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Shared Resource Laboratory

“Deciphering the black box”: leveraging shared-resource laboratory resources to tackle spectral flow cytometry’s quirks

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UMGCCC Flow Cytometry Shared Resource
Baltimore, MD

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Slides

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Spectral Flow Cytometry is amazing!!!



- Capable of resolving remarkably similar fluorophores → **increased number of markers per panel** compared to conventional flow cytometry (CFC)

- Permits rapid acquisition of millions of events in a few minutes → **greater number of acquired cells per sample** compared to mass cytometry (MC)

- Spectral flow cytometry (SFC) datasets can have an unique combination of **width and depth** compared to preceding technologies

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Making previously difficult studies possible

- Acquire greater number of cells → identify **rare cell subsets** that may have previously been overlooked

- Invaluable for clinical studies with limited biospecimen → build one panel, evaluate multiple cell types

- Expand our understanding of immune system heterogeneity in response to infection



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Sharing community best practices drives SFC success

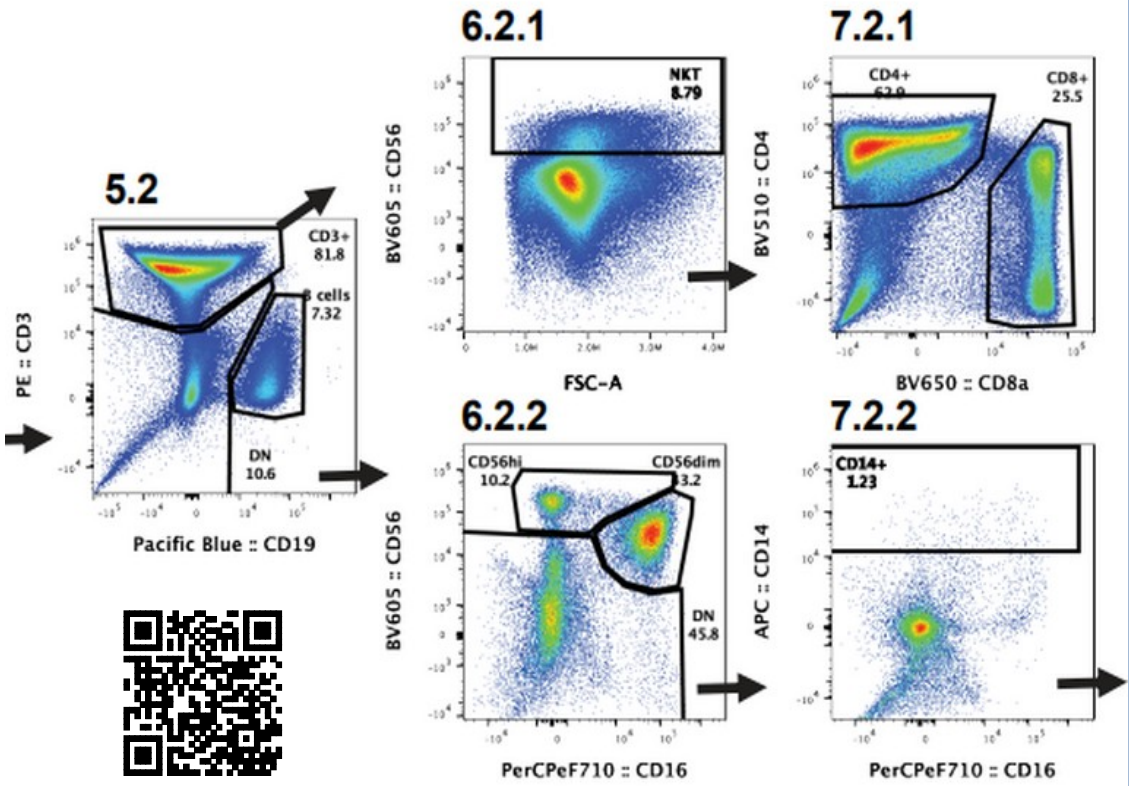


- SFC rapid and widespread adoption was not driven solely by the technology
- Community sharing of best practices is what enables the acquisition of good quality data
- If we hoarded our unmixing tips-and-tricks, fewer people would have switched

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SFC's quirks are a force of nature

- SFC requires synthesis of a lot of factors to get good quality data out of
- Not something you can expect to throw random markers and fluorophores and do well
- Some of these quirks are still not well defined, and consequently no solutions exist.



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Co-expression in unmixing

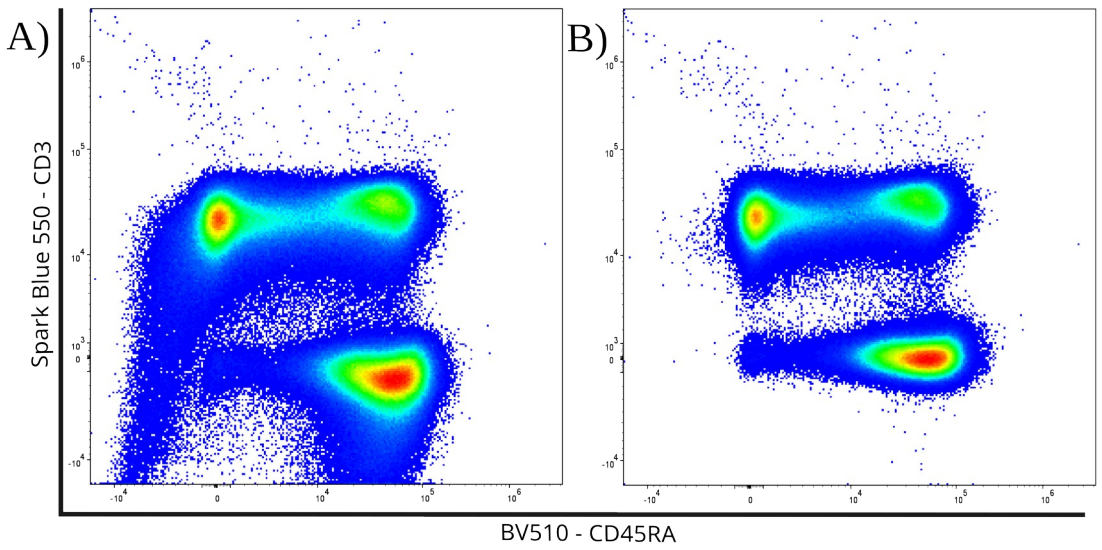


- Unmixing occurs on a cell-by-cell basis
- Unmixing same cell with increasingly larger unmixing matrices affects resolution
- When unmixing with the same matrix, co-expression of markers can drive similar effect
- Explains why different population of cells are shifted vs. each other for certain markers

