

# ISS Figures and Tables

Single Drop Cytometry Onboard the International Space Station

January 28, 2024



Featured Image

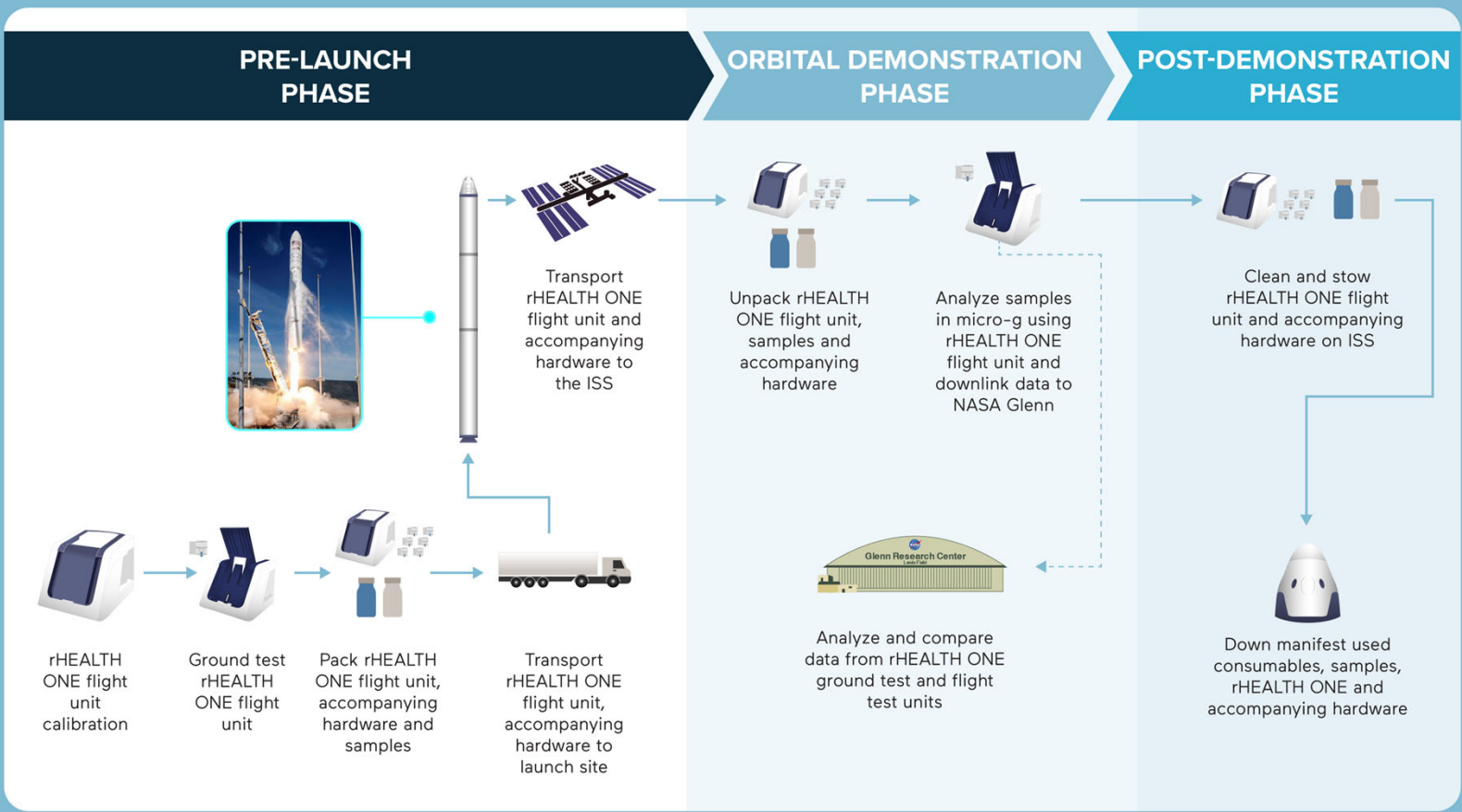


Figure 1

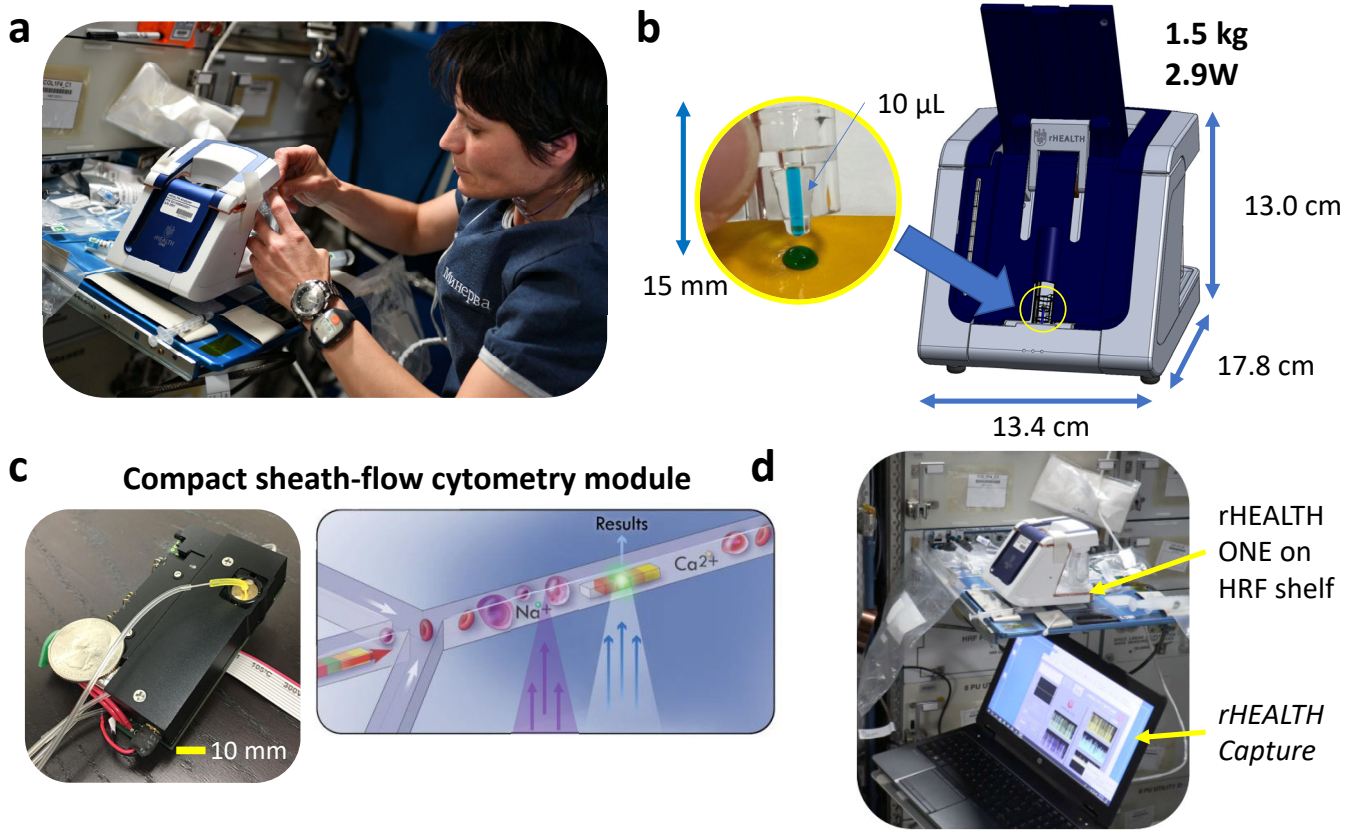


Figure 2



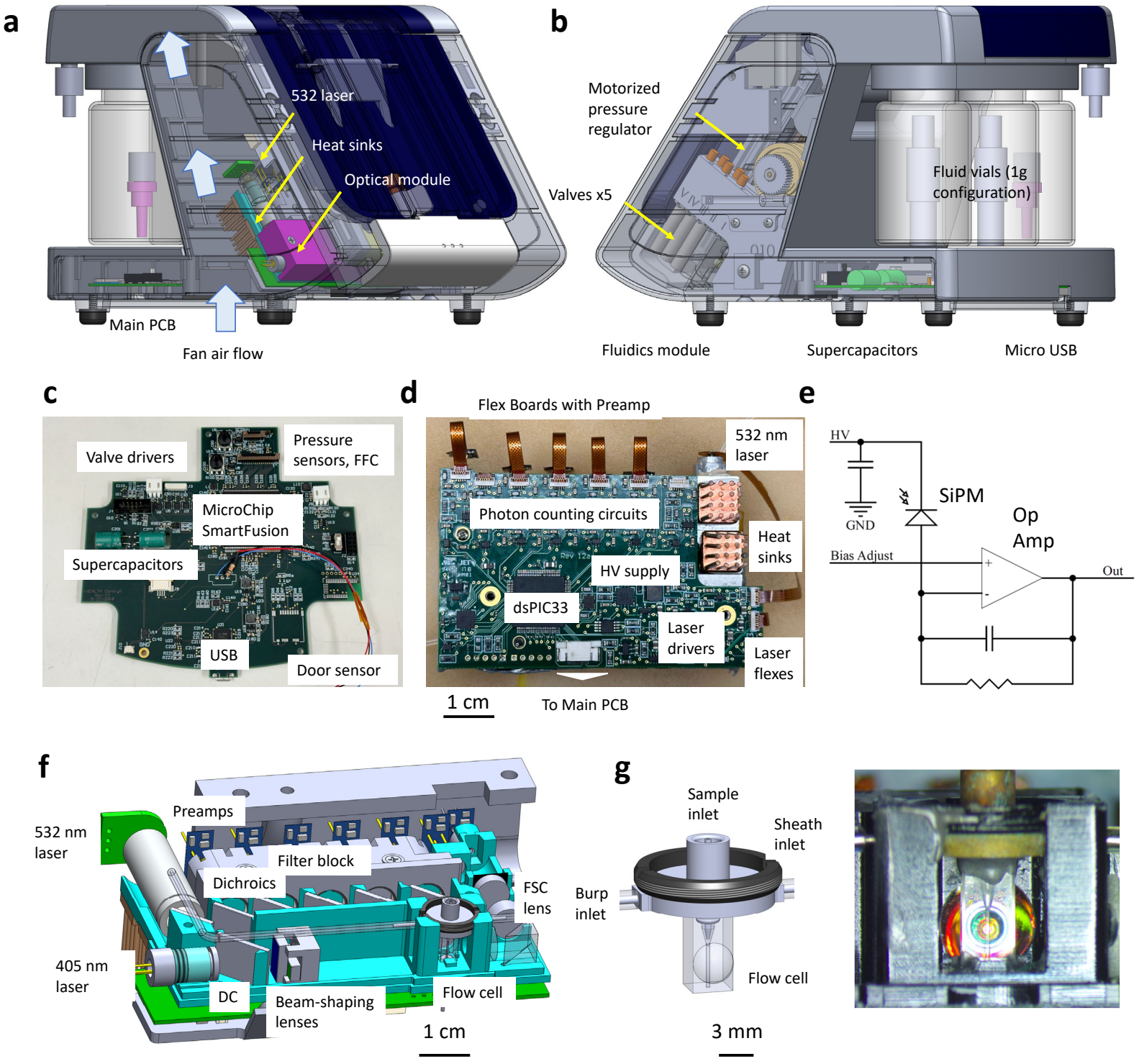


