

**SYNTHESIS AND
EVALUATION OF ANTI-
NONSPECIFIC BINDING
COATING IN
MICROFLUIDIC DEVICES
FOR ELISA BIOASSAYS**

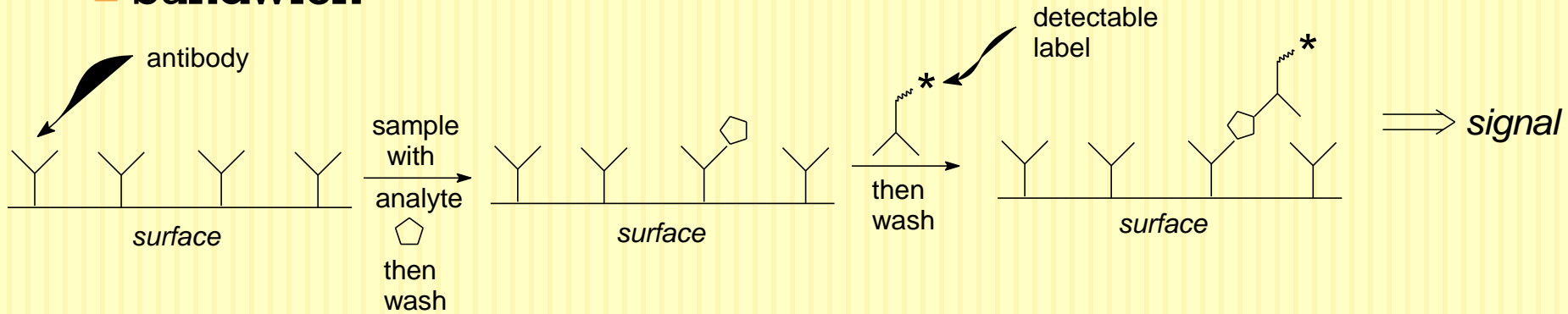
Melissa J. Gelwicks

ELISA- Enzyme-Linked Immunosorbent Assay

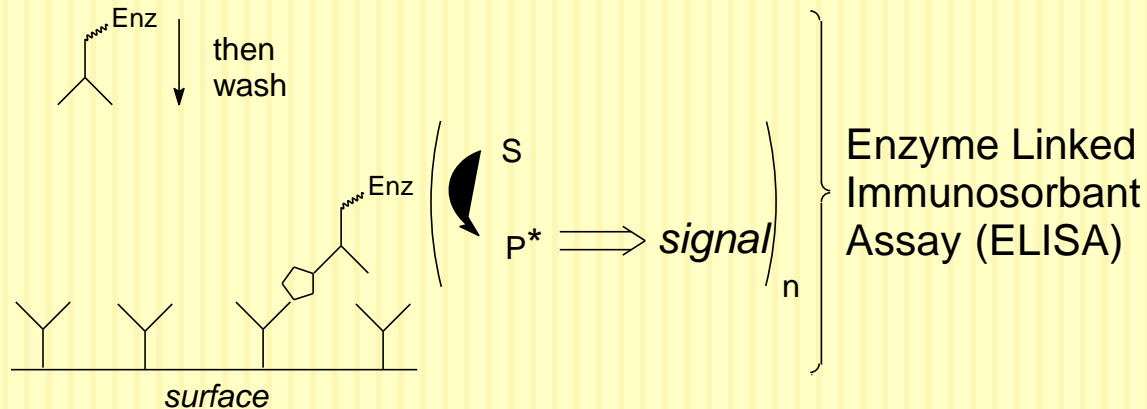
- ELISA is used to determine the presence of and to quantify biologically important compounds.
- Utilizes antibody coatings on surfaces to bind specific antigens, which are then complexed to enzyme-antibody conjugates, followed by a substrate that is converted by the enzyme to a detectable product.
- Amount of target biomolecule may be inferred from intensity of the signal.

Common Immunoassays

■ Sandwich

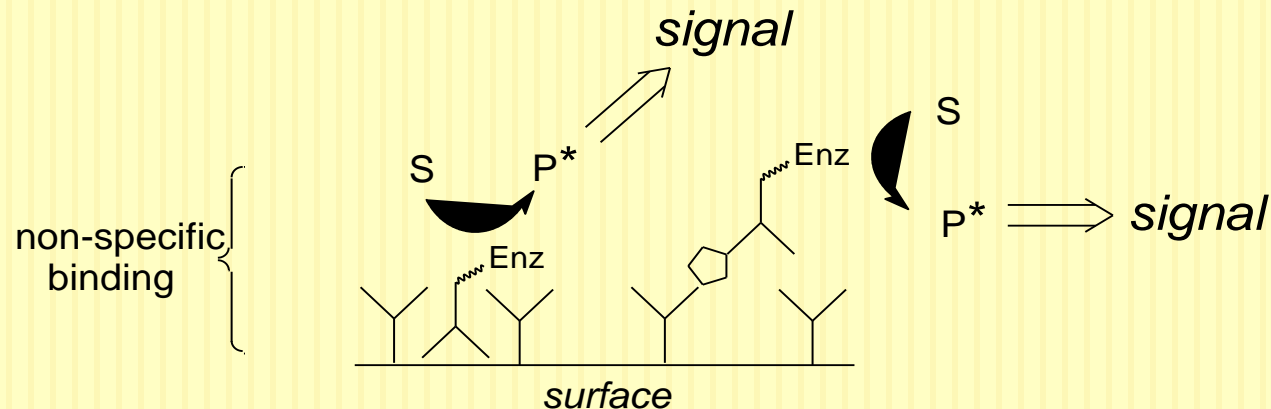


■ ELISA



Non-specific Binding (NSB)

- The sensitivity and reliability of these assays may be compromised by the phenomenon of non-specific binding.
- This is when the enzyme-antibody conjugate binds not only to the target antigen but also to other species on the surface.



