Development of two-color fluorescent imager and integrated fluidic system for nanosatellite biology applications

Diana Wu et al
NASA Ames Research Center
ASGSR
December 1, 2012
Microsatellite in-situ Space Technologies (MisST)

NanoSatellite Science Payload

Fluidic System

22 cm

10 cm

2-color Fluorescence
Payload Science & Technology

Overview

Objective: Technology demonstration of 2-color cent imager for space biology applications

- model organisms, resolve major features/organs, interest & control

- System: 2-cube integrated culture/imaging setup

- C. elegans model organism

- Fluidics: culture wells, imaging zone, reagent storage

- Imager: fixed focus, 300 kpixel CMOS chip

- Image storage: 2 GB/6,000 images/8 bits per color

- Telemetry of 1-20 images

- Hermetic containment vessel
C. elegans Overview

- Adult (1110-1150 μm) capable of egg laying
- Young adult (900-940 μm)
- L4 (620-650 μm)
- L3 (490-510 μm)
- L2 (360-380 μm)
- L1 (250 μm)
- L1/L2 molt
- Hatching
- 8 hr L1/L2 molt
- 12 hr L1 molt
- 13 hr L2 molt
- 10 hr L4/adult molt
- 150 min in utero development
- 40 min first cleavage
- Eggs laid at Gastrula (approximately 30-cell)
- Ex utero development (8 hr)
- Dauer (400 μm)
- L4 (900-940 μm)
- L3/L4 molt
- L2/L3 molt
- L1/L2 molt
- Dauer (400 μm)

The small size and short life cycle make it inexpensive to maintain and ideal for research. C. elegans is often used to study spaceflight effects and experiments using C. elegans.
<table>
<thead>
<tr>
<th>C. elegans Imaging</th>
<th>White</th>
<th>Amber</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td><img src="white" alt="tomato" /></td>
<td><img src="amber" alt="tomato" /></td>
<td><img src="blue" alt="tomato" /></td>
</tr>
<tr>
<td>Muscle cells</td>
<td><img src="white" alt="muscle" /></td>
<td><img src="amber" alt="muscle" /></td>
<td><img src="blue" alt="muscle" /></td>
</tr>
<tr>
<td>Nerve cells</td>
<td><img src="white" alt="nerve" /></td>
<td><img src="amber" alt="nerve" /></td>
<td><img src="blue" alt="nerve" /></td>
</tr>
<tr>
<td>Intestinal cells</td>
<td><img src="white" alt="intestinal" /></td>
<td><img src="amber" alt="intestinal" /></td>
<td><img src="blue" alt="intestinal" /></td>
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Fluidics Overview

Each: Multilayer polymer microfluidic card
- Multi-layer lamination to form 3-D fluidic network
- Laser-cut fluidic channels in pressure-sensitive adhesive (PSA)
- Lamination of machined polycarbonate sheets with via holes, filters, polystyrene using PSA

Materials, adhesives, sterilization, biocompatibility, all have GeneSat, PharmaSat, O/OREOS-EcAMSat heritage
Fluidic Card and Reservoir

- fluorescent beads
- imaging chamber (10 µL, 3 x 4 x 0.17 mm)
- Reservoir
  - peristaltic pump
  - solenoid valves
  - CEMMI bags, 5 - 10 mL
Load Card – young larvae in growth media, fluorescent beads in imaging field

Take photo to check focus

Launch/deploy

Take photos of beads

Displace beads in imager field with beads from well

Take photos of beads

Flush beads out of imaging field

Supply fresh CeMM to all *C. elegans*

Pump worms from well into imaging region

Take photos

Repeat for all 3 wells
Fluidic Sequence - Beads

- **In/out hole under filter**
- **In/out hole above filter**
- **Non Latching Valves**
- **Latching Valves**
- **Detection Chamber**
- **Buffer/Waste**
- **CeMM**
- **Bead loading port**
- **Pump**

