Single Drop Cytometry Onboard the International Space Station

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Abstract

Real-time lab analysis is needed to support clinical decision making and research on human missions to the Moon and Mars. Powerful laboratory instruments, such as flow cytometers, are generally too cumbersome for spaceflight. Here, we show that scant test samples can be measured in microgravity, by a trained astronaut, using a miniature cytometry-based analyzer, the rHEALTH ONE, modified specifically for spaceflight. The base device addresses critical spaceflight requirements including minimal resource utilization and alignment-free optics for surviving rocket launch. To fully enable reduced gravity operation onboard the space station, we incorporated bubble-free fluidics, electromagnetic shielding, and gravity-independent sample introduction. We show microvolume flow cytometry from 10 µL sample drops, with data from five simultaneous channels using 10 µs bin intervals during each sample run, yielding an average of 72 million raw data points in approximately 2 minutes. We demonstrate the device measures each test sample repeatably, including correct identification of a sample that degraded in transit to the International Space Station. This approach can be utilized to further our understanding of spaceflight biology and provide immediate, actionable diagnostic information for management of astronaut health without the need for Earth-dependent analysis.

Introduction

Under National Aeronautics and Space Administration (NASA) plans, humans are to travel to Mars in the 2030s [1]. For humans to explore Mars, the journey is a 1.8 billion kilometer round-trip journey, requiring a total of 760 to 850 days [2]. This is greater than 1000x more distance travelled and close to 100x more duration than Apollo 11, which was 1.534 million kilometers round-trip and just over 8 days [3]. The human health risks are significant and include space radiation exposure [4], bone loss [5], circadian rhythm changes [6], spaceflight psychological hazards [7], cardiac remodeling [8], vision changes [9], hematological dysfunction [10], and neurological changes [11]. Unexpected, acute, life-threatening medical conditions can arise that require emergent diagnostic assessment and medical intervention, such as the case of an obstructive jugular venous thrombosis on the International Space Station (ISS)[12]. The risk of inflight medical conditions is among the most concerning for a Mars mission per NASA's Human System Risk Board [13]. For humans to become an interplanetary species, these risks need to be studied and managed. In-flight clinical decision-making can benefit from immediate and abundant diagnostic information, available from drops of blood or other biological specimens that can be easily and frequently obtained. Separately, analysis of biological samples is a component of spaceflight research, but currently the samples are downmassed, which can result in sample transportation artifacts. This process takes months and during this time, the samples can be subject to unpredictable storage conditions, resulting in degradation or alteration. This was highlighted in the monozygotic twin study where samples had unavoidable transit time and

42 unknown transit conditions (vibration and temperature) [14]. Our current understanding of spaceflight medicine and
43 biology are thus limited by this approach, and while sample downmass, however imperfect, is an option for studies in
44 Low-Earth Orbit (LEO), it would be near impossible for long-duration, deep space exploration missions.

45 There has been a longstanding interest in developing and implementing a cytometer for routine spaceflight use.
46 This would allow for immediate sample analysis, without the risk of sample transport artifacts or delays in results. Flow
47 cytometry capabilities in space have been desired for decades. Jett et al., in 1985, described the ability to leverage
48 cytometry on the space station, a lunar base, or a voyage to Mars[15]. This approach is powerful enough to measure a
49 broad range of test classes, such as blood counts [16], hormones [17], chemistry [18], enzymes [19], nucleic acids [20,21], proteins
50 and biomarkers [15,22]. Furthermore, cytometry can allow for high levels of assay multiplexing, allowing simultaneous
51 measurement of diagnostic and biological parameters, thus increasing throughput and content of each sample analysis.
52 For instance, multiplexing over a 100 analytes is possible with differentially-dyed microspheres [23] and multiplexing in the
53 1000s is possible with barcoded hydrogel microparticles and nanostrips [24-26]. Thus, cytometry with multiplexing
54 capabilities would provide the required data density for detailed insights into biological systems and astronaut health.
55 This breadth and depth of applications makes a cytometer highly desirable for spaceflight applications.

56 There are multiple challenges, however, to implementing a cytometer in space for routine use[27,28]. The first is
57 obvious, which is the mass, volume, and power constraints. For instance, a BD LSRFortessa X-20 is large at 159 kg, 76.2 x
58 73.7 x 76.2 cm, and 1500 watts. For context, this would exceed the mass and volume allocation of the entire spacecraft
59 medical system. A smaller cytometer, such as the BD Accuri C6 is 13.6 kg, 27.9 x 37.5 x 41.9 cm, and 150 watts. Even
60 this smaller, capable cytometer would consume significant resources. In addition to the resource issue, cytometers
61 require sensitive laser alignment, generally focused down to the sample stream core, which is about 1/10th the width of
62 a 200 µm diameter human hair. The ability of this delicate system to survive a rocket launch, with high g-loads and
63 vibration would be very challenging. Even if transported to space safely, the cytometer would need to be able to
64 operate properly in microgravity. The fluidics in the system would be prone to air bubbles since there is no buoyancy in
65 space. Air bubbles would occupy the middle of the liquids and be likely to interfere with sheath flow operation. This can
66 manifest in sheath flow stream drift relative to the laser, degrading the performance of the system. Cytometers
67 generally also require a significant level of calibration and routine weekly maintenance to keep them running so optimal
68 data can be obtained. Any payload going to space is likely to be shipped to the launch facility months in advance,
69 precluding any servicing during this time. Training the astronaut crew members to service these complex instruments
70 and the performance of the calibration procedures would be significantly time consuming.

71 Several groups have made advancements in demonstrating cytometry in space or a space analog environment.
72 The MicroFlow1 was demonstrated onboard the ISS by the Canadian Space Agency (CSA) [29]. It achieved suitable
73 performance but lacked a fluidic system for loading small samples in microgravity. The authors from DNA Medicine
74 Institute demonstrated a miniaturized solid-state flow cytometer (an early version of the rHEALTH ONE), onboard
75 parabolic flights for cell and nanoscale test strip (nanostrip) measurements [25,26,30], together with a microvolume in-line
76 capillary sample loader [31-33] and a microfluidic spiral vortexer for mixing and dilution [34]. Others have also shown promise
77 in addressing the challenges of cytometry in reduced gravity environments, including a Guava cytometer significantly
78 modified for microgravity operations [35], a 460 nm blue LED-based cytometer [36], and a plastic chip-based fiber optic
79 cytometer[37]. All these technologies, including the rHEALTH ONE precursor, leveraged a sheathless approach. Sheath-
80 flow under hydrodynamic focusing, however, is the standard approach on larger cytometers. This approach brings the
81 sample off the walls of the channel for less channel fouling and avoids the zero-flow condition at the flow channel's
82 walls. Sheath-flow further allows the sample core stream to be centered in the channel, where the Poiseuille flow rate is
83 most uniform.

84 Cytometers are generally designed for environments with a gravity vector and a stable work surface, preventing
85 their off-the-shelf use in space. Gravity assists with minimizing air bubbles in fluidic systems by relying on buoyancy. In
86 1*g*, fluids are in predictable locations, at the bottom of a vial or a vessel. In microgravity, the locations of fluids can be

unpredictable and dictated by the simple movement and/or surface forces. This necessitates development of microgravity methods for handling cytometry fluids and sample introduction. These approaches should prevent or minimize microbubble formation, which degrades cytometry data. On the optical side, commercial cytometers generally have optomechanical positioners that allow technicians to fine-tune the performance and alignment of the laser(s) relative to the flow cell and detectors. Since the positioning tolerance is less than the width of a human hair, systems need to be realigned routinely. Some cytometers employ a fixed alignment approach, but these would not be rated to the *g*'s and vibrations experienced on rocket launch. Rocket launch conditions may also result in loose electrical, optical, or mechanical conditions, resulting in catastrophic failure of equipment. Furthermore, critical spacecraft communications may be impacted by electromagnetic interference (EMI) coming from high-powered microprocessors required for data collection and analysis. These reasons as well as significant resource limitations for mass, volume, power, and fluids ultimately preclude launching a commercial cytometer to space and having it yield useful information.

