HTGAA Final Project

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Abstract

Directed evolution is a process in which organisms are exposed to selective pressure for them to become more performing in a certain aspect. In this project we describe a device for the automated mutation of bacteria and detection of the best strains. The machine is capable of measuring chromophore concentrations using a spectrophotometer in order to select samples with the highest expression.

Introduction

The rapid and efficient mutation of bacterial strains is a topic of vital interest for industry, as it could be used to optimize a variety of manufacturing processes when coupled with

mechanisms for rapid selection. Hackuarium has been involved in this endeavor for about a year. The core concept behind the device ties in very well to the synthetic biology concepts given during some of the HTGAA lectures, in particular with the lectures on bio design, bio production and genome engineering which also cover some top down approaches to optimization problems.

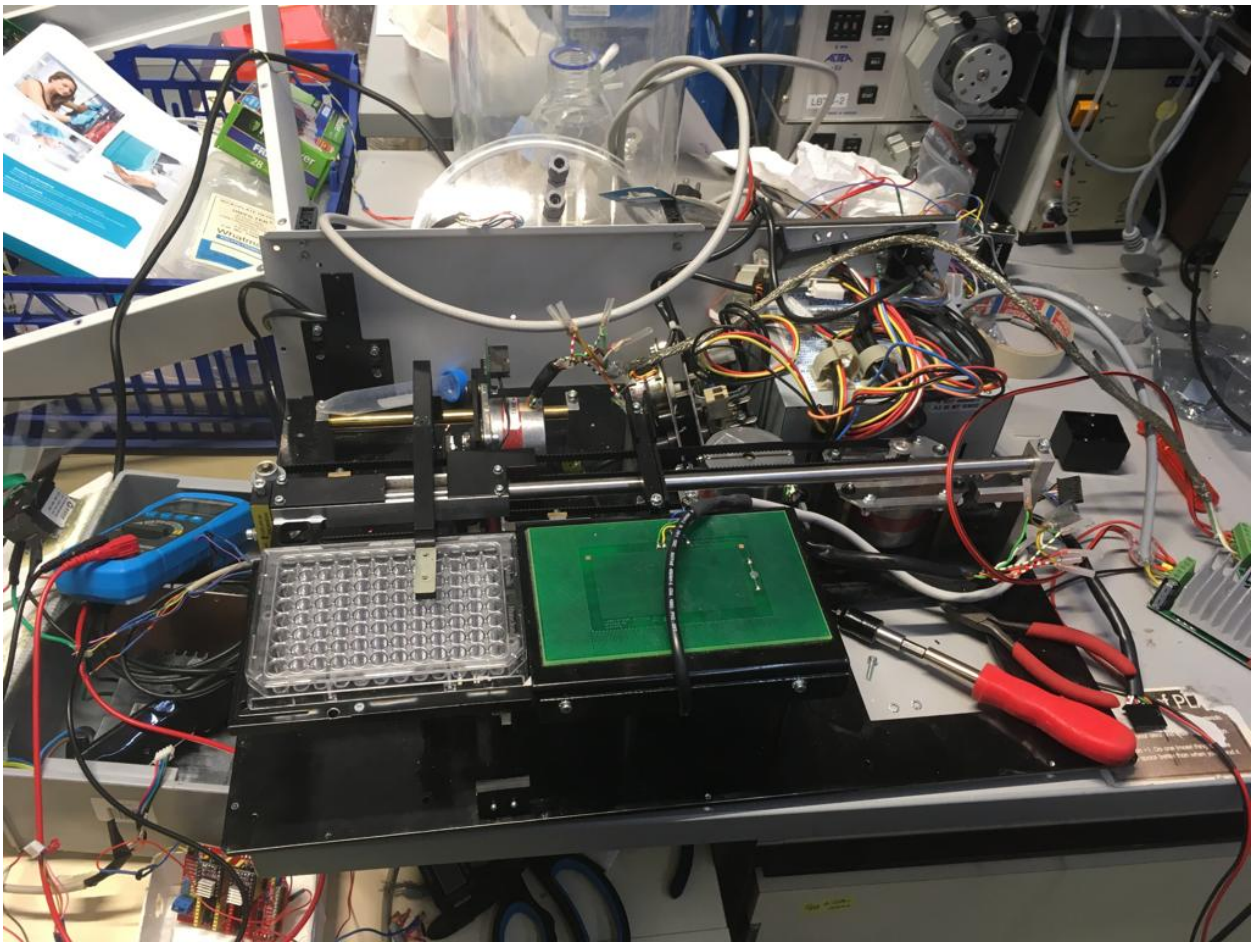

Figure 1: Overview of the machine, note that the spectrophotometer and pipettor are not yet present on the arm

Setup

The chassis for the apparatus is an old plate reader, the moving arms were kept and modified. The arms are moved by step motors and those are in turn controlled by drivers connected

to Arduinos. Optical fibers connected to a spectrophotometer were used for the detection of the samples with the information being fed to a raspberry pi. The raspberry pi controls the entire process using python scripts.