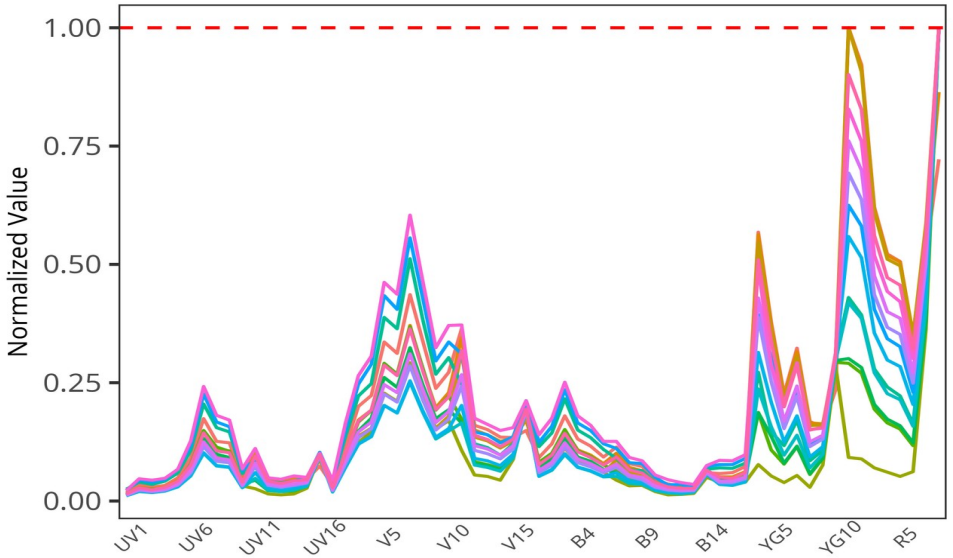
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Unstained unmixing controls

- All cells have autofluorescence
- No cell shares the exact same number of autofluorescent compounds
- Single autofluorescence as a running average
- When the running average differs, two autofluorescence form.
- Unaccounting for additional autofluorescence introduces unmixing errors



Single-color unmixing controls



- Fluorophores have characteristic spectra
- Fluorophores can be damaged or break apart
- Matching the spectra better unmixing results

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Instrumental changes over time

- Instruments change over time

- Attempts to stably maintain instrument performance with QC beads

- Severe enough changes in laser, array or fluidics can escape fixing

- Damage to the QC beads can lead to badly set settings



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Mix art, science and hoodoo



- When issues arise, how each individual troubleshoots varies
- Community best practices arise from a mix of forces
- Some are pretty well understood
- Some are not well understood, and the “fix” may be doing more harm than good

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SRLs as force for good

- Shared resource laboratories play important roles for the community
- Wealth of technical expertise, broad perspective arising from experience with broad array of panels
- Provide access to better instruments for a wider pool of researchers
- Help catch and correct instrumental issues before it becomes a $300,000 paperweight



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Wide range of panels and cell types



- Wide range of users using facilities
- Variation in panels, tissues, goals
- Context that often individual researchers may miss

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Instruments over lifespan

- Interact with instruments over their lifespan

- In good times, and bad

- Data at the individual instrument wealth of technical data if tools to leverage are present



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Who spots the issues



- When issues arise, who identifies them vary
- Staff more likely to encounter the automated flagged errors due to startup
- Users that consistently work with a panel more likely to see perturbations

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Im tired maaannn

- Staff remain overworked

- Asking to take on another role requiring effort is a tall ask

- Better ways to enable detection



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Automate the detection



- Within existing practice, data that could be used to flag issues is already being collected
- Implementing and refining systems to flag the issues could raise awareness
- Upfront cost, uncertain gain, what benefit long run

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And decipher some underlying unanswered

- Beyond identifying issues, set up framework to interrogate unknown
- Systematically identify the source of the quirks
- Improve the practice, and our understanding of the underlying technology



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Manufacturer accessability

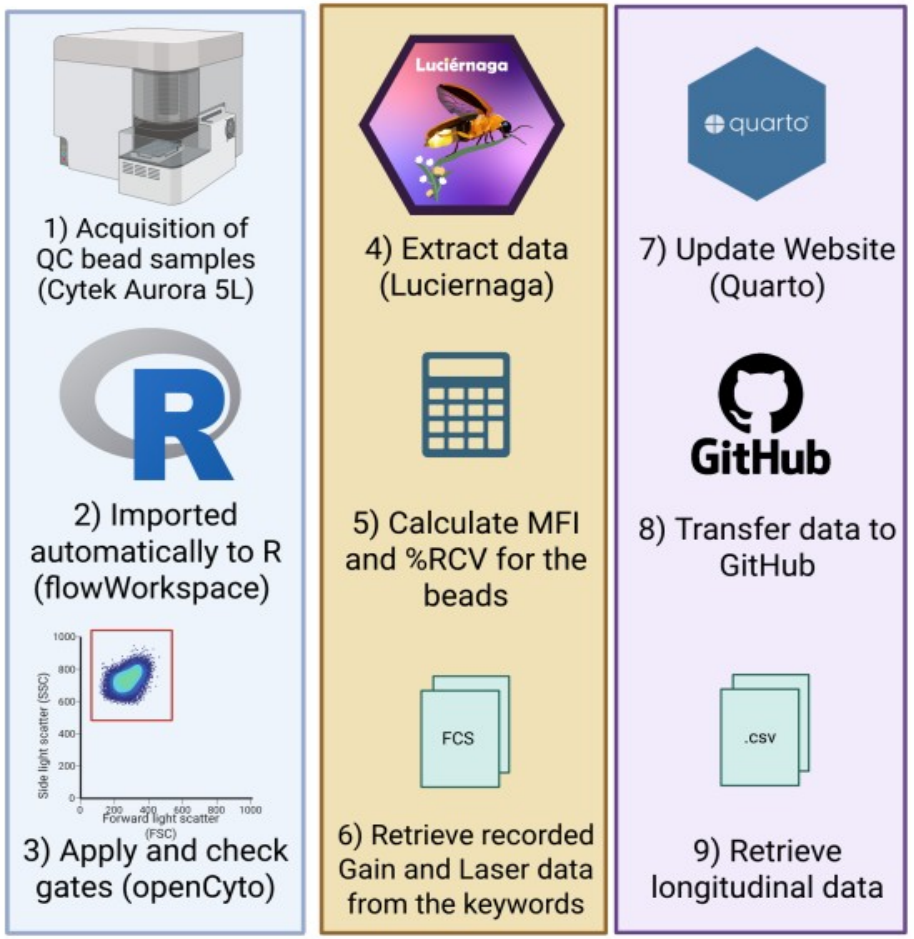


- Approach depends on manufacturer externalizing certain elements
- If no access to underlying raw data, not going to get as far
- Having parsable experiment .xml helps

