Guide to the See3 Viewing/Scoring/Optimisation tool in C3

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Introduction:

See3 is the software used by the Collaborative Crystallisation Centre (C3) in Melbourne to view and to score images. The software also allows users to create reports and design optimisations. The software is based on the "XtalTrak" application developed by Rigaku, but we have worked extensively on it to add functionality (and get rid of some niggles). Ideas for further improvements always welcome!

Geek stuff:

The program is written in the ASP MVC .NET Framework using KendoUI for the user interface. Alex Kruger, Nick Rosa and Marko Ristic have all worked to transform the original Rigaku code into See3.

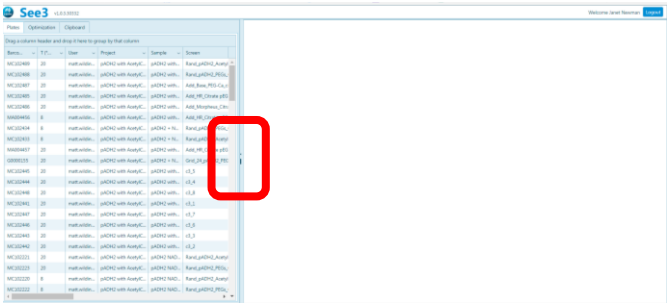
About the Login page:

Our Twitter feed is on the login page, this is where you will find the latest info from C3 – look here if something doesn't seem to be working: if we haven't tweeted about it, we probably don't know about it – please let us know (email c3@csiro.au) about any problems you are having.

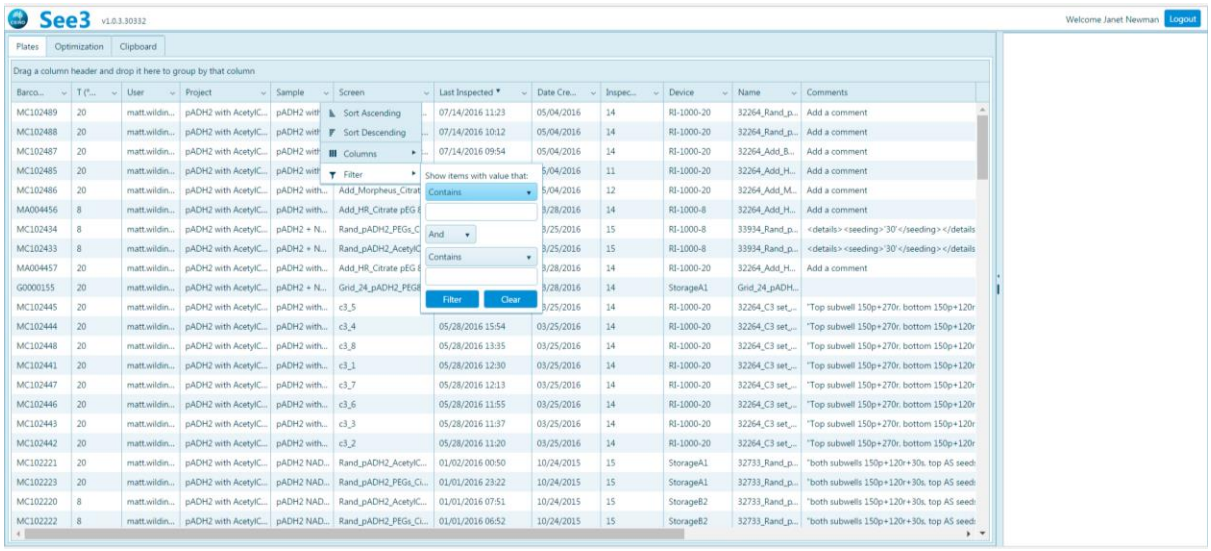
Using the software:

Navigate to See3.csiro.au in the browser of your choice, and log on with your C3 username and password.

This will open up the "Plates" tab, which is dominated by a grid on the left hand side of the page. The grid can take several seconds to load. The bar to the right of the grid may be moved by dragging the middle of the bar (circled in red, below) with the left mouse button depressed. Many of the panes can be resized the same way.



The grid can be modified as you wish – the changes will only persist for the current session. You can change the order of the grid columns, sort by a column, group by a column, or filter a column for a particular string (filtering shown below).



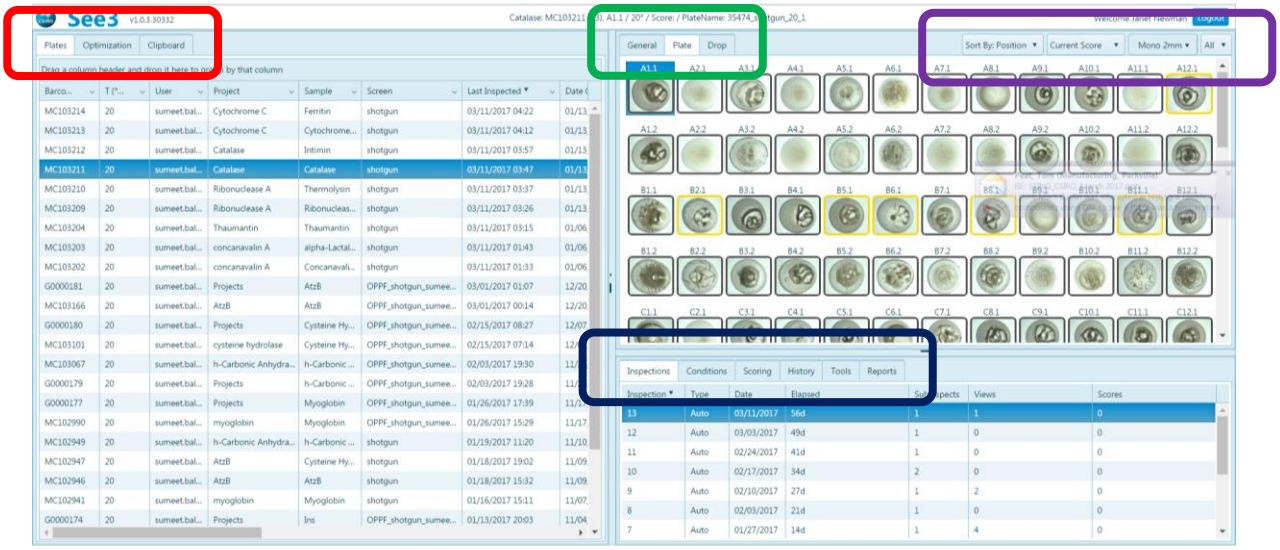
Clicking on a row in the grid will open up the latest inspection for that plate. There are a number of options on this view:

There are three tabs on the upper, left hand side (circled in red, below) – ‘Plates’, ‘Optimisation’ and ‘Clipboard’. Call these *VIEWS*

There are three tabs in the upper, middle screen (circled in green) ‘General’, ‘Plate’, ‘Drop’. Call these *TABS*

There are four pulldown menus on the upper, right hand side (circled in purple) ‘Sort by’, ‘Score’, ‘Type’ ‘Subwells’

There are 6 tabs in the middle of the screen, below the drops (circled in navy blue) ‘Inspections’, ’Conditions’, ’Scoring’, ‘History’, ’Tools’ and ‘Reports’. Call these *OPTIONS*



Plates *VIEW*, Plate *TAB*:

Sort by

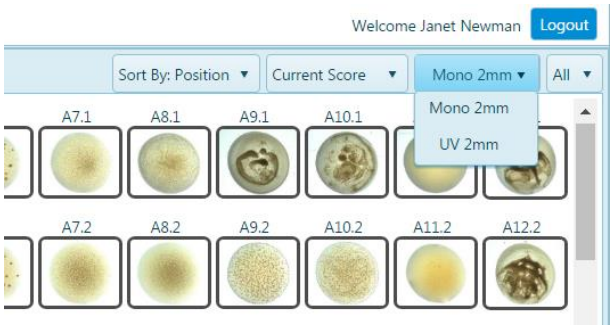
'Sort by' allows you to arrange the images either by position in the plate, or by an assigned score. By default, human scores are shown first, and better scores are shown first. Below is the same plate as above, but with the 'Sort by Score' option selected:

Score

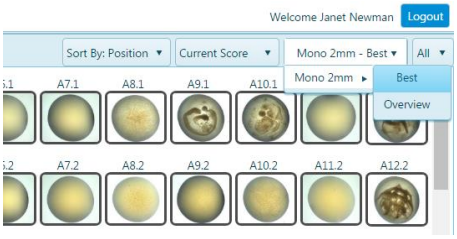
In the C3 database, inspections rather than drops are scored. This gives us the ability in See3 to choose between displaying the 'Current Score' (i.e., the most recent score associated with the inspection being viewed) and the 'Last Score', which is the most recent score associated with any inspection of that drop. By default, the 'Last Score' is shown. If there are both machine and human scores for a droplet, one can view only human scores by selecting the 'Last User Score' option.

