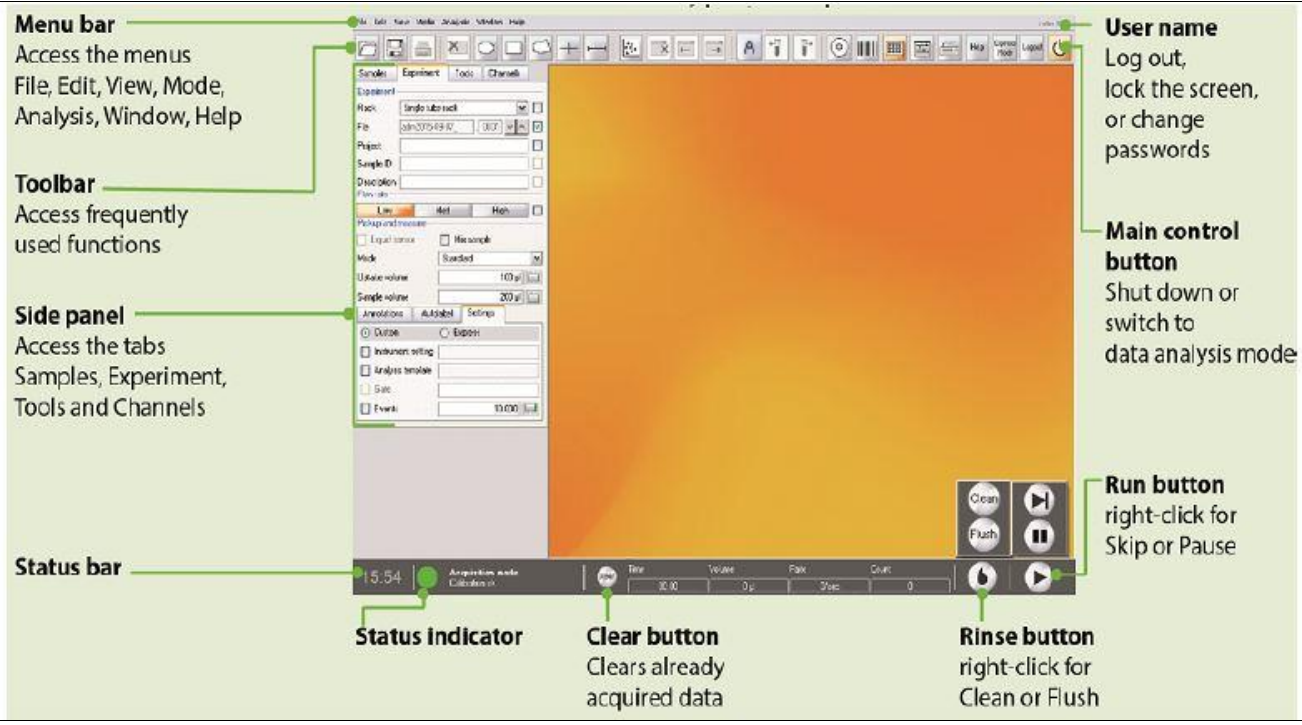
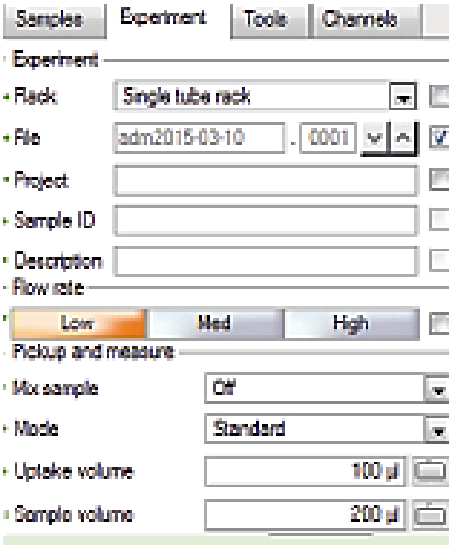
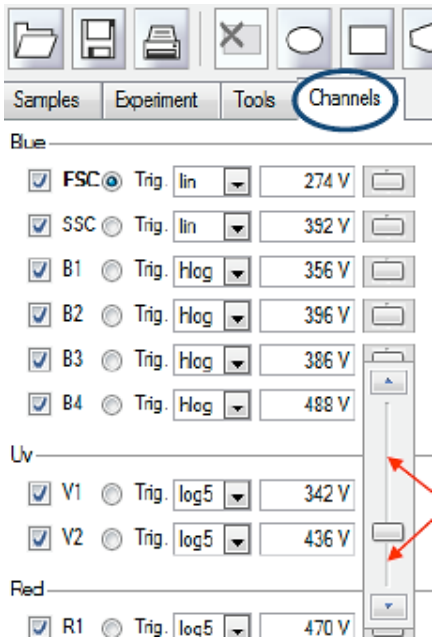
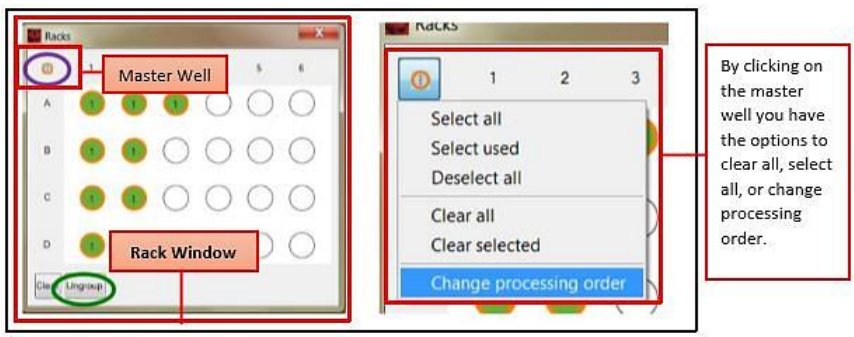
VYB Quick Start Guide

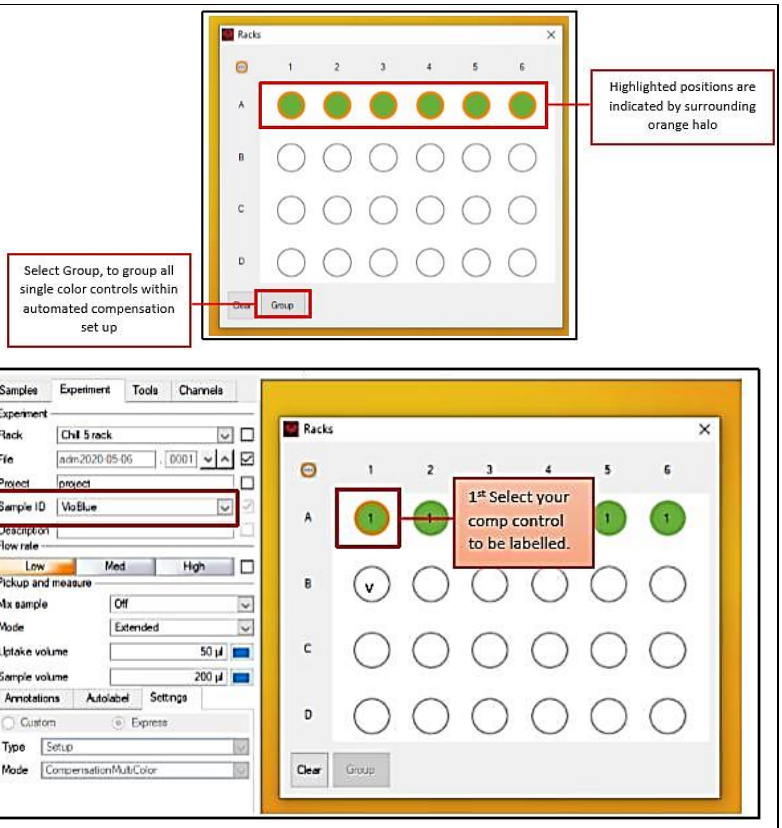
*__Download a copy of this instrument Help File__: salk.edu/fccfaccess > Help Files
**8 fluorescence channels (405nm (2), 488nm (2), 561nm (4 + FSC and SSC channel) See chart on side for filter/detectors

GREEN: Start up, turning on
YELLOW: Running bead QC/calibration/software overview
ORANGE: Running Samples and HTS
PINK: Loading Analysis and Instrument settings
BLUE: Cleaning up and Shutting down.

All FCCF instrument documents can be downloaded from our **Help Files** section on Salkland (salk.edu/fccfaccess, a shortcut has been placed on the workstation desktop).

