CELLISTA USER MANUAL

cellista

Document number : 3056-D214

Software Version: 4.2

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1 SAFETY INFORMATION

Retain and follow all product safety and operating instructions. Observe all warnings on the product and in the operating instructions. Please read the accompanying documents *7699* ***acumen*** *Instrument Manual* or *3056-D133* ***mirrorball*** *instrument manual* for further safety information pertaining to **mirrorball** and **acumen** respectively.

2 INTRODUCTION

2.1 About Cellista

The proprietary software algorithms provided allow measurements of cell morphology, size and spectral characteristics using either single or multiple fluorescent dyes. This versatility provides scientists within therapeutic research or assay development areas with the tools to investigate cellular biochemistry. Assay applications have been developed around such assay areas as Cell proliferation, cytotoxicity, , 3D biology such as spheroids and colonies, nuclear translocation, GFP reporting systems, cell morphology (e.g. angiogenesis), to name a few.

2.2 Software Version

This manual is designed to be used in conjunction with the Cellista training course. The version of software described in this manual is Version 4.2.

2.3 Licence Agreement

The system and software described in this manual is furnished under a licence agreement and may only be used in accordance with the terms of that agreement.

3 GETTING STARTED

At the logon box, fill in the box as shown below, unless your IT department have set up specific logins.

3.1 Logging on

At the PC logon box, fill in the details as shown below:

Username: ttplabtech

Password: ttplabtech

Note: in Windows 7 multiple users can be logged on simultaneously, but only the first login running cellista will control the **acumen** or **mirrorball** instruments, i.e. when the software is started by the first person to log onto the PC. All subsequent users will be running in analysis mode.

It is possible to configure additional users on the PC; please see **Appendix B: Application Security** .

3.2 Turning on acumen

To start up the instrument:

- Switch on the host PC and log on.
- Check the power to the instrument is switched on at the socket. Click on the switch under the power cord
- Turn on the laser(s) by turning the key switch clockwise from the 12 o'clock position to the 3 o'clock position
- Wait 5 seconds
- Double click on the Cellista software icon  on the PC desktop to start the software. The program window will show toolbars and an empty work area until a new .Plate file is opened
- Once the software has started, the drawer mechanism initialises

Note: The instrument will not be damaged if this start-up procedure is not followed exactly. However safety procedures require the laser unit to be switched on prior to the software being opened in order for the software to scan. If you do not wait 5 seconds then there may be a communication error. Just close the software and restart.

3.3 Turning on mirrorball

To start up the instrument:

- Switch on the host PC and log on
- Check the power to the instrument is switched on at the socket. Click on the switch at the rear of the instrument
- Wait 10 seconds
- Double click on the Cellista™ software icon  on the PC desktop to start the software.
- Once the software has started, the drawer mechanism initialises.

Note: The instrument will not be damaged if this start-up procedure is not followed exactly. However safety procedures require the scan unit to be switched on prior to the software being opened in order for the electronics to stabilise. Without a wait of 10 seconds, there may be a communication error. If this should occur, then close the software and restart.

3.4 Loading Plates

To insert a plate to be scanned, the Cellista software must be running.

- Open the drawer using the eject icon on the software 

- Ensure the plate is located correctly. Well A1 should be at the back left hand corner of the drawer as you face the machine for **acumen**, or at the front left hand corner of the drawer as you face the machine for **mirrorball**
- Close the drawer, using the eject icon on the software

3.5 Unloading Plates

To unload a plate:

- Open the drawer using the eject icon on the software 
- Remove the plate from the drawer
- Close the drawer using the eject icon on the software  .

3.6 Resetting the System

If an error occurs with the hardware the system may require resetting.

- Select **Run | Control** | **Reset** or click the **Reset** icon  on the toolbar.

It is possible to configure the system so that it does not automatically home and reset when Cellista starts. In this case you will also need to reset the system as above. Note that attempting to start a scan will also reset the system in this case.

3.7 Shutting down acumen

To shut down the instrument:

- Close the Cellista software
- Shutdown the PC.
- Turn off the lasers by turning the key switch anti-clockwise from the 3 o'clock position to the 12 o'clock position. Click off the switch under the power cord.

3.8 Shutting down mirrorball

To shut down the instrument:

- Close the Cellista software.
- Shutdown the PC
- Click off the switch under the power cord.

3.9 Analysis-only mode

If Cellista is already running – perhaps under a different user account on the same PC – then the second and subsequent instances of Cellista will run in analysis-only mode, in which it is possible to open, analyse and save files but not to control the machine. If Cellista starts in analysis-only mode then it will display the splash screen show in section 6.4.1.

4 ADJUSTING THE SOFTWARE WORKSPACE

The Cellista software provides many ways of viewing the data generated from scanning in multiple windows. These windows can be managed and arranged to suit the user.

4.1 Arranging Multiple Windows

Cellista software sub-windows can be “docked” within the main window, rather than floating in layers. The boundaries between the panes may then be altered to suit the content of each window.

4.1.1 Docking a Window

To dock a window:

- Grab the window to dock by clicking and holding down the left mouse button on the title bar.
- Drag the window around the screen by moving the mouse while holding down the left mouse button. A grey outline of the window moves as the window is dragged.
- Move the mouse over one of the docking guide boxes that appear.
- Release the mouse button to dock the window.

4.1.2 Undocking a Window

To undock a window, double click on the docked window’s title bar.

To undock a window from a layered set of windows, double click on the name tab to float the window in the main window.

A window can be moved around the screen without docking by keeping the CTRL key pressed.

4.1.3 Docking a Window on Top of another Window

Windows may be docked in layers to produce a “card index” of windows, with the most recently viewed window on top.

To tab a window with another open window:

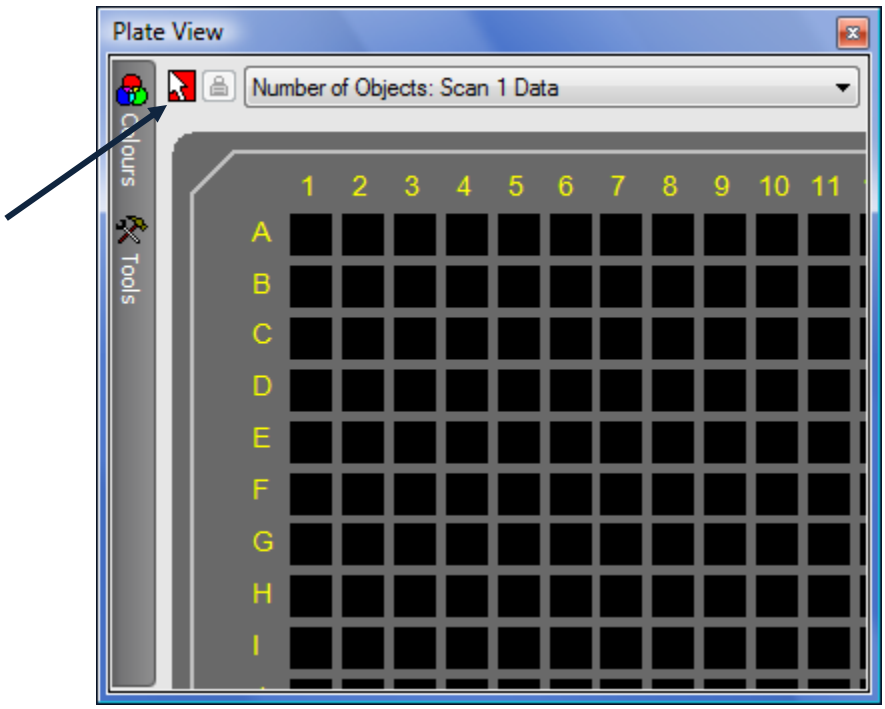
- Click and hold down the left mouse button on the title bar of the floating window.
- Drag the mouse over the central window in the docking guide box of the target window
- Release the mouse button.

Each window can be brought to the front of the index by clicking on the tab bearing its name in the bottom of the window. If there are more tabs in a window than are currently visible, use the arrows to the left of the tabs to scroll through the additional tabs.

4.2 Linking Windows

It is sometimes necessary to set up a link between different windows.

- Click and hold down the left mouse button on the link icon  on the **source** window toolbar, then drag the cursor across to the **destination** window
- Release the mouse button to link



The link icons in linked windows show the same background colour. To lock the selection in a given window so that it is not altered by the selection of objects or wells in other windows, click the open padlock . The icon changes to a locked padlock showing that the window is locked.

Note that in version 4.1.7 when creating assays on acumen which involve multiple scans, it is possible to link from the ribbon toolbar to the settings window using the grey link icon, so that the current scan can be changed in all open settings windows, or the settings for each scan can be displayed simultaneously.

5 CELLISTA SOFTWARE FILE TYPES

5.1 CData Files

When Cellista software saves data it creates a .CData file. A CData file contains all the data generated from a scan of a microplate and subsequent data analysis. The information that the file contains and the size of the file is very dependent on the Data Collection Level selected for the scan (see Section 7.2).

5.2 CTemplate Files

Once assay settings have been optimised, they can be saved as a CTemplate file for future scanning. CTemplate files contain only analysis and acquisition settings but no data. The document format is identical to that of .CData files but the template contains no object data. Assay settings can be saved as a CTemplate file by selecting **File** | **Save | Save as Template**.

To start a new scan using a plate template, select **File | New** | **New from Template**.

5.3 CBatch

This is a file that contains batch information for use with a robot or for repeat scanning of plates. See section see section 12.

5.4 CReprocess

CReprocess files define batch reprocessing jobs which alter and reanalyse multiple CData files at once. See section 13.

5.5 tif

8- or 16-bit open source, OME compliant TIFF files that can be used in third party software analysis packages. Refer to section 7.5.3

5.6 XML

Cellista can generate xml files summarising the settings used to perform scans.

6 FILE TAB

6.1 File Group

6.1.1 Recent

Displays a list of previously viewed files.

6.1.2 New

When selecting New, there are four options available.

New assay	This creates a blank template to start to set up new assay parameters
Browse for Template	This opens up Windows Explorer to find a previously saved Template file
Recent Template	Opens up a recently saved Template
Predefined Template	Open up a predefined Template set up by TTP Labtech

When using **Predefined Template** in acumen and mirrorball, it is important to check that the channels are set correctly for the instrument and dye setup being used. Due to multiple configurations of machines it is possible that detection channels will have to be changed for the **Predefined Template** to work correctly on a particular instrument.

6.1.3 Open

Opens an existing saved document.

6.1.4 Close

Closes the current open document.

6.2 Save Group

6.2.1 Save

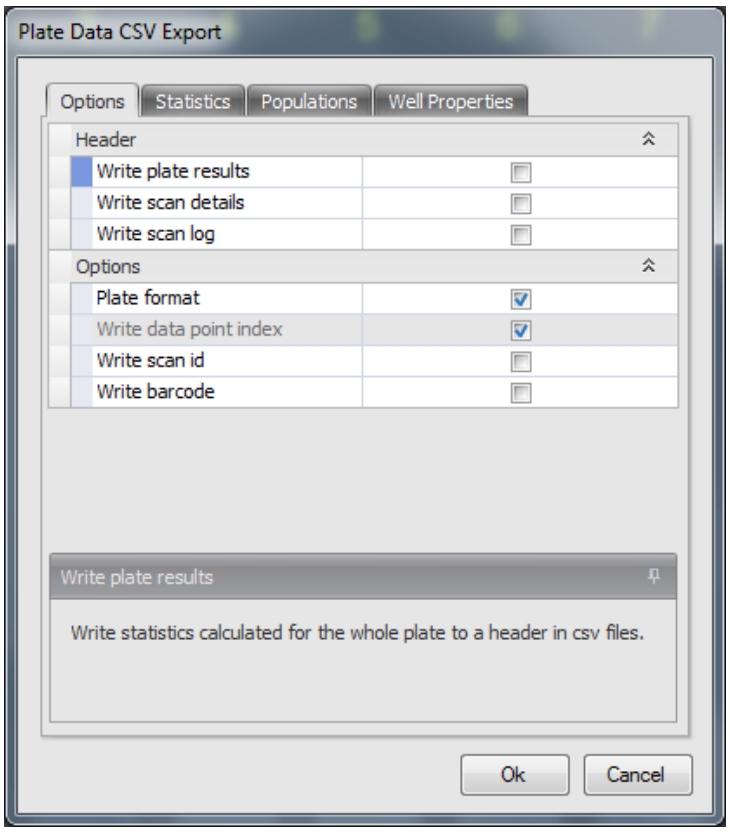
Saves the current open document, overwriting the original document on the disk.

6.2.2 Save As

Saves the current document as a new document or template.

6.2.3 Export

Clicking on Export opens the following box.

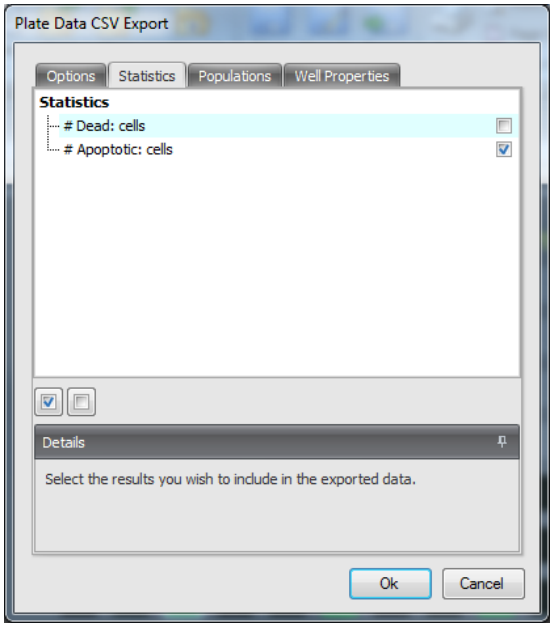


6.2.3.1.1 Options

Write plate results	Write statistics calculated for the whole plate to a header in csv files.
Write scan details	Write user-created scan details to csv files.
Write scan log	Write information from scan logs to csv files.
Plate format	If this box is ticked, the plate-level CSV file is arrayed as a series of tables in the format of a plate rather than one continuous list.
Write data point index	Writes a unique index for each data point (i.e. well) in a scan or batch.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.

6.2.3.1.2 Statistics

Selects if only data from certain populations are to be exported. In the example below, only data from Apoptosis cells will be exported.



6.3 Output Group

6.3.1 Print

Prints the active window

6.3.2 Print Preview

Display a preview of the active window prior to printing

6.3.3 Page Setup

Setup the page layout for printing

6.3.4 Copy to Clipboard

Copy data from the active window to the clipboard

6.4 Help Group

6.4.1 Startup Screen

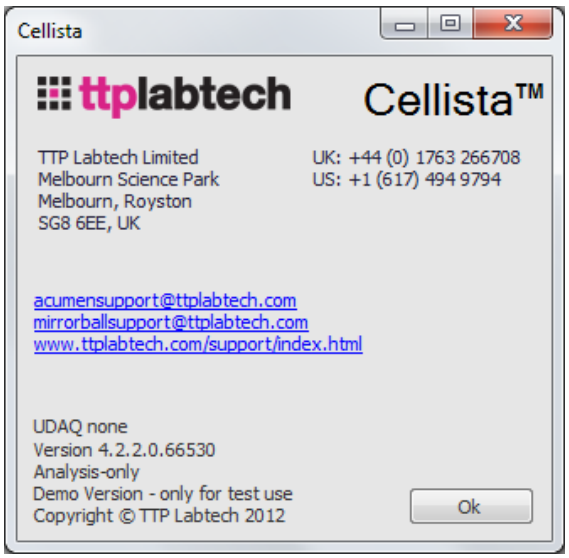
Displays the startup screen.

The following options on the startup screen are given

New assay	This creates a blank template to start to set up a new assay
Use Template	Opens up either a predefined Template file or opens up Windows Explorer to find a previously saved Template file
Load Results	Opens up an existing data file
Batch Run	Opens up the Batch Run controller
Batch Reanalysis	Opens the Batch Reanalysis controller

6.4.2 Contact Support

Shows contact information to get technical support. . Please ensure that you email the correct address for the instrument you are experiencing problems with. It also displays the current software version, and instrument serial number.



6.5 Application Group

6.5.1 Exit

Exits the application. If the file has not been saved, a prompt will be given to save the data before closing the file.

7 SCAN SETUP TAB

The Cellista software enables configuration of the system for each assay. A number of parameters must be set prior to scanning to ensure optimal performance. These can be subsequently saved in a template for repeated use.

7.1 Assay Group

7.1.1 Details

Opens a free text box where the user can paste or write in specific assay information.

7.2 Data Collection Level

Select **Settings | Data Collection Level**

Cellista software applies TTP Labtech's proprietary cytometric analysis to the identification and classification of scanned objects. Such analysis permits the storage of different quantities of data as assays are developed, validated and applied.

Assay Development (High)	Retains all the intensities measured. New object characteristics can be calculated from the data. Caution: very large files may cause data handling problems, or the PC to become unresponsive
Assay Validation (Medium)	Stores the characteristics of each object in the plate. Allows separation of objects into sub-populations based upon available characteristics, but new characteristics cannot be added after acquisition as the source (intensity) data has been discarded. True colour Well View is also lost
Secondary Screening (Low)	Stores the characteristics of each object in the plate. Allows separation of objects into sub-populations based upon available characteristics, but new characteristics cannot be calculated as the source (intensity) data has been discarded. All Well Views are lost
High Throughput Screening (Very Low)	Stores only the population statistics for each well and the settings used to scan the plate. Only Plate and Spreadsheet Plate views are available

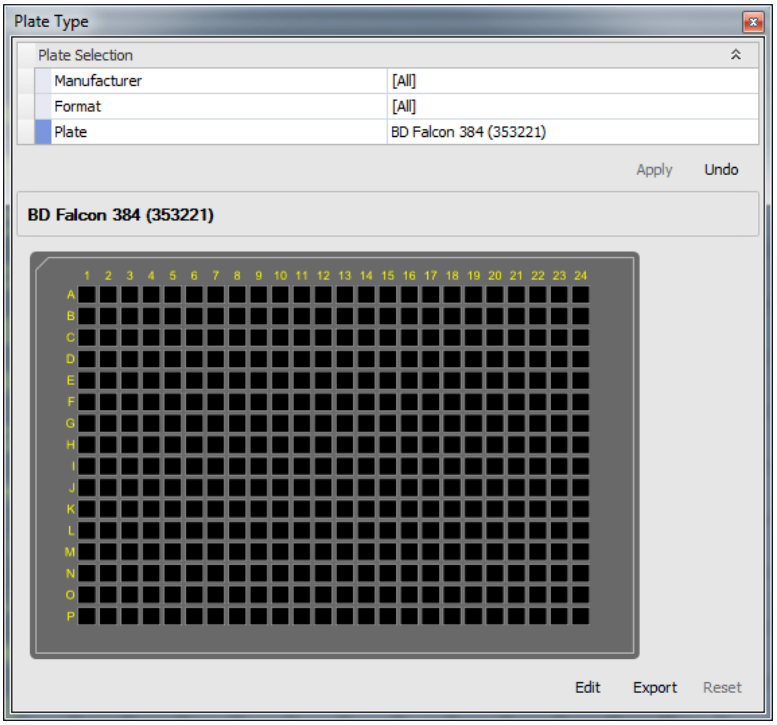
The data collection level can be reduced once a scan is completed to reduce file size but this action is irreversible. Any existing file on disk will be left intact unless it is overwritten by selecting **File | Save** to overwrite it.

7.3 Dimensions Group

7.3.1 Plate

Select **Settings | Dimensions | Plate**

Selects the plate type used in the assay. It is very important to set this correctly as it defines the number of wells and focus setting for the microplate.



Select the plate manufacturer, format and catalogue number for the plate being used, then click Apply. The plate name and ID will then appear on the bottom of the **Plate View**

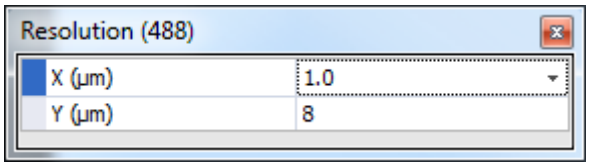
7.3.2 Resolution

Select **Scan Setup | Dimensions | Resolution**

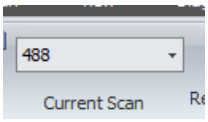
Controls data sampling resolution that is the spacing between intensity measurements taken by the instrument. Resolutions are set in μm.

7.3.2.1 Setting Scan Resolution for acumen

For **acumen** you can set both X and Y resolutions independently:

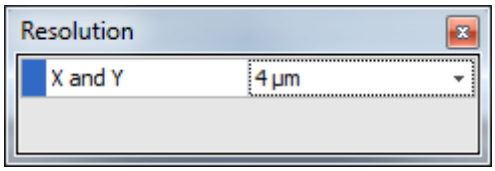


A setting of 1 by 8 μm is appropriate for most applications in **acumen** but each laser can be set with a different resolution. This is done by selecting which laser the setting is applied to above the Current Scan label (**Scan Setup | Detection | Current Scan**)



7.3.2.2 Setting Scan Resolution for mirrorball

For **mirrorball** you can set only the X and Y resolutions together so that they have the same value.

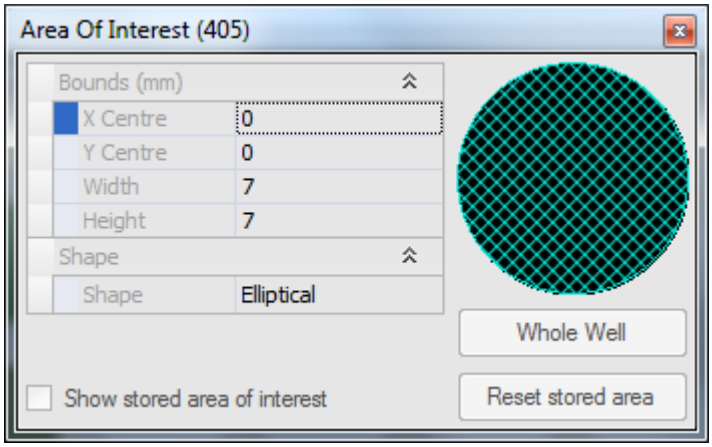


A setting of 4-9 μm is appropriate for most applications in **mirrorball**.

7.3.3 Scan Area

Select **Settings | Dimensions | Scan Area**

Sets the area to be scanned within each well, also known as the area of interest.



There are two methods of changing the area. Either the centre co-ordinates and size of the scan area can be entered or the area can be chosen by clicking the well graphic with left mouse button and dragging.

Clicking the **Whole Well** button will automatically scan the whole well. This is the default.

Note: For **acumen**, it is possible to scan different areas for each laser.

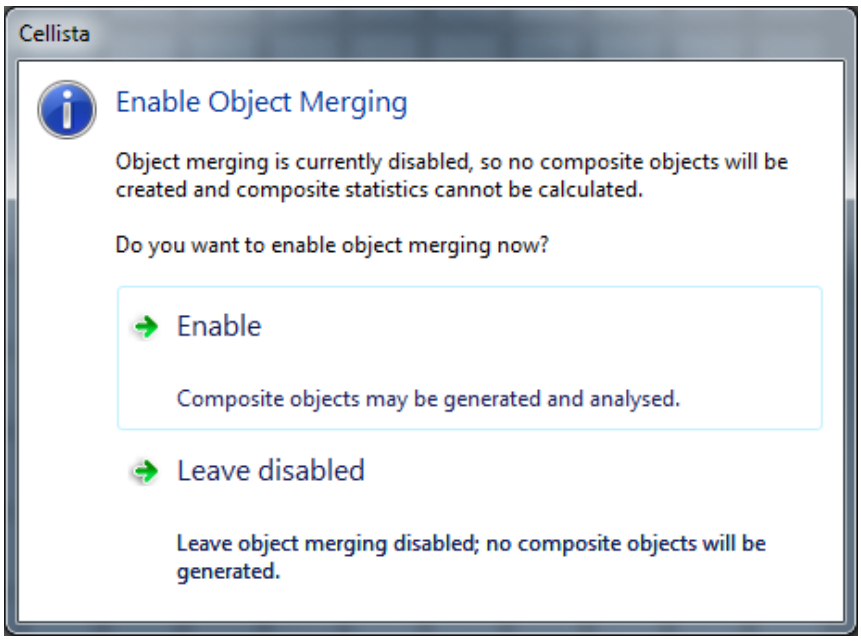
7.4 Detection Group

7.4.1 Current Scan

This is only available to **acumen**. To Select multiple laser scans (if fitted) Select **Scan Setup** | **Detection** | **Current Scan.**

Use this window to configure multiple scans of a single plate, storing the data from all scans in a single data file. This window also shows which settings are currently being displayed in the whole **Scan Setup** tab.

The 488nm laser is selected by default. To run a single laser scan and use a different laser, then select a different laser by selecting **Scan Setup** | **Detection** | **Lasers** and selecting the correct laser. If a second laser is to be added to the **Scan Setup**, click on the + icon to add a laser. The following box will be shown asking if **Object Merging** should be enabled.

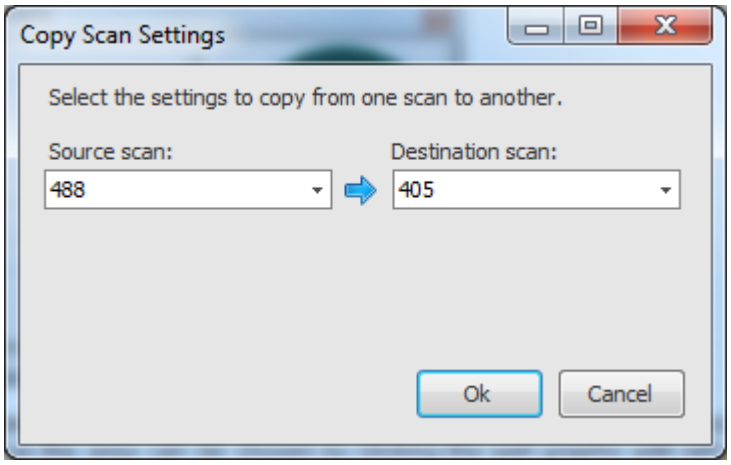


To enable or disable object merging at a later time open the 'current scan' dropdown and select the object merging button indicated:

See **section 8.3** for further information on **Object Merging**.

Once this box is closed, the laser has been added. The order of the laser displayed can be moved by using the or icons.

To copy the scan settings from one laser to another, then select the icon. The following box opens up:



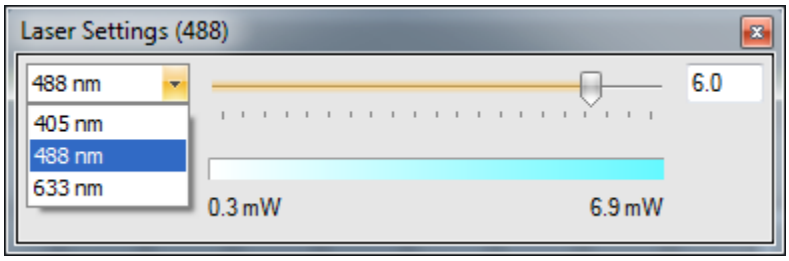
,

Select which laser is the source scan and which is the destination scan, click OK and the scan settings are copied across.

7.4.2 Lasers

7.4.2.1 Laser Settings for acumen

acumen systems contain one, two or three lasers for fluorophore excitation. To select a laser to be used, Select **Scan Setup** | **Detection** | **Lasers**

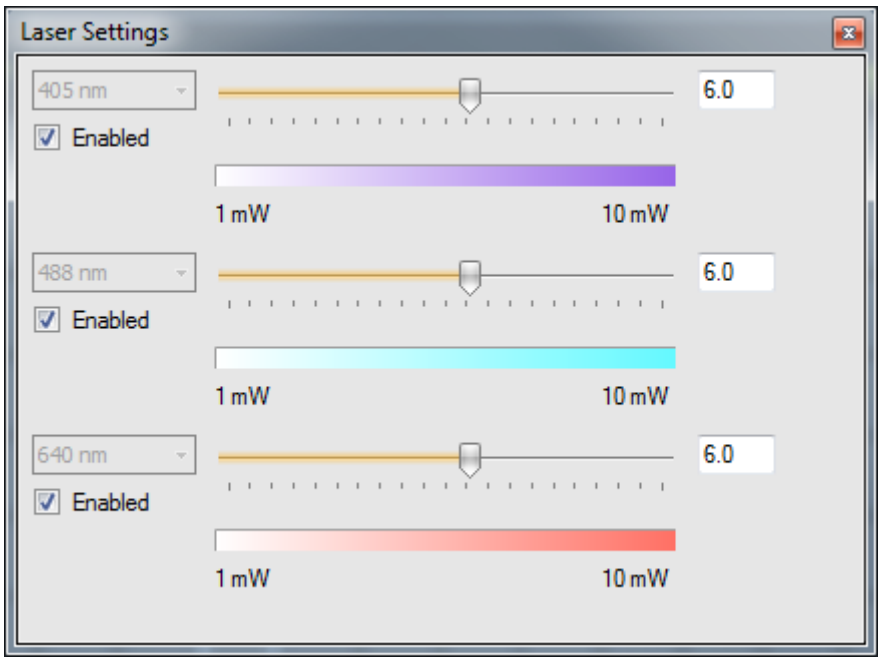


Here click on the drop down box to select which laser is to be turned on. The laser power can also be adjusted here, however, this is typically unnecessary, and 6mW will suffice for most assays.

7.4.2.2 Laser Settings for mirrorball

mirrorball systems contain one, two or three lasers for fluorophore excitation. On dual or triple laser systems, simultaneous scanning with multiple lasers is possible.

Select **Scan Setup | Detection | Lasers**



To enable a laser, check the **Enabled** box.

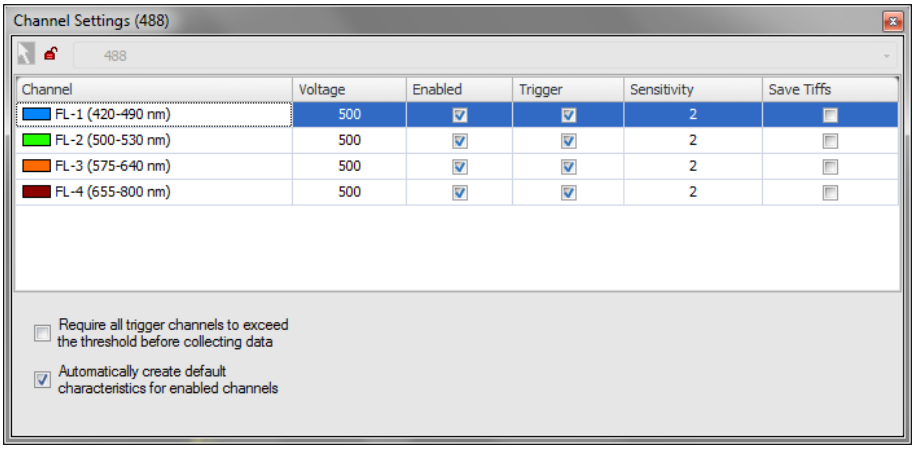
To change the laser power, either move the slider bar or enter the required value. A laser power of 6 mW should be suitable for most assays.

7.4.3 Channels -acumen

7.4.3.1 Channel Settings

acumen systems are available with four fluorescence detection channels. Detection is achieved using a sensitive photomultiplier tube (PMT) for each channel.

To select the **Detection Channels** to be used in the assay, click on the **Channels** button next to **Laser** name. Here you can select which channels are **Enabled**, the **Voltage** for the PMT and the **Detection Sensitivity.**



Voltage	The PMT (photo multiplier tube) voltage setting behaves much like the brightness control on a television – too low and no picture is seen, too bright and objects appear white. The voltage can be set and verified for each channel as appropriate. The permitted range is 0 – 1,000V for each channel. The default values are 500V for each channel. Steps of 20V are sufficient to set the voltage adequately.
Enabled	Selects a channel for data collection. If not ticked, then no data is recorded in that channel.
Trigger	Defines the amount of fluorescence from an object required to begin data recording in all enabled channels. Selecting the appropriate Trigger channel(s) for the laser scan depends on the dyes used in the assay. All channels are set as the default trigger. Channels are set to trigger when a tick is displayed in the Trigger column.
Sensitivity	See Section 7.4.5.1
Save TIFFs	Automatically exports an open source 8/16-bit TIFF

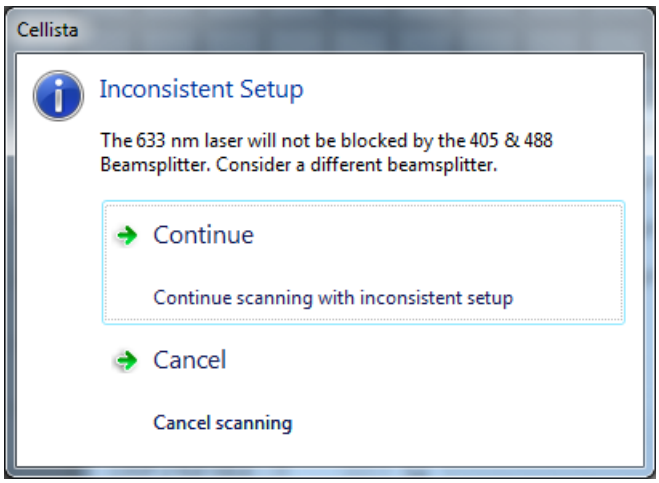
The channel settings need to be defined for each laser scan that has been set up in **Scan Setup | Detection | Lasers**.

7.4.3.2 Automatic filter set recognition

Cellista can detect the filters currently fitted to an **acumen** instrument. It will show a warning if the current filter set may reduce the data quality in any way. If a template was created on an **acumen** instrument with one filter set and another set is now installed then this will raise a warning too – see also 7.6.1. To overwrite the setup for the current instrument being used see section 7.6.1

7.4.3.2.1 Incorrect beam splitter fitted

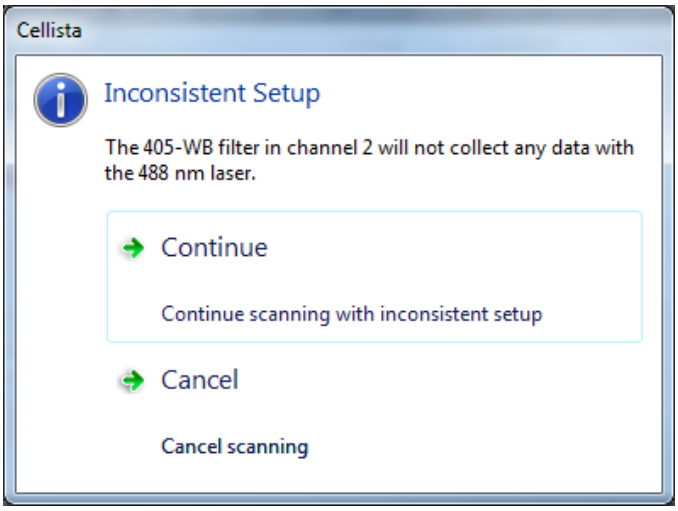
If the wrong beam splitter is in the system, for the laser selected, a warning message will be displayed to indicate the problem to the user.



To override the message, click on Continue, or click on Cancel and change the beam splitter to the correct one for the laser being used.

7.4.3.2.2 Incorrect filters fitted

If the filters are fitted in the incorrect order, or the wrong filters are selected for the laser being used, e.g. a Hoechst filter block is triggered but the 488nm laser is selected, a warning message will be displayed to indicate the problem to the user.



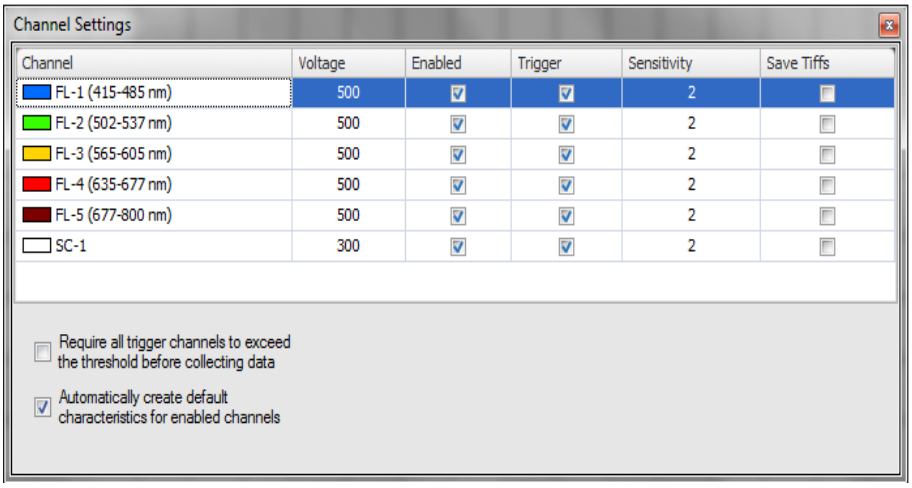
To override the message, click on Continue, or click on Cancel and change the filters to the correct order.

7.4.4 Channels - mirrorball

7.4.4.1 Channel Settings

mirrorball systems are available with up to five detection channels. Detection is achieved using a sensitive photomultiplier tubes (PMT) for each channel.

Select **Scan Setup | Detection | Channels**



Each Detection Channel can be configured to suit the assay.

