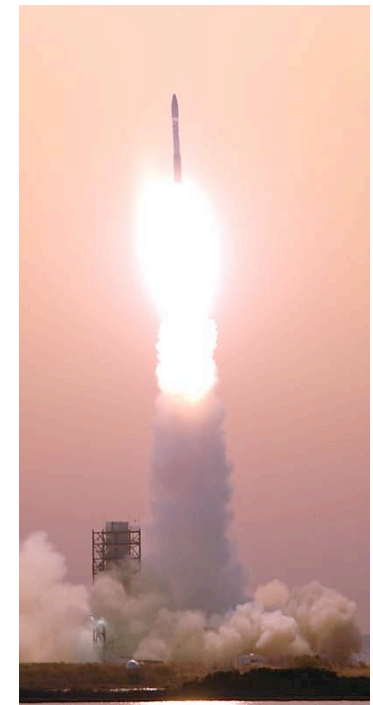
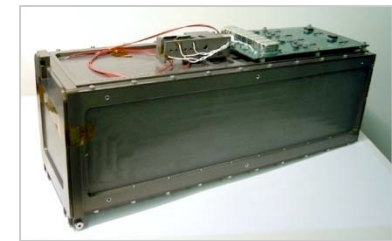
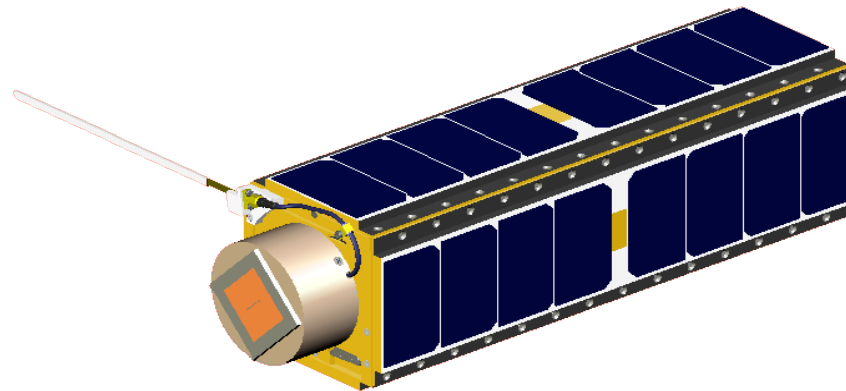
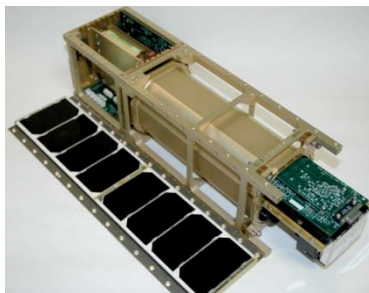
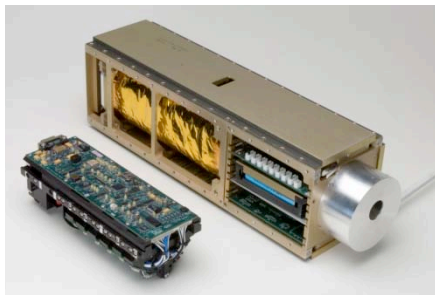




Studying Space Effects on Microorganisms Autonomously: GeneSat, PharmaSat and the Future of Bio-nanosatellites



**Macarena Parra, Michael R.
McGinnis, Antonio J. Ricco, Bruce
Yost, John W. Hines**



Overview

- GeneSat
 - Purpose
 - Hardware Design and Features
 - Tech Demo Experiment
 - Experiment Results
 - Summary
- PharmaSat
 - PI driven experiment
 - Hardware upgrades



GeneSat Tech Demo

Purpose of GeneSat:

Make a platform that can be used for gene expression studies in microorganisms using GFP promoter reporter constructs

For Tech Demo, we chose

- E. coli* strain
- Expresses GFP
- Survive long-term storage

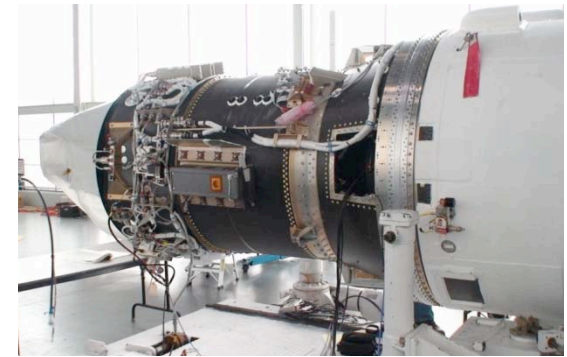
SCENARIO

- Prepare cells in storage buffer at a known cell density
 - Cells are in stasis
- Load cells into fluidics card and transfer to flight hardware
- Launch
- Attain stable orbit
- Activate cell culture by replacing the storage buffer with growth medium and adjusting temperature to 34 °C
- Monitor growth and fluorescence (take readings every 12 minutes)



SmallSat Limitations

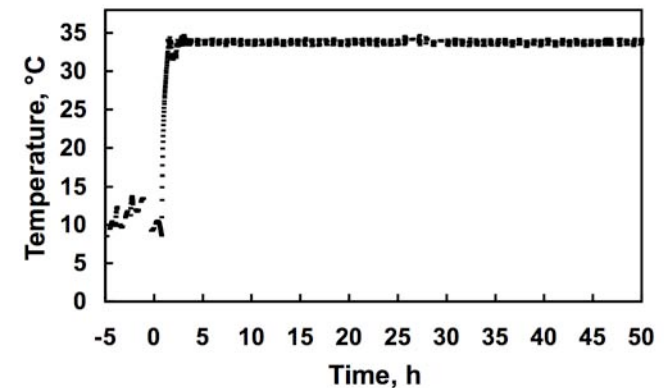
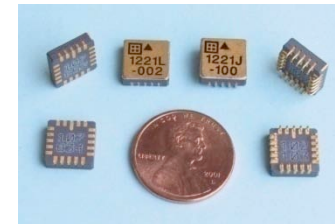
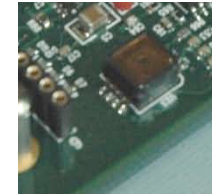
- Expendable vehicle – No sample return
- Secondary payload
 - Late load not likely
 - Re-loads due to launch slips not likely
- All operations must occur autonomously
 - Experiment start
 - Data collection
- Communications with satellite not continuous
 - Data download not instantaneous
 - 3-5 contacts/day, each lasting 5-12 min
 - Primary Communication: 18-m SRI Station, Palo Alto CA
 - Secondary Communication : Ham Radio Beacon
- Size





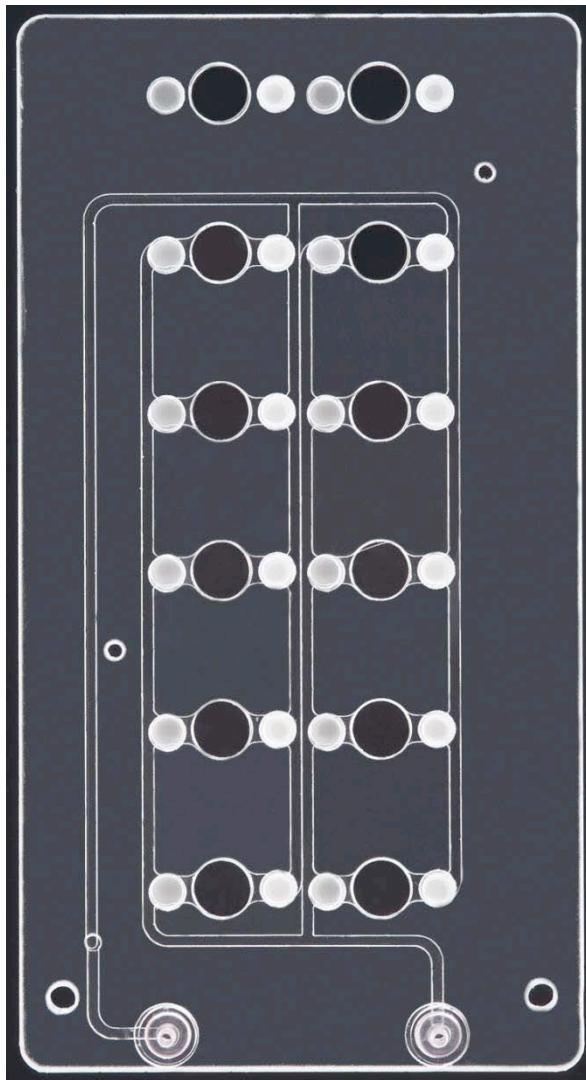
GeneSat Capabilities

- Fully autonomous operations
 - Experiment start
 - Increase temperature to experiment set point
 - Feed cells (add media)
 - OD and Fluorescence readings
- Sensors in satellites
 - Temperature
 - Pressure
 - Relative humidity
 - Acceleration (g-forces)
 - Radiation
- Fine temperature control
 - Reached experiment set point in one hour (34 °C)
 - Temperature kept to within 0.5 °C of set point throughout experiment

