

Who is ChemoMetec?

- Company founded by scientists for scientists, aiming to bring the best cell analysis technology possible into every lab
- ChemoMetec develops and manufactures automated cell counting and image cytometry equipment for labs working with Eukaryotic cells
- ChemoMetec's technology works through the principles of fluorescence microscopy to ensure that every cell can be identified while minimizing the influence of debris.



One-step Cell Count and Analysis - Using the Via-1 Cassette™

- Via1-Cassette™ stains:
 - All cells with Acridine Orange
 - Non-viable cells with DAPI
- Each Via1-Cassette™ is calibrated using a **dot code** for high precision counting
- **Integrated piston and pipette tip** of the Via1-Cassette™ simplify preparation by removing human error
- Large **counting chamber** volume ensures precise data acquisition



NC-200 Specifications

Loading volume:

60 μ l into the Via1-Cassette™

Measurement volume:

1.4 μ l in the measurement chamber of the Via1-Cassette™

Analysis time:

50 seconds (one step)

• Measurement range:

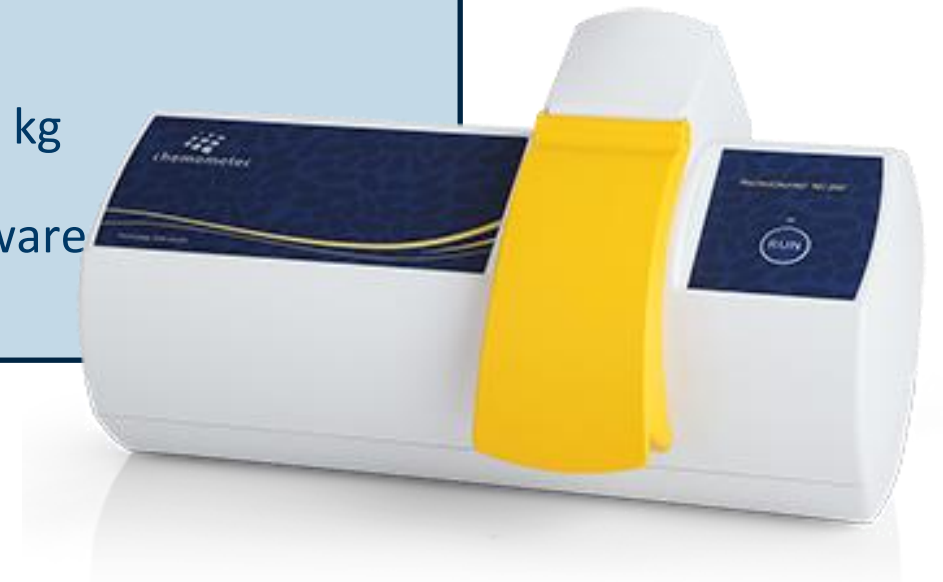
5×10^4 to 5×10^6 cells/ml

• Size:

38 × 26 × 22 cm (W × H × D), weight 3 kg

Software:

NucleoView™ NC-200 computer software



Topics for the Day

- Software Introduction
- Best Practices
- Gating Changes
- Optimizing Protocols
- Performing a Comparison Study



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Software Introduction

The screenshot displays the ChemoMetec NucleoView NC-200 software interface. The main window shows a dark field with green fluorescent spots. To the right, a file list contains various assay files. Below the image, there are input fields for sample and dilution volumes, and multiplication factors. On the right side, there are fields for sample ID, operator, and assay results including total cells/ml, viability percentage, and diameter.

ChemoMetec NucleoView NC-200

File Tools View Help

Image Result

1/1

Data folder and files

:\Documents\ChemoMetec\NucleoView NC-200\results\20200302

- 20200302-0001-c-CHO-S P2D2 NO DULTION.cm
- 20200302-0001-c-CHO-S PASSAGE 2 DAY 2.cm
- 20200302-0002-c-CHO-S P2D2 NO DILUTION.cm
- 20200302-0002-c-CHO-S P2D2 NO DULTION.cm
- 20200302-0003-c-CHO-S P2D2 NO DILUTION.cm
- 20200302-0003-c-CHO-S P2D2 NO DULTION.cm
- 20200302-0004-c-CHO-S P2D2 2X DILUTION.cm
- 20200302-0004-c-CHO-S P2D2 2X DULTION.cm
- 20200302-0005-c-CHO-S P2D2 2X DILUTION.cm
- 20200302-0005-c-CHO-S P2D2 2X DULTION.cm
- 20200302-0006-c-CHO-S P2D2 2X DILUTION.cm

Protocol Viability and Cell Count Assay

Via1-Cassette

Sample ID: CHO-S PASSAGE 2 DAY 2 Operator: NC-200 Demo

Total cells/ml: 3.48E6

Viability %: 99.1

Diameter um: 12.5

Via1: Sample [ul]: 200 Dilution [ul]: 0 Multiplication Factors: 1 # 1.00



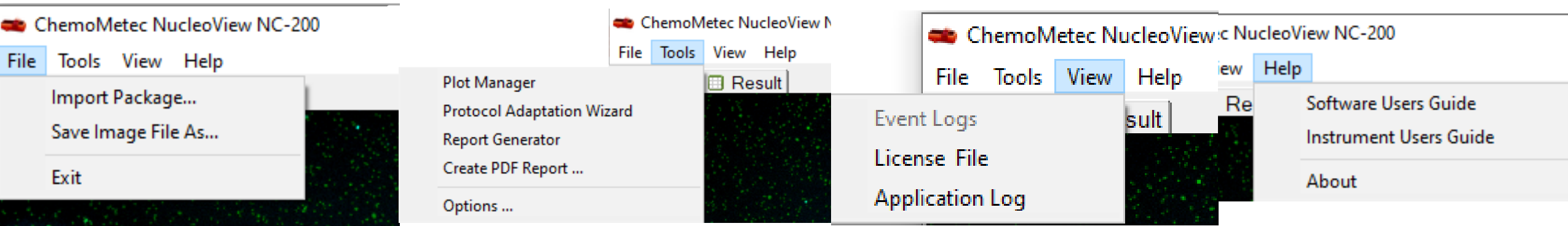
Software Introduction

Menu Bars

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. A red box highlights the menu bar at the top, which includes 'File', 'Tools', 'View', and 'Help'. Below the menu bar, there are tabs for 'Image' and 'Result'. The main area displays a dark field with green spots, representing a cell count assay. To the right, there is a file list showing various data files. Below the file list, there are fields for 'Protocol' (Viability and Cell Count Assay), 'Sample ID' (CHO-S PASSAGE 2 DAY 2), and 'Operator' (NC-200 Demo). At the bottom, there are input fields for 'Sample [ul]' (200), 'Dilution [ul]' (0), and 'Multiplication Factors' (1 # 1.0). On the right side, there are three buttons labeled 'F1', 'F2', and 'F3'. Below these buttons, there are three data fields: 'Total cells/ml' (3.48E6), 'Viability %' (99.1), and 'Diameter um' (12.5).



Menu Bars



File

- Import adapted protocols
- Export images

Tools

- Opens Plot Manager
- Starts Protocol Adaption Wizard
- Create PDFs
- Open the options menu

View

- Access Log files

Help

- Access digital user guides
- About screen, instrument firmware information for support



Software Introduction

Image and Results Window

The screenshot displays the ChemoMetec NucleoView NC-200 software interface. The main window is titled "ChemoMetec NucleoView NC-200" and has a menu bar with "File", "Tools", "View", and "Help". Below the menu bar are two tabs: "Image" and "Result". The "Image" tab is active, showing a large fluorescence image of a cell culture well with green spots on a dark background. A red box highlights this image and the "Result" tab. To the right of the image is a file list window titled "Data folder and files" showing a directory path and a list of files. Below the file list are buttons for "F1", "F2", and "F3". The "Protocol" section shows "Viability and Cell Count Assay" and "Via1-Cassette". The "Sample ID" field contains "CHO-S PASSAGE 2 DAY 2" and the "Operator" field contains "NC-200 Demo". At the bottom right, a "RETURN" button is visible. The results panel at the bottom right shows the following data:

Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

At the bottom left, there are input fields for "Sample [ul]" (200) and "Dilution [ul]" (0). Below these is a "Multiplication Factors" section with a field containing "1 # 1.00".



Software Introduction

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. The main window displays a fluorescence image of a cell culture well. To the right, a file list shows various data files. Below the file list, the protocol is set to 'Viability and Cell Count Assay' and the sample ID is 'CHO-S PASSAGE 2 DAY 2'. The operator is 'NC-200 Demo'. At the bottom right, a summary table displays the following data:

Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

At the bottom left, a 'Dilution Calculator' window is highlighted with a red box. It contains the following fields:

	Sample [ul]	Dilution [ul]	Multiplication Factors
Via1:	200	0	1 # 1.00

Dilution Calculator



Software Introduction

The screenshot displays the ChemoMetec NucleoView NC-200 software interface. On the left, a large fluorescence image shows green spots on a dark background. Below the image are input fields for 'Sample [ul]' (200) and 'Dilution [ul]' (0). The bottom center shows 'Multiplication Factors' as '1 # 1.00'. On the right, a file list shows various .cm files. Below the file list, the 'Protocol' section is highlighted with a red box and contains the following information:

Protocol	Viability and Cell Count Assay
Via1-Cassette	Via1-Cassette
Sample ID	CHO-S PASSAGE 2 DAY 2
Operator	NC-200 Demo

Below the protocol information, a 'RETURN' button is visible. At the bottom right, assay results are displayed:

Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

Protocol Information



Protocol Selection

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. On the left is a large dark image area with green spots. Below it are input fields for 'Sample [ul]' (200) and 'Dilution [ul]' (0). To the right is a file list window titled 'Data folder and files' showing a directory of .cm files. Below the file list are function keys F1, F2, and F3. A red box highlights the F3 key, with a red arrow pointing to the text 'Protocol Select Menu'. Below the function keys are fields for 'Protocol' (Viability and Cell Count Assay), 'Via1-Cassette', 'Sample ID' (CHO-S PASSAGE 2 DAY 2), and 'Operator' (NC-200 Demo). At the bottom right, a table displays assay results: Total cells/ml (3.48E6), Viability % (99.1), and Diameter um (12.5).

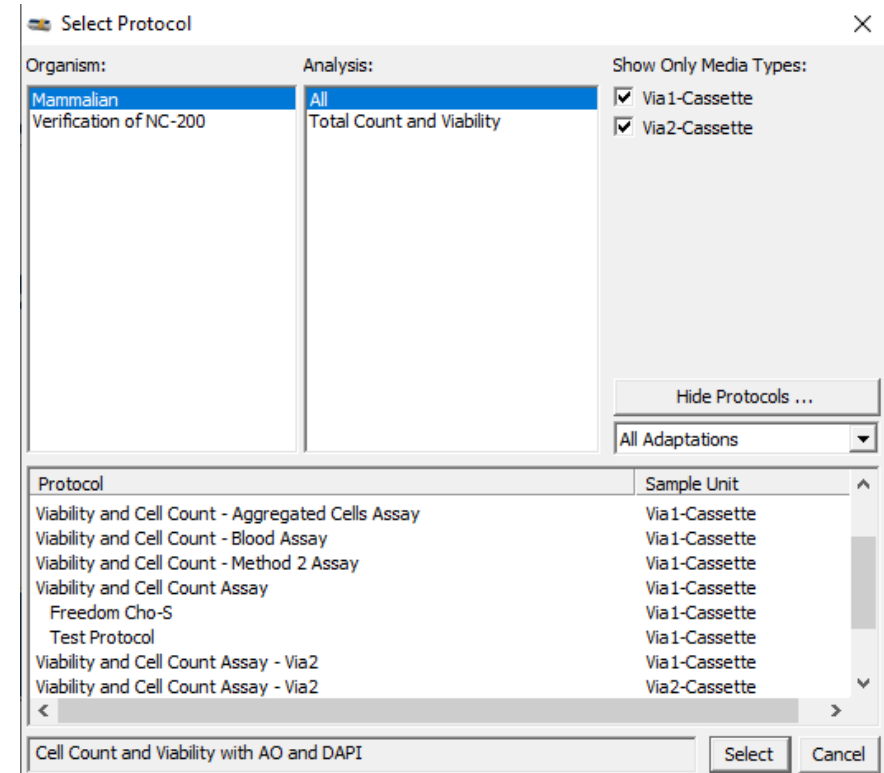
Parameter	Value
Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

