SEA MYSTERY

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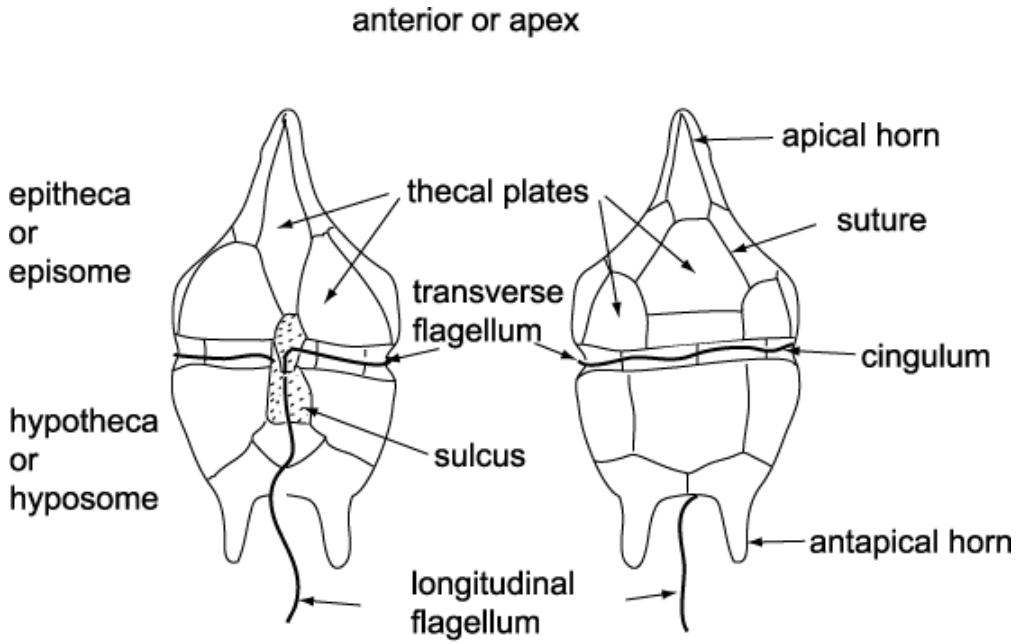
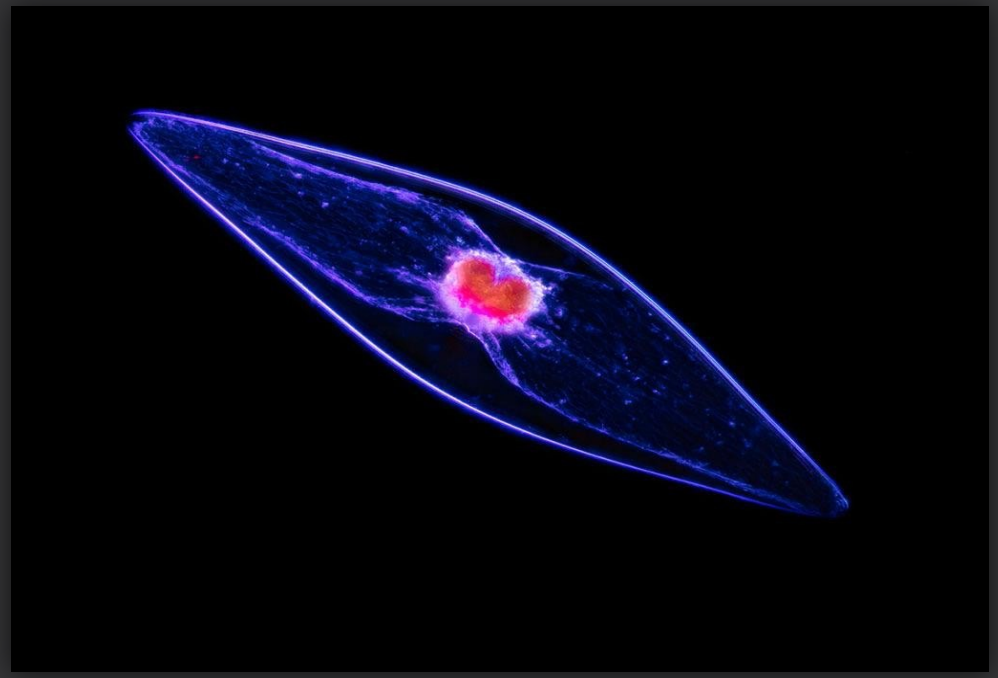
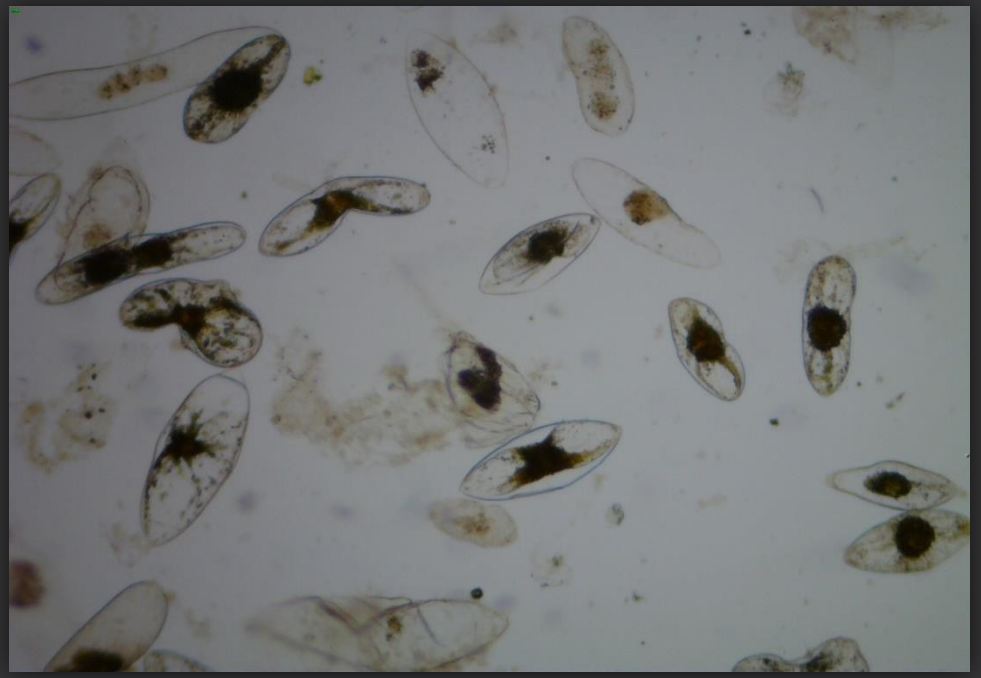
Pyrocystis fusformis is a tropical, epipelagic, marine dinoflagellate (flagellate microorganisms), reaching lengths of up to 1 mm. If chemically or mechanically irritated, it demonstrates the bioluminescence which is produced throughout the cytoplasm of this unicellular protist as a result of the *luciferin-luciferase reaction* in thousands of spherical-shaped organelles called *scintillons*. This produces bright blue color.

