



Phototaxis of *Chlamydomonas reinhardtii* in Microgravity Condition

Present By

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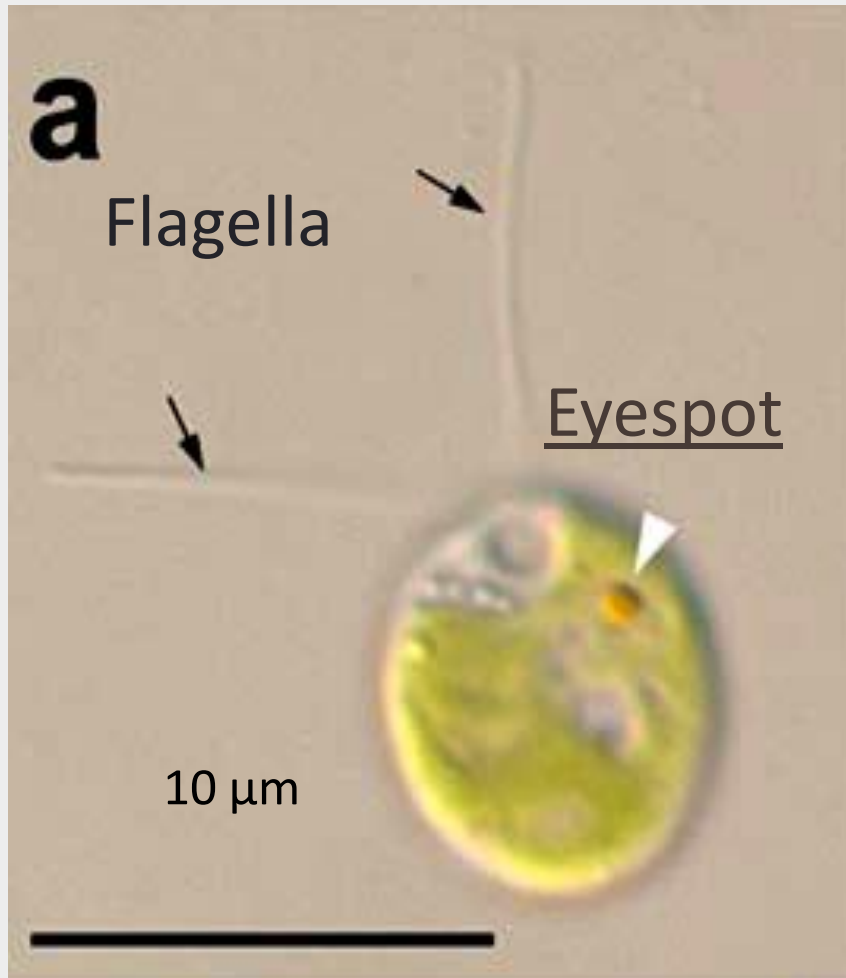


***Mentor* : Dr. Sawat Tantiphanwadi**

Introduction



***Chlamydomonas reinhardtii* is a unicellular green algae.**



Scientific classification

Protista

Chlorophyta

Chlorophyceae

Volvocales

Chlamydomonadaceae

Chlamydomonas

Chlamydomonas reinhardtii

Introduction

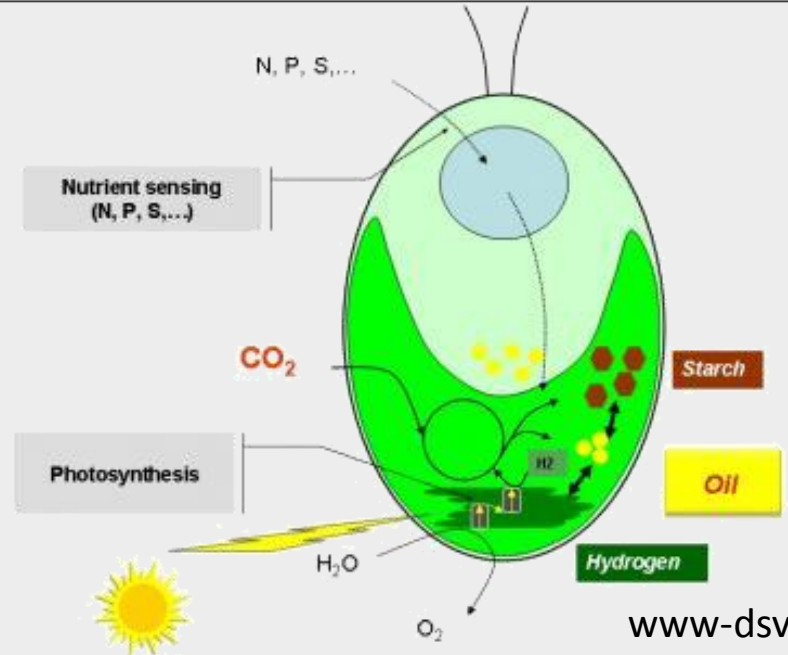
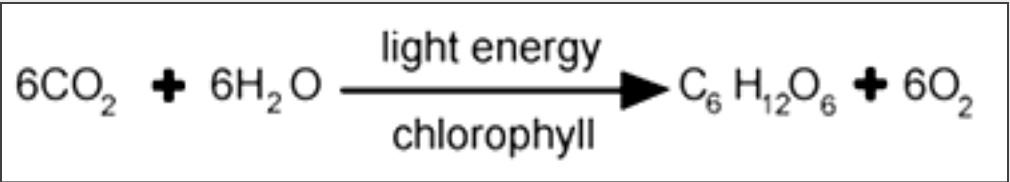


Why we study in *Chlamydomonas reinhardtii* ?

Algae model

Photosynthesis

Hydrogen producer

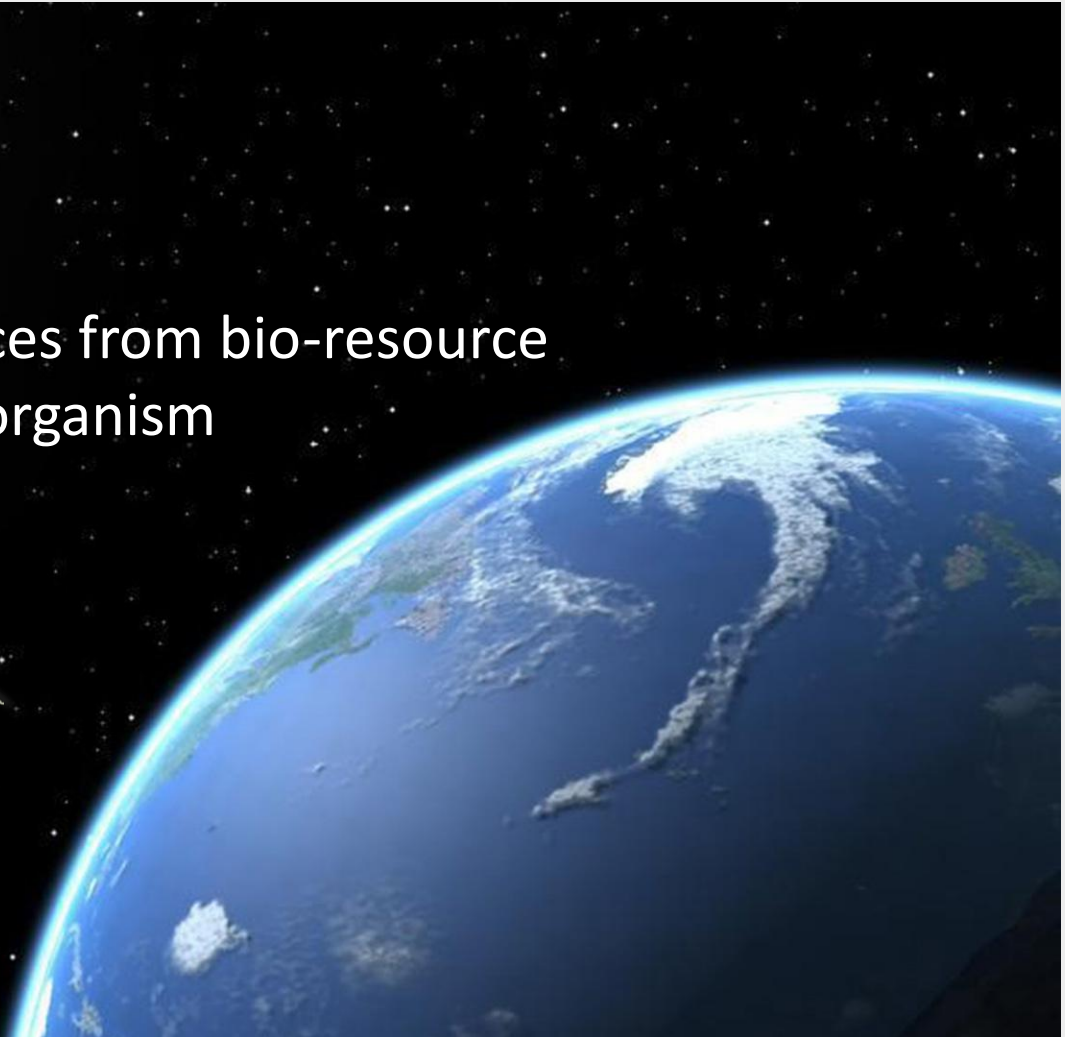
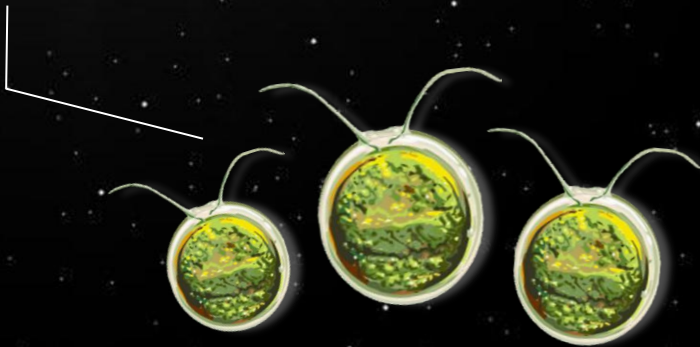


Application



In the future, If *Chlamydomonas* go to space. !!!

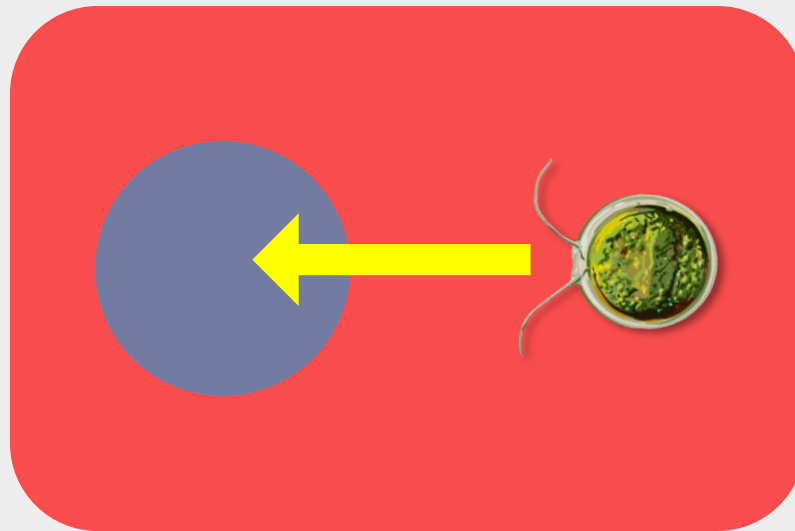
- Renewable energy resources from bio-resource
- Studying biology in small organism
- Material for food



Objective



To study the movement of *Chlamydomonas reinhardtii* to light under microgravity.



