# OD calibration

## Introduction

The purpose of this experiment is to establish a relation between the Arduino input voltage registered in the photodiode in response to the LED light going through the culture and the optical density (OD) of the culture obtained in the spectrophotometer. Once this transfer function has been obtained the turbidostat can act automatically to maintain the OD into the vial at a fixed level set by the user.

## Materials

- Turbidostat (including the electronics module)
- Turbidostat box
- ~50 ml of LB medium
- ~100 ml of cell culture (cell type does not matter)

## Procedure

First of all, some considerations have to be stated in order to understand how OD is obtained in our turbidostat. It has to be taken into account that the angle between the LED and the photodiode is 135º, avoiding therefore a direct impact of the 600 nm light emitted by the LED in the photocurrent produced by the photodiode. Moreover, in the photodiode that is being used for our turbidostat the photocurrent is directly proportional to the received illuminance. Bearing in mind this information, the photocurrent will depend on the refraction of the emitted light when passing through the culture. As the turbidity of the culture increases, this is the concentration of cells in it is higher, the refraction will increase and subsequently the incident light on the surface of the photodiode will also increase. To sum up, we can conclude that the OD of a certain culture is related with the registered photocurrent in a directly proportional way due to the refraction of light.
Furthermore, before starting the calibration some setup has to be done. Since the 100 ml cell culture grown after two overnights has an OD way higher than 1 (1.9 in our case) which we are not interested about, it has to be diluted before starting with the experiment, this dilution will depend on the value obtained at the spectrophotometer and will aim to obtain a sample of about 1 point of OD. Finally, a vial containing 14 ml (to know the reason of the selected volume check *Volume effect* document) of this first dilution (which in our case was 60/40 entailing an OD of ~1) is introduced into the turbidostat. Before starting the measurements, the turbidostat is covered with its box to avoid external visible light falling upon the photodiode and disturbing its performance.

Once everything is prepared the process of calibration can start. Firstly, the fan is switched on for a while to homogenize the sample. Then it is turned off and the OD LED (600 nm of wavelength) and the photodiode (300-820 nm of detection range) are turned on during 10 seconds. One value per second is registered at the Arduino input pin connected with the photodiode, leading thus to the registration of ten values per sample, each of these values can go from 0 to 1023 which is the same as from 0 to 5 V. Afterwards, the average of these ten values is calculated and taken as the representative value for that sample, being then associated with the real OD measured at the spectrophotometer. This process is performed for each dilution until a proper characterization of the behaviour of the turbidostat is achieved, yielding a table that correlates the OD at the spectrophotometer and the mean value at the Arduino input pin. As many dilutions as needed can be done, ours can be appreciated in the next section, *Results*. It also has to be taken into account that in order to calculate the intra-variability of the turbidostat two samples are prepared for each dilution.

At last, a scatter plot of Arduino input versus OD is drawn in Excel, where linear regression is performed to establish a transfer function. The inverse of this transfer function is computed, yielding a function that correlates OD with respect to Arduino input voltage, which will be introduced into the final code of the turbidostat so that it can work automatically.

## Results

| Dilution | OD | Arduino INPUT pin |
|---|---|---|
| *BLANK 1* | 0 | 292,7 |
| *BLANK 2* | 0 | 288,5 |
| *"2/98" (1)* | 0,208 | 333,8 |
| *"2/98" (2)* | 0,201 | 339,4 |
| *"5/95" (1)* | 0,261 | 370 |
| *"5/95" (2)* | 0,258 | 361,2 |
| *"10/90" (1)* | 0,346 | 401,9 |
| *"10/90" (2)* | 0,337 | 391,3 |
| *15/85 (1)* | 0,37 | 413,9 |
| *15/85 (2)* | 0,368 | 416,5 |
| *20/80 (1)* | 0,466 | 415,7 |
| *20/80 (2)* | 0,467 | 429,4 |
| *25/75 (1)* | 0,617 | 460,7 |
| *25/75 (2)* | 0,597 | 474,3 |
| *30/70 (1)* | 0,727 | 478 |
| *30/70 (2)* | 0,713 | 475,1 |
| *50/50 (1)* | 1,155 | 497,4 |
| *50/50 (2)* | 1,129 | 501,1 |
| *60/40 (1)* | 1,053 | 508,5 |

*Table1: performed dilutions and their results, both in the spectrophotometer and in the Arduino input pin.*