Voltage	The PMT (photo multiplier tube) Voltage setting behaves much like the brightness control on a television – too low and no picture is seen, too bright and objects appear white. The voltage can be set and verified for each channel as appropriate. The permitted range is 0 – 1,000V for each channel. The default values are 500V for each channel. Steps of 20V are sufficient to set the voltage adequately.
Enabled	Selects a channel for data collection. If not ticked, then no data is recorded in that channel.
Trigger	Defines the amount of fluorescence from an object required to begin data recording in all enabled channels. Selecting the appropriate Trigger channel(s) for the laser scan depends on the dyes used in the assay. All channels are set as the default trigger. Channels are set to trigger when a tick is displayed in the Trigger column.
Sensitivity	See Section 7.4.5.1
Save TIFFs	Automatically exports an open source 16-bit TIFF

7.4.5 Acquisition

These allow the user to adjust the sensitivity of the scan and determine which data are kept and which are discarded. Their correct setting is key for identification of objects using cytometric analysis.

7.4.5.1 Threshold Type

Select **Scan Setup | Detection | Acquisition**

Shot	Retains only the signal (object) data and discards background fluorescence. It tracks the midpoint of the sample's background noise and sets a dividing line or threshold above this to separate object data from noise. This option is the default and allows adjustment of the sensitivity of the scan above the background. This sensitivity is set in the Channel Settings dialog under Sensitivity. A low value gives a more sensitive scan (good for dim objects), a higher number requires more light to initiate data collection (good for very bright objects to discard dim unwanted data). Values between 1 and 4 are suitable for most assays with 2 being the default.
Constant	Retains only the signal (object) data and discards background fluorescence above a constant value defined in the Channel Settings dialog (Constant Threshold).
Aggregate	Retains signal and background fluorescence for the scanned area and is used to produce aggregate fluorescent measurements. There is no Object Characteristic data in this mode. Should be applied to limited number of wells due to the amount of data and is useful for comparing object and background fluorescence.

7.4.5.2 Features

A feature is defined as a single line of data in an object. These are collected when applying shot or

constant thresholding.

Feature Length	Controls the smallest and largest lines (features) through an object for which the instrument will collect data. Features smaller than the minimum are discarded and features larger than the maximum are truncated to the maximum value. Feature Length is set in μm.
Feature Extension	Under some circumstances it may be desirable to extend features beyond the limits of thresholding. This will result in larger objects. Permissible values for Feature Extension are multiples of the X scan resolution and are therefore set in μm. The default value of 0 disables the function.

7.4.5.3 Shot Thresholding

Meniscus Smoothing length	Controls the size of the Meniscus Smoothing Length. This is set to 301 μm by default but can be increased when looking at large objects, such as spheroids.. Meniscus Smoothing Length is set in μm.
Noise Smoothing Length	Controls the size of the Noise Smoothing Length. Set this to 0 to disable noise smoothing. Note though that data may be more noisy.

7.4.5.4 Object Identification

X Separation	The minimum separation between objects in the x direction.
Y Separation	The minimum separation between objects in the y direction.
Minimum Object Depth	The minimum size of an object across the scan lines. Objects smaller than this will be discarded.

7.4.6 Smoothing

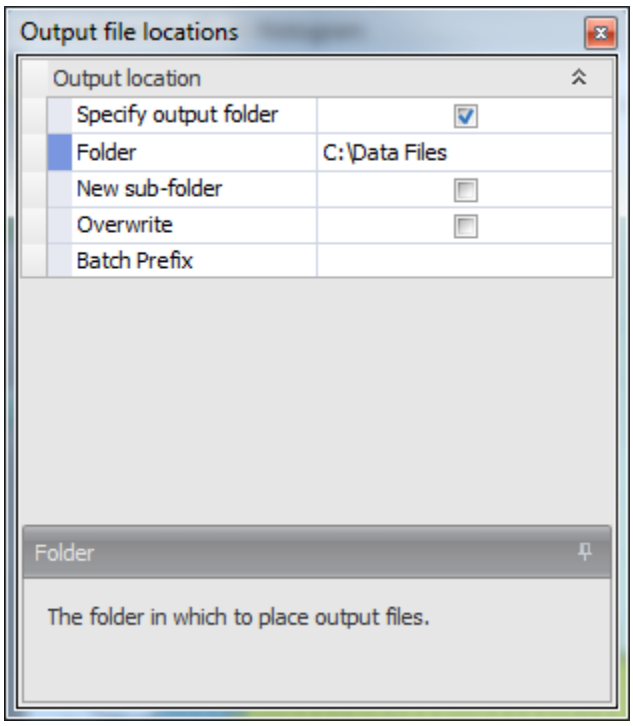
Select **Scan Setup | Detection | Smoothing**

Enabled Filtering	When turned on the sample data points are averaged over an area to produce a smoother result.

7.5 Output Options Group

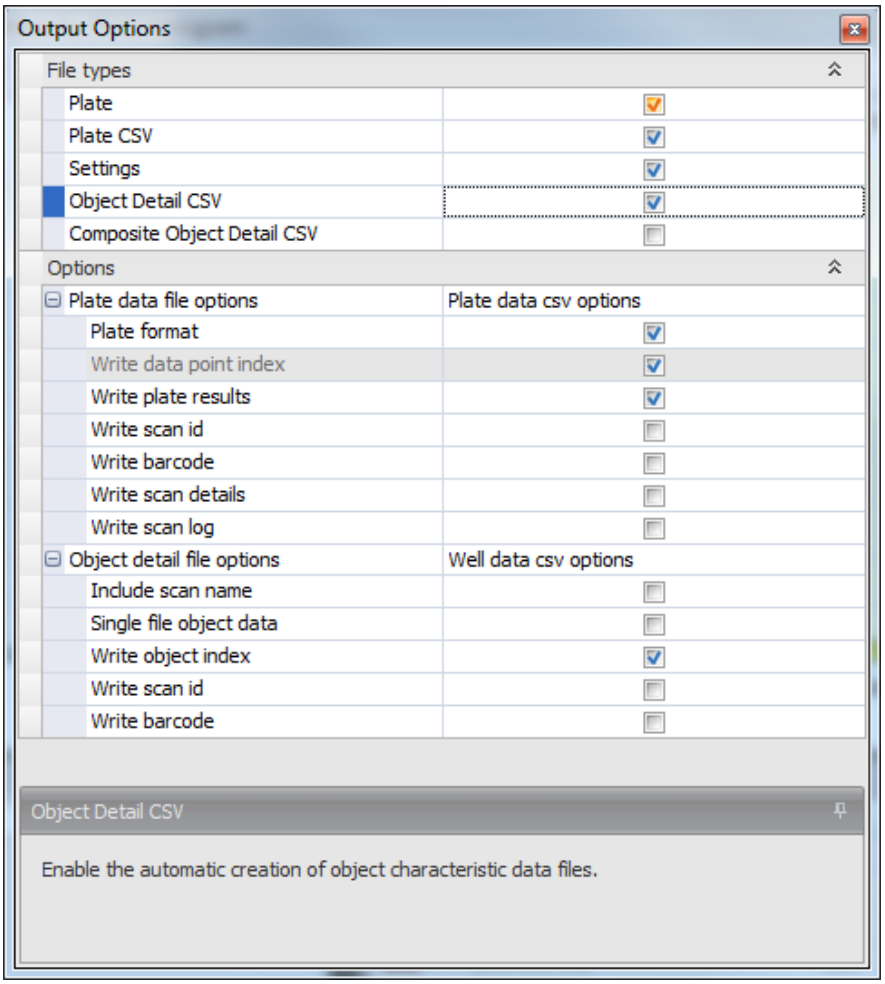
7.5.1 Location

Specifies the location where the scanned data is stored.



7.5.2 Options

When enabled every time the plate is scanned interactively, the data files asked for are automatically saved in the location specified in the Location tab (see section 7.5.1).



7.5.2.1 File Types

Plate	Tick box to save a cellista data file for each plate scanned.
Plate CSV	Saves a CSV file per plate containing population statistics values.
Settings	Saves text files which summarise the settings used to scan the plate.
Object Detail CSV	Creates one CSV file per plate which lists all collected object characteristics for every individual fluorescent object detected per plate.
Composite Object Detail CSV	Creates one CSV file per plate which lists all collected composite object characteristics for composite fluorescent objects when merging multiple scans.

7.5.2.2 Options

7.5.2.3 Plate date file options

Plate format	If this box is ticked, the plate-level CSV file is arrayed as a series of tables in the format of a plate rather than one continuous list**.**
Write data point index	Writes a unique index for each data point (i.e. well) in a scan or batch.
Write plate results	Write statistics calculated for the whole plate to a header in csv files.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.
Write scan details	Write user-created scan details to csv files.
Write scan log	Write information from scan logs to csv files.

7.5.2.4 Object detail file options

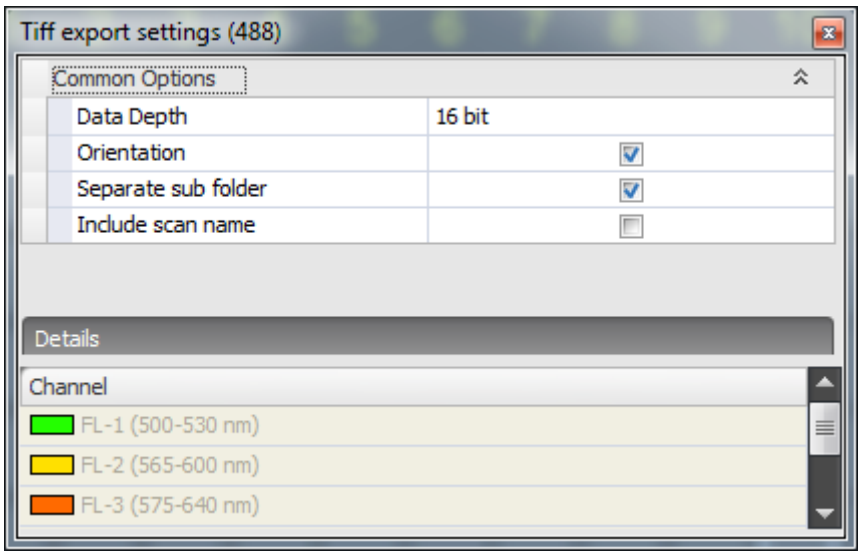
Include scan name	Set this option to include the name of the scan which found an object on each line of the detailed csv files even when not doing multiple scans or object merging. This will ensure that well data csv files have the same format regardless of the number of scans but does not affect file naming.
Single file object data	Write detailed object of composite object data to single file for all wells.
Write object index	Writes the index of each object with respect to the well which contains it.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.

7.5.3 TIFF

The **Scan Setup | Detection | Channels** box specifies options determining how TIFF image files are generated during a scan..

You can choose to export 16-bit or 8-bit TIFFS in the Data Depth box. It is recommended to use 16-bit TIFFs where possible.

Select **Scan Setup | Output Options | Tiff**



Data Depth	Selects to export 16-bit or 8-bit TIFFS in the Data Depth box. It is recommended to use 16-bit TIFFs.
Orientation	This is on by default. The generated TIFFS may not be displayed in the true orientation in some image analysis packages. However all the wells are displayed with the same change in orientation. To display all TIFFS in the correct orientation as viewed under a microscope in all analysis packages turn this off, however this significantly slows down the scan time.
Separate sub folder	Specifies whether exported TIFFS are stored in a dedicated subfolder. This will be a subfolder of the location specified for any automatically generated output files.
Include scan name	Set this option to include the name of the scan generated a TIFF even when not doing multiple scans or object merging.

7.5.4 External

It is possible to specify an external program to execute at the end of an interactive scan of a plate. The **Instancing** option can take one of the following values:

None	No external command will be executed
Per file	Once for each results file passing the results file name as a parameter. This should normally be a path to an executable file.
Parameters	Once only specifying the given command line parameters.
Results folder	Once only specifying the root output folder as the command line parameter.
Command	gives the command to be executed, normally the path to an external executable
Parameters	gives the parameters to be passed to the external command when using the **Parameters** option above

7.6 Advanced Group

7.6.1 Overwrite Setup

This overwrites the machine setup. For example, if a template was created on a 2 laser **acumen**, then loaded onto a 3 laser **acumen**, the template would adjust to account for the change. Similarly if a template was created on an **acumen** instrument with one filter set and another set is now installed then this will attempt to make the template compatible with the current setup.

7.6.1.1 Overwrite Setup – In Detail

It is sometimes desirable to transfer templates from one machine to another. However, machines can have widely different setups. Indeed, the configuration of a single machine can vary for instance by changing the filter set or by disabling lasers in the configuration file.

When a file is loaded in Cellista which is incompatible with the current machine setup all the settings – e.g. channel wavelengths – used to create the file will be displayed correctly. This broadly applies whether the file is a data file or a template, but note that the details below.

A file cannot be scanned while it is incompatible with the current machine setup. To make a file compatible with the current configuration, press the **Scan Setup | Advanced | Overwrite Setup** button. Note that this button is only enabled when there is no data in the file; it is not possible to scan two or more parts of a plate with different configurations.

Pressing **Overwrite Setup** stores the current machine configuration – including filter set – in the data file or template. It does not modify the original file on disk unless the user elects to save and overwrite it.

Factors which determine whether Cellista regards files as incompatible with the current configuration include:

- The type of instrument which created the file - it is not yet possible to convert a mirrorball template for use on an acumen system and vice versa
- Whether object merging (aka composite spot mode) is enabled in a file but not on a machine and vice versa
- The channel wavelengths and ordering on the current machine
- The lasers present on the machine
- The scan profiles and resolutions available
- The filters present in the system (acumen only)
- Scan head radius (mirrorball only)

The **Overwrite Setup** function is executed – sometimes automatically - in the following circumstances:

- When the user presses **Scan Setup | Advanced | Overwrite Setup**
- There are some circumstances under which a scan can be started even though it is apparently incompatible. The commonest case is when the filters on an acumen system do not match those in the file. In these cases the user will be prompted to overwrite the setup after pressing the **Scan** button if scanning interactively, or **Overwrite Setup** will be executed automatically in batch mode.
- When performing **New From Template** in interactive mode - since a new data file will being created
- When batch scanning
- A legacy acumen 3.4 template is opened via **New From Template**
- A Cellista template is opened from Windows Explorer (e.g. via double-clicking).

Overwrite Setup is *not* performed automatically when

- Templates are loaded during batch reanalysis operations
- A template is opened for editing via **File | Open** rather than **File | New From Template**
- A data file is opened
- A legacy acumen 3.4 template is opened for editing via **File | Open**
- When the filter set is changed which a file is loaded.
- Opening a legacy template file from Windows Explorer – this functionality is not available in any case

- When the current instance of Cellista is running in Analysis-Only mode.

Note that there are two cases may lead to behaviour which diverts from that documented above:

- Filter recognition not working correctly, perhaps due to being miscalibrated or grounding failure. This can sometimes be spotted by opening the **Diagnostics | Filters** window and seeing whether any filter values appear unstable (acumen only)
- Cellista incorrectly assuming that the user is not present due to some combination of scan settings.

Note that there are circumstances where **Overwrite Setup** may fail to make a document compatible with that of the instrument; if the configurations are just too different it may not be possible.

7.6.2 Settings Text

Displays an xml summary of all the settings used to perform a scan.

8 ANALYSIS TAB

8.1 Standard Group

8.1.1 Object Characteristics

A set of characteristics can be reported for each object identified by the software. Single or multiple Object Characteristics can be used as assay readouts or to define populations of objects within a well.

8.1.1.1 Adding Object Characteristics

- Select **Analysis | Standard | Object Characteristics**
- To add new **Object Characteristics**, click on  to open the selection dialog
- Select **Object Characteristics** by clicking the  icon. Multiple characteristics can be made by repeatedly clicking the  icon next to the Object Characteristic.

8.1.1.2 Deleting Object Characteristics

- Select **Analysis | Standard | Object Characteristics**
- Select **Object Characteristic** in the list on the left hand side.
- Delete by clicking the Delete icon  .

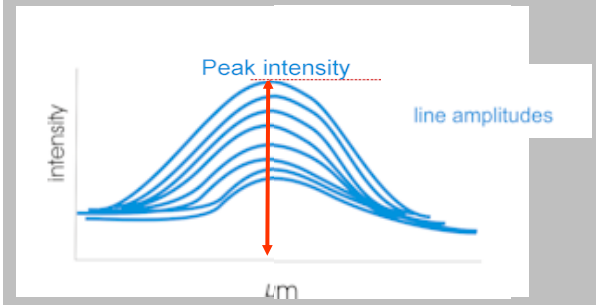
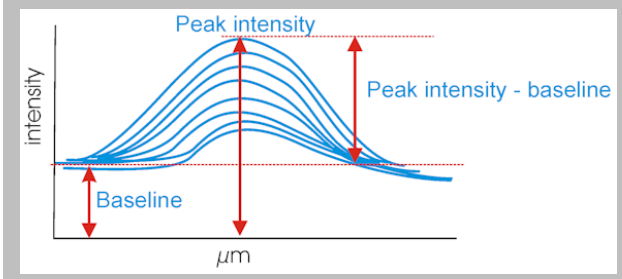
8.1.1.3 Editing Object Characteristics

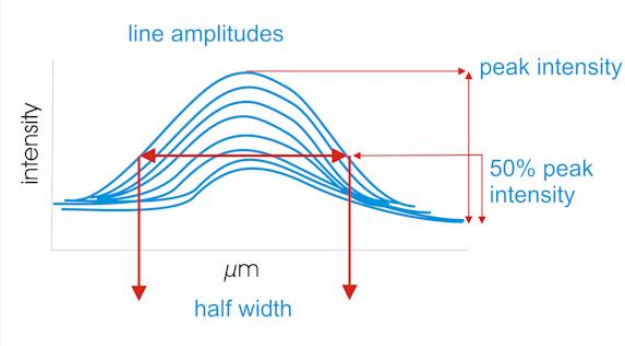
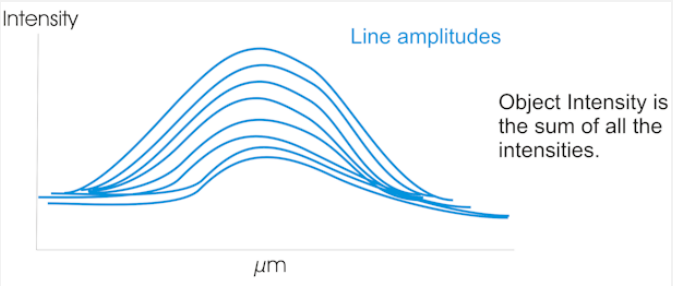
- Select **Analysis | Standard | Object Characteristics**
- Select the **Object Characteristic** in the list on the left hand side.
- Edit the settings on the right hand side.

Name	This can be edited to be more meaningful (e.g. FITC Peak Intensity)
Channel	Defines the data collection channel.
Contour	A threshold set on the object's fluorescent Peak Intensity. A contour can be set at a particular percentage of Peak Intensity and applied to determine the Width, Depth, Perimeter, Area, Intensity and Gaussian values of that object at that Peak Intensity percentage value.
Include in csv exports	If checked, the characteristic is included in the automatic export of well data. This setting can be overridden when exporting manually.

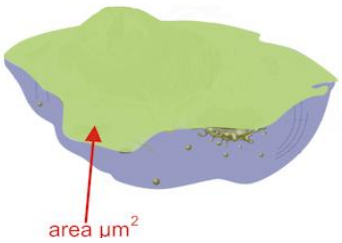
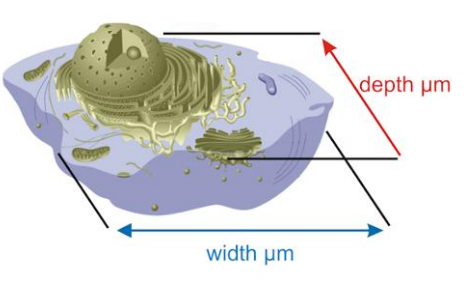
8.1.1.4 Definition of Object Characteristics

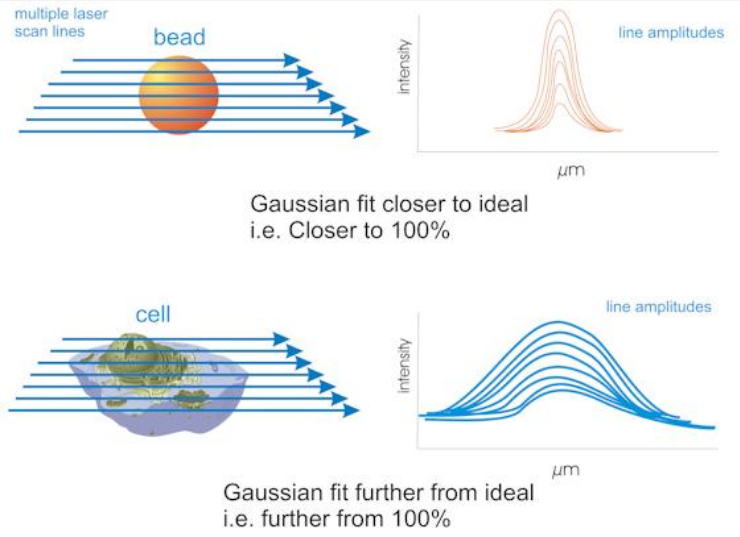
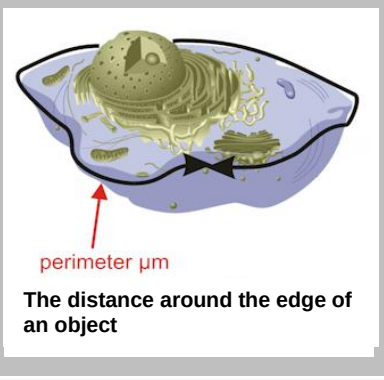
8.1.1.4.1 Intensity Characteristics

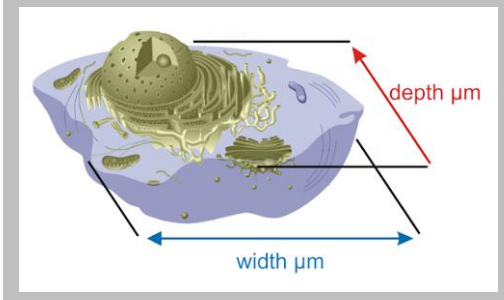
Peak Intensity	Measures the maximal intensity for that object 
Absolute Mean Intensity	The total of all intensities for an object divided by the number of data points in the area covered by the object and is measured in FLUs. No baseline subtraction is applied.
Baseline	The background fluorescence reading of the solution surrounding fluorescent objects. Knowing how much fluorescence is in solution helps understand how much dye to add for a particular assay. A high baseline fluorescence will limit the detection range of the instrument. 

Half width Intensity	The half width of the object is calculated at 50% of Peak Intensity (see diagram). Half width intensity is calculated as Peak Intensity divided by this half width. It can be useful in separating populations of objects that are morphologically distinct or have sharply defined intensities, for example, to measure the relocation of fluorescent dye in a defined object area (translocation assay). 
Mean Intensity	The total of all intensities for an object divided by the number of data points in the area covered by the object and is measured in FLUs. The Baseline is subtracted from each intensity. This is the primary report for homogeneous assays.
Peak Intensity - Baseline	Calculates the baseline intensity for fluorescence in the well and reports peak intensities with the baseline subtracted. Allowance is made for the effects of the meniscus at the well edge.
Standard Deviation	The standard deviation of the intensities of the sample points in an object, excluding the baseline
Total Intensity	Measures the sum of all the intensities for that object. 

8.1.1.4.2 Morphology

Area	Area measures the space within the perimeter of a given object in μm^2.  **The space within the perimeter of a given object**
Aspect Ratio	Divides the object's major axis length by its minor axis length to produce a rotationally independent value for the object. This characteristic is useful for measuring morphological changes such as cell detachment.
Compactness	A measure of the compactness of an object by comparing the relative length of the perimeter with the degree of fit of an object to an idealised 2d Gaussian distribution
Density Factor	Measures translocation and localisation events within cells.
Depth	Depth used for separating populations of cells or beads from fluorescent debris. Most cells and beads are homogenous single objects, so have a comparable size which can be used to derive a minimum and maximum range for a filter. Unit is μm. 

Gaussian	A measure of the fit of the intensity profile of an object to an idealised Gaussian shape. It is measured as a percentage. A small sphere produces Gaussian line amplitudes (close to 100%), while a larger, flatter object produces flatter line amplitudes (further from 100%). 
Major Axis Length	Is the longest length within an object that goes through the centre point of the object.
Minor Axis Length	Is the shortest length within an object that goes through the centre point of the object
Perimeter	Perimeter measures the distance around the edge of an object in µm.  **The distance around the edge of an object**
Spherical Volume	Calculates the mean volume of objects using the formula: Spherical Volume = $4/3\pi$ x $Radius_{min}$ x $Radius_{max}$ x $Radius_{depth}$ Where $Radius_{min}$ = Minor axis length/2, $Radius_{max}$ = Major axis length/2 and $Radius_{depth}$ = ($Radius_{min}$ + $Radius_{max}$)/2

Width	Width used together for separating populations of cells or beads from fluorescent debris. Most cells and beads are homogenous single objects, so have a comparable size which can be used to derive a minimum and maximum range for a filter. Unit is μm. 
X-Coordinate **Y- Coordinate**	Co-ordinates designate the location of an object within a well by giving the bottom left hand corner of a rectangle that encapsulates the entire object. The bottom left hand corner of the well is the origin. Location can be used to define an area within a well e.g. to eliminate edge effects.

8.1.1.4.3 Calculations

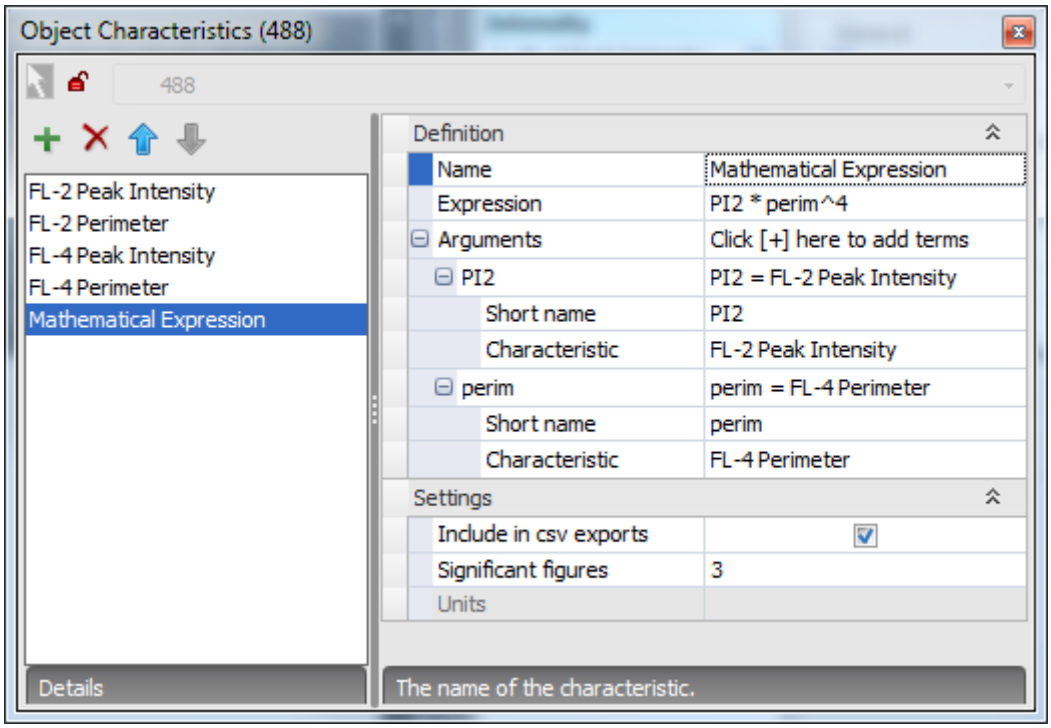
Add	Adds two Object Statistics together.
Antilog	The antilogarithm of a given characteristic in the specific base
Colour Compensation	Compensates a fluorescence Object Characteristic for spillage from another data collection channel.
Constant Value	Adds a user-defined Constant Value that can be used in secondary calculations.
Distance	The distance of the centre of an object from a given point in a well.
Log	Calculates Log values to Base 10, Base 2 or the natural logarithm of any given statistic.
Mathematical Expression	Allows formulae to be entered which are functions of other characteristics. To use it, add characteristics to the list below and enter a unique short name for each one. Enter the formula in the Expression box above, referring to the referenced characteristics by their short names. See section 8.1.1.4.4 below.
Multiply	Multiplies two Object Statistics. To use Multiply, both Object Statistics to be used must first be selected.
Percentage	Determines the percentage of two Object Characteristics.

Power	Raises an Object Characteristic to a user defined Power.
Ratio	Divides two Object Characteristics by each other. For example, it allows determination of the relationship between fluorescence across channels. To use Ratio, both the Characteristics must first be selected individually in the channel of interest.
Subtract	Calculates the difference between Object Statistics. To use subtract, both the Population Statistics to be used must first be selected.
Well Property	Allows definition of the contents of a well as a statistic for use in secondary calculations e.g. sample type, drug concentration, etc for IC_{50} determination.

8.1.1.4.4 Mathematical expressions

Mathematical expression characteristics allow the user to enter arbitrary mathematical functions of the other characteristics in an assay. The example below creates a characteristic MyCalc so:

$$MyCalc = FL2\ Peak\ Intensity\ \times (FL4\ Perimeter)^2$$



There is no need to include an equals (=) sign in the Expression. Mathematical expressions may prove slower to calculate than constructing the equivalent characteristic from a combination of the other primitive characteristics such as the Multiply and Power characteristic but are more concise and afford a greater range of calculations. Any performance penalty would normally only be noticed when applied to Object Characteristics and Composite Object Characteristics. The equivalent mathematical expression population statistic will not normally introduce any appreciable reduction in calculation times.

8.1.1.4.5 Mathematical Expression Language definition

The Mathematical expression parser implements the following operators:

-	**Unary Negation**
+	**Addition**
-	**Subtraction**
*****	**Multiplication. In some circumstances this may be omitted,** **e.g. 2 * (3 + 1) is equivalent to 2(3+1)**
/	**Division**
%	**Modulo**
^	**Exponentiation**

Identifiers – i.e. short names for characteristics – can contain only letters, digits and underscore symbols. They may not start with a digit.

The constants E and π may be used in calculations, but note that the case must be correct; E is uppercase and Pi is uppercase P followed by lowercase i. The constants Inf (infinity) and NaN (not a number) are also available.

Scientific notation may be used in any constants in expressions, e.g. 10.2E-3 or 1e4.

The mathematical expression language implements the following built-in unary functions:

Abs	The absolute value of a number
Sin(x)	The sine of an angle specified in radians
Cos(x)	The cosine of an angle specified in radians
Tan(x)	The tangent of an angle specified in radians
Sinh(x)	The hyperbolic sine of an angle specified in radians
Cosh(x)	The hyperbolic cosine of an angle specified in radians
Tanh(x)	The hyperbolic tangent of an angle specified in radians
Acos(x)	The angle in radians whose cosine is the given number
Asin(x)	The angle in radians whose sine is the given number
Atan(x)	The angle in radians whose tangent is the given number
Atan2(x, y)	The angle in radians whose tangent is the quotient (x/y) of two specified numbers.
Ceil(x)	The smallest integer greater than or equal to the specified number.
Floor(x)	The largest integer less than or equal to the specified number.
Round(x)	Rounds a value to the nearest even integer. Note that this uses Bankers' rounding; it does not always round away from zero.
Trunc(x)	Gives the integral part of a number, i.e. rounds down to the nearest integer
Log(x)	The natural (base e) logarithm of a specified number.
Log10(x)	The base 10 logarithm of a specified number.
Min(x,y)	The smaller of x and y
Max(x,y)	The larger of x and y

Exp(x)	Gives e to the specified power
Pow(x,y)	The number x raised to the power y
Sqrt(x)	The square root of a number

Note that not all valid functions available in mathematical expression characteristics in **Explorer version** 3.4 software – in particular conditional expressions – have direct equivalents in Cellista.

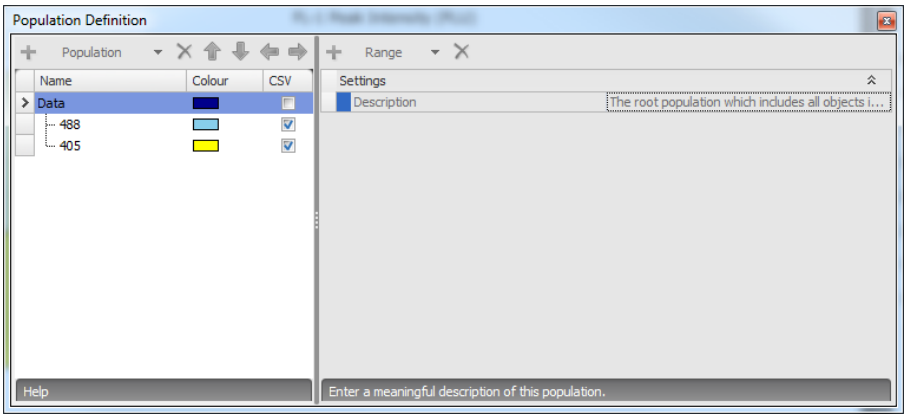
If a syntax error is detected than a message will appear in the expression editor and if calculated the characteristic will give the result 'not a number' or NaN, displayed as a dash (-).

8.1.2 Population Definition

Cellista software permits identification of a population of interest, by the user creating a population name and then applying a selection of filters to it. This can remove irrelevant material like cellular or experimental debris from the collected data.

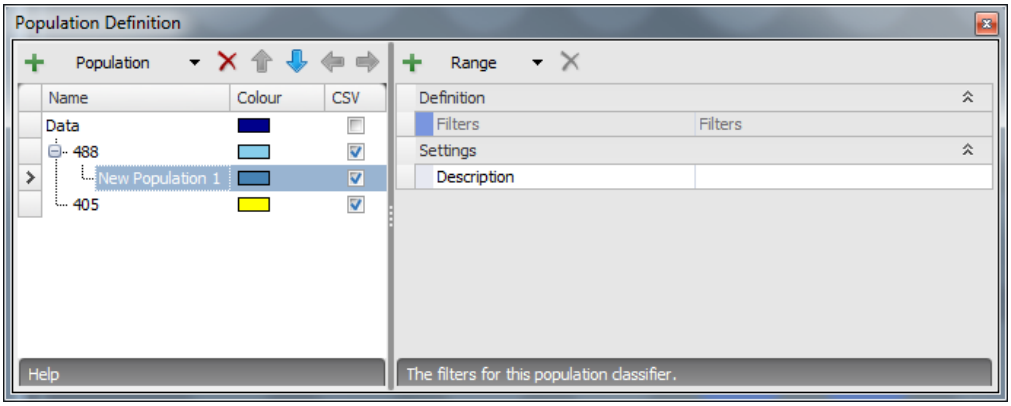
Population Definition is used to create and define sets of populations from within the scanned data set.

Note: the views below may be different to the one shown depending on the number of lasers used in the scan



8.1.2.1 Create a New Population

- Open **Analysis | Standard | Population Definition**
- The window will already contain a population called "Data". The Laser Data population is all data gathered for all wells in the scan from that laser.
- Select the laser data to which you want to add a new population. Click on the + symbol at the top of the window .
- Type in the name of the new population.



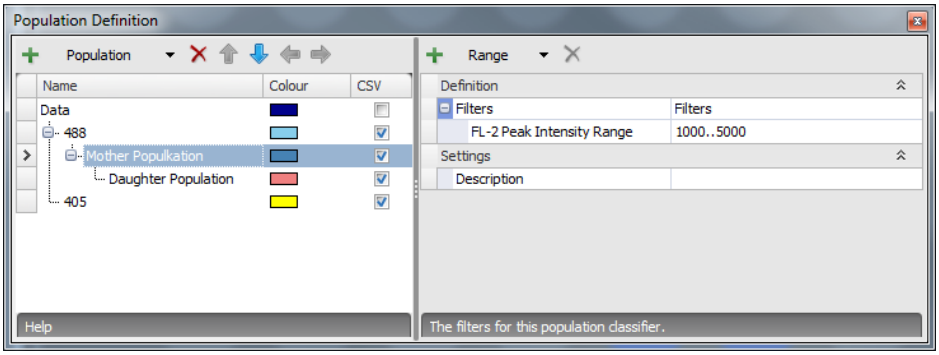
8.1.2.2 Creating a Sub-Population

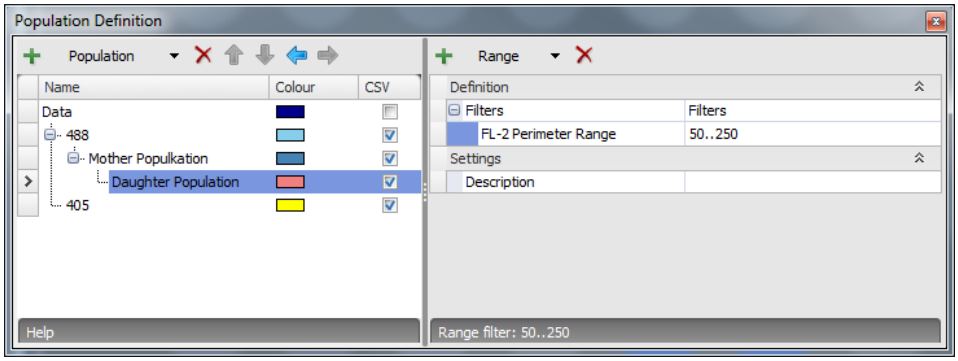
Populations may have other populations contained inside them. To reflect this, the user may create sub-populations and define filters for them.