Diagram showing fluidic sequence involving valves, tubing, and other components.
Fluidic Sequence - Worms
Imager Components:

A) Luxeon Rebel LEDs:
   2 amber, 1 blue, 1 white

B) Edmunds relay lens

C) Semrock filters:
   blue excitation, amber excitation,
   dual band-pass emission

D) Camera:
   COMedia C328-7221

Imager Specifications:

- 2-color fluorescence:
  Green excite/emit peaks at: 487/511
  Red excite/emit peaks at: 587/611
- Cool white for standard imaging
- Lateral resolution: ~ 8 μm
- Magnification: 1:1
- Field of View: ~3 x 4 mm
- Depth of Focus: ~150 μm
Fluidic well depth: 0.17 mm

- Fine pitch thread of S-mount (0.5 mm/thread)
- Camera adjustment range: ±5 mm
- Locknut/set screw...
COMedia 3326 T224
Camera chip

Fluorescently labeled *C. elegans*

Resolves ~8 µm feature size
CCD imaging chip

Camera options are compatible with a lens system.
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass</td>
<td>~1.5 – 2kg</td>
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<td>Sensors</td>
<td>Pressure: accurate to within 2% of 1 atm.</td>
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<tr>
<td></td>
<td>Humidity: accurate to within 4% from true value after temp. correction</td>
</tr>
<tr>
<td></td>
<td>Temperature accurate to within +/- 0.5ºC.</td>
</tr>
<tr>
<td>Processor</td>
<td>PIC 32</td>
</tr>
<tr>
<td>Temperature Control</td>
<td>Kept at 4°C to 30°C during the experiment phase.</td>
</tr>
<tr>
<td>Environmental Chamber</td>
<td>Maintains an atmospheric pressure in the pressurized payload chamber volume within 11.7 to 15.7 psia.</td>
</tr>
<tr>
<td>System Sterilization</td>
<td>Material selections have heritage to past missions for ability to be sterilized and low outgassing.</td>
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Payload Design Overview

Integrated Payload
- Exploded View -
Integrated Payload
- Sectional View -

Led entry

Imaging field

Focal point
LED Assembly

LED heat sink
Camera Assembly

Camera Adapter/Focus Adjust
Electronics Assembly

- Analog PCB
  - LED
  - Thermal

- Digital PCB
  - Camera
  - Voltage regulator
Conclusion

Technology built:
- integrated fluidics system that maintains *C. elegans* viability and supports growth
- fixed-focus imager with fluorescence and scattered-light imaging capabilities

Biocompatibility testing complete

Waiting to partner with a principle investigator and launch
Acknowledgements

Editors: Ming Tan, Matthew Piccini

Policy: Matthew Lera, Macarena Parra

Designing: Linda Timucin

Mechanical: Abraham Rademacher, Giovanni Minelli, Chris Beasler

Electrical: Aaron Schooley

Management: Andres Martinez

Technology: Antonio Ricco

Funding: NASA/Exploration Systems Mission Directorate
Thank You

Questions?
## Miss: P-38 Flyer Payload

### ConOps

<table>
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<tr>
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<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Off</td>
<td>Move Red Out</td>
<td>Off</td>
<td>Move Green In</td>
<td>Off</td>
<td>Feed all worms</td>
<td>Move Green Out</td>
<td>Off</td>
<td>Move Worms Out</td>
<td>Off</td>
<td>Move Worms In</td>
<td>No Imaging</td>
<td>Move Worms Out</td>
<td>Off</td>
<td>Move Worms In</td>
<td>No Imaging</td>
<td>Move Worms Out</td>
<td>Off</td>
</tr>
</tbody>
</table>

- **Validate Imager Cycle**
  - No imaging
  - 3 Cycles A, B, W
  - 1 Cycle A, B, W
  - 4 Cycles A, B, W
  - No Imaging
  - 1 Cycle A, B, W
  - No Imaging
  - ~ 6 Cycles & back to back with same color
  - No Imaging
  - 1 Cycle A, B, W

