Electrochemical Sensor Centrifuge Platform for Single-cell Study

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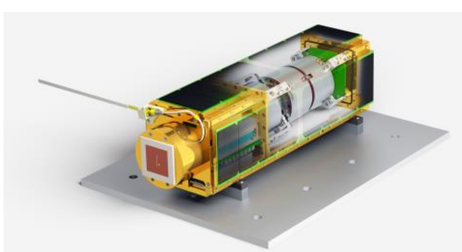
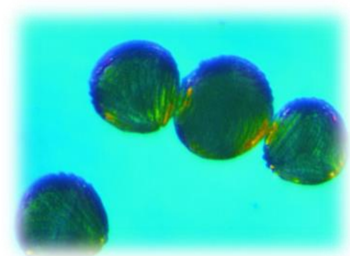




Motivation: It starts out with Space Biology

- Gravity has remained constant, continuously influencing the evolution of all organisms. Gravity impacts many aspects of the biophysical transport and exchange necessary for assimilation and metabolism in membrane-enclosed biochemical systems.
- Our understanding of response of microorganism available to a space environment is at its primitive level, especially those of photosynthetic species.
- Photosynthetic microorganism offer great promise to technologies critical for space explorations.
- **There is a need to understand the changes in their biophysical mechanism when exposed to space environment, such as altered gravity.**

Biological model system

Microalgae
Chlorella vulgaris

Photosynthetic activity:
– H^+,CO_3^{2-}

Fern spore
Ceratopteris richardii

Artificial gravity effect on onset of polarity development of fern spore *C. Richardii*
– Ca^{2+}

The Technology: Electrochemical Biosensors

- Electrochemical sensors are based upon potentiometric, amperometric, or conductivity measurements.
- Electrochemical biosensors provide an attractive means to analyze the content of a biological sample due to the direct conversion of a biological event to an electronic signal.

Samples:

- Cell culture
- Human samples (blood, urine, saliva, tissue)
- Food samples
- Environmental samples (air, water, soil, vegetation)

Transducer layer

Bioreceptor(s)

- Nucleic acid
- Cells
- Antibodies
- Enzymes

Electrical interface(s)

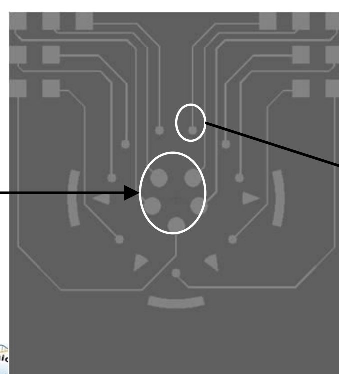
- FET devices
- Nanowire
- Nanoparticles
- Electrodes

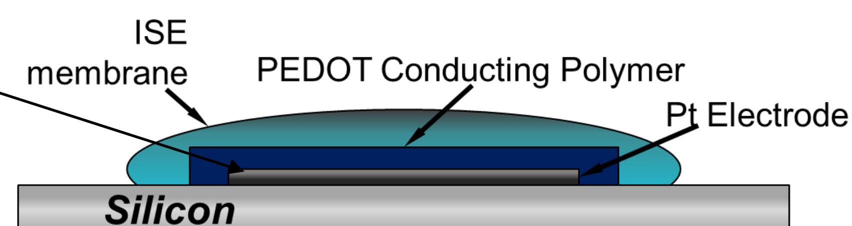
Electronic System

Signal amplifier

Signal processor

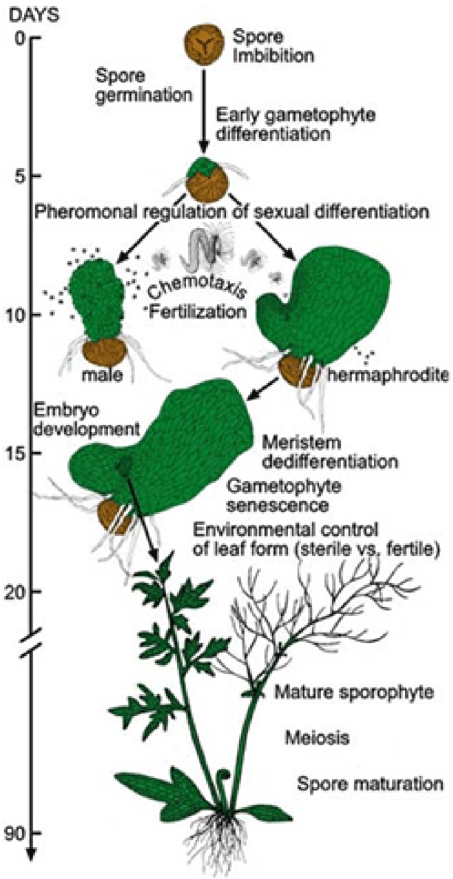
Display





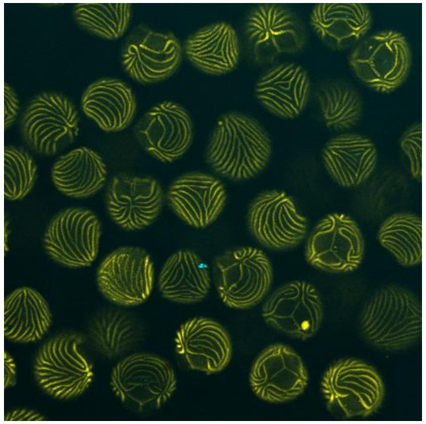
Single Cell: Spores of *Ceratopteris richardii*

Biologists have studied for decades, through hypothesis and experiment, how certain plants and their spores sense and respond to gravity.



Spores of the fern *Ceratopteris richardii* are among the most studied.

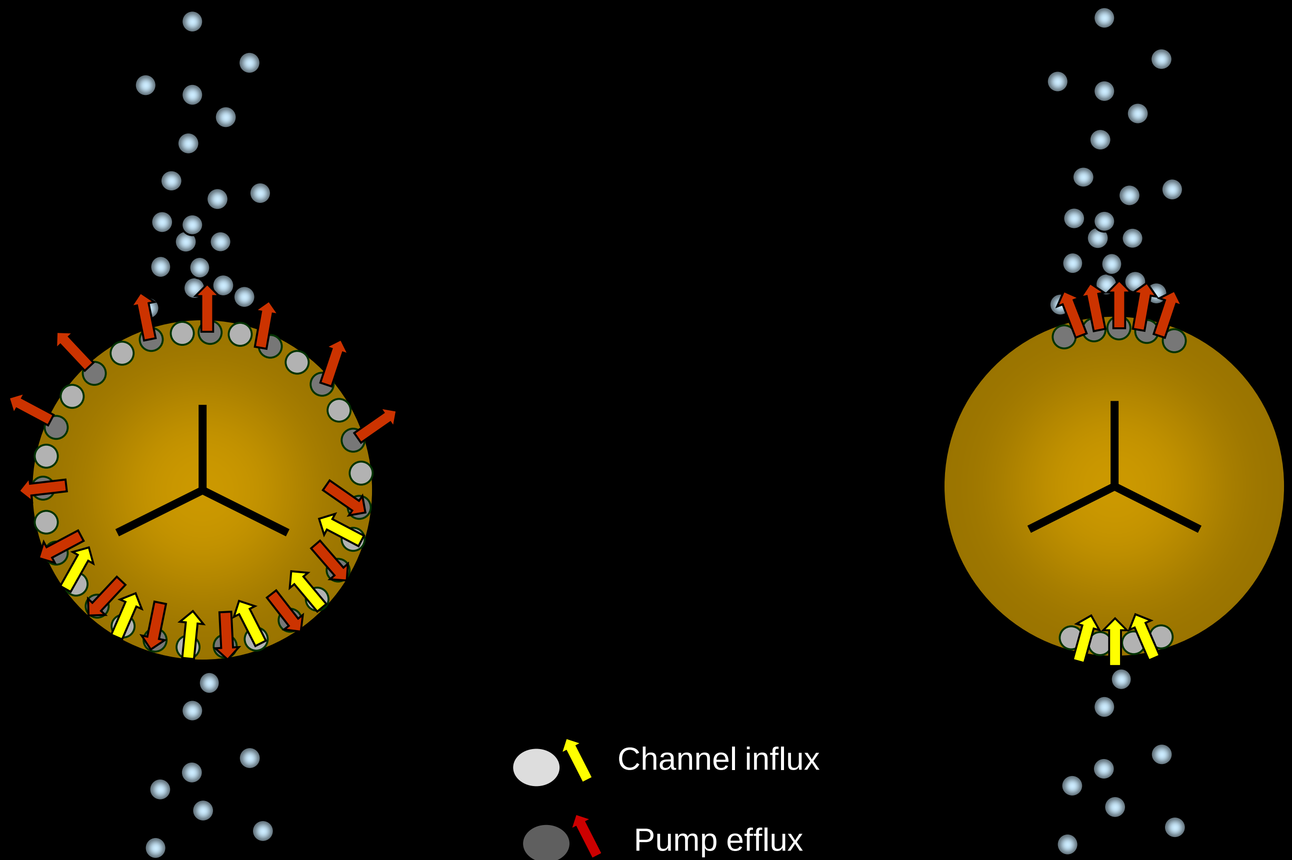
But they have not yet been studied in the microgravity environment of outer space, nor across a range of gravity levels lower than Earth's -- like gravity fields equivalent to the Moon or Mars.



How fast does it sense?

Localized Activation

Membrane Redistribution



Near instantaneous

Requires time

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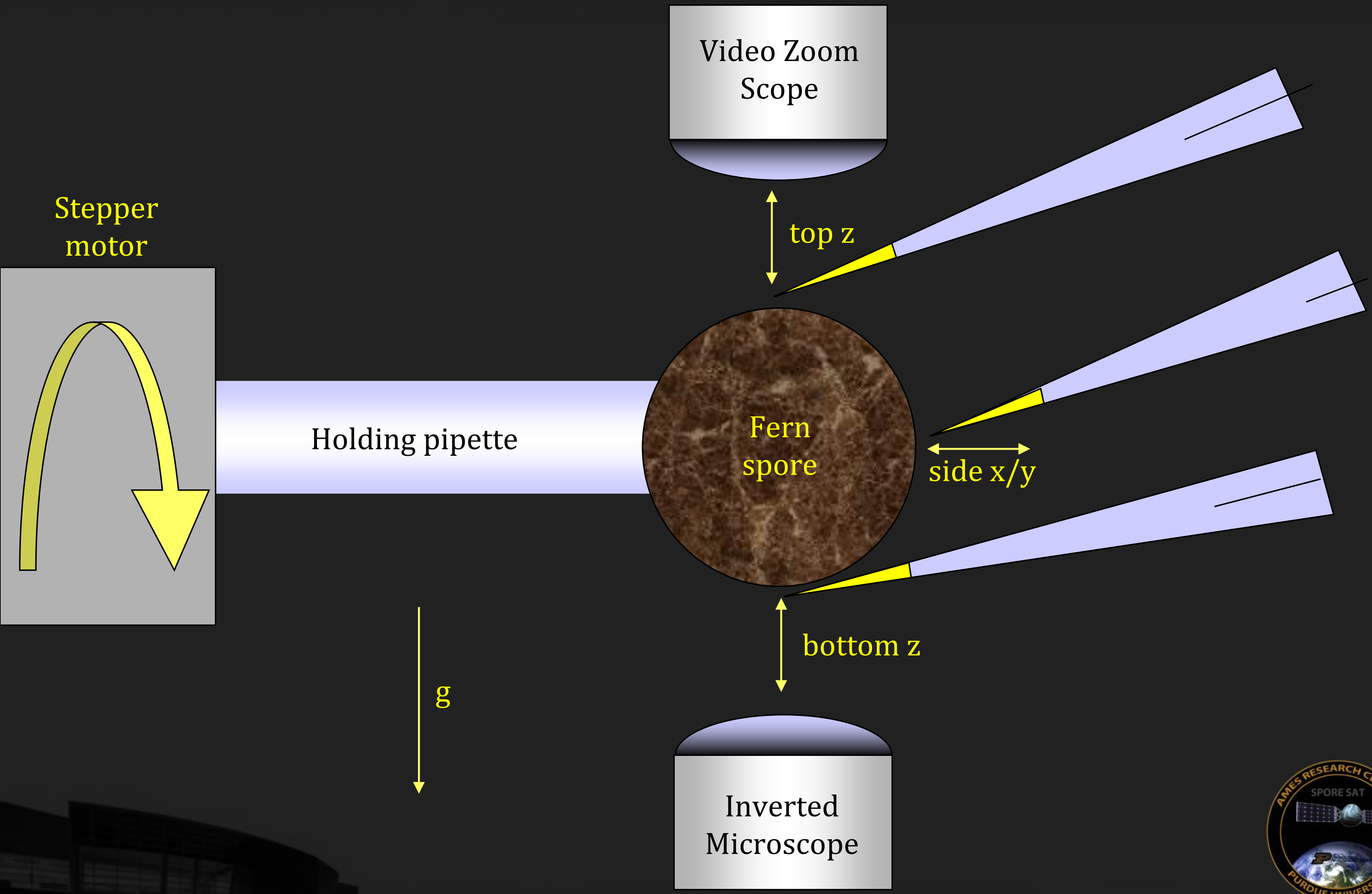
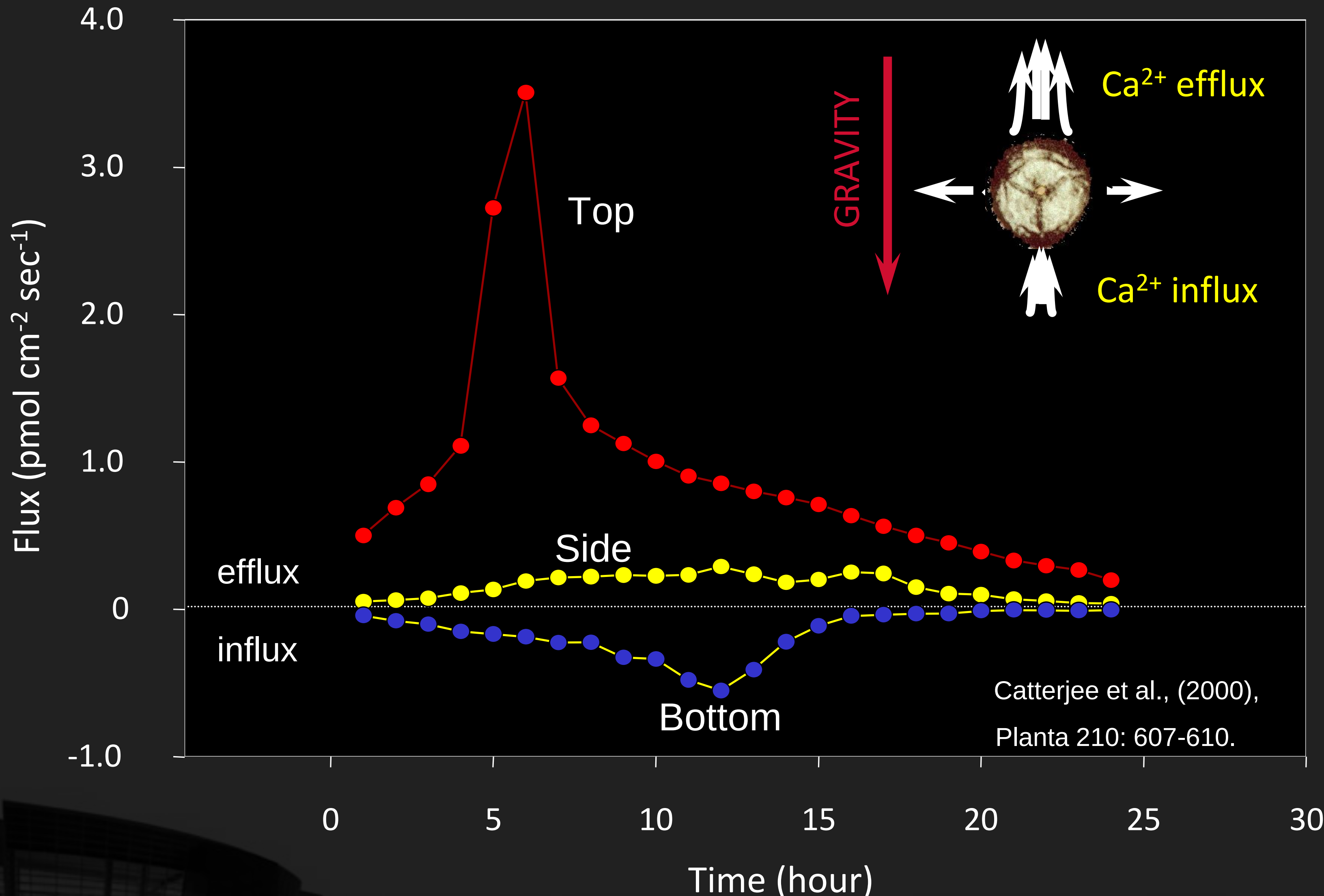
Salmi et al, Planta, 2011)

