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# Theremino DNA Meter

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Fluorometer for DNA Measurements

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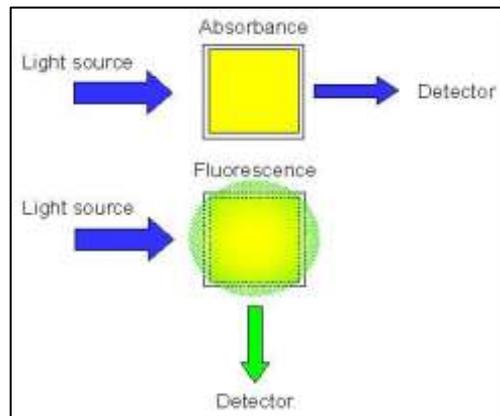
## Principle of Operation

For the measurement of the concentration of nucleic acids (DNA , RNA) different methods are available.

The first method is based on the **absorption in the near ultraviolet** : these molecules absorb selectively in a band of wavelengths around 200nm . Thus measuring the absorbance curve of the sample make it possible to determine the concentration of nucleic acids present in the sample. This method however requires the availability of a light source in the UV region and a sensitive spectrometer at these wavelengths.

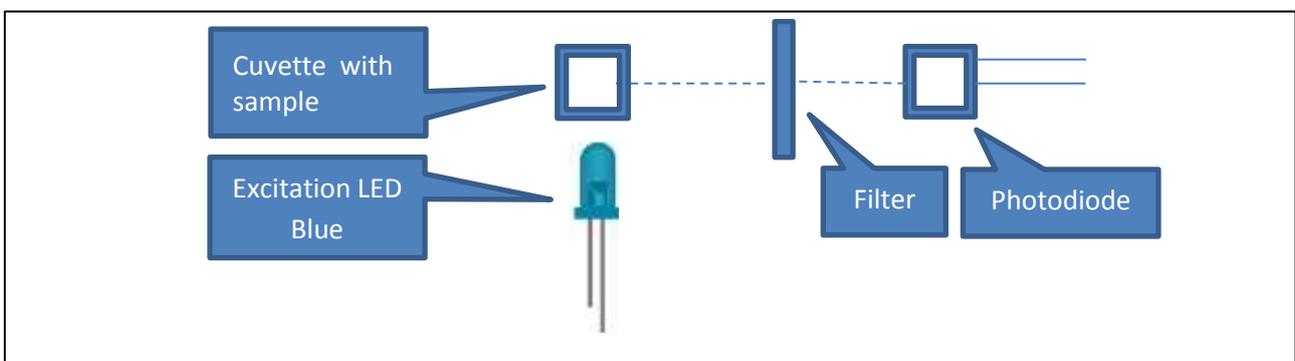
A simpler method is based instead on the **fluorescence** that some dye molecules acquire when they bind to molecules of nucleic acids.

Adding to the sample a known quantity of dye and measuring the intensity of fluorescence is possible to determine the concentration of nucleic acids present in the sample.



The prototype molecule of dye is ethidium bromide which is harmful, however, being carcinogenic. Recently other substances that act in the same way but are safer compared to ethidium bromide have become available.

In our case it was adopted the dye known commercially as **MidoriGreen**. In the picture below it is shown the block diagram of the Fluorometer.



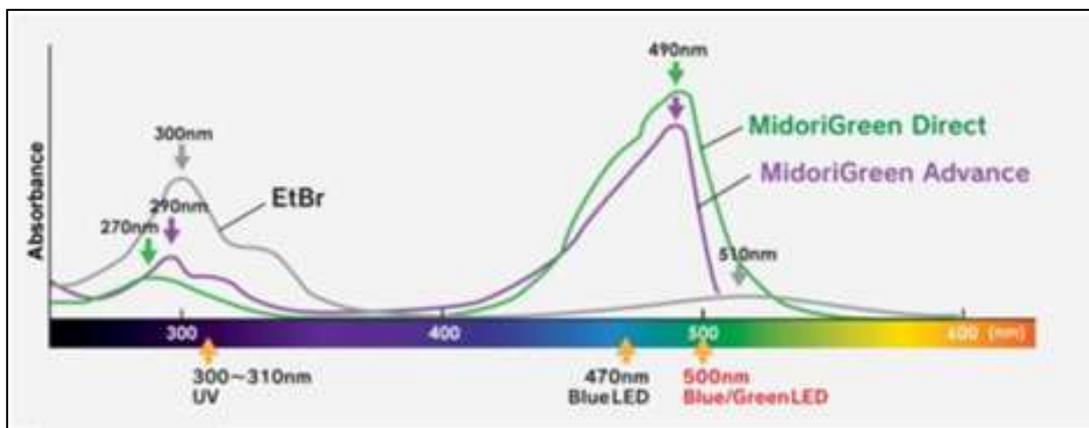
The cuvette with sample is illuminated by the excitation source (blue LED) and orthogonally with respect to the direction of illumination is placed the photodiode that collects the fluorescence

emission. The light from the LED is filtered so that the photodiode is reached only by the light coming from the fluorescence of the sample.

## Fluorescent Dye “MidoriGreen”

Midori Green Nucleic Acid Staining Solution is a new and safe alternative to traditional ethidium bromide(EtBr) stain for detecting dsDNA, ssDNA and RNA in agarose gels. Nearly identical to EtBr in performance and use, Midori Green is much less harmful to living organisms.

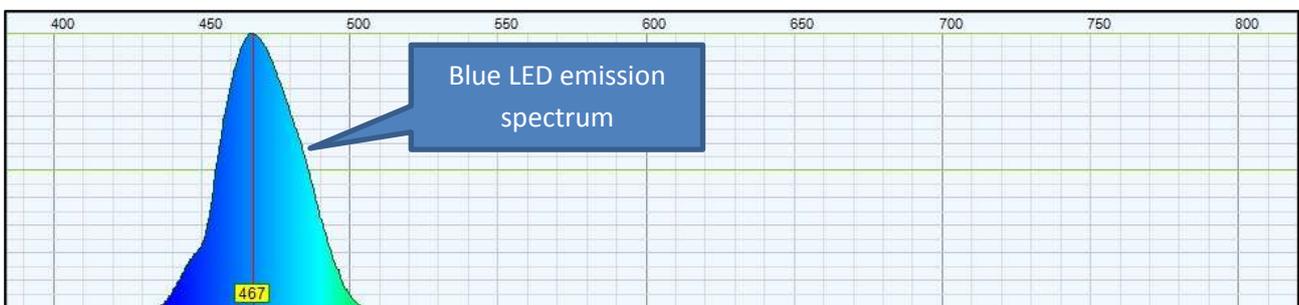
Midori Green has two secondary fluorescence excitation peaks (~300nm; ~400nm) and one strong excitation peak centered around 500nm. The fluorescence emission is centered around 540nm. In the picture below it is shown the absorption spectrum of MidoriGreen compared to the spectrum of ethidium bromide.



## Excitation Source

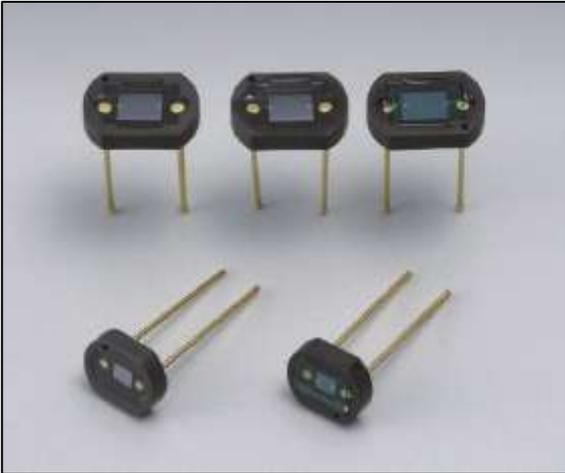
As a fluorescence excitation source a simple blue LED has been used. The Blue LED emits light of a wavelength centered around **470nm**, then close to the absorption peak of the MidoriGreen. In the figure below it is shown the LED emission spectrum. It is clear that the spectrum of the LED emission goes from about 440 to 500nm.

