# USER MANUAL

# HYDRO INSIGHT

## Vision Analytical Inc.

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# INTRODUCTION

## System Description and Operation

The Hydro Insight particle size and shape analyzer uses Dynamic Image Analysis for measuring particles. It is capable of measuring particles (organic and inorganic) ranging from 1 to 300 µm and 10 to 800 µm in diameter, depending on the optical configuration.

The Hydro Insight's operation is based on a simple, yet efficient process. The sample being analyzed, is recirculated by the Mastersizer 3000 system, and silhouette images are captured and analyzed. The Hydro Insight particle size and shape analyzer is designed to acquire statistically valid measurements of a sample in a very short amount of time; a capability that is essential for quality control purposes in many manufacturing processes. It differs from many microscopy-based systems in that it emphasizes speed and statistical assurance.

Although the Hydro Insight's focus is on speed and simplicity, it offers all the particle characterization capabilities that are needed for most industrial processes. The system offers up to 30 different size and shape measures analyzed in real-time. The results are given instantaneously. No need to wait for data processing after analysis means that users have almost instant feedback on their process.

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## Hydro Insight Advantages

- *Real-time results* - Shape analysis & display shown in real-time as images are captured, providing instant results the moment analysis begins.

- *Obtain meaningful results* – Offering 30 size / shape measures. The ability to correlate multiple shape measures gives unprecedented power to single out key particle shapes of interest.

- *Multi-Run sample trending* - Ability to track size and shape changes over user defined time intervals, for statistical process monitoring and control.

- *Particle Tracking* - Ability to track particles as they pass though the sensing zone.

- *Particle Classification & Identification* - Ability to classify and identify particles based on shape criteria of interest. This is also applicable to the ISO4406 Oil Cleanliness requirements.

- *Real Time backup* - Data mirroring feature to store all analysis data in multiple locations simultaneously offering a real-time backup of data as well as giving lab managers the ability to review results remotely.

- *Statistical assurance* - Recirculating sample and unique optics assures statistical accuracy without the need of costly sheath fluids or complex optical components.

- *Data reporting flexibility* - Data for a sample is saved in the sample file and may be printed or exported to Microsoft XLS (Excel) format. A data summary from each of several runs may be combined into one worksheet.

- *Simulated sieve data* - Size distributions may be reported as fractional parts on a set of sieves, allowing comparison to real sieve data.

- *Rare event detection* - Particle Thumbnail feature extracts individual particle silhouettes from captured images that meet user-defined shape parameters.

- *Sample handling & flexibility* - Aqueous and Organic solvent-compatible system.

- *Security and Regulatory Compliance* - Compliant with the FDA 21 CFR Part 11 regulations with multiple levels of security and robust audit log that tracks all security-related actions.

- *Post Run Processing* – Advanced feature that captures **all** measured particles and saves them for additional viewing and processing after the analysis.

- *Auto-Classification* – Feature allows the analysis of sub-components within the same mixed particle at the click of one button.

- *Time series chart* -- This feature allows the evaluation of behavior from particles suspended in a solution by plotting results of pre-selected measures and statistics from a collection of run files, over a period of time under same conditions and settings.  The run series settings: run duration, delays between runs, measures and statistic can be set in advance. The charts created for each combination of parameters reflects the behavior of particles and can be used in different application and processes like dissolution and crystallization in pharmaceutical industries.

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## Specifications

**Size Range:** depending on optics configuration.

- STANDARD Magnification --- 1 to 300 µm
- LOW Magnification --- 10 to 800 µm

**Image rate:** The image rate of the camera can be up to 127 frames per second depending on camera resolution settings.

**Shape Models:**

- Circle Measures:
  Equivalent circular area diameter, Equivalent circular perimeter diameter, Bounding circle diameter, Mean diameter, Circularity, Smoothness, Compactness

- Ellipse Measures:
  Equivalent elliptical area width, length, Bounding ellipse width, Length, Elliptical aspect ratio, Ellipticity.

- Rectangle Measures:
  Bounding rectangle length, Width, Bounding rectangle aspect ratio; Rectangularity.

- Polygon Measures:
  Polygon order, Interior angles.

- Fiber Measures:
  Fiber length, Width, Fiber aspect ratio, Fiber curl

- Irregular Measures:
  Ferret length, Width, Ferret aspect ratio, Smoothness

- Pixel Intensity:
  Opacity and White fraction

**Fluid compatibility**: Compatible with most common suspension fluids (aqueous and organic). For details, please check the Application Note: Chemical Compatibility of Tygon® sample tubing used on the Mastersizer family of instruments (document MRK1458-02).

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**Voltage**: 100 - 240 VAC, 240 Watts

**Frequency:** 50/60 Hz

**Temperature:** 10 to 45 °C for operation.  -10 to 55 °C for storing or shipping.

**Humidity**: 20 to 80% relative, without condensation

**Width:** 25.4 cm (10 in.)

**Height:** 38.1 cm (15 in.)

**Depth**: 63.5 cm (25 in.)

**Weight:** 13.2 kg (29 lbs.)

**Computer minimum Requirement:**

- 2 GHz CPU speed or better
- 2GB RAM (minimum)
- 250 GB hard drive (minimum)
- Writable CD or DVD drive (optional)
- USB interface (two USB 3.0 connectors, minimum)
- 24" HD LED display.

## Before you start: Warnings and Cautions.

⚠ **WARNING**

A warning message describes either a potentially hazardous situation which, if not avoided, could result in death or serious injury to the operator.

### Electrical Warnings

- Always disconnect the instrument from the main power supply before removing any cover.

- Do not remove any board while instrument is ON.

- The instrument must be grounded correctly.

- Operator shall use appropriately rated and approved supply cord-set in accordance with the regulations of the country it is used in.

### Mechanical Warning

- Do not use force to remove or replace any glass item. In the event of difficulty, consult a service representative from Vision Analytical for assistance.

- This instrument must be used in the manner specified in this operator's manual. Any operation of this instrument outside the specified manner in this operator's manual may impair the instrument and any protection provided, cause damage to the instrument or operator and is, therefore, prohibited.

### Chemical Warning

- Proper handling procedures for diluents and reagents used in particle analysis should be adhered to at all times. Consult appropriate safety manuals and Material Safety Data Sheets for all samples, diluents and reagents used.

- Flammable solutions should be prepared for use in an appropriate environment and brought to the instrument only when required for analysis.

- Take care disconnecting diluent lines. Open-ended tubing may allow liquid to spill out of the vessel.

- Fluid input pressure:
    - Minimum input pressure: 1 bar g (100 kPa g) @ 25 mL/s-1
    - Maximum input pressure: 4 bar g (400 kPa g) @ 25 mL/s-1

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| Label | Text | Location | Meaning |
|---|---|---|---|
|  | None | Fluid Inlet | Samples to be measured may be hazardous if misused. Before use, users must read the Health and Safety information in the Mastersizer 3000 Basic Guide.<br><br>Max input pressure: 4 bar g (400 KPa g) @ 25 mL/s-1 |

- Always handle all substances in accordance with the COSHH (Control Of Substances Hazardous to Health) regulations (UK) or any local regulations concerning sample handling safety.

- Before using any substance, check the Safety Data Sheets (SDS) for safe handling information.

- Use the instrument in a well ventilated room, or preferably within a fume cupboard, if the fumes from the sample or dispersant are toxic or noxious.

- Wear personal protective equipment as recommended by the Safety Data Sheets if toxic or hazardous samples are being handled, particularly during sample preparation and measurement.

- Wear protective gloves when handling hazardous materials, or those that cause skin infections or irritations.

- Do not smoke during measurement procedures, particularly where inflammable samples are used or stored.

- Do not eat or drink during measurement procedures, particularly where hazardous samples are used or stored.

- Take care when handling glass (e.g. microscope slides and beakers). Hazardous materials may enter a wound caused by broken glass.

- Always test a new sample or dispersant for chemical compatibility before use.

- After measuring hazardous samples, scrupulously clean the system to remove any contaminants before making another measurement.

- Always label samples for analysis using industry standard labelling, particularly if they are handled by a number of staff or stored for long periods. Clearly mark any operator hazard and associated safety precautions that are required for the handling of dangerous materials.

- Keep a record of all hazardous substances used in the system for protection of service and maintenance personnel.

- Always adopt responsible procedures for the disposal of waste samples. Most local laws forbid the disposal of many chemicals in such a manner as to allow their entry into the water system. The user is advised to seek local advice as to the means available for disposal of chemical wastes in the area of use. Refer to the Safety Data Sheets.

- The surfaces of the system may be permanently damaged if samples are spilt on them. If a spillage does occur, disconnect the system from the power supply before scrupulously cleaning up the spillage.

## Fire Warning

- Many non-aqueous solutions are flammable. Where possible choose less flammable alternatives.

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⚠ **CAUTION**

A caution message describes a potentially hazardous situation, which if not avoided, may result in minor or moderate injury. It can also be used to alert against unsafe practices.

## Electrical Caution

- If the instrument is exposed to unwanted electrical interferences, the use of a constant voltage transformer or regulator would result in a benefit.

## Chemical Caution

- Drain and Flush the instrument through before and after carrying out a decontamination procedure with bleach.

- Never place containers of liquids on top of the instrument. Repair of instruments damaged or affected by spilled liquids will not be covered by any warranty.

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## Conventions

- The word "instrument" unless otherwise specified, refers to Hydro Insight.

- The PC, keyboard, monitor and printer, etc., are all referred to collectively as "the computer".

- **Bold** and *Italics* type letters refers to either a menu selection, a tool bar item or button text appearing on the screen of the computer that can be selected with the mouse or by keystrokes.

- *Italics* type letters are used to indicate screen messages.

- The symbol "►" indicates the software options you need to select.  e.g.  At the tool bar select **File ► Save.**

- Keys at the computer keyboard are boxed.

- Keys that are to be pressed simultaneously are printed with a sign "+" between the keys.
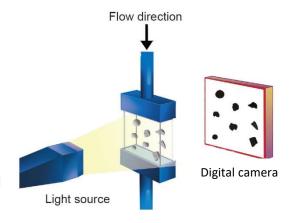
# Chapter 1 - ANALYZER OVERVIEW

## Principle of Operation

Particles flow through a view cell. An illumination source on one side of the flow cell shines light through the cell to a lens and digital camera on the other side. The camera records dark silhouettes of the particles and sends the images to the PC computer in grayscale format.



Software characterizes each particle based on the size and shape of the particle shadows using a pre-selected shape model that is appropriate for the kind of particles being analyzed.

In run mode, the camera images are analyzed as they come in, and the software accumulates statistics on the results of each image analysis. Data on individual particles can be saved while running. The statistical plots and values are shown on-screen while running and updated as the analysis is occurring. At the end of a run the accumulated particle statistics are available for display, printing, or export to Microsoft XLS format.

The image rate of the camera can be up to 127 frames per second depending on camera resolution settings. The software attempts to capture and analyze every camera image but may run at a slower rate depending on the number and type of measures selected and particle density. Higher frame rates can be achieved if the resolution setting of the camera is reduced.

In addition to the normal number histograms, the software generates surface area-weighted and volume-weighted histograms for some of the shape models, making certain assumptions about particle size and shape in the third (unseen) dimension. The recirculating fluidic system presents particles for analysis in a random orientation. This is critical to ensure all dimensions of the particles in question are being measured, not just two dimensions.
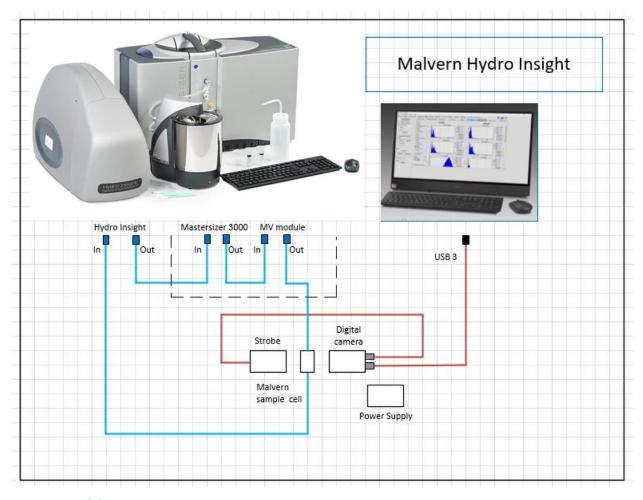
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## System Overview

The Hydro Insight is a full featured Dynamic Image Analyzer that is coupled to a Mastersizer 3000 systems as a complementary measurement accessory. The original sample cell from the Malvern Hydro Sight has been adapted (with minor modifications) to keep the same fluidics properties.

Particles suspended in fluid coming from the Mastersizer 3000, flow through the Malvern Hydro Sight sample Cell. The speed of the pump can be adjusted via software on the Mastersizer 3000.

An illumination source Strobe module on one side of the sample cell shines light through the cell to a lens and Digital Camera on the other side. The camera records dark silhouettes of the particles and sends the images to the PC computer via USB 3.0 serial connection in grayscale format. The application software processes the data and provide the shape information about the particles. A 24V DC Power Supply provides the required voltage for the instrument operation.



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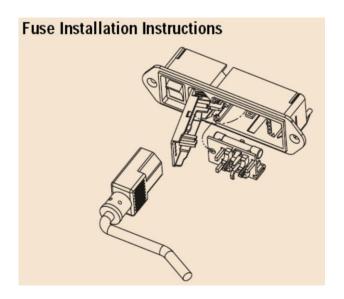
## Initial setup

The Hydro Insight can operate on input voltage from 100 to 240V AC. Therefore, there is no specific voltage configuration. Only the proper fuse arrangement should be observed per the specific country.

Ensure the proper fuse installation for the local AC Voltage as follows:
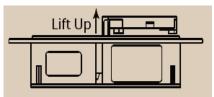
- FUSE, 2.0 Amp, AG, 250V, SLOBLO for 100/120V Operation          Qty. 1
- FUSE, 2.0 Amp, 250V, 5X20MM SLOBLO for 220/240V Operation          Qty. 2

**Fuse Installation Instructions**

- Pry door open at socket.



Insert a pocket screwdriver at point "X" as shown.

Section A-A

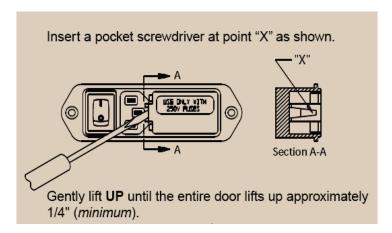Gently lift **UP** until the entire door lifts up approximately 1/4" (*minimum*).

Lift Up
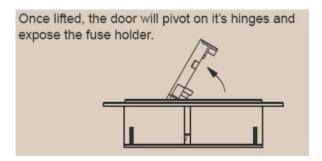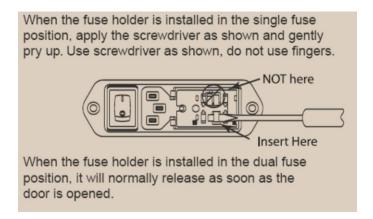
- Lift and swing door into socket.



Once lifted, the door will pivot on it's hinges and expose the fuse holder.

- Lift fuse holder out of housing.



When the fuse holder is installed in the single fuse position, apply the screwdriver as shown and gently pry up. Use screwdriver as shown, do not use fingers.

NOT here

Insert Here

When the fuse holder is installed in the dual fuse position, it will normally release as soon as the door is opened.

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- Install one (1) AG fuse OR two (2) metric fuses according to the country.  Install fuses on one side only, do not install both AG and metric fuses at the same time.



- Replace the fuse holder into housing.
- Swing and snap door back in place.
- Connect the two USB 3.0 cables between the Hydro Insight and Computer.

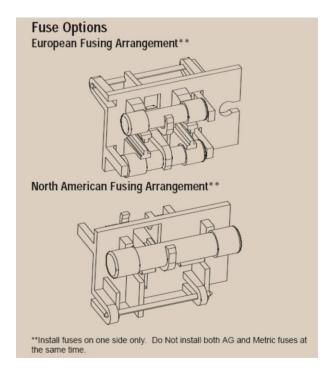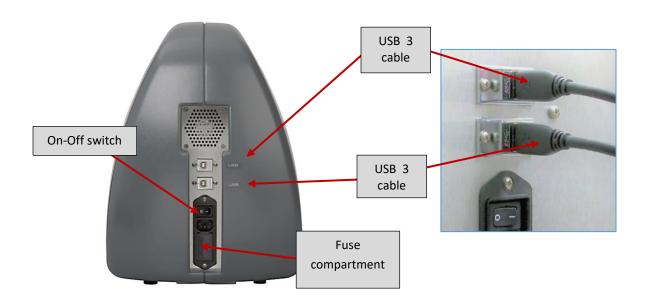## PLUMBING THE SYSTEM

The Hydro Insight must be properly attached to a Mastersizer 3000 system for normal operation.

| No | Connection | No | Connection |
|---|---|---|---|
| 6 | Hydro Insight – Output | 10 | Mastersizer 3000 – Output (Blue) |
| 7 | Hydro Insight – Input (Left) | 11 | Mastersizer 3000 – Input (Yellow) |
| 8 | Hydro MV – Input (Blue) | 12 | Water line |
| 9 | Hydro MV – Output (Yellow) | 13 | Drain |
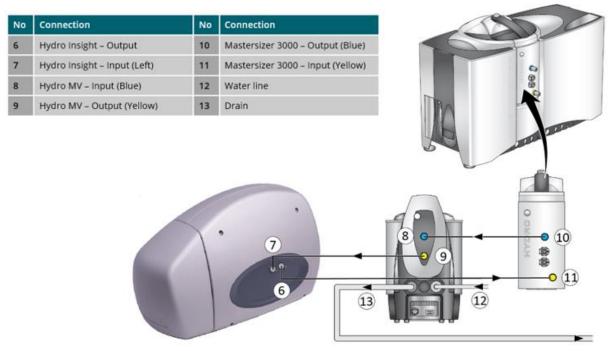
Figure 2. Tubing connections.

If a new installation of the whole system:
- Connect the Hydro MV-Output (9) to the Hydro Insight-Input (7).
- Connect the Hydro Insight-Output (6) to the Mastersizer-Input (11).
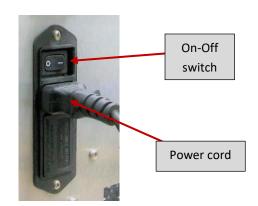
If installation is on existing Mastersizer 3000 customer system:
- Remove the Hydro MV-Output (9) and connect to the Hydro Insight-Input (7).
- Connect the Hydro Insight-Output (6) to the Mastersizer-Input (11).

In summary, the Hydro Insight has been inserted into the yellow line from the Mastersizer 3000 to the MV module.

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On-Off switch

Power cord

- **Connect** the power cable between the Hydro Insight and to the power source 115V AC in the wall.

- Turn the Mastersizer 3000 **On**, then open the application software.
- **Plug** the USB 3 cables between the computer and the Hydro Insight.
- **Verify** the computer recognizes the camera and the internal Memory stick by beeping.
- Turn the Hydro Insight **On**.
- **Open** the Hydro Insight Application software and verify the camera is recognized and the button **Continuous** is activated (not gray out). Once the program starts and finishes initializing, check the bottom status bar for any error numbers.  If no eror numbers are shown, your system should be ready to run.
- **Fill** the system using the Mastersizer 3000 application software.
- Perform a **Clean** system to eliminate any residual debris. The system will remains filled.
- Start circulating clean water in the system.

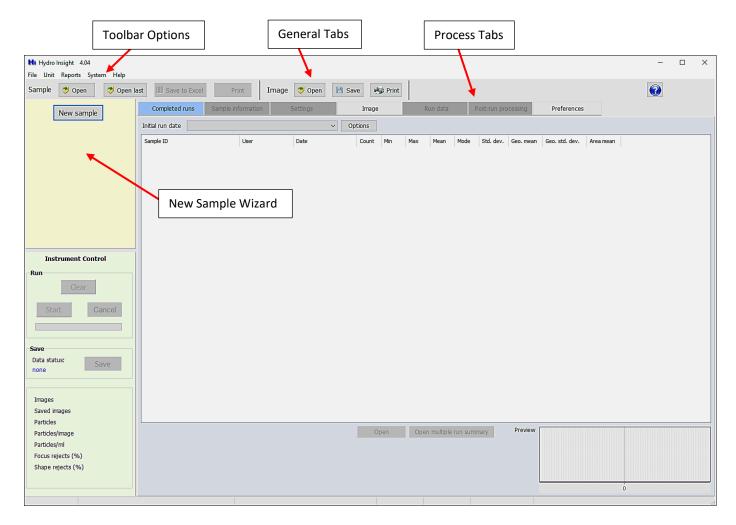At this point, your system is ready and operational.

# Chapter 2 - SOFTWARE OVERVIEW

## User Interface

The software covered in this User Manual is version 4.04. Below is the User Interface used by the Hydro Insight module.
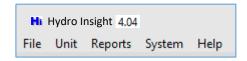


Toolbar Options · General Tabs · Process Tabs

New Sample Wizard



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## Toolbar options

Hi Hydro Insight 4.04
File  Unit  Reports  System  Help

### File                4.04

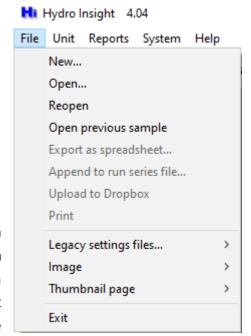**New...** Create a new sample file ready to accept data.

**Open...** Open a sample file or template file.  The current Analysis specifications and Report Options settings will be overwritten with the values stored in the sample file.

**Reopen:** This option reopens the original file if you have made changes to the currently open file**.**

**Save as ...** Save an open sample file under different name.

**Open previous sample:** Open the previously saved sample file.

**Export as spreadsheet ...** Test results will be written to a file in a Microsoft Excel format.  The first worksheet contains the run name, plus the sample documentation.  It then lists system performance data (from the System Data window), and the most important settings affecting the run.  Then there is a separate worksheet for each measure. Each of these worksheets present the statistical means and percentiles, followed by the complete bin-by-bin distribution table.

**Append to run series file ...** A one-line statistical summary of sample data currently showing will be appended to the file specified in *Settings* ➔ *Run Control Specifications* ➔ *Completion Actions* ➔ *Append to run series file* This feature is useful to add statistics from different runs to do comparisons in Excel.

**Upload to Dropbox ...** The current sample file can be uploaded into Dropbox for use with the Hydro Insight smartphone and iPad app.  More information on this discussed further in the manual.

**Print ...** This option prints a report for the currently open sample. The contents of the report are controlled by the settings in *Preferences* ➔ *Printed report*.

**Legacy settings files ...**

**Open** a file with extension *.ac containing analysis specifications previously saved.

**Open** a file with extension *.rc containing run control specifications previously saved.

**Open** a file with extension *.ro that contains the report options.

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**Image:**

**Open Single Image ...** Read in a stored image. The image can be analyzed with the current image analysis settings that are in effect.

**Save Single Image ...** Save the image now showing on the main screen to a disk file, in 8-bit TIFF format. A dialog box will appear, allowing you to enter three lines of descriptive information that will be saved with the image.

**Print Single Image ...** Print the image currently on the main screen. The image will be printed as it is showing on the main screen, using the currently selected image display mode, i.e. full grayscale or black/white. A dialog box allows entering descriptive information that will appear under the printed image. The print may take from a few seconds to several minutes, depending on the amount of darkness in the image.

**Thumbnail page:**

**Open ...** Read in a stored thumbnail page.

**Save ...** Save the currently open thumbnail page.

**Save to Cloud …** Save the currently open thumbnail page to ParticleShape cloud.

**Print ...** Print the thumbnail page currently on the main screen. The image will be printed as it is showing on the main screen.
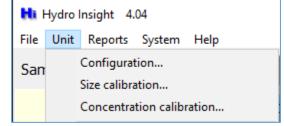
**Exit:** Exit the program.

## Unit

**Configuration ...** Opens the *Settings* tab and shows the *Analysis specificactions*, *Run Control specifications* and *Harware settings.*

**Size Calibration ...** Opens the *Size Calibration* dialog box (see **Calibrate** section of this manual for more information).

**Concentration calibration ...** Open the *Concentration calibration* dialog box. (see **Concentration Calibration** section of this manual for more information).

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## Reports

**Statistic summary file …** Open a sample run file. Select the subrange for size measures to be included in the summary file and then click on *Create file* to save the info in Excel format file. The output file will be a spreadsheet, and the default filename is the current sample name with "ss1" appended.
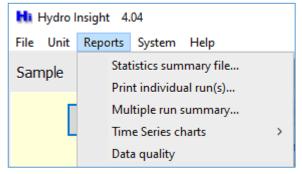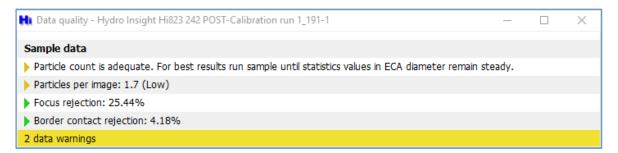
**Print Individual run(s) …** Select one or more sample run files to be printed.

**Multiple run summary …** Select one or more sample run files to be used to create a Run Series file, containing a line of statistical data for each sample. See Creating a multi-run summary section in this manual.

**Time series chart:** This feature allows the evaluation of behavior from particles suspended in a solution by plotting results of pre-selected measures and statistics from a collection of samples run files, over a period of time under same conditions and settings. The run series settings: run duration, delays between runs, measures and statistic can be set in advance. The charts created for each combination of parameters reflects the behavior of particles and can be used in different application and processes like dissolution and crystallization in pharmaceutical industries.

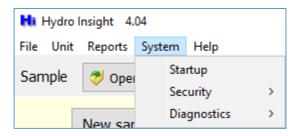Create a *New* series chart, *Open* an existing one and *Close* the currently open series chart can be done.

**Data quality:** This option offers information regarding quality aspects of a currently open runfile.

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## System

**Startup:** Allows you to select the Settings and Preferences files before running a sample.
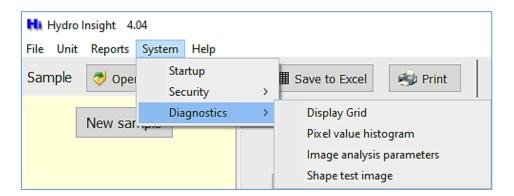


**Security:** Allows to turn the security options **On** or **Off**.

For more detailed information, click HERE to visit the Chapter 8 - *Security*.

**Diagnostics:** Below is an overview of Diagnostic options.

> **Display Grid**: Displays a grid over the current image.  The spacing of the grid is displayed in the main window status bar. To remove the grid, click on this menu item again.