- Signals from NSB are indistinguishable from those produced by the antigen-antibody complex, resulting in a signal that does not accurately reflect the presence or quantity of the target biomolecule.

Microfluidic Applications of ELISA

- Desirable to shrink the scale of bioassays-
 - Greater sensitivity
 - Smaller sample size required
 - Less reagent
 - Faster
- NSB issues have been addressed to a significant extent on a macroscale, but not in microfluidic devices.

Spring 2010 Research

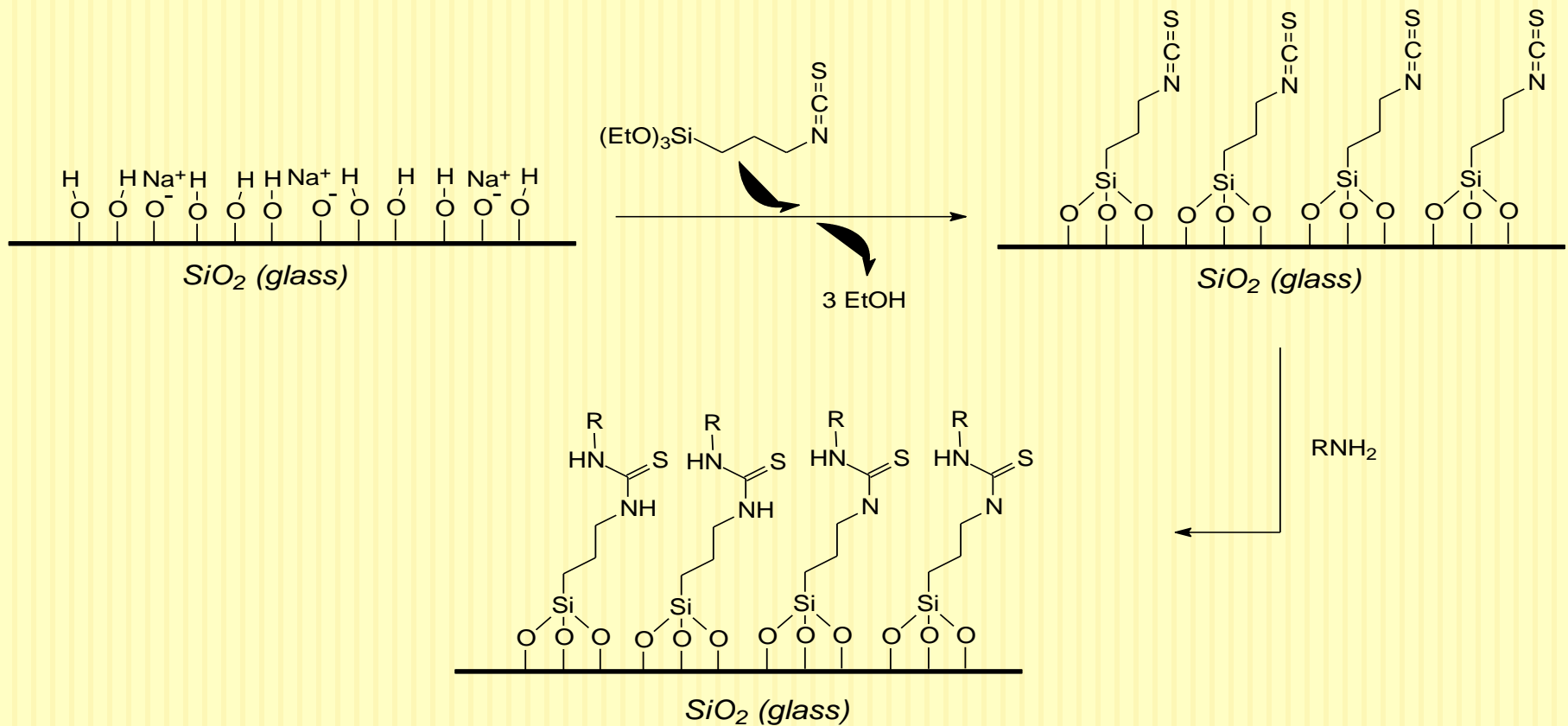
- Microchip fabrication
- Synthesis of coatings designed to inhibit NSB in microchannels
- Evaluation of coatings

Microchip Fabrication

- Complex, and yet fascinating process
- Isotropic etching of glass to create channels that are covered with a “ceiling” in order to create channels in which a variety of chemical processes may be carried out
- Straight line channels, etched to a depth of $\sim 30\mu\text{m}$

