



## Atmospheres in a Test Tube: state of the art at the Astronomical Observatory of Padova

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**Abstract.** At the Astronomical observatory of Padova we are trying to answer some questions about the detectability of biosignatures in the exoplanetary atmospheres, working in the framework of the project Atmosphere in a Test Tube. In particular we are investigating how the presence of photosynthetic biota living on the surface of a planet orbiting in the HZ of an M type star may modify the atmospheric gas abundances. This can be achieved in laboratory with an environmental simulator called MINI - LISA. The simulator allows to modify the temperature and the pressure inside a test chamber, where a selected population of photosynthetic bacteria is arranged. We'll focalize our experiments on the following bacteria: *Acaryochloris marina*, *Halomicronema hongdechloris*, *Leptolyngbya sp.1* and *Chlorogloeopsis fritschii*. The first two bacteria are naturally provided with NIR light metabolizers, like Chl-d and Chl-f, while the last two can develop such pigments if grown in NIR light. The experiment will lead us to obtain useful data to be compared with the ones expected either by the future space missions (JWST, ARIEL) and ground based new instrumentation (SPHERE@VLT; GPI@GEMINI; PCS@E-ELT). In this talk we discuss the layout of the experiment and its state of art.

**Key words.** Exoplanet – Atmosphere Characterization – Climate simulation – photosynthetic bacteria

### 1. Introduction

In the last years, a lot of extrasolar planets have been discovered in any direction of the

Galaxy. More interesting, some of them have been found in the habitable zone of their host stars. A lot of efforts are done in order to find habitable planets. Our research will focus on F, G, K, and M stars. In fact these stellar types are long-lived enough to support an habitable zone (HZ) capable of evolving life. By habitable zone, we mean the range of orbital distances from a star that will allow for the existence of liquid water on a planet (Hart et al. 1978; Kasting et al. 1993). Moreover, we'll focus on terrestrial-type planets, since the existence of life forms on gas giants requires speculation beyond analogous examples on Earth. In particular planets orbiting around M stars are very interesting because of their high statistic density and because of their capability of hosting earth-like planets. Nowadays, some habitable super-earths orbiting these type of stars have already been found. In this framework, "Atmosphere in a Test Tube", a project started at Astronomical observatory of Padua, simulates planetary environmental condition in order to understand how and how much the behavior of photosynthetic bacteria in different planetary/star scenarios can modify the planet atmosphere. The particular case of an habitable planet orbiting an M dwarf star is under study for the time being. The irradiation of an M star, due to its lower photospheric temperature is very different in quality and quantity by the irradiation of a star like our Sun. Because of their dimness, the habitable zone of M stars is very close to the star, such that planets may become tidally locked (one side constantly facing the star Joshi et al. 1997). The different radiation regimes of F, K, and M stars lead to different atmospheric photochemistry<sup>3</sup> as well as a much different photon density. This feature can strongly impact with the metabolic cycle of photosynthetic organisms. In the following of the paper a description of the experiment and of its aim is given.

## 2. Scientific requirements

The guide parameters in order to perform laboratory experiments are the temperature, and the knowledge the processes that build the chemical composition of its atmosphere. As said be-

fore, the focus of our research is to simulate super Earths orbiting in the habitable zone of its host star. The temperature interval depends by the pressure of the atmosphere is dependent on the greenhouse effect, that is effective in maintaining the superficial temperature of the planet as high as it can maintain the water in liquid state. Moreover another parameter is the distance at which the planet have liquid water, that depends by the host star, the orbital distance of the planet and the planet itself (Kasting et al. 1993; Selsis et al. 2007).

For example, for a super Earth orbiting an M star, using both the "Venus" and "Mars" criterion this range is enclosed between 0.47 and 0.88 A.U. and corresponds respectively to 216 K, (the first condensation of CO<sub>2</sub>) and 373 K (the water loss limit). The pressure and the gaseous mixture can regulate the air flows (Joshi et al. 2003, 1997; Edson et al. 2011) and shift the habitable zone forward and inward the Habitable Zone (HZ) limits.

