

# MIRACLE Tutorial

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A thorough introduction to the features of “Microarray R-based Analysis of Complex Lysate Experiments”

*Note: All screenshots were created by MLP and IB. The MIRACLE logo was created by ML.*

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## 1. Getting Started

### 1.1. Login

Go to <http://www.nanocan.org/miracle/demo> (31/03/2014) and login with username “demo” and the password “demo0815”.



Please Login


Username:

Password:

☐ Remember me

Login

Welcome to MIRACLE!




You are logged in as  
**demo**  
Logout

Organize ▾ Plate Layout ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter ▾ Data Analysis

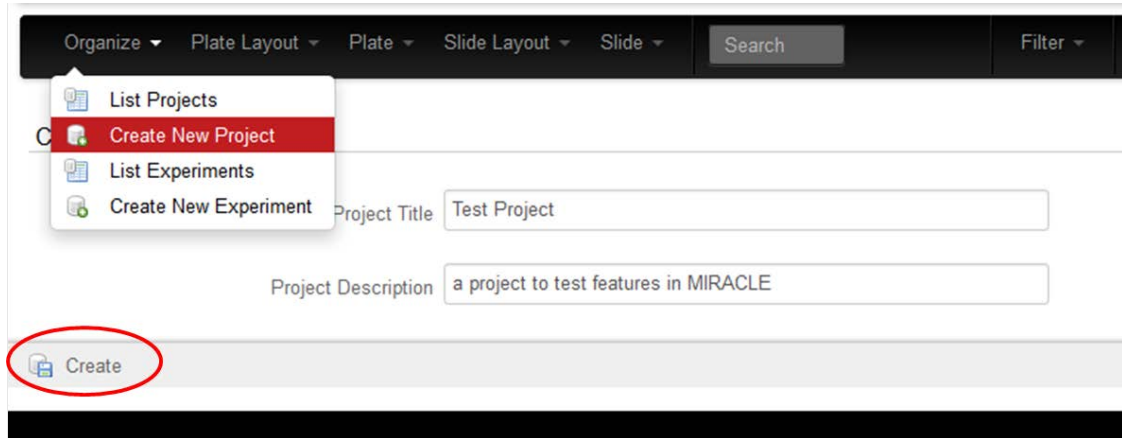
**APPLICATION STATUS**  
App version: 0.8  
Grails version: 2.2.5  
JVM version: 1.7.0\_51  
Reloading active: false  
  
Slides in the database: 3  
Spots in the database: 38880

**Welcome to MIRACLE**  
MIRACLE is a web-tool for **M**icroarray **R**-based **A**nalysis of **C**omplex **L**ysate **E**xperiments. It allows you to upload microarray files produced by a scanner (in either XLS, CSV or TXT format) and to add the experimental information, e.g. the spot signal, to a SQL database. In order to organize your results, you can assign layout information for each spot and perform further analysis by using inbuilt R functionality for plotting, normalization and quantification of the results. If you wish to perform further analysis you can export the combined layout / signal information as CSV file or directly to R, where a number of R functions help the user in performing a manual analysis of the data.



## 1.2. Organizing the Work

Start by organizing future results and data by creating projects with corresponding experiments. Use the drop-down menu, Organize > Create New Project, click “Create” (red circle) in the bottom left corner to save your changes.



Organize ▾ Plate Layout ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter ▾

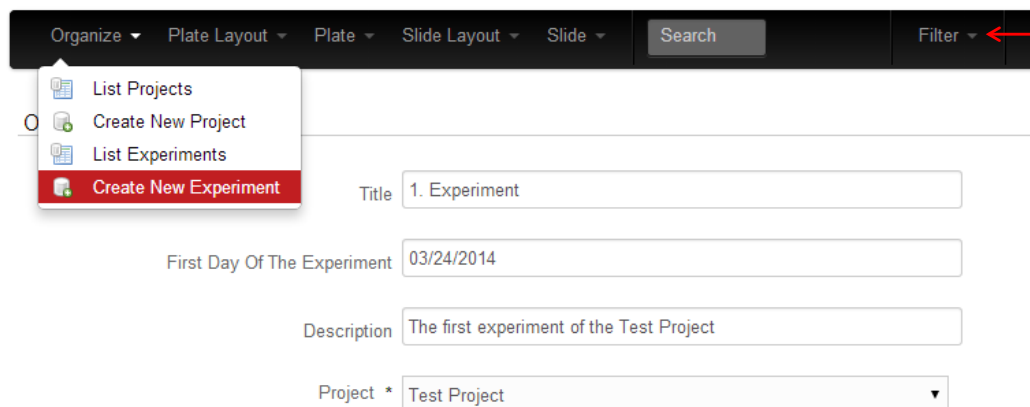
Organize ▾  
List Projects  
Create New Project  
List Experiments  
Create New Experiment

Project Title Test Project

Project Description a project to test features in MIRACLE

Create

Within each project, several experiments can be listed. Use the drop-down menu Organize > Create New Experiment, fill in experimental information and select a project subfolder from the “Project” drop-down menu; click “Create” in the bottom left corner to save your changes as shown above.



Organize ▾ Plate Layout ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter ▾

Organize ▾  
List Projects  
Create New Project  
List Experiments  
Create New Experiment

Title 1. Experiment

First Day Of The Experiment 03/24/2014

Description The first experiment of the Test Project

Project \* Test Project ▾

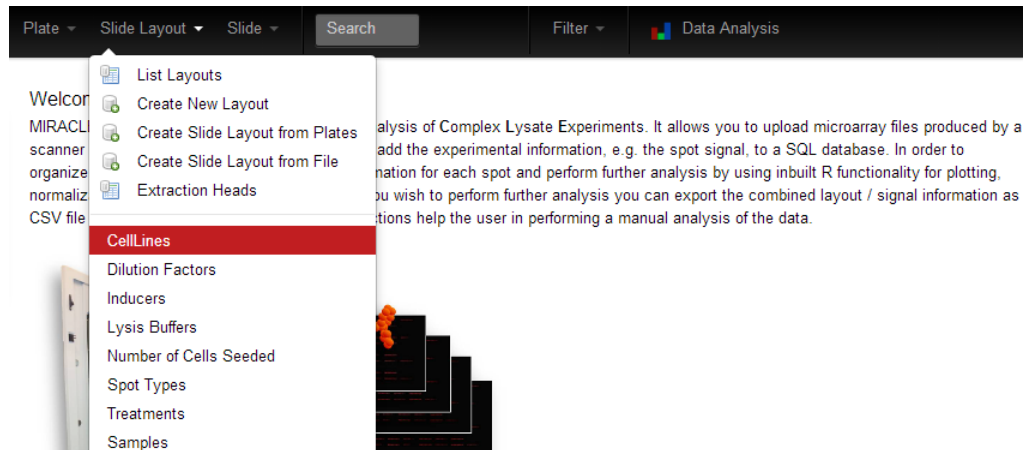
Filter ▾

Creation of projects and experiments will be important later for sorting and comparison of data. The “Filter” (red arrow) allows the user to display only those projects or experiments important for a particular analysis.

