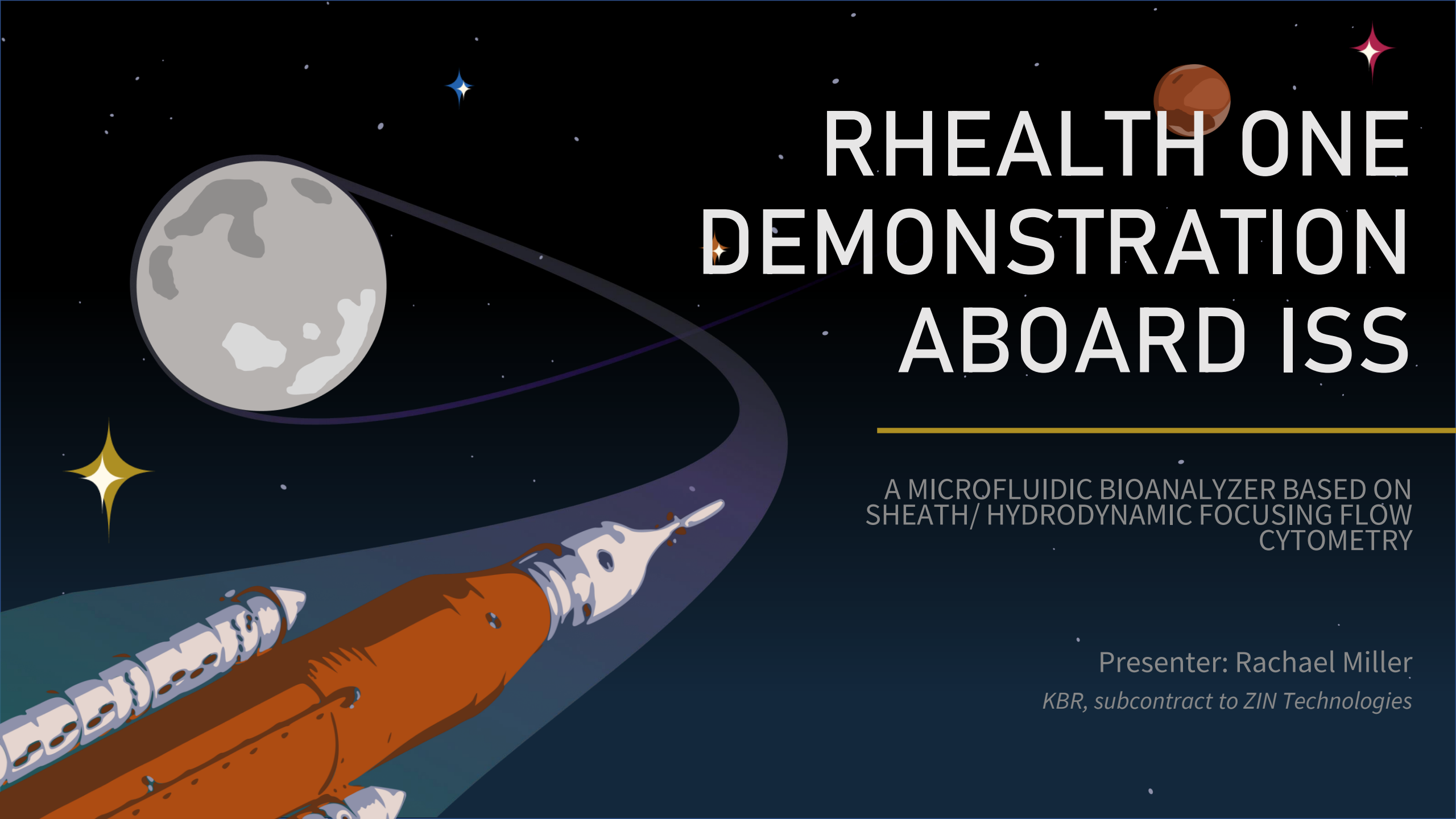

RHEALTH ONE DEMONSTRATION ABOARD ISS

A MICROFLUIDIC BIOANALYZER BASED ON SHEATH/ HYDRODYNAMIC FOCUSING FLOW CYTOMETRY

Presenter: Rachael Miller

KBR, subcontract to ZIN Technologies

RHEALTH ONE DEMONSTRATION ABOARD ISS

A MICROFLUIDIC BIOANALYZER BASED ON SHEATH/ HYDRODYNAMIC FOCUSING FLOW CYTOMETRY

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Overview

- As space flight ventures further from earth, the need for autonomous medical care increases under greater constraints on mass and volume. The Exploration Medical Capability (ExMC) element aims to provide astronauts with the means for their own health monitoring, diagnosis, and treatment. One avenue is to assess bioanalyzers that are miniaturizing lab technology.

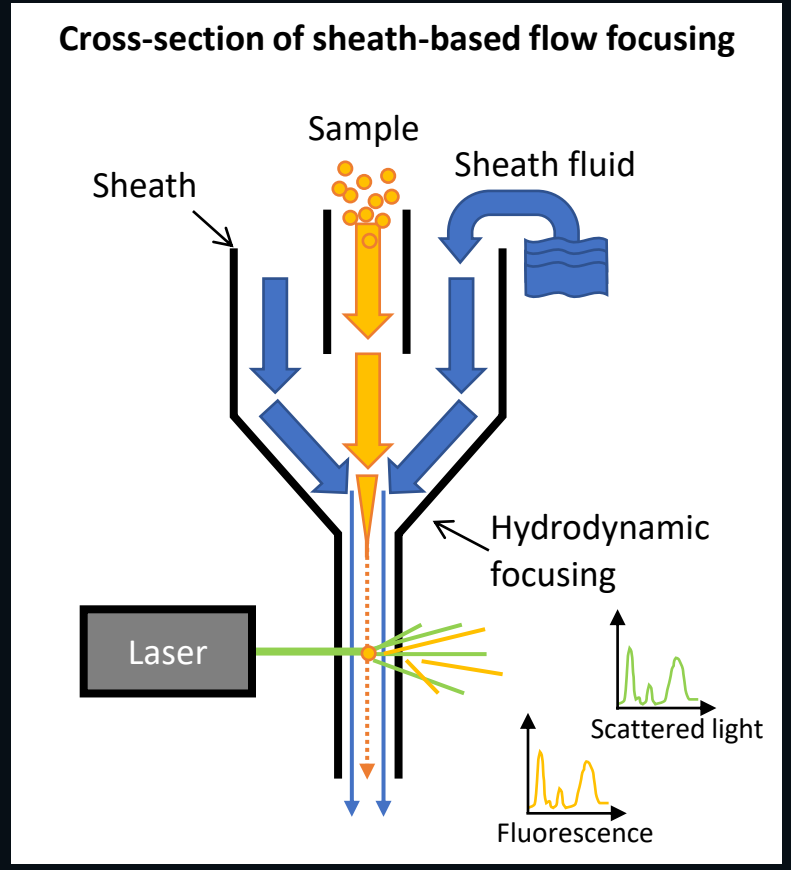
- rHEALTH LLC is developing the next generation of rHEALTH as a sample-to-answer solution for medical diagnosis. rHEALTH ONE is an interim version available now, functional as a sheath-based benchtop flow cytometer and test bed for the next generation of rHEALTH in development.

- In partnership with NASA Johnson Space Center (JSC) Immunology Lab and the Research Operations and Integration (ROI) element, ExMC conducted a technology demonstration on ISS of the rHEALTH ONE. This demonstration focused on the rHEALTH ONE's ability to function in microgravity, testing the underlying fluid dynamics of sheath-based hydrodynamic focusing in microgravity, and its performance compared to ground.

- Modifications were made to the rHEALTH ONE analyzer for operation in microgravity. For function, fluid management was key. In May 2022, European Space Agency astronaut Samantha Cristoforetti demonstrated the rHEALTH ONE aboard ISS for its sample loading, flow cytometry, and data collection capability in microgravity.

