

D1.2 Methods for simulation and computation of light, temperature and (in FACE) CO₂ in individual plants

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Executive Summary

Despite the general usage of the term “controlled environment”, the environment is generally highly variable in phenotyping installations. Because many processes that determine the plant phenotype are sensitive to the environment (i.e. phenotypic plasticity or reaction norms), often in a genotype-dependent manner (i.e. genotype-by-environment interactions), this lack of control results in a loss of accuracy in the phenotype assessment or, worse, bias the evaluation of genotypes or modalities. Except in fully controlled growth chambers, it is not possible to fully control environmental conditions, but it is possible to measure actual environmental conditions sensed by plants, to keep track of the environment variability during experiments and to store this information together with phenotype data. This is particularly important in a FAIR perspective where data from different installations are meant to be reused in other contexts. During the last decade, partners of the EPPN²⁰²⁰ project have demonstrated that the spatial and temporal variability of the environment within phenotyping installations that provide time series of plant phenotypes can be beneficially exploited to evaluate the responses of genotypes to environment variables. In several instances, these responses have been shown to better translate to field conditions than point phenotypic assessments.

The Joint Research Activity 1.1 has been designed on the premises that an accurate characterization of the environmental variability is a pre-requisite of any phenotyping experiment. This document presents the means used in JRA1.1 to progress towards light, temperature and CO₂ characterisation in all EPPN²⁰²⁰ installations. This includes (i) the development of specific methods for environmental characterization in installations, (ii) surveys of existing environmental characterization practices in every installation, (iii) decisions on common target standards and (iv) monitoring of progress towards these standards during the project.

In particular, methods have been developed and tested to map light and temperature within greenhouses. Temperature tends to display a continuous variation within the installation. The situation is different for light which presents discontinuous spatial patterns due to the presence of lit and shadow patches, as well as rapid temporal variations. We therefore developed a method based on a combination of sensors and simulations to estimate incident light at every position in the installation. Tutorials have been prepared and are currently used for the deployment of these methods in all EPPN²⁰²⁰ installations and potentially beyond.

The context is different in FACE installations aiming at controlling CO₂ concentration in open-field setups. The challenge here relies more in the design of the CO₂ supply equipment in order to ensure homogeneous gas concentration over the experimental plot. Different designs have been tested and evaluated, from which recommendations can be formulated for other experimental fields and installations.

Authors/Teams involved: Sixtine Passot (UCL) , Xavier Draye (UCL), Jacques Le Gouis, ... Llorenç Cabrera Bosquet, François Tardieu (INRA).

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1. COMPUTING INCIDENT LIGHT DISTRIBUTION IN GREENHOUSES

1.1. General principle

Incident light is highly heterogeneous in greenhouses in time and in space, due to the changes of sun position in the sky and the shadowing effect of the greenhouse structure. Due to the dependence of plant growth on incident light, being able to estimate or compute the amount of light received by individual plants is an important asset to interpret phenotypic differences within the greenhouse.

While statistical methods can account for the effect of spatial variability that tend to be stable over time, the variability of light inside a greenhouse presents complex patterns that are unlikely to be stable over time. In addition, light patterns are discontinuous due to the alternation of lit and shadow patches caused by the greenhouse structure, so that a grid of light sensors may not be accurate enough to estimate incident light correctly between sensors. The method developed within EPPN²⁰²⁰ computes maps of incident light in the greenhouse based on the measurement of solar radiation outside the greenhouse corrected by simulated values of light transmission through the greenhouse structure parameterized on the basis of hemispheric pictures of the sky acquired at plant positions.

The method estimates the proportion of sun light that reaches plants at any location. For this, the solar radiation is split into direct and diffuse light. Direct light is composed of light rays that travel in straight line from the sun and reach the plant only if there is no opaque obstacle on its way. Opaque obstacles, such as the greenhouse structure (e.g. beams, columns), create shadow patches that move in the greenhouse along with the sun trajectory. A hemispheric picture of the sky acquired at a plant position can be used to compute the set of directions for which incident rays of direct light can reach the plant. In the currently implemented method, pictures are captured from a subset of the possible plant positions in the greenhouse, and incident light at each plant position is estimated with an interpolation function. Diffuse light, produced by the reflection of light rays by air molecules, is assumed to be uniformly distributed and independent from the sun position. The proportion of direct and diffuse light depends on the date and time and on the atmospheric conditions. During cloudy days, 100% of the light is diffuse light, whereas during a sunny day, proportion changes. This proportion can be measured by specific sensors, but a prediction algorithm is proposed in the method.

In addition to the solar radiation and the greenhouse structure, steerable equipment such as light fixtures and curtains may affect the incident radiation at the plant position. The effect of curtains is to attenuate light transmission by a certain proportion that is estimated from the curtain state (open, half-closed, closed), itself controlled by the climate unit of the greenhouse and recorded in real time. Additional incident light brought by the fixtures is also obtained from the climate unit of the greenhouse and is added to the final result assuming it is homogeneously distributed.

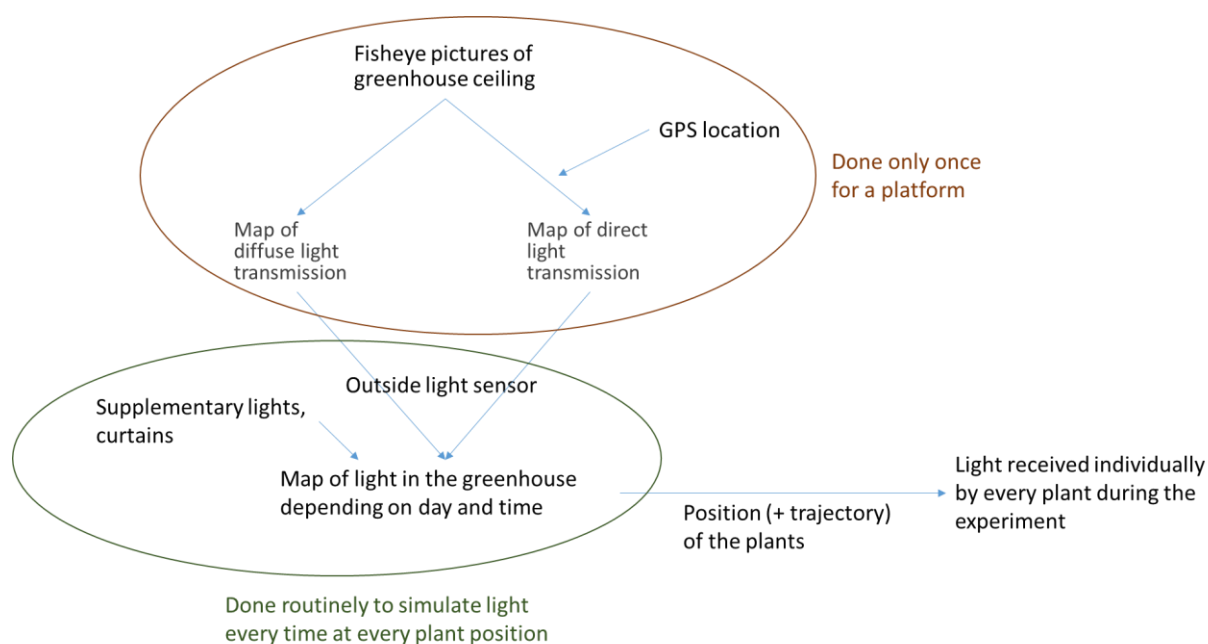


Figure 1 : Flowchart of the method to simulate incident light for all plants in a phenotyping platform.