Here, we describe analysis of individual drops of test suspensions with a spaceflight-modified rHEALTH ONE, a sheath-flow, cytometry-based biomedical analyzer [38], onboard the ISS. The device was designed and built with spaceflight considerations by the authors from DMI and rHEALTH, leveraging on previous successes with reduced gravity testing onboard parabolic flights[30]. The base rHEALTH ONE addresses the need for minimal mass volume and power (1.5 kg, 12 x 13 x 18 cm, and 2.9W), alignment-free optics, and single drop sample handling. This base device, considered a Commercial-Off-The-Shelf (COTS) device by NASA, was further developed as a payload for the space station, which included addressing the conditions of rocket launch, microgravity sample loading techniques, microgravity fluid bottles, safety, and EMI. The resulting device was fully operational in space. It leveraged sheath-flow for precise measurements of test microspheres that were designed to comprehensively assess system performance. Sample loading from individual sample drops was achieved with a zero dead volume sample loading system for analysis of precious samples. Preflight, in-flight, and post-flight analysis verified the robust operation of the device on-orbit.

Results

rHEALTH ONE Experiment and Device Description

The rHEALTH ONE is a portable, microvolume sampling, dual laser, five channel cytometer employing hydrodynamic focusing (specifications, **Supplementary Table 1**). The rHEALTH ONE was sent to the ISS as part of the NASA Commercial Resupply Mission NG-17. An Antares rocket carried the NG-17 Cygnus spacecraft to the ISS, after launching off the Mid-Atlantic Regional Spaceport (MARS) Pad 0A on Wallops Island, Virginia on February 19, 2022. The details of the pre-launch, orbital demonstration, and post-demonstration phases are outlined in **Fig. 1**. SpaceX Crew-4 Commander and European Space Agency (ESA) astronaut Samantha Cristoforetti unstowed the system contents (**Supplementary Fig. 1**) and performed experiments to characterize the device on May 13, 2022 between 09:00 and 17:00 GMT and again on May 16, 2022 between 10:45 and 18:30 GMT [39,40].

The device was mounted on a Human Research Facility (HRF) shelf (**Fig. 2a**). Four samples were flown to test the device's optical alignment, precision, intensity resolution, size resolution, and spectral separation (see sample details in **Supplementary Table 2**). These samples were blinded to the authors at DNA Medicine Institute and rHEALTH until the day of on-orbit operations. The samples were designed to be safe for use in the cabin without the need for additional levels of containment. Sample drops were dispensed from dropper bottles onto polyimide tape, selected for its ability to form a beaded drop. Capillary action was utilized to load the rHEALTH ONE sample consumable (**Fig. 2b**). Microgravity assisted the filling of the sample consumable as capillary forces did not have to compete with the sample's hydrostatic pressure, as would be the case in 1g. Using this approach, the astronaut operator was able to consistently fill the 10 µL volume of the sample consumable each time. This wicking approach was similar to that used for testing the Hemocue WBC DIFF cuvette on-orbit [41]. The sample was loaded into the in-line sample loader of the device that allows

130 the entire sample to be analyzed. This approach is in contrast to conventional flow cytometers that require more
131 volume than is analyzed, which results in a significant unanalyzed dead volume. Once loaded within the analyzer, the
132 sample is delivered via pressure-driven, sheath-flow based hydrodynamic focusing to the laser illumination region of the
133 cytometry module (**Fig. 2c**, **Supplementary Fig. 2**). In order to meet the minimal resource requirements, the optical
134 cytometry module occupies a volume less than 80 x 50 x 10 mm, requiring no more than 1W power. This palm-sized
135 module has all solid-state components including a 405 nm laser, a 532 Nd:YAG laser, and five silicon photomultipliers
136 (SiPM). Sheath-flow encases the sample, bringing the sample off the walls of the detection channel, into the channel
137 center for uniform flow. The sample passes through the 405 nm laser spot first, followed by the second 532 nm laser
138 spot. During the runs, data are streamed via the USB cable to an ISS laptop running the *rHEALTH Capture* software
139 program, which reports the number of photons collected by the five detectors in 10 µs intervals. Each sample run was
140 approximately 2 minutes, yielding an average of 71.55 million raw data points for each run. The data were streamed in
141 real-time, allowing visualization of each run by the astronaut (**Fig. 2d**).

142 In order to successfully perform cytometry onboard the ISS, unique engineering requirements had to be met.
143 The base device, developed with NASA high-level requirements but prior to the payload development process, is shown
144 in **Fig. 3.** The optical module and fluidics module are mounted inside the unit on both sides (**Figs. 3a-b**). A cooling fan
145 on heat sinks mounted to the optical module. Since there is no natural convective flow in microgravity, the fan is a must
146 for proper device operation. The fluidics module has valving and a pressure regulator for controlling the sample, sheath,
147 and cleaning fluids[42]. Custom printed circuit boards (PCBs) include the main board and detector board with parallel
148 SiPM circuits (**Figs. 3c-e**). The optical module has fixed alignment with all components epoxied in place. No adjustable
149 positioners were used in the design (**Fig. 3f**)[43]. The flow cell is fabricated from low-autofluorescence fused silica with an
150 integrated half ball lens (**Fig. 3g**). A brass flow cell top with press fit hypodermic gauge tubes allow for sheath, burp, and
151 sample connections. This flow cell is integrated into the optical module to allow precise positioning relative to the
152 lasers. The result is a plug-and-play cytometry module that is readily integrated with the rest of the system. Unlike
153 conventional cytometer optics and flow cell, the result is an alignment-free module that maintains relative positioning,
154 at the micron-scale, between the lasers, flow cell, and detectors.

155

156 ***Spaceflight ruggedization and modifications***

157 Specific spaceflight modifications were required as part of the payload development process. The fluidic
158 system had to operate without buoyancy, have minimal air bubbles, and accommodate a 10 µL sample. A pressurized
159 fluidic system specifically designed for microgravity was implemented by using a flexible fluid-filled bag within the
160 sheath and cleaning bottles in the back of the instrument. Using a syringe, the astronaut filled the bags with filtered
161 water and utilized a figure-eight swinging maneuver to remove any air bubbles. The bag was a 0.014 mm thick, easily
162 deformable medical grade balloon, allowing the system to operate at its intended pressure of 70 mbar. Pressure
163 external (P_{vial}) to the fluid bag provides the driving force (P_w) into the device (**Fig. 4a**). Other microgravity considerations
164 included using a disposable waste bag with a unidirectional check valve to contain the test fluids after the runs. This
165 replaced the standard, gravity-based waste bottle. Crew instructions provided key details on achieving bubble-free fluid
166 bags and samples (**Supplementary Fig. 3**). The sample wicking procedure developed for spaceflight required testing
167 with the sample loader. The loader has in-line mechanism that forms a seal around both ends of the sample
168 consumable, which has a 10 µL internal capillary volume (**Fig. 4b**). It generates a defined sample loading fluid profile
169 with a properly wicked sample, marked with Poiseuille flow in the sample's leading edge and a fluid bubble in the back
170 of the sample to allow the entire sample to be delivered to the cytometry module for absolute volumetric particle
171 counts. This specific fluid loading profile is critically important for full analysis of the sample when compared with other
172 loading profiles (**Supplementary Fig. 4**). To meet the ISS electrical requirements, copper tape was applied to the inside
173 walls of the device for electrical shielding and an additional grounding cable was added (**Fig. 4c**). Finally, the system was
174 fully ruggedized to withstand the high vibration and g-loads experienced on launches and returns (see **Supplementary**

175 **Tables 3-4, Supplementary Fig. S5**). All fluidic joints were reinforced with either zip ties or waterproof heat shrink
176 tubing. Electrical connections were reinforced with silicone. These additional modifications, on top of the base design,
177 enabled the device to be space worthy.

