



Terahertz Radiation Stimulates Neurite Growth in PC12 Derived Neurons :

Preliminary Study

Sergii Romanenko^a, Dominique Appadoo^b, Nicholas Lawler^a, Stuart I. Hodgetts^{a,c}, Alan R. Harvey^{a,c} Vincent P. Wallace^{a*}

^a The University of Western Australia, Perth, 6009 Australia

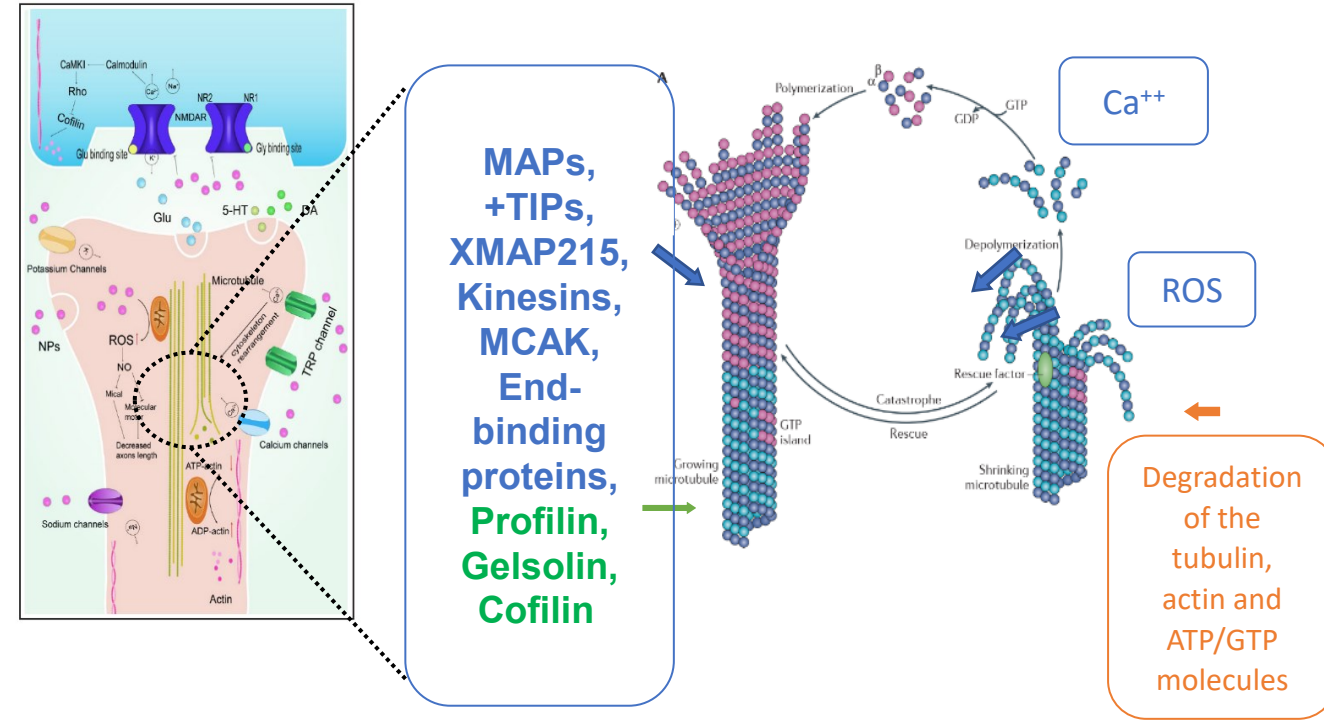
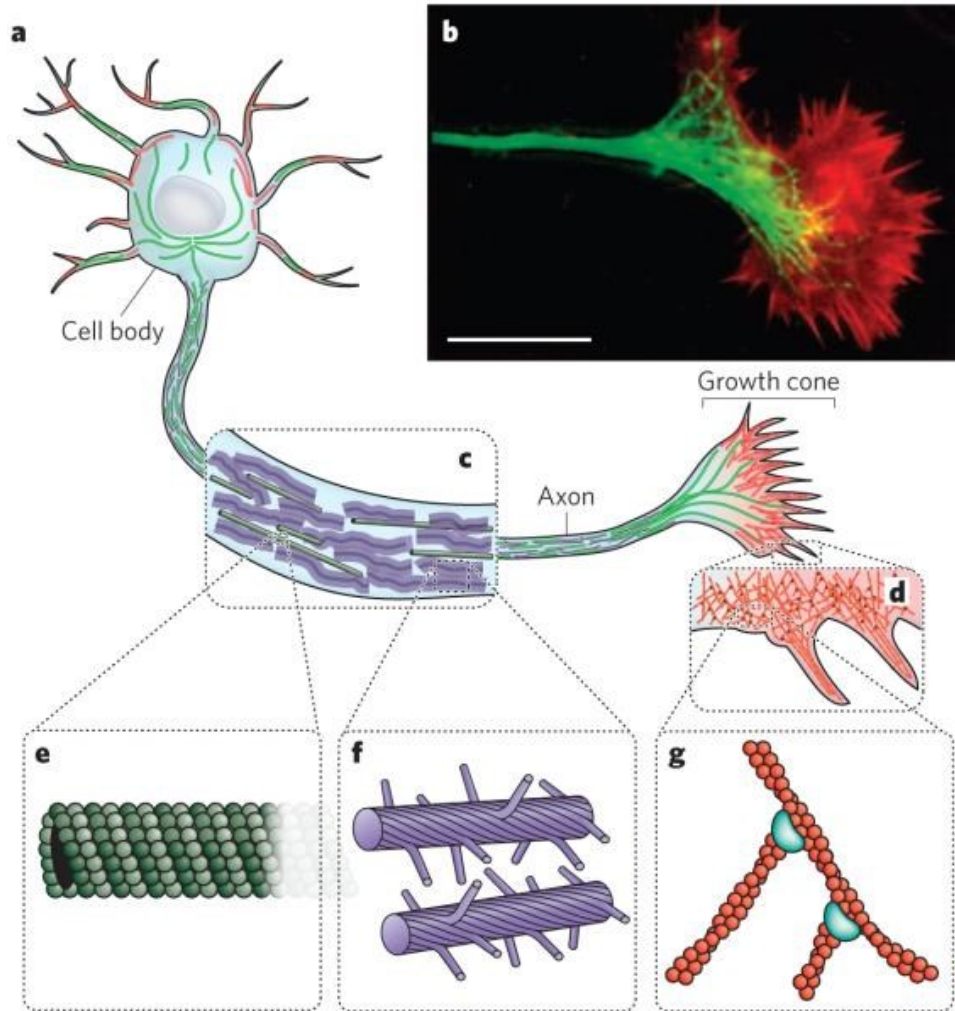
^b Australian Synchrotron (ANSTO), Clayton, VIC 3168, Australia

^c Perron Institute for Neurological and Translational Science, Perth, 6009 Australia

*vincent.wallace@uwa.edu.au

INTRODUCTION

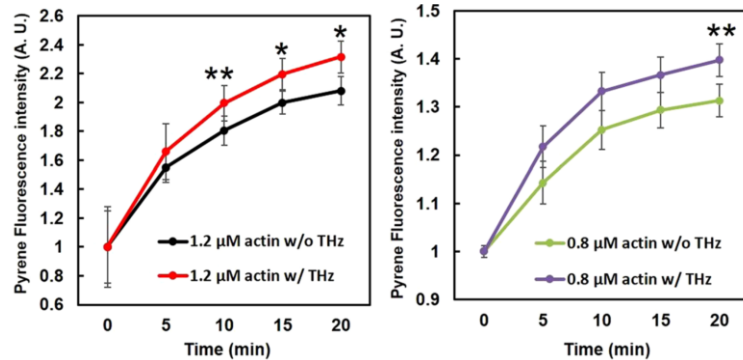
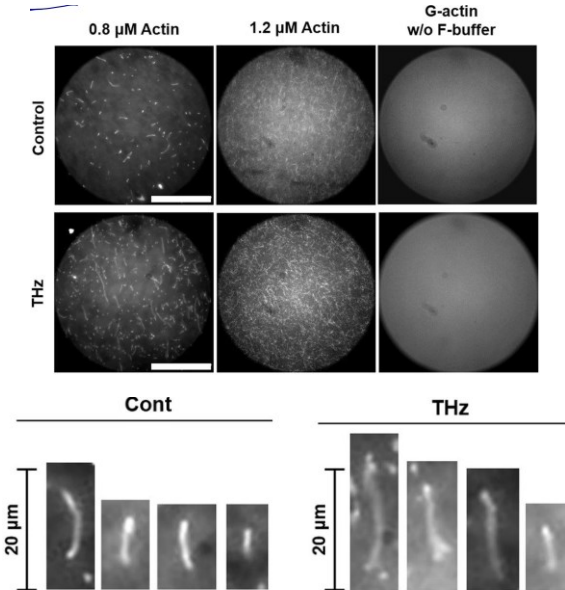
Cytoskeleton: tubulin microtubules and actin microfilaments



Adapted from: D. A. Fletcher, and R. D. Mullins, "Cell mechanics and the cytoskeleton," *Nature*, vol. 463, no. 7280, pp. 485-92, Jan 28, 2010.

