Near-Infrared Photobiomodulation of Living Cells, Tubulin, and Microtubules *In Vitro*

Michael Staelens (staelens@ualberta.ca), Ph.D.
Department of Physics, University of Alberta

E. Di Gregorio, A. P. Kalra, H. T. Le, N. Hosseinkhah, M. Karimpoor, L. Lim, J. A. Tuszyński

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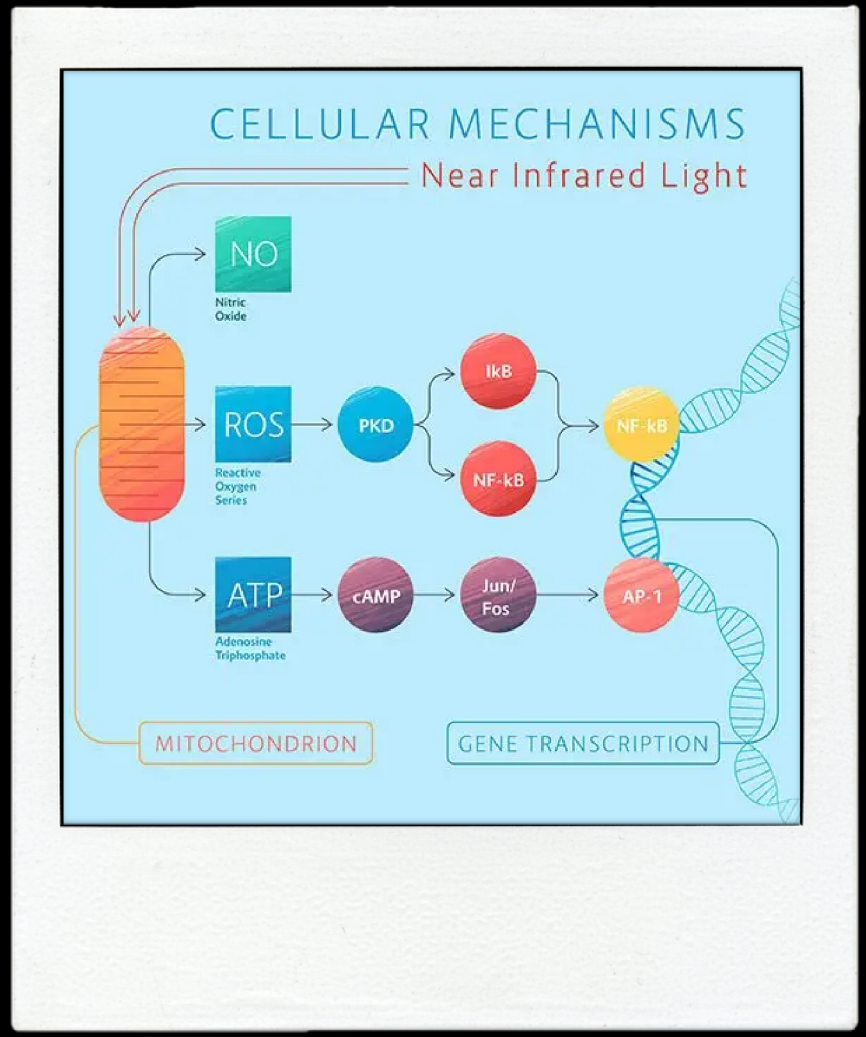

Near-Infrared (NIR) PBM — Clinical Results

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Alzheimer's Disease:

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Traumatic Brain Injury & Concussion:

- *A single concussion case study found positive changes in behavioural and neuroimaging measures (e.g., increased cerebral perfusion) after 8 weeks of home PBM treatments. Frontiers in Neurology* **11**, 952 (2020)

Experimental Device Information

Experimental Device Information

Vielight Neuro Alpha Brain PBM Device

Transcranial-Intranasal NIR PBM

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Transcranial-Intranasal NIR PBM

Parameter	Value (Intranasal)	Value (Transcranial)
Light source	810 nm LED × 1	810 nm LED × 4 (3 posterior, 1 anterior)
LED output power	25 mW	100 mW (posterior) and 75 mW (anterior)
LED pulse frequency	10 Hz	10 Hz
Pulse duty cycle	50%	50%
Beam spot-size	~ 1 cm^2	~ 1 cm^2
LED power density	25 mW/cm^2	100 mW/cm^2 (posterior) and 75 mW/cm^2 (anterior)
Application time (default)	20 min	20 min
E_{Net} delivered (per LED)	15 J	60 J (posterior) and 45 J (anterior)
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- *Proprietary LEDs used produce non-thermal, non-ionizing, and incoherent radiation*
- *LEDs target the DMN (disrupted in Alzheimer's cases)*
- *Low-level NIR photons have been shown to be able to penetrate the skull (both ex vivo & in silico)*

In Vitro Experiments with NIR PBM

Probing the Effects on Cells & Cellular Components

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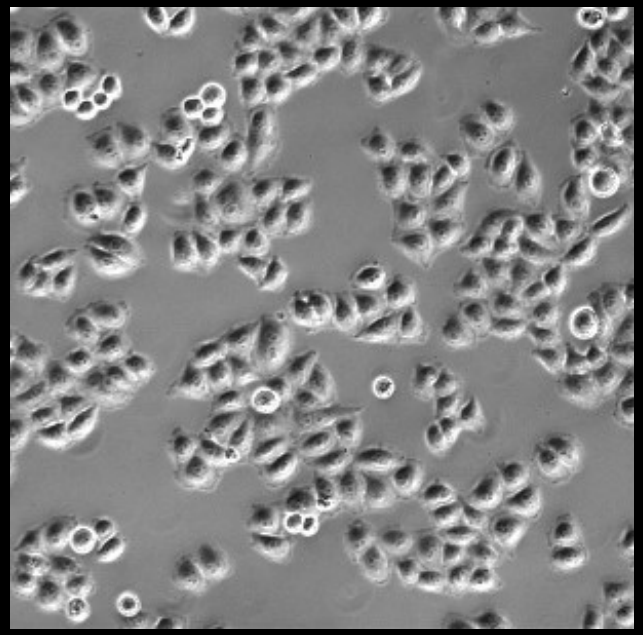

Living Cells (HeLa & U251)

Ionic Conductivity Measurements

In Vitro Experiments with NIR PBM

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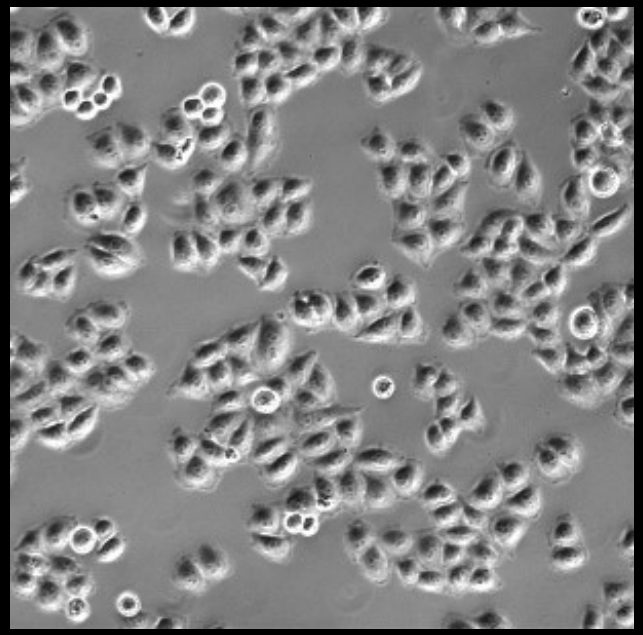

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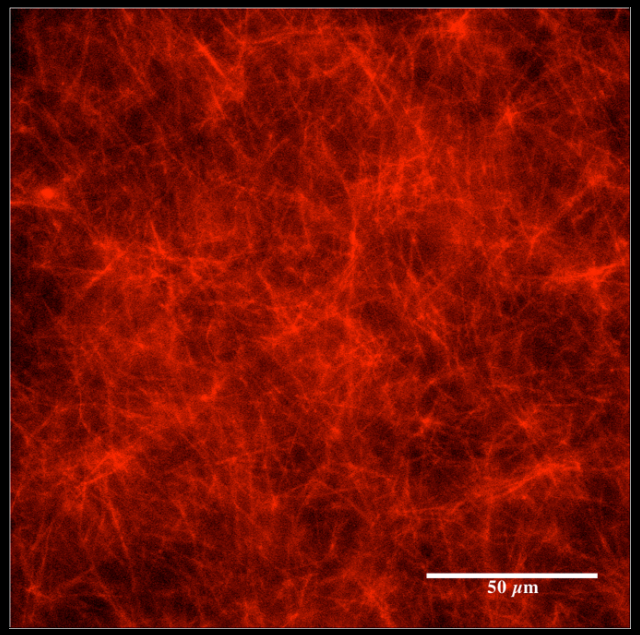

Microtubules in Buffer Solutions

Fluorescence Microscopy Analysis

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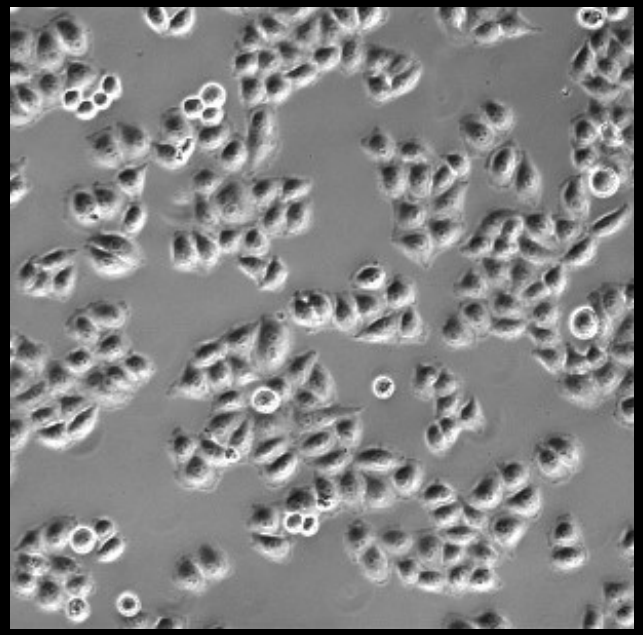