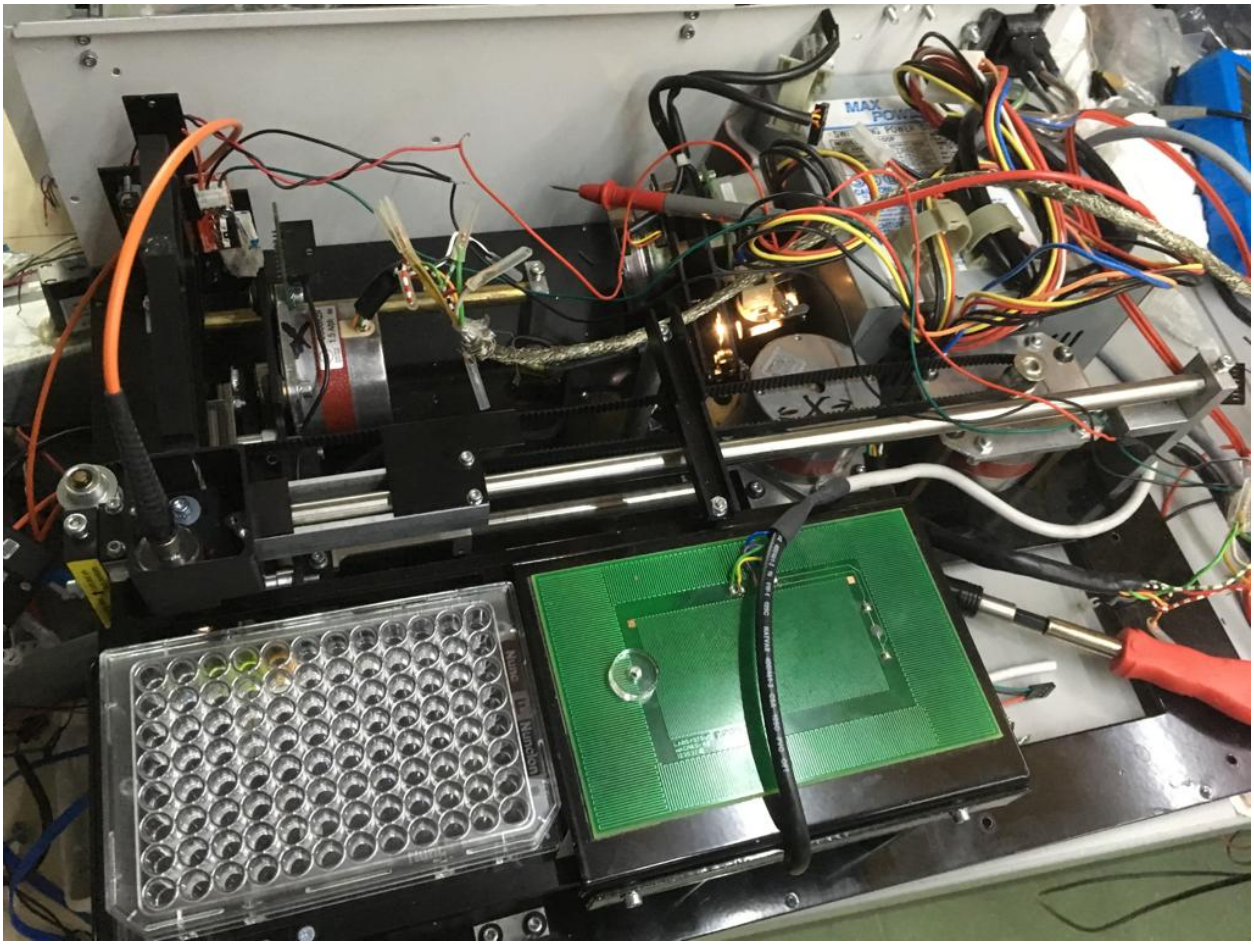

Figure 2: Top view of the apparatus with the spectrophotometer attached

In silico Modeling Coupled to Adaptive Laboratory Evolution for the generation of Cell Factories

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What is the Motivation?

