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SPARC - Analysis of multiplexed bead data using MPLEX software

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ABSTRACT

This protocol describes the process of achieving analysis of multiplexed bead data that was collected by flow cytometry and analyzed using the MPLEX software.

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PROTOCOL CITATION

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Analysis, software, gating conditions, hormone assay, beads, fluorescence

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Aug 30, 2022

PROTOCOL INTEGER ID

31081

PARENT PROTOCOLS

In steps of

SPARC - Attune NxT Set-up for Milli-metabolic bead assay Acquisition

GUIDELINES

These assays are frequently run as "half-384 well plates". Why? Good question. The fact is all the cytokine and hormone kits are available as 96 well plate options. They are very expensive generally and this makes the per-sample cost very high. We prefer to run 384 well plates for a good reason. Most of the sample volumes we have are very small, and we cannot get enough plasma to run 96 well plates. So we re-designed the assays to operate on 384 well plates - BUT, it takes a few weeks to generate sufficient samples, so the compromise is to run a half-384 well plate. It should not be lost however, that you effectively get 3.5 x 96 well plates now from one 96 well kit!! So you are reducing your operating costs, and reducing the volume of sample to about 8 microliters from about 30 microliters.

1 Transfer all the data frills from the flow cytometer to the appropriate computer directory

2 This directory must be available to the MPLEX software. The MPLEX software requires a license file to be placed in the same directory as the EXE file before the software will operate. The software is only available to academic and non-profit institutions.

3 Data files must be in FCS format (see FCS data standard here). It is critical that the instrument that collected the files name them properly. So for example, each well of a plate, must have somewhere within the filename a location address, e.g. A1, B2, F12, etc. It can be in any position of the filename, the sofware will find each file and associate it with the correct well.

4 All files must be properly identified by the row and column ID of the 96 or 384 well plate with at minimum a format which has in the file name A1, A2, etc.

5 All the FCS files must be in the same directory. MPLEX ONLY loads directories (although it is possible to load individual files and place them into any desired well if you want to)

6 Run MPLX (see image (blankMPLEX.gif below)

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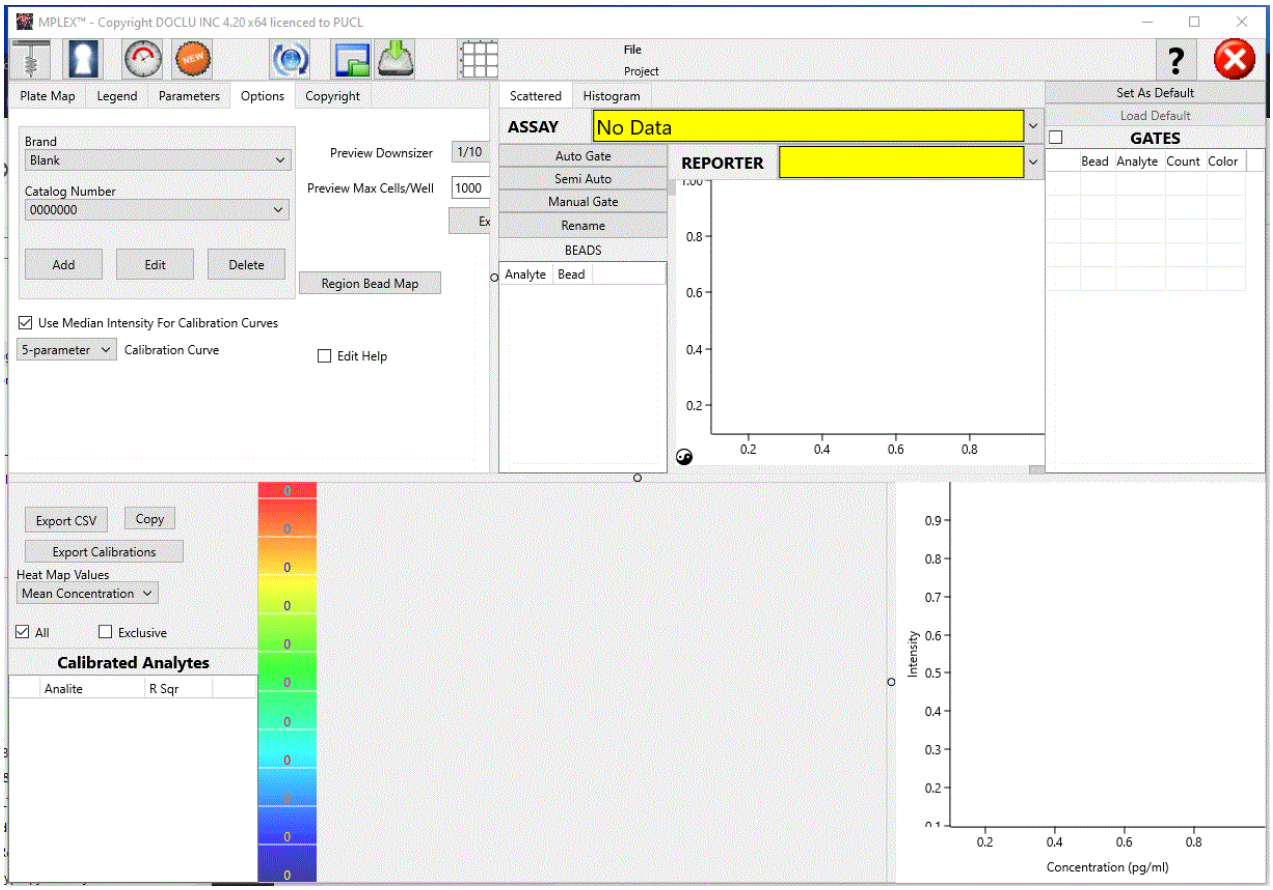