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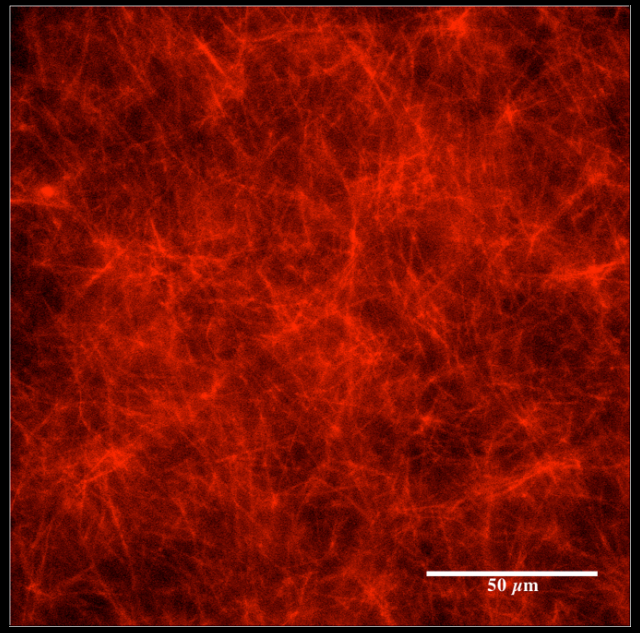

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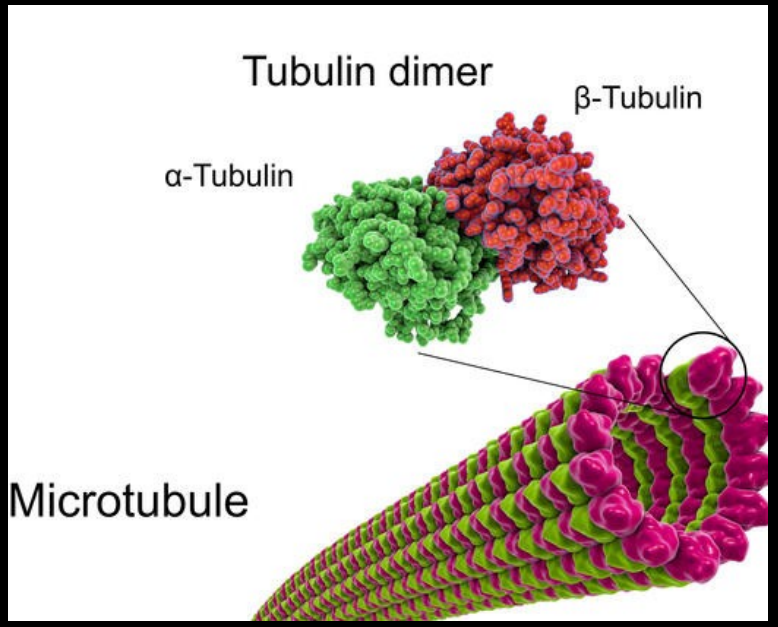

Tubulin in Buffer Solutions

Turbidity Measurements

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NIR PBM—Experiment 1:

Living Cells

Experimental Procedures

Experimental Procedures

Cell Cultures:

Experimental Procedures

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- *Measurements of T, R, and I are recorded every 3 s by the system*

Conductivity Measurement Results

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50 kHz TTFields & NIR PBM

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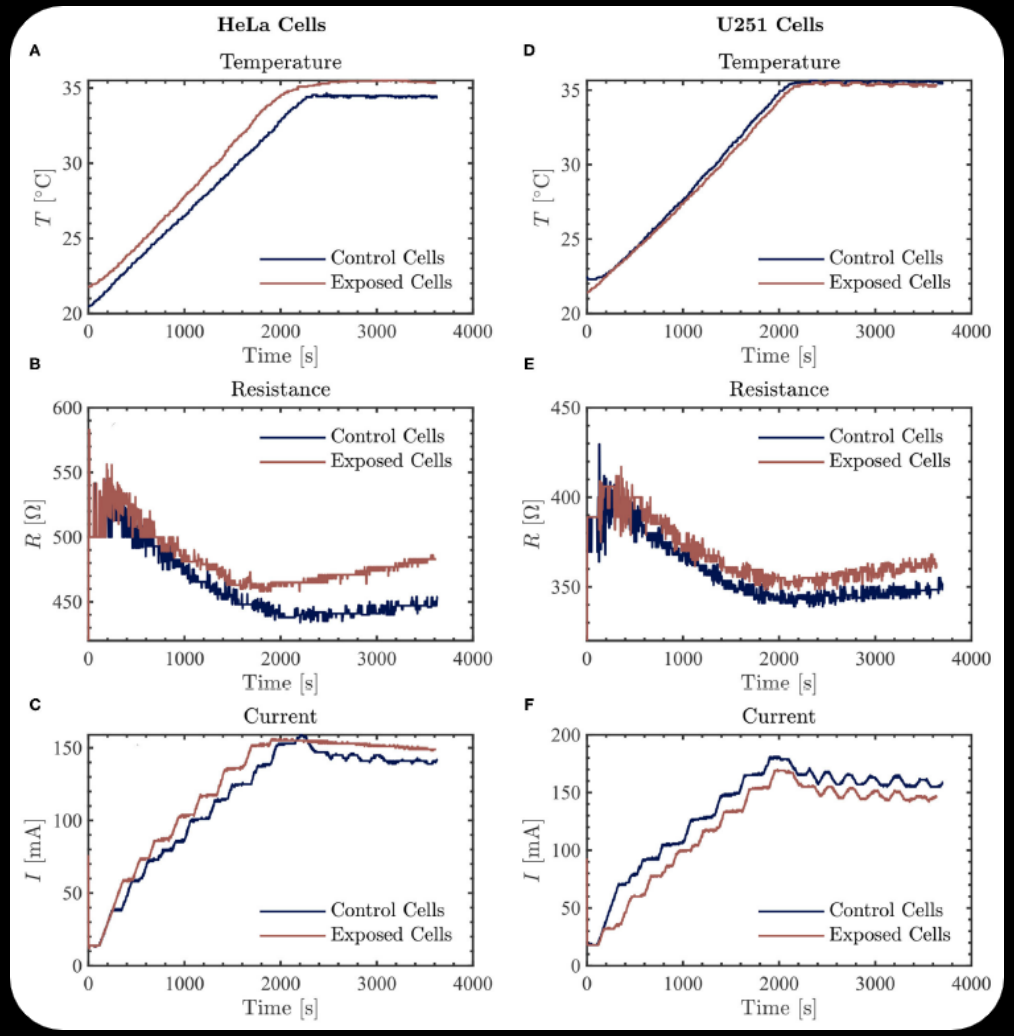

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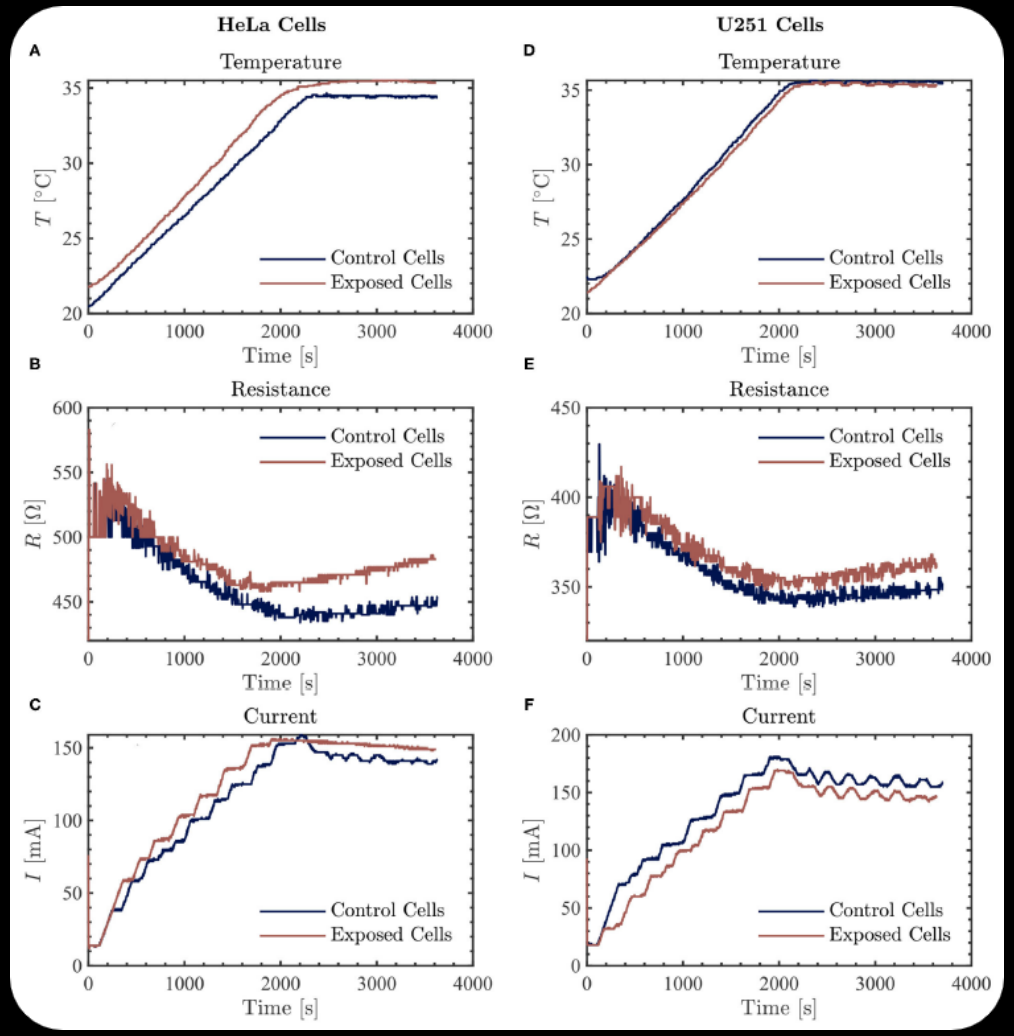