To create a sub-population, either:

- Create a population as normal and then drag it onto the population it is to be a subset of
- Highlight the 'mother' population and click on the + button to create a daughter population.

The bounds of the sub-population can be set in the same way as for normal populations.





A sub-population inherits any changes to the bounds of a higher level population it is a part of. Therefore in the above example, the daughter population will be objects that have a FL-2 Peak Intensity of 1,000-5,000 FLU and a FL-2 Perimeter between 50-250 μm^2.

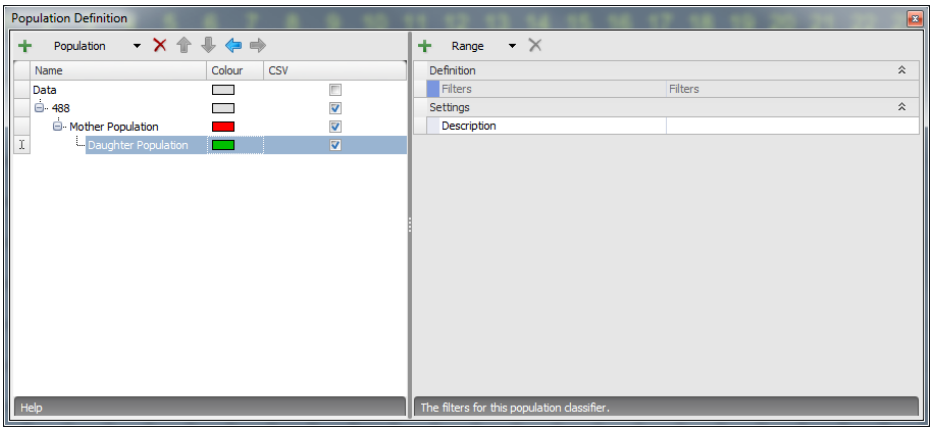
8.1.2.3 Deleting a Population

To delete a population:

- In the **Population Definition** window
- Select the population to remove by clicking once on the name of the population.
- Click on ✕ at the top of the window to remove the population.

8.1.2.4 Change Population Colour

- Select **Analysis | Standard | Population Definition** or select the icon from the toolbar to launch the **Population Definition** window
- Edit the population colour by double-clicking on its box then picking a new colour in the pop-up window.
- Press **OK** to close the window.



Click anywhere in the box to register the changes. Close this box down. At this stage, the filters for the population have not been specified: a label for the population is all that has been created.

When the data is reanalysed using the icon, after adding a population, the views show all data the same colour. This is because there are no filters or limits on each population.

If the **Include in csv exports** box is checked, then this population is included in automatically exported plate data. This setting can be overridden when exporting manually.

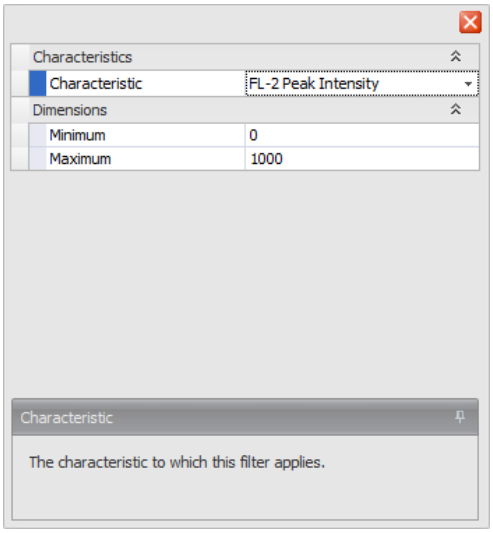
8.1.2.5 Population Filters

There are three main methods for adding, editing and deleting **Object Characteristics** used to define populations. These are using Ranges in **Population Definition** directly, or indirectly by using histograms or scatter charts.

8.1.2.6 Adding a Population Range

To add a range to a population using the **Population Definition** window:

- Select the population you want to define.
- Click on the Range button. This opens a new box and adds a default range for FL-2 Peak Intensity



- The characteristic can be changed to use another object characteristic by selecting one from the drop-down list. Only Object Characteristics that have been set can be used.
- Select the Minimum and Maximum values to describe the Population
- Close the box.
- Reanalyse the data.

8.1.2.6.1 Additional Population Filter Types

In addition to the simple *Range* filter described above it is possible to add filters which apply to two characteristics at once. Click the small arrow to the right of the add filter button:

A menu of the available filter types will appear – the full list is Splitter, Polygon, Ellipse or Range. See also section 10.2.3.3.1.

8.1.2.7 Deleting a Population Filter

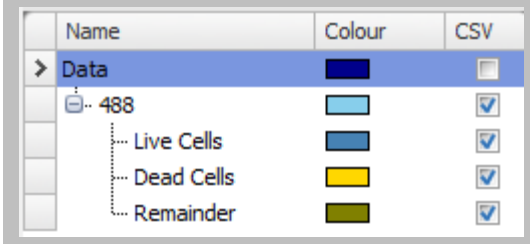
A population filter can be removed using the **Population Definition** window:

- Select the population range to remove by clicking on it.
- Select the icon next to the Tange Button to delete.

8.1.2.7.1 Additional Population Types

In addition to the simple filtered sub-population described above, it is possible to define populations which filter objects based on something other than characteristic values. Click the small arrow to the right of the add population button:

A menu will appear listing the types of populations which may be added. Note that not all will be available under all circumstances. The full list is explained in the table below.

Well Property	Filters objects based on whether the well to which they belong has a textual well property with a particular value – see also section 8.2.1. Intended principally for use with Well Definitions and Plate Statistics.
Controls	Filters objects based on whether the well to which they belong has been marked as a control well. Intended principally for use with Well Definitions and Plate Statistics **section 8.2.1.**
Remainder	Includes all objects excluded from all other populations at the same level. For example in the case below, the Remainder population includes all objects from the 488 population which are not in either the Live Cells or Dead Cells populations.  This is of particular use with the Splitter filter type; the splitter filter defines objects one side of a line on a scatter plot. The remainder will include all objects on the other side of the line.
Population	The default population type. Filters objects based on the values of object characteristics.
Location	Filters objects based on the location of the well to which they belong. Intended principally for use with Well Definitions and Plate Statistics **section 8.2.1**.

8.1.3 Population Statistics

Population Statistics report parameters for populations of objects within each well. Therefore they normally represent the primary assay readouts.

8.1.3.1 Adding Population Statistics

- Select **Analysis | Standard | Population Statistics**
- To add new Population Statistics, click on + to open the selection dialog.
- Select Population Statistics by clicking the Add icon. Multiple statistics can be made by repeatedly clicking the relevant + icon.

8.1.3.1.1 Statistics

Number of Objects	A count of objects belonging to a population.
Confidence Interval (95%)	Equals the Mean ± (1.96 X Standard Error of the Mean).
CV	The CV (Coefficient of Variance) is the standard deviation expressed as a percentage of the mean. It can be used to provide confidence that the results obtained from a population are statistically significant.
Geometric Mean	Obtained by multiplying together the values for the statistic for every spot, and calculating this value to the power (1/number of spots). If the statistics multiply together to give a value X, the geometric mean is the value each statistic would have if they all had the same value but still multiplied together to give X.
Maximum	The maximum value for objects in a population.
Mean	The mean value for an Object Characteristic for a given population (the total value for the characteristic divided by the number of objects).
Median	The median value for an Object Characteristic for a given population (the mean of the middle values for the characteristic in a frequency distribution).
Median Absolute Deviation	The median absolute value is the average absolute deviation from the mean and is a common measure of forecast error in time series analysis.
Minimum	The minimum value for objects in a population.
Standard Deviation	Square root of the variance of a Population Statistic. Variance is calculated by taking the sum of the squares of deviation of each value from the mean, and dividing by the number of samples.
Standard Error	Standard deviation/square root (number of samples). Measures how close the sample mean is to the real population mean.
Total	The sum of values for a Population Statistic.

8.1.3.1.2 Calculations

Add	Adds two Population Statistics.

Antilog	The antilogarithm of a given characteristic in the specific base
Constant Value	A user-defined value that can be used in secondary calculations.
Hit Picker	Apply a filter to a Population Statistic. This information can be used to identify specific wells of interest as defined by the user for example wells contained hit compounds, or cytotoxic compounds. For multi-parameter hit identification, users should consider defining a Well Population (see section 13).
Log	Calculates Log values to Base 10, Base 2 or the natural logarithm of any given statistic.
Mathematical Expression	Allows formulae to be entered which are functions of other statistics. To use it, add statistics to the list below and enter a unique short name for each one. Enter the formula in the Expression box above, referring to the referenced statistics by their short names. See section 8.1.1.4.4 for more information.
Multiply	Multiplies two Population Statistics. To use Multiply, both Population Statistics to be used must first be selected.
Percentage	The percentage of two Population Statistics by each other e.g. to determine the percentage of labelled cells relative to total cell number.
Power	Raises the Population Statistics to a user defined exponent power.
Ratio	Divides two Population Statistics.
Subtract	Calculates the difference between Population Statistics. To use Subtract, both the Population Statistics to be used must first be selected.
Well Number	Generates a unique index for each well starting from A1. If **one-based** is selecting then A1 is 1, A2 is 2 and so on, otherwise the numbering will start from 0.
Well Property	Allows definition of the contents of a well as a statistic for use in secondary calculations e.g. sample type, drug concentration, etc for IC_{50} determination.

8.1.3.2 Deleting Population Statistics

- Select **Analysis | Standard | Population Statistics**
- Select the Population Statistic in the list on the left hand side.
- Delete by clicking the ✕ icon.

8.1.3.3 Editing Population Statistics

- Select **Analysis | Standard | Population Statistics**
- Select the Population Statistic in the list on the left hand side.
- Edit the settings on the right hand side.

8.1.3.4 Definition

Name	This can be edited to be more meaningful (e.g. % positive cells)
Population	Selects the population for which to export the parameter.

8.1.3.5 Settings

Include in csv exports	If checked, the statistic is included in the automatic export of well data. This setting can be overridden when exporting manually.
Significant Figures	The number of significant figures used to display the characteristic
Suppress Calculation Errors	If a divide by 0 occurs during the calculation of this characteristic then enabling this setting will convert the result to a 0. Leaving it disabled will display a hyphen (-).

8.2 Plate Group

8.2.1 Well Properties

Define custom properties for which you can enter well specific data. This can be useful for entering plate map information, e.g. drug name or concentration.

Well properties have a unique value for each well, and can be numeric, boolean or textual. They are included in the OME metadata in tiff files and can appear in exported csv data. Numeric well properties can participate in Object Characteristic, Population Statistic and Well Statistic calculations. Textual well properties can be used to define groups or populations of wells for Well Statistic calculations.

8.2.1.1 Creating Well Properties

To create a numeric well property, click **Analysis | Plate | Well Properties**. Ensure that the drop down button reads **Numeric** and press the + button. Edit the name and units if required. The CSV checkbox indicates whether this property is included in automatically generated CSV export files, though this setting can be overridden when exporting manually.

To create a textual well property, click **Analysis | Plate | Well Properties**. Ensure that the drop down button reads **Text,** pressing the arrow if required, and then press the + button. Edit the name of this property as required.

To edit the values of these properties for each well, go to **View | Plate | Spreadsheet** and select the relevant property in the combo box. You may then edit the value of the property for each well. Note that you can copy and paste values into a range of wells, e.g. from Excel.

8.2.1.2 Using Numeric Well Properties

Numeric well properties can participate in characteristics and statistics calculations. For instance, it is possible to create a population statistic which is the result of some other value multiplied by the concentration. To do so,

- Add a numeric well property as described above and name it **Concentration**.
- Use the plate spreadsheet view to enter appropriate values for the concentration for the wells of interest.
- Add a **Well Property Population Statistic**. Go to **Population Statistics**, click add, and then add a **Well Property** statistic. Then edit the 'Property' value of this statistic to select 'Concentration'. This creates a population statistic called concentration which takes the value of the underlying numeric property for each well. The statistic will have the same name as the underlying property by default. Note that this statistic will be created automatically and this step can be omitted if the 'Add Statistic' box is checked in the **Well Properties** dialog.
- Add a multiplication statistic; set one argument to be the concentration statistic and the other argument to whatever you require.

8.2.1.3 Using Textual Well Properties

Textual well properties can be used to define populations in either **Analysis | Standard | Population Definition** or **Analysis | Plate | Well Definition.** Thus it is possible to create a population of wells which contain a particular drug. For example,

- Add a text well property as described above and name it **Drug**
- Use the plate spreadsheet view to enter appropriate values for the drug name for the wells of interest, e.g. Drug1.
- In **Analysis | Plate | Well Definition** select the **Sample Wells** population. Click the drop-down arrow to the right of the add button and select **Well Property**. This adds a new Well Property sub-population to Sample Wells. Call this population **MyDrug**
- In the Property dropdown on the right select the **Drug** property.
- In the Value dropdown on the right enter the drug name Drug1 for those wells you wish to include in the MyDrug population. All wells for which the Drug property matches this value will be included in the MyDrug population. The comparison can be case sensitive or not as required.
- You can now create a **Well Statistic** which uses this population e.g. Go to plate statistics and add a 'Number Of Objects' statistic. Set the population to MyDrug. This statistic will report – in Plate Results – the number of wells containing Drug1.

This idea can be extended to perform more sophisticated calculations on a per-drug basis, or to generate Plate Statistics which compare the results of two drugs across a plate.

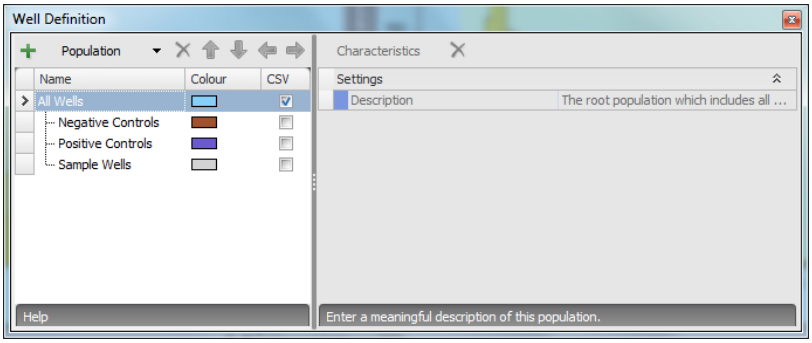
8.2.1.4 Using Boolean Well Properties

Boolean well properties allow each well to take a true or false value for a given property. They can be used to define populations in either **Analysis | Standard | Population Definition** or **Analysis | Plate | Well Definition.** Thus it is possible to create a population of wells which satisfy a particular condition. For instance, rather than specifying reagent names as in 8.2.1.3 above, you might create a series of Boolean properties, one for each drug, e.g. reagent 1, reagent 2, reagent 3. Then for each well you would tick the checkboxes to say whether a given reagent was present. This allows for the creation of populations based on the various combinations of reagents in wells.

8.2.2 Well Definition

Cellista software permits identification of a population of wells defined by a set of Population Statistics. Populations of wells can be viewed in the **Plate View** (10.1.1) or **Spreadsheet View** (10.1.3). This acts as a hit picker tool to identify hit wells using Population Statistic data.

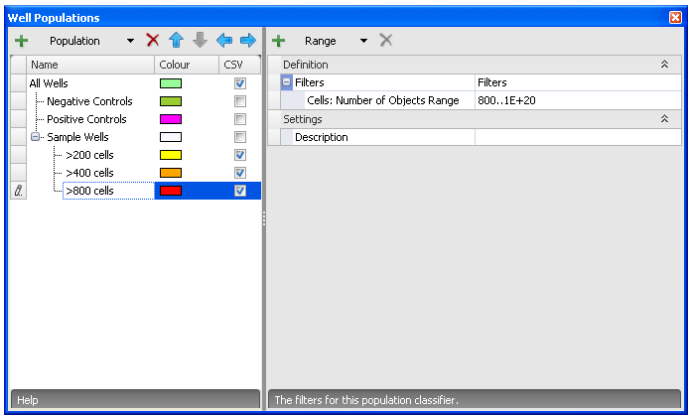
Select **Analysis| Wells | Well Definition**.



8.2.2.1 Adding a Well Population

It is recommended that new Well Populations are added as sub-populations of Sample Wells. This avoids inclusion of control wells. Once Populations have been created and defined, they can be viewed easily in plate views and histograms (see section 10.1.1.2).

- Select **Analysis| Wells | Well Definition**.
- Highlight Sample Wells and select the + icon to add a population.
- Change its name and colour as appropriate.
- Select the reanalyse icon on the top toolbar.



8.2.2.2 Deleting a Well Population

- Select **Analysis| Wells | Well Definition**.
- Select the population to be deleted
- Select the ✕ icon
- Select the reanalyse icon on the top toolbar

8.2.2.3 Defining a Well Population

Well Populations are defined using Population Statistics.

8.2.2.4 Adding a Population Filter

- Select **Analysis| Wells | Well Definition**.
- Select the population you want to define.
- Click on the + Range icon.
- Select the required **Population Statistics** from the drop-down list.
- Enter Minimum and Maximum values for the filter.
- Close the box.

- Select the reanalyse icon on the top toolbar.

8.2.2.5 Deleting a Population Filter

- Select **Analysis| Wells | Well Definition**.
- Select the population filter to remove.
- Click on the **Delete** Filter icon .
- Select the reanalyse icon on the top toolbar

8.2.3 Plate Statistics

Plate Statistics report parameters for an entire plate or subpopulation of wells (e.g. control or samples). They can be used to provide summary information on assay performance, for instance %CV, Z' and Number of Hits. Wells must be defined as positive and negative wells. See section **10.1.1.1**

8.2.3.1 Adding Well Characteristics:

- Select **Analysis | Plate | Plate Statistics**
- To add new Well Characteristics, click on to open the selection dialog.
- Select Well Characteristics by clicking the icon. Multiple characteristics can be made by repeatedly clicking the icon.

8.2.3.1.1 Statistics

Number of Objects	Number of objects belonging to a specified population.
Assay Window	Assay window of control wells. Calculated by mean of +ve controls/ mean –ve controls
Confidence Interval (95%)	Confidence Interval for the objects in a collection which belong to a specified population
CV	Sample covariance of the values of a given characteristic for a specified population
Geometric Mean	Geometric mean of a characteristic for a specified population.
Maximum	Maximum value a given characteristic for a specified population.
Mean	Mean value of a given characteristic for a specified population
Median	Median value of a given characteristic for a specified population
Median Absolute Deviation	Median Absolute Deviation of a population $MAD = \mathrm{K} \times \mathrm{median}\ (|\mathrm{Yi} - \mathrm{median}\ (\mathrm{Y})|)$ where K = 1.4826 modelling a normal distribution
Minimum	Minimum value of a given characteristic for a specified population.
Signal to Noise	S:N ratio of a given set of wells. $\mathrm{S{:}N} = (\frac{(mean\ of\ +ve\ controls - mean - ve\ controls)}{\sqrt{(sample\ variance\ of\ +ve\ controls + sample\ variance\ of\ -ve\ controls}}$
Standard Deviation	Sample Standard Deviation of a given characteristic for a specified population
Standard Error	Sample Standard error of the Mean of the values of a given characteristic for a specified population
Total	The sum of specified characteristic's value for all the objects in a population.
Z'	Z factor of a given set of wells Calculated: $Z' = 1 - 3 * \frac{(sample\ SD\ of\ +ve\ controls + sample\ SD\ of\ -ve\ controls)}{(mean\ of\ +ve\ controls - mean - ve\ controls}$

8.2.3.1.2 Calculations

Add	Adds the values of the given statistics.
Antilog	Antilog of a given characteristic in the specified base
Constant Value	Defines a constant value for use in calculations in other traits
Hit Picker	Returns a specified value – 'the weight' – if an underlying population statistic lies in the given range
Log	Logarithm of a given characteristic for a specified population in the specified base.
Mathematical Expression	Calculates an arbitrary mathematical function of other characteristics. See section 8.1.1.4.4 for more information.
Multiply	Multiplies the values of the given statistics..
Percentage	Calculates the ratio between two given characteristics as a percentage.
Power	Raises the given characteristic value to a specified power.
Ratio	Calculates the ration between two given statistics
Subtract	Calculates the difference between two given statistics.
Focus Offset	The batch focus position used to scan the plate. See section 11.2.1.3 for more details.

8.2.3.2 Deleting Plate Characteristics

- Select **Analysis | Plate | Plate Statistics**
- Select the **Well Characteristic** in the list on the left hand side.
- Delete by clicking the ✕ icon.

8.2.3.3 Editing Plate Characteristics

- Select **Analysis | Plate | Plate Statistics**
- Select the **Plate Characteristic** in the list on the left hand side.
- Edit the settings on the right hand side.

Name	This can be edited to be more meaningful (e.g. Number of Hit Wells).
Population	Defines the Population
Characteristic	Selects the required Population Characteristic

8.3 Composite Group

The **Composite Mode** must be set prior to scanning. Refer to section 7.4.1 for more details. It is assumed that users fully understand the basic operation of the cellista software prior to using the advanced mode. Please refer to previous sections of this User Manual for information on the basic operation of the system.

To obtain data from merged scans, it is first necessary to run through the software as normal (**see section 8.1.1.1):**

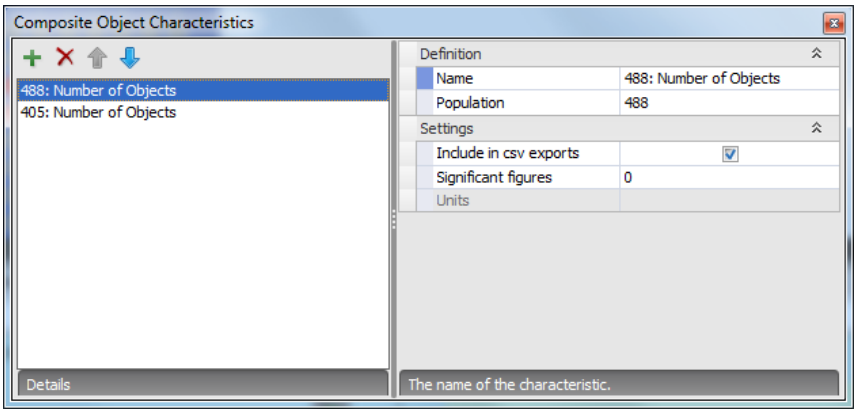
- Scan the wells of interest
- Set up **Object Characteristics** as required for the assay
- Create and define populations in **Population Definition**
- Set up **Population Statistics** (this is optional)

It is important to set up **Object Characteristics** and **Population Definition** correctly as these are required for the composite mode.

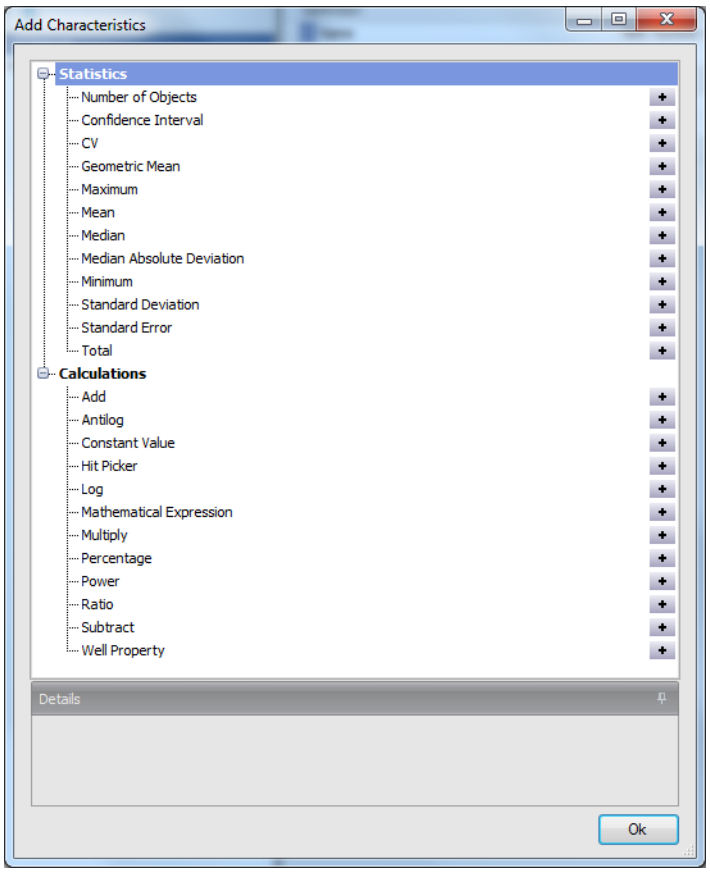
8.3.1 Composite Object Characteristics

To define **Composite Object Characteristics**, go to **Analysis | Composite | Object Characteristics**

The following dialogue box opens:



To add a new **Composite Object Characteristic** click on the + button and the following selections appear from the drop down menu.



Note that his box shows the same characteristics as the **Population Statistics** window (**section 8.1.3**) - this is because each composite object can be regarded as a small population of single-scan objects.

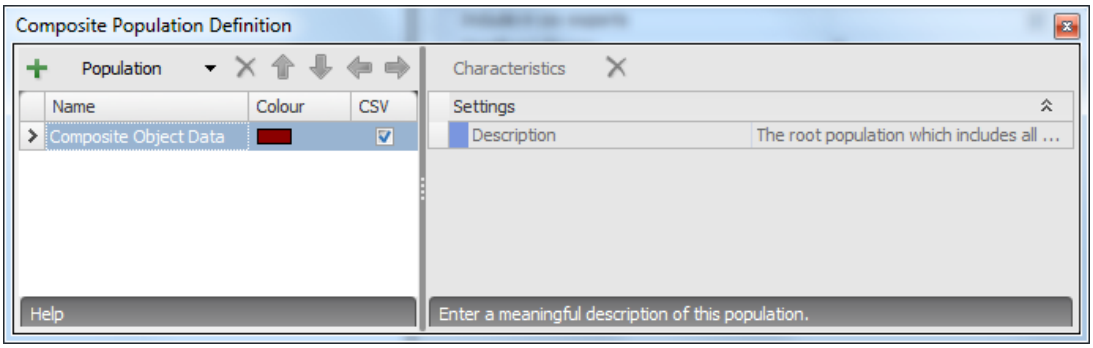
8.3.2 Composite Population Definition

cellista software permits identification of a composite population of interest, by the user creating a composite population name and then applying a selection of filters to it. This can remove irrelevant material like cellular or experimental debris from the collected data. **Composite Population Definition** is used to create and define sets of composite populations from within the scanned data set.

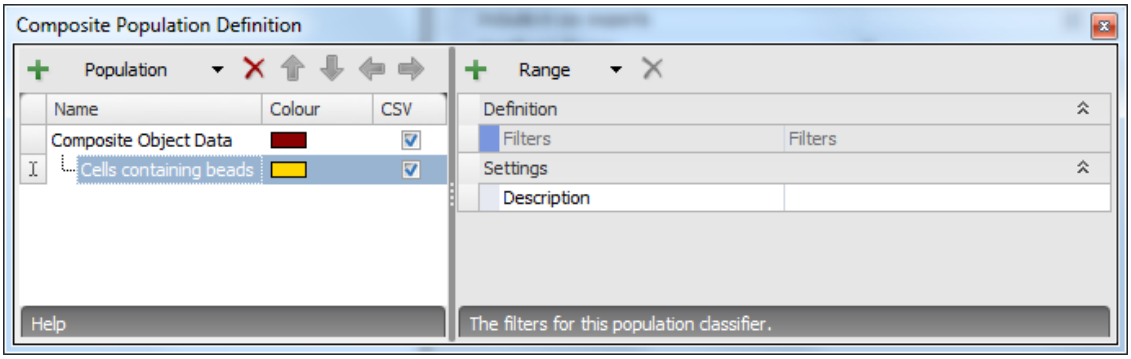
To open:

Select **Analysis | Composite | Population Definition**

The following box opens



Click on the **Population** symbol at the top of the window and enter the name of the new composite population.



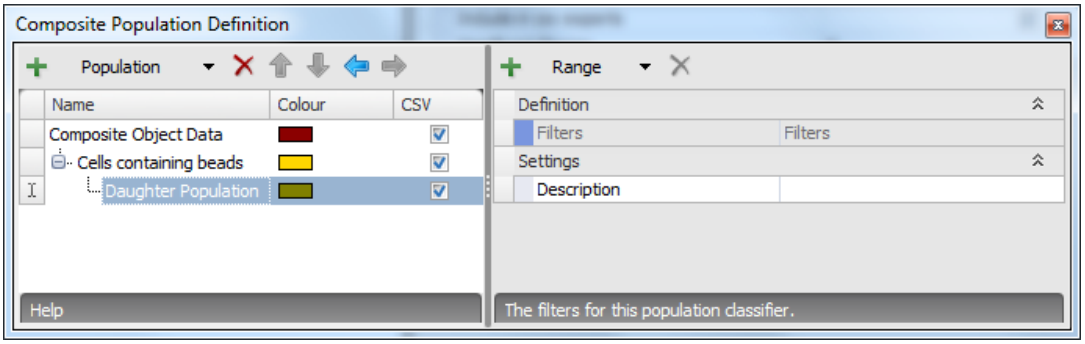
8.3.2.1 Creating a Sub-Composite Population

Composite populations may have other sub-composite populations contained inside them. To reflect this, the user may create sub-composite populations and define filters for them.

To create a sub-composite population:

- Highlight the 'mother' composite population and click on the **Population** symbol to create a 'daughter' composite population.

The bounds of the sub-composite population can be set in the same way as for normal populations.



A sub-composite population inherits any changes to the bounds of a higher level composite population it is a part of.

Close this box down. At this stage, the filters for the composite population have not been specified: a label for the composite population is all that has been created.

When the data is reanalysed using the icon, after adding a composite population, the views show all data the same colour. This is because there are no filters or limits on each composite population.

If the **Include in csv exports** box is checked, then when plate data is automatically exported, this composite population is included in the exported data. This setting can be overridden when exporting manually.

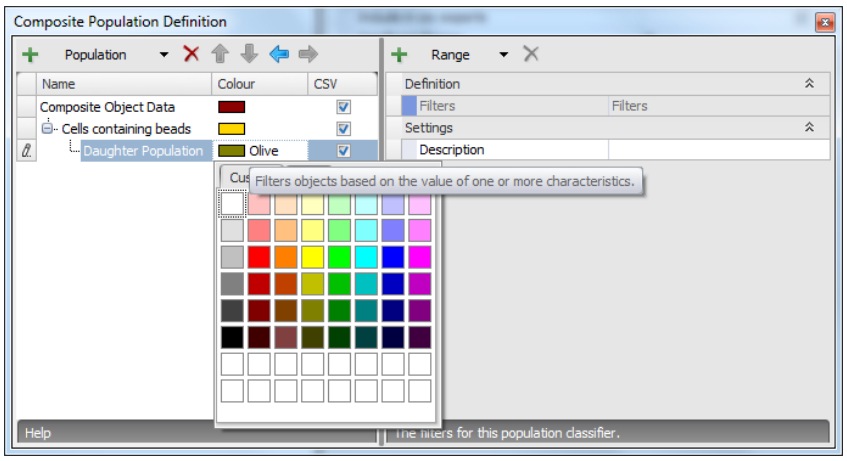
8.3.2.2 Deleting a Composite Population

To delete a composite population:

- In the **Composite Population Definition** window.
- Select the composite population to remove by clicking once on the name of the composite population.
- Click on at the top of the window to remove the composite population.

8.3.3 Change Composite Population Colour

- Select **Analysis | Composite | Population Definition**
- Edit the composite population colour by -clicking on its box then picking a new colour in the pop-up window.



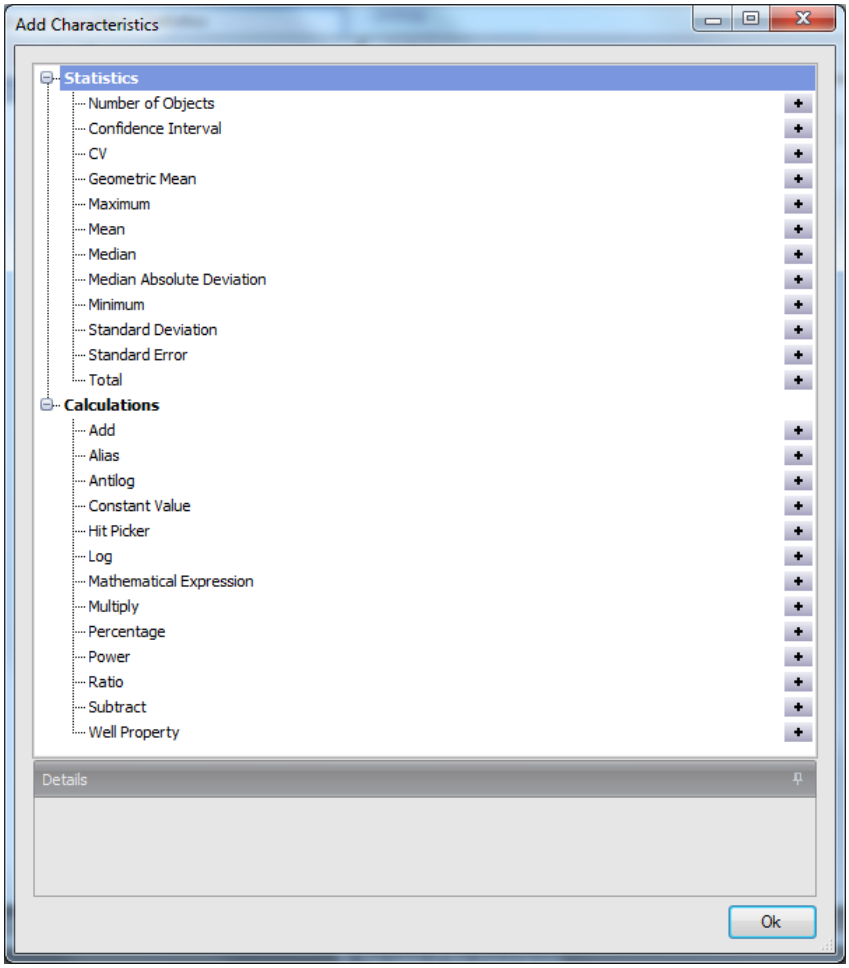
Click anywhere in the box to register the changes

8.3.4 Composite Population Statistics

Adding a **Composite Population Statistic** allows the characterisation of a composite population using a chosen parameter. Using multiple parameters can further refine composite population separation.

8.3.4.1 Adding Composite Population Statistic:

- Select **Analysis | Composite | Population Statistics**
- To add new **Composite Population Statistic**, click on + to open the selection dialog.



- Select Characteristics by clicking the + icon. Multiple characteristics can be made by repeatedly clicking the + icon.

8.3.4.1.1 Statistics

Number of Objects	A count of objects belonging to a population.
Confidence Interval (95%)	Equals the Mean ± (1.96 X Standard Error of the Mean).
CV	The CV (Coefficient of Variance) is the standard deviation expressed as a percentage of the mean. It can be used to provide confidence that the results obtained from a population are statistically significant.
Geometric Mean	Obtained by multiplying together the values for the statistic for every spot, and calculating this value to the power (1/number of spots). If the statistics multiply together to give a value X, the geometric mean is the value each statistic would have if they all had the same value but still multiplied together to give X.
Maximum	The maximum value for objects in a population.
Mean	The mean value for an Object Characteristic for a given population (the total value for the characteristic divided by the number of objects).
Median	The median value for an Object Characteristic for a given population (the mean of the middle values for the characteristic in a frequency distribution).
Median Absolute Deviation	The median absolute value is the average absolute deviation from the mean and is a common measure of forecast error in time series analysis.
Minimum	The minimum value for objects in a population.
Standard Deviation	Square root of the variance of a Population Statistic. Variance is calculated by taking the sum of the squares of deviation of each value from the mean, and dividing by the number of samples.
Standard Error	Standard deviation/square root (number of samples). Measures how close the sample mean is to the real population mean.
Total	The sum of values for a Population Statistic.

8.3.4.1.2 Calculations

Add	Adds two Population Statistics.

Alias	Converts a simple (single scan) population statistic result to a composite population statistic value for use in calculations and comparisons
Antilog	Antilog of a given characteristic in the specified base
Constant Value	A user-defined value that can be used in secondary calculations.
Hit Picker	Apply a filter to a Population Statistic. This information can be used to identify specific wells of interest as defined by the user for example wells contained hit compounds, or cytotoxic compounds. For multi-parameter hit identification, users should consider defining a Well Population (see Section 13).
Log	Calculates Log values to Base 10, Base 2 or the natural logarithm of any given statistic.
Mathematical Expression	Allows formulae to be entered which are functions of other statistics. To use it, add statistics to the list below and enter a unique short name for each one. Enter the formula in the Expression box above, referring to the referenced statistics by their short names. See section 8.1.1.4.4 for more information.
Multiply	Multiplies two Population Statistics. To use Multiply, both Population Statistics to be used must first be selected.
Percentage	The percentage of two Population Statistics by each other. E.g. to determine the percentage of labelled cells relative to total cell number.
Power	Raises the Population Statistics to a user defined exponent power.
Ratio	Divides two Population Statistics.
Subtract	Calculates the difference between Population Statistics. To use Subtract, both the Population Statistics to be used must first be selected.
Well Number	Generates a unique index for each well starting from A1. If **one-based** is selecting then A1 is 1, A2 is 2 and so on, otherwise the numbering will start from 0.
Well Property	Allows definition of the contents of a well as a statistic for use in secondary calculations e.g. sample type, drug concentration, etc for IC_{50} determination.

