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Abstract

Sustained deep-space exploration under space programs places critical constraints on pharmaceutical and nutritional resupply, motivating fluid-processing systems, including emulsification, that can operate reliably in microgravity. Conventional terrestrial emulsification relies on high-shear, buoyancy-assisted processes unavailable in free fall, while passive, capillary-driven static mixers offer a compact, low-power alternative better suited to spaceflight constraints. This paper reports the design, ground verification, and on-orbit validation of TIGERS-X, a low-cost, teleoperated lab-on-chip experiment cube for the International Space Station (ISS). Ground testing, including chemical safety, electromagnetic compatibility, and structural verification, confirmed compliance with ISS pressurized-payload requirements. TIGERS-X uses multi-channel Tesla-valve microfluidic devices and optical diagnostics to observe oil-water mixing and emulsification behavior, operating under ground control via YAMCS-based command and telemetry with no astronaut interaction required beyond installation and removal. Across a 10-day flight campaign, TIGERS-X returned near-real-time video of fluid behavior under sustained microgravity, resolving a duration and environment inaccessible to prior parabolic-flight studies. Quantitative colorimetric analysis revealed substantial, geometry-dependent oil-water phase redistribution within short microfluidic channel lengths, extending prior ground- and parabolic-flight-based findings to realistic orbital conditions. These results establish TIGERS-X as a validated, accessible platform for microgravity fluid science, with potential extension to more complex biological applications such as cell culture systems in future missions.

Keywords: Emulsification, Fluids Science, ICE Cubes Facility, International Space Station, Lab-on-Chip, Microgravity.

Introduction

The prospect of sustained human exploration of the Moon and Mars under the Artemis program and NASA's Moon to Mars architecture demands a fundamental reassessment of in-situ pharmaceutical and nutritional systems. A round-trip Mars mission may last 400–1000 days¹, during which resupply is infeasible and conventional pharmaceutical shelf lives of one to two years become limiting². Elevated radiation, thermal cycling, microgravity, and reduced convection pose combined physical and chemical challenges to astronaut health^{3–5}, with documented effects on bone density, fluid redistribution, and immune function⁶. These challenges motivate in-situ manufacturing not only of pharmaceutical-grade emulsions but, more broadly, of fluid- and cell-based systems for deep-space vehicles.

Emulsification — the controlled dispersion of an immiscible oil phase within an aqueous phase — is central to lipid-based intravenous nutrition (TPN) and many injectable formulations⁷. Terrestrial production relies on high-shear homogenizers to meet parenteral droplet-size standards ($d_{50} < 500$ nm, USP <729>), but neither turbulent dissipation nor buoyancy-driven separation is available in microgravity, making conventional methods unsuitable for deep-space use. Nano-emulsions, which enhance drug solubility and bioavailability, are a promising alternative for space-adapted drug delivery⁸.

In microgravity, the absence of gravitational body forces suppresses buoyancy-driven separation and sedimentation, allowing interfacial tension and capillary forces to dominate dispersed-phase behavior^{9,10}. This may lower the energetic threshold for emulsification: if capillary instabilities can form at low Reynolds numbers without turbulent input, simple passive mixers could replace high-shear machinery. Static mixers are well suited to microgravity, eliminating mechanical failure risk while handling diverse viscosities and flow rates¹¹, with geometric optimization critical to mixing efficiency and stability^{12,13}. Such systems could enable compact, low-power, inherently safe production compliant with deep-space constraints¹⁴.

Several ISS experiments have examined emulsion behavior in sustained microgravity: differential scanning calorimetry has shown altered coalescence kinetics¹⁵; in-situ optical imaging has captured real-time emulsion morphology and demulsification¹⁶; and dedicated devices have measured emulsion stability while highlighting the difficulty of adapting ground-based methods to flight platforms¹⁷. These confirm ISS-based emulsion research is feasible, but none has examined low-energy static-mixer emulsification at parenteral-relevant volumes, and none has addressed microfluidic, chip-scale mixing specifically.

The prior work is the parabolic-flight study by Seehanam et al.¹⁸, which deployed a compact 20 kg lab-on-chip platform to study colloidal mixing during ~20-second reduced-gravity intervals. Across 29 trials, immiscible fluid pairs through a passive static mixer showed enhanced interfacial oscillations and dispersed-phase nucleation versus terrestrial conditions; via Color Distribution Entropy analysis¹⁹, the best-performing emulsion showed 24.2% better mixing uniformity than the microgravity control¹⁸. However, the brief reduced-gravity window could not capture droplet evolution beyond initial mixing, and parabolic-flight g-jitter (0.01–0.05 g) differs substantially from the ISS's sustained microgravity environment; post-mixing quiescent observation was also impossible within the flight timeline. Comparing brief- versus sustained-microgravity dynamics thus remained unresolved — a gap essential to close for model validation and deep-space manufacturing design²⁰.

Here, we present TIGERS-X, a low-cost, teleoperated lab-on-chip platform for microgravity science, validated through on-orbit operation. Hosted within the ICE Cubes Facility (ICF)²¹, TIGERS-X performs fully ground-controlled, multi-channel emulsification studies with optical diagnostics; once installed, it requires no astronaut interaction until removal. By demonstrating reliable teleoperated performance under sustained microgravity, TIGERS-X offers an accessible platform for space-based fluid mixing research — and, more broadly, microgravity life-science applications — extendable toward more complex systems such as cell culture in future missions.

Methods

Concept of Operation

The TIGERS-X mission is structured in three sequential stages, as illustrated in Figure 1, consistent with standardized deployment protocols for ICE Cubes Facility payload. In Stage 1, materials are prepared under sterile conditions in a biosafety cabinet class 2, prior to integration into the experiment cube. Hence, the fully assembled and ground-verified experiment cube is transported to the launch site in a depowered. Upon arrival, a crew member installs the cube into the ICF, which simultaneously establishes all power, data, and grounding interfaces. Following power-on, the CRA ground team conducts remote commissioning checks and telecommand rehearsal sequences via the YAMCS telemetry and telecommand link.

In Stage 2, all scientific operations are executed under full ground control from the CRA User Home Base (UHB) in Bangkok, Thailand via YAMCS telecommand and telemetry without crew involvement. Scientific video results and log data are near real-time downlinked via secure SFTP over the ICF communication network following completion of each experimental cycle, with preliminary results analysis of the downlinked video commencing on the same day.

In Stage 3, the experiment cube is deactivated and returned to Earth aboard a cargo vehicle. The TIGERS-X experiment cube is subjected to recovering and powering-on when arriving at ground facility. Post-flight scientific results will be investigated and benchmarked against on-orbit datasets.