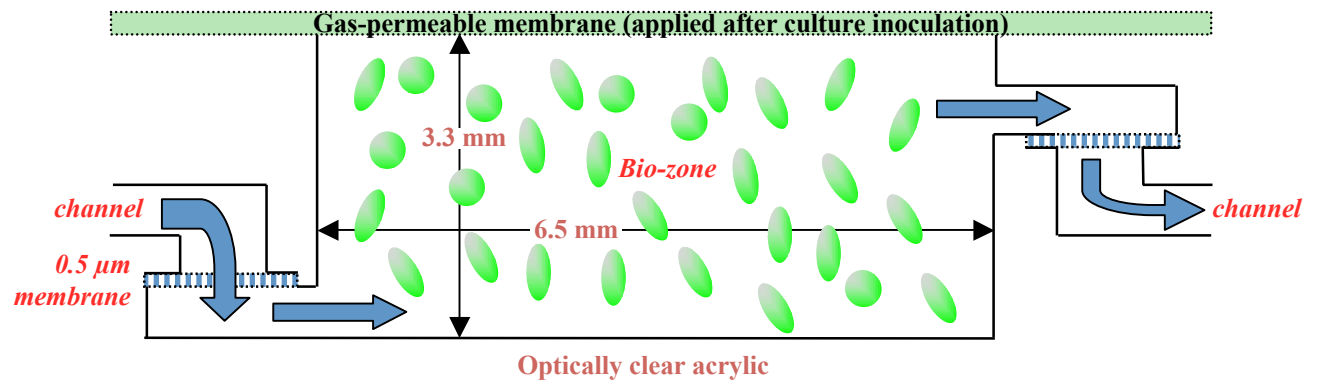




GeneSat Fluidic Card



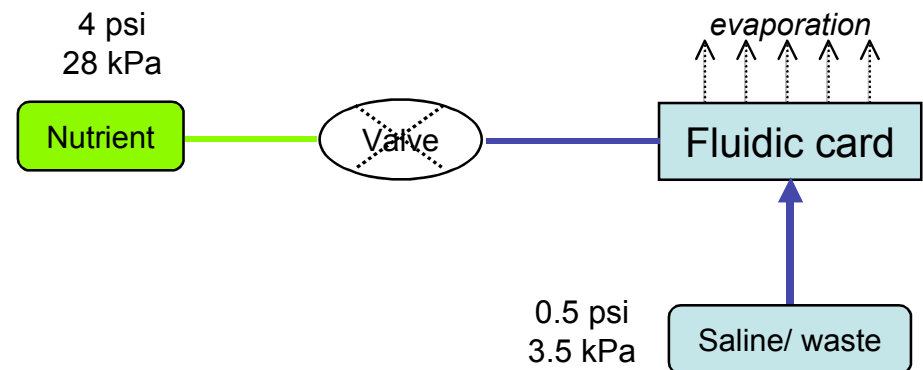
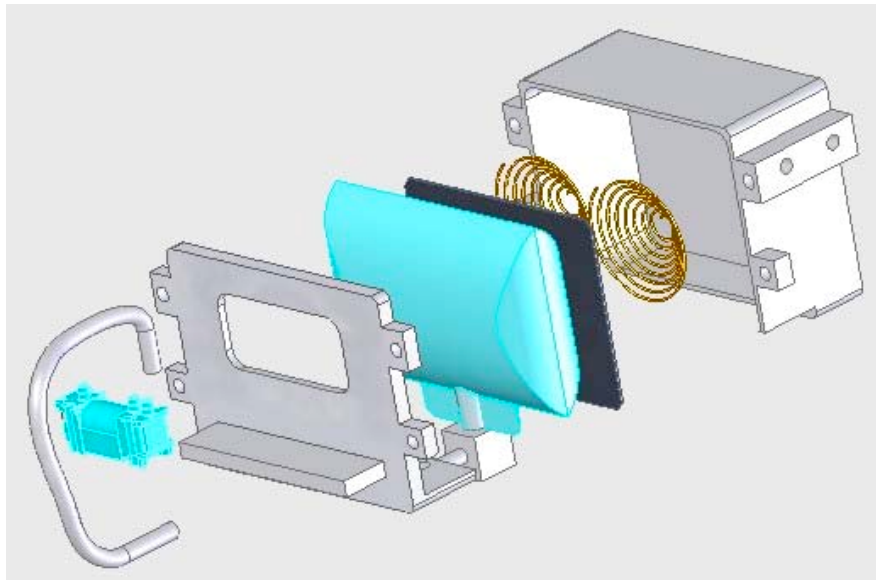
- 12-well culture-and-analysis plate
 - 10 biology wells, 2 control/standard wells
 - 110 μL /well \Rightarrow 1.1 mL total on-card volume
- Membrane filter at each well inlet and outlet
- Loaded pre-launch with *E. coli* in stasis medium
- Infused upon stable (**g**, **T**) orbit with glucose solution to initiate growth





Fluidic System

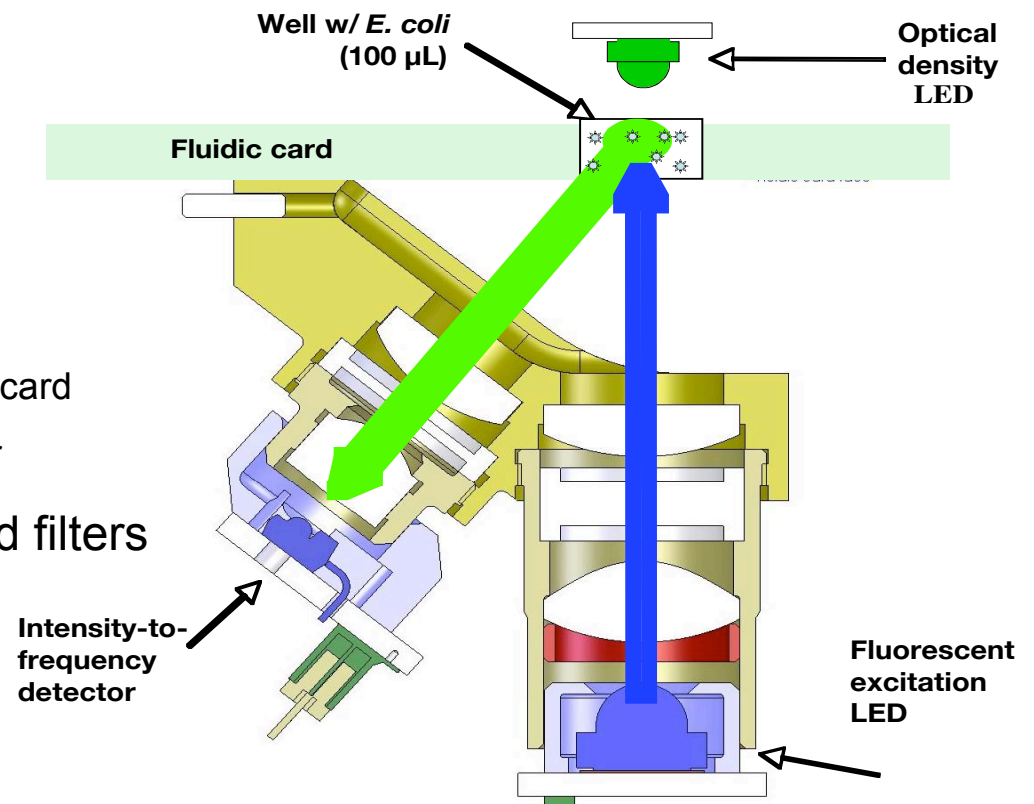
- Fluidic system consists of two bags under pressure from springs
 - One contains growth media, the other contains stasis media (saline)
- Reservoir capacity ~ 15 ml
- Growth media cannot enter the card until valve is opened
- Slight pressure on saline bag allows for replenishing of fluid loss from card due to evaporation





Optics System

- Optics system consists of 12 separate units, each is capable of measuring both Fluorescence and Optical Density
- Fluorescence measurement
 - Blue LED used for excitation (460-490 nm)
 - Detector reads green fluorescence (505-530 nm)
- Optical Density measurement
 - Indirect measurement
 - Green LED on opposite side of fluidic card
 - Scatter is measured by same detector
- Readings optimized by lenses and filters





Ground Testing

- Strain selection
 - Good GFP expression
 - Good long term survival
- Viability
 - Long term storage at ambient temperatures
- Biocompatibility
 - Cell growth in hardware is comparable to standard laboratory methods
 - Long term storage in contact with materials
- Long term stability of all reagents
 - Stasis buffer
 - Growth media
 - Additives



“Experiment” – Strains

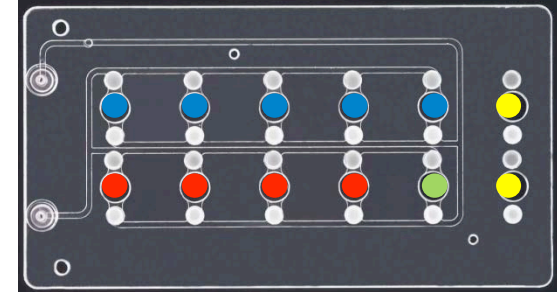
Two strains were chosen

- pGREEN in MM294 from Carolina Biological Supply Company
 - Green Gene Colony Transformation Kit
 - BUT the constitutive promoter was unknown
- AcGFP in DH5 α from Arizona State University
 - Clontech AcGFP plasmid under constitutive promoter (CMV)
- Slightly different GFP expression characteristics
 - pGREEN: more steady but not as bright
 - AcGFP: very bright but GFP expression may be lost under stress (i.e. non-biocompatible material)
- Slightly different survival characteristics
 - Stasis prep
 - Biocompatibility