Adapted from: Y. Kang, J. Liu, B. Song, X. Feng, L. Ou, L. Wei, X. Lai, and L. Shao, "Potential Links between Cytoskeletal Disturbances and Electrophysiological Dysfunctions Induced in the Central Nervous System by Inorganic Nanoparticles," *Cellular Physiology and Biochemistry*, vol. 40, no. 6, pp. 1487-1505, 2016. DOI: [10.1159/000453200](https://doi.org/10.1159/000453200)

Terahertz (THz) radiation facilitates elongation of cytoskeletal filaments



Monitoring of actin polymerization using pyrene actin.

Adapted from: S. Yamazaki, M. Harata, T. Idehara, K. Konagaya, G. Yokoyama, H. Hoshina, and Y. Ogawa, "Actin polymerization is activated by terahertz irradiation," *Scientific Reports*, vol. 8, no. 1, 2018.

Impact of Sub-Millimeter Waves on the Assembly Kinetics of Microtubules

Xomalin G. Peralta^{1,2}, Jody C. Cantu³, Cesario Z. Cerna³, Ibtissam Echchgadda²

¹ National Academy of Sciences NRC Senior Research Associateship, 4141 Petroleum Road, Joint Base San Antonio Fort Sam Houston, Texas 78234, USA

² Air Force Research Laboratory, Radio Frequency Bioeffects Branch, JBSA Fort Sam Houston, TX, 78234 USA

³ General Dynamics Information Technology, JBSA Fort Sam Houston, TX, 78234, USA

Abstract—Microtubules are highly dynamic intracellular structures critical to many biological functions in eukaryotic cells. They grow and shrink via the addition or subtraction of tubulin heterodimers and, given their polar nature, are expected to interact with electromagnetic fields. We evaluated the effect that externally applied submillimeter (sub-MM) waves have on the assembly kinetics of MTs. We observed a difference in MT formation due to exposure to sub-MM waves of MTs in solution and in cultured cells. Preliminary results indicate an effect on cell morphology and cell metabolism. Understanding the dynamic behavior of MTs opens an avenue to control various fundamental cellular processes.

In this study, we seek to assess and quantify the effect that externally applied sub-MM waves have on the assembly kinetics of MTs both in solution and inside living cells. In particular, we will evaluate the impact of sub-MM waves on cell morphology and cell division.

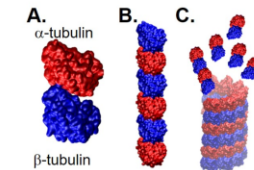
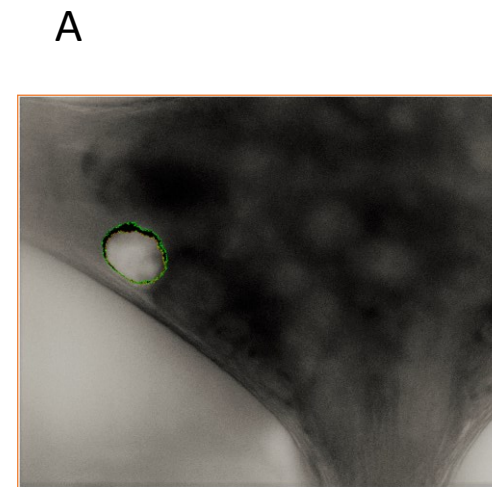


Fig. 1. Microtubule (MT) Formation. (A) Tubulin heterodimer. (B) Protofilament. (C) MT assembly from tubulin heterodimers. Red: α -tubulin, Blue: β -tubulin.

I. INTRODUCTION

Microtubules (MTs) are tube-shaped intracellular structures that are known to provide cells with mechanical stability and play a major role in many critical cellular functions such as cell motility, development, division, and communication [1]. MTs are formed by the assembly of



A: The fluorescent photograph of representative ganglia with NI neuron loaded with Lucifer Yellow (LY).
 B: photograph is enlarged picture NI neuron outlines from the same ganglia before and after MMW. Green fill corresponds to the cell size after 2 min MMW exposure showing the cell shrinkage from control size. Data obtained in Dr. V. Wallace lab.