<table>
<thead>
<tr>
<th>Alive C</th>
<th>Heat to (22C)</th>
<th>Temp Maintain (22C)</th>
</tr>
</thead>
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<td>Alive C</td>
<td>Heat to (22C)</td>
<td>Temp Maintain (22C)</td>
</tr>
<tr>
<td>Alive C</td>
<td>Heat to (17C)</td>
<td>Temp Maintain (17C)</td>
</tr>
</tbody>
</table>
After pumping worms into imaging chamber
Payload ConOps

Payload Builds/Satellite Integration

Workmanship/Functional Checkout (Data/Images)

Travel

Functional Checkout/Data/Images

Integration/Stasis/Launch

System Initiate
Keep alive mode

Set Temp
Payload Data Check and Images

Max 6 weeks in temp controlled environment

Temp Control to 21C
Beads ~2-6 hrs
Beads ~2-6 hrs
C.elegans ~ 2 days
C.elegans ~ 2 days

Experiment Arm

Experiment 8 Days
**Contact with C. elegans**
- (machined)
- porous membrane
- pre-sensitive adhesive (PSA) (cut edges only)
- acrylic adhesive, polyester carrier
  - laser-cut edges
- polyethylene gas-permeable cover film

**Contact with growth medium only (reservoir/tubing)**
- polyethylene vinylacetate) [EVA] bag
- one barbed ports
- ethane tubing

**Contact with C. elegans and growth medium**
- wetted valve materials
  - PPS = Polyphenylene sulfide ("Fortron")
  - PBT = Polybutylene terephthalate ("Valox")
  - 316 SS
  - FeCr Alloy
  - Silicone Rubber

**Thermal control**
- fluoresc. beads
Fluidics Stack Pins
- Detail -
Back-Cover Assembly

- Viton O-Ring
- Activated Carbon
Camera Mount
- Mated to Back Panel -

Ultem Mount

Ultem Washers
Camera Assembly
- Mated to Camera Mount -
Fluidics Assembly
- Mated to Camera Assembly -

PSA Layer
Front Panel Assembly

Hermetic Connector

• Laser Welded
Electronics Assembly
- Mated to Fluidics Stack Pins -
## Payload Electrical Overview

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
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<tr>
<td><strong>0 Imager Interface Board</strong></td>
<td>Translates the bus 50pin cable to the hermetic connection of the payload can. Also has connection for the remove by flight “kill switch”</td>
</tr>
<tr>
<td><strong>0 Imager Payload Analog</strong></td>
<td>Contains all analog payload circuits. Temperature sensor circuits, LED current drivers, RH sensor, pressure sensor circuitry, Heater circuitry</td>
</tr>
<tr>
<td><strong>2 Imager Payload Digital</strong></td>
<td>Contains all digital and inductive load circuits. Valve bridges, motor switches, memory chips, camera interface</td>
</tr>
<tr>
<td><strong>0 Imager LED</strong></td>
<td>Contains the 4 high power imager illumination led’s. Attached to a heat sink and devices are properly thermally sunk for continuous use.</td>
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<tr>
<td><strong>0 Imager Processor</strong></td>
<td>Contains the payload microprocessor and associated uC LDO, RTC, FRAM, ADC reference</td>
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Fluidic System

2-color Fluorescence imager
Payload Science & Technology Overview

Objective: Technology demonstration of 2-color fluorescent imager for space biology applications

Target: model organisms, resolve major features/organs, gene of interest & control

Implementation: 2-cube integrated culture/imaging instrument

- C. elegans model organism
- Fluidics: culture wells, imaging zone, reagent storage
- Imager: fixed focus, 300 kpixel CMOS chip
- Image storage: 2 GB/6,000 images/8 bits per color (telemetry of 1 - 20 images)
- Hermetic containment vessel
C. elegans Overview