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What we do as far as QC

- At our core, QC is run in the morning, also acquire pre-post bead samples
- Automated systems that process and send to GitHub
- Data available for querying, as well as for use in interactive dashboards



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A little programming knowledge makes you dangerous



- Programming not part of a regular job descriptions
- But beyond bioinformatics, knowledge of principles can make you lethal
- From reports, to automation, to analysis

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Things you may have not known about your Aurora

- Lets use a Cytek Aurora at our core as principle for what I argue

- Most outputs as their own .fcs file type

- .Expt etc files as .xml



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Sit Flushes in App Log



- Every sit flush ever occurs is recorded in the AppLog
- Parse this txt file
- And do useful things

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Usage Plots vs. hour

- Condense and show usage over the course of 15 minute periods
- Identify when the instrument actually being used
- Cross compare to actual schedule



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Commando Users



- No monitoring system based on a log-in
- Still able to identify users going commando on usage
- Address individually

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Usage Pattern Over Time

- Track individual users and their labs experiment patterns over time
- Monitor busy times for a particular instrument
- Identify whether need is present for another similar instrument



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QC CSV folder

- Likewise, QC is returned as .csv files
- Not tidy, but still parseable
- Wealth of technical data not directly exposed



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Temperature



- Instrument temperature over time
- Room changes
- Impact on the rcvs?

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QC Time

- How early are staff getting in
- Change over time
- Vs. when actually acquired



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Parse for errors, sequences



- Errors also stored in the AppLog
- Combinations indicative of when clean flow cells etc are run
- Longer tracking of instrument performance

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FCS files undefined

- Beyond manufacturer provided, .fcs files hold a wealth of information

- Additional parsing set up required to take full use

- Ability to wield a bunch of other datasets towards our automation goals



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QC doing what is says undefined



- Does instrument QC actually track over time?
- What happens when things fail
- Can we do this with actual user data and predict what is occuring?

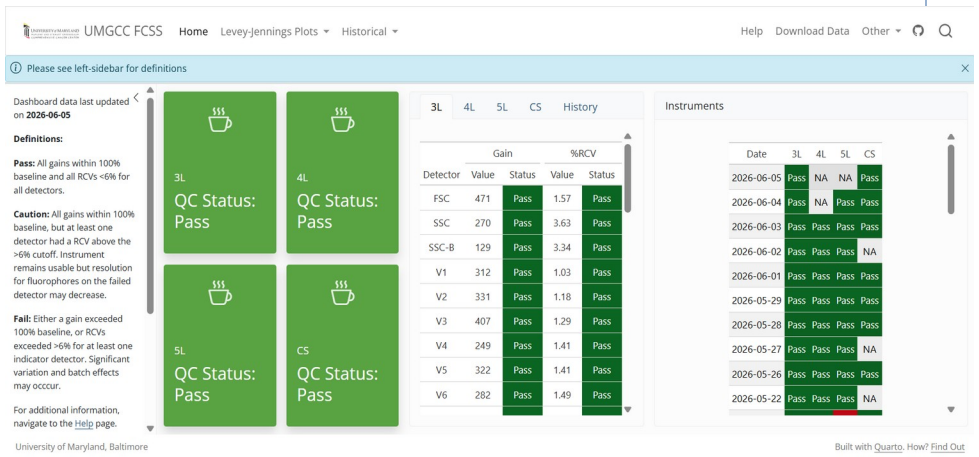
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CSVs to dashboard undefined

- Beyond processed data, can we leverage it
- InstrumentQC dashboards for interactive levy jenning plots
- Stage interventions a month before issues due to the lasers arise

Dash board

How -to



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Damaged QC beads



- Lot 2006 and bleach
- Loss of an APC-Fire 810 like molecule
- Incorrect settings, bad unmixing outcomes

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Expt files undefined

- .Expt files as .xml

- Every setting on your setup encoded starting in version 3

- What are all your facility users up to?



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Users not knowing about threshold



- Do the users actually set thresholds?
- Most don’t
- Easy enough intervention

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Correct antibody

- Does the antibody match the reference

- Or in a bad habit carrying over from conventional

- Ay ya yay



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Stain index and prediction



- From each, can we identify stain index
- Can we make predictions to better allow for panel building
- Rather than forgotten, what gold lies sitting forgotten in our external hard drives

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Correlating variation

- Identify issues
- See differential unmixing
- How do we tie these two together



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Is my unstained zombied?



- Users that keep staining in plates staining their unstained with other fluors
- Identify and stage interventions
- Plates vs. Tubes generally quantified

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Future works in progress

- Need for systematic if then frameworks for decision making
- Able to go beyond single-staff driven
- Need to generalize beyond a single-platform



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Regardless, what one person can do



- End of the day, what you can do is driven by your own curiosity
- Only so much good trouble that any one individual can get into
- But what if that wasn't the case

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But what if we had a community soapbox

- Adoption SFC was thanks to the community

- What if we leveraged our community resources together to drive a stake through the quirks heart

- Waiting on companies to implement things… not a fast lane to success historically



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Manufacturers can help or get out of the way



- Manufacturers that make it easier for us to work will attract more tinkering
- Benefit from the improved reliance, can integrate at their own pace if they wish
- Others that are not so cooperative will be forced to also move or get left behind

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Rise together stronger

- Be the change that we want in our community

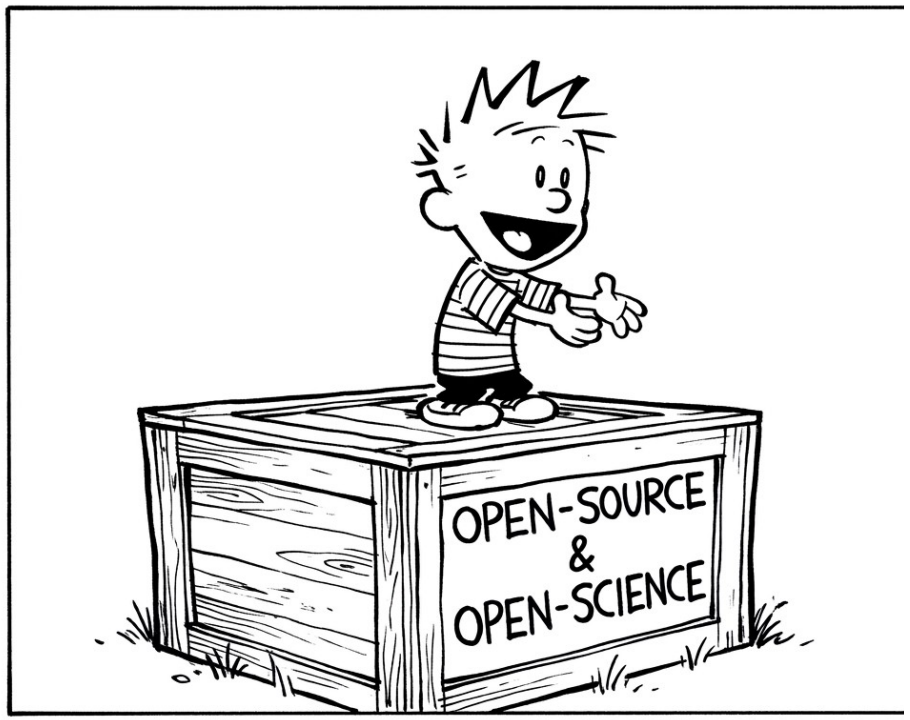
- Why wait for others when you can collaborate and change things now