Log in	Your lab user name, no password. Or Calibration (no password) if you have any issues.
Fluidics Sheath / Waste	Top up the sheath tank if needed, install an empty waste tank if needed **Start with a full tank/empty waste for a 96w plate run**. **Sheath**: Fill with 1 x Running Buffer (RB) from the carboy. Make up more by diluting the 16x RB with MQ H2O at the Stem Core sink if you empty the carboy (do this during the 18min Flush/10min Clean cycles). **Waste:** Bleach needs to be added to final volume 10% before disposing (can leave to sit in the FCCF sink during regular hours, staff will empty).
Wake Up VYB Hit Acquisition Mode	**Touch/click top left corner power icon** (power switch should be left ON: bottom right side of VYB). Select Acquisition Mode when prompted (instrument status will be indicated at the botton of the screen.
Do **Flush** (18min) & **Clean** (10min)	Left click droplet icon in lower right of screen to start each cycle. **Clean** prompts you to install single tube holder with tube of 10% bleach: the holder is sometimes stowed at the right side of Vyb near the power switch; "pull" it to the right to unplug (has 2 pins holding it to unit). Install in front of VYB (has 2 holes for the pins) near barcode reader window/by the sampler device.
QC (scan vial barcode to start)	Hold vial barcode near the yellow tape marked on the single tube holder (when installed in front) and then click on barcode icon at the top of screen to trigger reading. When prompted, add 1 drop to an empty tube and click "yes". Beads are kept in Parker Room fridge (outside the entryway). Check report is successful (notify staff otherwise) then REMEMBER to do File > Open > New Workspace after it is completed ahead of running any samples. This is to exit the QC template.
Software Overview Note Run, Clean, Flush buttons in lower right hand corner	
Load Template/ Instrument settings (or open these from a previous file)	File > Open > Analysis Template ("Analysis" file = plots, gates, stat windows), and then File > Open > Instrument Settings (IS) These are detector (PMT) voltages, threshold (aka "trigger") values, and any compensation previously calculated. Note: After loading IS, a IS window can also be opened for visual verification; in here the comp matrix values can be read. If you forgot to save the Analysis or the IS, but have an old .mqd file from that run, you can open those from that file (both are saved with every file. To do this Click File > Select Open > Select data file > Click public > Then open MQT file > Click the Samples tab > Then Right click and Select apply instrument settings > Then apply analysis template > then save both). If you are running exactly the same experiment as last time, you may have additionally have an experiment saved (contains info about sample volume, mixing, uptake volume, how many events to record etc). Load these by doing File > Open > Experiment. **The "Workspace" comprises all 3 including data, and is less useful to save as a whole as it can become corrupted: best to save each of data, Analysis, IS and Experiment separately**
Create an Analysis file (data plots) and Stats window if this is a new experiment	If you had nothing saved or it is a new experiment, you will need to start by loading a preset Analysis page with plots/stats tables. Click analysis template icon > Use the instrument settings icon (two icons to the right of the barcode icon) to select a page with a preset number of plots/stats table. Change axes on the plots to display relevant optical channels by clicking on the axis label and select the Area (-A) version of each parameter unless creating plots for aggregates exclusion where you will use Area as well as Height (–A and –H). First create a viability plot for live cells if this will be used (FSC vs DAPI for instance), a scatter plot to select your populations apart from any debris (FSC vs SSC), and two aggregate exclusion plots (SSC-A vs SSC-H and FSC-A vs FSC-H), followed by any plots needed to look at the fluors in your experiment. A Stats table is

	optional, and can be edited later to report on regions you draw; a useful measurement unique to the VYB is the cell concentration count for any region/population analyzed. This can be selected by editing the stats table (click on the "i" next to the table. Save the Analysis File to reuse it (especially before running any automated process such as the Multicolor Compensation where you will need to reload (or redraw!!) the Analysis afterwards
Prepare to run samples in tubes to optimize PMTs: (1) Make a new **Project** and (2) Define the sampling parameters in **Experiment** tab	 Experiment Tab (left of screen, choose the tab). Recommend to do set up in single tubes with a larger volume to enough time to adjust PMTs. **(1)** Choose the rack (Single Tube). **(2)** Project field must be labeled before starting e.g. DDMMYY-Caz-Setup. **(3)** Recommend Sample ID be filled in later to saved time, after the voltages are optimized, controls are checked (i.e. when the samples are ready to record); description is not necessary – leave blank. **(4)** Choose a flow rate based on concentration (if events populate slowly due to dilute samples, run in HI for set up to save time) bearing in mind that for actual recording best resolution is obtained by concentrating cells and running as slowly as possible. **(5)** Pick up and Measure settings: Important to choose these! Set mixing as desired (eg gentle, or medium for live (or for later wells of a automation run where cells could have pelleted over time; high for fixed cells). Mode: Choose for probe rinse (eg Standard; use Extended for very stringent low carry over washing for rare events/sticky samples – note it will takes longer to execute). Uptake volume: enter how much to commit to each run (DON'T RUN ALL THE SAMPLE NOW, this is just for setting up and you may need to run little bits several times depending how fast you are at setting up). Sample volume: Enter the volume in the tube/well (be sure to adjust for loss after setting up before starting any automation run. OK to enter a little less than what is there but be "fairly" close as this is used to calculate half the volume taken up (will be returned to tube/well) to ensure proper mixing of the sample. This is important for plates/rack of tubes run with automation to prevent issues due to cells settling out of solution.