Self-referencing probes



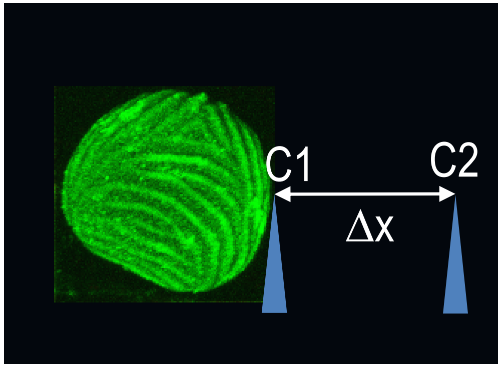
Salmi et al, Planta, 2011)

Gravitational Polarity and Ca^{2+} Flux in *C. richardii*

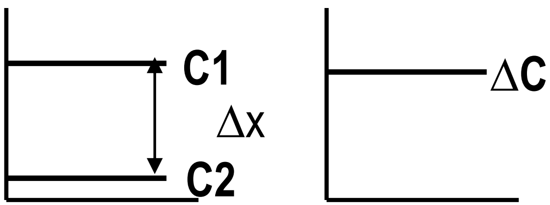


Salmi et al, Planta, 2011)

Self-Referencing



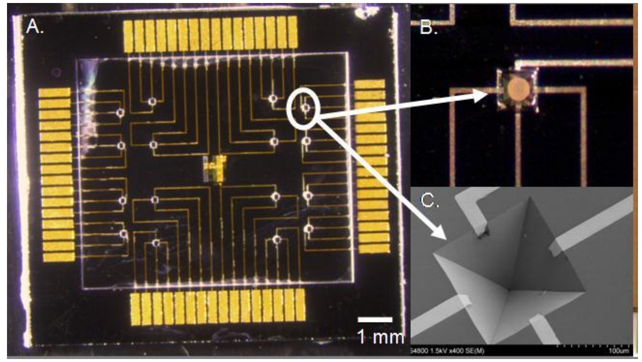
Chatterjee, 2000



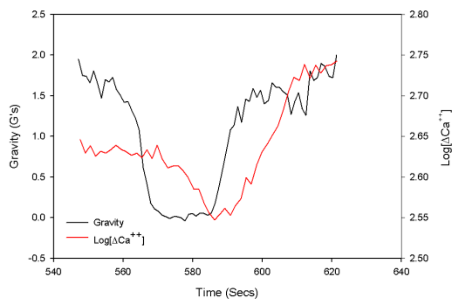
Fick's Law of Diffusion:

$$J = - D = \frac{\Delta C}{\Delta X}$$

CEL-C Biochip

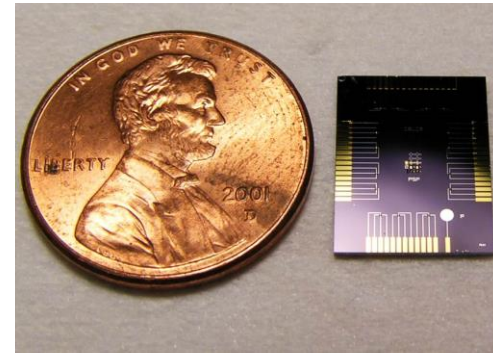


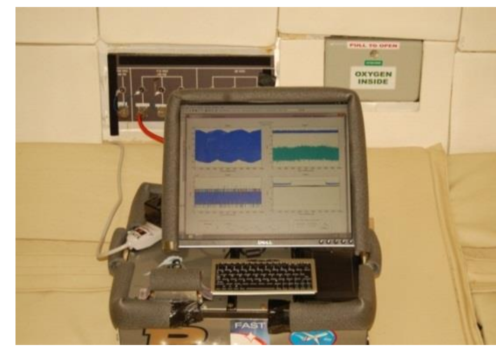
ul Haque et al., 2007, 2008



External reference electrodes
Microgravity experiment in NASA C9

CEL-C2 Biochip





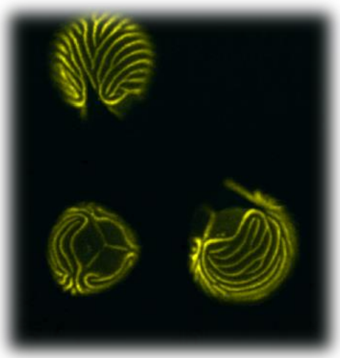
Integrated reference electrodes
Working electrodes with PEDOT

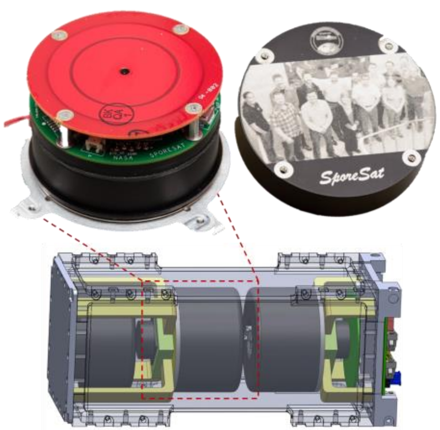
Wan W. Amani Wan Salim et. al (in review)

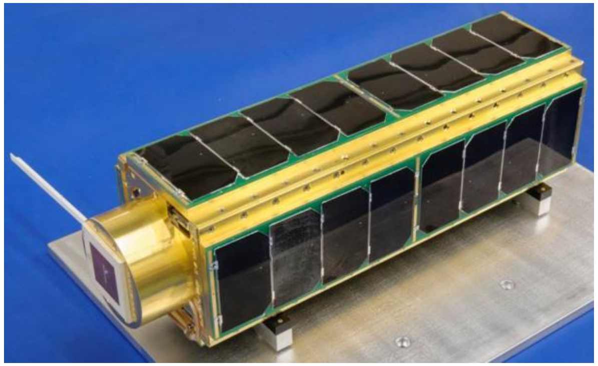
Project Overview

SporeSat is

- a fundamental space biology science space mission to investigate biophysical mechanisms of plant gravity sensing.
  - ➢ for studying unicellular germinating *Ceratopteris richardii* fern spores in outer space.
- a "lab-on-a-chip" centrifuge platform integrated as the payload of a small (5.5 kg), free-flying satellite.
- funded as a "Small Complete Mission" on a "nanosatellite" (< 10 kg) by NASA's Human Exploration and Operations Mission Directorate.







SporeSat Objectives

Science Objective: Determine gravity thresholds for calcium ion (Ca^{2+}) channel activation, which is a known component of the gravity sensing response in wild-type fern spores.

Technical Objectives:

- Provide a range of levels of less-than-terrestrial gravity to fern spores.
- Provide illumination to initiate spore germination.
- Measure local Ca^{2+} concentrations from germinating spores.
- Provide an environment conducive to spore stasis, then germination on command.

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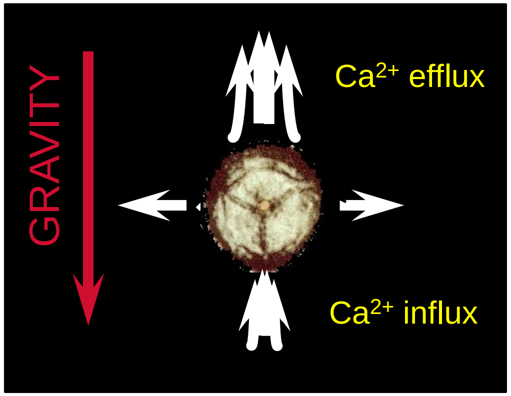
SporeSat Relevance

- Use of plants for food and life-support functions on future long-duration space, lunar, or planetary missions
- Ion channels are critical to the functioning of biological organisms, including humans.
- Ion channels are key components of the nervous system as well as cardiac, skeletal, and smooth muscle function, transport of nutrients and ions, T-cell activation, and pancreatic beta-cell insulin release.
- *Ion channels are often the target of the search for new drugs.*

Single cell (spore) from the fern *Ceratopteris richardii*

- Spore strain: RN5, wild type; cell polarity due to transcelluar Ca^{2+} current is controlled by gravity (not light)
- Single cell (Ø = 90-146 μm)
- Short life cycle (120 days)
- Germination initiated by red light
- Results from previous parabolic flight (NASA C-9B)
  - Transcellular Ca^{2+} flux tracks the rapid changes in gravity (μ-*g* to 2 *g*)
  - Hyper-*g* increases Ca^{2+} flow across spores and polarizes the spore
  - Hyper-*g* likely to open more stretch-activated channels
  - Micro-*g* depolarizes the spore: gravity-induced Ca^{2+} signal disappears



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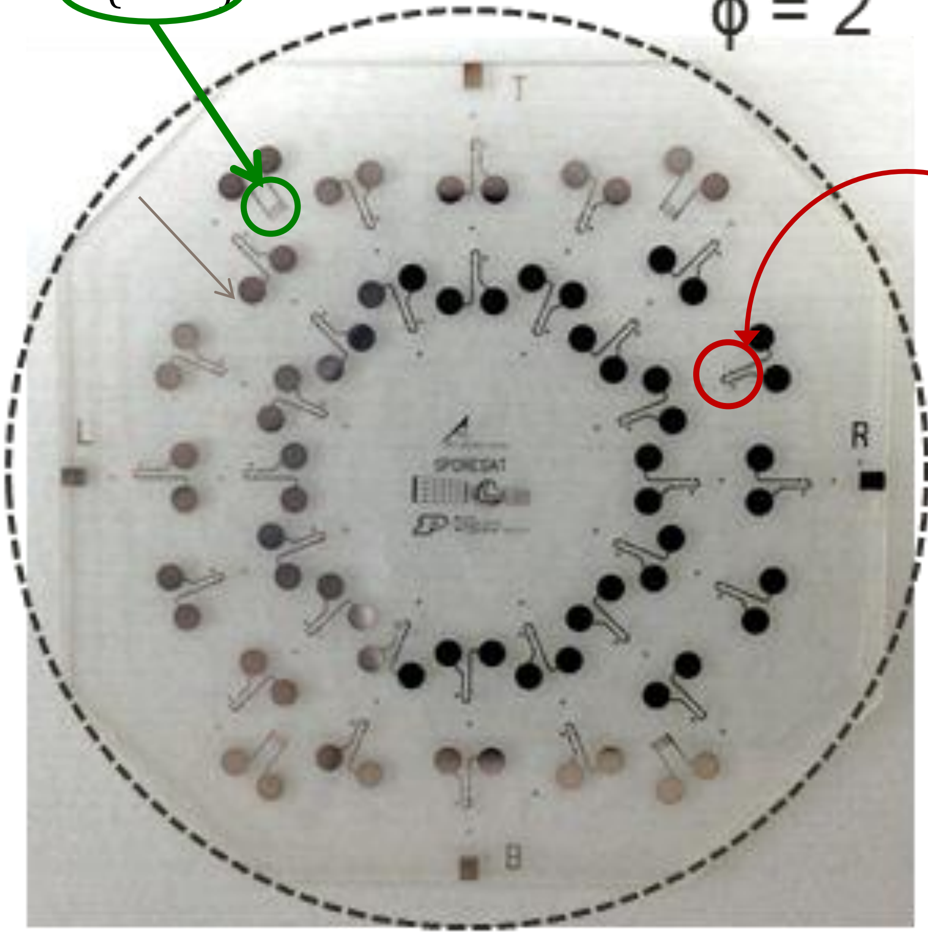
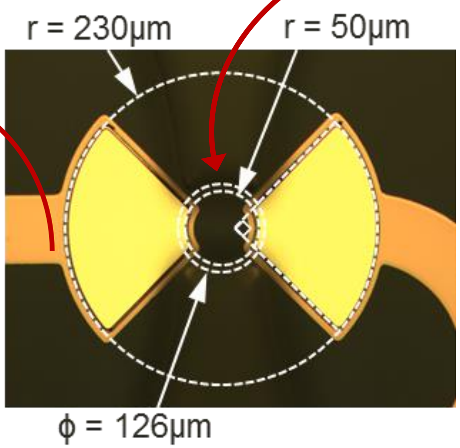
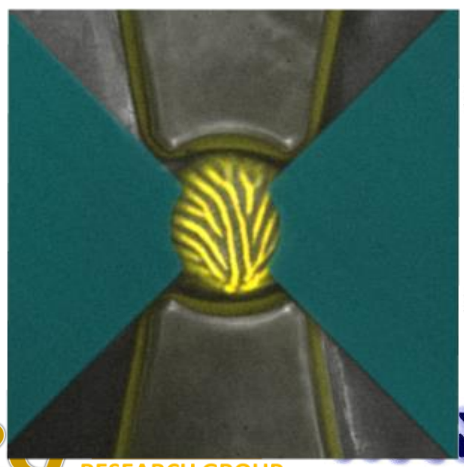
Biology Compact Disc (bioCD)