To speed up and drive Cell Factory creation.

We propose that by Combining Modeling and Natural selection we can speed up the phenotype optimization process

What is In silico Metabolic Modeling?

A computational tool to understand metabolism.

It is a workflow which allows the integration of experimental data, collects all known information on the strain, and mathematical analysis tools to guide hypothesis generation and design in the face of large uncertainty situations

Why Adaptive Laboratory Evolution?

It allows evolution of the production strain in the laboratory. Evolution is necessary:

- Working with cells involves very high complexity and uncertainty
- We can rationalize the main components, but the fine tuning is a mystery
- Even the perfect strain it will be out competed / have stability issues

Conditions for Evolution

If you have
- Variability
- Selection and
- Heredability

Then you must have evolution.
You get what you select for

Rational Design

Fine tuning Expression and Regulatory factors

- Identify Factors that could be limiting and search interaction networks for potential targets

Molecule, Pathway and Host evaluation

- Identify target compound
- Identify biochemical routes
  - Novel heterologous pathways
- Choose host(s)
- Screen for enzymes
  - Enzyme Engineering
- Evaluate and Rank

Strain Design

- Genome-scale Metabolic Reconstructions
- Metabolic Flux Analysis
- Thermodynamic information
- Kinetic Models
- Metabolic Control Analysis

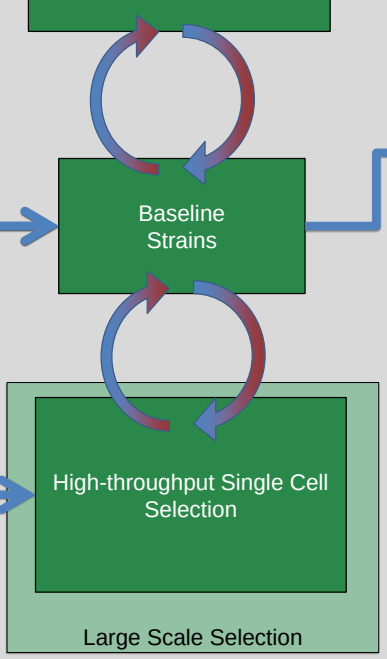
Optimal Phenotype Indicators

In addition to product formation, other indicators may be used to select cells e.g. metabolomic levels

Single Cell Selection

- Can we maintain a constant selective pressure?
- You get what you select for
- Can we do it at a fine enough resolution to distinguish high performers from low

Evolutionary cycle Applied to Industrial Biotechnology Cells



Coupled Modules

- Together, these modules speed up the process of natural selection
- The Selection Module combines microfluidics and micro-sensors to select individuals with favorable mutations

Data Analysis and Integration

Genome-scale Metabolic Reconstruction

- Omics Integration

Experimental Measurements

- transcriptomics, proteomics, metabolomics, fluxomics, regulomics, GPR, can be integrated in a model.
- Genomics must track the evolutionary changes

Data Analysis

- Design of Experiment
- Biostatistics
- Bioinformatics

Optimal Bioprocess conditions

Modeling can be used to define optimal conditions to initialize

Microfluidic Scale

- Microfluidic system separates cells spatially and in time
- Micro-sensors measures phenotype of one(few) cells
- Keep or trash cells according to phenotype read out
- End result is selective pressure directly on desired phenotype
- Parallelization allows unprecedented throughput