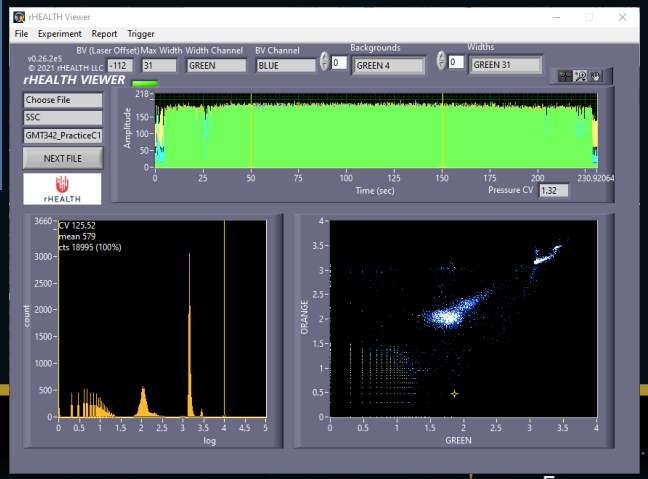
Flow Cytometry

- A method to analyze a liquid sample (blood, saliva, urine - even dissolved plant matter and mold) via tagging cells or biomarkers with fluorescent dyes and interrogating them with a laser for the light they emit and scatter
  - Fluidics focus the sample into a single file stream of cells (or particles) to pass through the laser(s)
  - Additional optics separate and focus the light onto individual detectors which convert the light into a digital signal
  - Each signal from each channel is processed to characterize and count the particles from the pulses produced
- This technique is widely used for medical lab testing and is involved in diagnosing numerous health conditions including those of interest to ExMC involving hematology, bone health, and radiation exposure



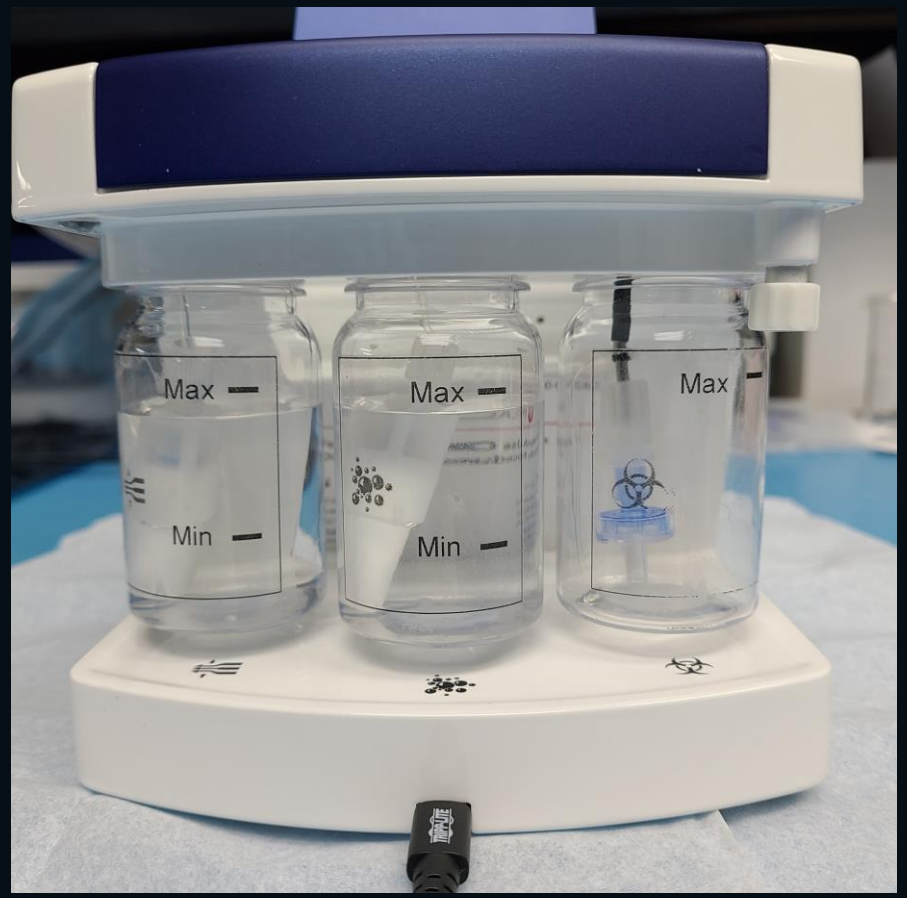
rHEALTH ONE

- Commercial–Off–The–Shelf (COTS)
- Interim version of rHEALTH technology for benchtop research, no automation of sample preparation or analysis yet
- Miniature sheath-based flow cytometer with fixed calibration (not adjustable by user)
- 2 lasers, 5 detectors
  - 2 scatter channels (FSC, SSC)
  - 3 color channels (BLUE, GREEN, ORANGE)
- 3 bottles dependent on gravity
  - Sheath fluid to flow the sample
  - Cleaning fluid to prevent contamination
  - Reservoir to collect the liquid waste
- 5uL-10uL sample
- Powered and controlled from laptop, software requires user processing to create Flow Cytometry Standard (FCS) file and provides histogram and scatterplot with some gating



Modifications

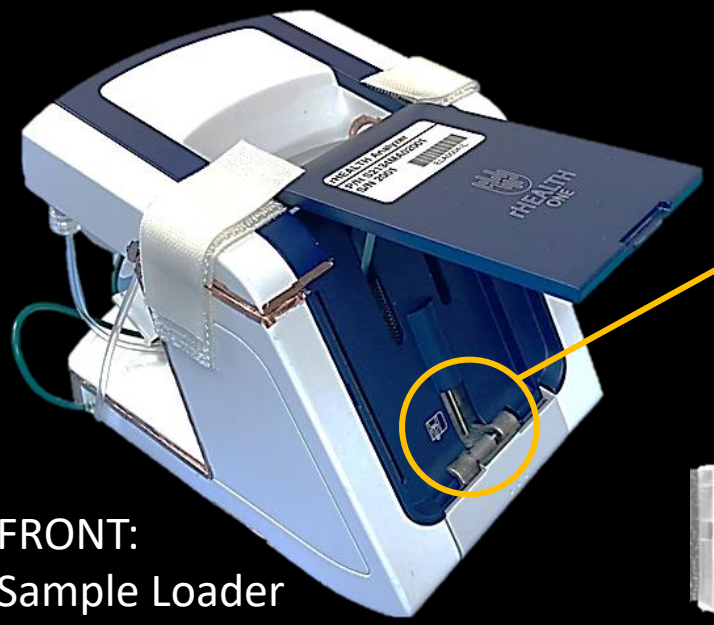
Before



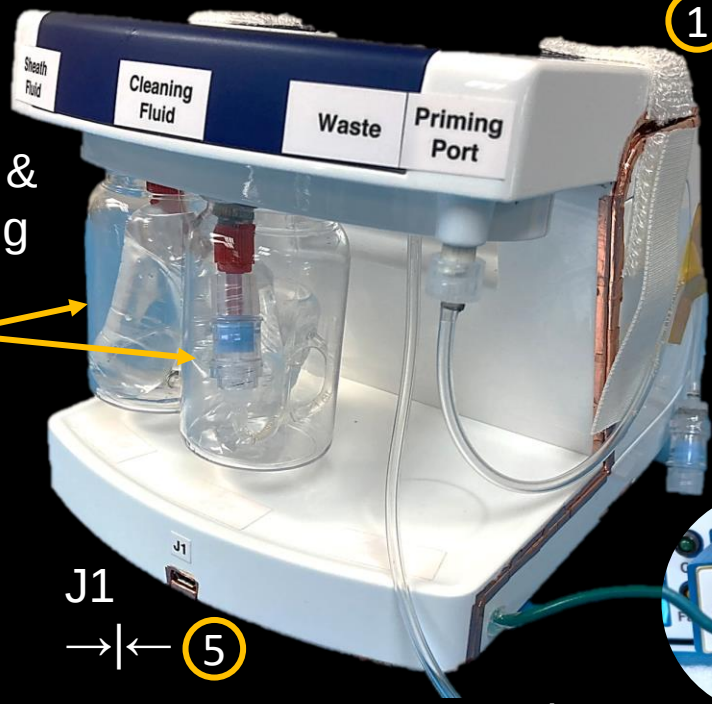
After

Flight Hardware

1 rHEALTH Analyzer



2 rHEALTH Sample Cup



1 BACK: Bottle Area

Sheath & Cleaning →|← 3 4 x 2

Priming Port Line →|← 7

J1 →|← 5

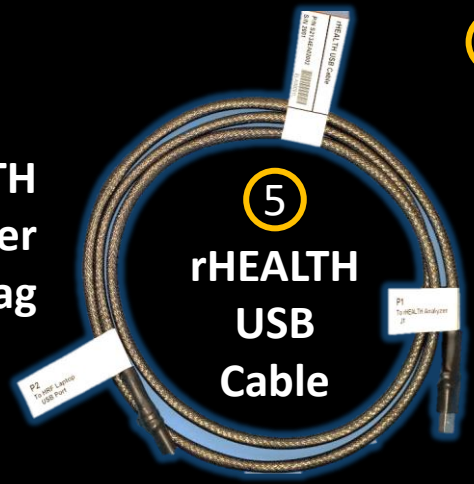
Waste Line →|← 6

Grounding Cable →|←

3 rHEALTH Bottle

4 rHEALTH Water Bag

5 rHEALTH USB Cable



6 rHEALTH Waste Bag (empty)

7 rHEALTH 3mL Syringe

8 rHEALTH Reservoir Bag (filled, water)