THz/Far-IR Beamline capabilities & applications at the Australian Synchrotron

Dom Appadoo

*dom.appadoo@ansto.gov.au
as-farir@ansto.gov.au*



Bruker IFS125/HR FT spectrometer

Beamsplitters Energy/cm⁻¹

- Multi/Mylar 5 – 25
 15 – 40
 30 – 630
- Ge/KBr 450 – 4800

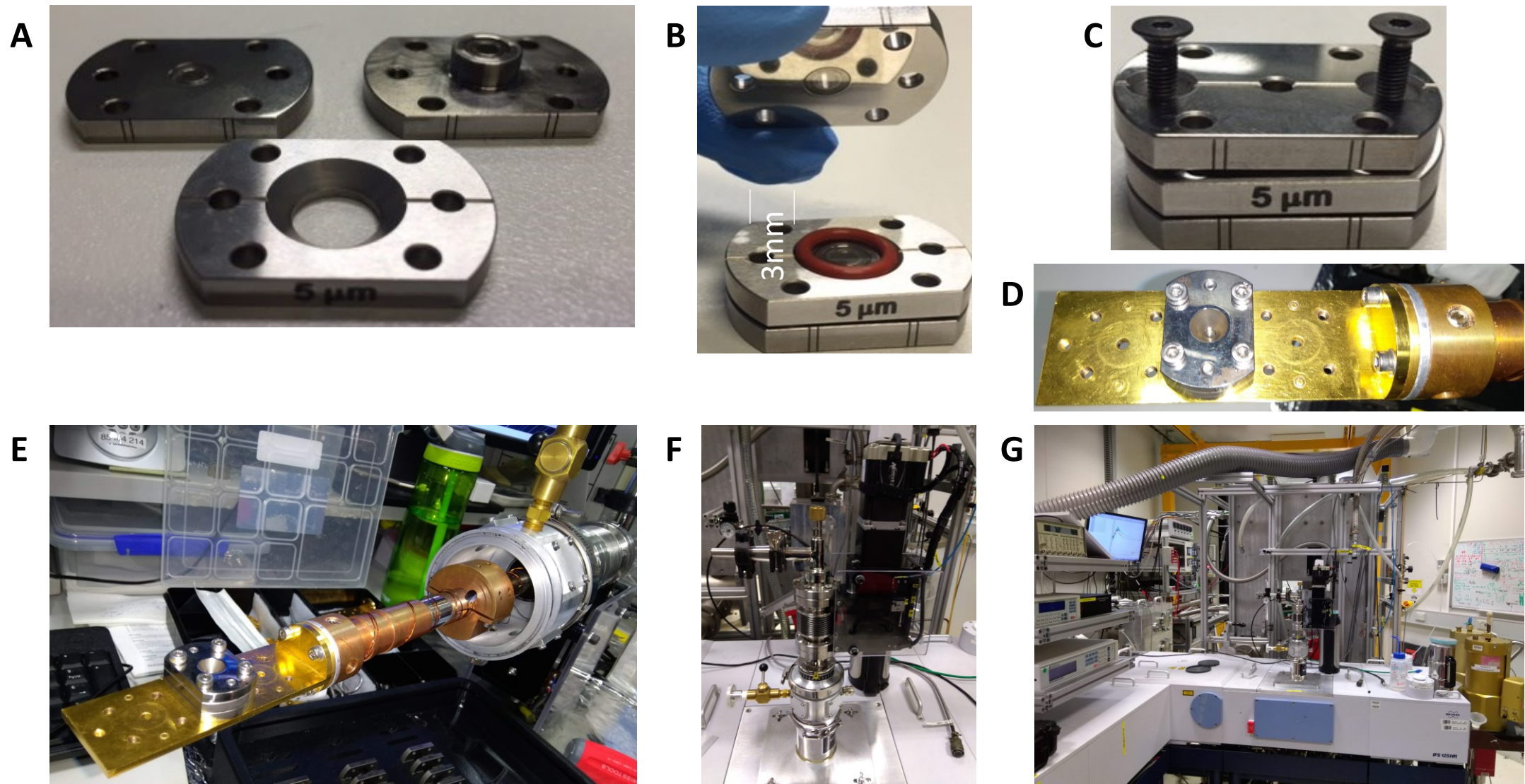
Detectors Energy/cm⁻¹

- Si bolometer 10 – 370
- Si:B photodetector 300 – 1850
- DTGS 100 – 3000
- MCT_N 700 – 5000
- MCT_M 600 – 5000

The Bruker IFS125 spectrometer is equipped with a 6 μm Mylar beam splitter.

The synchrotron is a broadband source emits THz radiation is limited to 10 - 650 cm^{-1} (0.3347 – 19.5 THz, $\lambda \sim 15.38 - 1000 \mu\text{m}$) by the beam splitter.

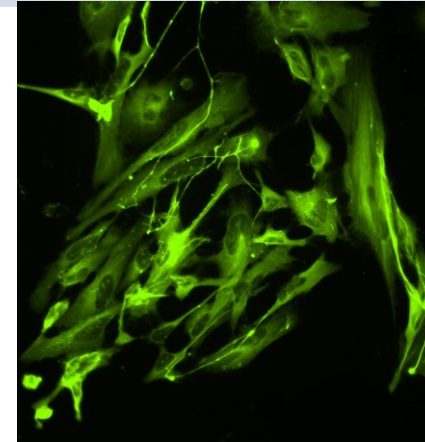
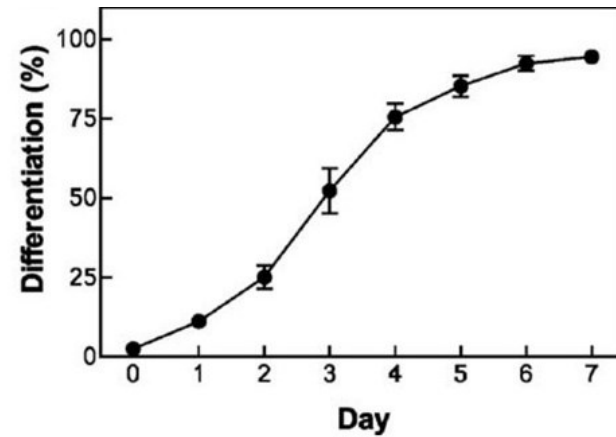
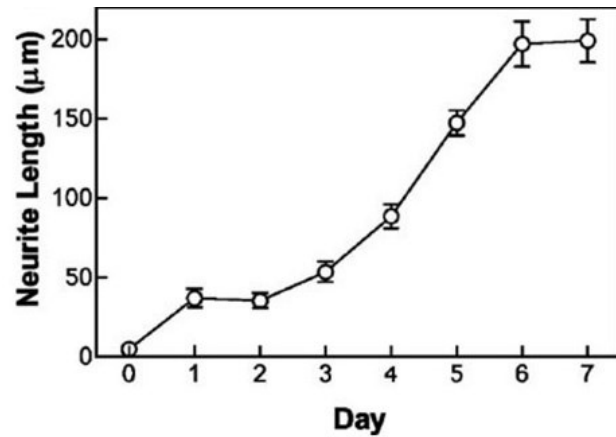
Methods



THz exposure setup: A – Diamond liquid cells (DLC) of three metal parts; B – DLC assembly with 5 mkm spacer and seal ring protecting the content of the DLC being evaporated in the vacuum environment of the spectrometer; (C) – complete DLC assembly; (E) – DLC on placed Janis ST100 cryostat multi-sample holder; (F) - Janis ST100 cryostat multi-sample holder mounted on Bruker IFS125 spectrometer; (G) – the side view of the Bruker IFS125 spectrometer chamber with mounted motorized Janis ST100 cryostat and connected to far-IR/THz beamline at the Australian Synchrotron (ANSTO).

Methods:

fast development of PC12 into neurons is a convenient model to study the cytoskeletal dynamics

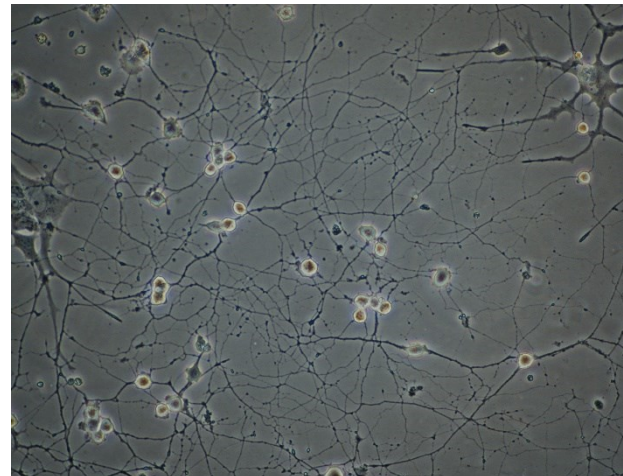
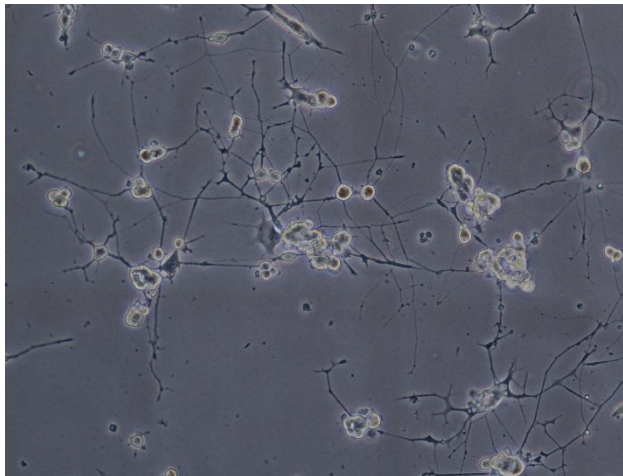


Effect of NGF treatment on neurite growth

K. Das, "Assessment of PC12 cell differentiation and neurite growth: a comparison of morphological and neurochemical measures," *Neurotoxicology and Teratology*, vol. 26, no. 3, pp. 397-406, 2004.

