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

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NASA Ames Research Center
ASGSR
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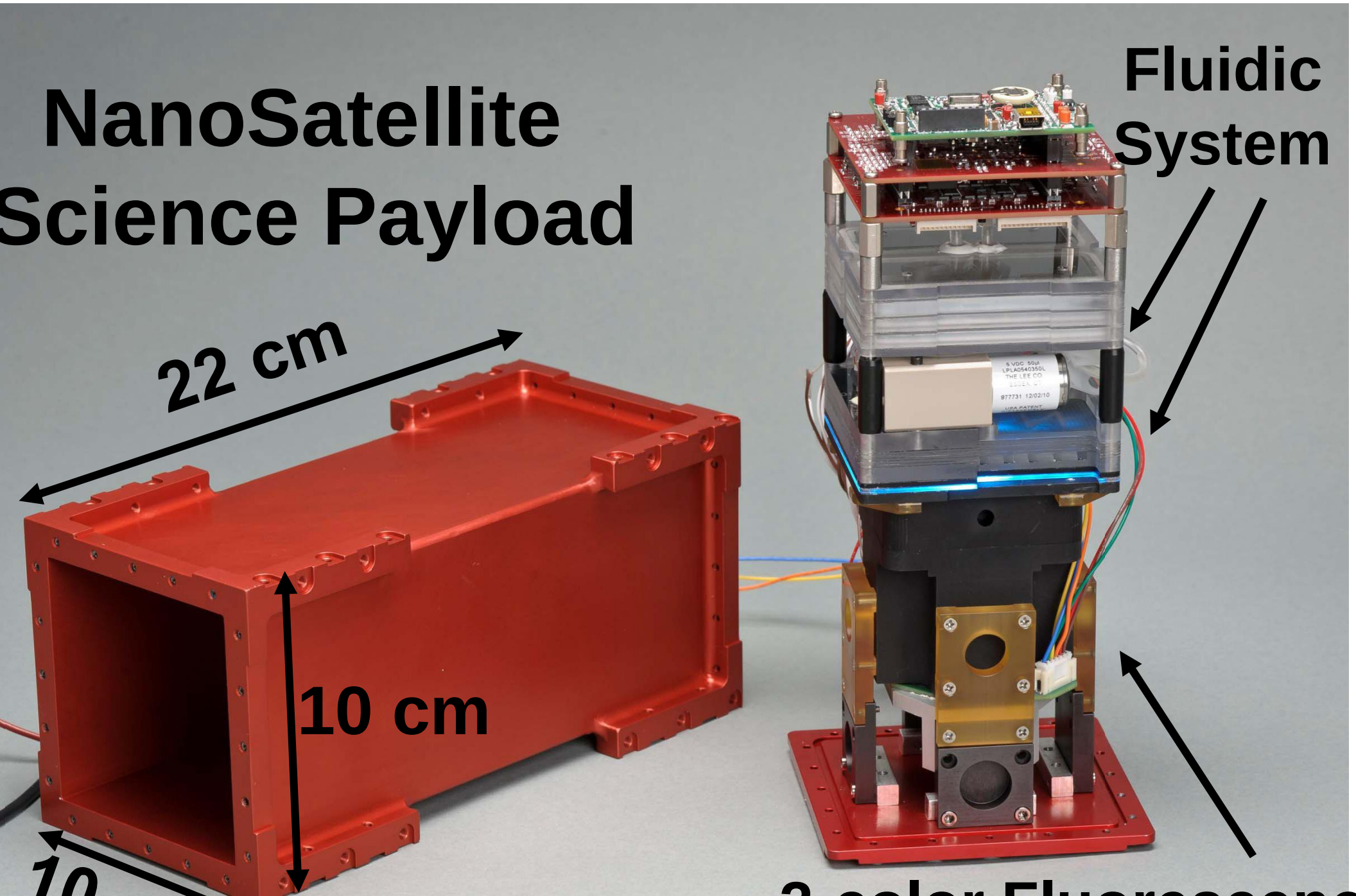
Technologies (MisST)



Payload Science & Technology Overview

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

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

entation: 2-cube integrated culture/imaging
ent

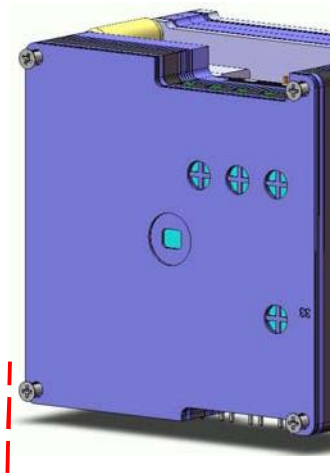
. elegans model organism

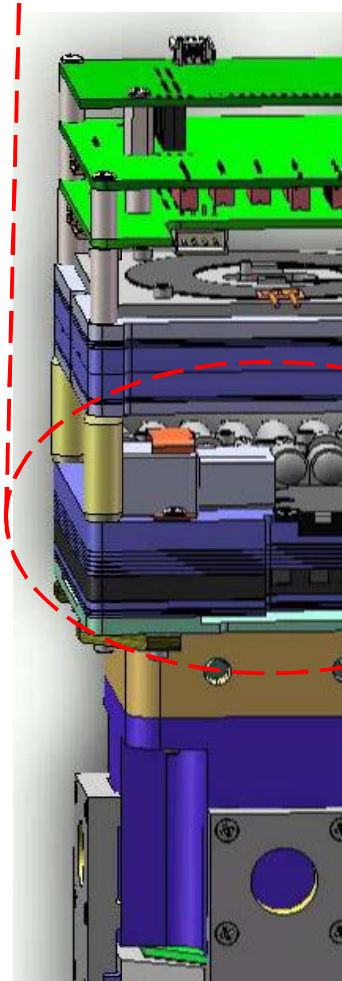
uidics: culture wells, imaging zone, reagent storage

nager: fixed focus, 300 kpixel CMOS chip

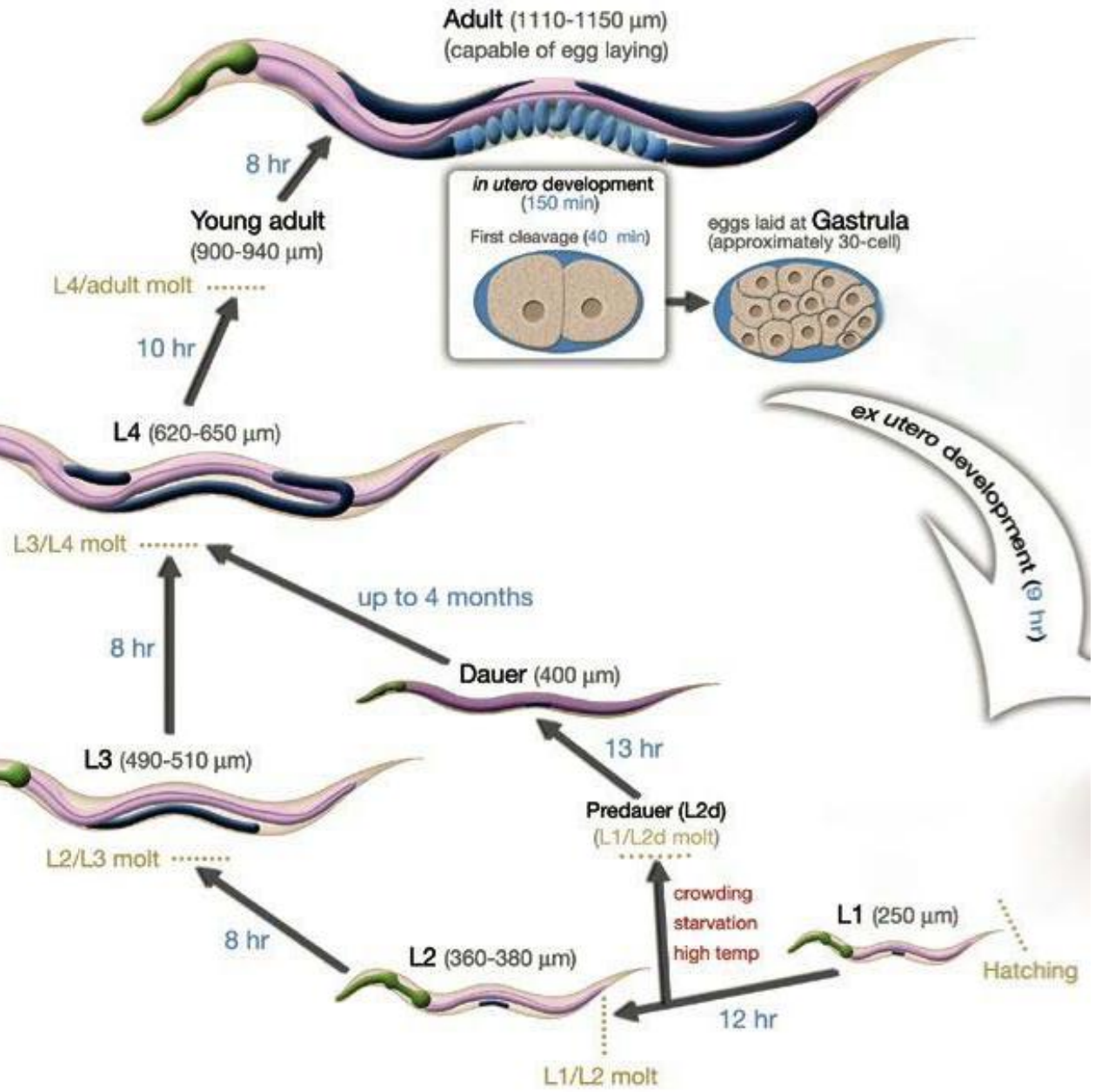
nage storage: 2 GB/6,000 images/8 bits per color
elemetry of 1 - 20 images)

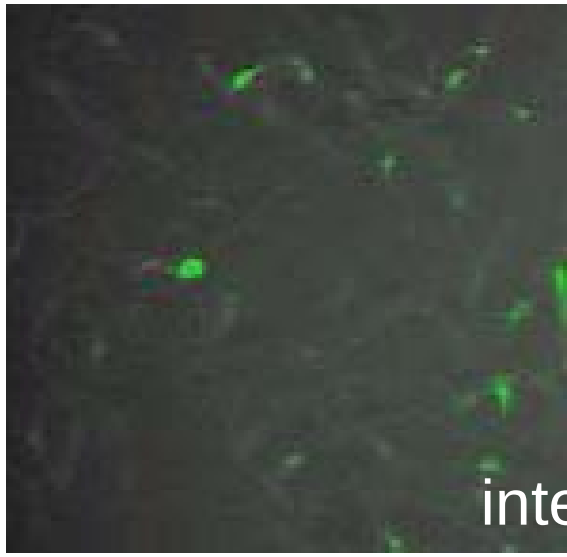
lermetic containment vessel

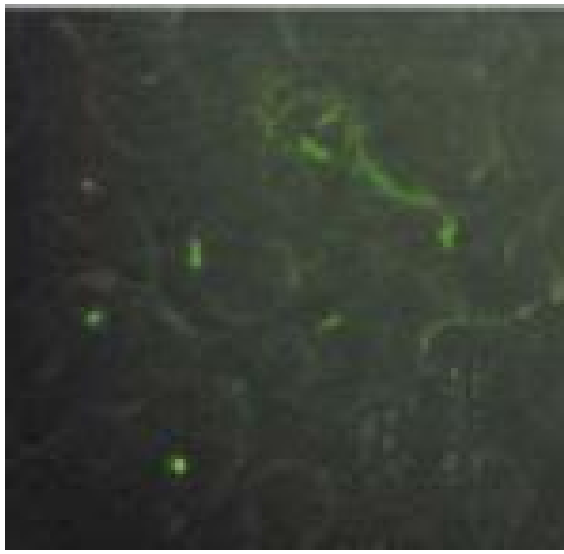


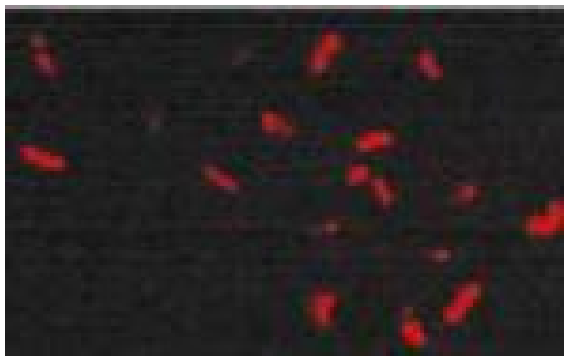


C. elegans Overview



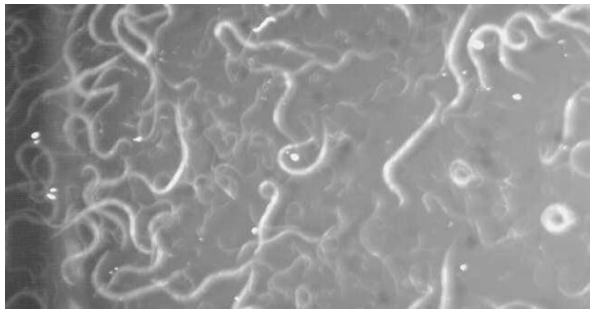
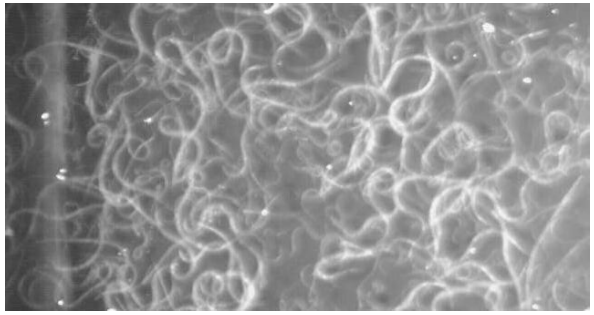
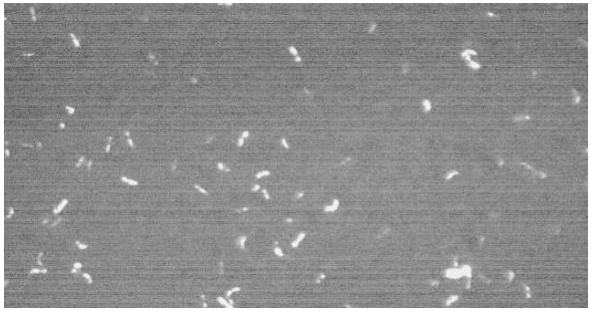
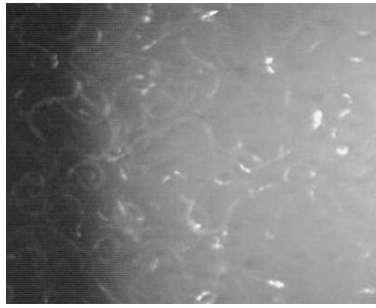
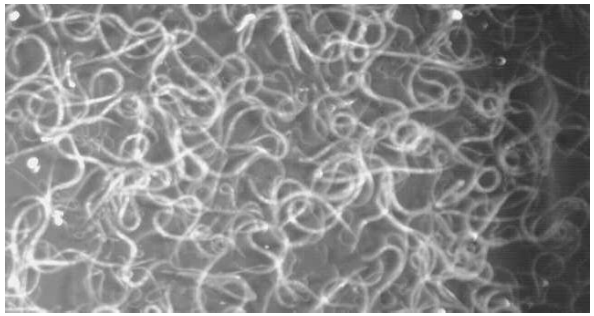
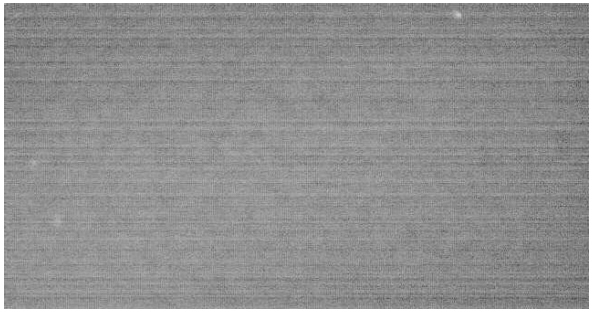
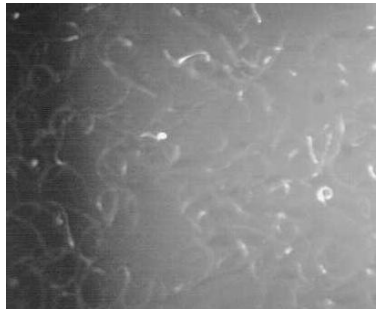
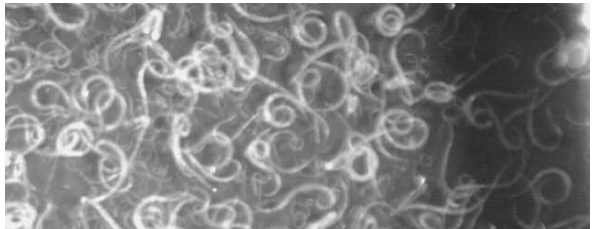






ding and short life cycle make it inexpensive to
and ideal for research
study spaceflight effects and experiments using *C.*

C. elegans Imaging

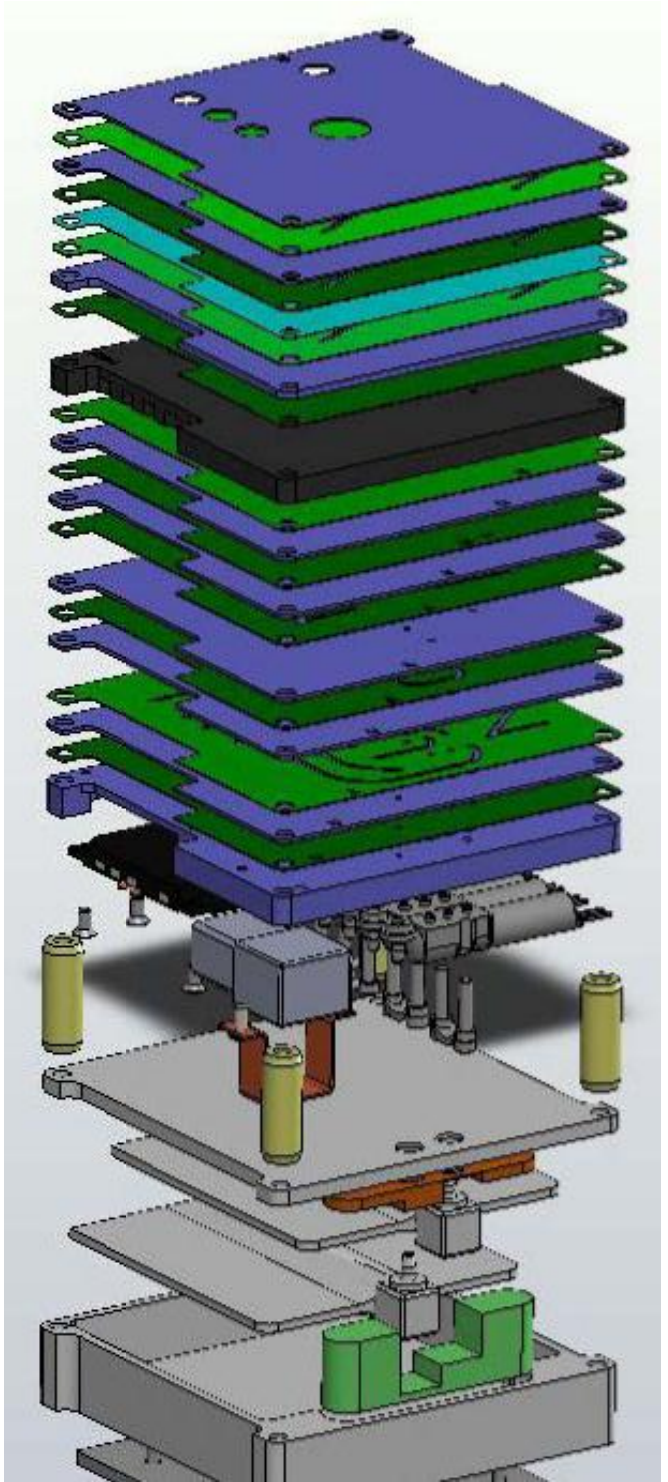
	White	Amber	Blue
t) rescent			
nato uscle cells			
4 nerve cells			
42 intestinal cells			

Fluidics Overview

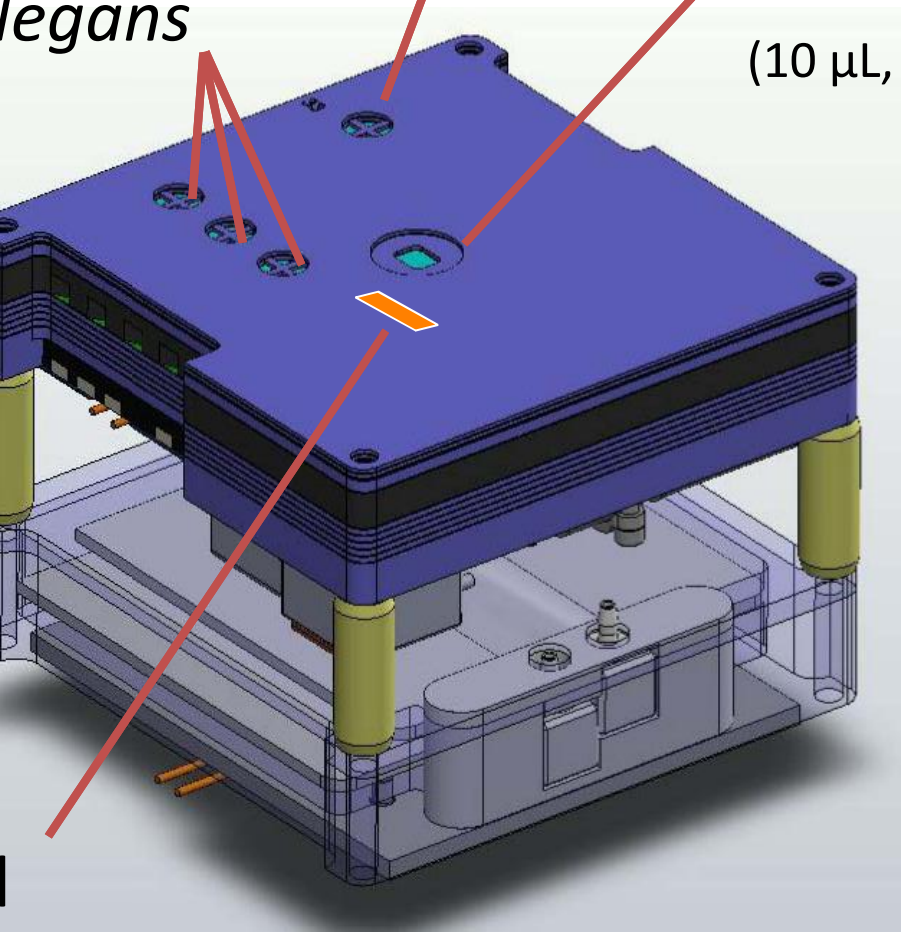
ach: Multilayer polymer microfluidic card

- 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

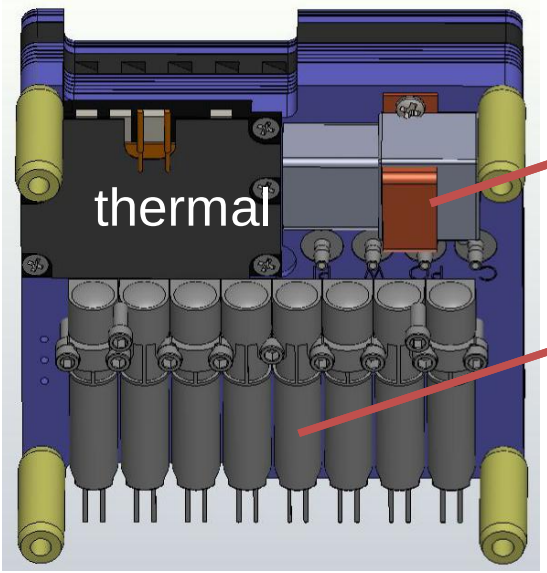
ers, adhesives, sterilization, biocompatibility, all have GeneSat, PharmaSat, O/OREOS-EcAMSat heritage