178 ***Results on each sample type***

179 The on-orbit raw data for the 3 µm diameter pan-fluorescent Flow-Set Pro Fluorospheres (Beckman Coulter, CA)
180 samples are shown in **Fig. 5a**. The data shows expected results for the five channels: blue, green, orange, forward
181 scatter (FSC), and side scatter (SSC). This particular run lasted 155 seconds before the end bubble showed up in multiple
182 packets. In the first zoom-in, multiple fluorescence and light scattering bursts are seen on a sample data trace. Further
183 zoom-in shows a single event, marked by a particle transiting through the two spatially separated 405 nm and 532 nm
184 lasers. The 405 nm laser is paired with the blue and green channels while the 532 nm laser is paired with orange, FSC,
185 and SSC (**Fig. 5b**). The spatial separation allows any fluorescence not paired with the specific laser to be excluded,
186 minimizing the need for fluorescence compensation due to spectral crosstalk. The peaks are identified by a threshold
187 and then integrated to the baseline, yielding each peak's burst intensity. Each detector channel has its own set of
188 analysis parameters. The FSC channel has a higher baseline than the other channels due to the nature of FSC, which is
189 on-axis with incident 532 nm laser light, with angles between 0 – 2 degrees masked with a beam block. In contrast to
190 most conventional cytometers, the data collected is all digital, allowing for visualization of all the collected signals and
191 greater flexibility in post-processing without *a priori* need to optimize instrument settings. **Fig. 5c** shows the analyzed
192 data presented as a histogram of counts versus log10 burst intensity for the Flow-Set Pro Fluorospheres. A single peak is
193 shown, as expected with a percent robust coefficient of variance (%RCV) of 5.84%. **Fig. 5d** shows the XY scatterplot of
194 blue versus green channels. A primary population shows the majority of the beads (BG1 gate) and a second smaller
195 population shows doublets (BG2).

196 A comparison of data collected preflight on the ground and in-flight on ISS for each detector channel during
197 benchmark commercial cytometer and rHEALTH ONE sample runs with Flow-Set Pro microspheres is shown in **Figs. 6a-c**.
198 The rHEALTH ONE shows similar single peak populations for each of the channels for both ground and flight runs. The
199 fluorescent channel %RCVs for ground and flight were within 2% of each other, with a lower %RCVs for flight SSC and
200 blue channels (**Supplementary Table 5**, all runs **Supplementary Table 6**). The mean bead intensities were similar for
201 green, blue, and FSC channels (± 15 detected photons) whereas the orange and SSC channels were brighter during flight
202 (> + 100 detected photons). The flight FSC %RCV was 1.44% greater than ground and also had a slightly higher
203 background noise. The raw counts for each channel are within < 1% for each channel to one another for both ground
204 and flight, indicating that each pan-fluorescent microsphere is equally detected in all five channels. Prior to flight, the
205 samples were analyzed with a commercial Gallios cytometer, which has multiple lasers (405, 488, 561 nm, 638 nm) and
206 comprises a 104 kg, 95 x 61 x 70 cm main unit, 4 kg 561 nm laser system, and a 30 kg, 72 x 30 x 50 cm supply cart.
207 Similar to the rHEALTH ONE data, the Gallios data shows broader histograms for the green and blue channels and a
208 more uniform orange channel. The rHEALTH ONE showed a predominant single peak for each channel as well as
209 presence of doublets and triplets.
210 The fluorescence resolution and linearity of the system was tested using differentially dyed pan-fluorescent
211 microspheres with three different intensities. This allowed us to characterize fluorescence measurements of the blue,
212 green, and orange channels. The ground and flight data are shown in **Figs. 7a-b**. The low, medium, and high
213 fluorescence beads are distinct for each color channel. For each set, a green channel versus forward scatter scatterplot
214 is included. The scatterplot shows the three populations as well as any coincident events, which can arise when any two
215 beads are in the laser spot together. **Supplementary Fig. 6** describes the counting methodology of each of the
216 populations and includes a Bland-Altman difference plot for the counts for each of the populations. The difference plot
217 shows minimal changes in the relative count numbers, with all counts within -4 to +4% difference, indicating the relative
218 population counts remained consistent. The relative count percentages match well with the Gallios data
219 (**Supplementary Table 7**, **Supplementary Fig. 7**, all rHEALTH ONE runs **Supplementary Table 8**), where the low
220 population is the most abundant population at > 33% of all the beads, as measured on both platforms on ground and
221 on-orbit. The log10 peak burst intensities plotted against the log10 MEF (Molecules of Equivalent Fluorochrome) show

222 linear relationships on all the channels for ground and flight. The dim populations had between 25-164 MEFL. The
223 ability to fully resolve the dim population indicates the system maintained high fluorescence detection sensitivity
224 performance on-orbit.

225 To test the FSC's ability to discriminate between different sizes of microspheres, a mixture of 4, 6, 10, and 15 μm
226 diameter beads (Spherotech PPS-6K) was analyzed. The results of these are shown in **Figs. 8a-b** (**Supplementary Table
227 9**, all runs **Supplementary Table 10**), which shows the scatter channels. In our system, FSC signal strength increases with
228 microsphere size and measures laser scattering around the microspheres. Forward scattered light between 2 and 20
229 degrees is collected, whereas direct laser illumination (0-2 deg) is blocked from reaching the detector. The SSC detector
230 collects light orthogonal to the laser beam (88 to 92 degrees) and, at these angles, is a measure of particle granularity
231 with less size dependence. XY scatterplots FSC versus SSC show four populations that, when gated, show mean FSC
232 intensity to increase with bead size. The on-orbit data has slightly less separation between the 10 and 15 μm
233 microspheres, but more separation between the 4 and 6 μm ones. The Gallios data also shows an increasing
234 relationship for FSC in the XY scatterplot, and good separation for the individual beads (**Supplementary Fig. 8**).

235 Fluorescent compensation beads (OneComp eBeads, ThermoFisher Scientific, MA) were conjugated to
236 fluorophore-labeled antibodies (anti-CD3 V500, anti-CD14 V450, and anti-CD19 PE) to determine the device's ability to
237 resolve multiple colors simultaneously. Both the V500 and V450 dyes are excited off the violet 405 nm laser and the PE
238 with the green 532 nm laser. The ground data shows correct quadrant-based separation of the three combinations of
239 colors (**Figs. 9a-b**). The orange channel shows the unlabeled bead population and a single PE-labeled population. During
240 flight, however, a lower intensity second orange peak unexpectedly appeared, along with increases in orange-green and
241 orange-blue coincidence populations associated with this peak (**Fig. 9c**). The total number of beads remained similar to
242 ground, including labeled and unlabeled fractions (**Supplementary Table 11**, all runs **Supplementary Table 12**). This on-
243 orbit observation prompted the return of the samples back to Earth from the ISS for subsequent terrestrial evaluation.
244 The samples were received 3 months later at Johnson Space Center and post-flight ground Gallios analysis confirmed
245 changes in the sample measured by the rHEALTH ONE on-orbit (**Fig. 9d, Supplementary Table 11**). The quadrant
246 analysis shows an overall decrease in the percentage of unlabeled beads, an increase in the orange percentage and
247 similar proportions of blue and green beads (**Figs 9b-c**). When the quadrant analysis is moved to the right to include the
248 new dim orange peak, the starting percentages are recovered (**Fig. 9d**).