- Tonight we shall steal the moon



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Open-source and shared



- Hoarding our tools and knowledge has not served the community well

- We can go further when we work together, and when the exchange of ideas is open and free

- Open-source and open-science

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R not just for the analyst

- R as not just the bionformaticians domain
- Wider ecosystem that can be of use
- Who knows what the next cool idea will come from



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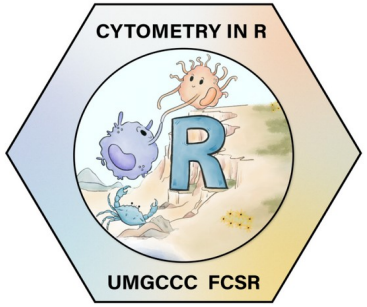
What good is any tool if the community is not able to use it?

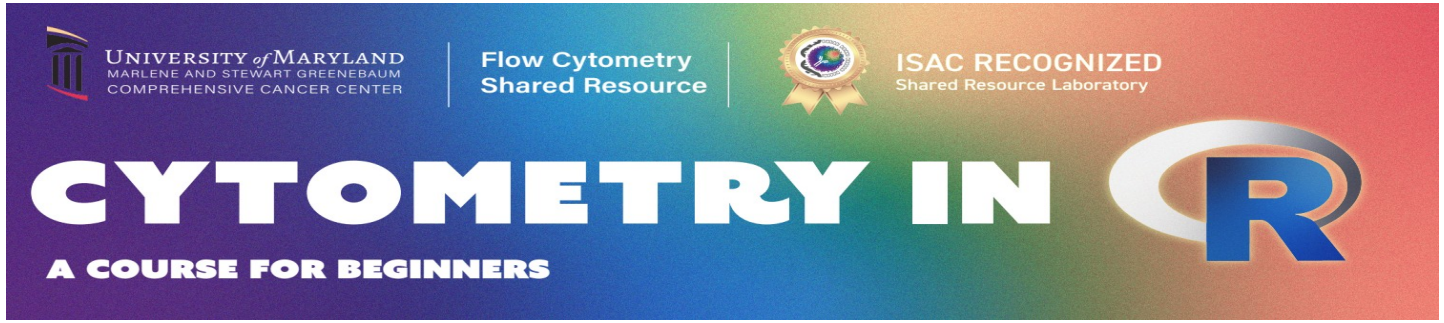
- Few resources to learn Cytometry in R to begin with
- Those that exist aimed at those with intermediate-advanced R skills, not beginners
- Know-how concentrated at a few institutions
- Most self-learning attempts end in frustration



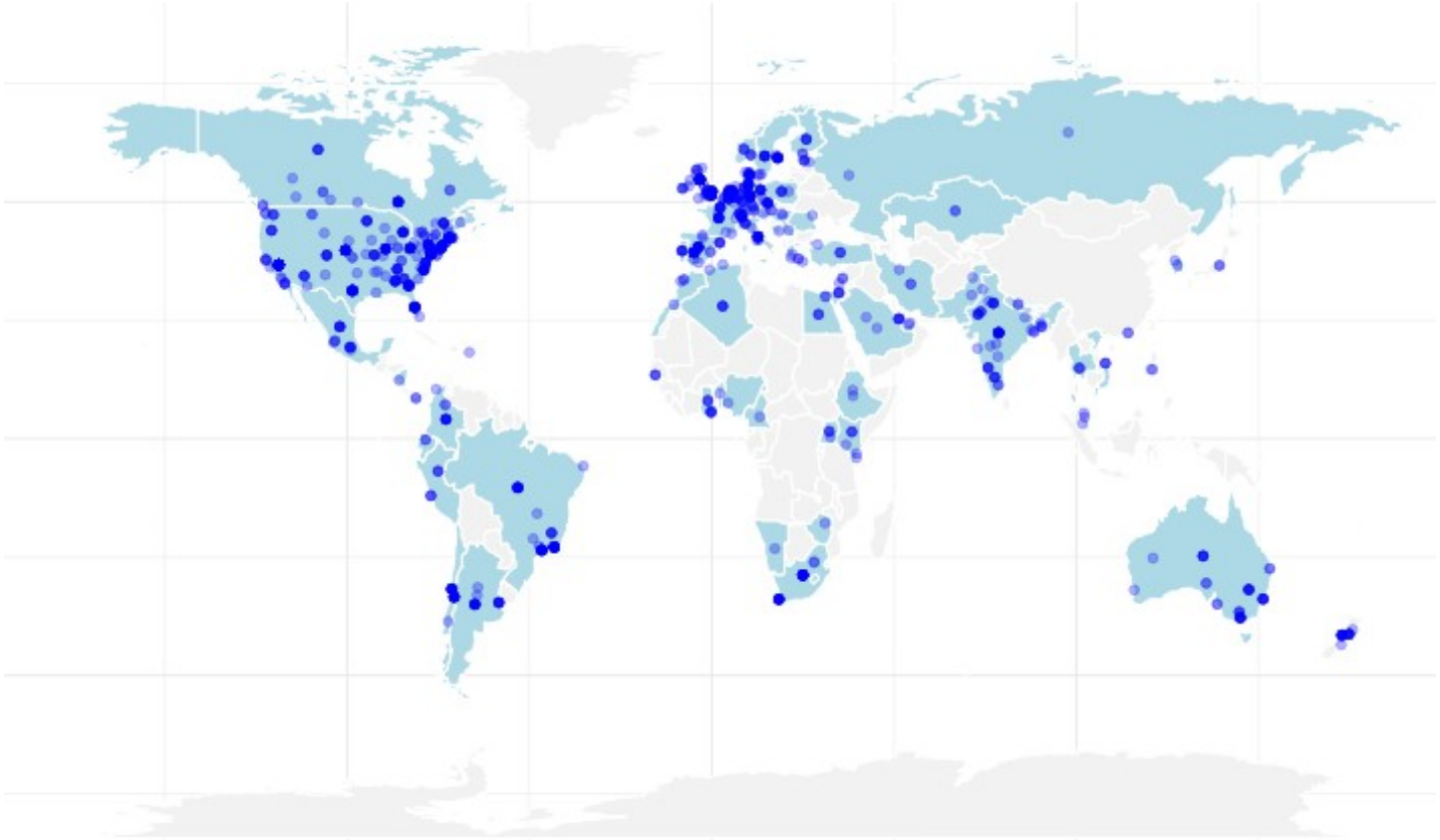
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Cytometry in R: A free weekly course for flow cytometrist with no-to-little coding experience