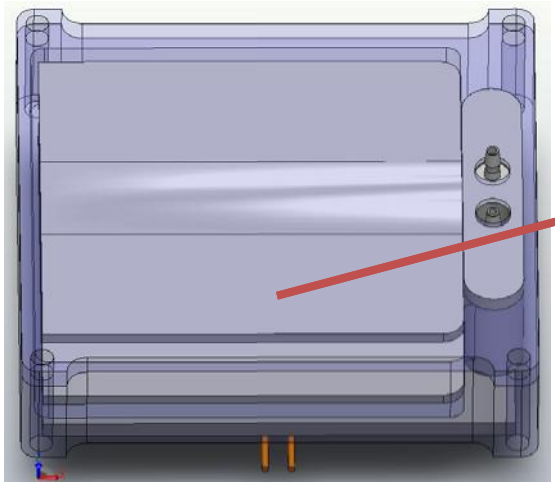
Fluidic Card and Reservoir



Fluidic Card

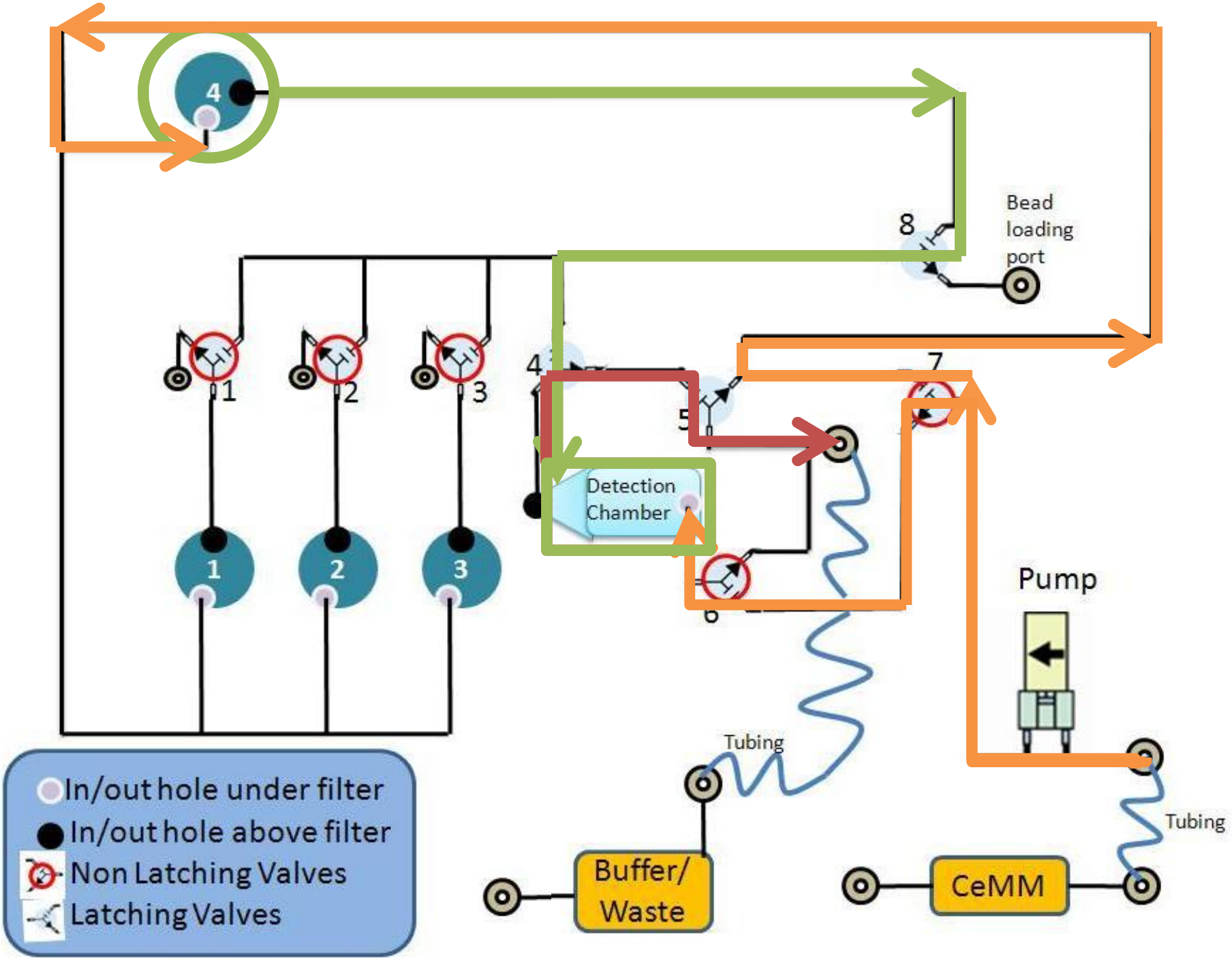


Reservoir

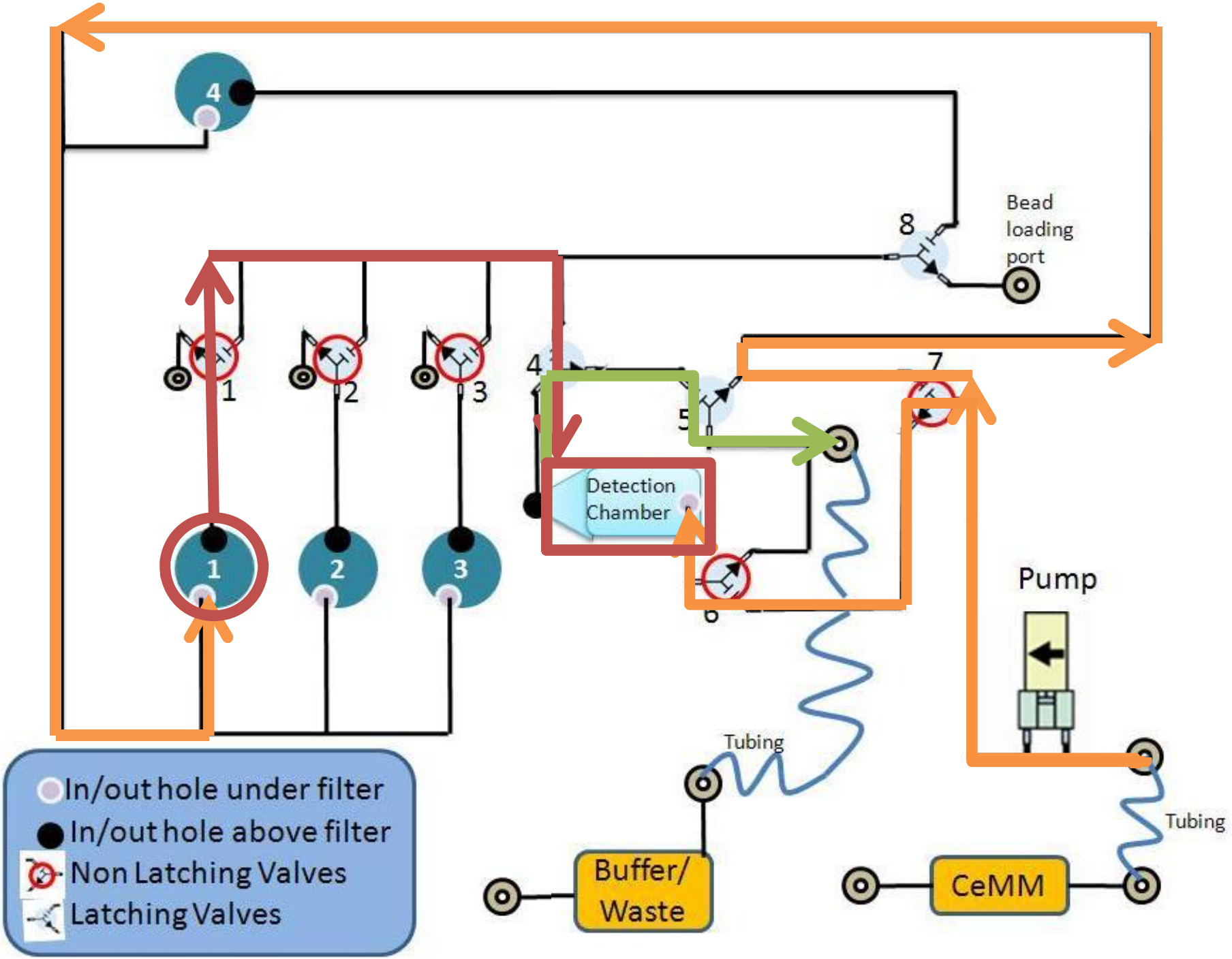


Experimental Timeline

Fluidic Sequence - Beads



Fluidic Sequence- Worms



Fluorescence Imager

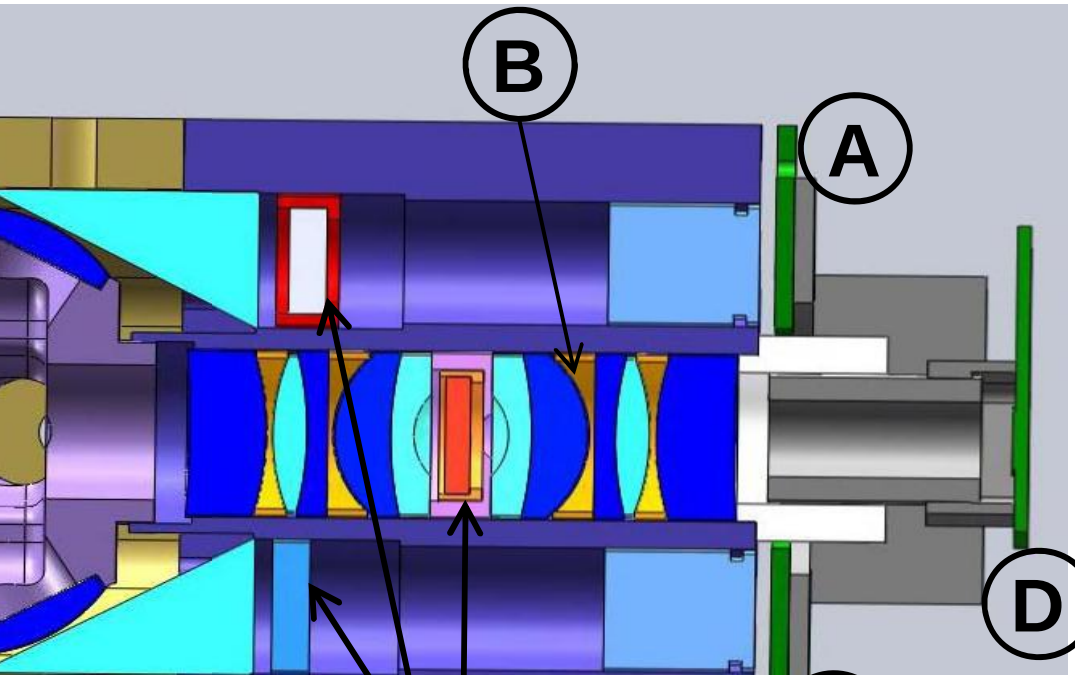
Imager Components:

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

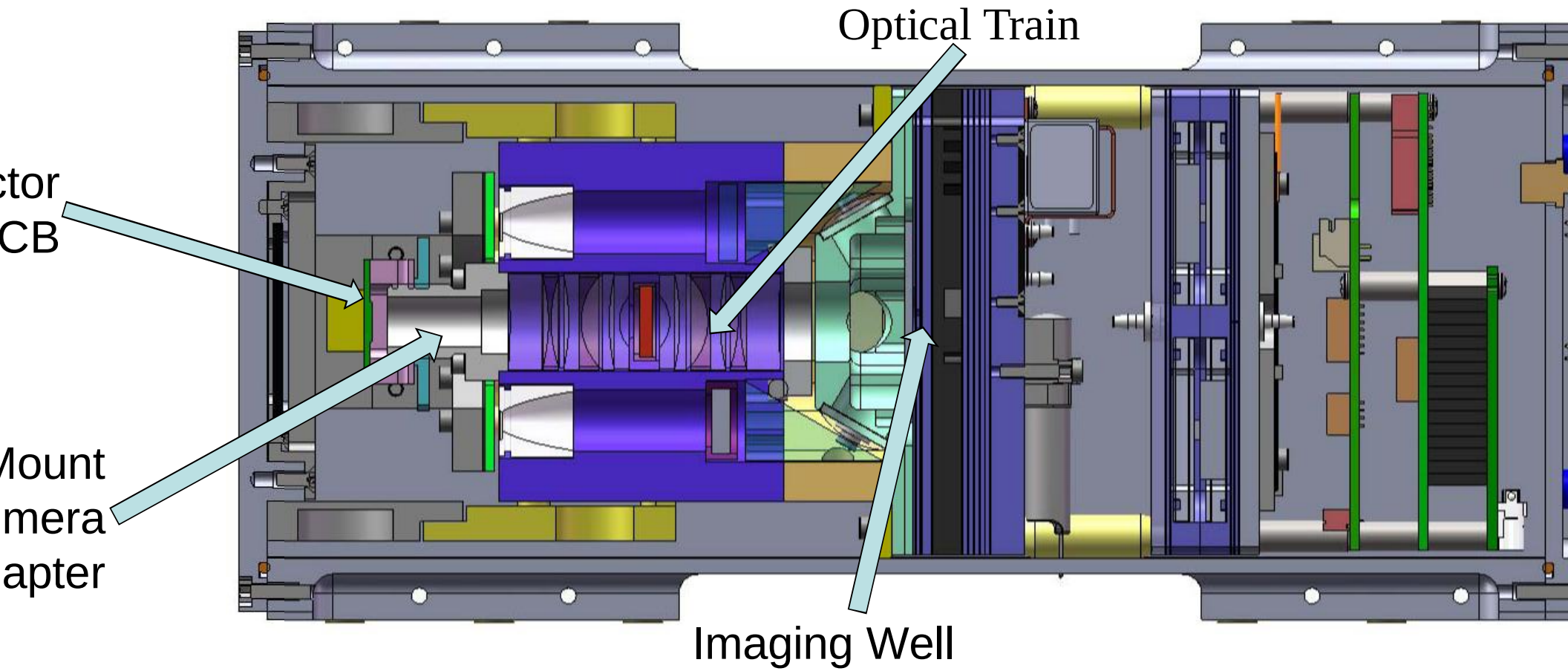
D Camera:
COMedia C328-7221

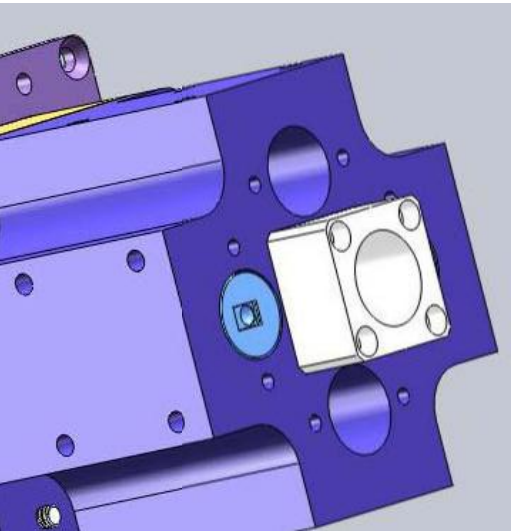


Imager Specifications:

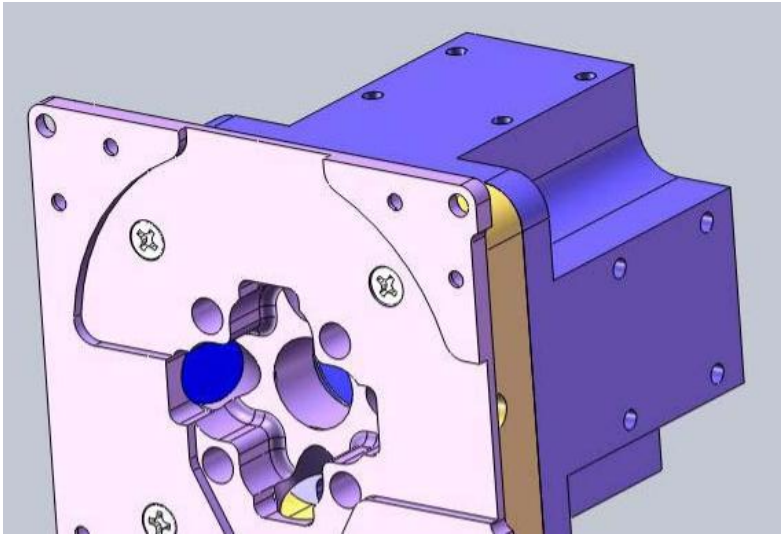
- 2-color fluorescence:
  Green excite/emit peaks at: 487/
  Red excite/emit peaks at: 587/61
- Cool white for standard imaging
- Lateral resolution: ~ 8 µm
- Magnification: 1:1
- Field of View: ~3 x 4 mm
- Depth of Focus: ~150 µm

Fluorescence Imager





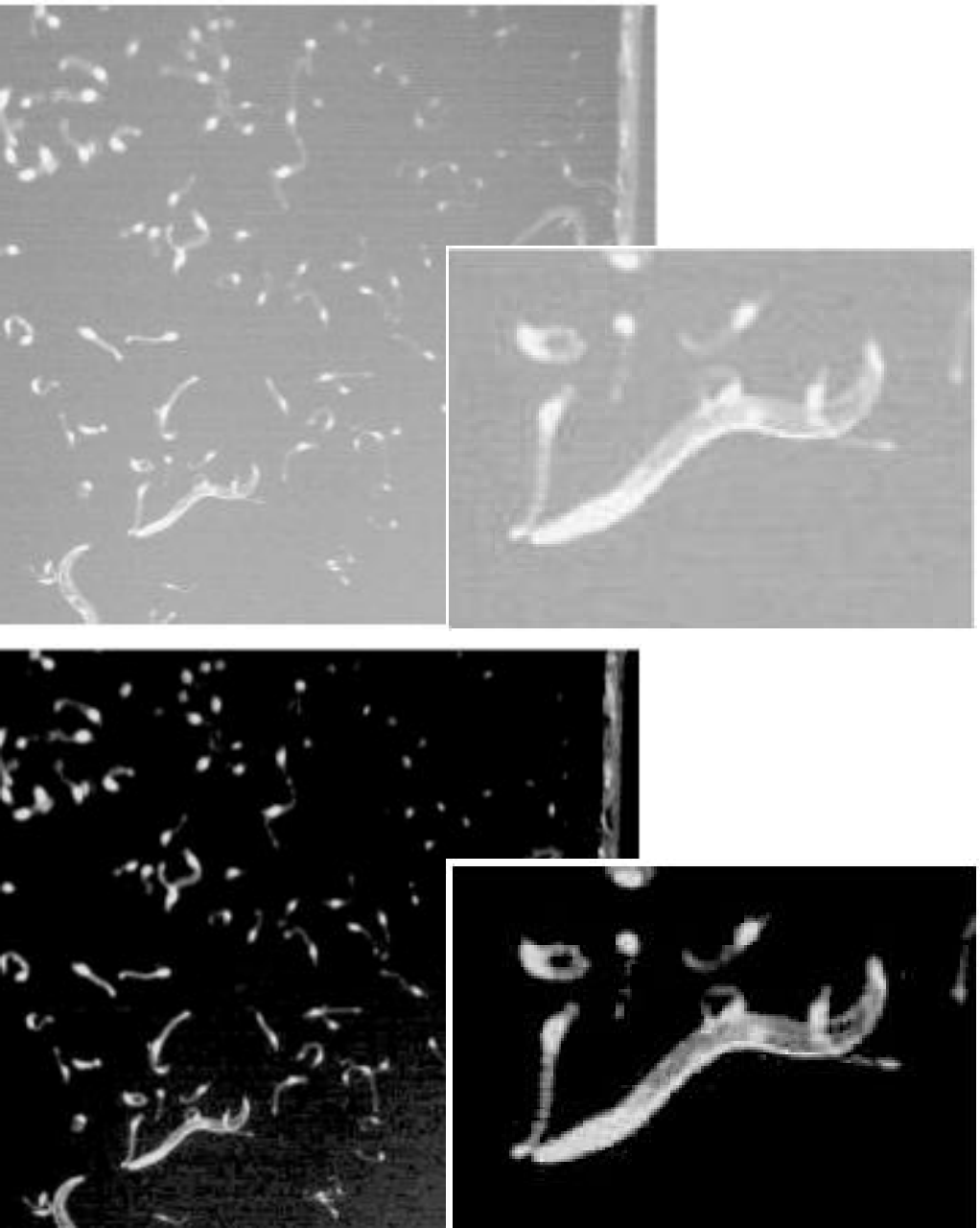
Imager Assy.

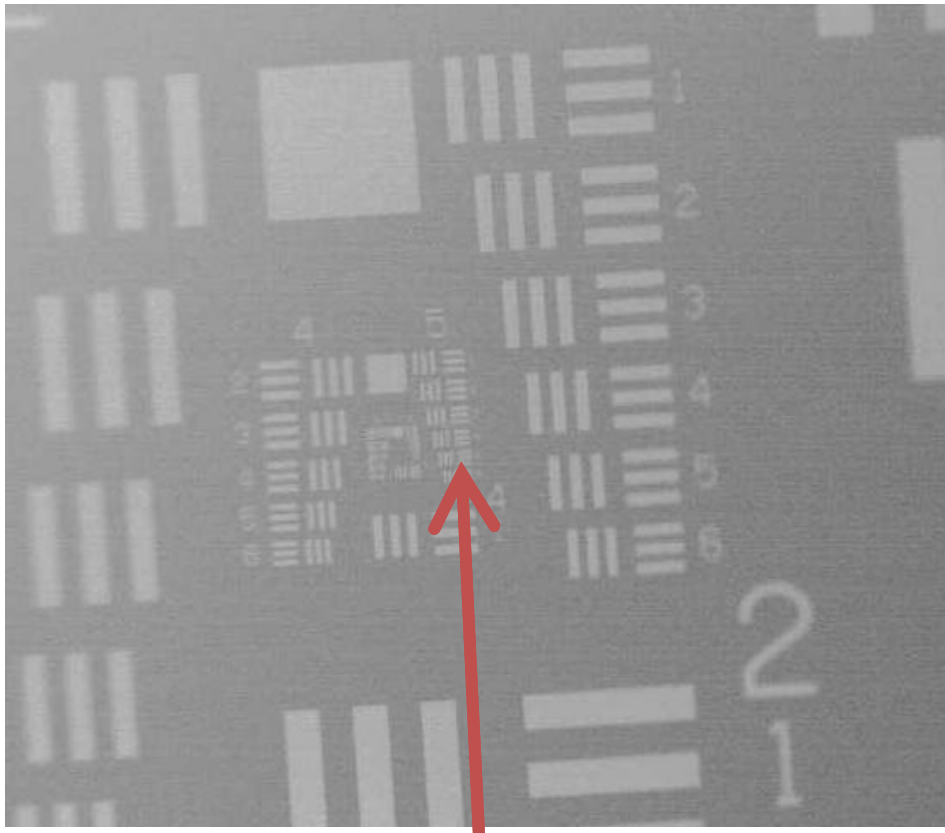


- Fluidic well depth: **0.17 mm**
- Fine pitch thread c S-mount (**0.5mm/th**
- Camera adjustmer **±5 mm**
- Locknut/set screw

Camera chip

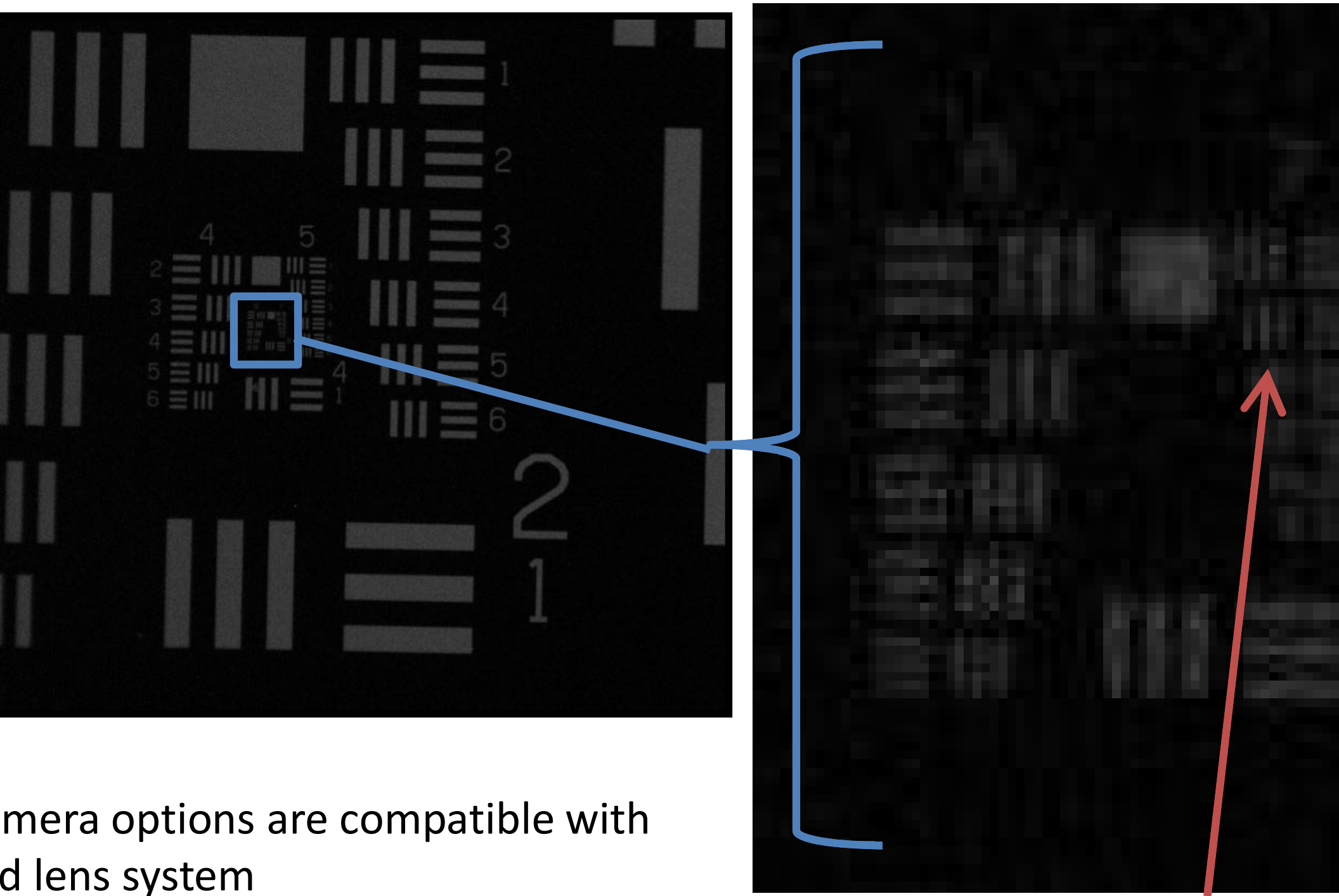
orescently labeled *C. elegans*





Resolves ~8 µm feature size

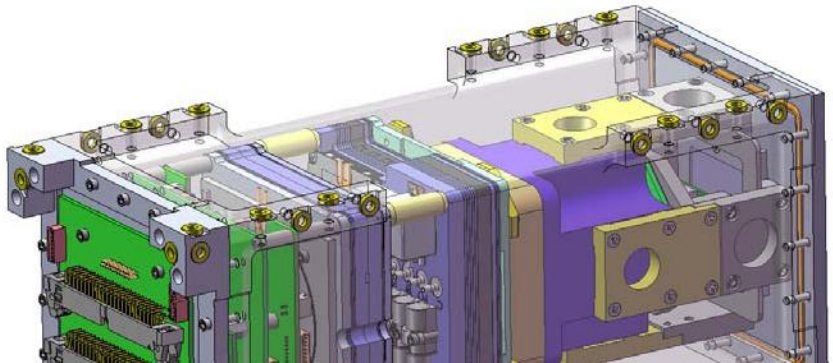
CCD imaging chip



mera options are compatible with
d lens system

Payload Functional Overview

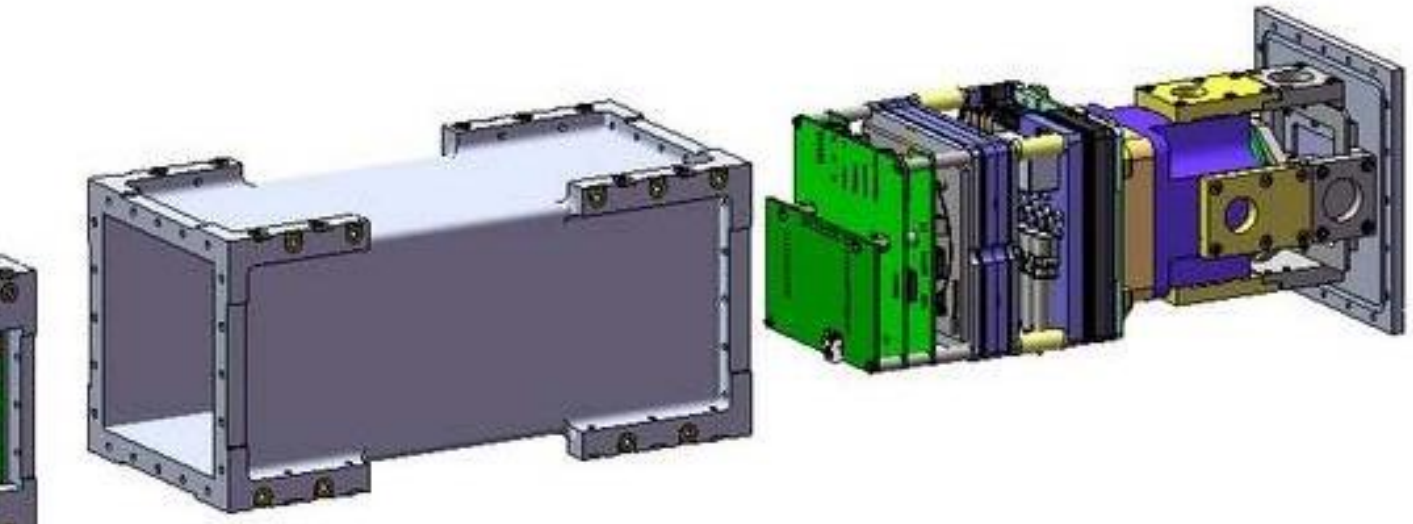
s	~1.5 – 2kg
sors	Pressure : accurate to within 2% of 1 atm. Humidity: accurate to within 4% from true value after temp. correc Temperature accurate to within +/- 0.5ºC.
essor	PIC 32
perature trol	Kept at 4°C to 30°C during the experiment phase.
ronmental mber	Maintains an atmospheric pressure in the pressurized payload cham volume within 11.7 to 15.7 psia.
em lization	Material selections have heritage to past missions for ability to be sterilized and low outgassing.

