## I. System overview

We are using an updated version of a photometric instrument that allows for the quantification of transmission changes due to chloroplast movement (Berg et al. 2006; http://academics.wellesley.edu/Physics/Rberg/papers/LogoChipLeaf.pdf). The basic idea of the instrument is to use a blue/red LED to shine blue light of defined intensity onto a leaf and induce chloroplast movement, before switching to red light for a brief moment to measure how much of the red light is transmitted through the leaf. The red light is detected by a red-light sensitive phototransistor that is positioned on the opposite side of the leaf. The original instrument was controlled by a LogoChip.
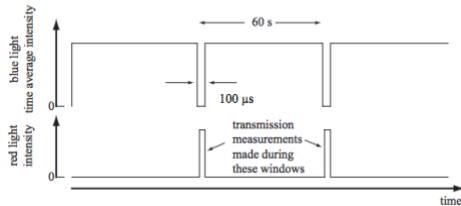
The updated instrument uses an Arduino microcontroller and readily available Arduino accessories, but the basics are the same. The extensive Arduino documentation, accessibility of components and low cost mean that this instrument can be relatively easily built. The instrument is connected to an iPad, and an app (LeafSensor.ipa) is used to set-up the experimental protocol and to record data. The data are also stored on an SD card as a backup. Leaf, LED and phototransistor are held in place by a 3D-printed leaf clip, which is positioned in a 3D-printed boat to which water can be added to keep the leaf from drying out. The entire set-up allows for 8 leaves to be tested.

II. Instructions for building the instrument

1. List of parts

Arduino Mega 2560 R3 SKU: DEV-11061 (SparkFun) https://www.sparkfun.com/products/11061

SparkFun microSD Shield SKU: DEV-12761 (SparkFun) and **microSD card** for extra memory
https://www.sparkfun.com/products/12761

SparkFun microSD Shield communicates using the SPI interface
https://www.arduino.cc/en/Reference/SPI and uses four digital I/O pins
https://learn.sparkfun.com/tutorials/microsd-shield-and-sd-breakout-hookup-guide

Adafruit Bluefruit LE - Bluetooth Low Energy (BLE 4.0) - **nRF8001** Breakout - v1.0
https://www.adafruit.com/product/1697

USB Cable to power the Arduino
https://www.amazon.com/AmazonBasics-USB-2-0-Cable-Male/dp/B00NH11N5A

2. Wiring
• Bend out or cut off pins D11-13 on the red **SparkFun microSD shield** before plugging in the shield.

• Solder wires into the holes for the D11-13 on the **SparkFun microSD shield board** and connect them as follows to the **Arduino Mega 2560:**

SparkFun 11 -> Mega 51 (MOSI)
SparkFun 12 -> Mega 50 (MISO)
SparkFun 13 -> Mega 52 (CLK)

• Next connect **Bluetooth nRF8001** to **SparkFun microSD shield**:

nRF8001   SparkFun microSD shield (modified)
CLK   -> 13
MISO   -> 12
MOSI   -> 11
REQ   -> 10
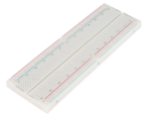RDY   -> 2
RST   -> 9
RESET   -> 8
GND   -> GND
Vin   -> 5V

• Use a breadboard to connect the LEDs, phototransistors, and resistors for the 8 sensors:

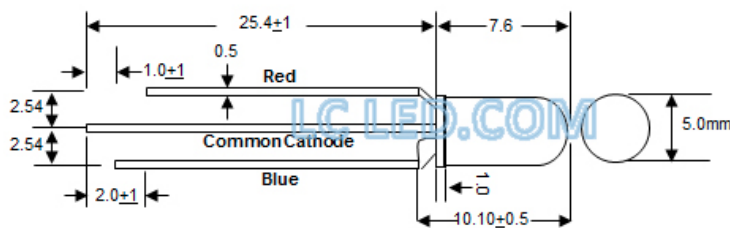**External board** to connect LEDs and phototransistors
https://www.sparkfun.com/products/12615

5 mm Blue/Red LED: LC LED-N500TBR4D

http://www.lc-led.com/View/itemNumber/150

Electro-optical Characteristics (Ta = 25⁰C)

