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

22 cm

10 cm

2-color Fluorescence

Fluidic System
Overview

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

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

- Instrumentation: 2-cube integrated culture/imaging system
  - 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

Owing and short life cycle make it inexpensive to keep and ideal for research study spaceflight effects and experiments using C.
<table>
<thead>
<tr>
<th>C. elegans Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White</strong></td>
</tr>
<tr>
<td>Tomato autofluorescent</td>
</tr>
<tr>
<td>muscle cells</td>
</tr>
<tr>
<td><strong>Amber</strong></td>
</tr>
<tr>
<td><strong>Blue</strong></td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>nerve cells</td>
</tr>
<tr>
<td>42</td>
</tr>
<tr>
<td>intestinal cells</td>
</tr>
</tbody>
</table>
**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)
- **Slugs of elegans**

**Fluidic Card**

**Reservoir**

- **Peristaltic pump**
- **Thermal pump**
- **Solenoid valves**
- **CEMME bags, 5 - 10 mm**
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

Repeat for all 3 wells
Fluidic Sequence - Worms

- In/out hole under filter
- In/out hole above filter
- Non Latching Valves
- Latching Valves

Detection Chamber
Buffer/Waste
CeMM
**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: 487/611
  - 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: ±5 mm
- Locknut/set screw
COMedia 5528 T224
Camera chip

Fluorescently labeled *C. elegans*

Resolves ~8 µm feature size
Camera options are compatible with lens system
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<th>Specification</th>
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<td>Mass</td>
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<tr>
<td>Sensors</td>
<td>Pressure: accurate to within 2% of 1 atm.</td>
</tr>
<tr>
<td></td>
<td>Humidity: accurate to within 4% from true value</td>
</tr>
<tr>
<td></td>
<td>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>
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<td>Environmental chamber</td>
<td>Maintains an atmospheric pressure in the pressurized</td>
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<tr>
<td></td>
<td>payload chamber volume within 11.7 to 15.7 psia.</td>
</tr>
<tr>
<td>Material selection</td>
<td>Material selections have heritage to past missions</td>
</tr>
<tr>
<td></td>
<td>for ability to be sterilized and low outgassing.</td>
</tr>
</tbody>
</table>
Payload Design Overview

Integrated Payload
- Exploded View -
Integrated Payload
- Sectional View -
LED Assembly

LED heat sink
Camera Assembly

Camera Adapter/Focus Adj
Fluidics Assembly

Dolomite Pump

Reservoir  Lee Co. Surface  Fluidics Block
Electronics Assembly
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

Mechanical: Abraham Rademacher, Giovanni Minelli, Chris Beasle
Electrical: Aaron Schooley
Management: Andres Martinez
Technology: Antonio Ricco

Funding: NASA/Exploration Systems Mission Directorate
Thank You

Questions?
**ConOps**

1. **Imager Test (A, B, W)**
   - Heating
   - Image Red Beads
   - Move Red Beads Out
   - Image Green Beads
   - Move Green Beads In
   - Feed all worms
   - Move Green Beads Out
   - Image Red Worms
   - Move Red Worms In
   - Red Worm Imaging
   - Move Red Worms Out
   - Green Worm Imaging
   - Move Green Worms In
   - Green Worm Imaging
   - Move Green Worms Out

2. **Off**
   - Move Red Out
   - Off
   - Move Green In
   - Off
   - Feed all worms
   - Move Green Out
   - Off
   - Move Worms In
   - Off
   - Move Worms Out
   - Off
   - Move Worms In
   - Off
   - Move Worms Out
   - Off

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

4. **Alive (C)**
   - Heat to (22C)
   - Temp Maintain (22C)

5. **Alive (C)**
   - Heat to (22C)
   - Temp Maintain (22C)

6. **Alive (C)**
   - Heat to (17C)
   - Temp Maintain (17C)
After pumping worms into imaging chamber.
Payload ConOps

Payload Builds/Satellite Integration

Workmanship/Functional Checkout (Data/Images)

Travel

Functional Checkout/Data/Images

Experiment Arm

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 8 Days
Act with *C. elegans*

b. (machined)
b. porous membrane
t. sensitive adhesive (PSA) (cut edges only)
acrylic adhesive, polyester carrier
laser-cut edges
trene gas-permeable cover film

**Contact with growth medium only (reservoir/tubing)**
ethylene vinylacetate) [EVA] bag
eone barbed ports
thane tubing

**Contact with C. elegans and growth medium**
wetted valve materials

1. PPS = Polyphenylene sulfide (“Fortron”)
1. PBT = Polybutylene terephthalate (“Valox”)
1. 316 SS
1. FeCr Alloy
1. Silicone Rubber

C. elegans
fluoresc. beads
thermal control
Fluidics Stack Pins
- Detail -
Back-Cover Assembly

Viton O-Ring

Activated Carbon
Camera Mount
- Mated to Back Panel -
Camera Assembly
- Mated to Camera Mount -
Front Panel Assembly

Hermetic Connector
- Laser Welded

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

<table>
<thead>
<tr>
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<tr>
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<td>Translates the bus 50pin cable to the hermetic connection for the payload can. Also has connection for the remove by flight “kill switch”</td>
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<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>
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<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>
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<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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<td><strong>0 Imager Processor</strong></td>
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NanoSatellite Science Payload

Fluidic System

22 cm

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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>
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<tbody>
<tr>
<td><strong>N2</strong> (wt)</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" 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="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" 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="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
<td><img src="image9" 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="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
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<td>green – intestinal cells</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
COMedia C328-7221
Camera chip

green fluorescently labeled *C. elegans*

Post Processing [Contrast]

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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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
Fluidics Assembly

Dolomite Pump

Reservoir
Lee Co. Surface Mount Valves
Fluidics Block
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
Acknowledgements

Fluidics: Ming Tan, Matthew Piccini
Biology: Matthew Lera, Macarena Parra
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

System Initiate
Keep alive mode

Set Temp
Payload Data Check and Images

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

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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<td>M450 Imager Processor</td>
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