A smart-sensing AI-driven platform for scalable, low-cost hydroponic units

D2.2 Prototype of Nutrient-content Kit

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ACRONYMS LIST

BB-MZI	Broad-band Mach-Zehnder Interferometry (or Interferometer)
CHSK	Crop-Helath Sensor Kit
CSU	Communications and Storage Unit
DIY	Do it yourself
MMSK	Multi-modal Sensor Kit
NCK	Nutrient-Content Kit
SW-MZI	Single-wavelength Mach-Zehnder Interferometry (or Interferometer)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

GOhydro aims at developing a cost-efficient smart-sensing ICT platform capable of monitoring the crops' health and nutrient content of hydroponically cultivated microgreens in order to optimise the cultivation process and allow the harvest of the best possible products. GOhydro aspires to culminate in the production of a platform that will be a shifting paradigm of how AI-driven technological innovation can become an affordable, accessible-by-all tool applicable to all forms of urban farming. Towards this, the project will produce a multi-modal sensor kit driven by a thorough analysis of nutritional and lighting requirements of microgreens and combine it with a multi-model machine learning solution that will guide growers to optimise microgreens tending in accordance with the environment where their hydroponic unit is installed and operates. In a nutshell, the project aims at creating a form of an easy-to-use e-agronomist which will assist any grower to fine-tune and optimise her hydroponic production.

One of the main technical objectives of the GOhydro project is the development of a Multi-modal Sensor Kit (MMSK) capable of monitoring at pre-determined intervals the environmental factors affecting the hydroponic cultures of microgreens, the quality of the water feeding the plants as well as the nutrient content of the microgreens. This kit has as a main task to collect information about the cultivation conditions and the quality of the microgreens and communicate the relevant information to the mobile application of the agronomist that will notify the user about the health of the crops and provide suggestions about necessary condition arrangements. Towards that end the MMSK is divided into 3 major components: **(1) the Crop Heath Sensor Kit (CHSK)** responsible for monitoring the temperature, humidity, light intensity at the installation as well as the electroconducitvity and PH of the water in the tank (indicating the quality of the watering), (**2**) a photonics-based kit that will determine the **nutrient content** of the microgreens through their pulps (**Nutrient-content Kit, NCK**), and (3) **the communications and storage unit (CSU)** that stores and transmits the data collected by the kits to the GOhydro data-driven platform. The MMSK is developed through a dedicated work package (WP2), which has received input from WP1 "Nutrient and environmental needs for microgreens" with regards to the specific parameters that must be monitored for a successful hydroponic installation.

This deliverable entails the development of the second component of the MMSK, namely the Nutrient-content Kit (NCK) and is the outcome of Task 2.2. Even though the actual deliverable D2.2 is a DEMONSTRATOR, this accompanying document analyses how the proprietary photonics-based sensor of NCSRD is explored as an alternative method that may provide indirect information about the nutrient-content of hydroponically-cultivated microgreens. D2.2 contains three basic chapters. The first one describes the underlying concept and hypothesis, the second the laboratory prototype developed and the first experiments with the NCK and the final chapter the future actions necessary for the completion of the system and to the validation of the working hypothesis as well as its possible limitations.

1 NUTRIENT-CONTENT KIT UNDERLYING CONCEPT

GOhydro as a whole aims at developing a cost-efficient smart-sensing ICT platform capable of monitoring the crops' health and nutrient content of hydroponically cultivated microgreens in order to optimize the cultivation process and allow the harvest of the best possible products in an urban setting. GOhydro aspires to culminate in the production of a platform that will be a shifting paradigm of how AI-driven technological innovation can become an affordable, accessible-by-all tool, applicable to all forms of urban farming. As already described in detail in D2.1, the GOhydro platform has a "dual core", one that consists of the platform's hardware and one that consists of the AI component. The former, collectively described as the MMSK, is the core that in essence monitors, collects and transmits the data pertinent to the health and nutrient content of the hydroponically cultivated microgreens and can be envisaged as the "front-end" of the GOhydro platform responsible for the continuous monitoring of all parameters for the successful cultivation of plants at the hydroponic installation (Fig. 1.1). Apart from the monitoring and collection of data, this front-end will also be responsible for the data transmission to the computational backend of the platform.

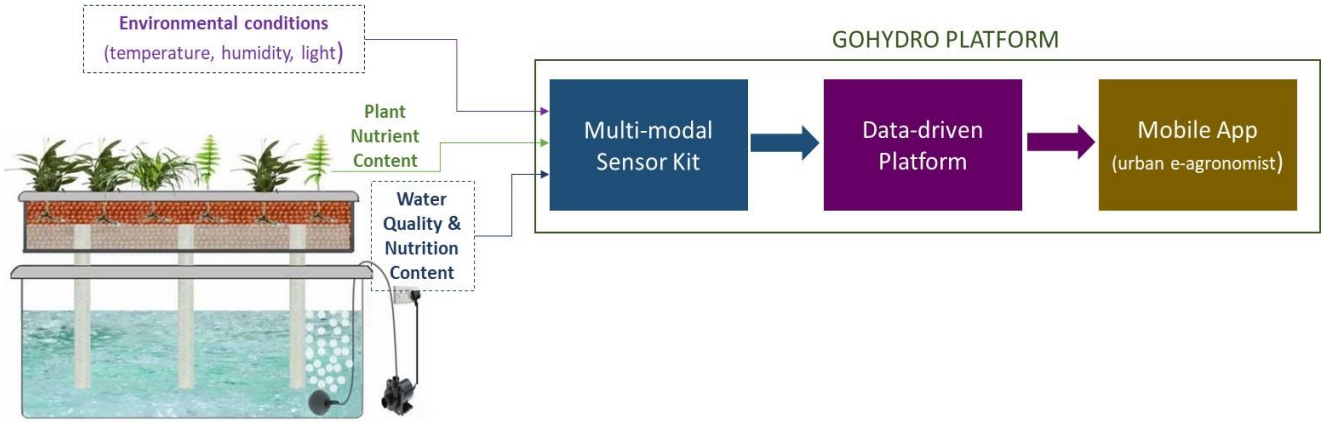


Figure 1.1. Schematic Representation of the GOhydro platform concept. The NCK is indicated with the green colour and complements the CHSK, represented by the dashed boxes. The NCK along with the CHSK and the CSU collectively serve as the front-end (input) of the GOhydro platform.

One of the major ambitions of GOhydro was to include within this frontend MMSK a radically innovative component, namely the NCK. Even though the first half of the GOhydro MMSK responsible for the environmental parameter monitoring is based around the concept of an affordable, DIY kit that the end-user can assemble himself/herself from off-the-shelf components, the NCK due to its innovative concept is the component that verges on "blue sky" research and is currently exploring the capability of state-of-the-art photonic circuits to provide indirect information about the nutrient content of microgreens through the dynamic evolution of spectra obtained via microgreen pulps. The principle of operation and the details of specifications and requirements were presented in detail in D2.1. For the sake of clarity though, these basic concepts are revisited briefly in this introduction.