7 Select the LOAD ICON (top left corner) and select a directory to load all the data files: (loadICON,gif) This icon will read the windows directory and offer you the option of selecting any directory

8 The screen should look as the following: (NEW datsetMPLEX.gif)

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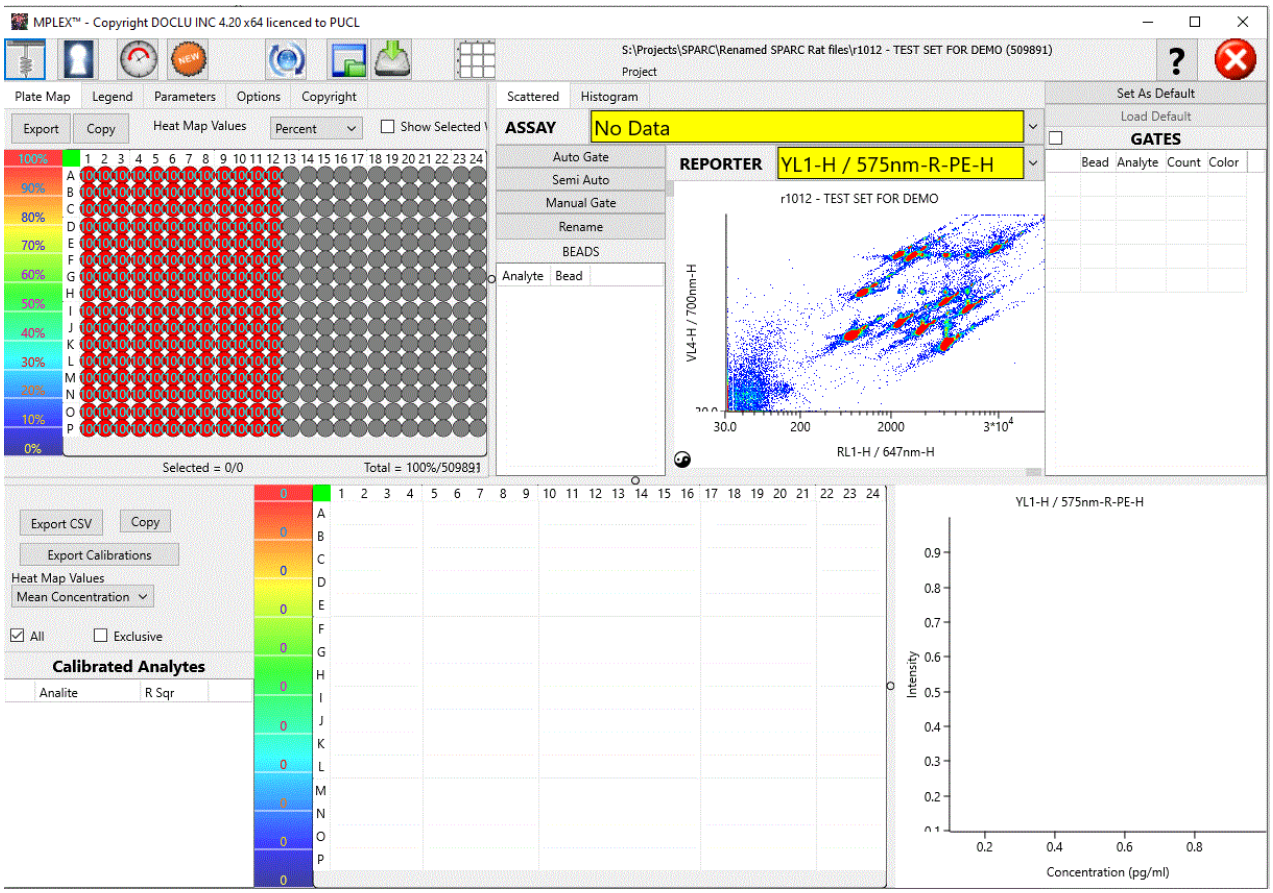

This example shows a half 384 well plate which is a very economical method for running these bead assays

9 Select a protocol from the OPtions Menu (optionsMPLEX.giif)

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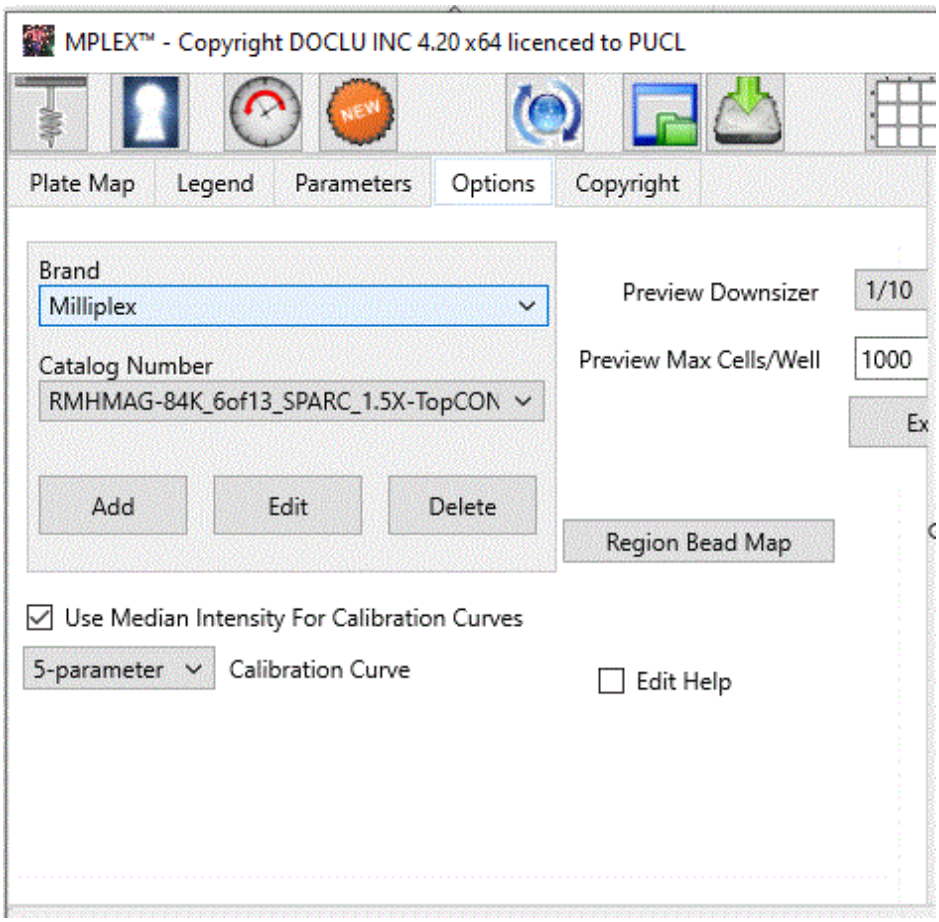