Type

In C3 we generally collect zoomed colour images collected with visible light. Our imaging schedules include other types of images – for example, images collected with UV light, or with polarising filters in place. If there is more than one type of image collected at the same time (i.e. in the same inspection), they can be accessed by choosing the appropriate image type from the 'Type' pulldown. The example below shows that there were both visible and UV images for a particular inspection, and the visible option has been selected.

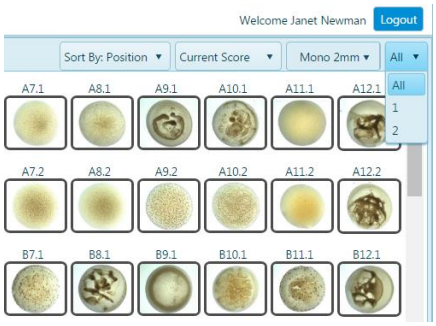


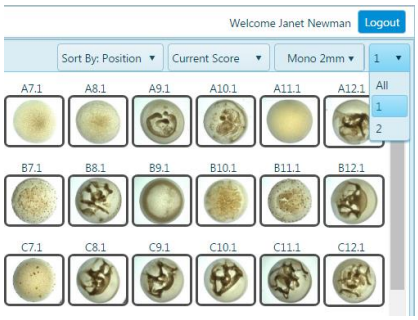
The first inspection of a plate will show both an overview inspection and the zoomed inspection.



Subwells

C3 uses two subwell plates (SwissSci SD-2 plates, aka 'MRC plates'); by default all drops are shown. If you want to only view the drops in the top (or the bottom) subwell then choose the appropriate subwell set from this pulldown





Plates *VIEW*, Drop *TAB*:

In this arrangement, the 'Sort' menu changes to 'Split', which allows the user to see two images from the same inspection (for example, Visible and UV images) side by side.

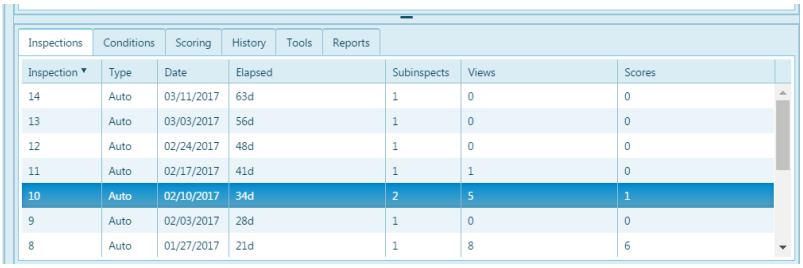
Most of the six *OPTIONS* give information or functions the Drop *TAB*. In the Drop *TAB*, the image can be zoomed in using the mouse wheel, and the green scale bar will adjust according to the zoom

level. Once zoomed, holding down the left mouse button allows you to drag the drop around. The zooming and re-centring on the image persist until another plate is loaded from the plate grid.

Plates *VIEW*, Drop *TAB,*

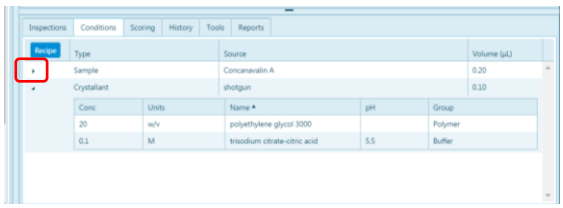
Inspections OPTION

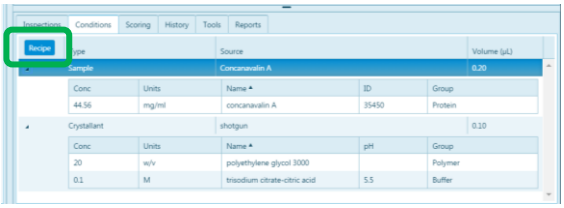
Shows the list of inspections for a selected plate, and gives the Inspection Number, the Date of Inspection, the time since the plate was set up, the number of sub-inspections, how many times an inspection has been viewed, and if any scores were associated with an inspection. This is the default *OPTION.*



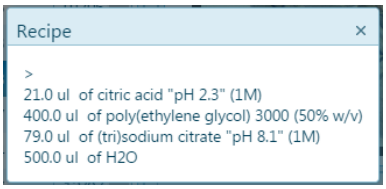
Conditions OPTION

Shows details about the chemistry of the droplet (what is in the reservoir), information about the sample (concentration, sample_ID), the ratio of the sample to the reservoir and information about additives, if appropriate. By default, the treeview for the reservoir contents is open, to see more information about the sample, click on the small arrow to the left of the sample (circled in red, below)





Click the Recipe button (circled green, above) to find out how to make up 1 mL of reservoir solution using C3 stocks. Use this recipe when booking a 'One Tube' request on the TECAN



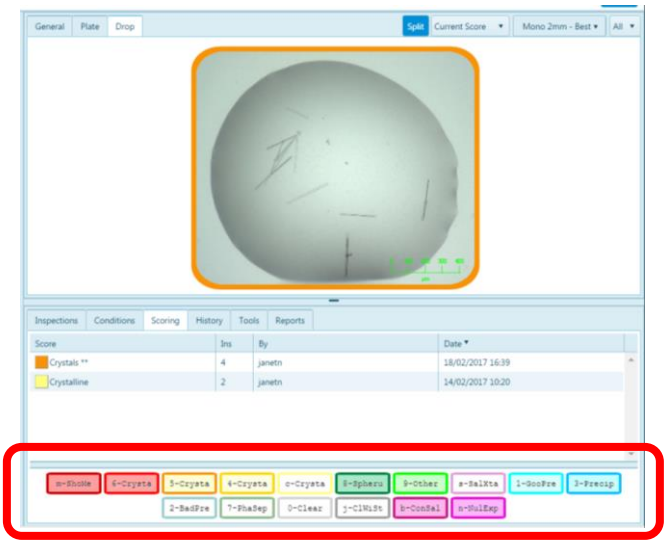
Scoring OPTION

There are 16 possible user scores one can associate with a droplet – see the table below. The order of the scores in the table below shows the ordering of scores when the 'Sort by Score' menu option is chosen. The descriptions associated with a score are somewhat arbitrary, as one project's 'Crystals***' might be another project's 'Crystal*', but the idea is that the more stars there are, the better. When a score is associated with a droplet, a coloured rim is drawn around the crystal image. The colours are grouped – yellow/red colours are associated with crystals, blue colours with precipitate, green colours for other results.

