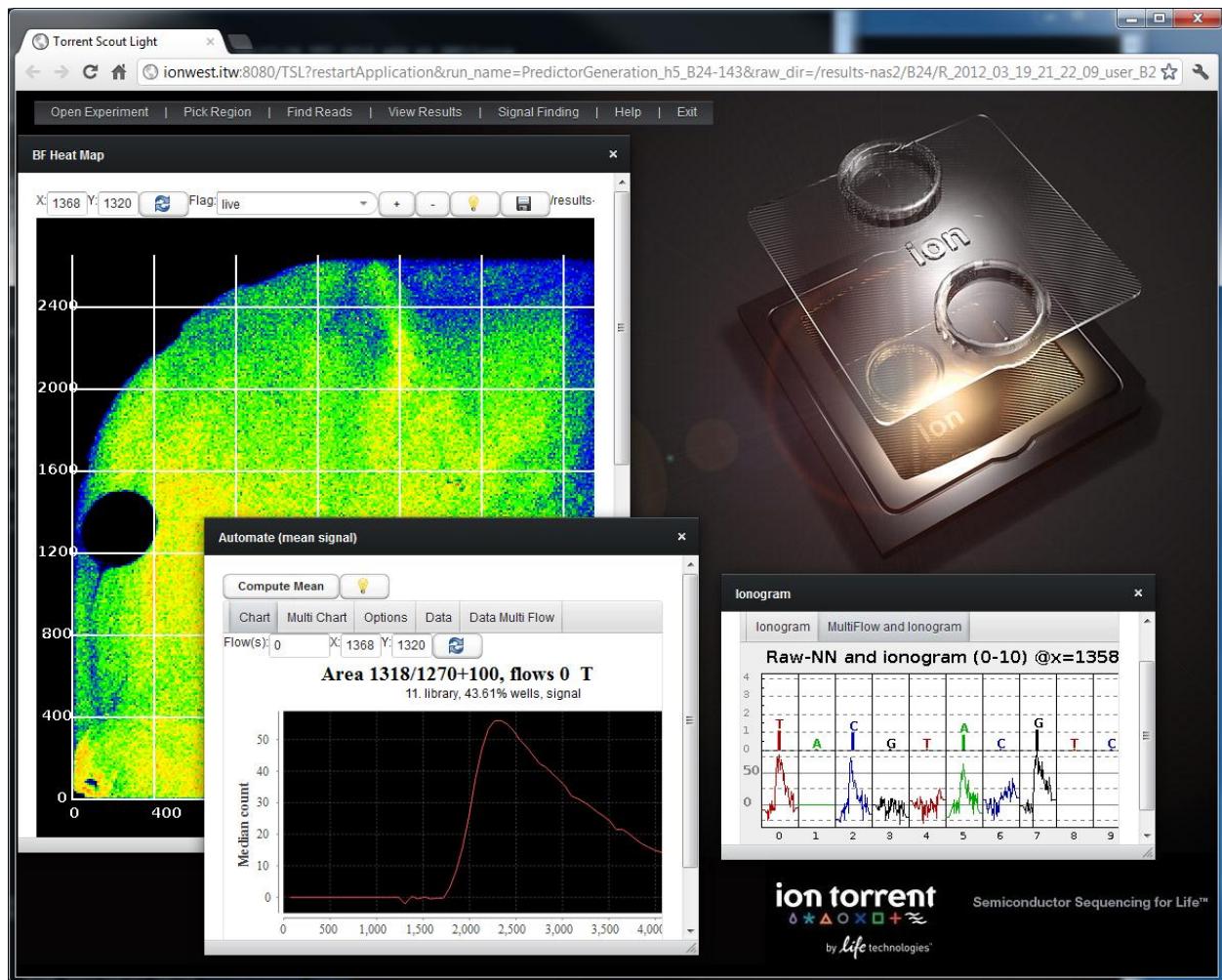


Torrent Scout Light

V3.0.6, Chantal Roth
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Please go to the [LifeTech Community](#) or [GitHub](#) page for more documentation and source code, or send an email to [Chantal Roth](mailto:Chantal.Roth@iontorrent.com) for help or feedback.

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Description

Torrent Scout Light is a pure web application written using Vaadin. The server side is written in Java and is the same code as used in the full Torrent Scout. Vaadin is a software framework that allows the developer to write 100% Java code, and then translates the gui for the web pages into Java Script, and uses the Google toolkit.

Functionality includes:

- Viewing ionograms and alignments
- Viewing raw traces of multiple flows of one well, or of multiple wells and one flow

- Export both raw and NN subtracted data of one of multiple flows, and of an entire area on the chip (optionally filtered by a mask)
- Find reads based on alignment (indels for instance), both via the interactive plugin (suggested if you are running multiple searches), and also via the TSL application
- View regional empty traces (if data is available)
- View XYZ charts of any read property (such as bead find metric versus Q20 values)
- Load and save your searches (stored as heat maps)
- Find/filter reads based on scores (such as % identity, Q lengths etc)
- Compute the mean signal of an area of multiple flows
- Find reads based on genome position
- Calculate your own masks using the dropdown and command line mask calculator (allows saving/loading of masks)
- Compute your own masks based on the raw data and using various histogram functions

Installation Instructions:

Install via Debian package

`sudo apt-get install ion-torrentscout-light`

Manual Installation

- `sudo apt-get install tomcat6 tomcat6-admin tomcat6-common`
- The TSL.war file is in the etc folder in this zip package
- `sudo nano /etc/default/tomcat6`
change line with JAVA_OPTS to:
`JAVA_OPTS="-Djava.awt.headless=true -server -Xms2048m -Xmx8000m -XX:MaxPermSize=512M"`
(to allow for more concurrent users, increase -Xmx setting. One user requires about 3-4 GB at peak time, such as when loading raw data)
- `sudo nano /etc/tomcat6/tomcat-users.xml`
Uncomment user section so that it contains:
`<user username="tomcat" password="tomcat" roles="admin, manager, manager-gui"/>`
- `sudo nano /var/lib/tomcat6/conf/logging.properties`
In there, change all the log levels to SEVERE to reduce log output – or else the log file can get quite large
- `cp TSL.war /var/lib/tomcat6/webapps`
- To use the desktop Torrent Scout version: `cp torrentscout.war /var/lib/tomcat6/webapps`
- `sudo /etc/init.d/tomcat6 restart`
- To start TSL : <http://yourserver.com:8080/TSL>
- To restart TSL : `http://yourserver.com:8080/TSL?restartApplication`
- To download the desktop version of TS : <http://yourserver.com:8080/torrentscout>

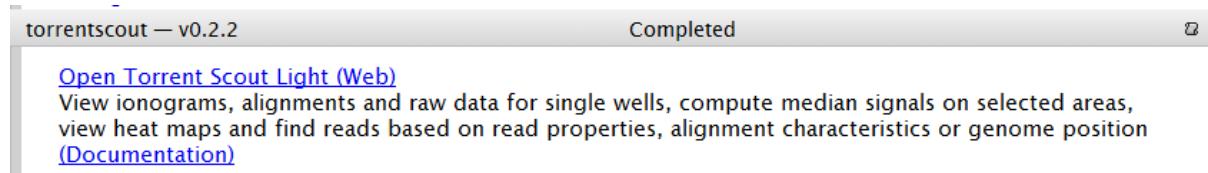
Source Code

- All of the code is open source (GPL 3). You can download the latest version of the source code and the compiled .jar files for deployment at any time at:
<https://github.com/chenopodium/TorrentScoutLight>
- The Plugin code is also in that repository, in the subfolder ion-torrentscout-light/plugins

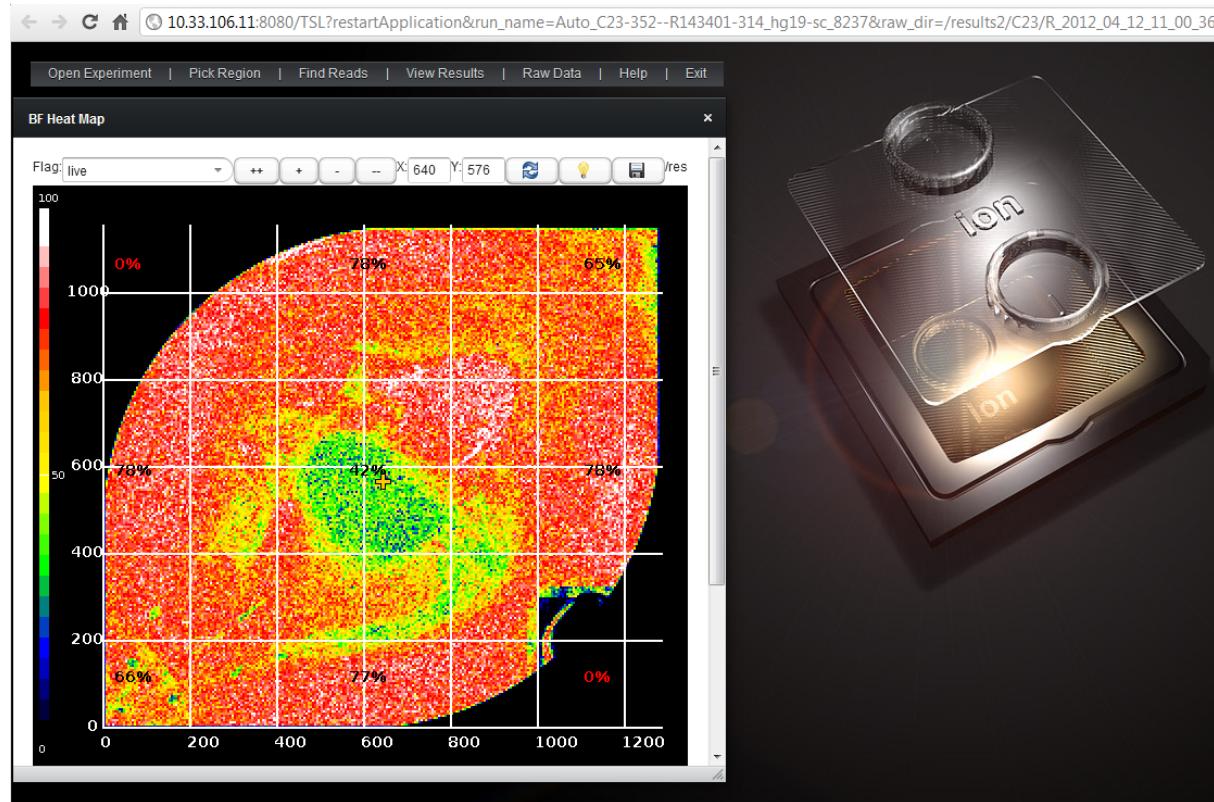
Torrent Scout Plugin

The **Torrent Scout Plugin** prepares the results data for viewing by creating index files on the .sff and .BAM file, by computing various heat maps and by pre-computing alignments searches when the plugin is used interactively. The plugin should always be run (ideally automatically) before launching the viewer so that the viewer does not have to do all the calculations on the head node.

By default the plugin computes those index files and presents the user with a link to the application:



The link both launches the application and loads the data set for the particular run.



In some cases, if a lot of alignment searches are to be done, the plugin also has an interactive part that allows a user to precompute the alignment searches. Simply select the plugin to run (again) and it will prompt you with a form where you can have up to 20 alignment searches done all at once:

TorrentScout Alignment Search Parameters

Search for alignment patterns for the following flows (end=0 means all):
 Start flow: End flow:

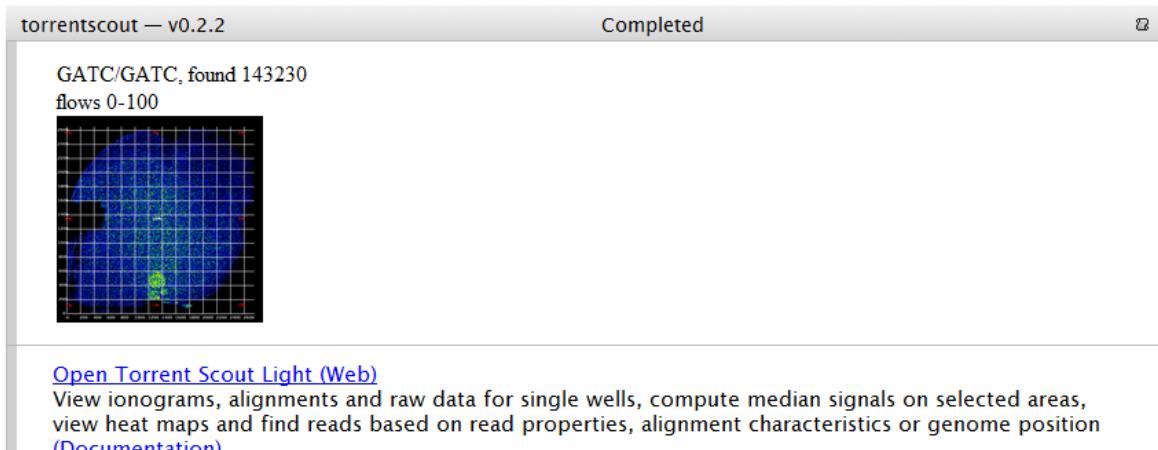
Read sequence (eg TT_) (X = 4 searches with G/A/T/C)	Alignment sequence (eg TTT) (? = any base or gap, _ =gap)	Name of heat map (X will be replaced with G/A/T/C)
<input type="text" value="TT_"/>	<input type="text" value="TTT"/>	<input type="text" value="missed_T"/>
<input type="text" value="XX_"/>	<input type="text" value="XXX"/>	<input type="text" value="missed_X"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>

The following special characters are used:

X denotes a G, A, T or C. Whenever an X appears in a search, this will correspond to 4 searches, for X=G, X=A, X=T and X=C (including in the name of the heat map)

? denotes any base or a gap. Each ? will match a G, A, T, C or a _ character

'-' is a gap (as in the .BAM alignment)

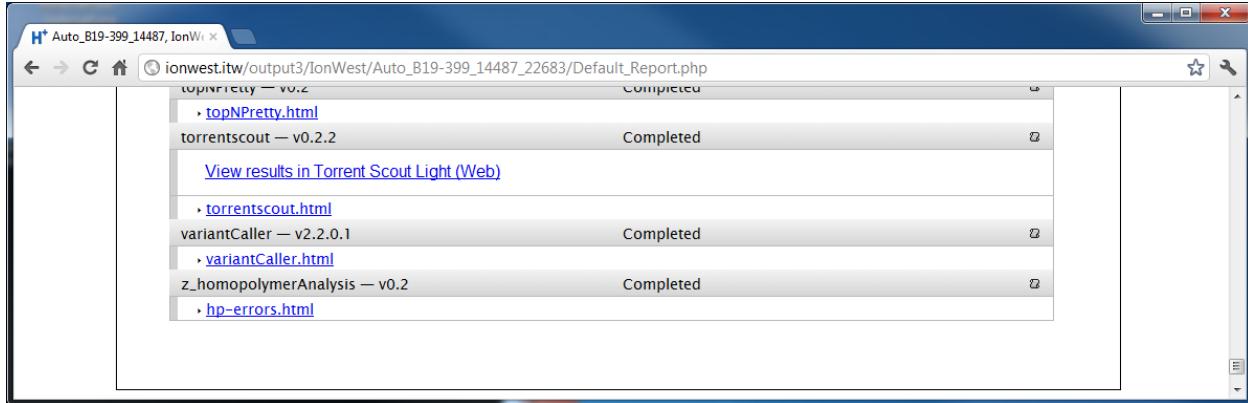


The alignment searches are stored as a heat map similar to the image above and can be loaded in the application under “Find Reads by Scores and Alignments”. Click on the “Load previous search results”

and pick a .bmp file that is stored in the plugin_out/torrentscout_out folder of your run (see in “Find reads” section for more details)

Starting

The most convenient way is to enable the TorrentScout plugin, and to view the results directly from the Default Report page. The plugin computes all the heat maps index files, so that you don’t have to wait when you want to look at the data:



Just click on the View results in Torrent Scout Light link. This will automatically load this run and show you a heat map.

Alternatively, you can open a browser and type in the URL of your Torrent Server, followed by :8080/TSL. For instance:

<http://myserver.com:8080/TSL>

In most cases you can now also use the URL without the port (if you also have ion-jk installed)

<http://myserver.com/TSL>

Opening an Experiment

You can select an experiment by browsing in the database. Open a PGM node, then an experiment node, and select an analysis result as shown in the image.

Browse DB

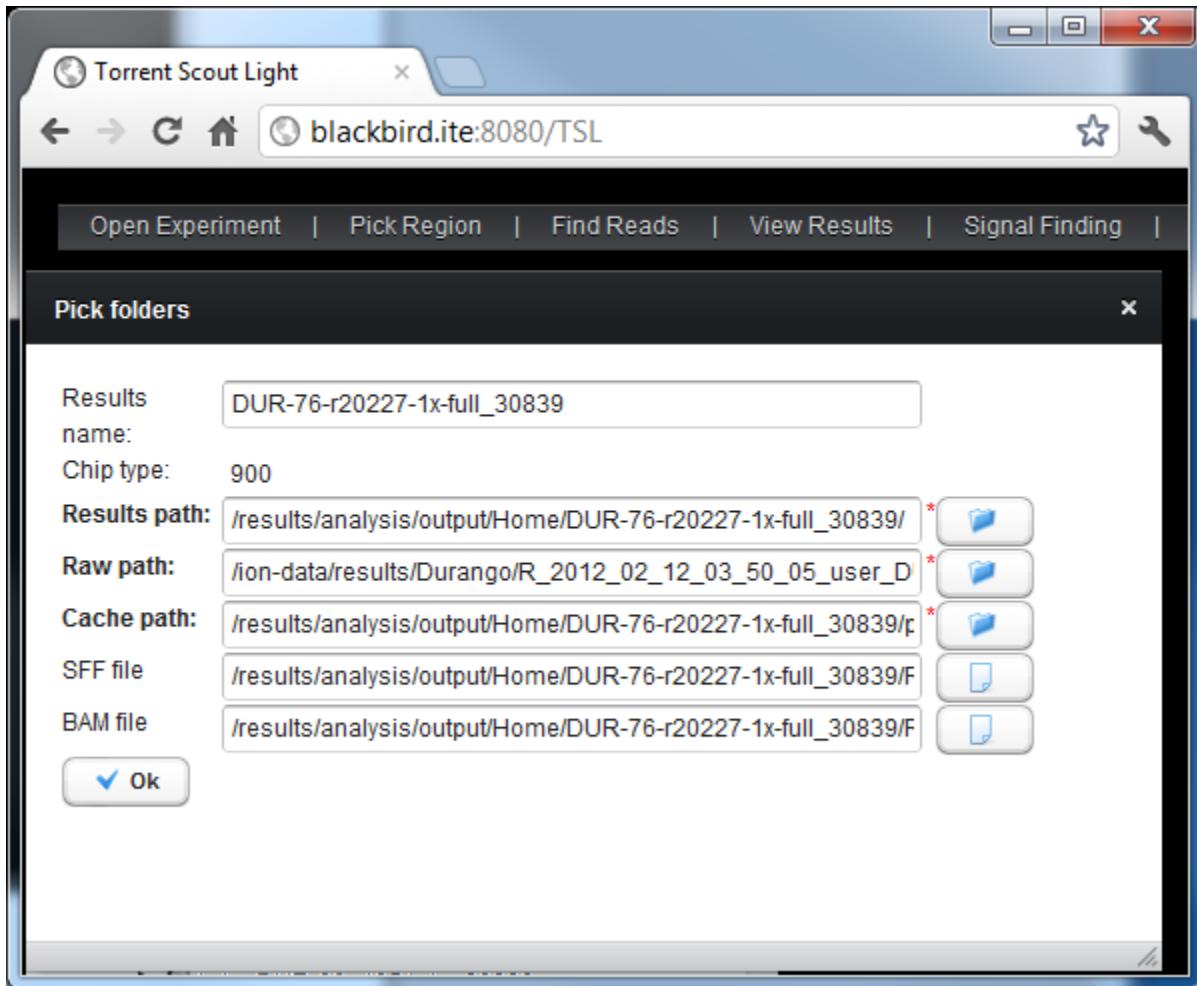
DB url: ionwest.itw:5432/iondb Connect Selected: Load  

Past 14 days	Last top Q17 bases	Last max Q17 len
--------------	--------------------	------------------