50 kHz TTFields & NIR PBM

Total exposure time was ~1 h

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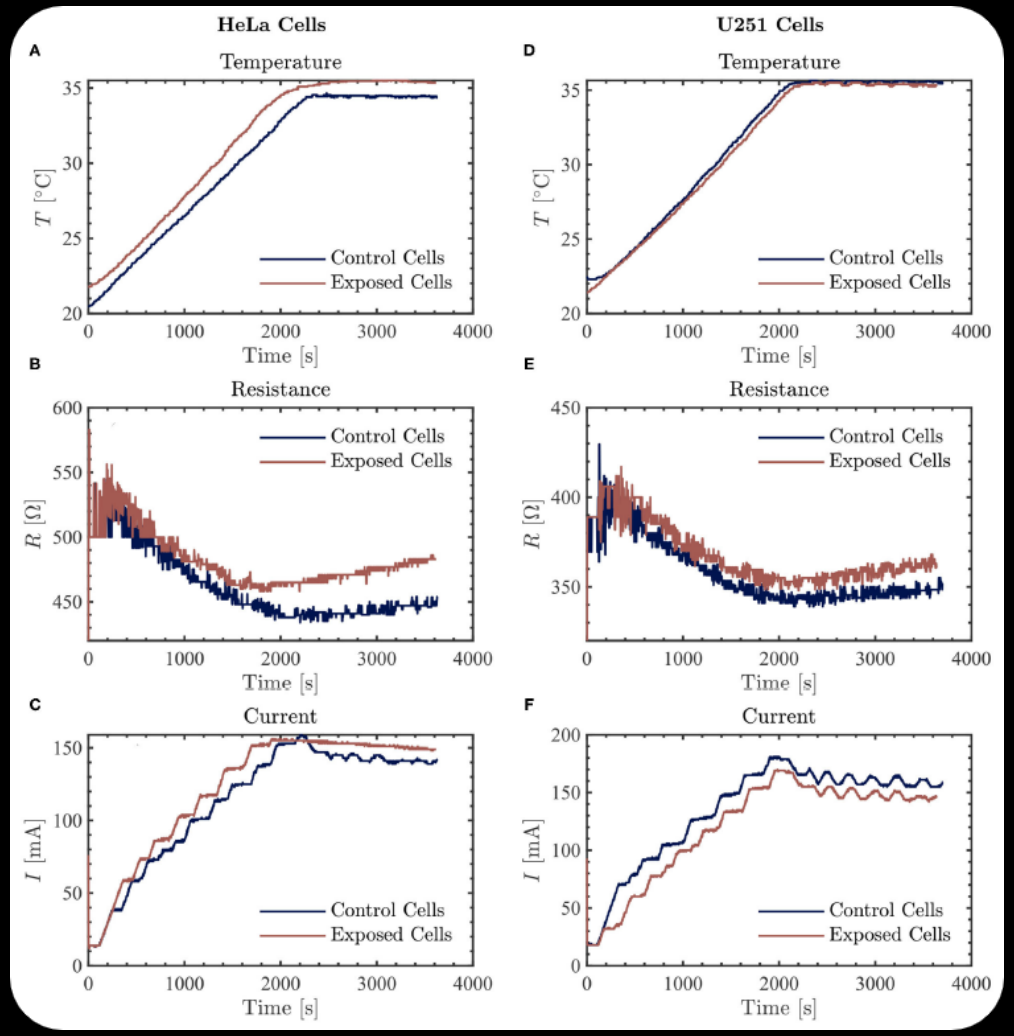

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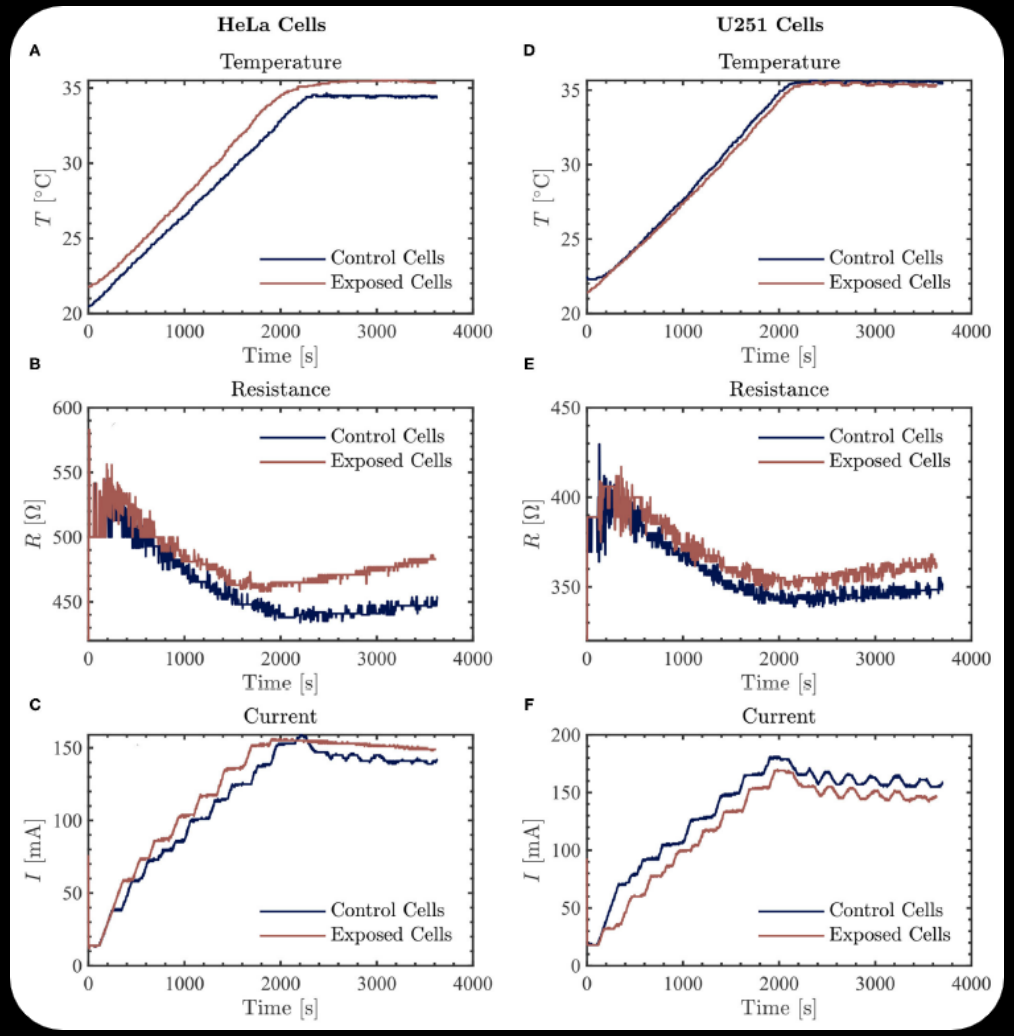

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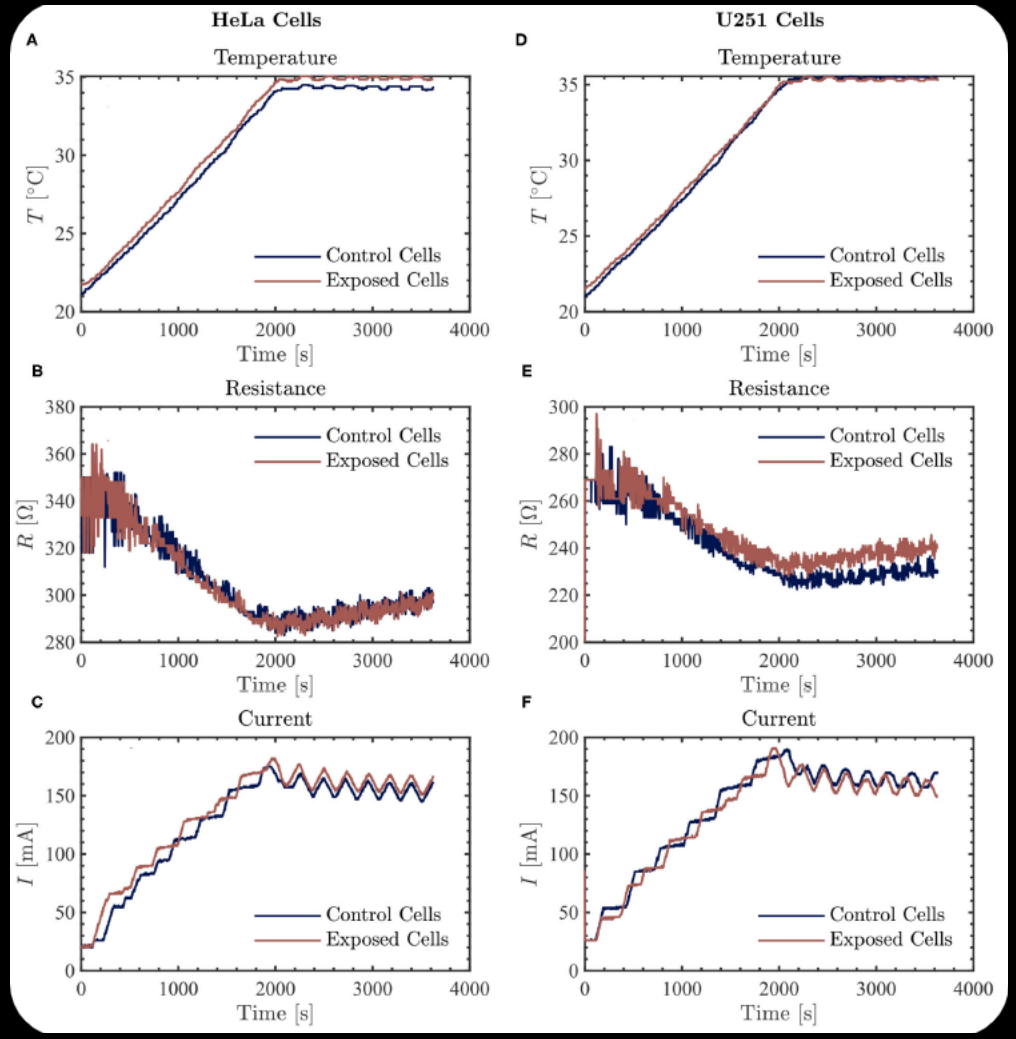

100 kHz TTFields & NIR PBM

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3

NIR PBM—Experiment 2:

Microtubules

Experimental Procedures

Experimental Procedures

Reconstitution of rhodamine-labeled MTs:

Experimental Procedures

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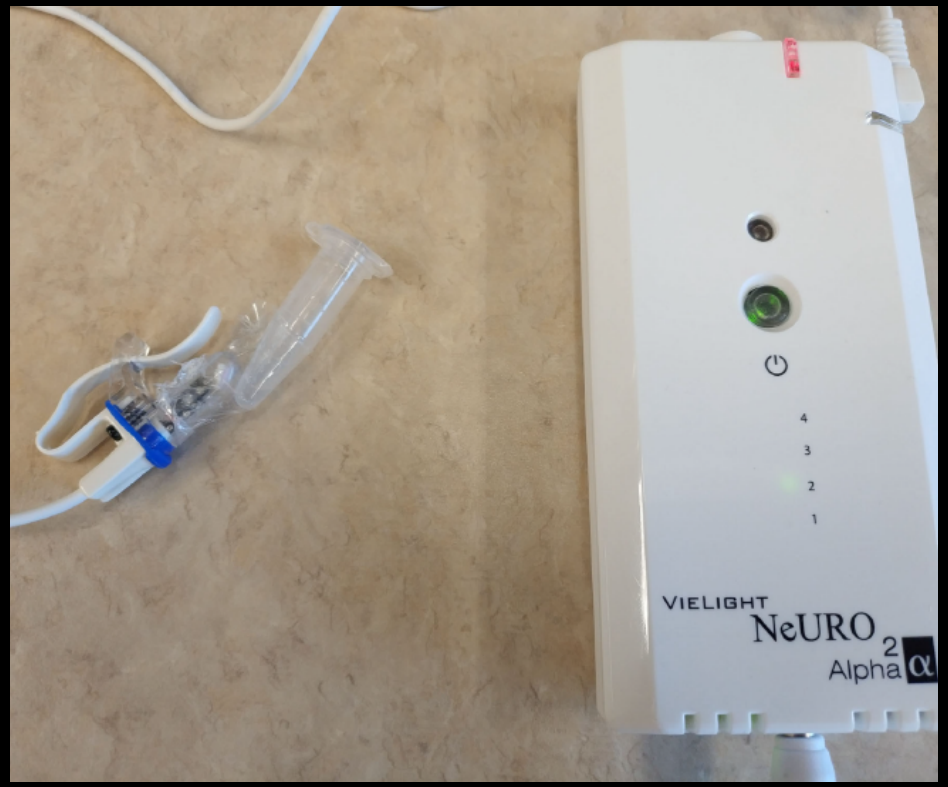

An example of one of the exposures (all performed at room temp)

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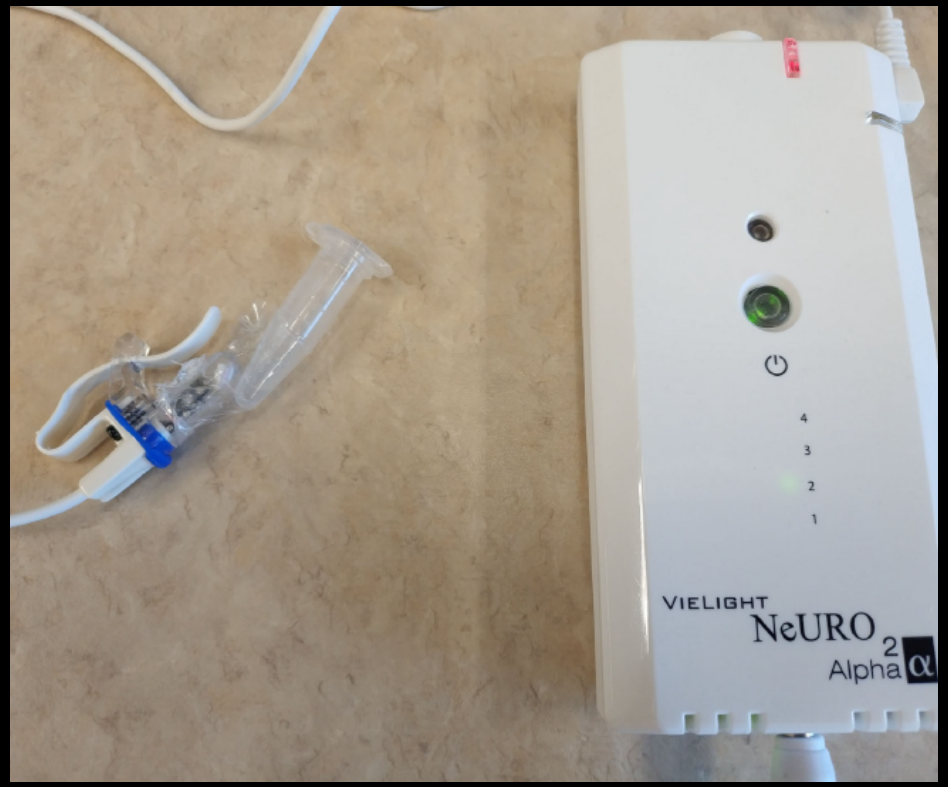

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Imaging Results 1

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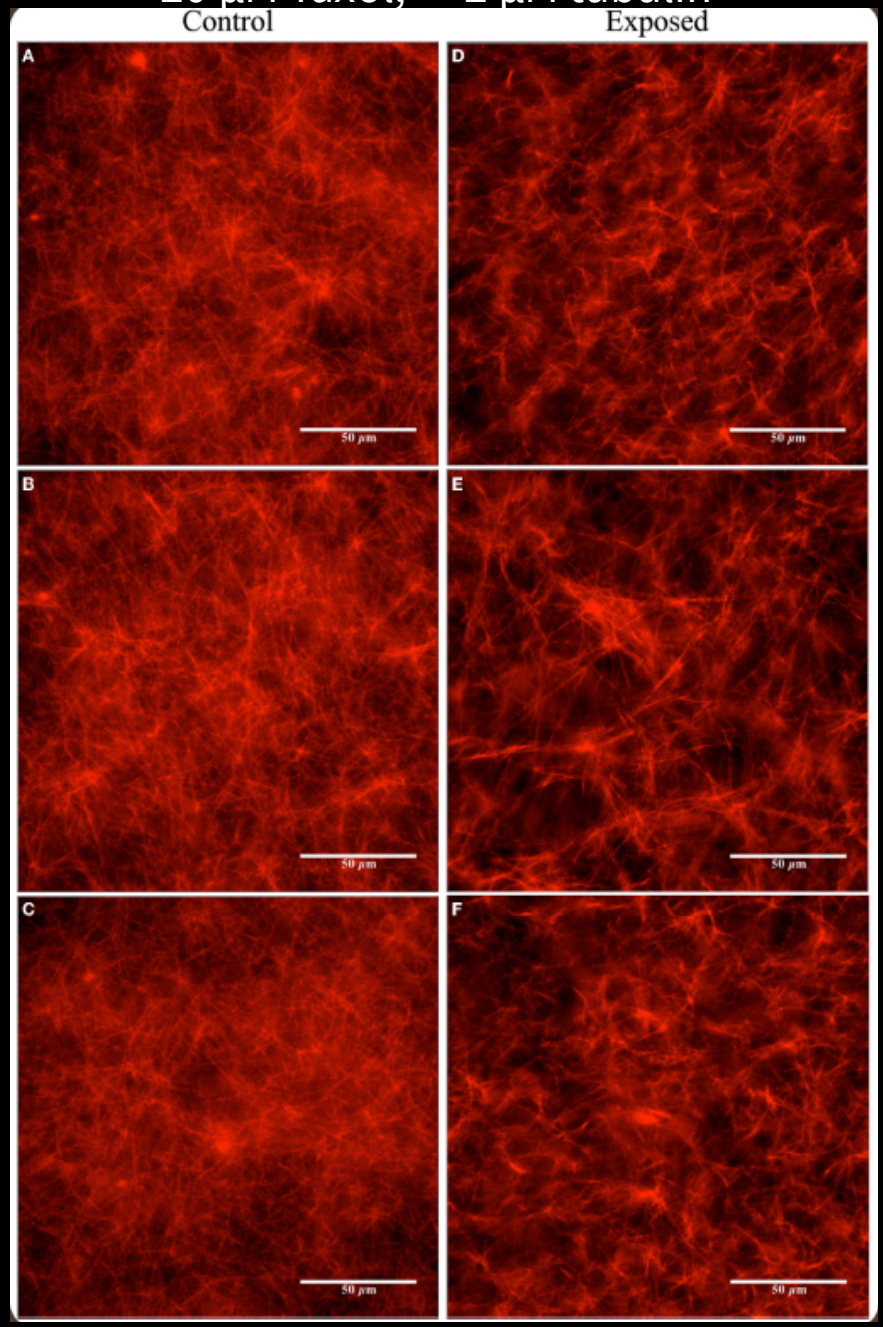
20 µM Taxol, ~ 2 µM tubulin



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- *Notably, a lower total polymer mass appears to be remaining in the PBM-exposed group (N = 1)*

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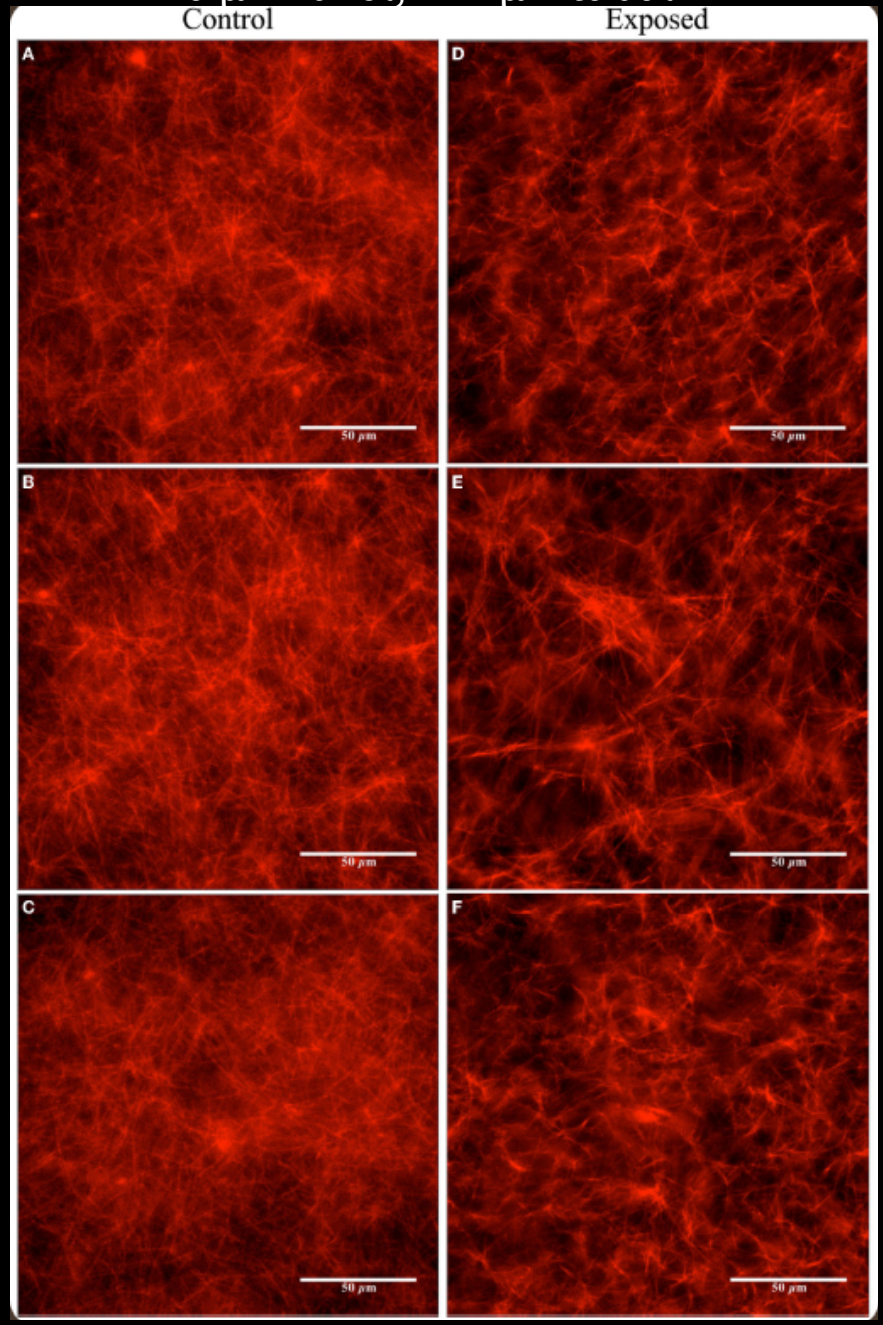