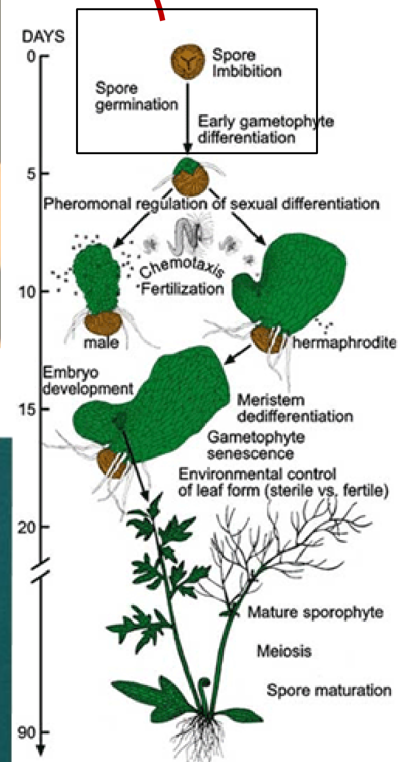
32x single-spore wells, each with differential measurement pair of electrodes: Pt/Ag-AgCl/Ca^{2+} ion selective membrane (ISM)

4x Pt Resistance Temperature Detectors (RTDs)









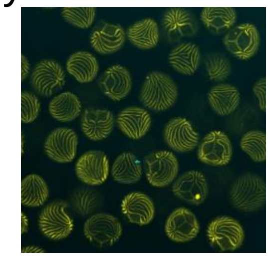
Static SU8 “pincer” holds each spore in place

Experimental Steps

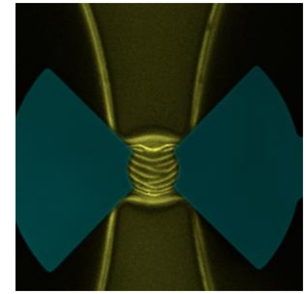
Measurements are conducted for ~ 20 hours after germination

1) Filter dry spores; select Ø = 126 µm

2) Presoak spores for 6 days in DI water (synchronization)

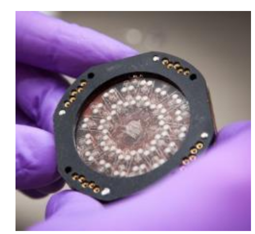


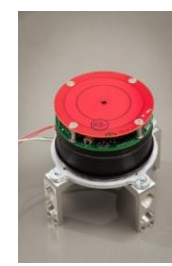
3) Load 1 spore in each of 32 bioCD wells



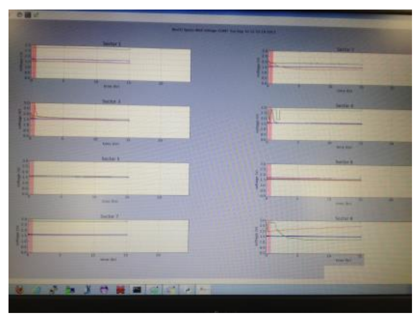
Secured by SU8 pincer + agar gel

4) Assemble bioCD Enclosed and Rotating Assembly

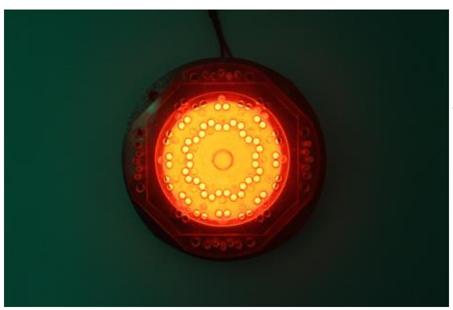




5) Initiate measurements to get a signal baseline



6) Initiate spore germination by RED light



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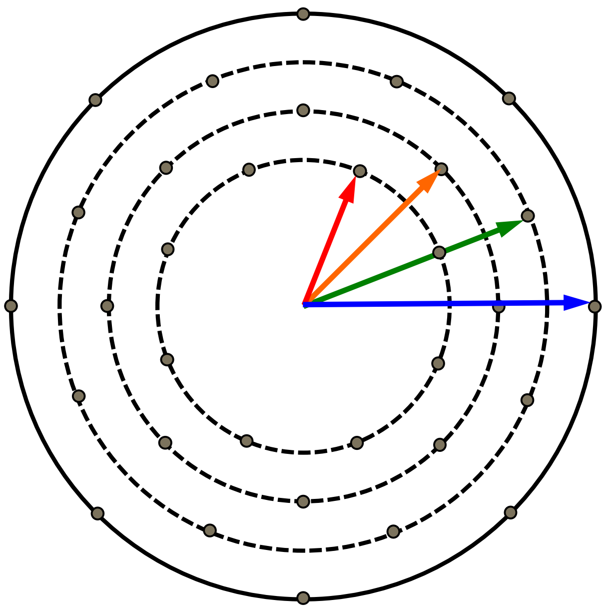
Centrifugal Gravity on bioCD

$$g = \frac{R}{9810}\left(\frac{\pi}{30}w\right)^2$$

- g: decimal fraction of Earth gravity
- R: radius from rotation axis (mm)
- ω: rotating speed (rpm)

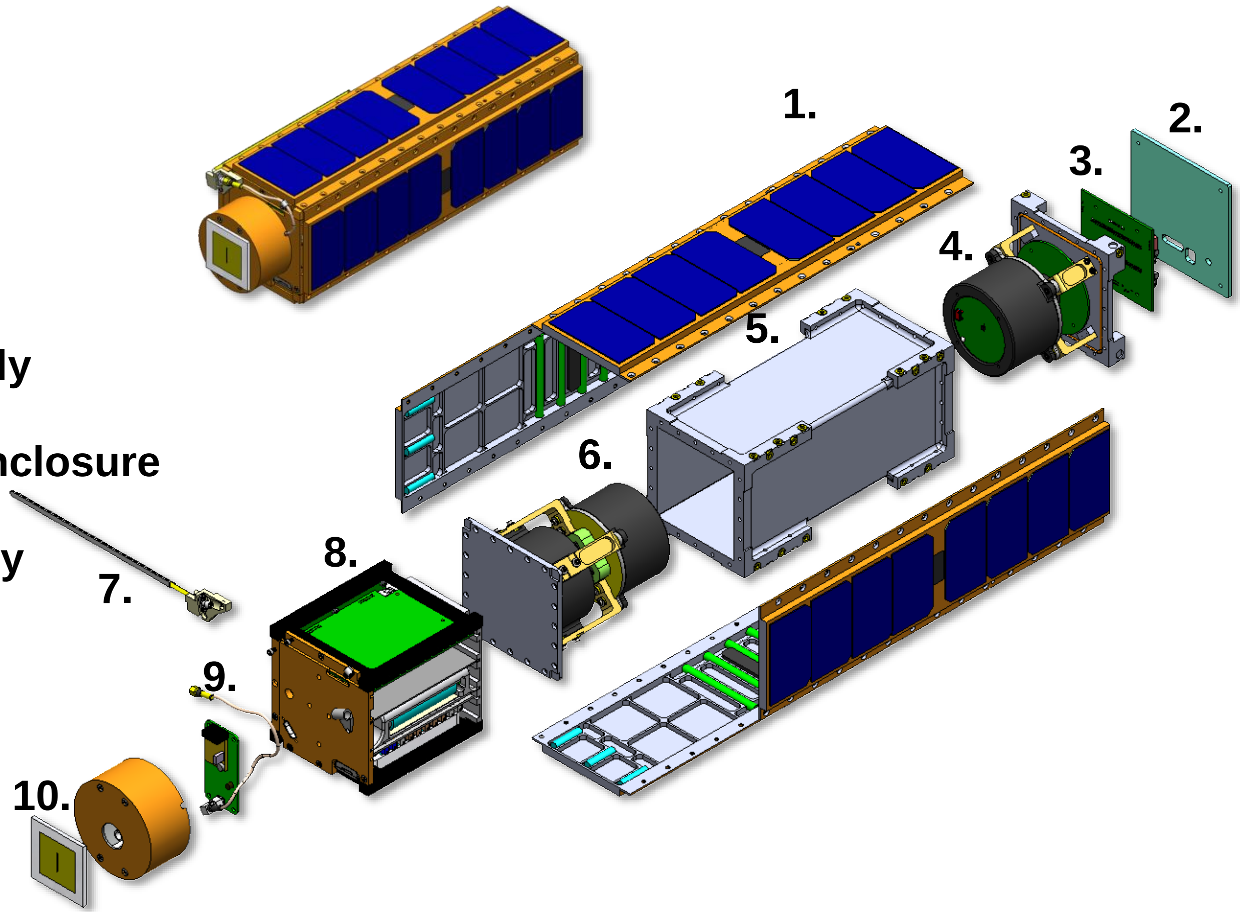


32 spores - 8 spores at each radius

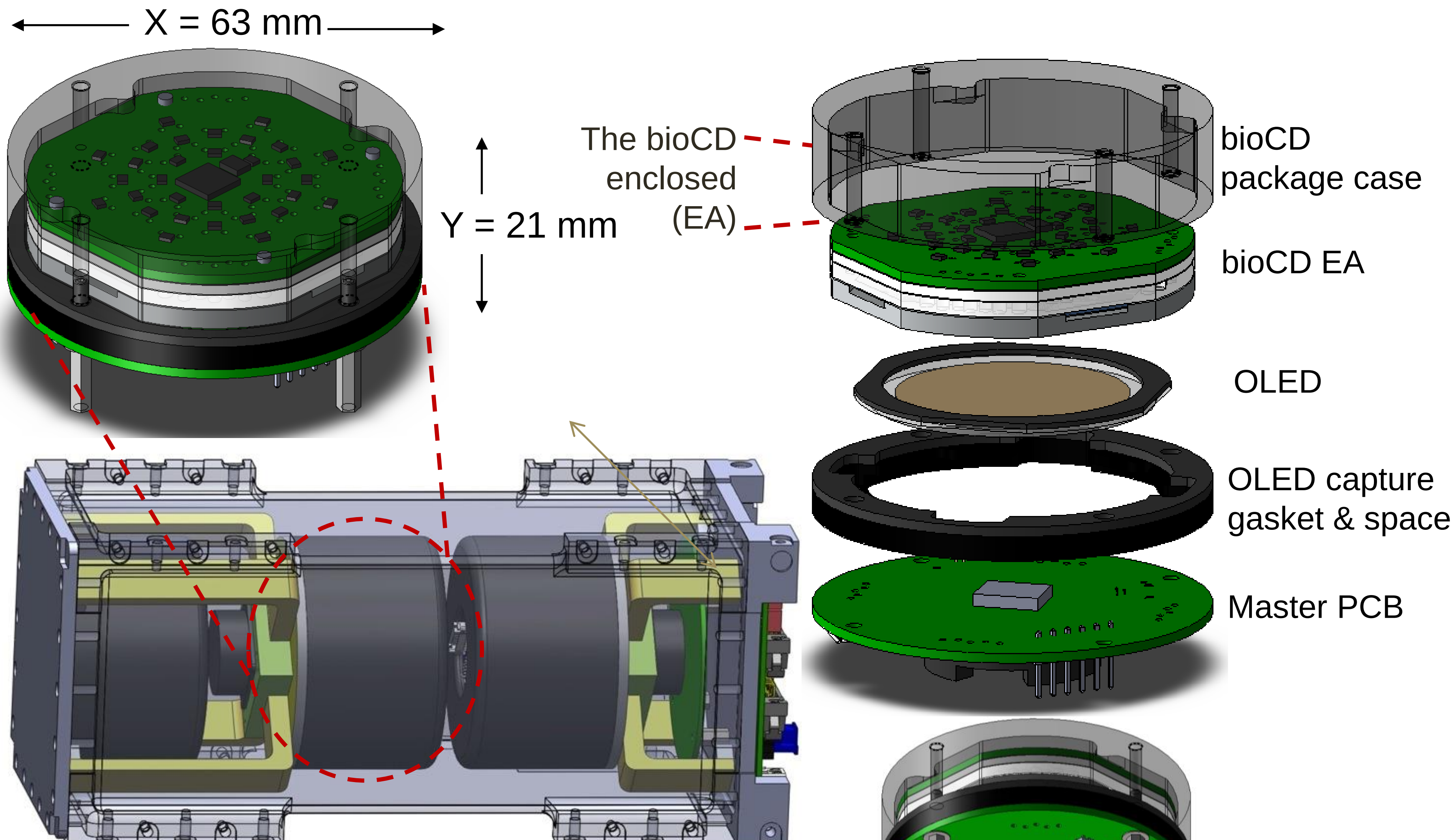
Rotation rate		Gravity at R (mm)			
(rpm)	(Hz)	R_1=9	R_2=12	R_3=15	R_4=18
80	1.38	0.06 x g	0.09 x g	0.11 x g	0.13 x g
112	1.87	0.13 x g	0.17 x g	0.21 x g	0.25 x g
158	2.63	0.25 x g	0.34 x g	0.42 x g	0.50 x g
223	3.72	0.50 x g	0.67 x g	0.83 x g	1.00 x g
315	5.25	1.00 x g	1.33 x g	1.66 x g	2.00 x g

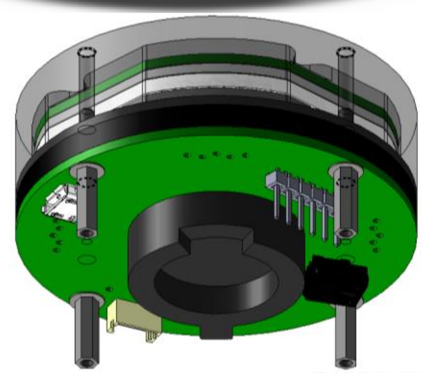
Exploded View: Spacecraft including Payload

1. Solar Panels (4 ea.)
2. Close-out Panel
3. Bus Interface PCB
4. **Front Panel Assembly**
5. **Hermetic Payload Enclosure**
6. **Back Panel Assembly**
7. Transponder Antenna
8. Avionics Bus
9. Transponder PCB
10. Patch Antenna (2.4 GHz)



