Microorganisms suitable for studying biomarkers within the atmosphere in a test tube project

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Research Group
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AIMS Study the metabolism, vitality and gaseous production of photosynthetic organisms when forced to live in a different environment, miming an earth-like planet orbiting around the HZ of an M type star.

Timeline of experiments

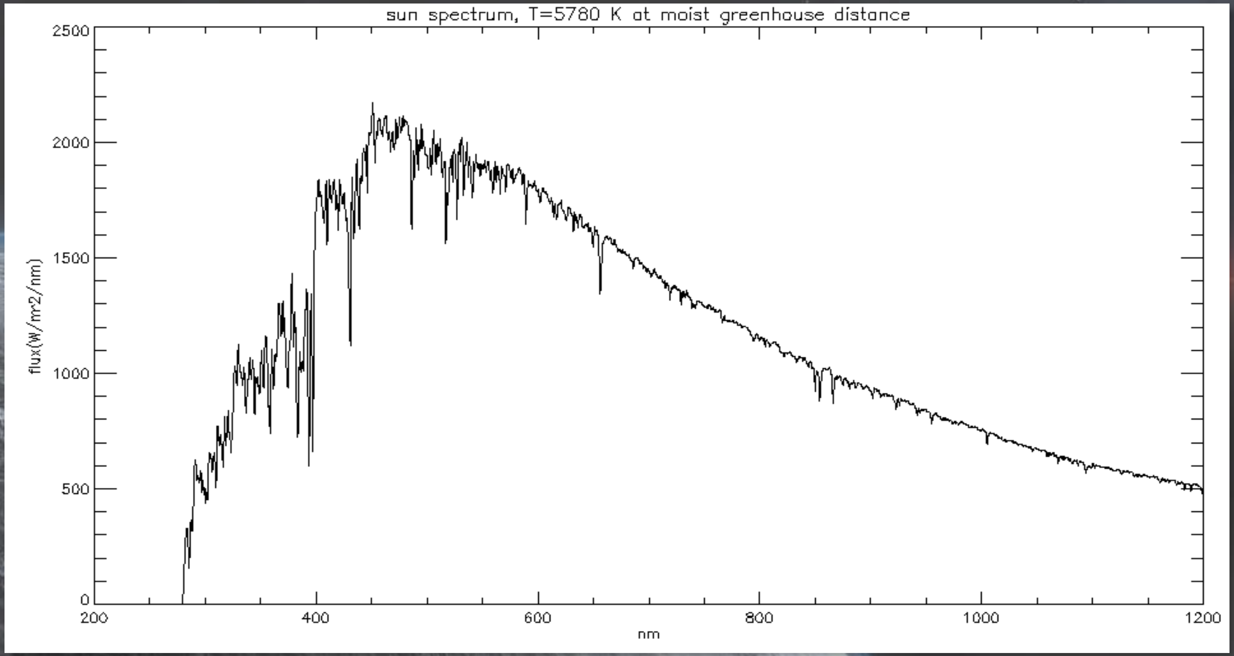
First step:
- M starlight simulator and Incubator build-up
- Choice of organisms

Second step:
- Irradiation of selected organisms with terrestrial conditions,
- Optimization of physiological analyses for the selected orgnisms

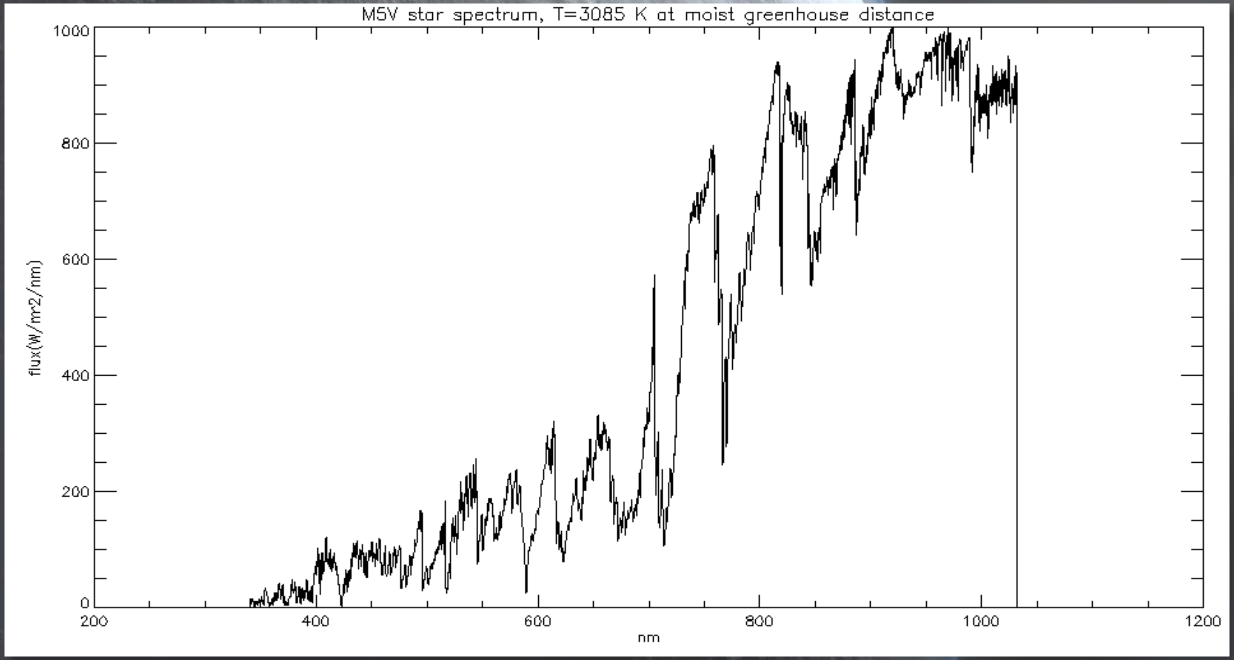
Third step:
- Irradiation of the samples with M star radiation,
- Analysis of photosynthetic performances.

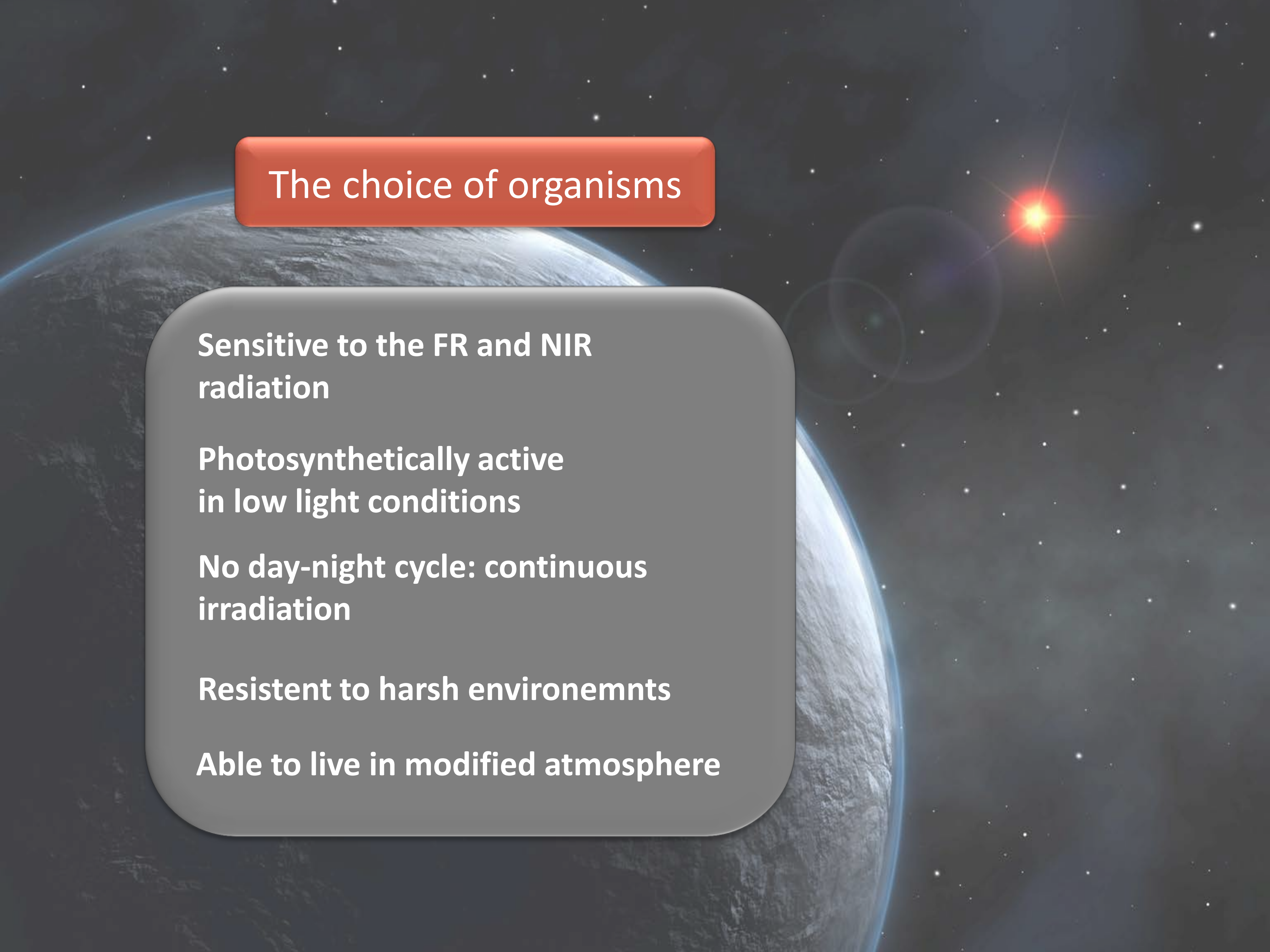
Fourth step:
- Irradiation of organisms with M star radiation at terrestrial temperature and extrasolar planet gaseous mixture.
- Analysis of photosynthetic performances.

