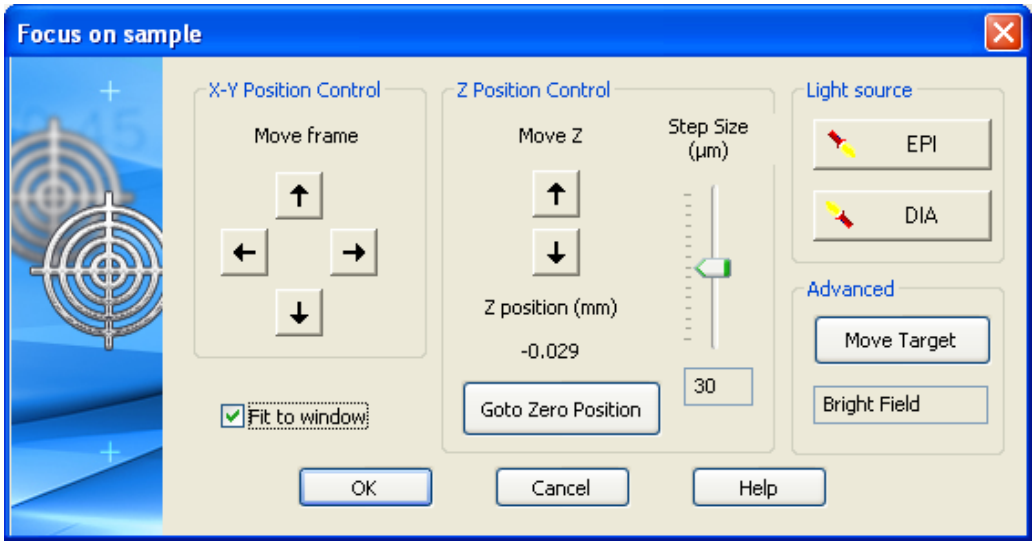
6. If the four/two slide holder or filter application is in use, the user is prompted to select the relevant slide(s), which become green in the dialogue:



Select the required slide(s) by clicking on them. The SOP's Measurement control settings specify how this produces the result. If multiple slides are selected, for example, this can produce a record for each slide or one combined record. Click **OK**.

7. If the integrated SDU is being used, the sample will now be dispersed. Wait for the settling time to pass.

   If **Refine area at run time** was selected in the SOP, the **Focus on sample** control dialogue appears:



8. The **Microscope Manager** performs initial focusing, using the plate holder's gratings and Z reference target. The Live Display window and the second monitor show these briefly.

9. Use the joystick to redefine the scan area. This is often needed if a cover slip is used with an oil dispersion sample; the sample may be pushed from the central area of the slide by the weight of the cover slip.

10. If the SOP specifies the **Fixed focus option** for the objective, there is no need for manual focusing so skip to step 13.

11. If the **Fixed focus** option is not selected in the SOP, the **Focus on sample** control dialogue appears as shown above. Use the joystick to focus the microscope.

12. Use the **X-Y Position** control buttons to find a frame containing one or more appropriate particles. If two or more objectives are being merged, focus for each in turn, starting with the lowest magnification objective. For low magnifications focus on big particles, for 10X and above focus on small particles. **Fit to window** allows the user to navigate the preview image smoothly in order to identify a region of interest on the sample. Once centred the region of interest in the preview, de-select Fit to window. This provides a closer view of the sample, making it easier to obtain a more precise level of focus.

    If needed, the **Reset Focus** button resets the Z position to its reference point using the Z switch.