249 A total of 32 runs (4 blank and 28 samples) were performed across the two days of on-orbit operation
250 (**Supplementary Table 13**). Excluding the blank runs, we were able to perform double the number of planned runs (28
251 versus 14) since sample running required less time than anticipated. Of these runs, 26/28 yielded good quality data in
252 all channels. One sample without data was a designated practice run on the first day of operation. An incomplete run
253 also occurred when the sheath fluid ran low. The runs had an average duration of 143.10s and an average of 71.55
254 million raw data points.
255
256 **Discussion**

257 A microgravity-capable microvolume flow cytometer demonstration unit, a spaceflight modified rHEALTH ONE,
258 was demonstrated on the ISS. The device utilized 517x less power, 183x less volume, 92x less mass, and 166x smaller
259 sheath reservoir than the ground-based benchmark Gallios cytometer (**Table 1**). This low-resource utilization allowed it
260 to be launched to space. It was tested over two days on-orbit with samples that characterized its performance. More
261 sample runs were performed than originally planned (**Supplementary Table 14**). The device was able to meet the
262 predefined criteria for a successful hardware technology demonstration (**Supplementary Table 15**): data collection in all
263 five channels, greater than or equal to three runs per sample, demonstration of sheath-based hydrodynamic focusing,
264 complete analysis of each sample, and data comparable to ground data.

265 The successful operation of the device highlighted several important technical developments that enable single
266 drop cytometry in microgravity. The use of all solid-state lasers and detectors with fixed alignment optics allowed
267 achievement of a highly miniaturized cytometry module that maintained sensitivity to dim events. This module
268 tolerated the complex vibration and g-profiles on rocket launch and subsequently, once onboard the ISS, allowed
269 collection of cytometry data simultaneously from the five photon counting detectors in microgravity. The microgravity
270 loading procedures were used to minimize the amount of air bubbles in the system, and the engineering of the fluidic
271 system allowed sheath flow-based hydrodynamic focusing, the standard of conventional cytometers, to be performed.

272 An in-line microvolume sample loader that worked with a microgravity wicking procedure enabled the repeated loading
273 of microvolumes of sample into the device, without any excess unused volume.
274 The rHEALTH ONE ISS demonstration advanced the current knowledge about performing flow cytometry in
275 microgravity. Small drops of sample, that were manifested separately in dropper bottles, were wicked into sample
276 consumables and successfully analyzed over the course of multiple on-orbit runs with the rHEALTH ONE device. This is
277 in contrast to the Microflow1, where cartridges had to be preloaded with a larger volume of sample (1.6 mL) prior to
278 flight and were susceptible to micro-bubbles which rendered some of the samples unusable [29]. The rHEALTH ONE device
279 increases the possibility for the inclusion of cytometry capabilities during space exploration missions by offering a
280 miniaturized, free-space laser optics approach that offers greater flexibility than the Microflow1's integrated fiber-optic
281 flow cell. The Microflow1's fixed geometry limited the total number of fluorescent channels and prevented the addition
282 of the critical FSC channel. The rHEALTH ONE's cytometry module allowed for a second laser, two more detector
283 channels, including FSC. The rHEALTH ONE demonstrates sheath-flow hydrodynamic focusing cytometry in microgravity.
284 To do this, a fluidic system was developed to minimize bubble interference and to control microvolume sample loading.
285 The benefits of a sheath-based system is one-by-one delivery of cells and particles through the laser excitation region.
286 The sheath-based system allows the sample to be pulled away from the wall of the analysis channel, which increases
287 sample velocity uniformity and decreases the risk of cell aggregation at the zero-velocity boundary condition of the
288 channel wall. The low pressures used to push the fluids through the system (70 mbar) minimizes the required amount
289 of filtered water sheath per run. The minimal, best-case amounts of required sheath water include 2.44 mL for the
290 startup prime and 1.13 mL for each subsequent run (**Supplementary Table 16**). The use of water as the sheath allows
291 the approach to be compatible with potable water sources on spacecraft, as long as it is adequately filtered at the level
292 used in the experiments.
293 Future improvements or additions are envisioned that can further improve or augment performance. Over an
294 hour of each of the test sessions was used for filling the fluid bags and removing the air bubbles, the success of which is
295 operator-dependent and where improper filling can result in undesired air bubbles. Pre-filled, gas impermeable fluid
296 bags could streamline instrument operation. Additionally, the instrument setup requires manual priming of the system
297 through the burp port. While this maneuver is short in duration, it could be automated to improve usability, especially
298 since it is required during device startup after having been stowed, facilitating start-up after launch or between
299 extended on-orbit uses. Incorporation of even more rigid optical elements will be important since a slight movement
300 likely resulted in the higher FSC channel noise observed on-orbit compared to on the ground. Biological test samples
301 could be evaluated in the future. This would require meeting NASA's biohazard containment constraints and also
302 necessitate the use of microgravity-compatible sample preparation devices, such as the easy-to-use Whole Blood
303 Staining Device [44], or automated microfluidic methods [37], both of which utilize sample volumes compatible with capillary
304 blood sampling. Assay capabilities can be expanded to include key tests envisioned for exploration missions, including
305 blood chemistry, blood counts, cardiac biomarkers, urine analysis, liver function, kidney health, and coagulation[45]. The
306 software could be improved with automatic processing of raw data into burst intensities and addition of features
307 familiar to flow cytometry scientists such as user-friendly approaches for gating, thresholding, compensation, detector
308 voltage adjustment, and laser intensity control. The data analysis could be upgraded to provide results in a readily
309 interpretable format for astronaut users, especially for cell counts, cell subpopulations, cell parameters, highly-
310 multiplexed biomarkers with differentially-dyed microparticles [23] or nanostrips [26], and other test panels used to guide
311 clinical decision making.
312 The ability to analyze biomedical samples via in-flight lab analysis throughout a mission has been a long-standing
313 aim of NASA's Human Research Program [46]. The vast majority of our understanding of space medicine biomarkers,
314 blood cell changes, immune function, and cell-based biology is derived from downmassed samples, which require a long
315 journey back to Earth prior to analysis in a central lab. Samples may degrade, change, and become unreliable during
316 transit. This was observed with the fluorescent compensation beads, which was the only sample used in the rHEALTH
317 ONE ISS demonstration that had a biological component to it. Varying sample storage conditions likely led to the
318 observed changes in the sample. This could have been from room temperature sample storage en route to and onboard
319 the space station and/or from the higher doses of radiation (1 mSv per day on the ISS[47] versus 2.4 mSv per year on
320 Earth[48]). Either of these could have led to desorption of the antibodies and subsequent reattachment to the unlabeled
321 fraction of the fluorescent compensation beads. Ionizing radiation could have altered the surface charges on the beads