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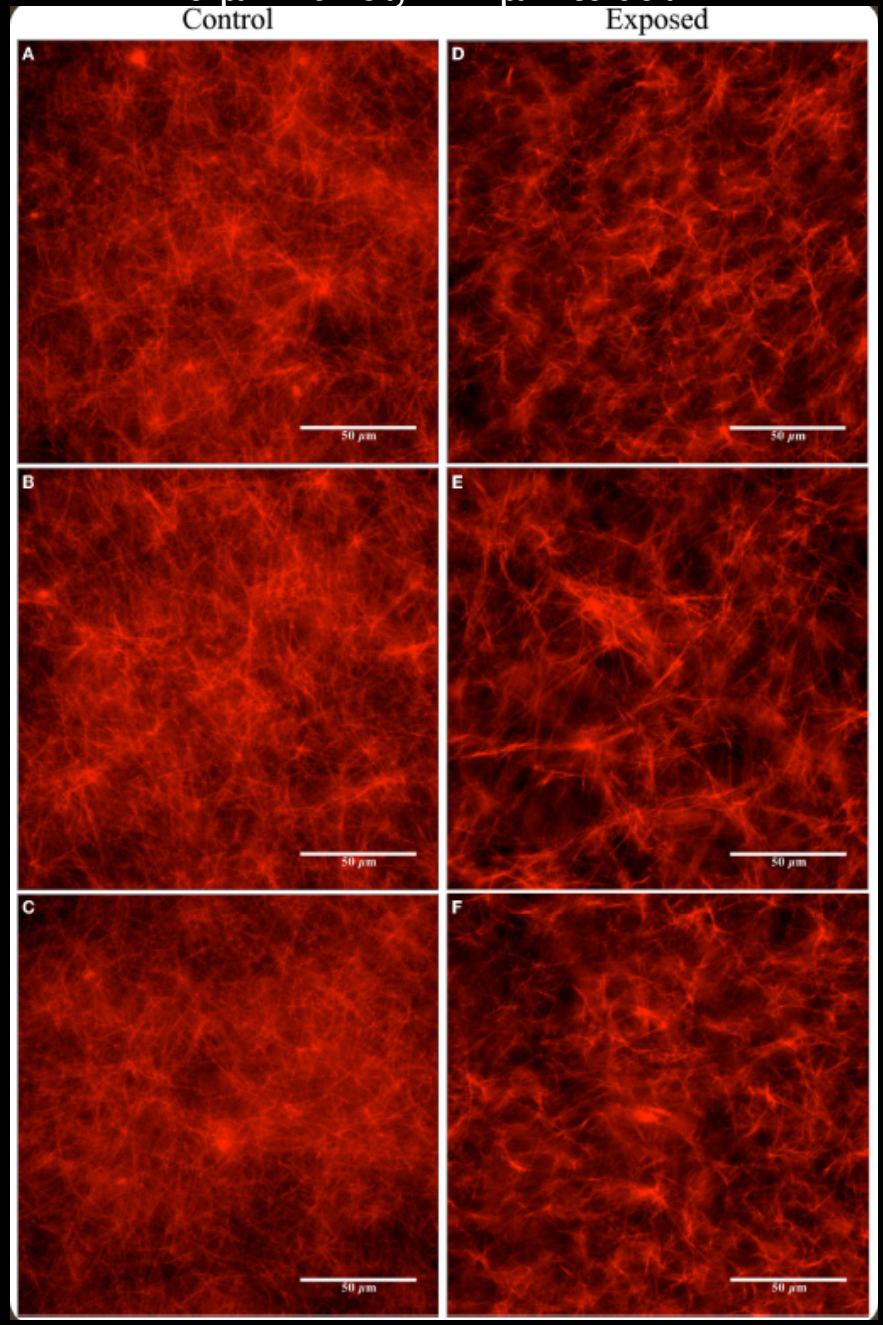

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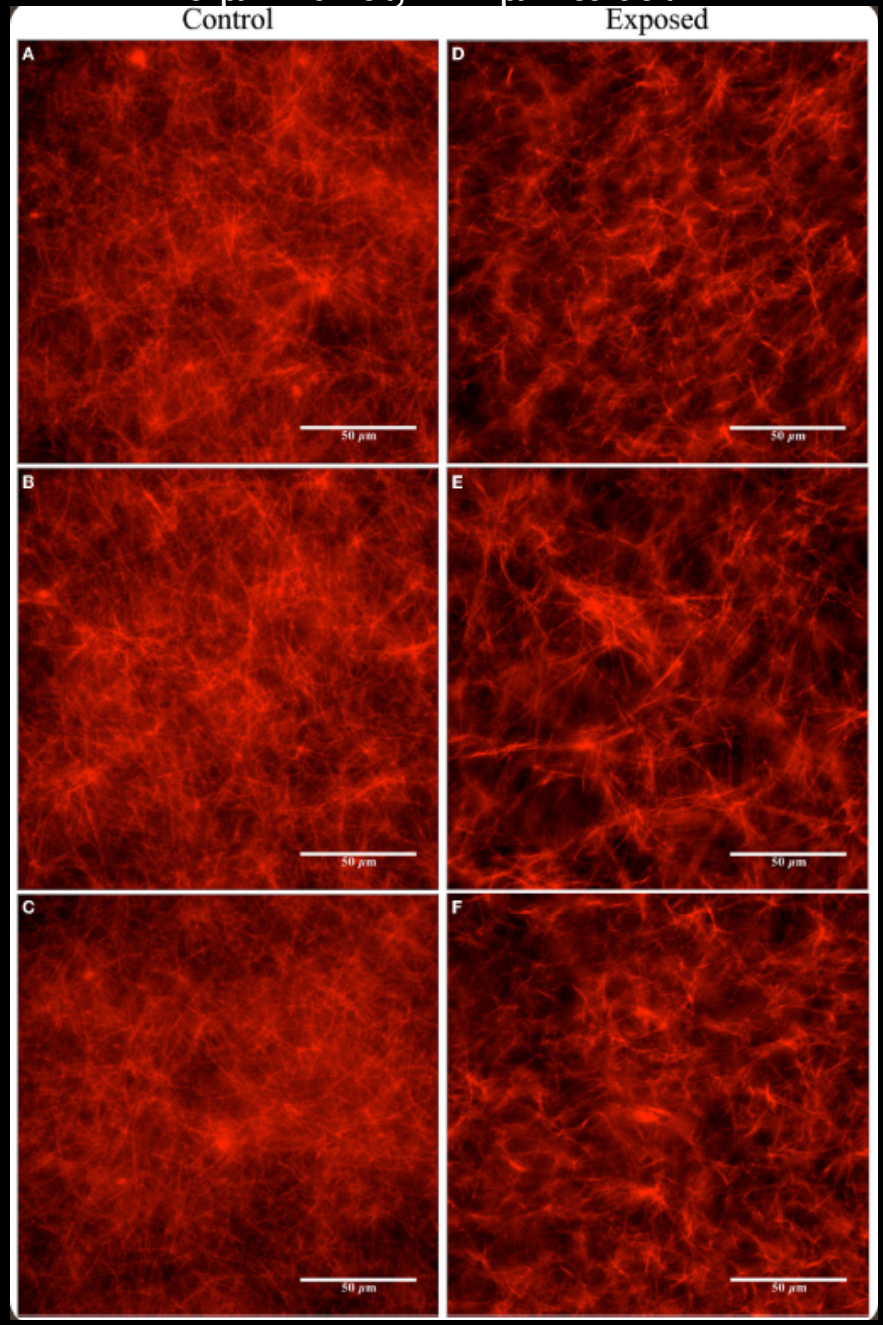