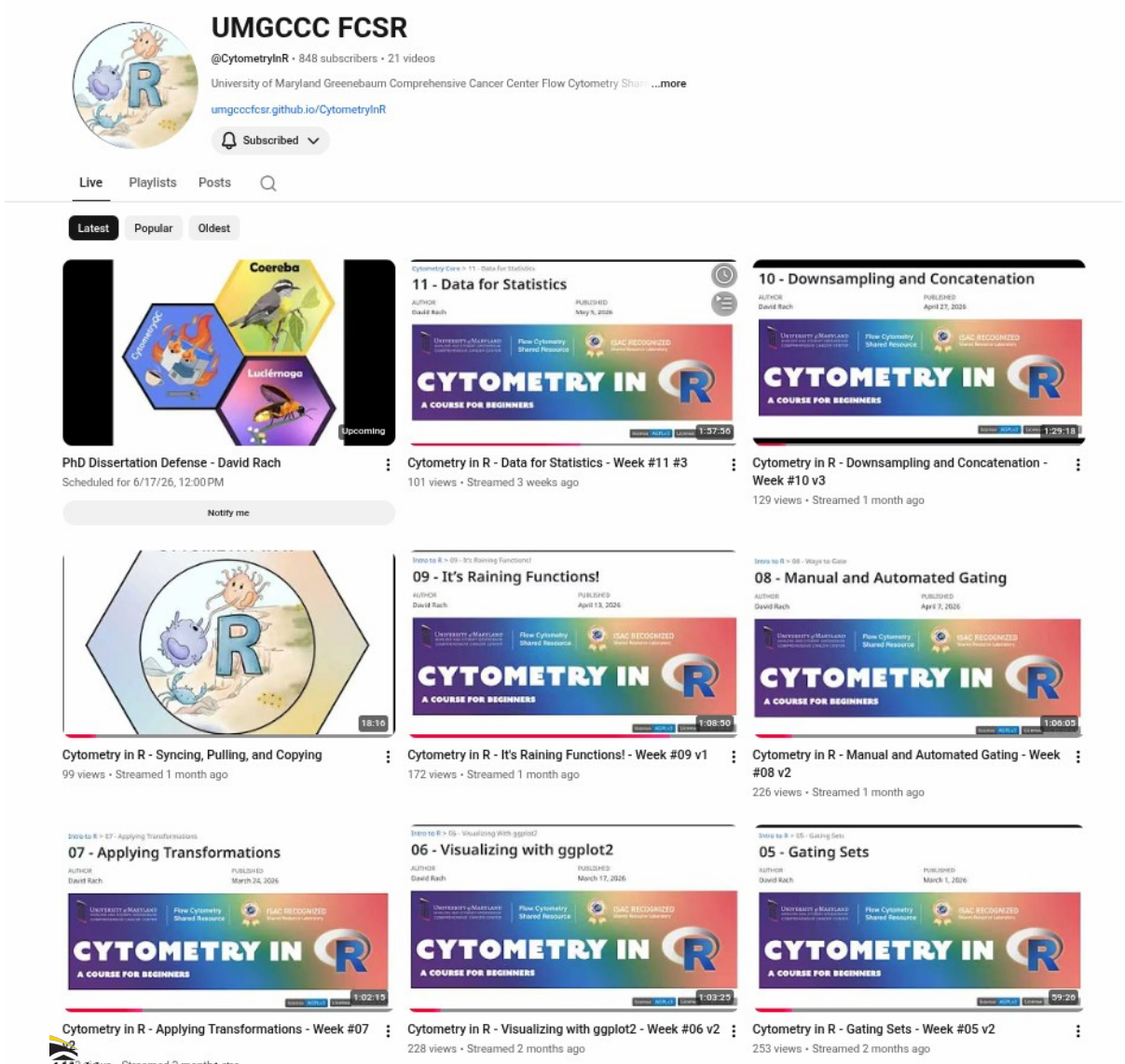
Website



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YouTube

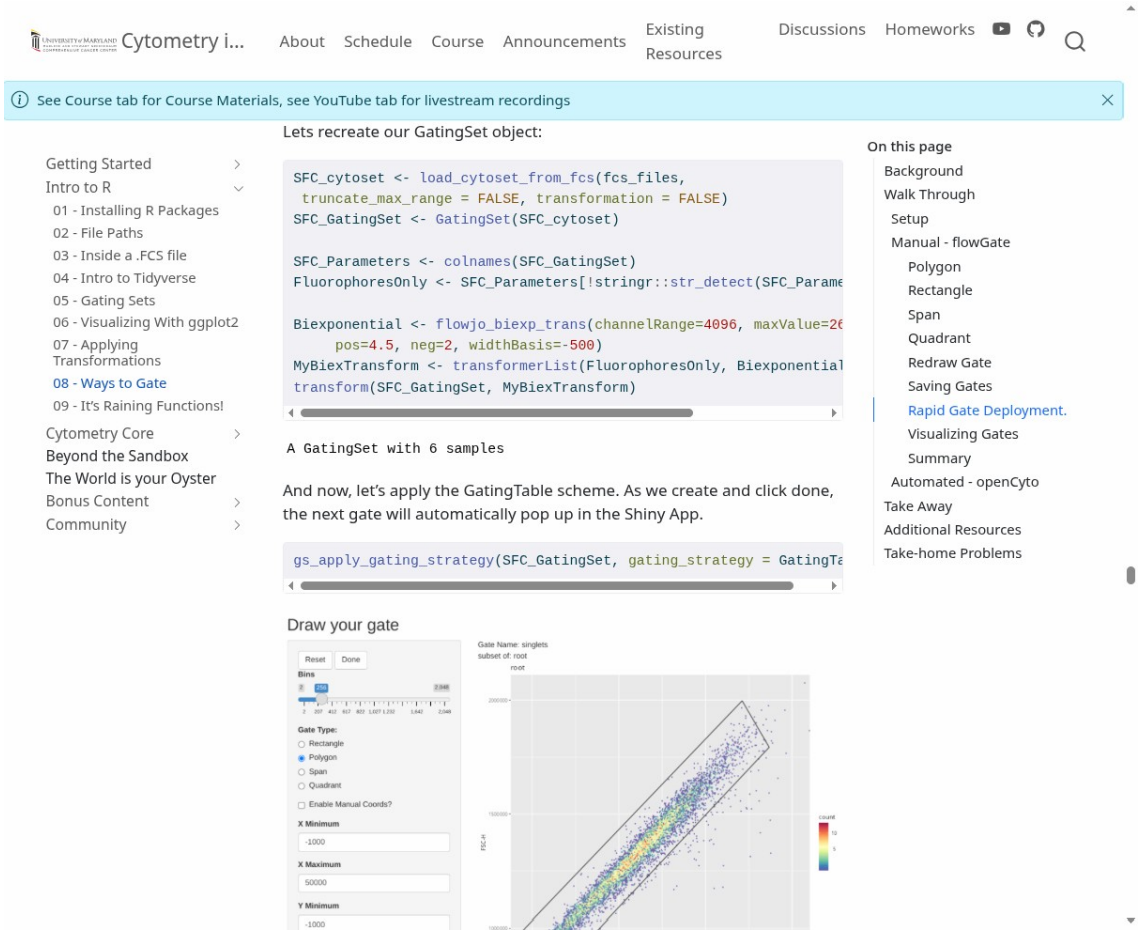


- Three live-streams a week (accommodating different timezones and schedules)
- Recordings available immediately after
