

SINGLE CELL LEVEL SEQUENTIAL GLYCAN PROFILING ON THE MICROFLUIDIC LAB-IN-A-TRENCH PLATFORM

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AIMS & OBJECTIVES

- Detect surface glycosylation patterns on live cells
- Enabled by:
 - Fluorescently labelled lectins
 - Lab-in-a-Trench (LiaT) microfluidic platform

CELL SURFACE GLYCOSYLATION

- Hypothesis: Changes in cell glycosylation patterns correlated to cell condition
- Cells undergoing apoptosis show increased levels of GlcNAc and Mannose

LAB-IN-A-TRENCH PLATFORM

Technological Concepts

- Gravity
 - Flow
 - Sedimentation
- Advection – diffusion
 - Sequential addition & removal of reagents
- Two layer PDMS chip

Conditions

- Laminarity
- Stopped velocity in the trench

Features

- Highly efficient cell capture (> 95%)
- Shear-free treatment
- On-site detection
- Real-time monitoring

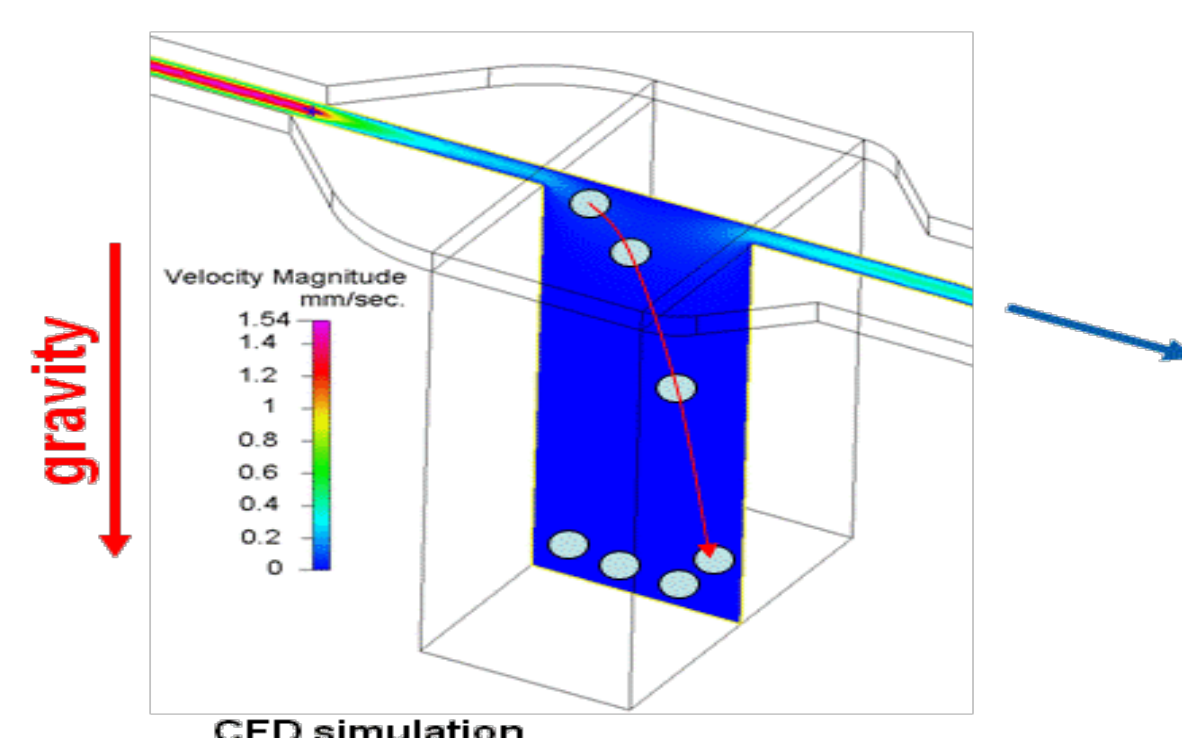


Figure 1: Cell capture based on gravity-driven cell sedimentation to bottom of trench structure.