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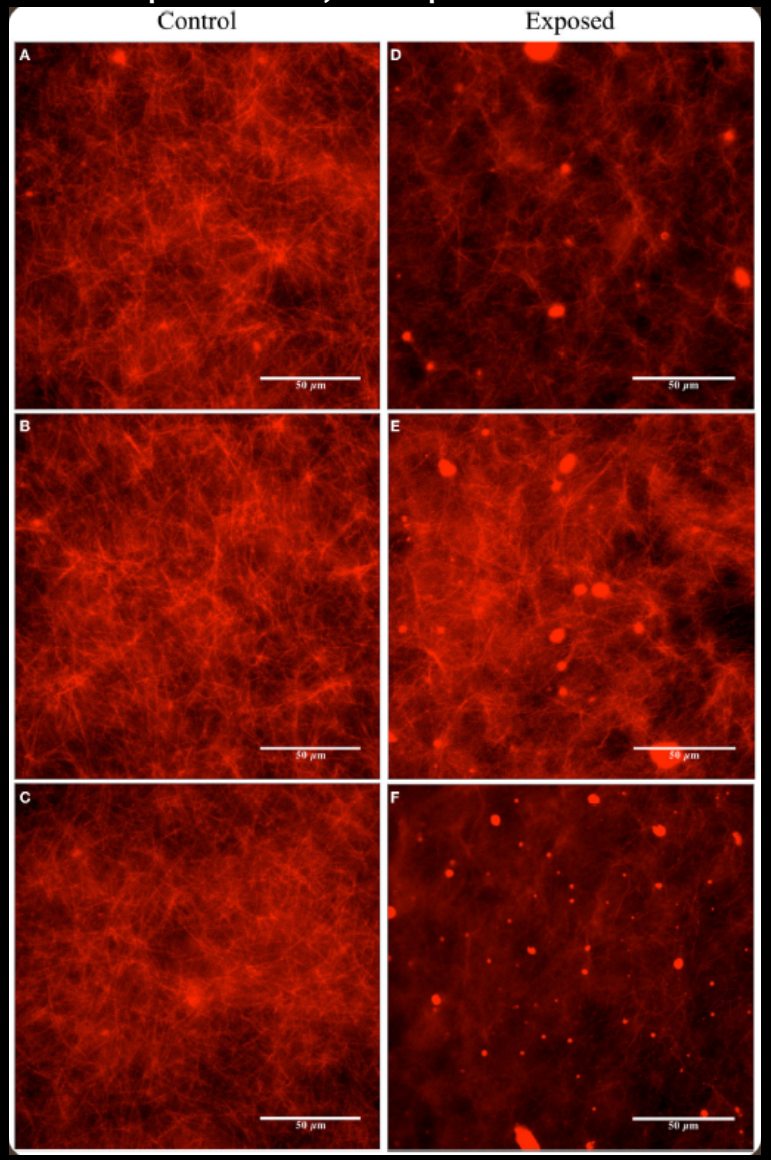

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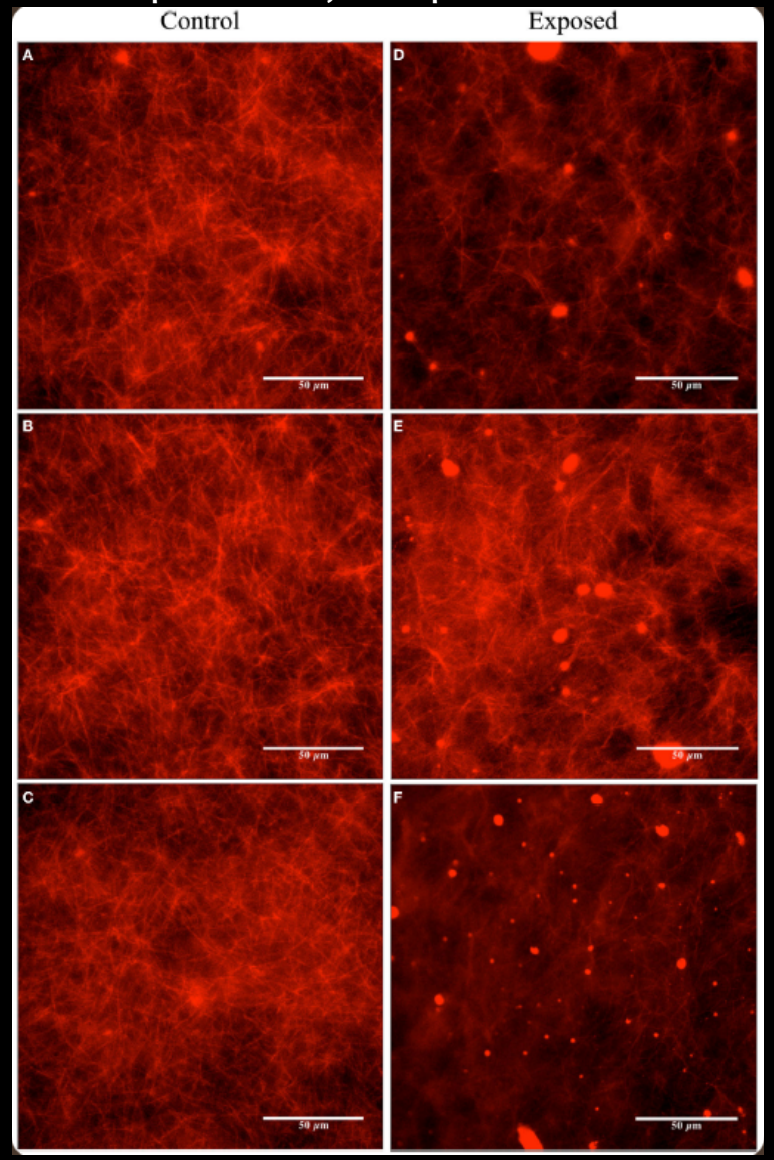

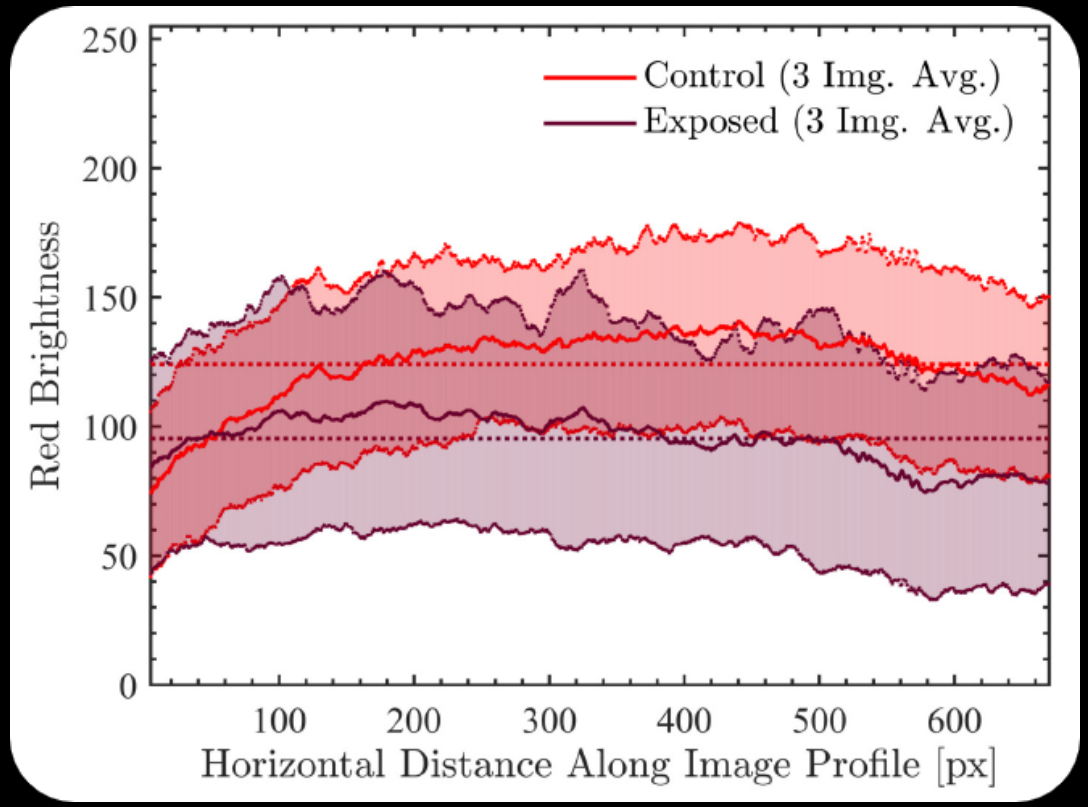

Results of a quick image analysis performed over the red band

4

NIR PBM—Experiment 3:

Tubulin

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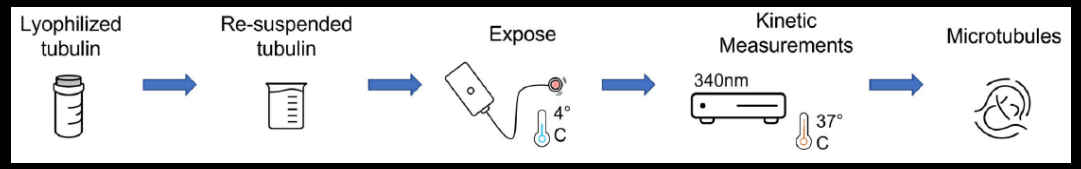

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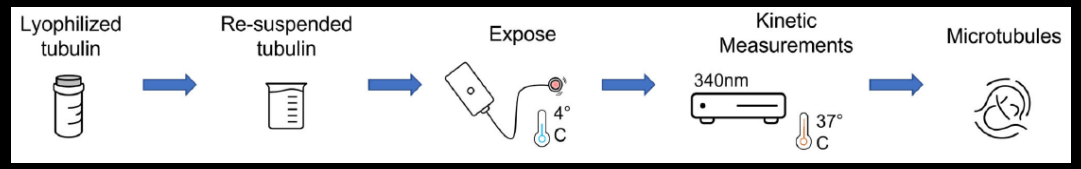

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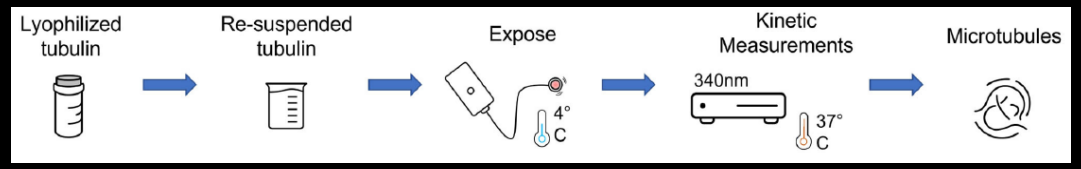

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Turbidity Measurements (Absorbance at 340 nm):

Parameter	Value
t_{total}	2400 s
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N_{reads}	81
Plate type	96 well standard (clear bottom)
Well height/depth	14.6 mm
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Shake before	Yes, 5 s orbital, medium
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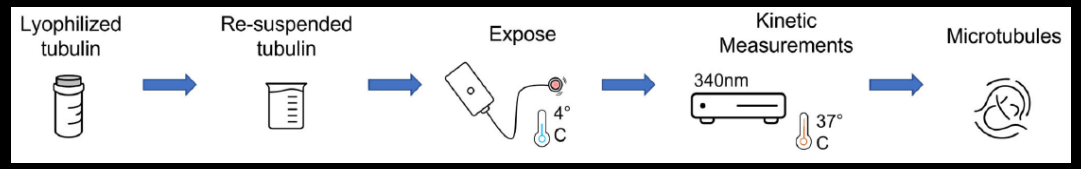

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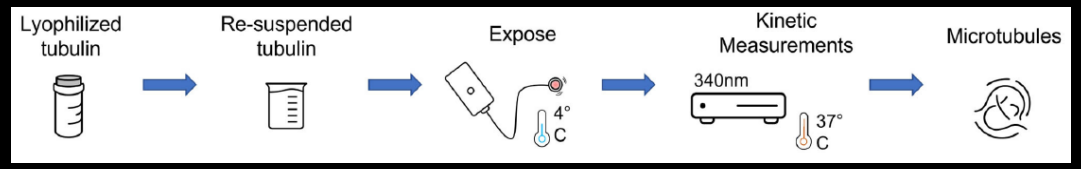

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Measurements were performed with ~100 μl of each sample per well.