During the past two decades, the progress in micro/nano-fabrication techniques and in-depth understanding of photonic circuits have allowed the "transfer" of optical biosensing modules into Silicon-based photonic integrated circuits (PICs). The key driver for such a choice is the growing -but still unmet- need for practical biosensors satisfying the growing demand for effective medical diagnostic technologies. Silicon-based solutions take advantage of three major parameters: (1) due to the compatibility with complementary metal-oxide semiconductor (CMOS) foundry processes, silicon PICs can be manufactured with great efficiency at high volume and relatively-low cost; (2) the high refractive index contrast between silicon and silicon dioxide/silicon nitride, or other surrounding media, enables the development of miniaturized compact sensing devices, with the additional possibility of fabricating multiple sensors on one single chip; and (3) silicon photonics are excellent transducers for continuous and quantitative label-free biosensing, which can directly respond to affinity interactions between analyte and receptor molecules in real-time.

Nonetheless, despite the impressive progress the existing Si PICs have not managed to escape the laboratory settings and satisfy the need for efficient point-of-interest solutions. The reason is that Silicon inherently does not emit light and there is always the need to find a way to couple light in- and out-of-the PIC chips. Thus, even though the chips themselves are miniaturized and compact, the fact that their driving and readout system requires a laboratory setup makes the chip miniaturization and improved analytical performance almost seem futile and the use of PIC sensors in everyday life impractical. The need for large laboratory equipment gives rise to the so called "chip-in-a-lab dilemma" for PIC sensors. To transform them from laboratory-based demonstrations into practical devices that can be commercialized and used easily in everyday life, it is necessary to develop a compact PIC sensor system, where not only the sensor chip itself, but its readout system is also compact and easy to operate by a non-expert.

NCSRD has found a way out of the above dilemma by developing and patented (Patents: GR20160100552A; US2020064260A1; PCT: WO2018078404A1; EPO Patent Application: EP3532825A1) a way to transform the photonic chips into re-usable consumables that could be used in a similar manner to immunochromatographic strips. These was achieved in a two-fold way: (1) by appropriate photonic engineering of the PICs that allow their operation in a dip-stick manner and alleviate the need for microfluidic compartments, pumps and wires (Fig. 1.2), and (2) by developing a new principle of operation, the so-called Broad-band Mach-Zehnder Interferometry (BB-MZI), which allows the system to function with a simple high-brightness LED and for each chip to be self-referenced. As a result, the system basically relies on coupling light to the photonic chip, which is used as an immersible probe, reading a spectrum with a portable spectrophotometer and analysing in real-time (a few minutes) the recorded spectra. The dynamic behaviour of the recorded spectra is directly related to the concentration of the targeted analyte upon customisable surface activation of the sensor surface with the appropriate probe molecules (allowing thus label-free detection formats).

Figure 1.2. Photograph of the photonic chip immersed directly in 2μl of a liquid sample demonstrating the dip-'n'-read measuring format

The innovative approach that this technology wishes to follow in the GOhydro project is to employ the immersible photonic chips containing BB-MZIs without any surface activation and record the dynamically-evolving interferometric spectra that are created due to adhesion of the various molecules contained in a plant pulp. With the aid of the GOhydro AI component a correlation between the shape and time evolution of the spectra and the nutrient content or crop health of the microgreens may be established. Therefore, the envisioned application is that at regular intervals microgreens will be taken from the hydroponic installation, mashed into pulp in a very regular solvent (acetone), the silicon chips will be immersed in the pulps and the recorded spectra will be fed to the algorithm that already contains the information from the CHSK establishing a connection between plant health and environmental conditions and water quality.

As such, the NCK does not follow the DIY concept that the rest of the platform has, and it represents a more "sophisticated" tool of comparatively higher cost. Nonetheless, the system is designed around the following four basic concepts:

(1) the system is user-friendly, and its deployment is based on simple steps that anyone can perform at any urban setting,

(2) the photonic components (chips) are re-usable (at least 50 times or until broken) compressing the initial cost and reducing the *cost per analysis* to a very small fraction of the purchase cost of the kit,

(3) the NCK can be envisaged as a shared kit among a community that can collectively purchase it and use it in turns (e.g., in a condominium that collectively grows microgreens in a communal space or in each apartment). That way the cost could be split among the users when purchased and which can be used for years without need for any consumables.

(4) the most costly component of the NCK is the spectrophotometer used for the recording of the dynamically-evolving spectra (see details in D2.1). However, this cost is expected to decrease because (a) of the rapid progress of photonic systems that is expected to reduce the prices in the future, (b) if the NCK is uptaken for production, the cost will decrease as the number of orders goes up (economy of scale), and (c) it might be proven that a lower performance (and hence lower price) spectrophotometer might be adequate for the envisioned scope. Therefore, it is expected that the final cost of the NCK will be reduced in the foreseeable future.

Keeping these concepts in mind, a proof-of-concept setup was first developed and used to test the hypothesis of the correlation of the output spectra to the nature and nutrient content of the microgreens using basil leaves from the first GOhydro hydroponic installation (SCiO premises, Greece). This is described in Section 2.

It should be noted at this point that D2.2 faced some delays and is not 100% completed by M12 as foreseen in the proposal. This delay is due to severe delays in funding of NCSRD by GRSI, which have not allowed the purchase of dedicated equipment and hiring of personnel. All activities of WP2 have been so far realized with some allocation of internal funds (covering mainly the fabrication of the photonic chips), equipment borrowed from other experimental setups and partial allocation of manpower. As a consequence, there is a shift in the validation experiments of about 3 months and the full validation is expected to be finished by M15. The planning and concepts to be tested are analysed in Section 3.1.

In addition, in terms of the design it was decided by the GOhydro partners and especially by the 3 partners heavily involved in WP2 (NCSRD, nr21 and SCiO) to prioritize the design of the Crop Health Sensor Kit (D2.3) over the design of the NCK. The reasons behind this choice were: (1) The CHSK is the GOhydro component is the essential DIY kit, which can be more readily transferred to commercialization, while the NCK is a high-risk component that first needs to be validated as a proof-of-concept. Hence, it was deemed more efficient and productive to finalize the first. (2) The proposal foresaw the delivery of 3 CHSK at M15 for the 3 installations in Greece, Denmark and Romania (USAMV). However, there was an expressed interest by one more partner (Holisun) to create an additional hydroponic installation in Romania, therefore the number of CHSK to be assembled increased to 4 and some increased effort will be required on that front. (3) A preliminary ideation on the NCK was realized and initial designs are under consideration. However, since the NCK needs to be validated first as a concept and since the protocols will be developed during the validation experiments, it was realized that the user experience from the proof-of-concept setup and the subsequent finalization of protocols would be a better guide for the kit design in order to render it efficient and user-friendly. The ideation results and initial designs are presented in Section 3.2.