Click on the **Channels** tab: Prepare to run small amounts of your sample and make adjustments to the Channels tab as it runs. See the **fluor labels** we stuck on left of VYB screen as a channel guide! Need enough sample volume/cells to have time to set up comfortably	**Before you press start** to run the sample, try out the channel slider: Choose one channel (e.g. FSC), take a note of the starting value so you can come back to it if needed. (2) Click on the FSC slider icon (button to the right of it's set channel voltage value). This opens the Channel slider. Note that once the sample is going, you should adjust (while checking the effects to the sample) by clicking on the "slider line" above (e.g. to raise) or below the slider's current set position (indicated by the small rectangle on the vertical slider line). This method makes nice 10 V adjustments. DO NOT GRAB THE SLIDER and move it, it is too sensitive (can lose your populations altogether). (1) **Start the sample and make adjustments while looking at plots:** Install the tube and press the START/PLAY icon . Adjust PMTS to: **(1)** put scatter populations on the plot and **(2)** put any fluorescent label signals "on scale" for their axes (i.e. no events should be off the high end of the range or they will be lost). Be sure to use the Clear button very regularly (located at the bottom of the screen) to refresh the events accumulating on the plots. Note that It may be easier to evaluate fluorescent signals after gating on live cells/scatter plot first (to "clean up" the plots). **(3)** To draw regions and form an appropriate hierarchy of connected regions and show these events on a plot: Select a gating tools > such as the polygon and draw (click around) around populations of interest. Form a gating hierarchy by first showing the "parent" region on a plot (click at the plot name > choose the region) and then proceed to draw the next region (becomes the "child" in hierarchy). **(4)** Repeat this process to check any control samples you have (e.g. negative, single colors) and be sure to check your brightest sample(s) also. This is to ensure you have an appropriate voltage for the entire set of samples to be on scale. **(5) Edit stats table** if needed: once various regions are drawn, edit any stats/regions displayed in the table (e.g. cell count, or choose other specific stats). **(5) When all PMTs are optimized** you can proceed to setting to run everything (set up a rack/automation run). 
Compensation Multicolor: run using overlapping colors	***Skip to next section if not needed*** Make sure to have **saved the Analysis** before starting. After running it, be sure to File > Save > IS, and then open a new Workspace (File > Open) to clear the compensation template. Open your saved Analysis and Open the IS to load the comps etc. **(1)** Under Experiment Tab select "Chill 5 Rack". **(2)** Label project as the folder name for your experiment (remember that this is where your recorded files will be saved). **(3)** Click on "rack" icon > and rack window will appear. Click on the "master well" located in the upper left corner of the rack window to adjust orientation of acquisition from up to down vs left to right. Add your unstained and single stain samples into rack, beginning with A1 and adding samples in consecutive spots in columns or rows (depending on what you have selected for acquisition orientation) (ex. A1, B1, C1, D1 OR A1, A2, A3, A4). 