10 The next step is to assign GATES to the light scatter. The scatter without gates looks like this: (scatterMPLEX.gif) It should be noted that there are almost always "satelite" beads that are doublets and sometimes form small populations that are not the key populations and these should be ignored. The best technique for determining the correct location of any bead population is to simply take a single bead and run a few hundred on the cytometer and identify its proper location. This can be done directly from the original bead populations and does not require you to perform any assay or incubations. You are only checking the bead locations.

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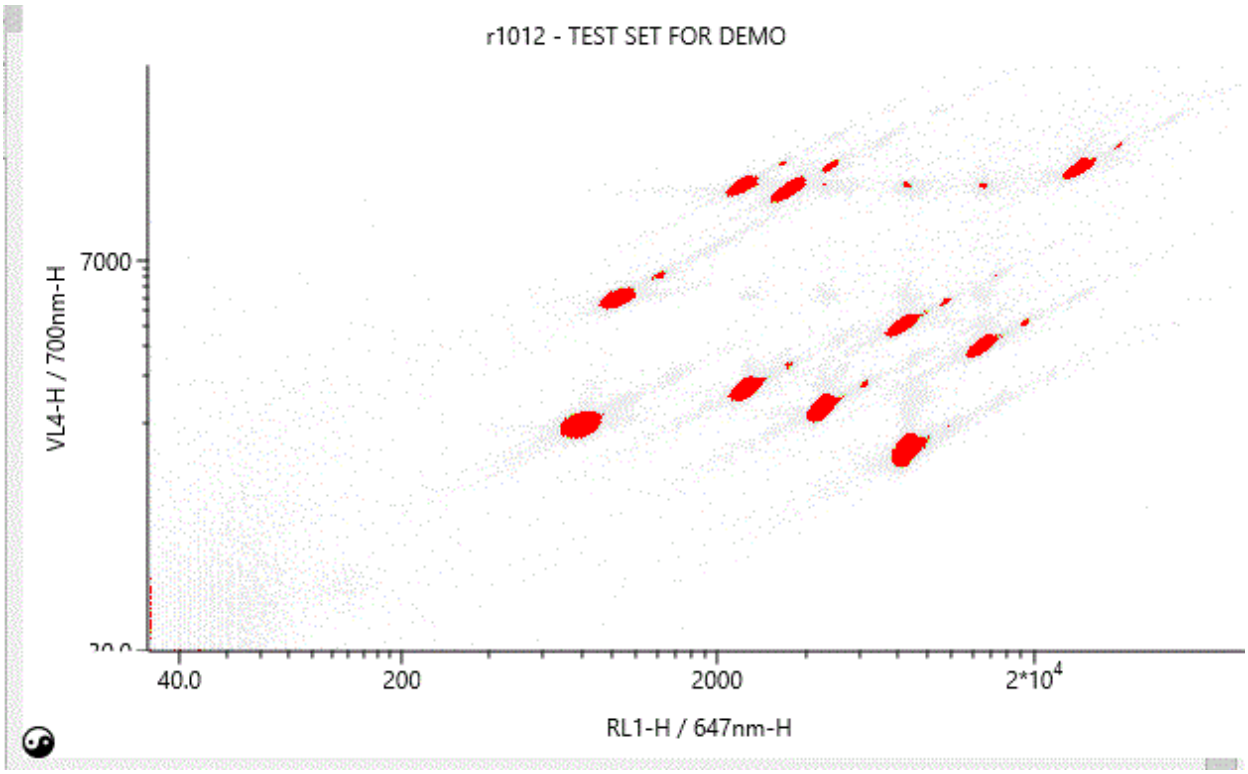

11 The goal is to identify the bead populations and assign them. Users will know the bead number for each hormone as this will be designated by the manufacturer and the pattern of display is closely related to the following map: (beadmapMPLEX.gif) Each bead should approximately fall within this map. However, when you run these beads on a standard flow cytometer (and not the Luminex instrument) there will be some variation of the location as it is not necessary to perfectly align them into the below map.