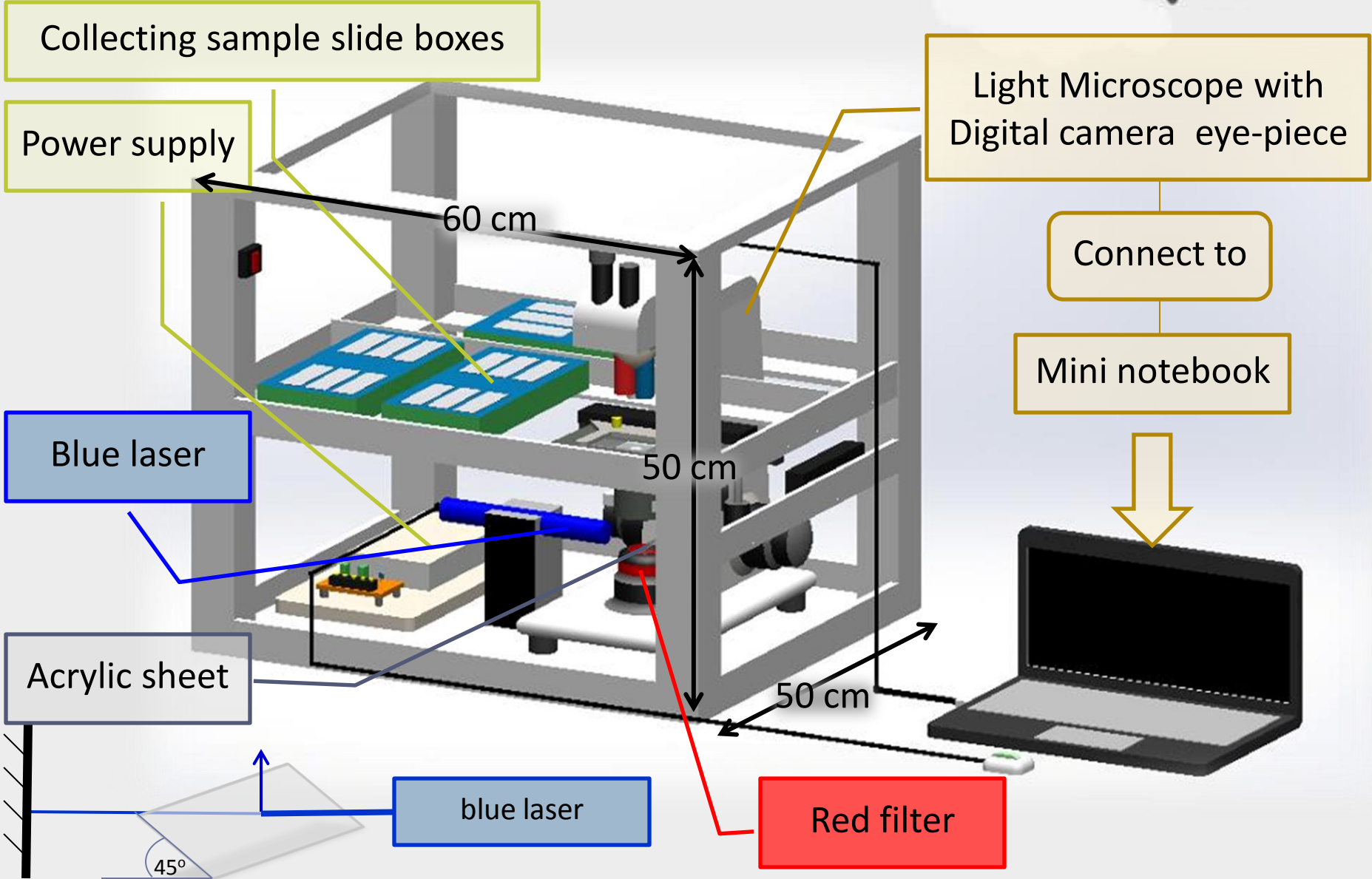
Method



Chlamydomonas reinhardtii in algal culture (10^5 - 10^6 cells/ml)

Genetics laboratory, Nagoya University, Japan (Prof. Masahiro Ishiura and Assi.Prof. Takuya Matsuo)

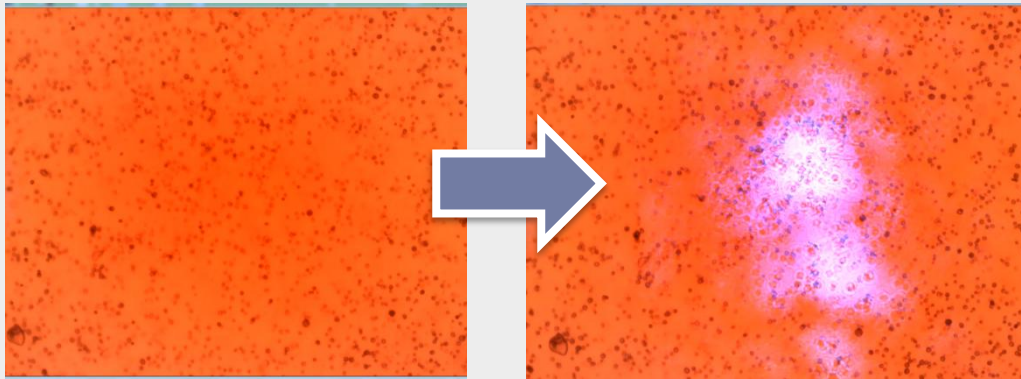
Experiment Designed



Method

Study the movement of *C.reinhardtii* to blue light

Light through the **red filter** is background.
Blue laser is a point



To observe number of *C.reinhardtii* moves to blue light in each time.
(0, 5, 10, 15, 20 seconds)



Do the experiment
in 2 conditions



Microgravity
Parabolic Flight
Test date : 26-27 Dec. 2012

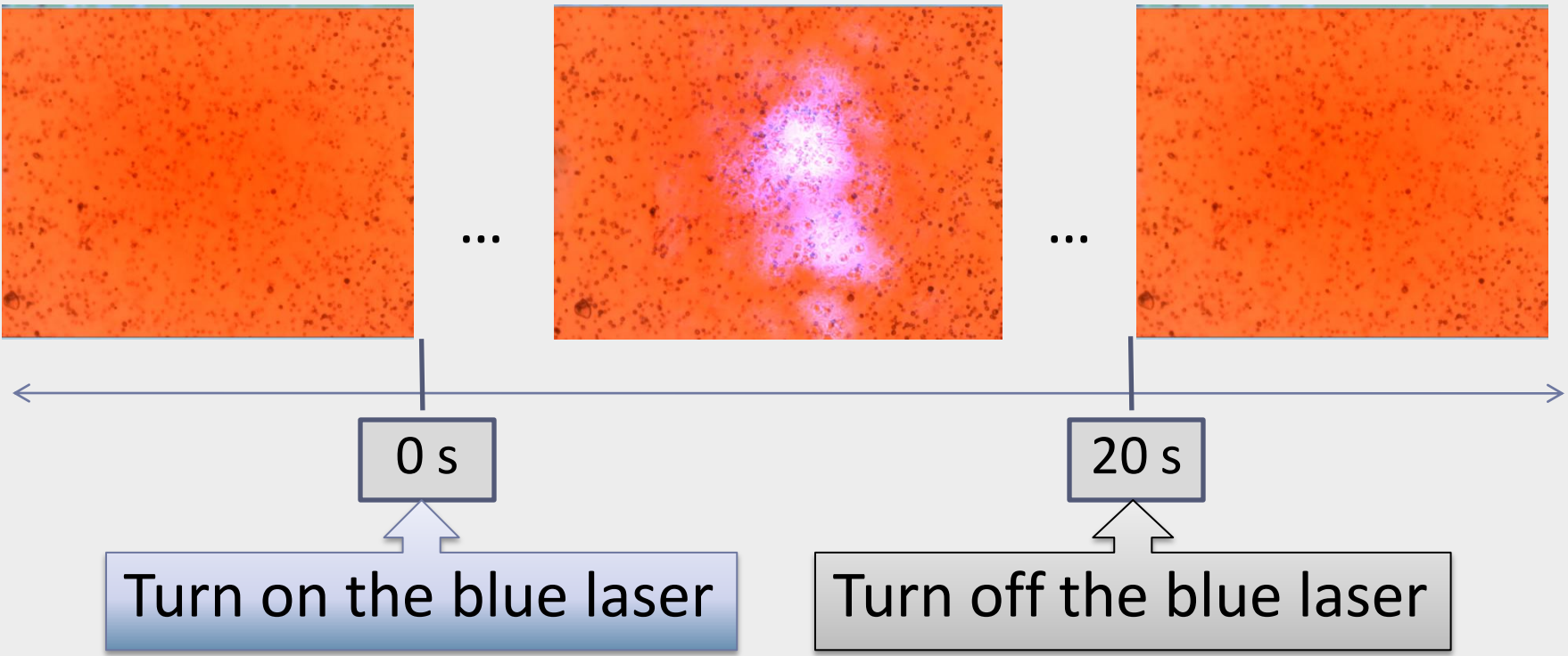


Normal gravity



Method

- To observe by light microscope with magnify 40x
- To record the video by microscope eye-piece with program in mini notebook

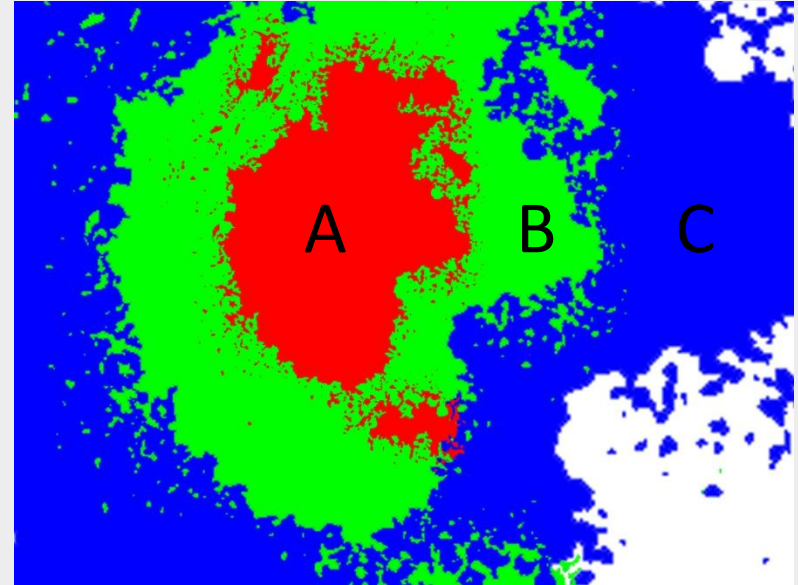
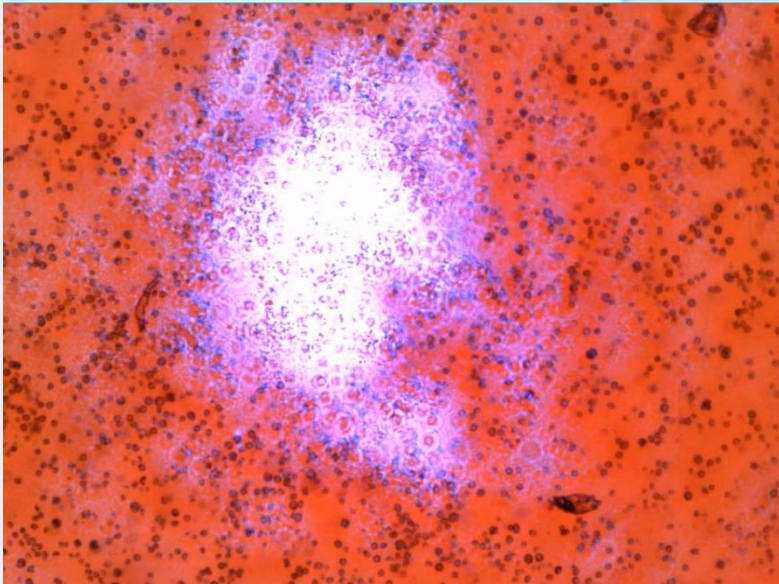


- capture the picture from the video every 5 seconds (at 0, 5, 10, 15, 20 seconds)

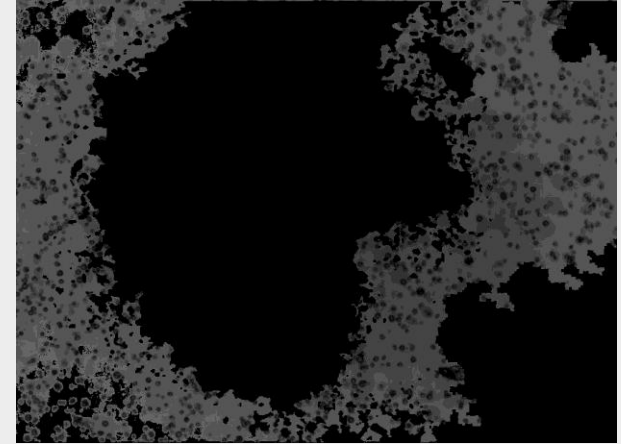
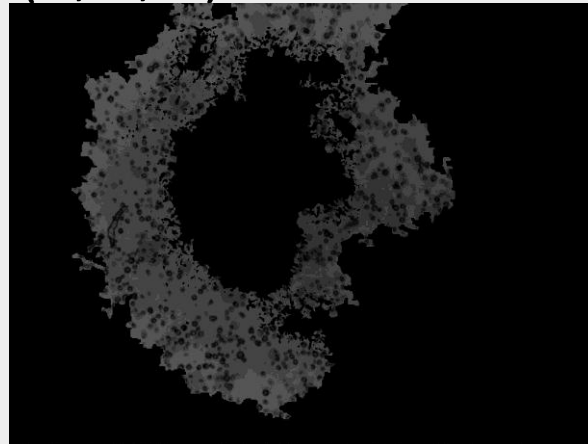
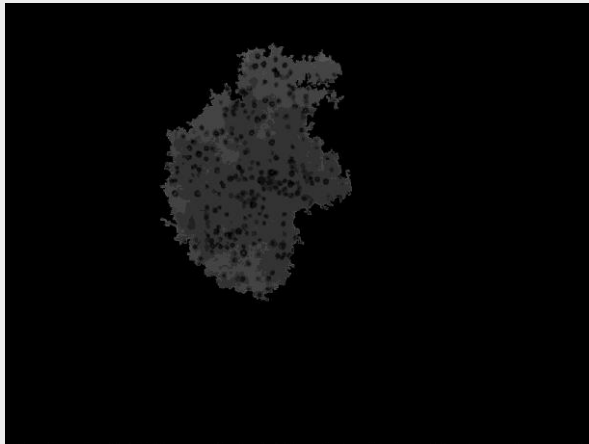
Analysis



