# Project 2.2: "On-chip" Real-time Polymerase Chain Reaction: Optical Detection

BE/EE/MedE 189a: Design and Construction of Biodevices

Fall 2017

## Instructions

1. Your thermocycling should be working by now.
2. Handle all optics very carefully. Do NOT touch the surfaces of the lenses, filters, objective lenses and camera sensors. When in doubt, please ask.
3. Please feel free to ask for demonstrations of how the optics should be mounted. When in doubt (and if there's no one to consult), visit the Thor Labs website for some ideas. We got all our opto-mechanic components there. It should get more intuitive as you start playing around with the components.
4. Your lab report should document clearly but concisely how your device and VI work. The lab report will be graded partially on functionality of the system. However, if your device doesn't function perfectly, do not fret. A large emphasis will be placed on understanding of the shortcomings and points of failure (if any) of your system.
    - Submit relevant VIs.
    - Write a brief outline of the lab any modification/addition to the lab instructions. Include answer to the circuit design questions embedded in the lab instructions.
    - Emphasis is placed on the analysis and demonstration of understanding of the functionality, performance and limitations of the system.

## Optical detection

Epifluorescence detection will be used. The optical detection system (essentially a homemade microscope) consists of an LED light source (see spec sheet attached), a filter set (excitation, dichroic and emission), an objective lens (infinite conjugate, spec sheet attached), a tube lens and a camera.

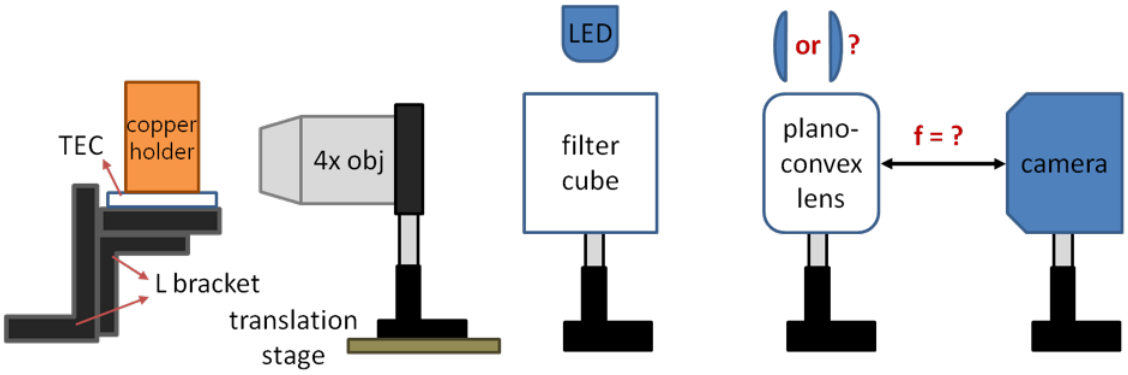


Figure 1: Suggested setup

**Q1**. What is the focal length of the plano-convex lens (50 mm, 75 mm or 100 mm)? How did you find it out (without referring to its part number)?

**Q2**. How would you orient the plano-convex lens? Should the flat surface face the objective or the camera? Hint: One orientation works better at focusing a collimated light into a point. You may draw an illustrative figure.

**Q3**. What is the magnification of the microscope system in the setup? How did you find out?

**Q4**. At which part of the cycle should the signal acquisition be done?

**Q5**. How would you determine the background signal and eliminate/consider that in your data?

**Q6**. Since background light contributes to noise, how would you maximize the signal-to-noise ratio?

## Tips

1. Since fluorophore bleaching can occur, it is prudent to turn on the LED only during signal acquisition.
2. The thermistor's temperature calibration needs to be performed so that it accurately represents the temperature of the copper holder.
3. To initialize, access and change camera's settings, type `imaqtool` in the Matlab window, and copy the lines of code displayed in `Session Log` according to your desired settings.
4. To capture images from the camera, type `var = getsnapshot(vid)` in the Matlab Script VI after initialization of the camera, where `var` is the name of the variable to which you assign the captured image.
5. The camera should be focused on to the tube in the copper holder visible through the small hole. A trick is to place a small piece of paper with letters on it in the copper holder so that the letters are visible through the hole, shine light on the paper, and adjust the lenses' and camera's position until you can read the letters in the camera's image.
6. Feel free to use the provided black tarp to cover your setup to block any stray light into your setup. You may also bring your own box for a more secure light isolation.

Your LabVIEW program should be able to acquire and plot a replication curve. A replication curve is required. A melt curve analysis is not required.

### System test

A tube of complete reaction mix (containing template DNA, DNA primers, polymerase, dNTPs, SYBR etc) will be given to you at the beginning of class. We're to test for the presence of a GMO gene. Here's an excerpt from the BioRad GMO investigator kit we're using, just so we have an idea of what we're trying to amplify:

> The GMO Investigator kit is designed to test for the presence of two different GMO-associated DNA sequences: the 35S promoter of the cauliflower mosaic virus, and the terminator of the nopaline synthase gene of Agrobacterium tumefaciens. These DNA sequences are present in most of the GM crops that are approved for distribution worldwide.

Test the functionality of your system during the class. A typical amplification plot looks like the graph in Fig. 2.

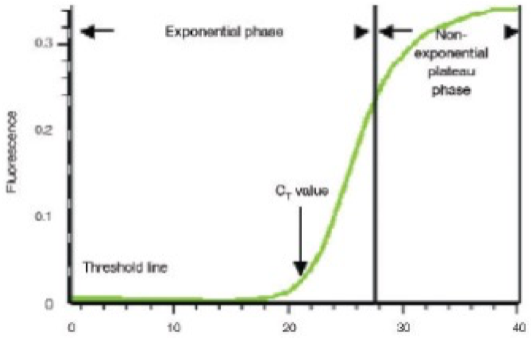


Figure 2: Typical amplification plot

**Q7**. Why would the curve look like this?

**Q8**. Did you get a typical amplification curve? What are the sources of noise and design inadequacies? How can the system be improved?

## Postlab Questions

**Q9**. Due to the time and resource limitations, we are only able to do a very rough simulation of what an "on-chip" real time PCR device should be able to. (We probably shouldn't even call it an on-chip system.) What are the potential advantages of a true on-chip platform? Think along the lines of sample preparation, reagent mixing, reagent volume, portability, etc ...

**Q10**. Without consulting current literature, design a fully on-chip PCR device. (A concise block diagram of design features will do. A complete blueprint is not required.) This part of the exercise is not graded based on feasibility (but it cannot be completely out of this world). Just be imaginative and have fun! Start from an intact tissue sample. The end point in mind would be the determination of the absence or presence of a specific DNA sequence.

**Q11**. Read some recent papers on on-chip PCR. Which part of your imaginary design has already been tackled? (Cite the papers you referred to.) What are the remaining engineering challenges? (If there isn't any remaining, think about any design opportunities. Include assumptions made (e.g. availability of technologies and/or processes).