Using a blue LED for excitation is cheaper than the UV source, it is better also because in this way damages to the DNA molecules that are sensitive to ultraviolet radiation are prevented.



## Photodiode

For the measurement of the intensity of the fluorescence is used a "general purpose" ceramic photodiode Hamamatsu S1133-14 type . Characterized by a good sensitivity over a wide range of wavelengths and by a low " dark current ", useful when you make low brightness measurements.



Here are the main features of the photodiode :

**type** : S1133-14

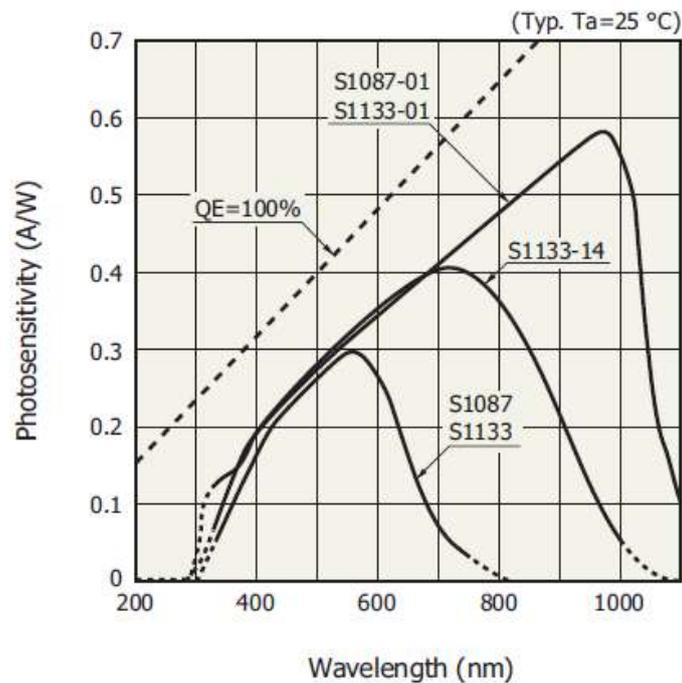
**Spectral response range  $\lambda$ (nm)** : 320 – 1000

**Peak sensitivity (nm)** : 720

**Dark Current  $I_d$  (pA)** : 20

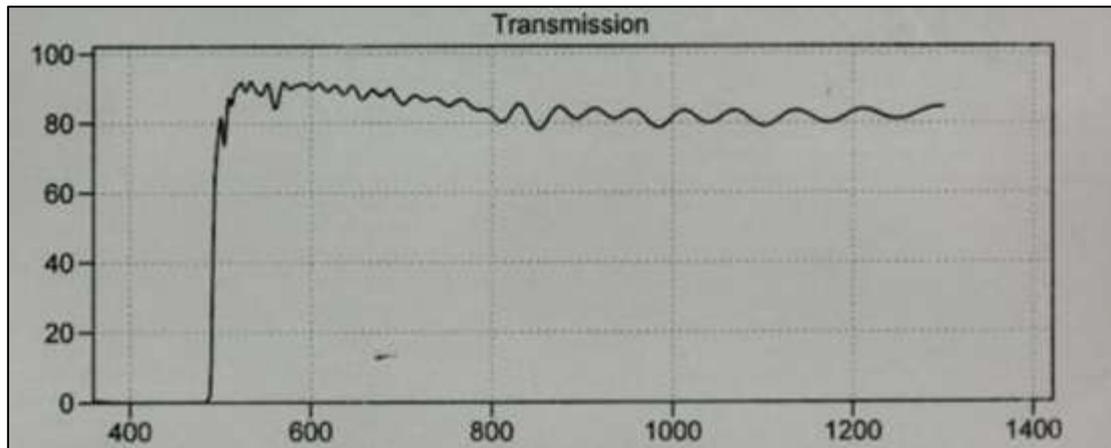
**Terminal Capacitance (pF)** : 200

in the image below the sensitivity spectrum is shown.



## Optical Filter

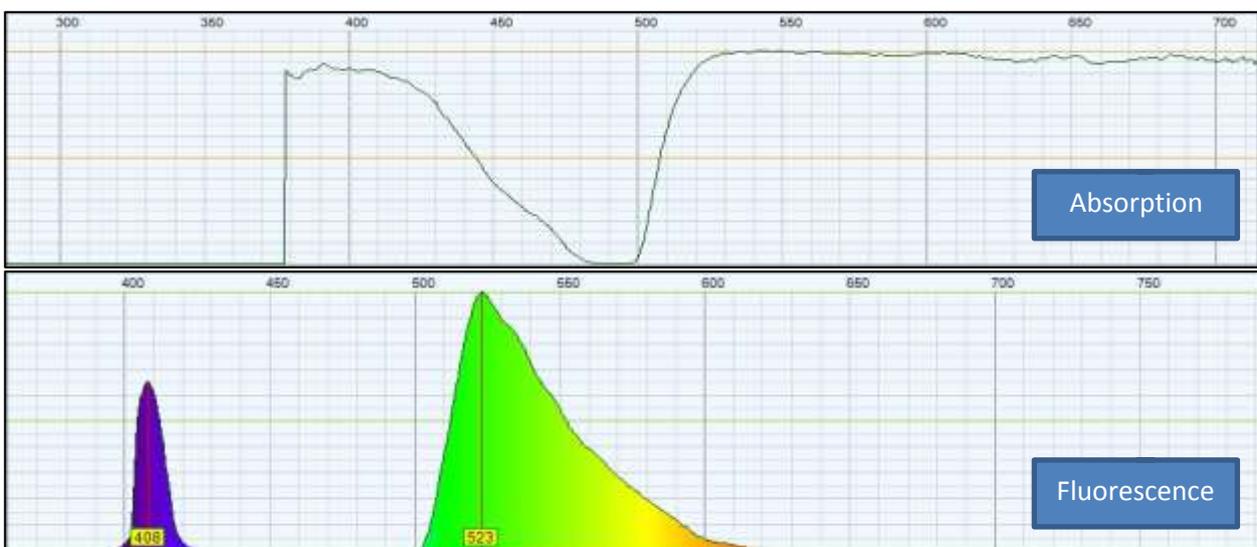
To prevent the photodiode is also reached by the light from the excitation source (blue LED with emission at 470nm), the photodiode has been shielded by an "low pass" optical filter that only passes light with wavelength longer than **490nm**. Doing so, it stops the emission of excitation at 470nm but not the emission of fluorescence at 540nm. Given that the LED emits a bit even beyond 490nm the photodiode is also achieved by a small part of the excitation emission, which is taken into account in the data acquisition software.



## Fluorescein as substitute of DNA + MidoriGreen

For testing and calibration the fluorometer is not strictly necessary to use DNA samples with relative dye : it is sufficient to have a dilute solution of fluorescein, with which it is possible to prepare the samples that quite faithfully simulate the behavior of a MidoriGreen solution.