To define areas by distance from blue laser light



In each time has 3 Area (A, B, C)



Analysis



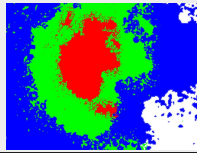
- To calculate number of *C.reinhardtii* by Image J program

The screenshot shows the ImageJ interface. On the left, a window titled '8-4_A.jpg (75%)' displays a grayscale image of a cluster of *C. reinhardtii* cells. The cells are represented by numerous small yellow plus signs (+) on a dark background. On the right, the 'Find Maxima...' dialog box is open. The 'Noise tolerance' is set to 5, and the 'Output type' is 'Point Selection'. The following options are checked: 'Exclude edge maxima', 'Light background', and 'Preview point selection'. The text '141 Maxima' is displayed at the bottom of the dialog box and is circled in red. Below the dialog box, a yellow callout box contains the text: '= 141 cells in A area at this time'.

Result and discussion



- Find the number of *C. reinhardtii* in area at the each time
- From the numbers of *C. reinhardtii*, were calculated to be percentage of differentiation rate of *C. reinhardtii*



Normal gravity

Microgravity

Time (seconds)	Differentiation rate of <i>C.reinhardtii</i> (%)		
	A	B	C
0	0	0	0
5	6.26	1.38	-4.87
10	8.37	-0.53	-4.22
15	10.48	-2.44	-3.57
20	19.05	-1.12	-4.68

Time (seconds)	Differentiation rate of <i>C.reinhardtii</i> (%)		
	A	B	C
0	0	0	0
5	28.94	-0.61	-3.34
10	31.02	1.34	-0.08
15	35.19	5.25	6.43
20	37.27	7.21	9.69

Table 2 Differentiation rate of population of *C.reinhardtii* (%) in normal gravity and microgravity condition.

Result and discussion



- The differentiations of *C. reinhardtii* were related in graph.

Normal gravity

Microgravity

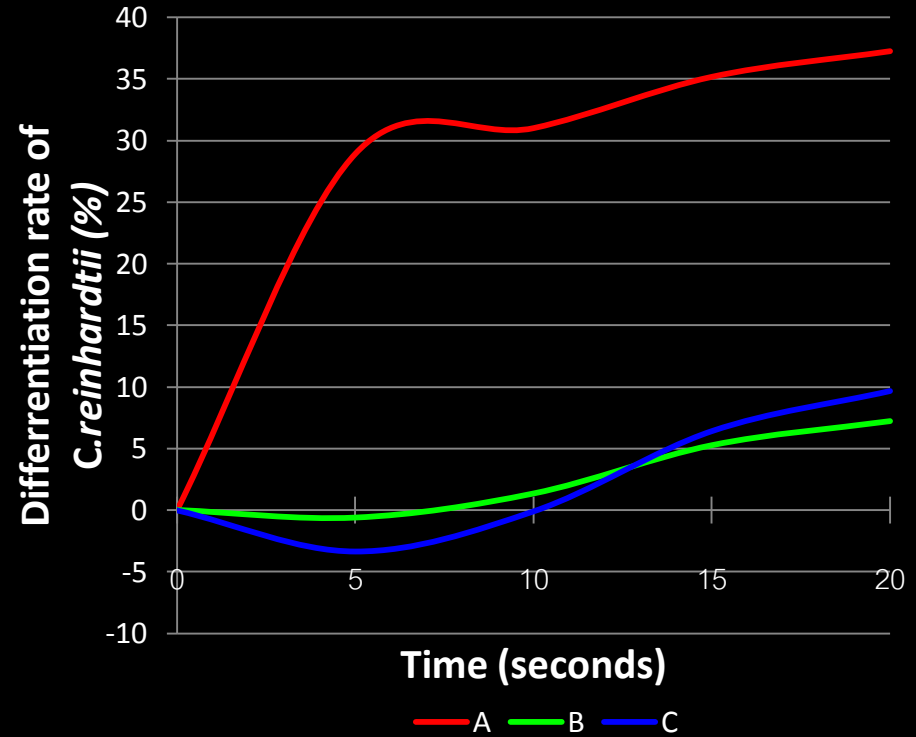
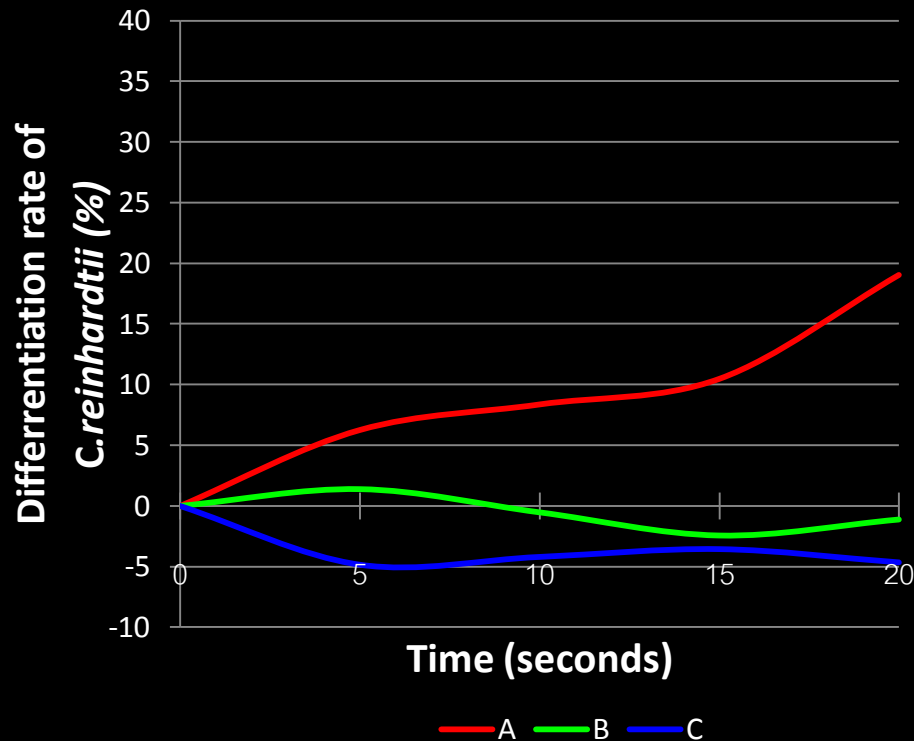
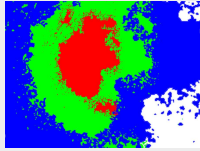
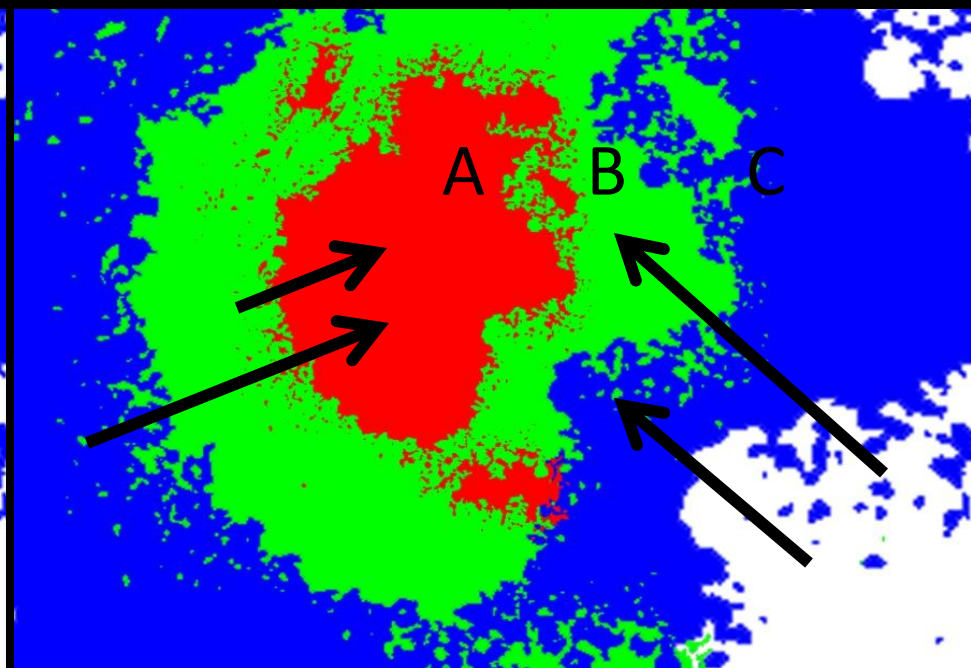
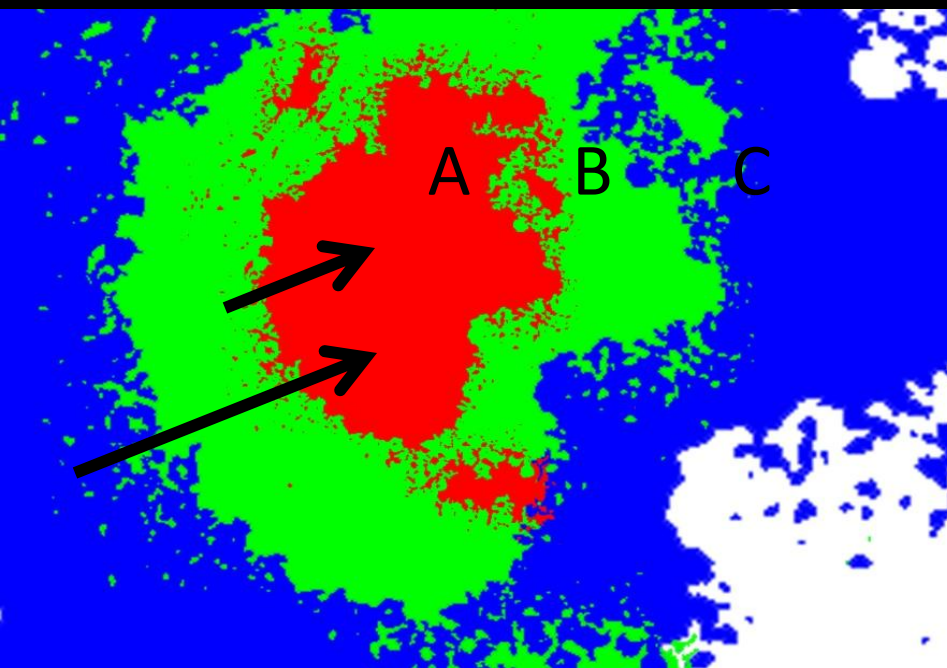
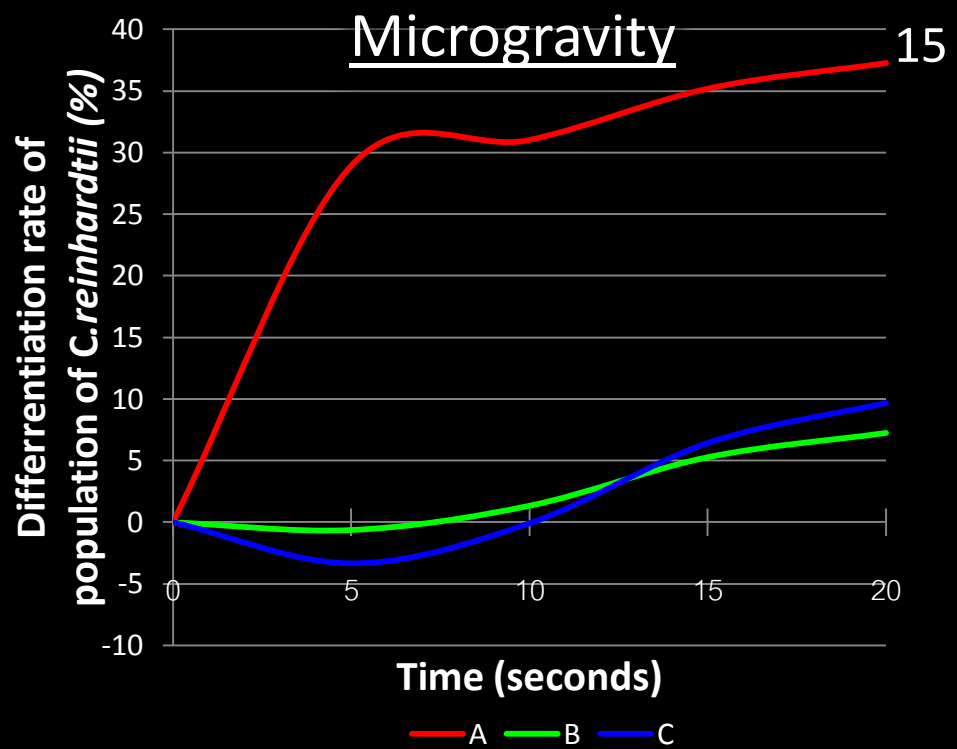
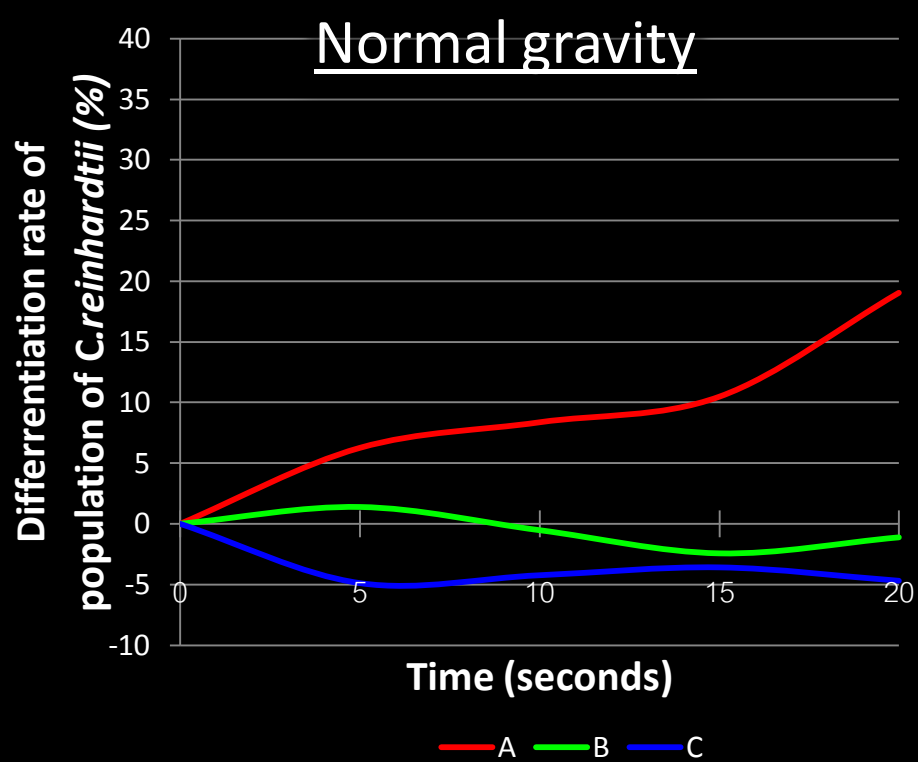
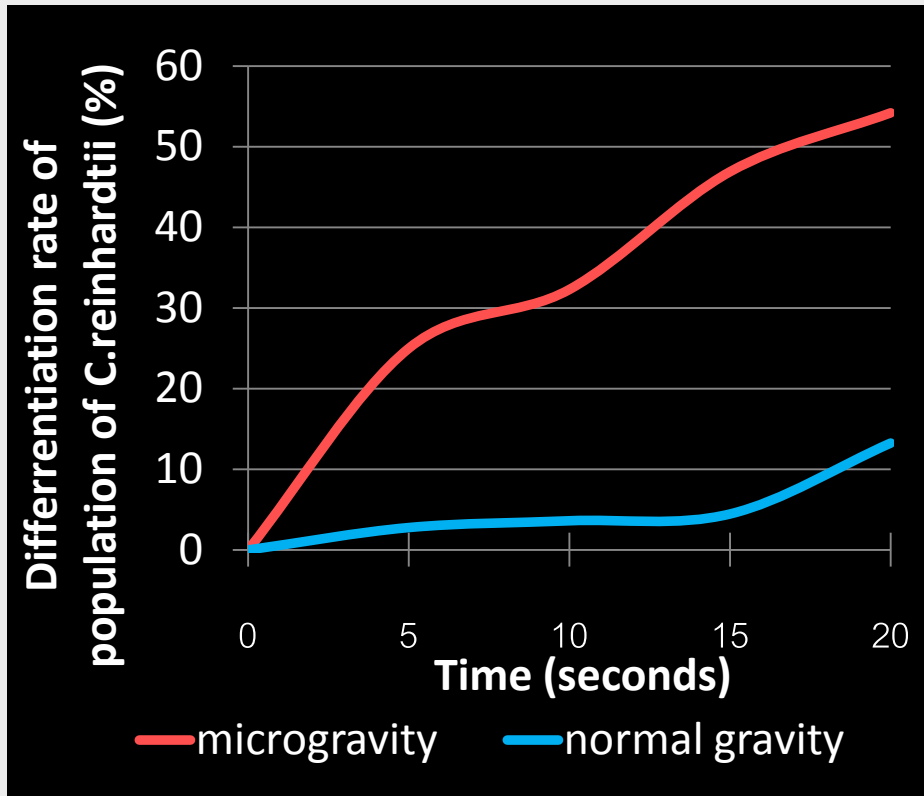