> **Pixel value histogram:** Generates a histogram of gray levels in the image; that is, a graph that shows, for every gray level from 0 to 255, the number of pixels in the image having that value.
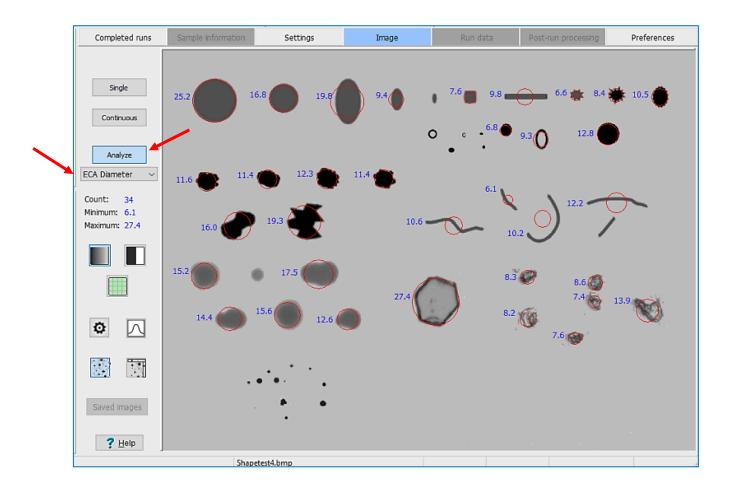
> **Image analysis parameters:** This option allows quick changes to camera parameters. This menu option is the same as clicking the ⚙ gear icon on the user interface.

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**Shape test image**: This image shows the differences among the various measures.

To see the differences visually, click on **Analyze**, to do a single frame analysis. The screen will show the measures selected in the pull-down menu.

To see a different measure, click on the **pull-down menu**, select other measure then click **Analyze** again.

## Help

**Hydro Insight User Manual (pdf):** Opens the Hydro Insight User Manual in Acrobat.

**Getting started:** Shows basic instructions to start running a sample. **BUG**

**Using he Hydro Insight (mp4):** Allows to open a few Video clips showing how to use the instrument. **BUG**

**Post-run Processing Guide (pdf):** Opens a guide on how to use the Post-run Processing options. **BUG**

**Analyzing powders using imaging: BUG**

**Application Notes (web page):** This option contains a link to Vision Analytical website where you can open an Application Notes of your interest.

**Application videos (web page):** This option contains a link to Vision Analytical website where you can open an Application video of your interest.

**Error codes:** This option shows a list of codes for errors that happens during the execution of a command.
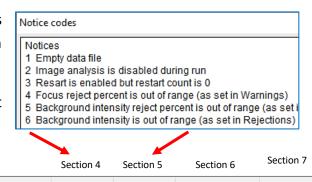
Those codes are shown in the section 4 of the Status bat at the bottom of the screen.

**Notices codes:** This option shows a list of warning codes for notifications delivered to clarify the situation when any action, function or command cannot be completed.

Those codes are shown in the section 5 of the Status bat at the bottom of the screen.

Section 1   tus bar   Section 2          Section 3          Section 4   Section 5   Section 6          Section 7

arm number 2-1

**About Hydro Insight:** Opens the software name and version window.

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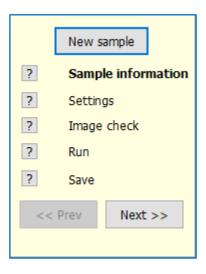
## Wizard for running a new sample.

This wizard walks you through the process to run a new sample.

Click on **New sample** to start the Wizard.

Then, click on **Next** after each step to navigate through the wizard.

If a correction is needed on any information previously entered, click on **Prev** to go to the previous step.

## Step 1 - Sample information

The user should enter a sample name, the file location if other than default, User name and location are optional.

## Next ➜ Step 2 - Settings

The user should select the set of parameters to be included in the analysis.
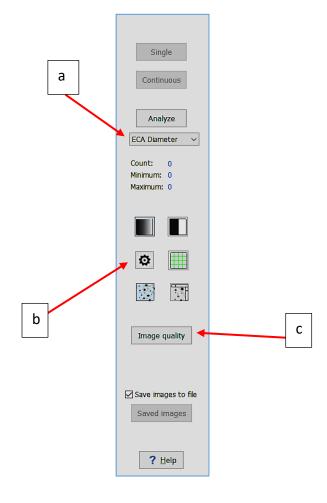
## Next ➜ Step 3 - **Image check**

This step allows the user to select different parameters related to the image like:

a. type of Measurements
b. quick adjust parameters,
c. verify the background histogram.



## Next ➜ **Step 4 - Run**
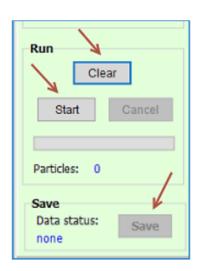
Start the analysis in the Mastersizer 3000.

To start the analysis in the Hydro Insight, click on *Clear* then click on *Start* and the run should start.



## Next ➜ **Step 5 - Save**

After the run is completed, click on *Save* to save the run.

For more detailed information about navigation through the *Wizard*, click HERE

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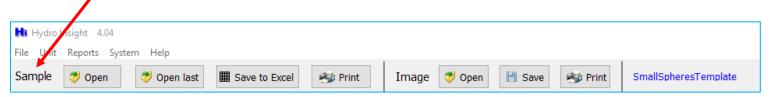
## General Tabs

General tabs are the high levels tabs divided in two categories: Samples and Image.

### Sample



**Open:**  Open a sample file or template file.  The current Analysis Conditions and Report Options settings will be overwritten with the values stored in the sample file.
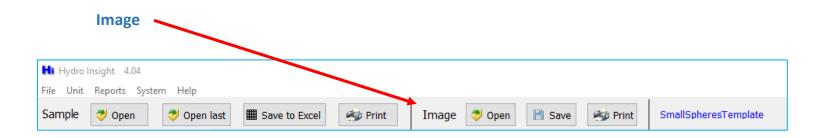
**Open last**: Open the previously saved sample file.

**Save to Excel**: Test results will be written to a file in a Microsoft Excel format. This tab function is the same as **Export as spreadsheet** under **File** in the toolbar.

**Print**: This option prints a report for the currently open sample. The contents of the report are controlled by the settings in *Preferences tab* ➔ *Printed and .xlsx report* ➔ *Report format*.

This tab function is the same as **Print** under **File** in the toolbar.

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## Image



**Open**: This tab function opens an image file. The image can be analyzed with the current image analysis settings that are in effect.

This tab function is the same as **File ➔ Image ➔ Open single image** in the toolbar.

**Save**: Save the image now showing on the main screen to a disk file, in 8-bit TIFF format.

This tab function is the same as **File ➔ Image ➔ Save single image** in the toolbar.

**Print**: Print the image currently on the image monitor. The image will be printed as it is showing on the image monitor, using the currently selected image display mode, i.e. full grayscale or black/white. A dialog box allows entering descriptive information (comments) that will appear under the printed image. The print may take from a few seconds to several minutes, depending on the amount of darkness in the image.

This tab function is the same as **File ➔ Image ➔ Print single image** in the toolbar.

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## Process Tabs

<u>Process tabs</u> are the high-level functions: ***Completed runs, Sample information, Settings, Image, Run data, Post-run processing*** and ***Preferences.***

### Completed runs.
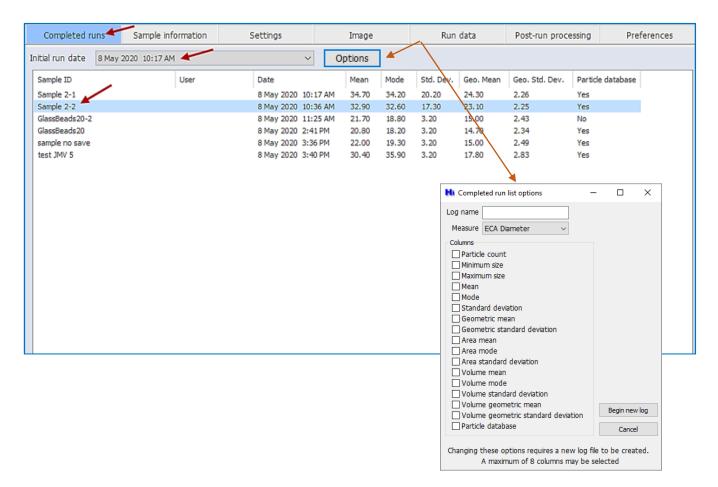
This Tab shows a list of runs completed along with relevant info.

The user can select the ***initial run date*** for that list and the measurements that will be shown by opening ***Options***.

Click on a sample name and see the ***Preview*** of the histogram of the ECA Diameter before open the run.

Double-click on the sample name in the ***Completed runs*** list to open a run.
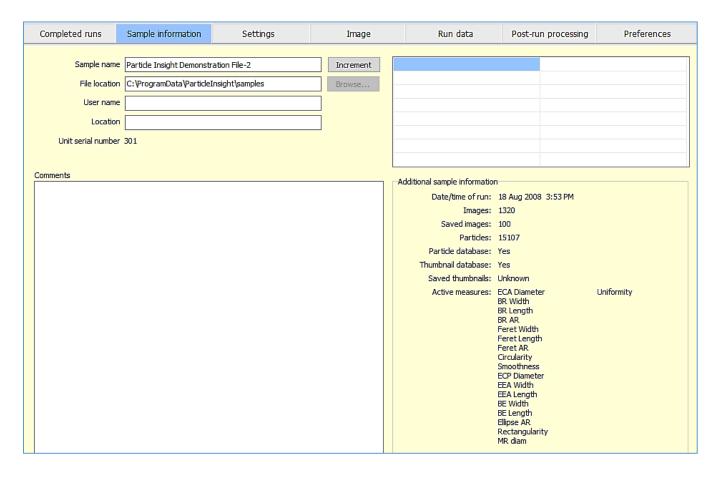
Results will be displayed in the ***Run data*** tab. If <u>individual particle data</u> was saved during the run, the ***Post-run Processing*** tab will also be enabled.

## Sample information

The user can enter the Sample name, the file location where to save the file and add comments.

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## Settings

| Completed runs | Sample information | Settings | Image | Run data | Post-run processing | Preferences |
|---|---|---|---|---|---|---|

```
⊟ Analysis specification
   ⊟ Measures
      • Circle model
      • Ellipse model
      • Rectangle model
      • Polygon model
      • Fiber model
      • Irregular model
      • Pixel intensity
      Image analysis
      Rejections
      Size ranges
      Percentiles
      Sieve sizes
   ⊟ Run control specification
      File locations and mirroring
      Run control
      Completion actions
      Warnings
      Special features
   Hardware configuration
   Hardware settings
```

The user should select the **Analysis specifications** that will apply to the analysis regarding **Measures**, *Image Analysis, Rejections, Size ranges, Percentiles, Sieve sizes, Run Control specification* and *Hardware configuration* and *settings*.

**Measures:**
In this section you select which measures to take data for. The measures are grouped by **Shape models**. Usually a set of analysis conditions is geared toward a particular type of sample and measures that are appropriate for the selected material. For example, spherical beads would normally use only the **Circle model**. It is recommended that no more than 20 measures be active to any specific sample, for speed and file size efficiency.

**Image analysis:** This section, together with Rejections, lists all the parameters that affect the raw results of an image analysis before the shape measures are calculated. These settings let you optimize the analysis for a certain particle type, particle density, and image features (brightness etc.).

**Rejections:** The various rejections are for bypassing particles or image artifacts that would not produce accurate data, such as out-of-focus particles, particles intersecting a border or non-particles ("debris"). The ranges can be restricted in *Rejections ➔ Shape rejection* separately for each FRACTION measure.

**Size ranges:** For size measure types (DIAMETER, LENGTH, WIDTH), specify the sizes range (min-max) that will be accepted. FRACTION type (circularity etc.) normally specifies 0 to 1.

**Percentiles**: The user can select the default percentiles (5) for the measurement.

**Sieve sizes:** In this section, the user must select the sieve sizes to implement on the simulated Sieve axis.

For more detailed information about **simulated Sieve axis**, click HERE.

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**Run Control Specifications:**

These specifications contain run control settings such as number of images or particles to take, and actions to carry out during and at end of run.

**File locations and mirroing:** Show the default file locations for sample files, XLS data files, text data files and database. Also, enable mirroing for sample, XLS and database files.

**Run control:**
- Append a run number to data file name: Append a run number (-n) to the data filename, where "**n**" is initially 1. "**n**" increments whenever ***Increment*** is clicked, or an auto-increment is done in a run series.
- Stop when count reaches limit: Set the limits for when to stop a run according to: number of images, number of particles, or elapsed time.
- Save images to files while running: All images up to the limit previously set, are saved to files on a storage device as they come in. After the maximum count is reached the run continues but no further images are saved. ***'Frame skip count'*** is the number of frames to bypass between captured frames. If ***'Require particle count > 0'*** is Yes, only frames with accepted particles are saved. This option may affect the frames/second rate. Saved images may be reanalyzed post-run under different analysis settings. Each image uses between 1MB and 5 MB of disk or drive storage, depending on camera resolution. For this reason, it may be impractical to save all images that are taken during the analysis. For instance, 100 images could represent about 500 MB of storage space.
- Save individual particle data: The values of all selected measures for each particle are saved while running and may be queried in ***Post-run processing***.
- Save particles thumbnail images: Thumbnail images for each particle are saved to a disk file while running. The image database may be queried in ***Post-run processing***. Take note that the size of this file will be 1 to 2K per particle, depending on particle size. Thus, a run with 20,000 particles will have a thumbnail file size of at least 20 MB if the option is enabled.

**Completion actions:** Set the actions that will be completed automatically at the end of the run. This includes saving sample files and export Excel run reports. The user can select if they want the system to print results at the end of a run. This page allows the user to have several automatic re-starts of the analysis using the same aliquot that is recirculating. Each restarted run represents a separate sample file, with a different number suffix. This is a useful tool to do studies on how particle shape changes over time. It is also useful for repeatability verification. Also, the user can append the run to a Run series file.

**Warnings**: You may specify allowable ranges for ***Focus reject percent, Background intensity reject percent***, and/or ***Dark pixel percent***. Also, ***Inappropriate Threshold*** has default limits.
If the option is enabled and the variable goes outside the allowable range, the value is shown in red in the lower right portion of the display. In all other respects, the run continues normally.

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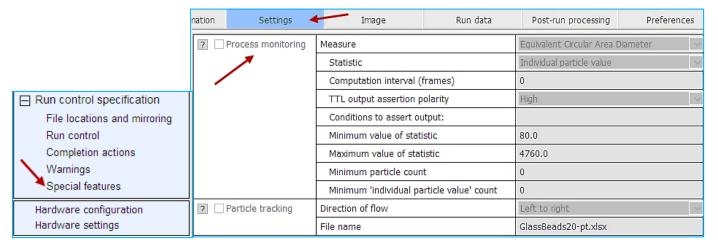
**Special Features**: Contains settings for special features like ***Process monitoring*** and ***Particle tracking.***

### Process Monitoring and Control

This feature can track the ECA Diameter value, or any other active measure value of particles, and generate a TTL output level when any of the following values falls within a specified range:

- Any individual particle measure value.
- Smallest particle measure value over the last N frames (images)
- Largest particle measure value over the last N frames
- Mean or standard deviation of the measure over the last N frames
- D10, D50, D90, DV10, DV50 or DV90 over the last N frames

Statistics (smallest, largest etc.) are recomputed and tested after each computation interval. Specify the computation interval in terms of a frame count (N in the list above).



***Minimum particle count*** refers to the count of all particles in each computation interval (frames count).

- If the count in a specific computation interval does not meet the minimum, the output does not change. In other words, will remain in the current TTL level.
- If the count meets the minimum, the output will change the TTL level and will stay in that level until a subsequent computation interval does not meet the minimum.

***Minimum 'individual particle value' count*** applies only if ***Individual particle value*** is the selected ***Statistic***.

- The count of all particles is reset to 0 after each computation interval is completed.
- To trigger the output on a single particle, set the ***Minimum individual particle count*** to 1.

**FOR MORE INFORMATION ON THIS FEATURE, PLEASE CONTACT VISION ANALYTICAL INC.**

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## Particle Tracking

This feature is used when we wish to determine the speed of relatively slow-moving particles and possibly the change in their size over time. Particle Tracking tracks particles across successive frames, and at the end of run, writes an output text file showing data about the tracked particles.

Assumptions and features:

- Particle concentration should be low.
- Particle size should not change appreciably from one frame to the next.
- To catch all particles, the speed of flow should be less than the image width/frame time. (Frame time can be determined by inverting the frames per second value.)
- The software can track up to 64 particles simultaneously; however, the particle density should be lower than 64 particles per frame for best results.
- All the normal analysis parameters apply, such as size limits and focus rejection.
- If normal size histogram data is also taken, the size data will contain multiple counts for tracked particles. Therefore, generating the normal size statistics is not recommended. The particle tracking data file contains information about particle size (but no histograms are computed).
- The direction of flow (left to right, right to left) must be defined if other than default direction (top to bottom).

The direction of flow and output filename should be entered before the run in **Settings ➔ Run control specification ➔ Special Features**.



For every tracked particle, the output file lists:
- the frame number in which it was first seen.
- the offset (in microns) from the centerline of the flow.
- the ECA diameter (in microns) of the particle the last time it was seen.
- the velocity of it as determined by the last two times it was seen.
- the number of images it was seen in.
- Up to four active measures previously selected before starting the run, right after click on **Clear** and before click on **Start**.

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The tracking file is written automatically when the sample is saved. This is what a typical tracking file looks like:

| | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | | | | | | | |
| 2 | 2 | 2 | | | | | | | |
| 3 | 2 | 2 | | | | | | | |
| 4 | Total images: | | 29 | | | | | | |
| 5 | Elapsed seconds: | | 6.2 | | | | | | |
| 6 | Tracked particles: | | 484 | | | | | | |
| 7 | | | | | | | | | |
| 8 | frame | offset | diam | velocity | frames | BR Width | BR Length | BR AR | Rect |
| 9 | 2 | 0.818 | 14.23 | 3.85 | 2 | 9.8 | 19.7 | 2.0 | -1.0 |
| 10 | 1 | 0.020 | 30.34 | 1.02 | 3 | 25.1 | 41.7 | 1.7 | 0.7 |
| 11 | 2 | 0.040 | 126.23 | 2.53 | 2 | 139.9 | 170.0 | 1.2 | 0.5 |
| 12 | 1 | 0.629 | 84.85 | 4.17 | 3 | 80.3 | 106.4 | 1.3 | 0.7 |
| 13 | 2 | 0.764 | 33.36 | 3.15 | 2 | 27.8 | 47.1 | 1.7 | 0.7 |
| 14 | 2 | 0.757 | 6.77 | 0.06 | 3 | 6.3 | 7.3 | 1.1 | -1.0 |
| 15 | 2 | 0.592 | 8.88 | 0.06 | 3 | 8.0 | 9.6 | 1.2 | -1.0 |
| 16 | 2 | 0.012 | 32.33 | 0.07 | 3 | 34.6 | 40.4 | 1.2 | 0.6 |
| 17 | 3 | 0.163 | 10.09 | 0.06 | 2 | 9.1 | 12.4 | 1.4 | -1.0 |
| 18 | 2 | 0.701 | 9.71 | 0.06 | 3 | 8.6 | 10.6 | 1.2 | -1.0 |
| 19 | 4 | 0.289 | 8.81 | 0.04 | 2 | 6.5 | 12.5 | 1.9 | -1.0 |
| 20 | 2 | 0.026 | 29.79 | 0.04 | 4 | 24.3 | 41.3 | 1.7 | 0.7 |
| 21 | 4 | 0.074 | 10.22 | 0.04 | 2 | 7.5 | 13.1 | 1.7 | -1.0 |
| 22 | 2 | 0.693 | 93.37 | 9.73 | 5 | 92.1 | 121.1 | 1.3 | 0.6 |
| 23 | 4 | 0.232 | 9.10 | 0.24 | 3 | 7.5 | 10.6 | 1.4 | -1.0 |
| 24 | 5 | 0.856 | 163.32 | 0.24 | 2 | 179.7 | 229.3 | 1.3 | 0.5 |
| 25 | 5 | 0.916 | 85.30 | 3.53 | 2 | 103.6 | 110.0 | 1.1 | 0.5 |
| 26 | 4 | 0.295 | 53.39 | 3.36 | 4 | 53.1 | 64.2 | 1.2 | 0.6 |
| 27 | 2 | 0.170 | 69.91 | 2.37 | 6 | 54.8 | 112.1 | 2.0 | 0.6 |
| 28 | 3 | 0.196 | 11.45 | 0.22 | 7 | 7.7 | 17.6 | 2.3 | -1.0 |
| 29 | 4 | 0.106 | 83.72 | 2.36 | 6 | 85.5 | 100.2 | 1.2 | 0.6 |
| 30 | 4 | 0.109 | 13.68 | 0.15 | 7 | 12.9 | 14.6 | 1.1 | -1.0 |

**Hardware configuration & Hardware settings**

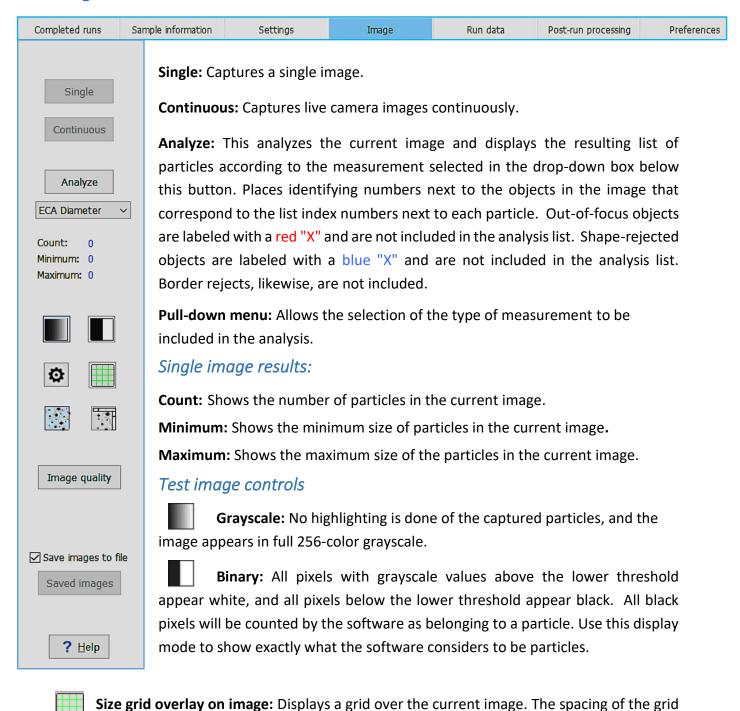Contains Hardware settings related to Camera and Dilution.

**Open settings …**       Open a previously saved Setting file with extension ".psf"
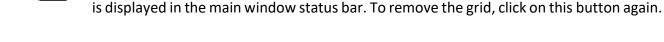
**Save settings**         Save the changes in settings to the currently open setting file.

**Save settings as …**    Save the current settings into a file you designate with different filename.

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## Image

| Completed runs | Sample information | Settings | Image | Run data | Post-run processing | Preferences |
|---|---|---|---|---|---|---|

**Single:** Captures a single image.

**Continuous:** Captures live camera images continuously.

**Analyze:** This analyzes the current image and displays the resulting list of particles according to the measurement selected in the drop-down box below this button. Places identifying numbers next to the objects in the image that correspond to the list index numbers next to each particle. Out-of-focus objects are labeled with a red "X" and are not included in the analysis list. Shape-rejected objects are labeled with a blue "X" and are not included in the analysis list. Border rejects, likewise, are not included.

**Pull-down menu:** Allows the selection of the type of measurement to be included in the analysis.

### Single image results:

**Count:** Shows the number of particles in the current image.

**Minimum:** Shows the minimum size of particles in the current image.

**Maximum:** Shows the maximum size of the particles in the current image.

### Test image controls

**Grayscale:** No highlighting is done of the captured particles, and the image appears in full 256-color grayscale.

**Binary:** All pixels with grayscale values above the lower threshold appear white, and all pixels below the lower threshold appear black. All black pixels will be counted by the software as belonging to a particle. Use this display mode to show exactly what the software considers to be particles.

**Size grid overlay on image:** Displays a grid over the current image. The spacing of the grid is displayed in the main window status bar. To remove the grid, click on this button again.

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Single

Continuous

Analyze

ECA Diameter

Count: 0
Minimum: 0
Maximum: 0

Image quality

☑ Save images to file
Saved images

? Help

**Camera Parameters quick adjust settings:**



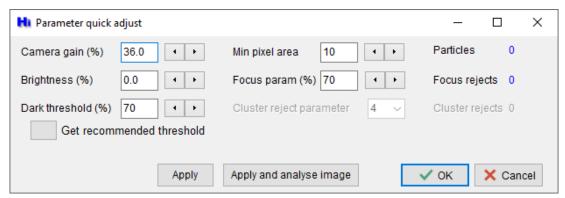**Image quality:** Displays a histogram graph of all pixel values in the image. This is useful for checking uniformity of brightness and background gray level. For more on this subject, refer to the Advanced Tasks and Procedures sections of this manual.

**Zoom in** the image.

**Zoom out** the image.

Save images to file.

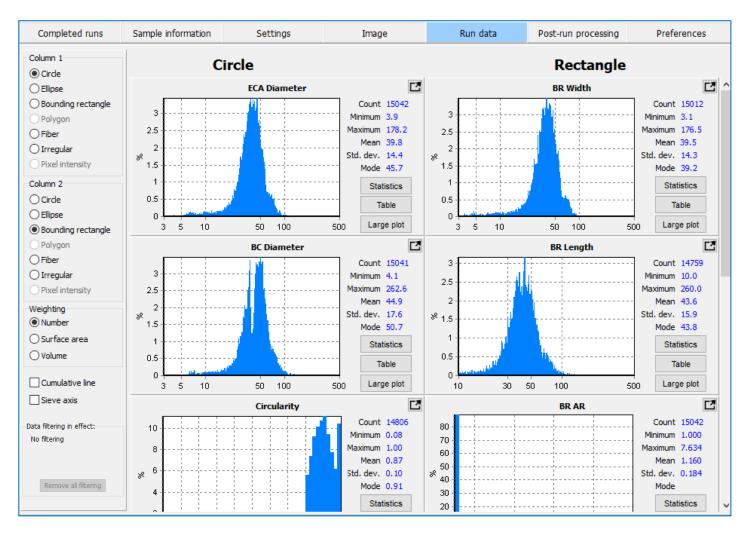Present images in a Show mode for images previously analyzed and saved.

Additional help related to imaging processing and parameters.

