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 µl into the Via1-Cassette™
Measurement volume:	1.4 µl 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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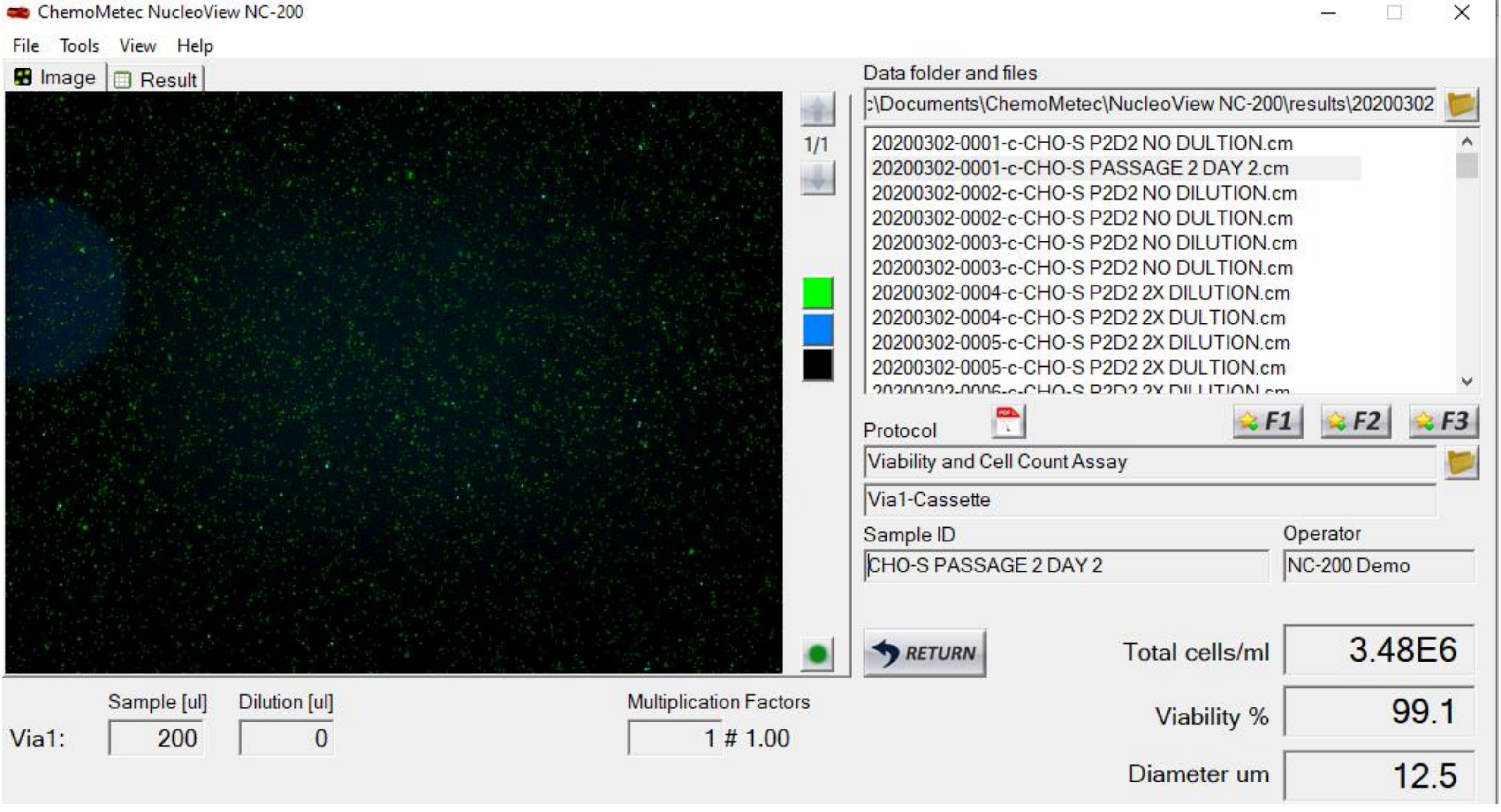
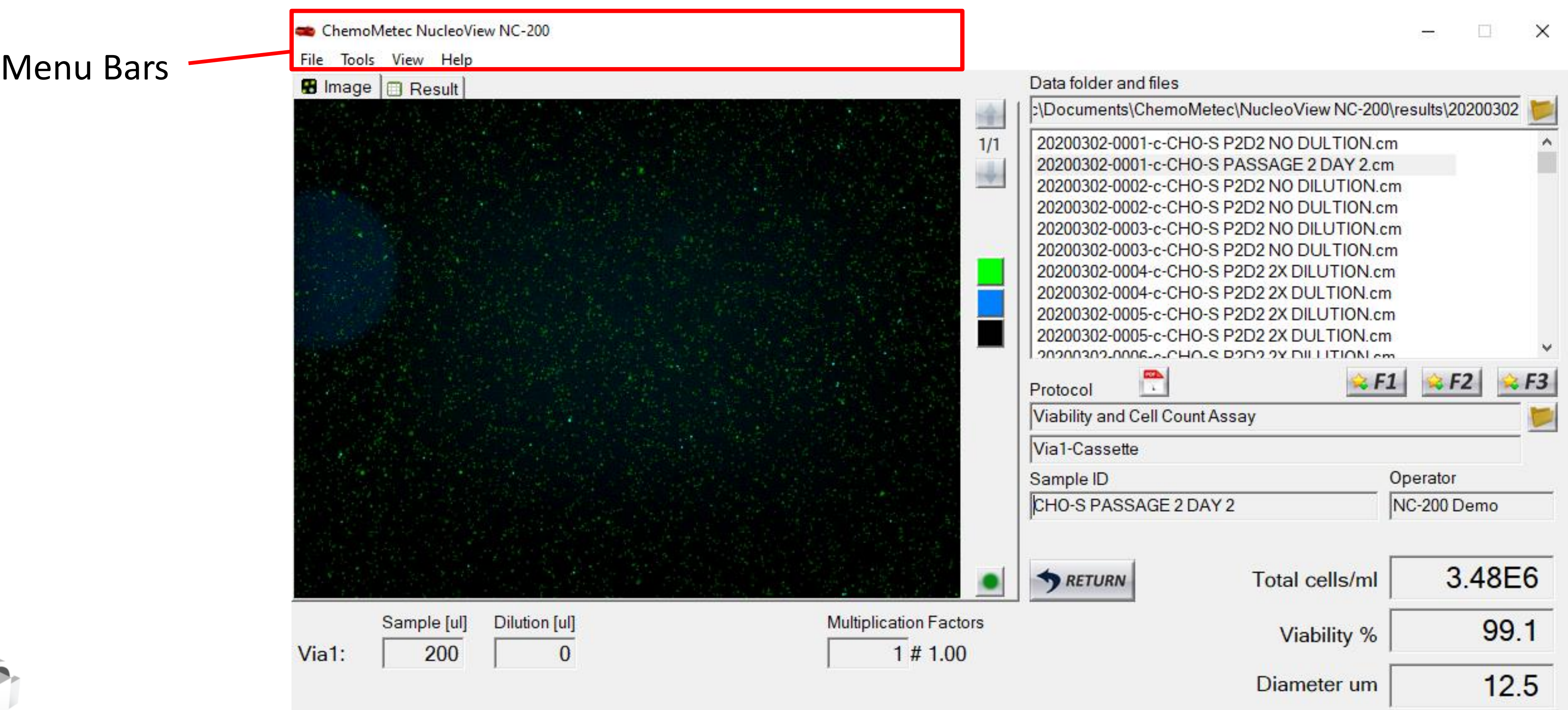
Software Introduction

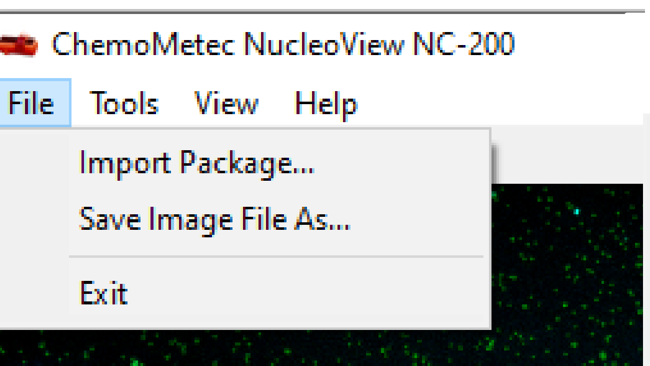


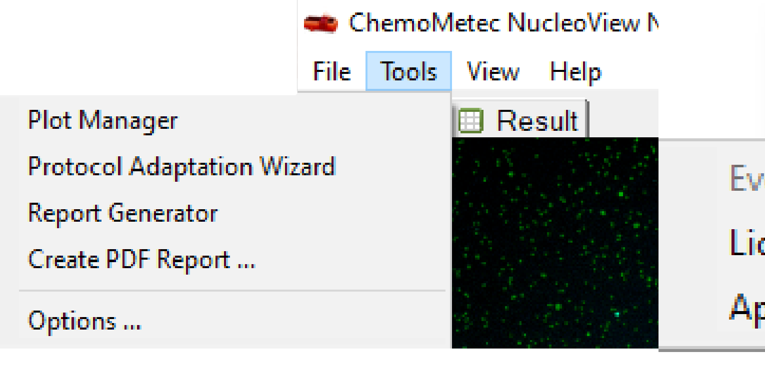
Software Introduction

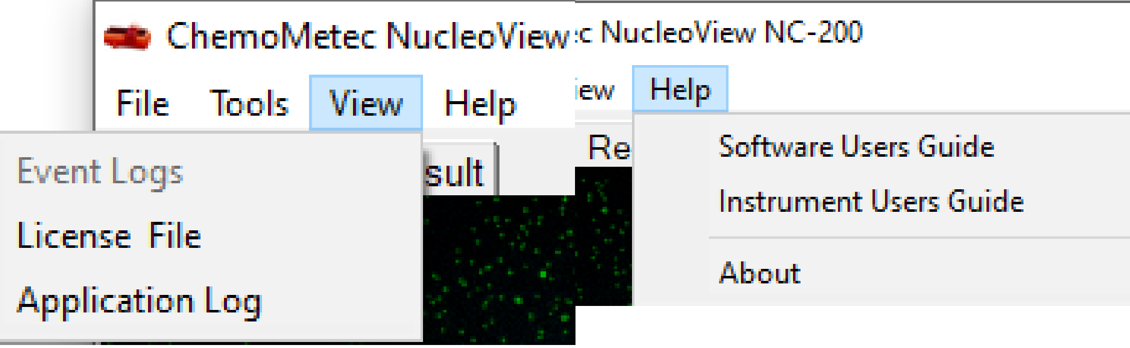
Menu Bars



Menu Bars







File
- Import adapted protocols
- Export images

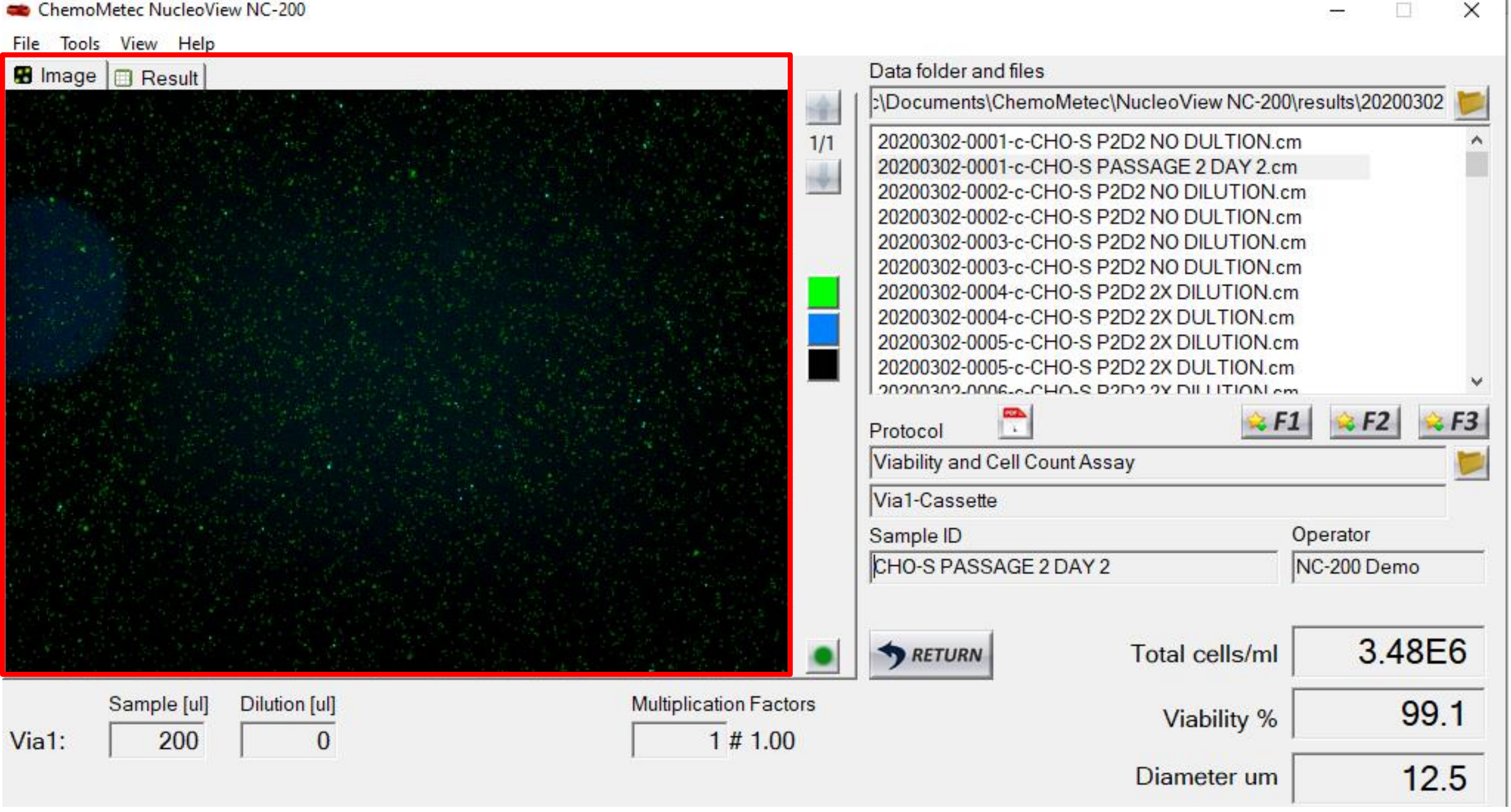
Tools
- Opens Plot Manager
- Starts Protocol Adaption Wizard
- Create PDFs
- Open the options menu