SEQUENTIAL GLYCAN PROFILING

- Surface glycosylation of Ramos B lymphoma cells
- Probed by a panel of four fluorescent labelled lectins
- Binding categories:
 - Mannose: LCA, NPL and Con A.
 - Galactose: ECL
 - N-acetylglucosamine (GlcNAc): WGA

EXPERIMENTAL SEQUENCES

- **Lectin** → *Elution*
- **LCA** → *Mannose* → **EPL** → *Galactose* (Figs. 2-3)
- **LCA** → *Mannose* → *Buffer Wash* → **Con A** → *Mannose* → *Buffer Wash* → **NPL** → *Mannose* (Fig. 4)
- **LCA** → *Mannose* → **ECL** → *Galactose* → **Con A** → *Mannose* → **WGA** → *GlcNAc* (Fig. 5)

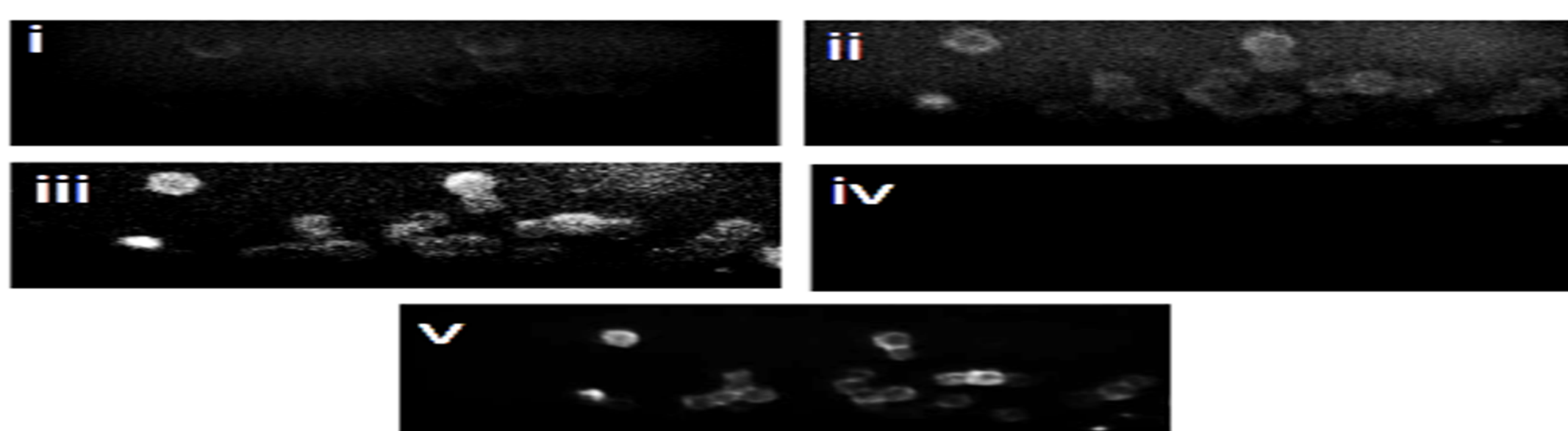


Figure 2: Sequential elution of fluorescent labelled lectin. (i-iii) LCA addition. (iv) Mannose elution. (v) ECL addition.