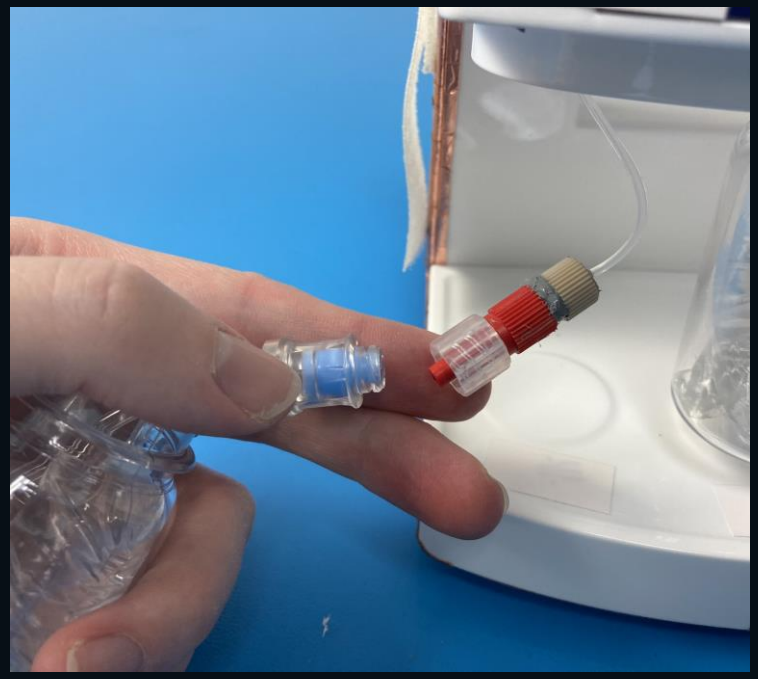
9 rHEALTH 30mL Syringe

10 rHEALTH Sample

④ rHEALTH Water Bag

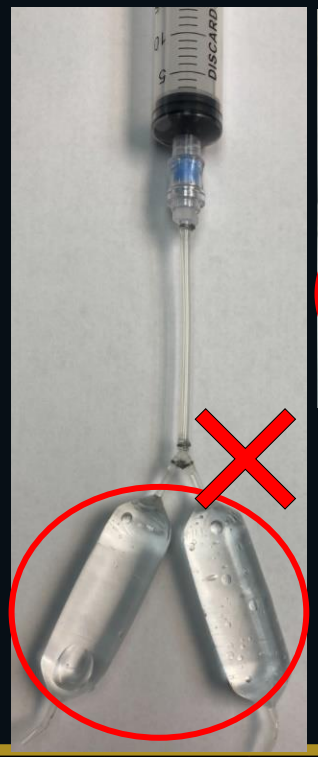
Water Bag Design

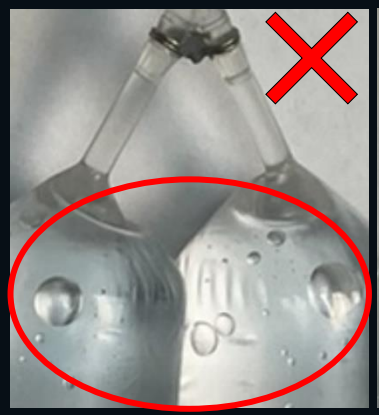
- Durable medical balloons only 0.014mm thick to collapse without resistance under 1psi air pressure
- Blue self-sealing luer valve contains water and seals out air
- Easy connect/ disconnect with luer lock fittings and syringes



Preventing Air Bubbles

- Air bubbles can obscure particles (poor data) or entirely disrupt flow (no data)
- Utilizing human centrifuge techniques and priming methods to separate air/water and remove bubbles

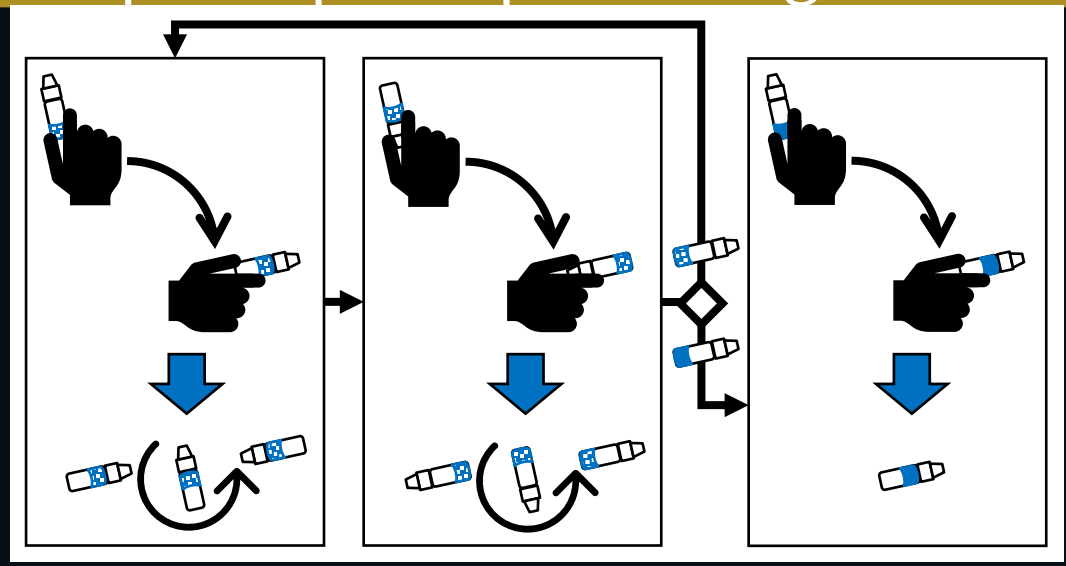




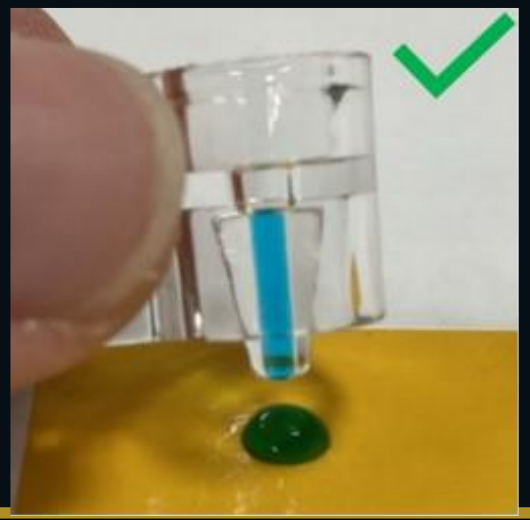
✓ Air separated to top through nunchuck swing and

✓ pulled into syringe

Whip & Flip sample mixing



✓ Filled, No bubbles



rHEALTH Ops

- 4 control samples (A,B,C,D)
  - Made and verified by NASA JSC Immunology Lab
  - TOX 0 polystyrene microbeads
- 3 test points for each sample
- Water for the sheath and cleaning fluid
- Flight Hardware tested on ground, then launched dry
- On-orbit crew operations across 2 days with remote guidance from ground team
- Results graphed in rHEALTH software and compared to ground

ESA Astronaut Samantha Cristoforetti operates the rHEALTH analyzer on the International Space Station in May 2022. *Credits: NASA*

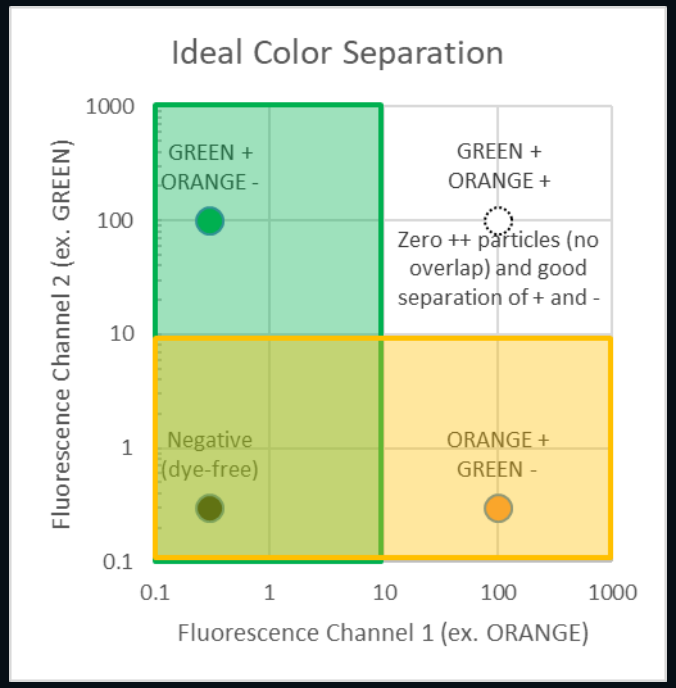
Operational Steps

- rHEALTH Water Bags are inserted in rHEALTH Bottles, filled with water, and connected to the rHEALTH Analyzer
- rHEALTH Analyzer is powered on, pressurizing the rHEALTH Bottles to push water from the rHEALTH Water Bags through the Analyzer to the rHEALTH Waste Bag
- rHEALTH Analyzer is primed to remove air
- rHEALTH Sample is mixed, wicked into rHEALTH Sample Cup, and placed in rHEALTH Analyzer
- Sample Run is started. Water pushes the sample through the optical block where the particles will be drawn single file past the laser then out the waste line. Signal is displayed on 5 graphs in the software.
- Sample Run is stopped. File automatically saves, rHEALTH Sample Cup is removed and short prime is run before repeating for next sample.
- rHEALTH Water Bags are refilled when near empty