View
- Access Log files

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

Software Introduction

Image and Results Window



Software Introduction

Dilution Calculator

Software Introduction

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

Protocol F1 F2 F3

Viability and Cell Count Assay

Via1-Cassette

Sample ID: CHO-S PASSAGE 2 DAY 2

Operator: NC-200 Demo

Protocol Information

RETURN

Total cells/ml 3.48E6

Viability % 99.1

Diameter um 12.5

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

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Protocol Selection

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
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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
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RETURN

Total cells/ml 3.48E6

Viability % 99.1

Diameter um 12.5

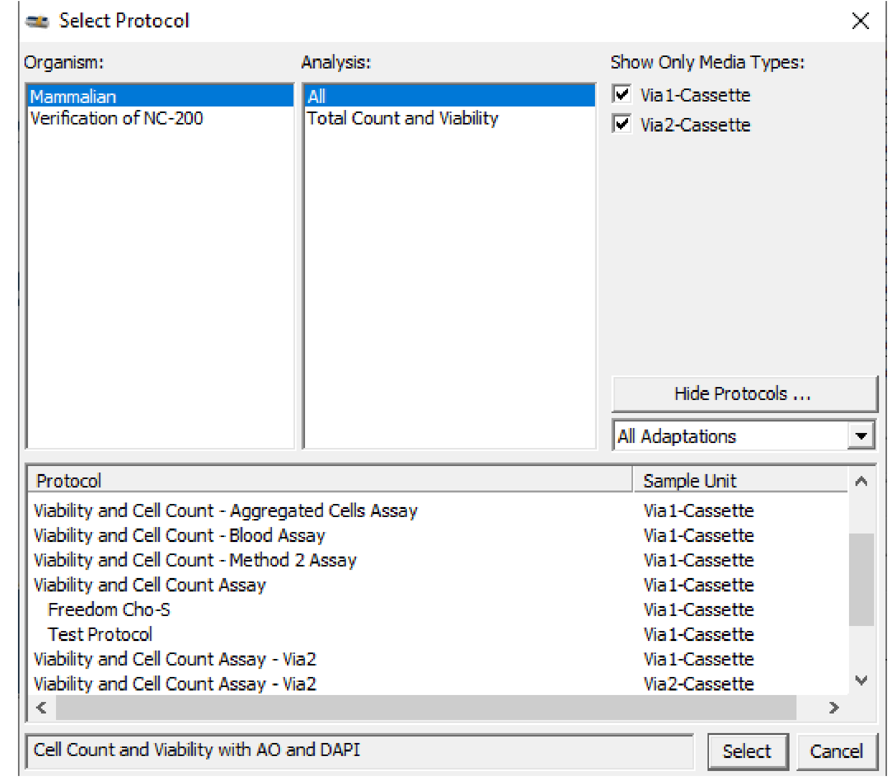
Sample [ul] Dilution [ul] Multiplication Factors

Via1: 200 0 1 # 1.00

Protocol Select Menu

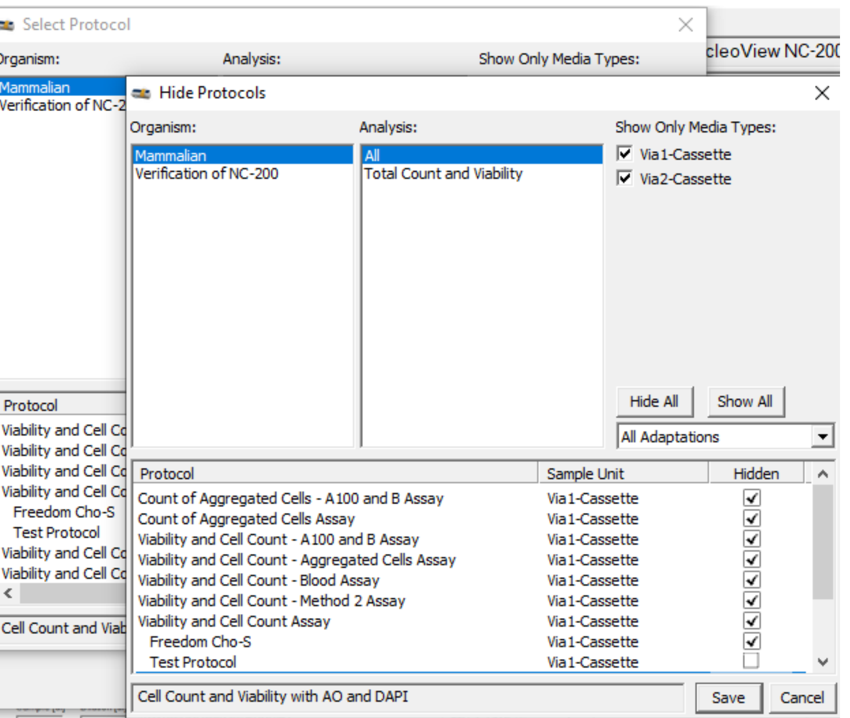
Protocol Selection

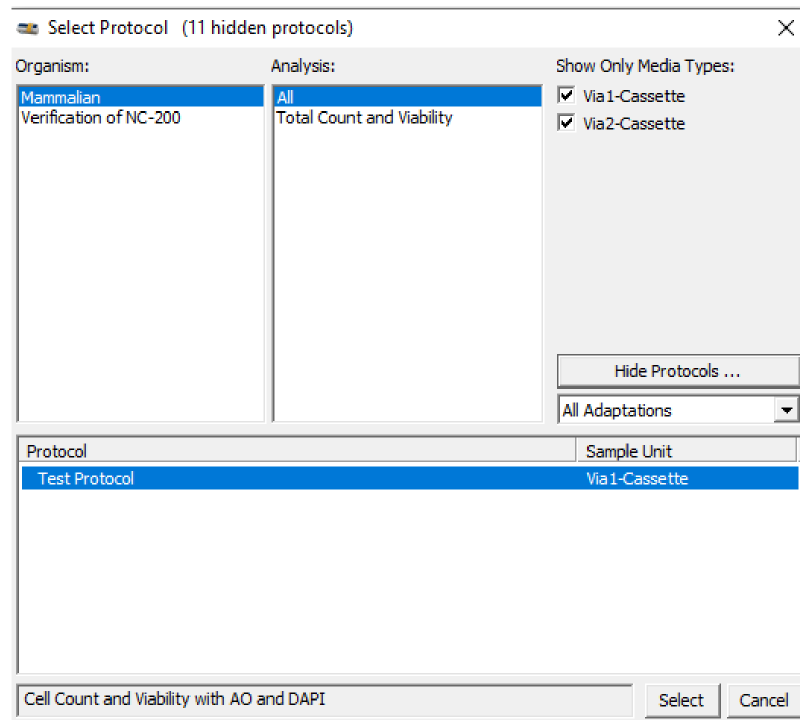
- The protocol select screen will allow you to browse all protocols created or uploaded onto the instrument you are using
- User generated protocols can be exported or deleted by right clicking on the protocol name from this list.
- To reduce the number of protocols shown here without deleting them,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

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

Quick Access Data Files

Protocol F1 F2 F3

Viability and Cell Count Assay

Via1-Cassette

Sample ID: CHO-S PASSAGE 2 DAY 2

Operator: NC-200 Demo

RETURN

Total cells/ml: 3.48E6

Viability %: 99.1

Diameter um: 12.5

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

File Browser and Exporting Data to Excel

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
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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
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20200302-0005-c-CHO-S P2D2 2X DULTION.cm

File Browser

Protocol F1 F2 F3

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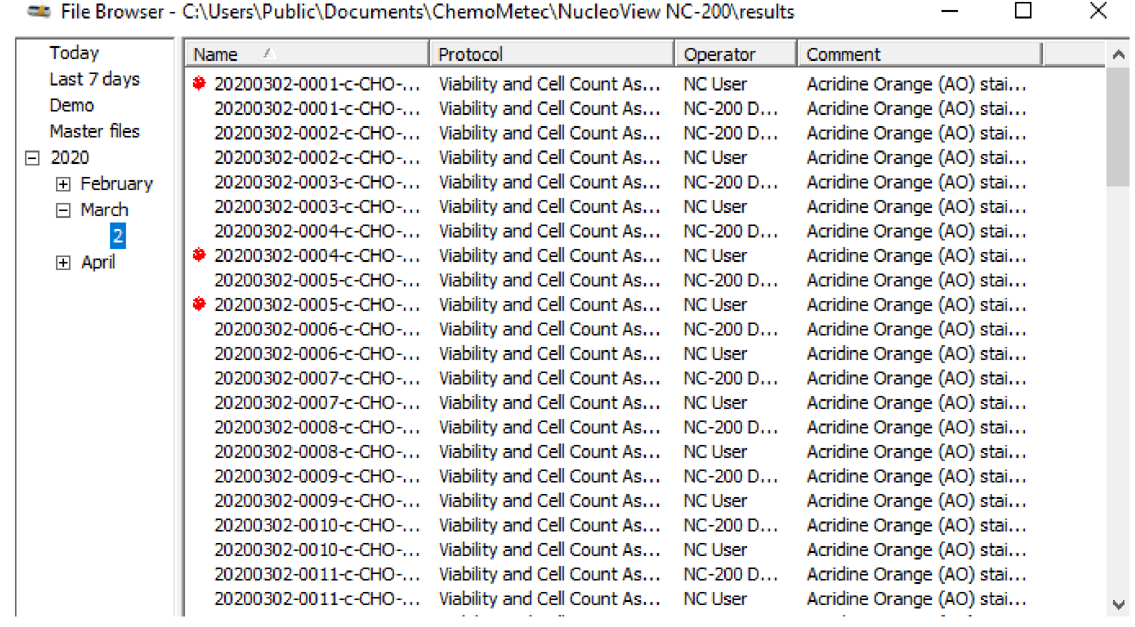
Diameter um: 12.5

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

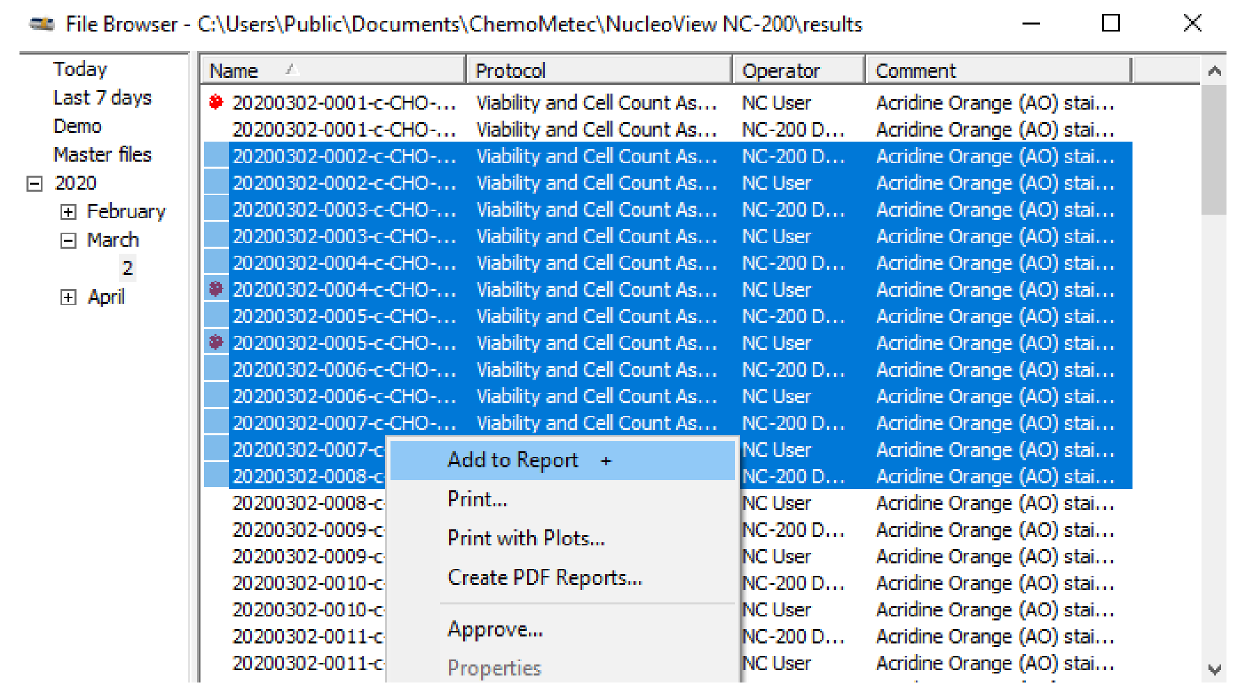
File Browser and Exporting Data to Excel