Day 5

Day 10



Progress in development of pheochromocytoma PC 12 cell culture on day 5 and day 10 after treatment with the differentiation cocktail (100 ng/ml NGF, NT3 and BDNF). Cell were grown on Geltrex basement membrane matrix. Data obtained in Prof. Stuart I. Hodgetts lab

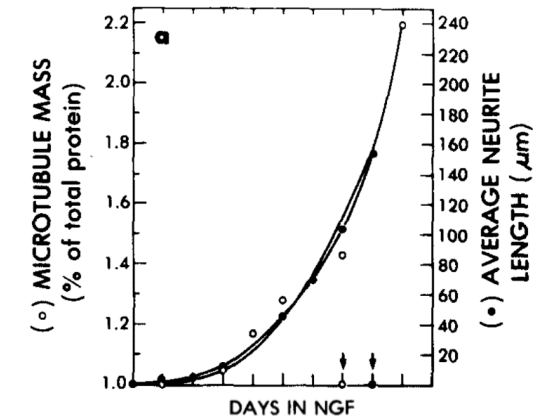
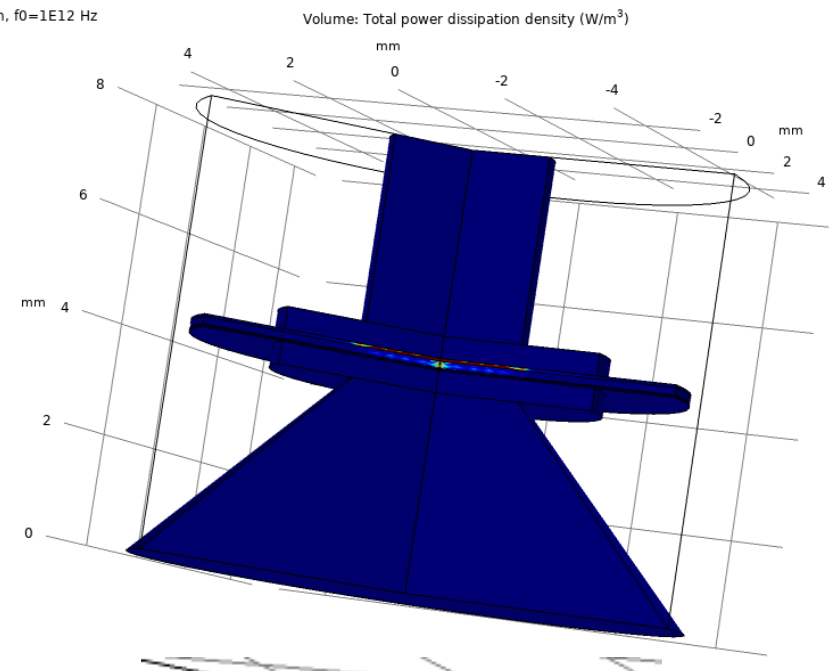


FIGURE 2 Quantitative analysis of neurite length, microtubule mass, and microtubule protein levels during PC12 cell neurite extension. (a) Microtubule mass, determined by quantitative immunoblotting of 15 µg detergent-extracted cytoskeleton protein with antitubulin monoclonal antibodies, and average neurite length for 200 neurites measured each day, are plotted as a function of culture time in the presence of NGF. Arrows indicate data points collected after 2 d (○) or 3 d (●) of NGF withdrawal. (b) Total

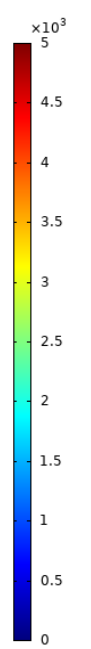
D. G. Drubin, S. C. Feinstein, E. M. Shooter, and M. W. Kirschner, "Nerve growth factor-induced neurite outgrowth in PC12 cells involves the coordinate induction of microtubule assembly and assembly-promoting factors," *Journal of Cell Biology*, vol. 101, no. 5, pp. 1799-1807, 1985.

Simulations: Evaluation of power absorbed and potential effect of sample temperature alteration