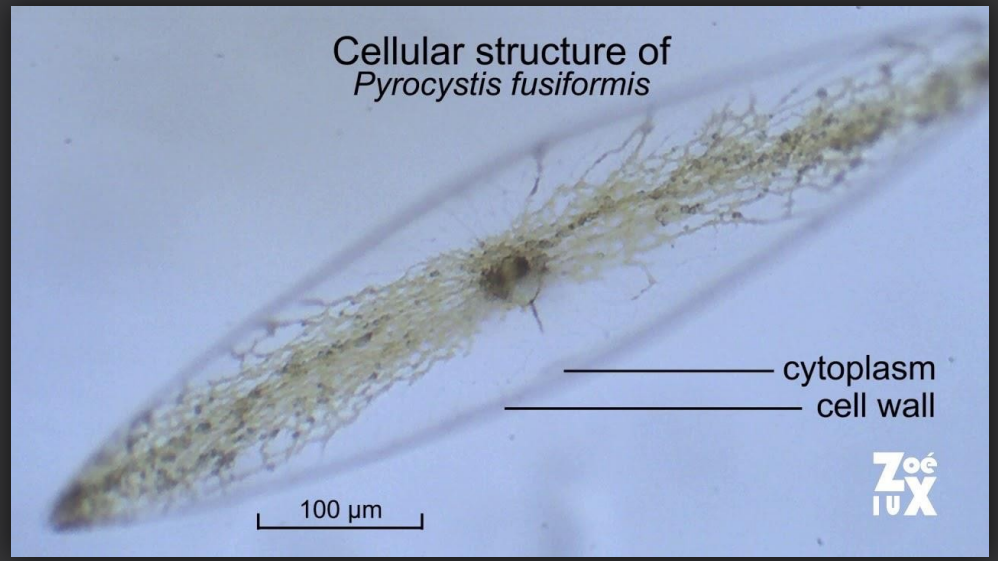


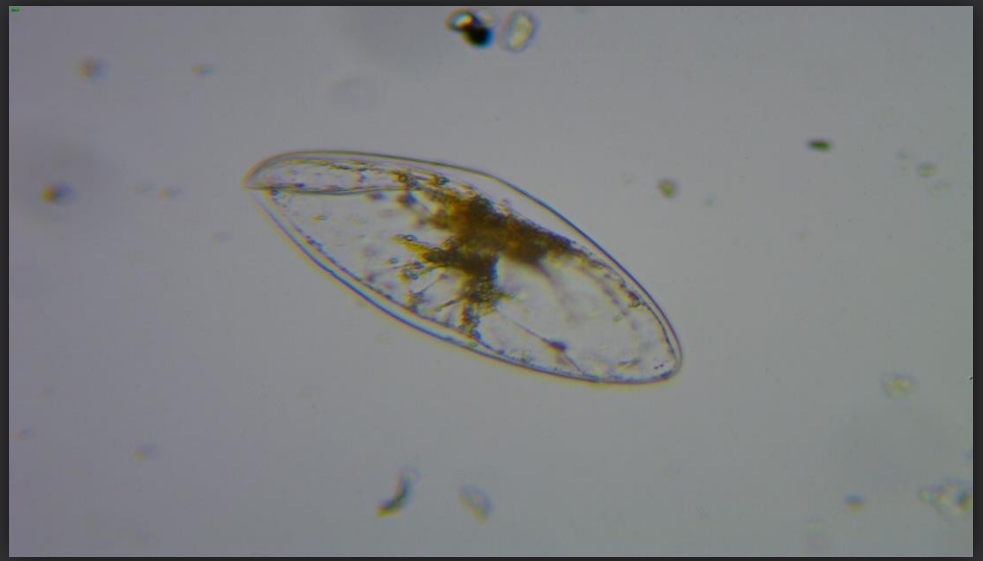
Principle features and terminology of a thecate, motile peridinialean dinoflagellate.

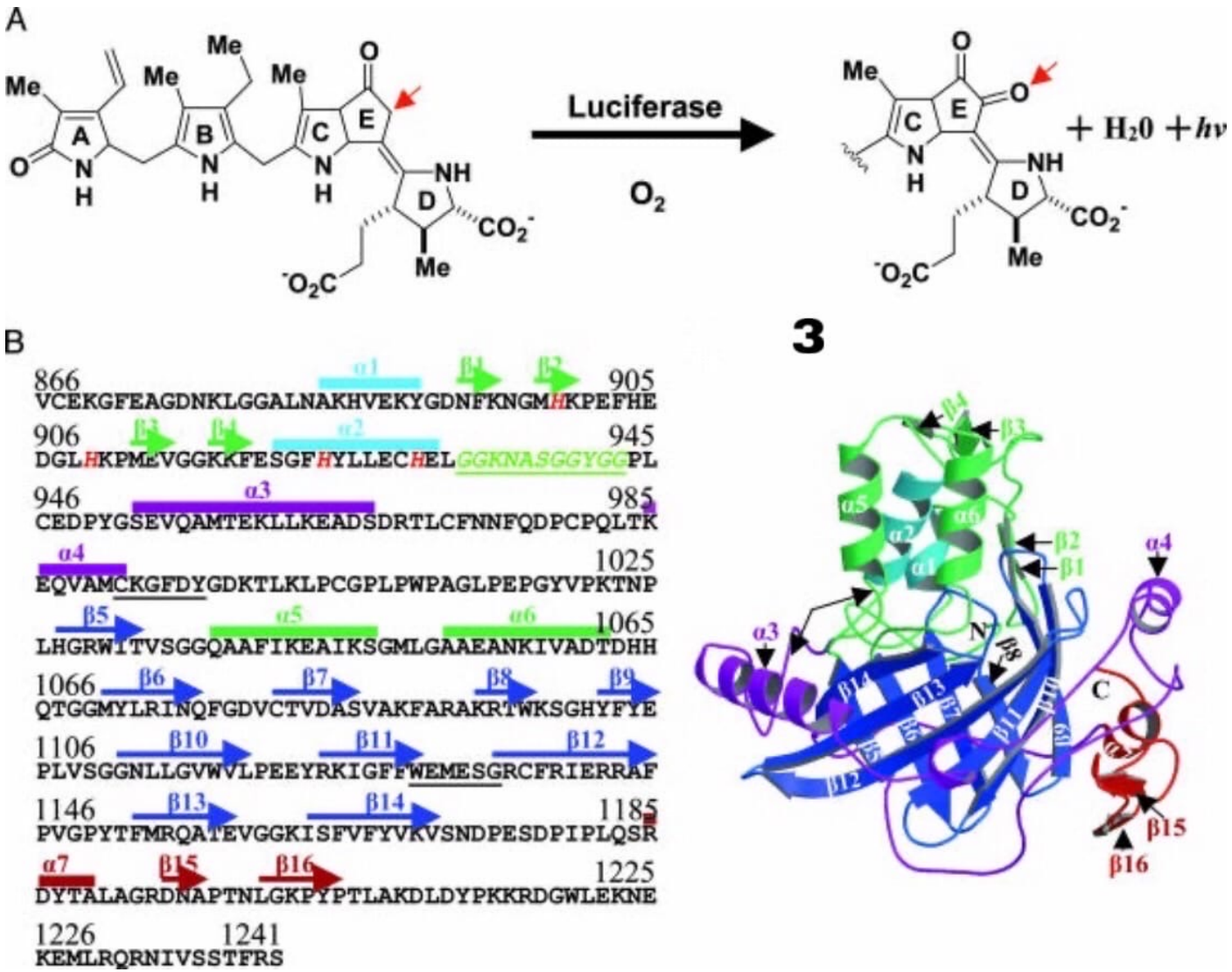
Redrawn from Fensome et al. 1996.