8.3.4.2 Editing Composite Population Statistic

- Select **Analysis | Composite | Population Statistics**
- Select the **Population Characteristic** in the list on the left hand side.
- Edit the settings on the right hand side.

8.3.4.3 Deleting Composite Population Statistic

- Select **Analysis | Composite | Population Statistics**
- Select the **Population Characteristic** in the list on the left hand side.
- Delete by clicking the  icon.

9 RUN TAB

9.1 Control Group

Functions within this group are used for basic instrument operation.

9.1.1 Scan

To begin a scan (when wells are marked for scanning), click on the scan icon: 

9.1.2 Abort

To manually stop a scan that is already in progress, click on the abort button: 

9.1.3 Load/Eject

The Load/Eject button () is used to open and close the drawer to allow the placement or removal of a microtitre plate.

9.1.4 Reset

The Reset button () is used to initialise the system if it has gone into error state.

9.2 Data Group

9.2.1 Reanalyse

The Reanalyse button () is used to recalculate assay results after making changes to any of the parameters within the “Analysis” tab.

10 VIEW TAB

10.1 Plate Group

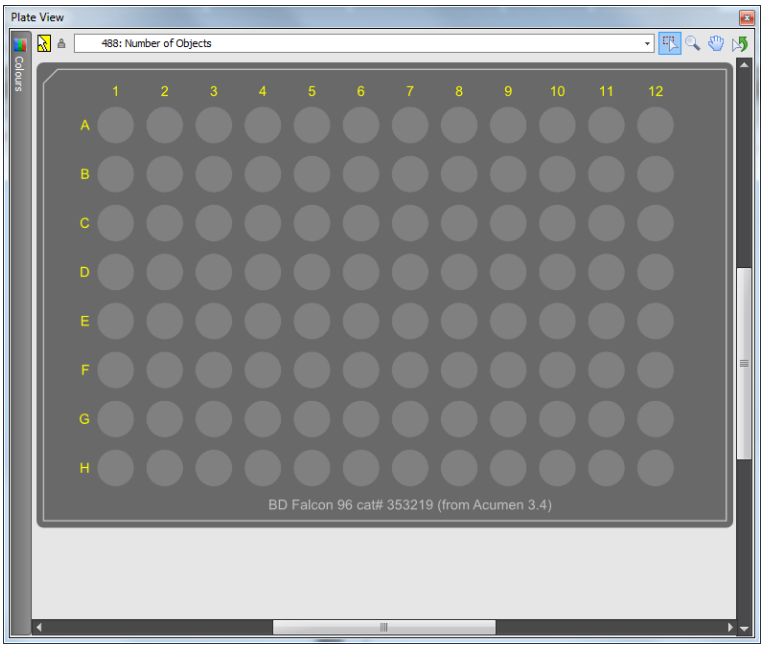
10.1.1 Plate

Opens up a Plate View. This is used to select wells for scanning or viewing plate heat maps.

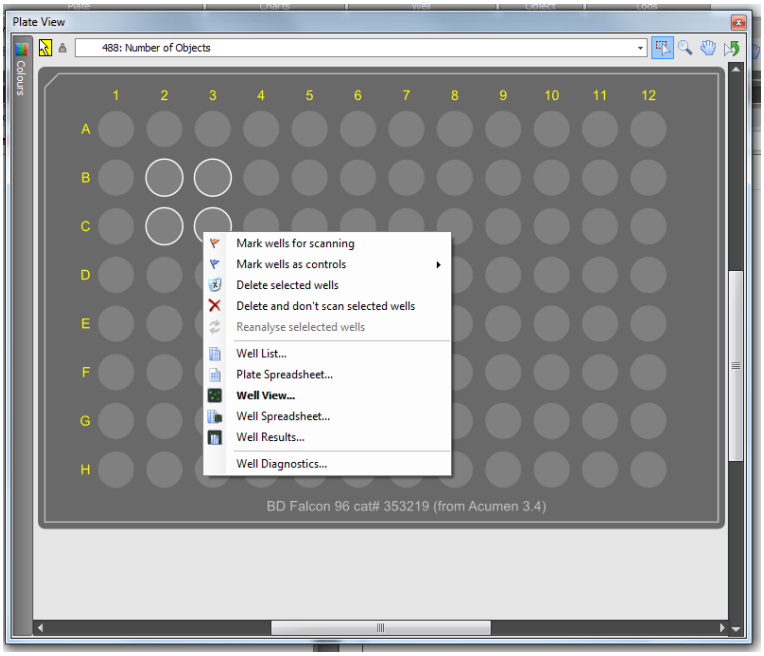
10.1.1.1 Selecting wells for scanning:

Go to: **View | Plate | Plate**

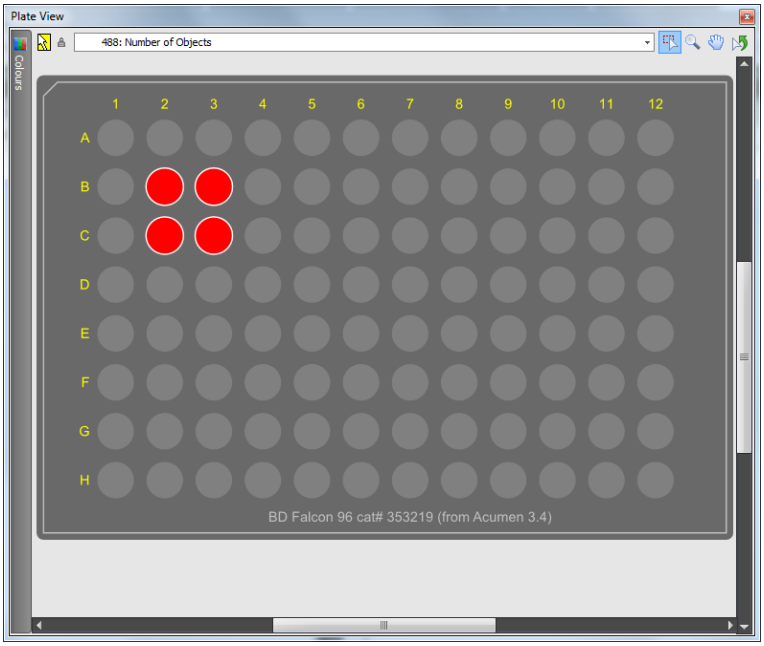
The plate view opens:



To select wells for scanning, select the wells to be scanned, then right click on the mouse.



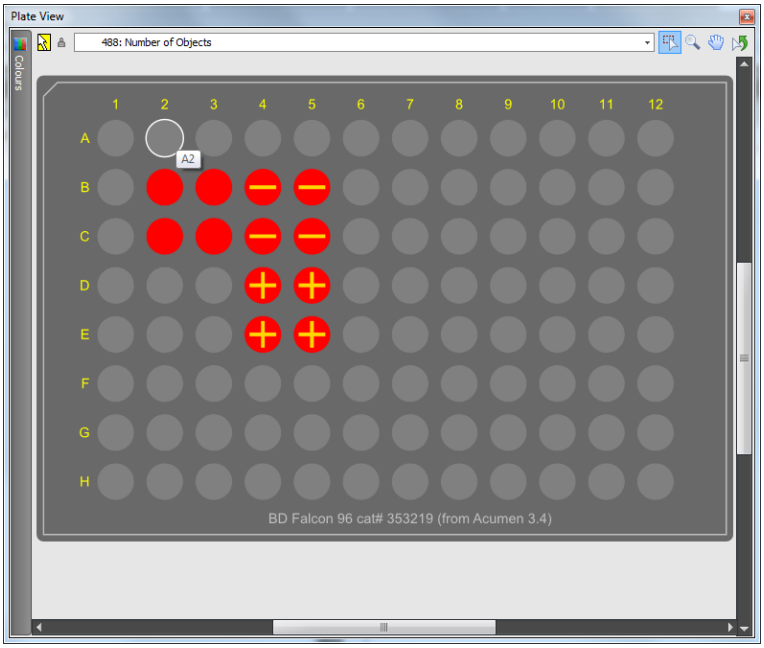
Select **Mark Wells for Scanning**. The wells then turn red indicating they will be scanned:



It is also possible to select positive and negative wells. This is used for automatically determining plate based statistical data such as Z' - see section **8.2.3**.

To select control wells:

- Select the wells
- Right click to access **Mark wells as Controls.**
- Select whether the wells are positive controls or negative controls
- The wells will be indicated as shown below:



Once the wells are scanned, the wells are shaded different levels of green corresponding to the well value of the selected Population Statistic in the title.

10.1.1.2 Viewing Well Definitions in Plate View

Once well Definitions have been set in **Analysis | Well | Well Definitions (section 8.2.2)** these can be viewed as a heat map in the **Plate View**.

- Select **View | Plate | Plate**.

- Expand the Colours option box on left side.
- Select the Population Colour check box at top.

10.1.2 Well List

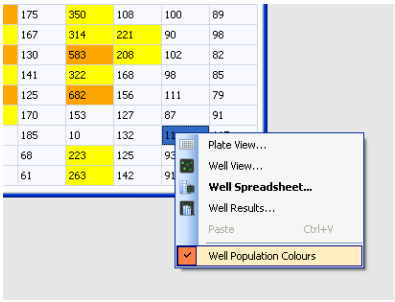
The **View | Plate | Well List** view displays the **Population Statistics** and **Well Definition** results for all wells in a single table.

10.1.3 Spreadsheet

The **View | Plate | Spreadsheet view** is a view of the **Population Statistic** results of the wells in the plate in a plate format. It shows the same data as the well list but for only a single population statistic at a time.

Once **Well Definitions** have been set in **Analysis | Well | Well Definitions (section 8.2.2)** these can be viewed as a heat map in the **Plate View**.

- Select **View | Plate | Spreadsheet**.
- Right mouse click on spreadsheet and select Well Population Colours.



This view is additionally used to edit well properties for each individual well once properties have been configured via **Analysis | Plate | Well Properties**.

10.1.4 Results

The **View | Plate | Statistics** view shows the plate-level results, i.e. the values of the plate statistics configured via **Analysis | Plate | Plate Statistics**. This reports out information such as plate Z', signal to noise and so on – see 8.2.2 and 8.2.3. By default this view shows the results for the entire plate but it can show the results for a selection of wells by linking from a plate view or plate spreadsheet view which has only a limited number of wells selected.

10.1.5 Population Details

The **View |Plate | Population Details** view shows the value of the various population statistics defined through **Analysis | Standard | Population Statistics**, but calculated over all of the wells selected. For example, by selecting wells A1-D4 in a plate view and linking that to a population details window it is possible to show the total number of objects in the 16 wells selected.

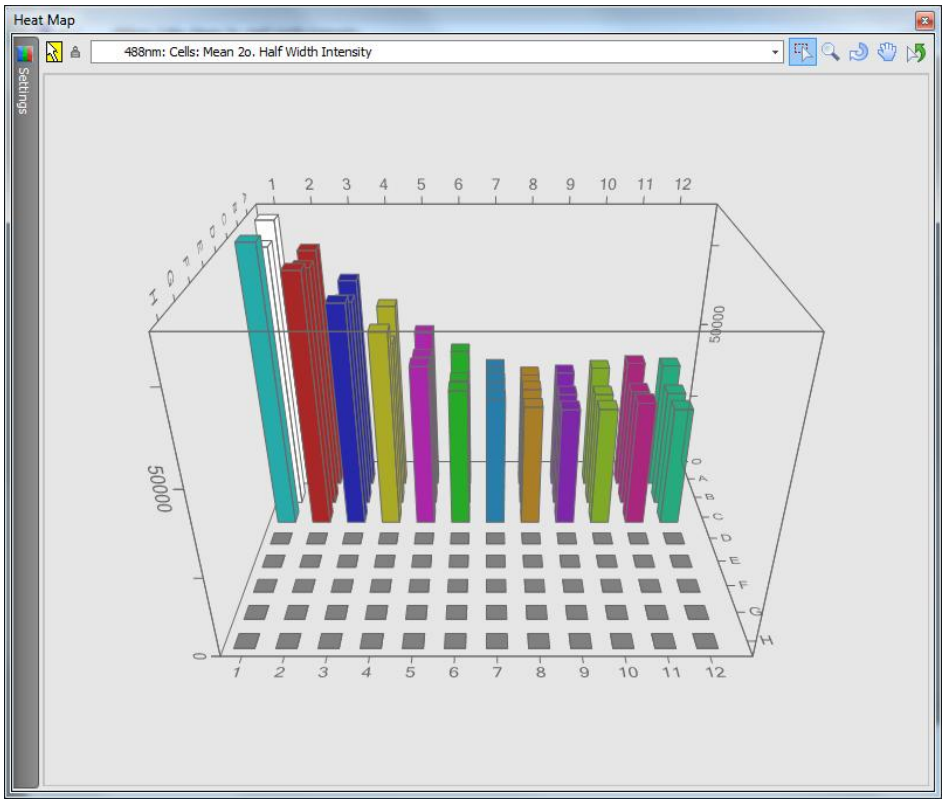
10.2 Charts Group

10.2.1 3D Heat Map

A 3D Heat Map provides a visual representation of a **Population Statistic** in the whole plate. It is available for any defined **Population Statistic**.

10.2.1.1 Viewing a 3D Heat Map

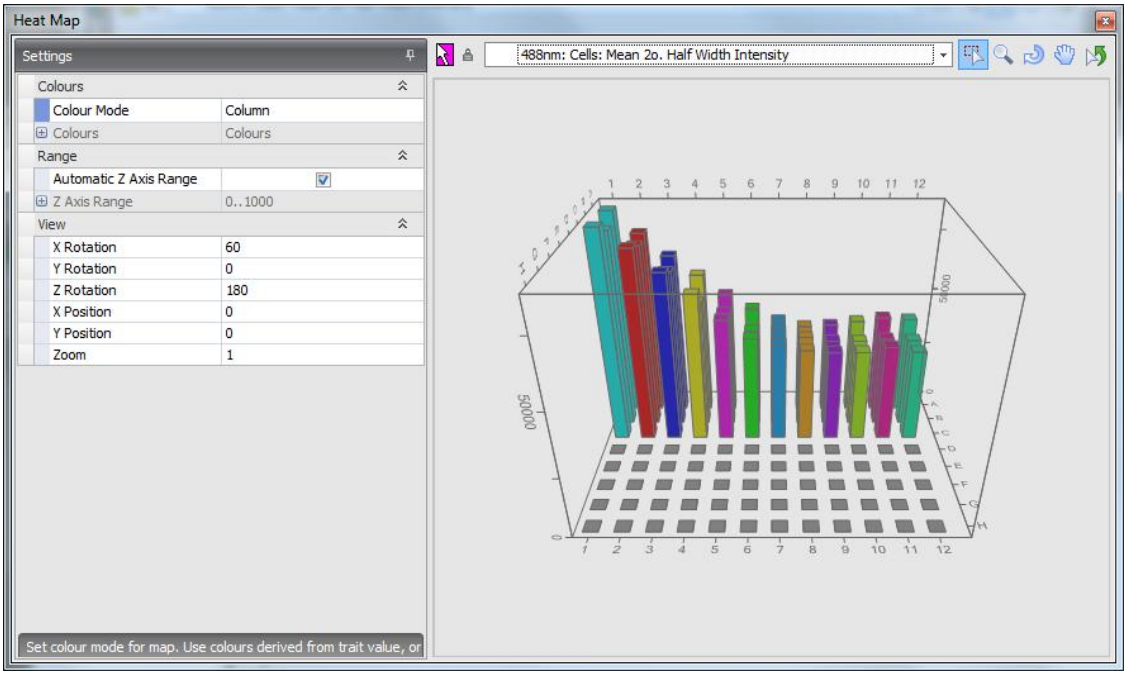
Select **View | Charts | 3D Heat Map**



Different **Population Statistics** can be viewed by selecting them from the Drop Down Box. The Heat Map can be also be rotated by clicking on the tool on the toolbar and then moving the mouse around within the main chart body while holding the left mouse button down.

10.2.1.2 3D Heat Map Settings

These are displayed by selecting the settings box on the left hand side of the Head Map window.



Colour Mode	Selects the format for colouring the vertical bars
Colours	Allows formatting of bars according to scan status. Selecting the Population Colour check box colours bars according to their designated population
Automatic Z Axis range	Sets the Z range based on the current data set

10.2.2 Histogram

The histogram chart provides a visual representation of an Object Characteristic for objects in selected wells. It is available for any defined Object Characteristic.

10.2.2.1 Viewing a Histogram

Select **View | Charts | Histogram**

To display the data, you need to link the source data to the histogram.

- Select the required scanned wells.
- Click on the  icon on the **Plate View** window.
- Drag and drop the mouse into the histogram.

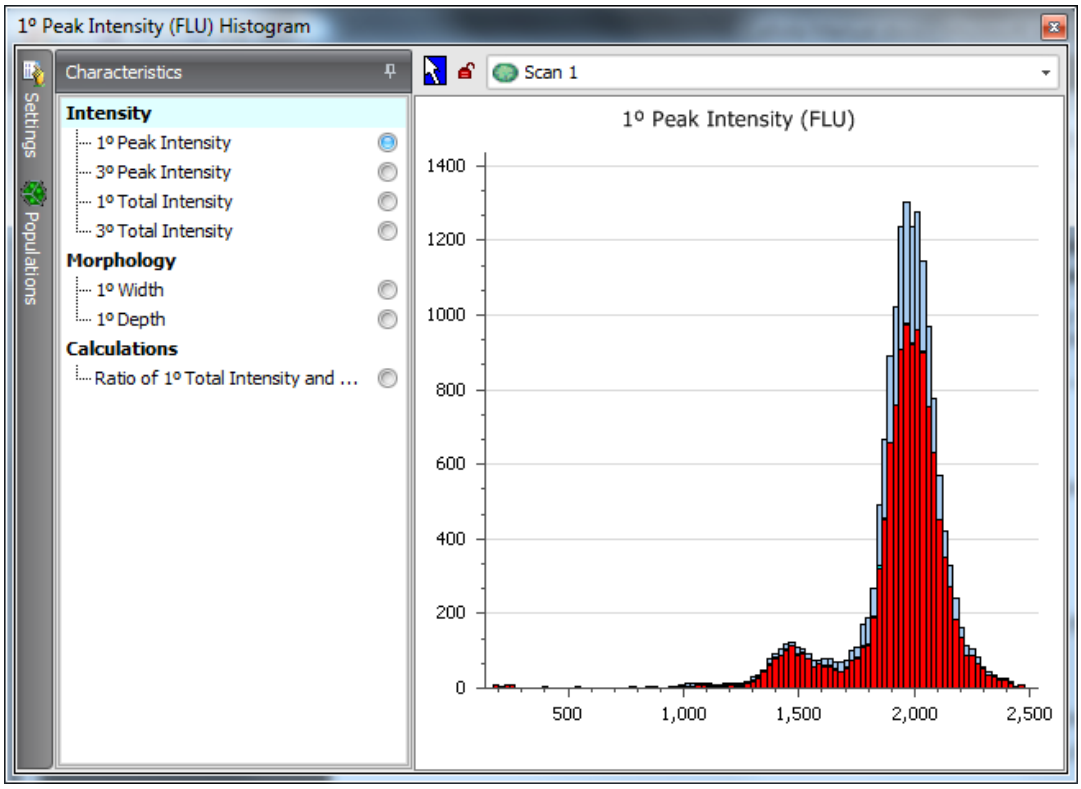
10.2.2.2 Histogram Options

These are displayed by selecting the tabs on the left hand side of the histogram window.

Characteristics	Select the Object Characteristic to be displayed. Note that Object Characteristics must added before they can be viewed. This is displayed by default.
Settings	Changes how the data are plotted. The resolution of the histogram can be altered by changing the number of bars.
Populations	Allows selection of the populations displayed.

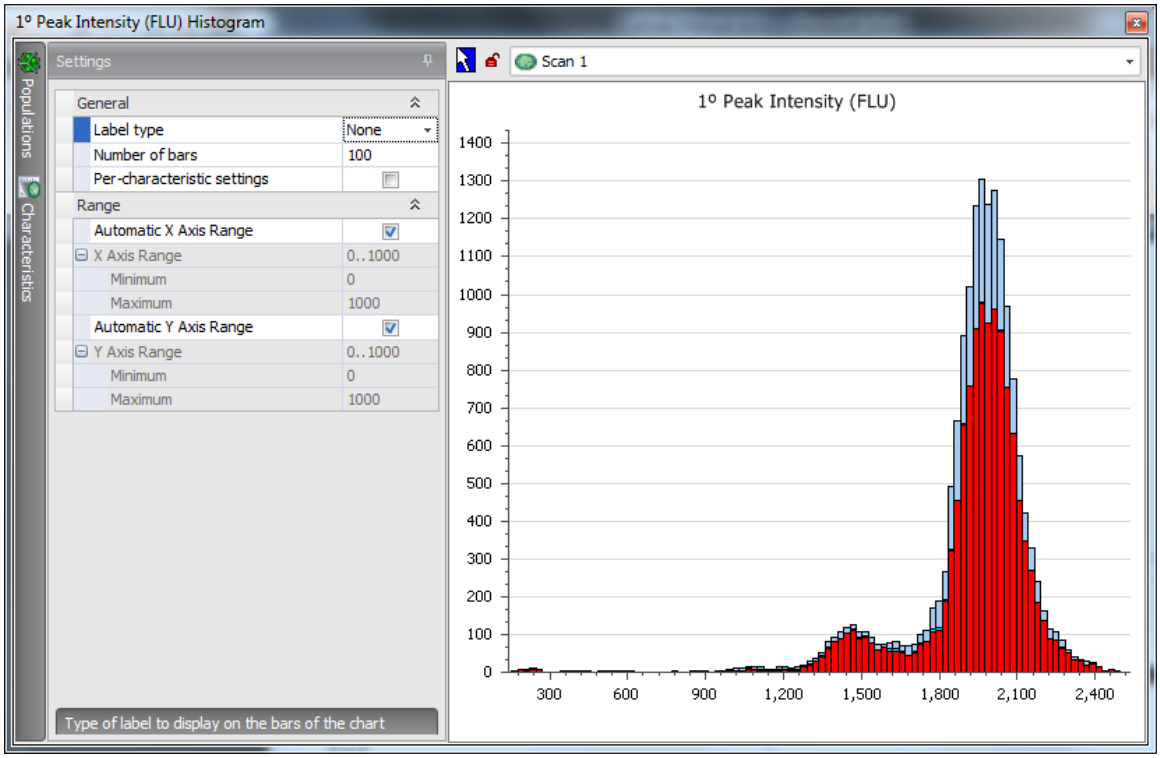
10.2.2.3 Characteristics

By selecting the different radial buttons, the display in the histogram displays that Object Characteristic.



10.2.2.4 Histogram Settings

To Edit the Histogram settings, click the  settings icon. To Lock the view so it doesn't auto-hide, click on the drawing pin icon  .

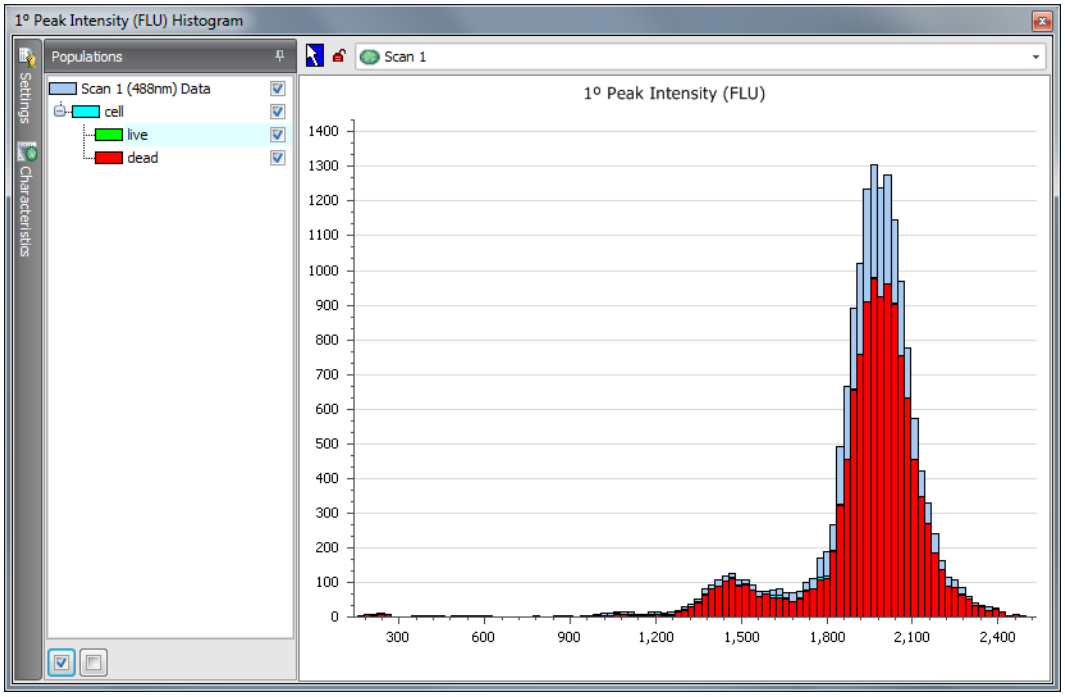


Label Type	Selects the type of label to display on the chart, such as values or frequencies.
Number of bars	Determines the size of buckets for histogram frequencies.
Pre-characteristic settings	Use separate axis settings for each characteristic. A characteristic's axis ranges etc will be restored when swapping between characteristics.
Automatic X Axis Range	Sets the range automatically based on current data set. Can be adjusted by checking the box, then selecting the Minimum and Maximum values.
Automatic Y Axis Range	Sets the range automatically based on current data set. Can be adjusted by checking the box, then selecting the Minimum and Maximum values.

10.2.2.5 Population Settings

To Alter which Populations are displayed on the histogram, click the Populations icon. To Lock the view so it doesn't auto hide, click on the drawing pin icon .

To Select or deselect populations to be shown, check or uncheck the boxes by the population name.



10.2.2.6 Editing Population Definitions Using Histograms

The following options are displayed by clicking the right mouse button on the histogram window.

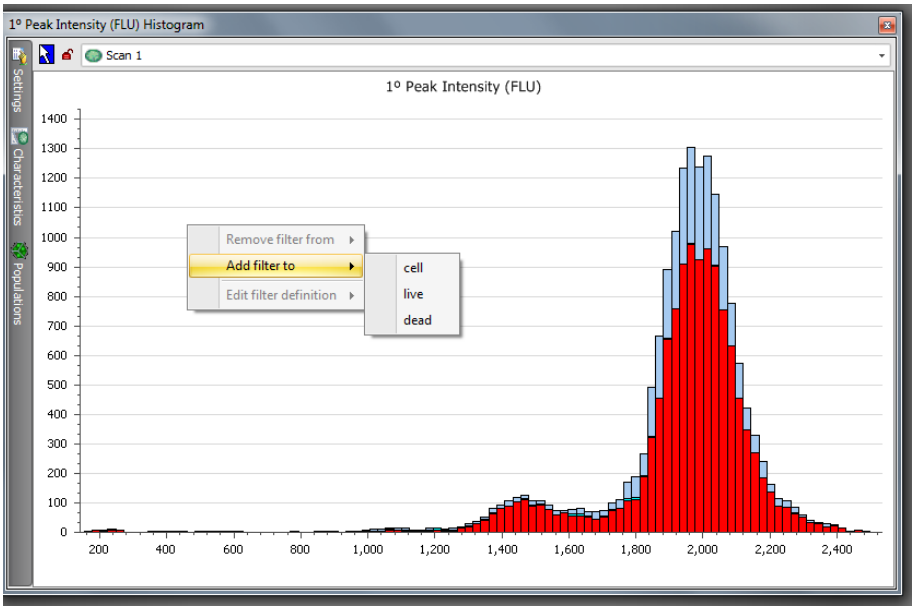
Add filter to	Allows definition of a population using the histogram
Remove filter from	Enables removal of a filter from a population.

10.2.2.7 Adding a Population Filter Using a Histogram

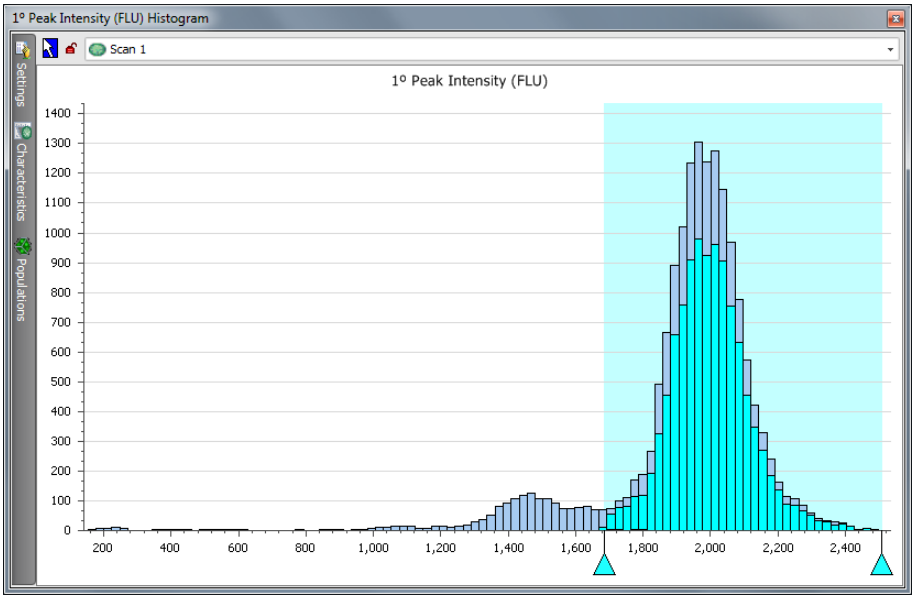
Two adjustable arrow markers can be added to a histogram chart for any population that has already been created in Population Definition. These markers can used to set the minimum and maximum values for the characteristic for a population range.

To add a range to a population for the characteristic displayed in the histogram:

- Right-click on the histogram to access the pop-up menu and select **Add as filter to…**
- The list of available populations to add the filter to will match the populations created by the user.
- Select the population name.



- Arrows appear on the histogram. These are the same colour assigned to the population.
- Use the cursor to drag the arrows to enclose the desired population range.
- Reanalyse the data by selecting the **Reanalyse** button from the toolbar or the **Tools** menu.



NOTE: Altering the filter values using the histogram chart will alter the values shown in the **Population Definition** window.

10.2.2.8 Removing a Population Filter Using Histograms

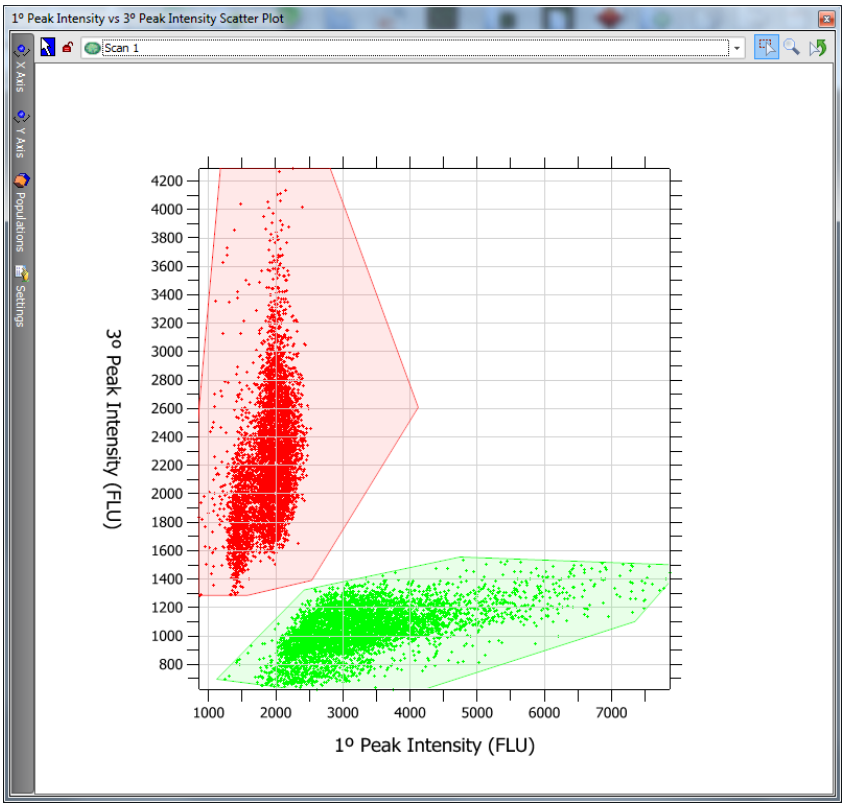
A population filter can be removed using the histogram view. The **Remove Filter From…** menu option removes the two arrow markers (and associated lines) previously added to the histogram chart, for the specified population.

To remove a filter:

- Right-click on the chart to access the pop-up menu and select **Remove Filter From…**
- Choose the population name to remove the filter from.
- Once a filter has been removed, the data must be reanalysed to reflect the changes. Select the **Reanalyse** button (from the toolbar or the **Tools** menu) to re-colour the histogram.

10.2.3 Scatter Plot

A Scatter Plot provides a graphical means of identifying relationships between two **Object Characteristics**.



10.2.3.1 Viewing a Scatter Plot

Select **View | Charts | Scatter Plot**

To display the data, you need to link the source data to the scatter plot.

- Select the required scanned wells
- Click on the  icon on the **Plate View**.
- Drag and drop the mouse into the scatter plot

10.2.3.2 Scatter Plot Options

These are displayed by selecting the tabs on the left hand side of the scatter plot window.

X Axis	Select the Object Characteristic displayed on the X axis
Y Axis	Select the Object Characteristic displayed on the Y axis.
Settings	Allows the range of each axis to set manually
Populations	Allows selection of the populations displayed

These are displayed by clicking the right mouse button on the scatter plot window.

Remove selected filter	Removes a selected filter for a population
Remove filter	Remove a filter from a population without prior selection
Add filter	Allows definition of a population using the scatter plot
Edit filter definition	Provides a link to the Populations window to allow manual definition of a population

10.2.3.3 Defining Population Filters on a Scatter Plot

A scatter chart may be used to set a population filter directly, for any population that has already been created in **Population Definition**. To do this:

- Click the right mouse button on the scatter plot window.
- Select Add filter.
- Select Population.
- Select Type of Filter.
- Position the filter over the target population using the object holders.

If certain types of filter are not displayed, it is because one or both of the **Object Characteristics** shown on the X- or Y- axis are already in use in another filter definition.

10.2.3.3.1 Types of filter

Range (X)	The **Object Characteristic** displayed on the X axis
Range (Y)	The **Object Characteristic** displayed on the X axis.
Draw polygon	Allows freehand drawing of polygon by pressing and holding down left hand mouse button. Note start and end positions must be the same.
Ellipse	Draws an ellipse.
Splitter	Defines population based on straight line ($y = mx + c$). The population may be defines as being above or below the line (signified by shading in population colour). The objects NOT contained in the population are termed Remainder and can be selected in the Population window
Rectangle	Draw a rectangle comprising ranges in both X and Y axes.

10.2.3.4 Editing a Filter Using Scatter Charts

To edit a population filter using a scatter chart:

- Open the scatter chart and click on the line/ellipse, polygon, whichever type of filter was selected. Then use the yellow dots to change the position, or boundaries.
- Press the Reanalyse Button

10.2.3.5 Removing a Filter Using Scatter Charts

To remove a population filter using a scatter chart:

- Right click and select **Remove Filter**.
- Choose the population name to remove the filter from.
- Press the Reanalyse Button

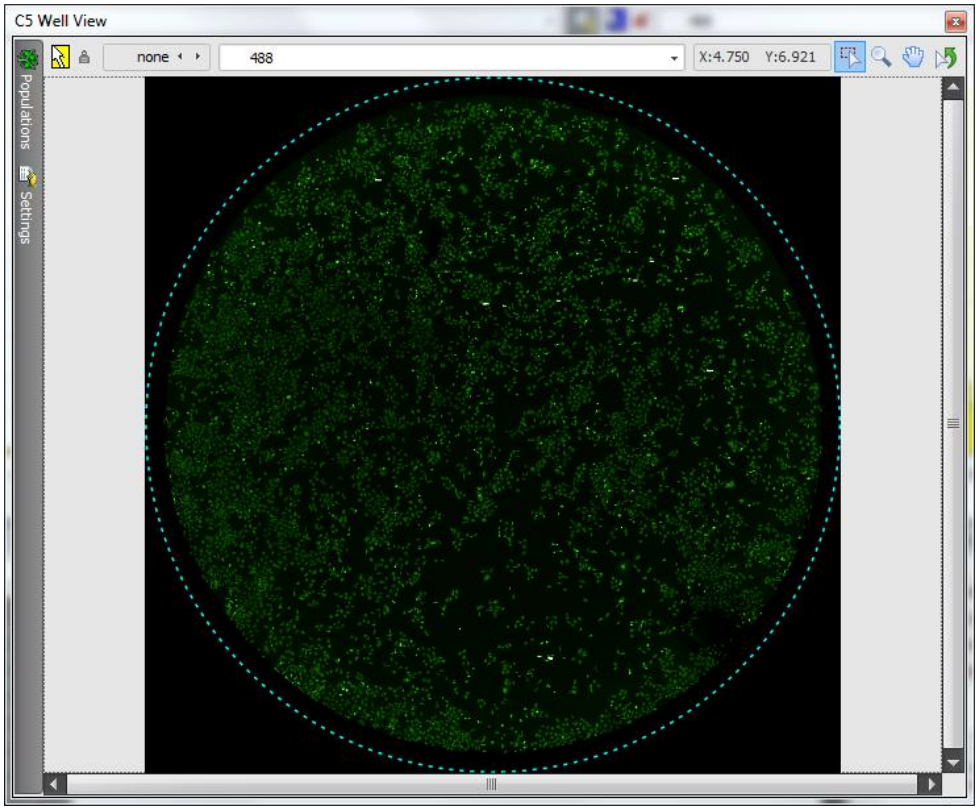
10.3 Well Group

10.3.1 Well

Displays a visual representation of all the objects in the selected well. It gives the user a visual link to the data.

