Non-Photochemical Quenching (NPQ) Protocol

The NPQ protocol is the most typically used measuring approach to quantify photochemical and non-photochemical quenching. The measurement should be performed with a dark-adapted sample. Thereby, it may not be appropriate under field conditions.

The NPQ protocol starts by giving a measuring light to acquire minimal level of fluorescence F_0**.** A short saturating flash of light is then applied to reduce the plastoquinone pool and measure maximum fluorescence in the dark-adapted state, F_m. After a short dark relaxation, the sample is exposed to actinic irradiance for tens to hundreds of seconds to elicit a transient of the Kautsky effect. Moreover, a sequence of saturating flashes is applied on top of the actinic light to probe the non-photochemical quenching *NPQ* and effective quantum yield of photosynthesis *QY* in light adapted state. After exposure to continuous illumination, the relaxation of non-photochemical quenching is determined by means of saturating pulses applied in dark (Fig.).

Three NPQ protocols, NPQ1, NPQ2 and NPQ3 are predefined. The protocols differ in the duration of the light exposure and the dark recovery phase, in the number and interval between pulses. See Tab. 1:

	Phase	Duration	# of pulses	1st pulse	Pulse interval
NPQ1	Light	60 s	5	7 s	12 s
	Dark recovery	88 s	3	11 s	26 s
NPQ2	Light	200 s	10	10 s	20 s
	Dark recovery	390 s	7	20 s	60 s
NPQ3	Light	200 s	10	11 s	21 s
	Dark recovery	60 s	2	20 s	21 s

Tab. 1 NPQ Protocols.

The protocol includes following measured and calculated parameters:

Abbreviation	Explanation
F_0	minimum fluorescence in dark-adapted state
F_m	maximum fluorescence in dark-adapted state, measured during the first saturation flash after dark adaptation
F_p	fluorescence in the peak of fast Kautsky induction
F_m_L, Lss, D, Dss[1]	maximum fluorescence
QYmax[2]	maximum quantum yield of PSII in dark-adapted state - F_v/F_m
QY_L, Lss, D, Dss[1,3]	effective quantum yield of PSII
NPQ_L, Lss, D, Dss[1,4]	non-photochemical chlorophyll fluorescence quenching
Qp_L, Lss, D, Dss[1,5]	coefficient of photochemical quenching, an estimate of open PSII reaction centers

[1] *L* - indicates light adapted parameters; *D* - refers to dark recovery phase after switching of the actinic illumination; *n* - represents a sequential number of light phase; *ss* - steady state

[2] Calculated as $(F_m – F_0) / F_m$

[3] Calculated as $(F_m\_Ln – F_t\_Ln) / F_m\_Ln$ or of corresponding steady state or dark recovery parameters

[4] Calculated as $(F_m – F_m\_Ln) / F_m\_Ln$ or of corresponding ss, Dn or Dss parameters

[5] Calculated as $(F_m\_Ln – F_t\_Ln) / (F_m\_Ln – F_0\_Ln)$ or of corresponding ss, Dn or Dss parameters

F_0_Ln is calculated as $F_0 / ((F_m – F_0) / F_m + F_0 / F_m\_Ln)$.

For more details please refer to: Oxborough K., Baker N.R. (1997): Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components: calculation of qP and F_v'/F_m' without measuring F_0'. Photosynthesis Research 54: 135-142.

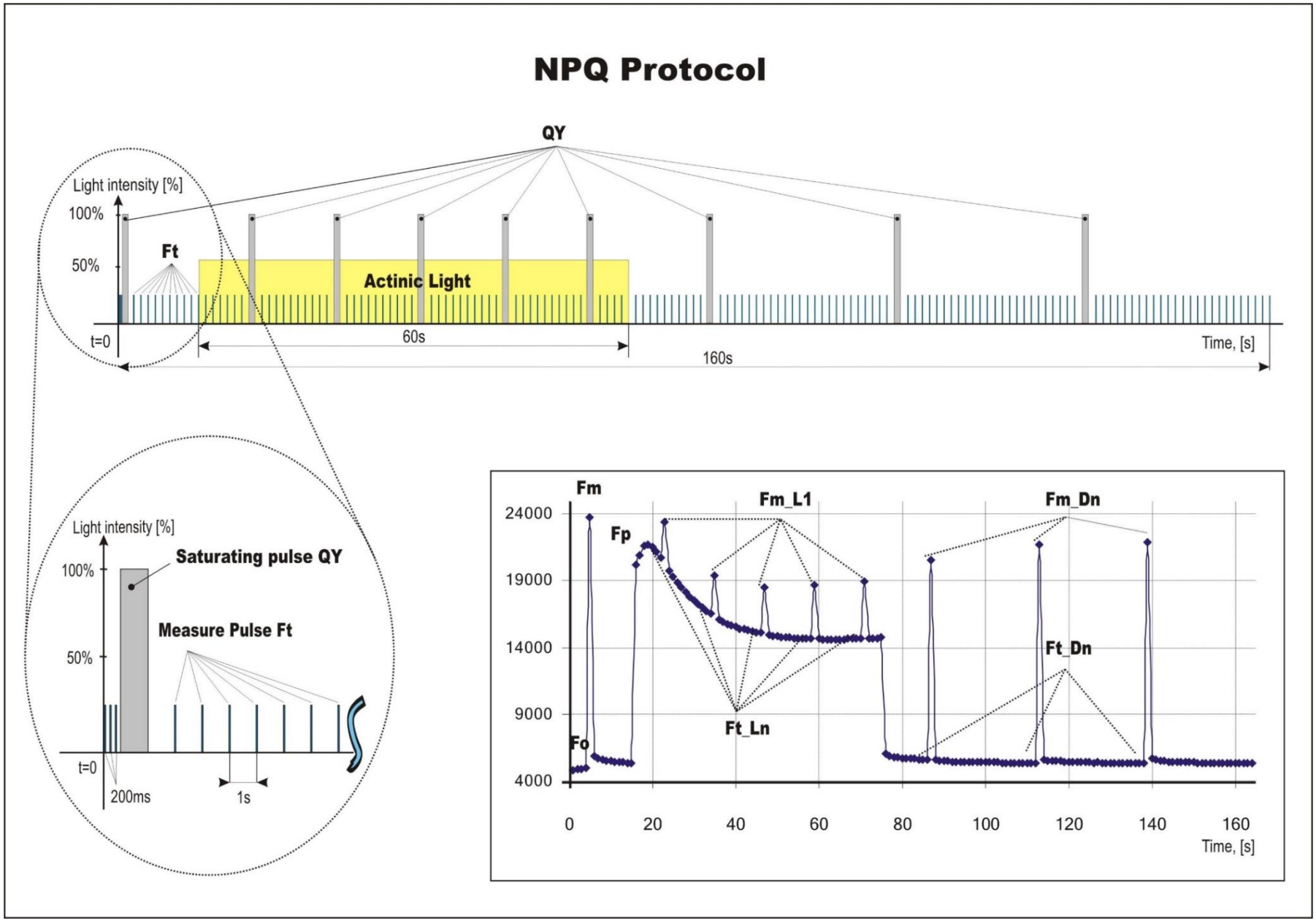

Fig. 1 NPQ Protocol.