## **BD LSRFortessa Standard Operation Protocol**

# **Basic Operation – Tube mode**

## A. Log in the Window

- 1. Press Ctrl+Alt+Delete
- 2. Key in Password **BDIS#3\$\$** \*No need to switch user

## B. Log in the BD FACSDiva Software

Key in user name and password and click OK to log in.
 \*Please contact the Technical Staff in charge if you do not have an account.
 \*\*Please contact the Technical Staff in charge if you forget password.



2. Click Use CST Settings when pop-up message as below is shown.



## C. Experiment Setup

- 1. Setup New Experiment
  - 1.1 Go to Browser, Click New Experiment icon. A new experiment will be created

| Name                    | Date                 |
|-------------------------|----------------------|
| 🗐 🧏 Ocleaning           |                      |
| 🕀 🔚 Experiment_001      | 12/30/19 11:16:15 AM |
| 🕀 📔 Accudrop Drop Delay | 12/30/19 11:21:30 AM |
| Experiment_002          | 3/3/20 2:22:43 PM    |
| 🕀 🔚 Experiment_003      | 3/9/20 5:11:11 PM    |
| 🖻 🛄 Experiment_004      | 3/11/20 11:57:10 AM  |
| Cytometer Settings      |                      |
| 🕀 🤷 Global Worksheets   |                      |
| 🕀 🦂 Shared View         |                      |

1.2 Click Cytometer Settings under the newly created Experiment

| 😡 Bro | owser - Experiment_004  | ×                    |
|-------|-------------------------|----------------------|
|       | 🖬 餐 🏴 📝 🜌 📓 -           |                      |
|       |                         |                      |
|       | Name                    | Date                 |
| (     | ⊒… 🚴 Ocleaning          |                      |
|       | 🕀 🔚 Experiment_001      | 12/30/19 11:16:15 AM |
|       | 🖶 🔚 Accudrop Drop Delay | 12/30/19 11:21:30 AM |
|       | 🕀 🔚 Experiment_002      | 3/3/20 2:22:43 PM    |
|       | 🕀 🔚 Experiment_003      | 3/9/20 5:11:11 PM    |
|       | 🖨 🛄 Experiment_004      | 3/11/20 11:57:10 AM  |
|       | Cytometer Settinos      |                      |
|       | 🕀 👸 Global Worksheets   |                      |
| (     | nared view              |                      |

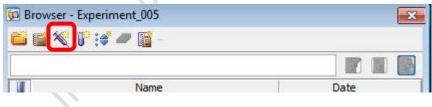
1.3 Go to Inspector Window, select unwanted channels and click Delete

| rameters Threshold Ratio C | ompensation |     |     |        |      |   |  |
|----------------------------|-------------|-----|-----|--------|------|---|--|
| Parameter                  | Voltage     | Log | A   | н      | w    |   |  |
| FSC                        | 353         |     |     |        |      | - |  |
| SSC                        | 292         | 1   |     |        | V    |   |  |
| ETTO                       | 247         |     |     | -      |      |   |  |
| PerCP-Cy5-5                | 483         |     | V   |        |      |   |  |
| PE                         | 446         | V   | V   |        |      |   |  |
| PE-Texas Red               | 414         | V   |     |        |      |   |  |
| PE-Cy5                     | 536         | V   |     |        |      |   |  |
| PE-Cy5-5                   | 386         |     |     |        |      | 8 |  |
| PE-Cy7                     | 440         | V   |     |        |      |   |  |
| APC                        | 395         | V   |     |        |      |   |  |
| Alexa Fluor 700            | 361         | V   |     |        |      |   |  |
| APC-Cy7                    | 368         | V   |     |        |      |   |  |
| DAPI                       | 343         | V   | V   |        |      |   |  |
| AmCyan                     | 383         | V   |     |        |      |   |  |
| BV605                      | 567         | V   |     |        |      |   |  |
| BV650                      | 536         |     |     | (m)    |      |   |  |
| Add                        |             |     | 240 | Delete | - V2 |   |  |