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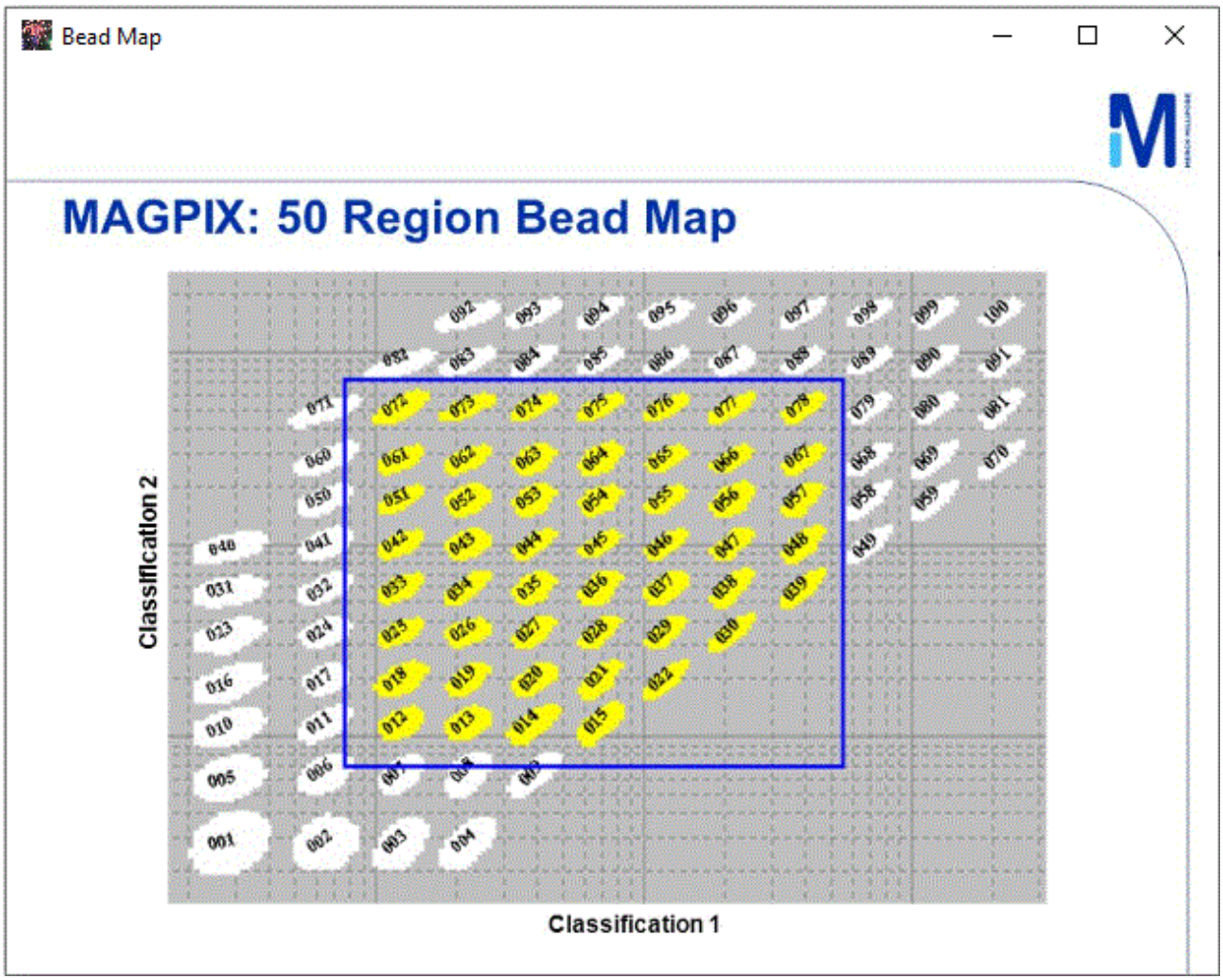

12 To create the gate regions, it is best to use the SEMIAutomatic Function (although the automatic can also be used). When using semiauto, the following screen is observed: (semiautoMPLEX.gif)
When the black screen appears, move the scroll bard on the right side up or down to show the exact number of bead populations you have selected – in the current assay there are 10 beads in the set so there should be 10 populations clearly identified. Once you have 10 populations, select the “CREATE GATES” button on the top left.

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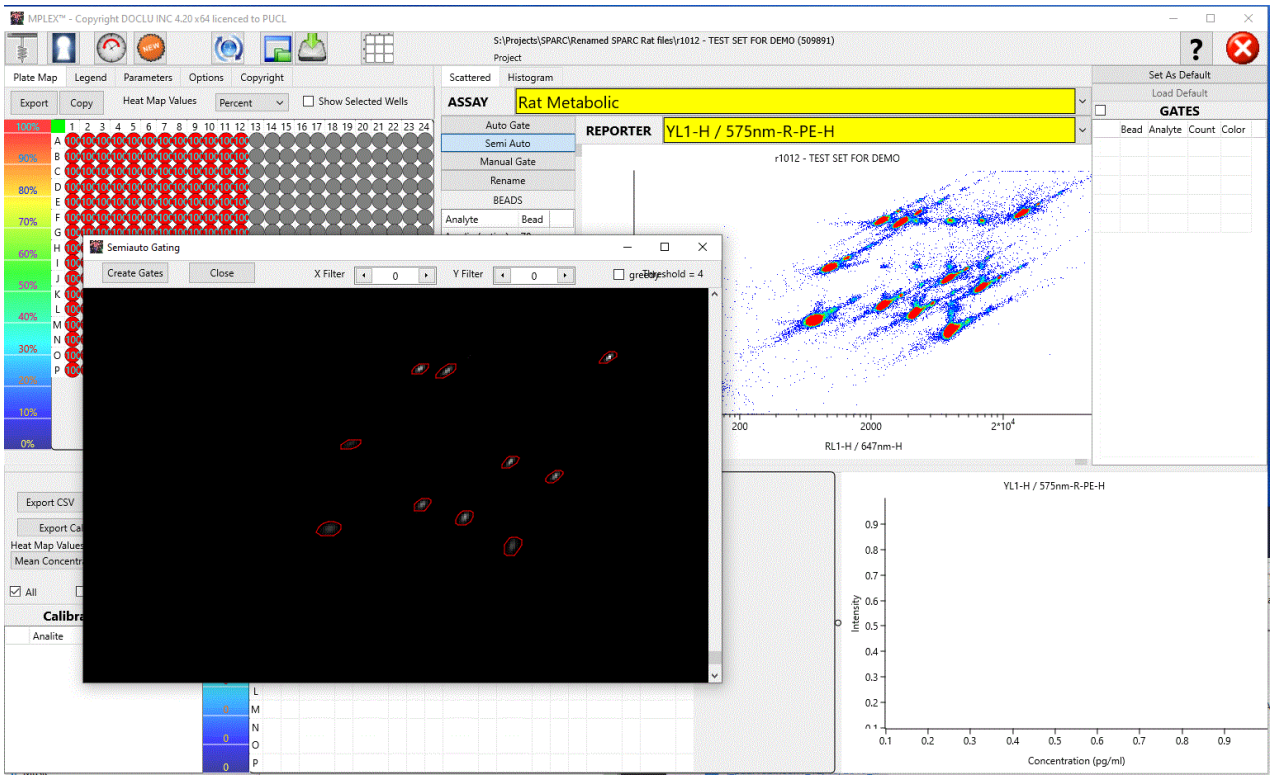