SCORE	DESCRIPTION	SHORTCUT
SHOOT ME	In plate screening marker	m

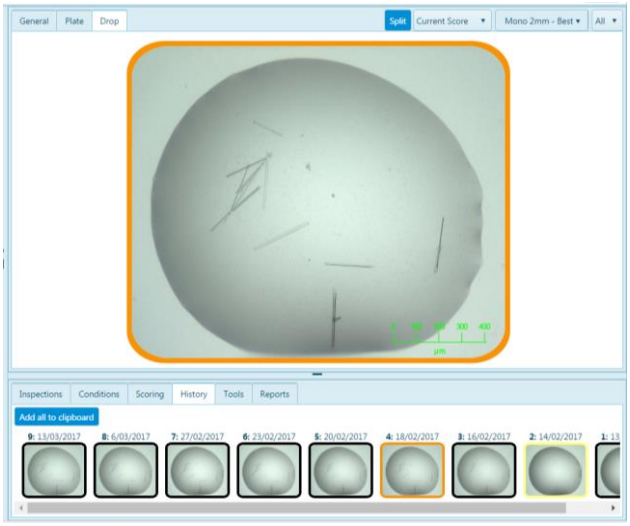
CRYSTALS ***	Worth harvesting	6
CRYSTALS **	Needs some work, but very promising	5
CRYSTALS *	too many, too small, needs a lot of optimising	4
CRYSTALLINE	Microcrystalline, optimise only if desperate	c
SPHERULITES	Things that look like meteors	8
OTHER	Unknown	9
SALT XTAL?	Putative salt crystals	s
GOOD PRECIPITATE	Grainy, shiny, birefringent	1
PRECIPITATE	All other precipitates	3
BAD PRECIPITATE	Brown, smoky, denatured	2
PHASE SEPARATION	Oily, immiscible	7
CLEAR	Clear droplet	0
CLEAR WITH STUFF	Clear drops with dust, fibres, or other non-crystalline matter	j
CONFIRMED SALT	Confirmed by UV or diffraction	b
NULL EXPERIMENT	This experiment didn't work (dried, not put down well)	n

One can assign a score to a droplet by clicking on one of the coloured boxes in the *Scoring OPTIONS* (circled red below), or by using the keyboard shortcut given in the table. When a score is associated with a drop, the viewing software will advance to the next image, and the scorer and score date will show up in the Scoring *OPTION*.



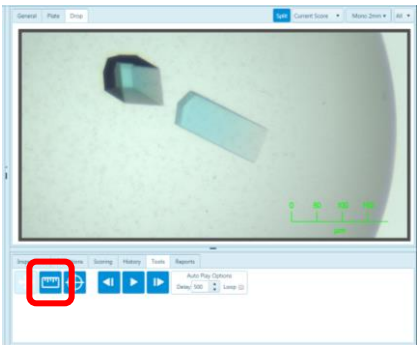
History OPTION

Shows the time-course of the droplet, that is, all the images associated with the droplet to date. Clicking on one of the thumbnails in the History OPTION will move it to the main viewing pane.



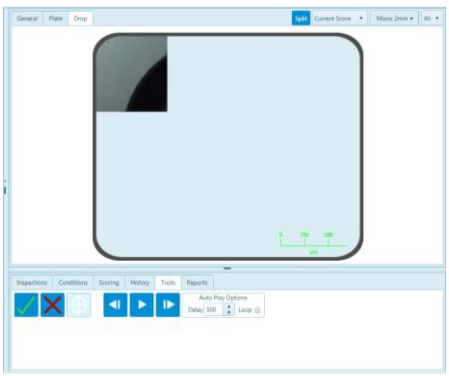
Tools OPTION

The measure tool is selected by clicking on the ruler icon (circled red, below left), and is de-selected by clicking on the four-arrow icon (circled green, below right). This option allows one to measure an object in a drop. The green ruler lines on the image persist until another plate is loaded from the plate grid.



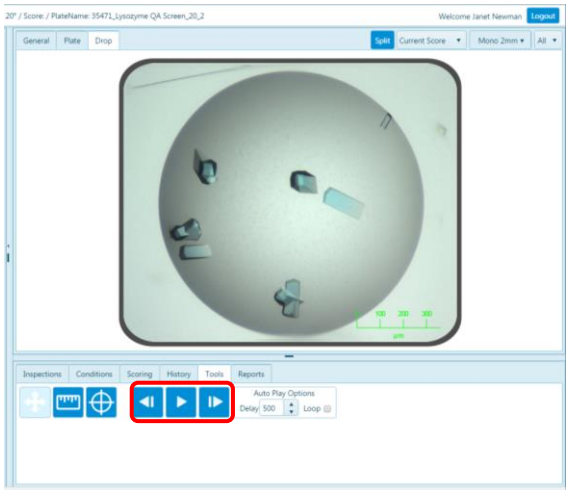
The re-centre/re-size tool is selected by clicking on the crosshair icon (circled in purple, below)

Once clicked, use the mouse to re-centre/re-zoom the droplet (some guesswork might be involved, as is the case above and below).



Click the green tick or the red cross to exit the re-centre tool. If you selected the tick the new centre and extent will be used for the next inspection of that drop, and the icon will appear in the drop image to confirm that the drop has been re-centred.

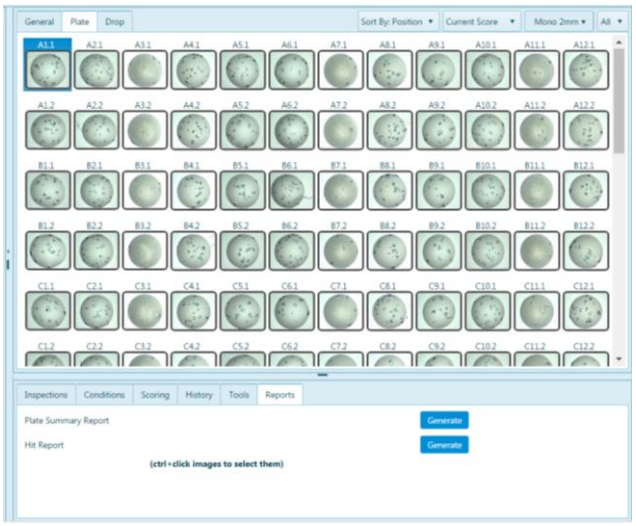
The forward, back and play buttons (circled below) move you through the images – the delay between the images can be set here as well.



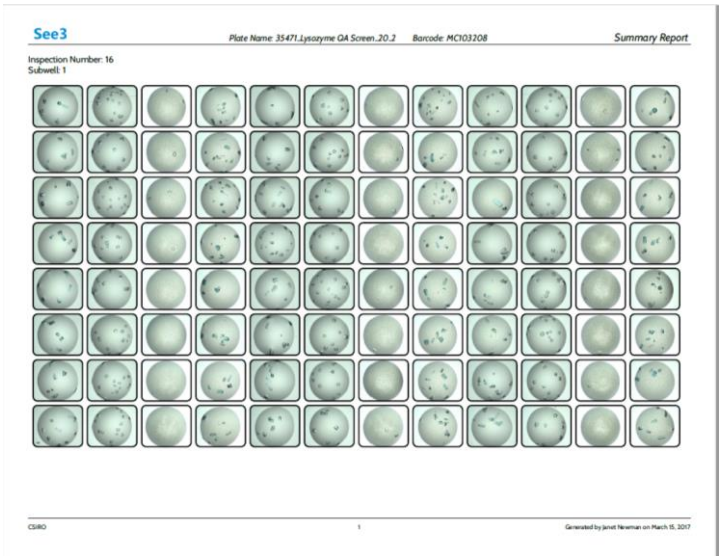
The autoplay function can also be toggled on and off with the space bar, and the keyboard arrow keys will also move you between images.

Reports OPTION

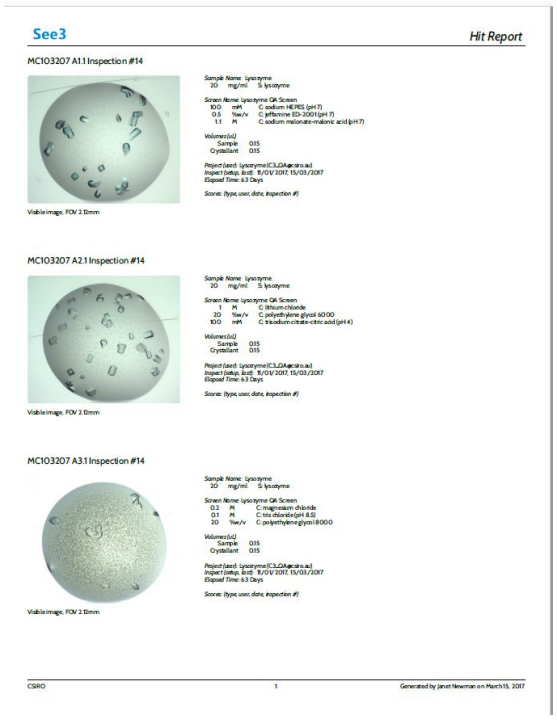
There are two reports, one which gives an overview of the selected plates, and one which gives a more detailed report of images that have been placed on the clipboard. Click on the “Generate” button opposite the report that you desire.