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## Run data

The user should select the **Shape Models** (*Circle, Ellipse, Bounding rectangle, Polygon, Fiber, Irregular and Pixel Intensity*) for each of the two columns of data, as well as the plot **_Weighting_** (*Number, Surface area and Volume*) that will apply to the analysis result shown in screen below.



Use the vertical scroll bar to view ALL active measures (Shape models).

- Circle model:
  ECA diameter (Equivalent Circular Area), BC diameter (Bounding Circle),  ECP diameter (Equivalent Circular Perimeter), Perim Circ (Perimeter Circularity), MR diameter (Mean radius), Circularity, Smoothness, Compactness.
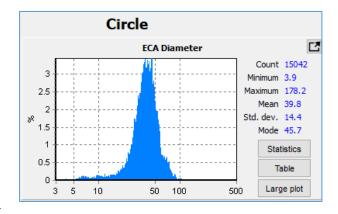
- Ellipse model:
  EEA Width, EEA Length (Equivalent elliptical area) width, length, BE Width, BE Length (Bounding ellipse) width, Length, Ellipse AR (Ellipse aspect ratio), Ellipticity.

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- Bounding Rectangle model:
  BR Width, BR Length (Bounding rectangle) Width, Length, BR AR (Bounding rectangle aspect ratio); Rectangularity
- Polygon model:
  Polygon order, Interior angles.
- Fiber model:
  Fiber Width, Length, Fiber aspect ratio, Fiber curl and Third dimension shape.
- Irregular model:
  Ferret Width, Ferret Length, Ferret aspect ratio, Martin Width, Martin Length, Surface uniformity.
- Pixel intensity:
  Opacity and White fraction

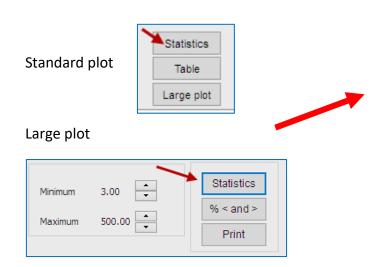For more detailed information, click HERE to go to the **Shape Models** section.

To view the results in detail, click on **Statistic**, **Table** or **Large plot**.
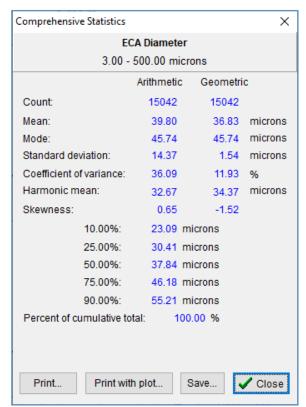
## Statistic

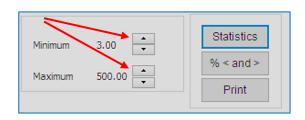Click **Statistics** button either in the standard plot OR the Large plot to open the **Comprehensive Statistics** window. This window displays statistics for the defined range.

Standard plot

Large plot



**Circle**

ECA Diameter

| | |
|---|---|
| Count | 15042 |
| Minimum | 3.9 |
| Maximum | 178.2 |
| Mean | 39.8 |
| Std. dev. | 14.4 |
| Mode | 45.7 |

**Comprehensive Statistics**

ECA Diameter
3.00 - 500.00 microns

| | Arithmetic | Geometric | |
|---|---|---|---|
| Count: | 15042 | 15042 | |
| Mean: | 39.80 | 36.83 | microns |
| Mode: | 45.74 | 45.74 | microns |
| Standard deviation: | 14.37 | 1.54 | microns |
| Coefficient of variance: | 36.09 | 11.93 | % |
| Harmonic mean: | 32.67 | 34.37 | microns |
| Skewness: | 0.65 | -1.52 | |
| 10.00%: | 23.09 | microns | |
| 25.00%: | 30.41 | microns | |
| 50.00%: | 37.84 | microns | |
| 75.00%: | 46.18 | microns | |
| 90.00%: | 55.21 | microns | |
| Percent of cumulative total: | 100.00 % | | |

Minimum 3.00
Maximum 500.00

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If the size axis is not a sieve size axis, you can compute these statistics for a user-defined sub range of the Large plot. When first opening a plot, the endpoints contain the entire data range for the current plot, as defined in the settings. To change the analysis range, edit the endpoints above the plot, then click **Statistics**. You can also change the sub range by dragging the red cursor lines.
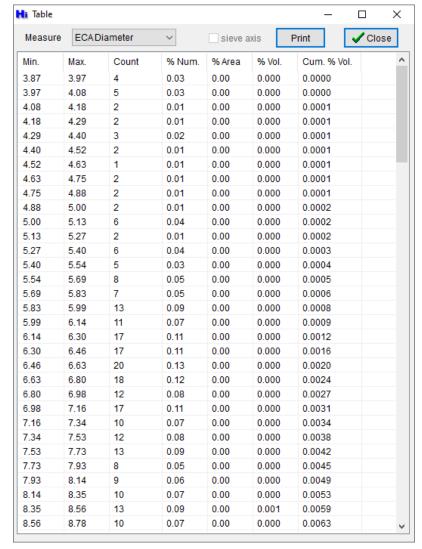
Using buttons at the bottom of the statistics window, you can save this data to a text file with suffix .CSV, print it, or print it along with a small version of the plot.

## Distribution Table
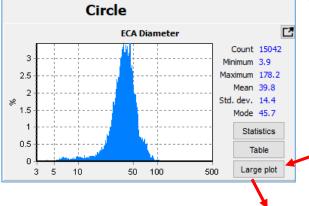
Click **Table** to the right of a data plot to get a bin-by-bin listing of histogram data.

**Table** — Measure: ECA Diameter — sieve axis — Print — Close

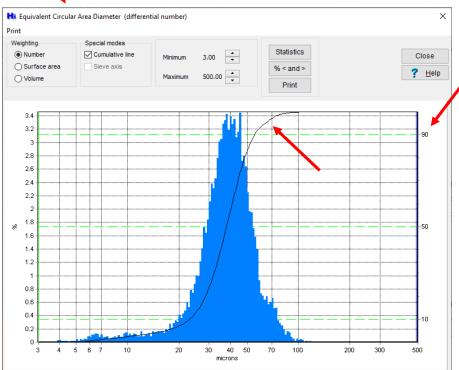| Min. | Max. | Count | % Num. | % Area | % Vol. | Cum. % Vol. |
|------|------|-------|--------|--------|--------|-------------|
| 3.87 | 3.97 | 4 | 0.03 | 0.00 | 0.000 | 0.0000 |
| 3.97 | 4.08 | 5 | 0.03 | 0.00 | 0.000 | 0.0000 |
| 4.08 | 4.18 | 2 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.18 | 4.29 | 2 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.29 | 4.40 | 3 | 0.02 | 0.00 | 0.000 | 0.0001 |
| 4.40 | 4.52 | 2 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.52 | 4.63 | 1 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.63 | 4.75 | 2 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.75 | 4.88 | 2 | 0.01 | 0.00 | 0.000 | 0.0001 |
| 4.88 | 5.00 | 2 | 0.01 | 0.00 | 0.000 | 0.0002 |
| 5.00 | 5.13 | 6 | 0.04 | 0.00 | 0.000 | 0.0002 |
| 5.13 | 5.27 | 2 | 0.01 | 0.00 | 0.000 | 0.0002 |
| 5.27 | 5.40 | 6 | 0.04 | 0.00 | 0.000 | 0.0003 |
| 5.40 | 5.54 | 5 | 0.03 | 0.00 | 0.000 | 0.0004 |
| 5.54 | 5.69 | 8 | 0.05 | 0.00 | 0.000 | 0.0005 |
| 5.69 | 5.83 | 7 | 0.05 | 0.00 | 0.000 | 0.0006 |
| 5.83 | 5.99 | 13 | 0.09 | 0.00 | 0.000 | 0.0008 |
| 5.99 | 6.14 | 11 | 0.07 | 0.00 | 0.000 | 0.0009 |
| 6.14 | 6.30 | 17 | 0.11 | 0.00 | 0.000 | 0.0012 |
| 6.30 | 6.46 | 17 | 0.11 | 0.00 | 0.000 | 0.0016 |
| 6.46 | 6.63 | 20 | 0.13 | 0.00 | 0.000 | 0.0020 |
| 6.63 | 6.80 | 18 | 0.12 | 0.00 | 0.000 | 0.0024 |
| 6.80 | 6.98 | 12 | 0.08 | 0.00 | 0.000 | 0.0027 |
| 6.98 | 7.16 | 17 | 0.11 | 0.00 | 0.000 | 0.0031 |
| 7.16 | 7.34 | 10 | 0.07 | 0.00 | 0.000 | 0.0034 |
| 7.34 | 7.53 | 12 | 0.08 | 0.00 | 0.000 | 0.0038 |
| 7.53 | 7.73 | 13 | 0.09 | 0.00 | 0.000 | 0.0042 |
| 7.73 | 7.93 | 8 | 0.05 | 0.00 | 0.000 | 0.0045 |
| 7.93 | 8.14 | 9 | 0.06 | 0.00 | 0.000 | 0.0049 |
| 8.14 | 8.35 | 10 | 0.07 | 0.00 | 0.000 | 0.0053 |
| 8.35 | 8.56 | 13 | 0.09 | 0.00 | 0.001 | 0.0059 |
| 8.56 | 8.78 | 10 | 0.07 | 0.00 | 0.000 | 0.0063 |

## Large plot

Click on **Large plot** to the right of a data plot to get a larger display of analysis results with more details can be achieved by click on Large plot.



You can have a maximum of eight large windows open at once. The window can be resized by dragging any edge or corner of the window.
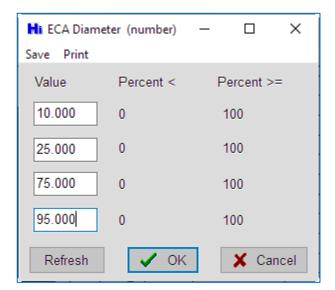


The **Number, Surface area**, and **Volume** options control the moment weighting of the size distribution. The **Cumulative line** option overlays a cumulative line on the plot, which is essentially the integral of the density distribution. The percent scale on the right axis relates to this line, and any point on the line can be interpreted as a percentile. For example, the 50th percentile size is the point on the x axis where the line reaches 50% on the y axis.

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**% <= and > :**



Enter up to four measure values, and the percent of the total by number that is less than or equal to, and greater than, each entered value will be presented. The calculation is over the entire allowed range of the measure. The calculation is only as precise as the histogram bin boundaries.



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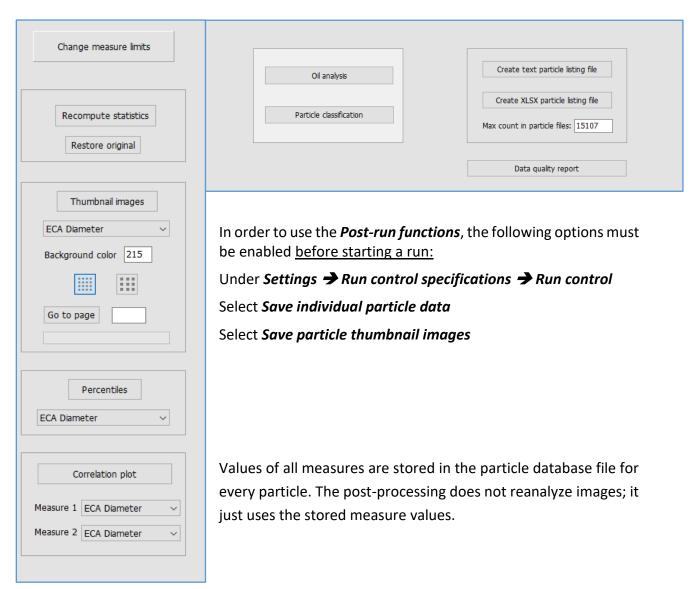
## Post-run processing

The Hydro Insight provides several ways to present data from a run beyond the normal data windows if the particle data and the thumbnail images have been saved. With each of these operations, you can request that the operation present a subset of the run particles, based on entered selection criteria.
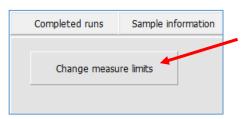
These presentations include a text file which lists data for each particle, thumbnail images of all particles, correlation data between two measures, and a re-creation of the distribution graphs using only the selected subset of particles.



In order to use the **Post-run functions**, the following options must be enabled <u>before starting a run</u>:

Under **Settings** ➜ **Run control specifications** ➜ **Run control**

Select **Save individual particle data**

Select **Save particle thumbnail images**

Values of all measures are stored in the particle database file for every particle. The post-processing does not reanalyze images; it just uses the stored measure values.

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## Change Measure Limits

Click on **Change Measure Limits** to specify a reduced range for any measure. Check the box in front of the desired measure and enter the new limits. Only the checked measures will be filtered; all others will be included at their full range. Be aware though, that limiting the range of one measure may reduce the observed range of other measures.

| | | |
|---|---|---|
| Completed runs | | Sample information |
| | Change measure limits | |

**Filter criteria**

| Measure | Min | Max | Measure | Min | Max |
|---|---|---|---|---|---|
| ☑ ECA Diameter | 3.0 | 500.0 | ☐ BR AR | 1.0 | 10.0 |
| ☐ ECP Diameter | 3.0 | 500.0 | ☐ Rectangularity | 0 | 1.000 |
| ☐ MR diam | 3.0 | 500.0 | ☐ Feret Width | 3.0 | 500.0 |
| ☐ Circularity | 0 | 1.000 | ☐ Feret Length | 10.0 | 500.0 |
| ☐ Smoothness | 0 | 1.000 | ☐ Feret AR | 1.0 | 10.0 |
| ☐ EEA Width | 3.0 | 500.0 | ☐ | | |
| ☐ EEA Length | 10.0 | 500.0 | ☐ | | |
| ☐ BE Width | 3.0 | 500.0 | ☐ | | |
| ☐ BE Length | 10.0 | 500.0 | ☐ | | |
| ☐ Ellipse AR | 1.0 | 10.0 | ☐ | | |
| ☐ BR Width | 3.0 | 500.0 | ☐ | | |
| ☐ BR Length | 10.0 | 500.0 | ☐ | | |

☑ Focus parameter   20   %       ✗ Cancel    ✓ OK

New Statistics and Percentiles change the results showing in the data windows, if any of the measures are given a reduced range.

A * in front of the sample name means that the results showing may have been altered from the original results for the sample due to filtering. To save the filtered results, use **Save As. BUG**
However, the sample file still contains the original results, which may be restored on screen using **Reopen**.

**Max particle count** is an upper limit on the number of selected particles that is used. The actual number of particles selected may be smaller than this number. The number of particles selected will always be equal to or smaller than the count in the particle database, which is shown at the top right of the dialog box.

| |
|---|
| Create text particle listing file |
| Create XLSX particle listing file |
| Max count in particle files: 15107 |

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## Recompute statistics
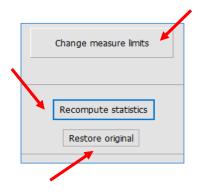
This option will clear the data graphs and statistics and regenerate them using the limits set for the measures.

To enable this action, select the option below, <u>before starting the run</u>:
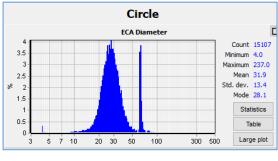
Under **Settings ➔ Run control specifications ➔ Run control**

Check **Save individual particle data**

**Note:** Polygon Angles data cannot be regenerated because the particle database does not save that information.



**Run data**



**Post-run processing**
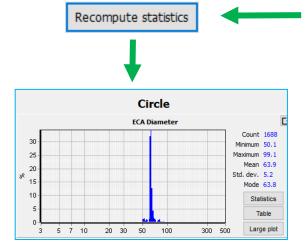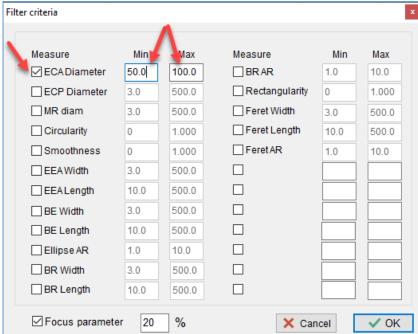


The newly created results data may be printed and/or saved under a new sample name using **Save As**. **BUG**

The sample name in the user interface will have an asterisk (*) prepended, indicating that the results for that sample name have changed.

To restore the original sample data in the user interface, use **Restore original**.

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## Displaying particle thumbnail images

By selecting the **Thumbnail image** feature in the Post Run Processing window, you view small images of all accepted particles, even if the full run images are not saved.

To enable this action, select the option below, <u>before starting the run</u>:
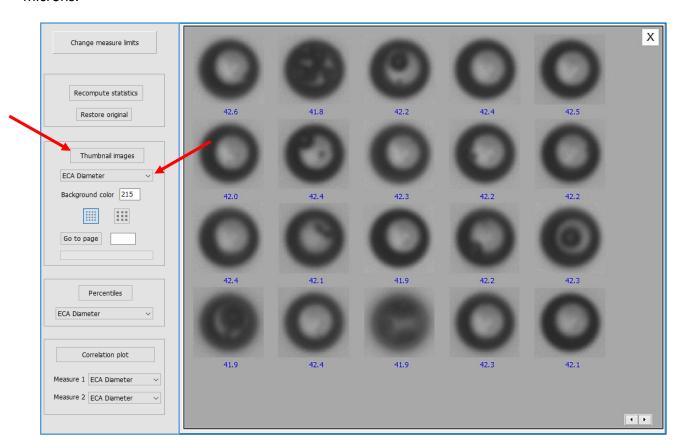
Under **Settings ➔ Run control specifications ➔ Run**

Check **Save particle thumbnail images**.

There is an upper size limit of 200 MB on the file that saves the thumbnails.
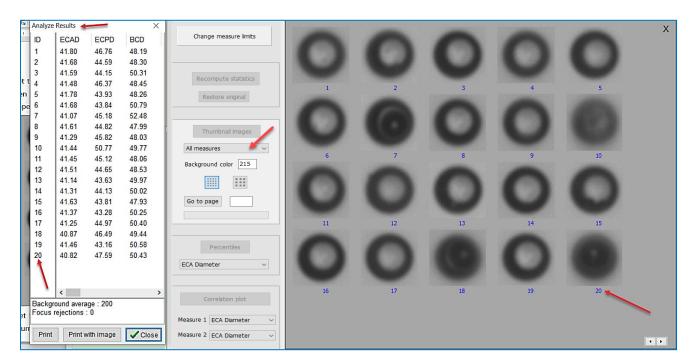
Click on **Thumbnail images** to display the selected particles in decreasing size order. Select the **Measure** to be applied to the current data in the **Pull-down menu**.

The example below shows particle thumbnails of Glass beads with an ECA diameter around 42 microns.



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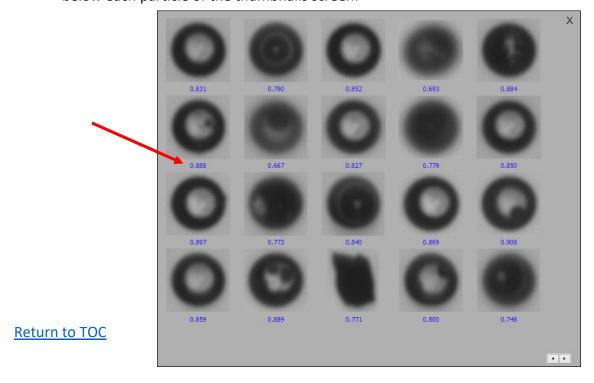
1. If the drop-down menu is set to "**All measures**" before opening the ***Thumbnail images*** button, the thumbnails screen has an **ID** numbers below each particle, referencing the **Analyze Results** list which appears at the left of the screen.
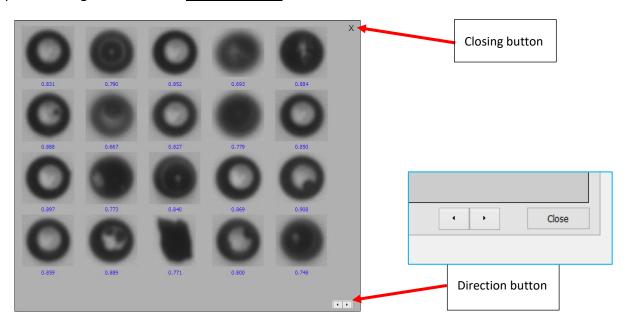


2. If the drop-down menu is set to a particular measure, the values of that measure are below each particle of the thumbnails screen.



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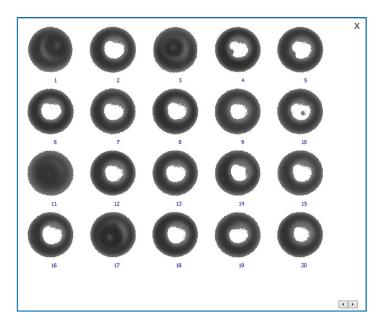
Use the **Direction button** to step through the pages of thumbnails. There is an upper limit of 256 thumbnail pages, and the limit on the number of thumbnails is 100,000.

Any page may be saved as a TIFF image using *File* ➔ *Image* ➔ *Save single image ...* in the toolbar options or printed using *Print*. The TIFF file will not have the number overlays, but they will show on the printed image. Use the "**X**" **Closing button** to close out the thumbnail screen.



Closing button

Direction button
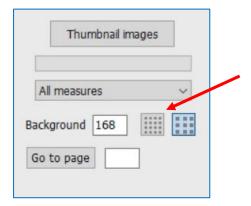
You can select a white background in *Preference* ➔ *User interface* ➔ *White Background in Thumbnail pages*



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Thumbnails may be displayed at a **reduced** size or at **full** size
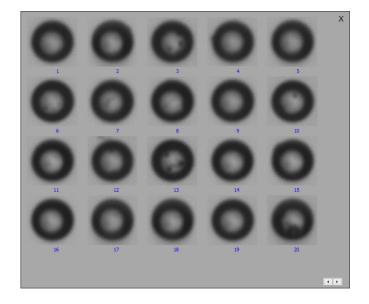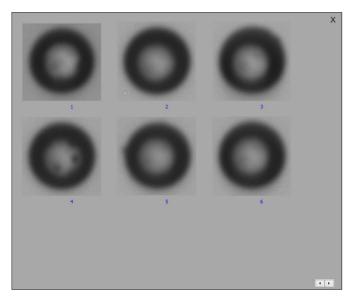


For reduced size: click the icon           For full size: click the icon 



Reduced size                                      Full size
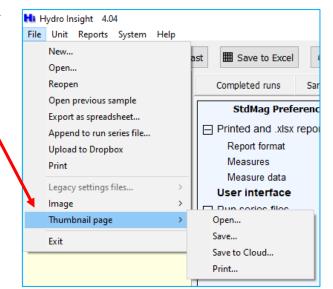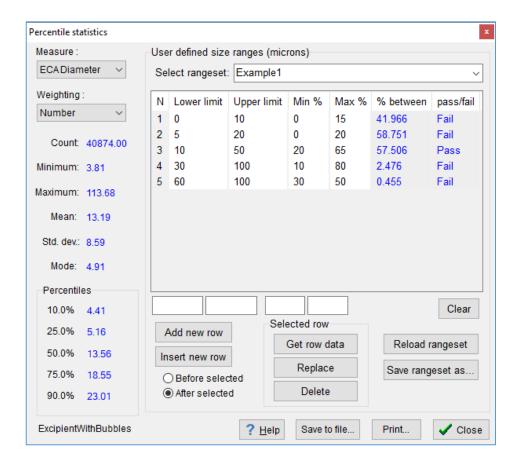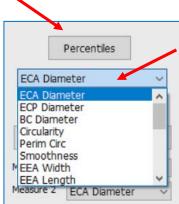
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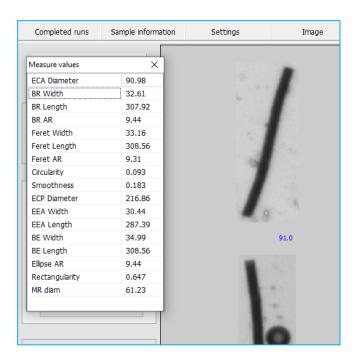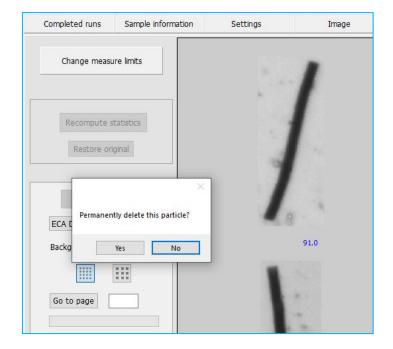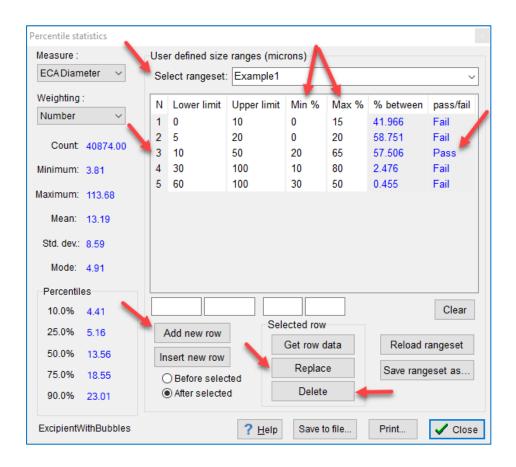
## Using Percentiles statistics

In the production of powders, it is often necessary to find out how much of a sample lies between two cumulative percentile points in the size distribution, and often several such determinations are desired. The **Percentiles** feature can do this determination. In addition, if you specify upper and lower bounds for the percent in each percentile band, the screen will show whether the sample lies within the bounds of acceptance **Pass/Fail**.   Select the measurement to be applied to the current data in the **Pull-down menu** before pressing **Percentiles**.
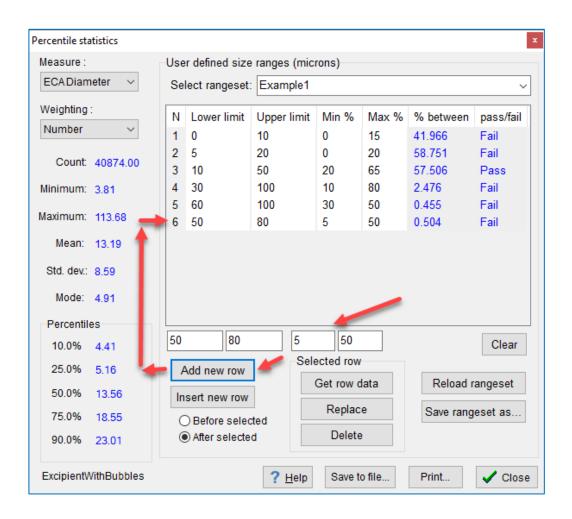
- Each row of the table represents a percentile band, with the upper and lower limits of acceptance.