- 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



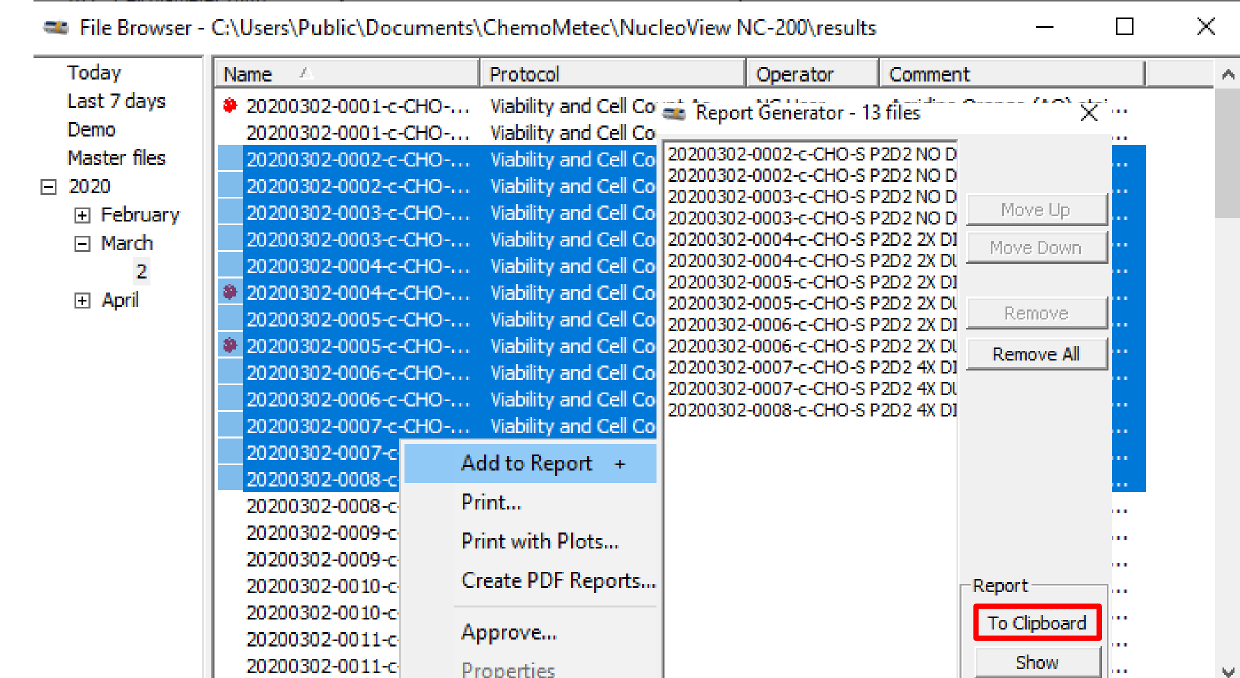
File Browser and Exporting Data to Excel

- 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

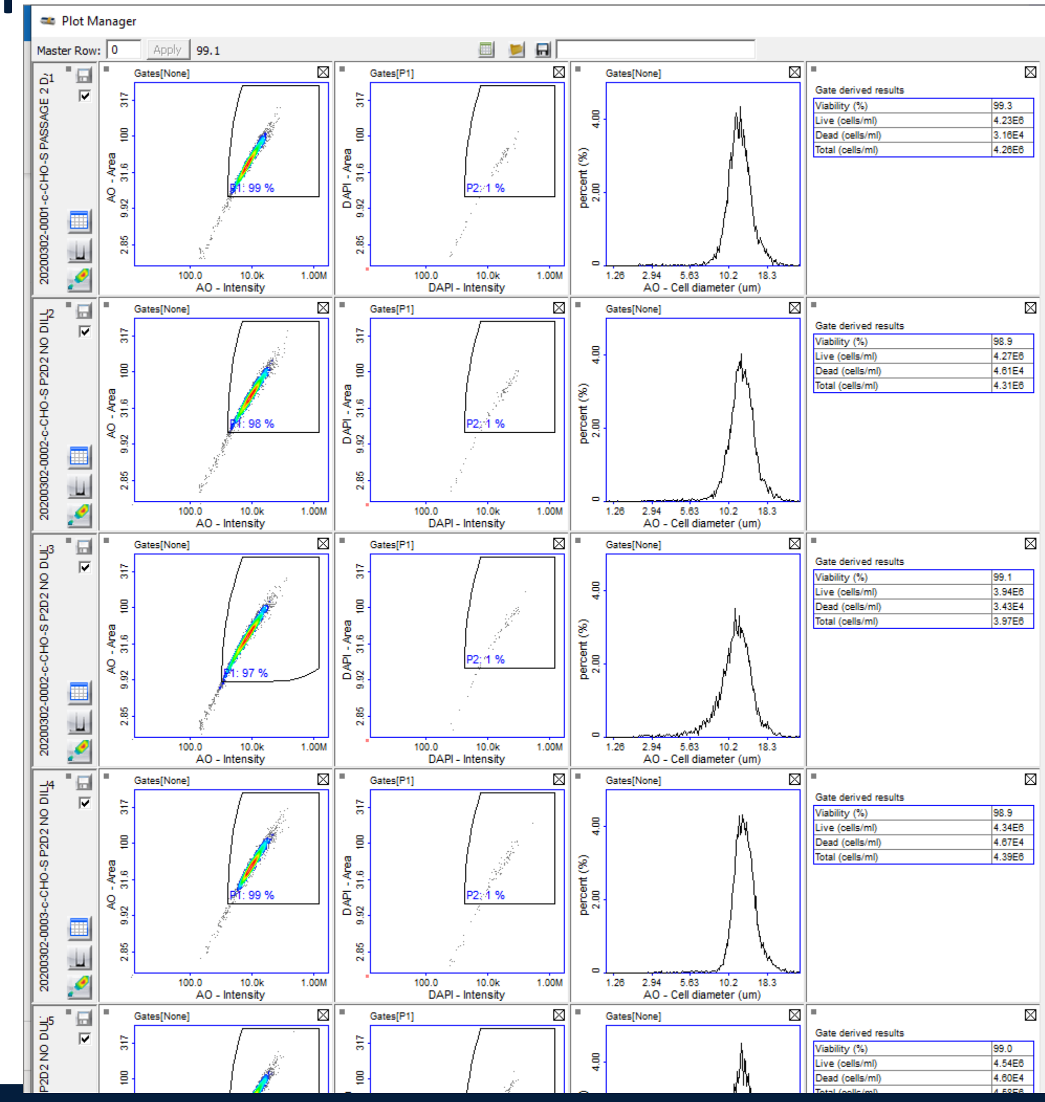


File Browser and Mass Import to the Plot Manager

- 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



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Topics for the Day

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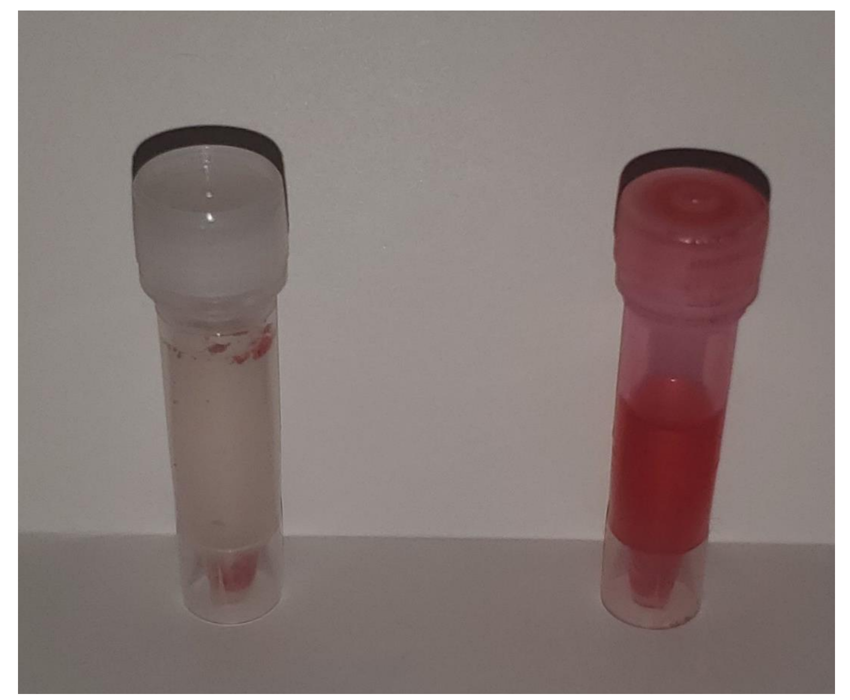
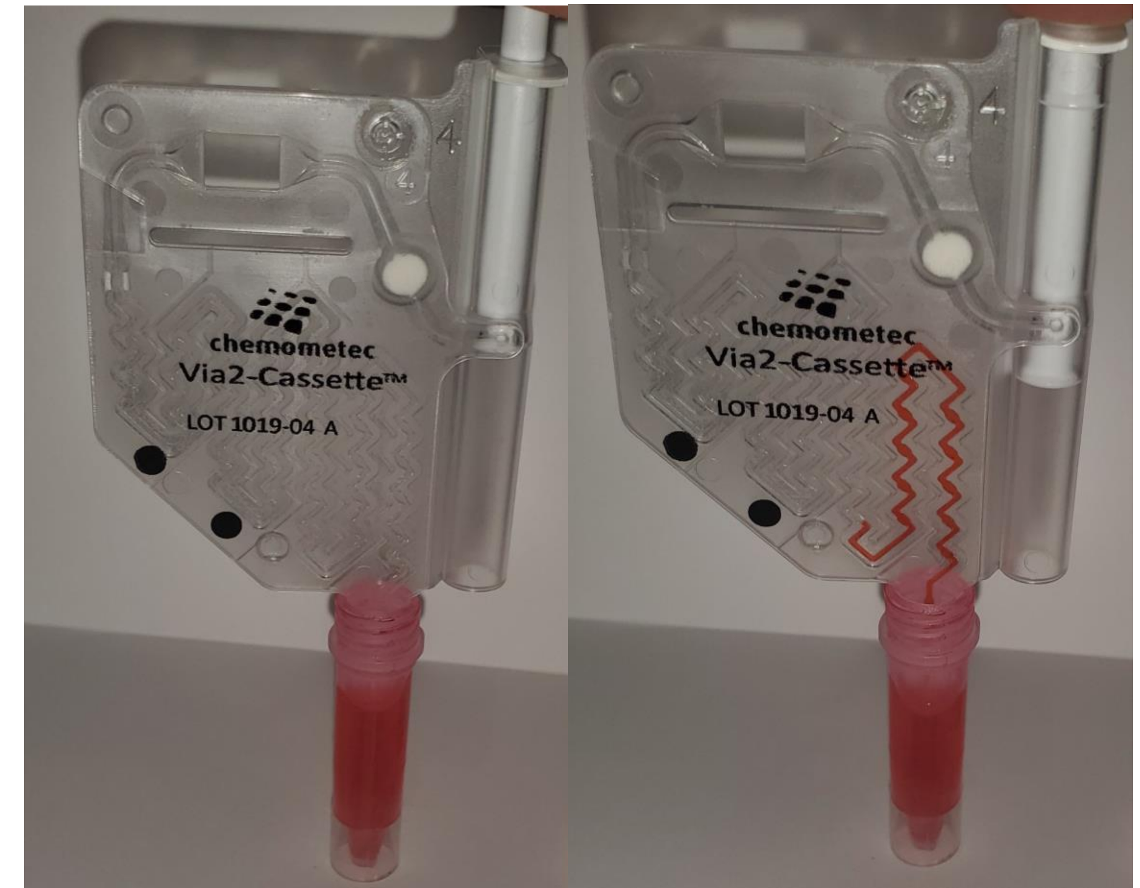
Best Practices