The Overview Report gives a .pdf report of the plate as it is laid out



The Hit Report gives details about individual droplets that have been selected. At the end of the Hit Report, a summary of the chemistry found in the drops selected for the report is given, an example is shown below.



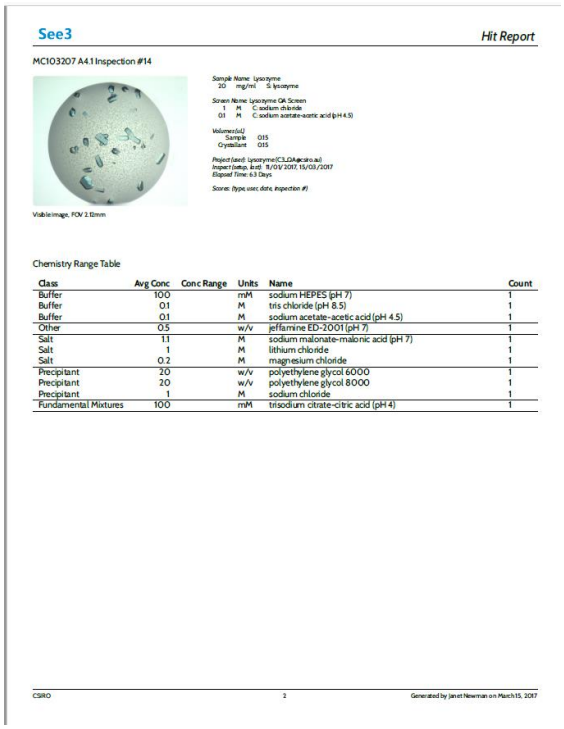
See3 Hit Report

MC103207 A1.1 Inspection #14

MC103207 A2.1 Inspection #14

MC103207 A3.1 Inspection #14

CSIRO 1 Generated by Janet Newman on March 15, 2017



See3 Hit Report

MC103207 A4.1 Inspection #14

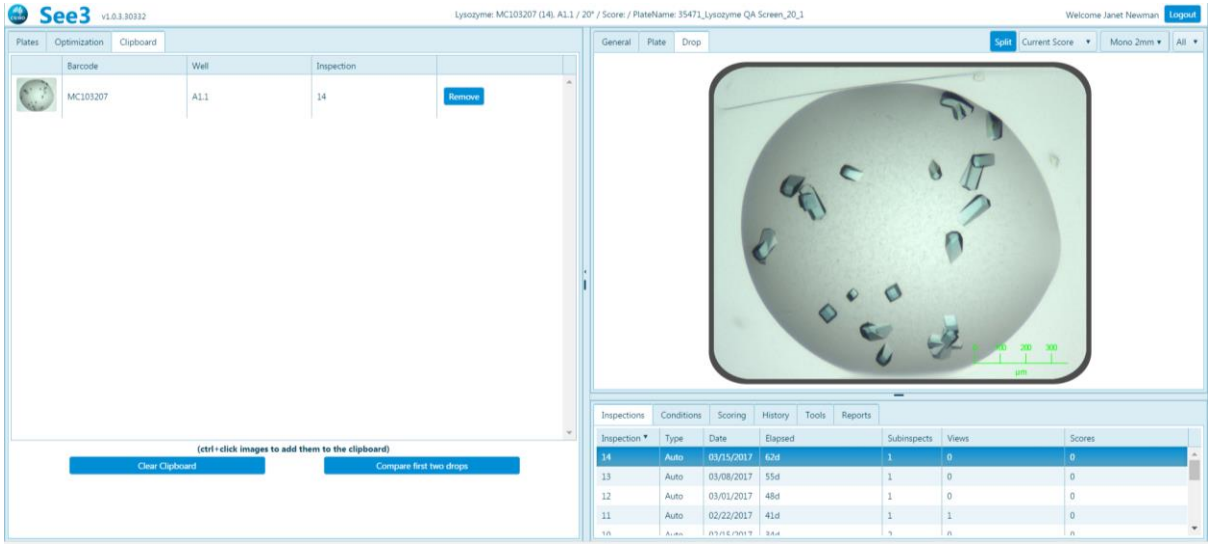
Chemistry Range Table

Class	Avg Conc	Conc Range	Units	Name	Count
Buffer	100		mM	sodium HEPES (pH 7)	1
Buffer	0.1		M	tris chloride (pH 8.5)	1
Buffer	0.1		M	sodium acetate-acetic acid (pH 4.5)	1
Other	0.5		w/v	jeffamine ED-2001 (pH 7)	1
Salt	1.1		M	sodium malonate-malonic acid (pH 7)	1
Salt	1		M	lithium chloride	1
Salt	0.2		M	magnesium chloride	1
Precipitant	20		w/v	polyethylene glycol 6000	1
Precipitant	20		w/v	polyethylene glycol 8000	1
Precipitant	1		M	sodium chloride	1
Fundamental Mixtures	100		mM	trisodium citrate-citric acid (pH 4)	1

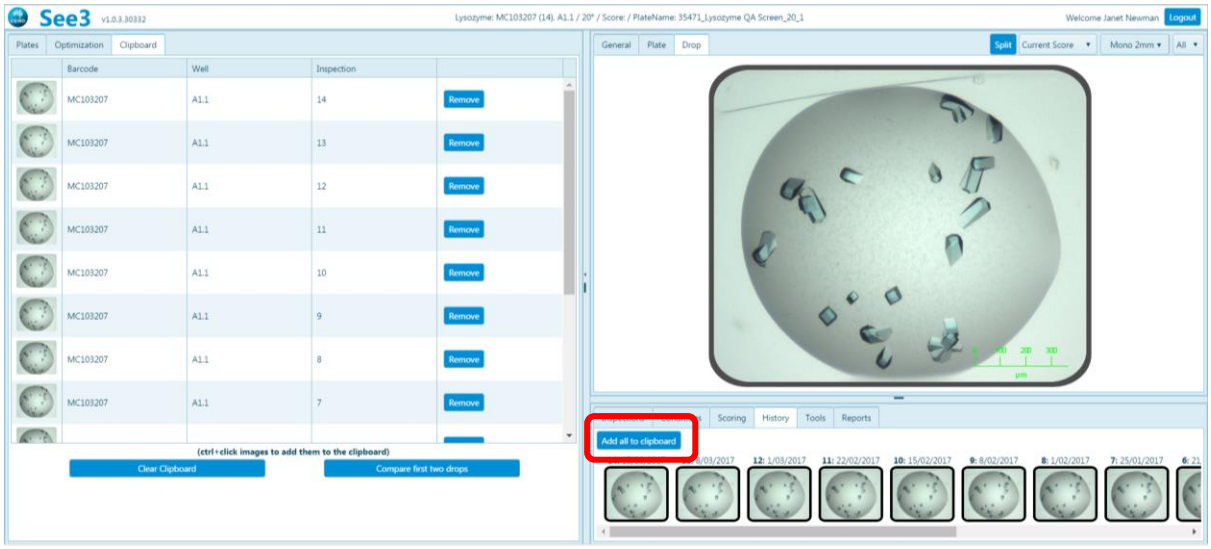
CSIRO 2 Generated by Janet Newman on March 15, 2017

Clipboard *VIEW*

The clipboard allows the user to assemble a set of images (droplets) that are interesting – these can be from one or more plates. The clipboard is cleared each time a user logs out of the system. A drop is added to the clipboard by holding down the CTRL key whilst clicking the appropriate image. This works both from the Plate or the Drop VIEW.

