Team Results Document
SenseWURk

Team members:

Kimm Borst
Sleka Buis
Inge Braak
Martijn Ekhart
Iris Janssen
Mengyichen Long
Lutger Rutten
Eleni Meuffels
Xueyi Yang

SenseWURk

Supervisor:

Johannes Holhbein

Coaches:

Aart van Amerongen
Vittorio Saggiomo

12th of August 2022
Wageningen University & Research

SensUs

SensUs 2022

Acute Inflammation with a focus on sepsis

Index

1. Summary for the SensUs website

Team SenseWURk developed a lateral flow-based biosensor which works similar as a COVID-19 test. However, our test is implemented in a cartridge which can be read out by the DiRa-Lab digital LFT Demo Reader from ams OSRAM, resulting in qualitative IL-6 concentrations. We optimized the antibody conjugate and membrane combination to achieve maximal sensitivity: L137 as the conjugated antibody and L519 as the capture antibody on the FF 120HP membrane from Cytiva was the most promising combination to meet our objectives. Europium nanoparticles (EuNPs-L137) are conjugated resulting in fluorescent emission upon IL-6 binding, solving the problems of low sensitivity of the traditional immunoassay methods. The DiRa-Lab digital LFT Demo Reader translates the fluorescent signal to digital data, which is processed in an Excel file. The biosensor would improve sepsis detection and monitoring as it shows results within minutes instead of the hours or days lab tests in hospitals take. Furthermore, the one-time purchase of the reader device and the interchangeable cartridges result in a feasible business case and a stakeholder analysis shows that hospitals are a promising market. The SenseWURk biosensor design is straightforward, compact, easy-to-use and there are still multiple options for future optimalization to increase its performance.

2. Biosensor system and assay

The biosensor system that is used by our team is a lateral flow assay. In this assay, the sample first flows over a pad which contains the colouring agent. In our case the colouring agent consists of fluorescent particles to which antibodies are attached: conjugates. These antibodies are specific for the analyte so with the help of affinity, the analyte will attach to the fluorescent conjugate. Next, the sample is flown over the surface of the assay, on which analyte specific antibodies are present. Again, with the help of affinity the analyte can attach to the antibodies. A reader can then quantify the amount of fluorescence to determine the amount of analyte in the sample. In the figure below a schematic representation of the test can be found.

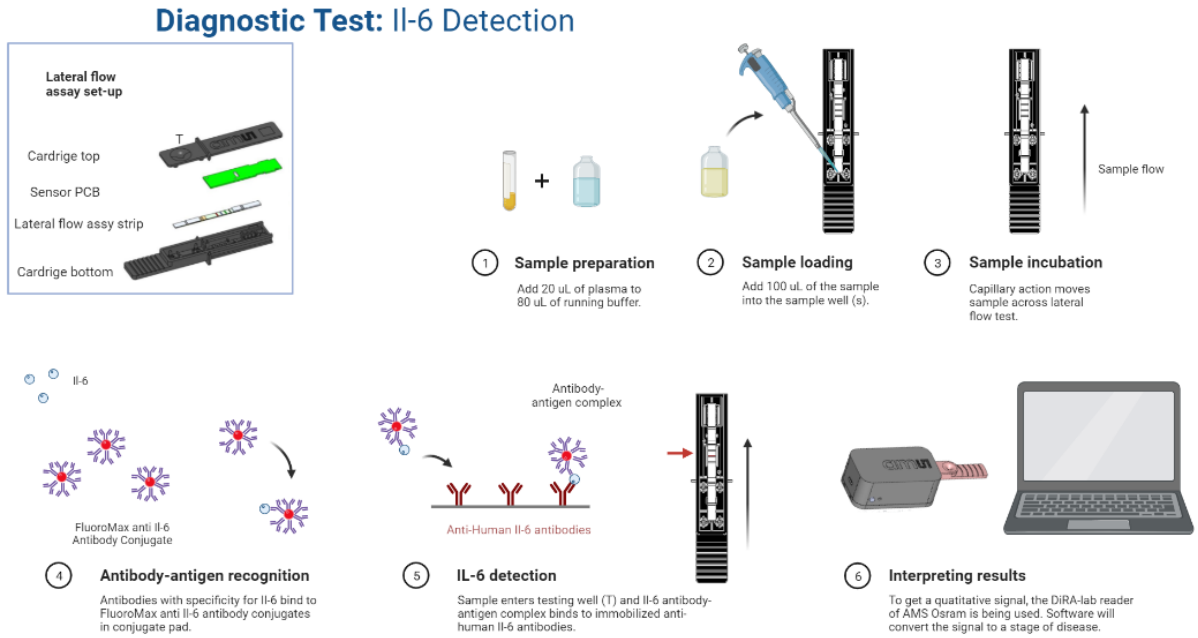


Figure 1: Overview of the testing procedure.

2.1 Molecular recognition and assay reagents

Antibody:

Conjugate antibody: L137. This is a monoclonal mouse antibody expressed in a hybridized cell line (Sp2/0 myeloma cells and Balb/c mice spleen cells).

Capture antibody: L519: Recombinant chimeric antibody expressed in a mammalian cell line, composed of original wild type variable domains of rat derived MAb and human IgG1 constant domains.

Label:

A fluorescent immunoassay based on europium nanoparticles (EuNPs-L137) was developed for the final detection of IL-6, solving the problems of low sensitivity of the traditional immunoassay methods. Fluorescent europium chelate doped nanoparticle has many advantages such as long fluorescence lifetimes, narrow emission spectra, large stokes shifts, extremely sharp emission profile with the full width at half-maximum of about 10 nm and emission from atomic states (Dezhi Huang et al., 2019). The coupling of the label is achieved using the reagents described in the protocol “Covalent coupling of fluoromax” that can be found in the appendix. It uses the EDC-Sulfo-NHS coupling reaction shown below:

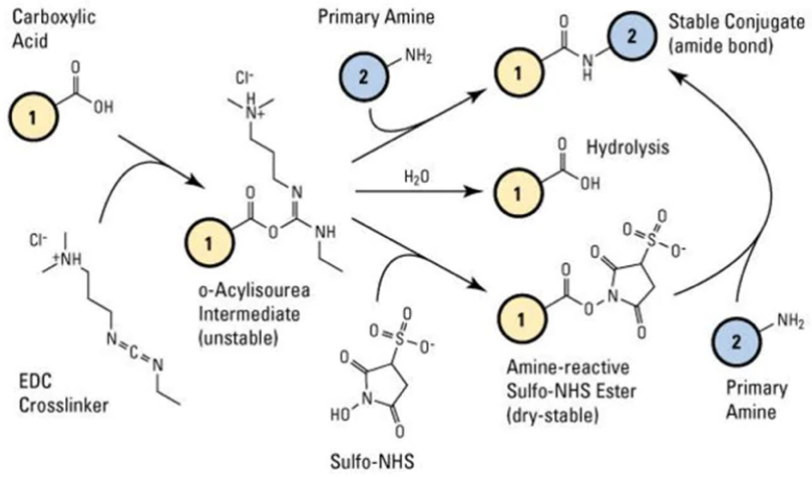


Figure 2: Overview of the EDC-Sulfo-NHS coupling reaction used to form the fluorescence antibody conjugates (ThermoFisher Scientific, 2012).

Membrane:
FF 120 HP nitrocellulose (Whatmann)

Test	Specification range	Mean results
Total thickness[µm]	180 - 220	202
Flow rate [s/ 4 cm]	90 - 140	108
Dustfree	dust-free surface is rated according to the specification	Conforms

Running buffer:
At this point in time 2 possible options for the running buffer remain:
0.1M borate buffer with 1% w/v BSA and 0.05% v/v Tween 20
1X PBS + 0.5% w/v skimmed milk + 0.05% v/v Tween 20

Enzymes:
At this point in time the use of enzymes is still being evaluated. The use of Fibrinolysin is now being tested and this may or may not be used in the final product.

2.2 Physical transduction

The nitrocellulose membrane in the lateral flow assay has been modified by spraying a line of capture antibodies onto the membrane, forming the test line. These capture antibodies bind IL-6 that passes over the test line, immobilizing the fluorescent europium nanoparticle conjugates (EuNPs) that are attached to the IL-6. The EuNPs that are immobilized on the test line are subsequently irradiated by UV light (365nm), exciting them to a higher electronic state. As the europium returns to its ground state it releases a photon (615 nm). This photon is detected by a photodiode located in the testing device above the test line, which generates an electrical signal. The numerical data produced by the testing device is exported to an Excel document where the data is interpreted to an IL-6 concentration in the sample.

2.3 Cartridge technology

We are currently still working on the optimization of our sample pre-treatment. One of our strategies consists of mixing the sample in a disposable Eppendorf tube with either a borate-based or a phosphate-based running buffer, in the ratio 1:4. The Eppendorf tube is subsequently placed in a heated water bath at 56°C for thirty minutes, after which 100 µL is taken out of the Eppendorf tube to run the lateral flow assay. Another strategy that we are looking into involves the use of enzymes to degrade components of the plasma that are otherwise interfering with our lateral flow assay. These enzymes are added to the running buffer, which is added to the sample in the aforementioned ratio. The lateral flow assay is performed in a disposable, 3D printed plastic cartridge that contains the lateral flow test and a PCB chip with the photodiodes required for the signal transduction as shown in Figure 1.

2.4 Reader instrument and user interaction

For the analysis of our test strips, we use the DiRa-Lab 2 reader instrument provided by ams OSRAM. The device is roughly 15cm long and 5cm high and wide excluding the cartridge. Test strips are kept in cartridges for use. When a test is performed, the sample is placed in the cartridge on top of the test strip and the cartridge is placed in the reader as shown in the image below. The reader excites the sample and based on how much fluorescent material is bound together with IL-6 to the test strip, a signal is measured. The device contains multiple sensors to measure different wavelengths as seen below. Our fluorescent material fluoresces around 615 nm, therefore we use the (as depicted below) yellow and orange sensors. After application of the sample on the test strip and turning on the device, the measurement is done fully automated. The device generates an excel csv file which is then loaded into an excel file to calculate the IL-6 concentration from the fluorescence intensity.

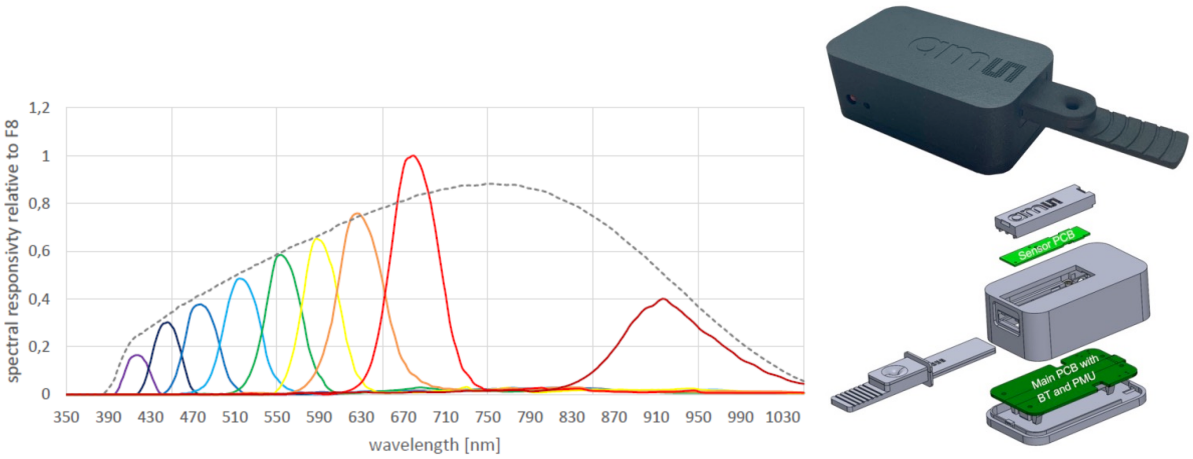


Figure 3: (left) The different sensors contained within the reader instrument and their corresponding wavelengths. (right) Overview of the different hardware components that together form the reader instrument (see Appendix: DiRa-Lab FAE Training & ams OSRAM DiRa-Lab 2 manual).

3. Technological feasibility

In this chapter the technological feasibility of our biosensor will be assesed based on scientific data and it will be evaluated if the new biosensor has the potential to achieve the required analytical performance in blood plasma.

3.1 Scientific results

Our team first attempted to construct the framework of lateral flow assay with amorphous carbon black particles and screened out the most effective combination from a variety of nitrocellulose membranes and a variety of IL-6 antibodies. Seven nitrocellulose membranes with varying pore sizes were made into strips by our team: FF 120HP, FF80HP, FF80HP+, FF120HP+, Immunopore XP, Immunopore RP, AE99, and sprayed them with seven kinds of capture antibodies (L137, L106, L152, L143, L519, L395 and mixed antibodies) and these antibodies were respectively tested on the strips as conjugated antibodies. This led to the screening out of several sets of membrane-antibody combinations with clear signals as combinations that matched our requirements (120HP, 120HP+, 80HP+). The best performing nitrocellulose membrane was then chosen by further testing the LFA's sensitivity to interleukin-6. After validating the effectiveness of the HP120+ membrane and L519 as capture antibodies, the relationship between the quantity of capture antibody sprayed on the membrane (800, 400, 200ug/mL) and the sensitivity of IL-6 detection (100, 200, 400pg/mL) was investigated. It was decided that using L137 as the conjugated antibody and L519 as the capture antibody on the FF 120HP membrane was the most promising combination to meet our objectives. Then dose response curve was made via IL-6 dilution range, shown in Figure 4. By serial dilutions of high concentrations of IL-6, we prepared a dilution range starting up to 6000 pg/mL. The signal intensity of each data point was tested twice and averaged, and the blank point (without IL-6) was repeated three times. The results shows that the lateral flow assay can effectively exhibit apparent fluctuation between concentrations ranging from 6000 to 24.7 pg/mL that can be recognized by reader. However, strips provide signal intensities similar to a blank sample for IL-6 concentrations below 24.7 pg/mL.

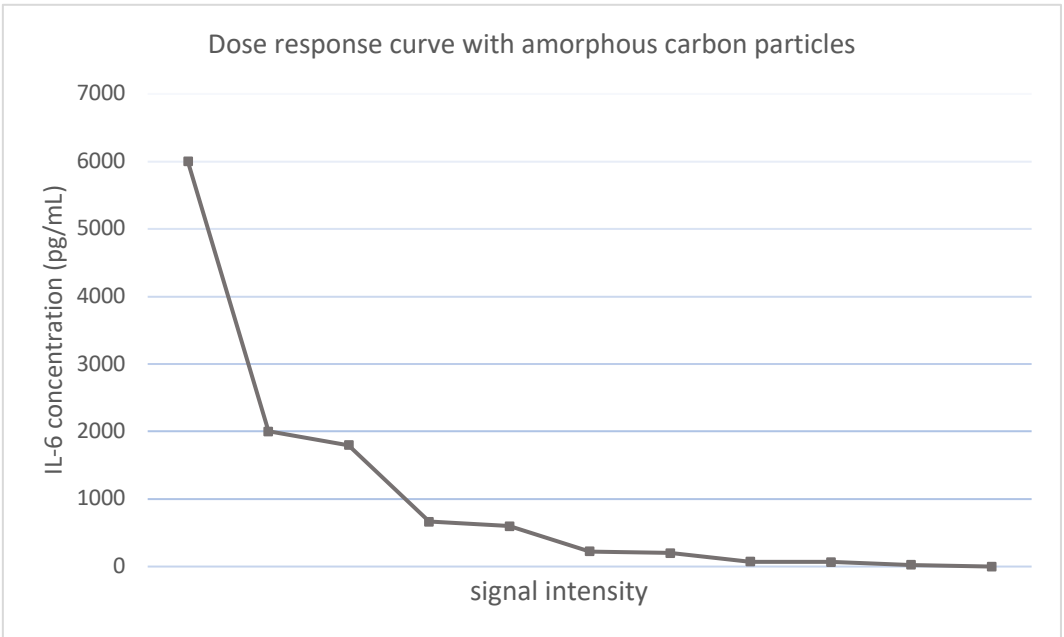


Figure 4: The dose response curve using amorphous carbon particle labelled L137 antibody as conjugate antibody, L519 as capture antibody on FF120HP membrane.

Next, we assessed the sensitivity of our sensor to IL-6 in plasma according to the detection criteria of the competition. The outcomes demonstrated that the plasma protein interfered with the conjugate antibody's ability to bind to the antigen (IL-6), causing a poor signal intensity and strong background interference during the test. Therefore, we altered the conjugated labelling particles from amorphous carbon particles to fluorescent europium and investigated the responsiveness of fluorescence europium conjugated with L137 antibody to the detection of IL-6 in plasma. The outcome of the compatibility test indicates that strips can identify the IL-6 level (2000pg/mL) in the sample within five minutes under plasma-free conditions, as shown in Figure 5 (left).

Additionally, under various circumstances, we examined the effects of plasma (heated at 56°C for varying lengths of time) on the fluorescence signal. We noticed that the background interference signal was weaker in plasma that had taken a 30-minute water bath at 56°C. The binding of IL-6 to the conjugated antibody in plasma is also influenced by the various running buffers used for the test, along with varying concentrations (10X, 20X diluted) of conjugate antibody. As shown in Figure 5 (right), 56℃ 30 minutes of heating plasma can significantly lessen the strips' background interference signal.

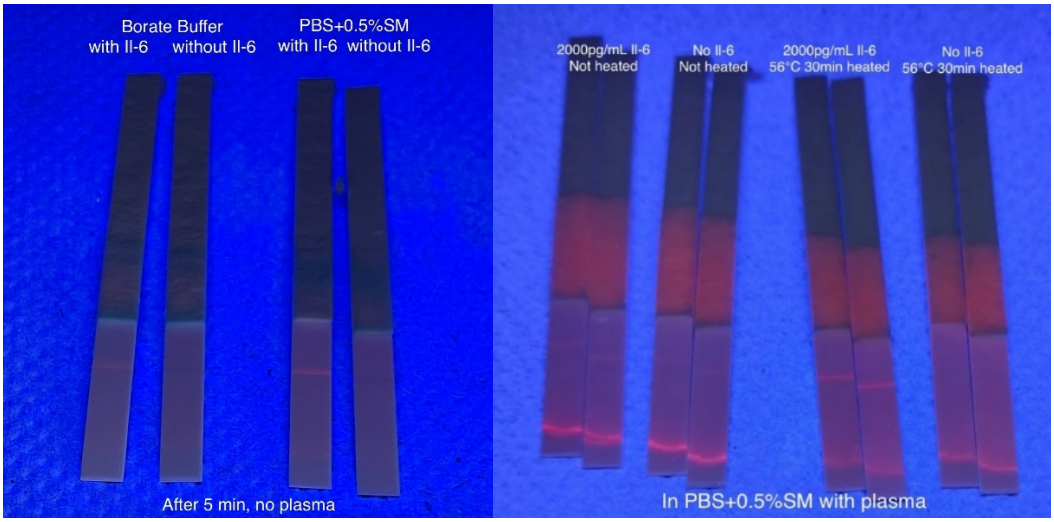


Figure 5: (left) Compatibility test of Europium particles and L137 conjugated antibody for IL-6 detection. (right) 30 minutes heating of sample gives less background signal compared to non-heating groups.

Due to the interference of plasma on the whole test system, our sensor cannot currently quantitatively analyse the fluorescent intensity of IL-6 in the band. Therefore, most of the results of fluorescence experiments can only be observed with the naked eye. The fluorescence sensor and detection strip will be incorporated in the following phase, followed by the quantification of the fluorescence signal and the development of a dose response curve utilizing fluorescent europium particles. On the other hand, we will keep researching strategies like degrading enzymes that can proficiently minimize the plasma interference in the background signal, enhancing our sensor's sensitivity and detection accuracy.

3.2 Potentiality and remaining problems

Due to its straightforward operation and rapid detection, lateral flow assay has demonstrated significant potential as a speedy detection approach during the COVID outbreak. The use of lateral flow assay to swiftly identify the amount of the sepsis marker (IL-6) in patients' plasma can effectively allow clinicians to easily assess the patient's physical status in the early diagnosis of Sepsis.

Based on the current experiments carried out by our team, we initially developed the sensor of lateral flow assay for sepsis diagnosis, and we acquired the preliminary detection findings for the sepsis marker (IL-6), which can accurately identify IL-6 from 6000-24.7 pg/ml in the absence of plasma. The present technological barriers, however, are in lowering the interference to IL-6 signalling in the presence of plasma and SenseWURk will continue to try multiple approaches further to enhance the sensitivity of biosensors to IL-6 in the presence of plasma.

4. Originality

SenseWURk developed a biosensor for detection of IL-6 based on a lateral flow assay. The principle of such a lateral flow assay is common and established. Still, many novel aspects can be found within every lateral flow assay e.g., its antibody combination, the used membrane or the different sample and conjugate pads. The combination of these creates a novel lateral flow assay which is optimized for the detection of IL-6 in our case. We as a team tested six different antibodies and seven lateral flow membranes with all these antibody combinations. Finally, the most sensitive combination was found and chosen. During this work, Cytiva supplied all membranes and pads. Moreover, issues during development where discussed and knowledge was exchanged between Cytiva and SenseWURk during regular meetings. Furthermore, to create the biosensor the BioSensing & Diagnostics group from Wageningen University & Research contributed vastly by providing basic knowledge and help during daily laboratory work. he concept of the reader of the SenseWURk biosensor was conceived by ams OSRAM. Demonstrators were supplied for both colorimetric signals as well as fluorescent signals. SenseWURk optimized and adjusted both readers together with ams OSRAM. Biweekly discussions assured knowledge was exchanged and bugs were solved.

Written and signed by co-team captain Iris Janssen

The team is using a diagnostic platform, the lateral flow test, that is well-known nowadays as this platform is widely used for assessing SARS-CoV-2 infections and immune responses. However, the novelty is in the search for optimizing test parameters to push sensitivity levels to new, very low concentrations. Thereto, the team has performed cross-incubations with all available antibodies (six) by using each antibody both as capture and as detection ligand. In addition, seven different lateral flow membranes were tested with all the antibody combinations. From this broad screening the best, i.e. most sensitive couple of capture and detection antibodies in combination with the most suited membrane were chosen.
Moreover, new readers for lateral flow tests, still demonstrators from ams OSRAM and equipped with a multi-spectral sensor, were applied to measure colorimetric (carbon nanoparticles) and fluorescent (Europium nanoparticles) signals. A new feature of these readers is the possibility to measure in transmissive mode, which means that all nanoparticles binding at the test zone and throughout the whole membrane thickness can be measured to increase the sensitivity of the test. The team has implemented this new reader, optimized the assemblage of test strips in the accompanying cartridges, analyzed the performance of the software and indicated and discussed bugs with ams OSRAM for further improvement.
Basic knowledge for developing lateral flow tests was contributed by the group BioSensing & Diagnostics from Wageningen Food & Biobased Research. Cytiva contributed by supplying lateral flow membranes and pads and by discussing assay-development issues with the team.

Written and signed by supervisor dr. Aart van Amerongen

5. Translation potential

In this chapter the translational potential of our biosensor will be discussed based on the proposed business model, market description, stakeholder desirability, business feasibility and financial viability.

5.1 Business model canvas

	Sepsis POC	SenseWURk	2022.08.12

Problem	**Solution**	**Unique Value Proposition**	**Unfair Advantage**	**Customer Segments**
- Sepsis is difficult to diagnose, leading to a high misdiagnosis rate (4,8). - Needs fast detection to prevent further disease progress hence less antibiotics administration when detection in earlier stage (8). - Complex and elaborate monitoring of disease progress is missing (4).	- Specific detection of IL- 6 resulting in detection of an inflammatory response. - On the one hand, ruling out sepsis disease when IL-6 are too low. On the other hand, establishing state of sepsis disease by the IL-6 value making specific antibiotic dosing possible. - Also, cheap POC device.	Currently, CRP is executed when suspecting sepsis disease which takes a about 1 hour. We use nanoparticles to detect IL-6, which can be done within minutes.	- We use real time measurement of fluorescence increasing sensitivity. - Later, carbon laser cutting could be used to increase sensitivity.	Medical professionals, especially emergency department doctors.
Existing Alternatives	**Key Metrics**	**High-Level Concept**	**Channels**	**Early Adopters**
- Sepsis prognosis requires elaborate monitoring of the patient. First, CRP monitoring followed by blood sample analysis for detection of viruses or bacteria (5).	- Use of sepsis rapid test in POC medical setting. - Decreasing misdiagnosis of sepsis. - Improving sepsis prognosis. - Lessing antibiotics use as well as its resistance.	Sepsis POC test is a quantitative lateral flow test for sepsis detection and prognosis.	- Aim to reach a key opinion leader. This person has lots of support can encourage hospitals sales and doctors to invest. - Reach insurances willing to cover the costs of the biosensor making it more accessible for hospitals to invest.	Academic hospitals due to their willingness to be open to student start-up concepts.

Cost Structure	**Revenue Structure**
- Main costs are related to strip manufacturing: antibodies, membranes.	- Revenue is based on cartridge buying (1 cartridge for every test needed). - Selling the reader is a 1-time investment for every hospital.

5.2 Market description

Classification of sepsis is commonly done with the use of the Systemic Inflammatory Response Syndrome (SIRS) criteria shown in Table 1. When a patient meets 2 or more criteria (i.e. score of ≥ 2) sepsis is defined as a probable infection (van der Woude et al., 2018). Also, qSOFA critieria are commonly used consisting of three main observations: a respiratory rate of higher than 22, altered mental status, and systolic blood pressure of lower than 100 mmHg (Koch et al., 2020).

Table 1: Systemic Inflammatory Response Syndrome (SIRS) criteria (1)

Variable	Criteria
Body temperature	> 38.0 ºC or < 36.0 ºC
Heart rate	> 90 /min
Respiratory rate	> 20 breaths / min or $PaCO_2$ of < 4.3 kPa
White blood cell count	< 4000 cells / mm^3 or > 12,000 cells/mm^3 or >10% immature bands

Singer et al. (2016) showed the presence of limitations of the SIRS criteria which included a disproportionate focus on inflammation. The fact that sepsis was defined by a model following a continuum through severe sepsis to shock resulted in inadequacy. This study resulted in updated definitions and stricter restrictions (Singer et al., 2016). However, up and till today concerns regarding the strict selection hold back the use of these criteria in practice. The risk of undertreatment and increase in mortality cause this legitimate concern (van der Woude et al., 2018). As a result, currently in Dutch hospitals SIRS criteria are often used at emergency departments determining the patients' antibiotic treatment. Despite the use of SIRS criteria or other scoring systems, accurate sepsis recognition in the Netherlands is low (van der Wekken et al., 2016). The use of a rapid biosensor with high specificity would increase the identification of sepsis patients as well as their crucial early initiation of treatment (van der Wekken et al., 2016).

Currently, the care pathway of sepsis in emergency departments often consists of a 1-hour bundle (Chen et al., 2019). Here, lactate levels are measured, and blood cultures are obtained before antibiotic administration. Next, a broad-spectrum of antibiotics is administrated as well as 30 ml of crystalloid per kg of bodyweight if the patient has a lactate level higher than 4 mmol per liter or hypotension. If fluid resuscitation does not redress the hypotensive state of the patient vasopressors are administrated (Chen et al., 2019). Moreover, two interviews with medical professionals at Dutch hospitals proved the importance of C-reactive protein (CRP) tests. Both doctors informed that this value is widely used in the clinical routine despite its limited specificity. Another drawback of the use of CRP tests is the late increase in CRP-level since its synthesis is induced by pro-inflammatory cytokines. Hence, the level of IL-6, being one of these pro-inflammatory cytokines, would rise earlier ensuring a potential faster recognition of sepsis (Ma et al., 2016).

When the SenseWURk biosensor reaches the market, the patients IL-6 levels can be determined within minutes. As a result, diagnosticating inflammation will be fast as the value of IL-6 increases within 1 hour after the infectious stimulus (Ma et al., 2016). It should be noted that a patients IL-6 levels could not exclusively be used as sepsis diagnosis tool as literature shows disputed findings of its accuracy (Ma et al., 2016)(Iwase et al., 2019). However, the biosensor would certainly assure a negative discrimination since patients with IL-6 concentrations less than 5 pg / mL are not infected by a stimulus hence do not require antibiotic treatment (Ma et al., 2016). Nowadays, broad-spectrum of antibiotics are administrated to uninfected patients, increasing the risk for antibiotic resistance, wasting nurses' and doctors' time, consuming blood culture bottles as well as other supplies (Chen et al., 2019). The use of an IL-6 biosensor would cast aside these problems. Above that, sepsis can be diagnosed in every hospital with more ease due to this parameter showing inflammation.

Customers of the biosensor would be emergency medical staff in every hospital with a potential start in the Netherlands. As the Netherlands counted 618 hospitals in 2020, market size is large enough to make the product economically viable (Michas, 2022). However, to reach the market the biosensor needs to enter hospital procedures which might be a challenge since medical staff education is required to use the sensor. A solution could be reaching the encouragement of a key opinion leader in sepsis in emergency departments. This would assure support of the use of the biosensor showing the need of equation of the biosensor manual.

5.3 Stakeholder desirability

Currently, there is a clear market need for our IL-6 biosensor hi. When doctors suspect a case of sepsis in patients in the ER, a probability diagnosis is made based on qSOFA or SIRS criteria. These consist of three observations: a respiratory rate of higher than 22, altered mental status, and systolic blood pressure of lower than 100 mmHg (Koch et al., 2020). Afterwards, the C-Reactive Protein (CRP) point of care testing is performed. Our IL-6 biosensor is more desirable compared to CRP since the results can be obtained within five minutes instead of an

hour for CRP. Also, IL-6 is a more useful biomarker for early detection of sepsis compared to CRP as IL-6 can be detected earlier in the course of a bacterial infection (Noor et al., 2008).

The IL-6 biosensor is beneficial on different levels of patients' care. Firstly, the need of knowing the level of IL-6 to determine patient state already in the ambulance on the way to the hospital. This can be used to administer the necessary antibiotics earlier instead of upon arrival at the hospital. Currently, it is recommended to administer the necessary antibiotics as soon as possible after sepsis diagnosis since survival is negatively impacted by poor source control (Schmidt et al., 2018).

Another application at which our biosensor would be useful is during treatment of a patient with sepsis. Usually, there are two treatment phases which the patients who have developed sepsis will go through. In the first phase, the medical personnel will try to suppress the immune reaction. In the second phase, the immune system is stimulated to decrease chance of secondary infection after the immune system has suffered a major fallback. Medical personnel might also be able to use our IL-6 biosensor to determine in which of the two phases the patient is in, and what would be the good timing to move on to the next phase of the treatment.

Looking at the usability of the IL-6 biosensor for a GP, the purpose would not be to diagnose the patient with sepsis, but rather to avoid administering unnecessary antibiotics based on the test results. This will help preventing antibiotic resistance.

As explained above, hospitals, especially emergency department doctors and patients, are a big stakeholder of the SenseWURk biosensor. However, it is good to note that insurance companies would also experience positive effects of the biosensor application. Reasoning, less antibiotic administration, fewer patients that require treatment due to belated diagnosis and subsequently fewer number of deaths, insurance companies would benefit considerably.

5.4 Business feasibility

In the process of developing our biosensor we had biweekly meetings with the collaborators to discuss the progress and possible hurdles. Our partner Cytiva provided us with membrane material. Kenosha provided the transparent backing materials for the membranes. The calorimetric and fluorescence measurement readers were provided by ams OSRAM. Additionally, ams OSRAM provided software to translate the measurement values from the reader to a quantitative value that can be read on remote devices such as a smart phone. Hytest supplied us with detection antibodies. We also have a collaboration with Highfield Diagnostics which can help the scale up process by supplying us with complete membrane strips by combining the materials from Cytiva and Kenosha.

Additionally, OnePlanet Research Center, which is a multidisciplinary collaboration between different universities in the Netherlands, is a partner who is ready to help us with questions regarding the upscaling and business plan. Surfix Diagnostics is a spin-off company of Wageningen University & Research which has expertise in biosensor development and commercialisation. The expertise of these two partners with innovative start-up companies has been very helpful in the development of the business model and with possible future upscaling of the process.

To conclude, we have established a strong team of collaborators that are willing to assist us in development, manufacturing, and scaling up of the biosensor. The materials provided by our collaborators are considered in the financial viability analysis (see 5.5). These partners see a healthy profit margin. Together, this ensures continuity of the production of the biosensor.

Our commercialization strategy emprises mainly selling the membrane strips which are required to be purchased with every new test. Readers only need to be invested in once by the costumer. This is sufficient to make the biosensor commercially viable (see 5.5).

The reusability of the reader and the low waste material of the cartridge are the most important sustainability factors. The exterior material from the cartridges and reader can be made carbon neutral and entirely from recycled plastic or cardboard, and the waste can also be recycled. This contributes to the low waste and sustainability of our business model. Adding to that, we are still working on a new system in which the laser and

detection chip are placed in the reader instead of the cartridge. Currently, we are experimenting together with our partner ams OSRAM with readers that use this system, but further optimisation is needed. Furthermore, the overall goal of obtaining the IL-6 detector will lead to a more sustainable healthcare chain, since it will prevent unnecessary antibiotic use, waste of medical equipment and materials, and lastly time of the medical personnel.

5.5 Financial viability

For our IL-6 biosensor, the main costs are related to strip manufacturing including antibodies, membranes. Revenue is based on cartridge buying (1 cartridge for every test needed). Selling the reader is a 1-time investment for every hospital. Seven different nitrocellulose membranes from Cytiva are tested during development. One reader Kit from ams OSRAM contains 1 x DiRa-Lab digital LFT Demo Reader and 25 x disposable test cartridges with strips. The price of one kit is € 25 and reader is € 3.5 per each (retrieved from AS7341L-DLGM ams - Distributors, Price Comparison, and Datasheets | Octopart component search)

Table 2: Costs of Development

Costs of Development		
Categories		Amount
Antibodies		€ 1195
Membrane	Immunopore XP	€ 232
	FF 80 HP	€ 128
	FF 80 HP Plus	€ 186
	FF 120 HP	€ 128
	FF 120 HP Plus	€ 168
	Immunopore RP	€ 325
	AE 99	€ 213
Human plasma		€ 342
Other chemicals		€ 844
Reader		€ 50
Total		€ 3811
Sales Price		
Biosensor kit with 1 x DiRa-Lab digital LFT Demo Reader and 25 x disposable test cartridges with strips.		€ 30
DiRa-Lab digital LFT Demo Reader		€ 4
Cartridges kits and reagent		€ 1
Expected Revenues		
Selling one biosensor kit to one hospital		€ 18,000

Constant changes in the healthcare industry have led to an increase in the need for hospitals with enhanced diagnostic services. An increase in healthcare expenditure globally has significantly contributed to the growth of the market. The hospitals and clinics segment dominated the lateral flow assays market and accounted for the largest revenue share of 39.5 % and the global market size is $7.2 billion in 2020 (“Lateral Flow Assays Market Size, Share & Trends Analysis Report By Product (Kits & Reagents, Benchtop Readers), By Application (Clinical Testing, Veterinary Diagnostics), By Technique, By End-use, By Region, And Segment Forecasts, 2021 – 2028”, 2019). Hospitals and clinics are primary care settings for the diagnosis and treatment of medical conditions. Most of the population relies on these long-term facilities for the diagnosis, treatment, and management of diseases. For our product, early adopters would be academic hospitals due to their willingness to be open to student start-up concepts and our main customers of the biosensor would be emergency medical staff in every hospital with a potential start in the Netherlands. In the Netherlands, there are over 618 hospitals in 2020 as mentioned in previous chapter. Therefore, the market size is large enough to achieve financial feasibility. Every year, about 15,000 people in the Netherlands get sepsis. Sepsis is more common in the elderly(retrieved from Sepsis (umcg.nl)). If our product can be launched by the main target customers that each hospital purchases one biosensor kit, around € 18,000 revenue would be achieved (Table 2). Notably, selling the reader is a one-time investment for hospitals and one new cartridge are needed for every test. One biosensor kit which contain 25 cartridges is able to satisfy the patients in the Netherlands per each year. After that, our main revenue would be based on new cartridges purchasing.