13 The following scatter pattern may appear where the hormonebead gates are applied. However, it is critical now to review the bead locations and match them to the known locations from the above bead map. bead populations. NOTE THAT THE BEAD GATES DO NOT MATCH THE MAP CORRECTLY AND 63, 63, AND 33 ARE IN THE WRONG POSITION. It is easy to re-assign them (incorrectbeadgatesMPLEX.gif)

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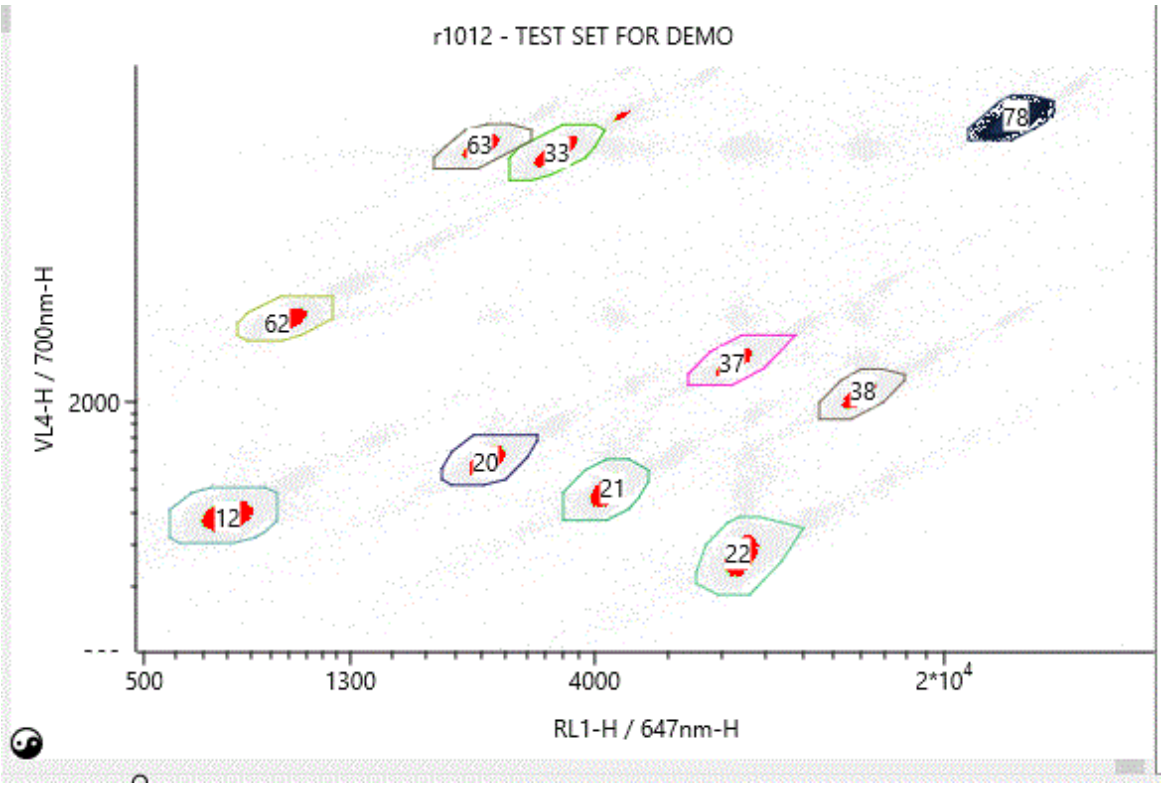

14 To reassign bead populations simply grab the correct number from the bead list and drag it to the correct population as in the following figure.

The process is very simple, just grab the number of the list if hormones and drag that number to the correct population as shown here.In this case, bead 33, 62 and 63 were not correctly placed by the semiauto algorithm.
(correctbeadMPLEX.gif). To move them, you move the mouse to the proper bead number listed in the assay list on the left, and "drag" that number to the correct gate. You can see this below where bead#33 was dragged to the top left bead location and beads #62 and #63 were properly placed as per the map shown in STEP 11.