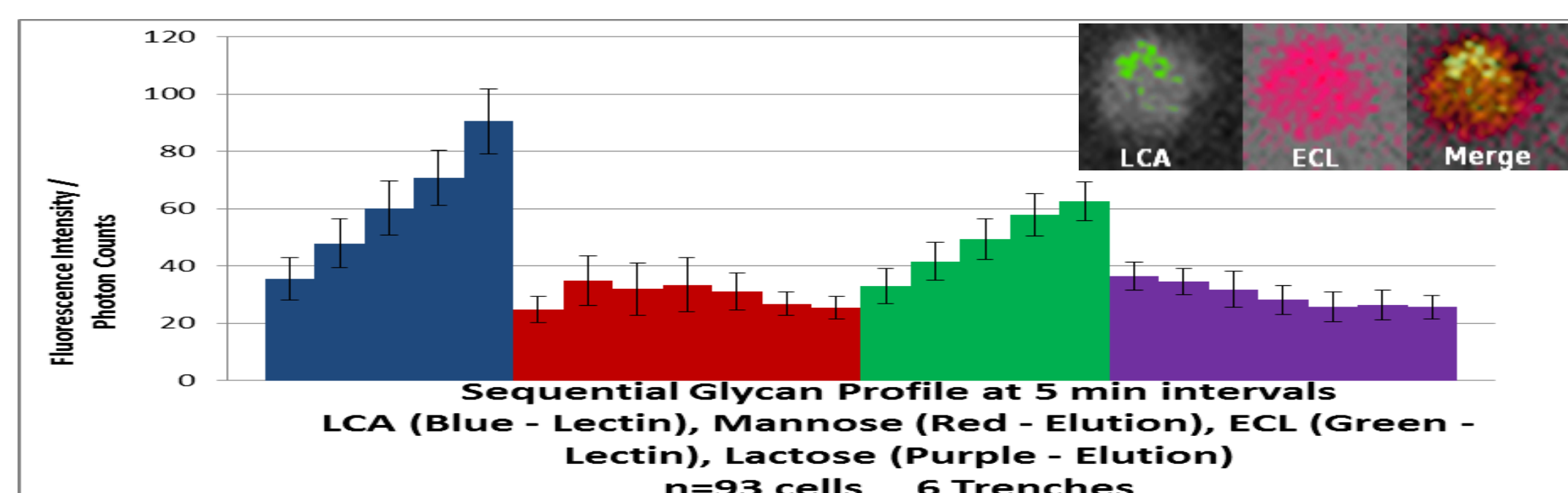


Figure 3: Sequential profile LCA – ECL.

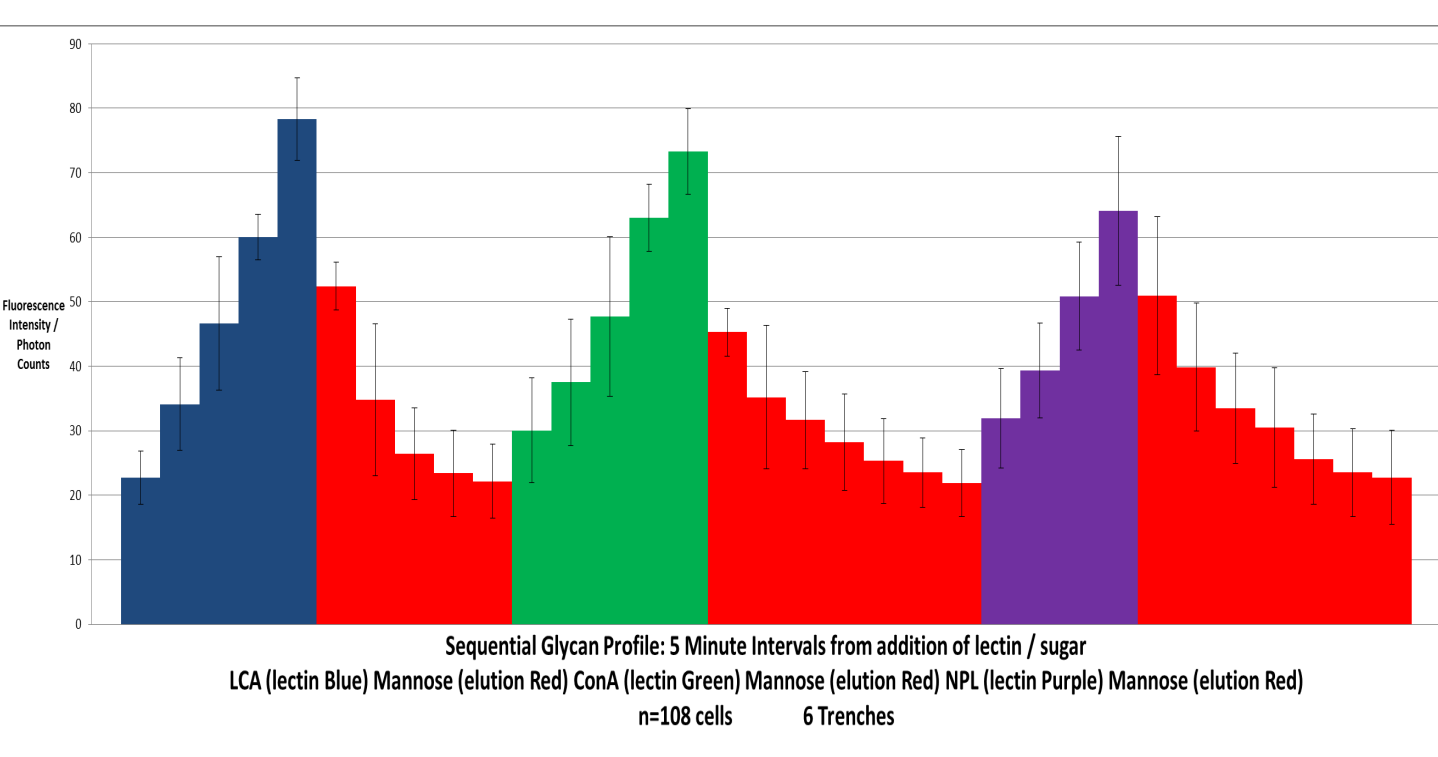


Figure 4: Sequential profile three mannose binders.