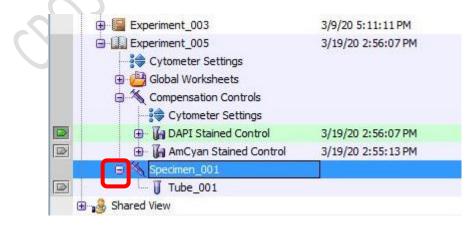
| arameters Threshold Ratio  |         |          |       |        |   |   |   |
|----------------------------|---------|----------|-------|--------|---|---|---|
| Parameter                  | Voltage | Log      | A     | Н      | W |   |   |
| FSC                        | 353     |          | V     | V      |   |   |   |
| SSC                        | 292     | E        | V     | V      | V |   |   |
| - me                       | 547     | V        | V     |        |   |   |   |
| PerCP-Cy5-5                | + 483   | V        | V     |        |   |   |   |
| PE                         | 446     | V        |       |        |   |   |   |
| PE-Texas Red               | 414     | V        | V     |        |   |   |   |
| PE-Cy5                     | 536     | V        |       |        |   |   |   |
| PE-Cy5-5                   | 386     | <b>V</b> |       |        |   | E |   |
| PE-Cy7                     | 440     | V        |       |        |   |   |   |
| APC                        | 395     | 1        |       |        |   |   | J |
| Alexa Fluor 700            | 361     | V        |       |        |   |   |   |
| APC-Cy7                    | 368     | V        |       |        |   |   |   |
| e DAPI                     | 343     | V        |       |        |   |   |   |
| <ul> <li>AmCyan</li> </ul> | 383     | 1        |       |        |   |   |   |
| # BV605                    | 567     | V        |       |        |   |   |   |
| BV650                      | 536     | V        | V     |        |   |   |   |
| Add                        |         |          | lde - | Delete |   |   |   |

#### 1.4 Check H and W boxes of FSC and SSC

- 1.5 Keep *Log* boxes of FSC and SSC **unchecked**
- 1.6 Keep Log boxes of all fluorescence channels checked
  - \* If you are doing **cell cycle or DNA content** analysis, please keep **log box** of your DNA specific fluorescence channel **unchecked**.
- 2. Setup Compensation Please refer to FACSDiva compensation automated protocol
- 3. Setup Plots and Tables
  - 3.1 Go to Browser, Click New Specimen icon



3.2 Expand Specimen\_001





| Globa            | al Worksheet - Global Sheet1          Image: Specimen_001-Tube_001         Icon         Type         Icon         Type         Dot Ph |                    |     |
|------------------|---|--------------------|-----|
| Globa            | bal Sheet1  |                    |     |
| Globa            | bal Sheet1<br>Specimen_001-Tube_001   |                    |     |
| Globa            | bal Sheet1  | KOMPE'L'           |     |
| 🖹 Globa<br>🔛 🏼 🍛 | • ™ ⊂ • <u>• ⊿ @ %</u> √ = #  |                    |     |
|                  |   | e e                |     |
|                  |   |                    |     |
|                  |   |                    |     |
|                  | obal Sheet Window, Click the gra  | pri type icon      |     |
|                  | -1 -1 Ob ( W/in dama Oli -1- (b   |                    |     |
|                  |   | ()                 | 2   |
| €8               |   |                    | Yr. |
|                  |   |                    |     |
|                  | 🕀 🖟 AmCyan Stained Control  | 3/19/20 2:55:13 PM |     |
|                  | General DAPI Stained Control  | 3/19/20 2:56:07 PM |     |
|                  | - 😝 Cytometer Settings  |                    |     |
|                  | 🖨 📉 Compensation Controls   |                    |     |
|                  | 🕀 🥶 Global Worksheets   |                    |     |
|                  | Cytometer Settings  |                    |     |
| 1                | Experiment 005  | 3/19/20 2:56:07 PM |     |
|                  | Experiment_005  | 3/19/20 2:56:07 PM |     |

#### 3.4 Go to Global Sheet Window, Click the graph type icon

| Image: Solution of the solut |          |
|--|----------|
|  | <b>.</b> |
|  |          |
| _ <u>Specimen_001-Tube_001</u>   |          |
|  |          |

| Ison | Turne        |
|------|--------------|
| Icon | Туре         |
|      | Dot Plot     |
| 6    | Contour Plot |
|      | Histogram    |