Figure 3

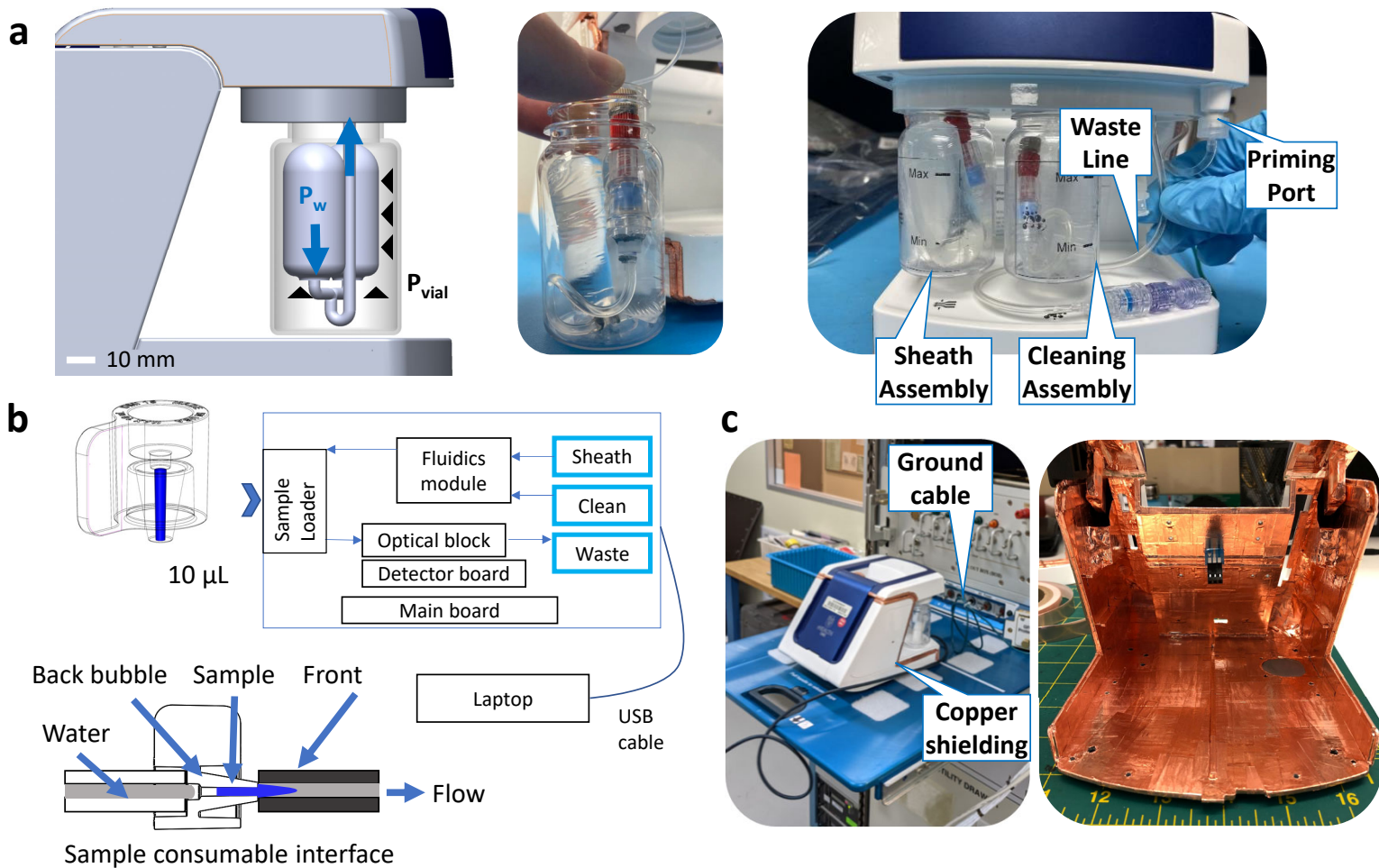


Figure 4

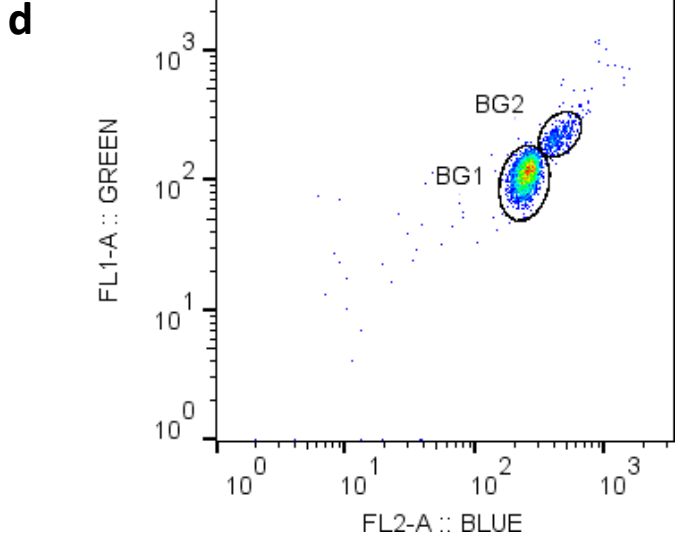
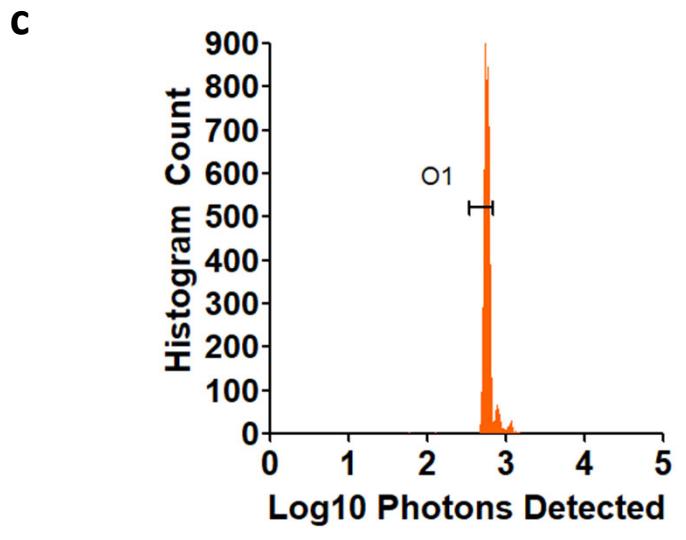
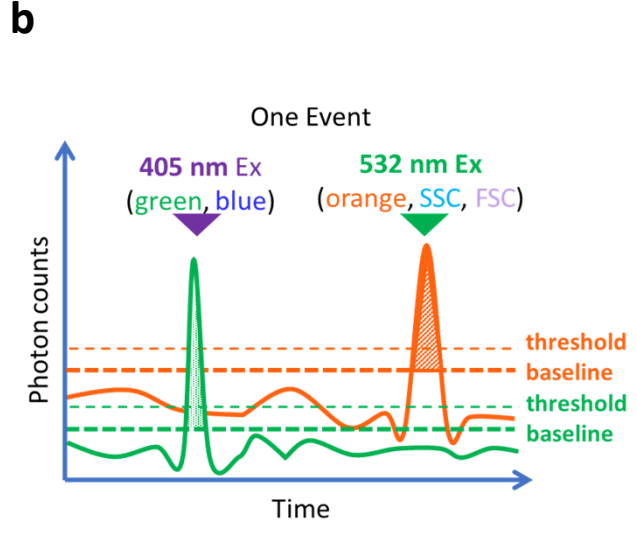
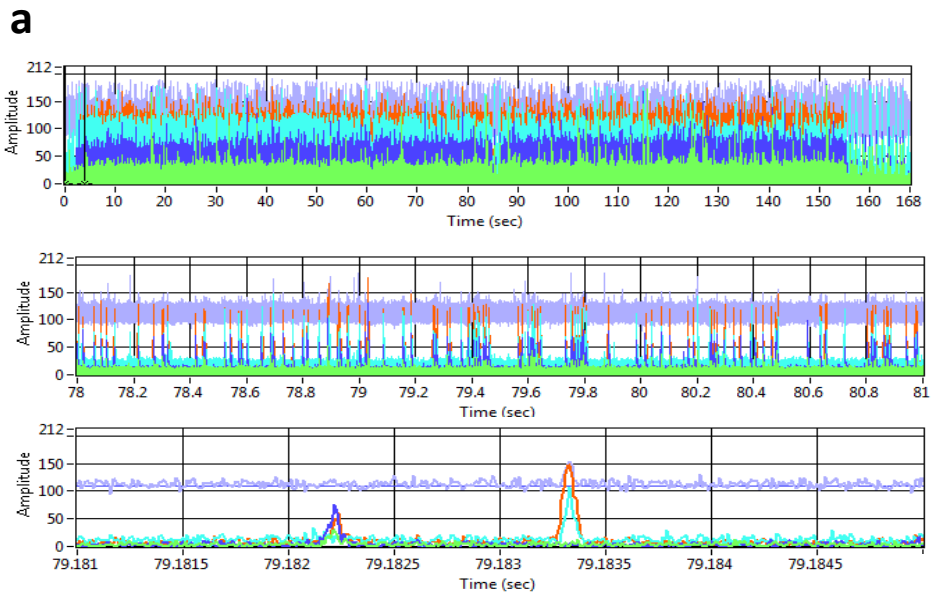
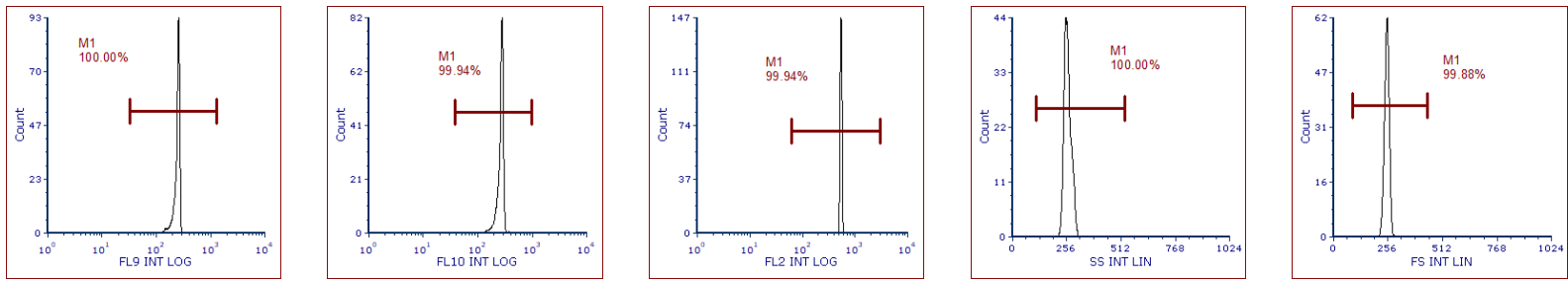