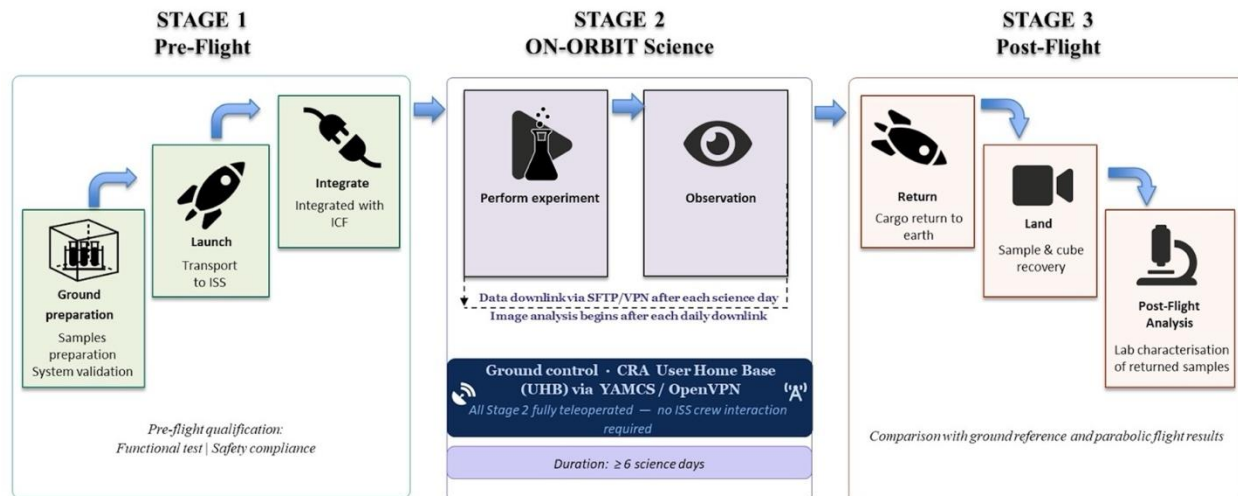


Figure 1: TIGERS-X concept of operations. Three-stage mission timeline: ground preparation, launch, and ICF integration (Stage 1); ground-controlled on-orbit emulsification science executed via YAMCS with no ISS crew interaction (Stage 2); and sample return, hardware investigation, and post-flight analysis (Stage 3).

Flight Hardware Overview

Figure 2 illustrates the system diagram of an experiment cube. At the sub-system level, the device integrates 3 main components including fluidic sub-system, electronic sub-system, and structural frame (Figure 2A). The device is designed to transfer the different fluids from reservoirs to be mixed in a passive mixer module by using multiple pumps. The high-resolution camera module is selected as the main data acquisition tool and allows it to capture fluid interactions. Therefore, custom-made controllers integrated with micro-computers are used to command the actuators and communicate with camera systems. Hence, the integrated sub-system is displayed in Figure 3. Figure 3A shows the assembly of the TIGERS-X payload. Hence, experimental devices are designed to separate experiment and electronics modules as seen in Figure 3B. Consequently, to satisfy the functional requirements, the experimental device consists of peristaltic pumps, fluid containers, a lab-on-chip device, a camera module, and an electronics system, which are seen in Figure 3C-D. Thus, the standard dimensions of $200 \times 200 \times 100 \text{ mm}^3$ with a total weight of $\sim 2.4 \text{ kg}$ following the ICE Cubes interface requirement document (IRD)²². Additionally, the CAD file is available in the supplementary materials.

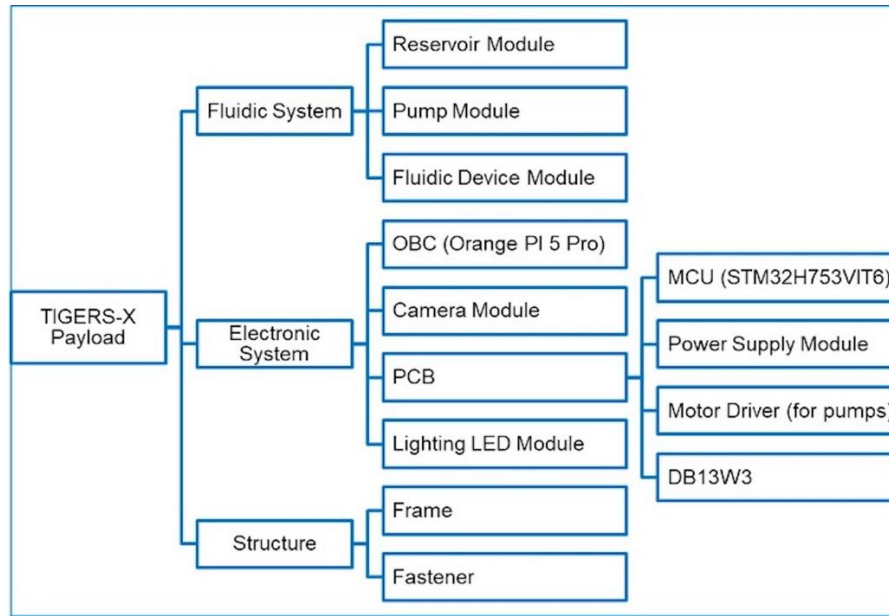


Figure 2: Schematic of experiment conceptual function.

