Portable & Inexpensive Fluorescence Imaging for Traumatic Brain Injury Diagnosis

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Background & Objectives

- Traumatic brain injury (TBI) occurs when sudden trauma to the head or body causes brain damage.
- Currently, TBI is diagnosed
  - Qualitatively via the Glasgow Coma Scale and general injury information
  - Quantitatively by imaging tests such as CT scans or an MRI
- Unfortunately, the quantitative methods for diagnosing TBI are **time-intensive** and **immobile**, while the qualitative methods have frequent disagreements between on-site physicians and expert reviewers.
- The Kwon Lab has laid the groundwork for an alternative approach based upon the detection of increased enzymatic activity of Calpain-1 (CAPN1) and Matrix Metallopeptidase 9 (MMP9), which are responsible for TBI-specific responses in the brain.

We are developing a novel quantitative technique for diagnosing TBI by measuring the activity of TBI-associated biomarkers: CAPN1 and MMP9. This approach aspires to result in indisputable diagnoses of TBI while addressing the deficiencies of current quantitative diagnostic procedures in an **intuitive** and **affordable** manner.

Project Overview

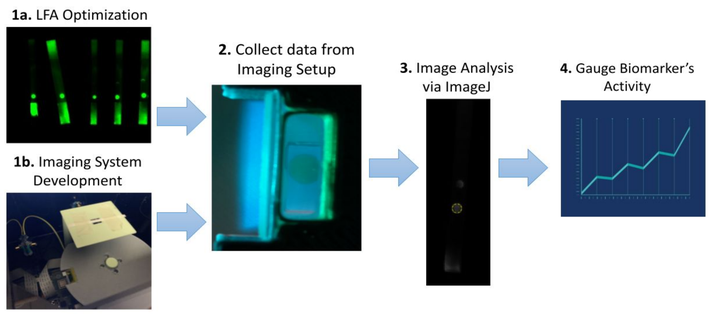


Figure 1: Project Scope

1a. A fluorescent lateral flow assay (LFA) is used to isolate analytes generated by the biomarkers from a blood or urine sample and tag them with their associated fluorophore.

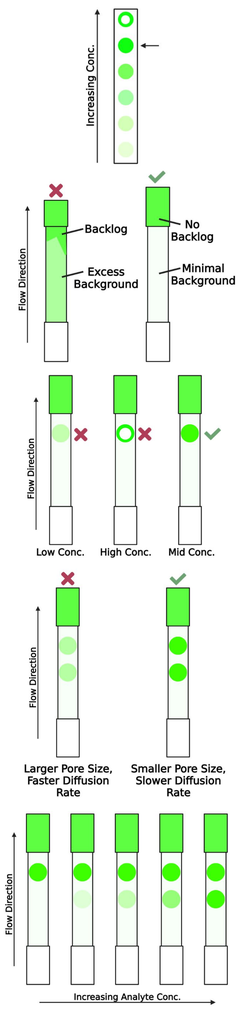
1b. The development of an imaging system from dichroic filters, LEDs, camera, Raspberry Pi 4, and 3D-printed parts.

2. The imaging system captures a snapshot of the emission of an excited fluorophore.

3. Image analysis via ImageJ can yield the fluorophore emission intensity.

4. A model relating fluorophore emission intensity to analyte concentration is used to gauge the biomarkers' activity in the patient.

LFA Development



I. Reporter Molecule Characterization
Find dynamic range of reporter molecule to determine optimal concentration used in conjugate pad for maximum signal without oversaturation. The ideal concentration for our reporter molecule was 30 µg/mL.

II. Flow Optimization
Ensure absorbent pad is large enough to absorb entire sample to avoid backlog of reagents and excess background. A length of 8mm was used to eliminate backlog and minimize background signal.

III. Control Zone
Determine the optimal concentration of BSA-Biotin used in control zone for strong and consistent signal without oversaturation. A concentration of 50 µg/mL consistently had strong signal in the control zone without excess binding.

IV. Binding Time
Determine the optimal pore size and length of nitrocellulose membrane to allow for adequate binding and capture of reagents. Our assay uses a membrane with a slower capillary flow rate of 105-150s/3cm to improve signal quality.

V. Assay Characterization
Assemble LFA using finalized dimensions and concentrations of components and determine the limit of detection and dynamic range of the assay. Our assay was able to measure concentrations from 20-100 nM.