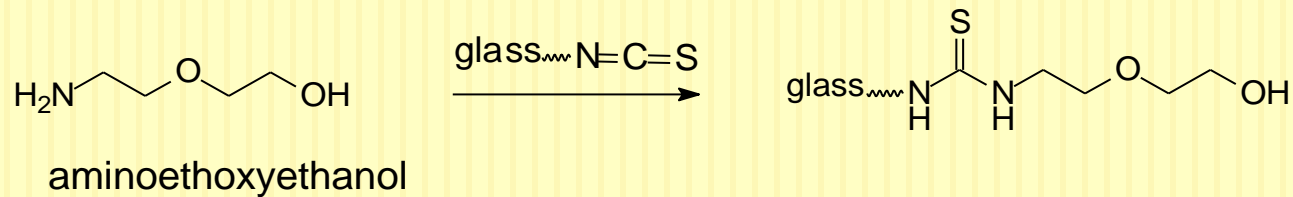
NSB Coatings

- Covalently bonded coatings
- Surface coating procedure

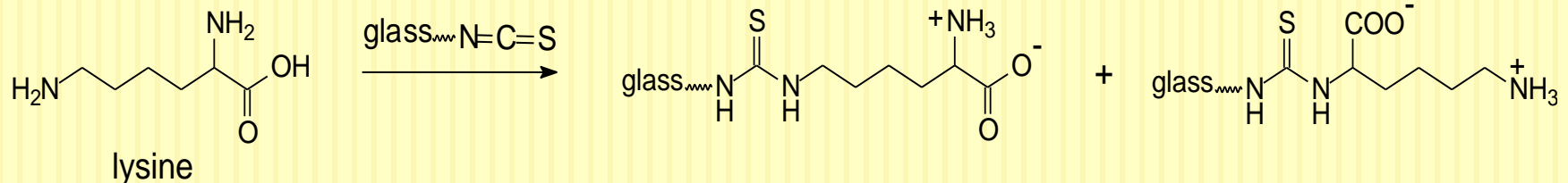


Coatings

- 1) aminoethoxyethanol- a simple, commercially available chemical

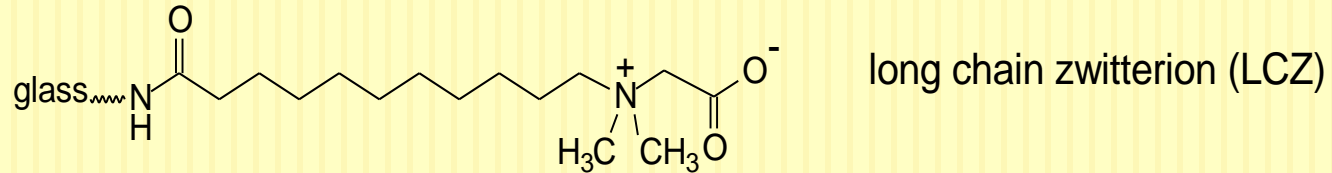


- 2) lysine- short chain zwitterion, which is resistant to NSB



Coatings

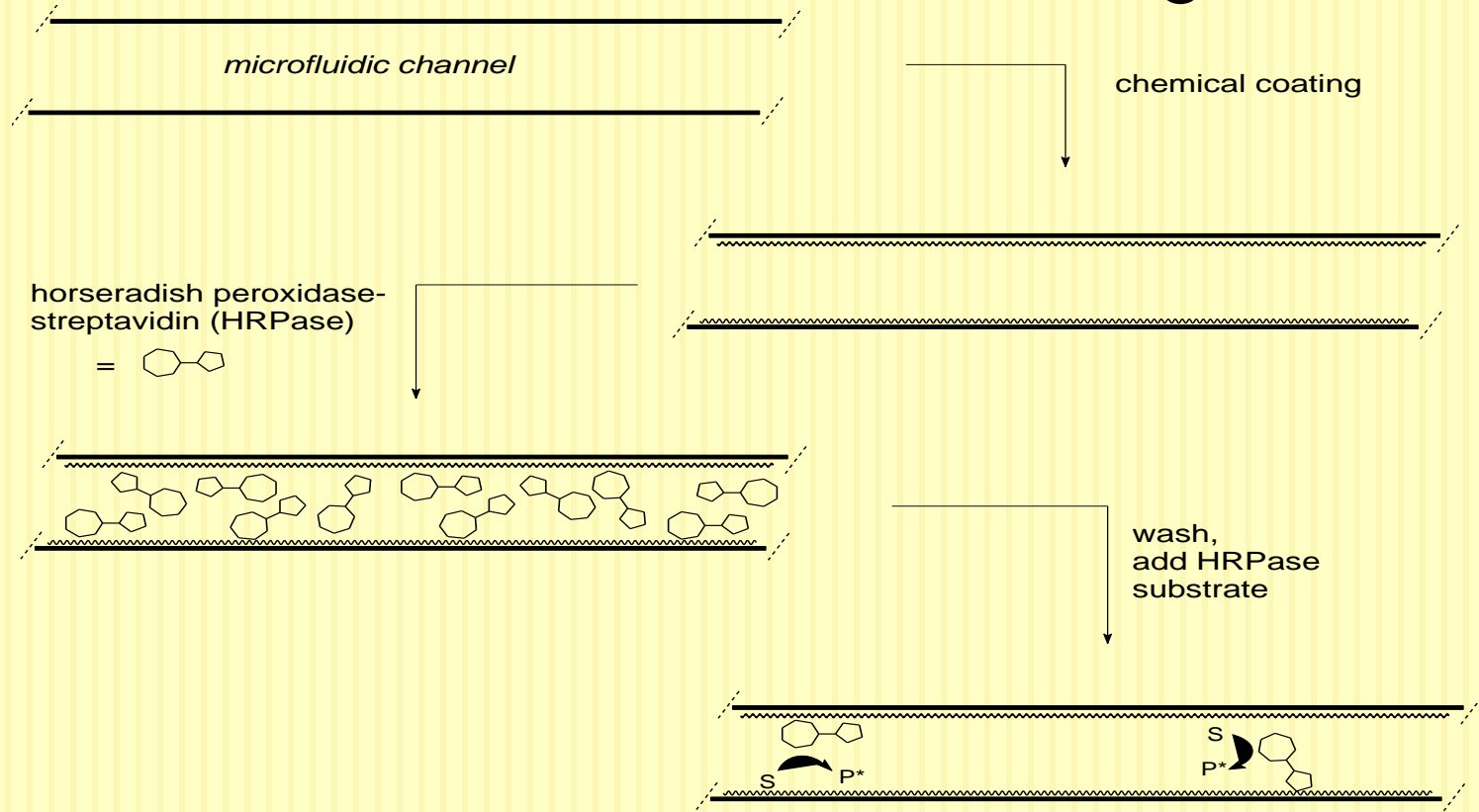
- 3) Long chain zwitterion- with a controlled distance between charges and large offset from surface.



