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## Smartphone-Based Optical Fiber Sensor for the Assessment of a Fed-Batch Bioreactor

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# 1. Introduction

## Industry 4.0:

- Application of novel mathematical and computer-based methods for the **optimization and monitoring of systems**, with social, economic, and environmental repercussions on the activities.
- This new period relies on the **development of new sensor technologies** capable of collecting, distributing, and delivering information by themselves.



# 1. Introduction

## Industry 4.0 in Chemical and Biochemical Industries:

- In such industries, **the increase on the data availability and of the portability of the monitoring devices** has potential for enhancing:
  - Safety;
  - Productivity;
  - Energy-use efficiency;
  - Environmental sustainability;
  - Product quality;
  - General process performance.



# 1. Introduction – Chemical and Biochemical Assessment

- Still based on:
  - High-performance liquid chromatography (HPLC);
  - Gas chromatography (GC);
  - Coupled techniques like GC coupled to mass spectrometry (GC-MS) and enzyme-linked immunosorbent assay (ELISA).



UV-Vis spectrophotometer



HPLC system (liquid chromatograph)



Centrifuge



# 1. Introduction – Chemical and Biochemical Assessment

Such methods are sensitive, reliable, and precise, but:

- Demand expensive and bulky instrumentation;
- Highly trained technicians;
- Procedures that require a long analysis time.

***The use of compact, real-time sensors allows the monitoring, control, and screening of the best process conditions.***



# 1. Introduction – Chemical and Biochemical Assessment

**Optical Fiber Sensors (OFSs) on Chemical and Biochemical systems: many advantages**

- Reduced mass;
- Low energy costs;
- Resistance to electromagnetic interference;
- Remote sensing capability;
- High environmental resistance, being inert to both chemical and biological agents;
- Reduced fabrication costs, making the **sensors suitable for mass-fabrication.**



# 1. Introduction – Approach of this Work

***A portable smartphone-based optical fiber sensing platform is designed for the monitoring of fed-batch fermentation systems.***

- The fed-batch mode was chosen for the study because *most of the alcoholic fermentation industrial systems in Brazil operate with this methodology.*
- The results were validated by:
  - 1) Comparison with a handheld refractometer;
  - 2) Comparison with the mathematical model.



## 2. Fermentation Monitoring and Modelling

- Monitoring and control focused on the **maintenance of the adequate conditions for the microorganisms.**
- It is based on the evaluation of the *cells' concentration X*.
- **Many of the traditional measurements are usually based on manual time-consuming procedures, e.g:**
  - *Counting with Neubauer chamber;*
  - *Dry mass evaluation;*
  - *Surface plating method to determine viable cell number.*





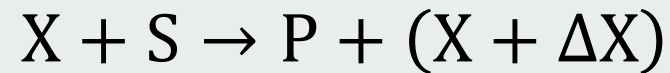
## 2. Fermentation Monitoring and Modelling

- In most of the practical and industrial cases, *the measurement and control are actually based on the quantification of a specific property*, which may be either:
  - Physical (e.g., variation of the medium's refractive index, viscosity, or electrical conductivity); or
  - Biochemical (concentration of proteins, carbohydrates, DNA or RNA, for example).

These properties are posteriorly ***correlated to the concentration of cells by an appropriate model derived from the general fermentation reaction.***



## 2. General Fermentation Reaction



$X$  = Concentration of Cells -  $\text{g}\cdot\text{L}^{-1}$

$S$  = Concentration of Substrate (e.g., Sucrose) -  $\text{g}\cdot\text{L}^{-1}$

$P$  = Concentration of Product (e.g., Ethanol) -  $\text{g}\cdot\text{L}^{-1}$

***Autocatalytic Process***



## 2. Fermentation Model Applied: Monod Equation

$$\mu = \frac{1}{X} \frac{dX}{dt}$$

$$\mu = (\mu_m) \frac{S}{K_M + S}$$

$$q_p = Y_{P/X} \mu + m_p$$

$$\mu_s = \frac{1}{Y_{X/S}} \mu + m_s$$

$t$  = Time from the Beginning of Fermentation - h

$\mu$  = Specific Growth Rate -  $h^{-1}$

$\mu_m$  = Constant of Maximum Specific Growth Rate -  $h^{-1}$

$K_M$  = Monod Constant -  $g \cdot L^{-1}$

$q_p$  = Specific Product Formation Rate -  $h^{-1}$

$Y_{P/X}$  = Theoretical Yield of Product Formation per Cell  
Reproduction - g/g

$m_p$  = Product Formation Rate Not Associated to Cell  
Growth -  $h^{-1}$

$\mu_s$  = Specific Substrate Consumption Rate -  $h^{-1}$

$Y_{X/S}$  = Theoretical Yield of Cell Reproduction per  
Substrate Uptake - g/g

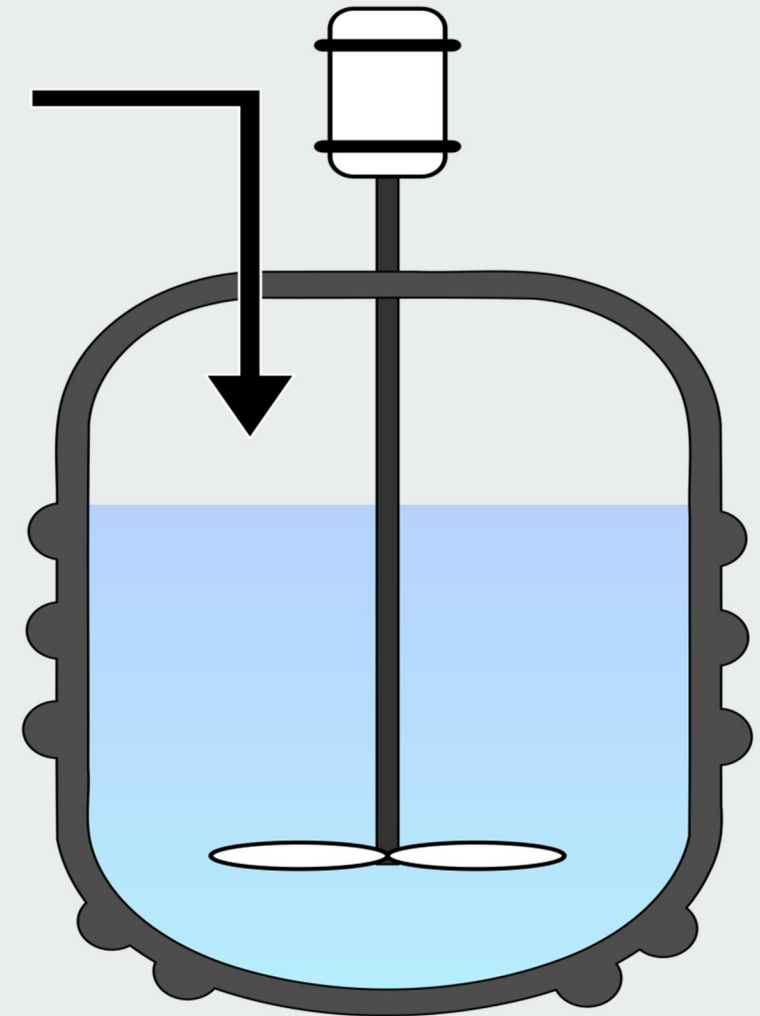
$m_s$  = Substrate Consumption Rate Associated to  
Metabolic Activities -  $h^{-1}$



## 2. Fed-Batch Reactor

- On fed-batch operation mode, the reaction is started with:
  - Initial concentrations  $X_0$ ,  $P_0$  and  $S_0$ , ( $P_0$  is usually zero);
  - An initial volume of fermentation broth  $V_0$ .

