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Report and fabrication of a dissolved oxygen sensors based on fluorescence quenching

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NAUTILOS - New Approach to Underwater Technologies for Innovative, Low-cost Ocean observation is an H2020 project funded under the Future of Seas and Oceans Flagship Initiative, coordinated by the National Research Council of Italy (CNR, Consiglio Nazionale delle Ricerche). It brings together a group of 21 entities from 11 European countries with multidisciplinary expertise ranging from ocean instrumentation development and integration, ocean sensing and sampling instrumentation, data processing, modelling and control, operational oceanography and biology and ecosystems and biogeochemistry such, water and climate change science, technological marine applications and research infrastructures.

NAUTILOS will fill-in marine observation and modelling gaps for chemical, biological and deep ocean physics variables through the development of a new generation of cost-effective sensors and samplers, the integration of the aforementioned technologies within observing platforms and their deployment in large-scale demonstrations in European seas. The fundamental aim of the project will be to complement and expand current European observation tools and services, to obtain a collection of data at a much higher spatial resolution, temporal regularity and length than currently available at the European scale, and to further enable and democratise the monitoring of the marine environment to both traditional and non-traditional data users.

NAUTILOS is one of two projects included in the EU´s efforts to support of the European Strategy for Plastics in a Circular Economy by supporting the demonstration of new and innovative technologies to measure the Essential Ocean Variables (EOV).

More information on the project can be found at: https://www.nautilos-h2020.eu/

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EXECUTIVE SUMMARY

Task 3.1 is devoted to the design and fabrication of a dissolved oxygen measurement system based on optical measurement of fluorescence quenching of specific fluorophores which are sensible to oxygen. This report presents a general introduction on the physical aspects of the oxygen-induced, fluorescence quenching and hence our unique solution for the implementation without requiring a selective optical filter to separate excitation light from the fluorescence itself.

A careful selection of the optimal fluorophore has been performed and hence the details of the electrical implementation are provided.

A first prototype, designed and fabricated early in the project, provided a reference tool for the characterization of the principle. Then two newer versions have been designed, in accordance with NKE, to fit in their waterproof casing, which would end up in a solution fully compatible with their current system. The latter step, not required in the context of the project, was considered worthful to implement in order to facilitate the technology transfer to the final user (i.e. NKE).

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Definition
Pd-TFPP	palladium(II) 5,10,15,20-meso-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin
Pt-TFPP	platinum(II) 5,10,15,20-meso-tetrakis-(2,3,4,5,6-pentafluorophenyl)-porphyrin

I. INTRODUCTION

Dissolved oxygen sensing through fluorescence quenching measurement.

Sensors able to measure oxygen rates in fluids exist since decades [1]. The first sensors used the Clark electrode [2] to quantify oxygen amount either in gas or in liquids. This kind of electrode and its evolutions are reliable but have a disadvantage; they consume small amounts of oxygen in order to measure the oxygen rate which can lead to measurement errors when the fluid is static.

Nowadays, the most precise and popular oxygen sensors are optical based. Specifically, they use the fluorescent properties of certain material to determine the oxygen rate. This approach does not consume any oxygen and can achieve measurements with a precision around the ppm. Certain molecules based on atoms such as Ruthenium, Palladium, Platinum, Carbon, etc… [3-6] are naturally fluorescent which means that they are emitting light in certain wavelength (usually in the red field) when they are excited by light in other wavelength (usually in the blue field). The particularity of the previously mentioned molecules is that their fluorescence is affected by oxygen. In other words, the more oxygen is in contact with these molecules, the less fluorescent they are. This phenomenon is called the quenching of the fluorescence.

This quenching can be computed with the Stern-Volmer equation as follows [3]:

$$\frac{I_0}{I} = 1 + K_q \cdot \tau_0 \cdot [O_2] = 1 + K_{SV} \cdot [O_2]$$

Where:

I_0	:	Fluorescence intensity at 0% O_2
I	:	Fluorescence intensity with actual O_2 rate
K_q	:	Quencher rate coefficient
T_0	:	Fluorescence lifetime at 0% O_2
$[O_2]$	:	Oxygen rate
K_{SV}	:	Stern-Volmer constant ($K_q \cdot T_0$)

The Stern-Volmer equation shows the physical and direct link between the presence of oxygen and the intensity of the fluorescence. This approach of measuring the oxygen rate has many advantages because it has a fast response time, does not consume oxygen and is not easily poisoned [7].

Intensity based - Intuitively, as the Stern-Volmer equation relies on fluorescence intensity, several applications use this parameter to measure the oxygen rate as done in [8]. This approach benefits of the above-mentioned advantages compared to the classical use of the Clark electrode. Unfortunately, the intensity is dependent on external perturbations and on the degradation of the fluorescent material [3].

This inconstancy of the fluorescence intensity over time involves that the sensors using this property need to be calibrated frequently. Moreover, the measuring setup has to be very robust because a small change on the intensity of the emitting light induces a different fluorescent light intensity. This problem is the same for the light losses between the emitter, the fluorescent material and the receptor. Finally, the sensitivity of the receptor also needs to be very stable, otherwise, the sensor will have to be calibrated even more often.

Phase shift based - As using the fluorescence intensity involves many disadvantages, many works explored the possibility of measuring the fluorescence's lifetime. Measuring the lifetime, τ, of the fluorescent material is very useful because the Stern-Volmer equation can be derived as follows [3]:

$$\frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$

Where:

T_0	:	Fluorescence lifetime at 0% O_2
T	:	Fluorescence lifetime with actual O_2 rate
$[O_2]$	:	Oxygen rate
K_{SV}	:	Stern-Volmer constant

To be able to measure the fluorescence lifetime, the phase shift between the exciting signal and the fluorescent signal can be used. This can be done with the following equation [7]:

$$\tau = \frac{\tan\theta}{2 \cdot \pi \cdot f}$$

Where:

T	:	Fluorescence lifetime with actual O_2 rate
θ	:	Phase shift between exciting and fluorescent signals
f	:	Signal frequency

The phase shift between the two signals, θ, can be defined as done in the following picture:

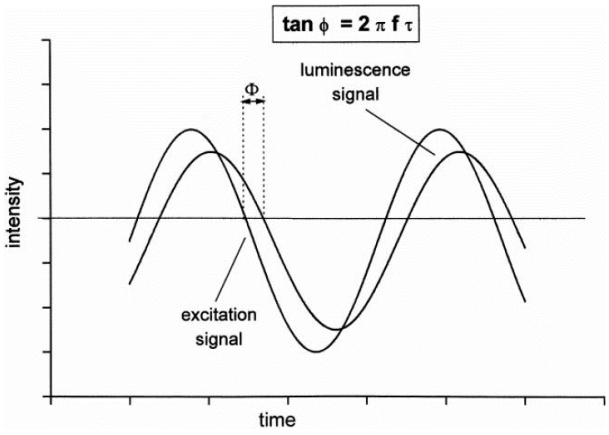


Figure 1: Definition of the phase shift between the exciting and the fluorescent signals [7]

This approach keeps all the advantages obtained by using the fluorescence intensity and adds a lot of reliability to the system. This is because the fluorescence lifetime is an intrinsic property of the fluorophore which is virtually independent of external perturbations [7].
However, as the fluorescent light is a lot weaker than the excitation light, it is necessary to filter the undesired wavelength to isolate the signal of the fluorescent light. The picture below shows an example of a measurement setup using the phase shift.

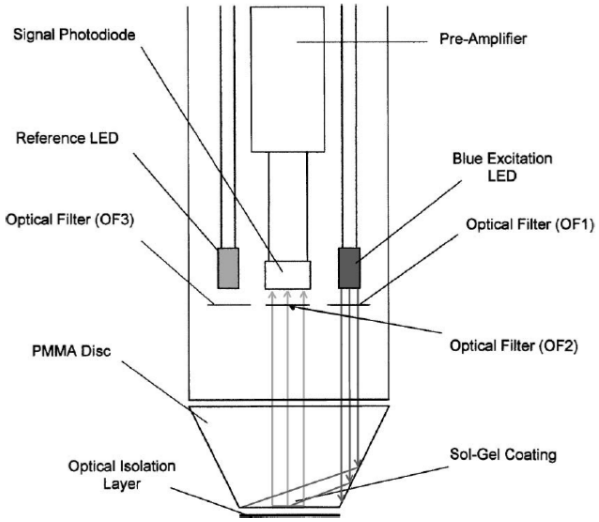


Figure 2: Example of a setup to measure fluorescence's phase shift [7]

This kind of setup needs at least two optical filters to be functional. The first one (OF1) is a band pass filter that lets only the excitation wavelength go to the fluorescent material. The second one (OF2) is also a band pass filter and lets only the fluorescence wavelength go to the photo sensor which usually is a photodiode. These filters represent some non-negligible constraints for the mechanical design of the sensor. Moreover, this kind of filter adds usually some losses on the signals even if it is designed to be exactly at the right wavelength. This involves that excitation light needs to reach a minimum intensity which is quite high to be able to excite significantly the fluorescent material to "generate" afterwards enough fluorescent light.

It is interesting to mention that the design represented on the picture above includes a second light source which is also filtered (OF3). This second light source has the same wavelength as the fluorescent light. Its purpose is to measure the delay induced by the light emission and the electronic processing allowing then to reach more precise results.

1. Instrument Description

Fluorescence decay based, dissolved oxygen sensing principle - The previous methods showed many advantages and already proved a great efficiency once the critical aspect such as time variance and mechanical setups are correctly considered. Nevertheless, another approach has been explored and showed very good results. This approach is also based on the fluorescence lifetime which allows this kind of design to keep the advantages of the previously mentioned approaches. The idea is to directly measure the lifetime constant τ. As many natural phenomena, the fluorescence, when no longer excited by a light at the right wavelength, decreases exponentially. This can be observed in the graph below. This graph shows the photodiode signal directly measured by an oscilloscope.