- 4) Glass control- antibody-enzyme conjugate allowed to come into contact with glass surface
- 5) Optical control- no coating, just fluorescent substrate
- Coatings evaluated by performing marker → signal reaction in all channels

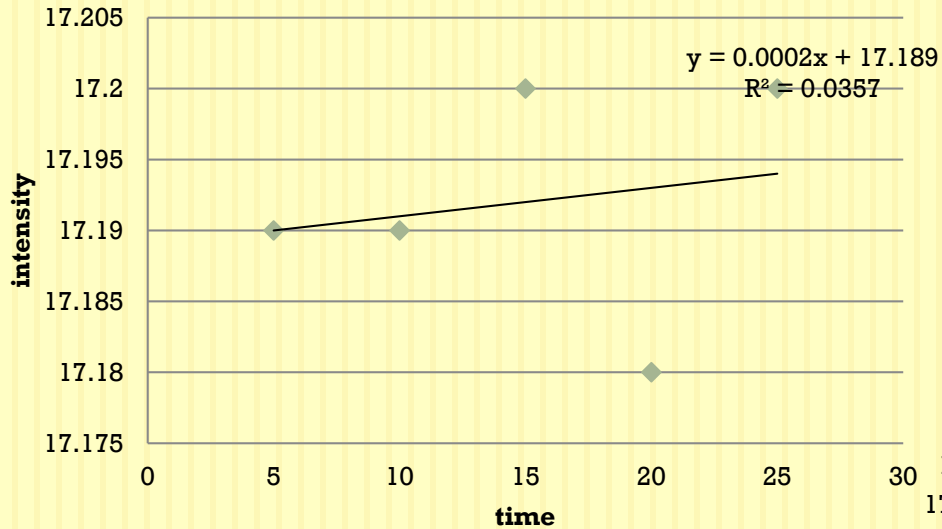
Evaluation of Coatings

- Microchip coatings evaluated with the use of a protein-enzyme conjugate in order to evaluate whether NSB is occurring