***A constant feed flow  $F$  supplies the reactor with an aqueous solution of fresh substrate (feed solution concentration:  $S_F$ ).***





## 2. Fed-Batch Differential Equations

$$\frac{dV}{dt} = F \Rightarrow V = V_0 + Ft$$

$$\frac{dX}{dt} = \mu X - \frac{F}{V} X$$

$$\frac{dP}{dt} = q_p X - \frac{F}{V} P$$

$$\frac{dS}{dt} = -\mu_s X + \frac{F}{V} (S_F - S)$$

$X_0, P_0, S_0$  = Initial Concentrations on Fed-Batch Operation -  $g \cdot L^{-1}$

$V_0$  = Initial Volume of Fermentation Broth on Fed-Batch Operation - L

$V$  = Instant Volume of Fermentation Broth – L

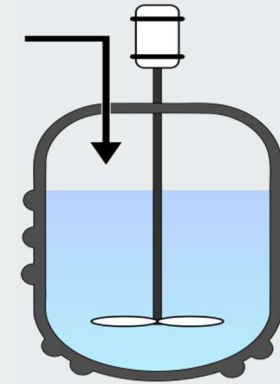
$F$  = Feed Flow -  $L \cdot h^{-1}$

$S_F$  = Concentration of Substrate in Feed Flow -  $g \cdot L^{-1}$



## 2. Working Conditions

- *Saccharomyces cerevisiae* ATCC 7754 cells were cultivated in yeast-peptone-dextrose (YPD) medium (yeast extract, peptone and dextrose with concentrations in proportions of 1:2:2)
- pH =  $6.5 \pm 0.2$ .
- Feed solution: sucrose dissolved in deionized (DI) water ( $S_F = 30.0 \text{ g.L}^{-1}$ ).
- Fed performed with a peristaltic pump.



Reactor design: high ratio of surface area per liquid column height.

- Surface kept free to the atmospheric air and under magnetic stirring, ***guaranteeing the saturation of the liquid medium with air.***
- Aerobic conditions: cell reproduction is favored over the ethanol production.



## 2. Working Conditions

Equation Parameter	Value for Working Temperature (33 °C)
$X_0$	1.5 g.L <sup>-1</sup>
$P_0$	0
$S_0$	10.0 g.L <sup>-1</sup>
$V_0$	0.1 L
$\mu_m$	0.49 h <sup>-1</sup>
$K_m$	4.1 g.L <sup>-1</sup>
$Y_{P/X}$	2.660 g.g <sup>-1</sup>
$m_p$	0.010 h <sup>-1</sup>
$Y_{X/S}$	0.2880 g.g <sup>-1</sup>
$m_s$	0.290 h <sup>-1</sup>
V (Maximum)	2 L
F	6.67 x 10 <sup>-2</sup> L.h <sup>-1</sup>
$S_F$	30.0 g.L <sup>-1</sup>

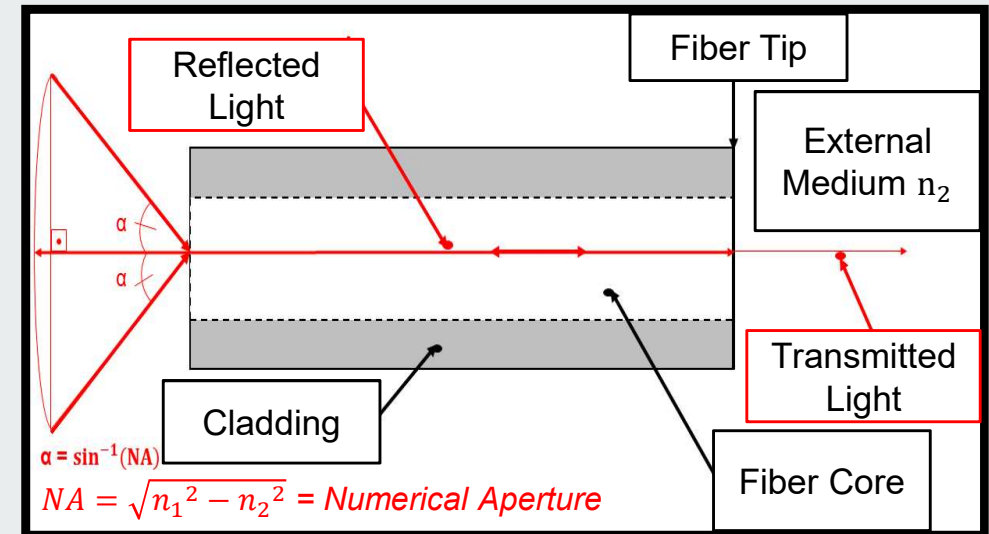
Soares *et al.*, *Sensors* **2019**, 19, 2493. doi:10.3390/s19112493

Soares *et al.*, *Blucher Chem. Eng. Proc.* **2018**, 1, 2010-2014. doi: 10.5151/cobeq2018-PT.0532



## 2. Optical Fiber Smartphone Sensor

- The optical fiber sensor is based on the modulation of the power reflectance caused by differences in the refractive index of the liquid medium.
- These differences are originated by the consumption of sucrose by the cells.
- Refractive index (RI) of sucrose is higher than water's - the substrate uptake lowers the medium's RI.



(Light being transmitted, refracted in an external medium and reflected back. Reflected intensity modulated by Fresnel Law).

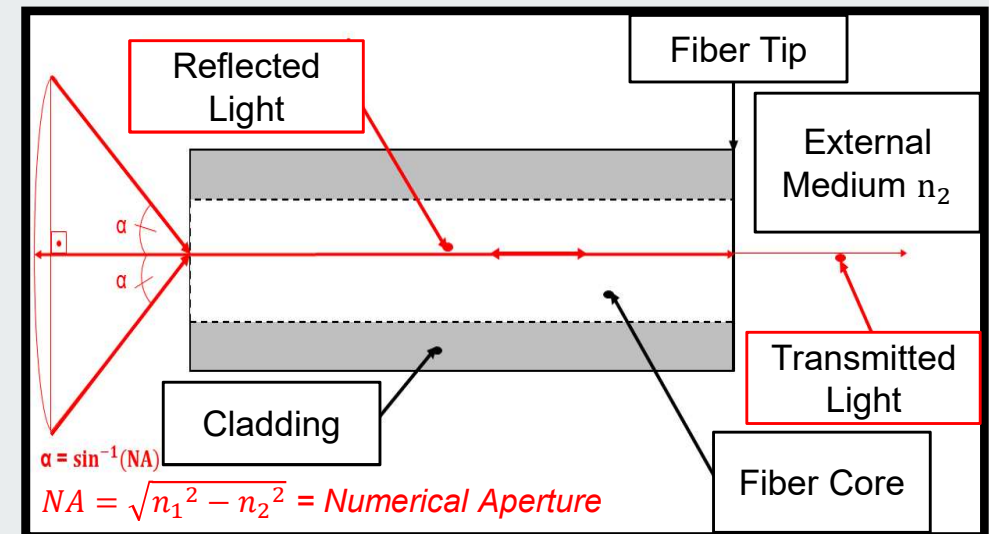
M.C.P. Soares, *School of Mechanical Engineering (University of Campinas, SP, Brazil)*, 2020. doi: 10.13140/RG.2.2.35146.93122/1





## 2. Optical Fiber Smartphone Sensor

- Light is launched by a LED source (the smartphone's camera LED or an external source) through a multi-mode silica optical fiber (MMF) and it is directed by a 2x1 coupler to the liquid medium.
- When light reaches the fiber-liquid interface, part of it is transmitted and part is reflected (**intensity modulated in accordance with Fresnel law**).
- Reflected light is redirected by the coupler to the smartphone's camera, and a developed application acquires and processes the data.



(Light being transmitted, refracted in an external medium and reflected back. Reflected intensity modulated by Fresnel Law).