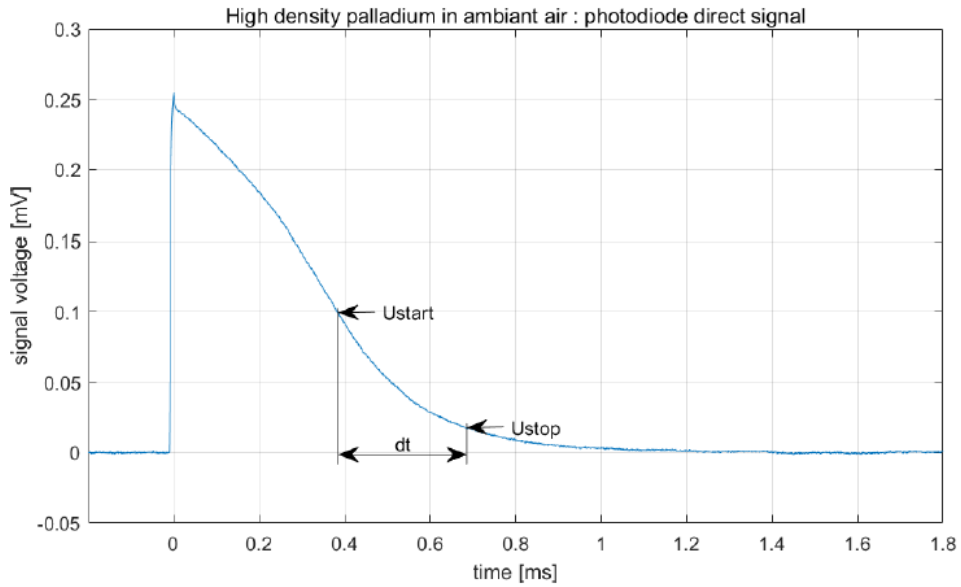


Figure 3: Typical exponential fluorescence's decay after excitation light extinction

To measure this signal, the fluorescent material which, in this case, is a palladium-based molecule solution, is excited by a UV light impulse of 10µs. The excitation light is shut down at 0ms on the graph timeline. Almost instantly, the photodiode is saturated by the excitation light. After the extinction, only the fluorescent light, which has a certain lifetime remains. As the above graph shows, a few hundreds of microseconds before reaching complete darkness, the decay is perfectly exponential. The function of this exponential decay can be described with the following equation:

$$U(t) = U_{SAT} \cdot e^{-t/\tau}$$

This means that the actual lifetime of the fluorescence, τ, which is directly related to the oxygen rate is accessible once the exponential has been characterized. This characterization can be easily done by taking two arbitrary points on the curve; (t_{start}, U_{start}) and (t_{stop}, U_{stop}), like it is done in the graph below.
Once these two points are chosen and recorded, it gives two equations with two unknown variables.

$$U_{start} = U_{SAT} \cdot e^{-t_{start}/\tau} \qquad U_{stop} = U_{SAT} \cdot e^{-t_{stop}/\tau}$$

Hence:

$$\frac{U_{start}}{e^{-t_{start}/\tau}} = \frac{U_{stop}}{e^{-t_{stop}/\tau}} \quad \rightarrow \quad \tau = \frac{t_{stop} - t_{start}}{ln\left(\frac{U_{start}}{U_{stop}}\right)} = \frac{dt}{ln\left(\frac{U_{start}}{U_{stop}}\right)}$$

With the previous equation, it is possible, in a fast computation to get the actual lifetime of the fluorescence and therefore, the actual oxygen rate. At this point, this approach can be defined as suitable as the one analysing the phase shift. But there are two big advantages to focus on the fluorescence decay instead of the phase shift. The first one is that there is no longer any need of using optical filters as when it is necessary to measure the fluorescence light, there is no more excitation light [3]. This simplifies drastically the mechanical setup of the sensor. The second one is that this approach needs very short periods of light excitation, which means less energy to excite the fluorescent material. This might be insignificant in several setups and use cases but, in the case of an embedded system, it is mandatory to target a design with the lowest possible power consumption to obtain the maximum working duration on batteries. This third approach, presenting the most advantages, is the one that will be used further in the project to develop the DO sensor.

Prototype design

The proposed prototype integrates the following functionalities:

- Light excitation driving (UV LED)
- Fluorescent light acquisition (Photodiode and analogic pre-treatment)
- Time measurement (TDC)
- Data processing (MCU)

The block diagram of the complete prototype is shown in the figure below.

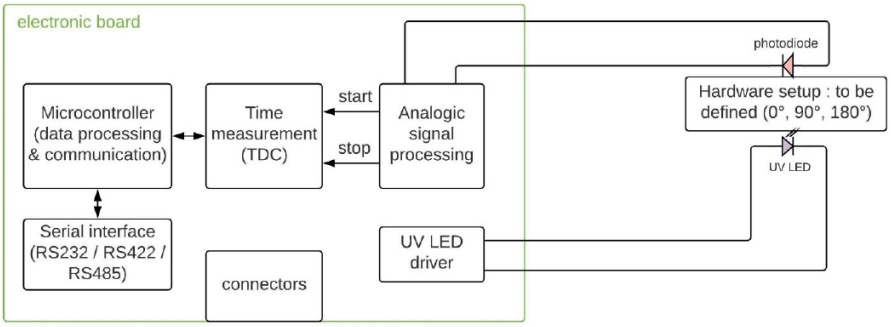


Figure 4: Block diagram of the prototype

LED driver - The whole measurement starts always with a UV light flash. Therefore, the board must have the possibility to drive an LED. As it has been observed, depending on the test setup, that light intensity losses can be significant. It is then necessary to have the possibility to drive an LED with different voltages. The board will generate its own 12V as default LED voltage as it is the voltage used during the preliminary tests. However, the board will have the possibility to be driven by an external voltage. It is also interesting to have the option of

selecting different sources as trigger for the flashes. The user will then have the choice to trigger the LED from the MCU, the TDC or from an external signal. It is mandatory to take care of the EMC aspect of the driver. Indeed, such circuit can be very disturbing especially for analogic circuits such as the photodiode signal pre-treatment. This is due to the switching of higher voltages and high currents drawn by the LED.

Analog signal preconditioning - Once the fluorophore has been excited by the UV light flash, the next step is to acquire the fluorescent light. As already explained, this is achieved with the help of a photodiode. As the photodiode signals are very small, it is necessary to amplify them. A more detailed explanation of this amplification circuit is available in the next chapter. But it can already be specified that it will be crucial to have the possibility to tune this amplification as the signal intensity is dependent of many parameters and can, therefore, vary over the different tests. Finally, as the signal amplitude will change, it will be needed to be able to adjust the voltage thresholds that allow to generate the “start” and “stop” signals. All these adjustments will be possible by adding some potentiometers to the circuit.

Time-to-Digital Converter (TDC) – Measurement of the tie interval between the start and stop signals is performed through a TDC. In our previous works, we identify the TDC GP22 formerly from ACAM, now from ScioSense, as the best in terms of performances. This TDC was intended for measuring liquid flow through ultrasonic excitation and measurement of the Doppler frequency deviation. This component resulted to be of particular interest for our application due to the very high time resolution, 22ps on two channels, the compactness (QFN32 package) and the PT1000 readout circuit for temperature measurement already integrated.

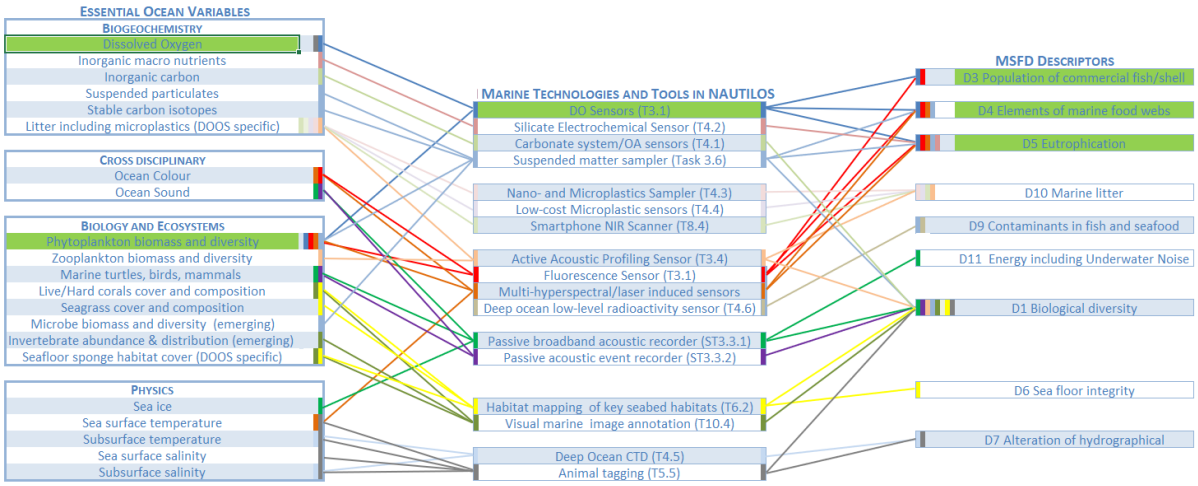
Digital stage - After the analog treatment of the optic measurement, only two digital signals remain: start and stop. From this point, all the next steps will be done with a digital circuitry. The two edges are transmitted to the TDC inputs that will compute the elapsed time during the fluorescence decay. Once this time is measured, it will be read by the MCU through a serial communication bus. From this point, lots of development options can be made. Therefore, this board will be a good basis for further improvements. This can be done by letting access to various MCU’s interfaces such as GPIOs, SPI and UART interfaces. Finally, as discussed with NKE, the board will be able to communicate via RS232 and/or RS485 protocols. The board will then integrate a chip that will be compatible with these two protocols and that can interact with the MCU via a standard UART.

Power supplies - As for the final product, the prototype will be power supplied by a single voltage. All other voltages needed for the various parts of the circuit will be generated internally. To remain as much flexible as possible, as mentioned previously, there will have two different options to supply it: 5V or a voltage that can vary between 8V and 16V. From this single input voltage, several other voltages will be generated such as -3.3V, +3.3V and +12V. Nevertheless, both the analogic and the digital circuit being power supplied with a +3.3V, the voltage will be generated twice to avoid having noises induced by the digital components on the analog power supply. Finally, a +3.3V reference voltage will be used to generate the start and stop thresholds voltages.