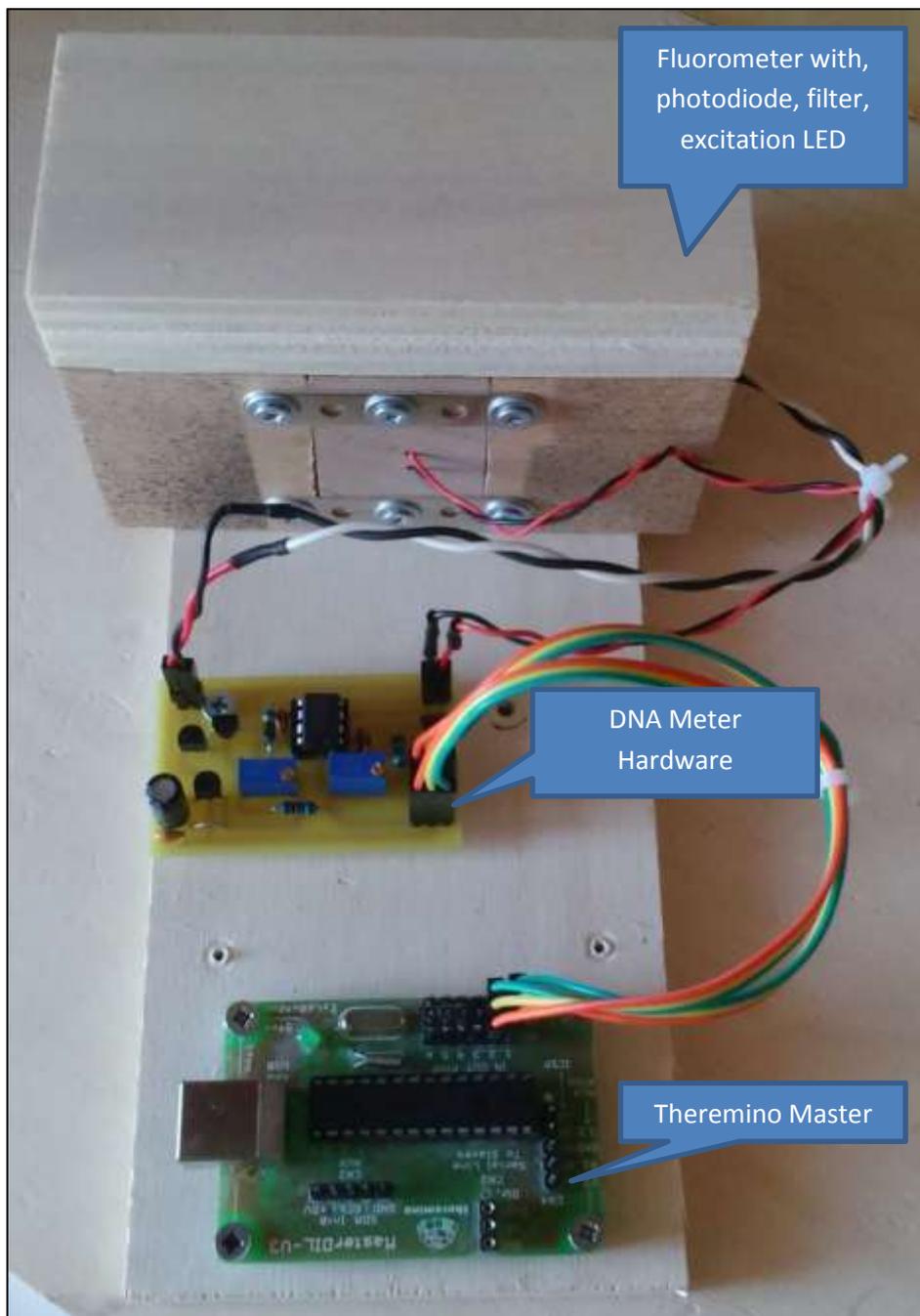
In the images there are the absorption and fluorescence spectra of fluorescein and you see how closely follow those of the MidoriGreen dye.



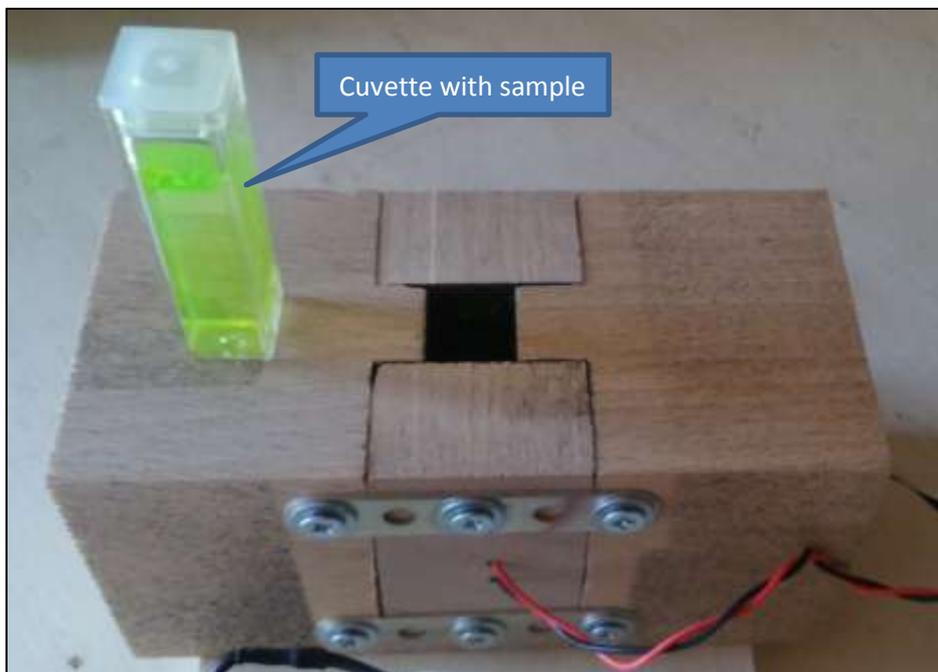
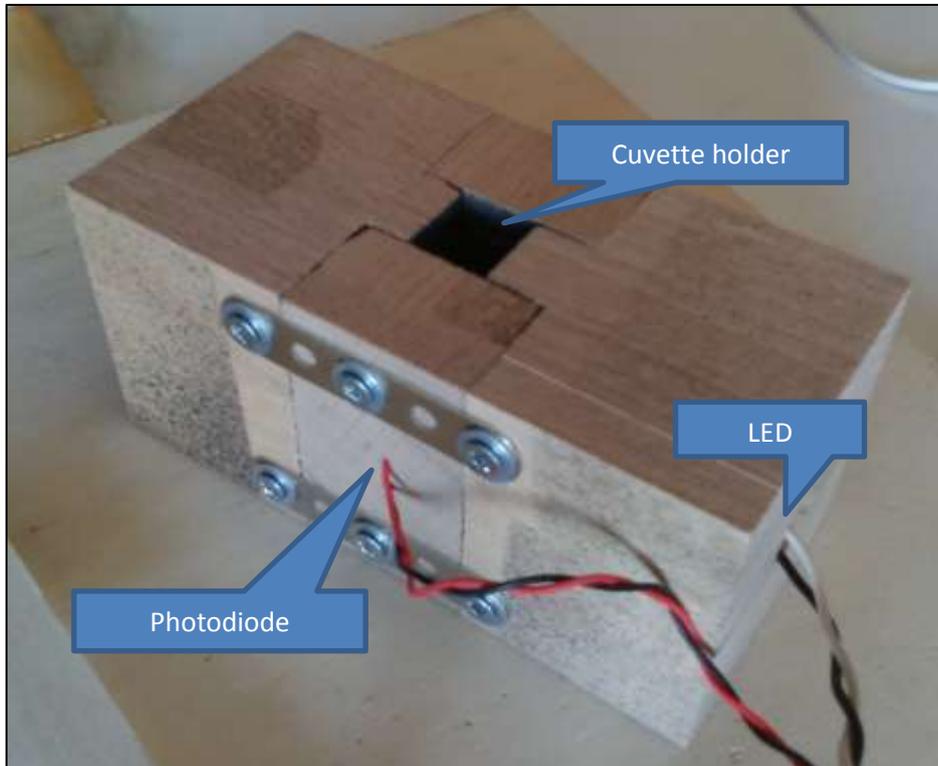
## Building the Fluorometer