1.2. Application to evaluate radiation use efficiency in maize plants

The amount of light effectively captured by a plant depends on the amount of light arriving at its place in the greenhouse but also of the plant architecture and of the shadow caused by neighbour plants. Once the spatial distribution of incident light has been computed, the amount of light intercepted by each plant can be simulated using a virtual scene of the canopy around the plant, constructed with a three-dimensional structural model of each plant based on 3D-plant reconstructions obtained from image analysis. This amount of intercepted radiation is a valuable information from which the radiation use efficiency (RUE, ratio of plant biomass to intercepted light) of individual plants can be estimated. This data analysis pipeline has been developed and tested on a maize experiment. Light interception varied largely between maize lines that differed in leaf angles (nearly stable between experiments) and area (highly variable between experiments). Estimated RUEs varied between maize lines, but were similar in two experiments with contrasting incident light. In addition, they correlated closely with measured gas exchanges (Cabrera-Bosquet et al., 2016) (Fig. 2). These results highlight the benefit of accounting for the spatial variation of environmental variables.

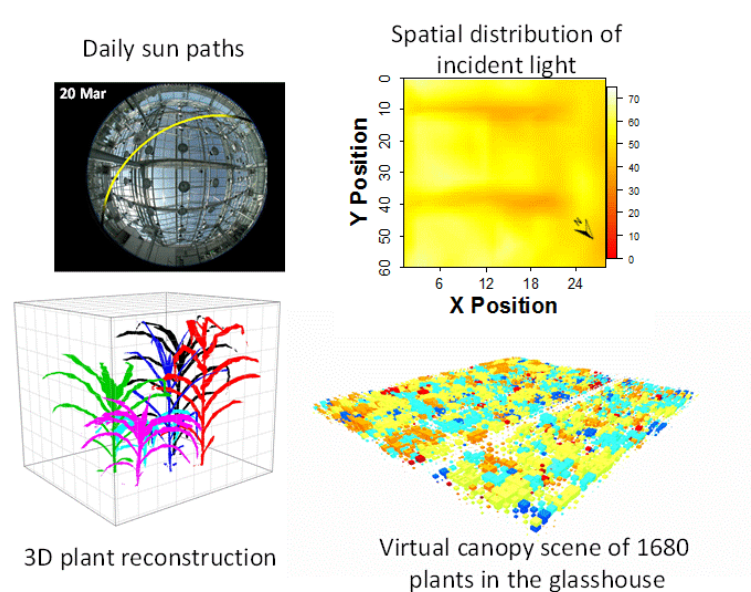


Figure 2. Data analysis pipeline for spatial incident light and light interception estimation.

1.3. Deployment of the method within EPPN²⁰²⁰

1.3.1. Extension of the method to a second installation

The method of mapping incident light with a single-plant definition was applied to the RootPhAir installation in UCLouvain. This required few adaptations of the method, which contributed to progressing towards the generalization to other installations. Indeed, several features of the RootPhAir installation differ from those in PhenoArch, in which the method was initially tested. The greenhouse measures only 64m² and the surface area on which plants actually grow is even smaller (Figure 3). In addition, plants are only grown three weeks and are small at the end of the experiment. Consequently, only 25 pictures were used to cover the area, at only one height. The opaque elements were segmented and maps of direct and diffuse light transmission were generated. Direct light transmission maps showed a clear spatial pattern, varying with day of the year.

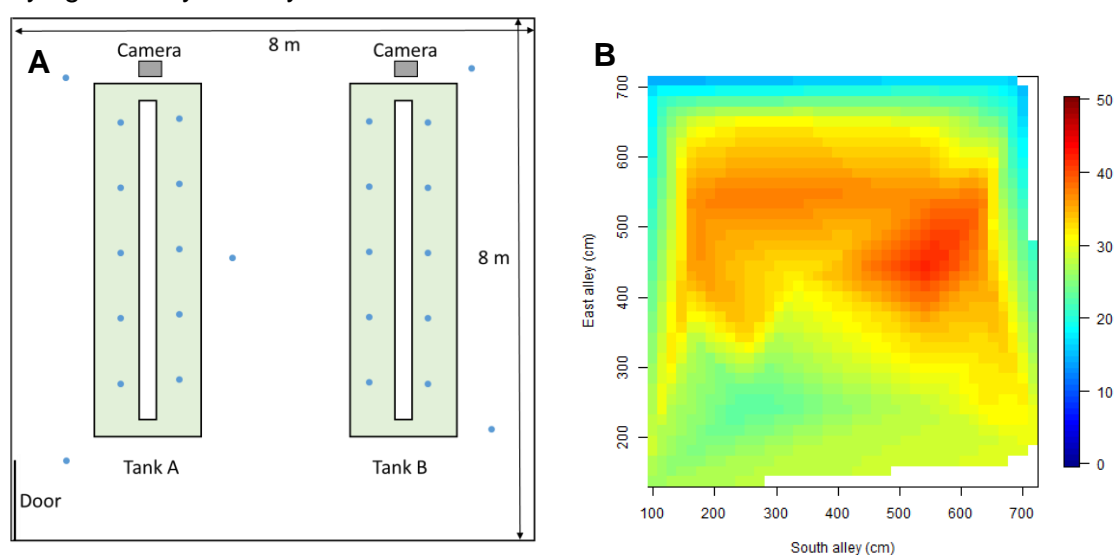


Figure 3 : A: Schematic representation of the RootPhAir platform. The place where plant grow is in light green. The position of the fisheye pictures are in blue. B: Direct light transmission (% of outside direct light) on July 28. A clear spatial pattern is visible, affecting differently the two tanks.

The simulated and measured radiation data during a three-week long experiment were analysed to validate the method (Figure 4). The correlation between measured and simulated light was 0.86 over the whole duration of the experiment.

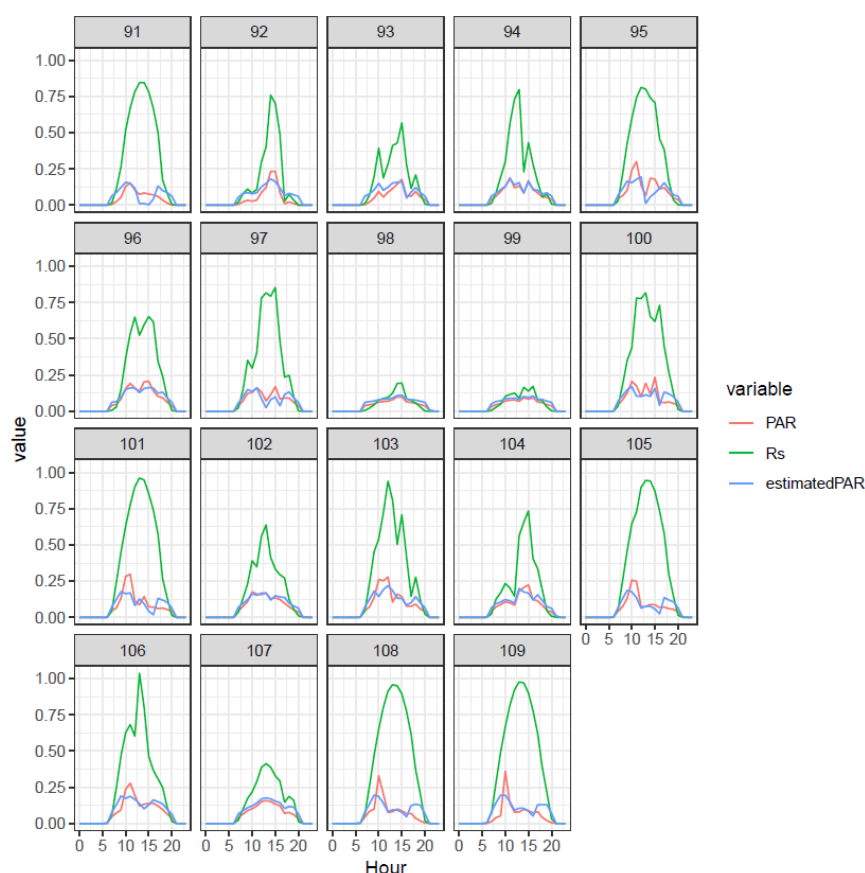


Figure 4 : Comparison of measured PAR (red), simulated PAR (blue) and outside solar PAR (green). Estimated PAR follows measured PAR irrespective of outside radiation.