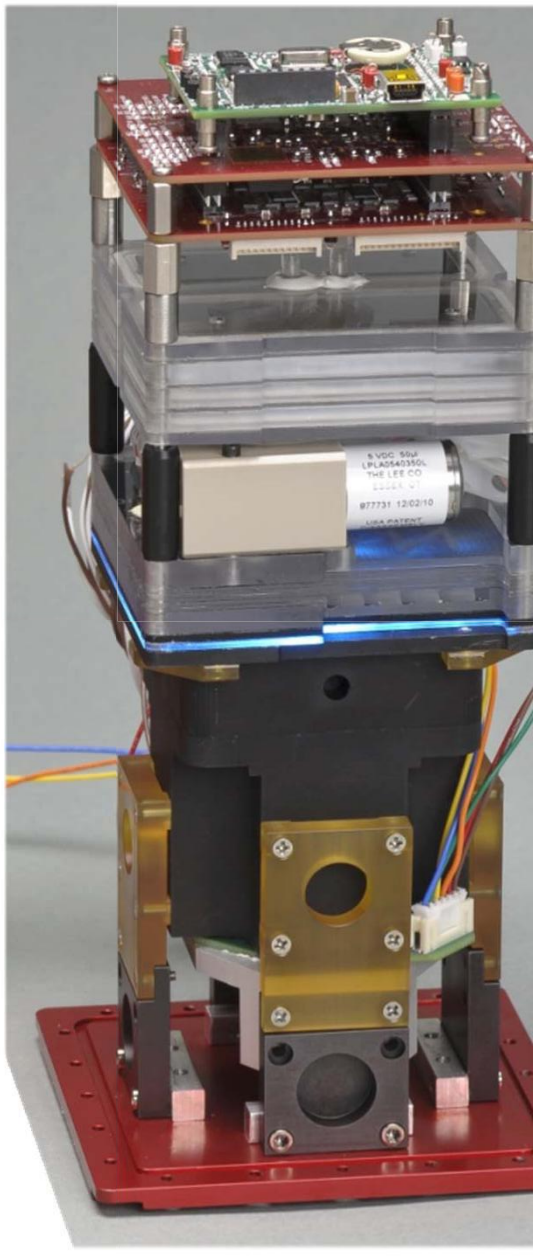


Payload Design Overview

Integrated Payload

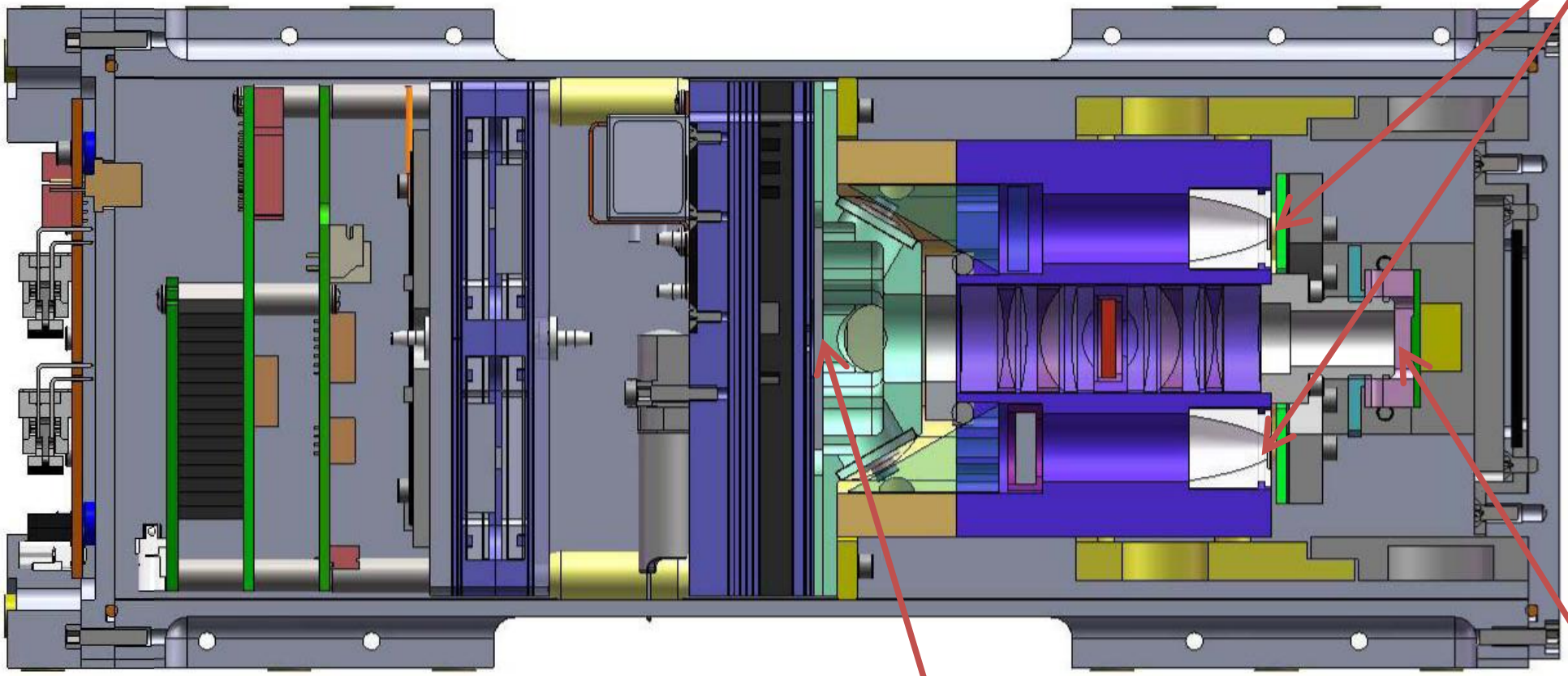
- Exploded View -



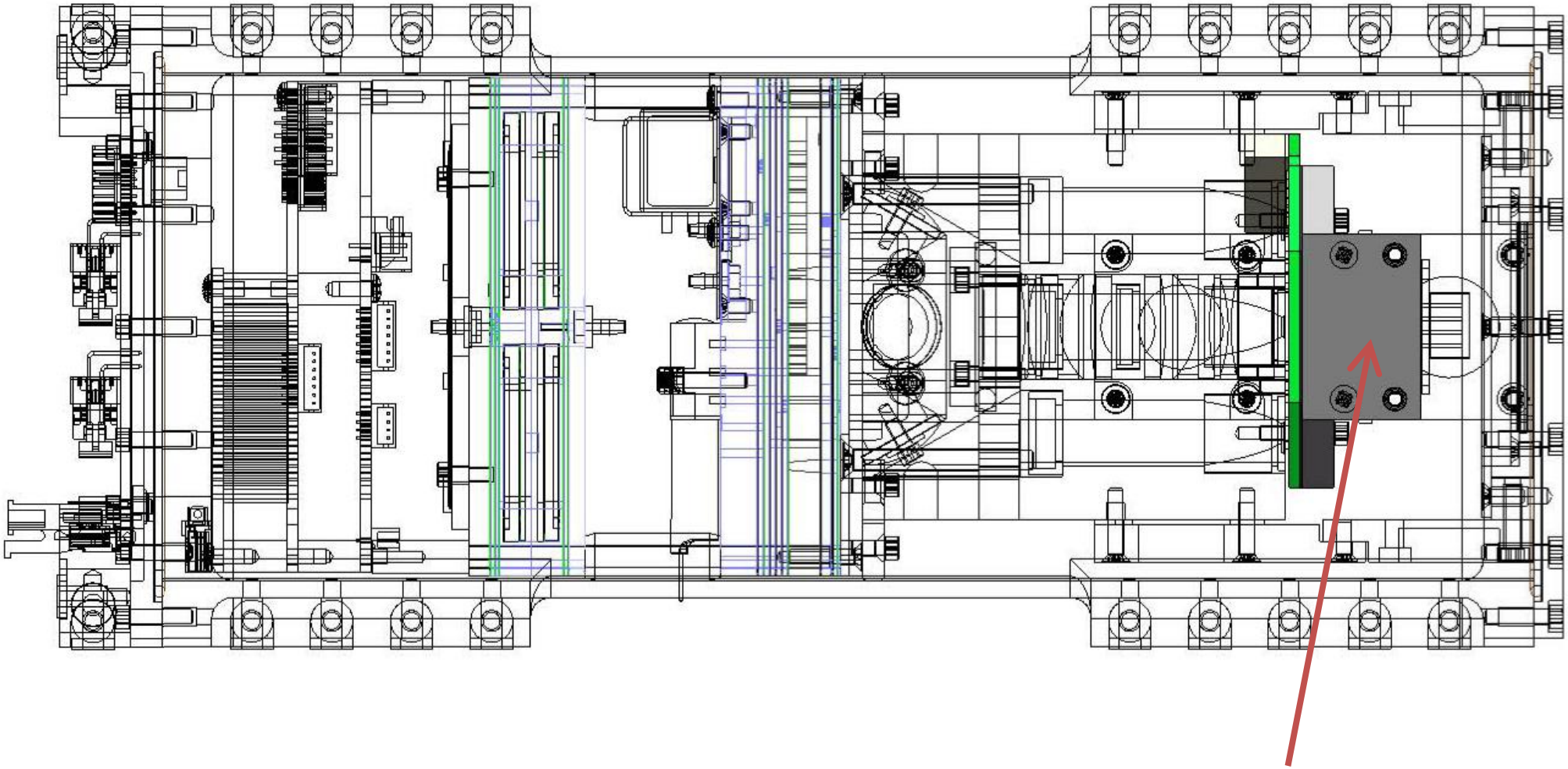


Integrated Payload

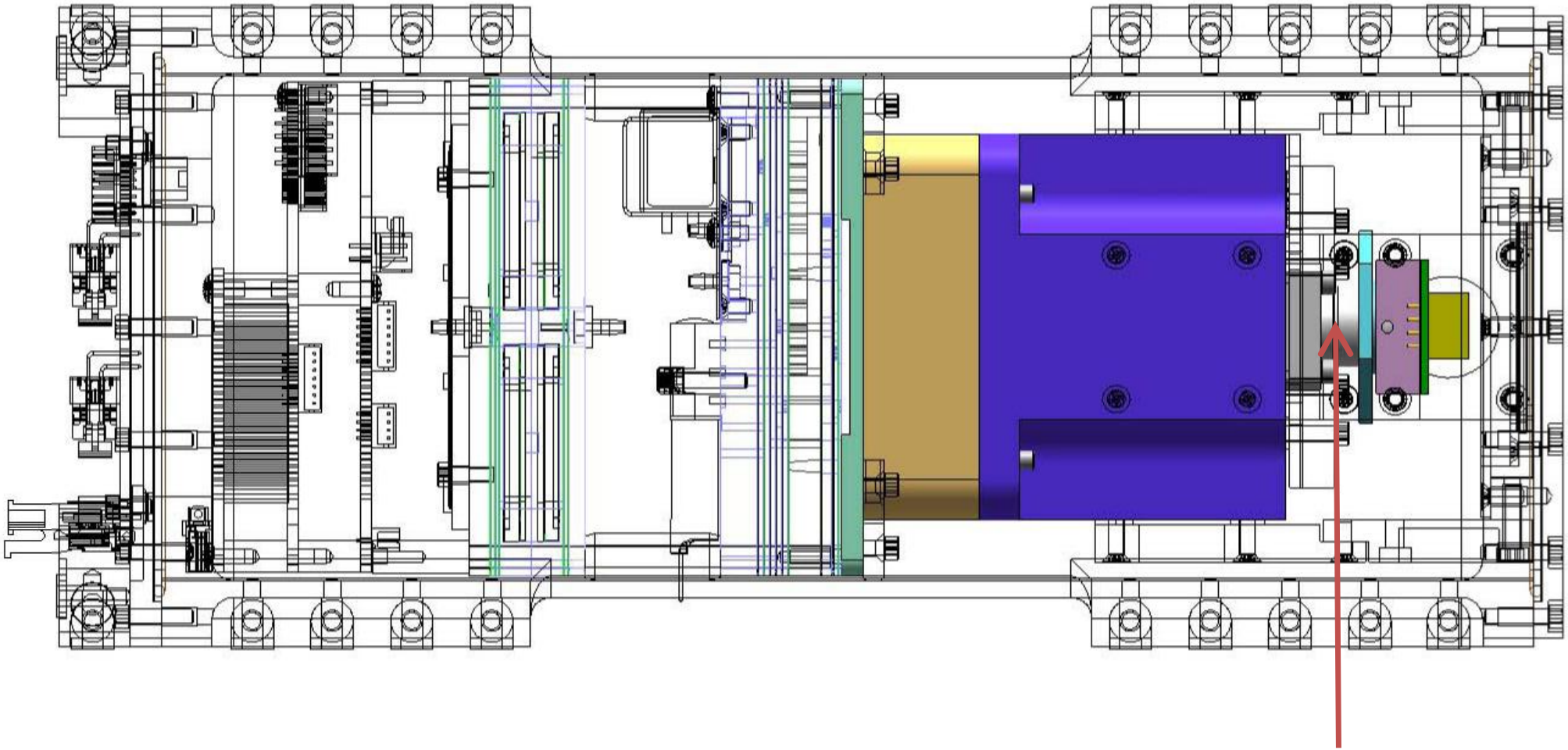
- Sectional View -



LED Assembly

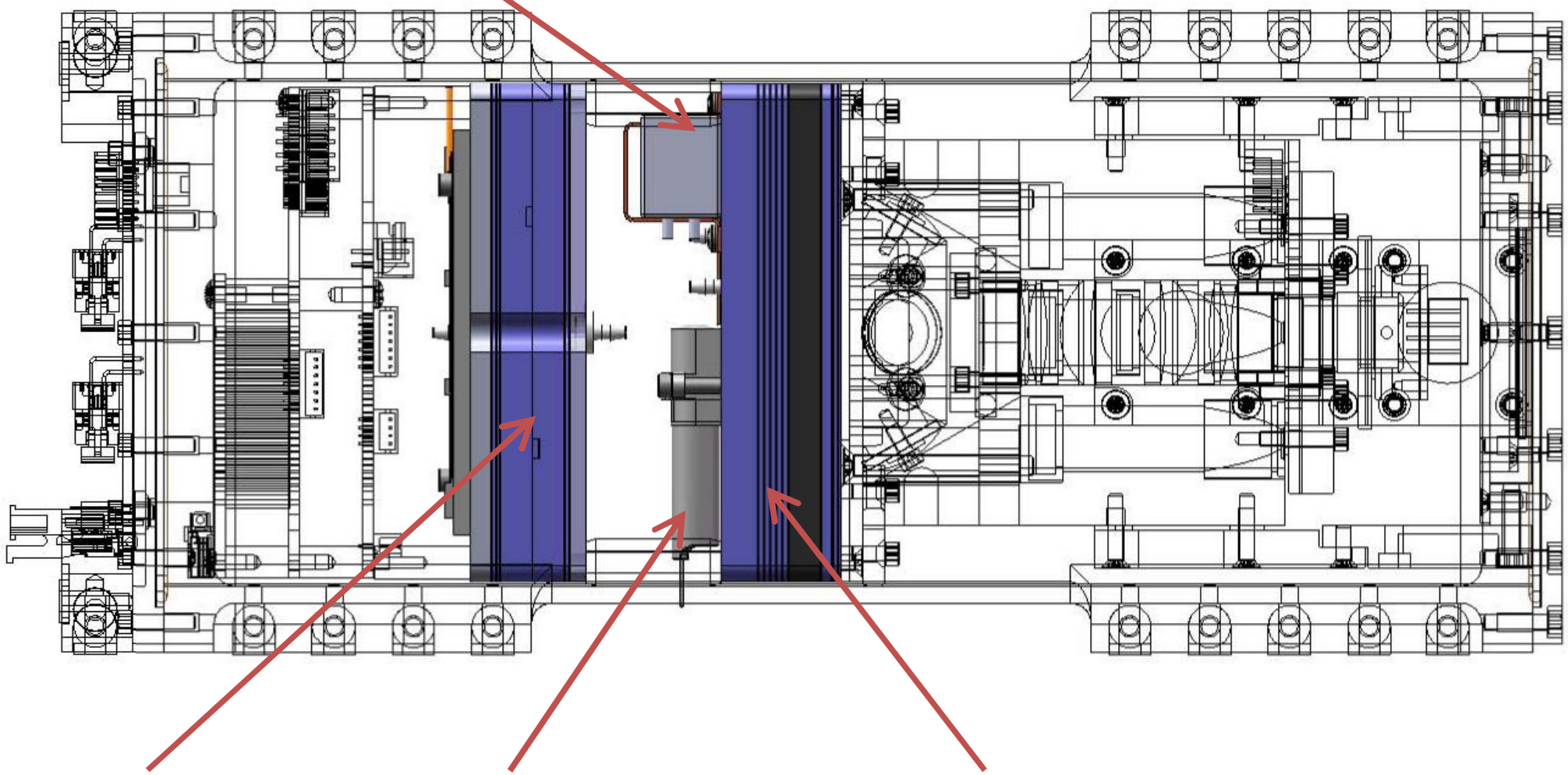


Camera Assembly

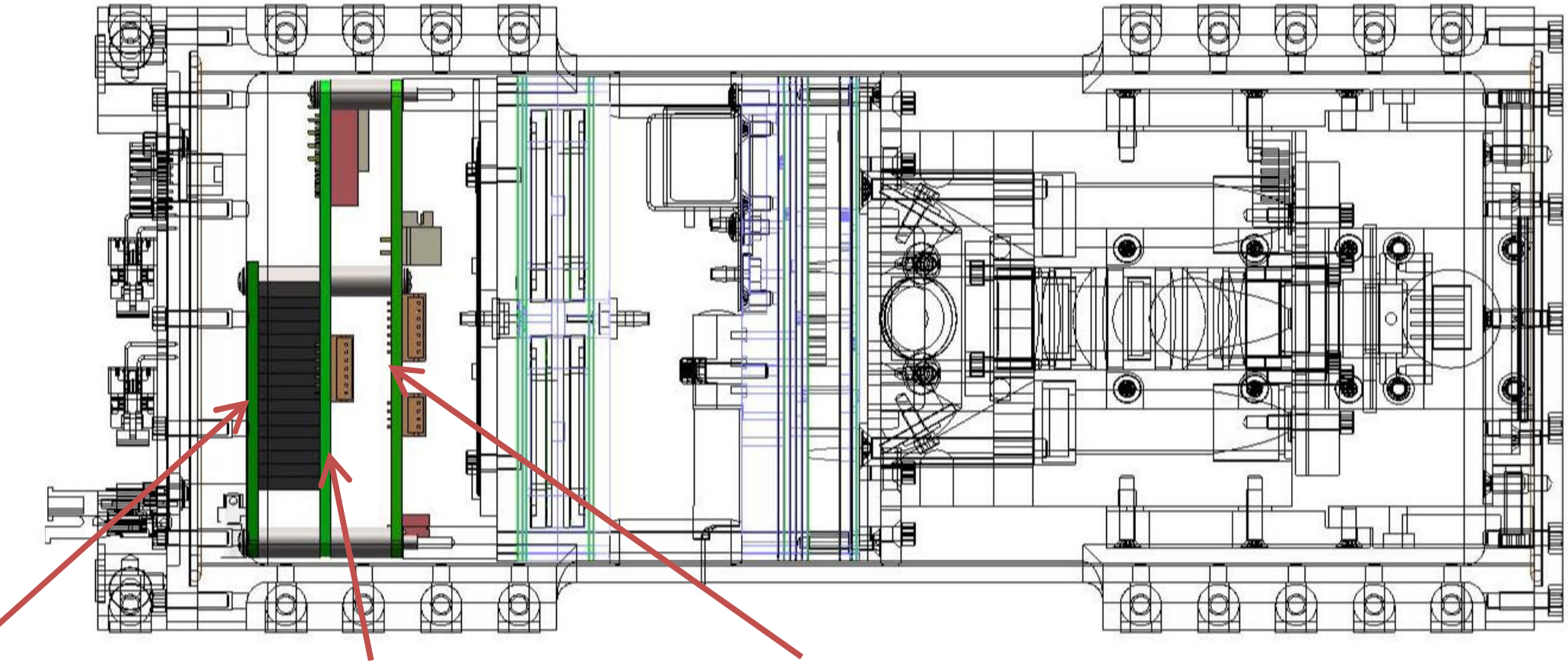


Camera Adapter/Focus Adju

Fluidics Assembly



Electronics Assembly



sor PCB

Analog PCB
- LED
- Thermal

Digital PCB
- Camera

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

dics: Ming Tan, Matthew Piccini

ogy: Matthew Lera, Macarena Parra

ging: Linda Timucin

chanical: Abraham Rademacher, Giovanni Minelli, Chris Beasle

trical: Aaron Schooley

nagement: Andres Martinez

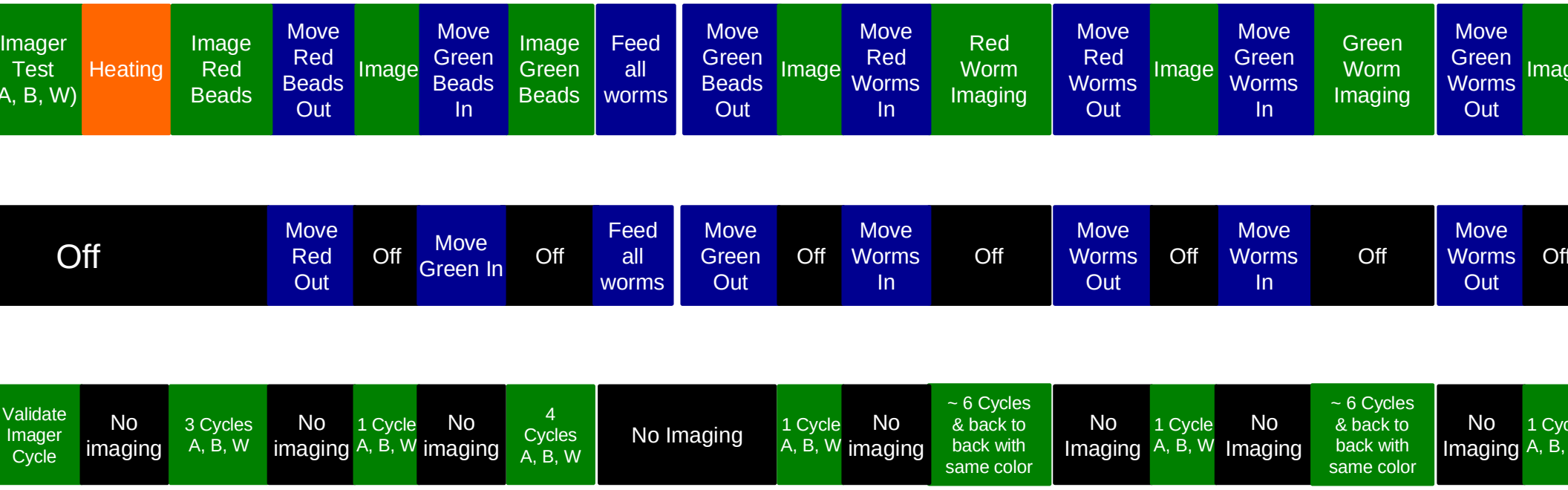
nology: Antonio Ricco

ding: NASA/Exploration Systems Mission Directorate

Thank You

Questions?

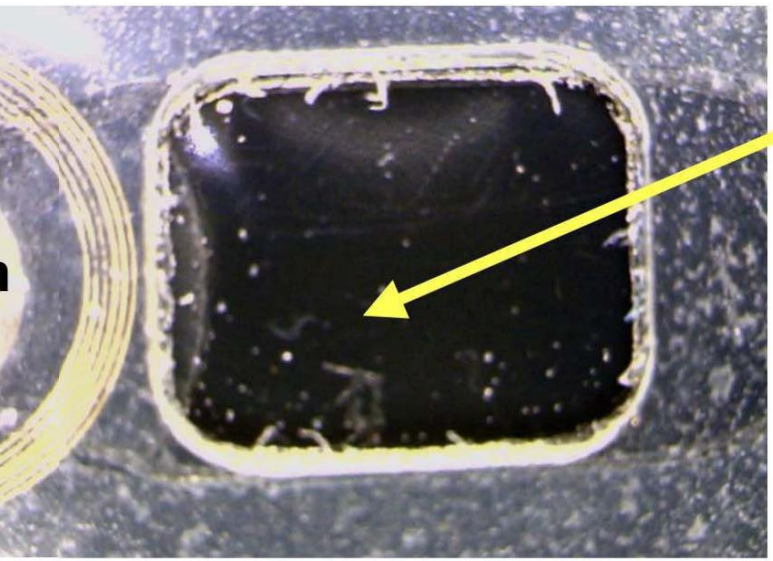
ConOps


Imager Test A, B, W)
Heating
Image Red Beads
Move Red Beads Out
Image
Move Green Beads In
Image Green Beads
Feed all worms
Move Green Beads Out
Image
Move Red Worms In
Red Worm Imaging
Move Red Worms Out
Image
Move Green Worms In
Green Worm Imaging
Move Green Worms Out
Imag
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
Validate Imager 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 Cyc A, B,

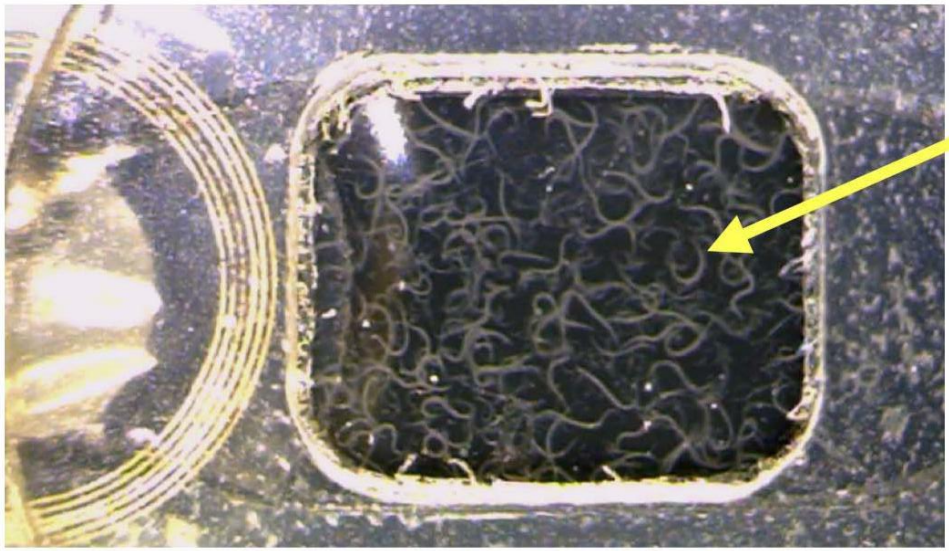

Alive C)
Heat to (22C)
Temp Maintain (22C)


Alive C)
Heat to (22C)
Temp Maintain (22C)

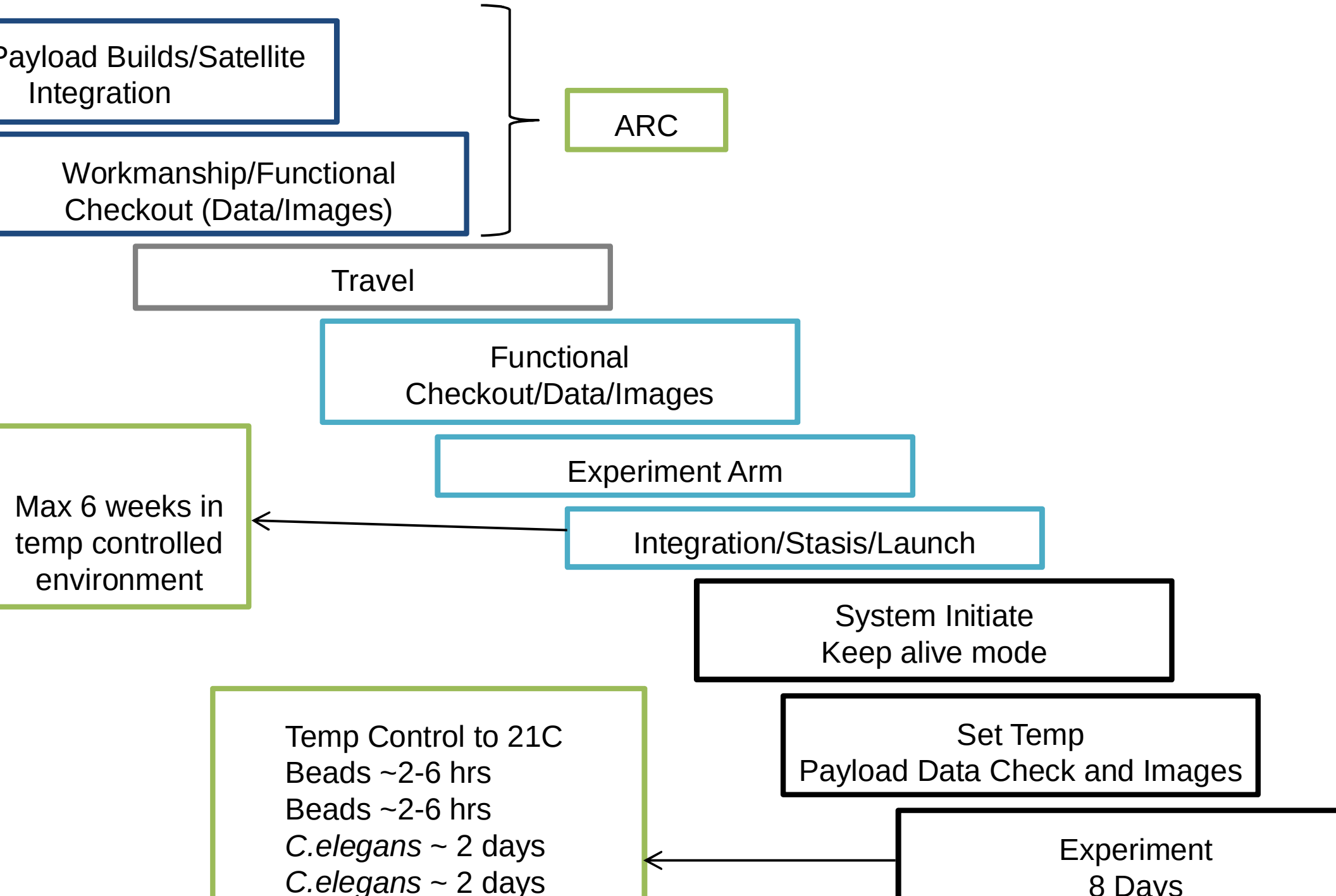

Alive C)
Heat to (17C)
Temp Maintain (17C)



After pumping worms into imaging chamber



Payload ConOps



act *with C. elegans*

b. (machined)

b. porous membrane

re-sensitive adhesive (PSA) (cut edges only)

acrylic adhesive, polyester carrier

laser-cut edges

rene gas-permeable cover film

with growth medium only (reservoir/tubing)

nylene vinylacetate) [EVA] bag

fone barbed ports

thane tubing

ntact with C. elegans and growth medium

wetted valve materials

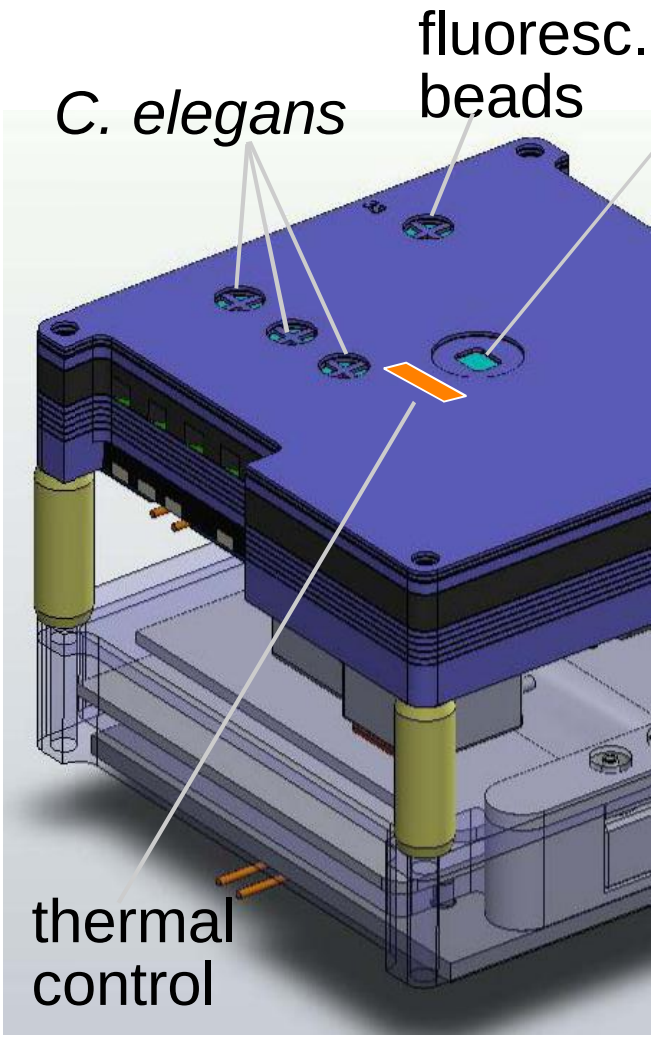
.PPS = Polyphenylene sulfide (“Fortron”)

