

Solentim

# Cell Metric™ user guide

How to operate the Cell Metric™



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## Solentim Cell Metric

### Introduction

The Cell Metric from Solentim is a cell imaging instrument designed for identifying live cells growing in media in cell culture microplates and other selected labware.

The system supports a number of applications and workflows which are described in this guide.

### Basic concepts

Each **application workflow** is presented separately in the Cell Metric software, so you only need to learn the software features specific to the workflows you want to use.

You can modify the settings for a particular workflow so that the application meets your requirements, for example by specifying the microplate type you want to use and parameters specific to your cell line. You can then save these settings into a **scan profile** so that you can easily use them later.

Often, you need to scan lots of plates all with the same scan settings, and you may have to scan the plates repeatedly over several days as the cells grow. The software provides two concepts to assist you. Firstly, you can group a number of plates into a **batch**, which you can then manage separately from other, unrelated, batches.

Secondly, the software allows you to use a stored scan profile to scan a batch of plates without being able to alter the scan settings. This ensures that the plates are all scanned with identical scan settings. To set up a new scan profile or change the scan settings in

an existing scan profile, you must start a **trial scan**. Once the scan settings have been defined and you are ready to use them to scan a batch of plates, you start a **normal scan**.

These concepts are discussed in more detail later in this guide.

## Starting the software

To open the software, click the 'Cell Metric' icon on the Windows desktop of the PC.

## Turning on the Cell Metric

There are several ways to start scanning a microplate using the Cell Metric software. For each of them, the Cell Metric instrument must first be switched on using the follow sequence.

First turn on the Cell Metric instrument and wait 30 seconds for it to fully initialise. Start the Cell Metric software on the computer by clicking the 'Cell Metric' icon, and then press either (a) the 'Scan plate' button on the software dashboard page, (b) either of the scan buttons on the scan page or (c) the scan button on the profiles page. Follow the on-screen instructions as explained below.

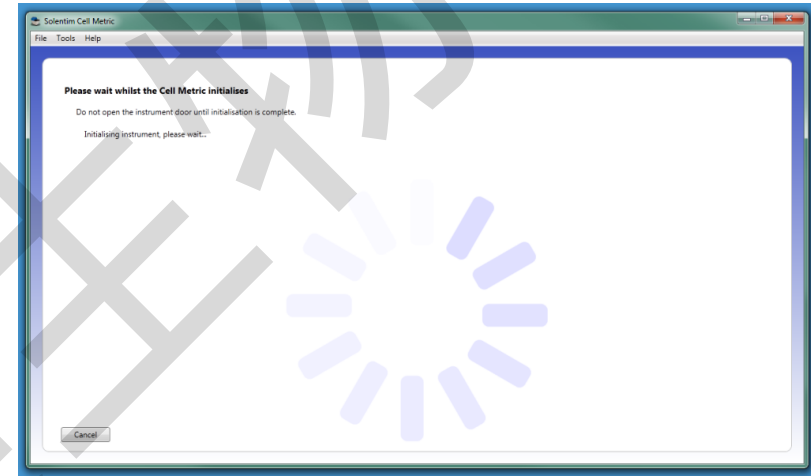
## Instrument initialisation

Important: turn on the Cell Metric instrument and wait at least 30 seconds for the instrument to fully initialise before you click any of the 'Scan' buttons, otherwise the instrument may not initialise and an error message will be displayed.

The first time the software is run, it will initialise the Cell Metric. This process includes several stages of initialisation and auto-calibration, and it is important to ensure that the instrument door remains closed throughout the process. The initialisation sequence can take a minute or so. Once the initialisation has completed, the microplate carriage in the instrument will be in the 'Eject' position,

and you can load a microplate. The microplate selection screen will automatically be displayed at this point (see next section).

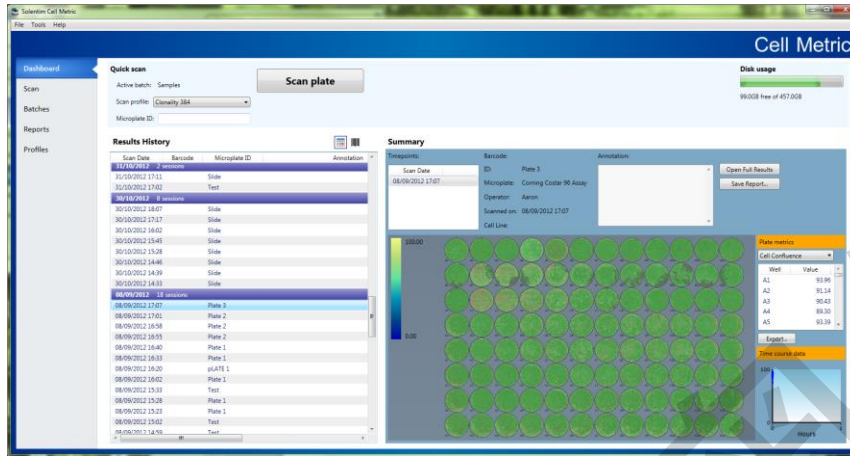
If you restart the software without powering down the instrument, a shorter initialisation sequence will run, which completes with the microplate carriage in the 'Eject' location.



For information on starting your first scan, see the section Scan profiles on page 6.

## Dashboard

The **dashboard** is the first screen that appears when the software opens. It groups together some of the most commonly-used features of the software, and it allows you to scan microplates and view results from previous scans.



## Selecting pages

The software is divided into **pages** which you can access using the navigation list on the left of the dashboard. The pages available include:

### Scan

Use this page to start a new scan. You can see a list of the currently-defined scan profiles and you can scan a plate using an existing scan profile without changing any settings.

### Batches

Use this page to view the list of batches you have created, to create a new batch and also to see the list of plates and scan results in each batch.

### Reports

This page allows you to generate reports based on all of the plates in a batch.

### Profiles

This page lets you view the currently defined scan profiles, create new scan profiles and change the settings in existing scan profiles.

### Quick scan

The **quick scan** controls at the top centre of the dashboard allow you to quickly scan a plate using an existing scan profile. Simply select the scan profile from the list and, unless the scan profile is set to read plate barcodes, provide a 'Microplate ID' to identify the plate. Finally, click the 'Scan Plate' button. The instrument will initialise if necessary, and then scan the plate. For more details on the scan process, see the Scan section later in this guide.

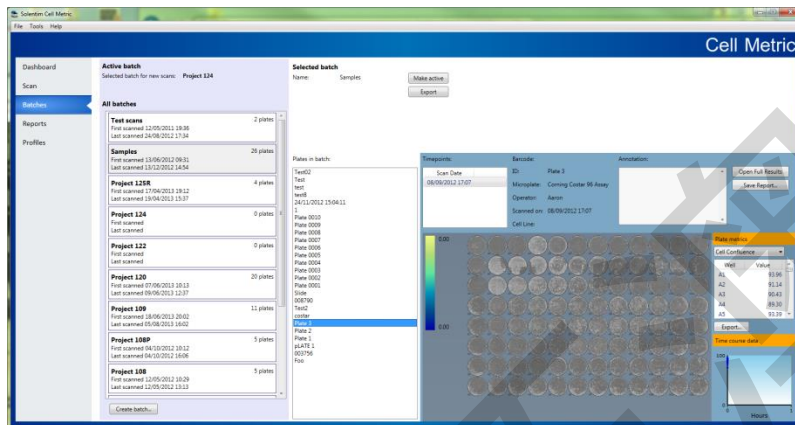
### Results history

This section takes up most of the dashboard and shows the list of scan sessions for the active batch on the left. Clicking on any of these sessions displays the results summary for that session of the right, including a thumbnail view of each well if applicable. Right clicking on a session allows you to exclude it from the reports.

## Batches

A **batch** is a group of plates that are in some way related and are managed separately from other groups of plates. Examples of batches include the plates for a particular project, plates that have been seeded at the same time, or plates seeded by the same person.

You can perform operations on all of the plates in a batch, such as generating a report to find wells that match specific criteria. You can also perform some operations just on individual plates in a batch, such as rescanning a single plate.



## Batches page

The batches page lists the batches you have defined on the left, and lets you view the results of the scan sessions on the right.

## Active batch

When you scan a plate, the results are stored in the **active batch**. Only one batch can be the active batch at a time, but you can

quickly change which is the active batch if you need to scan plates from more than one batch during the course of a day.

To change the active batch, simply select the batch in the list on the left and click the **'Make active'** button. All subsequent scans will be assigned to the batch you have selected until you make a different batch active.

## Exporting metrics

You can export metrics for all of the plates in a batch. Simply select the batch from the list on the left, then click the **'Export'** button. You can then select the metrics you want to export.

## Viewing results

Once you have selected a batch, you can view a summary of the scan results for the plates in the batch. Once you have selected a summary for a specific time point, you can view the full results for that plate by clicking the **'Open Full Results'** button. You can also generate a summary report for the selected time point by clicking the **'Save Report...'** button. Right clicking on a session allows you to exclude it from the reports.

## Creating batches

To create a new batch, click the **'Create batch...'** button and provide a description name for the batch.

Creating a new batch creates a new folder on the computer's hard drive to store the new results in. You can't delete a batch from the software, but you can move the folder to an archive disk or delete the folder and all of its results if you need to make space for more scan results.

## Scan profiles

A **scan profile** is the group of settings used to scan a plate. The Cell Metric supports a number of different applications. Each of these applications is defined by a particular **scan type**. The software includes a library of scan types, organised into various application areas. Not all of the scan types listed here may be available in your software.

## Cell line development

This category includes profiles to support single cell seeding and monitoring clone growth rates over time.

### Verify clonality

This scan type is used on the day that single cells are seeded into individual wells, either by FACS or by limiting dilution. Each well can then be checked for the presence of a single cell.

### Monitor clone growth

This scan type helps you monitor the rate of growth in each well in the days following seeding.

## Cell culture

This category is for general-purpose scan profiles to help you monitor cell growth over time.

### Monitor cell growth

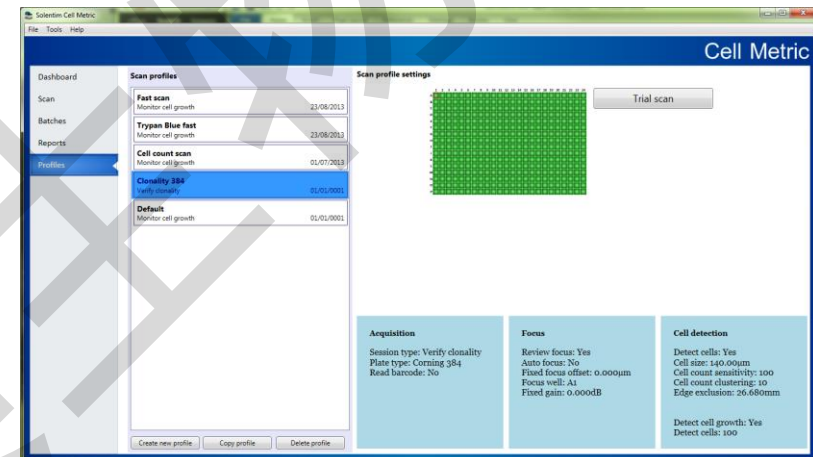
This scan type lets you monitor the change in confluence of each well over time.