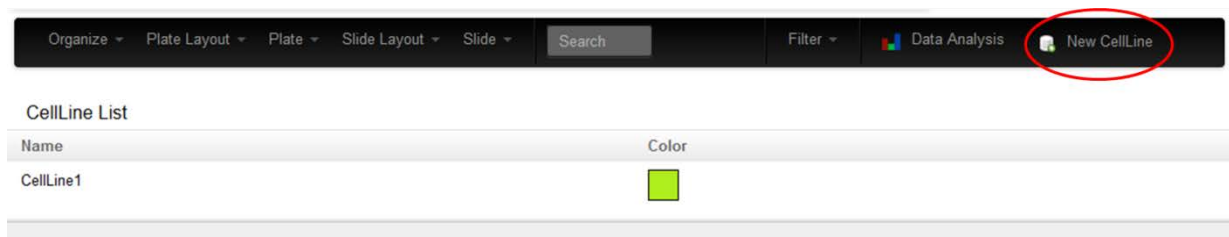
## 2. Plate and Slide Layouts

### 2.1. Creating Experimental Properties

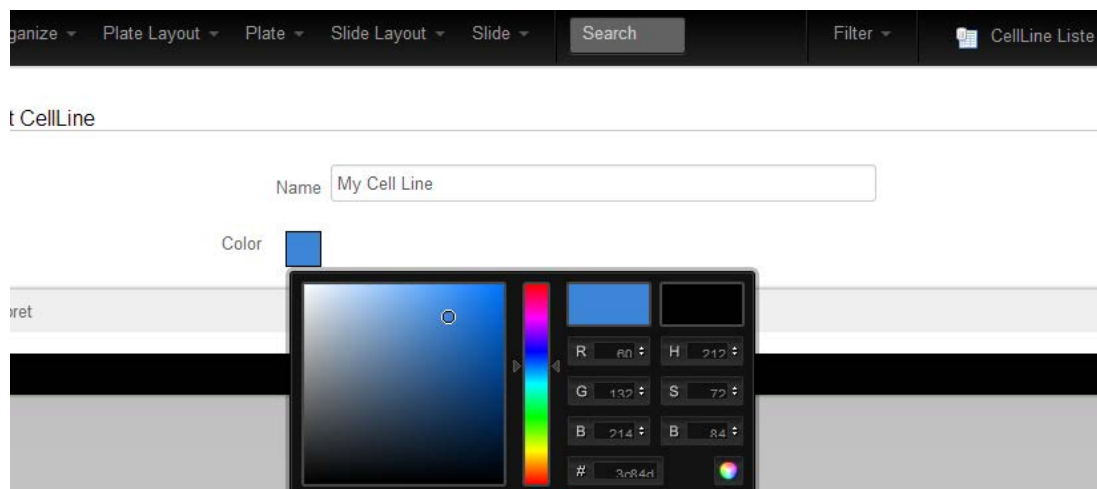
Before entering actual experimental plates, please enter properties such as cell lines, dilution factors, and inducers etc., used in your experiments, which may vary from sample to sample.






As a first example, select Slide Layout > CellLines. All annotated cell lines used in prior experiments will be displayed. Select “New Cell line” (red circle) in the top right corner to annotate further cell lines.



Give the cell line a name and select a colour for displaying the property. Click “Create” in the bottom left corner.



As a second example select Slide Layout > Samples. Select “New Sample” (red circle) to annotate samples.

New Sample Sample List Controls			
Sample List			
Name	Type	Target	Color
A_NC1	compound		
A1	compound		
A2	compound		

Specify the sample name, the type of sample and if the sample has a known target. It is also possible to select if the sample is a control and whether it is a positive or negative control. Click “Create” in the bottom left corner.

Sample

Name

Type

Target

Control

Control Type

Color

Prepare all the properties needed for your experiment; Cell lines, dilution factors, inducers, lysis buffer, number of cells seeded, spot types, treatments, samples.

## 2.2. Plate Layouts

Now the layouts of the experimental plates can be created. Select Plate Layout > Create New Layout. Enter the name of the plate, the format and which experiment the plate belongs to. Click “Create” in the bottom left corner.

Plate Layout Plate Slide Layout Slide Search Filter

List Layouts

Create New Layout

Name \*

Format \*

Experiments

Next, please use the drop-down property menu to select and assign the different properties of your plate, which were created in section 2.1 (red arrow). First, select the property (e.g. sample) in the legend on the left. Afterwards locate a sample that should have this property on the right, where wells of a plate are shown schematically. The colour code will reflect the loading of your plate. Make sure to save your work (red circle) every time a new property is entered to the layout. If needed, it is possible to create copies of plate layouts in case only few properties change from experiment to experiment, such as number of cells seeded or cell line. Give the copy a proper name and press “Create Copy of this layout” (Blue arrow). Now change the property, which is different between the original and the copy layout and save your changes (red circle) in the layout. *NB: It should be emphasized that you will now be working on the copy unless switched back to the original plate!*

You can list all created layouts by experimental plate(s) clicking Plate Layout > List Layouts.

Name	Format
Plate1	96-well
Plate2	96-well
Plate3	96-well
Plate4	96-well
Plate5	96-well

### 2.3. Plate Readouts

Next, the plate layouts must be linked to actual plates, possibly containing different primary readouts. First, create the type of plate that was used. Plate > Plate Types, select New Plate Type in the top right corner. Enter the well shape, name of the plate and optionally, the vendor.

Next select Plate > Create New Plate and enter details of the plate such as barcode, name, plate type, format etc. Tick the box to select whether this is a plate carrying samples or a so-called “Control Plate”. The plate must/should further be assigned to an already created experiment in section 1.2 (drop-down menu, red circle) and a plate layout, as created in section 2.2 (drop-down menu, blue circle).

The screenshot shows a software interface for creating a new plate. At the top, there is a navigation bar with tabs: 'Organize', 'Plate Layout', 'Plate', 'Slide Layout', and 'Slide'. A 'Search' button and a 'Filter' dropdown are also present. Below the navigation bar, a dropdown menu is open for the 'Plate' tab, showing options: 'List Plates', 'Create New Plate' (highlighted in red), and 'Plate Types'. The 'Create New Plate' form contains the following fields:

- Barcode: 1234
- Name: Date
- Plate Type: (empty dropdown)
- Format \*: 96-well
- Replicate \*: 1
- Experiment \*: 1. Experiment (highlighted with a red circle)
- Plate Layout: Plate1 (highlighted with a blue circle)

Afterwards, different readouts can be selected and assigned to this plate. Click the pane “Readouts” and the “Add readout”. Here it is possible to specify the data and type of readout along with which plate this readout belongs to. Furthermore, it is possible to upload a result file in .xlsx or tab separated .txt format along with an image and an experimental protocol. Click “Create” in the lower left corner.

Readout

Day Of Readout

06/25/2013

Type Of Readout

Fluorescence

Assay Type

Cell Titer Blue

Plate \*

Plate1111

Wavelength \*

560

Result File \*

Choose existing file:

...or upload new file:

Choose File C...

Result Image \*

Choose existing file:

...or upload new file:

Choose File N...

Experiment Protocol \*

Choose existing file:

...or upload new file:

Choose File N...



Next, the readout data should be linked to the information of the plate layout. Subsequently, you need to select the individual columns of your file corresponding to one of row, column, wellPosition or measuredValue. To specify the position of the well you need to select either row and column, or wellPosition (e.g. in the format of “A01” etc.). The measuredValue corresponds to the signal obtained during the readout. In addition, you may specify how many lines (if any) should be skipped from the readout document. Furthermore select the type of document (CSV or custom), the column separator and numerical separator(s). Press “Read the selected sheet” (red arrow). Match all columns needed to the columns of the result file using the drop-down menu. Next, press “Add readout data” (blue arrow).

**Import Readout Data**

*Select a sheet and configuration*

Select a suitable mapping of column names to properties (optional):

Skip lines (overrules config if checked): ☐


Minimum number of columns to read:

Select between CSV (comma-separated) and CSV2 (semicolon-separated):

Select a column separator:


Select a decimal separator:

Select a thousand separator:

Read selected sheet 

**Match columns and database properties:**

Plate	<input type="text" value="Do not use"/>
Repeat	<input type="text" value="Do not use"/>
Well	<input type="text" value="wellPosition"/>
Type	<input type="text" value="Do not use"/>
Time	<input type="text" value="Do not use"/>
CellTiter Blue 560/590 nm (Counts)	<input type="text" value="measuredValue"/>

Add readout data 

Subsequently, the readout data is displayed in a heatmap for visual inspection.

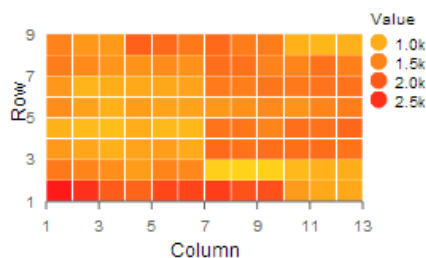
Type Of Readout    Fluorescence

Assay Type        Cell Titer Blue

Plate                Plate1111

Wavelength        560

Result File        CellLine1 Plate1 Readout.txt

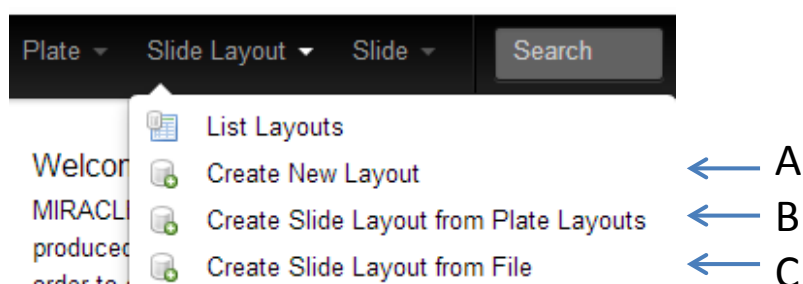


When finished, the plates can be listed via the Plate> List Plates function.