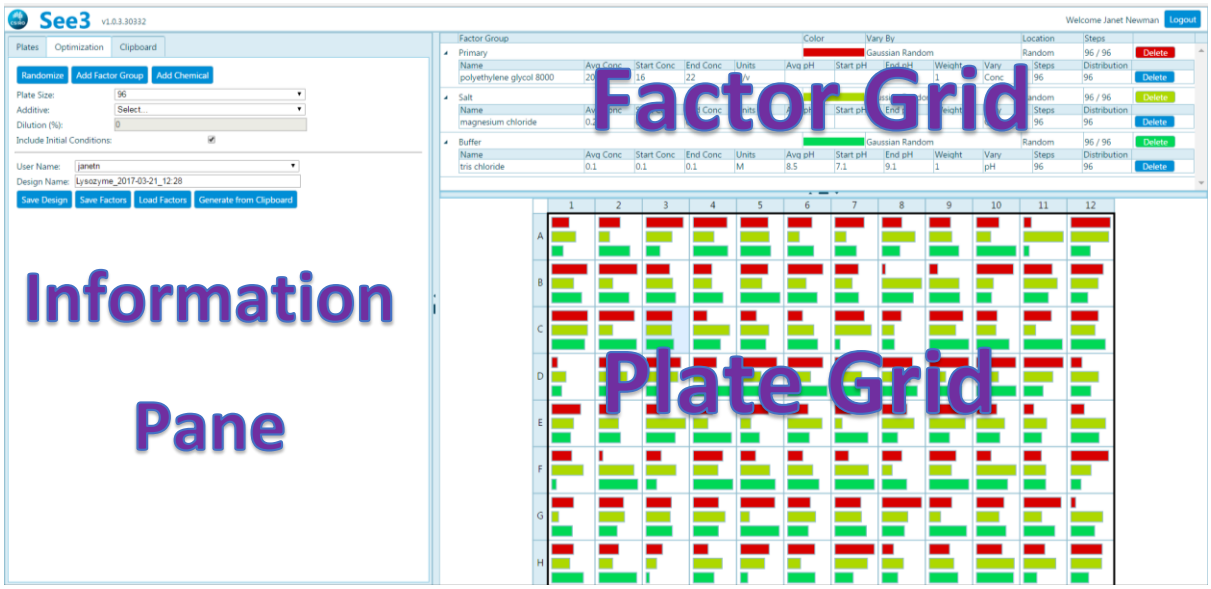


Once the appropriate set of drops has been assembled, these can be used in either optimisation or to generate a Hit report. One can add all the images from the History *OPTION* to the clipboard, then use the Hit Report to get a record of the time course of a droplet


See3
Lysozyme: MC103207 (14), A1.1 / 20° / Score: / PlateName: 35471_Lysozyme QA Screen_20_1
Welcome Janet Newman
Logout
Plates
Optimization
Clipboard
Barcode
Well
Inspection
MC103207
A1.1
14
13
12
11
10
9
8
7
Remove
(ctrl+click images to add them to the clipboard)
Clear Clipboard
Compare first two drops
General
Plate
Drop
Split
Current Score
Mono 2mm
All
Scoring
History
Tools
Reports
Add all to clipboard

Optimisation *VIEW*

The Optimisation VIEW has three areas, the **Information Pane**, found on the left hand side of the VIEW, the **Factor Grid**, on the upper right hand side of the VIEW and the **Plate Grid**, on the lower right side of the VIEW.

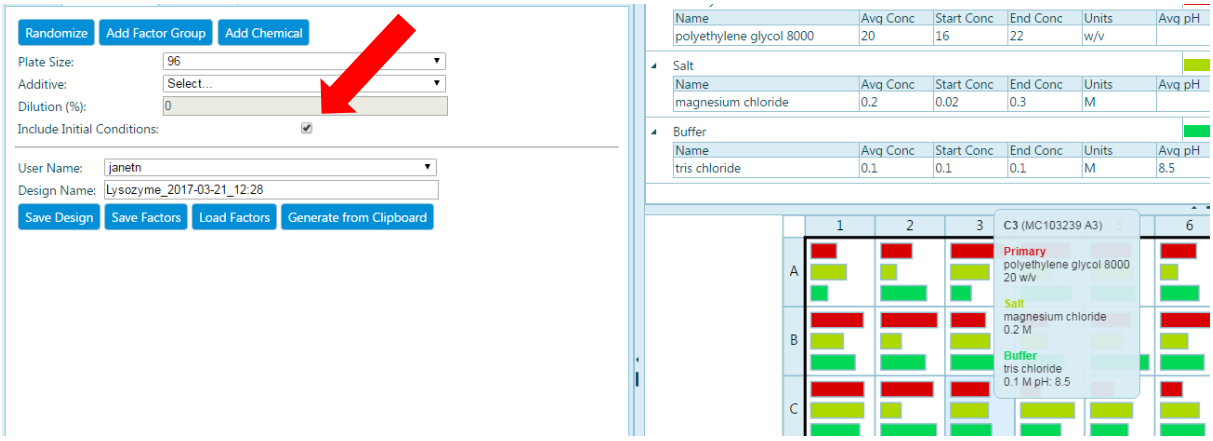


The **Information Pane** reads from and writes to the underlying database. This pane allows one to select the 'Plate Size' - the size of the optimisation: 24 well (4 rows x 6 columns); 48 well (6 rows x 8 columns) or most commonly 96 well (8 rows x 12 columns). Following standard convention, rows are lettered and columns are numbered and is always shown in portrait orientation with well A1 in the upper left hand corner. Thus a 96-well plate has rows A-H, columns 1-12.

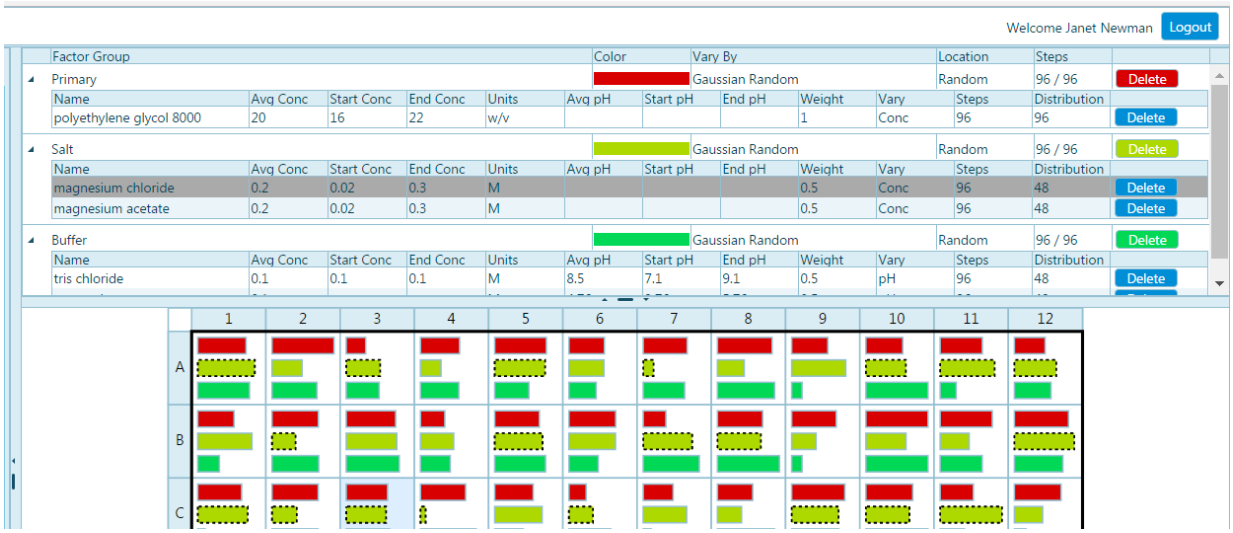
In the **Information Pane** one can populate the **Factor Grid** by adding Factor Groups and Chemicals to the optimisation - manually or from the clipboard, select designs to be used as additive screens, set the Design name and save the Design and Factor Grid.