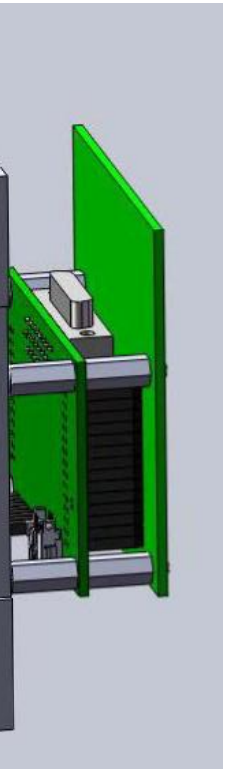
.PBT = Polybutylene terephthalate (“Valox”)

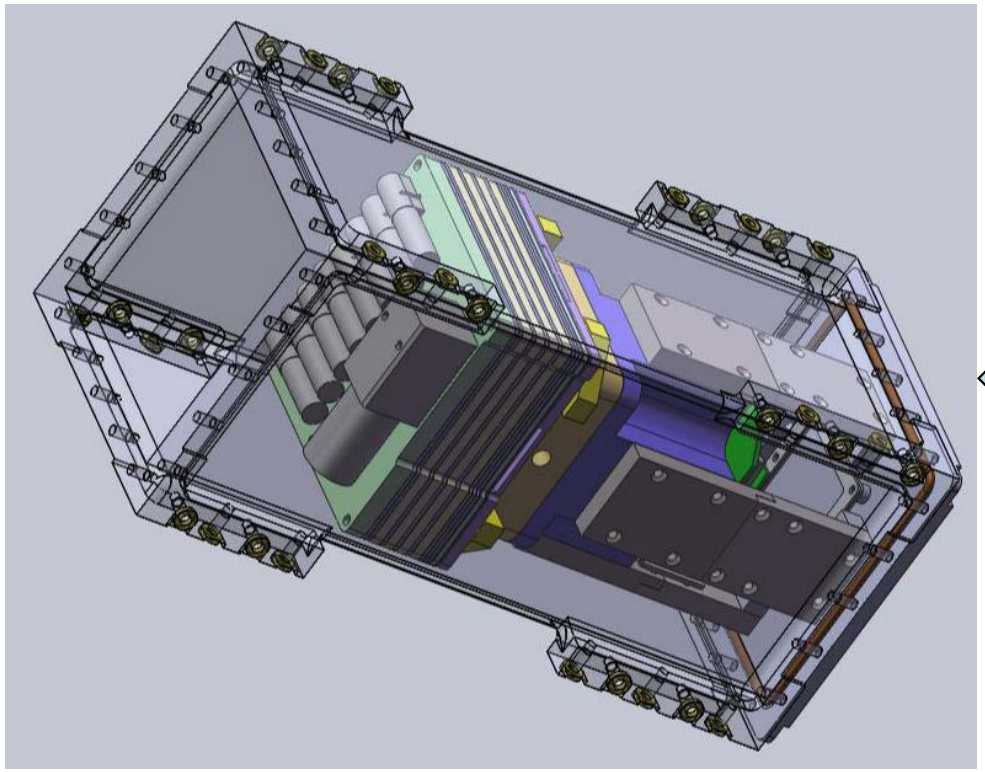
.316 SS

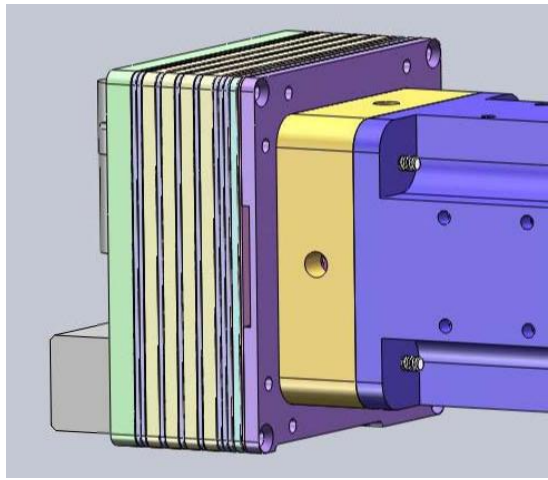
.FeCr Alloy

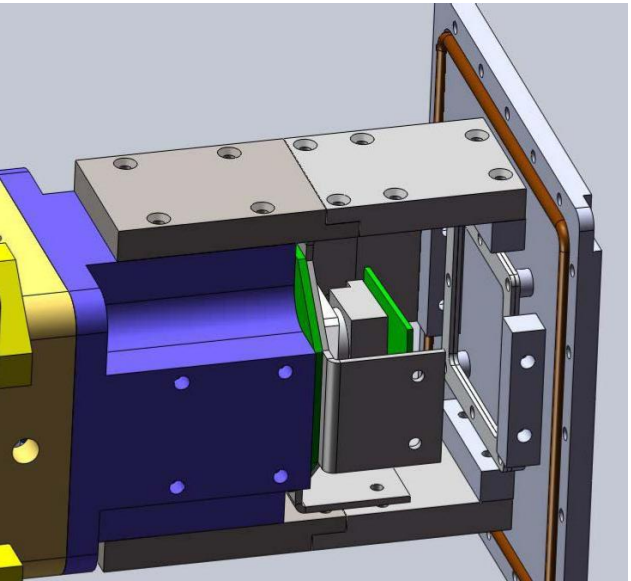
Silicone Rubber

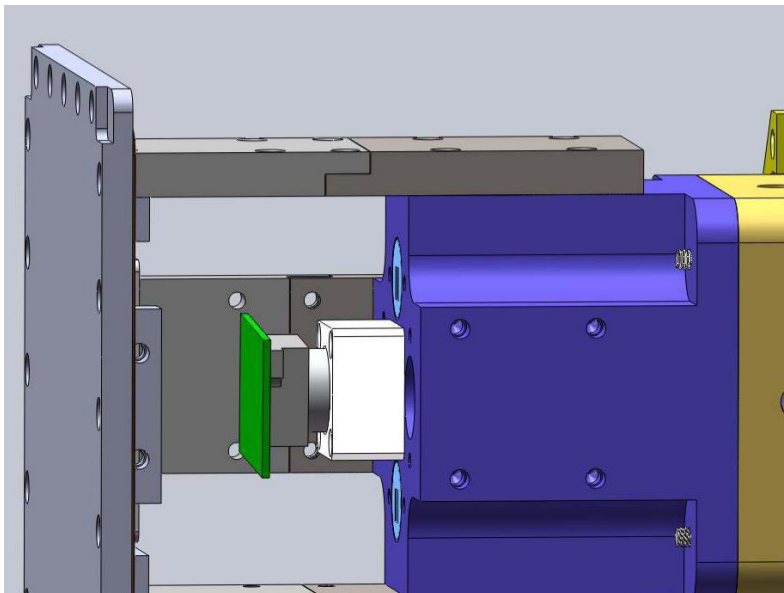






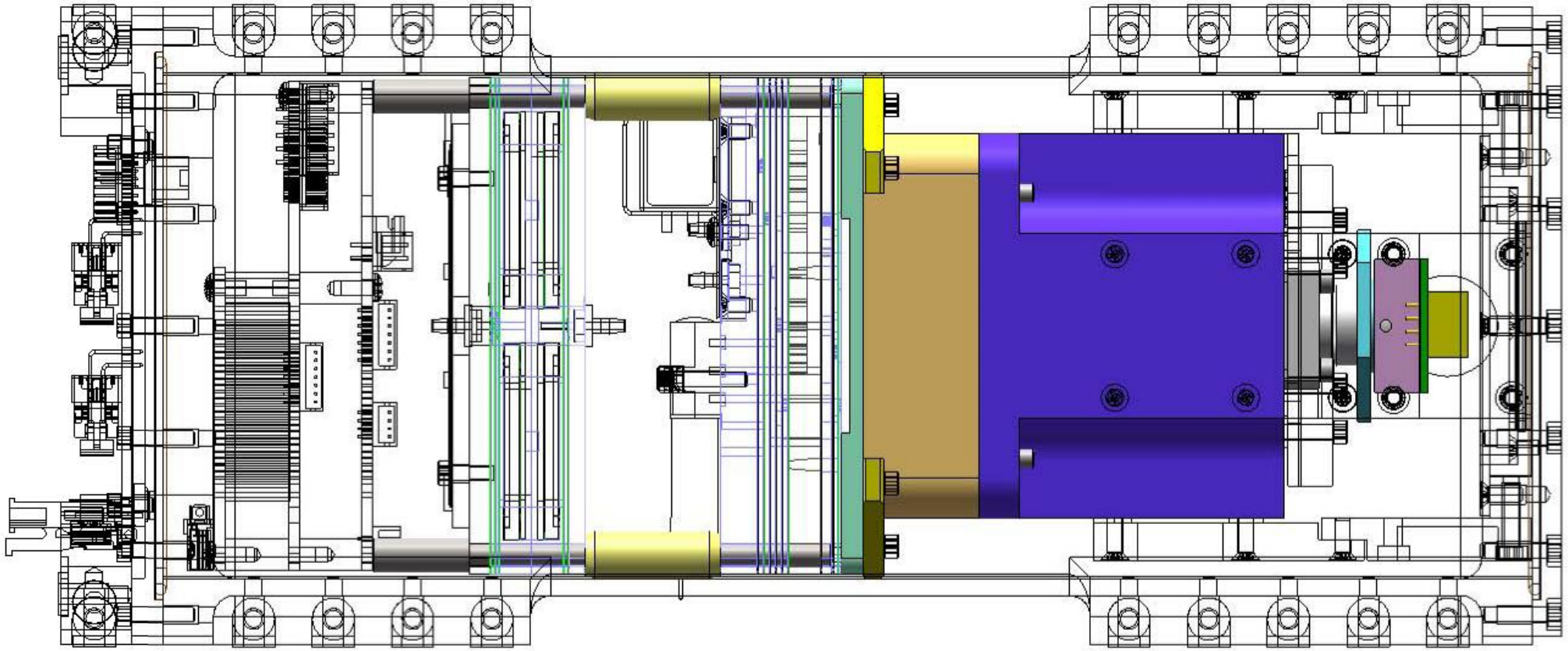




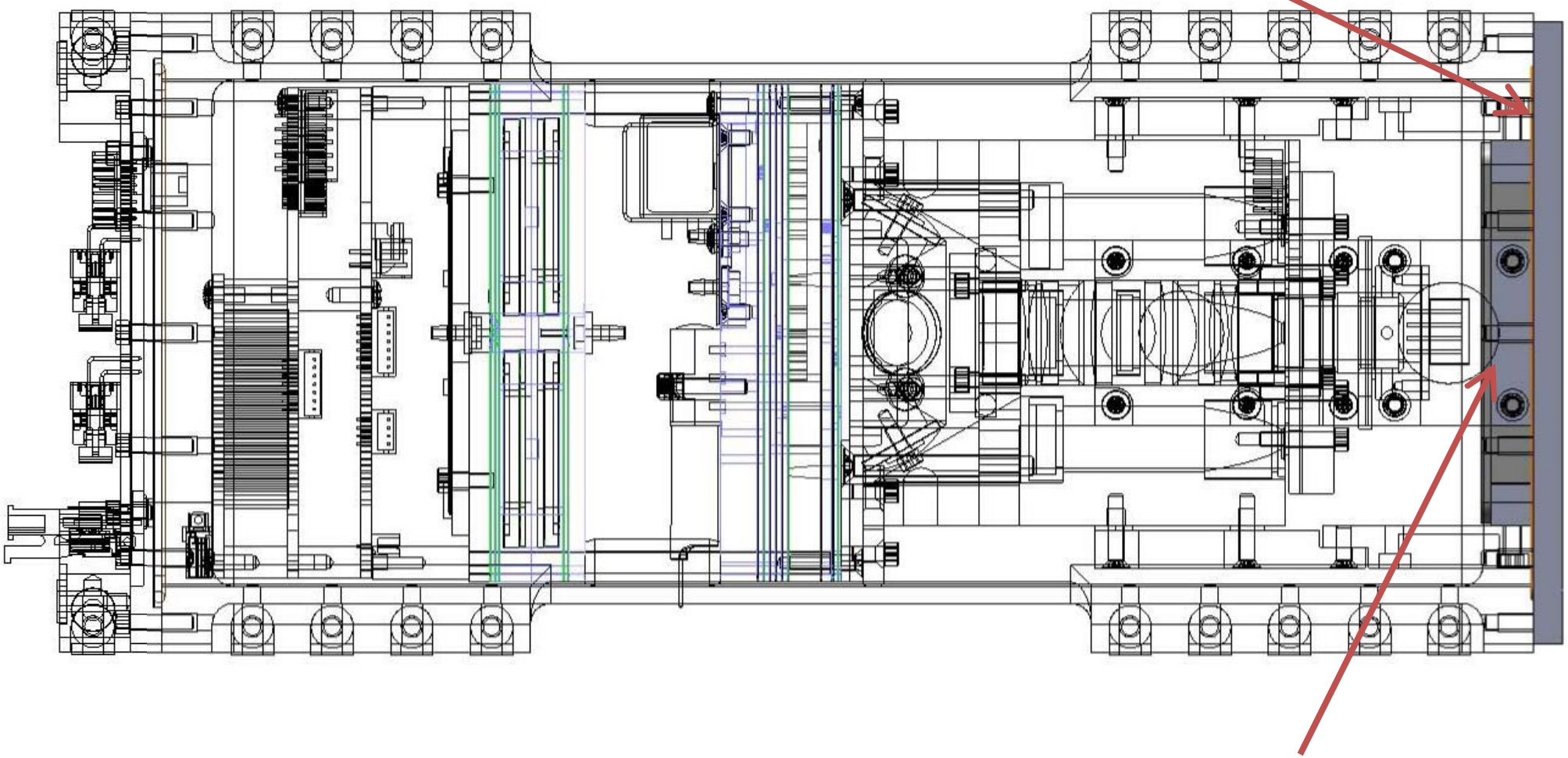


Fluidics Stack Pins

- Detail -

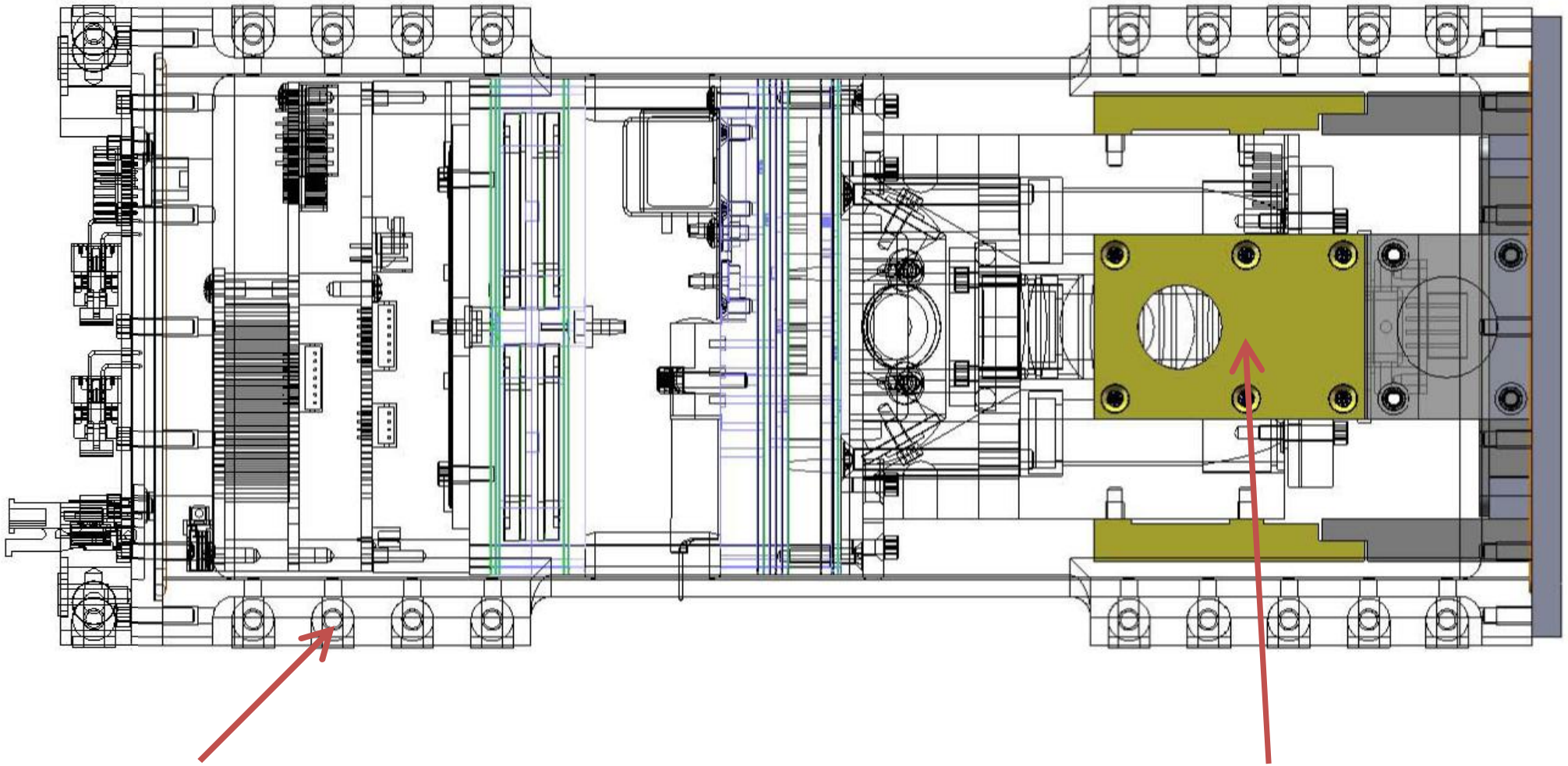


Back-Cover Assembly



Camera Mount

- Mated to Back Panel -

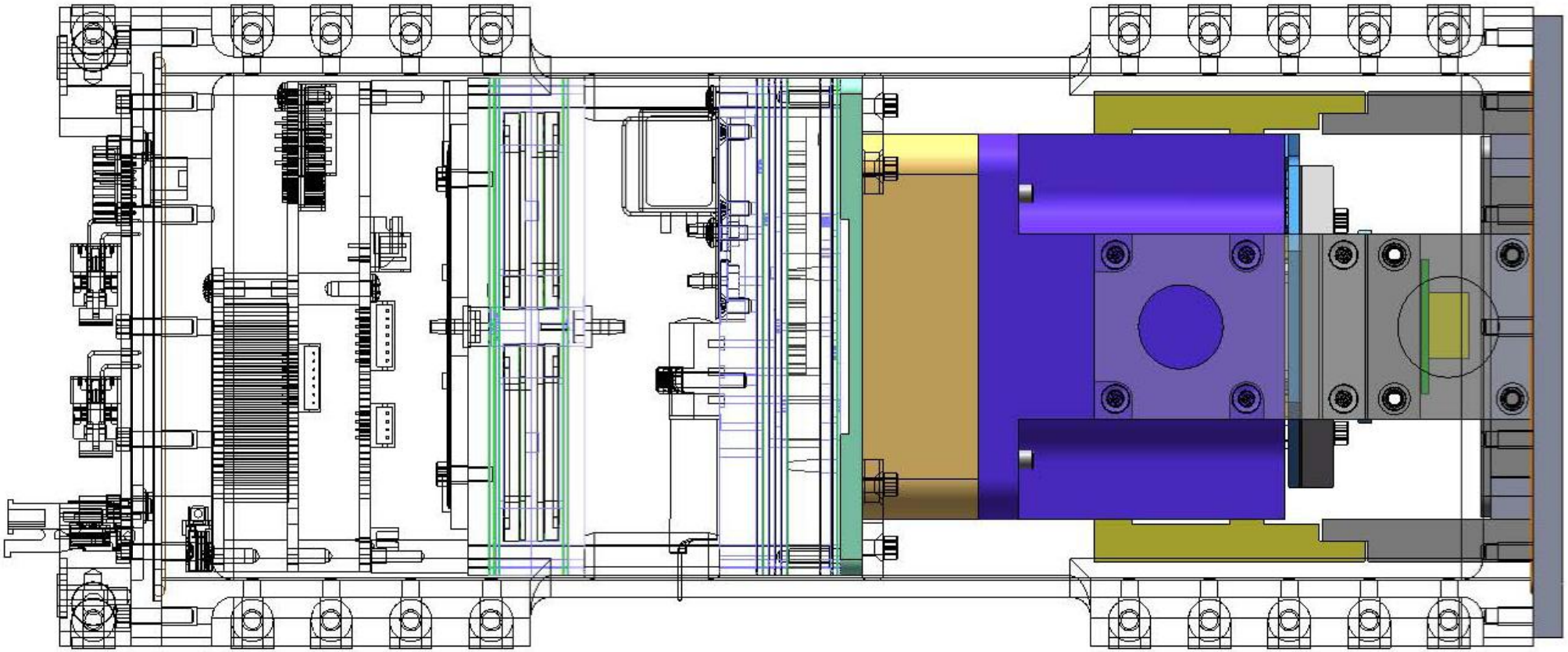


Ultem Washers

Ultem Mount

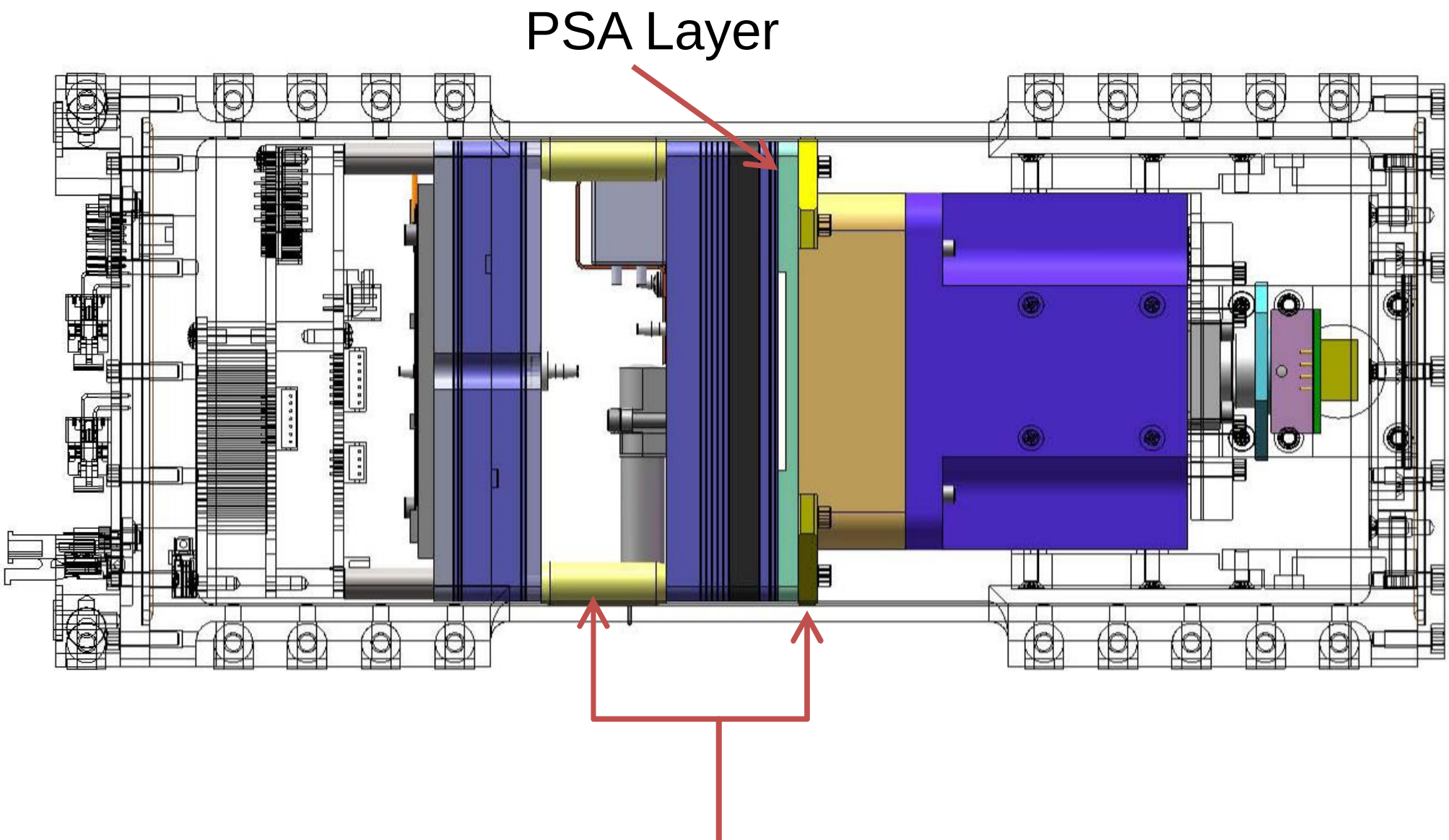
Camera Assembly

- Mated to Camera Mount -



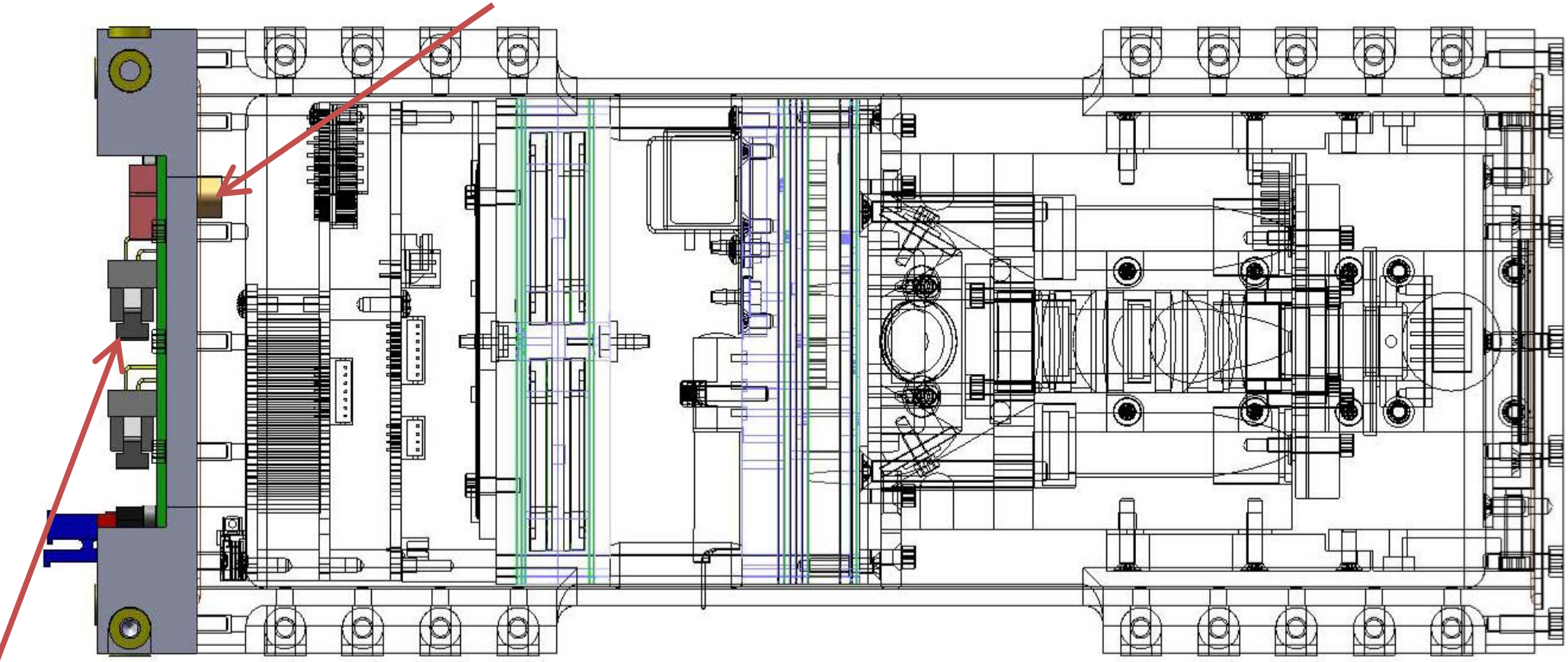
Fluidics Assembly

- Mated to Camera Assembly -



Front Panel Assembly

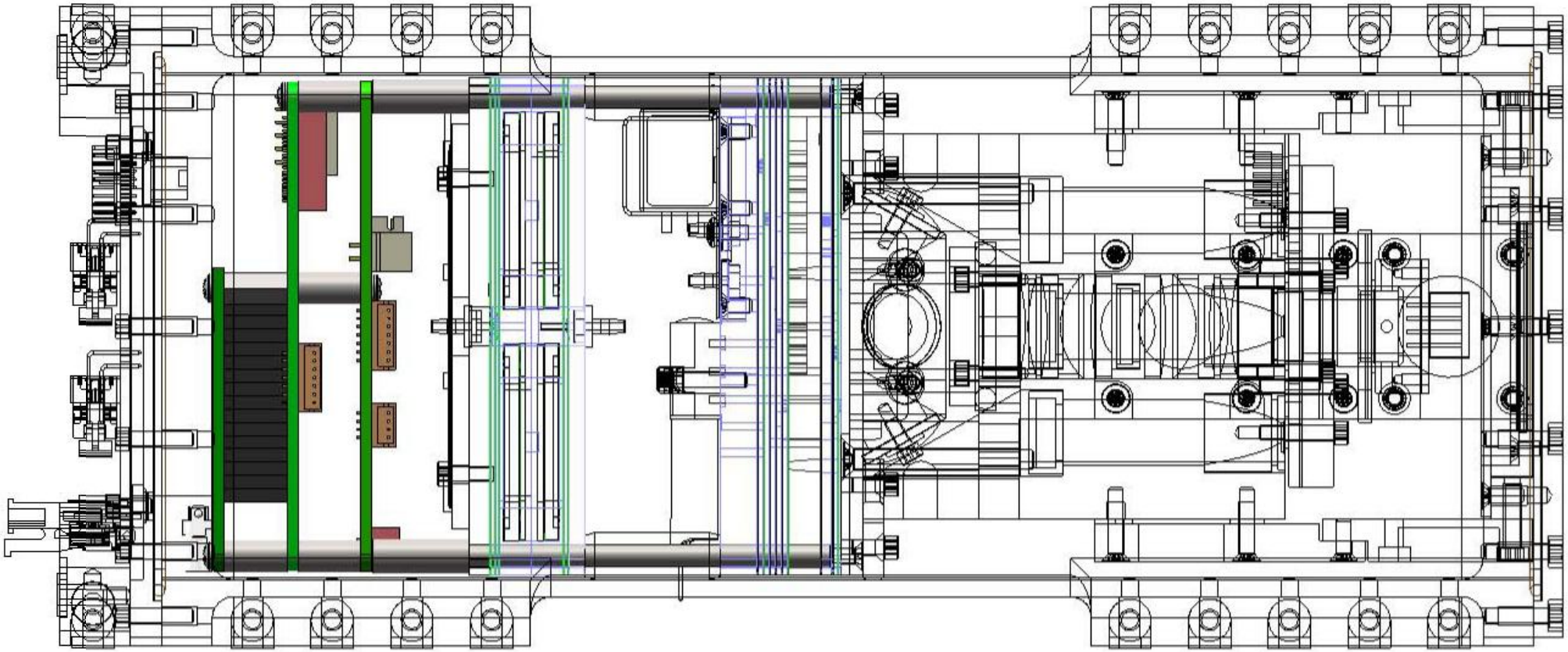
Hermetic Connector
•Laser Welded



er Interface Board

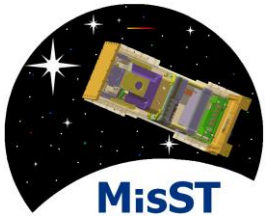
Electronics Assembly

- Mated to Fluidics Stack Pins -



Payload Electrical Overview

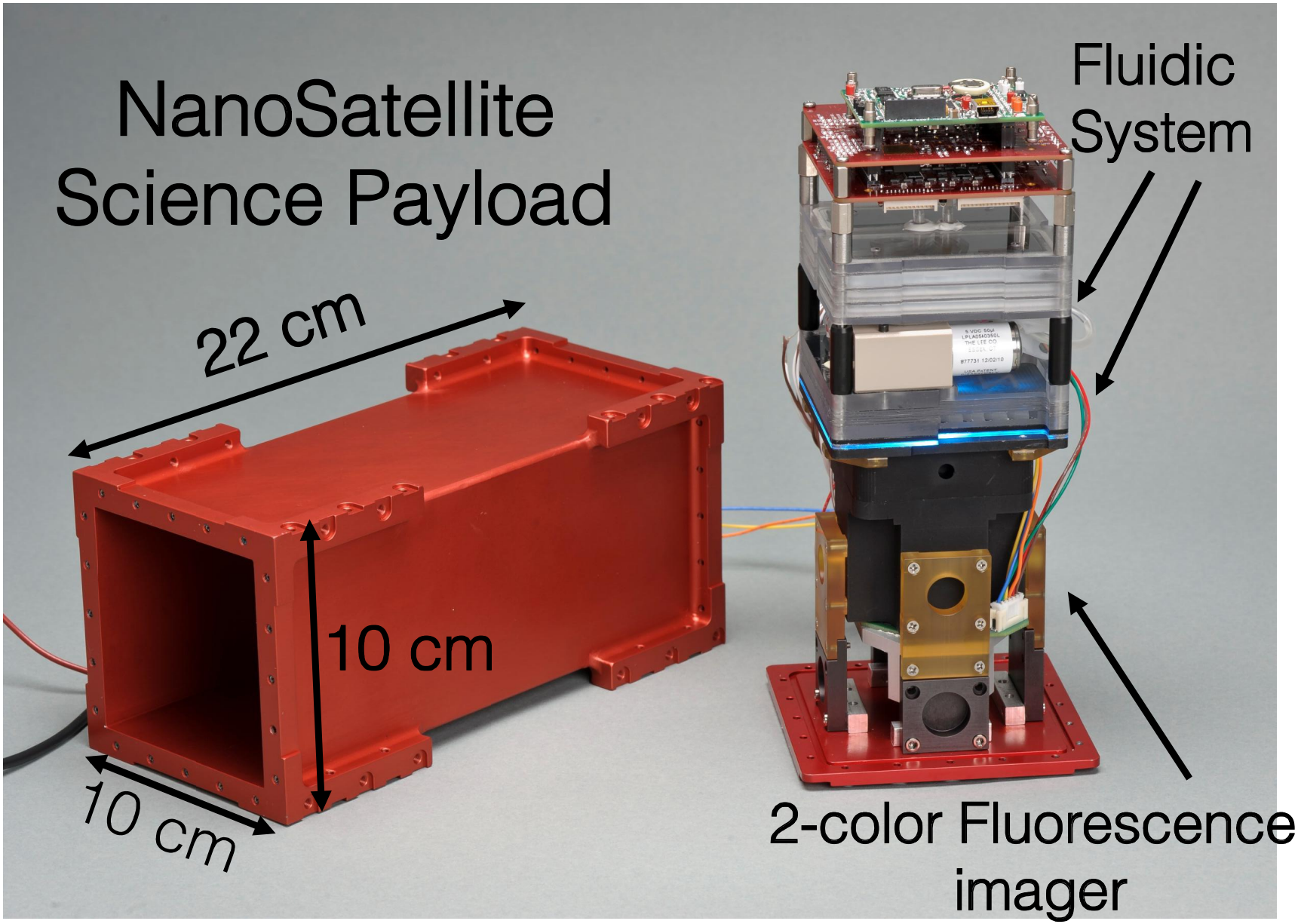
	Description
0 Imager Interface Board	**Translates the bus 50pin cable to the hermetic connect the payload can. Also has connection for the remove b flight "kill switch"**
0 Imager Payload Analog	**Contains all analog payload circuits. Temperature sen circuits, LED current drivers, RH sensor, pressure sen circuitry, Heater circuitry**
2 Imager Payload Digital	**Contains all digital and inductive load circuits. Valve bridges, motor switches, memory chips, camera interf**
0 Imager LED	**Contains the 4 high power imager illumination led's A to a heat sink and devices are properly thermally sunk for continuous use.**
0 Imager Processor	**Contains the payload microprocessor and associated uC LDO, RTC, FRAM, ADC reference**



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)