- There are features that allow **adding** a new row and **replacing** or **deleting** a row.

- Additionally, for convenience the whole table of limits is savable as a **Rangeset** file.

## Adding a new row

- Enter the values for: Lower limit, Upper limit, Min % and Max % at the four blank fields below the table.
- Click on **Add new row.**
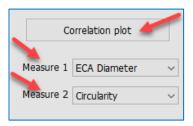- The new row number 6, will be added to the table
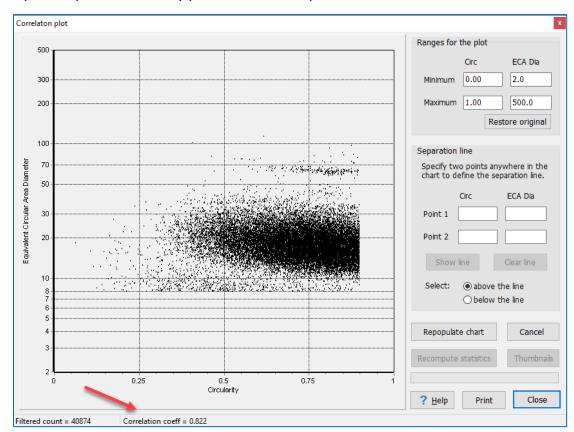
## Using correlation plots

In the Correlation plot a point is plotted in the scatter chart for every particle in the particle database. Correlations plots allow to view how one measure changes in relation to another measure among the particles of a sample. This a powerful tool useful in fully characterizing a sample.

The example below shows how **Circularity** varies in relation to **Size** (ECA). A Correlation coefficient greater than about 0.7 in absolute value indicates the two measures are related.

In the **Processing Tabs** area, click on **Post- run processing.** Select the two measures to be correlated, **Measures 1** and **Measure 2** then click on **Correlation plot** to generate the plot.
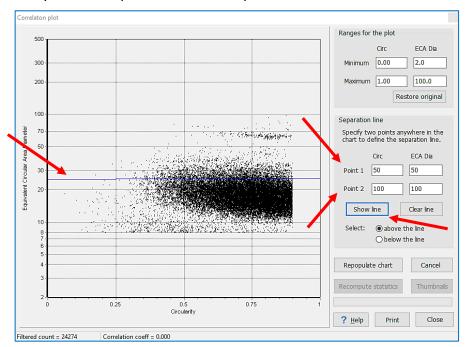
The Correlation window is a scatter plot of the two variables specified under the Correlation Plot button. A point is plotted for every particle in the sample
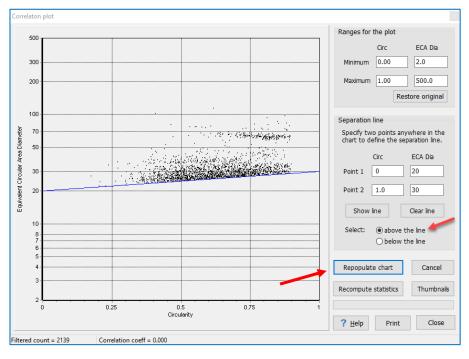
The **Separation Line** feature (blue line) can split a sample into two parts, and each part can be analyzed separately. The example below shows a plot of **ECA Diameter** vs. **Circularity**.
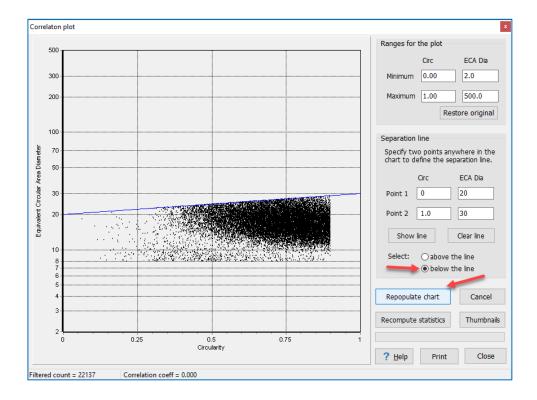


The **Separation line** is defined by the values for **Point 1** and **Point2**. When *Show Line* is clicked, a line is drawn through the two points.



*Repopulate Chart* will replot the chart with only the points on one side of the line, either above or below it.

---

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In statistics, the **Pearson product-moment correlation coefficient** (sometimes referred to as the **MCV** or **PMCC**) (*r*) is a common measure of the linear correlation between two variables *X* and *Y*. When measured in a population, the Pearson Product Moment correlation is designated by the Greek letter rho (ρ).

When computed in a sample, it is designated by the letter "r" and is sometimes called "Pearson's r."  Pearson's correlation reflects the degree of linear relationship between two variables.  It ranges from +1 to -1.

A correlation of +1 means that there is a perfect positive linear relationship between variables.

A correlation of -1 means that there is a perfect negative linear relationship between variables.

A correlation of 0 means there is no linear relationship between the two variables.

Correlations are rarely if ever 0, 1, or -1

Only Number data are used in creating a correlation report.

Click on *File* ➜ *Print* in the toolbar options to print the graph. Again, for best results enlarge the window before printing.

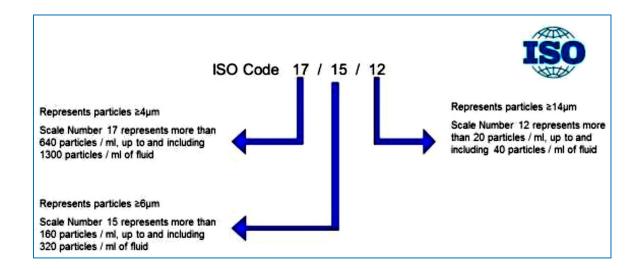Return to TOC

## Oil Analysis (ISO 4406)

Early detection of wear particles in lubricating and hydraulic fluids is critical to having a proper predictive maintenance program. It is this early detection and identification of wear particles that permits the extension of engine life and can minimize down-time of equipment.

The Hydro Insight combines the classification of particles required by industry standards (ISO 4406, NAS 1638) with the reporting of up to 30 size and shape measures for all particles identified.

The instrument also provides particle thumbnails for each particle allowing the user to make more educated decisions on the quality of their lubricating fluids.
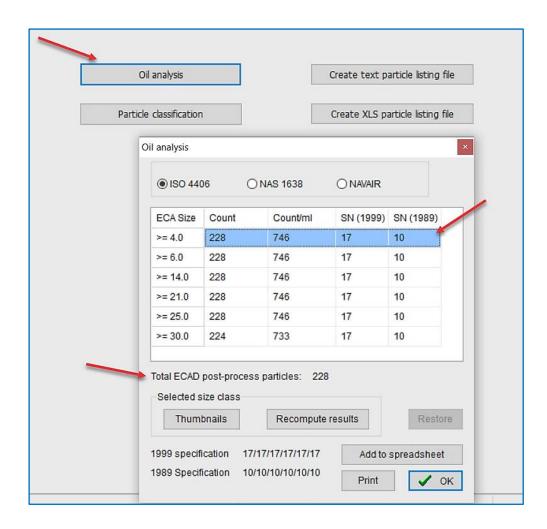
ISO 4406 established a method of coding the level of particle contamination of hydraulic fluids into a set of "scale numbers". The process begins by finding the particle counts above each of a set of fixed sizes: 4, 6, 14, 21, 26 and 30 microns. Then based on the particle concentrations, scale numbers are assigned.

This diagram gives an idea of how they are determined: The ISO 4406 standard was established as a method of coding the level of particle contamination of hydraulic fluids. The Hydro Insight provide results correlated to the ISO standard 4406.



ISO Code 17 / 15 / 12

Represents particles ≥4µm

Scale Number 17 represents more than 640 particles / ml, up to and including 1300 particles / ml of fluid

Represents particles ≥6µm

Scale Number 15 represents more than 160 particles / ml, up to and including 320 particles / ml of fluid

Represents particles ≥14µm

Scale Number 12 represents more than 20 particles / ml, up to and including 40 particles / ml of fluid

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**ISO 4406**: The protocol for determining the scale numbers changed in 1999. The Particle Insight lists scale numbers for the 1989 and 1999 standards, along with the particle counts and concentrations.



The oil analysis window has buttons for displaying **Thumbnails** for the particles in any selected row, or **Recomputing** ECAD graphs and statistics for any selected row. You can also save the table to an **Excel file**, or **Print** the table.
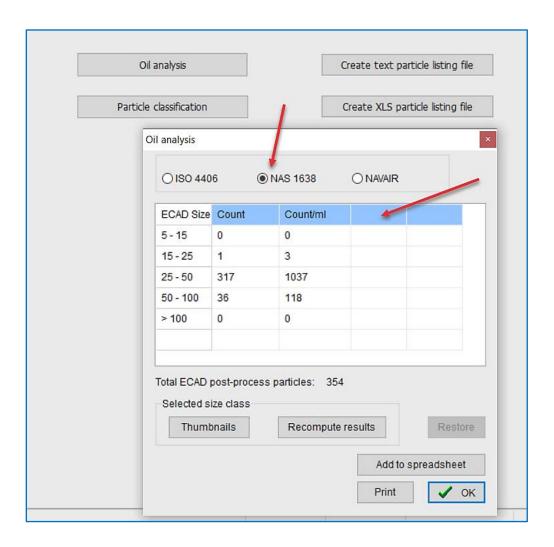
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**_NAS 1638:_** This protocol is similar but older standard developed in 1964. It also provides a way of documenting contamination levels in hydraulic fluid.

Differential rather than cumulative particle counts are determined for each size bin in the standard. In this sense it is like the Percentiles tool described earlier, because it finds out the amount of sample between a pair of breakpoints.
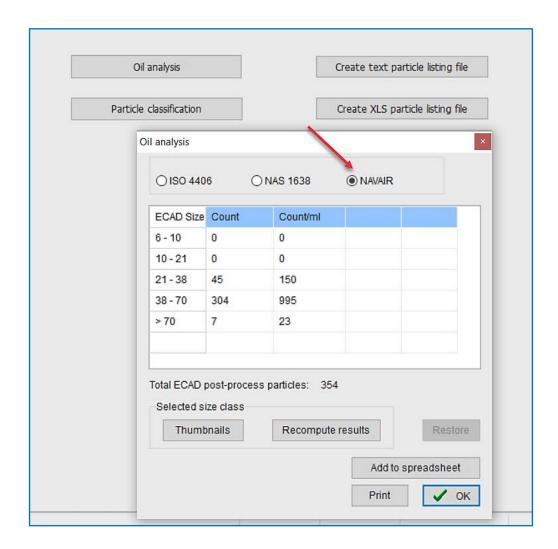
The size breakpoints are different from ISO 4406.

Enable NAS 1638 at the top of the form to show the results for this standard.

**NAVAIR:** This is also a standard protocol similar tool for describing contamination in hydraulic oil and other fluids. Like NAS 1638, it uses differential counts.
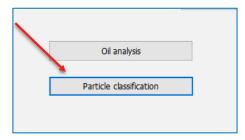
## Using Particle classification

With this tool, <u>you can define several types or classes of particles by name</u>. Each class is defined by specifying minimum and maximum limits for any or all active particle measures. The software will then determine the particle count in each class. Classes can overlap if the min/max limits are not mutually exclusive among the different classes. If there is overlap, the sum of counts for all classes may be greater than the total particle count in the sample.

Particles are classified by imposing limits on the values of all active measures. A particle that meets all the limits for a *Type* is classified as belonging to that *Type*. A particle can belong to more than one *Type*.
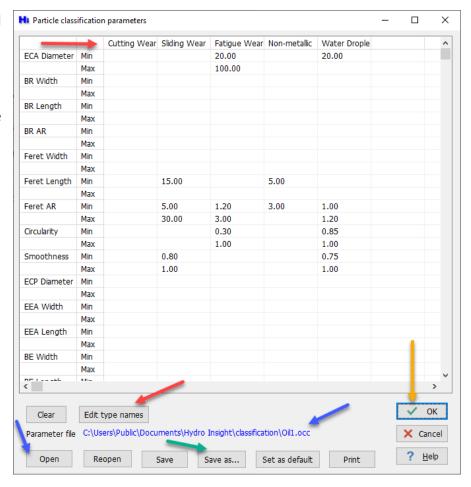
Click on *Particle classification* to begin the classification process.

The **Parameters form** will open, where limits are set for the several classification types.

- Open a parameter file (*.occ) or fill in the table.
- If the names need to be modified, click on *Edit type names.*
- Save the form as a parameter file w/(*.occ).
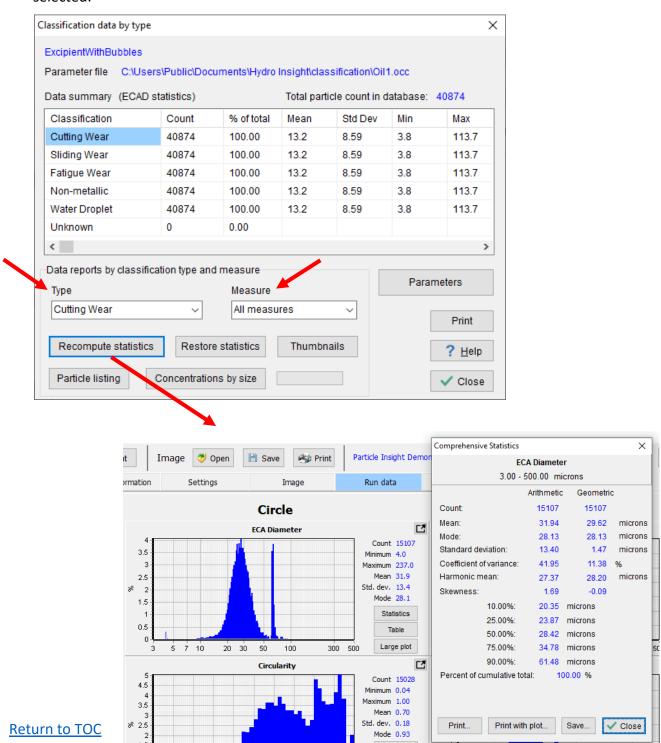- When limits and names are set, click on *OK* to jump to *Classification data by type.*

| | | Cutting Wear | Sliding Wear | Fatigue Wear | Non-metallic | Water Drople |
|---|---|---|---|---|---|---|
| ECA Diameter | Min | | | 20.00 | | 20.00 |
| | Max | | | 100.00 | | |
| BR Width | Min | | | | | |
| | Max | | | | | |
| BR Length | Min | | | | | |
| | Max | | | | | |
| BR AR | Min | | | | | |
| | Max | | | | | |
| Feret Width | Min | | | | | |
| | Max | | | | | |
| Feret Length | Min | | 15.00 | | 5.00 | |
| | Max | | | | | |
| Feret AR | Min | | 5.00 | 1.20 | 3.00 | 1.00 |
| | Max | | 30.00 | 3.00 | | 1.20 |
| Circularity | Min | | | 0.30 | | 0.85 |
| | Max | | | 1.00 | | 1.00 |
| Smoothness | Min | | 0.80 | | | 0.75 |
| | Max | | 1.00 | | | 1.00 |
| ECP Diameter | Min | | | | | |
| | Max | | | | | |
| EEA Width | Min | | | | | |
| | Max | | | | | |
| EEA Length | Min | | | | | |
| | Max | | | | | |
| BE Width | Min | | | | | |
| | Max | | | | | |

**Particle classification parameters**

Clear  Edit type names
Parameter file  C:\Users\Public\Documents\Hydro Insight\classification\Oil1.occ
Open  Reopen  Save  Save as...  Set as default  Print

OK  Cancel  ? Help

**User-defined type names**
Enter up to five type names:

| 1 | Cutting Wear |
| 2 | Sliding Wear |
| 3 | Fatigue Wear |
| 4 | Non-metallic |
| 5 | Water Droplet |

OK  Cancel

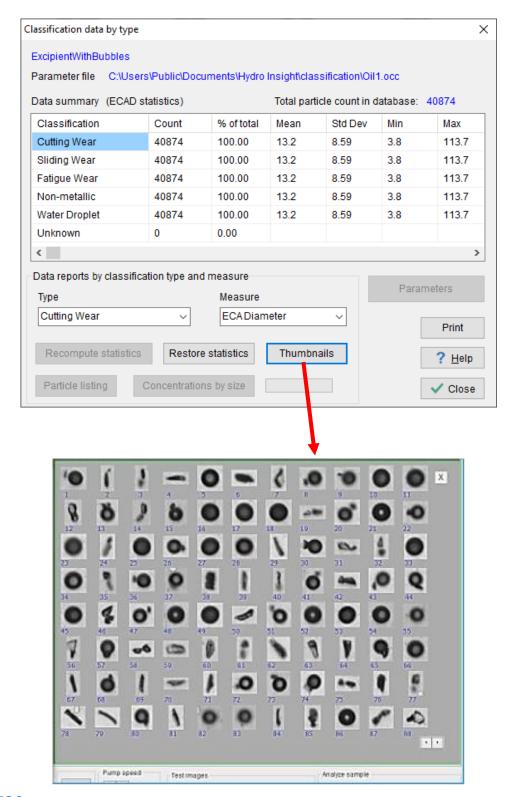[Return to TOC](#)

## Classification data by type

Use this option to report particles already defined.

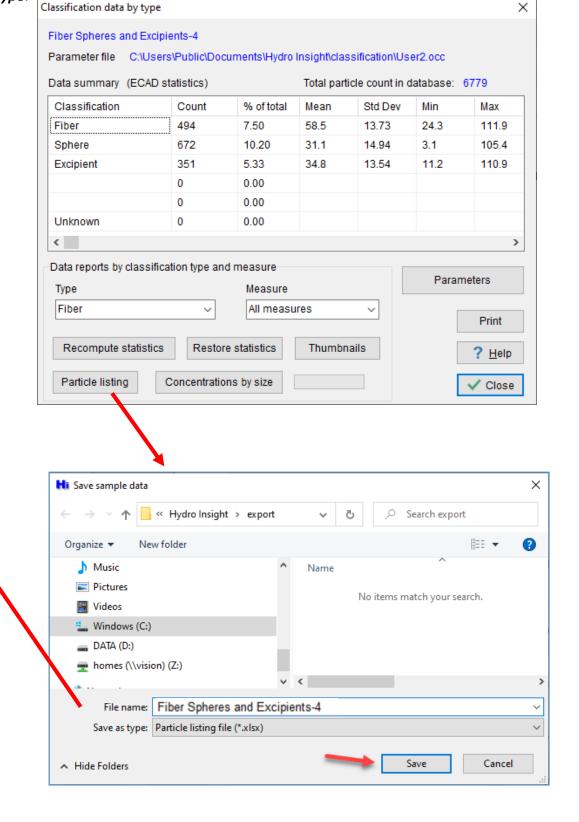- Click on **Recompute statistic** to report the Statistic by classification of **Type** and **Measure** selected.

- Click on **Thumbnails** to report the thumbnails by classification of **Type** and **Measure** selected.

- Click on **Particle listing** button to create a list in excel format (.xls or .xlsx) of Particle data <u>only</u> for the selected **Type**.

- To return to the Classification form click on **Parameters**.

## Classification data by type

ExcipientWithBubbles

Parameter file    C:\Users\Public\Documents\Hydro Insight\classification\Oil1withTypesFilename.occ

Data summary  (ECAD statistics)              Total particle count in database:  40874

| Classification | Count | % of total | Mean | Std Dev | Min | Max |
|---|---|---|---|---|---|---|
| Marsh | 40874 | 100.00 | 13.2 | 8.59 | 3.8 | 113.7 |
| Hoke | 0 | 0.00 | | | | |
| Squeaky | 7021 | 17.18 | 24.7 | 5.63 | 20.0 | 80.5 |
| Daisy | 101 | 0.25 | 11.7 | 5.41 | 5.5 | 34.4 |
| | 0 | 0.00 | | | | |
| Unknown | 0 | 0.00 | | | | |

### Data reports by classification type and measure

Type: Marsh

Measure: All measures

Recompute statistics    Restore statistics    Thumbnails

Particle listing    Concentrations by size

Parameters

Print

? Help

✓ Close

## Particle classification parameters

| | | Cutting Wear | Sliding Wear | Fatigue Wear | Non-metallic | Water Drople |
|---|---|---|---|---|---|---|
| ECA Diameter | Min | | | 20.00 | | 20.00 |
| | Max | | | 100.00 | | |
| BR Width | Min | | | | | |
| | Max | | | | | |
| BR Length | Min | | | | | |
| | Max | | | | | |
| BR AR | Min | | | | | |
| | Max | | | | | |
| Feret Width | Min | | | | | |
| | Max | | | | | |
| Feret Length | Min | | 15.00 | | 5.00 | |
| | Max | | | | | |
| Feret AR | Min | | 5.00 | 1.20 | 3.00 | 1.00 |
| | Max | | 30.00 | 3.00 | | 1.20 |
| Circularity | Min | | | 0.30 | | 0.85 |
| | Max | | | 1.00 | | 1.00 |
| Smoothness | Min | | 0.80 | | | 0.75 |
| | Max | | 1.00 | | | 1.00 |
| ECP Diameter | Min | | | | | |
| | Max | | | | | |
| EEA Width | Min | | | | | |
| | Max | | | | | |
| EEA Length | Min | | | | | |
| | Max | | | | | |
| BE Width | Min | | | | | |
| | Max | | | | | |

Clear    Edit type names

Parameter file  C:\Users\Public\Documents\Hydro Insight\classification\Oil1.occ

Open    Reopen    Save    Save as...    Set as default    Print

✓ OK

✗ Cancel

? Help

In the example of **Parameters form** below, limits from the parameters file: **User2.occ** are set to define three types of particles: **fibers**, **spheres,** and **excipients**.

**Fibers**: Any particle with a Bounding Rectangular Aspect Ratio (**BR AR**) between 3.5 and 10, is classed as a fiber.
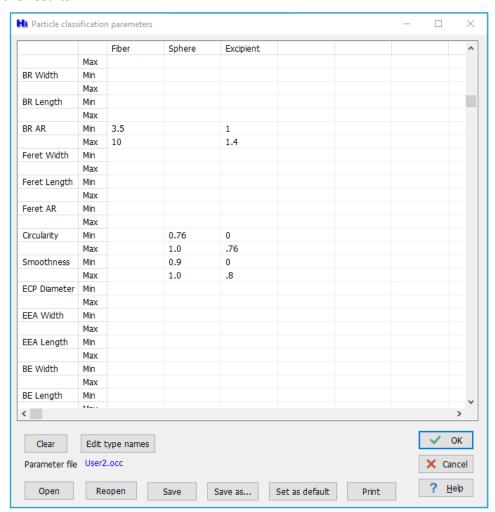
**Sphere**: To be a Sphere a particle must have **Circularity** between 0.76 and 1 and **Smoothness** between 0.9 and 1.0.

**Excipients**: Excipients have **BR AR** between 1 and 1.4, **Circularity** between 0 and 0.76 and Smoothness between 0 and 0.8. This classification does not necessarily encompass all particles. There may be some that are not in any of these categories.

For any measure, the original maximum and minimum and any further limits defined in *Measure Limits* also apply.

If an entry is blank, no limit test is done for that setting. If all entries in the whole table were left blank, only the original limits as set in Settings would apply and all particles would be counted for all classes.

Click *OK* to view the results.

**Hi** Particle classification parameters

| | | Fiber | Sphere | Excipient | | | |
|---|---|---|---|---|---|---|---|
| BR Width | Max | | | | | | |
| | Min | | | | | | |
| BR Length | Max | | | | | | |
| | Min | | | | | | |
| BR AR | Max | | | | | | |
| | Min | 3.5 | | 1 | | | |
| Feret Width | Max | 10 | | 1.4 | | | |
| | Min | | | | | | |
| Feret Length | Max | | | | | | |
| | Min | | | | | | |
| Feret AR | Max | | | | | | |
| | Min | | | | | | |
| Circularity | Max | | | | | | |
| | Min | | 0.76 | 0 | | | |
| Smoothness | Max | | 1.0 | .76 | | | |
| | Min | | 0.9 | 0 | | | |
| ECP Diameter | Max | | 1.0 | .8 | | | |
| | Min | | | | | | |
| EEA Width | Max | | | | | | |
| | Min | | | | | | |
| EEA Length | Max | | | | | | |
| | Min | | | | | | |
| BE Width | Max | | | | | | |
| | Min | | | | | | |
| BE Length | Max | | | | | | |
| | Min | | | | | | |

Clear    Edit type names    ✓ OK

Parameter file   User2.occ    ✗ Cancel

Open    Reopen    Save    Save as...    Set as default    Print    ? Help

To show results for **Fibers**, select that row in the table then:

- Click on **Recompute Statistics to see the** numerical values.
- Click on **Thumbnails** to see the images of only objects classed as Fibers.
- Click on **Particle Listing** to create an Excel file listing of measure values for all particles in that type.
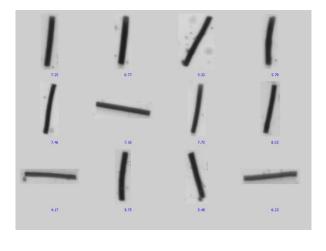


*Recomputed statistic*　　　　　*Thumbnails*　　　　　*Particle Listing*



Return to TOC
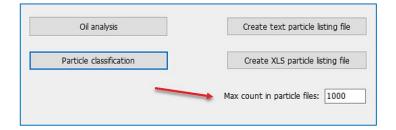
If the Counts column in the table adds up to more than the total particle count, it means that some particles were counted in more than one type. The types are not necessarily mutually exclusive unless the parameters are set up to make them exclusive.

Also, the defined **Types** do not necessarily cover all particles in the sample. In that case, the **counts** and **percents** will add up to less than 100% of the total.

The **Unknown** count includes only particles that are within the original ECAD limits. Therefore, the sum including Unknown can still be less than the total for the sample.

**Max count in particle files** in the **Post-run Processing** tab applies here.
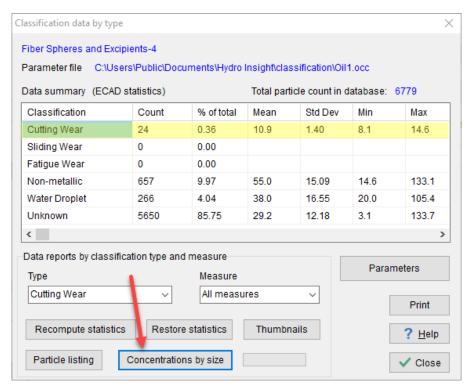


To return to the Classification form click on **Parameters**.
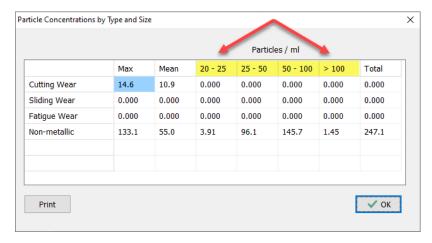
## Particle Concentration by Type and Size

Sometimes it is necessary to know about the particle concentration for each type of sample. At the ***Classification data by type*** window below, click on ***Size distributions.***



Below, the table with particle concentration values distributed in 4-size bins for each type of sample.