To open a Well View, either double click a well on the **Plate View**, or go to: **View | Well | Well**

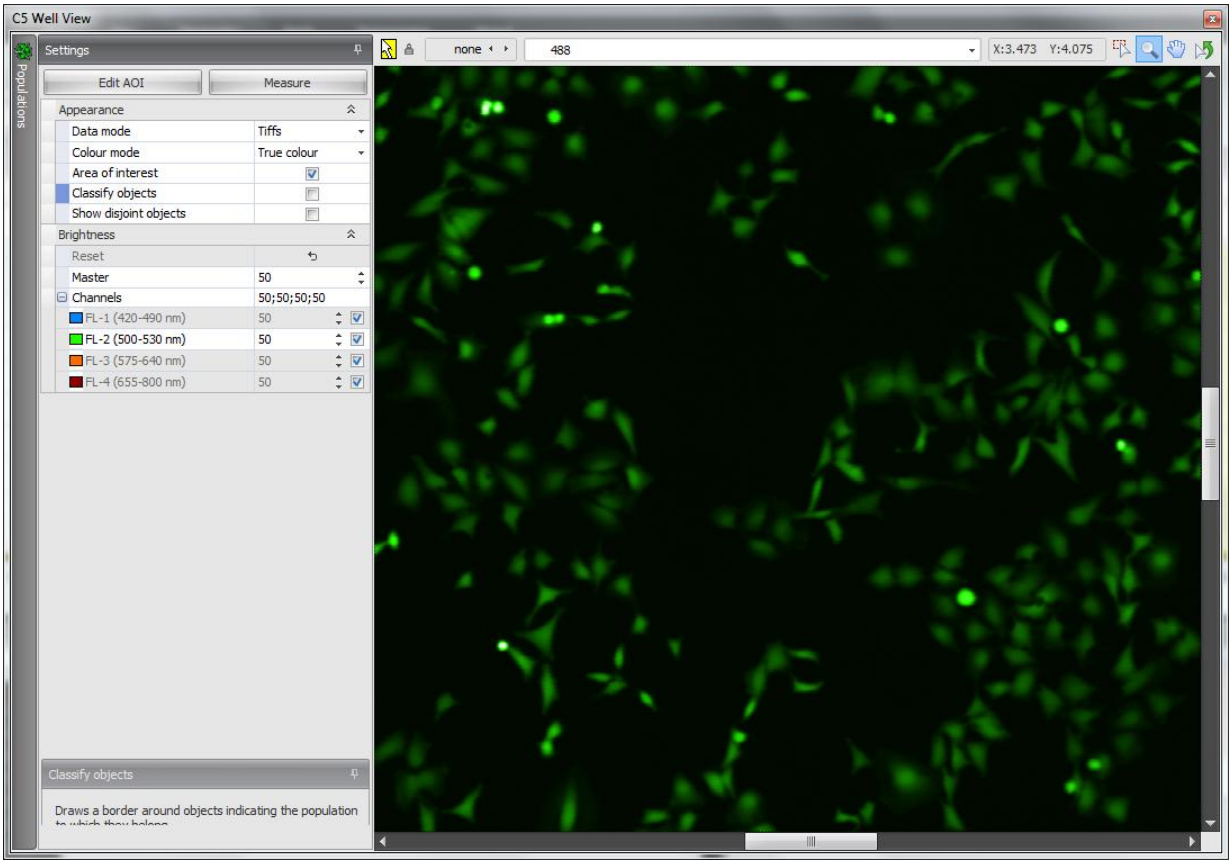
A **Well View** then opens



To zoom into the well view, click on the  tool and either left click to zoom in, and right click to zoom out, or draw an area of interest to zoom in to that area. To navigate around the **Well View**, select the  tool.

10.3.1.1 Well View Settings

To change what is being displayed, click the  settings icon. To Lock the view so it doesn't auto hide, click on the drawing pin icon .



Data Mode	Selects whether to display the raw data, the threshold objects, or both. This is only available when the corresponding TIFF files are in the plate's output file list (see section 7.5.3 to see how to enable TIFF files)
Colour Mode	Determines whether objects are shown using the solid colour of the population to which they belong or their true colour. 'Enhanced' draws objects in the population colour but uses the actual intensity information rather than a solid colour.
Area of Interest	Shows the bounds of the area of interest.
Classify Spots	Draws a border around objects indicating the population to which they belong.
Brightness	Overall image brightness, applied to all channels in addition to channel brightness.
Channel Brightness	The brightness of the individual channels to be applied in addition to the overall brightness. Individual channels can be turned off completely via the check boxes.

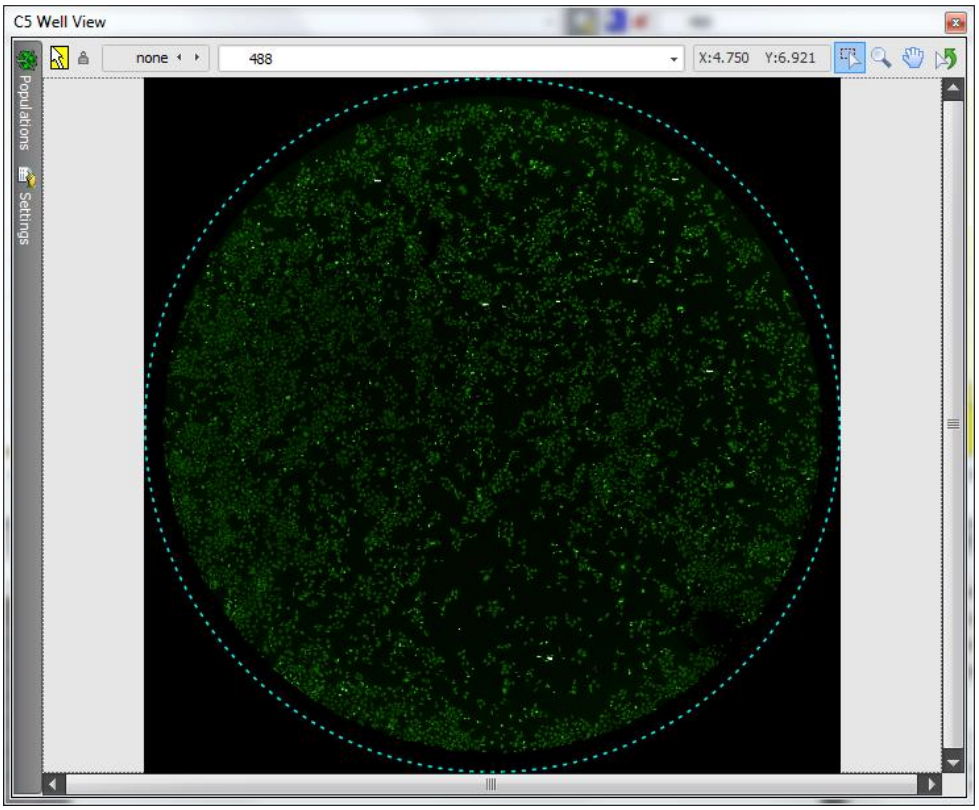
10.3.1.2 Well Appearance

Once filters assigned to populations are set up, further analysis is required to ensure that the filters' definitions are exact.

Population filters can be verified visually by using the **Well View**, which allows the user to view a pseudo-image of the contents of a well and determine how populations have been classified:

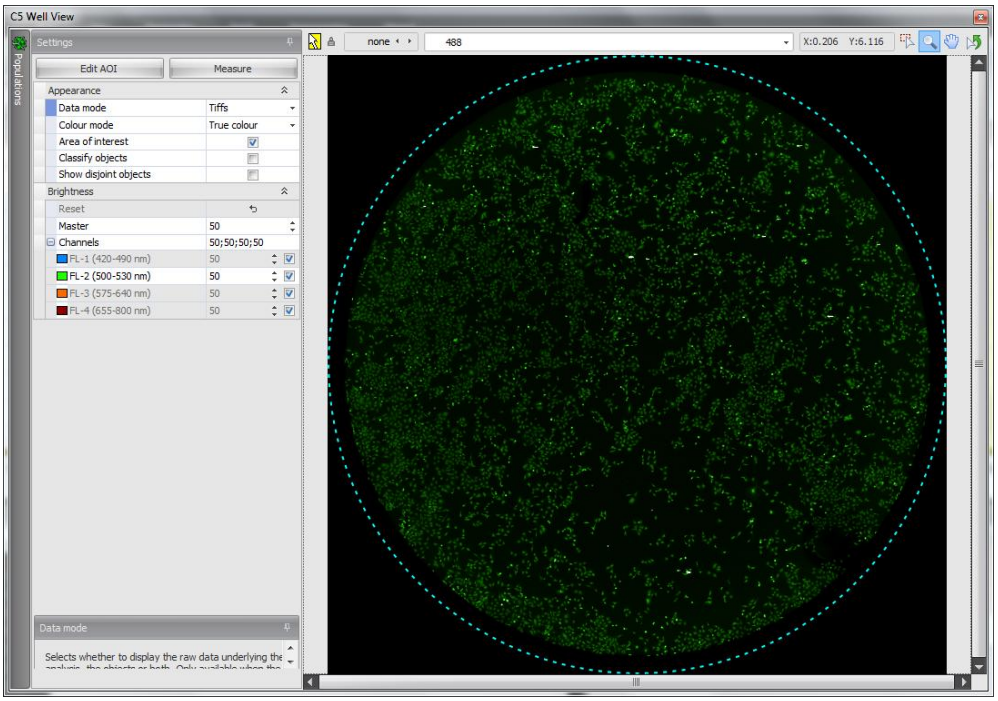
To open a well view, double click on a well in **Plate View.**

A **Well View** opens as shown below.

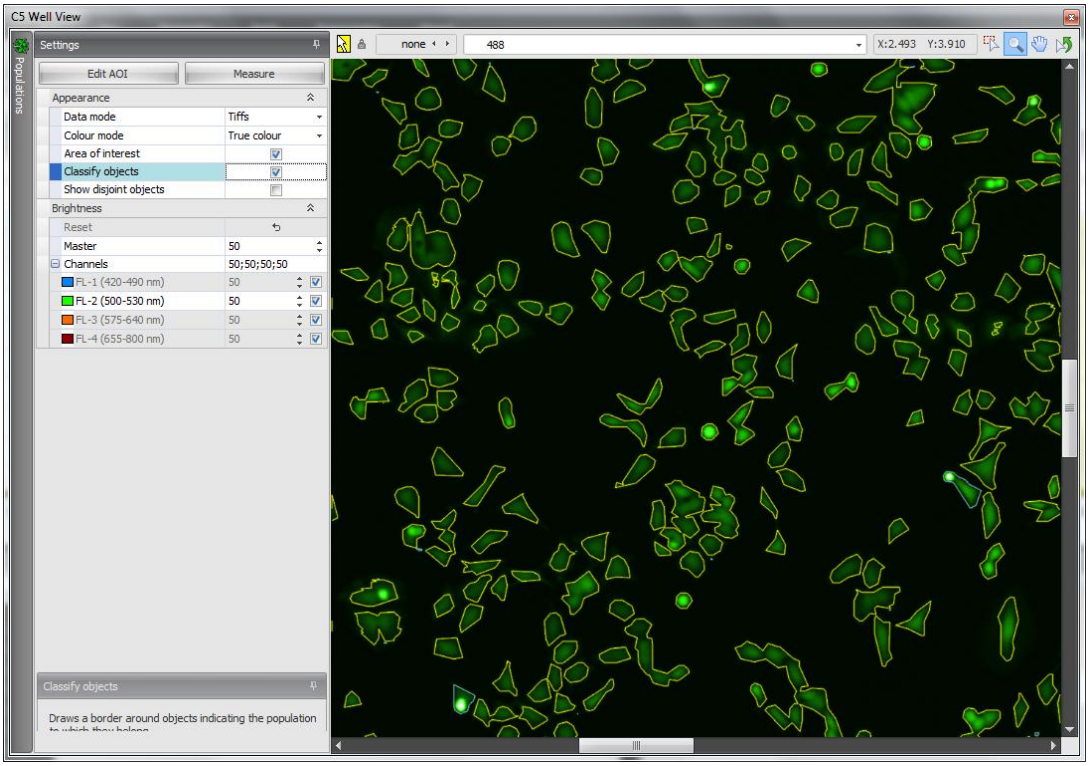


Ensure you are looking at the scan from the correct laser. If not, this can be changed by clicking on the name box button.

- In Data Mode, ensure iffs is selected. For this option to be available, TIFF files must be exported at the point of scanning. See section 7.5.3 for more information.

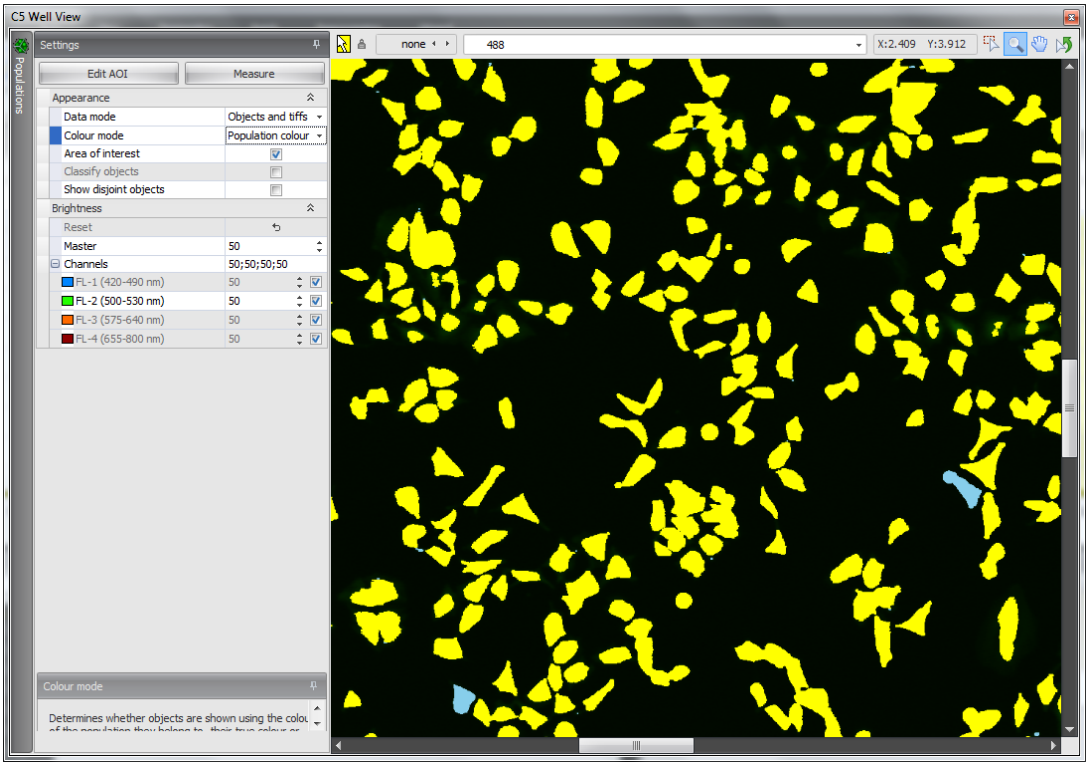


- Check the Classify Objects Box. Each object will be outlined in the colour allocated to the population to which it has been classified.



The objects in the image file are classified and an outline is drawn around the objects that fall within the set **Population Definition**.

- To View a mask of the objects, in Data Mode, select Objects and Tiffs, then Colour Mode, change this from **True Colour** to **Population Colour**, and a mask is placed on the cells. The colour corresponds to the population definition colour in the population definition - see section 8.1.2.To view the thresholded data, change th**e Data mode** t**o Object data** and the objects that are above the set threshold are displayed.

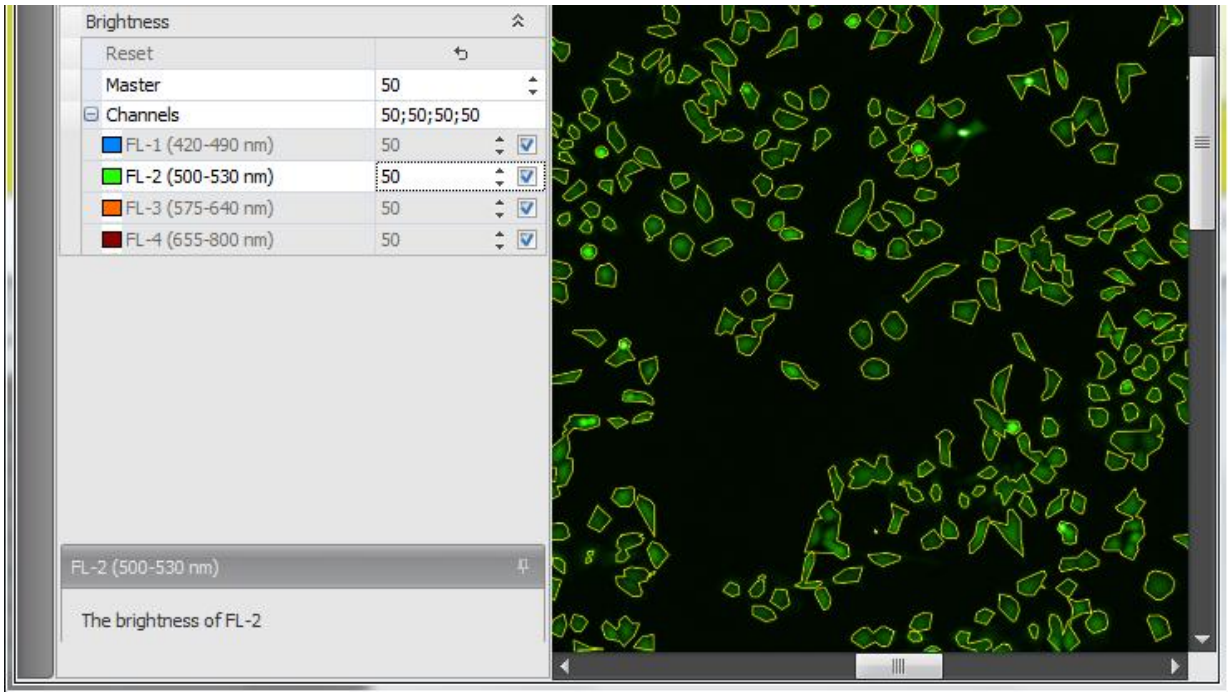


10.3.1.3 Adjusting Individual Channel Brightness in Well View

In **Well View**, there is the option to obtain well views for each channel that is enabled. The default is that all enabled channels are turned on. By sequentially reducing the number from 100% and down, the brightness is adjusted in that channel.

In Settings, channels are turned on and off by selecting/deselecting the checkbox.

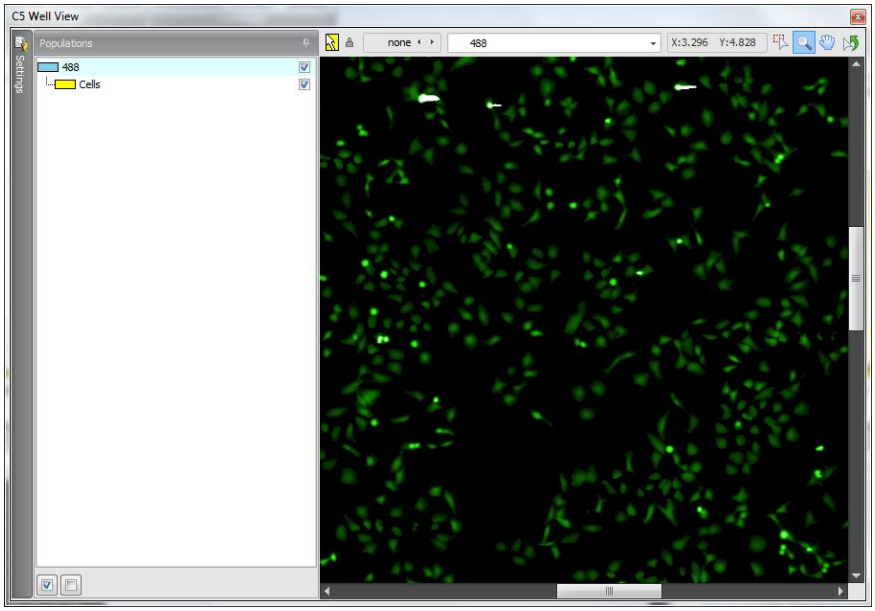
To adjust the brightness, adjust either the global brightness or the individual channel brightness using the up and down arrows, or typing a number as shown below.

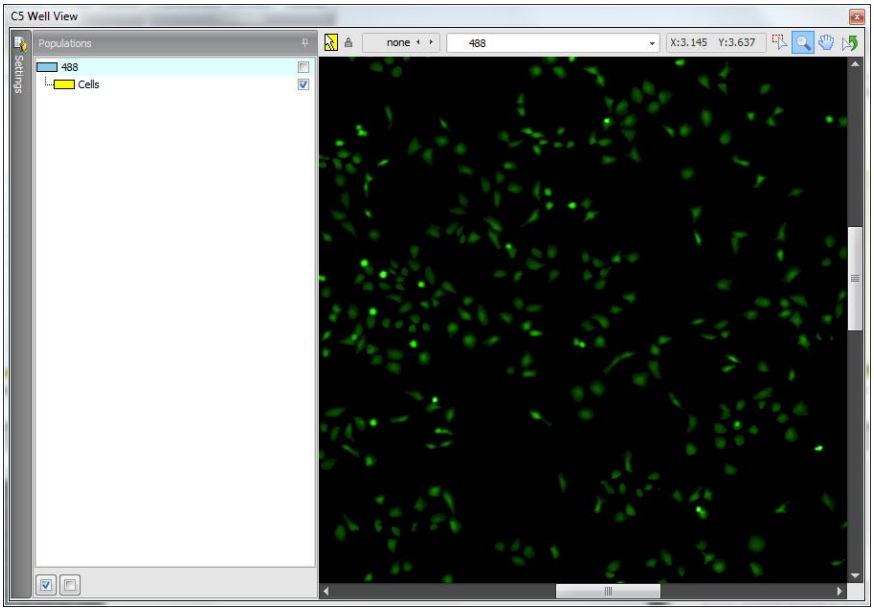


10.3.1.4 Viewing Distinct Populations in Well View

Once cells have been defined into Populations using **Population Definitions** distinct populations can be viewed individually when viewing Object data.

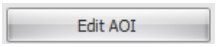
- To alter which Populations are displayed on the Well View, click the  Populations icon. Select the population you wish to view. The example below shows the same well view with different cell populations being displayed



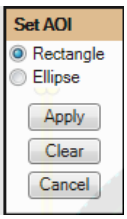


10.3.1.5 Setting Area of Interest in Well View

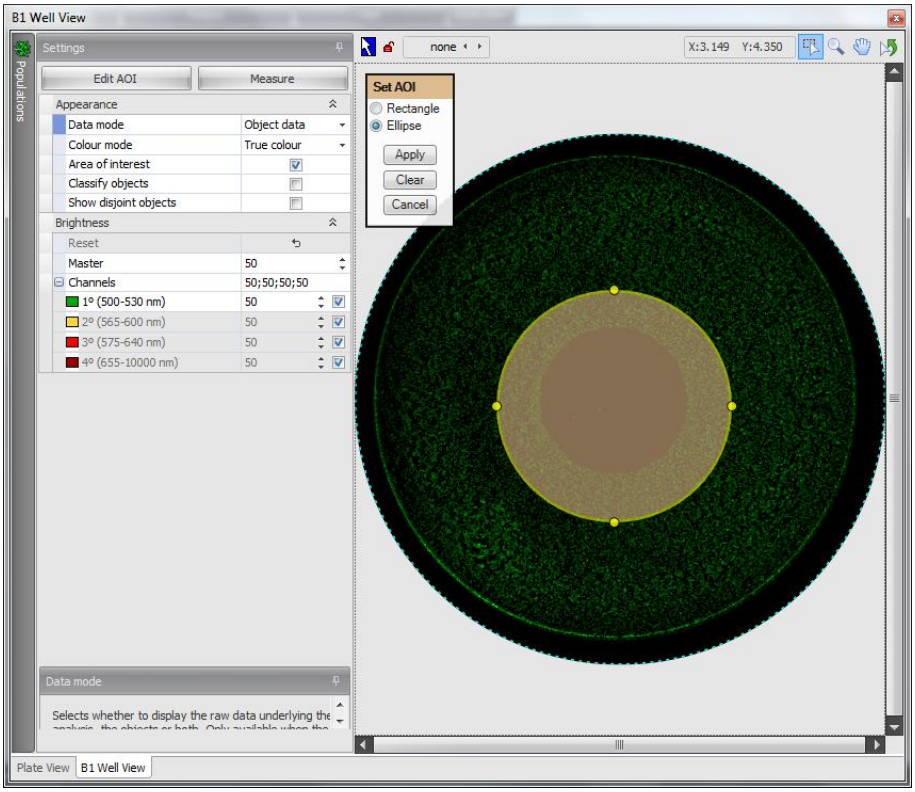
Pick a well for scanning and open up a well view.

In well view, click on the **Settings Tab** then at the top, click on 

. The following box appears:



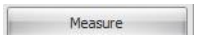
Select either a Rectangular or Elliptical shape to draw the area to be to rescanned. Draw the shape on the Well View:



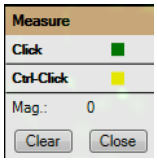
Once the correct area is selected, click on Apply. The next time the plate is scanned; this new Area of Interest will be used as the Scan Area.

10.3.1.6 Measure Size in Well View

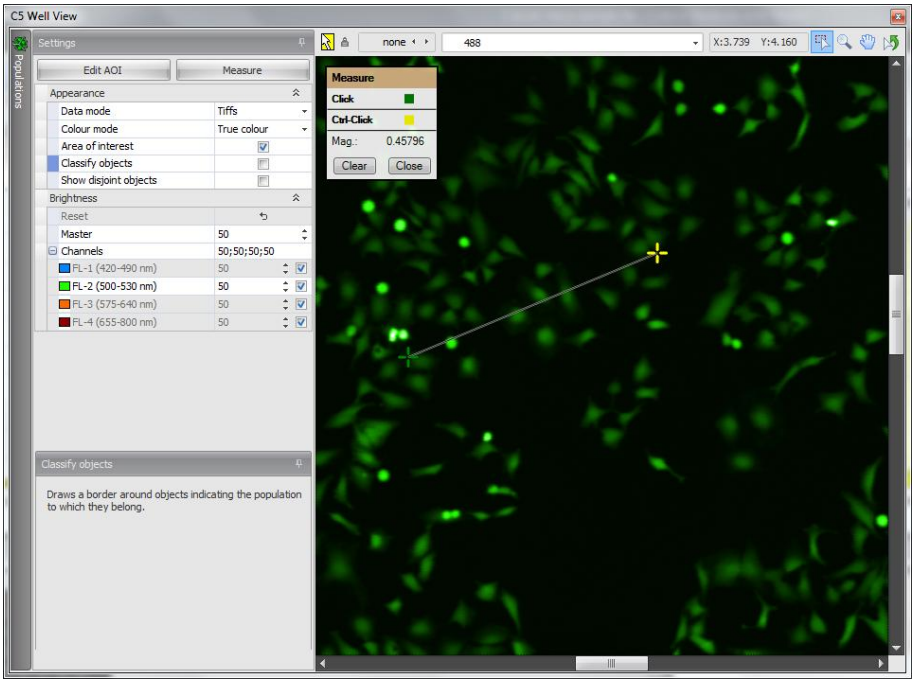
The measure feature is a function that can measure the length or width or a object, or the distance between two distinct points in the **Well View**.

Open up **Well View**, and select **Settings**, then click on the  button.

The following window opens up inside **Well View**:

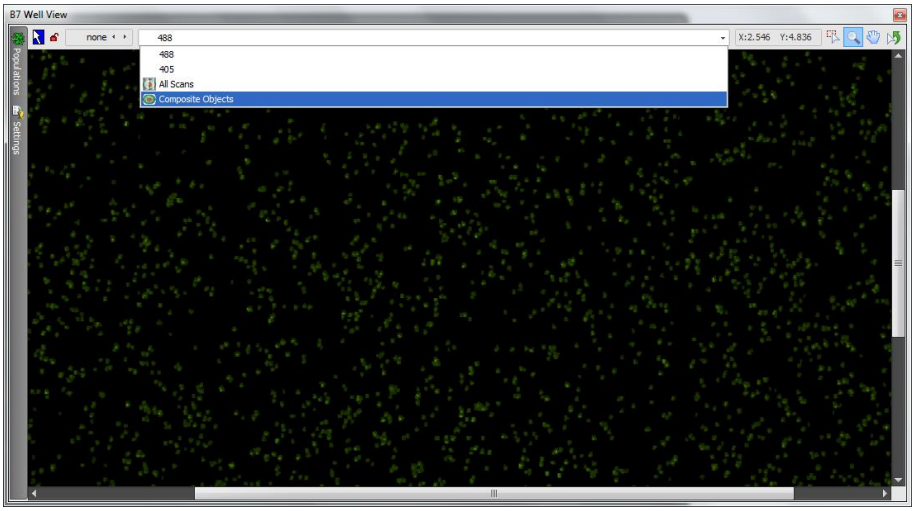


Click with the mouse to set the starting point, then hold the Ctrl key on the keyboard and that will set the destination point. The **Measure** box then automatically says the length of the line in millimetres, in the case below, 0.45796mm or 457.96µm



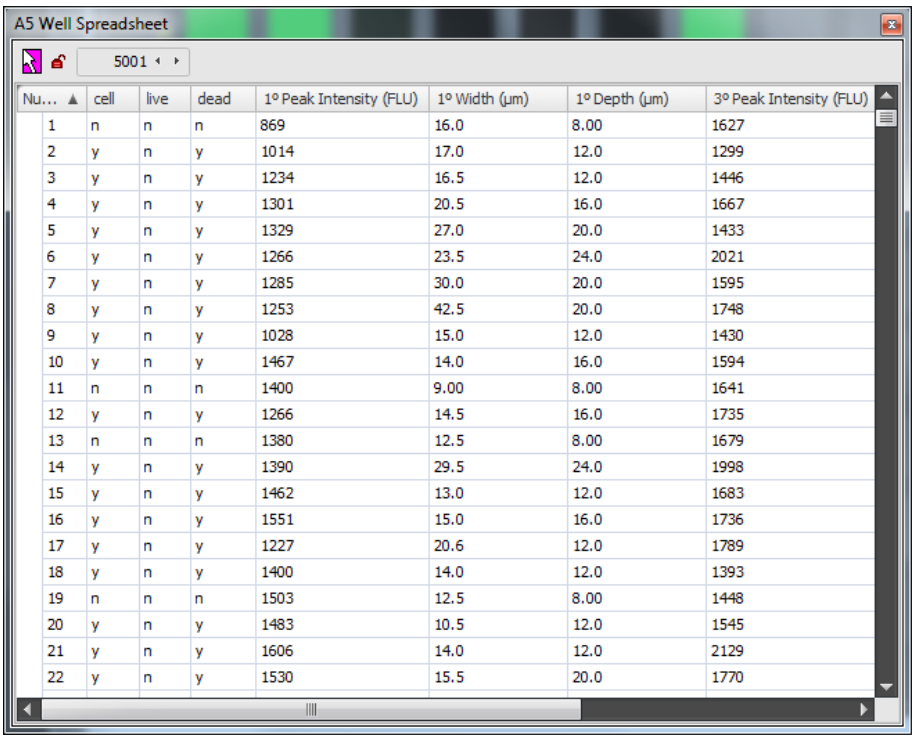
10.3.1.7 Viewing Composite Objects (acumen only)

If the plate has been scanned in Composite Mode, then to view the composite data in the name box of the Well View, select Composite Objects as shown below:



10.3.2 Spreadsheet

This displays a spreadsheet of the all the data in the current well. It details every object in the well and identifies which Population they are within and also gives that object's value for each of the **Object Characteristics** set.



Nu...	cell	live	dead	1º Peak Intensity (FLU)	1º Width (µm)	1º Depth (µm)	3º Peak Intensity (FLU)
1	n	n	n	869	16.0	8.00	1627
2	y	n	y	1014	17.0	12.0	1299
3	y	n	y	1234	16.5	12.0	1446
4	y	n	y	1301	20.5	16.0	1667
5	y	n	y	1329	27.0	20.0	1433
6	y	n	y	1266	23.5	24.0	2021
7	y	n	y	1285	30.0	20.0	1595
8	y	n	y	1253	42.5	20.0	1748
9	y	n	y	1028	15.0	12.0	1430
10	y	n	y	1467	14.0	16.0	1594
11	n	n	n	1400	9.00	8.00	1641
12	y	n	y	1266	14.5	16.0	1735
13	n	n	n	1380	12.5	8.00	1679
14	y	n	y	1390	29.5	24.0	1998
15	y	n	y	1462	13.0	12.0	1683
16	y	n	y	1551	15.0	16.0	1736
17	y	n	y	1227	20.6	12.0	1789
18	y	n	y	1400	14.0	12.0	1393
19	n	n	n	1503	12.5	8.00	1448
20	y	n	y	1483	10.5	12.0	1545
21	y	n	y	1606	14.0	12.0	2129
22	y	n	y	1530	15.5	20.0	1770

10.3.3 Results

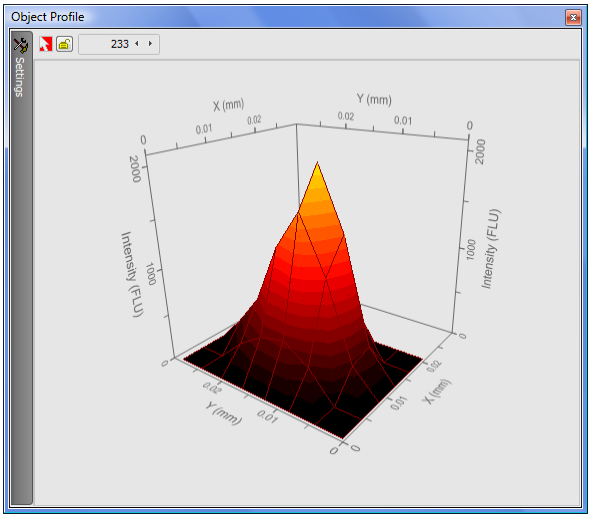
Displays the statistics and population membership results for a well.

10.4 Object Group

10.4.1 Profile

Displays a 3D intensity plot of the selected object. The 3D profile of an object can be used to differentiate beads/cells from irrelevant objects such as cell fragments or scan artefacts.

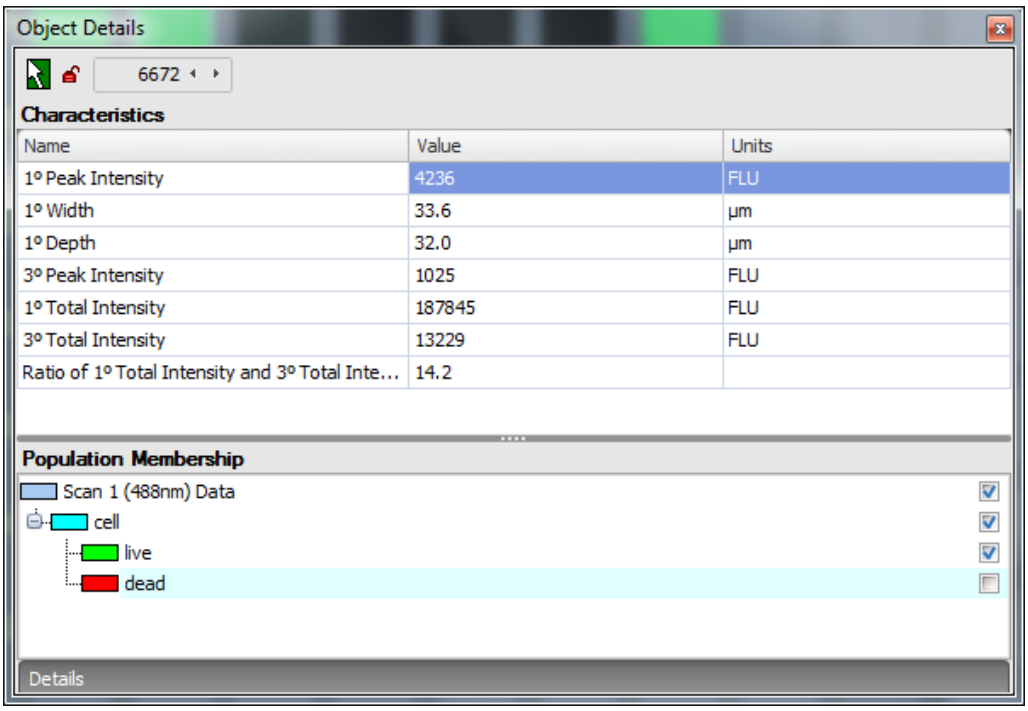
To view an object in 3D, in **Well View**, select an object with the left mouse button – a blue border will be displayed around the selected object. Right mouse click over the selected object and select **Object Profile**. Alternatively open the window in **View | Object | Profile**



Select data channels by clicking the **Settings** tab and selecting from the drop-down list. The 3D profile can be rotated by selecting the **Rotate** icon on the toolbar and holding down the left mouse button.