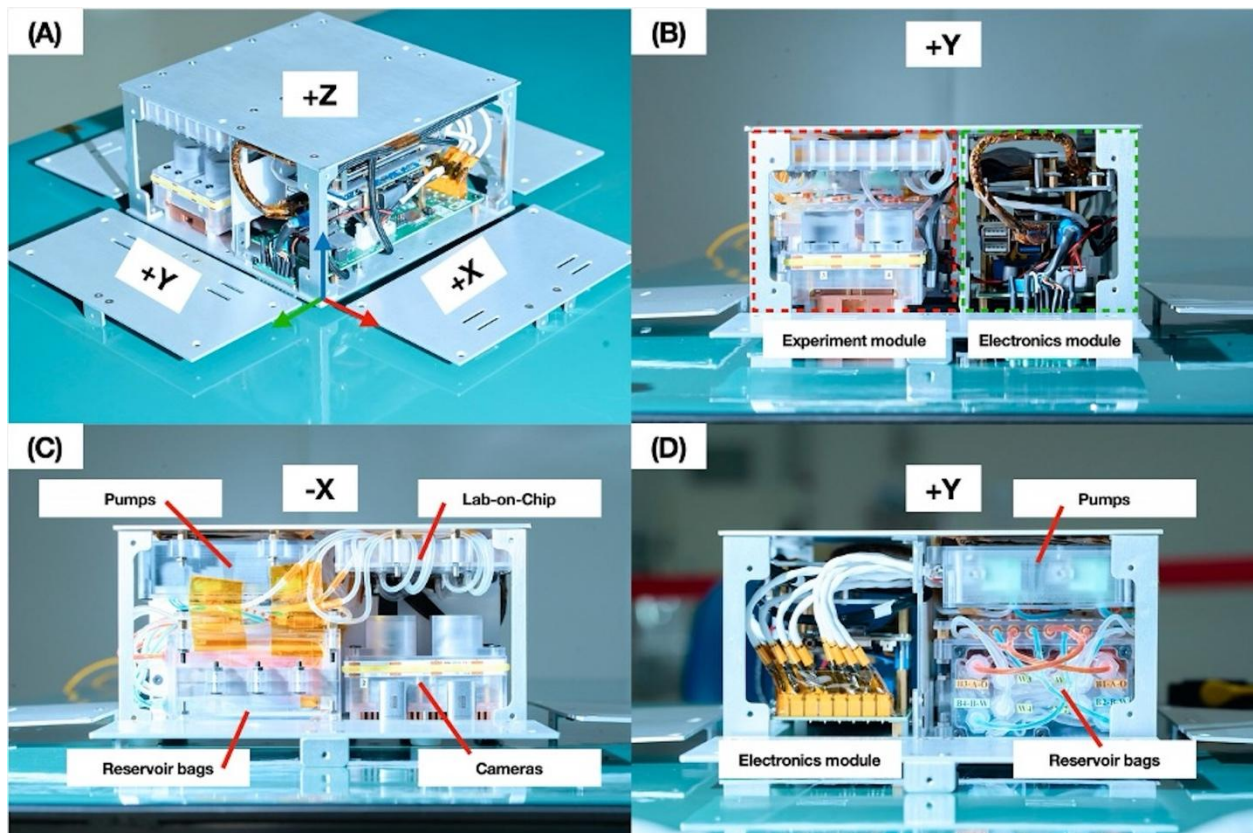


Figure 3: TIGERS-X experiment cube assembly. (A) isometric view with a reference coordinate, illustrating the overall component of experiment cube. (B) +Y face shows separated experiment and electronics modules. (C) -X face, the integrated of pump, Lab-on-Chip, Reservoir bags, and cameras. (D) +Y face, the connection between peristaltic pumps and electronics system.

Fluids Management

The fluidic management system is designed as a fully sealed and primarily double-contained circuit, with the goal of enabling safe and reliable mixing of fluids under microgravity conditions. A schematic as presented in Figure 4 representation of the complete fluidic system, including reservoir bags, waste or mixture bag, pumps, and fluidic device flow channel. The 8 Takasago® RP-QIII peristaltic pumps were used as fluid flow actuators. The fluids are stored in CryoStore® EVA bags CS25N, specifically 10 mL of oil-phase mixture (Fluid A: 10 mL per bag and total 2 bags - 20 mL), 15 mL of BFluid® (Fluid B: 15 mL per bag and total 2 bags - 30 mL), and 4 empty mixture bags with a maximum volume of 25 mL per bag. All 8 supply and waste bags are housed within a dedicated reservoir container for a double-containment strategy. A Tesla valve channel is implemented to control fluid flow in one direction, with high resistance to reverse flow. Its complex, asymmetric geometry generates turbulent flow, which functions as a passive mixer²³. Therefore, the outlet of the Tesla valve was modified into a rectangular chamber that allows observation of the fluid behavior after mixing throughout the flow channel.

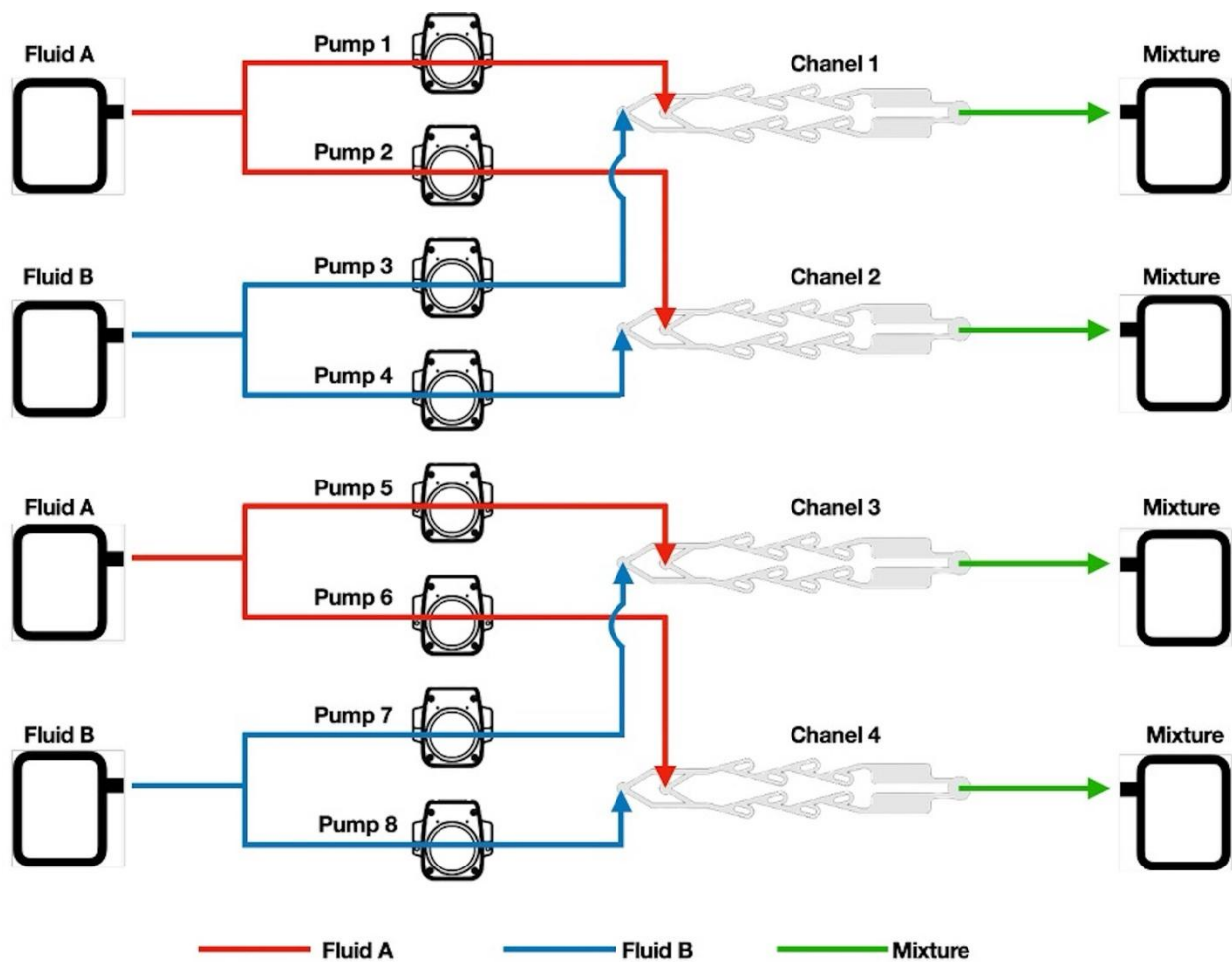


Figure 4: Schematic of the fluid management system. Fluid A and Fluid B reservoirs each supply two peristaltic pumps per channel row (Pumps 1–8 total), feeding four independent Tesla-valve mixing channels (Channel 1–4) arranged as two parallel rows per device (CH1: Channels 1–2; CH2: Channels 3–4). Red lines indicate Fluid A flow, blue lines indicate Fluid B flow, and green lines indicate the mixed output collected in the mixture bags following passage through each channel's static mixer.

Assembly of Electronics system

The electronic system manages power distribution, data handling, and control, following the DB13W3 pin diagram in Figure 5. According to Figure 5A, the DB13W3P mates with the ICF-side DB13W3S. The cube uses only +12 V (Pin A1) and ground (Pin A2); +5 V (Pin A3) is left unconnected (>10 MΩ isolation). Pins 1–4 and 6–9 form four differential pairs for gigabit Ethernet, pin 5 detects the cube, and pin 10 grounds the connector shield to the structure. Overcurrent protection is provided by the ICF²². Telemetry and telecommand data are transmitted via TCP/IP over a bi-directional Gigabit Ethernet link (1000Base-T) through the DB13W3 differential pairs, connecting directly to the ICF network.

