# 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:

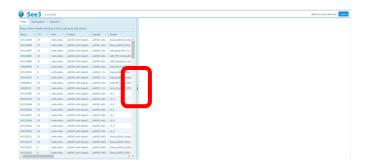
Our Twitter feed is on the login page, this is where you will find the latest info from C3 – look here if something doesn't seem to be working: if we haven't tweeted about it, we probably don't know about it – please let us know (email c3@csiro.au) about any problems you are having.

# 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).

Se	e3 v1.0.3	3.30332											Welcome Jane
Plates Opt	imization	Clipboard											
Drag a column	header and d	trop it here to g	roup by that column										
Barco ~	T (* ~	User ~	Project ~	Sample ~	Screen	Last Inspected * ~	Date Cre ~	Inspec ~	Device ~	Name ~	Comments		
MC102489	20	matt.wildin	pADH2 with AcetylC	pADH2 with	Sort Ascending	07/14/2016 11:23	05/04/2016	14	RI-1000-20	32264_Rand_p	Add a comment		
MC102488	20	matt.wildin	pADH2 with AcetylC	pADH2 with	F Sort Descending	07/14/2016 10:12	05/04/2016	14	RI-1000-20	32264_Rand_p	Add a comment		
MC102487	20	matt.wildin	pADH2 with AcetylC	pADH2 with	Columns	07/14/2016 09:54	05/04/2016	14	RI-1000-20	32264_Add_B.,	Add a comment		
MC102485	20	matt.wildin	pADH2 with AcetylC	pADH2 with	Filter •	how items with value that:	5/04/2016	11	RI-1000-20	32264_Add_H	Add a comment		
MC102486	20	matt.wildin	pADH2 with AcetylC	pADH2 with		Contains •	5/04/2016	12	RI-1000-20	32264_Add_M	Add a comment		
MA004456	8	matt.wildin	pADH2 with AcetylC	pADH2 with	Add_HR_Citrate pEG 8		3/28/2016	14	RI-1000-8	32264_Add_H	Add a comment		
MC102434	8	matt.wildin	pADH2 with AcetylC	pADH2 + N	Rand_pADH2_PEGs_C	And v	3/25/2016	15	RI-1000-8	33934_Rand_p	<details><seeding>'30'</seeding><td></td><td></td></details>		
MC102433	8	matt.wildin	pADH2 with AcetylC	pADH2 + N	Rand_pADH2_AcetyIC	Contains •	8/25/2016	15	RI-1000-8	33934_Rand_p	<details><seeding>'30'</seeding><td></td><td></td></details>		
MA004457	20	matt.wildin	pADH2 with AcetylC	pADH2 with	Add_HR_Citrate pEG 8	contails	3/28/2016	14	RI-1000-20	32264_Add_H	Add a comment		
G0000155	20	matt.wildin	pADH2 with AcetylC	pADH2 + N	Grid_24_pADH2_PEG8		3/28/2016	14	StorageA1	Grid_24_pADH		i.	
MC102445	20	matt.wildin	pADH2 with AcetylC	pADH2 with	c3_5	Filter Clear	3/25/2016	14	RI-1000-20	32264_C3 set	'Top subwell 150p+270r. bottom 150p+120r		
MC102444	20	matt.wildin	pADH2 with AcetyIC	pADH2 with	c3_4	05/28/2016 15:54	03/25/2016	14	RI-1000-20	32264_C3 set,	"Top subwell 150p+270r. bottom 150p+120r		
MC102448	20	matt.wildin	pADH2 with Acety/C	pADH2 with	c3_8	05/28/2016 13:35	03/25/2016	14	RI-1000-20	32264_C3 set	"Top subwell 150p+270r. bottom 150p+120r		
MC102441	20	matt.wildin	pADH2 with AcetylC	pADH2 with	c3_1	05/28/2016 12:30	03/25/2016	14	RI-1000-20	32264_C3 set	"Top subwell 150p+270r. bottom 150p+120r		
MC102447	20	matt.wildin	pADH2 with AcetylC	pADH2 with	c3_7	05/28/2016 12:13	03/25/2016	14	RI-1000-20	32264_C3 set	'Top subwell 150p+270r. bottom 150p+120r		
MC102446	20	matt.wildin	pADH2 with AcetylC	pADH2 with	c3_6	05/28/2016 11:55	03/25/2016	14	RI-1000-20	32264_C3 set	"Top subwell 150p+270r, bottom 150p+120r		
MC102443	20	matt.wildin	pADH2 with AcetyIC	pADH2 with	c3_3	05/28/2016 11:37	03/25/2016	14	RI-1000-20	32264_C3 set	"Top subwell 150p+270r. bottom 150p+120r		
MC102442	20	matt.wildin	pADH2 with AcetylC	pADH2 with	c3_2	05/28/2016 11:20	03/25/2016	14	RI-1000-20	32264_C3 set	"Top subwell 150p+270r. bottom 150p+120r		
MC102221	20	matt.wildin	pADH2 with AcetylC	pADH2 NAD	Rand_pADH2_AcetyIC	01/02/2016 00:50	10/24/2015	15	StorageA1	32733_Rand_p	"both subwells 150p+120r+30s. top AS seed:		
MC102223	20	matt.wildin	pADH2 with AcetylC	pADH2 NAD	Rand_pADH2_PEGs_Ci	01/01/2016 23:22	10/24/2015	15	StorageA1	32733_Rand_p	'both subwells 150p+120r+30s, top AS seed:		
MC102220	8	matt.wildin	pADH2 with AcetylC	pADH2 NAD	Rand_pADH2_AcetyIC	01/01/2016 07:51	10/24/2015	15	StorageB2	32733_Rand_p	'both subwells 150p+120r+30s, top AS seed:		
MC102222	8	matt.wildin	pADH2 with AcetylC	pADH2 NAD	Rand_pADH2_PEGs_Ci	01/01/2016 06:52	10/24/2015	15	StorageB2	32733_Rand_p.,	"both subwells 150p+120r+30s. top AS seed:		