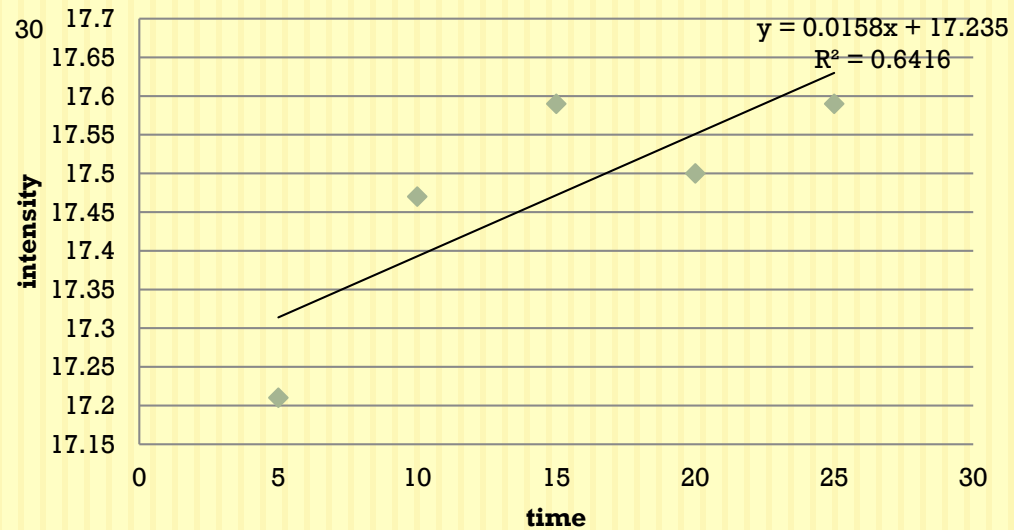


Results

Optical control

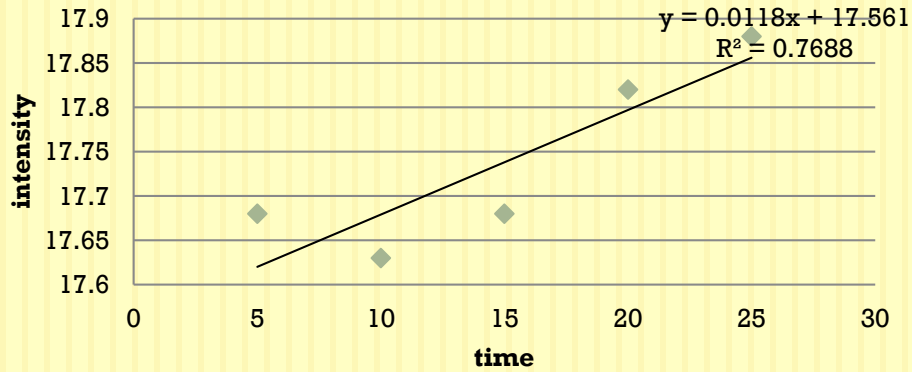


Glass control

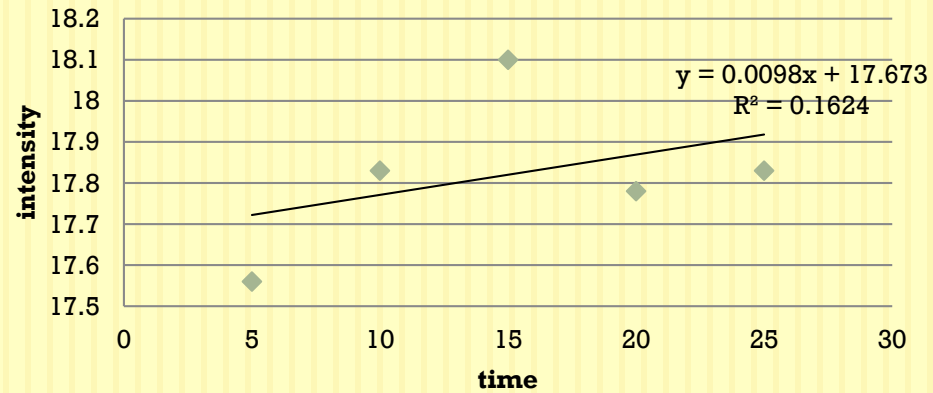


Results

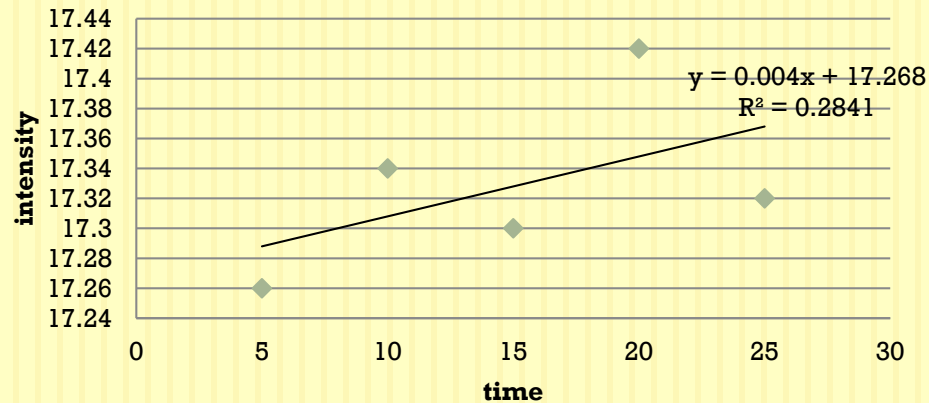
Lysine



Aminoethoxyethanol



Long-chain Zwitterion