## Monitor cell numbers

This scan type lets you monitor the change in the number of cells in each well over time for selected cell types.

## Creating a new scan profile

To create a new scan profile, first select the **Profiles** page and then click the **Create new profile** button.



A dialog box will appear listing each of the scan types that are available. Select the scan type appropriate to your application, then provide a **profile name** and optional description to help you identify the scan profile later.

Once you have created a new scan profile, you must then adjust the default scan settings in the profile to meet the needs of your biology. You will be prompted to do this once the new scan profile is created, or you can edit the scan profile at a later date.

## Editing an existing scan profile

To edit an existing scan profile, first select it from the list of scan profiles on the left. Once selected, the **Scan profile settings** display on the right of the screen shows a summary of many of the settings for the selected profile. You can use this to quickly check the settings, such as the microplate type, without having to scan a plate.

To edit the scan profile settings, you must scan a microplate. If you intend to use the scan profile to scan a number of plates, the plate you scan when editing the scan settings must be representative of the other plates, otherwise the scan settings you edit will not work well on the rest of the plate.

Click the **Trial scan** button to begin scanning a plate to edit the scan profile settings.

Each scan profile type is designed to support a specific application, so the exact settings that are required in each scan profile type vary. Most profile types are arranged into three stages: (a) select the wells to scan, (b) select the focus position and (c) set the cell detection parameters.

## Scan

The scan page is where you can start new scans using an existing scan profile. The scan results will be added to the active batch.

To image a microplate and generate cell metrics, first turn on the Cell Metric instrument and wait 30 seconds for it to fully initialise. Start the Cell Metric software on the computer by clicking the 'Cell Metric' icon.

Before you can scan a microplate, you must first set up a scan profile as described above in Profiles. Once a scan profile has been set up, you can use it to scan a batch of plates without making changes to the scan settings in either of the following ways.

### Quick scan

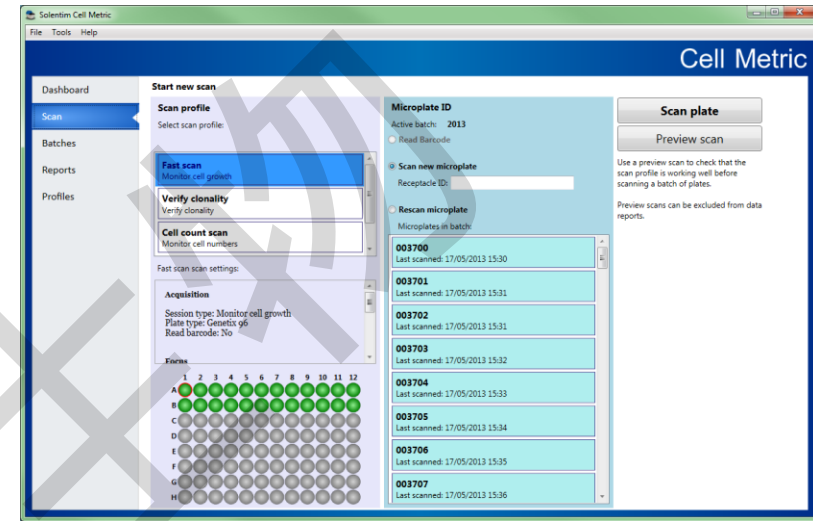
On the dashboard page, you can quickly start a scan by selecting the scan profile from the drop-down list, typing a microplate ID (if the scan profile does not include barcode reading) and then clicking the 'Scan plate' button.

### Scan page

The **Scan** page is accessed by clicking 'Scan' on the navigation panel of the software start screen. On the left of the scan screen the list of current scan profiles appears. When you select a scan profile, a summary of some of the scan settings appears under the list of scan profiles. You can use this to check that that scan profile you have selected is the one you want to use.

In the middle of the scan page you can specify the Microplate ID. If the scan profile has barcode reading enabled, this option will automatically be selected. Otherwise you can provide a Microplate

ID or, if some plates have already been scanned you can select an existing microplate ID to rescan that microplate.



Once you are ready to scan a microplate you can either scan the plate using a **normal scan** by clicking the 'Scan plate' button or using a **preview scan** by clicking the 'Preview scan' button.

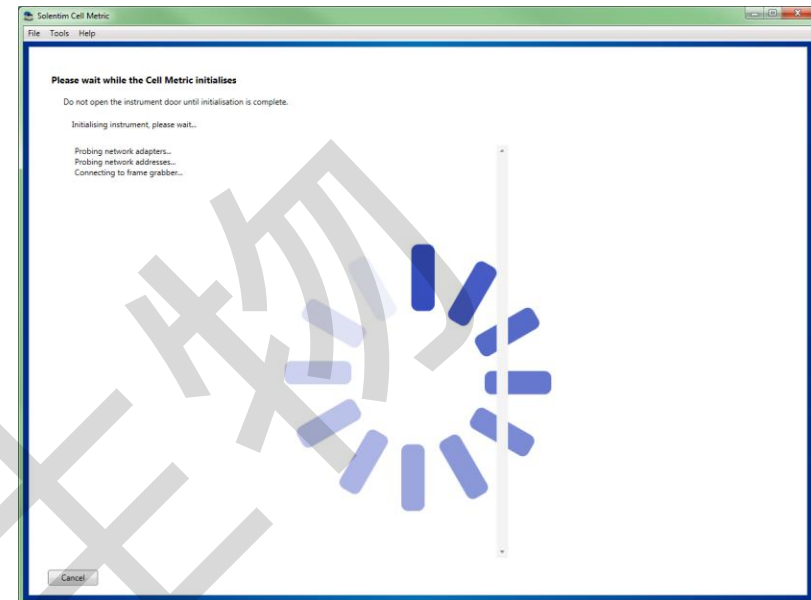
A preview scan allows you to quickly scan a plate to check that the scan settings are working correctly. Preview scans will automatically be marked as excluded, meaning they will not appear in reports by default. You can also choose which wells to scan in a preview scan, so for example you could just scan the first row of wells rather than the whole microplate.

## Instrument initialisation

Important: turn on the Cell Metric instrument and wait at least 30 seconds for the instrument to fully initialise before you click the 'Scan' buttons, otherwise the instrument may not initialise and an error message will be displayed.

The first time the software is run, it will initialise the Cell Metric. This process includes several stages of initialisation and auto-calibration, and it is important to ensure that the instrument door remains closed throughout the process. The initialisation sequence can take a minute or so. Once the initialisation has completed, the microplate carriage in the instrument will be in the 'Eject' position, and you can load a microplate. The microplate selection screen will automatically be displayed at this point (see next section).

If you restart the software without powering down the instrument, a shorter initialisation sequence will run, which completes with the microplate carriage in the 'Eject' location.

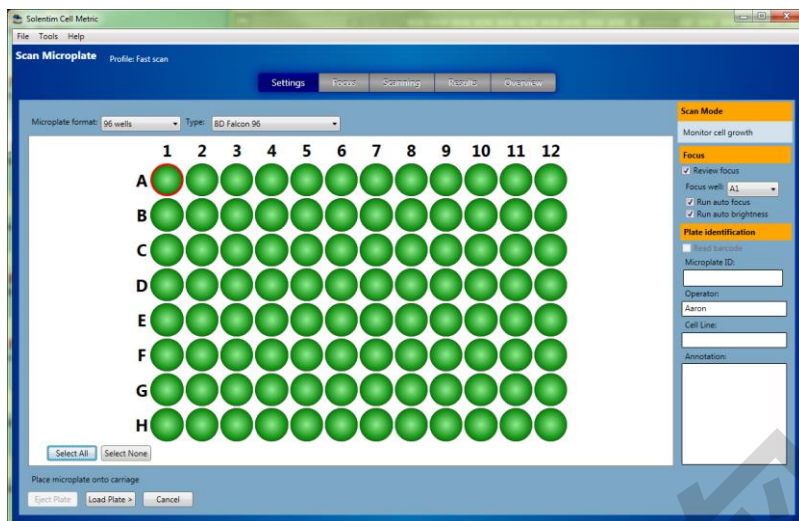


## Load plate

Once the Cell Metric has initialised, you will be prompted to load the microplate onto the plate carriage. Make sure the microplate is oriented correctly.

## Trial scan

When you start a trial scan, you can edit the scan settings stored in the current scan profile.



### Microplate format and well selection

Select the type of microplate to scan from the list of pre-configured plates. The microplate names are grouped by the number of wells in each plate, and flasks are grouped in their own category. To select a microplate, first select the microplate format from the first list – for example, '96 wells'. Next, select the microplate name from the second list, which only lists those microplates that match the format selected in the first list.

Only the specific microplates displayed in the list have been configured. If you have a microplate from a different manufacturer or of a different format, you will not get acceptable results by selecting an incorrect plate from the

list. If you want to use a plate type not listed, contact Solentim Support for a plate definition for the plate type.

If you have several microplates of the same format configured – for example, 3 different 96-well microplates – be sure to select the correct format. Each microplate has slightly different dimensions, so it is important that the selected microplate matches the type of plate you want to image.

You can also select which wells to image. By default, all wells are selected and are displayed in green. To toggle the selection of a well, simply click it with the mouse. You can toggle the selection of an entire row or column of wells by clicking the well or column labels. Deselected wells are displayed in grey and will not be imaged. You must select at least one well.

### Additional scan options

There are several options that you can adjust to affect the scan.

#### Read barcode

Check this checkbox to have the Cell Metric read the barcode on the microplate. If you are not using barcodes, you must supply a 'Microplate ID' instead (see below).

This option is only available if an automatic barcode reader is fitted inside the Cell Metric.

#### Review focus

Checking this option displays the focus screen so that you can check that the stored focus settings for this plate type are correct, and also allows you to adjust the cell detection

parameters. If you are scanning a batch of plates, you may not need to review the focus on each plate, so you can save time by unchecking this option after you have scanned the first plate.

### **Microplate ID**

To identify a microplate each time it is imaged it is necessary to supply an identification that is unique to that plate. If you are not using barcodes, you must instead supply a microplate ID. If you use the same microplate ID each time a given plate is imaged, the results can be aggregated together, for example to view the changes to cell growth over time.

Choose a plate ID like 'Plate 100' rather than 'Tuesday 10:am', so that the ID is the same each time you scan that plate. The software will automatically include the scan date and time.

### **Operator**

If there are several operators of the instrument, you can supply an operator name to help filter the stored results so that you can quickly find the results you are looking for.

### **Cell Line**

Type in a name of the cell line to help filter the stored results.

### **Annotation**

You can provide an annotation to store related information about each result set. This field can be

modified after the microplate has been imaged, so that you can comment on the results at any time.

Once you have selected the settings you want to use for the scan, click the 'Load Plate' button at the bottom of the screen to move to the next step in the process.

