

Testing the Diffusivity of Hydrogels By Fluorescence Recovery After Photobleaching

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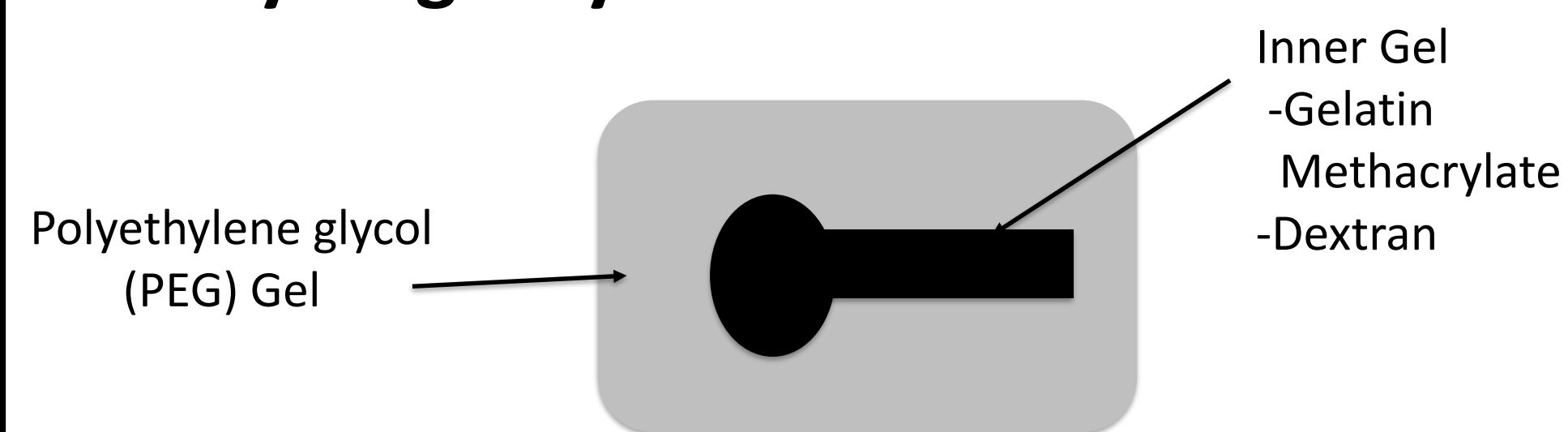
Objective

MOTIVATION: When compared to Dextran, Gelatin-Methacrylate has shown to promote more robust growth of dorsal root ganglions.

GOAL: To determine whether the diffusivities of Gelatin-Methacrylate and Dextran account for the differences of neurite growth in each gel.