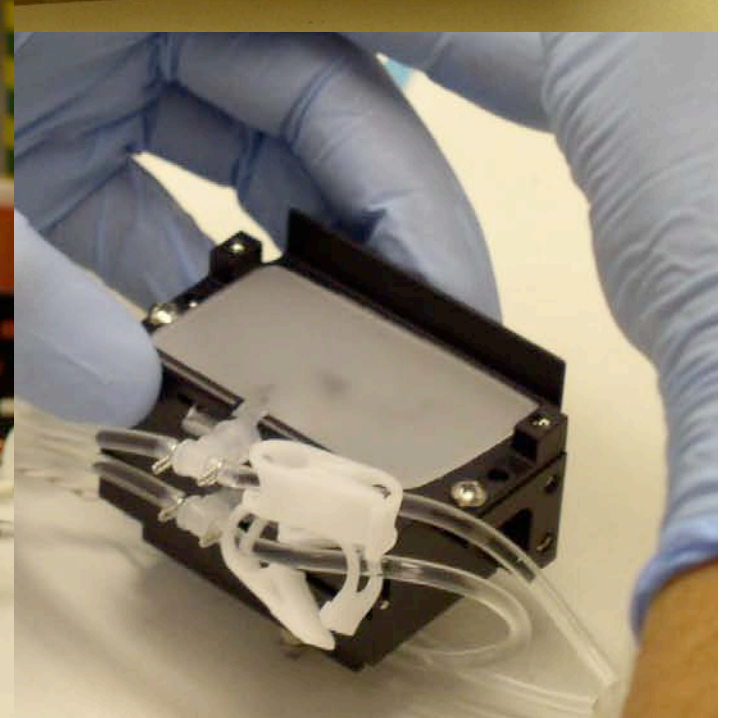
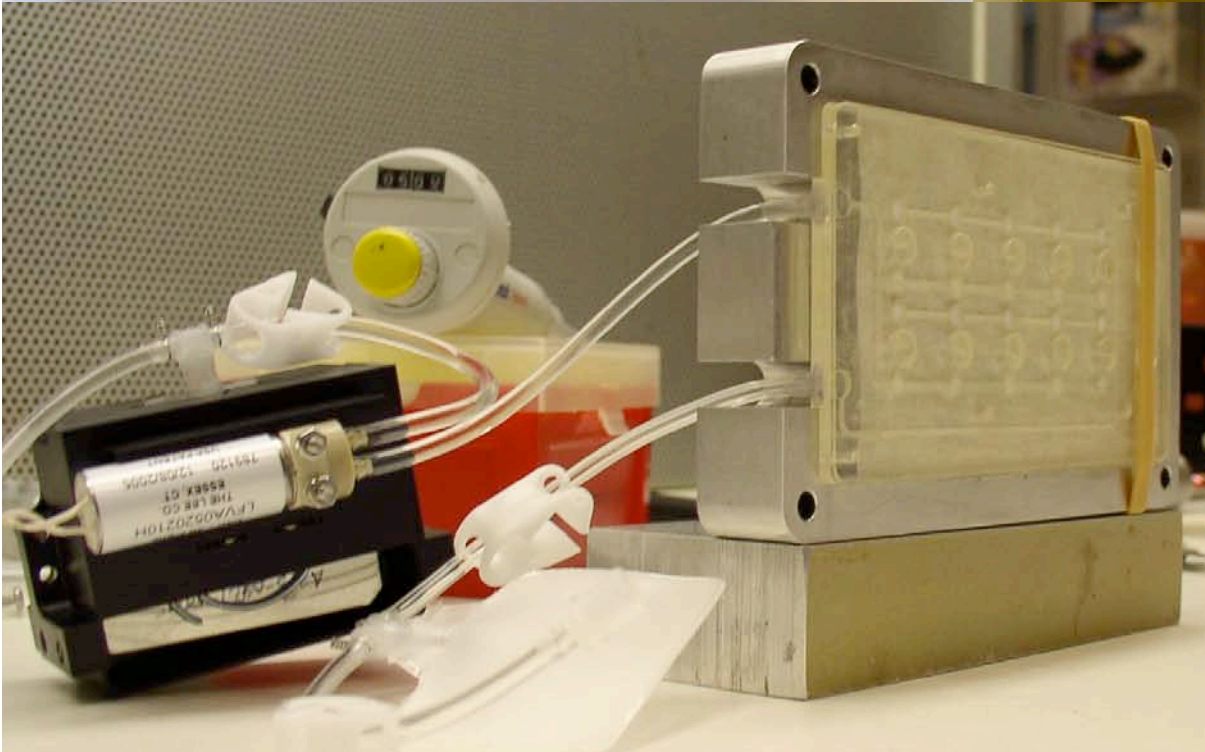
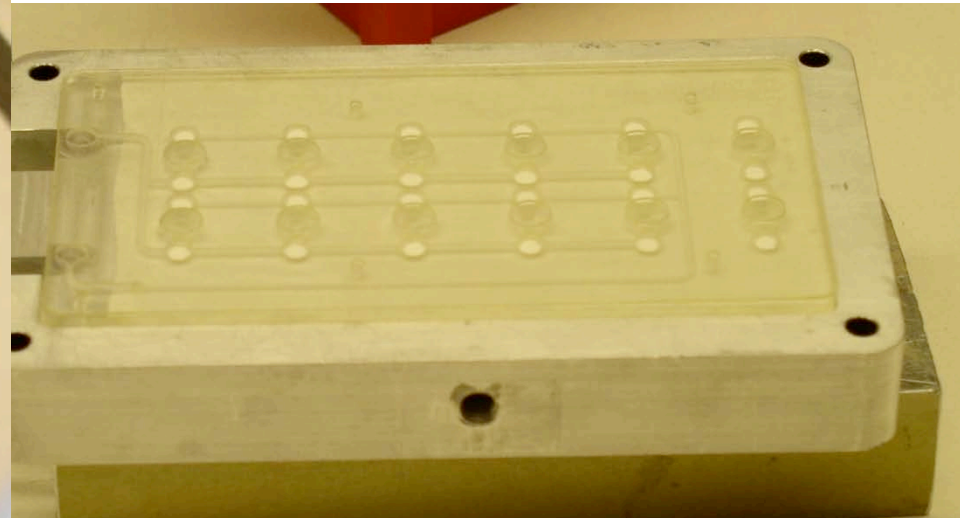
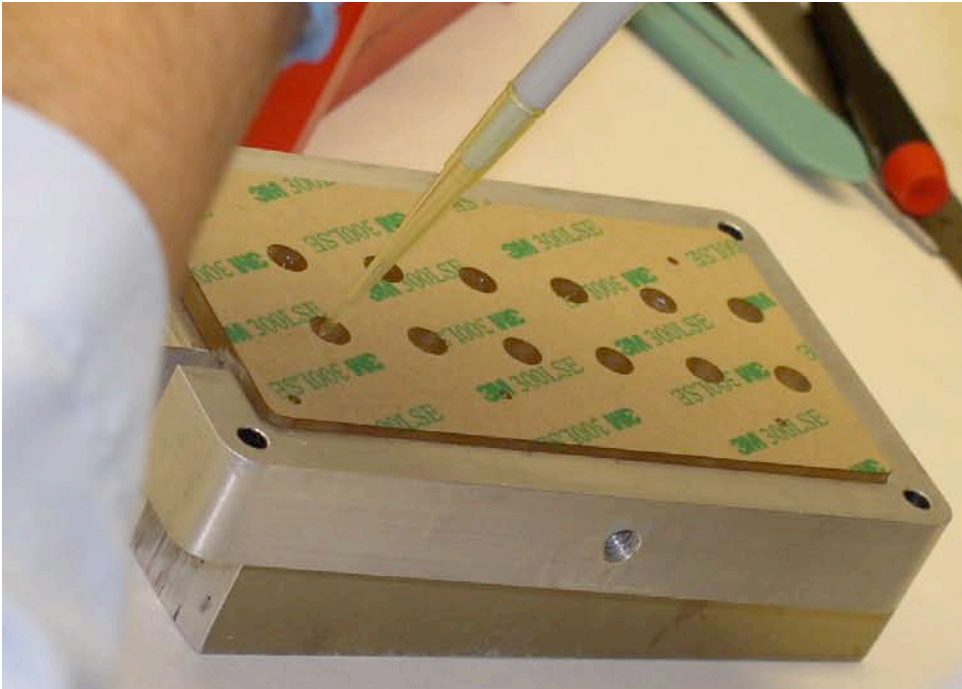


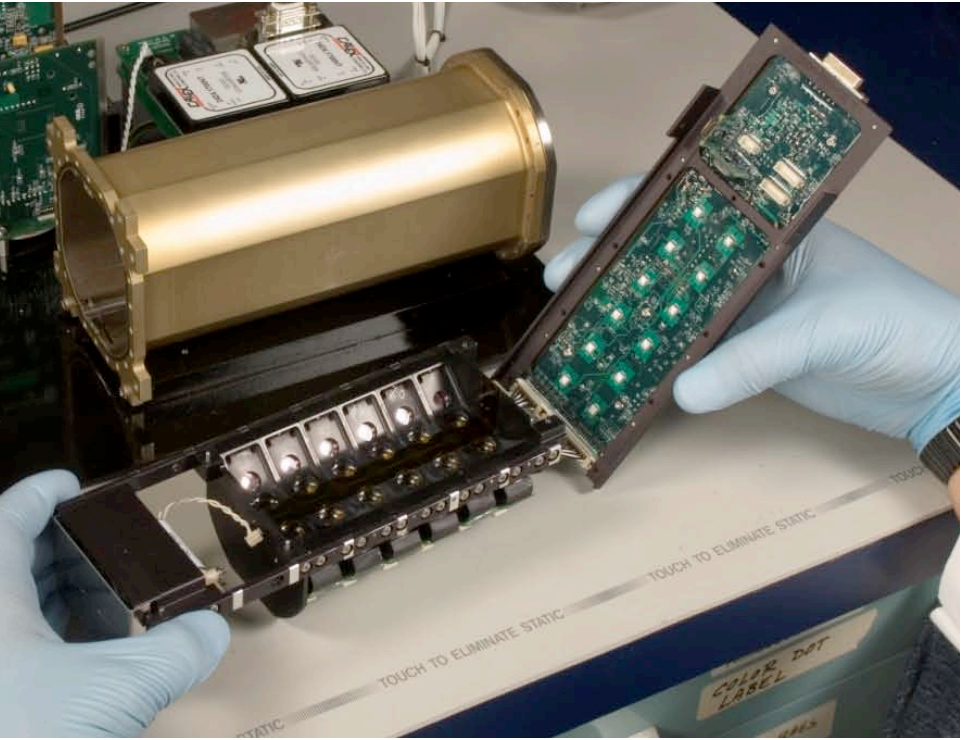
“Experiment” - Loading

- Card loaded with:
 - 5 wells with the pGREEN construct
 - 4 wells with the AcGFP construct
 - One control well (no cells)
 - Two non-biology wells loaded with fluorescence standards
- Loaded two satellites at Ames
- Both were hand-carried to Wallops Field
 - Run final functional checkouts on both systems
 - One was selected for integration
 - Other system was hand-carried back to Ames and served as ground control