10.4.2 Details

Displays the characteristic and population membership details of a selected object.



10.5 Logs Group

10.5.1 Output Files

View the automatically generated output files from the current plate. This view is also used to reattach tiff files which have become separated from the current CData file.

10.5.1.1 Tiff file locations

Tiff files generated during a scan are normally stored in a subfolder relative to the CData file but if this file is moved outside of Cellista – e.g. with Windows Explorer – then the paths to the relevant tiffs stored in this list may no longer be correct. Cellista will attempt to locate the tiffs files when any given CData file is opened but it may not always be able to do so. In this case, select all of the tiff files in the Output Files view and press the delete button ✕. This removes the files from the list but does not remove them from disk. Then press the add button + and select the relevant files in the dialog which appears. Note that you can select multiple files at once.

If the current data file is unsaved then the tiff files will normally be saved in a subfolder of the user's 'My Documents' folder. They will be moved when the data file is saved.

It is possible – but not recommended – to have the tiff files saved in the same folder as the CData file by deselecting **Scan Setup | Output Options | Tiff | Separate sub folder**.

Note that when you delete the data from a well Cellista will ask if you wish to delete any corresponding tiff files too. If you delete the data but elect to keep the tiff then when that well is rescanned, the tiff file *may be overwritten* in some circumstances.

10.5.2 Plate

Shows events which occurred during the scanning of the current plate file. This includes any significant warnings of events which may have lead to the data in the file being compromised, for instance if the user has elected to scan before the lasers are fully warmed up.

10.5.3 System

Displays the system log. Any informational, warning or errors messages generated during the operation of the system in the current session will appear here.

These messages also appear as popup notifications. They are categorised so:

	Informational message only. The popup will vanish after a short period. Any number of informational popups may be displayed at once.
	Warning message which may affect results or data quality. The popup will vanish after a longer period. If more than one warning occurs only the first will be displayed in a popup; the remainder will appear in the system log.
	An error which prevents system operation. The popup will not vanish until the user clicks the close button [X] on the alert. If more than one warning occurs only the first will be displayed. If more than one error occurs only the first will be displayed in a popup; the remainder will appear in the system log. In the event of an error please make a note of the time and date of the and contact TTP LabTech support for assistance. You may attempt to resume operation by pressing the reset  button.

The most recent warning or error will also be displayed in the status bar at the bottom of the screen. Messages describing the current state of the system and whether it is possible to run the current assay or batch are also displayed in the status bar.

11 DIAGNOSTICS

11.1 User Group

11.1.1 Logon

Logon as a different user, e.g. to acquire factory permissions.

11.1.2 Logoff

Reverts to the permissions of the user logged onto Windows. Note that if the current Windows users has greater privileges than the user logged on to Cellista then logging off will actually increase the privileges available to the current Cellista user.

11.2 Instrument Group

11.2.1 Plate Definitions

acumen can support most SBS plates (that is 96, 384 or 1536). **mirrorball** can support certain SBS plates (that is 96, 384 or 1536).

Plates must be:

- Flat, clear bottomed
- Of good optical quality

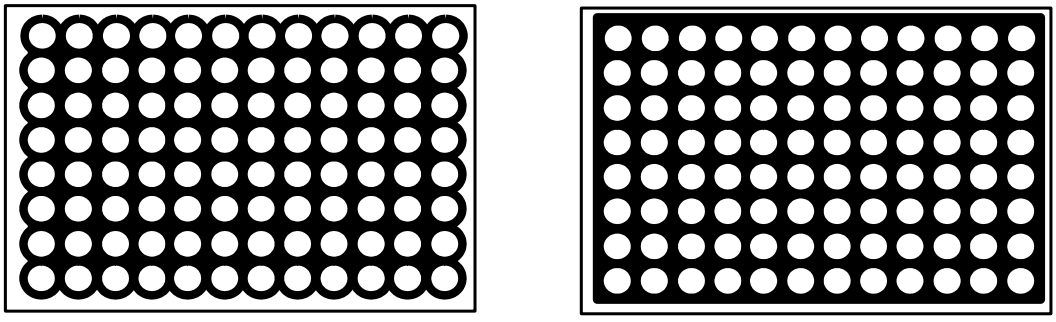
Preferably, plates should be black-walled. Clear walled plates can increase the light scatter and result in non-optimal scans.

Both acumen and mirrorball must have accurate information about the plate type in use in order to produce reliable scan data.

Do not change the information contained within the Plate Type information without first consulting **acumen**support@ttplabtech.com or mirrorballsupport@ttplabtech.com as any damage that is made to the instrument is not covered by the warranty or service contract

11.2.1.1 Recommended Plate Types

Cellista software comes with pre-installed settings for plate types that are compatible with the instrument (**acumen** or **mirrorball**). Some plate types are incompatible for use. A common example is shown below, looking at the base of the plate. The plate on the left can be scanned by the **acumen** , but the outer wells cannot be scanned, so it is advisable to use plates of the type which are shown on the right hand side.



PLEASE NOTE: If you wish to scan any other format of plate, or change the information contained within the Plate Definitions in the cellista software, first consult acumensupport@ttplabtech.com or mirrorballsupport@ttplabtech.com as any damage that is made to the instrument is not covered by the warranty or service contract. Note in particular that:

- Clear-walled plates do not work well with the Orbitor robotic plate loader. Additionally, such plate types do not always work well with either **acumen** or **mirrorball** due to the internal reflection of laser light.
- **mirrorball** is particularly sensitive to correct measurements.

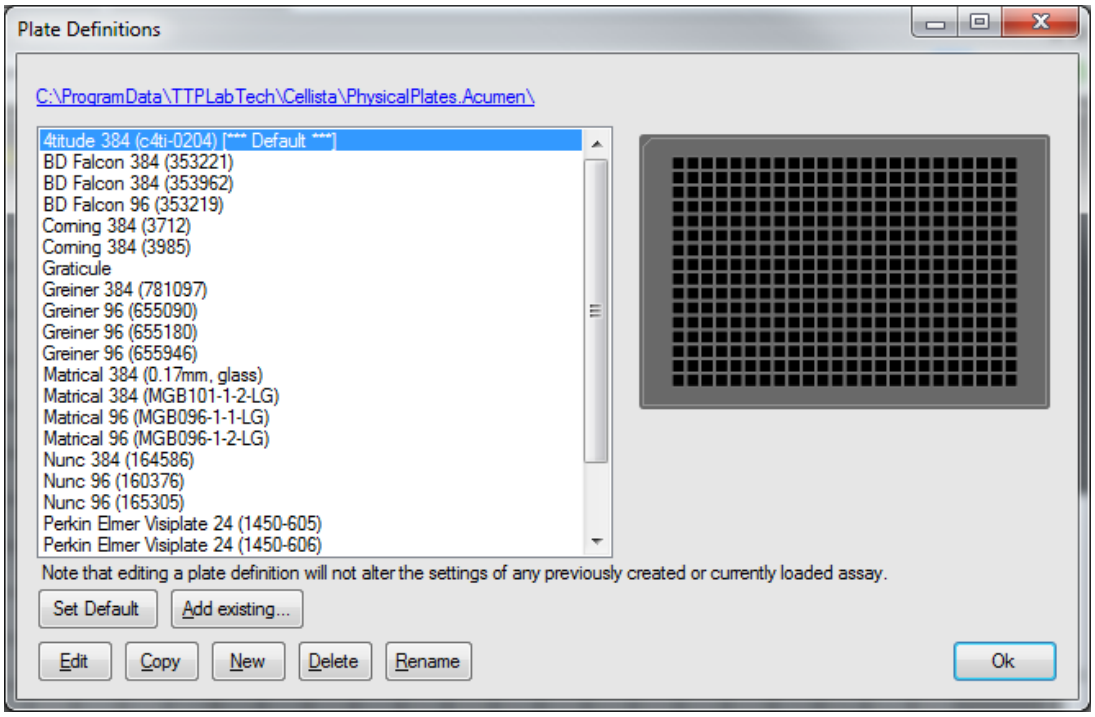
11.2.1.2 Defining Plates

This can only be done for acumen. It is not possible to define your own plates in mirrorball. Any attempt to do so, may result in serious damage to the instrument. Please contact mirrorball support if you wish to use a plate type nit surrently in the list.

Cellista software provides a Plate Definitions dialogue box to manage plate type information so that:

- New plate types can be set up for use on the system
- Current plate types can be edited or deleted

The menu can be accessed by: **Diagnostics** | **Instrument | Plate Definitions,** the following dialogue box opens:

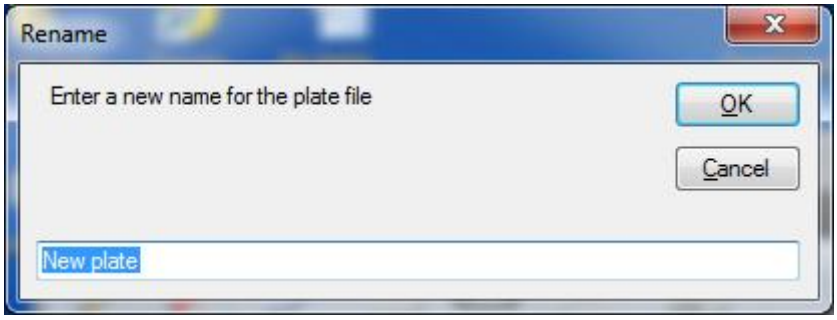


To set the default plate type, highlight the desired plate in the list and click on the “Set Default” button. This will now be the default plate type that is used when setting up a brand new scan.

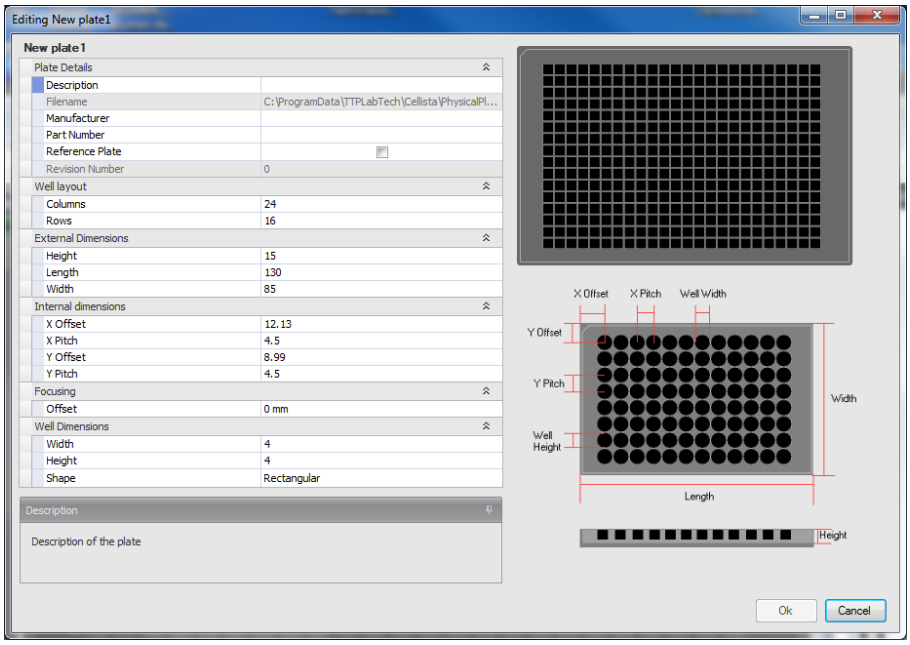
11.2.1.3 Adding a New Plate Type

For full details on how to set up and focus a new plate type on acumen, please refer to Appendix E: Setting up a New Plate Type.

- Select **Diagnostics | Instrument | Plate Definitions**
- Press the New button and give the new plate a name when the Rename box opens



- Fill in the plate type and all the fields with appropriate values for the new plate. Please note focus position is very important, and needs to be determined on a plate by plate basis. Please contact **acumen** or **mirrorball** support before changing this number.



- Press the OK button when finished to close the Edit window and add the plate to the Plates Definitions box.
- Press OK to close the Plates window.
- The new plate type will now be available from the drop down list available here: **Scan Setup | Dimensions | Plate**

11.2.1.4 Editing Plate Details

To alter plate details:

- Select **Diagnostics | Instrument | Plate Definitions**.
- Click on the plate to be edited and press **Edit**.
- Alter the fields as appropriate. Please note focus position is very important, and needs to be determined on a plate by plate basis. Please contact **acumen** or **mirrorball** support before changing this number.
- Press **OK** to close the **Edit** window.

Editing a plate definition will not affect any existing templates or data files. The original plate definition is stored in those files. Any such files can be altered to use the modified definition by selecting **Scan Setup | Plate** and pressing the **Reset** button.

11.2.1.5 Deleting a Plate Type

To remove a plate type from the list available to setup a scan:

- Select **Diagnostics | Instrument | Plate Definitions**.
- Highlight the plate type to remove and press the **Delete** button.
- OK the confirmation message.
- Press OK to close the window.

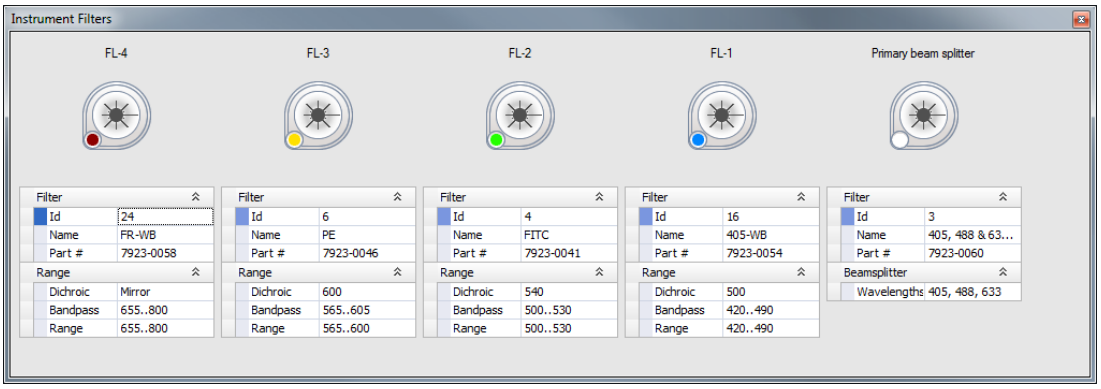
The deletion of a plate type from this list will not affect any existing templates or data files.

11.3 acumen Group

11.3.1 Filters

Displays the filters currently in the instrument. See also section 7.4.3.2.

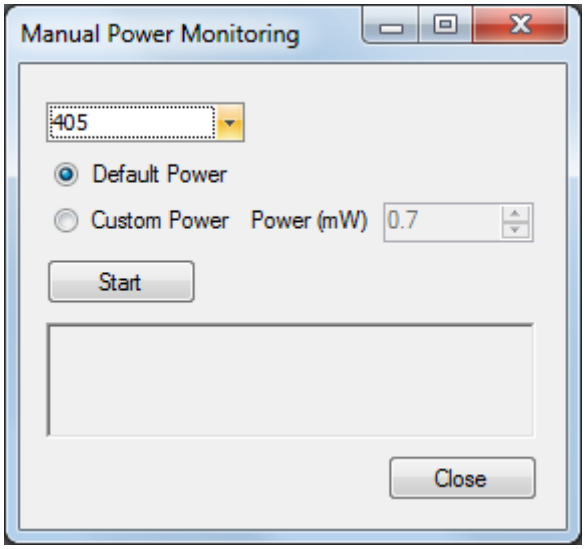
Select **Diagnostics | Acumen | Filters**



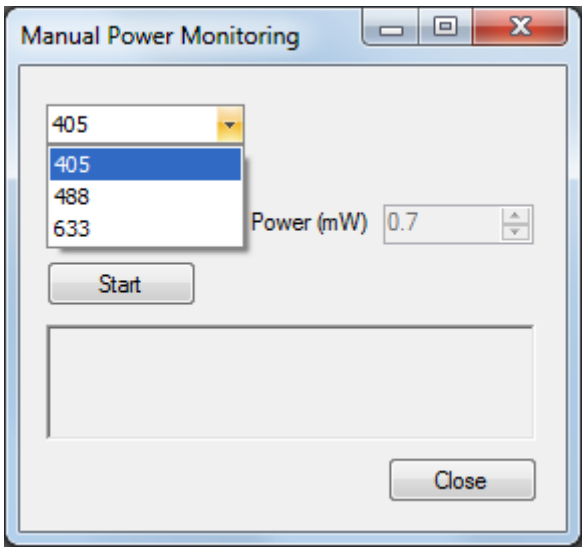
11.3.2 Laser Power Check

Manually check the power of one or more lasers.

Select **Diagnostics | acumen | Laser Power Check** the following box opens:



Use the laser drop down box to select which laser which is to be checked. Then click on Start:



The results of the laser power check will be displayed in the box below.

12 BATCH TAB

12.1 File Group

These functions are used to begin, load, save or close batch jobs which use a plate loader/stacker supplied by TTP Labtech to allow automated scanning of multiple plates unattended.

12.1.1 New

Opens dialogue box to begin defining a new batch scan.

12.1.2 Load

Load a pre-saved batch scan

12.1.3 Save

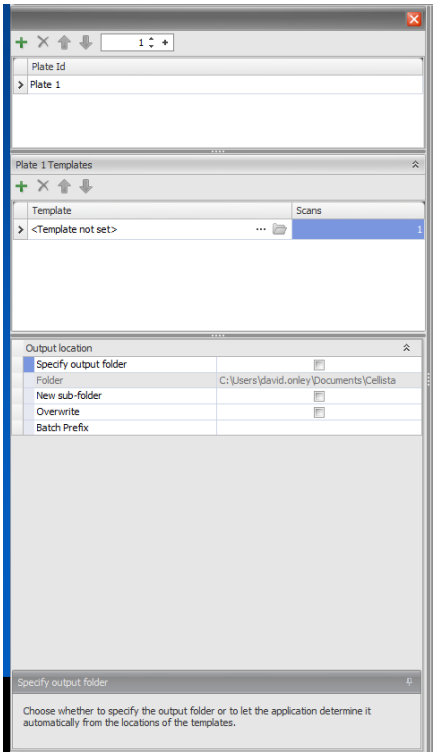
Save the batch scan.

12.1.4 Close

Close the batch scan.

12.2 Setting Up a New Batch Scan

- Click on the New button () to open the dialogue box shown below.

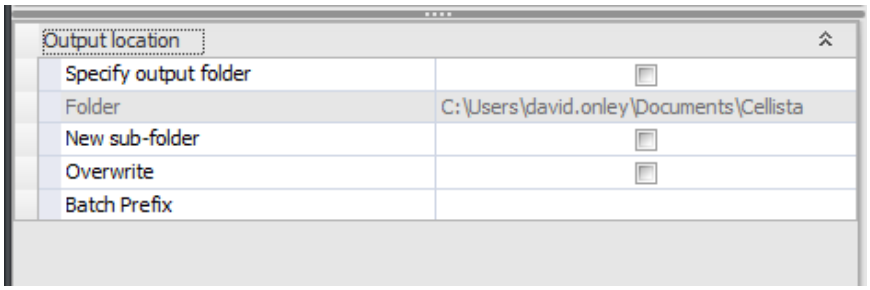


- If desired, enter a suitable description in the Plate Id field.
- Select an appropriate template to scan the plate.
- To scan multiple different plates using the same template (most common use of batch function), create copies of the plates by adjusting the number shown in the box and click on the + sign to the right of the number.
- For multiple scans of a single plate, adjust the number of scans in the “scans” field.
- It is possible to generate batches where a single plate or multiple plates are scanned using more than one template.

12.3 Settings Group

These settings allow the user to define information about the batch including types of output, where to save the output data and details on plate handling.

12.3.1 Location



Specify output folder	Ticking this box allows the user to specify the location where to save all files generated by the batch scan.
Folder	Shows the location where all files generated by the batch scan will be saved.
New Sub-folder	Creates a new subfolder for results files generated from each plate in the batch.
Overwrite	Overwrites existing output files.
Batch Prefix	Allows the user to specify a prefix for all files generated by scanning the current batch.

12.3.2 Files and Options

12.3.2.1 File types

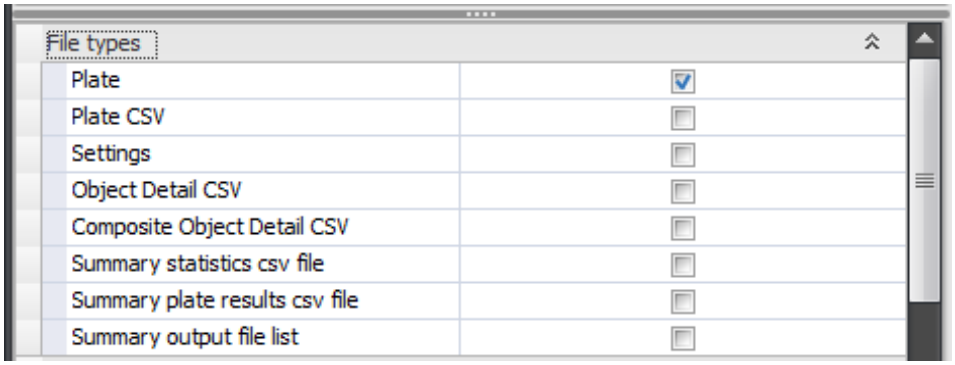
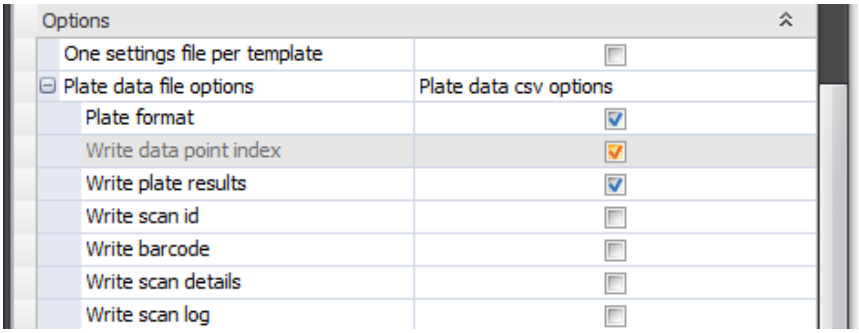


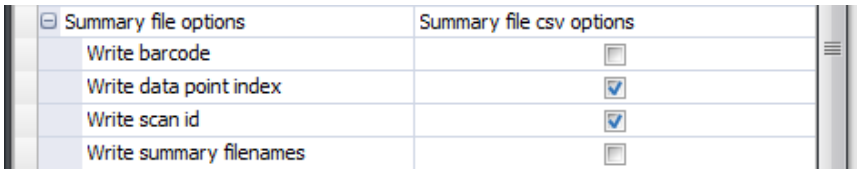
Plate	Tick box to save a cellista data file for each plate scanned.
Plate CSV	Saves a CSV file per plate containing population statistics values.
Settings	Saves text files which summarise the settings used to scan the plate.
Object Detail CSV	Creates one CSV file per plate which lists all collected object characteristics for every individual fluorescent object detected per plate.
Composite Object Detail CSV	Creates one CSV file per plate which lists all collected composite object characteristics for composite fluorescent objects when merging multiple scans.
Summary statistics csv file	Generates a single concatenated CSV file containing well-level data from all scans in the batch.
Summary plate results csv file	Generates a single concatenated CSV file containing plate-level csv data from all scans in the batch.
Summary output file list	Creates a single CSV file containing a list of all of the output files from all scans in the batch.

12.3.2.2 Options – Plate data file options



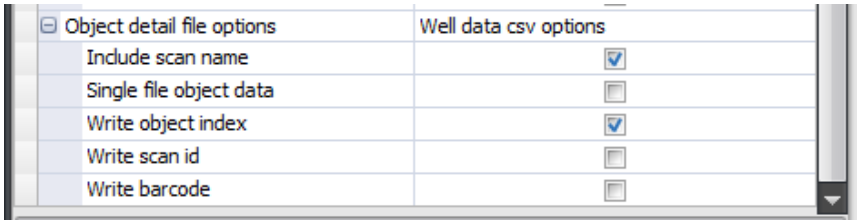
One settings file per template	Only write scan settings text files once per template instead of for each scan.
Plate format	If this box is ticked, the plate-level CSV file is arrayed as a series of tables in the format of a plate rather than one continuous list.
Write data point index	Writes a unique index for each data point (i.e. well) in a scan or batch.
Write plate results	Write statistics calculated for the whole plate to a header in csv files.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.
Write scan details	Write user-created scan details to csv files.
Write scan log	Write information from scan logs to csv files.

12.3.2.3 Options - Summary file options



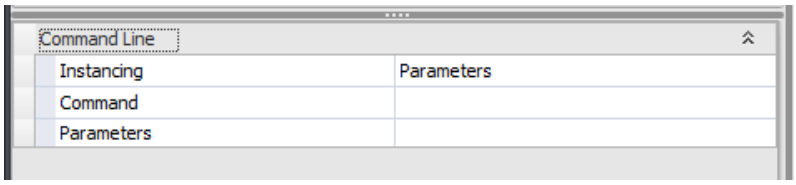
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.
Write data point index	Writes a unique index for each data point (i.e. well) in a scan or batch.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write summary filenames	Enable this option to write the summary files' names at the top of those files.

12.3.2.4 Options – Object detail file options



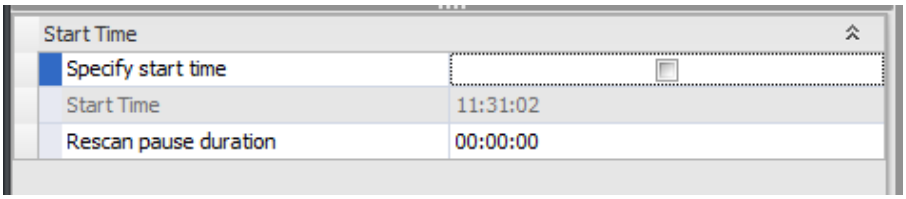
Include scan name	Set this option to include the name of the scan which found an object on each line of the detailed csv files even when not doing multiple scans or object merging. This will ensure that well data csv files have the same format regardless of the number of scans but does not affect file naming.
Single file object data	Write detailed object of composite object data to single file for all wells.
Write object index	Writes the index of each object with respect to the well which contains it.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.

12.3.3 Command Line



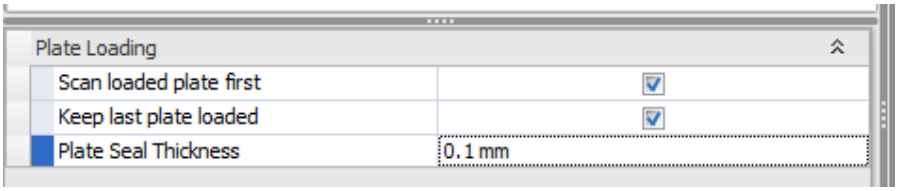
Instancing	Specifies an external command to execute at the end of a batch. The parameters are as follows: None: No external command will be executed. Per file: Once for each results file passing the results file as the name as a parameter. Parameters: Once only specifying the given command line parameters. Results folder: Once only specifying the root output folder as the command line parameter.
Command	The command to execute at the end of a batch job. This should normally be a path to an executable file. The working folder of the external command will be set to the root output folder for this batch.
Parameters	The parameters passed to the external command, when using the “Parameters” option for the Instancing type.

12.3.4 Start Time



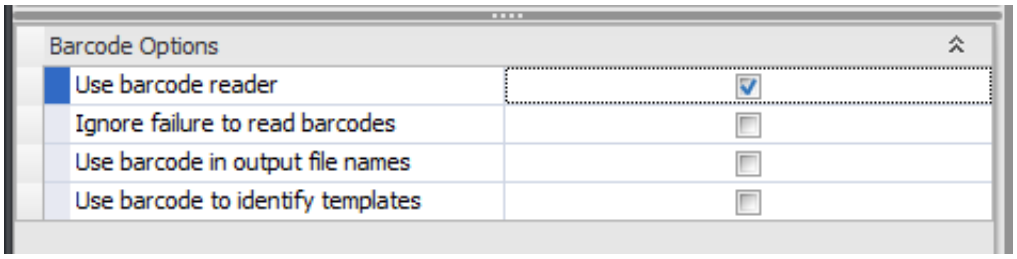
Specify start time	Enable this option to specify when the batch will start running after pressing the scan button.
Start Time	Time at which to start scanning, specified in 24 hour clock format (hh:mm:ss).
Rescan pause duration	Specifies the minimum time between the start of one scan and the start of the next scan of the same plate in the format hh:mm:ss. Note – this option is only relevant when scanning the same plate multiple times.

12.3.5 Plate Loading



Scan loaded plate first	Enable this option to scan the currently loaded plate as the first plate of a batch, when not using a robot.
Keep last plate loaded	Enable this option to keep the last plate of a batch loaded in the instrument, when not using a robot.
Plate Seal Thickness	Specify the thickness of the seals used on the plates in the batch. This is required for accurate robot picking.

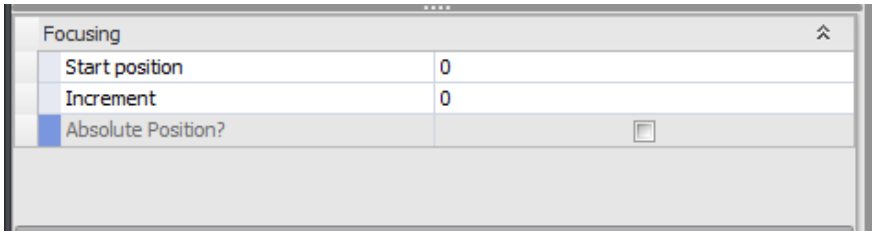
12.3.6 Barcode



Use barcode reader	If enabled then barcodes will be read as each plate is loaded.
Ignore failure to read barcodes	If enabled this will ignore failures to read barcodes and generate a dummy barcode for output filenames. This option only applies when using barcodes in output filenames but not using them to lookup the template to use to scan a plate.
Use barcode in output file names	If enabled then output file names will include the barcode of plates scanned as part of their name.
Use barcode to identify templates	If enabled the plate barcode will be matched to the plate id in the list above to work out which template will be used to scan the plate. The ordering of the plates in the list above will be ignored. If disabled then templates will be used in the order specified above. To use, the barcode IDs must be manually entered into the Plate ID box and the template correctly assigned to that particular barcode.

12.3.7 Focusing

For a full SOP on how to focus a plate on acumen, refer to Appendix E: Setting up a New Plate Type for Acumen



Start Position	This is the first focus position when performing a batch focussing run.
Increment	When doing repeated scans of the same plate, increments the focus position by this amount between scans.
Absolute Position?	If enabled then the position generated is treated as absolute, otherwise it is added to the base focus position for the instrument. Typically this is set to on to determine an instrument's base focus position from a reference plate and off when determining the focus position for a new plate type on a correctly focused instrument.

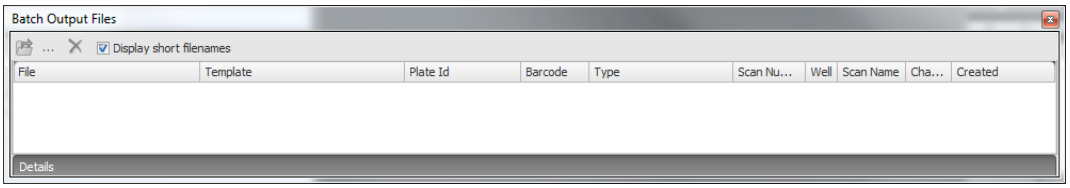
12.4 Control Group

Scan	Start scanning the current batch or plate.
Fast Forward	Abort the current scan and skip to the next one in the batch.
Abort Batch	Abort processing of the whole batch immediately.
Stop after this scan	Finish scanning the current plate and then stop the batch.

12.5 Results Group

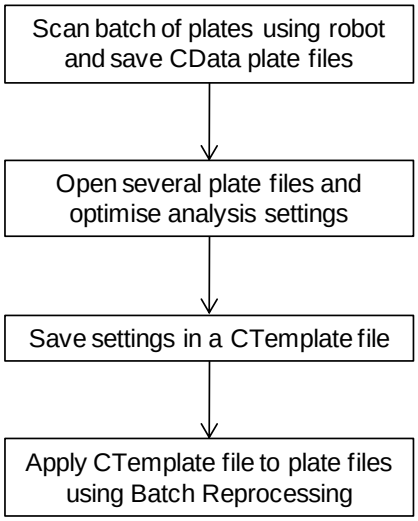
12.5.1 Output files

Clicking on the Output files button () opens the batch output files window (shown below) which allows the user to view the automatically generated output files from the current batch.



13 REPROCESSING TAB

This utility is for users who wish apply analysis changes to a batch of plate files after scanning. This feature avoids the need for repetitive opening, modification and reanalysis of large numbers of Cellista CData files.



A typical workflow involving automated file reprocessing.

13.1 File Group

13.1.1 New

Begins a new batch reprocessing job by clicking this button

13.1.2 Load

Load a batch reprocessing job definition

13.1.3 Save

Saves the current batch reprocessing job definition.

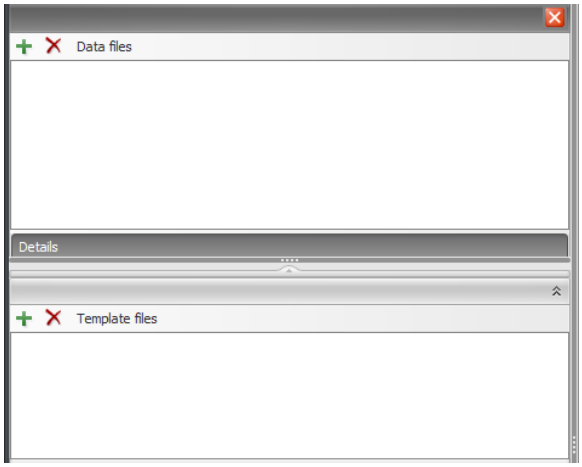
13.1.4 Close

Closes the batch reprocessing editor.

13.2 Setup a new Batch Reprocess

To setup file reprocessing, select **Reprocessing | File | New**.

The following Reprocessing window opens:



13.2.1.1 Data files

Add all plate files (CData) requiring reprocessing here. The Data Collection Level used to generate the plate files must be equal or greater than that in the CTemplate file used for reprocessing.

CData files can be dragged to this window from Windows Explorer.

13.2.1.2 Template files

Add the CTemplate files that are to be applied to each plate file. Normally a single template will be used.

The Data Collection Level defined in the CTemplate file must be equal or lower than that used to generate the plate files. This offers an effective means to reduce the sizes of the reprocessed plate files.

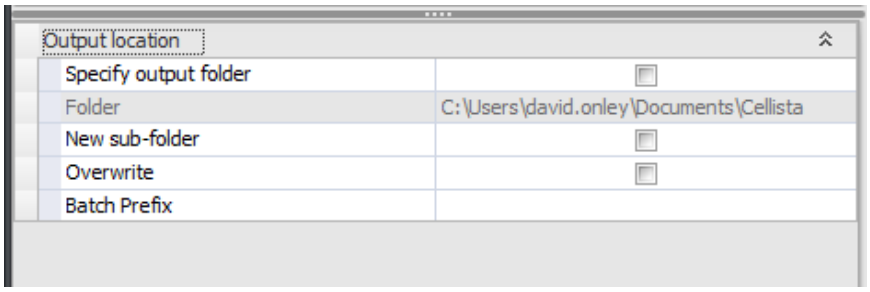
CTemplate files can be dragged to this window from Windows Explorer.

13.2.1.3 Re-exporting data

It is possible to re-export the data from a number of CData files as csv files - with different export settings – without reprocessing the data at all. To do so, specify the data files to be reprocessed as normal but do not add any templates at all to templates dialog. Then specify the required export options as described below.

13.3 Settings Group

13.3.1 Location



Specify output folder	Ticking this box allows the user to specify the location where to save all files generated by the batch scan.
Folder	Shows the location where all files generated by the batch scan will be saved.
New Sub-folder	Creates a new subfolder for results files generated from each plate in the batch.
Overwrite	Overwrites existing output files.
Batch Prefix	Allows the user to specify a prefix for all files generated by scanning the current batch.

