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Shared Resource Laboratory

Everything, Everywhere, All at Once: Open-source Automation for Situational Awareness in Shared Resource Laboratories

David Rach, Natarajan Ayithan, Xiaoxuan Fan

UMGCCC Flow Cytometry Shared Resource

June 6th, 2026

Slides Here

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On a good day at a small SRL

Everything is normal

- Show up
- Change sheathe and waste
- Flush out the fluidic lines
- Run QC

Drink your fourth cup of coffee

- Cell Sorting
- Training new users
- Building panels
- Deep diving interesting data

Go home, rinse and repeat

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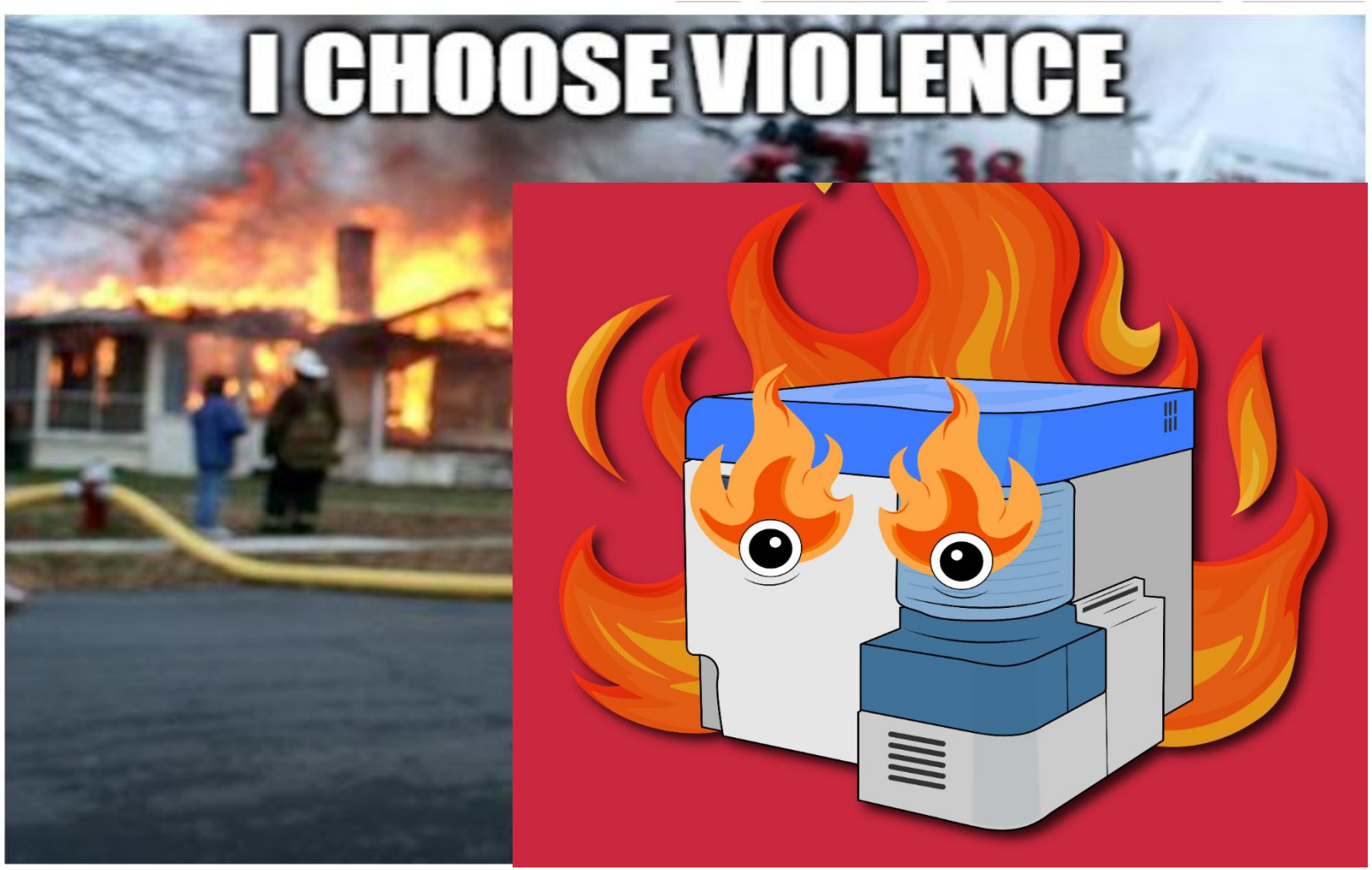
However, on a bad day at a small SRL…



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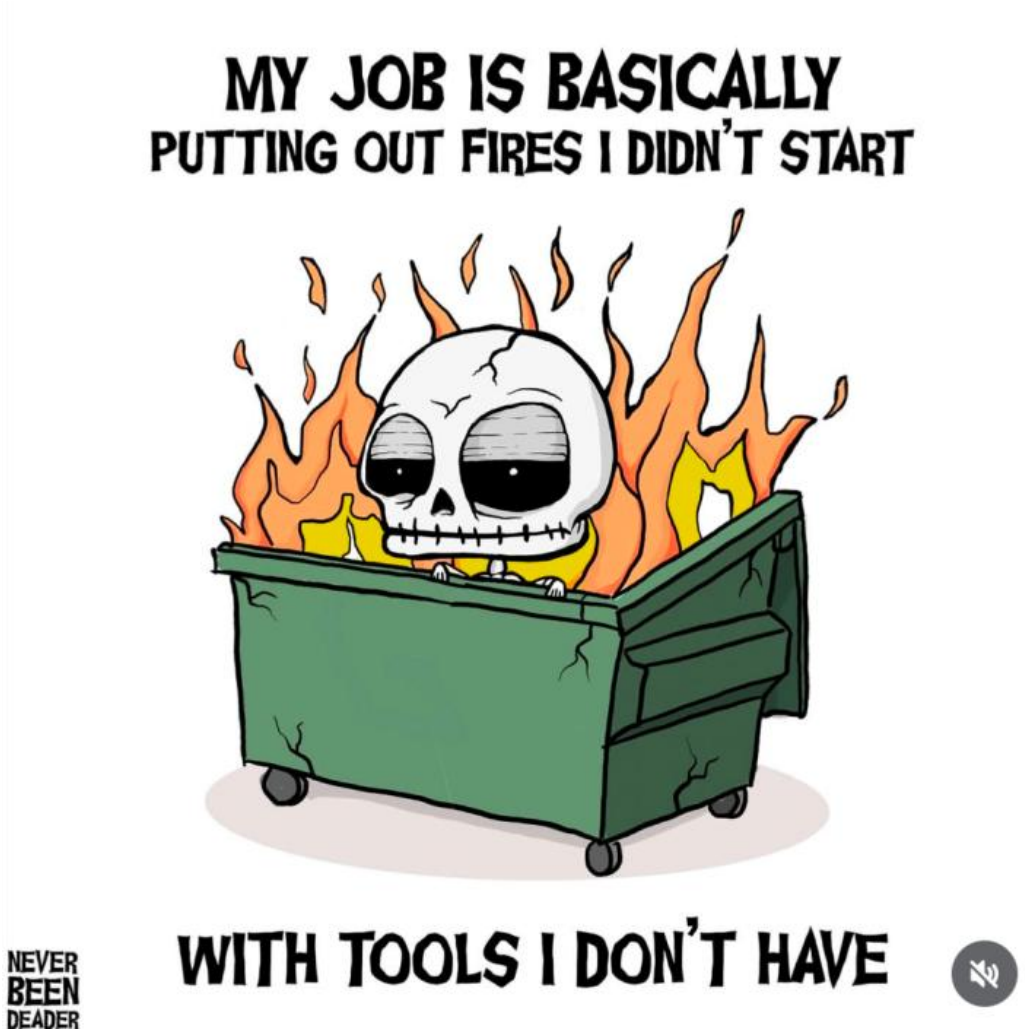
New Goal for the Day: Simply Survive



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Visible and Hidden Costs

Issue was detected, cytometer not in use

- Staff time
- Lost Revenue
- Delayed User Experiments

Issue undetected, cytometer still in use

- Poor Quality Data
- More complex analysis
- Need for experiment repeats

- Reputational Costs

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Are you treading water or drowning?

https://homeschooling2e.com/2021/04/11/treading-water/

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Can we anticipate the waves?

- Laser/Detector Issues
- Bleach-damaged QC Beads
- Debris Accumulation in fluidic lines
- Degrading tandem dyes
- Additional autofluorescences

What stories can the data already being acquired daily at out cores tell us?

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UMGCCC Flow Cytometry Shared Resource

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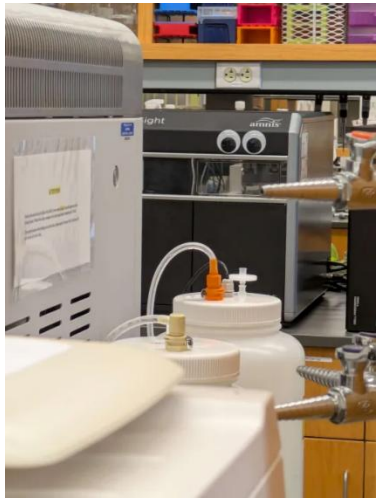

Analyzers:

- 3 Cytek Aurora
- 1 Amnis FlowSight
- 1 MacsQuant10
- 1 BD LSR-II
- 1 BD Canto

Cell Sorters:

- 1 Cytek Aurora CS
- 1 BD Aria II

Flow Cytometry Staff: 3

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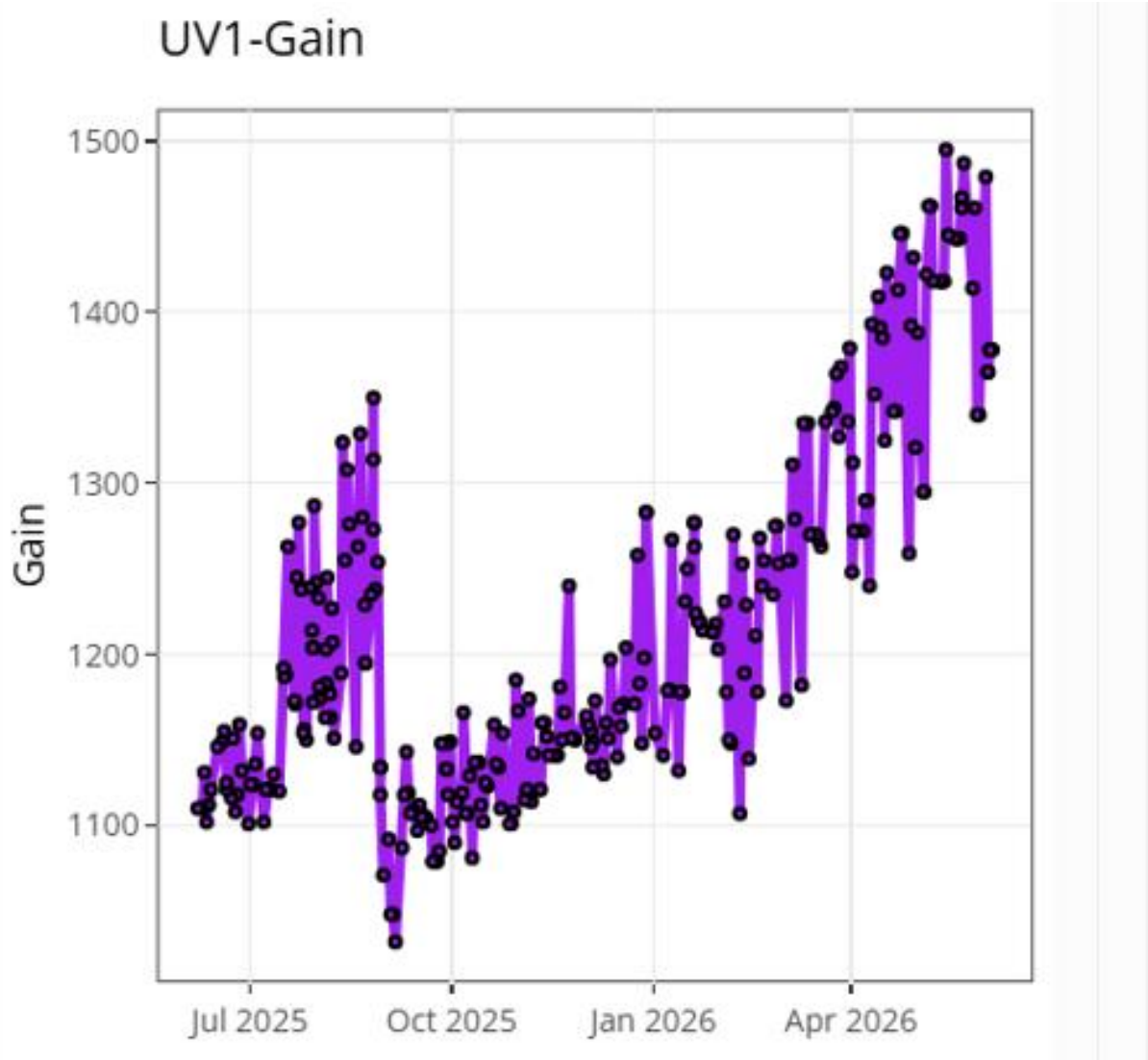
Our Goals



- Develop the tools
- Find bandwidth to leverage them
- Keep expenses minimal
- Share our code

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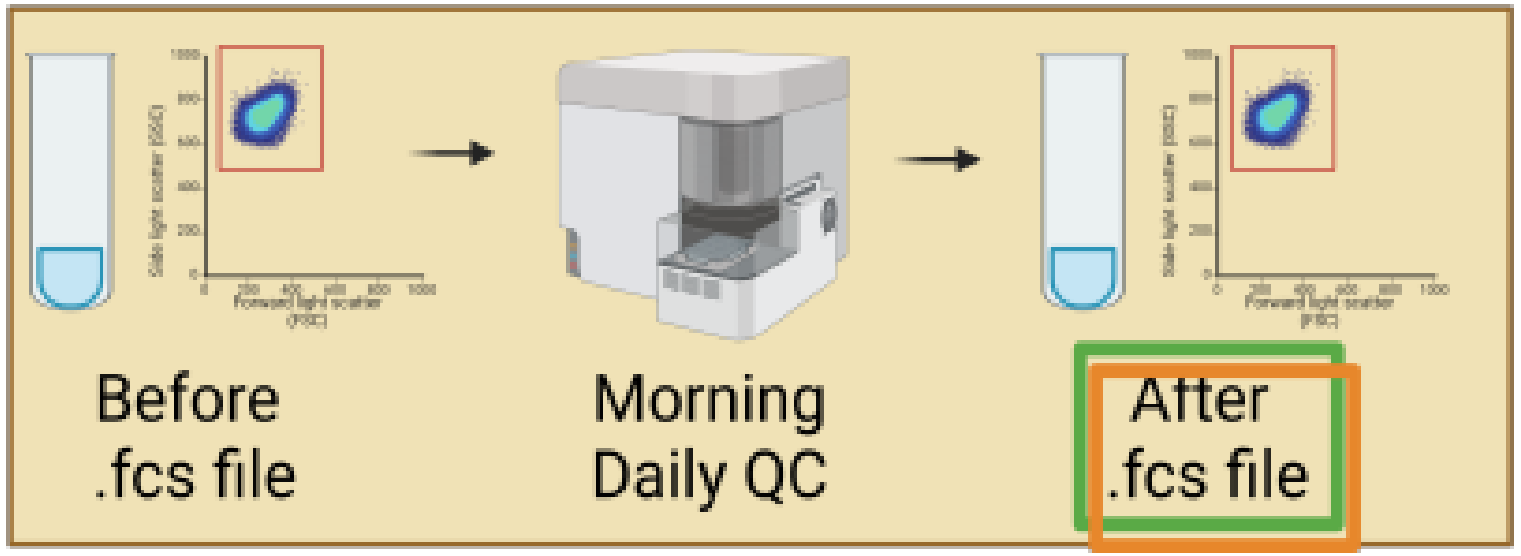
What data is already sitting around?