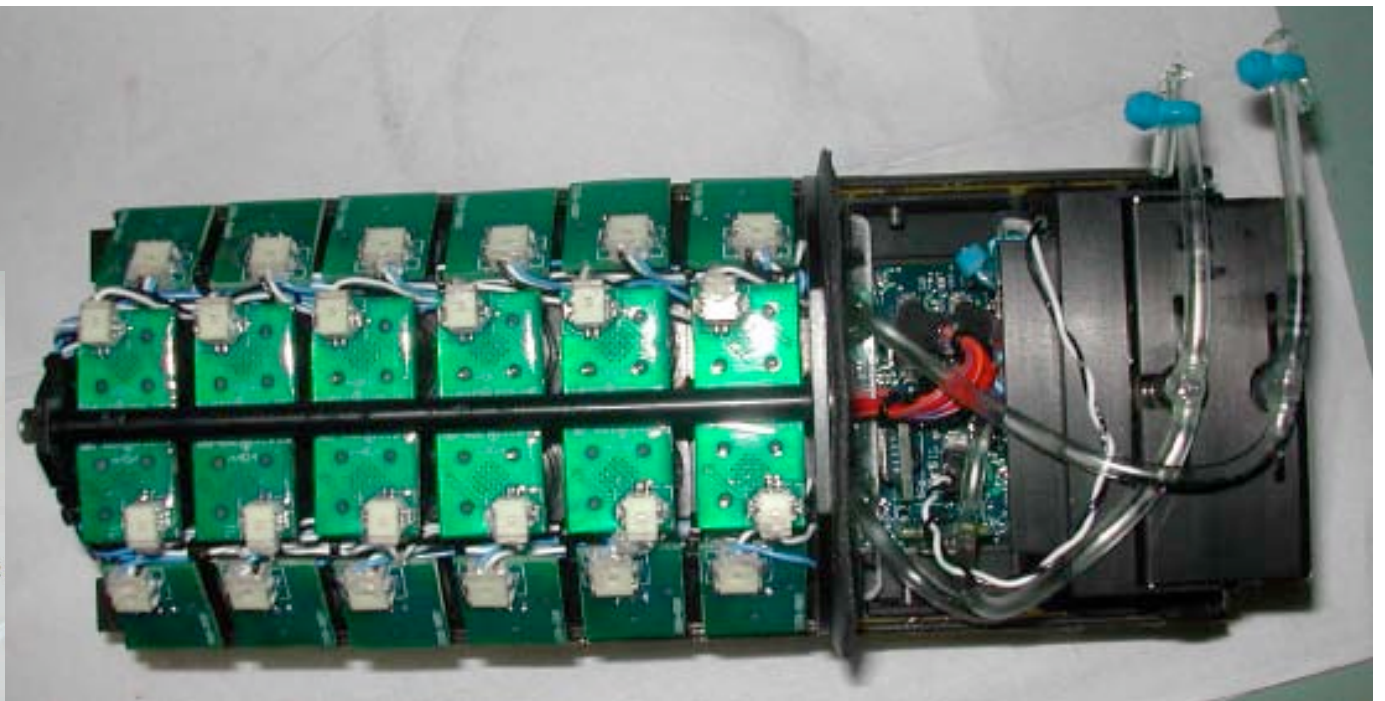
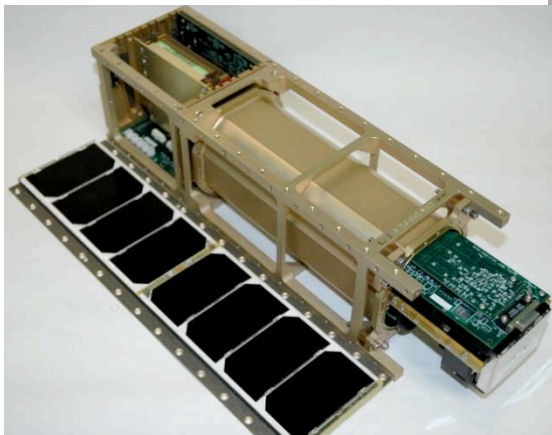


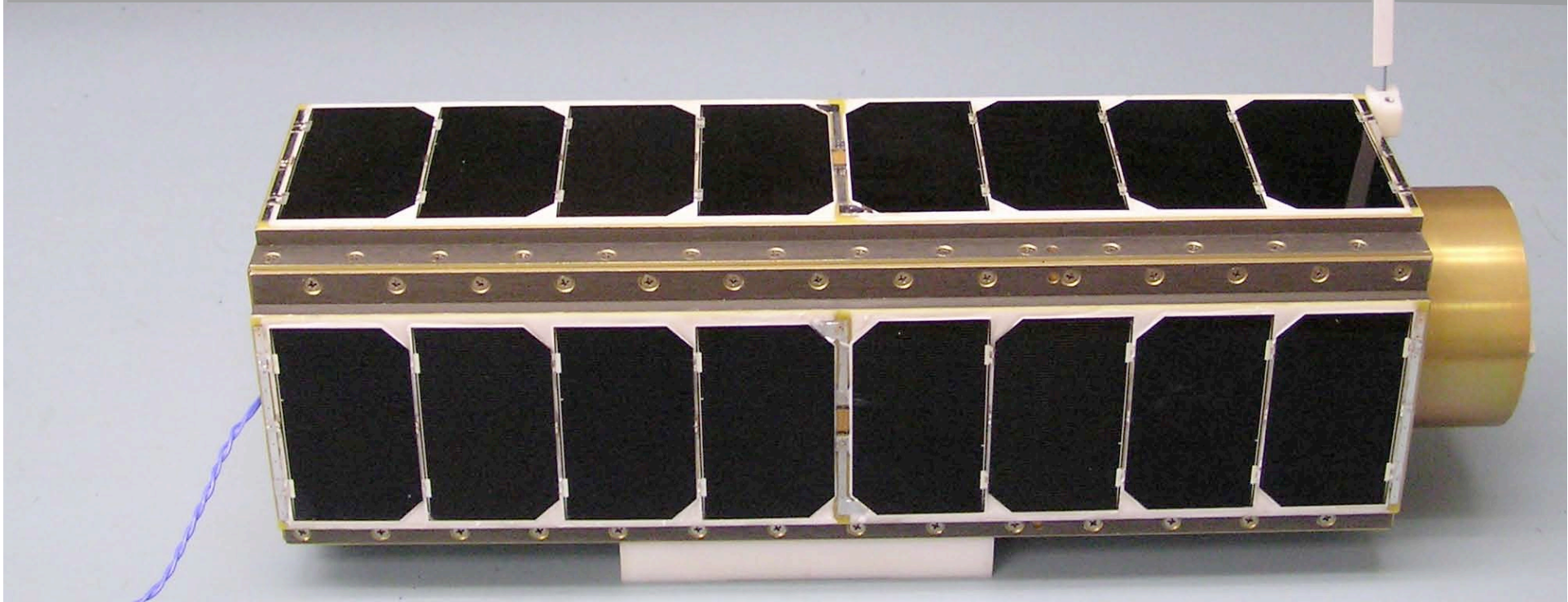
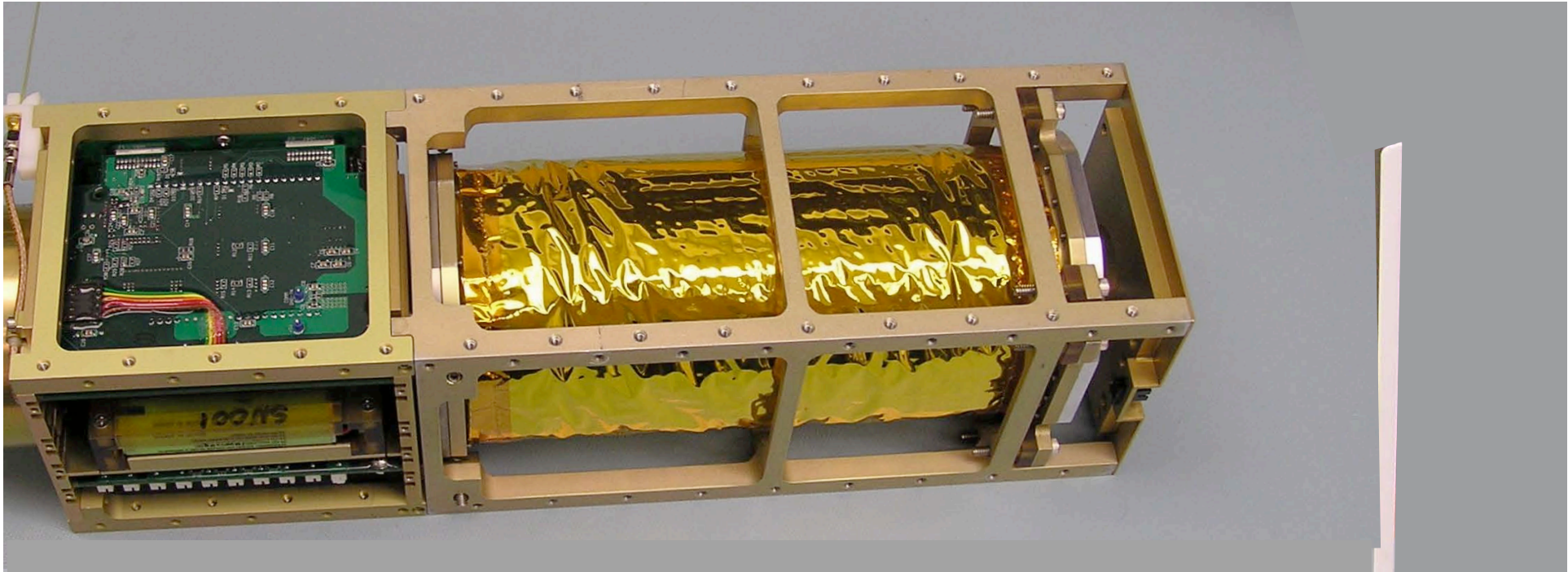
Biology and Fluidics Load





Payload System Assembly

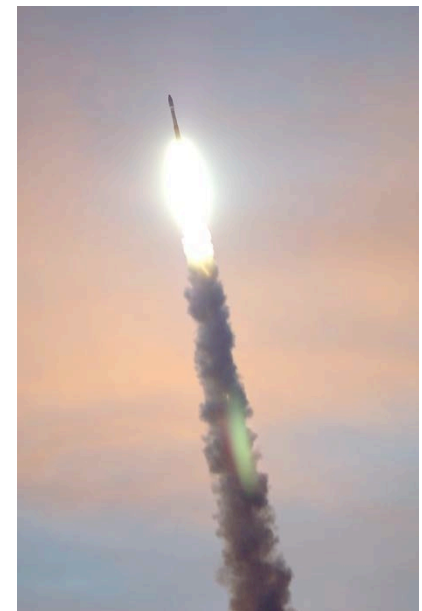
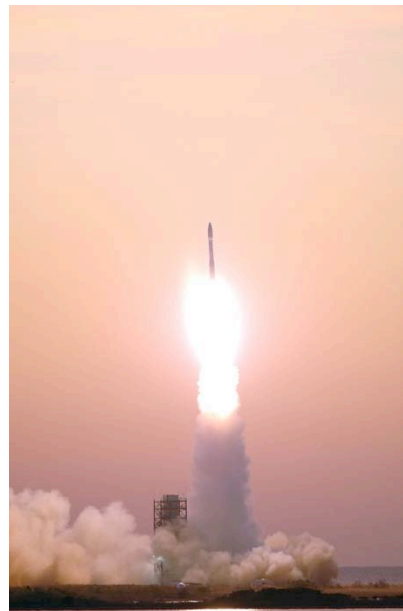






