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PSI Open Fluor CAM script for measuring qE component of NPQ in *Chlorella vulgaris*

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1 Works for me



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dx.doi.org/10.17504/protocols.io.byn9pvh6

Climate Change Cluster

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ABSTRACT

This is a simple protocol that consists of

- 1) 10 minutes preillumination with far red light
- 2) 5 minutes of illumination with actinic light
- 3) 5 minutes of dark adaptation with far red light

qE is calculated as the difference between NPQ_Lss and NPQ_D5

$$qE = NPQ_Lss - NPQ_D5$$
$$qI = NPQ_D5$$

Protocol to be used with FluorCAM 7.0 on a PSI Open FC 800-O/1010-S.

Act 2 - are the white light LED arrays

ADD2 - is the far red LED array

Camera is placed at ~20 cm above the measured sample. Light intensity uniformity across the 96 well plate was measured according to manufacturer instructions.

!Important - protocol only works under weak far red light. Intense far red will interfere with the fluorescence measurement.

DOI

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PROTOCOL CITATION

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protocols.io
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KEYWORDS

NPQ, qE, FluorCAM

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LAST MODIFIED

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Script for FluorCAM software

```

1 ; Quenching protocol with Actinic2
;with FilterWheel
;version November 11, 2020
;high-resolution CCD TOMI-2
;optimized number of measured frames
;Protocol duration 183s
;
ADD1=0
ADD2=10
Act1=0
TS=50ms
include default.inc ;Includes standard options, do not remove it !
include light.inc ;Includes standard options, do not remove it !
include FW.inc ;Includes standard options, do not remove it !
;
Shutter=2
Sensitivity=29.3
Act2=18
Super=69.6
LightA=29.069
LightB=-34.732
;
<0s>=>SET_FILTER(CHL)
Preillumination=600s
<0s>=>add2(Preillumination)
start = Preillumination;
;
*** Fo Measurement *****
;
F0duration=5s
F0period=1s
start + <0,F0period...F0duration>=>mfmsub
;
;Fo definition
start + <0s>=>checkPoint,"startFo"
start + <F0duration-F0period>=>checkPoint,"endFo"
;
*** Saturating Pulse & Fm Measurement *****
;
PulseDuration=800ms
a1 = start + F0duration+2mfmsub_length
;
<a1>=>SatPulse(PulseDuration)
<a1>=>act2(PulseDuration)
;
<a1>=>mpulse2
;
;Fm definition
<a1+PulseDuration/2>=>checkPoint,"startFm"
<a1+PulseDuration-mfmsub_length>=>checkPoint,"endFm"
;
;Visual frame definition; Imagee shown in pre-processing window

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<a1+PulseDuration/2+TS>=>checkPoint,"timeVisual"
;
;-----
;***** Dark Relaxation Measurement *****
;-----
DarkRelaxation1=17s
b1= a1+PulseDuration+2mfmsub_length
b2=2s
<b1, b1+2mfmsub_length... b1+b2>=>mfmsub
<b1+2b2, b1+4b2... b1+DarkRelaxation1>=>mfmsub
;
;-----
;***** Kautsky Effect Measurement *****
;-----
;
;***** Actinic light Exposure *****
ALPeriod=300s
c1=a1+PulseDuration+DarkRelaxation1+mfmsub_length
<c1>=>act2(ALPeriod)
;
;Fast Kautsky kinetics*****
c2=2s
;
<c1+TS, c1+TS+2 TS... c1+c2>=>mfmsub;
;
;Slow Kautsky kinetics*****
;
c3=4s
;
<c1+c2+c3/10, c1+c2+c3/5, c1+c2+c3/2>=>mfmsub
;
<c1+c2+c3, c1+c2+2 c3... c1+ALPeriod>=>mfmsub
;
;Fp definition
<c1>=>checkPoint,"startFp"
<c1+c2+c3>=>checkPoint,"endFp"
;
;-----
;***** Saturating Pulses - Fm' Quenching Analysis *****
;-----
;
;***** Saturating Pulses - Fm_L1 *****
f1=c1+<58s>
f11=f1#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
f11=>mfmsub
f1+mfmsub_length=>checkPoint,"startFt_L1"
f1+PulseDuration-mfmsub_length=>checkPoint,"endFt_L1"
;
f2=f1+PulseDuration
f2=>SatPulse(PulseDuration)
f2=>mpulse2
f2+PulseDuration/2=>checkPoint,"startFm_L1"
f2+PulseDuration-mfmsub_length=>checkPoint,"endFm_L1"
;
;***** Saturating Pulses - Fm_L2 *****
f3=c1+<118s>
f31=f3#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
f31=>mfmsub

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f3+mfmsub_length=>checkPoint,"startFt_L2"
f3+PulseDuration-mfmsub_length=>checkPoint,"endFt_L2"
;
f4=f3+PulseDuration
f4=>SatPulse(PulseDuration)
f4=>mpulse2
f4+PulseDuration/2=>checkPoint,"startFm_L2"
f4+PulseDuration-mfmsub_length=>checkPoint,"endFm_L2"
;
***** Saturating Pulses - Fm_L3 *****
f5=c1+<178s>
f51=f5#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
f51=>mfmsub
f5+mfmsub_length=>checkPoint,"startFt_L3"
f5+PulseDuration-mfmsub_length=>checkPoint,"endFt_L3"
;
f6=f5+PulseDuration
f6=>SatPulse(PulseDuration)
f6=>mpulse2
f6+PulseDuration/2=>checkPoint,"startFm_L3"
f6+PulseDuration-mfmsub_length=>checkPoint,"endFm_L3"
***** Saturating Pulses - Fm_L4 *****
f7=c1+<238s>
f71=f7#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
f71=>mfmsub
f7+mfmsub_length=>checkPoint,"startFt_L4"
f7+PulseDuration-mfmsub_length=>checkPoint,"endFt_L4"
;
f8=f7+PulseDuration
f8=>SatPulse(PulseDuration)
f8=>mpulse2
f8+PulseDuration/2=>checkPoint,"startFm_L4"
f8+PulseDuration-mfmsub_length=>checkPoint,"endFm_L4"
;
***** Saturating Pulses - Fm_Lss *****
f9=c1+<298s>
f91=f9#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
f91=>mfmsub
f9+mfmsub_length=>checkPoint,"startFt_Lss"
f9+PulseDuration-mfmsub_length=>checkPoint,"endFt_Lss"
;
f10=f9+PulseDuration
f10=>SatPulse(PulseDuration)
f10=>mpulse2
f10+PulseDuration/2=>checkPoint,"startFm_Lss"
f10+PulseDuration-mfmsub_length=>checkPoint,"endFm_Lss"
;-----
** Dark relaxation after actinic light period *****
;-----
DarkRelaxation2=300s
h1=c1+ALPeriod
<h1>=>add2(DarkRelaxation2)
h2=mfmsub_length
h3=10s
;
***** Relaxation measurement*****
;
<h1+h2, h1+2h2, h1+4h2, h1+8h2, h1+16h2>=>mfmsub;

