

# Detection and Quantification of Tunneling Nanotubes Using 3D Volume View Images

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## Materials

Name	Company	Catalog Number	Comments
35 mm dish with 14 mm well size made of #1.5 cover slip	Cellvis	D35-14-1.5-N	Imaging dish used to seed cells for staining experiments
A $\beta$ (1-42) 1 mg	AnaSpec	#64129	Oligomers of amyloid beta to treat the cells
Alexa flour 488 Goat Anti-rabbit IgG (H+L)	Invitrogen	A11070	Secondary antibody for phospho-PAK1
Biological Safety Cabinet	Thermo Scientific (MSC Advantage)	51025411	Provide asepetic conditions duirng cell culture
CO <sub>2</sub> Incubator	Thermo Electron Corporation (Heraeus Hera Cell 240)	51026556	For growing cells at or near body temperature
Confocal Laser Scanning Microscope	ZEISS (Carl Zeiss)	LSM 880	Able to generate three-dimensional images of large specimen at super-resolution
DABCO [1,4-Diazobicyclo-(2,2,2) octane]	Merck	8034560100	Anti-bleaching reagent
DAPI (4',6-diamidino-2-phenylindole)	Sigma	D9542-1MG	Neuclear stainer
DMEM media	Gibco	11965092	Used for the preparation of 100uM of A $\beta$ (1-42)
DMEM/F12 (1:1 mixture of DMEM and Ham's F12)	Gibco	12500062	Culture media for SH-SY5Y
DMSO (Dimethyl sulphide) Cell culture grade	Cryopur	CP-100	Cell culture grade used as dissolving agent for Retinoic acid
DMSO (Dimethyl sulphide) Molecular grade	Himedia	MB058	Used as one of the dissolving agent for the lyophilized A $\beta$ (1-42)
Fetal Bovine Serum	Gibco	16000044	Major supplement for Culture media (US origin)
Formalin Fixative (Neutral buffered 10%)	Sigma Aldrich	HT5014-120ML	Component in the Karnovsky's fixative solution
Glutaraldehyde (Grade I, 25% in H <sub>2</sub> O)	Sigma	G5882	Component in the Karnovsky's fixative solution
HFIP (1,1,1,3,3,3-hexafluoro-2-propanol ) solution	TCI	H024	Used to dissolve A $\beta$ (1-42) 1 mg

Image Processing/ Analysis Software: FIJI (ImageJ)	National Institute of Health (NIH)		Used to process/analyze the images and to differentiate the TNTs from neurites using its plugin named "volume viewer".
Lyophilizer	Christ, Alpha	2.4 LDplus	0.05 mg aliquots of A $\beta$ (1-42) can be stored in -20 °C after lyophilization only
Penicillin-Streptomycin-Neomycin Mixture	Thermo fisher Scientific	15640055	Antibiotic mixture
Phalloidin-iFlor 555	Abcam	ab176756	F-actin binding stain
Phospho-PAK1 (Thr423) /PAK2 (Thr402) [Rabbit]	CST	#2601	Primary antibody
Polyclonal Antibody to Tubulin Beta 3 (TUBb3)	Cloud clone	PAE711Hu01	Primary antibody
Retinoic acid	Sigma-Aldrich	R2625-50MG	Differentiating reagent
Saponin	Merck	8047-15-2	Detergent used in the Incubation buffer in immunostaining
Water bath sonicator (Quart, Drain valve Heater)	Ultrasonic Cleaner	3.0 L/3.2	Sonicator used to dissolve A $\beta$ (1-42) stock, after DMSO adding to it during the preparation of 100 $\mu$ M A $\beta$ (1-42)
ZEN Microscopy software	ZEISS (Carl Zeiss)		Imaging software to acquire confocal microscopy images with smart automation