This application showed that the method was easily transferable to an installation with very different features (greenhouse size and structure, geographic location ...). It also helped making the method easier to understand and more general.

1.3.2. Tutorial

A tutorial was written to enable all installations to apply this method. The tutorial explains all steps: image acquisition, image treatment, adaptation of pre-written R scripts and validation of the output. The target of JRA1.1 is that all installations will generate maps of light in every experiment. The method described above and its availability to all partners will support reaching this objective.

2. COMPUTING TEMPERATURE DISTRIBUTION IN GREENHOUSES

2.1. Simulation of temperature

Following the results obtained for mapping light variability, we considered the possibility of simulating temperature inside a greenhouse. A mathematical model of heat transfer was developed to predict the 3D time course of temperature in a greenhouse, based on the RootPhAir installation at UCL. The model takes into account the precise geometry of the greenhouse and heating pipes, the thermal properties of the greenhouse glass, the dynamic

regulation of the heating system, the heat conduction from the main sources (radiation, heating) and the heat convection within the greenhouse. The current model assumes that plants in the greenhouse modify the microclimate by their transpiration. Several configurations of heating pipes were simulated. The first results (Figure 55) suggest that the method can be extremely sensitive. However, its implementation in other installations is dependent on the availability of heating tubes real-time temperature records which may not be available. In addition, greenhouse specialists cast strong doubts on the ability of such models to account for complex effects of plants on the greenhouse climate. For these reasons, and because the variation of temperature in time and space is continuous, it was decided to move to interpolation-based methods.

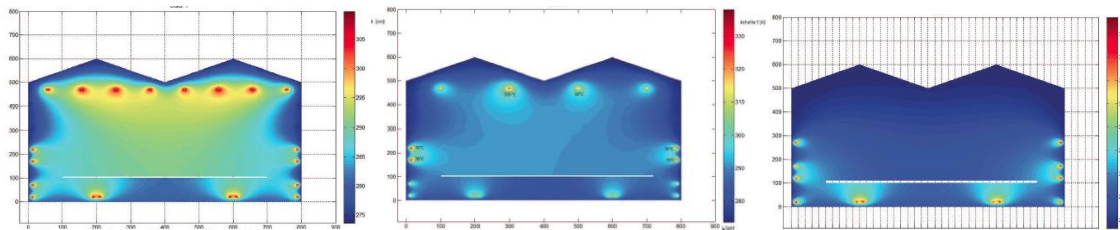
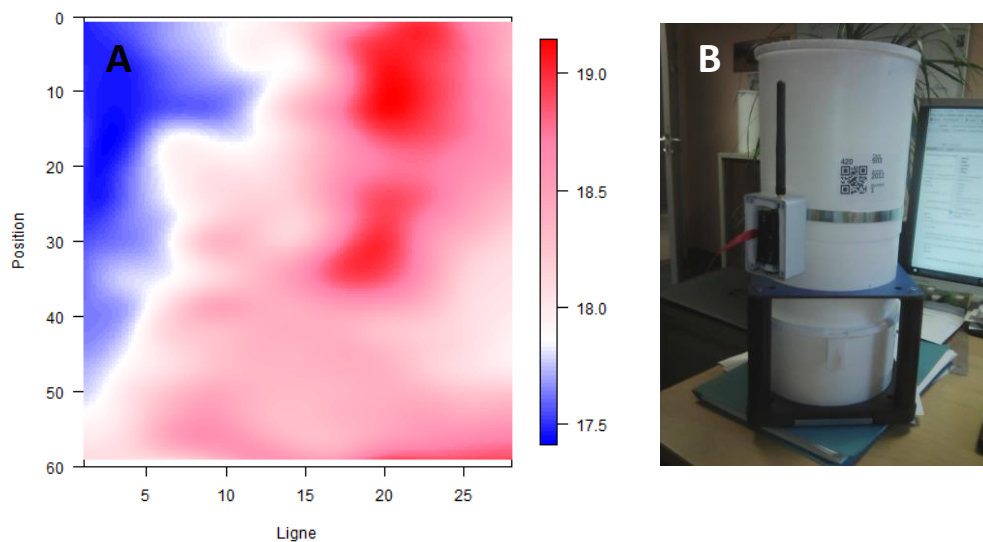


Figure 5 : 3D temperature simulations in a 64 m² greenhouse with three different configurations of heating tubes.

2.2. Creating temperature maps using a network of sensors

A geostatistical approach based on spatial interpolation of temperature based on the output of a grid of sensors distributed in the greenhouse was therefore developed. The method has been designed and implemented initially in the PhenoArch installation (INRA-Montpellier), based in a network of 60 wireless spatially distributed sensors.



A similar method has been evaluated in the RootPhAir platform, with 10 sensors distributed around the tanks containing the plants (Figure 66).

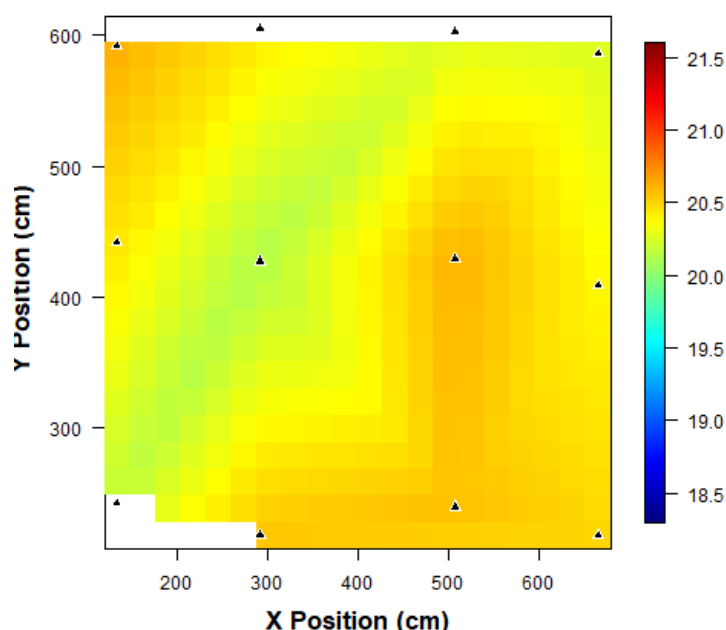


Figure 6 : Temperature in RootPhAir platform on 11-04-2019 at 7:00 AM (°C). Black triangles represent the locations of the sensors.

The method has also been deployed for the 4PMI platform (INRA-Dijon). In this greenhouse, some plants are located outside of the convex hull formed by the sensors. As it was not possible to infer the temperature of these plants by interpolation in the same way as for the other plants, the method has been adapted to attribute to these plants the temperature of the closest sensor. This method is actually rather simple to implement and adjust and there exists many interpolation methods. Installations are therefore likely to find local statistical competencies or support to develop in house solutions.