Return to TOC
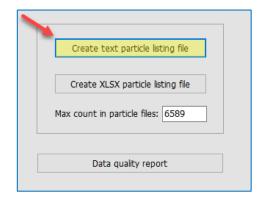
## Create a particle listing

You can create a list of all particles with their measure values to process the data in a spreadsheet in **.xlsx format** or may be generated as a simple **Text file.**
You can use **Max count in particle files** to limit the size of the file.

### Create text particle listing file
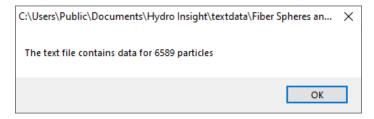
To create a text particle file, click on **Create text particle listing file** button.

The text file created will be saved in the following location:
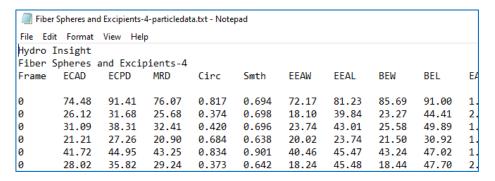
« Windows (C:) › Users › Public › Public Documents › Hydro Insight › textdata

The following message will show up after the file is saved:

C:\Users\Public\Documents\Hydro Insight\textdata\Fiber Spheres an...  ✕

The text file contains data for 6589 particles

OK

A text file will be generated, with each line in the file containing data for one particle.
The first entry in the line is the Frame number, then an entry for each active measure. Within each line, entries are comma delineated.

Fiber Spheres and Excipients-4-particledata.txt - Notepad

File  Edit  Format  View  Help

Hydro Insight
Fiber Spheres and Excipients-4

| Frame | ECAD | ECPD | MRD | Circ | Smth | EEAW | EEAL | BEW | BEL | EA |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 74.48 | 91.41 | 76.07 | 0.817 | 0.694 | 72.17 | 81.23 | 85.69 | 91.00 | 1. |
| 0 | 26.12 | 31.68 | 25.68 | 0.374 | 0.698 | 18.10 | 39.84 | 23.27 | 44.41 | 2. |
| 0 | 31.09 | 38.31 | 32.41 | 0.420 | 0.696 | 23.74 | 43.01 | 25.58 | 49.89 | 1. |
| 0 | 21.21 | 27.26 | 20.90 | 0.684 | 0.638 | 20.02 | 23.74 | 21.50 | 30.92 | 1. |
| 0 | 41.72 | 44.95 | 43.25 | 0.834 | 0.901 | 40.46 | 45.47 | 43.24 | 47.02 | 1. |
| 0 | 28.02 | 35.82 | 29.24 | 0.373 | 0.642 | 18.24 | 45.48 | 18.44 | 47.70 | 2. |

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## Create XLS particle listing file

To create an Excel file, click on **Create XLS particle listing file** button. Use this option to save particle data in excel format. The format **xls** or **xlsx** should be selected in **Preferences ➔ Spreadsheet files ➔** File type-format.
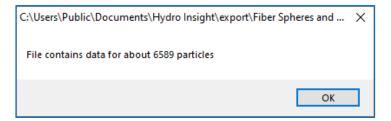
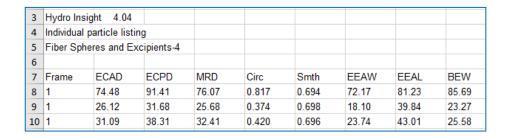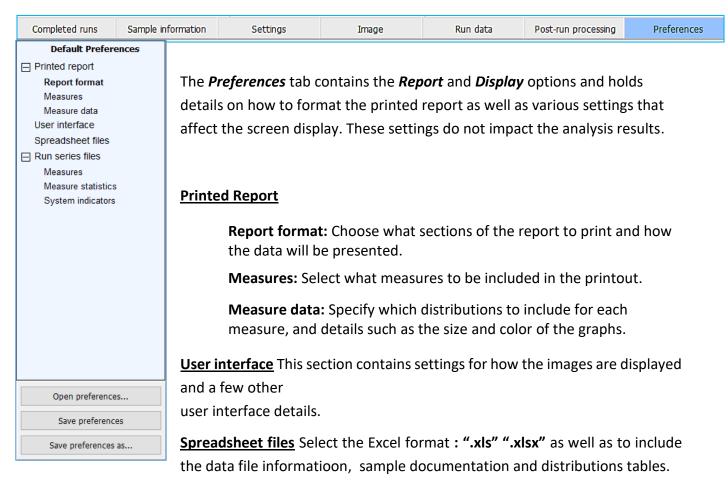You can use **Max count in particle files** to limit the size of the file.

The Excel file created will be saved in the following location:

« Windows (C:) › Users › Public › Public Documents › Hydro Insight › export

The following message will show up after the file is saved.

C:\Users\Public\Documents\Hydro Insight\export\Fiber Spheres and ... ✕

File contains data for about 6589 particles

OK

An Excel file will be generated, with each row in the file containing data for one particle. The first column in the file is the **Frame** number. Following that, there is a column for each active measure.

| | Frame | ECAD | ECPD | MRD | Circ | Smth | EEAW | EEAL | BEW |
|---|---|---|---|---|---|---|---|---|---|
| 3 | Hydro Insight 4.04 | | | | | | | | |
| 4 | Individual particle listing | | | | | | | | |
| 5 | Fiber Spheres and Excipients-4 | | | | | | | | |
| 6 | | | | | | | | | |
| 7 | Frame | ECAD | ECPD | MRD | Circ | Smth | EEAW | EEAL | BEW |
| 8 | 1 | 74.48 | 91.41 | 76.07 | 0.817 | 0.694 | 72.17 | 81.23 | 85.69 |
| 9 | 1 | 26.12 | 31.68 | 25.68 | 0.374 | 0.698 | 18.10 | 39.84 | 23.27 |
| 10 | 1 | 31.09 | 38.31 | 32.41 | 0.420 | 0.696 | 23.74 | 43.01 | 25.58 |

## Preferences

| Completed runs | Sample information | Settings | Image | Run data | Post-run processing | Preferences |
|---|---|---|---|---|---|---|

**Default Preferences**

Printed report
   **Report format**
   Measures
   Measure data
User interface
Spreadsheet files
Run series files
   Measures
   Measure statistics
   System indicators

Open preferences...

Save preferences

Save preferences as...

The **Preferences** tab contains the **Report** and **Display** options and holds details on how to format the printed report as well as various settings that affect the screen display. These settings do not impact the analysis results.

### Printed Report

**Report format:** Choose what sections of the report to print and how the data will be presented.

**Measures:** Select what measures to be included in the printout.

**Measure data:** Specify which distributions to include for each measure, and details such as the size and color of the graphs.

**User interface** This section contains settings for how the images are displayed and a few other
user interface details.

**Spreadsheet files** Select the Excel format **: ".xls" ".xlsx"** as well as to include the data file informatioon,  sample documentation and distributions tables.

### Run series files

**Measure:**  Select the measures to include in run series files.

**Measure statistic:** Select the statistics to include in run series files.

**System indicators:** Select the indicators like Date/time, Concentration, temperature to include in run series files.

**Open Preferences ...** Open a previously saved preferences file with extension ".prf"

**Save Preferences ...**  Save the changes in preferences to the currently open preferences file.
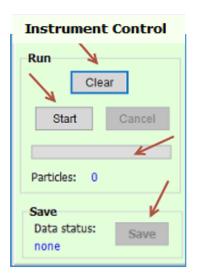
**Save preferences as ...**  Save the current preferences to a file you designate with a different filename**.**

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## Instrument Control

**Run:** This section controls the execution of an analysis:

- **Clear:**  To clear the statistic and the histogram of the current and unsaved data.
- **Start**: To start continuous data capture: strobe images continuously, analyze image, update the accumulated statistics after each frame, and periodically update the screen display.  After starting, this button changes to **Stop** run.

- **Stop:**  Suspends the run but does not carry out any save actions.  Changes to "**Resume**" run after being clicked.
- **Resume:**  Data acquisition resumes, with new data being added to what was taken previously.
- **Cancel** the run momentarily. The button will change to **Resume** to continue the run.
- A **progress bar** that shows the progress of the process.
- **Particles** shows the total count of the particles in the completed analysis.

**Save:** This section allows to save the data of a run that has not met the end condition.

**Note:** When the run reaches the end condition, the software automatically save the data If the option *Save sample file* under *Settings ➜ Completion actions* was enabled.

## Status bar

The status bar along the bottom of the main screen contains several sections.

Section 1                                                                                                    Section 7

| arm number 2-1 | | | | | | |
|---|---|---|---|---|---|---|

Section 1:

- The current username if security is enabled.
- *Filtered count* in *Correlation Form*.

Section 2:
- Sample filename
- *Correlation coefficient* in *Correlation Form*

Section 3:
- Image filename
- *Measure* name in *Thumbnails*.

Section 4: Shows error codes.

Section 5: Shows notice or warning codes.

Section 6:
- Shows the number of runs remaining in an auto-start run sequence.
- *COMPLETED* at end of a run.
- *Particle count* in *Thumbnails*
- Grid spacing when the grid overlay is present.
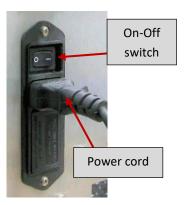
Section 7: *Page n* when *Thumbnails* are open.

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# Chapter 3 - GETTING STARTED

## Startup



On-Off switch

Power cord

- Turn the instrument **On** using switch at the rear of the instrument. Ensure USB3 and Power cable are all connected.



Hydro Insight 4.04

- Open the application software by double left-click the icon in the desktop.

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## Beginning a new run
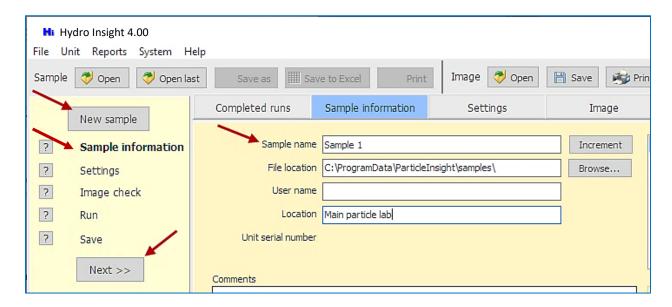
The analysis of a sample can be initiated in either of the two analyzers. The Mastersizer 3000 will be controlled by an SOP and the Hydro Insight manually.

To begin a new run, click on **New sample** (Wizard).

## Sample information                                                              4.04

- Enter a **Sample name** and the **File location** if other than default.
- **User name** and **Location** are optional.
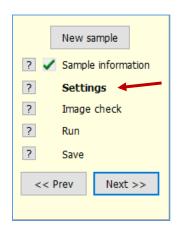


- When ready click **Next** in the left-hand panel to jump to the next step.

## Settings
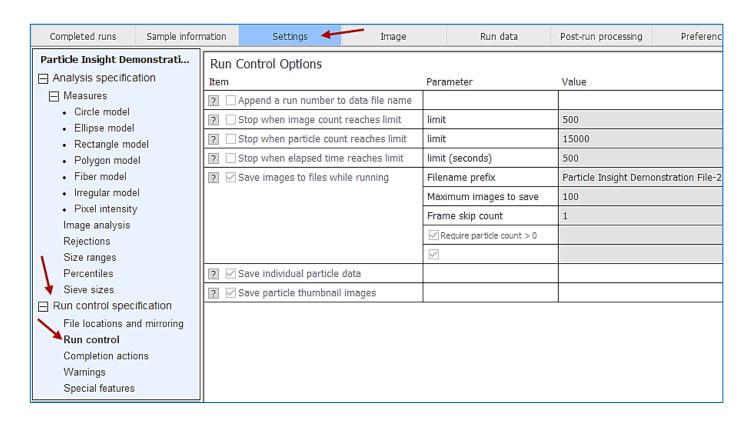
- Select the **Settings** tab.
- Open a specific Settings file or adjust the current settings under **Analysis Specification ➜ Measures** as well as the parameters to match the type of particles being measured. These settings affect the image analysis, and therefore the results of a run. This includes the measures to use and their ranges (minimum, maximum and divisions).
- You may need to return to this page during the **Image Check** section of **New Sample Wizard**.

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- Select **Run Control specifications ➔ Run control.**
- Set **Stop when image count reaches limit** to the maximum desired images to take.
- Set **Stop when particle count reaches limit** to the maximum number of particles count.
- Set **Stop when elapsed time reaches limit.**
  The run will stop when one of these limits is reached.
- Select **Save images to files while running** and set the maximum images to be saved.
- Select **Save individual particle data** If **Post-run processing** will be used.
- Select **Save particle thumbnail images** if thumbnail images will be used.

| Completed runs | Sample information | Settings | Image | Run data | Post-run processing | Preferenc |
|---|---|---|---|---|---|---|

**Particle Insight Demonstrati...**

- Analysis specification
  - Measures
    - Circle model
    - Ellipse model
    - Rectangle model
    - Polygon model
    - Fiber model
    - Irregular model
    - Pixel intensity
  - Image analysis
  - Rejections
  - Size ranges
  - Percentiles
  - Sieve sizes
- Run control specification
  - File locations and mirroring
  - **Run control**
  - Completion actions
  - Warnings
  - Special features

### Run Control Options

| Item | Parameter | Value |
|---|---|---|
| ? ☐ Append a run number to data file name | | |
| ? ☐ Stop when image count reaches limit | limit | 500 |
| ? ☐ Stop when particle count reaches limit | limit | 15000 |
| ? ☐ Stop when elapsed time reaches limit | limit (seconds) | 500 |
| ? ☑ Save images to files while running | Filename prefix | Particle Insight Demonstration File-2 |
| | Maximum images to save | 100 |
| | Frame skip count | 1 |
| | ☑ Require particle count > 0 | |
| | ☑ | |
| ? ☑ Save individual particle data | | |
| ? ☑ Save particle thumbnail images | | |

- Use the buttons at the bottom of the panel to Open or Save settings.

  Open settings...

  Save settings...

  Save settings as...

- When ready click **Next** in the left-hand panel to jump to the next step.

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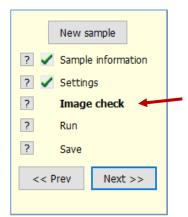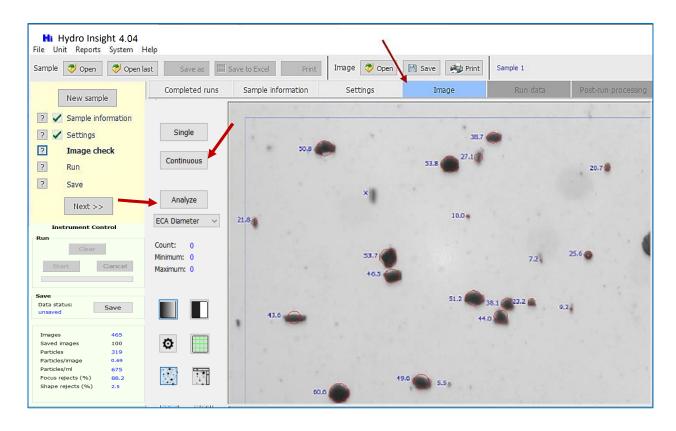
## Image check
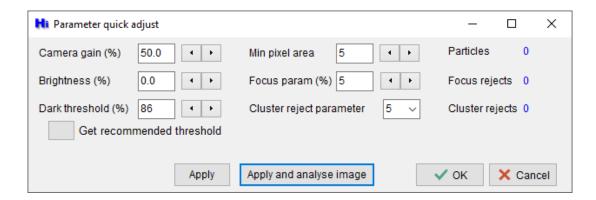
- Capture images using **Single** or **Continuous** to check image quality and particle density.
- Click **Analyze** to obtain the preliminary results.
- Check that the number of focus rejections (red X's) and shape rejections (blue X's) (if enabled) are reasonable.
- Adjust image analysis settings if necessary.





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- If necessary, adjust image analysis parameters in **Settings ➔ Analysis Specification** OR use **Parameter Quick Adjust** (gear icon).
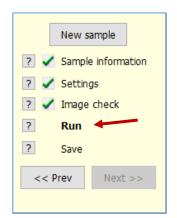




- Stop **Continuous** mode if it is running.

- When ready click **Next** in the left-hand panel to jump to the next step.

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## Run



1. Click on **Clear** then **Start** buttons to start the run.
2. Then, click **Stop** to end the run, or wait for a preset limit to be reached.
3. A run may be paused without loss of data by clicking **Stop**, then **Resume**.
4. To save a file before it reaches the set endpoint, click **Stop** the run and click **Save**.



Note: If additional runs are required, click on **Increment** under **Sample Information** dialog box and complete the Wizard again.

5. When ALL the runs are completed, perform a **Clean** system in the Mastersizer 3000.

- At any time, the status of the run is shown in the bottom status bar: RUNNING, PENDING, or COMPLETED.

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- To cancel a run before it has completed, click *Cancel*.
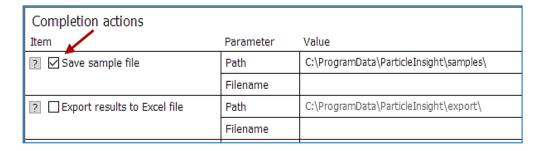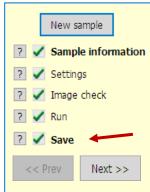
- After a run has been COMPLETED, click **Next** in the left-hand panel to jump to the next step.

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## Save

- If the option **Save sample file** under **Settings ➔ Completion actions** was enabled, the run will be saved automatically after run is completed.



- If **Save sample file** was not enabled, click **Save** to save the sample file. In the Save dialog box there are additional options for saving.

If **Save sample file** is not enabled



- If **individual particle data** was accumulated, post-run processing will become enabled.

- Perform a **Clean** system in the Mastersizer 3000, select mode: **Extensive.**
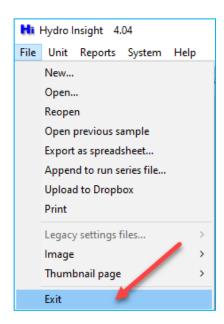
To return **Step 5 – Save**, click HERE

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## End-of-day shutdown procedure

- Complete a **Clean** operation in the Mastersizer 3000.

- To perform a **Clean**, select one of the three modes: **Quick,** **Normal** or **Extensive**, according to the sample used. Repeat, as necessary.

- **Exit** the Hydro Insight software.

SAVE AS

# Chapter 4 - SHAPE MODELS

## Circle model

**ECA Diameter (Equivalent Circular Area diameter)**

ECA Diameter characterizes the size of a non-spherical shape with a single number. With typical particle shapes that are not fibrous, ECA Diameter represents the diameter of a sphere that would have a volume close to the actual volume of the particle. Since the software has access only to a flat shadow or silhouette of the particle, ECA Diameter is defined in terms of the silhouette area. It is defined as the diameter of a circle that has the same area as the silhouette.

*Practical Use –* ECA Diameter is a measure that is commonly used to compare results of the **Hydro Insight** to results available from other particle size analyzers that report equivalent diameter.

Note that the ECA Diameter for the **Hydro Insight** can report:

Number weighted mean diameter (D [1,0])

Volume weighted mean diameter (D [4,3])

Surface weighted mean diameter (D [3,2])

Length weighted mean diameter (D [2,1])
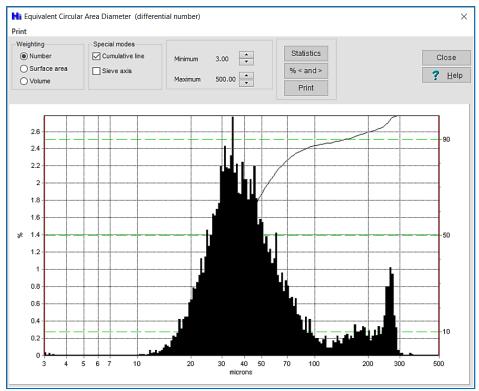
Mean volume (D [3,0])

Mean surface (D [2,0]).
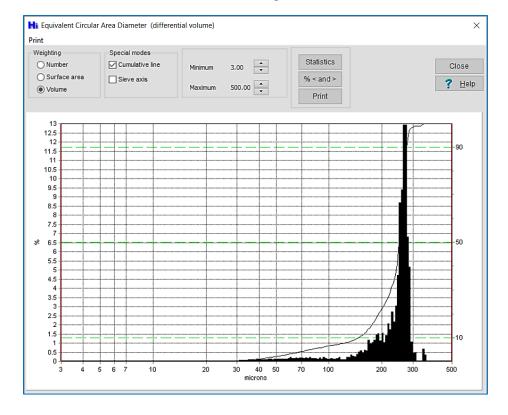
In addition to this, the ECA Diameter measurement in the **Hydro Insight** will show cumulative curves real-time and give real-time correlation to Sieve data.

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ECA Diameter graph shown with cumulative line for Number weighted mean.
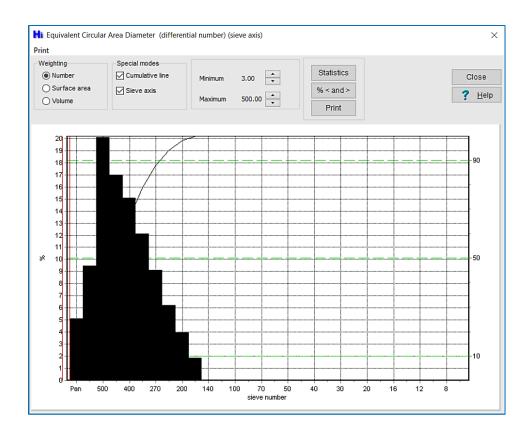


ECA Diameter graph shown with cumulative line for Volume weighted mean.



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ECA Diameter graph shown with cumulative line for Number weighted mean and Sieve Equivalent results. This is particularly useful when trying to correlate more modern methods, such as Image Analysis to historical Sieve data.
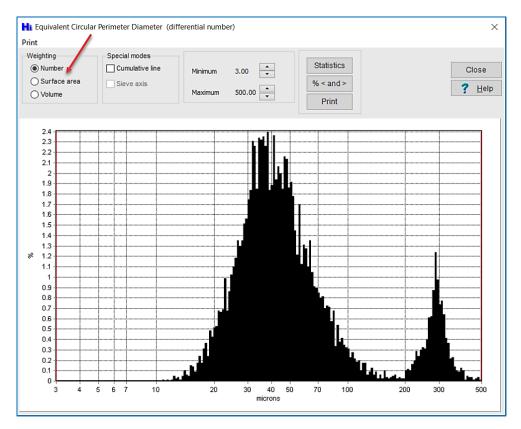
**ECP Diameter (Equivalent circular perimeter diameter)**



ECP Diameter is the diameter of a circle having the same perimeter as the actual shape. Whereas ECA Diameter relates to a particle's likely volume, ECP Diameter relates more to its surface area.

**Practical Use** – ECP Diameter is a measure that can be used to determine the surface area of the silhouette of a particle. A practical application of this would be particle coating. In such a case, the amount of coating a particle will require may be relatively controlled by monitoring the changes in ECP Diameter. If ECP Diameter is increasing in size, that means the greater the surface area of the particle and, therefore, the more required coating.



Typical ECP Diameter graph. Results can be displayed in Number weighted distribution, Volume weighted distribution as well as Surface Area weighted distribution. All can be displayed real-time with a Cumulative line.

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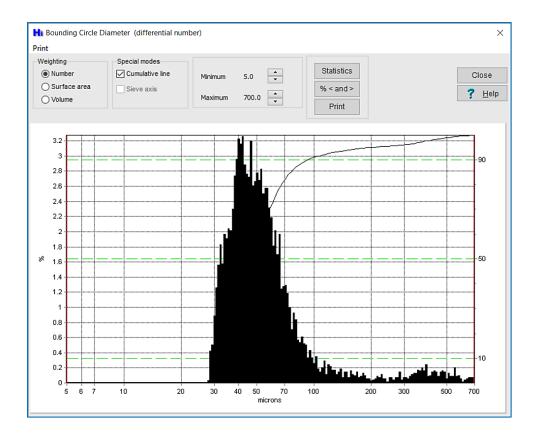
## BC Diameter (Bounding circle diameter)



BC Diameter is also a single diameter value. But instead of being an "average" or representative diameter, it characterizes the "largest diameter" of a particle. It is defined as the diameter of the smallest circle that encloses but does not intersect the particle.
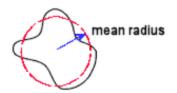
*Practical Use* – BC Diameter is a measure that can be used to monitor and control a process based on the maximum diameter of a particle. By monitoring the smallest circle that can encompass a particle in a process, the end user can ensure particle clogging or trapping in a process is controlled.



Typical BC Diameter graph. Results can be displayed in **Number** weighted distribution, **Volume** weighted distribution as well as **Surface Area** weighted distribution. All can be displayed real-time with a **Cumulative line**.

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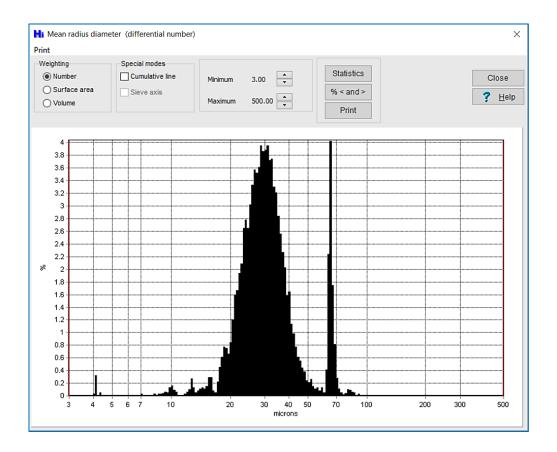
## MR Diameter (Mean Radius Diameter)



First the radius from the centroid to the actual perimeter is determined at 36 equally spaced angles. The average of these distances is the mean radius, and the mean radius diameter is defined as double the mean radius.

These measures use a spherical model for estimated surface area- and volume-weighted histograms, means, and percentiles. That is, the relative surface areas of particles are assumed to be proportional to the square of the diameter measure, and the volumes are proportional to the cube of the diameter measure.