Organize ▾ Plate Layout ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter ▾ Data Analysis Ny Plate						
Plate Liste						
Barcode	Name	Type	Format	Replicate	Rows	
1111	Plate1	Round bottom Plate	96-well	0	8	
2222	Plate2	Round bottom Plate	96-well	0	8	
3333	Plate3	Round bottom Plate	96-well	0	8	
4444	Plate4	Round bottom Plate	96-well	0	8	
5555	Plate5	Round bottom Plate	96-well	0	8	
C1111	Ctrl1	384-well v-bottom	384-well	0	16	
C2222	Ctrl2	384-well v-bottom	384-well	0	16	

## 2.4. Slide Layouts

Now, a slide layout can be created. This can be achieved in several ways. Slide Layout > A, B, C.



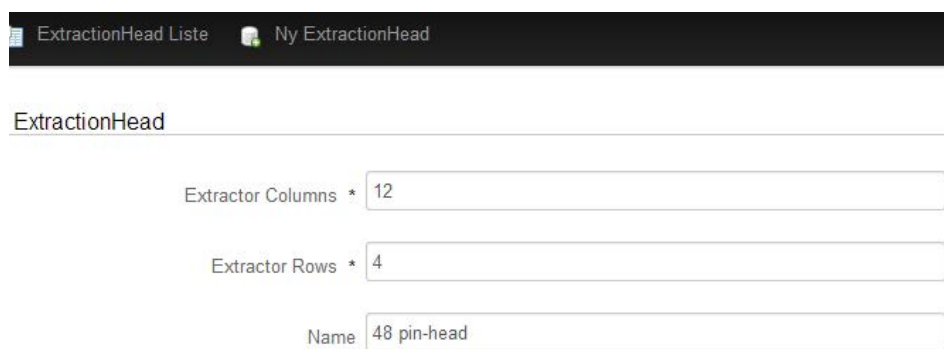
A – “Create New Layout”: No plate layouts are used. The user creates an empty layout meaning that all sample information must be assigned manually as illustrated previously for plate layouts.

B – “Create Slide Layout from Plate Layouts”: The user creates a slide layout through a “virtual spotting” by combining different source plates to a desired layout according to the applied spotting settings in an automated fashion.

C – “Create Slide Layout from File”: In this case the user creates the layout by uploading a file containing the sample information per spot position on a slide, for example, an excel file in MIRACLE compliant format (see Supplemental File 1).

In the following, the most complex application, namely the creation of a slide layout from a plate layout will be described.

Prior to creating a slide layout from a Plate Layout (B) it is important to specify the details of the spotter/array/printer extraction head used for the construction of the array. Select Slide Layout > Extraction Heads, Click “New ExtractionHead” in the top right corner. Give the extractor a name, and specify the rows and columns of the pins/pipets/nozzles of the print head. Press “Create” in the lower left corner. *NB: The program anticipates that neighboring pins/pipets/nozzles will transfer samples from neighboring wells onto the array!*



ExtractionHead

Extractor Columns \* 12

Extractor Rows \* 4

Name 48 pin-head

Next, please select Slide Layout > Create Slide Layout from Plate Layout. Here, plates must be selected for the virtual spotting in order to create the slide layout. Please, click the “Hints”-button if in doubt about how to proceed.

Plates are selected from “Available plate layouts” or from “Control plate layouts” and simply dragged to “Plates selected for spotting” in the desired order. *NB: Since the actual spotting process is mimicked, it is critical to use the right order in which the plates are processed in the respective spotter/arrayer/printer during array construction! In case a plate e.g. control plate is processed twice, insert the plate layout also twice in this application. If your plate(s) do not appear in the “Available plate layouts”, try to alter the filter setting to fit your project and experiment (red arrow) and then press “Refresh plate layouts” (blue arrow).* After selecting the plates, press continue.

Organize ▾
Plate Layout ▾
Plate ▾
Slide Layout ▾
Slide ▾
Search
Filter ▾

elect plate layouts for spotting

Hints

Select plates for new slide layout

Refresh plates
Continue

Plates selected for spotting	Available plate layouts	Control plate layouts
<div>Drop here</div> <div>PlateC1111</div> <div>Plate4444</div> <div>Plate5555</div> <div>Plate1111</div> <div>Plate2222</div> <div>Plate3333</div> <div>PlateC2222</div> <div>Trash Bin</div>	<div>Plate1111</div> <div>Plate2222</div> <div>Plate3333</div> <div>Plate4444</div> <div>Plate5555</div> <div>PlateC1111</div> <div>PlateC2222</div>	

Next, enter the title of your slide, and select properties such as default spot type and lysis buffer for the virtual spotting. Also, the setting for the virtual extractor must be set to match that of the actual extraction head used for spotting. Furthermore, enter information about the block spotting orientation, the number of layout columns and the pattern for depositing samples. *NB: Always consider the plate and slide orientation, which is fixed in MIRACLE as shown below. Select the extractor settings and the block orientation accordingly!*

Furthermore, in order for the virtual spotting to mimic the actual samples on the slide, in some cases a conversion from an original 96-well plate layout to the 384-well plate layout used in the array is necessary (red circle). In a HTS setting, for example, cells are seeded in 96-well plates and a primary readout is conducted. Subsequently, the treated cells are harvested and the resulting lysates are automatically diluted and transferred into a 384-well plate (the process is schematically depicted in the blue box). This reformatting step can be included in the creation of the slide layout.

▼ Spotter settings

Title for Layout:

Extraction head:

Default Spot Type for spotting:

Default Lysis Buffer for spotting:

Traverse settings for Extractor:

Block spotting orientation:

Number of layout columns per block

Deposition pattern:

**96-well plate conversion** ☒ A single spot in a 96 well plate is converted into a square of four spots in different dilutions. Please select the dilution pattern here.

1	5
2	6
3	7
4	8

1	2	3	4	5	...
6	7	8	9	...	

Plate orientation!

Slide orientation!

**Lysates, 96-well plate**

↓

**384-well plate**

Last, it is possible to exclude certain extractions from an experimental plate, in case not all the wells of a plate contain sample material, which should be spotted.

Columns and rows corresponding to extraction steps can be marked, e.g. extraction 3-8 in the shown example. By pressing “Continue” (red arrow) the marked areas are excluded from the virtual spotting process.

Spotter settings

- Exclude extractions in layout PlateC1111
- Exclude extractions in layout Plate4444
- Exclude extractions in layout Plate5555
- Exclude extractions in layout Plate1111
- Exclude extractions in layout Plate2222
- Exclude extractions in layout Plate3333
- Exclude extractions in layout PlateC2222