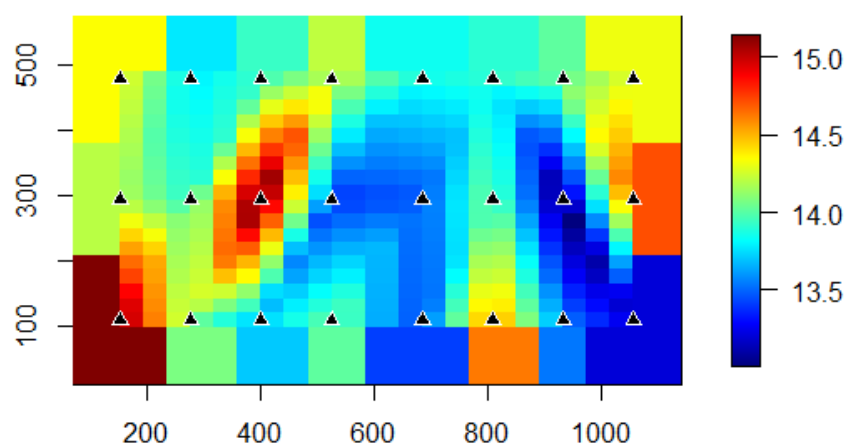


Figure 7 : Estimation of air temperature around individual plants in 4PMI platform (°C). The black triangles represent the location of the sensors. Each colored pixel corresponds to the location of one individual plant.

3. HOMOGENEITY OF CO₂ IN FACE EXPERIMENTS

3.1. Objective

The ability to increase atmospheric CO₂ in the field to mimic environmental scenarios corresponding to future climates is an important challenge that FACE (Free-Air CO₂

Enrichment) installations can address. The conventional FACE system relies on wind to mix pure CO₂ released on top of the canopy. With that system, experiments involving large number of genotypes (e.g. genetic studies) require a high spatial and temporal homogeneity at the scale of a micro-plot (~3 m²). The situation is further complicated in installations equipped with rainout shelters to analyse the interactions between CO₂ level and drought. Indeed, the equipment and the presence/absence of the rainout shelters are likely to disturb the homogeneity of CO₂ across the experimental area. Within JRA1.1, different designs have been elaborated and evaluated to propose settings that are suitable for large scale experiments involving rainout shelters.

3.2. Installation constraints

On the Pheno3C platform (INRA-Clermont), a block of 21 x 25 m is composed by 10 x 14 = 140 microplots, each about 1.5 x 2.0 m (Figure 8A). The material used for CO₂ release is a MiniFACE TEA (Italy) with eight channels, a set of Vaisala GPM 252 CO₂ sensors, and micro-perforated (0.2 mm) PVC tubes of 16 mm diameter. The design provides for eight injection channels on a 9-zone divided block (Figure 8B). The present CO₂ level is around 400 ppm while enrichments of 600-700 will be targeted.

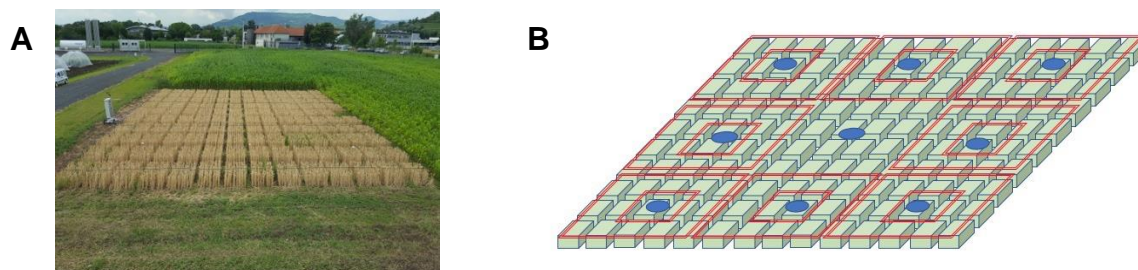


Figure 8 : A: a wheat block composed of 10 x 14 = 140 microplots. B: the FACE design with eight injection channels (red) on the 9-zone divided block.

3.3. The test in different steps

The FACE tests on the Pheno3C platform were performed in several steps, in collaboration with Franco Miglietta and Marin Tudoroiu (CNR, Italy). The homogeneity measurements were carried out using five CO₂ sensors distributed over the successive designs and acquiring CO₂ concentration at a rate of 1 measurement per minute. The reduced test area (3 x 4 m) required us to adapt the design to the larger block size (21 x 25 m).

1. Installation with the Italian team of vertical perforated tubes on a surface of 3 x 4 m (Figure 9A). On this limited area, we obtain a correct system response with a good spatial homogeneity for a 600 ppm setpoint (Figure 9B).

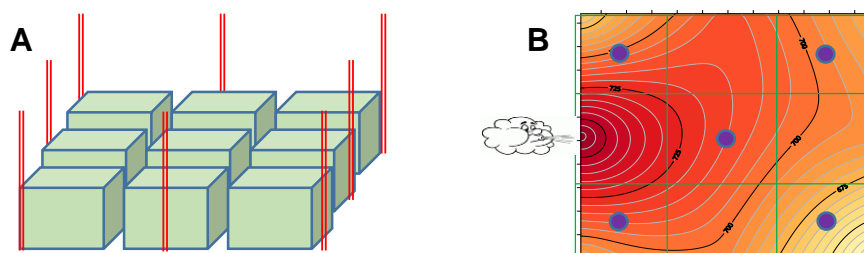


Figure 9 : A, the design to test the FACE system on a small area. The CO₂ is released on top of the canopy through vertical perforated tubes (red). B, a map of CO₂ concentration for a setpoint of 600 ppm extrapolated from measurements of five fixed sensors (blue dots) and one mobile sensor.

2. Extension of the system for a surface area of 8 x 7 m (1/9 of the block). With this size, homogeneity is no longer correct, the injection points are too far away from each other. The increase in the number of vertical tubes connected by flexible tubes would have posed practical problems for installation in the field.
3. The set-up was first changed with 8 x 7 m square rings of perforated tubes (Figure 10). Using this design, we obtain an improved homogeneity for a low setpoint (400 ppm) but not enough CO₂ flow to reach higher setpoints for this area.

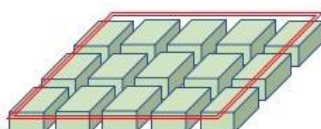


Figure 10 : the design to test the FACE system on a large area. The CO₂ is released on top of the canopy through one ring of horizontal perforated tubes (red).

4. The replacement of one of the injection solenoid valves (1000 l/min instead of 150 l/min flowrate) has allowed to reach and test a higher flowrate. The setpoint is now reached but a CO₂ gradient is detected between the different zones of the plot (Figure 11).

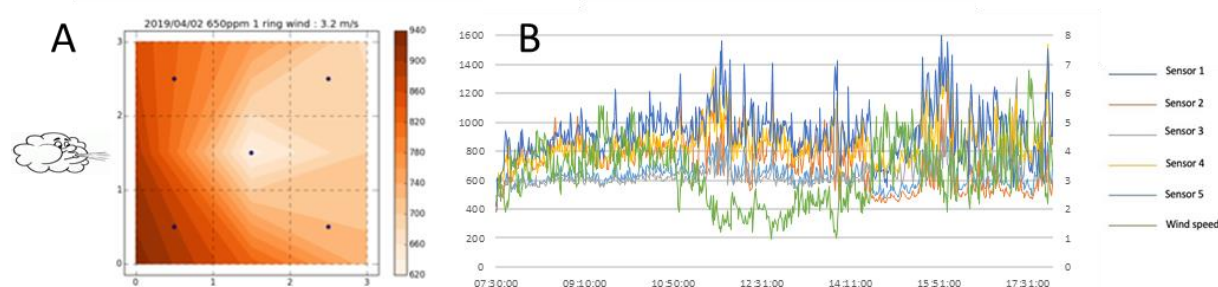


Figure 11 : A, a map of CO₂ concentration for a setpoint of 650 ppm (average on 1 day) extrapolated from measurements of five sensors (black dots). B, evolution of CO₂ concentrations measured by the five sensors on one day.

5. A central ring of 2 x 3 m was added (Figure 12). After a short period at the beginning of CO₂ release (morning) the setpoint is reached and the homogeneity is adequate (Figure 13).