For the first part of the experiment, in order to avoid extra-expensive laboratory infrastructure and simplify the work, we'll consider super Earths well inside the HZ of the host star with temperatures between 273 K and 288 K, that are the current temperature values on Earth.

Photosynthetic bacteria are the organisms chosen for this experiment and the key to understand how a hot, inhospitable planet can become a safe green and life friendly planet.

In fact photosynthetic organisms can produce gases, like O<sub>2</sub> (or O<sub>3</sub> from its photolysis), and nitric oxides like N<sub>2</sub>O<sub>2</sub> and other molecules formed from the breakdown of organic matter like NO<sub>x</sub>, CH<sub>3</sub>Cl or COS that can have modified exoplanets atmospheres in time and can be detected from Earth. Some of them are called biomarkers because they can outline the presence of biotic organisms on other planets.

But it is important to remember that biomarker measurements are affected by the problem of false positives. In fact, O<sub>2</sub> can be produced even abiotically through photolysis and the effects of carbon burial and hydrogen escape. Though, its simultaneous presence with other reduced gases as CH<sub>4</sub> can

be explained only with biotic processes that maintain chemical disequilibrium (Kiang et al. 2007b).

Detectability of photosynthetic bio-products depends on biotic productivity, which depends itself by several factors, like light, minerals, availability of resources like water, nutrients and electron donors. During the photosynthetic process, light impacts on photo-receptive organisms called antennae whose task is to split water molecules and produce proton gradients and energy useful for the cells. We can define  $O_2$ ,  $O_3$  and  $CH_4$  as the main biomarkers, as well as  $NH_3$  and  $N_2O$ . Though it is crucial to understand how organisms can photosynthesize on M star planets and if the metabolic processes can be influenced by different types of radiation. In fact, a detectable missing concentration of  $O_2$  and/or  $O_3$  is a strong signature of biologic activity, especially in combination with reduced gases like  $CH_4$ , as explained in Lammet et al. (2009). It is important to remember that not all bacteria respond equally to irradiation, but all of them have a typical response to light spectrum. The theoretical unicellular lower light photosynthetic limit, estimated by Raven et al. (1984) is  $0.1 \mu\text{mol}$  of photons  $\text{m}^{-2} \text{s}^{-1}$  ( $6 \times 10^{16}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ). For the upper limit of photon flux density, Wolstencroft, R.D. and Raven (2002) found that the theoretical tolerance for land plants against photo damage is 69 mmol of photons  $\text{m}^{-2} \text{s}^{-1}$  ( $3.654 \times 10^{21}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ) over the standard Photosynthetic Active Region of the spectrum (PAR, the part of the radiation spectrum between which organisms can operate photosynthesis), well above Earth's typical flux of 2 mmol photons  $\text{m}^{-2} \text{s}^{-1}$  ( $1.2 \times 10^{21}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ).

Wolstencroft and Raven conjectured a theoretical upper limit for land organisms living on Earth-like planets in general, to be 10 mmol of photons  $\text{m}^{-2} \text{s}^{-1}$  ( $6 \times 10^{21}$  photons  $\text{m}^{-2} \text{s}^{-1}$ ). Living organisms can organize their existence in order to fit as good as possible to their environmental niches. For example, cryptoendolithic organisms can live under rocks while aquatic organisms can live shielded under water, so they could exist for even higher