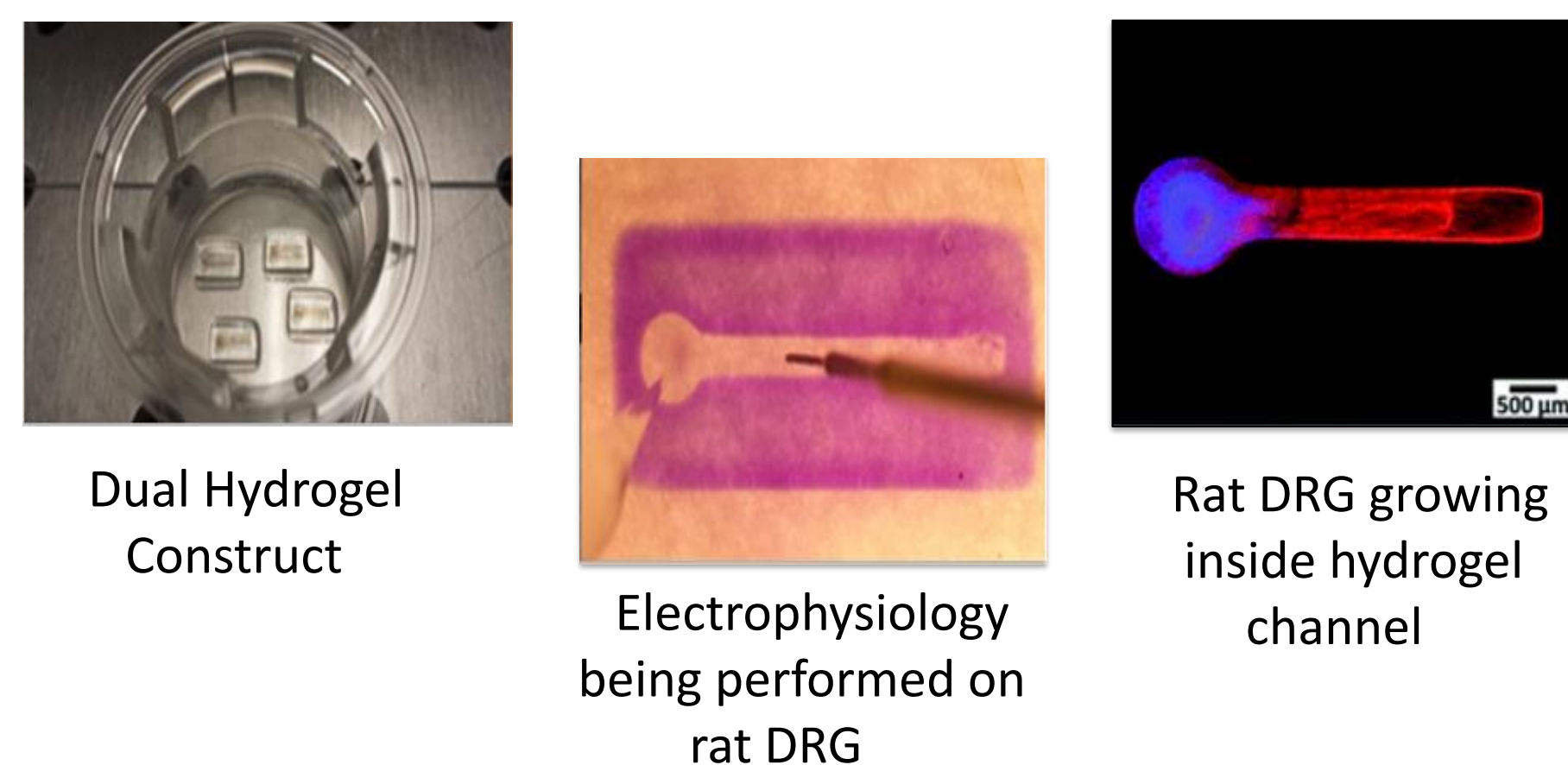
Background

Dual Hydrogel System:



- Inner Gel: Allows neural growth
- PEG Gel: Restricts neural growth

Nerve-on-a-Chip Model:



- Rat dorsal root ganglions (DRGs) inserted into dual hydrogel system to promote neurite growth in a 3D environment which better models physiology
- Mimics in vivo properties
- Able to perform electrophysiology and histology
- Allows for better studies on nerve development

Method

Preparing Hydrogel Samples:

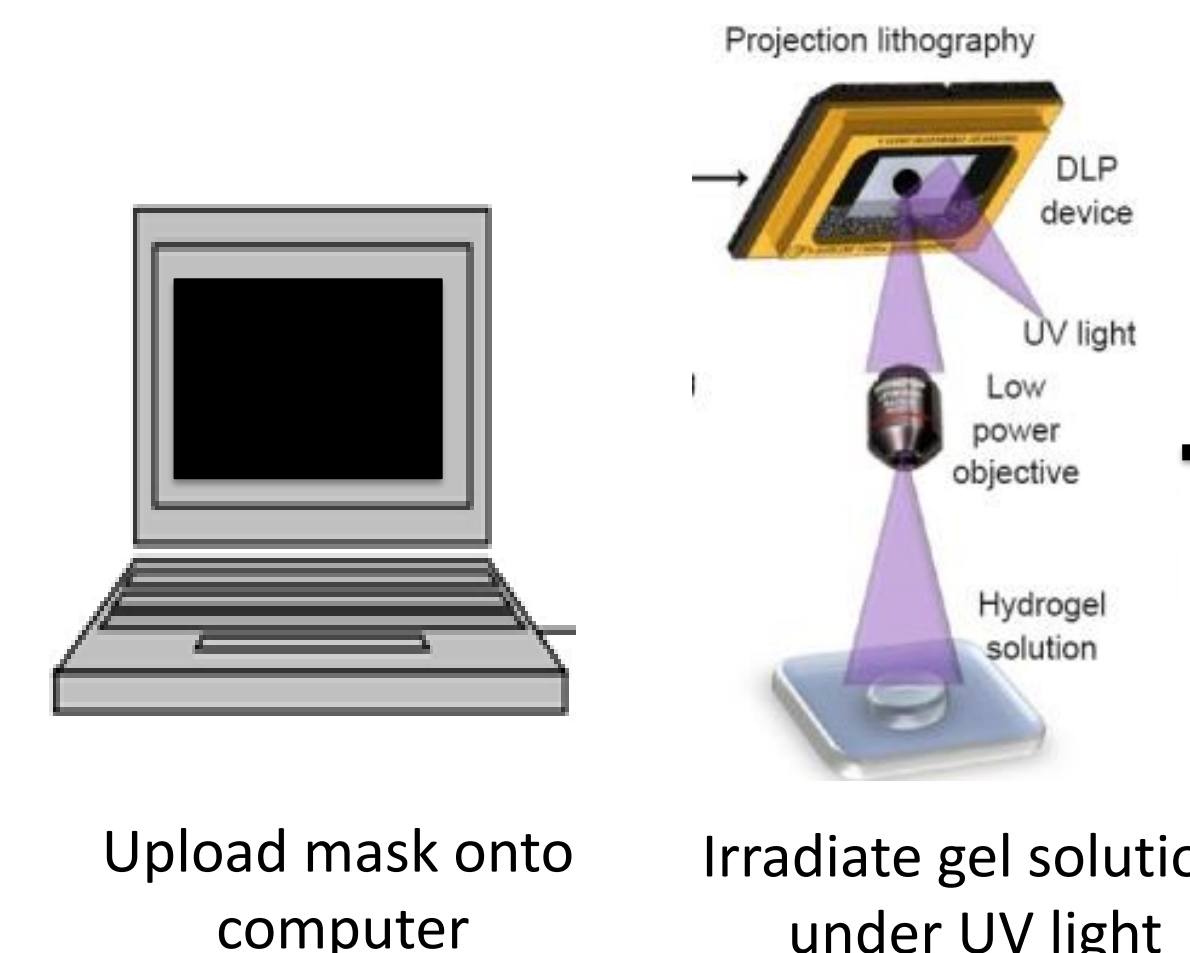
1)Set up Hydrogel System

Glue rubber o-rings onto cover slip and add inner gel inside area of ring



concentrations:
2%, 4%, 6%

2)Irradiate Gel Solution

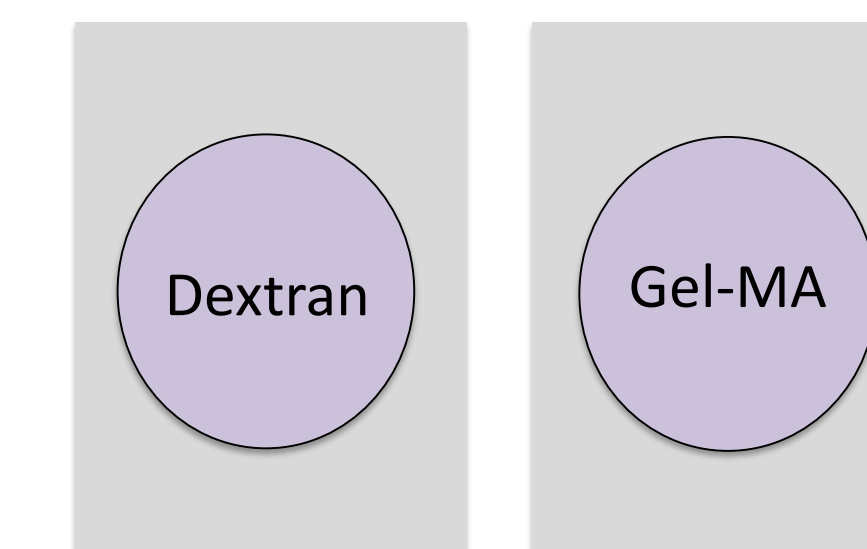


Create blank mask

Upload mask onto computer

Irradiate gel solution under UV light

3)Add Fluorescein-Conjugated Dextran



30ul of dye was used for each sample

FRAP Method:

1)Scan sample



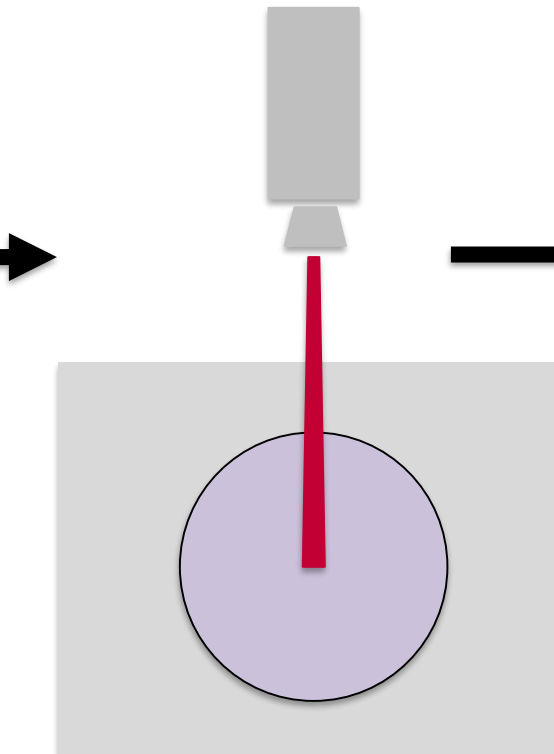
Picture of gel sample objective: 20x