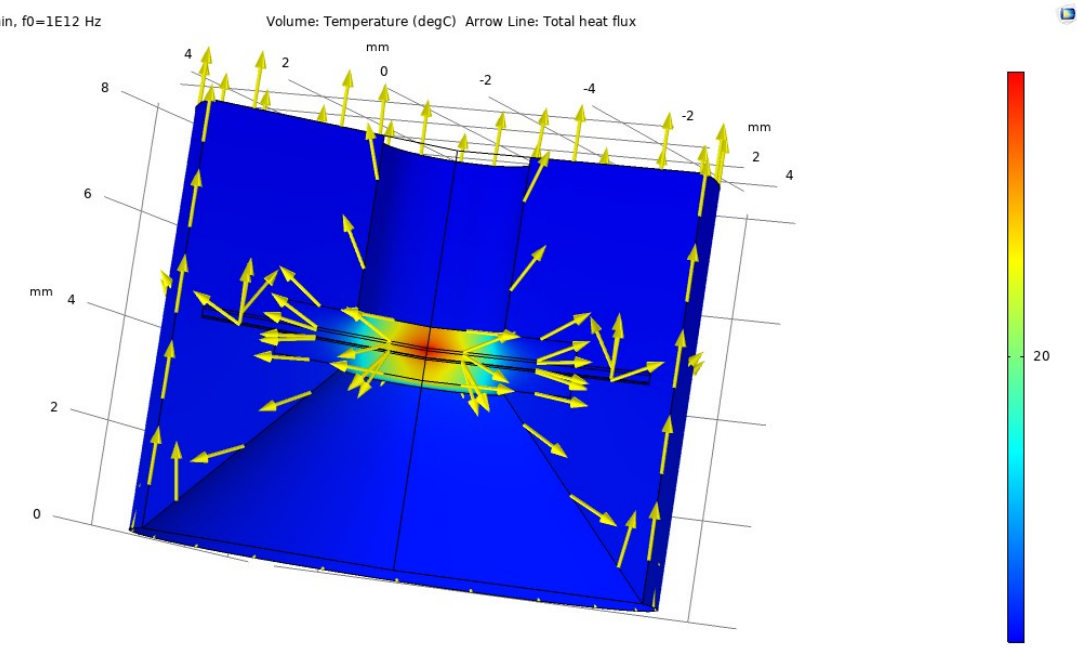
2706: Time=45 min, f0=1E12 Hz



2706: Time=45 min, f0=1E12 Hz



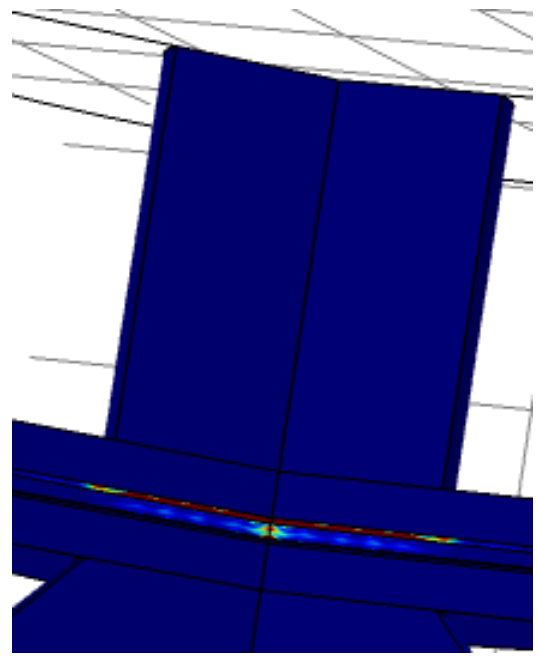
2706: Time=45 min, f0=1E12 Hz



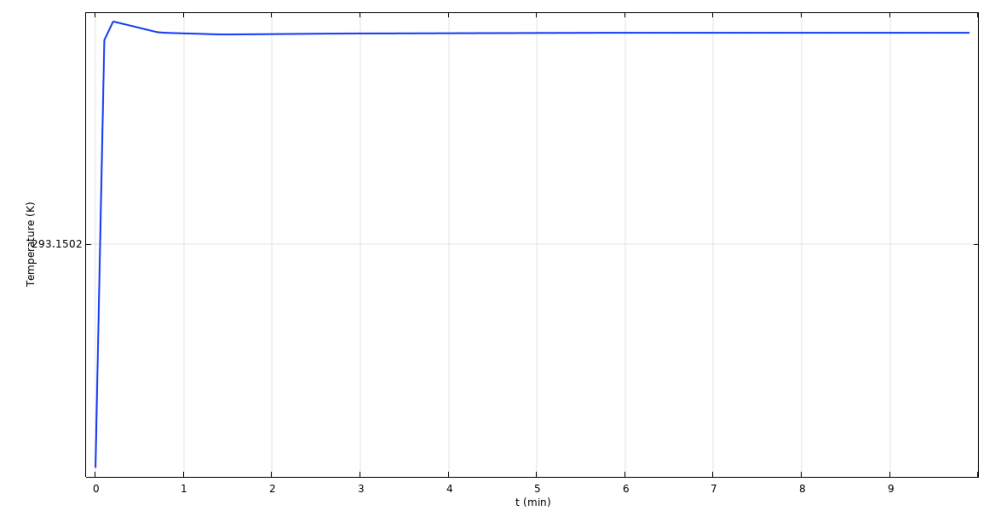
z
y
x

z
y
x

z
y
x



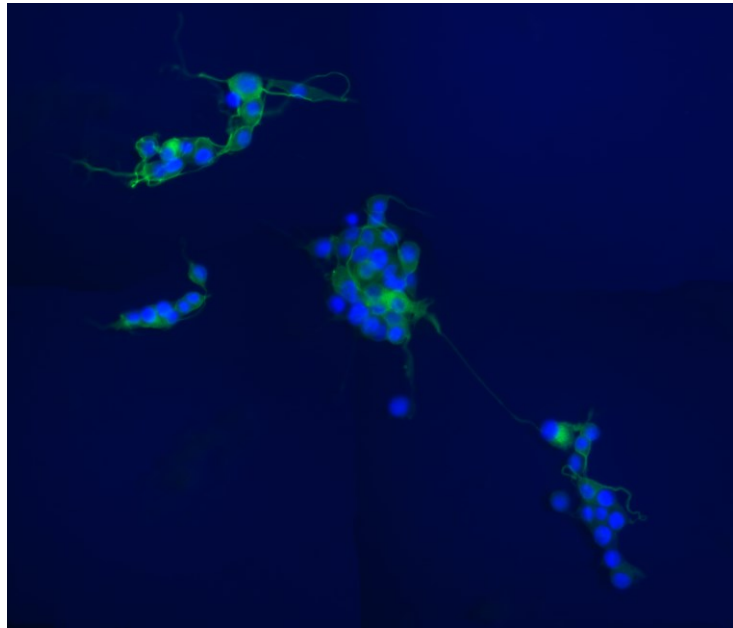
Temperature alterations caused by THz radiation were neglectable and stable over the time of the experiment



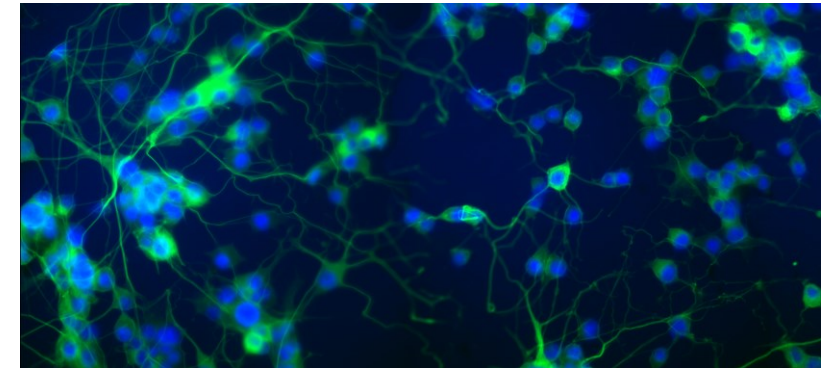
After ANSTO exposure fixed cells cytoskeleton staining

PC12 were set into differentiation and exposed every day beginning day 1 and up until day 3.
Different coverslips were fixed at different stages of differentiation. Cells were fixed 24 H after last THz exposure.

Cntrl: Sham



Test: 45 min exposure to THz radiation

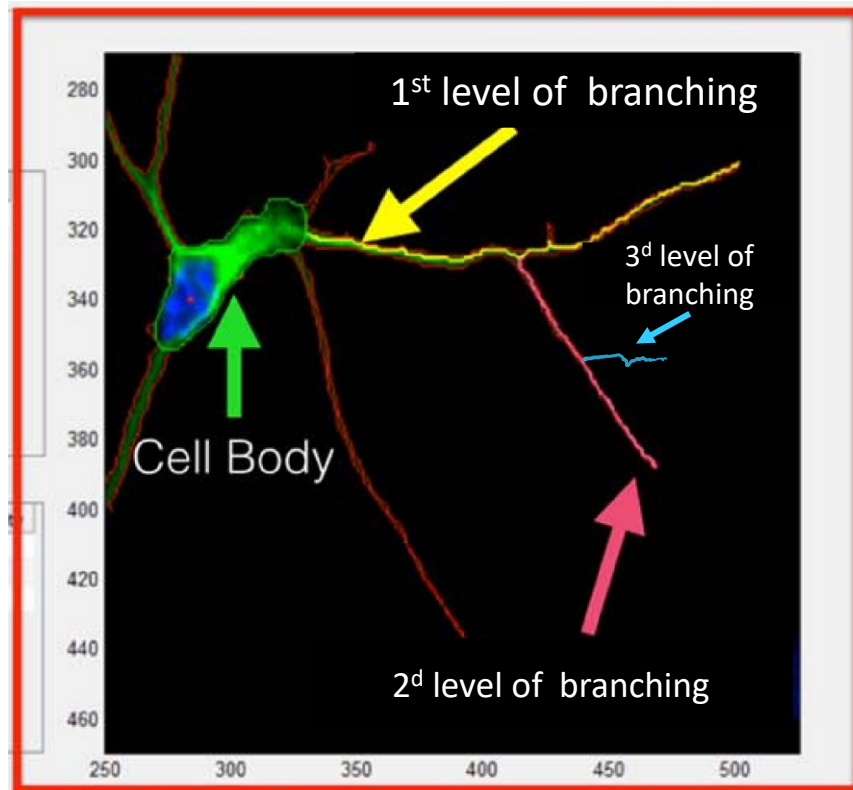


β III Tub / Hoechst

Sham – cells treated exactly same way as **Test** cells except being exposed to THz radiation

Neurite length estimation

β III -Tubulin

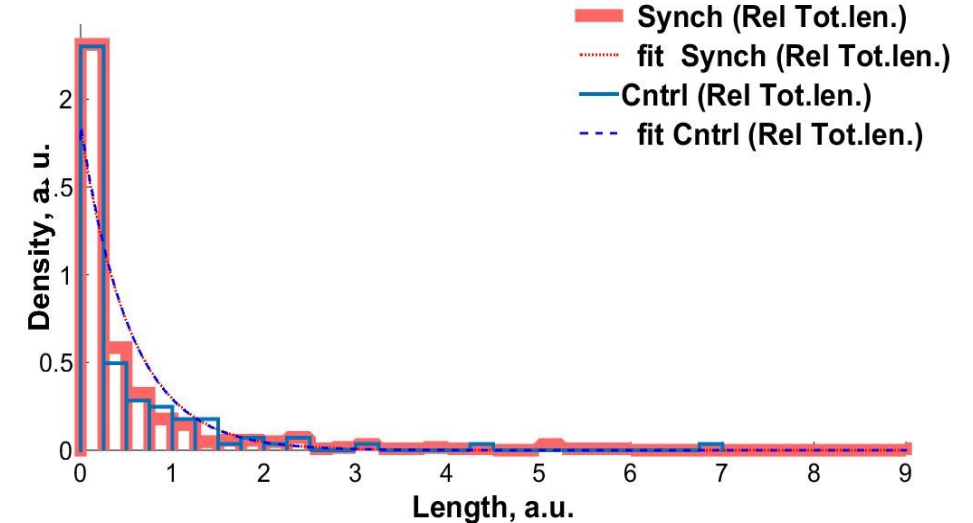


K. H. Ong, J. De, L. Cheng, S. Ahmed, and W. Yu, "NeuronCyto II: An automatic and quantitative solution for crossover neural cells in high throughput screening," *Cytometry Part A*, vol. 89, no. 8, pp. 747-754, 2016