Mission Overview

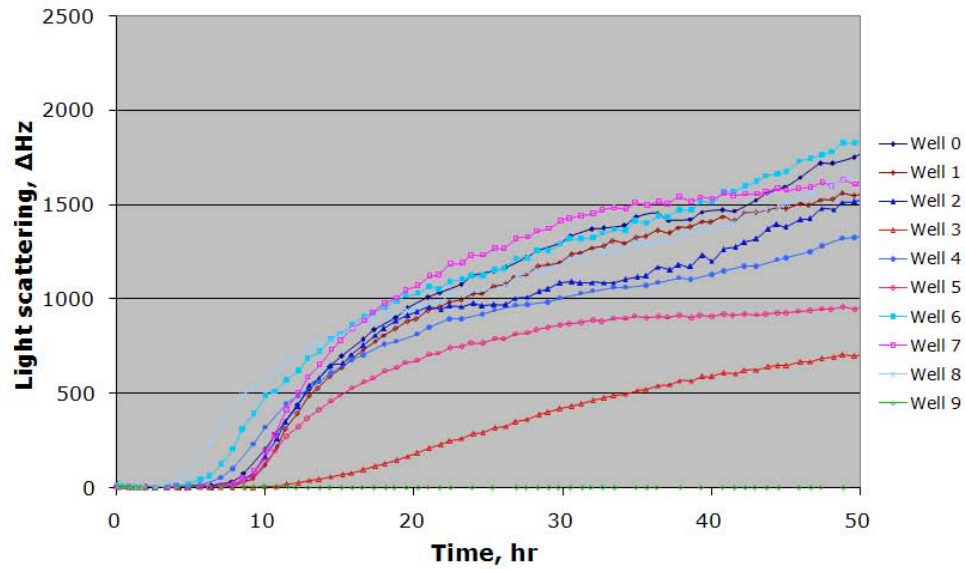
- **GeneSat Launch: 12-16-06**
- Communications established: 12-17-06
- Experiment Initiated: 12-18-06
- Due to launch slip and lack of late access, the cells were in stasis for a total of **48 days** before experiment initiation





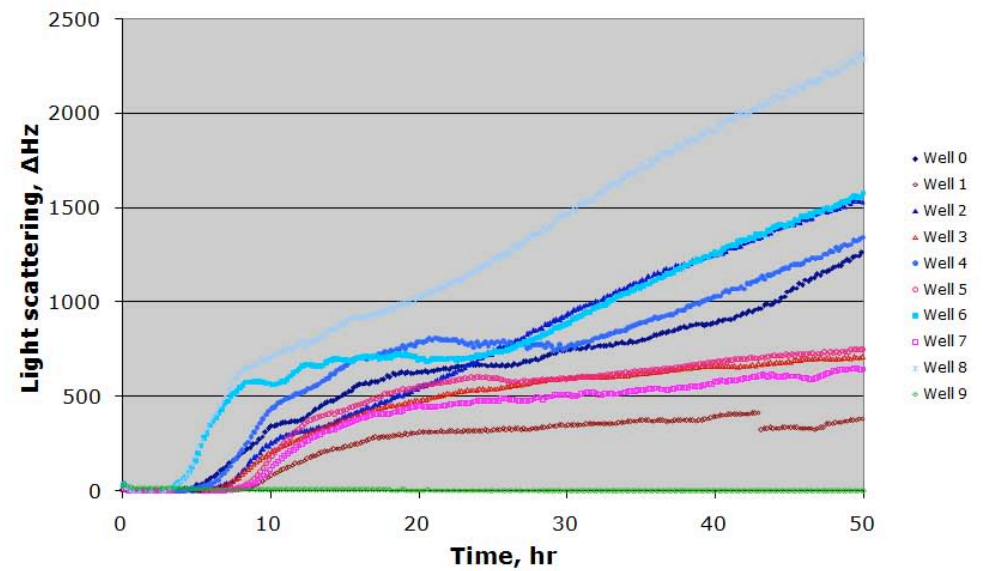
Results

FLIGHT OD - Zero'd



pGREEN
AcGFP
control

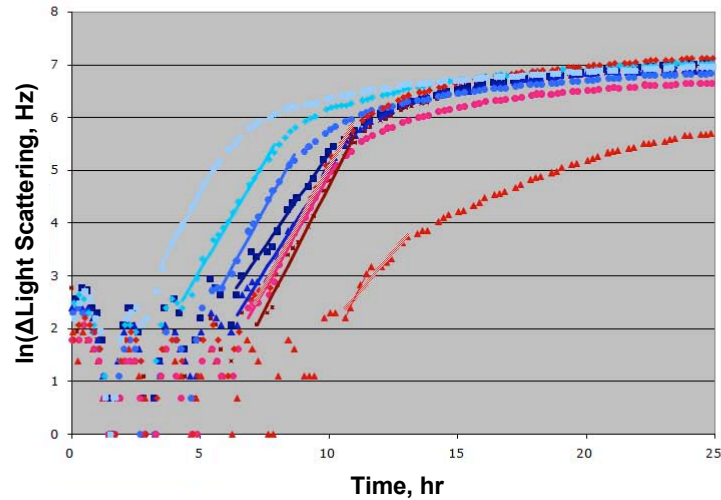
GROUND OD - Zero'd





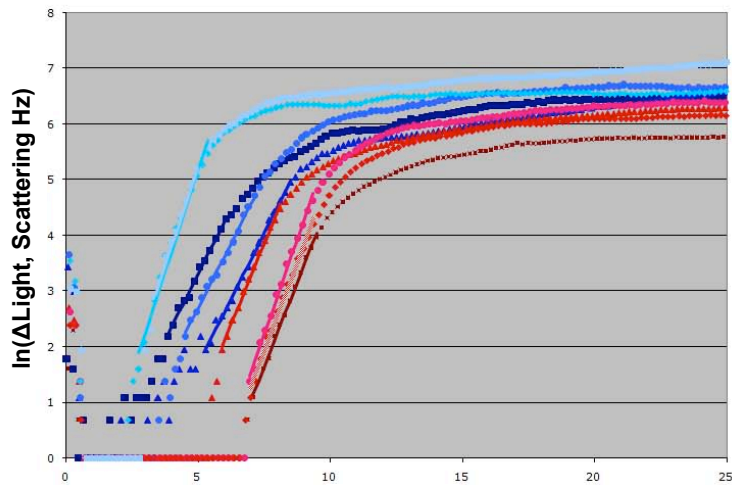
Results (continued)

Flight OD



Time, hr

Ground OD



Time, hr

pGREEN

DOUBLING TIMES (minutes) from semi-log PLOTS of OD vs. time

Well No.	Flight	Ground
0	59	44
2	54	44
4	47	45
6	51	28
8	46	29
Average:	51	38
SD:	5	8
	$p=0.018$	

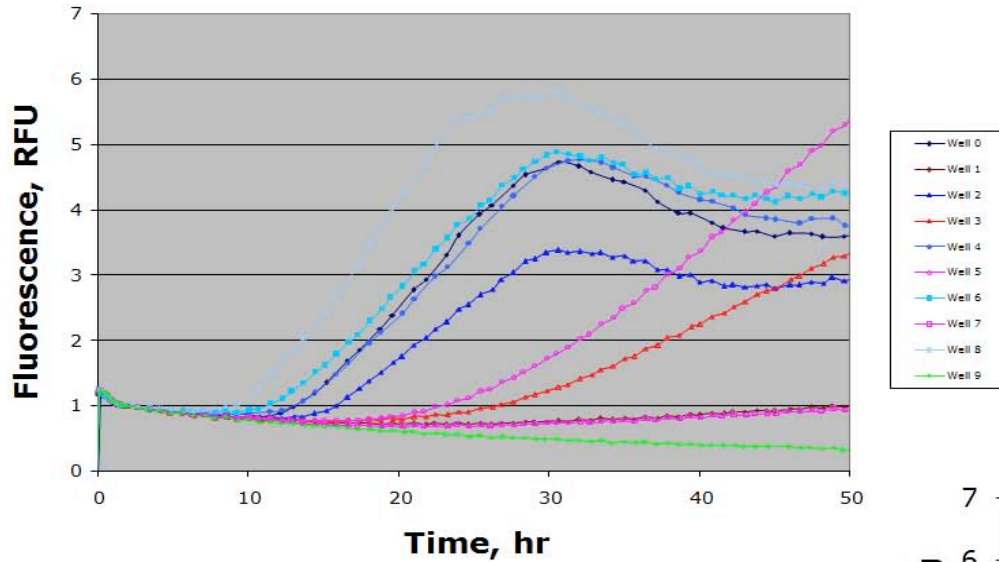
AcGFP

Well No.	Flight	Ground
1	44	34
3	41	36
5	48	30
7	47	33
Average:	45	33
SD:	3	2
	$p=0.0009$	



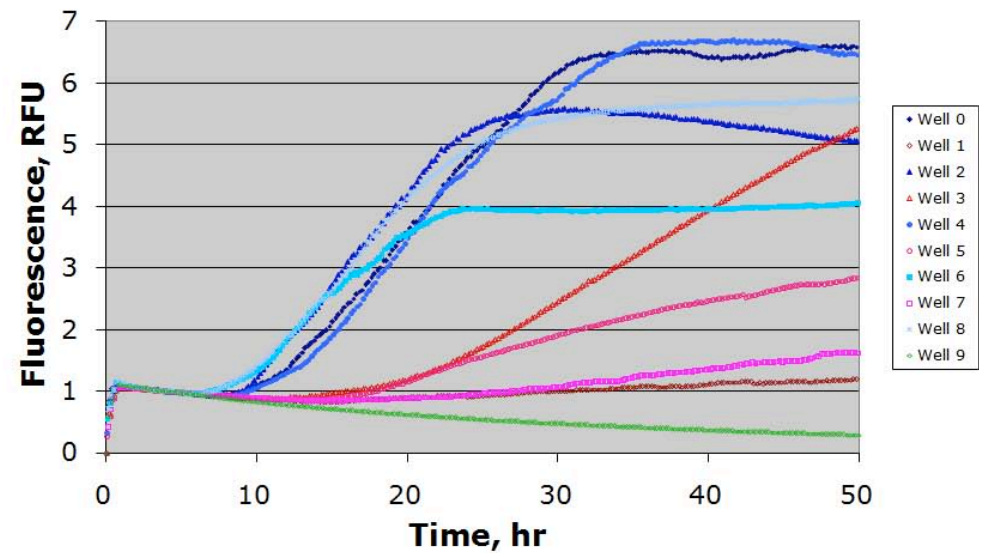
Results (continued)