6. Team and support

6.1 Contributions of the team members

- Iris Janssen: Iris Janssen has been the leading team captain for the team. She led most meetings and prepared the agendas. Furthermore, she was part of the PR subteam, reader subteam and innovation subteam.
- Inge Braak: Inge Braak took on the role of supportive co-captain, to keep the workload doable. She also handled communication and meetings. Furthermore, she was part of the PR subteam, reader subteam and innovation subteam.
- Sieka Buis: Sieka Buis was the secretary of the team. She took notes during all meetings and kept overview. Furthermore, she was part of the lab team and captain of the PR team.
- Mengyichen Long: Mengyichen Long was the treasurer of the team. She handled the finances of the team. Furthermore, she was part of the lab team.
- Kimm Borst: Kimm Borst was captain of the lab team. She made schedules dividing the lab work and worked most hours in the lab. She was end responsible for the lab work.
- Lutger Rutten: Lutger Rutten was captain of the reader team. He was responsible for the reader devices and their handling. Furthermore, he was part of the innovation team and worked a lot in the lab the last weeks.
- Xueyi Yang: Xueyi Yang was part of the lab team. She was not part of other subteams as most work needed to be done in the lab.
- Martijn Ekhart: Martijn Ekhart was part of the lab team and the reader team. He was needed to bridge between assay and digital read out. He worked on the software.
- Eleni Meuffels: Eleni Meuffels worked effectively abroad. She was part of the labteam (literature assistance), and the innovation team.

6.2 People who have given support

We would like to thank the following persons for their support during our project:

- Aart van Amerongen: for all his help as our supervisor, the use of his lab and providing us this much knowledge and materials.
- Ruben Massop: for all his close lab work assistance and helping us plan and learn from the experiments. He ensured we were on the right track to proceed lab work.
- Vittorio Saggiomo: for his help as coach and contribution from the BioNanoTech (BNT) lab.
- Johannes Hohlbein: for his coaching during the whole project.
- Rio Pals: for her help as WUR student challenge manager by setting up workshops, providing the work environment and meeting rooms for our team.

6.3 Sponsors

We would like to thank the following companies and organizations for their valuable input in our project:

- ams OSRAM for use of the reader device, help in troubleshooting, overall advice, financial contribution, and for helping keep us reminded to win.
- Cytiva for use of their membranes and conjugate and absorption pads, help in finding the right fit of materials for our project, sharing their knowledge, and their financial contribution.
- Highfield diagnostics for discussing the possible scale up of complete test strip manufacturing
- Hightest for supplying the antibodies with a discount.
- Kenosha for supplying the transparent backing cards.
- Surfix for valuable advice on our business plan and a financial contribution.
- BioNanoTech (BNT) lab of the WUR for their advice and financial contribution.
- Wageningen food and biobased research for use of their lab, experience and overall support.
- OnePlanet for their knowledge and input.

7. Final remarks

Although our biosensor design is still in the prototype phase, we believe that it could be an effective and applicable solution for sepsis detection in hospitals. Due to the simplicity and straightforwardness of the design, it could be realistically brought to the market. When implemented in existing treatment protocols, our biosensor could improve IL-6 quantification and sepsis detection. However, many steps still need to be taking during this road from prototype to actual use. With further professional help and more research done, we believe this is achievable.

The strip design can be improved in terms of design and biochemical aspects when more lab hours are made. Furthermore, the sensitivity can be increased by researching novel technologies. During our design process we had contact with Highfield Diagnostics, experts in lateral flow strip manufacturing. We discussed the possibility of using a laser-direct write (LDW) technique to enhance limit of detection and sensitivity (Katis et al., 2018). This polymer-based technique could be potentially used as a filter as well when adjusting porosity characteristics. This technique was proven to counter the Hook effect, which is limiting in many lateral flow assays (Galanis et al., 2022). In the future these novel techniques could significantly improve our lateral flow-based biosensor. When working together with Highfield Diagnostics the production could be scaled-up as well. However, for the Eindhoven Testing Days we decided on making or own strips to be able to adjust the design more easily and save on materials. The reader we used from ams OSRAM is a prototype as well, not brought on the market yet. During the process we had a lot of effective knowledge exchange which was useful for both our strip design as well as the reader and cartridge types ams OSRAM sent us. When working more closely together, the combination of their reader and the IL-6 detection strips can be more tailored towards sepsis detection. There is still a lot of potential there to improve that interaction. Not only in terms of design we think there is a lot of potential, we also talked about participating in the 4TU impact challenge for start-ups. Via the Wageningen University Student Challenges department and the StartHub community we have in Wageningen, there are a lot of opportunities to improve our entrepreneurial skills and lift off, as many startups have done before via StartHub.

In conclusion, with all the help and knowledge we gained via the WUR and our partners and sponsors, we believe in our design and think it has significant future potential. When putting more hours in the biosensor and making use of external resources and knowledge, this prototype can become a changing device in the field of sepsis detection.

8. References

Chen, A. X., Simpson, S. Q., & Pallin, D. J. (2019). Sepsis guidelines. *N Engl J Med*, *380*(14), 1369-1371.

Dezhi Huang, Haojun Ying, Dongneng Jiang, Fei Liu, Ye Tian, Chunlan Du, Liqun Zhang, Xiaoyun Pu. (2019). Rapid and sensitive detection of interleukin-6 in serum via time-resolved lateral flow immunoassay, Analytical Biochemistry, Volume 588, 2020, 113468, ISSN 0003-2697, https://doi.org/10.1016/j.ab.2019.113468.

Galanis, P. P., Katis, I. N., He, P. J. W., Iles, A. H., Kumar, A. J., Eason, R. W., & Sones, C. L. (2022). Laser-patterned paper-based flow-through filters and lateral flow immunoassays to enable the detection of C-reactive protein. *Talanta*, *238*, 123056.

Iwase, S., Nakada, T. A., Hattori, N., Takahashi, W., Takahashi, N., Aizimu, T., ... & Oda, S. (2019). Interleukin-6 as a diagnostic marker for infection in critically ill patients: a systematic review and meta-analysis. *The American Journal of Emergency Medicine*, *37*(2), 260-265.

Katis, I. N., He, P. J., Eason, R. W., & Sones, C. L. (2018). Improved sensitivity and limit-of-detection of lateral flow devices using spatial constrictions of the flow-path. *Biosensors and Bioelectronics*, *113*, 95-100.

Koch, C., Edinger, F., Fischer, T., Brenck, F., Hecker, A., Katzer, C., ... & Schneck, E. (2020). Comparison of qSOFA score, SOFA score, and SIRS criteria for the prediction of infection and mortality among surgical intermediate and intensive care patients. World Journal of Emergency Surgery, 15(1), 1-10.

Lateral Flow Assays Market Size, Share & Trends Analysis Report By Product (Kits & Reagents, Benchtop Readers), By Application (Clinical Testing, Veterinary Diagnostics), By Technique, By End-use, By Region, And Segment Forecasts, 2021 – 2028. (2019)

Ma, L., Zhang, H., Yin, Y. L., Guo, W. Z., Ma, Y. Q., Wang, Y. B., ... & Dong, L. Q. (2016). Role of interleukin-6 to differentiate sepsis from non-infectious systemic inflammatory response syndrome. *Cytokine*, *88*, 126-135.

Michas, F. (2022). Hospitals in the Netherlands 2005-2020. https://www.statista.com/statistics/949593/hospitals-in-the-netherlands/#:~:text=In%202020%2C%20there%20were%20618,resulting%20in%20this%20value%20surge

Noor, M. K., Shahidullah, M., Mutanabbi, M., Barua, C., Mannan, M. A., & Afroza, S. (2008). Comparison between CRP and IL-6 as early markers of neonatal sepsis. *Mymensingh medical journal: MMJ*, *17*(2 Suppl), S72-6.

Schmidt, G. A., Mandel, J., Parsons, P. E., Sexton, D. J., Hockberger, R. S., & Finlay, G. (2018). Evaluation and management of suspected sepsis and septic shock in adults. *Uptodate Online*, 1-29.

Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., ... & Angus, D. C. (2016). The third international consensus definitions for sepsis and septic shock (Sepsis-3). *Jama*, *315*(8), 801-810.

ThermoFischer Scientific. (2012). Carbodiimide Crosslinker Chemistry. https://www.thermofisher.com/nl/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/carbodiimide-crosslinker-chemistry.html

van der Wekken, L. C., Alam, N., Holleman, F., van Exter, P., Kramer, M. H., & Nanayakkara, P. W. (2016). Epidemiology of sepsis and its recognition by emergency medical services personnel in the Netherlands. *Prehospital Emergency Care*, *20*(1), 90-96.

van der Woude, S. W., Van Doormaal, F. F., Hutten, B. A., Nellen, F. J., & Holleman, F. (2018). Classifying sepsis patients in the emergency department using SIRS, qSOFA or MEWS. *Neth J Med*, *76*(4), 158-166.

9. Appendix

Covalent coupling protocol Fluoro-Max

Materials:

- Fluoro-Max Eu CM, 0.2 µm, 1%(w/v) suspension (Fisher Scientific, 11834213)
  (carboxyl modified, $\lambda_{exit.}$ = 333 nm, $\lambda_{emitt.}$ = 613 nm)

- 4-morpholineethanesulfonic acid MES (Merck Life Science)
  Activation-buffer 25 mM MES, pH6.1
  MES: mw = 195,20 g/mol, c.q. 25 mM/100 ml = 488 mg/100 ml
adjust pH to 6.1 using 5 N NaOH

  Binding-buffer 25 mM Phosphate buffer, pH7.0
  KH_2PO_4: mw = 136.09 g/mol, c.q. 25 mM/100 ml = 340.3 mg/100 ml
  K_2HPO_4 $3H_2O$: mw = 228.23 g/mol, c.q. 25 mM/100 ml = 570.5 mg/100 ml
  mix both solutions until pH7.0

  Blocking buffer 25 mM phosphate buffer, 2%(w/v) BSA, pH7.4

  Washing buffer 25 mM Tris-HCl, 0.9%(w/v) NaCl, 0.2%(v/v) Tween-20, pH7.8
  Tris: mw = 121.14 g/mol, c.q. 25 mM/100 ml = 302.9 mg/100 ml
  NaCl: 900 mg
  Tween-20: 200 µl
  adjust pH to 7.8 using diluted HCl

- EDC: 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride (Fisher Scientific)
prepare a fresh 1 mg/ml solution in activation buffer

- sulfo-NHS:N-hydroxysulfosuccinimide (Fisher Scientific)
prepare a fresh 1 mg/ml solution in activation buffer

Methods:

Shake vial with EuNPs thoroughly before withdrawing an aliquot with a sterile pipet tip (work for this step in PCR flow cabinet).

Take 100 µg EuNPs (= 10 µl of the 1%(w/v) suspension) and centrifuge for 15 minutes at 14.000 x g to remove glycerol and phosphate (discard supernatant).

Repeat this cleaning step once with 100 µl activation buffer.

Resuspend pellet EuNPs in 10 µl 1 mg/ml EDC + 10 µl 1 mg/ml s-NHS + 100 µl of MES (activation buffer) and stir for 45 minutes at room temperature for activation of -COOH groups.

Remove unbound EDC and s-NHS by centrifugation for 15 minutes at 14.000 x g.

Wash activated EuNPs twice with binding buffer and resuspend in 100 µl bindingbuffer.

Mix 20 µg of antibody into the solution and incubate for 3 hours under stirring at room temperature.

Remove uncoupled antibody by centrifugation for 15 minutes at 10.000 x g at
4 °C

Wash particles twice with 100 µl washing buffer

Resuspend particles in 100 µl blocking buffer to block unreacted active sites overnight at 4 °C

Wash the conjugate three times with washing buffer.

Resuspend the pellet in 100 µl of storage buffer; concentration is now 0.1%(w/v) EuNPs-Ab conjugate.

Sensing is life

DiRa-Lab

FAE Training

Horst Gether, Product Manager
01/02/2022

Agenda

1. System Block Diagram DiRa-Lab
2. Measurement Principles Colorimetric and Fluorescence
3. DiRa-Lab Phase Introduction
4. DiRa-Lab GUI Introduction
5. How to create a measurement sequence with DiRa-Lab
6. First Measurement with DiRa-Lab

DiRa-Lab System Block Diagram

ams spectral sensors do enable bench top reader performance in pocket size format

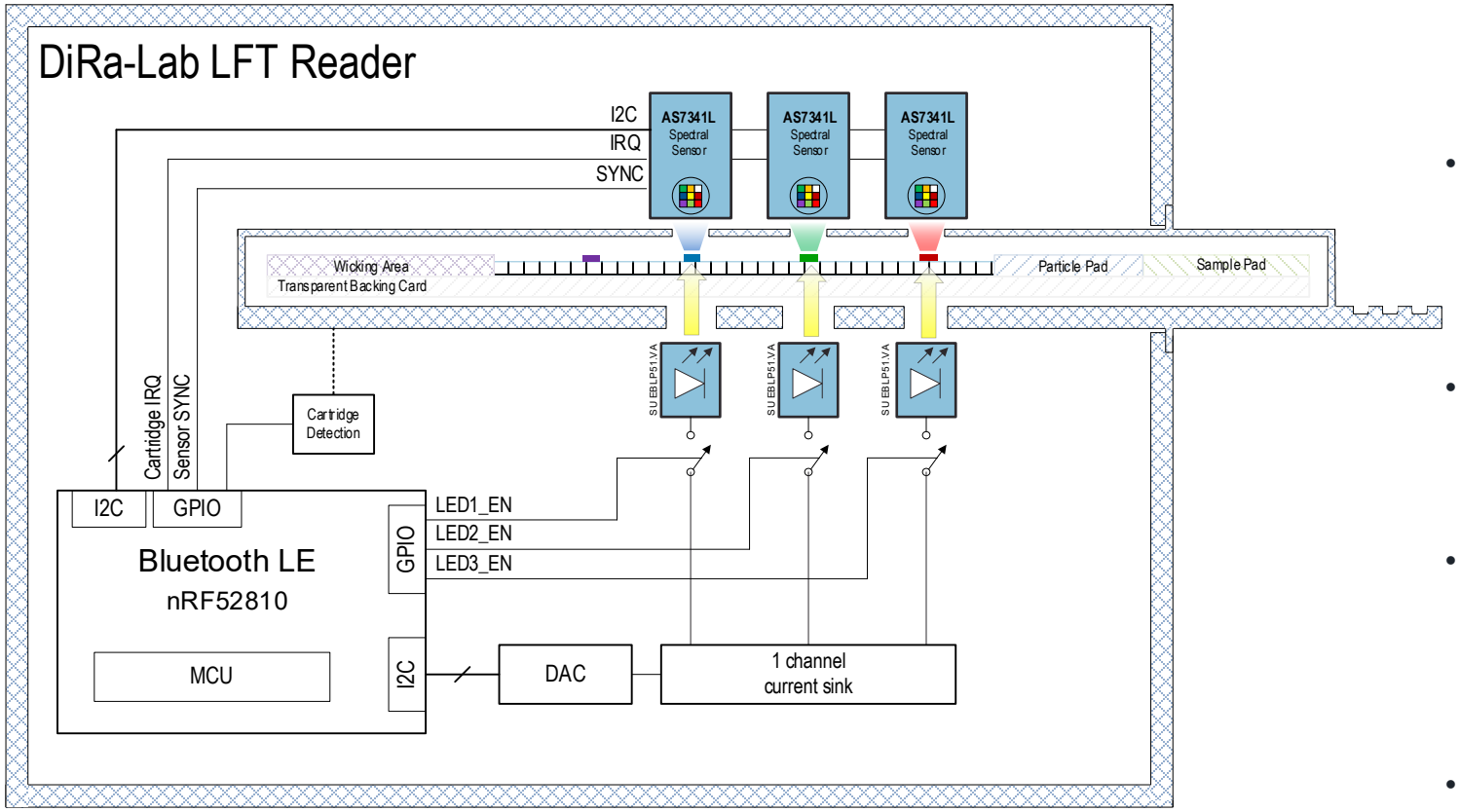


Figure 1: DiRa-Lab System Block Diagram

- Cost effective Bluetooth Low Energy devices can be the core processing element of DiRa-Lab.
- Up to 4 AS7341L sensors to reader out up to 4 LFT test lines with highest sensitivity controlled via I2C interface and GPIO
- LFT strip backside elimination via DAC controlled current sink (<1% accuracy)
- Precise measurement sequence control and synchronization to sensors ADCs via GPIO controlled LEDs
- Simple cartridge detection solution via optical or mechanical switch can be implemented

For internal use

DiRa-Lab Measurement Principles

Fluorescence Measurement Principle

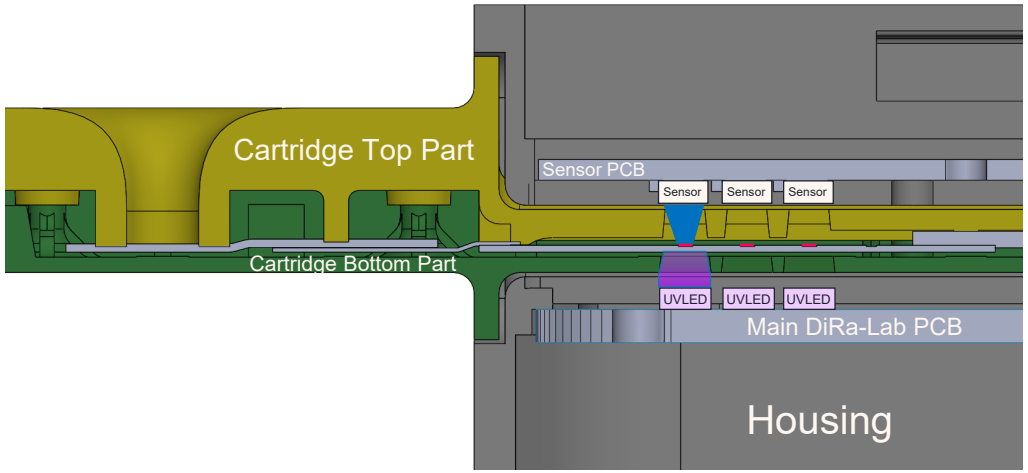


Figure 2: DiRa-Lab Sectional View

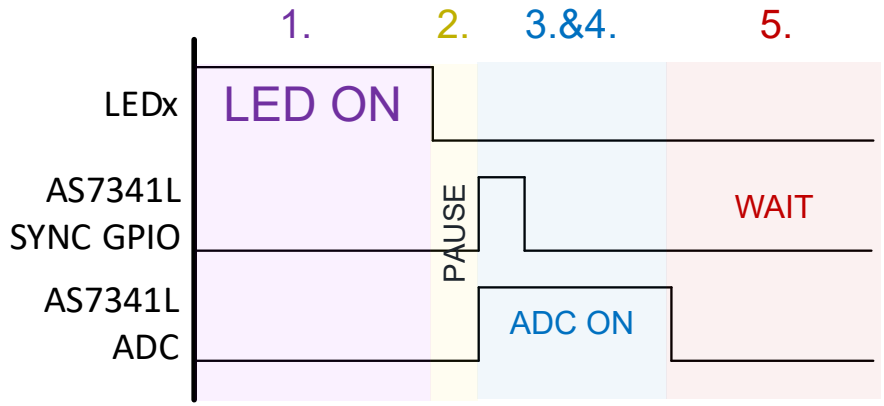


Figure 3: Optical Stack Timing Diagram Fluorescence

1. UV LED switch on to start illumination of test line
2. UV LED is switched off; Short pause time to make sure LED is not on any more
3. ADC of AS7341L sensor is enabled via SYNC pin of AS7341L
4. Emitted light of test line is picked up by AS7341L spectral sensor
5. Wait time until next sample periods starts again

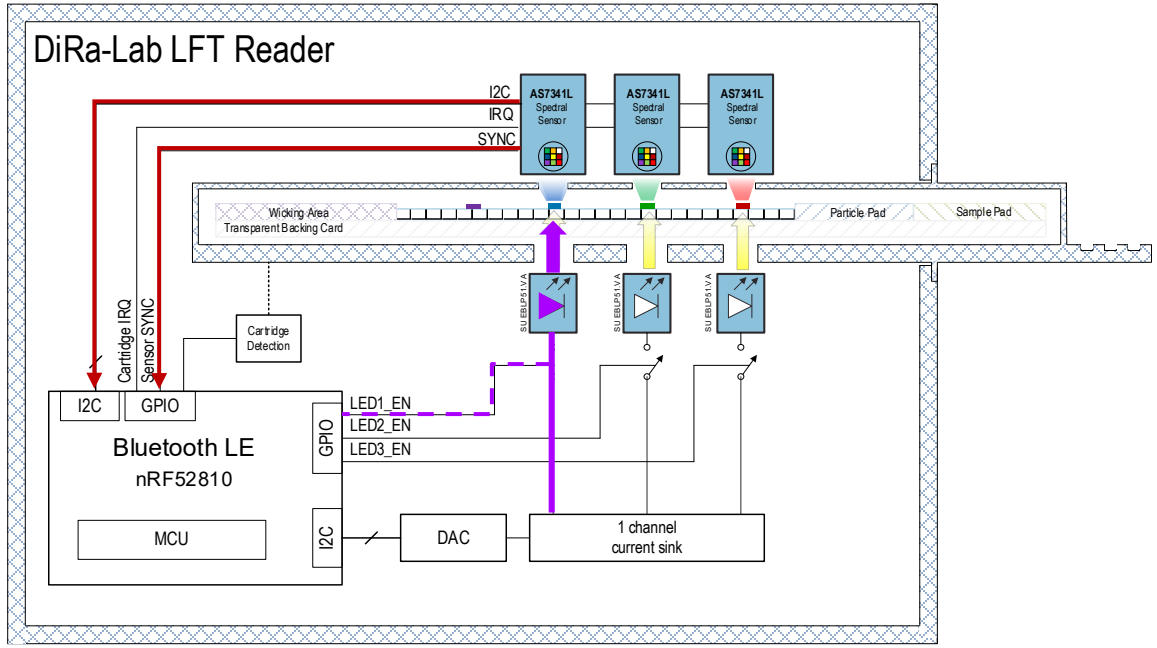


Figure 4: DiRa-Lab System Block Diagram

DiRa-Lab Measurement Principles

Colorimetric Measurement Principle

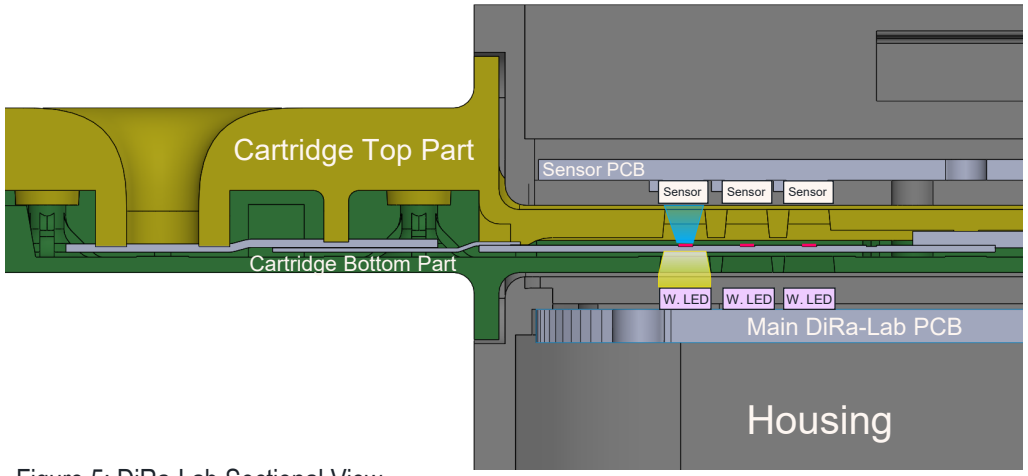


Figure 5: DiRa-Lab Sectional View

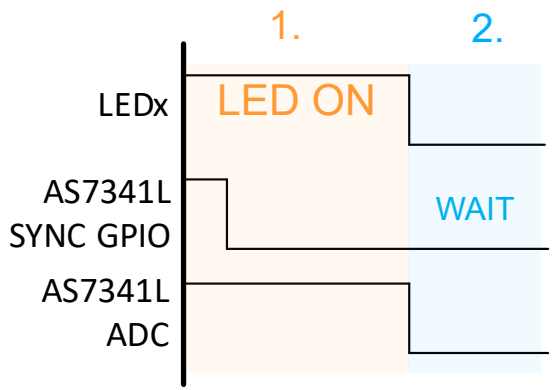


Figure 6: Optical Stack Timing Diagram Colorimetric

1. White LED switch on to start illumination of test line
   ADC of AS7341L sensor is enabled via SYNC pin of AS7341L
   Emitted light of test line is picked up by AS7341L spectral sensor
2. Wait time until next sample periods starts again

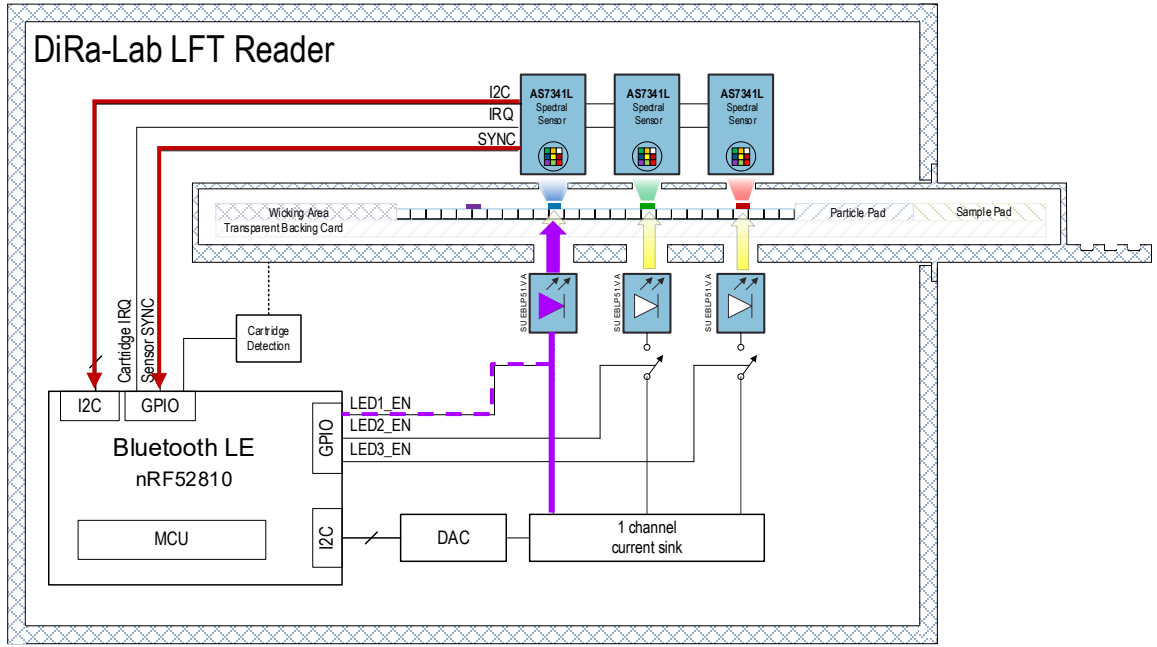
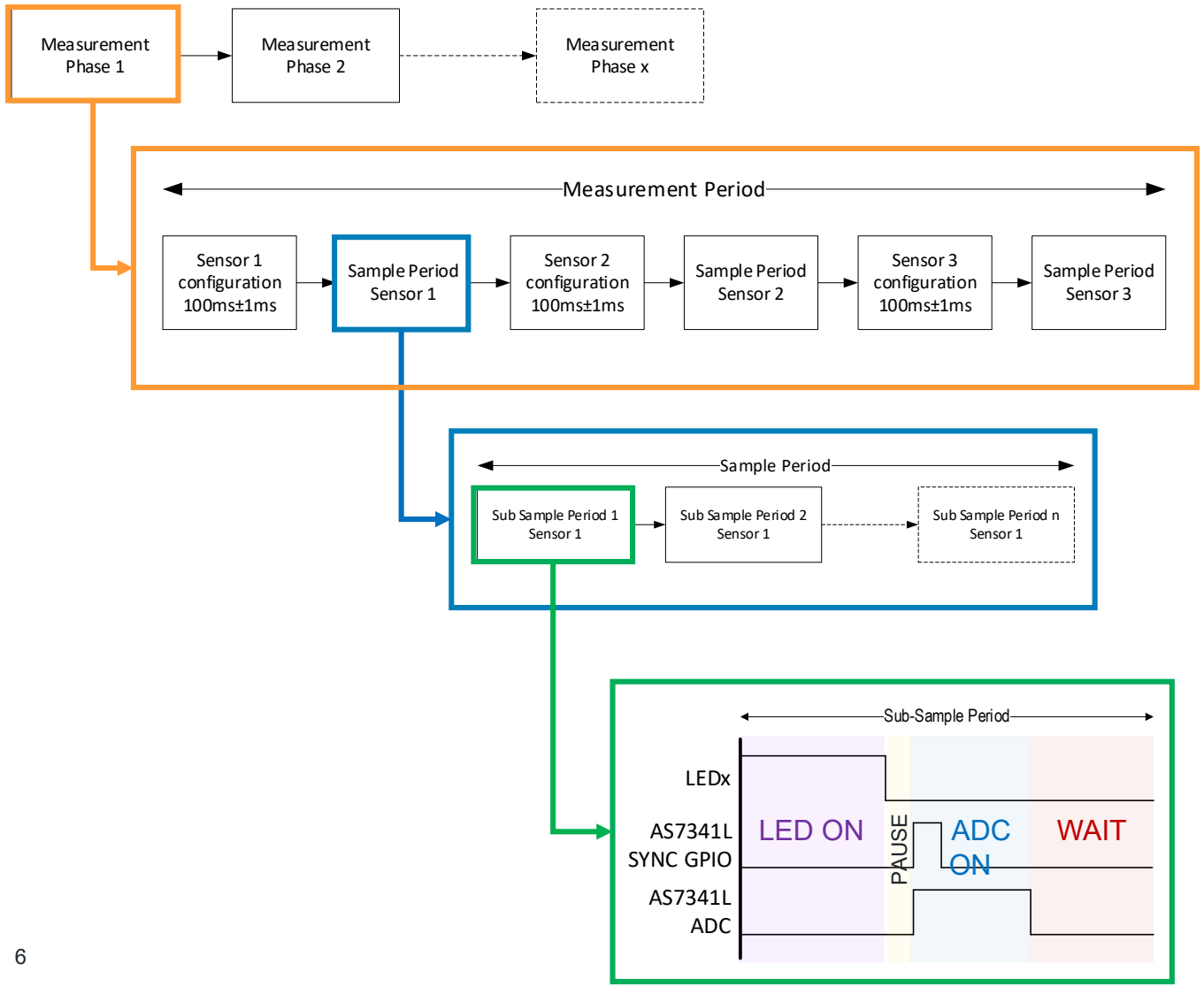


Figure 7: DiRa-Lab System Block Diagram

DiRa-Lab

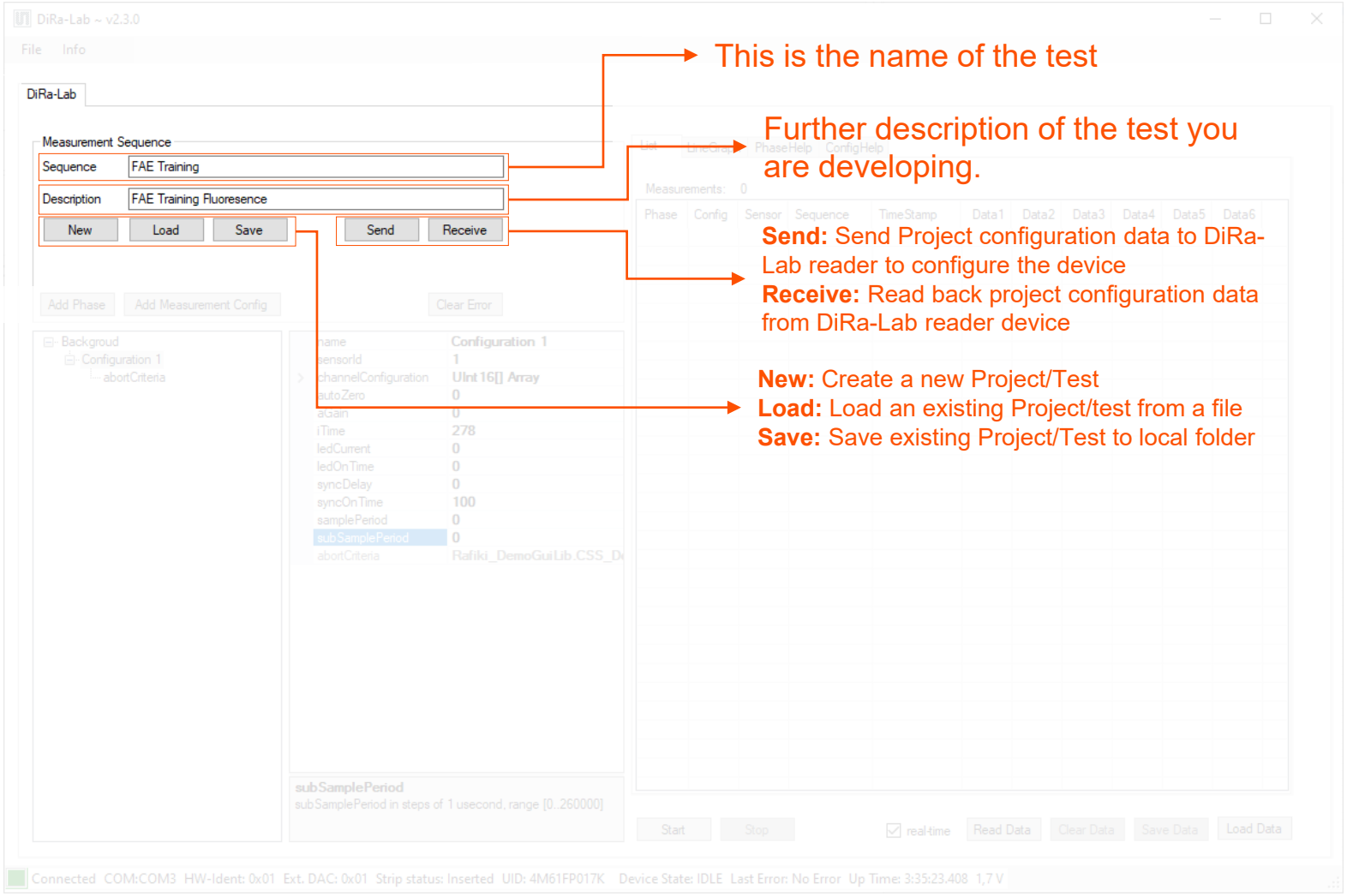
LFT Strip measurements consist our of multiple sub-sample measurements