SUN


sun spectrum, T=5780 K at moist greenhouse distance
flux(W/m^2/nm)
2500
2000
1500
1000
500
0
200
400
600
800
1000
1200
nm

M5V


M5V star spectrum, T=3085 K at moist greenhouse distance
flux(W/m^2/nm)
1000
800
600
400
200
0
200
400
600
800
1000
1200
nm



The choice of organisms

Sensitive to the FR and NIR radiation

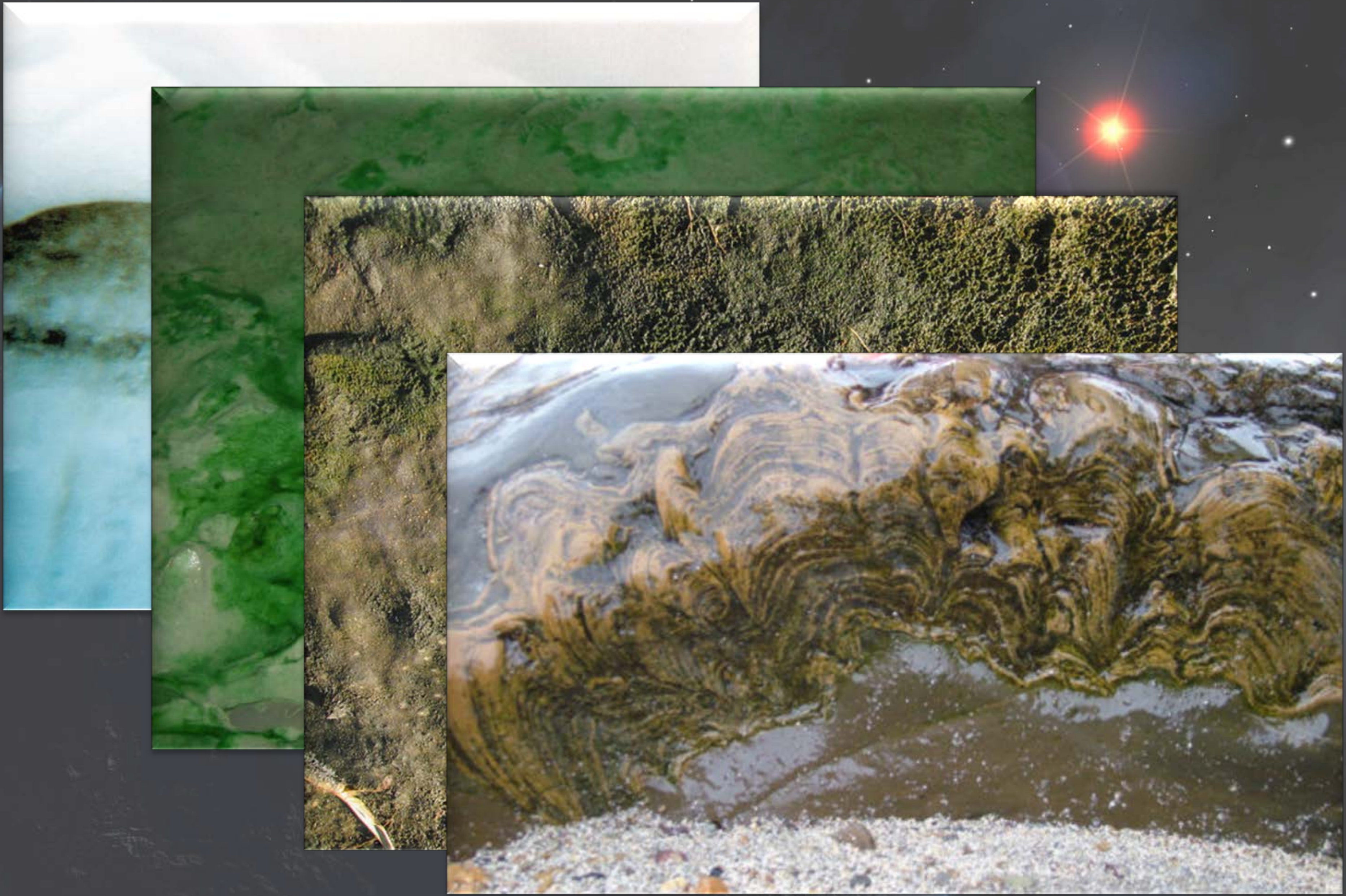
Photosynthetically active in low light conditions