surface photon flux densities (Kiang et al. 2007a). Photopigments that can harvest light are chlorophylls like Chl a that occurs in the core antenna, or other Chls, Chl b, c and d, that provide light harvesting roles. Chl d, recently discovered in cyanobacteria (Miyashita et al. 1996), (Miller et al. 2005), (Mielke et al. 2011), may replace Chl a in some cyanobacteria that live in environments with little visible light (Chen et al. 2005); (Larkum et al. 2005). Chl d has its absorbance peak in the NIR at 720 nm (Manning et al. 1943); (Larkum et al. 2005), and thus oxygenic photosynthesis is being performed in the NIR (Kiang et al. 2007a). Recently Chl f has been discovered which is able to capture light energy in the infrared spectrum, with an absorption peak at 706 nm. In non oxygenic bacteria, BChls play a primary role in electron donor and their peak can extend through the NIR part of the spectrum. Other photopigments are carotenoids and phycobilines. The first ones operate in the blue and green part of the spectrum and protect organisms against photooxidative stresses, high temperatures and the toxic presence of  $O_2$ . Phycobilines, that can be found in cyanobacteria and red algae, work in the green and yellow spectral regions. Other living organisms, such as green bacteria, purple bacteria and heliobacteria, can exploit solar light in slightly extended spectral regions or in ecological niches, such as the near-infrared. For example, purple bacteria have absorbance peak in the 1.013–1.025  $\mu\text{m}$  range using BChl b like *Blastochloris viridis* or *Rhodospseudomonas viridis* (that absorbs at 0.96  $\mu\text{m}$ ) and other bacteriochlorophylls in the range 0.7 – 0.9  $\mu\text{m}$  (Scheer et al. 2003). They don't use water as H donor, and then don't release oxygen as byproduct. Photosynthetic Active Region (PAR) on planets orbiting around M stars can be lower than the average terrestrial one even by an order of magnitude (Heath et al. 1999). Nevertheless this could not represent a problem because several marine organisms on Earth evolved to use only  $5 \times 10^{-4}$  times the average flux received at the Earth's surface, like sulfur bacteria that embed a large antenna complex, the chlorosome, that permit to use only small fractions of light intensities citepMcKay2000.

In these regions radiation is dominated by red or IR radiation.

## 2.1. Photosynthetic bacteria

In order to perform our experiment we will consider both model and atypical photosynthetic organisms. Between the model ones the moss *Physcomitrella patens*, the green microalga *Chlamydomonas reinhardtii* and the cyanobacterium *Synechococcus PCC 7002* will be tested. All of them are characterized by the presence of chlorophylls (chlorophyll a and b) with an in vivo absorption major peak in the Red (around 680 nm). We also selected a series of other peculiar photosynthetic microorganisms able to extend their in vivo absorption to the NIR (around 710 nm), due to particular rearrangement of the chlorophyll a in their photosystems or to the presence of other chlorophyll forms (chlorophyll d and f). To this second group of organisms belong the microalga *Ostreobium sp.* and the cyanobacteria *Acaryochloris marina*, *Halomicronema hongdechloris* and *Chlorogloeopsis fritschii*. *Physcomitrella patens* is an early colonist moss that can grow on exposed mud and earth around the edges of pools of water. *P. patens* has a disjunct distribution (separated from each other geographically) in temperate parts of the world, with the exception of South America. They contain Chl a and b. *Chlamydomonas* species are widely distributed worldwide in soil and fresh water. *Chlamydomonas reinhardtii* is a single-cell green alga about 10 micrometres in diameter. It has an "eyespot" that senses light and the organism is able to swim due to the presence of two flagella. *Chlamydomonas reinhardtii* is an especially well studied biological model organism, partly due to its ease of culturing and the ability to manipulate its genetics. When illuminated, *C. reinhardtii* can grow photoautotrophically, but it can also grow in the dark if supplied with organic carbon. They contain Chl a and b. *Synechococcus sp.* strain PCC 7002 is a unicellular (3-4  $\mu\text{m}$  of diameter), euryhaline cyanobacterium. It is a model organism for studies of cyanobacterial metabolism. It exhibits an exceptional tolerance of high-light ir-

radiation and shows very rapid growth. The habitats from which this and closely related strains were isolated are subject to changes in several environmental factors, including light, nutrient supply, temperature, and salinity. It contains Chla.