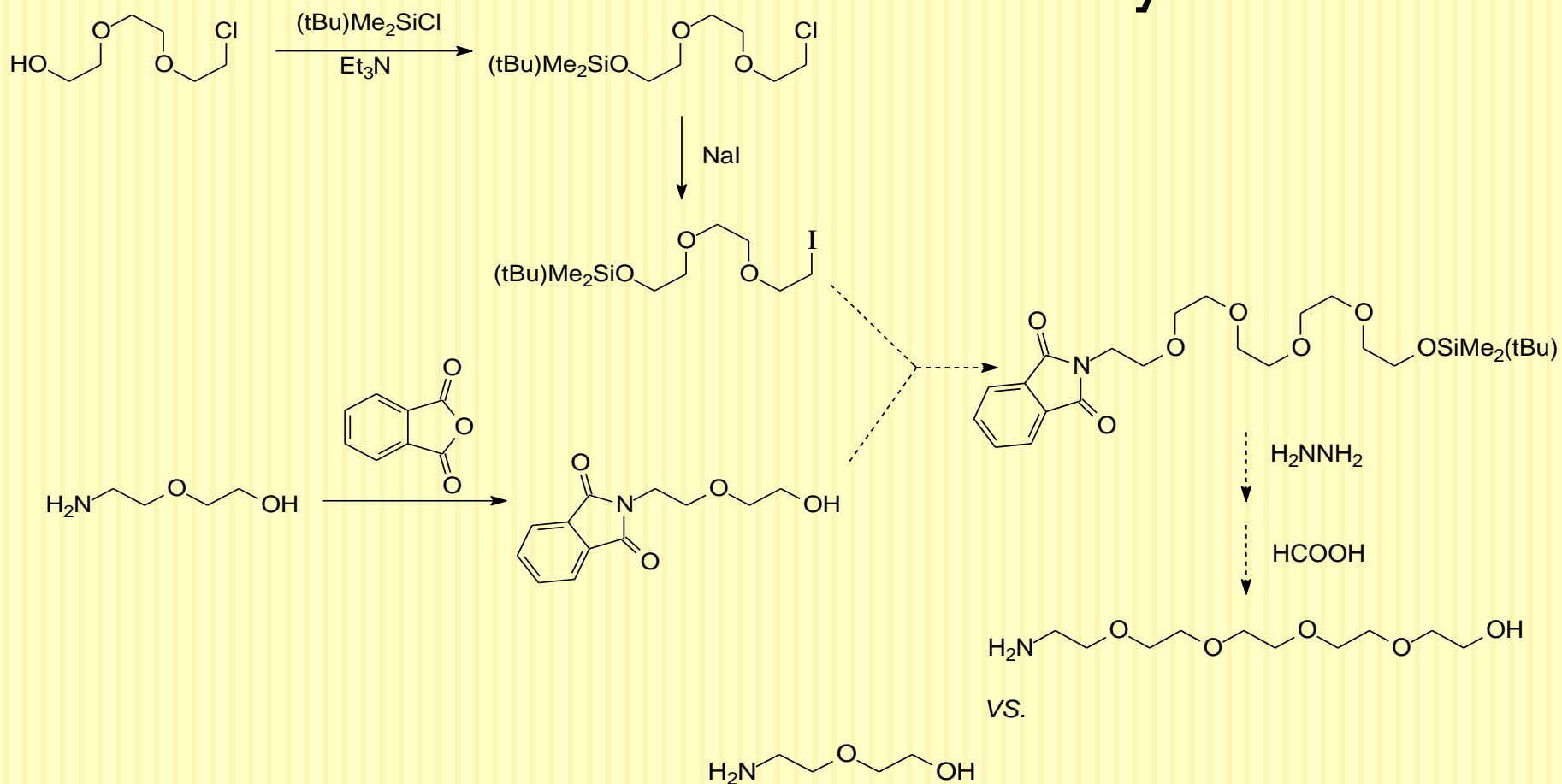


Conclusions

- The long-chain zwitterion provided the best results, with the least amount of NSB.
- Aminoethoxyethanol, a simple, off-the-shelf molecule was the surprise here, with the next least amount of NSB.

