FCRC December 2018 - October 2019
Status Report for Scientific Advisory Committee (October 25, 2019)

Staff and Operation

- Currently five full time staff positions:
  - Svetlana Mazel, FCRC Director, regular hours
  - Stanka Semova, Operations Manager Flow Cytometry Resource Center, full time, staggered hours
  - Songyan Han, FCRC Research Support Specialist, full time, staggered hours
  - Alena Keprova, FCRC Research Support Specialist, full time, regular hours
  - New Hire: Samer Shalaby, FCRC Research Support Associate, full time, staggered hours

- FCRC Staff operated instruments:
  - Cell Sorters - FACSAria-1, FACSAria-2, FACSAria-3, and ImageStream-X

Capital Equipment Update

Accomplished:

- Spectral Analyzer **Cytek Aurora** at FCRC planned upgrades:
  - FCRC has completed the commissioning process for the Cytek Aurora platform and starting February 1, 2019 it became fully available for use at the same hourly rate as conventional Advanced Analyzers
  - Automatic Micro-Sampling System (AMS) the Multi Loader was purchased in mid-March 2019 and installed in mid-May 2019
  - To show the Rockefeller University researchers capability of the Spectral Flow Cytometry, multiple experiments on Aurora are currently performed by FCRC Staff (Alena Keprova) free of charge
  - Since the hourly rate was applied (February-September 2019) Cytek Aurora was used by internal users and the User Credits fluctuated between $316 and $1342
  - Despite the multiple upgrades, the user fees for the Aurora (64 fluorescent channels, 40 colors) will remain at the same rate as for conventional Advanced Analyzers (with 16-18 fluorescent channels)

- Advanced Analyzer **Attune NxT**:
  - FCRC has completed the commissioning process for the Attune NxT platform and starting February 1, 2019 it became fully available for use at the rate of the Basic Analyzer, BD Accuri C6. It appeared to be possible because as a

POS for Attune NxT we were able to negotiate extremely low service contract fees

Capital equipment proposals status:

FCRC equipment needs FY19 to FY24_20190719_SM.xlsx

By type (without justification)

		Amount	Priority FY19	Priority FY20	Priority FY21	Priority FY22	Priority FY23
	Flow Cytometry						
Imaging Flow Cytometer	Upgrade ImageStream-X to ImageStream-Mark II	**$428,000**	High	Critical	**Essential**	too late	
Cell Sorters	SONY MA900	**$360,000**	Critical	**Essential**			
	Cell Sorter - high end, FCRC Operated (similar to BD FACSAria or Beckman Coulter ASTRIOS EQ)	**$900,000**		High	Critical	**Essential**	
	Additional SONY MA900 or similar mid-level cell sorter	**$400,000**		High	Critical	**Essential**	
	Cell Sorter - high end, FCRC Operated (similar to Beckman Coulter ASTRIOS EQ or BD FACSAria)	**$1,000,000**			High	Critical	**Essential**
Cell Analyzers	Cytek Aurora Upgrade with Ultra-Violet Laser	**$85,000**	Critical	**Essential**			
	Cell Analyzer - high end (similar to BD FACSymphony A3, five-laser configuration 23 colors, up to 28 colors)	**$331,665**	Critical	**Essential**			
	Cell Analyzer - high end (similar to BD FACSymphony A5, five-laser configuration up to 48 colors)	**$800,000**		Critical	**Essential**		
	Cell Analyzer - middle complexity (similar to Beckman Coulter CytoFLEX EX LX)	**$400,000**		Critical	**Essential**		
	Attune NxT Upgrade to add Violet and Red Lasers	**$76,000**	High	Critical	Critical		
Small Equipment (Essential for Improvement of the Sample Preparation)	Inverted fluorescent microscope	**$50,000**	High	**Essential**			
	gentleMACS™ Octo Dissociator with Heater	**$23,800**		**High / Essential**			