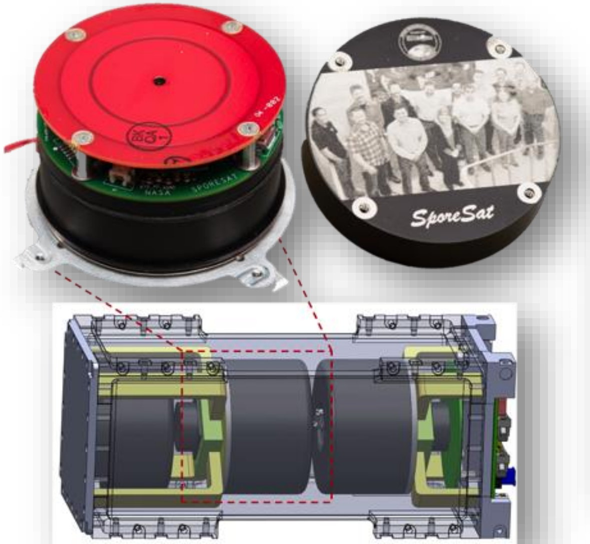
bioCD Design & Fabrication

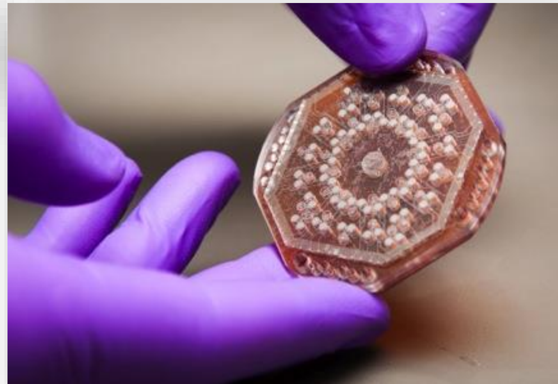




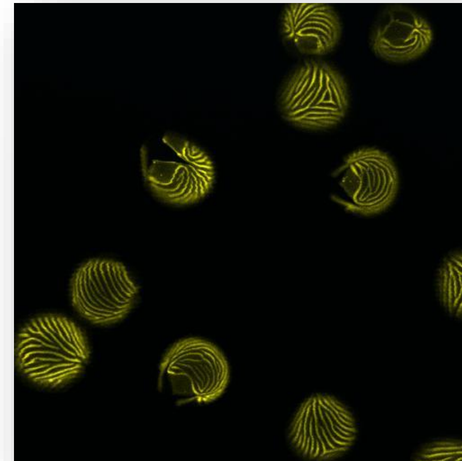
Two rotating & one stationary bioCD are within the hermetic container

Research Components

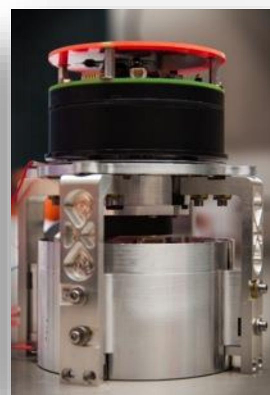




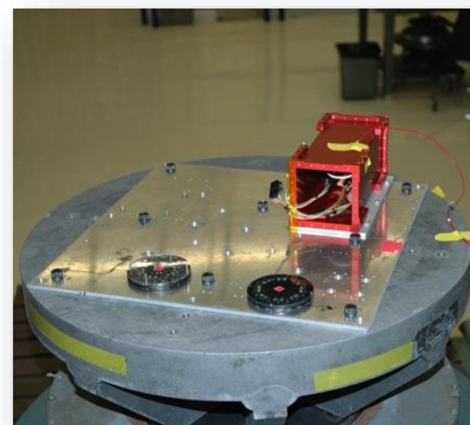
bioCD design and fabrication



Spore germination

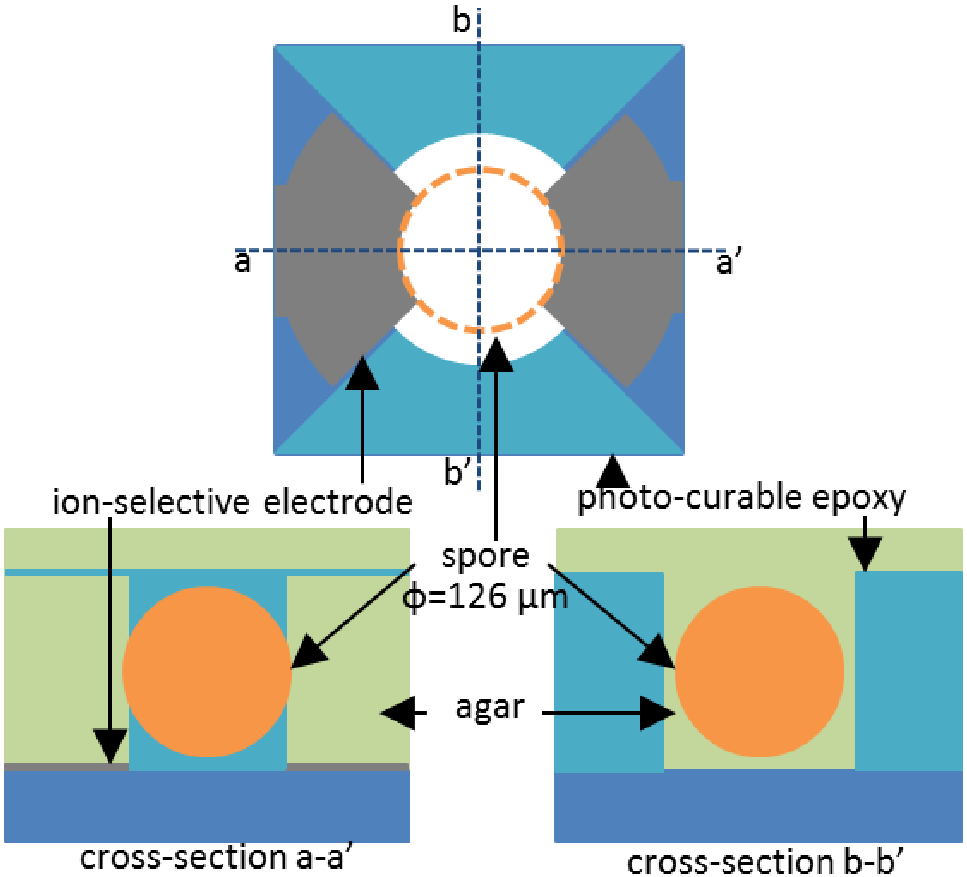


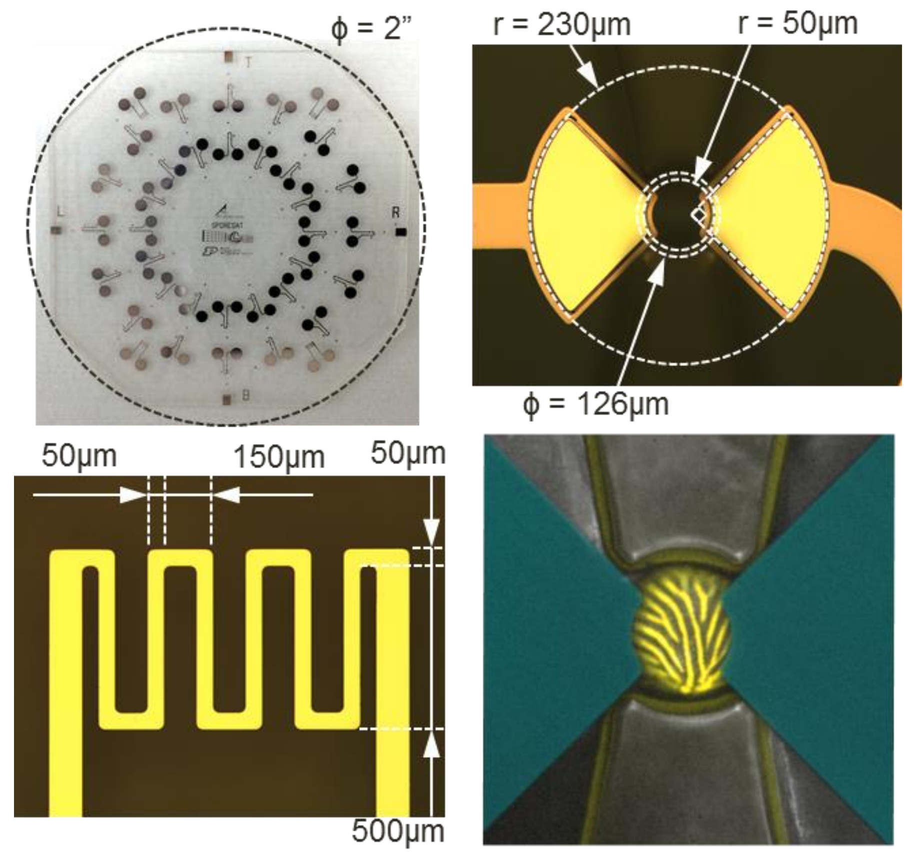
Ground experiments



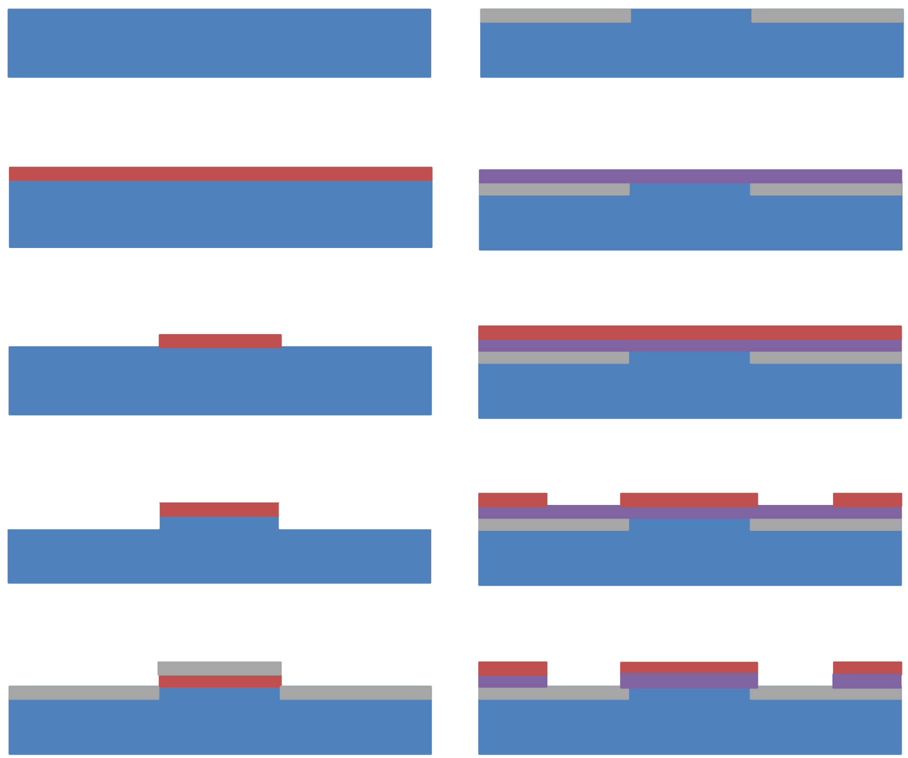
Risk analysis: biocompatibility, dislodge

bioCD Design & Fabrication





bioCD Fabrication Steps



Fused silica wafer (t=1 mm)

Photoresist spin coating

Photolithography for Ti-Pt lift-off (Mask 1)

BOE wet etching for Ti-Pt lift-off

Ti-Pt deposition (30-150 nm)

Lift-off

PECVD SiO_2 deposition (500nm)

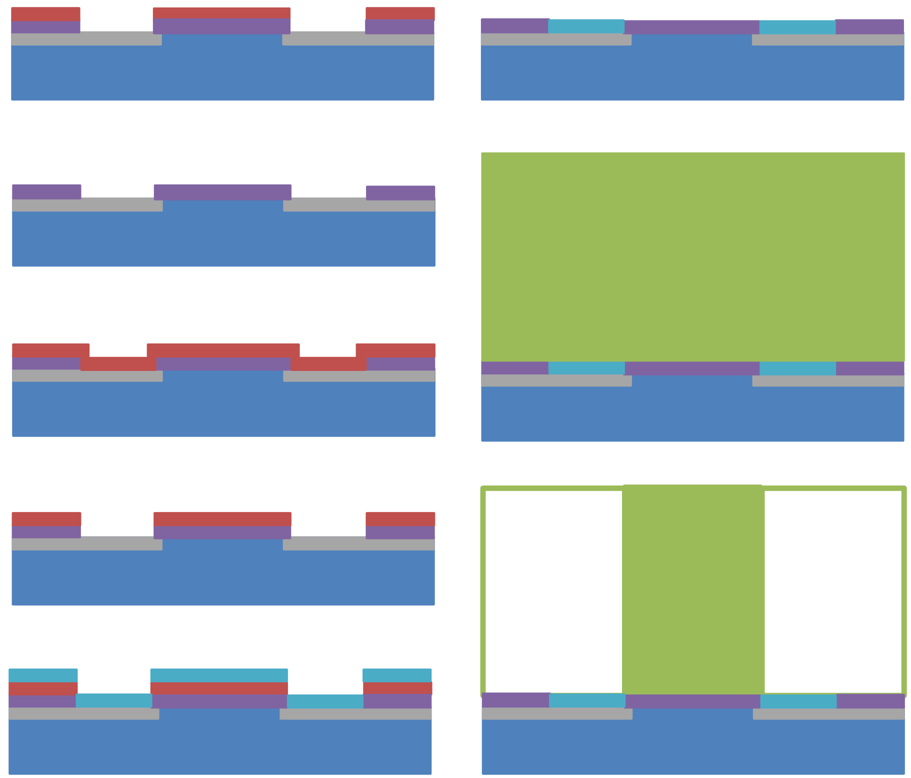
Photoresist spin coating

Photolithography for RIE (Mask 2)

RIE for exposing ISE and pad

fused silica | SiO_2 | SU-8 | Ti-Pt | Ag | photoresist

bioCD Fabrication Steps



Solvent cleaning

Photoresist
spin coating

Photolithography
for Ag lift-off
(Mask 3)

Ag deposition
(2.5 μm)

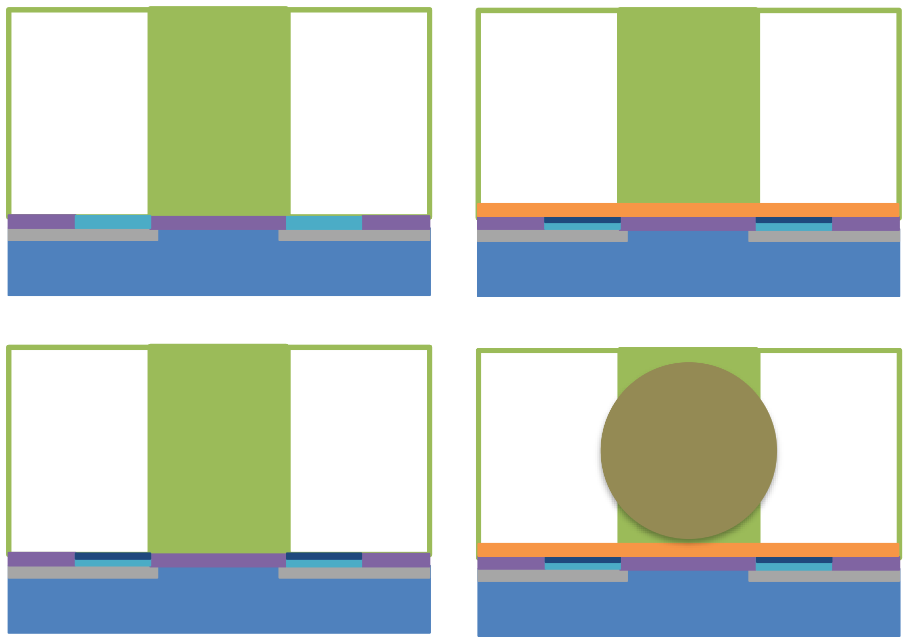
Lift-off

SU-8
spin coating
160 μm (80 μm x 2)