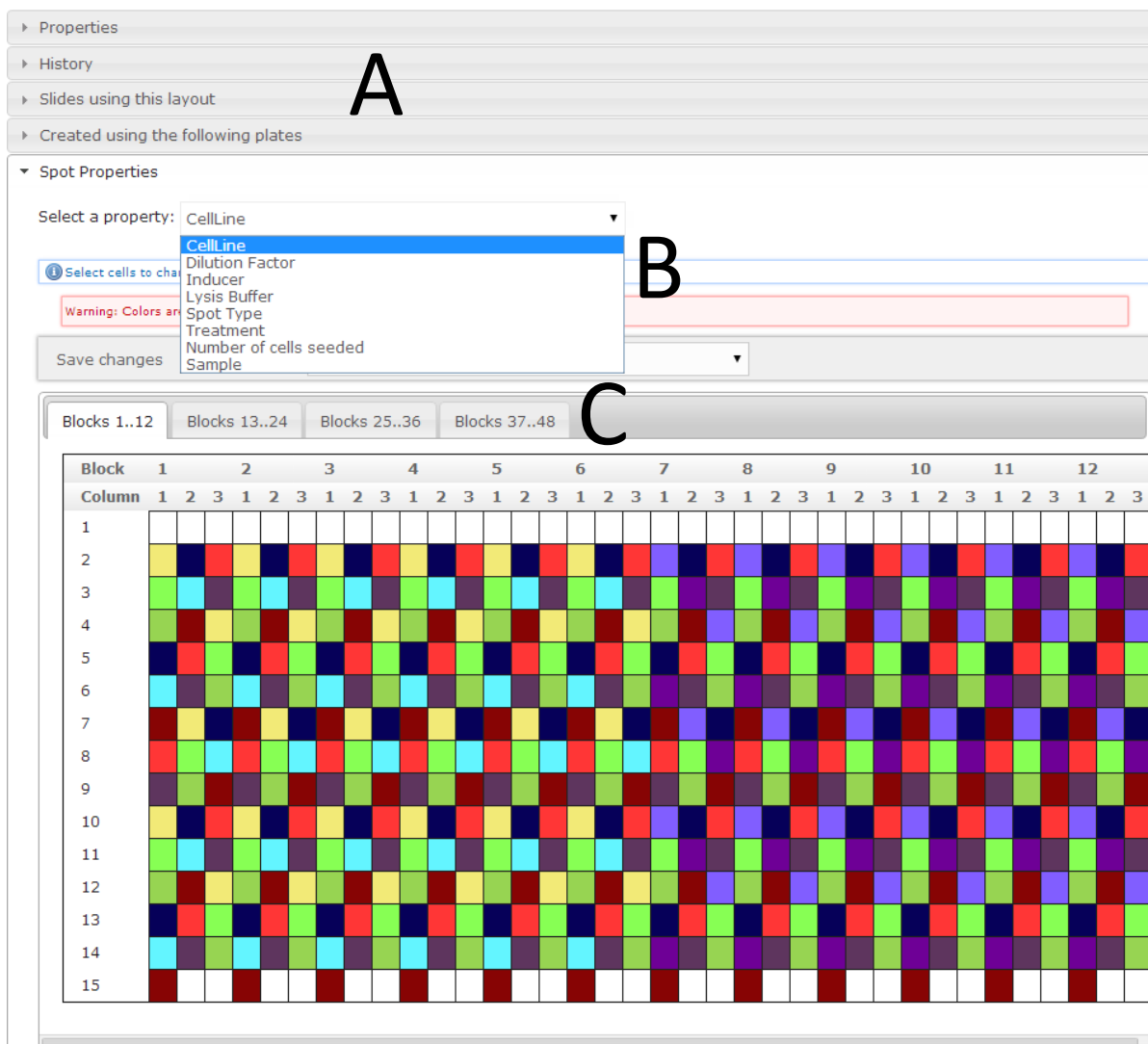
1	2
3	4
5	6
7	8

Continue ←

For visual inspection of the final layout, press “Show Layout”. Press “Properties” (A) to show the different properties of the slide layout, including the physical appearance of the layout (number of blocks, row, and columns). The sub-category “History” will display information on who created the layout and when it was created. Below in the next sub-category it is possible to see which slides use the particular layout and which source plates were used to create this layout.

Under “Spot Properties” the user can switch between the different properties, which were already assigned to the experimental plates in section 2.1 and 2.2 (B). It is also possible to switch between the layouts of the different blocks present on the array (C).

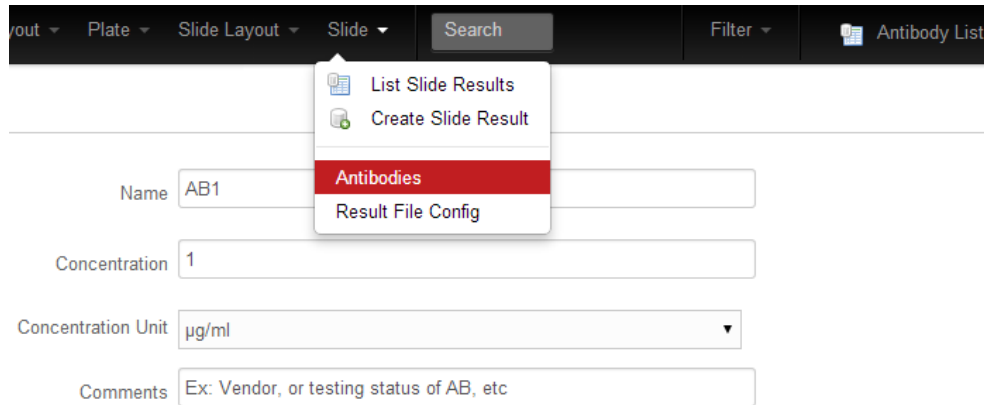
*Hint: By pointing the mouse over a certain spot (square) on the slide will give you all the information available for this particular sample in a separate pop-up box.*



Again it is possible to find the layout through Slide Layout > List Layouts.

## 2.5. Slide Readouts

Next, results of differentially stained slides (scanner files) must be connected to the corresponding slide layout. First, create the antibodies used for the staining. Slide > Antibodies, please click the top right corner, "New Antibody". Assign a specific name, used concentration as well as a comment to the antibody, and save your changes by clicking on the bottom left "Create" button. Once created, all used antibodies will be listed under Slide > Antibodies and will be available for further experiments.



out ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter ▾ Antibody List

- List Slide Results
- Create Slide Result
- Antibodies**
- Result File Config

Name

Concentration

Concentration Unit

Comments

Furthermore, to extract the critical information e.g. the positioning of spots as well as measured signal intensities from a scanner output file, a file configuration needs to be selected, which enables MIRACLE to match the scanner file information to the sample information saved in the slide layout. For this purpose, create a Result File Configuration by clicking Slide > Result File Configuration > NewResultFileConfig. Please give the configuration a name and select the type of grid applied by the scanner software, e.g. in cases signals are order according to their position in blocks of spots enter “Blocks”. Further enter the column numbers/names corresponding to the main columns and rows according to the names used in the header of your scanner file (red box; example txt result file). In case your scanner file contains a header with information about the scanner setting, count the rows of information until you reach your actual result table/list and insert the number in the “skip line” field (see also example txt result file). Save the configuration by clicking the “Create” button at the lower left corner.



Organize ▾ Plate Layout ▾ Plate ▾ Slide Layout ▾ Slide ▾ Search Filter

ResultFileConfig Liste Ny ResultFileConfig

ResultFileConfig

Name Config1

Select grid type Blocks ▾

Block Col Block

Column Col Column

Row Col Row

Fg Col F635 Mean

Bg Col B635 Mean

Diameter Col Dia.

Flag Col Flags

Skip Lines \* 25

X Col X

Y Col Y

List Slide Results  
Create Slide Result

Antibodies  
Result File Config

Example: txt result file

Skip lines


```
ATF 1.0
23 59
"Checksum_1
"Checksum_R
"Type=Mapix
"DateTime=2013/11/29 10:21:54
"ConfigFile=
"CalFile=
"PixelSize=5"
"Wavelengths=635 532"
"ImageFiles=M:\Scannings and charts\Fall 2013\29-11-13\pwt1\warlene_2013-11
"JpegImage=M:\Scannings and charts\Fall 2013\29-11-13\pwt1\warlene_2013-11
"RatioFormulations=w1/w2 (635/532)"
"FeatureType=Circular"
"Barcode=01128"
"BackgroundSubtraction=LocalFeature"
"Normalization=None"
"ImageOrigin=1502, 2033"
"JpegOrigin=7517, 4293"
"Creator=Mapix 5.0.0"
"Scanner=Is710 709046"
"Temperature=24.0"
"Comment="
"LaserPower=5.0 5.0"
"PinGain=1
"Block" "Column" "Row" "X" "Y" "Dia." "Flags" "F635 Mean"
1 2 1 7938.0 4478.0 171.43 0 64226.266 65
1 3 1 8185.0 4480.0 182.65 0 64226.266 65
1 4 1 8437.0 4480.0 181.9 0 64691.055 65
1 5 1 8681.0 4476.0 173.9 0 64288.617 65
1 6 1 8933.0 4483.0 170.25 0 64134.926 65
```

Now, the scanner result files can be connected to the slide layouts. Select Slide > Create Slide Result. Here you may enter information on the slide itself such as a barcode, title, comments to the experiment or the slide, date of staining etc.. Also an antibody must be specified along with scanner properties and slide layout information. Last, a result file bearing the configuration specified in the previous step is to be uploaded (Result File) along with a jpeg or tiff image of the scanned slide (Result Image). It is also possible to link an experimental protocol to the specific slide. Press “Create” in the lower left corner to save your information.