No day-night cycle: continuous irradiation

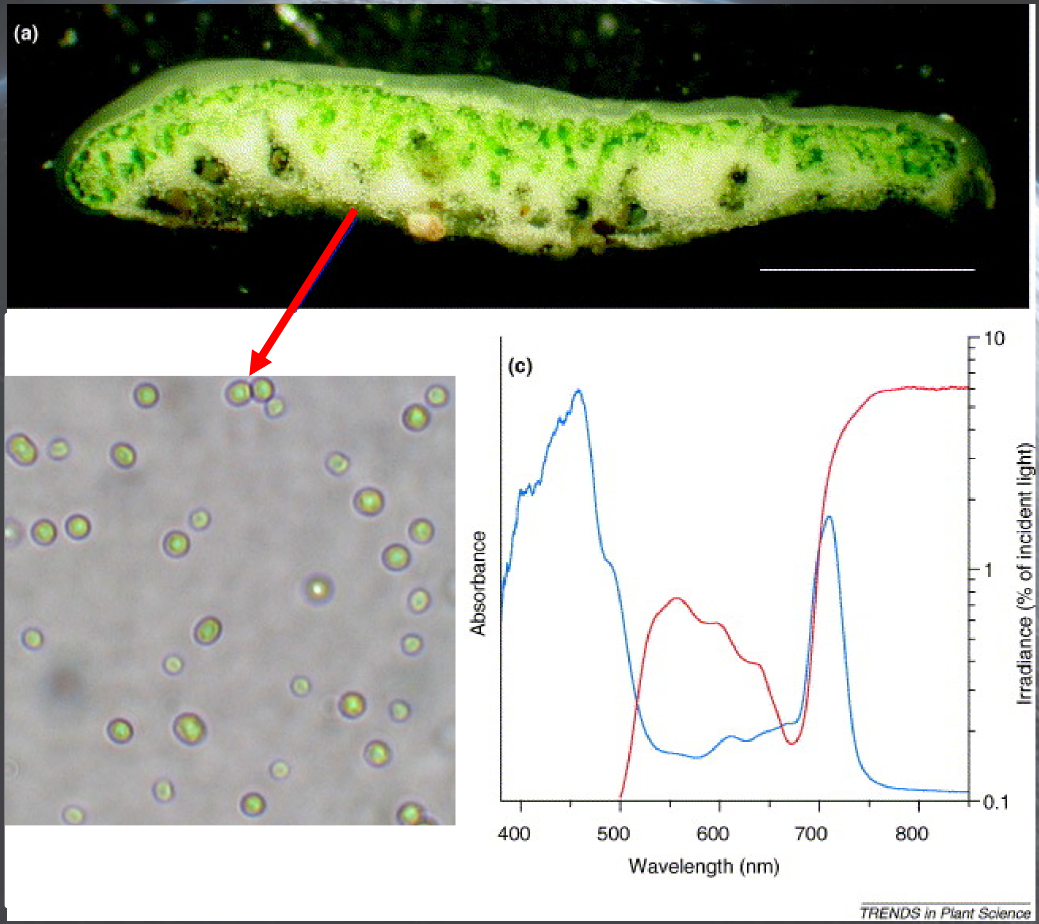
Resistent to harsh environemnts

Able to live in modified atmosphere

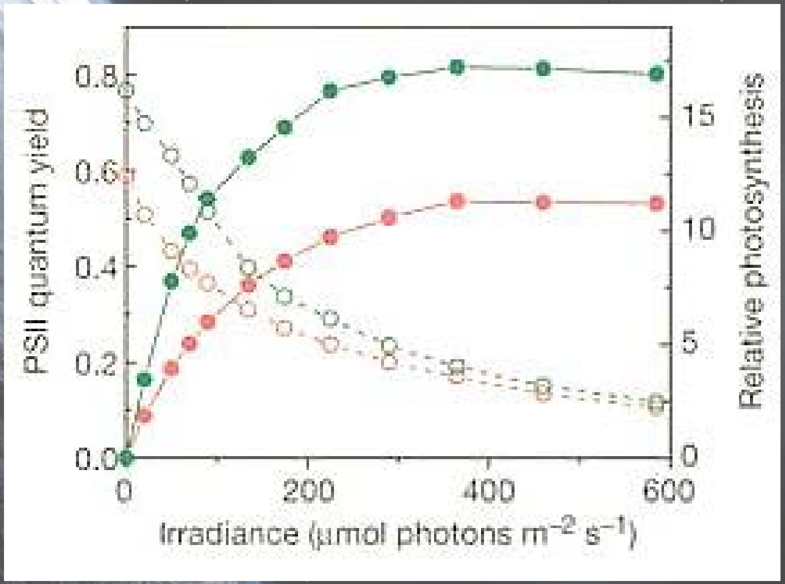
The high plasticity of cyanobacteria



Acaryochloris marina



TRENDS in Plant Science



Larkum research group, Nature 2005

Recent findings

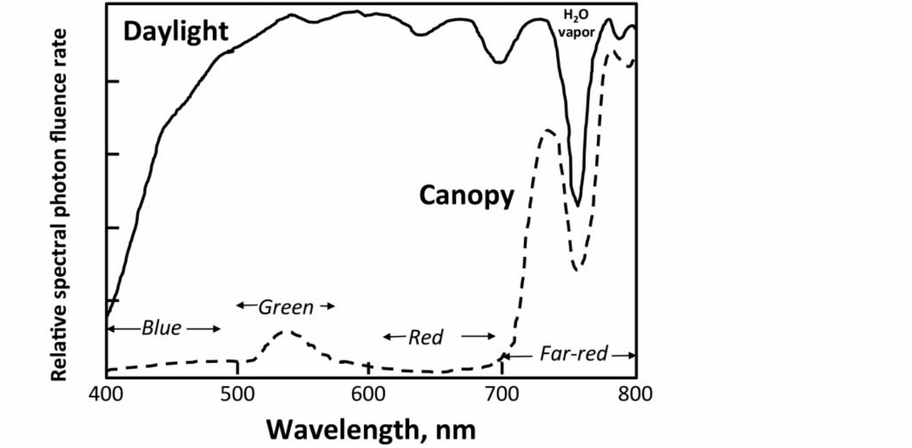
Gan et al., 2014 SCIENCE (September)
Extensive remodeling of a cyanobacterial photosynthetic apparatus in far-red light

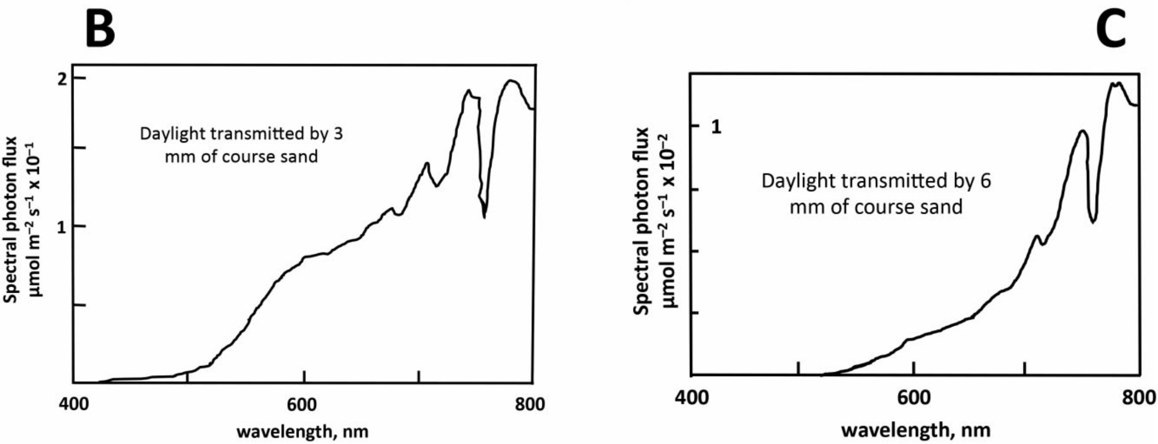
Gan et al., 2015 LIFE
Occurrence of Far-Red Light Photoacclimation (FaRLiP) in diverse cyanobacteria

Gan e Bryant., 2015 Environmental Microbiology
Olsen et al., 2015 Frontiers in Microbiology
Adaptive and acclimative responses of cyanobacteria to far-red light (FaRLiP) and low light (LoLiP)

Which kind of environments?

http://www.nhm.ac.uk/





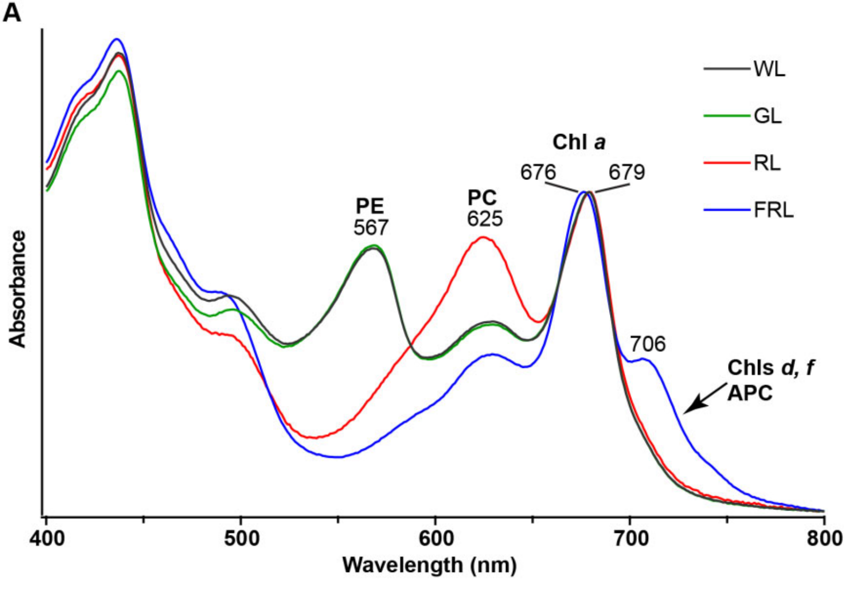
Gan and Bryant., 2015

FaRLiP cyanobacteria

After only **24h of FarRed light** (720nm, 15 µmol photons m^{-2} s^{-1})