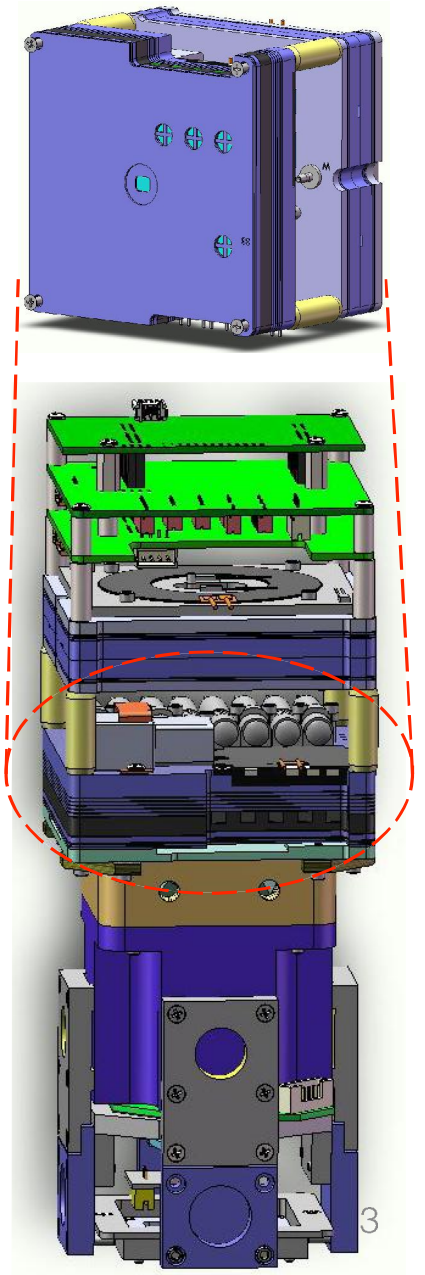
Payload Science & Technology Overview

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

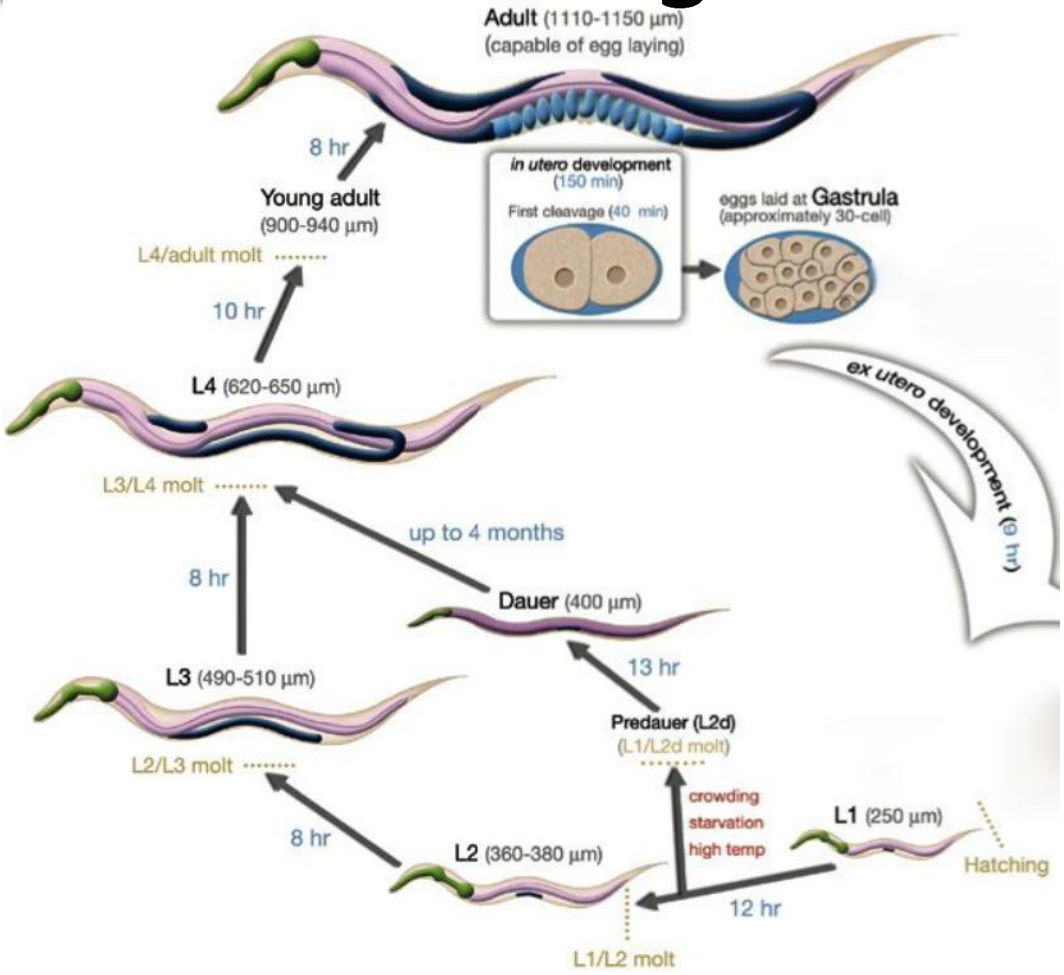
Target: model organisms, resolve major features/organs, gene of interest & control

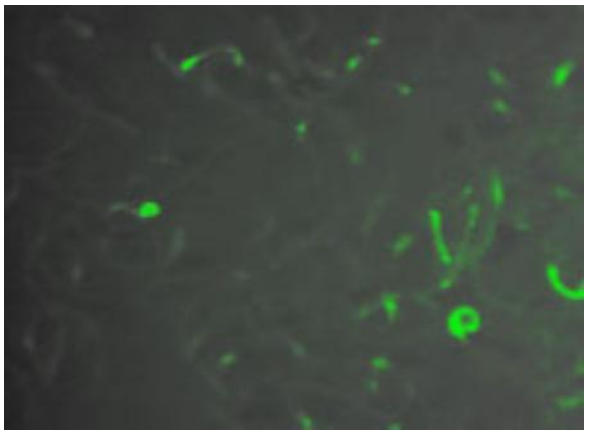
Implementation: 2-cube integrated culture/imaging instrument

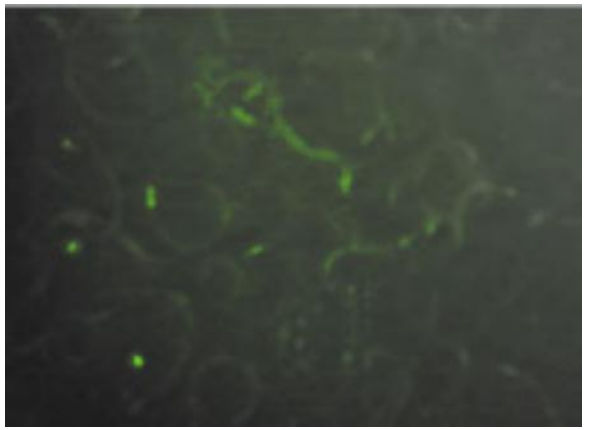
- C. elegans model organism
- Fluidics: culture wells, imaging zone, reagent storage
- Imager: fixed focus, 300 kpixel CMOS chip
- Image storage: 2 GB/6,000 images/8 bits per color (telemetry of 1 - 20 images)
- Hermetic containment vessel

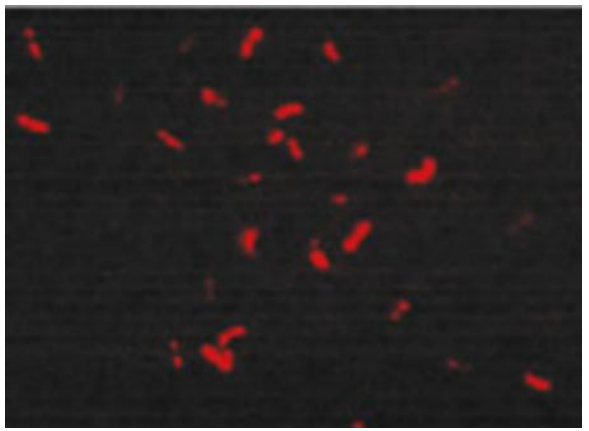


C. elegans Overview



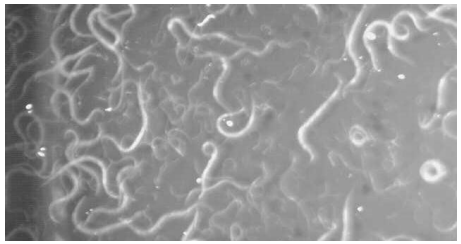
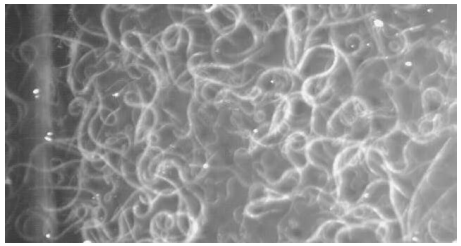
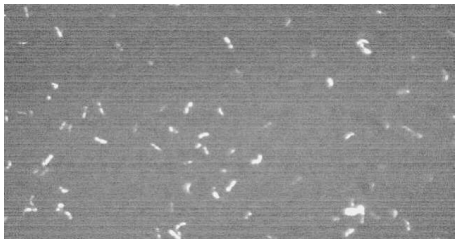
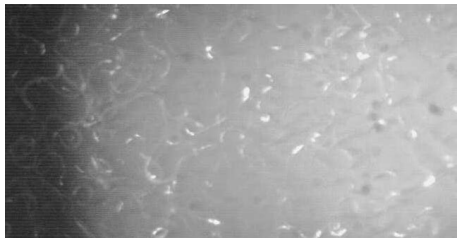
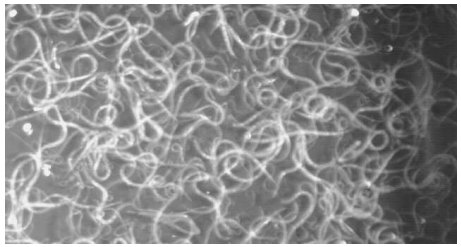
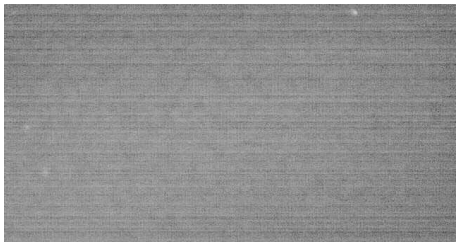
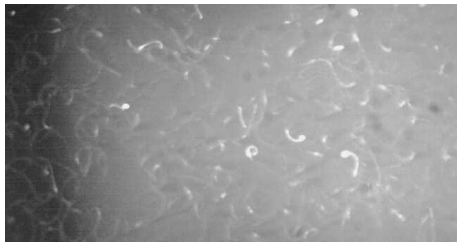
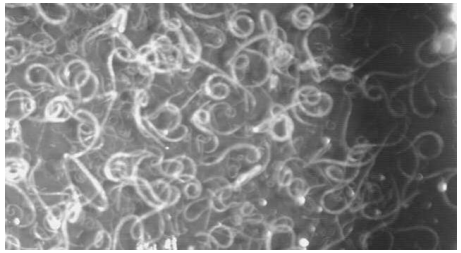
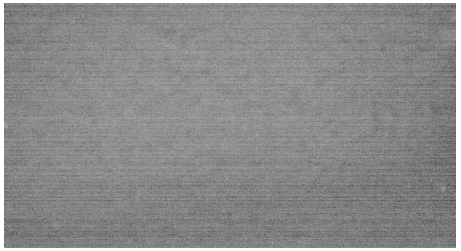
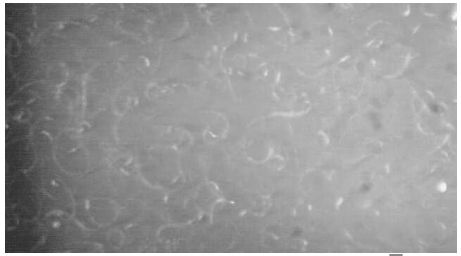






- Easy feeding and short life cycle make it inexpensive to maintain and ideal for research
- Used to study spaceflight effects and experiments using *C. elegans* have flown numerous times including on STS-107.

C. elegans Imaging

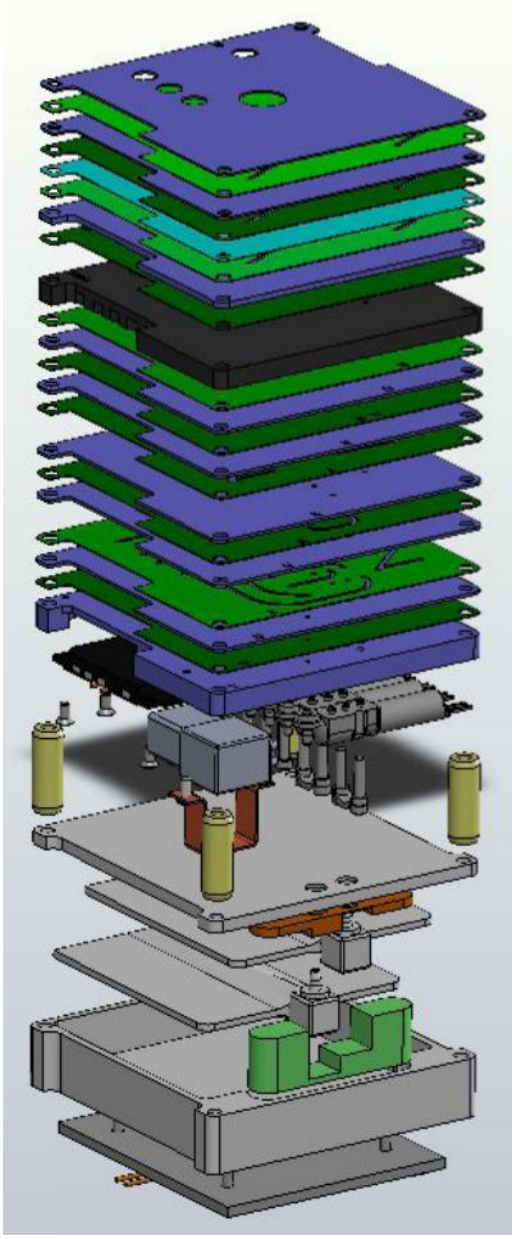
	White	Amber	Blue
N2 (wt) not fluorescent			
tdTomato red – muscle cells			
IM324 green – nerve cells			
MR142 green – intestinal cells			

Fluidics Overview

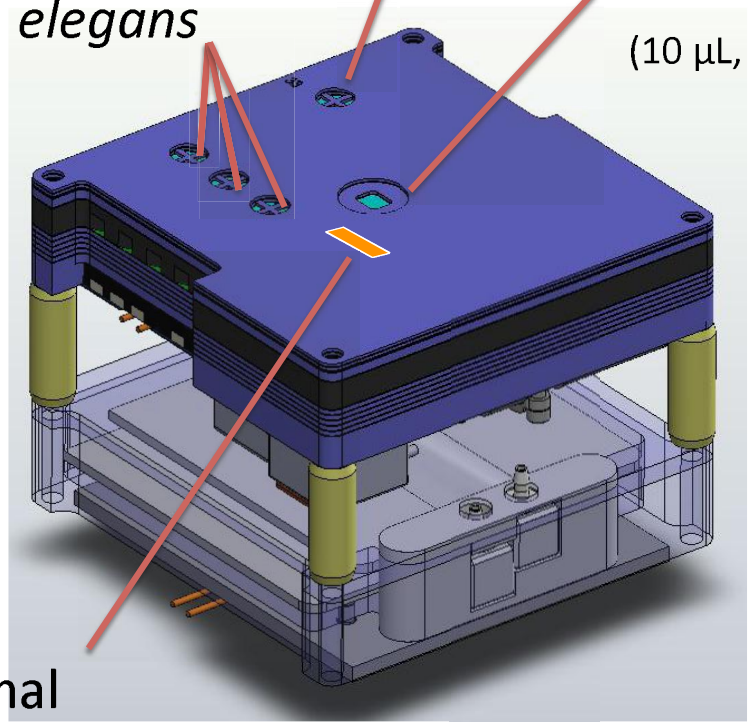
Approach: Multilayer polymer microfluidic card

- 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

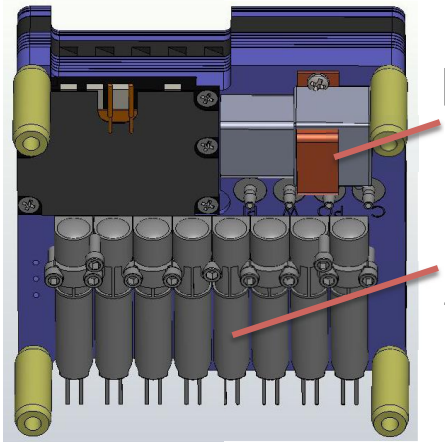
Polymers, adhesives, sterilization, biocompatibility, & fab. all have GeneSat, PharmaSat, O/OREOS-SESLO, EcAMSat heritage