Barcode *	<input type="text" value="1234"/>
Title *	<input type="text" value="Slide no1"/>
Comments *	<div><div></div></div>
Date Of Staining *	<input type="text" value="07/04/2013"/>
Experimenter *	<input type="text" value="mlpedersen"/>
Laser Wavelength (nm) *	<input type="text" value="635"/>
PhotoMultiplierTube (PMT) *	<input type="text" value="0"/>
Antibody *	<input type="text" value="AB1 1.0 µg/ml"/>
SlideLayout *	<input type="text" value="TestSlideLayout"/>
Experiments	<div>1. Experiment</div>
Result File *	<div>Choose existing file: <input type="text"/></div> <div>...or upload new file: <input type="button" value="Vælg fil"/> Mar...txt</div>
Result Image *	<div>Choose existing file: <input type="text"/></div> <div>...or upload new file: <input type="button" value="Vælg fil"/> Ma...pg</div>
Experiment Protocol *	<div>Choose existing file: <input type="text"/></div> <div>...or upload new file: <input type="button" value="Vælg fil"/> Der...fil</div>

Next, a message box will ask if you would like to add spots to the database, meaning if the signals of each spot should be linked to sample information from the slide layout. Press yes and select the configuration (Config1) for reading the result file. Press “Read the selected sheet”.

## Add Spots

 Select a sheet and configuration

Select a suitable mapping of column names to spot properties (optional):

Config1 ▼

Skip lines (overrides config if checked):

☐ 0

Minimum number of columns to read:

1

Select between CSV (comma-separated) and CSV2 (semicolon-separated):

custom ▼

Select a column separator:

tab ▼

Select a decimal separator:

. ▼

Select a thousand separator:

▼

Read selected sheet

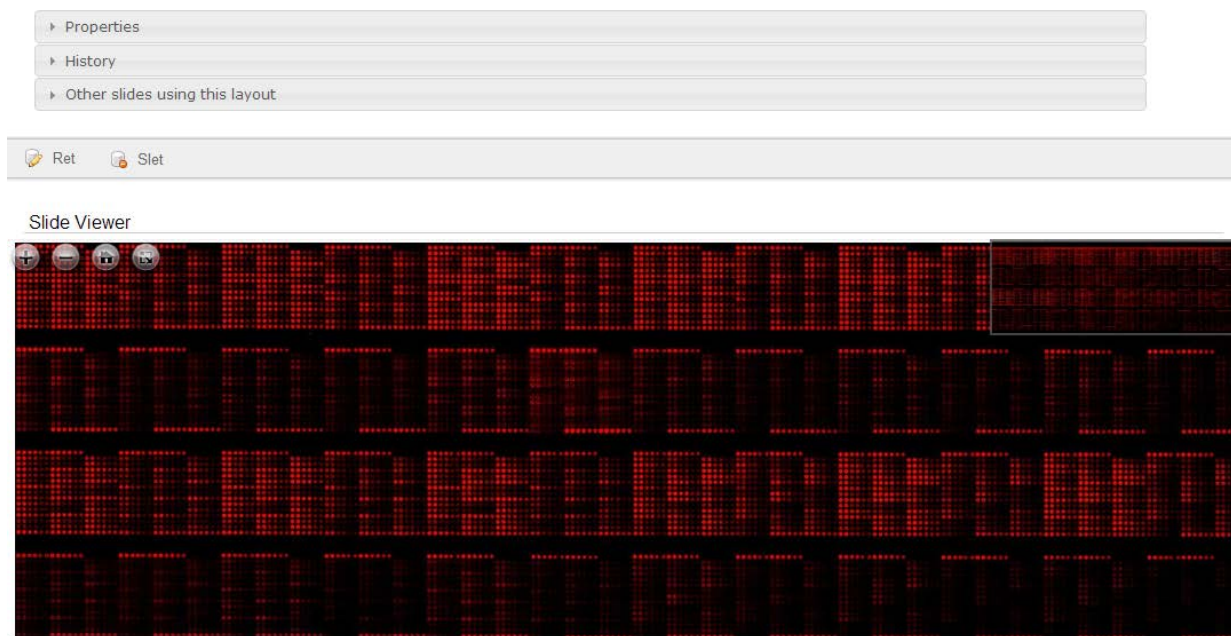
Now the columns of the configuration file must be matched to that of your scanner file. If selected, MIRACLE will try to match the columns of the file with its own database properties. However, since this step is critical, the result should briefly be checked here by the user. *NB: Should your configuration file not fit the data uploaded into MIRACLE, you can still match each column manually using the drop down boxes.*

### Match columns and database properties:

"Block"	block ▼
"Column"	column ▼
"Row"	row ▼
"X"	X ▼
"Y"	Y ▼
"Dia."	diameter ▼
"Flags"	flag ▼
"F635 Mean"	FG ▼
"F635 Median"	Do not use ▼
"F635 SD"	Do not use ▼
"F635 [log Mean/Median]"	Do not use ▼
"F635 CV"	Do not use ▼
"B635"	Do not use ▼
"B635 Mean"	BG ▼
"B635 Median"	Do not use ▼

Press the “Create” button in the lower left corner to create the slide. Once created, it is again possible to view the slide either by clicking “Back to Slide” in the top right corner, or in a list by selecting Slide > List Slide Results.

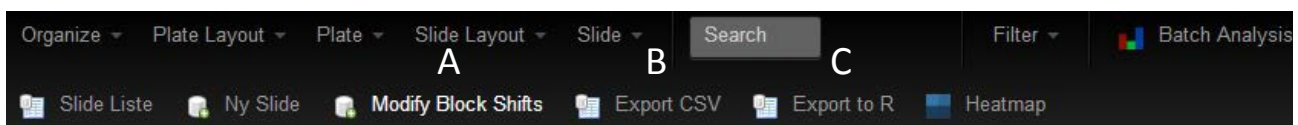
The newly created slide has all property information listed in sub-folders, along with the files applied and which experiments the slide belongs to. The last sub-folder displays other slides with identical layout, which vary with regards to the staining/antibody, e.g. normalizations slides stained with FastGreen or for a housekeeping protein. Last, a Slide Viewer displays the corresponding image file and allows quick navigation to inspect the quality of the staining and spot morphology.



### 3. Data Handling and Analysis

#### 3.1. Block Shifts and Export of Data

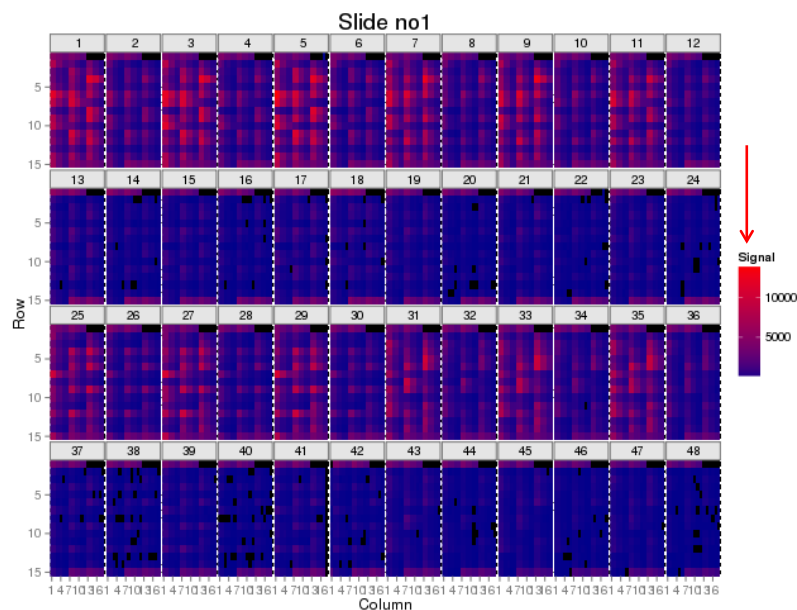
Artificial shifts in the data file may for instance be introduced by the scanner software by inserting a grid shift before signal quantification. It is possible to correct for such shifts on single slides and blocks separately. Select a slide in the slide list (Slide > List Slide Results) and press “Modify Block Shifts” (A). A heatmap will be generated (see next image), displaying the selected property (red arrow). Single blocks can be shifted in either in a horizontal or vertical manner by using the drop-down menu (blue arrow).



## Block shift correction:

Please enter shift correction values for each block.  
A block can be shifted horizontally (- : left, + : right) or vertically (- : up, + : down)

B	H	V	B	H	V
1	0	0	25	0	0
2	0	0	26	0	0
3	0	0	27	0	0
4	0	0	28	0	0
5	0	0	29	0	0
6	0	0	30	0	0
7	0	0	31	0	0
8	0	0	32	0	0
9	0	0	33	0	0



Furthermore, the sample information matched to the signals from the scanner file may be converted to CSV files or exported to R for other analysis not supported by this web-based application (B, C). Select Export CSV (B) to generate for instance a tab separated file. Press “Export and download” (red arrow) for download.