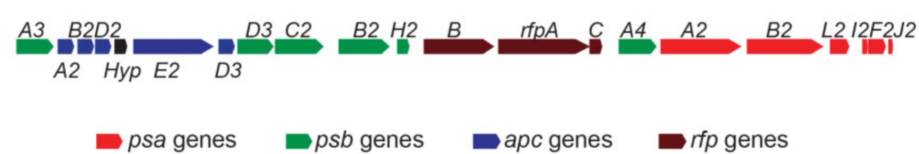
reorganize the photosynthetic apparatus

reaching a **photosynthetic activity up to 40% faster** then in Red light

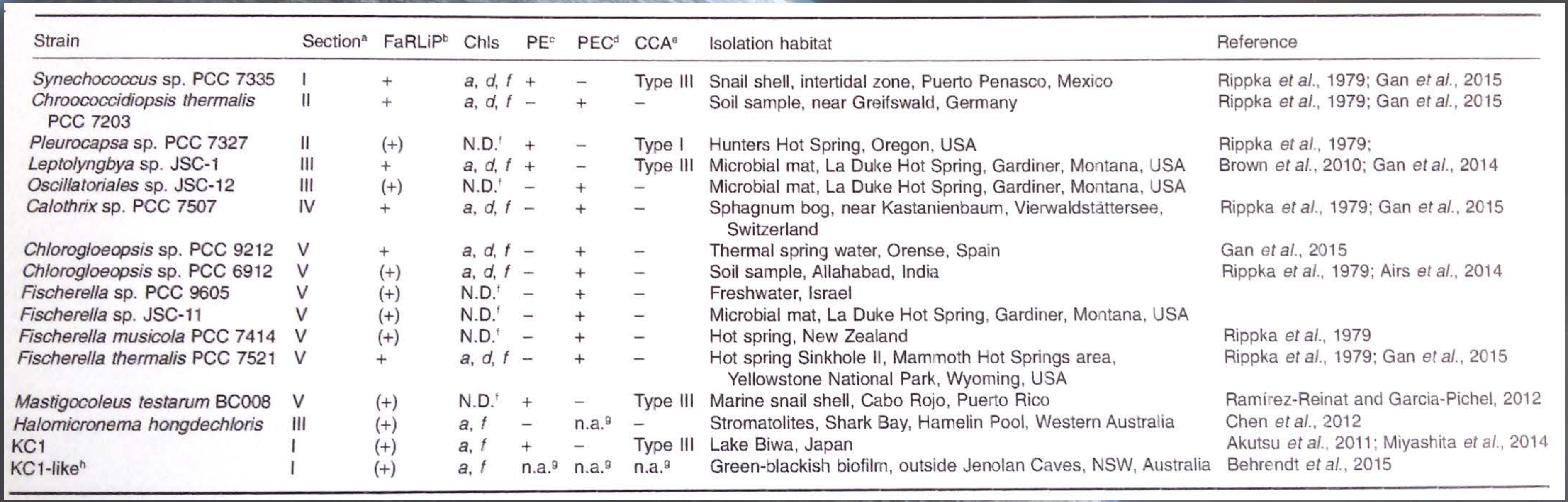


Gan and Bryant., 2015

They have a special cluster of 21 genes:



coding for a FR light photoreceptor and protein and pigment components of the major three complexes of the photosynthetic apparatus:
PSI, PSII and phycobilisomes



Strain	Section[a]	FaRLiP[b]	Chls	PE[c]	PEC[d]	CCA[e]	Isolation habitat	Reference
Synechococcus sp. PCC 7335	I	+	*a*, *d*, *f*	+	–	Type III	Snail shell, intertidal zone, Puerto Penasco, Mexico	Rippka *et al*., 1979; Gan *et al*., 2015
Chroococcidiopsis thermalis PCC 7203	II	+	*a*, *d*, *f*	–	+	–	Soil sample, near Greifswald, Germany	Rippka *et al*., 1979; Gan *et al*., 2015
Pleurocapsa sp. PCC 7327	II	(+)	N.D.[f]	+	–	Type I	Hunters Hot Spring, Oregon, USA	Rippka *et al*., 1979;
Leptolyngbya sp. JSC-1	III	+	*a*, *d*, *f*	+	–	Type III	Microbial mat, La Duke Hot Spring, Gardiner, Montana, USA	Brown *et al*., 2010; Gan *et al*., 2014
Oscillatoriales sp. JSC-12	III	(+)	N.D.[f]	–	+	–	Microbial mat, La Duke Hot Spring, Gardiner, Montana, USA	
Calothrix sp. PCC 7507	IV	+	*a*, *d*, *f*	–	+	–	Sphagnum bog, near Kastanienbaum, Vierwaldstättersee, Switzerland	Rippka *et al*., 1979; Gan *et al*., 2015
Chlorogloeopsis sp. PCC 9212	V	+	*a*, *d*, *f*	–	+	–	Thermal spring water, Orense, Spain	Gan *et al*., 2015
Chlorogloeopsis sp. PCC 6912	V	(+)	*a*, *d*, *f*	–	+	–	Soil sample, Allahabad, India	Rippka *et al*., 1979; Airs *et al*., 2014
Fischerella sp. PCC 9605	V	(+)	N.D.[f]	–	+	–	Freshwater, Israel	
Fischerella sp. JSC-11	V	(+)	N.D.[f]	–	+	–	Microbial mat, La Duke Hot Spring, Gardiner, Montana, USA	
Fischerella musicola PCC 7414	V	(+)	N.D.[f]	–	+	–	Hot spring, New Zealand	Rippka *et al*., 1979
Fischerella thermalis PCC 7521	V	+	*a*, *d*, *f*	–	+	–	Hot spring Sinkhole II, Mammoth Hot Springs area, Yellowstone National Park, Wyoming, USA	Rippka *et al*., 1979; Gan *et al*., 2015
Mastigocoleus testarum BC008	V	(+)	N.D.[f]	+	–	Type III	Marine snail shell, Cabo Rojo, Puerto Rico	Ramírez-Reinat and Garcia-Pichel, 2012
Halomicronema hongdechloris	III	(+)	*a*, *f*	–	n.a.[g]	–	Stromatolites, Shark Bay, Hamelin Pool, Western Australia	Chen *et al*., 2012
KC1	I	(+)	*a*, *f*	+	–	Type III	Lake Biwa, Japan	Akutsu *et al*., 2011; Miyashita *et al*., 2014
KC1-like[h]	I	(+)	*a*, *f*	n.a.[g]	n.a.[g]	n.a.[g]	Green-blackish biofilm, outside Jenolan Caves, NSW, Australia	Behrendt *et al*., 2015

Gan and Bryant., 2015

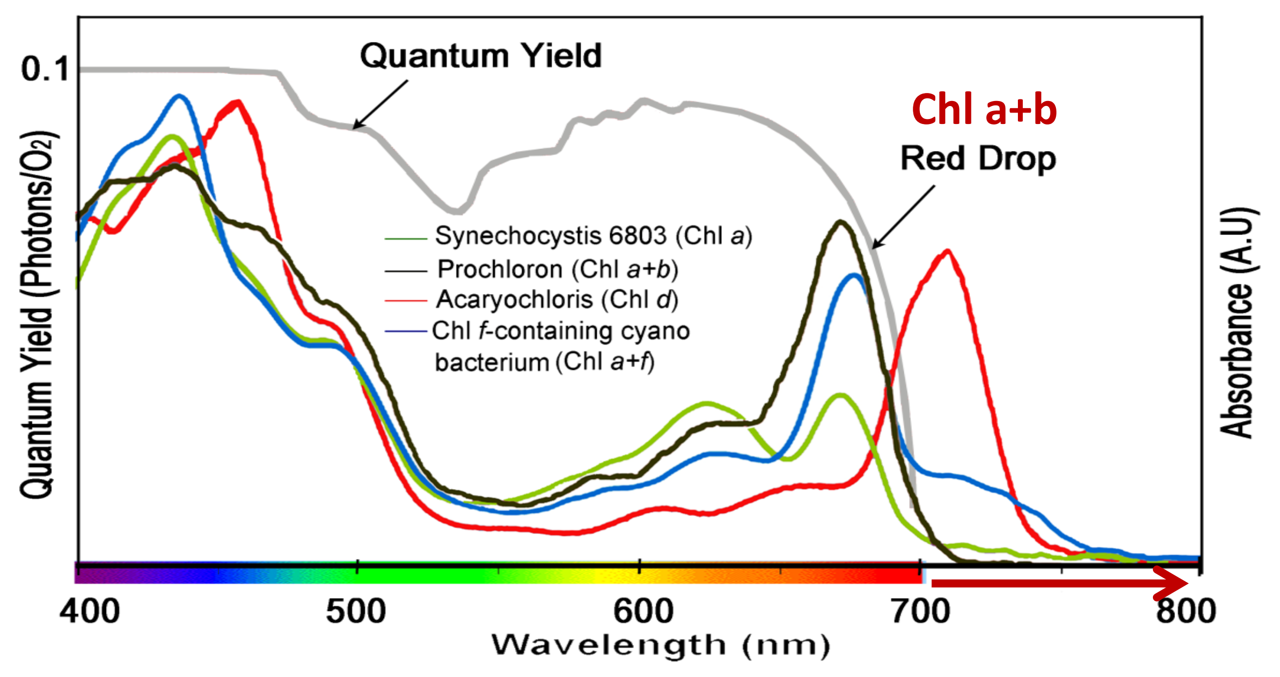
LoLiP cyanobacteria

First described for shaded *Synechococcus* sp. ecotypes of Thermal Spring,Yellowstone National Park