M.C.P. Soares, *School of Mechanical Engineering (University of Campinas, SP, Brazil)*, 2020. doi: 10.13140/RG.2.2.35146.93122/1



## 2. Fresnel Law

$$R = \frac{I_R}{I_0} = k_0 \left[ \frac{(n_1 - n_2)}{(n_1 + n_2)} \right]^2$$

$R$  = Power Reflectance

$I_R$  = Normalized Reflected Intensity Signal

$I_0$  = Normalized Reference Signal (light source)

$k_0$  = Correction Constant (accounting the power losses in the optoelectronic system)

$n_1$  = Fiber's Refractive Index (constant)

$n_2$  = Medium's Refractive Index



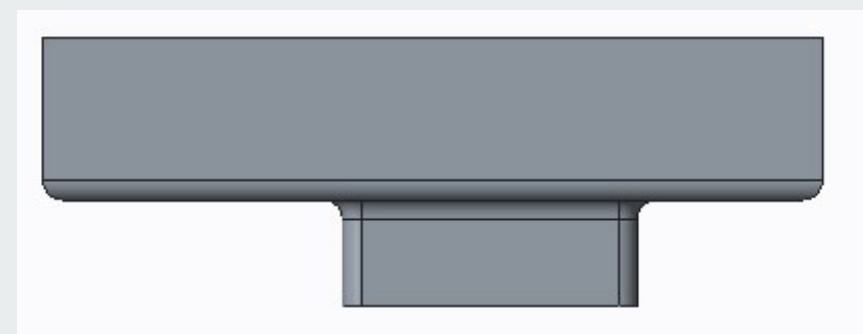
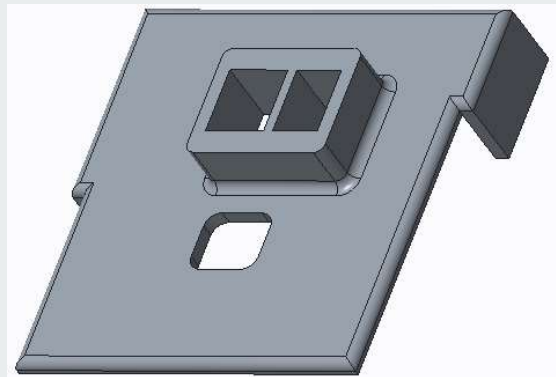
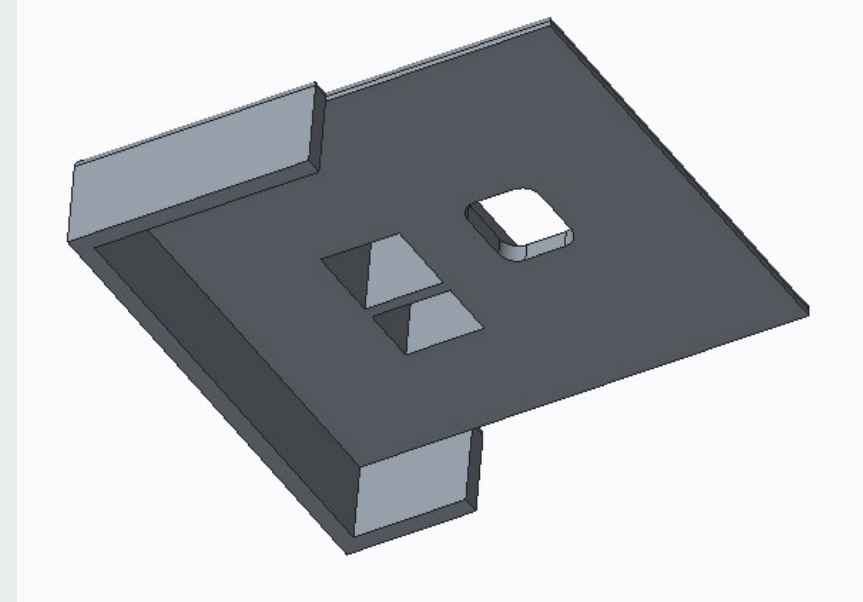
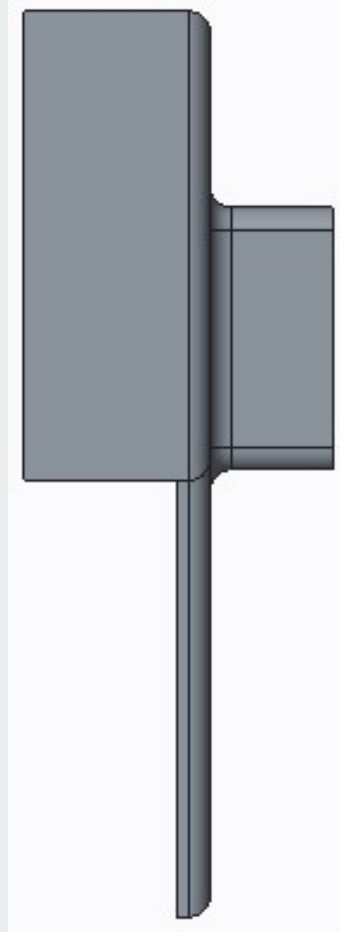
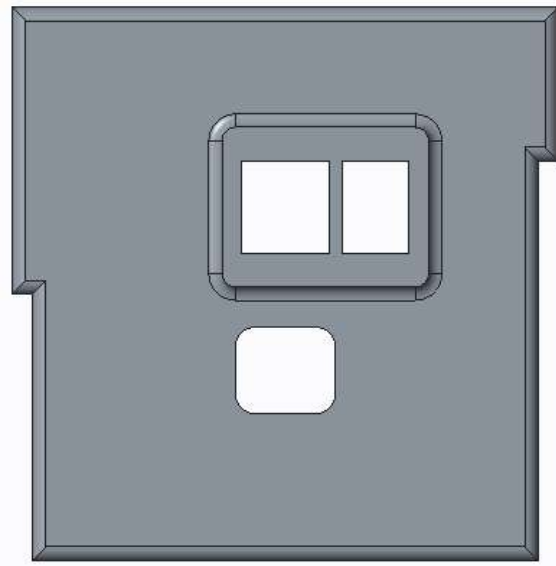
## 2. Smartphone Case

***Correct positioning of the optical fibers in relation to the smartphone's camera and the isolation from the environmental light are crucial for a reliable reading.***

- For this purpose, a **smartphone case** was developed with dimensions for fitting a Samsung Galaxy 9 Plus smartphone (76 mm x 83 mm x 14 mm, hardware setup: Snapdragon 845 2.8 GHz, 6 GB RAM, 12 MP resolution camera).
- The case **contains ports for connecting the optical fiber to the camera and LED.**
- It was manufactured by a **3D-printer** Ultimaker 2+ Extended (Ultimaker BV, Utrecht, The Netherlands) using *poly (ethyleneglycol) filament*.

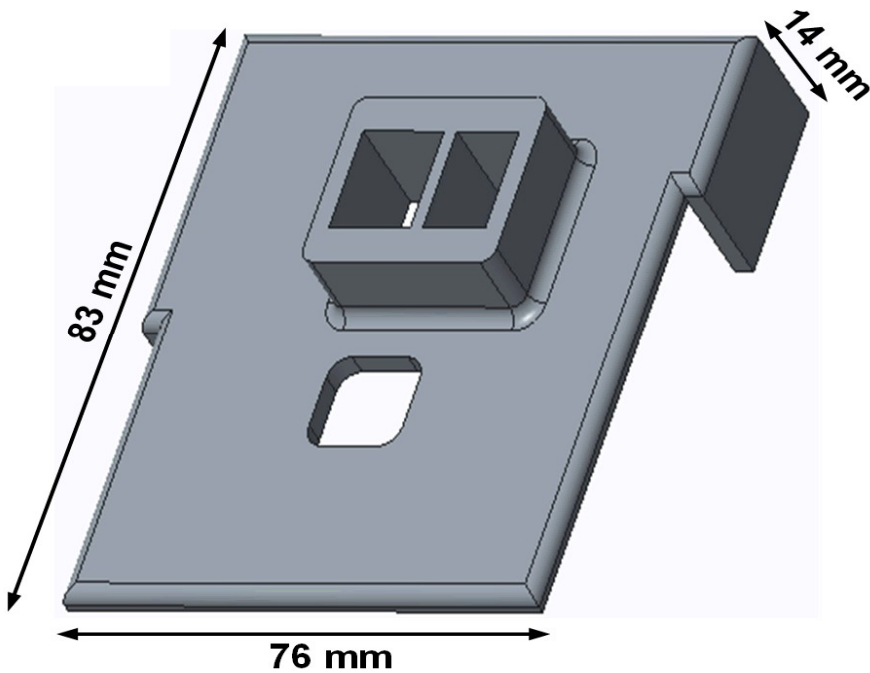
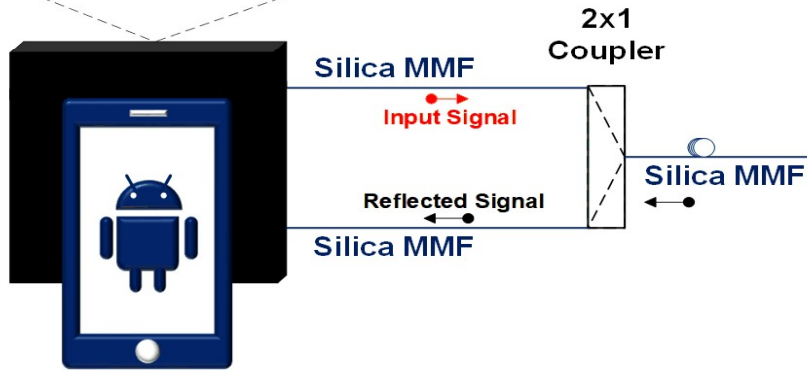
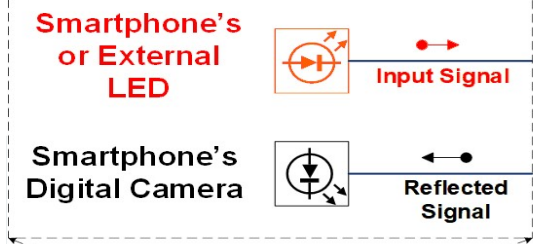


