

Materials List for

# Measurement of 3-Dimensional cAMP Distributions in Living Cells using 4-Dimensional (x, y, z, and $\lambda$ ) Hyperspectral FRET Imaging and Analysis

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## Materials

Name	Company	Catalog Number	Comments
Attofluor Cell Chamber	Invitrogen	A7816	Attofluor contains steel cell chambers and a rubber O-ring. Cell chamber holds the coverslip and O-ring provides a lock in mechanism to hold the buffer in cell chamber with out leakage
Dimethyl Sulfoxide (DMSO)	Fisher Scientific	BP231-100	Solvent used to prepare stock solution forskolin.
DRAQ5 Fluorescent Probe Solution	Thermo Scientific	62252	Nuclear label
Dulbecco Modified Eagle Medium (DMEM)	Gibco	11965-092	Contains nutrients and growth factors for the cells to grow and divide in the culture dishes.
Fetal Bovine Serum (FBS)	Sigma	F6178	Growth factor suppliment that is added to culture medium, DMEM
Forskolin	Sigma	F3917	Adenyly cyclase activator.
H188 Cyclic AMP FRET biosensor	Netherland Cancer Institute, Dr. K. Jalink	Gift	Plasmid encoding Turquoise (donor fluorophore), Venus (acceptor fluorophore), and binding domain obtained from Epac.
Image J	image.net	Free download	Another image processing platform used to extract spectral information and image processing.
Integrating Sphere	Ocean Optics	FOIS-1	Used to measure illumination intensity of the laser line at different laser intensities (?).
Laminin Mouse Protein, Natural	Invitrogen	23017-015	Coverslips are coated with laminin and this helps in cell attachment, growth and motility of the cell.

Lipofectamine 3000 Transfection Kit	Invitrogen	L3000-015	Transfection reagent used to transfect cells with H188 FRET biosensor
MATLAB	Mathworks	R2019a	Image processing operations (linear unmixing and FRET efficiency calculations) are performed by writing custom programs in MATLAB programming environment
Nikon A1R confocal microscope	Nikon Instruments	Nikon A1R	Spectral image acquisition is performed using confocal microscope.
Nikon Elements Software	Nikon Instruments	Software dongle	used to export and handle nd2 image files (multidimensional image files) that are acquired using Nikon A1R
NIST-Traceable Calibration Lamp	Ocean Optics	LS-1-CAL-INT	A lamp with a known spectrum for use as a standard
PBS pH 7.4 (1X)	Gibco	10010-023	commonly used buffer during cell culture
Pulmonary Microvascular Endothelial Cells (PMVECs)	In house - Cell culture core, University of South Alabama	Isolated from Rat pulmonary microvasculature	PMVECs form inner lining of a blood vessel.
Penicillin-Streptomycin (10,000 U/ml)	Gibco	15140-122	antibiotics are added to culture medium to prevent contamination of the cells.
Pre-Cleaned Gold Seal Micro Slides	Clay Adams	3010	Microscope slides used for cell fixation
ProLong Diamond Antifade Mounting Media	Invitrogen	P36961	If samples are fixed using antifade mountant, then the later protects fluorescent dyes and chromophores from fading.
Spectrometer	Ocean Optics	QE65000	Used to measure spectral response of the light source (?)
Trypsin-EDTA (0.25%)	Gibco	25200-056	Digests the protein-protein bond between the cell and cell matrix and helps to dissociate and lift the cells during cell plating.
Tyrodes Buffer	Made in-house	Made in-house	Tyrodes buffer is used to make working solutions and to maintain cells in aqueous solution during image acquisition.
6 Well Cell Culture Plate	Corning	3506	Laminin coated coverslips are placed in 6-well culture dish (one coverslips/well). Cells along with medium is added into each well.
25 mm Round Microscope Cover Slips	Fisher Scientific	12545102	Cells were grown on round glass coverslips
60X Objective	Nikon Instruments	Plan Apo VC 60X/1.2 Wl $\infty$ /0.15-0.18 WD 0.27	water immersion and commonly used objective for cells