- In a typ. measurement configuration the previously shown sub-sample is executed multiple times to average measurement result especially at little concentrations?
- Therefore the “Sample Period” can be divided into multiple sub-sample periods defining the number of sub-sample executions
  - *e.g. 200ms sample period and 10ms sub sample period*
    - *20 repetitions of the sub-sample period.*
- Since there are typ. multiple test lines, whereas each line is read with a dedicated spectral sensor, a complete measurement period is assembled with multiple sample periods
- Important to know is that prior to each sample period a Sensor Configuration is necessary which typ. takes 100ms
- Therefore, when defining the measurement period in the GUI its important to make sure that the sample configuration time is considered in the definition of the measurement period

DiRa-Lab GUI Introduction

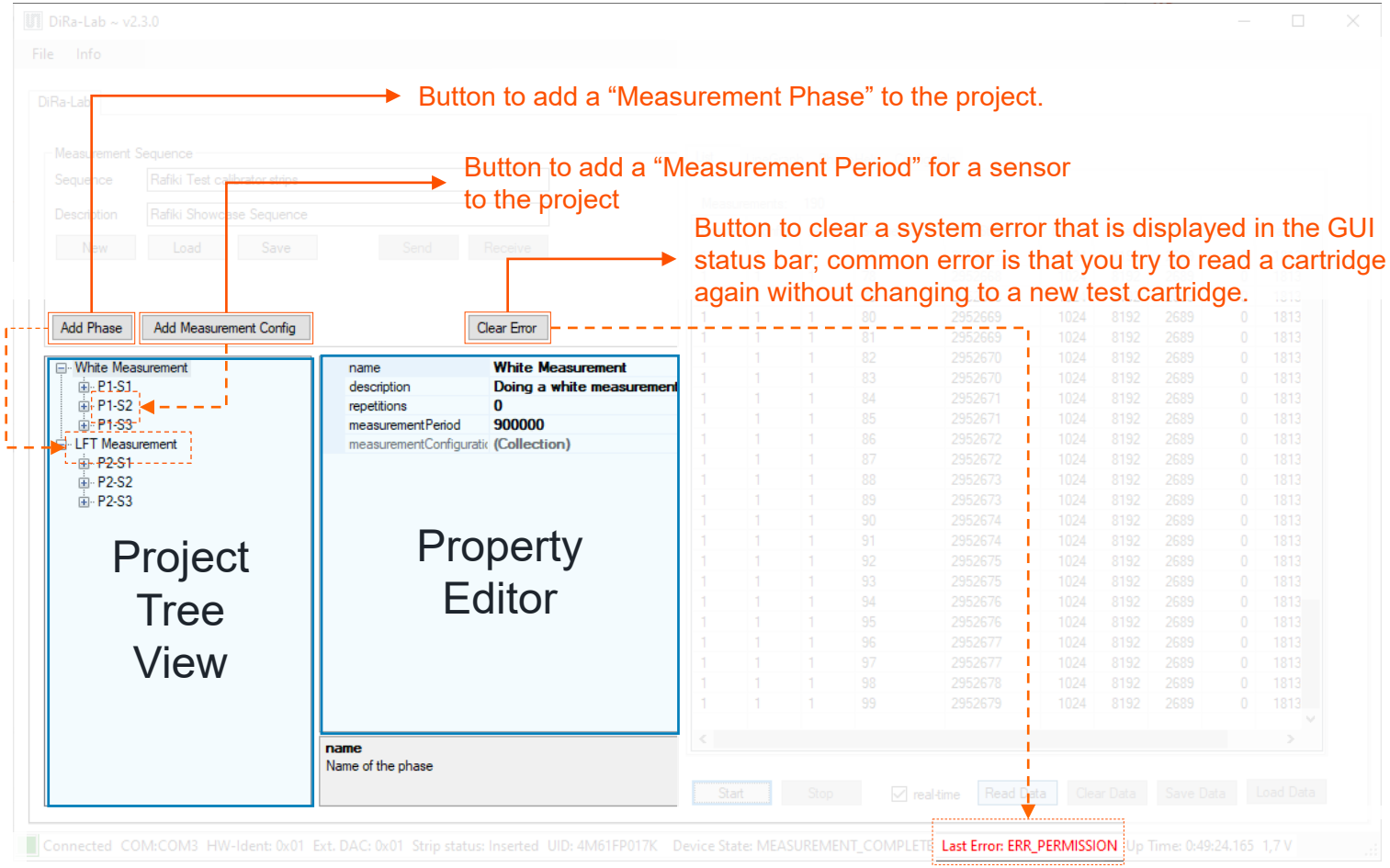
Control Area – New; Load; Save; Send; Receive; Sequence; Description



The “Control Area” of the DiRa-Lab software is used for project control, defines project names, load and save project etc.

DiRa-Lab GUI Introduction

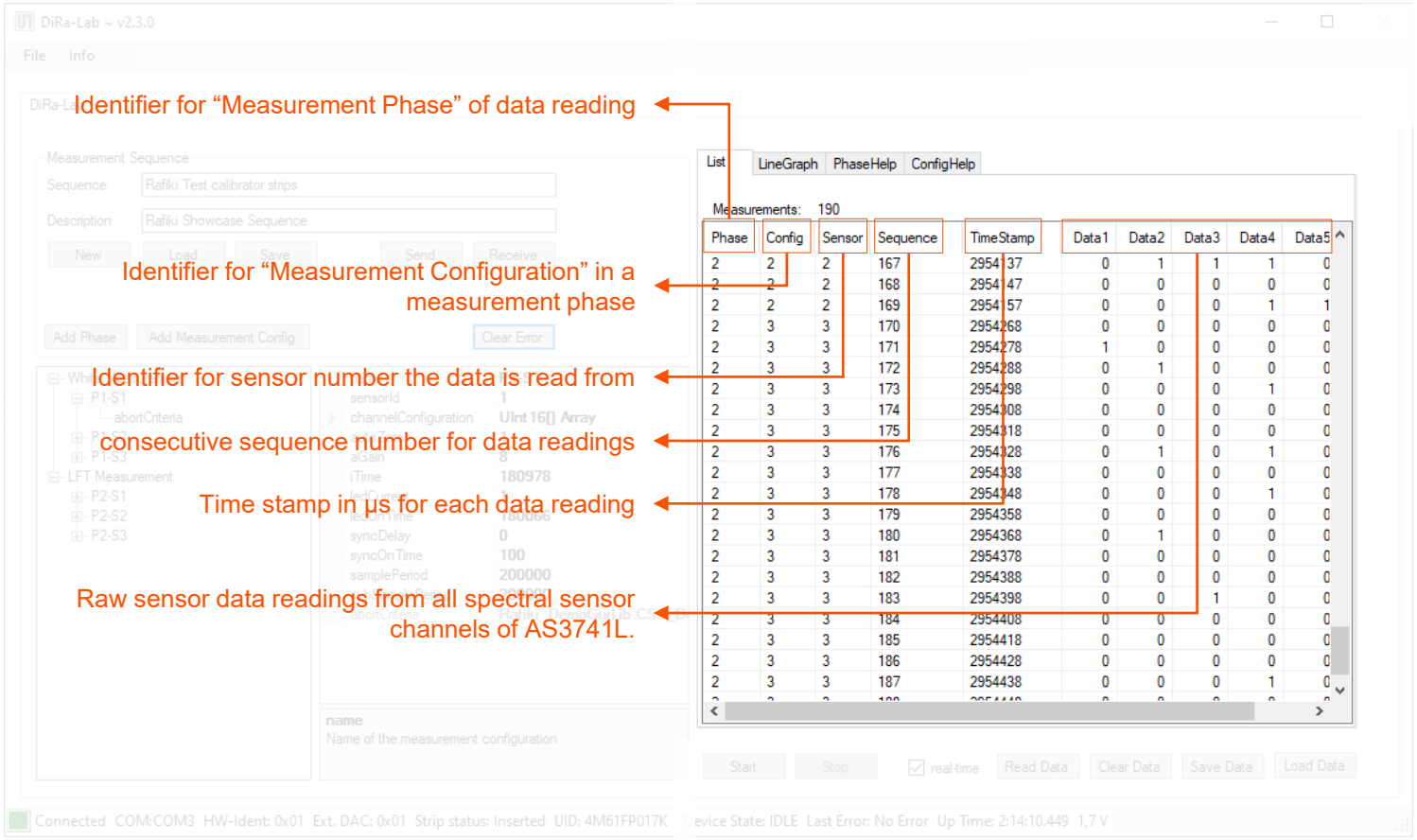
Project Area and Property Editor - Add Phase; Add Config



- The “Project Area” of the DiRa-Lab software is used to define and configure the different measurement phases and sequences
- The Project Tree summarizes the measurement phases and measurement periods for a loaded project
- The Property Editor is used to edit the relevant configuration parameters like timings, LED current, etc.

DiRa-Lab GUI Introduction

Data Explorer – List View

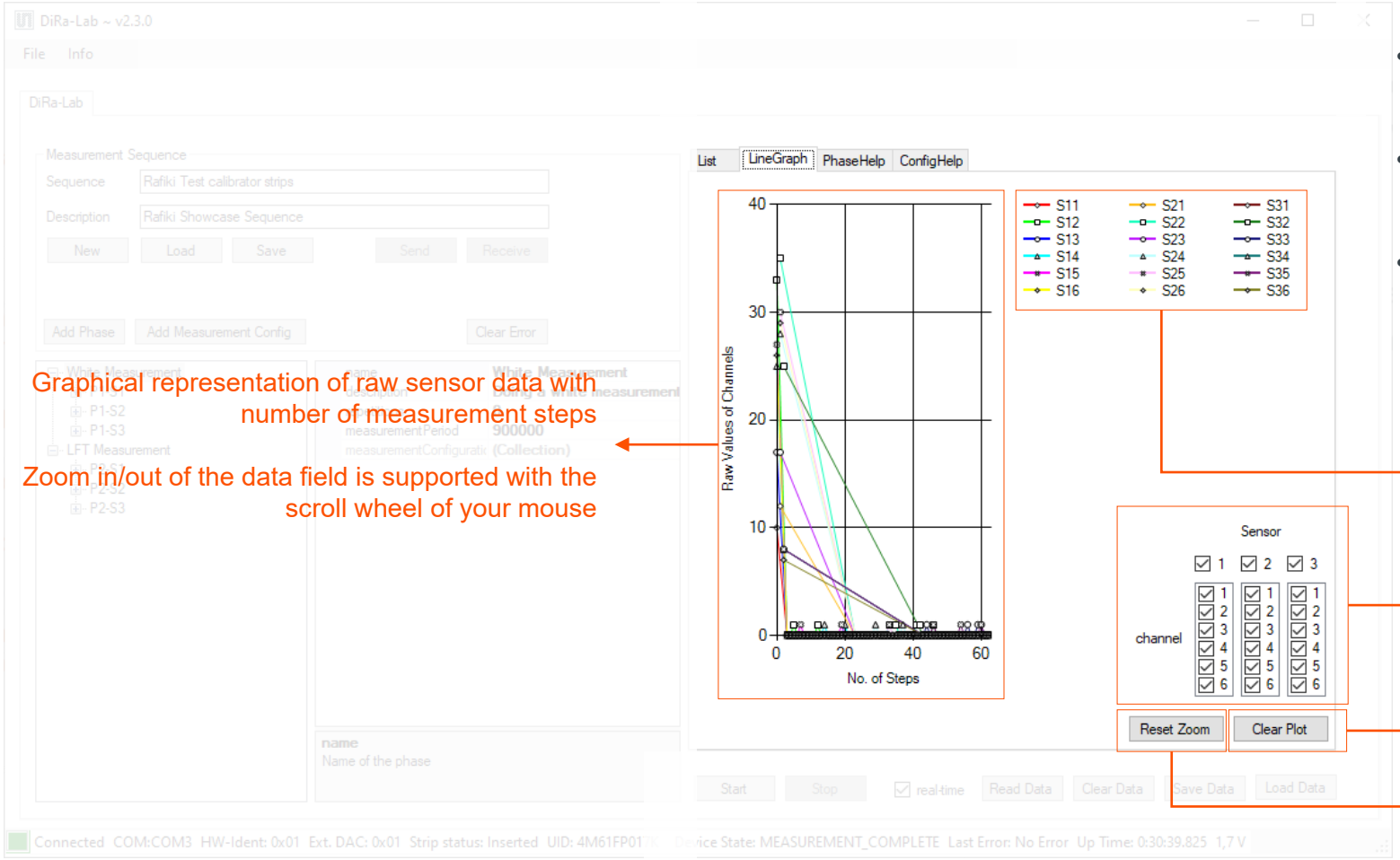


- The Data Explorer window provides the raw sensor data of all relevant spectral channels of AS7341L.
- Each data reading is labeled with time stamp, sensor ID as well as measurement phase information.

For internal use

DiRa-Lab GUI Introduction

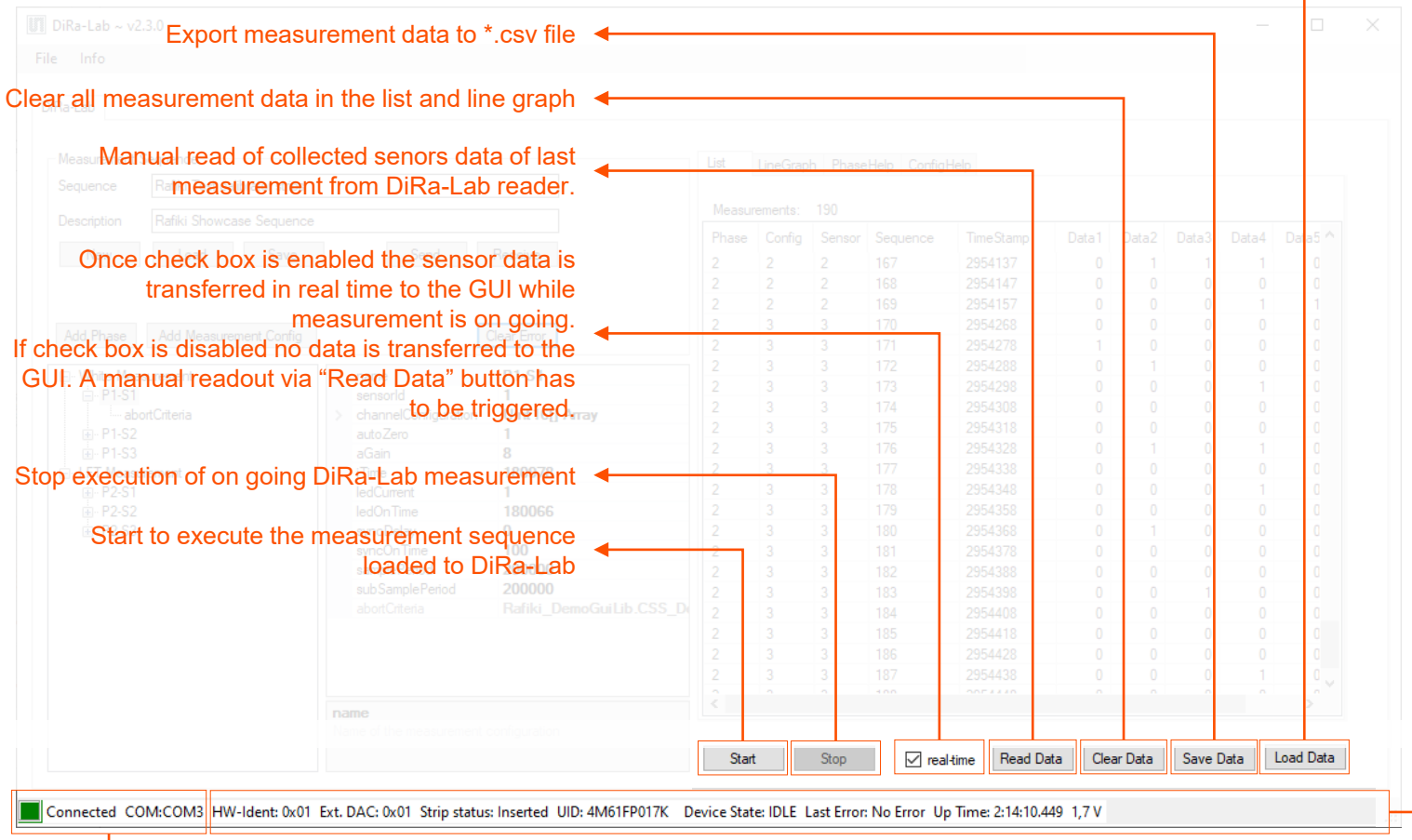
Data Explorer – Line Graph View



- The LineGraph menu allows also for graphical representation of the sensor data
- A zoom functionality is available for better readability of the graph
- Sensors and related sensor channels can be enabled and disabled depending on application requirements

DiRa-Lab GUI Introduction

Measurement Control and Status Bar



HW-Ident: provides information about the DiRa-Lab reader version. A value of 0x01 indicates that reader is equipped with white LEDs for colorimetric measurements. A value of 0x00 indicates that UV LEDs are installed to support fluorescent cartridges.

Ext.DAC: A value of 0x01 indicates the highly accurate external DAC current sink is in use with the reader. A value of 0x00 would indicate that the on board AS7341L chips are used.

Strip Status: provides feedback if a cartridge is detected in the reader

UID: This is the unique identifier of the DiRa-Lab

Device State: provides feedback about the reader status and which operation mode it is in.

Last Error: provides an error code message in case an error occurs

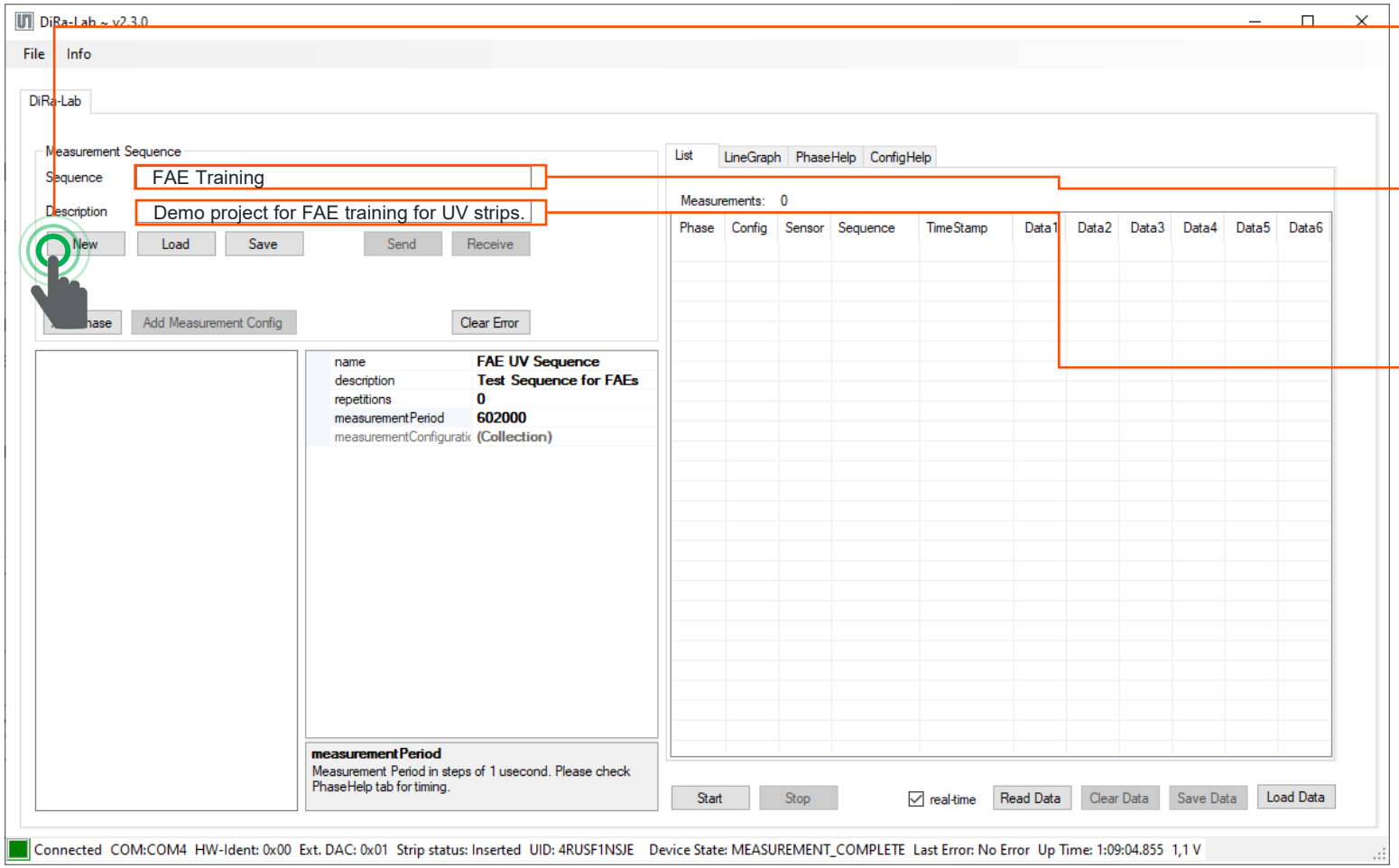
Up Time: timer how long the DiRa-Lab is switched on followed by an internal comparator reference voltage for low battery detection.

Green box: Device connected via serial interface COMx
Red box: No GUI connection to device found

ams OSRAM

How to create a measurement sequence with DiRa-Lab

1st Create a new Project



1. Click on “New” Button to start a new project

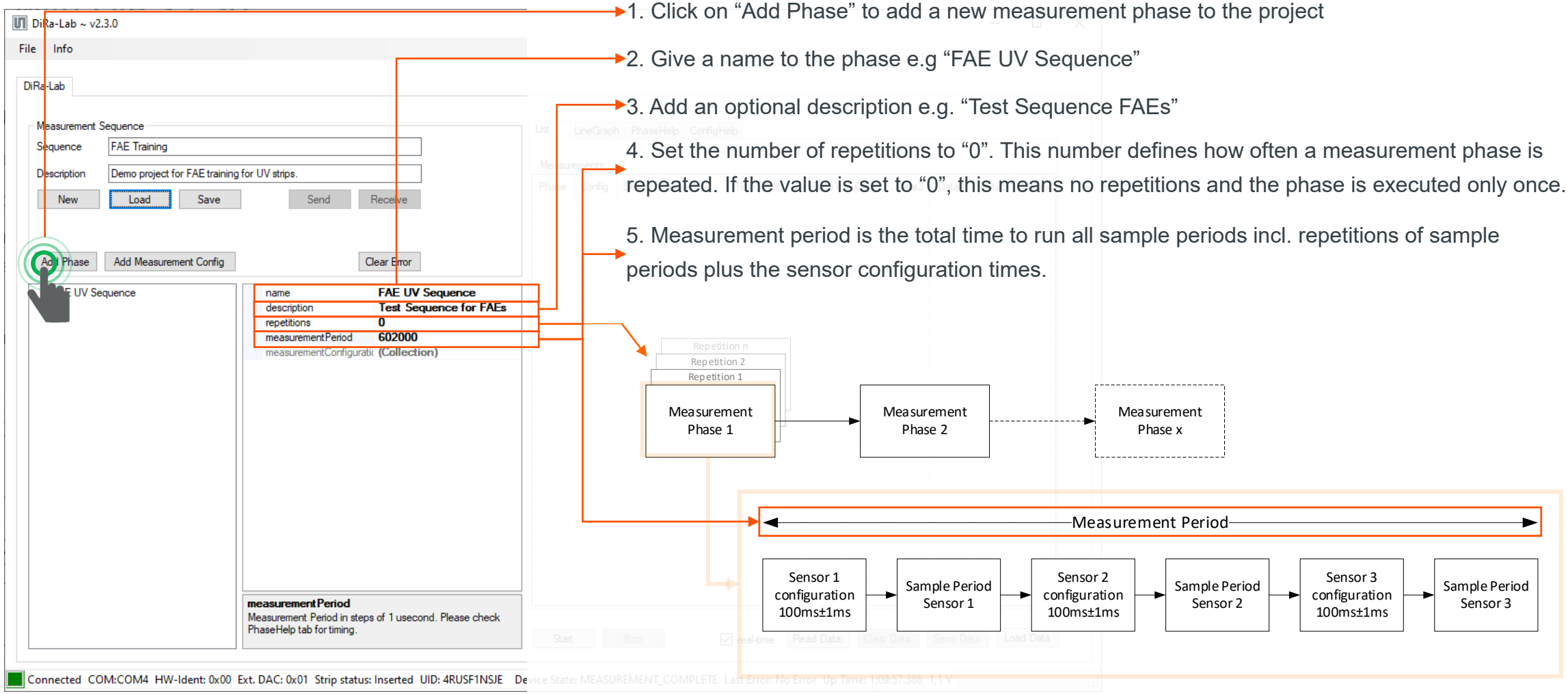
2. Enter project name and description to the control area

3. Next save the project by clicking the “Save” button under a name of your choice.

IMPORTANT: Make sure reader is connected, switched on and status bar is green with status “Connected”

How to create a measurement sequence with DiRa-Lab

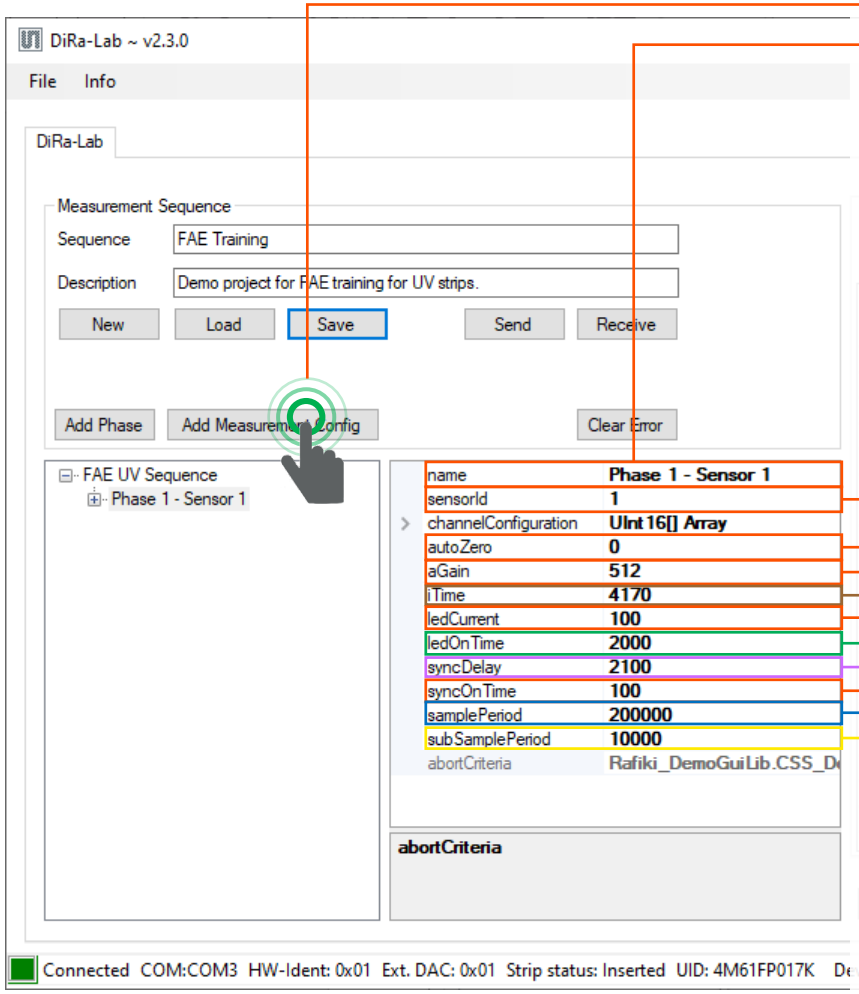
Add a Phase to the project and configure it correctly



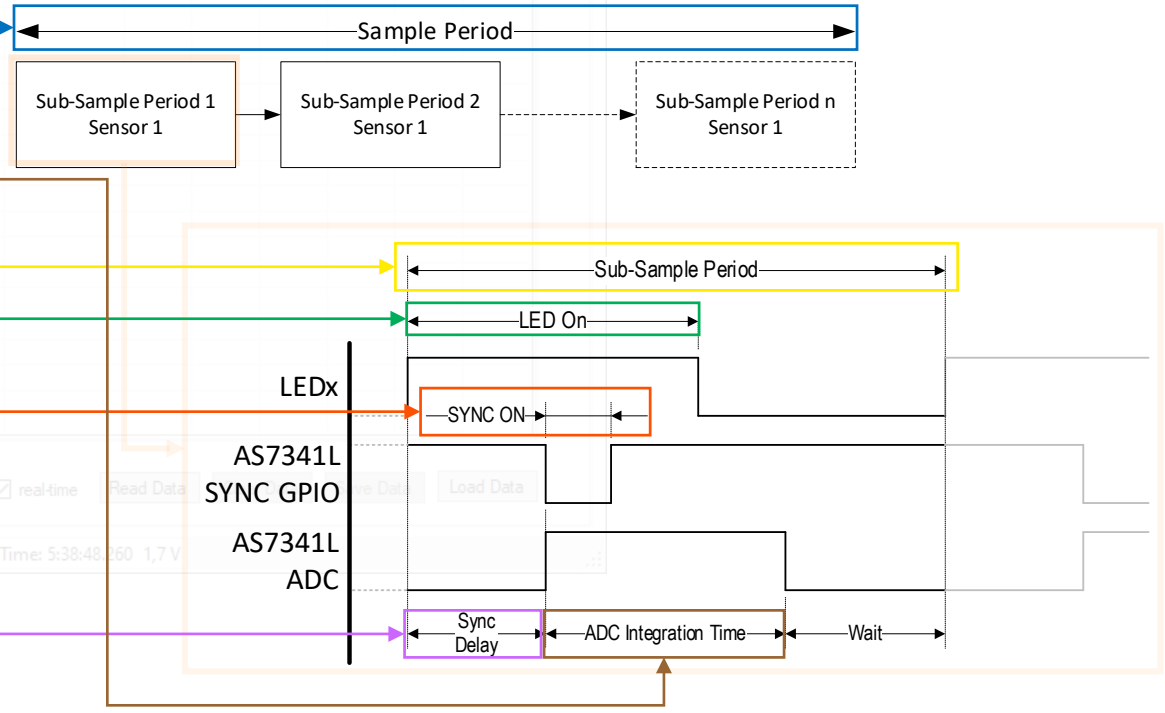
For internal use

How to create a measurement sequence with DiRa-Lab

Add a Sample Period for Sensor 1 to the project and configure the parameters accordingly



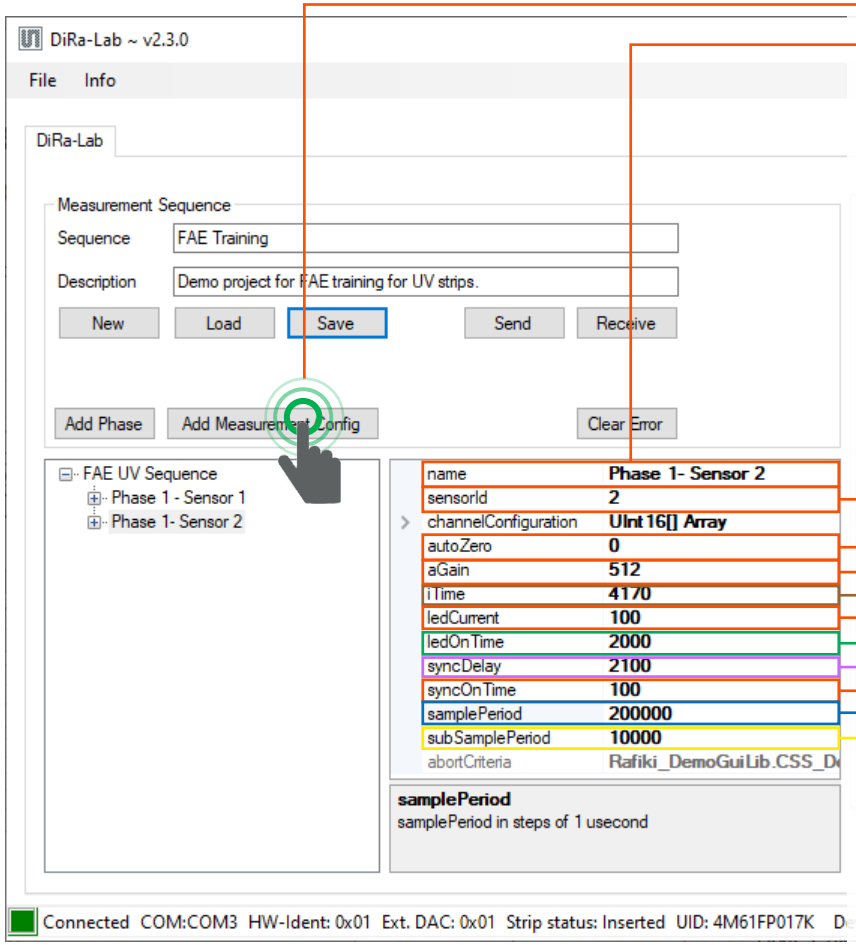
1. Click on “Add Measurement Config” to add a new Sample Period” for a sensor
2. Give a name to the Sample Period e.g. “Phase 1 – Sensor 1”
3. Defines the sensor number that is read out in this Sample Period. DiRa-Lab design supports 3 sensors, therefore *sensorID* can be values from 1 – 3;
4. Is the auto zero functionality to calibrate for dark counts to be eliminated.
5. This parameter defines the pre-amplifier gain before signal is measured with integrated ADC. *aGAIN* values supported: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512
6. The parameter *ledCurrent* defined the LED current in percent. A value of 100 delivers max. LED current of 30mA to the LED during the LED On phase.