A prototype fluorometer has been built (Images shown below) for measuring the concentration of nucleic acids. The instrument is based on the principle of fluorescence described in the previous chapter. To read the photodiode signal and to drive the excitation LED has been designed and implemented an electronic circuit, called DNA Meter, which is connected to the Master Theremino that performs the data read/write which are managed by a specific software described in the following sections.

In the figure below you see the prototype, which includes the photodiode, LED and optical filter, the DNA Meter board and the Theremino Master for data acquisition and for interfacing with computer.



In the image below you can see the detail of the cuvette place that is composed of four blocks of wood fixed with brackets and screws, the interior of the blocks is painted black to reduce glare. Two blocks are drilled and shaped to accommodate photodiode, filter and LED. In the second image it is also shown a cuvette with the fluorescein solution used for the test.

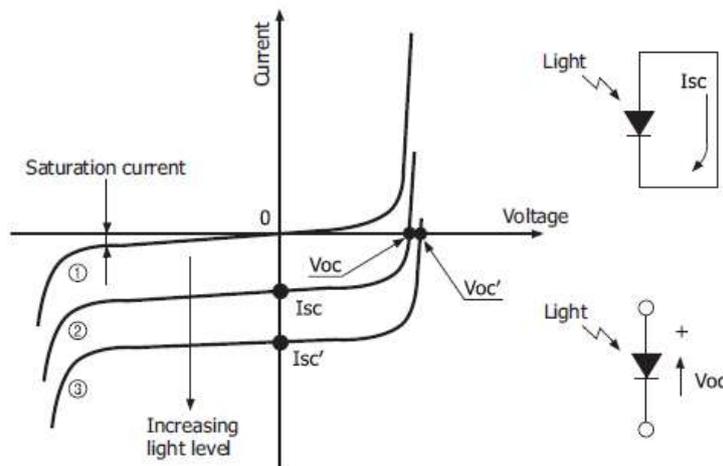


# Hardware

## Reading the Photodiode Signal

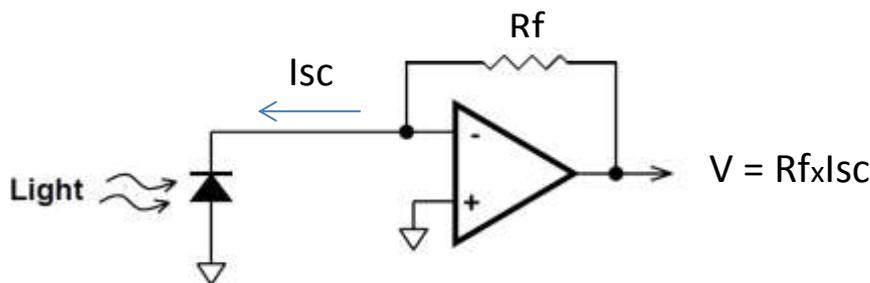
The hardware portion of the DNA Meter is constituted by the amplifier of the photodiode signal, the excitation LED drive circuit and the section which supplies the stabilized **3.3V** voltage for the operational amplifiers power supply. Everything is powered from the 5V supplied by Theremino Master board. The analog signal proportional to the brightness detected by the photodiode is sent to an **analog input** and then converted to digital and sent to the PC . Similarly, the driving signal of the excitation LED is produced by a digital output directly managed by the software.

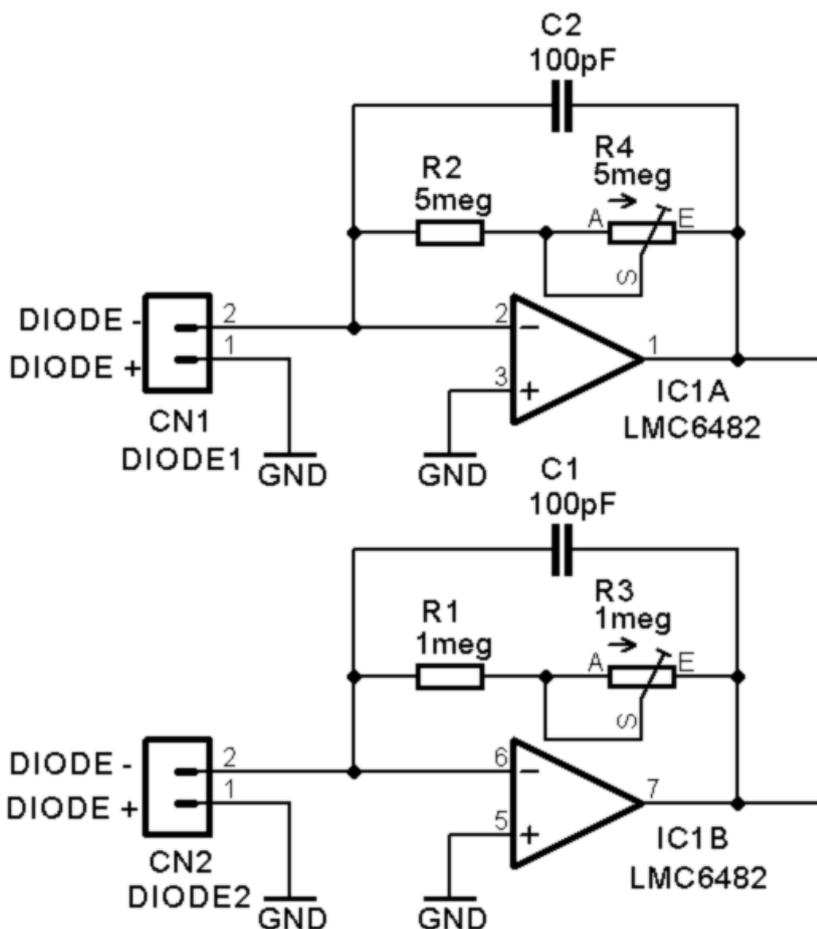
The heart of the instrument is the photodiode. The V - I characteristic is represented in the graphic below in which we see how the curve, in the fourth quadrant of the reverse bias, moves to higher currents in proportion to the light incident on the P- N junction.