According to Figure 5B, a power module with a soft-start circuit and LM61495Q1 DC-DC converter derives regulated 5 V and 3.3 V rails from the 12 V input, with built-in overcurrent, short-circuit, and undervoltage protection. The 5 V rail powers the Orange Pi 5 Pro (OBC) and a USB hub supplying 4 See3CAM cameras, since the OBC's native USB output cannot power all four; the hub also provides stable dedicated 5 V for the cameras under full load. The OBC handles telecommand decoding, experiment sequencing, and telemetry via Ethernet. The 3.3 V rail powers the STM32F746 MCU, which receives UART commands from the OBC, drives the LED module via I2C, and operates the micropumps via GPIO/PWM through the motor drivers. The OBC runs experiment software, stores images and telemetry, and communicates with ground via a YAMCS-based link through the ICE Cubes Mission Control Center (ICMCC) over VPN, with an onboard YAMCS instance on the Orange Pi 5 Pro managing local buffering, state control, and data serving.

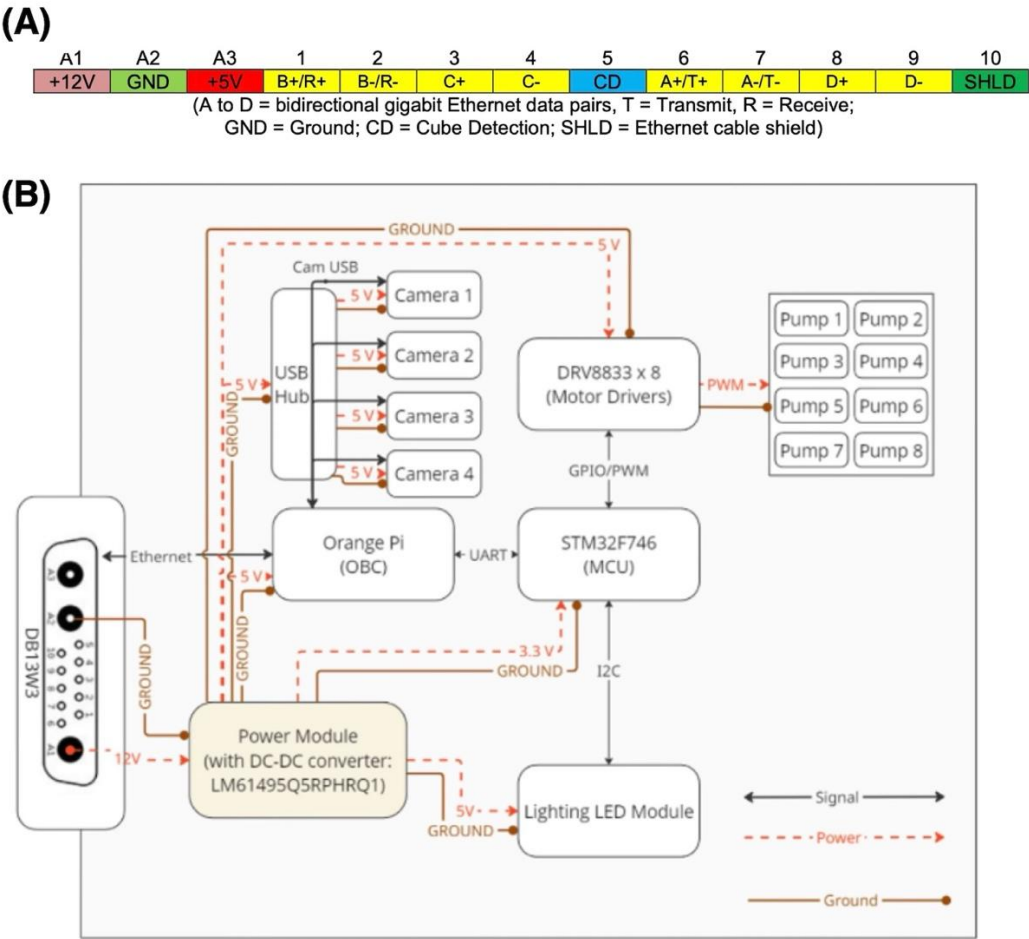


Figure 5: Systematic diagram of electrical connections. (A) DB13W3 pin assignment²⁴. (B) Experiment cube internal electronic system.

Hardware Verifications

Hardware and safety verification addressed three independent compliance domains: structural verification, electromagnetic compatibility, and chemical hazard. Structural verification and electromagnetic compatibility testing were conducted by the Electrical and Electronic Products Testing Center (PTEC), Thailand, while chemical and materials hazard assessment was conducted by NASA.

Structural verification qualified the physical structure and mechanical design of the TIGERS-X hardware against the acceptance mechanical loading and vibration test specifications required by the ICF²². Success criteria required physical survivability with no cracking, damage, component disintegration, or change in structural dynamic signature. Testing was performed individually along all three axes (X, Y, Z), with test types, levels, profiles, and durations given in Table 1. A low-level resonance search (LLRS) was carried out before and after each test sequence in every direction to detect structural changes following higher-level loading, and tested hardware was visually inspected afterward.

Electromagnetic compatibility (EMC) testing followed ICF requirements and applicable sections of MIL-STD-461^{22,25}, adapted for ISS pressurized payloads. The test campaign covered Radiated Emissions (RE102, 14 kHz–18 GHz), Magnetic Field Emissions (RE101, 30 Hz–250 kHz), Conducted Emissions on power leads (CE101, 30 Hz–10 kHz; CE102, 10 kHz–10 MHz), Conducted Susceptibility (CS114), and DC Magnetic Field (316 μ T limit at 7 cm standoff).

Chemical safety was assessed against the ICF chemical approval criteria for ISS pressurized payloads^{26,27}, evaluating the corrosivity, oxidizing potential, and flammability of all experimental reagents at the quantities used, including the trace colorants (Oleoresin Paprika and food-grade blue dye). The assessment also considered the double-barrier fluidic containment architecture described in the Fluid management section and the maximum releasable volume in the event of simultaneous failure of both containment layers.

Video Acquisition

Scientific events during operation were recorded using two synchronized cameras (e-con System, See3CAM 24CUG) positioned to capture different portions of the experimental region of interest. Due to the limited field of view of each camera, the two videos were combined into a single panoramic view. Images of each fluidic chip, including a four-quadrant color reference swatch (pure red, green, blue, and a neutral gray/white patch), were acquired under white-light illumination. Therefore, a true-color reference chart consisting of saturated red, green, and blue patches, along with a white patch with known RGB values, was imaged once and used as the exact color reference for all calibrations.