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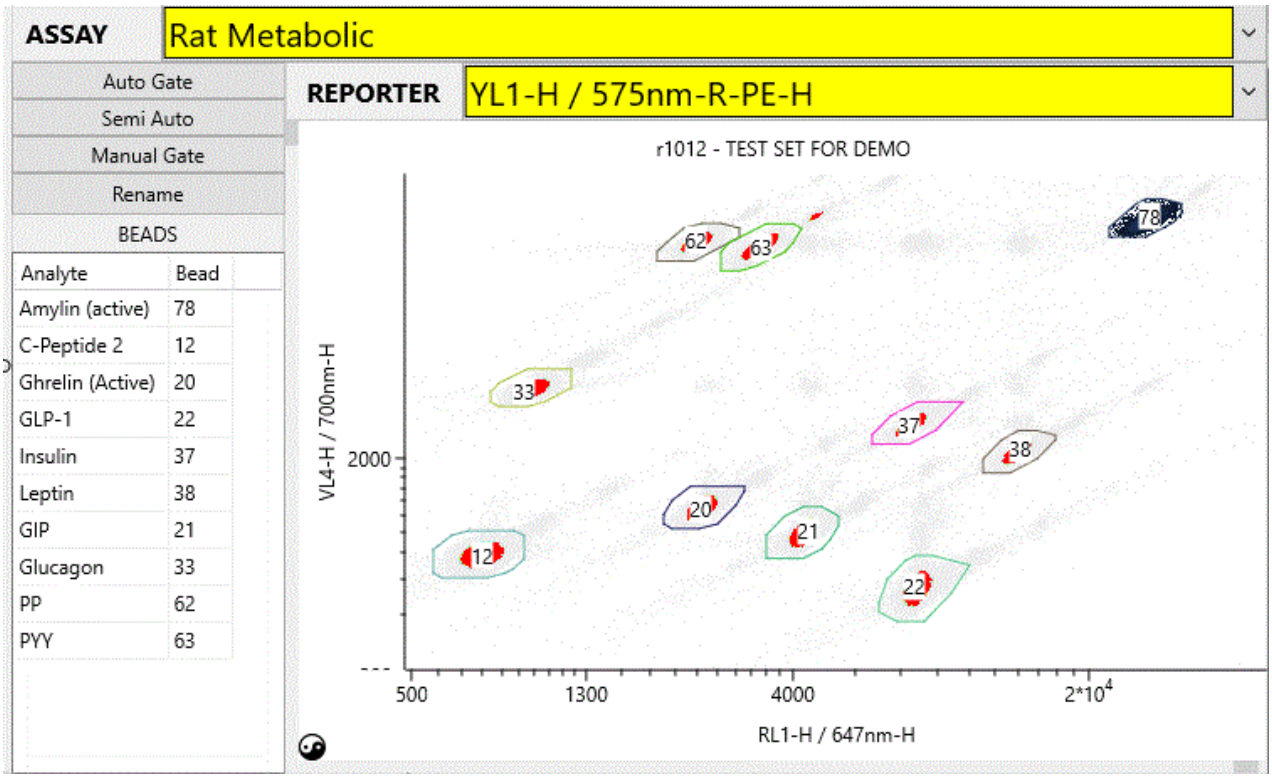
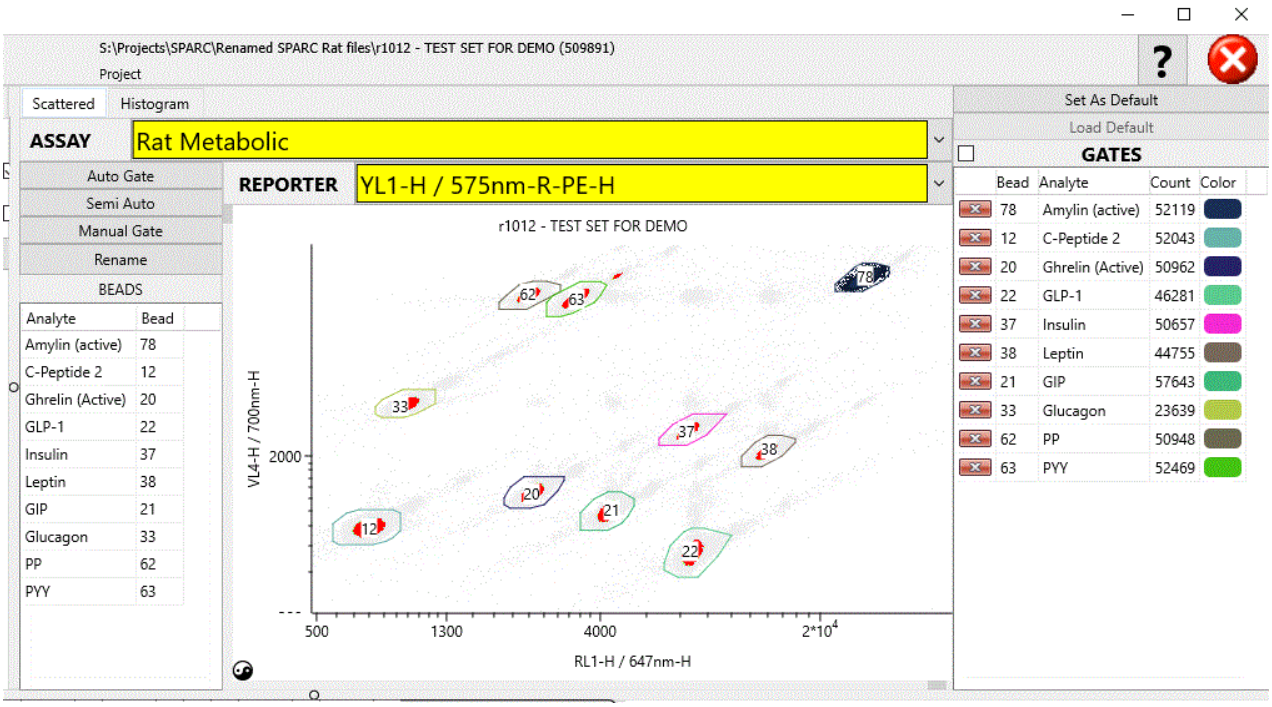


