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'CR1000 Series Datalogger
'Biofeedback system based on fluorescence measurements using a MINI-PAM and a PWM and frequency control for four
'100-W LEDs
'by Rhuanito Soranz Ferrarezi, Geoff Weaver and Marc van Iersel
'Note: program is available in compilable format from Marc van Iersel (mvanier@uga.edu)

'Variables
'DA: fluorescence yield in a dark-adapted measurement
'DA_Fmin: minimum fluorescence measured before a dark-adapted measurement (normally denoted by Fo)
'DA_Fmax: maximum fluorescence measured after a dark-adapted measurement (normally denoted by Fm)
'DA_Yield:  yield of photochemical energy conversion ain the dark
'Fmin: Minimum fluorescence measured before a saturation pulse in any given light state (normally denoted by F)
'Fmax: Maximum fluorescence measured after a saturation pulse in any given light state (normally denoted by Fm')
'Yield: Yield of photosystem II
'PARbfb: photosynthetic active radiation; PARbfbadjust: PAR after adjusitng PAR
'ETR: electron transport rate
'NPQ: non-photochemical quenching
'DA(50): 50 dark-adapted maximum fluorescence measurements
'LEDOut(2): analog voltages used for control of the LEDs
'LedOut(1): analog signal to control duty cycle of LEDs
'LedOut(2): analog signal to control frequency of LEDs

'DataOut: determines whether data is written to the output file
'FminTMP, Fmin_int: intermediate variables to determine Fmin

'Wiring

'Analog output from mini-PAM to Diff channel 1 on CR1000
'Li-190 quantum sensor to Diff channel 2 on CR1000
'C1, C2, and C3 on CR1000 to C1, C2, and C3 on SDM-AO4
'Ground and 12V on CR1000 to Ground and 12V on SDM-AO4
'Analog out 1 and 2 on SDM-AO4 to PWM control board

'Declare variables
Public DataOut, LEDOut(4), DA(50), Fm(50), DA_Fmin, DA_Fmax, DA_Yield, Fv, Fmin, Fmax, Yield
Public PARbfb, PARbfbTemp, PARbfbInt, ETR, NPQ, ETRt, FminTMP, Fmin_int, i, j

'Variables 'Dark' and 'Light' are used to control when to turn the LEDs on and off. This also affects how often
'fluorescence data is collected.
Public Dark, Light

'Units
Units PARbfb, ETR, ETRt = µmol m-2 s-1
Units LEDOut() = mV

'Data tables. Specify which data need to be collected. Data will be collected
'when 'DataOut is not equal to 0
DataTable (Biofeedback,DataOut,-1)
  'Collect data at 5 minute intervals
  DataInterval (0,5,Min,0)
  'Store the following values
  Sample (1,DA_Fmin,FP2)
  Sample (1,DA_Fmax,FP2)
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  Sample (1,DA_Yield,FP2)
  Sample (1,Fmin,FP2)
  Sample (1,Fmax,FP2)
  Sample (1,Yield,FP2)
  Sample (1,PARbfb,FP2)
  Sample (1,ETR,FP2)
  Sample (1,NPQ,FP2)
  Sample (1,ETRt,FP2)
  Sample (2,LEDOut(1),FP2)
EndTable

'Subroutine to turn the saturation pulse on the mini-PAM ON
Sub MINI-PAM
  'open communications with the mini-PAM (connected to COM3 on the CR1000
  SerialOpen (Com3,9600,0,0,195)
  'Send the signal for the saturing pulse
  SerialOut (Com3,"s","",0,1)
  'Wait for 50 msec
  Delay (1,50,mSec)
  'Send 'return'
  SerialOut (Com3,CHR(13),"",0,1)
  'Wait for 50 msec
  Delay (1,50,mSec)
  'Close communications with the mini-PAM
  SerialClose (Com3)
EndSub

'Main Program
BeginProg

  ' Set the frequency of the LEDs to 1000 Hz
  LEDOut(2) = 400 'Note: a 400 mV signal results in a 1000 Hz frequency with our PWM board
  'Send the analog signal to set the duty cycle and frequency of the LEDs
  SDMAO4 (LEDOut(1),2,0)

  'This program runs once every 5 minutes.
  Scan (5,Min,0,0)
    'Set output variable to zero
    DataOut = 0

    'The growth chamber is programmed to be dark from midnight to 8 am with the lights on from 8 am to midnight
    'The following statements determine whether it is 'day' or 'night'
    'At midnight
    If TimeIntoInterval (0,24,Hr) Then
      'Set variable 'dark' to 1 and 'light' to 0.
      Dark = 1
      Light = 0
      'When the dark period starts, turn off the LEDs
      LEDOut(1) = 0 'a 0 mV signal turns the LEDs off

      'Send the analog signal to set the duty cycle and frequency of the LEDs
      SDMAO4 (LEDOut(1),2,0)
      'End of 'If' loop
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EndIf

'At 8:00 am
If TimeIntoInterval (8,24,Hr) Then
  'Set variable 'dark' to 0 and 'light' to 1.
  Dark = 0
  Light = 1
  'When the Light first get turned on, set the light levels to their starting intensity
  'Note: 765 is just an estimate of the millivolt signal needed to get 250 umol/m2/s of PAR. This value can be adjusted.
  LEDOut(1) = 765
  SDMAO4 (LEDOut(1),2,0)
  'Now wait for five seconds to allow the LEDs to turn on
  ExciteV (Vx1,0,5000000)
EndIf