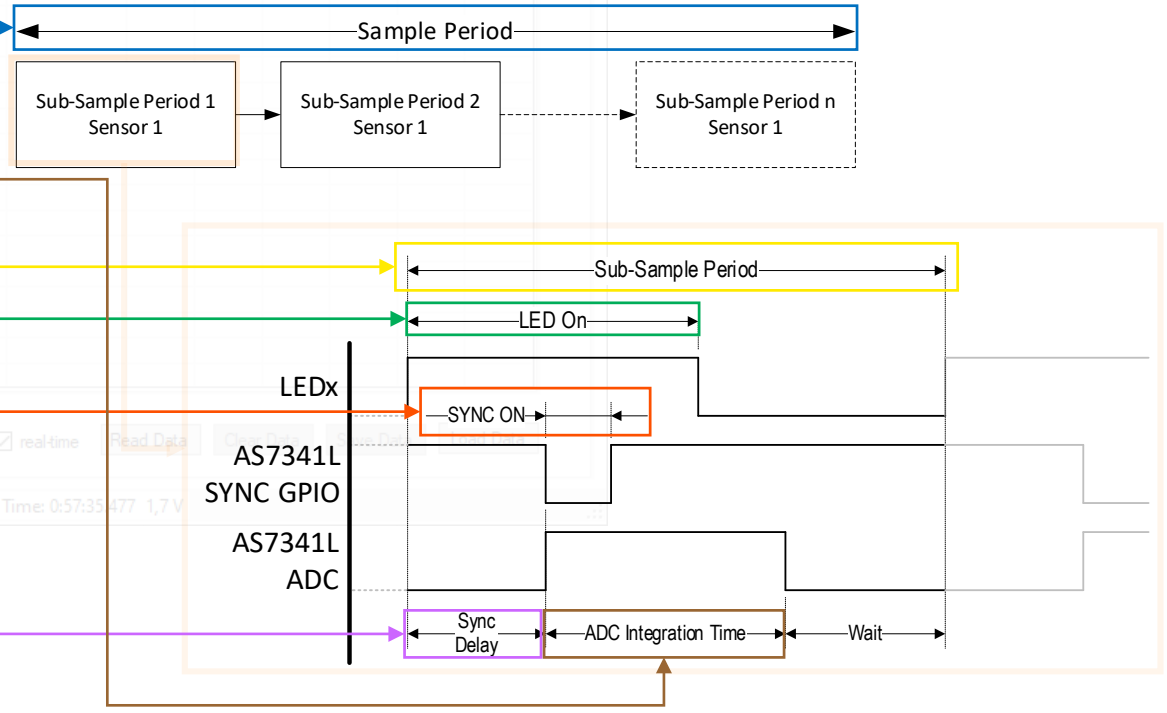
For internal use

How to create a measurement sequence with DiRa-Lab

Add a Sample Period for Sensor 2 to the project and configure the parameters accordingly



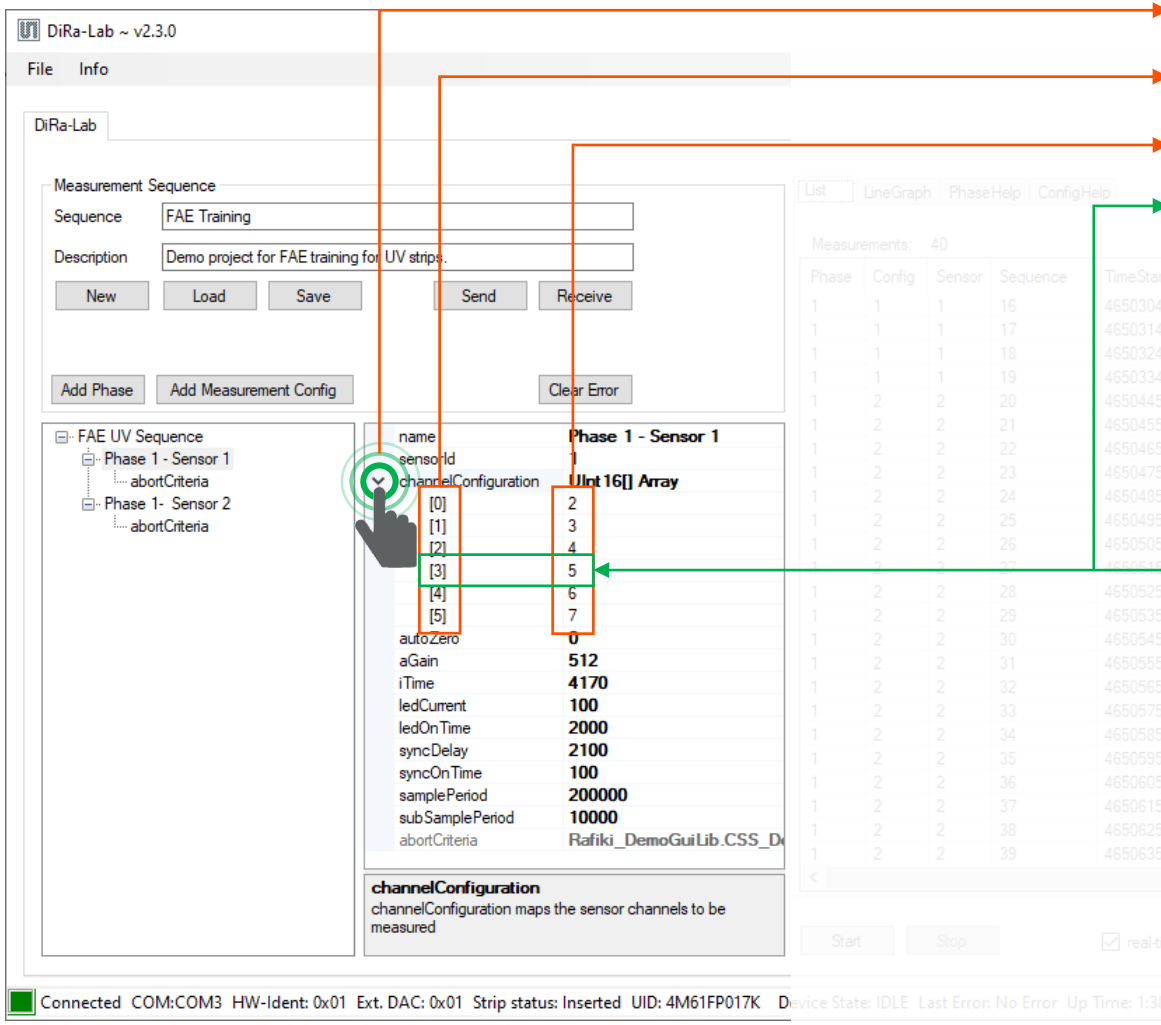
1. Click on “Add Measurement Config” to add a new Sample Period” for a sensor
2. Give a name to the Sample Period e.g. “Phase 1 – Sensor 2”
3. Defines the sensor number that is read out in this Sample Period. DiRa-Lab design supports 3 sensors, therefore *sensorID* can be values from 1 – 3;
4. Is the auto zero functionality to calibrate for dark counts to be eliminated.
5. This parameter defines the pre-amplifier gain before signal is measured with integrated ADC. *aGAIN* values supported: 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512
6. The parameter *ledCurrent* defined the LED current in percent. A value of 100 delivers max. LED current of 30mA to the LED during the LED On phase.



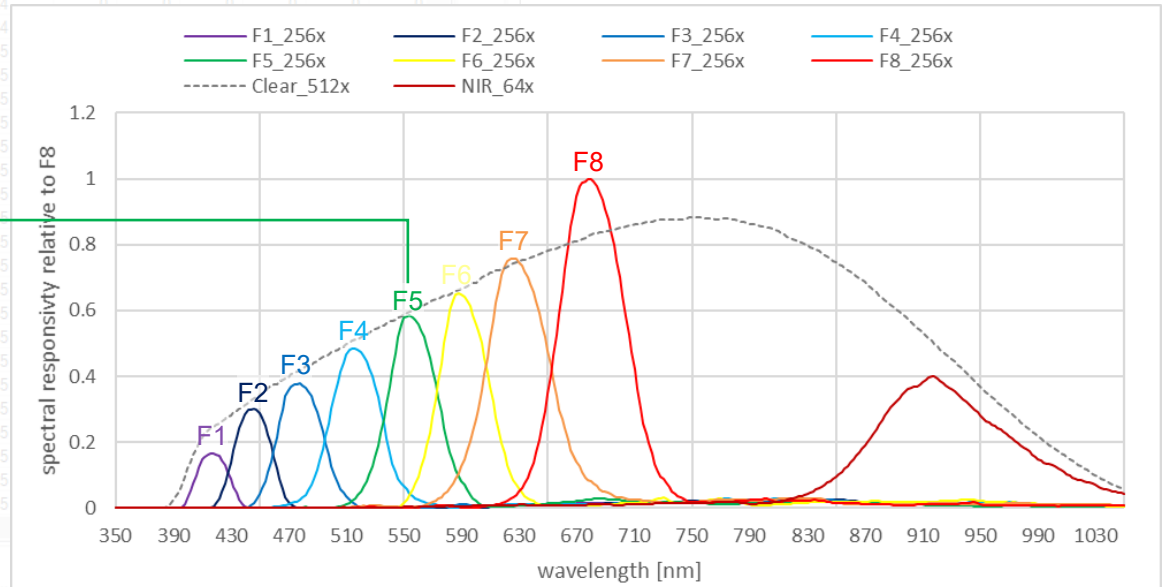
For internal use

How to create a measurement sequence with DiRa-Lab

Channel Configuration



1. Click on the down arrow at channel configuration to open the drop down menu
2. In the channel configuration settings the first column represents the 16 bit unsigned integer data array with six data elements inside the array ([0] ….[5]) which are filled with the raw sensor data.
3. The second column represents the assigned spectral color channel of AS7341L to the data array.
4. The raw data count provided by the sensor a e.g. spectral channel F5 is stored in the data array at position 3.
5. Repeat the channel assignment also for Phase 1 – Sensor 2 in order to finalized the example configuration of the training project.

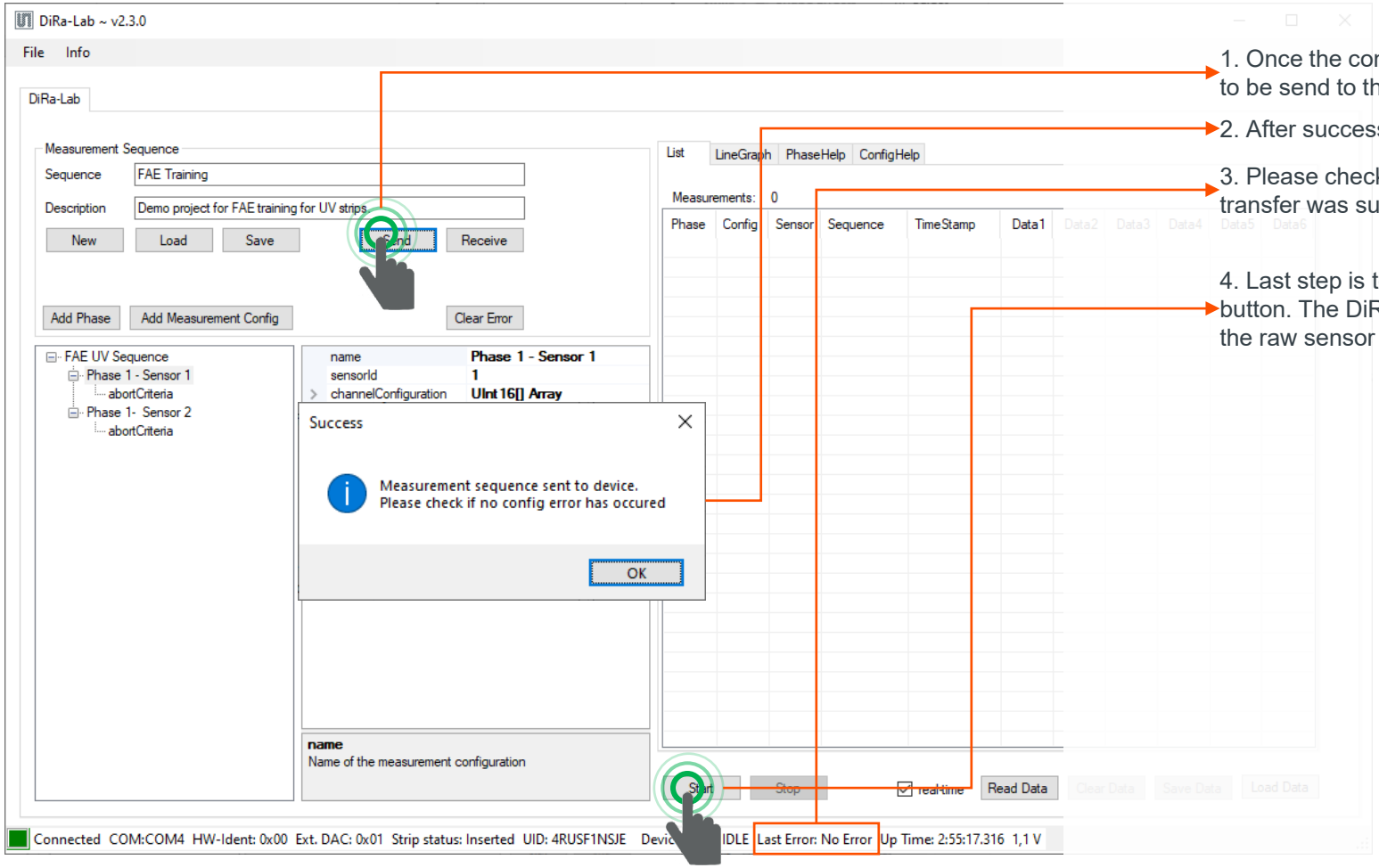


	F1	F2	F3	F4	F5	F6	F7	F8
Peak [nm]	415	445	480	515	555	590	630	680
FWHM [nm]	26	30	36	39	39	40	50	52

For internal use

First Measurement with DiRa-Lab

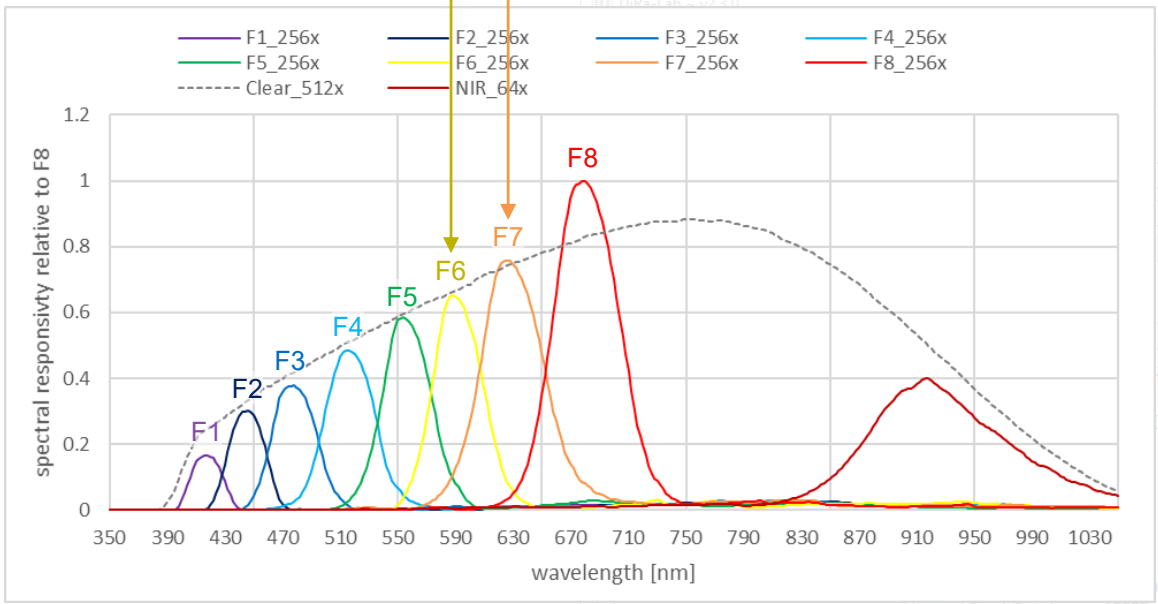
Send data to DiRa-Lab and start the measurement by pressing the “start” button

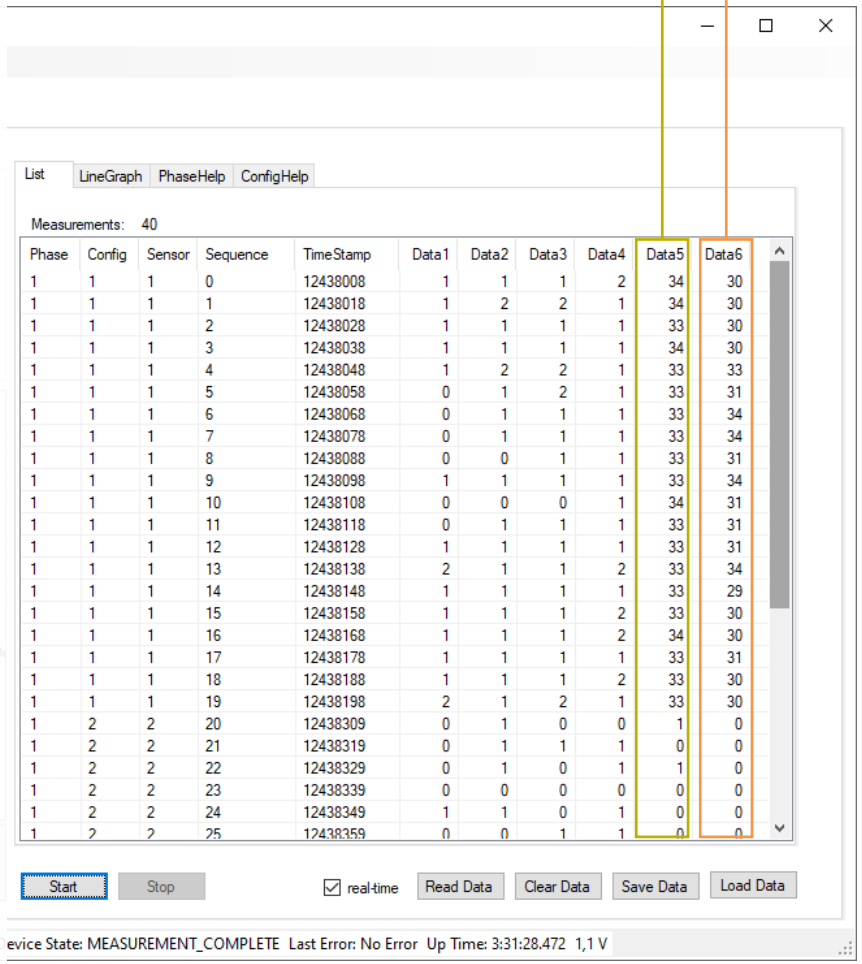


1. Once the configuration of the test sequence is complete the configuration needs to be send to the DiRa-Lab by pressing the “Send” button.
2. After successful transfer of the data a pop-up window appears.
3. Please check if there is an error code displayed at the status bar. In case the transfer was successful there should be “No Error” displayed.
4. Last step is to start the automatic measurement sequence by clicking the “Start” button. The DiRa-Lab is then executing the measurement sequence and provides the raw sensor data in the Data Explorer.

First Measurement with DiRa-Lab

Data Explorer - Measurement Results – List View





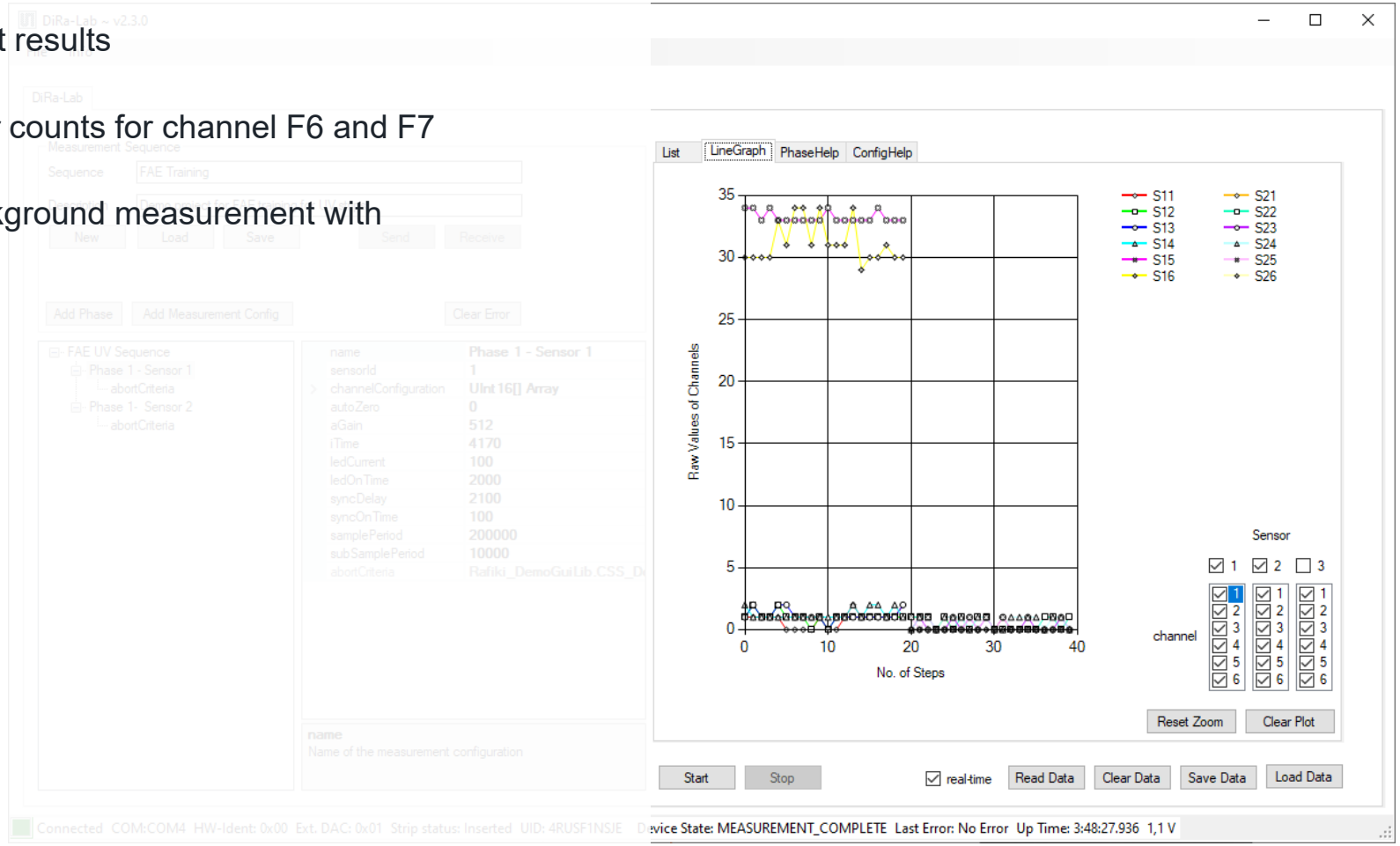
- Measurement results, as assigned in the channel configuration, is displayed in the Data Explorer.
- Each sensor is read out 20 times in this training example file (period time 200000µs/10000µs = 20 cycles)
- Sensor 1, which holds the calibrator test lines, provides sensor counts on channel F6 and F7 as expected
- Sensor 2, which is used for background noise measurement, provides little counts as expected.

For internal use

First Measurement with DiRa-Lab

Data Explorer - Measurement Results – List View

- Graphical representation of the measurement results
- First 20 measurement steps show the sensor counts for channel F6 and F7
- Step 21 to 40 represent sensor 2 for the background measurement with little sensor counts.



For internal use

Sensing is life

For internal use

User Guide

Document Number

DiRa-Lab Evaluation Kit User Manual

Evaluation Demo Kit

DiRa-Lab Demo Kit

v0-04 • 2022-Mar-08

Content Guide

1 Introduction

The LFT Reader Kit is a platform designed to evaluate the spectral identification of lateral flow tests using a digital reader system. The digital reader system is equipped with three **ams**-specific sensors of type AS7341L to achieve spectral identification. The AS7341L is a highly versatile 10-channel sensor targeted for various laboratory applications such as lateral flow tests, fluid or reagent analysis, color matching, and spectral identification in the visible range.

Two variants of the LFT Reader Kit are available, depending on the proposed application:

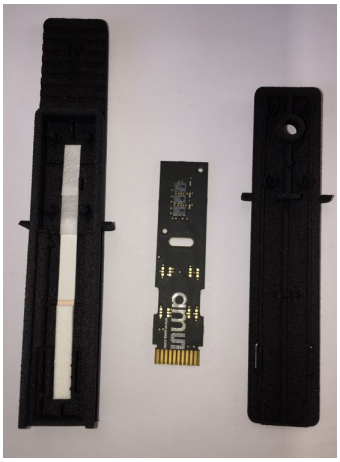
- DiRa-Lab Colorimetric LFT Reader Kit (White LEDs).
- DiRa-Lab Fluorescence LFT Reader Kit (UV LEDs).

This user manual describes the features and functions of the LFT Reader Kit, controlled via a Windows 10-based GUI. The Windows 10-based GUI application communicates with the reader via a USB interface, allowing the customer to configure their use cases. The Kit is designed in modules, which are intended to provide a head start for various lateral flow-based use cases. Customers have different technical backgrounds and support needs. Therefore, it is possible to use the complete **ams OSRAM** solution as a starting point, or only parts of it. The customer can ask **ams OSRAM** for such modules and support to develop a product.

1.1 Kit Content

The LFT Reader Kit consists of the following items, shown in Figure 1.

Figure 1:
Kit Content

Item 1 USB-C Cable	Item 2 LFT Reader	Item 3 USB Stick	Item 4 Cartridge
			

Item No.	Item	Comment
1	USB-C Cable	USB-C type cable with 16 pins, USB 2.0.
2	DiRa-Lab LFT Reader	LFT Reader with pre-assembled PCBA in the housing.
3	USB Data Stick	Documents, software, firmware, and drivers.
4	Cartridge	Content: 3D-printed housing PCBA sensor LFT strip

1.2 Ordering Information

SAP Ordering Code	Description
990601219	DiRa-Lab Fluorescence LFT Reader Kit
990601220	DiRa-Lab Colorimetric LFT Reader Kit
990601221	DiRa-Lab Fluorescence Cartridge Refill Kit
990601222	DiRa-Lab Colorimetric Cartridge Refill Kit
990601223	DiRa-Lab Empty Cartridge Kit

2 Getting Started

The LFT Reader Kit consists of a USB-C cable, LFT reader, and the cartridge, as shown in Figure 3. The cartridge is preassembled and equipped with a calibrator test strip - to minimize the alignment effort for customers and enable an intermediate start of the first measurements.

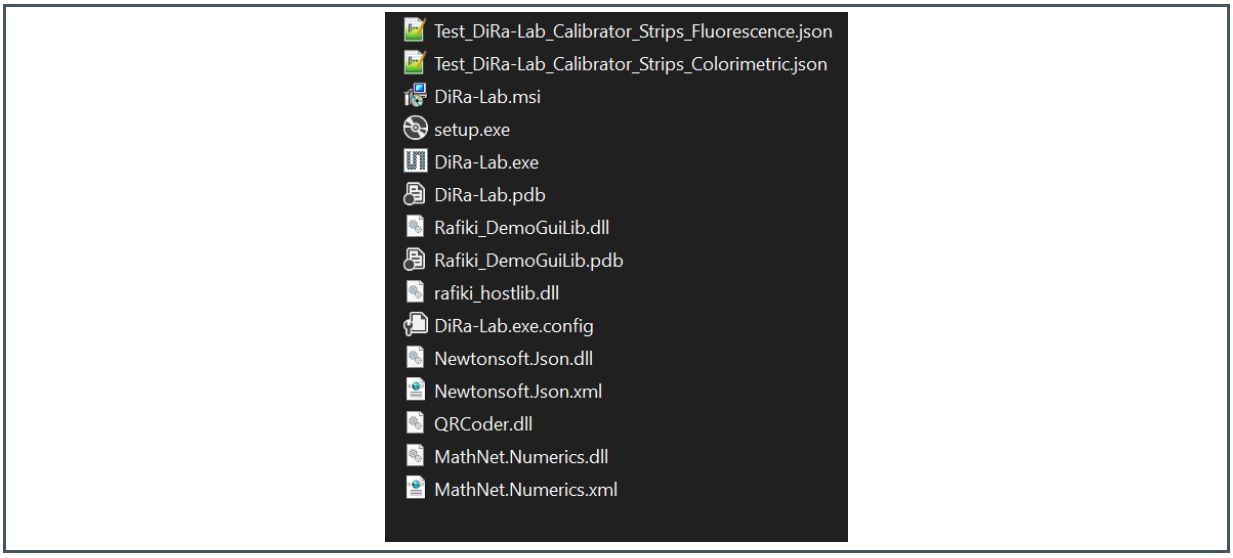
Figure 2:
LFT Reader Kit, Cartridge, and C-type USB cable

2.1 Software installation

To install the GUI, follow the steps outlined below.

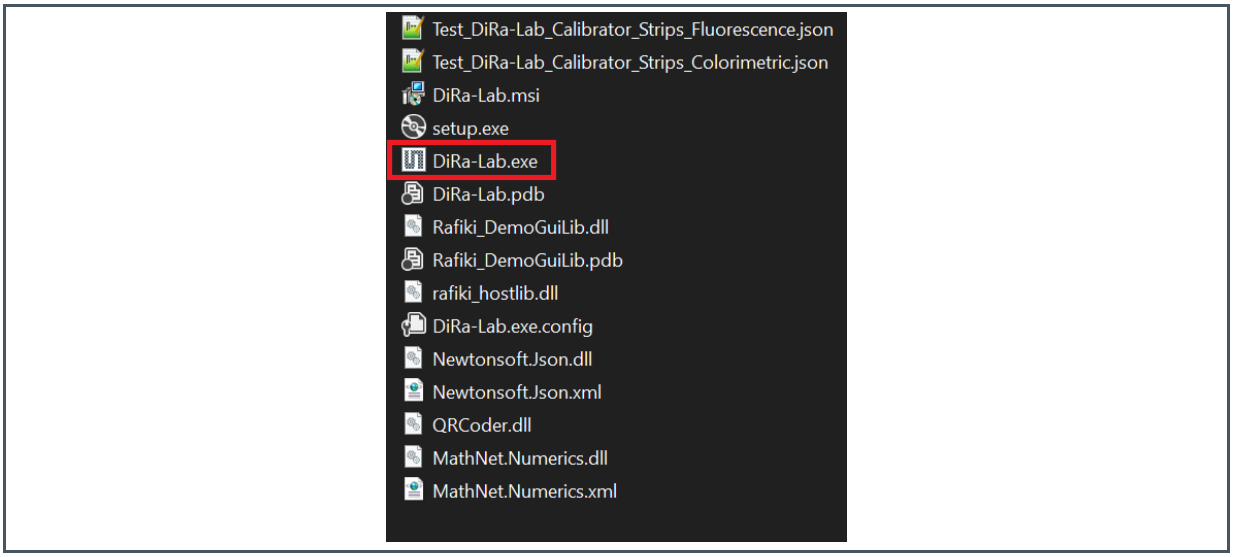
1. Connect the USB flash drive (from the Kit) to your computer with a preinstalled Windows operating system. Afterward, the folder, "*LFT_Reader_Kit*", will be displayed on the screen. Double-click on the file, "*LFT_Reader_Kit*", to display folders such as GUI, documentation, etc. Double-click on the "*GUI*" folder to find the software files, as shown in Figure 4.

Figure 3:
Contents of the GUI folder



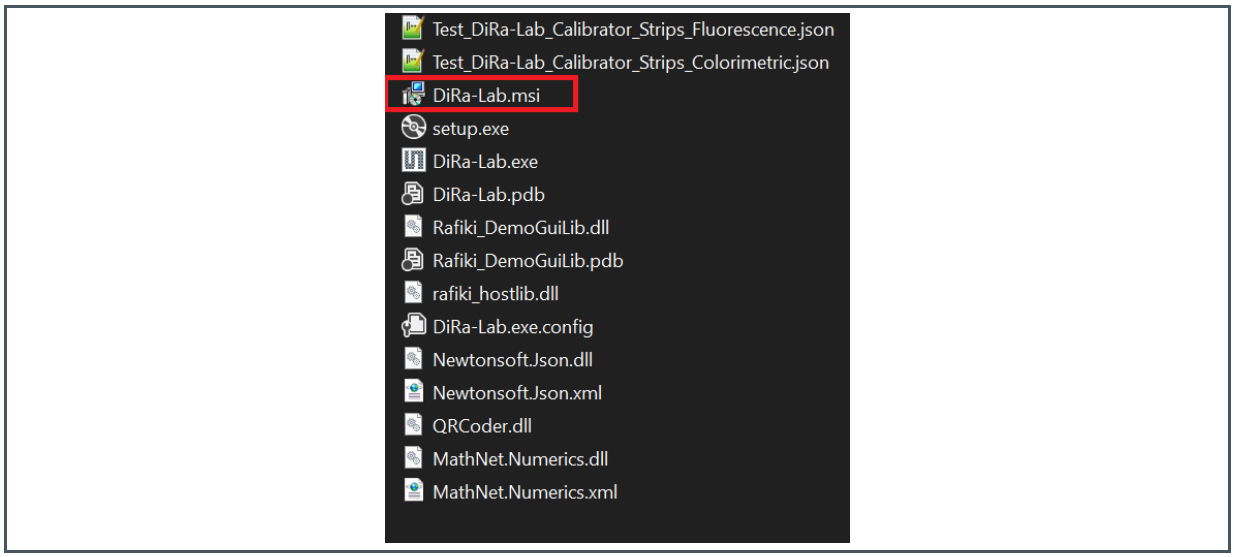
2. The GUI can be used directly without installation by double-clicking on the file "*DiRa-Lab.exe*" (Figure 5). To start the GUI without installation, please continue to chapter 2.2.

Figure 4:
Using the GUI without installation



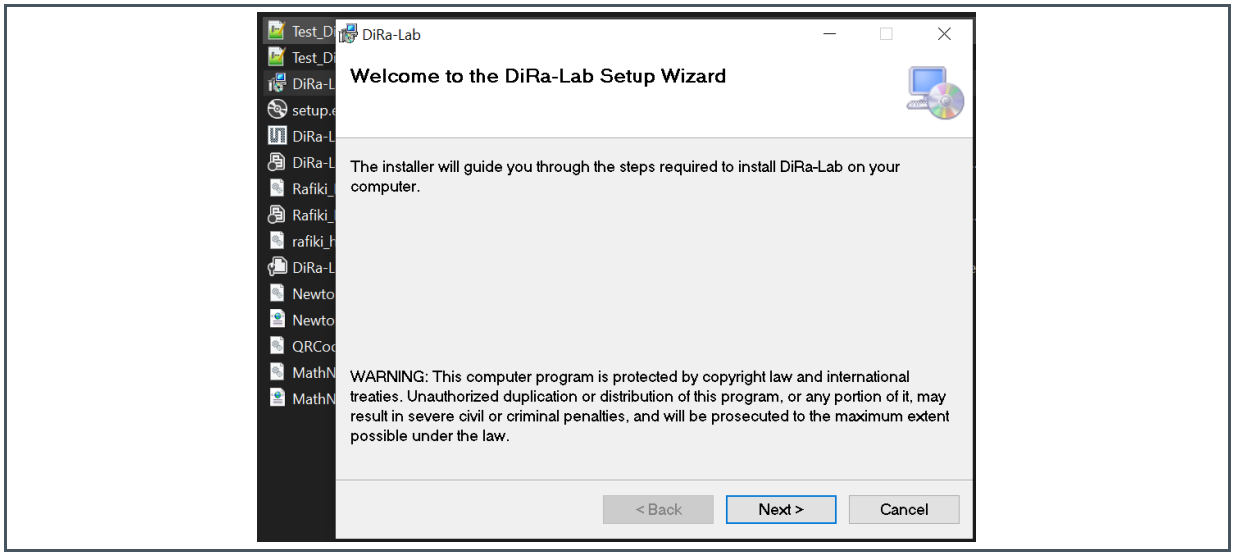
3. Tto install the GUI on your computer (recommended), double-click on the file "*DiRa-Lab.msi*" (Figure 6).