## 2. Smartphone Case



*Full blueprint with all dimensions (in millimeters) is given as Supplementary Material. Additional information (including the 3D-project file in “.stl” extension) by contacting the authors via email.*

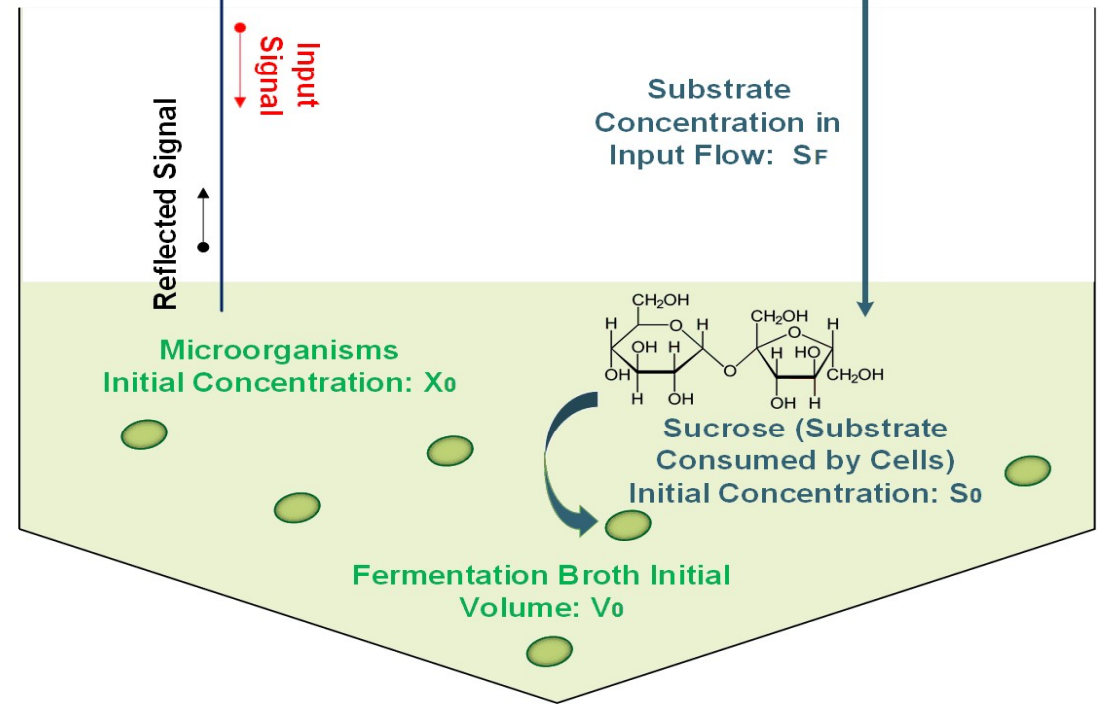
# 2. Full Experimental Setup



Substrate (Sucrose) Tank  
Concentration:  $S_F$

Peristaltic Pump

Substrate (Sucrose)  
Input Flow:  $F$



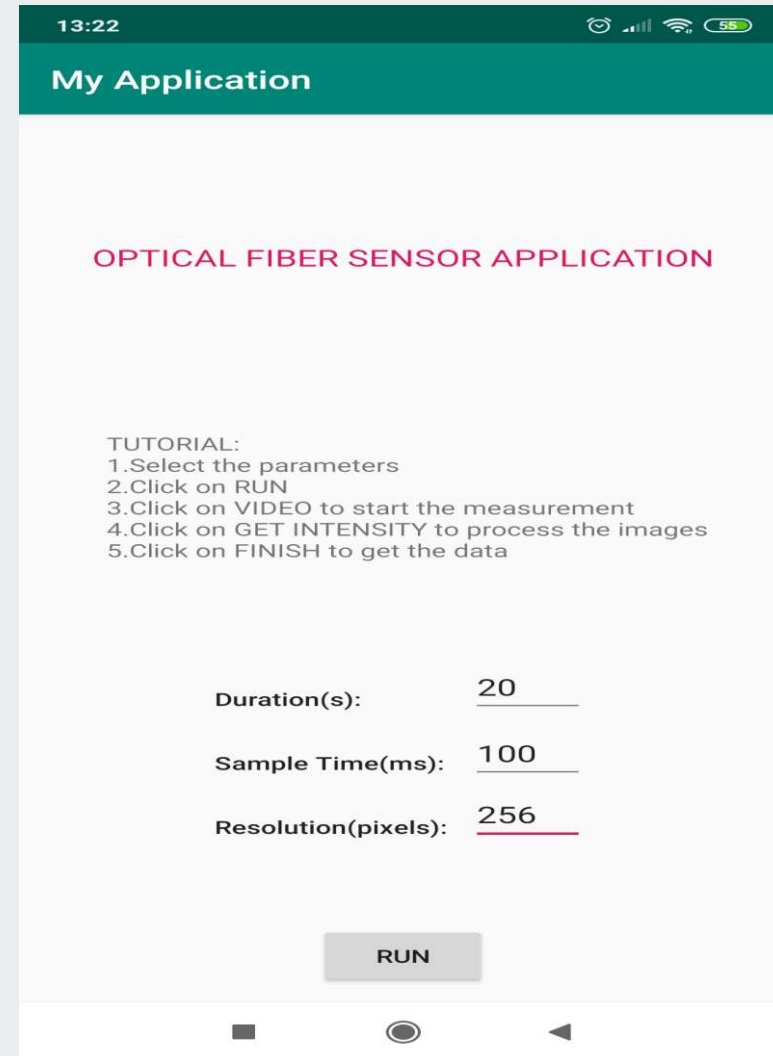


## 2. Android Application

*An application for processing the intensity signals* was developed in the environment “Android Studio” and implemented in Java language.

The operation of this software is **based on three steps**:

- (i) Choose of the image processing parameters;
- (ii) Acquisition of the optical data by video capture; and
- (iii) Analysis of the optical intensity on the recorded video’s frames.