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 Opti	mization	lipboard							General Plat	Drop	1				1	Sort By: Position	• Current	Score •	Mono 2mm *	All *
tan a column	header and d	oo it here to o	by that column						A1.1	A2.1	A3.1	A4.1	A5.1	A6.1	A7.1	A8.1	A9.1	A10.1 A1	1.1 A12.	1 .
Barco 🤟	T (° ~	User 🔍	Project ~	Sample ~	Screen ~	Last Inspected *	Date						(2)	(dk)			(0)	63	0	5
MC103214	20	sumeet.bal	Cytochrome C	Ferritin	shotgun	03/11/2017 04:22	01/13							allow a						
MC103213	20	sumeet.bal	Cytochrome C	Cytochrome	shotgun	03/11/2017 04:12	01/13		AL2	A2.2	A3.2	A4.2	A5.2	A6.2	A7.2	A8.2	A9.2	A10.2 A1	1.2 A12	2
MC103212	20	sumeet.bal	Catalase	Intimin	shotgun	03/11/2017 03:57	01/13		60		(54)		an	61.2	1000	1		6	63	6
MC103211							01/13				Caro			1000			Tom the lam	ofactoring, Park		2.
MC103210	20	sumeet.bal	Ribonuclease A	Thermolysin	shotgun	03/11/2017 03:37	01/13		81.1	82.1	B3.1	84.1	B5.1	B6.1	B7.1	88.1 PE	BAT CONO	810.1° 2017 181	11 812	1
MC103209	20	sumeet.bal	Ribonuclease A	Ribonucleas	shotgun	03/11/2017 03:26	01/13		1	150	6	Ra	(B)	23	6	B	000	(200 - Ca	8	200
MC103204	20	sumeet.bal	Thaumantin	Thaumantin	shotgun	03/11/2017 03:15	01/06			C.		9	C	0		C.			2	
MC103203	20	sumeet.bal	concanavalin A	alpha-Lactal	shotgun	03/11/2017 01:43	01/06		81.2	82.2	83.2	84.2	85.2	86.2	87.2	88.2	89.2	B10.2 B1	1.2 812.	2
MC103202	20	sumeet.bal	concanavalin A	Concanavali	shotgun	03/11/2017 01:33	01/06			R		(D)	(A)	1	(In)	60	6000			
G0000181	20	sumeet.bal	Projects	AtzB	OPPF_shotgun_sumee	03/01/2017 01:07	12/20	1	and			J.						Carl Carl	19	9
MC103166	20	sumeet.bal	AtzB	AtzB	OPPF_shotgun_sumee	03/01/2017 00:14	12/20		C1.1	C2.1	C3.1	C4.1	C5.1	C6.1	C7.1	C8.1	C9.1	C10.1 C1	1.1 C12	1
G0000180	20	sumeet.bal	Projects	Cysteine Hy	OPPF_shotgun_sumee	02/15/2017 08:27	12/07								TR	000	00	1		<b>.</b> .
MC103101	20	sumeet.bal	cysteine hydrolase	Cysteine Hy	OPPF_shotgun_sumee	02/15/2017 07:14	12/		T MILLOW II 1			10.659		-	102.39	T UNREALA TT	INSEL IN	NM3111100	2407110843	
MC103067	20	sumeet.bal	h-Carbonic Anhydra	h-Carbonic	OPPF_shotgun_sumee	02/03/2017 19:30	11/		Inspections	Conditions	Scoring	History	Tools R	eports						
G0000179	20	sumeet.bal	Projects	h-Carbonic	OPPF_shotgun_sumee	02/03/2017 19:28	11/		Inspection *	Tune	Date	Elapsed		0	up spects	Views		Scores		
G0000177	20	sumeet.bal	Projects	Myoglobin	OPPF_shotgun_sumee	01/26/2017 17:39	11/1.			Auto	03/11/2017			10	diam'r	1		0	-	
MC102990	20	sumeet.bal	myoglobin	Myoglobin	OPPF_shotgun_sumee	01/26/2017 15:29	11/17		12	Auto	03/03/2017	Provide a second		1		0		0	-	
MC102949	20	sumeet.bal	h-Carbonic Anhydra	h-Carbonic	shotgun	01/19/2017 11:20	11/10		11	Auto	02/24/2017			1		0		0		
MC102947	20	sumeet.bal	AtzB	Cysteine Hy	shotgun	01/18/2017 19:02	11/09		10	Auto	02/17/2017			2		0		0		
MC102946	20	sumeet.bal	AtzB	AtzB	shotgun	01/18/2017 15:32	11/09		9	Auto		27d		1		2		0		
MC102941	20	sumeet.bal	myoglobin	Myoglobin	shotgun	01/16/2017 15:11	11/07		8	Auto	02/03/2017			1		0		0		
G0000174	20	sumeet.bal	Projects	Ins	OPPF_shotgun_sumee	01/13/2017 20:03	11/04		-	Auto	01/27/2017					570		0		