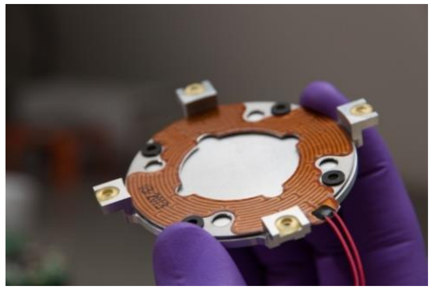
SU-8
photolithography
for spore well
(Mask 4)

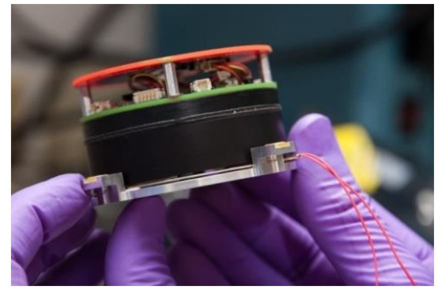
fused silica SiO_2 SU-8   Ti-Pt   Ag   photoresist

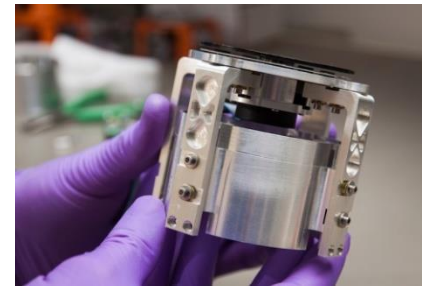
Surface Functionalization

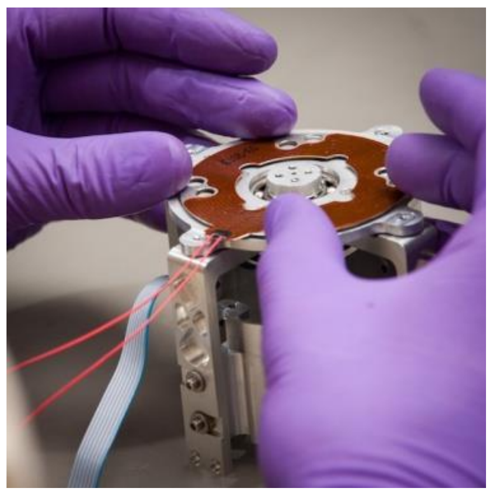


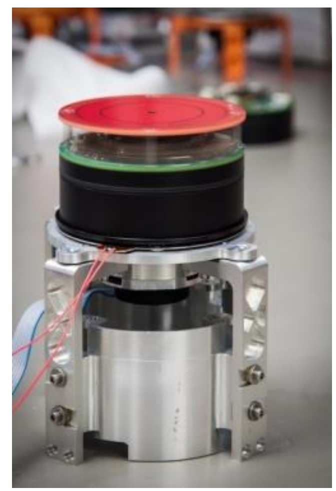
Integrating bioCD Rotating Assy. & Motor

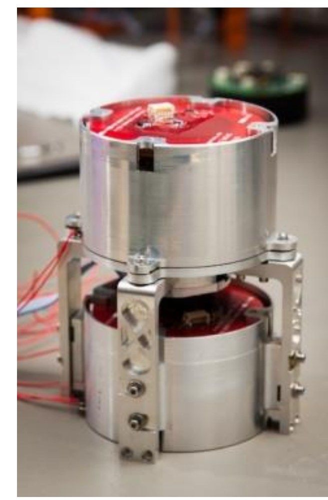






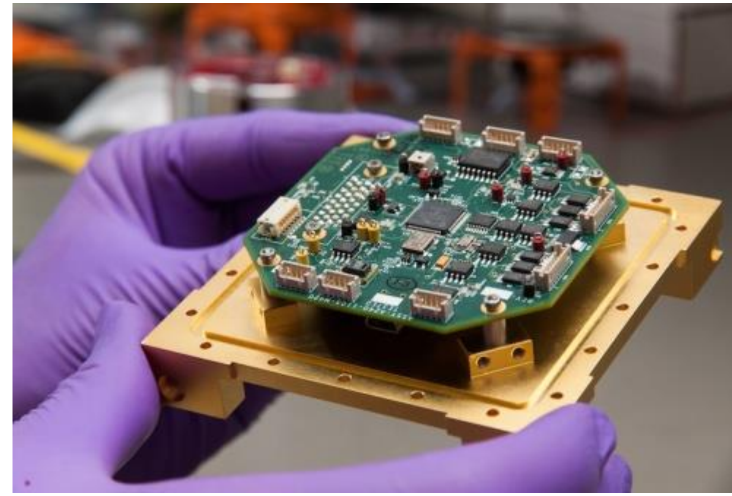






23

Integrating bioCD/Motor in Payload Vessel



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Ion-selective Electrode

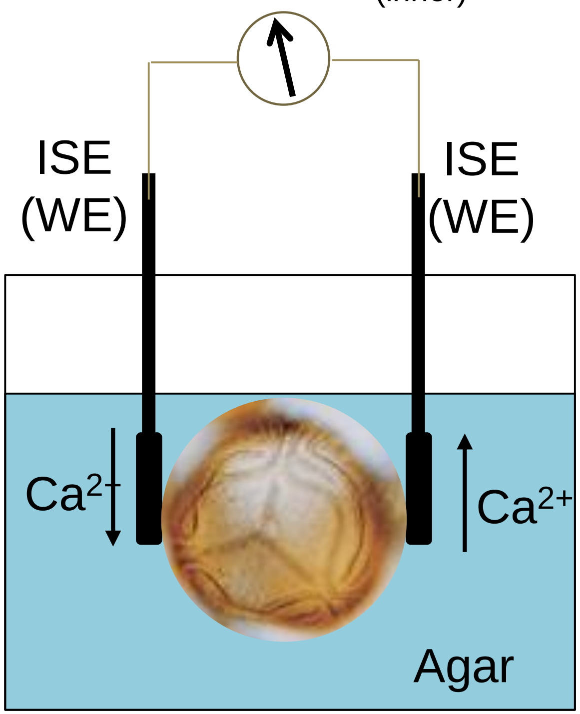
DEDC (Dual Electrode Differential Coupling)

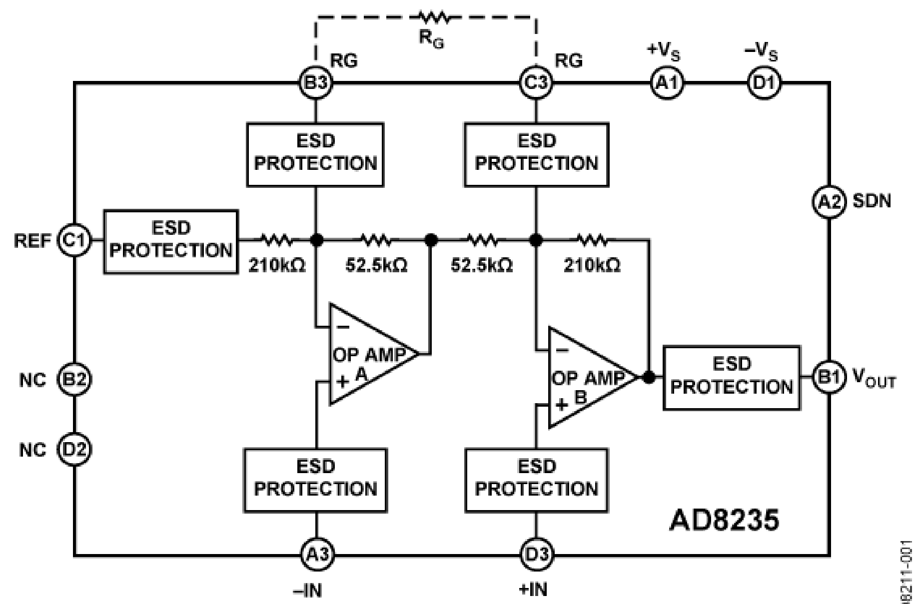
Simplified Nernst equation for Ca^{2+} concentration cell:

$\Delta E = 30 * \log\{ [Ca^{2+}_{(inner)}] / [Ca^{2+}_{(outer)}] \}$ mV

32 instrumentation amplifiers on PC board; pogo-pin connections to bioCD

- gain: 5
- offset voltage: 1.5 V





$E_{out} = 1.5 + 5 * 0.03 \log\{ [Ca^{2+}_{(inner)}] / [Ca^{2+}_{(outer)}] \}$ V

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Experiment Protocol

Total experiment duration < 62 hr

Spores in Stasis: 30 – 100 days

Pre-Launch
Load spores & media onto 3 BioCDs
(32 spores per BioCD)

Transfer, Integrate, Launch, Deploy in Earth Orbit, Satellite Stabilization

Begin Experiment

Data Acquisition Start

Heat On
29 °C

(2 hr)

Experiment ~ 20 hr per BioCD (3x)

Establish Artificial Gravity
(2 hrs)

Initiate Germination
Red light spore activation
(2 hrs)

Germination Wait Window

(9 hrs)

Germination Measure Window
5 rotation rates
(6 hrs)

Post-Germination
1 rotation rate

(1 hr)

Repeat for BioCD-3 from “Begin Experiment”
(no rotation)

Repeat for BioCD-3 from “Begin Experiment”

End

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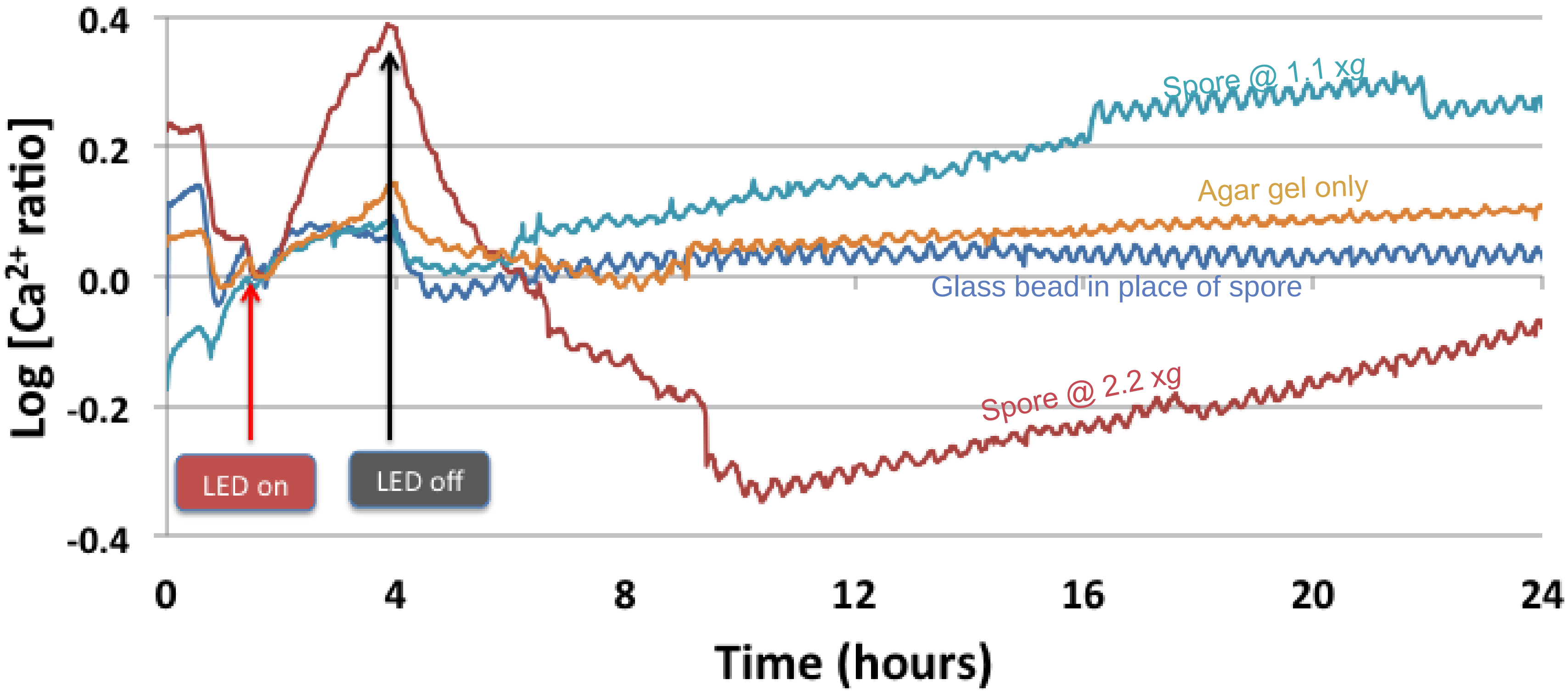
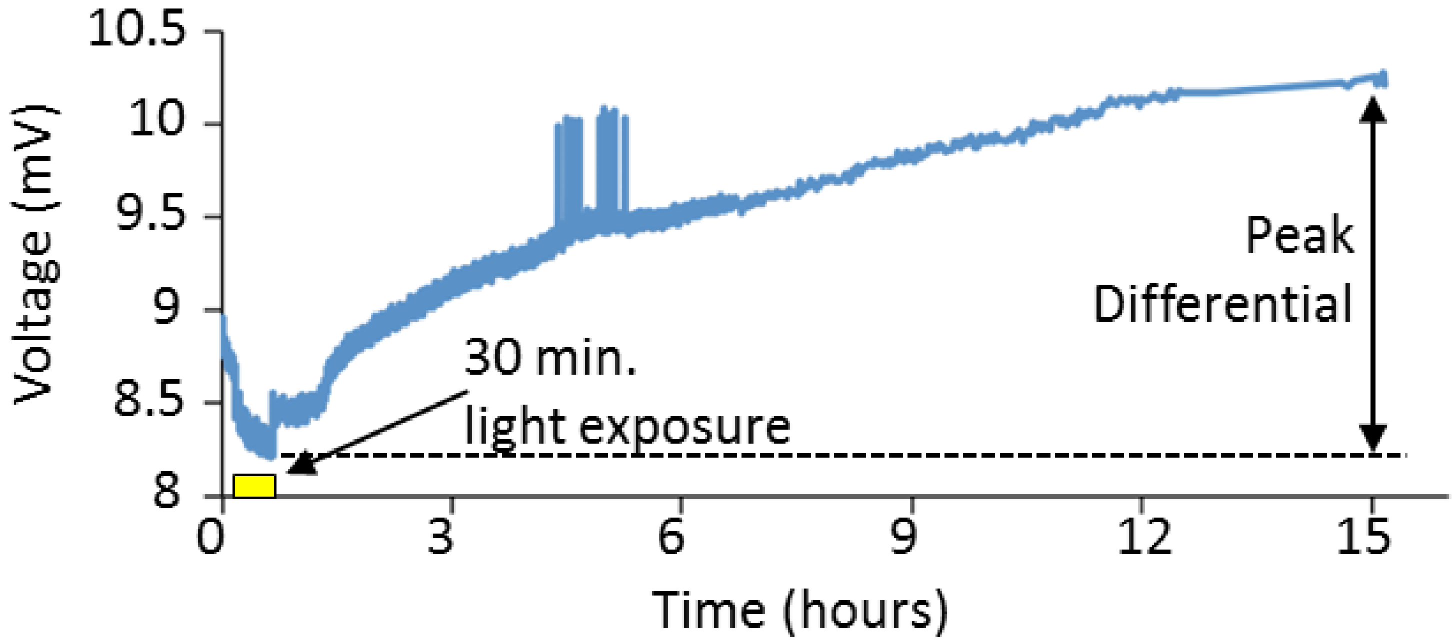
Laboratory Experiment (Minimal Stasis Time)