15 Once the correct populations are identified. It is critical to select “Set As Default” on the top right to lock in the assay design, bead assignments to the data set. This sets the analysis for the data set and appropriate default files are now saved in the INFO directory associated with the data directory. (setdefaultMPLEX.gif)



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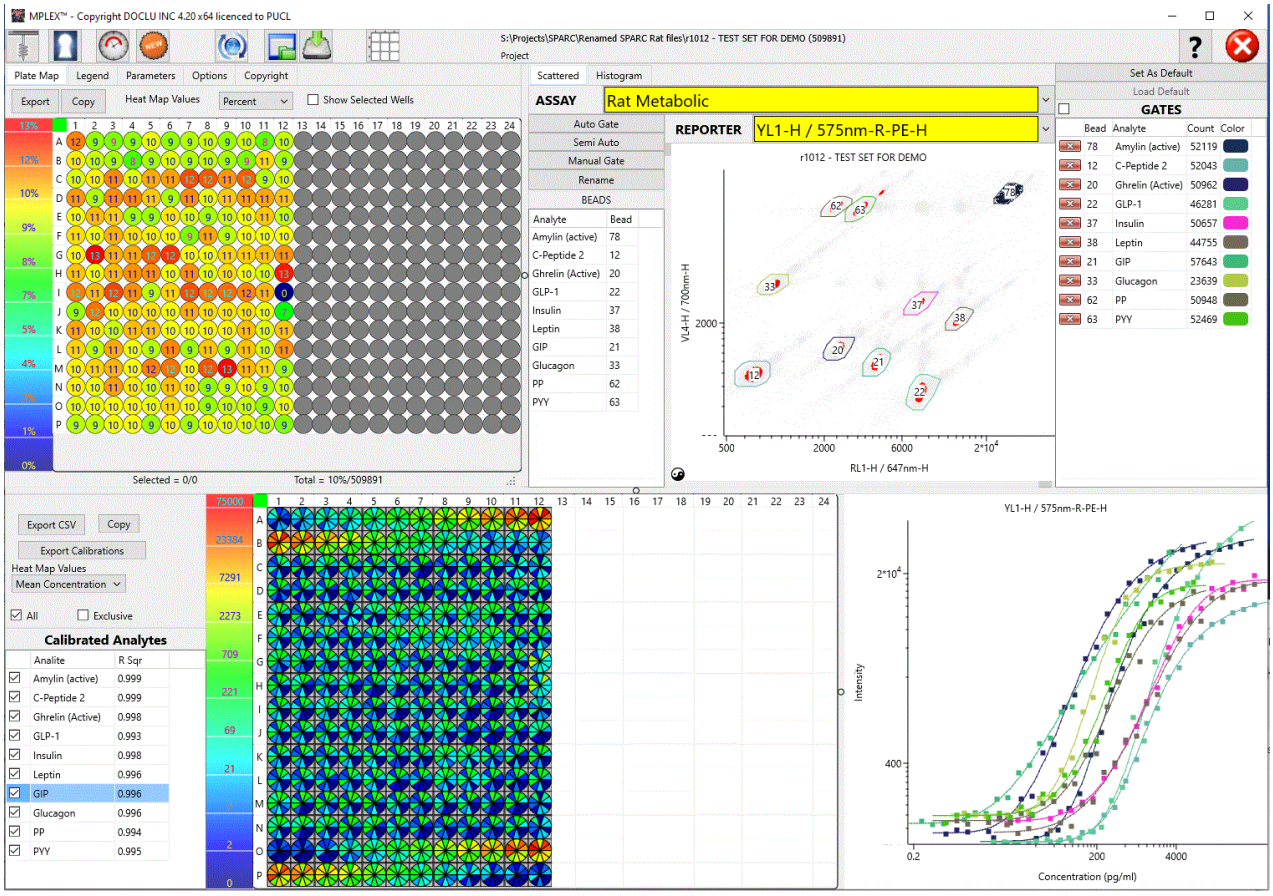
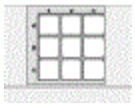
16 The final analysis screen should now look something like this: (finalanalysisMPLEX.gif) All the bead populations should properly match the bead map data from the manufacturer, the assay design should be correct and the standard curves should appear correctly. (finalanalysisMPLEX.gif)



Individual bead (analyte) concentrations can be plotted in the lower left window by selecting "concentration" in the dropdown. Alternatively bead intensity can be also selected.