Fig. 1 Differentiation rate of population of *C.reinhardtii* (%) in normal gravity and microgravity condition.



Conclusions



- Under microgravity condition, in every area have the higher increasing rate of population.
- The *C. reinhardtii* has ability to moving to light under microgravity condition better than normal gravity condition

>>Phototaxis behavior can tells that *C.reinhardtii* will have photosynthesis and produced the energy for cell living and be one of the ways to suppose a chance to survival of *C.reinhardtii*.

Acknowledgement



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 Department of Genetic : Dr. Anchalee Sirikhachornkit
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Department of Physic
 Faculty of science, Chulalongkorn University



THANK YOU FOR YOUR ATTENTION

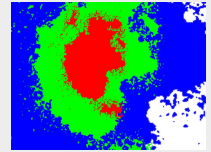
THANK YOU FOR YOUR ATTENTION



Result and discussion



- Find the number of population in area at the each time



Normal gravity

Time (second)	Population of <i>C.reinhardtii</i> in area (cell/mm ²)		
	A	B	C
0	197.71	354.06	312.97
5	210.10	358.95	297.72
10	214.27	352.18	299.75
15	218.44	345.41	301.77
20	235.39	350.08	298.32

Microgravity

Time (second)	Population of <i>C.reinhardtii</i> in area (cell/mm ²)		
	A	B	C
0	99.46	225.56	194.22
5	128.25	224.17	187.73
10	130.32	228.59	194.06
15	134.47	237.43	206.71
20	136.54	241.84	213.04

Table 1 Population of *C.reinhardtii* in area (cell/mm²) under normal gravity and microgravity condition.

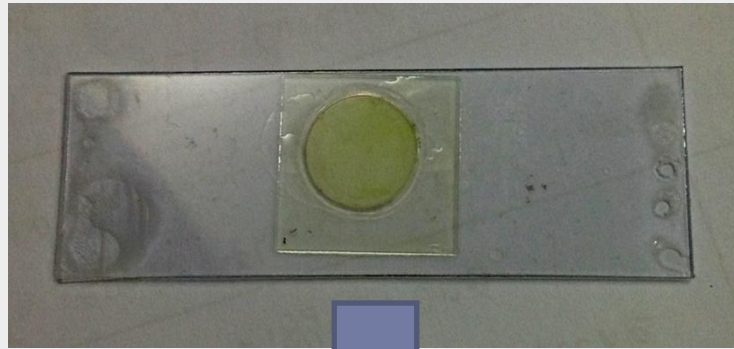
The populations of *C. reinhardtii* in each area have differentiated in every time. That's mean the population of *C. reinhardtii* have movement.

Method

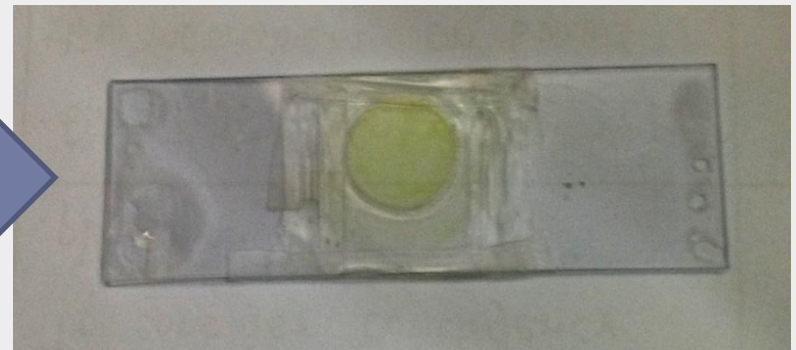
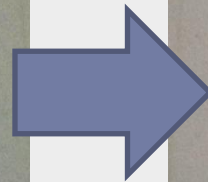
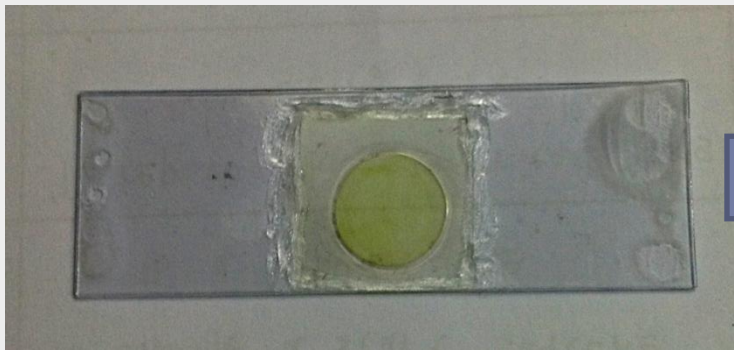
How to prepare the sample on slide