Protocol Select Menu



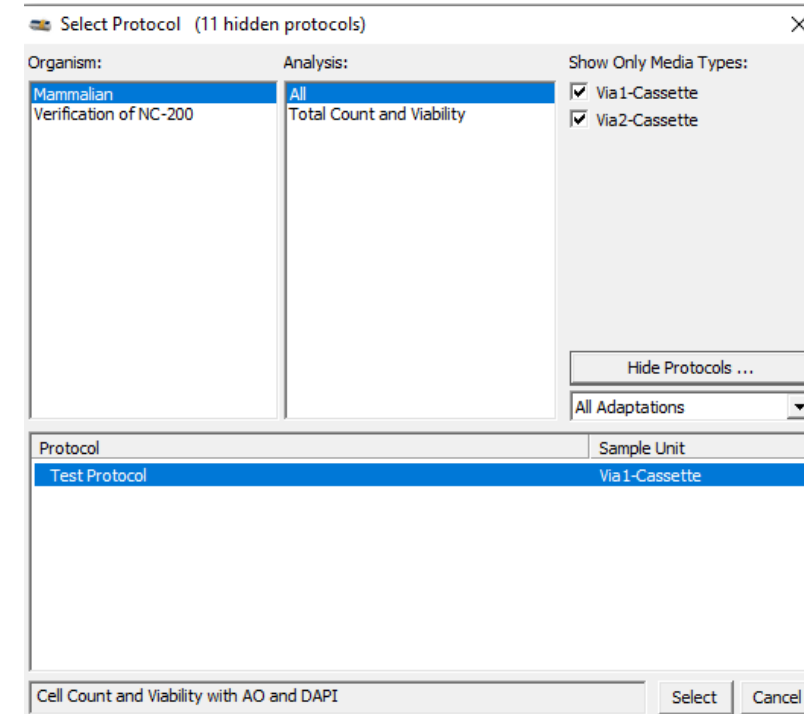
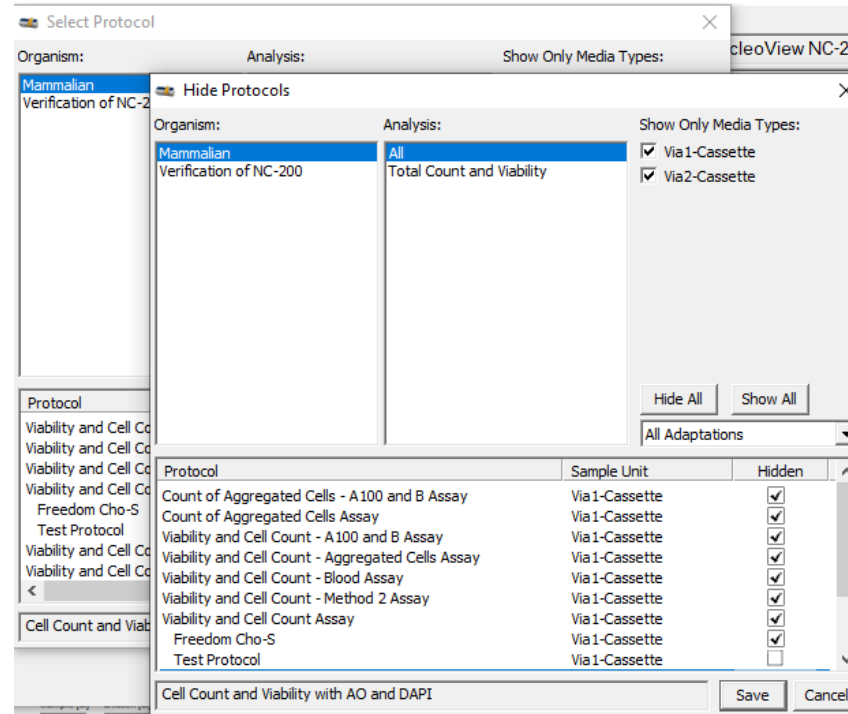
Protocol Selection

- The protocol select screen will allow you to browse all protocols created or uploaded onto the instrument you are using
- User generated protocols can be exported or deleted by right clicking on the protocol name from this list.
- To reduce the number of protocols shown here without deleting them, you simply have to click the “Hide Protocols” option



Protocol Selection

- To hide a protocol in the hide protocol menu, simply check the box next to the protocol you wish to hide, and click save!



Software Introduction

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. On the left is a large image window displaying a dark field with green fluorescent spots. Below the image are input fields for 'Sample [ul]' (200) and 'Dilution [ul]' (0). To the right of the image is a 'Data folder and files' window, outlined in red, showing a file list for the path 'C:\Documents\ChemoMetec\NucleoView NC-200\results\20200302'. The file list includes several .cm files with names like '20200302-0001-c-CHO-S P2D2 NO DULTION.cm'. Below the file list are fields for 'Protocol' (Viability and Cell Count Assay), 'Via1-Cassette', 'Sample ID' (CHO-S PASSAGE 2 DAY 2), and 'Operator' (NC-200 Demo). At the bottom right, a summary table displays results: Total cells/ml (3.48E6), Viability % (99.1), and Diameter um (12.5).

Parameter	Value
Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

Quick Access
Data Files



File Browser and Exporting Data to Excel

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. The main window displays a dark field with green fluorescent spots. On the right, a file browser window is open, showing a list of files in the directory `:\Documents\ChemoMetec\NucleoView NC-200\results\20200302`. A red box highlights the file browser window, with a red arrow pointing to it from the text "File Browser". Below the file list, the "Protocol" is set to "Viability and Cell Count Assay". The "Sample ID" is "CHO-S PASSAGE 2 DAY 2" and the "Operator" is "NC-200 Demo". At the bottom right, the assay results are displayed:

Total cells/ml	3.48E6
Viability %	99.1
Diameter um	12.5

At the bottom left, the assay parameters are shown:

Via1:	Sample [ul] 200	Dilution [ul] 0	Multiplication Factors 1 # 1.00
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File Browser



File Browser and Exporting Data to Excel

- The File Browser allows you to view data from previous days, as well as select multiple data files at once to export the data to both the plot manager, and to excel

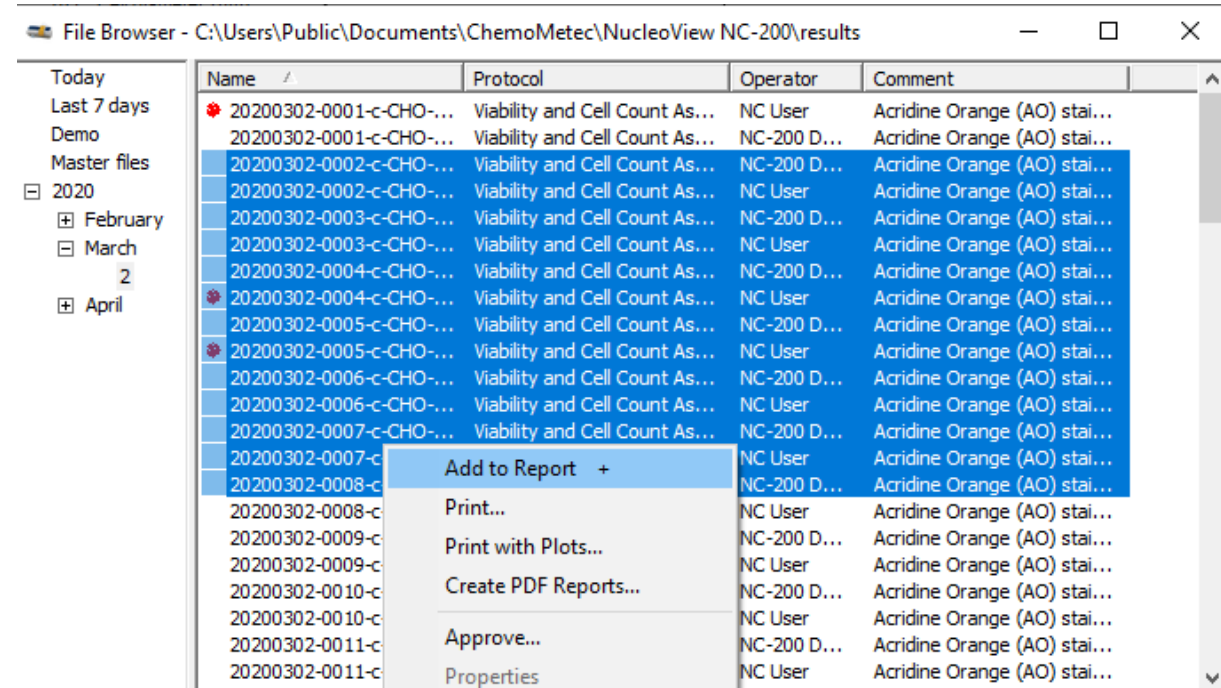
File Browser - C:\Users\Public\Documents\ChemoMetec\NucleoView NC-200\results

Today	Name	Protocol	Operator	Comment
Last 7 days	20200302-0001-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
Demo	20200302-0001-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
Master files	20200302-0002-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
2020	20200302-0002-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
February	20200302-0003-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
March	20200302-0003-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
2	20200302-0004-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
April	20200302-0004-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0005-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0005-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0006-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0006-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0007-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0007-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0008-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0008-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0009-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0009-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0010-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0010-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...
	20200302-0011-c-CHO-...	Viability and Cell Count As...	NC-200 D...	Acridine Orange (AO) stai...
	20200302-0011-c-CHO-...	Viability and Cell Count As...	NC User	Acridine Orange (AO) stai...



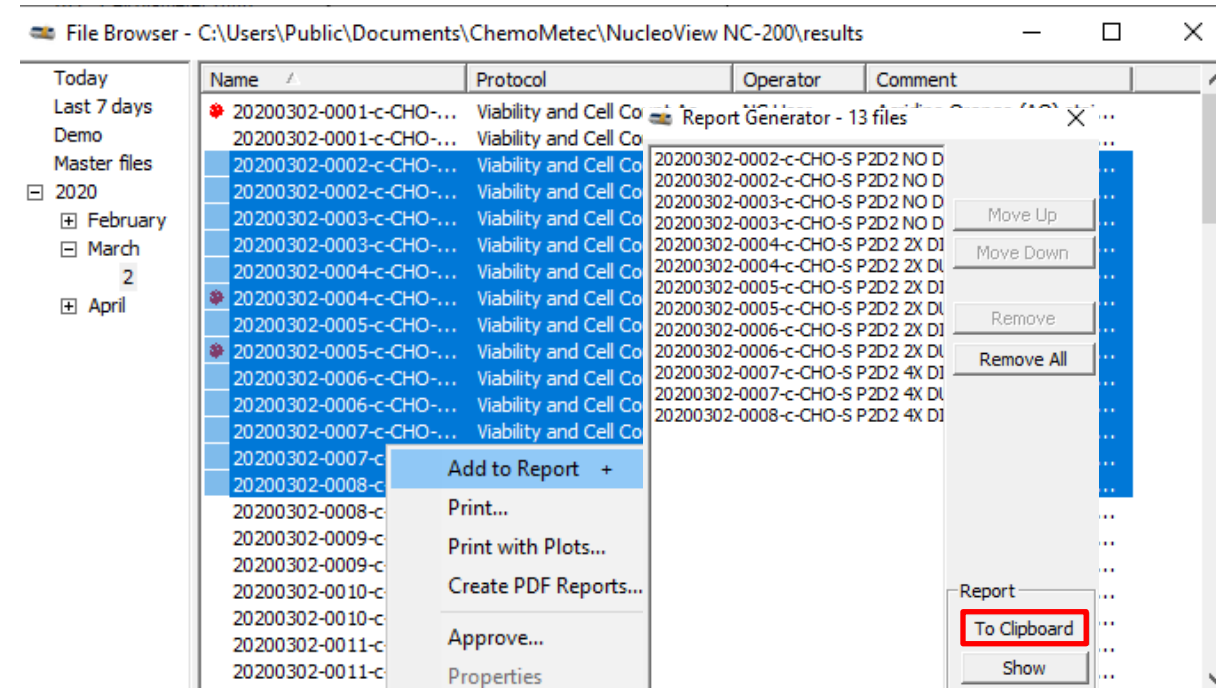
File Browser and Exporting Data to Excel

- The File Browser allows you to view data from previous days, as well as select multiple data files at once to export the data to both the plot manager, and to excel
- To export the data to excel, simply highlight the data you wish to export and then right click one of the highlighted files and click “Add to Report”



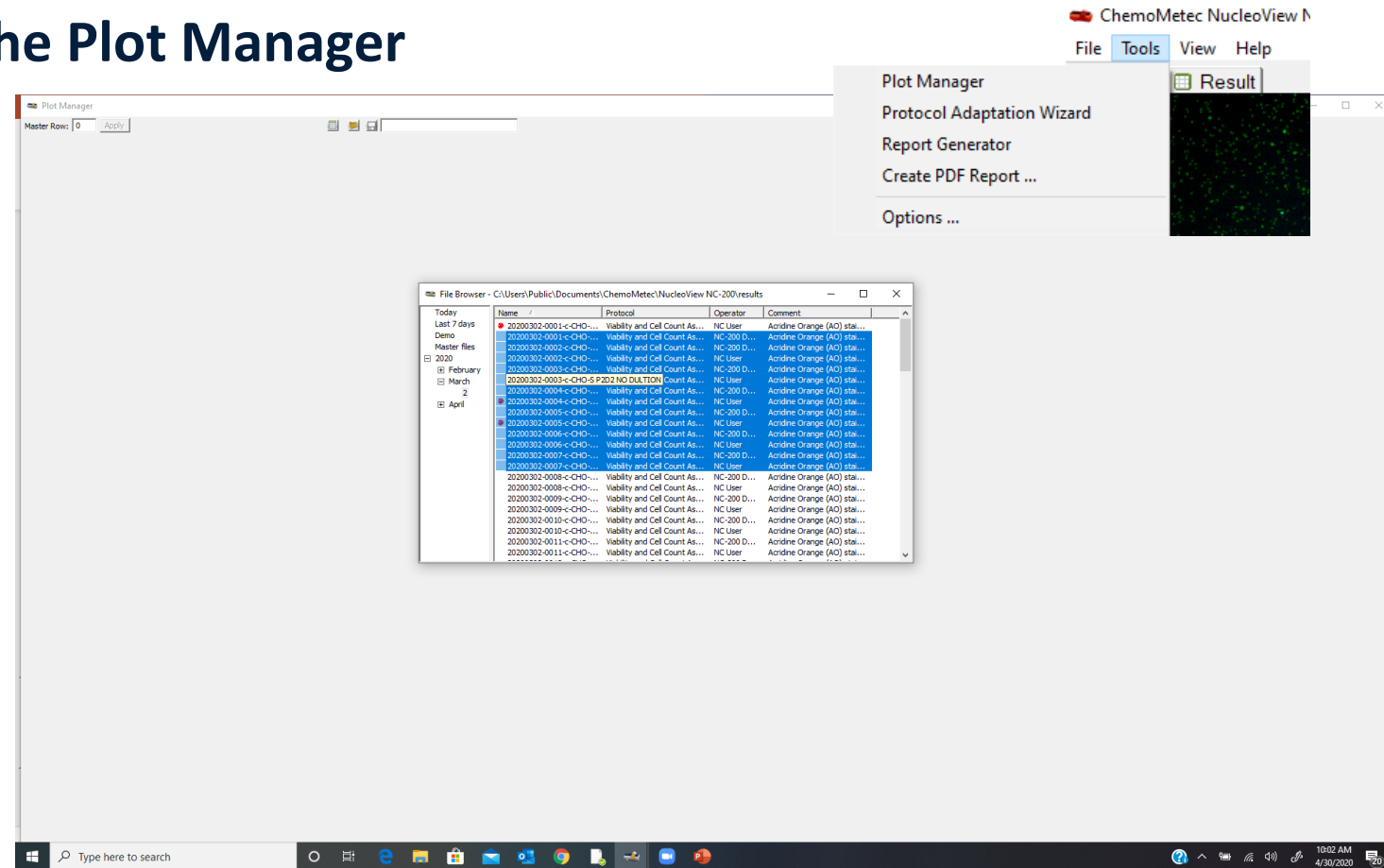
File Browser and Exporting Data to Excel