# 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:

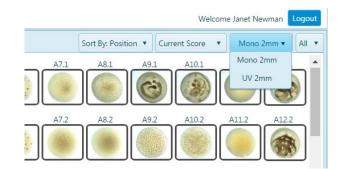
		General	Plate Drop	0					Sort By: Score	* Cu	rrent Score	<ul> <li>Mono</li> </ul>	2mm •	All
		A12.1	821	85.1	861	88.1	8111	ALI	A21	A3.1	A41	A5.1	A6.1	
st Inspected *	~ Date (	6	(Ga	(3)	2	D	6	O		6		(47)	100	
11/2017 04:22	01/13					C.				ale.			1000	
/11/2017 04:12	01/13	A7.1	A8.1	A9.1	A10.1	A111	A1.2	A2.2	A3.2	A4.2	A5.2	A6.2	A7.2	_
11/2017 03:57	01/13			0	3		Ro		(62)	6		60	600	L.
11/2017 03:47	01/13				10									J
11/2017 03:37	01/13	A8.2	A9.2	A10.2	A11.2	A12.2	B1.1	83.1	84.1	87.1	89.1	810.1	812.1	
11/2017 03:26	01/13			(00)		(FER	(She	6	Pa	6		6	6	
11/2017 03:15	01/06			and the second s		- CE			9	S			A	7
11/2017 01:43	01/06	81.2	82.2	83.2	84.2	85.2	B6.2	87.2	88.2	89.2	810.2	811.2	812.2	
11/2017 01:33	01/06				100				G	1		60		7

# 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 Score' option.

# Туре

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*.