*Halomicronema hongdechloris* is the first reported filamentous cyanobacterium containing Chl f together with Chl a (Chen et al. 2012). It was isolated from a stromatolite cyanobacterial community. The extremely slow growth rate of *H. hongdechloris* has hindered research on this newly isolated cyanobacterium and the investigation of chlorophyll f-photosynthesis. Chlf is Reported to be a "red-light-induced" chlorophyll with increased amount when *H. hongdechloris* is cultured under far-red light (Chen et al. 2012). *Acaryochloris marina* is a cyanobacterium containing Chlorophyll d, instead of Chlorophyll a allowing it to utilise far-red light, at 710 nm wavelength. It is a species leaving in symbiosis with invertebrate marine organisms. It was first discovered in 1993 from coastal isolates of coral in the Republic of Palau in the west Pacific Ocean. Scientists including NASA's Nancy Kiang have proposed that the existence of *Acaryochloris marina* suggests that organisms that use Chlorophyll d, rather than Chlorophyll a, may be able to perform oxygenic photosynthesis on exoplanets orbiting red dwarf stars (which emit much less light than the Sun). Because approximately 70 percent of the stars in the Milky Way galaxy are red dwarfs, the existence of *Acaryochloris marina* implies that oxygenic photosynthesis may be occurring on far more exoplanets than astrobiologists initially thought possible. *Chlorogloeopsis fritschii* is a terrestrial cyanobacterium that can grow in hot springs.

In 2014 the production of chlorophyll f and chlorophyll d in the cyanobacterium *Chlorogloeopsis fritschii* cultured under near-infrared and natural light conditions was reported. In the laboratory, the ratio of chlorophyll f to chlorophyll a changed from 1:15 under near-infrared, to an undetectable level of chlorophyll f under artificial white light. *C. fritschii* produced chlorophyll f and chlorophyll d when cultured under natural light to a high culture density in a 20 L bubble col-

umn photobioreactor. In figure 1 are shown different images of these biotypes. *Ostreobium sp.* is a green alga. The plants consists of endozoic (endolithic) branched siphonous filaments 1-160  $\mu\text{m}$  in diameter. Filaments are straight and sparsely branched or forming irregular, tangled networks. They form cylindrical portions and/or with inflated regions. Chloroplasts are small, spherical or polyhedral to reticulate with no presence of pyrenoids. Cell walls are thin and undifferentiated to thick and lamellose. Their reproduction is by quadri-flagellate zoospores known only *Ostreobium quekettii*. *Ostreobium marine* is widely distributed in tropical to temperate areas, growing primarily in calcified substrata including corals, calcified red algae and old mollusk shells. *Ostreobium* is among the deepest growing macroalgae in both temperate and tropical regions. Physiological studies on in situ photosynthesis by *Ostreobium* confirm low levels of light requirements and an ability to utilize near infra-red light greater than other green algae. A modified photosystem I reaction centre was hypothesized to account for this.

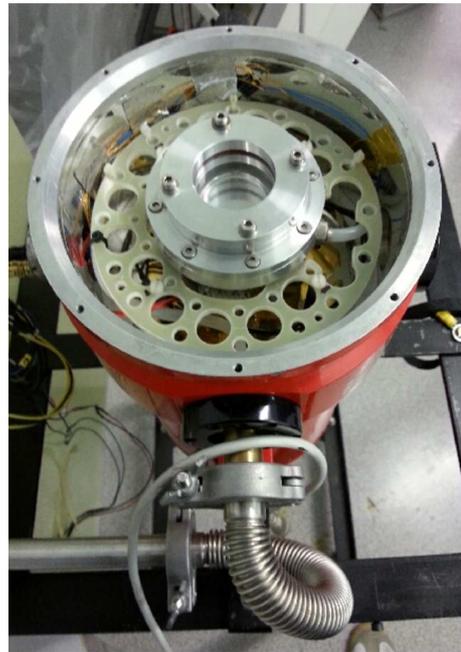


**Fig. 1.** In figure we can see samples of (a) *Physcomitrella* growing on agar plates by Sabisteb–Anja Martin from the Ralf\_Reski lab. (b) *Chlamydomonas reinhardtii*, (c) *Synechococcus sp.* strain PCC 7002, (d) *Halomicronema hongdechloris* (Chen et al. 2012), (e) *Acaryochloris marina*, (f) *Ostreobium quekettii* and (g) *Chlorogloeopsis fritschi*.