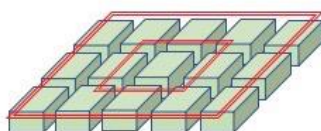


Figure 12 : the final design to test the FACE system on a large area. The CO₂ is released on top of the canopy through two rings of horizontal perforated tubes (red).

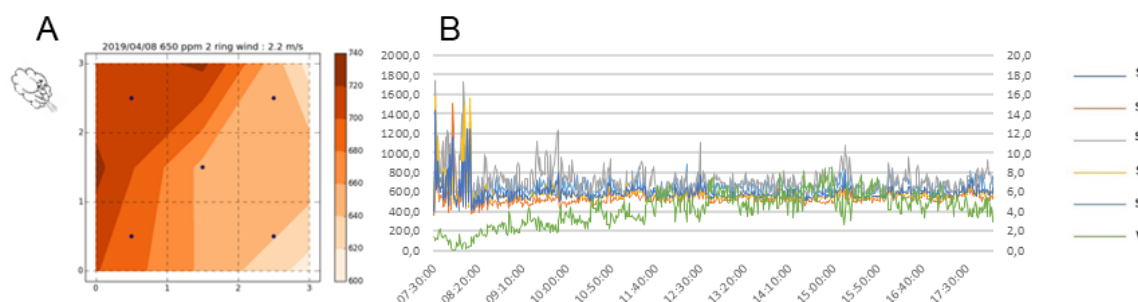


Figure 13 : A, a map of CO₂ concentration for a setpoint of 650 ppm (average on 1 day) extrapolated from measurements of five sensors (black dots). B, evolution of CO₂ concentrations measured by the five sensors on one day.

We are currently waiting for the change by our Italian colleagues of the other solenoid valves to finalize the tests on a complete block.

4. ENVIRONMENTAL CHARACTERIZATION ACROSS EPPN²⁰²⁰ INSTALLATIONS

4.1. Standards definition

Two levels of environmental characterization in phenotyping installations have been defined within EPPN²⁰²⁰, taking into account the actual practices across the phenotyping community that were considered as good practices at the beginning of the project. Under level 1, greenhouse conditions are monitored every hour (at least) using a minimum of two sensors of light intensity, temperature and relative humidity, in order to be able to realize the amplitude of spatial and temporal variability within the installation. CO₂ concentration is checked regularly. In FACE installations, CO₂ is monitored every hour (at least). As of M24, the survey of installations revealed that 21 installations complied with level 1 and 2 installations were progressing towards this level.

After training sessions organised during the annual meetings, the partners collectively agreed to raise the level of environmental characterization, with the use of additional sensors and scripts, in order to generate hourly maps of light intensity and temperature covering the whole installation area, with a resolution down to the individual plant. This was defined as level 2. As of M24, 3 installations were compliant with level 2 and 6 installations were progressing towards this level.

At the third annual meeting (April 2019), it was agreed that all controlled condition platforms should engage in a transition towards level 2.

4.2. Projected evolutions

We expect that more platforms will comply with level 2 before the next annual meeting, and nearly all of them by the end of the project. The following strategy has been setup since the beginning of EPPN²⁰²⁰ to support installations in their transition:

- Annual survey of installation status. Every year, we survey individually each platform on the sensors and methods that are used. The results of these surveys are presented to the community at the annual meetings.

- Diffusion of practices used in other installations. During 2019 annual meeting, we organized a training session on environmental characterization. In particular, the methods listed in this document have been presented to the participants.
- Compilation and sharing of sensor documentation. We will generate a list of all sensor brands and models currently used by platforms. This list will include technical and pricing data and, most importantly, feedbacks from the users.
- Development of a tutorial to interpolate temperature data. This tutorial is helping partners to use interpolation to obtain maps from a network of temperature sensors. It is based on examples developed in R. The possibility to extend the estimation outside the convex hull formed by the sensors, as done for 4PMI greenhouse, is included, along with advices to deal with time series.
- Development of a tutorial to generate radiation maps in greenhouses. This tutorial covers the successive steps of the computation: image acquisition, image treatment, adaptation of pre-written R scripts, validation of the output, progress towards routine usage. A first version has been provided to few volunteer platforms. We will closely follow they progress and will improve the tutorial accordingly.

5. CONCLUSIONS

JRA1.1 has achieved considerable progress in environmental characterization through a series of concrete actions involving several partners (serving as pilots) or all partners:

- Novel methods have been designed and validated to compute incident radiation at the level of individual plants within greenhouses.
- Interpolation methods have been validated to estimate air temperature at the level of individual plants within greenhouses.
- Adjustments have been made to conventional FACE systems to make them suitable to installations covering large surfaces and equipped with rainout shelters.
- Standard levels of environmental characterization have been decided and installations are transitioning towards the high standards.
- A continuous monitoring process has been put in place.
- Tutorials have been created to allow the deployment of the new methods within the EPPN²⁰²⁰ network.

As these actions progress towards completion, EPPN²⁰²⁰ installations are in a better position to contribute to a new generation of phenotype data usage, enabled by an accurate characterization of the environment during experiments. More elaborate combination of environmental and phenotype data has already made possible to compute complex traits or variables that are beyond the reach of conventional phenotyping.

References

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Glossary

EPPN²⁰²⁰: European Plant Phenotyping Network – 2020

RUE: Radiation Use Efficiency

FACE: Free Air Carbon dioxide Enrichment. Designates an experimental field where CO₂ is artificially added to the air

Annex 1: Tutorial “Mapping light in a greenhouse”

Mapping light in a greenhouse

Method developed by Llorenç Cabrera-Bosquet (INRA Montpellier)

Tutorial written by Sixtine Passot (UCLouvain)

Questions, remarks: sixtine.passot@uclouvain.be

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1. INTRODUCTION

1.1. Method presentation

This method aims at estimating the light effectively received by every plant in a greenhouse, depending of its position. It is based on the simulation of light transmission through the greenhouse ceiling, which structure is acquired by hemispheric pictures, taken at regular positions in the greenhouse. We estimate the proportion of light that can go from outside to inside and multiply it by the outside light intensity, instead of actually measuring light inside the greenhouse.

Sunlight is divided into two parts: direct and diffuse. Direct light is composed of light rays coming in straight line from the sun. This light beam passes only if there is no opaque obstacle between the sun and the point of interest. Opaque obstacles block direct light and creates a shadow. Even in the shadow, a uniform and soft light persists, which is diffuse light. It is produced by the reflection of light rays on air molecules. This light does not depends on the position of the sun, as it is ubiquitous. During a cloudy day, 100% of the light is diffuse light, whereas during a sunny day, some direct light is also present. The proportion of direct light that attains a certain point of the greenhouse varies with time and place. It depends on the position of the Sun in the sky, itself depending on the GPS location of the greenhouse, the day and the time. It also depends on the localization of opaque and transparent parts of the greenhouse structure. This information comes from the fisheye pictures of the greenhouse ceiling, that are specifically transformed to this end. For each picture location, we compute the transmission coefficient through the greenhouse of the two types of light (direct and diffuse) independently and sum them afterwards. Steerable equipment may change the light received by the plants: lamps and curtains. Light given by the lamps is added to light received by the plants. The effect of curtains is also applied to the final result (eg: 50 μ mol less light). Finally, to estimate light attaining each plant in the greenhouse, we use an interpolation function between the position of the pictures.

This tutorial details the steps to apply this method to your greenhouse. R scripts are available. They are currently written for one specific greenhouse but all the instructions to adapt them are described below.

Overall, simulating light for phenotyping experiments can be divided in two steps:

- The initial set up of the method: taking pictures, image treatment, adaptation of R scripts, method validation... This may be long but has to be done only once.
- The daily computation of light received by every plant: when the method is set up, this can be done very quickly and, ideally, should be automated.