PARAMETER	SYMBOL	CONDITIONS	MIN.	TYP.	MAX.	UNIT
Forward Voltage (Blue)	V_F	I_F = 20mA	3.0	3.0	3.8	V
Forward Voltage (Red)	V_F	I_F = 20mA	1.7		2.6	V
Reverse Voltage	V_R	I_R = 100mA			5	V
Dominant Wavelength (Blue)	I_D	I_F = 20mA	464		475	nm
Dominant Wavelength (Red)	I_D	I_F = 20mA	624		635	nm
Viewing Angle	2q1/2	I_F = 20mA		30		Deg.
Luminous Intensity (Blue)	I_V	I_F = 20mA	1,800		3,200	mcd
Luminous Intensity (Red)	I_V	I_F = 20mA	4,000		6,500	mcd

DEVICE DRAWING

Phototransistor Silicon NPN T-1: https://www.digikey.com/products/en/sensors-transducers/optical-sensors-phototransistors/544?k=480-1951-ND&pkeyword=&FV=ffe00220&mnonly=0&ColumnSort=0&page=1&quantity=0&ptm=0&fid=0&pageSize=25
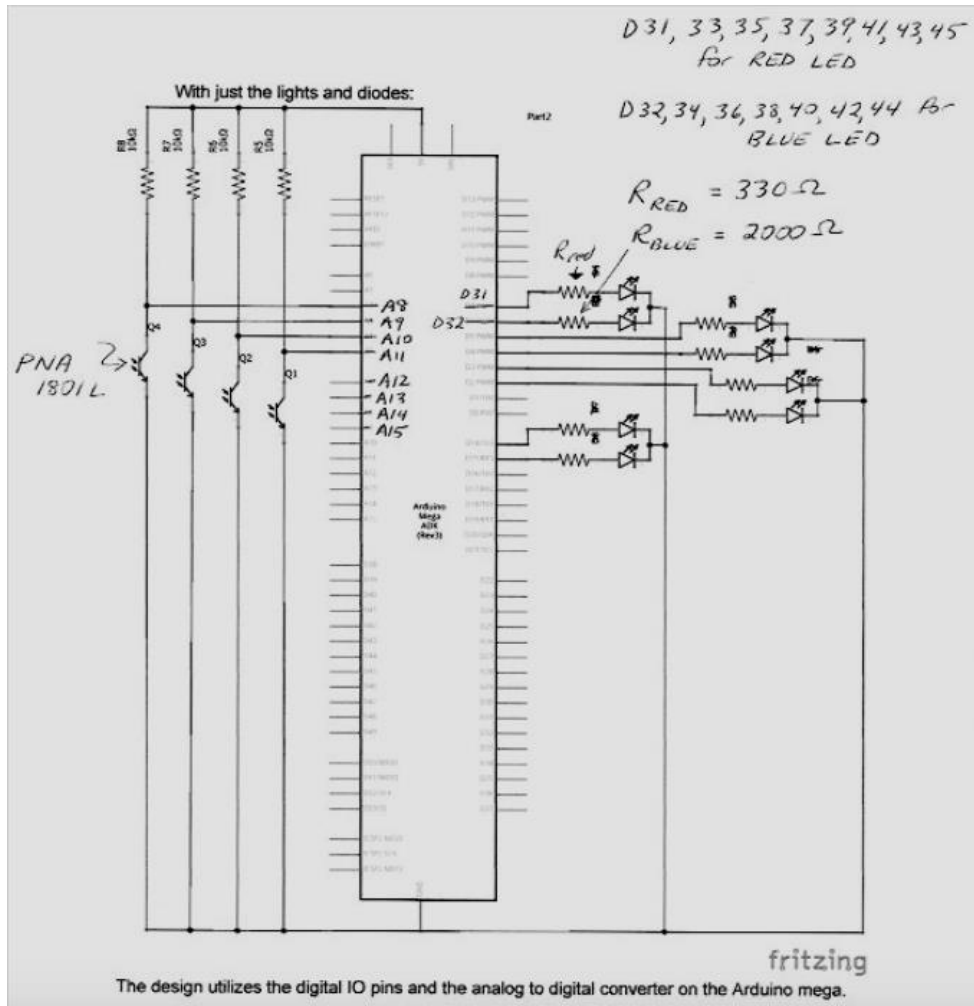
Resistors

resistors (upright) for the red LED lead 330 Ohm

resistors (horizontal) for the blue LED lead 1K Ohm

pull-up resistor between the 5V and the phototransistor 10K Ohm

Important to get a useful range of output values. For more info see:
https://learn.sparkfun.com/tutorials/pull-up-resistors