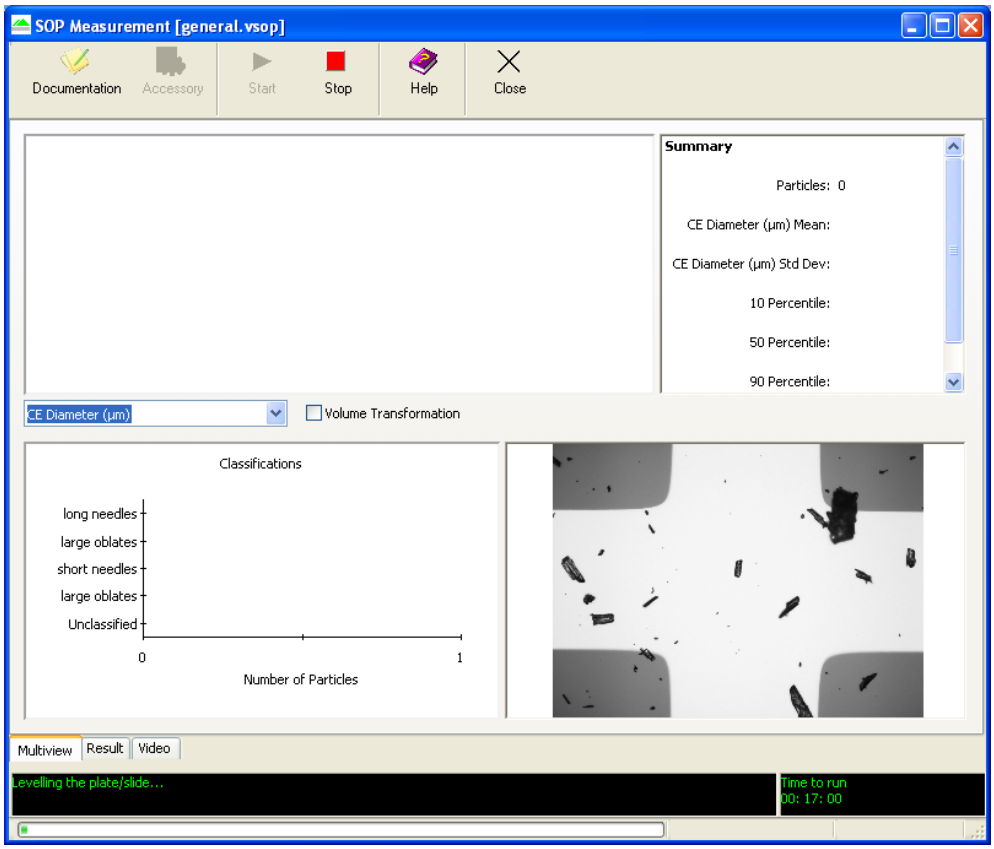
> **Note**
> If the sample is polydisperse, it is not possible to focus simultaneously on both small and large particles. We recommend focusing on the smaller particles, since a focus error on a large particle is less significant than focusing on a large particle and possibly losing the small particle altogether.

13. When the particles are in focus click **OK**.

14. When multiple objectives are selected, steps 10 to 12 are repeated for each objective.

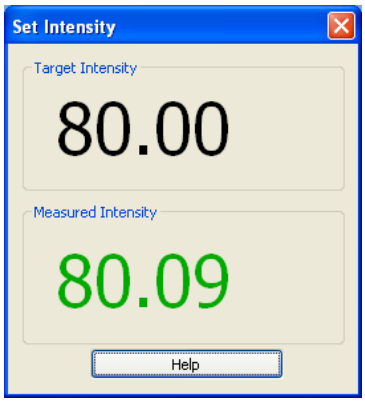
15. The microscope goes to the gratings first, then possibly the Z reference target, before finally moving to the slide scan area.

16. If tilt compensation self-levelling is selected in the SOP, the Live display window and monitor show the instrument focusing on the focusing cross, like this:



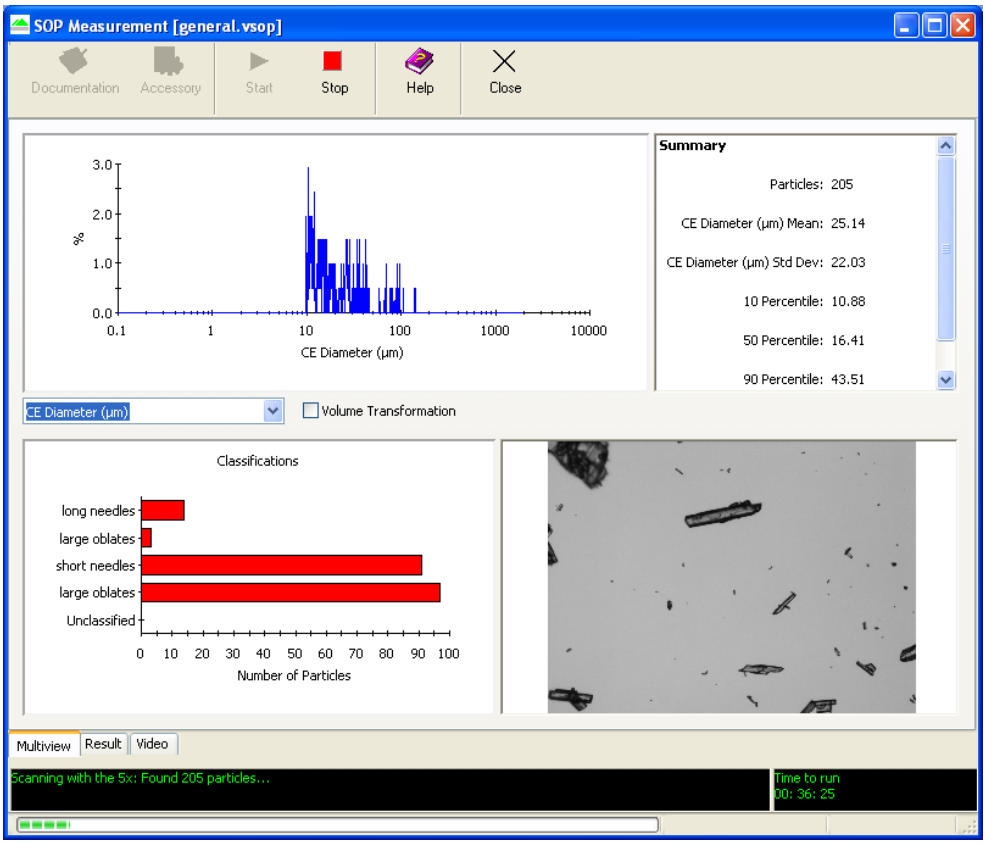
During this step, which takes about three minutes, the status bar says “Levelling the plate/slide...”. Otherwise, a single focusing cross appears for a short time.

17. The microscope moves to the light calibration position and begins calculating the optimum light intensity. The **Status bar** reports “Setting the intensity for the <objective>”. This dialogue shows its progress towards the target intensity:



When the microscope reaches the target intensity it moves straight to the next step.

18. The measurement starts. It may take anything from two minutes to over an hour, depending on the SOP settings. The Status bar and Summary window should show the number of particles analysed increasing.



If more than one optic is selected in the SOP, the light intensity check (step 16) is repeated for each optic.

If more than one slide is required, the user is prompted for this.

19. Once the measurement sequence is complete, any post-measurement information for the SOP is displayed. The operator may need to enter documentation at this stage, as in step 6.

20. The **Measurement Manager** now prompts the user to click **Start** to make another measurement or **Close** to shut the measurement display and return to the main Morphologi G3 application.

The new record will be shown in the **Records tab**. It will be the last record listed.

Part 5: Viewing the results

Introduction

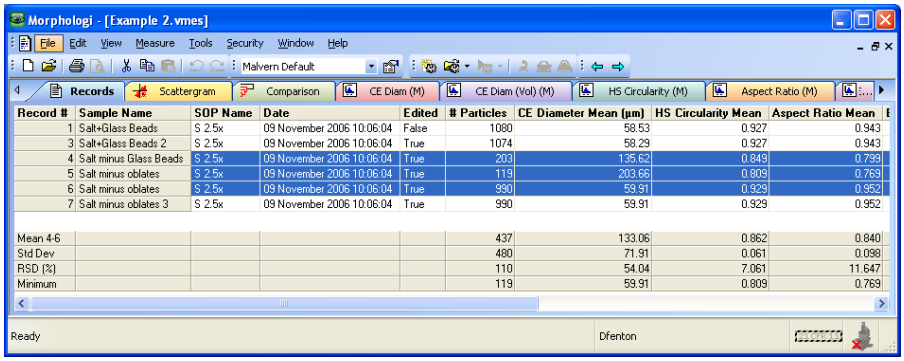
The instructions above show how to test the installation by running a macro and using the supplied sample. Once the instrument is set up, most measurements are made by running a **Standard Operating Procedure** (SOP) as described here.

Malvern® Instruments supplies some default SOPs, others may be created by supervisors/advanced users.