- Drop a sample on acrylic slide by micropipette and cover



- Seal with petroleum jelly and bind with parafilm for preserve the sample



Introduction

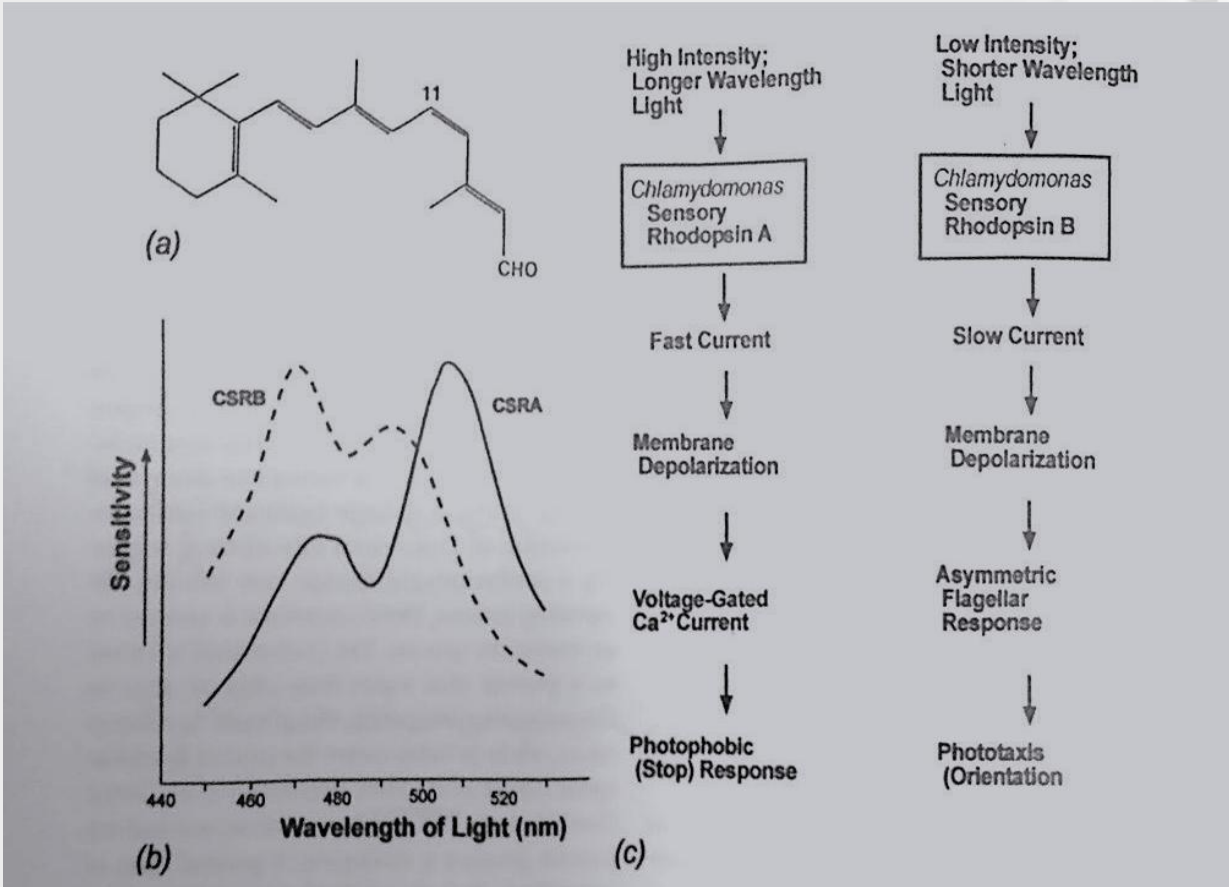


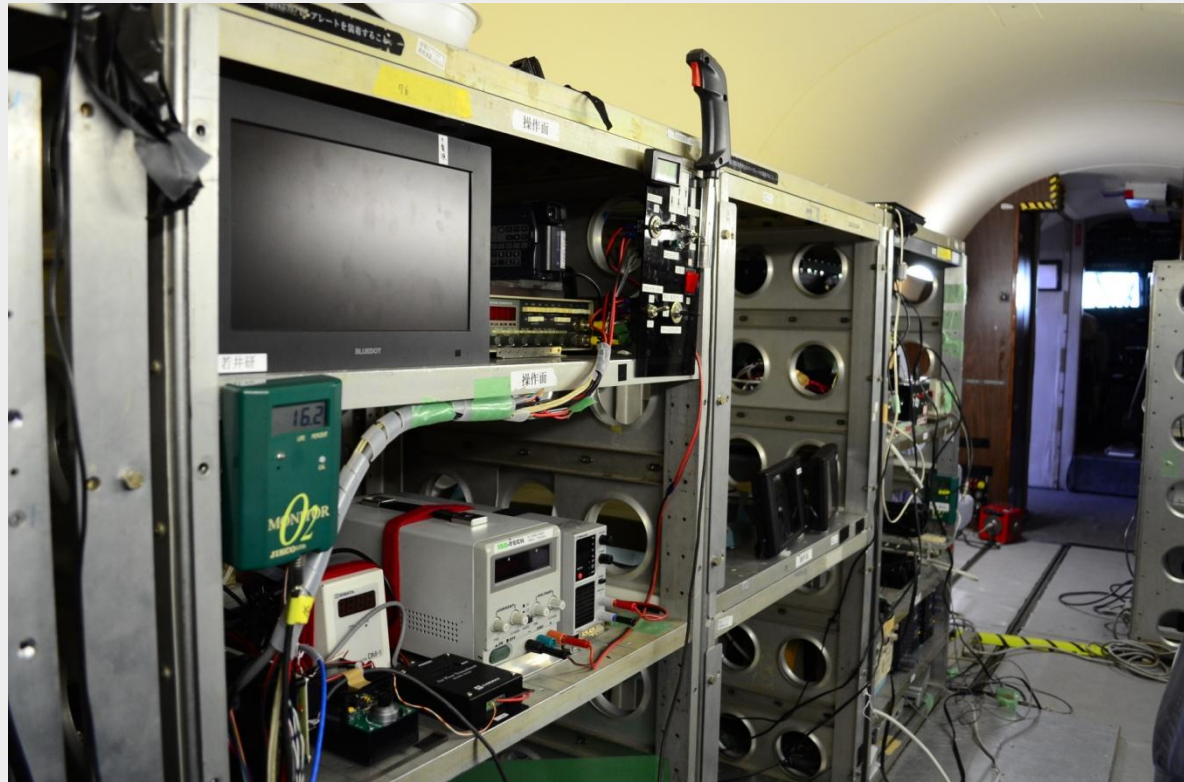
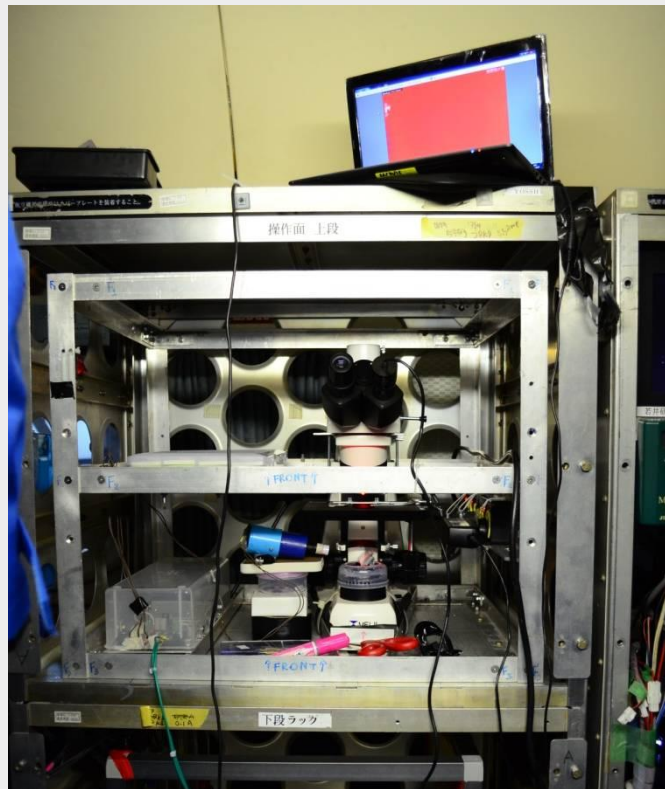
Fig.2 (a) the part of structure photoreceptor molecule in *Chlamydomonas*.

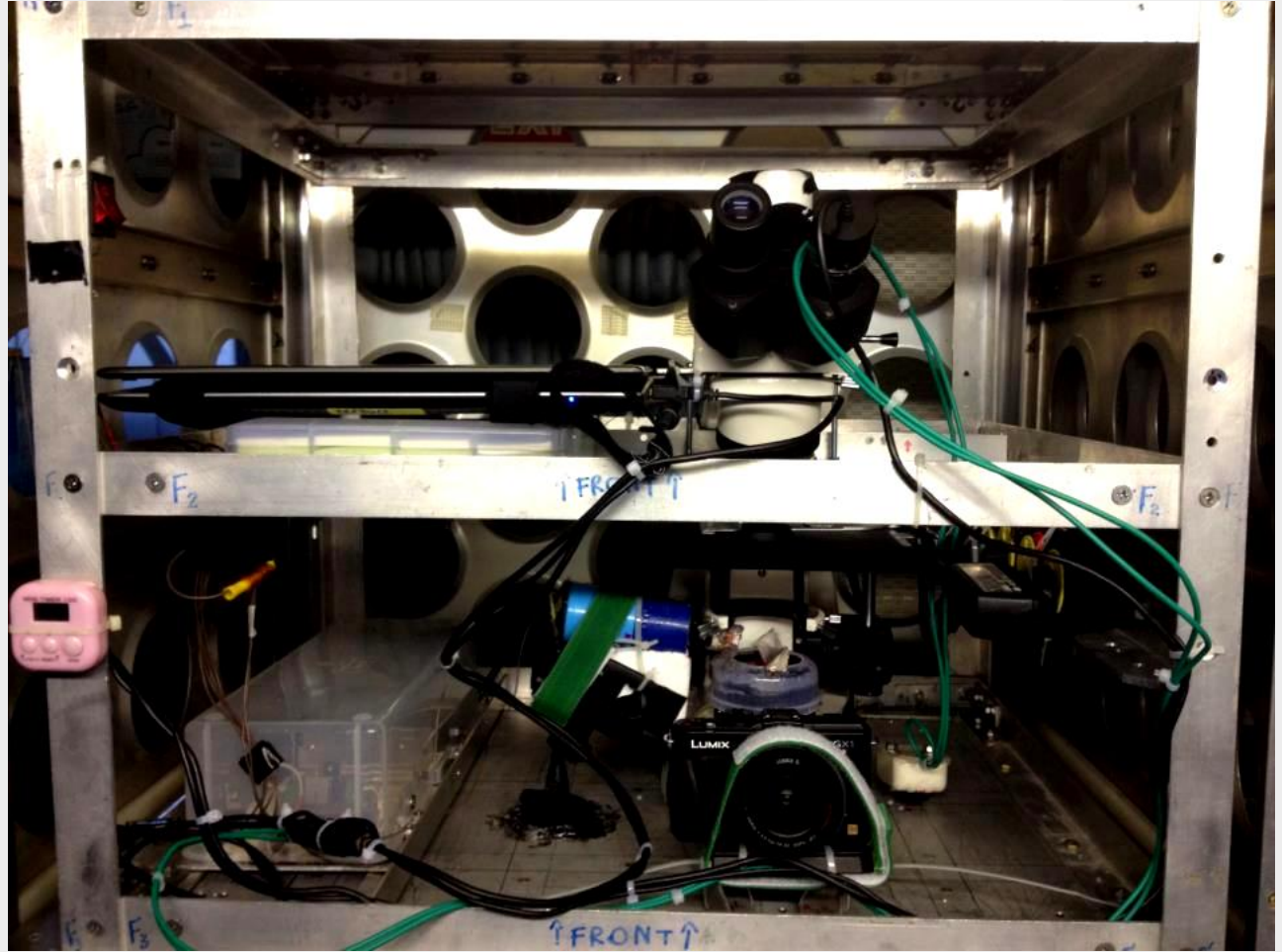
(b) action spectra showing the sensitivity of photoreceptors *Chlamydomonas* sensory rhodopsin A and B.

(c) scheme of light signal transduction initiated by the two sensory rhodopsin pigments in *Chlamydomonas*. (Robert Edward lee, 2008)

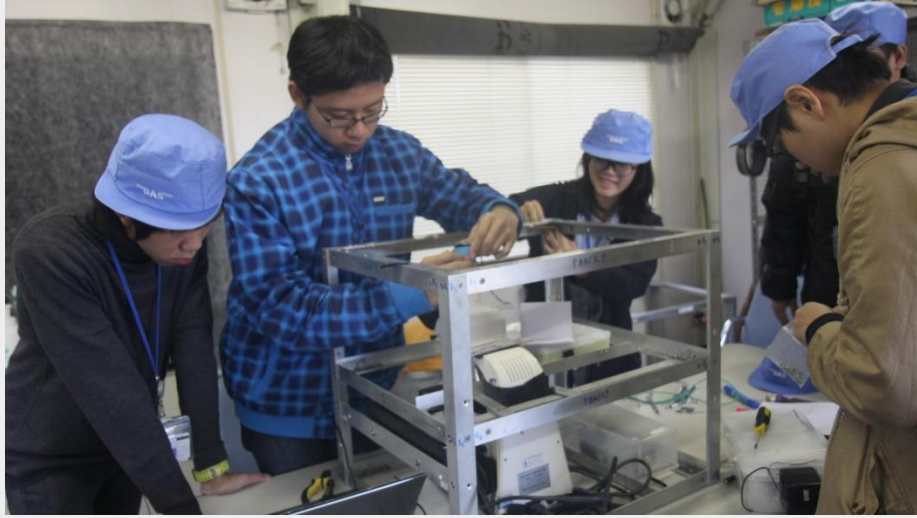


Time (seconds)	Differentiation rate of population of <i>C.reinhardtii</i> (%)	
	normal gravity	microgravity
0	0	0
5	2.77	24.98
10	3.61	32.28
15	4.46	46.88
20	13.25	54.18













**The study of *Chlamydomonas reinhardtii*'s ability
to movement in microgravity condition.**

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2012

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We are greatly thankful to Professor Masahiro Ishiura and Assistant Professor Takuya Matsuo for the algal sample, in experiment we use a sample, *Chlamydomonas reinhardtii* was cultured from genetic laboratory, Nagoya University and was contacted by Professor Muneo Takaoki.