Results

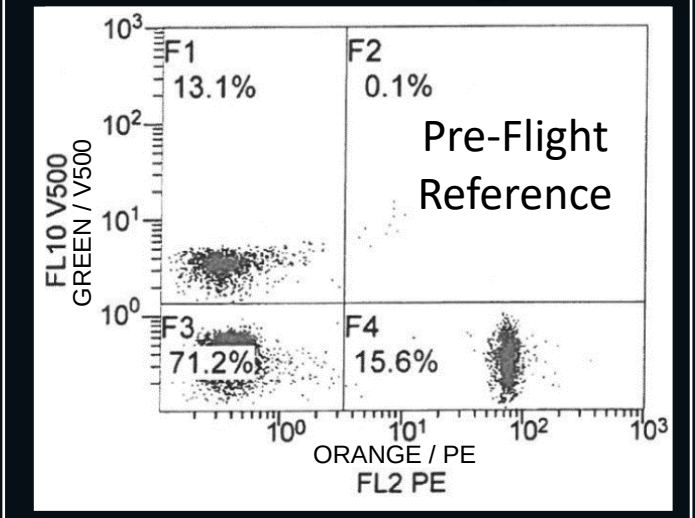
Selected graphs from the best runs to represent each sample

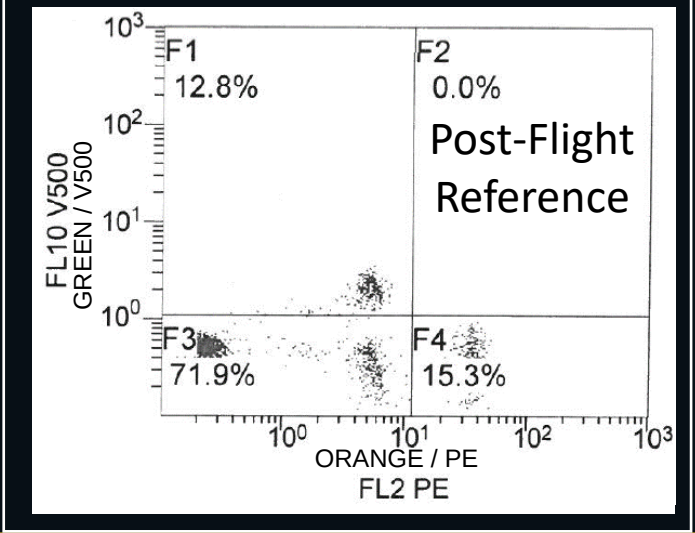
Sample A - Spectral Overlap

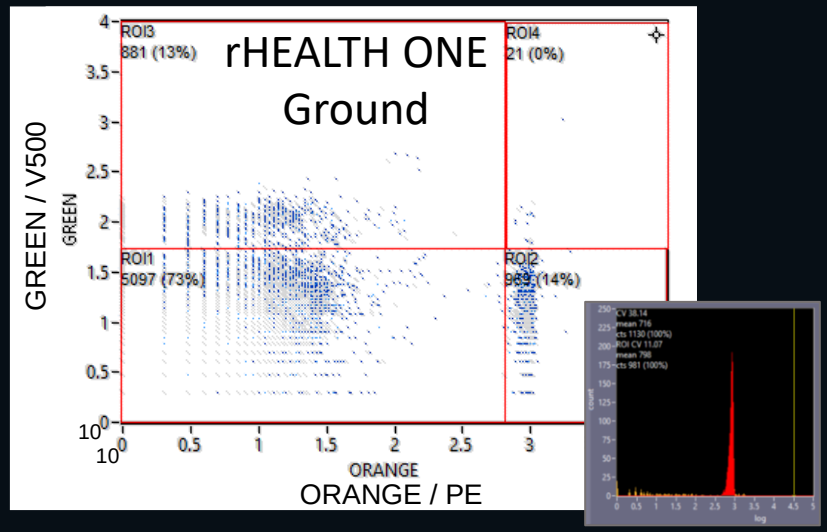


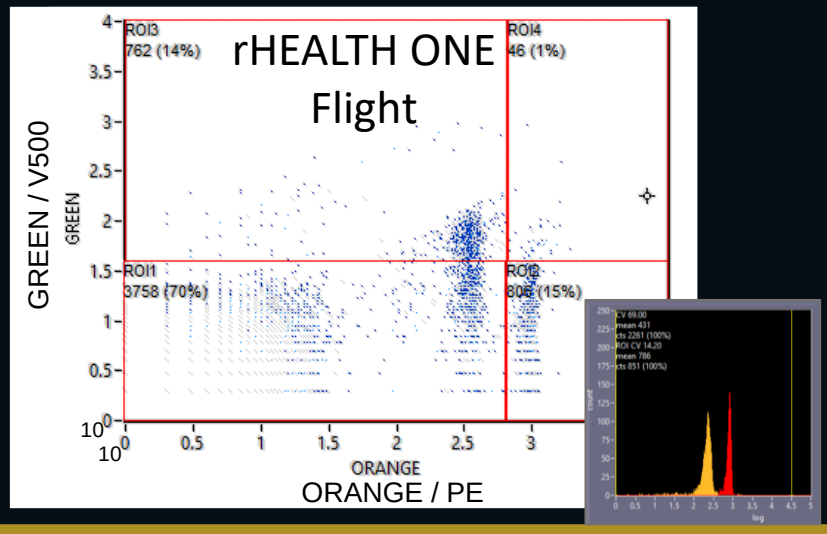
Tests the analyzer's ability to sort a mixed sample into its separate colors on the light spectrum