For a good calculation of the metabolic evolution in time there would need many parameters. In fact, studies show that bacteria  $\text{O}_2$  productivity is directly dependent on bacteria growth and pigment concentration as well as the light irradiation. Bacterial growth can be fitted with a Gompertz curve with three freedom degrees.

## 2.2. The experiment

The instrument that will be used to carry out the experiment is MINI-LISA. It has originally been created by the Astronomy Department of University of Padua to study how living bacteria, mosses and lichens could survive in a Martian atmosphere. The main structure is a steel cylinder inside which are located one cell (inside which biological samples can be placed) with a  $600 \text{ cm}^3$  capacity (0.6 l) and topped by a Borofloat glass window transparent from UV to NIR figure 2.



**Fig. 2.** Internal part of MINI-LISA

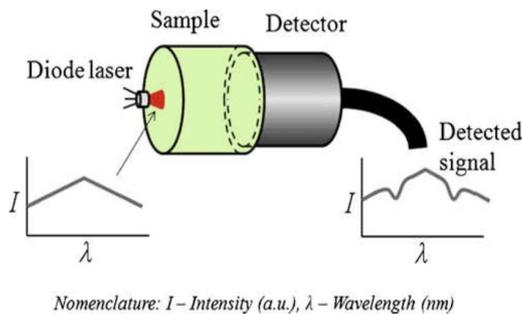
The cell is connected with the outer part by pipes at the end of which are implemented mechanical filters to let gas mixture to course and at the same time avoid biological material to go through the pipes inside the cryostatic chamber located at the base of LISA-SAM. Until now, the cooling process have been made by contact, with an aluminium plate located on the liquid nitrogen reserve (Ranger Air Liquide), but as biological samples should be kept at a mean temperature of 293 K, there is no need of liquid nitrogen to reach this temperature and this goal could be easily achieved with a Peltier cell kept under the cells. This way the temperature can be raised or lowered. For practical and energetic reasons we are trying to understand how to customize MINI-LISA to perform a single sample experiment. As shown in Figure 2 the reaction cell is isolated from the rest of the Dewar. This allow to evacuate the space between the cell and the Dewar walls by the use of a vacuum turbo-pump.

The first preparatory and preliminary tests will test the hardware, in particular to understand how the cells keep pressure and void air tightness. This gave us an estimate of the limits in sensibility our system can reach. Done this, will begin the main part of the experiment, that consists of three steps. In the first step, called the fiduciary experiment, measurements of photosynthetic bacteria products will be taken in terrestrial conditions. It's obvious that the choice of these bacteria will be done depending on their productivity rate and metabolic processes as previously said. The other choice parameter is their resistance to extreme environments. This part of the experiment will be surely done in a biological laboratory because of the need of a controlled environment chamber to lodge bacteria inside the cells. In the part of the experiment, cells will be filled with a gaseous mix that will reproduce Earth's atmospheric composition. During the first step, the pressure will be kept constant at 1.013 bar (Earth's mean pressure at sea level) and the temperature will be around 298 K, a temperature suitable for oxygenic photosynthetic bacteria. The irradiation will reproduce the solar irradiation spectrum. The samples will be irradiated with a tunable led stellar-