Figure 5:
Double-click to install the GUI



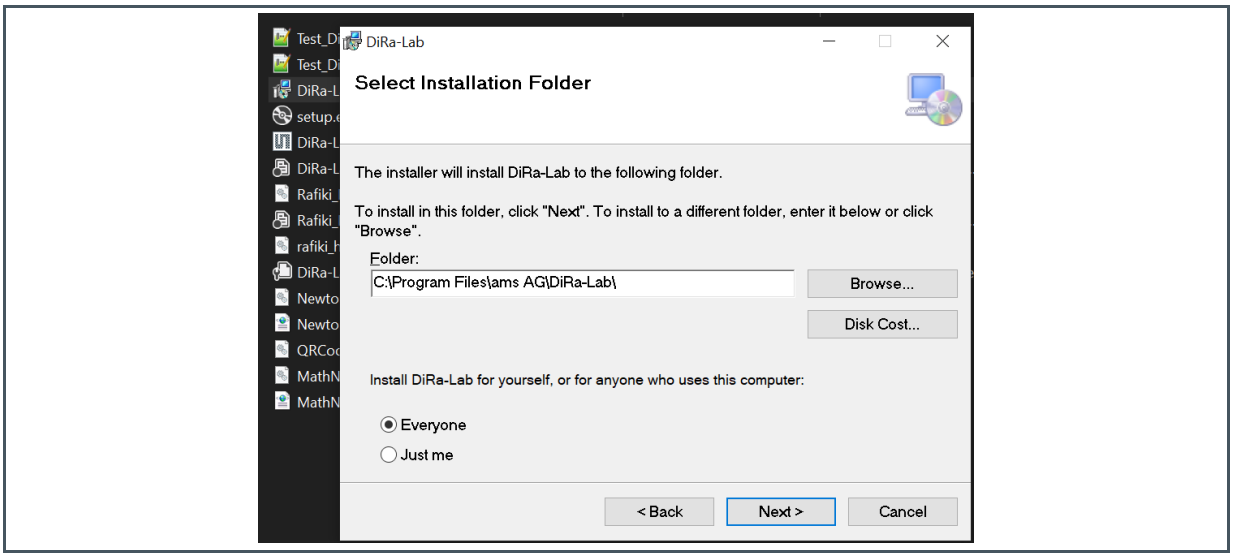
4. A pop-up with a welcome message will display on the screen (Figure 7). Click "Next" to continue.

Figure 6:
Pop-Up with Welcome message for GUI installation



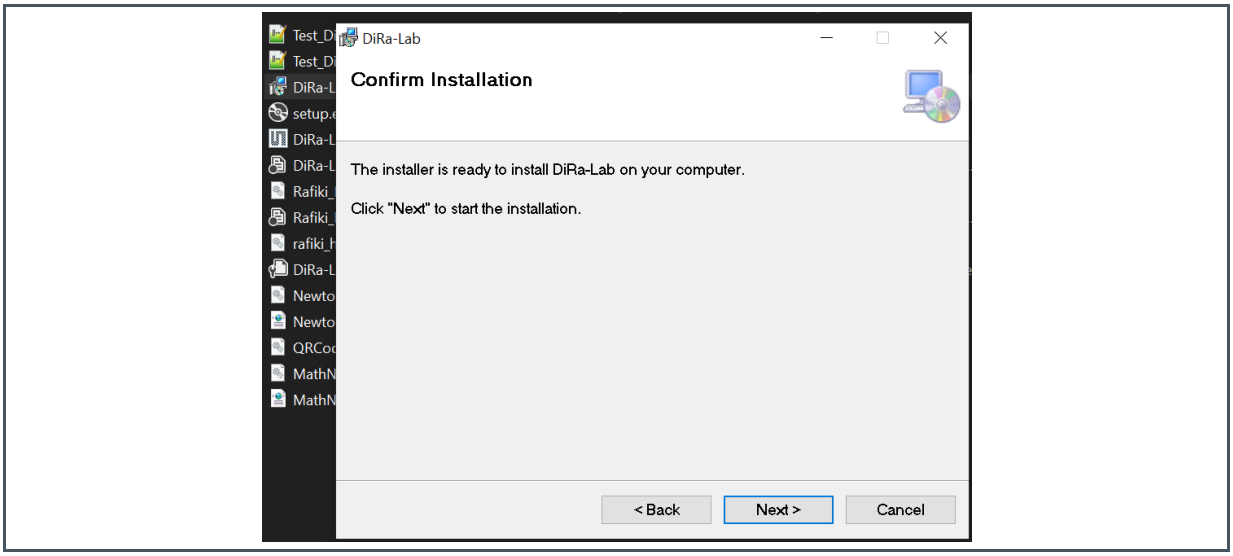
5. This leads to a new pop-up window for choosing the location to install the GUI, as well as which users will have access to it (Figure 8). Afterward, click “Next” to continue.

Figure 7:
Choose the location to install the GUI



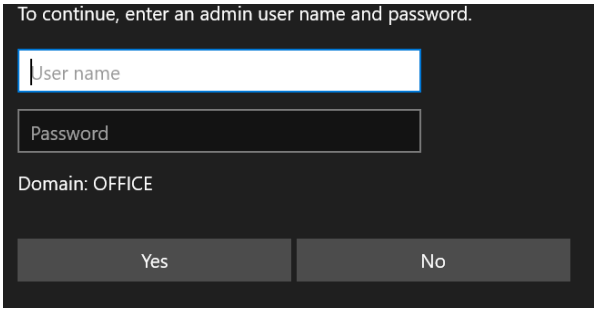
6. A confirmation pop-up will appear (Figure 9). Click “Next” to continue and start the installation.

Figure 8:
Confirm the DiRa-Lab installation



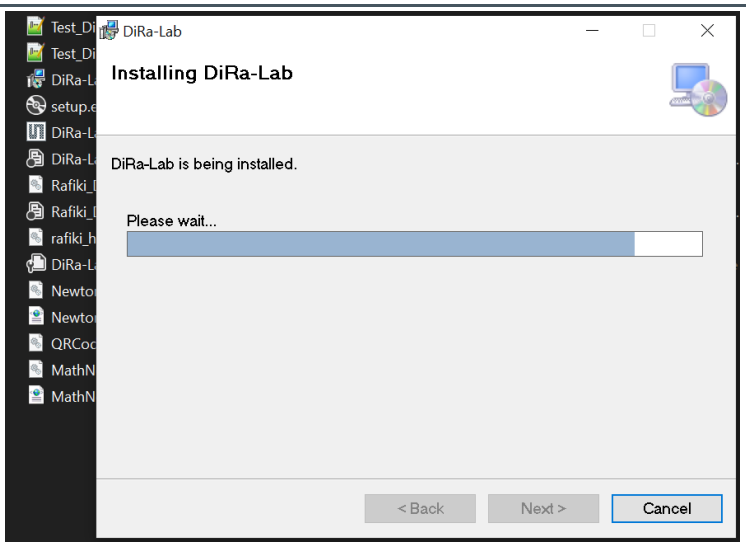
7. Installation of the GUI might need, on some systems, administrative privileges to continue with the installation (Figure 8). You will be asked to enter your “username” and “password” before proceeding.

Figure 9:
Administrative privileges required to proceed with the GUI installation



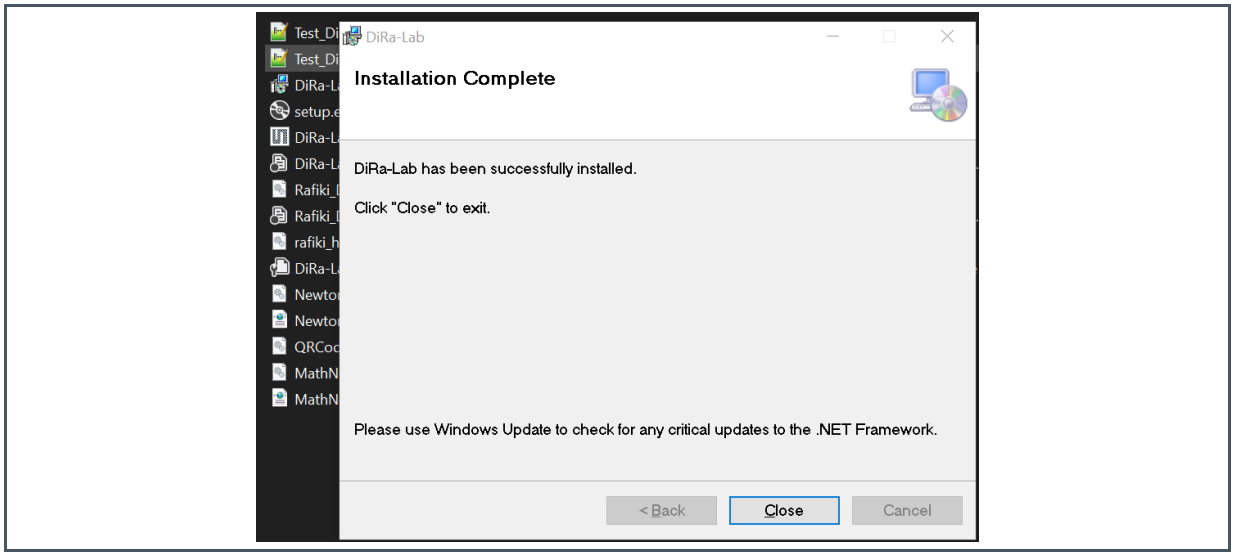
8. After inserting the administrative credentials, the installation will begin, and a status bar will display the status (Figure 11).

Figure 10:
Installation in progress



9. When the installation finishes, click “Close” (Figure 12) to complete the installation of the GUI.

Figure 11:
Completion of the GUI installation



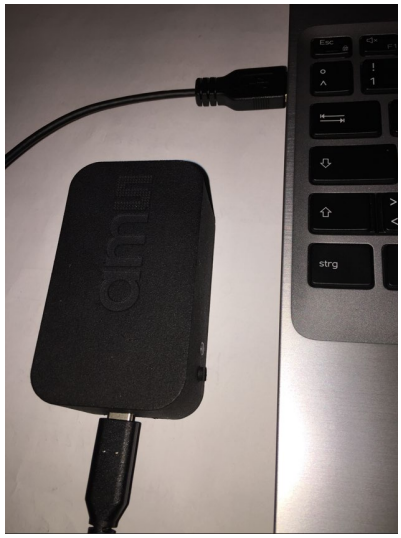
2.2 Working with the LFT Reader

The LFT Reader should be ready for use before starting the GUI since the GUI automatically detects the LFT Reader without the need to search for it.

2.2.1 Preparing the LFT Reader

1. Connect the USB-C cable to the reader, as shown in Figure 13. Then, connect the normal USB to your computer.

Figure 12:
LFT Reader connected to the PC



2. Afterward, push the button shown in Figure 14 to power up the LFT reader. Once the reader is powered, a blue LED will start blinking, meaning the device is trying to connect via Bluetooth (Figure 15). In this example, we are connecting the LFT Reader via USB interface, thus the blue LEDs function is just to indicate the reader is powered.

Figure 13:
Push button on LFT reader

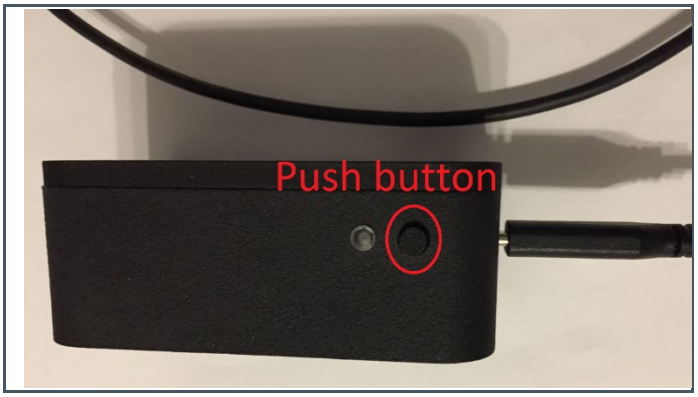
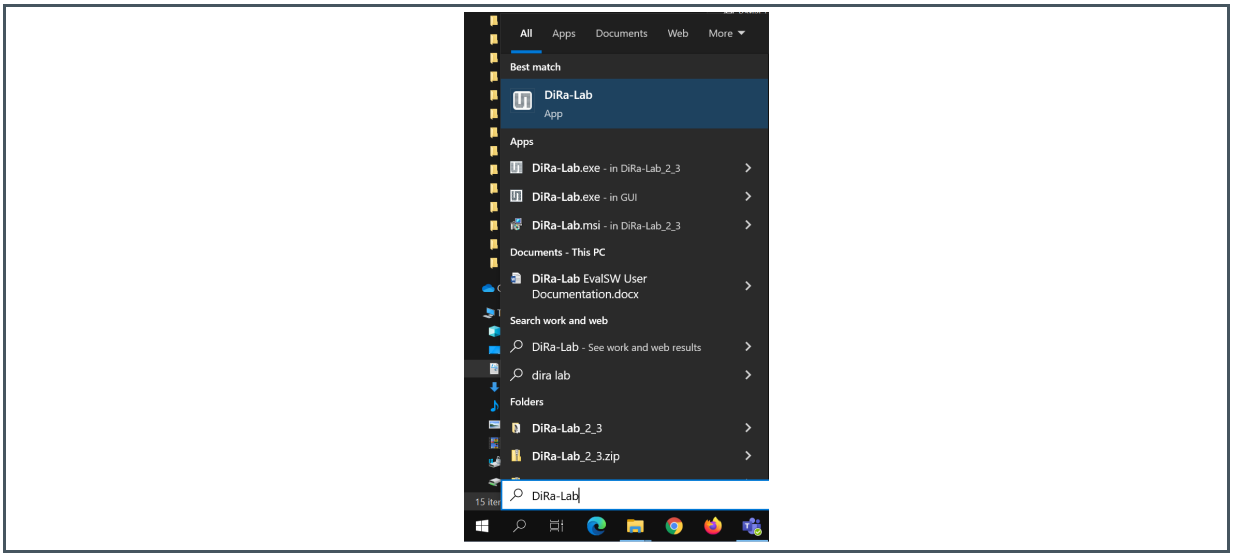


Figure 14:
Blue LED for pairing

3. Open the GUI as shown in Figure 5. The GUI can also be opened from the Start menu after installation, by clicking the Windows symbol on the left-hand side of the taskbar and searching for “DiRa-Lab” (Figure 16). Then, click on “DiRa-Lab Demo” to start the GUI, as shown in Figure 16.

Figure 15:
DiRa-Lab Demo GUI from the Windows Start menu



4. The GUI will pop up as illustrated in Figure 17, with the green LED blinking, which indicates that the reader is connected via USB and ready for use (Figure 18).

Figure 16:
DiRa-Lab GUI window

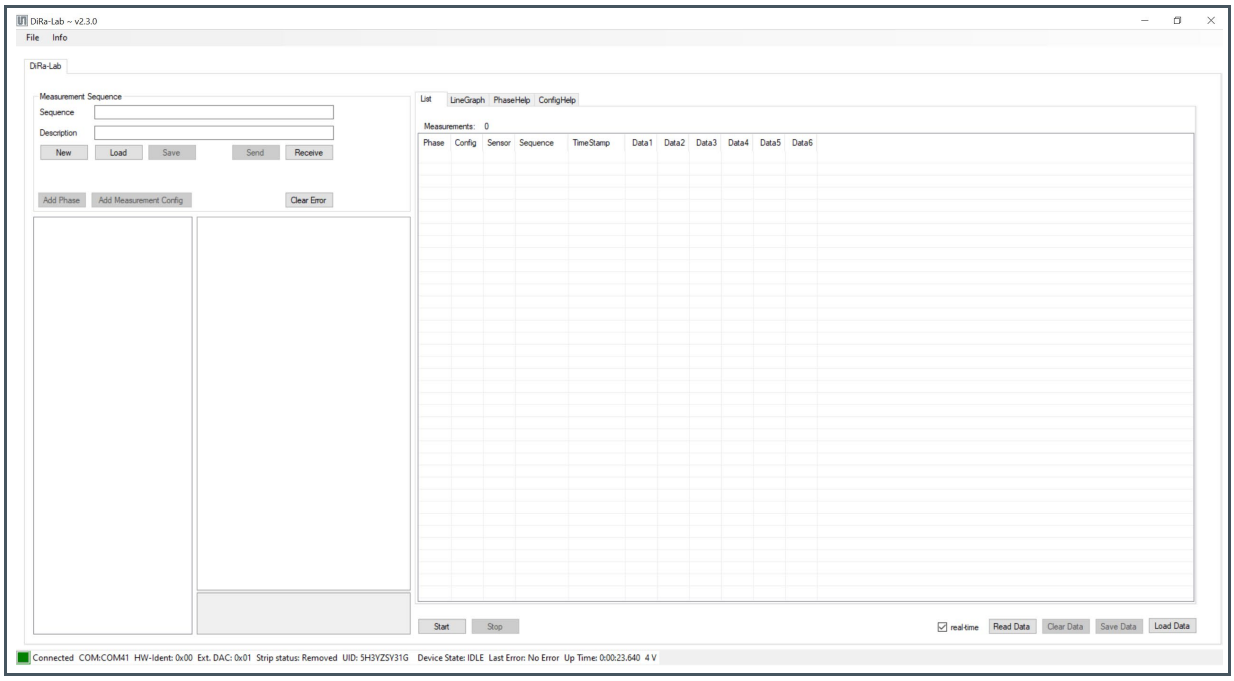


Figure 17:
Green LED on the LFT Reader blinks when connected via USB to the GUI

5. The green box in the status bar of the GUI (Figure 19) indicates that the LFT Reader is correctly connected.

Figure 18:
GUI Status bar

6. From Figure 19, it can be seen that the “Strip status” is removed. By inserting the cartridge (from the Kit) as shown in Figure 20, the status will be changed to “inserted” (Figure 21).

Figure 19:
Inserting the cartridge

Figure 20:
Status bar confirming connected cartridge

2.2.2 Sending a configuration file to the LFT Reader

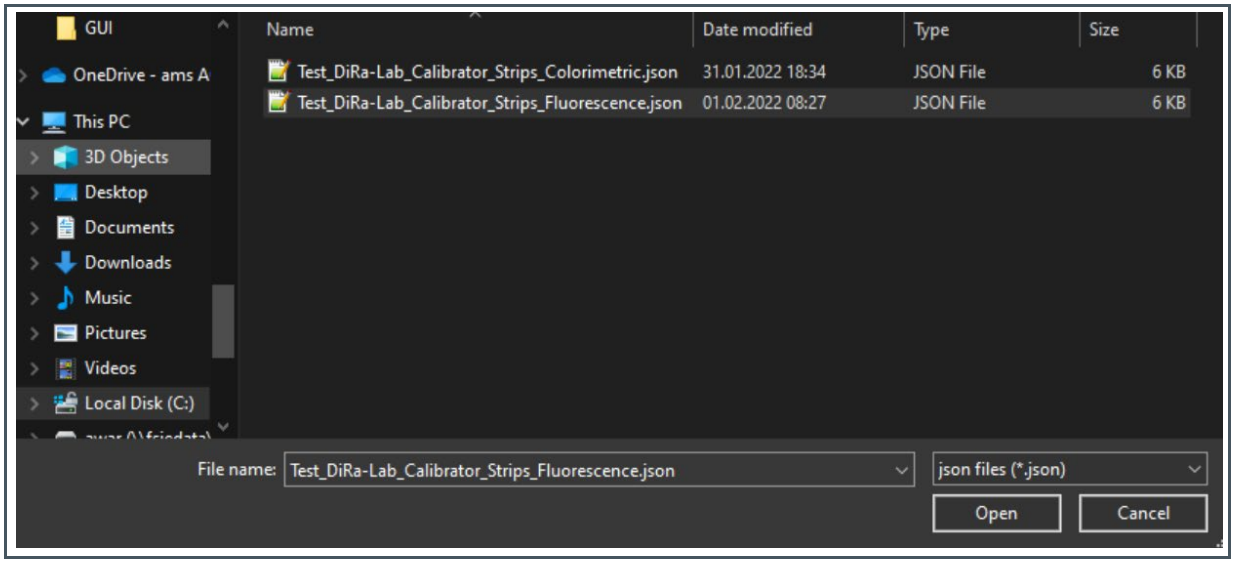
After completing the steps in chapter 2.2.1, a configuration file can be loaded, sent to the reader, and measurement can be started.

1. To load a configuration file click “Load” in the GUI, which opens a pop-up window for selecting the configuration file.
2. Select the correct configuration file from below (depending on the type of kit) and click “open”:

- DiRa-Lab Colorimetric Kit ► “Test_DiRa-Lab_Calibrator_Strips_Colorimetric.json”
- DiRa-Lab Fluorescence Kit ► “Test_DiRa-Lab_Calibrator_Strips_Fluorescence.json”

3. In the example in Figure 22, a UV kit is used. Therefore, the configuration file “*Test_DiRa-Lab_Calibrator_Strips_Fluorescence.json*” is selected.

Figure 21:
Loading a configuration file



4. The configuration is shown on the left-hand side of the GUI (Figure 23). Click “Send” to send the configuration to the LFT Reader. A notification window will pop up to confirm the success of sending the configuration (Figure 24). Click “Ok” and begin the measurement by clicking “Start”.

Figure 22:
Configuration file loaded

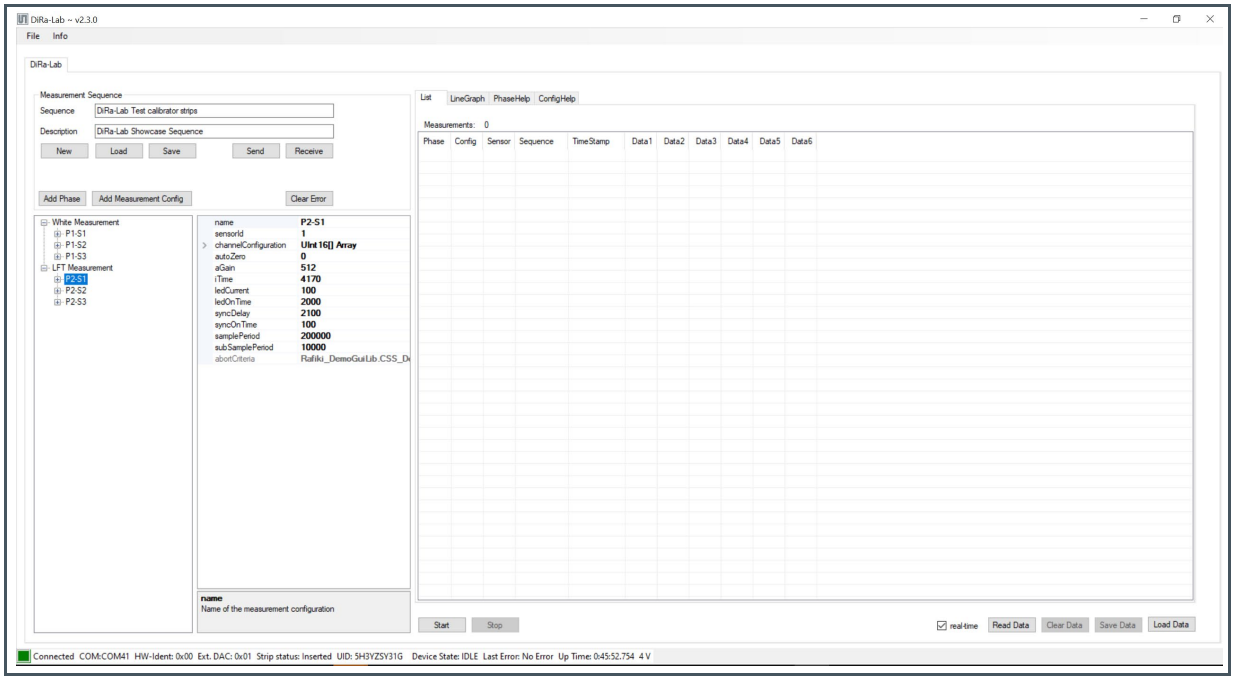
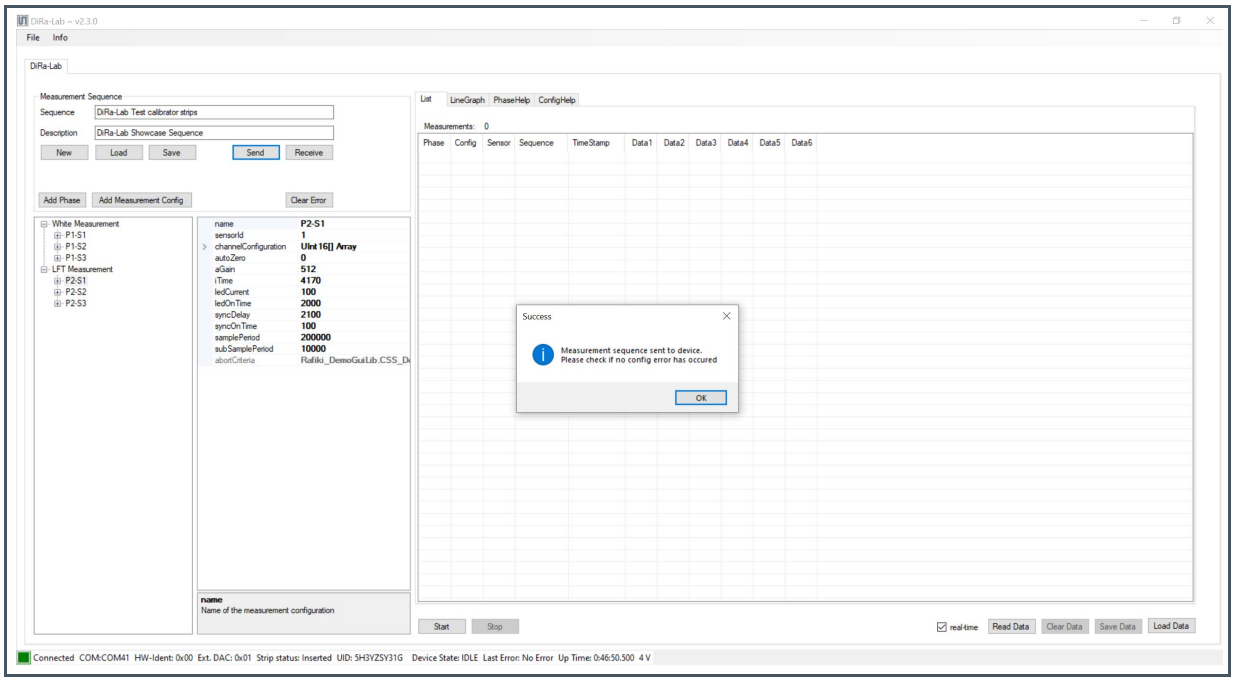
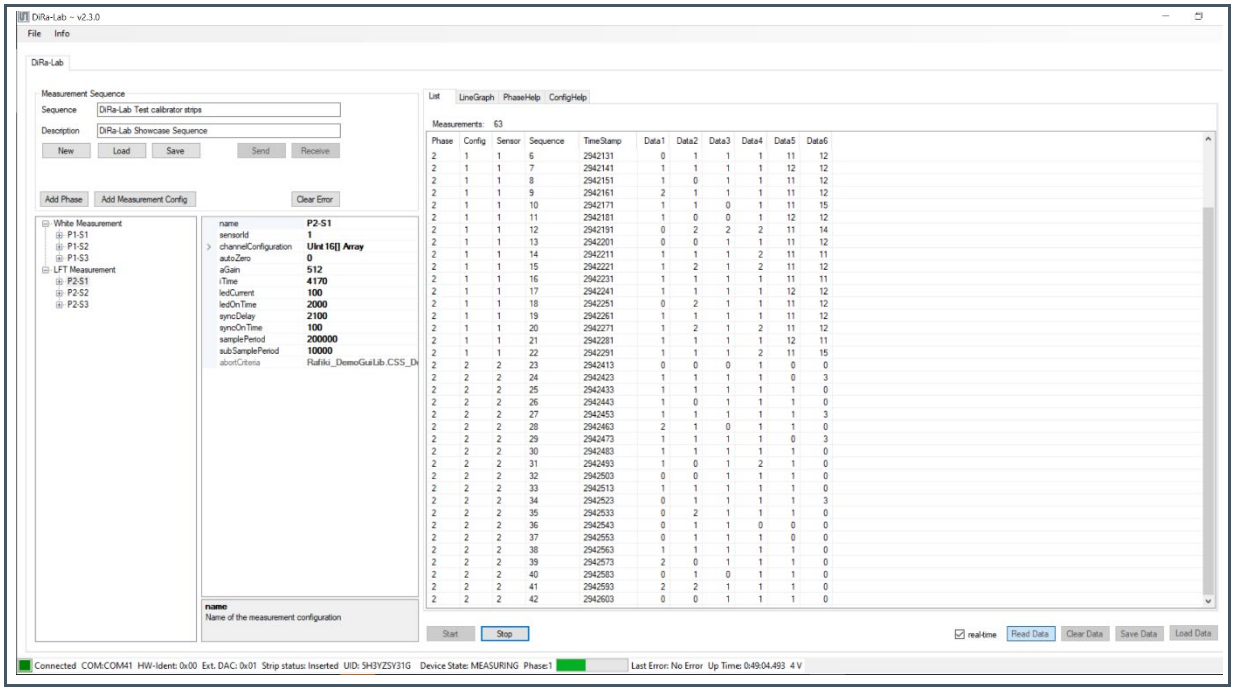


Figure 23:
Configuration sent to the device



5. Afterward, the raw measurement data can be seen on the right-hand side of the GUI (Figure 25). In the status bar, the status of the proceeding measurements can be seen in the “Device Status”.

Figure 24:
Performing the measurement

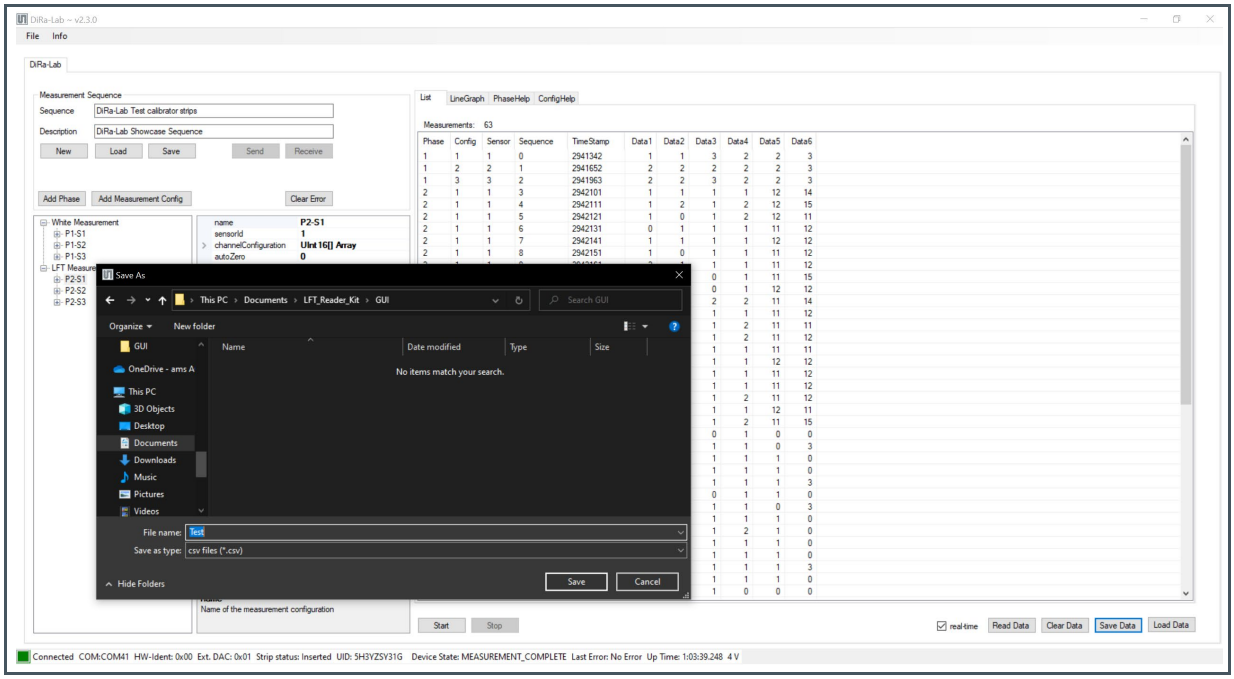


6. When the measurements are completed, the status bar will display the message “MEASUREMENT_COMPLETE” (Figure 26).

Figure 25:
Measurement complete in the device status

7. The measurements can be saved in a .csv file by clicking “Save Data”, which gives a prompt for a file name to be added, and then click “Save”. An example of saving a measurement to a CSV file is shown in Figure 27.

Figure 26:
Saving measurement to “Test.csv” file



3 Hardware Description

The PCBA Reader mainly contains the microcontroller with an integrated BLE module, RGB diode for status indication, and three LEDs as part of the optical system. Depending on the type of LEDs mounted on the PCBA Reader, there are two variants of the LFT Reader Kit: the colorimetric variant and the fluorescence variant. For colorimetric application, the DiRa-Lab Colorimetric LFT Reader Kit uses white LEDs (LW Q38E-Q2OO-3K5L) with a broad spectrum from 380 -780 nm. In the case of fluorescence applications, the DiRa-Lab Fluorescence LFT Reader Kit uses UV LEDs (SU EBLP51.VA) with a peak wavelength of 365 nm. The firmware automatically detects which variant is connected with the GUI. This can be seen in Figure 26, where “HW-Ident: 0x00” means the fluorescence variant is connected.