2)Select area to bleach

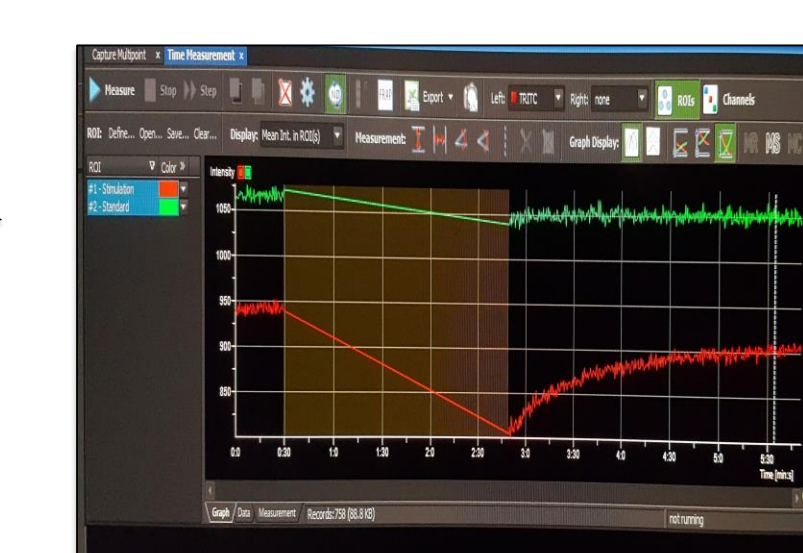


Red= area to be bleached