Connectors - It is also very important that this board provides clean interfaces with the other parts of the system. This can be done by choosing the right connectors for the right application. As already mentioned, most of the final product's connectors are not defined, especially because there is no need to have a fully waterproof setup at this stage of development. For prototyping, usually, the most practical connectors are simple pin headers that easily allow to wire the board with another. However, for certain sensitive signals, it is better to have other connectors that keep a better signal integrity. On this board, there are two critical signals: the LED supply and the photodiode signal. As already mentioned, the LED flashing can be very disruptive, therefore, an isolated and short wire is recommended. For the photodiode signal it is also better to have an isolated wire such as a coaxial cable. For these purposes, both for the LED and the photodiode, it has been decided to use coaxial cables connected to the board via SMA connectors.

2. NAUTILOS CONTEXT

In the context of the NAUTILOS project, the current system will provide a measurement of the dissolved oxygen as a primary ocean variable and consequently information on Phytoplankton biomass and diversity. In terms of Marine Strategy Framework Directive (MSFD), three main descriptors will be addressed: (i) population of commercial fish/shell, (ii) elements of marine food webs and (iii) eutrophication.



3. PERFORMANCE SPECIFICATIONS

According to our measurements and consequent calculations we expect our system to reach a precision smaller than 10ng/L for oxygen concentrations lower than 5mg/L, i.e. a 1-2 ppm resolution.

4. INTERFACE SPECIFICATION

Our system is compliant both with RS232 and RS485 communication protocol and compliant with any ASCII-based interface protocol, such as NMEA.
A 12V power supply line is required.

5. DATA MANAGEMENT AND FORMAT

Our system can work in different modes: continuous measurements and on-demand measurement. Data are delivered to the communication bus and managed by the AUV central unit.

II. ETHICAL CONSIDERATIONS

1. DATA PROTECTION

Nothing intrinsic in stored data unless user tags free text with personal/confidential data

2. ENVIRONMENTAL PROTECTION

The product is not considered hazardous for environment.

2.1. WEEE considerations

NKE is not directly linked to the WEEE regulations, but NKE is committed to sustainable development by working with local companies to manage obsolete electronic cards.

3. HEALTH AND SAFETY

3.1. Dangerous goods (if Lithium is used)

Fullerene C_{70}

- No known hazard

Pd-TFPP

- Skin irritation, H315
- Serious eye irritation, H319

Pt-TFPP

- No known hazard

3.2. Pressure housing safety issues

The sensor is subjected to external pressures induced by the marine environment. The waterproof housing is at atmospheric pressure. The enclosure is not dangerous as such. To be compliant for deployment at sea, Dissolved Oxygen sensor must meet the AFNOR standard: NF X10-812. The latter is a document devoted to the tests to be performed on oceanographic equipment immersed in a marine environment.

3.3. RoHS

All components selected for the system are RoHS compliant.

4. PROTECTION OF MARINE LIFE

Device emits a flashing blue light for a very short time (few milliseconds) to induce fluorescence on the sensing layer. Duty cycle of the light emission can be tuned accordingly to the measurement frequency rate and, in case, can be shielded not to bother surrounding environment.

5. DUAL USE POTENTIAL

Not applicable.

III. Lab Test and Validation References

1. Reference material

- **Oxygen**
  - **Ref.:** I1001M30R2A001
  - **Purity:** 99.5%
- **Nitrogen**
  - **Ref.:** I4092L50R2A001
  - **Purity:** 99.999%
- **Fullerene C_{70}**
  - **Manufacturer:** Sigma-Aldrich
  - **CAS-Nbr:** 115383-22-7
- **Pd-TFPP**
  - **Manufacturer:** Sigma-Aldrich
  - **CAS-Nbr:** 72076-09-6
- **Pt-TFPP**
  - **Manufacturer:** Combi-Blocks
  - **CAS-Nbr:** 109781-47-7

2. Reference equipment

- **Bar coater**
  - **Manufacturer:** Zehntner
  - **Model:** ZAA 2300
- **Magnetic stirrer**
  - **Manufacturer:** INTLLAB
  - **Model:** MS-500
- **Power supply**
  - **Manufacturer:** RND LAB
  - **Model:** RND 320-KA3005D
- **Water pump**
  - **Manufacturer:** KNF
  - **Model:** NF 1.60 KPDC

3. Reference instruments for lab tests

- **Oscilloscope**
  - **Manufacturer:** LeCroy
  - **Model:** Waverunner 44MXi-A
- **Oxygen sensor**

- **Manufacturer:** Oxyscan
- **Model:** 300

IV. LABORATORY TESTS

1. DESCRIPTION

Our oxygen sensor has been tested in our labs using the testbench sketched in the figure below. Samples of water have been prepared by bubbling nitrogen and hence adding ascorbic acid to reduce the oxygen concentration. This solution is our starting point for testing the system; then we started bubbling some oxygen into the solution to increase the oxygen partial pressure.

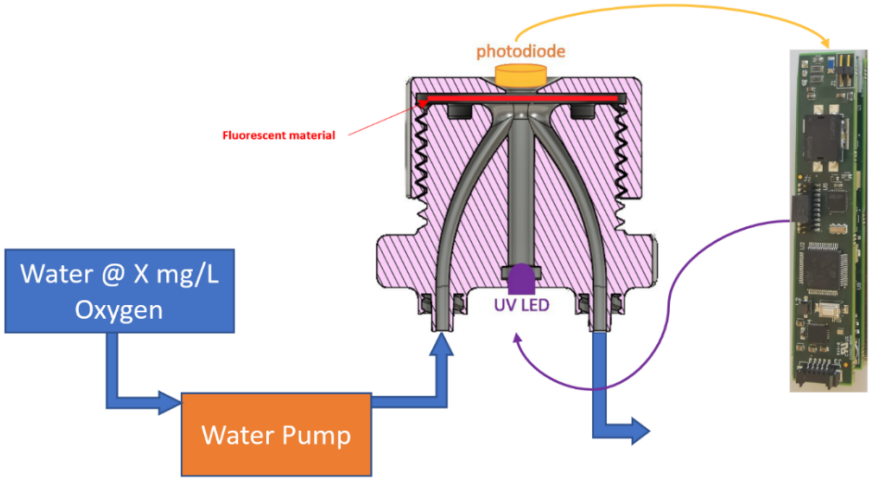


Figure 5: Block diagram of the system used to test the DO sensor

Before any measurement performed by our system, dissolved oxygen was determined using a Clark-cell based system, the OxyScan 300. This equipment presents the following performances:

DO < 15mg/L:	0.01mg/L precision
15mg/L < DO < 150 mg/L	0.02mg/L precision
150mg/L < DO < 750 mg/L	0.1mg/L precision

Although the theoretical precision of our system should be considerably higher, we estimate this preliminary calibration good enough for the testing phase in WP5.

1.1. DO sensitivity range and precision

DO sensitivity range and precision have been evaluated according to the system described above.

1.2. Response speed

From a system point of view, the fluorescence decay time measurement is very fast, in the order of few hundreds of microseconds. In order to get rid of the electrical noise in the measurements, averaging results on 64 up to 256 samples is applied.

The physical limitation of the sensor is related to the diffusion of the water into the fluorophore; this parameter can be improved by modifying the fluorophore layer thickness and/or increasing porosity.

1.3. Fluorophores comparisons

Many fluorophores sensible to oxygen are commercially available. The selection has been based on two main parameters:

a) Fluorescence decay time in presence of no oxygen
b) Stern-Volmer constant for the oxygen quenching

The first parameter defines the time scale need for the measurement, since the precision of the Time-to-Digital Converter (TDC) is fixed, the longer the decay time, the better the precision.
The Stern-Volmer constant defines the sensibility to the oxygen; this parameter must be carefully be taken into considerations with respect to the oxygen measurement range we are targeting.

2. RESULTS

2.1. DO sensitivity range and precision

A series of tests have been performed to evaluate system performances in terms of range and precision. In Figure 6 the main results are resumed, fluorophore decay time is presented with respect to the dissolved oxygen concentration. The negative exponential dependence is clearly visible, the Pd-TFPP performs at its best at lower oxygen concentration, closer to the typical values found in marine water.
In the same picture, the estimated resolution of our device is presented in a logarithmic scale to enhance the value at lower concentration and higher precision.
In short, the precision is calculated as follow:

$$resolution\ \left[\frac{ng}{L}\right] = DOrate\ \left[\frac{mg}{L}\right] \cdot \frac{TDC\ precision\ [22\ ps]}{decay\ time\ [\mu s]}$$

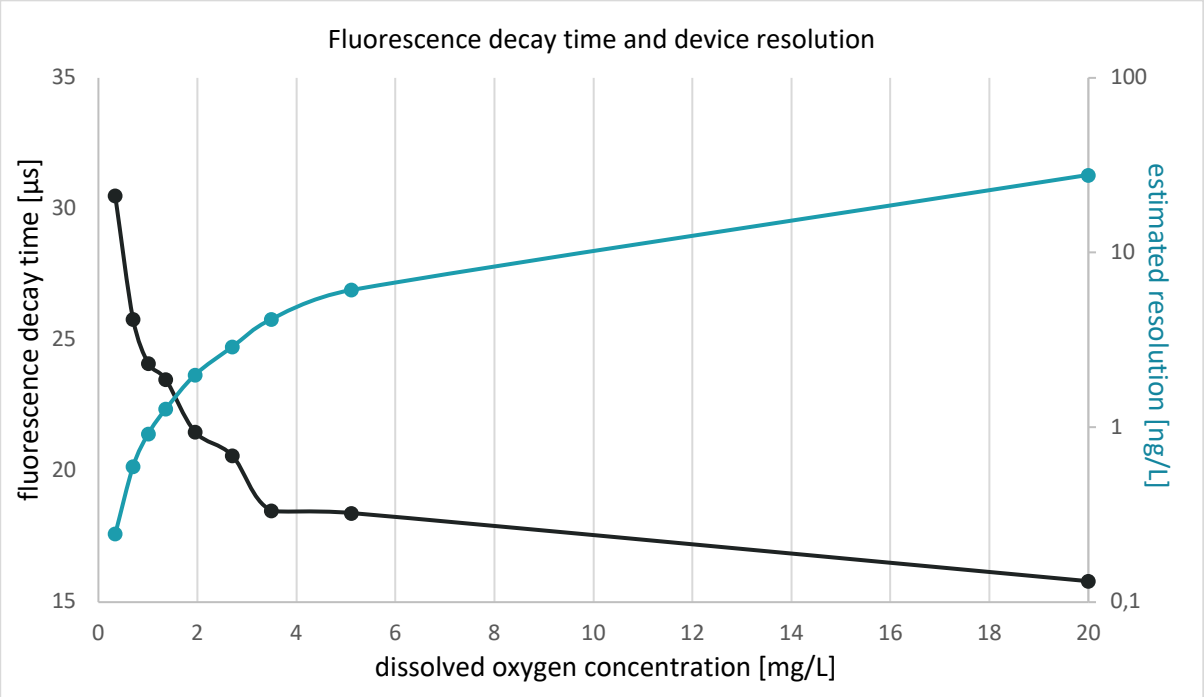


Figure 6: fluorescence decay time and device measurement resolution

The presented system will have extremely high sensitivity in water with very poor concentration of dissolved oxygen. However, compared to the performance of a commercial system such as the OxyScan 300, we theoretically increase the performance of several orders of magnitude.

