Optical Digital Assay for High-Sensitivity Quantification of Protein Protofibril Disassembly

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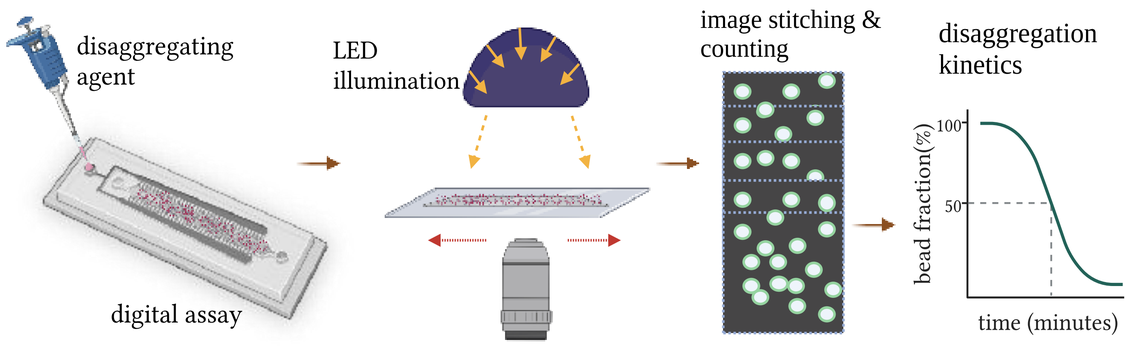
Introduction

We present a latex bead-based digital molecular assay for sensitive monitoring of small molecule treatment-induced disaggregation of surface-tethered early-stage $A\beta_{42}$ aggregates. The assay provides a digital readout of aggregate detachment kinetics, enabling quantitative detection of early disaggregation events that are difficult to resolve using conventional ensemble-averaged assays.

Schematic of the Latex Bead Assay



Methodology

- Early-stage $A\beta_{42}$ aggregates were covalently immobilized onto latex beads.
- Aggregates were treated with the small molecules EPPS, HEPES, and PIPES.
- Dark-field mosaicking was used to generate large-field-of-view datasets for single-bead level digital bead counting.
- Bead-retention kinetics were determined by measuring the time-dependent decrease in retained bead counts using digital bead counting.
- Unbinding times and spatiotemporal dynamics of aggregate detachment were characterized.

Results - Digital Bead Assay

Representative dark-field images and progressive bead loss

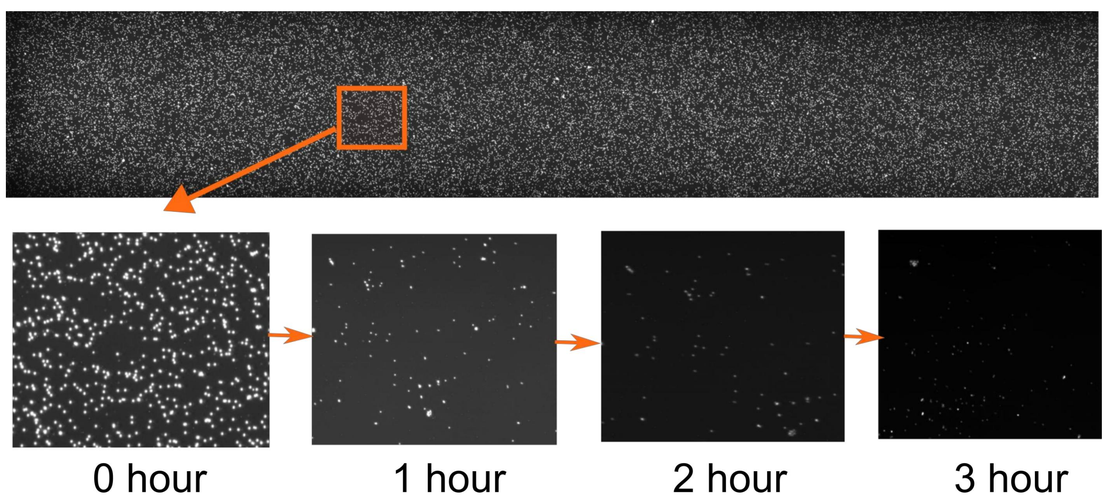
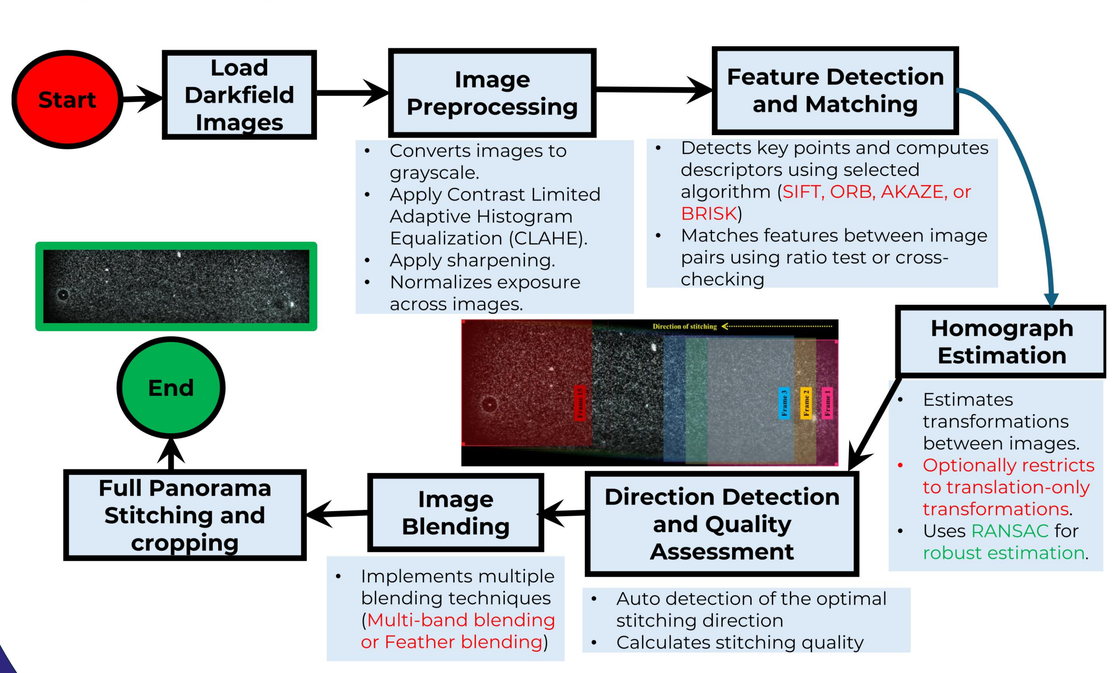


