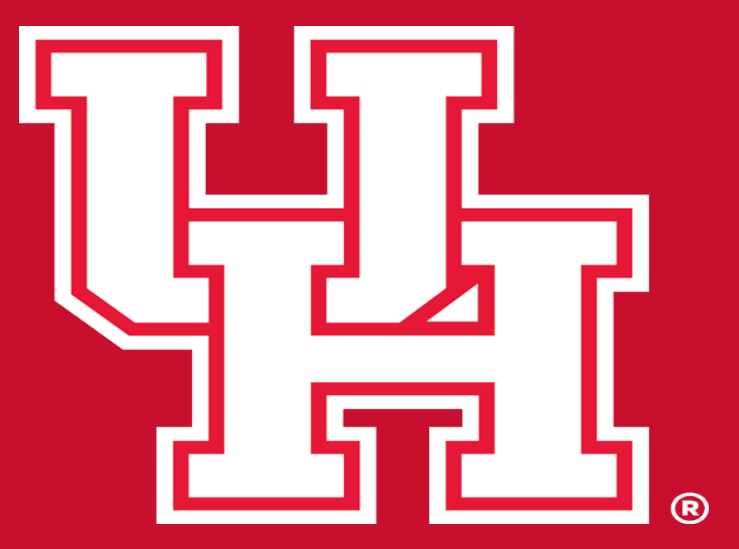




Point of Care Enzyme-Based Assays For Lupus Nephritis



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Background

Lupus Nephritis (LN) is a life-threatening kidney complication of systemic lupus erythematosus, affecting 50% of adults and up to 80% of children with lupus. Current diagnostic methods are invasive or non-specific. Enzymes are dynamic biomarkers that reflect ongoing inflammation, tissue remodeling, and immune responses in LN. Serine proteases, like Cathepsin S (CTSS), and matrix metalloproteinases, like MMP-13, are highly active in LN, making them excellent targets for real-time detection via enzyme-substrate assays.

Objective: We aim to develop two point-of-care diagnostic assays for lupus nephritis: a fluorogenic rapid test targeting CTSS, and an at-home chromogenic assay targeting MMP-13.

Methods

MMP-13 Chromogenic Dipstick Assay

1. This dipstick detects MMP-13 levels from 0–140 mU and protein levels from 0–10 g/L.
2. Protein absorbent pads were soaked overnight in 2 mM MMP-13 substrate and a bromophenol blue substrate, then dried.
3. The dipstick was assembled (Figure 1), and synthetic urine spiked with varying MMP-13 and Albumin concentrations was applied.
4. After brief dipping and incubation in the dark, the resulting color change was compared to a reference chart.

CTSS Fluorogenic Smartphone-Based Assay

1. This assay detects Cathepsin S (CTSS) levels from 0–1000 pg/mL.
2. The OmniCathepsin substrate was diluted to 1 mM in assay buffer, then enzyme was added and incubated at 37 °C for 1.5 hours.
3. Fluorescence was measured every 15 minutes using a TECAN Infinite M-plex reader and a 3D printed Light-Isolating Chamber