Cellular structure of
Pyrocystis fusiformis
cytoplasm
cell wall
100 µm




A
Luciferase
O_2
+ H_20 + hv
B
3
866 905
VCEKGFEAGDNKLGGALNAKHVEKYGDNFKNGMHKPEFHE
906 945
DGLHKPMEVGGKKFESGFHYLLECHELGGKNASGGYGGPL
946 985
CEDPYGSEVQAMTEKLLKEADSDRTLCFNNFQDPCPQLTK
1025
EQVAMCKGFDYGDKTLKLPCGPLPWPAGLPEPGYVPKTNP
1065
LHGRWITVSGGQAAFIKEAIKSGMLGAAEANKIVADTDHH
1066
QTGGMYLRINQFGDVCTVDASVAKFARAKRTWKSGHYFYE
1106
PLVSGGNLLGVWVLPEEYRKIGFFWEMESGRCFRIERRAF
1146 1185
PVGPYTFMRQATEVGGKISFVFYVKVSNDPESDPIPLQSR
1225
DYTALAGRDNAPTNLGKPYPTLAKDLDYPKKRDGWLEKNE
1226 1241
KEMLRQRNIVSSTFRS

Work aim and main tasks

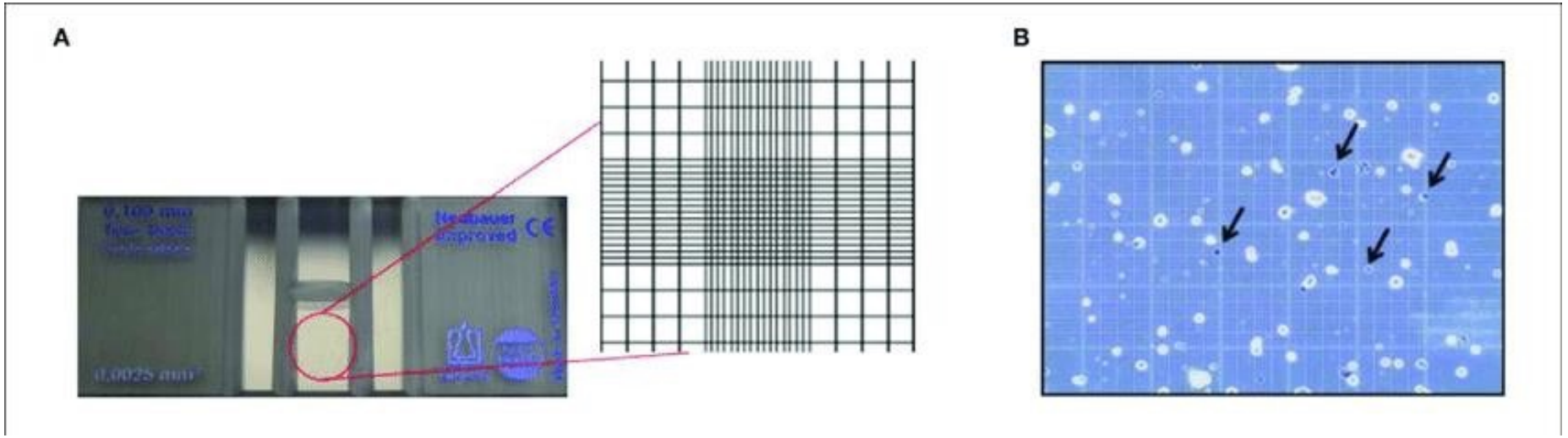
Work aim: to develop a way of demonstrating Pyrocystis fusiformis in the conditions of ‘Moskvarium’

Work tasks:

- Grow a culture of Pyrocystis fusiformis
- Develop a method for counting the population density
- Identify ways to safely
- activate bioluminescence ability Pyrocystis fusiformis
- Examine and analyze the bioluminiscent ability of Pyrocystis fusiformis
- Find out the recharging time
- Develop a model of mechanism which can effectively demonstrate P yrocystis fusiformis’ ability to exhibit bioluminescence in the condit ionf of ‘Moskvarium’

Counting the population density

- At first, we planned to use special cameras, such as the hemocytometer (the Goryaev's camera). It didn't work for us for some technical and financial reasons. Also the large size of the cells did not allow to put the cover slip without damage hemocytometer to Pyrocystis fusiformis.