## Export Spots to CSV

▼ Export settings

Select a column separator:

Decimal separator:

Decimal precision:

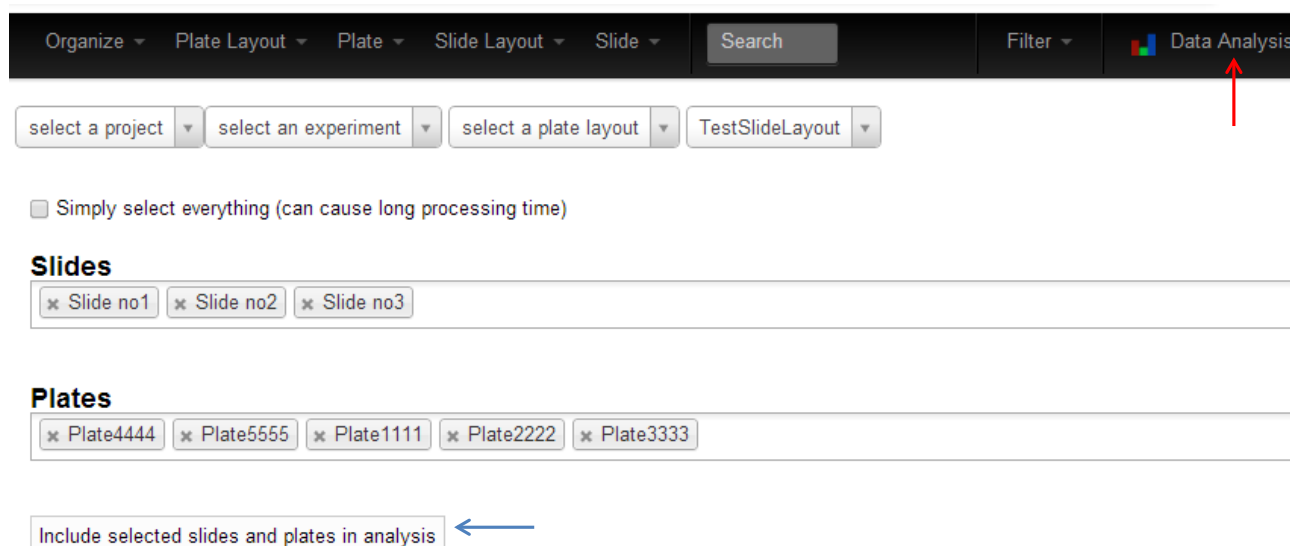
Include block shifts (columns hshift and vshift) ☒

Export and download

**NB:** A tab-separated file is the required for Rmiracle in case you wish to upload data for your analysis without using a connected MIRACLE. Please see section 3.3. for details.

### 3.2. Data Analysis

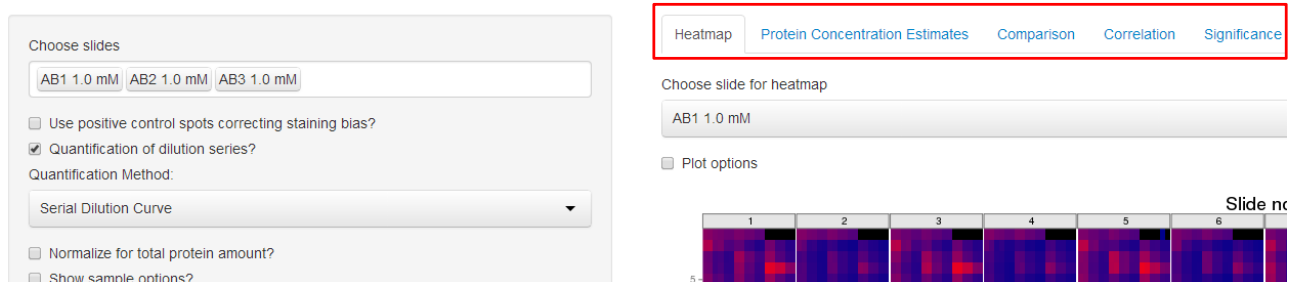
Once all slides of interest are uploaded, it is possible to click the upper right button, “Data Analysis” (red arrow) in order to access the different tools for analysis (Rmiracle). Here, please again specify, which project and experiments you would wish to include in the analysis. Then select a slide layout, e.g. “Test slide Layout”. By clicking the “Slides” or “Plates” rows, available slides will become visible and can be selected. Selection of multiple slides is feasible. Finally press “Include selected slides and plates in analysis” (blue arrow).



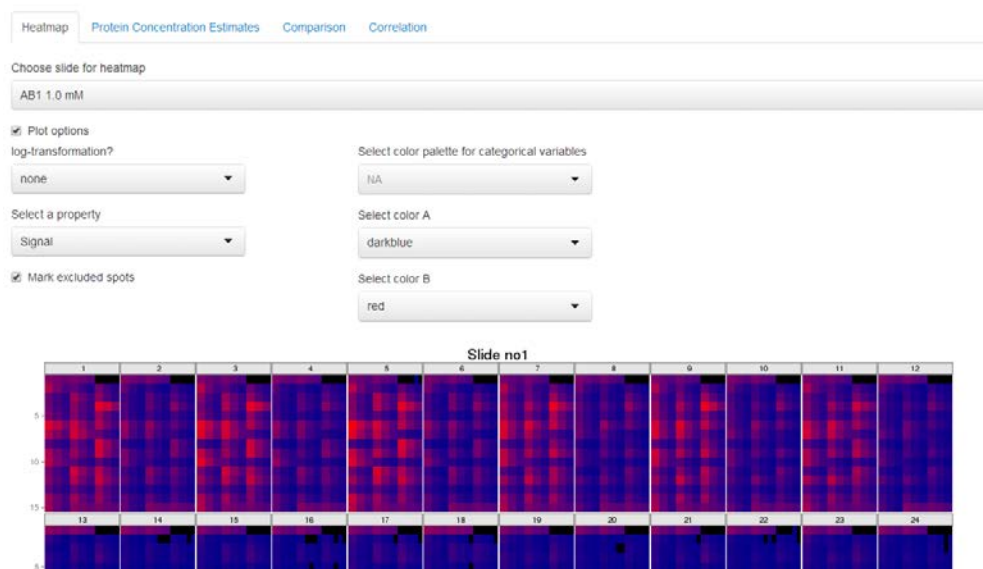
Rmiracle will open, which enables data analysis based on R using information from MIRACLE. The first step is to retrieve the information from MIRACLE, which will take some seconds.

Once loaded, it is possible to choose between different tabs to display heatmaps, protein concentration estimates, comparison of different slide data to each other or to other data, calculation of correlation between data and calculation of significance (red box).