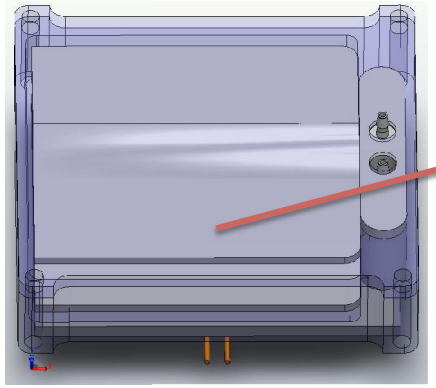
Fluidic Card and Reservoir



Fluidic Card

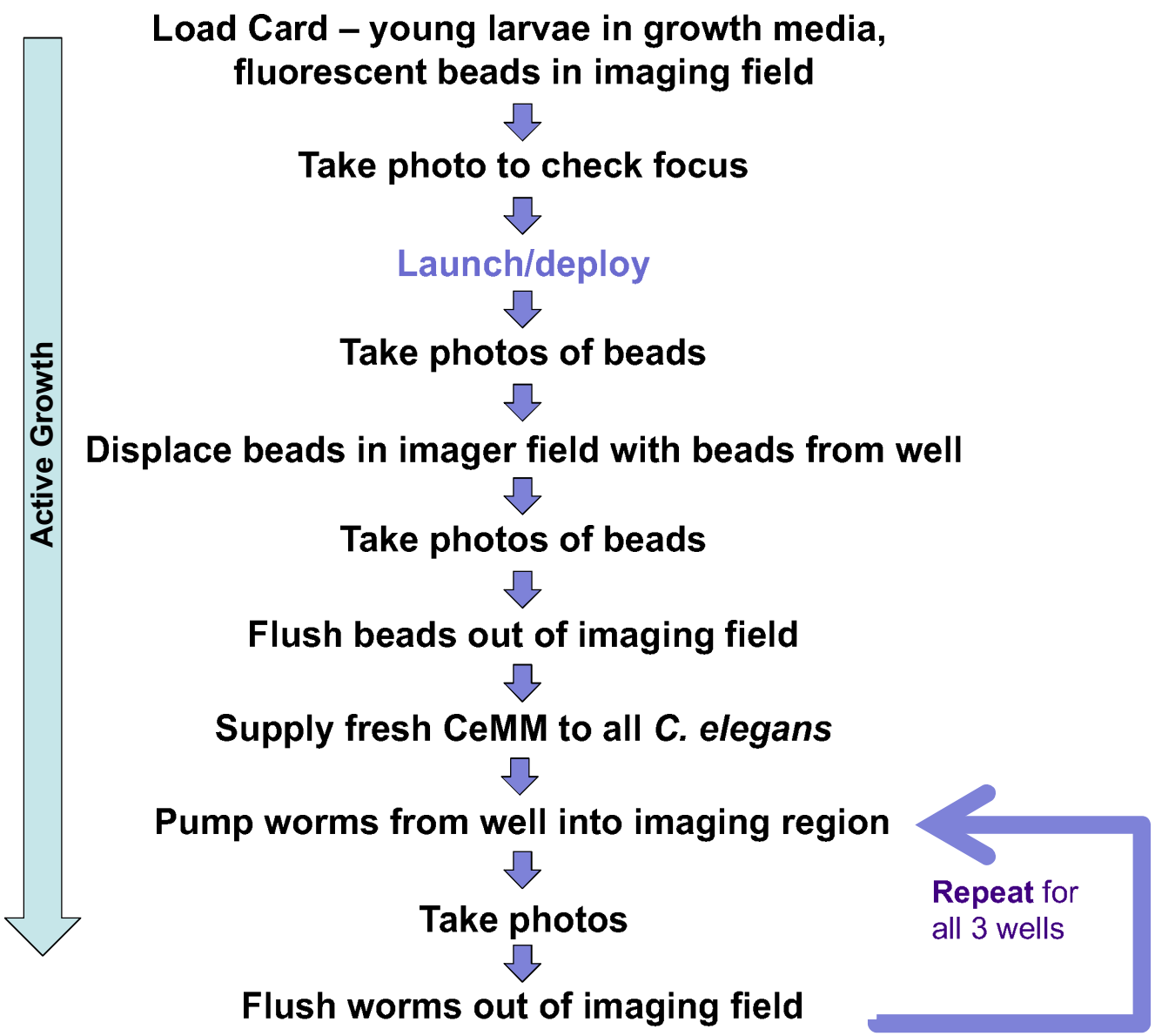


Reservoir

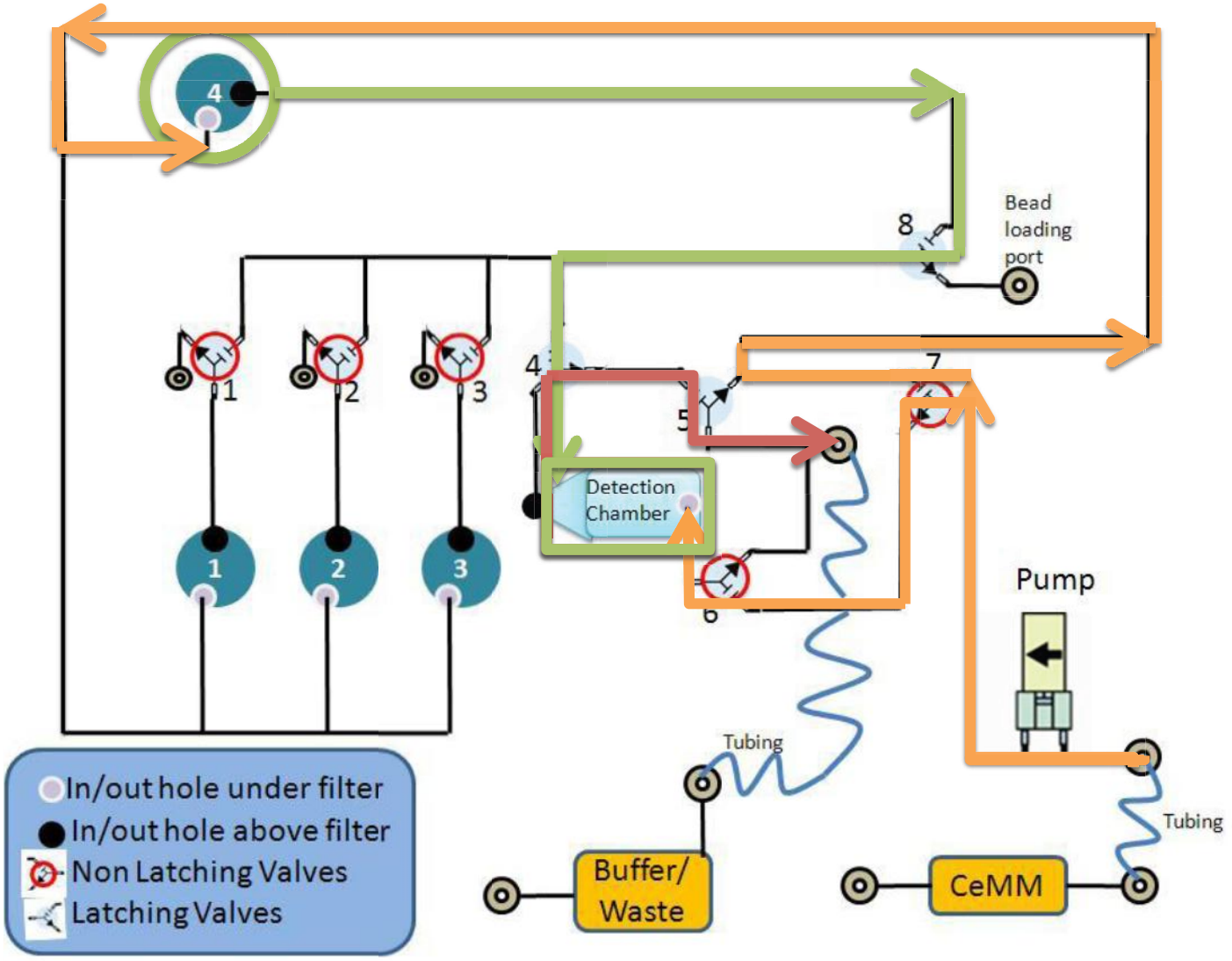


**C. elegans* maintenance medium

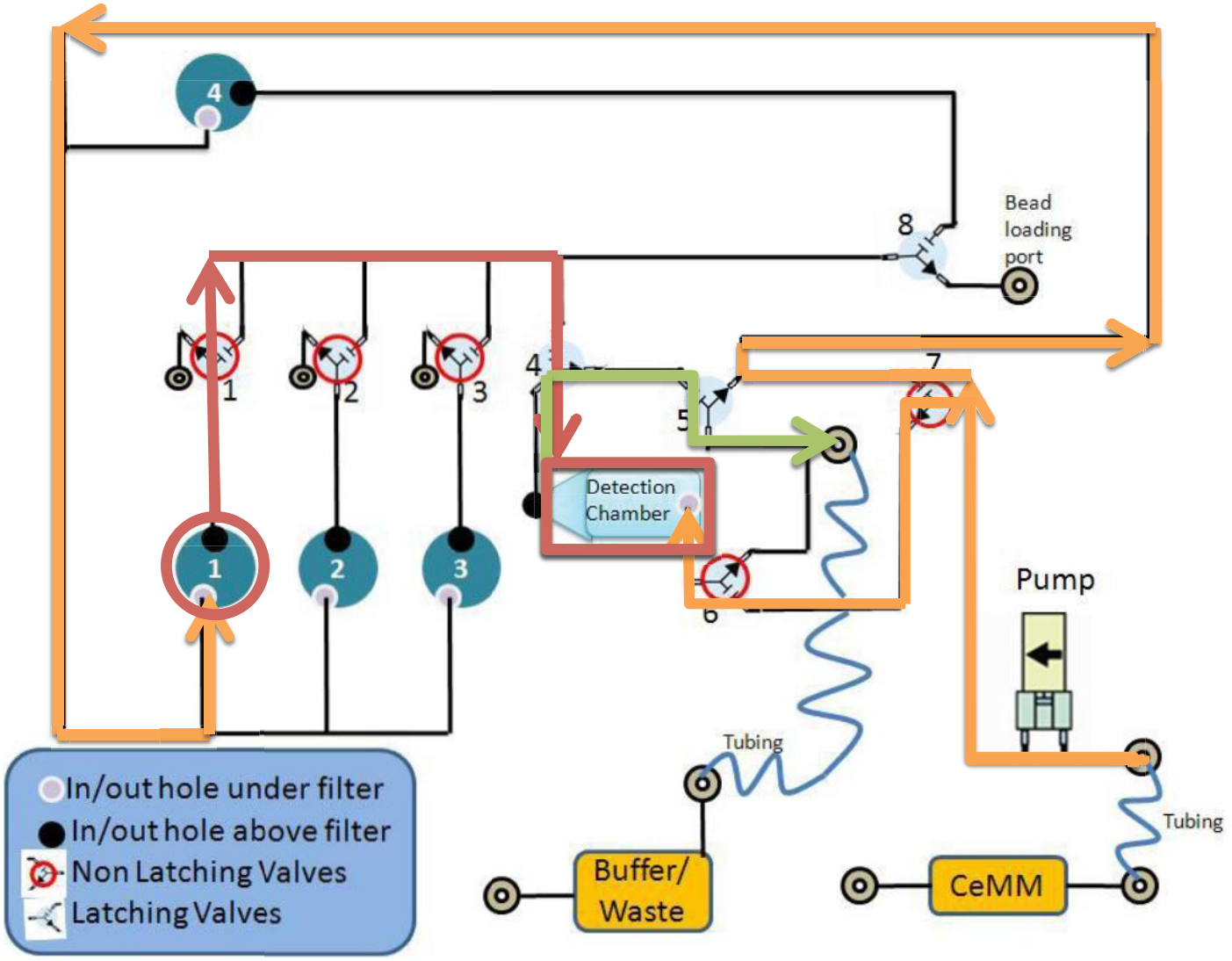
Experimental Timeline



Fluidic Sequence - Beads



Fluidic Sequence- Worms



Fluorescence Imager

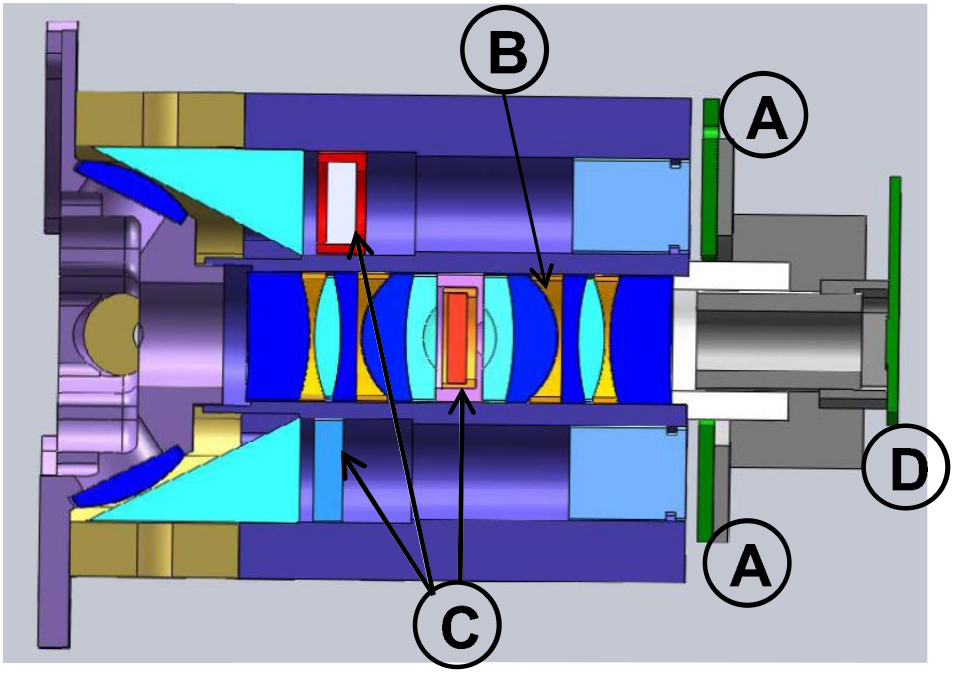
Imager Components:

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

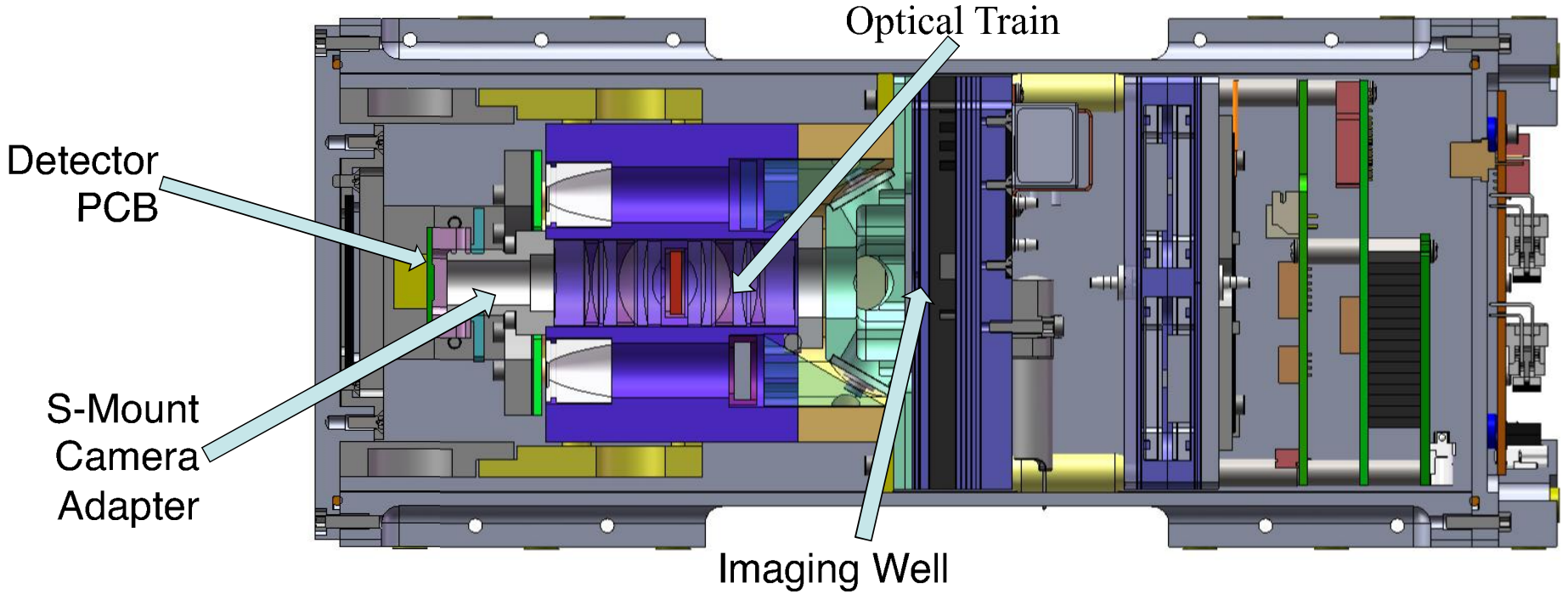
D Camera:
COMedia C328-7221

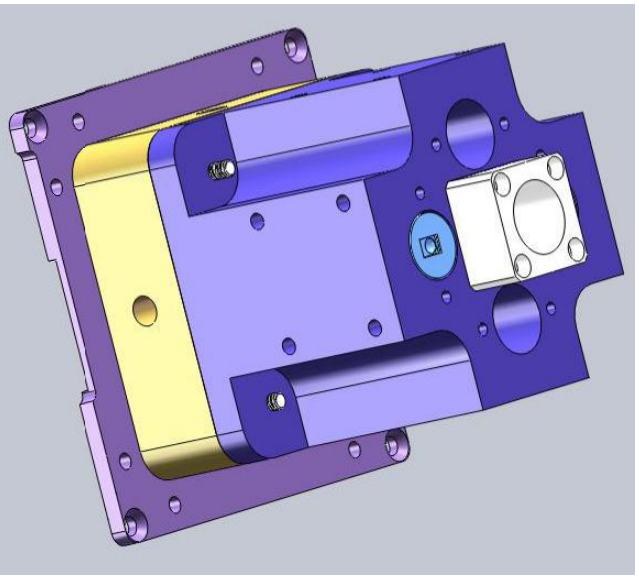


Imager Specifications:

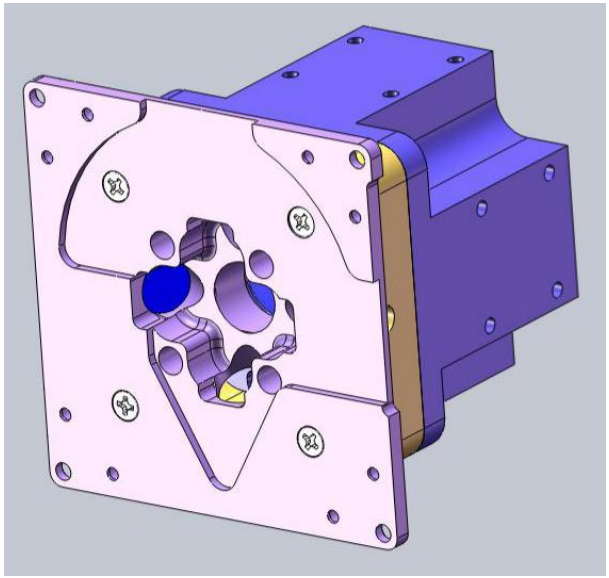
- 2-color fluorescence:
  Green excite/emit peaks at: 487/509nm
  Red excite/emit peaks at: 587/610nm
- Cool white for standard imaging
- Lateral resolution: ~ 8 µm
- Magnification: 1:1
- Field of View: ~3 x 4 mm
- Depth of Focus: ~150 µm

Fluorescence Imager





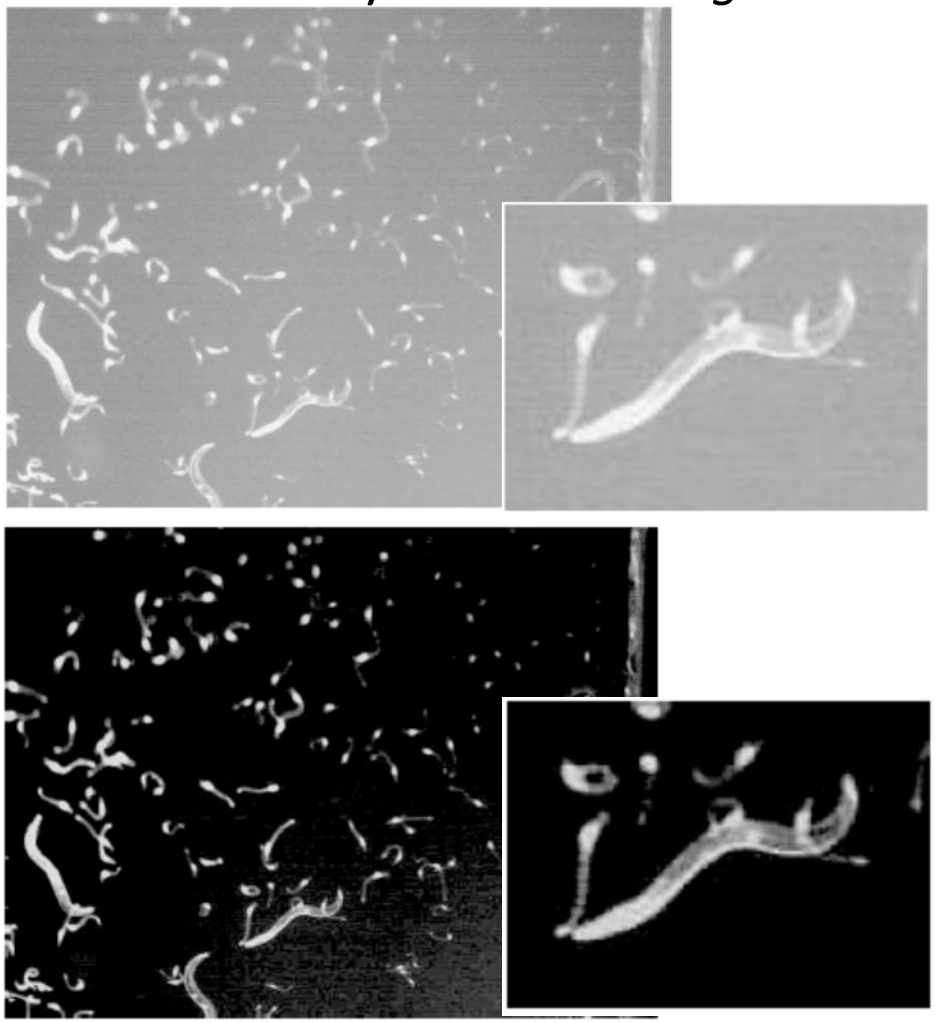
Imager Assy.



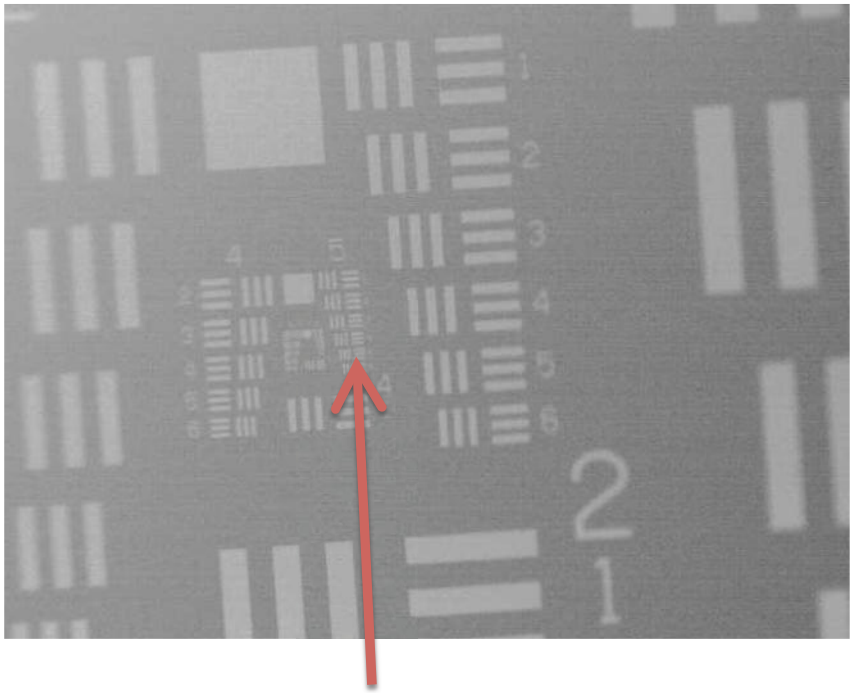
- Fluidic well depth: **0.17 mm**
- Fine pitch thread on S-mount (**0.5mm/thread**)
- Camera adjustment: **±5 mm**
- Locknut/set screws hold detector in place

COMedia C328-7221 Camera chip

green fluorescently labeled *C. elegans*

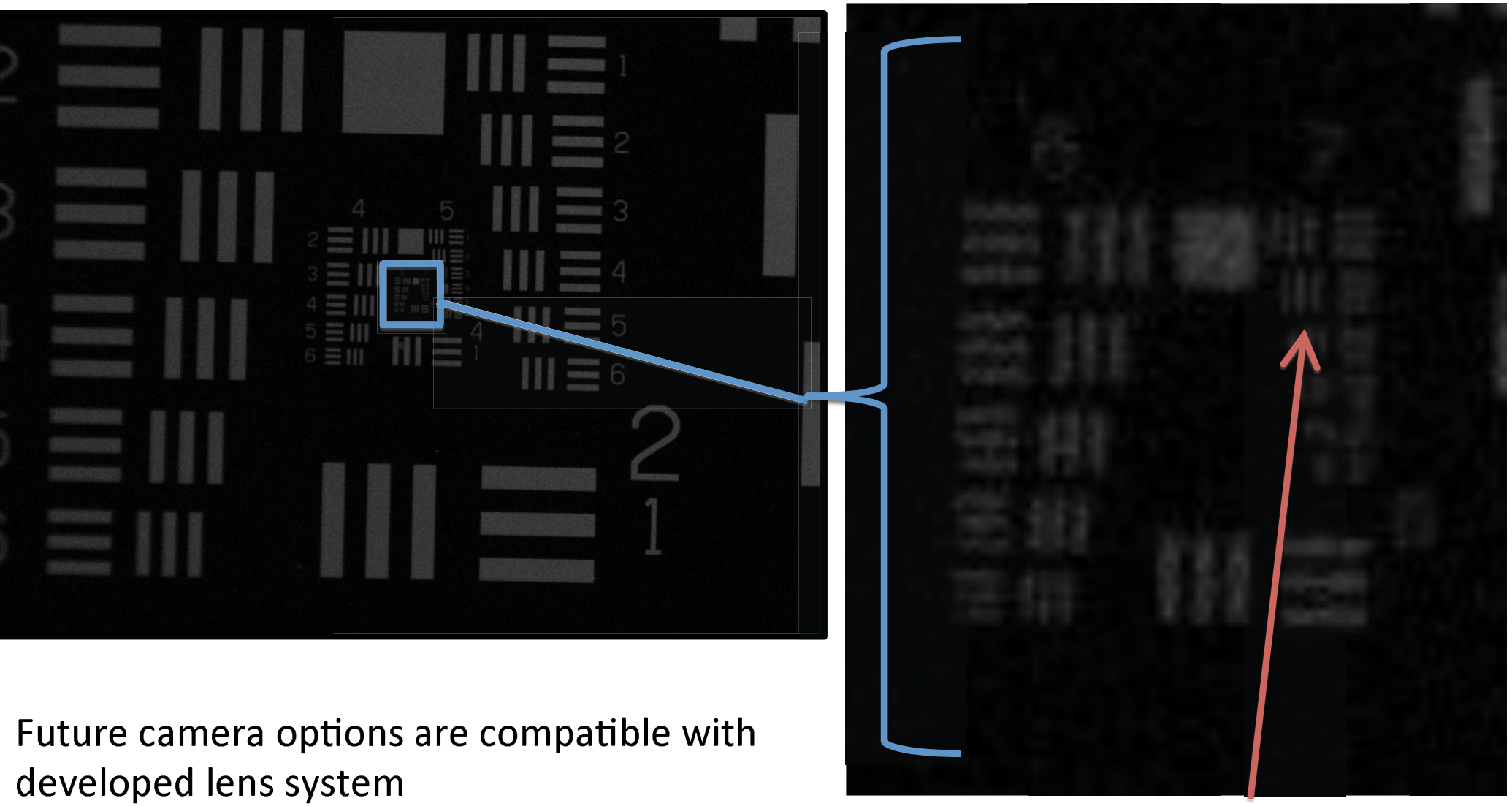


Post Processing [Contrast]



Resolves ~8 µm feature size

CCD imaging chip

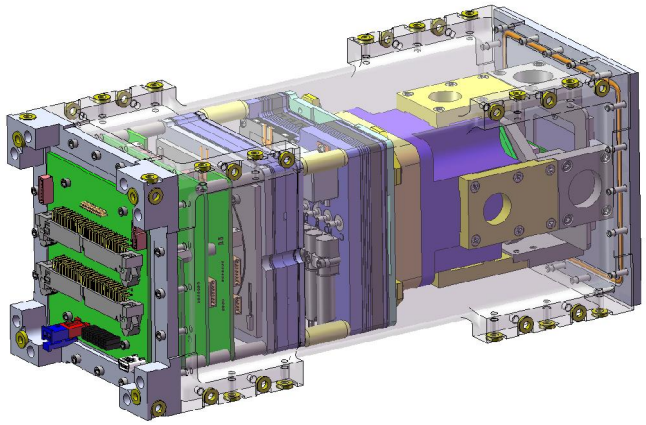


Future camera options are compatible with developed lens system
(e.g., Omnivision 7141 CMOS)

Resolves ~ 4 µm feature size

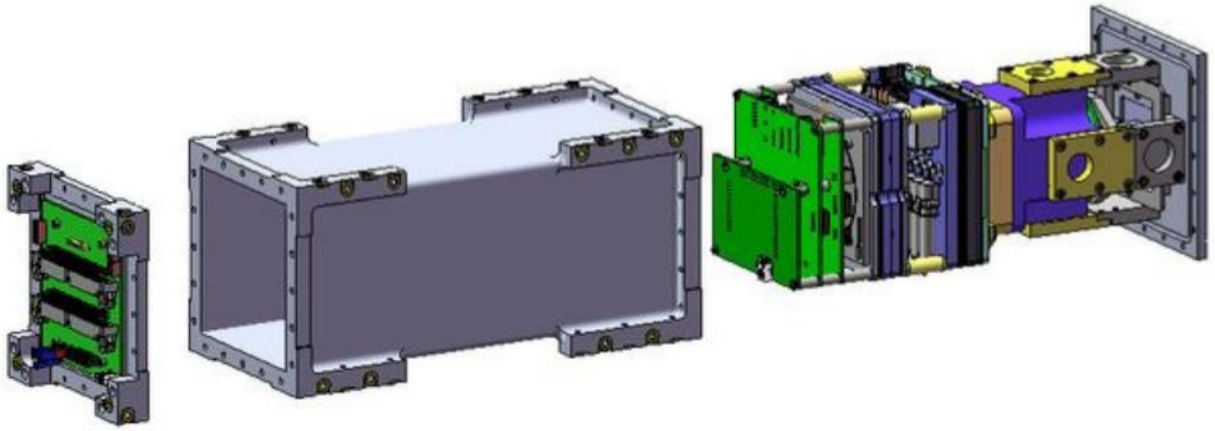
Payload Functional Overview

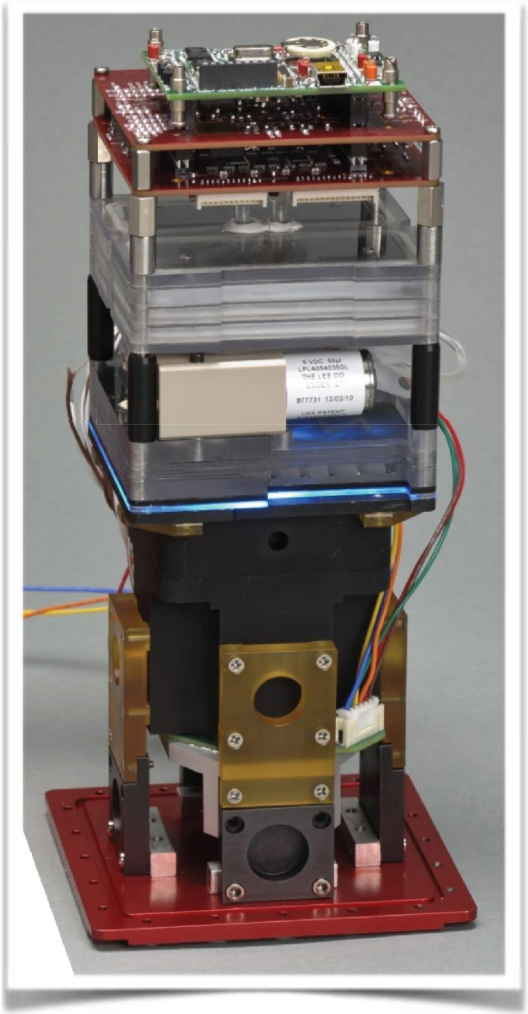
Mass	~1.5 – 2kg
Sensors	Pressure : accurate to within 2% of 1 atm. Humidity: accurate to within 4% from true value after temp. correction Temperature accurate to within +/- 0.5ºC.
Processor	PIC 32
Temperature Control	Kept at 4°C to 30°C during the experiment phase.
Environmental Chamber	Maintains an 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