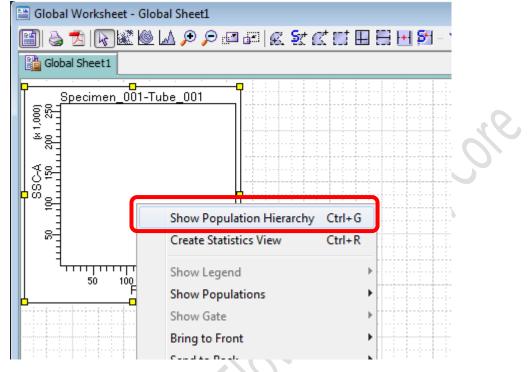
- 3.5 Click on the blank area of Global Worksheet window to create a new plot.
- 3.6 Mouse over the axis label and right click. Select the parameters of interest from the list.

| 🗎 Global Worksheet - Global Sheet1  |    |
|---|----|
| i 🖾 😞 🔁 💽 🌌 🕲 🖾 🗩 🔎 i 🗖   | 21 |
| Global Sheet1   |    |
| Specimen_001-Tube_001   |    |
|   |    |
| 200 K 1000  |    |
|   |    |
| Kara Sector Sec |    |
| FSC-H   |    |
| FSC-W   |    |
| SSC-A TTTT  |    |
| SSC-H × 1,000   |    |
| SSC-W   |    |

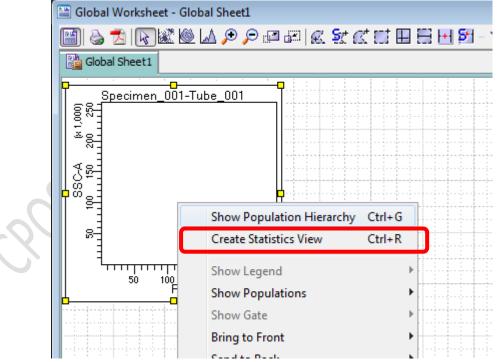
3.7 Repeat step 3.4 - 3.6 until all plots needed is created.

## \* Essential Plots: FSC-A VS SSC-A; FSC-H VS FSC-W; SSC-H VS SSC-W

3.8 Click on any plot and right click. Click Show Population Hierarchy



3.9 Click on any plot and right click. Click Create Statistics View



3.10 Right Click on Statistics View table, select **Edit Statistics View** to select statistics of interest to be shown in the table.

| Tube: Tube_001   |            |               |         |         |        |                     |        |
|------------------|------------|---------------|---------|---------|--------|---------------------|--------|
| Population       |            |               | #Events | %Parent | %Total |                     |        |
| All Events       |            |               | 0       | ****    | ****   |                     |        |
| Experiment Name: | • •        | -             |         |         |        | <b>1</b>            |        |
| Specimen Name:   | Specimen_I | 001           |         |         |        |                     |        |
| Tube Name:       | Tube_001   |               |         |         |        |                     |        |
| Record Date:     |            |               |         |         |        |                     |        |
| \$OP:            |            |               |         |         |        | Edit Statistics Vie |        |
| GUID:            |            |               |         |         |        | Export Statistics   |        |
|                  |            |               | F       | SC-A    | SSC    | 🔏 Cut               | Ctrl+X |
| Population       | #Events    | %Parent       | 1 1     | Mean    | Me     | 🗈 Сору              | Ctrl+C |
| All Events       | D          | _ <i>****</i> | : ;     | ****    | ##     | Delete              | Delete |

3.11 Click Statistics Tab, check the boxes of interested statistics and then click OK

| Parameters     | Al   | Min | Max | Geo M | Mean   | Median | 50 | 150 | 200 | 2<br>%/CV | Mode |
|----------------|--|-----|-----|-------|--|--------|----|-----|-----|-----------|------|
| PSC-A          |  |     |     |       |  |        |    |     |     |           |      |
| PSC-H          |  |     |     |       | <b>V</b>   |        |    |     |     |           |      |
| PSC-W          |  |     |     |       |  |        |    |     |     |           |      |
| SSC-A          |  |     |     |       | ¥  |        |    |     |     |           |      |
| SSC-H          |  |     |     |       |  |        |    |     |     |           |      |
| 99C-W          |  |     |     |       |  |        |    |     |     |           |      |
| FITC-A         |  |     |     |       | <b>V</b>   |        |    |     |     |           |      |
| PE-A           |  |     |     |       | Image: A start and a start |        |    |     |     |           |      |
| APC-A          |  |     |     |       | <b>V</b>   |        |    |     |     |           |      |
| Time           |  |     |     |       |  |        |    |     |     |           |      |
| Decimal Places |  | 0   | 0   | 0     | 0  | 0      | 0  | 0   | 1   | t         | 0    |
|                | <ul> <li>Sort by Perameter</li> <li>Sort by Formula</li> <li>Display Range</li> <li>Linear Scale (0-10,000)</li> </ul> |     |     |       |  |        |    |     |     |           |      |

S. Mar

#### D. Sample Acquisition

1. Gently tab or pulse vortex your sample and put your sample tube on SIP (Sample injection port).

\*Run the unstained/negative control sample before stained samples.