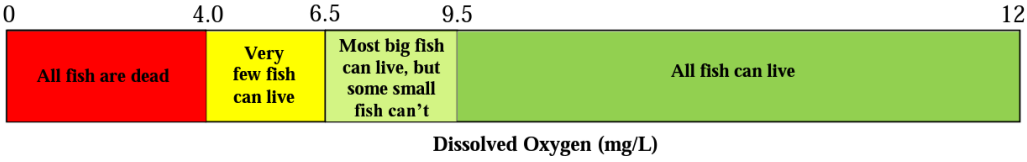


Figure 7: Fish survival according to different dissolved oxygen rate

2.2. Response speed

No measurement have been performed on this specific parameter.

2.3. Fluorophores comparisons

Three fluorophores have been tested for this application:

- Fullerene C_{70}
- Palladium based porphyrin (Pd-TFPP)
- Platinum based porphyrin (Pt-TFPP)

Fullerene C_{70} – this molecule was selected because of its attractive fluorescence lifetime estimated between 20ms and 25ms, however, the quenching of this molecule is quite high at 75 $ppmv^{-1}$ [4]. The molecule was prepared by following the same steps explained in the work of Nagl, S., Baleizão, C. & Borisov, S. M. [6].
The fullerene molecules were embedded in an ethyl cellulose according to the following proportions: C_{70} : 0.5 mg / Ethyl cellulose : 50 mg / Toluene : 1 g.
The solution was then coated onto polyester films, even pre-treated with O_2-plasma, PET and PVC.
Unfortunately, no fluorescence was detected, neither in air, nor in deoxygenized water.

Palladium based porphyrin (Pd-TFPP) – this molecule was proposed in [4], as well as the previous one, and it's characterized by a shorter lifetime (estimated at 978 µs in [5]) and a smaller K_{SV}, if compared to fullerene [4]. For the preparation of the sensing layer, we strictly applied the process described in [5].
Prof. Sergey Borisov, co-author of [5], was contacted and suggested an improvement by replacing the silica-gel with polystyrene for the fluorophore embedding.
The final proportions adopted in our solution are: Pd-TFPP : 67 mg / Polystyrene : 393 mg / Chloroform : 5900 mg. As before, this solution was coated on polyester film.

Figure 8: High density Pd-TFPP emitting fluorescent light in water under UV light

Pd-TFPP presents a nice fluorescence in water but completely quenched in ambient air.

Platinum based porphyrin (Pt-TFPP) – for this molecule, as well, we based our preparation on [5] and from advices directly received from the above-mentioned prof. Borisov.
The final proportions used for our tests are: Pt-TFPP : 20.4 mg / Polystyrene : 394 mg / Chloroform : 5170 mg.

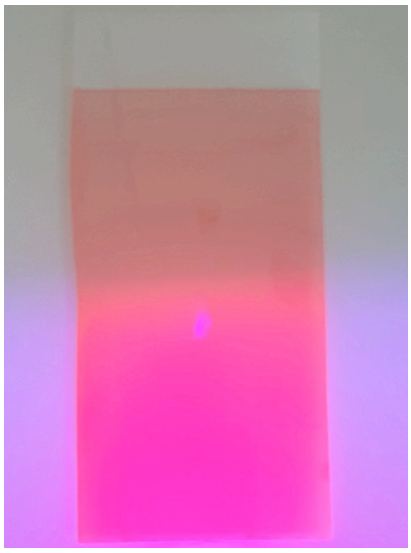


Figure 9: Pt-TFPP coated sample partially exposed to UV light in ambient air (high density solution)

Pt-TFPP presents a nice fluorescence not completely quenched in air, resulting to be not enough sensible to the dissolved oxygen concentration in water.

Conclusion - Pd-TFPP resulted to be the best option for our device.

V. MEASUREMENT SYSTEM VERSIONS

1. FIRST PROTOTYPE

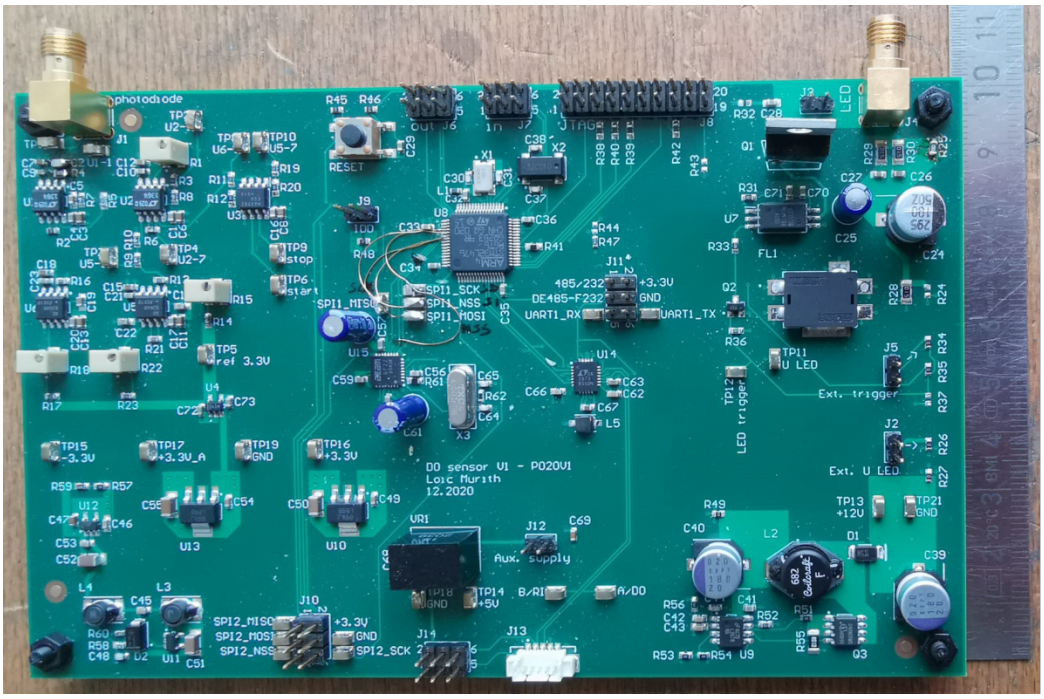


Figure 10: prototype #1

Our first prototype was a large PCB, 160mm x 100mm in size, intended for preliminary lab tests. Plenty of test points have been added to ease system debugging; the large size also allowed us to detect and correct some early stage mistakes (visible in the picture above).

The larger design was also considered at this stage for a faster PCB design.

During the successful testing of this prototype, some improvement included in the following design have been tested, such as:

- threshold voltage levels for START and STOP signal provided by microcontroller internal DAC
- modification to the SPI bus wiring (necessary because of the previous point)

The system proved to be fully functional.

2. SECOND PROTOTYPE

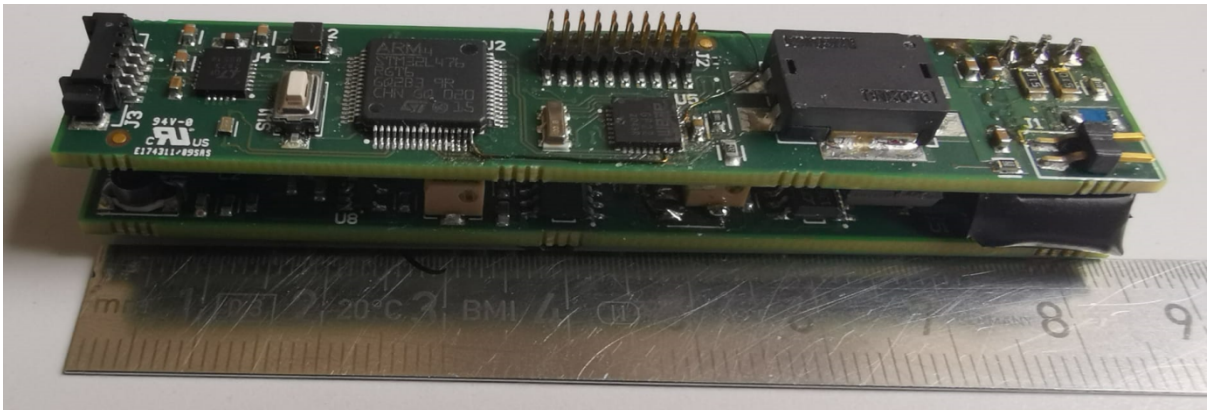


Figure 11: prototype #2

The second prototype presents a notable reduction in terms of size, compared to the previous version. NKE suggested us to modify the prototype #1 in order to fit it within one of their standard waterproof casing; the available volume was a cylinder of 20mm in diameter and 90mm in length.