Manual method

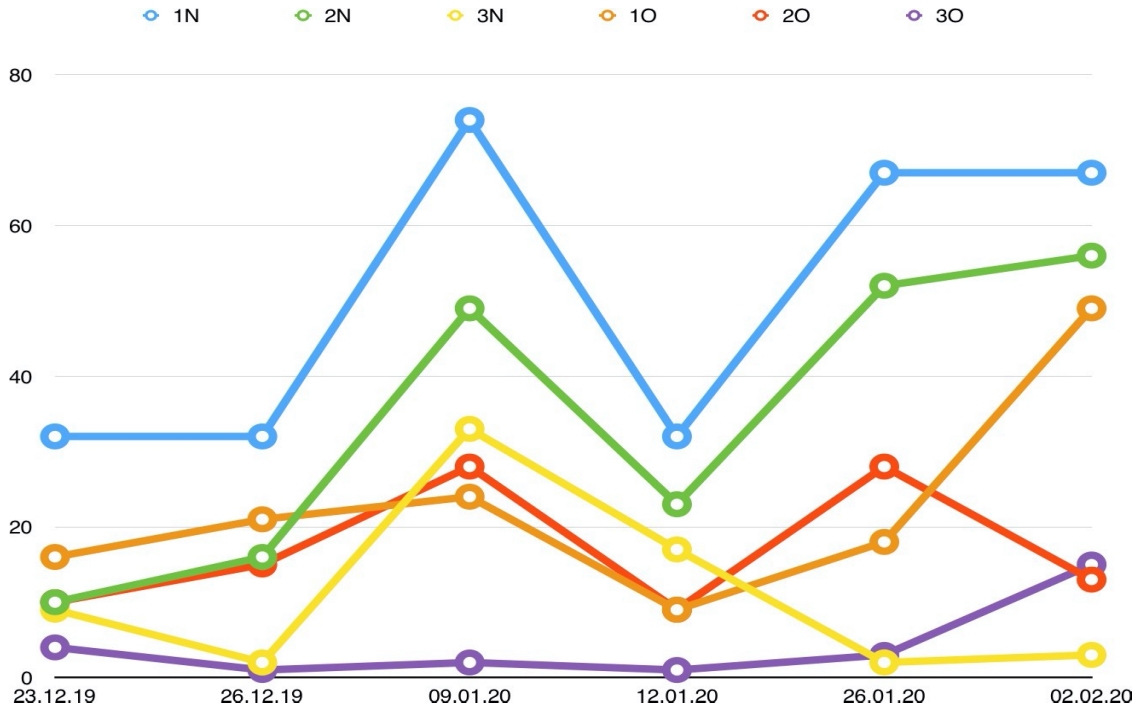
- In the end, we decided to count the density manually. We used automatic single channel pipettes and a Petri dish. The volume of 20 µl drop fit well, as such a drop was clearly visible on the binocular and the cells were also well visible. N stands for 'New', O stands for 'Old'
- '1 N' – 30 ml pure water + 30 ml Pyrocystis fusiformis
- '2 N' - 60 ml pure water + 60 ml Pyrocystis fusiformis
- '3 N' - 60 ml pure water + 60 ml Pyrocystis fusiformis
- '1 O' - 15 ml pure water + 15 ml Pyrocystis fusiformis
- '2 O' - 30 ml pure water + 30 ml Pyrocystis fusiformis
- '3 O' - 30 ml pure water + 30 ml Pyrocystis fusiformis

Date/ Concentration of Pyrocystis f.	23.12.19	26.12.19	09.01.20	12.01.20 #	16.01.20	26.01.20	02.02.2020
1 N	32	32	74	32	41	67	67
2 N	10	16	49	23	6	52	56
3 N	9	2	33	17	17	2*	3*
1 O	16	21	24	9	11	18	49
2 O	10	15	28	9	17	28	13
3 O	4	1	2	1	0	3	15

Table 1. Dynamics of Pyrocystis fusiformis culture growth in the condition of the "Phystech-lyceum"

*- organisms no more have an ability to demonstrate the bioluminescence

#-the dilution in the 1:1 ratio occurred
In other words, the greatest concentration of organisms was observed in the "1 N" vial, the lowest - in "3 O" vial



From this data we have come to a conclusion that '1N' concentration is the optimal one. In this particular concentration Pyrocystis fusiformis showed the highest reproduction ability. That is why we have chosen '1 N' concentration for the subsequent cultivation of Pyrocystis fusiformis as well as for the subsequent experiments,

Graph 1. Dynamics of Pyrocystis fusiformis culture growth in the condition of the 'Phystech-lyceum'