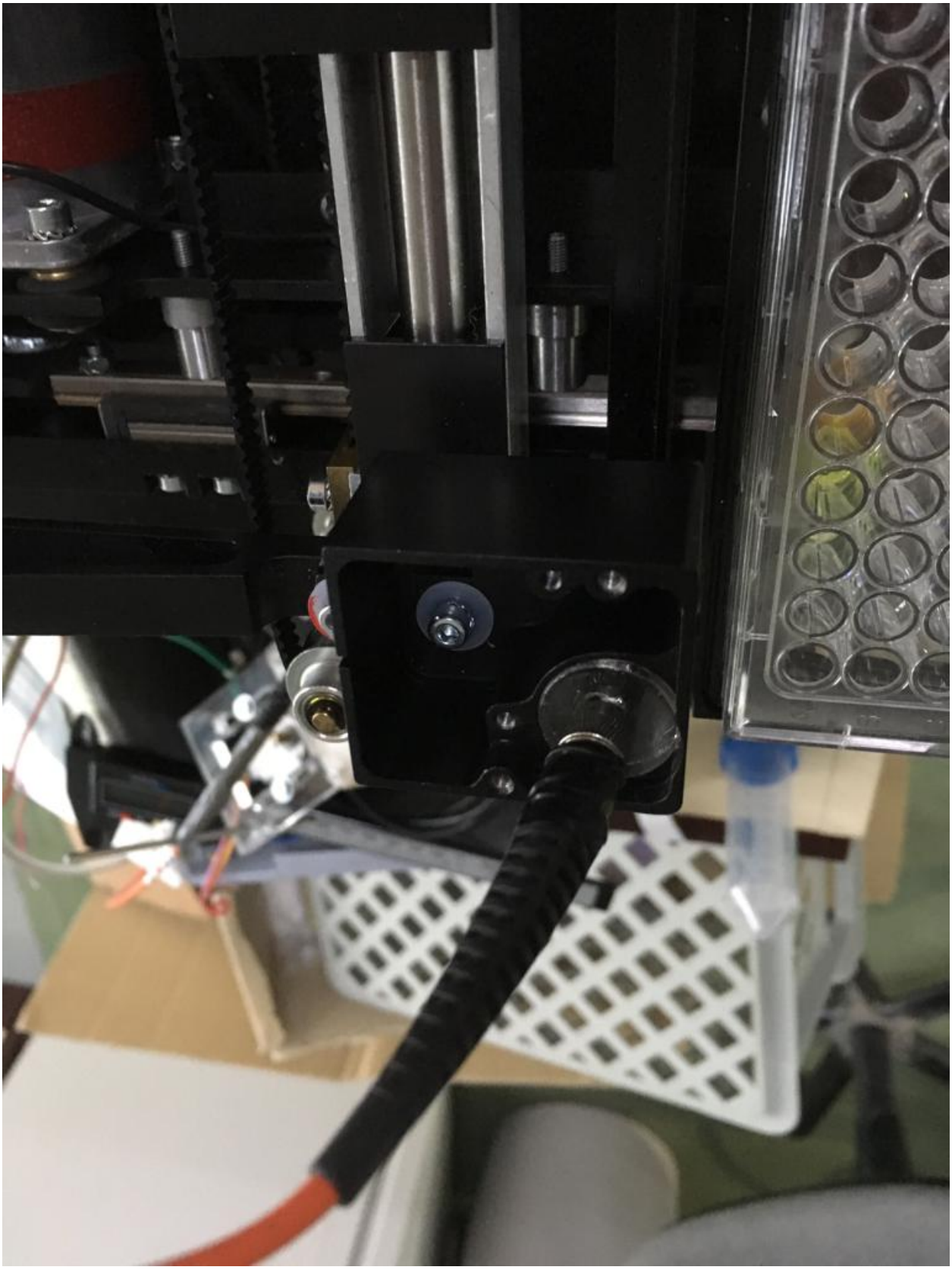


Figure 3: Closeup of the spectrophotometer head

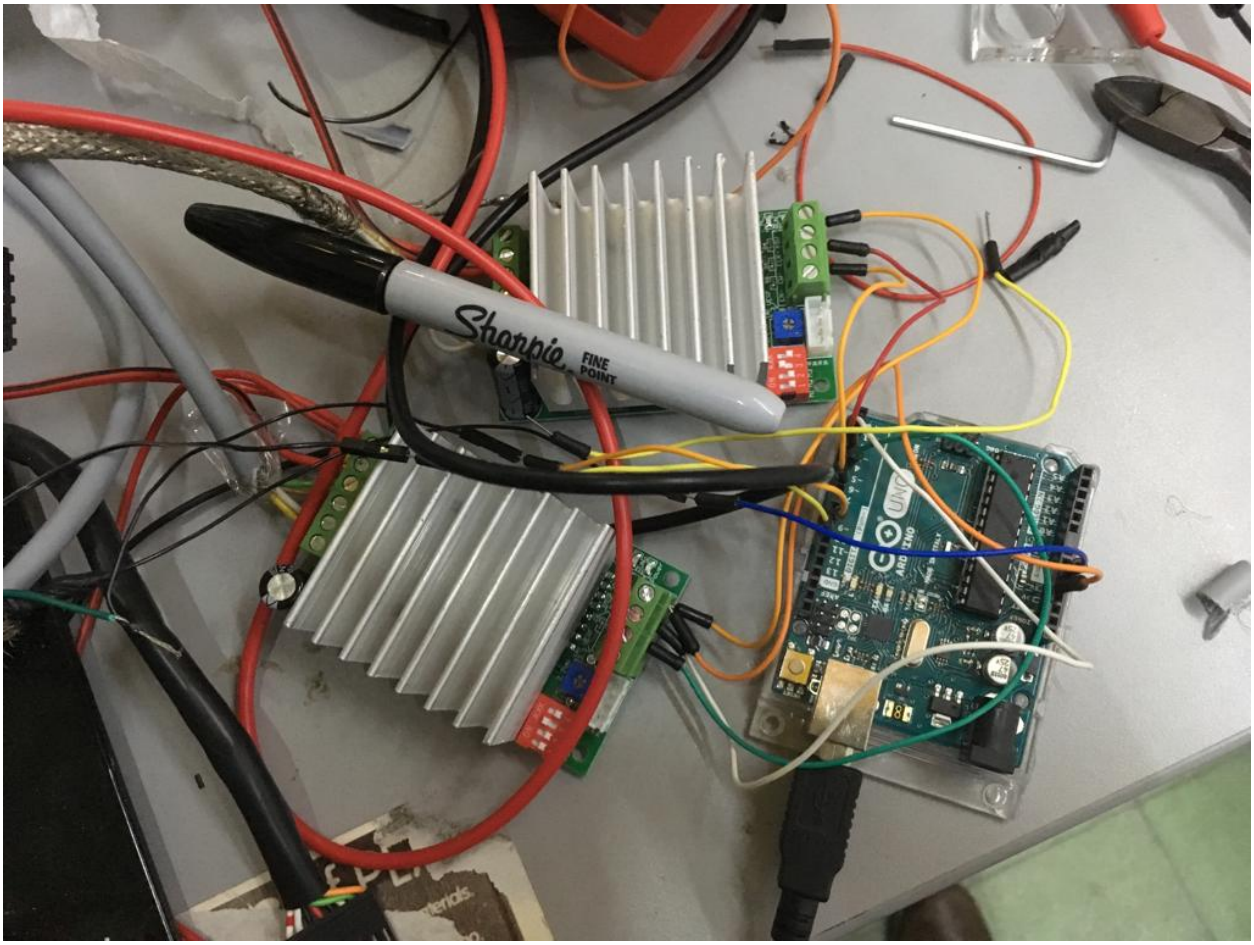


Figure 4: The computing equipment controlling the device

Results and future implementations

The machine is able to autonomously collect data on the different cell populations using the spectrophotometer. The pipettor has been proven to work as a proof of concept. It will still take quite some time until the plates are filled automatically and the mutation process is implemented to work by itself. Additionally it would be very interesting to have the plates loaded into the apparatus automatically so that a larger quantity of bacteria can be examined in parallel.

The experiments performed consisted of detecting the evolution of orange and yellow bacterium strains over a period of seven hours where the spectrophotometer took measurements repeatedly. The evolution of the bacterial population was observed corresponding to an exponential decay in the transmittance at maximum absorption wavelength (Figure6).