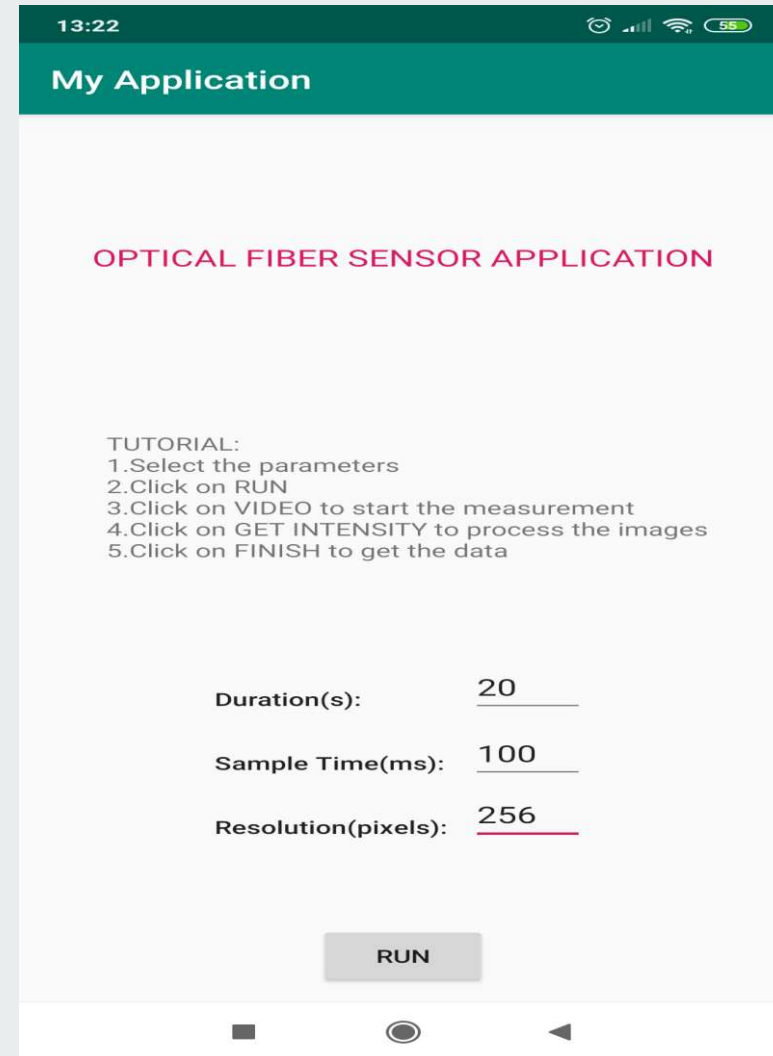




## 2. Android Application

The main menu also *has fields for the inputs of the image processing parameters*:

- Total duration of the measurement (in seconds);
- Interval between frames to be analyzed (in ms);
- Dimension (resolution) of the window where the user wants to effectively analyze the image (determines the total number of pixels that will be evaluated).

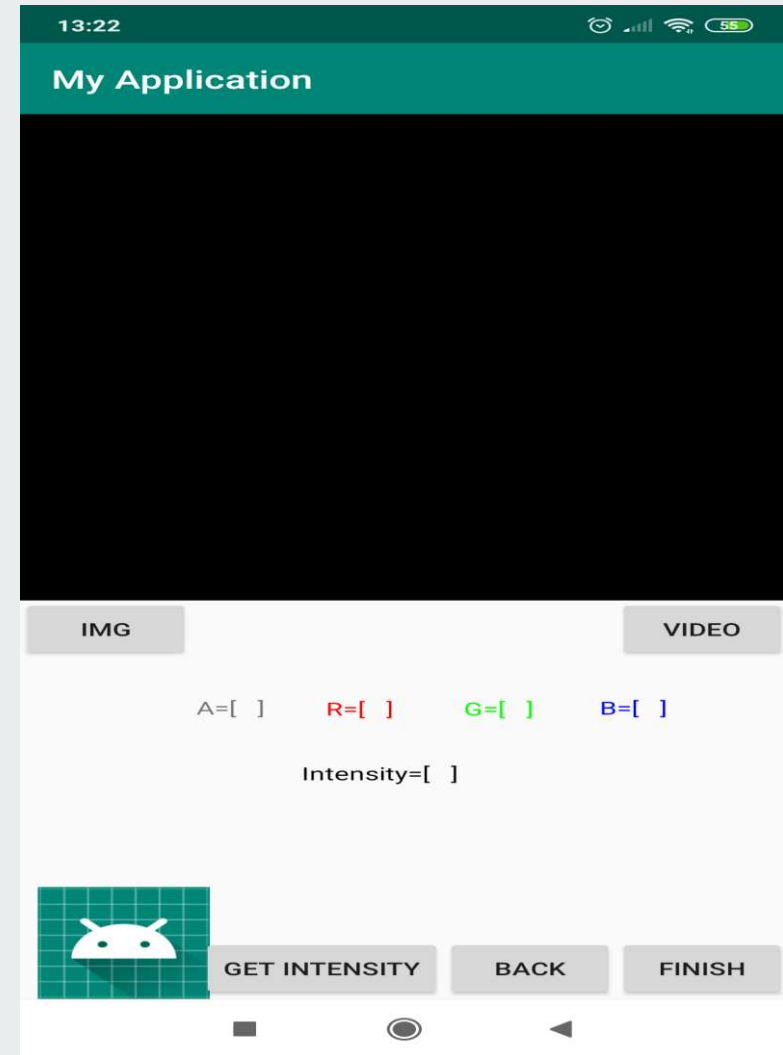




## 2. Android Application

The application presents **two operation modes**:

- The first and simpler one is for evaluating the light intensity (pixels intensity) for a single image received from the camera (this mode is supposed to be used for adjustments of calibration and setup positioning).
- The second mode performs the effective acquisition of data by recording a video with the extension “.mp4”.

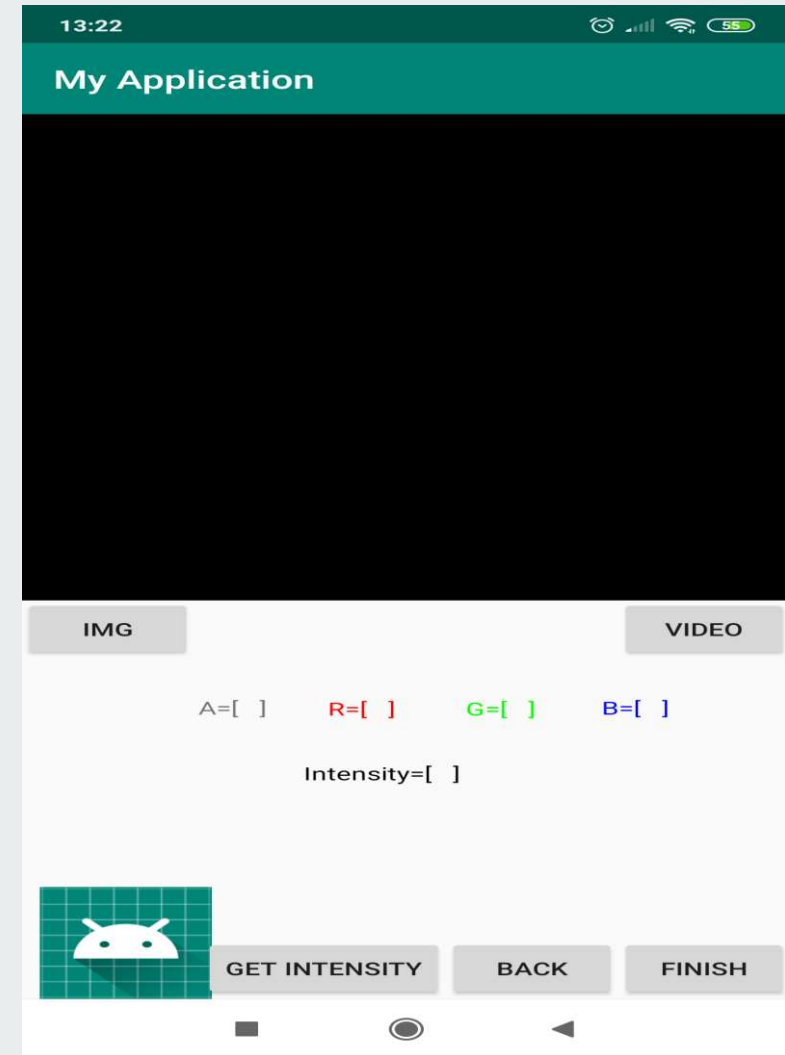






## 2. Android Application

- After recording the video, its frames are discretized by the software and analyzed.
- The application reads the camera images.
- Collected images consist of dark images containing an illuminated spot referent to the optical fiber section.
- The average pixel luminous intensity  $I_m$  on the window region is calculated for each frame.