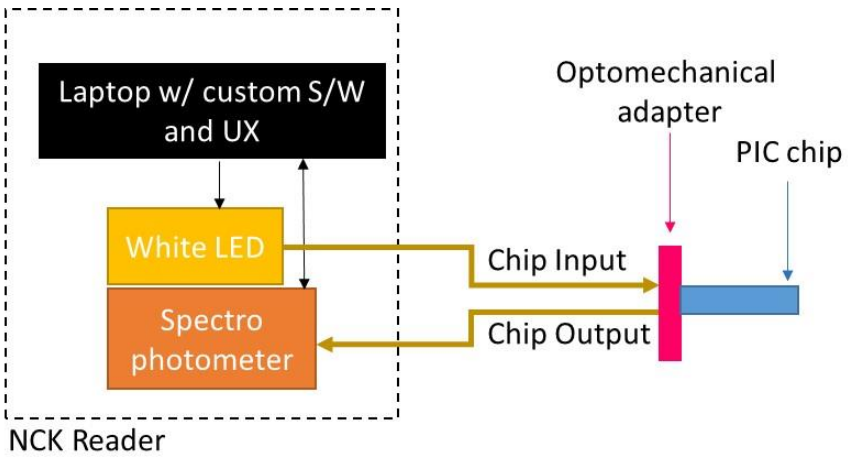
2 NUTRIENT-CONTENT KIT PRELIMINARY EXPERIMENTS

2.1 NUTRIENT-CONTENT KIT DESCRIPTION

The NCK components were selected and specified in D2.1 and its chosen architecture is schematically depicted in Fig. 2.1a. At the moment the system is not being operated though by the foreseen battery (power bank), nor is it controlled by a microprocessor. Powering is taken of through a USB connection a laptop that contains the operating software and user interface (UX). The reason is that this "dis-assembled" format is more efficient to conduct the experiments and process the data until the sampling and measuring protocols are established (see section 2.2 and Chapter 3). Data recording, extraction and post-processing is more straightforward through the dedicated software and UX and the collective results will enable the final programming of the microcontroller, the format of the output data as well as the specifics of the AI-based processing algorithm that will be developed. Table 1 compiles the components of the NCK as they are used in the dis-assembled format until the establishment of protocols and as they will be in the final version of the GOhydro MMSK. Photographs or schematics of the various components are shown in Figures 2.2-2.4, while Figure 2.5 shows the current proof-of-concept version employed for the preliminary and validation experiments.

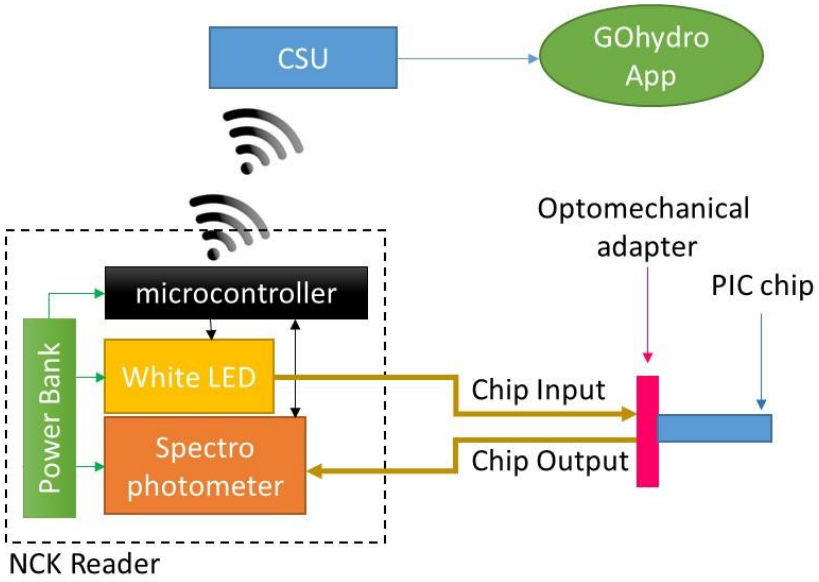
Table 1 NCK Components

	Current (Proof-of-Concept) Version	***Final MMSK Version***
1. Optical Source[1]	White LED with custom-made PCB and integrated heat sink	White LED with custom-made PCB and integrated heat sink
2. Spectrophotometer[1]	Portable spectrophotometer	Portable spectrophotometer
3. Bi-furcated Optical Fiber	Ocean Insight QBIF400-VIS-BX	Ocean Insight QBIF400-VIS-BX
4. Optical Coupling	Proprietary optomechanical adapter (fabricated with 3d printing)	Proprietary optomechanical adapter (fabricated with 3d printing)
5. Power Supply	USB connection to laptop	Xiaomi Redmi 18W Fast Charge 20000mAh
6. Control	Custom S/W and UX installed in laptop and operated in MatLab environment	Raspberry Pi Zero W
7. Data recording	Stored in laptop and post-processed using commercial software	No recording but data transmission directly to CSU



(a)

[1] The precise models and manufacturers of the LED and the spectrophotometer are not disclosed in this document (which is PUBLIC) because they constitute part of NCSRD's technological know-how.

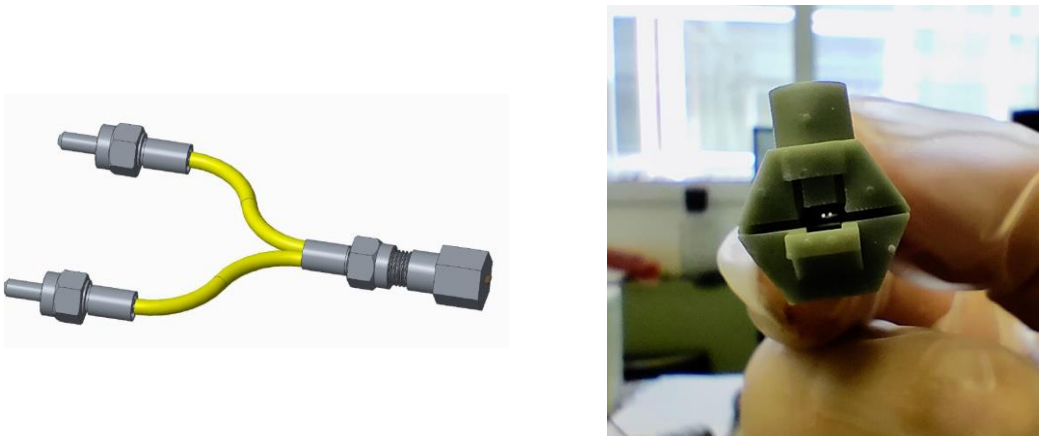


(b)

Figure 2.1. Schematic representations of the NCK system architecture (a) current version for validation experiments and (b) final GOhydro version

(a) **(b)**

Figure 2.2 Photographs of the(a) handheld spectrometer with respect to a palm and (b) the in-house custom housing of the high-brightness white LED on top of the handheld spectrometer. The metallic plate is the heat sink. The PCB is the green plate in the middle.A short bi-furcated fiber is connected to the LED and the spectrometer for demosntration purposes. A lighter is included in the phtograph to better demonstrate the actual size of the components



(a) (b)

Figure 2.3. (a) Schematic of the optomechanical adapter attached to the bi-furcated optical fiber and (b) close-up photograph of the optical adapter where the light from the two branches of the bi-furcated fiber can be seen. The chips are inserted in the recession shown at the front.