Are characterized by peculiar genes coding for light harvesting complexes coponents of the photosynthetic apparatus absorbing FR light: photosystemIICP43protein homolog *isiX*/ Allophycocyanin subunit *apcB3*/ Allophycocyanin subunit *apcD4*/Hypothetical protein (putative low light photoreceptor)

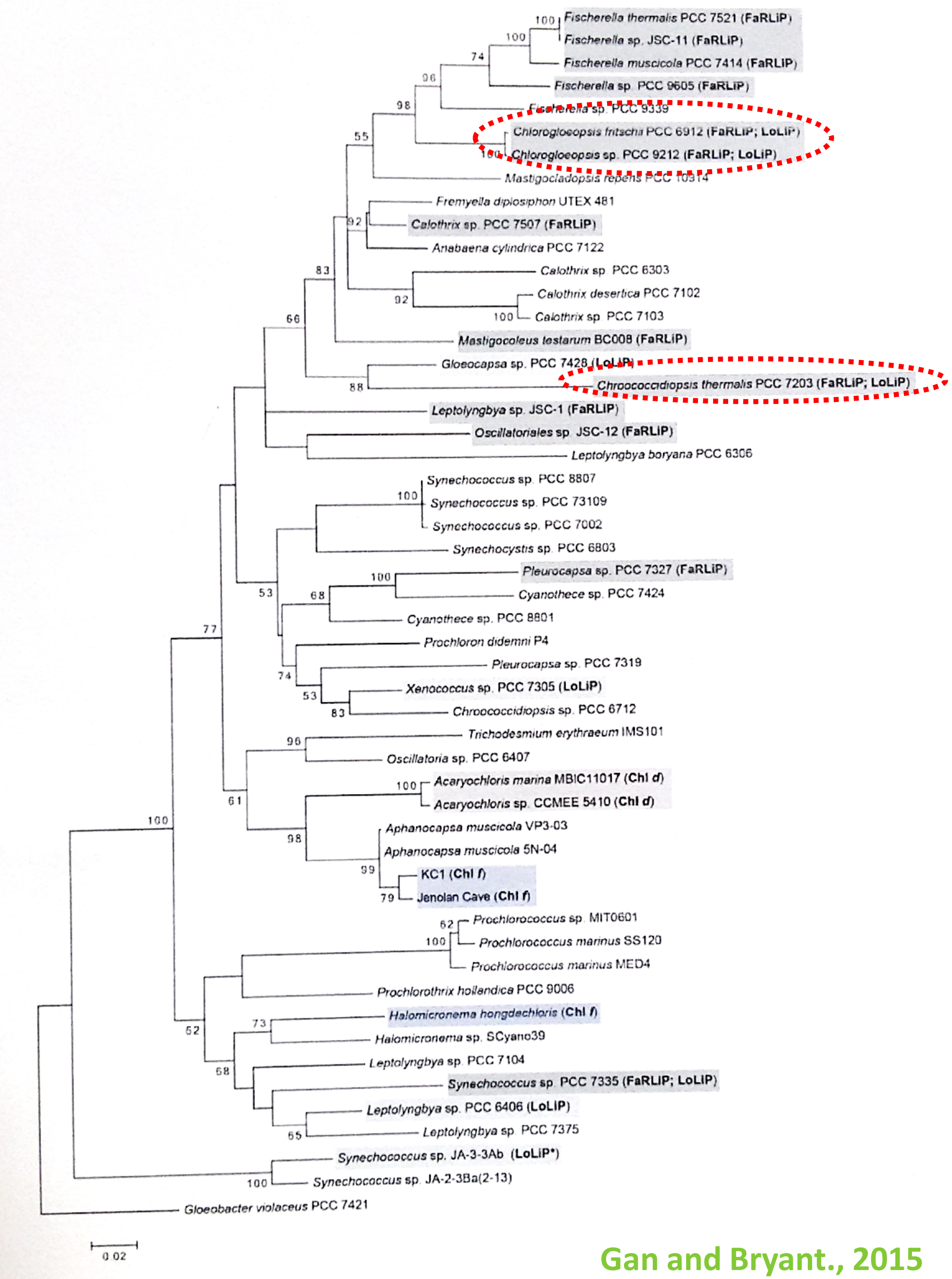
Synechococcus sp., Chlorogloeopsis spp.PCC6912andPCC9212, Fischerella sp. PCC9605, Chroococcidiopsis thermalis PCC7203, Gloeocapsa sp. PCC7428, Xenococcus sp.PCC7305,and Leptolyngbya sp.PCC 6406.

This discovery lead to enlarge the PAR radiation range



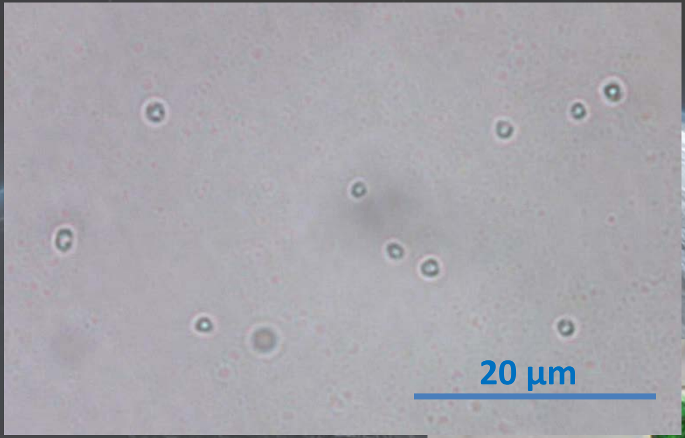
http://plantsinaction.science.uq.edu.au/book

In vivo absorption spectra of photosynthetic organisms containing different chlorophylls and the quantum yield of photosynthesis using only chlorophylls *a* and *b* (grey line).