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Table of Contents

Experiment background.....	4
Introduction	
Purpose	
Expected results	
Application	
Materials and Method.....	9
On board experiment procedure.....	15
Result and discussion.....	17
Conclusion and further perspective.....	21
Reference.....	22

Experiment background

Introduction

In everyday life, the energy likes a one part of our life. Moreover, in the globalization, technology rapidly growth and most of it have to use energy. That emphasizes the important of energy. In contrast, human use more energy, energy resources are rapidly less. Now, we have to concern about management the energy and find the new energy resources. The space exploration is one that use huge amount of energy, we have to contain enough energy to launching, living and come to earth again. H₂ from Chlamydomonas is the one choice of the alternative clean energy that is a renewable energy resource. Then we choose to study Chlamydomonas in microgravity as a model for using in space.

Chlamydomonas is a unicellular, biflagellate organism that can be easily grown and manipulated in culture. The uninucleate cells have a cup-shaped, basal chloroplast with a central pyrenoid. There are two contractile vacuoles at the base of the flagella, and there is an anterior eyespot in the chloroplast. (Fig.1) Most flagellate green algae exhibiting phototaxis posses a singular specialized light sensitive organelle. (Robert Edward lee, 2008)

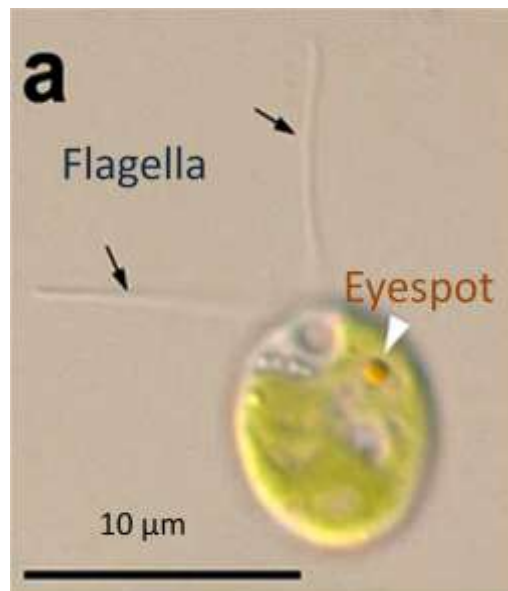


Fig.1 *Chlamydomonas reinhardtii* with biflagellate and eyespot (Georg Kreimer, 2008)

Chlamydomonas reinhardtii was used since this species had been widely studied and would serve as a reasonably good model for other algae. The eyespot apparatus of *C. reinhardtii* has emerged as the model organism for investigating H₂ metabolism in water-oxidizing, photosynthetic eukaryotes, and substantial progress has been made in explaining the mechanisms of H₂ production, as well as in improving H₂ photoproduction activity (Matthew C. Posewitz et al., 2008)

Green algae can also show geotactic response to gravity. *Chlamydomonas* exhibits negative geotaxis by swimming against gravity. Such a feature would be selected for in evolution because when the algal cell (which is heavier than water) is confronted with darkness, it must move up to the surface in order to obtain light for growth and reproduction. (Robert Edward lee, 2008)

C. reinhardtii contains both chlorophyll a and b as well as associated pigments with the greatest amount of light energy being absorbed in the red and blue regions of the spectrum. Rhodopsin, a blue–green photoreceptor with peak absorption around 400-500 nm, has been determined to be used in the phototaxis of algae and is largely contained in the distinctive “eye-spot” for *C. reinhardtii*. There are known to have a blue light receptor that controls the production of several essential enzymes and catalyst specific stages in reproduction and gamete formation. It has been suggested that a red light receptor exists but one has yet to be found, despite it being demonstrated that red light plays a role in gene regulation and development, while having a lesser role in gamete formation (Sara S. Kuwahara et al., 2011).

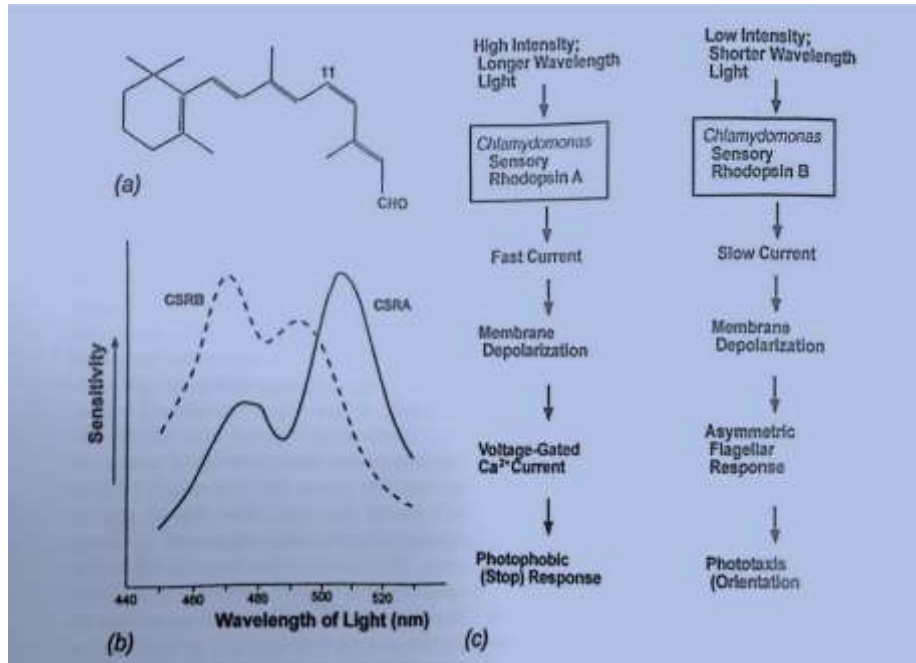


Fig.2 (a) The part of structure photoreceptor molecule in Chlamydomonas.
 (b) Action spectra showing the sensitivity of photoreceptors Chlamydomonas sensory rhodopsin A and B.
 (c) Scheme of light signal transduction initiated by the two sensory rhodopsin pigments in Chlamydomonas. (Robert Edward lee, 2008)

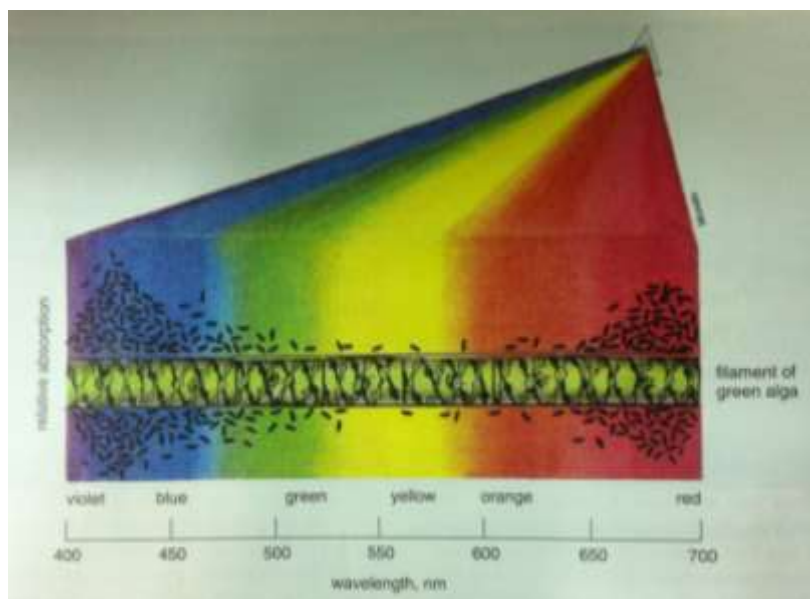


Fig. 3 Engelmann's experiment. A tinny spectrum of light was focused on a microscope slide with a row of algal cells suspended in water containing bacteria that move toward to an oxygen source. The bacteria assembled mostly in the areas exposed to the blue and red portions of the spectrum. But the blue portion is wildly wavelength has the bacteria lives. (Stern, Kingsley Rowland, 2003)

In this project, we will study the movement of *C.reinhardtii* to the light by using blue laser light makes a point on center of field and make a background light is red. We chose the blue light because Chlamydomonas has two rhodopsins: Chlamydomonas sensory rhodospin A and B that can absorbs light maximally at 510 and 470 nm. (Robert Edward lee, 2008) The result was analyzed from the population of *C.reinhardtii* to that move in responded to blue laser light

In the result, we aim to know the chance to survival of *C.reinhardtii*. By the way, phototaxis behavior can tells that *C.reinhardtii* will have photosynthesis and produced the food for cell living and that mean opportunity to culture *C.reinhardtii* in the space for use the benefit from it such as the bio-resource that can produce clean energy such as Hydrogen, biodiesel and material for food.

Purpose

To study how gravity affect movement of *Chlamydomonas reinhardtii* to light for photosynthesis and survival. These study lead to know the probability to culture the *C. reinhardtii* in microgravity for investing H₂ metabolism.

Expected result

Quantitative data of the difference of the *Chlamydomonas reinhardtii*'s movement between in microgravity and normal gravity

Application

To understand the movement of *Chlamydomonas reinhardtii* to light under microgravity condition. This would help we know possibility to culture *C. reinhrdtii* in space as the suitable renewable energy resources from bio-resource because the *C. reinhardtii* can produce H₂, the clean energy.

Materials and Method

Materials

The experiment set up consists of three main part as organism part, observing part and light source part.

1. Experiment/test item

1.1 Organism part

1.1.1 *Chlamydomonas reinhardtii* (mt+)

1.1.2 TAP medium for culturing *C. reinhardtii*

- Tris base
- FBS (Filner's Beijerincks Solution)
- KPO_4 mix
- Trace mineral solution
- Glacial acetic acid
- H_2O

1.1.3 Collecting boxes for keeping the sample slides

1.2 Observing part

1.2.1 Light microscope (MEJI MT4200L)

1.2.2 Microscope eye-piece camera (Dino-eye AM7023, 5megapixels USB2.0)

1.2.3 Mini Notebook w/10" screen (acer)

1.3 Light source part

1.3.1 Blue laser pointer 5mW

1.3.2 Red filter (HOYA 25a 58mm) for changing the color light from light microscope to be red background

1.3.3 Power supply

1.3.4 Laser base

1.3.5 Adaptor

1.3.6 Acrylic sheet for reflex the blue laser

1.3.7 Power strip

1.3.8 Switch of laser

1.3.9 White LED for lighting while working

1.3.10 Switch of white LED

2. Mechanical design

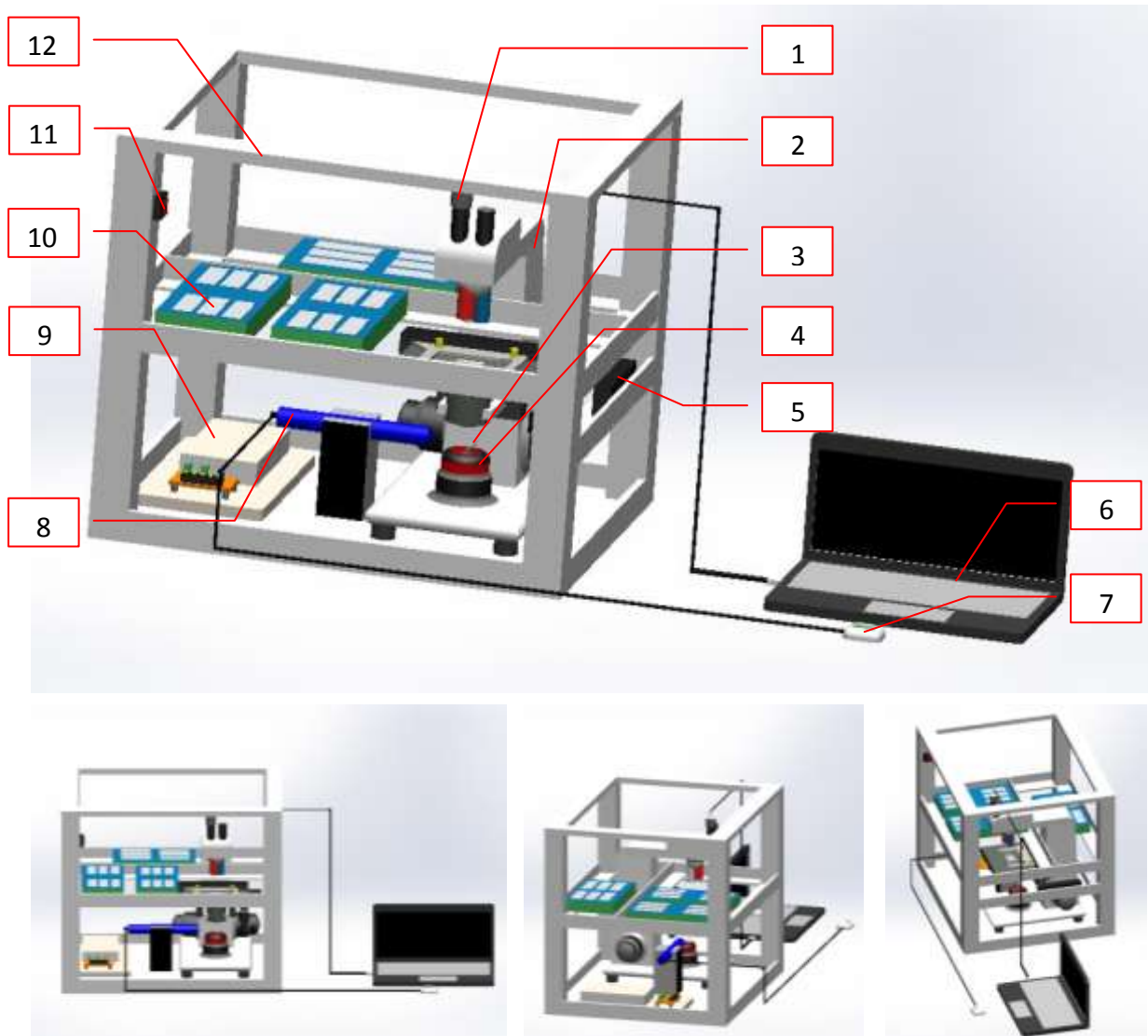


Fig. 4 Diagram of the mechanical design.