Inspections	Conditions	Scoring	History	Tools	Reports				
Inspection <b>*</b>	Туре	Date	Elapsed			Subinspects	Views	Scores	
14	Auto	03/11/2017	63d			1	0	0	
13	Auto	03/03/2017	56d			1	0	0	
12	Auto	02/24/2017	48d			1	0	0	
11	Auto	02/17/2017	41d			1	1	0	
	Auto								
9	Auto	02/03/2017	28d			1	0	0	
8	Auto	01/27/2017	21d			1	8	6	

#### **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)

			-									-			
Inspection	s Conditions S	coring History Too	is Reports					Inse	pections	Conditions Sco	oring History 1	ools Reports			
Recipe	Туре		Source			Volume (µL)		Re	ecipe	ype		Source			Volume (µL)
•	Sample		Concanavalin A			0.20	-	1.1		Sample		Concanavalin A			0.20
<u> </u>	Crystallant		shotgun			0.10				Conc	Units	Name *	ID Grou		
	Conc	Conc Units Name *		pH	Group					44.56	mg/ml	concanavalin A	35450	Protein	
	Conc         Units         Name #           20         w/v         polyethylene glycol 3000           0.1         M         transdum obrate-obric acid		polyethylene glycol 3000		Polymer				Crystallant		shotgun			0.10	
			trisodium citrate-citric acid	5.5	Buffer					Conc	Units	Name *	pН	Group	
										20	w/v	polyethylene glycol 3000		Polymer	
										0.1	м	trisodium citrate-citric acid	5.5	Buffer	
							*								

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

Recipe	×
> 21.0 ul of citric acid "pH 2.3" (1M) 400.0 ul of poly(ethylene glycol) 3000 (50% v 79.0 ul of (tri)sodium citrate "pH 8.1" (1M) 500.0 ul of H2O	w/v)

#### 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

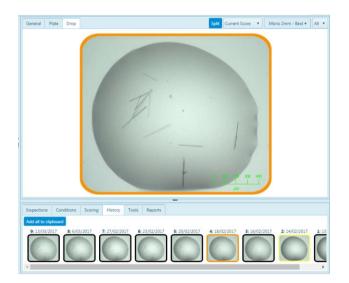
CRYSTALS ***	Worth harvesting	6
CRYSTALS **	Needs some work, but very promising	5
CRYSTALS *	too many, too small, needs a lot of optimising	4
CRYSTALLINE	Microcrystalline, optimise only if desperate	С
SPHERULITES	Things that look like meteors	8
OTHER	Unknown	9
SALT XTAL?	Putative salt crystals	S
GOOD 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
<b>CLEAR WITH STUFF</b>	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*.

Inspections Conditions Scoring Hist	ory Tools Reports	- AT	
	Ins By	Date *	
Score		18/02/2017 16:39	
Score Crystals **	4 janetn		
	4 janetn 2 janetn	14/02/2017 10:20	

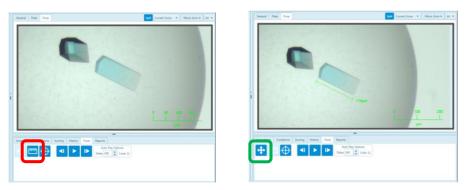
# History OPTION

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

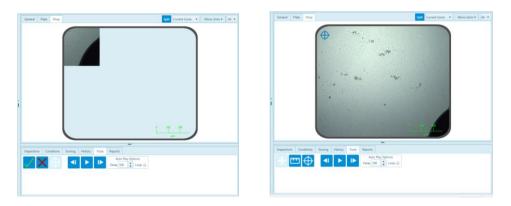
The measure tool is selected by clicking on the ruler icon (circled red, below left), and is de-selected by clicking on the four-arrow icon (circled green, below right). This option allows one to measure an object in a drop. The green ruler lines on the image persist until another plate is loaded from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

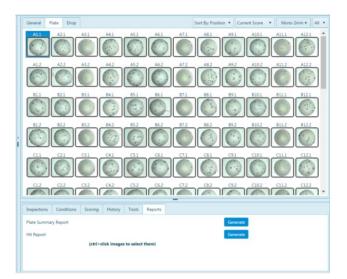
The forward, back and play buttons (circled below) move you through the images – the delay between the images can be set here as well.



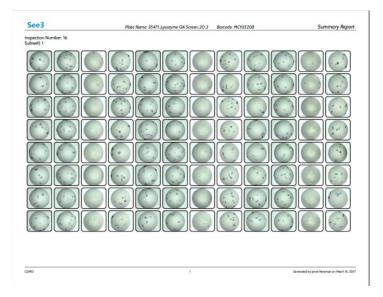
The autoplay function can also be toggled on and off with the space bar, and the keyboard arrow keys will also move you between images.