Image stitching workflow

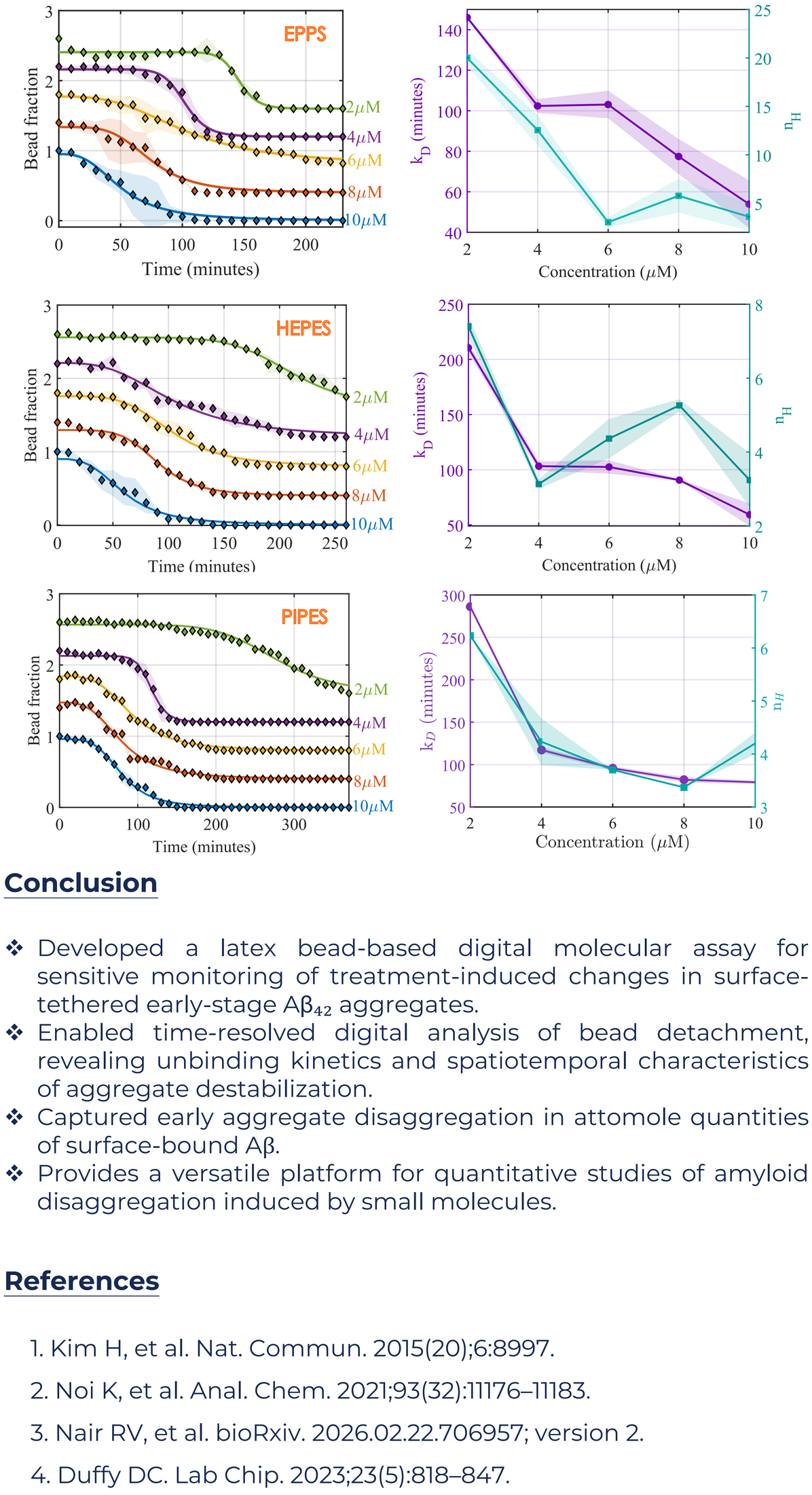


Results - Bead Detachment Analysis

Hill Model: $$F(t) = F_{\text{final}} + (1 - F_{\text{final}}) \left(\frac{1}{1 + (t/k_d)^{n_H}} \right)$$

Hill fits of temporal bead loss at different disaggregation agent concentrations

Extracted Hill parameters
k_d = half time
n_H = cooperativity



Conclusion

- Developed a latex bead-based digital molecular assay for sensitive monitoring of treatment-induced changes in surface-tethered early-stage $A\beta_{42}$ aggregates.
- Enabled time-resolved digital analysis of bead detachment, revealing unbinding kinetics and spatiotemporal characteristics of aggregate destabilization.
- Captured early aggregate disaggregation in attomole quantities of surface-bound Aβ.
- Provides a versatile platform for quantitative studies of amyloid disaggregation induced by small molecules.

References

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