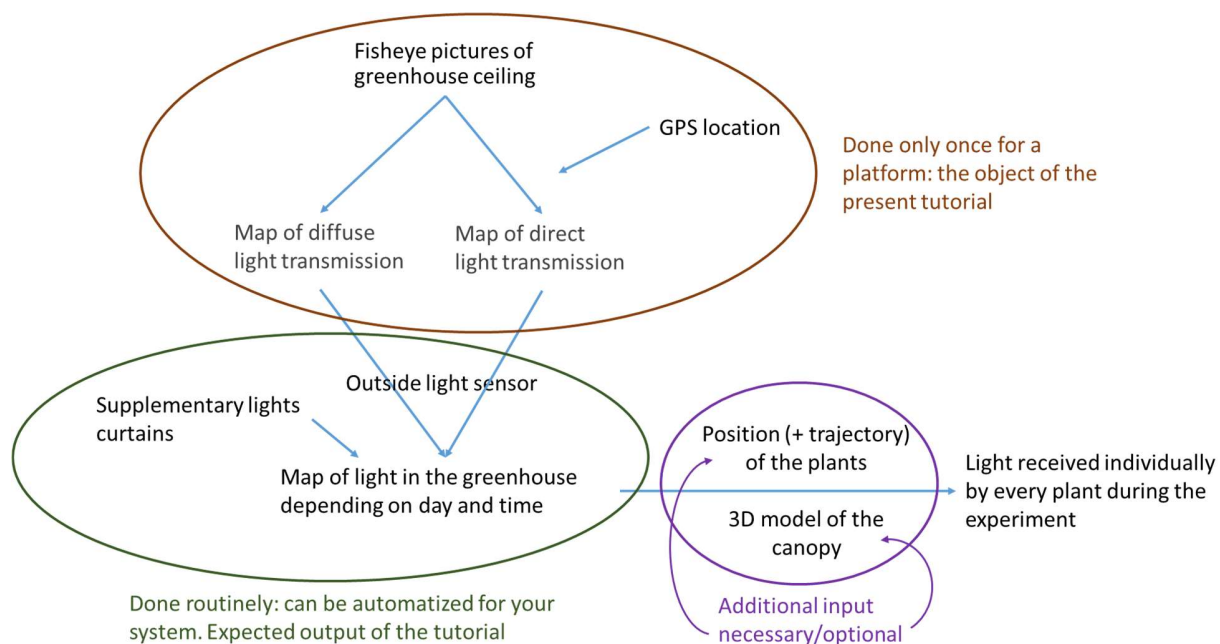


Figure 1 : Flowchart of the method to simulate incident light for all plants in a phenotyping platform

1.2. Requirements

1.2.1. Equipment needed temporarily

Part of the hardware is only needed for the initial step. This equipment can be borrowed if your lab don't own them:

- Camera with fisheye lens: the program is designed to work with "real fisheye" pictures, meaning the image is a circle surrounded by black borders. Semi-fisheyes and fisheye adaptors do not produce suitable pictures for the pipeline as it is designed now.
- Stand that can be oriented nicely (camera lens must face the ceiling). May be optional, depending on the configuration of your greenhouse. Is it possible to put the camera on the pots with the lens horizontal?
- Spirit level: to check that the fisheye lens is really horizontal (doable with a smartphone: open google and type "spirit level", or download an app. But a real one is more convenient)
- Compass to orientate the lens (smartphone app can do that)
- Light sensor inside the greenhouse: can be pyranometer or quantum sensor (PAR). At least one, to compare predicted light with actual light.

Ideally, one or two light sensors should stay in the greenhouse during every experiment. However, the method does not use the information given by these sensors to simulate current light in the greenhouse. Thus it is conceivable to check the accuracy of the simulation at the beginning, during a duration sufficient to encounter a variety of weather. Then a regular check at different places in

the greenhouse should insure that the simulation is still valid throughout seasons and at different places, but would not require that the sensors stay permanently in the greenhouse.

1.2.2. Equipment needed permanently

- Light sensor outside the greenhouse like pyranometer. This sensor measures the total solar radiation flux density, in W/m^2 . Ideally, this sensor should give the proportion of direct and diffuse light. However, this kind of sensor is expensive and generally not present in standard weather station. A formula allows to estimate the proportion of direct and diffuse light so the package is still usable with a standard sensor.

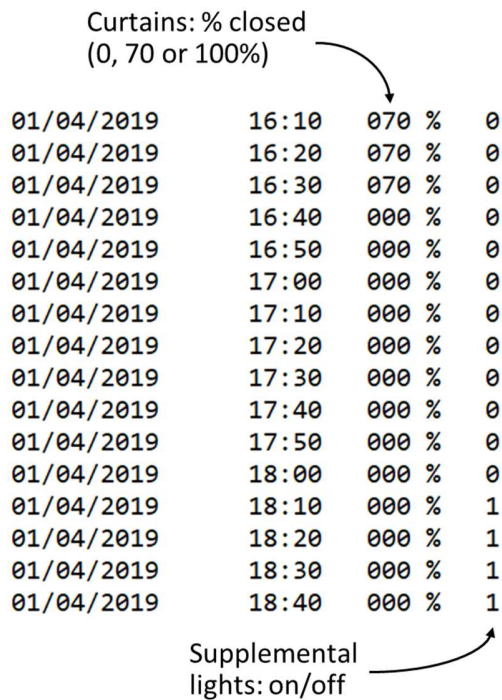
This sensor is the only hardware absolutely necessary for a routine usage.

Measurements must be made regularly (eg: every 10 minute) and stored in a file (.txt or .csv) like this one:

01/04/2019	10:00	0336 W/m^2
01/04/2019	10:10	0362 W/m^2
01/04/2019	10:20	0385 W/m^2
01/04/2019	10:30	0402 W/m^2
01/04/2019	10:40	0425 W/m^2
01/04/2019	10:50	0445 W/m^2
01/04/2019	11:00	0465 W/m^2
01/04/2019	11:10	0483 W/m^2
01/04/2019	11:20	0502 W/m^2
01/04/2019	11:30	0521 W/m^2
01/04/2019	11:40	0536 W/m^2
01/04/2019	11:50	0552 W/m^2
01/04/2019	12:00	0566 W/m^2

- if your greenhouse has artificial lights and curtains, similar files must record their status (on/off, open/close). You may want to evaluate their impact on incident light with the inside sensor, by computing the difference between incident light with these equipments on or off. The current version of the script applies a unique correction for the whole greenhouse. A mean of values measured at different places should fit.

Curtains: % closed
(0, 70 or 100%)



01/04/2019	16:10	070 %	0
01/04/2019	16:20	070 %	0
01/04/2019	16:30	070 %	0
01/04/2019	16:40	000 %	0
01/04/2019	16:50	000 %	0
01/04/2019	17:00	000 %	0
01/04/2019	17:10	000 %	0
01/04/2019	17:20	000 %	0
01/04/2019	17:30	000 %	0
01/04/2019	17:40	000 %	0
01/04/2019	17:50	000 %	0
01/04/2019	18:00	000 %	0
01/04/2019	18:10	000 %	1
01/04/2019	18:20	000 %	1
01/04/2019	18:30	000 %	1
01/04/2019	18:40	000 %	1

Supplemental
lights: on/off

- R software. A basic knowledge will be helpful. A graphical user interface (RStudio for example) renders things more comfortable, but this will not change the output of the code.

2. IMAGES OF THE GREENHOUSE STRUCTURE

2.1. Image acquisition

Chose a cloudy day to avoid sun reflection on metallic parts. Draw a small mark at the base of the lens: this mark will have to be orientated toward the North in each picture. Set the focus on infinity.

You can either put the camera on a pot or use a stand. The lens must be horizontal, check it with the spirit level.