**!!** DO NOT return the support arm to the centre without putting a tube filled with liquid on SIP!!

2. Press **RUN** and **LO** on the fluidics control panel.



3. Go to Acquisition Dashboard, Click Acquire Data

| Current Activity  |            |                 |             |                |             |                      |  |
|-------------------|------------|-----------------|-------------|----------------|-------------|----------------------|--|
| Active Tube/Well  |            | Threshold Rate  | Stoppin     | ng Gate Events | Elapsed Tir |                      |  |
| Tube              | _001       | 0 evt/s         |             | 0 evt          |             | 0:00:00              |  |
| Basic Controls    | _          |                 |             |                |             |                      |  |
| eij Next Tube     |            | Acquire Data    | Record Data |                |             | Restart              |  |
| Acquisition Setup |            |                 |             |                |             |                      |  |
| Stopping Gate:    | All Events | V Events To Re  | cord:       | 10000 evt      | ~           | Stopping Time (sec): |  |
| Storage Gate:     | All Events | V Events To Dis | nlav:       | 1000 evt       | ~           |                      |  |

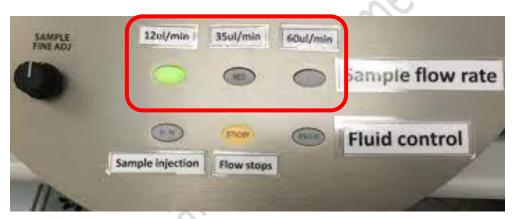
4. When Threshold rate > 0, go to Cytometry window and Click **Parameters** tab.

| Statu Parameters Threshold Laser | Compensation | Ratio |   |          |     |    |
|----------------------------------|--------------|-------|---|----------|-----|----|
| Parameter                        | Voltage      | Log   | A | н        | W   | -1 |
| + FSC                            | 179          | Ð 🗆   |   | <b>V</b> |     | 1  |
| • SSC                            | 240          |       |   | 1        |     |    |
| • DAPI                           | 343          |       |   |          |     |    |
| • AmCyan                         | 383          | 1     | V |          | 100 |    |

5. Adjust PMT Voltage accordingly

| Status Parameters Thresho | ld Laser Compensation | Ratio     |           |          |         |   |
|---------------------------|-----------------------|-----------|-----------|----------|---------|---|
| Parameter                 | Voltage               | Log       | A         | н        | W       |   |
| • FSC                     | 179                   |           |           |          |         |   |
| • SSC                     | 240                   |           |           | <b>V</b> | V       |   |
| • DAPI                    | 343                   |           |           |          |         |   |
| • AmCyan                  | 383                   | 1         |           |          | 0       |   |
| Channel                   | Suggested V           | oltage ra | nge for n | nammalia | n cells |   |
| SC                        | 450-600               |           |           |          |         | 5 |
| SC                        | 250-310               |           |           |          | - ()    |   |
| luorescence               | 300-850               |           |           |          |         |   |