Image analysis

All image processing, calibration, classification, and quantification steps were implemented in Python for connected-component analysis, morphological operations, and region-filling operations. Color calibration was used due to ambient lighting varying between imaging sessions. Each session's captured swatch was used to derive a session-specific hue correction prior to phase classification, following the general approach of embedding printed color reference markers alongside the sample and computing a correction transforming from the observed-versus-true color relationship²⁸. Images were converted to Hue-Saturation-Value (HSV) space^{29,30}, which separates color identity (hue) from brightness and colorfulness, allowing correction independent of local shading. Hence, image analysis tools can be found in the supplementary material.

For each swatch quadrant, mean hue was computed and matched to the nearest reference hue (0 = red, 1/3 = green, 2/3 = blue); quadrants with saturation below 0.2 (the neutral gray/white patch) were excluded, as hue is undefined for near-achromatic colors. The signed circular deviation between observed and true hue (Eq. 1):

$$\Delta H = \text{mod}(H_{\text{Observed}} - H_{\text{Reference}} + 0.5, 1) - 0.5 \quad (1)$$

Where $H_{Observed}$ is the hue value measured from the camera and $H_{Reference}$ is true hue of that same swatch quadrant. Therefore, Eq. (1) was averaged across the three chromatic patches to yield a single hue-correction offset per imaging session, then subtracted (modulo 1) from the hue channel of all chip images in that session. A full affine RGB correction was also tested but rejected: since the captured swatch was systematically darker than the chip surface, the fitted correction caused severe clipping (>60% of pixels outside the valid range). We used hue-only correction instead, as hue is bounded and circular and thus cannot clip, while still removing the systematic color shift. Residual swatch-like fragments within the chip were identified as compact clusters of highly saturated red-, green-, or blue-like pixels, excluded from downstream analysis, and not used for calibration.

Phase segmentation & Continuous Mixing Index

Pixels with saturation below 0.15 were classified as air per background (index = 0). Remaining pixels were assigned a continuous mixing-index value between 1 (pure water) and 2 (pure oil) based on circular hue distance to each phase's reference center (oil: $h \approx 0.11$; water: $h \approx 0.465$) as described in Eq. (2):

$$Index = 1 + \left(\frac{D_{Water}}{D_{Water} + D_{Oil}} \right) \quad (2)$$

where D_{Oil} and D_{Water} are the circular hue distances to the respective reference centers of oil and water phase, respectively. This continuous formulation avoids an arbitrary hard boundary between fluids, letting interfacial or partially mixed regions take intermediate values, consistent with prior image-based colorimetric mixing-extent measurements in microfluidic mixer characterization and the classical definition of mixing quality as a continuous measure of local composition relative to full homogeneity^{31,32}. Consequently, the per-pixel index map was smoothed with a normal (box/moving-average, not Gaussian) filter applied sequentially along each dimension, then rendered as a filled contour plot (25–30 levels) for a continuous visual gradient matching the underlying index definition. Where relevant, each device's two parallel channel rows were split at the row of minimum fluid-phase coverage in the central image height, corresponding to the physical gap between rows, with contour maps generated per row and in combination.

For each channel, the fluid-phase index values were averaged over each pixel column to obtain a local per-column mean index representing the degree of mixing at that axial position. The cumulative (running) mean index along the flow direction was computed as Eq. (3):

$$Index_{cum}(x) = \frac{\sum_{x' \leq x} n(x') \cdot Index_{mean}(x')}{\sum_{x' \leq x} n(x')} \quad (3)$$

where $n(x')$ is the number of fluid pixels in column x' . This running mean converges to the channel's overall mean mixing index by the outlet and was used both to visualize the progressive development of mixing along individual channels and, after normalizing the axial coordinate to a fractional channel position (0 = inlet, 1 = outlet), to compare mixing development across channels of different pixel length on a common axis.

Results

Hardware Safety Verification

Structural verification was successfully completed in all three axes (X, Y, Z) in accordance with the specifications in Table 1S, with no cracking, damage, disintegration, or structural dynamic signature change observed. Inspection results for the TIGERS-X hardware are summarized in Table 1. Therefore, all EMC

tests except RE102 passed without hardware modification on the initial measurement. The RE102 non-conformance, attributable to broadband switching noise from the Gigabit Ethernet interface and the 12 V DC/DC converter, was resolved by installing ferrite suppression cores on the LAN and power supply cables. Subsequent measurements confirmed full compliance across the entire 14 kHz–18 GHz range without further modification. Complete results are summarized in Table 2. Hence, chemical safety assessment confirmed that all experimental reagents are non-corrosive, non-oxidizing, and non-flammable at the quantities used, and comply with ICF chemical approval criteria. The trace colorants were confirmed physiologically benign at the volumes used. In the unlikely event of simultaneous failure of both primary and secondary containment layers, the total releasable volume is limited to ~60 mL — insufficient to present a credible fire, toxicological, or electrical hazard to crew or onboard systems, satisfying ISS safety requirements.

Table 1: Structural verification summary

Category	Sub-category	Qualification criteria	Measurement results			Result
			Initial test	During Test	Final test	
Mechanical Performance	Enclosure (Visual inspection)	Not broken No loose parts	Not broken No loose parts	-	Not broken No loose parts	PASS
	Components (Visual inspection)	Not broken No loose parts	Not broken No loose parts	-	Not broken No loose parts	PASS

Table 2: EMC/EMI Verification Summary

Test	Limit	Initial Result	Final Result	Mitigation Measures
RE102	14 kHz–18 GHz	FAIL	PASS	Ferrite cores applied to LAN and power cables
RE101	30 Hz–250 kHz	PASS	PASS	None required
CE101	30 Hz–10 kHz	PASS	PASS	None required
CE102	10 kHz–10 MHz	PASS	PASS	None required
CS114	Conducted susceptibility	PASS	PASS	None required
DC Magnetic Field	≤ 316 μ T at 7 cm	PASS	PASS	None required

In-orbit operation

TIGERS-X hardware was launched on 16 May 2026 via SpaceX CRS-34 and successfully operated for 10 days between 26 May and 5 June 2026. Figure 6 displays the TIGERS-X flight hardware preparation and on-orbit installation. According to Figure 6A-C, prior to launch, the Experiment Cube underwent a formal pre-handover verification, confirming overall dimensions of 20 × 20 × 10 cm, a cube mass of 2.32 kg (2.52 kg including the launch bag), intact DB13W3P connector pins, secure labeling, and good cosmetic condition across all six faces, with no residual adhesive, damage, or protrusions noted.

Following ICF integration (Figure 6D), the TIGERS-X payload was successfully installed within the ICE Cubes Facility on the Columbus module, and established YAMCS telemetry contact with the CRA User Home Base in Bangkok. The mission operated successfully within the available Acquisition of Signal (AOS) windows throughout the 10-day campaign, with only a brief power interruption of approximately 2–3 hours recorded during the operational period. Heartbeat telemetry otherwise confirmed continuous +12 V power delivery, processor responsiveness, and Ethernet connectivity throughout the campaign. Representative video frames captured within the lab-on-chip channels under sustained microgravity conditions are presented in Figure 7. As a result, images obtained in Figure 7 will be used to quantify the mixing performance of 2 liquids in microgravity. Additionally, raw video can be found in the supplementary materials.

