A Real-Time Non-Invasive Sensor for Monitoring Laser-Induced Temperature in Medical Applications

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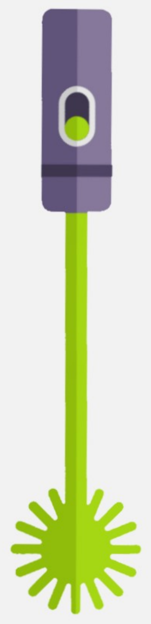
Motivation

Many surgical procedures such as retinal surgery use high power lasers [1]. The laser-generated heat is often hard to monitor [1] [2]. Molecular beacons are compounds that can exhibit temperature dependent fluorescence [3]. To be representative of the human eye, they are suspended in a gel matrix and can act as an **accurate, real time, non-invasive temperature sensor.**



Materials

Molecular beacons are self-hybridizing oligonucleotides, with a fluorophore and quencher, that exhibit fluorescence proportional to temperature [3]. This is shown in Figure 1.

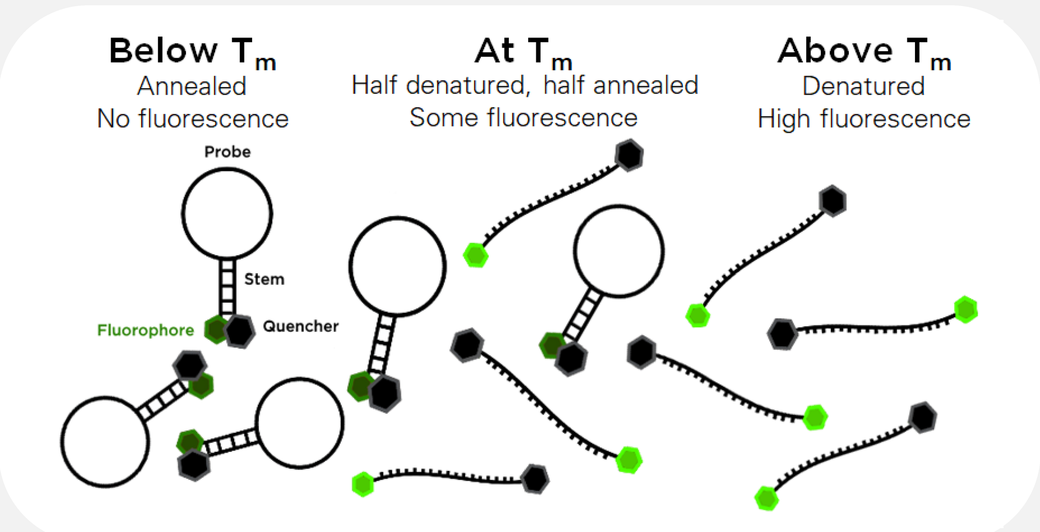


Figure 1. Molecular beacon behaviour before, at, and above T_m

The fluorophore chosen in our design is 6-Carboxyfluorescein (6-FAM) and the quencher is Black Hole Quencher 1 (BHQ-1), which are both illustrated in Figure 2 [4] [5]. BHQ-1 quenches the fluorescence of 6-FAM when in proximity [6].