FLIGHT Fluorescence - Zero'd & Scaled



pGREEN
AcGFP
control

GROUND Fluorescence - Zero'd & Scaled





GeneSat – Summary

Successful Mission

- Evidence of cell growth and fluorescence in all nine bio-wells
- All parameters within requirements
 - Temperature controlled to within set point limits (34 +/- 0.5 °C)
 - Fluidic system operated successfully
 - All optical detectors operated nominally
 - Sensors for relative humidity, pressure, acceleration, and radiation all returned data showing nominal operation & conditions
 - Sufficient power throughout experiment to power heaters, sensors, etc.
 - Data collection and storage throughout experiment was successful
 - Data download from satellite to Ames was successful
- GeneSat system is capable of:
 - Fully autonomous operations
 - Temperature control
 - OD measurement to track growth
 - Fluorescence measurements to track GFP production (gene expression)

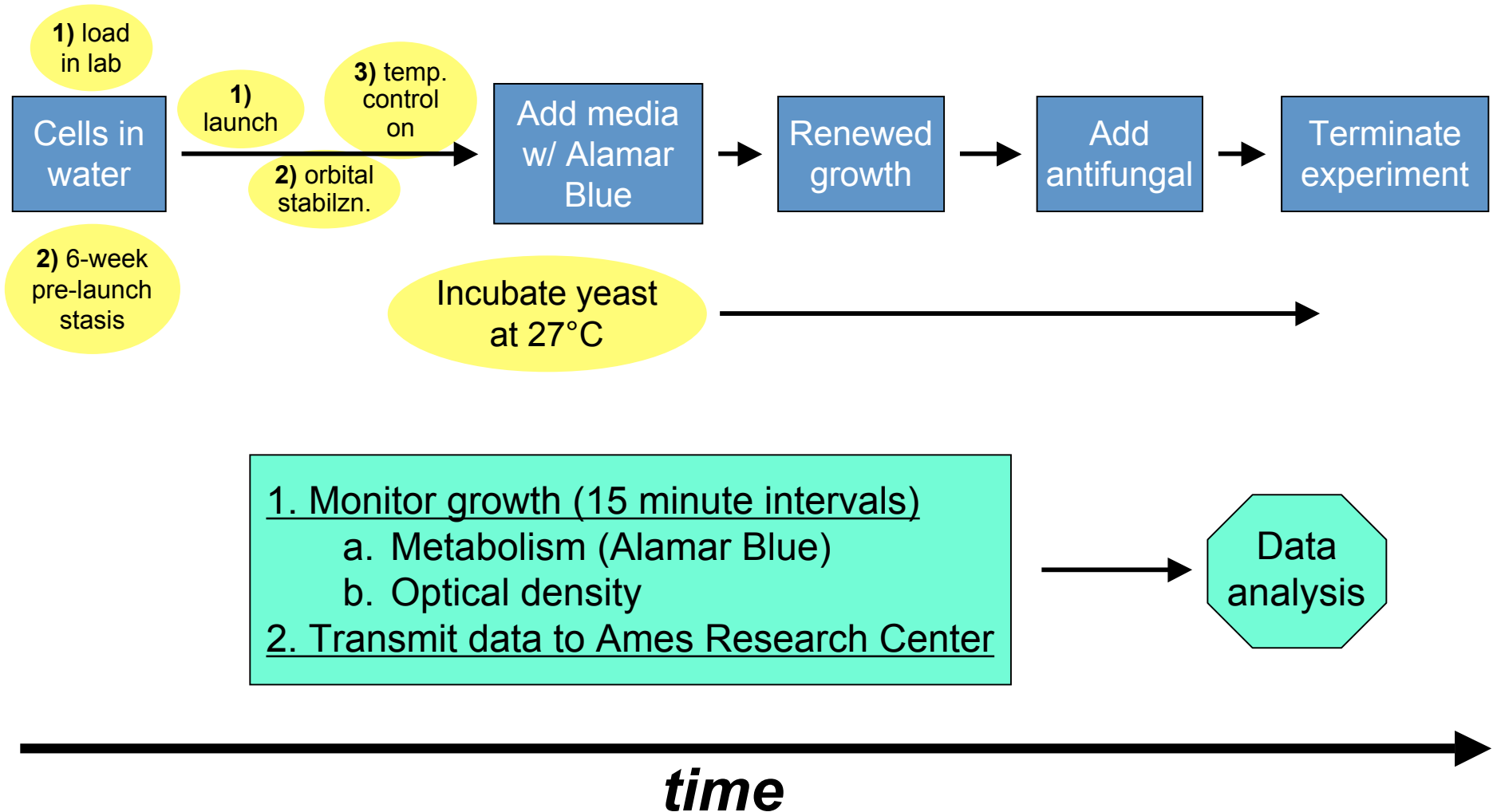


PharmaSat

- PI driven experiment:
Effect of Microgravity upon Yeast Susceptibility to Antifungal Drugs for Countermeasure Development
Principal Investigator: Michael R. McGinnis, Ph.D.
University of Texas Medical Branch (UTMB)
- Grow yeast cells in fluidics card
 - Use one antifungal agent
 - 3 different concentrations of antifungal
 - Control
- Measure cell health two different ways
 - Absorbance (OD)
 - Alamar Blue reduction
 - Colorimetric assay: blue-oxidized, pink-reduced
 - Normal metabolic products of living cells will reduce Alamar Blue



PharmaSat Experiment

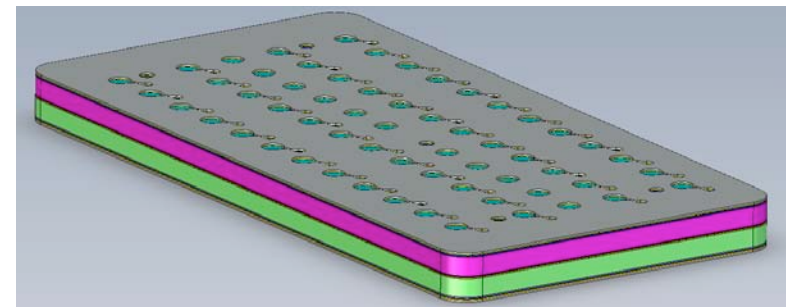
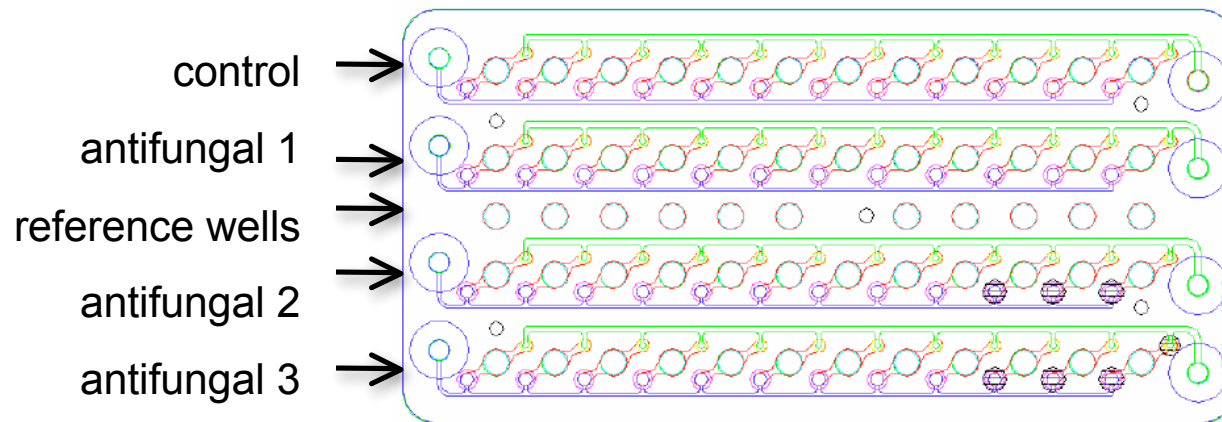




PharmaSat Technology

GeneSat technology as starting point

- Larger n - from 12 wells to **60 wells** in ~ same fluidics card footprint
 - 4 independent sets of 12 wells
 - 10 - 12 reference wells

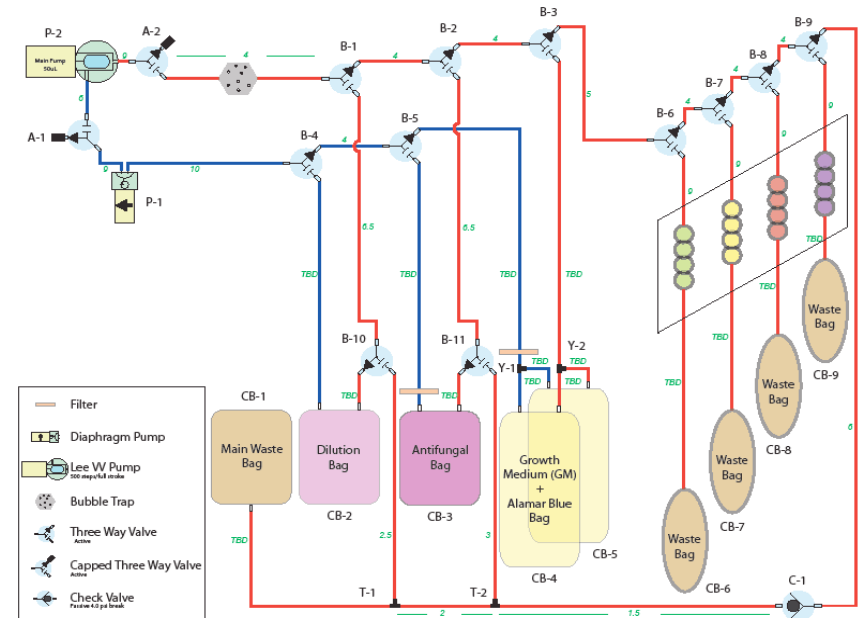
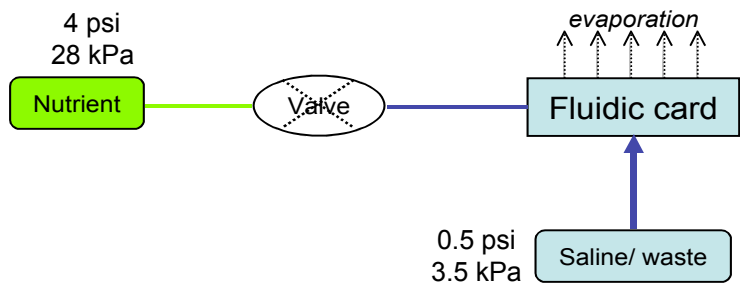




PharmaSat Technology - 2

GeneSat technology as starting point

- Greater fluidic processing **complexity**
- System must allow for two fluid exchanges
 - Feed cells and allow them to recover from stasis
 - Add fresh media with 3 conc. of antifungal (or none for control)
- Dilutions of antifungal required
- Circulation of liquids for dilutions and anti-freeze purposes required
- Many more pumps, valves, and connections