	(4) Highlight all the positions selected for running controls on the software rack layout and select "group". The highlighted positions should have an orange halo surrounding them. **(5)** Under Experiment Tab, Click on Settings tab and select "express". For Type, select "setup". For Mode, select "compensationMultiColor". For Mix Sample, select "gentle mix". Type in the Uptake volume and Sample volume appropriate for your samples. Assign proper fluorochrome lavel to each compensation control within rack window by clicking on each comp one at a time, then selecting corresponding color/channel under Experiment Tab ID drop-down menu. **(6)** Once all wells are labelled properly, start acquisition by clicking play icon. When prompted by the software, draw a gate around the cells of interest in the scatter plot, then click continue. When automated compensation finishes, create a name for the instrument settings, then click "save". **These settings will automatically be applied to the current acquisition session until you change them, load another instrument setting, or log-out. *Before moving on, right click on the "master well" icon and "clear", then go to Settings Tab under Experiment main tab and select "custom" mode.* 
Automation: Prepare to run all samples Make a new **Project** Choose **rack** > open viewer Add **Experiment** info: **SampleID** (do one at a time) followed by shared info (**select all samples**). Check the **Experiment Table.**	**(1)** Go to Experiment tab> Select the rack (eg. Chill5 for 5ml tubes) or plate. **(2)** Create a new Project: change the name (e.g. DDMMYY-Name-ExperimentName). This makes a new folder so it easy to find all the final data files. **(3)** Open up the rack icon to view a layout of the individual positions within the rack, as you will need to label/assign with information such as Sample ID, fluor names, how many events to record, sample uptake info etc. **(4)** Note the running orientation (default is vertical) of the automation rack, indicated in the top left of the viewer (vertical dots vs horzontal dots > click to change if needed). Each position turns green when clicked (becomes added/activated), and should show an orange circle when it is active for labeling. **(5)** Add individual SampleID (Experiment Tab): Click on one green dot (only that green dot should now be orange ringed once "selected") and enter information. Deselect green dot (click to remove orange ring) and add next SampleID untill all samples are added. **(6)** Add information common to all the samples: click each green dot one after another (i.e. several will then be orange ringed), then add information to Experiment tab fields (see previous section if needed), regarding Flow Rate, Mixing, Mode (probe rinse), Uptake volume (how much to commit to the run) and Sample Volume (what is left in the sample tube/well, i.e. adjust for loss if needed, after setting up). **(7)** Add fluor labels if desired: Experiment Tab > Annotations Tab (at bottom of main tab). **(8)** Enter # events to record or stopping gate info: Experiment Tab > Settings Tab (bottom of main tab). **(9)** Do a final check for all the labels and info entered by opening the Experiment Table: View > Experiment Table, scroll through and verify the information in the different tab sections. **(10)** If running with a Chill5 rack and you have space, after the samples **the 3 cleaning tubes can be added as part of the automation** rack (select the 3 positions together in rack viewer first before adding the Experiment Tab "common" running info to all). Run in Flow Rate = HI, Sample Uptake = 450ul (sampleID not really needed, optional) loading the tubes in this order: 1ml Rinse (contrad detergent), followed by 1ml Bleach ("Clean", or 10% bleach), finishing with 1ml filtered H2O. (11) Press START/PLAY and walk away!
Clean	*Skip to next step if already added to automation rack. If you ran a plate, set up a Chill5 rack and run as per step (10 and 11) of previous section to load 1ml of Rinse (detergent) > Clean (bleach) > Water to run 450ul ("Sample Uptake") on flow rate HI. Otherwise, Right click Rinse button and select Flush. Right Click Rinse Button and Select Clean, then follow prompts to place bleach on the sample port.
Export Data, delete last session data	(1) Insert your USB into back left side of YVB (labeled with tape!). (2) Do File > Copy: Find your files and copy TO USB (either take just FCS files, or both versions but will double your server space). (3) Eject USB. (4) Do File > Copy: Check last session's data and delete.
Make screen grab/print to Document writer	Load recorded samples into plots for viewing by clicking on them in the Samples Tab. Hit the PrintScrn button on the keyboard. Repeat for each sample needed. Alternatively, if you have a PC, use File > Print > Microsoft Document printer to create .xps files that can later be converted to PDF. Retrieve these images/files under File > Open > Other Files. *Data analysis option: Sign up for Flowjo (ask fccf@salk.edu) through the Salk site license.*
Save IS, Experiment	File > Save > IS (label with name/date etc), File > Save > Experiment (again, label appropriately).