322 and non-refrigerated temperatures could have increased the desorption kinetics. Protein degradation could have also
323 been accelerated. High g-loads and vibration during transit is less likely a possibility given that laboratory samples
324 typically are subject to mixing and vortexing without issues. Our challenges with this sample has been observed with
325 other space-based biological samples. For instance, the harsh transit conditions during sample transport for the NASA
326 twins study resulted in loss of telomerase activity from samples [14].
327 On-orbit biomedical analysis would aid a more complete understanding of spaceflight biology by providing
328 timely information on freshly acquired samples. Flow cytometry was used as the core analytical modality since it has
329 versatile and diverse applications, ranging from blood cell counts, immunophenotyping, multiplexed biomarker assays,
330 bacteria/virus analysis, and general particle sizing (which could be used for lunar or Martian dust). Given the highly
331 limited in-cabin resources and the inability to rely on Earth for analysis support or resupply, a single instrument that can
332 achieve the greatest assay diversity and multiplexing is desirable. Aside from research studies, a microvolume
333 cytometer that can analyze self-collected capillary samples can guide critical preventative and emergent medical
334 decision making. Sample return challenges become exponentially more daunting, if not impossible, as we perform
335 missions that return us to the Moon and travel deeper into space to Mars. These challenges of space are also analogous
336 to those on Earth in minimally resourced settings such as developing countries, satellite labs, pharmacies, and homes,
337 where point-of-care analysis is desirable. The rHEALTH ONE ISS demonstration provided a step forward in realizing
338 immediate and actionable biomedical information in environments where no cytometer has gone before.
339
340 **Methods**

341 *Flight sample preparation.* All samples were prepared in sterile conditions, bottled separately as 1 mL of
342 solution in 3 mL dropper bottles (United States Plastic Corp. P/N 66529), protected from direct light, and stored at 2-8°C
343 for longest shelf-life before being delivered for launch at ambient temperature. Samples A: OneComp eBeads, B:
344 Spherotech PPS-6K, and C: Spherotech 3-peak fluorescent standards were prepared by the JSC Immunology lab. Sample
345 D: Flow-Set beads was prepared at ZIN technologies since it was a simple process and reduced shipment of materials.
346 Samples were diluted to a concentration of beads (polystyrene microspheres) that allowed the rHEALTH ONE analyzer to
347 be set to one target pressure (1 psig, 70 mbar) throughout testing.
348 For Sample A, 5.0 mL of OneComp eBeads (Thermo Fisher P/N 01-1111-42, Lot 2297369) ~4µm in diameter were
349 stained in three separate batches with one color of fluorophore-conjugated antibodies each, washed thoroughly, then
350 resuspended together in 20.0 mL Cell Culture Grade Water (Sigma Aldrich W3500-100ML, Lot RNBK3069). 50 µL of Food
351 Color & Egg Dye - Blue (McCormick UPC 52100071077, Lot FEB 10 25 H 03:48) was added then 1 mL of the final solution
352 was transferred into each dropper bottle. Antibodies used for staining (all are mouse IgG1, kappa isotype control):
353 CD19 PE (Tonbo Biosciences P/N 50-0199-T100, Lot C0199110320503), CD14 V450 (Tonbo Biosciences P/N 75-0149
354 T100, Lot C0149092019753), CD3 V500 (Tonbo Biosciences P/N 85-0038 T100, Lot C0038012221853). Each drop of
355 beads has a positive population that captures the mouse antibodies and a negative population that does not react with
356 the antibodies.
357 For Sample B, 1.0 mL of each size - 4, 6, 10 and 15 µm in diameter - from the Polystyrene Bead Particles, Size Mix
358 (Spherotech P/N PPS-6K, Lot AM02) were mixed then diluted by the addition of 16.0 mL Cell Culture Grade Water (Sigma
359 Aldrich W3500-100ML, Lot RNBK3069). 50 µL of Food Color & Egg Dye - Blue (McCormick UPC 52100071077, Lot FEB 10
360 25 H 03:48) was added then 1 mL of the final solution was transferred into each dropper bottle.
361 For Sample C, 5.0 mL of Rainbow QC Calibration Particles, 3 peaks (Spherotech P/N RQC-30-5, Lot AL01) were
362 diluted by the addition of 15.0 mL Cell Culture Grade Water (Sigma Aldrich W3500-100ML, Lot RNBK3069). 50 µL of Food
363 Color & Egg Dye - Blue (McCormick UPC 52100071077, Lot FEB 10 25 H 03:48) was added then 1 mL of the final solution
364 was transferred into each dropper bottle.
365 For Sample D, 2 µL of Food Color & Egg Dye - Blue (McCormick UPC 52100071077, Lot FEB 10 25 H 03:48) was
366 added to each dropper bottle then 1 mL of Flow-Set Pro Fluorospheres (Beckman Coulter P/N A63492, Lot 3941176F) at
367 full concentration was transferred into each dropper bottle.
368

369 *Sample loading and running.* After a gentle inversion process, the individual test samples in dropper bottles
370 were dispensed onto polyimide tape adhered to the work surface. The rHEALTH ONE sample consumable tip was
371 tapped against the drop to fill it by capillary action. The presence of blue dye in the samples allowed visualization of a
372 proper 10 µL capillary fill. The door to the rHEALTH ONE was actuated to allow the sample consumable to be loaded.
373 Closing the door forms seals around the end of the sample consumable via gaskets, allowing it to be in-line with the
374 fluidic system. The sample loading mechanism forms a fluid-fluid interface at the leading edge of the consumable and
375 an air-fluid interface at the trailing edge. Pressure-driven flow at 70 mbar (1.0 psig), actuated through the *rHEALTH*
376 *Capture v59e4* was utilized to drive the sample through the device's optical block. The full 10 µL of sample was analyzed
377 within 3 minutes and the files stored in a .TDMS format suitable for *rHEALTH Viewer v.26.2f_exporthistogram* analysis.
378 FCS files were exported for analysis and visualization on FlowJo (Becton Dickinson, OR).
379
380 *rHEALTH ONE device description.* The base rHEALTH ONE device (purchased from rHEALTH Inc., MA), developed
381 with NASA support by authors from DMI and rHEALTH, has its specifications listed in **Supplementary Table S1.** The
382 device measures 13.4 x 17.8 x 13.0 cm and is 1.5 kg. Power and data are supplied with a USB 2.0 port on the back of the
383 device. Pressure to the vials is provided by a small DC-powered eccentric diaphragm pump inside the unit. The lasers
384 are 405 nm violet 5 mW and 532 nm green 20 mW lasers. Each of these have a rate > 5000 hours of operational life.
385 The sample consumable allows a minimum of 5 µL per sample and up to 10 µL (as demonstrated on the ISS). The sample
386 flow rate can be adjusted by changing the pressure on the device and it can range from 2 – 10 µL per minute. As
387 operated on the ISS, the sample flow rate is approximately 3-4 µL per minute, which is considered a very low flow rate.
388 The system on the ISS operated with 70 mbar to achieve this low flow rate. Low pressure operation allowed
389 minimization of the use of fluids. The particle throughput can be up to 1000 events a second. As operated on the ISS,
390 the event rate was below 100 per second. The event rate is dictated in part by the sample concentration, which can
391 range from 10^4 to 10^7 particles per mL. The unit has a dedicated in-line, zero dead volume sample loader. The fluidic
392 system is rinsed with the fluid in the cleaning bottle after each use. On the ISS, the cleaning compartment was water to
393 remove the need for double containment of the device. The device supports up to a five-log assay dynamic range and
394 has two software modules, the *rHEALTH Viewer* for visualization and the *rHEALTH Capture* for data capture and device
395 operation. The data output is all digital, which is different than analog-based cytometers. This allows for rethresholding
396 and also changing analysis parameters. This flexibility bypasses the need to optimize run parameters prior to runs and
397 offers additional thresholding capabilities after the runs. The device supports the use of nanostrips, lumibeads,
398 multiplexed microspheres, and cell-based assays. On the ISS, contrived samples had to be utilized to avoid the need to
399 address biosafety considerations.
400 The optical module is mounted on the inside wall of the unit, in close proximity to the sample loader to minimize
401 the transit time of the sample to the flow cell. This mounting configuration also allows the optical module to be cooled
402 by a fan placed at the base of the instrument. This provides cooling to the heat sinks, which are thermally coupled to
403 the optical module and both lasers. The fan intake is from the bottom, blowing up through the top of the hinged bottle
404 assembly. The lack of buoyancy in zero gravity necessitates fan-based cooling since warm air does not rise to the top.
405 No peltier-based cooling is needed if the device is operated within the bounds of 15° to 35°C. The system requires
406 warm-up to stabilize the lasers. The heat generated by the printed circuit boards (PCBs), lasers within the enclosure
407 allows a steady-state to be attained after 20 minutes of power applied to the lasers. On the other side of the unit is a
408 fluidics module that has integrated low-power solenoid valves and a two-stage pressure regulator that is motor-
409 controlled. Closed loop feedback with a pressure sensor allows for precise pressure regulation.
410 The electronics inside the unit consists of multiple custom PCBs, including a main, detector, and LED PCB. The
411 main PCB is at the bottom of the unit and manages the control of the device, including valving, fan control, pressure
412 sensors, and data. A Microchip SmartFusion System on a Chip (SoC) with Field Programmable Gate Array (FPGA)
413 controls the primary functions, including data transfer from the detector PCB to the main board and from the main
414 board to the PC connected via USB. Wires connect the main PCB to a door sensor, which determines the door state
415 (open or closed). The optical module has its own separate detector PCB, which is mounted in close proximity to the