Figure 5

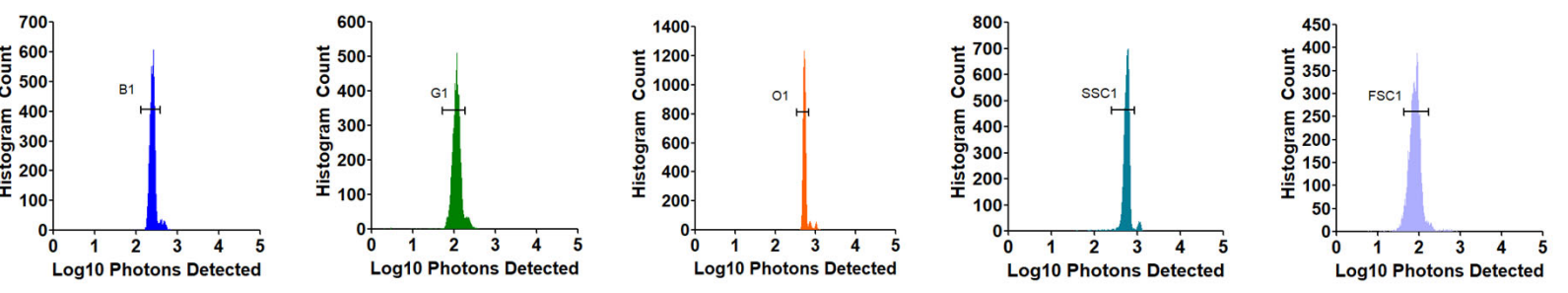
**a**

Preflight Ground Data - Gallios



**b**

Preflight Ground Data - rHEALTH ONE



**c**

On-Orbit Flight Data - rHEALTH ONE

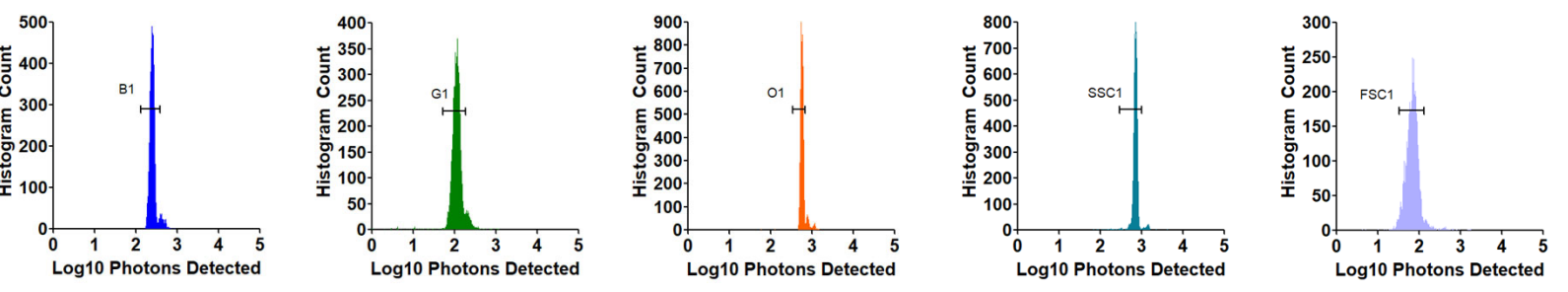
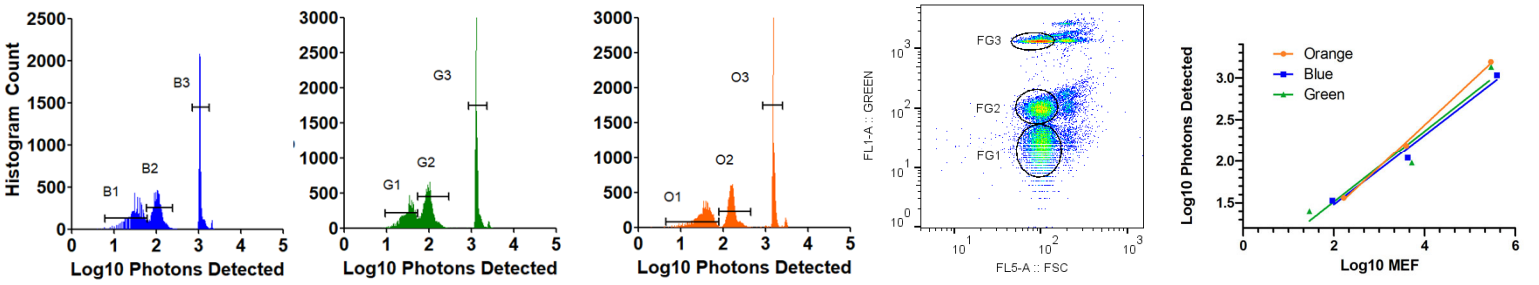