# Reports OPTION

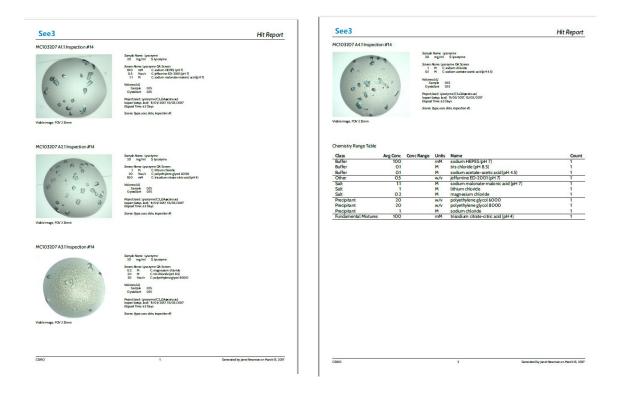
There are two reports, one which gives an overview of the selected plates, and one which gives a more detailed report of images that have been placed on the clipboard. Click on the "Generate" button opposite the report that you desire.



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

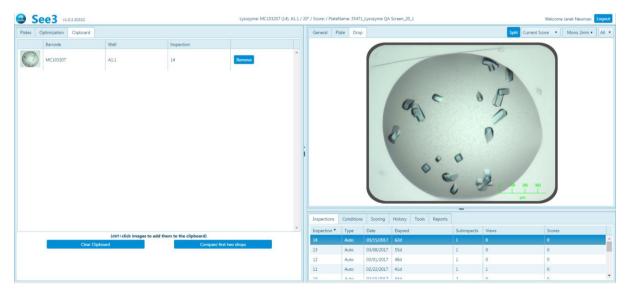


The Hit Report gives details about individual droplets that have been selected. At the end of the Hit Report, a summary of the chemistry found in the drops selected for the report is given, an example is shown below.



# 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

Se	v1.0.3.30332			Lysozyme: MC103207 (14). A1.1 / 2	/ 20° / Score: / PlateName: 35471_Lysiozyme QA Screen_20_1 Welcome Janet Newman Logov
Plates C	Optimization Clipboard				General Plate Drop Split Current Score • Mono 2mm • All
	Barcode	Well	Inspection		
0	MC103207	ALL	14	Remove	
0	MC103207	A1.1	13	Remove	
0	MC103207	A1.1	12	Remove	a con
0	MC103207	A1.1	11	Remove	
0	MC103207	A1.1	10	Remove	
0	MC103207	A1.1	9	Remove	0° 0 0
0	MC103207	A1.1	8	Remove	0 2
0	MC103207	A1.1	7	Remove	Scoring History Tools Reports
6.0				-	Add all to clipboard
	Clear Clipbe	(ctrl+click images to add th bard	em to the clipboard) Compare first ty	vo drops	

# 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)

			2 C							
			Name		Avg (	Conc	Start Conc	End Conc	Units	Avg pH
Randomize Add Fac	tor Group Add Chemical		polyethylene glycol	8000	20		16	22	w/v	
Plate Size:	96	4	Salt							
Additive:	Select		Name		Avg (	Conc	Start Conc	End Conc	Units	Avg pH
Dilution (%):			magnesium chloride		0.2		0.02	0.3	м	
Include Initial Condition	s: 🗹	4	Buffer							
			Name		Avg (	Conc	Start Conc	End Conc	Units	Avg pH
User Name: janetn	T		tris chloride		0.1		0.1	0.1	М	8.5
Design Name: Lysozyn	ne_2017-03-21_12:28									
Save Design Save F	actors Load Factors Generate from Clipboard									
Save Design Save P	actors Load Pactors Generate from Clipboard				1	2	3	C3 (MC10323	9 A3) 5	6
								Primary		
				A				polyethylene g 20 w/v	glycol 8000	
								Salt		
								magnesium ch	nloride	
				в				0.2 M		
						_		Buffer		
								tris chloride		
								0.1 M pH: 8.5		
				C						

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**.