- Two essential pieces from the list above were funded in the FY20 budget – Sony MA900 and the Cytek Aurora upgrade with the Ultra-Violet laser (marked in green):
  - Sony MA900:
    - During the month of August 2019, in anticipation of the arrival of the Sony MA900:
      - Room DWB211 went through comprehensive (lab bench, shelves and cabinets rearrangements by RU Plant Operations
      - FCRC Staff has relocated several of FCRC Analyzers (BD LSR-Fortessa; ThermoFisher Attune NxT and BD Accuri C6) within the DWB211
    - Sony MA900 in the Baker Safety Cabinet was installed on 8/28/19
    - First step of FCRC Staff training was performed on 9/19/19 by Gisele Knowles, application specialist from Sony Biotechnology Inc.
    - FCRC Staff started running the test experiments on the instrument
    - Second step of FCRC Staff training is scheduled for 11/19/19 and 11/20/19
    - After Thanksgiving Holidays, the FCRC Staff will start performing sorts for users on Sony MA900

- Cytek Aurora upgrade with Ultra-violet laser:
    - The UV-laser upgrade was purchased in May 2019
    - To the advantage of the FCRC at RU it was negotiated with Cytek Biosciences that instead of the upgrade with the UV (which will require the 3-4 weeks downtime to send the Aurora 4L for the in-house retrofit laser to Aurora 5L), the Rockefeller University got the new Aurora 5L
    - Cytek Aurora 5L (5-laser) was delivered in September 2019:
        - Aurora 5L has been installed on October 9-11, 2019 in order to replace Aurora 4L
        - For the length of the transition period (three weeks) both Auroras (Aurora 4L and Aurora 5L) – will be installed side-by-side in the FCRC Analysis Room (DWB211)
        - FCRC Staff and Users will perform the experiments on both instruments in order to ensure smooth transition and reproducibility of data between the instruments

Future Capital Equipment Concerns:

- Basic Analyzer **BD Accuri C6** is in constant, but low usage by several labs. In the future we plan most of the experiments from BD Accuri C6 to be transferred to the Attune NxT with alteration of the fluorochrome panel if the red-excyted dyes are in use. Accuri Taking in account the limitation with the absence of the red laser on Attune NxT, in the long run we plan for the laser upgrade (to be discussed for red and violet lasers)

- Image Analyzer, **ImageStream-X**:
    - Usage of the ImageStream-X stays extremely low and fluctuates on the monthly basis. In the reporting period (December 2018 - September 2019) user experiments were run five out of the ten months and User Credits (with no external users) were fluctuating between $300 and $1000
    - Taking in account current development of the situation, FCRC keeps this aging instrument in a good shape and didn't lose the hope for its upgrade to ImageStream MarkII
    - Bringing in very low User Credits, this instrument is still extremely valuable and essential for several types of experiments performed by different labs. Main usage of it is for the sample evaluation purposes, when the conventional flow cytometry or imaging representation of the data are not sufficient (Fuchs, Hang, Nussenzweig, Brivanlou, Greengard and Smogorzewska Labs)

Highest Priority Needs for FY21:

- Upgrade ImageSteamX to Mark II ($440K): While the instrument is not heavily used, it is a unique and powerful technology. If not upgraded in FY21, the technology will become de facto obsolete. It still uses the Windows XP OS, which is no longer supported
- Attune NxT Upgrade to add Violet and Red lasers ($76K): Recommended by SAC, to increase its capacity.
- BD Symphony ($800K) or promotion for BD Symphony A3 SORP with the BD LSRII trade-in ($427K): To maintain FCRC analyzers state of the art and diversified for High Parameter (HP = more than 18 colors). Note that if BD Symphony will be acquired one or both of the BD LSRs could be decommissioned in FY21-FY22

Space Planning

On request of Amy Wilkerson the floor plan proposal for the FCRC relocation to half of a floor in WRB for expansion from current 1900 sq ft to 3500 sq ft space and for more convenient access to the GRC in support of single cell sequencing needs was developed on 1/14/2019 and was attached to the corresponding FCRC January 2019 Quarterly Status Report