To read the photodiode signal it has been chosen a photovoltaic mode configuration in which the anode of the photodiode is connected to ground, while the cathode is connected to the inverting terminal of the operational amplifier, which is also virtually grounded. Thus in the photodiode there is no voltage, and then the operating point moves on the axis of the voltage to zero current (Isc of the above diagram ): in this way the "dark current " is null. The main advantages of this configuration are :

- **Low noise**
- **Good Linearity**
- **High Precision**



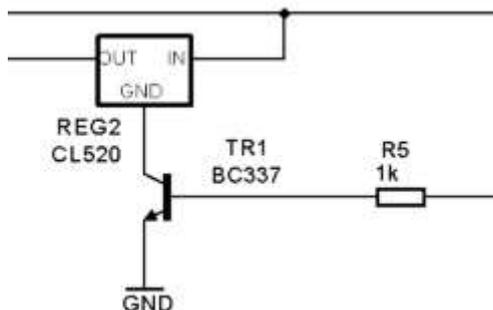
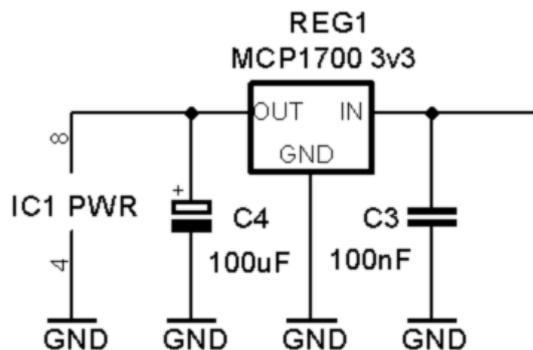


The scheme that has been adopted is shown in the figure on the left. The integrated circuit **LMC6482** includes two operational amplifiers with rail-to-rail input stage CMOS characterized by low current input. The supply voltage is single and has been adopted the value of 3.3V.

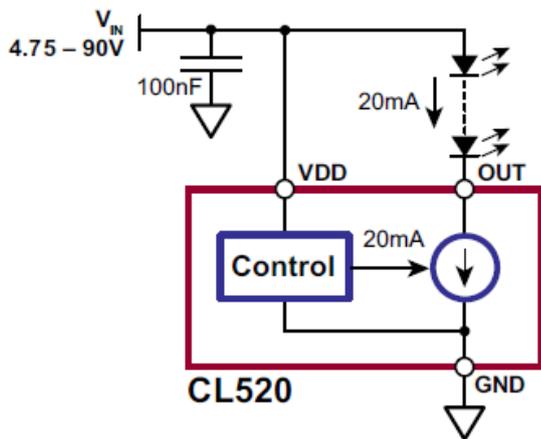
In parallel to the feedback resistor  $R_f$  it was placed a 100pF capacitor with the aim to stabilize the amplifier.  $C_f$  and  $R_f$  constitute a low-pass cell. Since the IC provides two amplifiers, two channels have been prepared: one with middle gain ( $R_f = 1\text{MW}$ ) and the other with a higher gain ( $R_f = 5\text{M}\Omega$ ), in both cases in series with the feedback resistor a trimmer of the same value was placed.

### Power Supply and LED driving

The power supply of the IC LMC6482 is obtained by the MCP1700 regulator that supplies a stabilized voltage of 3.3V. The input of the regulator voltage is 5V and is taken directly by the master board.



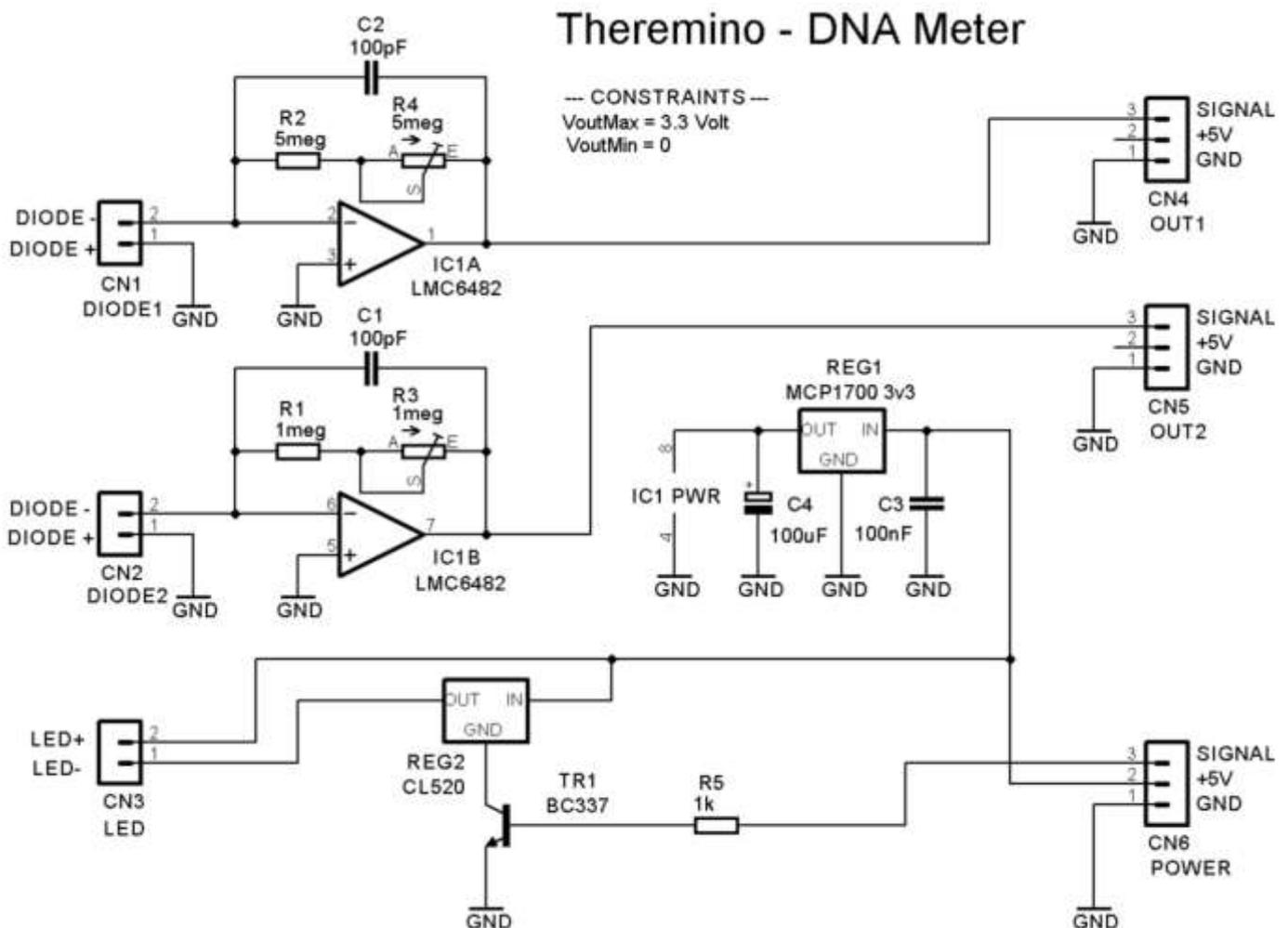
The LED is driven at a constant current so as to maintain constant its light emission, the regulator is the CL520 that provides a constant current of 20mA. The LED on / off is obtained from the BC337 transistors controlled by a digital output of the master board .