The Records tab

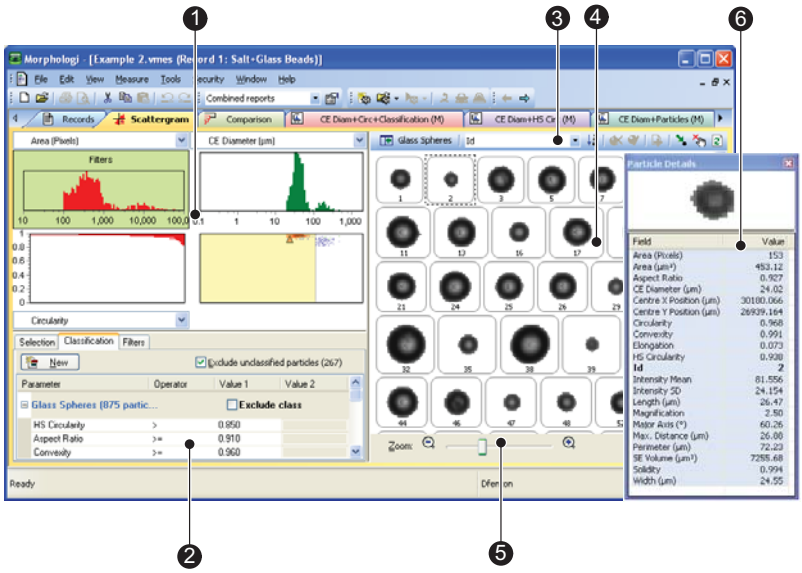
When a measurement is complete, the new measurement record appears in the **Records tab**. By default records appear in the order that they were measured.

The parameters shown are those defined by the workspace. The tab below uses the **Malvern Default** workspace:



The Scattergram tab

With a record selected in the **Records tab**, click the **Scattergram tab** to display images of particles in the sample. Initially all particles are shown, in the order they were detected. This section shows how to refine the selection of particles displayed in various ways. The new particle set produced can be saved as a new record, if required. The **Scattergram tab** displays size distributions, class and filter information and particle images.



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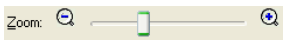
The components are:

① **Scattergram pane** – used to select the parameters used, define filters quickly and select particles of interest. It shows the distribution of particles on a plot of any two selected parameters.

② **Selection**, **Classes and Filters pane** – used to make quick selections based on parameter values, also create or modify classes and filters. (These can also be set up as part of an SOP, as described in **Chapter 6**.)

③ **Particles toolbar** – controls the particle display.

④ **Particles pane** – shows the particle images. The images initially appear in the order that they were detected (i.e. based on their **Id**). The selected parameter value for each particle is shown. This pane initially displays all particles, but as filters are applied and classes defined some may be excluded. A red "X" is used to show an excluded particle, as shown for particles **3** and **4** above. It can also display particles in selected areas of the scattergram only.

⑤ **Zoom control** – drag the slider as required 

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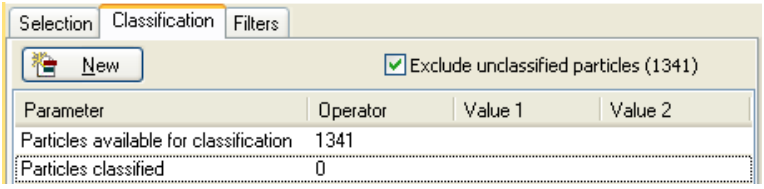
⑥ **Particle Details window** – shows a zoomed image of the selected particle and also its parameter values. Double-click on a particle in the **Particles pane** to open this.

Creating particle filters

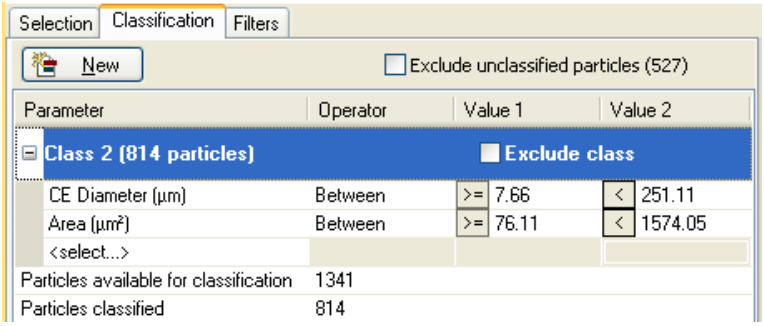
1. Select the parameter to filter on above the major filter graph.
2. Select the **Filters tab**. Any filters already defined in the SOP will be shown.
3. Drag the left and/or right margins inward to filter out the particles in the darker area that this draws.
4. If it's necessary to change the filter, drag again or type a value into the **Value n** box on the Filters tab.
5. A red cross watermark marks the particles excluded by filters in the **Particles pane**. Clicking the  button removes these particles from view in the display.