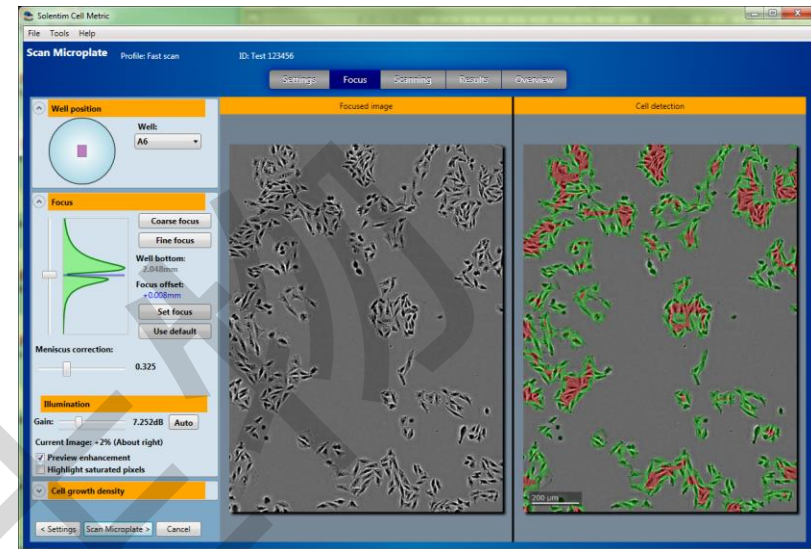
## Review focus

The next steps are to review the focus and cell detection parameters. If you are scanning a batch of microplates, you may be able to skip these steps once you have verified the settings for the first plate.

Once the microplate has loaded, the instrument takes a stack of images at different focal planes around the last used focus position for the current microplate type. The in-focus image is then displayed so that you can verify the focus is correct. This usually means that the first image displayed in the Review Focus screen is an image of cells correctly focussed.

It is usually only necessary to adjust the focus the first time a microplate type is used. The focus offset that you set is then used each time that microplate type is imaged, so the first image displayed on the focus review screen is usually already the optimal focus image.

You can only verify the focus offset if there are cells to focus on. If you have a microplate with very few cells, it is better to pre-focus using a plate with lots of cells and not adjust the focus when you later image the plate with few cells in. Alternatively, you can create a control well with a higher density of cells so that you can focus on that well.



You can cycle through the z-stack of images by sliding the **vertical slider** in the 'Focus' group up and down. The image updates as you move the slider, allowing you to select the best image.

The **coarse focus** button takes a stack of images through the entire focal range of the instrument. This is useful when you first try to focus a plate, or if the stored focus for a plate is wrong. You will typically see several distinct focal planes, corresponding to the bottom and top surfaces of the well bottom. Always select the *top* surface of the *bottom* of the plate (i.e. the surface the cells are attached to) – this is easiest if there are at least some cells in at least one well in the plate to focus on. Adjust the focus slider until the cells appear approximately in focus, then click the **fine focus** button to accurately set the focus as described below.

### Well position

By default, the Cell Metric takes the focus images in the centre of the well. If you have a low seeding density, there may not be any cells in this location. In this case, you can use the well position selector to move the focus position around the well. Simply click on the picture of the well in the **well position** box, and a new stack of focus images will be taken in that location.

### Focus score

The green graph next to the focus slider represents the focus 'score', which is a measure of how sharp each image is. Because cells are three-dimensional objects and the depth of field of the optical system is quite shallow (about 10µm), it is possible to adjust the focus so that focal plane slices through only a section of the cell. For example, you can focus on the 'top' of the cell or on the 'bottom' of the cell. Typically, the brightness of the cell changes as you do this, in addition to small sub-cellular features coming into and out of focus. When there is a monolayer of cells in the field of view, the focus graph typically shows two peaks, which correspond to the top and bottom sections through the cells. There is also a pronounced drop in focus score between these two peaks which corresponds to a section through the centre of the cell.

The Cell Metric instrument performs best when you select the focal plane through the centre of the cells. It uses the position you select to determine the optimal focal position to generate the highest-contrast images.

When you click the **fine focus** button a stack of images is taken at very fine intervals. The images are processed and if the two high

focus scores typical of cells are found then the software automatically selects the position between these focal planes as the optimal focus position, and displays the image to you. If there are no cells in the field of view, this procedure will not operate correctly and you may have to manually select a focus position. This is why it is important not to adjust the stored focus setting if there are no cells to focus on.

The z-stack positions of the stored focus offset and of the current image (in mm) are displayed just above the Coarse Focus button.

### Meniscus correction

The horizontal slider under the focus slider allows you to correct the error caused by the meniscus in the liquid in each well. This error is minimised if the correct level of media for the well type is used, as described in the section Guide to successful imaging using the Cell Metric on page 30.

To determine the correct setting for meniscus correction, first slide the slider to the left so that the value on the right of the slider reads 0. Next, ensure the Preview Enhancement checkbox (described below) is unchecked. Now slide the focus selector up and down several times whilst looking at the cell image. If you see the cells in the image appear to move towards and away from the centre of the image as you do this, then you need to adjust the meniscus correction setting. Increase the meniscus correction setting in small increments by dragging it to the right, and repeat the movement of the focus position up and down. There should come a point when the cells no longer appear to move in and out as you move the focus slider. Ignore any slight movement of cells

that is consistent across the whole image (such as if all cells in the image move slightly left and right as you adjust the focus).

For most wells, a meniscus correction value of between 0.15 and 0.4 usually gives good results. Flasks have no meniscus and the setting should be zero. Avoid setting the meniscus correction whilst focused on a well at the edge of a microplate (such as well A1), since edge wells typically have higher evaporation and the meniscus will be different to wells closer to the centre of the microplate.

Be sure to return the focus slider to the optimal focus position once you have finished adjusting the meniscus correction.

### Illumination

You can adjust the brightness of the image by changing the camera gain. This affects how much the output from the digital camera is amplified, and therefore how bright the image appears. The software is able to suggest whether the current image is too dark or too bright. You can then move the **gain slider** before clicking the Fine Focus button to acquire a new set of z-stack images at the new camera gain. See also the discussion in the section 'Highlight Saturated Pixels', below.

Take care not to adjust the focus offset when the fine focus button is pressed, as explained above.

When using the cell counting scan profiles, images acquired by the Cell Metric system are automatically enhanced to improve image contrast and to enable the images to be processed for cell detection. You can adjust the parameters of the image

enhancement both to improve the visual appearance of the images and to optimise the cell detecting process.

#### Preview enhancement

Check this checkbox to display a preview of the enhanced image. The previewed image can make it difficult to determine the correct focus position, so this checkbox is unchecked by default. However, all images will be processed using the enhancement parameters, so you should preview the enhanced image to verify that the parameters are acceptable.

#### Highlight saturated pixels

To display saturated pixels, click the **highlight saturated pixels** checkbox. If there are any pure white pixels in the image, they will instead be shaded red. If there are any pure black pixels, they will be shaded blue.

#### Saturated pixels

When adjusting the Brightness of the image very often the image data is shifted outside of the range of values that can be represented in the image. For example, if you brighten an image enough all of the pixels will turn pure white and there will be no detail in the image at all. Conversely, an image can be darkened so much that all pixels are pure black and similarly there is no detail.

Since the range of shades of grey that can be represented in the image is finite, values that lie outside this range cannot be accurately represented, so instead are just represented as pure black or pure white. By analogy, if you have a set of weighing

scales with a capacity of 1Kg you can only accurately weight things lighter than 1Kg. Anything heavier than that you can only say 'weighs at least 1Kg'. Similarly, the range of shades of grey in the image cannot represent all of the possible variations in the world. Anything brighter than the lightest shade is 'off the scale' and we do not really know how bright it is, relative to the other shades of grey in the image.

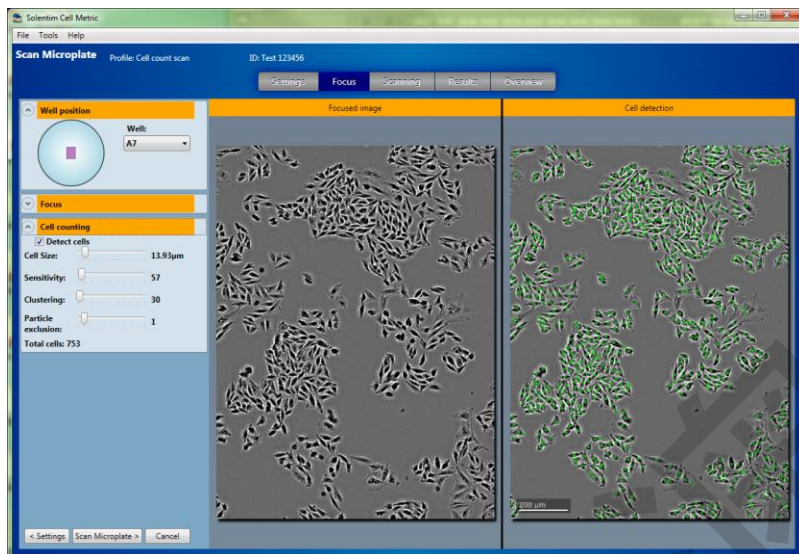
Pixels that are either pure white or pure black are both described here (for simplicity) as 'saturated'. If large areas of the image contain many saturated pixels then the detail in those areas is lost, which in turn limits the accuracy of the cell detection algorithm. For this reason it is important to check whether there are any saturated pixels in the image and if they are obscuring detail in the image.

Besides the inherent nature of the cells themselves, several parameters can cause an increase or decrease in the number of saturated pixels in the image. These include the focus offset position, camera gain, enhancement strength and both contrast and brightness. However, by adjusting each parameter in turn it is usually straightforward to obtain an acceptable image.

It is acceptable for there to be some saturated pixels in the image. If, however, there are large areas of adjacent pixels that are saturated and are obscuring the detail in the image, you should adjust one or more of the parameters mentioned above to improve the image.

## Cell detection parameters (cell counting scan profiles)

Once you have obtained a properly focused image that is well illuminated you can preview the cell detection result for the currently selected focus image.



### Cell Counting

Check the **detect cells** checkbox to display the cell detection overlay. Each detected cell is drawn as a small circle over the location of the detected cell (the circle is not necessarily in the centre of the cell). As described in the previous section, both the focus offset and the acquisition settings affect the performance of the cell detection algorithm, but there are also some more direct parameters that you can adjust.

### Cell size

This parameter adjusts the area over which detection of each individual cell occurs – the optimal value is typically related to the actual size of the cells in the image, but this parameter does not prevent smaller or larger cells from being detected. It is rather an optimisation parameter that allows cell detection to favour larger or smaller cells.

Typically, suspension cells or cells that have not yet adhered to the microplate surface are detected accurately when the cell size parameter is set to a smaller size – less than 14µm. Adherent cells are usually detected better when the cell size is around 20-24µm. However, changes in the cell morphology may require you to select a different value.

Note that this parameter is also used to detect cell growth (see next section). This is an *input* parameter that affects how the cell finding algorithm is performed.

### Sensitivity

This parameter is related to the morphology of the detected cell. Small, point-like objects tend to have lower values whereas bright, voluminous objects tend to have higher values. Increasing this value will make some detected cells disappear.

This is an *output* parameter that you can also adjust after the microplate has been imaged to improve the accuracy of the cell detection algorithm across the whole well.

### Clustering

This parameter affects how clustered cells are detected. Adjusting it has an indirect effect on how close together cells can be whilst still being considered distinct. This also has an effect on the location of the detected cells, so as you adjust this parameter you may see the orange circles move around slightly on the image.

This is an *input* parameter that affects how the cell finding algorithm is performed.

### Particle exclusion

This parameter reduces the effects of image noise and debris in the well. The default value of 1 is typically optimal.