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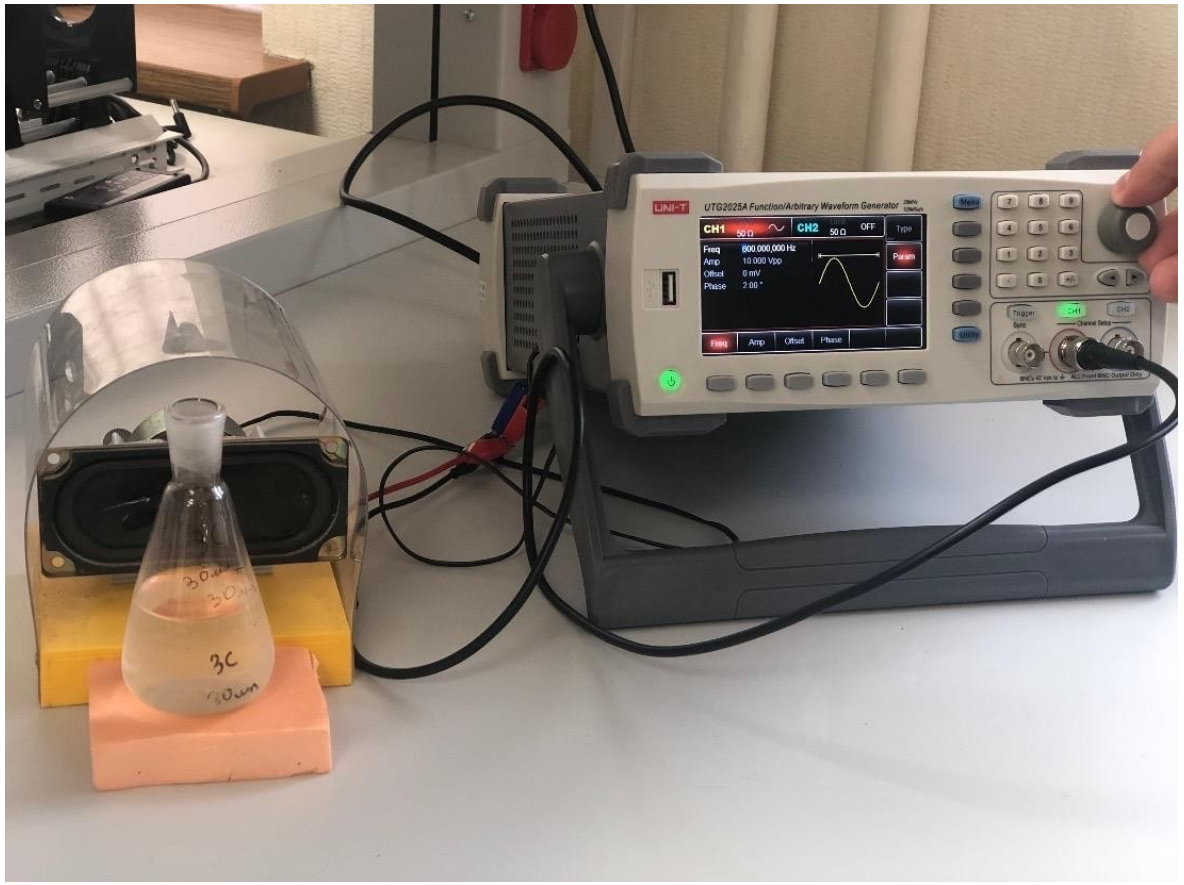
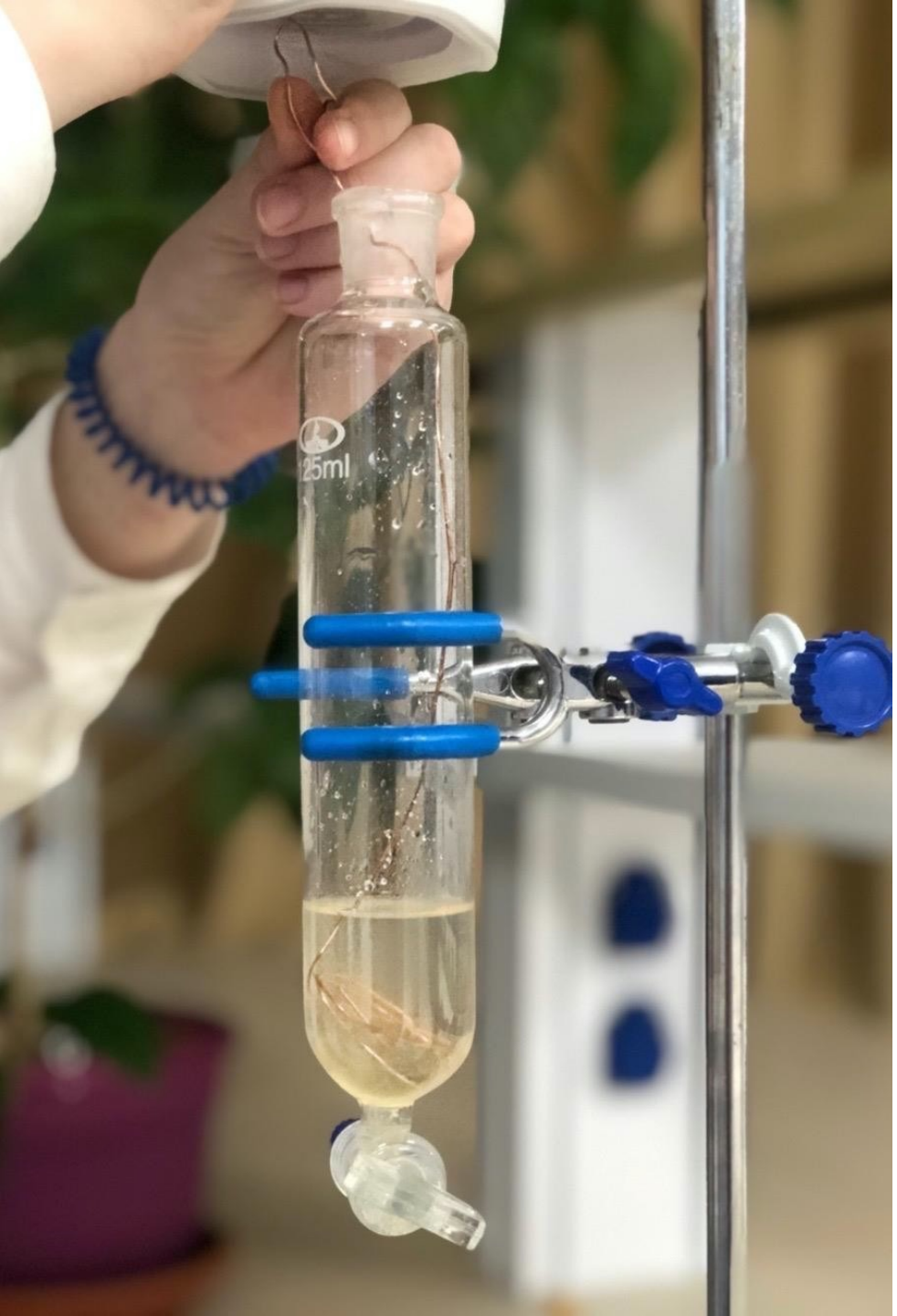
Experiment №1

- At first we have tried to change a flow of water. Here we have faced a big problem – the pump with the help of which we had wanted to change a flow did not work the way we wanted it to. Pyrocystis fusiformis exhibited bioluminescence only when squeezed out of the pump. Moreover, there is a lack of oxygen in the pump itself, so Pyrocystis fusiformis can not survive in these conditions