Validation of Turbidity Protocol and Methodology

Prior to performing any exposures, we tested our equipment and protocol.

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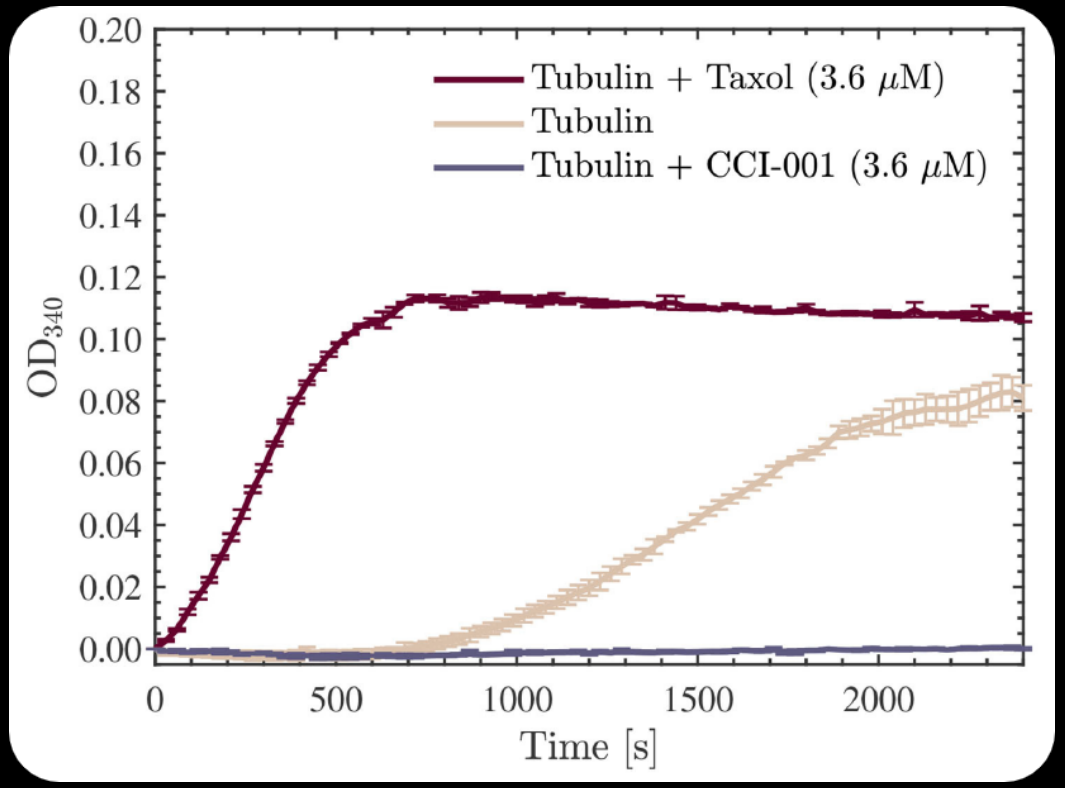

Results of turbidity measurements performed on 22.7 μM tubulin (*N* = 1)

Curves shown are the average of 3 wells measured separately

Turbidity Results for PBM-Exposed Tubulin

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22.7 µM tubulin

PBM-Exposed vs. Unexposed

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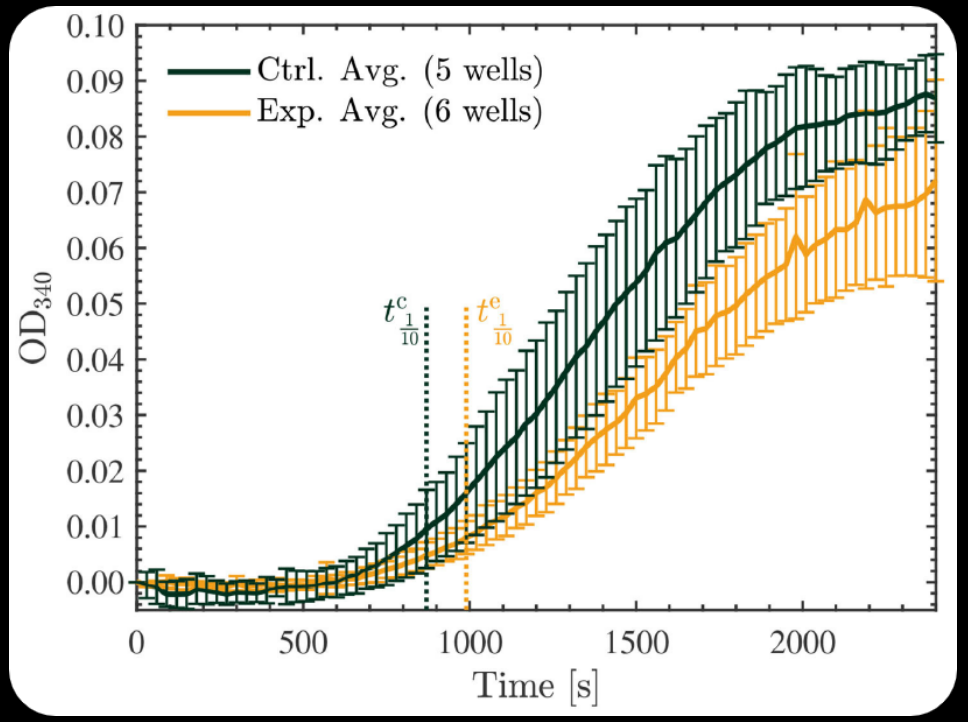

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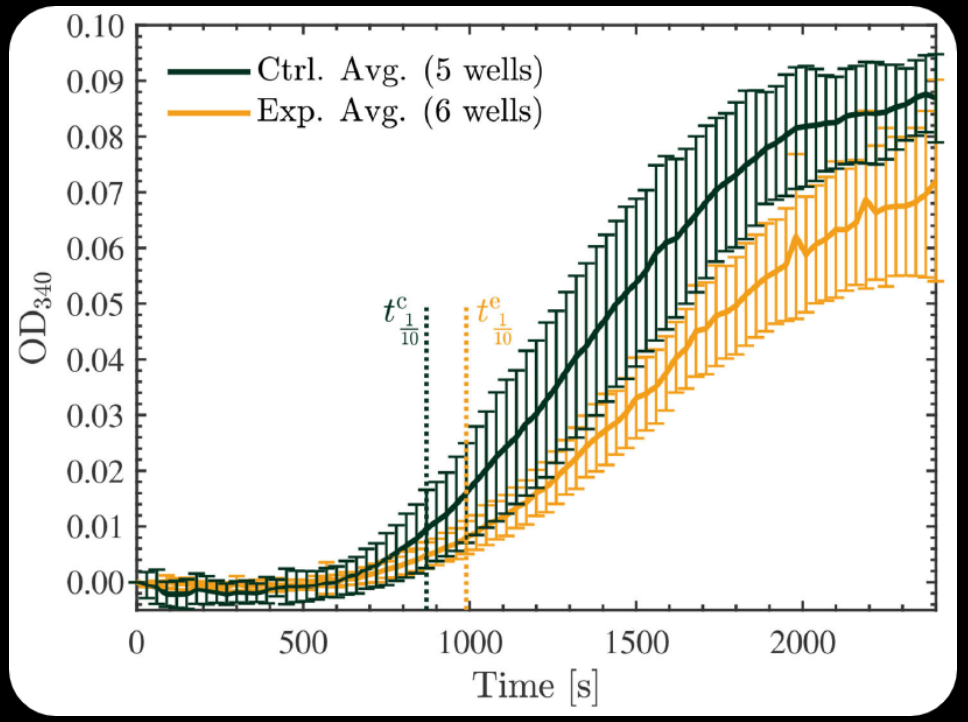

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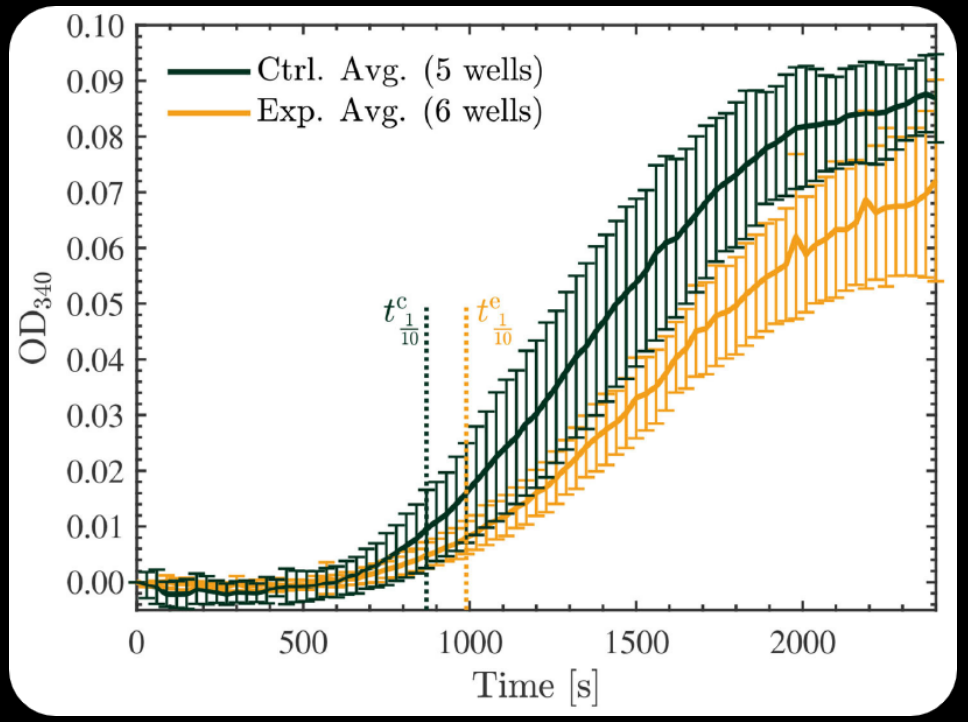