light simulator capable to recreate the radiation spectrum of M type stars (but with the potential to be expanded even to F, G, K star spectra types) incident on the planet. The radiation source is a multiple LED matrix cooled by means of air fan technology. In order to endow it with modularity this device will be composed by a mosaic of circuit boards arranged in a pie-chart shape, on the surface of which will be welded the LEDs. The device can be driven by a PC to raise or lower the intensity of both each LED and the lamp, in order to simulate as close as possible a portion of the star spectrum. The wavelength intervals overlap the limits of photosynthetic pigment absorption range (280-850 nm), while the range of the radiation source will be between 365 nm and over 1200 nm. The reason why we chose a higher outer limit is that M stars have the emission peak at about 1000 nm and we want to study the effects of low-light radiation on bacterial vitality (Erculiani et al. 2015). Before and during sample irradiation will be taken measurements of gas composition, expecting a peak of gas concentration variations at the end of bacteria metabolic and photosynthetic process. The measurements of gas concentrations inside the cells will be done with a Tunable Diode Laser Absorption Spectroscopy setup (TDLAS) and is based on absorption energy following Beers law. This measurement method provides a Vertical Cavity Surface Emitting laser (VCSEL) source shot through the cell environment and to a photodiode diode laser tuned to a particular narrow emission band. The source wave number is selected in order to match a single absorption line on a molecule of interest and the laser emission is scanned several times across the whole spectral width of the absorption feature. Usually, the line width of the laser emission is much maller than the molecular absorption line width allowing the instrument to be selective among components of a gas mixture and have no interferences from other gases, especially at low pressure (the absorption lines are narrower). The sensitivity of the analyzer is dependent on the absorption strength of the line chosen and on the absorption path length (Erculiani et al. 2015). Many gases of biologi-

cal interest can be sensed in this way, for example HF (detection limit 0.2 ppm.m), H<sub>2</sub>S (detection limit 20.0 ppm.m), NH<sub>3</sub> (detection limit 5.0 ppm.m), H<sub>2</sub>O (detection limit 1.0 ppm.m), CH<sub>4</sub> (detection limit 1.0 ppm.m), HCl (detection limit 0.15 ppm.m), HCN (detection limit 1.0 ppm.m), CO (detection limit 40.0 ppm.m), CO<sub>2</sub> (detection limit 40.0 ppm.m), NO (detection limit 30.0 ppm.m), NO<sub>7</sub> (detection limit 0.2 ppm.m), O<sub>2</sub> (detection limit 50.0 ppm.m). A Wavelength Modulation Spectroscopy scheme will be used to improve detection of weak absorptions from the low concentrations obtained. The measurement setup will be based on a PC with a DAQ card for synchronous modulation and demodulation of the WMS waveforms as well as for fitting the absorption signals. In figure 3 is shown a scheme of its working (Erculiani et al. 2014).

The second step of the experiment will consist to change the irradiation source to simulate an M star's one on the surface of the planet. This irradiation lamp still need to be realized. M star spectra have strong absorbance lines, so it is difficult to reproduce exactly it as they are not good black body emitters. Finally, in the third step we will change even the gas mixture inside the cells, keeping the irradiation source as in the previous step. The gas composition will come by theoretical simulation of super earths atmospheres.



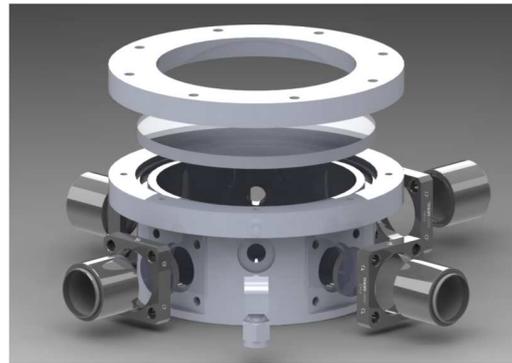
**Fig. 3.** Working scheme of the gas detector

### 3. State of the art

The experiment is evolving on more than one front, the more challenging of which are the test and customization of existing hardware (the step zero), the development of the irradiation source and the choice of the samples.