- Use at least 200µL 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

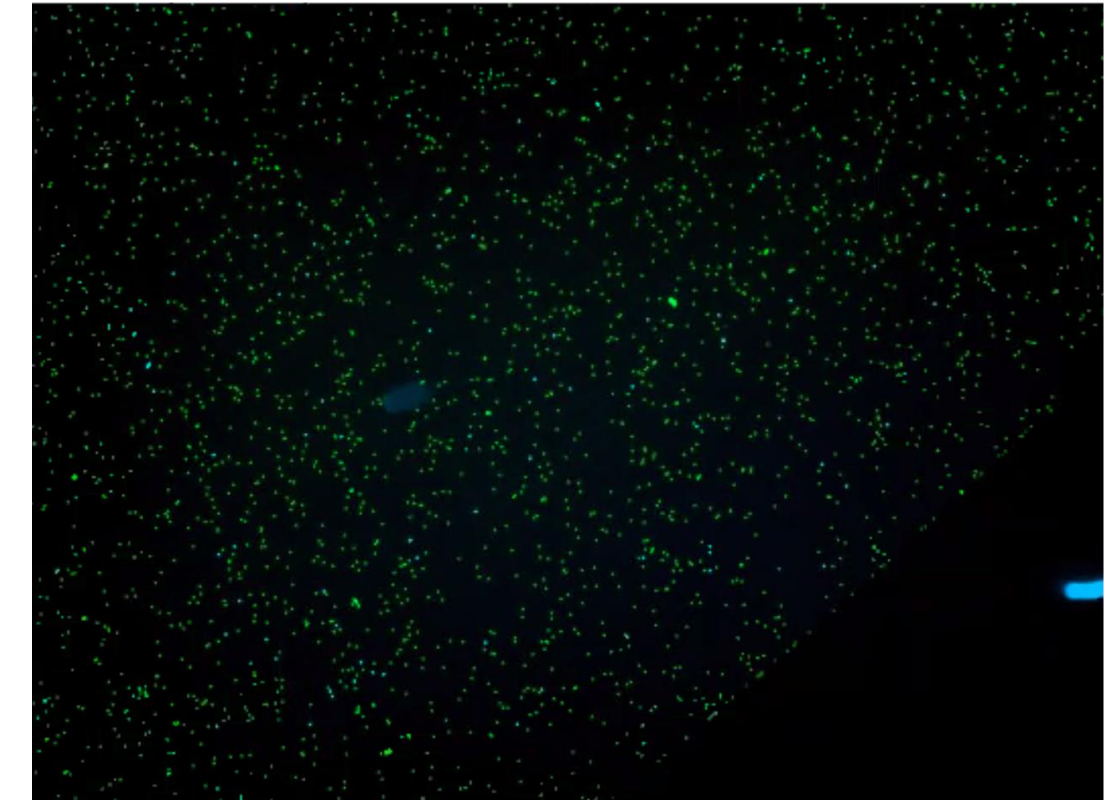


Best Practices

- 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

Microcarrier suspension sample

1

Reagent A100 added

Reagent B added

Load cells in Via-1 Cassette™

Run sample in NC-200™

2

No reagents added
Load cells in Via-1 Cassette™

Topics for the Day

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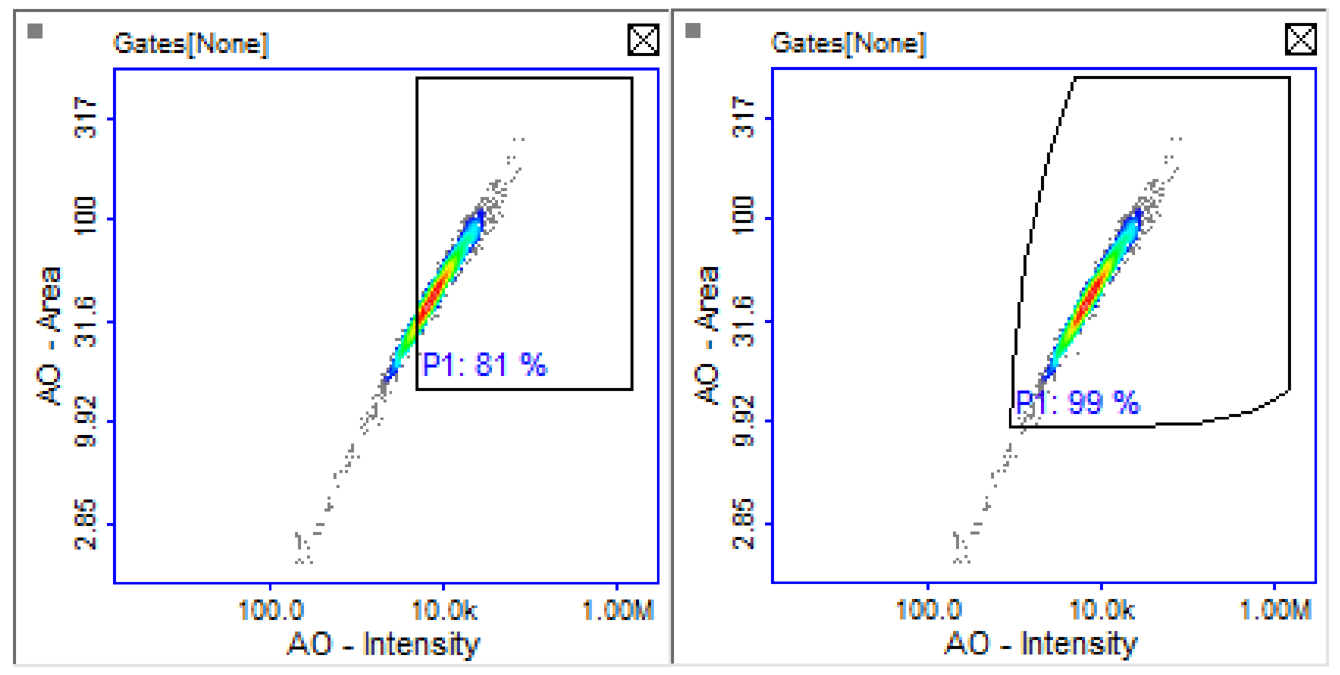
Gating Changes

- 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



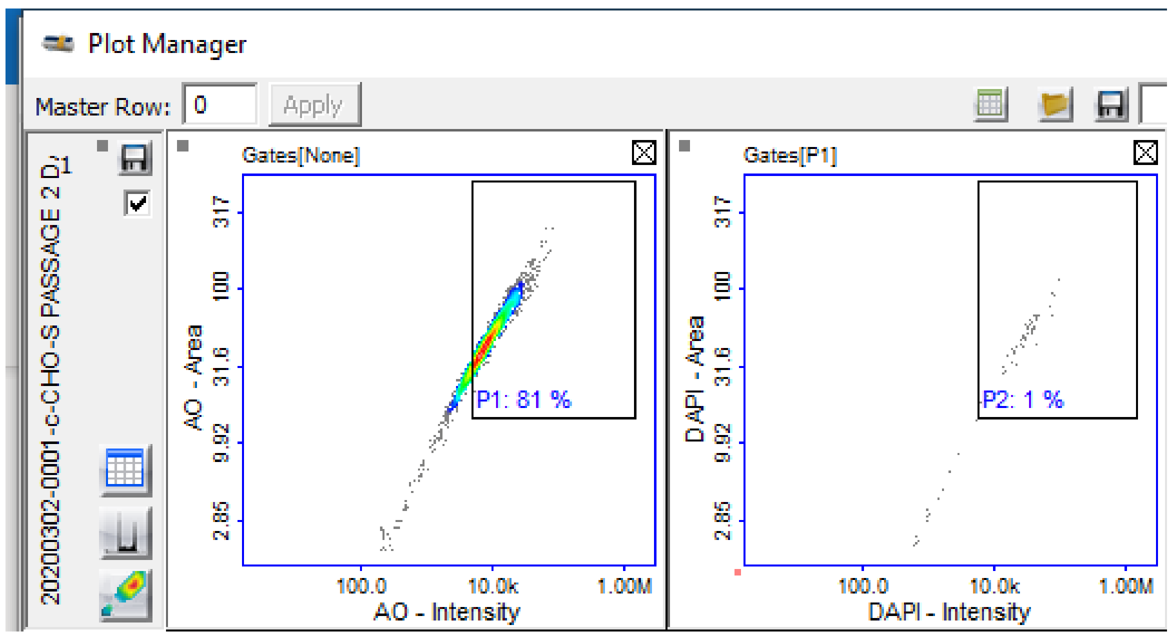
Gating Changes

- 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

ChemoMetec NucleoView NC-200
File Tools View Help
Image Result
1/1
Data folder and files
:\Documents\ChemoMetec\NucleoView NC-200\results\20180817
20180817-0001-c-293-1.cm
20180817-0002-c-29
20180817-0003-c-29
20180817-0004-c-29
20180817-0005-c-29
20180817-0006-c-29
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
Properties
Protocol
Viability and Cell Co
Via1-Cassette
Sample ID
293-1
Operator
NucleoCounter
RETURN
Total cells/ml 1.86E6
Viability % 91.0
Diameter um 14.8
Via1: Sample [ul] 200 Dilution [ul] 0
Multiplication Factors 1 # 1.00