Before and After QC .fcs files

- For Every Instrument
- Back to 2017

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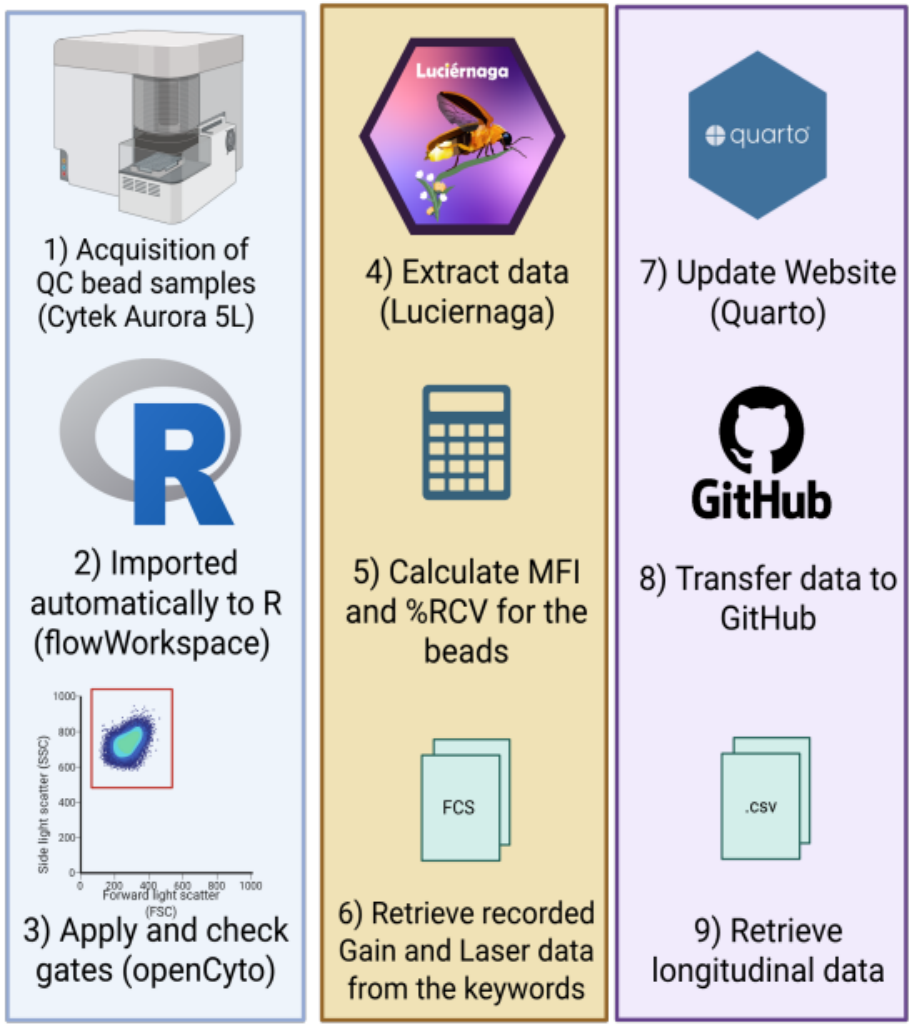
Automated Daily Processing



Behind-the-scenes:

- Using R (and increasingly Rust) to process the .fcs files
- Automated transfer to GitHub via Windows Task Scheduler
- Our Quarto Website gets updated twice daily via a GitHub action

Eclectic Mix of Computation:

- Existing Instrument Computers
- Repurposed Windows 10 End-of-Life computers running Linux
- Small motherboard computers (Raspberry Pi 4b)

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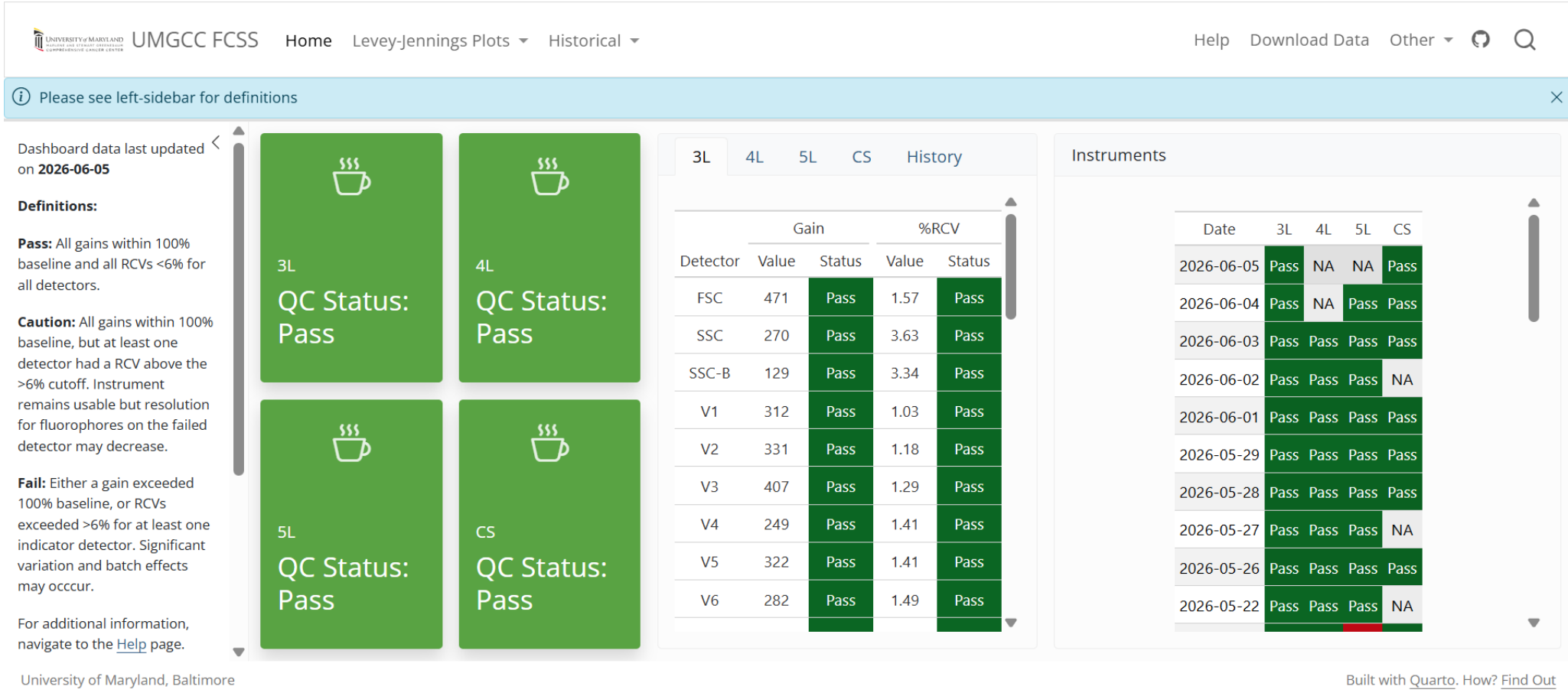
Instrument Quality Control Dashboard

Dashboard

How-To



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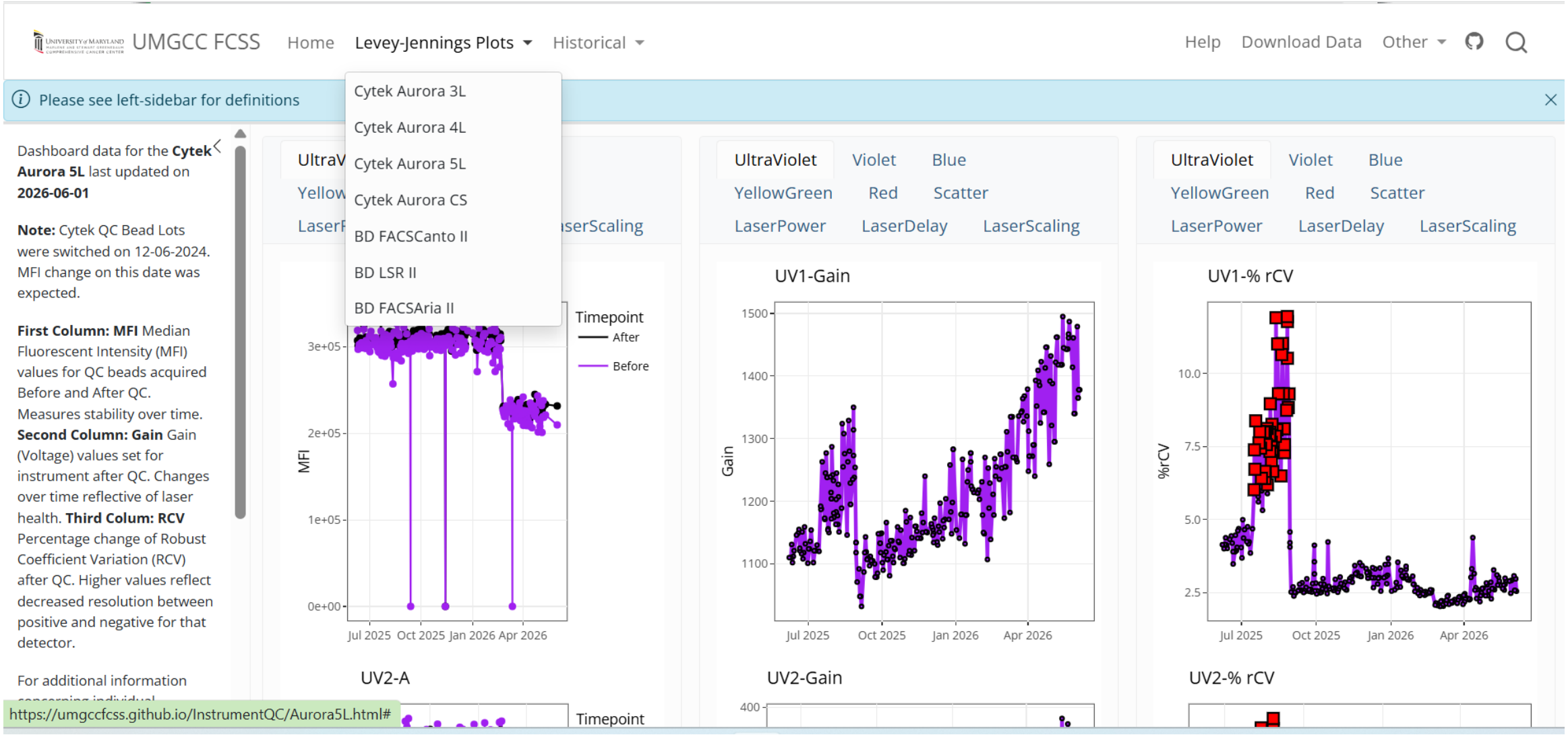
Instrument Quality Control Dashboard



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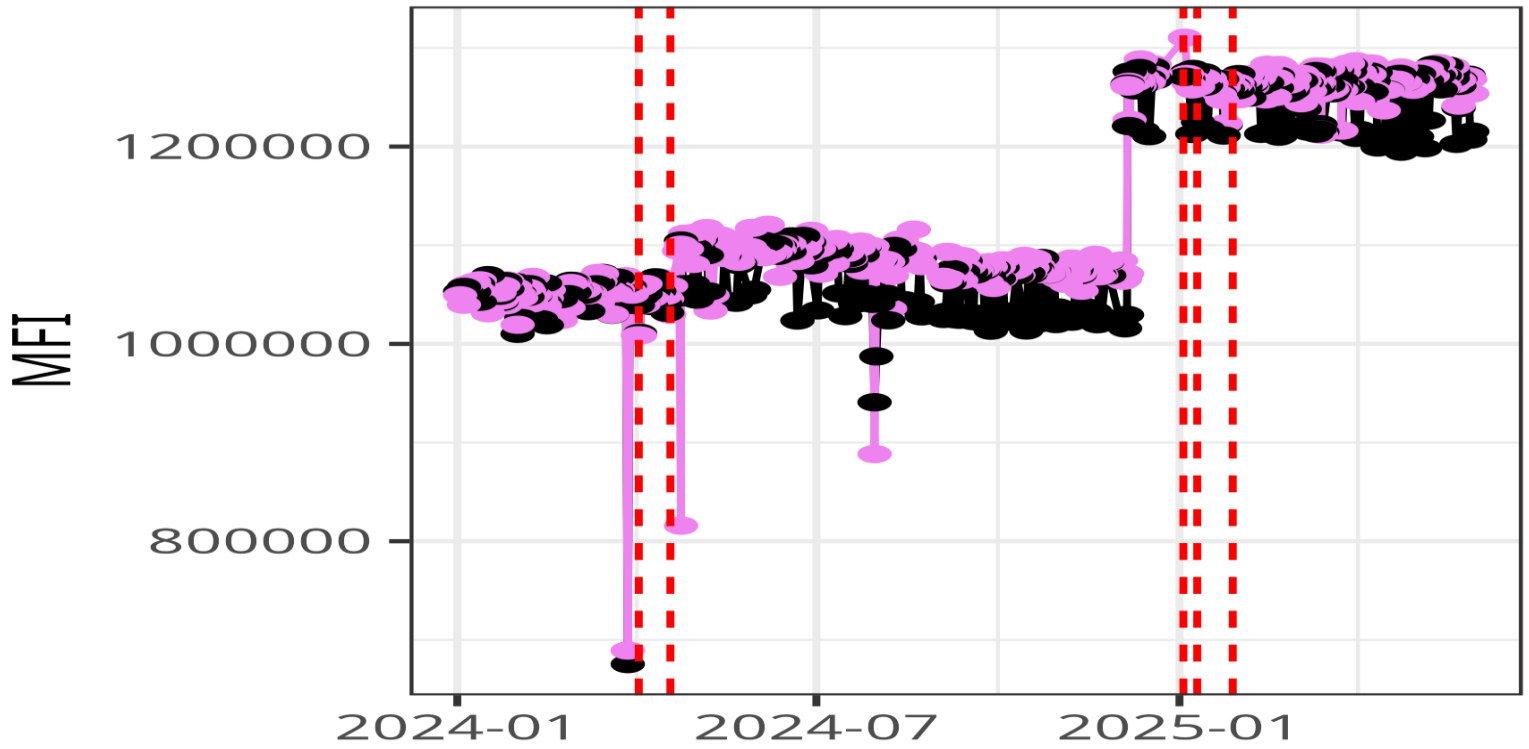
Instrument Stability over Time