- Easy feeding and short life cycle make it inexpensive to maintain and ideal for research.
- Used to study spaceflight effects and experiments using C. elegans have flown numerous times including on STS-107.
**C. elegans Imaging**

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>Amber</th>
<th>Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N2 (wt)</strong></td>
<td><img src="white_n2.png" alt="Image" /></td>
<td><img src="amber_n2.png" alt="Image" /></td>
<td><img src="blue_n2.png" alt="Image" /></td>
</tr>
<tr>
<td>not fluorescent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>tdTomato</strong></td>
<td><img src="white_tdt.png" alt="Image" /></td>
<td><img src="amber_tdt.png" alt="Image" /></td>
<td><img src="blue_tdt.png" alt="Image" /></td>
</tr>
<tr>
<td>red – muscle cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IM324</strong></td>
<td><img src="white_im.png" alt="Image" /></td>
<td><img src="amber_im.png" alt="Image" /></td>
<td><img src="blue_im.png" alt="Image" /></td>
</tr>
<tr>
<td>green – nerve cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MR142</strong></td>
<td><img src="white_mr.png" alt="Image" /></td>
<td><img src="amber_mr.png" alt="Image" /></td>
<td><img src="blue_mr.png" alt="Image" /></td>
</tr>
<tr>
<td>green – intestinal cells</td>
<td></td>
<td></td>
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Fluidics Overview

Approach: Multilayer polymer microfluidic card

- Multi-layer lamination to form 3-D fluidic network
- Laser-cut fluidic channels in pressure-sensitive adhesive (PSA)
- Lamination of machined polycarbonate sheets with via holes, filters, polystyrene using PSA

Polymers, adhesives, sterilization, biocompatibility, & fab. all have GeneSat, PharmaSat, O/OREOS-SESLO, EcAMSat heritage
Fluidic Card and Reservoir

**Fluidic Card**
- C. elegans
- fluorescent beads
- imaging chamber (10 µL, 3 x 4 x 0.17 mm)
- thermal control

**Reservoir**
- peristaltic pump
- solenoid valves
- CEMM* & waste bags, 5 - 10 mL

*C. elegans maintenance medium*
Experimental Timeline

Load Card – young larvae in growth media, fluorescent beads in imaging field

Take photo to check focus

Launch/deploy

Take photos of beads

Displace beads in imager field with beads from well

Take photos of beads

Flush beads out of imaging field

Supply fresh CeMM to all C. elegans

Pump worms from well into imaging region

Take photos

Flush worms out of imaging field

Repeat for all 3 wells
Fluidic Sequence - Beads
Fluidic Sequence - Worms
Fluorescence Imager

**Imager Components:**

- **A** Luxeon Rebel LEDs:
  - 2 amber, 1 blue, 1 white

- **B** Edmunds relay lens

- **C** Semrock filters:
  - Blue excitation, amber excitation,
  - Dual band-pass emission

- **D** Camera:
  - COMedia C328-7221

**Imager Specifications:**

- 2-color fluorescence:
  - Green excite/emit peaks at: 487/509nm
  - Red excite/emit peaks at: 587/610nm

- Cool white for standard imaging
- Lateral resolution: ~8 µm
- Magnification: 1:1
- Field of View: ~3 x 4 mm
- Depth of Focus: ~150 µm
Fluorescence Imager

- Fluidic well depth: 0.17 mm
- Fine pitch thread on S-mount (0.5mm/thread)
- Camera adjustment: ±5 mm
- Locknut/set screws hold detector in place
green fluorescently labeled *C. elegans*

Resolves ~8 μm feature size
CCD imaging chip

Future camera options are compatible with developed lens system (e.g., Omnivision 7141 CMOS)

Resolves ~ 4 μm feature size
## Payload Functional Overview

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</table>
| Sensors    | Pressure: accurate to within 2% of 1 atm.  
              Humidity: accurate to within 4% from true value after temp. correction  
              Temperature accurate to within +/- 0.5°C. |
| Processor  | PIC 32                   |
| Temperature Control | Kept at 4°C to 30°C during the experiment phase. |
| Environmental Chamber | Maintains an atmospheric pressure in the pressurized payload chamber volume within 11.7 to 15.7 psia. |
| System Sterilization | Material selections have heritage to past missions for ability to be sterilized and low outgassing. |
Payload Design Overview