## 2. Android Application: Evaluation of Pixel Intensity

$$I_m = \frac{\sum_{i=1}^N I_w(x_i, y_i)}{N} \quad I_w(x_i, y_i) = \left| \frac{I_{\text{Red}}(x_i, y_i) + I_{\text{Green}}(x_i, y_i) + I_{\text{Blue}}(x_i, y_i)}{3} \right|$$

$$|[0,0,0]| \leq I_w(x_i, y_i) = |[R, G, B]| \leq |[255,255,255]|$$

$[R,G,B]$  = Color of Pixel in the Coordinate  $(x_i, y_i)$  in RGB-System

$[0,0,0]$  = Black Color (RGB-System)

$[255,255,255]$  = White Color (RGB-System)

$I_m$  = Average Pixels' Luminous Intensity

$N$  = Total Number of Pixels Evaluated

$I_w(x_i, y_i)$  = Average Intensity of the Pixel in the Coordinate  $(x_i, y_i)$  (White Color)

$I_{\text{Red}}(x_i, y_i)$  = Intensity of Pixel in Red Color and Coordinate  $(x_i, y_i)$

$I_{\text{Green}}(x_i, y_i)$  = Intensity of Pixel in Green Color and Coordinate  $(x_i, y_i)$

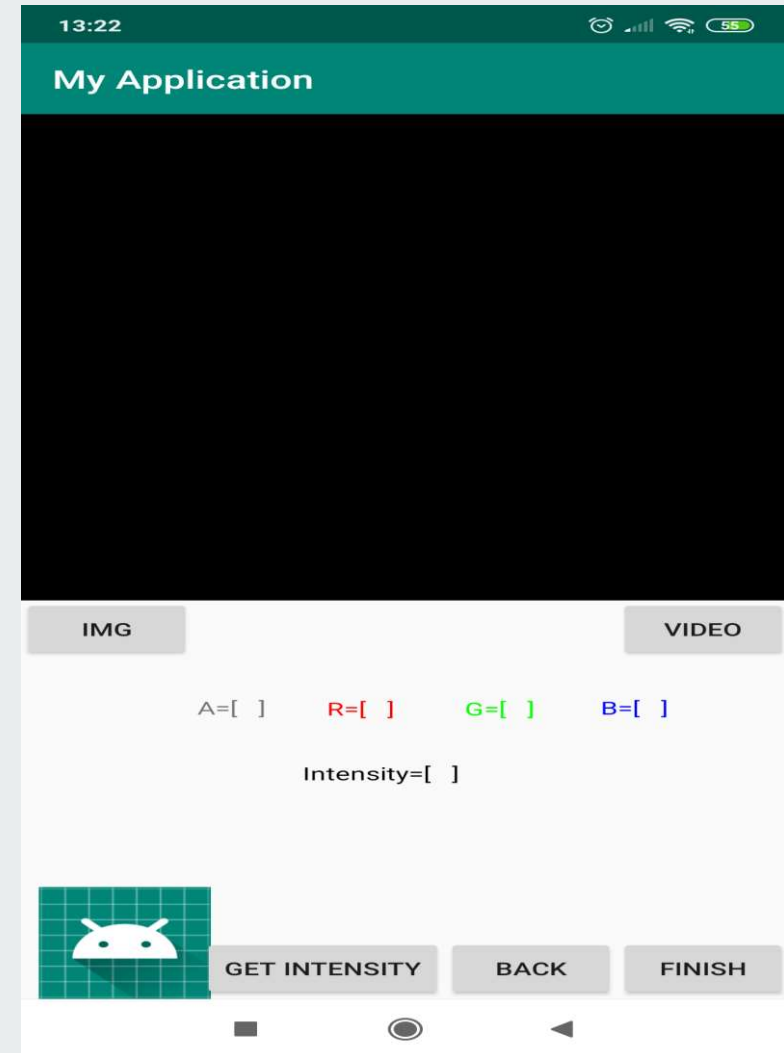
$I_{\text{Blue}}(x_i, y_i)$  = Intensity of Pixel in Blue Color and Coordinate  $(x_i, y_i)$



## 2. Android Application: Evaluation of Pixel Intensity

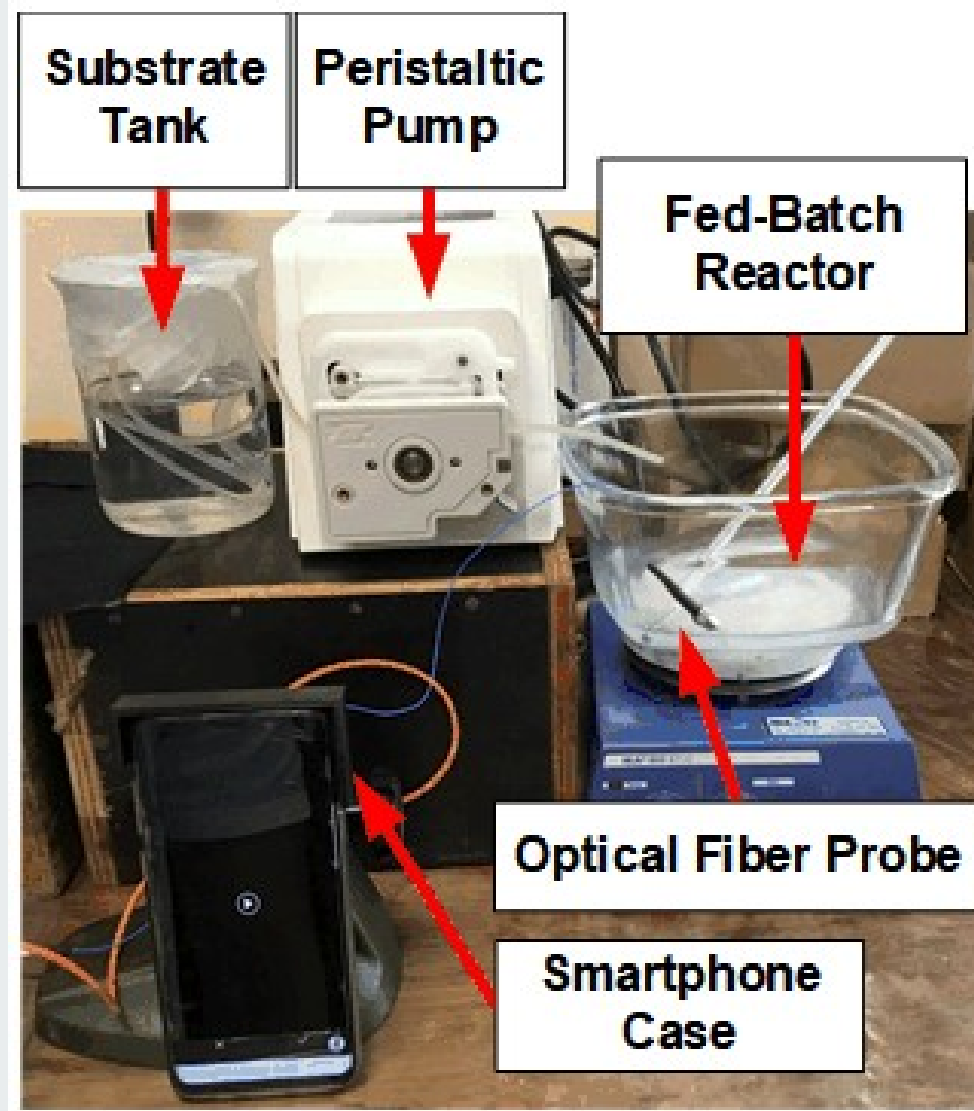
- When evaluating the expression  $I_m = \sum_{i=1}^N I_w(x_i, y_i) / N$ :
- **The dark region is not considered for the calculus;**
- N is the total number of pixels present on the region-of-interest, and  $I_w(x_i, y_i)$  is the intensity level of the pixel in the coordinate  $(x_i, y_i)$ , given by the average of RGB channels.

Finally, **the processed light intensity data can be exported to a text file**. Then, the normalized data are compared to a calibration curve for giving the value of P.