- Clicking “Add to Report” will open the report generator
- Once open, you simply need to click “To Clipboard” and the data will be copied to your clipboard, enabling you to paste it into Microsoft excel, or your spreadsheet program of choice



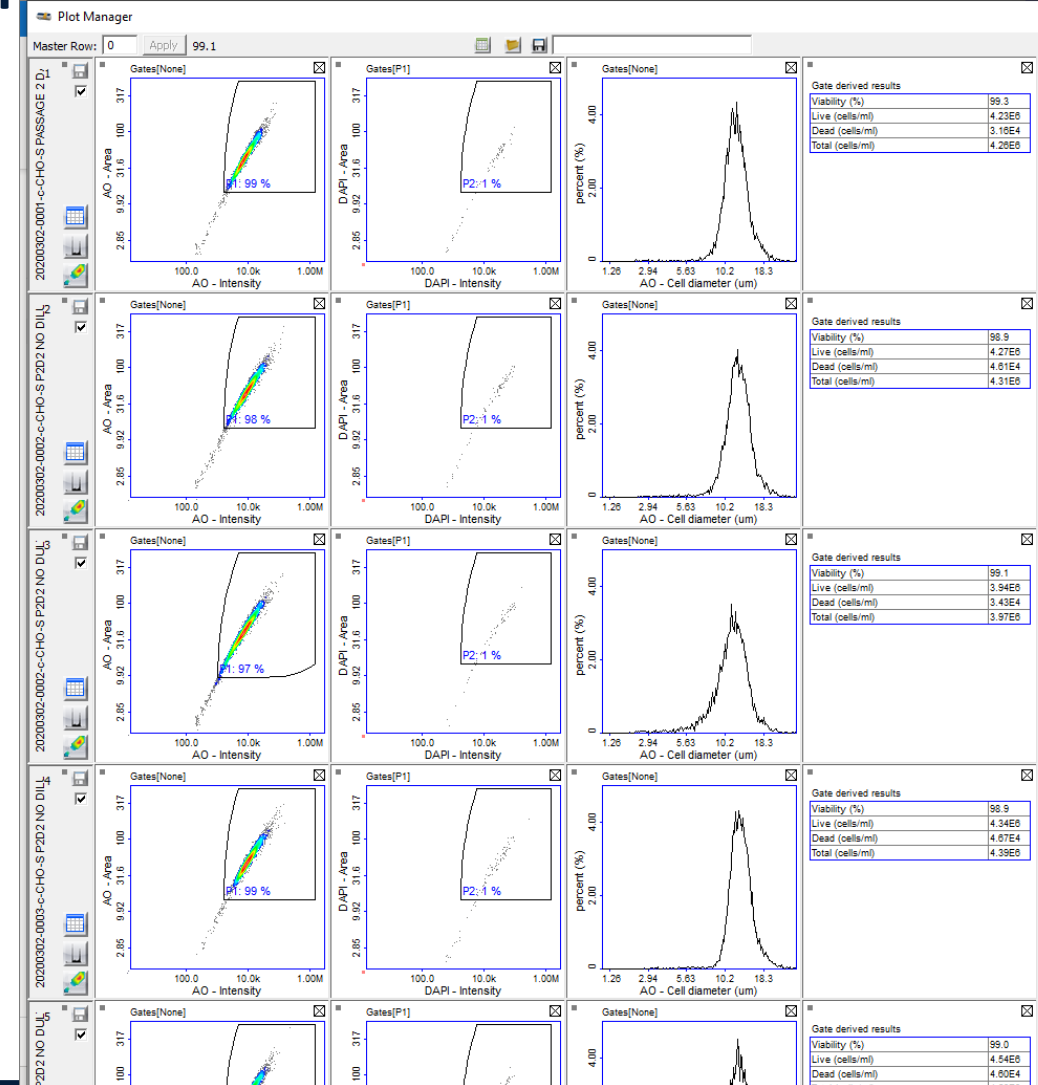
File Browser and Mass Import to the Plot Manager

- To mass import files to the plot manager, first open the plot manager by going to Tools>Options
- Then, follow the previous steps to open the File Browser and highlight the data you wish to analyze



File Browser and Mass Import to the Plot Manager

- Then simply drag the highlighted files into the blank File Browser and everything will load automatically



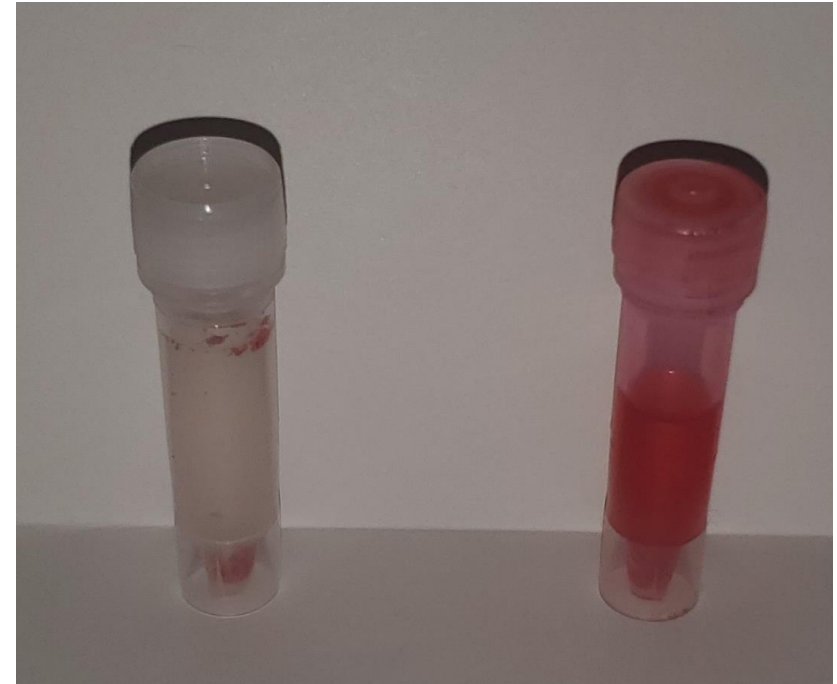
Topics for the Day

- Software Introduction
- **Best Practices**
- Gating Changes
- Optimizing Protocols
- Performing a Comparison Study



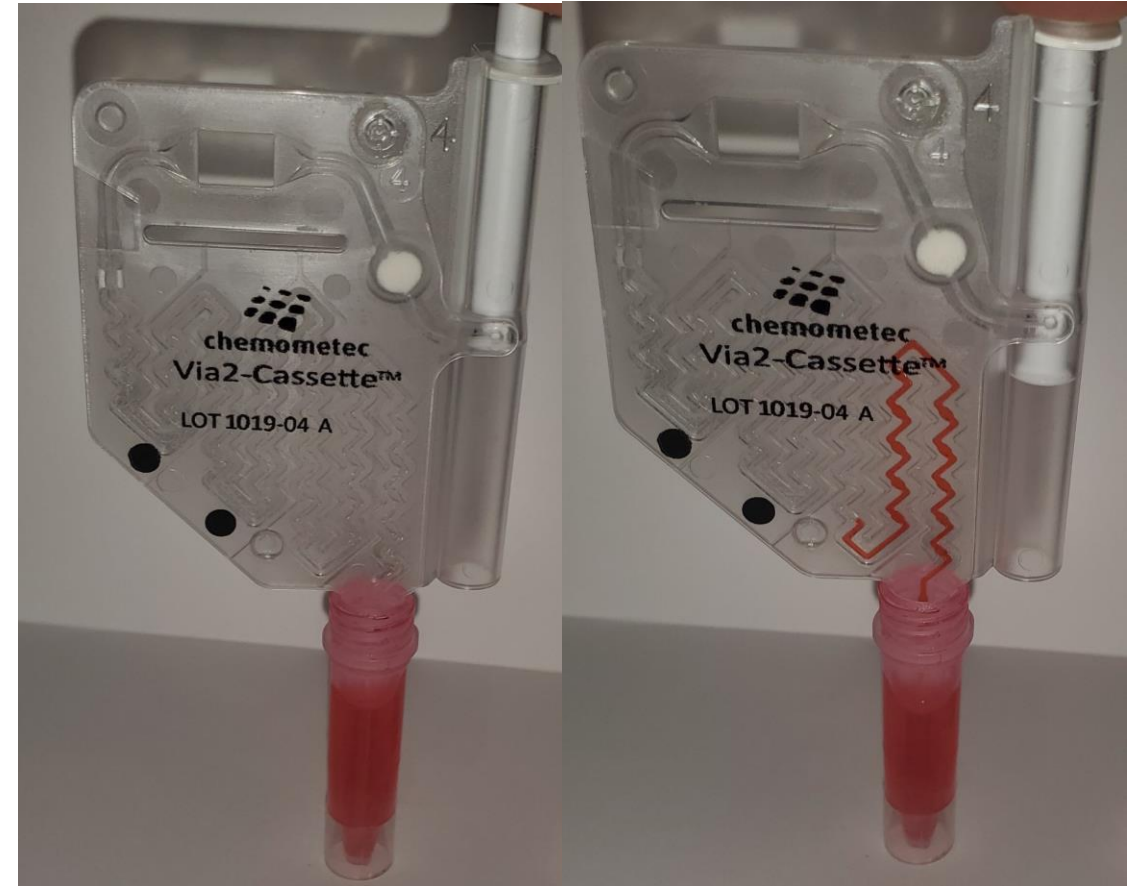
Best Practices

- Use at least 200 μ L of sample, if you must use less, be very very careful to avoid bubbles!
- Make sure your sample is well mixed!
- Please ensure DMSO concentration is below 5% or viability results may be skewed



Best Practices

- Make sure to always load from the same part of the sample tube to ensure consistency
- It is easiest to do this by placing the cassette at the very bottom of the tube
- Depress the plunger until it is flat and even with the top of the cassette



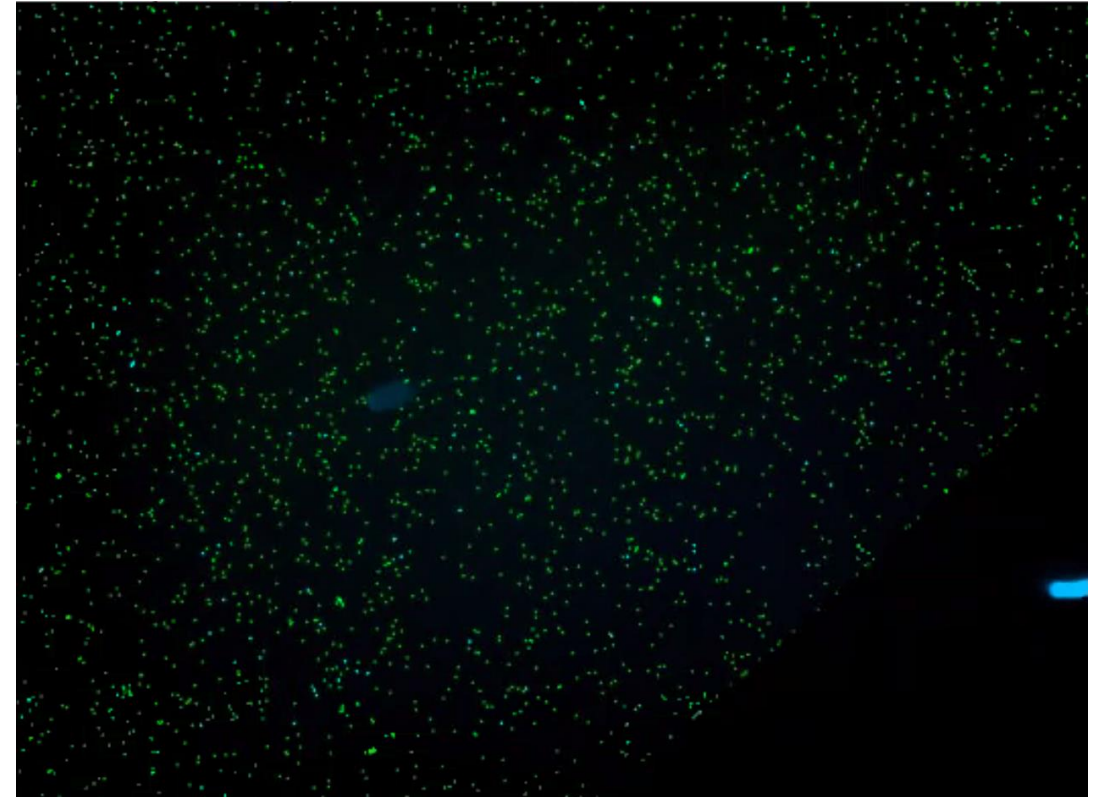
Best Practices

- Insert your cassette into the instrument until you feel it “click” past the ball bearing, locking it into place
- After a run don’t forget to remove your used cassette and properly dispose of it!