- Best of the weekly livestreams added to the playlist, cutting down on editing

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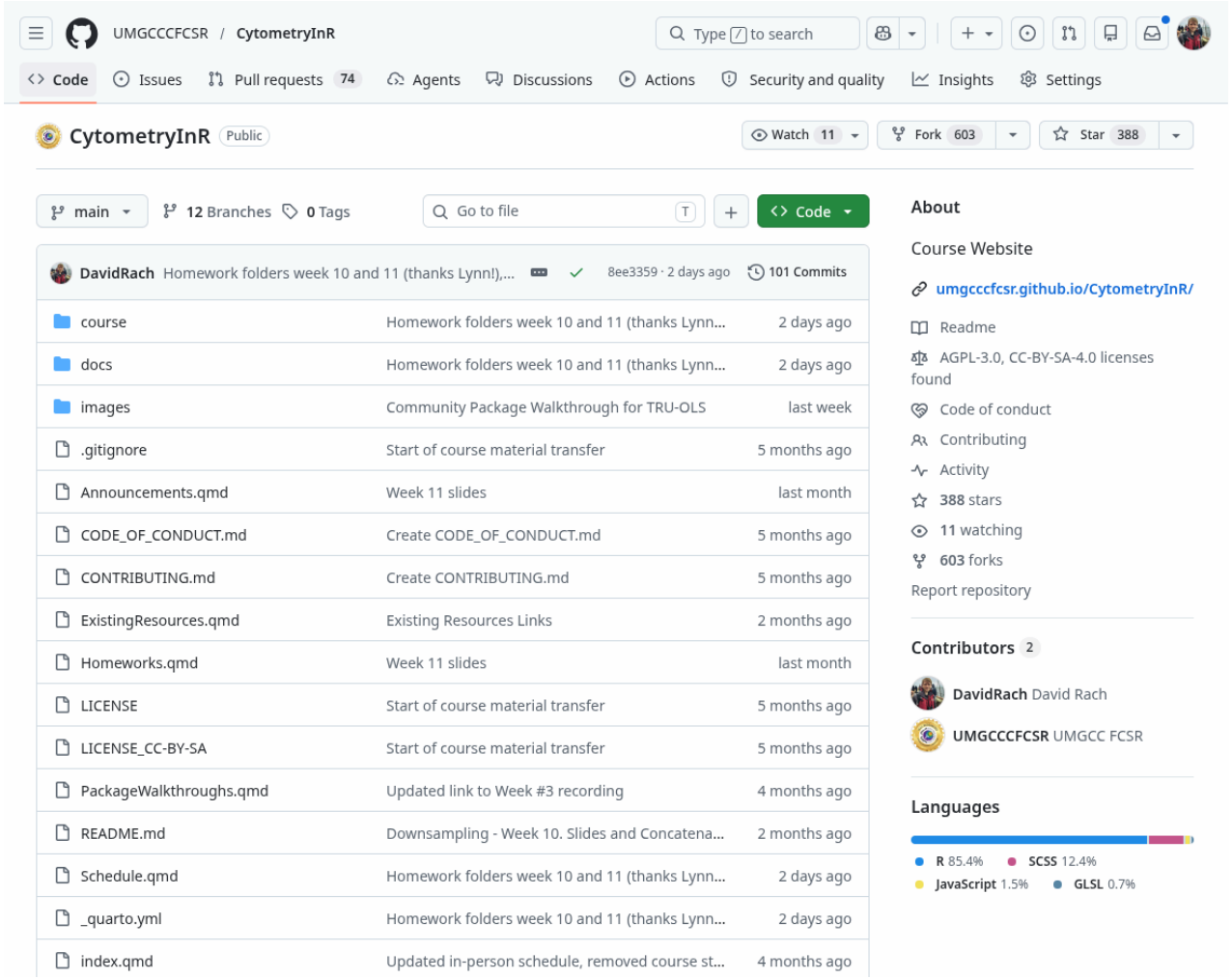
Website

- Created with Quarto, hosted via GitHub pages.
- Code, code outputs, screenshots and explanations
- Searchable
- New weekly material added as a new .qmd file