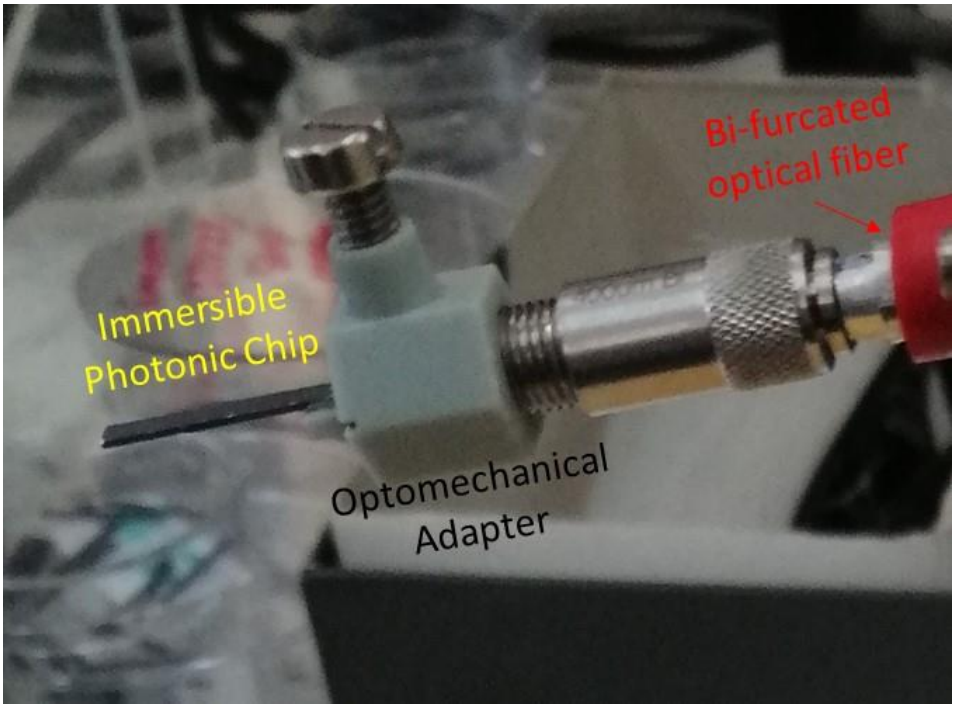


Figure 2.4. Close-up photograph of the immersible photonic chips mounted on the optomechanical adapter that ensures the optical coupling of the input/output ports of the chip (located on the same side of the chip) to the bi-furcated optical fiber

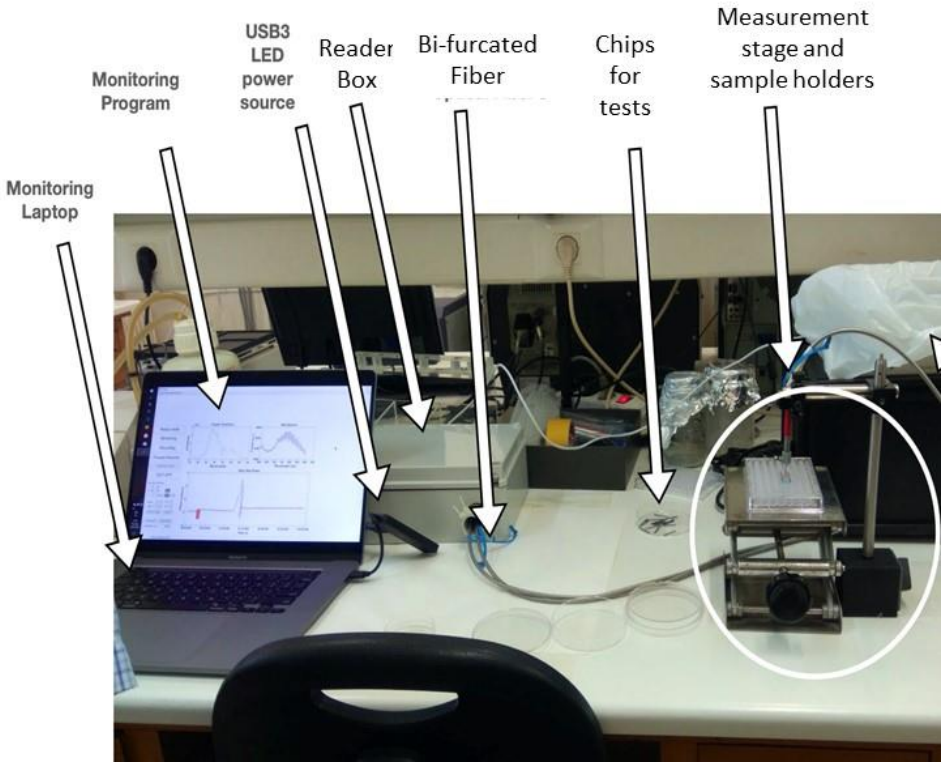


Figure 2.5. Photograph of the proof-of-concept NCK experimental setup

The existing experimental setup *emulates as closely as possible* -in a laboratory setting and with the so far available components- *the envisioned concept of the NCK.* It is used in a dip-stick format with the photonic probes being directly immersed into the sample container. The majority of the components of the proof-of-concept system are the same with the ones that will be used in the final prototype. As already stated, power supply is achieved through a USB connection with a laptop, which also contains the S/W for the system operation. In summary, the proof-of-concept system consists of the following parts:

1. A Reader Box that contains (i) the LED (source), (ii) a spectrophotometer (recording medium), and (iii) a USB hub.
2. A Bi-furcated Optical Fiber, pre-connected to the LED and spectrometers inside the Reader Box. Its "free-end"" is attached to
3. An Optical Adapter, where the chips are to be inserted
4. A L-shaped Fiber Holder (consisted of components routinely unemployed in optical setups) used to rigidly hold the fiber and chip to vertical position. It also allows for simple rotation to the horizontal axis for alignment and chip removal/re-insertion of chips
5. A Lab-jack that serves as a z-positioning stage
6. A Laptop that both powers up the LED and Spectrophotometer and contains the analysis S/W.

In more detail, the optical fiber is fixed in a vertical position with parts routinely used for optical setups. The movable z-stage is used to make the sample approach to the free-end of the Photostick chip and achieve full immersion in the liquid sample. The samples are placed in 1.5ml Eppendorfs. The change from well to well is performed manually with simultaneous lowering of the moveable stage and lateral movement of the tubes and a sequential re-dipping of the chips into the selected tube by raising the stage. The setup is built in such a way that allows the rotation of the fiber with a single move to the horizontal position to exchange the chips. After replacement of the chip, the fiber is returned to its vertical position to begin the measurements with another single move.

The signal recording from the spectrophotometer and partial real-time processing (aka the Fast Fourier Transform and the real time monitoring of the peak phases) are performed through a commercial laptop, which is also used to power up the LED and the spectrometer. A USB hub is used to multiplex the power sources of the LED and spectrometer as well as the spectrometer control and data readout by the laptop. The S/W at this stage shows on screen all "hidden" parts that no end-user will see, aka the raw data (real-time spectra), the FFT real-time monitoring and the time-evolution of the peak phases (Figure 2.6). This is imperative so that the investigators can assess, evaluate and post-process the data and develop the appropriate signal processing algorithm specifically designed for the studies of microgreen pulps. The post-processing of data will result at the end of all evaluation studies to the creation of the appropriate data collection protocol which will be embedded as an add-on to the core of the future version of the S/W.