Best Practices

- After a run it is always a good idea to check for bubbles
- If a bubble is present, the sample must be re-run as the count will be inaccurate
- It is also a good idea to check the gating of your sample to ensure the majority of your cell population falls within the gate

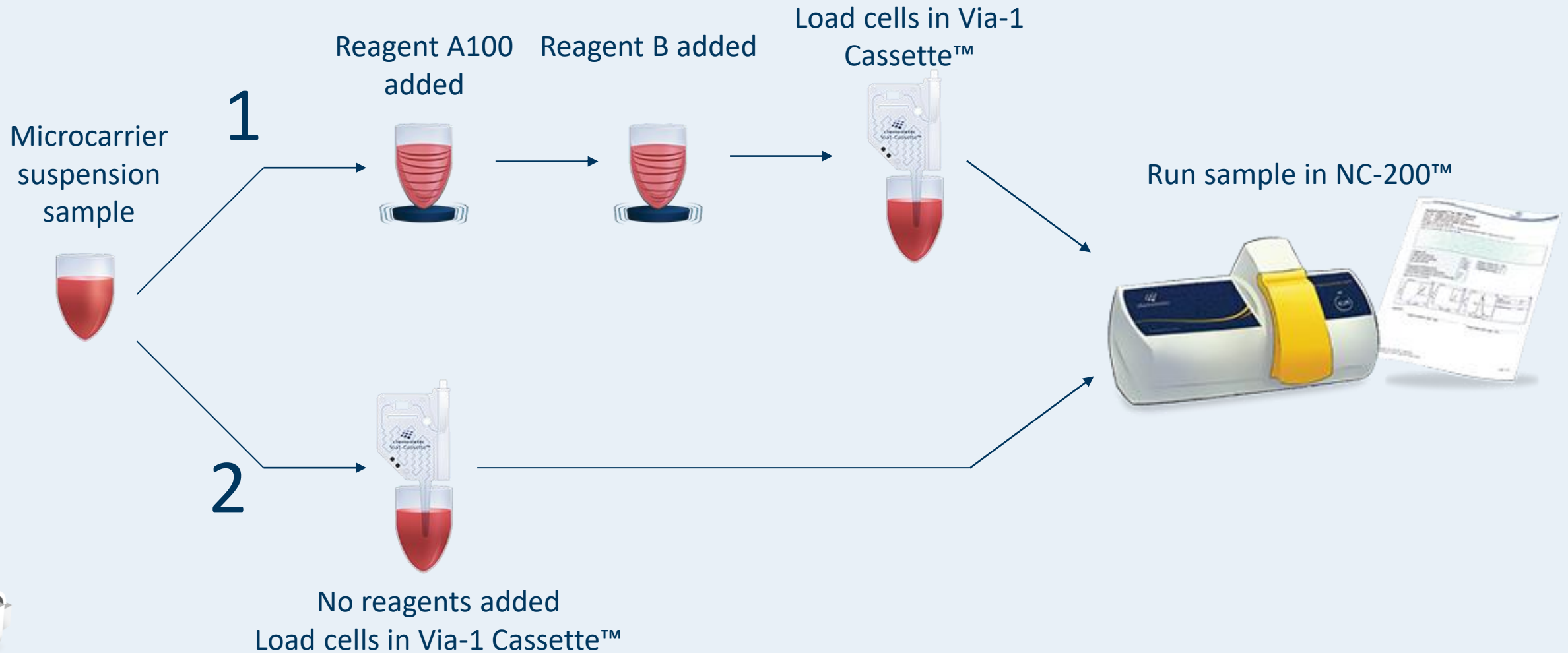


Best Practices – Using Other Built In Assays

Sample Type	Normal Suspension Cells	Aggregated/Adherent cells (% aggregation >20%)	Whole blood or other RBC containing samples	Cells Grown on Microcarriers
Protocol to Use	Viability and Cell Count	Viability and Cell Count – Aggregated Cells Assay	Viability and Cell Count – Blood Assay	Viability and Cell Count – A100 and B Assay
Lysis Solution to Use	None	Solution 10	Solution 17	Solutions A100 & B



Microcarrier Cultured Cells



PLYMOUTH
MEDICAL

www.PlymouthMedical.com

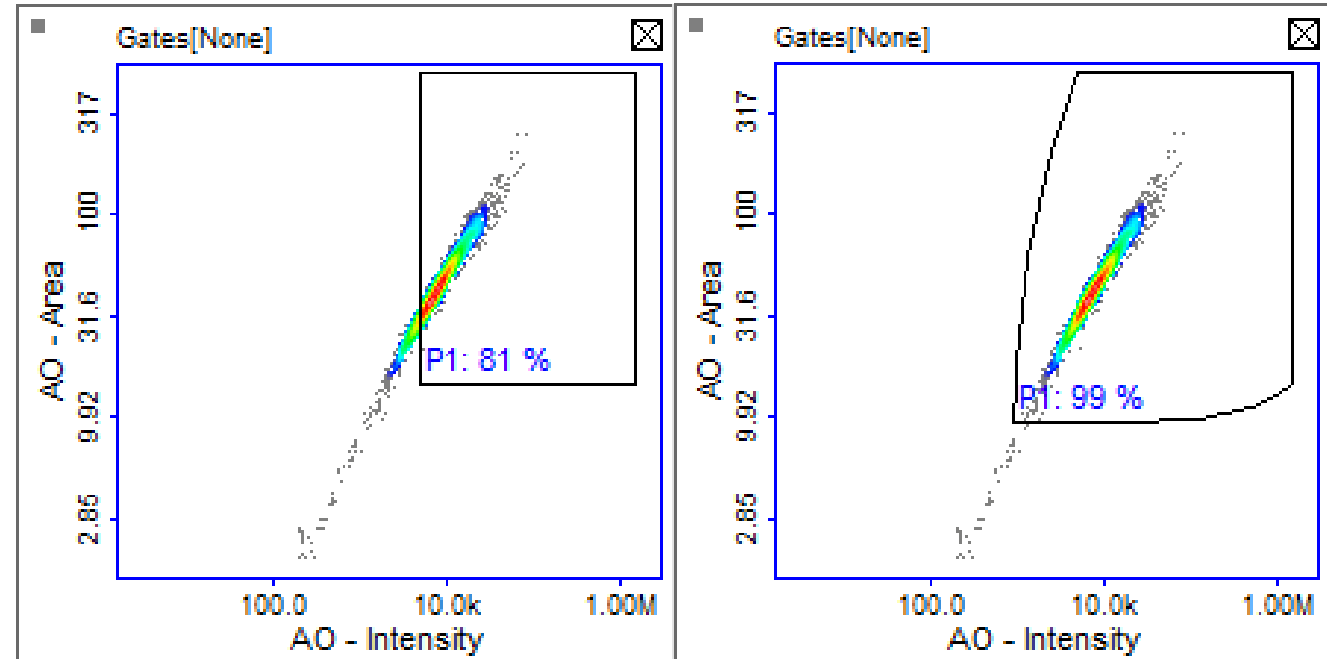
Topics for the Day

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Gating Changes

- For some cell types, you may see a shift in the cell population on the AO scatterplot.
- This is especially prevalent in patient samples as real world cells tend to have more variability than cultured cells
- To ensure that your counts are as accurate as possible as well as reproducible, we recommend changing the gating in these cases



Gating Changes

- First you will want to bring your data into the plot manager
- This can be done by either right clicking a single data file and selecting “Show Data” or by opening the plot manager in Tools> Plot Manager, and dragging multiple files in from the file browser

The screenshot shows the Chemometec NucleoView NC-200 software interface. The main window displays a dark field with green fluorescent spots. A file browser on the right shows a list of files, with '20180817-0001-c-293-1.cm' selected. A context menu is open over this file, listing options such as 'Show Data', 'Show Raw Data', and 'Reanalyze Image File with Selected Protocol'. Below the file browser, there are fields for 'Sample ID' (293-1) and 'Operator' (NucleoCounter). At the bottom right, a summary table displays the following data:

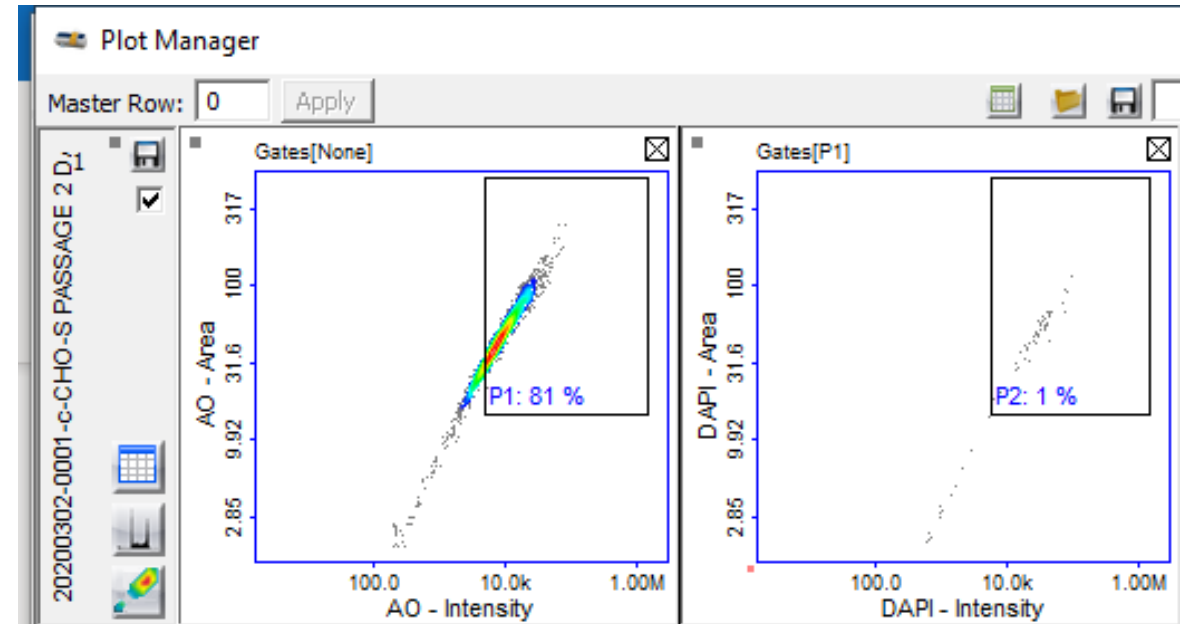
Total cells/ml	1.86E6
Viability %	91.0
Diameter um	14.8

At the bottom left, there are input fields for 'Sample [ul]' (200) and 'Dilution [ul]' (0), and a 'Multiplication Factors' field showing '1 # 1.00'. A 'RETURN' button is also visible.



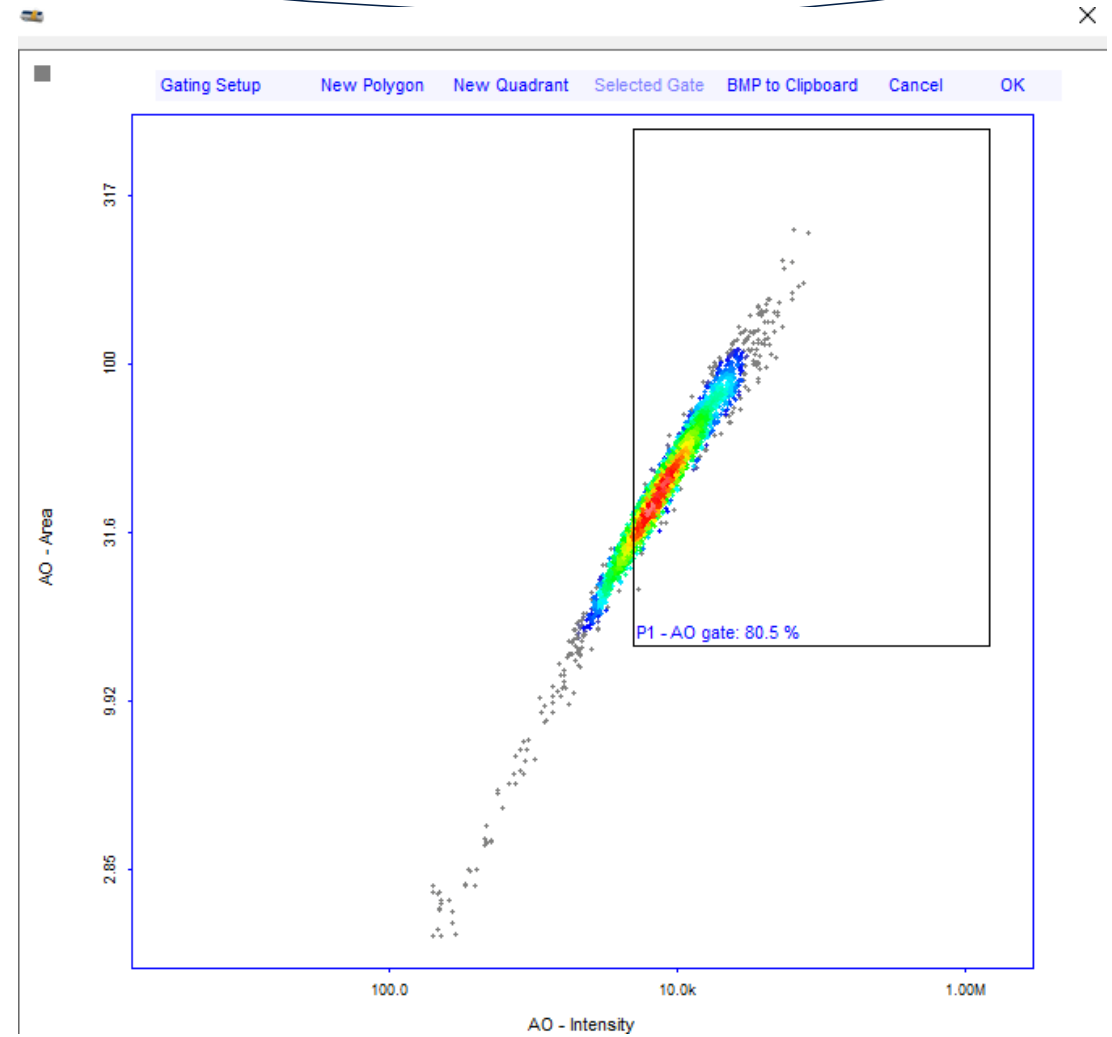
Gating Changes

- Once the plot manager is open, double click on the scatterplot you wish to edit to enlarge it