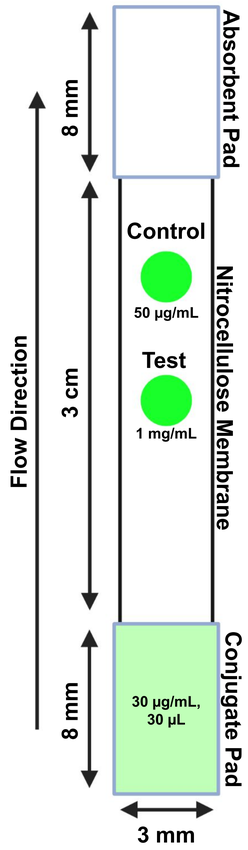


Figure 2: Finalized LFA

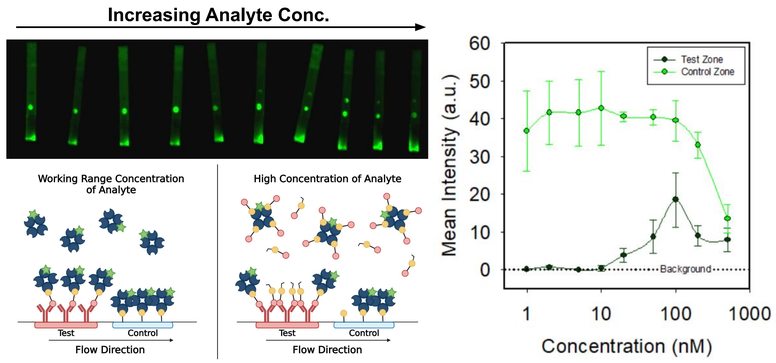
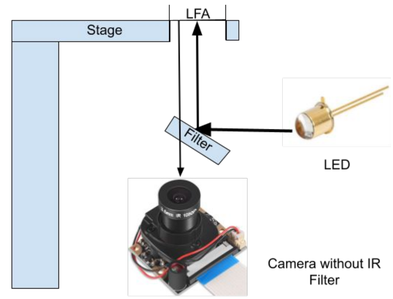


Figure 3: Fluorescent images of LFAs and data plot of fluorescent signal as a function of analyte concentration with mechanism illustration

Imaging Setup Optimization



Components	Price
Raspberry PI	$97
Dichroic Filter (495 nm)	$165
LED (490 nm)	$12.24
Camera	$16.99
3D Printed Parts	$0
Acrylic Sheets for Box	$46.20
	Total: $337.43

Figure 4: Imaging Setup Schematic

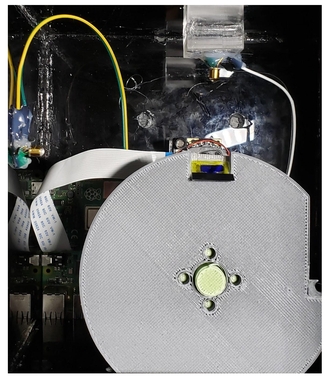


Figure 5: Imaging Setup without Stage

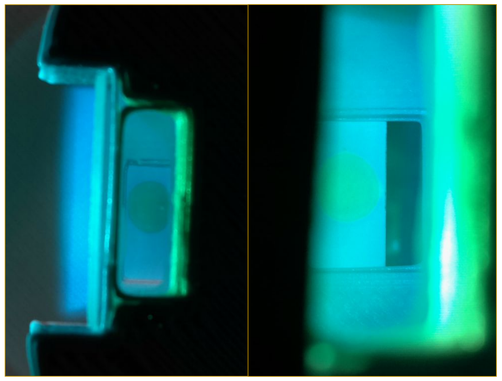


Figure 6: Before & After Moving the Camera and LED closer to LFA

Conclusions & Future Directions

This project has resulted in a solid foundation for a portable imaging setup that works seamlessly with a LFA for TBI diagnosis. The next major step would be to determine the limit of detection and dynamic range of the portable imaging system with the LFA. Progress can also be made by increasing the lateral flow assay's dynamic range and limit of detection; or automating the image capture and analysis pipeline to streamline the data acquisition workflow. Lastly, future groups should multiplex the imaging device and LFA for other target analytes using additional reporter molecules or additional filters for multiple wavelengths.

Acknowledgements & References

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