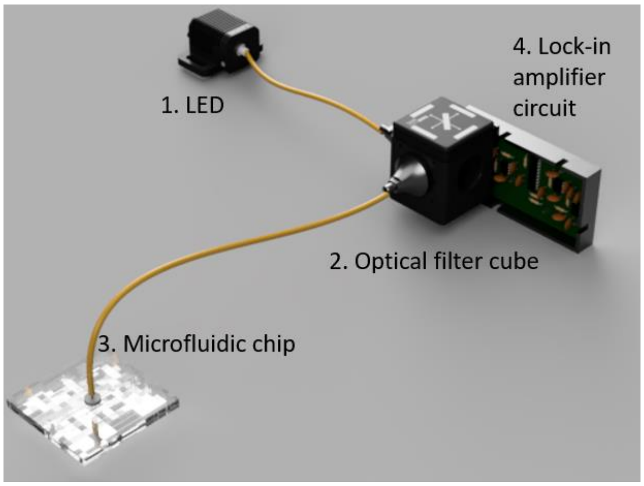
Fluorescence Detector on Chip Handover Notes

Shachi Khadilkar
Ph.: 6173787579
Email: shachivk@bu.edu

The Fluorescence detector on chip uses a lock-in amplification technique to amplify weak fluorescent signals obscured by noise. The object of this text is to briefly describe the different parts of this system and how to use them.

Big picture:



Electronics:

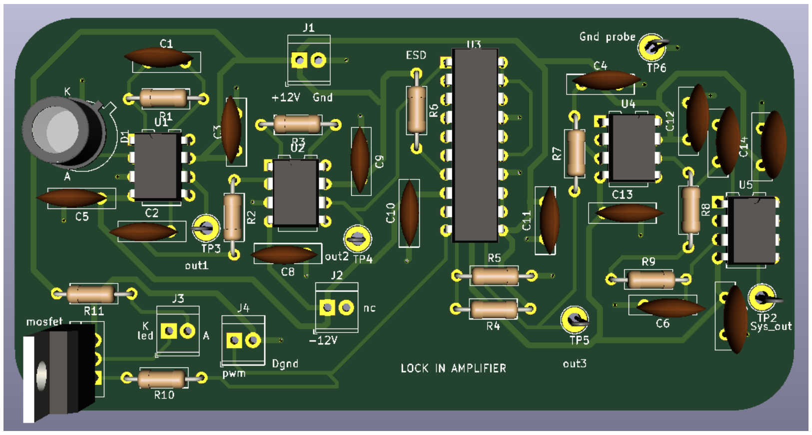
Lock-in amplification requires a modulated light source, pre-amplifiers and a demodulator + low pass filter circuit. Three PCBs were made – an LED modulator, a lock-in amplifier and an A to D board.

Lock-in amplifier PCB:

Components:

1. TL081 (4 ICs) – operational amplifiers
2. AD630 (1 IC) – Demodulator
3. Photodiode (1) – BPW21
4. Passives
5. Screw Terminals
6. Test points

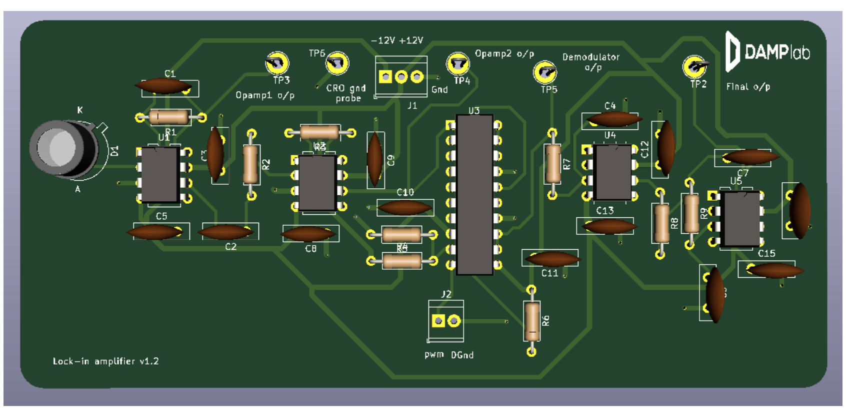
Lock-in amplifier v1.1:



Using the board:

- Connect the power supply in series mode and supply power to the board at screw terminals with appropriate marking (+12V, -12V, Gnd). DO NOT exceed +-18V.
- Fluorescent light incident on the photodiode is the signal input to the PCB.
- The demodulator needs a PWM signal (at the screw term marked accordingly). Connect the Arduino ground to the Dgnd pin on the PCB. PWM signal is 5kHz, 10 % duty cycle for the current design.
- Output signal is obtained at test point Sys_out (or Final o/p for v1.2).
  If the output signal is being measured by an oscilloscope, connect the scope probe to Sys_out and the ground clip to the test point labelled Gnd probe.
  If ADC is used, connect Sys_out to ADC
- The test-point after each stage can be used for hardware debugging.

Lock-in amplifier v1.2:

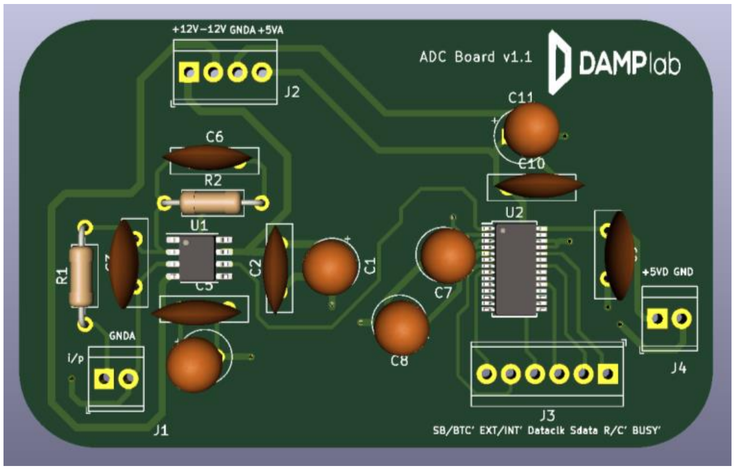


Using the board:

- Similar to v1.1.

A to D Converter:

Components:
1. AD8517 (1 IC) – A/D converter
2. OPA132 (1 IC) – Operational amplifier
3. Passives
4. Screw terminals

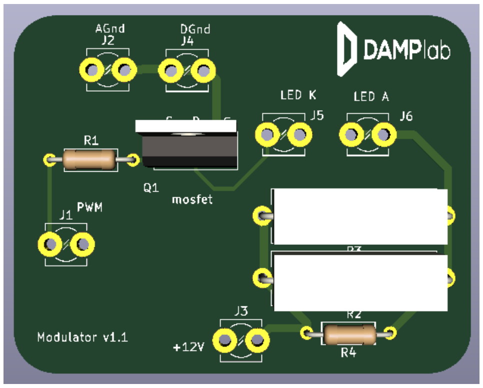


Using the board:

- Connect Sys_out (or Final o/p) of the lock-in amplifier board to appropriate screw terminal (marked i/p) for ADC analog input.
- Supply +-12V, ground, +5V analog and +1.8V digital power inputs to the board at the appropriate screw terminals. (Use 1.8VD where +5VD is marked)
- Connect digital ground to screw terminal J4 where indicated.
- SB/BTC' (input): Set to high for straight binary format output. (Low gives binary twos complement)
- EXT/INT' (input): Set to high to use the ADC as SPI slave. (Low to use ADC as SPI master).
- Dataclk (input): Clock from microcontroller.
- R/C' (input): Use PWM to generate required signal (Refer to ADS8517 datasheet)
- Sdata (output): Serial data output synchronized to Dataclk.
- Busy' (output): Busy' goes low and stays low until conversion is complete.