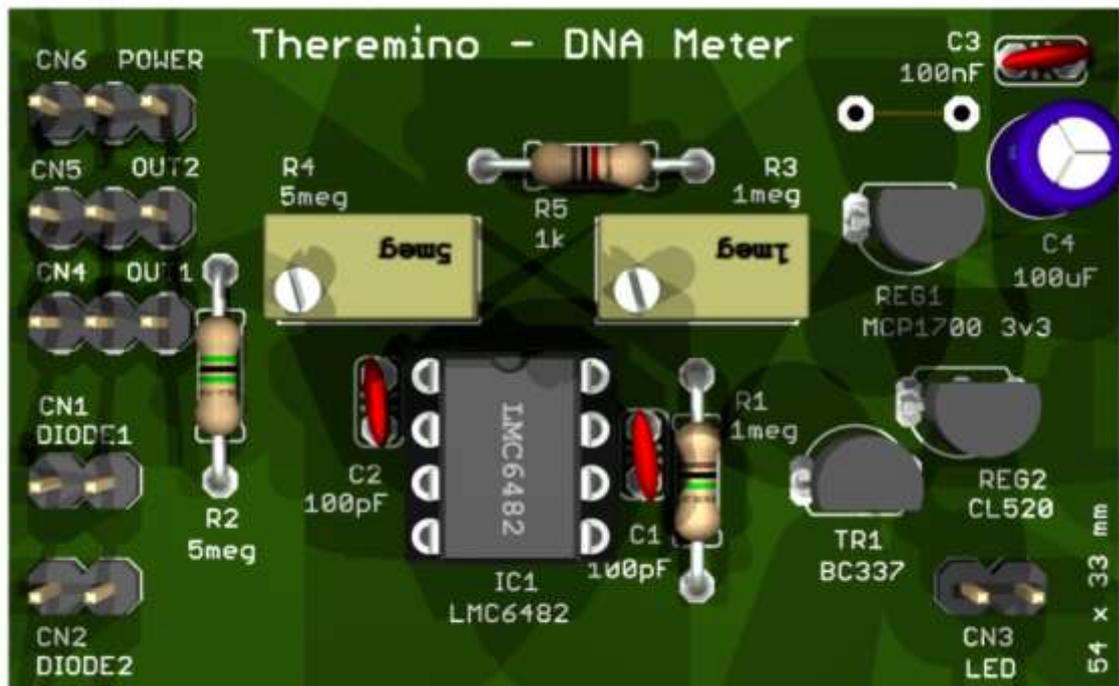
In the diagram to the right is shown schematically the operation of the constant current LED driver CL520.

### PCB and Complete Scheme

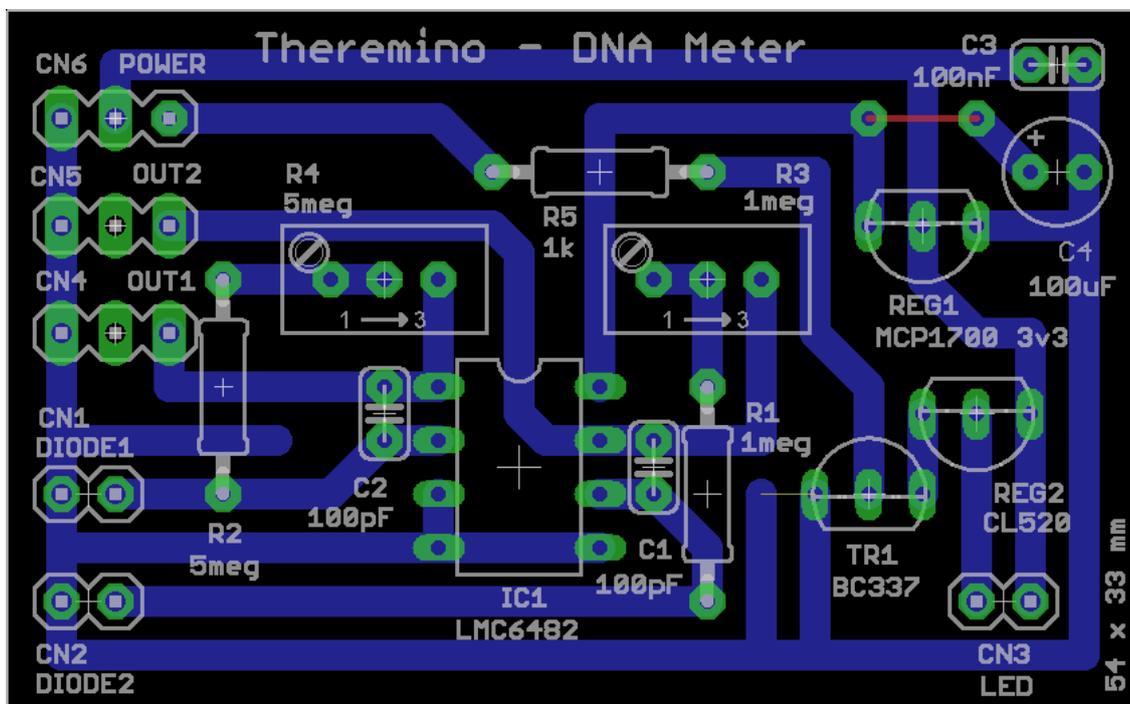
The following figure shows the complete diagram comprising the two channels for the reading of the photodiode signal, the power supply section and the section for the constant-current control of the excitation LED :



PCB image with components :



PCB image with detail of connections :





DNA Meter PCB

PCB image of Theremino Master :