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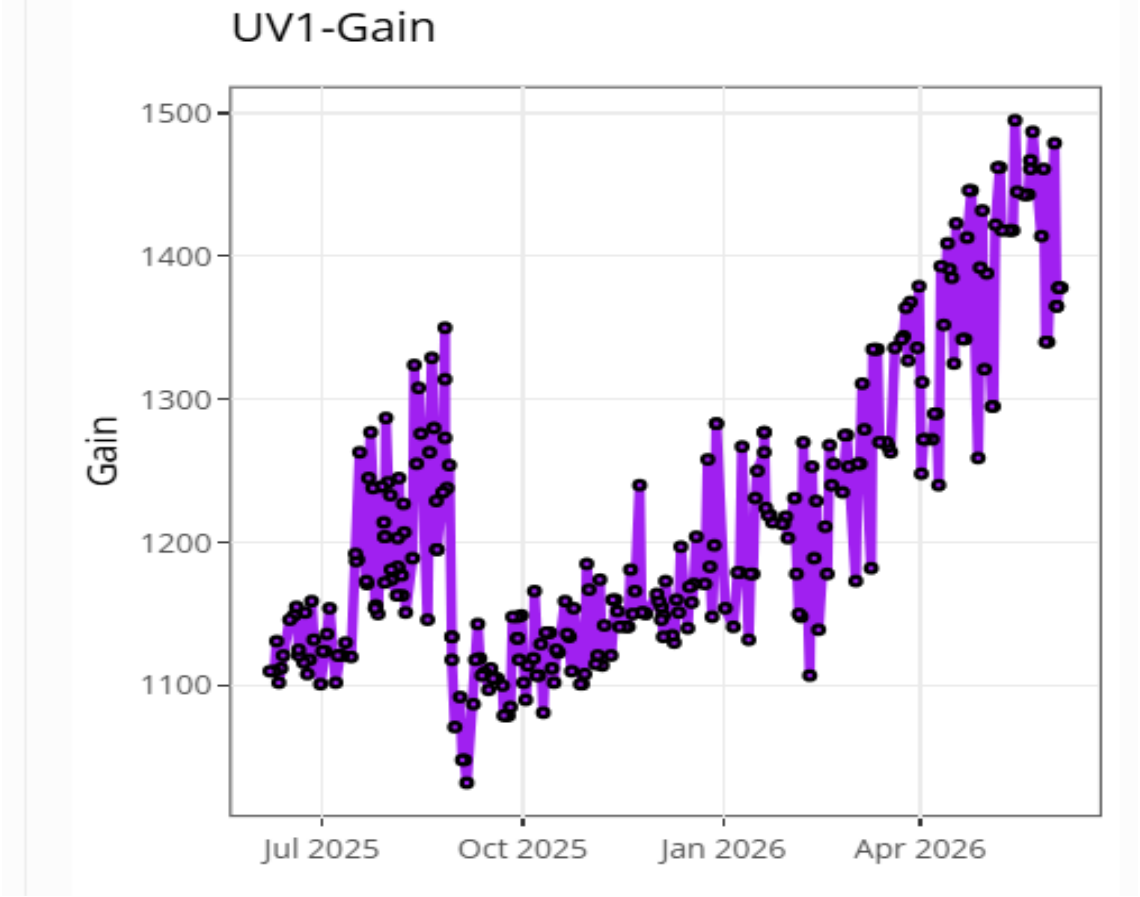
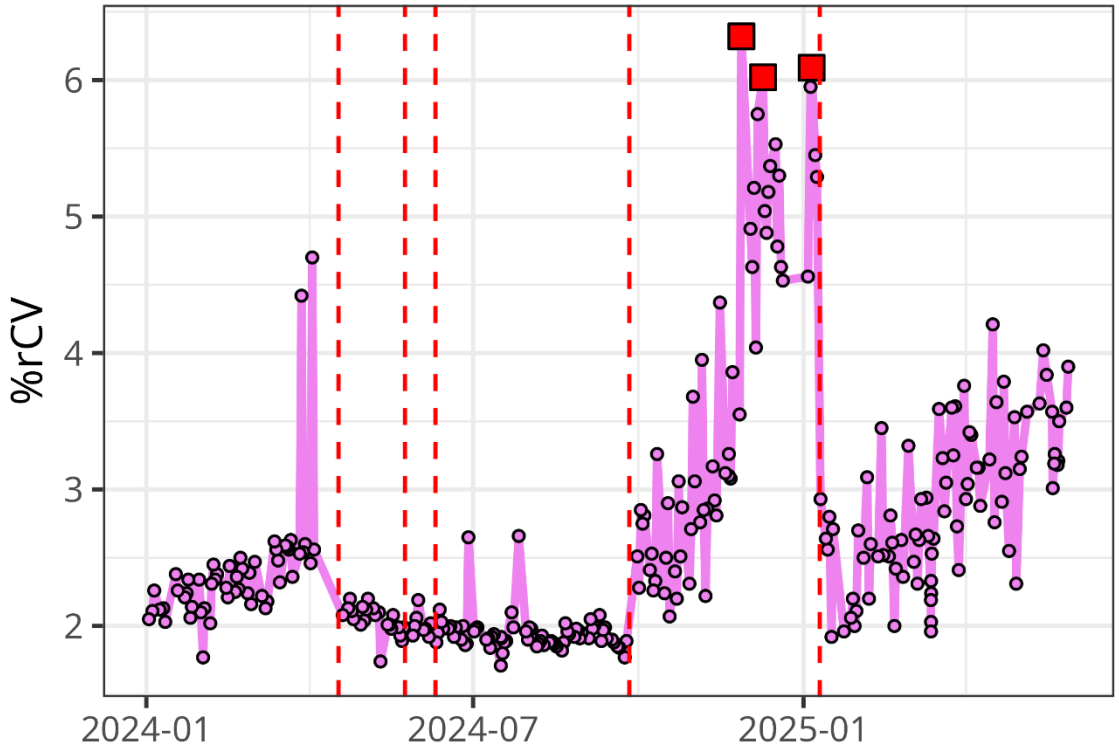
Early warning on Lasers/Detectors





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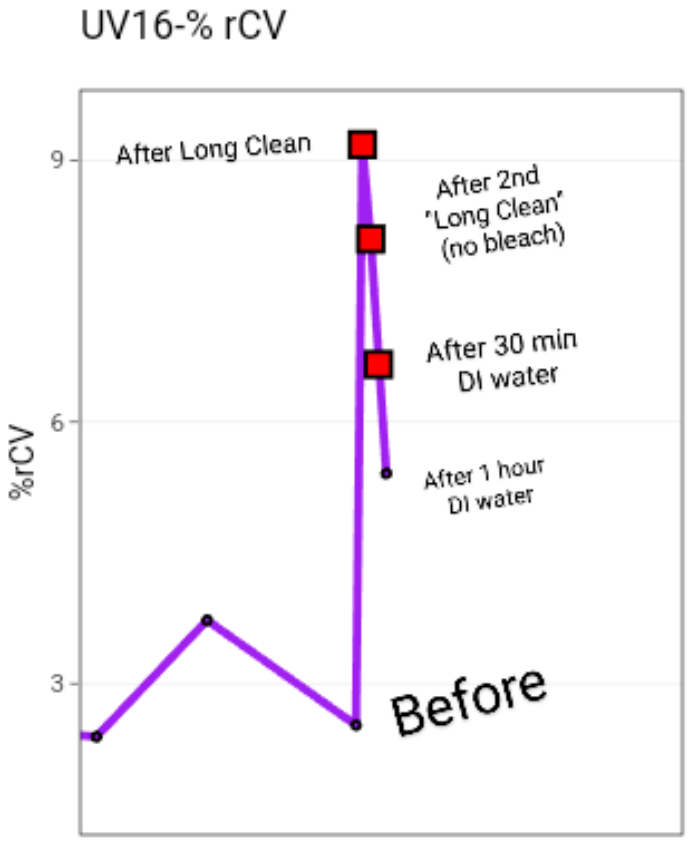
Front row seats to Bleach-Sensitive QC Bead Lots



Data

"Wait, when was QC last run???" Evaluating MFI drift after morning QC and its impact on unmixing.

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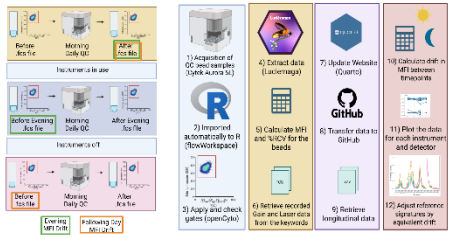
Introduction

At our core, quality control (QC) beads are run daily on Cytek Aurora instruments as part of the instrument startup process. Based on the beads initial observed MFI values for each detector, the SpectroFlo software adjusts the gains and laser settings of the instrument to ensure that the after-QC MFI values match lot-specific thresholds. This accounts for instrumental changes, allowing specimens acquired on different days to be comparable, and reducing the frequency of MFI-based batch effects in large spectral flow cytometry panels. We recently implemented a website to monitor daily QC for our instruments at our core (https://umgccfcss.github.io/InstrumentQC/), visualizing longitudinal changes in gain, RCV, and bead MFI values before and after daily QC. In the process, we observed various detectors for which MFI values pre-QC were consistently different from the MFI values observed after QC the day before. While MFI was reset to baseline at the following QC, we were curious whether these observed MFI drifts would have already occurred by the evening before, or were a result of the instrument shutdown. Additionally, we wanted to evaluate whether these drifts were sufficient to impact unmixing when samples were acquired in the evening compared to shortly after morning QC.

Methods



Results

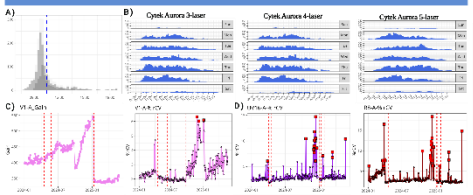


Figure 1. Automated QC processing, tracking and visualization allows for long-term monitoring of instrument performance. A) The histogram depicts when daily QC was performed, as recorded in the .fcs file metadata. **B)** The histogram plots depict the typical user hours shown by hour and weekday for each instrument, based on total recorded instrumental sit flushes in the Application Log. **C)** The Levey-Jennings plots depicts change in gain and %RCV representative of a faiting laser, with field service engineer visits appearing as red dashed lines. **D)** The Levey-Jennings shows spikes in %RCV associated with QC beads exposure to bleach.

Results

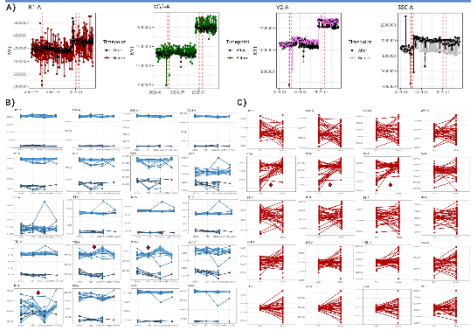


Figure 2. Drift in MFI can be observed by evening, but are more pronounced the following day. A) Typical MFI drift pattern observed for the Cytek Aurora 5-laser. **B)** The lineplots depict the MFI measurements of QC beads acquired before and after both morning and evening QC. **C)** The lineplots depict the recorded MFI measurements of QC beads after morning QC, and before morning QC the following day.

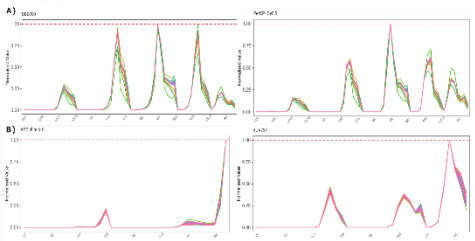


Figure 3. Simulated shifts in reference signatures arising from observed MFI drifts. Evening and morning after MFI drifts were calculated for each instrument for a six-month period, and fluorophore reference signatures were adjusted to acccount for the observed drift in detector. Line plots with each day a different color. **A)** shows two examples from the 5L instrument were variation was observed, while **B)** shows more typical minimal changes

Acknowledgements

This publication was supported by funds through the Maryland Department of Health's Cigarette Restitution Fund Program – GH-640-GRF and the National Cancer Institute - Cancer Center Support Grant (CCSG) - P30CA134274 d Service

https://github.com/UMGCCFCSS xiaoxuanfan@som.umaryland.edu

Results

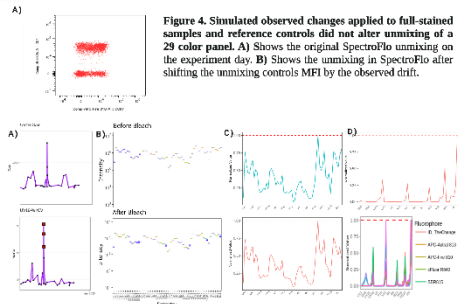


Figure 4. Simulated observed changes applied to full-stained samples and reference controls did not alter unmixing of a 29 color panel. A) Shows the original SpectroFlo unmixing on the experiment day. **B)** Shows the unmixing in SpectroFlo after shifting the unmixing controls MFI by the observed drift.

Figure 5. Avoiding bleach exposure with SpectroFlo Lot 2006 beads. Following a fluidics shutdown with bleach, fresh QC beads were run. **A)** Levey-jennings plots show spike in gain and %RCV. In **B)** spectrum plots show bead MFI before (top) and after (bottom) bleach exposure. **C)** lineplots depict grouped normalized signatures from individual cells before and after bleach exposure. In **D),** the after bleach MFI was subtracted from before bleach bead MFI, and the difference normalized to identifty suspected stripped fluorophore.

Takeaways

Leveraging our access to processed quality control data for each of our instruments, we were able to quantitatively access whether the observed drifts in MFI visible in the Levey-Jennings tracking plots were insignigificant or of concern. We found that drifts in detector MFI do occur by evening for a few detectors unique to each instrument, they are not to the same extent as if the instrument were shut down overnight. When simulating changes to reference controls resulting from these, the changes were minimal, only being visible in a few fluorophores on secondary and tertiary peaks. When we simulated the changes in actual experimental controls and unmixed a full-stained 29-color panel, we did not observe any significant differences. Additionally, using our instruments data we identified when lasers began to fail, and issues with the new QC bead lot so that preventive measures could be taken.