'Take light sensor measurements
PARbfbInt = 0
' Because the light measurements under the LEDs are noisy, we take 20 measurements and average them
For i = 1 To 20
  'Photosynthetic active radiation (PAR) measurement on the biofeedback side of the growth chamber used to
  ' calculate ETR
  VoltDiff (PARbfbTemp,1,AutoRange,2,True,0,_60Hz,-244.27,0) 'Note: check calibration of this light sensor
  'Calculate the sum of the PAR measurements
  PARbfbInt = PARbfbInt+PARbfbTemp
  'And calculate the average of the PAR measurements
  PARbfb = PARbfbInt/i
Next i

'Dark-adapted Fmax and Fmin measurements are collected hourly in the dark.
'If it is dark
If Dark = 1 Then
  'And on the whole hour
  If TimeIntoInterval (0,60,Min) Then
    'Collect output
    DataOut=1
    DA_Fmax=0 'Set Dark -adapted "Fmax" to zero
    For j=1 To 50 'take 50 fluorescence measurements
      VoltDiff (DA(j),1,mV2500,1,True,0,250,2.0,0)
      If DA(j) > DA_Fmax
        DA_Fmax = DA(j)  'The highest measurement is recorded as DA-Fmax
      EndIf
      DA_Fmin=DA(3)   'The third fluorescence measurement (with saturating light off) is recorded as DA-Fmin
      If j=5 'When we get to the 5th measurement
        Call MINI-PAM 'Turn on saturating pulse
      EndIf
      Delay(1,100,mSec) ' Wait 100 msec before the next measurement
    Next j
    'End of loop that runs at the whole hour
  EndIf
  'Dark-adapted yield calculation
  DA_Yield=(DA_Fmax-DA_Fmin)/DA_Fmax
  'End if the 'If it is dark' loop
EndIf
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'If the lights are on
If Light = 1 Then
 'Collect output
 DataOut=1

 Fmax=0 'Set "Fmax" to zero to allow for new measurements.
 'To calculate "Fmin", 1000 measurements are taken at 20 msec intervals and the average of these is recorded.
 ' Set Fmin_into to 0
 Fmin_int=0
 'measure fluorescence signal 1000x at 20 msec intervals
 SubScan (20,mSec,1000)
  VoltDiff (FminTMP,1,mV2500,1,True,0,_60Hz,2.0,0)
  'Calculate the sum of the 1000 measurements
  Fmin_int = Fmin_int+FminTMP
  'Take the next reading
 NextSubScan
 'Calculate the average of the 1000 measurements and store this average as Fmin
 Fmin=Fmin_int/1000

 'Turn off the LED to take "Fmax" measurements. Through trial and error it was found that
 'the best way to eliminate excessive noise from this measurement is to briefly turn off the LEDs
 SDMAO4(0,1,0)

 'Collect voltage measurement for "Fmax". 50 measurements are taken with the saturating pulse on and the LEDs off,
 ' and the highest value measured is recorded as "Fmax"
 Call MINI-PAM  'Call the mini-PAM subroutine to turn on saturating pulse
 '0.2 second delay to give the mini-PAM time to respond
 Delay (0,200,mSec)

 'Take 50 measurements at 20 mSec intervals.
 For j=1 To 50 'take 50 measurements
  VoltDiff (Fm(j),1,mV2500,1,True,0,250,2.0,0)
  If Fm(j) > Fmax Then Fmax = Fm(j) 'The highest measurement is recorded as Fmax
  'Wait 20 msec
  Delay(1,20,mSec)
  'Take the next measurement
 Next j

 'Turns LEDs back on according to the last "LEDOut1= " instruction (from previous scan)
 SDMAO4(LEDOut(1),2,0)
 'yield calculation: Quantum Yield of PSII = Fv/Fm
 Yield=(Fmax-Fmin)/Fmax
 'Electron transport rate (ETR) calculation, where 0.5 is the efficiency of PSII
 ' and 0.84 is the ETR-factor (fraction of light absorbed by the leaf)
 ETR=Yield*PARbfb*0.5*0.84

 'Non-photochemical quenching (NPQ) calculation (result range from 0 to 4)
 NPQ=(DA_Fmax-Fmax)/Fmax

 'Adjust the voltage signal controlling the duty cycle, based mon the ratio between ETRt and measured ETR
 If ETR>=5 Then LEDOut(1) = LEDOut(1)*ETRt/ETR
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    'Prevents LEDOut1 from exceeding its operational range. Value has to be between 0 and 2000 mV
    If LEDOut(1) > 2000 Then LEDOut(1)=2000 'Send the maximal voltage of 2000 mv to power the LEDs

    'Sends a voltage signal to the LED lamp to adjust the duty cycle
    SDMAO4 (LEDOut(2),2,0)
   EndIf

   'The following statement makes sure ETR also gets calculated when it is dark
   ETR=Yield*PARbfb*0.5*0.84

   'Delay of 5 seconds to allow the LEDs to reach the new light intensity.
   Delay(0,5,Sec)

   'Call the data table to store all the data
   CallTable Biofeedback

   'And run the program again
  NextScan

EndProg
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