Figure 6



**a**

## Preflight Ground Data - rHEALTH ONE

**b**

## On-Orbit Flight Data - rHEALTH ONE

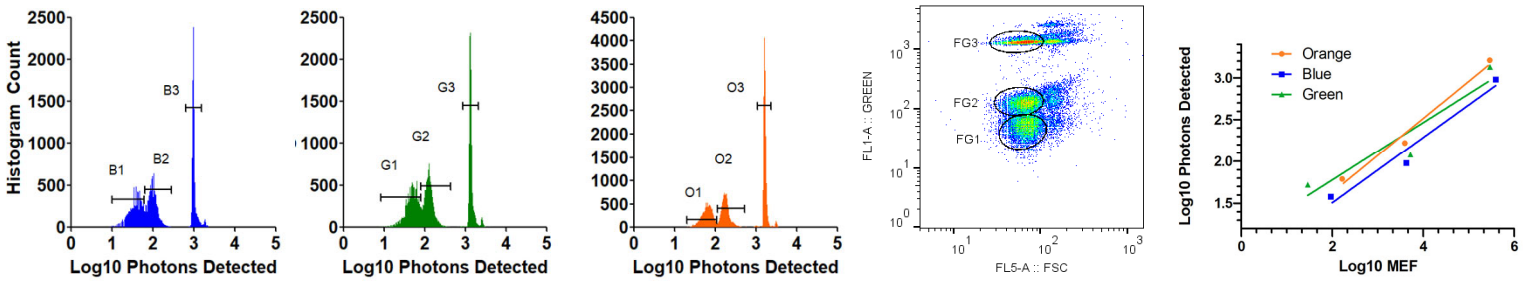


Figure 7

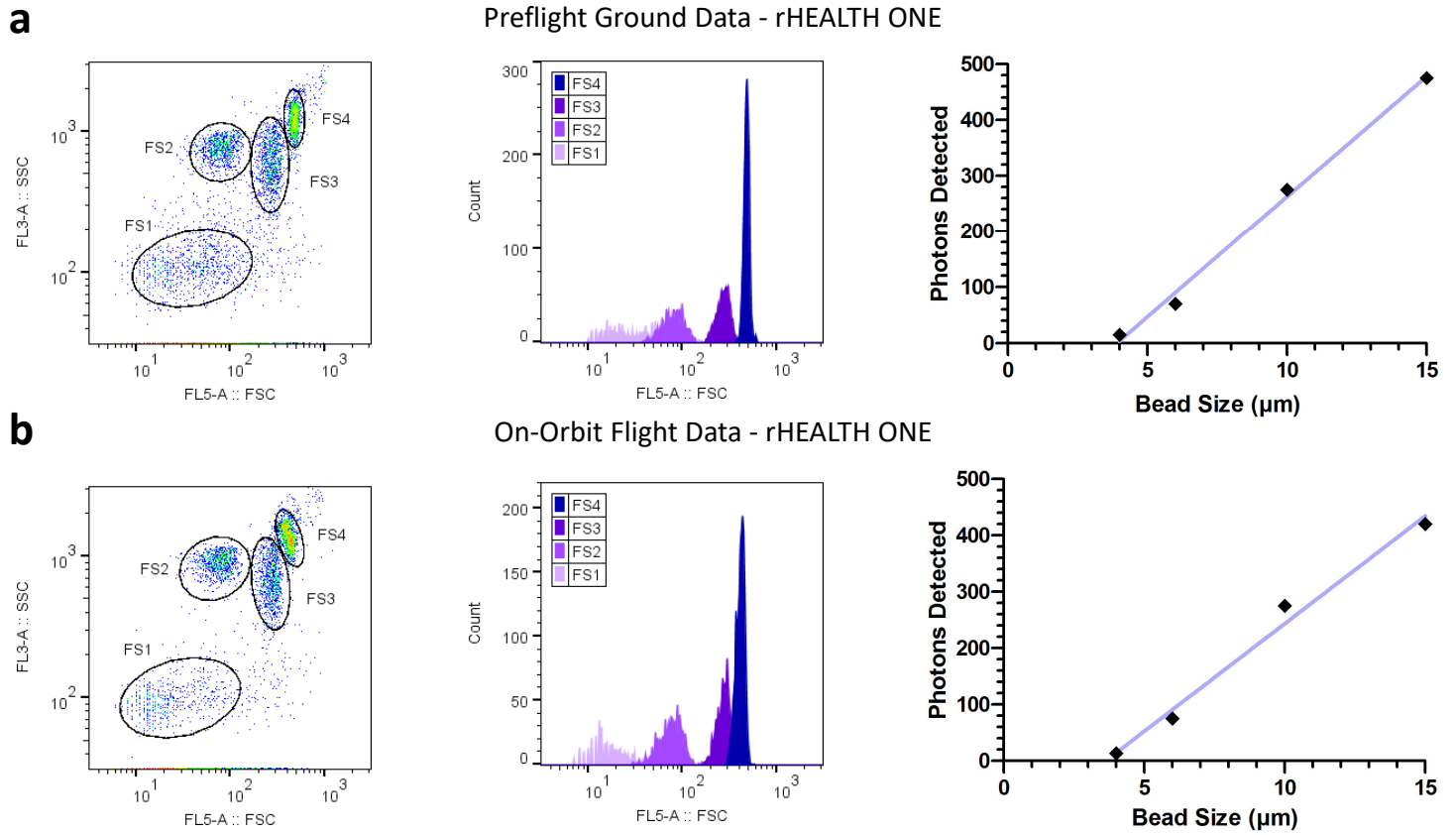


Figure 8

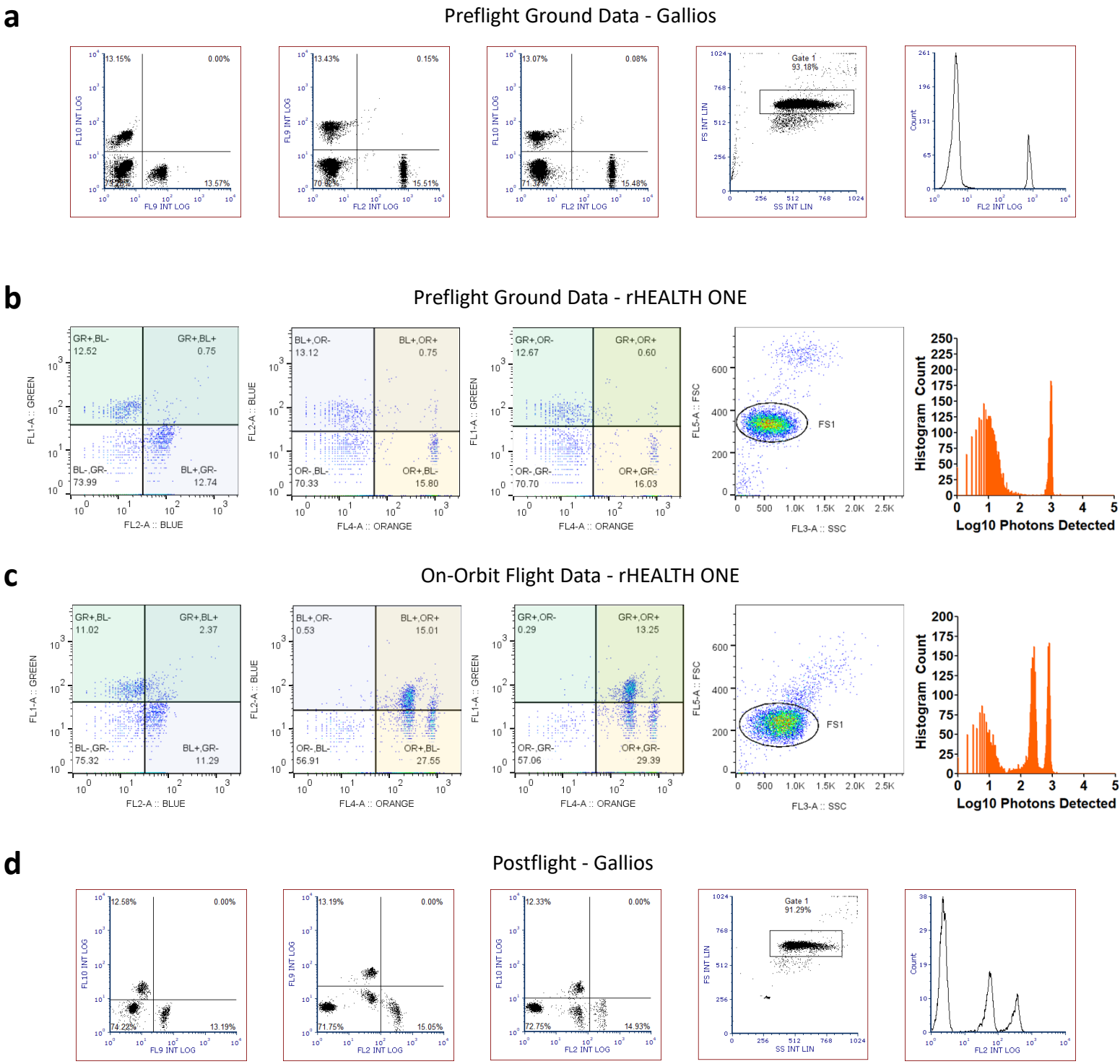


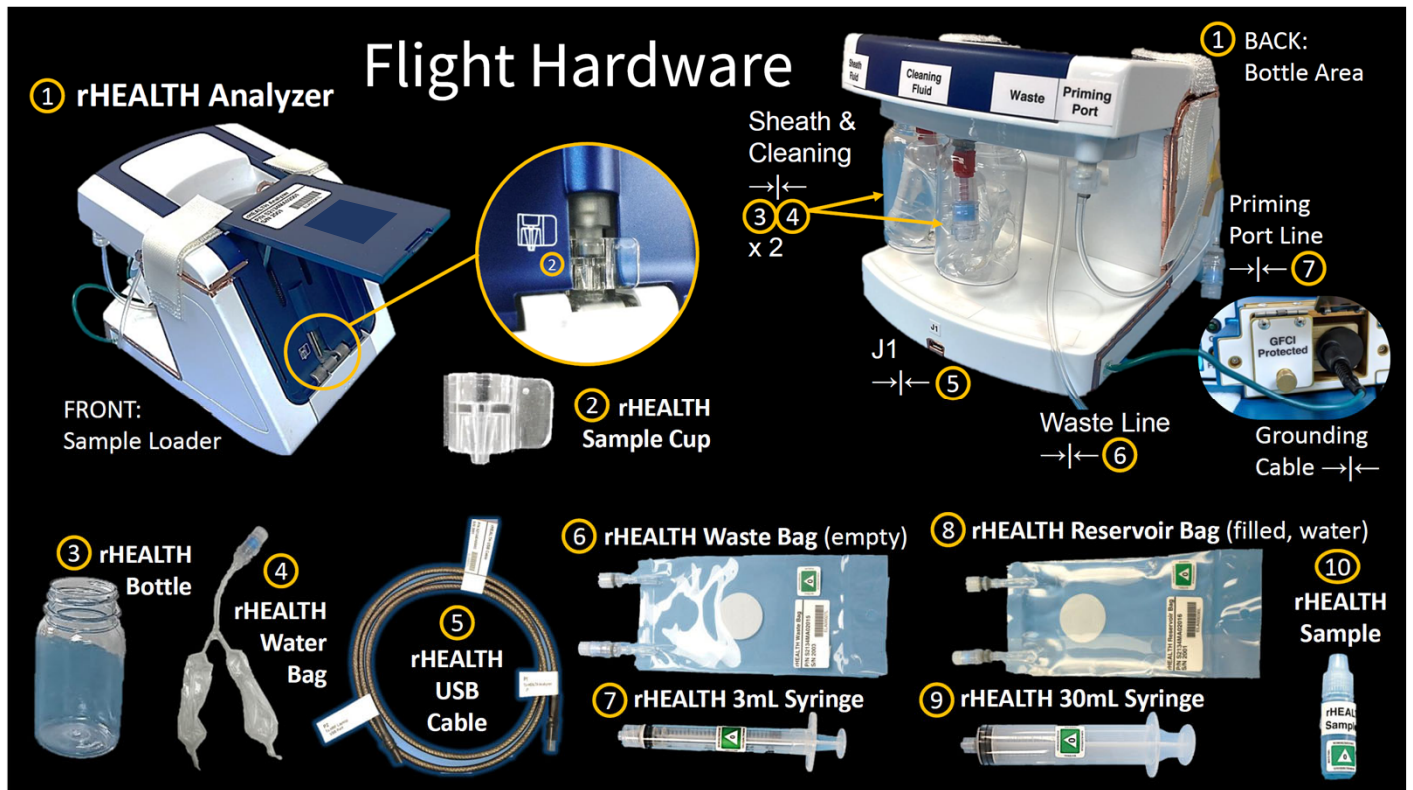
Figure 9

<b>Instrument Resource Utilization Comparison</b>			
<b>Metric</b>	<b>rHEALTH ONE</b>	<b>Gallios</b>	<b>Fold-Reduction</b>
<b>Volume</b>	2,808 cm <sup>3</sup>	513,650 cm <sup>3</sup>	183x
<b>Power</b>	2.9W	1,500W	517x
<b>Mass</b>	1.5 kg	138 kg	92x
<b>Sheath Volume</b>	0.060 L	10 L	166x

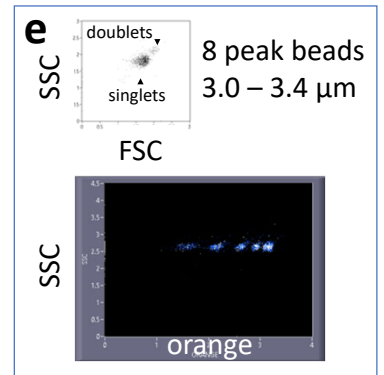
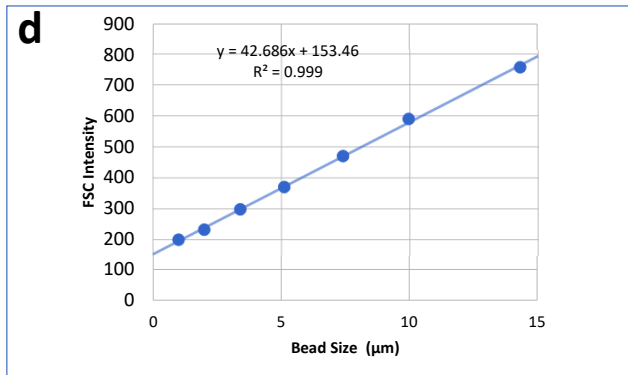
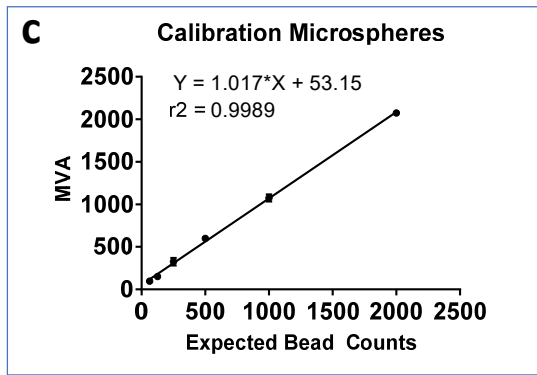
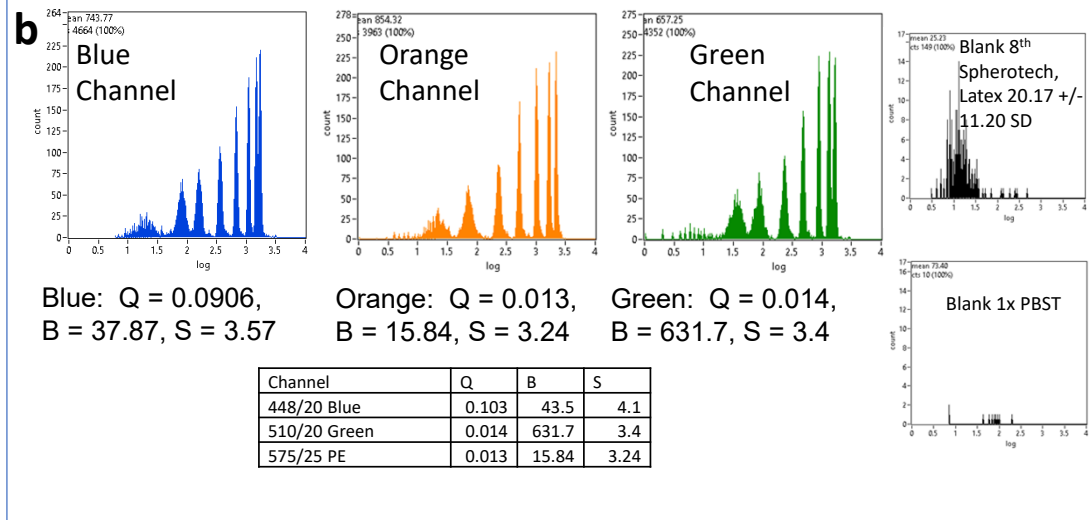
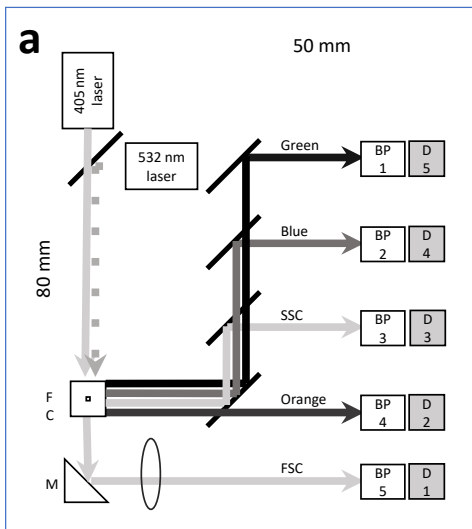
Table 1

EXTENDED FIGURES

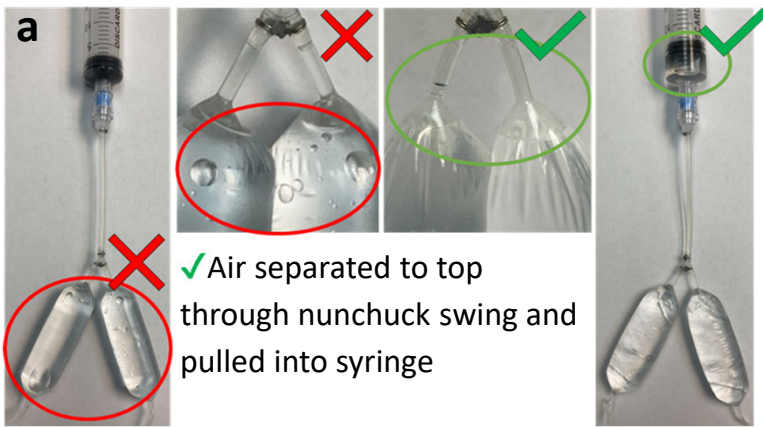




**Supplementary Fig. 1. Detailed view of the flight hardware.** The flight kit for the rHEALTH ONE Analyzer (1, logo digitally covered in dark blue on the door) included the following: rHEALTH sample cup (or sample consumable) (2), plastic bottles for sheath and cleaning (3), flexible water bag for inside of sheath and cleaning bottles (4), micro-USB cable and connection (5), waste bag and connection (6), burp syringe and connection (7), master rHEALTH reservoir bag (8), 30 mL syringe (9), and samples (10).



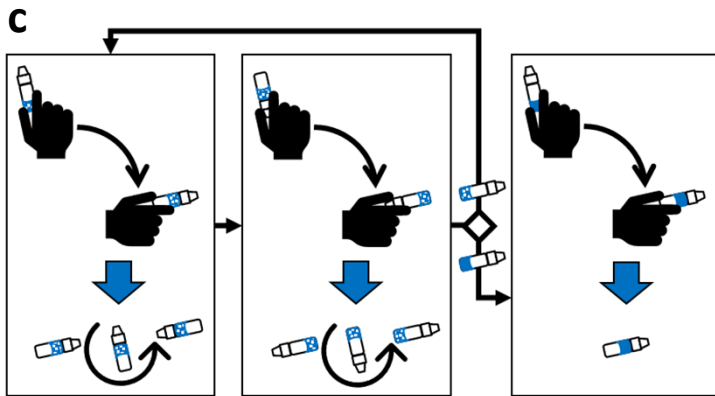
**Supplementary Fig. 2. Optical cytometry module performance.** **a**, Layout of the 50 mm x 80 mm optical block. The 405 nm and 532 nm lasers are focused onto the flow cell (FC). Orthogonal fluorescence light is split by dichroic filters and filtered by bandpass (BP) filters and captured by detectors (D1-5). The channels are green, blue, side scatter channel (SSC), orange, and forward scatter channel (FSC). **b**, Q (detection efficiency), B (background), and S (separation) analysis of the fluorescence channels. *Top right*, histogram for detection of blank autofluorescent beads is shown. *Bottom right*, run with 1x PBST negative control, showing confirmation that the 8<sup>th</sup> autofluorescent peak can be detected. **c**, The Molecules of Equivalent Cascade Blue (MECSB) is plotted versus 256 channels. **d**, FSC intensity is plotted versus bead size ( $\mu\text{m}$ ). This size histogram for 1.0-1.4  $\mu\text{m}$  beads in the SSC channel is displayed, showing resolution from noise. **e**, Scatterplots of FSC and SSC for 3.0-3.4  $\mu\text{m}$  beads showing singlets and doublets. SSC plotted versus orange for calibration beads.