Green= reference area

3)Bleach selected area

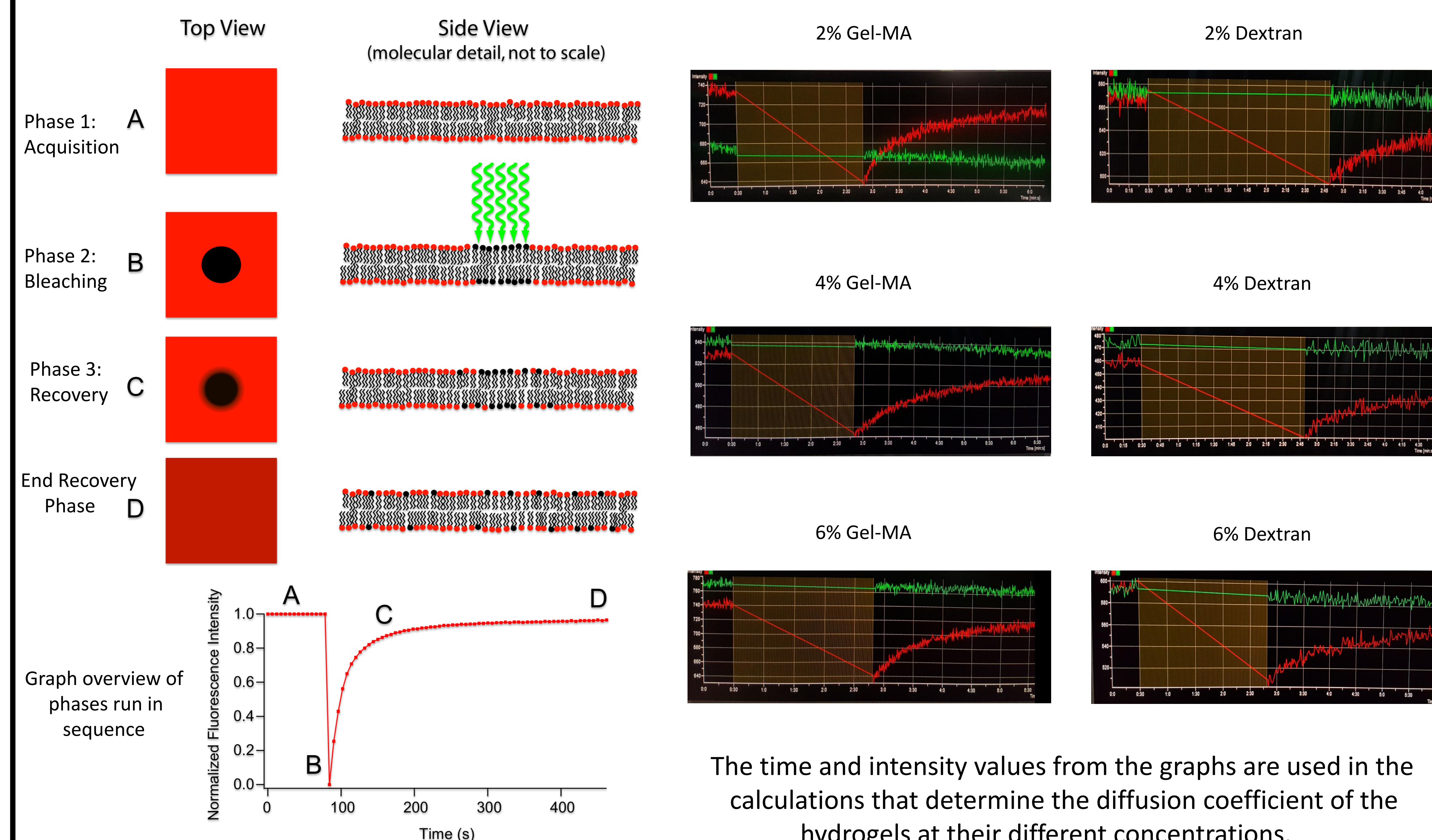