Wiring scheme:

The design utilizes the digital IO pins and the analog to digital converter on the Arduino mega.

Eight phototransistors (PNA 180IL) are connected to A8-A15 and eight LEDs
connected to pins D31-45 on the Arduino Mega 2560 as listed above in the wiring scheme.

Connect 5V to 5V on the red board.
Connect Vin to Vin on blue board.

3. Arduino program
• See Supplemental coding Arduino

• Make sure to install all of the libraries needed for the SD card shield and the BLE peripheral.
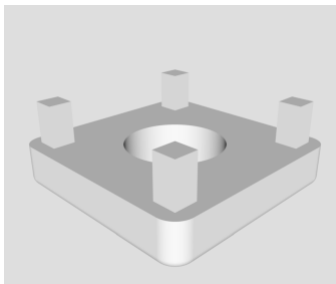
4. Instructions for making leaf clips

Use a 3D printer to make the leaf clips. They need to be made out of black filament (e.g., Black PLA Filament -1.75mm Matter Hackers) otherwise light from the LEDs will be picked up by other leaf clip phototransistors.

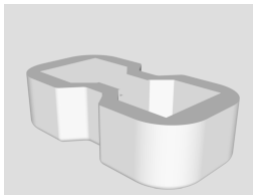There are 2 parts to it, one holding the phototransistor, one the LED.
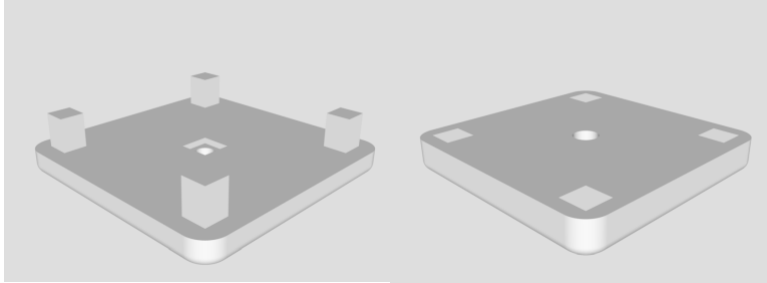
We also printed a clip to hold a light meter sensor that allows us to determine the blue light intensities.

The leaf clips were placed in 'boats' that can be filled with water to make sure the leaves do not dry out in longer runs.

Bend some of the wires so that there is not a cable mess.

5. Instructions for making the box

Black acrylic laser cut, then assembled with acrylic "glue" which dissolves the acrylic, basically chemically welding the sides together.

6. LeafSensor app
Contact Susan Kohler (skohler00@gmail.com) for info about the app.

To install the app:

• Hook up the iPad to your computer and open iTunes. You should see your iPad listed as a device in the panel on the left.

(Delete the old version of Leaf Sensor from the iPad if you have one)

• Drag the **LeafSensor.ipa** file over the iPad in the device list. It should install the app on the iPad.

III. Instructions for determining blue light intensities of LEDs

There may be slight difference in the leaf clips because of the inaccuracies of the 3D printer, the manufacturing of the LEDs, or because of the exact positioning of the LEDs in the leaf clips. Therefore, it is important to label each leaf clip and LED and to calibrate them separately.

• On the iPad, set **Setting** to **Auto-Lock, Never;** pick **Expt Name**: eg CAL; choose **Blue Intensities**: 1, 100, 1000, 2000, 3000; choose **Blue Duration (minutes)** 10, 10, 10, 10, 10

• Attach the part of leaf clip 1 with the LED to the matching leaf clip that holds the light sensor of a light meter (eg LICOR LI-190R Quantum Sensor and LI-250A).