This is an *input* parameter that affects how the cell finding algorithm is performed.

The count of total cells detected in the current image is also displayed.

### Optimising cell detection

To get the best results from the cell detection algorithm you must select optimal focus, image acquisition settings and cell detection parameters. The simplest way to get good results is to adjust each of these settings in turn. Once the focus and gain are adjusted as described in the section above, proceed to adjust the image enhancement strength and the cell detection settings until the results are satisfactory. Since the image is updated whenever any of the parameters are changed, it is straightforward to see what effect the adjustments are having.

Like any automatic detection, the cell detection algorithms can only give good accuracy under good conditions. See the section Guide to successful imaging using the Cell Metric on page 30 for advice on improving image quality and cell detection.

Determining the optimal parameters is a matter of trying various settings and seeing how well the detection parameters work, especially as the cell density changes. In general, cell counting will work best at densities of cell confluence between about 5% and 80%. At less than 5%, small debris and imperfections in the microplate can give false results, so accurate cell counts require clean images. At densities above about 80% confluence, the cells can crowd together and begin to stack on top of each other, which limits the accuracy of cell detection. In general, if you cannot accurately count the cells in an image by eye, you will be unlikely to achieve good cell detection automatically.

### Detecting cell growth (cell growth scan profiles)

The cell growth detection metrics provide a means of measuring the confluence of each well (that is, the percentage of the well bottom that is covered in cells). The confluence metric can give more accurate results for some cell morphologies, but at high confluence values (> 70%) it may not increase in proportion to the number of cells in the well. Once cells are at 100% confluence, they completely cover the surface of the well but of course it is still possible for more cells to grow, either by packing together more tightly or by stacking on top of each other. By this point, different wells can be at 100% confluence but can have considerable variation in the number of cells in them.

In addition to confluence, cell growth detection provides metrics that help quantify the distribution of cell growth within each well. To turn on cell growth measurement, check the **Detect cell growth** checkbox. There are some parameters you can adjust to optimise cell growth detection.



### Shade background

This checkbox toggles shading of the background in the well, where no cells have been detected instead of shading the area where cells have grown. It makes seeing the cells clearer as the false colour overlay does not obscure the cells.

### Cell Size

This parameter is used from the Detect Cells parameters, described above.

### Sensitivity

This parameter has the greatest effect on cell growth detection. A lower value will give a higher confluence reading. Values between 80 and 120 are usually a good starting point.

This is an *input* parameter that affects how the cell growth detection algorithm is performed.

### Dilation

This parameter is used to 'fill the holes' between areas of detected confluence. Higher values 'fill' more of the holes. Depending on cell morphology, adjusting this parameter can improve accuracy significantly.

This is an *input* parameter that affects how the cell growth detection algorithm is performed.

### Minimum density

This parameter can be used to exclude small regions of detection caused by debris, plate imperfections, etc.

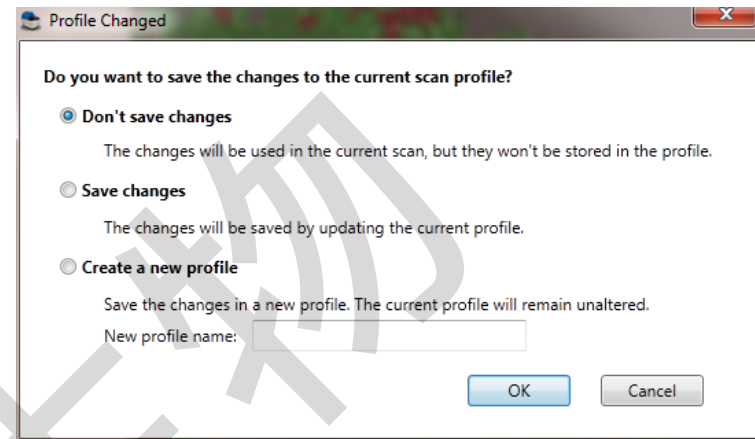
This is an *output* parameter that you can also adjust after the microplate has been imaged to improve the accuracy of the cell growth detection algorithm across the whole well.

A histogram of cell growth is displayed under the cell growth parameter adjustment controls. In this histogram, all of the pixels in the image are displayed. Those pixels that the cell growth detection algorithm did not determine as cell growth are displayed on the left, in grey. The rest of the pixels represent increasing levels of density of cell growth, up to the maximum which is shown in red. These measures of cell growth density is also drawn over the cell image, with areas of cell growth shaded in increasingly darker shades of green, up until the highest density which is shown shaded red.

The statistics below the histogram quantify the amount of cell growth in the current image.

### Saving changes to settings

Once you are satisfied with the imaging settings, click the **Scan Microplate** button to image the microplate. You will be prompted to save the changes to the settings you have made:

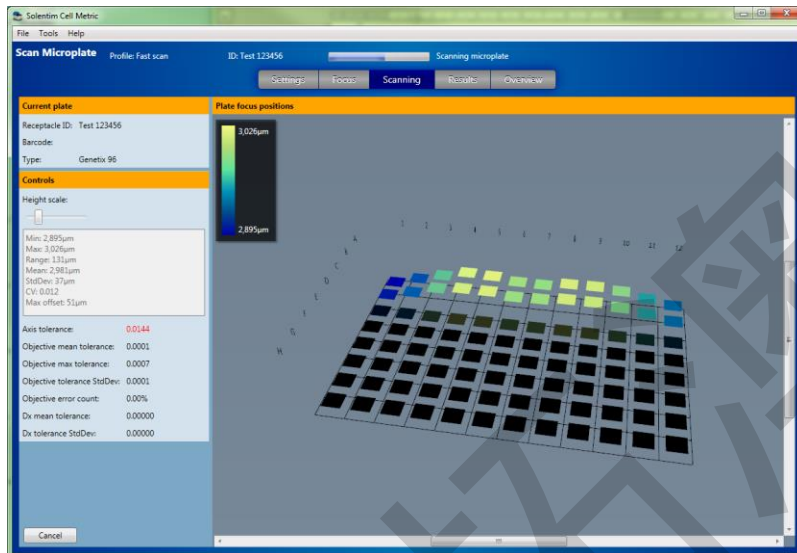


Using scan profiles is explained in detail in the section Using scan profiles on page 27. For now, select the default option 'Don't save changes', and click 'OK'.

## Scan progress

Once the scan has started, a screen is displayed showing the focus measurements across the microplate. There are no parameters that can be adjusted at this point – you can leave the instrument to complete the scan without intervention. The Cell Metric will eject the microplate once the image acquisition phase has completed.

A status bar indicating progress along with a status message is displayed at the top of the screen.

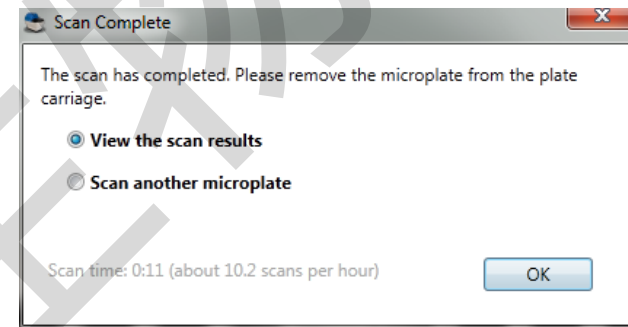


Do not open the instrument door while the scan is in progress. Doing so will abort the scan and cause the results to be discarded. If the scan is aborted, in some

circumstances you may have to power down and restart the software, the instrument or both to recover.

## Normal Scans

Once the scan has completed, you have the option to either view the results for this scan, or to scan another microplate using the same settings:



If you have a batch of microplates to scan, you may want to review the results from the first plate to check that the cell detecting is giving good results, and then simply scan the rest of the microplates without checking the results as you go. You can then review the results later at your leisure, once the microplates are back in the incubator.

## Trial scans

Once the scan has completed, you can view the scan results. Some of the scan settings stored in the scan results can only be edited when you are viewing the scan results. If you make changes to these settings when you view the results after a trial scan, they will be saved back into the scan profile so that they can be used on the next scan.

## Viewing results

Once a scan is complete, the results for that microplate are displayed. You can also view the results at a later date by double-clicking them in the list in the home page, which is displayed when the software first opens.

## Results overview

The results overview screen is divided into three sections. The left-hand section displays information about the results such as the date and time the results were scanned, the barcode and plate identification etc. The middle section contains a series of thumbnail images of the microplate, giving an at-a-glance view of the selected metric (such as cell count). Lastly, a table on the right-hand side lists the number for the selected metric for each well, with the metric selector just above it.



## Results information

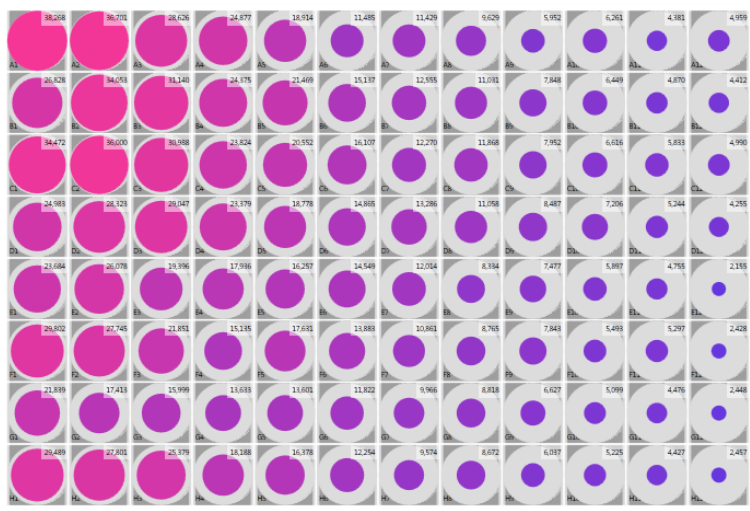
This section lists various details about the current results including the date and time the microplate was scanned, the operator who

scanned the plate and other details. In addition, the 'annotation' field can be edited at any time. This enables you to scan a microplate and then add commentary and references at a later time when you review the results.

## Thumbnail images

The thumbnail images provide a quick way of seeing the growth pattern of cells across the whole microplate. Each well image is annotated with the well name. Clicking on each well thumbnail image takes you to a screen with the high resolution image of the well (see the section **Results image** on page 22, below).

If you chose to detect cells when the microplate was scanned, when you move the mouse cursor over the well images a circle is drawn over each well that was scanned. The well with the highest value for the selected metric, for example the most cells in it, has a large circle overlaid on it that fills the well thumbnail image. All of the other wells have smaller circles, proportionally showing the value of the selected metric in each well relative to the well with the highest value. The image below shows a microplate containing a dilution series of cell density. The wells on the left have the most cells and therefore have large circles drawn over them; the wells to the right have decreasing numbers of cells in them and therefore the circles drawn over them get smaller across the microplate.



Whilst this graphic is a good way of seeing the relative distribution of cell counts etc. within a microplate, it will not be very useful if there are only a very few cells in each well.

Along with the circle, a small label appears at the top of each well reporting the value of the metric for the well.

### *Data table and metric selector*

On the right hand side of the screen is a data table which lists out all of the wells in the microplate along with the value for the selected metric in each well. Just above the table is a drop-down list that lets you select which metric to display in the thumbnail view and data table.