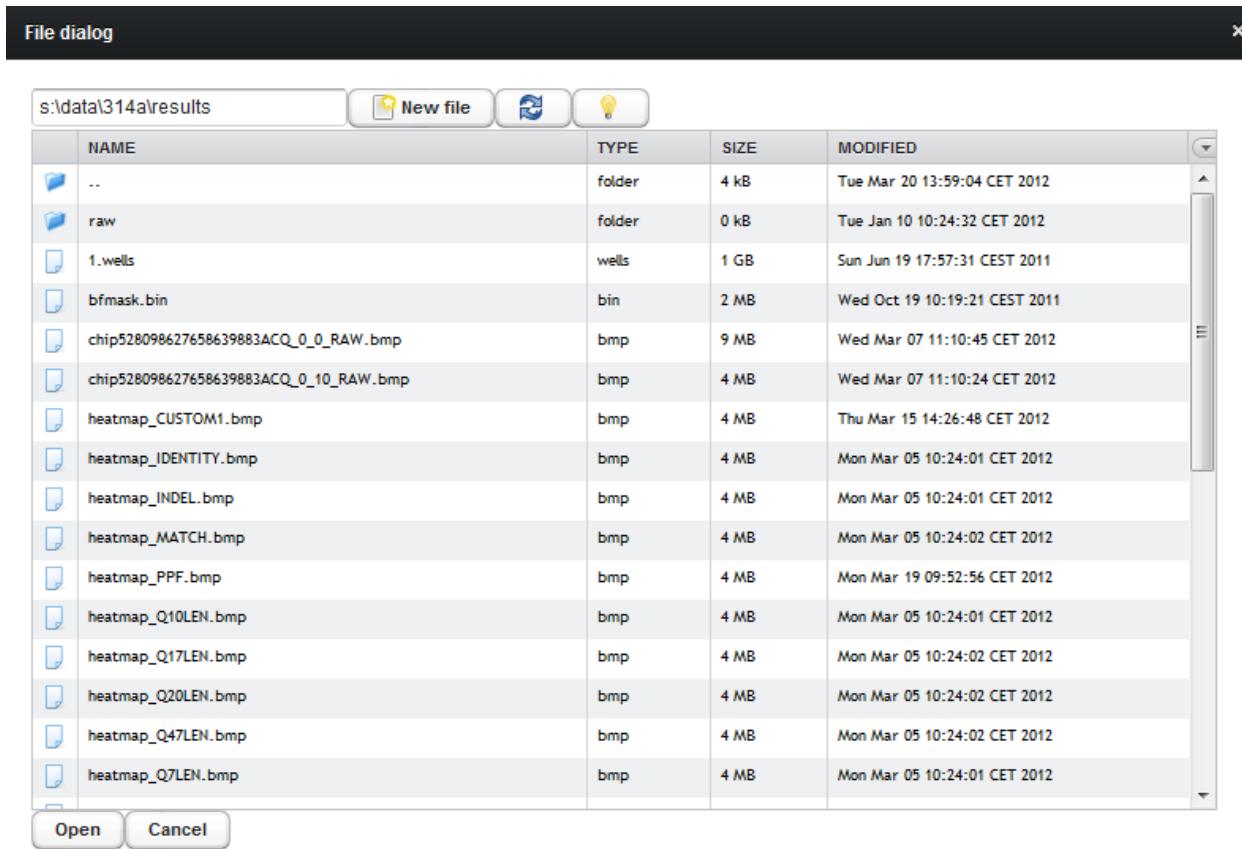
- ▶  B15
- ▶  B17
- ▼  B18
 - ▶  316D: R_2012_06_05_19_37_22_user_B18-562-R147772-control_for_cleaning-BG
 - ▶  Auto_B18-562-R147772-control_for_cleaning-BG_15969 (713.25 MB)
 - ▶  B18-562-R147772-control_for_cleaning-BG_TGC (8922 Q17 bases)
 - ▶  314R: R_2012_06_04_21_49_49_user_B18-561
 - ▶  314R: R_2012_06_03_20_06_26_user_B18-560
 - ▶  314R: R_2012_06_03_17_44_50_user_B18-559
 - ▶  316D: R_2012_06_02_22_54_44_user_B18-558-R147443-Lot_26B03_12_GL
 - ▶  316D: R_2012_06_02_19_26_59_user_B18-557-R147442-Lot_26B03_12_GL
 - ▶  316D: R_2012_06_01_15_49_45_user_B18-556-R147368-Exp464_QC_nIDP_r2-BK
 - ▶  318B: R_2012_05_30_14_44_40_user_B18-555-R147102-EXP457_ASHG_CCP409_N-DJ
 - ▶  316D: R_2012_05_25_16_17_34_user_B18-554-R146958-checking_cleaning_wi-NJF

- Click on an experiment or run node to see a description popup.
- To load a run, click on a run node (such as Auto_xyz) and click on Load.
- You can specify a different db in the DB url – but keep in mind that you can only view the data if the server has access to the files. One scenario that makes sense is if you have an rnd machine where you run TSL, but that machine is on the same network as the head node has access to the exact same files using the same paths as the head node.

After you click OK (the button is on top), it will show you the paths it has found from the database and will show you the details.



If the paths are not correct, you can change it here manually or by clicking the folder/document icon, which opens a file browser:



You can also open other experiments that way that are not in the database, by selecting “Open Experiment / Pick Folders”.

Searching for Runs

The database window has two additional tabs where you can see the latest runs sorted by top Q17 bases and the longest Q17 reads:

Browse DB

DB url: ionwest.itw:5432/iondb Connect
Selected:   

Past 14 days	Last top Q17 bases	Last max Q17 len			
PGM	RUN		DATE	Q17 BASES	Q17 M.
B32	Auto_B32-162--R147530-PES_3xTiN_40M_lot103-C_fwd_15912_24889		Tue Jun 05 11:36:01 PDT 2012	1148057424	347
B31	Auto_B31-174--R146885-d2_clear_26b03_r1__-EKL_15792_24674		Fri May 25 10:36:01 PDT 2012	1249406447	368
B31	Auto_B31-175--R146889-d2_clear_26b03_r2__-JT_15807_24692		Fri May 25 15:58:43 PDT 2012	1239883447	351
B31	Auto_B31-183--R147528-PES_3xTiN_40M_lot103-C_fwd_15909_24887		Tue Jun 05 11:36:50 PDT 2012	1207736031	326
B31	Auto_B31-186--R147610-R2_20_LR2_Std_B31-EKL_15952_24952		Wed Jun 06 16:30:42 PDT 2012	1266705137	361
B23	Auto_B23-417--R146888-d2_opaque_26b02_r1__-JT_15794_24676		Fri May 25 07:56:28 PDT 2012	1227160609	321

In case you are looking for an older run, or a specific run where you know part of the name, you can also use the filter icon:

How would you like to filter the db runs? 

Filter by...

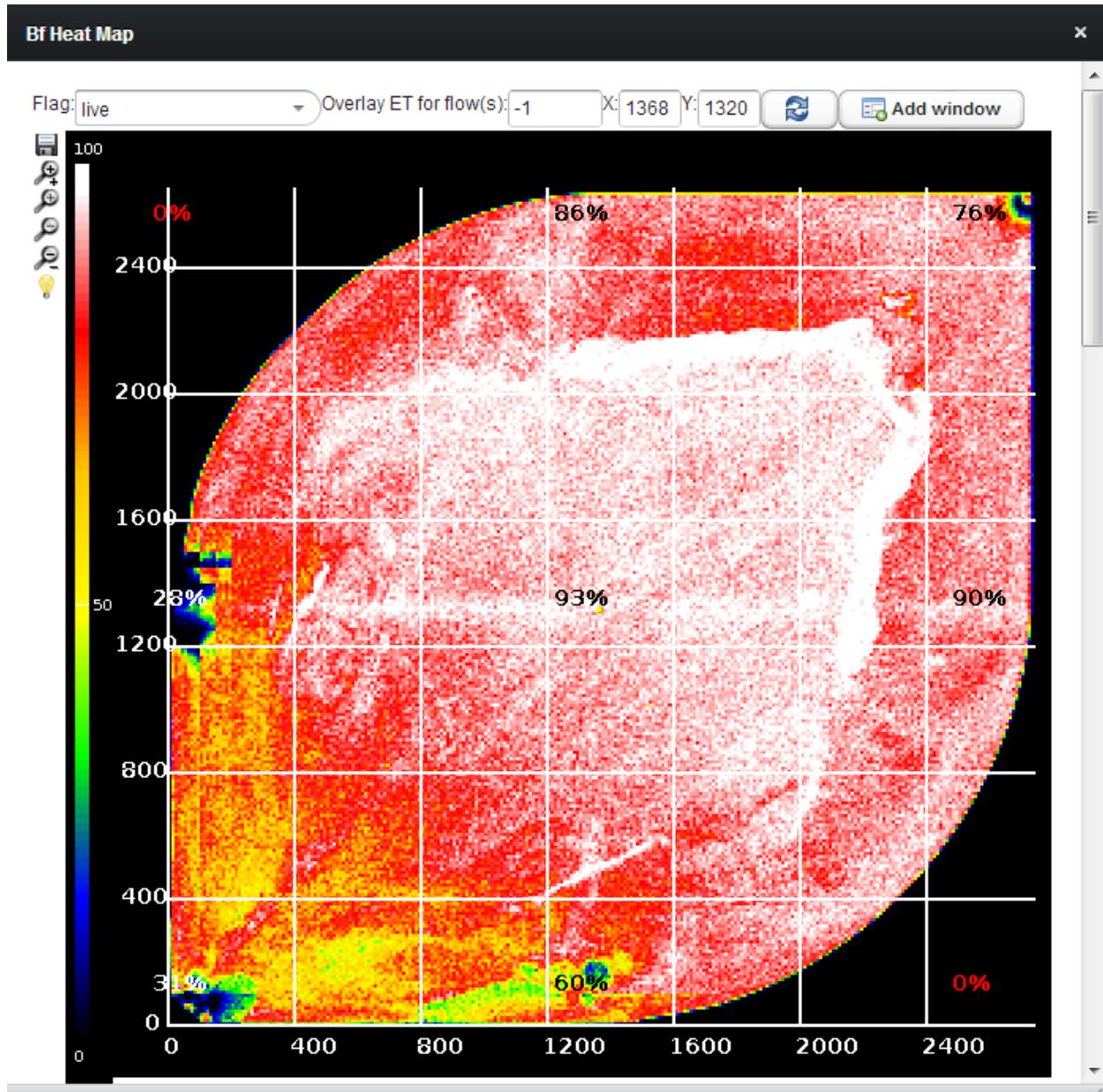
1) ... runs of last X days
 2) ... experiments containing string X

Ok

Picking a region on the chip

BfMask Heat Map

If there is a bfmkask.bin file, it will open the BfMask Heat Map view that shows you the various flags such as bead, live, dud, ambiguous etc in a heat map:



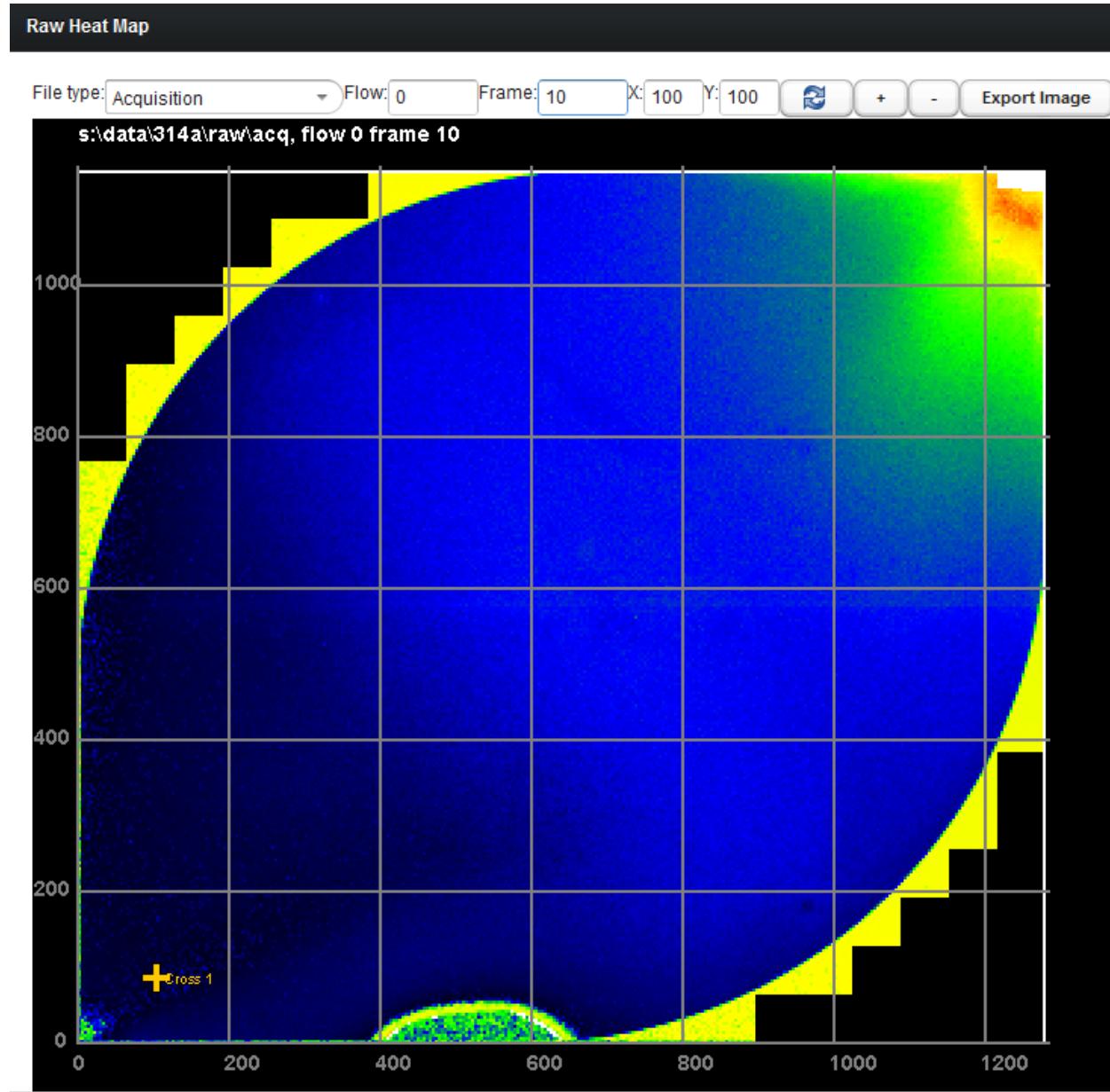
- To pick an area just click into the heat map
- You can also enter the coordinates manually
- The +- buttons (in all components) allow you to zoom in and out
- The save icon lets you save the image
- The light bulb icon shows some info on what you can do (available in most components)
- You can pick any flag from the dropdown box
- You can also create a second or third window to view additional masks side by side (with the "Add window" button)

To pick a coordinate, move the orange cursor, or enter a coordinate in the X/Y text boxes.

In the flag drop down box you can chose different flags (such as bead, keypasse, dud etc) to view.

Raw Chip View

If there is no bfmask.bin file, or if you select “Pick region/ Raw heat map” it will open the WholeChip view that displays one frame of a raw .dat file:

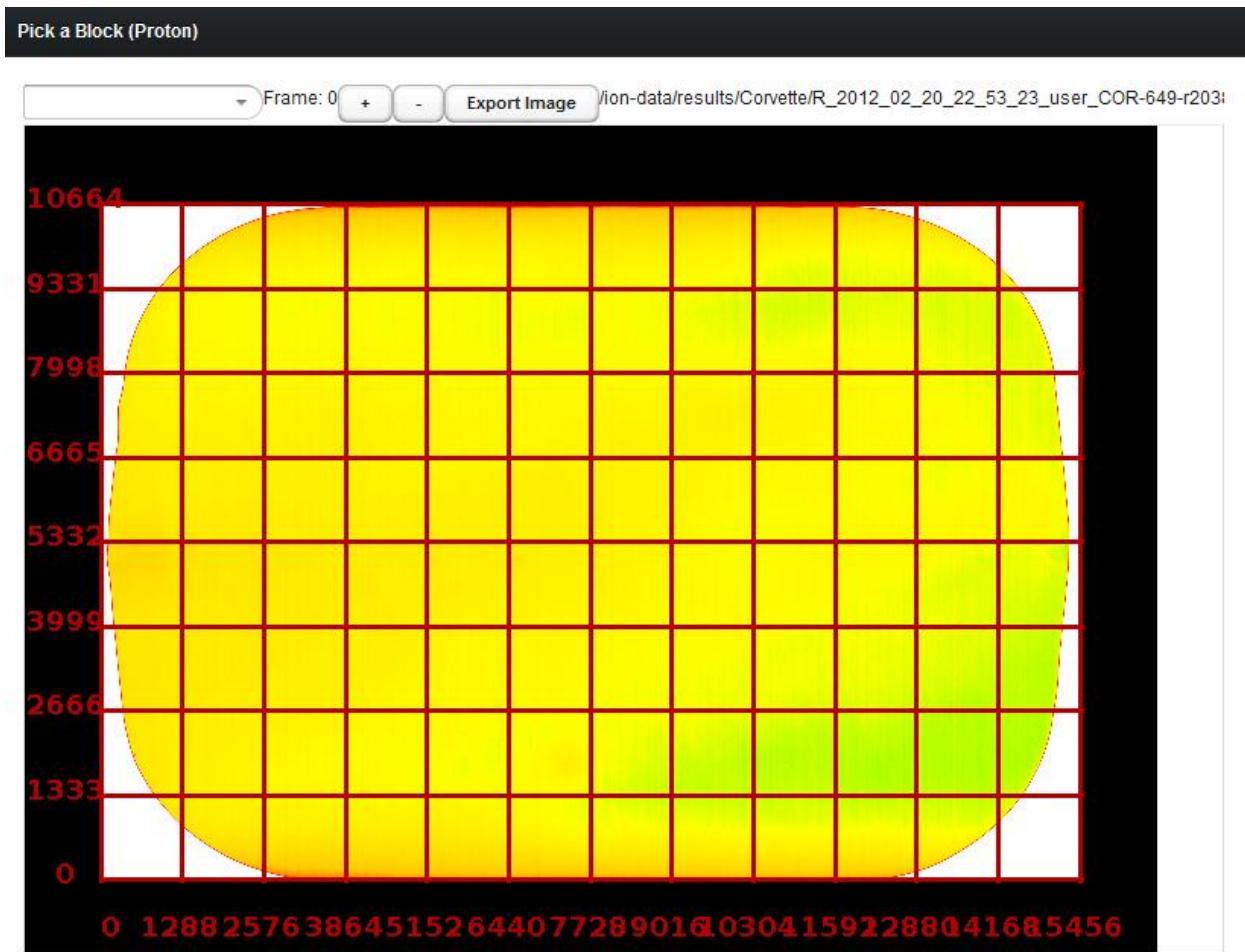


You can change the file type (acquisition, prerun bead find pre/post), the flow (any flow number starting from 0) and any frame (default is frame 10).

To pick a coordinate, move the orange cursor or enter the coordinates as in the bf mask view, and you can also zoom in and out with the +/- buttons.

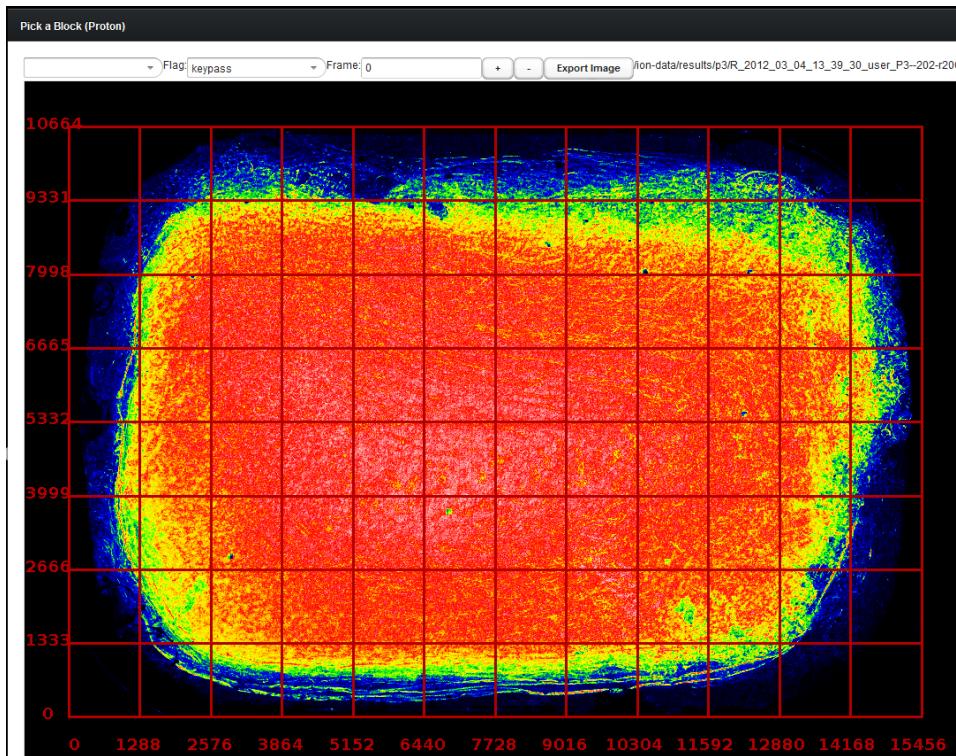
Proton View

For proton chips, where the result is composed of multiple sub-blocks, you can pick a block for viewing with the “Pick Region/ Pick a block” menu:



The Proton view also lets you pick other heatmaps as in the BF heat map component.