NASA JSC Immunology Lab

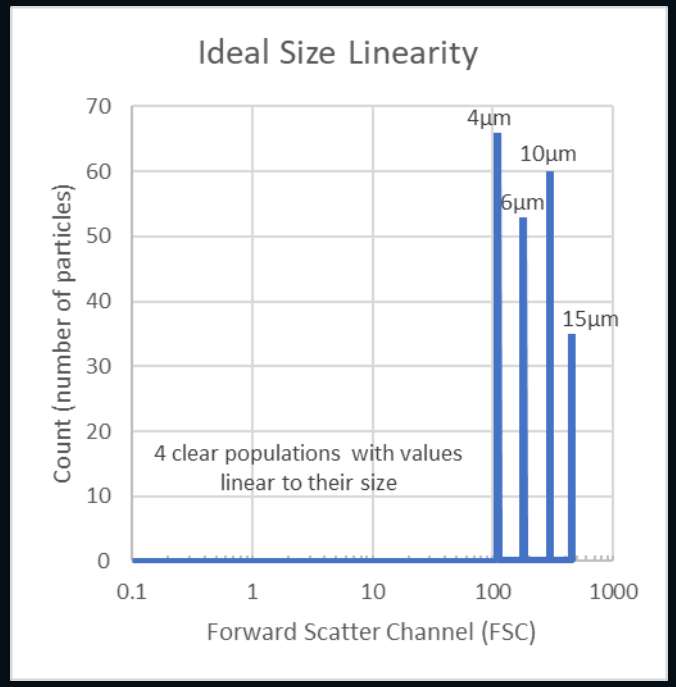




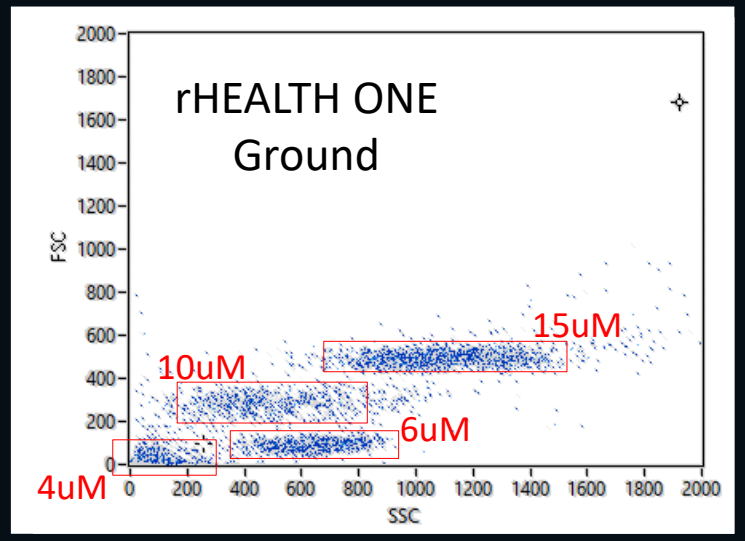


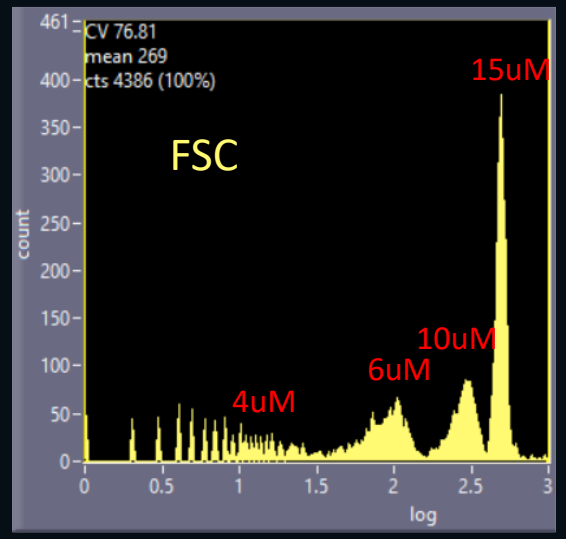


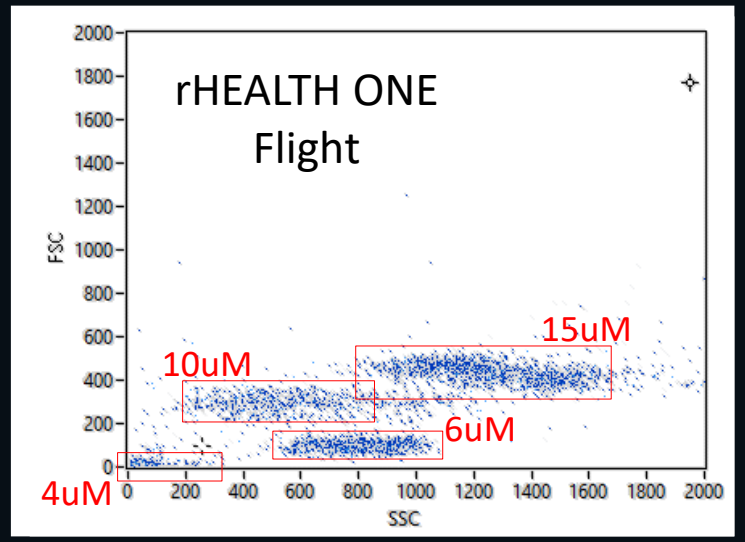
Sample B – Particle Size

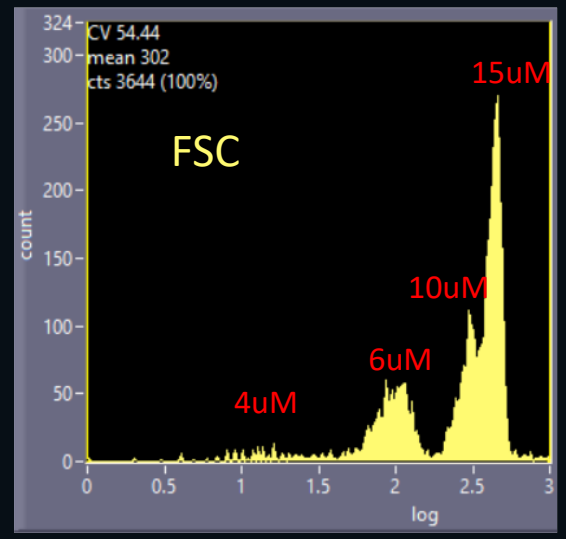


Tests the analyzer's ability to resolve different sizes of particles using FSC

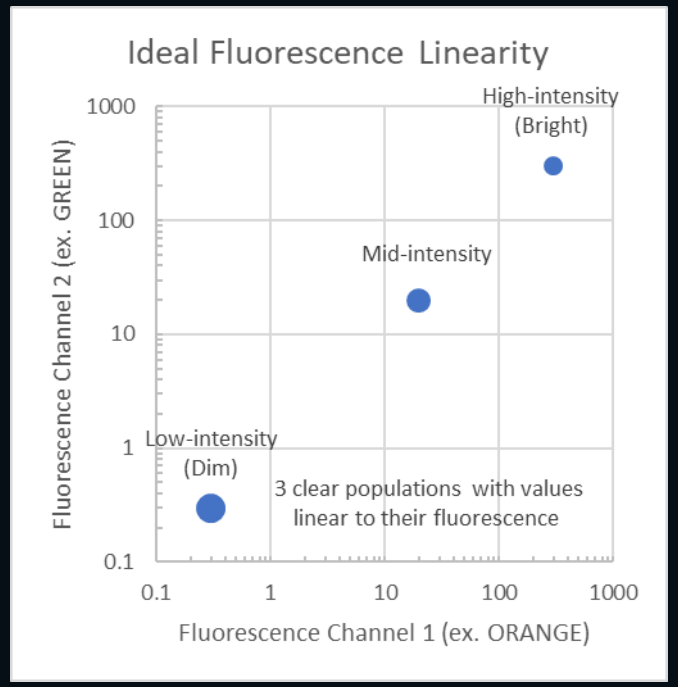




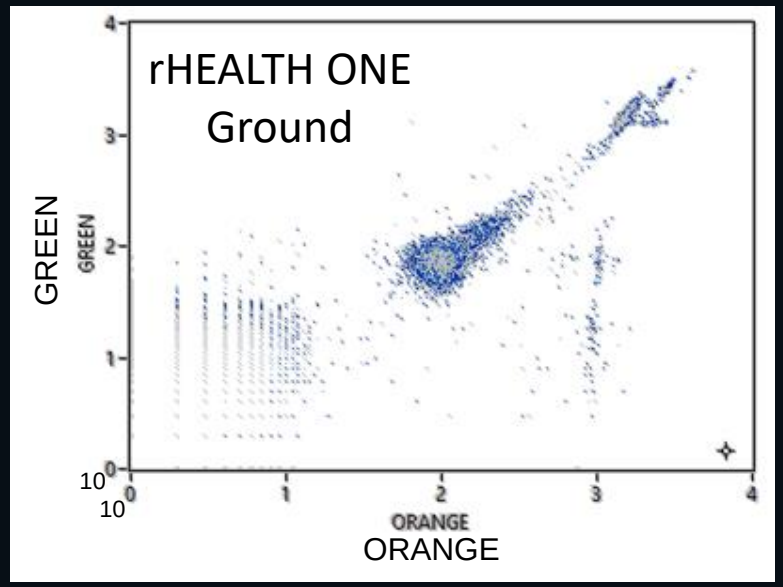


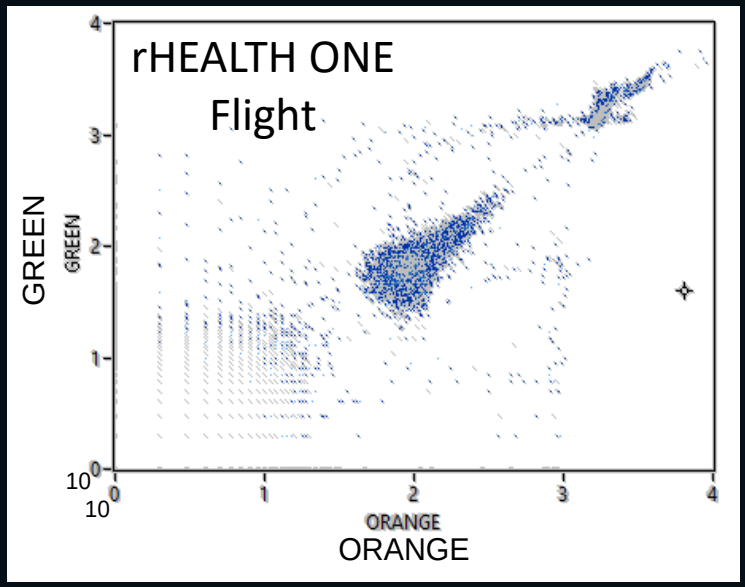


Sample C – Linearity

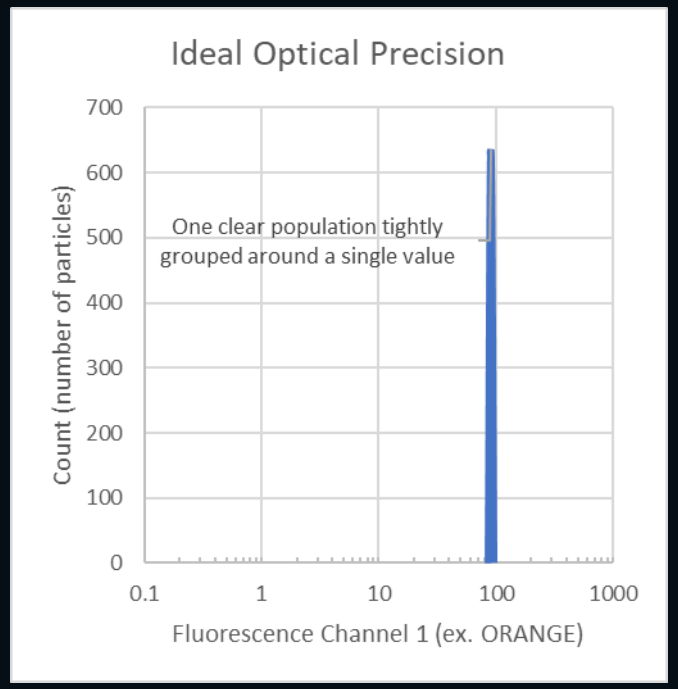


Tests the analyzer's ability to resolve different intensities of fluorescent light