Research and Development Projects at FCRC

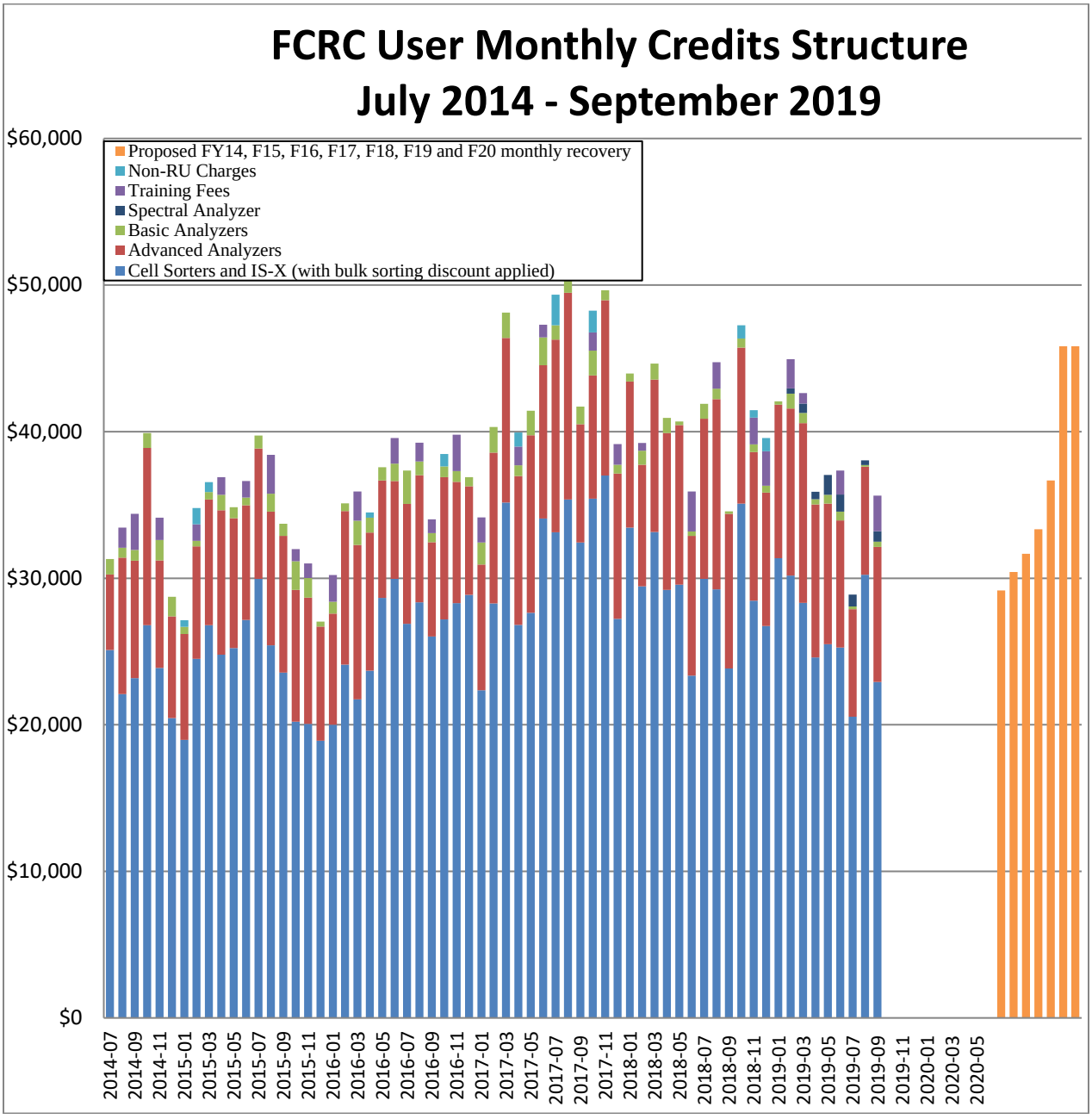
- As an initiative from FCRC Director, Svetlana Mazel, the Big Apple Flow Network (BAFN) Group has been founded in April 2015. Participants - Staff of the NYC Flow Cytometry Shared Resource Facilities. 33 out of 41 BAFN Meetings were held at the Rockefeller University, while only nine were hosted by Flow Cytometry Core Facilities at other institutions (New York Blood Center, MSKCC, Columbia University Medical School, Albert Einstein College of Medicine, WCMC)
- The main, originally planned goal for this group was to encourage and enhance the essential networking and sharing, which includes but not limited to exchange news, practical findings, get in the cutting edge theoretical presentations and demos, discuss new installations, encourage distribution of free samples, enhance communication with the companies, voice out the hot issues to the vendors and other society groups
- As of October 2019, the BAFN Group has 58 signed-up members. Between 15 and 25 people regularly show up for the monthly meetings. BAFN Meetings held in the reporting period:
  - BAFN-35, 1/1/19, 5 pm (at Rockefeller University)
    "Simultaneous Proteomics and Transcriptomics - TotalSeq™ and The Future of Single Cell Analysis" by Ashley Cornett, Ph.D., Technical Application Scientist / Mid-Atlantic Team Lead, BioLegend
  - BAFN-36, 2/21/19, 5 pm (at Rockefeller University)