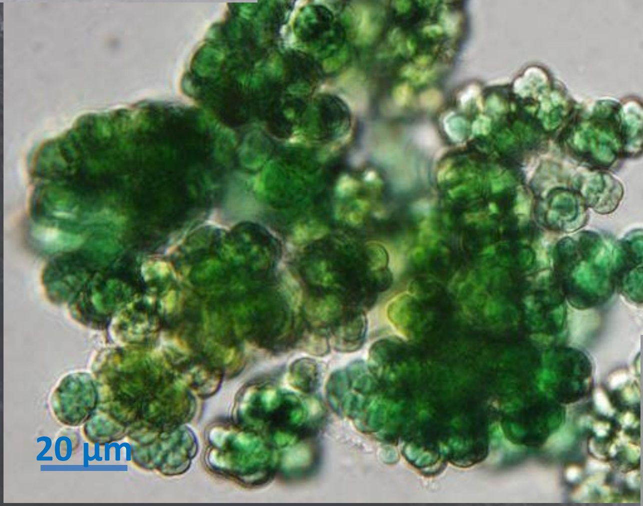


Gan and Bryant., 2015

The selected organisms



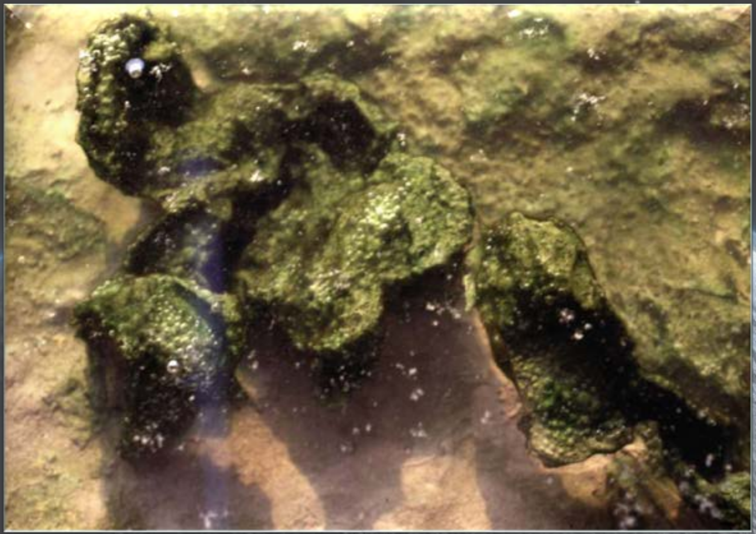
Acaryochloris marina

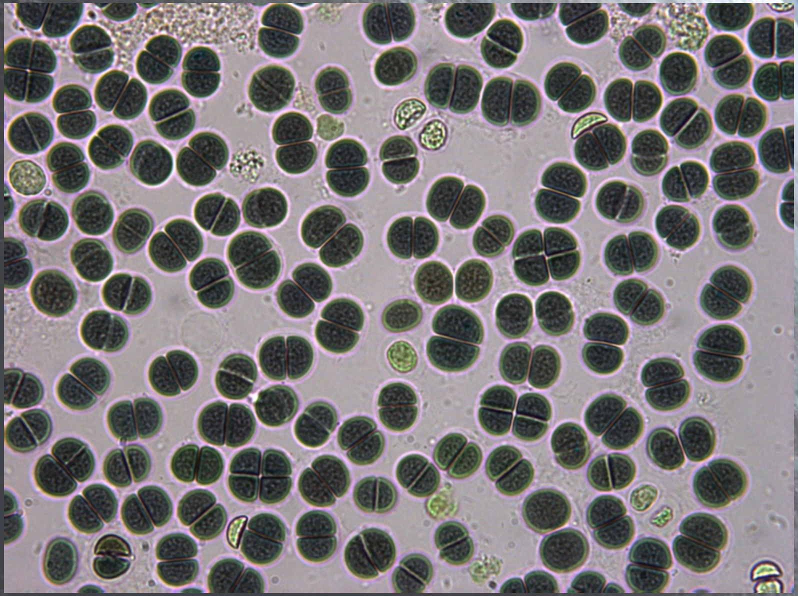


Chroococcidiopsis thermalis PCC7203

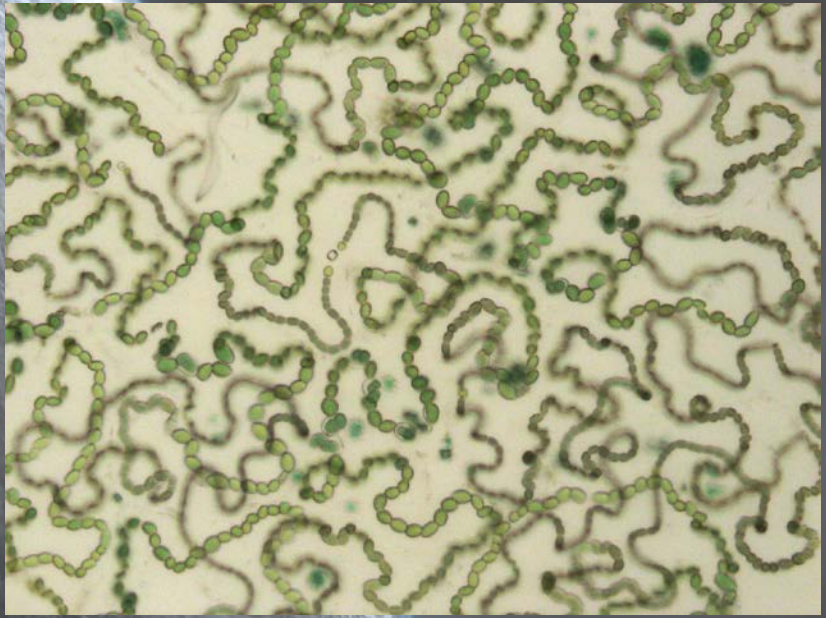
Chlorogloeopsis frischii PCC 6912

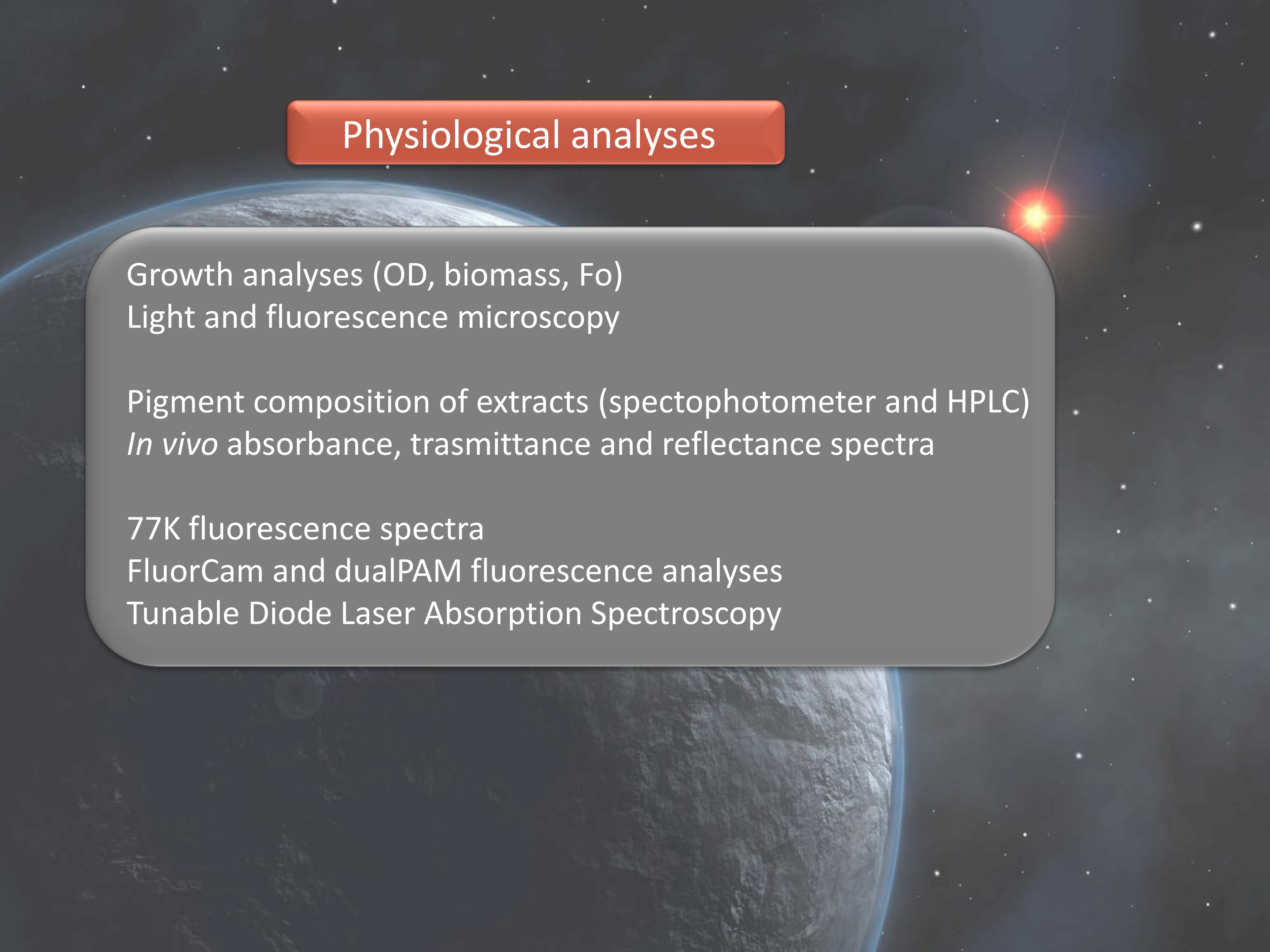
Cyanobacteria from thermal springs





Cyanobacteria from caves





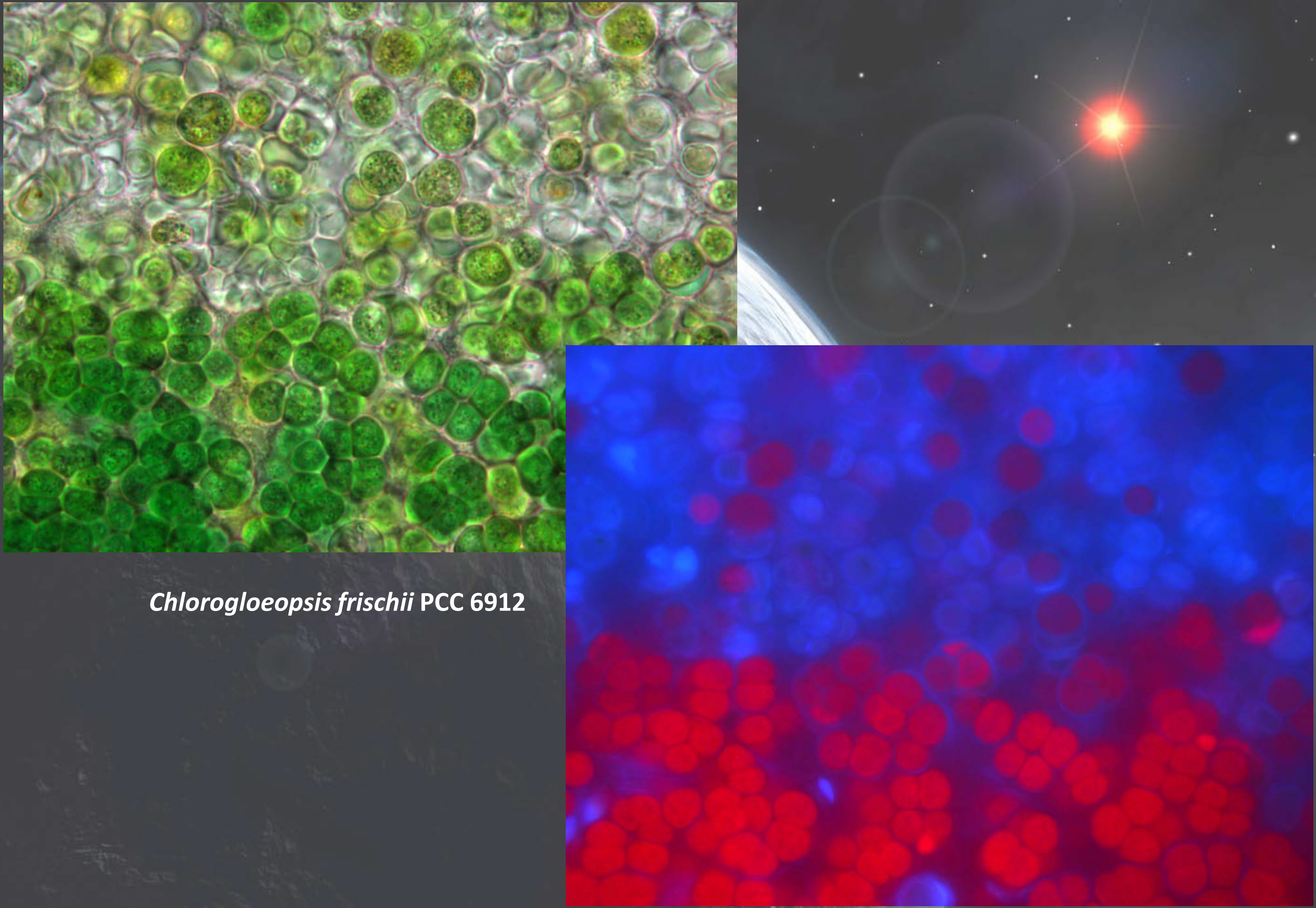
Physiological analyses

Growth analyses (OD, biomass, Fo)