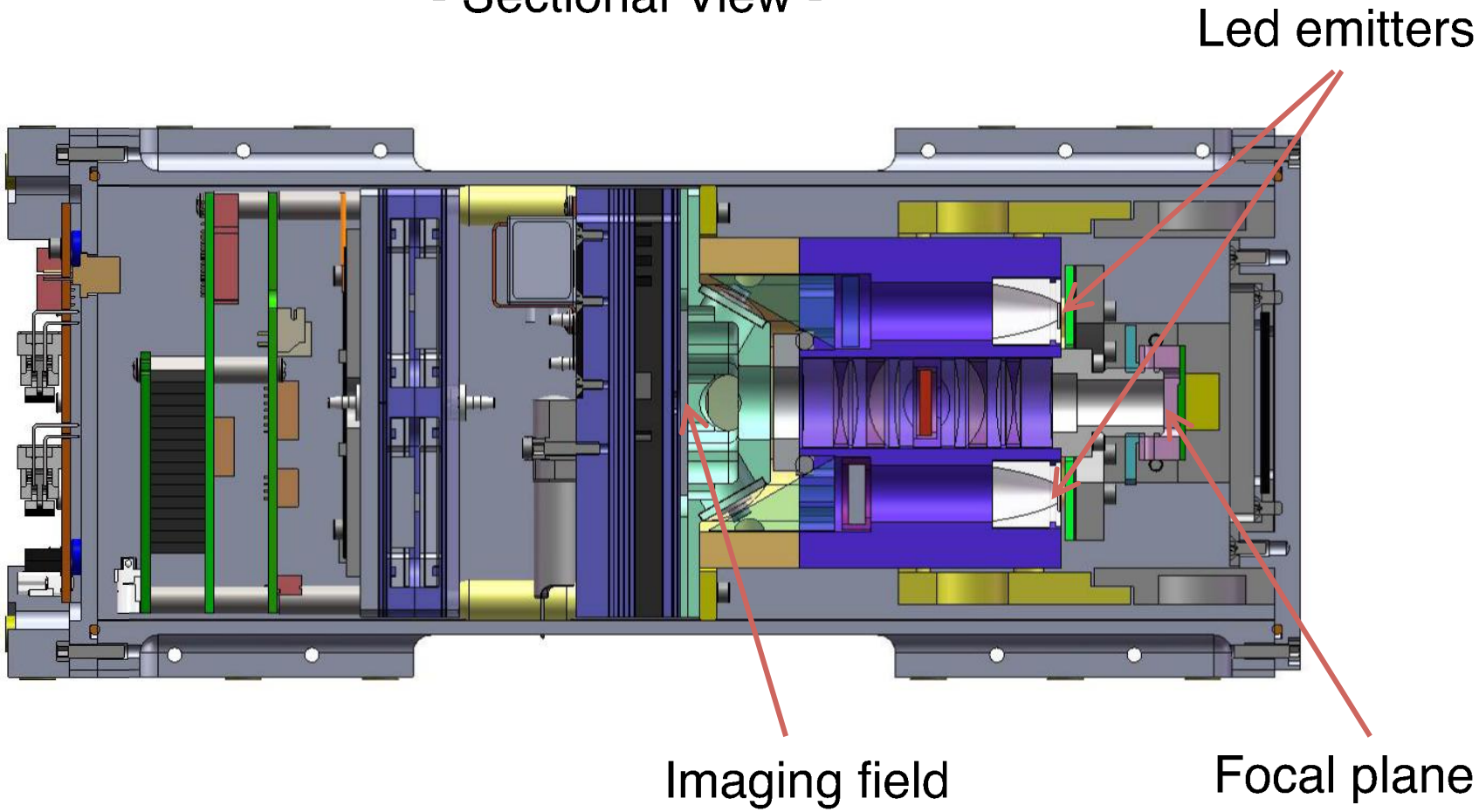
Integrated Payload
- Exploded View -



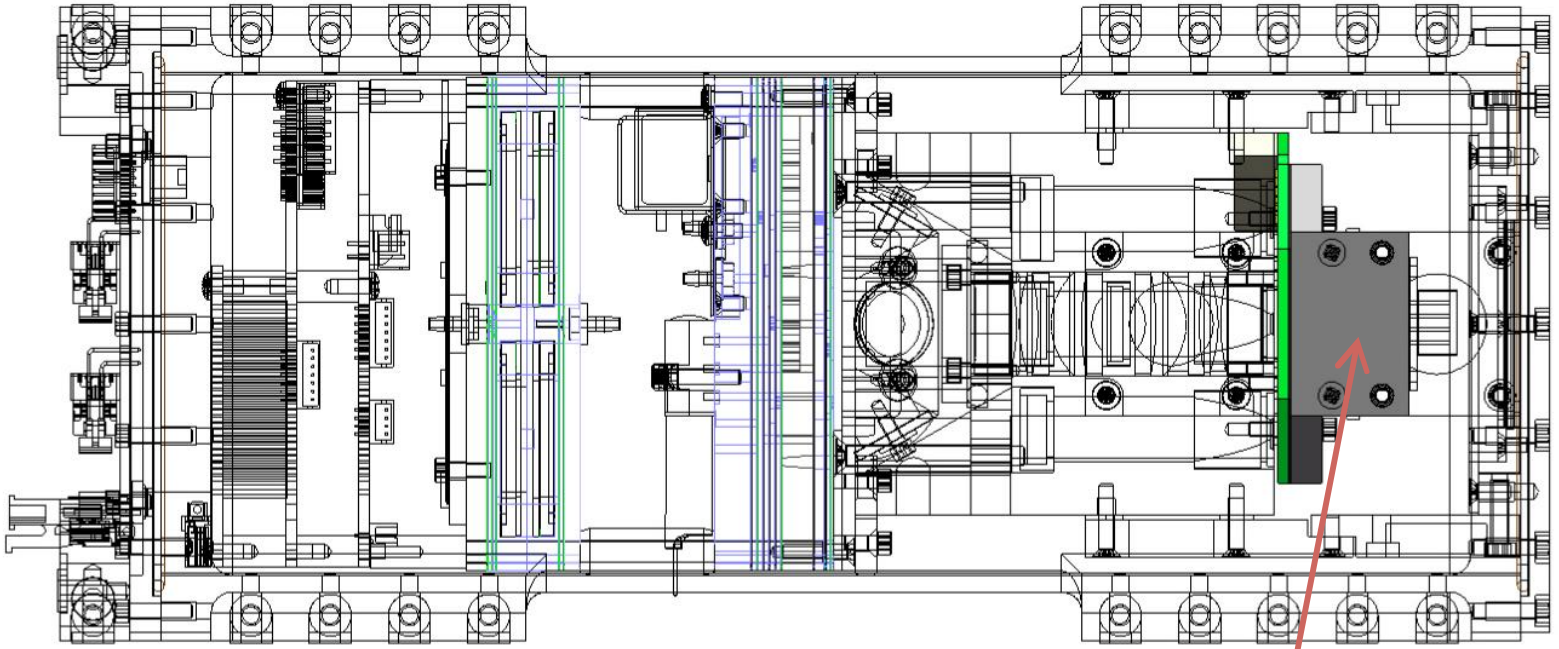


Integrated Payload

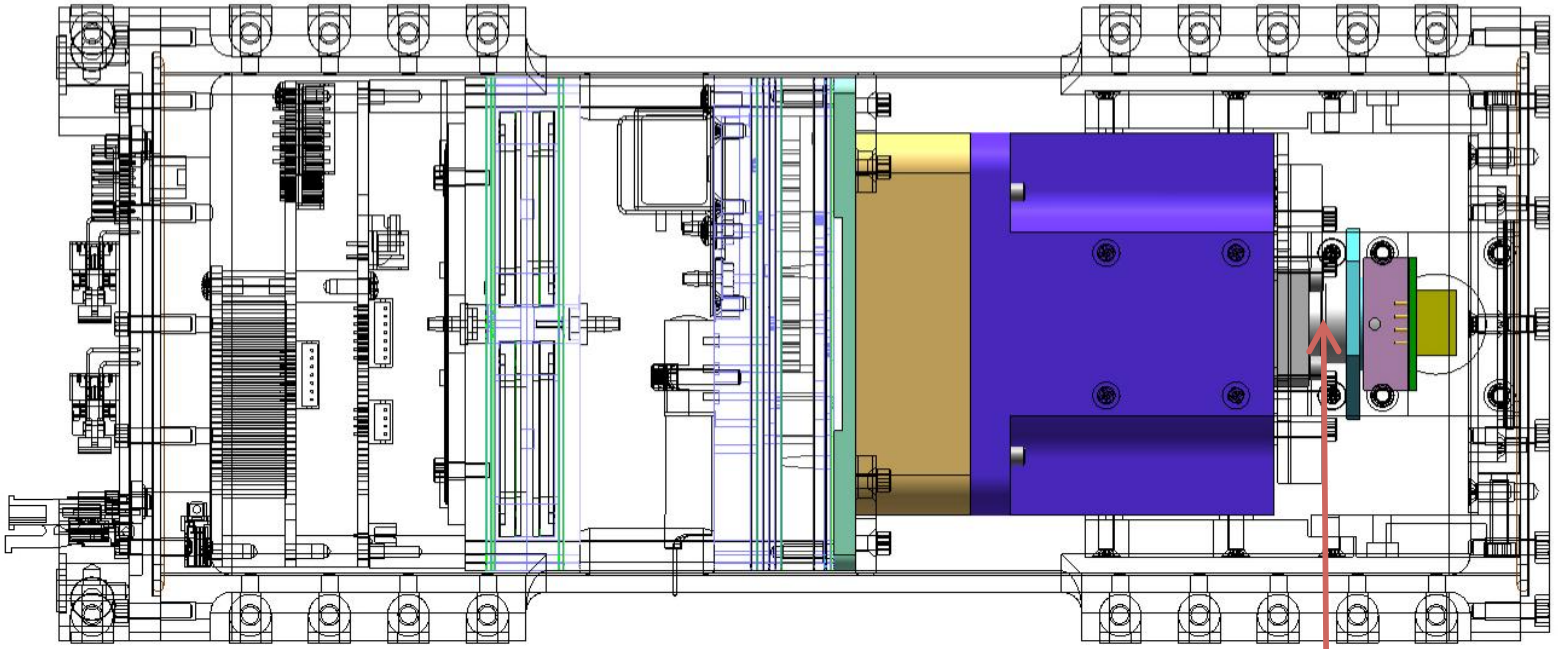
- Sectional View -



LED Assembly

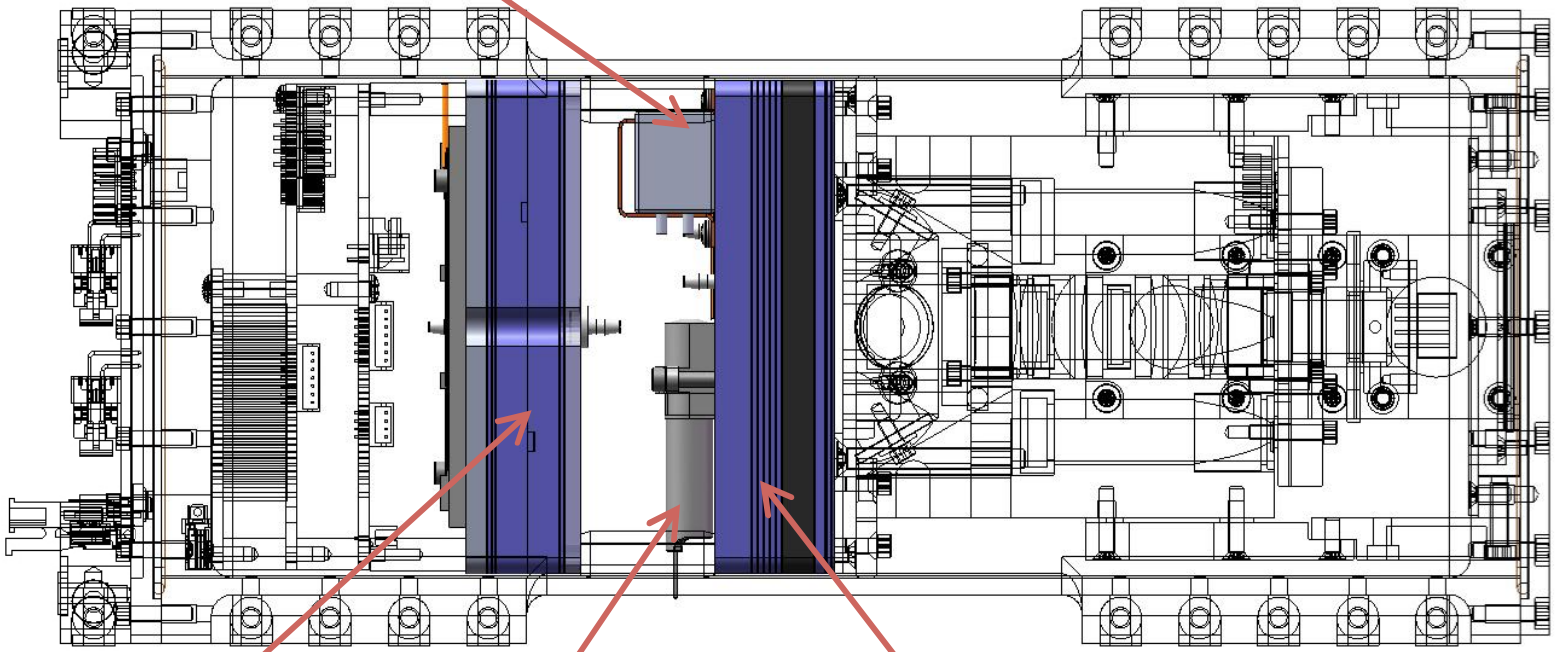


Camera Assembly

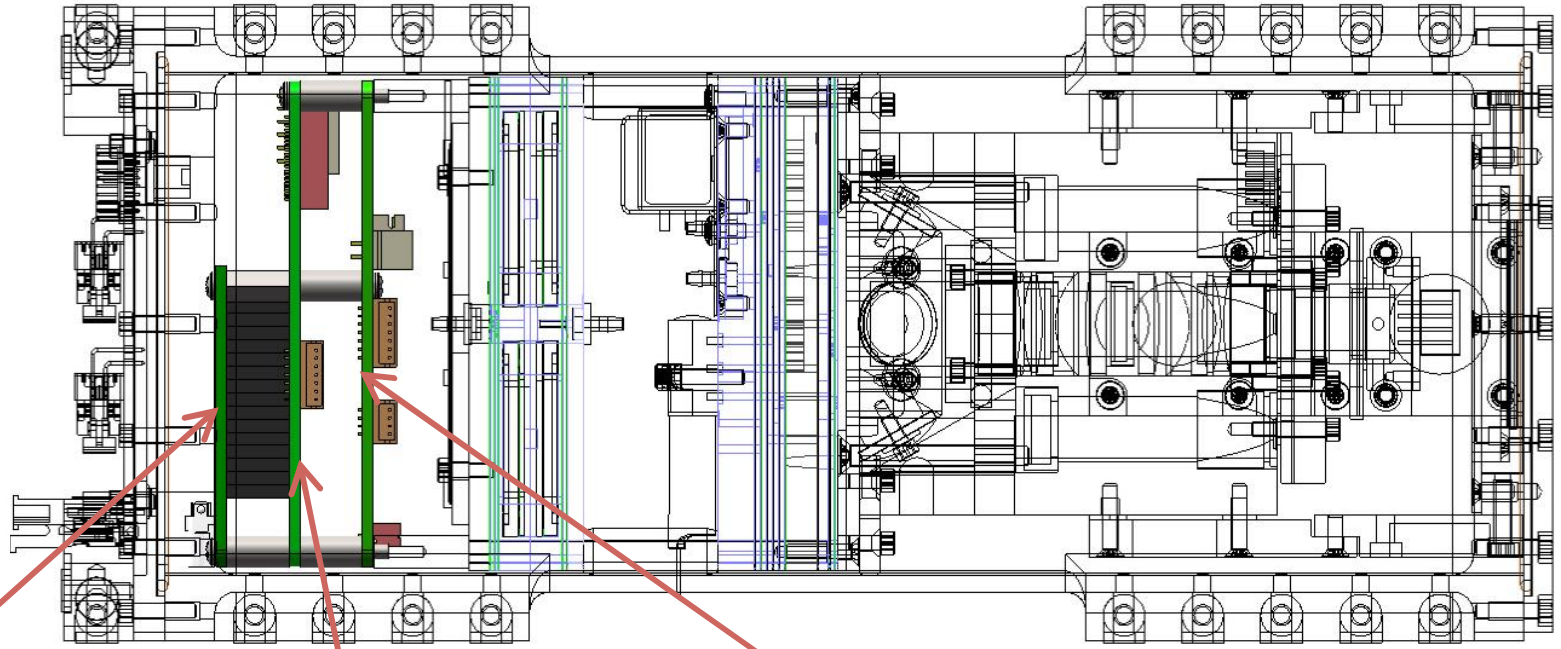


Camera Adapter/Focus Adjustment
With Locknut

Fluidics Assembly



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

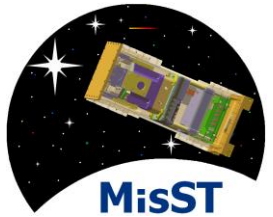
Mechanical: Abraham Rademacher, Giovanni Minelli, Chris Beasley

Electrical: Aaron Schooley

Management: Andres Martinez

Technology: Antonio Ricco

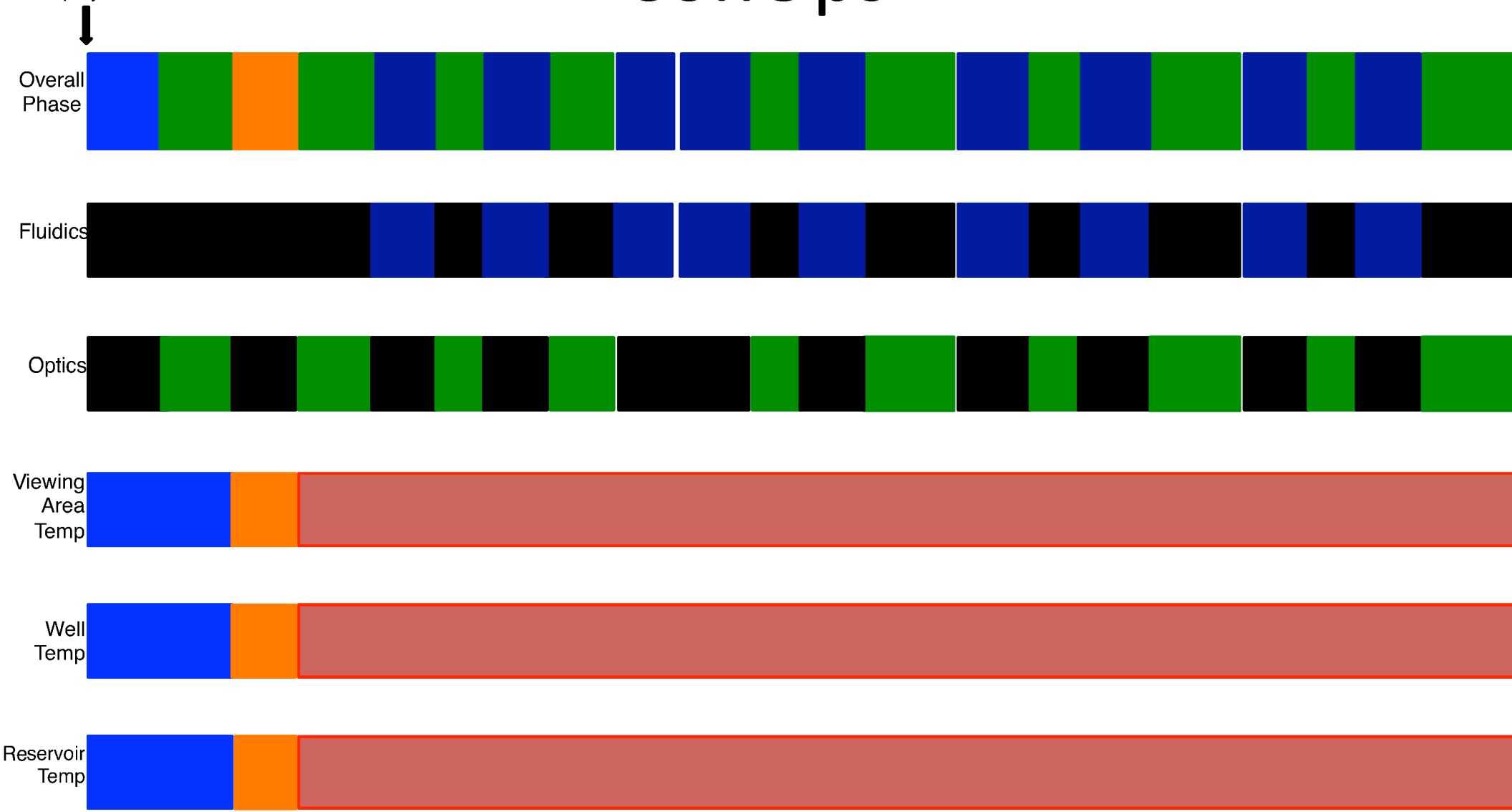
Funding: NASA/Exploration Systems Mission Directorate



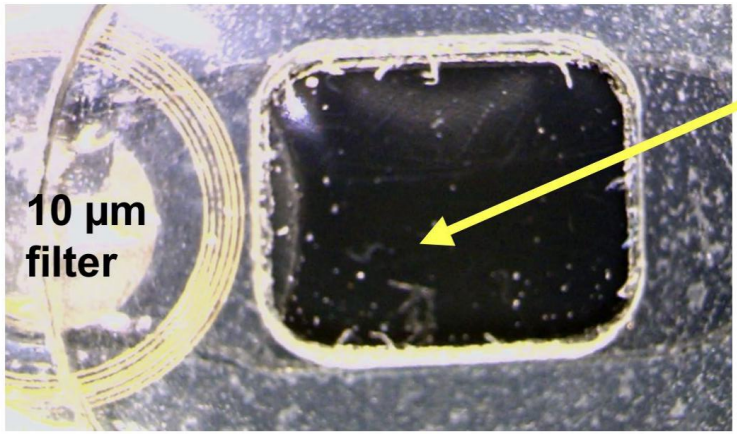
Thank You

Questions?

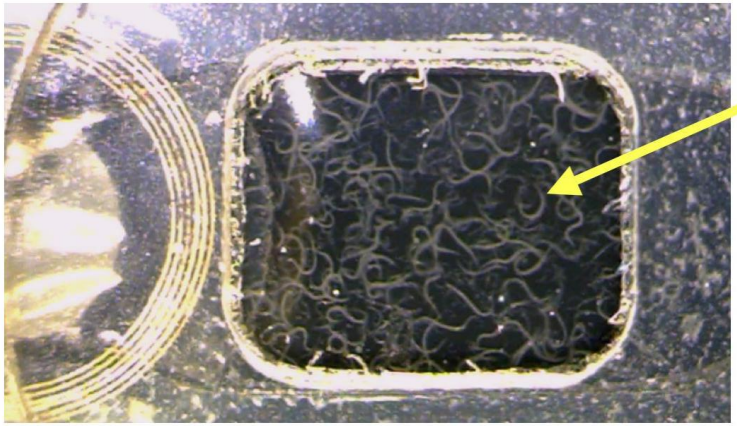
MisST Free-Flyer Payload ConOps



Note: A = amber, B = blue, W = white

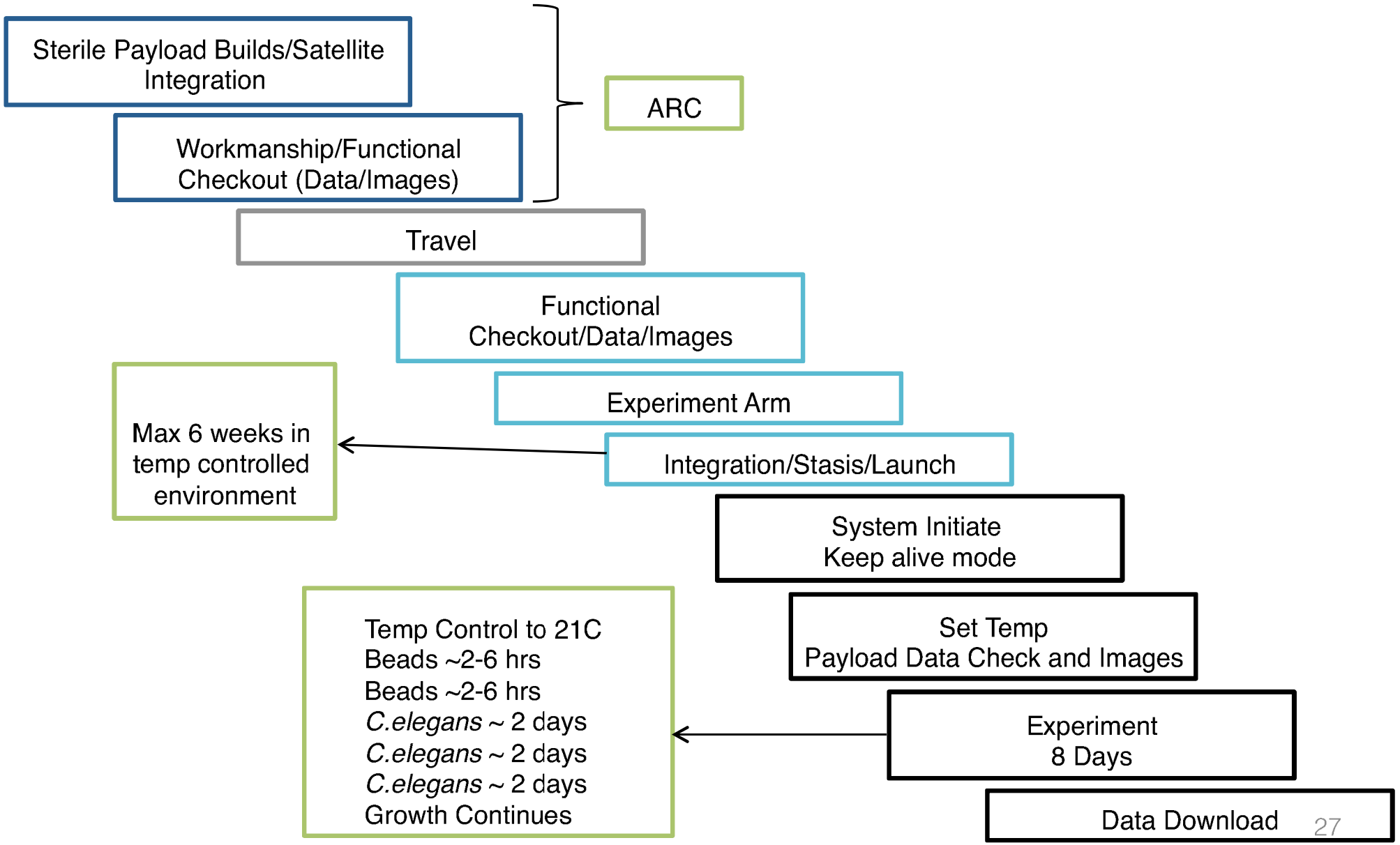


After pumping worms into imaging chamber



C. elegans growth after 1 week

Payload ConOps



Payload 1 (Imager): *Fluidics materials*

Direct contact *with C. elegans*

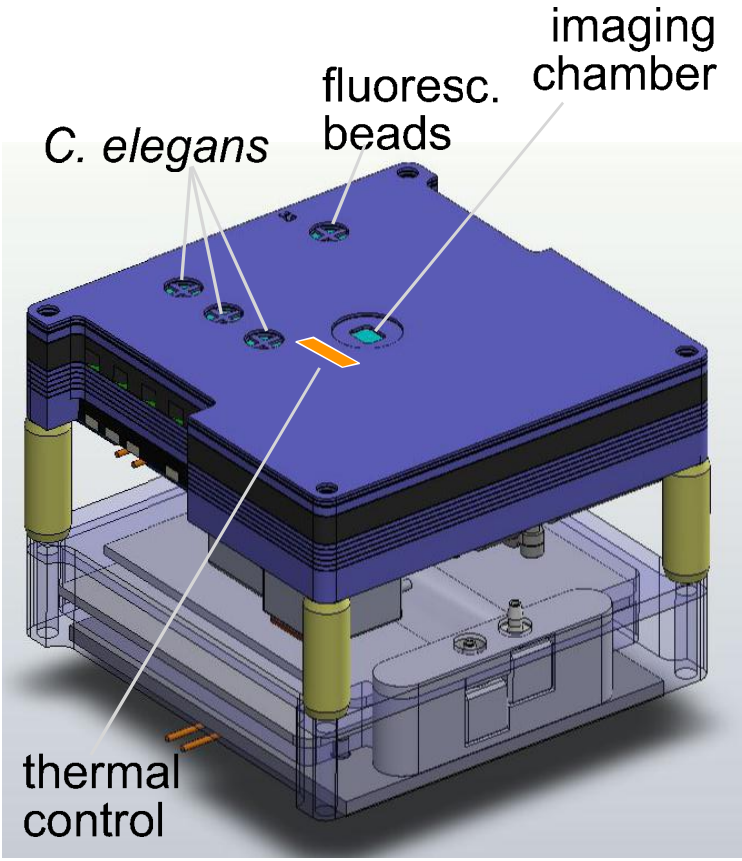
1. Polycarb. (machined)
2. Polycarb. porous membrane
3. Pressure-sensitive adhesive (PSA) (cut edges only)
    - acrylic adhesive, polyester carrier
    - laser-cut edges
4. Polystyrene gas-permeable cover film

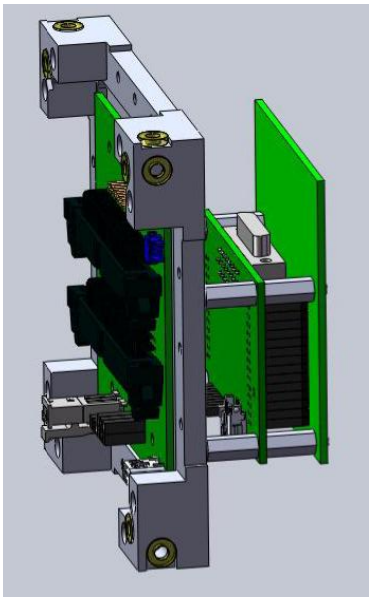
Contact with growth medium only (reservoir/tubing)

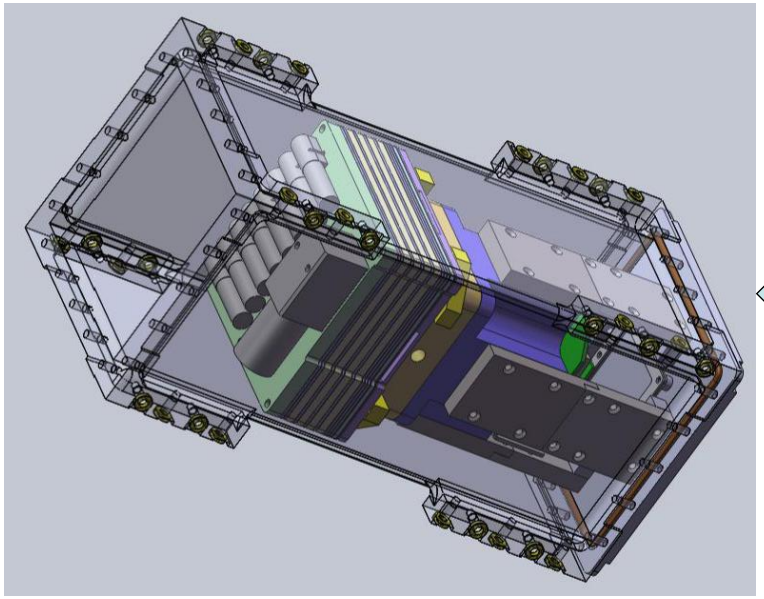
1. Poly(ethylene vinylacetate) [EVA] bag
2. Polysulfone barbed ports
3. Polyurethane tubing