✓ Air separated to top through nunchuck swing and pulled into syringe

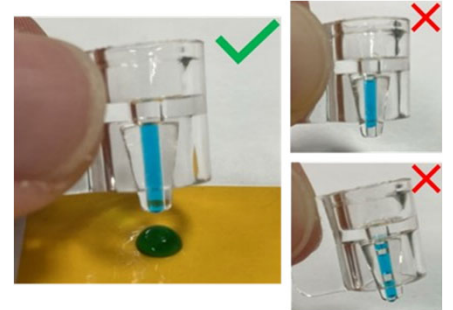


Whip & flip sample mixing

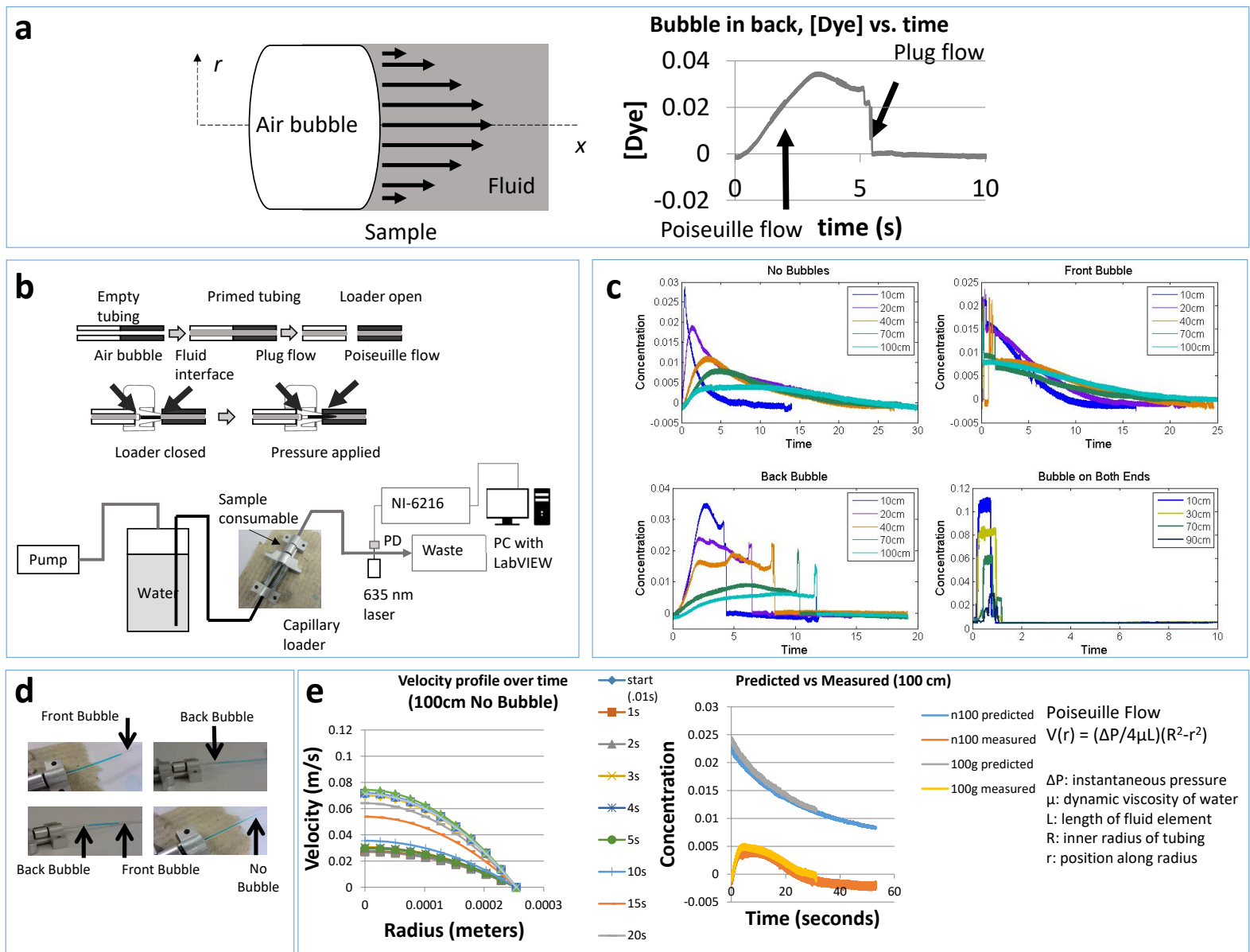


d

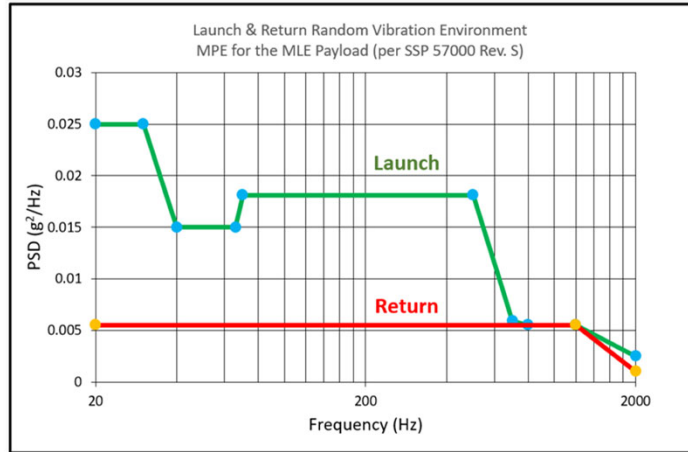
✓ Filled, No bubbles



**Supplementary Fig. 3. Techniques for bubble-free filling and loading in microgravity.** *a*, Left two images, improperly filled fluid bag with air bubbles after a nunchuck swing, which creates a force vector to localize bubbles near the syringe injection area. Right two images, correctly filled fluid bag with no air bubbles. *b*, rHEALTH ONE with the correctly filled fluid bag inside the plastic bottles. *c*, Left-to-right, description of the whip and flip sample mixing where the sample was whipped and flipped with the cap pointing out, then with the cap pointing in, then the cap pointing out just prior to dispensing. *d*, Left, correctly filled sample consumable without any air bubbles. Right two images, two examples of improperly filled air bubbles, one with air bubbles at the top and another with air bubbles in the middle.



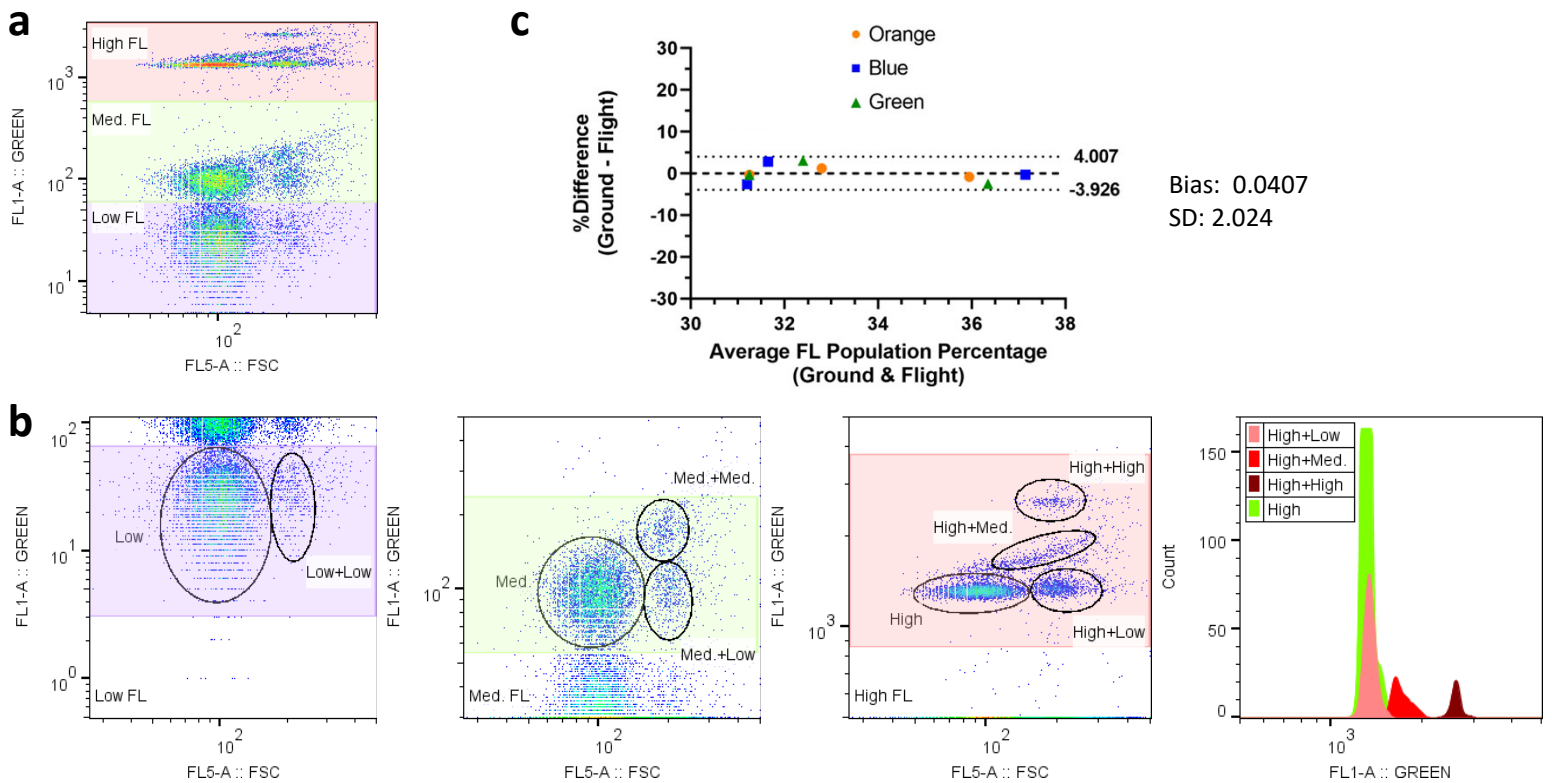
**Supplementary Fig. 4. Sample loader characterization.** **a**, Sample loading profile showing Poiseuille flow at the leading edge and plug flow in the back where the air bubble is. The graph shows relative blue dye concentration [dye] versus time of transit through the detector, showing the different sample profiles at the leading and trailing edges. **b**, Sample loading sequence, starting with empty tubing, which is primed with buffer. The interface is opened and the sample consumable is loaded into the system. Pressure is applied, resulting in a fluid interface at the leading edge and an air bubble at the trailing edge. The in-line loading approach allows for controlled sample loading with a fluid-fluid interface at the leading edge and an air-fluid interface at the trailing edge. The sample loading test apparatus is also shown, which was utilized to test various bubble loading cases. An air pump is utilized to pressurize a water container, which then pushes water through tubing through the sample loader. A 635 nm laser is utilized to measure the loading profiles, which are captured by a photodiode connected to an NI-6216 DAQ card attached to a computer running LabVIEW. **c**, Loading profiles of the four cases: (1) no bubbles, (2) front bubble, (3) back bubble, and (4) bubble on both ends. In all cases, tubing lengths from 10 to 100 cm, in increments of 10 cm, were tested. **d**, Various cases of sample loading testing with blue dye. The bubble can be at the front, back, both ends, or none on either side. **e**, The velocity profiles along the radius of 100 cm tubing, where  $r = 0$  is the center axis of the tubing. The fastest velocity is found at the center of the tubing. The calculation can be utilized to predict the relative dilution of the sample at the leading edge of the sample. Poiseuille flow describes a parabolic velocity profile of a fluid which matches the observed data.