Defining classes of particle

1. Select the **Classification tab**. This will initially show no classes (unless the SOP defined any):



2. In the scattergram draw a box around the required particles. The **Classification tab** shows the parameter criteria, like this:

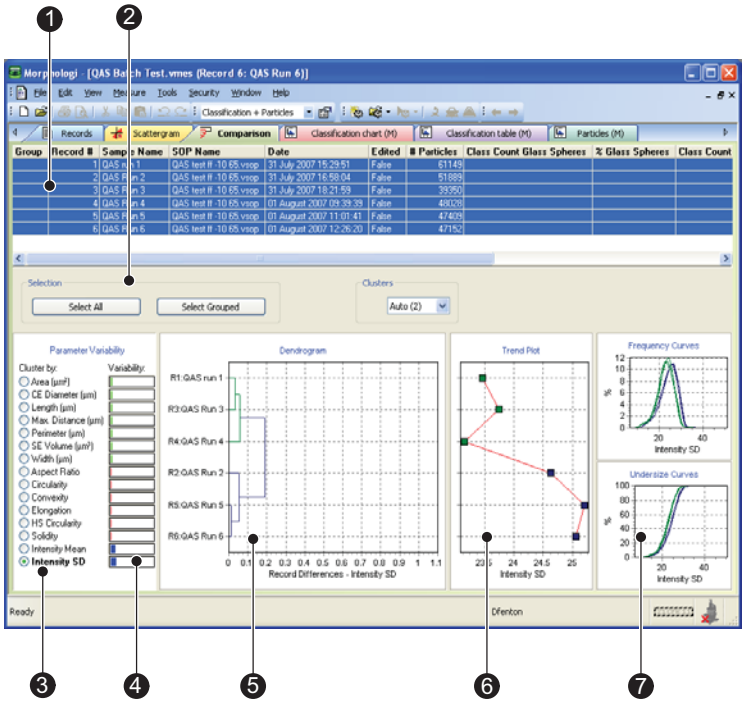


3. If required, change the values by re-dragging the box or by typing values into the Value 1 and Value 2 fields.
4. When the class is defined correctly, rename it as required. Over the default name (Class 2 in the above example) type a name for the class which makes clear what the particles are.

Comparison tab

The **Comparison tab** provides a powerful means of comparing all the morphological distributions of multiple measurements. Use it to:

- Automatically find the morphological parameter that is varying the most between measurements.
- See at a glance how all morphological parameters vary across a set of measurements.
- Automatically cluster measurements according to their similarity.
- Establish how results cluster, based on a chosen morphological parameter.
- Analyse correlations between expected similarities and actual measurements.
- Easily find appropriate morphological pass/fail criteria.



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The components are:

① **Records selected** – shows the records selected in the **Records tab** before the **Comparison tab** was selected.

② **Selection area** – use this to select the record groups to use. Records can be grouped initially by the user, then the groups changed on the basis of the results displayed.

③ **Parameter Variability** radio buttons – used to specify the morphological parameter to display. Use the radio buttons to specify which parameter to base the clustering on.

④ **Parameter Variability** bar charts – show the morphological variability of the selected records. The widths of the bars show which parameter differentiates the most.

⑤ **Dendrogram** – clusters the selected records, showing the degree of similarity between them.

⑥ **Trend plot** – this is useful for setting Process Control thresholds.

⑦ **Frequency and Undersize Curves** – initially the morphological parameter that shows the biggest difference value between two records is selected for showing these two distribution plots. If the user selects a different parameter in -, the curves for that are displayed. The Undersize Curve is also known as a cumulative oversize curve or result-under plot.