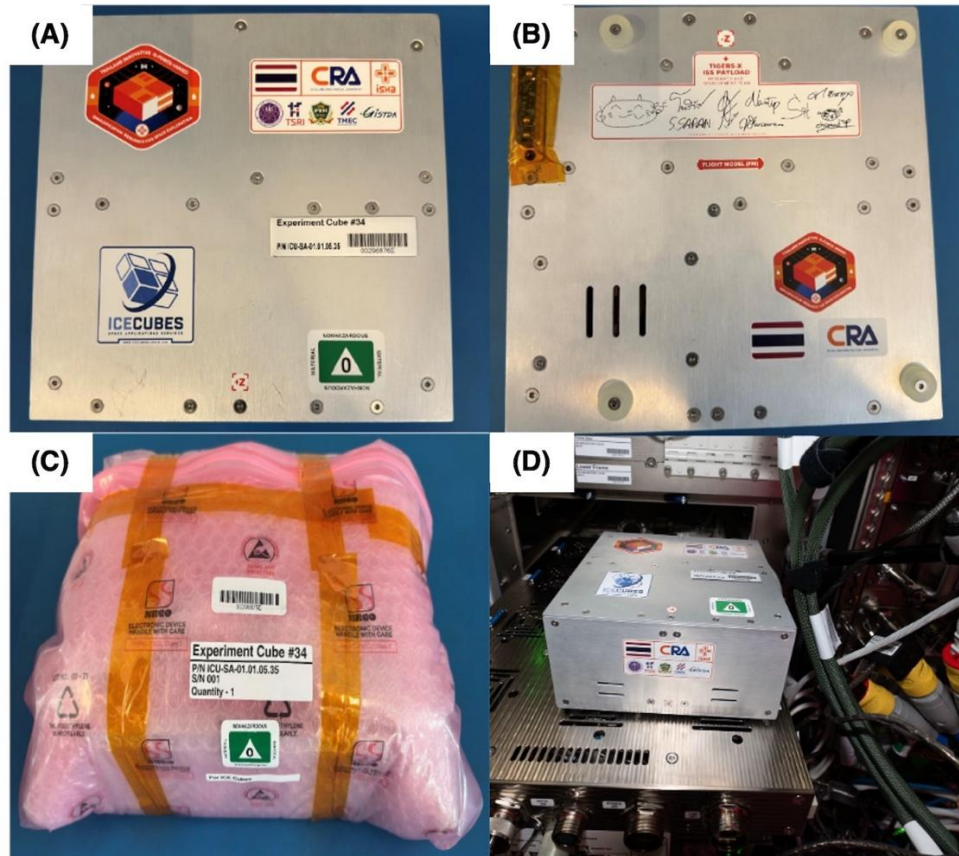


Figure 6: TIGERS-X flight hardware and on-orbit installation. (A) TIGERS-X Experiment Cube (+Z face), showing mission patch, partner agency logos, ICE Cubes identification label, and non-hazardous material marking. (B) DB13W3P connector protected with Kapton (polyimide) tape prior to installation. (C) TIGERS-X flight hardware under bubble wrapped. (D) TIGERS-X hardware installed within the ICE Cubes Facility (ICF) on the Columbus module aboard the ISS. Image courtesy by ESA/NASA – S. Adenot.

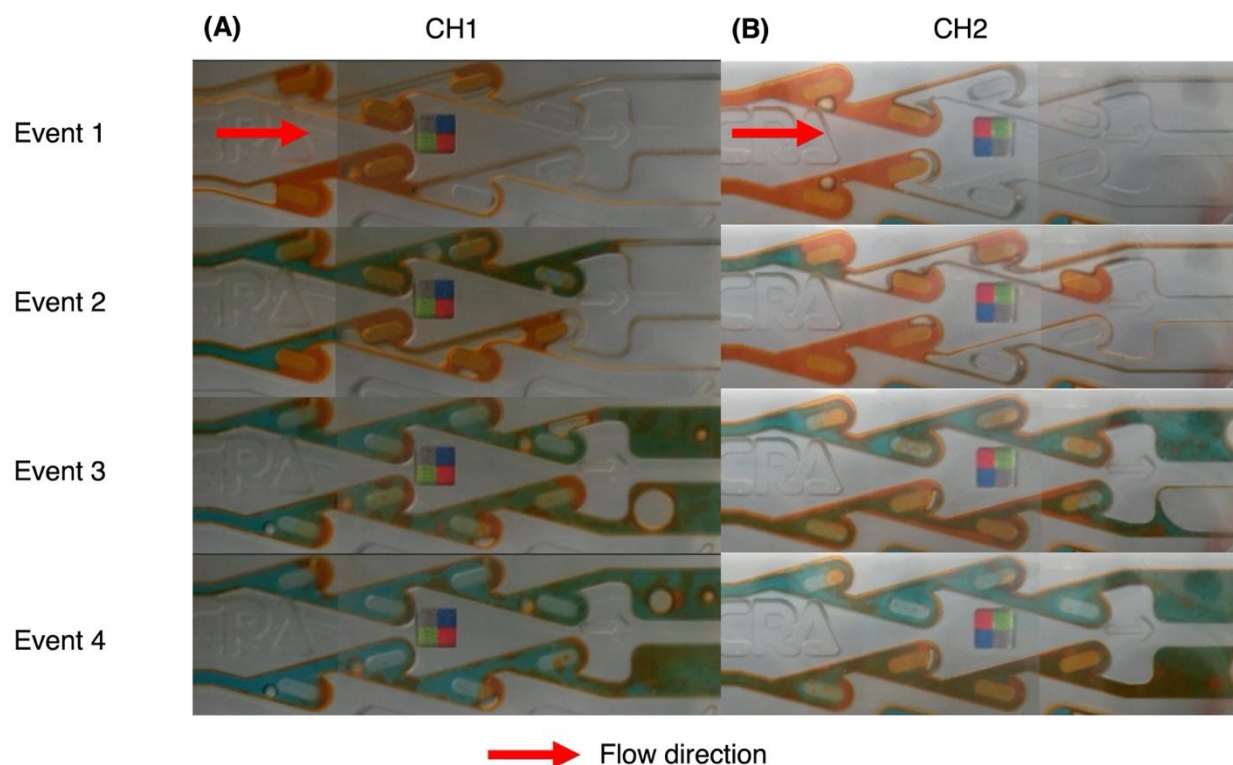


Figure 7: Time-resolved optical images of oil-water emulsification in the two Tesla-valve microchannels (CH1, CH2) acquired by the in-line optical camera of the space experiment hardware. (A) CH1 and (B) CH2 at events 1, 2, 3, and 4 after flow initiation. Red arrows indicate the direction of flow; the four-quadrant color reference swatch visible in each frame was used for downstream color calibration (see Methods). Orange/brown coloration indicates the oil phase, teal/cyan-green indicates the water phase, and white/light-gray regions correspond to air or unfilled channels.