Figure 27:
Hardware identification in the GUI

HW-Ident	Code
Fluorescence	0x00
Colorimetric	0x01

Figure 28:
Open device by pushing in direction of the arrow

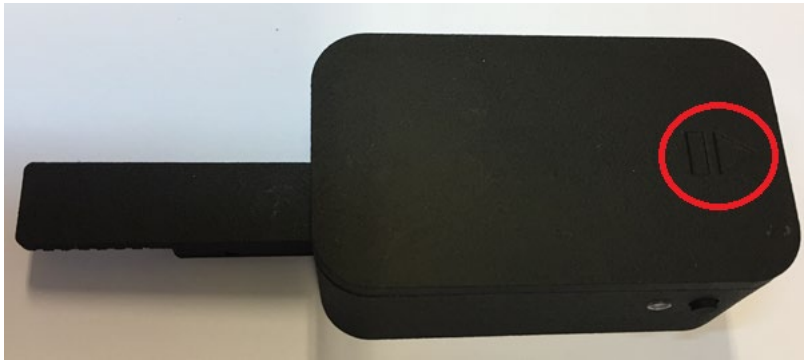
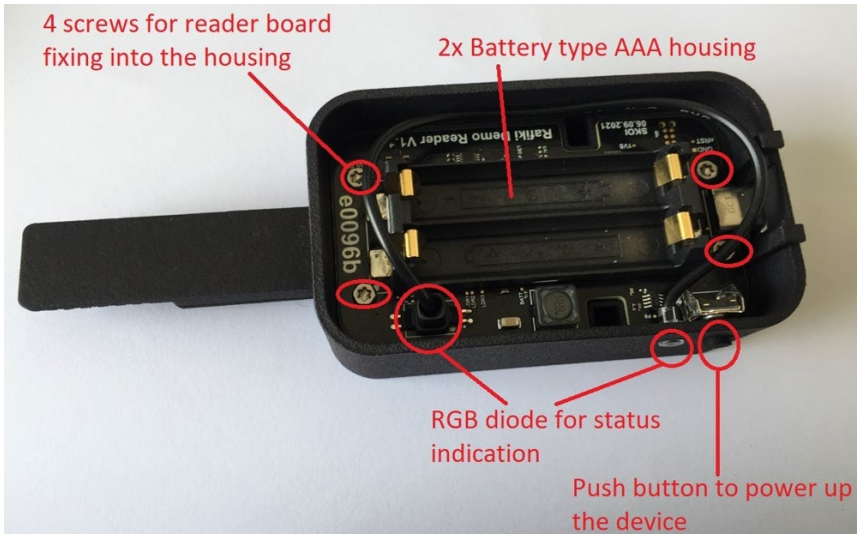


Figure 29:
PCBA Reader fixed within the housing



The firmware selects the sensor via its address and controls the communication between the microcontroller and the sensors. The microcontroller integrates a BLE module (nRF52840). The customer can flash the firmware or update via the BLE on demand. The firmware also controls the LED ON/OFF timing and starts the measurement with the corresponding sensor. When a sensor finishes its measurements, it sends a signal to the microcontroller to indicate that the measurements are done and data is ready to be readout.

The LFT Reader has an RGB LED to indicate the status of the reader. The green LED will be enabled when the device is switched on and connected with the GUI. The red LED blinks at 0.5 Hz to indicate a low battery voltage. In this case, the batteries must be replaced before continuing any measurements. If the LED is continuously doing a measurement, the red LED will be switched off after the completion of the measurement. The blue LED indicates the pairing status with a host device if there is no USB connection to the reader. If the reader is not paired with a Bluetooth host device, the blue LED will blink with a 1 Hz scheme. The blue LED is “static on” if the device is paired with a host.

The device is in advertising mode if it is connected via USB without opening the GUI. The same happens if batteries are plugged in. In advertising mode, the blue LED periodically flashes at 1Hz. If a BLE device wants to connect to the DiRa-Lab, it asks to establish a connection. Then, the DiRa-Lab enters pairing mode, bonds to the device, and sets up the connection. If a connection is established, the blue LED to be “static on”. However, if it fails, it goes back to advertising mode.

Figure 30:
RGB LED Colour Indicator

Colour	Blink mode	Indication
Green	Static on	Reader connected to the GUI.
Red	0.5 Hz	Low battery voltage.

Colour	Blink mode	Indication
	Static on	Ongoing measurements.
Blue	1 Hz	Not paired.
	Static on	Paired with a host device.

The PCBA Reader is connected to the PCBA Sensor through a 10-pin connector, as shown in Figure 32. The sensors have the I2C addresses: 0x29, 0x39, and 0x49. The dimensions of the PCBA Reader are 41 mm x 74.25 mm (Figure 33). The dimensions of the PCBA Sensor are 12.20 mm x 50.20 mm (Figure 34).

Figure 31:
PCBA Reader

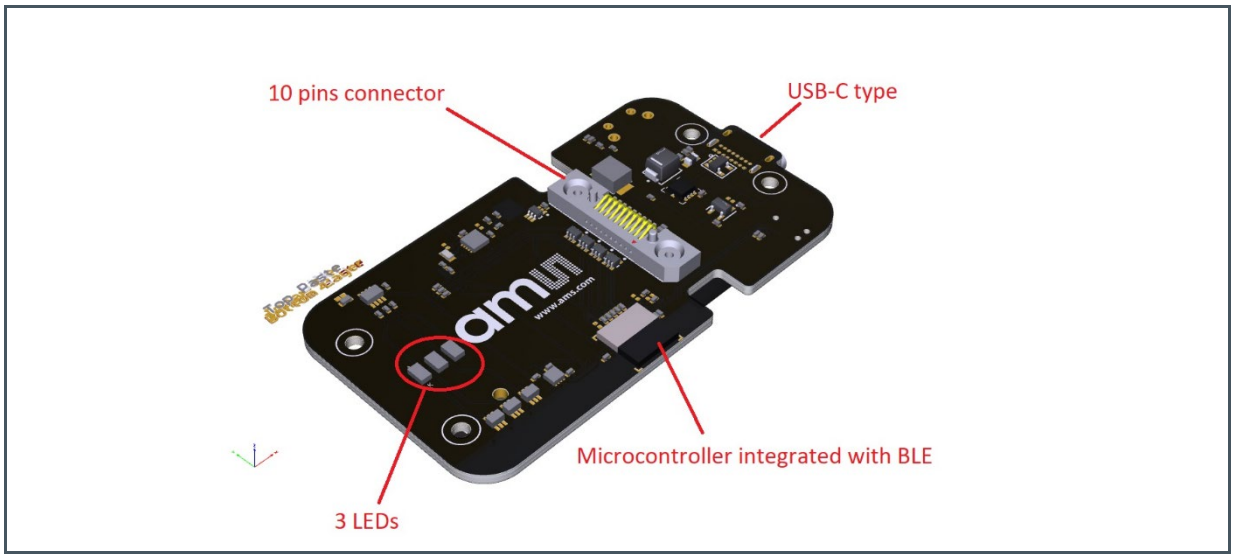
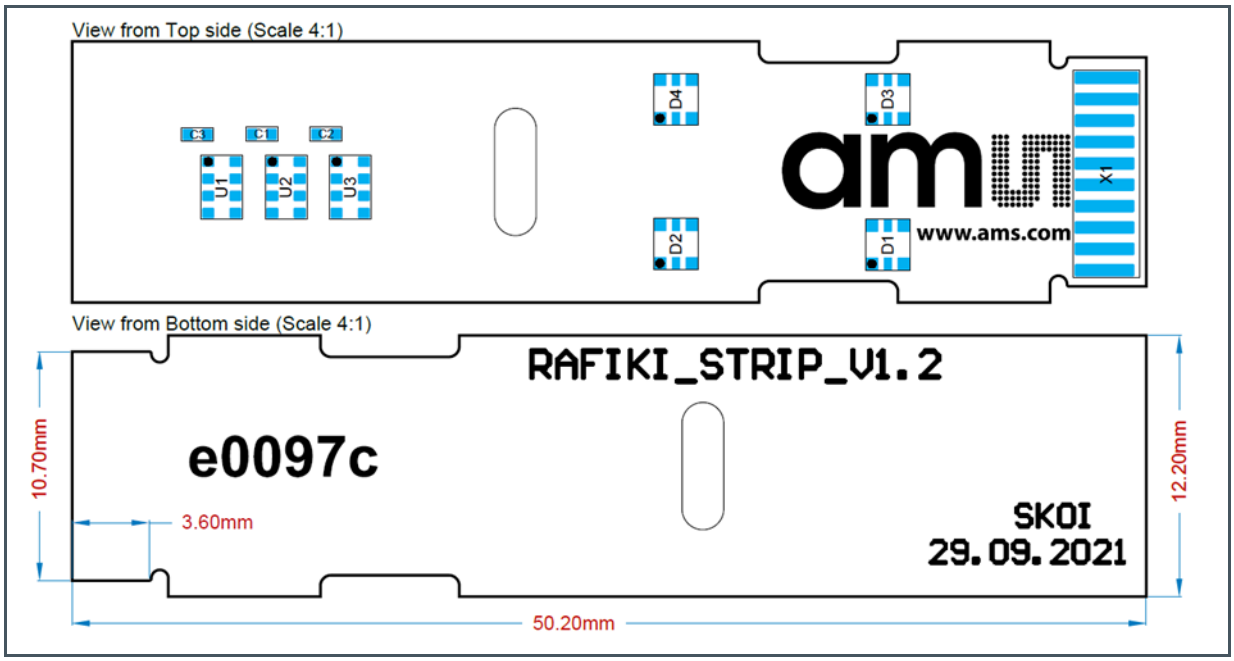


Figure 32:
Detailed view of both sides of the PCBA Reader

Figure 33:
Detailed view of both sides of the PCBA Sensor



3.1 Hardware Assembly

The PCBA Sensor and the LFT strip should be assembled, as shown in Figure 35. The PCBA Sensor has two holes for fixing it into the cartridge housing through two small pins (Figure 36). The assembly of these parts should look as shown in Figure 37. For the precise alignment of the LFT strip into the cartridge, please refer to chapter 5 of this document.

After correctly assembling the cartridge components, carefully press the top part of the housing with the bottom part, ensuring the cartridge is locked properly. The cartridge is then ready to be inserted into the reader, and the measurement can be made after configuring the device. Inserting the cartridge should be done gently, to avoid breaking the connector mounted on the PCBA Reader. If the cartridge does not fit into the reader, ensure the cartridge parts are assembled correctly. The three sensors can be configured individually using the GUI. Please refer to chapter 4.1 for more details.

Figure 34:
Assembling the PCBA Sensors with the LFT strip in the cartridge

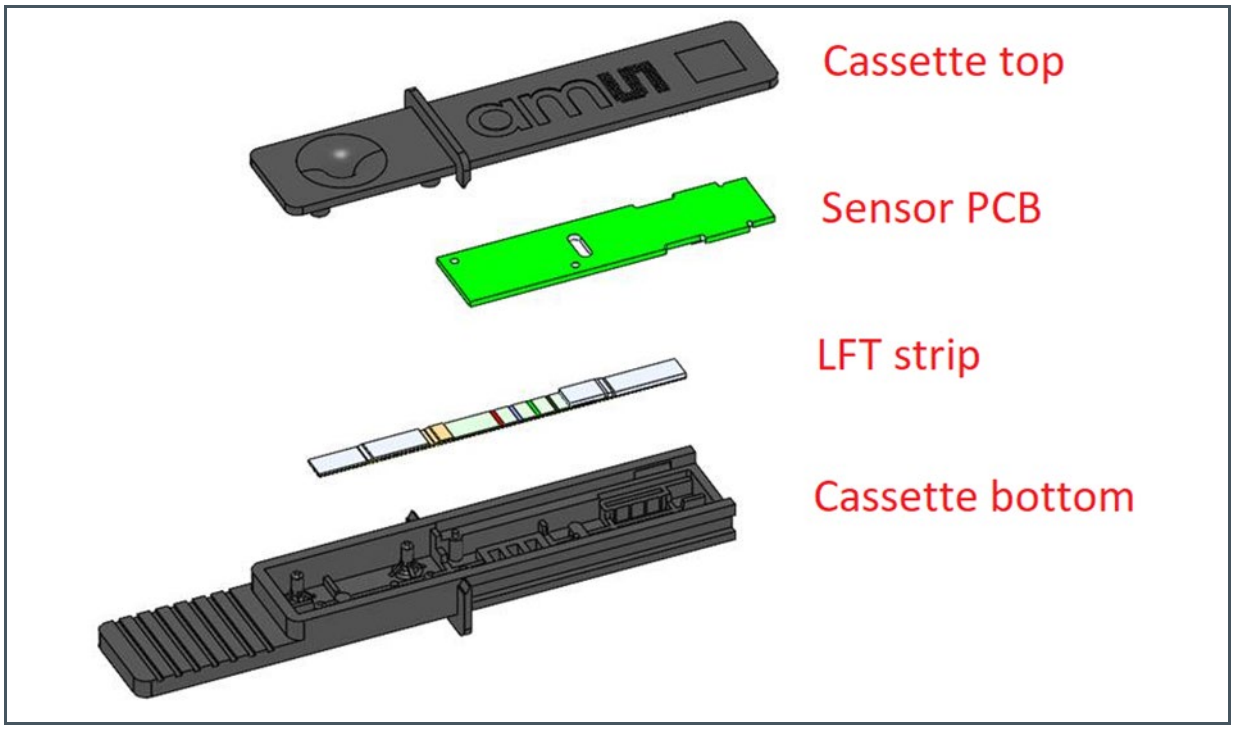


Figure 35:
PCBA Sensor

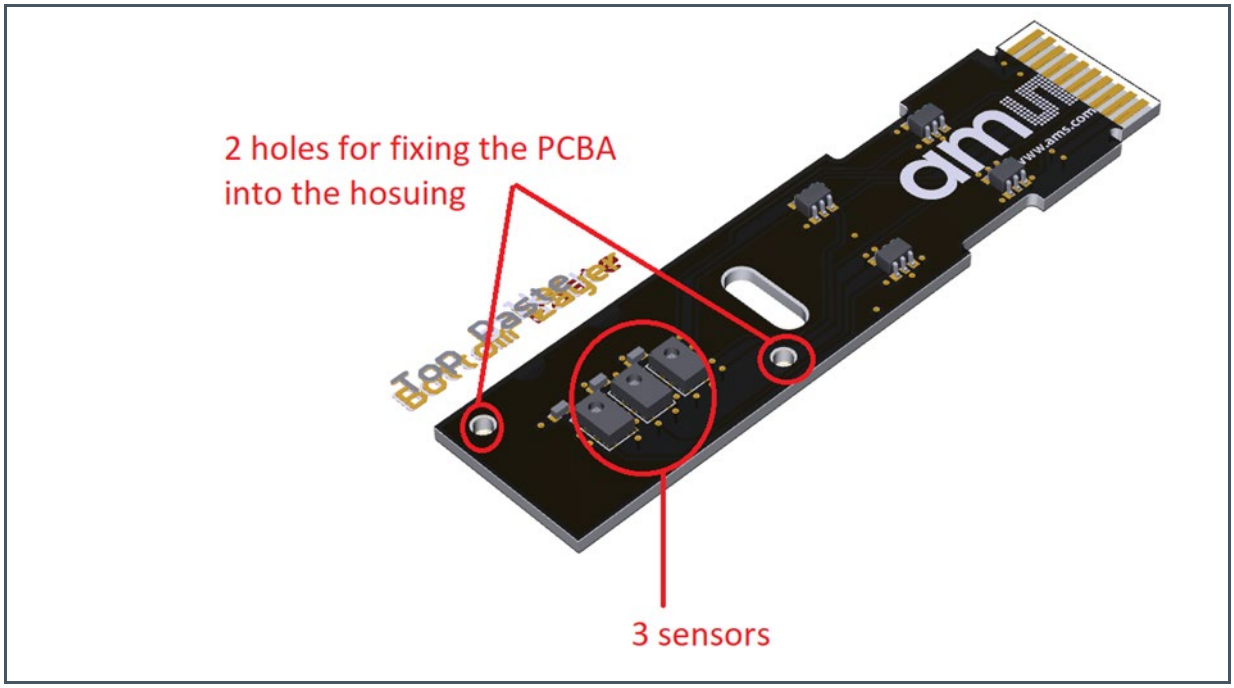


Figure 36:
Assembly of the PCBA Sensor with the LFT strip in the cartridge

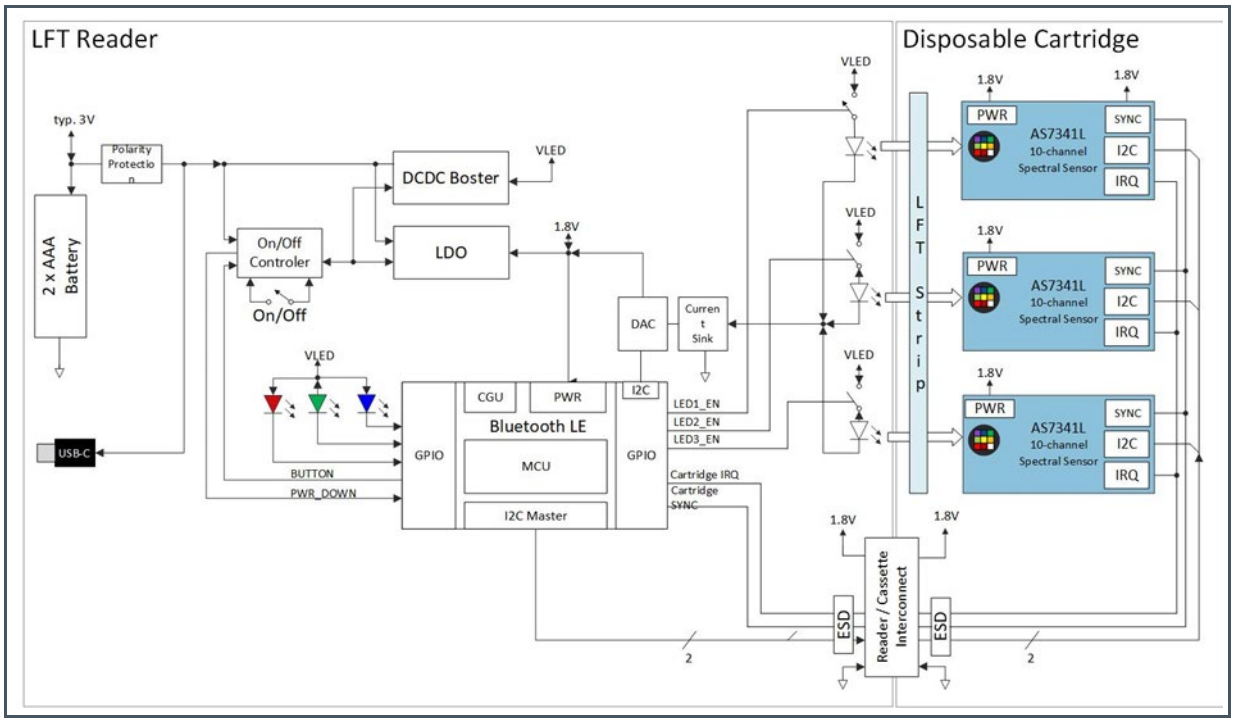
3.2 Hardware Architecture

The high-level hardware architecture of the LFT Reader is presented in Figure 38. The LFT strip, which is a transparent backing card, is located between the LEDs and the sensors. The LFT Reader contains the microcontroller (MCU), power, LED Driver, and the connection to the sensor strip.

The MCU contains a Bluetooth module, GPIOs, and an I2C master, to control which sensor to communicate with at a time and switch ON/OFF its related LED. The firmware controls the RGB LED, which shows the various conditions of the device. It also controls the LEDx signal emitted to AS7341L sensors and a Digital-to-Analog circuit (DAC) via the I2C, which generates the voltage supplying the required LED current with high accuracy. The firmware detects the strip insertion and the start of the measurements for the configured integration time (ITIME). After the measurements are completed, the sensor sends a signal to the microcontroller, indicating that the measurements are completed and data are ready to be read.

For controlling the LED current, the DAC generates a high accuracy voltage. This voltage is to be converted to a current with a current sink IC, which will generate the required current for turning ON/OFF the LEDs.

Figure 37:
Block Diagram of the LFT Reader



3.3 Power Supply

The USB-C cable provides the power supply for operating the LFT Reader. This cable is a part of the original package of the LFT Reader Kit.

3.4 Connector Pinout Description

An overview of the mounted connectors and interfaces on the PCBA Reader are shown in Figure 39.

Figure 38:
Overview of the connectors and interfaces

Designator	Comment
J1	USB 2.0 – 16-pin connector (USB4085-GF-A)
J2	10-pin connector (FSI-110-06-L-S-M-AD) to connect the PCBA Reader with the PCBA Sensor.

Designator	Comment
J4	6-pin connector for flashing the PCBA Reader (needs a 8.19.00 J-LINK BASE COMPACT programmer with a TC2030-IDC-NL cable).

The following figures (Figure 40 and Figure 41) describe the pinout of the FSI-110-06-L-S-M-AD connector, which connects the PCBA Reader with the PCBA Sensor. The interface between the PCBA Reader and the PCBA Sensor is described in Figure 40, and the pin signal details are mentioned in Figure 41.

Figure 39:
J2 Pinout Connector

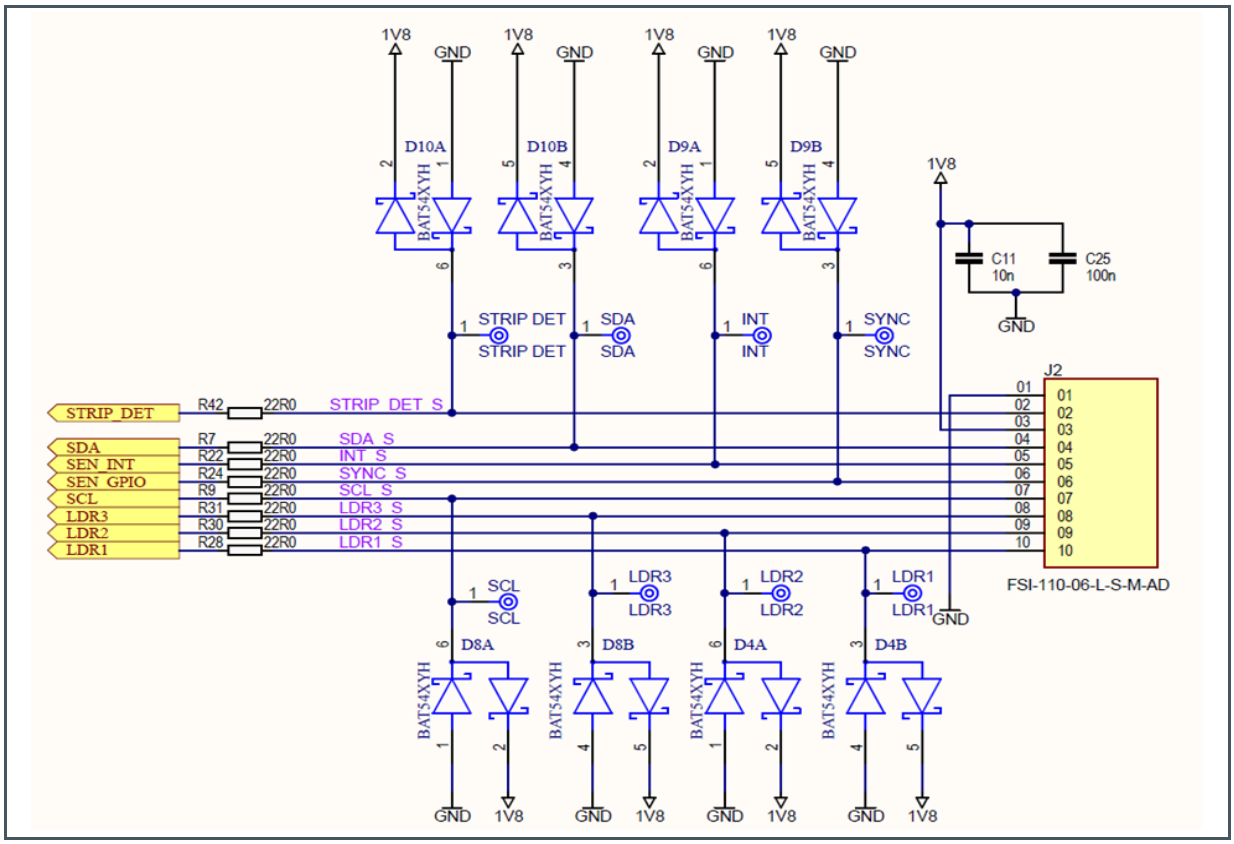


Figure 40:
J2 Connector Pin Description

Pin Number	Net Name	Description
1	GND	Ground
2	STRIP_DET_S	Pin for detection if the strip is inserted or not.
3	Supply voltage	Supply voltage to the sensors (1.8V).
4	SDA_S	I²C Data Signal.

Pin Number	Net Name	Description
5	INT_S	AS7341L Interrupt Signal.
6	SYNC_S	Synchronization Signal for the AS7341L to start measurements.
7	SCL_S	I²C Clock Signal.
8	LDR3_S	Constant current sink from Sensor 3.
9	LDR2_S	Constant current sink from Sensor 2.
10	LDR1_S	Constant current sink from Sensor 1.

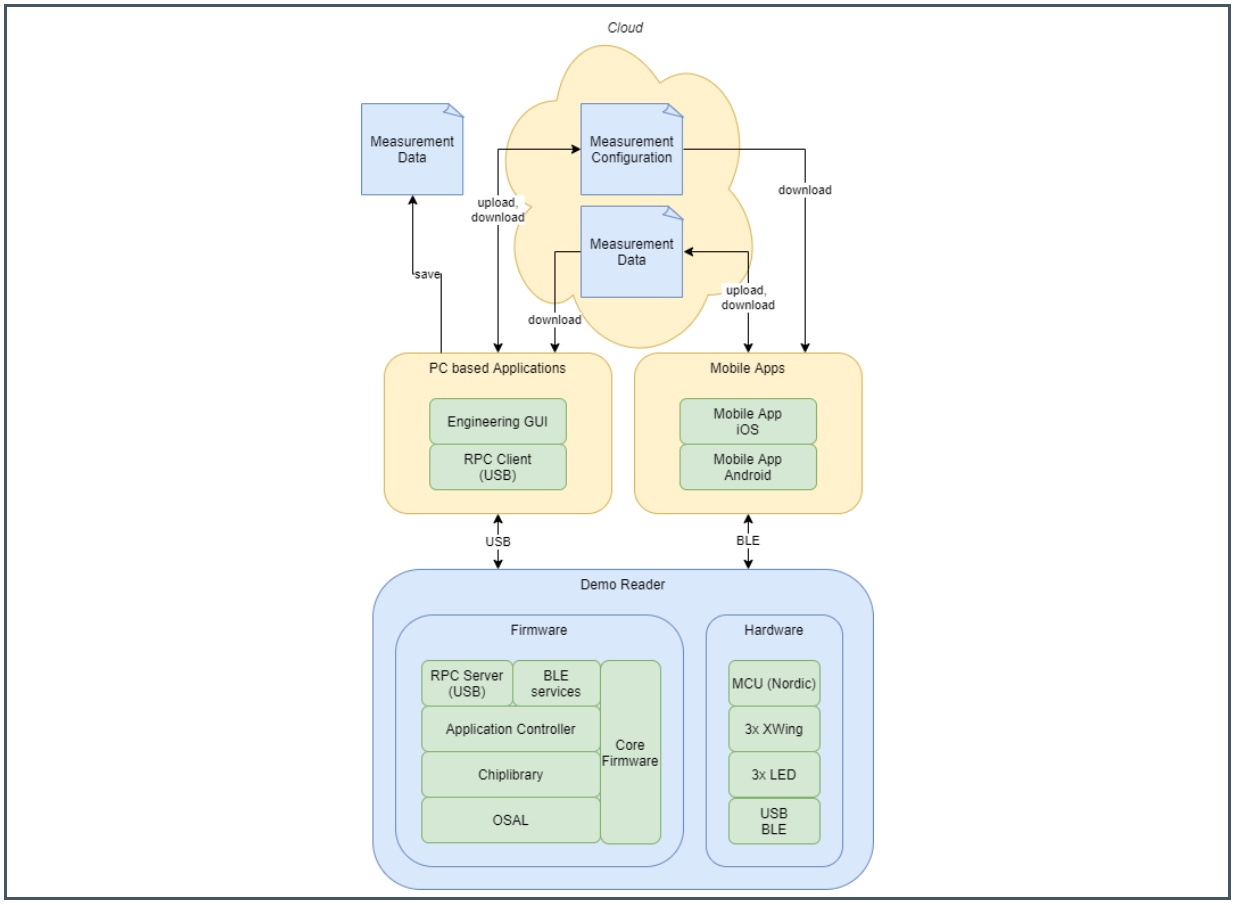
4 Software Description

The software system consists of the architecture of separate modules, spanning across multiple domains. In between domains, there are interfaces specified to ensure compatibility, independent of the customer use case.

The overall software system defines the following main components:

- Firmware for the LFT Reader.
- PC-based Engineering GUI, communicating via USB with the LFT Reader.

Figure 41:
Software Overview – System Architecture



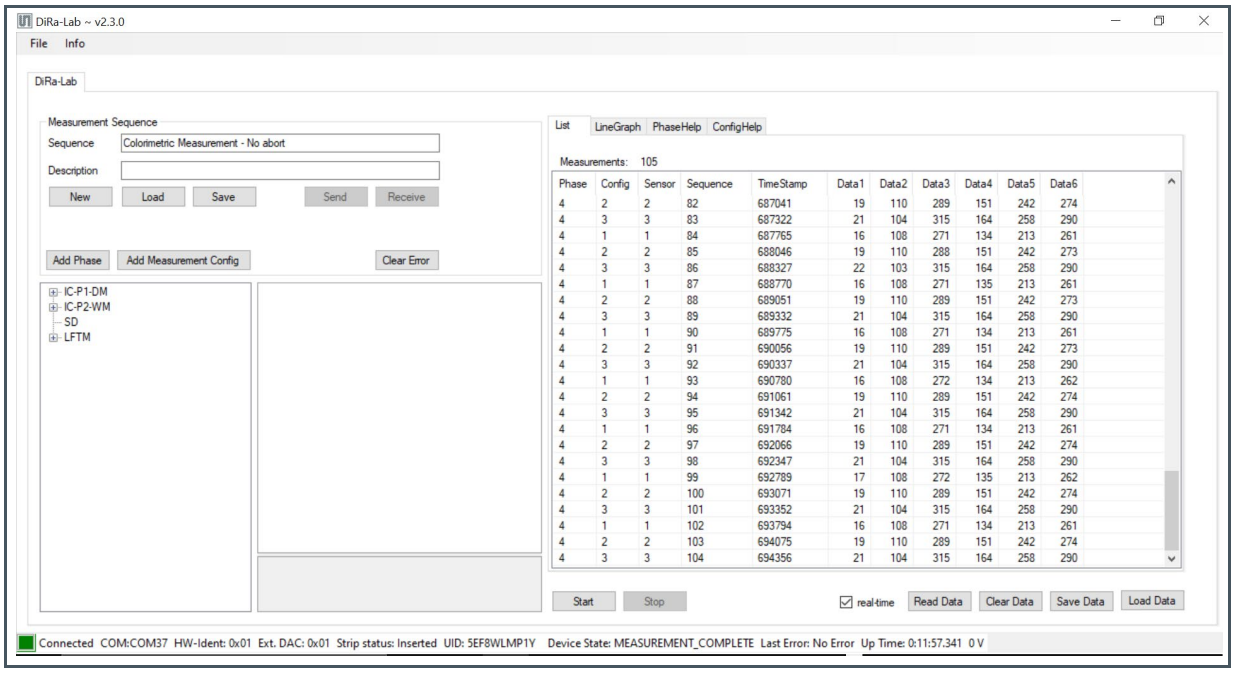
The system is designed in modules, which are intended to provide a head start for various use cases. Customers have different technical backgrounds and different support needs. Therefore, it is possible to use the complete **ams OSRAM** solution as a starting point, or only parts of it.

4.1 Graphical User Interface

The LFT Reader software is a Windows 10 (64 bit) based software. To install the application on a Windows system or run it directly, please refer to chapter 2.1.

Running the application will display a user interface, as shown in Figure 43. This window allows the user to connect to an attached LFT Reader device. The status bar at the bottom of the GUI shows the status of the device. The GUI gives the user freedom to configure and run measurements. The measurements' output can be visually monitored and logged to a CSV file with the help of the GUI.

Figure 42:
Main window of the application after a measurement



4.1.1 Connecting to a Device

To connect an LFT Reader, insert the cartridge into the Reader. Afterward, connect the reader via the USB-C cable to the PC with the installed software. Press the power button once to power the device. If only a single device is connected to the computer, it will automatically open when the GUI starts. If more devices are connected, you will need to choose one from the pop-up list. When successfully connected, the status bar at the bottom of the GUI will show, as displayed in Figure 44.

Figure 43:
GUI Status Bar

The following information is available on the status bar:

- Connected status: Connected, Disconnected
- COM port
- Hardware identification: 0x00 (Fluorescence), 0x01 (Colorimetric)
- External DAC flag
- Strip status: Removed, Inserted
- Device UID
- Device state: Idle, Measuring, Measurement complete, Error
- Last error
- Up Time in hours: minutes:seconds.milliseconds
- Battery voltage: only for debugging purposes

If a red box is displayed in the status bar of the GUI, as in Figure 45, it means the device is not connected.

Figure 44:
Status bar with a disconnected device

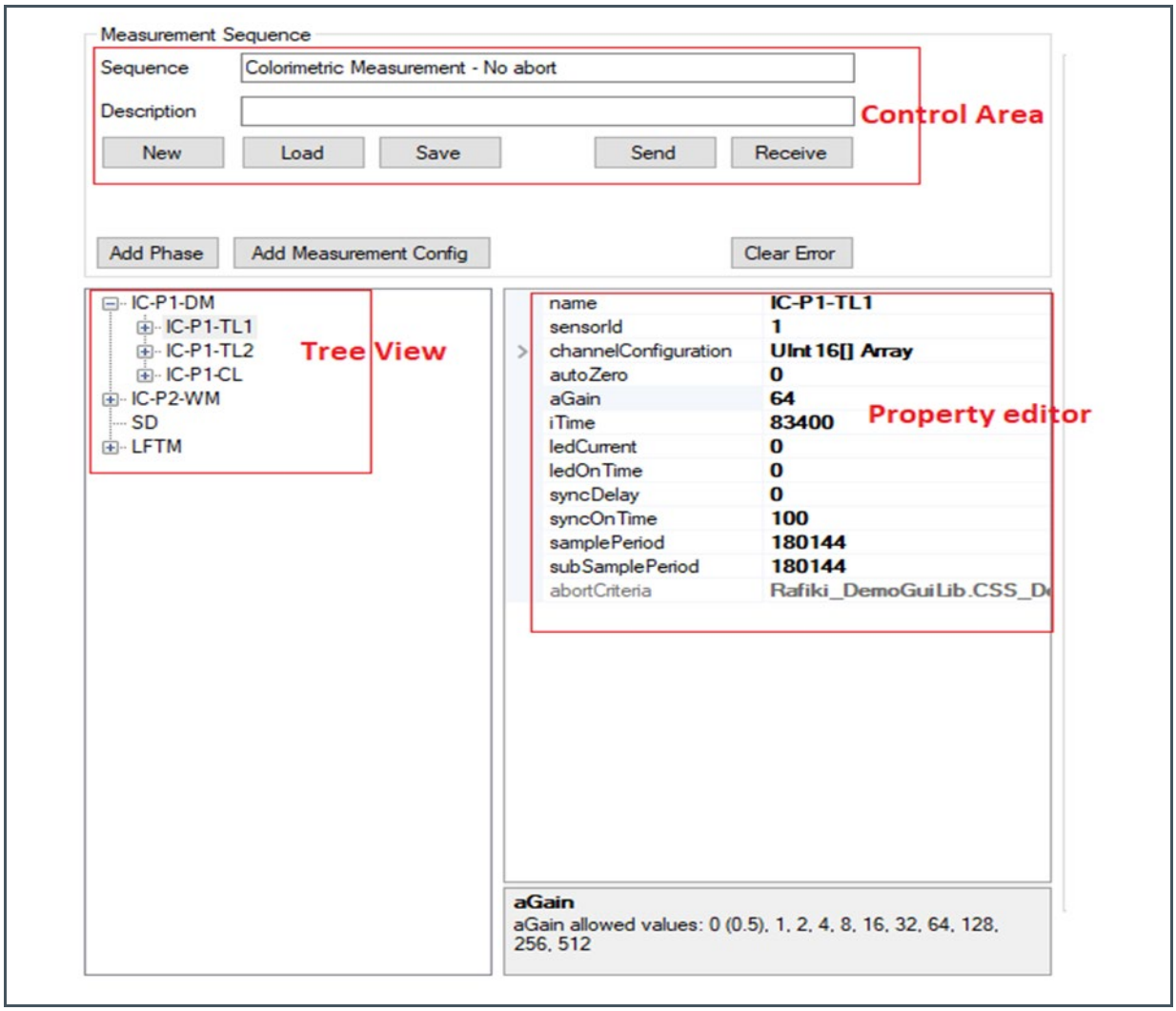
In this case, check the “Device Manager” for an active COM port. If there is no active COM port in the device manager, check the power cable and ensure that you pressed the push button to switch on the reader. The status bar of the GUI gives information about the error status, board information, etc.