Take pictures of the ceiling of the greenhouse on a regular grid (eg 1 pic/m²). Cover all the surface where plants go, and include the corners. The light estimation will be interpolated between the pictures but not extrapolated outside this zone.

The lens should be at about the canopy height. If you grow plants with very different size (young vs fully grown maize?), you may consider doing more than 1 picture per spot, at corresponding heights.

Record the position of each picture in the greenhouse. Think about the system coordinate you use for that.

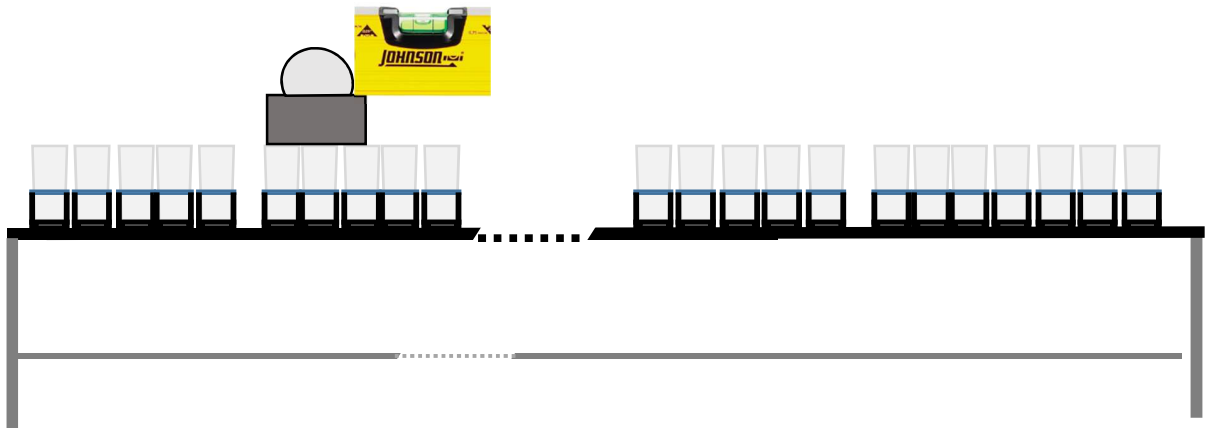


Figure 2 : Position of the camera for the pictures

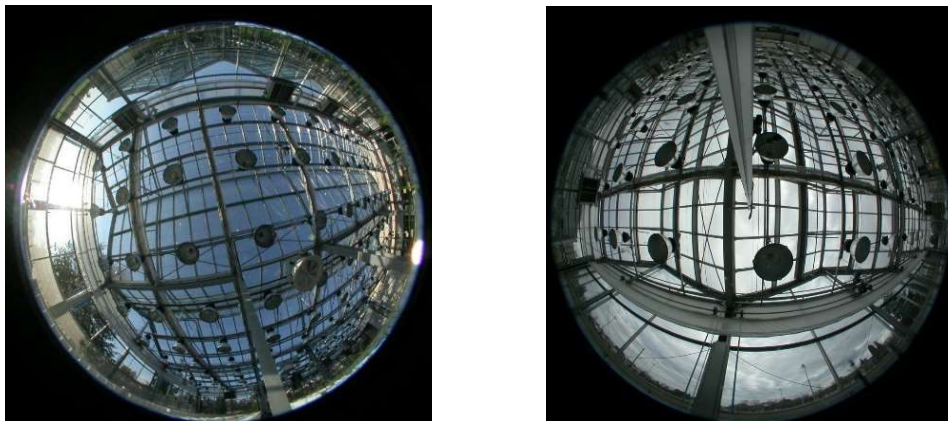


Figure 3 : Examples of pictures taken during a sunny day (left) and a cloudy day (right).

2.2. Coordinate file

Prepare a file (.txt or .csv) with the name of the final images and the corresponding coordinates. The coordinates correspond to the distance (here, in cm) to an origin point, set appropriately considering the organization of the greenhouse. The file should look like this. You can add a supplementary column for height in case you took several pictures at the same position.

image	x	y
DSC_0005_cropped_segmented.jpg	256	246
DSC_0006_cropped_segmented.jpg	256	346
DSC_0007_cropped_segmented.jpg	256	446
DSC_0008_cropped_segmented.jpg	256	546

DSC_0009_cropped_segmented.jpg	256	646
DSC_0010_cropped_segmented.jpg	166	240
DSC_0011_cropped_segmented.jpg	166	340
Etc...		

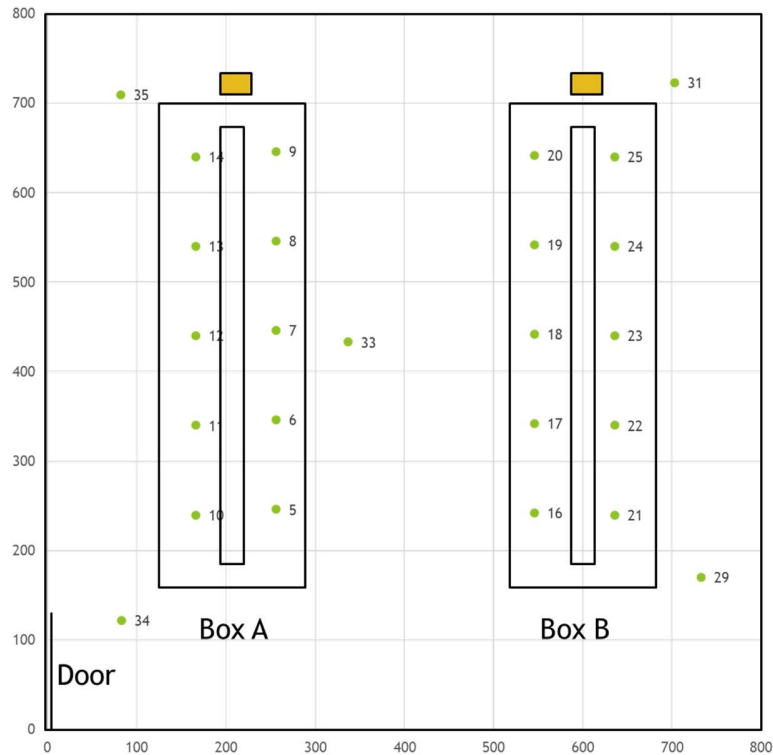


Figure 4 : Position of the pictures taken in RootPhAir. The plants are grown inside the two boxes. The origin of the coordinate system is set in the left bottom corner of the greenhouse, next to the door. Notice that some pictures are taken outside the zone where plants grow to enable interpolation.

2.3. Image processing

The objective is to get binary images with transparent parts in white, opaque parts in black. The original method performed this step with Ilastik (<https://www.ilastik.org/>). This software uses machine learning to perform image analysis on a batch of images. Other software can be used to perform this treatment. The following part presents all the steps to process the images with ImageJ. Feel free to use another software if you prefer.

2.3.1. Using ImageJ to process images: step by step

Original image: notice the round shape + black border, red tick is oriented towards North



Figure 5 : Original picture

Crop the image: trace a rectangle then Image > Crop

Turn the image to black and white: Image > Type > 8-bits

To automatize this process on all your images, you may want to create a macro and run it.

Plugin > Macro > Record

This will track all the changes you are doing on an image. Then you can run exactly the same operation on all your images (typically, cropping).

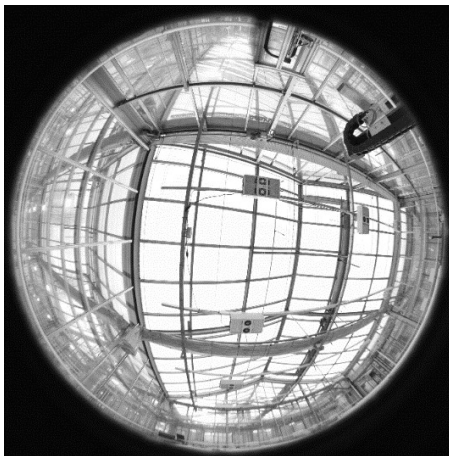


Figure 6 : Picture was cropped and turned to 8 bits (black and white)

Segment the image: Image > Adjust > Threshold, my threshold was 230



Figure 7 : Picture with transparent parts segmented, with a threshold of 230.