Results

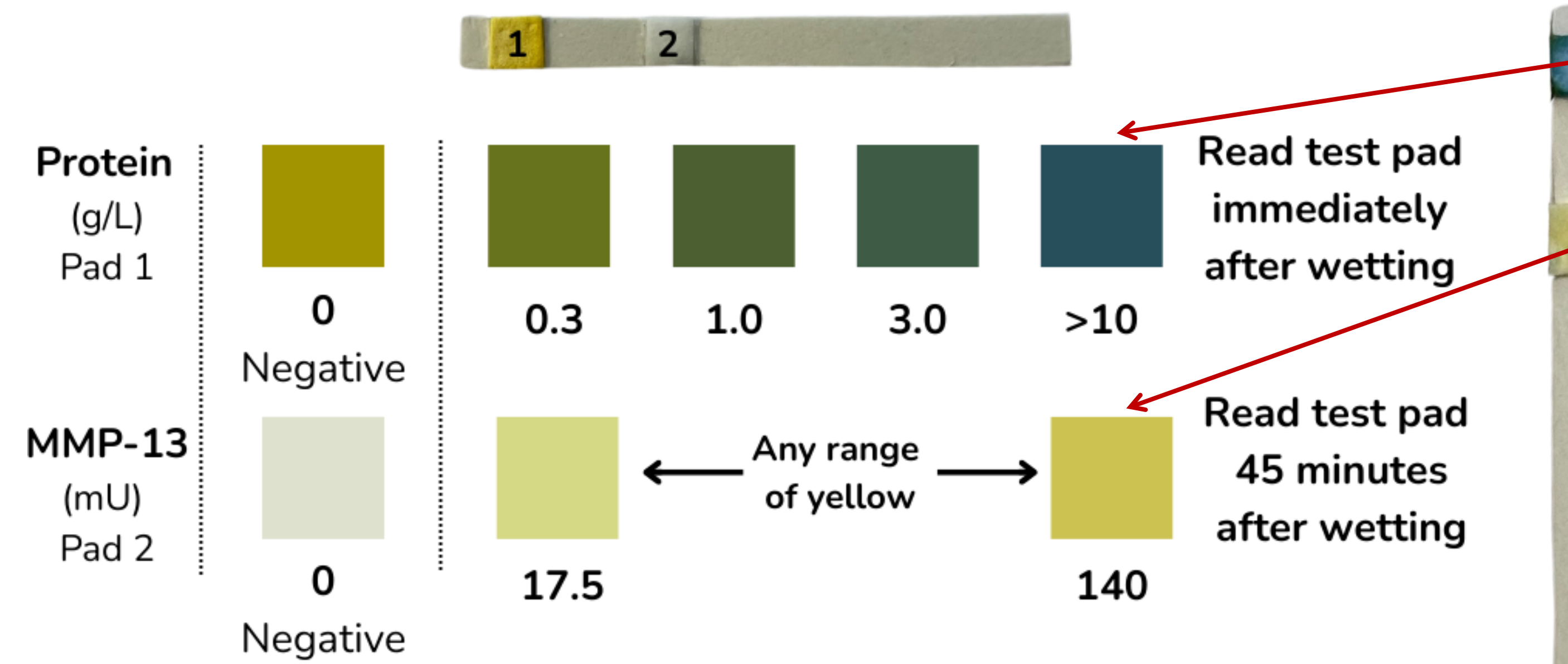


Figure 1. Reference-Guided Visual Readout of MMP-13 and Albumin Levels

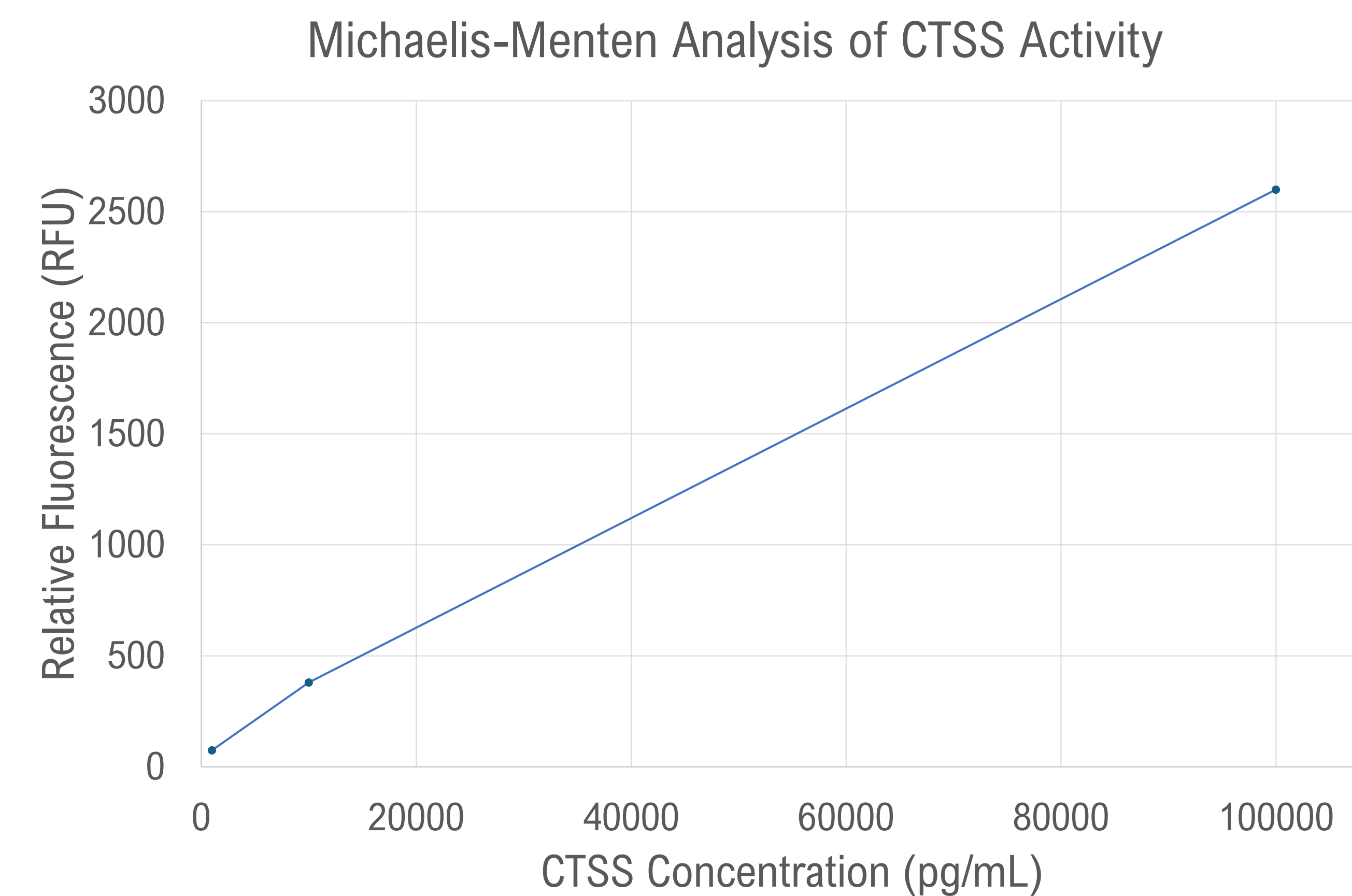


Figure 2. The rate of the CTSS-catalyzed reaction