#### 3.1. Cells build-up

The cell incubator will be composed by a stainless steel main body topped with a Borofloat window capable to transmit the spectral range useful for our purpose. On the lateral surface will open four wedge windows that will be used to probe the gas content, in particular O<sub>2</sub> and CO<sub>2</sub>. The two elbow connectors will need for the gas flowing, in order to select the desired gas mixture inside the cell. In figure 4 is shown a picture of the internal part of internal cell.

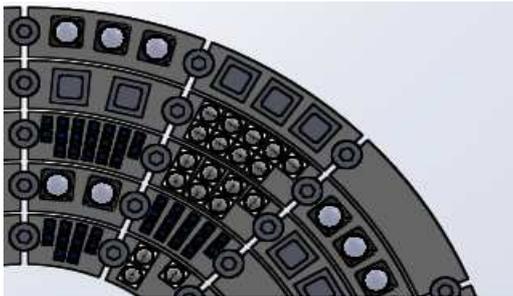


**Fig. 4.** Inner steel cells for bacterial lodging

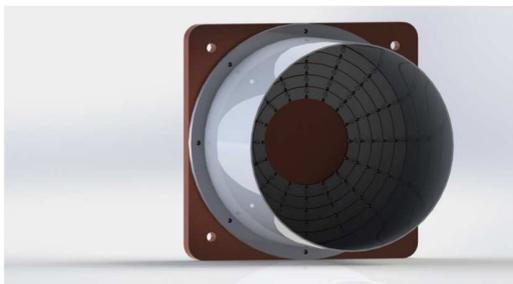
#### 3.2. Illumination system

The radiation source will be composed of a multiple LED matrix cooled by means of fan cooling technology. In order to generate a dynamical spectrum we evaluated different kind of radiation sources, but LEDs have been chosen instead of lamps because they are cheap, very small and dynamic. This last feature can allow a non-static characteristic of this novel radiation source, that can be tuned by a PC and

a dedicated software in order to match the desired radiative spectrum (enclosed in the technical limits. As already said, the wavelength intervals (365nm-940nm) will overlap the limits of the most common photosynthetic pigment absorption range (280-850 nm). For this radiation source we were driven by the concept was the idea of modularity, thinking at a multi-plate system shaped in the form of annuli, with mosaic of circuit boards arranged in a pie-chart shape, on the surface of which will be welded the LEDs. Modularity is a successful idea in case of LED damage or wrong working. Moreover dividing the tool in plates can allow future implementations, such as to reproduce other spectral types of stars just changing some kind of LEDs without building a new lamp ex-novo. In figure 5 and 6 is shown the concept design of the radiative system made with solid works.



**Fig. 5.** Main radiative system concept design



**Fig. 6.** Reflective cylinder assembled with the main radiative system.



**Fig. 7.** DALI USB channel multiplier from Tridonic Labs and buck led drivers.

On the back of the lamp will be fixed a copper plate that will be used to join the main body with the cooling system and will need to carry the heat to the dissipation device. In order to drive the radiation source by a PC such as to raise or lower the intensity of each LED and to set their luminosity, we chose a 15 channels expandable up to 63 DALI USB channel multiplier from Tridonic Labs and buck led drivers, illustrated in figure 7. Each channel can host a maximum of 45 V. In order to monitor the LEDs' work an STS-VIS spectrograph from Ocean Optics with cosine corrector will be used. This spectrograph has a spectral range falling between 380 and 900 nm, an integration time varying from 1 ms and 10 s and a SNR of 2000:1. It has a dedicated diffraction grating with 600 rows  $\text{mm}^{-1}$  and a linear CMOS ELIS1024 with 1024 elements. In figure 8 can be seen a picture of it.

#### 4. Conclusions

In this work we have described the ongoing project of an experiment focused on the study of biosignatures in the atmosphere of a super Earth orbiting an M star well inside its habitable zone. We have underlined the scientific re-



**Fig. 8.** STS-VIS spectrograph from Ocean Optics.

quirements and choice of the bacteria. We have therefore analyzed the aspects of hardware customizing and the development of a source of irradiation.

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