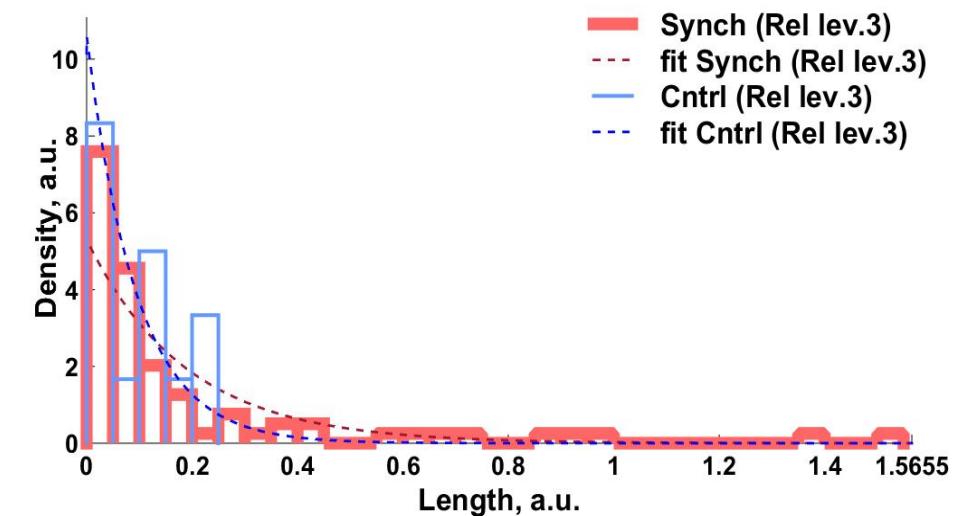
Histograms representing density distributions and exponential distribution fits for neurite length estimation in control (Cntrl, blue) and test conditions (ANSTO-THz, red). Cell clustering and neurite tracing performed with NeuronCyto II software [4]. Neurite length was estimated in relation to the total cell length: (A) – Distribution of the total neurite normalized length (length of all traced neurites emerging from soma); (B) – Distribution of neurite length estimated on the third level of branching (length of branches originating from a primary, then secondary neurites attached to soma).

Note, while total relative neurite length distributions for both conditions overlap significantly (i.e. there is little effect on already formed neurites), the length distribution of third level neurites (most intensely undergoing developmental perturbations) demonstrates a subset of much longer neurites (in the range 0.2 and longer).

A



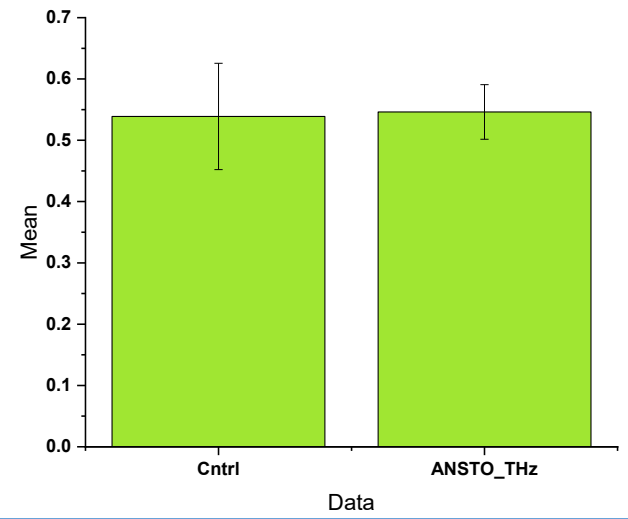
B



β III -Tubulin

Total Relative length

Mean length



Level 3 Relative length

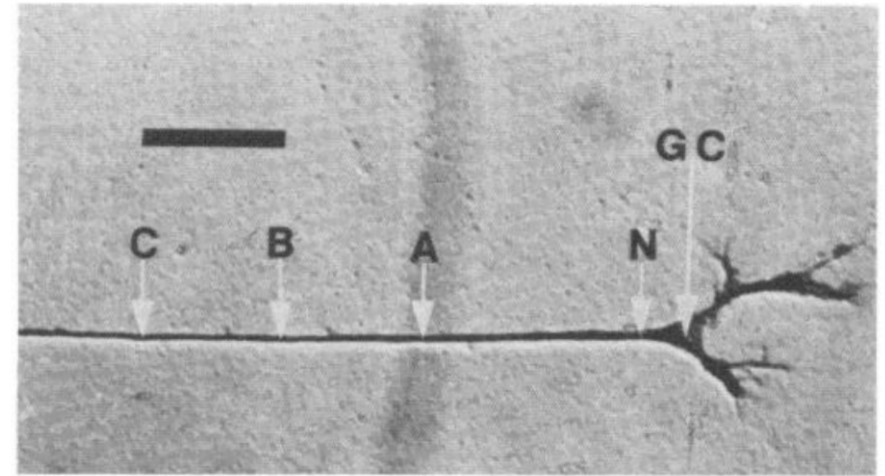
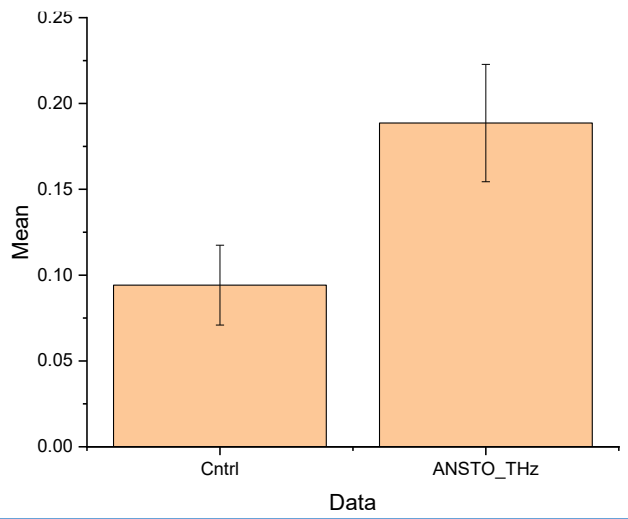
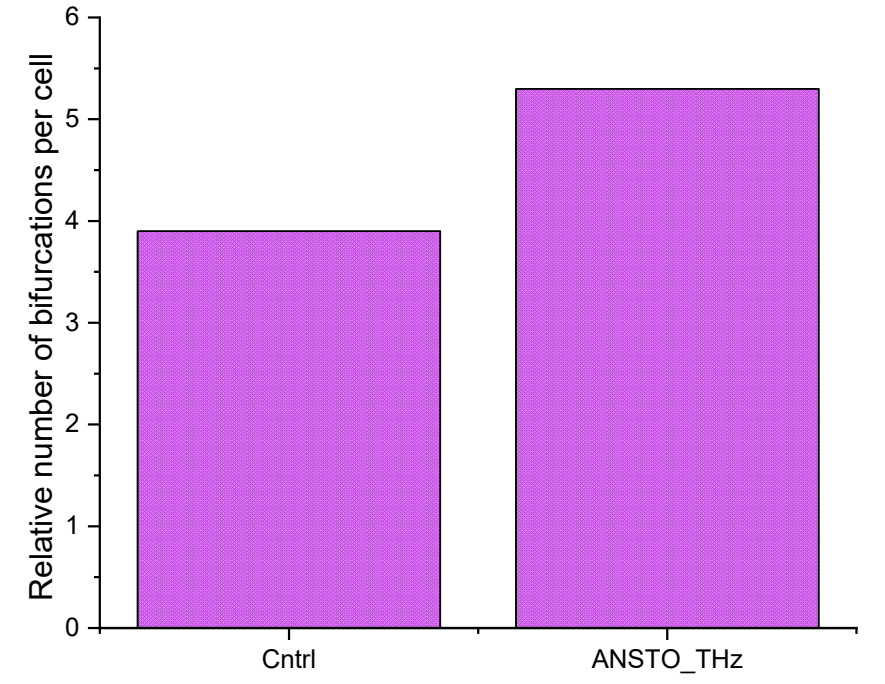
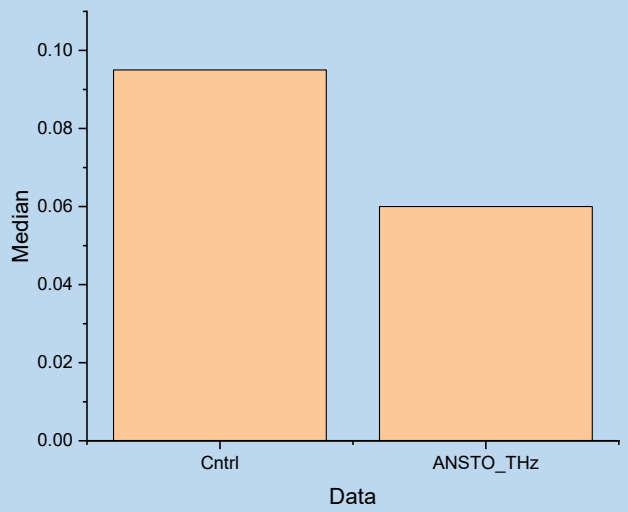
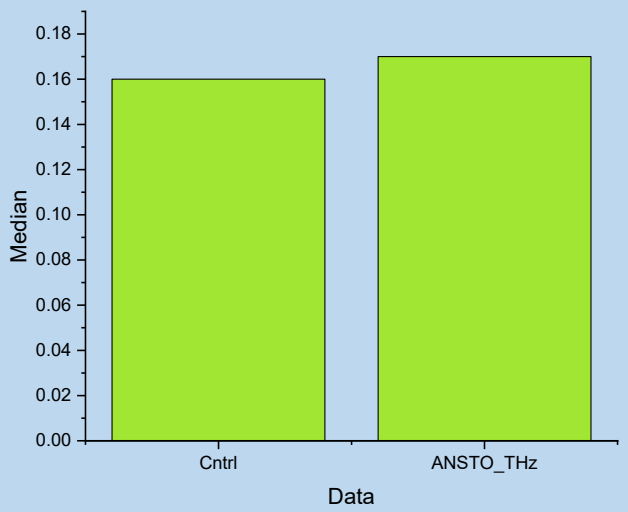