silicon photomultiplier detectors (SiPMs, Hamamatsu, JP). The detector PCB has its own microcontroller, a dsPIC33 operating at 70 MHz with 128 kb program memory size, 4 Direct Memory Access (DMA) channels, multiple analog-to-digital converters (ADCs), and pulse-width modulation (PWM) channels. This dsPIC33 takes data coming from the silicon photomultipliers and shuttles them to the main board. This allows operation of the detector board at the desired data rates of 10 µS bin intervals. The five SiPMs are supplied from a common high voltage. The individual channels have their bias voltage set by adjusting the positive input to the opamp, so that actual bias voltage is the common high voltage minus the adjustment voltage. The output is a negative outgoing pulse that is AC coupled to the gain stage. The SiPMs are capable of detecting single photons and they can self-quench within about 60 ns. The SiPMs are mounted on individual flex circuits to allow each detector to be individually aligned. Each flex board has a separate pre-amplifier. The pulse output from the preamps are further amplified with an opamp set for a gain of ten. These amplified signals are then fed into comparators, and the outputs of the comparators are used as triggers for counters within the dsPIC33. The counts are stored with sample periods of 10 µS, which is reset every 10 µS for continuous measurement. The counts are stored as a single byte of data for each channel. This data is sent to the main PCB in real-time over an SPI channel, allowing collection and real-time visualization of this data on the PC using the *rHEALTH Viewer.* The detector PCB also contains laser driver circuits that allow the output of the lasers to be adjusted and monitored. The laser outputs are controlled by an active circuit that adjusts the current passing through each laser diode. Each laser also has a thermistor mounted on its flex board to track the temperature of the laser and correct brightness variations with temperature.

The optical module has two compact, commercially-available diode lasers (405 nm 5mW, Egismos, Taiwan and 532 nm 20 mW Snake Creek Lasers, PA). These are at right angles to each other and combined using a dichroic filter. The collimated lasers are shaped into elliptical laser spots (200 µm x 10 µm) by a pair of cross cylindrical lenses. An achromat focuses the lasers into the rectangular fused silica flow cell (Hamamatsu, JP). The laser spots are offset by 400 µm (approximately two channel's width) to decrease spectral crosstalk and decrease the need for fluorescence compensation. Fluorescence and side scatter is collected by an integrated lens that is fabricated with the flow cell. This allows for high numerical aperture light collection. This light is directed to a set of dichroic and bandpass filters that color separate the three color channels and the side scatter laser light. This light is focused onto the SiPMs connected to the preamp flex boards. The forward scatter channel has separate optics and light collection. A neutral density filter and beam block attenuates the direct laser light, allowing it to be measured also by a photon-counting SiPM.

Several design considerations made this optical module tolerate the vibration and g-profiles during launch and also in zero gravity. These include the low mass of each of the components in the module. This minimizes the amount of force exerted on fasteners and epoxy joints. None of the components were adjustable. This in contrast to conventional cytometers that generally have screw adjusters and motion control for aligning the system in the field. The use of all solid-state components meant the greatest level of miniaturization possible. Conventional photomultiplier tubes have a photocathode with multiple physical stages of anodes and dynodes for signal amplification. In SiPMs, this amplification is within the silicon. The flow cell is integrated into the optical cytometry module. This was possible since this flow cell and connectors were miniaturized with a custom flow cell top fitted with hypodermic gauge pins. This approach minimizes fluid volume usage, air bubble trapping within connectors, and allows for integration with the optical module. Typically, the flow cell is separate from the optics in larger cytometry systems, but in our system, because of the fixed alignment approach, it necessitated an integration with the module.

Specific device modifications required for spaceflight. A number of modifications were made to the rHEALTH ONE device to ensure safe and functional operation in the microgravity environment aboard the ISS. To address any potential fluid leaks from the fluidics system, 1.80mm wide zip ties (PLT.6SM, Panduit, IL) were used to secure polymer tubing to pins and barbed connections. Additionally, any plastic tubing-to-tubing connections were strengthened with 3.175mm wide, adhesive-lined 3:1 heat shrink tubing. To safeguard the PCBsagainst water damage, DOWSIL^{TM} 3140 clear RTV was thinned with an epoxy thinner (xylene) and applied to the PCBs using a high-volume low pressure, gravity fed spray gun. DOWSIL^{TM} 3145 gray RTV silicone was dispensed on to all fasteners, threaded parts, and PCB connections for additional strength to withstand the forces/vibrations at launch and return. This same epoxy was also used to seal