4.1.2 Configuring a Measurement

The left-hand side of the screen, shown in Figure 46, allows you to configure new measurements. It has three distinct areas:

- Control area
- Tree view
- Property editor

Figure 45:
Measurement configuration with the GUI



4.1.3 Creating a new measurement sequence

To create a new measurement sequence, click the “New” button in the “control area” in Figure 46. Phases can be added to this measurement sequence by clicking the “Add Phase” button. Clicking the “Add Measurement Config” button will add a new measurement configuration to the selected phase.

Left-clicking on a node in the “Tree view” will select the phase, configuration, or abort the criteria. Right-clicking on a phase or configuration node opens a menu with the option to Copy, Paste, or Delete.

4.1.4 Editing a measurement sequence

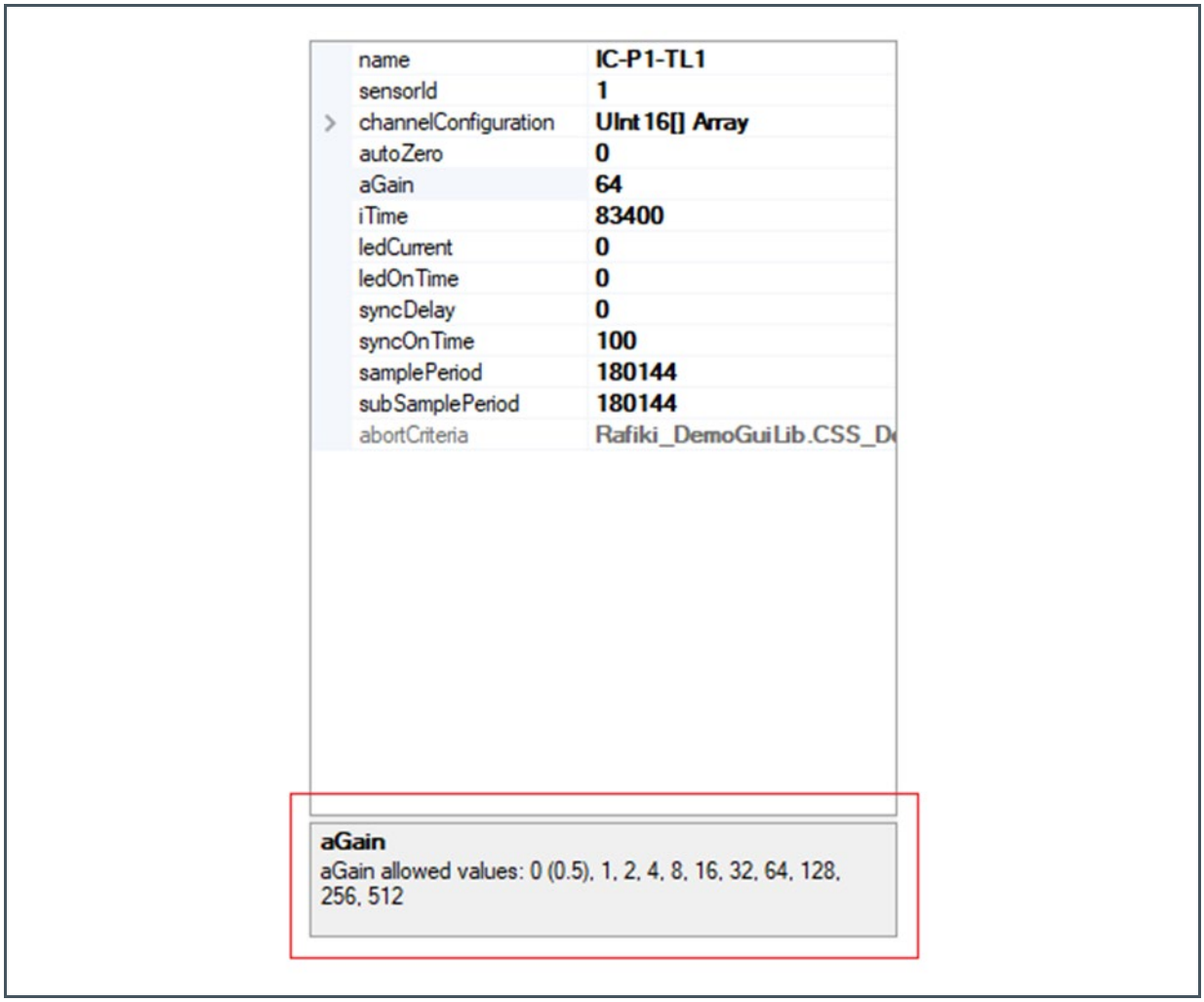
Clicking a phase, measurement configuration, or abort criteria node in the Tree view, will show its properties in the Property editor.

In the Property editor, a property can be selected with a mouse click. Afterward, type the new value for the property. Pressing <Enter> on the keyboard accepts the new value and moves to the next property. Press <Esc> on the keyboard to discard any changes.

Going forward in the properties is also possible with the keys <Tab>, <arrow down>, or <arrow right>. Going backward can be done with the keys <Shift><Tab>, <arrow up>, or <arrow left>.

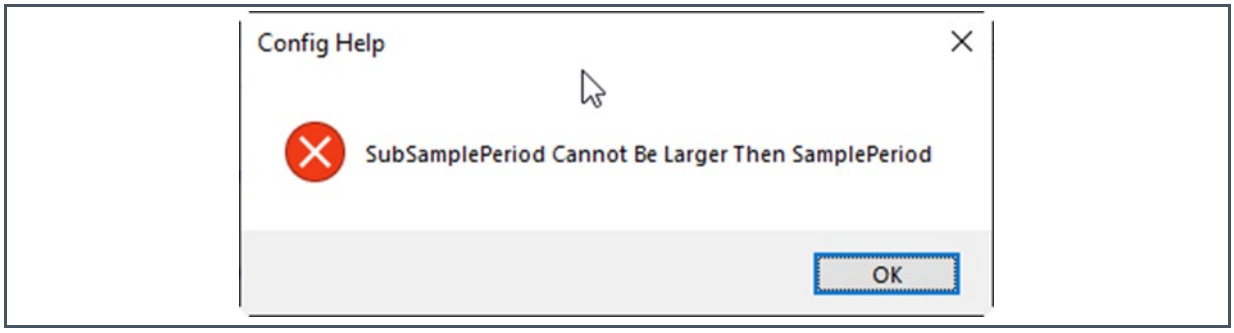
At the bottom of the Property editor, a short explanation about the selected property is given, such as the units used and the valid ranges.

Figure 46:
Property description



If a value outside the valid range is entered, it will be automatically clipped to its lower or upper boundary without any warning. If a combination of the entered values is invalid, an error message will pop up (Figure 48).

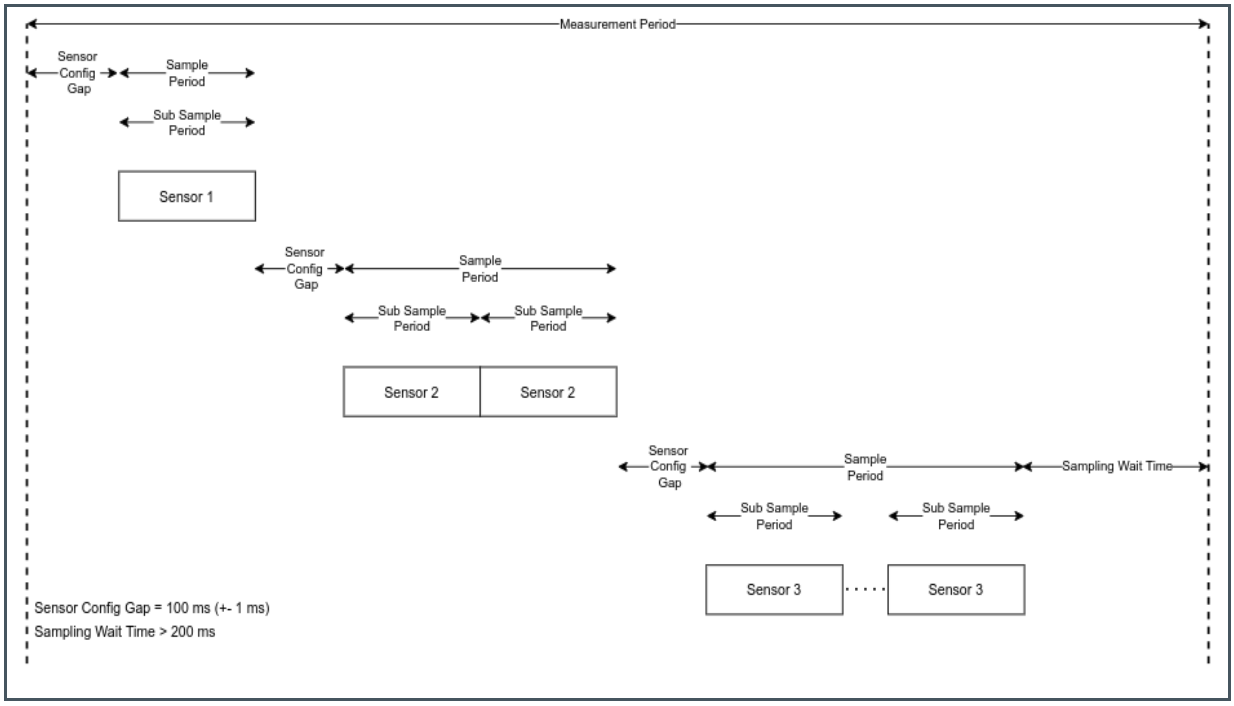
Figure 47:
Error of Sample Period



Extra help for setting the correct property values can be shown in the tab-view, at the right-hand side of the screen:

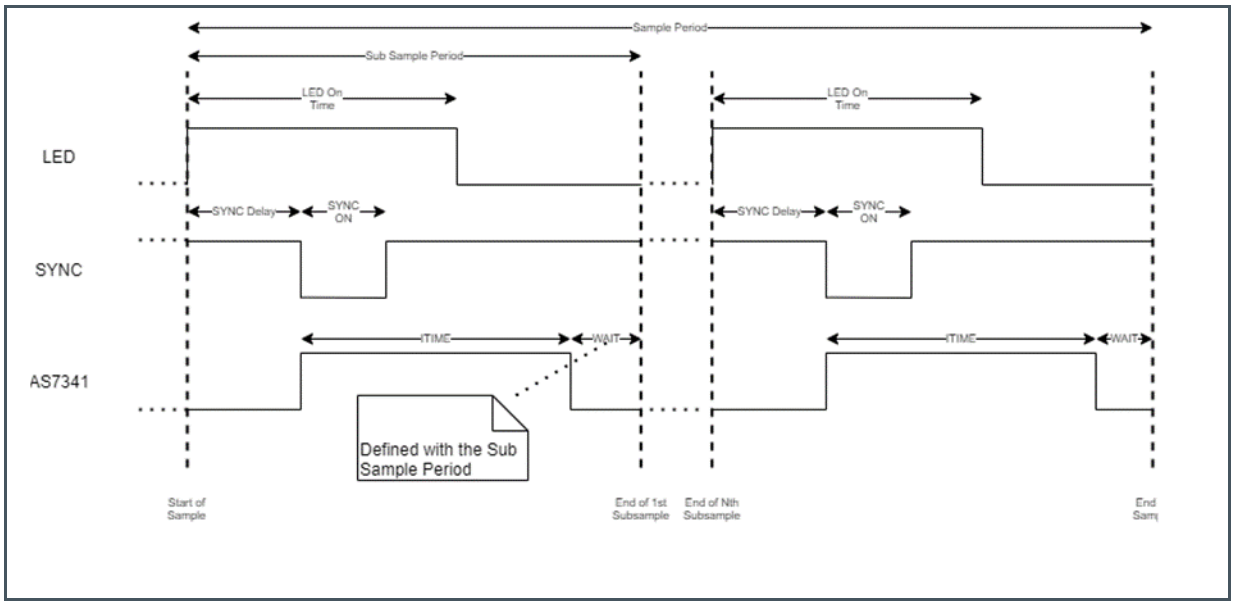
- The “PhaseHelp” tab shows the measurement period (sampling period).

Figure 48:
Measurement period



- The “ConfigHelp” tab explains the detailed timing parameters (subsampling).

Figure 49:
Sampling period



4.1.5 Operations on Measurement sequences

Save a measurement sequence to a file by clicking the “Save” button in the control area. It will be saved as a JSON file in a readable format, as shown in Figure 51 below.

Figure 50:
JSON file example of a saved measurement

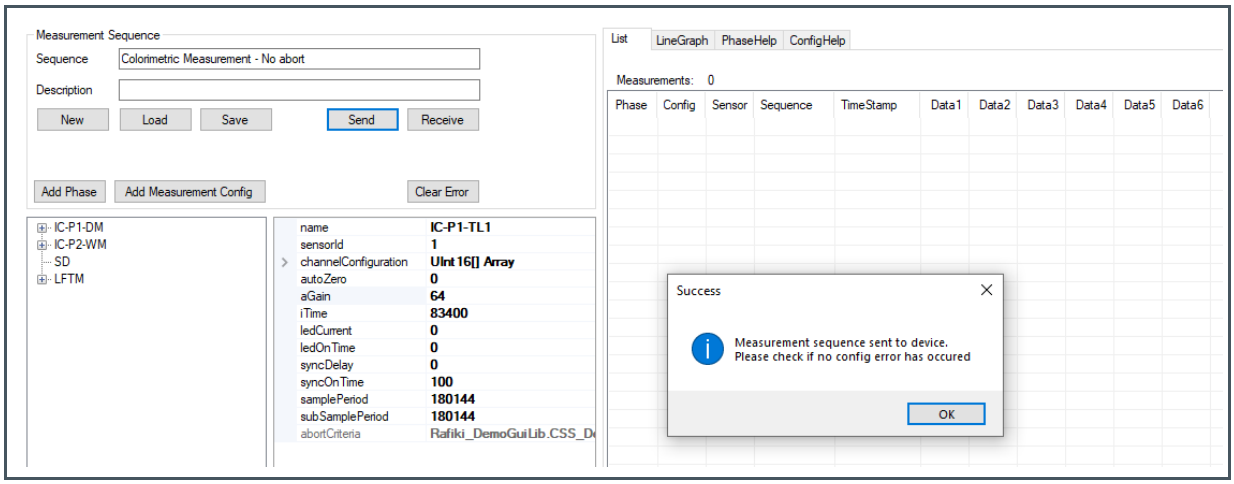

```
{
  "name": "Colorimetric Measurement",
  "description": "",
  "measurementPhases": [
    {
      "name": "IC-P1-DM",
      "description": "IC-DM",
      "repetitions": 1,
      "measurementPeriod": 1000000,
      "measurementConfigurations": [
        {
          "name": "IC-P1-TL1",
          "sensorId": 1,
          "channelConfiguration": [
            1,
            2,
            3,
            4,
            5,
            6
          ],
          "autoZero": 0,
          "aGain": 64,
          "iTime": 83400,
          "ledCurrent": 3,
          "ledOnTime": 0,
          "syncDelay": 0,
          "syncOnTime": 100,
          "samplePeriod": 180144,
          "subSamplePeriod": 180144,
          "abortCriteria": {
            "mode": 0,
            "operation": 0,
            "triggerAmount": 0,
            "timeout": 0,
            "channelCondition": [
              0,
              0,
              0,
              0,
              0,
              0
            ],
            "channelActive": 0
          }
        },
...
```


Clicking the “Load” button starts a file dialog to load an existing JSON measurement configuration file. After selecting the file and opening the file dialog, it will display the Tree view of the configuration saved in the JSON file.

After installing the GUI, users can find the example configuration files in the folder “*C:\Program Files\ams AG\Rafiki Demo\Example Configurations”*.

To start a measurement, the measurement configuration needs to be transferred to the connected device by clicking “Send”. This is only allowed if the device is in an idle state.

Figure 51:
Configuration file loaded from the file directory and sent to the connected device



It is also possible to reread the measurement sequence from the connected device by clicking the “Receive” button. This is particularly useful if the measurement sequence is loaded onto the device using the BLE. Receiving a measurement sequence is also only possible if the device is in an idle state.

The measurement configuration defines all measurement-related parameters for a specific sensor. Additionally, an abort criterion should be defined, which allows external events to cause the measurement to abort (e.g. the sensor channel reaches a specific value or a sample is not detected, etc.). Please see Figure 53 and Figure 54 for a complete list of the parameters.

Figure 52:
Parameters for measurement configurations

Parameter Name	Type	Range	Description
SensorID	uchar	1 to 3	The Sensor ID for which the configuration shall be used.
ChannelConfiguration	uchar[6]	0 to 11	Configure up to six channels, to be measured (one byte for each channel). 0 – Channel disabled 1 to 8 – select Channel F1 to F8 for measurement 9 – select Channel NIR for measurement 10 – select Channel CLEAR for measurement 11 – select Channel FLICKER for measurement
AutoZero	uchar	0 to 255	Configure autozero, to be run with every nth subsample. 0 – never 1 to 254 – every nth subsample 255 – only with the first subsample

Parameter Name	Type	Range	Description
AGAIN	ushort	0 (0.5), 1, 2, 4, 8, 16, 32, 64, 128, 256, 512	Sets the gain of the ALS ADC.
ITIME	uint	278 to 260000	Configures the integration time to be used for the measurement in steps of 278us.
LEDCurrent	uchar	0 to 100	Configures the LED current in percent, to be driven during the measurement. *Note: The resulting current depends on the LEDs mounted on the PCB.*
LEDOnTime	uint	0 to 260000	Configures the On-time of the LED in steps of 1us. A maximum value of 260000us is allowed. *Note: see the sampling period in Figure 50.*
SyncDelay	uint	0 to 260000	Configures a delay in steps of 1us, after which the SYNC pulse shall be generated. A maximum value of 260000us is allowed. *Note: see the sampling period in Figure 50.*
SyncOnTime	uint	100 to 10000	Configures the width of the SYNC pulse in steps of 100us. *Note: see the sampling period in Figure 50.*
SamplePeriod	Uint	-	Configures the sampling period for the measurement in steps of 1us. *Note: see the sampling period in Figure 50.*
SubSamplePeriod	uint	0 to 260000	Configures the subsample period for the measurement in steps of 1us. A maximum value of 260000us is allowed. *Note: see the sampling period in Figure 50.*

Figure 53:
Parameters for Abort criteria

Parameter Name	Type	Range	Description
Mode	uchar	0 to 2	Configures the abort criteria to wait for a measurement to continue. A measurement will only continue if the abort criteria is met. 0 – disable the abort criteria 1 – Logical AND. All channels activated for the abort criteria shall trigger the abort criteria during the run of a measurement configuration. 2 – Logical OR. One channel activated for the abort criteria will trigger the abort criteria during the run of a measurement configuration. *Note: The value needed for the abort criteria is given as a parameter for each channel (see Operation and ChannelCondition).*

Parameter Name	Type	Range	Description
Operation	uchar	0 to 1	0 – abort upon specific channel deviation in percent 1 – abort upon specific channel deviation by an absolute value
TriggerAmount	ushort	0 to 255	The number of samples the defined abort criteria needs to be triggered to end the measurement phase. If subsamples are used, then the last measurement value will be used for evaluation.
Timeout	uint	-	A timeout in seconds cancels the overall measurement if the configured amount (TriggerAmount) of the abort criteria trigger events did not occur.
ChannelCondition	ushort[6]	0 to 65535	Configures the abort parameter for each channel and the specified abort criteria. If the abort criteria “deviation in percentage” is selected, the parameter will contain values from 0 to 100. If the abort criteria “deviation by an absolute value” is selected, the parameter will contain values from 0 to 65535.
ChannelActive	uchar	-	Defines which channel is activated for the abort criteria. Active channels are defined as a bit array. 0x01 – use Channel 1 for the abort criteria 0x02 – use Channel 2 for the abort criteria 0x04 – use Channel 3 for the abort criteria 0x08 – use Channel 4 for the abort criteria 0x10 – use Channel 5 for the abort criteria 0x20 – use Channel 6 for the abort criteria

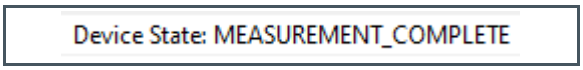
4.1.6 Executing a measurement sequence

The right-hand side of the main window (Figure 43) controls the execution of a measurement sequence. When the connected LFT Reader is in the idle state, clicking the Start button will start the measurement sequence, which is loaded onto the device.

The measurement progress can be visually monitored in the status bar with “Device State”, as shown in Figure 55. After the measurement finishes, “Device State” will update to “MEASUREMENT_COMPLETE”, as shown in Figure 56.

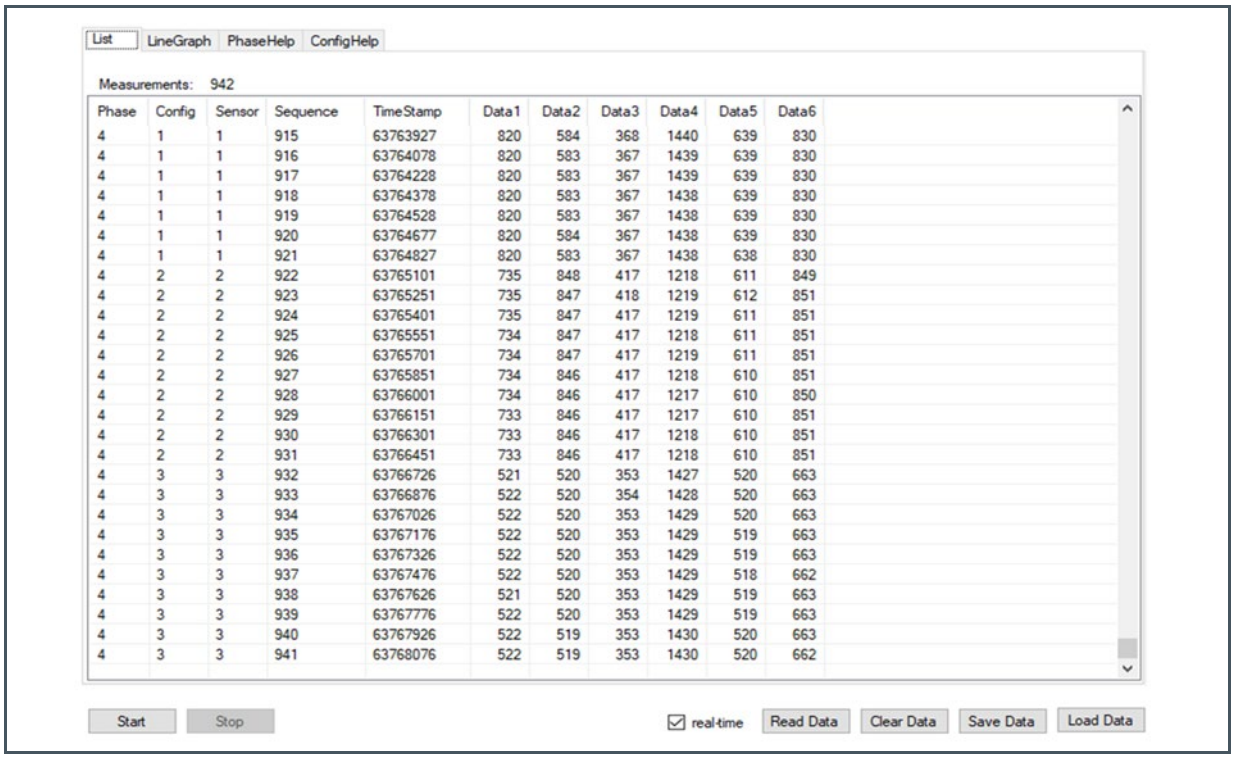
Figure 54:
Device State during the measurement

Figure 55:
Device State after completing measurement



At any time during the execution, the measurement sequence can be stopped by clicking the “Stop” button.

Figure 56:
List of measurements



Phase	Config	Sensor	Sequence	TimeStamp	Data1	Data2	Data3	Data4	Data5	Data6
4	1	1	915	63763927	820	584	368	1440	639	830
4	1	1	916	63764078	820	583	367	1439	639	830
4	1	1	917	63764228	820	583	367	1439	639	830
4	1	1	918	63764378	820	583	367	1438	639	830
4	1	1	919	63764528	820	583	367	1438	639	830
4	1	1	920	63764677	820	584	367	1438	639	830
4	1	1	921	63764827	820	583	367	1438	638	830
4	2	2	922	63765101	735	848	417	1218	611	849
4	2	2	923	63765251	735	847	418	1219	612	851
4	2	2	924	63765401	735	847	417	1219	611	851
4	2	2	925	63765551	734	847	417	1218	611	851
4	2	2	926	63765701	734	847	417	1219	611	851
4	2	2	927	63765851	734	846	417	1218	610	851
4	2	2	928	63766001	734	846	417	1217	610	850
4	2	2	929	63766151	733	846	417	1217	610	851
4	2	2	930	63766301	733	846	417	1218	610	851
4	2	2	931	63766451	733	846	417	1218	610	851
4	3	3	932	63766726	521	520	353	1427	520	663
4	3	3	933	63766876	522	520	354	1428	520	663
4	3	3	934	63767026	522	520	353	1429	520	663
4	3	3	935	63767176	522	520	353	1429	519	663
4	3	3	936	63767326	522	520	353	1429	519	663
4	3	3	937	63767476	522	520	353	1429	518	662
4	3	3	938	63767626	521	520	353	1429	519	663
4	3	3	939	63767776	522	520	353	1429	519	663
4	3	3	940	63767926	522	519	353	1430	520	663
4	3	3	941	63768076	522	519	353	1430	520	662

The first tab in the tab view shows a list of the measurement data. When the real-time checkbox is checked, measurement data will be read from the device in real-time, and the list will be updated whilst the measurement is running. If the “real-time" checkbox is unchecked, no measurement data will be updated during the measurements (in order to not disturb the measurement process). Please note to uncheck the “real-time” box if fast measurements are executed. Otherwise, transfer via USB will cause data loss.

The list of measurement data is stored in flash memory on the device. Hence, after connection to the device, the stored measurement data can always be read out by clicking the “Read Data” button.

The “Save Data” button allows you to save the measured data into a CSV file.

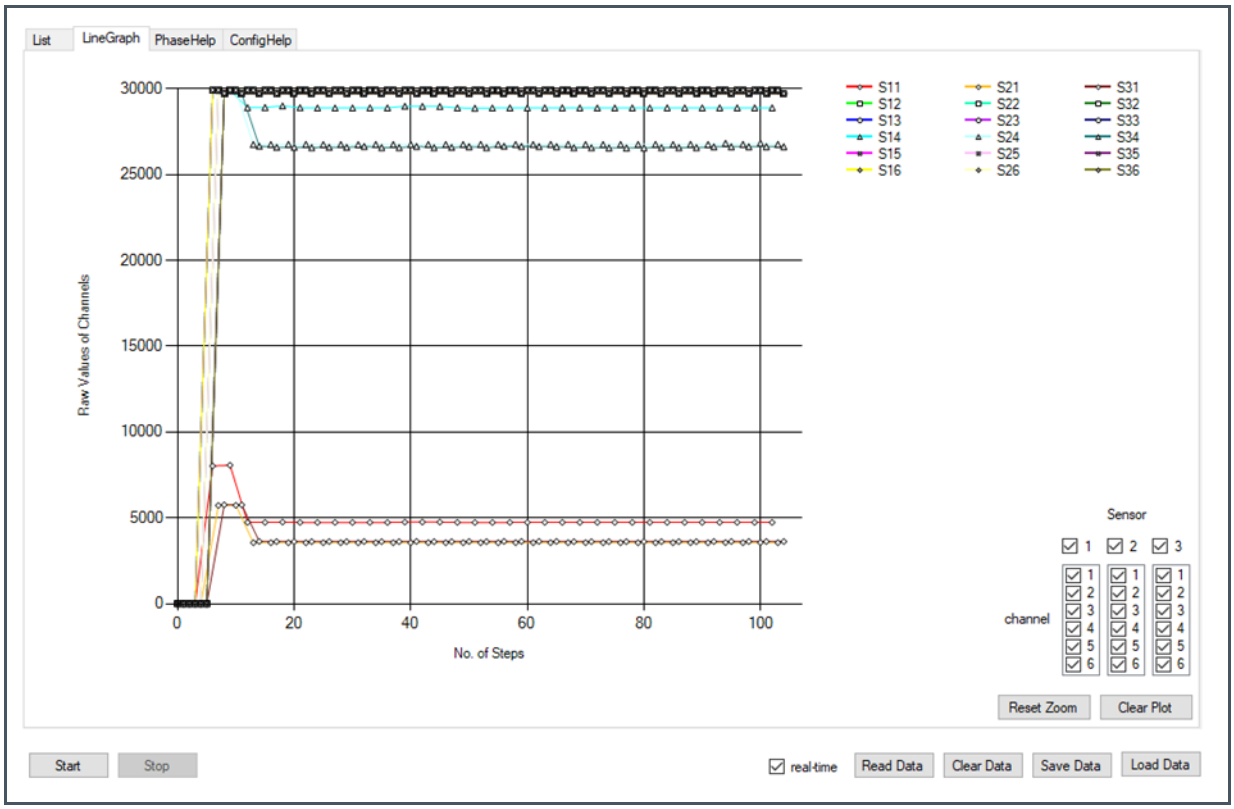
The “Load Data” button can be used to load the data back from this file.

The “Clear Data” button will clear all the displayed values from the GUI.

4.1.7 Graphical Output

The second tab in the tab view shows a line graph of the measured data.

Figure 57:
Line Graph



The line graph will show the raw ADC values of a sensor's channels on the Y-axis and the sample number on the X-axis. When the "real-time" checkbox is checked, the line graph will be updated in real-time while the measurement sequences are running on the device.

It is also possible to reread the data from the device when the measurement sequence has been completed or to read the data from a saved file.

Filtering the data

The checkboxes at the lower right corner of Figure 58 are for filtering sensor data, and the checklist boxes are for filtering the ADC channels of each sensor. Selecting the checkbox on a particular sensor will show the data, or deselecting the checkbox will hide the data of the selected channels of that sensor. The sensor channels can be selected by checking channels 1 to 6 in the checklist box below the sensor.

It is possible to select or deselect all the sensor channels at once by pressing the <CTRL> key while clicking the sensor checkbox.

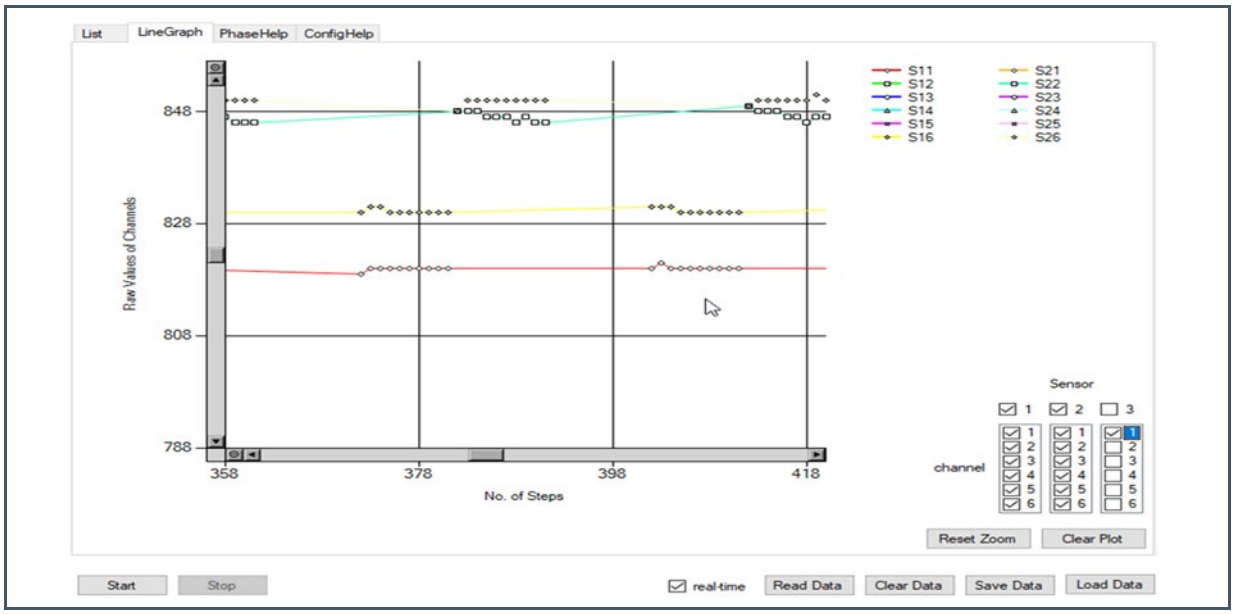
By default, all the sensors and all the channels are selected.

Zooming

There are two ways of zooming into the details of the line graph:

- The quickest way is to use the mouse wheel to zoom in or out around a pivot point. The pivot point is where the mouse is located. Zooming creates horizontal and vertical scroll bars that can be used for panning across the line graph.

Figure 58:
Zooming with the mouse wheel



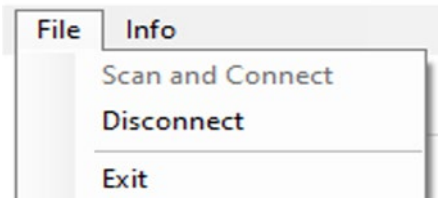
- The second way is to zoom in by drawing a rectangle whilst holding down the <CTRL> key. This rectangle will be shown magnified on the screen. This operation can be repeated to zoom in further.
  Note that this way of zooming in does not work correctly while the line graph is being updated in real-time and the measurement sequences are running on the device.