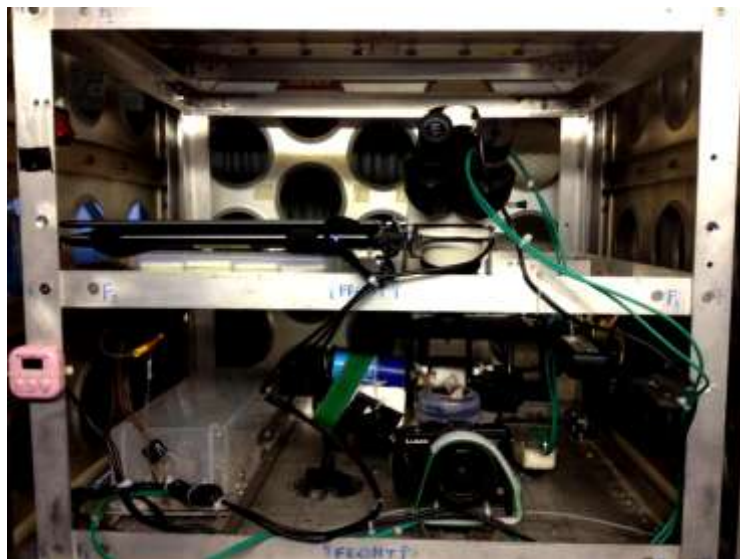


Fig. 5 Mechanical part of experiment

Part of Mechanical design

1. Microscope eye-piece camera (Dino-eye AM7023, 5megapixels USB2.0)
2. Light microscope (MEJI MT4200L)
3. Acrylic sheet for reflex the blue laser
4. Red filter (HOYA 25a 58mm) for changing the color light from light microscope to be red background
5. Power strip
6. Mini Notebook w/10" screen (acer)
7. Switch of laser
8. Blue laser pen 5mW
9. Power supply
10. Collecting boxes for keeping the sample slides
11. Switch of white LED
12. White LED for lighting while working

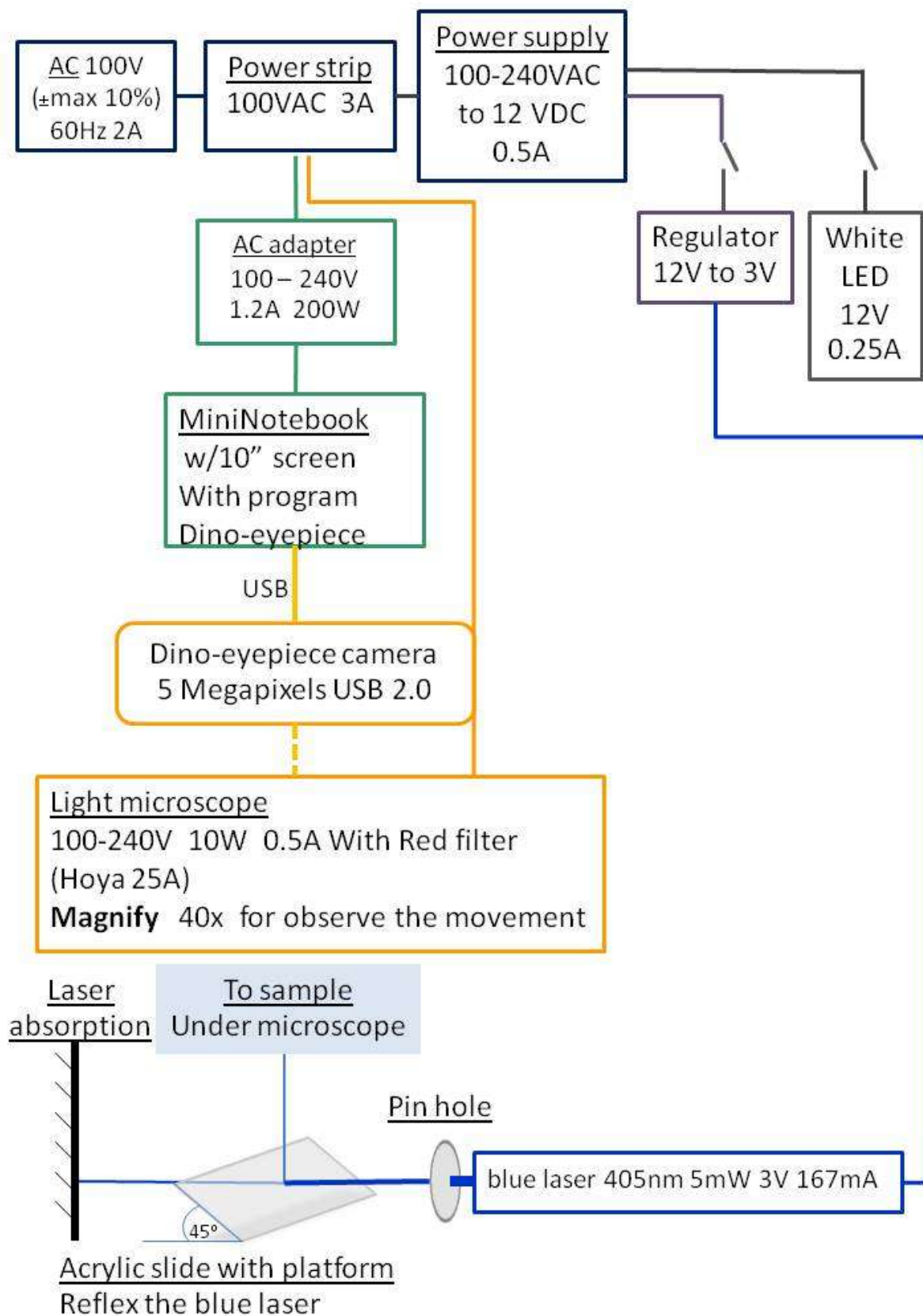


Fig. 6 Block diagram of the experiment

Method

Cell culture

Chlamydomonas reinhardtii was obtained from algal culture collection of Nagoya University, and grown in static culture in Tris-Acetate-Phosphate (TAP) medium. Algal are kept in 100 ml Erlenmeyer flasks at 25°C with continuous light from fluorescent lamps, the flasks are placed. Cell density was about 10^5 - 10^6 cell/ml.

Parabolic flight

For obtaining microgravity (μG ; the term microgravity does not strictly refer to 10^{-6}g but is used in general for diminished gravity), a modified airplane JA8431 gulfstream was used. The aircraft was operated by the Japan cooperation Diamond Air Service (DAS). The 20 times parabolas were carried out on two flight days.

During the flight, cells were kept in a custom-made acrylic slide. A cover slip was glued with petroleum gel and bind with parafilm for saving moisture. The slide dept was about 0.5 mm and area approximately 1.5 mm^2 , yielding a total volume about 0.75 mm^3 . The custom-made acrylic slide was kept in collecting boxes with spongy for reducing the vibration in the flight.

The laser beams are high density distribution of energy, high directivity, mono wave length light and coherency. In consequence of this suitable ability, the laser was used as light source because it certainly controlled. Laser light is mono wavelength, the frequency that effective for algal were used for getting high response rate of algal. Basic biological study shows that the wavelength between 400-500 nm is the most effective for algal. Red filter was used for background illumination since light over 600 nm is not affected to algal (Akito et al., 2005)

For observation the experiment, we used the light microscope connected with microscope eye-piece camera. The microscope eye-piece camera was connected to computer and collecting the data with dino capture 2.0 programs in video capturing mode and capture the picture from video to analyze.

Analysis

Capture the picture from video in JPEG format file in every 5 seconds (0, 5, 10, 15, 20 s). Each pictures were specify the area in 3 parts as A, B and C by Photoshop CS3. The 3 parts of each picture are A as laser area, B as the ring area around the laser area and C as the ring area around B area. The numbers of population of *C. reinhardtii* in each area, that we specify, were found with Image J program. The differentiations of population of *C. reinhardtii* were related in line graph. The differentiations of population of *C. reinhardtii* were compared between under micro gravity and normal gravity condition.

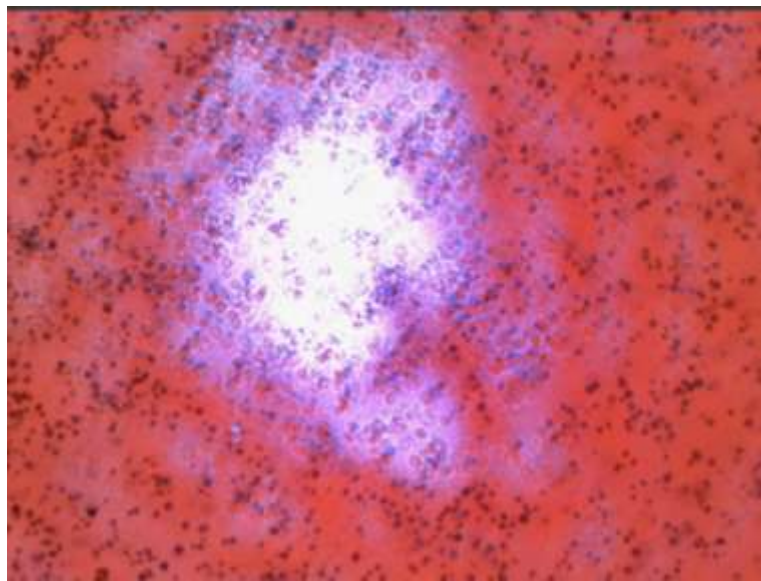


Fig. 7 The picture captured from video for specifying area.

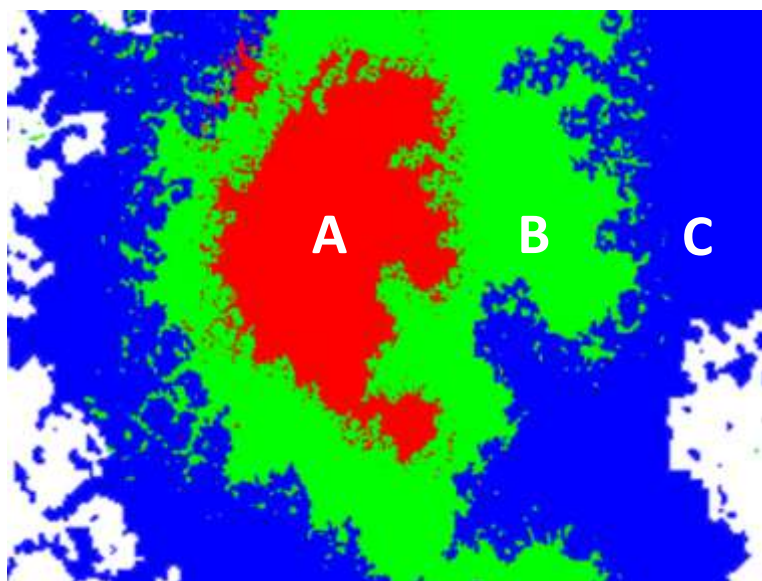


Fig. 8 The areas that were specified in 3 parts as A, B and C

Onboard experiment procedure

In microgravity, the experiment was recorded video clip for observing the movement of *C. reinhardtii* to the blue laser light and in normal gravity, we used the same experiment procedure.

From the video clip were analyzed and compared the experiment how the movement of *C. reinhardtii* different in microgravity and normal gravity.