6. Adjust **Sample flow rate** on the fluidics control panel if needed (optimum Threshold rate 2000 – 5000 evt/s)





## E. Create Gates

1. Go to Global Sheet Window, Click the type of gate needed

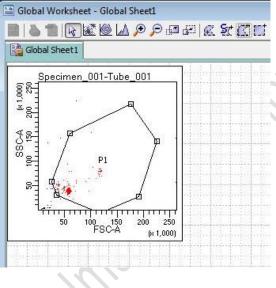
 Global Worksheet - Global Sheet1

 Image: Second Sheet1

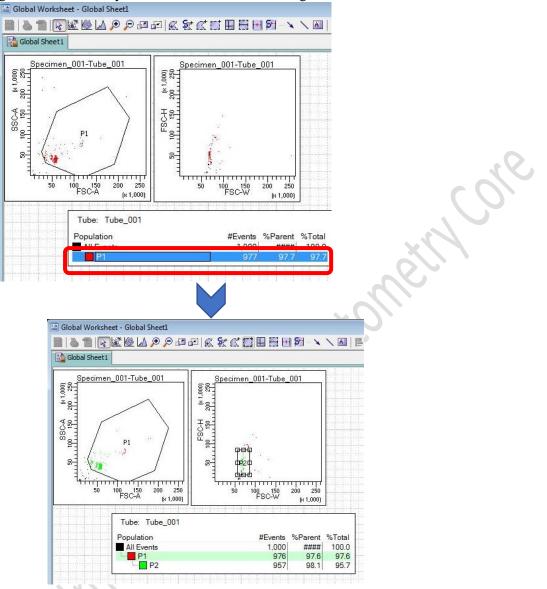
 Image: Sheet1

| Icon | Туре                |
|------|---------------------|
| €¢   | Polygon Area Gate   |
| EET  | Rectangle Area Gate |
|      | Quantrad Gate       |
| 8    | Interval Gate       |

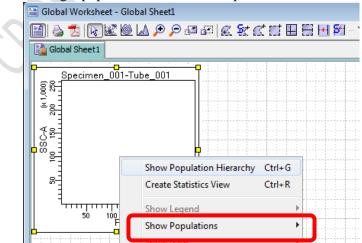
2. Draw the gate on the plot of interest to gate out target cluster /peak



3. If you want to create a new population out of particular parent population, highlight the parent gate on the hierarchy table first and then create the gate.



4. Click on target plot and right click, Click *Show Population* > *Target population* to visualize the target population ONLY in that plot.



## F. Data Recording

1. Go to Acquisition Dashboard, set Stopping gate to singlet gate or live cell gate

| Current Activity     |                             |                         |                 |   |
|----------------------|-----------------------------|-------------------------|-----------------|---|
| Active Tube/Well     | Threshold Rate              | Stopping Gate Events    | Elapsed Time    |   |
| Tube_001             | 0 evt/s                     | 0 evt                   | 00:00:00        |   |
| Basic Controls       |                             |                         |                 |   |
| <b>∳</b> ] Next Tube | Acquire Data                | Record Data             | start           |   |
| Acquisition Setup    |                             |                         |                 |   |
| Stopping Gate: XP2   | vents To Record:            | 20000 evt 🗸 Stopp       | ing Time (sec): | 0 |
| Storage Gate:        | Events 🗸 Events To Display: | 1000 evt $\sim$         |                 |   |
| Acquisition Status   |                             |                         |                 |   |
| Processed Events:    |                             | Electronic Abort Rate:  |                 |   |
| Threshold Count:     |                             | Electronic Abort Count: |                 |   |

## 2. Set the Storage gate to All Events

| 🔢 Acquisition Dashboard |                          |                         |                          |
|-------------------------|--------------------------|-------------------------|--------------------------|
| Current Activity        |                          |                         |                          |
| Active Tube/Well        | Threshold Rate           | Stopping Gate Event     | s Elapsed Time           |
| Tube_001                | 0 evt/s                  | 0 evt                   | 00:00:00                 |
| Basic Controls          |                          |                         |                          |
| <b>∛</b> Next Tube      | Acquire Data             | Record Data             | Restart                  |
| Acquisition Setup       |                          |                         |                          |
| Stopping Gate: P2       | v Events To Record:      | 20000 evt               | ✓ Stopping Time (sec): 0 |
| Storage Gate: All Ev    | ents 🗸 vents To Display: | 1000 evt                | ~                        |
| Acquisition Status      |                          |                         |                          |
| Processed Events:       |                          | Electronic Abort Rate:  |                          |
| Threshold Count:        |                          | Electronic Abort Count: |                          |
|                         |                          |                         |                          |