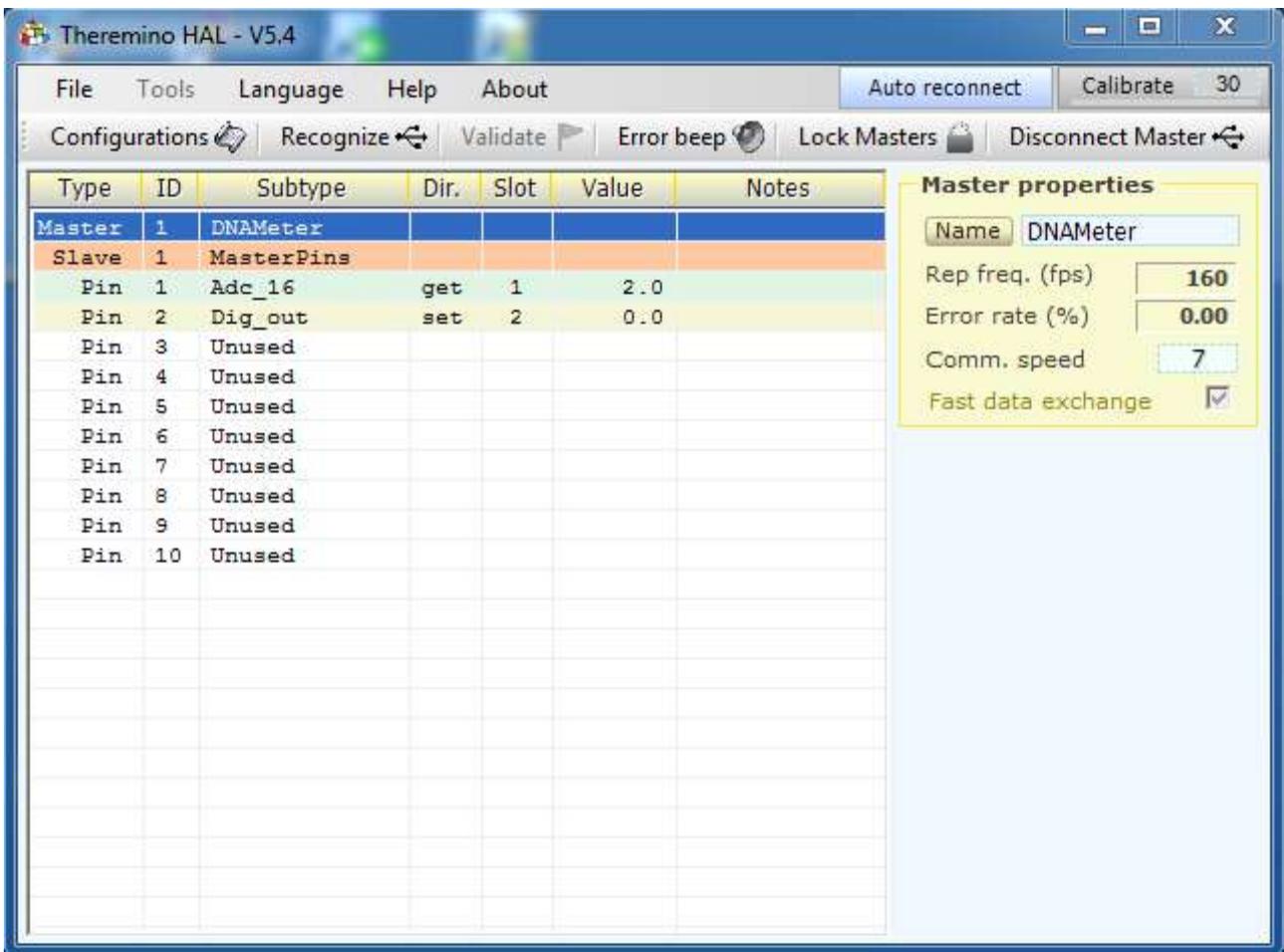
# Software

## Theremino HAL

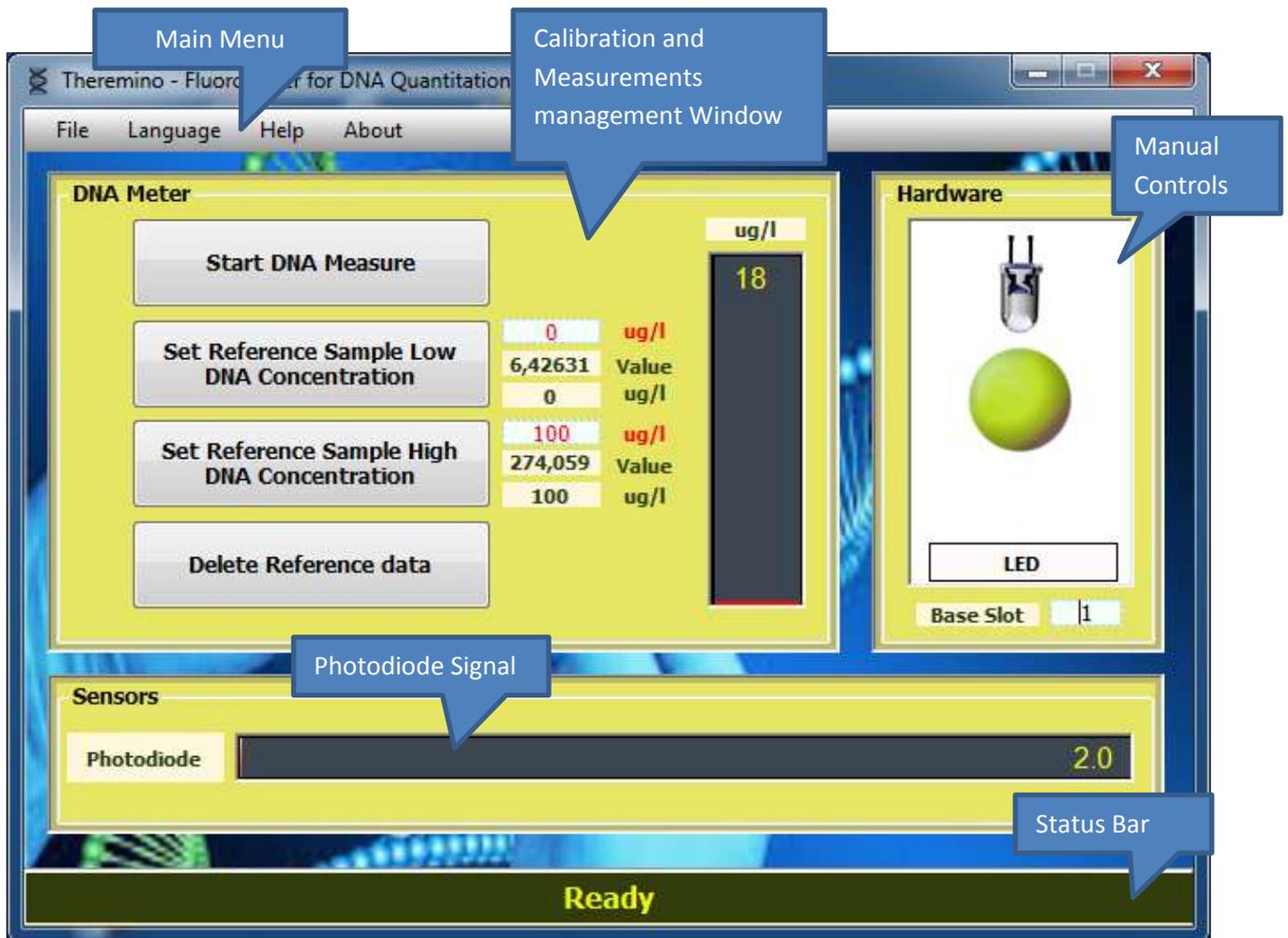
The DNA Meter is based on Theremino software suites. The application that is responsible for communication with the hardware is **Theremino HAL**, whose window is shown in the figure below. HAL takes care of the communication between the PC and the Master. Communication takes place via the USB interface.

HAL is automatically launched when you start the DNA application Meter and has to remain to be active.

As seen in the figure below the **pin 1** is reserved for the reading of the analog signal of the photodiode (16-bit ADC), while the **pin 2** is the digital signal that drives the excitation LED (Digital Output).



## Theremino DNA Meter



In the image above the main windows of the application **DNA Meter** is shown.

### Main Menu :

- **File** : application management;
- **Language** : change the language (Italian, English);
- **Help** : read the documentation;
- **About** : general author information;

**Status Bar** : Displays the status of the application. In particular, the " Ready" message means that the application is ready to perform the measuring operations.

**Manual Controls** : from this window you can turn on / off the excitation LED. It also set to the HAL basic slots on which is sent the signal of the photodiode.