13.3.2 Files and Options

13.3.2.1 File types

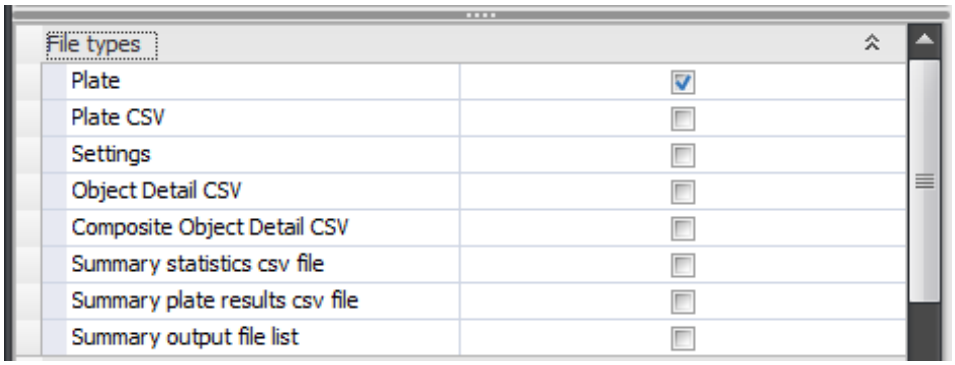
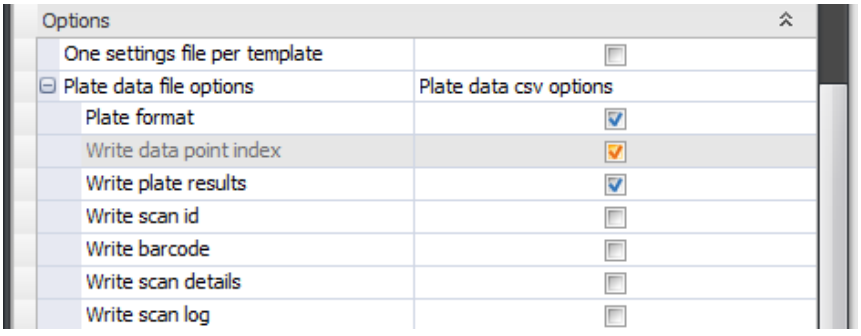


Plate	Tick box to save a cellista data file for each plate scanned.
Plate CSV	Saves a CSV file per plate containing population statistics values.
Settings	Saves text files which summarise the settings used to scan the plate.
Object Detail CSV	Creates one CSV file per plate which lists all collected object characteristics for every individual fluorescent object detected per plate.
Composite Object Detail CSV	Creates one CSV file per plate which lists all collected composite object characteristics for composite fluorescent objects when merging multiple scans.
Summary statistics csv file	Generates a single concatenated CSV file containing well-level data from all scans in the batch.
Summary plate results csv file	Generates a single concatenated CSV file containing plate-level csv data from all scans in the batch.
Summary output file list	Creates a single CSV file containing a list of all of the output files from all scans in the batch.

13.3.2.2 Plate date file options

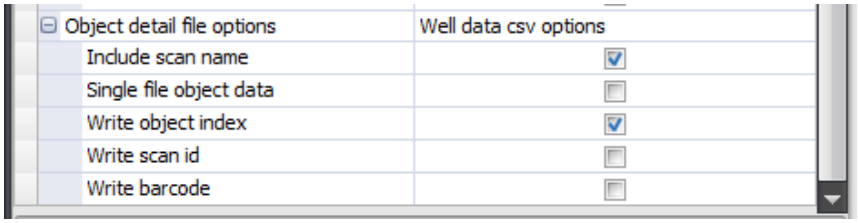


One settings file per template	Only write scan settings text files once per template instead of for each scan.
Plate format	If this box is ticked, the plate-level CSV file is arrayed as a series of tables in the format of a plate rather than one continuous list.
Write data point index	Writes a unique index for each data point (i.e. well) in a scan or batch.
Write plate results	Write statistics calculated for the whole plate to a header in csv files.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.
Write scan details	Write user-created scan details to csv files.
Write scan log	Write information from scan logs to csv files.

13.3.2.3 Summary file options

Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.
Write data point index	Writes a unique index for each data point (i.e., well) in a scan or batch.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write summary filenames	Enable this option to write the summary files' names at the top of those files.

13.3.2.4 Object detail file options



Include scan name	Set this option to include the name of the scan which found an object on each line of the detailed csv files even when not doing multiple scans or object merging. This will ensure that well data csv files have the same format regardless of the number of scans but does not affect file naming.
Single file object data	Write detailed object of composite object data to single file for all wells.
Write object index	Writes the index of each object with respect to the well which contains it.
Write scan id	Write the scan identifier to each row of list-format csv files or the header of plate-format csv files.
Write barcode	Write the plate barcode to each row of list-format csv files or the header of plate-format csv files.

13.4 Control Group

To begin running a batch reprocessing job, click on **Reprocessing | Control | Start Reanalysis.**

Reprocessing of the whole batch can be stopped by selecting **Reprocessing | Control | Abort Batch.**

13.4.1 Start Reanalysis

Starts the reanalysis process

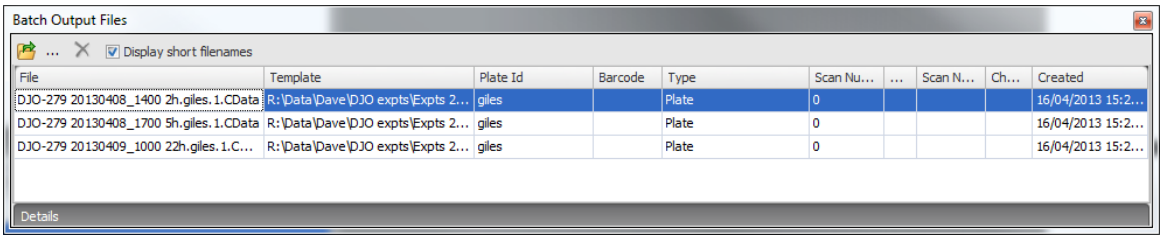
13.4.2 Abort Reanalysis

Aborts the reanalysis process. Previously converted files will be stored, but plate files not analysed will not be processed.

13.5 Results Group

13.5.1 Output files

View automatically generated output files from the reprocessing job by selecting **Reprocessing | Results | Output Files**



14 APPENDIX A: CELLISTA SOFTWARE SUPPORT

TTP Labtech offers software and assay development support and training materials to enable Cellista software usage in research and development laboratories.

14.1 Assay Protocols

A comprehensive range of standard assay protocols are available in PDF format to all Cellista software users. These cover bead and cell-based antibody binding assays, and also a number of high content cellular assays.

14.2 Additional Training

Supplementary training focussing on specific aspects of Cellista software usage not covered during the basic introduction is available. Costs are available upon request.

14.3 Application Development

TTP Labtech's Application Team are able to provide their expert knowledge on a contract basis to speed up development of user-specific applications not covered by the standard assay protocols. Costs are available upon request.

14.4 Contacting Customer Support

TTP Labtech are available to answer customer support queries during the company's UK working hours.

Contact details are:

Catherine Boddington
TTP Labtech Ltd
Melbourn Science Park
Melbourn
Royston
Herts SG8 6EE
United Kingdom

Tel: +44 (0) 1763 266708
Fax: +44 (0) 1763 261964

acumensupport@ttplabtech.com
mirrorballsupport@ttplabtech.com
http://www.ttplabtech.com/

15 APPENDIX B: APPLICATION SECURITY

Cellista software includes facilities to prevent unauthorised users performing such tasks as recalibrating the instrument or modifying plate definition files. This security is implemented via Microsoft Windows users and groups, and managed via the Users and Groups tool in the control panel.

Note that all users of Cellista will require read/write access to the folder C:\ProgramData\TTPLabtech\Cellista\.

15.1 Users and Groups

When the Cellista software is registered on the PC three new groups are configured on the local PC as follows:

TTP_Factory	Members have access to all control and diagnostic functions of the software

Note that Cellista also makes use of three groups which are preconfigured on the PC:

TTP_Factory	Members have access to all control and diagnostic functions of the software
Administrators	Members can administer the PC and modify users and groups privileges, but perform no diagnostics functions
Users	Members can load, edit and run assays as well as use the PC. They can normally only save documents to 'My Documents'

Additionally, a number of users are created on the PC so:

TTPFactory	Member of the TTP_Factory group and Administrators group
ttplabtech	The default user of the system. The default password for this user is ttplabtech
Administrator	Member of the Administrators group

15.2 Logging on and off

When Cellista software starts it inherits the rights of the user who is currently logged on to Windows. However, by using the **File | User | Log on** menu it is possible to change the user logged onto Cellista. This does not alter who is logged on to Windows.

To change the currently logged on user, either

- Exit Cellista software
- Select **Start | Shutdown | Log off** and then log on to Windows as a different user

or

- From within the Cellista software, select **File | User | Log on** and enter the new username and password.

In this way the current user level can be increased from within the software to perform diagnostic functions without logging off from Windows. Note that the new user name only affects the Cellista

software and not any other Windows functions.

To log off a Service or Factory level user and revert to the previous logged on user level, select **File | User | Log off**.

15.3 Automatically Logging On and Off

It is possible to configure the software so that after a period of inactivity where the machine is not used, the current Cellista user will be logged off automatically if they have greater privileges than the currently logged on Windows user, and the currently logged on Windows user will be logged on instead. This behaviour can be modified if required; contact TTP Labtech Support for further details.

15.4 Creating Additional Users

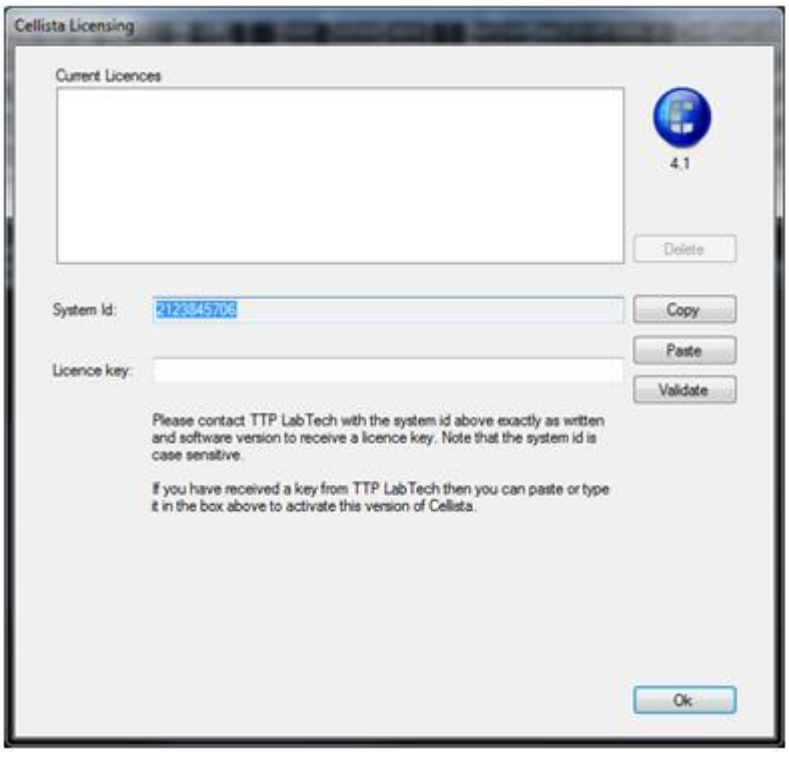
Where systems have large numbers of users it is recommended that each user has their own username and password on the control PC. If the PC is to be networked then this could be their normal network id. These users should be made members only of the Users group, or possibly the Power Users group.

If you are not familiar with configuring user accounts and permissions in Windows then please seek the assistance of your local IT department.

15.5 Licensing

Version 4.2 Cellista requires a licence key before the application will run. Licence keys are specific to an individual PC and software version. Installing Cellista on a new PC will require a new key. Minor upgrades for maintenance releases – e.g. 4.2.1 to 4.2.2 – will not require a new key but major upgrades introducing new functionality – e.g. 4.2.1 to 5.0.0 – will require a new key.

On starting Cellista if no valid licence key is present the following dialog will be displayed:



To obtain a key, send the system id to TTPLabtech or call TTPLabtech support ready to quote the id. TTPLabtech will be able to email you a suitable licence key; paste it into the Licence key text box and press Validate.

Cellista will then run normally.

Note that some licence keys may be time-limited. If you are running Cellista using such a key then a warning will displayed as the application starts. It is possible to add a new licence at a later date; to do so go to **File | Help | Licensing**. The dialog above will be shown – process as described above.

16 APPENDIX C: INTEGRATING WITH CELLISTA

16.1 Introduction

Cellista exposes a WCF (Windows Communication Foundation) interface to allow third party integrators to control an **acumen** or **mirrorball** instrument and integrate with a robot arm or other systems.

The WCF interface is normally configured to use TCP endpoints but can be configured to use HTTP if required. Thus it is possible to expose the Cellista WCF interface as a SOAP service. This is done in the usual way via the Cellista.exe.config file in C:\Program Files\TTPLabTech\Cellista\.

The automation interface is geared towards loading and unloading plates, scanning plates using pre-defined templates and specifying the format of output data files. It does not provide access direct to either the raw or processed scan data, nor to the acquisition settings. Similarly, it does not provide access to the batch reprocessing functionality.

Note also that the automation interface does not provide access to the robot functionality natively provided by Cellista. Either Cellista controls a robot – if present – or a client application controls the robot and Cellista.

- When running under automation Cellista should be configured to auto-reset the instrument when it starts; ensure the IsAutoStart entry in HardwareEnable.config is set to “true”.
- To enable the automation interface ensure that the IsIntegrated entry in HardwareEnable.config is set to “true”.
- When Cellista is first run with this configuration it may cause the Windows Firewall prompt to appear. Denying access will cause the automation interface to be inaccessible but Cellista will otherwise function normally.

The automation interface assumes that the system will be processing batches of plates – though this batch could have a size of 1. This is because Cellista can generate output files which summarise a whole batch, and options determining which output files to produce apply to the batch as a whole.

16.2 Compatibility with acumen 3.4

Cellista’s automation interface is not directly compatible with the COM or DLL interfaces exposed by **acumen** Explorer 3.4 and earlier versions of the software. It has a broadly similar structure so migrating should not be overly onerous. ExplorerCOM (see section 20) is provided for existing COM integrations.

The format of the csv files generated by Cellista is different from that generated by **acumen** 3.4. This may affect tools which automatically parse or import the results. The intent has been to aim for greater consistency between the various files which Cellista can generate and to make them more easily machine-readable.

16.3 Installing Cellista in Simulation Mode

Cellista can be configured to perform simulated scans, emulating either an **acumen** or a **mirrorball** system. Cellista runs only on 64 bit versions of Windows 7. It requires the .NET framework version 4.5.1 (included on the install CD). This can allow integration to be tested without an instrument.

16.3.1 Installation

To install Cellista and configure it to run in simulation mode:

- Log on to the PC as an administrator
- Install the .NET framework version 4.5.1 full version from the CD by running dotNetFx451.exe
- Run the CellistaFactorySetup64.msi file from the CD
- You will be asked whether you want to create the TTPFactory and Admin users who will have access to the diagnostics functionality of Cellista. This step is optional. If you do choose to create these users then the installer will also attempt to set the desktop wallpaper to the TTP Labtech wallpaper for all users.
- Then follow the on-screen prompts to complete installation.

- Navigate to C:\ProgramData\TTPLabTech\Cellista\Config.**acumen**\ or \Config.**mirrorball**\ (according to which type of system you wish to simulate).
- Copy HardwareEnable.simulation.config and either Instrument.config (for **acumen**) or Instrument.simulation.config (for **mirrorball**) to C:\ProgramData\TTPLabTech\Cellista\ (up one folder) and rename to HardwareEnable.config and Instrument.config respectively.
- Edit HardwareEnable.config; change the IsAutoStart entry to “true” to ensure that the ‘instrument’ homes automatically on starting the software
- To enable the automation interface ensure that the IsIntegrated entry in HardwareEnable.config is set to “true”.

If you have set the desktop wallpaper in step 4 above and wish to undo this change then either use the group policy editor gpedit.msc or delete the registry keys below:

<!-- Set the desktop for the current user -->

HKCU\Control Panel\Desktop\Wallpaper

HKCU\Control Panel\Desktop\WallpaperStyle

<!-- Set the default desktop for new users -->

<!-- Note that this can be overridden by local policies, active directory and so on -->

HKCU\SOFTWARE\Microsoft\Windows\CurrentVersion\Policies\System\Wallpaper

HKCU\SOFTWARE\Microsoft\Windows\CurrentVersion\Policies\System\WallpaperStyle

16.3.2 Configuring Simulated Scanning

To run in simulation mode you will need a raw data file to serve as a source of the scan data. Two are provided on the disk image in the Tools\Sample mcf files folder, one each for **acumen** and **mirrorball**.

- Copy the correct file from the CD to a suitable location on the hard disk of the PC. The normal location is C:\ProgramData\TTPLabTech\Cellista\
- Edit the DefaultDataSource key of the HardwareEnable.config file to point at the correct mcf file. The <appsettings> key should contain a subkey in this format:

```
<DefaultDataSource
Filename="C:\ProgramData\TTPLabTech\Cellista\acumenSample.mcf"
                        DataSourceType="SingleWellFile"
                        UseScanResolution="false"
                        IsKeepPartialWells="false" />

Or
<DefaultDataSource
Filename="C:\ProgramData\TTPLabTech\Cellista\mirrorballSample.mcf"
                        DataSourceType="MultiWellFile"
                        UseScanResolution="false"
                        IsKeepPartialWells="false" />
```

You can now perform a simulated scan:

- Do **File | New From Template**
- Browse to the file C:\ProgramData\TTPLabTech\Config.**mirrorball**\Sample.CTemplate or C:\ProgramData\TTPLabTech\Config.**acumen**\Sample.CTemplate
- Press the scan button

Note that running with templates file which do not match the mcf files, or mixing **acumen** and **mirrorball** configuration, may well lead to variety of errors or exceptions in the software. This simulation

mode exists as a mechanism to insert any arbitrary raw data into the acquisition path regardless of validity and not possible all inputs are sanitised.

You are now in a position to create a WCF client to perform simulated scans using this template file and data source.

16.4 Sample Client Application

A sample WCF automation client application is provided; it is very extensively commented complete with sequence diagrams showing how to use Cellista's automation interface. The example client source code is installed by the factory installer (CellistaFactorySetup64.msi) to C:\Program Files\TTPLabTech\Cellista\Automation Example\.

To build the client application once Cellista is installed:

- Start Cellista
- In Visual Studio right-click in the Solution Explorer and 'Add Service Reference'
- Select Cellista.exe from the drop-down. At present the Automation Service is not discoverable.

For reference, the automation service is implemented by Burton.Automation.dll.

16.4.1 Threading Considerations

The automation interface has a number of outgoing events. For this reason it is normal to run the client from within a worker thread which is distinct from the thread which handles callbacks from Cellista.

Please note however that the interface is not re-entrant, so a client application may not call Cellista's interface methods from within the callback handler.

These issues are illustrated and explained in the sample client code.

16.4.2 Multiple PCs

It is possible to use the automation interface with Cellista running on a different PC from the client application, with each application running under a different user account. If both PCs are on the same domain then typically the only change you would need to make is to change the default config file, replacing localhost with the ip address of the machine connected to the instrument. So if the client ip was 172.20.6.25 on a subnet with mask is 255.255.0.0 then you'd need the following:

So the Cellista config contains:

```
    <services>
            <service name="Cellista.Automation.AutomationService">
                <endpoint name="TcpIInstrument" binding="netTcpBinding"
contract="Cellista.Automation.IInstrument"/>
                <endpoint name="TcpMetaData" kind="mexEndpoint" address="mex"
binding="mexTcpBinding"/>
                <host>
                    <baseAddresses>
                        <add
baseAddress="net.tcp://172.20.5.76:50000/Cellista.Automation/AutomationService/" />
                    </baseAddresses>
                </host>
            </service>
        </services>
```

And the client config contains:

```
        <client>
            <endpoint
address="net.tcp://172.20.5.76:50000/Cellista.Automation/AutomationService"
                binding="netTcpBinding" contract="AutomationService.IInstrument"
                name="TcpBinding" />
```

```
</client>
```

If either PC does not have a static IP address then you'd need to configure the endpoints for automatic discovery as described in http://msdn.microsoft.com/en-us/library/dd456792.aspx. This may require the use of x509 certificates as described in http://wcfsecurityguide.codeplex.com/.

16.5 Hardware Integration

Dimensioned drawings – in .dwg and .pdf format of the **acumen** and **mirrorball** systems are installed to C:\ProgramData\TTPLabTech\Cellista\Layout.**Acumen** and .\Layout.**Mirrorball**. They are also included in the Tools\Drawings folder on the installation CD.

16.6 Running an external application

It is possible to automatically start an application external to Cellista on completion of an interactive scan or a batch scan. Please see sections 7.5.4 and 12.3.3 for details.

16.7 Barcode reader integration

When not using the automation interface, Cellista can control an attached barcode reader. The default configuration is for the barcode reader to be used only in batch mode, either to identify which template to use to scan a plate or to name the output files – see section 12.3.6. The barcode can appear in exported csv files and in OME metadata too.

It is also possible to configure the barcode reader to scan every plate as it is loaded, even when running interactively. To do so edit the `BarcodeReader` section of the HardwareEnable.config file and set `ReadOnLoad="true".`

Limited support for barcodes is provided through the automation interface – see section 16.8. To use an attached barcode reader `Options.IsReadBarcode` should be true. For the client software to specify a barcode `Options.IsReadBarcode` should be false and the alternate `ScanWithBarcode` method should be used.

16.8 IInstrument interface

The `IInstrument` interface allows automation clients to control scanning of individual plates. The interface assumes the automation client is responsible for loading plates (i.e. robot and, if required, barcode control).

All scan configuration (e.g. channels, resolution etc. must be preset in Cellista and stored in template files that will be used to scan the plates).

16.8.1 Methods

void Connect();	Initiate an automation session. **NOTE:** This must be the first call on the automation interface. The application's user interface will be disabled while an automation session is in progress. Only one session is allowed.
void Disconnect();	End an automation session. This must be called after Connect() to release the automation interface; until this is called no other automation client may connect to Cellista. **NOTE:** no further calls on the automation interface should be made after this is called.
void StartBatch();	Start running a batch. Will throw an InvalidOperationException if a batch has already been started.
void Scan(string plateId, string templateFile);	Initiate the scan of the next plate in the batch. The batch will open the drawer at the end of a scan and raise an IInstrumentCallback.ReadyForPlate event. It is the responsibility of the automation client to unload the previous plate (if any) and load a new plate, before calling Scan. **Parameters:** plateId: Unique identifier for plate; will be used in all reports generated. templateFile: Template to use for scan; containing all scan configuration for the loaded plate.
void ScanWithBarcode(string plateId, string templateFile, string barcode);	An alternative to Scan; provides the same functionality but allows the barcode field in reports to be set by the client software. The barcode will be ignored if Options.IsReadBarcode is set to true.
void PlateUnloaded();	Should be called by the client to indicate that a plate has been removed from the drawer When a plate has been scanned the drawer will be ejected. The system waits for an indication that the plate has been removed by the client, at which point it can proceed to the next plate, or end the batch.
void EndBatch();	Indicate a batch has finished. This will generate any summary files specified in the configuration options. Will throw an InvalidOperationException if a batch has not been started.
bool IsOk();	Returns true if the automation server okay.
bool IsReady();	Returns true if the automation server ready.

bool IsReadyForPlate();	Returns true if the instrument is ready for a plate to be placed into the drawer.
bool IsWaitingForPlateRemoval();	Returns true if the instrument is waiting for a plate to be removed from the drawer.
ulong GetTotal();	Get the total time that the current task will take.
ulong GetElapsed();	Get the elapsed time of the current task.
string GetTaskName();	Get the name of the task which is in progress.
List<OutputFile> GetOutputFiles();	Get the list of output files generated during the most recent batch.
void Reset();	Reset the automation service.
void AbortBatch();	Abort the current batch.
void SetLogLevel(LogLevel level);	Set the minimum log level to be reported to client. All log levels above the set level will be reported. **Parameters:** level: Minimum log level to report (or None). LogLevel can be one of: • None • Exception • Error • Warning • Info
bool IsRunning();	Returns true if the automation server is running a scan.
string GetBarcode();	Returns the last read barcode (either from an attached barcode reader or set through ScanWithBarcode). It is best to read this when IsPlateLoaded returns True to ensure it is for the current plate.

There are a separate set of methods that can be used to control the drawer. These fall outside the normal sequence of control for running a batch. **NOTE:** they will be ignored while within a batch (between calls to StartBatch, EndBatch).

void OpenDrawer();	Open the drawer.
void CloseDrawer();	Close the drawer.

16.8.2 Properties

Options Options {get; set;}	Configure the automation session. Will throw an ArgumentException if the set configuration is not valid.

There are a separate set of properties that can be used to query the drawer state. These fall outside the normal sequence of control for running a batch. They all return a TriState enumeration (with values of False, True or Unknown); in some situations the instrument sensors can not determine the actual state.

TriState IsDrawerOpen {get;}	Returns True if the drawer is fully open. Can return Unknown during start-up/reset or if instrument is in error.
TriState IsDrawerClosed {get;}	Returns True if the drawer is fully closed. Can return Unknown during start-up/reset or if instrument is in error.
TriState IsPlateLoaded {get;}	Returns True if a plate is loaded into the drawer. Will return Unknown if the drawer is not fully closed; the drawer itself does not have any sensors to determine if a plate is in the drawer.

16.8.3 Options

The Options class consists only of properties with both get and set methods, and is used to configure the automation session.

NOTE: these settings are used throughout the batch run; they should only be set outside of a batch, i.e. before `StartBatch` or after `EndBatch` is called.

Since they configure the reporting format and barcode interaction, custom integrations should expose any relevant settings through their UI; or agree a pre-defined set with the customer.

16.8.3.1 Properties

`string SessionPrefix`	Specify a prefix for all files generated in this session.
`string OutputDirectory`	The folder in which to place output files.
`bool IsSpecifyOutputDirectory`	Choose whether to specify the output folder or to let the application determine it automatically from the locations of the templates.
`bool IsMakeNewSubDirectory`	Creates a new subfolder for results files generated from each plate in the batch.
`bool IsOverwriteOutputFiles`	Overwrites existing output files.
`bool IsOnlyOneSettingsFilePerTemplate`	Only write scan settings text files once per template instead of for each scan.
`bool IsSaveSummaryFile`	Enable this option to create a single concatenated CSV file containing well-level CSV data from all the scans in the batch.
`bool IsWritePlateResultsFile`	Enable this option to create a single concatenated CSV file containing plate-level CSV data from all the scans in the batch.
`bool IsWriteSummaryFilenames`	Enable this option to write the summary files' names at the top of those files.
`bool IsSavePlateFile`	Enable the automatic creation of plate data files which contain all of the retained scan data, population and characteristic definitions and so on.
`bool IsSaveCSVFile`	Enable the automatic creation of CSV files of plate-level data, e.g. population statistic values.
`bool IsSaveSettingsTextFile`	Enable the automatic creation of textual files which summarise the settings used to scan the plate.
`bool IsSaveWellDataFile`	Enable the automatic creation of object characteristic data files.
`bool IsSaveCompositeSpotCSVFile`	Enable the automatic creation of composite object characteristic data files when merging multiple scans.

bool IsPlateFormat	Write plate-level CSV data as a series of tables in the format of a plate rather than one continuous list.
bool IsWriteScanLog	Write information from scan logs to CSV files.
bool IsWriteScanComments	Write user-created annotations to CSV files.
bool IsWritePlateResults	Write statistics calculated for the whole plate to a header in CSV files.
bool IsWellDataCSVSingleFile	Write detailed object or composite object data to single files for all wells.
bool IsWriteScanName	Set this option to include the name of the scan which found an object even when not doing multiple scans or object merging. This will ensure that well data CSV files have the same format regardless of the number of scans.
bool IsWriteObjectIndex	Writes the index of each object with respect to the well which contains it.
bool IsWriteScanId	Write the scan identifier to each row of CSV files.
bool IsReadBarcode	Use an attached barcode reader to read the plate barcode.
bool IsUseBarcodeToNameOutputFiles	Use the barcode as part of the output file name.
bool IsIgnoreBarcodeFail	This will create a dummy barcode (containing date and time) if the barcode reader fails to read a barcode.
bool IsWriteBarcode	Write the plate barcode to each row of CSV files.
bool IsWriteDataNumber	Writes a unique index for each data point (i.e. well) in a scan or batch.

16.8.4 IInstrumentCallback

All automation clients connecting to Cellista must implement the IInstrumentCallback interface.

Methods

`void Notify();`	Notifications from the current session. This will be called to indicate a change in session state. The client should query the IInstrument interface, if interested in state changes.
`void ReadyForPlate();`	Indicates the session is paused, the drawer is open and waiting for a plate to be loaded. Once a plate is loaded call IInstrument.Scan(…).
`void RemovePlate();`	Indicates the session is paused, the drawer is open and waiting for a plate to be removed.
`void LogEvent(LogLevel level, string message);`	Report log event. All log events meeting the specified log level will be reported through this interface. **Parameters:** level: Severity level of log. message: Message contained in log.

16.9 Sequence Diagram

Detailed sequence diagrams are provided in the Sample Client Applications (see section 16.4) comments.

16.10 Automation Settings

16.10.1 Defaults

It is possible to configure a set of default settings used when running in Automation mode. They will override Cellista defaults but can be changed later through the automation interface. This allows a system wide change, such as what CSV files to generate, without changing any 3rd party integration code.

They are configured in HardwareEnable.config, in a subkey of <appsettings> called <Automation>. Each attribute in the <Automation> key is optional and named after the properties in the Options class (see section 16.8.3.1). Boolean properties are specified with one of 3 values (note: case sensitive):

True	Cellista setting will default to true
False	Cellista setting will default to false
DoNotOverride	Cellista will use its own default setting. This is used as a placeholder, so that attributes can be defined but have no effect.

There are 2 additional boolean settings:

IsKeepFirstPlateLoaded	When set to True, if a plate is loaded when a batch is started this plate will be scanned. When set to False, if a plate is loaded when a batch is started the plate will be ejected and a RemovePlate event generated.
IsKeepLastPlateLoaded	When set to True, the loaded plate will not be ejected when EndBatch is called. NOTE: EndBatch must be called while scanning for this behaviour; the automation interface does not know how many scans are in a batch. When set to false, the loaded plate will be ejected after each scan.

Example configuration:

<Automation IsKeepFirstPlateLoaded="True" IsKeepLastPlateLoaded="False" SessionPrefix=”Test” IsSaveSummaryFile=”True” />

16.10.2 Fixed

There is a corresponding <AutomationFixed> configuration available in HardwareEnable.config. This takes the same format as <Automation>. This allows automation options being set through 3rd party integrations to be overridden. Automation options set through <AutomationFixed> can not be changed.

16.11 Additional Diagnostics

To check that Cellista has started the automation host check the log file. It should contain entries similar to:

```
AutomationHost.Create: Automation service started.
AutomationHost.Create:     channel[0]:
net.tcp://localhost:50000/Cellista.Automation/AutomationService/
AutomationHost.Create:     channel[1]:
net.tcp://localhost:50000/Cellista.Automation/AutomationService/mex
```

This indicates that Cellista is ready for automation and the endpoint addresses it is listening for connections on.

While running the log should contain entries from the `AutomationService` and the `AutomationServiceManager` for events related to interaction with the automation client. For example, when a client connects to Cellista:

```
AutomationService.Connect: Automation session started.
AutomationServiceManager_c.ClientConnected: ENTRY
```

The `AutomationServiceManager` indicates the internal state, while the `AutomationService` indicates actions taken on the automation interface.

For additional diagnostics the `system.diagnostics` section of the `app.config` file and the `Cellista.exe.config` file have been configured to generate two log files:

```
CellistaAutomation_Client.svclog
CellistaAutomation_Host.svclog
```

NOTE: you may need to change these filenames if the current user can not write to the directory containing the .exe files.

For example, it may be easier to set them to `"C:\logs\Client.svclog"` and `"C:\logs\Host.svclog".`

These logs can be viewed using Microsoft's Service Trace Viewer Tool (SvcTraceViewer.exe). By opening both logs in a single SvcTraceViewer project you should see the end to end WCF transactions between Cellista and your client.

Search Microsoft's MSDN library for "WCF diagnostics" for additional information on configuring trace and using SvcTraceViewer.

17 APPENDIX D: OME METADATA

The TIFF images generated by Cellitsa include OMETiff meta data describing the instrument setup used to produce those images. For an overview of the OMETiff standard, please see http://ome-xml.org/wiki/OmeTiff.

The OME xml-format metadata is included in the ImageDescription tag of the TIFF files. Cellista targets the 2011-06 version of the standard as documented at http://www.openmicroscopy.org/Schemas/OME/2011-06.

The OMETiff standard is a broad one aimed at documenting images acquired through traditional imaging systems. Consequently Cellista does not attempt to implement all parts of the standard and there are a small number of minor deviations from the strict interpretation of the standard. Cellista additionally makes use of OME StructedAnnotations to include extra information in the metadata.

This section describes how Cellista implements the standard.

- The Creator attribute is a comma separated list giving the software manufacturer name, application name and full version number.
- The UUID attribute is a UUID unique to this file.

```
<?xml version="1.0" encoding="UTF-8"?>

<!-- Warning: this comment is an OME-XML metadata block, which contains
crucial dimensional parameters and other important metadata. Please edit
cautiously (if at all), and back up the original data before doing so. For
more information, see the OME-Tiff web site: http://ome-xml.org/wiki/OmeTiff.
-->

<ome:OME xmlns:spw="http://www.openmicroscopy.org/Schemas/SPW/2011-06"
         xmlns:ome="http://www.openmicroscopy.org/Schemas/OME/2011-06"
         xmlns:sa="http://www.openmicroscopy.org/Schemas/SA/2011-06"
         xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance"
         xsi:schemaLocation="http://www.openmicroscopy.org/Schemas/OME/2011-
06 http://www.openmicroscopy.org/Schemas/OME/2011-06/ome.xsd"
         Creator="TTP Labtech Ltd., Cellista, V4.1.5.57095"
         UUID="urn:uuid:a2221c1a-aa70-4af2-a326-5d729f20a03f">
```

- Plate:ID is the root of the names of output files generated by the current scan combined with a UUID which is unique to the most recent scan and well.
- Plate:Name is the name of the plate and is used as the root of the names of output files generated by the most recent scan of the plate.
- Plate:ExternalIdentifier attribute is the barcode of the plate, if known, a dummy id if the relevant option is set and barcode reading failed or an empty string if no attempt was made to read the barcode.
- Plate:Description is a comma separated list giving the short name of the physical plate file used in the scan followed by the plate manufacturer's part number.
- The naming conventions indicate that in the UI and in filenames the convention is to use well names like A1 for (row, column).
- Cellista does not make use of the group, image, screen, ROI and project attributes

```
  <spw:Plate ID="Plate:ome.1:dc7d6539-3a36-42d7-8468-a00e701b6c7f"
       ColumnNamingConvention="number"
       RowNamingConvention="letter"
       ExternalIdentifier=""
       Name="ome.1"
       Rows="16"
       Columns="24">
    <spw:Description>4titude 384 (c4ti-0204),4ti-0204/IND</spw:Description>
```

- Well:ID gives the user-friendly name of the well
- Well:Column gives the 0-based index of the column of the well from the top-left of the plate.
- Well:Row gives the 0-based index of the column of the well from the top-left of the plate.
- The Well:Scan and Well:WellProperties refer to entries in the StructuredAnnotations section of the metadata.

```
    <spw:Well ID="Well:A1"
              Column="0"
              Row="0" />
    <sa:AnnotationRef ID="Annotation:Scan" />
    <sa:AnnotationRef ID="Annotation:WellProperties" />
  </spw:Plate>
```

- Experimenter:ID gives the unique identity of the user logged on to Cellista at the time of the scan in the form of the fullname:SSID. Note: this may be different from the current Windows user.
- Experimenter:DisplayName gives the Windows 'friendly' name of the user logged on to Cellista at the time of the scan.

```
  <ome:Experimenter ID="Experimenter:TTPGROUP\\simon.carter:S-1-5-21-
1547161642-854245398-682003330"
                    DisplayName="simon.carter" />
```

- Instrument:ID is always Instrument:0
- Detector:ID gives the instrument name, with spaces replaced by underscores, prefixed by Detector:.
- Detector:Model gives the instrument type, either **acumen** or **mirrorball**.
- Detector:SerialNumber is the same as the Detector:ID without the Detector: prefix.
- Detector:Type is always PMT.
- Cellista does not include Dichroic, Lightsource, Power, and filter information as OME-TIFF is not well set up for multiple light sources per channel at present, which occurs in the **mirrorball** case.

```
  <ome:Instrument ID="Instrument:0">
    <ome:Detector ID="Detector:acumen_00000"
                  Model="acumen"
                  SerialNumber="acumen_00000"
                  Type="PMT" />
  </ome:Instrument>
```

Image:ID is always 0. While OMETiff supports multiple image sets with either shared or duplicated OMETiff meta data Cellista does not implement this functionality.