To fit in such a tiny space, we needed to split the circuit in two superposed PCB resulting in a final size of 16.5mm x 17.5mm x 85mm.

Although some minor design errors have been detected, the system, again, proved to be fully functional.

3. FINAL PROTOTYPE

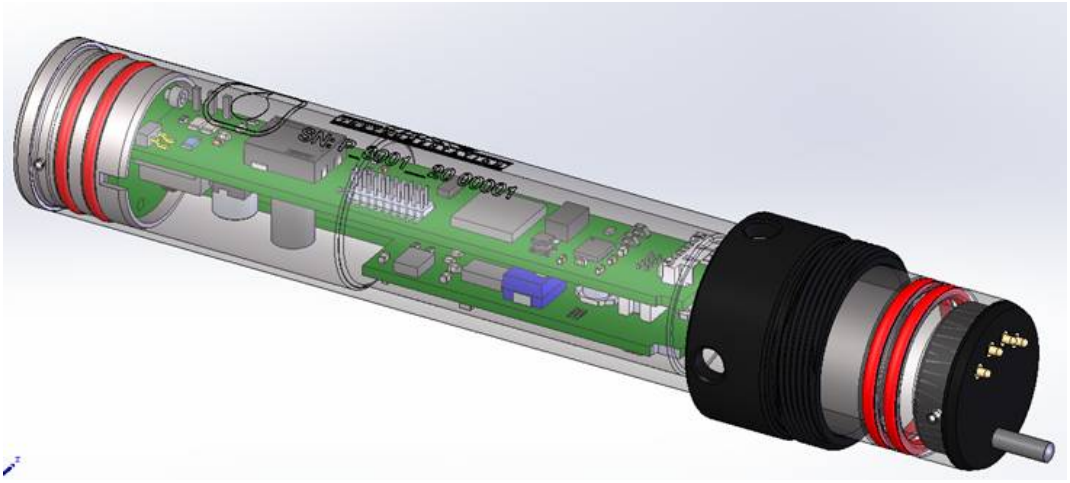


Figure 12: the final prototype

The final version of the PCB is based on minor modifications of the previous one:

- PCB connectors have been changed from SMD to THT ones to increase the global robustness of the system
- A third round PCB has been designed to host LED diode and photodiode

The schematic of the current solution are included in the annexes of this document.
This final prototype is currently under test.

VI. SUMMARY

In this task we developed a dissolved oxygen measurement system based on optical measurement of fluorescence quenching. The innovations related to the proposed solution are mainly based on:

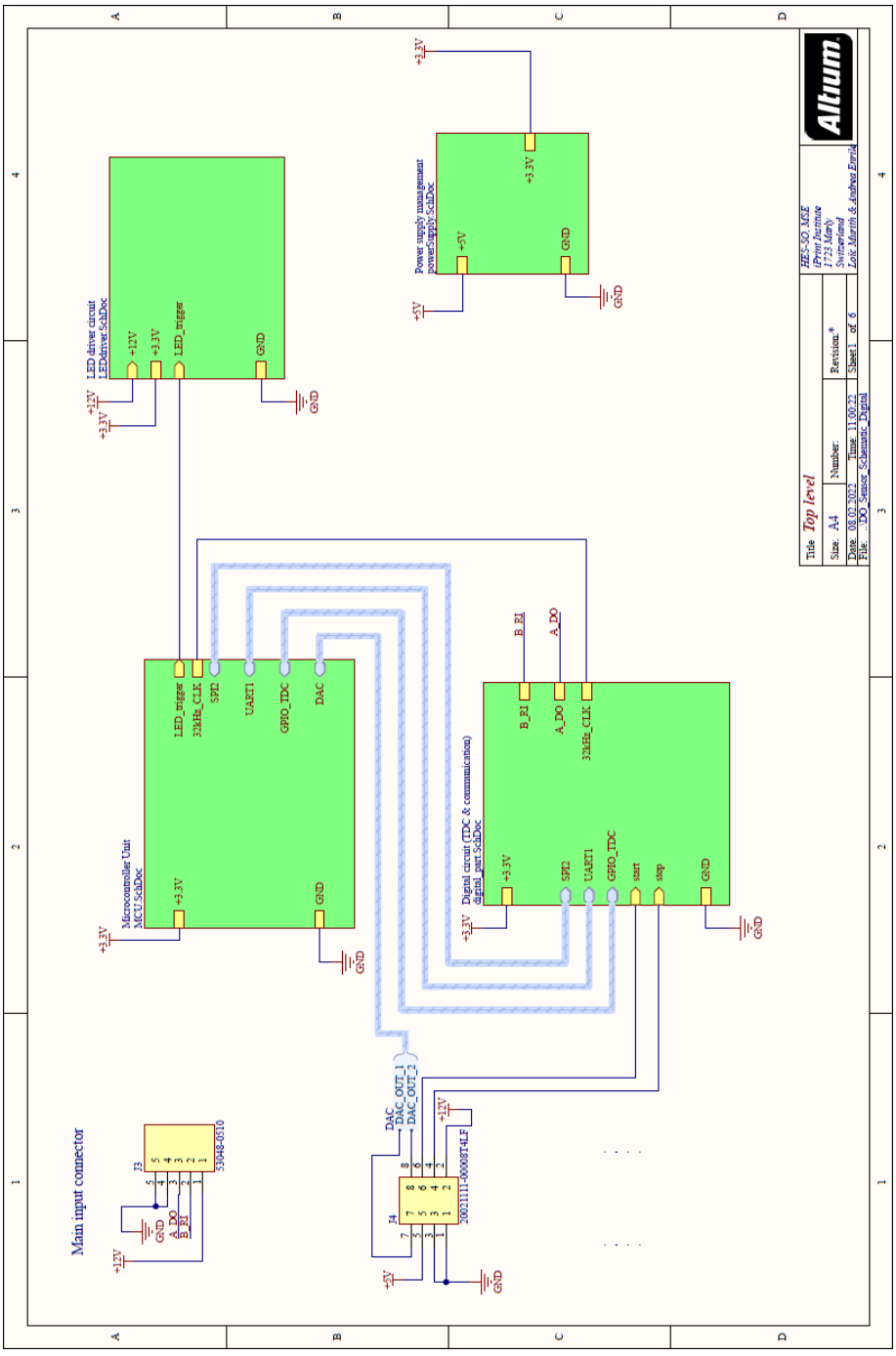
- Use of a novel technique, for estimating the fluorescence decay time directly from the exponential pulse response instead of a standard phase difference measurement; this solution removes the need for a selective optical filter to remove the contribution of the excitation light.
- Use of a high performance TDC, such as the ACAM GP22, with a time resolution of 22ps, resulting in a considerable increase of the sensitivity of the system.

Aggressive reduction of the final size was not intended for this project; however, we attempt and achieved a considerable shrinking in the size of the system capable to fit within a standard waterproof casing provided by NKE.
Three different versions of the system have been designed, fabricated and successfully tested.
We consider for the electrical part of this system to have reached a TRL level of 9. For the fluorophores handling, preparation and coating, we consider to be at a TRL level of 8.

APPENDIX 1: REFERENCES AND RELATED DOCUMENTS

ID	Reference or Related Document	Source or Link/Location
1	Chodavarapu V., Shubin D., Bukowski R., Titus A., Cartwright A., Bright F., "CMOS-Based Phase Fluorometric Oxygen Sensor System", 2007	*https://ieeexplore.ieee.org/document/4061008*
2	*Wikipedia on Clark Cells*	*https://en.wikipedia.org/wiki/Clark_electrode*
3	Mazza, M., Pirrami, L., Wicht, J., Rosspeinter, A., Debrot, F. & Aebisher J.-N. "Oxygen sensor for indirect calorimetry based on ruthenium fluorescence quenching" 2011	*https://ieeexplore.ieee.org/document/6095733*
4	Lehner, P., Staudinger, C., Borisov, S. M. & Klimant, I. "Ultra-sensitive optical oxygen sensors for characterization of nearly anoxic systems" 2014	*https://www.nature.com/articles/ncomms5460*
5	Borisov, S. M., Lehner, P. & Klimant, I. "Novel optical trace oxygen sensors based on platinum(II) and palladium(II) complexes with 5,10,15,20-meso-tatrakis-(2,3,4,5,6-pentafluorphenyl)-porphyrin covalently immobilized on silica-gel particles." 2011	*https://pubmed.ncbi.nlm.nih.gov/21414443/#:~:text=New%20optical%20sensors%20for%20trace,are%20dispersed%20in%20silicone%20rubber.*
6	Nagl, S., Baleizão, C. & Borisov, S. M. "Optical sensing and imaging of trace oxygen with record response" 2007	*https://pubmed.ncbi.nlm.nih.gov/17323397/*
7	McDonagh, C., Kolle, C., McEvoy, A. K., Dowling, D. L., Cafolla, A. A., Cullen, S.J. & MacCraith, B. D. "Phase fluorometric dissolved oxygen sensor" 2001	*https://www.sciencedirect.com/science/article/abs/pii/S0925400500007218*
8	Davenport, J. J., Hickey, M., Phillips, J. P. & Kyriacou P. A. "Fiber-optic fluorescence-quenching oxygen partial pressure sensor using platinum actaethylporphyrin" 2016	*https://pubmed.ncbi.nlm.nih.gov/27463913/*