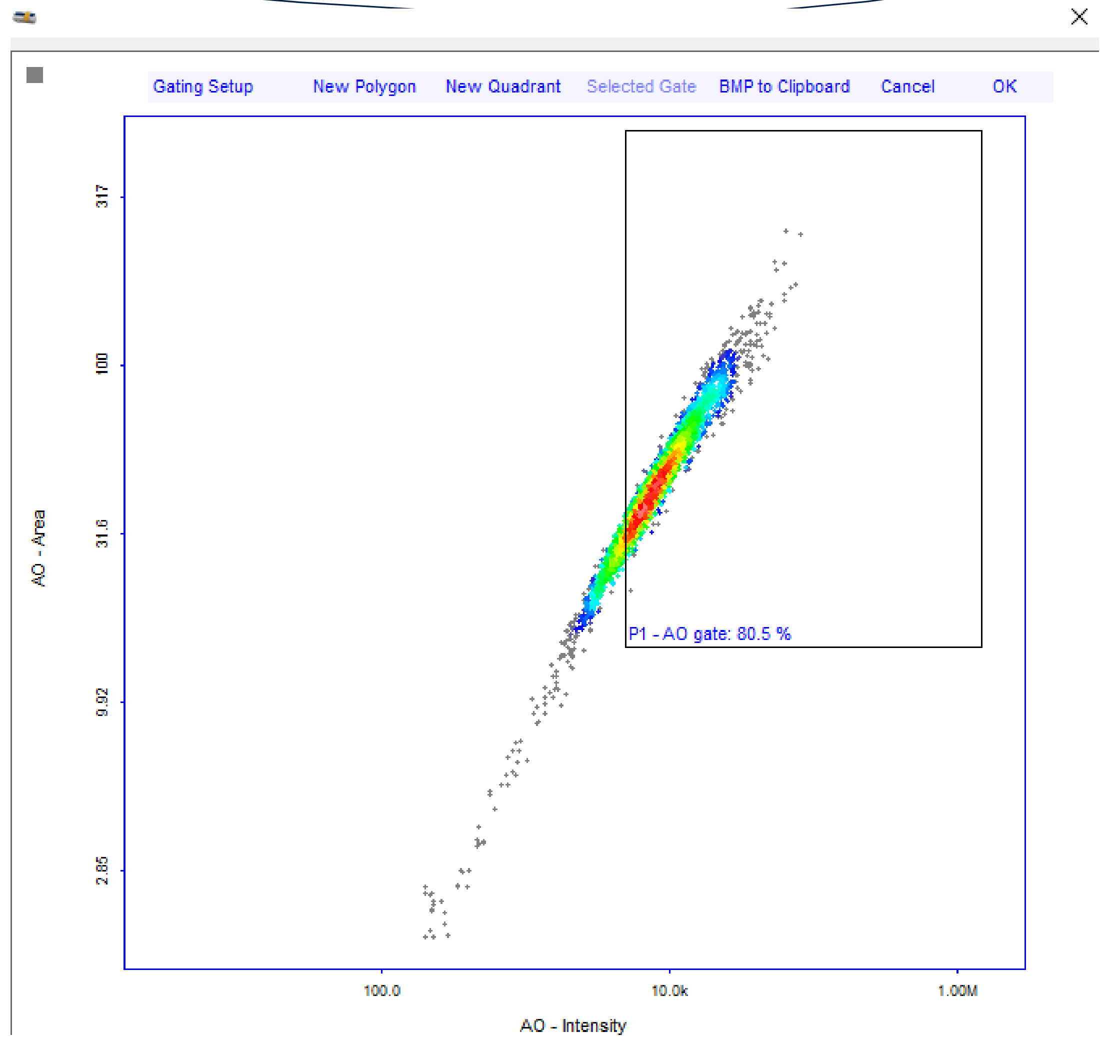
Gating Changes

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



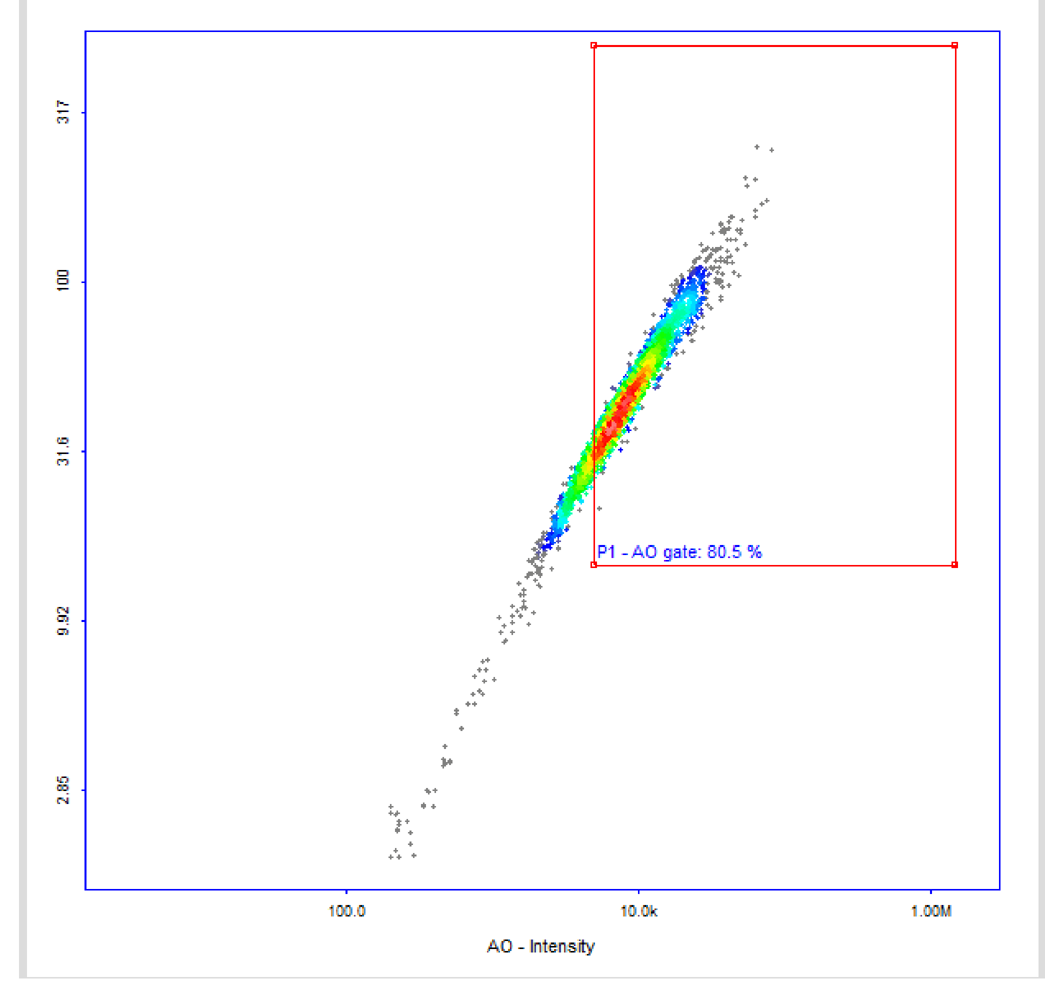
Gating Changes

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



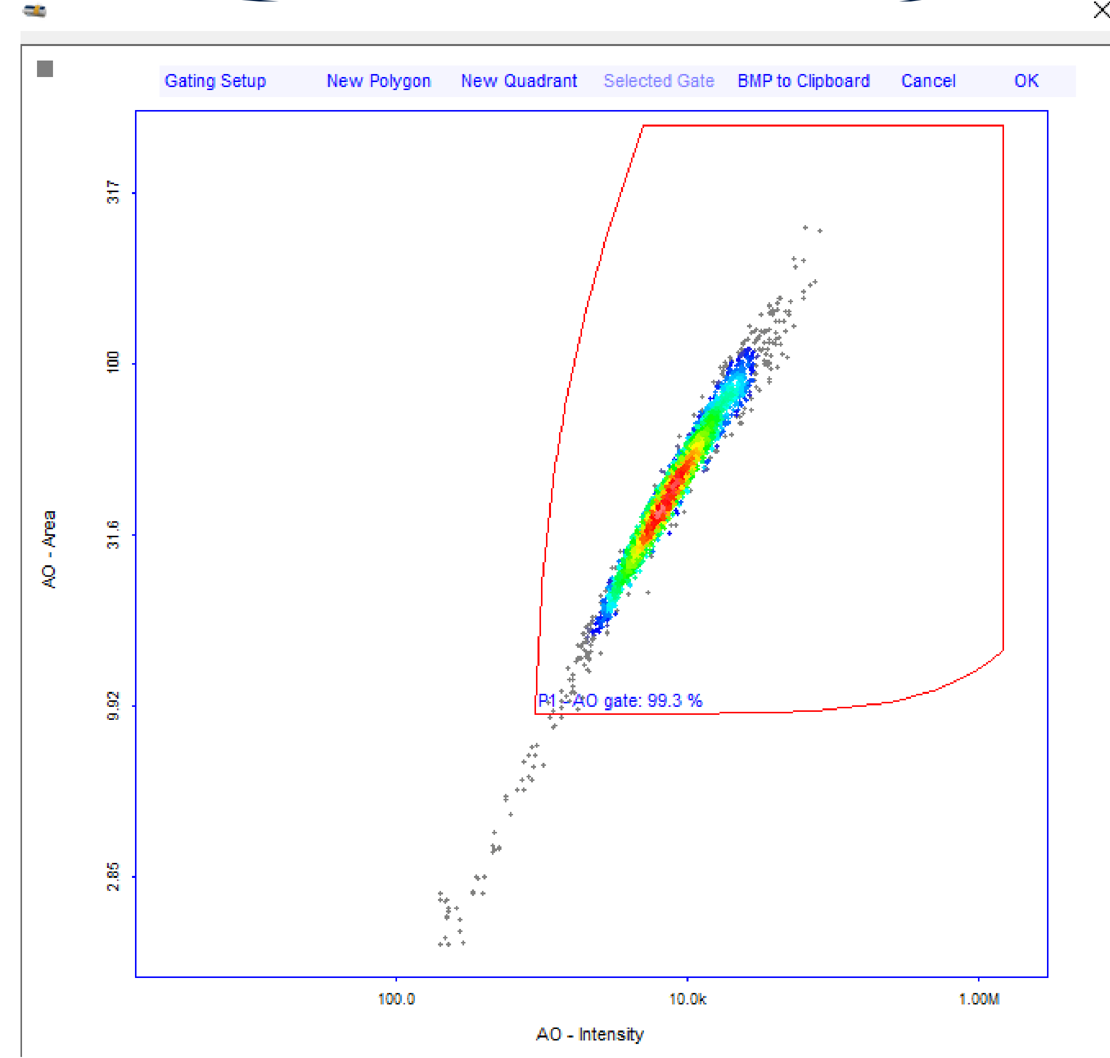
Gating Changes

- 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

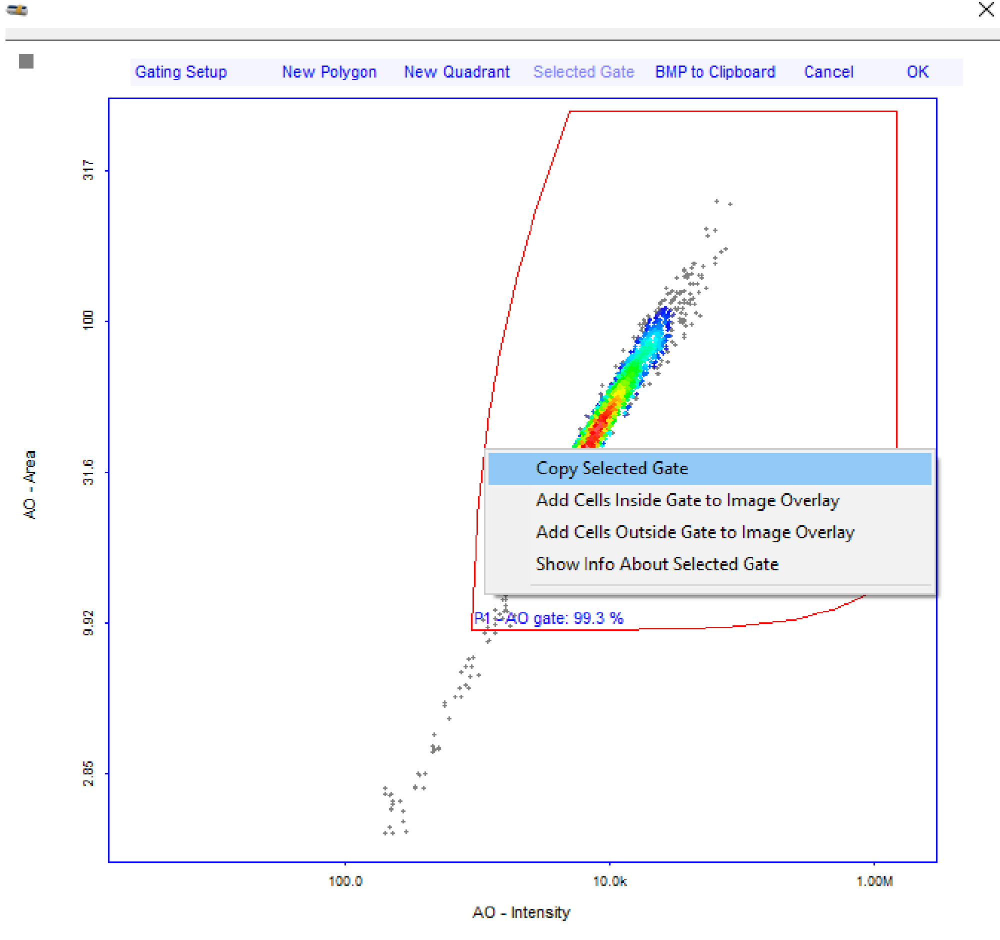
- Begin by double clicking the gate to turn it red and give it little handles on each corner
- Using the handles, we can drag the corners of the gate to a desired position
- Once the gate is placed, you must click in the white space to de-select the gate and save your changes



Gating Changes

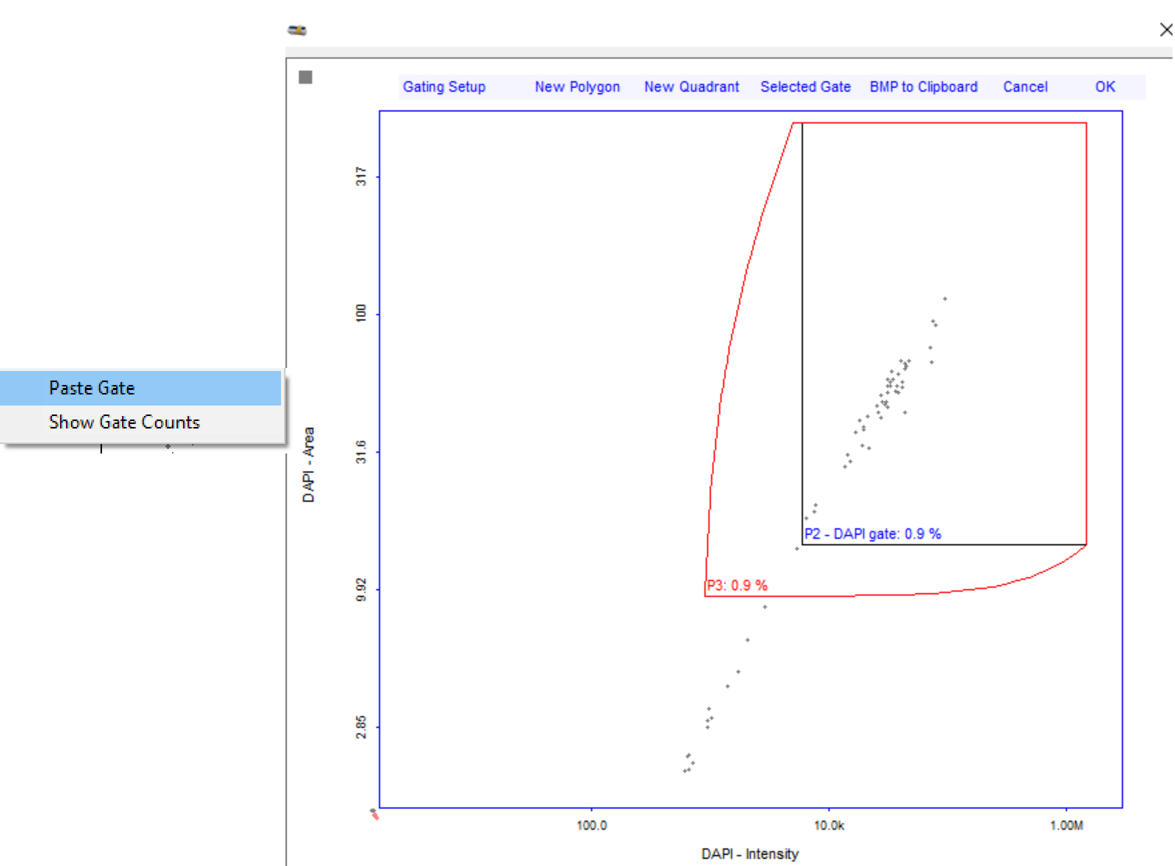
- To copy your gate to transfer it to your DAPI channel, click the gate once to turn it red without handles, and then right click it to open a small drop down menu