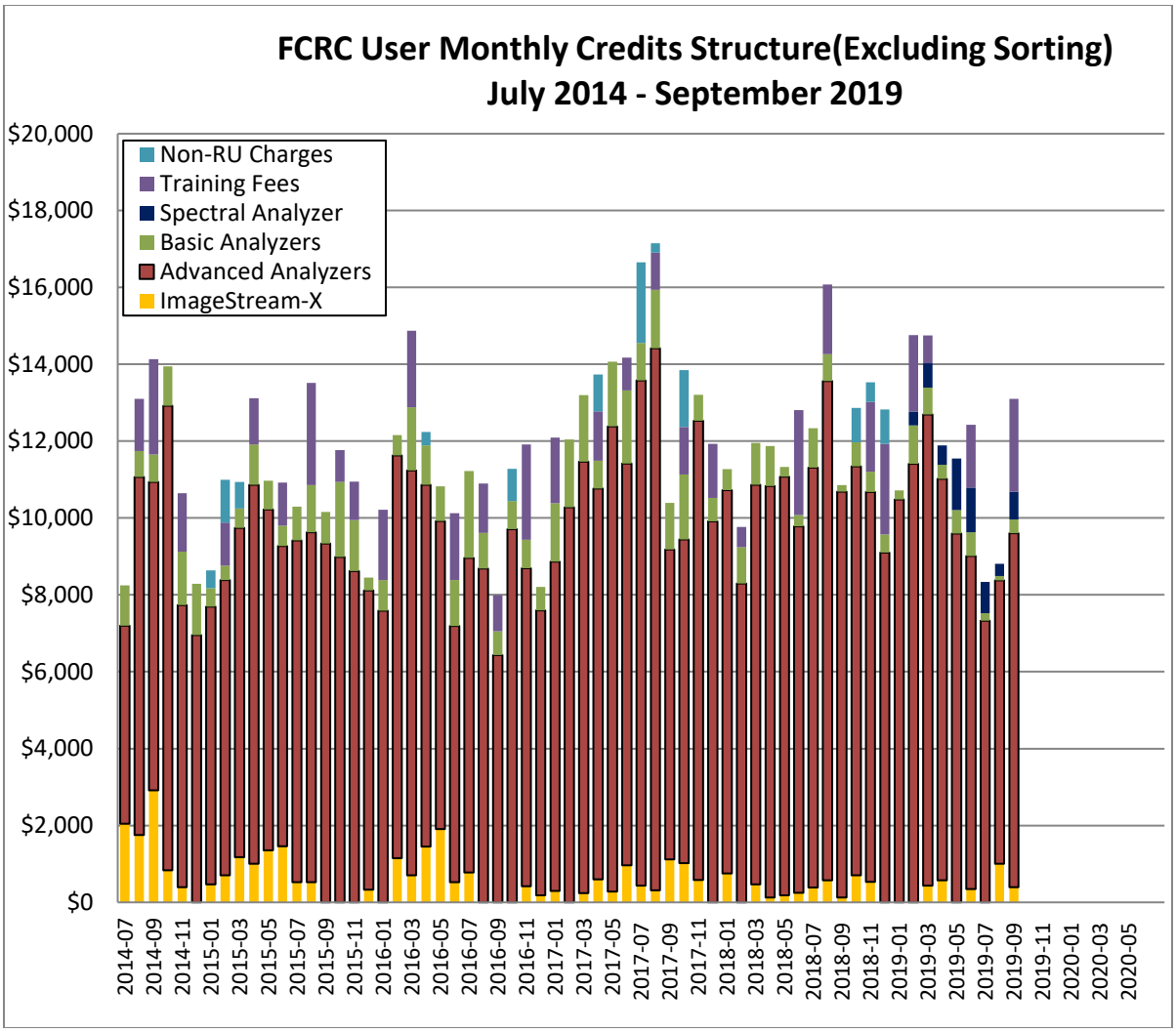
"Best practices for developing and managing SRLs: feedback from experience what's been done in Europe", presenter and moderator Alena Keprova, Research Support Specialist, FCRC at the Rockefeller University