Future Research

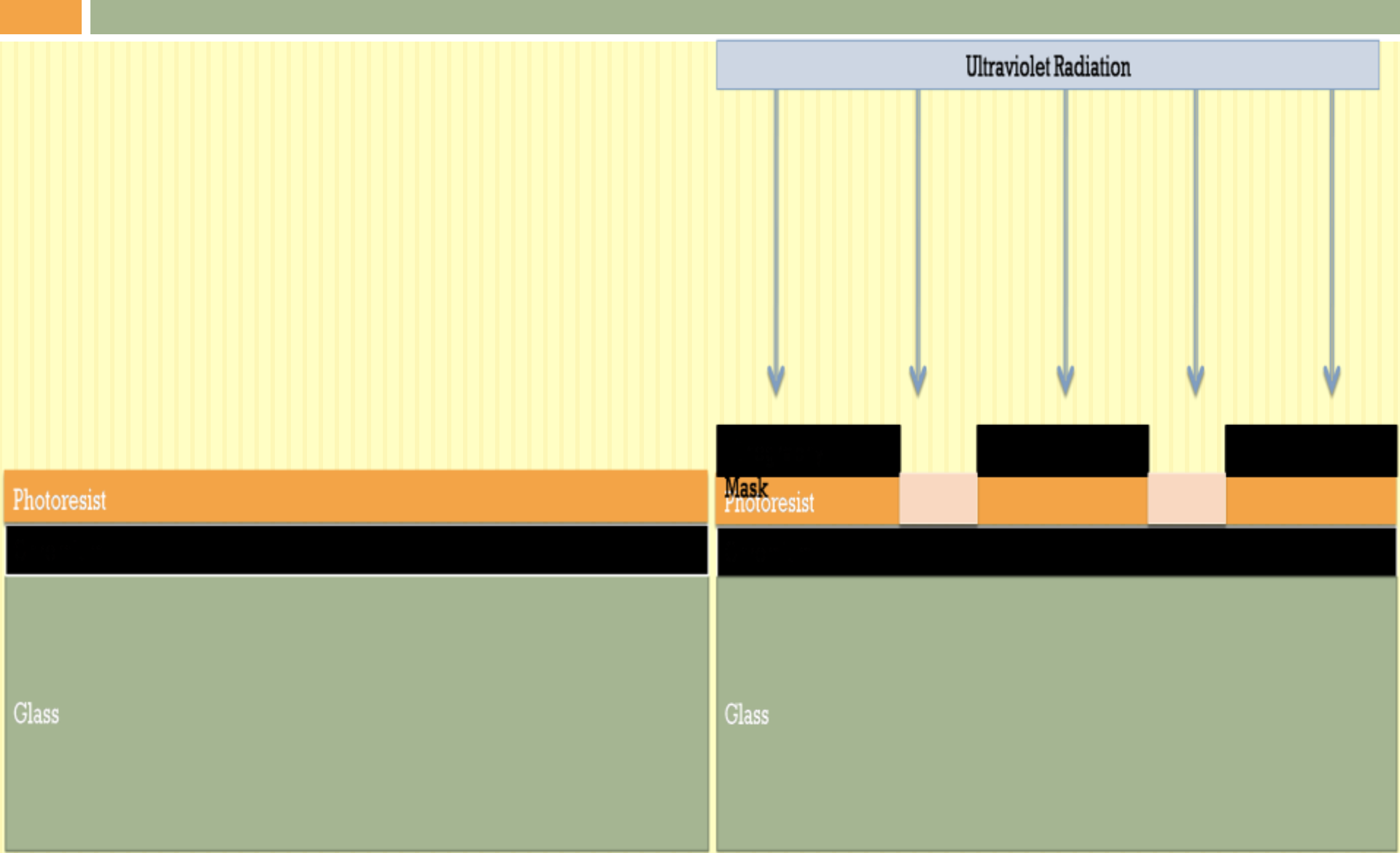
- Currently in the process of synthesizing an extended version of aminoethoxyethanol.