Modulator:

Components:
1. FQP30N06L (1 IC) – Mosfet
2. Passives
3. Screw Terminals



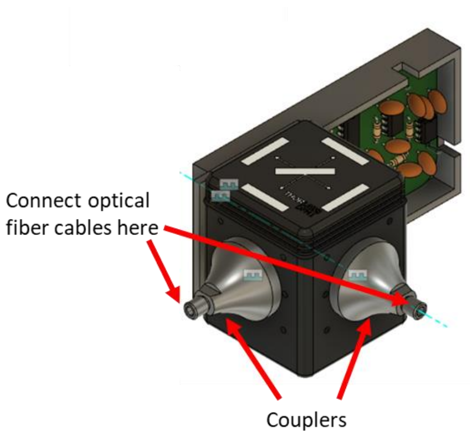
Using the board:

- Supply 12V, analog and digital ground to the board at appropriate screw terminal. The two high wattage resistors are 50 ohms each.
- Connect LED Anode and Cathode as indicated on the board.
- PWM for modulating the LED (5khz, 10 % duty cycle) should be given at screw terminal J1.
- Maximum allowable LED current is 700mA for M565F3.

Optics:

Components:

1. DFM1 (1) - Kinematic Fluorescence Filter Cube
2. CVH100-COL (2) – Couplers
3. LA1074 (2) – Collimators
4. MF620-52 (1) - TRITC/CY3.5 Emission Filter CWL = 620 nm, BW = 52 nm
5. MD568 (1) - TRITC Dichroic Filter, Refl. Band = 525-556 nm, Trans. Band = 580-650 nm
6. M28L01 (2) – SMA – SMA fiber patch cable
7. M565F3 (1) – 565 nm LED



- A 13mW optical power LED is used as the light source for the system.
- A 30 mm kinematic fluorescence filter cube with an emission filter and a dichroic mirror is used. The filter cube is mounted on the case that holds the PCB. To change filters/ dichroic in the filter cube, refer to https://youtu.be/qWIfiwuL-gQ.
- Use retaining rings to fix the filters in place. (DAMP lab South has a spanner wrench for standard size filters)
- Optical couplers are compatible with filter cube and can be screwed in easily. Both couplers have been fitted with collimators.
- Use optical fiber cables to connect filter cube to microfluidic chip and light source (through the couplers).

Instructions for experiments:

- The setup should be placed on the ESD mat and user must wear the wrist strap to avoid damage to the ESD sensitive demodulator IC AD630 and photodiode BPW 21.
- Make connections to the modulator board and the lock-in amplifier board as described above.
- Connect LED to optical filter cube using fiber cable and coupler.
- Depending on number of optical interfaces to be used for the experiment use optical fibers, couplers etc. to capture fluorescent light from the sample.
- The current optical setup is chosen for reagent Amplex UltraRed. For spectrum see https://www.thermofisher.com/order/catalog/product/A36006#/A36006
- The final goal is to detect fluorescence from droplets in microfluidic chips. To find a lower limit of detection, a stationary cuvette (or droplets on a slide) may be used for testing by serially

diluting the sample and measuring fluorescent signals for each concentration. (Eg. 1mM to 100nM)
- Connect the oscilloscope (as mentioned above) to measure the voltage proportional to each concentration.

Software used for PCB design: KiCad EDA (https://kicad-pcb.org/)
Software used for simulating the circuit: LTspice (https://www.analog.com/en/design-center/design-tools-and-calculators/ltspice-simulator.html)

For LTspice Analysis refer to http://ltwiki.org/index.php?title=Simulation_Command, https://www.analog.com/en/technical-articles/ltspice-using-the-step-command-to-perform-repeated-analysis.html

Status and Next Steps:

Part	Status	Issues	Potential Next Steps
Lock-in Amplifier Circuit	**Working prototype** Circuit output increases with increase in light incident on photodiode. (Expected behavior)	1. Noise-signal trade-off (Low pass filtering cutoff frequency) 2. Low frequency noise from amplifiers, passives. 3. Inability to operate in ambient light.	1. Testing with different low pass filter frequencies (eg 20Hz to 2.5kHz) 2. Replacing existing opamps with lower noise alternatives (eg. OPA145) 3. Lockin amplifier multiplexing
Optical setup	**Prototyping**	1. LED power is very low (max 13mW) 2. Losses at each optical interface 3. LED is broadband and hence a very small fraction of light reaches the photodiode.	1. Light source must have a high optical power and should be narrow band (eg. LASER diode)