4.1.8 File Menu

The File menu contains the following options:

- Scan and Connect – To scan and connect to LFT Reader devices.
- Disconnect - To disconnect from the current LFT Reader device.
- Exit - To quit this program.

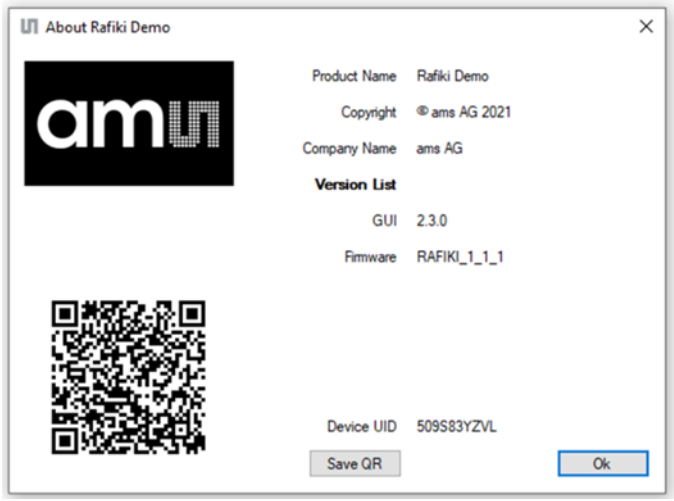
Figure 59:
File Menu



4.1.9 Info – About DiRa-Lab Demo

The “Info” menu contains an “About” option that shows a dialog of information about the application version and the connected device (Figure 61). It also contains a QR code generated for the connected device; this QR code can be saved as a bitmap file by clicking the “Save QR” button.

Figure 60:
DiRa-Lab Demo About window

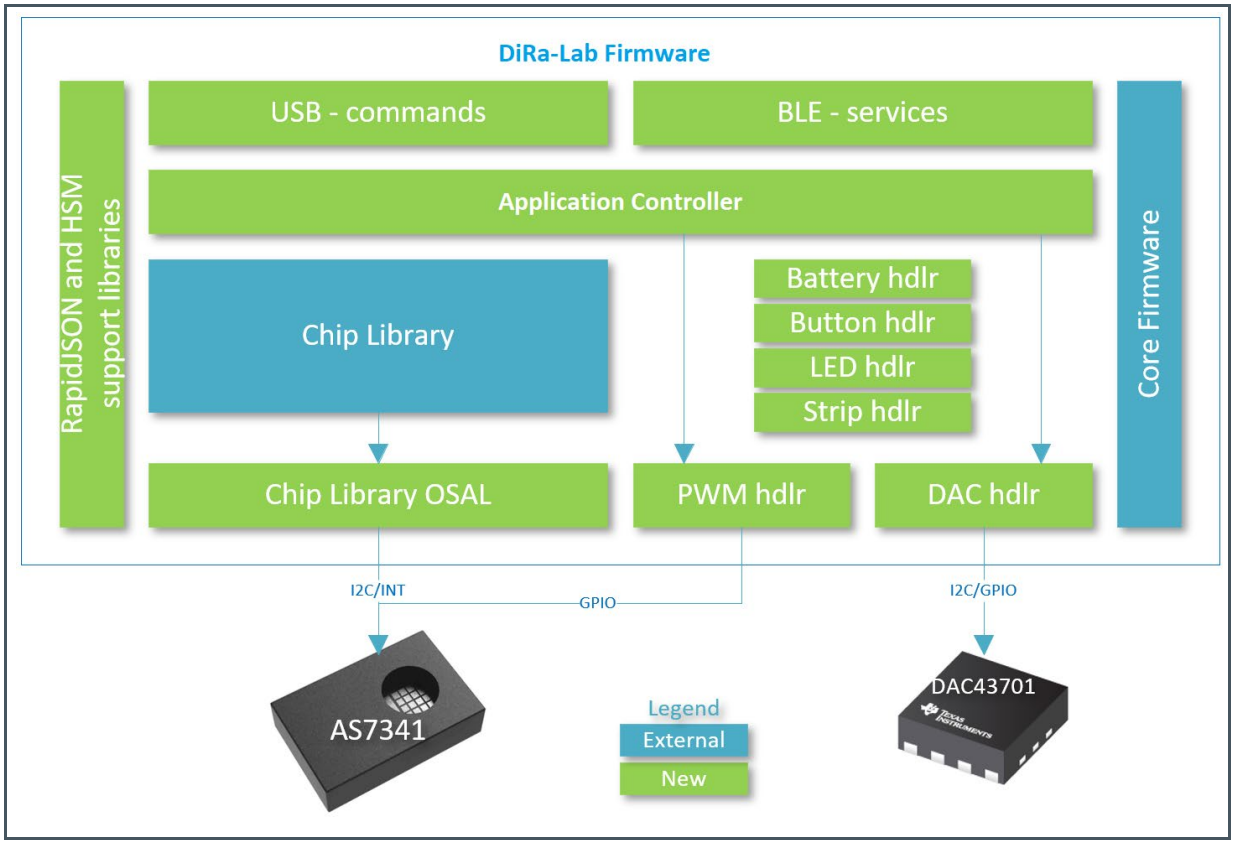


4.2 Firmware Overview

The firmware is part of the embedded system shown in the LFT Reader domain. It resides within the system’s flash memory and is executed by the microcontroller. The firmware manages the onboard peripherals, such as the optical system (three AS7341L sensors and three LEDs), the system’s power management, the memory, etc. It also implements the system’s main interfaces: the USB Interface (meant for use in engineering and validation) and the Bluetooth Low Energy (BLE) Interface. A dedicated RGB LED is used to indicate the firmware status to the user.

The firmware's main role is to control the module's peripherals, including the optical sensors, AS7341L, to measure the visual response during a lateral flow test (LFT). The firmware acts as a BLE peripheral and requires a central BLE to initiate the communication over BLE. The firmware components are mentioned in Figure 62.

Figure 61:
Firmware components of the LFT Reader



The internal measurement control engine is designed as a sequencer, which processes configurable measurement sequences. The firmware performs the measurement patterns described in the configuration and stores the raw data on the internal flash memory.

5 Lateral Flow Strip Requirements

The strip has the possibility of a maximum of three test/control lines. The current optical design is optimized for three sensors and three LEDs, to achieve the detection ability in three windows. The LFT Reader Kit, based on a white LED and an AS7341L spectral sensor, can be used for a wide range of visible wavelength particles, such as a gold Nanoparticle, colored latex particles, cellulose Nano pads, etc. The UV LED-based (365 nm peak wavelength) LFT Reader Kit can be used for detecting fluorescent particles, such as Europium, etc.

5.1 Strip Dimensions

The LFT strip consists of a sample pad, a conjugate pad, a transparent packing card, and a wicking pad (Figure 64). It has the dimensions 4 x 60 mm. According to the LFT Reader design, the LFT strip has three lines that can be used as test or control lines, plus one alignment line. A combination of test/ control lines can be, for example, two test lines and one control line. In this case, you can detect two different targets with the LFT Reader system. If you do not want to use a control line, you can skip it to compensate for the background (in colorimetric application) or add a third target to the LFT strip. There is also the possibility to have more than one target in one test line since the AS7341L sensor has eight channels in the visible range, plus one clear channel and one infrared channel (350 – 1000 nm spectrum). The AS7341L enables you to detect different colors on the same test line. This possibility shows the flexibility of designing the LFT strip with different targets.

5.1.1 Strip requirements

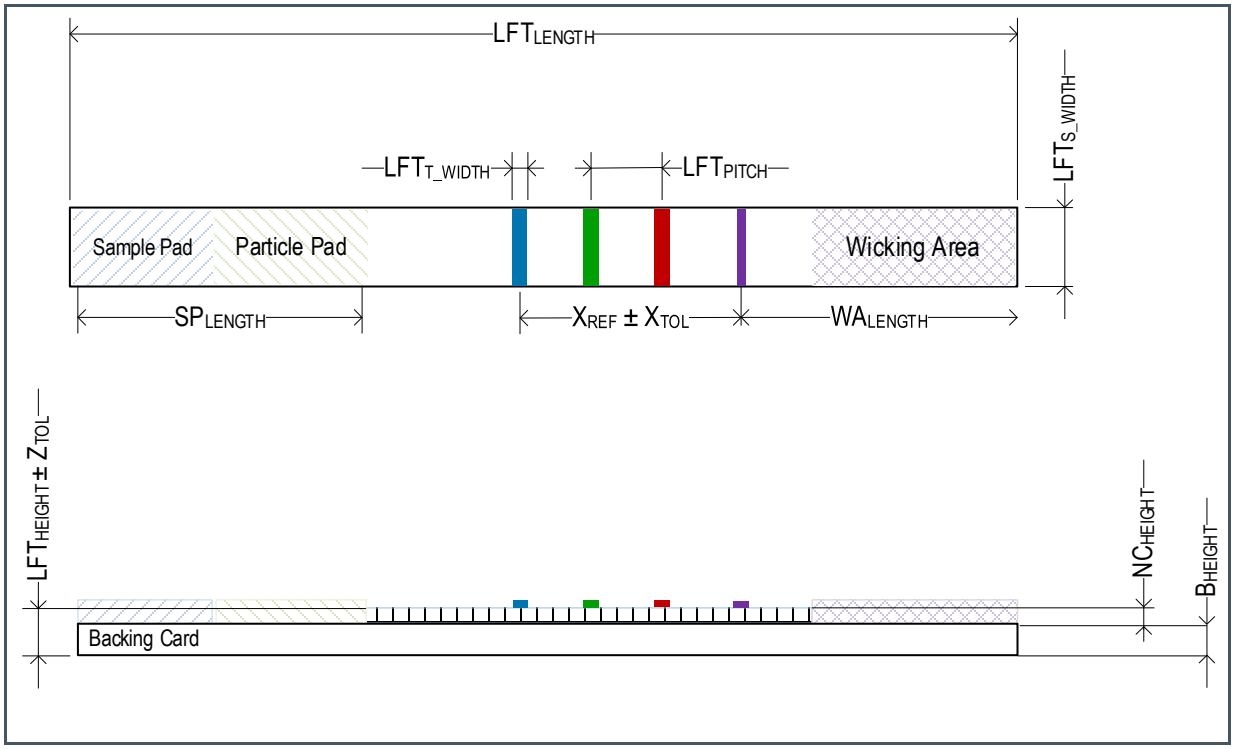
Strip dimensions can be taken from Figure 63 below according to Figure 64.

Figure 62:
LFT strip dimensions

Symbol	Parameter Description	Min	Typ	Max	Unit	Conditions
LFT_{LENGTH}	Total length of LFT strip.		60		mm	To be defined by the strip manufacturer and the customer.
LFT_{S_WIDTH}	Total width of LFT strip.	3.9	4	4.5	mm	Assuming a 4mm strip.
B_{HEIGHT}	Backing card height.	0.15	0.25	0.35	mm	
NC_{HEIGHT}	NC height.	0.17	0.27	0.37	mm	
LFT_{HEIGHT}	LFT strip height.		0.52		mm	
LFT_{PITCH}	Distance between the test and control lines.	3	3	5	mm	
LFT_{T_WIDTH}	Test/Control line width.	300	700	1000	µm	
X_{TOL}	Test line tolerance related to the mechanical alignment hole/line.			±100	µm	
Z_{TOL}	Height tolerance of the LFT strip.			±200	µm	100µm backing card and 100µm NC membrane.

Symbol	Parameter Description	Min	Typ	Max	Unit	Conditions
X_{REF}	Test line distance, referencing the mechanical alignment hole/line		9			To be defined by the strip manufacturer and the customer.
WA_{LENGTH}	Wicking area length.		21		mm	
SP_{LENGTH}	Sample and particle pad length.		22		mm	

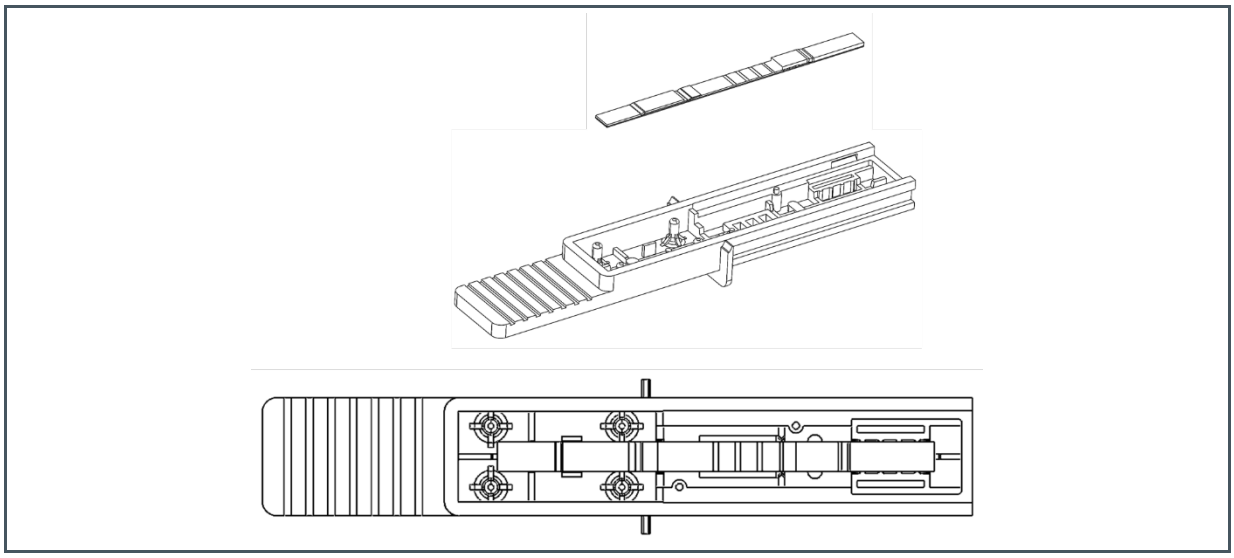
Figure 63:
LFT strip structure (Drawing not true to scale)



5.2 Strip Alignment

The LFT strip alignment is the main key to getting useful results after performing the measurements. This chapter explains how to align the LFT strip. Firstly, hold the strip correctly from the wicking pad and avoid touching the sensitive area with the test lines. The sample pad should face your direction, as shown in Figure 63. In this case, the LEDs will illuminate the strip from the backside, and the sensors will measure while facing the test lines directly from the other side.

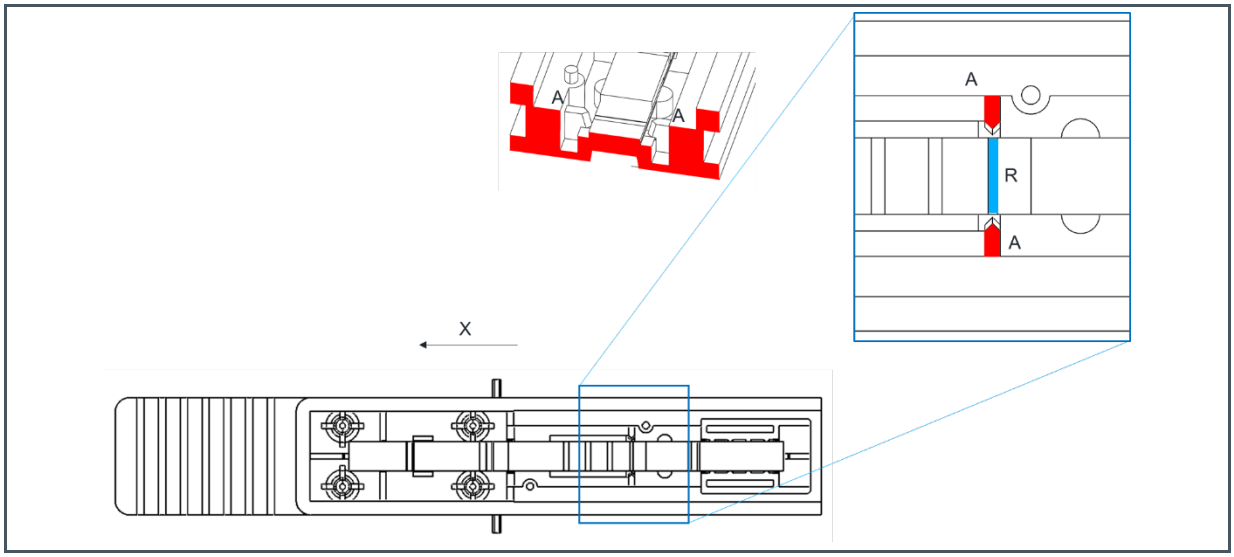
Figure 64:
Correct alignment of the LFT strip



5.2.1 Strip alignment in the x-direction

1. Place the strip on the surface.
2. Gently move in the positive or negative x-direction, to centre the strip reference line R (in blue) with the cartridge references, A (in red). This is illustrated in Figure 66.
3. Align centerline to centerline, using a magnifying glass or microscope.

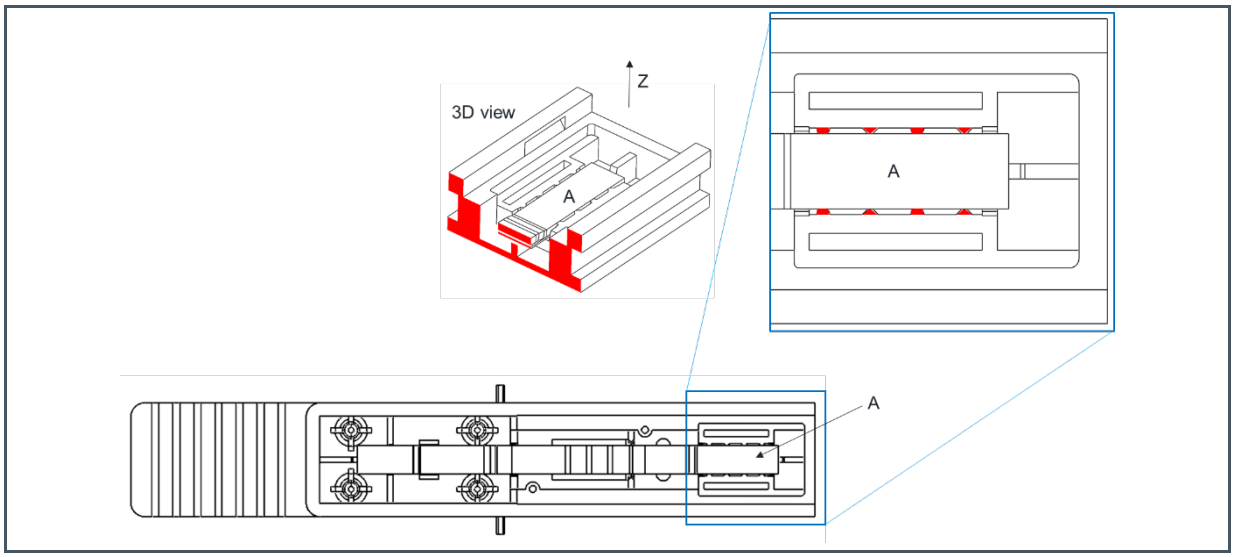
Figure 65:
LFT strip alignment in the X-direction



5.2.2 Secure strip in the x-direction

1. Align the strip in the positive or negative x-direction.
2. Gently press down in the negative z-direction of the strip at the indicated area, A, to secure the strip between the features on the cartridge (marked in red in Figure 67).
3. Report any issue if the strip is not locked properly. Free strips are not suitable for measurement.

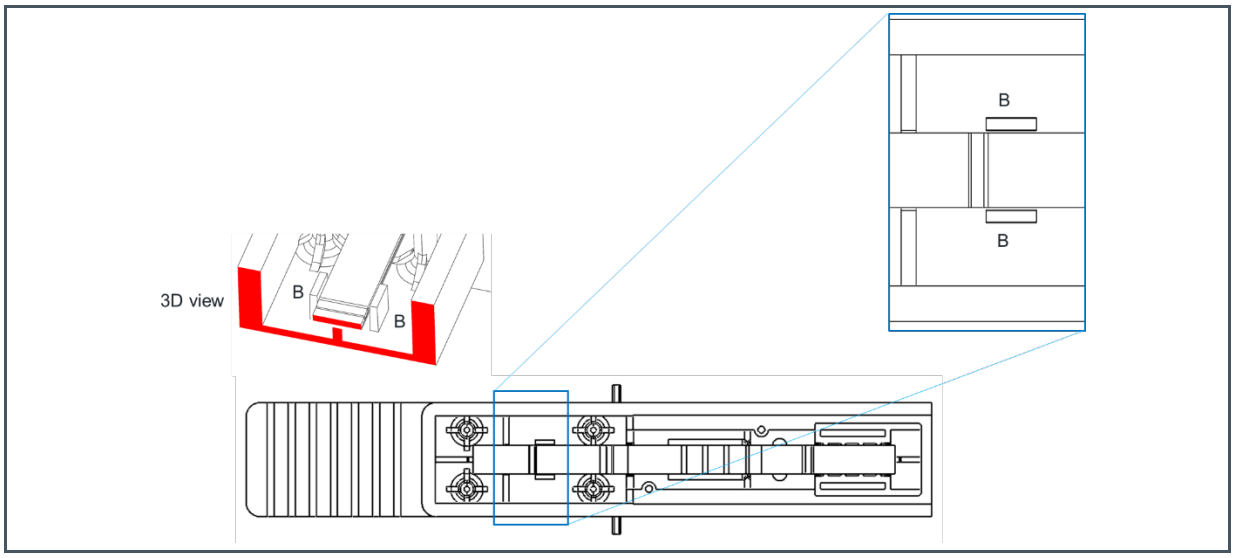
Figure 66:
Secure strip in the x-direction



5.2.3 Rotation lock

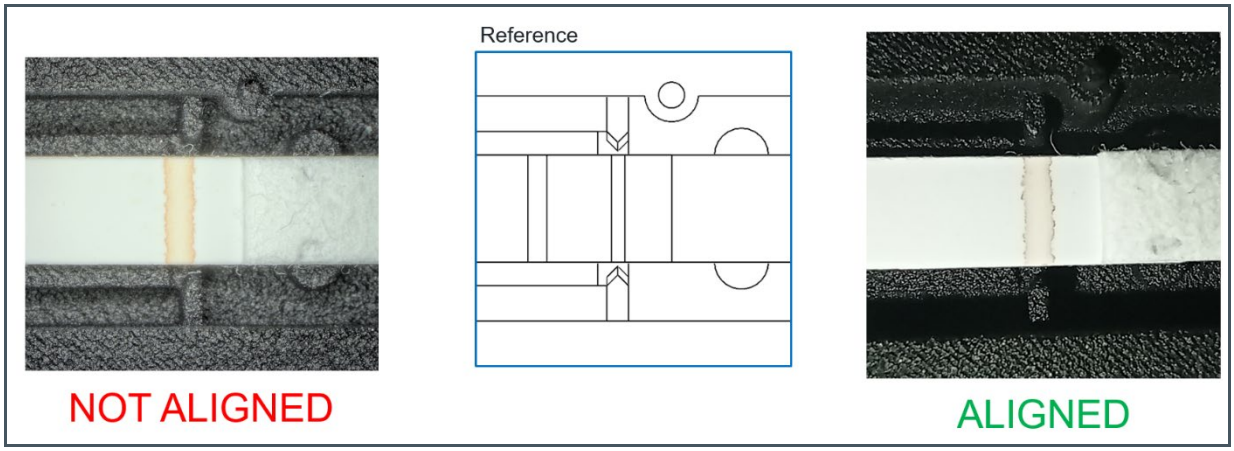
The B features are in place to reduce the rotation of the strip around the z-axis (Figure 65). No action from the operator is required.

Figure 67:
Strip rotation lock



5.2.4 Alignment Examples

Figure 68:
Useful Caption



5.3 Cartridge Design

The cartridge has the dimensions 16.2 x 106 x 8 mm, as shown in Figure 70. The length of the top part is 76.3 mm. The distance between the sensor and PCBAs reader is around 6 mm, and the cartridge has four features to hold the strip stable within it (the labeled red parts in Figure 71).

Figure 69:
Cartridge parts

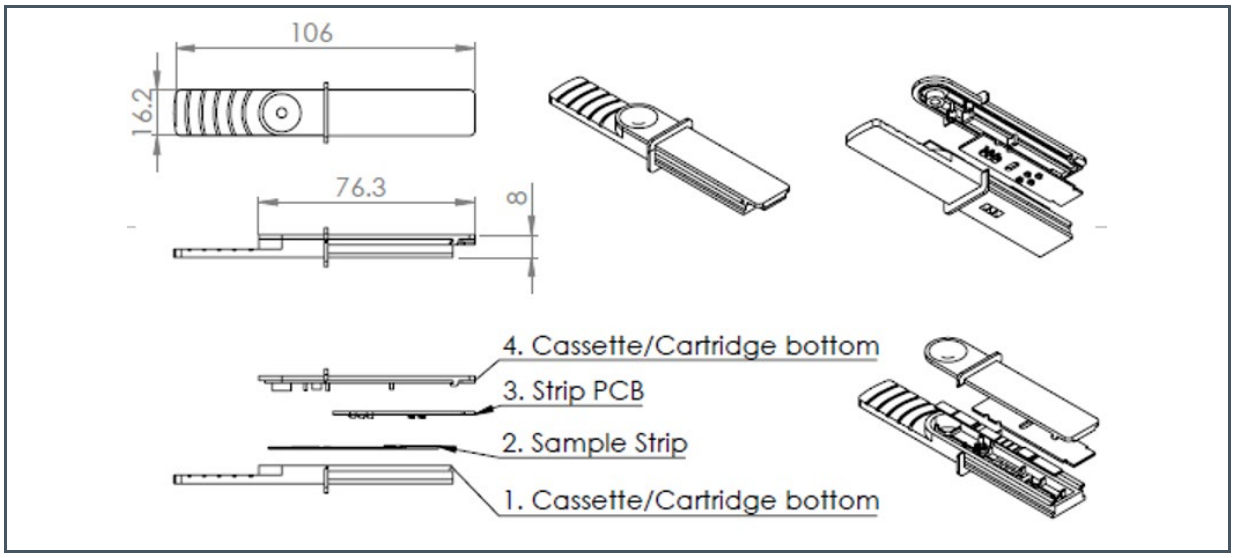
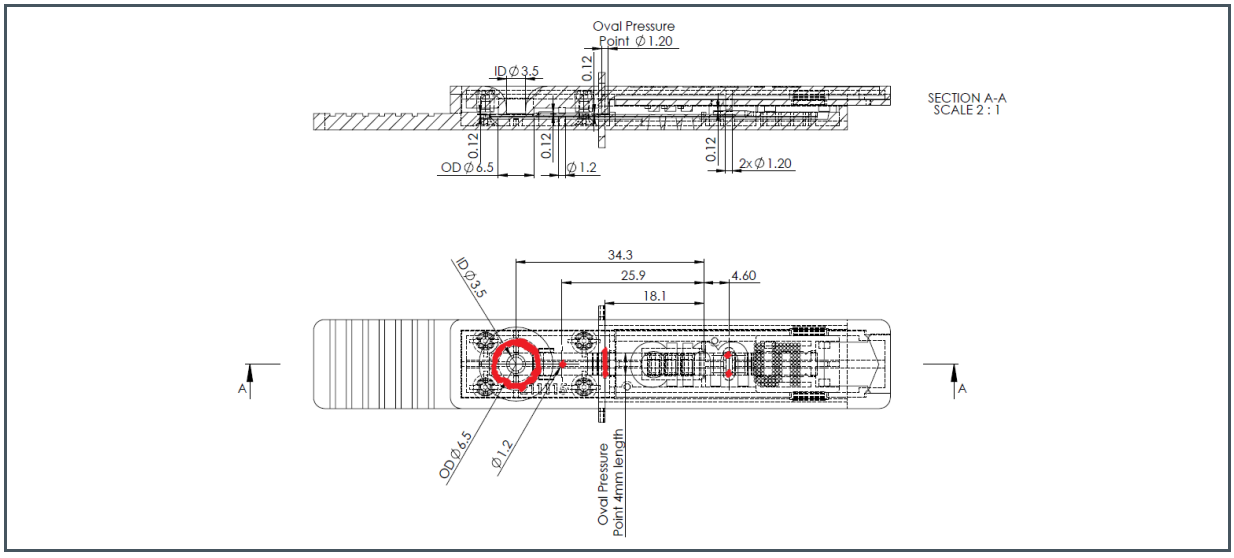


Figure 70:
Detailed cartridge structure



6 Algorithm

After performing the measurements, and obtaining the raw data (counts) from the sensors, configure the number and position of the data points as explained in chapter 4.1.5. To obtain a measurement result, implement an evaluation algorithm, which depends on the chosen chemistry, pad and matrix materials, sample type and volume, line configuration, etc. **ams OSRAM** assists customers in developing such algorithms for specific applications. For this, please contact the support team of **ams OSRAM**. For example, Figure 72 and Figure 73 show the raw data of two COVID-19 samples: a strong positive sample and a negative sample. In this experiment, the test line on the LFT strip was under the sensor labeled S2 and the measurement time was about 16 minutes. As shown in Figure 72, the signal of the S2 sensor is very high compared to the signals of the S1 and S3 sensors. This gives a strong indication that the sample was strongly positive with the COVID-19 virus. In Figure 73, all the signals were almost from the same order, which indicates that the sample is negative. The F7 Channel in the three sensors (AS7341L) is considered in this experiment. To decide if the sample is positive or negative, consider the last measurement points of the S2 and S3 sensors or the S2 and S1 sensors for evaluation of the results. The LFT Reader system can track the difference between the target line and the background over time, so you can identify between the positive and negative samples. Depending on your application, a combination of the sensor channels can also be used: for example, using the F6 + F7 channels to develop your algorithm.

Figure 71:
Strong positive sample

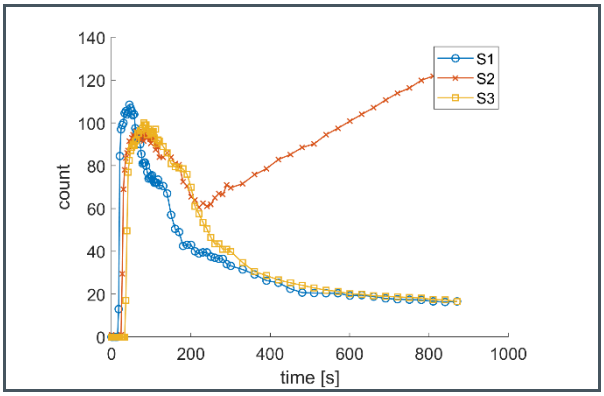
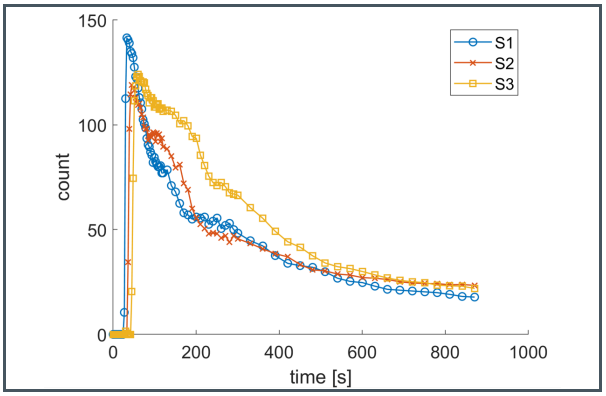


Figure 72:
Negative sample



7 Revision Information

Changes from previous version to current revision v0-04	Page
Initial version	all

- Page and figure numbers for the previous version may differ from page and figure numbers in the current revision.
- Correction of typographical errors is not explicitly mentioned.

8 Legal Information

Copyrights & Disclaimer

Copyright ams AG, Tobelbader Strasse 30, 8141 Premstaetten, Austria-Europe. Trademarks Registered. All rights reserved. The material herein may not be reproduced, adapted, merged, translated, stored, or used without the prior written consent of the copyright owner.

Demo Kits, Evaluation Kits and Reference Designs are provided to recipient on an “as is” basis for demonstration and evaluation purposes only and are not considered to be finished end-products intended and fit for general consumer use, commercial applications and applications with special requirements such as but not limited to medical equipment or automotive applications. Demo Kits, Evaluation Kits and Reference Designs have not been tested for compliance with electromagnetic compatibility (EMC) standards and directives, unless otherwise specified. Demo Kits, Evaluation Kits and Reference Designs shall be used by qualified personnel only.

ams AG reserves the right to change functionality and price of Demo Kits, Evaluation Kits and Reference Designs at any time and without notice.

Any express or implied warranties, including, but not limited to the implied warranties of merchantability and fitness for a particular purpose are disclaimed. Any claims and demands and any direct, indirect, incidental, special, exemplary or consequential damages arising from the inadequacy of the provided Demo Kits, Evaluation Kits and Reference Designs or incurred losses of any kind (e.g. loss of use, data or profits or business interruption however caused) as a consequence of their use are excluded.

ams AG shall not be liable to recipient or any third party for any damages, including but not limited to personal injury, property damage, loss of profits, loss of use, interruption of business or indirect, special, incidental or consequential damages, of any kind, in connection with or arising out of the furnishing, performance or use of the technical data herein. No obligation or liability to recipient or any third party shall arise or flow out of ams AG rendering of technical or other services.

RoHS Compliant & ams Green Statement

RoHS Compliant: The term RoHS compliant means that ams AG products fully comply with current RoHS directives. Our semiconductor products do not contain any chemicals for all 6 substance categories plus additional 4 substance categories (per amendment EU 2015/863), including the requirement that lead not exceed 0.1% by weight in homogeneous materials. Where designed to be soldered at high temperatures, RoHS compliant products are suitable for use in specified lead-free processes.

ams Green (RoHS compliant and no Sb/Br/Cl): ams Green defines that in addition to RoHS compliance, our products are free of Bromine (Br) and Antimony (Sb) based flame retardants (Br or Sb do not exceed 0.1% by weight in homogeneous material) and do not contain Chlorine (Cl not exceed 0.1% by weight in homogeneous material).

Important Information: The information provided in this statement represents ams AG knowledge and belief as of the date that it is provided. ams AG bases its knowledge and belief on information provided by third parties, and makes no representation or warranty as to the accuracy of such information. Efforts are underway to better integrate information from third parties. ams AG has taken and continues to take reasonable steps to provide representative and accurate information but may not have conducted destructive testing or chemical analysis on incoming materials and chemicals. ams AG and ams AG suppliers consider certain information to be proprietary, and thus CAS numbers and other limited information may not be available for release.

Headquarters
ams AG
Tobelbader Strasse 30
8141 Premstaetten
Austria, Europe
Tel: +43 (0) 3136 500 0

Please visit our website at www.ams.com
Buy our products or get free samples online at www.ams.com/Products
Technical Support is available at www.ams.com/Technical-Support
Provide feedback about this document at www.ams.com/Document-Feedback
For sales offices, distributors and representatives go to www.ams.com/Contact
For further information and requests, e-mail us at ams_sales@ams.com