4)Result Graph

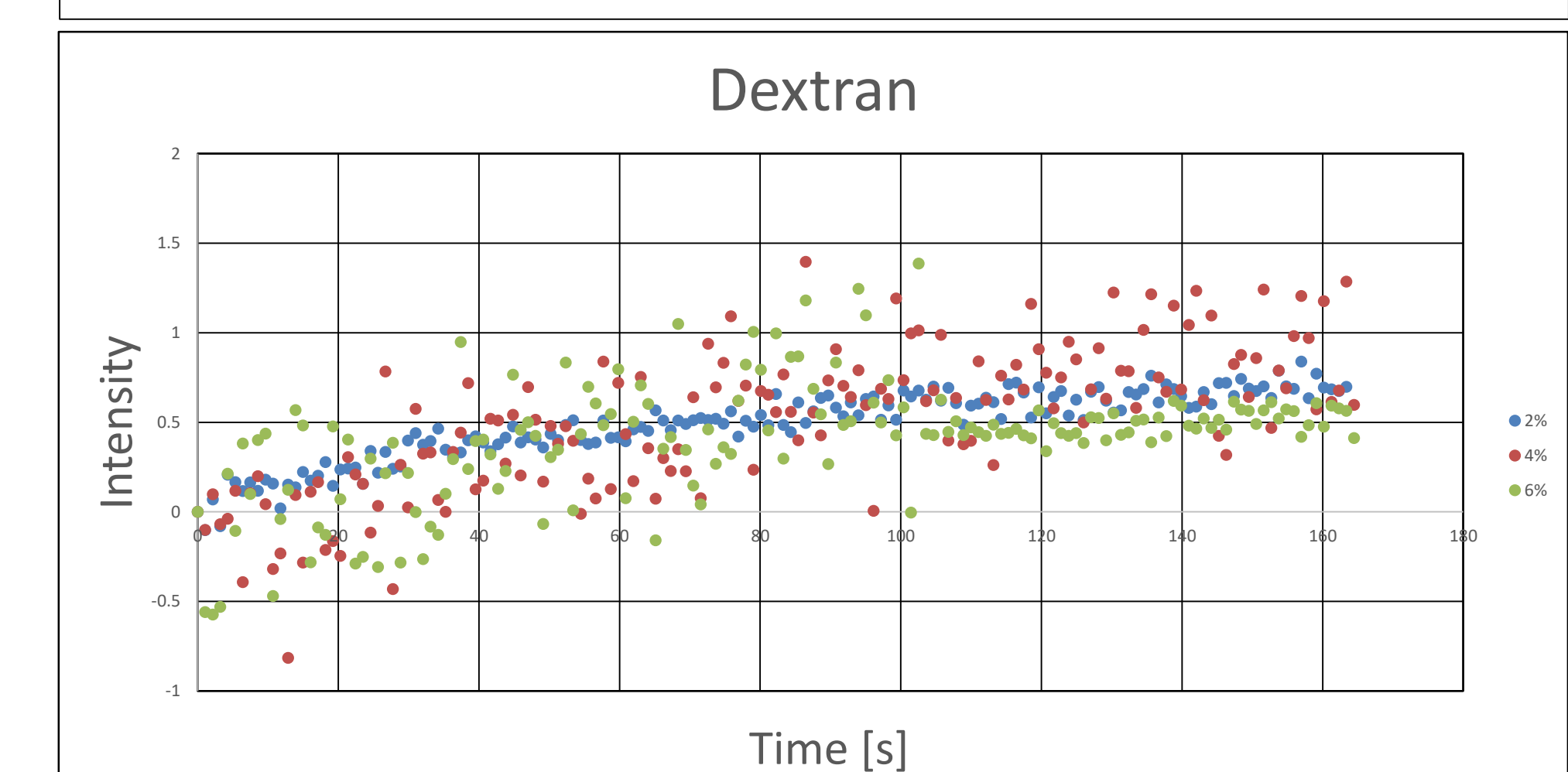
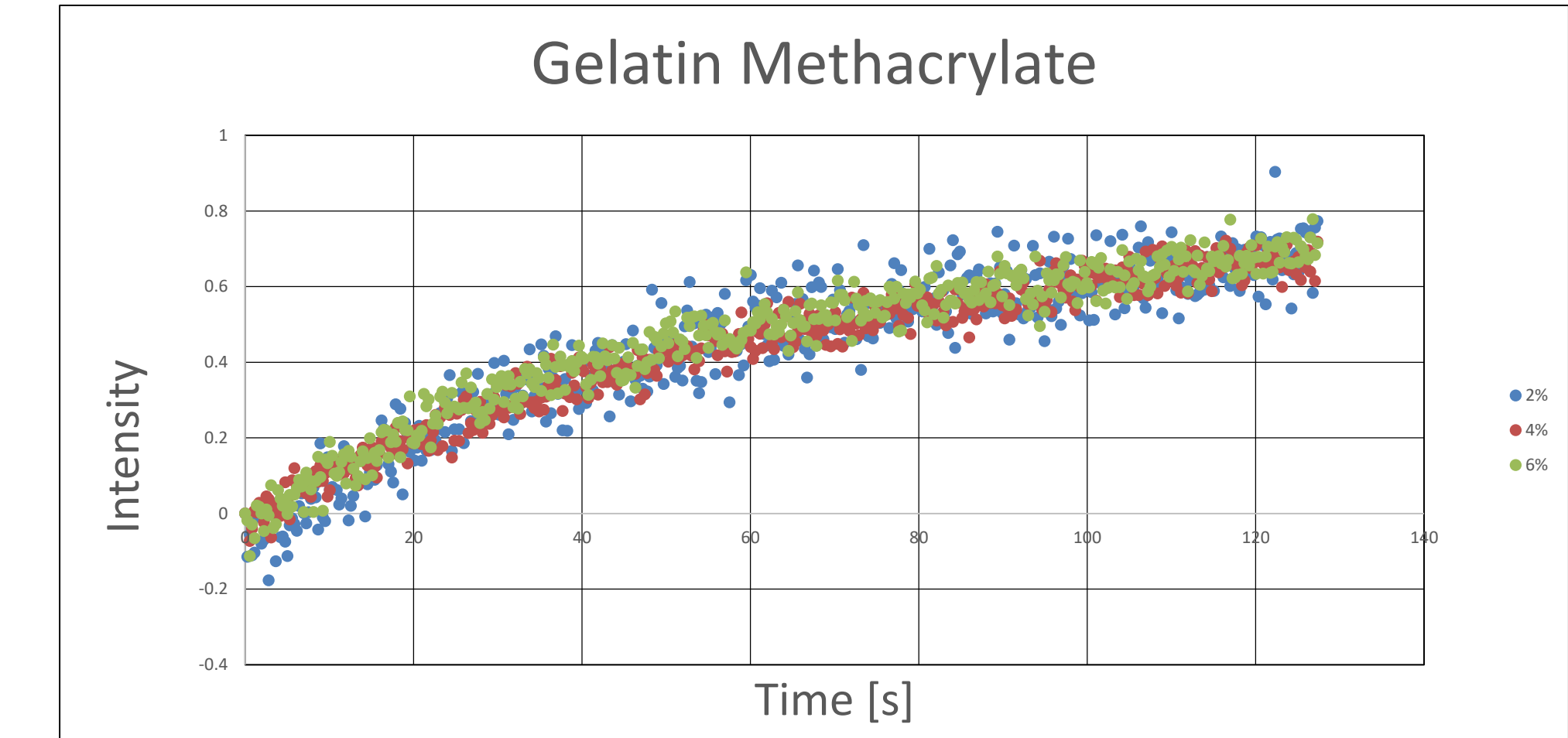