Data availability

Would you like to do this your own dataset? The R code used for the analysis and figure generation is available on our GitHub (https://github.com/DavidRach/EveningQC_Cyto2025). The current and historical QC data for all our instruments is available via our website (https://umgccfcss.github.io/InstrumentQC/Data).

Interested in setting up an automated QC tracking website for your own instruments? We have a how-to tutorial for those with some coding literacy (https://github.com/DavidRach/InstrumentQC_Install), and for those without, we are actively finishing an R package that will automatically set one up for Cytek and BD instruments with a single click (https://github.com/DavidRach/CytometryQC).

Take some stickers and track our progress!

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Beyond .fcs files, what else can we leverage?

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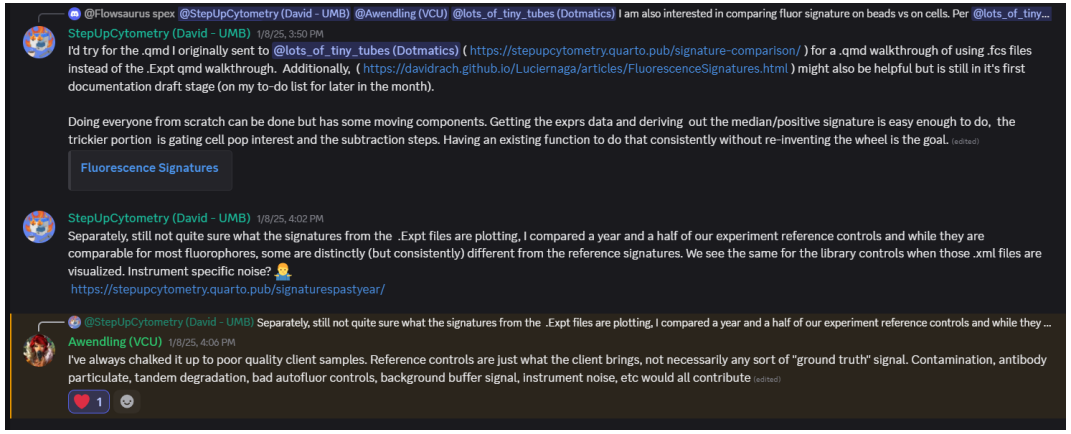
SpectroFlo .Expt Files



Every .Expt file

- Read in XML to R
- Access Experimental Metadata

Useful

- Fluorophores
- Markers

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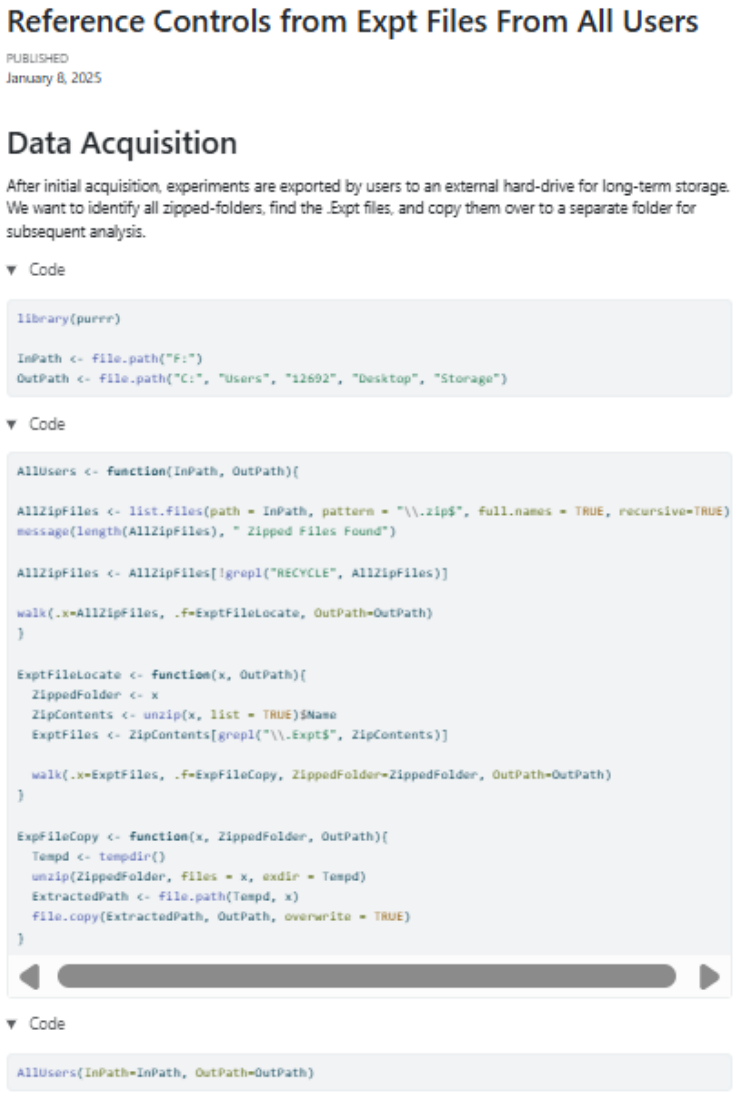
SpectroFlo .Expt files

Code



Reference Controls from Expt Files From All Users

PUBLISHED
January 8, 2025

Data Acquisition

After initial acquisition, experiments are exported by users to an external hard-drive for long-term storage. We want to identify all zipped-folders, find the .Expt files, and copy them over to a separate folder for subsequent analysis.

▼ Code

```
library(purrr)

InPath <- file.path("F:")
OutPath <- file.path("C:", "Users", "12692", "Desktop", "Storage")
```

▼ Code

```
AllUsers <- function(InPath, OutPath){

AllZipFiles <- list.files(path = InPath, pattern = "\\.zip$", full.names = TRUE, recursive=TRUE)
message(length(AllZipFiles), " Zipped Files Found")

AllZipFiles <- AllZipFiles[!grepl("RECYCLE", AllZipFiles)]

walk(.x=AllZipFiles, .f=ExptFileLocate, OutPath=OutPath)
}

ExptFileLocate <- function(x, OutPath){
  ZippedFolder <- x
  ZipContents <- unzip(x, list = TRUE)$Name
  ExptFiles <- ZipContents[grepl("\\.Expt$", ZipContents)]

  walk(.x=ExptFiles, .f=ExpFileCopy, ZippedFolder=ZippedFolder, OutPath=OutPath)
}

ExpFileCopy <- function(x, ZippedFolder, OutPath){
  Tempd <- tempdir()
  unzip(ZippedFolder, files = x, exdir = Tempd)
  ExtractedPath <- file.path(Tempd, x)
  file.copy(ExtractedPath, OutPath, overwrite = TRUE)
}
```

▼ Code

```
AllUsers(InPath=InPath, OutPath=OutPath)
```

Gather

- Identify the Zipped Folders
- Unzip
- Copy out the .Expt File
- Re-zip

Process

- Parse for Fluorophores
- Parse for Markers
- Save outputs by user and experiment

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What can we do with that data?

- How many fluorophores in our largest user panel?
- “Does anyone have an anti-human Alexa Fluor 647 CD163 that we can borrow?”

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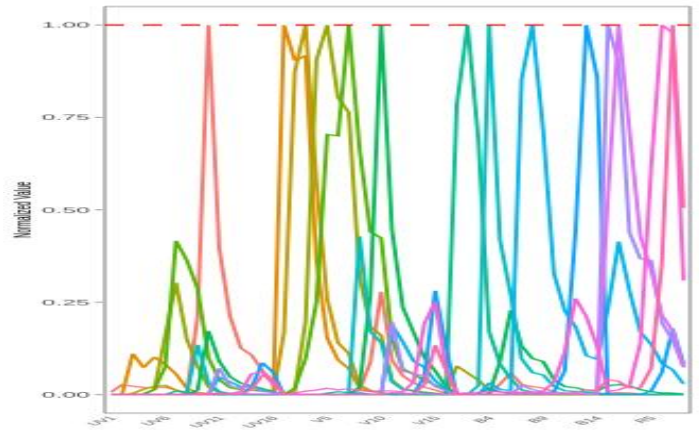
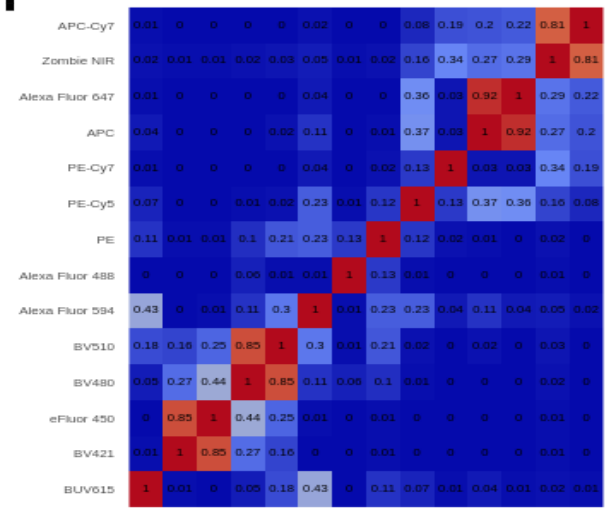
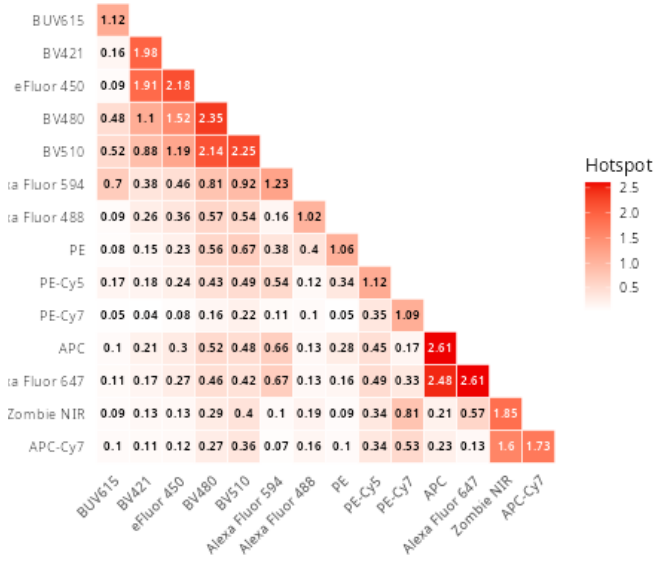
Evaluate User Panels



D	WV	Fluorophore	Antigen	D	Fluorophore	Antigen	D	Fluorophore	Antigen
V1	428								
V2	443								
V3	458								
V4	473								
V5	508	BV480	CD11b	B1					
V6	525			B2	FITC	MHC II			
V7	542	BV510	CD8	B3	Spark Blue 550	CD3			
V8	581	BV570	Ly6C	B4	PE	F4-80			
V9	598			B5					
V10	615	BV605	CD4	B6					
V11	662	BV650	CD317	B7	7-AAD	Viability	R1		
	679			B8	PE-Cy5	CD25	R2		
V12	695			B9	PerCP-Cy5.5	CD11c	R3		
V13	718	BV711	Ly6G	B10			R4	Alexa Fluor 700	CD45
	738			B11			R5		
V14	750								
	760			B12			R6		
V15	781	BV785	CD127	B13	PE-Cy7	PD-1	R7	APC-Fire 750	LAG-3
V16	812			B14			R8		

Code





Not all user panels are “good”

Once we have parsed the panels fluorophores

- Signatures (based on references)
- Signature Worksheet
- Cosine Values (Similarity Matrix)
- Matrix Condition Number (Complexity Index)
- Collinearity (Unmixing-dependent Spread)

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What about the quality of the actual data?

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Christopher Hall's previous attempts

ProactiveCoreDefense Public

Watch 1 | Fork 0 | Star 1

master | 1 Branch | 0 Tags | Go to file | Add file | Code

hally166 corrected variable name error — 00a8692 · 5 years ago — 15 Commits

LICENSE	Initial commit	6 years ago
QCScriptPwrShell.ps1	Update QCScriptPwrShell.ps1	6 years ago
QCscriptv23.R	corrected variable name error	5 years ago
README.md	Update README.md	6 years ago
example.PNG	Add files via upload	6 years ago

README | GPL-3.0 license

ProactiveCoreDefense

Protect your flow cytometry core from bad data.

Autonomously monitor user sample quality for flow cytometers.

This is an "improved" version of the 'Sample Quality Monitor' repository I developed at Sanger (https://github.com/hally166/SampleQualityMonitor).

About

Protect your flow cytometry core from bad data.