Note that if the heatmap has not been computed by the plugin, it will have to do it on the fly, which will take several minutes and use a lot of memory/CPU on the server...



Viewing results

Once you have chosen an experiment and a location (and a block in Proton experiments), you can now view results.

Well Table

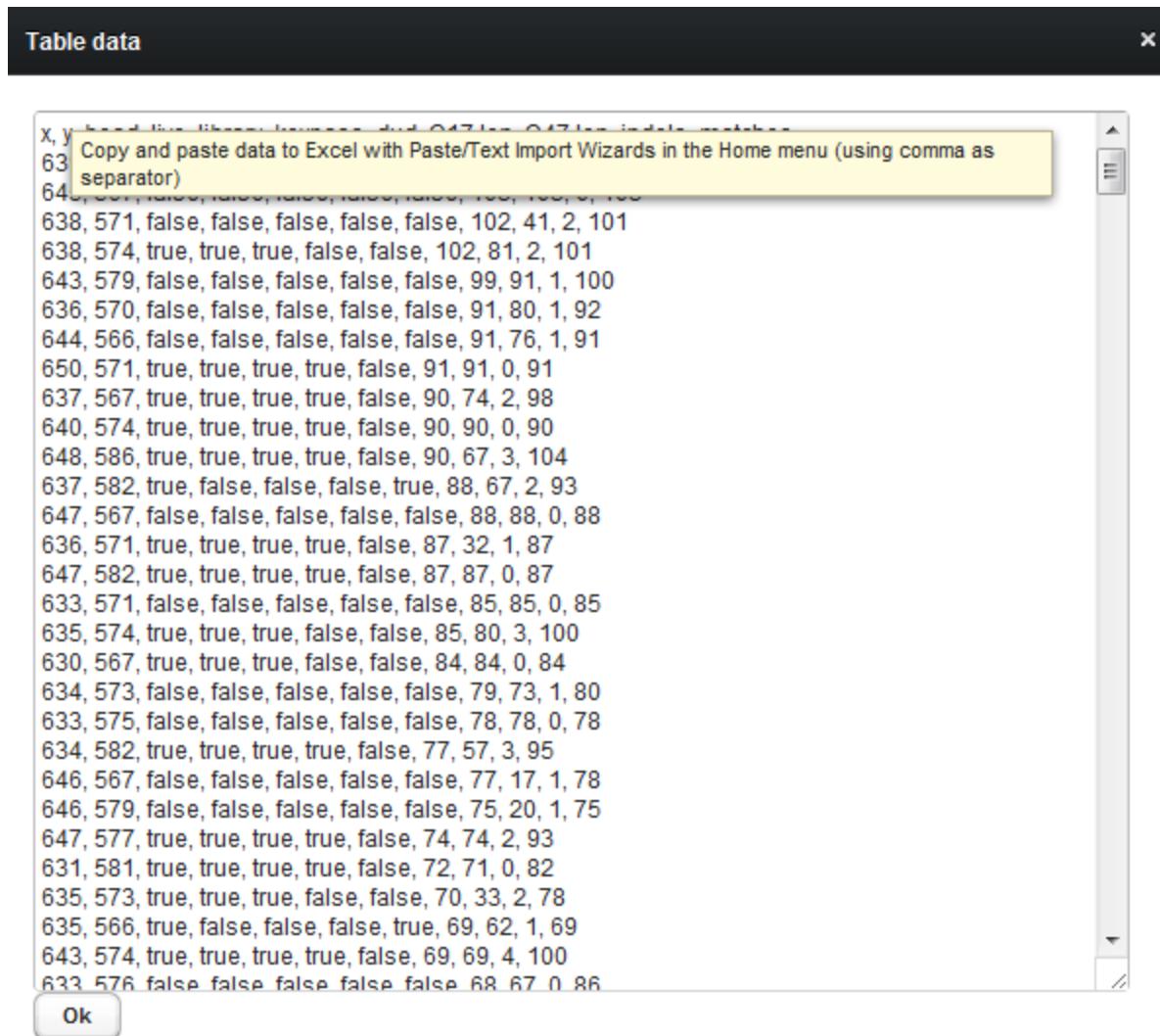
The well table shows a few thousand wells surrounding the currently selected well. It shows a few flags and scores such as Q17 lengths, indels and so on. The scores might not always load if the index files have not been created yet. To force them to load, click on Load Scores (it might take a few minutes if there are no index files yet).

You can sort the table by clicking on the table header.

You can select a well by either clicking on a row, or by entering the coordinates on top. To view the results, select any of the viewers from the menu “View Results” (such as Ionogram, Alignment, Raw View etc)

Well Table											
X:	1700	Y:	1893		Load Scores			/var/www/output13/IonEast/Auto_FLO-507-R140608-30m.318.fs2-A			
X	Y	BEAD	LIVE	LIBRARY	KEYPASS	DUD	Q17 LEN	Q47 LEN	INDELS	MATCHES	
1704	1894	true	true	true	true	false	256	256	0	256	
1694	1887	true	true	true	true	false	253	253	0	253	
1698	1884	true	true	true	true	false	252	252	0	252	
1695	1902	true	true	true	true	false	251	251	0	251	
1706	1884	true	true	true	true	false	250	250	0	250	
1709	1894	true	true	true	true	false	250	250	0	250	
1697	1897	true	true	true	true	false	248	248	0	248	
1707	1889	true	true	true	true	false	248	248	0	248	
1690	1892	true	true	true	true	false	247	247	0	247	
1695	1897	true	true	true	true	false	247	247	0	247	
1697	1892	true	true	true	true	false	247	247	0	247	
1696	1884	true	true	true	true	false	246	246	0	246	
1691	1884	true	true	true	true	false	244	244	0	244	
1694	1889	true	true	true	true	false	244	244	0	244	
1694	1890	true	true	true	true	false	244	244	0	244	

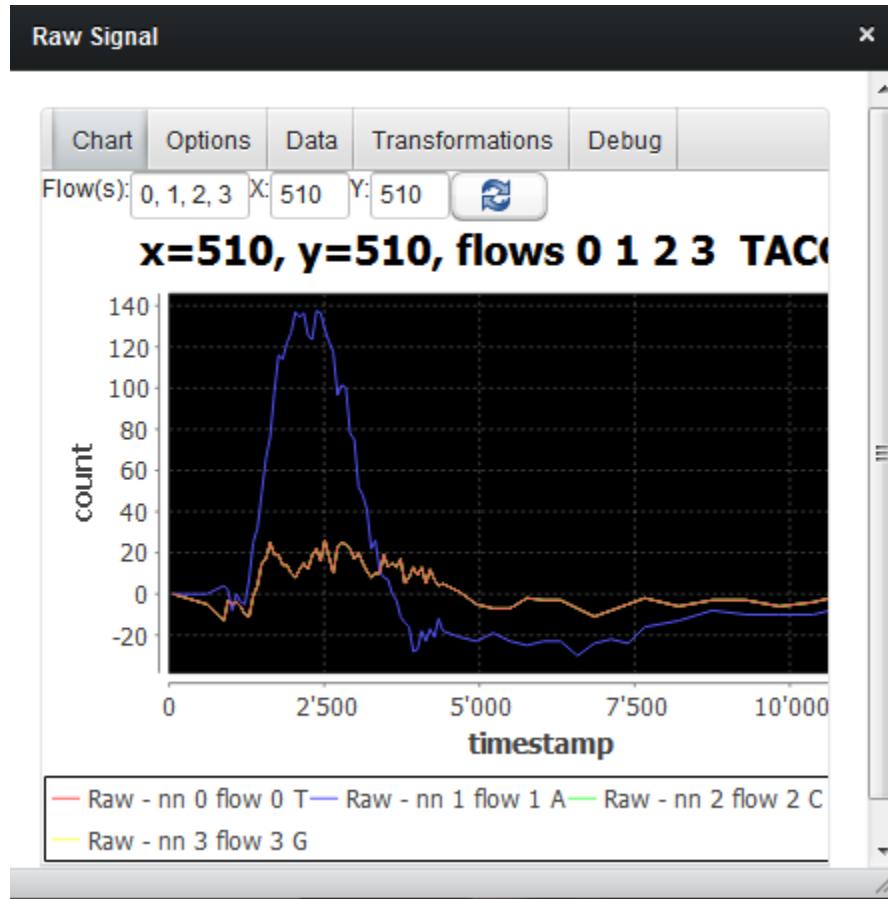
The export button lets you export the table data (from all tables):



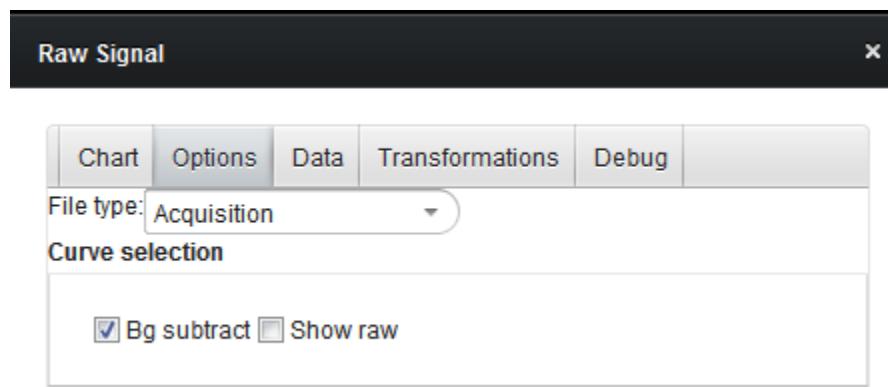
Raw Data View

You can enter one or more flow numbers (separated by comma) into the flows text field. You can also enter a coordinate right there. Note that sometimes it takes time to load the raw data, in particular as this all goes to the torrent server first (which might be busy). For better performance and better

response/interactivity, you might want to use the full Torrent Scout client.



In the options tab you can pick to view the raw data and/or the bg subtracted data and the file type. The bg subtraction simply subtracts the average signal of the surrounding wells that are considered empty.



The data tab shows the currently loaded data:

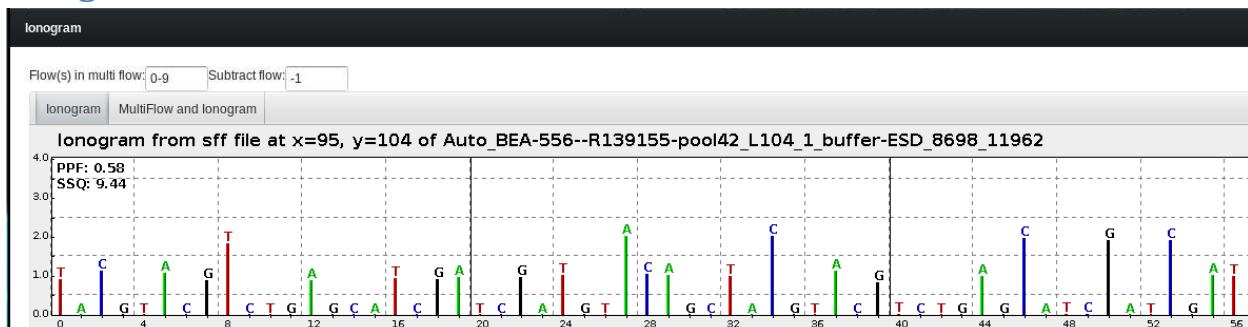
Raw Signal

Chart	Options	Data	Transformations	Debug
-------	---------	------	-----------------	-------

```
Experiment name: null
Raw data dir: s:/data/314a/raw/
x=510, y=510
Raw - nn 0 flow 0 T, Raw - nn 1 flow 1 A, Raw - nn 2 flow 2 C, Raw -
nn 3 flow 3 G
68.0,0.0,0.0,0.0,0.0
610.0,-5.0,0.0,-5.0,-5.0
882.0,-13.0,4.0,-13.0,-13.0
950.0,-3.0,2.0,-3.0,-3.0
1017.0,-5.0,-8.0,-5.0,-5.0
1085.0,-4.0,0.0,-4.0,-4.0
1153.0,-6.0,-4.0,-6.0,-6.0
1221.0,-10.0,-5.0,-10.0,-10.0
1289.0 -11.0 7.0 -11.0 -11.0
```

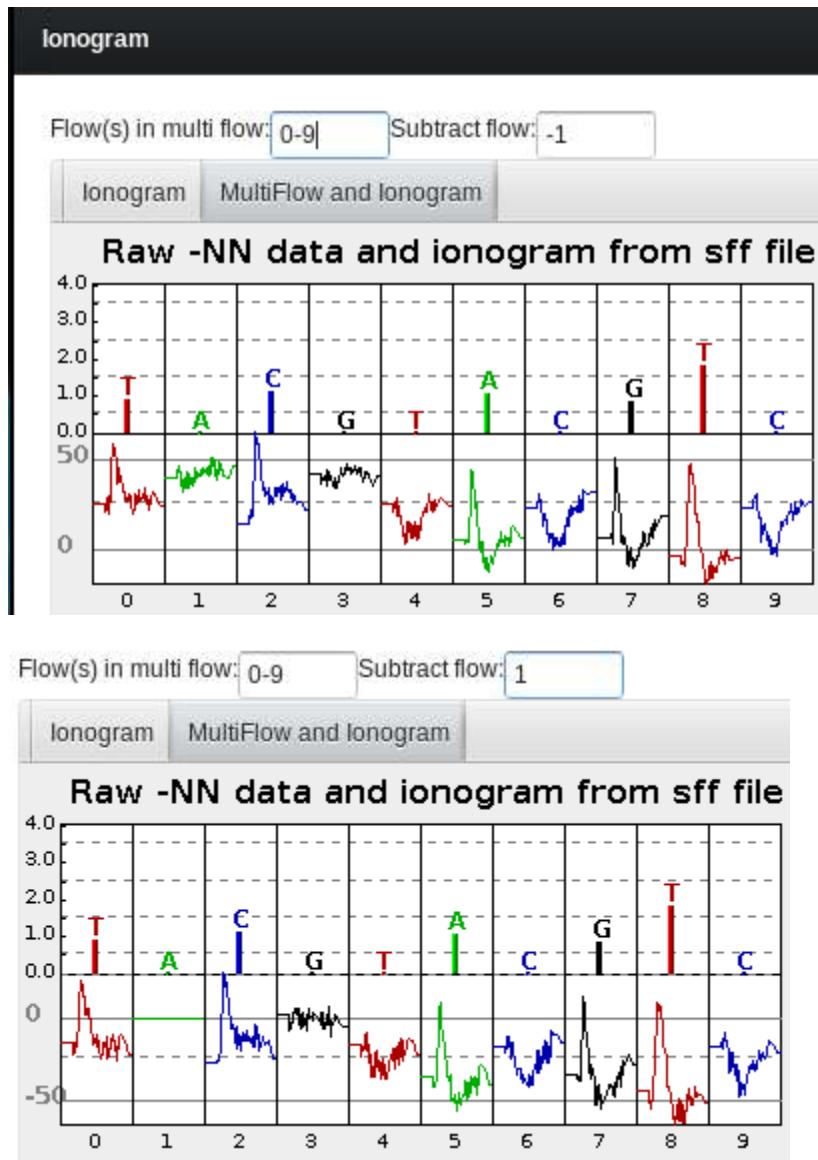
You can copy and paste this to Excel (Use Paste/Text import wizard, comma as separator) to create your own charts.

Ionogram



Viewing raw signals in ionogram view

The second tab shows any number of nearest neighbor subtracted raw signals for this well: just enter the flow numbers (starting at 0) into the text field and hit enter:



Genome To Read

In the component Find Reads/ Find reads by genome position you can locate any read that maps to a certain genome position.

Pick the reference sequence in the drop down box and enter a genome location in the text box. This will return a list of all reads (including the flow number) that map to that position. To view the ionogram or alignment of any read you can just open that component and select the read in the table.

Genome to Read										
gi 170079663 ref NC_0			1234		Find Reads		💡	💾	s:\data\314\results\	
X	Y	REVERS	READ LEN	FLOW	POS IN	POS I	BASE	REFERENCE	ALIGNMENT INFO	
388	908	true	147	62	25	85	G	gi 170079663 ref NC_010473.1	% ident=95.78947368421052, nr in	
1168	518	false	145	39	18	18	G	gi 170079663 ref NC_010473.1	% ident=97.82608695652173, nr in	
1210	709	false	206	11	1	1	G	gi 170079663 ref NC_010473.1	% ident=98.9795918367347, nr in	

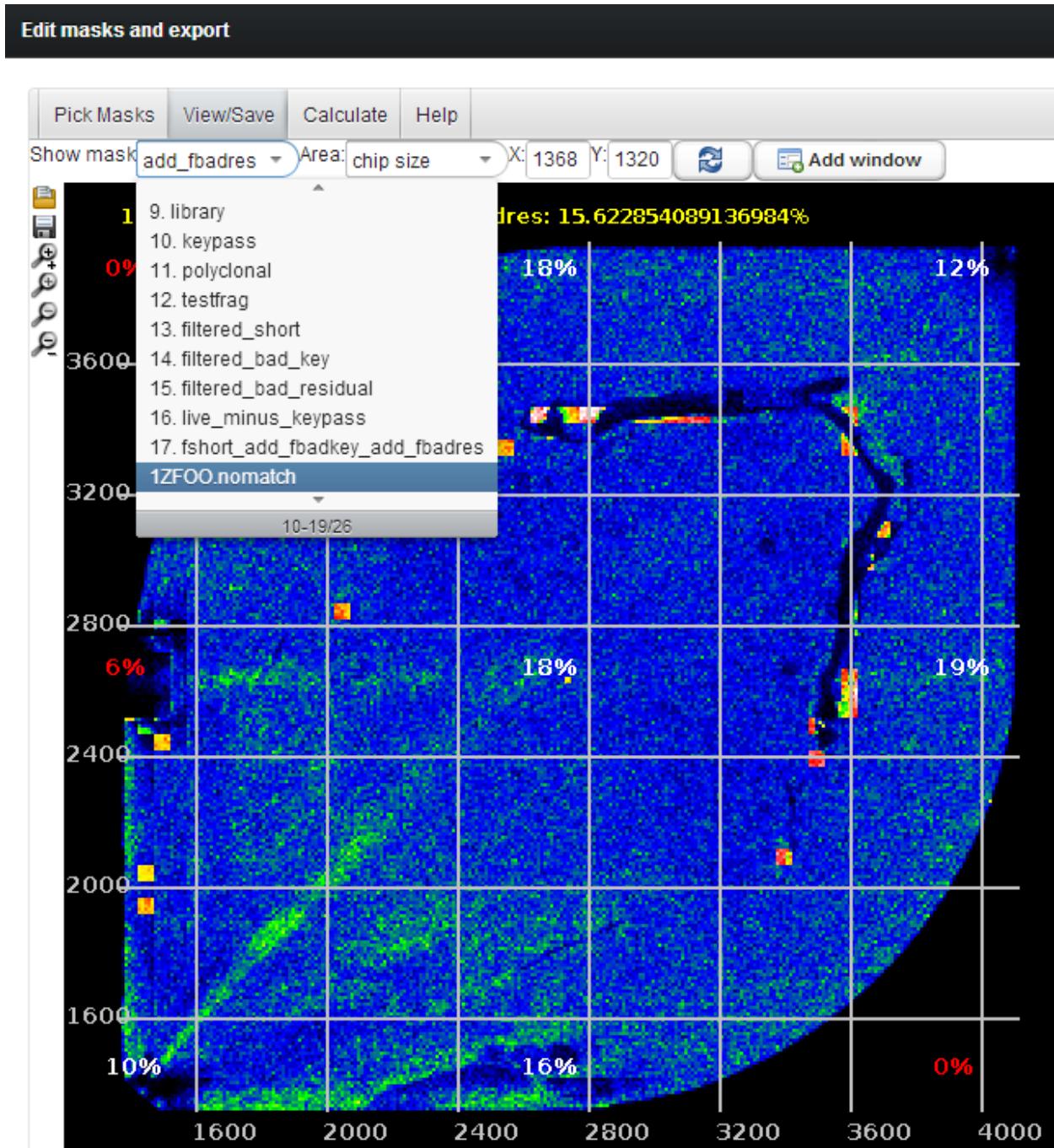
Custom and Barcode Masks

View Results | Regional Views |

- Ionogram
- Alignment
- Create masks and find reads from read
- Raw Signal (one well)
- XYZ Charts
- Export data (ionograms, alignments, raw)
- Compute and view custom heat maps**
- View barcode table
- View barcode heat maps

The “Edit Masks” and “Compute and view custom heat maps” and “View barcode heat maps” components let you view and do calculations with both barcode and custom masks. It lets you view the entire chip, or zoom into a particular area and see the single wells in that area.