- Larger current at 2.2xg
- Lower g-levels signals trend closely to that of glass bead and agar

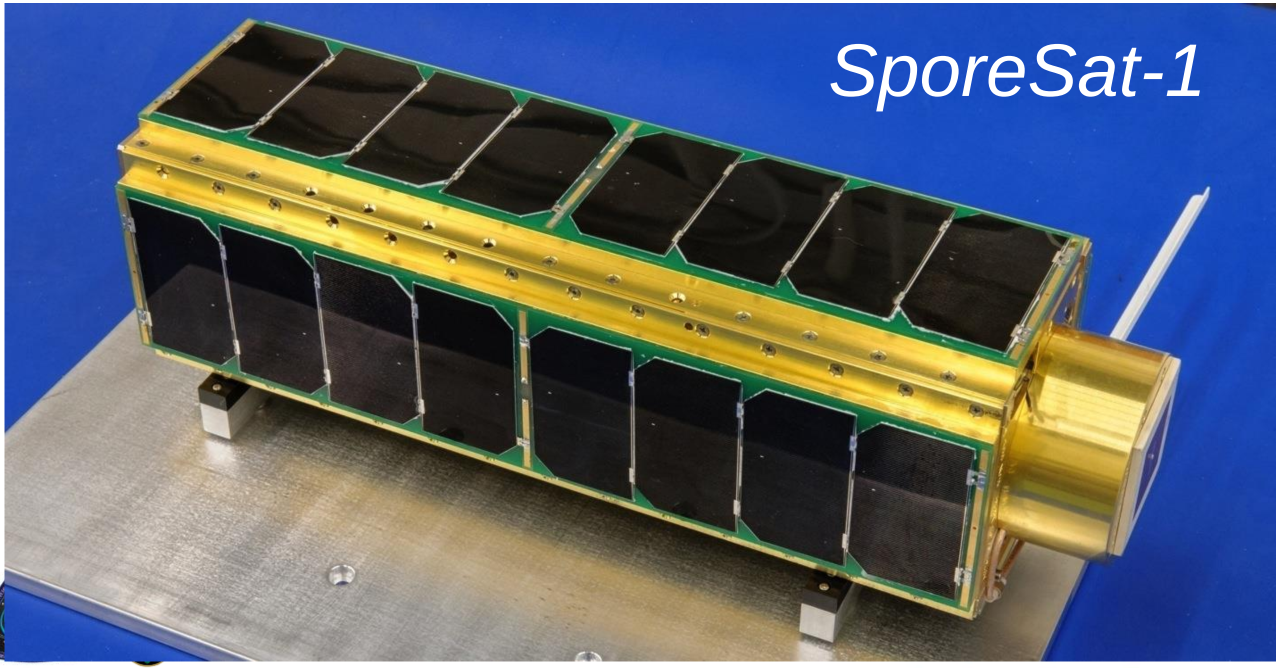
Long Duration Stasis (37 days)



- Confirmed germinated spores have similar signals

Project Timeline

JAN 2011	DEC 2011	APR 2013	FEB 2014	MAR 2014	APR 2014	JUNE 2014
✓ Project Kick off	✓ System Requirements Review	✓ Critical Design Review	✓ Flight Readiness Review / ✓ Operational Readiness Review	✓ Mission Readiness Review	✓Launch #1	✓De-orbit



Summary & Conclusions

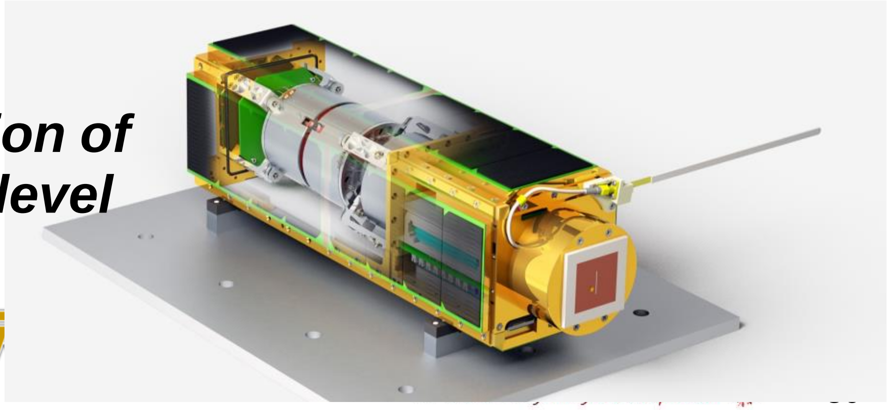
- Sophisticated science experiments and measurement instrumentation can be implemented in small spacecraft to study phenomena uniquely accessible in outer space.

- SporeSat is a first-of-its-kind small science satellite that couples novel miniaturized technology to novel biological science: a variable-rate centrifuge enables biological studies from microgravity to hypergravity!

  - *Including gravity levels corresponding to moon, Mars, outer space*

- SporeSat measures the responses of Ca^{2+} ion channels, which are common to many biological organisms, including humans**.**

  - ***SporeSat results will link modulation of Ca^{2+} ion channel activity to the level of gravitation for fern spores***



CURRENT WORK AT IIUM

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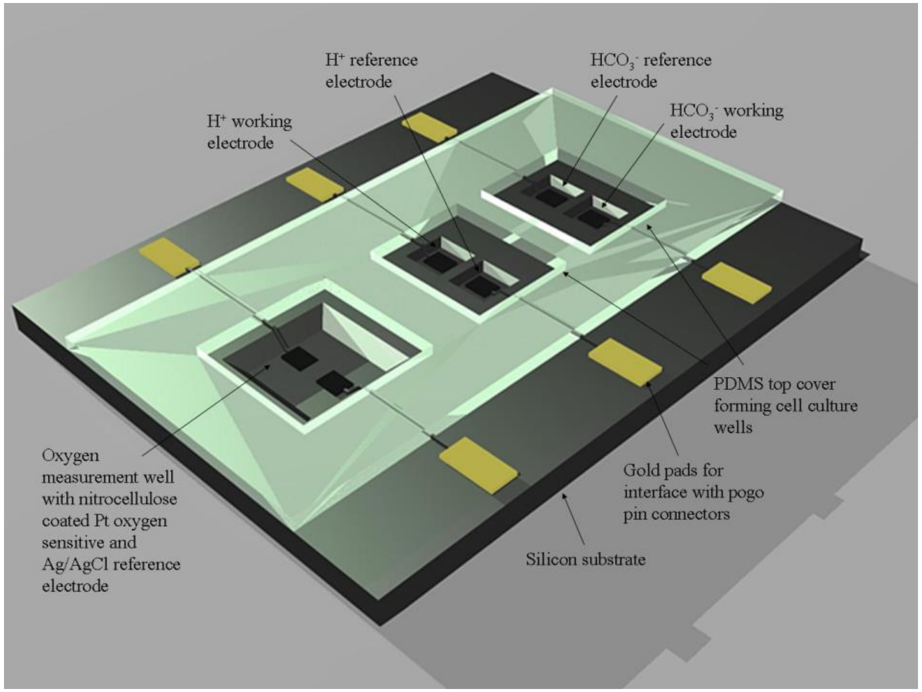
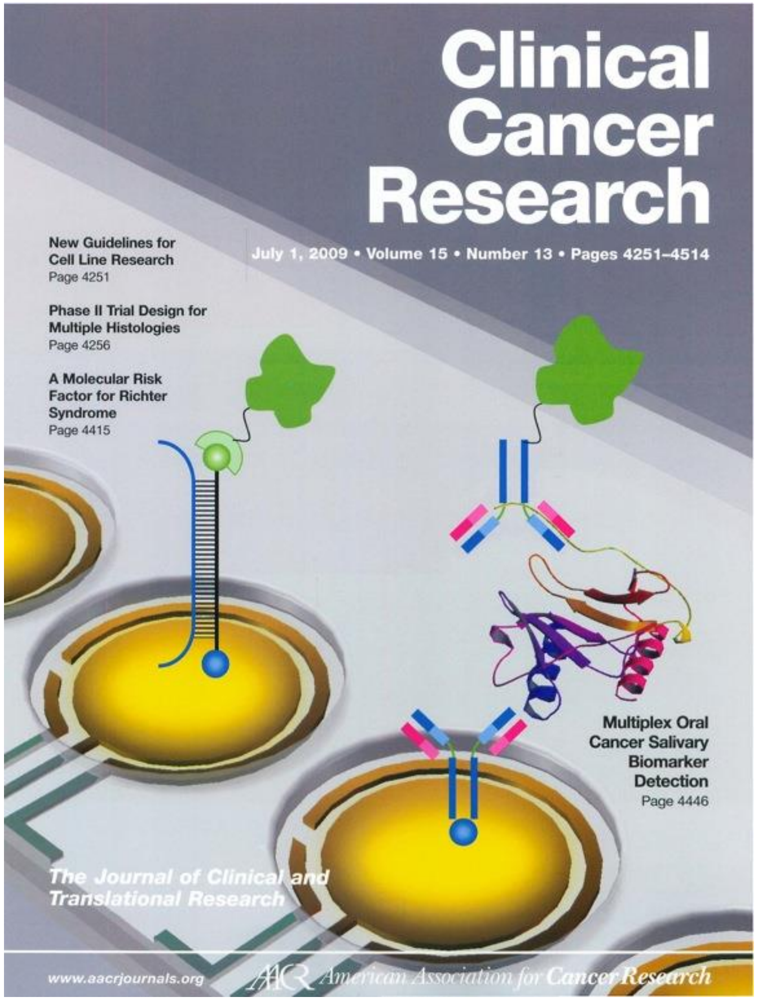
Envisioned multiplex platform





Problem Statement

- Cancer leading cause of death in the world (no brainer why its important!)
- Globally, it is responsible for 1 out of every 4 deaths annually, and was responsible for ~ 580, 350 deaths, or more than 1600 deaths a day (American Cancer Society, facts and figures for 2013)
- Most common type of cancer in men and women is prostate and breast cancer respectively, with 192,000 new cases reported annually.
- ACS estimates the average survival rate for all cancers for the years 1996-2004 has increases to 66% (up from 50% for 1975-1977)
- Increase in survival rate is attributed to technological advances resulting in better treatment and early diagnosis.
- However, 5 year survival for certain cancers such as liver, pancreatic, and lung remains low (6%-16%)

Ames Research Center PURDUE UNIVERSITY PHYSIOLOGICAL SENSING FACILITY Santa Clara University School of Engineering ARG DR. AMANI RESEARCH GROUP NANORG The Future's Big Thing is Really Small

Current needs in cancer detection

- **Use of emerging bio-nanotechnology (biosensor technology) could be instrumental in early cancer detection and more effective treatments, particularly for those cancers that are typically diagnosed at late stages and respond poorly to treatment, resulting in improvements in patient quality of life and overall chance of survival.**

- **Current biosensors technology especially for cancer early detection are not readily-commercialized owing to several factors:**
  1. One method of biosensor transduction could not give a definite answer, hence cross-correlation between biosensor and biochemical results is needed
  2. Biosensor technology needs to have reliable repeatable results hence a confidence-user interface is required
  3. Results are often in physical values (mass, current, voltage) which data processing and data presentation is needed to yield results that make sense to practicing clinical oncologist (researchers need to focus on end user)
- **To increase confidence in current biosensor technology, a multiplex sensing approach is needed.**

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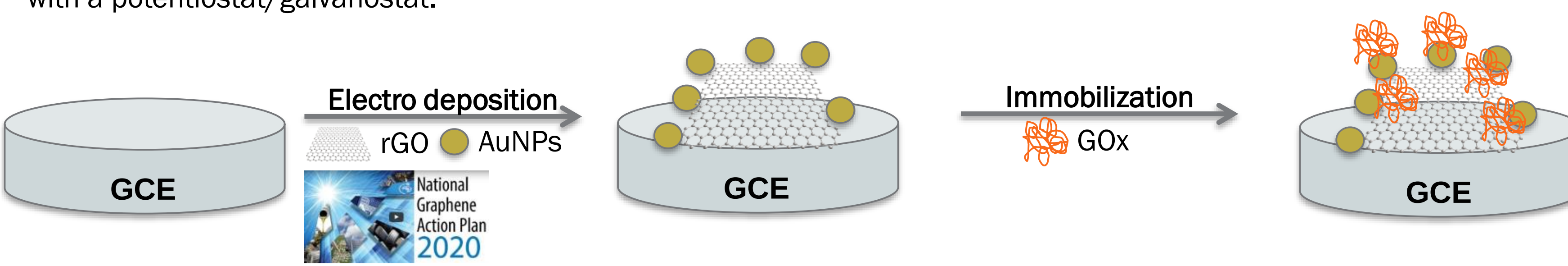
Research Title: Graphene-based Nanocomposites for the Development of Biosensors

Research Objectives:

Graphene is a two-dimensional, zero-band gap semiconductor monolayer sheet of sp^2-bonded carbon atoms, arranged in a perfect honeycomb network lattice and possesses remarkable and excellent optical, electrical, mechanical, and electrochemical properties. It is often called a wonder material due to its attractive properties. For the purpose of my research, I will seek to develop a non-invasive, wearable, cost-effective, and reliable glucose biosensor using graphene-based nanocomposites as the transducer. Glucose oxidase (GOx) enzyme as the bioreceptor will be immobilized using physical and chemical methods and characterization of the biosensor will be conducted using electrochemical methods with a potentiostat/galvanostat.