Integrated Payload
- Exploded View -
Integrated Payload
- Sectional View -

- Led emitters
- Imaging field
- Focal plane
LED Assembly

LED heat sink
Camera Assembly

Camera Adapter/Focus Adjustment With Locknut
Electronics Assembly

Processor PCB
- PIC 32

Analog PCB
- LED
- Thermal
- RH
- Pressure

Digital PCB
- Camera
- Valves
- Pump
Conclusion

• Technology built:
  – integrated fluidics system that maintains *C. elegans* viability and supports growth
  – fixed-focus imager with fluorescence and scattered-light imaging capabilities

• Biocompatibility testing complete

• Waiting to partner with a principle investigator and launch
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Imaging: Linda Timucin
Mechanical: Abraham Rademacher, Giovanni Minelli, Chris Beasley
Electrical: Aaron Schooley
Management: Andres Martinez
Technology: Antonio Ricco

Funding: NASA/Exploration Systems Mission Directorate
Thank You

Questions?
MisST Free-Flyer Payload ConOps

Note:  A = amber,  B = blue,  W = white
10 μm filter

After pumping worms into imaging chamber

C. elegans growth after 1 week
Payload ConOps

Sterile Payload Builds/Satellite Integration

Workmanship/Functional Checkout (Data/Images)

ARC

Travel

Functional Checkout/Data/Images

Experiment Arm

Integration/Stasis/Launch

Max 6 weeks in temp controlled environment

Temp Control to 21C
Beads ~2-6 hrs
Beads ~2-6 hrs
C.elegans ~ 2 days
C.elegans ~ 2 days
C.elegans ~ 2 days
Growth Continues

System Initiate
Keep alive mode

Set Temp
Payload Data Check and Images

Experiment 8 Days

Data Download
Payload 1 (Imager): *Fluidics materials*

**Direct contact with* C. elegans**
1. Polycarb. (machined)
2. Polycarb. porous membrane
3. Pressure-sensitive adhesive (PSA) (cut edges only)
   - acrylic adhesive, polyester carrier
   - laser-cut edges
4. Polystyrene gas-permeable cover film

**Contact with growth medium only (reservoir/tubing)**
1. Poly(ethylene vinylacetate) [EVA] bag
2. Polysulfone barbed ports
3. Polyurethane tubing

**Direct contact with* C. elegans* and growth medium**
1. Internal wetted valve materials
   1. PPS = Polyphenylene sulfide (“Fortron”)
   2. PBT = Polybutylene terephthalate (“Valox”)
   3. 316 SS
   4. FeCr Alloy
   5. Silicone Rubber
Fluidics Stack Pins
- Detail -
Back-Cover Assembly

Viton O-Ring

Activated Carbon Assembly
Camera Mount
- Mated to Back Panel -

Ultem Washers
Enclosure Thermal Isolation

Ultem Mount
Payload Thermal Isolation
Camera Assembly
- Mated to Camera Mount -
Fluidics Assembly
- Mated to Camera Assembly -

PSA Layer

Ultem Standoff
Front Panel Assembly

Hermetic Connector
• Laser Welded

Imager Interface Board
Electronics Assembly
- Mated to Fluidics Stack Pins -
## Payload Electrical Overview

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<th>Payload Module</th>
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<td>M420 Imager Payload Analog</td>
<td>Contains all analog payload circuits. Temperature sensor circuits, LED current drivers, RII sensor, pressure sensor and circuitry, Heater circuitry</td>
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<td>M430 Imager LED</td>
<td>Contains the 4 high power imager illumination led’s. Attached to a heat sink and devices are properly thermally sunk to allow for continuous use.</td>
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