463 the optical block and reduce any dangerous laser light leakage. Two Velcro strips were applied to the device's lid to
464 prevent unwanted movement in zero-g. To keep the device stationary during mission operations, standoffs with Velcro
465 on the underside were adhered to the bottom of the device.
466
467 *Fluidics for zero gravity bottles, waste, and reservoir.* Several modifications were made to the external fluidics
468 and procedures were developed to separate, direct, and contain the air and liquid during transfer and operation. Tubing
469 lines that handled liquids were adapted to Luer lock connections that would be securely connected to mating Luer lock
470 bags and plastic syringes but also easily connected and disconnected by crew. A Luer lock bag with a self-sealing valve
471 (Origen PL120-2G that includes an Origen NFV) was used to transport the liquid (Sigma-Aldrich W3500 cell culture grade
472 water) needed for operations to the ISS and the same part was used to replace the waste bottle in capturing liquid
473 waste. ISS safety requirements require the liquid to remain contained, not free-floating in the cabin. A self-sealing Luer
474 lock (Origen NFV) was added to the priming line and tubing extensions were added to both priming (IDEX P-850,
475 Masterflex 06407-71, IDEX P-857, Origen NFV) and waste (IDEX P-857, Masterflex 06464-90, Masterflex 30505-92) lines
476 for maneuverability during operations and visibility of the flow of liquids and air bubbles. The fluid bag assembly inside
477 the bottles was made from two medical balloons (Nordon Medical 20005500CA) with walls at 0.014 mm thick, but
478 durable across thousands of inflation/deflation cycles. These were connected by a barbed Y connected (IDEX P-860),
479 1/8" OD 1/16" ID Tygon tubing (Masterflex 06407-71), adapter (IDEX-P-857) and Origen NFV. This design directly utilized
480 as much of the bottle volume as possible (requiring less refilling) while still being compressible by 70 mbar (1 psig) of air,
481 small enough to pass through the bottle neck and accommodate the necessary Luer adaptors, and flexible enough to be
482 filled and connected through a complex sequence of steps over multiple uses without leaking. The original filters were
483 removed and modified to allow in-line Luer lock connection (IDEX P-235, IDEX P-200, IDEX P-675) between the device
484 and each bag assembly.
485
486 *Device and sample transport.* The unit was purged with air and packed dry for transport. Samples were
487 prepared 2021-10-29 and 2021-12-02, kept refrigerated at 4°C then transported at ambient temperature starting ~2021-
488 12-20 for launch, operations, and return to ground. Launch was 2022-02-19 and operations 2022-05-13 and 2022-05-16.
489 Samples were returned at ambient temperatures 2022-08-20 and analyzed at NASA's Johnson Space Center. The
490 rHEALTH ONE analyzer and the water bags were all packed dry for launch to avoid developing fluid bubbles. Filling the
491 rHEALTH Water Bags on ground and pre-loading them in the rHEALTH Bottles was considered but due to the permeable
492 materials they would develop air pockets that would require removing and refilling them on-orbit, negating the benefit.
493
494 *Payload development.* Payload development followed the NASA's experiment flight hardware development
495 process, for modified Commercial-Off-The-Shelf (COTS) devices. In order to qualify for this approach, the device had to
496 be commercially available. This included hazard and safety analysis unique to the payload to determine operational
497 requirements and containment then verification to ensure the requirements were met. This included vibration testing
498 according to launch loads to verify the lasers and frangible material inside the device remained contained and required
499 reducing the electromagnetic interference (EMI) over the specified ranges reserved for ISS systems by applying copper
500 tape. Specifically 0.04mm thick, RF EMI shielding copper foil PSA tape (Parker Chomerics, CCH-18-101-0100) was applied
501 to the interior of the unit's housing. The fan opening was covered with a 150 per 2.54 cm, stainless steel mesh screen to
502 complete this Faraday cage. Sections of the PCBs that came in contact with the housing were additionally protected
503 with Kapton® tape to insulate against electrical shorts. A one-meter-long ground wire was secured to the inside of the
504 device for adequate grounding.
505 Further modifications and controls set in the procedures ensured all fluids would remain contained according to
506 the level of containment required by their NASA toxicology and biohazard assessment. Since activities required great
507 dexterity and visual acuity with many small parts and clear materials, it was decided to tailor the experiment to allow
508 crew operations to occur in the cabin instead of a glovebox. Water as the sheath and cleaning fluid and TOX 0 samples
509 were used to reduce the biohazard risk to the crew. Cell culture grade water (de-ionized and sterile) and sterile samples

were chosen to prevent occurrence of clogs such as from minerals or biofilms. Biological samples such as blood or saliva were not used, which would have added uncertainty to the measurements and needed greater than TOX 0 cleaning fluid after running. This demonstration focused on the analyzer's ability to function in microgravity, testing the underlying fluid dynamics of sheath-based focusing in microgravity, and its performance compared to ground.

Crew training. Crew training was only for the data collection portion of using the device (no sample preparation, no adjustment of settings, no *rHEALTH Viewer* user processing of the data, etc) and was a 30-45min period to review a summary PowerPoint, the procedures, and short videos of main steps. Payloads are designed to minimize crew time required, including training. The crew was guided through all steps with the majority of the work done by the ground team (samples tailored to the device, sample preparation, tailored procedures, real-time verification and troubleshooting, post-run data processing and analysis, etc.).

Data analysis. The data analysis was performed using the *rHEALTH Viewer v26.2f_exporthistogram* (rHEALTH, MA) with peak calling settings that were preset for each of the samples. The same peak calling parameters were utilized for both the ground and the flight data to avoid any inconsistencies. The peak files were exported in Flow Cytometry Standard (FCS v3.1) for further visualization, gating, and statistics in FlowJo v10.8.1 (Becton Dickinson, CA). Graphs were either created in FlowJo or in GraphPad Prism 9.5.1 (Dotmatics, MA). Collected data was organized into tables for further analysis using Microsoft 365 Excel (Microsoft, WA) and Google Sheets (Google, CA).

Statistics & Reproducibility. The samples were selected to test the flow cytometry performance of the rHEALTH ONE for ability to resolve fluorescence intensity, microsphere size, fluorophores, and bead populations. At least N = 3 triplicates of the four samples were analyzed. The samples were blinded to the authors at DMI and rHEALTH until the day of the on-orbit operations. Two runs were excluded from analysis: one was a practice run that did not yield any data, another was an incomplete run that resulted from running out of sheath. The total number of samples was limited by astronaut crew time and the two days of operation was designed to attain at least minimum of triplicates. No statistical method was used to predetermine sample size. The sequence of the sample runs were not randomized.

Data Availability

All data supporting the findings of this study are available within the article and its supplementary files. The rHEALTH ONE Flow Cytometry Standard (FCS) source data for the ground and flight tests are available via FlowRepository[49], under accession FR-FCM-Z76L (http://flowrepository.org/id/FR-FCM-Z76L). Source data filenames are provided in the Source Data File. Any additional requests for information can be directed to, and will be fulfilled by, the corresponding authors.

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Author Contributions

DR performed final assembly, device testing, and data analysis. RM performed device testing and developed fluid bags, flight protocols, and data analysis. RM and RV performed flight qualification and protocols. SC performed the on-orbit experiments. ZS assembled and tested the optical cytometry module. JC graphed the data. RY performed device testing. KC managed the flight project. SB wrote the *rHEALTH Capture* and *rHEALTH Viewer*. DE designed the electronics and wrote the device firmware. BC developed the sample protocol, formulated the flight samples and performed benchmark testing. EN reviewed the project, BL reviewed the project, GP reviewed and managed the project, KL contributed to the conception of the project and reviewed the paper, BE contributed to the conception of the project and reviewed the paper, EC designed the device and wrote the paper.

652

653 **Competing Interests Statement**

654 EC is an equity holder of DMI, rHEALTH, and listed as an inventor on US Patents 9194780, 9617383, 9568425, 9835542,
655 D785781, 10180442, 10279347 B2 related to the technology. The remaining authors declare no competing interests.

656

657 **Table 1.** ***Instrument resource utilization comparison.*** The resource utilization of the rHEALTH ONE and benchmark
658 Gallios cytometer is listed as well as the fold-reduction in resource utilization.

659

660 **Fig. 1.** ***Summary of the logistics of the rHEALTH ONE on-orbit demonstration.*** *Left,* Pre-launch phase included
661 calibration of the unit, ground testing, packing, transportation to the launch site, launch and ISS docking. Antares rocket
662 image credit: Northop Grumman. *Middle*, Orbital demonstration phase included unpacking, analysis of the samples in
663 microgravity with the rHEALTH ONE, and data downlink to NASA Glenn Research Center. *Right,* Post-demonstration
664 phase included cleaning and stowage of the unit, downmass of test hardware and samples, and adjudication of
665 discrepant samples.

