PROJECT 5
FLUORESCENCE THERMOMETER

TUHH
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FISHING FOR EXPERIENCE

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Problem Statement

- Traditional methods lack precision for small biological samples, leading to inaccurate temperature data.
- The conventional temperature measurement techniques fail to provide non-evasive, spatially resolved temperature measurements because they cannot be scaled down to the same degree which is required by the sample sizes and amounts of reagents.
- This limitation hinders advancements in cell biology, microfluidics, and nanomedicine.

Why?

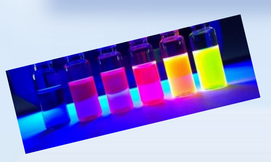
Solution

HOW TO!

Fluorescence-based thermometer

- Provides a robust method for temperature measurement in small biological samples.
- Temperature-dependency of fluorescence intensity: Utilizes specific fluorophores that change fluorescence intensity with temperature.
- Accurate and non-invasive: Delivers precise temperature data without disturbing the sample e.g. if encapsulated in biocompatible, transparent microspheres.
- Spatially resolved: Offers detailed temperature mapping at the microscale level.
- Overcomes limitations: Addresses the shortcomings of traditional measurement techniques.

Fluorophores



Fluorophores are molecules that absorb light at a specific wavelength and re-emit it at a longer wavelength, a process known as fluorescence.

Principles of Fluorescence

- Excitation: A fluorophore absorbs light energy, causing electrons to move from the ground state to an excited state.
- Vibrational relaxation: The excited electrons quickly lose some energy through non-radiative processes, settling into the lowest vibrational level of the excited state.
- Emission: As the electrons return to the ground state, they emit light (fluorescence) at a longer wavelength than the absorbed light due to the energy loss during vibrational relaxation.

Parameters

1. Excitation and Emission Spectra:

- The excitation spectrum of a fluorescent dye is the range of wavelengths it can absorb.
- The emission spectrum is the range of wavelengths emitted by the dye after absorbing light, with a specific emission maximum where the emission is most intense.

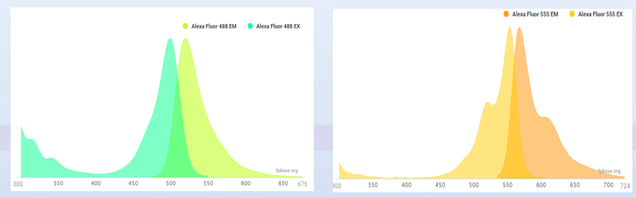
2. Temperature Sensitivity:

- Dyes used in fluorescence thermometry must exhibit predictable and reproducible changes in their fluorescence characteristics with temperature.

3. Thermal Degradation:

- Thermal degradation refers to the breakdown or loss of fluorescent properties of dyes due to exposure to elevated temperatures.

Selection of Dyes



- Both Alexa Fluor 555 and Alexa Fluor 488 are known for their exceptional thermal stability.
- They maintain their fluorescence properties over a wide temperature range, ensuring consistent and reliable temperature readings without significant degradation.
- Alexa Fluor 555 and Alexa Fluor 488 show different temperature dependencies: Positive for Alexa Fluor 555 and constant for Alexa Fluor 488.
- These dyes have well-defined excitation and emission spectra with minimal overlap.

Setup

1. Preparation of the fluorescent probe:

- Dissolve or disperse the fluorescent probe in a suitable solvent or embed it in a solid matrix if required. Ensure the probe is well-mixed and stable.

2. Calibration:

- Calibrate the fluorescence response of the probe as a function of temperature. This involves measuring the fluorescence intensity or lifetime at known temperatures to create a calibration curve.

3. Sample placement:

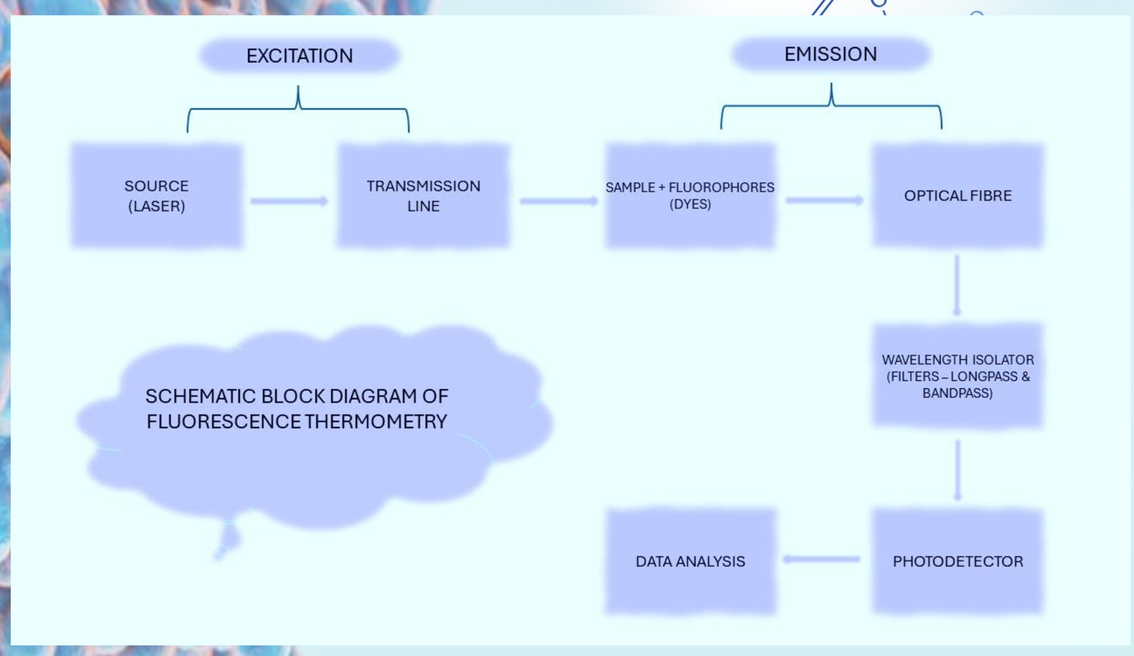
- Place the sample containing the fluorescent probe in the sample holder. Ensure that the sample is properly aligned with the excitation and detection optics.

4. Excitation:

- Illuminate the sample with the excitation source. Adjust the intensity and focus of the light to optimize the fluorescence signal without causing photobleaching.

Things to consider when designing the detection setup:

- Even if the sample itself does not show fluorescence the setup needs an optical detection system anyways. But there are more degrees of freedom in designing this detection system.
- If the sample does show fluorescence, one must make sure to either separate the light from the sample from the thermometer light (= different wavelength ranges) or must use at least two identical vessels next to each other (one for the sample, one for the "thermometer dyes") with no optical interference.



The concept of the setup is a temperature sensor or heating element which can detect changes in temperature and indicating the temperatures.