Light and fluorescence microscopy

Pigment composition of extracts (spectophotometer and HPLC)
In vivo absorbance, trasmittance and reflectance spectra

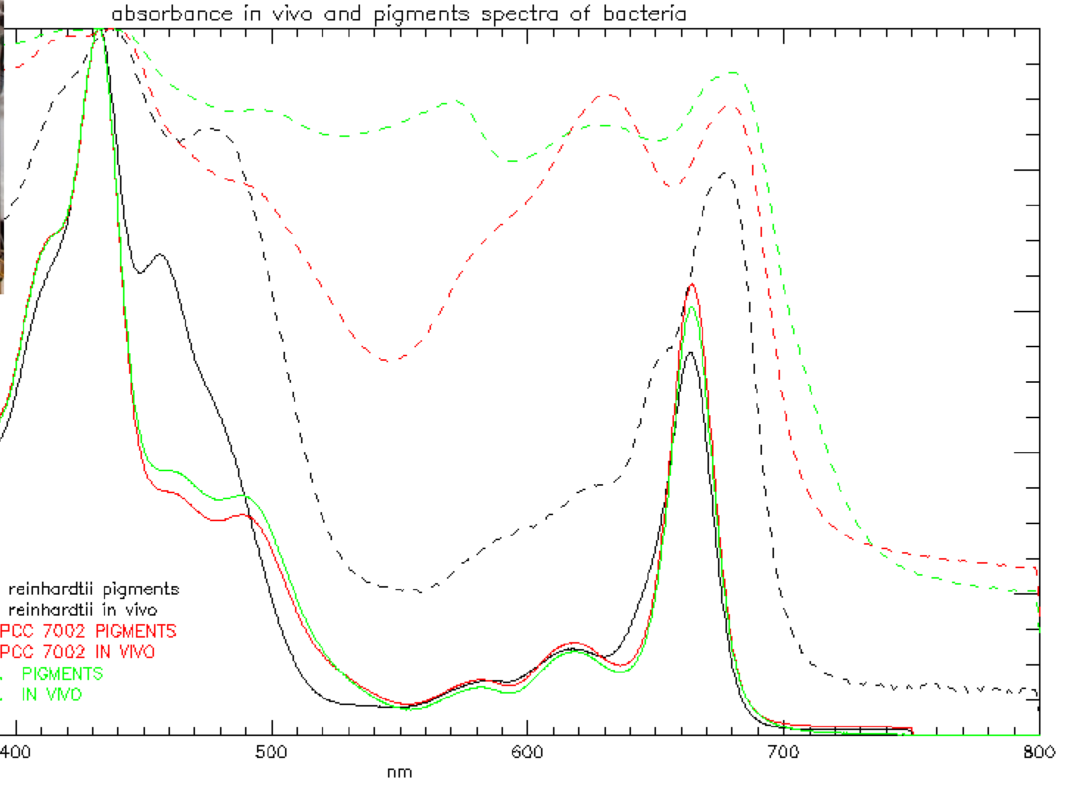
77K fluorescence spectra
FluorCam and dualPAM fluorescence analyses
Tunable Diode Laser Absorption Spectroscopy

Light and fluorescence microscopy

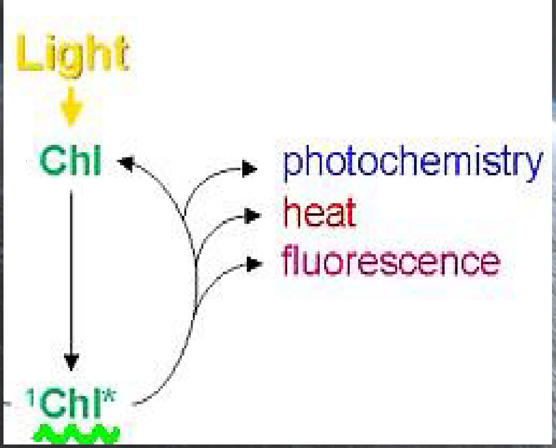


***Chlorogloeopsis frischii* PCC 6912**

In vivo spectra by Cary 5000 (collaboration with Prof. Pellizzo)