Deposition Methods

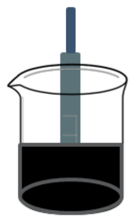
Deposition of rGO-AuNPs/matrix on glassy carbon electrode (GCE)

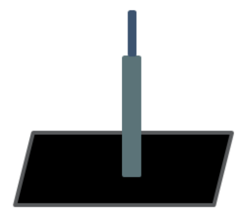
Physical

- Dip-coat in **UHC GO*** suspension & reduce
- Drop-cast **UHC GO*** suspension & reduce
  - Drop-cast **UHC GO* + AuNPs** composite & reduce
    - Drop-cast **UHC GO* + AuNPs + matrix** composite & reduce
- Rubbing on **GO sheet** & reduce

Electrochemical

- Electro-deposit **UHC GO*** suspension & reduce
  - Electrodeposit **UHC GO* + AuNPs** composite & reduce
    - Electrodeposit **UHC GO* + AuNPs + matrix** composite & reduce





***UHC GO**: Ultra highly concentrated single layer graphene oxide (concentration of 6.2 mg/ml)
rGO: Reduced graphene oxide
AuNPs: Gold nanoparticles

Utilization of matrix in the nanocomposites

3. Adding matrix

- 4.1 1,6-hexanediamine
  Chemical method
- 4.2 Nafion
  Physical method
- 4.3 Cellulose
  Physical method
- 4.4 Conductive polymers
  Physical & chemical methods

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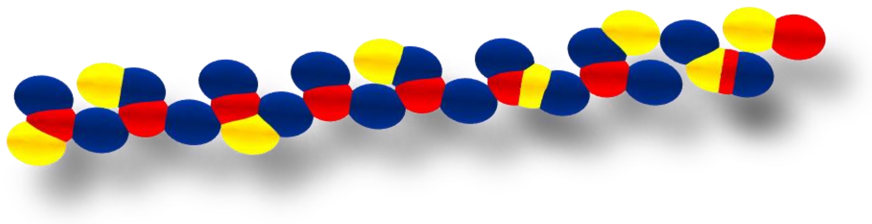
Research Title: Poly(3,4-ethylenedioxythiophene) – Poly(styrene sulfonic acid) (PEDOT:PSS) Composite As Transducer For Continuous Monitoring In Liquid Media



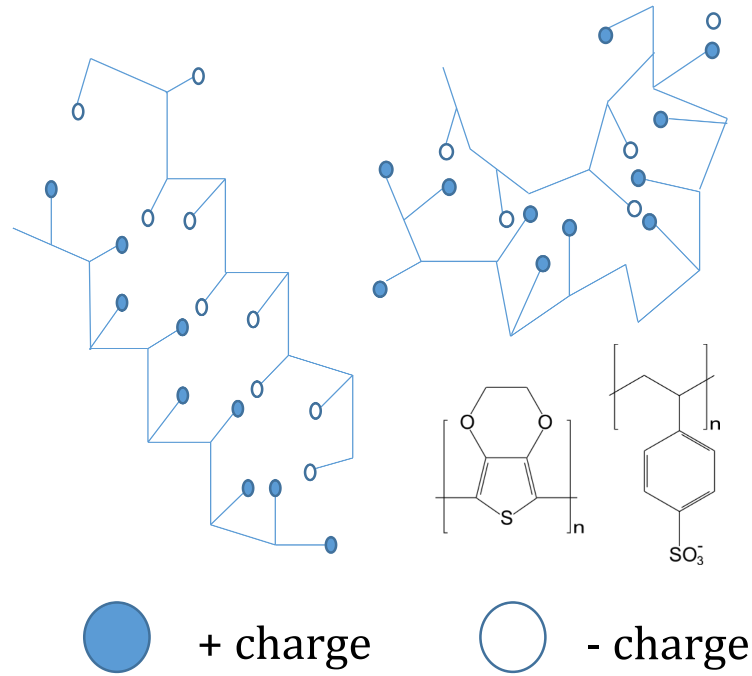
❖ We are optimizing methods to deposit conductive polymer Poly(3,4-ethylenedioxythiophene) – Poly(styrene sulfonic acid) (PEDOT:PSS) on platinum electrodes to increase electrode lifetime in liquid media. This is to enable long-term *in vivo* monitoring for biological and biomedical applications.

Conductive Polymers

Why conducting polymers?

- Electronically conducting like gold and platinum
- Rapid prototyping through electrochemical or chemical deposition
- Can be deposited from solution
- An electro-active material
- Tailored eletrochemical property
- Robust

Polyelectrolyte complex



Adapted from Philipp, W. Dawydoff et al., (2001) Makromoleküle Physikalische Srukturen und Eigenschaftenm Vol 2, 6th ed., Wiley-VCH

Conducting polymer conjugates such as PEDOT:PSS are promising materials for the development of electrochemical biosensors.

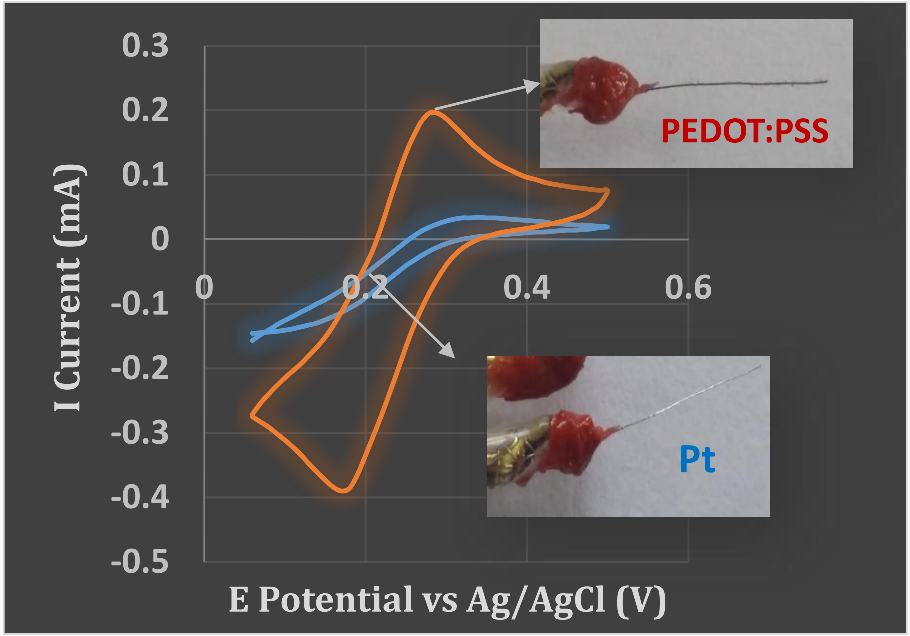


Fig 1. the peak current on Pt electrode before after Electropolymerization deposition of EDOT

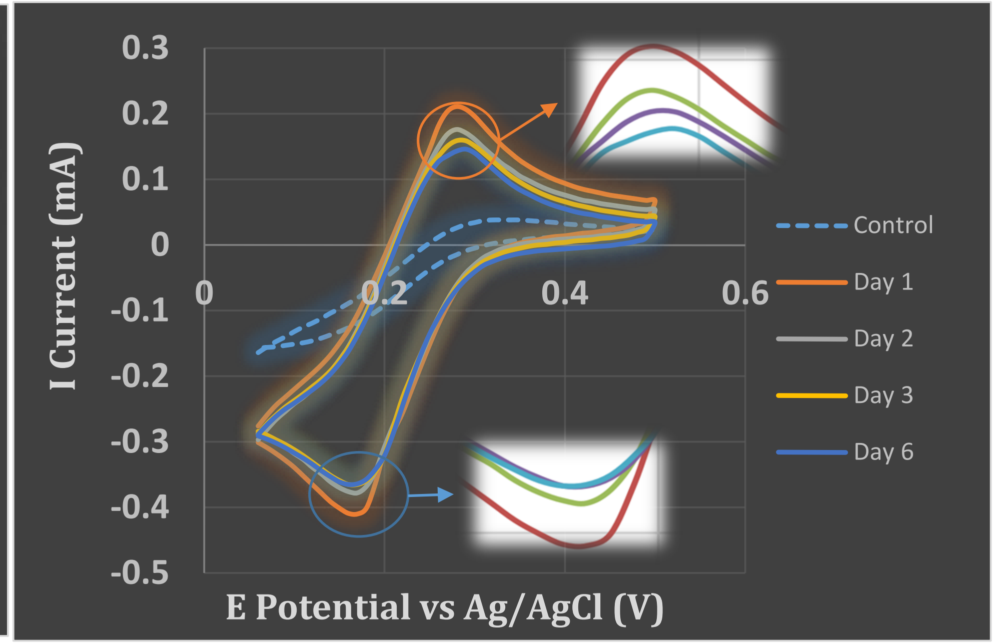


Fig 2. The peak current for (E1) during six days of measurements

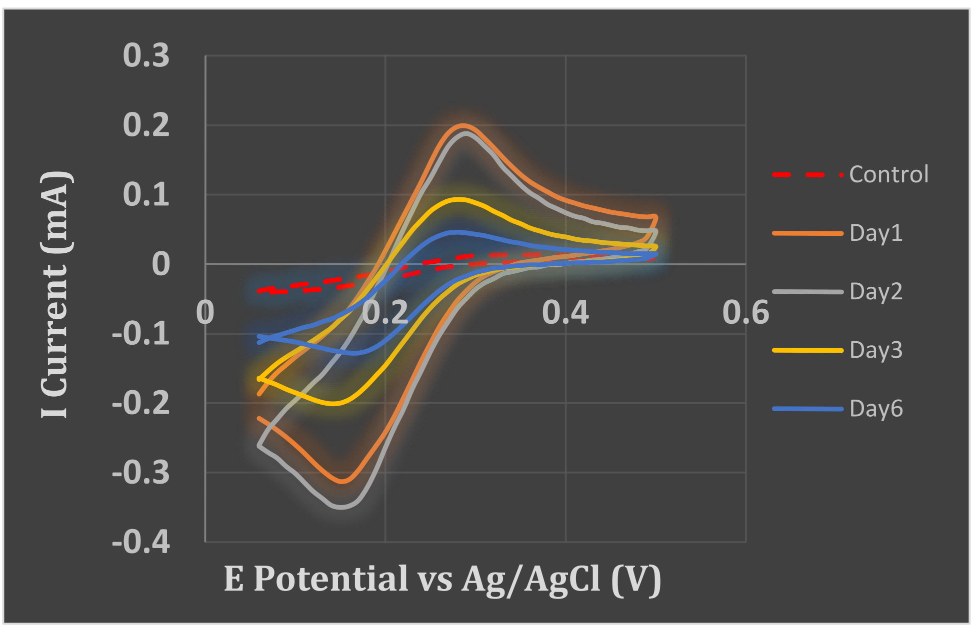


Fig 3. The peak current for (E2) during six days of measurements

These preliminary results PEDOT:PSS is suitable as a transducer for long-term monitoring measurements in liquid media.

ACKNOWLEDGEMENTS

- **NASA-HEOMD** for funding support
- All members of the **SporeSat Team**
- **All members of the Amani Research Group**

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- **Ministry of Education Malaysia**

  (FRGS and RAGS grants)

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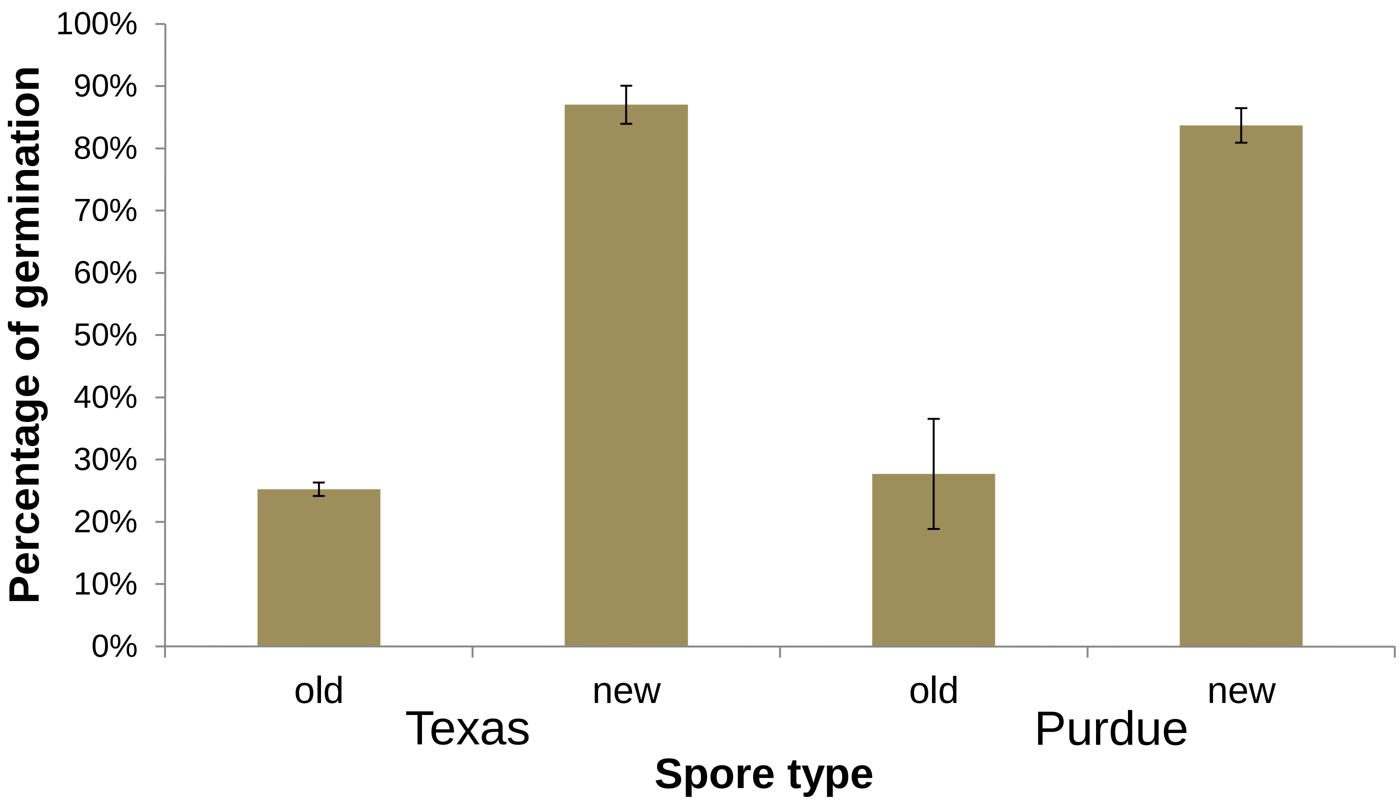
BACKUP SLIDES

Experiment	Dates	unit	Spores	Wells	Notes
14 day biocomp			Old	Isolated	30 min. OLED, No rotation
37 day biocomp			Old	Isolated	30 min. OLED, w/rotation
108 day biocomp			Old	Isolated	30 min. OLED, w/ rotation No sign of germination, Stable sensors
Zero Day I	1.13.14	bioCD5 4	New	Flight1	Pre-soaked for 6 days Excellent germination Peak activity may correlate with radii No negative controls
Zero Day II	1.16.14	bioCD5 5	New	Flight1	2 hr. OLED RTD failure lead to spike in temp on BioCD
Zero Day III	1.21.14	bioCD5 1	New	Flight1	2 hr. OLED Pre-soaked for 45 days
Zero Day IV	3.26.14	bioCD6 6	New	Flight2	Agar not loaded properly Negative Controls
Zero Day V	3.31.14	bioCD6 4	New	Flight1	Motor failure 1.5 hr past OLED off
Flight RA1			New	Flight1	
Flight RA2		bioCD5 7	New	Flight1	Negative Controls
Flight RA3			New	Flight1	
Flt-1 BioComp RA9 ground	5.2.14	bioCD6 2	New	Flight1	?Germination? → do new spores survive long term?

