# LI-COR 6400

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Gas exchange provides a direct measure of net rate of photosynthetic carbon assimilation of individual, whole plants or plant canopy. LI-COR 6400 is an open gas infrared analyzer system (Fig. 1) that allows instant measurements of photosynthesis, stomatal conductance and transpiration.

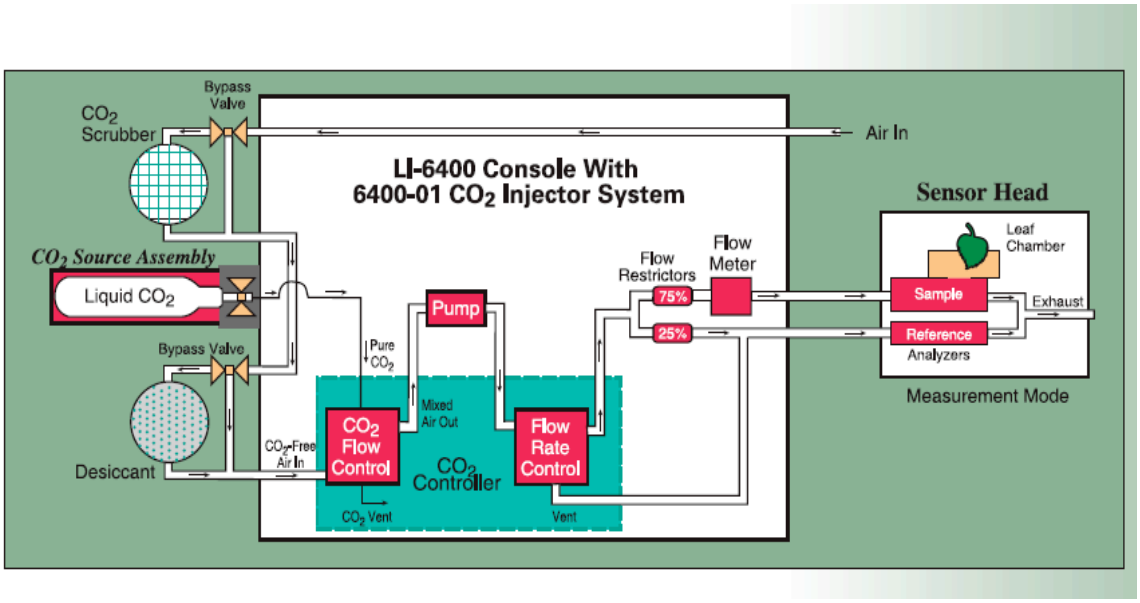


**Fig. 1.** Schematic Infrared gas analyzer system. The Li-Cor 6400 allows air to enter both the analysis and reference lines. Air is continuously passed through the leaf chamber and measurements of photosynthesis and transpiration are based on the difference in $CO_2$ and $H_2O$ in the air stream that is flowing into the leaf cuvette (reference), compared to the air stream flowing out of it (sample). The rate of $CO_2$ uptake is used to assess the rate of photosynthetic carbon assimilation, while the rate of water loss is used to assess the rate of transpiration.

**Main variables measured using LI-COR:**

Photosynthetic rate (A) µmol $CO_2$ $m^{-2}$ $s^{-1}$
Transpiration rate (E) mmol $H_2O$ $m^{-2}s^{-1}$
Stomatal conductance (gs) mol $H_2O$ $m^{-2}$ $s^{-1}$
Intercellular $CO_2$ concentration (Ci) µmol $CO_2$ $mol^{-1}$

For accurate photosynthesis measurement, it is highly recommended to construct a light response curve (Fig. 2). Increasing photosynthetically active radiation (PAR), which represents the

fraction of sunlight with a spectral range from 400 to 800 nm, induces increases of the light reactions of photosynthesis, thus increasing photosynthetic $CO_2$ assimilation.