	Factor Group							Color	Va	ry By			Location	Steps		
	Primary								Ga	ussian Rande	om		Random	96 / 96	Delete	
	Name			Avg Conc		End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	polyethylene glyc	0008 lo	2	20	16	22	w/v				1	Conc	96	96	Delete	
	Salt								Ga	ussian Rande	om		Random	96 / 96	Delete	
	Name		ł	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		Ĩ
	magnesium chlori	de	(	0.2	0.02	0.3	M				0.5	Conc	96	48	Delete	
	magnesium aceta	te	0	0.2	0.02	0.3	M				0.5	Conc	96	48	Delete	
1	Buffer								Ga	ussian Rando	om		Random	96 / 96	Delete	
	Name		ł	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	tris chloride		0	0.1	0.1	0.1	M	8.5	7.1	9.1	0.5	pH	96	48	Delete	
		_			-				7							1
			1	2	3	4	5	6	/	8	9	10	11	12		
		A							8							
														Steps     Delete       andom     96 / 96     Delete       Steps     Distribution     96       96     96     Delete       Steps     Distribution     96       96     48     Delete       96     48     Delete       Steps     Distribution     96       96     48     Delete       11     12		
		в	_						i i i i i i i i i i i i i i i i i i i	-			Location     Steps       Random     96/96       Steps     Distribution       96     96       Random     96/96       Delete       Steps     Distribution       96     48       96     48       96     48       96     48       96     48       96     48       96     11       11     12			
			_								-					
		C				8										

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.

											w	elcome Janet Ne	wman Logo	out
	Factor Group					Color	Va	ry By			Location	Steps		
4	Primary						Ga	ussian Rando	m		Random	96 / 96	Delete	<b>^</b>
	Name	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	polyethylene glycol 8000	20	16	22	w/v				0.5	Conc	96	48	Delete	
	sodium malonate-malonic acid	1.1	0.88	1.21	M	7	7	7	0.5	Conc	96	48	Delete	
4	Salt						Ga	ussian Rando	m		Random	48 / 96	Delete	
	Name	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	magnesium chloride	0.2	0.02	0.3	M				0.5	Conc	96	48	Delete	
4	Buffer						Ga	ussian Rando	m		Random	96 / 96	Delete	
	Name	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	tris chloride	0.1	0.1	0.1	M	8.5	7.1	9.1	0.5	pН	96	48	Delete	
	sodium HEPES	100	100	100	mM	7	6.55	8.55	0.5	рН	96	48	Delete	
4	Other						Ga	ussian Rando	m		Random	48 / 96	Delete	
	Name	Avg Conc	Start Conc	End Conc	Units	Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribution		
	jeffamine ED-2001	0.5	0.05	0.75	w/v	7	7	7	0.5	Conc	96	48	Delete	

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.

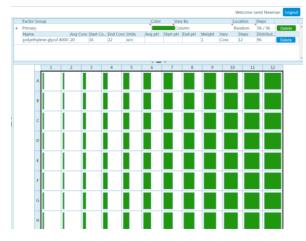
The 'Vary By' pulldown menu shows the different options for changing the concentration of the factors within the group. By default, the concentration is varied randomly between the upper (starting x 110%) and lower (starting x 80%) limits, but with a bias towards the starting value. This is called 'Gaussian 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.

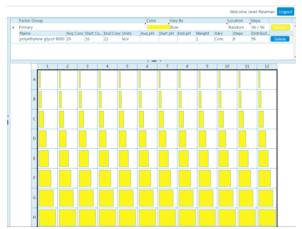


Both the Gaussian and Uniform random selections can be ordered, so that the smallest concentration value is found in the upper right hand corner of the selection, and the largest concentration is found in the lower right. Notice that the values don't change, but the arrangement does.

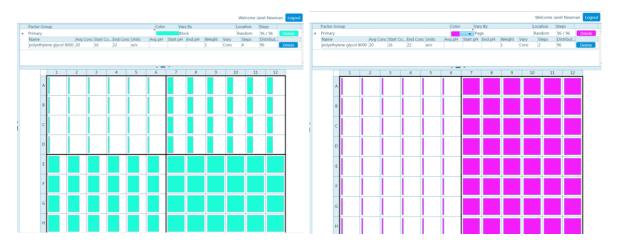


'Vary By' column sets up a grid from left to right across the columns of the plate (green, below) and 'Vary By' row sets up a grid from top to bottom across the rows of the plate (yellow, below)





'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)

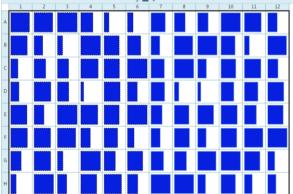


'Vary By' fixed keeps the value of the factor constant (brown, below)

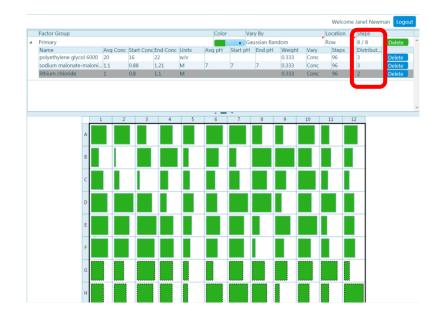


When there is more than one Chemical Factor in the Factor Group then the 'Location' option becomes relevant. This allows one to position the Chemical Factors on the plate, irrespective of how the concentration is being varied. By default, the distribution of the Chemical Factors on the plate is 'Random' (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 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.