- BAFN-37, 3/1/19, 5 pm (at WCMC)
  - Tour of the WCMC Flow Cytometry units run by Thomas Miller
  - Fluidigm Presentation "Exploring Discovery and Functional Profiling with Mass Cytometry" by Daniel Frederick, PhD, Field Applications Specialist – RI/MA/NH/VT/ME/NY
- BAFN-38, 4/18/19, 5 pm (at Rockefeller University)
  De Novo Software Presentation: "High Dimensional Data Reduction Basics for Research Scientists: Understanding how to perform and interpret tSNE and SPADE with FCS Express", presenter Sean Burke, Senior Product Manager
- BAFN-39, 5/1/19, 5 pm (at DrWatson)
  "ISAC SRL Recognition Program discussion", moderator Michael D. Gregory, Technical Director, Cytometry and Cell Sorting Lab, Division of Advanced Research Technologies, NYULMC
- BAFN-40, 7/18/19, 5 pm (at Columbia University Medical Center)
  "CYTO 2019 Highlights" by members of the BAFN Group, who have attended the CYTO 2019 in Vancouver
- BAFN-41, 9/26/19, 5 pm (at the Rockefeller University)
  "Advancing the Boundaries of High Parameter Cell Analysis: From HP Flow Cytometry to Genomic Cytometry" by Robert Balderas, Vice President of Biological Sciences and Market Development, BD Biosciences

- Together with Cytek Biosciences R&D team, headed by Maria C Jaimes, MD (Director, R&D, Applications and Reagents) FCRC runs the collaborative project. The goal of this project is to assess and possibly improve the performance of the FCRC instruments and develop the common SRL approach in the instrumental setup for the wide range of the experimental sample types for all FCRC instruments, including Cytek Aurora. As a follow-up of the FCRC on-site visits by Cytek Biosciences R&D and continuation of this collaboration Svetlana Mazel has been invited for a four-days visit of the Cytek Headquarters in Fremont, CA (Date – TBD)

Budget/Finances




FCRC User Monthly Credits Structure(Excluding Sorting)
July 2014 - September 2019
$20,000
$18,000
$16,000
$14,000
$12,000
$10,000
$8,000
$6,000
$4,000
$2,000
$0
Non-RU Charges
Training Fees
Spectral Analyzer
Basic Analyzers
Advanced Analyzers
ImageStream-X
2014-07
2014-09
2014-11
2015-01
2015-03
2015-05
2015-07
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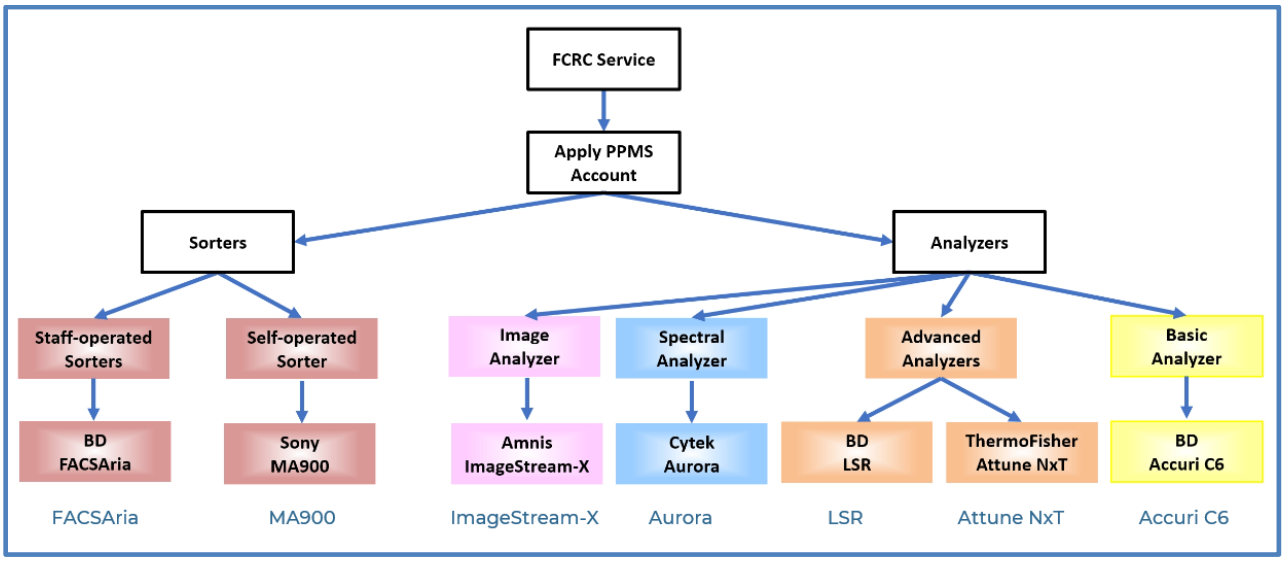
Customer/User Communications and Training