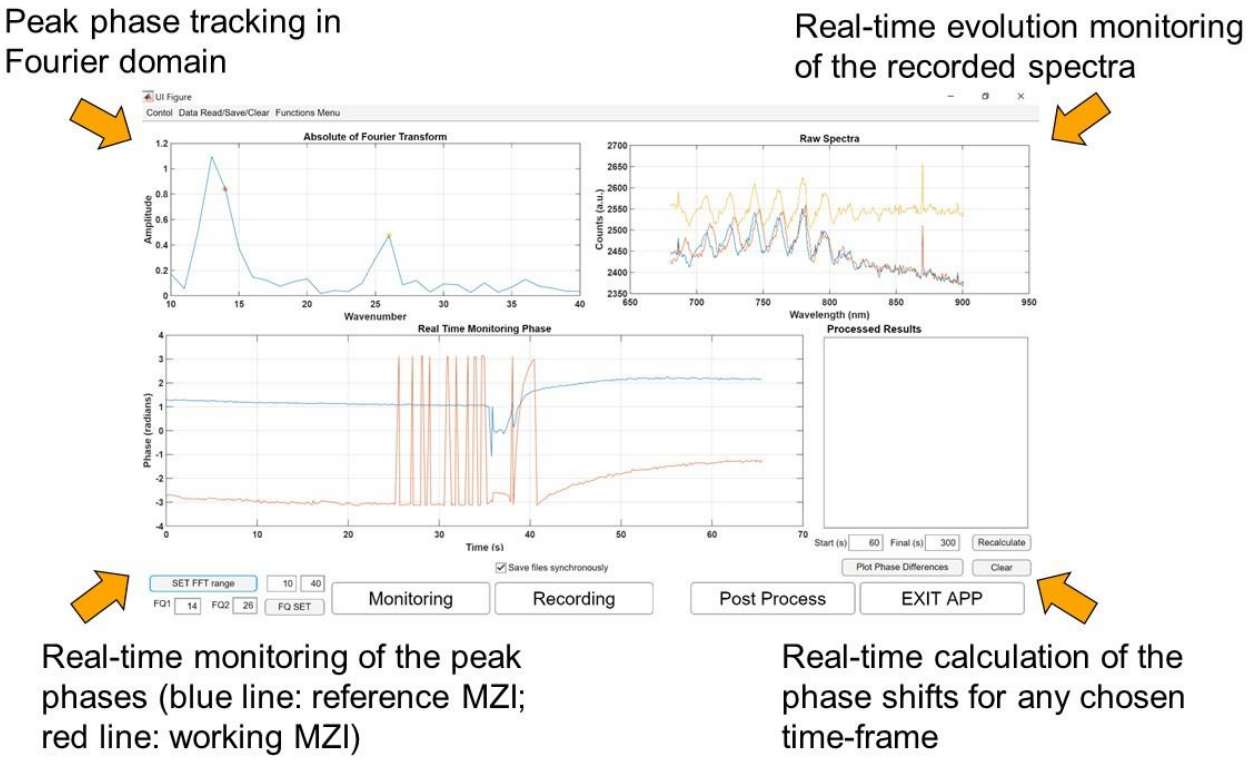


Figure 2.6 Screen-shot of the UX during operation

In more detail, the UX shows on screen in real-time:

(1) the chip output spectrum recorded every 100msec. The spectrum is de-convoluted with respect to the emission spectrum of the LED (top right frame in Fig. 2.6)
(b) the monitoring of the 2 FFT peaks (top left frame in Fig. 2.6). At present the photonic chips contain 2 MZIs, because they were based in older designs. In future versions, specifically fabricated for the GOhydro NCK, it will be decided whether one or two MZIs are necessary for reliable extraction of information. At this point it appears that a single operating MZI is more than enough.
(c) real-time monitoring of the FFT peak phases (bottom left frame in Fig. 2.6). At this point, monitoring the peak phase time evolution is necessary to understand the dynamic adhesion of the various molecules, the point when the MZI active surfaces are saturated by the adhered molecules and to what extent and under which sequential process the surface is thoroughly cleaned and ready to be used with a new sample. This function will not be included in the final UX version once the measuring protocols are established.
(d) the last frame is an extra function that allows to calculate the phase difference and the phase gradients. These calculations may be proven indicative of the time required for each measurement in conjunction with the real-time phase tracking. This variable may be also proven to be necessary for the extraction of information about the nutrient contents.

2.2 PRELIMANRY EXPERIMENTS

Preliminary experiments were carried out to see how the system may be operated. As a first step the goal was to establish whether it is possible through the simple time-evolution and shape of the spectra to distinguish between two different types of basil and specifically, *ocimum basilicum (small-leaf basil)* and *ocimum basilicum "mammoth" (wide-leaf basil)*. The leaves were taken from the GOhydro hydroponic installation in Greece (SciO premises, Fig. 2.7) and used within one hour of their harvesting.

Figure 2.7. Photogrpah of the two varieties of basil microgreen within the GOhydro hydroponic installation at Scio, Greece

The concept behind this experiment was to define whether one could distinguish the two plants by using simple utensils that could be found in any household and simple, low-cost "scientific" parts that any commercially available kit could contain with a minimum cost. Towards that end, the leaves were "mashed" with a simple kitchen pestle and mortar with the aid of acetone (Fig. 2.8). The ratio of leaves to acetone was 1gr of leaves per 3ml of acetone. This "recipe' was selected with the aid of UCHP and the literature review of WP1 among various protocols for sample preparation used in other analytical techniques for plant studies. The pulp was collected with a syringe and the liquid was dispensed into Eppendorf tubes after being filtered through a 400μm-pore filter attached to the syringe (Fig. 2.8). The extracted liquids may be seen in Fig. 2.9. The darker colour liquid corresponds to the small-leaf basil, while the lighter one to the wide-leaf basil (mammoth). The proof-of-concept setup is shown in Fig. 2.10

Figure 2.8. Mashing the basil leaves in acetone with a simple pestle and mortar. A syringe with a filter attached to it was used to dispense the liquid into Eppendorf tubes.

The initial measurements protocol was as follows (Fig. 2.9):

Step 1: The photonic chip was immersed for 60-320sec into acetone to obtain a baseline

Step 2: The chip was then immediately immersed into the basil liquid for 2-5 min and the spectral shifts and phase shifts were monitored

Step 3: The chip was then rinsed for 2min in fresh acetone to see whether the surface was properly rinsed and whether the signal returned to the baseline levels.

Step 4: Steps 1 to 4 were sometimes repeated or alternatively the two basil liquids were interchanged to verify whether the rinsing was adequate and the chip could be sequentially used for different samples.