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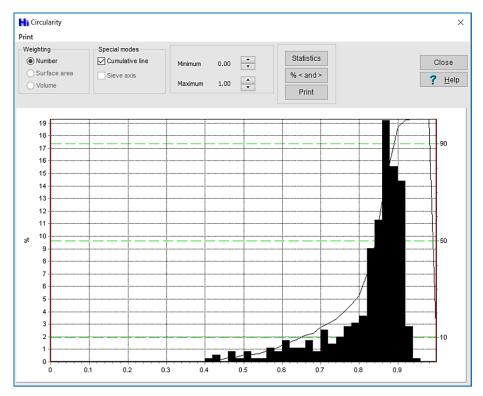
**Circularity, Smoothness,** and **Compactness** are commonly used measures of roundness and by inference, particle sphericity.

## Circularity

Circularity is a commonly used measure of roundness and by inference, particle sphericity. It is a fractional measure, equal to 1 for a perfect circle. It can be thought of as the fraction of the bounding circle's area covered by the actual shape. Circularity is not affected by small irregularities in the perimeter and errors in perimeter measurement. It is not affected by any systematic size bias in perimeter determination. Circularity is computed from area (A) and bounding circle diameter ($D_{BC}$):

$$\text{Circularity} = 4\,A\,/\,\pi\,D^2{}_{BC}$$

***Practical Use*** – Circularity is a measure that can be used to assess flowability of a particle in a process or, in the case of multi-component particles, how each particle will interact with others. Particles that are more circular in nature will flow and mix better. Particles with a lower circularity can get hung up with other particles or feeders which will impact mix-ability and flow-ability.



Typical Circularity graph. Results are shown in Number weighted distribution only and can be displayed real-time with a Cumulative line. Note that a distribution with circularity close to "1" would indicate mostly round particles in the sample population. In the case of these round glass beads particles, circularity is close to "1" which is expected.

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**Smoothness**

Smoothness is calculated from area (A) and perimeter (P) of the particle projection:

$$\text{Form factor} = 4\,\pi\,A\,/\,P^2$$
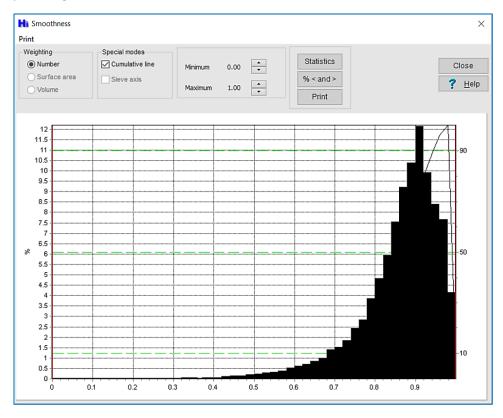
It is a number between 0 and 1, a perfect circle having Smoothness equal to 1.

Like circularity, Smoothness is affected by the degree of out-of-roundness of the general shape.

It is also affected by irregularity of the perimeter, which by inference is an indication of surface roughness. Particles having a pixel area smaller than 'minimum area' will not be smoothness-tested because they are too small for the procedure to work meaningfully. In general, 'minimum area' should be at least sensitivity*100; for example, 500 or more for sensitivity 5.

Particles should be in sharp focus; do not use Smoothness with out-of-focus particles.

 *Practical Use –* Smoothness is a measure that can be used to monitor surface roughness. This can affect the performance of an abrasive particle and can also have an impact on how pharmaceutical powders flow and mix prior to granulation



Typical Smoothness graph. Results can be displayed in Number weighted distribution, Volume weighted distribution as well as Surface Area weighted distribution. All can be displayed real-time with a Cumulative line.

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**Compactness**
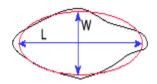
$$\text{Compactness} = 2 \, \sqrt{A} / \sqrt{\pi \, D2_{BC}}$$

Compactness is the square root of Circularity and does not vary as greatly as Circularity.
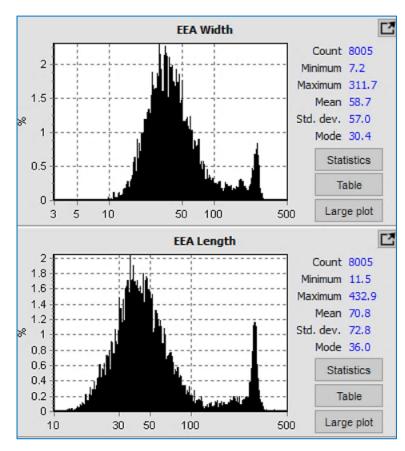
## Ellipse model

### EEA Width and EEA Length (Equivalent Elliptical Area Width and Length)
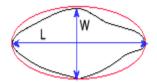


Of all equivalent area ellipses, the one chosen is the one that has the same aspect ratio as the bounding rectangle.
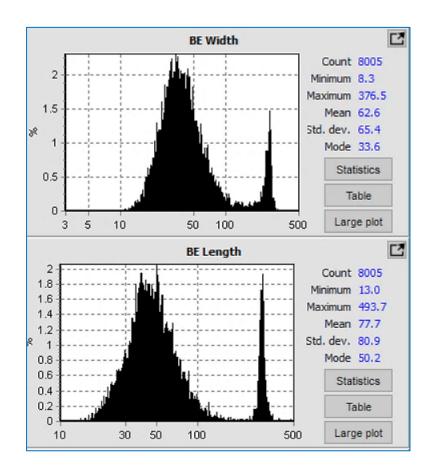


Typical EEA Width and Length graphs respectively. Results can be displayed in Number weighted distribution, Volume weighted distribution as well as Surface Area weighted distribution. All can be displayed real-time with a Cumulative line.

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**BE Width and BE Length (Bounding Ellipse Width and Length)**



The bounding ellipse is the ellipse of least area that bounds the shape.
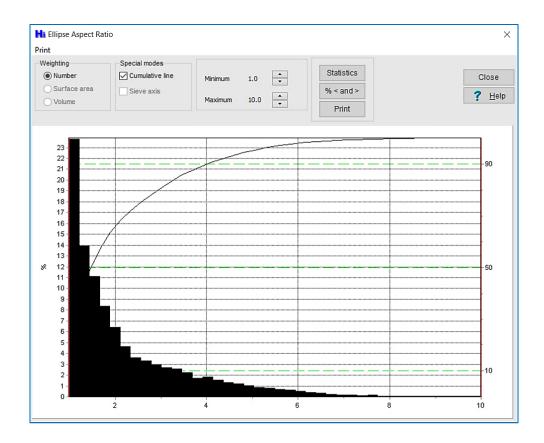


Typical BE Width and Length graphs, respectively. Results can be displayed in Number weighted distribution, Volume weighted distribution as well as Surface Area weighted distribution. All can be displayed real-time with a Cumulative line.

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**Ellipse AR (Ellipse Aspect Ratio)**

Computed as the ratio of EEA Width EEA Length.



Typical Ellipse AR graph. Results can be displayed in Number weighted distribution, Volume weighted distribution as well as Surface Area weighted distribution. All can be displayed real-time with a Cumulative line.

**Ellipsicity**

Ellipsicity is the ratio of the shape's area to the area of the bounding ellipse.

Calculation of estimated surface area (A) and volume (V):
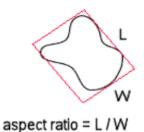
ecc = square root(1 - 1/(AR*AR))     where ecc is eccentricity and AR is the aspect ratio

A = (pi/2) * BEW*BEW*(1 + AR*arcsin(ecc)/ecc)

V = (4*pi/3) * BEL * BEW * BEW

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## Rectangle model



aspect ratio = L / W
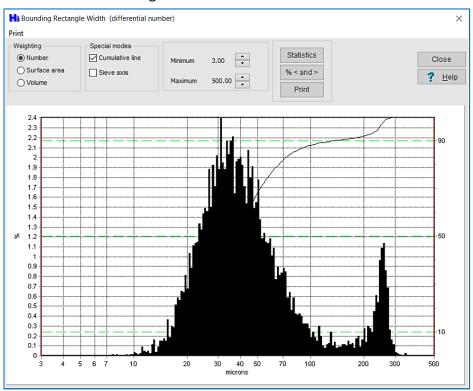
Bounding Rectangle is intended for non-fiber shaped objects, usually particles that are not perfectly round but still have low aspect ratio. It is defined as the rectangle of smallest area that encloses but does not intersect the object. The BR model assumes a thickness in the third dimension equal to the average of the other two distances, for purposes of calculating estimated surface area and volume.

***Practical Use –*** Bounding Rectangle measures is a measure that can be used to determine the width and length of a particle as well as aspect ratio. One powerful use of this measure is using BR Width and its Sieve equivalence calculation. This would allow the estimation of what would pass through each predetermined standard sieve mesh based on the narrowest portion of the particle (BR Width).
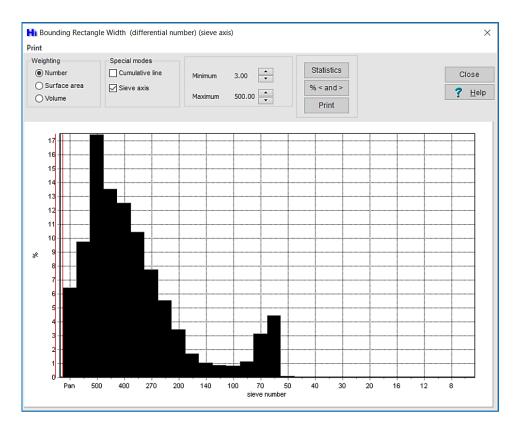
**BR Width (Bounding Rectangle Width)**

BR Width is the smaller side of the rectangle.



Typical BR Width graph. BR Width and Length results can be shown in Number, Volume and Surface Area weighted distribution and can be displayed real-time with a Cumulative line.

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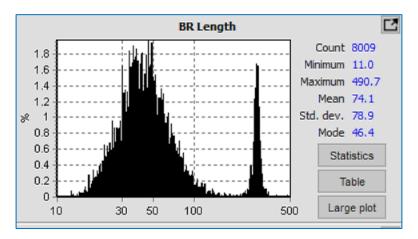
BR Width results can also be shown with Sieve equivalent results to enable comparable results to historical Sieve data. This would enable users to estimate which particles, if passed through a sieve via its narrowest dimension (BR Width), what would pass through each sieve.

**BR Length (Bounding Rectangle Length)**
BR Length is the larger side.
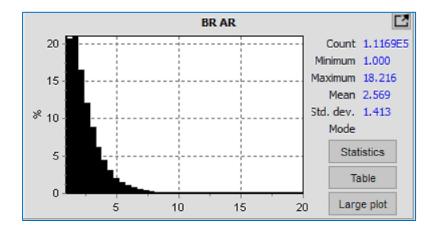
**BR AR (Bounding Rectangle Aspect Ratio)**

BR Aspect Ratio is the ratio of length to width.

Aspect Ratio is another very used measure in industry.



Typical BR Aspect Ratio graph. BR Aspect Ratio can be shown in Number, Volume and Surface Area weighted distribution and can be displayed real-time with a Cumulative line.

Rectangularity is a measure of how close to a rectangle the shape is, and can be useful with short, thick fibers for example.

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## Polygon model

The Polygon model can characterize particles or bio-organisms that have a polygonal shape, recognizable corners and sides that are straight or approximately straight. If a shape is not drawn for a particle it means that 3 or more corners could not be determined, or the shape is outside the minimum and maximum pixel area settings. There is an automatic border contact exclusion.

*Practical Use –* these measures are useful with abrasives, crystals, and some types of flakes

This model finds an n-sided convex polygon that approximately bounds the shape.

Parameters for this model are:

Minimum angle represented by a side:  sides that subtend a central angle smaller than this value are not counted in the number of sides.

Minimum area:  objects smaller than this number, in pixels, are not included in the polygon data because the polygon fit cannot be done accurately.
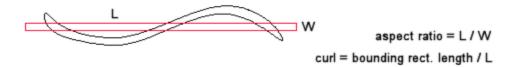
**Polygon order** is the number of sides of the fitted polygon.

**Polygon Interior Angles** will accumulate angle statistics on objects that are larger than the minimum size entered for Polygon Order. Since this is a multi-valued measure for each particle, it is not available in places that require a single value. For example, when analyzing a single image, it will not appear in the analysis table.

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## Fiber model

The Fiber model is optimal for long, thin particles that may be curved or bent, but having a fairly constant thickness over the entire length. If such a shape were straightened out and fit with a rectangle, that rectangle would represent the **Fiber Length** and **Fiber Width**.



$$\text{aspect ratio} = L \,/\, W$$
$$\text{curl} = \text{bounding rect. length} \,/\, L$$

The rectangle dimensions are computed only from the silhouette's area (A) and perimeter (P):

$D = (P^2 - 16\,A)\,/\,4$

If $D > 0$,

$L = (P + 2\,\sqrt{D})\,/\,4$      (Fiber Length)

$W = A\,/\,L$      (Fiber Width)

With fibers that are always straight, even long, thin ones, Bounding Rectangle may be more accurate than the Fiber model, which computes length and width indirectly from area and perimeter and is thus subject to error from non-smoothness in the perimeter.

Nor should the Fiber model be used with low aspect ratios since the calculation is imprecise for aspect ratios less than about 3.

The analysis algorithm in its current form cannot separate two crossed fibers. They are counted as one fiber; whose length is the combined lengths of the two actual fibers.

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## Fiber Aspect Ratio

Fiber Aspect ratio is the ratio of fiber length to fiber width.

## Fiber Curl

Fiber curl is the ratio of bounding rectangle length to fiber length (smaller values indicate greater amounts of curl, so the value is really a degree of straightness. We have kept the definition that is common in industry).
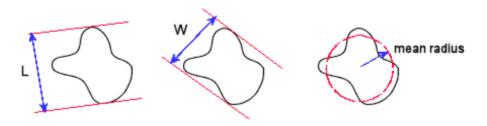
The fiber model assumes either a cylindrical or rectangular solid shape ("flat") for estimated surface area and volume calculations. In the case of the rectangular solid shape, the thickness in the third dimension is assumed to be constant and is entered as a parameter in the settings.

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## Irregular model

**Feret Width and Feret Length**

Feret Width and Feret Length are measures of the smallest possible and largest possible spacing between two parallel lines that contact but do not intersect the particle. These measures can be thought of as "caliper dimensions."  The Feret measures are sometimes close in value to Bounding Rectangle length and width, but not always since they are not always orthogonal (at right angles to each other).  There are no estimated surface area and volume statistics with the Feret measures since area and volume cannot be inferred from them.



**Feret AR (Feret Aspect Ratio)**

Feret Aspect ratio is Feret Length / Feret Width.

**Martin Width**

Martin Width is the narrowest width near the middle of the shape. It can be thought of as the "waist" of the particle shape.

**Martin Length**

Martin Length is the longest chord through the middle of the shape.

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## Pixel Intensity

**Intensity**

The particle's intensity mean is calculated as a value between 0 (black) and 255 (white).

**Opacity**

Opacity is then computed as (256 – (Intensity Mean))/256.

1 is fully opaque, 0 is fully transparent

**White Fraction**

White fraction is the fraction of the particle area that is lighter than *Dark Threshold* in *Settings* ➔ *Image Analysis* options.

## Further information regarding the models

The following models compute estimated surface area and volume distributions:

Circle
Ellipse
Rectangle
Fiber

The following measures can be used in simulated sieve mode:

ECA Diameter
Bounding Rectangle Width
Fiber Width
Feret Width

To return to **Run Data** section, click HERE.

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# Chapter 5 - CALIBRATION
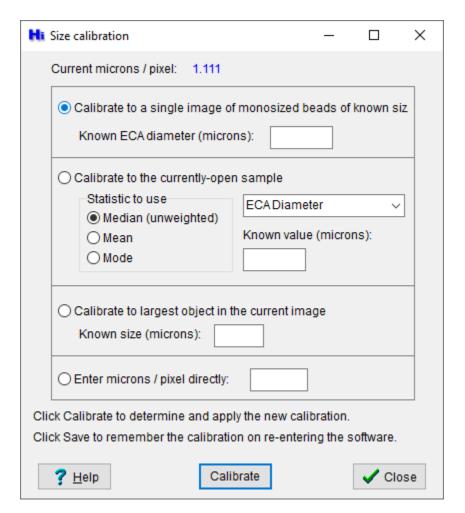
## Calibration

There are a few ways to calibrate the Hydro Insight by using: A single image of monosized beads of known size, the curently open sample, the largest object in the image or entering the microns/pixel ratio directly.

It is <u>highly recommended</u> to use the **Shape Control** sample for calibration, that has a narrow size distribution, and the particle size is known. The **Mode** size of the entire sample will be used for calibration. For best accuracy, do two or more iterations of run/calibrate, unless the change in calibration factor is small.

In the toolbar, click on **Unit ➔ Calibration** to change/perform the size calibration.
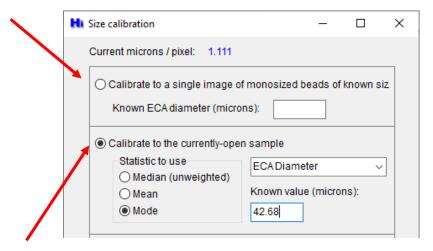
**<u>Note:</u>** Administrator level is required if **Security** is in effect.

## Calibrate to a single image:

Using monosize beads of known size, take an image containing at least 10 beads. Enter the micron size in **Known ECA diameter (microns)** and click on **Calibrate,** then **Close**.
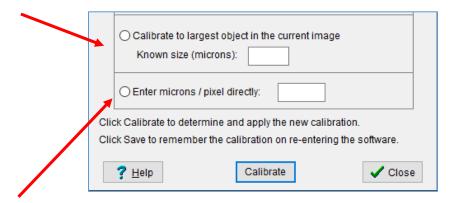


## Calibrate to the currently-open sample:  Recommended

Use a sample that has a narrow size distribution, and the particle size is known. The **Mean** or **Mode** size of the entire sample will be used as the standard. For best accuracy, do two or more iterations of run/calibrate, unless the change in calibration is small.    (**Mode** is strongly recommended), enter the **Known value (microns)** and click on **Calibrate** then **Close**.

## Calibrate to largest object:

Take an image in which that object is the largest object in the image. Enter the **Known size (microns),** click on **Calibrate** then **Close**.



## To enter a calibration factor directly:

Enter a new calibration value **microns/pixel** and click on **Calibrate** then **Close**.

The new Calibration factor (microns/pixel) will be shown (in blue) at the top of the dialog window.

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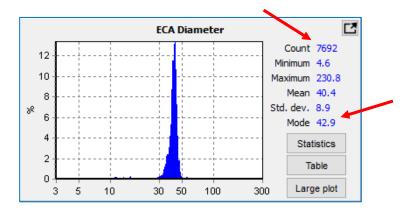
## Running Shape control

Use the Shape Control sample, that has a narrow size distribution, and the particle size is known. The **Mode** size of the entire sample will be used for calibration. For best accuracy, do two or more iterations of run/calibrate, unless the change in calibration factor is small.

1. Activate the pump on the Mastersizer 3000 and set the **Pump speed** to 3500 rpm.
2. Add Particle Shape control in the sample vessel of the Dispersion unit.
3. Click on **New sample** and follow the prompts from the Wizard.
   - Enter a **Sample name** and the **File location** if other than default.
   - Enter the **Sample parameters** according to the Shape Control Assay Sheet.
   - Check the images.
4. Click on **Clear** then **Start** buttons to start the run.
5. Accumulate more than 3000 particles Count, to become a representative sample.
6. Then, click **Stop** to end the run, or wait for a preset limit to be reached.



Below histogram belongs to 42 μm Shape Control.
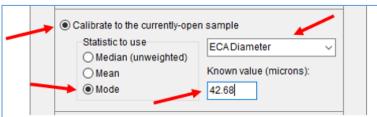


**Good concentration run**

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## Calibration with Shape Control

1. In the toolbar, click on **Unit ➔ Calibration** to perform the size calibration.
2. Select **Calibrate to the curently-open sample** option.
3. Select **Mode**.
4. Select **ECA Diameter** in the pull-down menu.
5. Enter the **Mode** value from the Assay Sheet into the **Known value (microns).**



6. Press **Calibrate** then **OK.**



7. The new Calibration factor (microns/pixel) will be shown (in blue) at the top of the dialog window.


## Verification

Perform a verification run right after calibration is completed using the same sample from the calibration process:
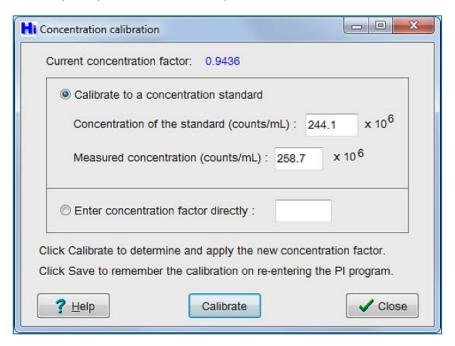
1. Activate the pump on the Mastersizer 3000 and set the **Pump speed** to 3500 rpm.
2. Click on **New sample** and follow the prompts from the Wizard.
   - Enter a **Sample name** and the **File location** if other than default.
   - Enter the **Sample parameters** according to the Shape Control Assay Sheet.
   - Check the images.
3. Click on **Clear** then **Start** buttons to start the verification run.
4. Check the **Mode** for **ECA Diameter** to verify the calibration.  The resulting **Mode** diameter should be within ± 10% of the Shape Control Assay value.
5. Perform a **Clean** system.
6. If not within ± 10%, perform another Calibration procedure until the verification passes.

Size calibration exists independently of all Settings. When a calibration is done, it will remain in effect even if a different sample is loaded to the user interface.

## Concentration calibration

Click **Unit ➔ Concentration calibration** to open a form where the concentration factor may be changed, either directly or by reference to a sample of known concentration.
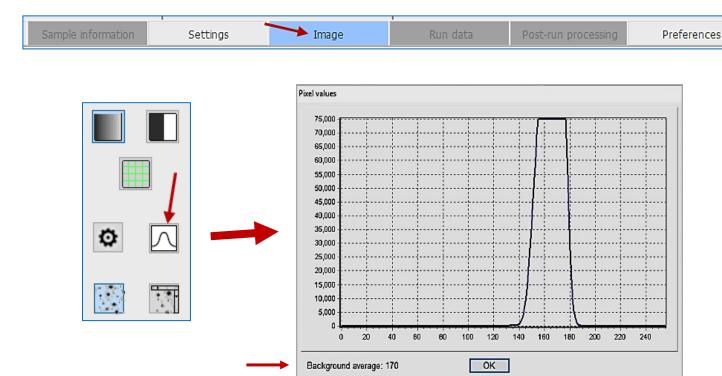
# Chapter 6 - WORKING WITH HARDWARE

## Check the Background intensity

Run the Hydro Insight instrument with clean water (no particles)

- Select the tab *Image*.
- Click on the *Pixel value histogram* icon.
- Note the *Background average* intensity at the bottom left of the histogram.



- If the Background intensity is less than 140, increase the camera gain in *Parameter quick adjust* using the gear icon.
- Check the *Background average* intensity again for the new gain setting. Verify it is within 140 and 200.
- If the background is higher than 200, decrease the camera gain.

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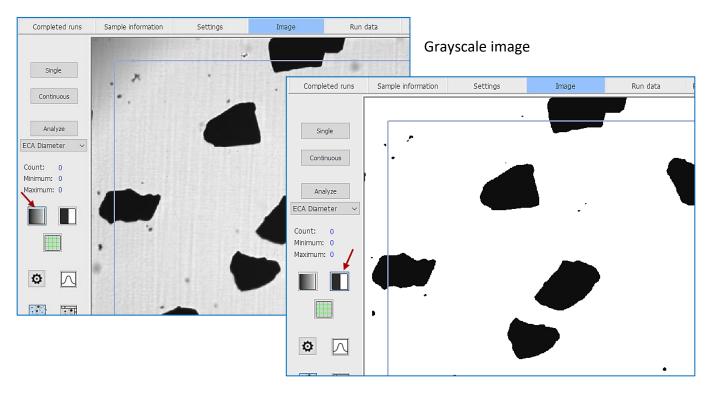
## Adjusting the threshold

The internal analysis threshold is a gray level value between 0 (black) and 255 (white). In the settings, threshold is specified as a percent (0 to 100%) of the background average intensity. This method makes the analysis less sensitive to changes in light intensity due to varying particle densities, for example.

The threshold percent is the most critical parameter in ensuring that images are correctly analyzed. If the threshold is set too low, tiny particles may not be counted. If set too high, dark areas of the background may be counted as objects and particles that almost contact each other may be counted as one particle. Nevertheless, there is usually a range of values that produces consistent results.

The threshold is calculated automatically in software on every frame, based on the background average intensity in that frame and the threshold percent. Normally the threshold setting should be kept within the range 55 % to 75 %.

To check the threshold value, use the **Binary image** mode to verify that only actual particles appear as black objects, and that all actual particles appear.



Grayscale image

Binary image

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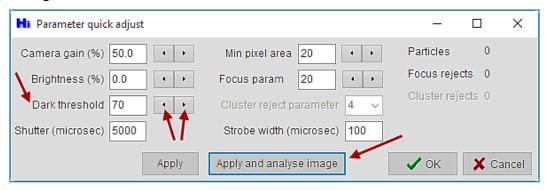
To adjust the **Threshold** setting, complete the following actions:
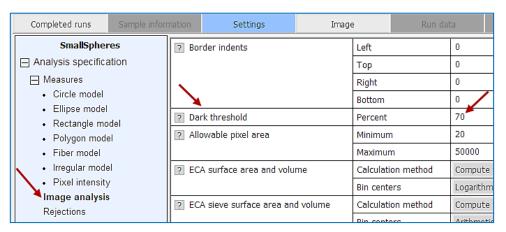
- Run **Shape Control** particles (nominal 42 um).
- Take an image using the ***Single*** button and click ***Analyze.***

- Click on the ***Binary*** button

- For an acceptable **Threshold** setting, particles should appear black, with no other areas of black in the image.
- If it appears incorrect, click on the ***Parameter Quick Adjust*** icon              to adjust the threshold (Dark threshold).
- Increase OR decrease ***Dark threshold*** then click on ***Apply and analyze image*** and verify the image.



- Repeat above step as needed, including pressing ***Analyze*** each time to verify ALL particles of interest have been analyzed.
- When finish, restore normal Grayscale image by pressing ***Grayscale*** icon.