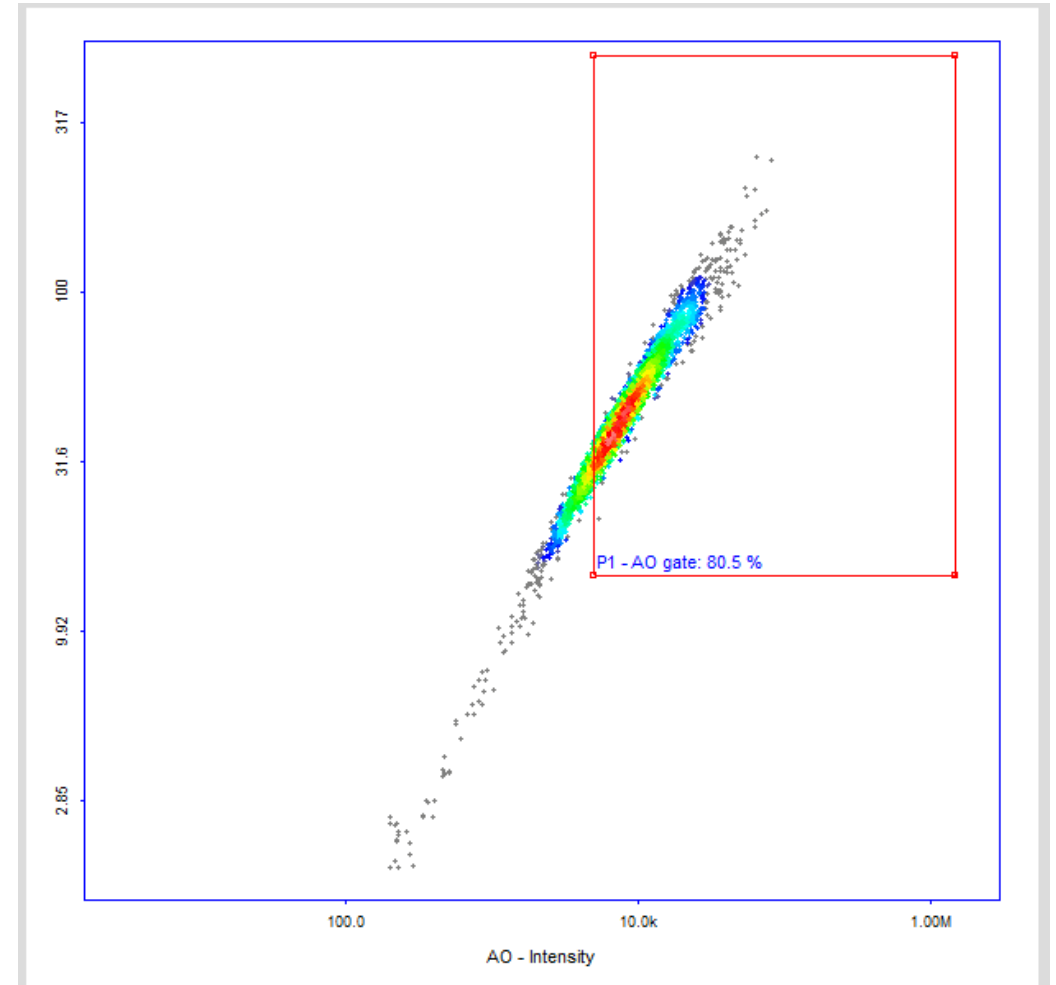
Gating Changes

- Begin by double clicking the gate to turn it red and give it little handles on each corner



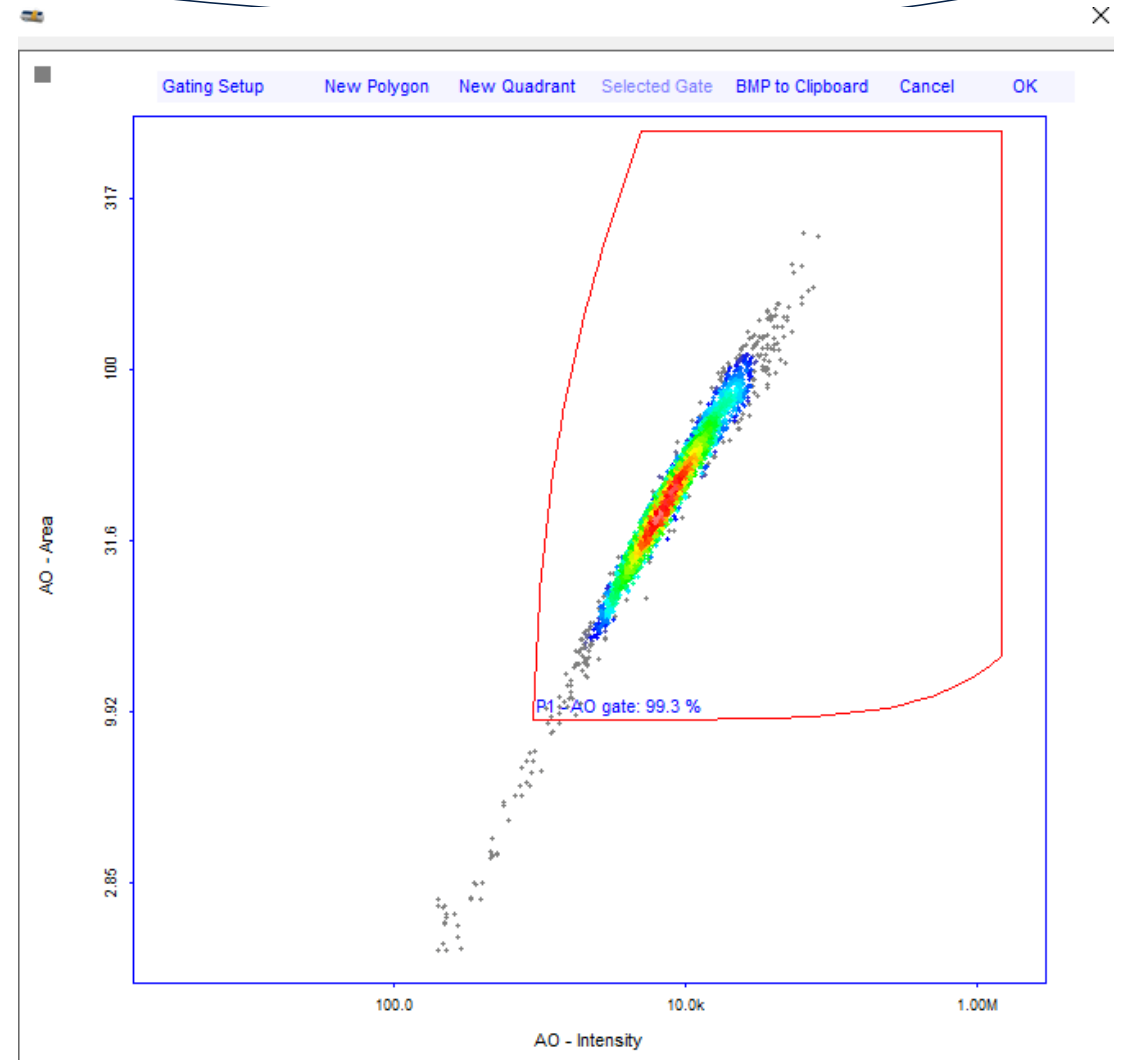
Gating Changes

- Begin by double clicking the gate to turn it red and give it little handles on each corner
- Using the handles, we can drag the corners of the gate to a desired position



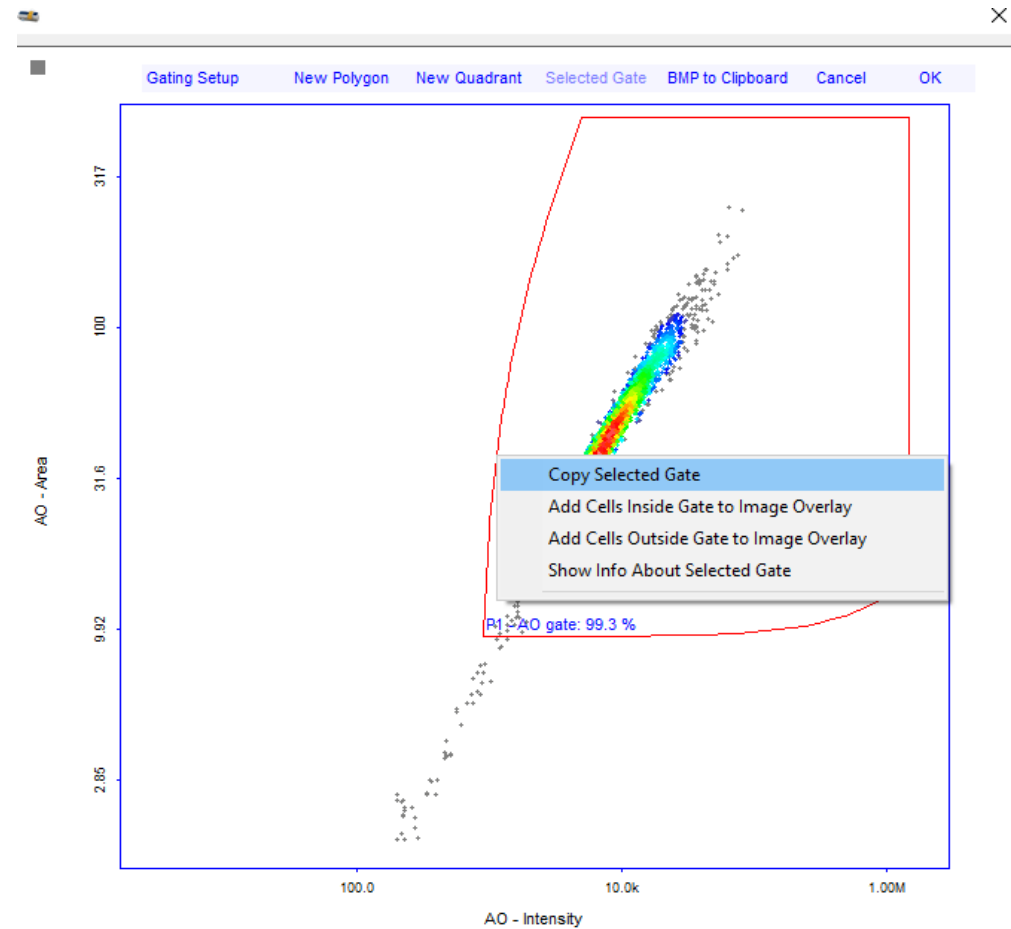
Gating Changes

- Begin by double clicking the gate to turn it red and give it little handles on each corner
- Using the handles, we can drag the corners of the gate to a desired position
- Once the gate is placed, you must click in the white space to de-select the gate and save your changes



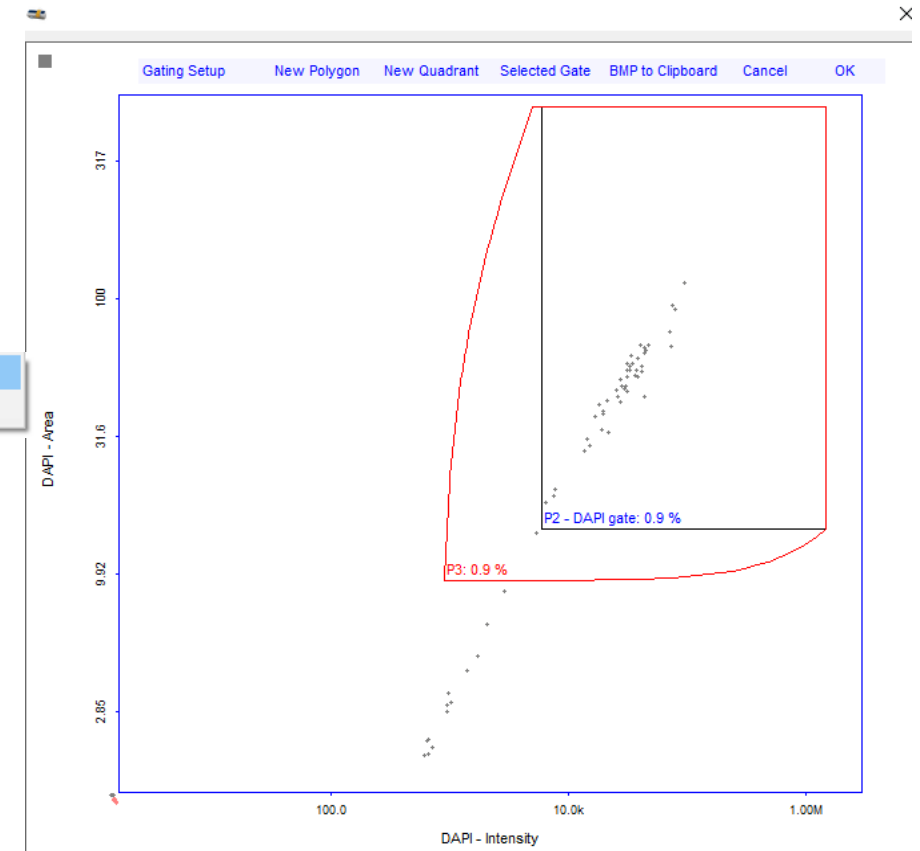
Gating Changes

- To copy your gate to transfer it to your DAPI channel, click the gate once to turn it red without handles, and then right click it to open a small drop down menu
- From here you may copy the gate and then press OK to save your change. This is just a temporary save, the gating changes as a whole must be saved in the plot manager