Experiment №2

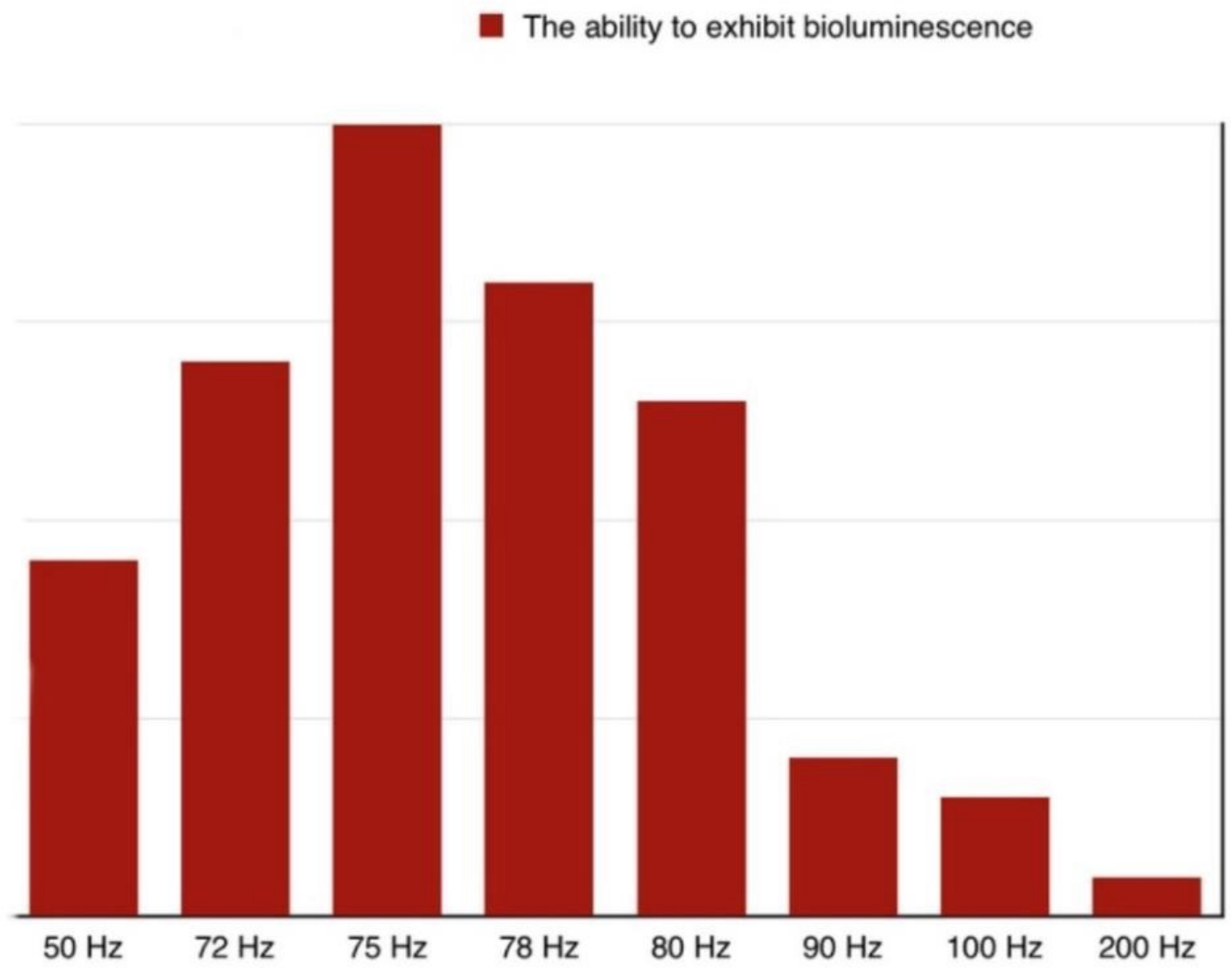
- Then we decided to try the ultrasound since it is also mechanical nature irritation. We took the ultrasound machine and affected Pyrocystis fusiformis with frequencies from infrasound to ultrasound. The experiment was conducted five times with absolutely no result. So, we have ascertained that this range has no impact on the ability of Pyrocystis fusiformis to emit light. '3 O' culture was used. Elaborating on this experiment, after 2 weeks the density of 3 'O' decreased from 15 to 0 algae per unit of water volume. There is no way for the cause of death but ultrasound frequency exposure. All other external factors always remained the same. Hence, we have revealed that ultrasound frequency is lethal for Pyrocystis fusiformis.





Experiment №3

- We took the separating funnel and then we placed there 25 ml of the ’1 N’ culture and a copper wire (vibration conductor). Afterwards we attached a portable Bluetooth speaker JBL Charge 4 which performed tracks of certain frequencies. We made a decision to proceed with 10 step. We have conducted the experiment with these frequencies: 200 Hz, 100 Hz, 90 Hz, 80 Hz, 78 Hz, 75 Hz, 72 Hz, 70 Hz, 50 Hz.



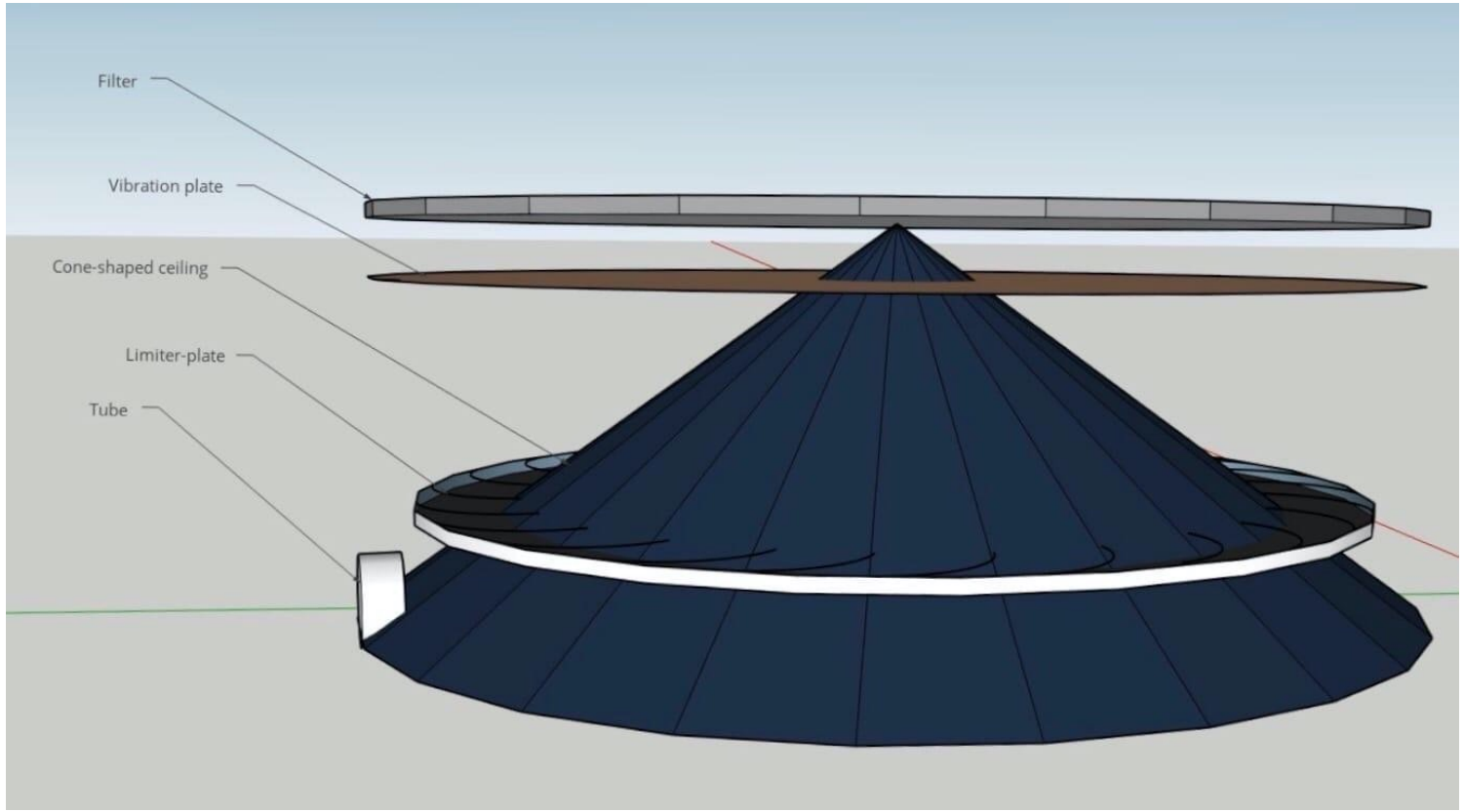
Accordingly, the brightest bioluminescence is observed under 75 Hz frequency, the most faint - under 200 Hz. Even a slight deviation from 75 Hz frequency results in a decrease in bioluminescence