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GitHub

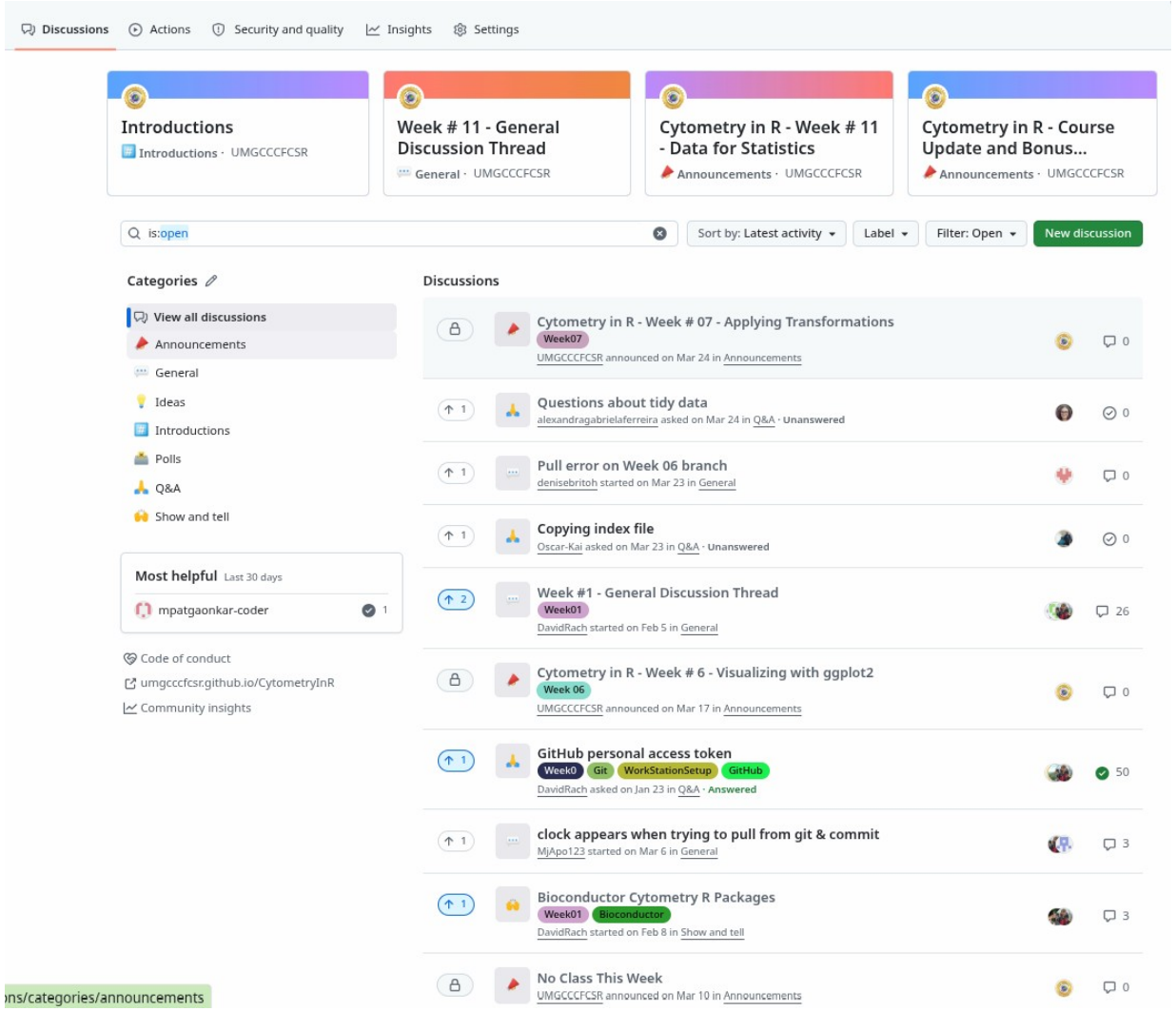


- Code and datasets available via the GitHub repository
- Course participants start course by creating GitHub account and forking the repository
- After Positron is linked to GitHub account, pull locally
- When new material posted each week, sync and pull

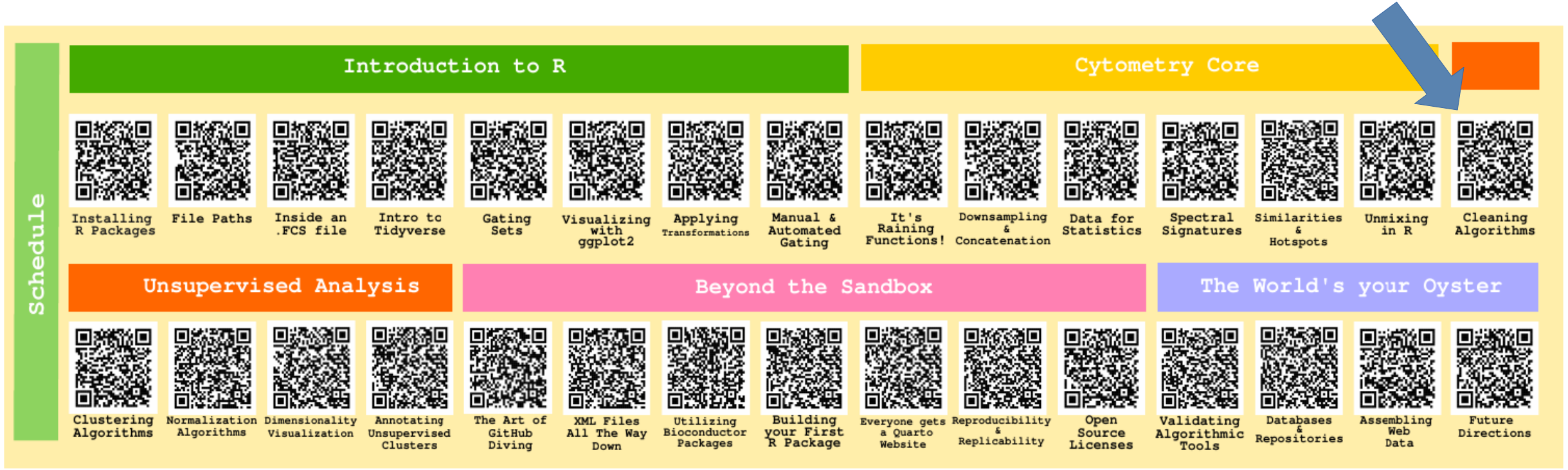
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Discussion Forum

- GitHub Discussions page has been repurposed as a community forum for questions, etc.



Half-way through this passion project



- Wrapping up Cytometry Core module
- Gathering steam to start on the Unsupervised Analysis section

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My scripts are here

- Actions where my mouth is

- Here is our brand new website with the scripts

- And video explanations are on their way to boot



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Call for Cytometry QC data



- An R package that writes the dashboards for you
- Seeking to generalize, but we only have certain instruments
- Contribute your QC data from different instruments, I will see that you can do set up as well

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Acknowledgments

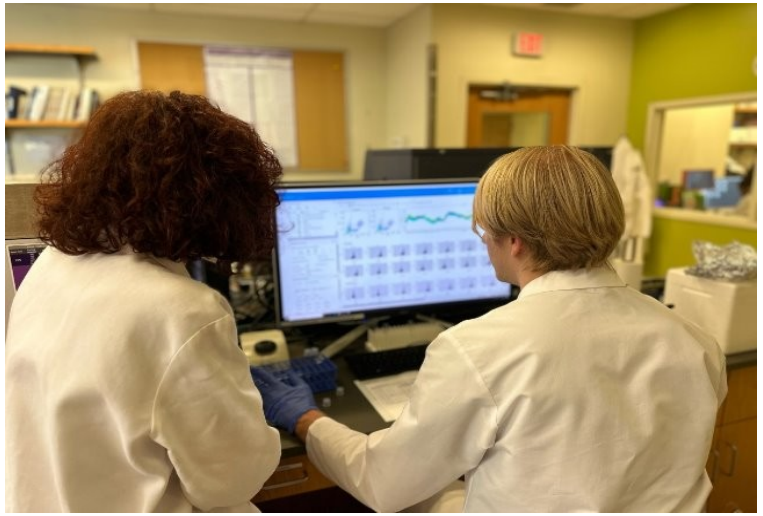
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OF HEALTH SCIENCES