APPENDIX 2: FINAL PCB VERSION SCHEMATICS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J4
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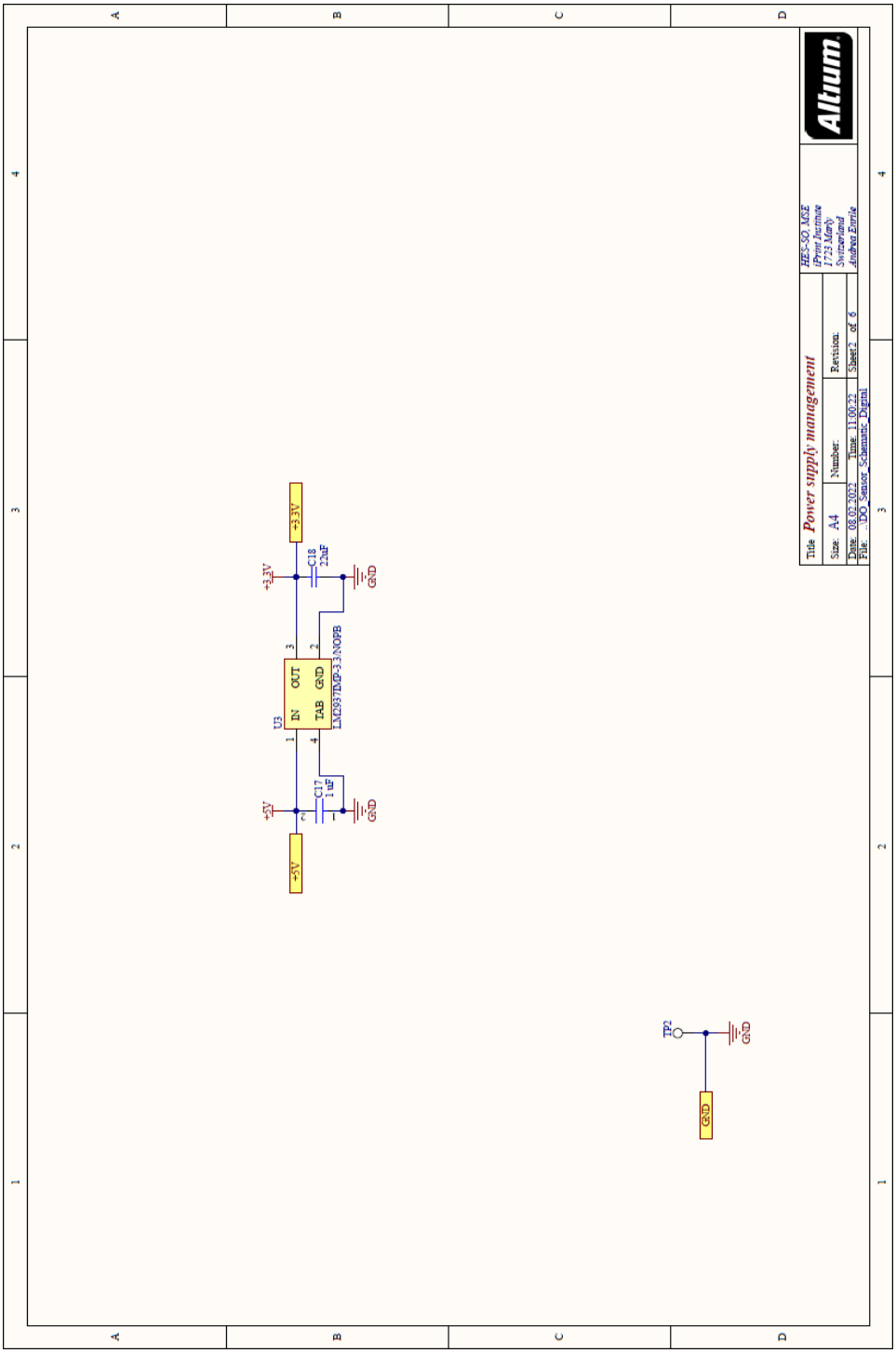
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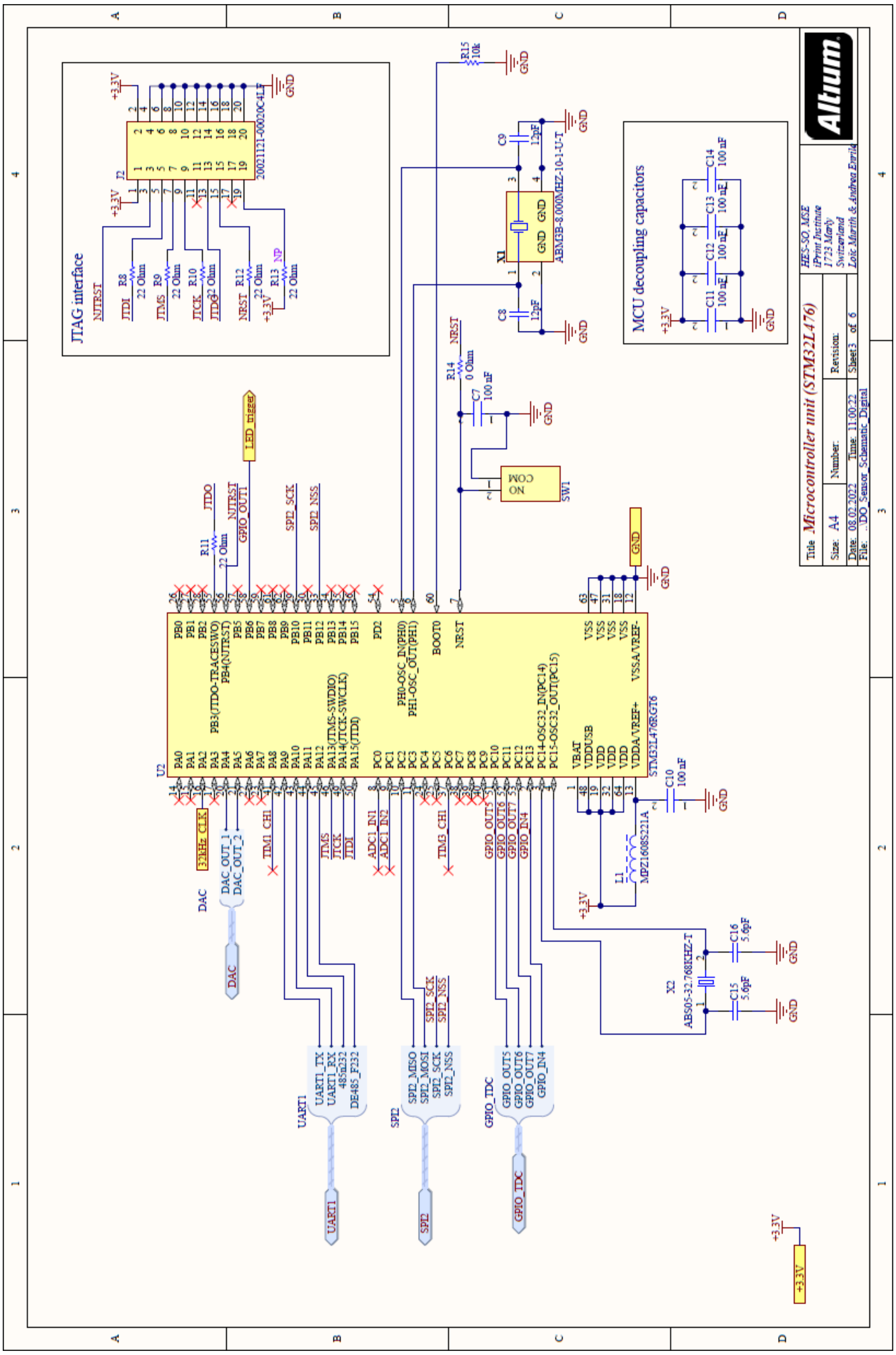