The **Factor Grid** encapsulates the concepts of the optimisation, whereas the **Plate Grid** shows the current instantiation generated by that particular **Factor Grid**. Thus the same **Factor Grid** will generate different **Plate Grids** if the 'Randomize' button on the **Information Pane** is clicked. If the 'Save Design' button on the **Information Pane** is pressed, the conditions currently shown in the **Plate Grid** will be saved. A previously saved **Factor Grid** can be reloaded by clicking the 'Load Factors' button – this will display a list of previously saved grids, which can be selected.

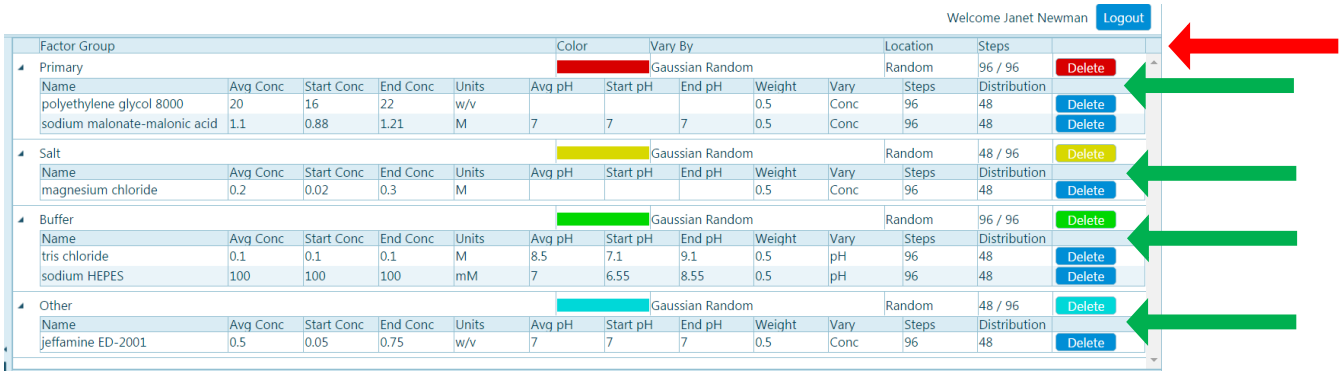
The **Plate Grid** gives a visual representation of the current instance of the **Factor Grid**, where the colour of the bar indicates from which Factor Group it comes, and the length of the bar gives an indication of the concentration or the pH (the distance from the lower limit). Mousing over a well will display the contents of the well. By default, any conditions used to define the optimisation will be transferred unchanged to the optimisation, and will be found in the wells starting at position C3. These wells have a pale blue background, and the starting condition is included in the information provided in the mouse over. Uncheck the 'Include Initial Conditions' checkbox in the **Information Pane** to turn off this feature (Red Arrow, below)



Mousing over a particular coloured bar will highlight the other wells which contain that same chemical (black dashed border), and will highlight where that chemical is found in the Factor Grid. Conversely, mousing over a chemical in the **Factor Grid** will highlight where that chemical is found in the **Plate Grid.**



The **Factor Grid** consists of one or more Factor Groups. Each Factor Group contains one or more Chemical Factors. A Chemical Factor is a chemical with an associated concentration, unit and (potentially) pH value. Thus ‘ammonium sulfate’ is a chemical, and ‘1 M ammonium sulfate’ is a Chemical Factor. The **Factor Grid** has a Header Bar (marked with a red arrow, below) which contains columns relevant to all Factor Groups. Each individual Factor Group has a secondary Header (green arrows) which contains columns relevant to the Chemical Factors within the Factor Group. The coloured ‘Delete’ buttons remove the entire Factor Group, whereas the blue ‘Delete’ buttons remove individual Chemical Factors from within a Factor Group.

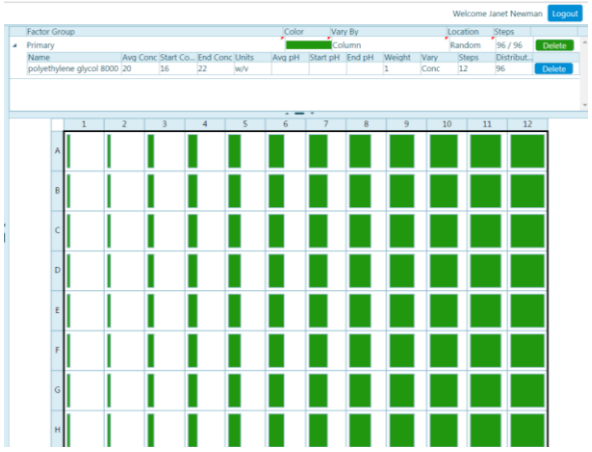
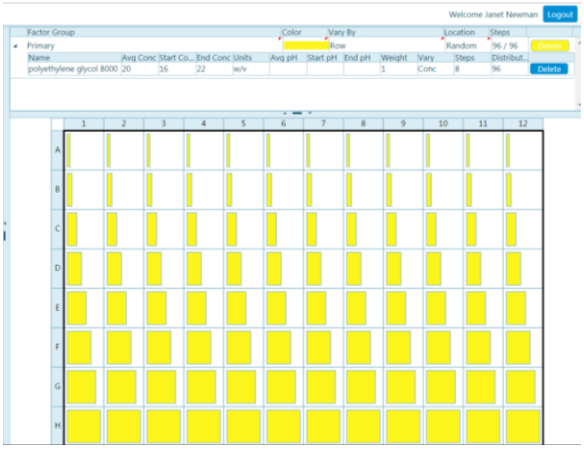


Within a Factor Group one can vary the colour, the way in which concentration varies ('concentration' will be used throughout, the same logic would apply to pH values if the pH rather than concentration is being varied), and the way in which the values are arrayed in the **Plate Grid**. All factors in the group will use the same 'Colour', 'Vary By' and 'Location' options.

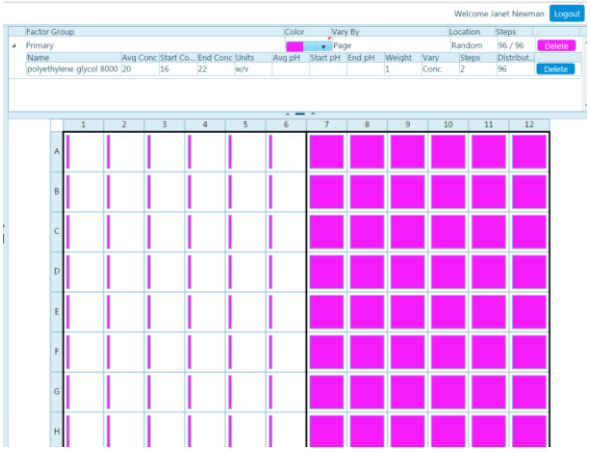
The 'Vary By' pulldown menu shows the different options for changing the concentration of the factors within the group. By default, the concentration is varied randomly between the upper (starting x 110%) and lower (starting x 80%) limits, but with a bias towards the starting value. This is called 'Gaussian Random'. An unbiased random selection of concentration between the upper and lower limits can be chosen with the 'Vary By' option 'Uniform Random' – these are shown as the red and blue images below.

Both the Gaussian and Uniform random selections can be ordered, so that the smallest concentration value is found in the upper right hand corner of the selection, and the largest concentration is found in the lower right. Notice that the values don't change, but the arrangement does.