```

```

h1+h3/2+<0s, h3... DarkRelaxation2=>mfmsub
;
;***** Saturating Pulses - Fm_D1 *****
g1=h1+<58s>
g11=g1#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
g11=>mfmsub
g1+mfmsub_length=>checkPoint,"startFt_D1"
g1+PulseDuration-mfmsub_length=>checkPoint,"endFt_D1"
;
g2=g1+PulseDuration
g2=>SatPulse(PulseDuration)
g2=>act2(PulseDuration)
g2=>mpulse2
g2+PulseDuration/2=>checkPoint,"startFm_D1"
g2+PulseDuration-mfmsub_length=>checkPoint,"endFm_D1"
;
;***** Saturating Pulses - Fm_D2 *****
g3=h1+<118s>
g31=g3#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
g31=>mfmsub
g3+mfmsub_length=>checkPoint,"startFt_D2"
g3+PulseDuration-mfmsub_length=>checkPoint,"endFt_D2"
;
g4=g3+PulseDuration
g4=>SatPulse(PulseDuration)
g4=>act2(PulseDuration)
g4=>mpulse2
g4+PulseDuration/2=>checkPoint,"startFm_D2"
g4+PulseDuration-mfmsub_length=>checkPoint,"endFm_D2"
;
;***** Saturating Pulses - Fm_D3 *****
g5=h1+<178s>
g51=g5#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
g51=>mfmsub
g5+mfmsub_length=>checkPoint,"startFt_D3"
g5+PulseDuration-mfmsub_length=>checkPoint,"endFt_D3"
;
g6=g5+PulseDuration
g6=>SatPulse(PulseDuration)
g6=>act2(PulseDuration)
g6=>mpulse2
g6+PulseDuration/2=>checkPoint,"startFm_D3"
g6+PulseDuration-mfmsub_length=>checkPoint,"endFm_D3"
;
;***** Saturating Pulses - Fm_D4 *****
g7=h1+<238s>
g71=g7#<mfmsub_length, 2mfmsub_length... PulseDuration-mfmsub_length>
g71=>mfmsub
g7+mfmsub_length=>checkPoint,"startFt_D4"
g7+PulseDuration-mfmsub_length=>checkPoint,"endFt_D4"
;
g8=g7+PulseDuration
g8=>SatPulse(PulseDuration)
g8=>act2(PulseDuration)
g8=>mpulse2
g8+PulseDuration/2=>checkPoint,"startFm_D4"
g8+PulseDuration-mfmsub_length=>checkPoint,"endFm_D4"
;

```

```
,***** Saturating Pulses - Fm_D5 *****
g9=h1+<298s>
g91=g9#<mfmsub_length, 2*mfmsub_length... PulseDuration-mfmsub_length>
g91=>mfmsub
g9+mfmsub_length=>checkPoint,"startFt_D5"
g9+PulseDuration-mfmsub_length=>checkPoint,"endFt_D5"
;
g10=g9+PulseDuration
g10=>SatPulse(PulseDuration)
g10=>act2(PulseDuration)
g10=>mpulse2
g10+PulseDuration/2=>checkPoint,"startFm_D5"
g10+PulseDuration-mfmsub_length=>checkPoint,"endFm_D5"
;
;END *****
```