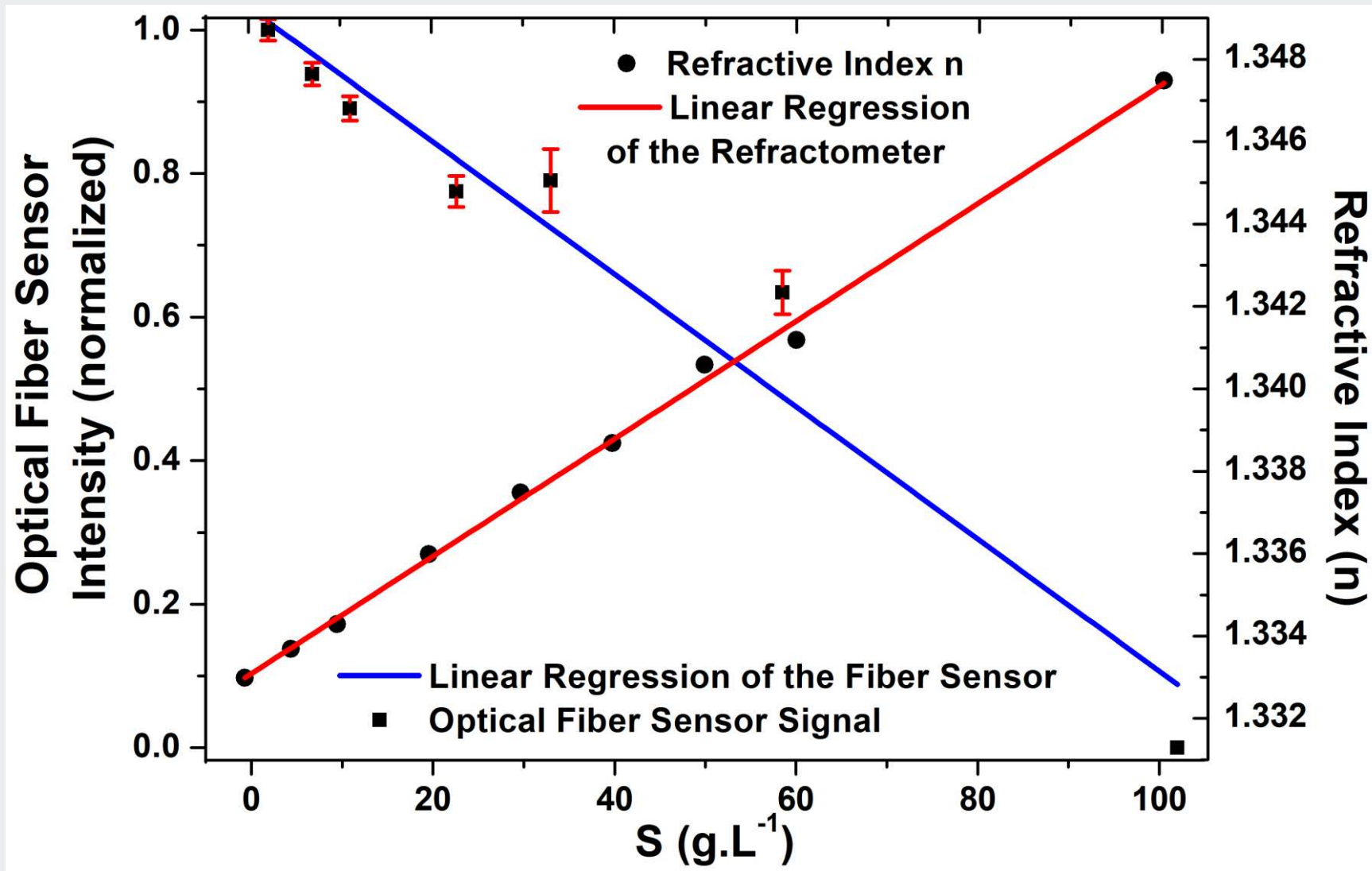


### 3. Experimental Results





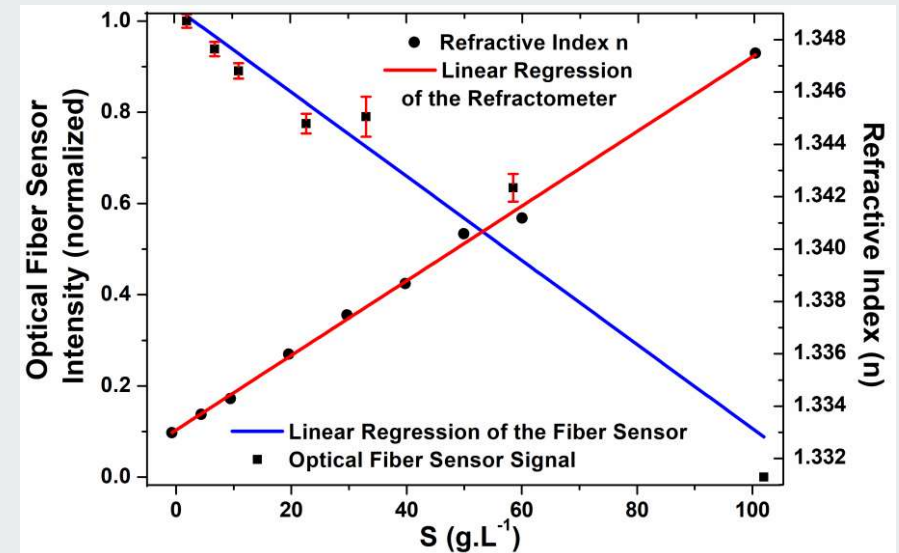
### 3. Experimental Results – Calibration Curve





### 3. Experimental Results – Calibration Curve

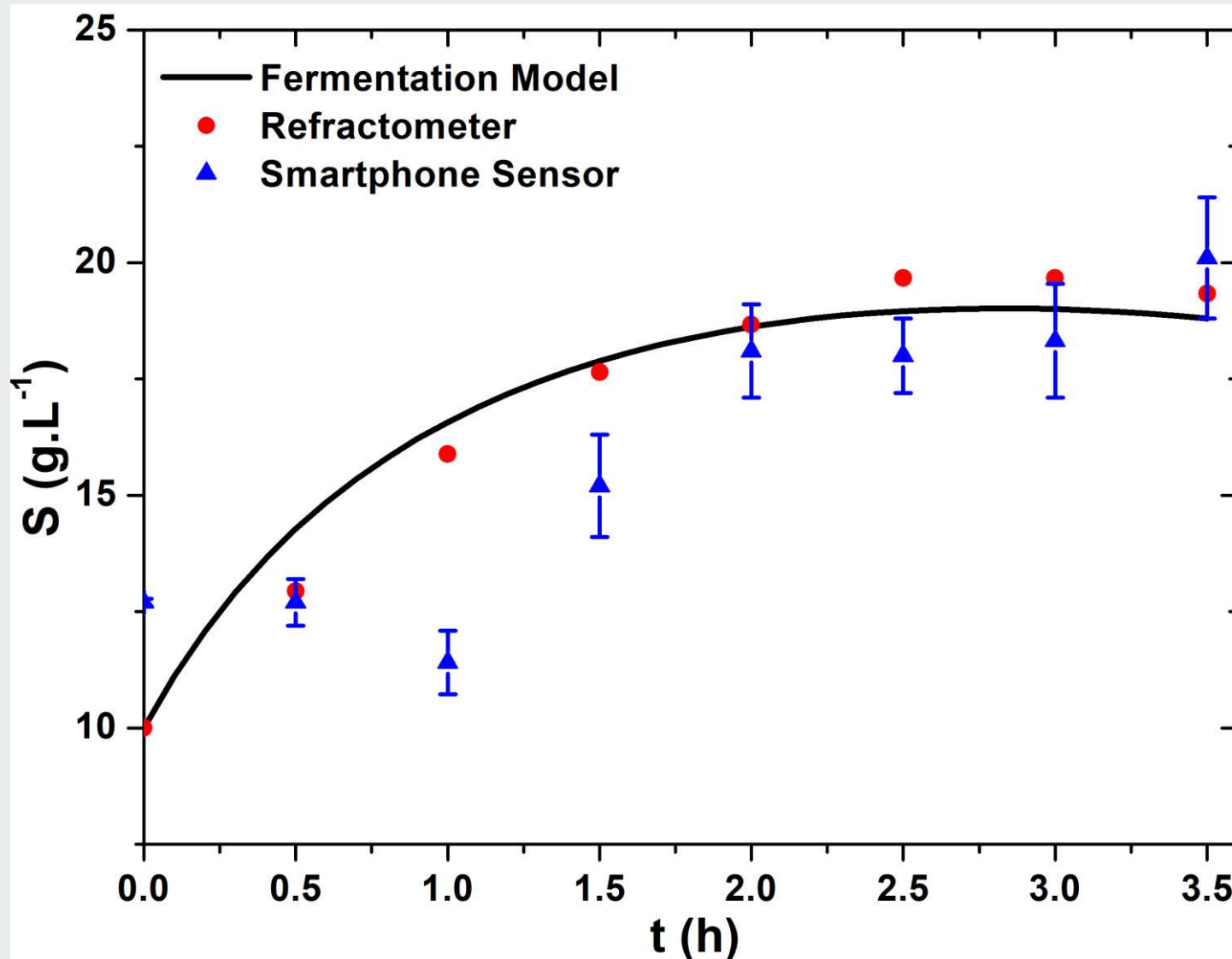
- Correlations between RI, average intensity and the sucrose concentrations ranging from 0 to 100 g.L<sup>-1</sup> were obtained.
- **Sensitivity** of the fiber sensor (ratio of variation of the intensity with the refractive index): 85.83 RIU<sup>-1</sup> (refractive index units).
- **Signal-to-noise ratios (SNRs)** were evaluated as the relation  $I_m^2 / \sigma^2$ , where  $\sigma$  is the signal standard variation: SNRs ranging from  $2.25 \times 10^2$  to  $2.4 \times 10^5$  were obtained.