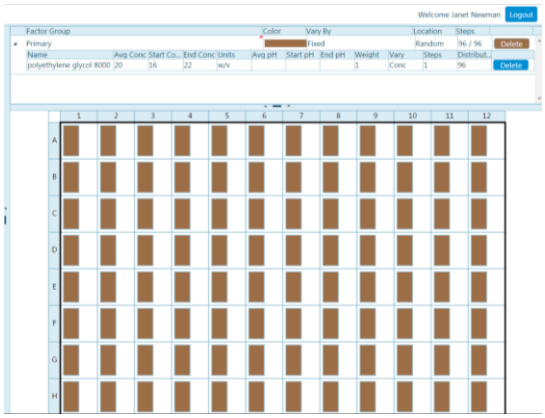

‘Vary By’ column sets up a grid from left to right across the columns of the plate (green, below) and ‘Vary By’ row sets up a grid from top to bottom across the rows of the plate (yellow, below)

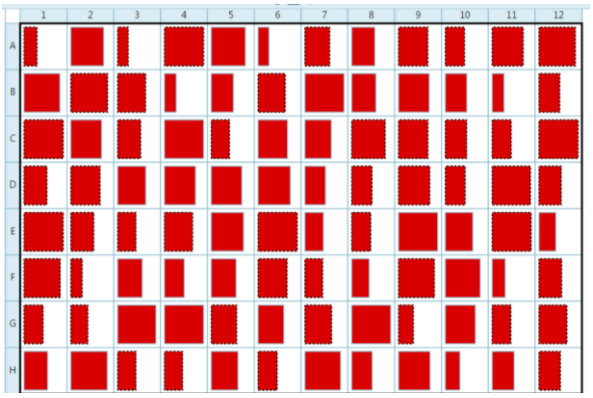
‘Vary By’ block gives four values spread over the quadrants of the plate (cyan, below), and ‘Vary By’ Page gives two values spread over the two halves of the plate (magenta, below)

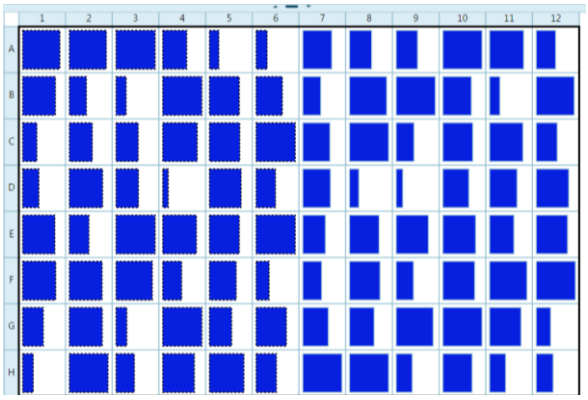



‘Vary By’ fixed keeps the value of the factor constant (brown, below)

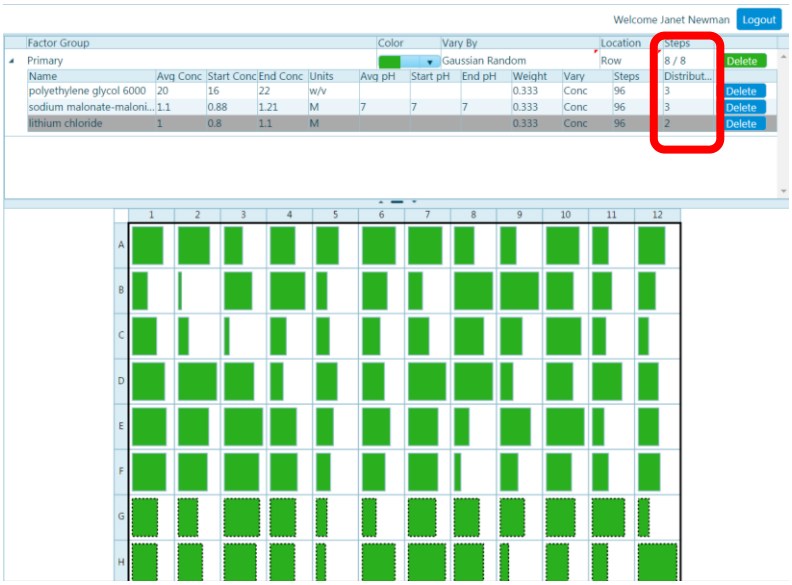


When there is more than one Chemical Factor in the Factor Group then the ‘Location’ option becomes relevant. This allows one to position the Chemical Factors on the plate, irrespective of how the concentration is being varied. By default, the distribution of the Chemical Factors on the plate is ‘Random’ (red, below - look to see which wells have the black dotted border). By selecting ‘Column’ under ‘Location’, the plate is sorted so that all the wells containing a particular Chemical Factor are co-located (blue, below – look to see which wells have the black dotted border). The distribution of the Chemical Factor across the plate is determined by the weight associated with the Chemical Factor. In the following example, each of the two Chemical Factors within the Factor group have an equal weighting of 0.5, so so each gets half the number of available columns. The order of the Chemical Factors within the group dictates where on the plate the clustering occurs – the first Chemical Factor in the Factor Group will be to the left (Location = Column or Plate) to the top (Location = Row), to the upper left (Location = Block). One can drag and drop Chemical Factors within a Factor Group to fine tune the location on the plate.

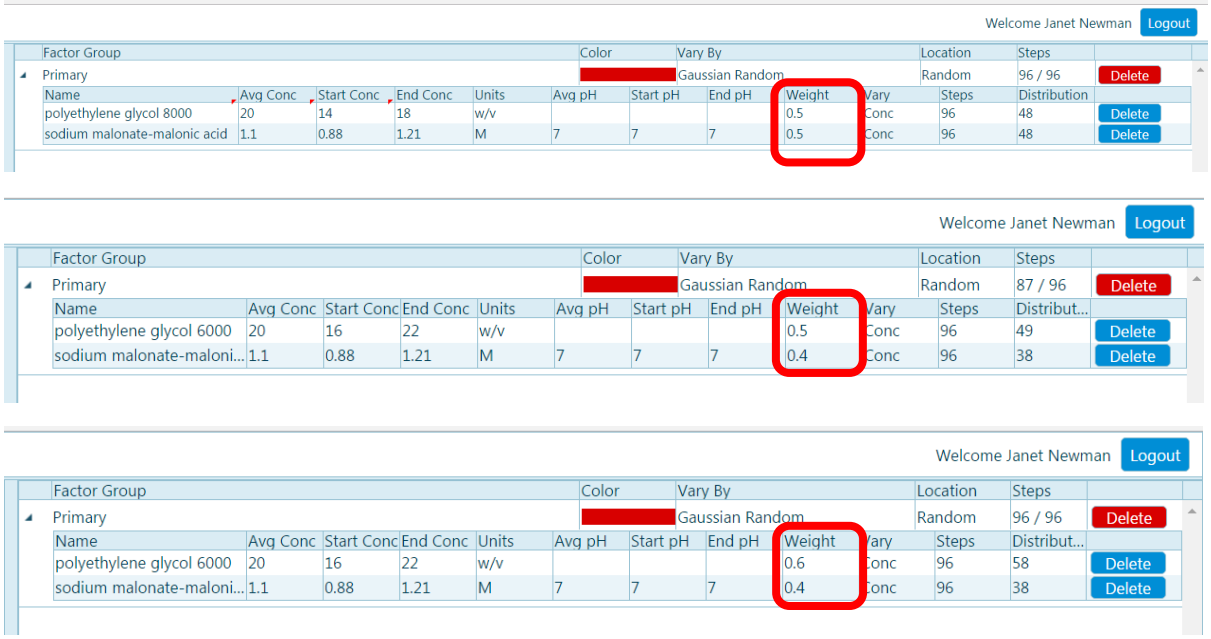




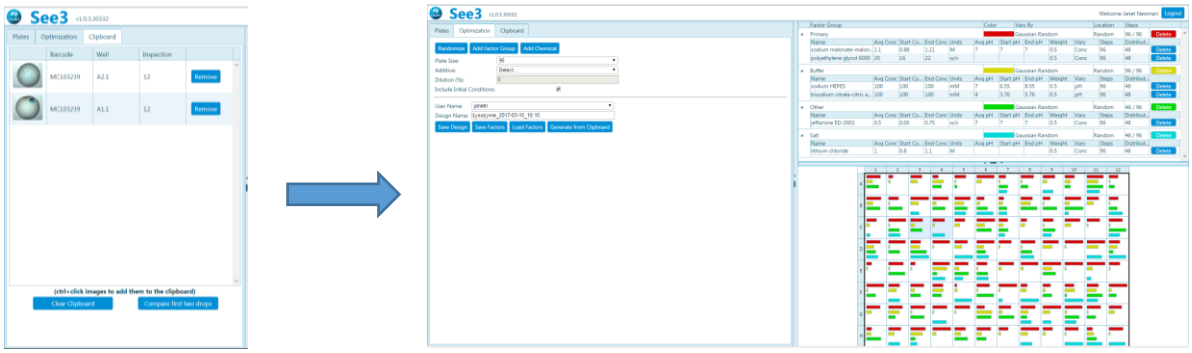
If the number of Chemical Factors or weighting of the Chemical Factors in a Factor Group does not divide evenly into the location (for example, below – three Chemical Factors of equal weight cannot be spread exactly into a Location = Row), the software will round as best it can to whole columns, rows, blocks or pages – and this will be shown in the ‘Distribution’ column of the Secondary Header – circled in red, below.



