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Calibration and validation of an experimental setup to study by remote sensing cyanobacteria responses under exo-Earth simulated environments

Mariano Battistuzzi[1,5], Lorenzo Cocola[2], Riccardo Claudi[3], Eleonora Alei[3,4], Luca Poletto[2], Tomas Morosinotto[5], Nicoletta La Rocca[5]

[1]*Centro di Ateneo di Studi e Attività Spaziali "Giuseppe Colombo" – CISAS, Via Venezia 15, 35131, Padova, Italy*
[2]*CNR-IFN, Via Trasea 7, 35131, Padova, Italy*
[3]*INAF – Astronomical Observatory, Vicolo Osservatorio 5, 35122, Padova, Italy*
[4]*Dept. Physic and Astronomy Galileo Galilei, Padova Univ., Vicolo Dell'Osservatorio 3, I-35122 Padova, Italy*
[5]*Dept. Biology, Padova Univ., Via U. Bassi 58/b, 35131, Padova, Italy*

E-mail: mariano.battistuzzi@phd.unipd.it

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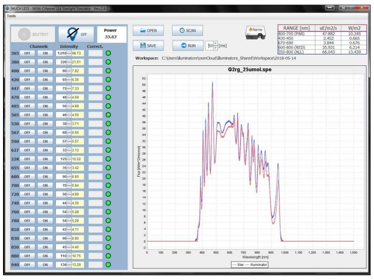
Aim of the project

In the last 10 years, a great number of exoplanets have been found orbiting the Habitable Zone (the distance of a planet from its host star at which water can be liquid on the surface of the planet) of M-type stars. These celestial bodies are less luminous than our Sun and have a different light spectrum, with a low emission in the visible and the major component in the far-red and infrared lights. These stars are interesting for astrobiology, as they are the most common stars of our galaxy and live long enough to potentially sustain life evolution.
A joint effort between INAF, CNR-IFN, and the department of Biology of Padova, let to the development of the Star Light Simulator (SLS) and of the Atmosphere Simulator Chamber (ASC). Using these devices, within the ASI project "LIFE in SPACE – Origin, Presence, Persistence of life in Space: from molecules to extremophiles", we simulate the light spectrum reaching the surface of exoplanets orbiting M-type stars, to investigate the survivability of cyanobacteria to that and other environmental conditions (e.g. Temperature, pressure, atmospheric composition), their oxygenic photosynthetic activity and furthermore understand which impact they could have on primeval atmospheres lacking oxygen.
Environmental conditions tested inside the ASC can thus be extremely different from terrestrial ones and imposes us to assess the physiological responses of the cultures only at the beginning and at the end of the experiments. To overcome this issue, here we present a novel experimental setup to follow by remote sensing the growth and photosynthetic activity of oxygenic photosynthetic microorganisms by means of reflectivity, spectroscopic and fluorescence measurements, respectively. The system is reliable and complements other invasive physiological measurements of the culture such as optical density (OD), pigment content, dry weight.

Star Light Simulator (SLS)

The Star Light Simulator is a device capable of reproducing the emission spectrum of different kinds of stars (F/G/K/M spectral types) from 365 nm to 940 nm, thanks to 273 air cooled diodes and 25 LED channels, each tunable through a custom made software.

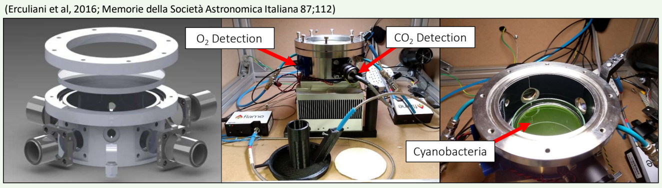
(Salasnich et al, 2018; DOI: 10.1117/12.2311436)



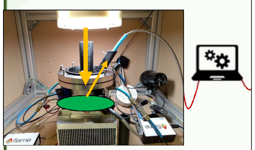
Atmosphere Simulator Chamber (ASC)

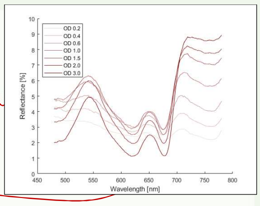
The Atmosphere Simulator Chamber is a stainless steel growth chamber in which it is possible to grow cyanobacteria, microalgae, and small plants inside Petri dishes, controlling temperature, pressure and atmospheric composition. On top of the chamber is placed a large Borofloat window through which the light coming from the SLS can be trasmitted to the sample. Oxygen production and carbon dioxide consumption of the organisms can be detected with different sensors, based upon *fluorescence quenching* and *Tunable Diode Laser Absorption Spectroscopy* (TDLAS).