All mothers and their infants, and their communities

All the open-source contributors, developers, and maintainers, that collectively made the tool sets without which all of this would not be feasible

Dr. Cristiana Cairo
Dr. Kirsten E. Lyke
Dr. Xiaoxuan Fan

Dr. Daniela Franco
Dr. Natarajan Ayithan



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Cytometry Discord

Bioconductor Zulip

https://github.com/DavidRach

@davidrach.bsky.social

www.linkedin.com/in/david-rach/

Want to collaborate? Reach out!

Hiring? 👀

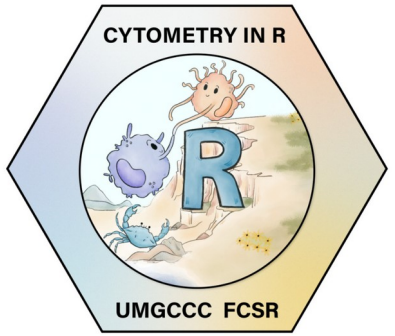
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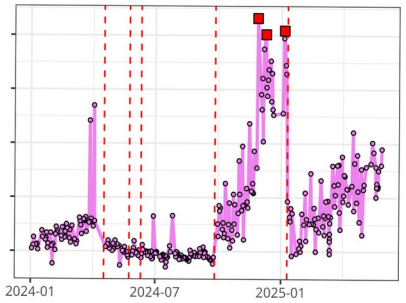
Questions?



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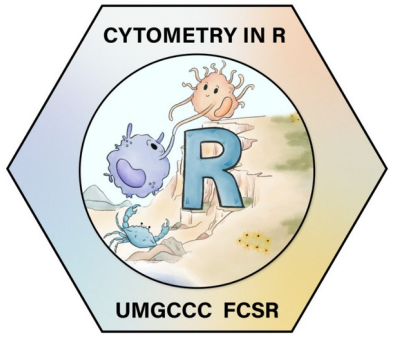
Website - CytometryInR



UMGCCC FCSR
InstrumentQC dashboard



Coereba



YouTube @**CytometryInR**



InstrumentQC How-To

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Building open-source tool-sets to address SFC's quirks



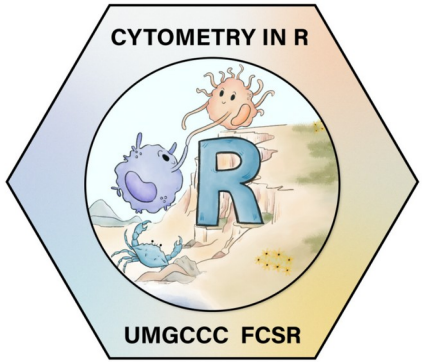
Spectral Signature Characterization and Isolation

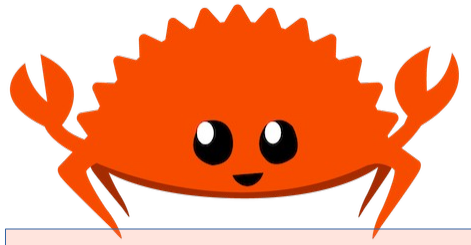


Incorporation of manually-annotated gating information



Automated Instrument Quality-Control Dashboards





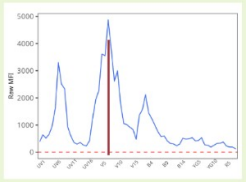
Rust

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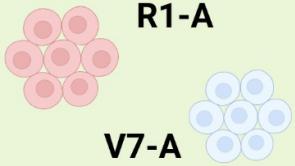
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Code



Identify detector with brightest MFI for each cell



Group cells with shared peak detectors



Subtract Out Autofluorescence

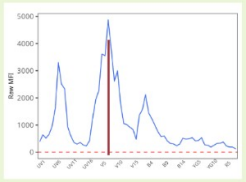
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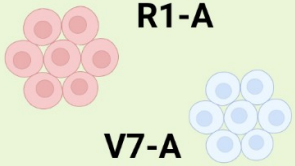
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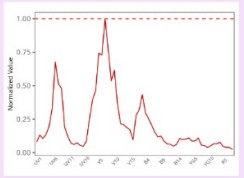
Identify detector with brightest MFI for each cell



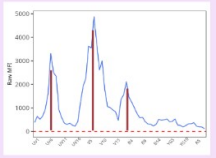
Group cells with shared peak detectors



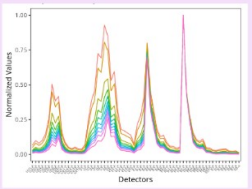
Subtract Out Autofluorescence



Normalize individual cell fluorescent signatures



Identify peaks using local maxima



Group cells with shared peaks & heights

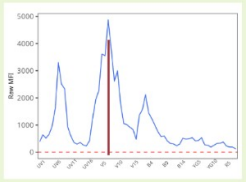
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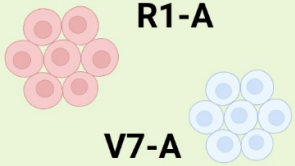
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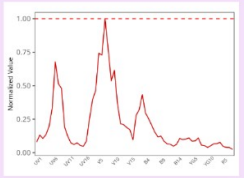
Identify detector with brightest MFI for each cell



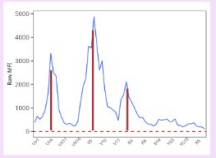
Group cells with shared peak detectors



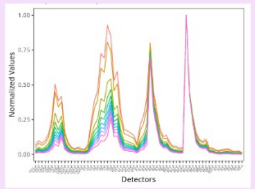
Subtract Out Autofluorescence



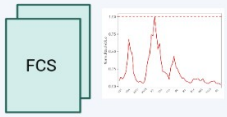
Normalize individual cell fluorescent signatures



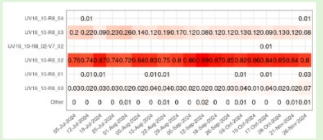
Identify peaks using local maxima



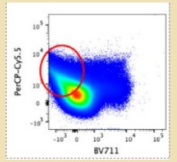
Group cells with shared peaks & heights



Enumerate and export out cells that share signature as .fcs files



Checks for tandem degradation



Autofluorescence Isolation

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