- Save the adjusted ***Threshold*** value in ***Settings*** ➔ ***Image analysis*** ➔ ***Dark threshold.***
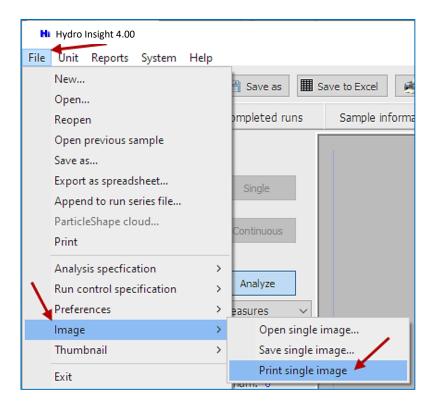
# Chapter 7 - WORKING WITH DATA FILES

## Printing a single image

- To print an image, use ***Image ➔ Print***.



- To print the image with the analysis list, use ***Print With Image*** in the analysis list box.
- To save as a TIFF file, use ***File ➔ Save single image***.
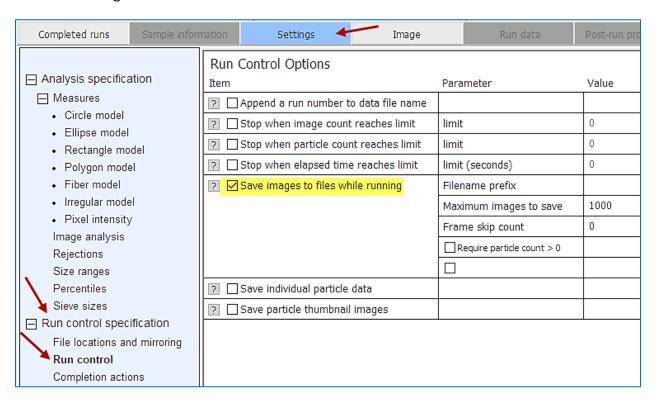
## Save run images

Normally images are not saved during a run since a run may involve thousands of images. However, the Hydro Insight system provides the option of saving run images while running the sample, which later may be reviewed and re-analyzed offline.

- To save the images while running the sample and before starting the run click on: **Settings ➔ Run Control specifications ➔ Run control.**
- Check **Save images to files while running**.
- Set the option **Maximum number to save** equal to or greater than the intended number of images in the run.



- **Frame Skip Count** is the number of frames to bypass between captured frames. Set **Frame Skip Count** to **"n"** and every (n+1) th image is going to be saved. Typical value for **Frame Skip Count** is 50. To save consecutive camera images, set **Frame skip count** to 0.
- If **Require particle count > 0** is enabled, only frames with accepted particles will be saved.

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Run images will be saved as Name-0001.TIF, Name-0002.TIF etc. where "Name" represents the current Run Name entry.

If saving to files on a disk or other storage device, the number is limited by the storage capacity of the device.  Saving images to files may slow down the frame rate of the run.

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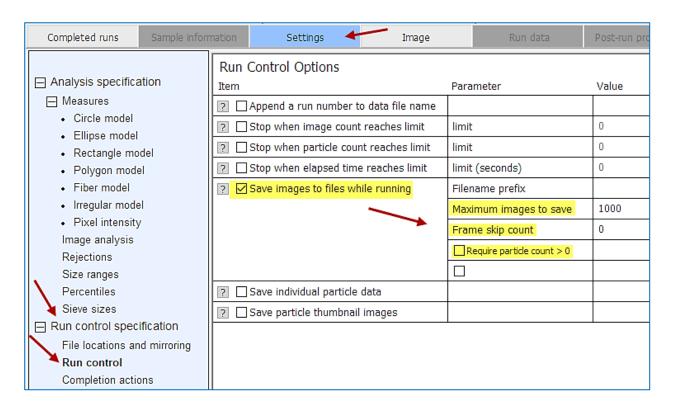
## Prepare a re-analyzable run

Images saved during a run may be reviewed later and analyzed individually or all together. To be fully re-analyzable, a run must save all its analyzed images.

Before initiate a run, prepare the following settings:

- Click on Settings ➔ *Run Control specifications* ➔ *Run control*

- Select *Save images to files while running.*

- Set the option *Maximum number to save* equal to or greater than the intended number of images in the run.

- Set *Frame skip count* to *0*.

- The *Require particle count > 0* should be turned off if you expect that the run may be re-analyzed under different settings.



If only some of a run's images are saved, they may still be re-analyzed offline, but the results will likely not agree exactly with the original run results.

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## Review or re-analyze stored run images.

Image Playback is a feature of the Hydro Insight that allows the user to replay saved images either step by step or in a continuous sequence. This feature can be used to simply examine the run images. If a significant number of images were saved, it can also be used to regenerate run results under different image analysis parameters or a different set of active measures.

To playback images is required to have the images saved during a run. Select the following option *Save images to files while running* under *Settings* ➔ **Run control specification** ➔ **Run control** before starting the run. It is important to note that it may be impractical to save all images that are taken during the analysis because of the image file size. Each image file can represent from 1 up to about 5 MB, depending on the camera in use. Thus, 100 images could represent about 500 MB of storage space.

The Hydro Insight system, when installed, comes with two sample demonstration files that have a sequence of 100 200 images.
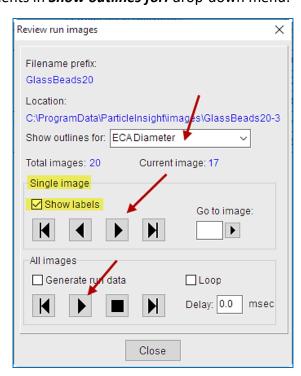
- *Open* the run if it is not already open.
- Click on the tab *Images*.

| Completed runs | Sample information | Settings | Image | Ru |
|---|---|---|---|---|
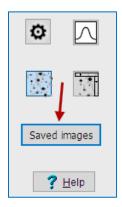
- Click on *Saved images.*

**To review stored run images**:

- Select the measurements in *Show outlines for:* drop-down menu.
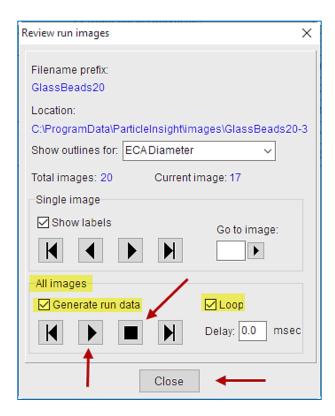
- Under *Single image* use the icon ▶ to review images individually. Select *Show labels* if you want to see the measurements previously selected attached to the particles.

- Under *All images* use the icon ▶ to start an automatic playback.

**To re-analyze stored run images:**

- To re-analyze the run images, click on *Clear*, make any desired changes to *Settings* then click *Clear* again.
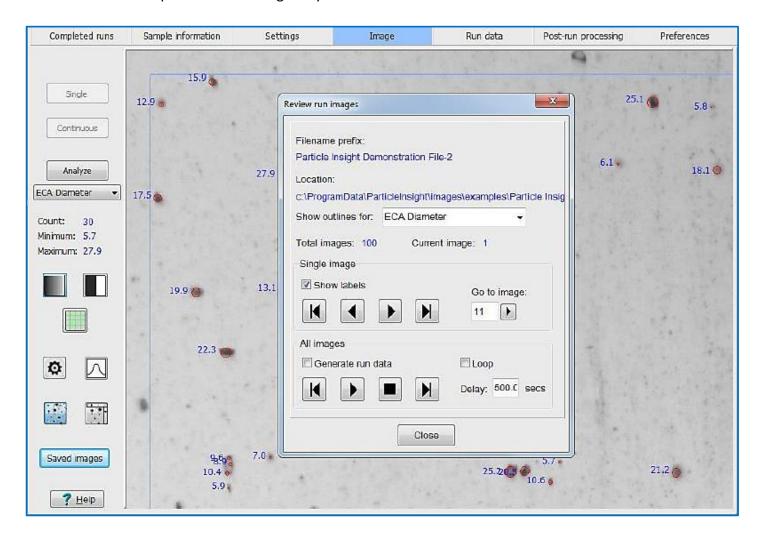- Under *All images* select *Generate run data* and *Loop.* Use the icon ▶ to start a continuous playback.



**Note**:

- You can always stop the playback manually by clicking the *Stop* button.
- Automatic run completion actions do not happen when re-analyze ends, but the results may be manually saved or printed.
- Images are not saved during re-analyze.

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Below a full picture of the image Playback feature:



Once done, you can re-load the original file and original results using **File** ➔ **Reopen**.

## Creating a Time series chart

Before starting a Time series chart, some settings should be set in advance, then save those settings as a Settings file for future use. Otherwise, open the Default settings file as a starting point then change the settings as needed.

**Selecting the Settings:**

- Click on **Settings ➔ Measures**
  - Select a **Shape Model** and the respective measures that will be selected later in the chart.
  - Repeat above step for all **Shape Models** that are to be included.
- Click on **Settings ➔ Run control**
  - Select <u>How to stop</u> the run: when image, particle or elapsed time count reaches the limt.



| Run Control Options | | |
|---|---|---|
| Item | Parameter | Value |
| [?] ☐ Append a run number to data file name | | |
| [?] ☐ Stop when image count reaches limit | limit | 0 |
| [?] ☑ Stop when particle count reaches limit | limit | 10000 |
| [?] ☐ Stop when elapsed time reaches limit | limit (seconds) | 0 |
| [?] ☐ Save images to files while running | Filename prefix | |
| | Maximum images to save | 1000 |

- Click on **Settings ➔ Completion actions**
  - Select **Start a new run**.
  - Under **Restart,** enter the <u>amount of runs</u> to be completed in the chart.   *e.g.* to complete 10 runs, enter 9 Restart. Initial run + 9 Restarts = total 10 runs.
  - Under **Delay (secs),** enter the delay in seconds between runs.



| Completion actions | | |
|---|---|---|
| Item | Parameter | Value |
| [?] ☐ Save sample file | Path | C:\ProgramData\ParticleInsight\samples\ |
| | Filename | |
| [?] ☐ Export results to Excel file | Path | C:\ProgramData\ParticleInsight\export\ |
| | Filename | |
| [?] ☐ Print results | | |
| [?] ☑ Start new run | Restarts | 9 |
| | Delay (sec) | 10 |
| [?] ☐ Append to run series file | Path | C:\ProgramData\ParticleInsight\export\ |
| | Filename | |

- Click on **Save Settings** to save the settings with a designated filename related to the experiment. This will also result useful if you would like to repeat the runs.
- Click on **Preferences ➔ Run Series files ➔ Statistics** to select the set of Statistics that will be selected later in the chart.
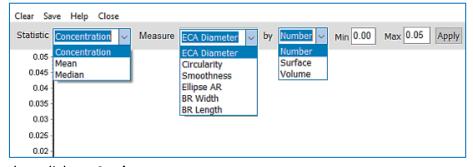
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The runs should be taken under identical settings and have the same root name, differing only in the number suffix, for example TestRun-1, TestRun-2 etc.

The Statistics, Measures and Min/Max values can be set different for each chart once opened. There is a maximum of 4 charts that can be initiated.

At the end, the final results enable the operator to complete an evaluation of behavior from particles suspended in a solution by plotting results of pre-selected measures and statistics from a collection of run files, over a period of time under same conditions and settings. This is the principal advantage of this feature mainly oriented to two key processeses in the pharmacautical industry: Cristalization and Disolution, where the behaviour of components must be closely monitored.

**Creating a Times series chart:**
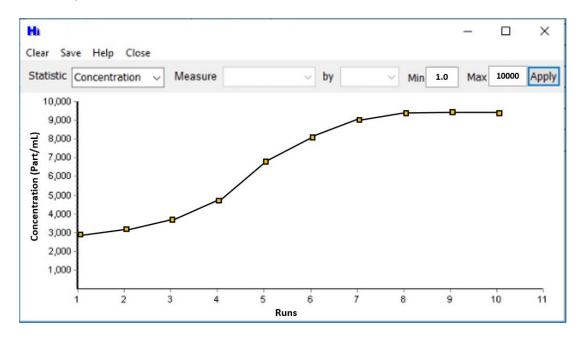
- Click on *New sample* button.
    - Enter *Sample information* including the sample name which will be the filename for the collection of runs.
    - Click on *Next* then open a Settings file if other than default file. Modify any parameter if needed, then save the file.
    - o  Activate the pump on the Mastersizer 3000.
    - o  Set the Pump speed to 3500 rpm.
    - Click on *Next*, click on *Continuous* button to check the images. Adjust any image parameter if needed. Turn the pump **Off**.
    - Click *Next* and start opening the charts.
    - Click on *Report* then select *Time series chart* to open the first chart.
    - Open the *Statistic* pull-down menu and select: Concentration, Mean or Median.
    - Open the *Measure* pull-down menu and select: One of the previously selected.
    - Open the *b*y pull-down menu and select Weighting: Number, Surface, or Volume.
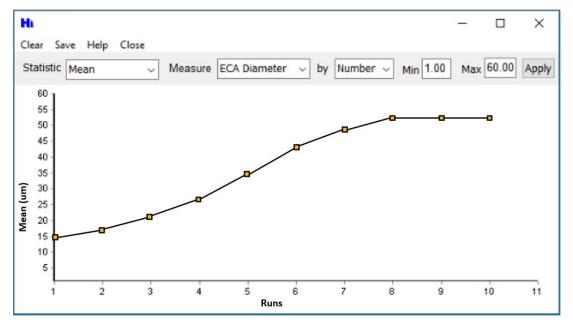    - Enter the *Min* and *Max* for the Measure selected*.*



- then click on *Apply.*
- Repeat above steps for any additional chart opened, until a maximum of 4.
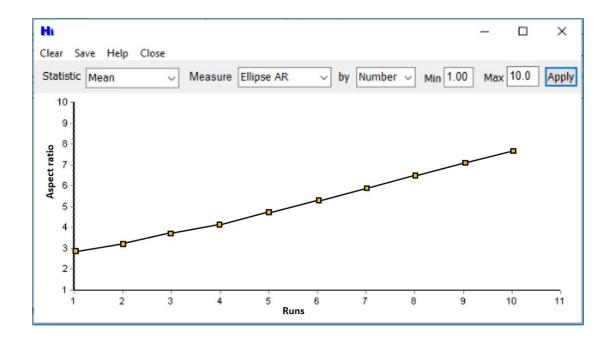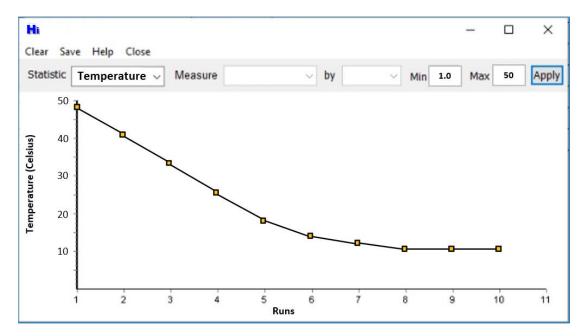
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- Activate the pump on the Mastersizer 3000.
- Click on **Clear** then **Start** to start the first Time series run.
- After the last Time series run is completed, turn the pump **Off**.
- All runs are saved in **ProgramData → particleinside → samples** folder.
- Below is an example how the charts look like, depending on the **Statistics**, **Measures** and **Min/Max** selected.





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- The following dialog window shows: **Save data to a spreadsheet?**
  - If **Yes,** an Excel listing file is saved in Programdata ➔ particleinside ➔ export folder. Otherwise, will not save.
- The following questions shows up: **Additional Time series charts?**
  - If **Yes,** repeat above steps for **Creating a Time series chart**. Otherwise, **Time series chart** function concludes.

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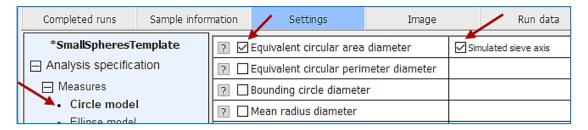
## Using simulated sieve mode
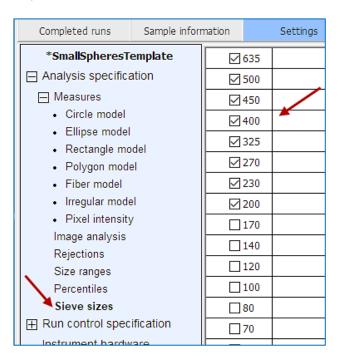
In this mode, the user must select the sieve sizes to implement on the <u>simulated Sieve axis</u>. In simulated sieve mode, the size axis of distribution plots is divided into size classes that correspond to standard sieve sizes. The sizes used are selectable.

Simulated sieve mode is available only for Circle model (ECA Diameter), Rectangular model (BR Width), Fiber model (Fiber Width), and Irregular model (Feret Width).

In *Settings* ➜ *Measures*, enable one or more measures that allows sieve mode, and enable the sieve option.  e.g.



In *Settings* ➜ *Sieve Sizes*, select all the sieve sizes that are to be simulated.
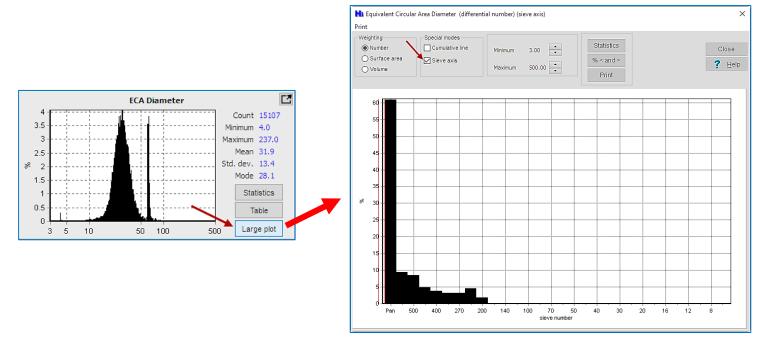


With this feature enabled and the user selected sieves chosen, several of the statistical histograms will report not only **Volume**, **Number** and **Surface Area** weighted results, but also will report results based on **Sieve** weigted data.

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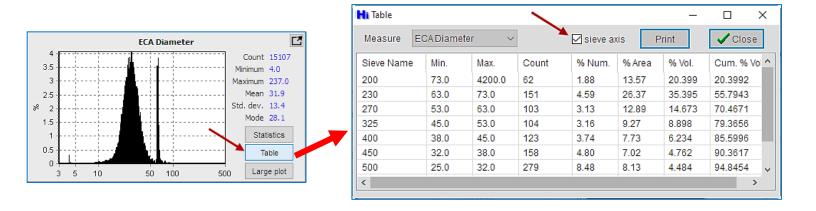
If sieve mode is in effect, both micron axis and sieve axis data are taken. Whether micron data or sieve data are shown on the screen plots during a run has no effect on what data are accumulated. Either or both kinds of plots may be viewed or printed after a run. If sieve mode is not enabled before starting a run, only micron data are accumulated.

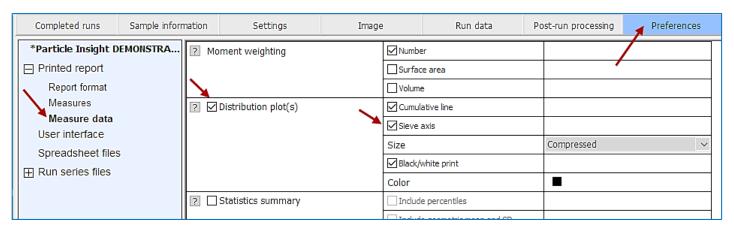In a large plot window, check the Sieve axis option at the top.
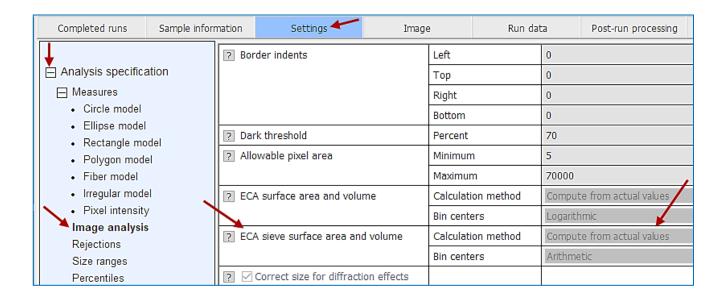


In a Table window, check the Sieve axis option at the top.



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To include sieve mode graphs in a printed report, go to **Preferences** ➜ **Printed Report** ➜ **Measure data** ➜ **Distribution plot(s)** and enable **Sieve axis**.



If the sieve mode data is to be compared to actual sieve results, use volume-weighted data for the comparison. Also, if the material being tested is spherical, in **Settings** ➜ **Analysis specification** ➜ **Image Analysis** make sure that Calculation Method for **ECA sieve surface area and volume** is set to "**compute from actual values**."



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When comparing sieve axis results to micron results, be aware that the sieve axis size bin boundaries and the micron bin boundaries are not aligned. A sieve boundary may fall in the middle of a micron size bin, and vice versa.
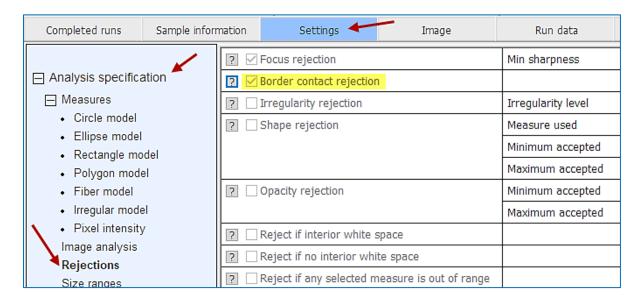
## Sieve size – micron correspondence

| Sieve size | micron size |
|---|---|
| 635 | 20 |
| 500 | 25 |
| 450 | 32 |
| 400 | 38 |
| 325 | 45 |
| 270 | 53 |
| 230 | 63 |
| 200 | 75 |
| 170 | 90 |
| 140 | 106 |
| 120 | 125 |
| 100 | 150 |
| 80 | 180 |
| 70 | 212 |
| 60 | 250 |
| 50 | 300 |
| 45 | 355 |
| 40 | 425 |
| 35 | 500 |
| 30 | 600 |
| 25 | 710 |
| 20 | 850 |

To return to *Sieve sizes* in *Settings*, click HERE.

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## Border contact rejection

Under **Settings** ➔ **Analysis specification** ➔ **Rejections** enable **Border contact rejection** to reject particles that contact or intersect the image border.

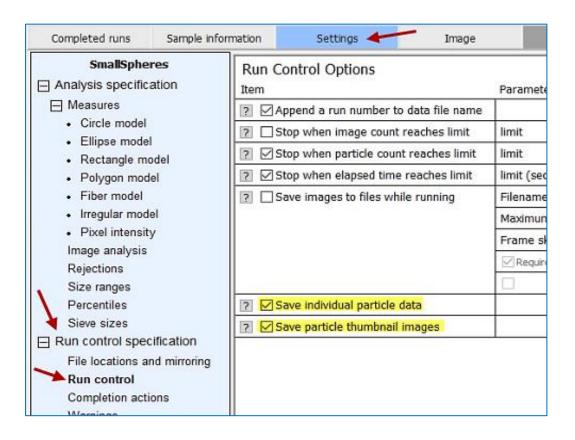## Saving data on individual particles

In a normal run, size and shape information is computed for each particle, but the data is not permanently saved; it gets added to the appropriate distribution arrays.

In order to use ALL the *Post-run functions*, the following options must be enabled <u>before starting a run:</u>

*Save individual particle data*

*Save particle thumbnail images*

under *Settings* ➔ *Run Control specifications* ➔ *Run Control.*

## Creating a multi-run summary

You can print a report or create an XLS file consisting of a row of data for each of several sample runs. The runs should have been taken under identical settings and have the same root name, differing only in the number suffix,  for example TestRun-1, TestRun-2 etc. The line of data contains selected statistics for each active measure.

The statistics to include should be selected beforehand in *Preferences* ➔ *Run Series files* ➔ *Statistics.*  Values for Mean, Standard Deviation, and Mode are always the "number weight" values, i.e., unweighted. The file name will be the series name with "-RS" as a suffix, for example TestRun-RS.xls. The runs series summary is in addition to the normal data files that are saved for each individual sample.
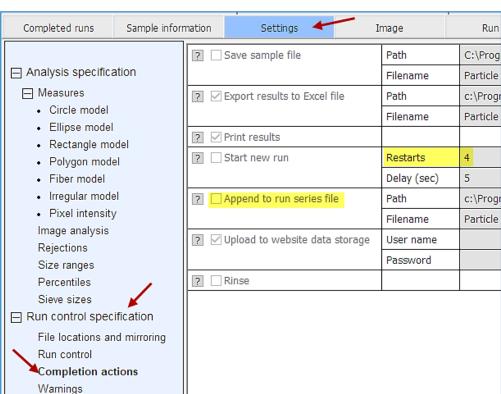
There are three ways to create a run series file:

1. **Automatic append**

To use the automatic method, enable the *Append to run series file* option under *Settings* ➔ *Run control specifications* ➔ *Completion actions.* A line is automatically added to the file upon completion of a run.

This method may be used in conjunction with the *Restarts* feature to create a summary file for a set of similar runs.
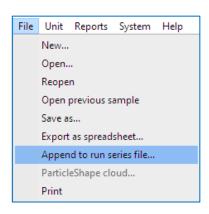
---

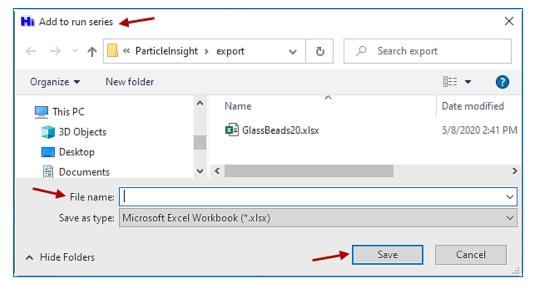**Example**: to take a data point every 30 minutes for five hours, using the series name "SampleXYZ":

- Create a sample named SampleXYZ.
- The software adds "-1" as a suffix to the name of the first run.
- Under **Settings ➔ Run control specifications ➔ Completion actions,** enable **Start new run** and set **Restarts** to 9 and **Delay (sec)** to 1800.
- Check that a run limit is set by Image count, particle count or elapsed time under **Run control**.
- Click **Clear**, then **Start**. After five hours, the run series file will be complete and will have the filename SampleXYZ-RS.xls and will be in the Export subdirectory.

Complete data from the individual runs will be saved under the names SampleXYZ-1, SampleXYZ-2 ……… SampleXYZ-9 . The run series may be ended before ten runs have been completed by simply clicking **Cancel** in the delay period between runs. The run series file will be retained and will have a line for each run up to that point in time.