Figure 1. Micrograph of fixed chick dorsal root ganglion axon in culture, indicating sites of measurement (C, B, A, and N) cf. Figure 2. Bar = 10 μ m. Bodian stain with intensifier (see Katz and Watson, 1984).

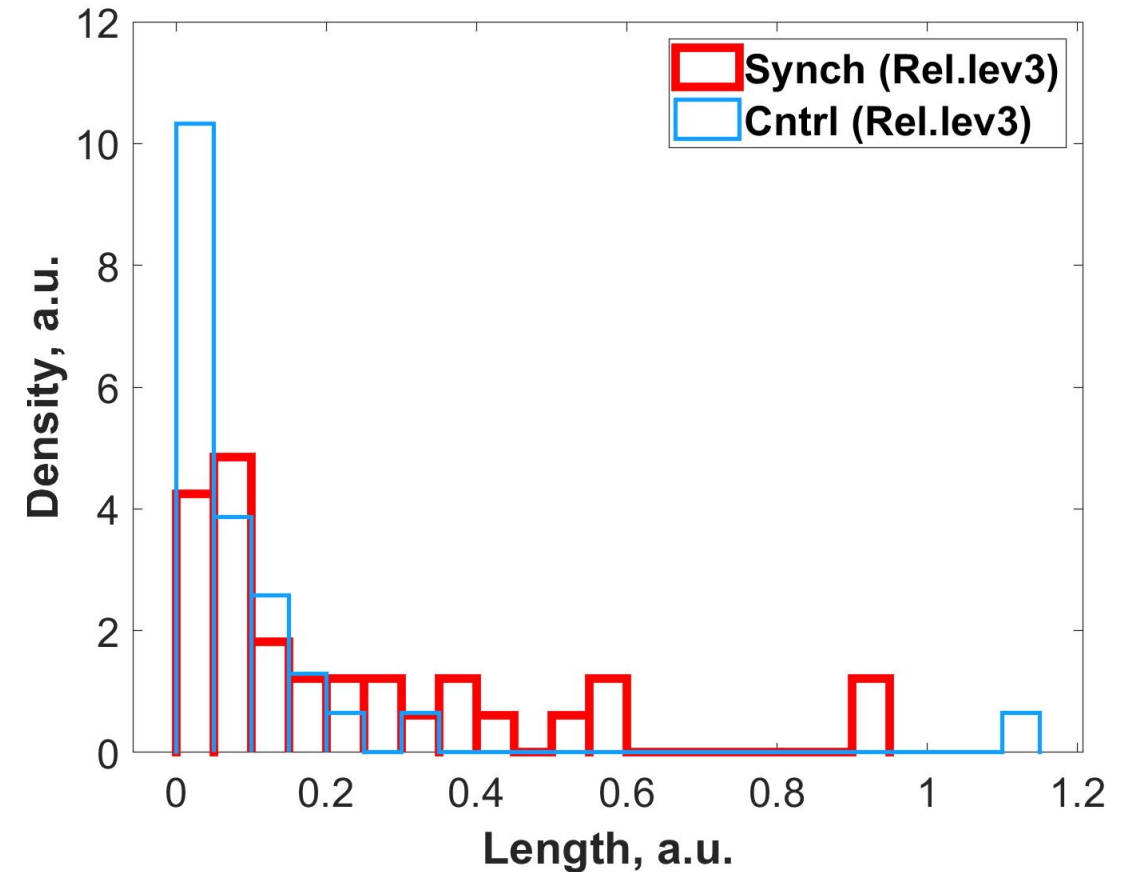
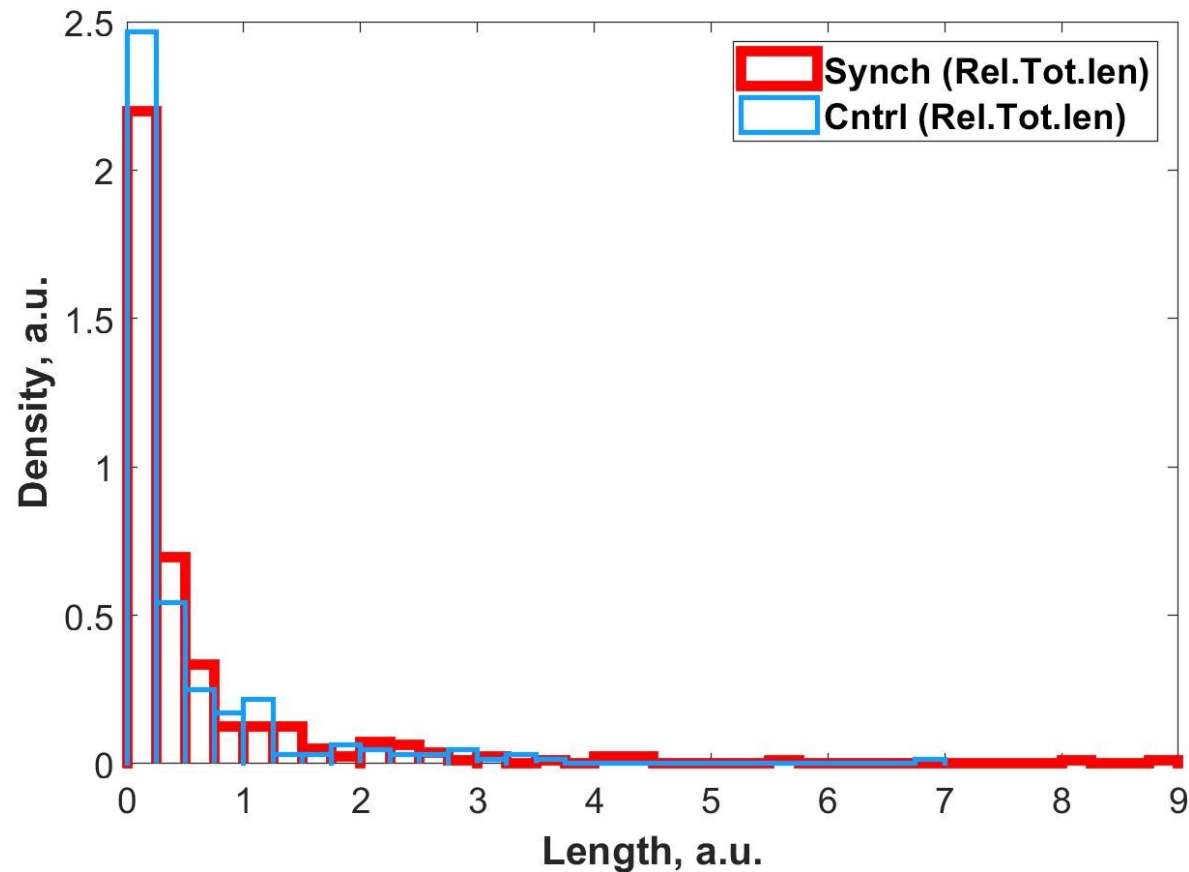
Median value of length