Flow Behavior

Qualitative comparison of 4 channels as seen in Figure 7 indicates visually consistent interfacial instability and dispersed-phase nucleation, providing initial evidence of the phenomena observed under microgravity conditions. According to Figure 7, at event 1, both CH1 and CH2 showed a similar flow front, with the oil phase (orange) leading into the channel network and the downstream region still largely unfilled (white/gray). By event 2, the two channels had begun to diverge visibly: CH1 developed extensive teal/green coloration through the serpentine channel, indicating substantial water-phase penetration, while CH2 retained a more continuous orange coloration, indicating a comparatively oil-dominated flow front. This divergence persisted and became more pronounced at event 3 and event 4, by which point CH1 showed the water phase occupying a large fraction of the visible channel network with the oil phase confined to the channel rim, whereas CH2 remained predominantly oil-colored across most of its length, with water-phase coloration largely restricted to the channel inlet region. This qualitative time series is consistent with the quantitative single-time-point mixing indices obtained from Figures 8-9, where CH2 exhibited a markedly higher (more oil-dominant) mixing index than CH1 at the imaged endpoint.

Emulsification behavior of this kind — where the balance between the dispersed and continuous phases evolves over the course of flow development — has similarly been reported for oil-in-water systems studied under microgravity, where the absence of buoyancy-driven creaming and sedimentation was found to slow phase separation and alter droplet interaction dynamics relative to ground-based emulsification^{33,34}. The present time series extends this observation to a microfluidic, Tesla-valve-structured mixing geometry rather than a bulk pulsatile emulsification cell, and to our knowledge represents one of relatively few reports of passive micromixer-based emulsification imaged in an active

space-flight hardware context, complementing ISS soft-matter-dynamics platforms designed for bulk emulsion stability studies²⁰.

Mixing and Emulsification

Figure 8 displays raw image, and the phase-processed maps recovered the spatial distribution of the water and oil phases throughout the Tesla-valve geometry for all four channel rows. In CH1-2, a bubble trapped within the channel — visible in the raw image as a bright, weakly colored circular region — was correctly reclassified as air (index ≈ 0) rather than being absorbed into the oil-phase classification, confirming that the bubble/reflection correction step performed as intended on real experimental data rather than only on validation images. Qualitatively, CH1-1 and CH1-2 showed a comparatively balanced spatial mixture of green (water-like) and orange-red (oil-like) regions distributed along the channel length, consistent with progressive, distributed mixing. In contrast, CH2-2 showed extensive high-index (orange-red) coverage, particularly in the downstream/outlet region of the channel, indicating a substantially oil-dominated composition by the point of imaging, while CH2-1 retained more extensive green (water-dominated) regions. This colorimetric, hue-based phase classification approach follows the general strategy of using printed/embedded color references to correct for illumination variability in optical quantification of microfluidic devices²⁸, combined with a continuous rather than binary mixing metric in the spirit of classical concentration-variance-based mixing index and prior optical mixing-extent measurements in microfluidic mixers^{31,32}.

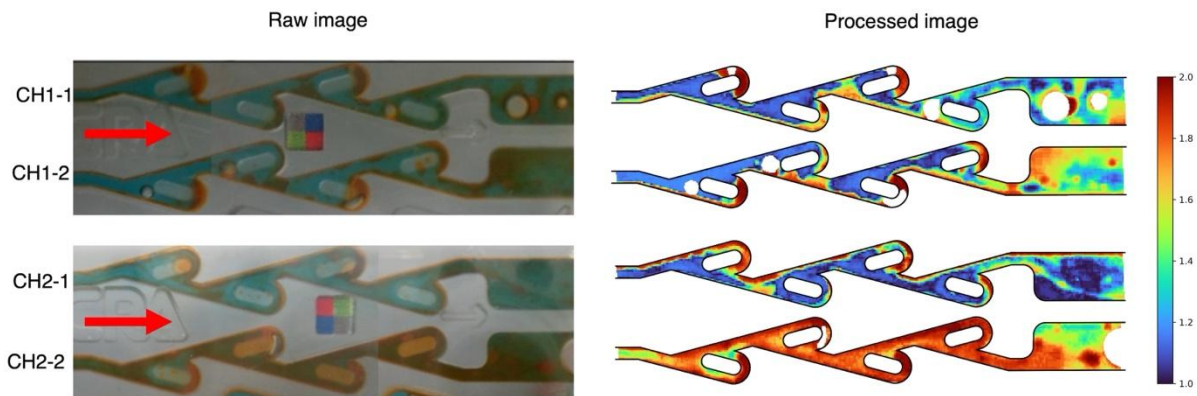


Figure 8: Representative raw images and corresponding phase-processed mixing index maps for both channel rows of CH1 and CH2. Raw images (left) were converted to HSV color space and calibrated against the in-frame color reference swatch (see Methods) to correct for session-specific illumination shifts. Each pixel was then classified as air (index = 0) or assigned a continuous index between 1 (pure water hue) and 2 (pure oil hue) based on its circular hue distance to the reference hue of each fluid phase. Bright, weakly saturated regions consistent with trapped air bubbles or specular reflection were reclassified as air regardless of hue. The resulting index maps (right) are displayed as a smooth false-color scale from 0 (air, dark blue) to 2 (oil, red), with water-dominated regions appearing green (~ 1) and oil-dominated regions appearing orange-red (~ 1.5 -2).

Quantification of Mixing Index

Quantification of the mixing index was analyzed from event 4 for all channels, as seen in Figure 9. The cumulative mixing index converged within the first 10–20% of channel length in all four rows (Figure 9A),

remaining comparatively flat thereafter, indicating that bulk phase composition was established early and changed little over the remaining Tesla-valve units. Final cumulative index values were 1.49 (CH1-1), 1.53 (CH1-2), 1.34 (CH2-1), and 1.81 (CH2-2). As shown across the full normalized channel length (Figure 9A), the two CH1 rows converged to similar values ($\Delta \approx 0.04$), indicating symmetric behavior, whereas the two CH2 rows diverged substantially ($\Delta \approx 0.47$), with CH2-1 the most water-dominated and CH2-2 the most oil-dominated channel overall. The average mixing index per channel as seen in Figure 9B reflected this: CH1-1 (1.47 ± 0.30) and CH1-2 (1.50 ± 0.30) were statistically indistinguishable, CH2-1 (1.33 ± 0.33) showed a similarly widespread but lower mean, and CH2-2 (1.80 ± 0.17) combined the highest mean with the narrowest spread — indicating CH2-2 was both oil-dominated and the most spatially homogeneous channel measured.

The pronounced asymmetry between CH2 is two rows, and CH1 is more balanced behavior, suggests unequal flow partitioning or a systematic difference in local mixing efficiency between nominally parallel channels — a structural or flow-distribution effect worth further investigation (e.g., inlet flow resistance matching) rather than a stochastic one, given its consistency across the full channel length (Figure 9A). All four channels remained at or only marginally below the notional “fully mixed” midpoint (1.5) except CH2-2, which sat well above it, indicating persistently oil-dominated rather than homogeneous composition.