The View tab lets you view and pick the masks:



Here you can also:

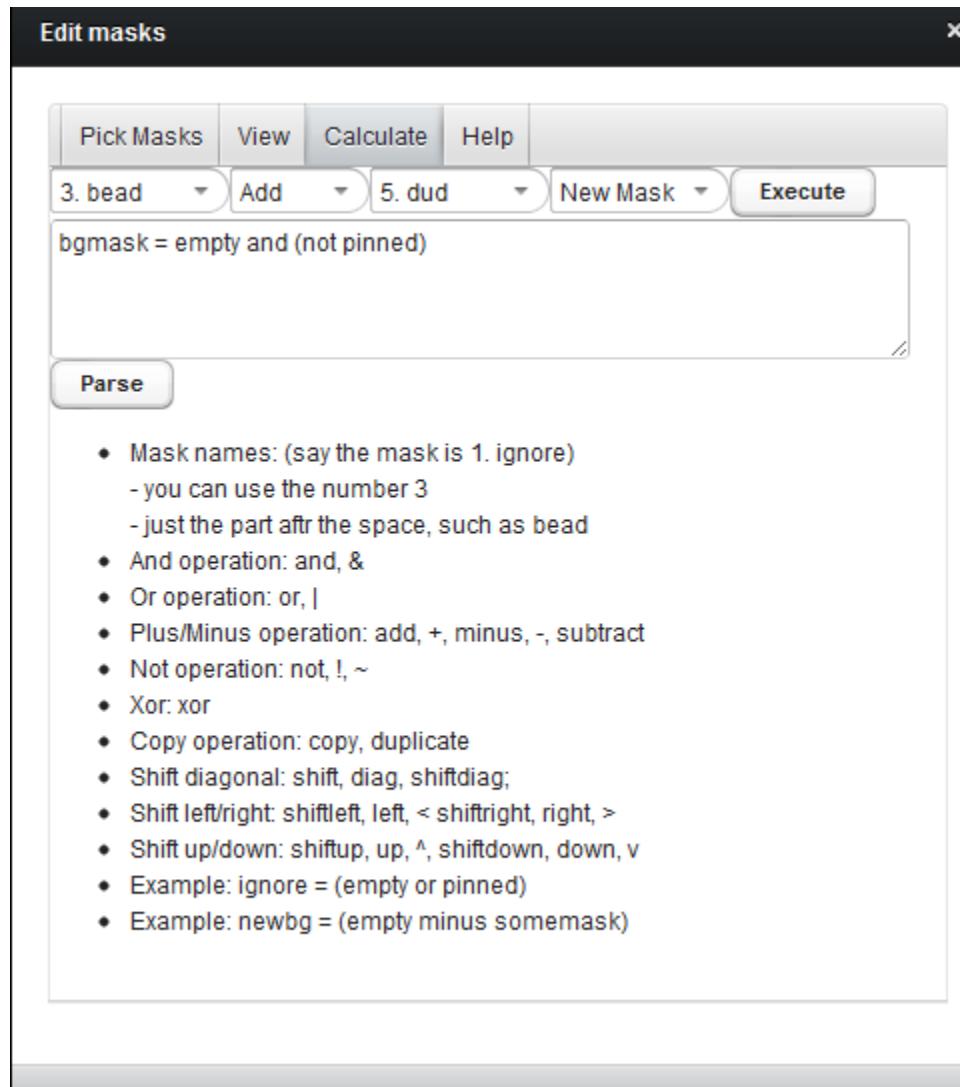
- Pick the viewable area (default is chip size)
- Pick barcode masks
- View default masks that are the result of some calculations, such as live minus keypass or the combination of all filtered masks

In the View tab you can save and load masks. It stores the coordinate in addition to all masks, so next time you load a masks file, it will automatically move the view to the coordinate of the mask. Note that you have to be in the correct experiment or block. For Proton experiment currently you have to load the block for which you created the mask.

The calculate tab has 2 calculators:

- a) A simple drop down calculator
- b) A parser

The drop down calculator is really simple: pick mask A as first operand, an operation such as add, subtract, xor etc, and if it makes sense, a second operator mask B, and finally either an existing mask as result (Which will be overwritten) or else a new mask.



After the computation, it will print the command line string into the text box.

The parser allows you to copy/paste frequent operations directly into the text box, so you don't have to pick the masks in the drop down box :-)

The Pick masks tab is important for the Process and Automate component Shown later)

Edit masks

Pick Masks	View	Calculate	Help	
Ignore mask:	0. pinned			
Background mask:	1. empty			
Signal mask:	11. library			
Notes: The signal mask is used in Automate to compute the mean signal The background mask is the one used in Process for NN bg subtraction				

Working with Raw data

Process

Basically it lets you view an area of say 100x100 wells, pick a few wells in there at the same time (the cursors), and view NN subtracted signal. This allows you to compare the signal of different wells, maybe look at problems, and to see where approximately the signal appears.

You can snap the cursors to any of the masks (also your own!).

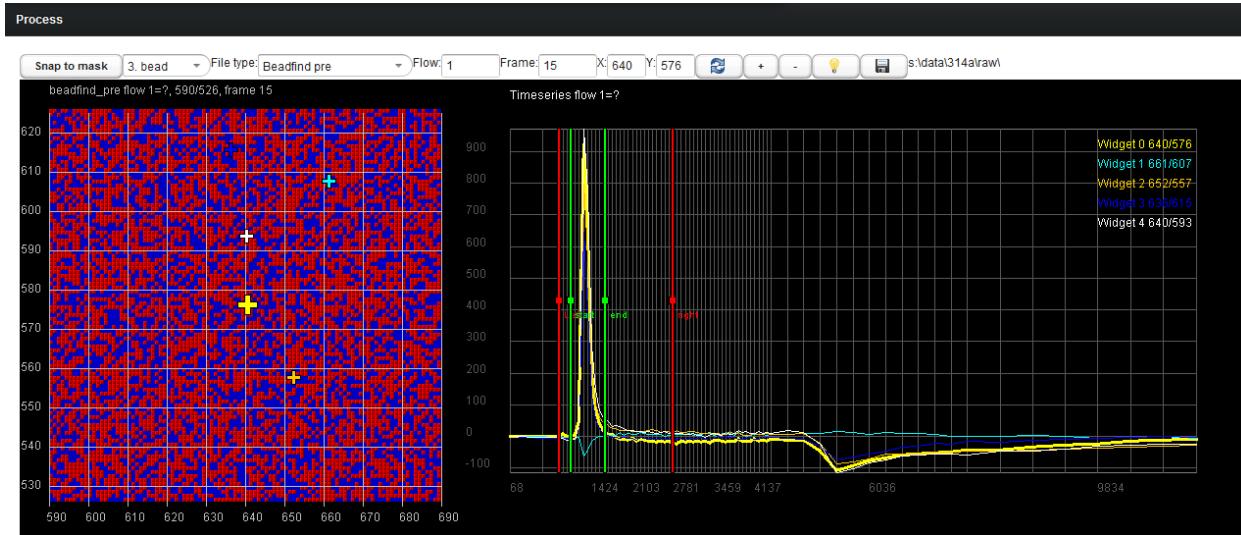
It automatically computes the NN bg subtraction, so make sure you have a reasonable mask selected in the Mask/Pick tab (default tis empty):

Edit masks

Pick Masks	View	Calculate	Help	
Ignore mask:	0. pinned			
Background mask:	1. empty			
Signal mask:	11. library			
Notes: The signal mask is used in Automate to compute the mean signal The background mask is the one used in Process for NN bg subtraction				

The zoom in magnifies the area, and reduces its size by a factor of 2 each time. **Note this currently reloads the data and masks.** So you have created your own masks, they would be lost (will think of a save feature soon, but in the meantime, you might want to use the **full Torrent Scout client** to do more professional mask editing as it allows you to save one or all masks!)

You can pick the type of data and flow – here the beadfind pre flow 1 is shown with a very clear bead find signal:



Note the vertical red and green lines. They can be moved around with the mouse.

Those positions are used in **the Fit component** below:

Fit Component

The fit component is a bit difficult to understand at first I think: basically it helps you to classify wells. That way you can for instance create a mask such as “all live wells with a very high signal”, or maybe “duds that don’t look like duds”, or “bead find with a low signal” etc.

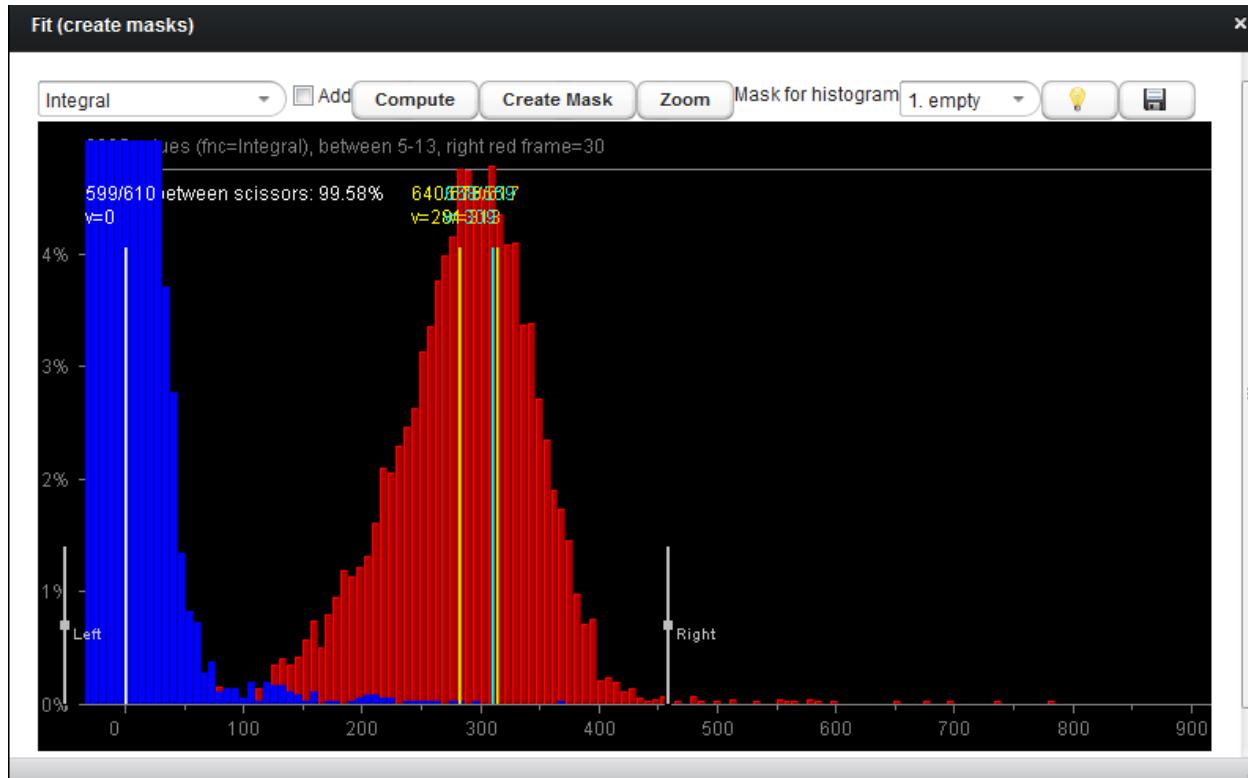
The histogram is calculated using all wells from the selected histogram mask (or all wells if none is selected). For all those wells, it computes the values using the green (and red) frames.

The integral computes the area under the curve between the green frames.

The max-end height computes the max between the green frames, and subtracts the value at the right red frame.

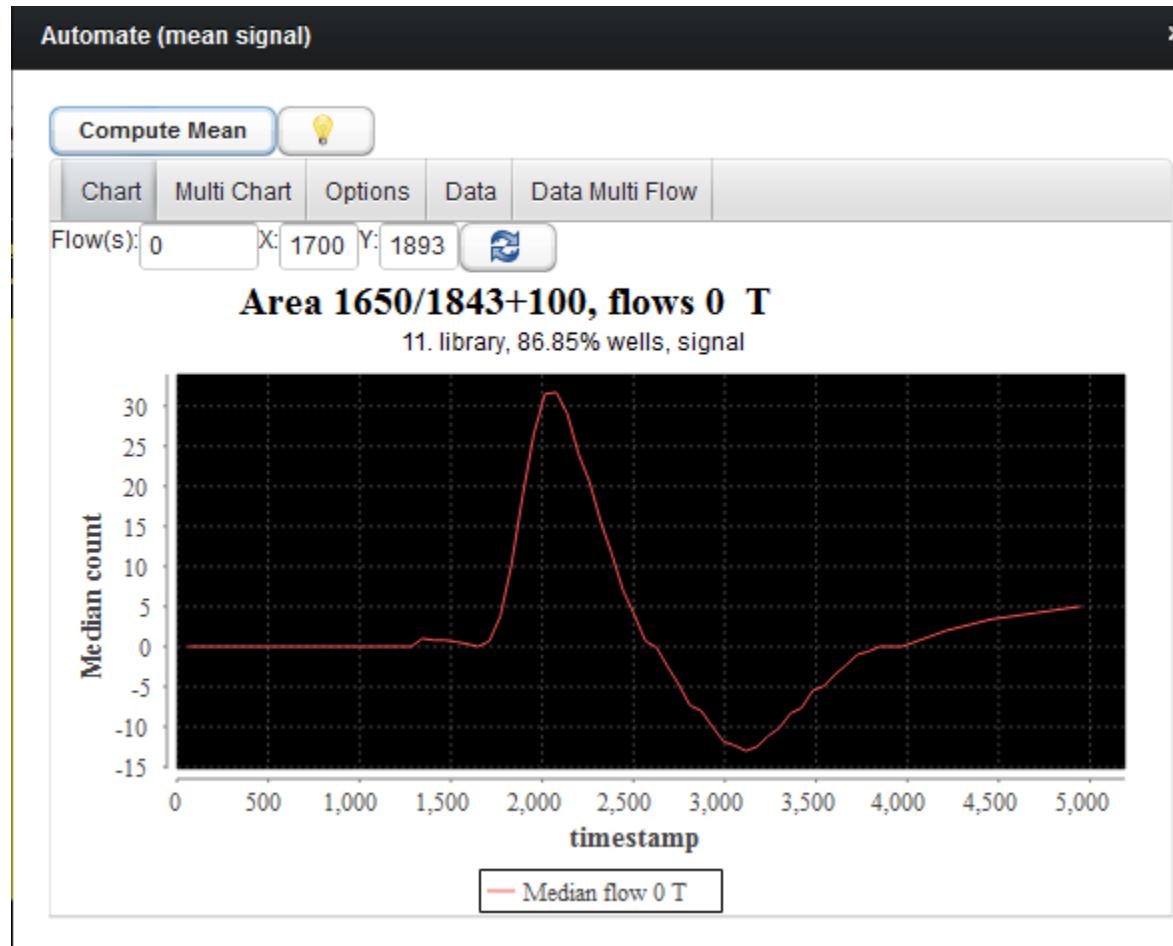
The add check box lets you add a second histogram the next time you compute it. So you might want to compare the data of the empty wells (blue) versus the bead wells (red) as in the example below.

The zoom button zooms into the area where the scissors are (it only shows the last data set currently...)

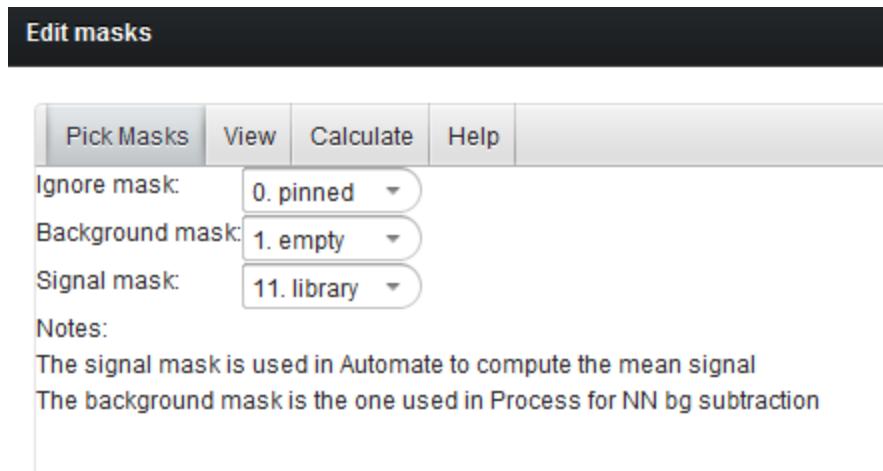


Automate (Compute Mean Signal)

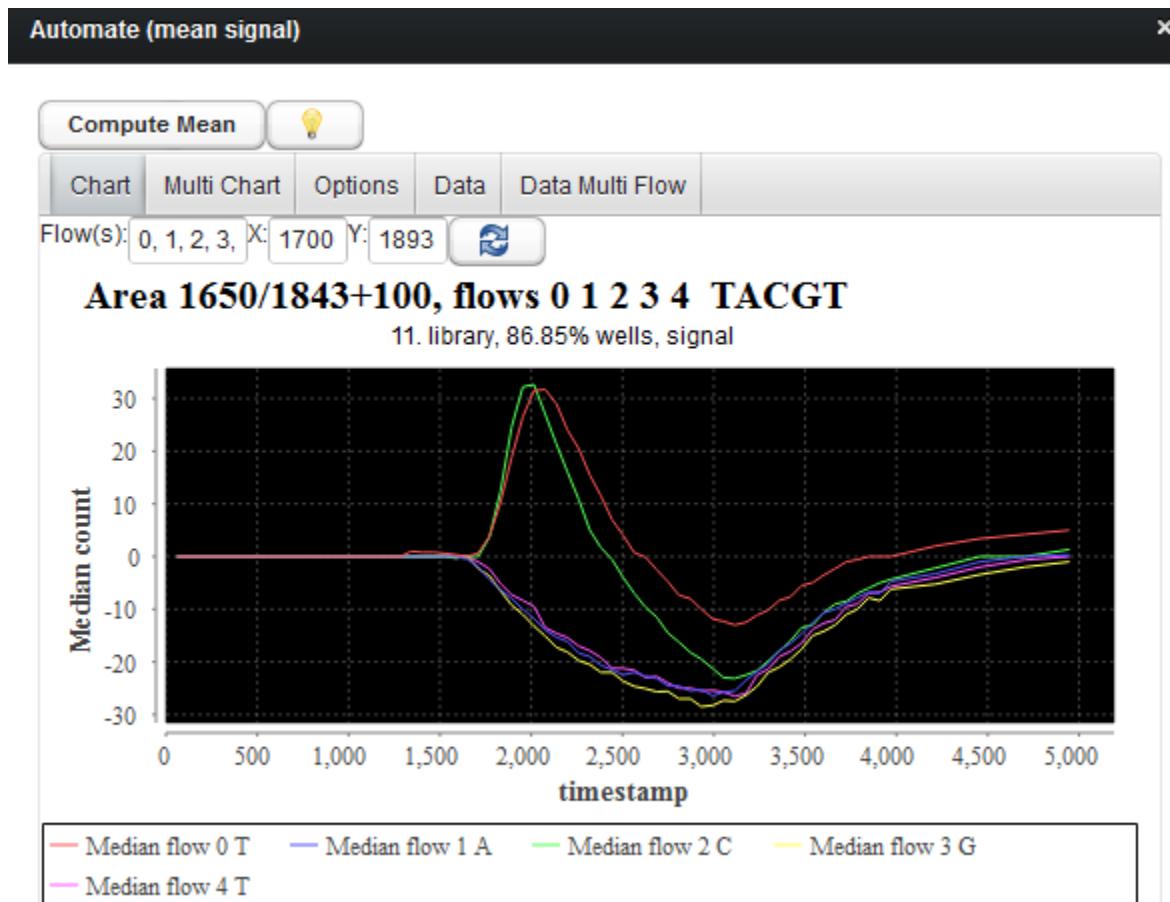
Maybe you want to compare the signal shape of different areas the chip, maybe using different masks. This is where the automate component comes in:



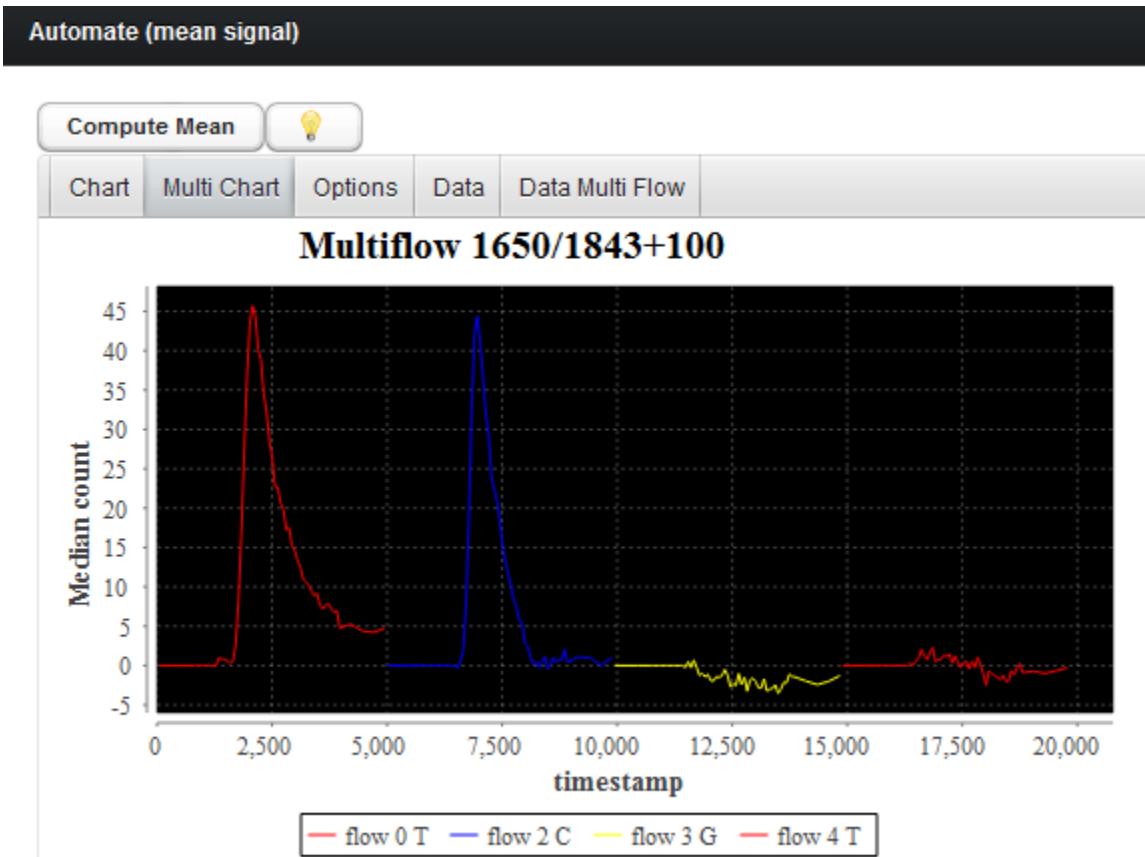
It takes all wells from the Process component (such as a 100x100 area, but it can be larger/smaller depending on the zoom level), and computes the NN subtraction and mean signal for the entire area. Make sure you pick the signal mask you want in the Mask/Pick tab:



You can enter multiple flows in the text box (such as 1-4 or 1,2,3,4 or any other flow). Select the file type for beadfind in the Options tab (there you can also decide to subtract the result from another (empty) flow).



The Multi Chart tab shows the same data, but the flows are spread along the x axis:



You can export the data to excel with the Data tabs. In Excel, use Paste/Text Import Wizard/comma as separator.

Automate (mean signal)

Compute Mean 

Chart Multi Chart Options Data Data Multi Flow

```
/results2/Floyd/R_2012_03_05_16_01_39_user_FLO-507--  
R140608-30m.318.fs2-AUT/  
Multiflow 1650/1843+100

time, median value  

61.0.0.0, flow 0 T  

550.0.0.0, flow 0 T  

794.0.0.0, flow 0 T  

855.0.0.0, flow 0 T  

916.0.0.0, flow 0 T  

977.0.0.0, flow 0 T  

1038.0.0.0, flow 0 T  

1099.0.0.0, flow 0 T  

1160.0.0.0, flow 0 T  

1222.0.0.0, flow 0 T  

1283.0.0.0, flow 0 T  

1344.0.1.0, flow 0 T  

1405.0.0.8000001907348633, flow 0 T  

1466.0.0.8000001907348633, flow 0 T  

1527.0.0.5999999046325684, flow 0 T  

1588.0.0.33333349227905273, flow 0 T  

1649.0.0.5, flow 0 T  

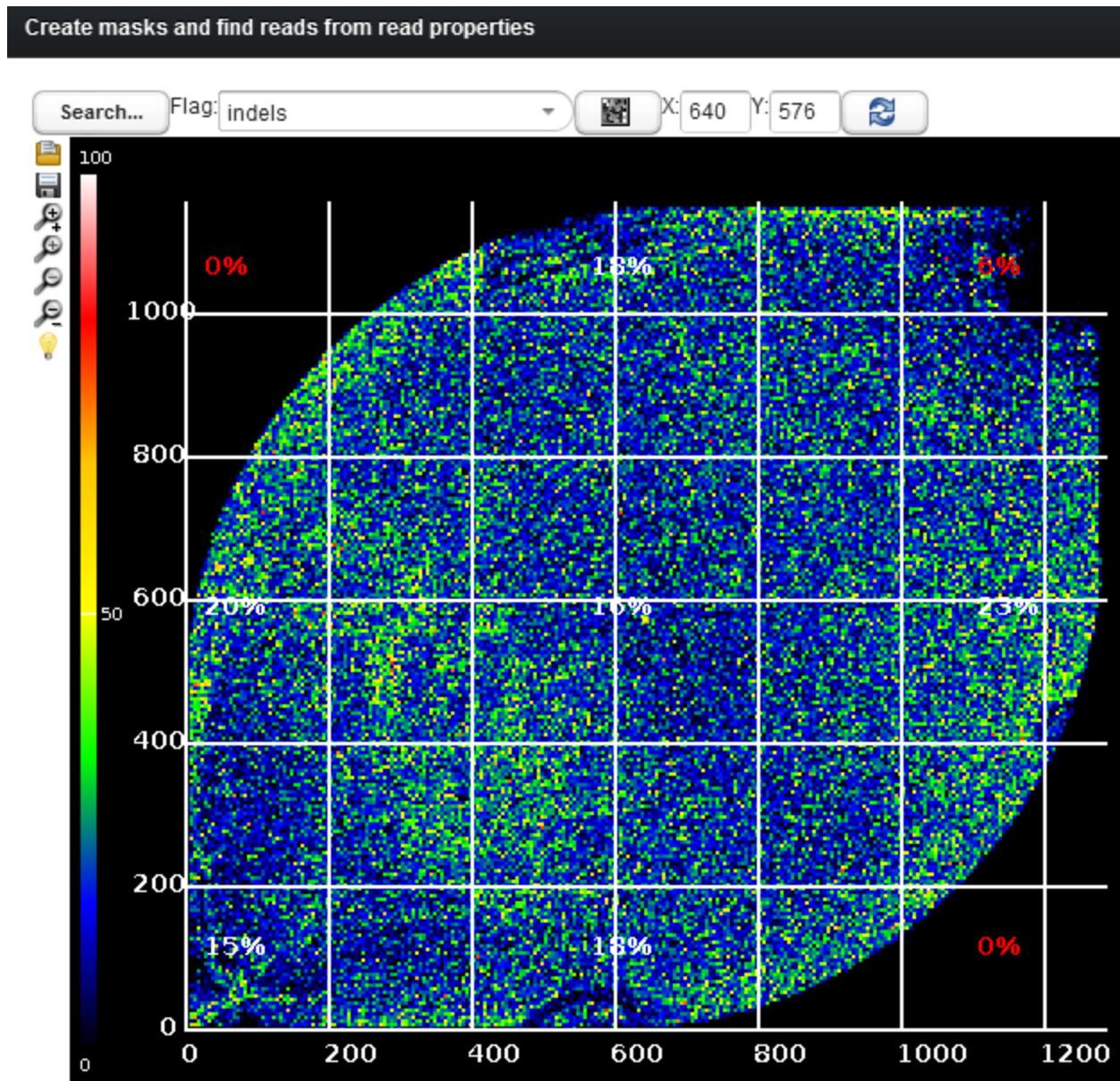
1710.0.2.6666669845581055, flow 0 T  

1771.0.8.15416669845581, flow 0 T  

1822.0.18.59222206011621, flow 0 T
```

Find reads

The find Reads component shows heat maps of scores such as Q17 length, and lets you filter the reads:

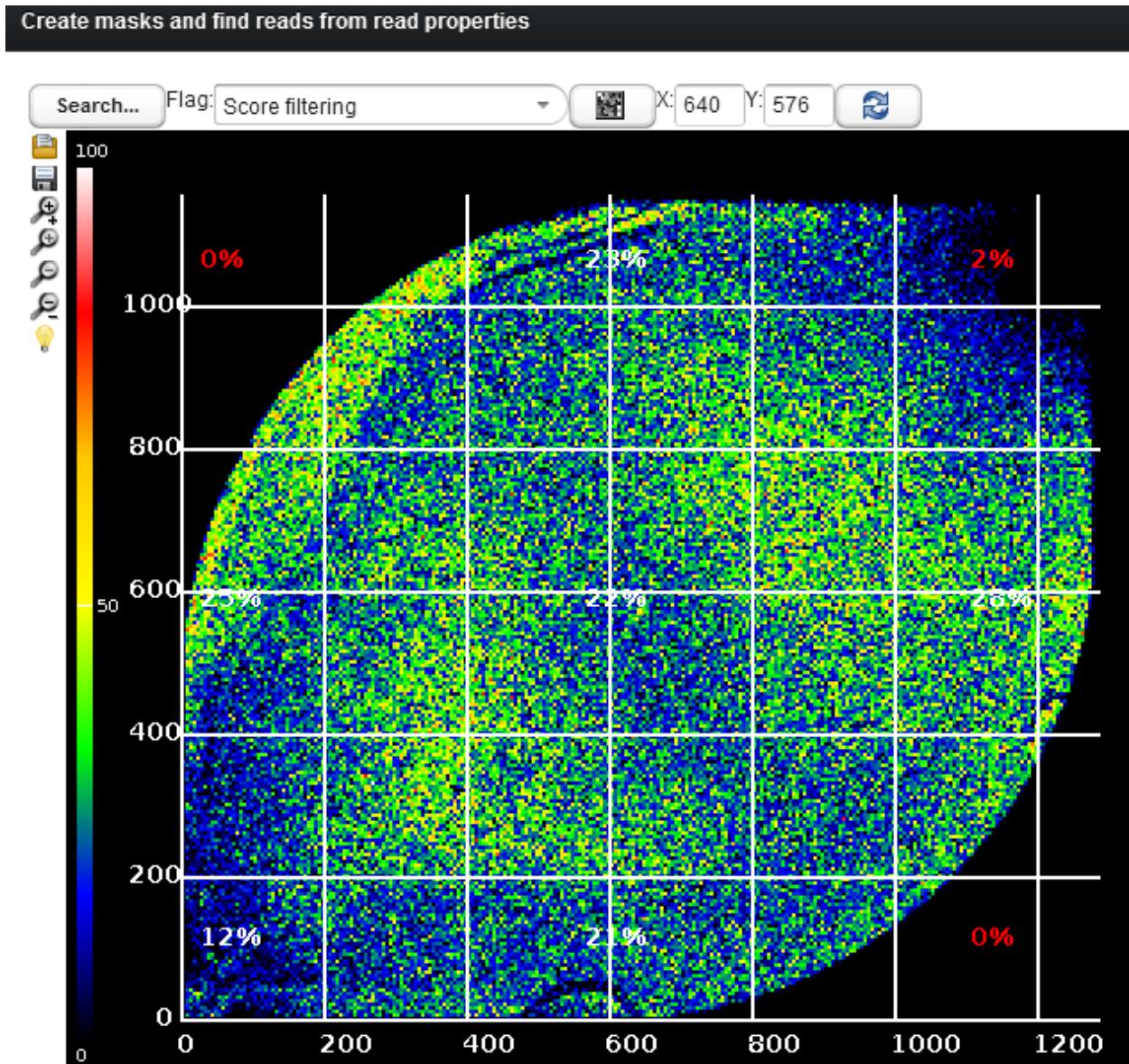


Select the filter button to only show wells that have certain values:

Enter a range of values for Q47 len

Enter values between 0.0 and 141.0

Min: 100 Max: 141



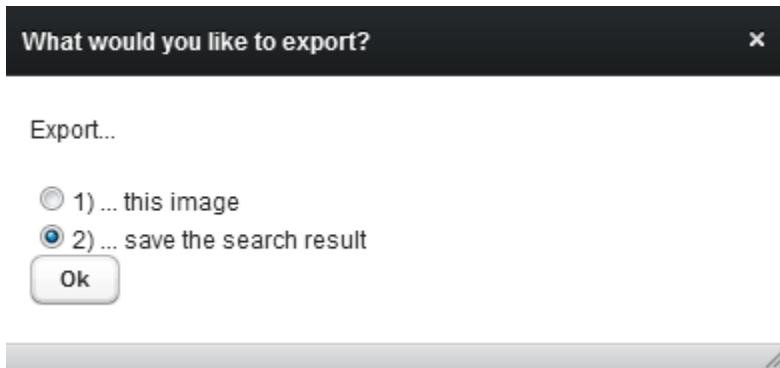
If you now select a well by moving the orange cursor, and open the well table, it will only select wells that fit the search criteria. In this case, only wells with a Q47 length of at least 100:

Well Table												
X:	640	Y:	576		Load Scores			s:\data\314a\results\				
X	Y	BEAD	LIVE	LIBRARY	KEYPASS	DUD	Q17 LEN	Q47 LEN	INDELS	MATCHES		
709	606	true	true	true	false	false	123	106	1	123		
669	620	true	true	true	false	false	120	120	0	120		
658	621	false	false	false	false	false	119	116	0	119		
690	619	true	true	true	false	false	118	100	2	117		
717	647	true	true	true	false	false	117	117	0	117		
702	688	false	false	false	false	false	115	107	1	115		
660	687	true	false	false	false	true	114	108	1	114		
693	690	true	true	true	false	false	114	102	1	114		

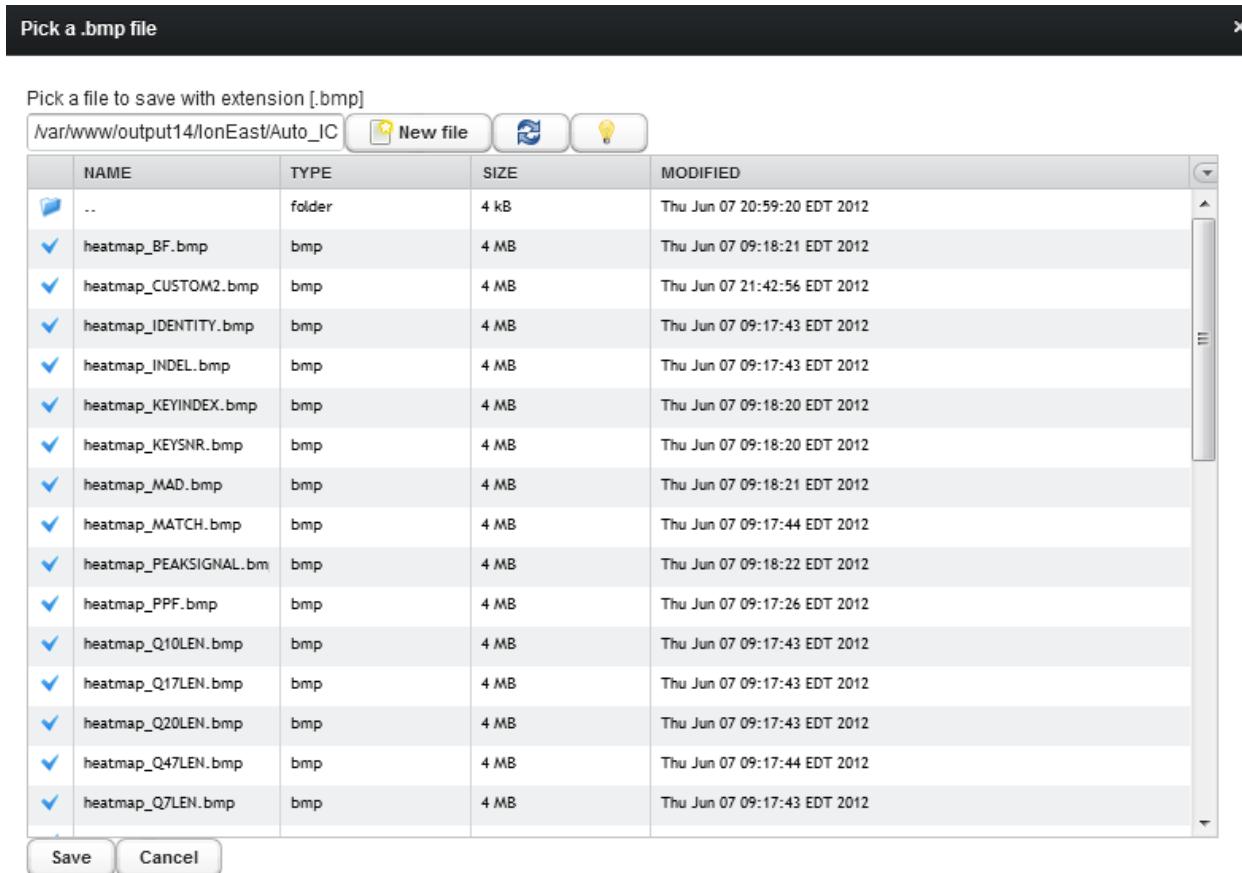
If there are fewer than 5000 wells in the entire chip that fit the result, it will load them all into the table.

Save your search result

You can save your search so that you can go back to it later – just click the disc icon:



And pick/create a .bmp file:



Create a mask from your search result

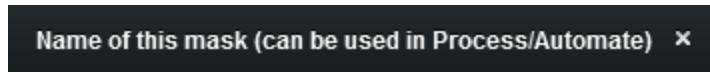
You can convert your search result into a mask just like any other mask (like live or empty), which you can then use in any other component.

For instance you can use it in the Process component to see the raw data of those wells.

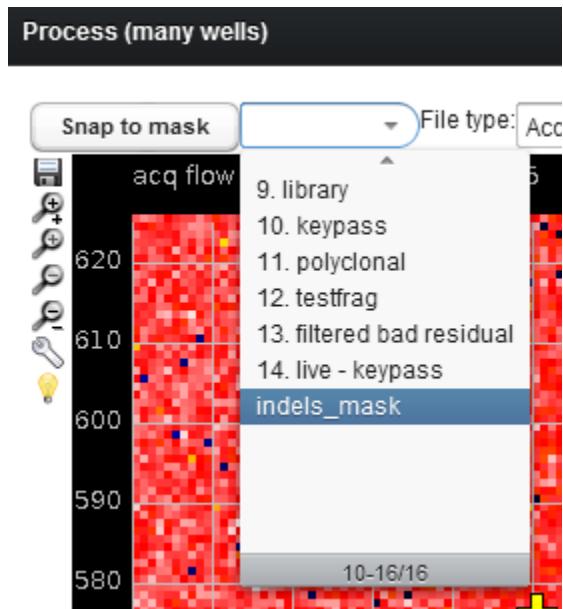
Or you can use it in the Automate component to compute the mean signal of all wells in that mask.

Or you can combine this mask with other Mask Editor.