- Readme
- GPL-3.0 license
- Activity
- 1 star
- 1 watching
- 0 forks

Report repository

Releases

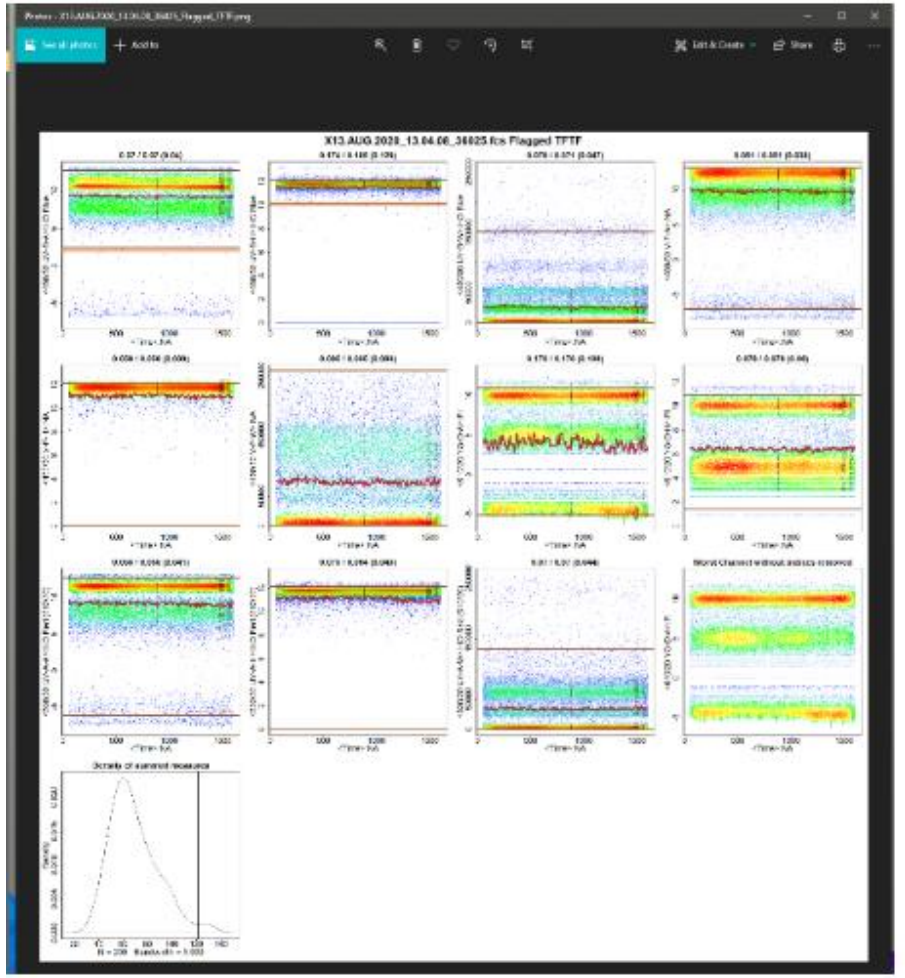
No releases published

Packages

No packages published

Contributors 1

hally166 Christopher



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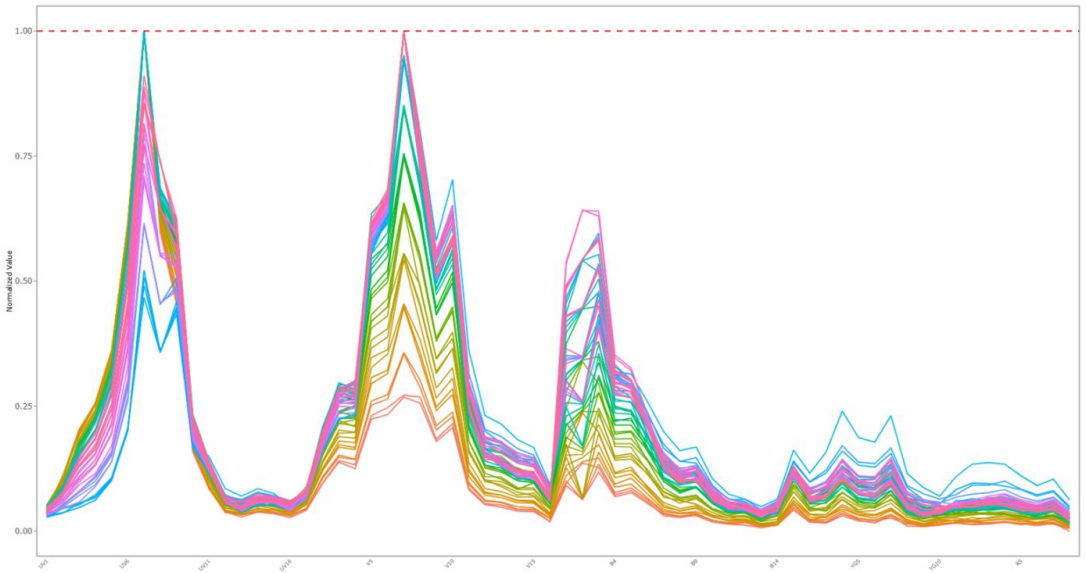
Evaluating Unmixing Controls



1) Acquisition of unmixing controls (Cytek Aurora 5L)

2) Import to R (flowWorkspace)

3) Apply and check gates (openCyto)

4) Extract data (Luciernaga)

5) Identify detector with brightest MFI for individual cells

6) Enumerate total cells for each peak detector, and identify location fluorophore and autofluorescence peak(s)

7) Group cells with shared peaks

8) Subtract Out Autofluorescence

9) Generate normalized signature of individual cells for identified fluorophore detector

10) Identify all peaks using local maxima

11) Group cells with shared primary peaks and similar heights other peaks

12) Retrieve original data for identified cells and export out new .fcs files containing purified signatures

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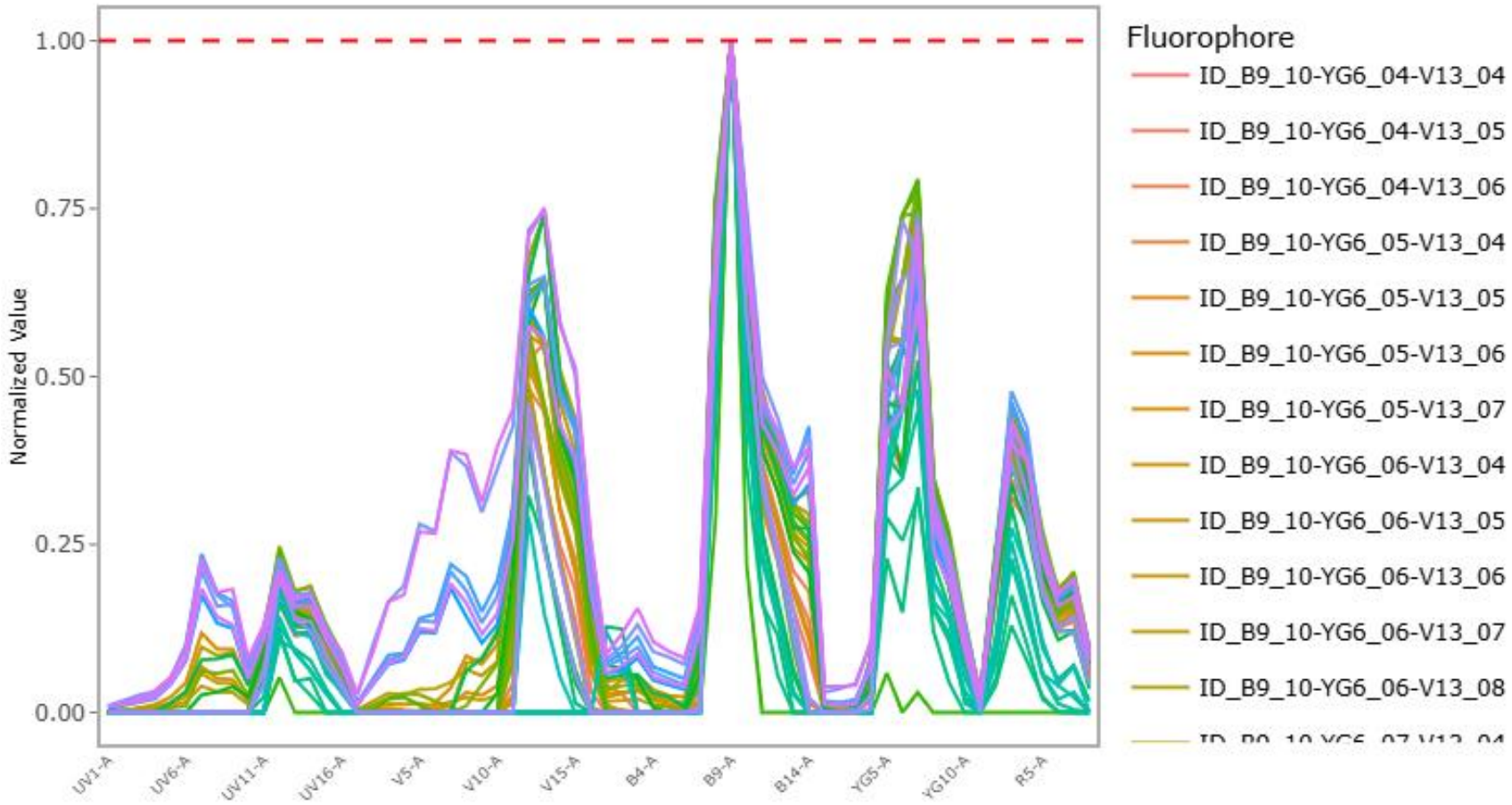
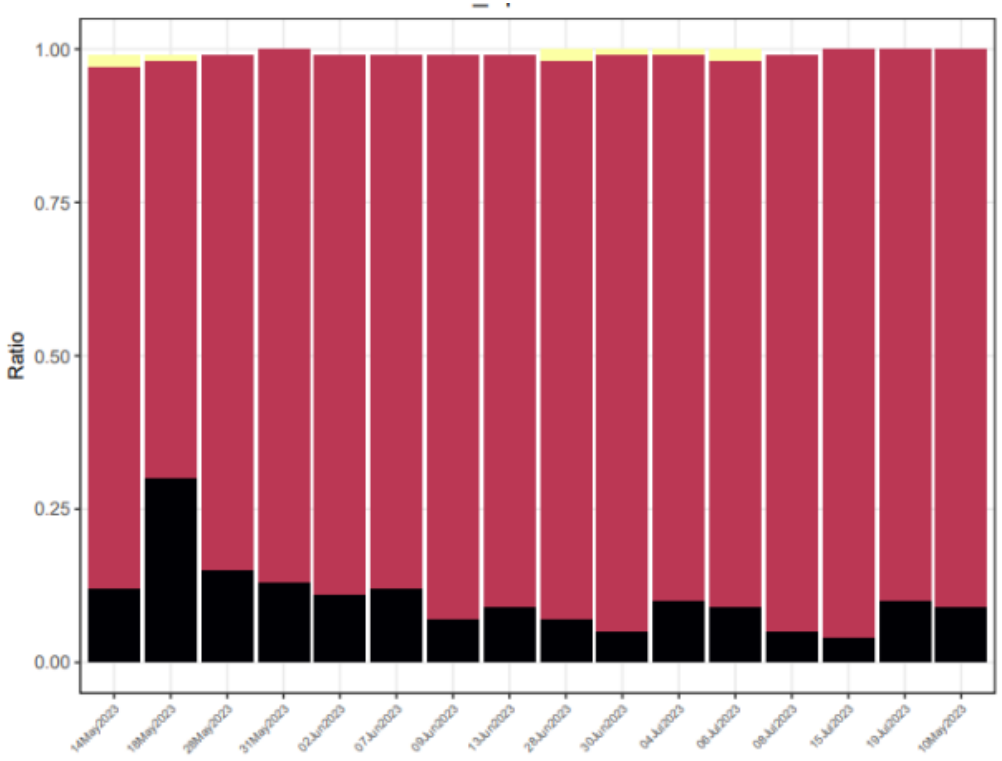
When PerCP-Cy5.5 degrades

Interactive Plots

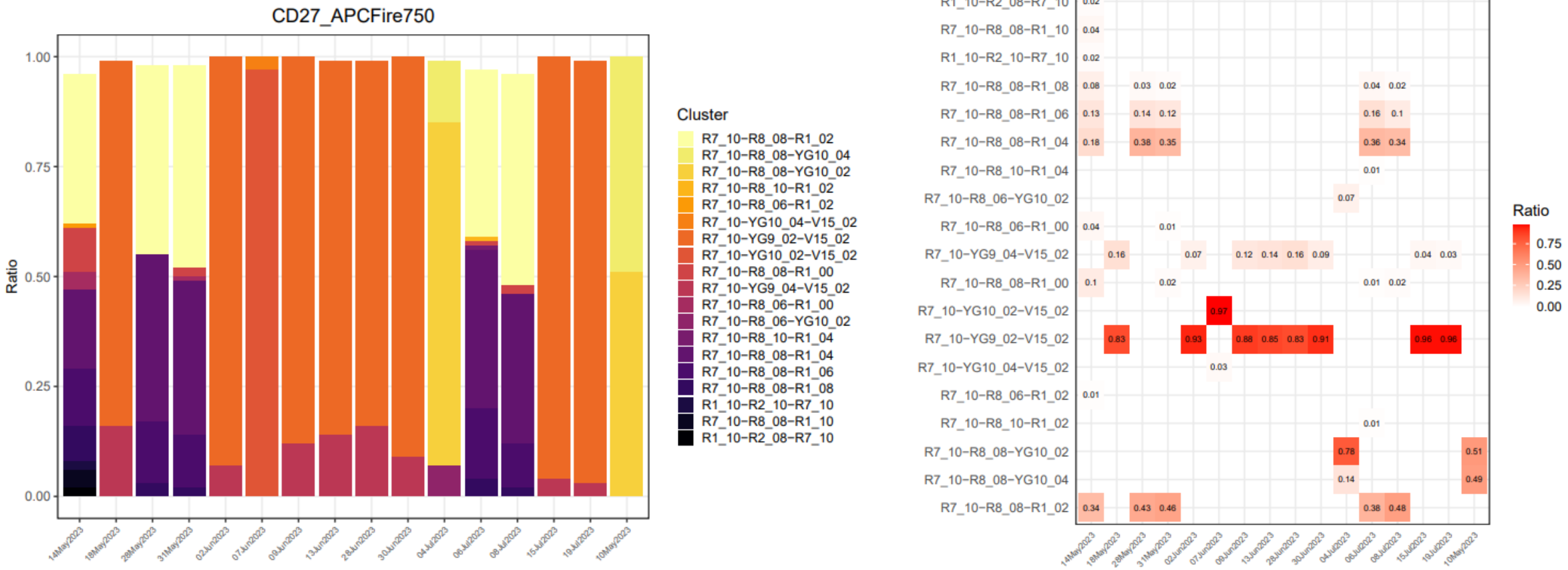


When antibody vials are in good condition



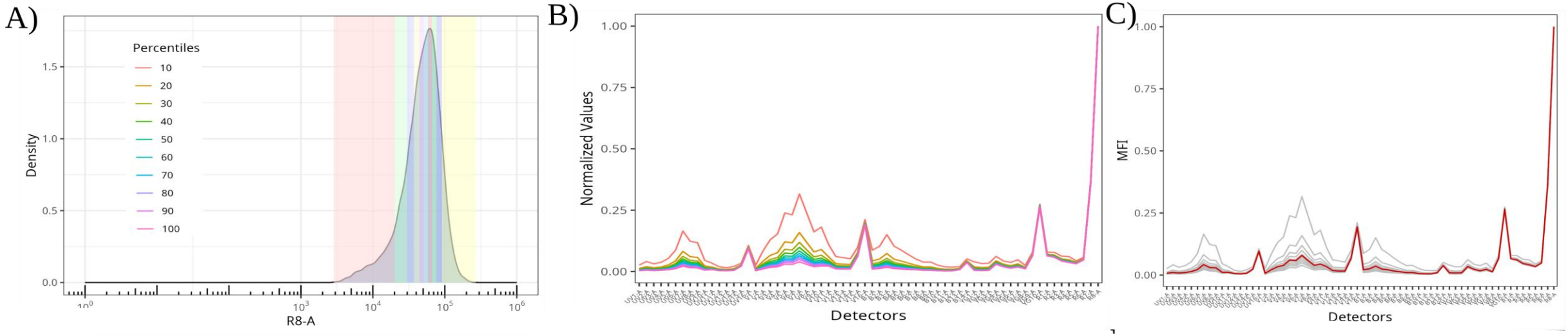
- Few normalized signature variants

When the tandem starts degrading



- Noticeable increase in normalized signature variants

Residual Autofluorescence peaks in Single-Color Signatures



For cell controls with a **<u>moderate density antigen</u>** on **<u>bright fluorophores</u>**, we see that any residual autofluorescence contributes minimally to the overall isolated single-color signature when gated according to best practices