State of aircraft	G state	Work item	Time required
After aircraft taking-out from hangar and before engine start	Ground	(With or without the request of power supply)	About 20 minutes
Engine start (No power supply during this time) ↓	1G		About 5 minutes
Takeoff ↓ Experiment airspace Arrival	1G	-Turn on all power strip switch -Turn on the light microscope -Turn on blue laser (for checking) -Connect Eye-piece Camera USB to notebook	20 minutes to K airspace 35 minutes to G airspace
" 2 minutes before starting 1st PF" ↓	1G	-Set the slide on microscope -Find the picture and focus	1 minute
"1 minute" ↓	0.5 - 1.2G	-lock adjustment knob -recheck again	30 seconds
"30 seconds" ↓	2G	-back to sit on chair	10 seconds in 0.5-1.2G 20 seconds in 2G

"NOW"	μG	-Start to record VDO on notebook -turn on blue laser	20 seconds
End of μG ↓ Normal flight	1.5G	-turn off blue laser -finish to record VDO on notebook, save file	20 seconds
The following experiment preparation ↓ (Returns to "2 minutes before 2nd PF" call. And repeat PF till predefined times.)	1G	-Change the slide on microscope -Find the picture and focus	3 minutes
Departure from experiment airspace ↓ Landing	1G	- Turn off the light microscope - Turn off all power strip switch	20 minutes from K airspace 35 minutes from G airspace

" " : Call from a pilot []: call from an experimenter

Result and Discussion

From the experiment, the pictures captured from video were specified in A, B and C (A is the laser area, B is the ring area around the laser area and C is the ring area around B area). Each area were found the population of *C. reinhardtii* in every 5 seconds (0, 5, 10, 15, 20 s).

Time (second)	population of <i>C.reinhardtii</i> in area (cell/mm ²)		
	A	B	C
0	99.4661	225.5690	194.2243
5	128.2539	224.1776	187.7370
10	130.3261	228.5952	194.0645
15	134.4705	237.4306	206.7198
20	136.5427	241.8483	213.0475

Table 1 Population of *C.reinhardtii* in area (cell/mm²) under microgravity condition.

Time (second)	population of <i>C.reinhardtii</i> in area (cell/mm ²)		
	A	B	C
0	197.7167	354.0619	312.9778
5	210.1095	358.9551	297.7295
10	214.2769	352.1842	299.7526
15	218.4444	345.4133	301.7758
20	235.3991	350.0894	298.3280

Table 2 Population of *C.reinhardtii* in area (cell/mm²) under normal gravity condition.

From the table 1 and 2, show the populations of *C. reinhardtii* in each area have differentiated in every time. That's mean the population of *C. reinhardtii* have movement. From this data, we calculate percentage of differentiation rate of population as show in table 3-4 and fig. 9-10.

Time (seconds)	Differentiation rate of population of <i>C.reinhardtii</i> (%)		
	A	B	C
0	0	0	0
5	28.9423	-0.6168	-3.3401
10	31.0256	1.3416	-0.0823
15	35.1923	5.2585	6.4336
20	37.2756	7.2170	9.6915

Table 3 Differentiation rate of population of *C.reinhardtii* (%) in microgravity condition.

Time (seconds)	Differentiation rate of population of <i>C.reinhardtii</i> (%)		
	A	B	C
0	0.0000	0.0000	0.0000
5	6.2680	1.3820	-4.8720
10	8.3758	-0.5303	-4.2256
15	10.4835	-2.4427	-3.5792
20	19.0588	-1.1220	-4.6808

Table 4 Differentiation rate of population of *C.reinhardtii* (%) in normal gravity condition.

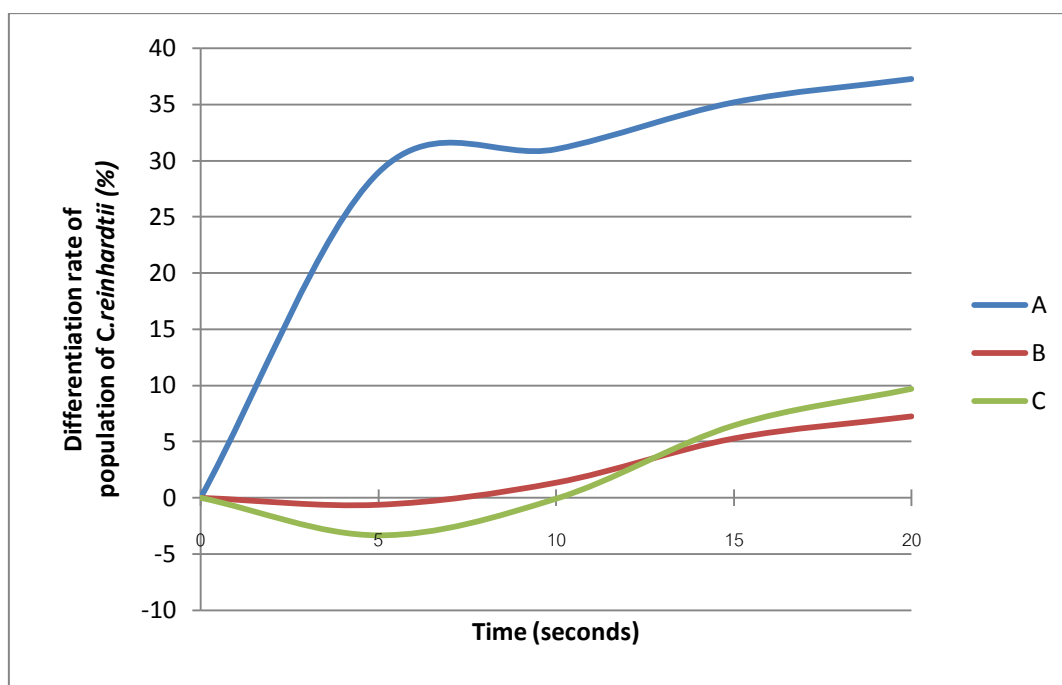


Fig. 9 Differentiation rate of population of *C.reinhardtii* (%) in microgravity condition.

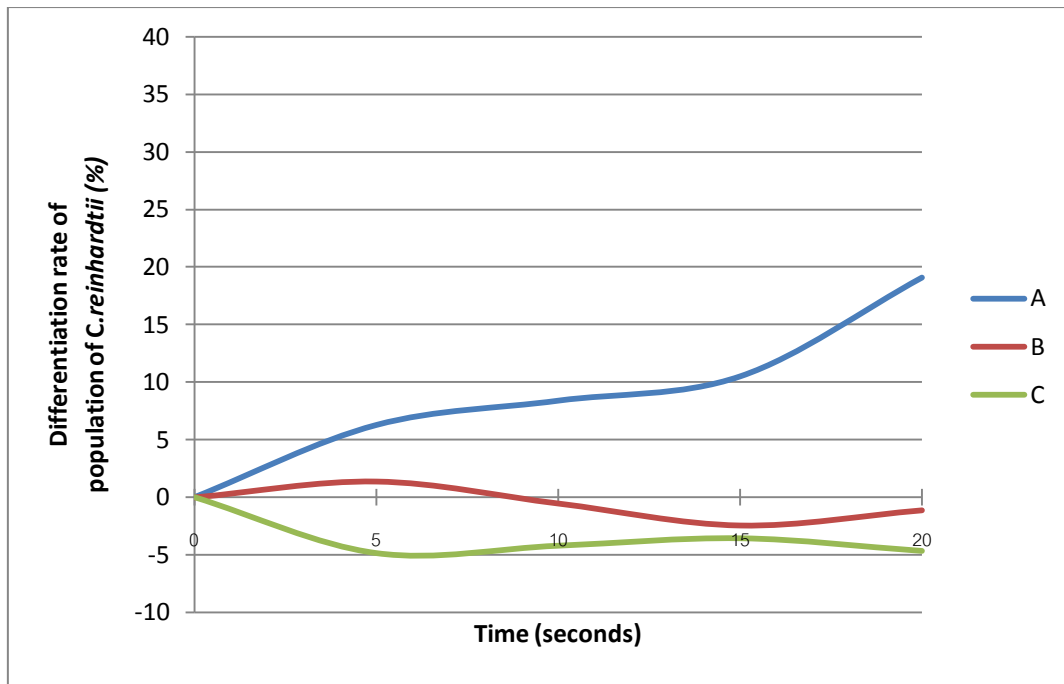


Fig. 10 Differentiation rate of population of *C.reinhardtii* (%) in normal gravity condition.

From the fig. 9 and 10, graphs show the result under microgravity and normal gravity condition. In A area, we found that the population rate of *C. reinhardtii* have increasing in order because A area is the blue laser area, the frequency that effective for algal, the algae have blue-green photoreceptor in eye spot that effect with the blue light from the laser, phototaxis, then *C. reinhardtii* move to A area. When 20 seconds ago, in microgravity differentiation rate of population of *C. reinhardtii* is 37.2756% and in normal gravity is 19.0588%.

From the fig. 9 and 10, graphs show the differentiation rate of population in B and C area under microgravity condition, that decrease in 5th second and increase in 10th -20th second, which in B area increase to 7.2170% and in C area increase to 9.6515%. In normal gravity, both B and C area have the differentiation of population decrease and increase also. In 20th second, the rate of population decrease from beginning time, which B area has the rate of population decreases to 1.1220% and C area decreases to 4.6808%.

From the result, in microgravity, the populations in B and C area decrease in 5th second because *C. reinhardtii* in B and C area move to blue laser in A area. In 10th -20th second, the *C. reinhardtii* out of area that we specified move to B and C area for be nearly blue laser light. That made population in B and C area increase. In normal gravity, when 20 seconds ago, the population in B and C area have the decreasing rate because the population in B and C area move to blue laser light in A area more than the *C. reinhardtii* out of area that we specified move to B and C area. Then, in 20th second the population in B and C were decreased.

In order that, the population in microgravity increases more than in normal gravity because movement of *C. reinhardtii* in microgravity is better than normal gravity as a result of in microgravity does not have gravity force affect to algae, then the algae can move more independent than in normal gravity.

Conclusion and further perspectives

The result shows that gravity affects the *Chlamydomonas reinhardtii* moving to light. Under microgravity condition, in every area have the higher increasing rate of population. This result indicated that the *C. reinhardtii* has ability to moving to light under microgravity condition better than normal gravity condition because movement of *C. reinhardtii* in microgravity does not have gravity force affect to algae, then the algae can move more independent than in normal gravity.

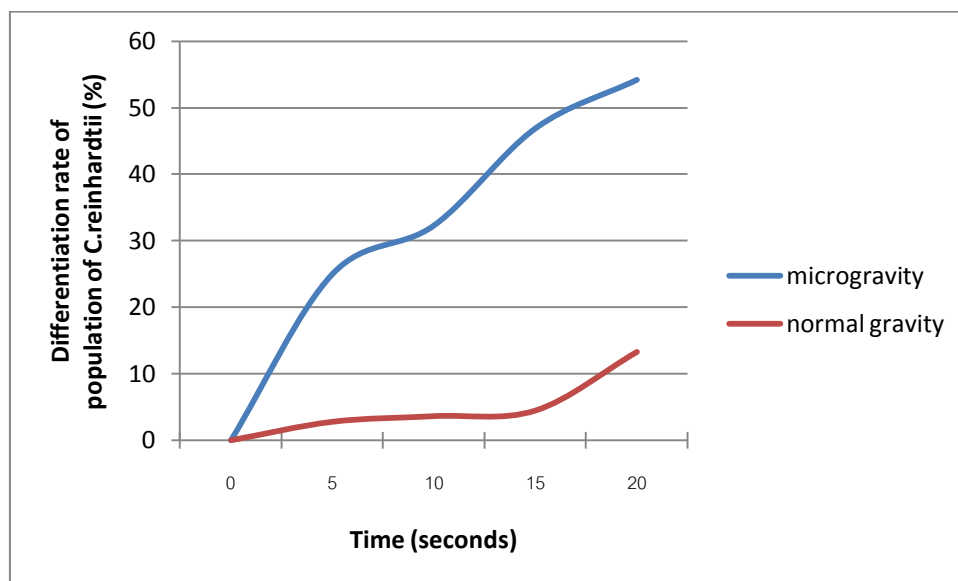


Fig. 11 Differentiation rate of population of *C.reinhardtii* (%) between in microgravity and normal gravity condition.

The experiment show that *C. reinhardtii* have behavior moving to light in microgravity, that mean *C. reinhardtii* probably growth in microgravity because phototaxis behavior for photosynthesis and survival mean opportunity to culture *C.reinhardtii* in the space for use the benefit from it such as Hydrogen, the clean energy. Moreover, *C. reinhradtii* is a unicellular algal as a beginning model organism before evolution to higher plant. Then, *C. reinhardtii* was interesting to study the evolution and adaptive behavior in microgravity and *C. reinhardtii* is the algae model that well known the genetic sequence.

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