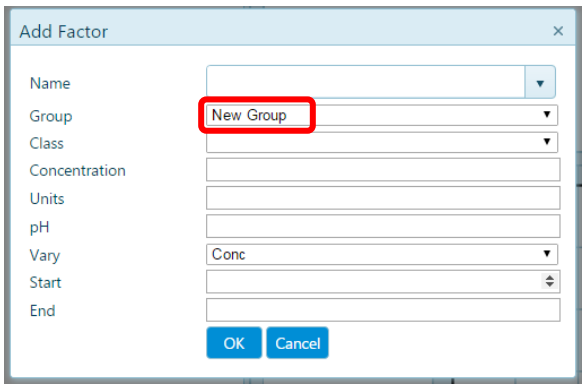
Most of the columns in the secondary header within a Factor Group can be edited for each Chemical Factor within the Factor Group, although 'Steps' and 'Distribution' are information columns which depend on either the 'Weight' or 'Weight' and 'Location'. Note that the sum of 'Weight' for a Factor Group may never exceed 1, even temporarily – so to change from having two Chemical Factors with equal (0.5) weight to having one at 0.6 and one at 0.4 requires changing the weight 0.5 -> 0.4 first, then changing the weight 0.5 -> 0.6, as shown below.

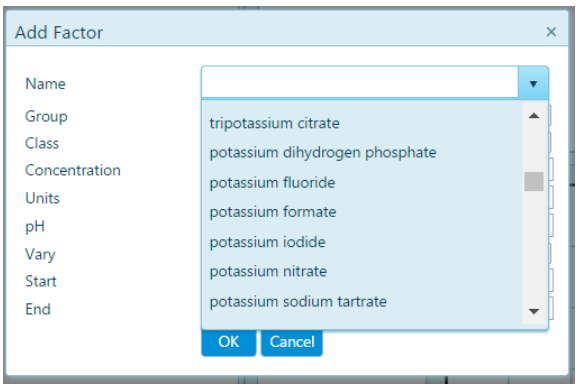


Optimisation experiments can be designed from scratch or from droplets placed on the clipboard. Click the 'Generate from Clipboard' button (circled in red below) to load the summary chemical table from the Hit Report into the **Factor Grid**

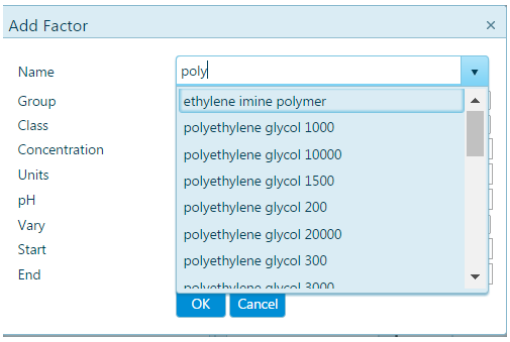


If starting from scratch use the ‘Add Chemical’ button to populate the Factor Grid – click on the ‘Add Chemical’ button, and a dialog will pop open





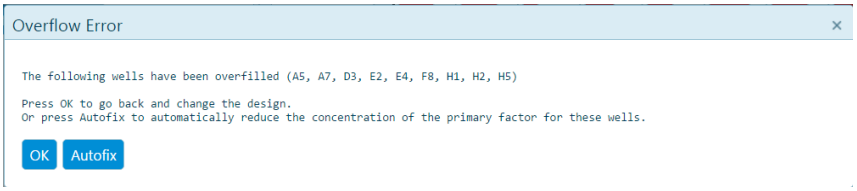
Starting from the top, populate the fields – the ‘Name’ field shows a list chemicals available in C3. The pulldown menu list can be filtered by including a string in the ‘Name’ Field. Notice that the full chemical name is used, not common abbreviations. The string ‘poly’ typed into the ‘Name’ field will return all the polyethylene glycols, but the string ‘PEG’ will not.



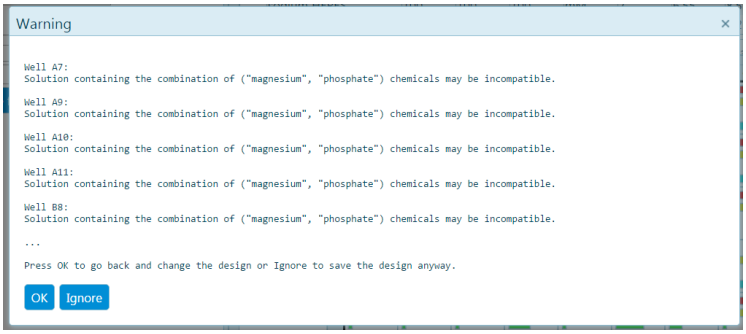
Once the chemical has been selected, chose the desired factor group (or just use the default ‘New Group’ – the name can be changed later) and the desired concentration. By default the limits of the chemical will be 80-100% of the given concentration UNLESS the chemical concentration is used at 0.2 M or less, in which case the default limits are set to 10-150% of the given concentration. If the pH is varied, then by default the limits are set to the [nearest pKa] +/- 1 pH unit. If these limits are not what you want, then the limits can be changed

Use the ‘New Factor Group’ button to create one or more Factor Groups in the Factor Grid, rename as required, then these new Factor Groups can be selected as the destination for the Chemical Factor.

A Design is saved into the database by pressing the ‘Save Design’ button on the **Information Pane.** When ‘Save Design’ is clicked, a check is made to ensure that all the stocks are available in C3 to make the required design. If the design has been made where there is a mismatch between the units of the required Chemical Factor and the units of the stock available in C3 this will cause an error. This happens when, say, 20 w/v polyethylene glycol 200 has been used as a Chemical Factor, and the stock in C3 is 80 v/v polyethylene glycol 200. More commonly, there may be ‘overfill’ errors, where the stocks available in C3 are not concentrated enough to be able to make the combination of chemical factors. For example, if a well was designed to contain 30 w/v polyethylene glycol 4000, 2M ammonium sulfate – this can’t be made with C3 stocks of 50 w/v polyethylene glycol 4000 and 3.5M ammonium sulfate (as 30/50+3/3.5 >1). If there are less than 20 overfill errors, the software will give the option of autofixing (Click ‘Autofix’), which it does by reducing the concentration of the primary factor until there is no error, if possible. Clicking ‘OK’ will return you to the Optimization VIEW to fix this manually.



The software will warn, but not error, if incompatible chemicals are co-located in wells. Combinations that are incompatible include magnesium salts with phosphate salts and zinc salts with sodium HEPES buffer. Press ‘OK’ to go back into the Optimization VIEW, or ‘Ignore’ to continue writing the design into the database.



The **Factor Grid** can be saved by clicking the ‘Save Factors’ button on the **Information Pane**. The saved **Factor Grid** will have the ‘Design Name’ as shown in the **Information Pane** at the time the button is clicked.