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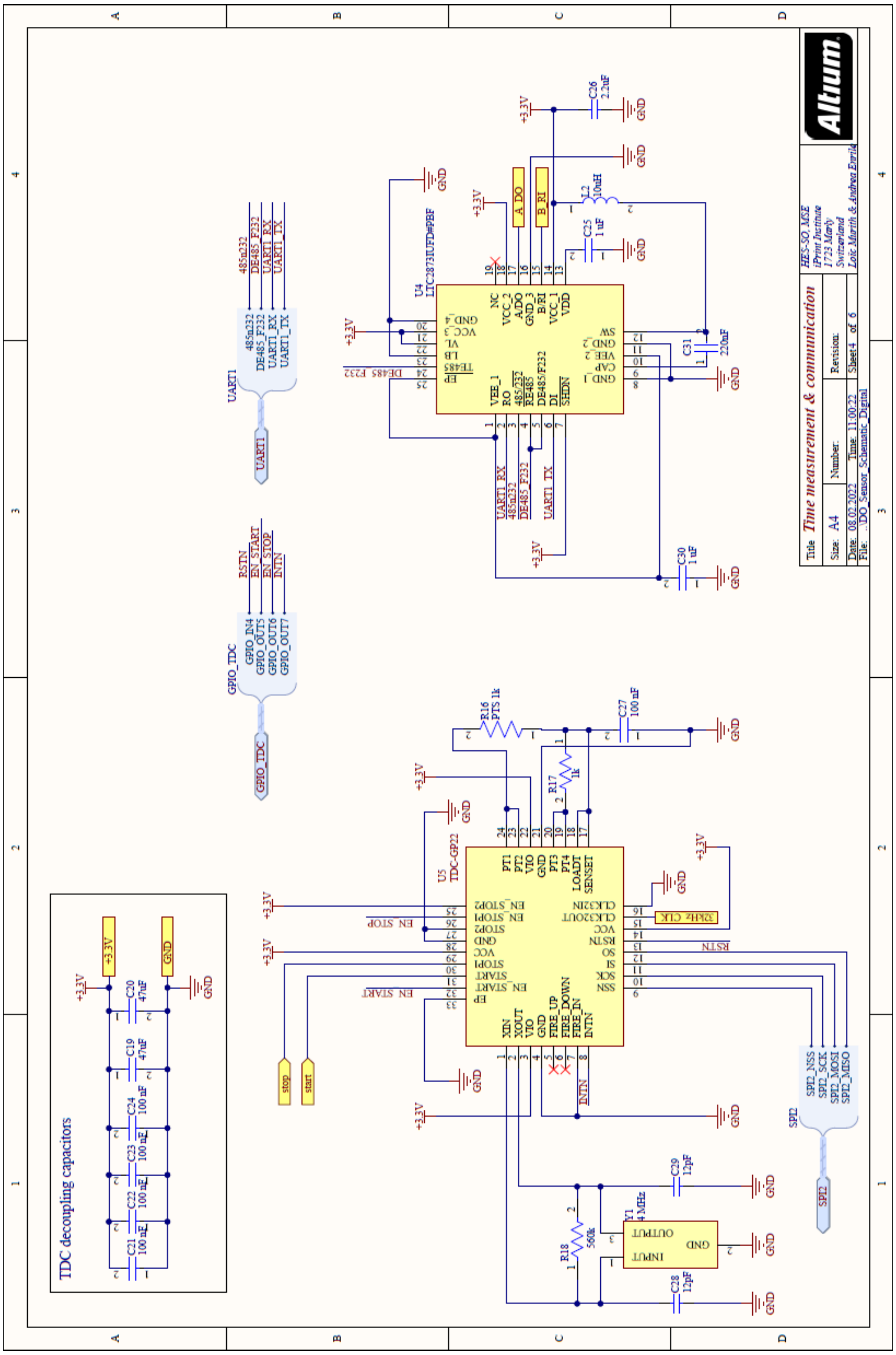
Loïc Marith & Andrea Ewrila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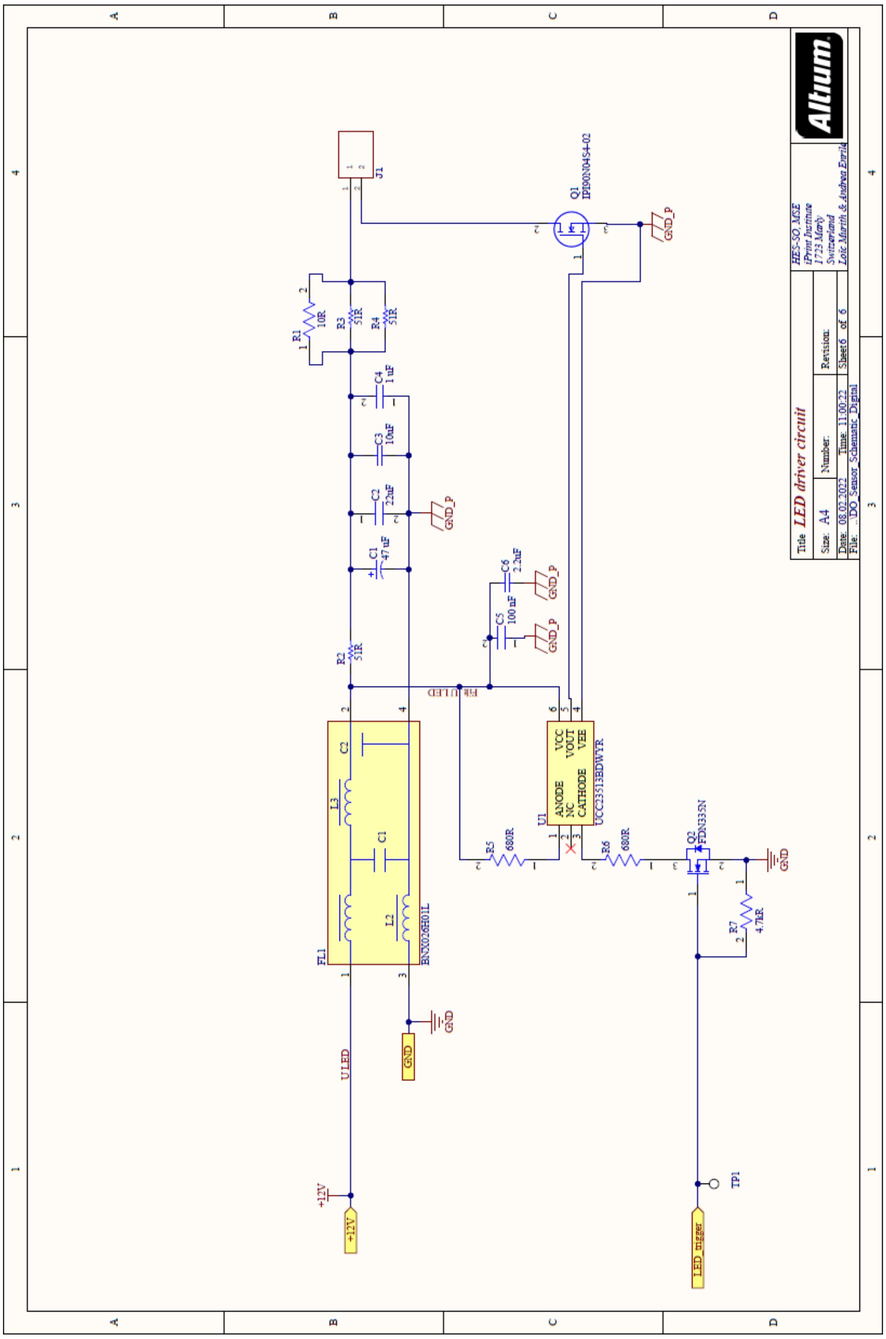
Switzerland
Altium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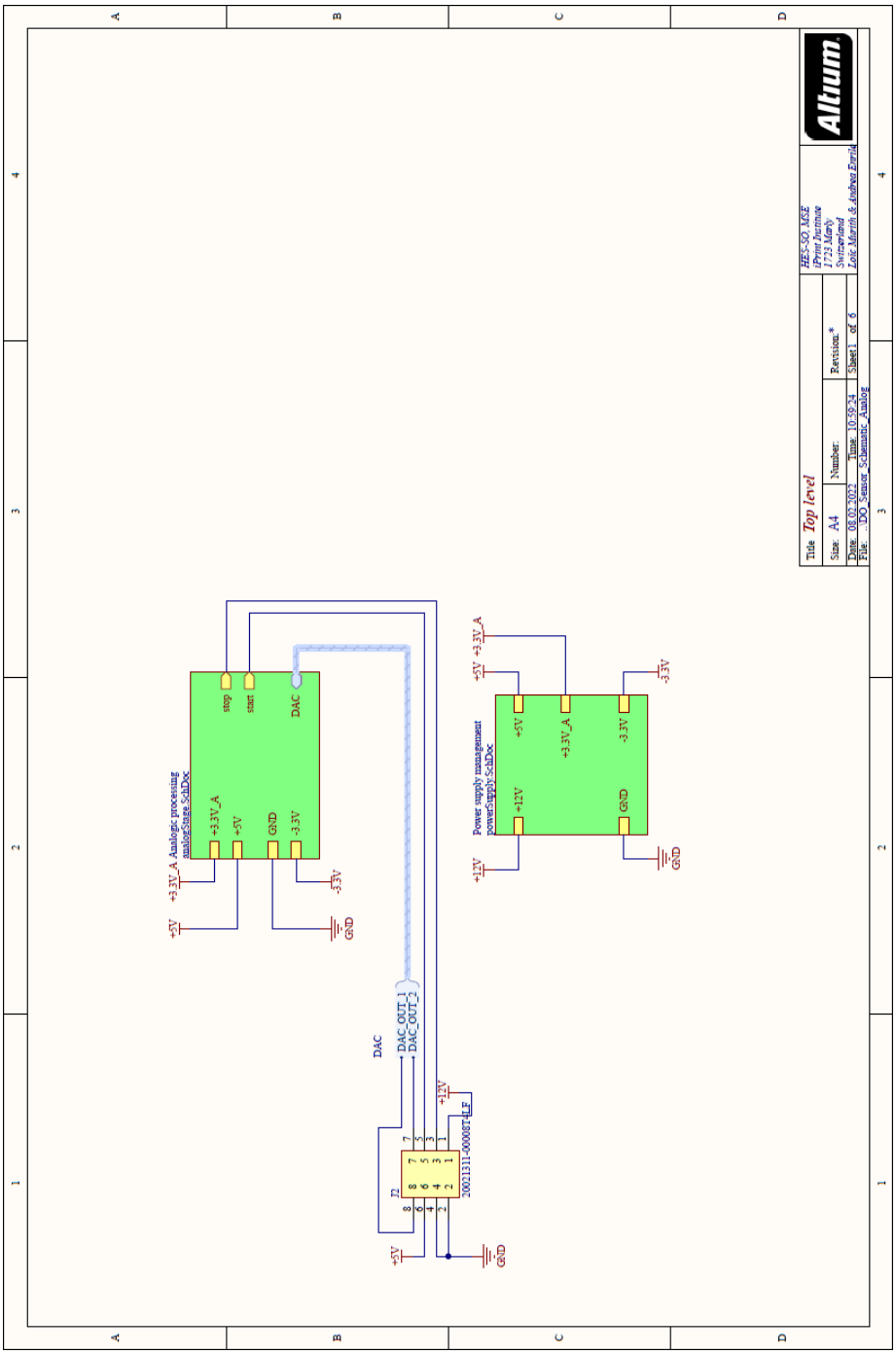