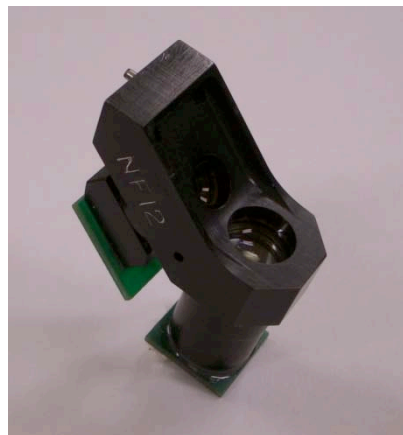




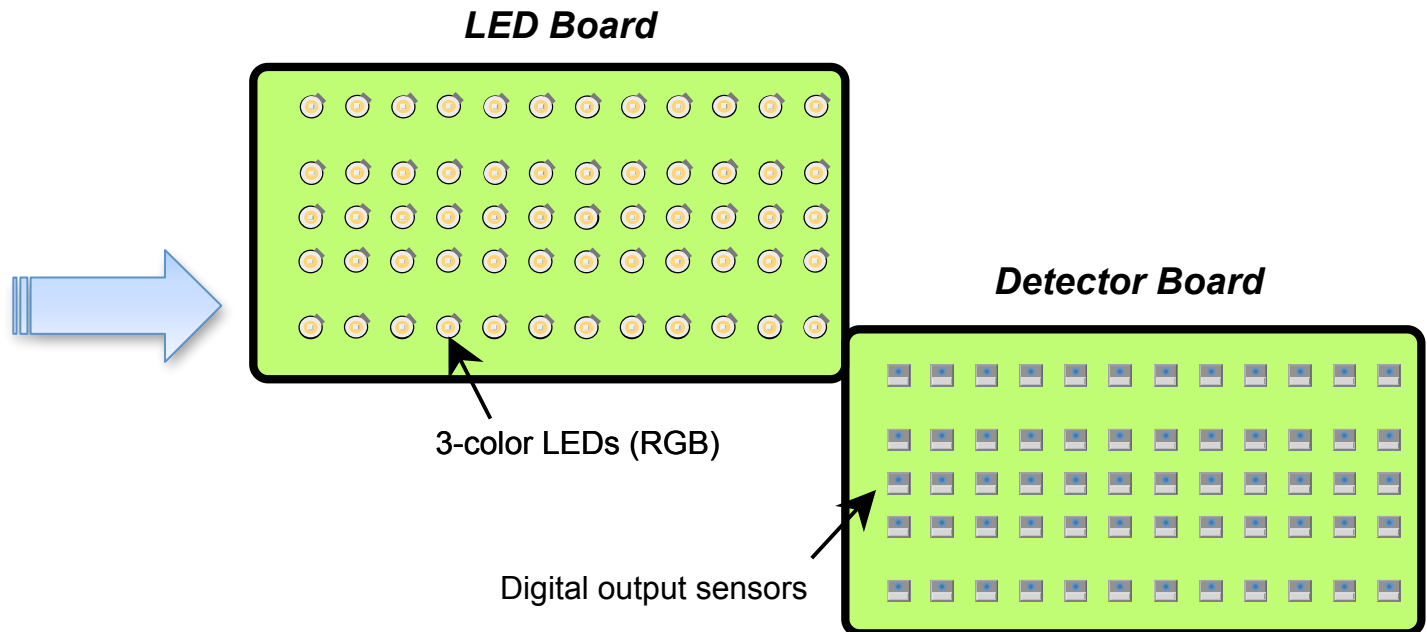
PharmaSat Technology - 3

GeneSat technology as starting point

- **3 color** optics: 1 for OD, 2 for viability assay (*GeneSat: fluorescence and scatter*)
 - One, 3-color LED (*GeneSat was 2 separate 1-color LEDs*)
 - True optical density (OD): absorbance
 - While each optics “unit” is simpler, the larger number of wells requires more units that are more closely spaced: denser LED & detector layouts

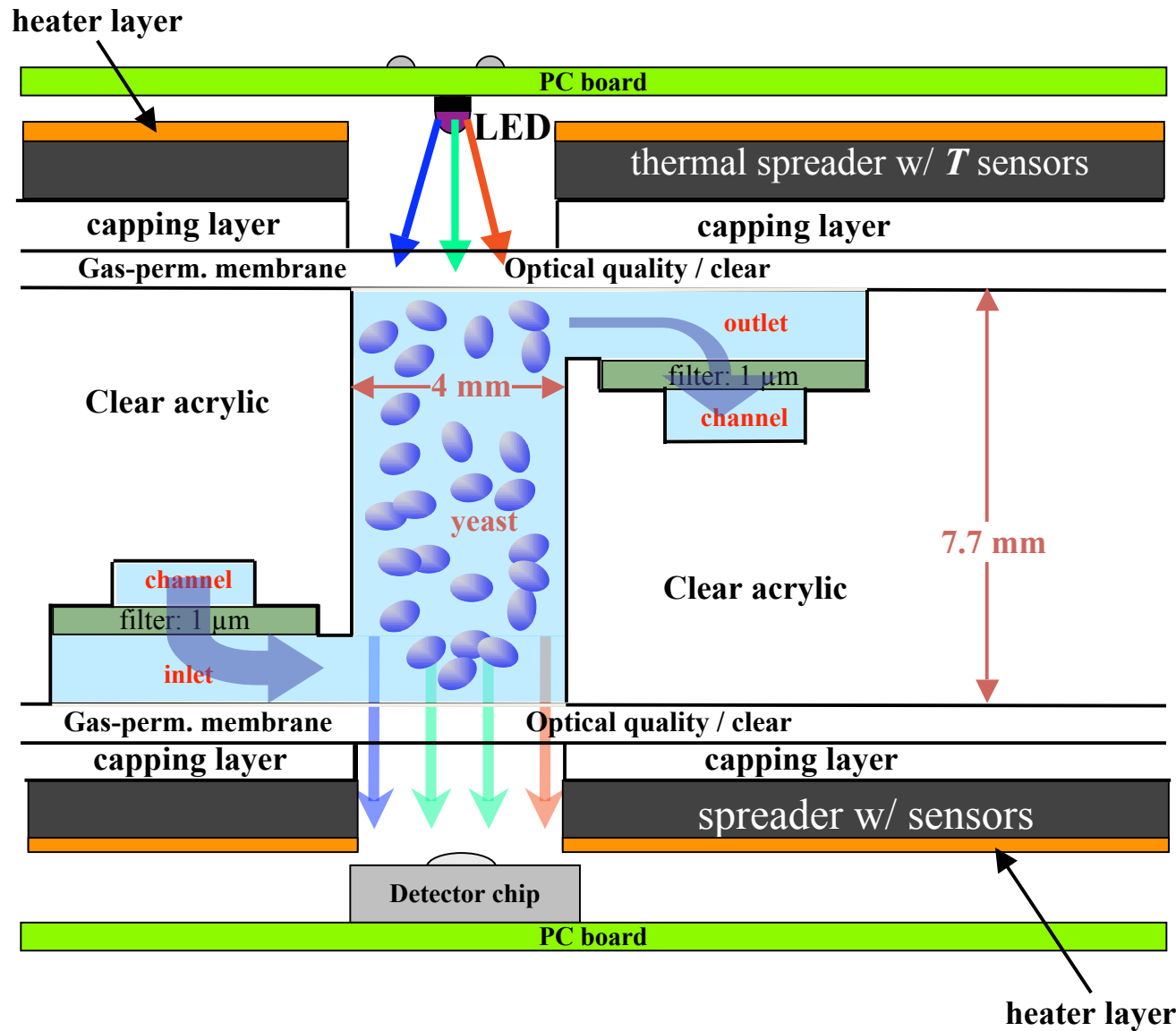


X 12





PharmaSat Card and Optics



3-color LED for OD and viability: track population during growth; viability using color-change reagent

*Fluidic/
optical/
thermal
cross-section*

Detector for OD and viability measurement using 3-color absorbance



What's next?



Accommodate higher complexity

- Higher sample number
10 → 48 → 96 → 384?
- Larger variety organisms
 - More complex model organisms (*C. elegans*, *Drosophila*, etc.)
 - Mammalian cells
- Greater variety of measurements
 - Luminescence
 - Imaging fluorescence
- Increasingly complex analytical technologies
 - Cytometry
 - Microarrays