If you didn't check either the Detect Cells checkbox or Detect Cell Growth checkbox before the microplate was

imaged, no cell measurements will have been generated and the list of cell metrics will be empty.

### *Exporting data*

You can export the cell metric data into a file, for analysis with other software programs such as Microsoft Excel. Click the **export** button and select the export format (choice of Comma Separated Values [csv] or Tab delimited values [txt]). For Microsoft Excel, Comma Separated Values are the best choice as they load straight in to the software when you double-click them.

### *Saving reports*

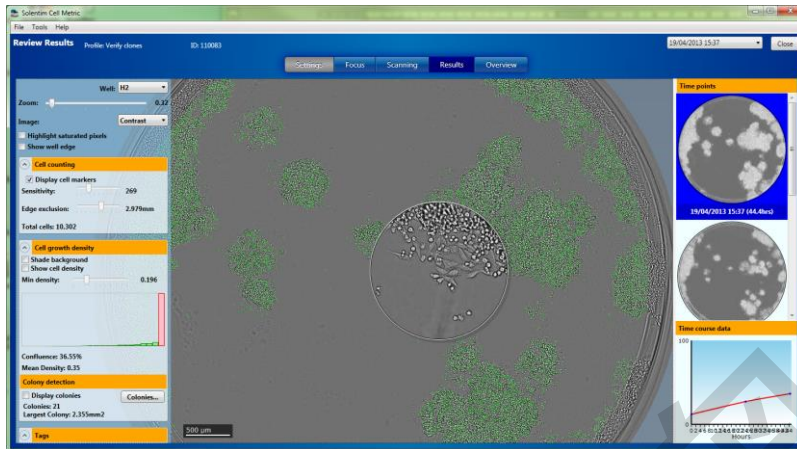
You can generate a report containing the information on the results summary screen, including scan details and annotation, image thumbnails, selected metric well graphic and metric value table. Click the **save report** button and select a location to save the report to. The report is saved in HTML format, which can be opened in a web browser. The report consists of a web page and a folder containing the relating images; Windows Explorer automatically moves the file and the folder when you copy and paste them on your computer.

Only the currently selected metric is included in the report. Select a different metric and generate another report if you want to report on more than one metric.

### *Results image*

When you click on a well in the well thumbnail images view, or click the 'Results' tab, a high-resolution whole-well image is displayed for the selected well. You can zoom in and out of this image by scrolling the wheel on the mouse. Pushing the wheel

forward zooms the image in; pulling the wheel back zooms the image out. When the image is zoomed in, you can click and drag the image to scroll around. Clicking the image a single time scrolls to the 'full' resolution, such that each pixel on the screen displays a single image pixel.



There are several options available using the right mouse button on the well image :

- Zoom in to the selected location
- View the whole well image
- Export a screenshot of what is currently displayed

### *Cell detection parameters*

If you ran the cell detection during scanning, you can toggle the detected cell count and cell growth overlays on or off to either see where the detected cells are or to get a clear view of the cells themselves.

You can adjust the cell sensitivity to optimise the cell detection results (see discussion in the section Cell Counting on page 16). You can also selectively exclude cells a specified distance from the centre of the well by using the **edge exclusion** slider. This is useful for circular wells, when the areas outside the wells or at the well border contain debris or extraneous noise that impacts the cell detection results. You can adjust the edge exclusion slider so once cells within the specified radius of the centre of the well are counted, all others are ignored.

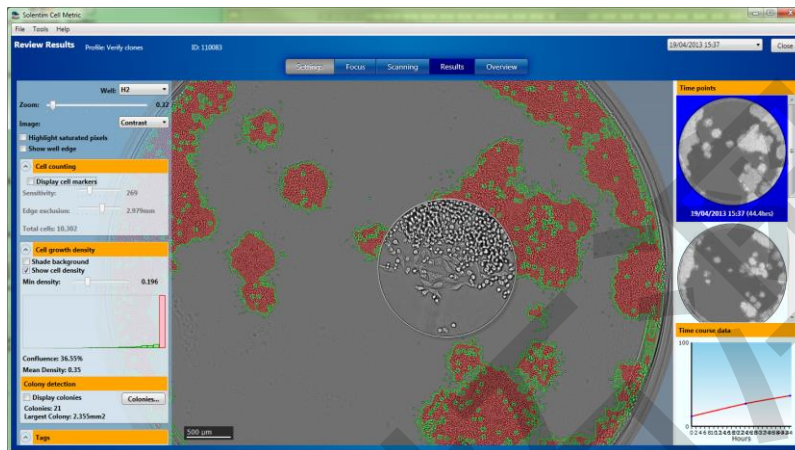
Adjusting the cell detection sensitivity or edge exclusion slider updates the cell count metric for every well in the microplate. If you have previously exported the metric values for this microplate, those values will no longer match what you now see in the software.

Adjusting the Min density parameter for cell growth has the same effect on the cell growth parameters in each well.

### *Additional viewing facilities*

Click the **full screen** button to switch to a full screen display to view the maximum image area. Press the Escape key on your keyboard (often labelled 'Esc' at the top left). You can select a different well to view from the list at the top left of the screen.

When you move the mouse over the well image, a **magnifying loupe** is automatically displayed when the image magnification is less than 100% (the image is zoomed out). This allows you to quickly scan the plate for cells without having the zoom in and pan the image around. This saves time if you have lots of wells that you want to look at.

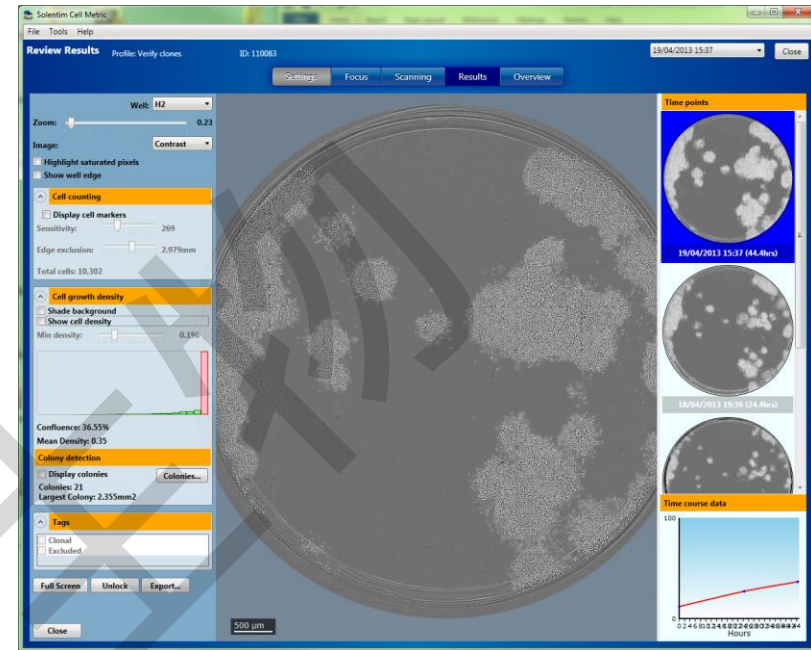


### Viewing changes to the plate over time

Once you have completed the scan, if you have imaged the plate before you can view the previous scans of this plate to monitor changes to cell growth over time. To do this, first close the results so that any changes to cell detection parameters take effect. Then you can re-open the results by clicking on them in the software home screen.

When opened in this way, if the plate has been scanned more than once you can select each time point from the drop-down list at the top right of the results view. Additionally, on the results image there is a column of thumbnail images displayed on the right of the screen, one image for each time point. You can click on each thumbnail to switch the image view to that time point, allowing you to cycle through the images to see how the cells have changed over time.

Each image has a caption below it showing the date and time the image was acquired, and a calculation of how many hours have elapsed since the plate was first imaged.



### Excluded sessions

Often there are scans in a batch that are not useful. There are several reasons why this might occur :

- It was a trial / preview scan
- The wrong microplate / settings were used
- It was just an experiment

Whilst it may be sensible to just delete these sessions, often they need to be kept, but in a way that does not clutter the batch. The software allows a user to mark a session as being **Excluded**. It will still appear in the results, and you can still view it, however it will not appear in any reports that are produced using the application.

### Excluding a session

- View the batch (either using the Dashboard or by selecting “Batches”, choosing the appropriate batch and selecting “View batch”)
- Right click on the session you wish to exclude and choose “Mark Excluded...”

Scan Date	Barcode	Microplate ID	Annotation
24/04/2015 19 sessions			
24/04/2015 15:09		NOBARC1509	
24/04/2015 14:56	SIM BARCODE 1455:38	SIM BARCODE 1455:38	
24/04/2015 14:54			
24/04/2015 14:45	SIM BARCODE 1444:58		:58
24/04/2015 14:43			
24/04/2015 14:40	SIM BARCODE 1439:55		:55
24/04/2015 14:38		NOBARC1437	
24/04/2015 14:36	SIM BARCODE 1436:47	SIM BARCODE 1436:47	

- The session will be greyed out in the list of sessions, all of the wells will have a red cross through them and the session will show “Excluded” as the Inclusion Status :

Scan Date	Barcode	Microplate ID	Annotation	Microplate
24/04/2015 19 sessions				
24/04/2015 15:09		NOBARC1509		Corning Costar 96 Mir
24/04/2015 14:56	SIM BARCODE 1455:38	SIM BARCODE 1455:38		Corning Costar 96 Mir
24/04/2015 14:54		NOBARC1453		Corning Costar 96 Mir
24/04/2015 14:45	SIM BARCODE 1444:58	SIM BARCODE 1444:58		Corning Costar 96 Mir
24/04/2015 14:43		NOBARC1444		Corning Costar 96 Mir
24/04/2015 14:40	SIM BARCODE 1439:55	SIM BARCODE 1439:55		Corning Costar 96 Mir
24/04/2015 14:38		NOBARC1437		Corning Costar 96 Mir
24/04/2015 14:36	SIM BARCODE 1436:47	SIM BARCODE 1436:47		Corning Costar 96 Mir
24/04/2015 14:35		NOBARC1435		Corning Costar 96 Mir

Summary	
Timepoints:	Scan Date: 24/04/2015 14:56
	Barcode: SIM BARCODE 1455:38
	Annotation: SIM BARCODE 1455:38
	Microplate: Corning Costar 96
	Cell Line:
	Scan Type: Normal
	Scanned on: 24/04/2015 14:56
	Operator: Ian Hunt
	Inclusion Status: Excluded

### Including a session

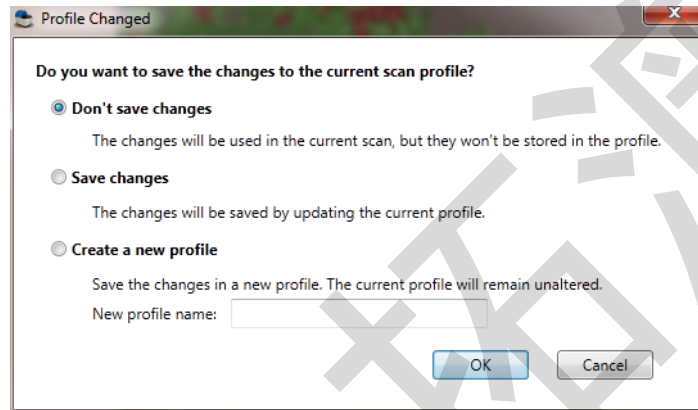
- View the batch (either using the Dashboard or using Batches → View batch)
- Right click on the session you wish to include and choose “Mark Not Excluded...”