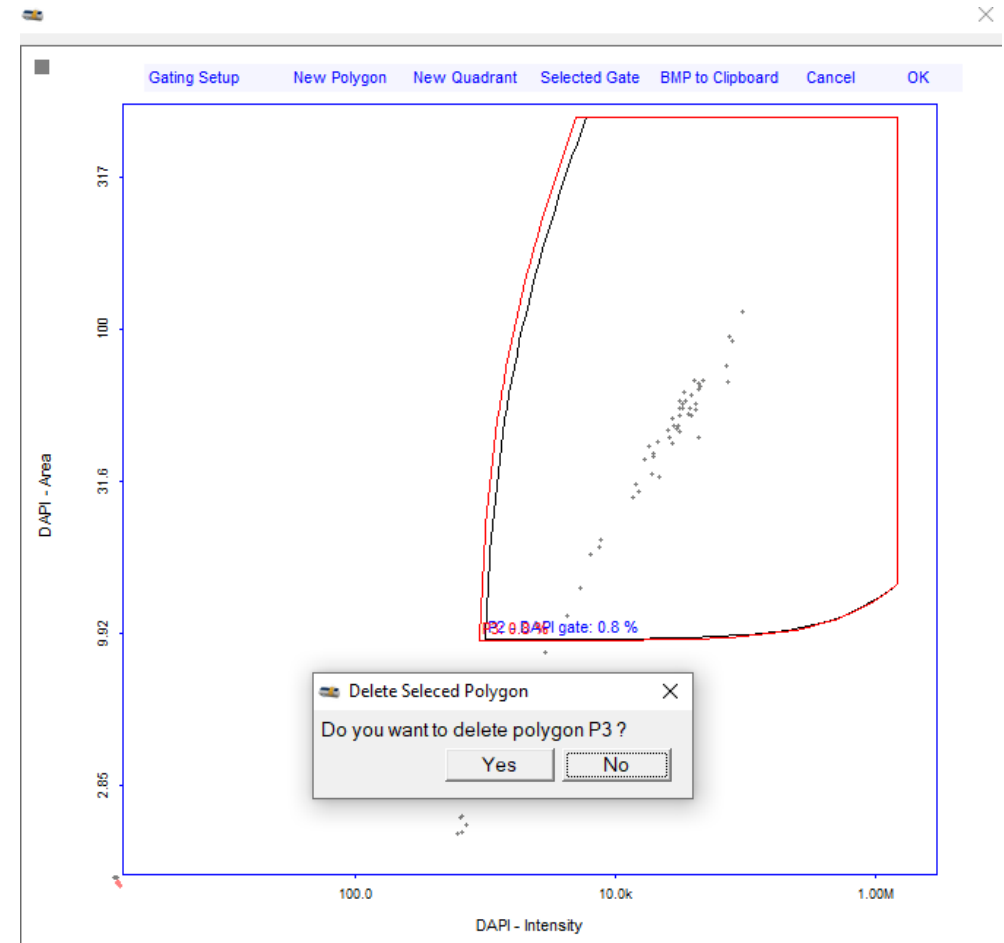
Gating Changes

- To paste the gate in your DAPI channel, open it by double clicking, and then right click in the white space
- This will open a small drop down menu that allows you to paste the gate in.



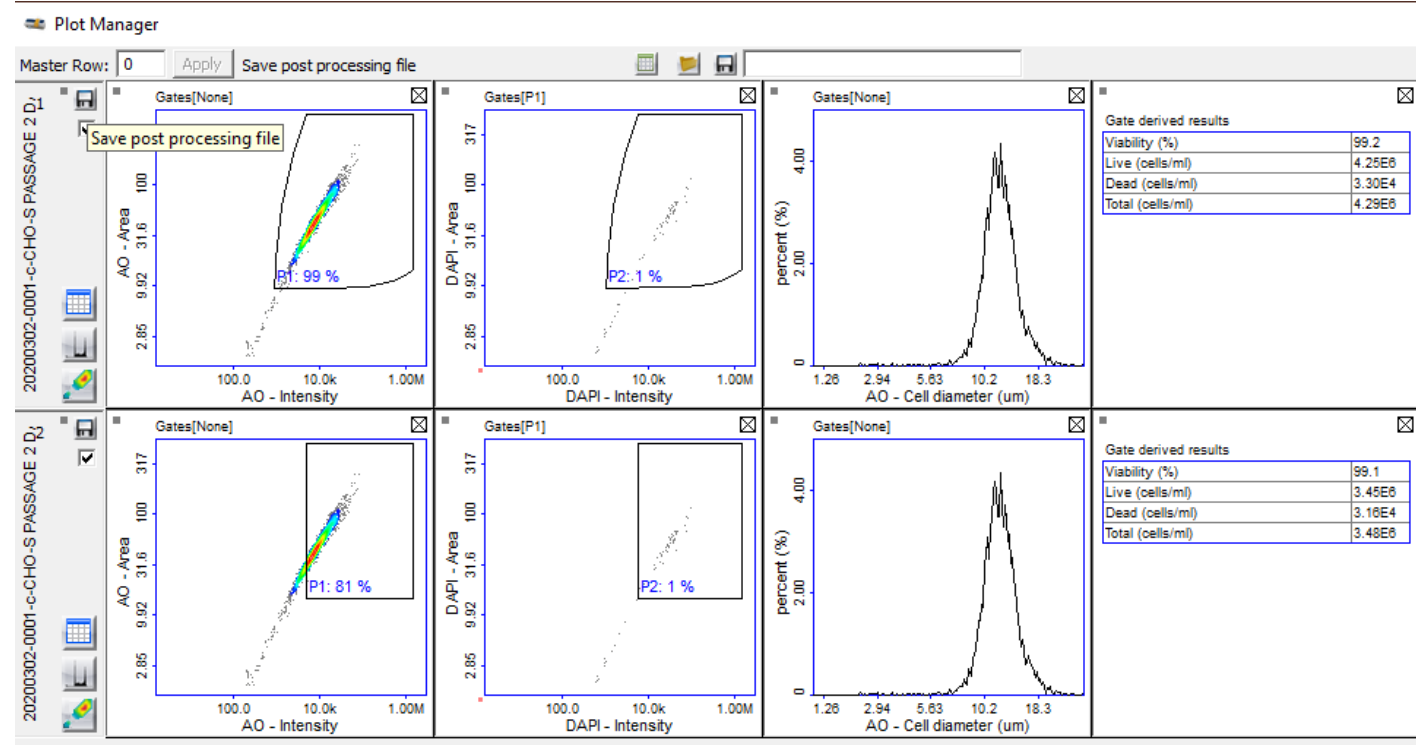
Gating Changes

- Once the gate is pasted, you will need to adjust the default DAPI gate to match closely with the pasted gate.
- To make your data look cleaner, you may then delete the pasted gate by clicking it once and pressing the Delete key on your keyboard
- Exit the gate window by pressing OK



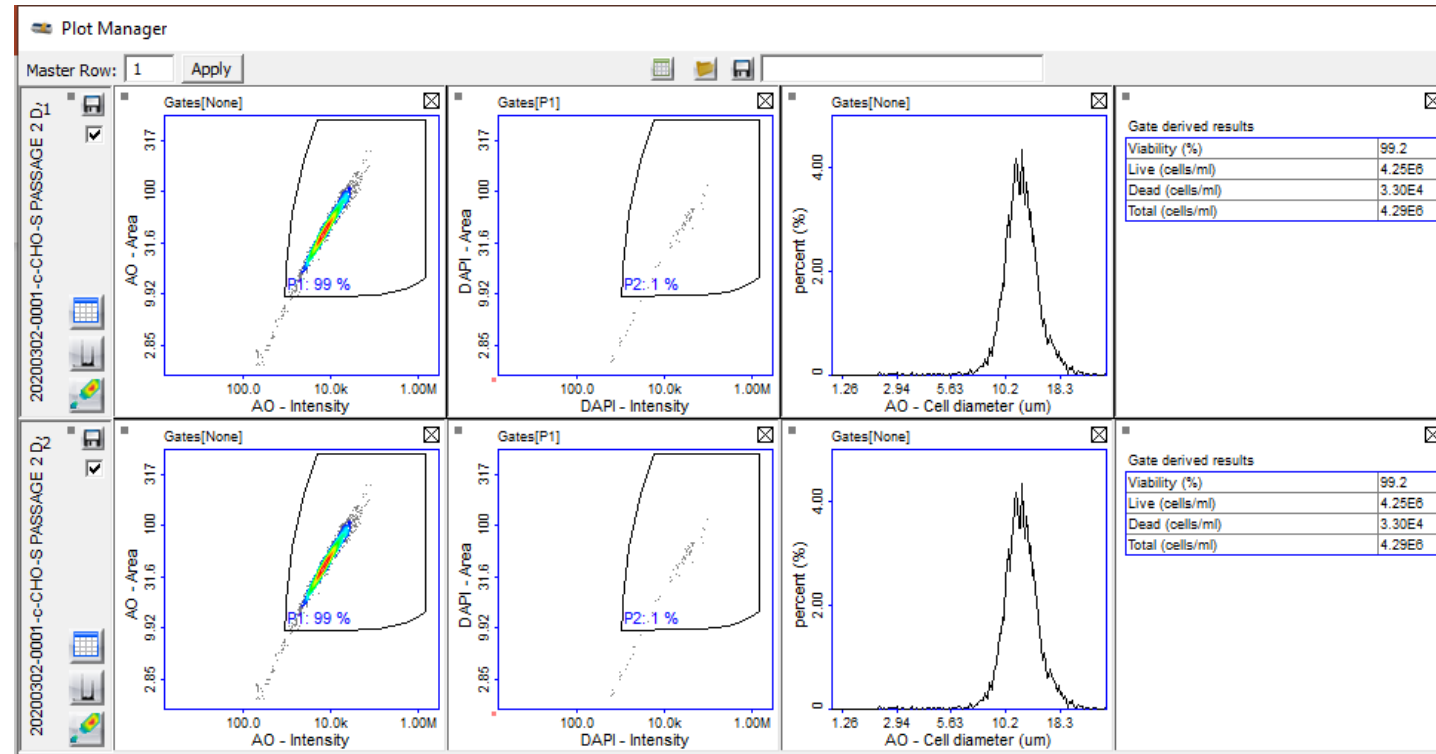
Gating Changes

- Now that your gates have been applied make sure you save your changes by clicking the floppy disk icon at the top left corner of the data row
- To copy your gating to multiple data sets at once to ensure it is a universal fit, enter the changed row in the master row field at the top of the window and hit apply.



Gating Changes

- Now that your gates have been applied make sure you save your changes by clicking the floppy disk icon at the top left corner of the data row
- To copy your gating to multiple data sets at once to ensure it is a universal fit, enter the changed row in the master row field at the top of the window and hit apply.



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- **Optimizing Protocols**
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Optimizing Protocols

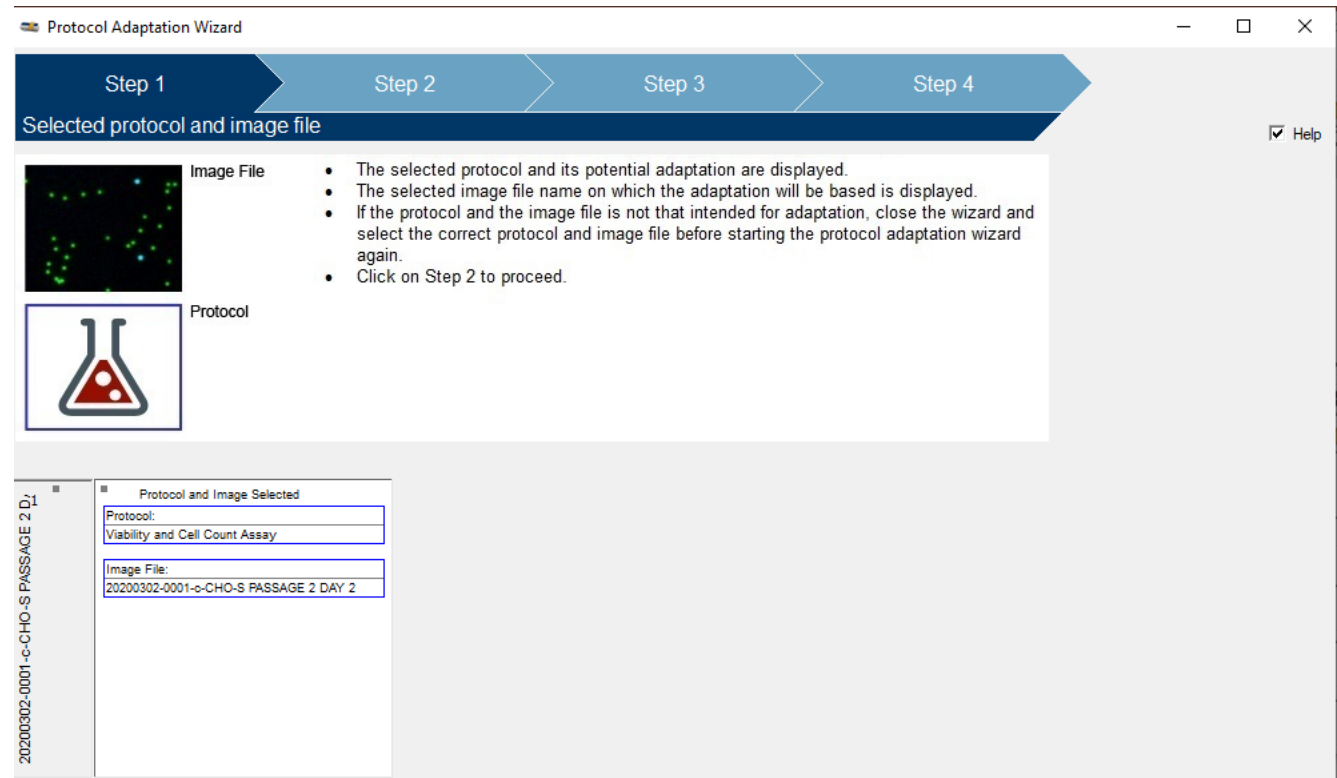
- Once your gating is set and it reliably covers your data set, you may wish to generate a protocol utilizing your custom gating
- To do this, first right click on the data set you have adjusted and saved the gating on and select Start Protocol Adaptation Wizard