Acknowledgements



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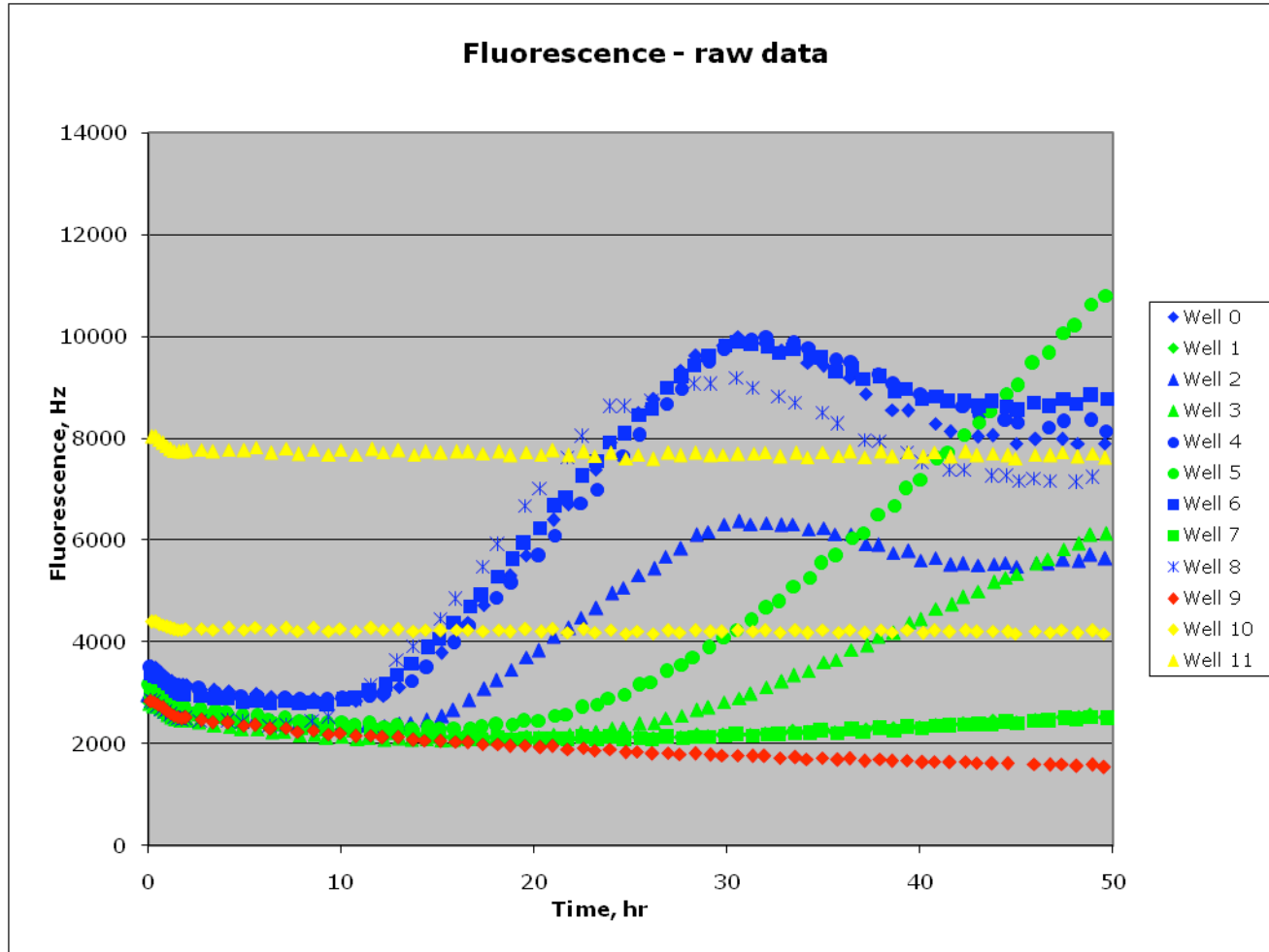
Wenschel Lan
Simon Lee
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Ignacio Mas
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Mike Miller
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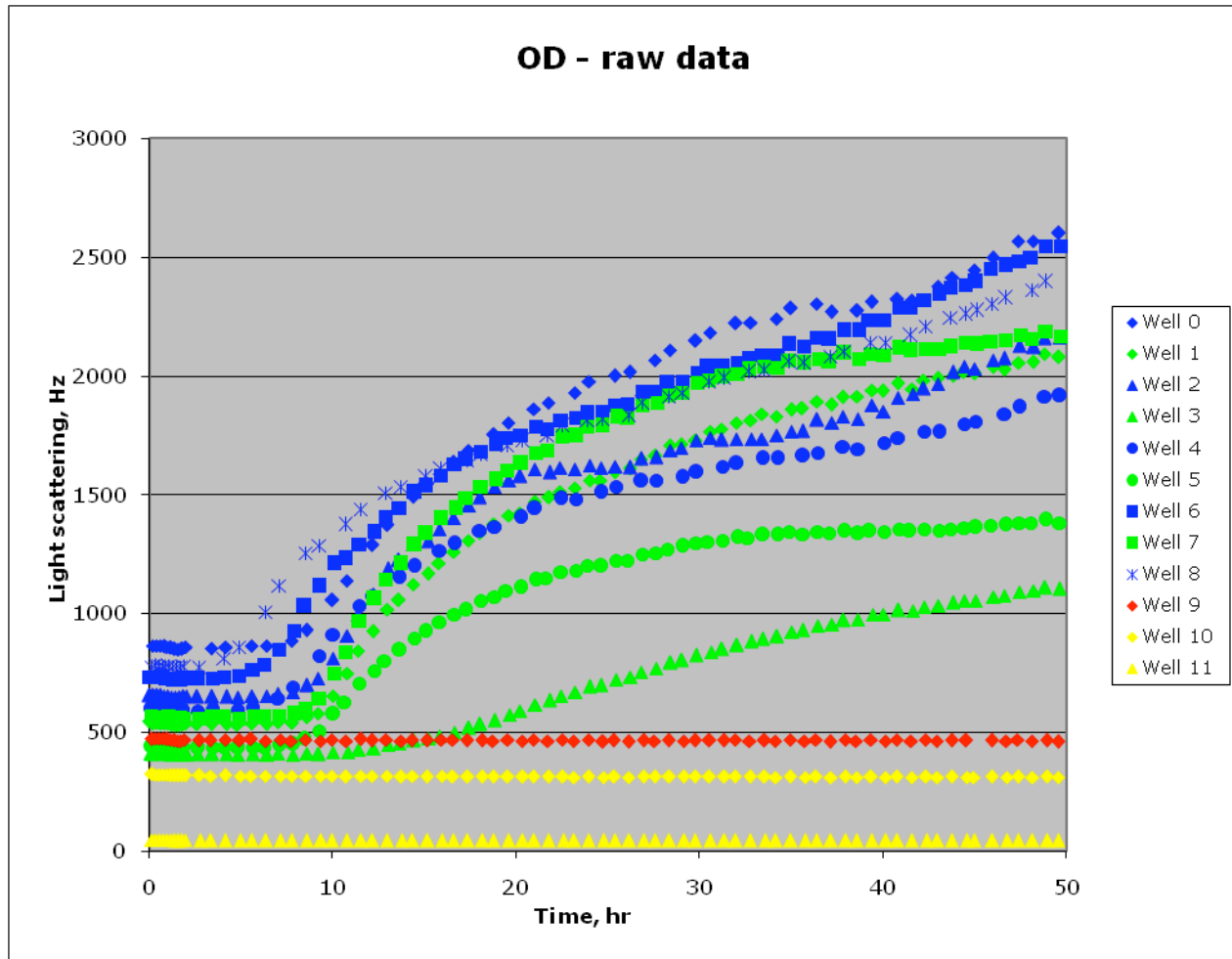
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Phelps Williams
Ken Zander





BACKUP SLIDES







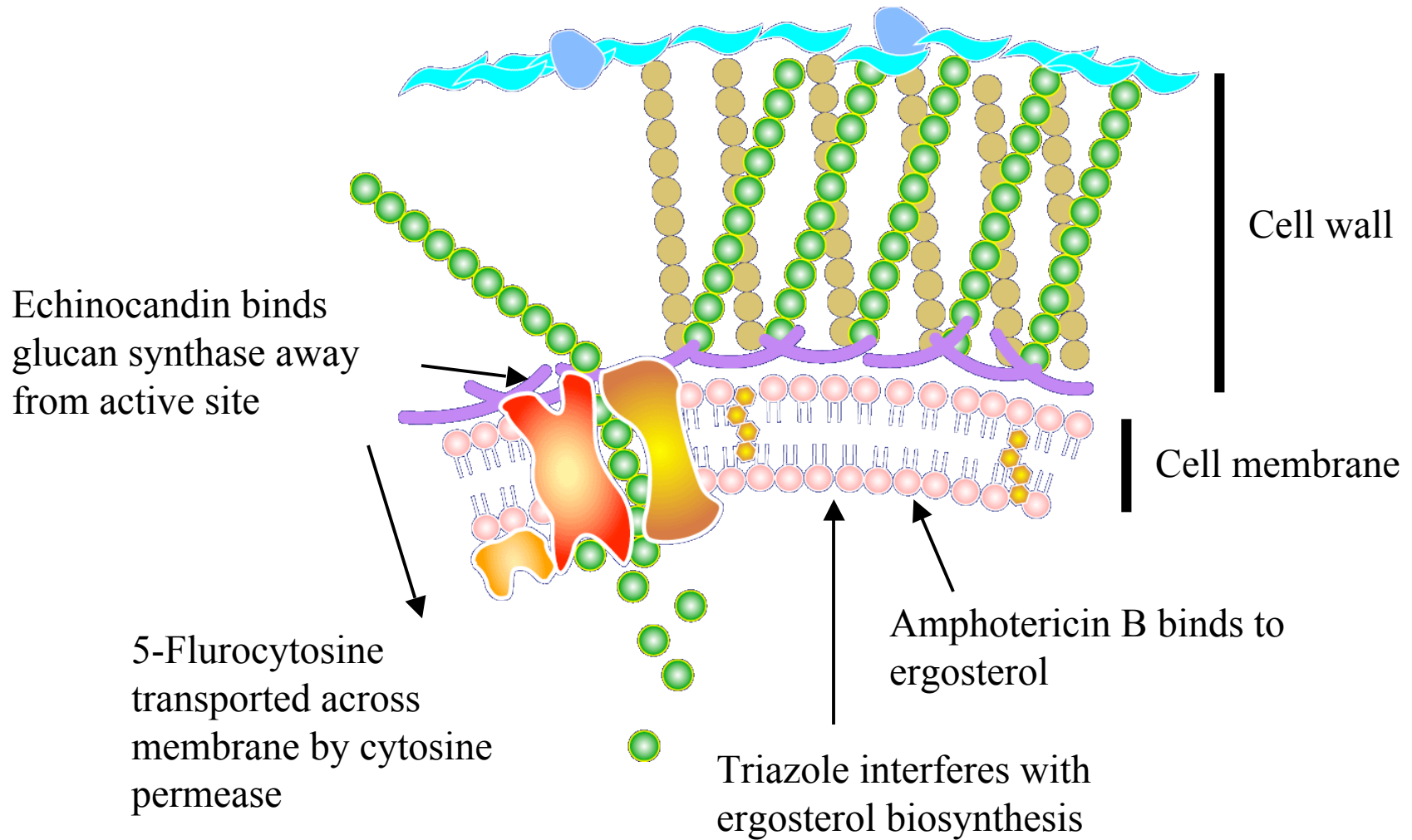
Hypothesis:

“Yeasts growing in microgravity have an altered resistance to azole antifungal agents”.

1. Measure the influence of microgravity upon yeast resistance to the azole antifungal agent voriconazole.
2. Microgravity and modeled microgravity data suggest that resistance to azoles is increased.
3. Experimental design is based upon an internationally recognized *in vitro* laboratory testing method.
4. Statistical analysis of the data will be based upon the methods used to analyze longitudinal data. This analysis methodology is more robust than the traditional end-point- based analysis.
5. Important implications for the prevention and management of fungal infections that may occur during space exploration.



Fungal Wall and Cell Membrane





Standardized Susceptibility Testing Method

- Determine medium for experiment.
- Determine voriconazole drug concentrations for experiment based upon MIC_{90} of $0.5\mu\text{g/ml}$.
- Determine inoculum concentration for experiment ($0.5-2.5 \times 10^3 \text{ CFU/mL}$).