## Using scan profiles

Scan profiles provide a means of grouping together all of the scan settings for a given microplate type and cell line, and saving them with a name you choose. You can have as many scan profiles as you wish. This makes it straightforward to have a scan profile for each of the various cell growth configurations that you use. You can quickly change between scan profiles without having to re-enter all of the scan parameters individually.

### Creating a new scan profile

Scan profiles are always available, but they only become useful once you create your own profiles. You do this by modifying an existing profile, and then saving it with a new name when the software asks you if you want to save changes just before it scans a microplate:



To save the settings as a new profile, click the **Create a new profile** option and type a descriptive name into the profile name box,

then click OK. The profile will be saved and automatically selected for the current scan.

The other options here are **don't save changes** which will use the changes you have made to the current profile in the next scan but will not save them into the scan profile. This is useful if you are experimenting with new parameters and are not yet sure if you want to keep them.

Although the changes aren't stored in the profile, they are retained until you either select a different profile or you close the software. For this reason, even if you choose not to save the parameters the software will display the prompt screen above every time you scan a new plate, to give you the option to save them if you want to and to remind you that the current scan profile has been modified.

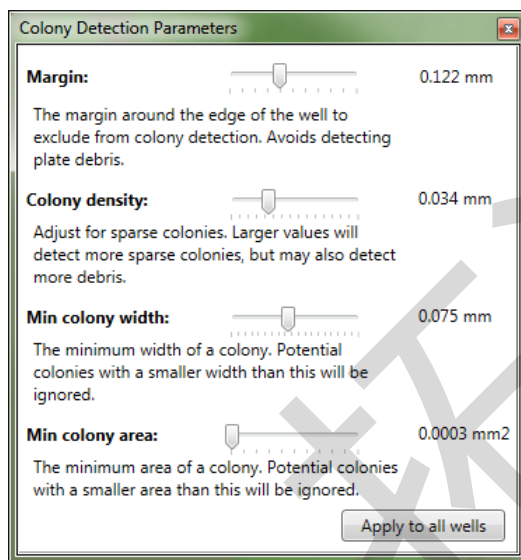
**Save changes** simply stores the changes you have made back in to the currently selected scan profile. Bear in mind that this overwrites the original parameter values in the profile. The changes will be retained even if you close the software.

## Cell growth based colony detection

The colony detection feature of the Cell Metric is only available for microplates that were scanned in the **monitor cell growth** scan type. The colony counting how many distinct 'islands' of confluence there are in each well.

To run the colony detection algorithm, first open the saved scan results. Click the 'Unlock' button so that you can edit the detection parameters, then click the 'Colonies' button in the 'Colony detection' section of 'Cell growth density'.

The colony detection dialog box appears. This allows you to adjust the colony detection parameters.



The correct parameters to use depend upon how many cells you have, how close together they are etc.

### Margin

This parameter lets you exclude a region around the edge of each well. Often, the well edge causes a small amount of confluence to be detected where there are no cells. My increasing the well margin, you can exclude this region.

### Colony density

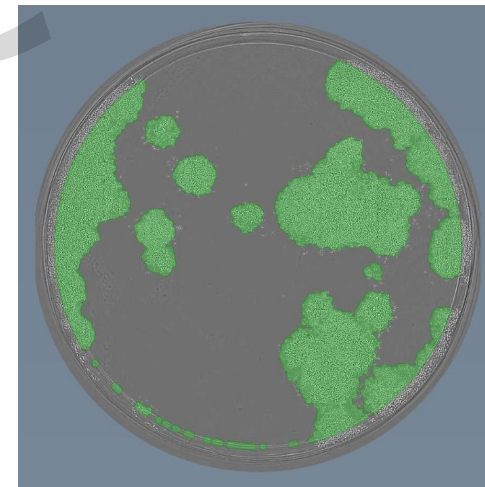
If you have a cell line that forms sparse, 'open' colonies, you can increase this parameter to improve colony detection.

### Min colony width

Sometimes debris is detected that is long and thin, but has quite a large area. You can increase this parameter to exclude colonies that are thin, without excluding small, round colonies.

### Min colony area

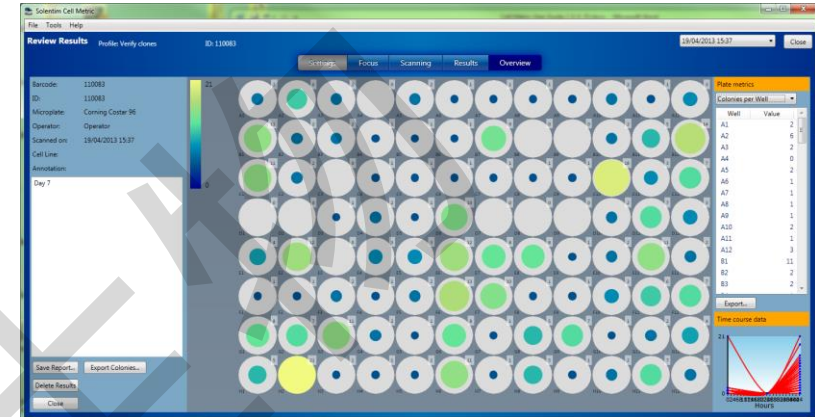
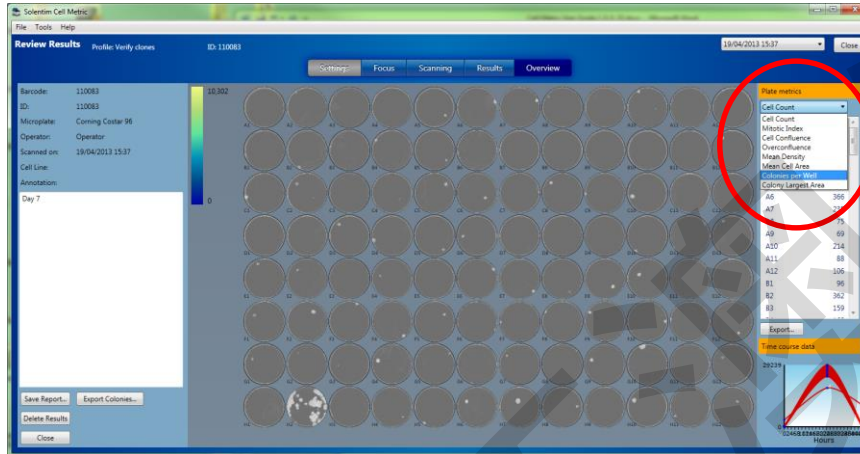
You can exclude debris that is smaller than a required size by increasing this parameter.



Each time you adjust any of the sliders, the current well is re-processed. To apply the settings to all wells, click the 'Apply to all wells' to see the effect of the changes.

Once you are satisfied with the colony detection, you can view the summary for the whole plate. First click the 'Overview' tab to return to the plate overview screen. Then select 'Colonies per well' from the metric selector:

Once the 'Colonies per well' metric is selected, the colony count is displayed for each well:



You can then export the colony count summary data.

## Preparing Results for Reports

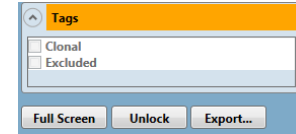
Before generating reports based on the data gathered during the imaging sessions, it is helpful to go through each well and apply extra information to it to make the report generation more automatic later on. There are two types of data that can be added:

- **Tags** can be added to a well to identify wells that are of interest, or wells that are excluded
- **Annotations** apply only to Verify Clonality projects. These can be added to individual images of a well, and allow reports to include relevant parts of the images

The following sections describe what can be done with each of these types of data.

## Tagging Wells

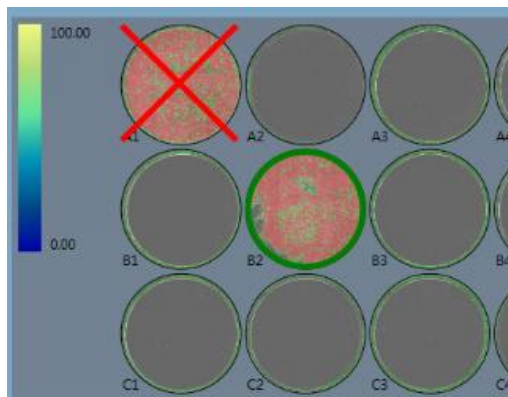
Whilst reviewing the results of a scan you may wish to associate one or more tags with the well in order to help with reporting later on. There are up to three tags available depending on which workflow you are using :



- **Clonal** : This is used in both the Clonality Report and the Clone Selection report to filter the list of wells to only those containing cells that are all clones.
- **Chosen** : This is used in the Colony Selection report to filter the list of wells down to only those wells that require further work on them
- **Excluded** : This is used in the Colony Selection and Clone Selection reports to filter out wells that should not appear in the reports.

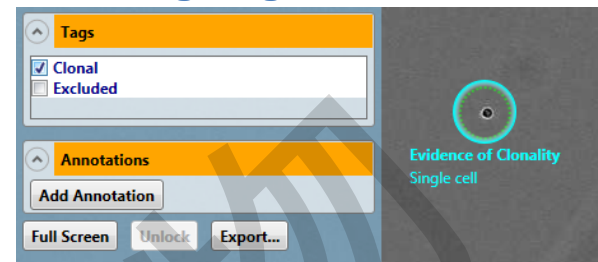
To tag a well :

- 1) View the whole well image of the most relevant image of the well. For a Verify Clonality project this would be the image that shows the single cell.
- 2) Select the “Unlock” button found on the bottom left of the screen
- 3) Enable or disable the relevant tag by checking or clearing its entry in the Tags panel



Tagged wells are shown differently in the results review screen – Clonal/Chosen wells have a green ring around them, Excluded wells have a red cross through them.

## Annotating Images



Annotating an image involves putting a marker onto the image to indicate something of interest. There are two types of annotation you can add to an image :

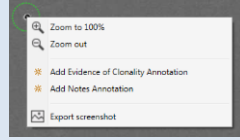
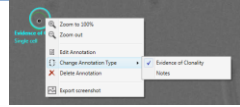

- **Evidence for Clonality** : This is used in the Clonality Report to indicate that the marked feature should appear in the report.
- **Notes** : This is used to indicate some other feature of the image that is of interest. These also appear in the Clonality Report, but not with the same prominence as the Evidence for Clonality

To annotate an image it must be unlocked, so ideally you would annotate the image at the same time you tag it as clonal, described in the previous section.


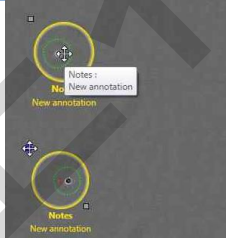

Once the timepoint is unlocked, there are 2 ways to annotate the cell. Either right click on the cell you wish to annotate in the main image and select the “Add Evidence of Clonality Annotation” option, or click the “Add Annotation” button and then click on the single cell. The annotation will appear. Then you can customise it to suit your requirements using the options describe in the following section.