Primary     Name     polyethylene glycol 8000     sodium malonate-malonic acid     1.1	Start Conc         Er           14         14           0.88         1.	8	Units w/v M	Avg pH 7	Gau Start pH 7	ussian Randor End pH 7	Weight 0.5 0.5	Vary Conc	Random Steps 96	96 / 96 Distribution 48	Delete
polyethylene glycol 8000 20	14 18	8	w/v		Start pH	End pH	0.5	· ·			
				7	7	7		Conc	96	48	
sodium malonate-malonic acid 1.1	0.88 1.	.21	М	7	7	7					Delete
							0.5	Conc	96	48	Delete
Factor Group				Color	Va	ry By			Welcom Location	e Janet Newn Steps	han Logo
	Gaussian Random										
Primary	<u>.</u>	5 1 6							Random	87 / 96	Delete
	Start Conc E			Avg pH	Start pH	End pH	Weight	Vary	Steps	Distribut	_
polyethylene glycol 6000 20		22	w/v				0.5	Conc	96 96	49 38	Delete Delete
sodium malonate-maloni 1.1	0.88 1	1.21	M	7	7	7	0.4	Conc			

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** 

7 7 7

М

1.21

0.88

sodium malonate-maloni... 1.1

0.4

96

Conc

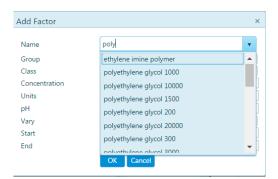
38

See3 v1013			See3 VL03.30932		
Optimization Cl			Planes Optimization Clipboard	Factor Group Color Primary Name Ang Conc (Start Co End Conc Units Ang pH	or Very By Gaussian Randi Start pH End pH
Barcode	Well Inspection		Randomae Add Easter Group Add Chemical Pinte Stree 196	sodium maloute-rusion, 11 0.88 122 M 7 polyethylene glycol 6000 20 16 22 w/v	7 7 0.1 0.5
MC103239	A2.1 12 Re		Additive Select. • Diluteo (Nil) B Include India Conditions IR	Aufler     Name     Ava Conc Start Co End Conc Units     Ava pH     codum HEPES     100     100     100     mM     7     Hysindam cityster-citric a 100     100     100     100     4	Gaussian Random Start pH End pH Wek 6.55 8.55 0.5 1.76 5.76 0.5
MC103239	AL1 12 Re		User Name: jørets   Dosign Name: [kjist@ymm_2017@sit]_16.15	Other     Name Aug Conc (Start Co. End Conc Units Aug PH     Jeffership ED-2001 0.5 0.05 0.75 uv/v 7	Gaussian Random
			Taes Dange See Factors Load Factors Generate from Diploard	Salt     Name     Avg Conc Start Co End Conc Units     Avg pH     Rthum chloride     1     0.8     1.1     M	Gautsian Random I Start pH End pH Wei 0.5
(ctrl+click ima Clear Clipboard	ges to add them to the clipboard) Compare first two dr	×			FE

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

Add Factor		×	Add Factor		
Name Group Class Concentration Units pH Vary Start End	New Group	▼ ▼ ▼ ■ ■ ■ ■	Name Group Class Concentration Units pH Vary Start End	tripotassium citrate potassium dihydrogen phosphate potassium fluoride potassium formate potassium iodide potassium nitrate potassium sodium tartrate OK Cancel	•

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.

Warning	×
Well A7:	
Solution containing the combination of ("magnesium", "phosphate") chemicals may be incompatible.	
Solution containing the combination of ("magnesium", "phosphate") chemicals may be incompatible.	
Well A10: Solution containing the combination of ("magnesium", "phosphate") chemicals may be incompatible.	
Well A11: Solution containing the combination of ("magnesium", "phosphate") chemicals may be incompatible.	
Well 88: Solution containing the combination of ("magnesium", "phosphate") chemicals may be incompatible.	
Press OK to go back and change the design or Ignore to save the design anyway.	
OK Ignore	

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.