666

667 **Fig. 2.** ***Overview of the rHEALTH ONE experiment on the ISS.*** **a**, The rHEALTH ONE during device setup by ESA astronaut
668 Samantha Cristoforetti. Image courtesy of NASA. **b**, Sample drop shown being wicked up using the capillary-based
669 sample consumable, which is then loaded into the sample loader, located in the front of the device. The device is 1.5 kg
670 with dimensions of 13.4 x 17.8 x 13.0 cm. **c**, A compact, sheath-flow cytometry module, with a US quarter for size
671 reference, performs the measurements. Sheath-flow hydrodynamic focusing aligns cells and particles for one-by-one
672 analysis through a 405 nm and a 532 nm laser. **d**, The on-orbit runs were performed on a Human Research Facility (HRF)
673 shelf and data streamed to an ISS laptop running the *rHEALTH Capture* software program.

674

675 **Fig. 3.** ***rHEALTH base device with detail of subsystems.*** **a**, Transparent image of the device, showing the location of the
676 optical module in the unit. The 532 nm laser and associated copper heat sinks are directly in the path of the fan. **b**,
677 Fluidics module is located on the opposite side from the optical module. It is a microfluidic assembly with a bank of five
678 low-power latching valves and a motorized pressure regulator. **c**, Main PCB that controls the device and sends data to
679 the USB-attached PCB. The valve drivers are on the side of the fluidics module. Supercapacitors allow for additional
680 power for valve switching. A field-programmable gate array (FPGA)-based system on a chip (SmartFusion) provides
681 computing. Wires lead to a door sensor. A flat flex connector (FFC) provides data and power between the main PCB and
682 the detector PCB. **d**, The back of the optical module has an integrated detector PCB with attached flex boards with
683 preamplifiers, powered by the high-voltage (HV) supply. The detector PCB has a separate microprocessor (dsPIC33) to
684 provides counters for photon counting. Additional features are labeled on the figure. **e**, Preamplifier circuit schematic.
685 Photons captured by the SiPM results in a detectable signal after the op amp. HV, ground (GND) provide power input
686 and the photon counting signal is the Out. **f**, Inside the optical module showing the locations of the 532 nm and 405 nm
687 lasers, bandpass filters, dichroic filters (DC), lenses, and overall layout. **g**, *Left,* detail of the flow cell showing the
688 retaining ring, flow cell top (with burp, sample, and sheath inlets), and fused silica flow cell with integrated lens. *Right,*
689 fused silica flow cell image with epoxied brass flow cell top as in the graphic.

690

691 **Fig. 4.** ***Spaceflight modifications required for on-orbit operation.*** **a**, Pressurized clear plastic bottles provide the driving
692 force for filtered water inside flexible bags. *Left image,* The air space between the bottle and the flexible bags is
693 pressurized (P_{vial}) and fluid pressure inside the bag (P_{water}) drives the sample into the cytometry module. *Middle image*,
694 Filled fluid bag with the connectors prior to assembly into the back of the unit. *Right image,* Back of the device showing
695 the sheath and cleaning bottle assemblies (each with filtered water), a waste line prior to connection to the waste bag,

696 and a priming port. **b**, Schematic of the sample consumable, sample loading, and instrument block diagram. The sample
697 consumable containing 10 µL sample (blue) is loaded into the sample loader that generates a fluid-to-fluid interface at
698 the leading edge and an intentional air bubble behind the sample. The sheath bottle holds water that drives the sample
699 into the optical block and to waste. The water in the cleaning bottle rinses the system between runs. **c**, The device
700 (shown on a ground HRF shelf) had an additional grounding cable attached. Electromagnetic shielding (copper tape)
701 installed on the inside of the device.
702
703 **Fig. 5.** ***Data output for a Flow-Set microsphere run.*** **a**, Raw data from five channels, collected in 10 µs intervals,
704 visualized in the *rHEALTH Viewer*. The sample is Flow-Set microspheres. The top shows the full run, which lasts for 155
705 seconds prior to the appearance of the trailing edge air bubbles. The channel colors are as follows: purple (FSC), cyan
706 (SSC), dark blue (blue channel), green (green channel), and orange (orange channel). The middle shows a zoom in to 3
707 seconds of data, allowing individual events to be seen. The bottom is a 4 ms window showing a single event. The first
708 peak is the transit of the 3 µm microsphere through the 405 nm laser (blue, green channels) and the second is through
709 the 532 nm laser (FSC, SSC, and orange channels). **b**, The software analyzes burst intensity for each peak after
710 determining a suitable threshold (dashed lines) and baseline (bold dashed lines). For illustrative purposes, only the
711 green and orange channels are shown. The burst intensity is integrated to the baseline. **c**, The orange burst intensities
712 are plotted on a histogram showing number of counts (y-axis) at each log10 burst intensity bin (x-axis). A histogram gate
713 (O1) highlights the singlet population. **d**, XY scatterplot of the blue and green channels on a log10 versus log10 burst
714 intensity plot. Individual populations are gated for statistics. BG1 outlines the single events and BG2 outlines doublet
715 events. Source data filenames are provided in the Source Data File.
716
717 **Fig. 6.** ***Flow-Set calibration beads data.*** **a**, Ground benchmark cytometer data for all five corresponding channels
718 showing, from left to right, blue, green, orange, SSC, and FSC channels. Horizontal gates are shown. **b**, Ground rHEALTH
719 ONE data for the same series. Gates are labeled based on their channel and exclude the coincident events. **c**, Flight
720 data for the same channel series. The log histograms are base 10. Source data filenames are provided in the Source
721 Data File.
722
723 **Fig. 7.** ***Results for rainbow calibration particles with three different fluorescence intensities, from dim to bright.*** **a**,
724 *Left-to-right,* Preflight ground rHEALTH ONE data for log10 blue, green, orange burst intensity histograms (low, medium,
725 high populations are identified with gates labeled with 1, 2, and 3 respectively); XY scatterplot of FSC versus green with
726 the singlets gated for low, medium, and high fluorescence with gates ending in 1-3, respectively); and a log10 expected
727 molecules of equivalent fluorochrome (MEF) versus log10 photons detected graph. **b**, On-orbit rHEALTH ONE data for
728 the same series. Source data filenames are provided in the Source Data File.
729
730 **Fig. 8.** ***Results for particle size standards of 4, 6, 10, and 15 um microspheres.*** **a**, *Left-to-right,* Preflight ground
731 rHEALTH ONE data for XY scatterplot of FSC versus SSC with gates numbered 1-4 from smallest to largest bead; FSC
732 histograms of the XY scatterplot gated populations; and graph of bead size (µm) versus photons detected. **b**,
733 Corresponding rHEALTH ONE on-orbit flight data for set of four microspheres. Source data filenames are provided in the
734 Source Data File.
735
736 **Fig. 9.** ***Fluorescence compensation standards with dye-conjugated antibodies, anti-CD3 V500, anti-CD14 V450, and***
737 ***anti-CD19 PE.*** **a**, *Left-to-right,* Ground preflight benchmark cytometer data showing scatterplots for blue-green, orange-
738 green, orange-blue, and a log10 histogram of orange FL2 burst intensities. **b**, Corresponding rHEALTH ONE preflight
739 ground sample analysis. A transparent color overlay is included to highlight the quadrants that are unlabeled (i.e. BL-,
740 GR-), labeled with a single color (i.e. GR+, BL-), or labeled with both colors (i.e. GR+, BL+). The color channel
741 abbreviations are green (GR), blue (BL), and orange (OR). **c**, Corresponding rHEALTH ONE on-orbit flight data. **d**, Ground
742 postflight benchmark flow cytometer data. The quadrant analysis is moved to match the percentage of beads seen in
743 the preflight analysis. Source data filenames are provided in the Source Data File.
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