- 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



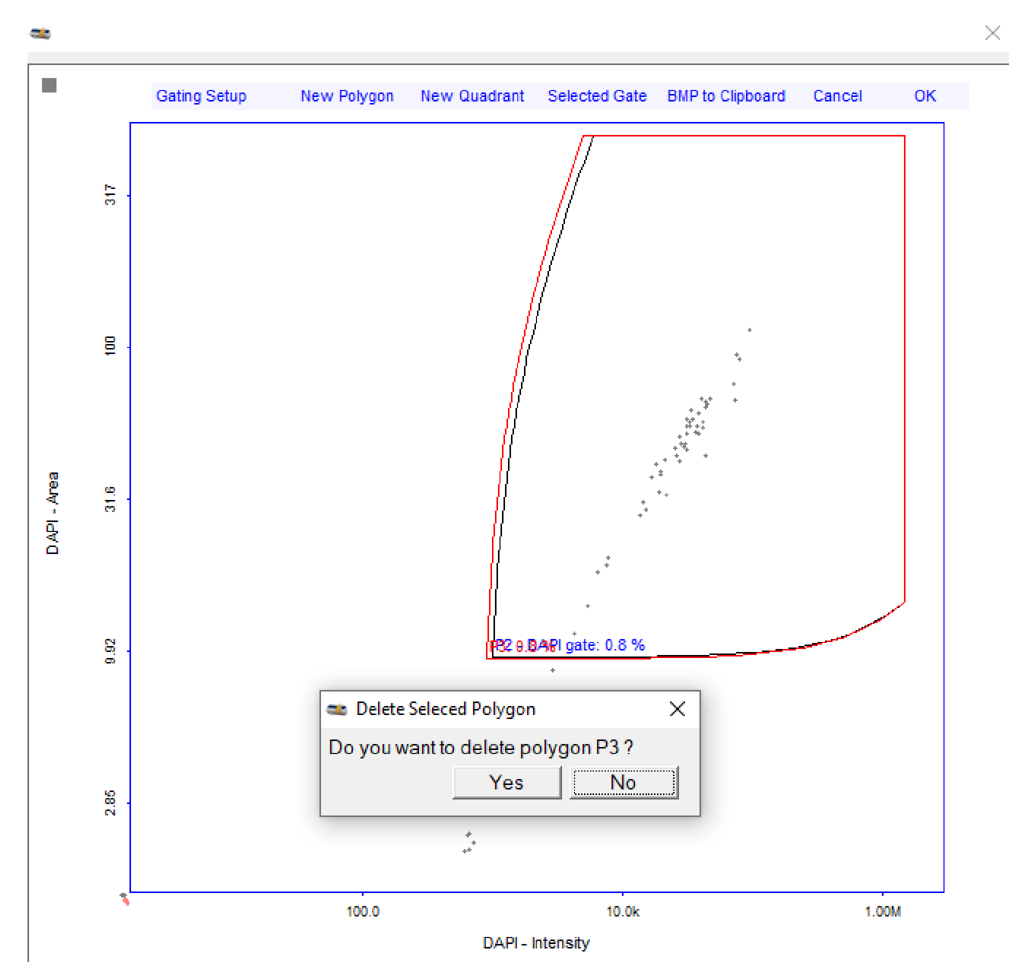
Gating Changes

- 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

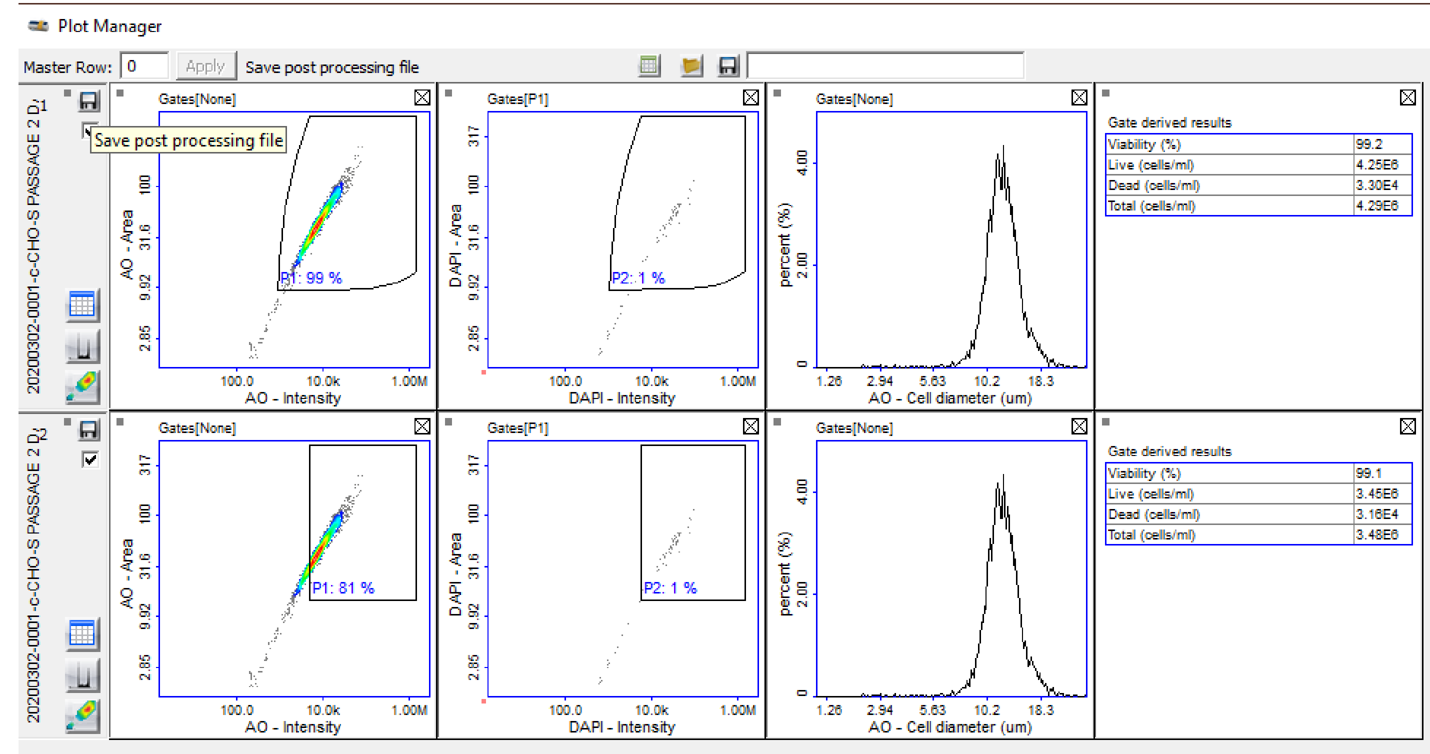
- Once the gate is pasted, you will need to adjust the default DAPI gate to match closely with the pasted gate.
- To make your data look cleaner, you may then delete the pasted gate by clicking it once and pressing the Delete key on your keyboard
- Exit the gate window by pressing OK



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.



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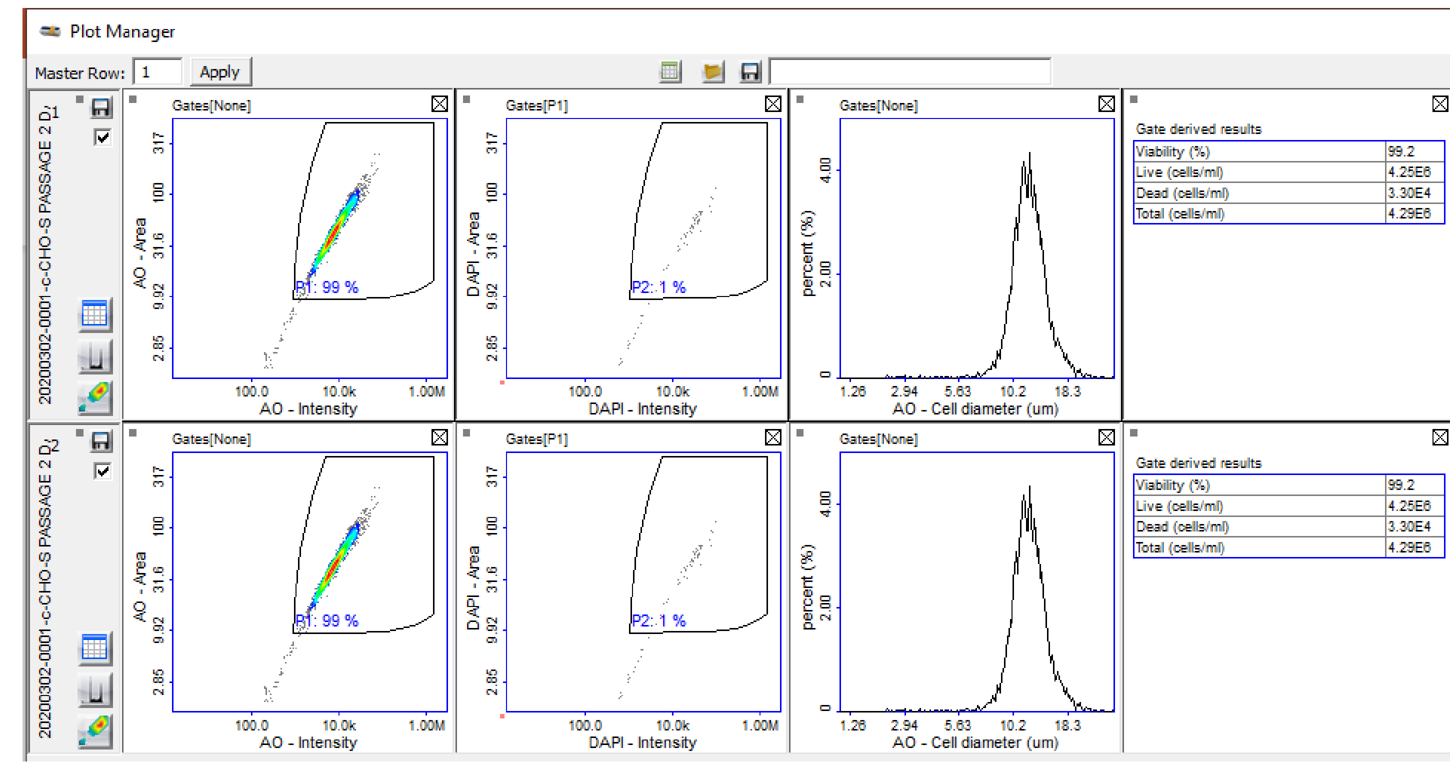

Topics for the Day

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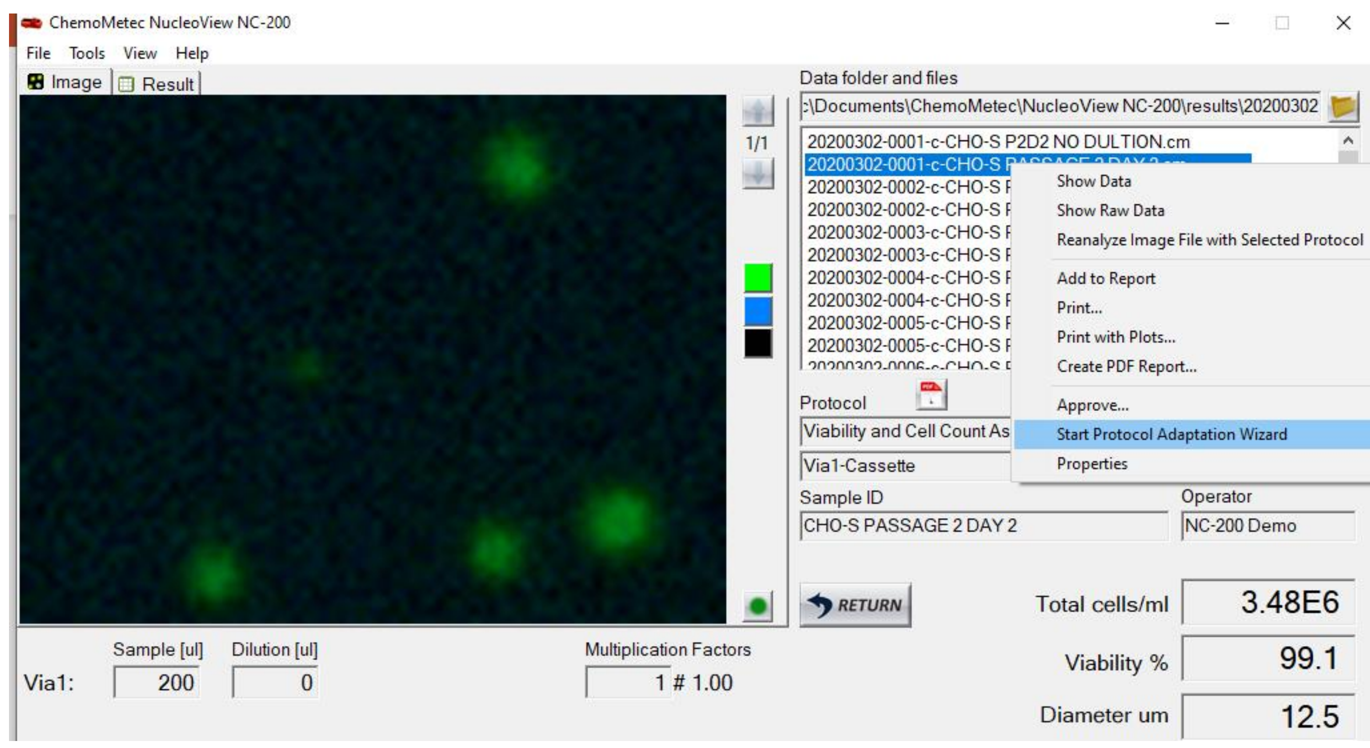
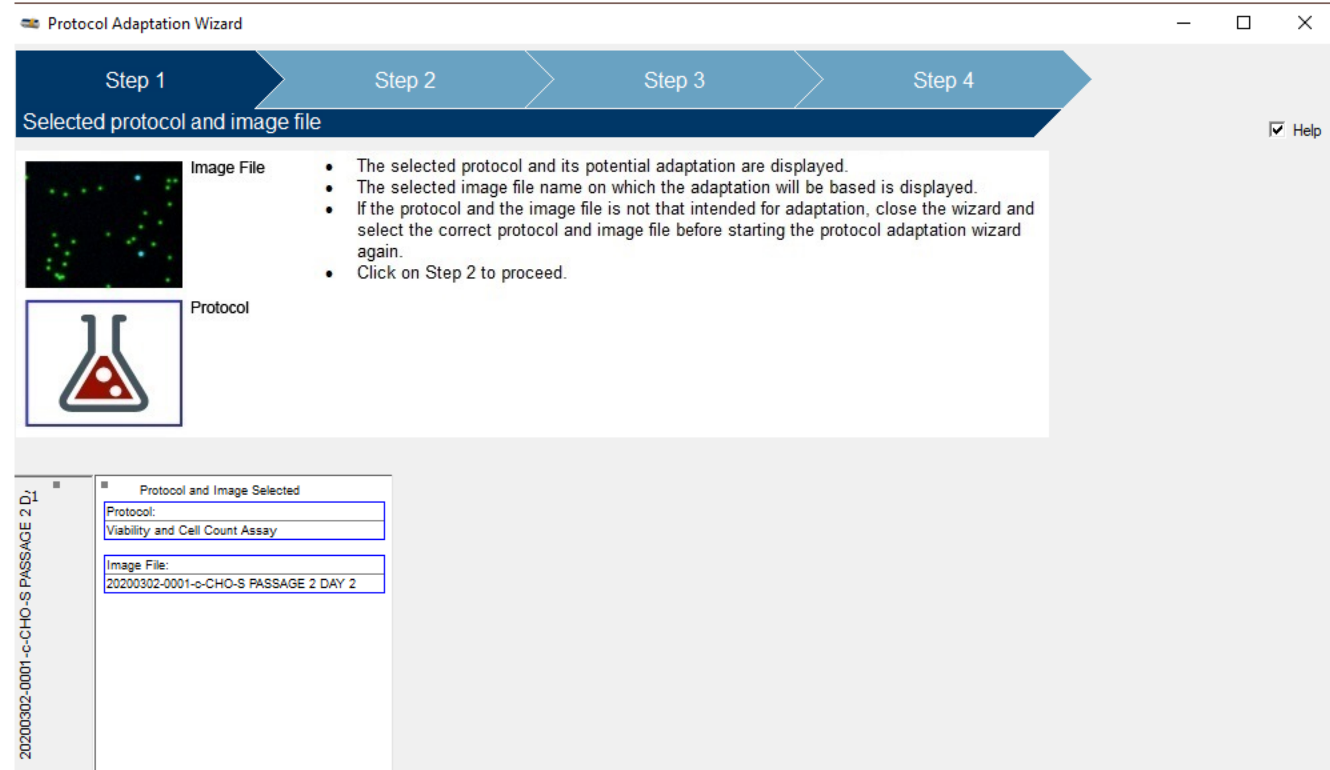
Optimizing Protocols