17 The next step is output of data sets. This requires a plate map and the PLATE MAP protocol should be run to create a PLATE MAP (platemapICON.gif)



18 Once a plate map is prepared, select the plate map ICON and this will open up the plate map that designates sample identities for any particular plate as shown below. In this case, the plate is an half-384 well plate and the plate name is shown at the top and this should mirror the data

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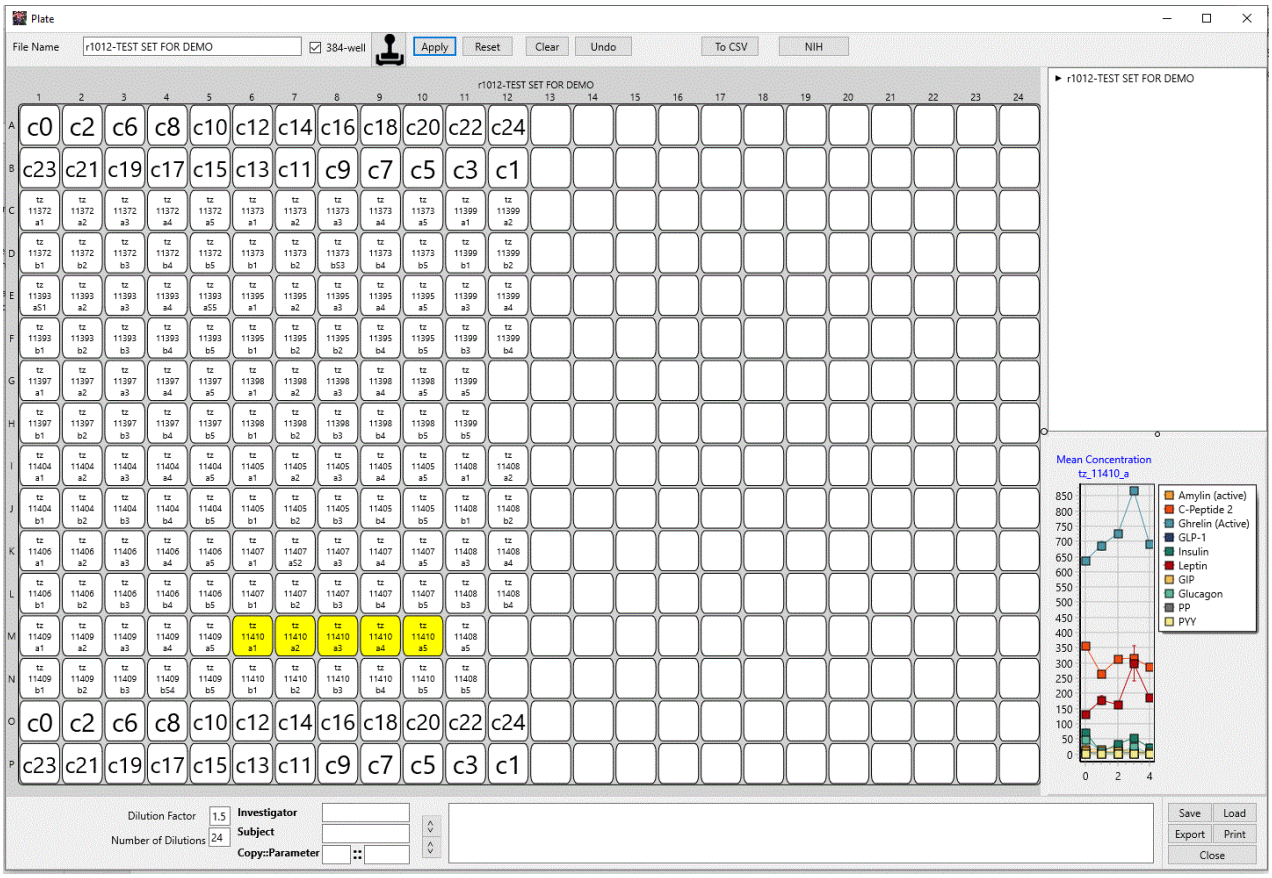
set directory name (in this case “r1012-TEST SET FOR DEMO" (platemapMOLEX.gif)



A video is available to show how to rapidly fill in this plate map. The map above shows a "half" 384-well plate as this is the most economical method of performing these assays in both cost and time. In particular, the sample volume can be reduced to 8 microliters per replicate.

19 Data output can be achieved using one of two methods depending on the output use. One is for NIH SPARC program and this is designated as “NIH” button and the other is “To CSV” (NIH_CSVoutputMPLEX.gif)

20 If the NIH button is selected severaloutput directories are created at the user direction and these make NOH required directories and file structures including “derivative”, “primary” and “source”. These contain all the required data formats, data and metadata for the NIH SPARC program. The INFO directory is for internal MPLEX use and creates a set of files that identifies the internal default analysis for the original data set. (NIHdefailtMPLEX.gif)

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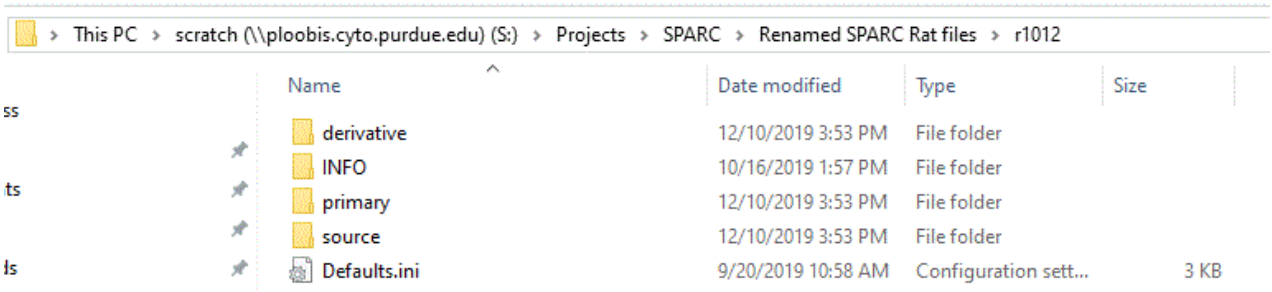

21 For additional information on the data processing options of MPLEX this link has a variety of video tutorials:

http://www.cyto.purdue.edu/mplex

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