3. Set Events To Record, i.e. events number out of stopping gate to be recorded

| 🚺 Acquisition Dashboard |                     |                       |                          | <b>—</b>         |
|-------------------------|---------------------|-----------------------|--------------------------|------------------|
| Current Activity        |                     |                       |                          |                  |
| Active Tube/Well        | Threshold Rate      | Stopping Ga           | te Events Elaps          | ed Time          |
| Tube_001                | 0 evt/s             | 0 evt                 | 00:0                     | 0:00             |
| Basic Controls          |                     |                       |                          |                  |
| <b>∳</b> ] Next Tube    | 📕 Acquire Data      | Record Data           | Restart                  |                  |
| Acquisition Setup       |                     |                       | _                        |                  |
| Stopping Gate:          | P2 V Events         | To Record: 20000 ev   | /t 🗸 Stopping Time (sec) | : • • <b>•</b> • |
| Storage Gate:           | All Events 🗸 Events | s To Display: 1000 ev | t v                      |                  |
| Acquisition Status      |                     |                       |                          |                  |
| Processed Events:       |                     | Electronic Abo        | rt Rate:                 |                  |
| Threshold Count:        |                     | Electronic Abo        |                          |                  |

| 4. CHER RECOLU Data | 1. | Click Record | Data |
|---------------------|----|--------------|------|
|---------------------|----|--------------|------|

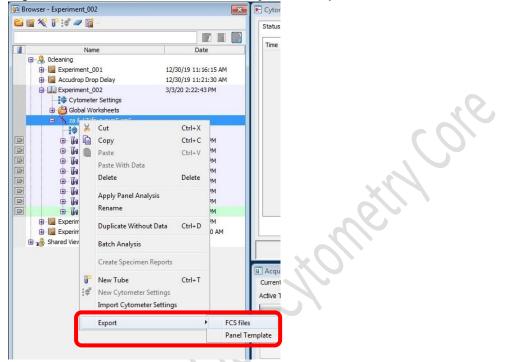
| <b>II:</b> Acquisition Dashboard   |                        |                   |                      | ×       |
|--|------------------------|-------------------|----------------------|---------|
| Current Activity   |                        |                   |                      |         |
| Active Tube/Well   | Threshold Rate         | Stopping Gate     | Events Elapse        | ed Time |
| Tube_001   | 0 evt/s                | 0 evt             | 00:00                | 0:00    |
| Basic Controls   |                        |                   |                      |         |
| <b>ø</b> investor value valu | Acquire Data           | Record Data       | Restart              |         |
| Acquisition Setup  |                        |                   |                      |         |
| Stopping Gate:   | P2 v Events To         | Record: 20000 evt | Stopping Time (sec): | 0 🖨 🕇   |
| Storage Gate:  | All Events V Events To | Display: 1000 evt | ~                    |         |
| Acquisition Status   |                        |                   |                      |         |
| Processed Events:  |                        | Electronic Abort  | Rate:                |         |
| Threshold Count:   |                        | Electronic Abort  | Count:               |         |
|  |                        |                   |                      |         |

# 5. Click **Next Tube** to create a new sample

| Active Tube/Well Tube_001 Basic Controls | Threshold Rate 0 evt/s | Stopping Gate E<br><b>0 evt</b> | Events Elapsed Time 00:00:00 |
|--|------------------------|---------------------------------|------------------------------|
| Basic Controls                           | 0 evt/s                | 0 evt                           | 00:00:00                     |
|  |                        |                                 |                              |
| 7  |                        |                                 |                              |
| ø <mark> </mark> Next Tube               | 📕 Acquire Data         | Record Data                     | Restart                      |
| Acquisition Setup                        |                        |                                 |                              |
| Stopping Gate:                           | P2 V Events To         | Record: 20000 evt               | Stopping Time (sec):         |
| Storage Gate:                            | All Events V Events To | Display: 1000 evt               | ~                            |
| Acquisition Status                       |                        | L                               |                              |
| Processed Events:                        |                        | Electronic Abort R              | ate:                         |
| Threshold Count:                         |                        | Electronic Abort C              | ount:                        |
| 11                                       | 06)                    |                                 |                              |

## G. Data Export

- 1. FCS file
  - 1.1 Go to Browser window, Select the Tubes / Specimen of interest.
  - 1.2 Right Click over the selection and click *Export* > *FCS file*



