It is now known that about 30% of fluorescence NPQ (nonphotochemical quenching), at high actinic light levels, is due to q_M (chloroplast migration). Chloroplast migration, as it occurs in nature, only occurs under intense white light, or intense blue light, not intense red light. (Cazzaniga 2013)

![Quenching relaxation test representation at high actinic light levels.](image)

After dark adaptation

After high light treatment

Definition of qM

qM - Chloroplast Migration – game changing research

What causes the non-photochemical quenching (NPQ) fluorescence relaxation change that is greater than a few minutes and less than thirty five minutes? Is it caused by state transitions q_T, a slow acting xanthophyll cycle mechanism q_Z, or chloroplast migrations q_M? Under higher light levels and near saturating light conditions, the latest data supports chloroplast migration as the source of fluorescence change and quenching relaxation. Since q_M is responsible for about 30% of NPQ in samples tested, it becomes a game changer for most chlorophyll fluorescence measuring parameters and protocols. This research affects the type of actinic light sources that should be used to measure most light adapted parameters under high light conditions, the times recommended for dark adaptation, and the time required to reach steady state photosynthesis under high light conditions.

Until recently, it was believed that as a plant goes from a dark adapted state to a high light level or from a high light adapted state to a dark adapted state, there were three basic mechanisms involved in chlorophyll fluorescence measurement of nonphotochemical quenching (NPQ) ; q_E, q_T, and q_I. q_E can be described as a rapid photo-protective adjustment of photosystem II caused by ΔpH of the thylakoid lumen and the xanthophyll cycle. It can take q_E several seconds to minutes to adjust, and it tends to be longer in field plants (Baker 2008, Murchie 2011, Nilkens 2010). Traditional q_T was thought to be caused by state transitions. q_T could take up to fifteen or twenty minutes has been described as a fluorescence change that overlapped somewhat with q_E. Changes that took longer were related to q_I or photoinhibition (Ruban 2009). There is now significant evidence to show that the fluorescence change measured as q_T in quenching relaxation measurements is likely due to other chloroplast migration at least under high actinic light conditions in many land plants.
State transitions – classical view:

According to classical state transition theory, state transitions are thought to be a low light level survival mechanism that allows balancing of light between photosystem II (PSII) and photosystem I (PSI). It was believed that LHCII antenna trimers, or peripheral phosphorylated light harvesting complex II antenna, migrated from PSII complexes, to PSI complexes. The movement would occur from one thylakoid membrane to another when they were very close or adjacent to one another. The movement and reaction took place on the stroma side of the thylakoid membrane allowing the LHCII antenna to serve as a PSI antenna. When dephosphorolated, the LHCII antenna favored movement back to PSII. The result was that at low light levels, movement was favored to PSI. LHCII phosphorylation was a prerequisite for dynamic regulation of relative balance of PSI/PSII excitation under artificially induced state transitions with different qualities of light. However, this process has not been viewed, without reservation, in PSII rich thylakoid membranes.

State transitions – a more recent view:

A more recent view of state transitions is as follows: There is no clear evidence to support the actual movement of LHCII (light harvesting complex II antenna) in stroma-exposed PSII rich thylakoid membranes, to PSI reaction centers. LHCII phosphorylation does not collect light energy for PSI in these cases. (Tikkanen 2012). Only in the margins of the grana thylakoid membrane do LHCII antenna behave according to the traditional view of phosphorylation-induced state transition (Tikkanen 2008). Tikkanen also states that there is substantial evidence to show that the classical mechanism of state transitions is not the sole method for energy balance between the two different photosystem types. There is also evidence to show that LHCII phosphorylation probably connects the regulation of light balance between PSII (photosystem II) and PSI (photosystem I) through unknown non-photochemical quenching mechanisms (Tikkanen 2012), that work not only with PSII but also PSI (Tikkanen 2010). It was also noticed that when light intensity is increased, the PsbS protein is protonated, turning the LHCII antenna into a dissipative state for PSII. (Li 2004, Tikkanen 2012). At lower light levels, LHCII activity is restored, and PSII activity is increased. Furthermore, phosphorylation is controlled by the enzymes STN7 and STN8 kinases and their opposing phosphatases, that are in turn, closely controlled by light intensity. These kinase functions are completely synchronized with PsbS and the xanthophyll cycle (Tikkanen 2012).