Image:Name is used to give the current image filename relative to any CData file. Note that the standard does not require that this attribute is used in this way.

```
<ome:Image ID="Image:0"
     Name="./ome.1.A1.FL-1 (500-530 nm).tif">
```

AcquiredData is the date and time at which the image was captured in UTC format, serialized using the .NET XmlDateTimeSerializationMode.RoundTripKind mode to preserve time zone information.

```
<ome:AcquiredDate>2013-04-26T13:31:01.1358824Z</ome:AcquiredDate>
```

- Pixels:SizeX is the number of samples per line in the tiff.
- Pixels:SizeY is the number of lines in the tiff image
- Pixels:PhysicalSizeX is the x resolution in microns
- Pixels:PhysicalSizeY is the y resolution in microns
- Note that the SizeX, SizeY, PhysicalSizeX and PhysicalSizeY are not strictly compliant with the OMETiff standard; the image may use the tiff orientation tag according to the options set and SizeX and SizeY are the dimensions along the rows of pixel data, not the actual physical direction.
- SizeZ, SizeC and SizeT are always 1. Cellista does not generate OME metadata corresponding to multi-file Z position, channel number or time points. Separate OMETiff files are linked by the plate and scan id but are not otherwise managed as a set.
- Type gives the numeric type of each pixel data. Cellista-generated tiffs are normally unsigned 16-bit integer greyscale images, but could contain unsigned 8-bit data.

```
<ome:Pixels  ID="Pixels:0"
             DimensionOrder="XYZCT"
             Type="uint16"
             SizeX="462"
             SizeY="3700"
             SizeZ="1"
             SizeC="1"
             SizeT="1"
             PhysicalSizeX="8"
             PhysicalSizeY="1">
```

- Channel:AcquisitionMode is WideField for both **acumen** and **mirrorball**.
- Channel:Id is the actual zero-based channel index rather than the index with respected to the generated tiffs. Note that this is not used to link separate channels from multiple tiff files.
- Channel:Name is the name of the channel as presented to the user
- Channel:IlluminationType is always Epifluorescence
- Channel:ExcitationWavelength in nm is only reported if a single laser enabled. The standard doesn't support multiple wavelengths, as can happen with **mirrorball**.
- Channel:EmissionWavelength is not reported for **mirrorball**'s scatter channels, otherwise it is set to the middle of the range of the filter for the given channel, in nm.
- Channel:ContrastMethod is Darkfield for **mirrorball**'s scatter channels and Fluorescence in all other cases.
- Channel:Color is specified in 32-bit RGBA format. Note that the OME documentation does not specify colours correctly, see http://www.openmicroscopy.org/community/viewtopic.php?f=6&t=1130
- DetectorSettings:ID is the same as the Detector element above
- DetectorSettings:Voltage gives the PMT Voltage for the channel used to acquire the image, in Volts.
- Annotation:FilterRange refers to a structured annotation below.

```
<ome:Channel AcquisitionMode="WideField"
             ID="Channel:0"
             Name="FL-1"
             IlluminationType="Epifluorescence"
             ExcitationWavelength="488"
             EmissionWavelength="515"
```

```
                    ContrastMethod="Fluorescence"
                    Color="620691711">
          <ome:DetectorSettings ID="Detector:acumen_00000"
                                Voltage="500" />
          <sa:AnnotationRef ID="Annotation:FilterRange" />
        </ome:Channel>
```

- TiffData:FirstC, FirstT, FirstZ and IFD are all 0; we do not implement the OMETiff standards multi-file set functionality.
- TiffData:PlaneCount is similarly always 1
- UUID:FileName gives the name of this image. It is the same as the Image:Name attribute above. The body of the UUID element is the same as the OME:UUID above.

```
      <ome:TiffData FirstC="0"
                    FirstT="0"
                    FirstZ="0"
                    IFD="0"
                    PlaneCount="1">
        <ome:UUID FileName="./ome.1.A1.FL-1 (500-530
nm).tif">urn:uuid:a2221c1a-aa70-4af2-a326-5d729f20a03f</ome:UUID>
      </ome:TiffData>

    </ome:Pixels>

  </ome:Image>
```

The structured annotations include some data additional to the OMETiff standard.

- FilterRange gives the range of the filter in the detection channel used to acquire this image
- Scan gives the name of the scan used to acquire this image. This will normally be the laser wavelength but **acumen** supports multiple scans of a well within a single scan of a plate and those scans can be arbitrarily renamed by the user. The name element gives that name.
- WellProperties (see section 8.2.1) lists the values of user-defined Well Properties for the well of interest, as well as whether they are textual or numeric.

```
  <sa:StructuredAnnotations>

    <sa:XMLAnnotation ID="Annotation:FilterRange">
      <sa:Description>Non OME-standard details of the range in nm of the data
channel used to acquire this image</sa:Description>
      <sa:Value>
        <Minimum>500</Minimum>
        <Maximum>530</Maximum>
      </sa:Value>
    </sa:XMLAnnotation>

    <sa:XMLAnnotation ID="Annotation:Scan">
      <sa:Description>Non OME-standard details of the scan used to acquire
this image; relevant when performing multiple scans within a single data
file</sa:Description>
      <sa:Value>
        <Name>488</Name>
      </sa:Value>
    </sa:XMLAnnotation>

    <sa:XMLAnnotation ID="Annotation:WellProperties">
```

```
      <sa:Description>Non OME-standard user defined well property
values</sa:Description>
      <sa:Value>
        <Concentration Type="Numeric">
          <Value>100</Value>
          <Units>mol/m3</Units>
        <Concentration>
        <Drug name Type="Text">
          <Value>Paracetamol</Value>
        <Drug name>
      </sa:Value>
    </sa:XMLAnnotation>

  </sa:StructuredAnnotations>
</ome:OME>
```

18 APPENDIX E: SETTING UP A NEW PLATE TYPE FOR ACUMEN

PLEASE NOTE: If you wish to add a new plate, or change the information contained within the Plate Definitions in the cellista software, first consult acumensupport@ttplabtech.com as any damage that is made to the instrument is not covered by the warranty or service contract.

New Plate types cannot be added to mirrorball, except by TTP Labtech Ltd.

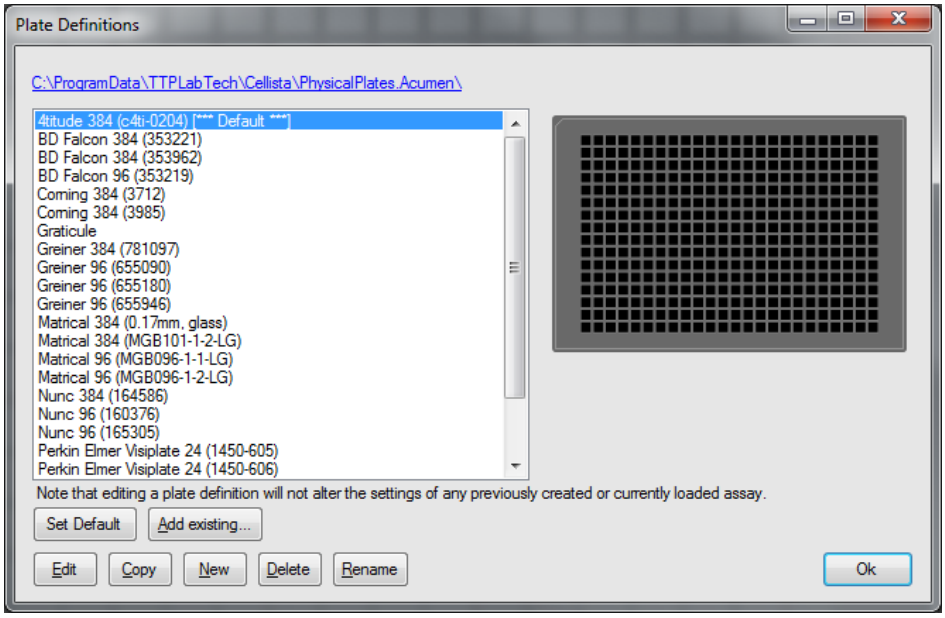
PLEASE NOTE: if you intend using the plate on a Thermo Orbitor robot, the plate stacking height must be determined by TTP Labtech. If this is not done, the Orbitor robot is liable to either not pick up plates in the stack or it can drop plates.

We recommend that if you want to use a plate not defined in cellista software, you send the plate to the UK TTP Labtech offices and we can optimally set up the plate definition for you and send the definition file. Should you wish to setup the plate definition yourself, please follow these instructions.

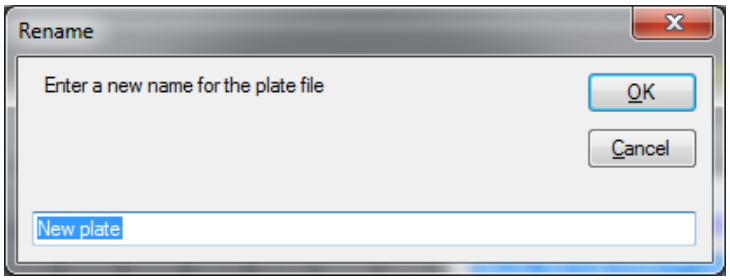
To obtain optimal results from a new plate type on the acumen, its settings must be correctly defined. This ensures that the wells are correctly aligned and that the appropriate focus values are used. Before beginning the procedure, see section 11.2.1 to confirm the plate type is compatible for use with acumen.

18.1 Step A – Enter Basic Plate Definitions

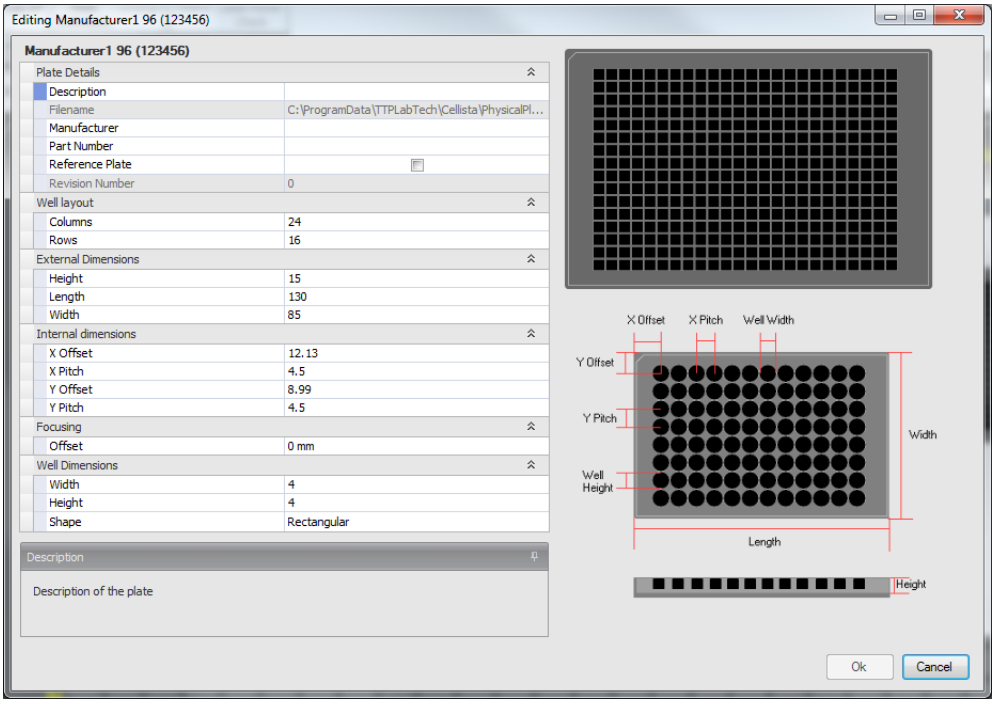
- Go to **Diagnostics | Instrument | Plate Definitions**.
- This opens the **Plate Definitions** window:



- Click on “New” and then enter a name for the new plate type in the “Rename” window that opens. TTP Labtech naming convention uses manufacturer_number of wells_(catalogue number) e.g. Corning 384 (3712). TTP Labtech would suggest this naming convention is adhered to for clarity although users are of course able to use any name they would like.



- Click “OK” and the editing window for the newly named plate type opens:



- Fill in the plate details from the manufacturer's technical drawings of the plate type. The description box can be used to enter information such as "tissue culture treated, Sterile, poly-lysine coated" etc – this information will then always be available to the user. One important point to note is that in the external dimensions section, the "height" the ***stacking height*** of the plate, not the overall plate height. This value must be correct if an orbitor plate stacker is connected otherwise this would cause errors in plate handling.
- At this stage, leave the Focussing Offset as 0mm.
- When all information has been entered, click "Ok" to close the editing window.
- Click "Ok" to close the "Plate Definitions" window.

18.2 Step B – Batch Focus Run

Fluorescent beads are used to determine optimal focus position. Please contact acumensupport@ttplabtech.com to obtain suitable beads.

- Add beads (from stocks at ~ 50,000 beads/ml) to wells of the new plate type as suggested in the table below:

Plate format	Wells to add beads	Volume per well (µL)	Number of beads per well
96 well plate	C3 – D4	100	5000
384 well plate	E5 – H8	50	2500
1536 well plate	I9 – P16	5	250

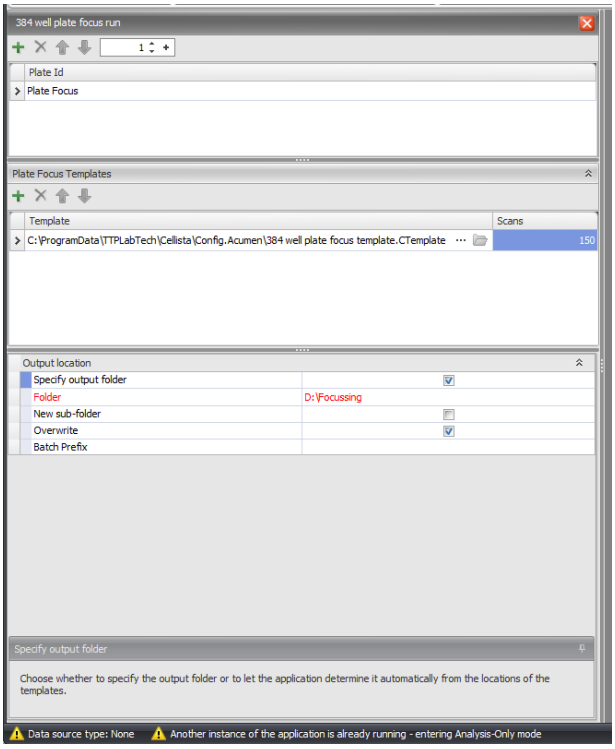
- Leave plate at room temperature for 1 hour or more in the dark to allow the beads to settle to the bottom of the plate. Alternatively, centrifuge the plate to bring the beads to the bottom of the wells.
- Cellista software comes preinstalled with a focus template for each of 96, 384 and 1536 format plates. Open the appropriate format (number of wells) template by going to

File | File | New | Browse for Template….

Navigate to: C:\ProgramData\TTPLabTech\Cellista\Config.Acumen and select the appropriate template from the list shown below:

- 96 well plate focus template.CTemplate
- 384 well plate focus template.CTemplate
- 1536 well plate focus template.CTemplate

- When the template is open, go to **Scan Setup | Dimensions | Plate** and select the new plate type to be focussed from the list. This changes the plate type only. Ensure that the appropriate wells are still marked for scanning.
- The preloaded focus templates have been setup for use on a four channel, three laser acumen system equipped with 405, 488 and 640nm lasers and channels FL1 (500-530nm), FL2 (530-585nm), FL3 (575-640nm) and FL4 (>655nm). Adjust the lasers/channels as appropriate for the instrument by manually changing channel options or by selecting **Scan Setup | Advanced | Overwrite Setup**.
- Go to **File | Save | Save As | Save As Template** and save the template.
- Open the drawer ( ), load the plate and close the drawer again ( ).
- Go to Batch tab
- Cellista software comes pre-loaded with batch focus template. Go to Batch | File | Load | Browse… Navigate to C:\ProgramData\TTPLabTech\Cellista\Config.Acumen and select the appropriate batch template from the list shown below:
    - 96 well plate focus run.CBatch
    - 384 well plate focus run.CBatch
    - 1536 well plate focus run.CBatch

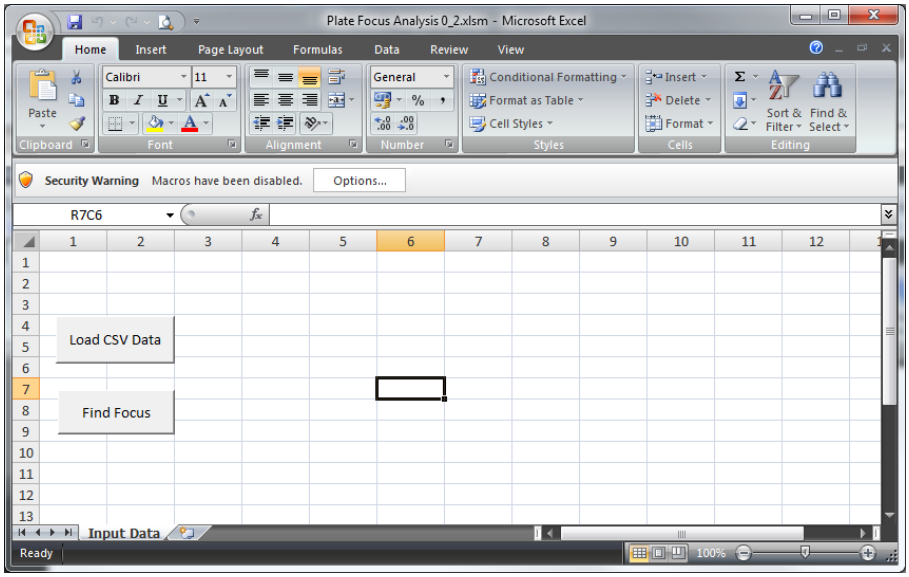


- When the appropriate batch template has loaded, go to the Templates section, click on "…" and browse to the location where the newly-created template was saved. Select the template created in earlier steps.

- In the Output Location section, folder section, define an appropriate location to save the data.
- Begin the batch focus scan by going to **Batch | Control | Scan**
- The system now performs a continuous batch run of scans, gradually working through the focus range of the instrument. This takes approximately 4 hours.
- When the batch focus run is completed, it will have generated a single summary plate results csv file in the location specified by the user.

18.3 Step C – Analyse Focus results to determine plate focus position

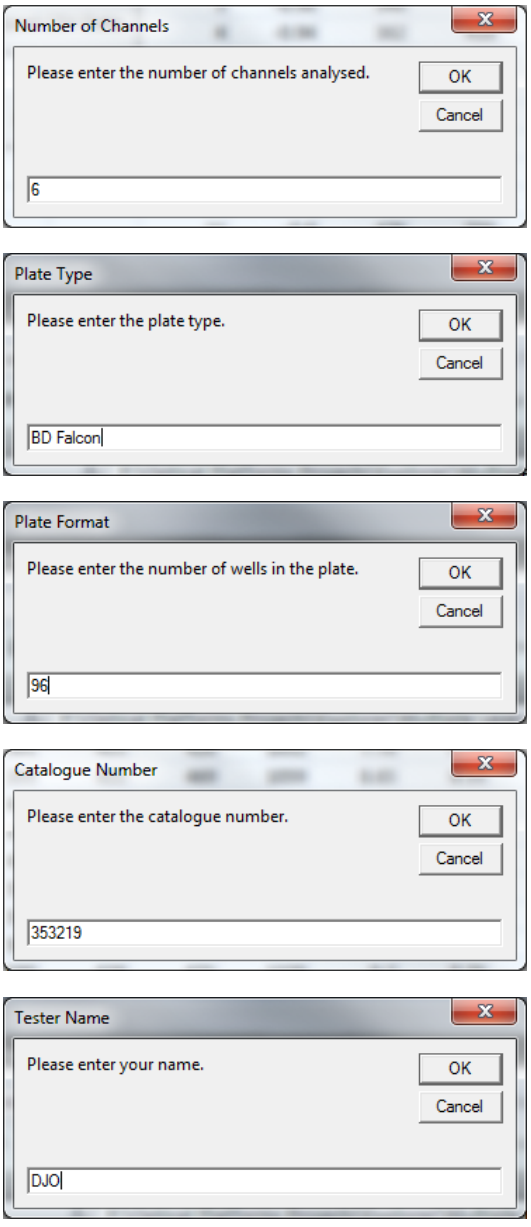
- Open Microsoft Excel.
- Go to open a new file and navigate to **C:\ProgramData\TTPLabTech\Cellista\Config.Acumen** and select the file “Plate Focus Analysis 0_2xlsm”.

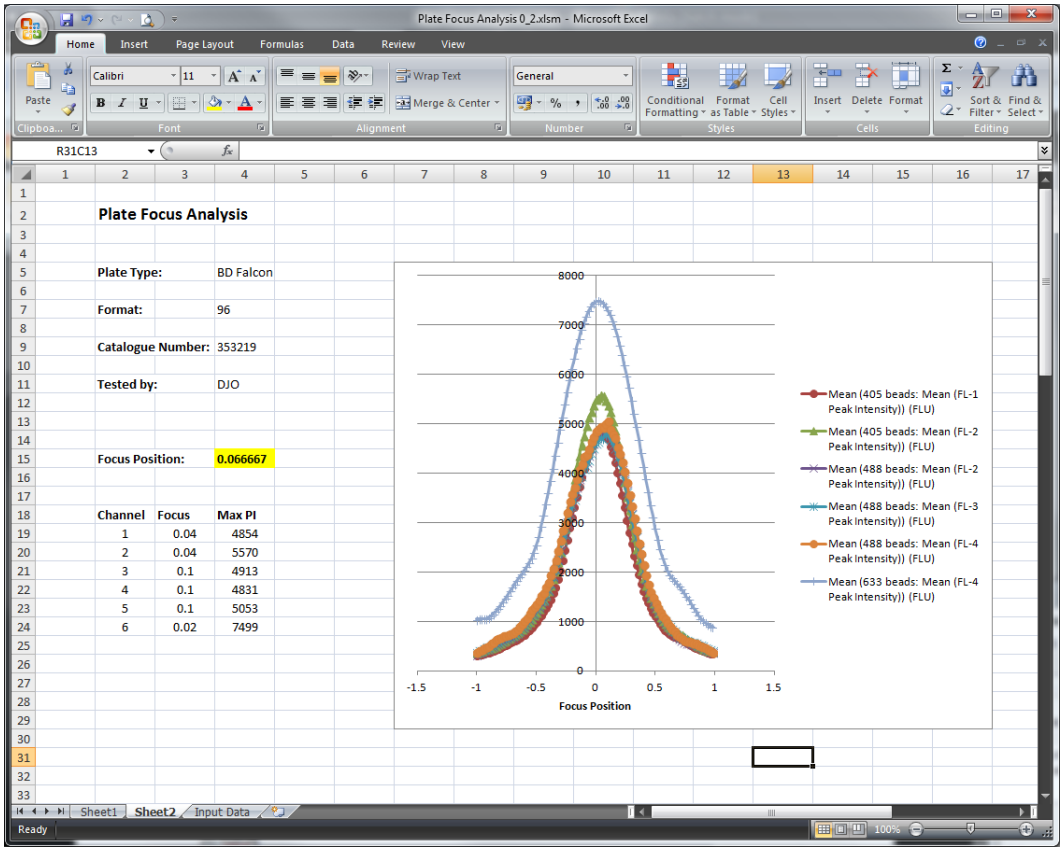


- You may need to change the excel security settings to allow the macro to run correctly. Click on the options button next to the “Security Warning” message and select “Enable this Content”, then click “ok”.

- Click on the “Load csv Data” button.
- Select the summary plate results csv file that was generated by the batch focus run and click “open”.

- When the data has been imported, click on the “Find Focus” button and this then leads the user through multiple dialogue boxes to enter information about the plate type that has just been focussed. The example below has been completed for BD Falcon 96 well plate catalogue number 353219 that was focussed by user DJO:





- On completion of these steps, the focus data is summarised as shown above. The focus position for the plate is shown highlighted yellow.

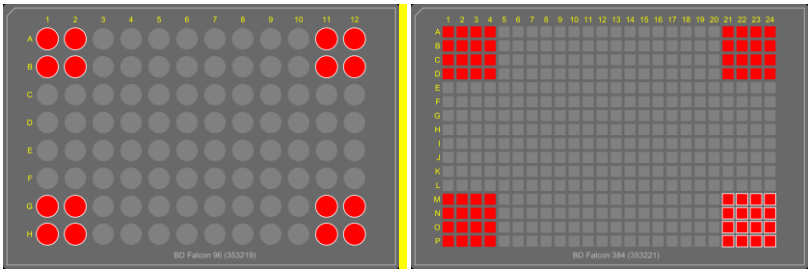
18.4 Step D – Finalise Plate Definitions

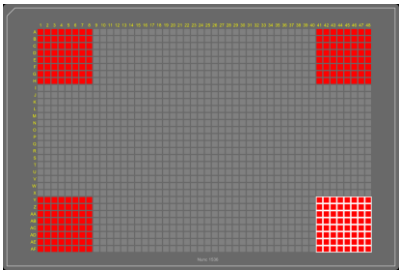
- Go back to Cellista.
- Go to **Diagnostics | Instrument | Plate Definitions.**
- Select the newly defined plate from the list and click “Edit”.
- In the Focussing Offset box, enter the Focus Position determined by the Plate Focus Analysis excel sheet.
- The well locations now need to be fine-tuned to ensure accurate well location

18.5 Optimising Well Alignment

Beads in the well are not required for this process. Correct alignment ensures that the whole well is scanned throughout the plate and any plate moulding inaccuracies are accounted for. Also a correct well alignment allows for faster scan times.

- Place the plate in the acumen.
- Select the plate type to be adjusted in **Scan Setup | Dimensions | Plate**.
- Scan at 1 by 20um resolution Select the 488nm laser and put the PMTs to 750V on all channels. Ensure Tiff files are enabled
- Scan the wells as shown below depending on which plate type you are using:



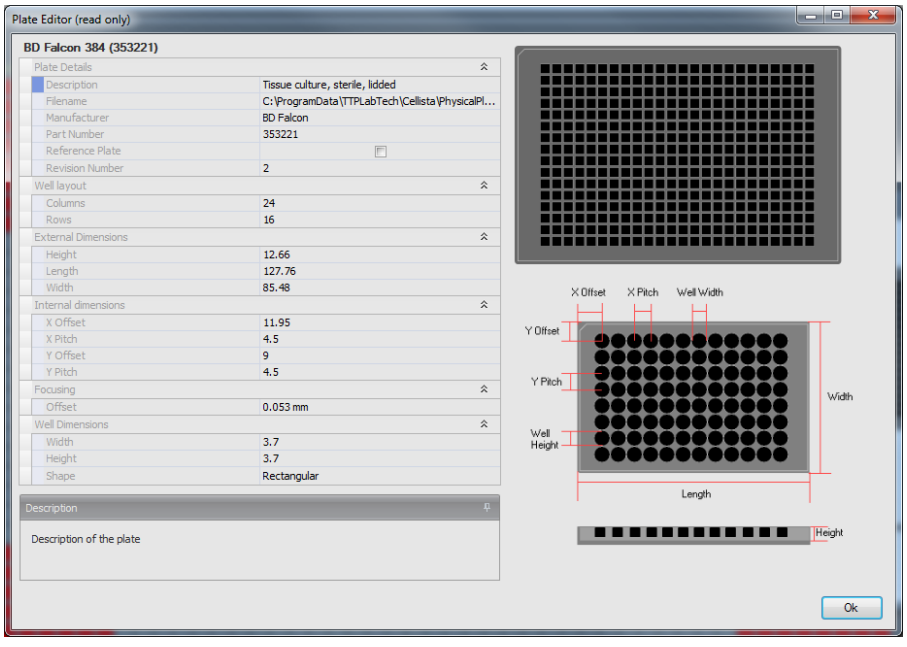


Open up a Well View and link the Plate view to the Well View. Ensure that in the **Well View | Settings | Data mode** that tiffs are selected.

Then View the position of the well in the scan area. If it is not central, the offsets need to be adjusted.

- Go to **Diagnostics | Instrument | Plate Definitions**
- Select the Plate Type to be edited. Select Edit

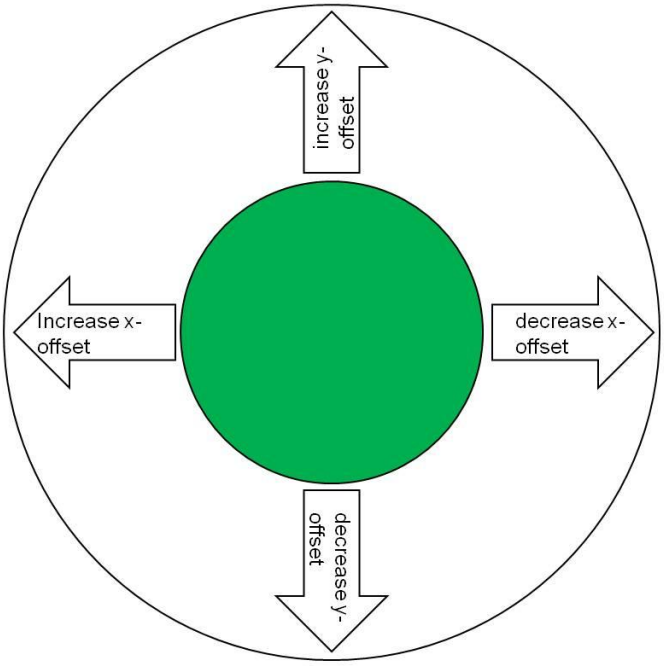
The following box opens.



To Adjust the well position, the X Offset and Y Offset **ONLY** need to be adjusted.

Internal dimensions	
X Offset	12.1
X Pitch	2.25
Y Offset	8.99
Y Pitch	2.25

To Adjust the X Offset and Y Offset in the correct direction, use the following graphic to move in the correct direction.



- Change the values at approximately 0.1µm steps. Click on OK.
- Reselect the plate type such that the adjusted offset values are used
- Rescan the wells
- Look at the well Views again and see if the change is correct.
- Repeat the above process until all the wells are within the scan area.

19 APPENDIX F: CONVERTING FROM ACUMEN EXPLORER

Cellista can open data and template files created by **acumen** Explorer software versions 3.2.13 onwards. This functionality is normally only available where an **acumen** system has been upgraded to Cellista, and will appear in the user interface only if the following key is present in the HardwareEnable.config file:

```
<add key="IsShowFileConverter"  value="true" />
```

19.1 Opening and saving files from acumen Explorer

Cellista can save modified **acumen** files but only in Cellista format, i.e. once modified by Cellista, **acumen** Explorer will not be able to open them.

To open a data file select **File | Open** as normal, then in the file type drop-down box select *Legacy **acumen** Data Files.*

Opening large **acumen** data files can be slow, and the progress indicator may show a scrolling green line without indicating the time remaining. It is suggested that you save the data file in Cellista format to speed up future access. Note that while Cellista can save modified **acumen** files it can only do so in Cellista format, i.e. once modified by Cellista, **acumen** Explorer will not be able to open them.

To create an assay from a legacy template select File | New | Browse for Template as normal, then in the file type drop-down box select *Legacy **acumen** Template Files*.

19.2 Batch Conversion

19.2.1 Converting Multiple Templates

It is possible to convert multiple **acumen** Explorer template files at once. To do so select **File | Convert Templates**, then navigate to the folder which contains the PlateTemplate files and select all of the templates to be converted and press Ok. Cellista CTemplate files will be created in the same folder, with the same filename as those you have selected.

The System Log (**View | Logs | System**) will display any warnings or errors raised.

19.2.2 Converting Multiple Data Files

At present it is not possible to convert multiple **acumen** data files at once.

19.3 Conversion Considerations

Not all scan settings in **acumen** Explorer have direct equivalents in Cellista. Cellista will warn you if it encounters any such settings, and then use the closest equivalent setting.

Some of the issues which may be encountered include the following:

- Cellista does not yet export FCS data files
- Cellista does not allow the user to specify the scan direction; it always uses Minimum Moves
- Cellista does not support the Locator integrated microscope system
- Certain obscure deprecated object characteristics will be deleted on conversion
- The decimation sampling filter has been deprecated
- All of the plate definitions included with Cellista has been reviewed. Consequently the plate definitions embedded in **acumen** data files and templates may differ from those used by Cellista. The embedded plate measurements will still be used in converted files but TTP LabTech recommend that you migrate to the new plate definitions. If a plate definition you require is not present then please contact TTP LabTech for assistance. If absolutely necessary it is possible to convert a physical plate used in an **acumen 3.4** template or data file to a Cellista .PhysicalPlate file by loading the legacy file and then selecting **Scan Setup | Plate | Export.**

- The compression scheme to generate 8 bit tiffs has changed, so tiff images may not be directly comparable
- When merging objects from separate scans the only strategy employed is *Accurate* merging. *Overlap* and other strategies have been deprecated
- The half-width specific intensity characteristic is calculated more accurately in Cellista and may give different results when data is reanalysed
- Various statistical calculations and characteristics based on standard deviations now use the corrected sample standard deviation rather than the uncorrected population standard deviation, which may lead to marginally different results
- The hit-picker characteristic is still available in Cellista but much greater functionality is afforded by Well Populations (see section 8.2.2)
- The 3rd party component used to implement mathematical expression characteristics has changed, which means that not all expressions implemented by **acumen** can be converted to Cellista. Expressions which made use of conditional operators (if statements) are the most likely to be compromised
- The format of csv output files has changed to improve consistency and machine-readability, as well as to support new functionality. This may affect tools which automatically parse output data such as those which import csv files into database systems
- The automation interface has changed. Systems integrated by 3rd parties may need to make arrangements with the integrator to migrate to the new interface. Please see section 16 for more details. Or they can use the supplied ExplorerCOM, see section 20.

20 APPENDIX G: EXPLORER COM

ExplorerCOM is designed to aid with upgrading from an existing **acumen** Explorer with a 3rd party automation integration. New automation integrations should use the WCF interface.

ExplorerCOM provides a COM to WCF shim that can be registered as the COM interface provider instead of the **acumen** Explorer software. It marshals the COM calls through to the Cellista WCF interface.

It implements the IScan, IScan2, IInstrument and IInstrument2 interfaces and provides the IAcumenExplorerEvents. Methods not available in WCF are stubbed and return `S_FALSE`;

It does **not** implement the IAcumenExplorer interface used by DLL integrations (all methods are stubbed and return `E_NOTIMPL`).

The IDL is binary compatible with **acumen** Explorer.

See the **acumen** Explorer manual for details of these interfaces.

20.1 Installation

To install ExplorerCOM follow the Cellista installation in section 16.3:

- Log on to the PC as an administrator
- Install the required 32bit Microsoft Visual C++ runtime from the CD by running vcredist_x86.exe
- ExplorerCOM.exe should be located in the same directory as the Cellista.exe (usually C:\Program Files\TTPLabTech\Cellista). To register for COM, open cmd.exe (as administrator) and run the command:
  - ExplorerCom /RegServer
- For existing **acumen** Explorer installations this will replace the registry entries with ones pointing to ExplorerCOM. To cleanly revert you would need to run the two commands:
  - ExplorerCOM /UnregServer
  - AcumenExplorer /RegServer

20.2 Remote PC

Although the WCF interface allows remoting, ExplorerCOM is designed to run on the same PC as Cellista. This is because it will attempt to start Cellista if it is not running.

For integrations that require remote control of the instrument, this should be done through DCOM; as it would have been with the **acumen** Explorer software.

20.3 Configuration

Cellista's automation interface must be configured to emulate the behaviour of **acumen** Explorer. To do this the following automation options must be set in HardwareEnable.config:

<Automation IsKeepFirstPlateLoaded="True" IsKeepLastPlateLoaded="False"/>

In the case where ExplorerCOM launches Cellista it will delay to allow the Cellista software to initialise. This delay can be configured by setting the appSetting "DelayAfterStartingCellista" that can be found in the ExplorerCOM.exe.config file. The value is specified in milliseconds.

If the existing integration expects IsRunning to return true as soon as the call to Start returns then the appSetting "RunningAsSoonAsStartCalled" should be set to true. If this is set to false IsRunning will only return true while scanning, i.e. the draw movement operations (load/eject) are excluded from the IsRunning state.

20.4 Diagnostics

ExplorerCOM will generate log files in the same directory as Cellista's logging. These logs will detail the interactions with the COM interface.

20.5 Behaviour Differences

The unsupported methods and properties are stubbed and will return `S_FALSE`, so the COM `SUCCEEDED` macro will still pass.

The methods Start and StartWhenWarmedUp will behave the same (start when warm). All their parameters are ignored and assumed to be false.

The following methods and properties have no corresponding mechanism in Cellista and are stubbed:

```
SaveFCSFile
CSVWriteFileHeader
SaveMCFFile
TiffScaleFactor
UseDestination
SetChannelTiffScaleFactor(LONG Channel, LONG ScaleFactor)
GetChannelTiffScaleFactor(LONG Channel, LONG* ScaleFactor)
```

These TIFF properties are stubbed and should instead be configured through the Cellista templates used:

```
SaveTiffFile
TiffIsCompressed
TiffIs8Bit
TiffIsWhiteBackground
```

20.6 VB.NET Example

The VB.NET Example project supplied with the **acumen** Explorer software can be used to verify ExplorerCOM is installed correctly.

Note: The VB.NET Example does not cleanly dispose of its COM connections. To rectify this, the `AcumenDemoForm_FormClosed` method in `AcumenDemo.vb` should be modified to include the additional two lines, before `GC.Collect()`:

```
Private Sub AcumenDemoForm_FormClosed(… )
    Scan_m = Nothing
    Instrument_m = Nothing
    GC.Collect()
End Sub
```

Without this modification the ExplorerCOM.exe will be left running when the VB.NET Example program is closed. This will prevent further connections from succeeding until the ExplorerCOM.exe process is ended (through TaskManager). Most likely this will be seen as an RPC_E_SERVERFAULT; ExplorerCOM will throw an exception if the Cellista WCF connection fails.

20.7 Template Filenames

Some existing integrations may automatically apply a `.PlateTemplate` extension to the specified templates. Provided this has been saved in the `.CTemplate` format used by Cellista this will work.

For example, if you have an existing template named `method.PlateTemplate`, which is specified through your automation interface as `method`. These are the steps to follow:

- Load `method.PlateTemplate` into Cellista. Cellista will convert this into the new format [or see 19].
- Review the settings for this template; all conversions should be reviewed [see 19.3].
- Save this as `method.PlateTemplate` (or whatever name is required by your integration system).
- Cellista's automation interface, and therefore ExplorerCOM, assumes that the template files provided are in `.CTemplate` format (regardless of their name) and will not perform any conversion.

NOTE:

Cellista however, will attempt to perform the conversion again if you try to reload a converted template with a `.PlateTemplate` extension. This will fail. If you need to modify these templates you will need to rename them back to `.CTemplate`, to prevent this automatic conversion process.

21 APPENDIX G: REVISION HISTORY

Revision	Date	Comments