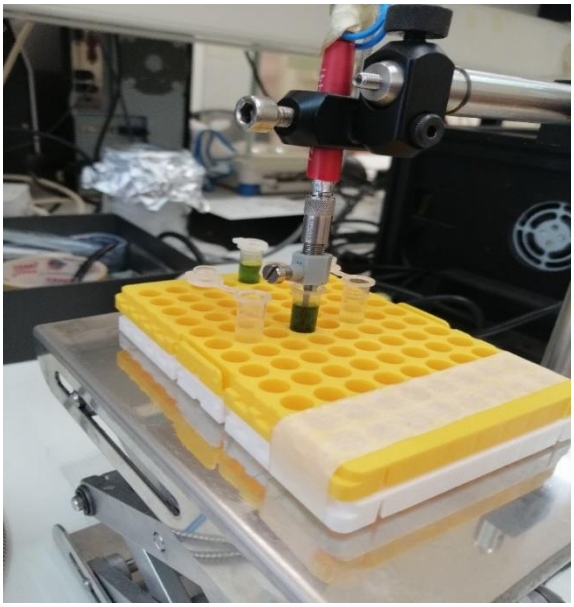


Figure 2.9. Photograph of the extracted basil liquids during a measurement. The sample being measured is the short-leaf basil, which is darker in colour, while in the background the second sample from the wide-leaf basil can be seen distinguished by its lighter colour

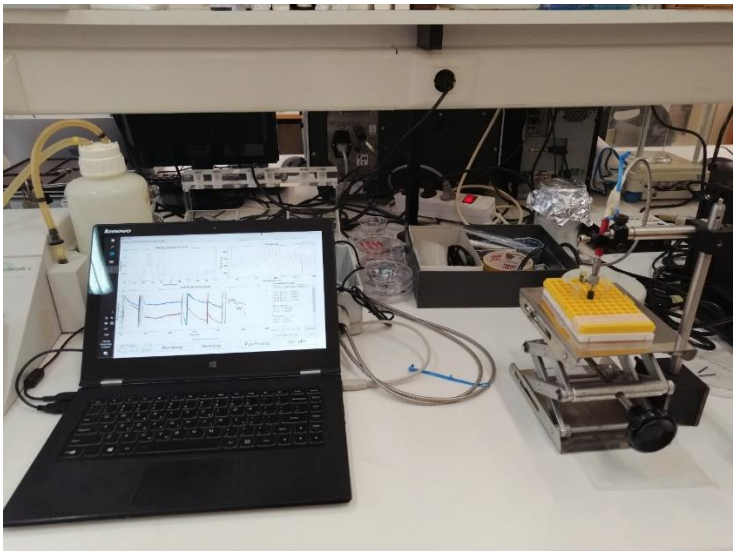
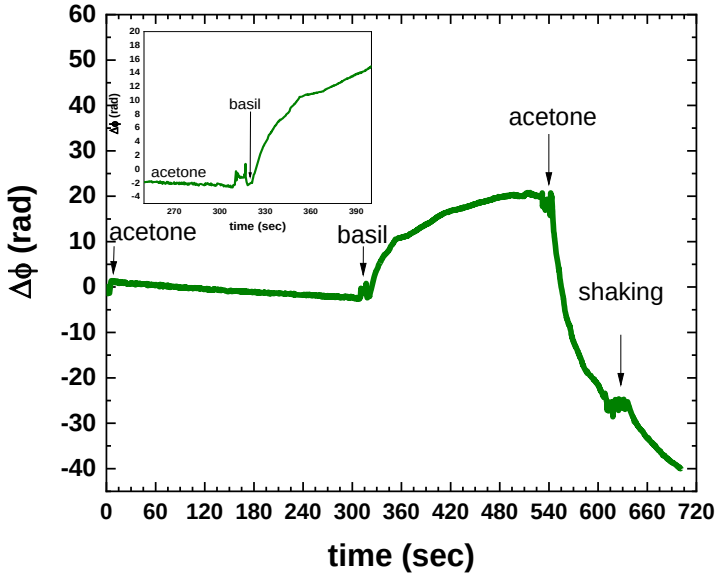
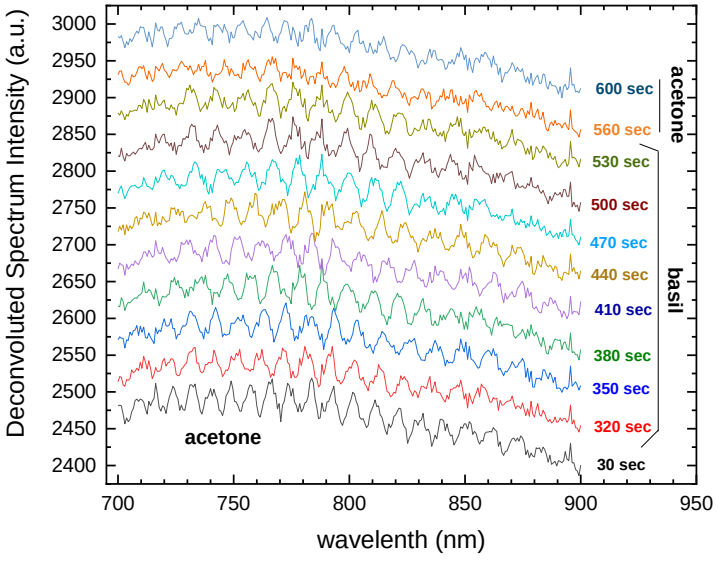


Figure 2.10. Photograph of the proof-of-concept setup during measurements

Figure 2.11 shows the very first measurement obtained with the NCK proof-of-concept setup. The NCK was tested with a wide-leaf (mammoth) basil leaf extract. The measurements included the monitoring of the shifting spectra and the phase changes during time. As can be seen there was a very large phase shift of nearly 20rad (~3.5 cycles), while the most abrupt change occurring within the very first 30sec (inset Fig. 2.11a). However, when trying to rinse the photonic chip of the pulp residues it appears that the spectrum and the phase signal did not revert to the original values and exhibited a rather counterintuitive behaviour.



(a)



(b)

Figure 2.11. (a) Real-time monitoring of the phase change: 0 to 320sec acetone (baseline); 320-540 sec basil extract; 540-720 sec rinsing in acetone. (b) De-convoluted spectra (to the LED shape) of the experiment with their time stamps indicated. The spectra have been vertically shifted for clarity. The 3.5 period blueshift is not evident and can only be observed in the recorded video