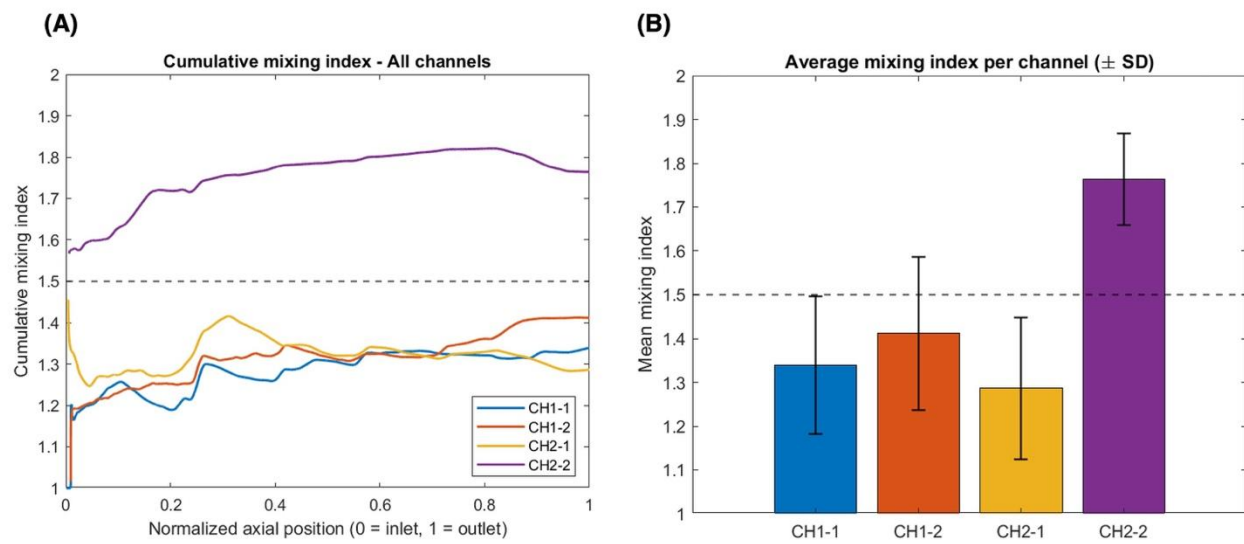


Figure 9: Cumulative mixing index analysis for all four channel rows. (A) Combined cumulative mixing index for all four channels plotted against normalized axial position (0 = inlet, 1 = outlet) to allow direct comparison across channels of different physical length. (B) Average mixing index per channel (mean $\pm$ SD across all fluid-phase pixels in each channel).

Discussion

TIGERS-X validated a complete mission lifecycle for a low-cost, teleoperated ISS lab-on-chip payload — from ground verification through 10 days of autonomous flight operation. Structural, EMC, and chemical safety verification followed the ICF IRD, MIL-STD-461-based EMC procedures, and ISS pressurized-payload safety requirements^{22,25,26}, with only a single RE102 non-conformance resolved via ferrite suppression, demonstrating a tractable qualification pathway for compact, university-scale hardware to reach ISS-certified status — a core aim of the ICE Cubes Facility. On orbit, the payload required no astronaut interaction beyond installation and removal, with all 10 days of operation conducted remotely via YAMCS telemetry from the User Home Base. This teleoperated architecture mirrors and extends that

validated for the VITA astro-pharmaceutical payload³⁶, suggesting such ground-controlled architectures generalize well across ICF payload types. A brief ~2–3 hour power interruption did not compromise the overall campaign, underscoring the robustness of the design. Critically, sustaining operation over days rather than the ~20 s afforded by parabolic flight¹⁸ directly resolves the limitations identified in that earlier work — namely, the inability to observe long-term stability, realistic microgravity conditions, and post-mixing quiescent behavior.

The observed divergence between CH1 and CH2 supports the hypothesis that absent buoyancy-driven separation, interfacial tension and capillary forces dominate immiscible-phase behavior in microgravity^{9,10}. That a purely passive Tesla-valve geometry^{12,13,23} produced substantial phase redistribution within a short channel length, without turbulent shear input, is consistent with the premise that capillary-driven instabilities can lower the energetic threshold for emulsification relative to terrestrial high-shear methods. The pronounced asymmetry between CH2's two nominally parallel rows, against CH1's more balanced behavior, points to unequal flow partitioning rather than stochastic variation, and is a practical design consideration (e.g., inlet resistance matching) for future chip iterations. Because our colorimetric mixing index is defined for an immiscible, hue-distinguished oil–water system, it cannot be numerically compared to variance-based indices reported for miscible-fluid Tesla micromixers³⁵. Nonetheless, both point to the same qualitative conclusion that Tesla-valve geometries drive substantial phase redistribution over short lengths. The time-resolved divergence in flow behavior (Figure 7) — where the balance between phases evolves over the course of flow development — echoes prior microgravity findings that the absence of buoyancy-driven creaming and sedimentation slows phase separation and alters droplet interaction dynamics^{17,33,34}, here extended from bulk emulsification cells to a microfluidic, passive-mixer context. To our knowledge, this is among the first demonstrations of passive micromixer-based emulsification imaged within active space-flight hardware, complementing ISS soft-matter-dynamics platforms built for bulk emulsion stability studies²⁰. Methodologically, the hue-based continuous mixing index — corrected via an in-frame color reference and grounded in the classical continuous definition of mixing quality— proved robust to illumination drift across the full mission without requiring recalibration hardware and may be broadly applicable to future colorimetric flight diagnostics^{28,31,32}.

Conclusion

TIGERS-X demonstrates a low-cost, fully teleoperated lab-on-chip platform capable of studying passive microfluidic emulsification under sustained microgravity aboard the ISS. Across a 10-day mission, the payload operated reliably with no astronaut interaction beyond installation and removal, and the Tesla-valve static-mixer geometry produced measurable, spatially resolved oil–water phase redistribution, extending the physical feasibility shown in prior parabolic-flight work to the realistic duration and gravitational environment relevant to deep-space applications. Beyond its scientific findings, TIGERS-X establishes a validated, accessible hardware and operational architecture for future microgravity fluid research and offers a foundation that can be extended toward more complex biological applications, such as cell culture systems in future missions.

Data availability

CAD file, experiment videos, and image analysis software package are available at <https://doi.org/10.5281/zenodo.22934189>.

Abbreviations

AOS Acquisition of Signal

430	CDE	Color Distribution Entropy
431	CRA	Chulabhorn Royal Academy
432	DSC	Differential Scanning Calorimetry
433	EMC	Electromagnetic Compatibility
434	EMI	Electromagnetic Interference
435	GPIO	General-Purpose Input/Output
436	HSV	Hue-Saturation-Value (color space)
437	ICF	ICE Cubes Facility
438	ICMCC	ICE Cubes Mission Control Centre
439	IRD	Interface Requirement Document
440	ISS	International Space Station
441	LLRS	Low-Level Resonance Search
442	MCU	Microcontroller Unit
443	OBC	On-Board Computer
444	PWM	Pulse-Width Modulation
445	SFTP	Secure File Transfer Protocol
446	TC	Telecommand
447	TM	Telemetry
448	TPN	Total Parenteral Nutrition
449	UART	Universal Asynchronous Receiver-Transmitter
450	UHB	User Home Base
451	USP	United States Pharmacopeia
452	VPN	Virtual Private Network
453	XTCE	XML Telemetric and Command Exchange
454	YAMCS	Yamcs Mission Control System

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552 Visualization. All authors have read and agreed to the published version of the manuscript.

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