### Working with Annotations

Annotations can be added or removed as required, and existing annotations can be made editable/read only as necessary :

Action	Example	Detail
<b>Add</b>		Using the mouse, right click on the main image. This allows you to add a new annotation centred on the clicked location.
<b>Edit / Delete</b>		Right click on an annotation to edit, change its type or remove it. By default when you first view a well any annotations on it will be read only, so you have to make them editable before you can move or resize them. If you have just added the annotation it will already be editable.
<b>Finish Editing</b>		Simply click somewhere away from an annotation in order to finish editing the annotations on the well.

Once an annotation is editable, the following operations are possible:

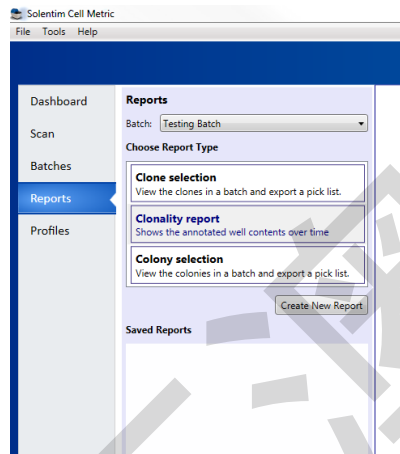
Action	Example	Detail
<b>Resize</b>		Using the mouse, drag the edge of the circle to resize it. Alternatively, grab the resize box on the bottom right hand corner, and drag that to resize. Note that there is a minimum size for the annotation.
<b>Move</b>		Using the mouse, drag the centre of the circle to move it around the image. Alternatively, grab the move box on the top left hand corner of the annotation, and drag that.
<b>Change Text</b>		Click on the text of the annotation to change what it says. This allow you to edit the text. Press "Enter" or click somewhere else when you are done. Note that removing all the text will remove the annotation.

## Reports

The Cell Metric software is capable of producing several different types of reports that describe the results of the imaging that the instrument has performed. The styles of reports are described in the following sections.

Generating a report is a 4 stage process :

- 1) **Report Batch** : Select the “Reports” page on the left hand side of the main application screen, and then the batch that you wish to create the report for.
- 2) **Report Type** : Select the type of report you wish to create from the list of options (which will depend on the applications configured for the device)
- 3) **Report Configuration** : Customise the report to match your requirements
- 4) **Export** : Export the report in a variety of formats



## Picklist Reports

### Overview

Picklist reports are designed to allow a user to produce lists of wells that contain cells that are of interest for further development. It then automates the creation of lists allowing the user to transfer samples from one plate to another.

### Report Type

The picklist report comes in two forms depending on which application you are using :

Application	Report Type
Colony Growth Assay	Colony selection
Verify Clonality	Clone selection

### Report Configuration

When the report is first selected, a table is generated showing all of the imaged wells in the batch. Note that any sessions that are marked as excluded will not be available.

**Clone selection**

Ignore wells tagged 'Excluded'

Only wells tagged 'Clonal'

Only wells imaged on 11/06/2015 with confluence over 10.00 %

Limit to 10 wells per microplate (highest confluence)

96 of 96 wells selected (100.0%)

Microplate ID	Well	Clonal	Confluence
003705	A1		45.95
003705	A2		48.60
003705	A3		30.19
003705	A4		44.23
003705	A5		42.06
003705	A6		36.18
003705	A7		51.54

The columns of data that appear depend on the type of report :

Column	Detail
Microplate ID	The plate identifier
Well	The well within the plate
Clonal	<b>Clone selection report only</b> Whether the well has been marked as clonal
Chosen	<b>Colony selection report only</b> Whether the well has been marked as chosen
Confluence	The confluence value for the well
Colony #	<b>Colony selection report only</b> The number of colonies detected in the well

There are various options at the top of the page that act to filter the list of wells down to those of interest.

Once the list of wells has been filtered down to those of interest, select the “Export pick list” option to go to the following screen.

**Export picklist**

Format: Tecan worklist

Leave edge wells empty in destination

Destination format: 96 wells  
Destination microplate name:

Prefix:  ID:  Suffix:

Volume:  µL

Source labware label	Source position	Destination labware label	Destination position	Volume
003705	1	1	1	100ul
003705	9	1	2	100ul
003705	17	1	3	100ul
003705	25	1	4	100ul
003705	33	1	5	100ul
003705	41	1	6	100ul
003705	49	1	7	100ul
003705	57	1	8	100ul

This screen allows you to control how the samples you selected on the previous screen are transferred into new receptacles for onward development. It contains various options along the top to

help define how the samples get transferred, and below those there is a preview of what the export will look like. To complete the export :

- Specify the name structure of the destination receptacle identifiers (in the form [Prefix][ID][Suffix]) with the ID starting with the selected value)
- Specify whether the edges of the wells should be left clear, and the volume of liquid required in the well

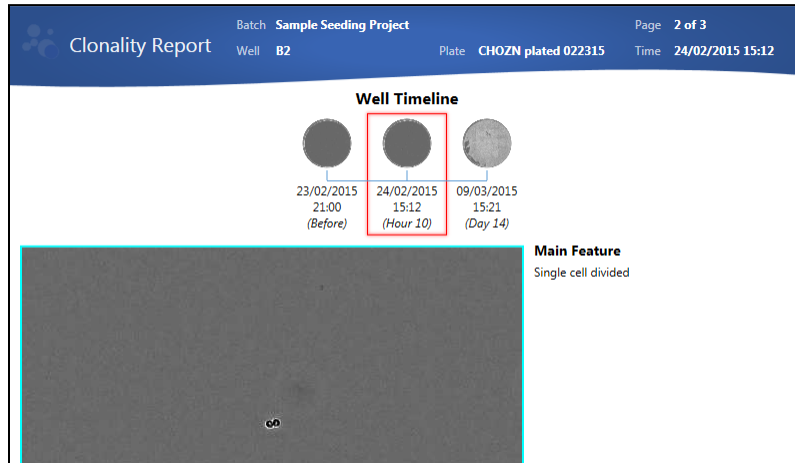
### Export

The data from the report can be exported in two different formats. Both formats present similar data.

Format	Usage
Tecan worklist	For import into a Tecan-compatible liquid handling system for automated transfer of cells from one well into a well in a new plate
Manual worklist	For opening in a spreadsheet application to allow for an operative to manually transfer the cells into new locations.

Once the preview shows the correct information, select the “Export” button and then select where you wish the output to be stored. The resulting file can be opened in a text editor or a spreadsheet application.

## Clonality Report



### Overview

The clonality report is designed to provide documentation containing the evidence that shows that specific wells contain colonies all derived from a single cell. The output provides large scale images of selected portions of each well of interest, and notes for the highlighted areas.

### Report Type

The clonality report is only available for the Verify Clonality application.

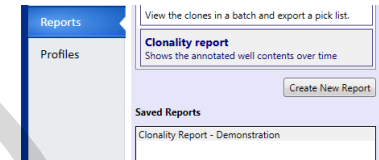
### Report Configuration

As with other types of report, the first step is to select the “Reports” tab in the application, choose the batch you wish to use and then choose “Clonality report” from the list of available reports.

Below this you will see a list of all of the clonality reports that have been saved for this batch with options to Delete, Rename and Load them.

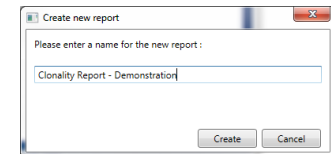
### Loading Existing Reports

If you have a saved report, you can simply double click it within the list of saved reports and it will load the report for configuration and exporting. Note that when you first load the report it will be read-only, you will need to select to edit the report in order to make any changes to it. However, you can export and print the report without editing it.



### Creating a New Report

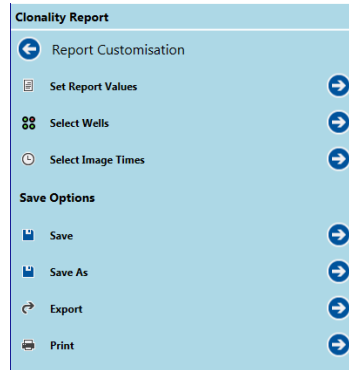
Select the “Create New Report” button, give it a suitable name and click “Create” :



### Working with the Report Generator

Whenever you work with the Clonality Report Generator, you will see a screen with two main parts :

- The configuration tool on the left which allows you to interact with the generator, make changes to the report and save / export it
- The main part of the screen contains a preview of what the report might look like with the settings as they are at the moment. As you change the settings, this will update the preview on the right hand side



There are two standard buttons that are used to help navigate through the options :

Button	Detail
	This is an action that can be clicked on
	Clicking on this button will take you logically “back” to where you were before you performed an action

### Defaults

When you first generate a new report, some default settings are applied to get you started :

- Wells that are marked as clonal, and have annotations, are automatically included in the report

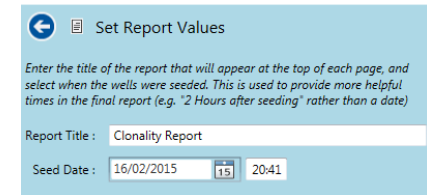
- Up to three imaging sessions are included for each well – the oldest two and the newest. This is intended to be “Before seeding”, “Immediately after seeding” and “Current state”
- The report title is automatically set to “Clonality Report”, and the date when the plates were seeded is defaulted to a date between the first and second set of scanning sessions.

Each of these settings can be changed by clicking on the relevant action in the report configuration as detailed in the following sections.

### Setting Report Values

Clicking on the “Set Report Values” option allows you to configure settings that affect the whole report:

- The report title that appears at the top of each page of the report
- The time that the batch was seeded, which is used throughout the report to interpret the dates of images in a more friendly way (i.e. “2 days after seeding” rather than a date and time)

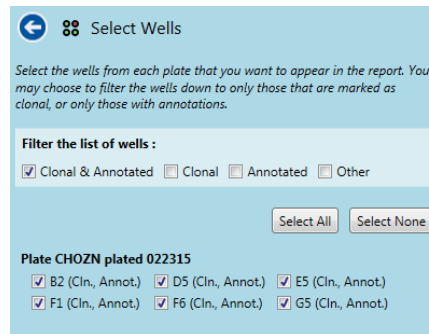


Changes made here will be reflected in the preview immediately

### Selecting Wells

Clicking on the “Select Wells” action allows you to choose which wells appear in the report. You can use the filters at the top to restrict the list of wells down to those of interest :

- Wells that are marked are clonal
- Wells that have annotations attached
- Wells that are both clonal and annotated
- Wells that are neither clonal nor annotated

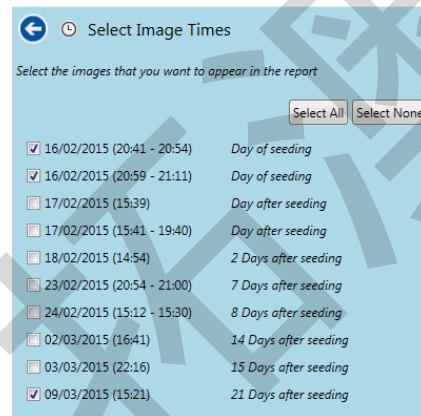


Checking the appropriate well will make it appear in the preview. Note that wells from sessions that have been excluded will not appear in the list.

### Selecting Image Times

Clicking the “Select Image Times” action allows you to pick which images to include for each well that you selected in the previous section. The imaging sessions are grouped such that

- Each day has its own group
- If a well has been imaged multiple times in a given day, there will be more than one group for that day.