1.3 Select *FCS 3.0* and keep all parameters Linear. Click *OK* 

| Gated Events      |                            |       |        |  |
|-------------------|----------------------------|-------|--------|--|
| File Version      | FCS3.1                     |       |        |  |
| Parameter         | Parameter Type             | U Log |        |  |
| FSC-H             | Linear                     | 🕐 Log | 🔘 None |  |
| FSC-W             | Linear                     | 🕐 Log | 🔘 None |  |
| SSC-A             | Iinear                     | 🕐 Log | None   |  |
| ssc-H             | Iinear                     | 🕐 Log | None   |  |
| SSC-W             | <ul> <li>Linear</li> </ul> | 🕑 Log | None   |  |
| FITC-A            | inear                      | 🖱 Log | None   |  |
| DAPI-A            | Linear                     | 🔘 Log | 🔘 None |  |
| APC-Cy7-A         | Linear                     | 🔵 Log | 🔘 None |  |
| PE-Cy7-A          | inear                      | 🖱 Log | None   |  |
| Alexa Fluor 700-A | Linear                     | 🕐 Log | 🔘 None |  |
| Time              | Linear                     | O Log | None   |  |

1.4 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

| 1.5 | Click | Save |
|-----|-------|------|
| 1.5 | CIICK | Save |

| irectory Path    |           |        |
|------------------|-----------|--------|
| D:\User\SCM\LinX |           | Browse |
| Save             | Details>> | Cancel |

- 2. PDF file
  - 2.1 To export pdf of multiple tubes, Click the tube pointer of any tubes

| I | Name  | Date                 |
|---|---|----------------------|
| G | ∋ 😓 Ocleaning   |                      |
|   | 🖶 📔 Experiment_001  | 12/30/19 11:16:15 AM |
|   | <ul> <li>Accudrop Drop Delay</li> <li>Cytometer Settings</li> <li>Specimen_001</li> </ul> | 12/30/19 11:21:30 AM |
|   | 🖽 🔰 Tube_001  |                      |
| _ | 🕀 🔚 Experiment_002  | 3/3/20 2:22:43 PM    |
|   | 🕀 🔚 Experiment_003  | 3/9/20 5:11:11 PM    |

- 2.2 Select the wanted tubes/ Specimen.
  - Browser Experiment\_002 🗶 Cytor ビ 🝯 餐 🧗 📝 🜌 📓 Status Time J Date Nam 🖃 🚴 Ocleaning 12/30/19 11:16:15 AM Experiment\_001
     Accudrop Drop Delay 12/30/19 11:21:30 AM Experiment\_002 3/3/20 2:22:43 PM Cytometer Settings Cut Ctrl+X X :0 🕀 🐻 🗋 Сору Ctrl+C - 6 PM Paste Ctrl+V PM Paste With Data ÞΜ Delete Delete M • **h** Apply Panel Analysis • 6 M Rename M 🕀 🔚 Experim Duplicate Without Data Ctrl+D 🕀 📔 Experim MAC Batch Analysis 🗄 🍰 Shared View Acqu 📔 New Tube Ctrl+T Curren
- 2.3 Right Click over the selection and click Batch Analysis

2.4 Check the boxes as picture below

| <ul> <li>Auto</li> <li>View Time:</li> <li>Manual</li> </ul> | 10 👻    | Output To Printer  Save as PDF Save as XML  Add Report | Sec. and   | s<br>Biexponentia<br>ferred Globa |          |
|--|---------|--|------------|-----------------------------------|----------|
| PDF Filename:  | t_003-B | atch_Analysis_19032020                                 | 143013.pdf | Browse                            | View PDF |
| (ML Filename;  | t_003-B | atch_Analysis_19032020                                 | 143013.xml | Browse                            |          |
| Stats Filename:  | t_003-B | atch_Analysis_19032020                                 | 143013.csv | Browse                            | ]        |
|  |         |  | 10         |                                   |          |

2.5 Click **Browse** to choose the destination (D:/User/Department/PersonalFolder)

| <ul> <li>Auto</li> <li>View Time: [</li> <li>Manual</li> </ul> | 10 👻    | <ul> <li>Output To Printer</li> <li>Save as PDF</li> <li>Save as XML</li> <li>Add Report</li> </ul> | Sec. and   | :s<br>Biexponentia<br>ferred Globa |          |
|--|---------|---|------------|------------------------------------|----------|
| PDF Filename:  | t_003-B | atch_Analysis_19032020  | 143013.pdf | Browse                             | View PDF |
| XML Filename:  | t_003-B | atch_Analysis_19032020  | 143013.xml | Browse                             |          |
| Stats Filename:  | t_003-B | atch_Analysis_19032020  | 143013.csv | Browse                             |          |
| Status:  |         | 0°  | /0         |                                    |          |

