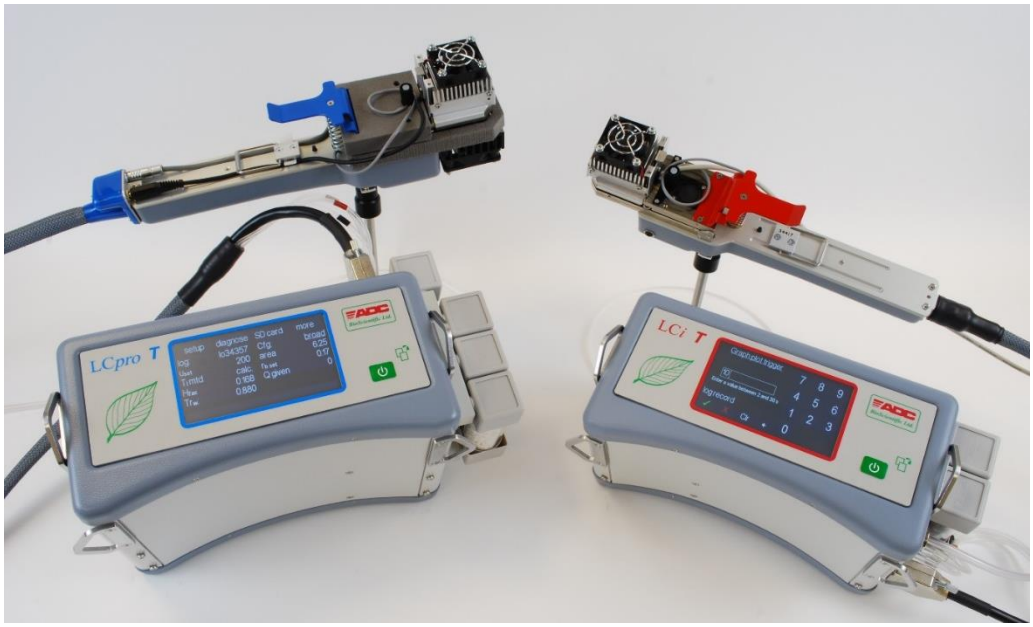
#AN053 When to use White light and when to use RGB light

The new LC*pro* ***T*** and LC*i* ***T*** photosynthesis systems are now supplied with either a White LED or RGB LED light unit. This provides researchers with a choice, for the first time, with a choice of light source:

White Light Unit: A broad spectrum light with an output of 2,500 µmol m^{-2} s^{-1} at 25˚C and a colour temperature of 4250 K.

RGB Light Unit: Total output range of 0 -2,400 µmol m^{-2} s^{-1} at 25˚C.
The maximum achievable outputs of Red, Green and Blue are 800 µmol m^{-2} s^{-1} at 25˚C.

So the question arises: Which type of light source is best for which application?



Why use a light unit?
For consistent measurements: A stable intensity and spectrum of light is widely used for making accurate gas exchange measurements of plants in a light-adapted state (see Useful References on final page). When working in a variable climate outdoors, or through greenhouse glass; or if growing plants under controlled lighting conditions; using a light source with matching or similar qualities to the ambient light will ensure that no additional variable is introduced to measurements of photosynthesis/transpiration.

For producing Light Response Curves (LRCs):

Light units which can be controlled in a stepwise sequence are essential when producing automated **LRCs** or **A/I curves.** Using gas exchange systems such as LC*pro* ***T*** and LC*i* ***T***, the parameter 'A' (rate of photosynthesis) is calculated as µmol m^{-2} s^{-1}. A is then plotted against I (irradiance), also referred to as PAR or Q, provided by a light unit.

The length of time a leaf is exposed to each successive light level is also controlled. LRC data quantifies and graphically presents how a leaf responds to changing light levels over time. A Light Response Curve can be performed as 'rapid' or 'slow'. Different plants (even different leaves on the same plant) show differences in the shape of their light response curves. The shape of LRCs reveals characteristics of the underlying photosynthetic processes including: the light-dependent and light-independent reactions, the efficiency at which light is utilised by photosynthesis, and even the rate of CO_2 evolution.