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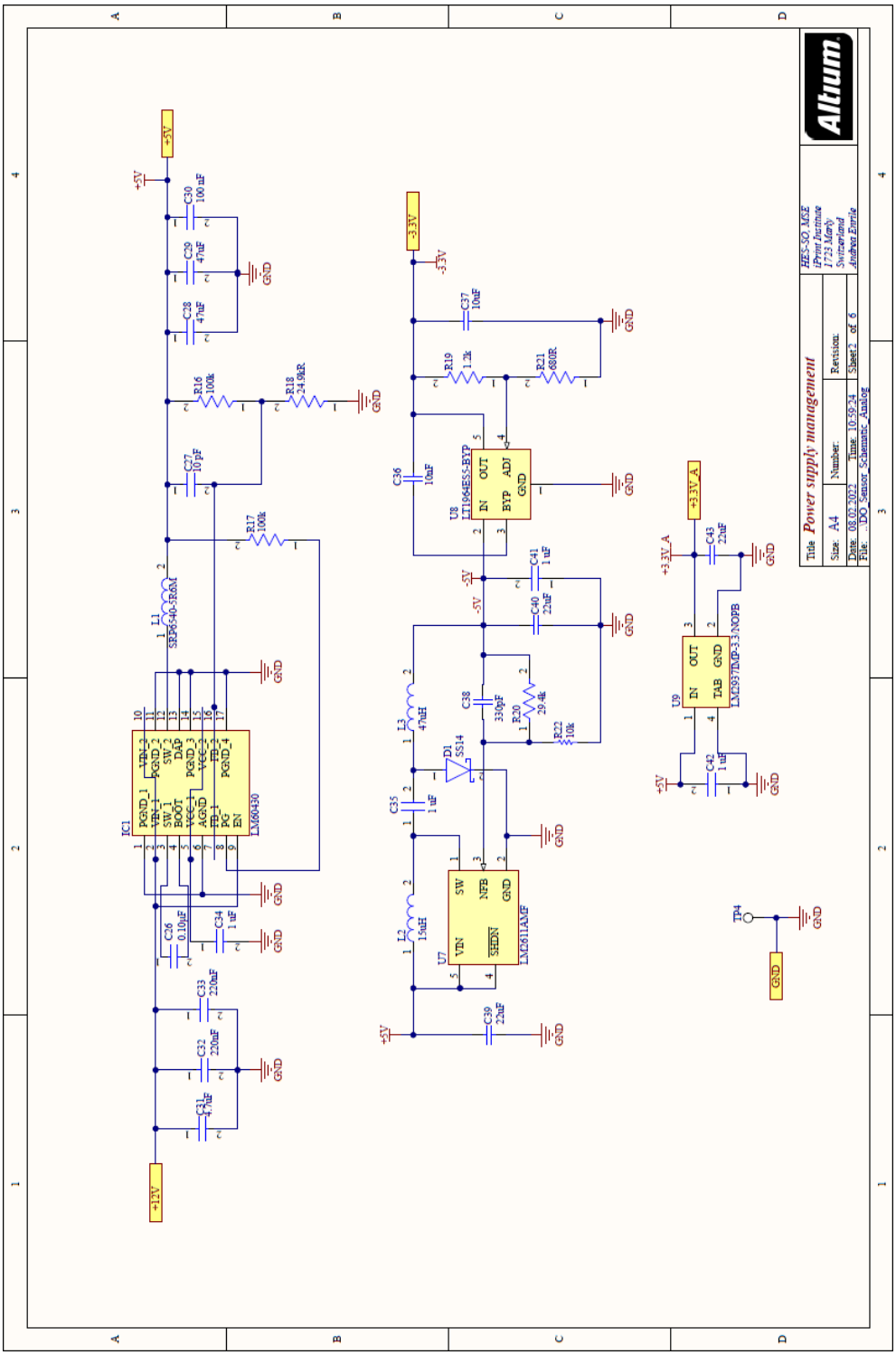
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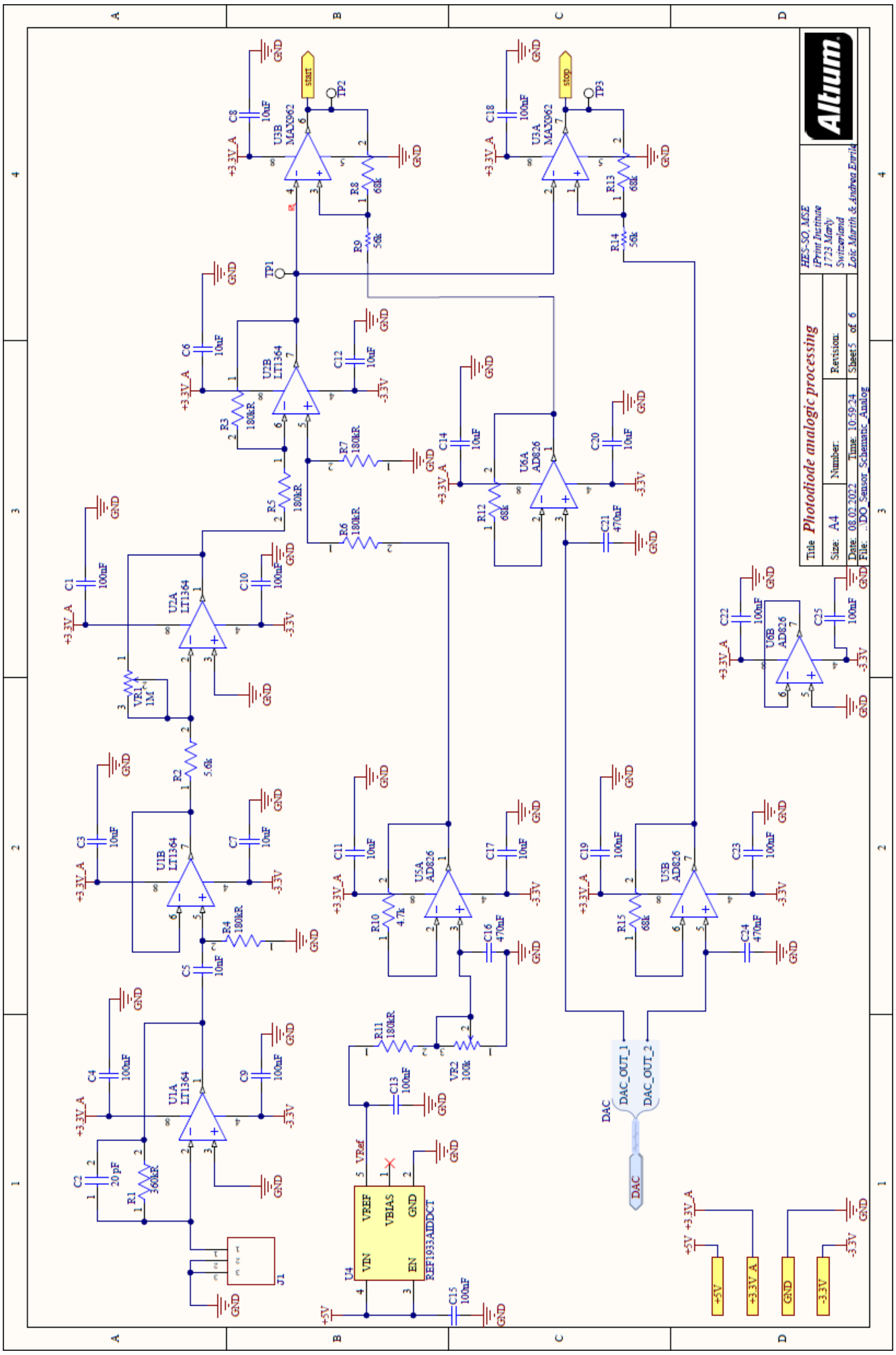
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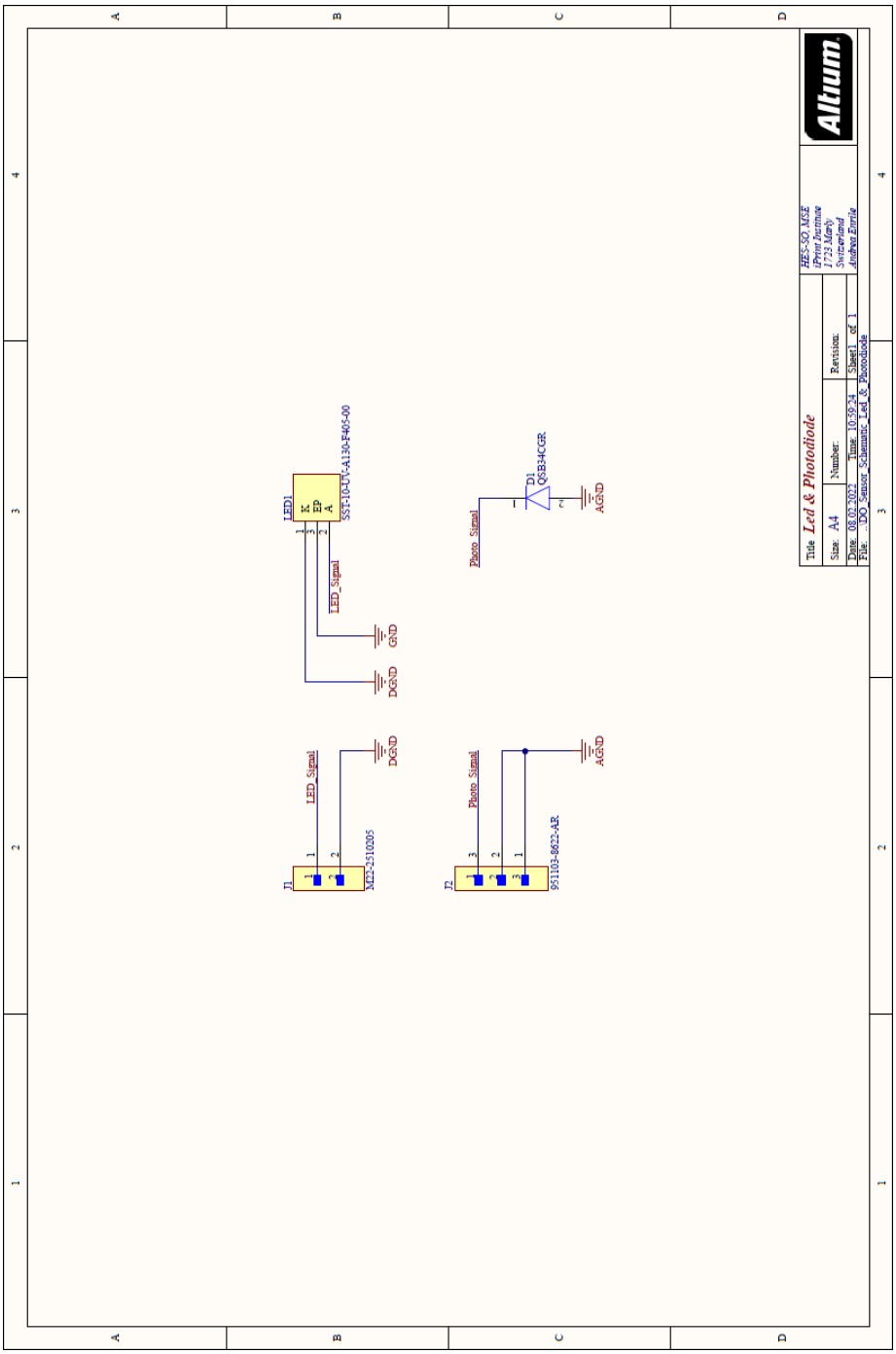
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