**Photodiode Signal** : This window displays the photodiode signal, with a value from 0 to 1000 and one decimal.

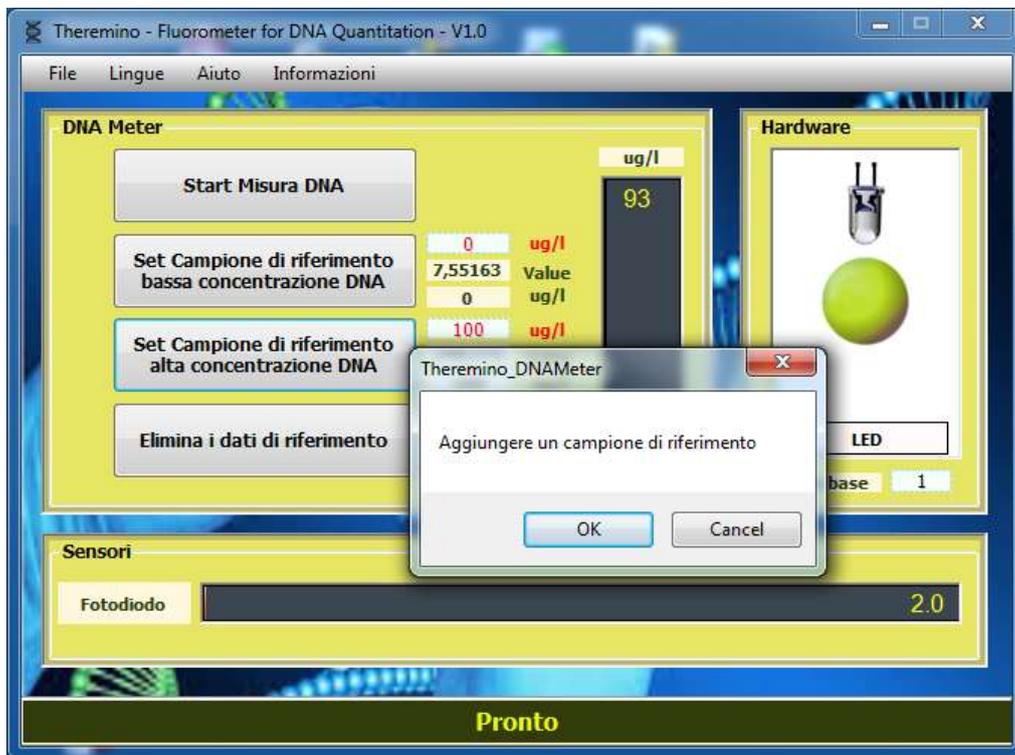
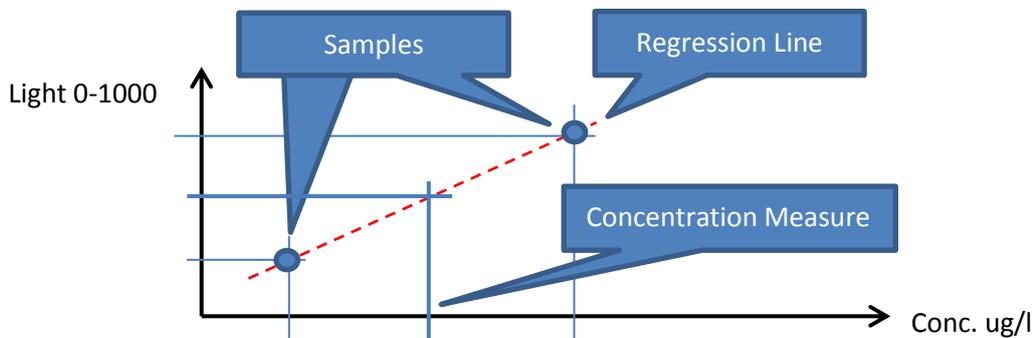
**Management window** : This window includes the main controls for the calibration and the measurement of the concentration of DNA.

## Calibration Procedure

The measurement that is made with the DNA Meter presupposes a **previous calibration** that is carried out with **two samples** : one with low DNA concentration and the other with high concentration of DNA. Both concentrations of the two samples used to calibrate the instrument **must be known**. The known concentration is written in the text box highlighted in red and then insert the cuvette with the sample to make the measurement with the control button.

The data of the reference samples can be deleted with the button indicated. without reference data is not possible to make any measurement.

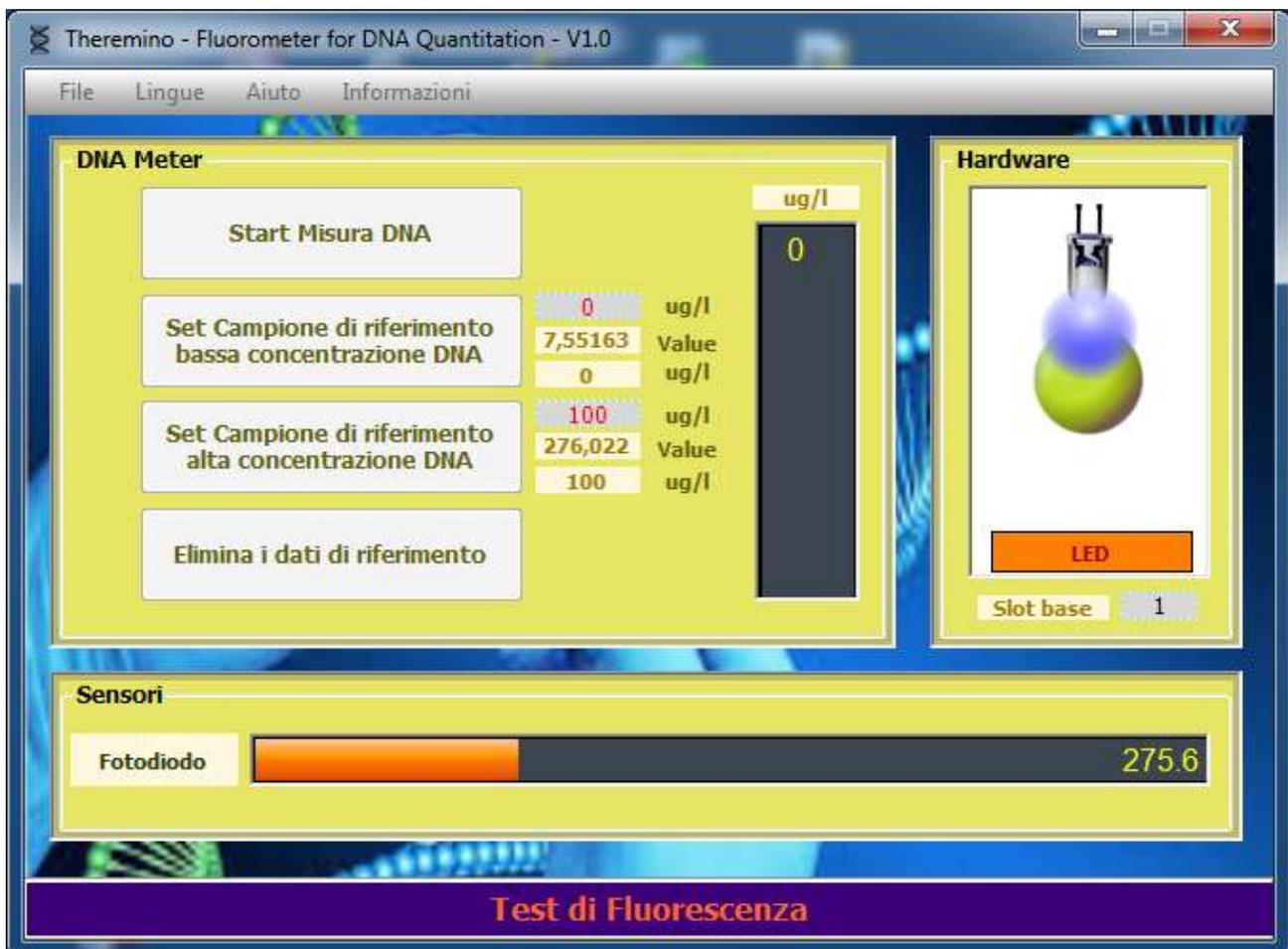
Knowing the concentrations of the reference samples and the corresponding photodiode signal, the software generates the regression line which will serve to determine the DNA concentration of the unknown sample.



In the above image the procedure to add a reference sample is shown

## Measurement procedure

After the calibration procedure has been completed, the instrument is ready to make the concentration measurements. The cuvette containing the sample to be measured is placed in the instrument and the measurement begins with the command "Start DNA Measure". During the measurement the excitation LED is activated and the photodiode signal is acquired. The measurement lasts a couple of seconds, just the time needed to have a stable value. The signal read from the photodiode is presented in the bottom bar, while the DNA concentration value, calculated based on the calibration reference samples, is presented in graphical and numeric form on the vertical bar.



## Manual Procedure

For testing purposes you can also manually activate and deactivate the excitation LED. This is done with the "LED" button located in the right window with the image of the LED graphics. The signal read from the photodiode is presented in the bottom bar, while in this case is not made any calculation of the DNA concentration.