**Supplementary Fig. 5. *Vibration profiles utilized to qualify the rHEALTH ONE for flight per NASA's Pressurized Payloads Interface Requirements Document SSP 57000 Rev. S.*** Vibration frequency (Hz) plotted versus the Power Spectral Density (PSD) in g<sup>2</sup>/Hz. The Maximum Permissible Exposure (MPE) is graphed for the Middeck Locker Equivalent (MLE). The launch is subject to higher PSD than the return.

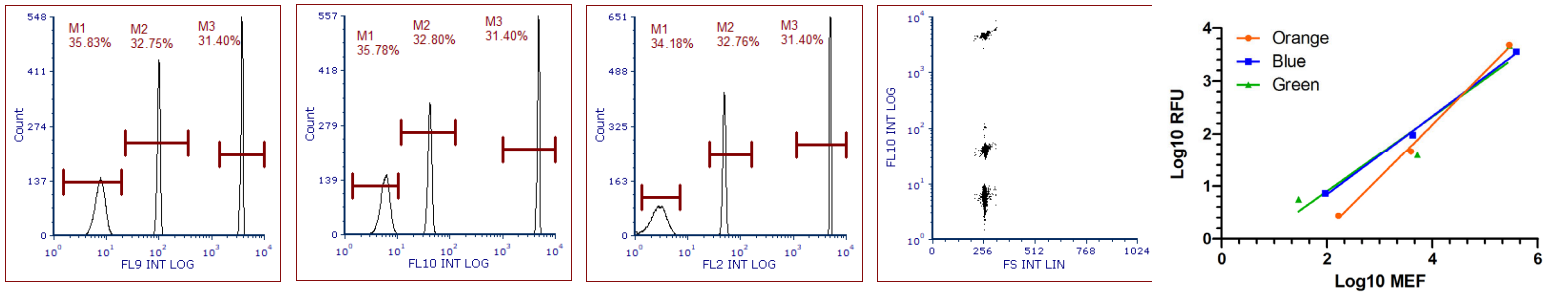


## Spherotech RQC-30-5: Counting Methodology



**Supplementary Fig. 6. Coincident event counting methodology for Spherotech RQC-30-5 beads.** **a**, FSC versus green scatterplot showing the three different low (Low), medium (Med), and high (High) fluorescent (FL) populations. The low events are boxed in purple, medium in green, and high in red. **b**, Zoom-in for each of the event boxes. *Left-to-right*, purple boxed low events showing singlet events (Low) and coincident events paired with other low events (Low + Low), green boxed medium events showing singlet medium events (Med) can pair with Med or Low events (Med + Low, Med + Med), red boxed high events showing singlet high events (High) and coincident pairing in all combinations (High + Low, High + Med, and High + High), and red boxed histogram view of only the high events with the green histogram as the singlet and the red histograms (in various shades) as doublets. **c**, The percentage of counted events for the three populations were compared using a Bland-Altman plot comparing the ground and flight percentage events. The x-axis shows the average percentage for flight and ground and the y-axis shows the % difference between the ground and flight.

## Preflight Ground Data - Gallios



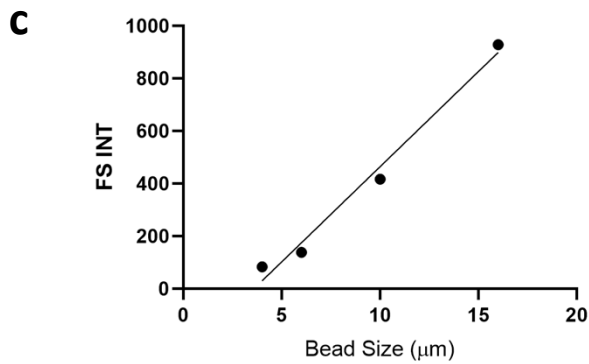
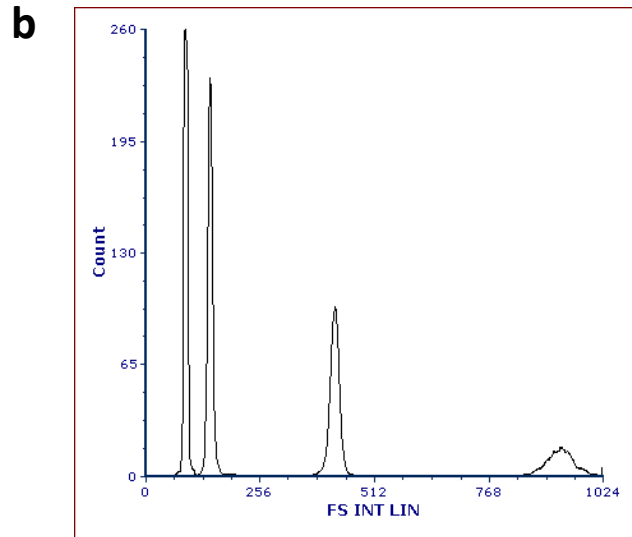
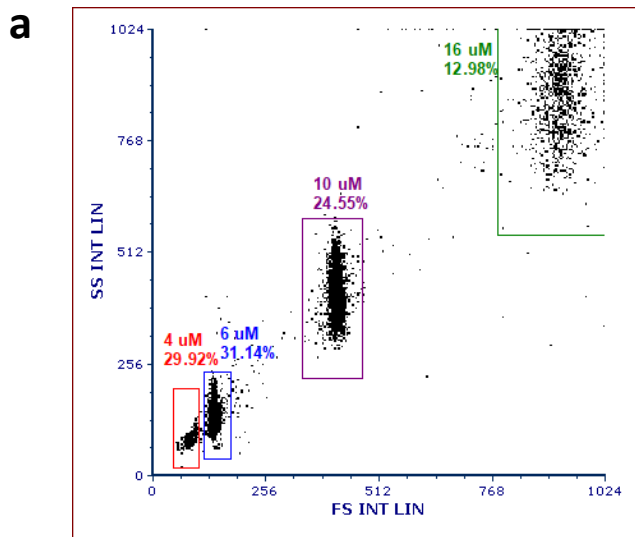
## GALLIOS GROUND DATA

LMD 16435

Date: Oct 29, 2021

Flt reagents assayed PRE flight check

**Supplementary Fig. 7. Benchmark preflight Gallios data for Spherotech RQC-30-5 beads.** Left-to-right, blue (FL9) histogram with gates M1 – M3 calculating the bead percentages, green (FL10) histogram showing gates M1 – M3, orange (FL2), and FSC versus green scatterplot, and Molecules of Equivalent Fluorochrome (MEF) versus median  $\log_{10}$  RFU for each population.



**d**

Gate	# of Events	X Geometric Mean	Y Geometric Mean	% of gated cells (from plot)	% of all cells
None	10125	202.74	189.55	100.00	100.00
4 uM	3029	83.90	75.46	29.92	29.92
6 uM	3153	138.93	128.90	31.14	31.14
10 uM	2486	417.18	407.20	24.55	24.55
16 uM	1314	928.59	881.55	12.98	12.98

## GALLIOS GROUND DATA

LMD 16436

Date: Oct 29, 2021

PRE flight check of flt reagents

**Supplementary Fig. 8. Benchmark preflight Gallios data for Spherotech PPS-6k beads.** (A) FSC versus SSC scatterplot for the beads. Various color gates are utilized to identify each population. (B) FSC histogram of the four populations. (C) Bead diameter in  $\mu\text{m}$  versus the mean FSC intensity (FS INT). (D) Summary statistics for each of the gates shown in A.

# EXTENDED TABLES

Features	Description
Product Name	rHEALTH ONE
Product Description	Compact small volume, high sensitivity biomarker analyzer
<b>General</b>	
Size	Height: 5.1" (13.0 cm) Width: 5.3" (13.4 cm) Depth: 7.0" (17.8 cm)
Weight	3.3 lb (1.5 kg)
Operating environment	15°C to 35°C
Power, data	USB 2.0
Water supply	None required
Air supply	None required
Laser safety	Class I laser product
<b>Optics</b>	
Lasers	405 nm violet (5 mW); 532 nm green (20 mW). Life expectancy > 5000 hrs for each laser
Detectors	Solid-state, high-gain photon counting detectors for all channels
Detection Channels	405 nm laser: Blue fluorescence (450 nm), Green fluorescence (510 nm). 532 nm laser: Yellow fluorescence (575 nm), FSC, SSC.
<b>Fluidics</b>	
Sample volume	5-10 µL
Sample flow rate	2 – 10 µL/minute
Fluid reservoirs	Sheath, cleaner, and waste at 60 mL each
Sample analysis time	< 3 minutes
Throughput	1000 events per second
Particle size	2-60 µm
Sample concentration	10 <sup>4</sup> to 10 <sup>7</sup> particles per mL
Sample loader	In-line, no dead-volume 10 µL plastic consumable
Cleaning	Automated
<b>Signal Processing and Analysis</b>	
Assay dynamic range	Five log decade range
Software	rHEALTH Viewer and Capture Software
Data format	All digital
Minimum computing requirements	2.8-GHz processor, 2 GB RAM, 256 GB storage.
<b>Assay Particles Supported</b>	
Assays	Ultra-high sensitivity Nanostrips and Lumibeads, cell analysis, multiplexed microsphere assays.

**Supplementary Table 1. rHEALTH ONE base device specifications.** The device, prior to payload development, for the NASA COTS program.



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) 85-0038-T100 75-0149-T100 50-0199-T100 (Tonbo Biosciences)	Antigen-capture OneComp eBeads stained with antibodies: CD3 V500, CD14 V450, CD19 PE	Spectral Overlap	GREEN BLUE 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 15µm	Particle size resolution	FSC	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	GREEN BLUE ORANGE	Target: 3 distinct amplitudes
D	A69184 (RUO) or A63492 (IVD) (Beckman Coulter)	Flow-Set Pro Fluorospheres uniform size and intensity	PMT Optical Precision, alignment	ALL	Target: Low CV

**Supplementary Table 2. Summary of samples tested.** Detailed descriptions of the four samples (A-D).

Frequency (Hz)	MPE PSD (g <sup>2</sup> /Hz)
20	0.0250
30	0.0250
40	0.0150
66	0.0150
70	0.0181
500	0.0181
700	0.0059
800	0.0055
1200	0.0055
2000	0.0025
Grms	4.063*
Duration (sec/axis)	60

\*Rounded to 4.07 in SSP 57000

**Supplementary Table 3. Simulated launch shock and vibration with frequency (Hz) and associated Maximum Permissible Exposure (MPE) g<sup>2</sup>/Hz.** The G<sub>rms</sub> is the root mean square gravity level for the various exposures. Based on NASA SSP 57000 Rev S.

Frequency (Hz)	MPE PSD (g <sup>2</sup> /Hz)
20	0.0055
1200	0.0055
2000	0.0010
Grms	2.908**
Duration (sec/axis)	60

\*\*Rounded to 2.09 in SSP 57000

**Supplementary Table 4. Simulated return shock and vibration with frequency (Hz) and associated Maximum Permissible Exposure (MPE) g<sup>2</sup>/Hz.** Based on NASA SSP 57000 Rev S.