was measured across a range of CTSS concentrations to evaluate enzyme kinetics.

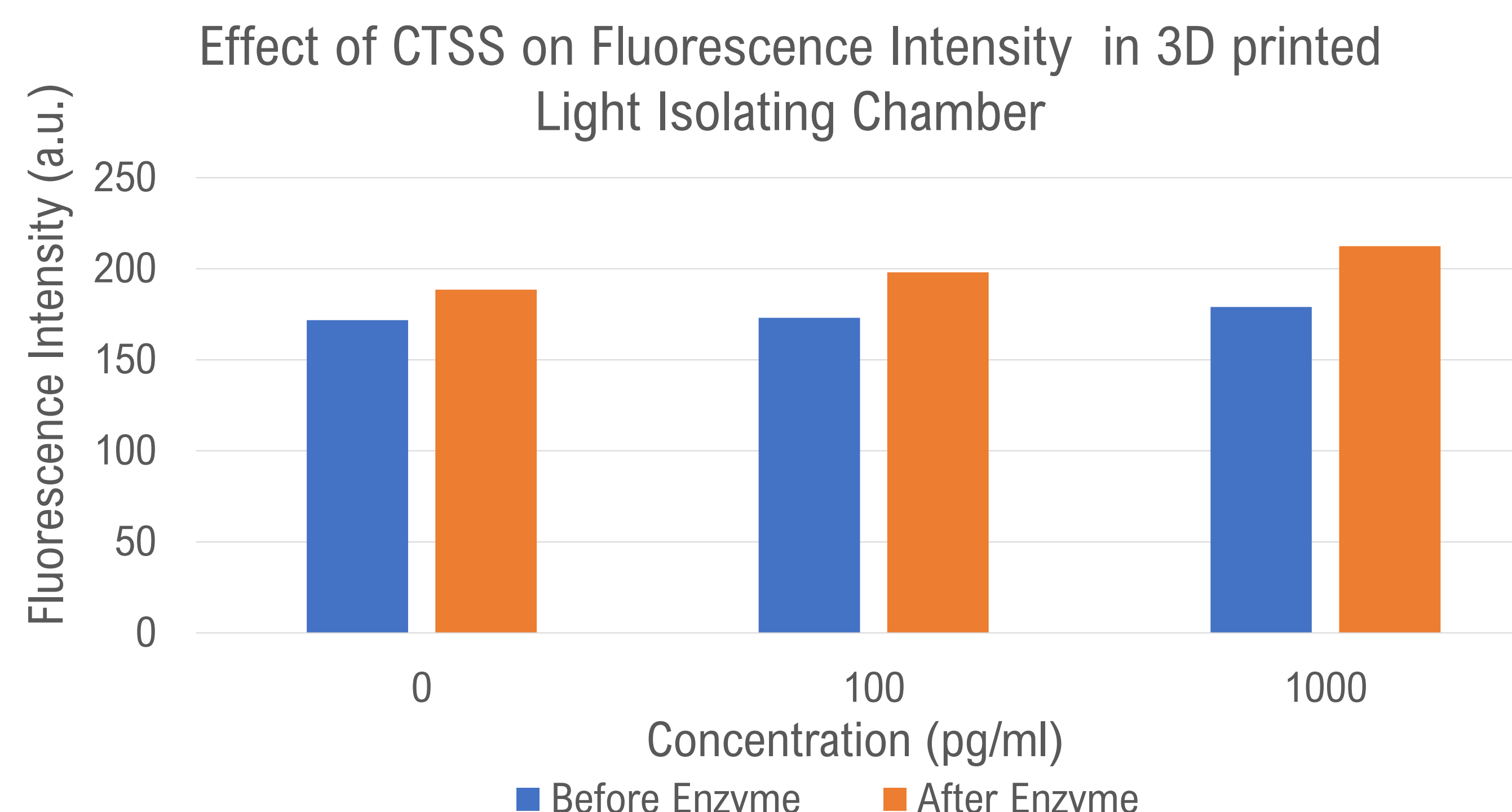


Figure 3. Differences in fluorescence obtained from ImageJ by varying enzyme concentrations.