2. **Manual append**

After a run finishes, you may select **File ➔ Append to run series file** to add data from the currently open run file into the Run Series file as a new line on the multi-run file.
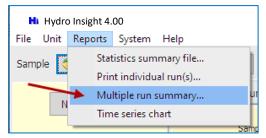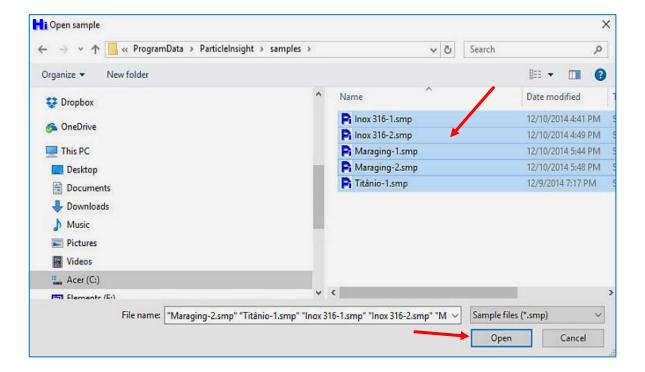
3. **All at once**

Use this utility to create a run series file from a previously saved set of runs, or to compare data values from a set of runs on-screen without saving as a file.

- Go to **Toolbar** options.
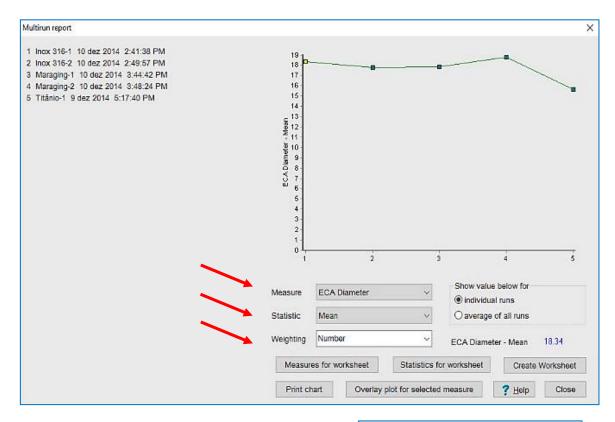- Click on **Reports** ➔ **Multiple run summary**.



- Select the runs to be included. Use your mouse and the **Ctrl key** to select multiple files and then click **Open**.
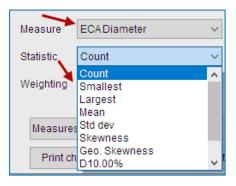
- Once the files are opened, a screen will show all the selected files on the **Multirun Report** screen, as showm below. The graph will show a set of nodes, one node for each run in the series.
- The left axis variable is determined by the **Measure** and **Statistic** options selected below in the chart. The selections will determine the statistical value that is plotted in the chart.



- If **System data** is selected in the **Measure** drop-down, the options for Statistic to plot are **Frames**, **Particle count**, and **Elapsed seconds**.
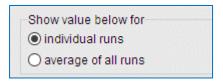


- If an actual M**easure** is selected, the options for Statistic to plot are Count, Smallest value, largest, Mean, Std Deviation, Geometric Skewness, and three percentiles (10%, 50%, 90%).
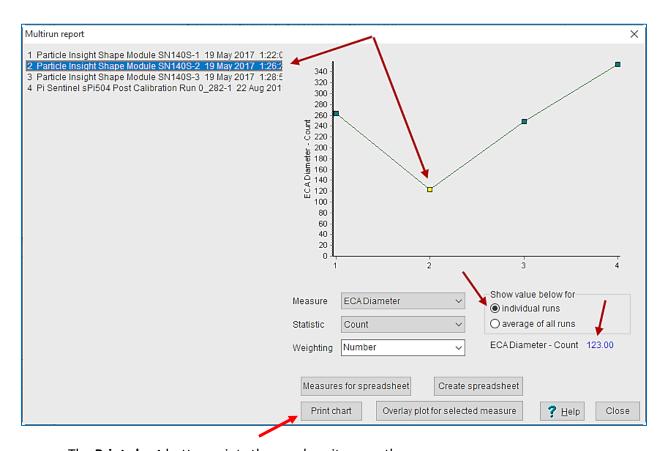


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- The **Show value for** box contains two options:
    - o **individual run**: click on one of the runs in the list to the left of the chart or one of the nodes in the graph. The node representing that run becomes yellow and the numeric value of the selected statistic is displayed at the bottom of the panel.
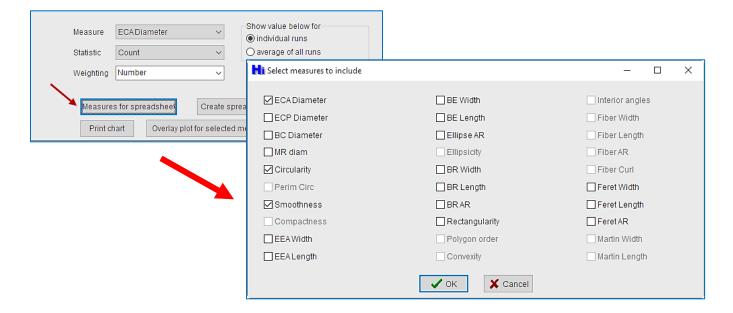    - o **average of all runs:** displays the averages of all the runs listed at the bottom of the panel. .
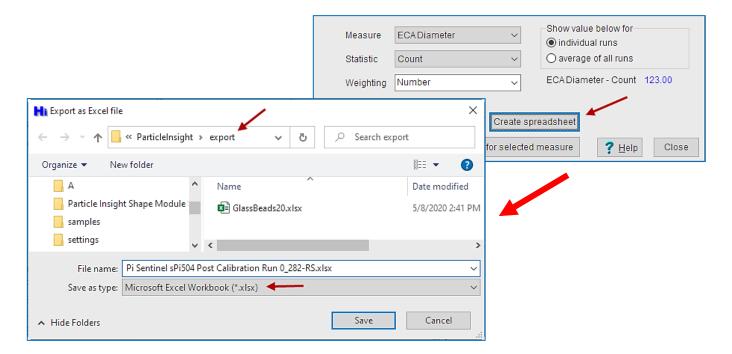


- The **Print chart** button prints the graph as it currently appears.

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- To create an Excel file:
  - Click on **Measures for spreadsheet** button to select the measures to be included in the Excel file.



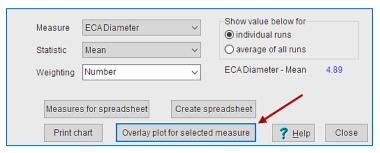  - Then, click on **Create spreadsheet** button to create and save the Excel file into folder **export** under **ParticleInsight** folder.
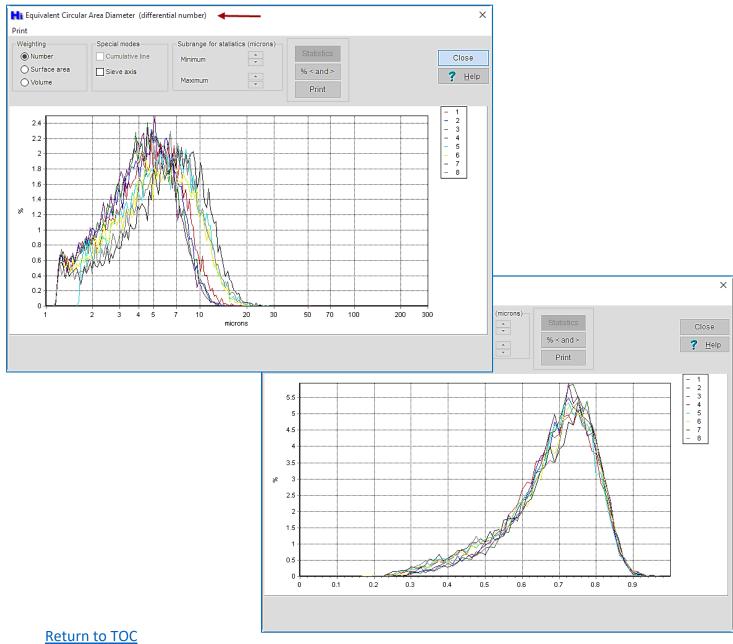
- Click on **Overlay plot for the Selected Measure** to compare the data histogram curves for all the selected files. The measure and statistic weighing is selected from the drop down menus.



- This is useful to compare not only size but all the different shape measures.

# Chapter 8 - SECURITY

## Security features

When security is on, there is always a current user (Normal User or Administrator), identified at the lower left of the screen.

- An Administrator can turn security On or Off, add a new user, disable, or enable any user, and change any user's password.
- A Normal user can change only his own password.

When a new user is added, a password should be created for the user. Passwords must contain at least 8 characters and must be renewed every 60 days.

User information is recorded in the file "users.txt."  This file contains for each user:

- date/time the user was added
- user's security level
- date/time of the last password change for that user
- user's password, in encrypted form
- whether the user is enabled or disabled

There can be any number of users of each level in users.txt. It will always contain at least one Administrator and at least one Service User. Users.txt contains a checksum which is read on program startup. If the file has been modified outside of the PI software, *Error 6* will be flagged.

When security is on, these features are enabled:
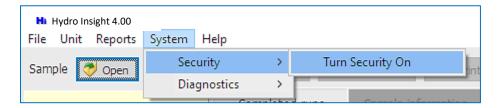
- Users must log in at startup, and when the software has been idle for more than 15 minutes.
- The following events are logged into the audit trail:
  - ➢ user login
  - ➢ login failure
  - ➢ switch to a different user
  - ➢ a user's password changed
  - ➢ checksum error reading users.txt
  - ➢ the audit trail was archived
  - ➢ security was turned on or turned off
  - ➢ a new user was added
  - ➢ a user was disabled or enabled
  - ➢ a user was removed (by an Administrator)

Maximum audit trail file size is 512 KB.

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## Security options

**Security:** Allows to turn the security options **On** or **Off**.



When turned **On,** the following dialog windows shows up:

You must enter the default values:
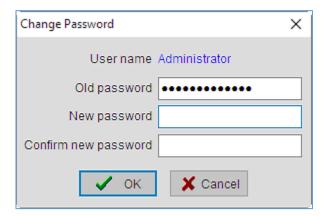
**Admin. Name**: Administrator

and
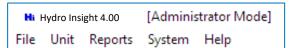
**Password**: Administrator



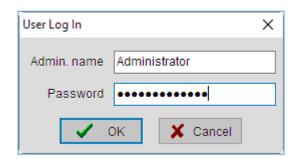Once accepted, the administrator must change the password to their own password.
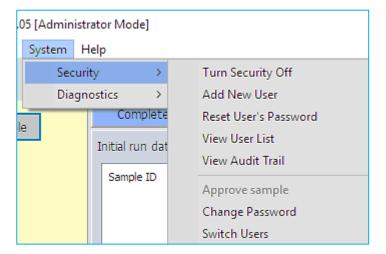


Then, the top left of the software interface will show:
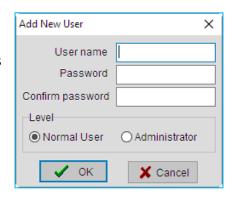
Below an overview of Security options. These menu items are available only to administrators:
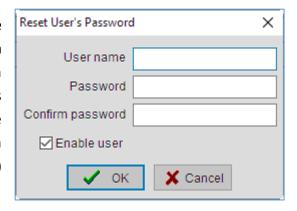


**Turn security off:** disables all security features. Security can be turned Off only while in Administrator mode.



**Add new user:** Administrator can add a new username as well as a password and the access level for that user.
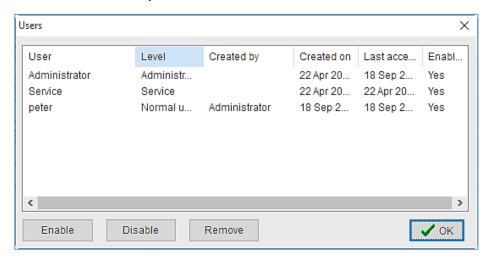
**Reset User's Password:** Administrator may change any user's password with this item. In the event a user forgets their password, the Administrator can change the user's password.   In the event a user is no longer active; the Administrator should disable the user.  This retains the username in the system (required to ensure audit trail shows user activity) but it will not be active.



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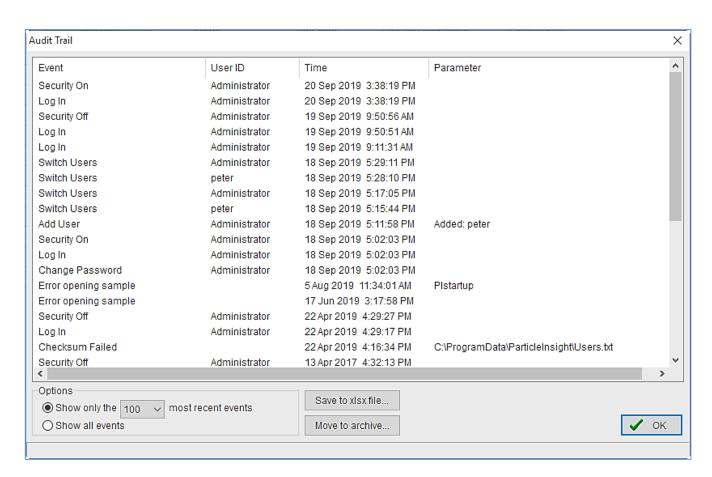
**View User's list:** Open a list of all users with status information. The administrator can Enable, Disable or Remove any user.



**View Audit Trail:** Opens a window showing events in the audit trail, most recent event first.



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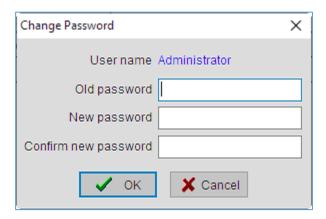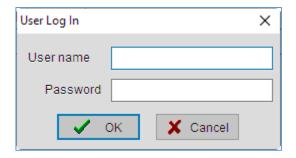
These menu items are available only for administrators:

**Approve sample …** A user may add a signature to a run file. This may be done when the file is saved, or at any later time. Only one signature may be added, and once a run is signed the signature is permanent. The signature consists of the username and date of signing.

**Change Password:** Administrator may change his password.



**Switch Users:** Change to a different active user.



To return to **Toolbar options ➜ System**, click HERE.

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## Chapter 9 – CONSUMABLE PARTS LIST

| P/N | Description | Picture |
|---|---|---|
| 6601133 | Disposable Pipette - 10 per pack |  |
| 6600990 | Disposable Spatulas - pack of 10 |  |
| 6601170 | Particle Shape Control - Glass Spheres 42 µm (nominal) |  |
| 6601395 | Particle Shape Control - Glass Spheres 242 µm (nominal) |  |
| 6601257 | Dispersant |  |
| 6601168 | Lens cleaner - Carl Zeiss Lens Cleaning Spray 2oz - 60ml |  |
| 6600989 | Lens Paper, 4" L x 6" W (10.1 cm x 15.2 cm) |  |

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| P/N | Description | Picture |
|---|---|---|
| 6601138 | Swabs - 5 per pack |  |
| 6600991 | Fuse - USA, 3AG SLO-BLO, 2A, 250V |  |
| 6600992 | Fuse - International, 5x20mm, SLO-BLO, 0.5A |  |
| 6601383 | Tygon Tubing S3-E-3603 Aqueous Compatible (Outside instrument) |  |
| | | |
| | | |
| | | |

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# APPENDIX A

## A.1 - File system reference

The following is the structure and location of the different files used in the Hydro Insight:

### Sample File

The software is organized around the concept of the **sample file**, which holds the data from an analysis along with information about the sample and settings of the analysis, plus reporting options.

**Template file** is a special kind of sample file. Several template files are provided in the samples folder to provide an easy way to create a sample file that is suitable for a standard particle type. Templates are provided for small spheres, large spheres, crystals, thin fibers, and thick fibers. To use a template file, open one in the software and then use *Save As* to create a copy of it under a new filename.

- Data cannot be taken into an original template file.
- A template file cannot be modified.
- A user-defined template file may be created by opening an existing template file and using *Save As* with "Template" as part of the new filename.
- If the new filename does not include "Template", the new file is not a template file.

**Filename structure**
**Sample filename:**       if number suffix option is Off <samplename>
                                        if number suffix option is On  <samplename>-n, where n = 1, 2, 3 …
**Template filename:**   <samplenameTemplate>
**File extension**: **\*.smp**
**Default location:**   This PC ＞ Windows (C:) ＞ Users ＞ Public ＞ Public Documents ＞ Hydro Insight ＞ samples

### Image files

Image files are monochrome 8-bit-per-pixel format, which encodes 256 gray levels.

**Filename structure:**
<samplename>-n-xxxx,       where n = 1, 2, 3, …       ; xxxx = 0001, 0002, …

**n**: increments every time you press the *Increment* button in *Sample information* OR you set *Start a new run* and including more than 1 restart under *Settings* ➔ *Run control specification* ➔ *Completion actions.*

**xxxx**: increments up to the number of images saved.

**File extension: \*.tif** or **\*.bmp.**
\*.tif files are TIFF format; \*.bmp files are Windows bitmap format.
**Default location**: This PC ＞ Windows (C:) ＞ Users ＞ Public ＞ Public Documents ＞ Hydro Insight ＞ images

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## Spreadsheet files

There are several types of spreadsheet files created from different files:

- From a <u>sample file</u> - contains the same information to be included in the printed report for a sample run. The first page is sample documentation, settings, and system values. Succeeding pages are one page per measure and contain histogram listings.

  **Filename structure:**
  <samplename>-n, where n = 1, 2, 3, …

- From a <u>Multirun or run series file</u> - contains a line of summary data for each run in the series.

  **Filename structure:**
  <samplename>-n-RS, where n = 1, 2, 3, …

- <u>Particle listing</u> - contains a line of data for every particle in the sample database.

  **Filename structure:**
  <samplename>-n-PF, where n = 1, 2, 3, …

- Listings from the <u>Percentiles, Oil Analysis</u> and <u>Classification features</u> in Post-run Processing.

  **Filename structure:**
  user-specified

- From the <u>Comprehensive Statistics</u> form. The values shown in the form are written as a .csv (comma-separated values) text file.

For all types of spreadsheet files:

**File extension**: *.xlsx* (Microsoft Excel file format) or *.csv* in the case of Comprehensive Statistics.

**Default location:** This PC > Windows (C:) > Users > Public > Public Documents > Hydro Insight > export

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## Particle database files

These are optional files for saving data on individual particles.

- Particle data
  **Filename structure:**
  <samplename>-n-**pd**, where n = 1, 2, 3, …

- Particle thumbnails
  **Filename structure:**
  <samplename>-n-**id**, where n = 1, 2, 3, …

**File extension**: **\*.dat**
**Default location:**  This PC  ›  Windows (C:)  ›  Users  ›  Public  ›  Public Documents  ›  Hydro Insight  ›  particledata

## System files

There are several files with different extensions that are used by the security system and at startup.

- **Users.txt**: contains usernames, dates of creation and encoded passwords.

- **AuditTrail.**txt: maintains the security audit trail.

- **PIstartup.smp**: contains certain values that load at program startup.

**Default location:**  This PC  ›  Windows (C:)  ›  Users  ›  Public  ›  Public Documents  ›  Hydro Insight  ›

## Settings files

These files hold Analysis Specification and Run Control Specification settings.

Usual filename: <particle type> where particle type is **"Small Spheres"** etc.
**File extension: \*.psf**
**Default location:**  This PC  ›  Windows (C:)  ›  Users  ›  Public  ›  Public Documents  ›  Hydro Insight  ›  settings

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## Preference files

**Filename structure:**
Usual filename: <name> where name is indicative of either a type of sample, or of a user's default preferences for reports and user interface.

**File extension:** *.prf
**Default location:** This PC > Windows (C:) > Users > Public > Public Documents > Hydro Insight > settings

## Text files

Certain features generate text files. These include individual <u>particle listings</u> and <u>particle tracking listings</u>.

**Filename structure:**
- particle listings - Usual filename: <samplename>-PF
- particle tracking files  - <samplename>-PT.

**File extension:** .txt
**Default location:** This PC > Windows (C:) > Users > Public > Public Documents > Hydro Insight > textdata

## Thumbnail image files

Thumbnail pages are able to be saved as image files. These images are of a smaller size than the normal camera images.

**Filename structure:**
Usual filename: <samplename>-n-thumbsX          where n = 1,2,3… and X = 1,2,3…
**File extension:** *.tif
**Default location:** This PC > Windows (C:) > Users > Public > Public Documents > Hydro Insight > thumbnails

## Classification files

The Particle Classification feature in Post-run Processing saves <u>classification limits</u> and <u>category names.</u>

Classification limits are saved in files with extension:  *.occ

Category names are saved in files with extension: *.ini

**Default location:** This PC > Windows (C:) > Users > Public > Public Documents > Hydro Insight > classification

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# APPENDIX B

## B.1 - GLOSARY

**Frames:** The number of images captured.

**Particles:** The particle count (after edge contact correction if enabled).

**Particles/frame**: Average particle count per frame.

**Particles/ml**: A concentration figure, equal to total particles/(probe volume * frames).

**Elapsed seconds:** The time duration of the actual data-taking.

**Frames per second:** Total frames/elapsed seconds

**Background average:** The average pixel value, from 0 to 255, of the image background.

**Contrast**: (Lb-Ld)/Lb    where Lb = background gray level; Ld = average particle gray level.

**Background reject:** Percent of images that was not counted because their intensity (background value) was outside the range specified in Rejections.

**Focus reject:** The number of particles not counted due to their being out of focus.

**Shape reject:** The number of particles not counted due to their failing the shape reject criteria.

**Border reject:** The number of particles rejected due to contacting the border.

**Dark pixels**: Percent of the total image area that was counted as particles.

**Depth of Focus**: This is the distance, centered at the focal plane, over which objects will be accepted, if Focus Rejection is in effect.  It is determined by the Focus Parameter and the current magnification but is limited to the view cell thickness.

**Probe volume**:  Volume of space in which particles are counted; equal to image size x depth of focus.

**Sample volume:** The total volume contained in all the particles included in the sample.

**Area density**: The average surface area density, expressed as square cm per cc.  The calculation assumes spherical particles.

**Volume density:** The average particle volume fraction in the probe volume.  This is a dimensionless fraction (cc per cc).

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# APPENDIX C

## C.1 - FILE SUFFIXES

| Suffix | File |
|--------|------|
| -dat | Particle ata file |
| -pd.dat | Particle Data file |
| -id.dat | Thumbnail Data file |
| -rs | Run series file |
| -rc | Run Condition file (legacy file) |
| -smp | Sample Run File |
| -prf | Preferences file |
| -psf | Settings file |
| -ro | Report Options file (legacy file) |
| -ac | Analysis Condition file |
| -xls, - xlsx | Microsoft Excel file |
| -tif | Tiff Image files |
| -cs | Comprehensive statistics file |
| -occ | Clasification file |
| -ini | Category names file |

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# APPENDIX D

## D.1 - STATISTICAL DEFINITIONS

**MEANS:** The $D_{pq}$ means are a way of characterizing a particle sample by a single number and are often used in particle technology. Each $D_{pq}$ mean characterizes the sample in a different way.

The standard method of computing Dpq from number diameter data is:

$$D_{pq} = [ \; \sum(n_i \; d_i^p) \; / \; \sum(n_i \; d_i^q) \; ]^{\; 1/(p-q)}$$

where i ranges over all particles in the sample and $d_i$ is the $i^{th}$ bin center   In this definition q is always smaller than p.

The $P^{th}$ power geometric mean, $D_{pq}$, is defined as

$$D_{pq} = \; \exp [\sum(n_i \; d_i^p \ln(d_i)) \; / \; \sum (n_i \; d_i^p) \; ]$$

The $D_{pq}$'s may be computed more accurately from the surface area and volume distributions. This calculation (for p not equal to q) is

$$D_{pq} = [\sum (p^{th} \text{ power array})[i] \; / \; \sum (q^{th} \text{ power array})[i] \; ]^{\; 1/(p-q)}$$

In Sample Types / Image Analysis, there is an option specifying how the higher power arrays are to be generated. This choice also dictates which $D_{pq}$ computation method will be used.

**The commonly used means are:**

**D10 (arithmetic mean):**  Average diameter (or other measure) of all particles in the sample.

**D20 (mean surface diameter):**  Diameter of a particle having the average surface area (total surface area in the sample divided by number of particles).

**D30 (mean volume diameter):**  Diameter of a particle having the average volume (total volume in the sample divided by number of particles).

**D32 (Sauter mean  or  surface-weighted mean diameter) :**  The moment mean of the surface area frequency distribution.  Also, the diameter of a particle having the same volume to surface ratio as the entire sample.

**D43 (volume-weighted mean diameter):**  The moment mean of the volume frequency distribution.

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**STANDARD DEVIATION:** The arithmetic standard deviation of any histogram is calculated as:

$$SD = \sqrt{\left( \sum (Y_i (X_i - X_m)^2) / \sum Y_i \right)}$$

WHERE:    $Y_i$ = the histogram y-axis values

$X_i$ = the x axis values

$X_m$ = the mean X

**PERCENTILES:  DVXX** : The XX$^{th}$ percentile by volume.  It is computed as the diameter such that the collection of particles having that size or less represents XX % of the total volume.  The commonly used ones are DV10, DV50 and DV90.

DV50 is also called the **volume median**.  It is the diameter that divides the sample into two equal halves, by mass or volume.

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# APPENDIX E

## E.1 ERROR CODES

| Code # | Description |
| --- | --- |
| 1 | Image file already exists |
| 2 | Image file path error |
| 3 | Error creating or writing sample file |
| 4 | Error creating or writing run series file |
| 5 | Error appending to run series file |
| 6 | Checksum error opening users.txt |
| 7 | Error creating or writing mirror sample file |
| 8 | Error creating or writing mirror image file |
| 9 | An image file prefix has not been entered in Run / Basic Settings |
| 10 | A limit must be set in Run Options / Run Control to use auto restart |
| 11 | Run name, measures, or statistics options does not match what is already in the run series |
| 12 | Error writing image file |
| 13 | Error writing TIFF file (unknown cause) |
| 14 | A sample file was not found |
| 15 | Camera not found or bad connection or adapter not powered |
| 16 | Could not create particle tracking file |
| 17 | Could not create or write sample xls file |
| 18 | No run series files were found |
| 19 | Individual particle data file not opened; possibly filename already exists |
| 20 | Error removing user |
| 21 | Error changing user attribute |
| 22 | Error writing rangeset fractions file |
| 23 | Error creating individual particle data file - name may already exist |
| 24 | Error creating individual particle thumbnail file - may already exist |
| 25 | Error creating particle text file |
| 26 | Error creating individual particle data file at mirror location |
| 27 | Error creating particle thumbnail file at mirror location |
| 28 | Filename error |
| 29 | Could not create stat summary xls file |
| 30 | Error during sample upload to website |
| 31 | File not created because no data exists |
| 32 | Syringe pump initialization or communication error |
| 40 | Unknown error |

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