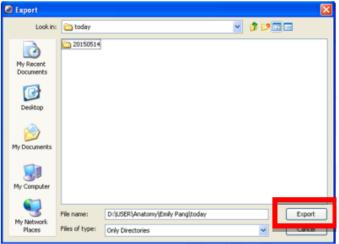
2.6 Click **Start** and then **OK**.

| <ul> <li>Auto</li> <li>View Time:</li> <li>Manual</li> </ul> | Output To Pr Output To Pr Save as PDF Save as XML Add Report | 🔽 Fr           | atistics<br>eeze Biexponent<br>e Preferred Glob |          |
|--|--|----------------|---|----------|
| PDF Filename:  | t_003-Batch_Analysis_190                                     | 032020143013.p | odf Browse                                      | View PDf |
| XML Filename:  | t_003-Batch_Analysis_190                                     | )32020143013.> | cml Browse                                      | Ĩ        |
| Stats Filename:  | t_003-Batch_Analysis_190                                     | )32020143013.c | sv Browse                                       | ī.       |
| Status:  |  | 0%             |   |          |
|  | Start  | Pause          | Continue  | Close    |
| Tube_(<br>Tube_(<br>Tube_(                                   | 02   | al worksheet:  |   |          |

- 3. Experiment
  - 3.1 Right click on the **Experiment > Export > Exerpiments** 3 20150514 5/14/15 4:26:44 PM 6/16/15 3:36:21 PM E 1 20150616 6/16/15 4:09:01 PM Ctrl+X Сору Ctrl+C ) ی 0.3 Paste Ctrl+V 0.5 Paste With Data 🕀 🔚 E 6/23/15 1:30:58 PM Delete Delete 🔒 Share Rename Duplicate Without Data Ctrl+D Open Experiment Ctrl+O Close Experiment Ctrl+W Batch Analysis... 👔 New Global Worksheet X New Specimen... Ctrl+M New Cytometer Settings Import Cytometer Settings Share Experiment Export Exp FCS files... Experiment Template...
  - 3.2 Click **Browse** to select the file destination

| 🙆 Export   | Experiments                          |                 |
|------------|--------------------------------------|-----------------|
|            | Delete experiments after export      |                 |
|            | ⊙ Directory Export ○ Zip File Export |                 |
| Directory: | D:\USER\Anatomy\Emily Pang\today     | Browse          |
| Experime   | nt Date                              |                 |
| 20150616   | 6/16/1                               | 5 4:09:01 PM  🔨 |
|            |                                      | ×               |
|            | ОК                                   | Cancel          |

3.3 Select the file destination and click **Export**.



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#### 3.4 Click **OK** to export

| Directory: | Delete experiment  Directory Export  D:\USER\Anatomy\Em | O Zip File Export | Browse    |                     |
|------------|---|-------------------|-----------|---------------------|
| Experime   |   | Date              | Croinsen. |                     |
| 20150616   |   | 6/16/15 4:0       | 09:01 PM  |                     |
|            |   |                   | ~         | $\langle O \rangle$ |
|            |   | ОК                | Cancel    |                     |

## H. Software Log out

1. To log out of FACSDiva software, go to File and click Log Out

| Save              | Ctrl+S |
|-------------------|--------|
| Page Setup        |        |
| A Print Preview   |        |
| la Print          | Ctrl+P |
| 🛃 Save as PDF     |        |
| Administration    |        |
| User Tracking Log |        |
| Import            | ٠      |
| Export            | •      |
| Log Out           |        |
| Quit              |        |

## I. System Cleaning

## \*Cleaning procedure will not interfere with the data export and analysis.

- 1. Prepare 3ml of each cleaning solution (Solution1: FACSClean; Solution 2: FACSRinse; Solution 3: MilliQ water)
- 2. Press HI (60ul/min) and RUN (Sample Injection) on fluidics control panel
- 3. Load the tube with Solution 1 on the SIP with the support arm on the side for 1 minute
- 4. Move the support arm under the tube and run for another 4 minutes
- 5. Repeat step 3 and 4 with Solution 2 and Solution 3
  \*If PI stain is used, please clean the system 9 minutes instead of 4 for Solution 2
- 6. After cleaning with Solution 3, press LO (12ul/min) and Standby (Flow Stop)