Examples from ADC users:

"The A/I curves were also determined at a CO_2 concentration of 370µmol mol^{-1}. Leaves were preadapted for 10min to darkness and then subjected to a sequence of increasing light intensity, rising stepwise from 50 to 100, 200, 300, 500, 800, 1100 and 1300µmol m^{-2} s^{-1} for 4min at each intensity. Intensity was set by an external controllable LED light unit (ADC BioScientific Ltd). Gas exchange measurements were registered every 60s at each irradiance to assure that the steady-state of A was achieved after 4min". **Nunes *et al*., 2009.**

"Light response curves were produced…using an IRGA (LC*pro* +, ADC BioScientific Ltd, Herts, UK) equipped with a temperature and light controlled cuvette. Attached leaves were…exposed to decreasing irradiances from 1740 to 0 µmol m^{-2} s^{-1} (1740, 1305, 870, 653, 435, 261, 174, 131, 87, 44 and 0µmol $m^{-2}s^{-1}$). A maximum radiation intensity of 1740 µmol m^{-2} s^{-1} was chosen because previous experience had shown that this was higher than the light conditions needed to achieve Amax (a complete light response curve was created in approximately 20 min)". **Tsormpatsidis *et al*., 2010.**

The following information is gained from analysing LRCs:

1. The Y axis intercept determines the dark Respiration (**Rd**) phase, when there is no light to enable photosynthesis.
2. Light compensation point (**LCP**), the point at which CO_2 uptake balances CO_2 released by respiration.
3. Apparent quantum yield (**Q**), determined from the initial slope of the curve.
4. **Amax** (light saturated photosynthetic capacity). Reached when C fixation rate becomes the limiting factor.
5. The final, Photo-oxidative phase occurs where the curve begins to decline after Amax.

RGB Light Unit advantages:
With the ability to change the spectrum of light through the **RGB light unit**, LRCs with different **spectral compositions can be compared**. For example, a composition of R = 40%, G = 20%, B = 40% may be set using the climate control 'Q%RGB' on either LC*i* ***T*** or LC*pro* ***T*** system. It is important to note that the study leaf (or plant) should have been allowed to acclimatize to the Q%RGB under investigation, prior to taking measurements.
A 'climate sequence file' can then be run to produce a graph of A against I (a Light Response Curve). A second 'climate sequence file' could then be created, setting Q%RGB to, say, R = 30%, G =30 %, B =40 %. Overlaying and comparing these curves for the same leaf, (or similar leaves) enables hypotheses to be tested regarding the photosynthetic efficiency of plants adapted or exposed to differing irradiance.

Temporal studies, whereby irradiance is adjusted to match those at different times of day, are used **outdoors or in greenhouses**. Over the course of a day, the spectral composition of irradiance (from sunlight) changes. Latitude and longitude, altitude, cloud cover and canopy density are all influencing factors (see Endler, 1993). The following website is a very useful tool for determining the PAR or irradiance at various wavelengths reaching the earth at any given location, date and time: (http://cprm.acom.ucar.edu/Models/TUV/Interactive_TUV/).

In addition, the irradiance spectrum can also be altered by greenhouse glass and coverings, creating a diffuse light from direct sunlight (Hemming et al., 2013).

Below is a diagram of the Blue-Green-Red wavelength spectrum of light, covered by the new RGB light unit. Details are included of the importance to plant physiology of each waveband:

•439nm is the blue absorption peak of chlorophyll a.

•450-460nm is the royal blue that is absorbed by one of the peaks in beta-carotene. It is a readily available LED wavelength commonly used to excite the remote-phosphor in white LED lamps.

•469nm is the blue absorption peak of chlorophyll b.