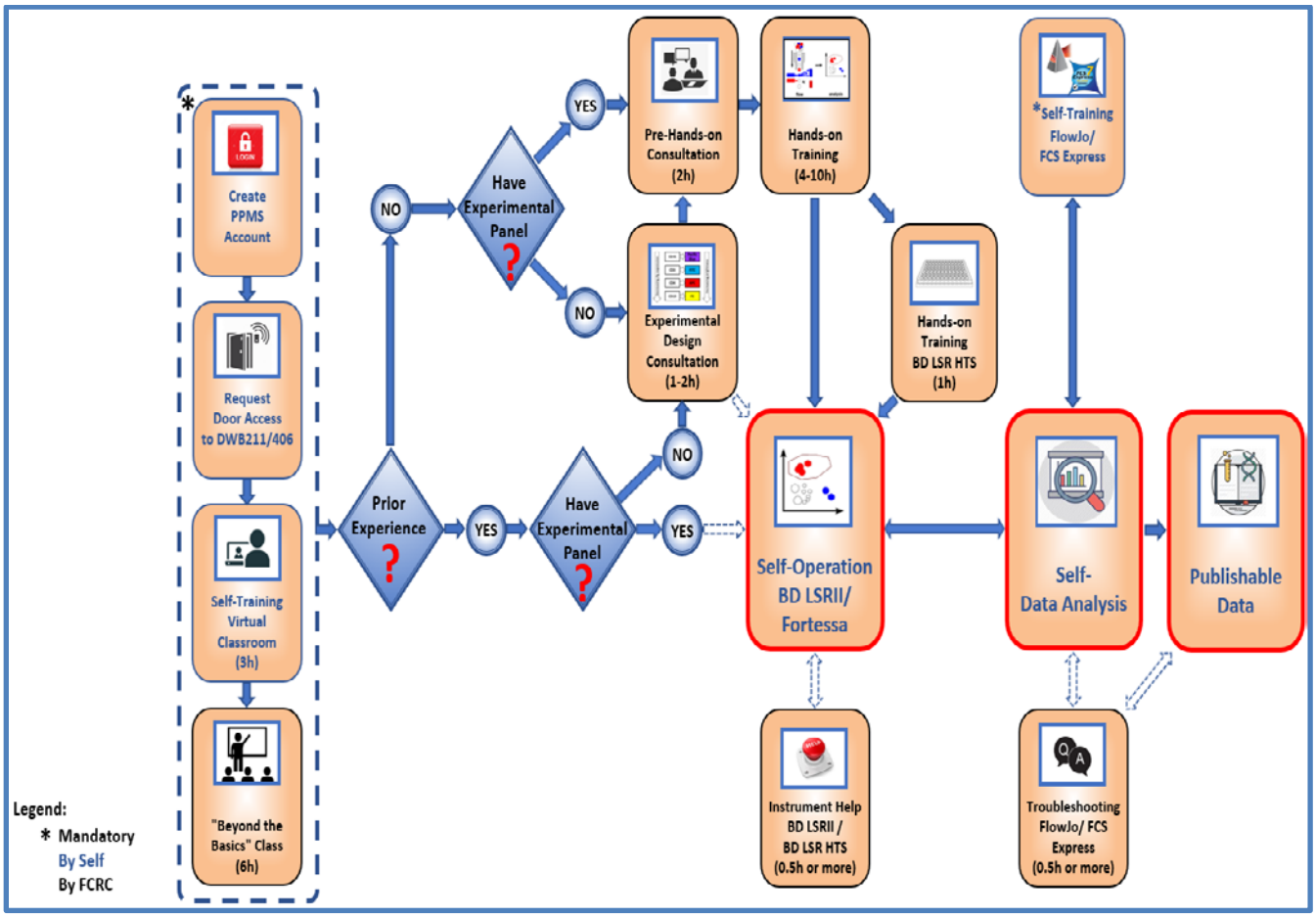
- Currently FCRC-PPMS has more than 385 active users and over 80 PIs registered
- FCRC User Training depends on the type of the instrument is in use