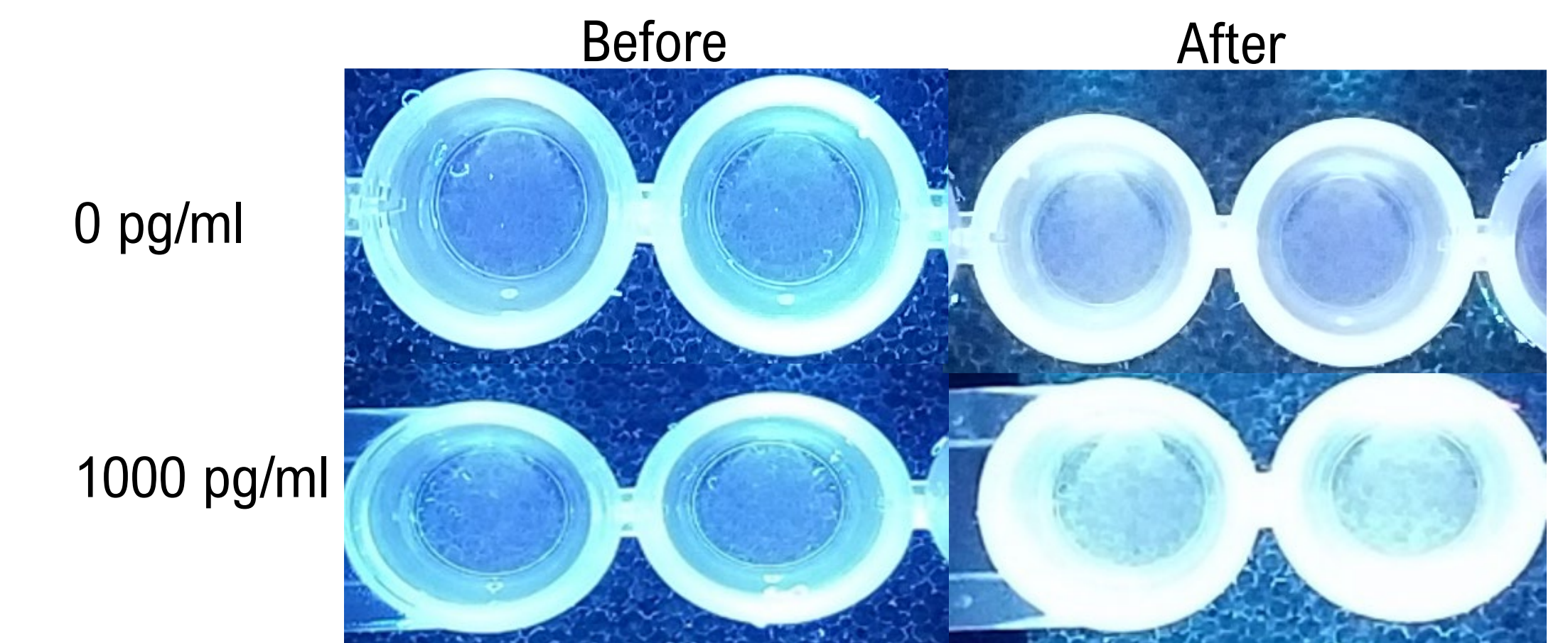


Figure 4. Images obtained from ImageJ showing the fluorescence of the 0 pg/ml (top) 1000 pg/ml (before) adding the enzyme (left) and after (right).

Conclusion

This study presents a strong foundation for a portable point-of-care diagnostic system. The chromogenic dipstick provides both MMP-13 and Albumin presence in an accurate, user-friendly manner. Fluorescence signals captured using our prototype showed high agreement with TECAN X fluorometer data, validated through ImageJ analysis. Additionally, future work should involve testing both assays with lupus nephritis patients. Design improvements, such as an adjustable phone slot, integrated wells for alignment, and expanded support for iOS devices, aim to enhance usability. Additionally, refining the fluorogenic standard curve to improve sensitivity at lower concentrations and integrating a colorimetric app for accurate chromogenic detection.

References

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