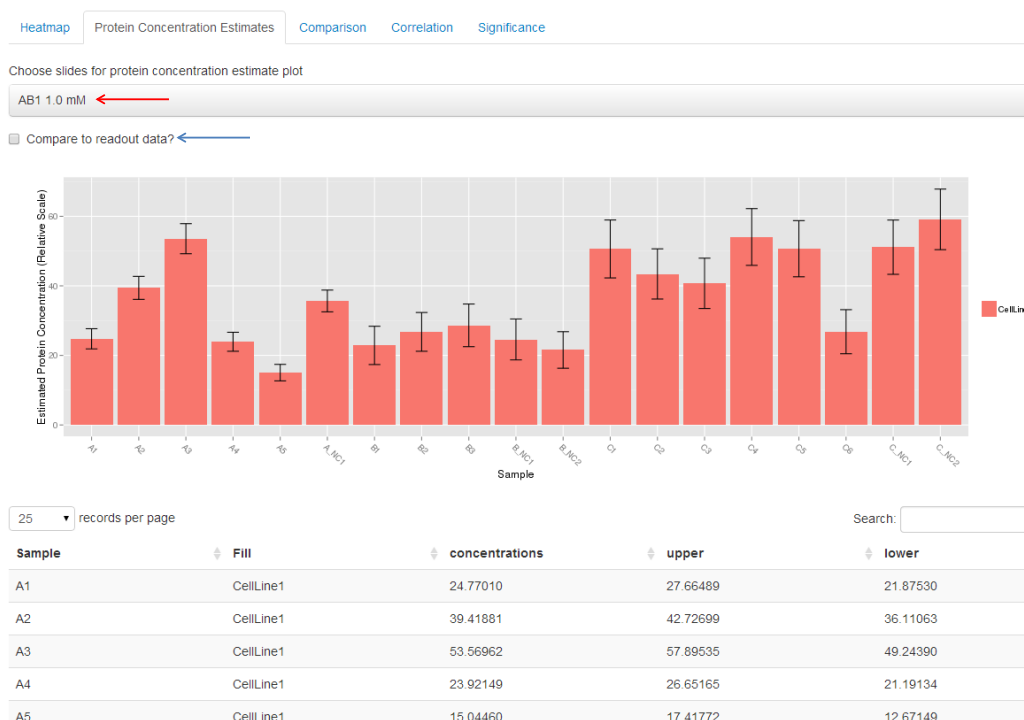
### Rmiracle

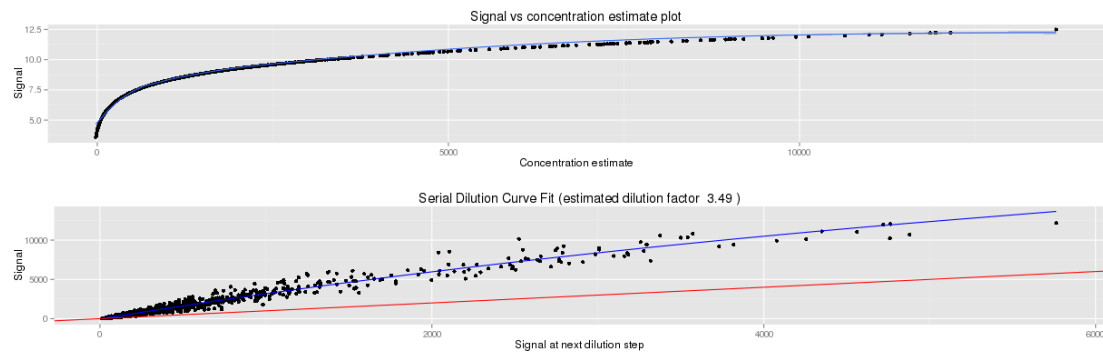


Start by selecting the “Heatmap” pane. Tick the box saying “Plot options” to change the physical properties of the heatmap (e.g. colour, scale) or to change the values (e.g. signal intensity, deposition, sample) displayed in the map.

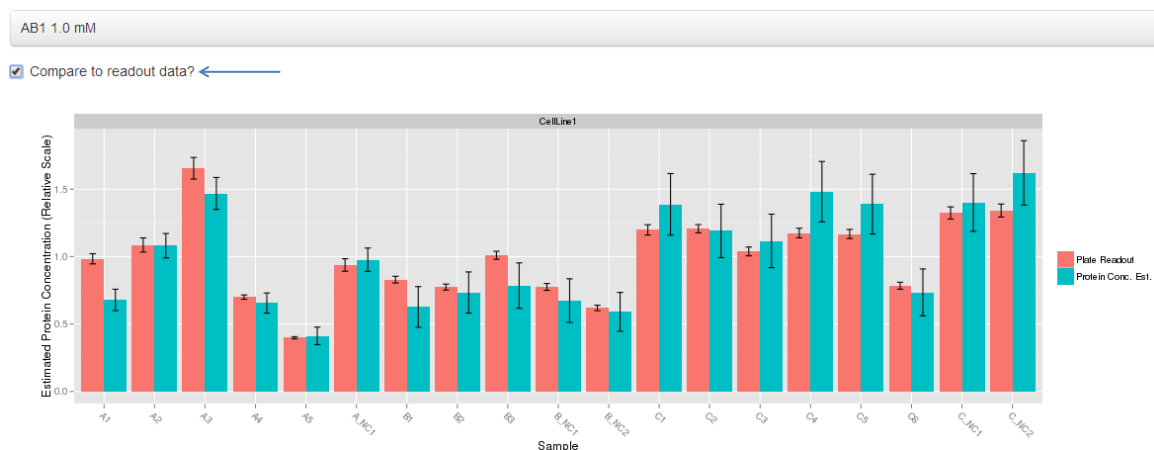


Next, select “Protein Concentration Estimation” (top panel) to analyse your array data. The data are shown for each antibody staining individually. Use the drop-down menu “Choose slide for protein concentration estimate plots” (red arrow) to switch between the different stainings. A bar-chart is generated with the estimated protein concentrations per samples along with an interactive table with estimates including errors. Furthermore, graphics of the signal vs concentration estimates and a curve displaying the serial dilution fit are generated automatically. *NB: The latter is only applicable in case lysates from a single sample were spotted in multiple dilutions on the array and the Serial Dilution Curve method was selected for quantification. For more information on selection in quantification, please read further.*





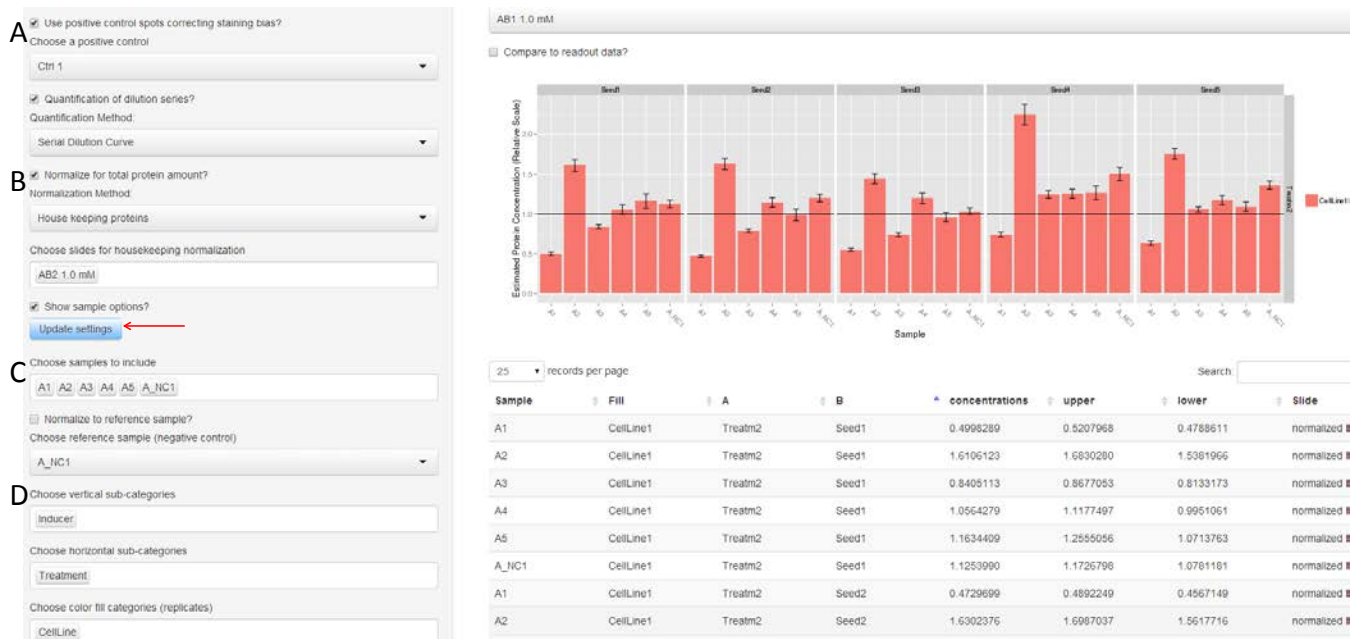
In addition, it is possible to view the protein concentration estimates along with the primary readout. For this, select “Compare to readout data?” (blue arrow). *NB: Prior to comparisons with primary readout data, result files need to be implemented as described in section 2.3.*



The selection-pane to the left efficiently allows the user to customize the different parameters for analysis, such as switching correction for staining bias on/off and selecting a quantification method (A). *NB: The Serial Dilution Curve method is the fastest and most robust method for the data uploaded in this tutorial. Generation of protein concentration estimates using the Tabus or Non-parametric methods demands a little patience and the SuperCurve method does not work with the uploaded test data, possibly due to the small number of dilution steps (4) used in these experiments.*

Furthermore, there is the option to normalize your protein values according to total protein amounts by selecting different methods such as housekeeping protein normalization or Median Loading or Variable Slope normalization (B). It is also possible to select only a sub-set of the samples included for analysis and data display, such as here the A-samples, and to select a reference sample (e.g. A-NC1 set to an average of 1 over all displayed 5 property subgroups) (C). In addition, for visualization, the data can be separated into different categories in a horizontal and vertical manner and a category can be selected for the colour fill of the bars (D). In the screenshot displayed below, the data are split based on “Treatment” and “Inducer” and the property “CellLine” (bar fill). *NB: Whenever changes are made in category C, you must press the “Update settings” (red arrow) button to execute the changes!*





Moreover, it is possible to select if results should be scaled as in the example below, where two different sample treatments provide values in different ranges (E). The example displays samples of the B-set and the scaling is selected for the vertical orientation only.