(Erculiani et al, 2016; Memorie della Società Astronomica Italiana 87;112)



Reflectivity Detection





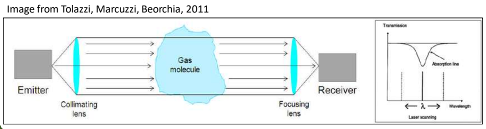
$$NDVI = \frac{Ref(745\ to\ 755nm) - Ref(675\ to\ 685nm)}{Ref(745\ to\ 755nm) + Ref(675\ to\ 685nm)}$$

- Probe collects reflected light from growing cyanobacteria culture
- Spectrometer acquires Photon Counts
- Data is elaborated into Reflection Spectra
- A Normalized Difference Vegetation Index (NDVI) is measured

CO_2 Detection

- *Tunable Diode Laser Absorption Spectroscopy (TDLAS)* Technique
- Selected a Single absorption line for CO_2 in the NIR
- Diode laser scans its wavelenght across the absorption line
- CO_2 molecules absorb emitted light at the specific absorption line
- Amount of % CO_2 in the measurment path is calculated

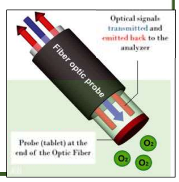
Image from Tolazzi, Marcuzzi, Beorchia, 2011



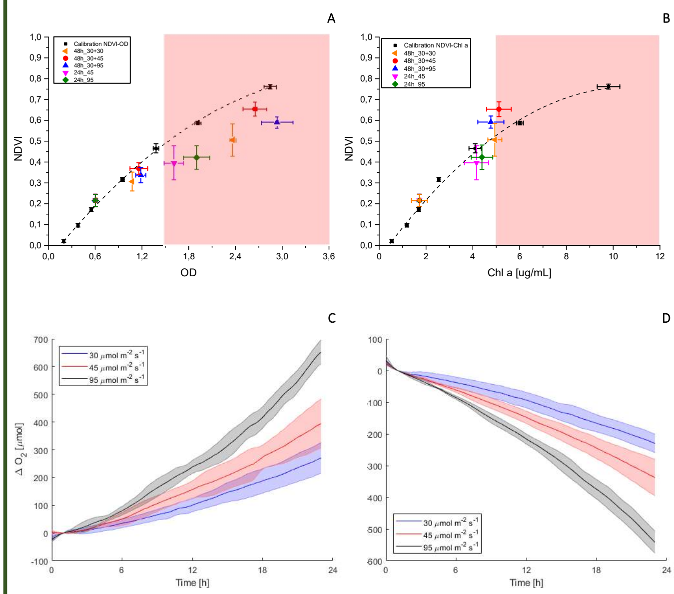
O_2 Detection

- *NomaSense O_2 P300* (Vinventions), used in wine quality assessment
- Based upon *fluorescence quenching* Technique
- Blue light transmitted through fiber to a tablet inside the ASC
- Tablet reacts according to dissolved O_2 in the ASC
- Red light emitted back
- Amount of % O_2 is measured
- Detects up to 15 μg/L of O_2

https://www.vinventions.com/it/wine-quality-solutions



Calibration and validation of the setup with cyanobacterium *Synechocystis sp. PCC6803*



Validation data fit within the linearity threshold, NDVI is a good proxy for growth measurment in the ASC

Calibration curve (Black dashed line) and Validation experiments (colored dots) are shown, relating NDVI to OD in figure **A**, and NDVI to Chl a content in **B**. The relation is linear up to an NDVI of ≈ 0,5, an OD ≈ 1,5 and a Chl a content ≈ 5 μg/ml; beyond those values the linear relationship is lost (red area), so NDVI isn't anymore a good proxy to follow the growth of this organism.
Validation experiments parameters:
- T = 30 °C
- P = 1 atm
- Atmospheric composition = air + 5% CO_2
- Starting cell concentration = 0,6 OD
- Spectrum: G2 (Sun) starlight

Esperiments (n=3) were carried on for 24 or 48 h, at three different light intensities: 30, 45, 95 μmol of photons $m^{-2}s^{-1}$. During 48 h experiments, the ASC was opened at 24 h to take samples for OD measurments, afterwards it was sealed again and refilled with air + 5% CO_2. As expected data from the validation experiments of OD and Chl a content fit well within the threshold, afterwards the relation fades and it is not possible to use the setup to evaluate growth with reliability.

Cyanobacteria growth and photosynthetic activity can be followed through the oxygen and carbon dioxide levels inside the ASC

Oxygen (**C**) and carbon dioxide (**D**) evolution of cyanobacteria during 24 h experiments in the three different light intensities.
Through the sensors equipped in the ASC it is possible to follow the photosynthetic performance of the cyanobacteria during growth. It is possible to clearly distinguish the different amounts of O_2 and CO_2 produced and consumed under different light intensities, furthermore by deriving the curves over time (not shown), it is possible to obtain the rate of O_2 production and CO_2 consumption for each light intensity. In the end, by subtracting the contribute of O2 production and CO2 consumption rate at t = 1 h to the whole production rate, it is possible to have information on the growth rate of the cyanobacteria.

Conclusions

The experimental setup works and gives real-time information on the growth and photosynthetic activity of cyanobacteria inside the ASC using remote sensing techniques.
Despite this, work has to be done to implement a mixing system to the chamber, as sedimentation of the culture is an issue that can lead to inaccurate NDVI measurments. To improve the accuracy of CO_2 and O_2 measurments, new sensors for both gasses are being explored.

This setup is currently being used to assess the growth and photosynthetic activity of different cyanobacteria under solar and red-dwarf star light spectra, along with terrestrial-like or O_2-depleted atmospheres.