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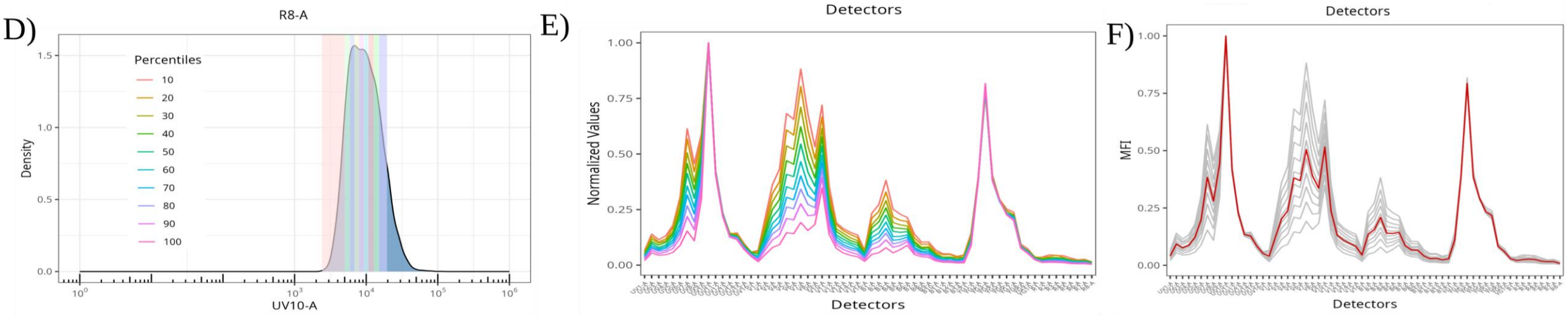
Residual Autofluorescence peaks in Single-Color Signatures



For cell controls with a **moderate density antigen** on **dim fluorophores,** we see substantial residual autofluorescence contributions to the isolated single-color normalized signatures even when gated according to best practices

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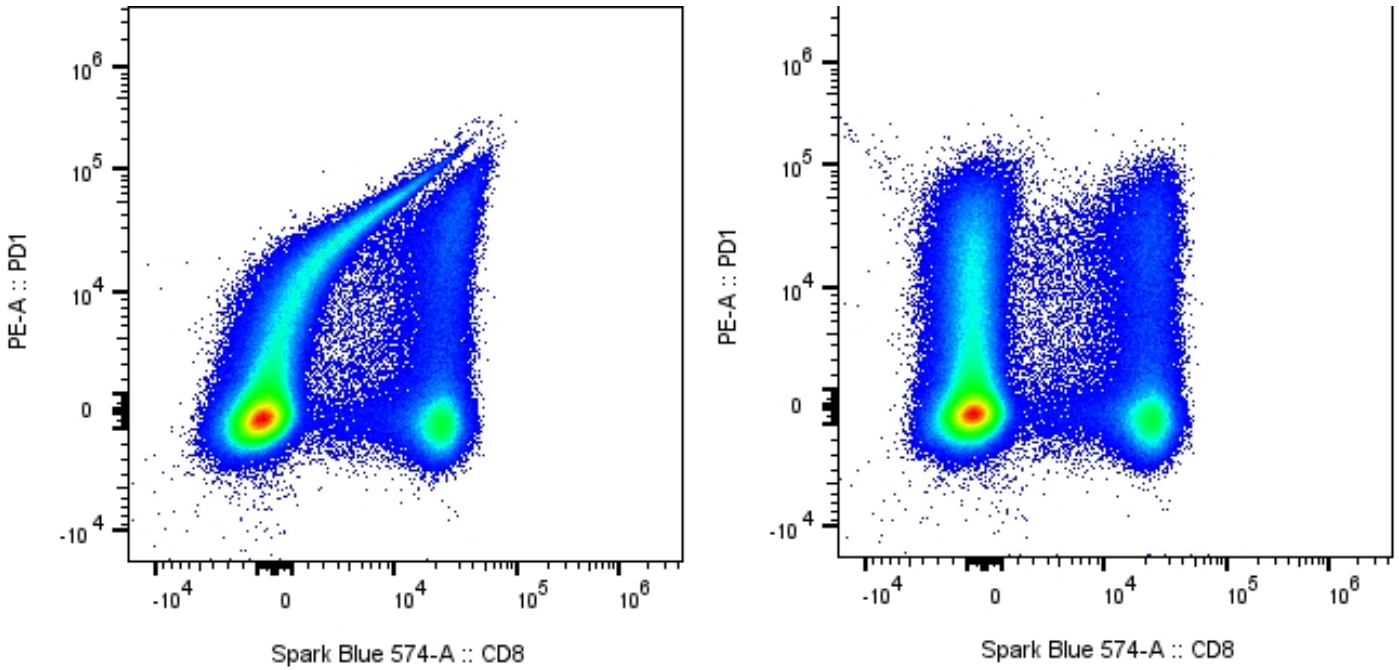
Residual Autofluorescence peaks in Single-Color Signatures

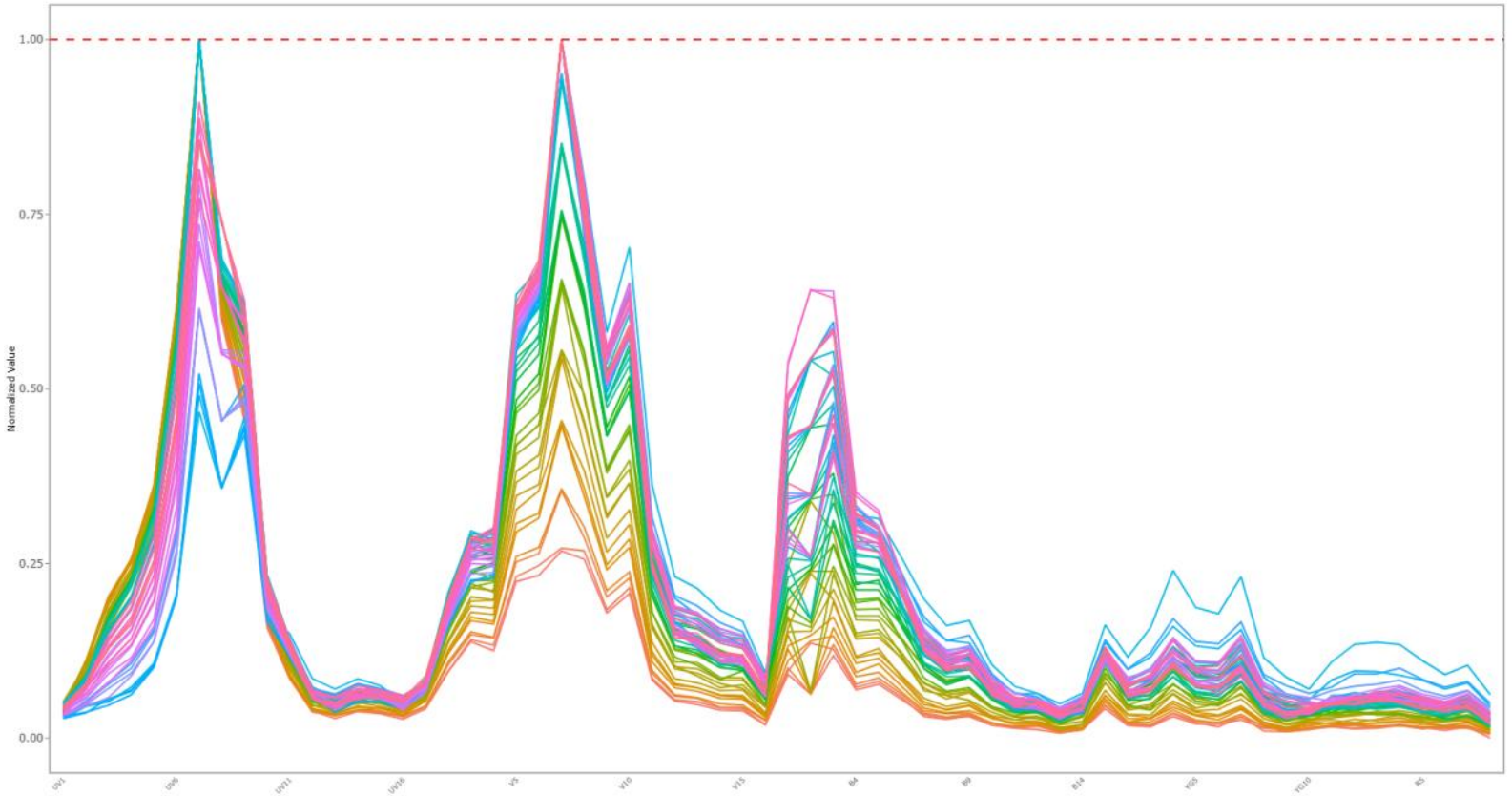
PE signature with residual AF



PE signature without residual AF

This residual autofluorescence contribution results in unmixing errors,
and the panel unmixing significantly improved when switched out for bead controls

Autofluorescence



- Same process to isolate autofluorescence variants
- Isolate cells with same normalized signature for use in unmixing

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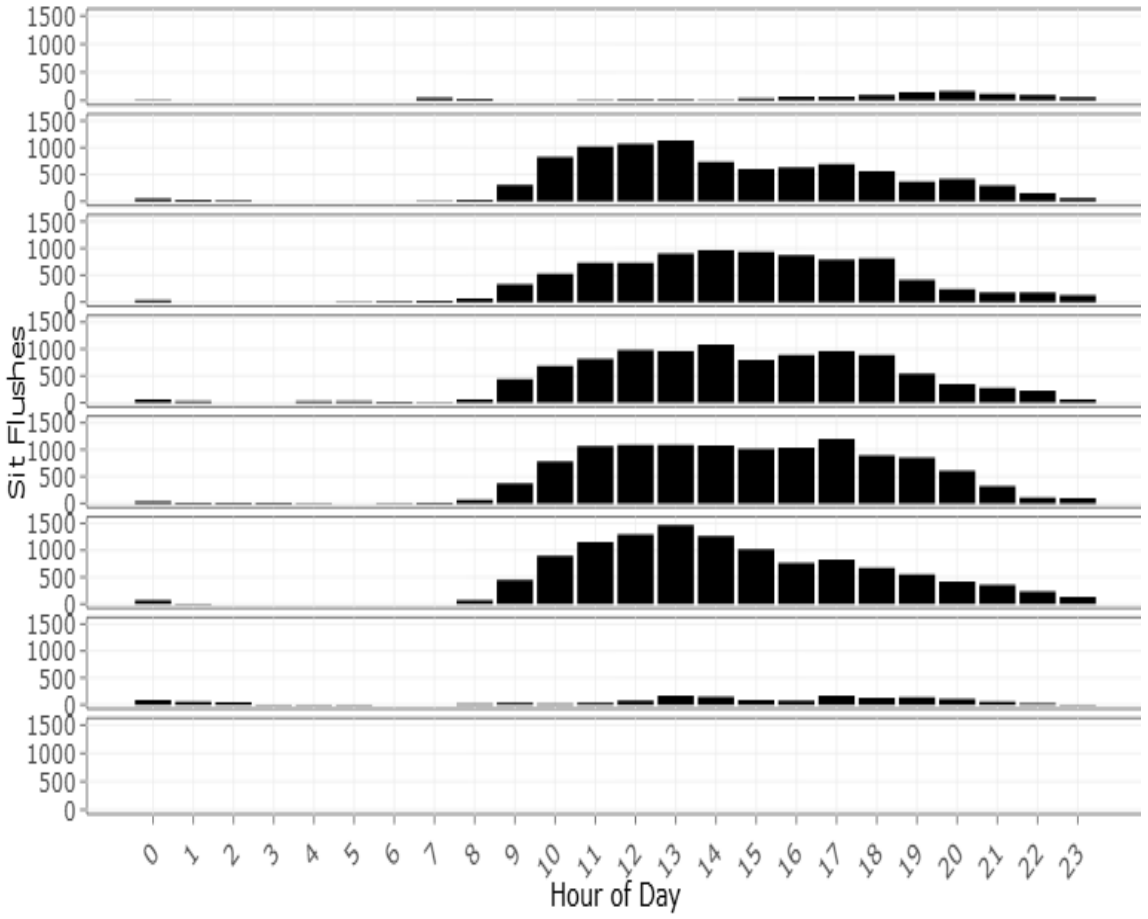
Application Log



- Every time a sit-flush occurs, its recorded
- Plotted over time, shows weekly usage patterns for instruments
- Including “off-schedule” usage

- Were clean flow cells and fluidic shut-downs performed?
- What software issues occurring?
- How many hours has each laser been left on?

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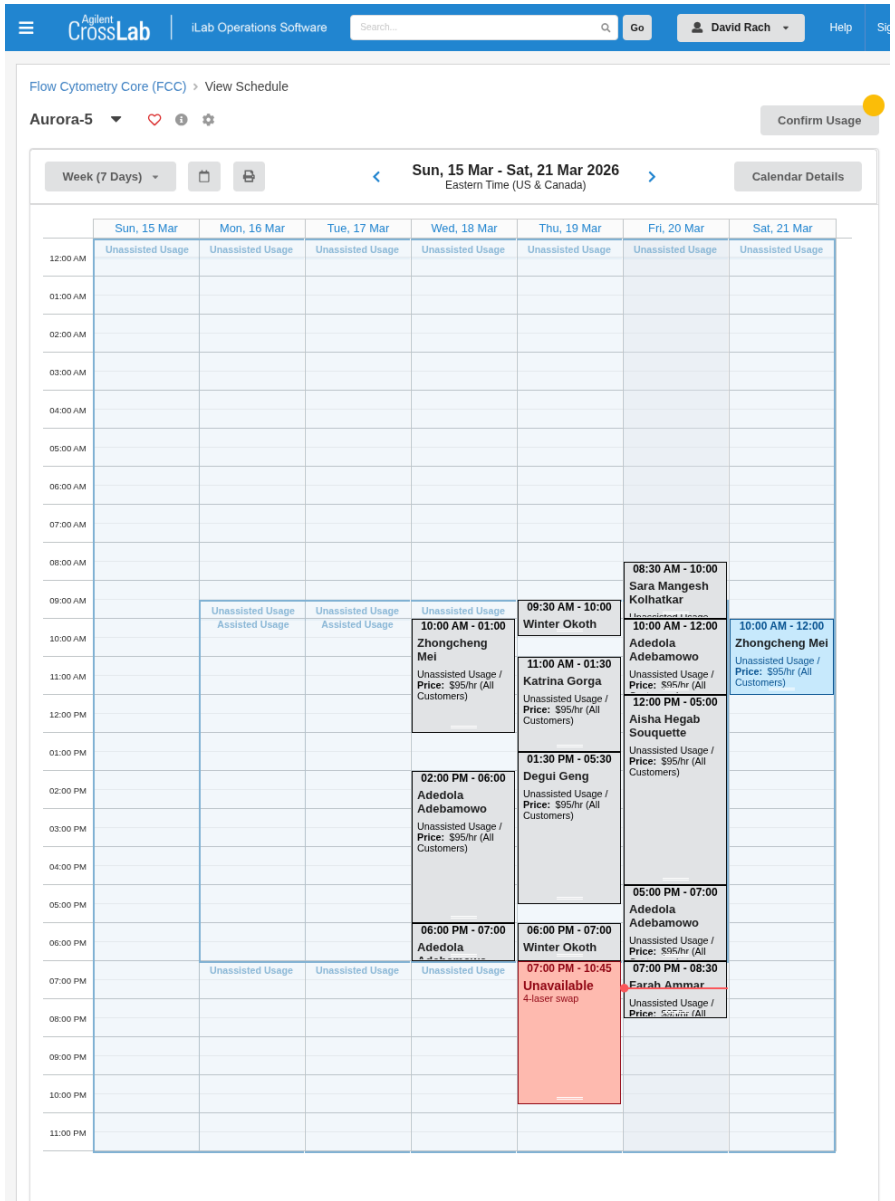
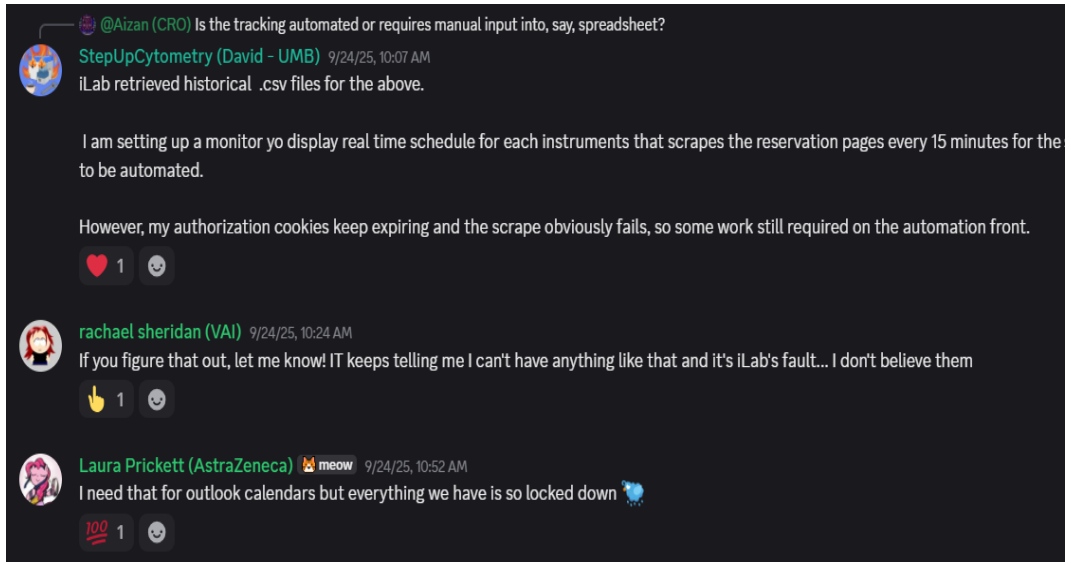
“Is anyone on after-me?”