Last, it is possible to swap categories, insert error bars, draw a line through 1, and to select a specific export data format (F) [CSV, CSV2, TAB].

**E** ☒ Show plotting options?

Should results be scaled?

Scale only vertical

**F** ☐ Swap categories?

☒ Draw a horizontal line through 1

☐ Include error bars

Select file type for table downloads

TAB

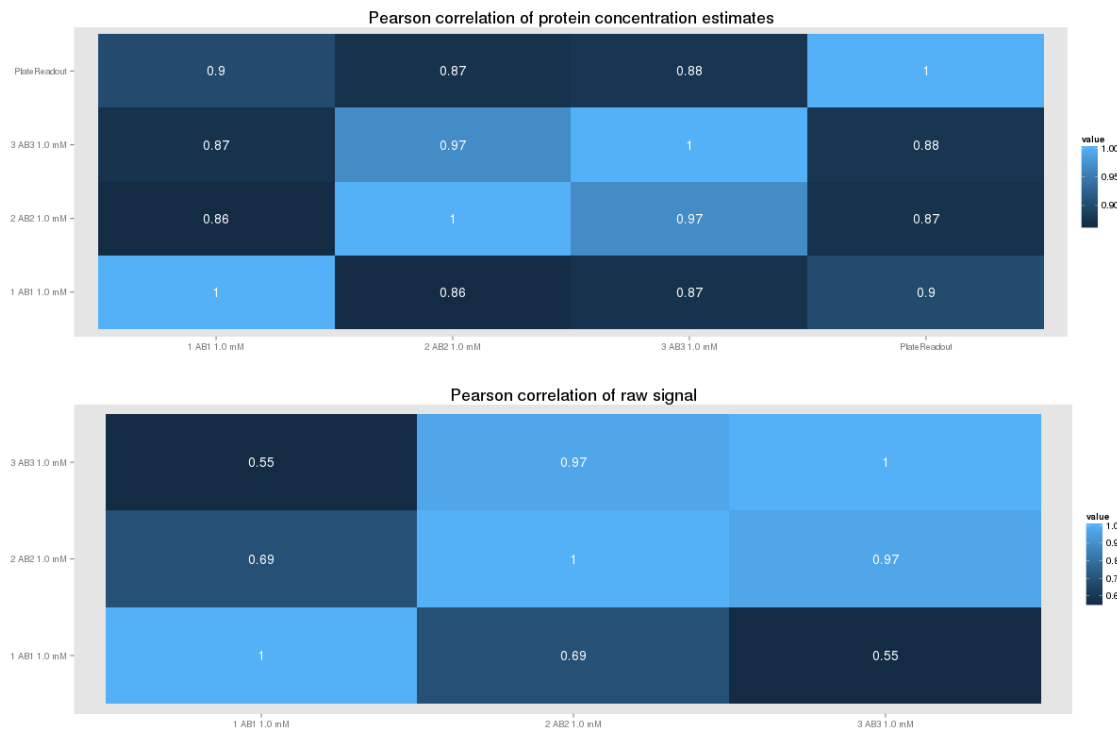
Furthermore, the protein concentration estimates for all three slides of the example data set can be compared. For this purpose select the “Comparison” pane. This feature can be applied on normalized or non-normalized protein estimates. Here it is also possible to compare to the readout data if such is existing. *NB: Be aware that the scaling of the protein- vs. readout data may differ so try to normalize to a reference sample.*



By selecting the “Correlation” pane it is possible to retrieve calculated Pearson correlation factors between protein concentration estimates from the different antibodies, but also between the different raw signals obtained from each staining. Here, it is also possible to correlate protein values to other primary readouts conducted in the original 96- or 384-well plates, e.g. a cell viability readout or total protein content determination.

Heatmap Protein Concentration Estimates Comparison Correlation Significance

☒ Include readout in correlation?



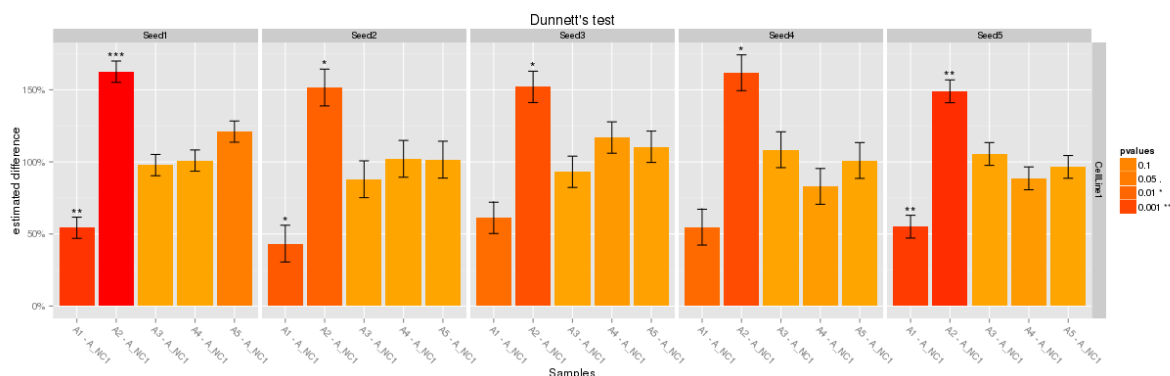
Another feature of Rmiracle is Dunnett’s test for significance of differences in protein concentration estimates, given a number of replicates is available. Select the pane “Significance”. Similar to the protein concentration estimation, it is possible to select different categories for displaying the data. In the example below, data are separated based on “CellLine” and “Inducer”. Most important for this part of the analysis is that a ‘fill’ property is selected that determines which of the samples should be treated as replicates. To this end, the “Replicate” property can be used if set during layout creation. In our example, however, we choose “PlateCol”, e.g. the column of the source plates. This suffices, since all replicates are located in a different column.

Heatmap Protein Concentration Estimates Comparison Correlation Significance

☒ Set reference (negative control) to 100%?

Choose slides for testing significance

AB1 1.0 mM



### 3.3. Uploading files to Rmiracle

As explained in section 3.1., it is also possible to upload data files into Rmiracle without using the MIRACLE web-platform itself. For this, please go to <http://www.nanocan.org/miracle/analysis> which will take you directly to Rmiracle.

Here files containing layout/sample- and signal information can be selected by using the browse function (red circle). In order to select several slides, e.g. use shift or control.

## Rmiracle

File data

Browse...

No files selected.

☐ Use positive control spots correcting staining bias?  
☒ Quantification of dilution series?  
Quantification Method:

Heatmap

Protein Concentration I

☐ Plot options  
**Error: No slides loaded**

The file format required is a tab-separated .txt file or .csv file and the layout must contain consecutive columns with information on the following:

hshift, vshift, id, Signal, Block, Row, Column, FG, BG, Flag, Diameter, SampleName, SampleType, TargetGene, SampleGroupA, SGADesc, SampleGroupB, SGBDesc, SampleGroupC, SGCDesc, CellLine, LysisBuffer, DilutionFactor, Inducer, Treatment, SpotType, SpotClass, NumberOfCellsSeeded, Replicate, PlateRow, PlateCol, PlateLayout.

Below is an example of such a file with 11 samples-rows and 32 information-columns (see also Supplemental File 1).

MIRACLE reverse phase protein array file format																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
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#### 4. Final Remarks

We intend to continue the development of MIRACLE to make it *the* state-of-the-art tool for RPPA data processing. Thus, in case you have any more questions and comments on how MIRACLE can be improved, please have a look at the project wiki page <https://github.com/NanoCAN/MIRACLE/wiki> (31/03/2014). Here, there is also the possibility to use a bug tracker allowing you to report any malfunctions you might have encountered and to request new features (<https://github.com/NanoCAN/MIRACLE/issues>) (31/03/2014).