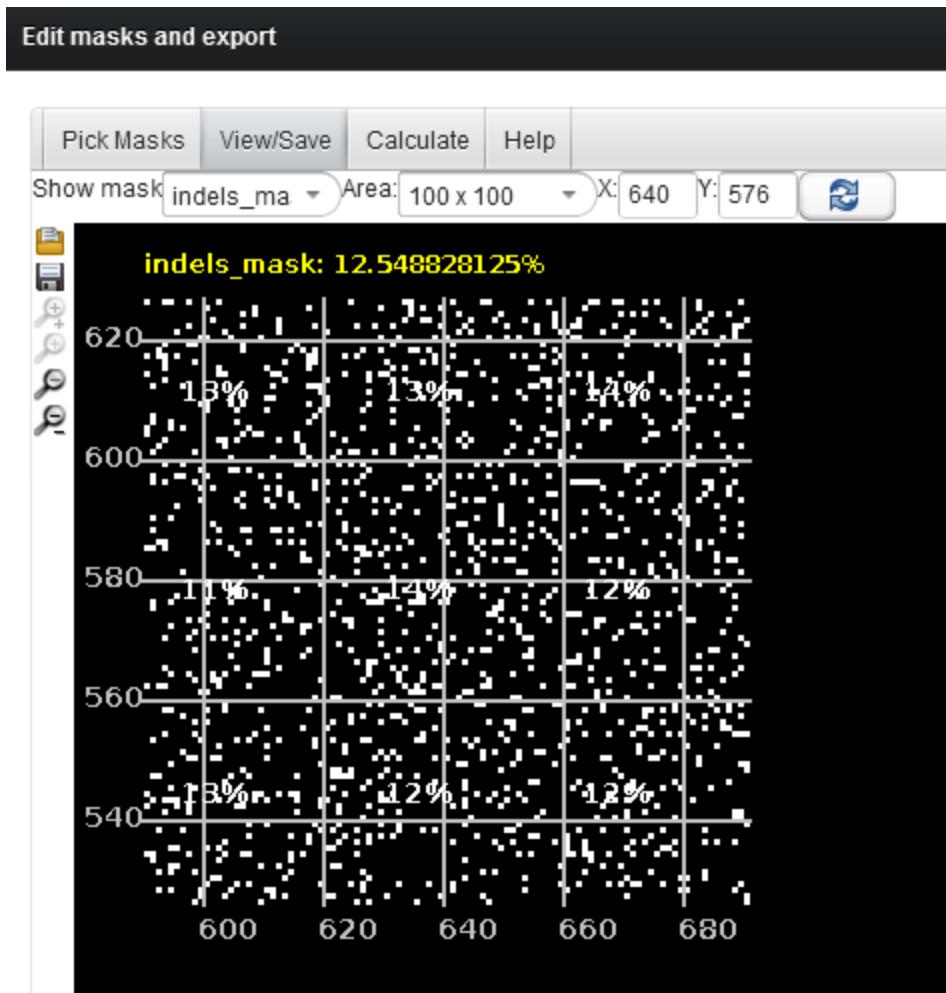




For instance you can now see it in the drop down of the Process component:



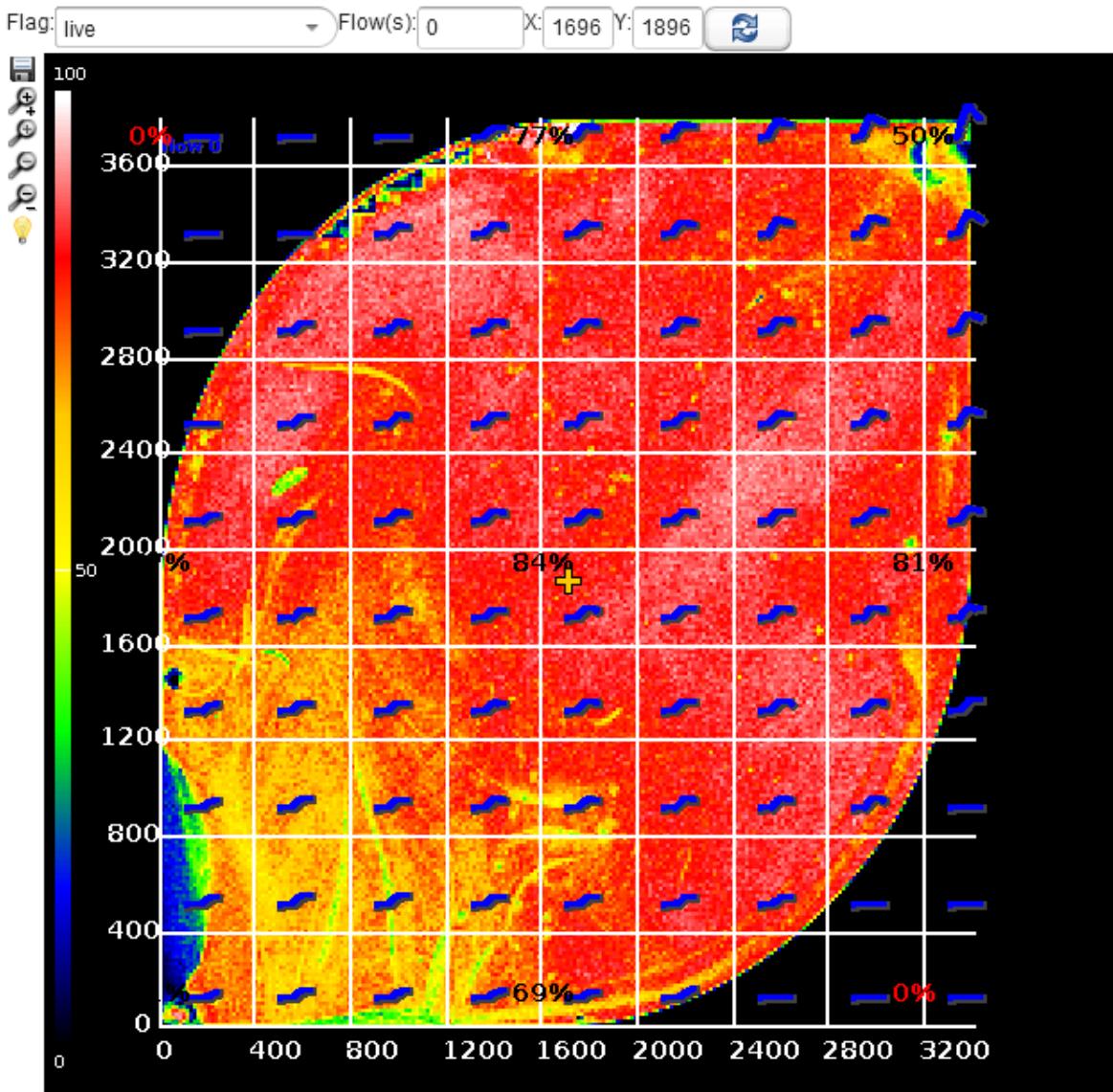
You can also work with the new mask in the mask editor:



Regional Charts

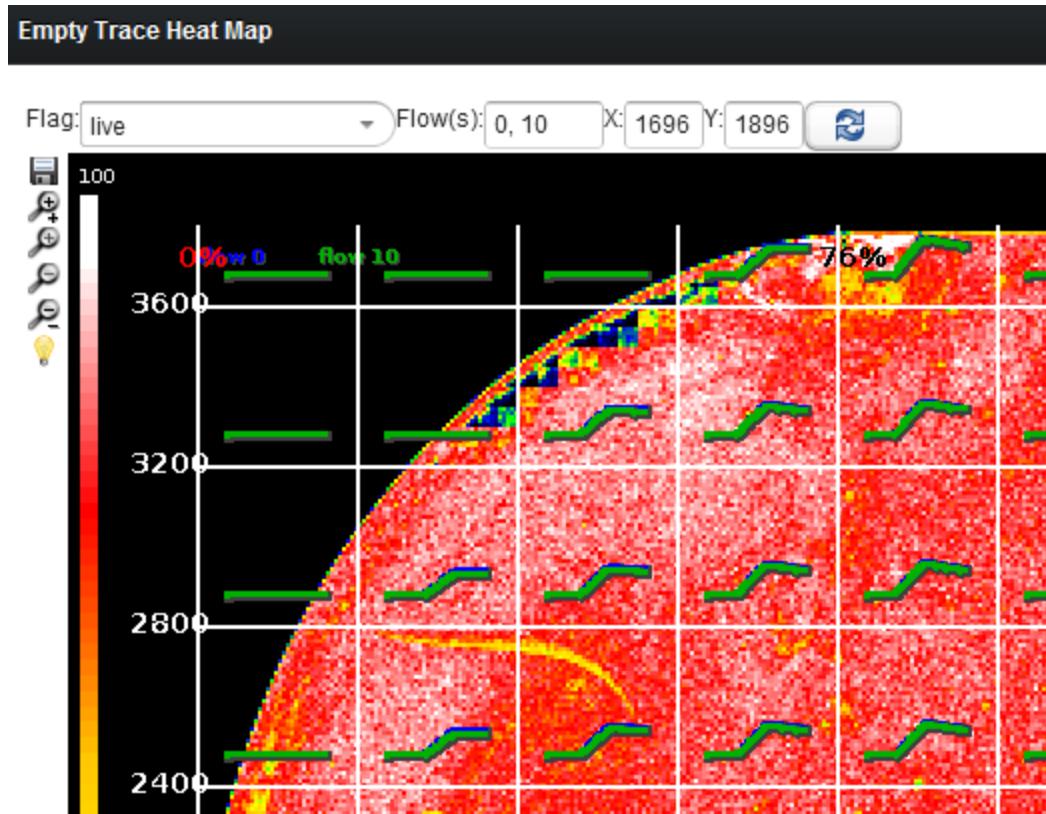
Regional Empty Traces

After you pick an experiment the bf heat map opens. It also shows the regional empty traces for the flow entered on top (multiple flows can be entered).



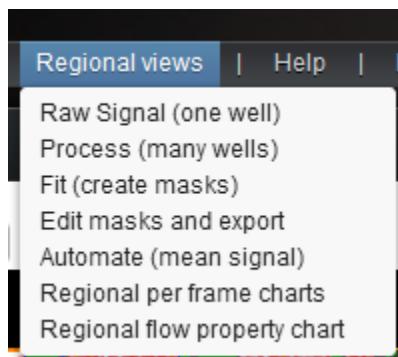
Zoom in to see more details. If you enter more than one flow, it will draw them on top of each other – the idea is to just get a general sense of the shape of the empty trace and relative sizes to each other.

Note: to hide the empty traces, just enter -1 for flow!



Regional per Frame Charts

To view any particular region in more detail, first double click on an area, and then pick one of the regional views :



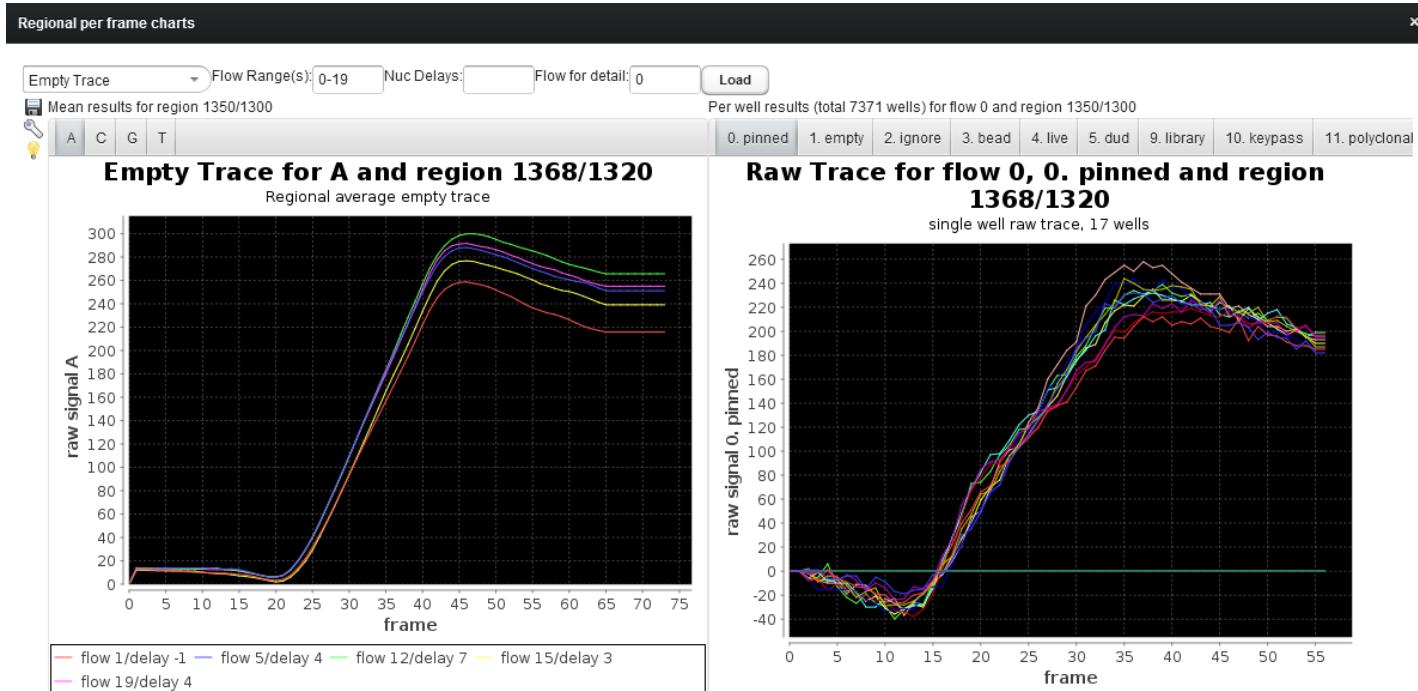
Pick the “Regional per frame charts” to see the empty traces (and other data) for a particular region:

On the top left drop down, you can pick the type of data to view (currently there are is just empty traces, but in the future there might be other types).

The chart on the left is partitioned by base (ACGT). You can pick one or more flow ranges, such as:

0-19,20-30, 34, 50-60

If you wish to further limit what is shown in the charts, you can also enter one or more nuc waits (such as 1, 7)

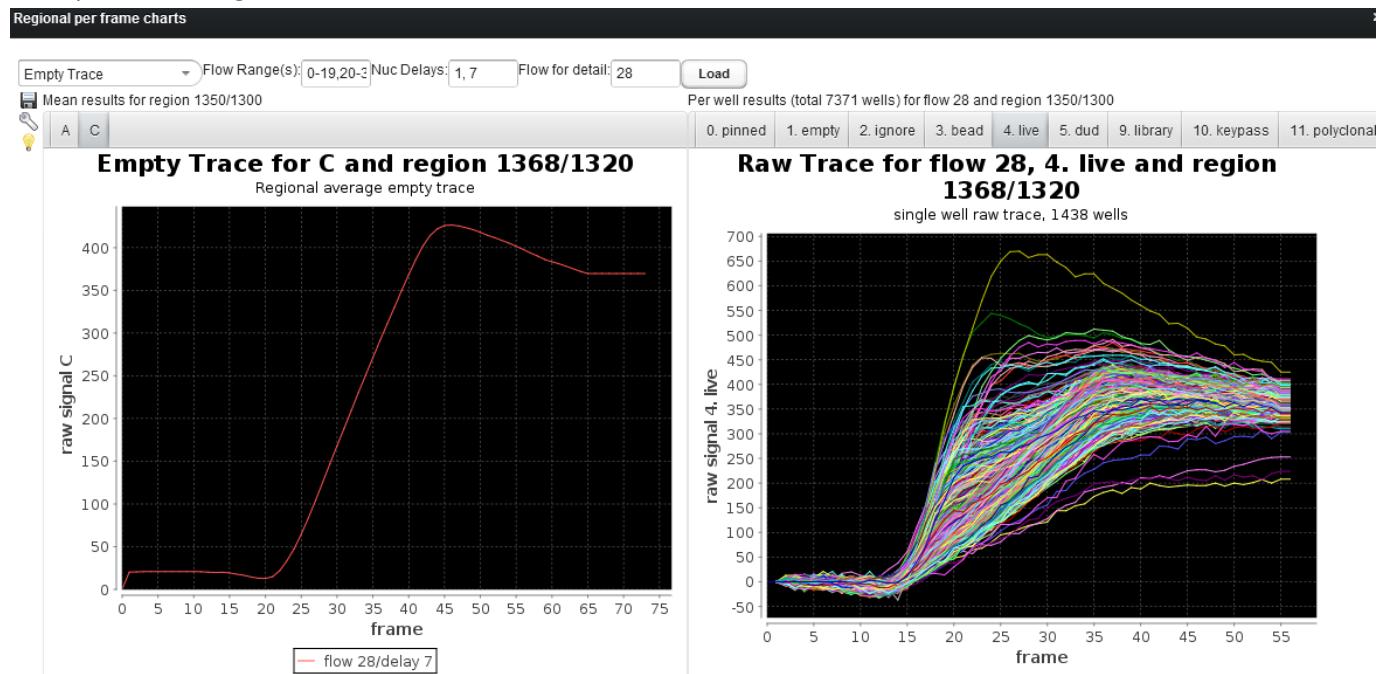


On the right side, it will show the actual raw data from the .dat file for any entered flow (flow for detail) (I will add the ability to pick the flow by clicking on a trace on the left shortly).

The right side is partitioned by bf flag. So in the pinned tab, it shows the raw for all wells that are flagged as pinned and so on.

The disc icon on the very left border allows you to save all charts and all data (in Excel format).

Example for looking at flow 28 with nuc wait 7



The second new regional view is the “Regional flow property chart”, which lets you see properties by flow:

In the left drop down you can pick the type of data to view. Currently it only contains step size, but in the future there might be additional types such as base call error and others.

Regional per Flow Charts

Again you can limit the data to see by nuc delay. The same data is shown in 3 charts, which are partitioned in different ways:

- Partitioning by base (ACGT)
- Partitioning by nuc wait
- Partitioning by both nuc wait and base

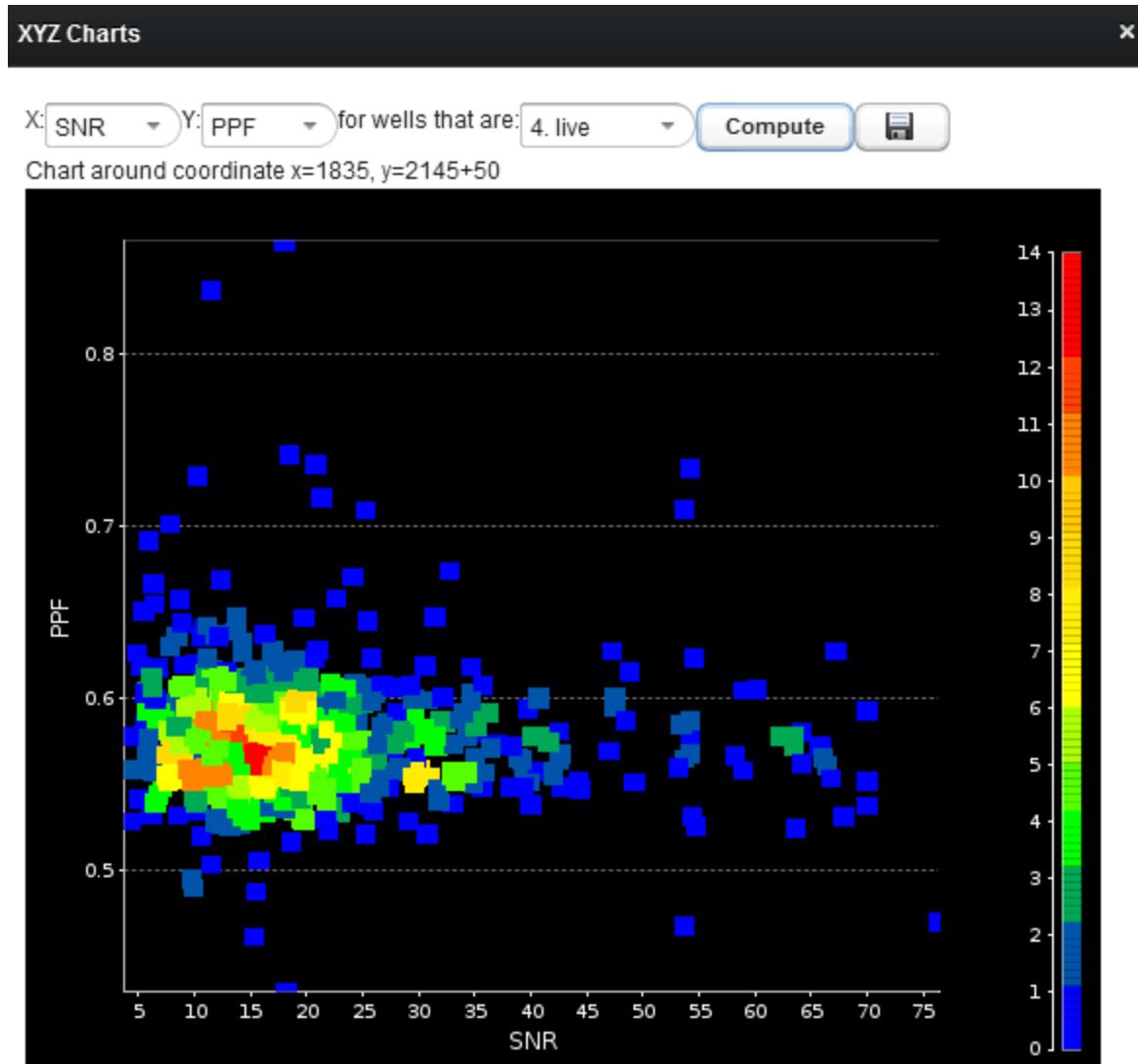
If you only wish to see certain bases (say AC), you can enter them in the Bases text field.