Mesuring Chls levels and photosynthetic efficiency by Fluor Cam


Light

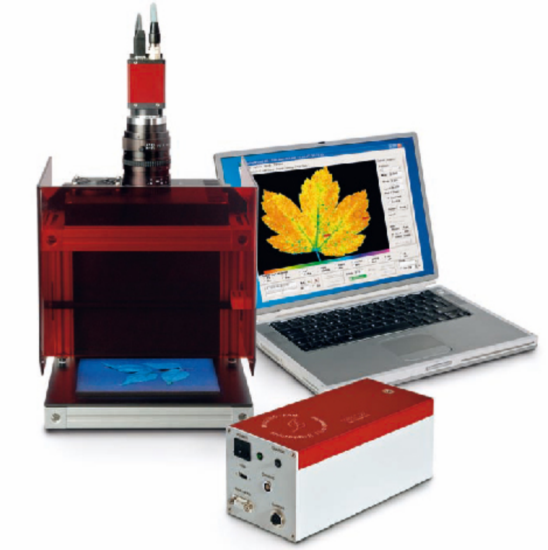
Chl

photochemistry

heat

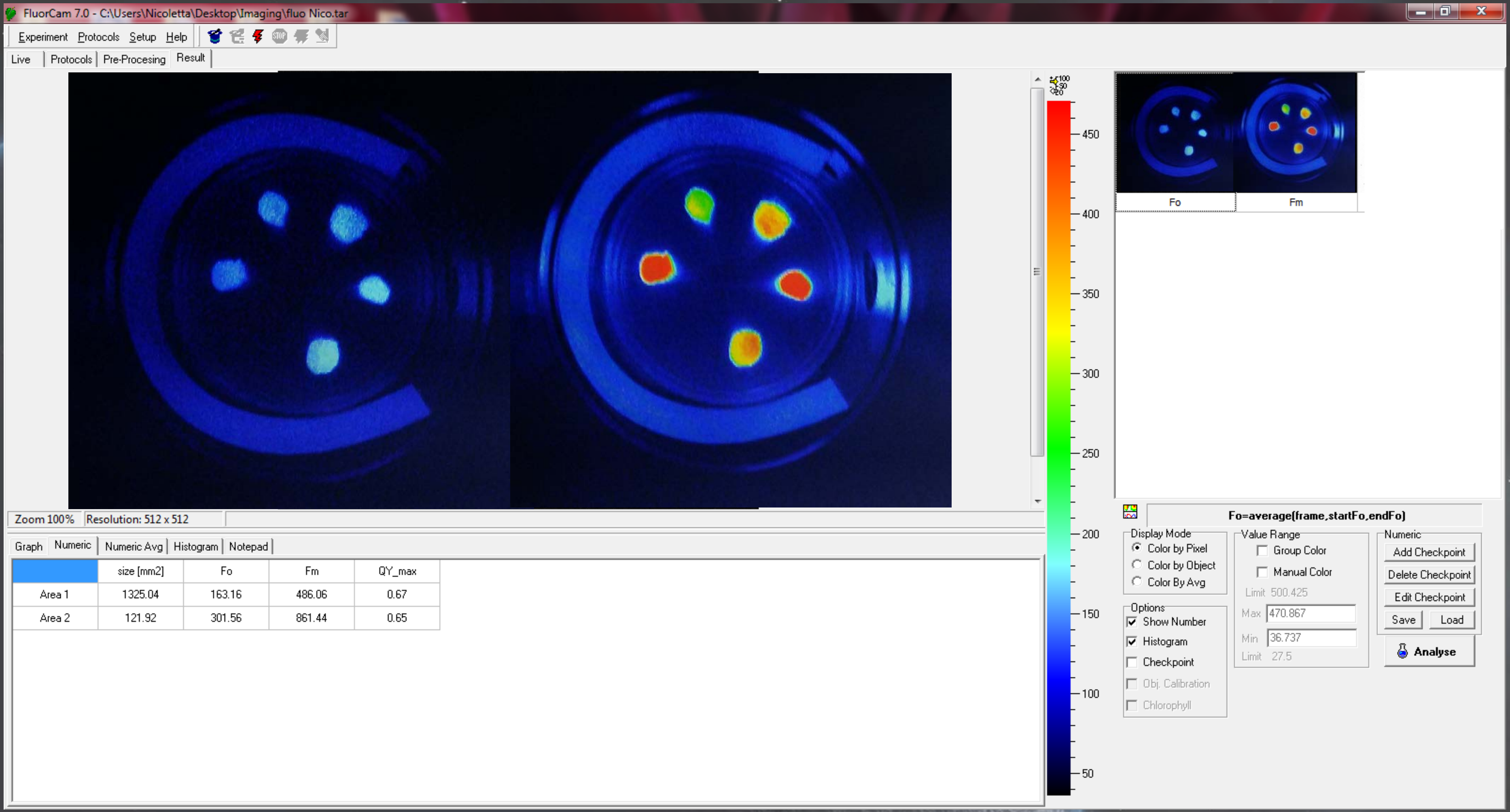
fluorescence

$^{1}\mathrm{Chl}^{*}$



The organisms in the MINI LISA incubator

FluorCam output



Zoom 100%
Resolution: 512 x 512

	size [mm2]	Fo	Fm	QY_max
Area 1	1325.04	163.16	486.06	0.67
Area 2	121.92	301.56	861.44	0.65

Fo=average(frame,startFo,endFo)

Display Mode
● Color by Pixel
○ Color by Object
○ Color By Avg

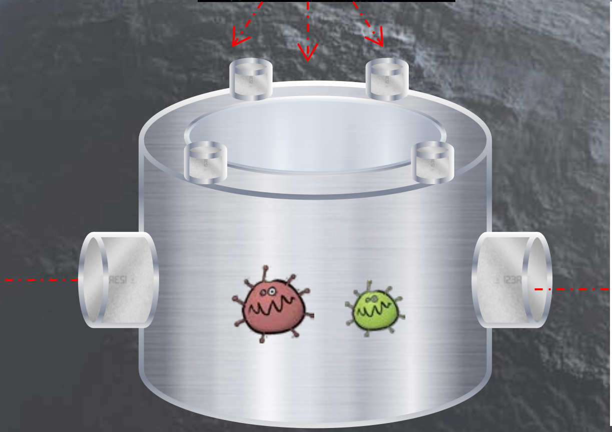
Options
☑ Show Number
☑ Histogram
☐ Checkpoint
☐ Obj. Calibration
☐ Chlorophyll

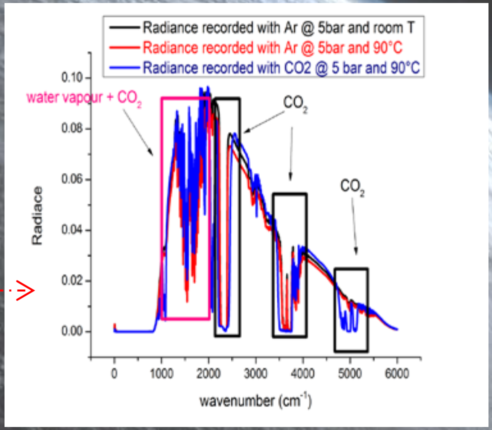
Value Range
☐ Group Color
☐ Manual Color
Limit 500.425
Max 470.867
Min 36.737
Limit 27.5

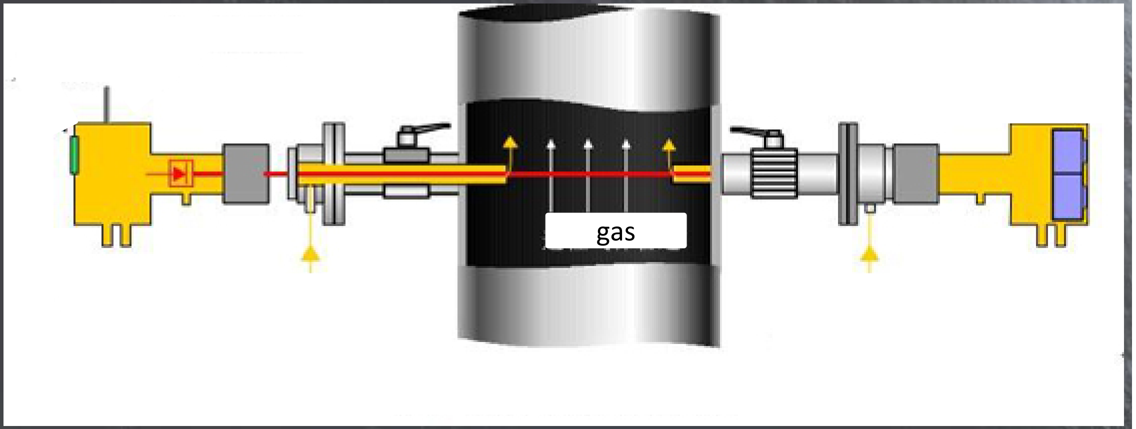
Numeric

Masuring CO_2 by Tunable Diode Laser Absorption Spectroscopy setup (TDLAS)

LUXOR







Accuracy: O2 <1% ; CO2 , CH4= 0.1%

UN NUOVO METODO PER LA MISURA DELLA CO2

L pro presenta L.sensor.CO_2, un afrometro elettronico di nuova concezione.
Utilizza la tecnologia laser per fornire la pressione totale ed il contenuto di CO_2 all'interno delle bottiglie di vino senza forare o togliere il tappo.

VANTAGGI

-misura non invasiva (non distrugge il campione)
-veloce (la misura viene effettuata in pochi secondi)
-preciso e ripetibile (la misura non viene influenzata dall'abilità dell'operatore)
-sicuro e facile da usare (non sono richiesti interventi sulle bottiglie e quindi si elimina il rischio di esplosione o rottura della bottiglia)
-ottengo due risultati: la pressione totale e la componente dovuta alla sola CO_2 (l'afrometro tradizionale fornisce solo la Ptot)
-si può misurare più volte lo stesso campione per misure (ad es.) sull'andamento della fermentazione o sulla tenuta dei tappi

LA TECNICA

L.sensor.CO_2 utilizza il passaggio di un raggio laser attraverso lo spazio di testa di una bottiglia per misurare, tramite la spettroscopia di assorbimento, la pressione totale dovuta a tutti i gas presenti all'interno della bottiglia e la pressione relativa dovuta alla sola CO_2 presente nel collo della bottiglia.

L PRO SRL
L.SENSOR.CO2

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