- Linear increases on  $n$ :  $n = 1.3330 + (1.4432 \times 10^{-4})S$ ,  $S$  is the sucrose concentration in g.L<sup>-1</sup>, adjusted  **$R^2 = 0.9971$** ;
- and on the average intensity  $I_m$ : normalized  $I_m = 85.8353n - 63.6340$ ,  **$R^2 = 0.9344$** .



# 3. Experimental Results – Fed-Batch Fermentation

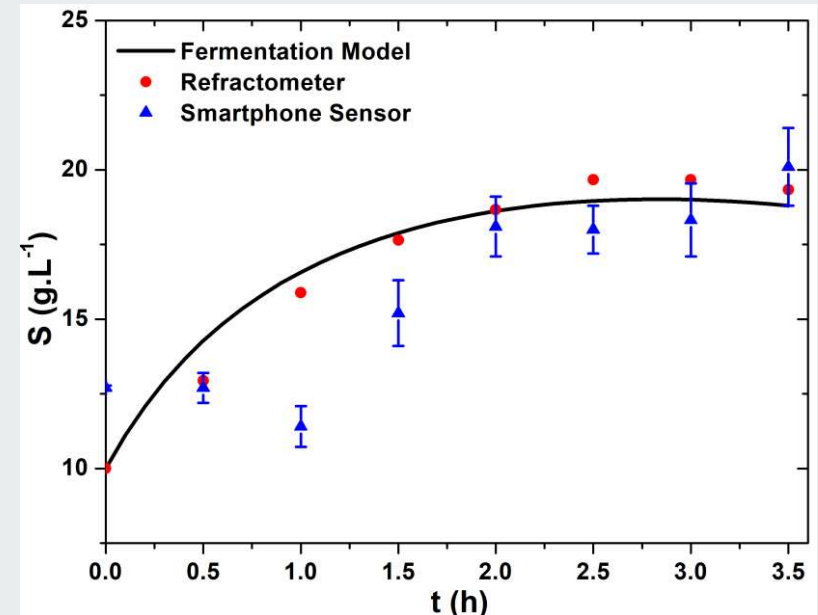




## 3. Experimental Results – Fed-Batch Fermentation

- In fermentation systems, ***relatively high deviations from the model are usually expected*** due to the uncertainties involved in representing different cells as a homogeneous population and to other different aspects of the bioprocess:
  - Temporal evolution of the microorganism;
  - Adaption to small differences of substrate;
  - Small oscillations of temperature throughout the experiment;
  - Differences on shear stresses, etc.

***The constant recalculating of the adjusted parameters is highly suggested.***



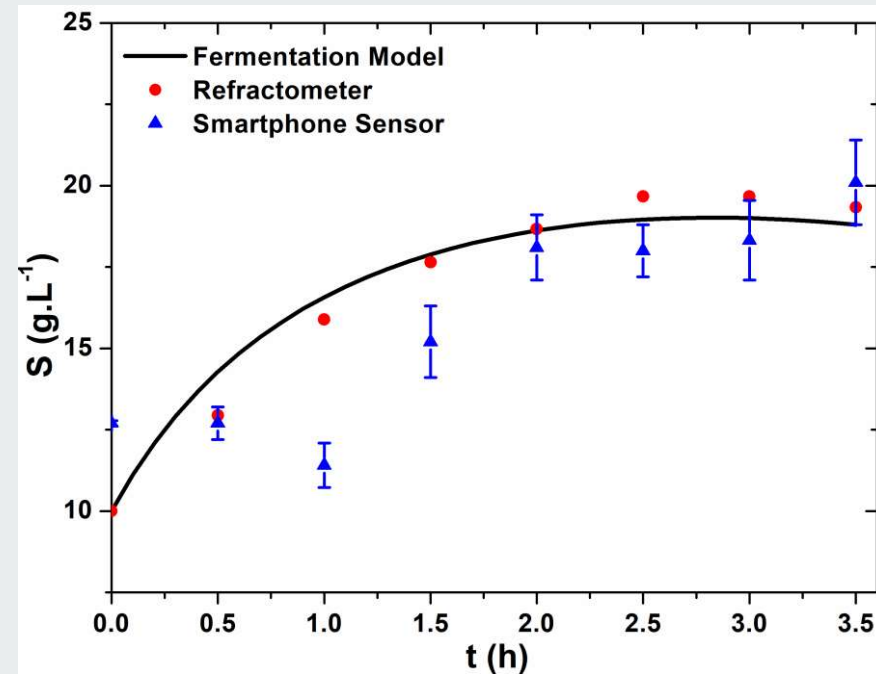
- Mathematical model predictions are represented by a solid black line.
- Results obtained by the smartphone sensor ***corroborates with the refractometer and with the theoretical model analyses.***





## 3. Experimental Results – Fed-Batch Fermentation

- The refractometer presents a high resolution of  $1 \times 10^{-4}$  RIU but is also a high-cost equipment *that requires sample collecting throughout the experiment.*
- In contrast, **the smartphone-based fiber sensor is available for online monitoring of the fermentation broth.**
- The model predicted a final concentration of  $P$  (ethanol) of only  $3.51 \text{ g.L}^{-1}$ , 5.36 times lower than the predicted concentration of sucrose  $S$  ( $18.80 \text{ g.L}^{-1}$ ).
- Therefore, ***the ethanol effect on the RI can be indeed neglected.***





## 4. Conclusions

- The design of a portable and low-cost smartphone-based optical fiber sensor for the monitoring of bioreactors was demonstrated.
- The system provided a sensitivity of  $85.83 \text{ RIU}^{-1}$  and a reliable assessment of the fermentation process.
- Due to the limited frequency of data collecting by the camera, the system is not capable of performing a quasi-elastic light scattering analysis.
- On the other hand, ***its production costs are quite inferior, it can be manufactured on-site by 3D-printing, and it can be easily integrated to an industrial line.*** This last characteristic is of major importance for the ***Industry 4.0.***
- **Such integration is not possible for the traditional assessment methods.**
- Future works will focus on enhancing the sensitivity of the sensor and to test it under higher fermentation scales for the evaluation of its performance in analyzing more complex systems. Special fibers and sensors will be also evaluated with the application.



# Thank you for your attention!

## Questions?

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