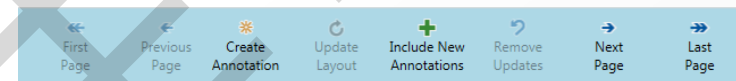


Once again, sessions that have been excluded will not appear in this list. Note that if the seeding date is not correctly set then the seeding data on this screen will not be accurate.

### Using the Report Preview

The report preview, on the main part of the screen, gives you an example of how the report might look when it is output. Note that because of the different sizes and styles of document that you can output, the reports generated will not be exactly the same, but will be as close as possible.

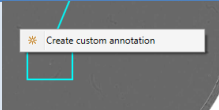
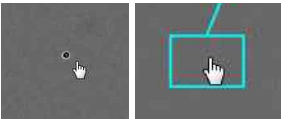
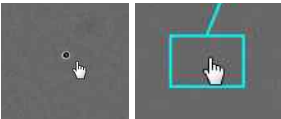
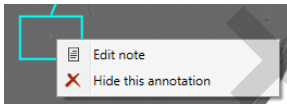
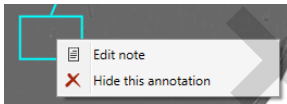
The navigation bar along the bottom has several options, and the preview screen itself allows you to customise the annotations themselves. Note that these customisations are specific to your report, they are not saved back to the well itself.



- Go to the first, previous, next and last pages of the report
- “Create Annotation” creates a new custom annotation on the current page of the report
- “Update Layout” rearranges the annotations to minimise crossed lines where possible
- “Include New Annotations” will include any extra annotations that you have added to the well since the report was created
- “Remove Updates” resets the page to how it was without any customisations (i.e. using the annotations as they were saved with the well)

### Working with Custom Annotations

In the report preview window itself you can do various operations described in the following table. Note that these are all saved with your report, and so will not appear when you view the well in the application.

Action	Example	Detail
<b>Add</b>		Right click on the image of the whole well (away from any existing annotations), and select “Create custom annotation.” This adds a new custom annotation centred on the clicked location.
<b>Move</b>		By clicking and dragging the annotation image, or the box on the whole well image, you can move the annotation
<b>Zoom</b>		By using the mouse wheel when the mouse is over an annotation image, or the box on the whole well image, you can zoom the annotation in or out
<b>Edit</b>		By right clicking on an annotation image, or on the box in the whole well image, you can choose to edit the annotation. This brings up a window that allows you to change it.
<b>Hide</b>		Also via the right click menu, you can choose to hide this annotation from the report. This stops it appearing in your report, or deletes it if it is a custom annotation.

### Saving and Exporting

Once you are happy with all the pages of the report, you can save and export the report :

- “Save” will save the report as the name you chose when you created the report
- “Save As” allows you to save the report as a different name, leaving the one you created untouched
- “Export” allows you to actually generate the report
- “Print” allows you to output the report to the printer



## Export

You have two main options when you export a report :


- By default, a single report file is generated. However, if you wish to have a separate report for each well, you can select that option on the Export screen
- There are 3 types of export possible, each with a different usage
  - XPS documents can be printed within windows
  - PDF documents can easily be shared with others
  - PPTX documents rearrange the report to make it show better in a presentation



Once you have selected what type of report you want to output, you need to give it a file name to use as the output :

- If you chose to generate a single report, it will save the report in the file you chose, and then open it if you have a suitable viewer installed on the PC
- If you chose to generate separate reports for each well, it will save all the files in the folder you chose, with names based around the file name you entered. It will then open the folder so you can easily get to the reports for viewing.

## Leaving the Report Generator

Once you are done with the report generator, simply use the back button repeatedly until you are out of the report configuration screens. 

If you have not saved your work, it will ask you if you are sure you want to lose the changes before you exit the configuration screens.

## Guide to successful imaging using the Cell Metric

The Cell Metric is a precise optical instrument, designed for live cell imaging of cells growing in microplates. Quite apart from the cells themselves, there are a number of factors that affect the quality of the results when using the Cell Metric. This guide explains some of the major factors to take into account.

### *Eliminate vibration and shocks*

Like any optical instrument, the image quality in the Cell Metric will be reduced (in some cases to the point of being unusable) if the instrument experiences vibration or external shocks (knocks and bumps) during operation. For best results:

1. Site the instrument on a stable, heavy duty bench, ideally away from walkways where people will be frequently passing by.
2. Avoid knocking the instrument or bench, for example leaning on it, putting items down on the bench etc. whilst the instrument is scanning a microplate.
3. Do not place the instrument near other instruments that themselves cause vibrations such as centrifuges, shakers, stirrers etc.
4. Avoid placing the unit near doors that slam and cause shocks, including lab doors, incubator doors, cupboards and drawers etc.

### *Use an optimal media volume in each well*

The meniscus that forms on the top surface of the media in a microplate well tends to act as a lens, and it can create uneven lighting towards the edge of the well. It is therefore important to

ensure that the correct range of volumes is maintained to achieve high quality images. Since the diameter and depth of wells in microplates from different manufacturers varies, the suggested amount of media is expressed as a depth rather than a volume.

1. For 384-well microplates, a volume sufficient to fill the well between one third and half full is usually optimal.
2. For 96-well microplates, a volume sufficient to fill the well between one half and two thirds full is usually optimal.
3. For other formats (48-well, 24-well, 12-well, 6-well) a volume that approximately half fills the well is usually optimal.
4. Flasks (T25, T75 etc.) should be completely filled so that any air gap is retained entirely in the neck of the flask when the flask is placed on a flat level surface.

Evaporation at the edge of the microplate will reduce the media volume and may affect image illumination. Corner wells are especially vulnerable – for 96-well and 384-well plates consider not using these wells at all. 1536-well plates might need two or more unused wells at the edge of the microplate. Be especially wary of using edge wells for control data.

Some well coatings may reduce or increase the effect of the meniscus – if image results are poor consider experimenting with different media volumes to determine the optimal range for the plate type you are using.

### ***Filter the cell culture media***

Any debris in the cell culture media will affect the accuracy of cell detection. For optimal results, filter the cell culture media to remove debris, for example with a 0.22 µm filter.

### ***Do not write on the microplate lids or flask tops***

The Cell Metric illuminates the microplate or flask from above during imaging. Any writing, labels or anything else placed on the upper surface of the lid may limit light transmission and adversely affect image quality.

### ***Limit condensation on microplate lids***

When a microplate is taken out of an incubator at 37°C into a lab environment typically of 20°C, the microplate lid will begin to cool. The humidity in the wells will start to condense on the inside of the microplate lid, initially forming a haze and then larger drops of liquid. These drops act like small lenses and can adversely affect image quality. It is therefore sensible to limit the time out of the incubator before imaging a microplate. For example, it is usually better to take plates out of the incubator one at a time rather than in a batch of three or four.

### ***Take care to keep the bottom surface of microplates clean and always wear gloves when handling microplates***

The Cell Metric imaging optics are below the microplate. It is therefore critical to ensure that the bottom surface of the microplate is free from debris, grease, scratches etc. For optimal image quality, it is important to always take especial care of the bottom surface of the microplate. Some microplates have a very thin, soft plastic well bottom (typically 384-well and 1536-well microplates). These plates are especially vulnerable, because

attempting to clean them simply scratches the plastic. Fingerprints on any plate type can reduce image quality, so *always* wear gloves when handling plates and try to avoid touching the bottom of the plate altogether.

### ***Select an optimal microplate for your workflow***

Whilst most cell culture microplate types from most manufacturers can be imaged on the Cell Metric, they don't all result in the same image quality. It is important to select a plate type that gives accurate results for your workflow, which typically involves balancing how well the cells grow in the plate against image quality and plate cost.

Don't assume that more expensive plates always give better image quality – some glass bottom plates have very poor image quality at the edge of the well, where traces of the glue that holds the glass bottom to the plastic plate top results in a poor image, even in an empty microplate. Conversely, the well diameter in some fully moulded (clear) plates varies considerably – a variation between wells of over 10% sometimes occurs (which means that the same number of cells would be at different confluence levels depending upon which well they were in).

Those microplates that have opaque well walls typically give better image contrast, as there is less stray light.

## Troubleshooting

This section describes some of the most common problems that might occur, and suggest steps to take to overcome them.

### *The instrument fails to initialise*

If you see an error message after you click the 'Scan Microplate' button, there might be a problem communicating with the instrument or the instrument may have entered a state from which it cannot automatically recover.

1. Check that the Cell Metric is plugged in to the power supply and the supply is switched on.
2. Check that the power switch on the Cell Metric is in the 'on' position, and the power indicator light is illuminated.
3. Check that the cable between the Cell Metric and the computer is plugged in at both ends. The computer has two network sockets: one for the Cell Metric and one for connecting the computer to a LAN. The Cell Metric should be plugged in to the PCI card network card; then on-board network socket is for LAN connections.
4. Next close the Cell Metric software, wait at least five seconds (to ensure it has completely closed) and then restart the software. Click the 'Scan Microplate' button and wait for the instrument to initialise.

If the initialisation still fails, power down the Cell Metric using the power switch on the instrument and close the software on the computer. Wait ten seconds, and then turn on the power to the Cell Metric. After a few seconds you should hear a (possibly faint) beep from the Cell Metric. Wait a further twenty seconds to allow the Cell Metric to initialise (approximate 30 seconds in total), then

start the Cell Metric software on the computer and click the 'Scan Microplate' button.

If the initialisation still fails, shutdown and restart the PC, and also power down the Cell Metric as in the previous steps, and try running the software as before.

In the unlikely event that the system still does not initialise, contact Solentim Support. See the section **Exporting Cell Metric log files** below for details on how to send us the logs files for further analysis.

### *A scan fails to complete*

The software will automatically attempt to retry if an error occurs during a scan. However, some conditions will cause a scan to fail. For example, putting a microplate into the microplate carriage incorrectly so that it is not flat, or so that it is rotated so that the well A1 is at the back of the instrument instead of the front can cause the scan to fail because the focus position cannot be maintained across the plate.

Remove and carefully replace the microplate, ensure that the correct plate type is selected in the plate selection screen and retry scanning the microplate.

Check that there is sufficient disk space on the drive that the scan results are being saved to (by default this is drive C:).

If the error persists, contact Solentim Support. . See the section **Exporting Cell Metric log files** for details on how to send us the logs files for further analysis.

***The images in a scan a dark, blurry or out of focus***

Check that the advice in the section Guide to successful imaging using the Cell Metric on page 30 is being followed. Dark images can be caused by low media level, writing on the microplate lid, grease or debris on the microplate bottom etc. Blurry images can also be caused because of debris or scratches on the underside of the microplate, wrong microplate type selection or incorrect placement of the microplate on the microplate carriage.

In some circumstances, better results can be obtained if the 'Fixed Cells (No media)' option is selected when scanning a plate, even when the plate does have media and the cells are not fixed. This option uses a less precise, but more tolerant auto focusing procedure, so may give better results.

***Exporting Cell Metric log files***

If you can send the logs files generated by the Cell Metric to Solentim to analyse, then we should be able to resolve any problems more quickly. To do this click the 'Tools' menu and select 'Export logs...', then save the logs to a location that you can then email the log file from, such as a network location or removable USB drive. Email the file to your Solentim support engineer.