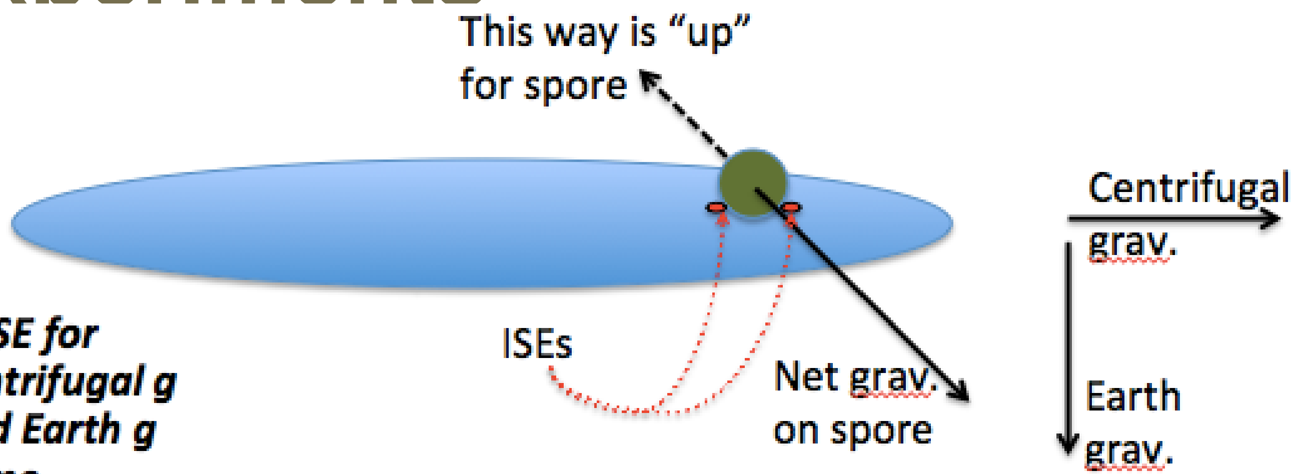
Germination Study

- Type of spores: RN5 strain, new spores are younger spores
- Spores were counted after 3 days after initiation of light

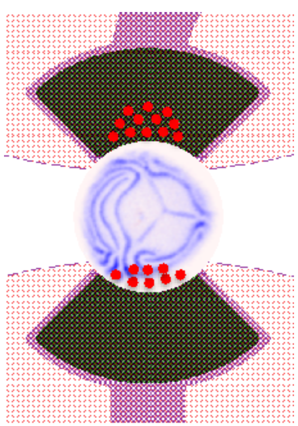


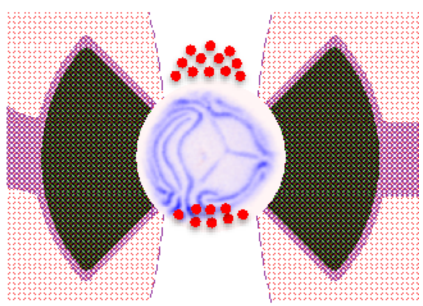
New spores show germination percentage of > 83%

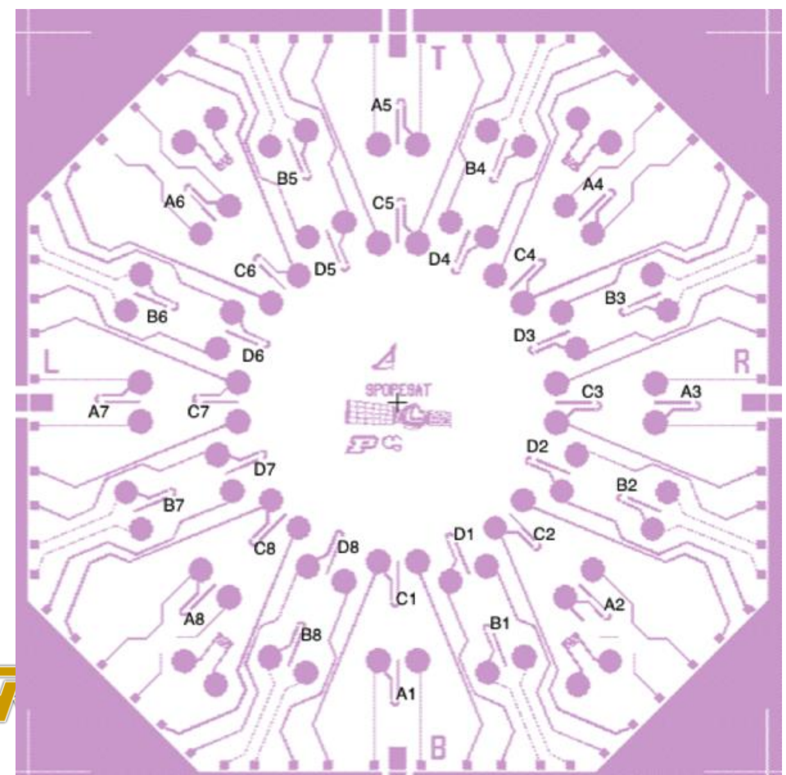
Experimental Context for Ground Experiments



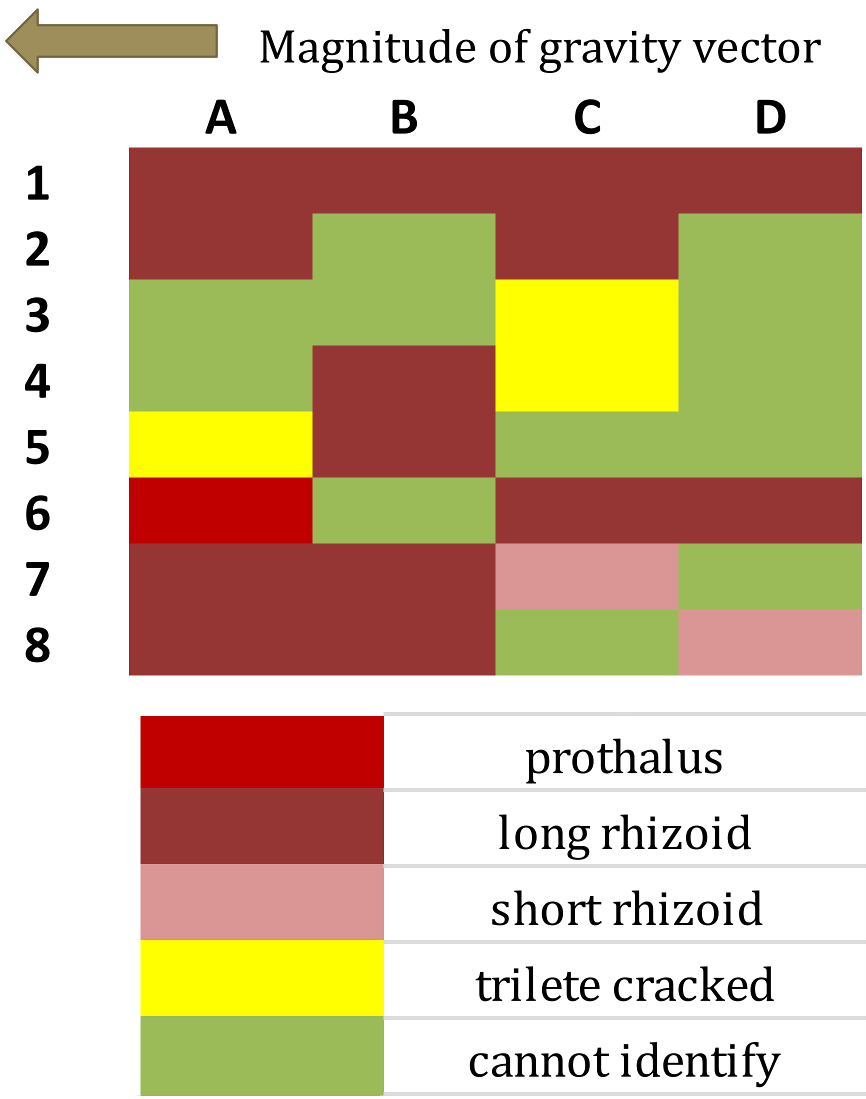
CASE for centrifugal g and Earth g same magnitude

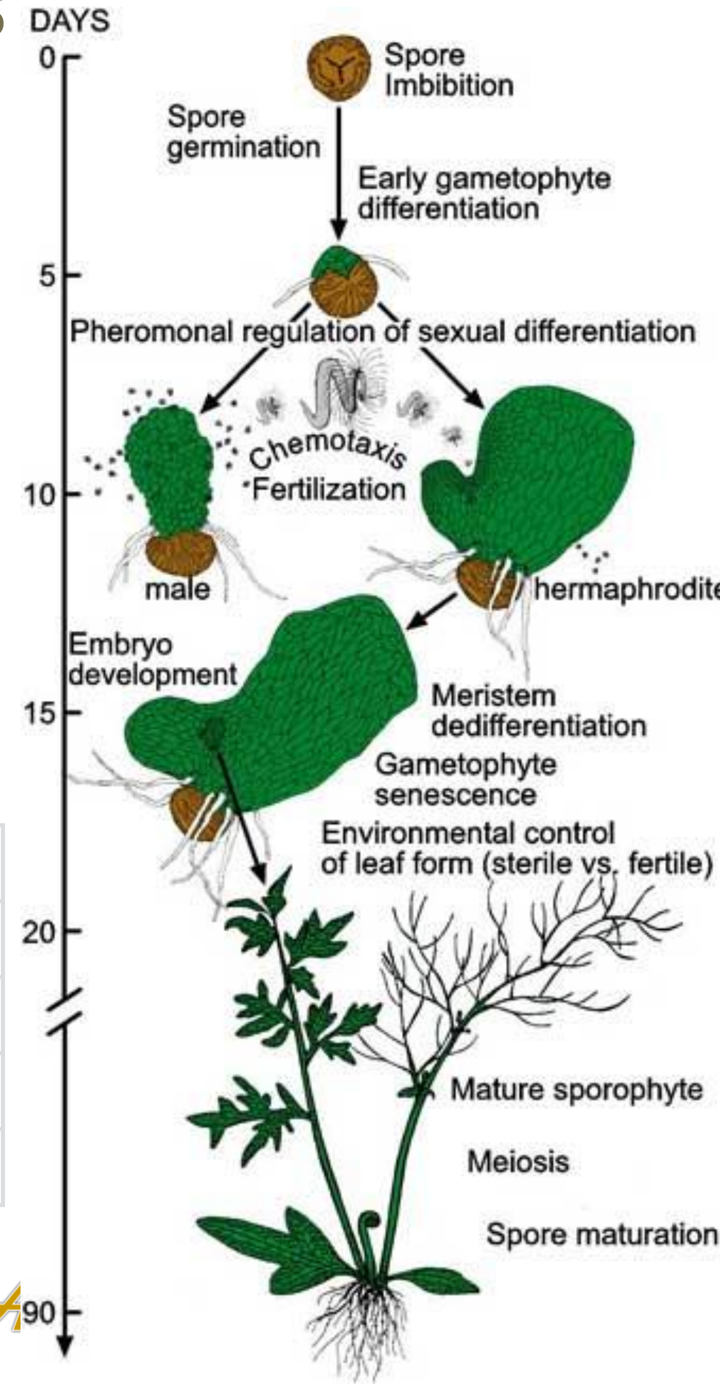






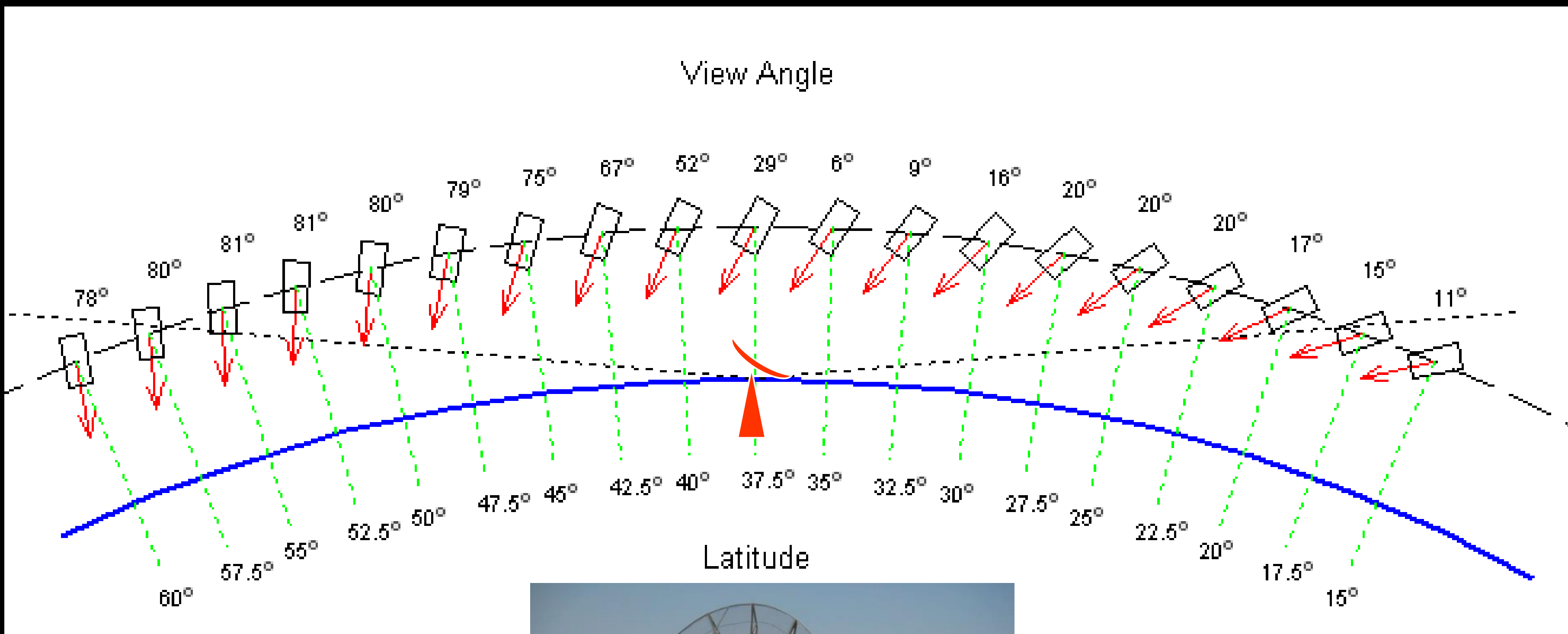
Germination Results





SporeSat Mission

Passive Magnetic Attitude Control Points Patch Antenna Toward Ground Station



North Pole

Santa Clara Univ. 3-meter antenna / ground station

Equator