qZ - due to an unknown longer term xanthophyll cycle mechanism

In 2010, Nilskens and others used NPQ Arabidopsis mutants to determine that under saturation light conditions, qT or state transitions did not significantly contribute to fluorescence change and resulting quenching relaxation in the dark. Furthermore, samples were tested at moderate illumination to rule out qI or photoinhibition as the contributor to this fluorescence change. The changes related to qZ were complete by 30 minutes. It was proposed that under steady state, saturating light conditions, NPQ should be divided into qE, qZ, and qI.

As described by others, qE is a process that is created and relaxes in the ten second to two hundred second time frame, and is depends on Δph of the thylakoid lumen, the PsbS protein and zeaxanthin formation. The longer portion of qE from one hundred seconds to about 200 seconds is limited by zeaxanthin synthesis. Relaxation of qE is regulate the Δph of the thylakoid lumen.

According to the Nilskens group, the proposed qZ is created in the ten to thirty minute time frame. Psbs is not involved in qZ, but is wholly dependant on zeaxanthin formation. Relaxation depends on the re-converson of zeaxanthin to violaxanthin. It should be stated that the group found a sample that seemed to contradict the
other qZ results; however, they said that is was likely due to a retarded relaxation of a large fraction of qE and not normal middle range fluorescence relaxation characteristics. The test plant was zeazanthin devoid mutant npq1.

Photoinhibition, qI, was shown to form after 30 minutes and was dependant on illumination time, intensity and genotype. It was also found the state transitions, qT, were not a significant contributor to NPQ at saturating light intensity.

The OS5p+ provides a stable white LED actinic light source with an intense blue spectrum. It also allows direct readout of qE, qM, qZ, qT and qI using both lake and puddle model protocols.

OS5p + screen using the Hendrickson & Klughammer lake model protocol with quenching relaxation
OS5p + screen using the Kramer lake model protocol with quenching relaxation

qM – due to chloroplast migration

Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) used multiple methods, Arabidopsis mutants and wild type Arabidopsis plants to determine that the fluorescence change, previously thought to be the result of state transitions, or more recently, thought to be a longer lasting xanthophyll cycle process, was caused, by chloroplast migration. They found, as others have, that under high light levels, chloroplasts move from the top of cells to the sides of cells, partially shading other chloroplasts. This was verified with light microscopy using samples treated to prevent migration after high light migration. They also found that leaf transmittance increased and therefore leaf absorbance was lowered with chloroplast migration. The research concludes that the cause of qM is a decrease in light photon absorption which creates lower fluorescence yield, rather that a true quenching process. This is thought to be another avoidance process to protect leaves from high light levels. They found that the time scales for adjustment and fluorescence intensity change mirrored the previously used qT, but extended up to 35 minutes with some plants. Chloroplast migration has been known and studied for a while, and it was stated by Brugnoli in 1992 that chloroplast migration affected chlorophyll fluorescence. The Cazzaniga paper is the first to name chloroplast migration as the source of the qT and qZ fluorescence change.

Researchers found that high white actinic light was more effective than high red actinic light at inducing the photoprotective functions of qM. Chloroplast migration was shown to be controlled by high blue light intensity (Kagawa T. 2001). Arabidopsis mutants that were devoid of qE (npq4) were tested and Arabidopsis mutants devoid of qE and chloroplast migration (npq4 photo2) were also tested along with other mutants. In addition, targeted reverse genetic analysis was used to eliminate other contending possibilities by creating a series of double and triple mutant that covered the remaining spectrum of mechanisms that affected chlorophyll fluorescence, including; the composition of various parts the photosynthetic apparatus, carotenoid biosynthesis, and state transitions as sources of qM.
In regard to \( q_T \) being replaced by \( q_Z \), it was found that by using mutants devoid of \( q_E \) and zeaxanthin, that the magnitude of \( q_M \) did not change but the recovery time in the dark was longer. Plants were grown at 150 \( \mu \text{mol} \) photons m\(^{-2} \) s\(^{-1} \), and tested at 400 \( \mu \text{mol} \) photons m\(^{-2} \) s\(^{-1} \), 800 \( \mu \text{mol} \) photons m\(^{-2} \) s\(^{-1} \), and 1,200 \( \mu \text{mol} \) photons m\(^{-2} \) s\(^{-1} \). The adjustment time for \( q_M \) ranged up to 35 minutes for some mutants.

The Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013) observation that the stn7 mutant, devoid of state transitions, has NPQ measurements very similar to the wild type in Arabidopsis, casts strong reservations in regard to the relevance of \( q_T \) in Arabidopsis.

A follow on article has also been published on chloroplast migration:


**Conclusion, and the ramifications for chlorophyll fluorescence:**

The relevance of state transitions measured as \( q_T \) fluorescence is highly questionable for a number of photosynthetic organisms including Arabidopsis. The evidence shows that changes previously reported as \( q_T \) in quenching relaxation tests are not due to state transitions at higher light levels or saturating actinic light intensities. The latest evidence points to chloroplast migration and the resulting reduced photon absorption as the source of fluorescence change during light adaptation and during quenching relaxation, in dicot land plants at the very least.

Some prominent researchers see evidence that intermediate component mechanisms that are part of an NPQ measurement, may not be the same in all photosynthetic organisms. There could be a relationship between the phosphorylation found in state transitions and NPQ regulation found in some monocots (corn, barley and rice). There is also some strong evidence that \( q_T \) fluorescence, from state transitions, exists in the green algae Chlamydomonas reinhardtii. (Depège N., Bellafiore S., Rochaix J-D., 2003) Current research will likely provide some additional surprises in this area, moving forward.

The fact that higher intensity white or high intensity blue actinic light is required to properly activate \( q_M \) and the fact that \( q_M \) represents about 30% of NPQ under these condition, indicates the need for either a white light source or a high intensity blue light and red light instead of using a high intensity red and lower intensity blue actinic light. This new research shows that substantial measuring artifacts are possible when using a low intensity blue light source for chlorophyll fluorescence measurement. ETR or \( J, Y(II) \) or \( \Phi_{PSII} \), NPQ, \( g_M \), \( C_C \) and \( q_I \) may all include measuring errors without the a reliable light source.

This also may change the times required for proper dark adaptation measurements, and the time to reach steady state photosynthesis under light adapted conditions. Until now, Maxwell K., Johnson G. N, (2000) has been the most sighted paper for reliable steady state photosynthesis conditions at any given light level. It lists 15 to 20 minutes as the time required for 20 wild land plants to reach steady state photosynthesis. Prominent researchers, Lichtenthaler (1999) and Ruban (2009), list the dark adaptation time required for quenching relaxation of \( q_T \) at the same 15 to 20 minutes. With this new evidence from Cazzaniga S, Osto L.D., Kong S-G., Wada M., Bassi R., (2013), it shows that dark adaption times and the time to reach steady state lighting conditions should be extended, at least at higher light levels. They show that it takes from 20 minutes to 35 minutes for chloroplast migration to adapt to higher light levels and for relaxation in the dark for measurement of chlorophyll fluorescence values. Most gas exchange protocols tend use the term steady state photosynthesis
to mean the adjustment of $q_E$ or the xanthophyll cycle. However, this too should be reviewed moving forward. It was thought that any change due to state transitions, at high actinic light levels, was very small and that acute photoinhibition was responsible for larger changes. Now that chloroplast migration replaces both mechanisms as the source of change, gas exchange measurements, at high actinic light levels, can contain very significant measuring artifacts if chloroplast migration is not considered.

**Notes:**

The OS5p+ chlorophyll fluorometer allows the measurement of $q_E$, $q_T$, $q_Z$, $q_M$ and $q_I$ for direct readout depending on samples and conditions. In addition, the iFL integrated fluorometer, the OS5p+, OS1p and the Y(II) meter use white light actinic light sources that allow chloroplast migration to occur.

The iFL also measures leaf absorptance, and it can demonstrate the change in leaf absorptance as chloroplast migration occurs.

**References:**