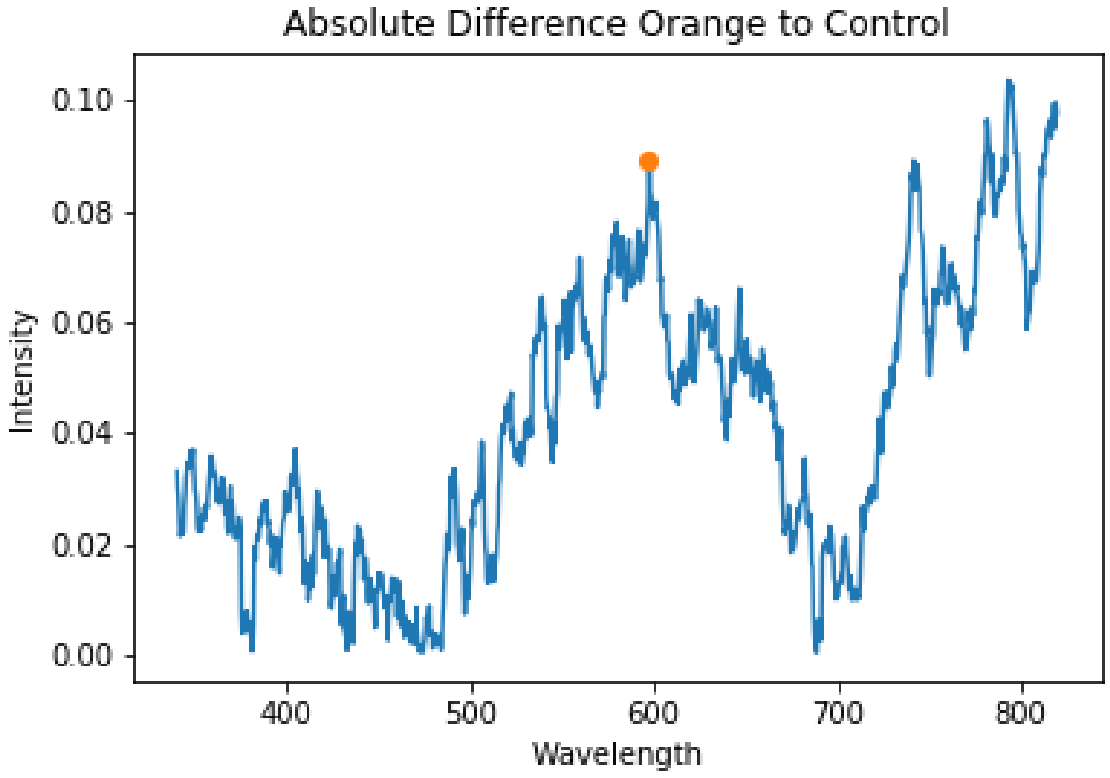


Figure 5: The absorbance of the orange pigment producing bacteria, note maximum at 597nm

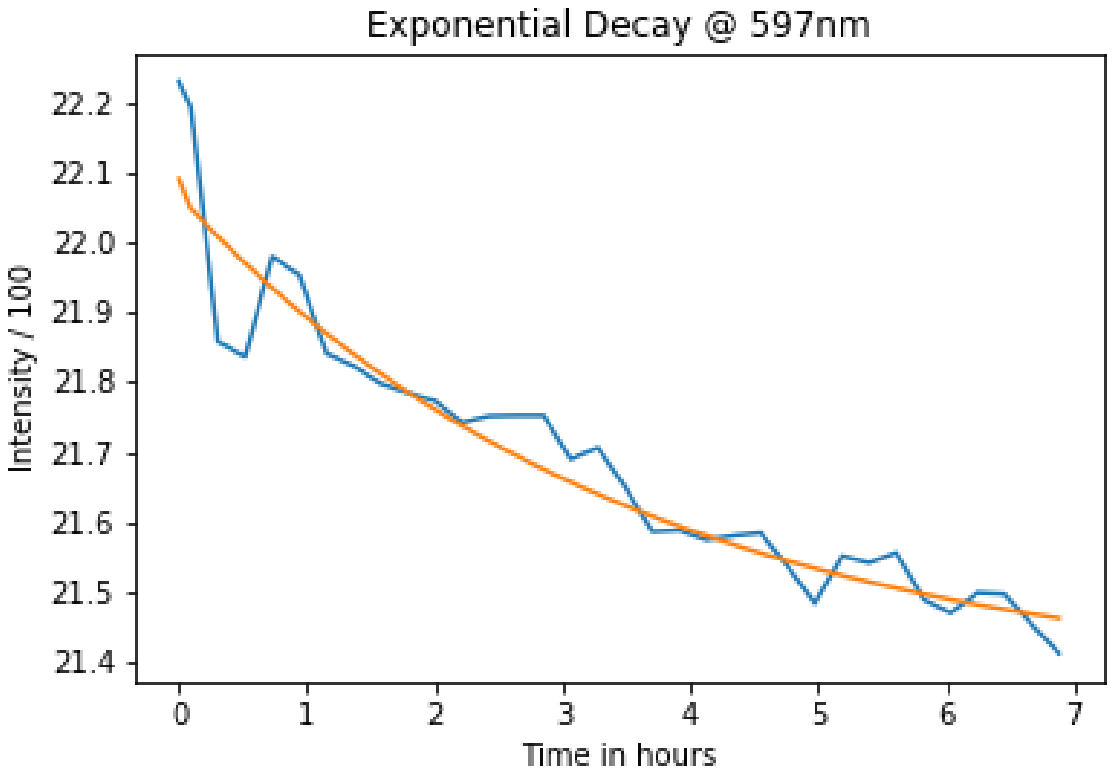


Figure 6: The exponential decay measured by the device of a population of pigmented cells