Under 75 Hz Pyrocystis fusiformis showed a scattered bioluminescence. Moreover, the tint of the shining was light azure, not blue as usual. We called this phenomena *The Starry Night effect*.

Recharging

- Elaborating on the 75 Hz frequency, the first flash (FF) in response to a mechanical stimulus was very bright and had a rise time of 10 ms.
- The form of subsequent flashes in response to further stimuli differed radically from the FF. They were dimmer and longer lasting than the FF with approximately 150 ms rise times and a monotonic decay.
- Cells that were stimulated to exhaustion recovered some bioluminescent capacity once stimulation ceased.
- Initial recovery was rapid and cells stimulated after only a 15 min recovery period produced as many flashes in the second stimulus series as in the first even though their TMSL (Total mechanically stimulated luminescence) was reduced.
- With a 24 h recovery period FF kinetics were more dependent on the cell receiving a normal 12 h day phase than was TMSL recovery.
- Mechanically triggered bioluminescence in Pyrocystis fusiformis appeared to be the result of at least two temporally distinct processes, one of which was dependent on a recharging period.

The Moskvarium mechanism



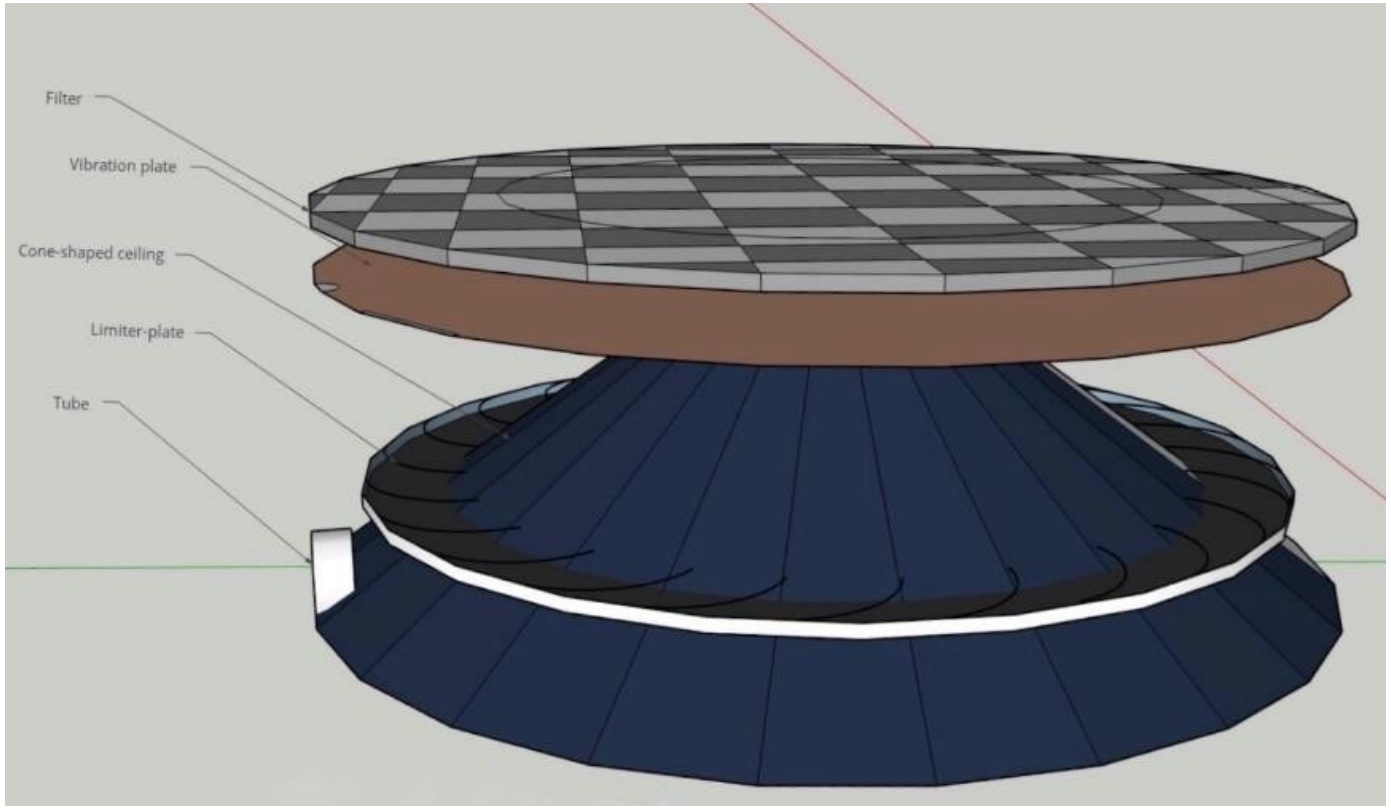
Generally, mechanism is a suspended ceiling. It consists of several parts:

1)Filter

It protects the water surface from contamination. Of course it should be located so that it can be cleaned or changed. Consequently, there should be a technical room above the filter. Thus, from the very beginning quite a high room should be selected for this mechanism. Fortunately, there are corresponding places in Moskvarium for such a room.

2)Vibration plate

This plate can be manipulated to generate vibration with the help of vibration motors which are to be located on the edges of the plate. The plate must be thin and also can even be reticulated for a greater vibration. An analog of the reticulated plate can serve as a wire (see experiment №3).