The save button lets you save both the data and the charts

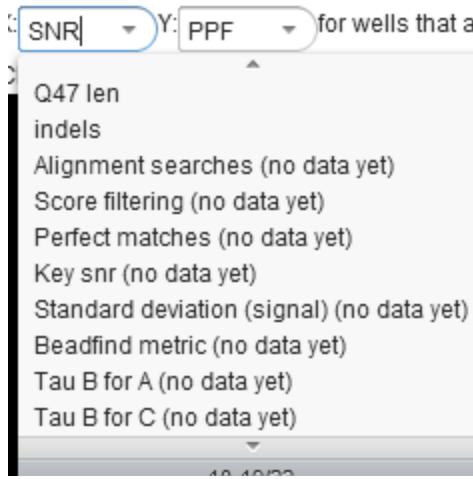
XYZ Charts

To see relationships between different read properties – say to see what kind of effect the signal to noise ratio has on the Q17 length, we can use the XYZ charts:



You can pick two properties (here SNR and PPF) and pick a mask. The colors indicate how many reads/wells it found for that particular combination of values. So in the chart above, there were lots of reads with SNR around 15 and PPF around 0.57

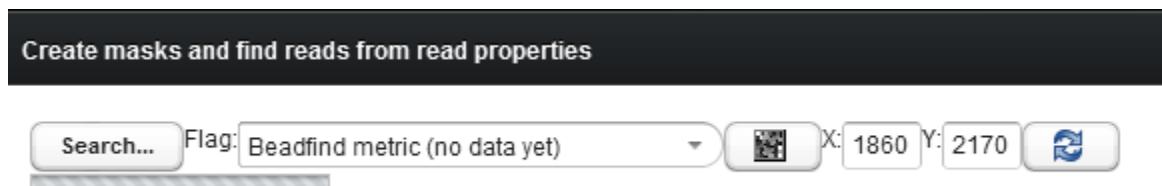
Depending on how/if the TS plugin was executed, there may be properties where it says “no data yet”. This simply means that if you chose to use it, it first has to compute that particular heat map, which may take up to 10 minutes (depending on the chip size).



A lot of the listed properties are from the Separator file (beadfind metric, Tau B values and others). So in case that file is missing, you won't be able to use those properties.

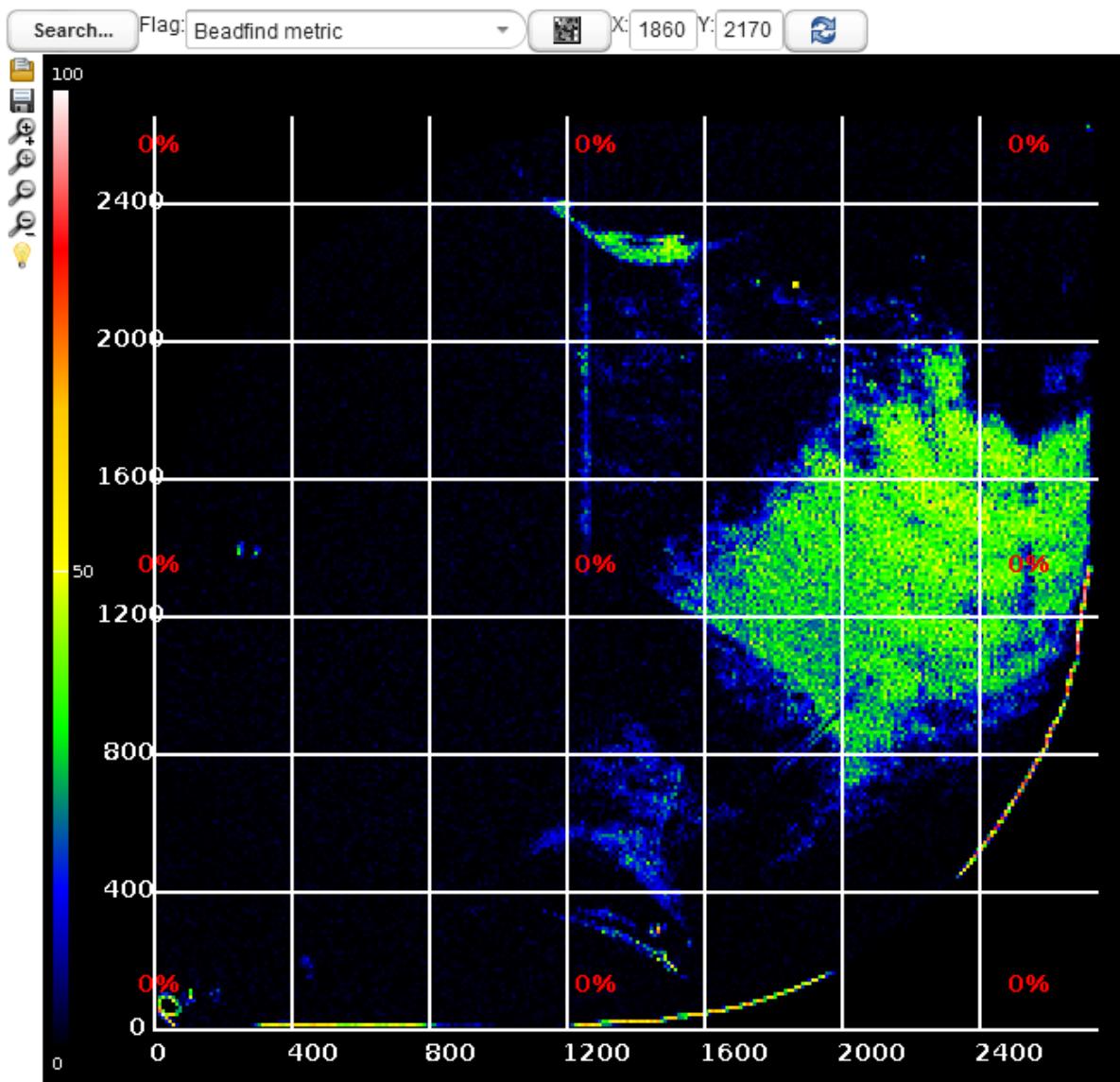
Computing missing data

The Score heat map lets you pick those heat maps and it will show you in a progress bar how far along it is in computing any missing data – in the example below the beadfind metric.

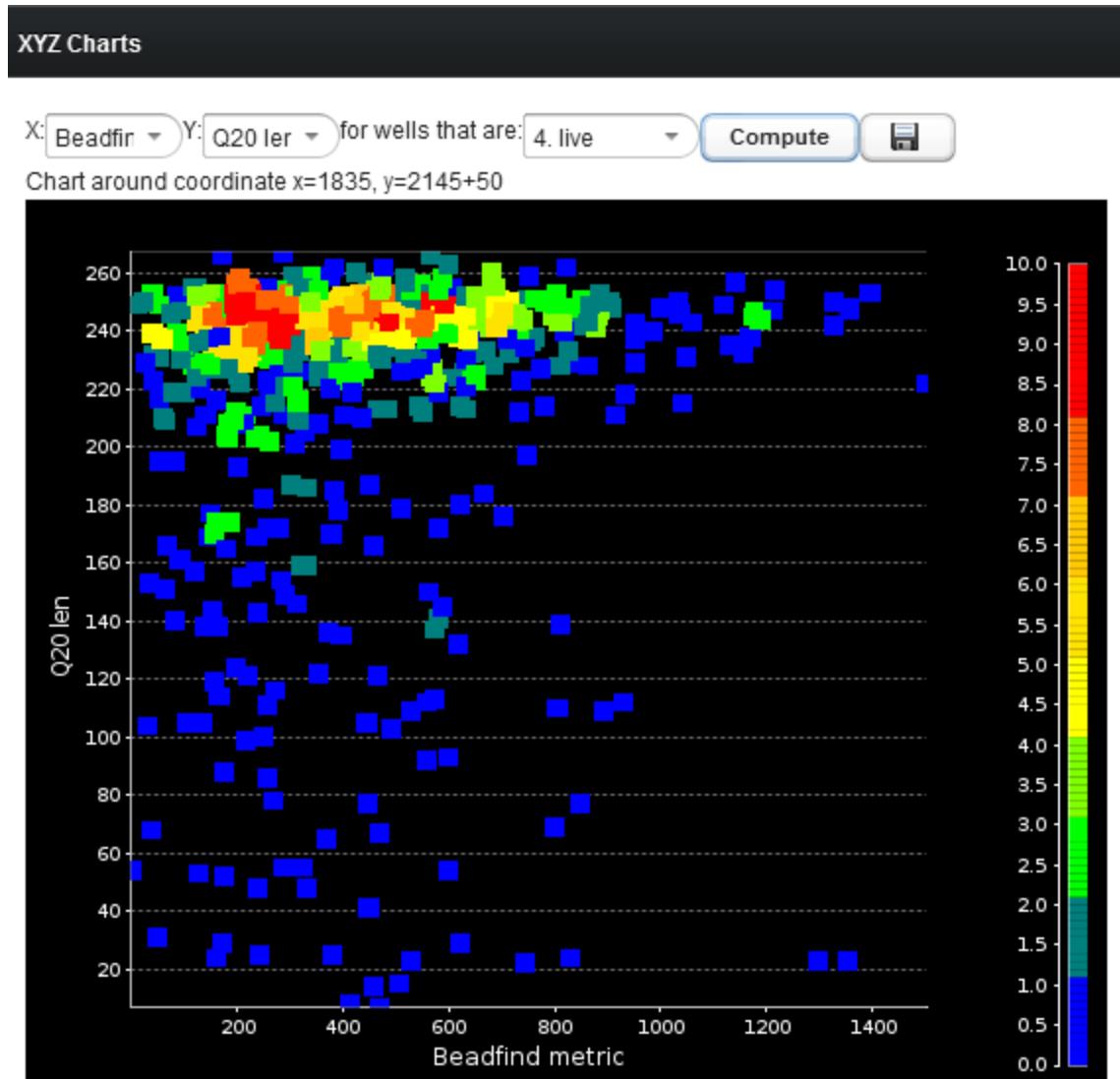


After the map has been computed, it can be used in the XYZ charts.

Create masks and find reads from read properties

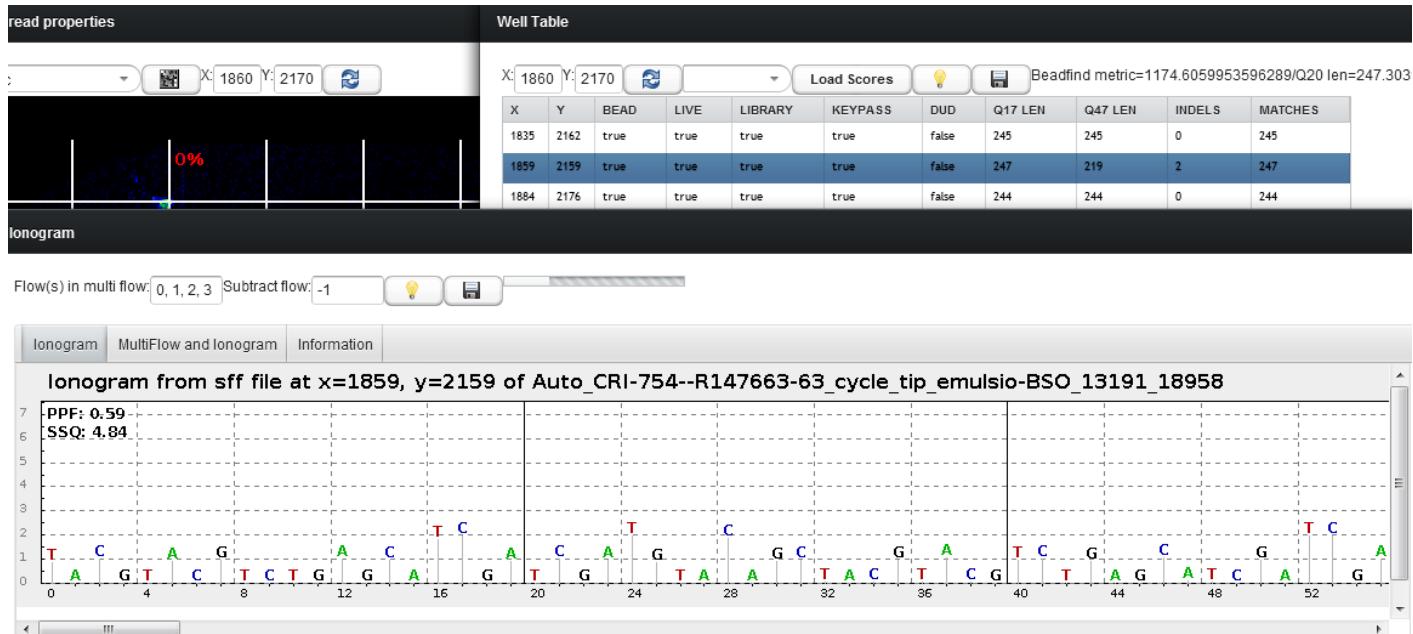


This chart below shows the relationship between beadfind metric and Q20 length (which is extracted from the .BAM file).



Picking data points

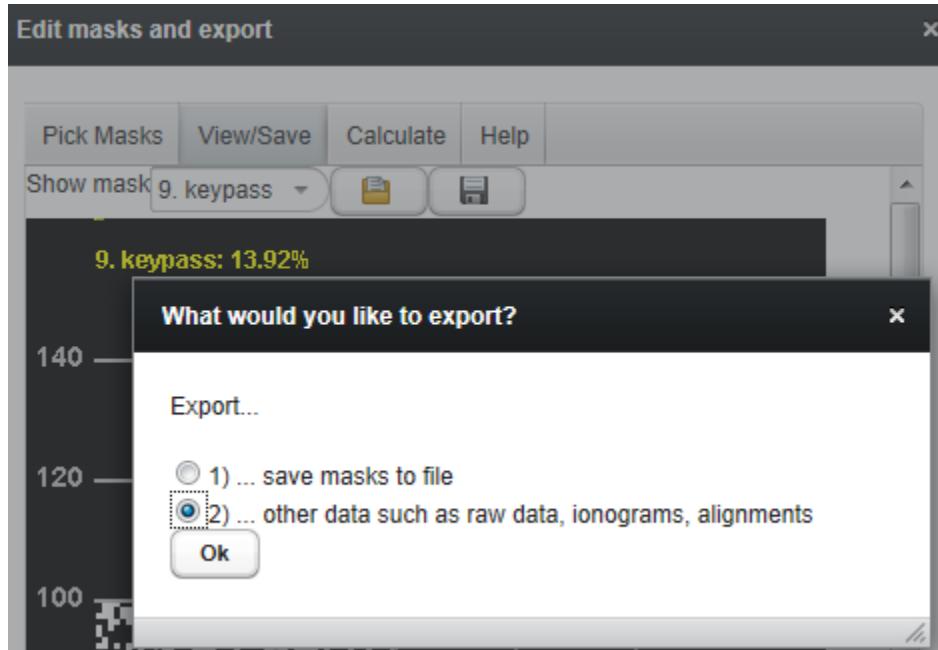
You can **click into the chart**, which will select all wells/read that have this particular set of properties. It will list those reads in the table – from which you can do anything you want, look at ionograms, alignments, look at raw signal etc (in this example I clicked on the top right green spots):

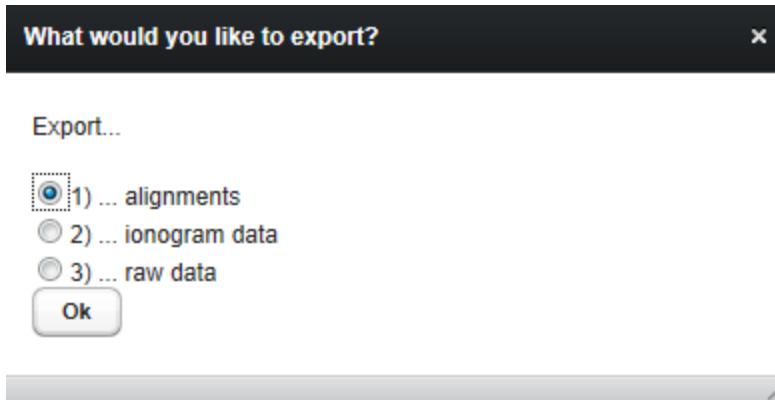


Exporting Data

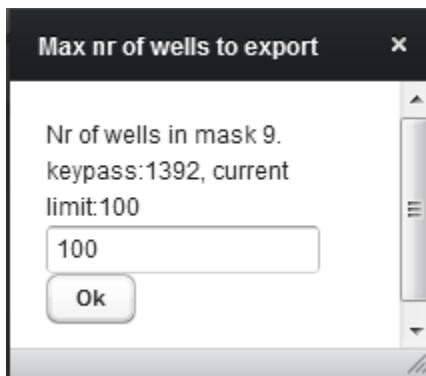
In both the Process and Mask Edit window you can export raw data, alignments and ionogram. Simply pick the save icon:

You can save the masks or other data:

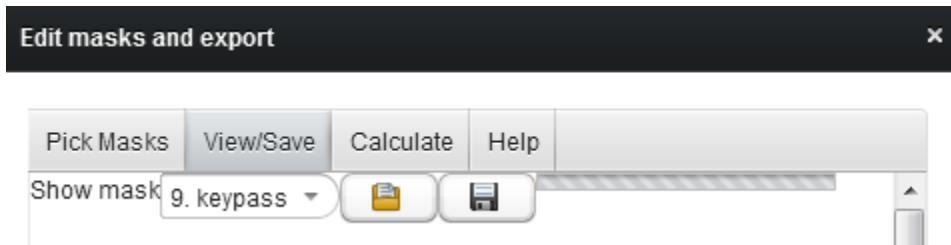




You can limit the nr of data points to export not only by mask, but you can also give it an absolute limit



A progress bar indicates how far along the export is



You can pick if you want .csv or .html. If you chose to export raw data, you can pick if you prefer to export the raw data as it is, or NN subtracted.