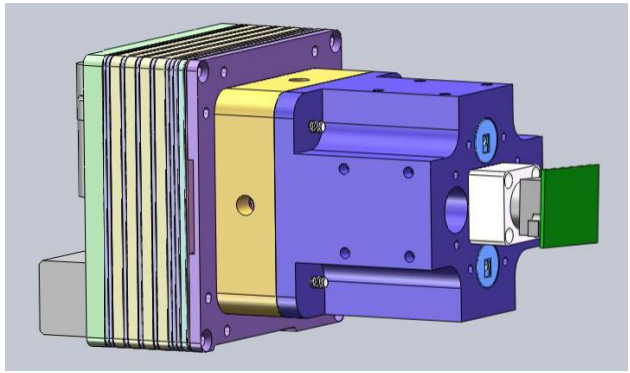
Direct contact with C. elegans and growth medium

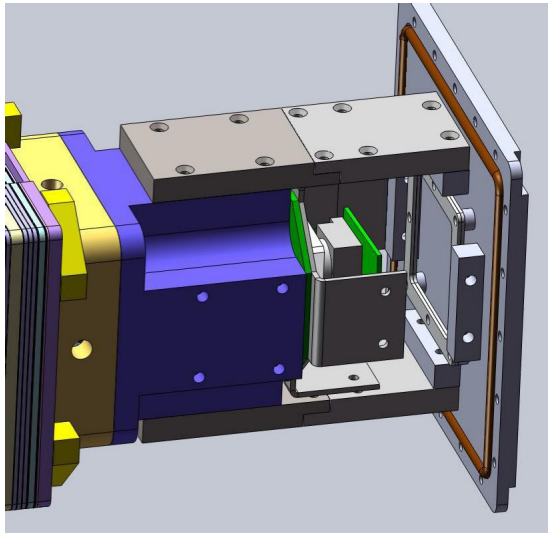
1. Internal wetted valve materials
    1. PPS = Polyphenylene sulfide ("Fortron")
    2. PBT = Polybutylene terephthalate ("Valox")
    3. 316 SS
    4. FeCr Alloy
    5. Silicone Rubber

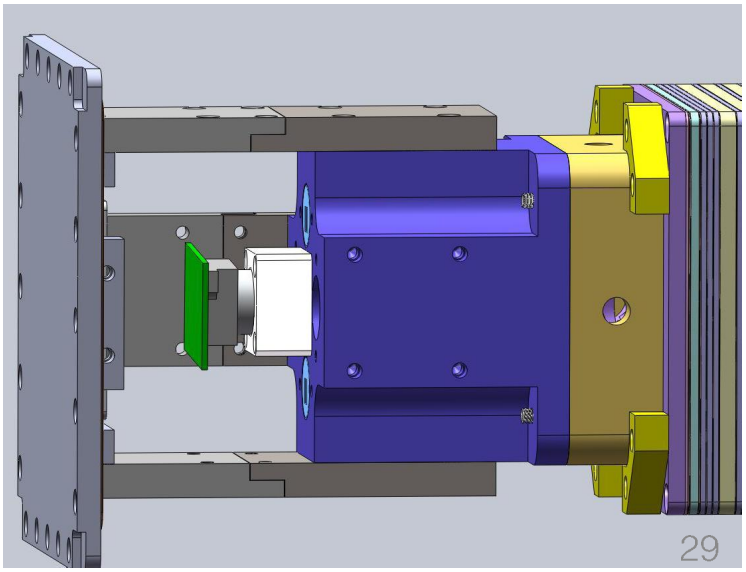






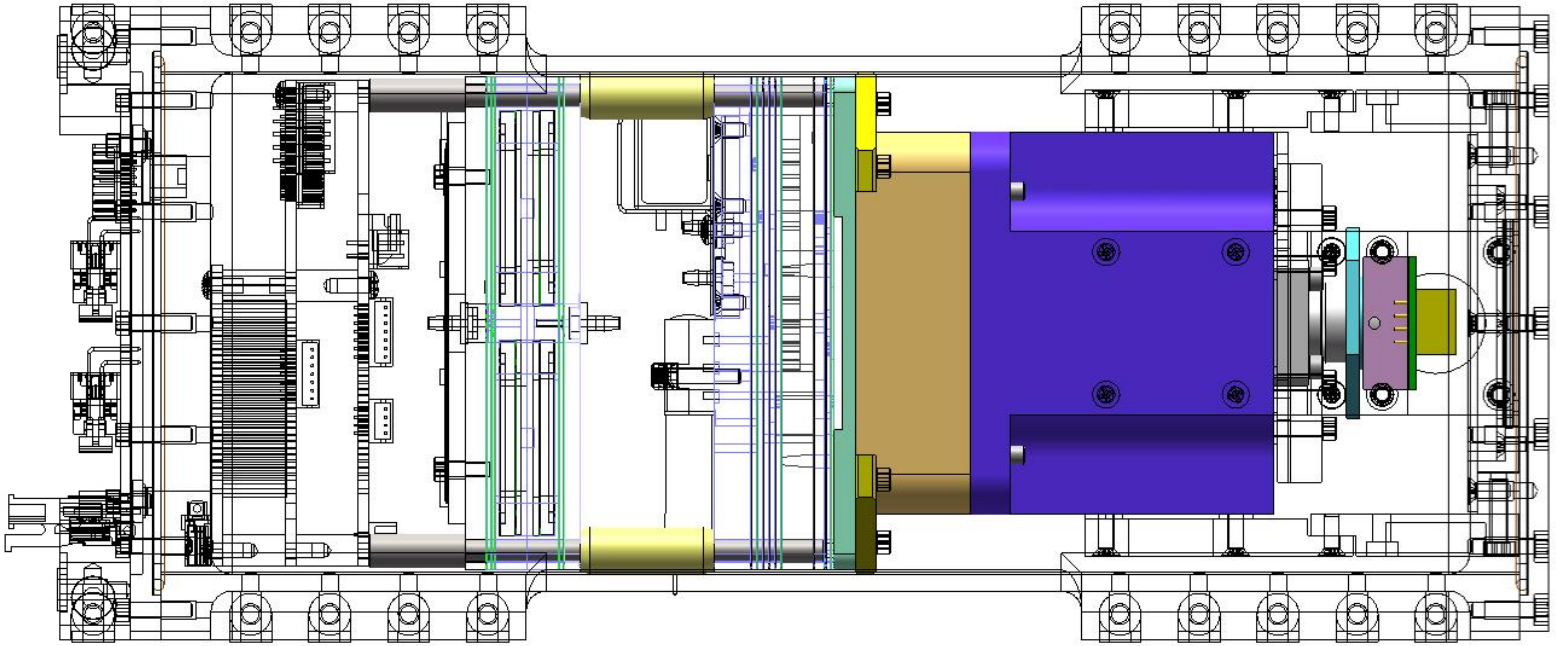




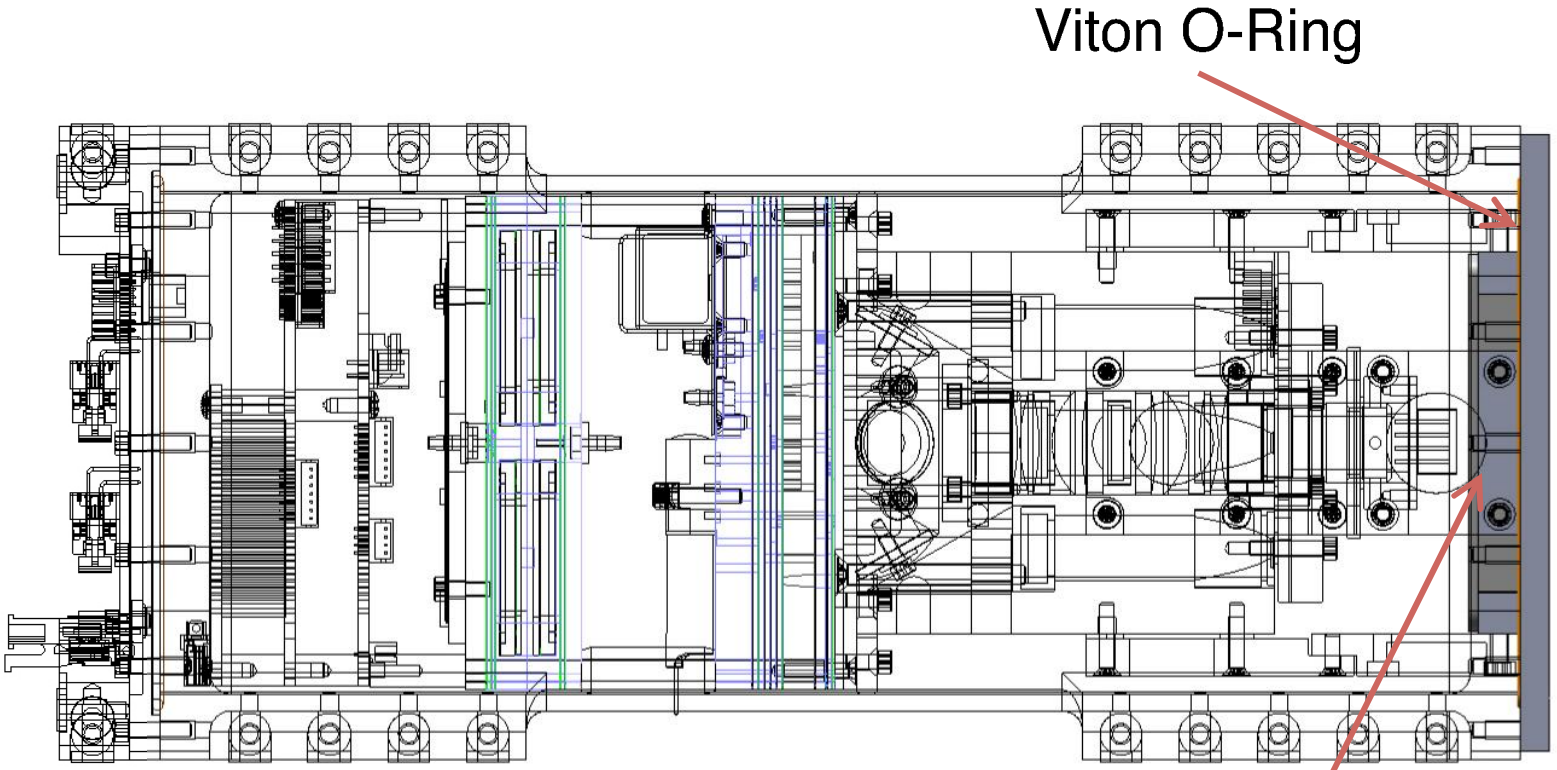


Fluidics Stack Pins

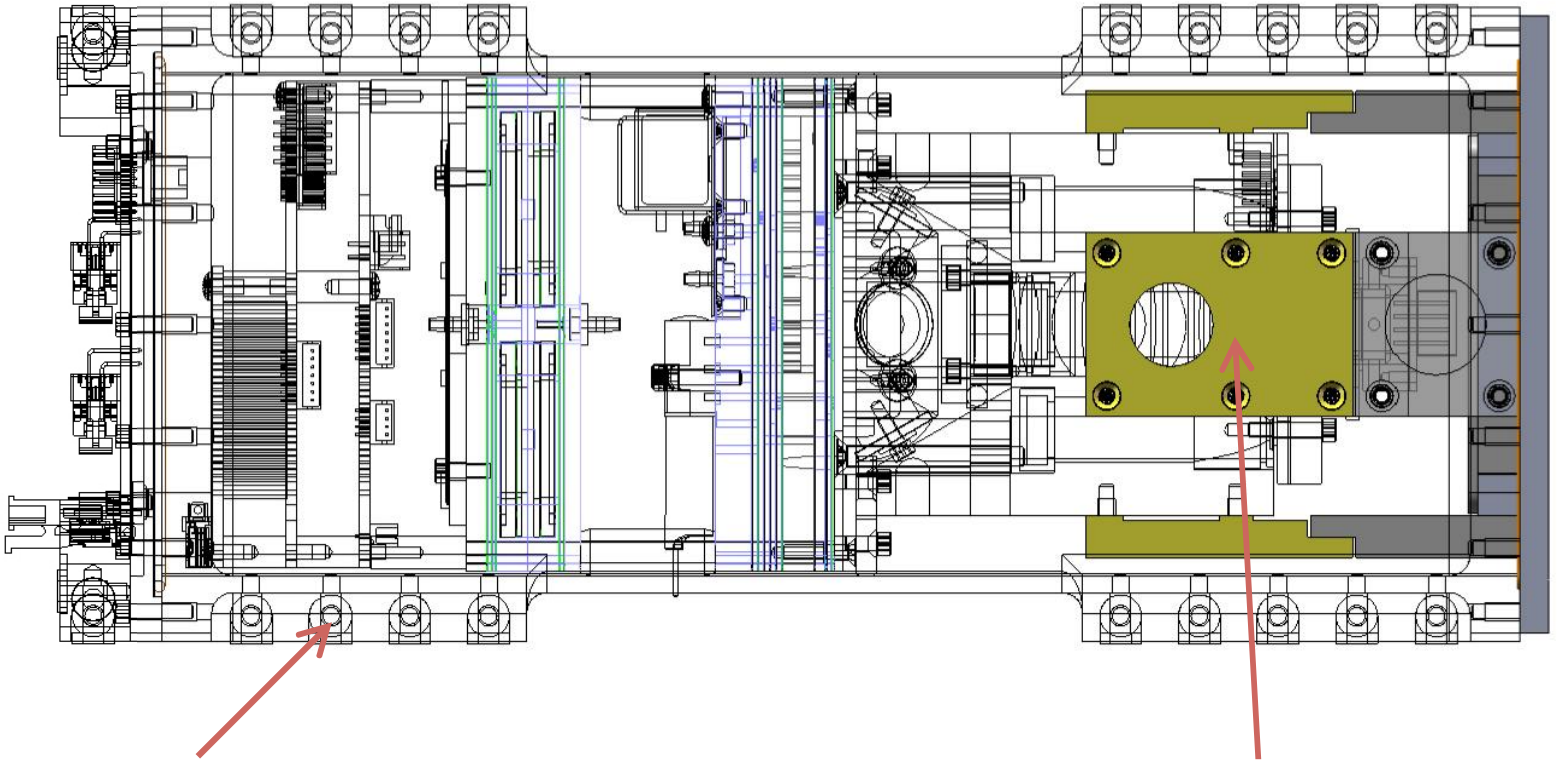
- Detail -



Back-Cover Assembly



Camera Mount
- Mated to Back Panel -

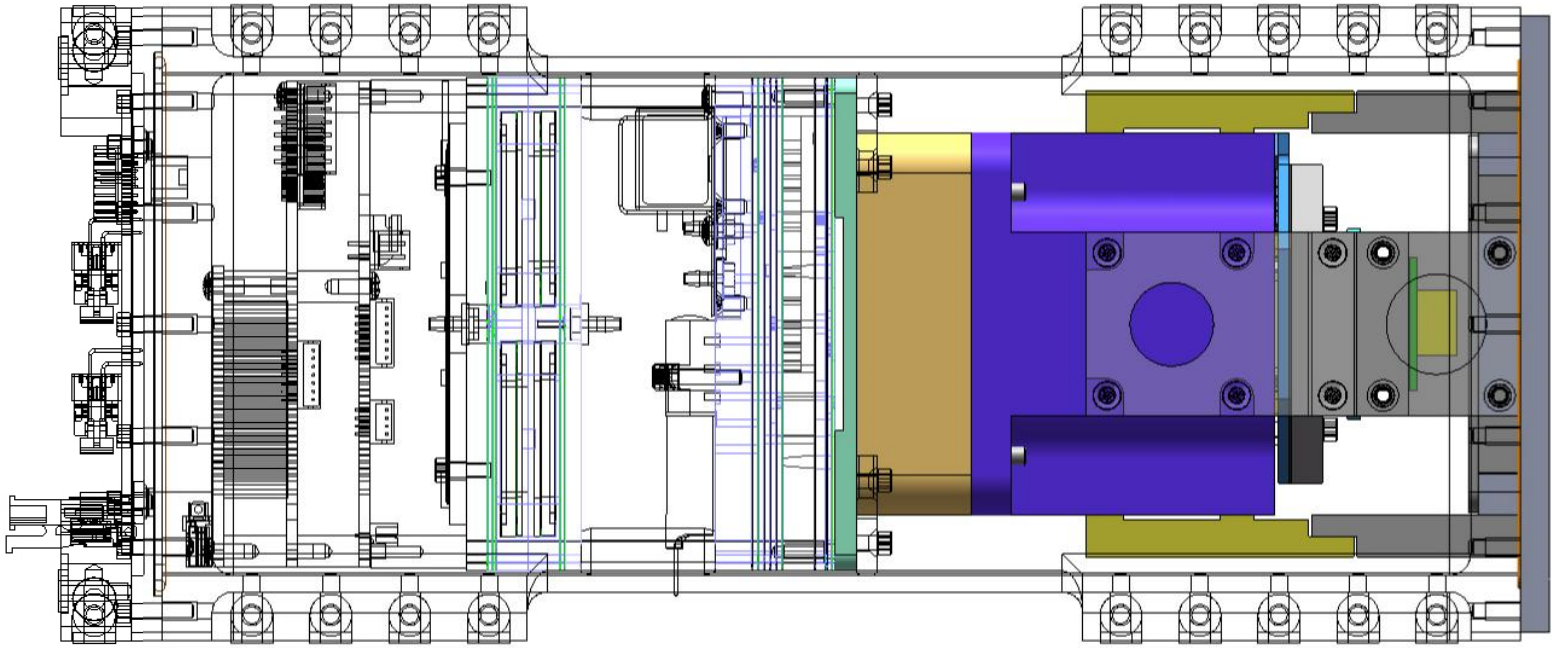


Ultem Washers
Enclosure Thermal Isolation

Ultem Mount
Payload Thermal Isolation

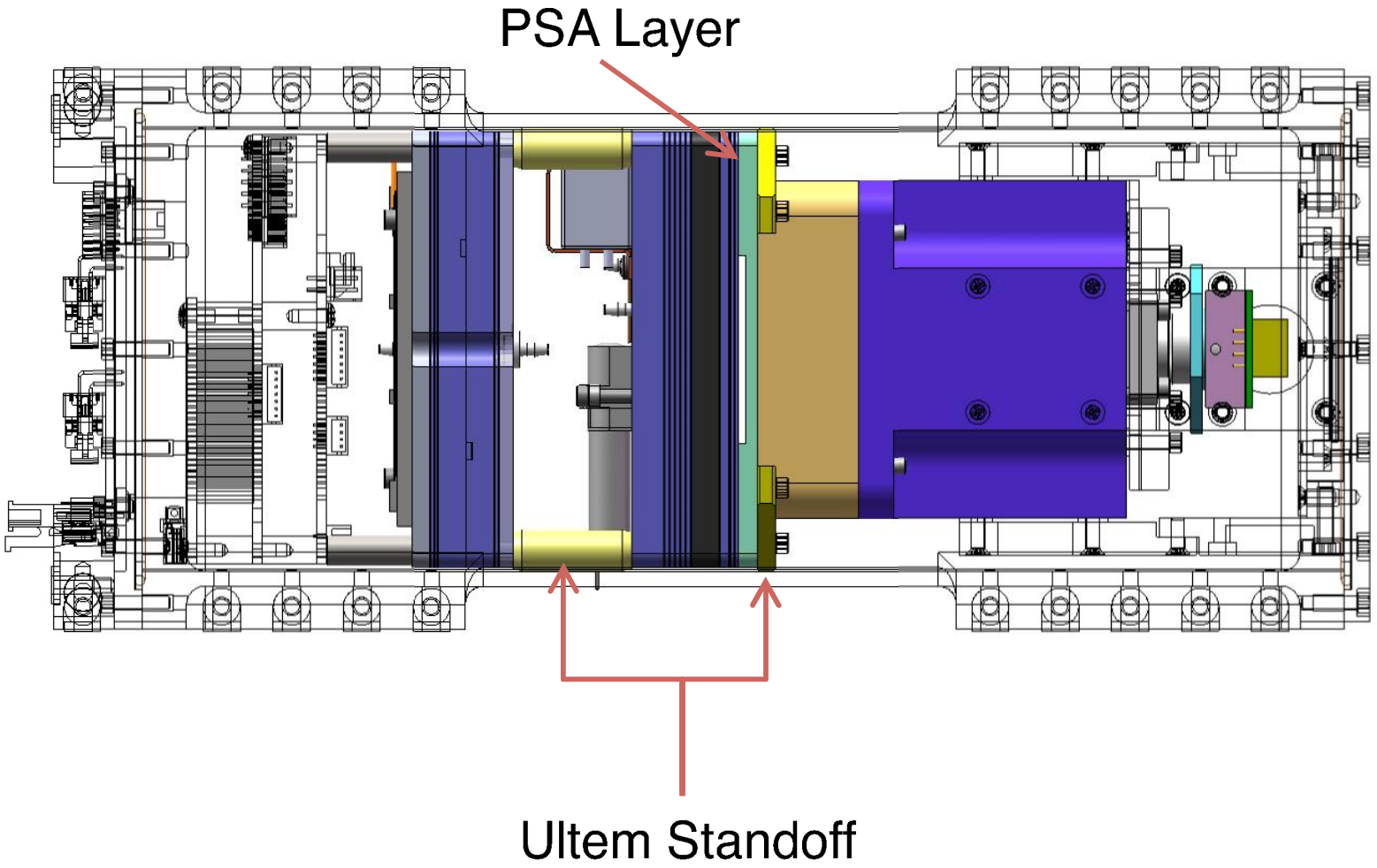
Camera Assembly

- Mated to Camera Mount -



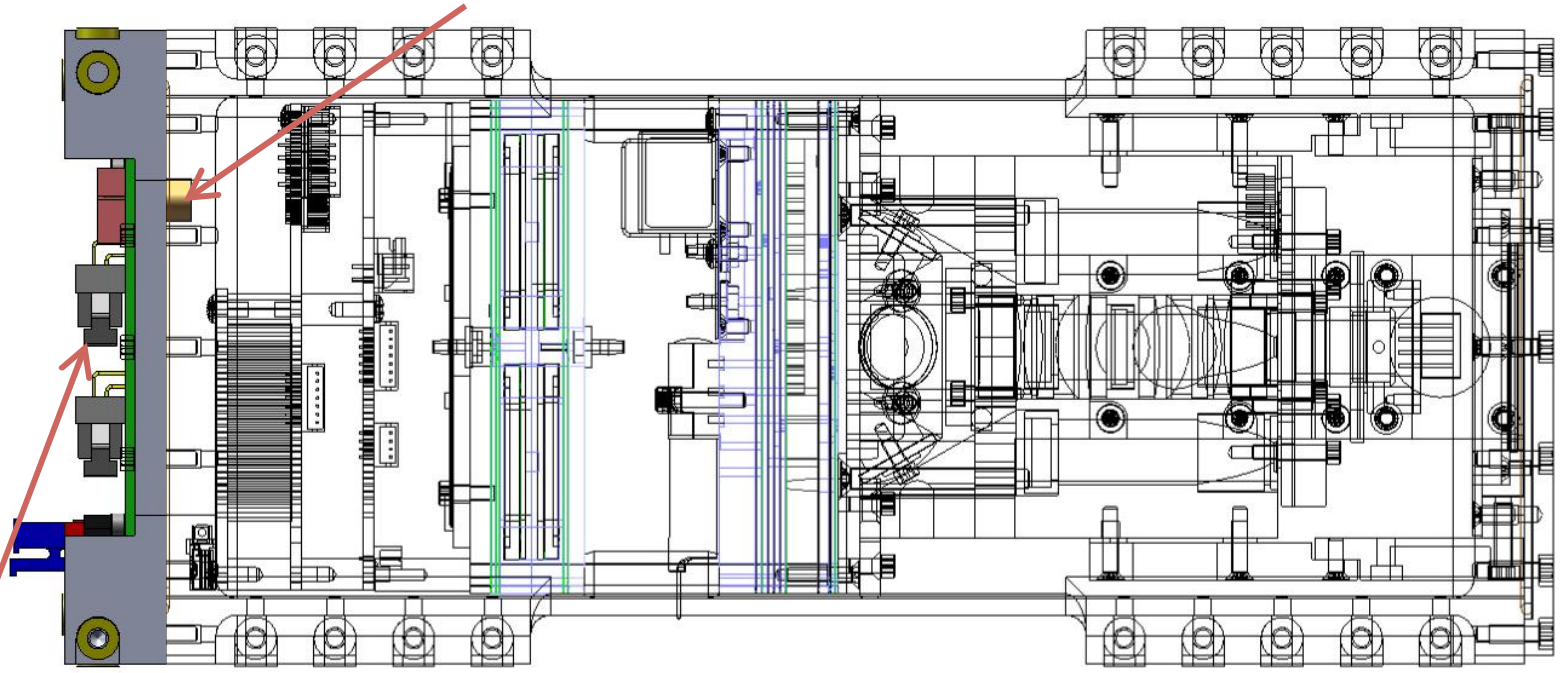
Fluidics Assembly

- Mated to Camera Assembly -



Front Panel Assembly

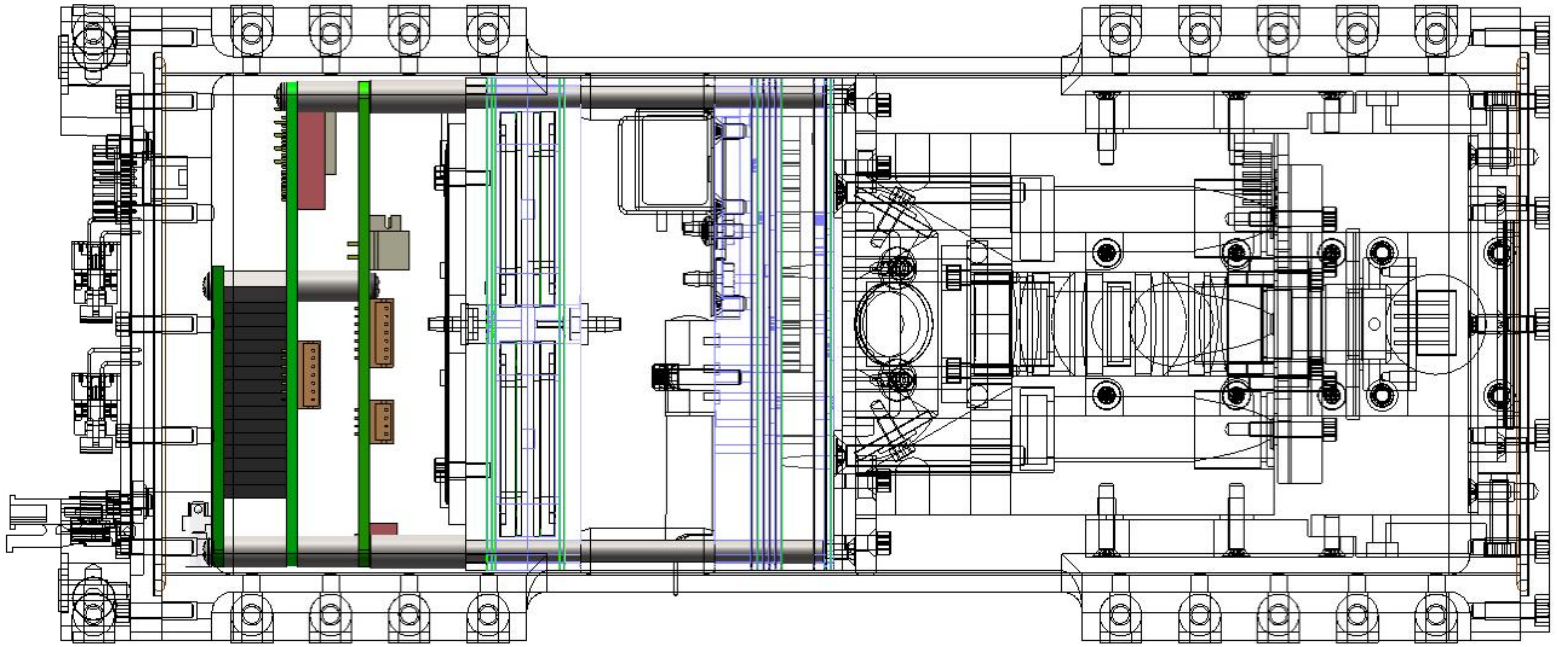
Hermetic Connector
•Laser Welded



Imager Interface Board

Electronics Assembly

- Mated to Fluidics Stack Pins -



Payload Electrical Overview

M410 Imager Interface Board	**Translates the bus 50pin cable to the hermetic connector on the payload can. Also has connection for the remove before flight "kill switch"**
M420 Imager Payload Analog	**Contains all analog payload circuits. Temperature sensor circuits, LED current drivers, RH sensor, pressure sensor and circuitry, Heater circuitry**
M422 Imager Payload Digital	**Contains all digital and inductive load circuits. Valve H-bridges, motor switches, memory chips, camera interface.**
M430 Imager LED	**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.**
M450 Imager Processor	**Contains the payload microprocessor and associated circuitry, uC LDO, RTC, FRAM, ADC reference**