The screenshot shows the ChemoMetec NucleoView NC-200 software interface. The main window displays a fluorescence image with green spots on a dark background. A context menu is open over the image, listing various actions such as 'Show Data', 'Show Raw Data', 'Reanalyze Image File with Selected Protocol', 'Add to Report', 'Print...', 'Print with Plots...', 'Create PDF Report...', 'Approve...', 'Start Protocol Adaptation Wizard', and 'Properties'. The 'Start Protocol Adaptation Wizard' option is highlighted. Below the image, there are input fields for 'Sample [ul]' (200), 'Dilution [ul]' (0), and 'Multiplication Factors' (1 # 1.00). On the right side, there are summary statistics: 'Total cells/ml' (3.48E6), 'Viability %' (99.1), and 'Diameter um' (12.5).



Optimizing Protocols

- Once opened, you will be guided through the protocol creation in four steps
- Step one allows you to double check to ensure you have the correct parent protocol and data file selected



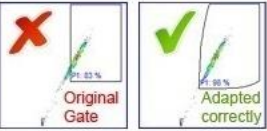
Optimizing Protocols

- Step two will allow you to import your custom gating as well as any changes to the analysis you have made
- When first coming to step two you will notice that the gating has reverted to its default setup

Protocol Adaptation Wizard

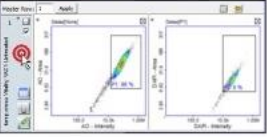
Step 1 Step 2 Step 3 Step 4

Adapt Gating and Setup Master File Help



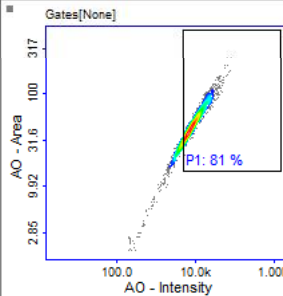
Original Gate: 81.8%

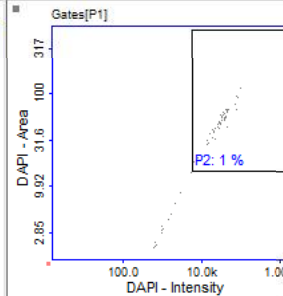
Adapted correctly: 99.1%

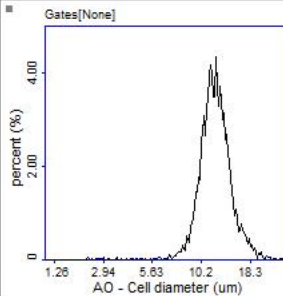


- To adapt a counting gate, double click on a scatter plot below to enlarge the plot.
- Click on the gate to select it, mark the corners of the gate by clicking on the gate again and move the corners of the gate by dragging the small squares.
- The best position of the gate is where the cell population is clearly separated from the debris. Make sure not to include debris in the gate or to place it too close to the main cell population.
- When the adaptation of the gate is satisfactory click "OK" in the upper right corner.
- Optionally setup data presentation in one row by adding the preferred scatter plots, histograms, table plots, markers, quadrants, polygons and tables. Note that changes in one row will be applied to all rows.
- To select a master file, click on the row dialog (the gray area in the left side around the scatter plot and histogram buttons). A click on the row dialog again deselects it as master file.
- Click on Step 3 to proceed.

20200302-0001-c-CHO-S PASSAGE 2 D₁







Gate derived results	
Viability (%)	99.1
Live (cells/ml)	3.45E8
Dead (cells/ml)	3.16E4
Total (cells/ml)	3.48E8



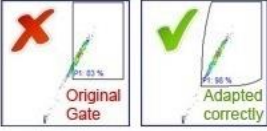
Optimizing Protocols

- To bring your custom gates in, simply right click on the grey space and select Reload Post Processing
- A pair of popups will appear to confirm you want to load the custom gating for both the AO and DAPI channels

Protocol Adaptation Wizard

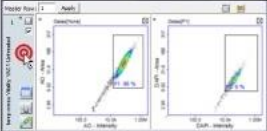
Step 1 Step 2 Step 3 Step 4

Adapt Gating and Setup Master File Help



Original Gate

Adapted correctly



- To adapt a counting gate, double click on a scatter plot below to enlarge the plot.
- Click on the gate to select it, mark the corners of the gate by clicking on the gate again and move the corners of the gate by dragging the small squares.
- The best position of the gate is where the cell population is clearly separated from the debris. Make sure not to include debris in the gate or to place it too close to the main cell population.
- When the adaptation of the gate is satisfactory click "OK" in the upper right corner.
- Optionally setup data presentation in one row by adding the preferred scatter plots, histograms, table plots, markers, quadrants, polygons and tables. Note that changes in one row will be applied to all rows.
- To select a master file, click on the row dialog (the gray area in the left side around the scatter plot and histogram buttons). A click on the row dialog again deselects it as master file.
- Click on Step 3 to proceed.

20200302-0001 -c-CHO -S PASSAGE 2 D1

Gates[None]

317

0

2.85

100.0 10.0k 1.00M

AO - Intensity

Gates[P1]

317

100 100

2.85 9.92 31.6

100.0 10.0k 1.00M

DAPI - Area

DAPI - Intensity

P2: 1%

Gates[None]

4.00

2.00

0

1.28 2.94 5.63 10.2 18.3

Percent (%)

AO - Cell diameter (um)

Gate derived results

Viability (%)	99.1
Live (cells/ml)	3.45E8
Dead (cells/ml)	3.16E4
Total (cells/ml)	3.48E8

Show Image

Save As Master...

Reload Post Processing



Optimizing Protocols

- After your custom gating has been loaded, left click the grey space to the right of the AO gate to select the row
- Without selecting the row to be used for the analysis of the protocol, the gating will revert to default

Protocol Adaptation Wizard

Step 1 Step 2 Step 3 Step 4

Adapt Gating and Setup Master File Help

- To adapt a counting gate, double click on a scatter plot below to enlarge the plot.
- Click on the gate to select it, mark the corners of the gate by clicking on the gate again and move the corners of the gate by dragging the small squares.
- The best position of the gate is where the cell population is clearly separated from the debris. Make sure not to include debris in the gate or to place it too close to the main cell population.
- When the adaptation of the gate is satisfactory click "OK" in the upper right corner.
- Optionally setup data presentation in one row by adding the preferred scatter plots, histograms, table plots, markers, quadrants, polygons and tables. Note that changes in one row will be applied to all rows.
- To select a master file, click on the row dialog (the gray area in the left side around the scatter plot and histogram buttons). A click on the row dialog again deselects it as master file.
- Click on Step 3 to proceed.

temp cmsu_20200302.0001-c-CHO-S-P1

Gates[None]

AO - Area: 317
AO - Intensity: 100.0, 10.0k, 1.00M

Gates[P1]

DAPI - Area: 317
DAPI - Intensity: 100.0, 10.0k, 1.00M

Gates[None]

percent (%): 4.00, 2.00, 0
AO - Cell diameter (um): 1.28, 2.94, 5.63, 10.2, 18.3

Gate derived results

Viability (%)	99.2
Live (cells/ml)	4.24E8
Dead (cells/ml)	3.23E4
Total (cells/ml)	4.27E8



Optimizing Protocols

- Step three will allow you to set a few variables for your protocol
- This will let you assign a default sample volume and dilution for the protocol
- You will also be able to customize the three results fields you see on the main nucleoview screen after completing a count

Protocol Adaptation Wizard

Step 1 Step 2 Step 3 Step 4

Adapt parameters Help

Parameter Selection	
Show count gates in Plot Manager	no
Sample Volume	200
Dilution Volume	0
Right result field	Total cells/ml
Left result field	Viability %

- Double click on the small table plot to open a large version.
- The counting gates can be displayed in the plot manager after each run of the adapted protocol.
- This is recommended in a phase when evaluating and validating new gate-settings, and to ensure that the new gating can contain the normal biologic variation of the cell line.
- The default volumes can be changed and the output in the result fields can be selected.
- When the parameters has been changed click "OK" in the upper right corner of the large table plot.
- Click on Step 4 to proceed.

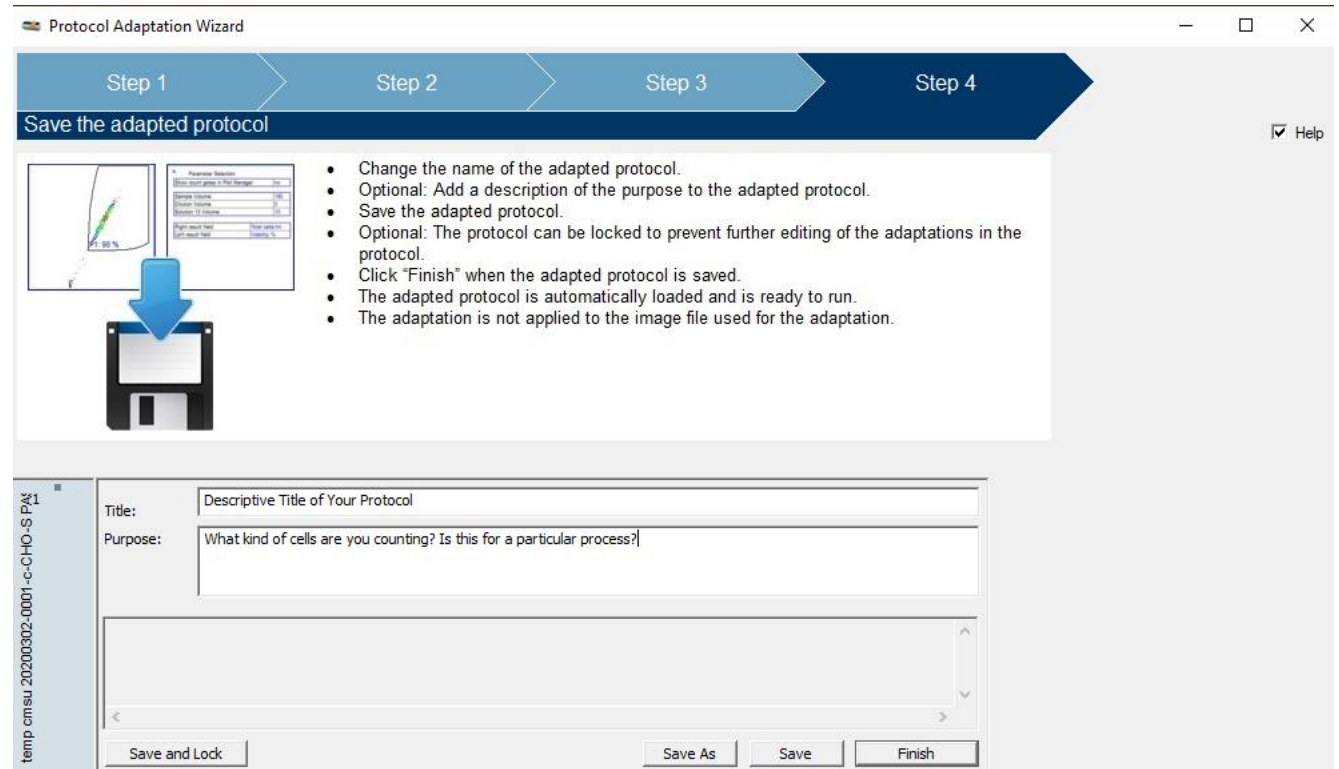
temp cmsu:20200302-0001-c-CHO-S-FX1

Parameter Selection	
Show count gates in Plot Manager	no
Sample Volume	200
Dilution Volume	0
Top result field	Total cells/ml
Middle result field	Viability %
Bottom result field	Diameter um



Optimizing Protocols

- Lastly, step four will allow you to give your protocol a name and a brief description
- Please ensure to save and finish your protocol before exiting the window
- For a protocol to be used on a 21 CFR enabled instrument you will be required to select the option Save and Lock, as only locked protocols may be used on a GMP instrument



Topics for the Day

- Software Introduction
- Best Practices
- Gating Changes
- Optimizing Protocols
- Performing a Comparison Study



Performing a Comparison Study

- When generating a new protocol, or evaluating a new method it is necessary to gather data to show that the integrity of your process is maintained
- It is critical to evaluate both your methods Reproducibility as well as its Linearity
- Reproducibility – The ability of your method or protocol to obtain consistent results when repeated. A good measure of this would be your samples CV%
- Linearity – The ability of your data to be described by a linear function. This is critical to evaluate whether your sample will be reliably counted within the linear range of the instrument.