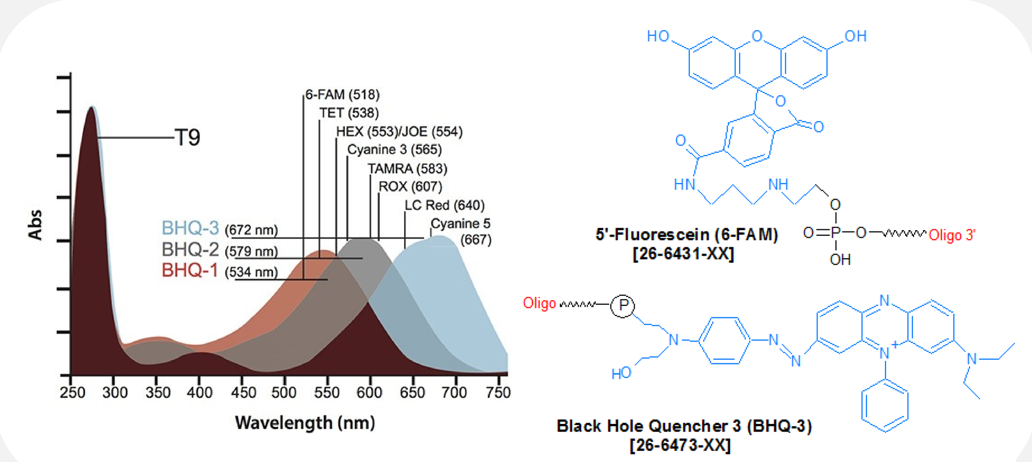
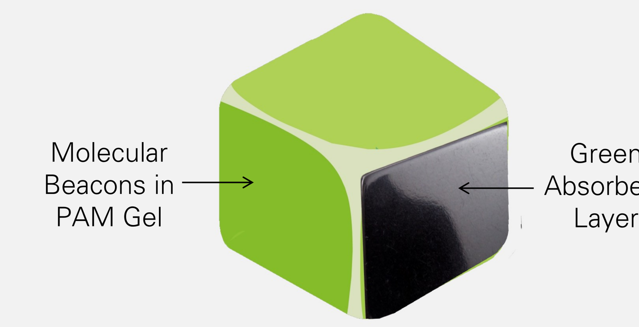


Figure 2. Absorption spectra and structure of BHQ-1 and 6-FAM

Design and Results

Requirements: 1) Exhibit real-time temperature dependent fluorescence.
2) Show thermal and material properties similar to an eye.

Design: The molecular beacons were suspended in a polyacrylamide (PAM) gel to create a vitreous humour-like thermal environment [7] [8]. A piece of green-absorbent material was attached to the back of the gel to simulate a retina.



Materials Validation

1) A fluorescein gel was heated to a variety of temperatures to discern temperature dependent fluorescent behaviour of the fluorophores.

2) A fluorophore gel was exposed to continued UV excitation for two hours to observe the extent of fluorophore degradation.

Prototype Testing

The final prototype was tested using a higher power green laser. Molecular beacons suspended in phosphate buffer solution were heated with this laser to ensure temperature dependent fluorescence of the system. A plot of molecular beacon fluorescence data against laser exposure was made.