The difficult part is here. How to choose a threshold?

Bad examples:

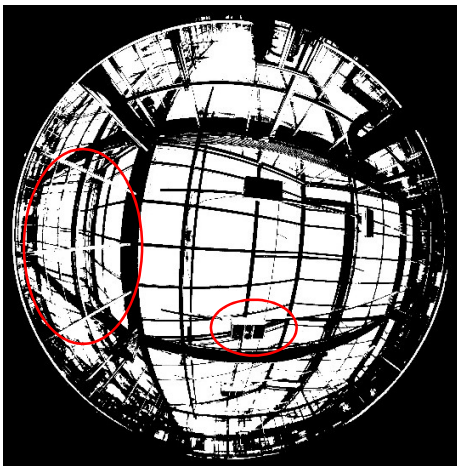


Figure 8 : Threshold was 170: some opaque parts are withe (red circles)

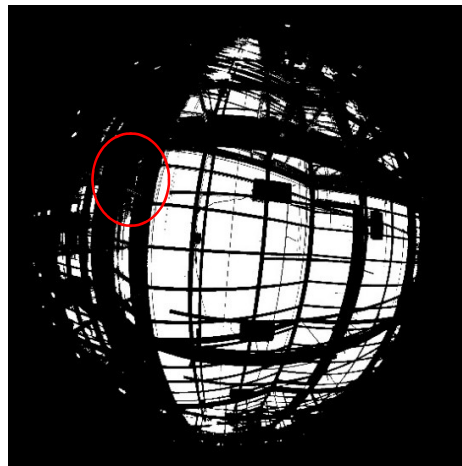


Figure 9 : Threshold was 255 some transparent parts appear in black (red circle)

The chosen threshold is not perfect either! You have to find a compromise that seems optimal for your situation. Also, you can keep the same threshold for all the images... Or adapt it for each image.

3. SIMULATIONS WITH R

This part describes what every script furnished with the tutorial does and explains you what to modify to fit your greenhouse.

3.1. 1_ImageAnalysis.R

This scripts produces a file that gives for each picture, each day of the year, every hour, the proportion of direct and diffuse light that passes from outside to the position where the picture was taken. The diffuse light does not depend of the sun position and the proportion of diffuse light transmitted by the structure is constant for one place at all time.

Requires: segmented images, GPS coordinates of the greenhouse

Modify

- the location of the images (l.18)
- the city of the installation (l.22 and 23)
- the location of the images again (l.33)
- the coordinates of the North (l.52 and 53: coordinates of the pixel corresponding to the North direction)
- the time frame for which you want the calculation (l.143)
- the directory and the name for the Transmission file (l.174) (here: TransmissionUCL.csv)

3.2. 2_TransmissionEstimation.R

This script makes a map of diffuse and direct light transmission in the greenhouse. There will be only one map for diffuse light (constant over time) and one map per day for direct light (the daily value representing a mean transmission). Light is not simulated everywhere but on a grid (x,y). You can consider simulating the light at exact plant positions. Note that the best grid to simulate light at plant locations may not be the nicest grid in terms of visualization.

Requires: transmission file from script 1 (TransmissionUCL.csv), coordinate file, greenhouse dimension

Modify:

- the loading of the transmission file that was produced by script n°1 (l.13)
- the name of the file with the coordinates of the images (l.27)
- the dimensions of the greenhouse (optional) (l.82 & 141)
- name + location of the map of transmitted direct light (l.127) (here: TransmissionDirect.csv)
- name + location of the map of transmitted diffuse light (l.168) (here: TransmissionDiffuse.csv)

In this script, there is a test from l.39 to 73 that will output the average transmission coefficient every month of the year. It outputs 12 maps of the greenhouse with light transmission represented

on a color scale. The second part (from l.76) is doing the average for every day. Its output is two table for direct and diffuse light, average every day, on every point of the grid defined.

3.3. 3_LocalPAREstimation.R

Uses outside light to simulate inside light at a certain point. Useful to compare simulated light with measure light.

Requires:

- inside light sensor (to compare simulated with measured light) giving a file of measured light at regular interval (similar to the files for outside light, artificial light and curtains),
- map of transmission coefficients of direct and diffuse light, produced by script n°2 (named TransmissionDirect.csv and TransmissionDiffus.csv),
- meteo file: light measured by the outside sensor regularly (here: every 10 minutes),
- file recording the state of artificial lights and curtains,
- script n°5

Modify:

- loading of the file containing transmission coefficients of direct and diffuse light (l.12 & 13)
- reading the meteo file + adjusting the formatting lines to read the dates correctly (l.18 and followings)
- l.28: this lines extends the instant PAR to the cumulative PAR on the considered period. Here the PAR is recorded every 10 minutes. Adjust the multiplicative coefficient if your PAR record is more or less frequent than that.
- l.32-34: considers the artificial light. This will be useful to simulate light at each plant position. Requires a file that indicates at each time point if lights are on or off + adjust the light intensity of your projectors if necessary
- l.45: change source file. This gives an estimation of the ratio between direct and diffuse light, via Spitters equation.
- l.46 extracts PAR from solar radiation. If your outside sensors gives PAR, remove this line.
- If your sensor gives you direct and diffuse radiation, remove l.44 and l.45 and put direct/diffuse radiation l.47 and l.48.
- change origin files (l.51 & l.54)
- l.61: same as l.28
- l.69: this line converts the energy from sunlight (what is measured with pyranometer) to the amount of photosynthetically active photons (which constitute the PAR). This line may be changed if your outside sensor directly measures PAR.
- l.72-87: manages shading and lamps. Change if necessary

- l.114-117: position of the sensor . Light is not simulated everywhere in the greenhouse but on each node of the grid we defined in script 2. Look for the node closest to your sensor.
- l.120 : transmission coefficient of glass. 0.7 corresponds to our double-glazing but this coefficient may be different for your greenhouse.
- l.136 & 179 & 200 change file directory

3.4. 4_MakeGIF.R

This script intends to make a GIF of the daily average direct light transmission in the greenhouse during a whole year. It requires some specific packages to produce the GIF and the file "TransmissionDirect.csv". Change directory (l.42), dates (l.49 & 50), xlab and ylab (l.51 & 52) and, if necessary, zlim (l.53). zlim should go from 0 to the maximum direct light transmission coefficient existing in the data.

The GIF only represents direct light transmission, as diffuse light transmission does not change over time. Producing a GIF is not necessary, however it is a nice output.

3.5. 5_suncourse.R

This script has the number 5 but it is needed for script n°3. It implements functions that will

- trace the sun course on the sky, depending on the gps position
- estimate direct and diffuse light (spitters function)

Modify:

- GPS coordinates of your city (l.7-8)

4. TOWARDS ROUTINE USAGE

This tutorial guides you to set up the method. There is still a bit of work to achieve the simulation for every plant during a full phenotyping experiment. The process may consist in simulating the light every day of the experiment at all places of the greenhouse with a modified version of the script n°3. If the plants keep a fixed position, it is enough to sum the light received during all the experiment. If the plants move, another algorithm will be necessary to sum the light received at each position occupied during the experiment.

The simulation of canopies in 3D goes far beyond this tutorial.

Depending on your greenhouse, the model can be improved by mapping the supplemental light brought by the lamps and the lowering of light due to the curtains. Here we supposed that these effects were constant over space, or that their spatial variations are negligible as compared to sunlight.