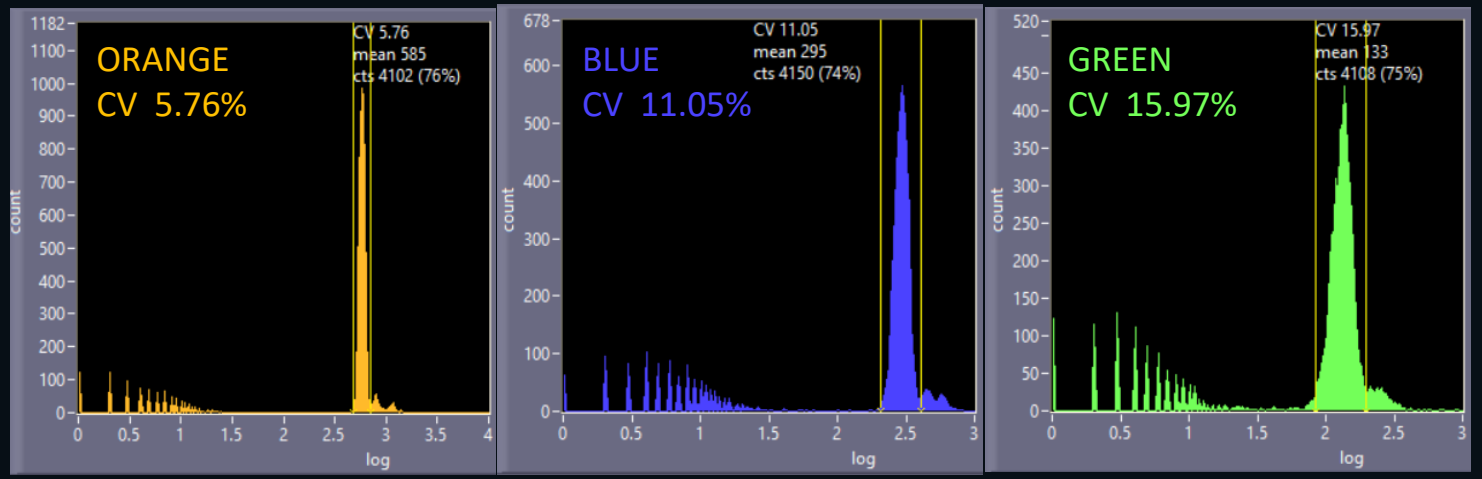


Sample D – Optical Precision

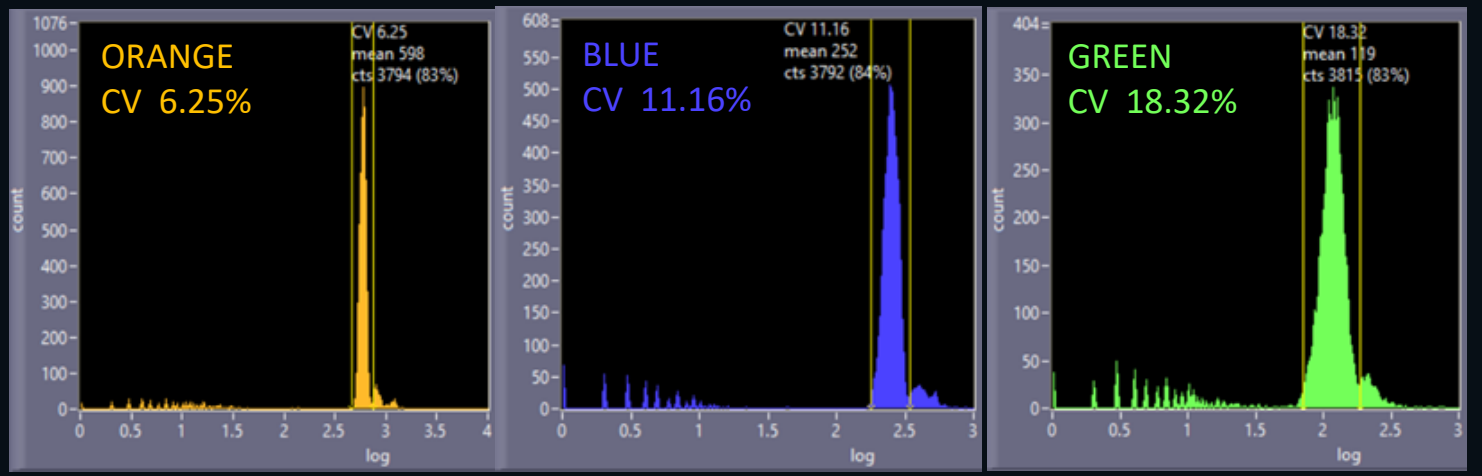


Tests the analyzer's optics are aligned and functioning optimally with low coefficient of variance (CV)

rHEALTH ONE Ground



rHEALTH ONE Flight



Mission Success Criteria Met:

- ✓ Signal was received on all photodetector channels
- ✓ 3 test points were completed for each sample (and more!)
- ✓ Each test point was ≥ 3 minutes or contained at least 1 minute of constant particle events equivalent to the minimum sample size (5uL)
- ✓ **Hydrodynamic focusing was demonstrated** (particles detected)
- ✓ The minimum number of expected histogram peaks (particle populations) for each sample were present
- ✓ Flight Data comparable to Ground Data – performance in microgravity matches ground
  - ✓ Sample A anomaly confirmed by NASA JSC Immunology Lab on returned samples post-flight, rHEALTH ONE correctly detected change in sample
  - ✓ Samples B, C, D

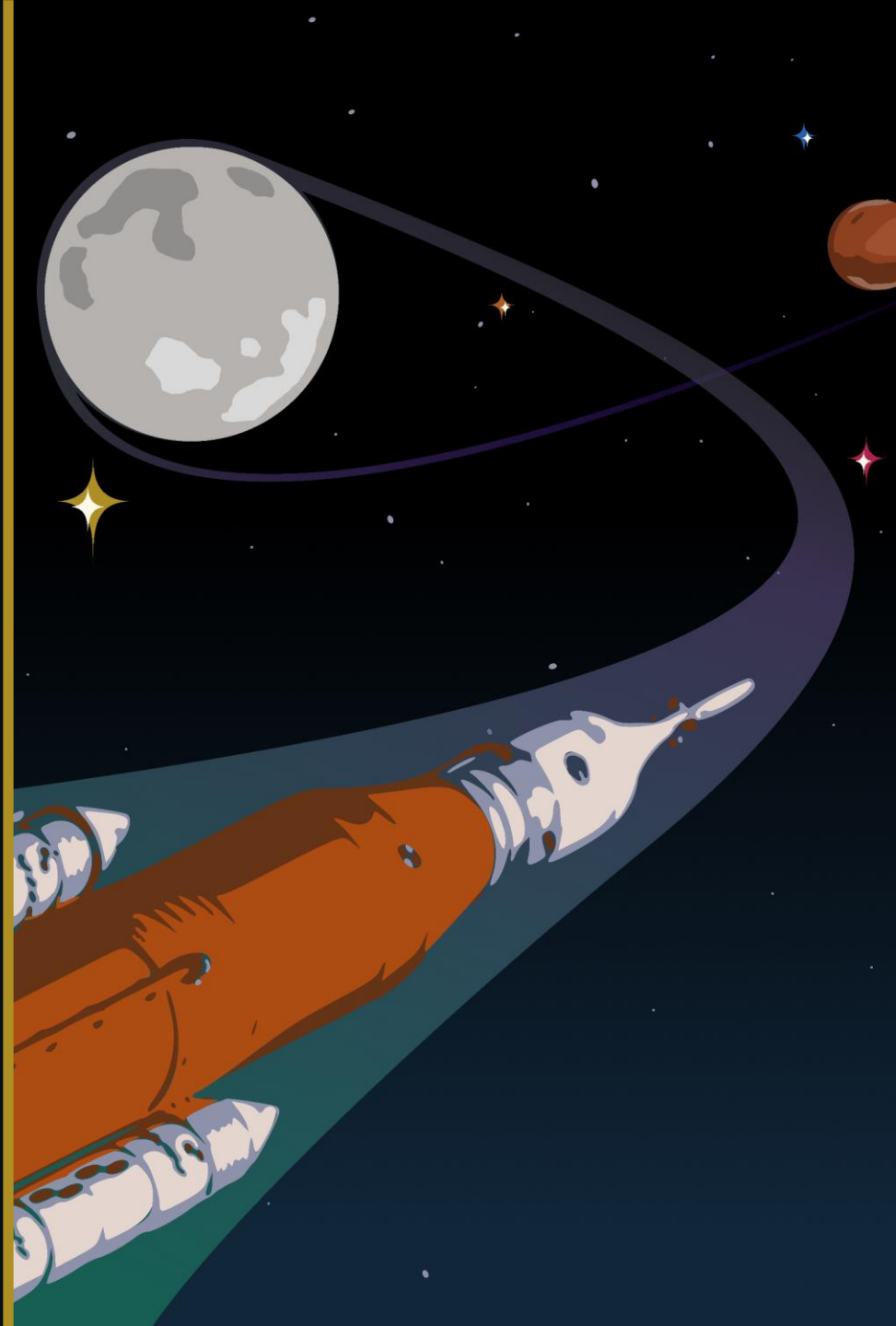
Summary

- Hydrodynamic focusing was accomplished, demonstrating that a sheath-based flow cytometry design is viable in microgravity
- With the post-flight verification of the anomaly in Sample A, the rHEALTH ONE ISS demonstration successfully met all mission success criteria, collecting sufficient data of good quality to show that flight performance in microgravity matched ground performance
- Overall performance as a flow cytometer was constrained by the fixed calibration and noise on the channels. Even with tightly controlled procedures the fluidics design introduces and traps air, disrupting good flow at any time between sample runs. Future experiments would benefit from a different fluidics design, such as the one already developed for the next generation of rHEALTH.
- Increased noise in Flight was also seen Post-Flight (cause not attributed to microgravity but to launch/return and/or priming/flow)