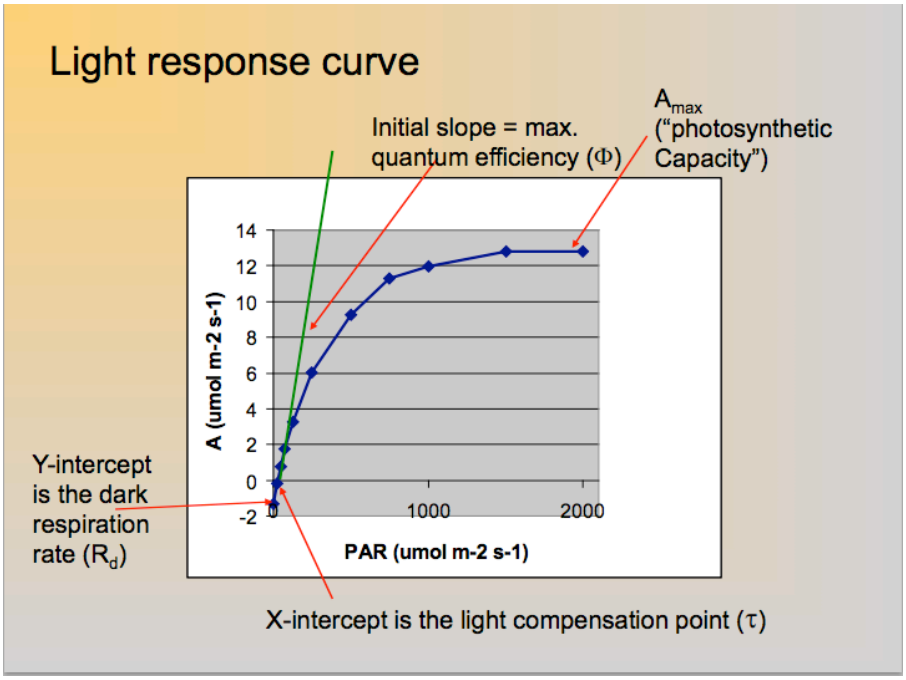


**Fig. 2.** Photosynthetic response to increasing absorbed light (PAR). Beyond the saturation point ($A_{max}$), any further increase in PAR will not result in more light fixation, therefore it's crucial to find the saturation point for the specie of your interest.

**Gas exchange measurements:**

**Things to check before using Li-COR 6400**

- If using batteries at 12.5 V optimum charge, they will last 1 hour approximately. When working in the field, make sure to bring 4-6 batteries.
- Ensure that the leaf chamber gaskets close tightly, otherwise environmental $CO_2$ will induce an increase in the sample $CO_2$ and measurements will not be accurate.
- Make sure that both soda lime (removes $CO_2$) and drierite (removes water vapor) are not saturated (Fig. 3). When changing soda lime or drierite make sure to leave 1 cm to the end of the tube.


**Fig. 3.** Tube showing the difference between active (blue) saturated (pink) drierite. This chemical is anhydrous 97% calcium sulfate ($CaSO_4$) and 3% cobalt chloride, and absorbs 6.6% its weight of water. When drierite changes to pink after water absorption, it should be replaced.

Replace the soda lime when the chemical decreases the capacity to reduce $CO_2$. How often the soda lime needs to be replaced depends upon how much $CO_2$ it has been forced to remove. Loss of capacity to scrub $CO_2$ can be recognized by an inability to reduce the $CO_2$ mole fraction to zero and hold it there.

**Using the LICOR 6400:**

Make sure of these instructions before starting your measurements!!!

1. Connect camera to equipment
2. Close leaf chamber
3. Turn on the equipment
4. Is the chamber connected? Y/N  Y ↲
5. **Calibration Menu**

   5.1 Flow meter zero ↲
   Flow meter signal: in MV (noise as low as possible)
   Adjust the noise if it's above 0.4 mV and wait until the number is stable.
   The flow meter signal has to be stable and close to zero in order to start the measurements.
   OK ↲

   5.2 Irga zero: In order to have the difference between sample and reference. Chamber has to be closed!!!
   YES ↲

   Note: it's better to zeroing both $CO_2$ and $H_2O$. Both knobs have to be in SCRUB ↲

When zeroing the IRGA, both Reference (R) and sample (S) for $CO_2$ and $H_2O$ have to be 0. This will assure you that $CO_2$ and $H_2O$ in the chamber and equipment are in equilibrium.
After 10-15 minutes press AUTO ALL to do zero in both $CO_2$ and $H_2O$
AUTO ALL ↲
QUIT ↲
ESCAPE to go to main menu

**6. Configuration Menu (this command is for light source)**

6.1 Light source control ↲

6.1.1 Pick source: Red/blue (inside growth chamber) or sun/sky (for measurements in the field)
DONE ↲
ESCAPE ↲

**7. New measurements**

7.1 Open a new file. OPEN LOGFILE ↲
Name the file and press ↲
7.1.1. Remark (give a name for each measured tree). Go to this window ***every time*** you measure a new tree.

7.2 Go to Menu 2. This is to configure
7.2.1. Lamp: 750 µmol PAR ↲
7.2.2. Temperature (Block temperature 20°C)
**Note**: Every time that any of the variables has an asterisk (*) is because the equipment is trying to get the specified value.
**7**.2.3. Mixer (how much $CO_2$ the reference has. Commonly is atmospheric). Check $CO_2$. Although, it can be assumed that $CO_2$ of the chamber is the same as atmospheric.
**7**.2.3.1 Ref $CO_2$ ↲
385 µmol $CO_2$ (check web page esrl.noaa.gov)
7.2.4. Flow. It is 400 µmol/s ↲
7.2.5 Leaf fan: slow ↲
7.2.6 Relative humidity (check the RH in the environment. The RH in the chamber can not be higher than environment, unless you induce another source of humidity).
Relative humidity is adjusted opening the $H_2O$ column "BYPASS"

AFTER MAKING SURE OF ALL THE PREVIOUS STEPS. YOU ARE READY TO START YOUR MEASUREMENTS!!!

**Tips to consider**:

- Once you start doing measurements, it is important to "**Match the IRGAS**" every other sample. This is important because transpiration and assimilation are computed by the concentration differences. And because these differences are measured by two independent infra-red gas analyzers, the IRGAS must be checked against each other. It is recommended to do "Mix matching" at least once

at the start of the measurements. 1 μmol $mol^{-1}$ difference between the two IRGAs can be significant when ΔCO2 is only 3 μmol $mol^{-1}$.