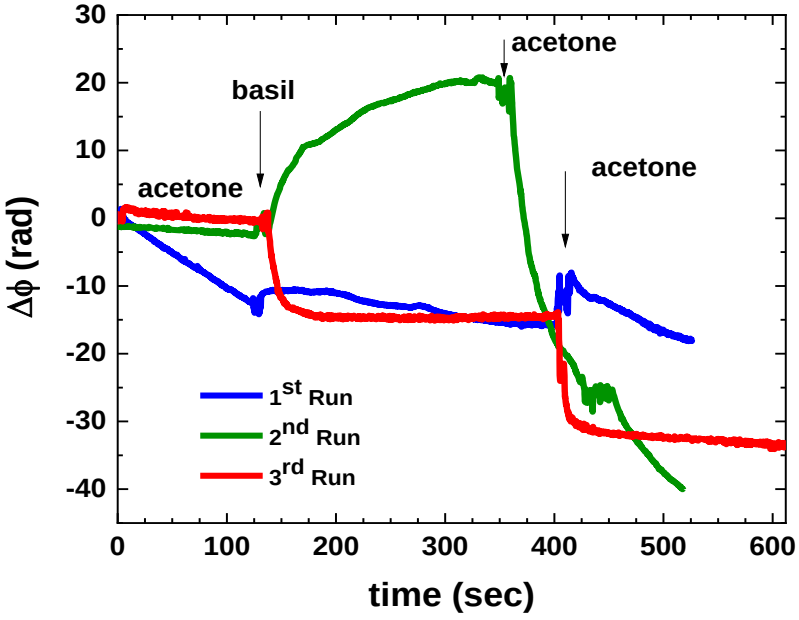
The same sequence of steps was repeated 2 more times to see whether rinsing with acetone is enough to adequately clean the surface of the sensor so the chip can be re-used. The results can be collectively seen in Fig. 2.12. Fig. 2.12a shows the phase tracking for all measurements. In all measurements at time t=0sec (i.e. when the chip is first dipped into acetone to obtain a baseline) the initial phase value is zero. This is automatically performed by the S/W. In Fig 2.12b the phase values from the measurement have been placed "sequentially" as obtained and "manually" displaced. This "manual" displacement was chosen in order to better demonstrate the phase gradients.

As can be deduced from Fig. 2.12 dipping in acetone even for several minutes cannot remove completely the various residues from the basil leaf extract. This is immediately demonstrated by two facts. Firstly, the phase signal after the first basil extract measurement does not revert to the original baseline when immersed in acetone (t=380sec→t=520 sec, green line, Fig. 2.12a, b), but instead it continues blueshifting, indicative of adlayer removal. Secondly, when immersing the chip in acetone to perform the second measurement instead of obtaining an almost straight line, the phase continues to decrease (t=0sec → t=120sec, blue line, Fig. 2.12.a; t=540sec-→ t= 660sec, blue line Fig. 2.12b) with the same gradient as the rinsing step of the first measurement. This also indicated that material is being removed from the sensor surface.

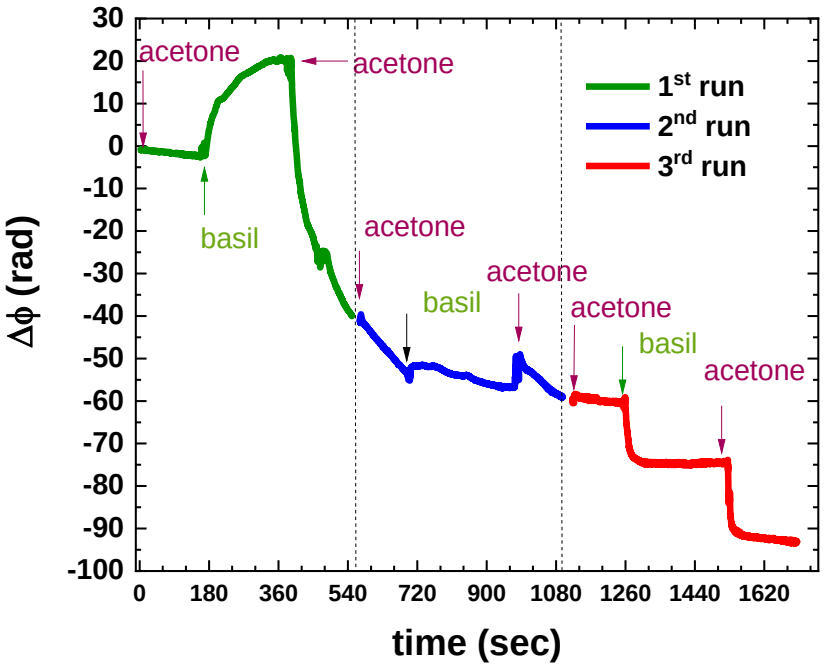
Moreover, when attempting to measure once more the same basil extract the phase change is merely 2 rads, aka 10-times smaller than the original value. This is an additional indication that an adlayer had formed on the sensor surface which could not be fully removed. Upon this second measurement, some more molecules re-instated themselves atop the sensor surface and the layer became thick resulting in almost a plateau in the phase signal (i.e. the adlayer is so thick with respect to the evanescent field of the propagating light that it is "viewed" by it as the upper cladding layer). This thick adlayer was again partially removed during the rinsing step (t=400sec →t= 560sec, blue line Fig. 2.12a ; t=960sec →t= 1080sec, blue line Fig.2.12b).

Moving on to the third measurement, an almost stable baseline was obtained, but when the chip was immersed in the basil extract there was a new and very abrupt redshift of the spectra, which is counterintuitive. A scenario that is explored -through simulations at the moment- is that the adlayer from the various basil molecules had "solidified" onto the sensing area and the immersion from acetone into the basil extract was "perceived" as a refractive index change of the cover medium. Nonetheless, when immersed into the rinsing acetone the redshift continued and the spectrum did not revert to the baseline. It might be that the first acetone –used as for the baseline- was already "contaminated" by basil residues and had a different refractive index than pure acetone (as a matter of fact it was observed that the "baseline" acetone had a slight greenish tint and was not fully transparent).

As a first conclusion, this first set of preliminary experiments showed that a more effective rinsing step is required to render the chips re-usable.



(a)

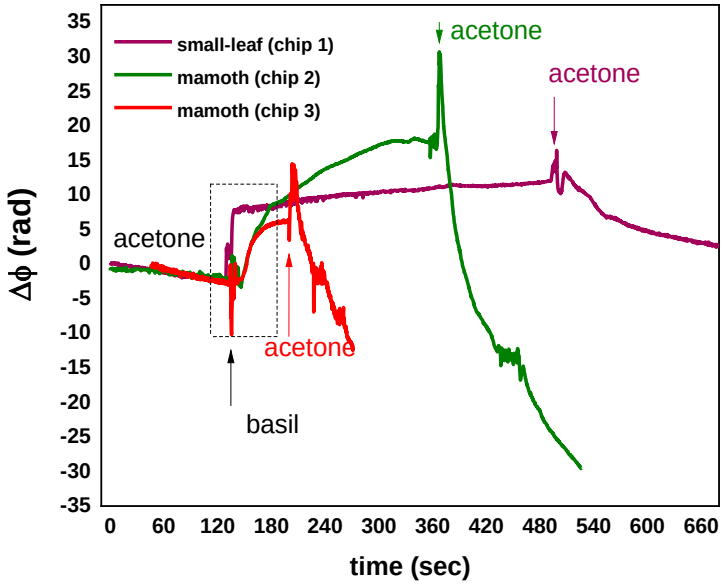


(b)