Acknowledgements

- ZIN Technologies
- NASA JSC Immunology Lab
- rHEALTH LLC
- NASA ROI with special thanks to our integrators Scott Humbert and Melissa Hennigan
- ESA astronaut Samantha Cristoforetti

This work was funded by the NASA Glenn Research Center for ExMC through the SpaceDOC II contract (NNC14CA02C DO-213)



Backup Slides

Subtitle

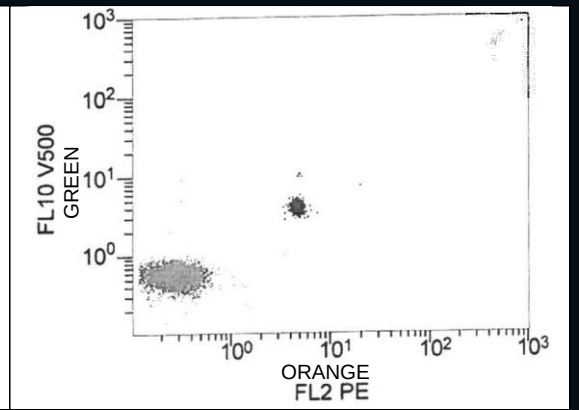
Flight Samples

Sample Letter for Flight	Control Solution P/N (Manufacturer)	Description	Feature Tested	Applicable Channel(s)	Feature in Raw Data
A	01-1111-42 (Thermo Fisher) 75-0149 T100 85-0038 T100 50-0199-T100 (Tonbo Biosciences)	OneComp eBeads (~4 µm) stained with fluorophore-conjugated antibodies: CD14 V450 CD3 V500 CD19 PE	Spectral Overlap	BLUE GREEN ORANGE (respectively to dyes)	Target: 1 distinct amplitude, peaks not repeated on other color channels
B	PPS-6K (Spherotech)	Particle Size Standard Kit - using 4, 6, 10, and 15 µm	Particle size resolution	FSC (SSC)	Target: 4 distinct amplitudes
C	RQC-30-5 (Spherotech)	Rainbow QC Calibration Particles, 3 peaks of intensity, all colors	Fluorescence resolution: sensitivity and linearity of the instrument	BLUE GREEN ORANGE	Target: 3 distinct amplitudes
D	A69184 (RUO) or A63492 (IVD) (Beckman Coulter)	Flow-Set Pro Fluorospheres, uniform size and intensity	Optical Precision, alignment	All	Target: Low CV

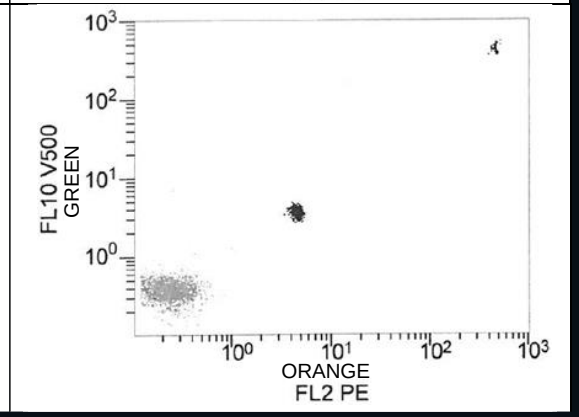
NASA JSC Immunology Lab Graphs

Sample B

Gallios
2022-10-29
Pre-flight
(master lot of flight samples)

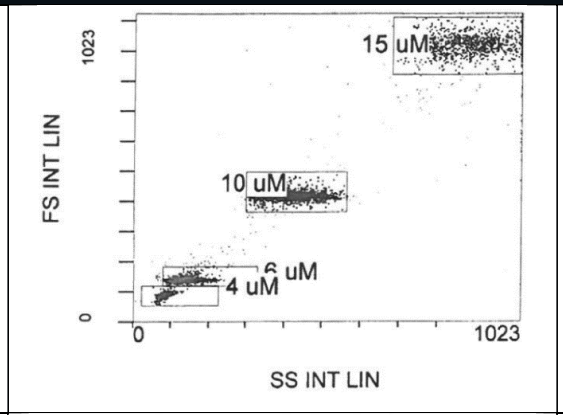


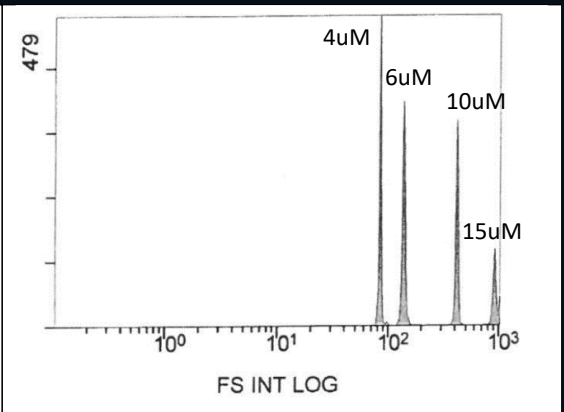
Gallios
2022-08-26
Post-flight, Returned flight sample kit SN 2002



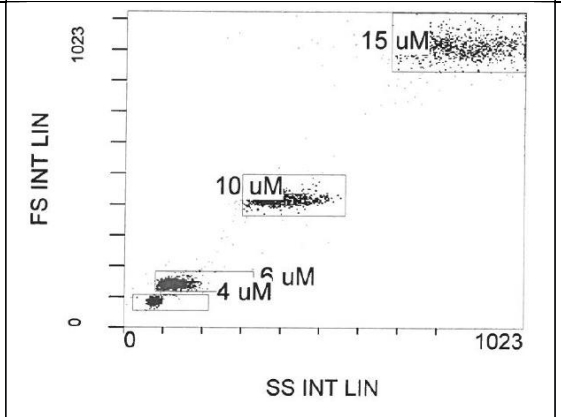
Sample C

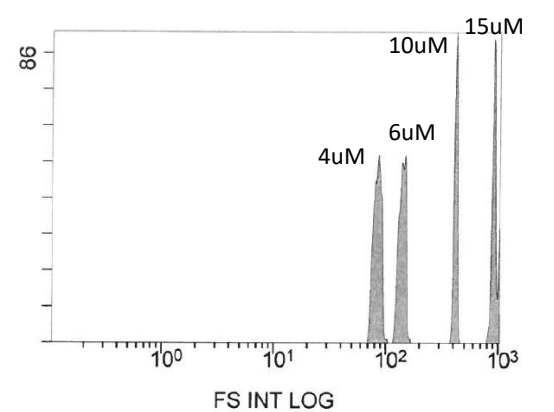
Gallios
2022-10-29
Pre-flight
(master lot of flight samples)





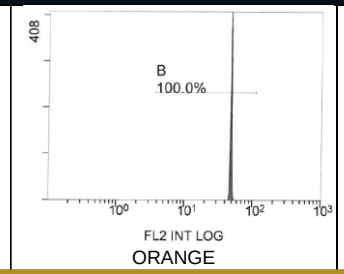
Gallios
2022-08-26
Post-flight, Returned flight sample kit SN 2001

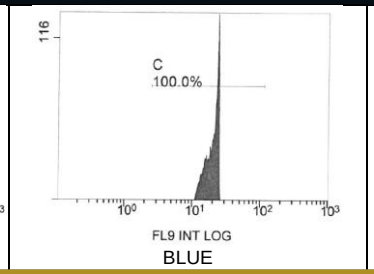


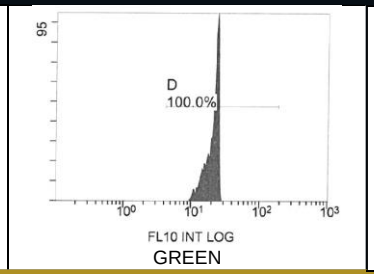


Sample D

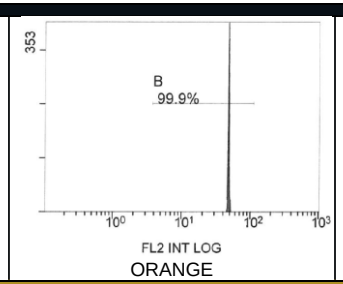
Gallios
2022-02-04
Pre-flight
Unkitted flight sample