<b>Preflight Ground Data - Gallios</b> Trigger: FSC			
<b>Channel</b>	<b>Counts</b>	<b>Mean</b>	<b>%CV</b>
Blue:	1682	232.71	12.61
Green:	1681	250.95	12.04
Orange:	1681	518.01	4.45
SSC:	1682	254.62	6.41
FSC:	1680	244.92	5.83
Mean Channel Counts	1681		
<b>Preflight Ground Data - rHEALTH ONE</b> Trigger: SSC			
<b>Channel</b>	<b>Counts</b>	<b>Mean</b>	<b>%RCV</b>
Blue:	5277	247.42	12.91
Green:	5263	113.20	19.02
Orange:	5257	520.80	6.33
SSC:	5380	557.20	12.26
FSC:	5390	78.54	26.89
Mean Channel Counts	5313		
<b>On-Orbit Flight Data - rHEALTH ONE</b> Trigger: SSC			
<b>Channel</b>	<b>Counts</b>	<b>Mean</b>	<b>%RCV</b>
Blue:	4072	245.88	11.77
Green:	4062	110.85	18.91
Orange:	4047	561.58	6.23
SSC:	4169	703.17	7.88
FSC:	4183	68.05	28.29
Mean Channel Counts	4107		

**Supplementary Table 5. Summary statistics for Flow-Set beads for ground (Gallios, rHEALTH ONE) and flight (rHEALTH ONE), as presented in Figure 5.** The counts, %CV, and mean are shown for each channel and experiment.

FILE	B1 MN	B1 SD	B1 CV	G1 MN	G1 SD	G1 CV	O1 MN	O1 SD	O1 CV	SSC1 MN	SSC1 SD	SSC1 CV	FSC1 MN	FSC1 SD	FSC1 CV
<b>GROUND</b>															
GMT343_SampleD4	247.66	30.99	12.52	113.13	20.98	18.54	520.70	31.95	6.14	556.85	68.35	12.28	78.49	20.12	25.64
GMT343_PracticeD1	264.07	42.33	16.03	67.91	17.82	26.24	642.12	33.53	5.22	182.08	40.24	22.10	113.44	18.98	16.73
GMT343_PracticeD2	314.73	41.99	13.34	93.08	18.70	20.09	673.99	31.72	4.71	203.99	33.15	16.25	112.68	19.63	17.42
GMT343_SampleD1	268.01	32.75	12.22	118.20	22.61	19.13	541.75	39.55	7.30	522.38	81.87	15.67	40.83	13.04	34.38
GMT343_SampleD2	273.79	30.50	11.14	127.54	21.25	16.66	565.24	33.71	5.96	589.43	70.62	11.98	90.52	21.18	23.40
GMT343_SampleD3	247.67	29.61	11.95	115.34	20.74	17.98	503.52	31.56	6.27	541.20	62.51	11.55	80.98	19.78	24.42
<b>FLIGHT</b>															
GMT136_Samplek1d1	245.92	28.22	11.47	111.08	20.50	18.45	561.59	34.84	6.20	702.88	59.38	8.45	67.93	18.45	27.16
GMT133_Sampled1	234.89	36.43	15.51	62.45	16.46	26.36	634.88	32.62	5.14	285.92	82.99	29.02	67.72	16.49	24.36
GMT133_Sampled2	285.74	54.66	19.13	78.07	22.28	28.54	665.92	30.19	4.53	236.00	41.52	17.59	62.49	16.32	26.12
GMT133_Sampled3	340.15	44.82	13.18	95.87	20.26	21.13	677.16	37.62	5.56	242.03	36.92	15.25	54.71	14.73	26.93
GMT136_Samplek1d2	279.64	31.34	11.21	121.08	21.78	17.99	656.94	41.87	6.37	756.56	62.45	8.25	79.03	20.23	25.60

**Supplementary Table 6. Flow-Set run statistics for all runs.** rHEALTH ground and flight run statistics, MN (mean), SD (standard deviation), and CV (coefficient of variation) for each channel, across all rHEALTH ONE experiments performed.

RQC-30-5 Spherotech Bead Analysis

Preflight Ground Data - Gallios															
	Blue					Green					Orange				
	# of Events	Population %	Median	Geometric Mean	CV	# of Events	Population %	Median	Geometric Mean	CV	# of Events	Population %	Median	Geometric Mean	CV
Low FL	8232	35.83%	7.23	7.14	20.87	8222	35.78%	5.57	5.50	19.43	7854	34.18%	2.74	2.71	30.76
Med. FL	7526	32.75%	94.75	93.88	5.69	7536	32.80%	39.60	39.35	10.47	7527	32.76%	46.56	46.59	5.92
High FL	7215	31.40%	3651.74	3579.75	4.70	7215	31.40%	4655.53	4573.21	4.19	7215	31.40%	4782.86	4766.75	1.69
Preflight Ground Data - rHEALTH ONE															
Trigger: SSC	Blue					Green					Orange				
	# of Event	Population %	Median	Geometric Mean	RCV	# of Events	Population %	Median	Geometric Mean	RCV	# of Events	Population %	Median	Geometric Mean	RCV
Low FL	9081	37.1%	31.98	30.59	37.46	8545	35.9%	26.03	24.96	42.24	8396	35.8%	35.98	34.55	38.94
Med. FL	7850	32.1%	101.02	99.99	21.72	7837	32.9%	96.05	95.38	20.32	7739	33.0%	155.13	155.20	16.14
High FL	7523	30.8%	1057.87	1068.84	3.69	7413	31.2%	1313.68	1326.43	3.06	7307	31.2%	1534.85	1542.39	2.95
On-Orbit Flight Data - rHEALTH ONE															
Trigger: SSC	Blue					Green					Orange				
	# of Events	Population %	Median	Geometric Mean	RCV	# of Events	Population %	Median	Geometric Mean	RCV	# of Events	Population %	Median	Geometric Mean	RCV
Low FL	10568	37.2%	35.98	34.95	31.94	10696	36.8%	50.05	48.92	27.96	10180	36.1%	60.99	59.44	28.66
Med. FL	8858	31.2%	93.07	92.06	23.57	9253	31.9%	122.07	121.18	19.64	9208	32.6%	169.10	168.36	16.55
High FL	8964	31.6%	954.31	963.59	5.06	9097	31.3%	1310.92	1321.99	4.85	8836	31.3%	1610.90	1623.06	2.85

**Supplementary Table 7. Summary statistics for Spherotech RQC-30-5 beads, as presented in Figure 6.** The summary statistics for each low, middle, high population for each color channel is summarized for the ground Gallios, ground rHEALTH ONE and flight rHEALTH ONE experiments.

FILE	B1 MN	B1 SD	B1 CV	B2 MN	B2 SD	B2 CV	B3 MN	B3 SD	B3 CV
<b>GROUND</b>									
GMT342_PracticeC1	33.94	12.13	35.75	104.56	20.52	19.62	1056.10	37.45	3.55
GMT342_PracticeC2	24.50	9.34	38.18	87.98	18.80	21.36	1047.57	38.50	3.68
GMT343_SampleC1	83.55	23.67	28.32	215.61	39.25	18.21	1491.08	167.93	11.26
GMT343_SampleC2	104.80	25.23	24.08	252.35	39.69	15.73	1695.52	170.87	10.30
GMT343_SampleC3	117.24	33.35	28.45	281.85	42.05	14.92	1739.75	140.17	8.06
<b>FLIGHT</b>									
GMT136_Samplek1c1	35.40	9.93	28.04	94.45	19.38	20.52	950.94	40.67	4.28
GMT133_practicec2	88.49	17.29	19.54	157.23	20.00	12.72	1075.59	42.68	3.97
GMT136_Samplec1	74.58	17.86	23.95	192.41	31.46	16.36	1321.56	130.41	9.87
GMT136_Samplec2	119.07	22.62	19.00	262.99	33.73	12.82	1600.32	136.04	8.50
GMT136_Samplec3	110.76	18.18	16.41	198.46	35.12	17.70	1511.13	143.77	9.51
GMT136_Samplec4	89.44	17.22	19.25	172.35	46.71	27.10	1624.92	146.92	9.05
GMT136_Samplec5	46.13	15.69	34.01	124.21	16.23	13.07	1086.08	78.52	7.23
GMT136_Samplec7	47.47	14.32	30.17	110.70	20.21	18.26	1057.26	42.73	4.04

FILE	G1 MN	G1 SD	G1 CV	G2 MN	G2 SD	G2 CV	G3 MN	G3 SD	G3 CV
<b>GROUND</b>									
GMT342_PracticeC1	27.88	10.71	38.42	98.15	18.41	18.75	1310.45	35.07	2.68
GMT342_PracticeC2	17.87	7.93	44.39	77.80	16.21	20.83	1267.24	41.40	3.27
GMT343_SampleC1	19.58	8.56	43.73	94.68	21.63	22.84	1422.99	152.31	10.70
GMT343_SampleC2	24.16	8.90	36.83	101.75	21.86	21.49	1508.19	111.42	7.39
GMT343_SampleC3	23.55	8.88	37.68	100.30	20.27	20.21	1521.95	102.39	6.73
<b>FLIGHT</b>									
GMT136_Samplek1c1	50.02	13.50	26.99	123.95	19.79	15.97	1304.7	53.12	4.07
GMT133_practicec2	110.77	15.75	14.22	176.14	19.59	11.12	1338.31	51.77	3.87
GMT136_Samplec1	74.39	18.22	24.49	158.91	14.26	8.97	1359.26	108.83	8.01
GMT136_Samplec2	110.70	18.43	16.65	199.81	20.87	10.44	1600.57	84.01	5.25
GMT136_Samplec3	88.65	16.66	18.79	166.78	26.69	16.00	1500.20	105.28	7.02
GMT136_Samplec4	78.92	15.08	19.11	142.45	28.22	19.81	1569.81	124.24	7.91
GMT136_Samplec5	79.38	18.03	22.72	172.91	23.42	13.54	1499.39	109.36	7.29
GMT136_Samplec7	69.28	16.64	24.02	148.7	22.74	15.29	1459.11	59.88	4.10

FILE	O1 MN	O1 SD	O1 CV	O2 MN	O2 SD	O2 CV	O3 MN	O3 SD	O3 CV
<b>GROUND</b>									
GMT342_PracticeC1	37.34	13.78	36.90	156.22	24.81	15.88	1536.14	45.40	2.96
GMT342_PracticeC2	27.99	11.35	40.54	151.70	22.13	14.59	1531.39	41.11	2.68
GMT343_SampleC1	75.46	22.78	30.19	260.55	32.23	12.37	2042.49	108.15	5.30
GMT343_SampleC2	83.23	28.52	34.26	261.30	42.89	16.41	2182.69	122.06	5.59
GMT343_SampleC3	112.59	33.05	29.36	300.83	42.88	14.25	2283.48	107.70	4.72
<b>FLIGHT</b>									
GMT136_Samplek1c1	62.46	15.28	24.46	169.41	26.75	15.79	1614.53	45.74	2.83
GMT133_practicec2	57.85	17.81	30.79	165.39	23.46	14.18	1741.81	48.58	2.79
GMT136_Samplec1	88.93	20.87	23.47	240.43	27.25	11.33	1817.37	91.13	5.01
GMT136_Samplec2	121.02	21.47	17.74	290.97	27.99	9.62	2069.50	75.78	3.66
GMT136_Samplec3	127.79	25.30	19.79	275.90	37.10	13.45	1961.00	76.01	3.88
GMT136_Samplec4	123.47	24.30	19.68	316.24	59.51	18.82	2340.78	247.29	10.56
GMT136_Samplec5	67.14	16.43	24.48	190.63	31.80	16.68	1813.61	67.40	3.72
GMT136_Samplec7	84.97	23.27	27.39	203.66	29.77	14.62	1807.50	51.04	2.82

**Supplementary Table 8. Spherotech RQC-30-5 run statistics for blue, green, and orange channels for all runs.** rHEALTH ground and flight run statistics, MN (mean), SD (standard deviation), and CV (coefficient of variation) for each of the three blue (B1-3), green (G1-G3), and orange populations (O1-3), across all rHEALTH ONE experiments performed.

Preflight Ground Data - rHEALTH ONE					
Bead Size	FSC				
	# of Events	Population %	Median	Geometric Mean	RCV
4µm	802	18.45	41.02	39.14	78.01
6µm	872	20.06	81.05	78.80	26.52
10µm	942	21.66	272.08	268.20	16.49
15µm	1732	39.83	474.03	473.58	6.37
On-Orbit Flight Data - rHEALTH ONE					
Bead Size	FSC				
	# of Events	Population %	Median	Geometric Mean	RCV
4µm	531	12.46	20.01	23.72	107.49
6µm	961	22.55	76.95	74.32	27.91
10µm	948	22.25	272.08	266.21	15.43
15µm	1821	42.74	411.73	406.00	10.41

**Supplementary Table 9. Summary statistics for Spherotech PPS-6K beads, as presented in Figure 7.** rHEALTH ground and flight summary statistics for each of the four gated populations (4, 6, 10, and 15 µm diameter beads).