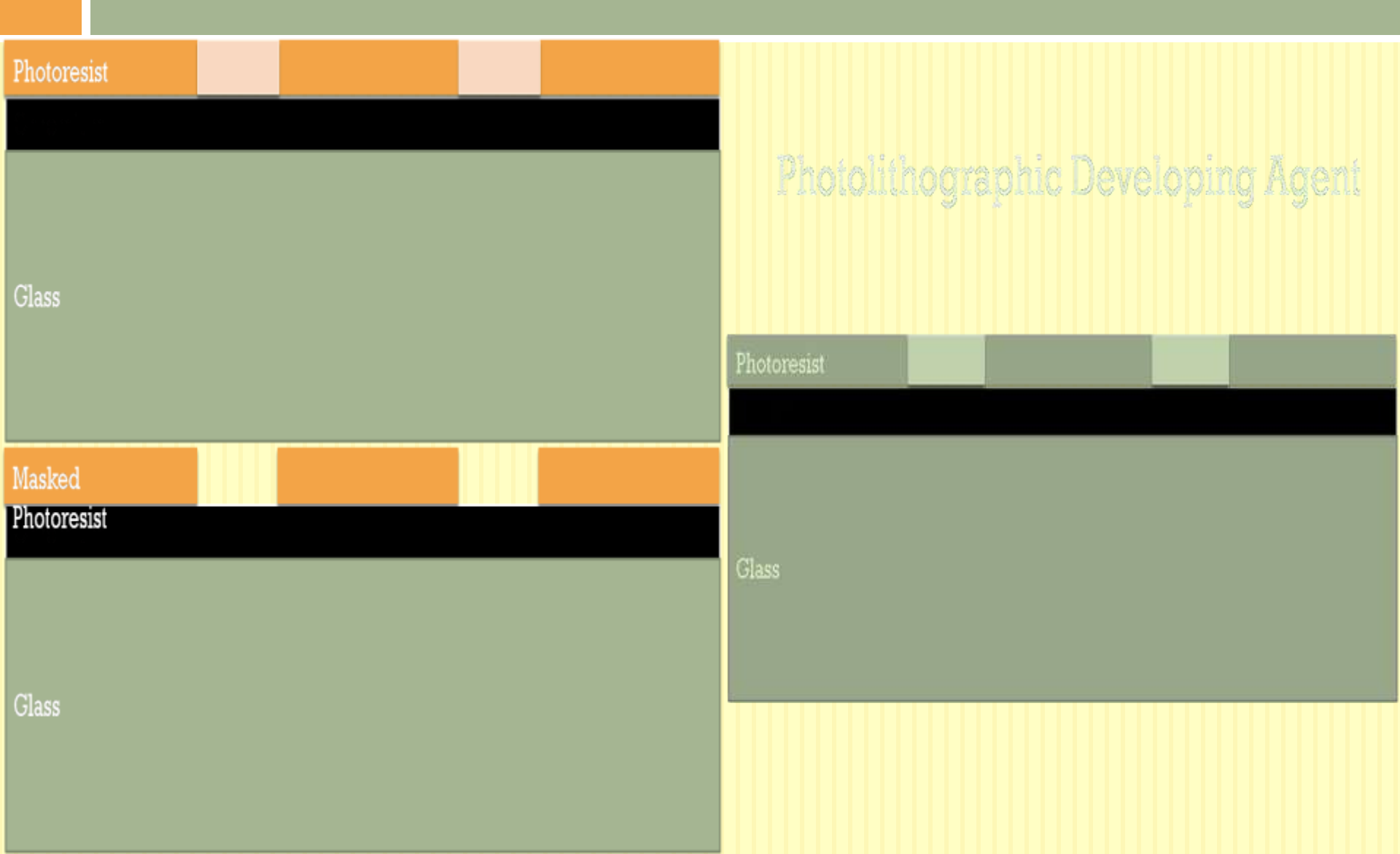
Acknowledgements

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 - INBRE

Fabrication of Microfluidic Devices

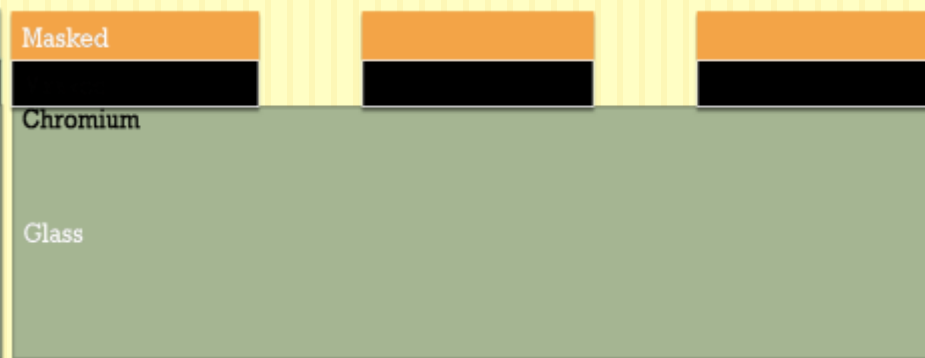


Microchip Fabrication

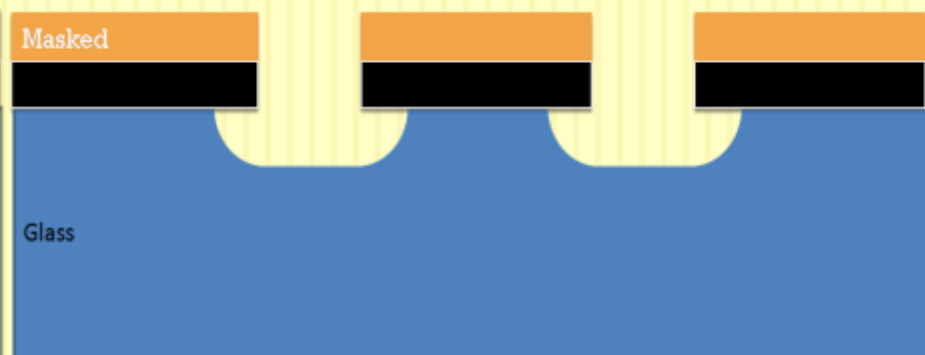


Microchip fabrication

Chromium Etchant

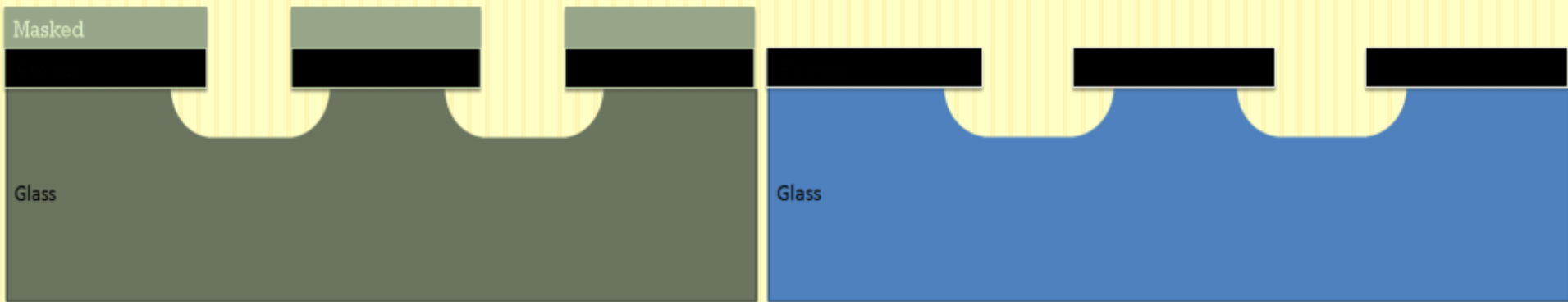


BOE Glass Etchant



Microchip Fabrication

Acetone Rinse



Chromium Etchant