Performing a Comparison Study

- The first thing to consider when performing a comparison study is the experimental design
- Sarkar et al. put forth a method using a single sample taken from your culture, and then aliquoted into various dilutions
- These dilutions are then run in triplicate for each method under scrutiny, allowing the calculation of a CV% to compare the statistical reproducibility of each method
- In addition, the average of each dilution triplicate can be plotted against its dilution factor to determine the Linearity of the method

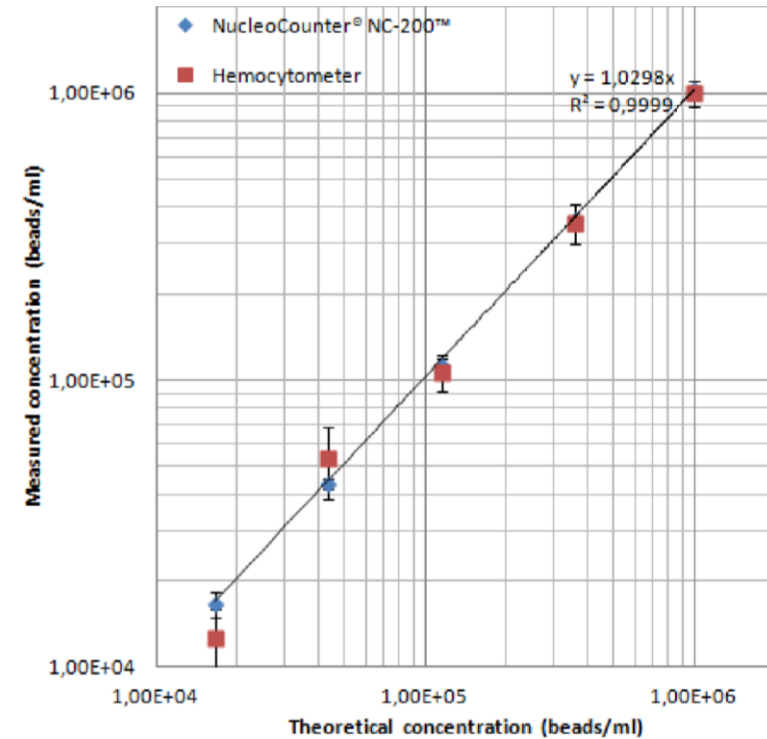


Figure 2: A dilution range of a bead solution was used to compare counting in triplicates with the NucleoCounter® NC-200™ and standard Burker-Turk hemocytometers.



Performing a Comparison Study

- In addition to the sampling and dilution schema, there are other important factors to take into consideration when designing your comparison
- Common Homogenous Stock Solution
- Independent Dilution Fractions
- Blinded random Labeling and randomized measurement order

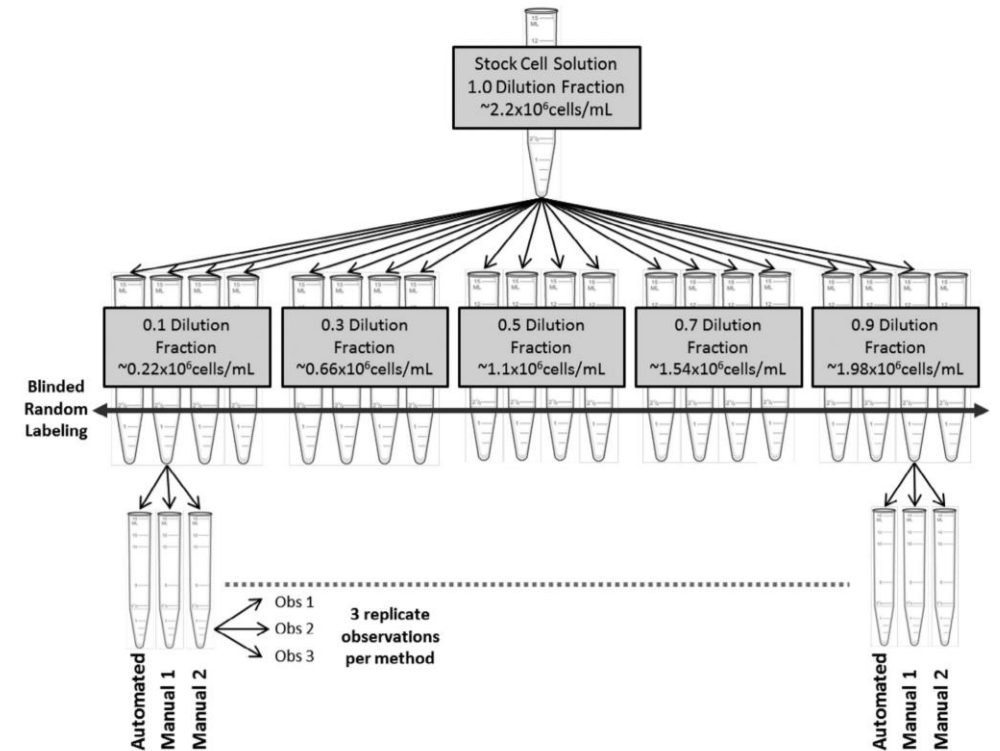


Figure 2. Schematic representation of the cell counting dilution series study experimental design.

Sarkar S, Lund SP, Vyzasatya R, et al. Evaluating the quality of a cell counting measurement process via a dilution series experimental design. *Cytotherapy*. 2017;19(12):1509-1521.



Performing a Comparison Study

- While performing the comparison study, a number of other factors must also be carefully controlled to ensure even and fair sampling between methods
- The stock solution must be homogeneously mixed prior to sampling
- Dilution Fractions are well controlled and evenly pipetted by a qualified pipette
- Samples should be unaffected by the dilution process, and most importantly, the diluent

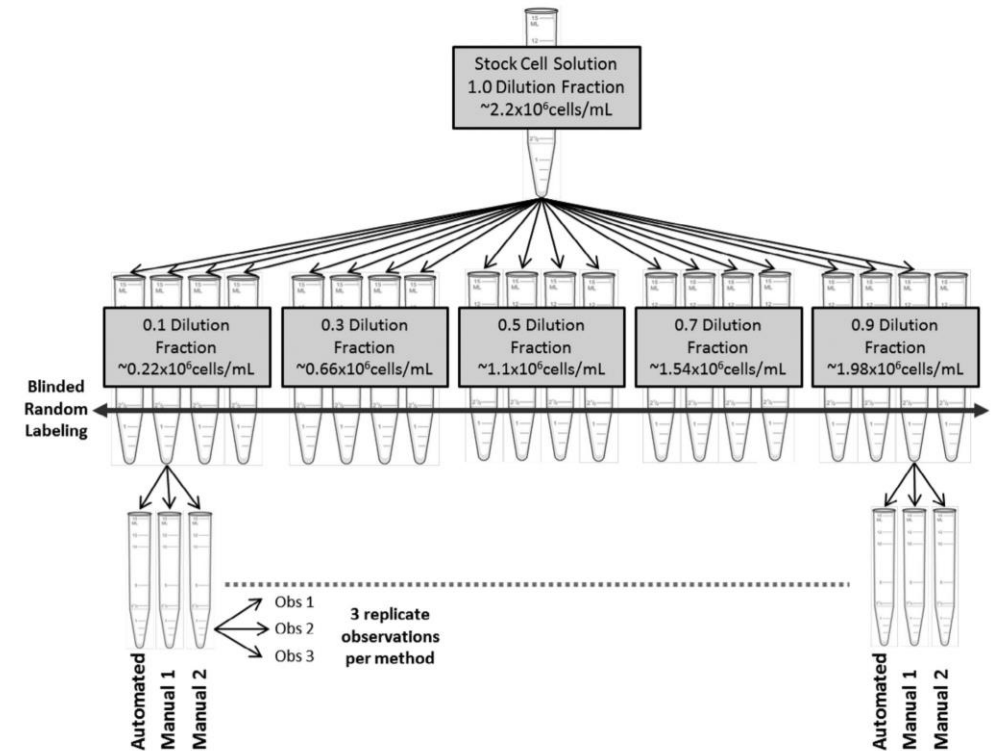


Figure 2. Schematic representation of the cell counting dilution series study experimental design.

Sarkar S, Lund SP, Vyzasatya R, et al. Evaluating the quality of a cell counting measurement process via a dilution series experimental design. *Cytotherapy*. 2017;19(12):1509-1521.



Practical Considerations When Performing a Comparison Study

- When comparing two methods it is important to take into consideration what is actually being measured so that a comparison may be drawn
- Trypan Blue staining for instance measures the ability of a particle to exclude the stain from its interior, so larger vesicles or apoptotic fragments may be counted as a cell, skewing your count and viability
- Florescence DNA staining however only considers a particle with a nucleus as a cell, limiting the influence of debris

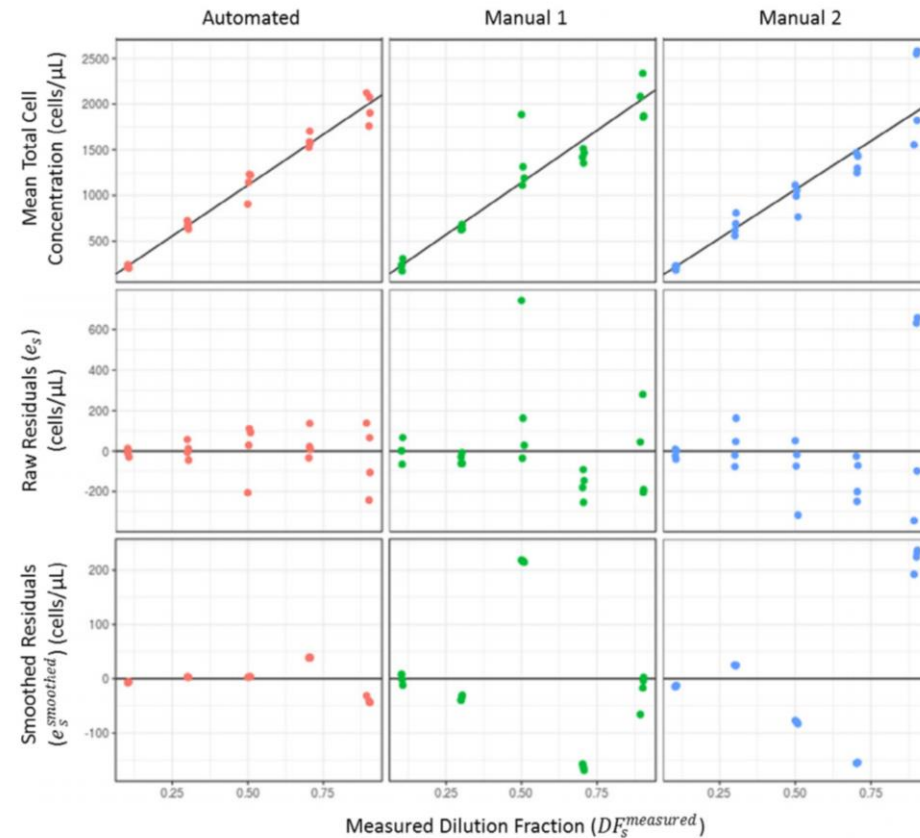


Figure 4. (Top panel) Mean of triplicate observations of cell concentration for four independent test samples over each of five dilution fractions for three devices (Automated [red], Manual 1 [green] and Manual 2 [blue]) with fitted proportional model (black line). (Middle panel) Raw residuals calculated from difference between total cell concentration for each sample and the proportional model fit. (Lower panel) Smoothed residual calculated from the difference between flexible model estimate of total cell concentration and the proportional model fit.

Sarkar S, Lund SP, Vyzasatya R, et al. Evaluating the quality of a cell counting measurement process via a dilution series experimental design. *Cytotherapy*. 2017;19(12):1509-1521.



Practical Considerations When Performing a Comparison Study

- It is also critical to consider the potential user to user variation when deciding on a method to adopt
- Any subjectivity is a potential source of error, so for manual counting extensive training is required to standardize what is considered a cell and what is not

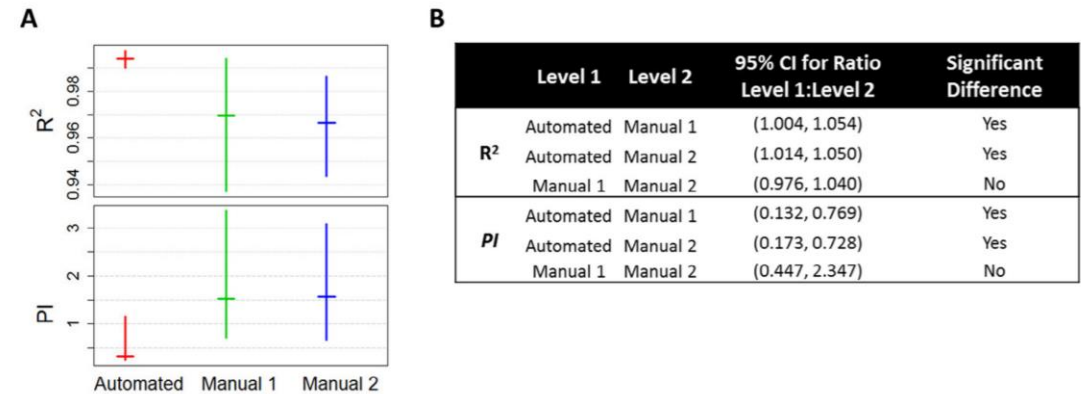


Figure 5. (A) R^2 and PI summary statistic (PI^{SabsSR}) for total cell concentration measurements with error bars representing nonparametric bootstrap estimate of 95% CIs for PI and R^2 . (B) Table indicating statistically significant differences between methods for R^2 values and PI values evaluated by examining the 95% CI for the ratio of R^2 values and PI values between methods.