FCRC Instruments' Diversity:



- FCRC Staff was working hard on the essential updates for the FCRC JumpStart page http://inside.rockefeller.edu/fcrc/General_Information_FCRC_JumpStart with the goal to make the information about the user training better organized. Please note that it is work in progress which will take some time
- FCRC have started adding the visual schemes for the training steps, which will help the researchers to streamline the FCRC training process

FCRC User Training Scheme for BD LSR Advanced Analyzers:



For internal RU Users the only training which has fee structure implemented is the "Beyond the Basics Class", which is performed in the groups of 5-15 people once in 2-3 months dependent on demand http://inside.rockefeller.edu/fcrc/chargesandfees

- All other trainings, consultations and help by FCRC Staff are free of charge, although, they take a lot of time and efforts from the FCRC Staff
- Currently we do not have a tracking system in place. Rough estimate of the time spent for the user training on average take ~ 50 hours a month

FCRC User Training Summary

FCRC User Training Summary for FY19:

Training			Number		Estimated number of hours			
Category	Type	Frequency & Format	Participants	Sessions	Per session	Total	Total per category	Per month
"Beyond the Basics" Flow Cytometry Class		Every 2.5 - 3.5 months (5 – 11 participants) 8/10/18; 12/7/18; 2/11/19; 3/29/19; 6/7/19	49	5	6	30	30	2.5
Consultations				130			297	24.8
	Pre-Sort Consultation	Every 2-3 weeks Small group; 1/23; 1/30; 3/6; 4/25; 5/1; 6/5/19	40	13	3	39		
	Experimental Design Consultation		70	64	2	128		
	Experimental Design Troubleshooting		23	22	3	66		
	Pre-ImageStream Consultation		5	4	2	8		
	Pre-ImageStream Consultation (external)				2	0		
	Pre-Hands-on Consultation BD LSR; ThermoFisher Attune NxT; BD Accuri C6		26	25	2	50		
	Pre-Hands-on Consultation Cytek Aurora		4	2	3	6		
Hands-on Training				41			190	15.8
	BD LSRII		36	36	5	180		
	HTS Module		1	1	1	1		
	Cytek Aurora		1	1	4	4		
	BD Accuri C6		3	3	1	3		
	ThermoFisher Attune NxT		2	1	2	2		
Help on the Instrument				45			44.5	3.7
	BD LSR		41	39	1	39		
	HTS Module		1	1	0.5	0.5		
	Cytek Aurora		4	4	1	4		
	BD Accuri C6		0	0	1	0		
	ThermoFisher Attune NxT		1	1	1	1		
TOTAL_FY19			Number of Participants	Number of Sessions		Total Hours		Total Hours/Month
			307	221		562		47

- When the FCRC-PPMS forms/booking for training will be implemented, we will able to provide more precise statistics on it (see "FCRC-WEBSITE and BOOKING" section below)

FCS Express 7 from DeNovo Software (site license):

- One-year FCS Express site license was purchased using the FCRC's budget which covers 25 seats and is in a free for the three-months trial period until November 30, 2019

- Starting December 1, 2019, the access to the FCS Express will be offered to the FCRC Users with deeply discounted rates, which will depend on the level of overall

RU usage (calculated Quarterly), and will range from $0 (if we do not exceed the number of seats already paid by FCRC) to max $50 per Quarter (three-month period).