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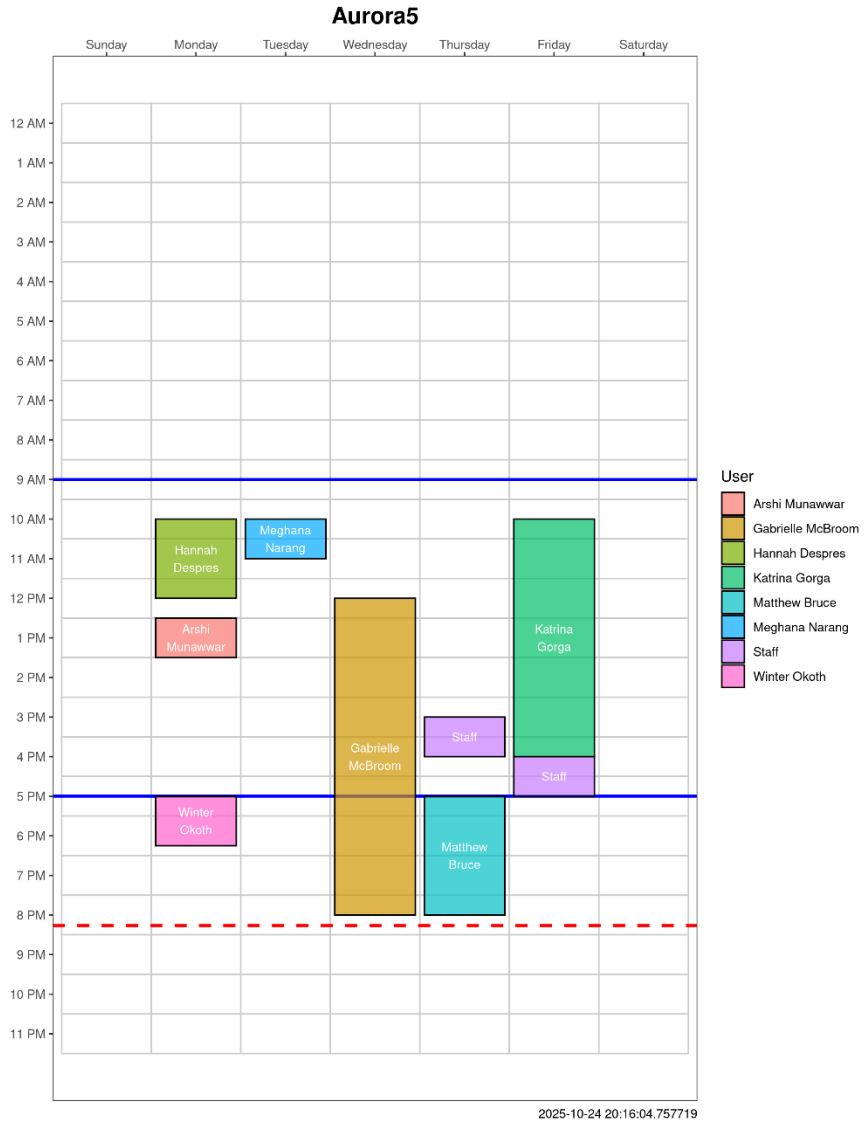
Updating Schedule to Display Monitor



- Displayed on a monitor to a Raspberry Pi 4b
- Cycles continuously through all the instrument schedules
- Refreshes every 15 minutes
- Sends booking data to GitHub

- Currently working on identifying late evening cancellations and sending emails to power down the instrument

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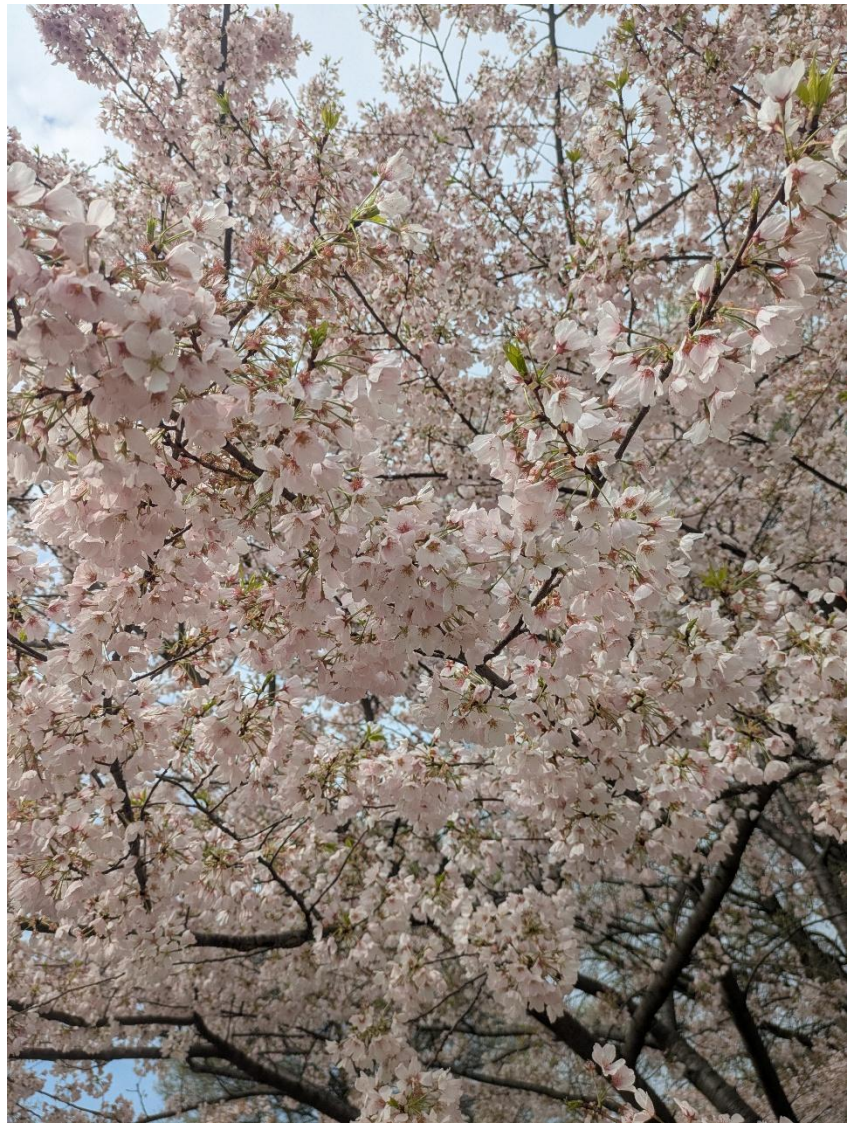
What have we learned?



- Small steps, sequentially implemented, have improved our awareness over time.
- With most instruments, useful information can be retrieved from unlikely sources
- Repurposing existing open-source software infrastructure speeds things up
- Set up for generalization requires additional planning

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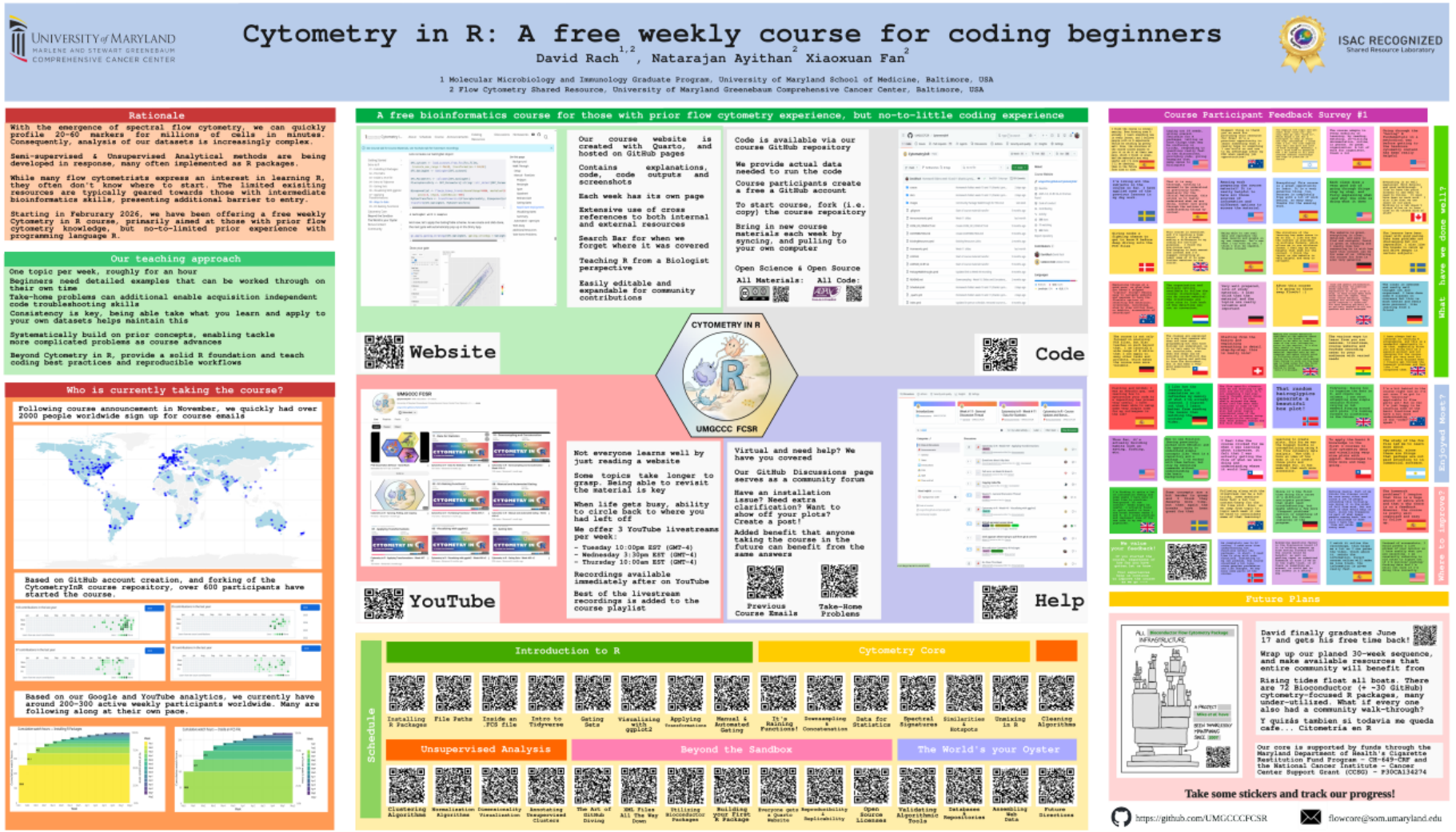
Learn R at your own pace

Website



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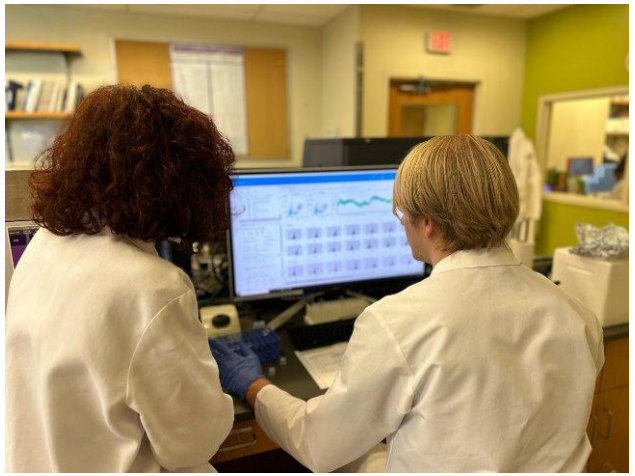
Bess Tracey



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Cytometry Discord

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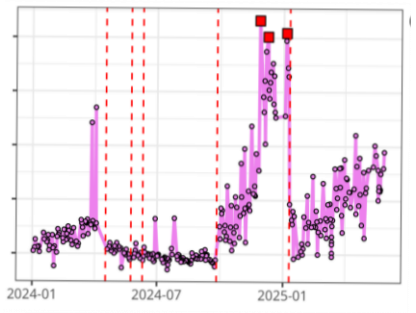
Questions?