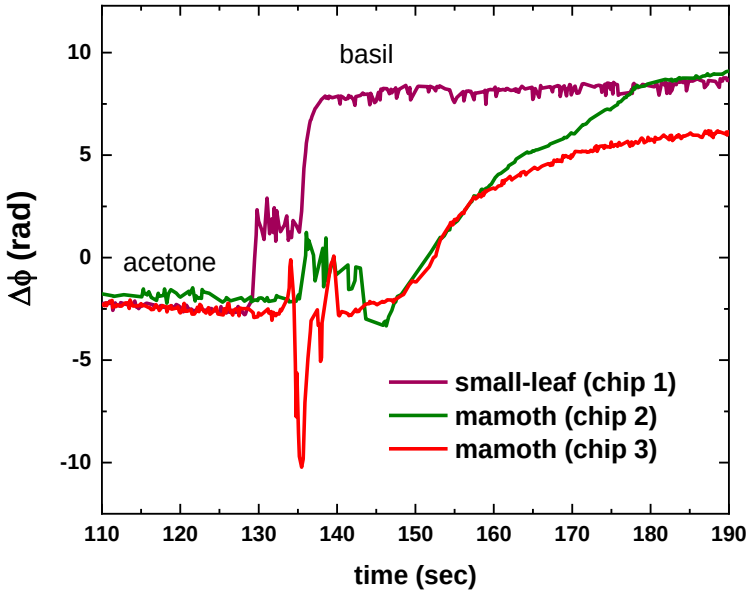
Figure 2.12. Phase tracking from the three measurements in *mammoth basil leaf* extract. The green line corresponds to the first measurement when the chip was pristine. The blue line corresponds to the second measurement and the red line to the third one. (a) the measurements shown as obtained with the first value at t=osec set to zero, and (b) the measurements are shown sequentially and "artificially" translated in the y-axis to show the phase gradients.

The second set of preliminary experiments wanted to see whether the chips can distinguish between two closely related plants. Towards that purpose a second basil extract from small-leaf basil was prepared and tested following the same procedure (acetone to obtain baseline → basil extract → rinsing in acetone). Three (3) different chips were employed, each one used pristine and only once, since the previous tests indicated that the rinsing procedure was not adequate and could allow re-use. The results are shown in Fig. 2.13.

It was very interesting to see that the small-leaf basil (which was much darker in coloro gave a distinctively different phase signal. While the mammoth basil resulted in a "smoother" phase change that was almost identical in rate within the first 15 sec for both chips, the small-leaf basil resulted into an almost step-like phase change of less than 3 sec. Even though this is definitely not an exhaustive study, this first indication that the two plants –even though they are closely related- can result in such distinct signals, it might be suggestive that the best observable might not necessarily be the shifting spectra, but rather the gradient of the phase within the first 30 sec of immersion in the plant extracts. Another observable may be how quickly the phase signal reaches a plateau and how this rate can be linked to the nutrient content, but it is too soon to say, and more experiments are required. Nonetheless, these findings will serve as a new guide in designing the next thorough experimentation with the NCK and to defining the best observables that will be used as the input to the GOhydro AI component.



(a)



(b)

Figure 2.13 (a) Phase signals from two chips immersed in mammoth basil extract (green and red lines) and one from a chip testing small-leaf basil extract (purple line). It should be noted that the chips were not immersed for the same amount of time in the basil extracts, therefore the removal from the extracts and placement in the rinsing acetone are indicated separately for each chip with the respective color coding. **(b)** Zoom-in of the area of the dashed frame in order to demonstrate the phase gradients upon insertion in the basil extracts.

3 FUTURE ACTIONS

3.1 PROTOCOL DEVELOPMENT & NCK VALIDATION

In order to fully assess the NCK it is imperative that the measurement protocol is optimized. Three critical parameters are to be examined in the forthcoming period:

1. The nature of the solvent for the basil extracts. It appears form the measurements so far that acetone may not be suitable since it may affect the chemical stability of the Eppendorf tubes. Ethanol –another "household" solvent- will be tested, Initial tests will use laboratory-grade ethanol and then it will be substituted with commercial ethanol from drug stores.
2. The concentration of the extracts was large as the preliminary experiments showed and evidenced by the fact that the sensor surfaces were not appropriately leaned. More dilute solutions will be tested.
3. The cleaning (regeneration) protocol so far that included simple rinsing in the solvent was proven inadequate. Therefore, the rinsing will be first tested with simple rinsing in the new solvent (ethanol) and if this is not adequate as well, more intense cleaning procedures will be explored. A candidate that will be tested is HCl which can be found commercially as a cleaning agent in stores and has not real effect on the final price.

3.2 NCK INITIAL DESIGNS

As already mentioned, it was decided to shift the design of the NCK after some first rounds of validation experiments so a better feeling of the user experience could be gained. This experience will serve as a better basis for the kit design under the 4 basic concepts described in Section 1. The most important concept though is that the final kit should be ergonomic and user-friendly.

The NCK initial design concepts are shown in Figure 3.1 and have mostly concentrated in how to render the reader part as compact as possible. These designs have not taken into account the most demanding element of the kit, which is the bi-furcated fiber. The fiber is the most "difficult' part to accommodate since it is relatively fragile and has a rather large bending radius. The validation experiments will guide what would be the most ergonomic way to place the fiber, whether it should be shortened and how it can be fixed without danger of damaging it during use outside a laboratory setting.

At the moment, there are three configurations which are slight variations of each other as shown in Fig. 3.1.. All three variations presuppose a "vertical" positioning of the reader with the bi-furcated fiber protruding from the top. Option 3 assumes that the power bank is placed externally or in general power supply is not included in the reader box. In the forthcoming period, a new round of designs will commence and it is expected to be finalized by M15.

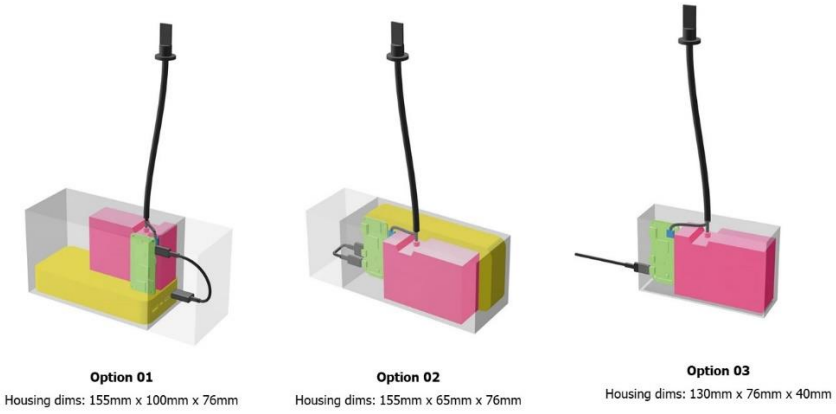


Figure 3.1. The 3 initial design concepts from the NCK reader along with their respective footprint

4 CONCLUSIONS

D2.2 has been partly completed mainly due to delays in funding. Nonetheless, the NCK has been realized as a proof-of-concept laboratory setup and initial validation experiments have been carried out. A choice was made to shift the final design after completion of the validation experiments in order to optimize the design exploiting the user experience.

Further validation experiments are being carried out that will culminate in a specific measurement protocol for basil microgreens, since there are numerous parameters that need to be fine-tuned.

An updated version of D2.2 providing the optimized protocols and final kit designs will be submitted in M15.