Materials Validation Setup

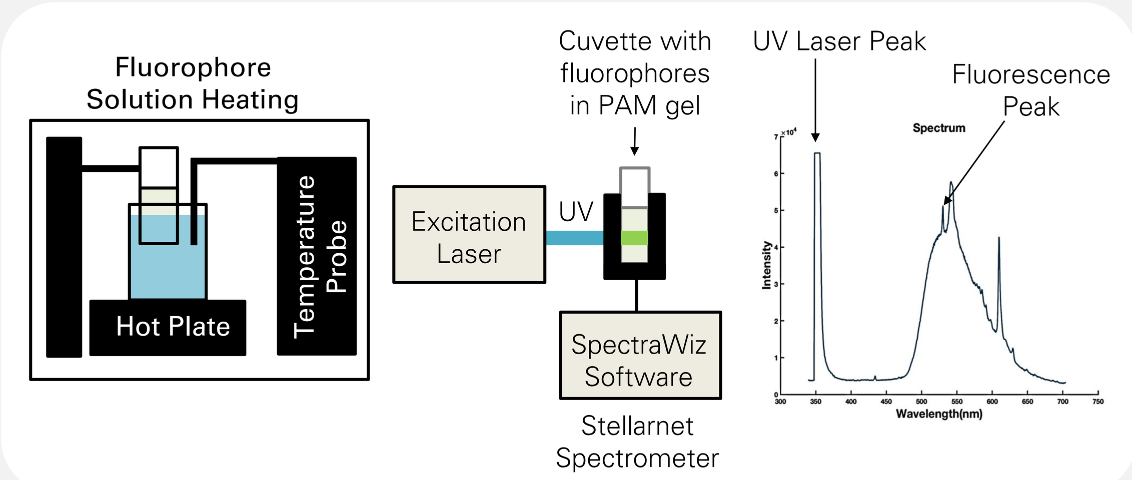


Figure 3. Testing setup used to collect emission spectra data

Prototype Testing Setup

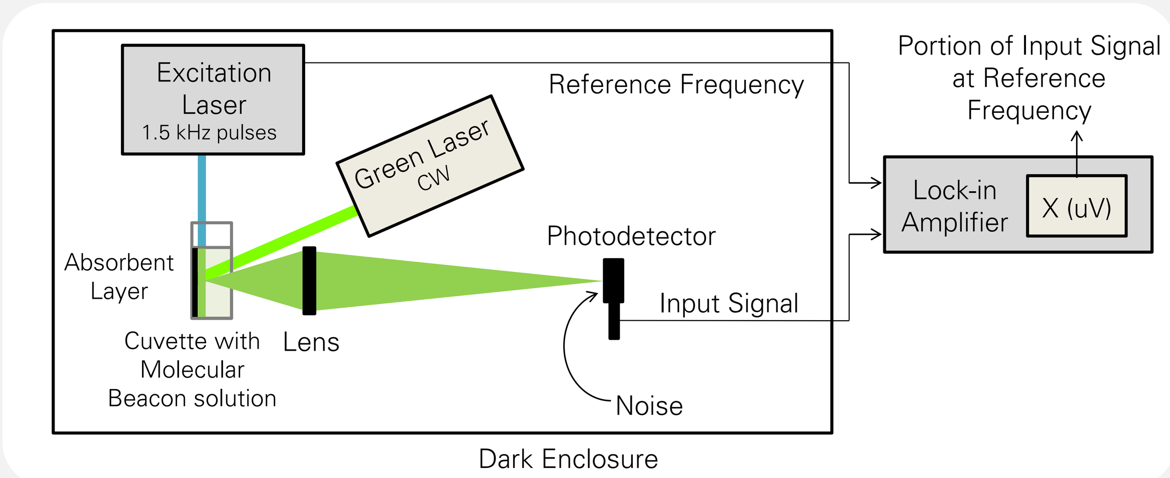


Figure 4. Testing setup used to collect real time fluorescence data

Materials Validation Results

Over 2 hours, fluorescence decreases in intensity by about 25%, suggesting photobleaching via UV excitation, as shown in Figure 5. Additionally, fluorescence is seen to be temperature dependent, with increasing temperatures inducing decreases in fluorescence intensity.

Prototype Testing Results

Exposure to the green laser induces heating, and results in an increase in fluorescence, as shown in Figure 6. The signal qualitatively corresponds to the temperature of the area exposed to the green laser in this proof of concept experiment. Future work will involve quantitatively defining system temperature.