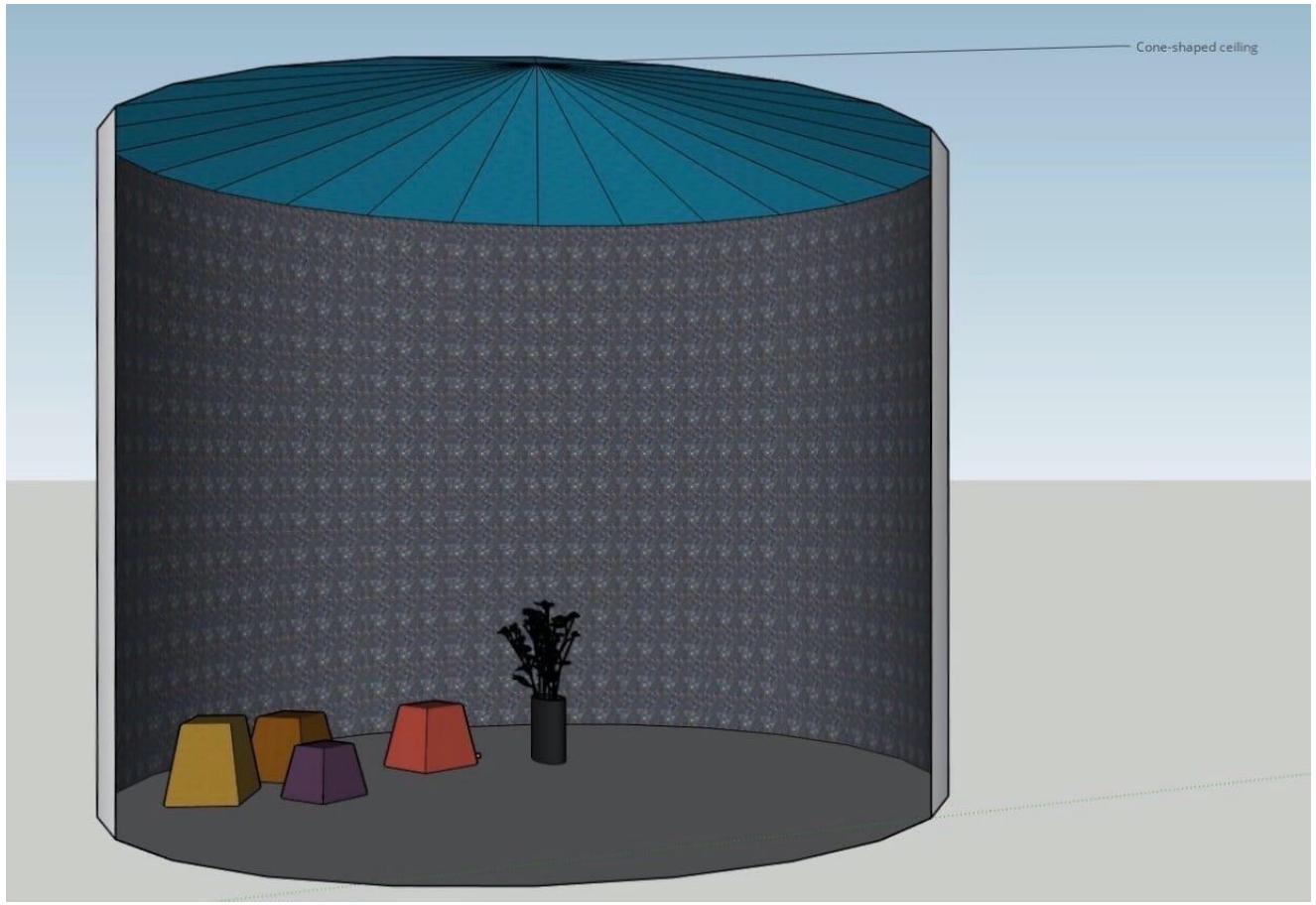
Cone-shaped ceiling of course is transparent and made of glass in order to demonstrate Pyrocystis fusiformis.

3)Limiter plate
Limiter plate is a circular plate consisting of separate fragments. It can be closed or opened by the pistons when filtering dead organisms from the alive ones.

4)Tube
The tubes are responsible for the removal of dead organisms. It is a significant part of the mechanism since the ceiling is transparent, so if there is a dead organisms cluster (on the ceiling's surface), it becomes somewhat difficult for visitors to discern Pyrocystis fusiformis

This mechanism is *beneficial in terms of its cleaning*. Having a culture of Pyrocystis fusiformis for several months, we have noticed that vials get dirty and become muddy every end of the week. This is in direct correlation with the life cycle of Pyrocystis fusiformis - 5-7 days. Thereby, a conjugate ‘limiter plate-tubes' system minimizes the number of dead Pyrocystis fusiformis on the ceiling surface. A real need to clean the surface comes with the official cleaning day – the last Monday of each month.

The rest area



The main idea is to effectively demonstrate Pyrocystis fusiformis in the conditions of Moskvarium, also this demonstration should be attractive for the visitors of Moskvarium. The best way to attract visitors is to organize a '*rest area*'. A space where people can relax in the deep darkness while examining the Starry night effect and listening to soothing melodies. A session system can be set up and people can be let into the room in portions. A particular session can last from five to ten minutes (considering Pyrocystis fusiformis' charging time). People can place themselves on the padded stools and look up at the glow of Pyrocystis fusiformis.

The main results

- During the preparation for the experiments, we increased our population of Pyrocystis fusiformis several times, which allowed us to conduct a sufficient number of experiments.
- A method that has been selected allows the simplest and most effective calculation of the density of the culture of Pyrocystis fusiformis. It is a manual method with the automatic single channel pipettes.
- We have proved that asexual reproduction occurs more rapidly when the culture of Pyrocystis fusiformis is moderately diluted.
- The hypothesis of prolonged mechanical irritation of Pyrocystis fusiformis and their ability to emit light was refuted for only a few seconds.
- Our team found out that Pyrocystis fusiformis recharge time does not exceed 30 minutes (in unfatigued cells; in rare cases - 60 minutes).
- According to the experiments the influence of ultrasound and infrasound was not justified and the population could not survive living in a closed pump system. Vibration on the other hand occurred to be the safest way to activate the bioluminescence of Pyrocystis fusiformis. Moreover, we found out that the Starry Night effect can only be achieved by using vibrations of 75 Hz.
- In the end we have developed a model of the mechanism that will most effectively demonstrate the ability of Pyrocystis fusiformis to exhibit bioluminescence in the conditions of the Moskvarium.

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THANK YOU FOR ATTENTION