MAP2

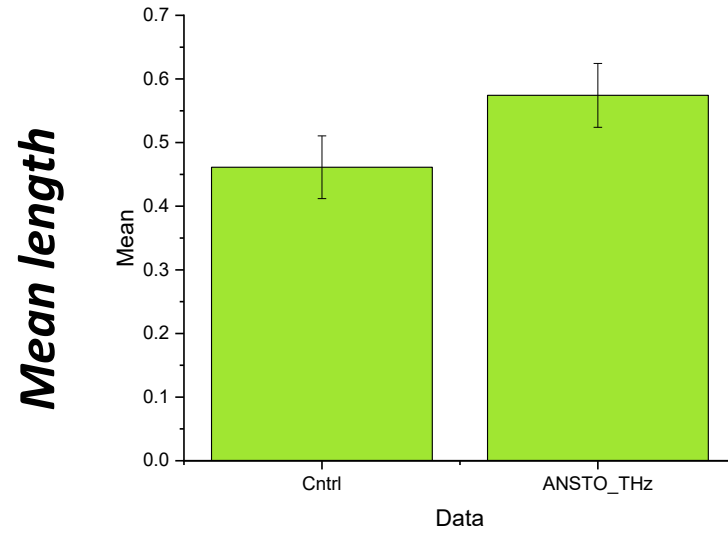
Microtubule-associated protein 2

MAP2 functions to stabilize neuronal shape by promoting microtubule synthesis and cross-linking with other components of the cytoskeleton (Murphy et al. 1977; Matus 1988)

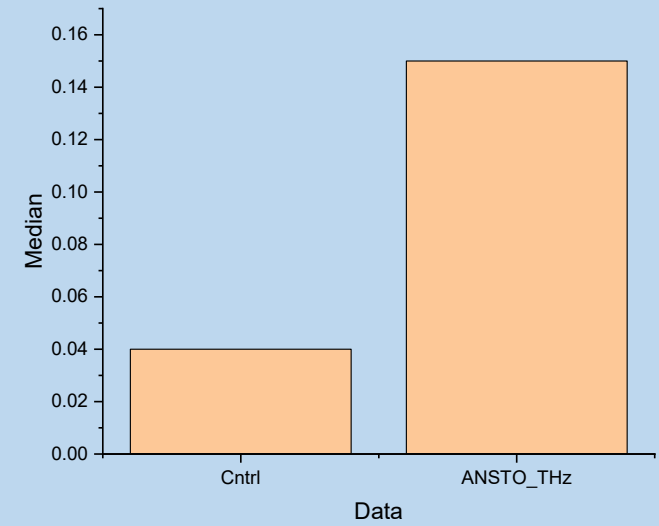
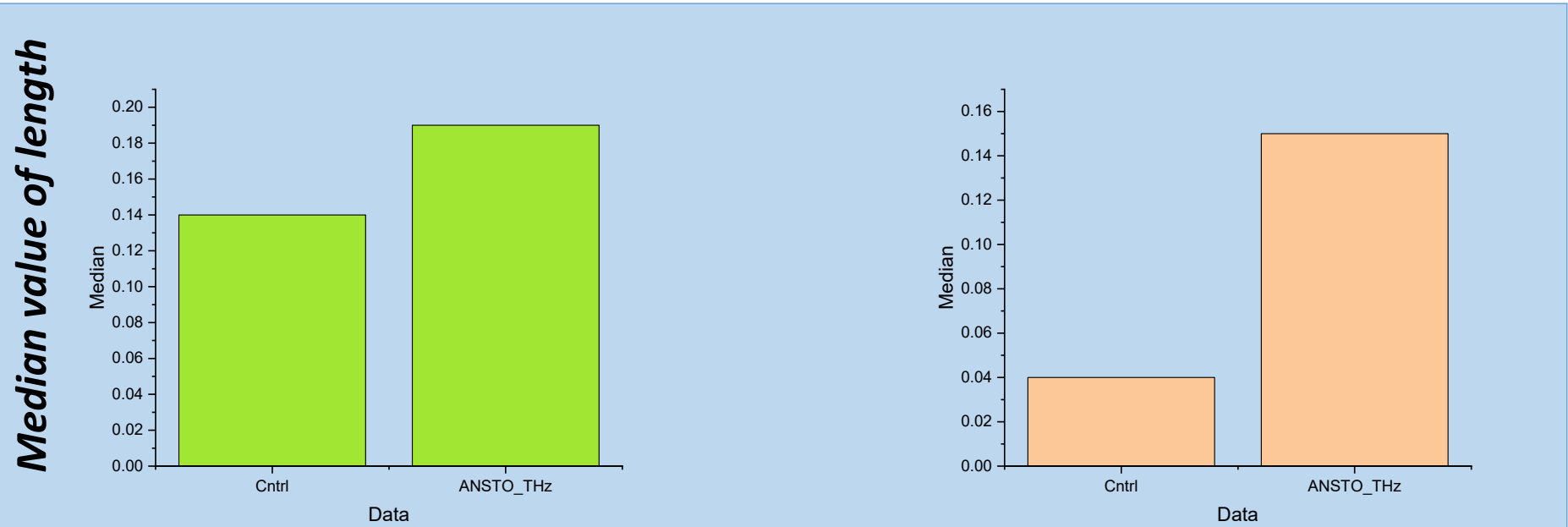
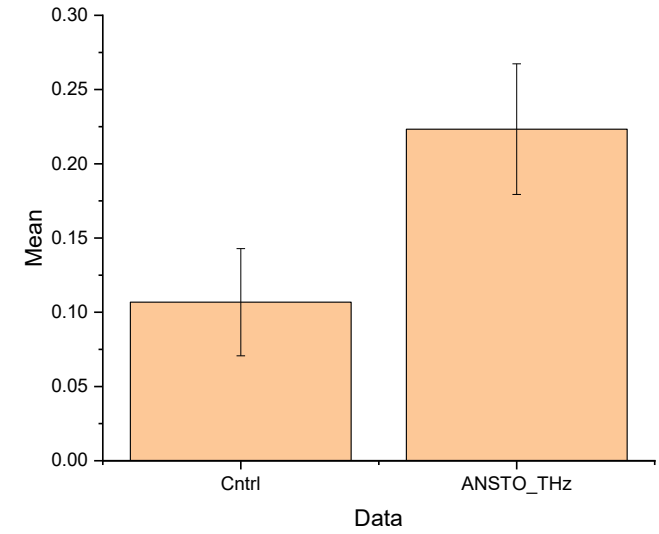


MAP2

Total Relative length



Level 3 Relative length



Conclusions:

- **Preliminary study demonstrated the effect of synchrotron THz radiation on differentiation and structural development of neuron-like cells *in vitro*.**
- **Results support previous experimental studies** (X.G. Peralta et.al. and S. Yamazaki et.al.), **where THz radiation facilitated polymerization of actin filaments and microtubules.**
- **Considering that both microfilaments and microtubules undergo intense restructuring during cell development, our results correlate with reports of enhanced *ex vivo* polymerization of cytoskeletal structures.**
- Study provides an initial insight into one of the potential mechanisms of synchrotron THz radiation interaction with living tissues, and neurons in particular. It lays out a basis for further research.

*

[1] X. G. Peralta, J. C. Cantu, C. Z. Cerna, and I. Echchgadda, "Impact of Sub-Millimeter Waves on the Assembly Kinetics of Microtubules," *43rd International Conference on Infrared, Millimeter, and Terahertz Waves (Irrmmw-Thz)*, 2018.

[2] S. Yamazaki, M. Harata, T. Idehara, K. Konagaya, G. Yokoyama, H. Hoshina, and Y. Ogawa, "Terahertz irradiation stimulates actin polymerization," *43rd International Conference on Infrared, Millimeter, and Terahertz Waves (Irrmmw-Thz)*, 2018.

LIMITATIONS OF IR SPECTROSCOPY