Outreach

- December 2018-January 2019 - “FCRC: Planned changes for BD LSRs and BD FACSArias”
- 2/27/19 at 4 pm - FCRC Presentation - "FCRC: Exciting Changes and Even More to Emit and Explore" presented by Svetlana Mazel
- 3/6/2019 – mass e-mail about the upgrade of the FCRC-PPMS booking system
- 4/10/19 - mass e-mail “GOOD NEWS at FCRC: New Staff Member - Samer Shalaby"
- 4/23/19 at 12 pm - "BioLegend Web Tools for Optimizing your Flow Cytometric Experiments” presented by Ashley Cornett, Ph.D., Technical Application Scientist / Mid-Atlantic Team Lead, BioLegend
- 6/10/19 - mass e-mail “GOOD NEWS at FCRC: Cell Sorting and ImageStream-X Schedule restoring to full staff levels” starting July 8, 2019
- 7/10/19 at 12:30 pm - FCRC: FCS Express Site License - "Lunch and Learn" Flow Cytometry Seminar "The FCS Express Difference – Moving Beyond FlowJo” presented by Sean Burke, MS, Senior Product Manager, De Novo Software
- 9/27/19 - mass e-mail “Aurora 5L system at RU"
- 10/15/19 at 12:30 pm - FCRC: FCS Express Site License - "Lunch and Learn" Flow Cytometry Seminar "The FCS Express Flow Cytometry Data Analysis Training” presented by Sean Burke, MS, Senior Product Manager, De Novo Software

FCRC Staff professional development/training

- Exchange between the two Flow Cytometry Centers - Dr. Iyadh Douagi from Karolinska Institutet visited FCRC at RU as a "2019 short-term Nicholson Exchange Fellow" – on January 7th -17th, 2019
- Absolute Counting Exploratory Study to perform at FCRC together with Janelle Shook and Huimin Gu (Cytek Biosciences, Inc.) - January 16th – 17th, 2019
- FlowTex Conference 2019 – February 6th - 7th, 2019, Mitchell Basic Sciences Research Building, Texas Medical Center, Houston, TX (Svetlana Mazel)
- 2019 Annual ABRF Meeting (Association of Biomolecular Resource Facilities), March 23nd - 26th, 2019 – San Antonio, TX (Stanka Semova)
- Regeneron Pharmaceuticals and MetroFlow - Flow Cytometry focused vendor day – April 30th, 2019 - at Regeneron Pharmaceuticals in Tarrytown, NY (Svetlana Mazel, Songyan Han, Samer Shalaby)
- Pre-CYTO 2018 – ISAC Council Meeting (June 20th, 2019); ISAC leadership development day for SRL Emerging Leaders (June 21st, 2019) - Vancouver, Canada (Svetlana Mazel)

- Pre-CYTO Cytek's Inaugural Aurora User Group Meeting, June 20th, 2019, Vancouver, Canada (Alena Keprova)
- CYTO 2019 – XXXIV ISAC Congress, June 22nd – June 26th, 2019 - Vancouver, Canada (Svetlana Mazel, Alena Keprova).
- BD Cell Sorting Roadshow - July 17, 2019, Apella - Alexandria Center, New York, NY (Songyan Han, Stanka Semova, Svetlana Mazel)
- GLIIFCA 2019 - September 27-29, 2019, Troy Marriott, outside Detroit (Songyan Han)
- NECyto 2019 – October 17th, 2019, New England Cytometry User Group fall meeting Ragon Institute in Cambridge, MA (Svetlana Mazel)
- MetroFlow 2019 "Flow Cytometry in Action: The Art of Single-Cell Analysis" – October 24th, 2019, The Graduate Center at CUNY, Manhattan, NY (Svetlana Mazel, Stanka Semova, Songyan Han, Alena Keprova, Samer Shalaby)