•430-470nm is a range that is important for the absorption of chlorophyll a and b, which is key for vegetative growth.

•480-485nm is the second absorption peak of beta-carotene.

•525nm (green light) is a phototropic activator whose chromophore is still unknown. Green light isn't important for photosynthesis, but it is apparent that plants are gaining direction and environmental signals from it, and that it affects internodal spacing. This is also the wavelength of GaN or InGaN green LEDs commonly used in RGB and tuneable applications.

•590nm is key for carotenoid absorption. Carotenoids are starch-storing, structural and nutritional compounds.

•590nm is additionally the phycoerythrin absorption wavelength. Phycoerythrin is a red protein-pigment complex from the light-harvesting phycobiliprotein family, present in red algae and cryptophytes, and is an accessory pigment to the main chlorophyll pigments responsible for photosynthesis.

•625nm is the phycocyanin absorption peak. Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin. It is also an accessory pigment to chlorophyll.

•642-645nm is the peak absorption point of chlorophyll b.

•660nm is often called the super-red LED wavelength and is important for flowering.

•666-667nm is the peak red absorption point for chlorophyll *a*.

Greenhouse

When measuring gas exchange of plants inside a greenhouse it is desirable to match the quality of light under which measurements are taken to the irradiance within the greenhouse. Alternatively, if the aim is to produce LRCs, these are carried out automatically by a light unit to guarantee the irradiance reaching each leaf under measurement, without needing to change the overall lighting conditions in the greenhouse by manual control.

If the greenhouse lighting is natural or broad spectrum white light, the **White light unit** will enable point measurements to be taken under matching and consistent irradiance. Greenhouse lighting spectrums are more commonly controlled using different coloured LEDs to encourage different plant responses. The **RGB light unit** provides flexibility in matching certain R:G:B ratios at different times. Both point measurements and LRCs could be made and compared at different plant growth stages, or at different times of day, to determine the effects of changing spectral quality on plant gas exchange.
The number of papers published using different ratios of RGB is rapidly increasing as researchers explore the effect of specific LED wavelengths on plant growth rate, yield, photosynthesis and physiology (see references on final page).

Outdoors

For outdoor measurements, where plants are growing in variable sunlight/shade, the PAR sensor located on either LC*i* ***T*** or LC*pro* ***T*** chamber handle can be used to accurately determine I (also referred to as Q) at the leaf plane (μmol m^{-2} s^{-1}). Using this information, a light unit can be set to match the total Q value.
Where Q is found to be greater than 2,400 μmol m^{-2} s^{-1} and the research aim is focused on total Q alone, the White light unit will be more suitable, with a higher total range of 0 to 2,500 μmol m^{-2} s^{-1}. If the ratio R:G:B is under study, the RGB light unit will provide more flexibility and allow an experimental approach.

Indoor laboratory/growth chamber

Laboratory-grown plants will be 'adapted' to the light conditions in which they are kept, typically in a highly controlled environment. Either light unit could be used to perform automatic LRCs, again depending on the research aim regarding the quantity/spectral quality of irradiance. Some researchers choose to control the light intensity of an entire growth chamber by reducing and increasing the ambient irradiance. However, this is a slow and manual method which cannot guarantee that all leaves measured are receiving exactly the same irradiance.

Plant leaves receive variable irradiance depending on their position in the plant canopy and the angle of their growth. A light unit is essential indoors (and outdoors) when comparing gas exchange in leaves growing at different levels on a plant. Leaves positioned at twice the distance from the light source will receive ¼ the irradiance, as stated by the inverse square law of light ($E=I/R^2$). Leaf growth angle also strongly influences the irradiance received by multiple leaf planes on the same plant.

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Website to calculate the amount of actinic light at various wavelengths that falls onto the earth at various latitudes, at different times of day, and at different times of year:
http://cprm.acom.ucar.edu/Models/TUV/Interactive_TUV/

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