Example of graph

Fluorescence Recovery After Photobleaching



Results



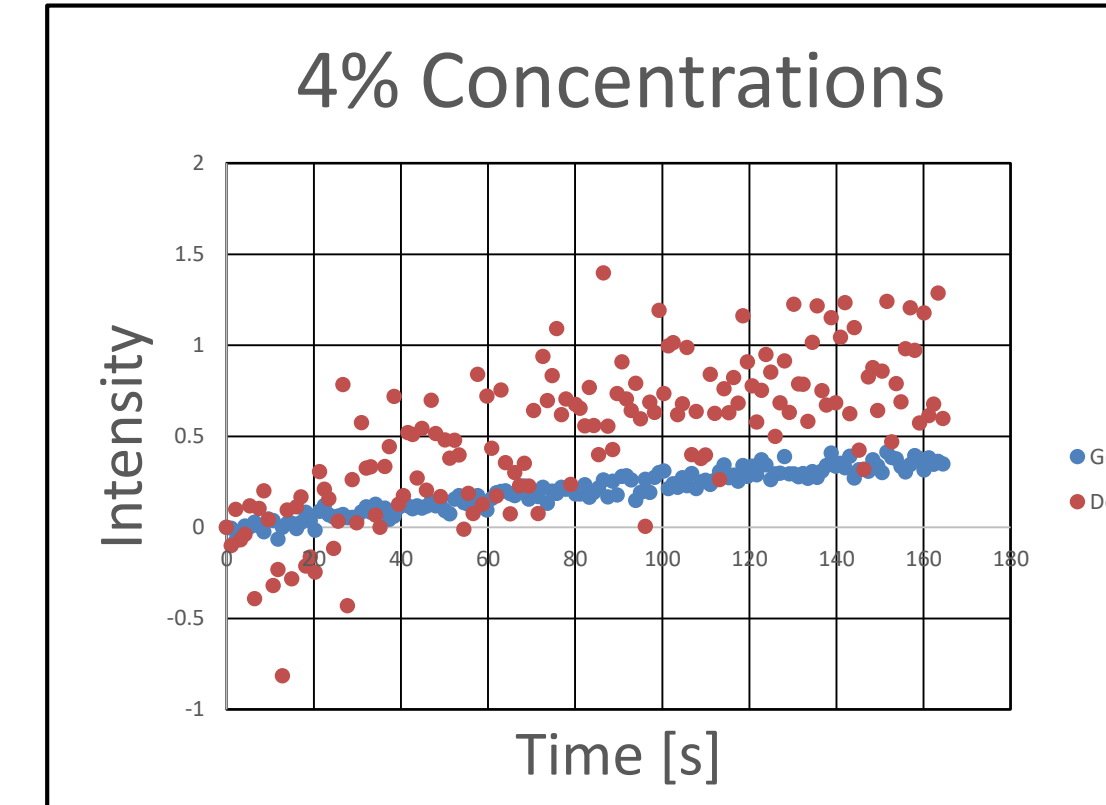
Equations used to normalize values

$$f(t) = \frac{I_{ref}(pre)}{I_{ref}(t)} \times \frac{I_{frap}(t)}{I_{frap}(pre)}$$

$$f(t) = \frac{f(t) - f(0)}{f(pre) - f(0)}$$

$$f(t) = k \cdot e^{-\frac{I_0}{2t}} \left[I_0 \left(\frac{I_0}{2t} \right) + I_1 \left(\frac{I_0}{2t} \right) \right]$$

$$D = \frac{w^2}{I_0}$$



There were no differences between the concentrations of each gel. Since neurite growth is observed at the 4% concentrations, both gels were compared at that concentration and Dextran showed a higher curve on the graph than Gel-MA.

Conclusion and Future Work

- Dextran showed a higher diffusivity than Gel-MA as seen by the curve on the graph
- Future Work:**
- Testing neurite growth in the different concentrations
 - Stiffness
 - Impedance

Acknowledgements

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