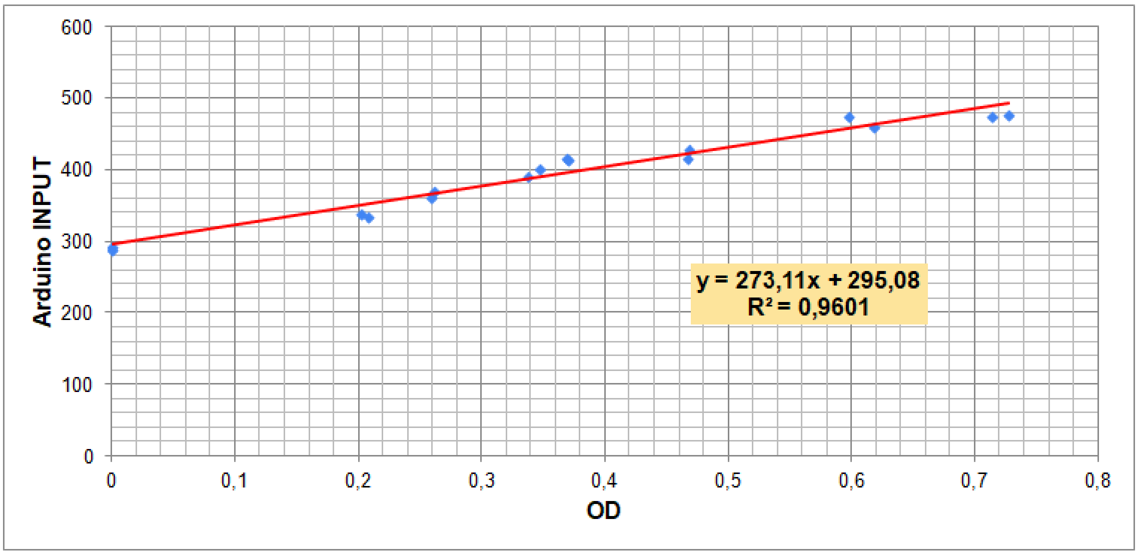


*Figure 1. Linear regression of the obtained data.*

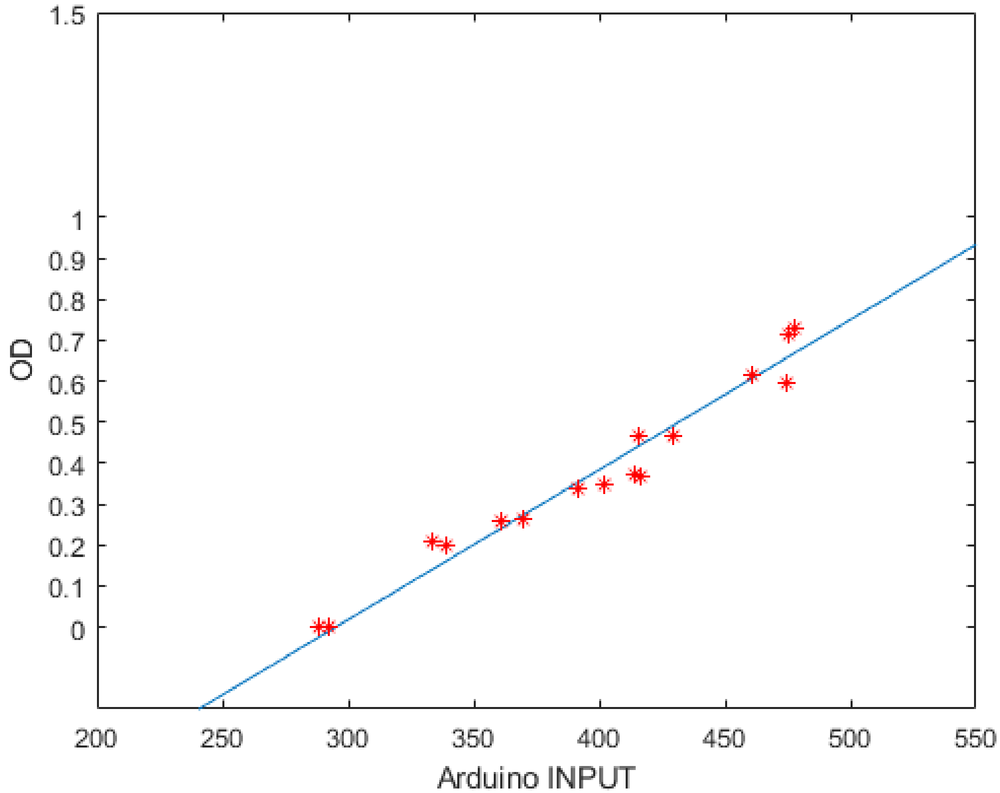


*Figure 2. Graph showing the inverse function (OD with respect to Arduino INPUT) to the previously attached in Figure 1.*