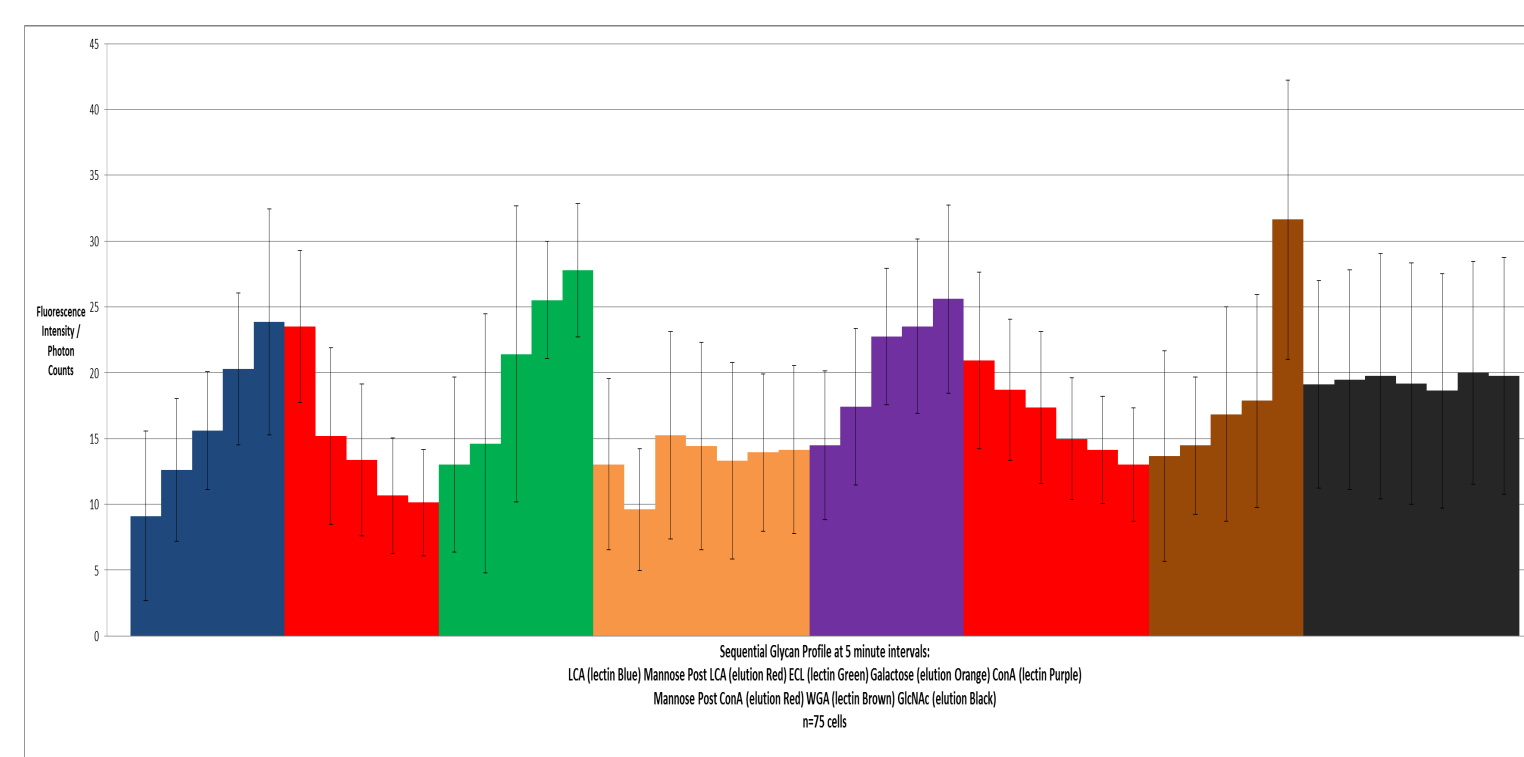


Figure 5: Sequential profile LCA – ECL – Con A - WGA.

LiaT provides

- ✓ High-efficiency capture and pumping driven by gravity
- ✓ Novel method to perform sequential glycan analysis
- ✓ Real-time monitoring of the lectin binding rate
- ✓ Elution with free sugars to confirm lectin specificity
- ✓ Widely shear-free analysis environment
- ✓ Cost efficient manufacture