• Press **Start Experiment**, write down the read out of the light meter in μmol photon m^{-2} s^{-1}; switch the leaf clip that holds the light sensor to LED 2; note the read out of the light meter; repeat until all eight LEDs have been measured.

• In an excel sheet plot **Light intensity** (read out of light meter) vs Blue Intensities (1, 100, 1000, 2000, 3000) for each sensor.

• The light intensities have to be similar for the eight set-ups. If they are different, check that you used the correct LEDs and if necessary adjust their position in the leaf clip until they are similar and then secure them in place with glue or patty.

• Average the light intensities for all 8 leaf clips and plot the values against Blue Intensities (1, 100, 1000, 2000, 3000); get a linear regression and the formula that describes the relationship.
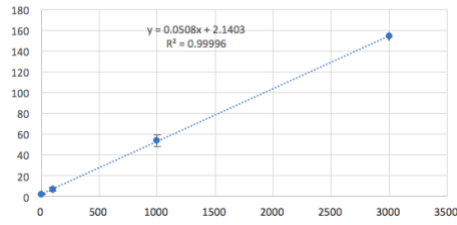
In our set-up, the true light intensity in μmol photon m^{-2} s^{-1} = 0.0508 * Blue Intensities + 2.14
1 = 2.2 μmol photon m^{-2} s^{-1}
100 = 7.2 μmol photon m^{-2} s^{-1}
1000 = 53 μmol photon m^{-2} s^{-1}
3000 = 155 μmol photon m^{-2} s^{-1}

IV. Instructions for calibrating phototransistors to convert voltage output into % transmission

Use filters e.g., https://www.barbizon.com/product_info/rosco-roscolux-light-grey.html?attr={8}161 or https://www.barbizon.com/search/browse/?psearch=site&keywords=Kelly+Green to achieve certain transmissions between 1% and 15%. To figure out which filters and filter combinations to use, set up a light source of known intensity e.g., 100 μmol photon m^{-2} s^{-1} (measured at a set distance from the light source with a light meter). Place filters in front of light source and determine the light intensity, which will allow you to determine the % transmission e.g., if the light intensity with filter is 10 μmol photon m^{-2} s^{-1}, then the transmission is 10%. Select at least 4 different filter combinations that generate a range of % transmissions:

3* #3 = 2.8%T
2* #3 = 7.9%T
3* #4 = 11.5%T
2* #4 = 15.9%T
#3 = our grey filter 1
#4 = our grey grey filter 2

Our instrument loses it linearity if %T is above 16.

If you are using several filters at the same time, tape them together and avoid touching them in the area that will be inserted into the leaf clip. Make sure the filters stick out on top and the bottom so that you can be sure that they were inserted correctly and are placed between the LED and the phototransistor.

Place the filters, one after the other, into each leaf clip, turn on program with BL at e.g., 1000 and write down the Voltage output that you see on the app screen.

Plot the data with Voltage on the X-axis and % transmission on Y-axis. Fit a linear regression line and get the formula and R^2. If the different leaf sensors are similar, average the values and use the formula in the excel sheet that will convert Voltage into %T. If not, keep the calibrations separate for each leaf sensor and use the respective formulas for each Excel sheet.

Sensor 5	%T
829.8	7.9
921.25	2.8
732.25	11.5

%T

y = -0.0459x + 45.42
R² = 0.98625
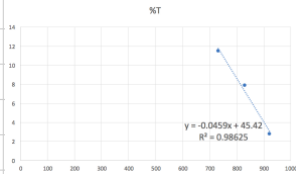
V. Excel sheet for data analysis

Make a master Excel sheet that has the results of the calibrations for each sensor in a separate sheet. Copy each data set into the respective data sheets: column A, contains the time; column C contains the data. Set up column B so that it converts time from seconds to minutes. Set up column D so that it contains the formula to convert voltage to % transmission using the equation from the calibration. The formula may be different for each sensor:

A	B	C	D	E
Time, s	Time, min	Sensor 1	% Transmission	
66.118108	1.1	8841	=-0.0459* $C2+45.42	
128.639216	2.1	883	4.9	