Example for alignment in .html format:

Data of area starting at x=50, y=50, size 100 "Only using data for mask 9. keypass, with 1392=13.92% wells"

Alignment at 500, 510

Sff read 4MOP0:51:50:CAGCCGTAAGCCGCTTCCAGTGCTCTTGTGCTGATAAACCGGACAAATAAATTCTGGCTGCAAAACGCGGATCATCACGGCGAACCT

Flags: 16 (reverse: true)
Genome position: 489343-489418

```

Alignment in sequencing order:
          10      20      30      40      50      60
seq: CAGCCGTAAGCGCGTCTCCAGTGCTCTTGTCTCTGGCTGATAAACCGGACCAATAAAT
      ||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
ref: CAGCCGTAAGCGCGTCTCCAGTGCTCTTGTCTCTGGCTGATAAACCGGACCAATAAAT

          70
seq: T_CTCCTGGCTGCACAA
      | | | | | | | | | | | |
ref: TTCTCTGGCTGCACAA

Identity:                      75 = 98.7%
Gaps in seq:                  1 = 1.3%

```

Example for ionogram in .csv format:

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	
1	Data of array y=50	size 100																						
2	Only using data for mask 9, keypass, with 1392=13.92% wells																							
3																								
4	50	510	normalize	107	5	88	0	0	100	1	106	1	102	0	0	90	107	204	0	0	0	93	0	9
5	50	540	normalize	93	0	90	1	0	97	5	285	1	0	0	5	97	0	197	0	0	0	99	103	
6	50	570	normalize	142	0	129	0	5	131	0	278	46	0	0	0	203	0	70	79	142	82	47	4	14
7	50	620	normalize	104	1	110	0	0	98	0	117	0	0	0	0	109	0	103	104	0	0	100	3	
8	50	630	normalize	115	0	108	0	0	108	0	306	9	105	0	0	100	78	0	195	301	2	101	103	
9	50	640	normalize	108	0	103	0	3	91	4	122	5	0	0	0	105	1	0	0	235	0	5	101	
10	50	650	normalize	98	0	97	0	1	96	0	303	4	0	0	0	190	182	0	0	107	3	89	6	9
11	50	660	normalize	103	0	105	0	0	102	0	117	101	0	3	0	106	0	0	0	98	0	7	105	
12	50	690	normalize	109	0	93	0	0	82	0	114	167	36	10	33	136	0	174	0	385	226	188	0	
13	50	710	normalize	107	0	96	0	0	104	0	107	215	327	1	96	0	0	111	3	89	0	0	322	32

Example of raw data in .csv format:

A1		Data from s:\data\raw\																								
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	
1	Data from s:\data\raw\																									
2	Coordinates: 50/50 - 150/150																									
3	filetype: Acquisition																									
4	NN subtracted: false																									
5	Data of area starting at 0/0, size 100																									
6	Only using data for mask 9, keypass, with 1392=13.92% wells																									
7																										
8																										
9	flow	time (ms)	68	610	882	950	1017	1085	1153	1221	1289	1356	1424	1492	1560	1628	1696	1763	1831	1899	1967	2035	2103	2171		
10	0	column	row	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
11	0	0	4	8639	8641	8637	8638	8634	8639	8640	8632	8637	8641	8630	8640	8648	8661	8695	8723	8746	8767	8776	8793	880	881	
12	0	0	7	9096	9094	9091	9084	9088	9082	9085	9097	9092	9094	9091	9091	9101	9127	9158	9197	9227	9248	9253	9269	927	928	
13	0	0	12	9190	9187	9185	9187	9175	9180	9184	9179	9175	9181	9181	9188	9194	9207	9228	9254	9275	9303	9318	9338	935	936	
14	0	0	13	8831	8829	8823	8827	8820	8826	8832	8827	8821	8824	8831	8832	8842	8876	8908	8941	8954	8970	8981	8997	900	901	
15	0	0	14	8828	8824	8820	8821	8820	8823	8820	8831	8818	8817	8824	8829	8867	8881	8910	8919	8941	8956	8982	898	899	900	
16	0	0	15	9142	9142	9139	9142	9141	9135	9142	9143	9149	9143	9137	9154	9185	9214	9249	9257	9271	9290	9302	931	932	933	
17	0	0	16	7481	7481	7478	7474	7471	7478	7483	7481	7480	7482	7477	7493	7515	7551	7578	7601	7614	7624	7632	764	765	766	
18	0	0	19	8812	8813	8806	8814	8805	8813	8814	8807	8805	8798	8800	8809	8831	8846	8872	8892	8915	8937	8950	897	898	899	
19	0	0	21	8881	8866	8864	8868	8857	8858	8854	8856	8856	8864	8852	8863	8869	8891	8915	8931	8952	8972	8993	9000	902	903	
20	0	0	22	9089	9089	9090	9090	9082	9087	9098	9095	9093	9087	9085	9089	9101	9130	9175	9209	9232	9245	9257	9269	927	928	
21	0	0	24	8945	8942	8939	8937	8933	8933	8942	8937	8936	8941	8932	8935	8953	8963	8992	9022	9044	9074	9099	9121	913	914	
22	0	0	25	8169	8164	8160	8157	8154	8161	8163	8165	8164	8163	8160	8162	8171	8204	8249	8278	8303	8324	8345	835	836	837	
23	0	0	27	8613	8608	8603	8607	8606	8602	8600	8607	8603	8601	8598	8605	8616	8639	8678	8705	8730	8760	8772	878	879	880	

Alignment searches via Plugin

Searching for alignment patterns can take 10 or more minutes for large chips. A more convenient way to do this is to run the Torrent Scout plugin:

You can enter up to 5 alignment searches and specify the range of flows in which to search the alignment pattern.

Any X will be replaced with 4 different searches, with another base each time. So if you search for

TXTTX, it will search for TATTA, TCTTC, TGTC and TTTT.

A question mark ? means that any base is accepted. So a TT?T results in just one search, where ? can be any base.

If you were to search for TXTT?, it would run 4 searches TATT?, TCTT?, TGTT? and TTTT?

TorrentScout Alignment Search Parameters

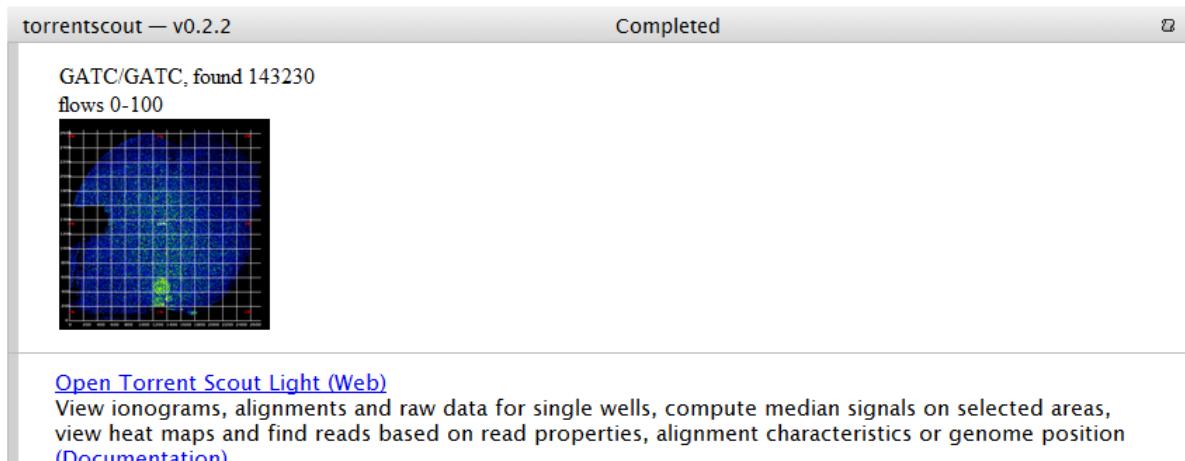
Search for alignment patterns for the following flows (end=0 means all):

Start flow: End flow:

Read sequence (eg TT_) (X = 4 searches with G/A/T/C) <input type="text" value="TT_"/> <input type="text" value="XX_"/> <input type="text"/> <input type="text"/> <input type="text"/>	Alignment sequence (eg TTT) (?) = any base or gap, _ =gap <input type="text" value="TTT"/> <input type="text" value="XXX"/> <input type="text"/> <input type="text"/> <input type="text"/>	Name of heat map (X will be replaced with G/A/T/C) <input type="text" value="missed_T"/> <input type="text" value="missed_X"/> <input type="text"/> <input type="text"/> <input type="text"/>
--	---	--

Viewing results in the report page

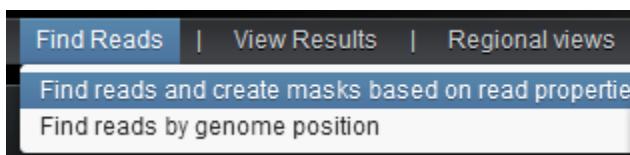
Once the alignment search is complete, it is displayed on the results page:



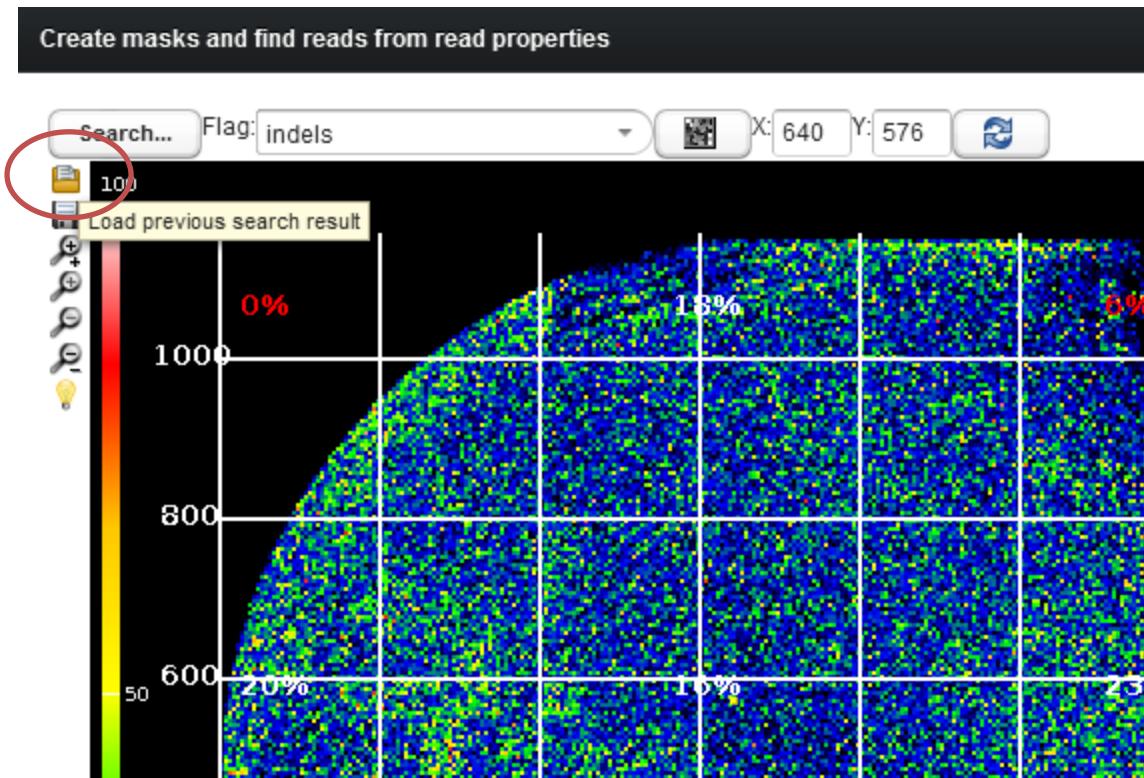
Viewing results interactively

But you can also interactively work with the results by opening it in Torrent Scout with the link from the plugin results page.

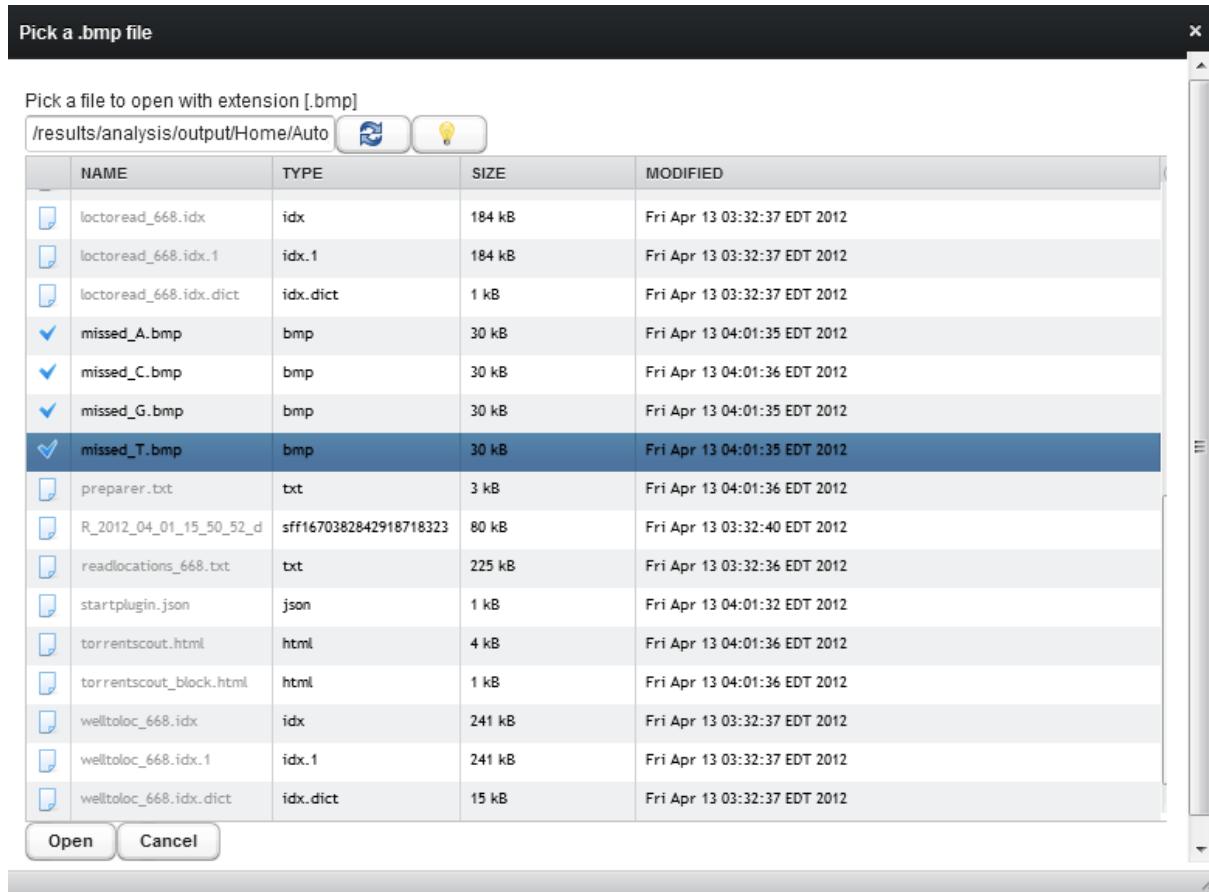
Once started, go to the Find/Find reads and create masks window:



and click the open button:



Your search results will have names as specified on the plugin page, such as missed_T:



Now you can work with this heat map as you would with any other.

For instance if you click anywhere on the heat map, it will show the wells with this flag set in the table (all of them if < 5000, or else in the area where you clicked), from which you can then view the ionograms, raw data, alignments etc.

Torrent Scout Light URL

The url for Torrent Scout is now simplified without the 8080 port:

<http://blackbird.itw/TSL>
<http://blackbird.ite/TSL>
<http://blackbird.bev/TSL>
<http://10.33.106.11/TSL>
<http://rnd1.ite/TSL>
<http://ioneast.ite/TSL>
<http://ionwest.itw/TSL>
<http://rnd3.itw/TSL>
<http://rndbev.apg.per.na.ab.applera.net/TSL>
<http://aruba.apg.per.na.ab.applera.net/TSL>

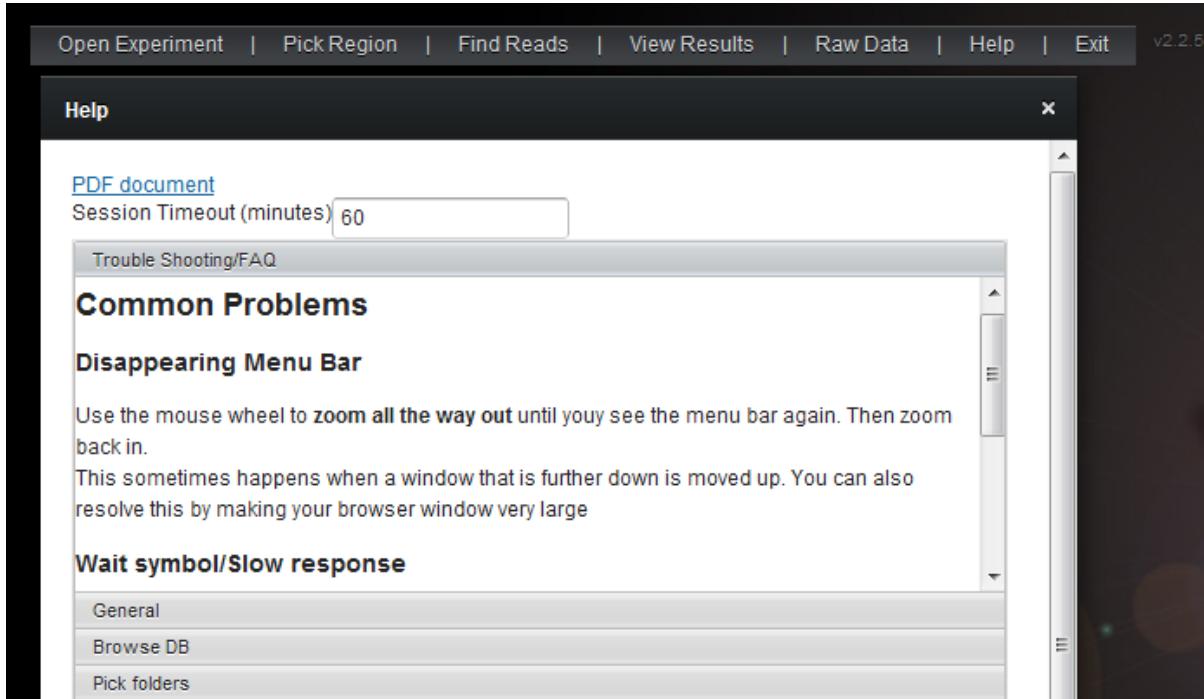
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Please go to the [LifeTech Community](#) or [GitHub](#) page for more documentation and source code, or send an email to [Chantal Roth](#) for help or feedback.

Help

The help menu item shows a short description for each component (same as the light bulb icon in each component).

The help pages also contains info about common problems (Please let me know so I can add them!), and you can also extend your session time (current limit is 60 minutes):



Glossary

Beadfind Mask Flags

empty 1<<0 Indicates if the well does not contain a bead

bead 1<<1 Indicates if the well contains a bead

live 1<<2 Indicates if the well contains a bead with live template

dud 1<<3 Indicates if the well contains a bead with no live template

ambiguous 1<<4 Indicates if the well contains a bead with live template that may be either library or test fragment

testFrag 1<<5 Indicates if the well contains a bead with live test fragment template

library 1<<6 Indicates if the well contains a bead with live library template

pinned 1<<7 Indicates if the well went out of the system dynamic range at some point during the run

ignore 1<<8 Indicates if the well should be ignored in various calculations such as background signal derivation from empty wells

washout 1<<9 Indicates if the well contained a bead that washed out at some point during the run

exclude 1<<10 Indicates if the well should be excluded from all analysis as it is not fluidically addressable

keypass1 1<<11 Indicates if the well generated a valid nucleotide sequence in the key flows

filteredBadKey 1<<12 Indicates if the well was filtered out due to generating an invalid nucleotide sequence in the key

filteredShort 1<<13 Indicates if the well was filtered out due to generating too short a nucleotide sequence

filteredBadPPF 1<<14 Indicates if the well was filtered out due to too many incorporating flows

filteredBadResidual 1<<15 Indicates if the well was filtered out due to the signal values fitting poorly

Troubleshooting and FAQ

I can't move the scroll bar

To move a scroll bar of any windows, first activate it by clicking on the title bar

I see a wait symbol and the GUI doesn't seem to respond

If your server is far away (network wise) then it might take a while to process clicks from the client. It is better to wait until the server response is back, otherwise it might make it worse :-). It could also indicate an error (is there a red exclamation point? If so move your mouse over it and you will see a stack trace)

I see a red exclamation point

This indicates an error. If you move your mouse over it, you will see the exception. If it is a memory error, see below on how to fix it. Otherwise, if you don't know what the issue is or it seems like a bug, let me know ☺.

The application stopped working, and there is an error message saying "OutOfHeapSpace"

Since the application runs on the server, it can only handle 1-2 users at the same time as all the data is loaded in the **server** memory. You could increase the server memory (if your server has enough) by changing the setting in **/etc/default/tomcat6** and restarting tomcat. It is the line with:

JAVA_OPTS="-Djava.awt.headless=true -Xms2048m -Xmx6096m -XX:MaxPermSize=512M"

How can I check the log file for errors?

`sudo tail -f /var/logs/tomcat6/catalina.out`

How do I restart tomcat?

`sudo /etc/init.d/tomcat6 restart`

How do I deploy the app?

Copy the TSL.war file to `/var/lib/tomcat6/webapps`
(you might have to restart with `sudo /etc/init.d/tomcat6 restart`)

How can I use the Tomcat web admin interface?

Edit the file `/etc/tomcat6/tomcat-users.xml`:

```
<tomcat-users>
  <user username="tomcat" password="tomcat" roles="admin, manager, manager-
gui"/>
</tomcat-users>
```

How do I access the Tomcat web admin interface?

<http://yourserver.com:8080/manager/html> (enter username/pw as specified in the file `tomcat-
users.xml`)