- 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



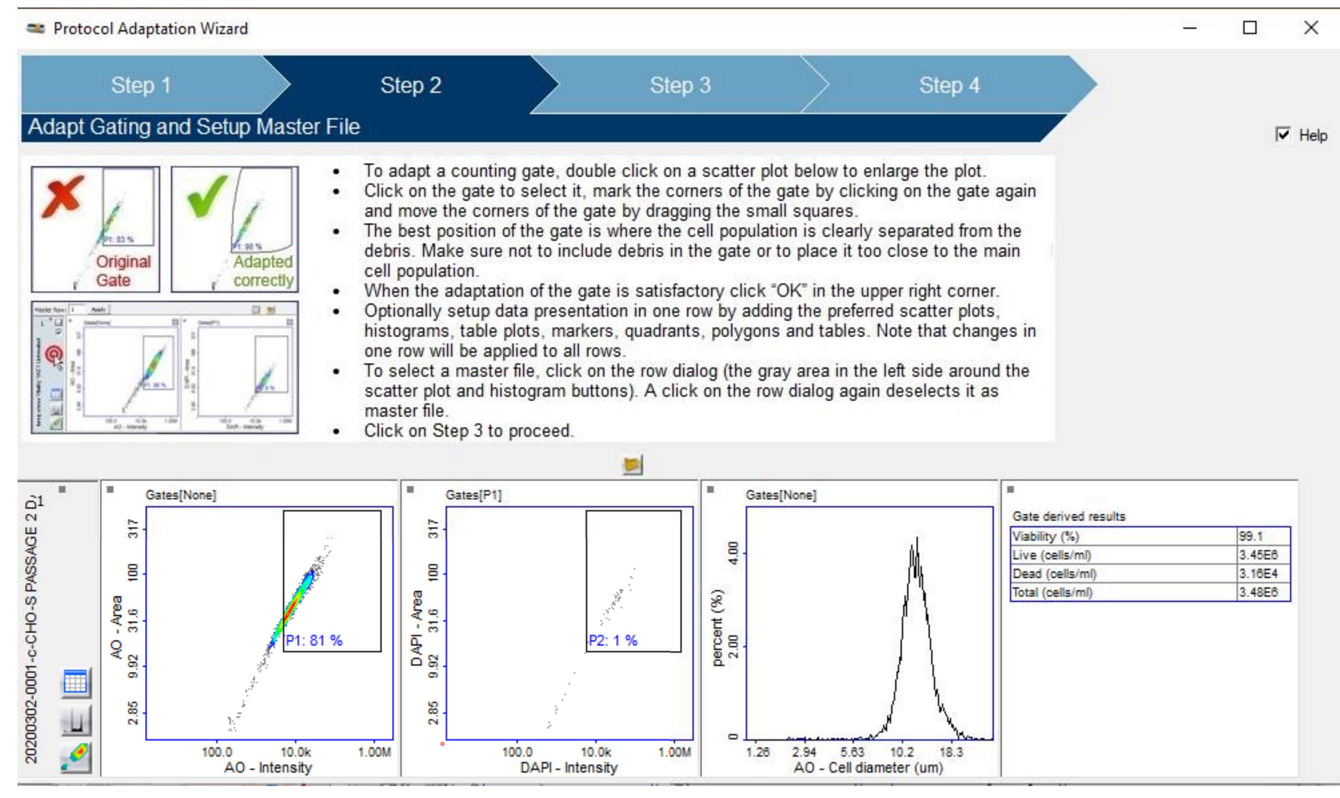
Optimizing Protocols

- 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



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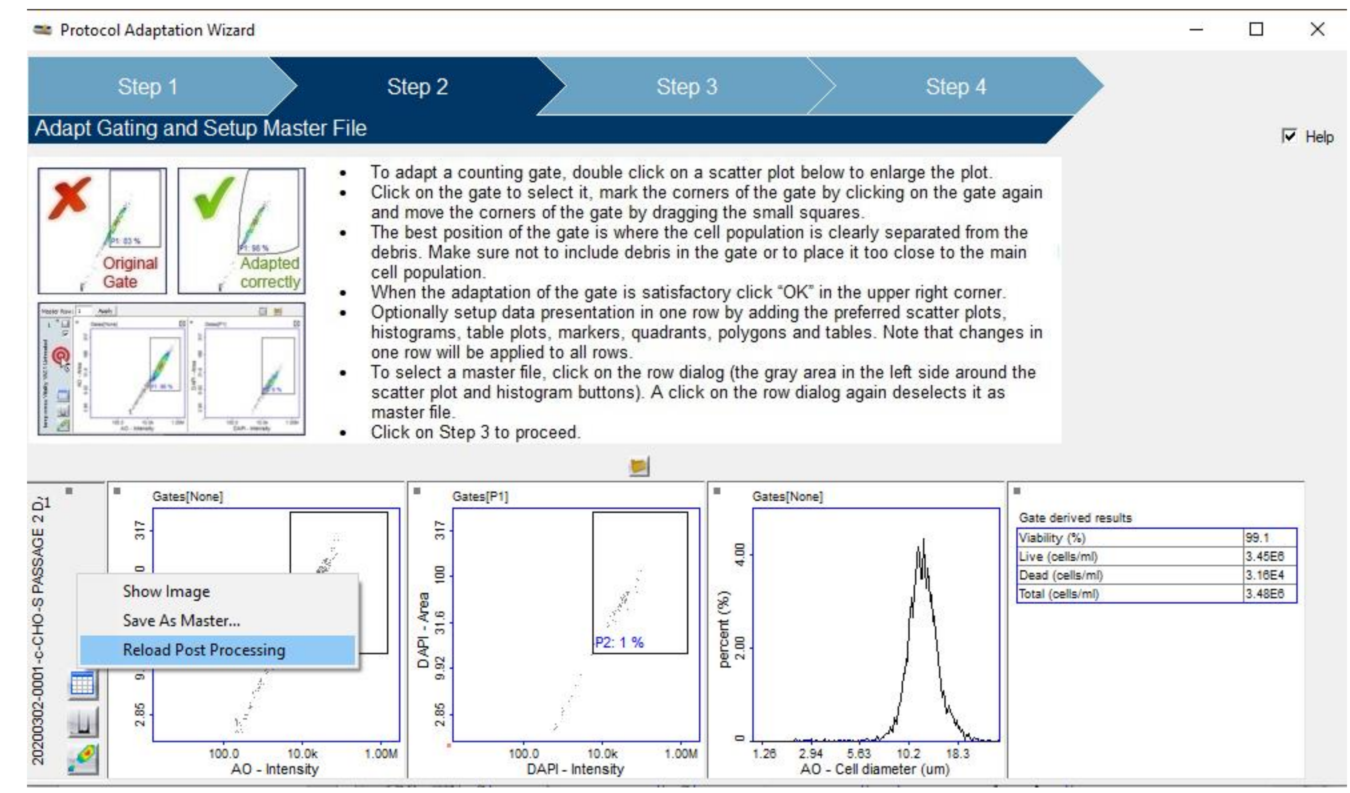
Optimizing Protocols

- 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



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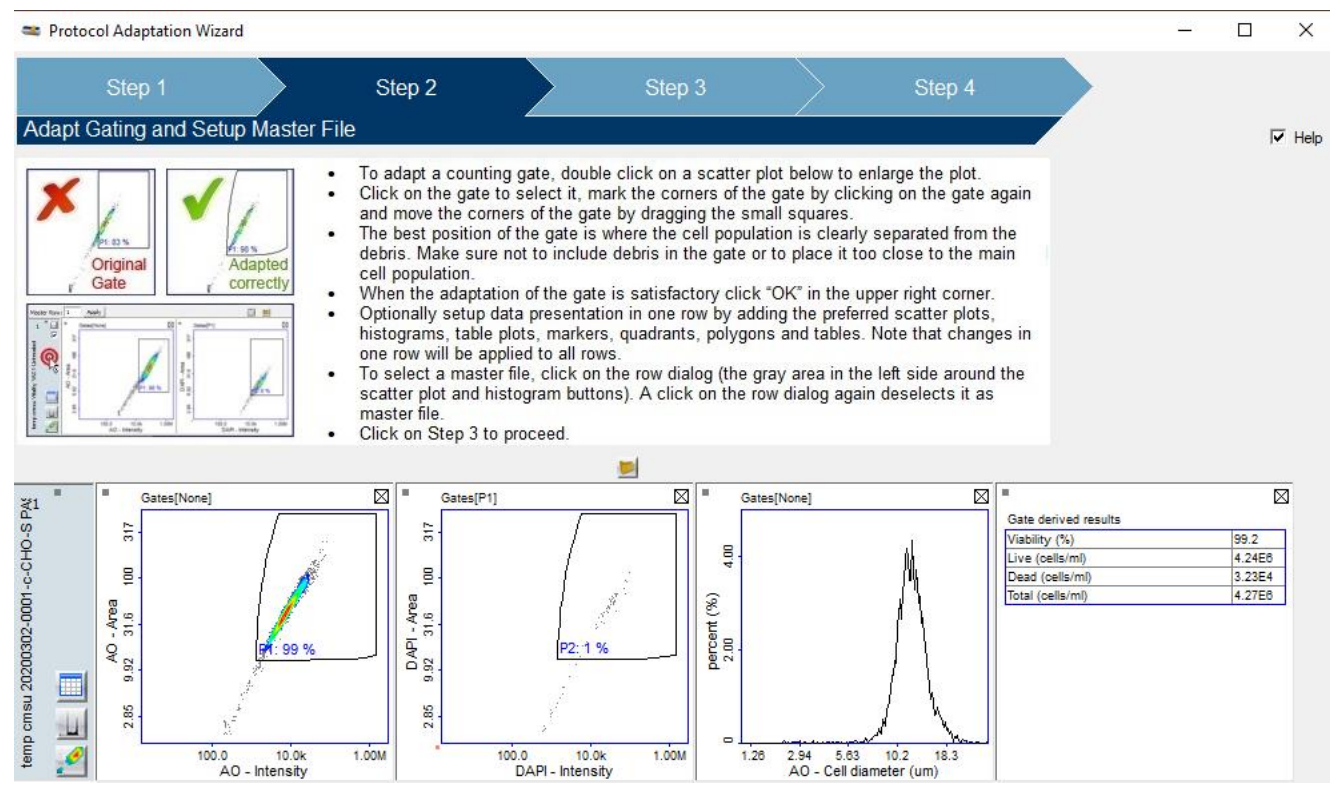
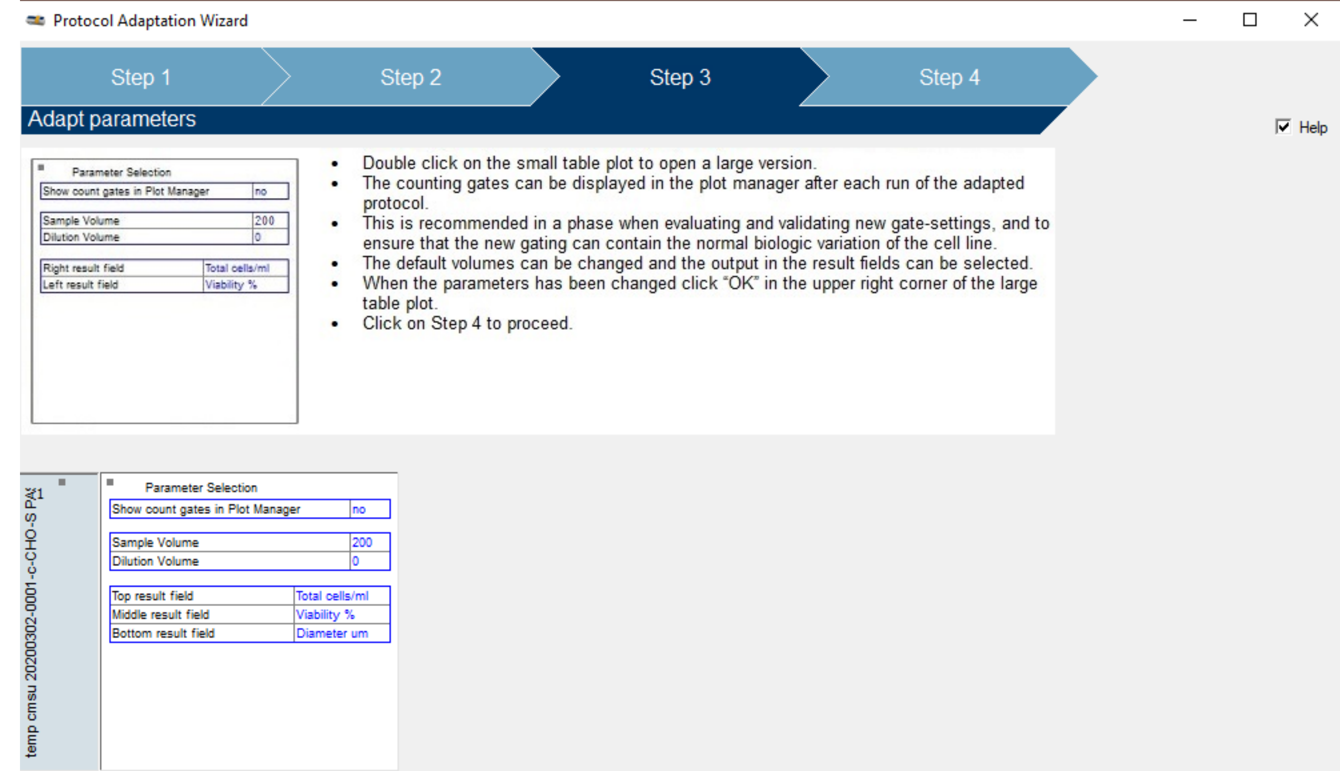
Optimizing Protocols