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PBM-Exposed vs. Unexposed

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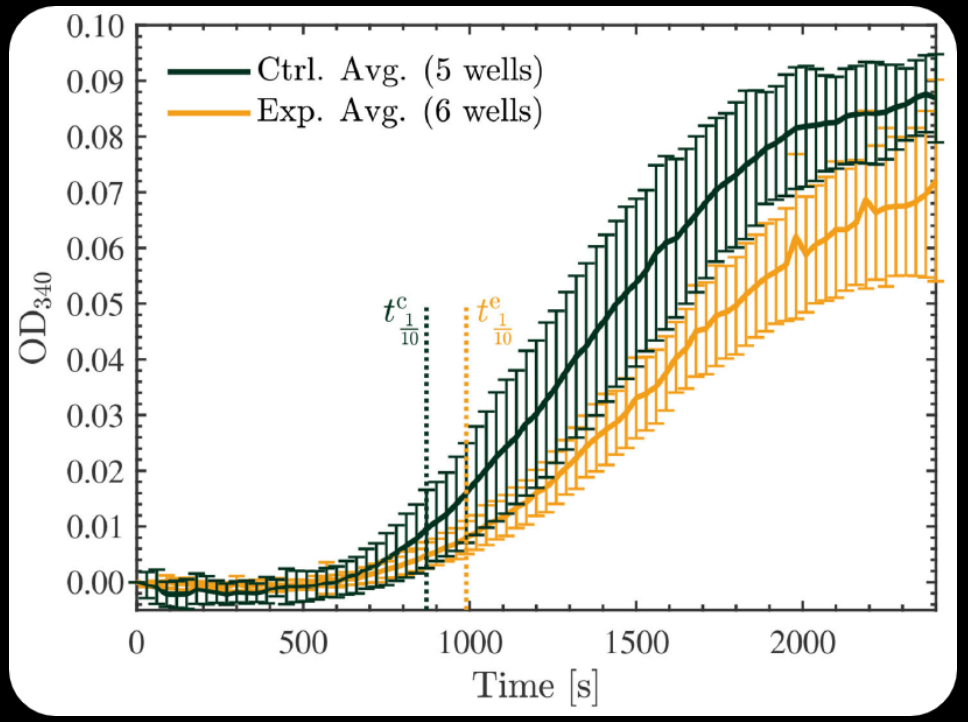

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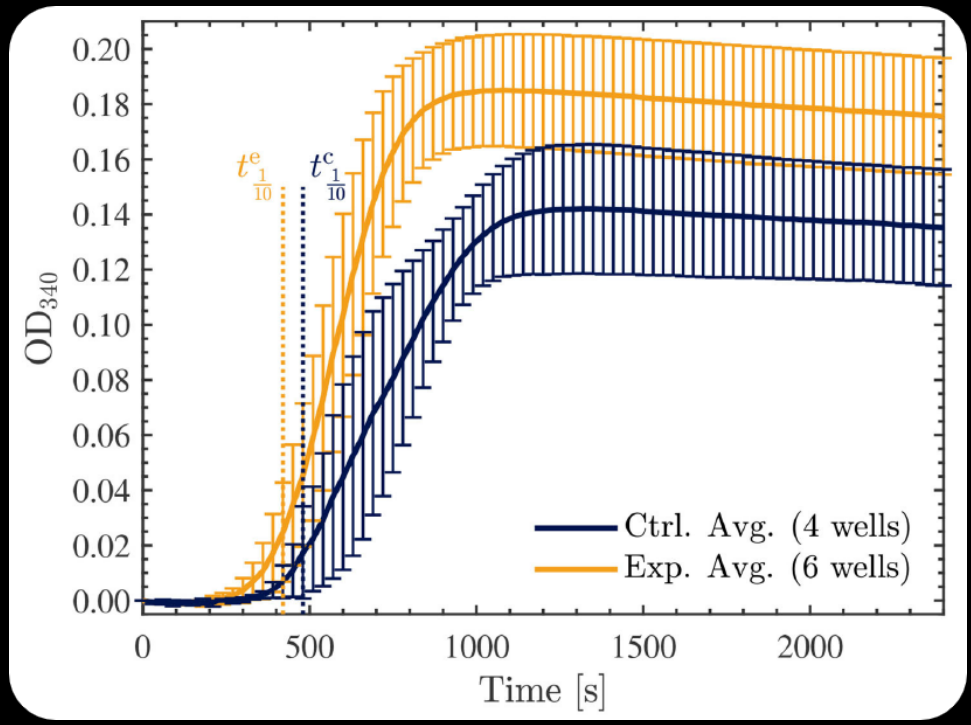

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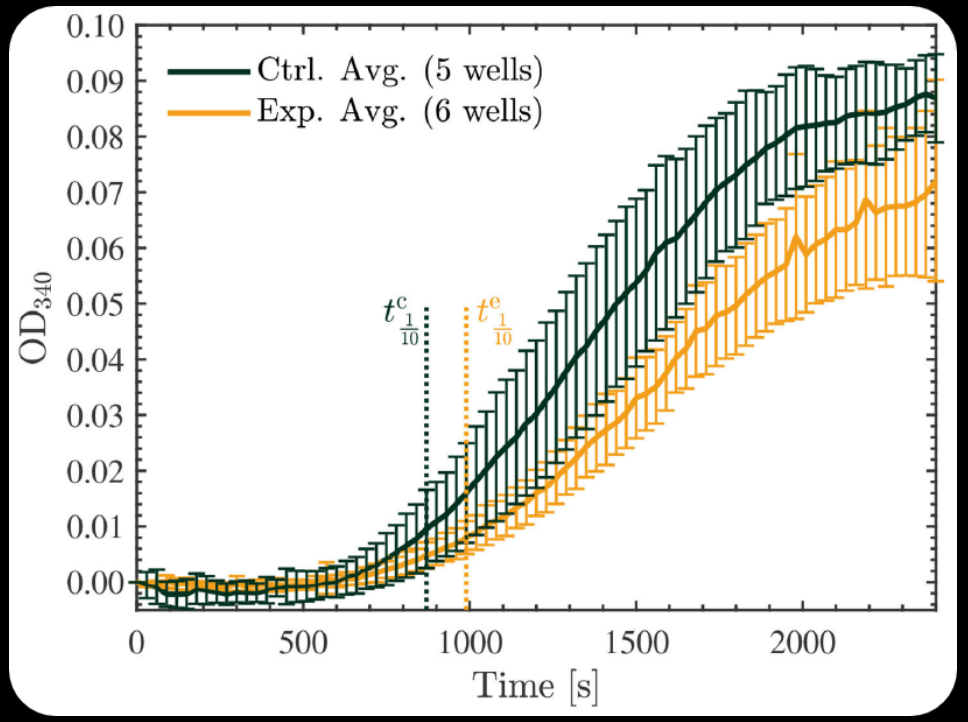

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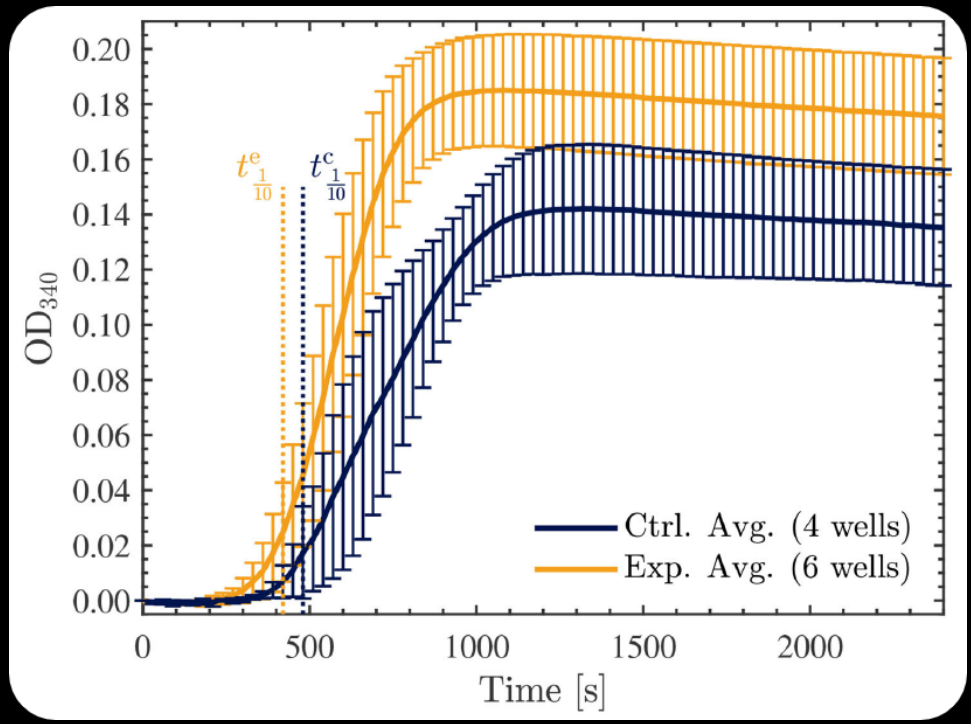

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PBM-Exposed vs. Unexposed

Tenth times calculated for the control and exposed curves were 480 s and 420 s, respectively. Maximal slopes were 17.6 ± 0.5 & 33.2 ± 0.8 mOD/min.

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Preliminary results w/ Raman Spectroscopy demonstrate an effect on secondary structures (α-helices & β-sheets)

Conclusions & Future Directions

Living Cells

An **increased *R*** in both cell lines at 50 kHz and in U251 cells only at 100 kHz: suggests an **inhibitory effect**, that in the case of HeLa cells, was balanced with an increased current.

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Tubulin

Tubulin concentrations representative of the cellular concentration that were exposed to NIR PBM showed a **measurable decrease polymerization rates and total polymer mass**.

Future Directions: (1) *in vitro* experiments that study **the effects of different PBM pulse frequencies**,
(2) in vitro experiments that study **the effects in an environment that more accurately resembles the cellular environment**

Thank you!

Questions?

Backup Slides

GTP Exposure