UMGCCC FCSR
InstrumentQC dashboard

Luciernaga

Website - CytometryInR

InstrumentQC How-To

Coereba

YouTube **@CytometryInR**

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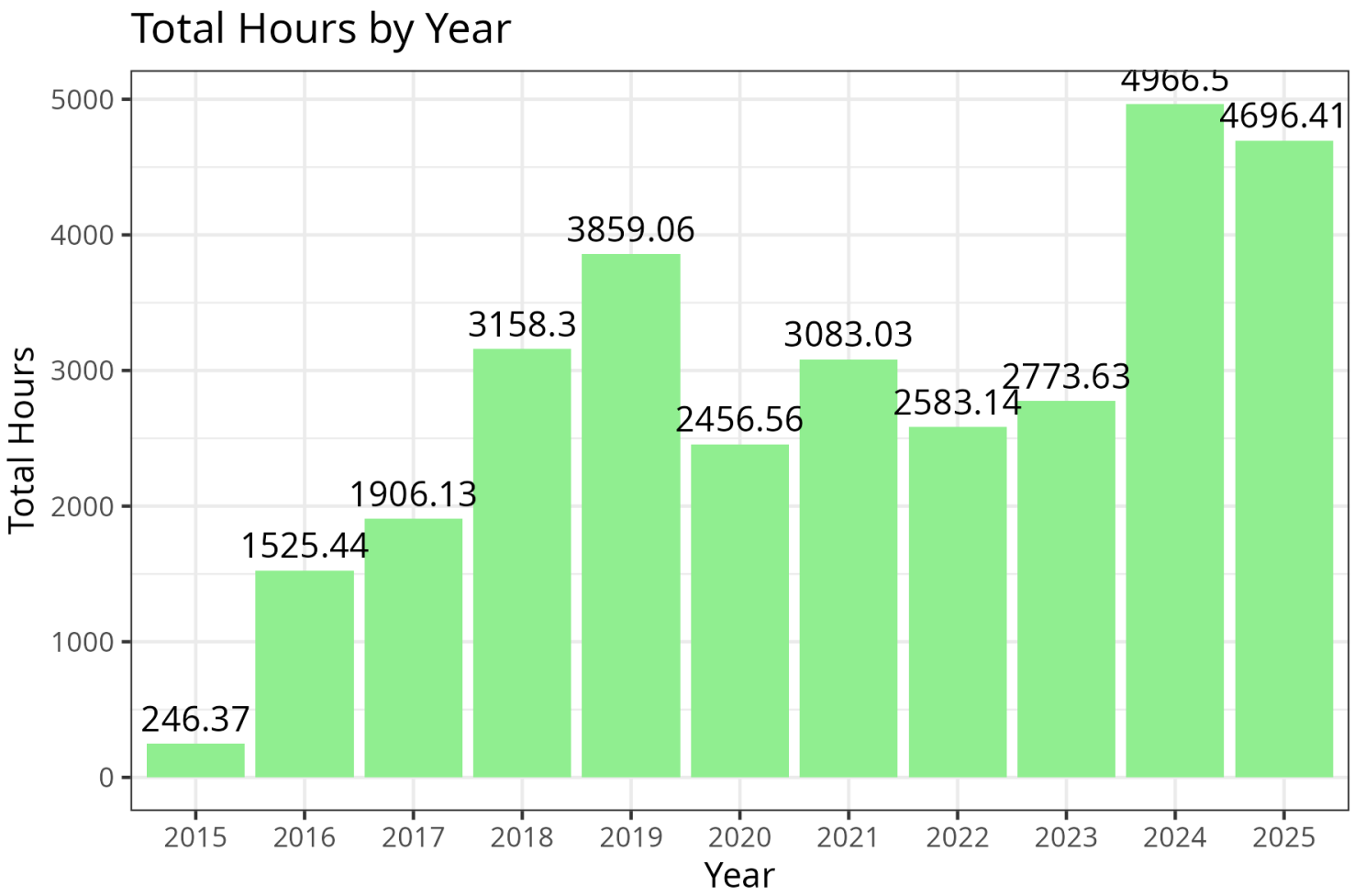
- Graduate Next Week
- All coding side-projects will then resume post-defense

Metrics from iLab



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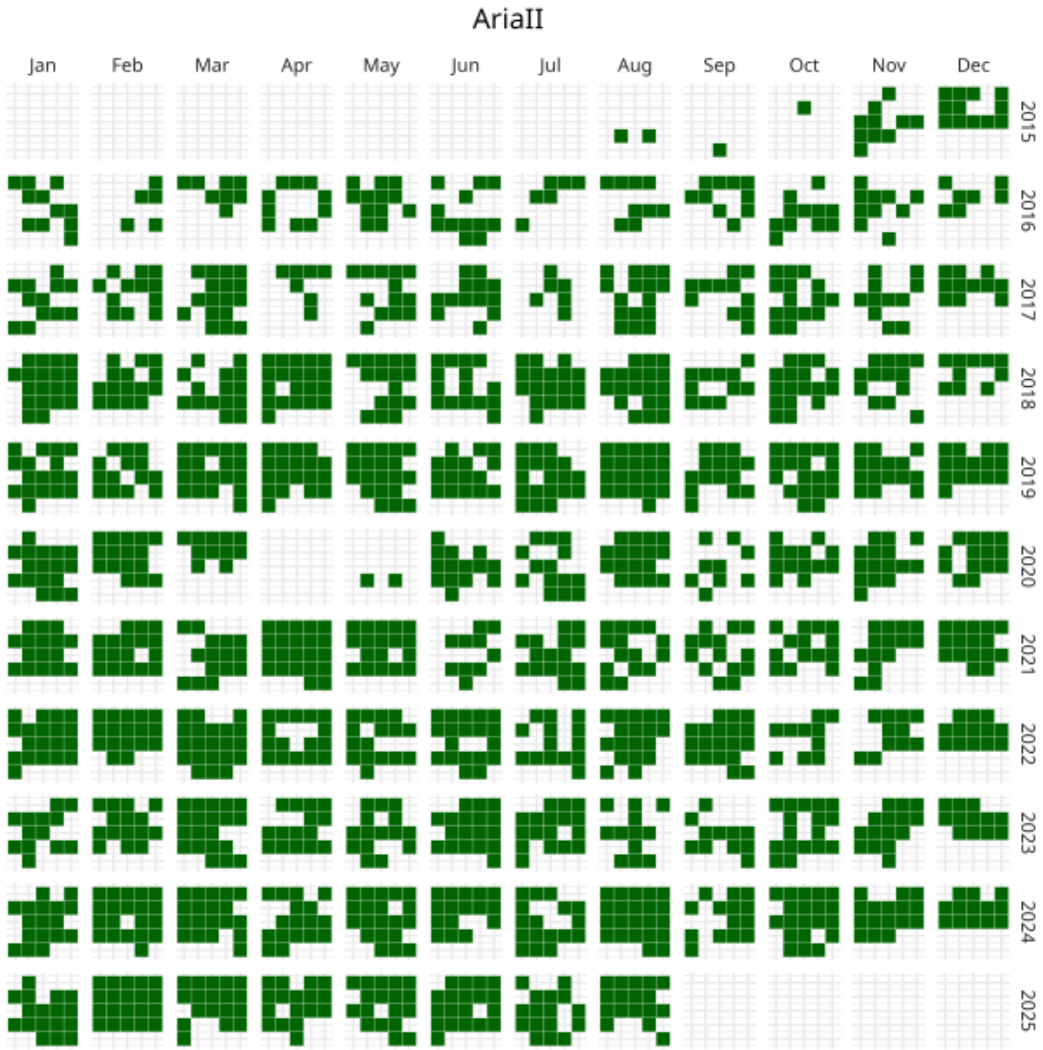
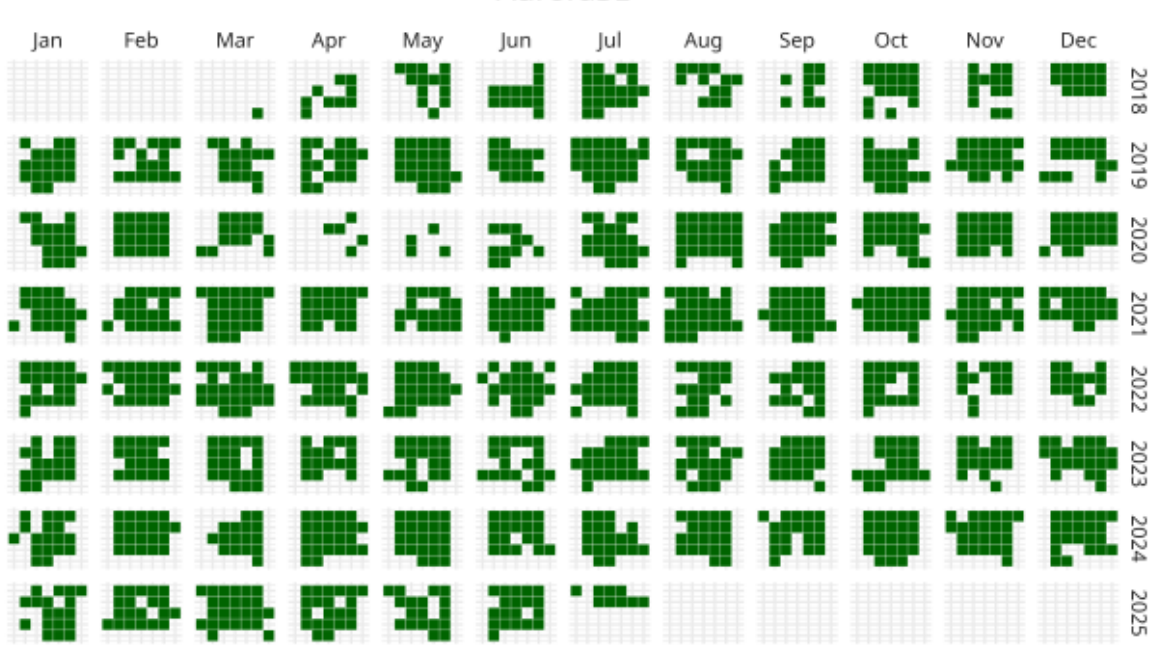
Instrument Use over Time





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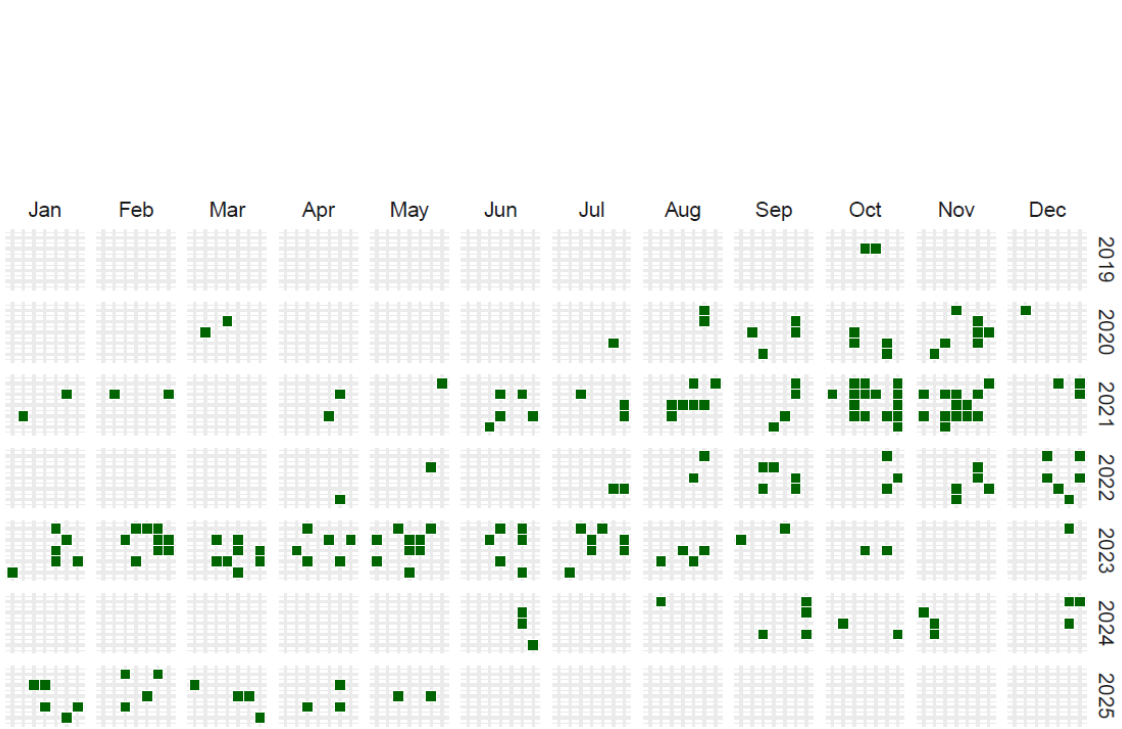
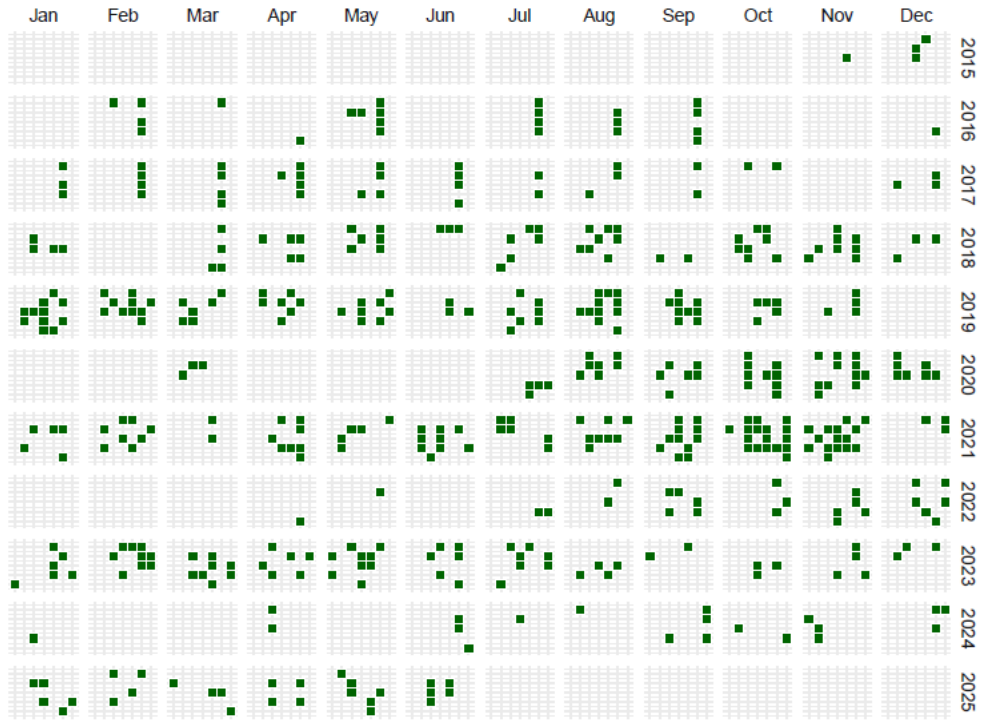
When did individual users or labs use the instruments?



David Rach



Cairo Lab

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