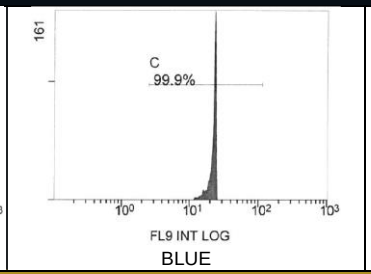


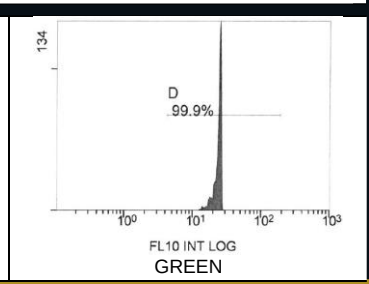




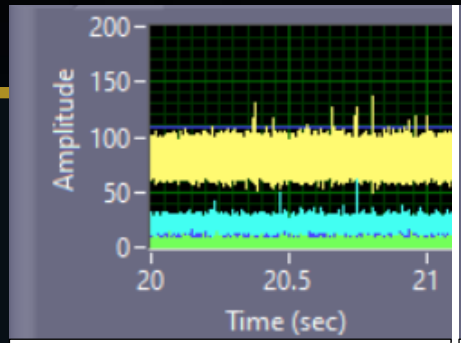
Gallios
2022-08-26
Post-flight, Returned flight sample kit SN 2001



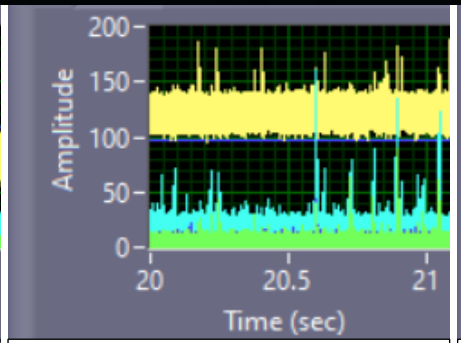




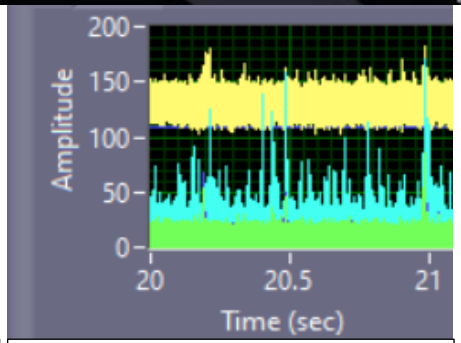
Raw Data Graphs, Startup Noise on Flight Hardware



Ground 2021-12-08 GMT342_Blank1

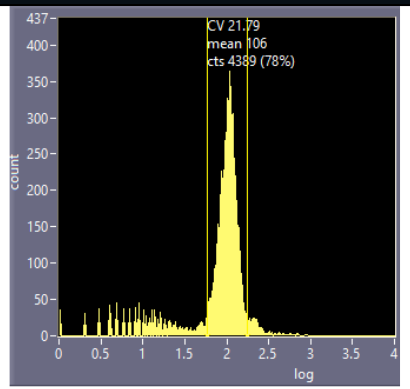
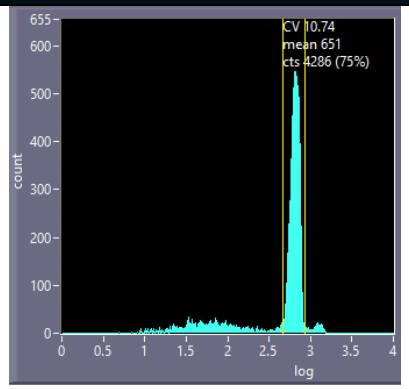
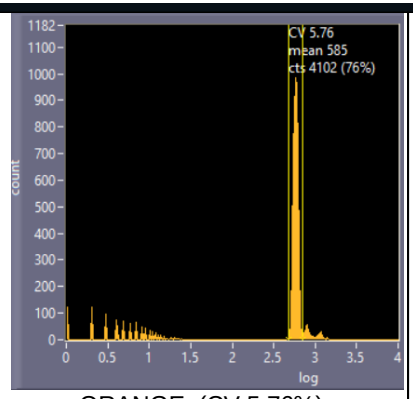
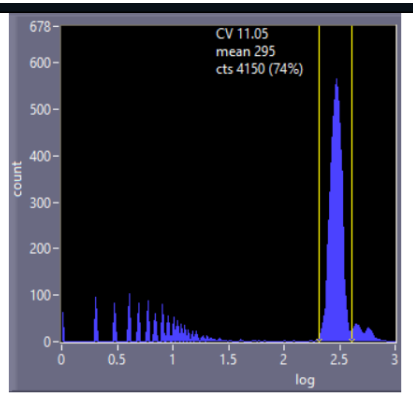
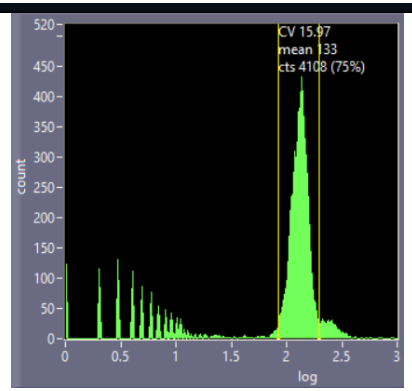
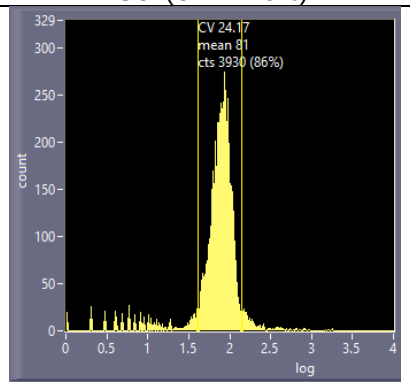
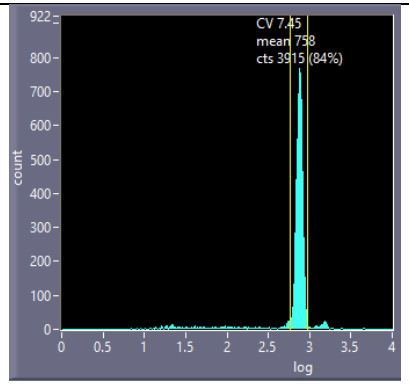
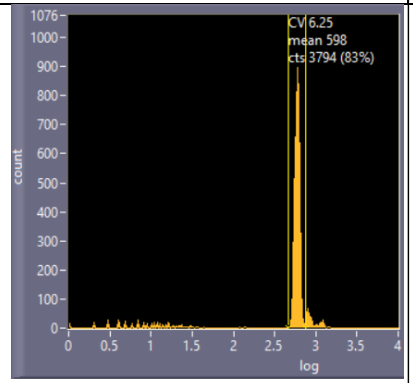
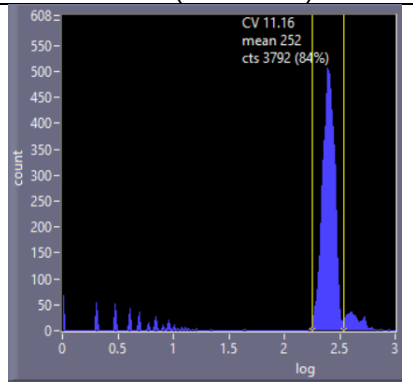
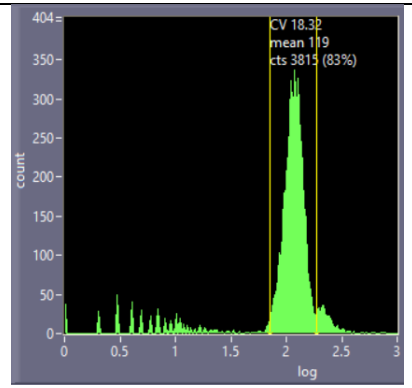


Flight 2021-05-13 GMT133_Blank1



Post-flight 2022-11-14 GMT318a_Blank1

All channels for Sample D

rHEALTH ONE 2021-12-09 **Pre-flight**, Run D2, Unkitted flight sample	 FSC (CV 21.79%)	 SSC (CV 10.74%)	 ORANGE (CV 5.76%)	 BLUE (CV 11.05%)	 GREEN (CV 15.97%)
rHEALTH ONE 2022-05-16, **Flight**, Run K1D1, Flight sample kit SN 2001	 FSC (CV 24.17%)	 SSC (CV 7.45%)	 ORANGE (CV 6.25%)	 BLUE (CV 11.16%)	 GREEN (CV 18.32%)