- 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



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

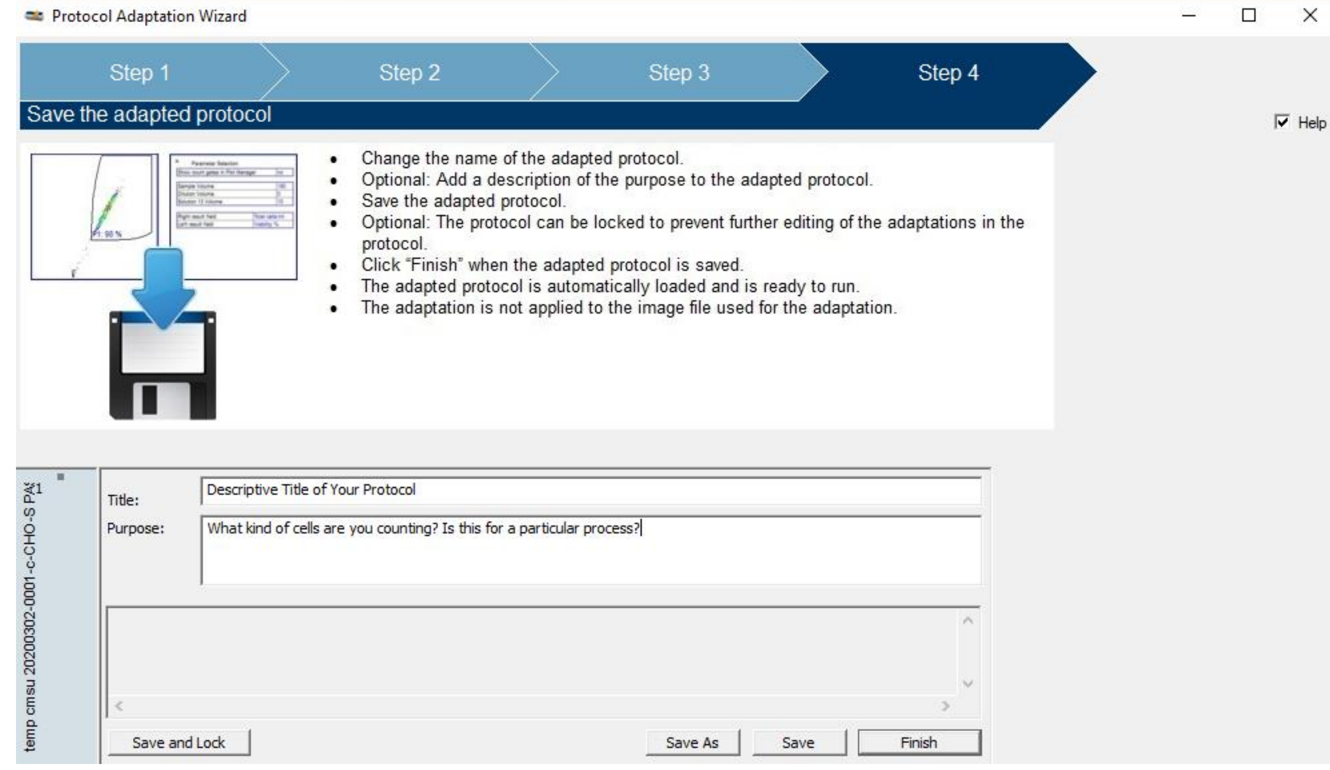


Optimizing Protocols

- 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



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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 ech method
- In addition, the average of each dilution triplicate can be plotted against its dilution factor to determine the Linearity of the method

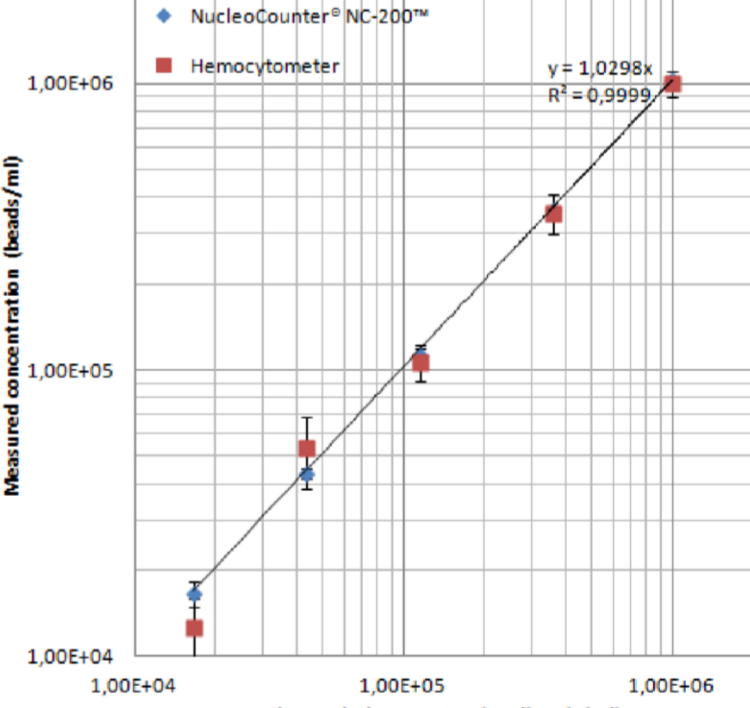

Figure 2: A dilution range of a bead solution was used to compare counting in triplicates with the NucleoCounter® NC-200™ and standard Burker-Turk hemocytometers.

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

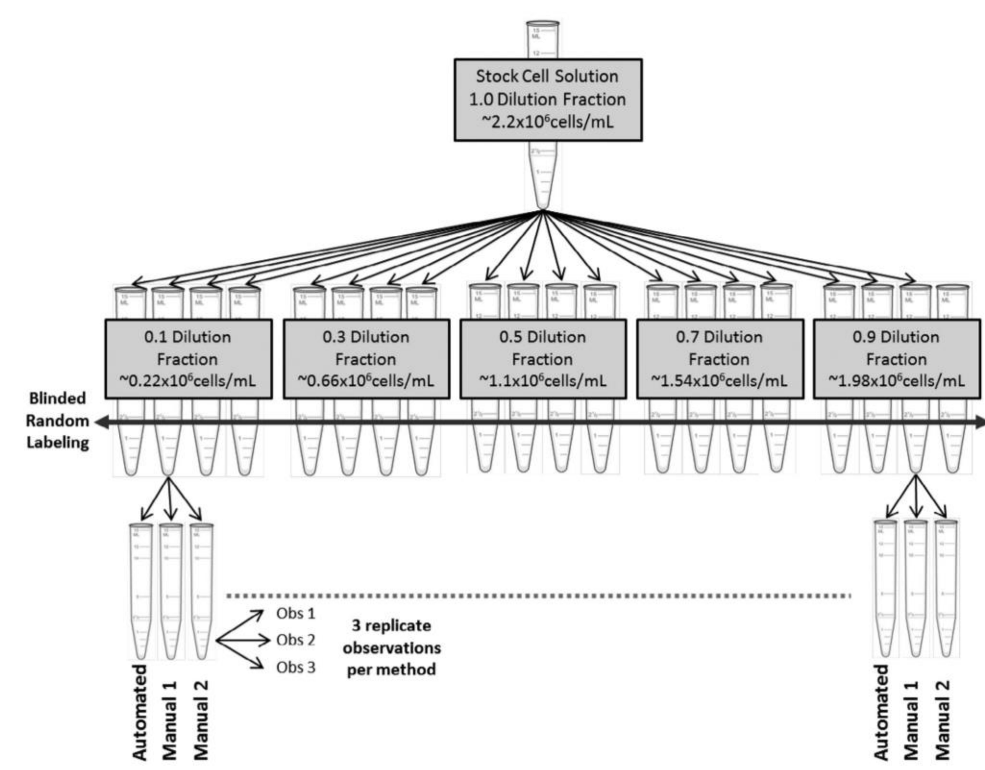

Figure 2. Schematic representation of the cell counting dilution series study experimental design.

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 homogenously 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

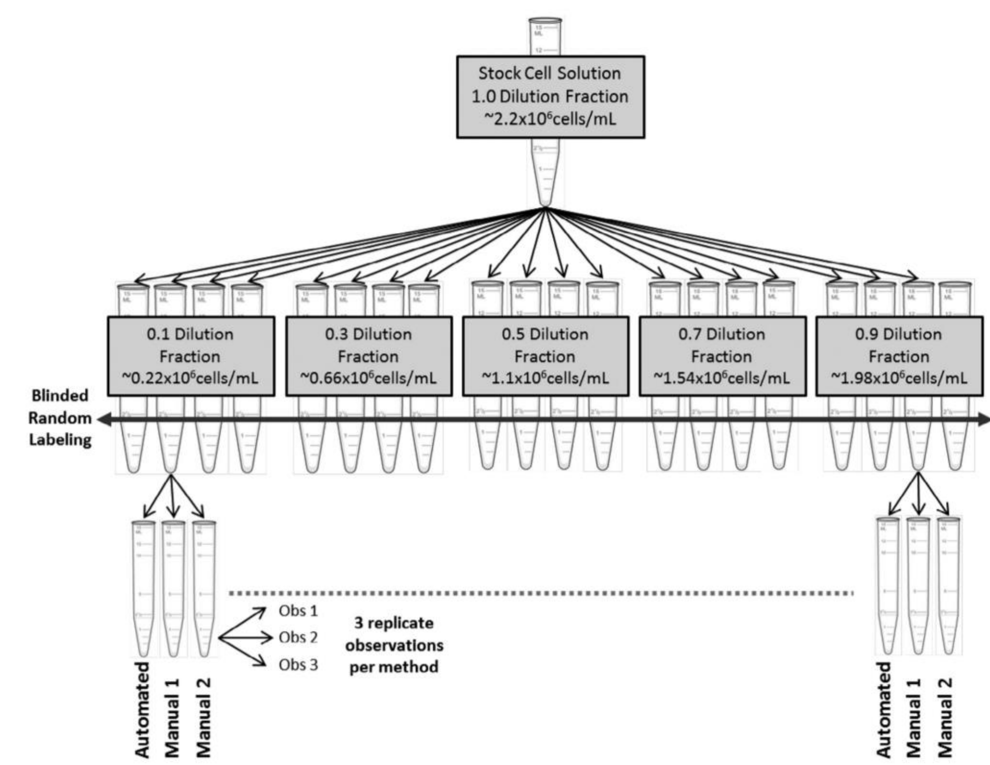

Figure 2. Schematic representation of the cell counting dilution series study experimental design.

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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

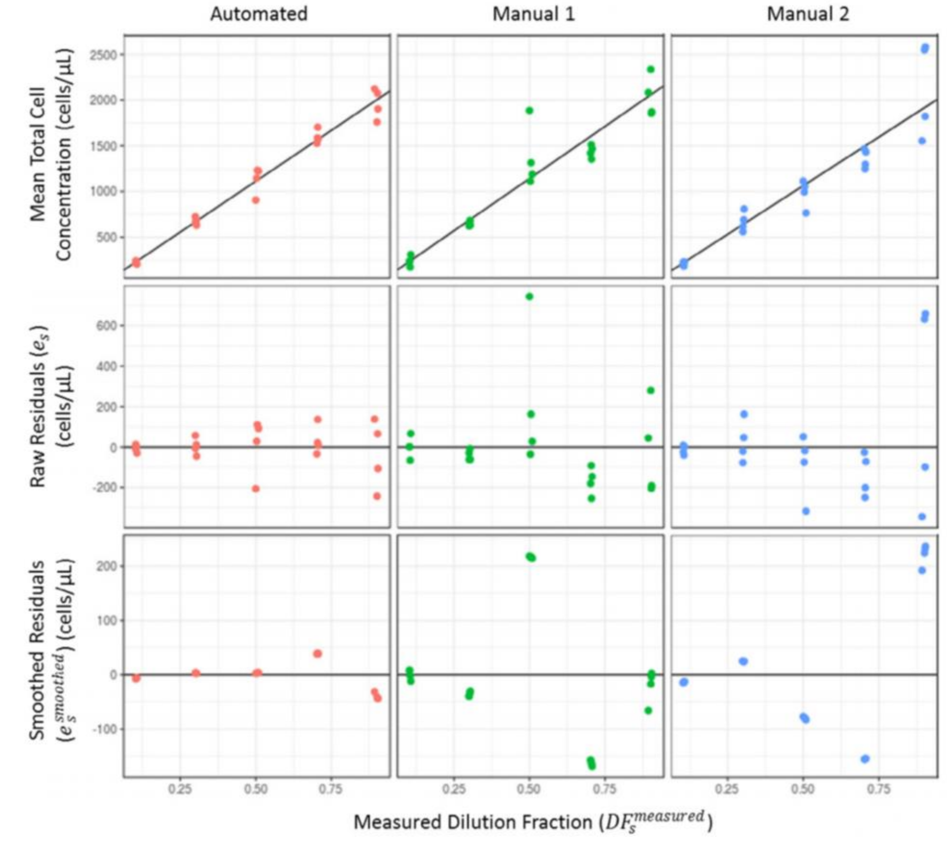

Figure 4. (Top panel) Mean of triplicate observations of cell concentration for four independent test samples over each of five dilution fractions for three devices (Automated [red], Manual 1 [green] and Manual 2 [blue]) with fitted proportional model (black line). (Middle panel) Raw residuals calculated 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.

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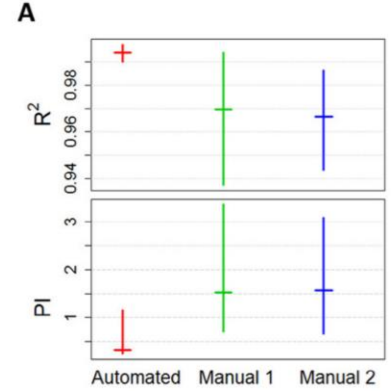
Practical Considerations When Performing a Comparison Study

- 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

A



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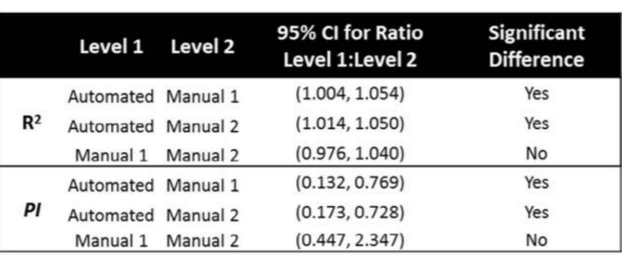

	Level 1	Level 2	95% CI for Ratio Level 1:Level 2	Significant Difference
R^2	Automated	Manual 1	(1.004, 1.054)	Yes
	Automated	Manual 2	(1.014, 1.050)	Yes
	Manual 1	Manual 2	(0.976, 1.040)	No
PI	Automated	Manual 1	(0.132, 0.769)	Yes
	Automated	Manual 2	(0.173, 0.728)	Yes
	Manual 1	Manual 2	(0.447, 2.347)	No

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.

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