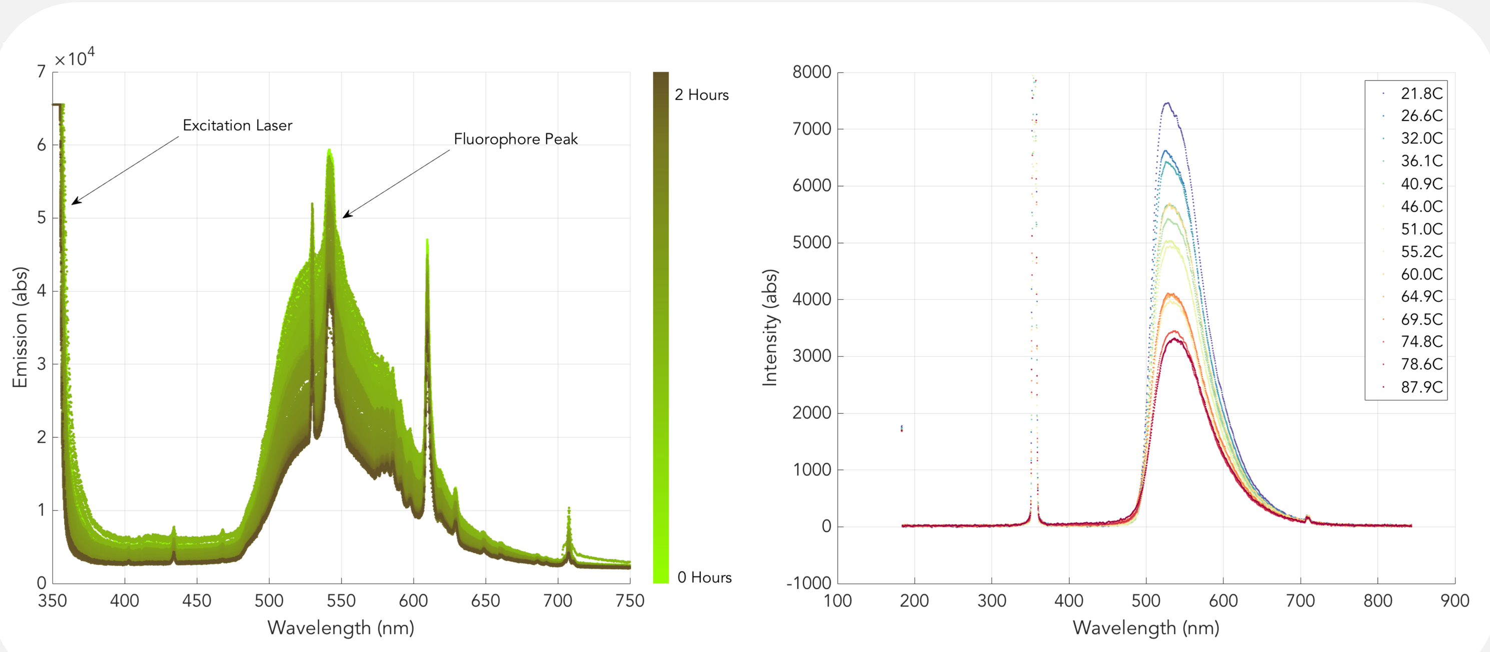


Figure 5. Left: Fluorescein reflectance spectra under continuous UV excitation laser exposure (100 μmol). Right: Fluorescence temperature dependence of 100 μmol fluorescein gel

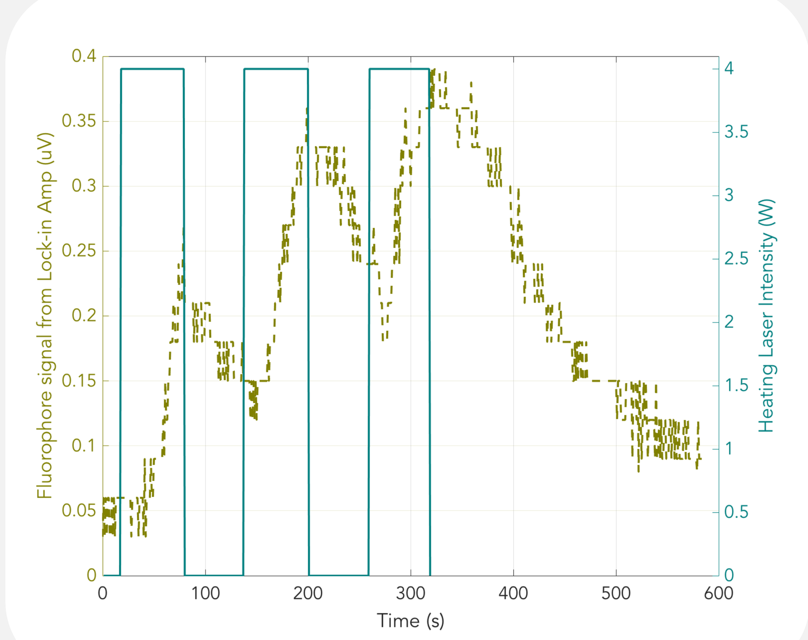
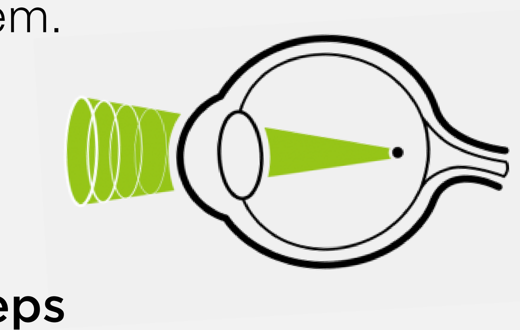


Figure 6. Fluorescence of MB solution heated by exposure to green laser

Conclusions

Overall, this demonstrates that with proper modulation of fluorophore photobleaching and improvement of the heat transfer properties of the suspension media, a molecular beacon system could be used to transduce temperature to a fluorescence signal. However, fluorophores also incurred significant bleaching from UV excitation, imposing a lifetime on the usage of this system.



Next Steps

- Performing laser heating experiments with molecular beacons suspended in PAM gel
- Creating a fluorescence versus temperature calibration curve
- Researching ways to further minimize photobleaching of the molecular beacons
- Optimizing hydrogel polymerization

Acknowledgements

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