FILE	FS1 MN	FS1 SD	FS1 CV	FS2 MN	FS2 SD	FS2 CV	FS3 MN	FS3 SD	FS3 CV	FS4 MN	FS4 SD	FS4 CV
<b>GROUND</b>												
GMT343_SampleB1	50.65	32.45	64.09	82.05	20.19	24.61	271.34	40.20	14.81	474.10	29.26	6.17
GMT342_SampleB1	40.84	32.53	79.66	71.37	18.22	25.53	232.54	34.91	15.01	434.55	38.33	8.82
GMT342_SampleB2	56.85	36.08	63.46	81.61	20.33	24.91	264.03	34.78	13.17	474.33	22.73	4.79
GMT342_SampleB4	53.12	38.14	71.81	54.63	20.63	37.75	234.77	46.07	19.62	640.05	108.35	16.93
GMT342_SampleB5	18.67	6.32	33.87	56.20	15.43	27.45	175.77	52.37	29.80	494.45	75.84	15.34
GMT343_SampleB2	53.59	38.05	71.00	75.38	21.66	28.73	261.51	42.04	16.08	478.22	31.37	6.56
<b>FLIGHT</b>												
GMT136_Samplek1b1	31.21	23.97	76.79	76.52	17.82	23.29	271.68	36.17	13.31	408.77	39.70	9.71
GMT136_Sampleb1	56.64	33.93	59.91	91.46	16.73	18.29	303.60	24.09	7.94	387.26	62.34	16.10
GMT136_Sampleb2	38.96	29.37	75.38	97.12	14.54	14.97	321.06	21.17	6.60	398.25	59.54	14.95
GMT136_Sampleb3	37.55	31.25	83.23	100.80	13.99	13.88	329.68	26.89	8.16	411.25	61.32	14.91
GMT136_Sampleb4	24.26	18.61	76.72	108.00	17.07	15.80	329.61	29.69	9.01	422.37	60.48	14.32
GMT136_Samplek1b2	30.79	35.72	116.03	161.34	54.60	33.84	606.82	119.6	19.71	1063.54	258.88	24.34

**Supplementary Table 10. Spherotech PPS-6K run statistics for all runs.** rHEALTH ONE ground and flight run statistics, MN (mean), SD (standard deviation), and CV (coefficient of variation) for each of the four gated populations (FS1, FS2, FS3, and FS4), across all experiments performed.



Preflight Ground Data - Gallios					
FL9 vs FL10	Percentages	FL2 vs FL9	Percentages	FL2 vs FL10	Percentages
TL (FL10+ , FL9-)	13.15%	TL (FL9+ , FL2-)	13.43%	TL (FL10+ , FL2-)	13.07%
TR (FL10+ , FL9+)	0%	TR (FL9+ , FL2+)	0.15%	TR (FL10+ , FL2+)	0.08%
LL (FL9- , FL10-)	73.28%	LL (FL2- , FL9-)	70.92%	LL (FL2- , FL10-)	71.37%
LR (FL9+ , FL10-)	13.57%	LR (FL2+ , FL9-)	15.51%	LR (FL2+ , FL10-)	15.48%
Preflight Ground Data - rHEALTH ONE					
Blue vs Green	Percentages & Counts	Orange vs Blue	Percentages & Counts	Orange vs Green	Percentages & Counts
TL (GR+ , BL-)	12.52% (956)	TL (BL+ , OR-)	13.12% (1002)	TL (GR+ , OR-)	12.67% (970)
TR (GR+ , BL+)	0.75% (57)	TR (BL+ , OR+)	0.75% (57)	TR (GR+ , OR+)	0.60% (46)LL
LL (BL- , GR- )	73.99% (5649)	LL (OR- , BL-)	70.33% (5370)	LL (OR- , GR- )	70.70% (5395)
LR (BL+ , GR-)	12.74% (973)	LR (OR+ , BL-)	15.80% (1206)	LR (OR+ , GR-)	16.03% (1224)
On-Orbit Flight Data - rHEALTH ONE					
Blue vs Green	Percentages & Counts	Orange vs Blue	Percentages & Counts	Orange vs Green	Percentages & Counts
TL	11.02% (795)	TL	0.53% (38)	TL	0.29% (21)
TR	2.37% (171)	TR	15.01% (1083)	TR	13.25% (956)
LL	75.32% (5433)	LL	56.91% (4105)	LL	57.06% (4116)
LR	11.29% (814)	LR	27.55% (1987)	LR	29.39% (2120)
Postflight - Gallios					
FL9 vs FL10	Percentages	FL2 vs FL9	Percentages	FL2 vs FL10	Percentages
TL	12.58%	TL	13.19%	TL	12.33%
TR	0%	TR	0%	TR	0%
LL	74.22%	LL	71.75%	LL	72.75%
LR	13.19%	LR	15.05%	LR	14.93%

**Supplementary Table 11. Summary statistics for OneComp eBeads with CD19 PE, CD14 V450, and CD3 V500-labeled antibodies, as presented in Figure 8.** The data for the quadrant analysis for preflight ground Gallios, ground rHEALTH ONE, flight rHEALTH ONE, and postflight Gallios are shown. The quadrant gates for the Gallios are denoted by TL (top left), TR (top right), LL (lower left), and LR (lower right). The Gallios gates postflight were moved to match the percentages in each quadrant.

FILE	B1 MN	B1 SD	B1 CV	G1 MN	G1 SD	G1 CV	O1 MN	O1 SD	O1 CV	O2 MN	O2 SD	O2 CV	SSC1 MN	SSC1 SD	SSC1 CV	FSC1 MN	FSC1 SD	FSC1 CV
<b>GROUND</b>																		
GMT342_SampleA1	73.74	27.82	37.73	81.79	24.51	29.96	NA	NA	NA	915.16	100.46	10.98	571.07	183.46	32.12	333.19	26.61	7.99
GMT342_SampleA2	87.97	24.07	27.36	79.32	22.72	28.64	NA	NA	NA	953.76	106.23	11.14	497.07	168.17	33.83	344.21	26.27	7.63
GMT342_SampleA3	92.48	27.80	30.06	78.70	23.72	30.13	NA	NA	NA	1007.21	107.27	10.65	436.77	155.42	35.58	361.47	24.45	6.76
<b>FLIGHT</b>																		
GMT136_Samplea2	53.07	17.47	32.91	75.77	23.52	31.04	243.35	45.98	18.90	751.37	82.55	10.99	769.82	194.75	25.30	234.44	34.54	14.73
GMT133_Samplea1	54.16	23.35	43.11	52.41	18.18	34.69	340.54	50.32	14.78	954.12	95.35	9.99	672.97	225.96	33.58	258.96	20.40	7.90
GMT133_Samplea2	60.70	22.76	37.50	52.71	16.80	31.88	344.33	59.61	17.31	989.24	103.46	10.46	612.72	222.60	36.33	272.86	21.11	7.74
GMT133_Samplea3	51.17	21.09	41.22	51.31	14.81	28.87	356.11	56.08	15.75	1015.67	105.42	10.38	558.64	218.48	39.11	297.98	22.68	7.61
GMT136_Samplea1	41.94	14.46	34.47	62.12	22.32	35.93	223.97	43.46	19.41	735.32	84.98	11.56	811.29	199.81	24.63	238.89	37.18	15.56
GMT136_Samplek1a1	50.54	18.66	36.92	73.66	25.40	34.47	232.18	58.82	25.34	791.08	114.22	14.44	904.09	217.94	24.11	280.17	43.88	15.66
GMT136_Samplek1a2	47.00	16.02	34.08	66.62	22.95	34.45	198.69	49.91	25.12	679.92	98.31	14.46	768.72	189.06	24.59	241.63	35.43	14.66

**Supplementary Table 12. OneComp eBeads run statistics for all runs.** rHEALTH ground and flight run statistics, MN (mean), SD (standard deviation), and CV (coefficient of variation) for each gated population (B1, G1, O1, O2, SSC1 and FSC1), across all experiments performed for the rHEALTH ONE.

Run Summary					
On-orbit run summary	Sample A	Sample B	Sample C	Sample D	Total
# planned runs	3	3	5 <sup>1</sup>	3	14 <sup>2</sup>
# runs performed	7 (233%)	6 (200%)	10 (200%)	5 (166%)	28 <sup>2</sup> (200%)
Runs yielding data	7 (233%)	6 (200%)	8 <sup>3</sup> (160%)	5 (166%)	26 (186%)
Mean run duration					143.10s
Mean raw data per run					71,550,000
<sup>1</sup> Includes 2 practice runs <sup>2</sup> Excludes blank runs to test instrument fluidics <sup>3</sup> 1 run had no peaks, 1 run was incomplete					

**Supplementary Table 13. Summary of on-orbit runs.** The total number of runs, including which runs yielded usable data, average run duration, and data points collected are shown. The (%) is the percentage of the planned runs.

TEST MATRIX	
OPS SESSION 1	OPS SESSION 2
Blank Run	Blank Run
Practice C1	Practice C1 (or D1*)
Practice C2	Practice C2 (or D2*)
Sample A1 (OneComp eBeads)	Sample B1 (Spherotech PPS-6K)
Sample A2	Sample B2
Sample A3	Sample B3
Sample D1 (Flow-Set Pro)	Sample C1 (Spherotech RQC-30-5)
Sample D2	Sample C2
Sample D3	Sample C3
No time: At least 1 good run each	Replace C with reruns
Extra time: run all 4 samples	Rerun samples as needed
* C or D shows up best to check the analyzer. Choose based on data needed.	
END Session 1	END Session 2

**Supplementary Table 14. Original test sequence for Ops Session 1 and Ops Session 2.** Planned test matrix for the two day operational sessions. Blank and practice runs start each session and then two of the four test samples were planned for each day.

MISSION SUCCESS				
CRITERIA		MINIMAL	SUBSTANTIAL	COMPLETE
Number of Channels with Signal:	Scatter channel	≥ 1	2	2
	Color channel	≥ 1	3	3
Number of Test Points:	A	N/A	≥ 1	≥ 3
	B	≥ 1	≥ 1	≥ 3
	C	≥ 1	≥ 1	≥ 3
	D	≥ 1	≥ 1	≥ 3
Hydrodynamic Flow Focusing:		Present	Present	Present
Post-Flight Analysis	A	N/A	1 distinct population per channel	Comparable to Ground Data (e.g. Number of peak, CV for optical precision, degree of spectral overlap)
	B		≥ 1 population on a scatter channel	
	C		1 distinct population per scatter channel, AND ≥ 1 population per color channel	
	D		1 distinct population per channel	
Data Collection per Sample:		≥ 20 events	≥ 1000 events	≥ 3 minutes OR full sample run (to end bubble) with 5μL worth of data (~1 minute of constant events)

**Supplementary Table 15. Predetermined success metrics for the experiments.** NASA-determined metrics for minimal, substantial, and complete success for the experiments.

<b>rHEALTH Fluids and Power Consumption</b>		
	<b>Startup Prime Sequence</b>	<b>Sample Run + Ending Prime</b>
<b>Sheath</b>	2.44 mL	1.13 mL
<b>Clean</b>	0.55 mL	0.02 mL
<b>Waste</b>	2.99 mL	1.15 mL
<b>Power Consumption</b>	0.147 Wh (528 J over 180 seconds, 2.93W)	0.156 Wh (563 J over 192 seconds, 2.93W)

**Supplementary Table 16. *rHEALTH fluids and power consumption.*** The system requires a startup full prime at the beginning of each experimental day/session. Afterwards, multiple individual sample runs (consuming less fluid) are performed. The power consumption is also shown.