ASP Online Seminar Series (May 11, 2021)

Evaluation of the Radioprotective Properties of *Curcuma longa* L. Extract on Biomechanical Changes in Irradiated Brain Cells

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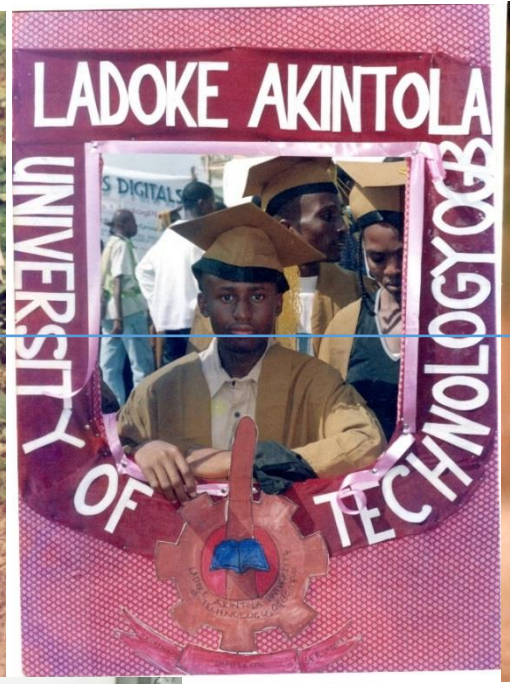
African School of Fundamental Physics and Applications

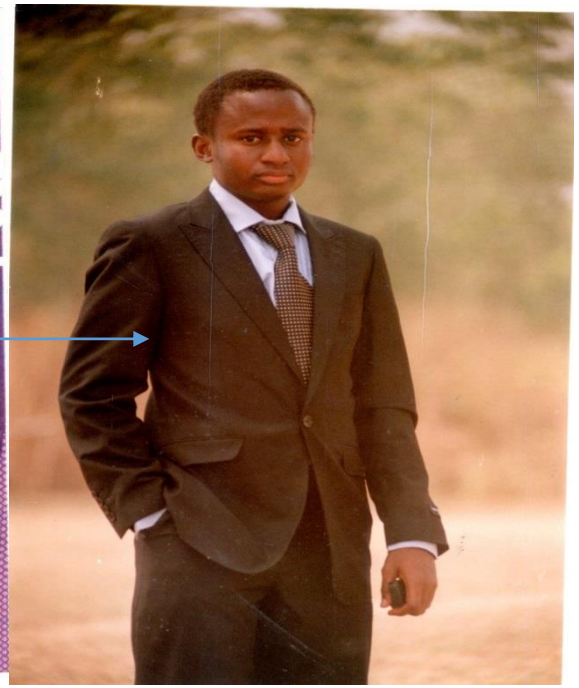
Outline

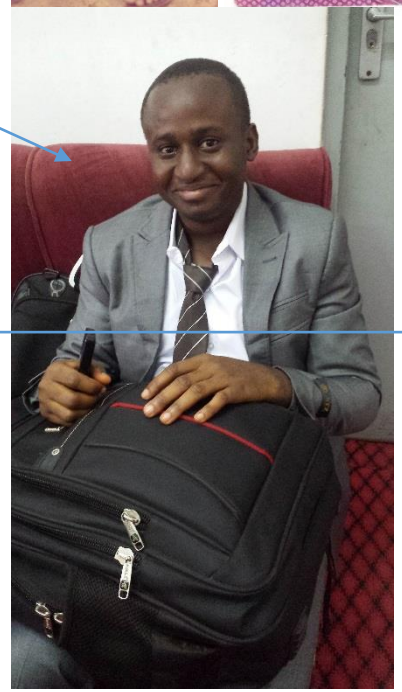
- The presenter
- Connection to ASP
- Background to study
- Statement of Research Problem
- Aim and Objectives of the Research
- Methods
- Results/Discussion
- Future Studies

The Presenter, the journey so far.

- B. Tech. (Pure and Applied Physics)-LAUTECH
- M. Sc. (Medical Physics)- OAU
- Ph.D. (Medical Physics)- Defended (March 2, 2021), administrative processes still ongoing.

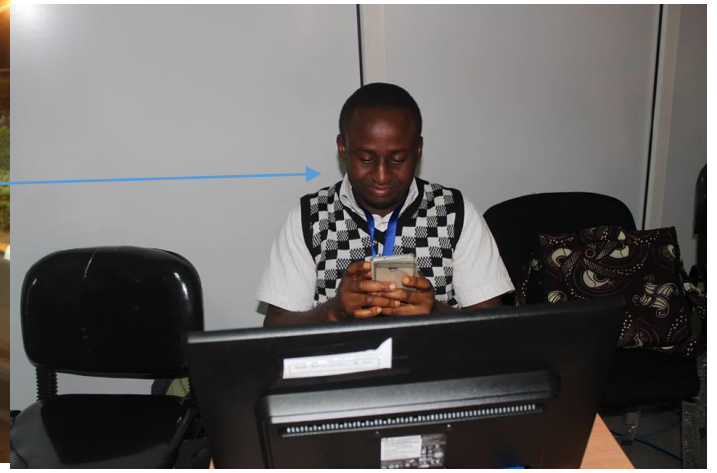






Connection to ASP- Memories

- 2016 alumni
- Application info: Internet
- Mentor: Dr. Esmeralda Yitamben

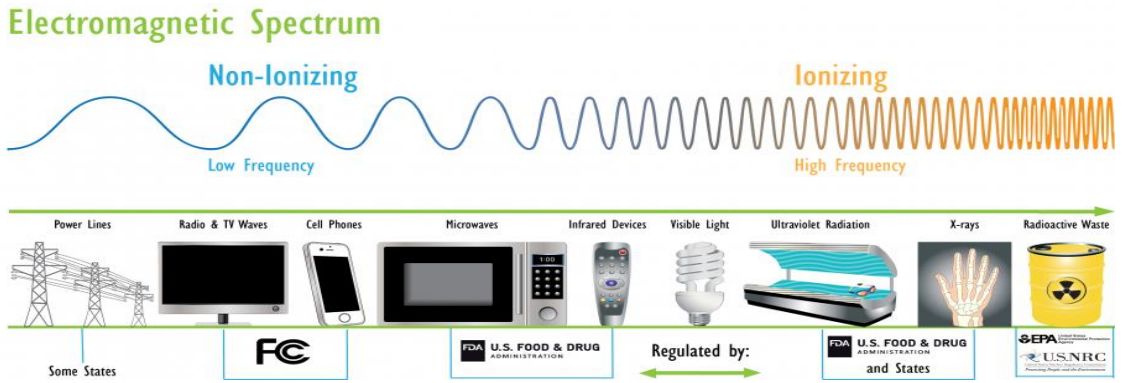


An Overview of my Doctoral Research

Background

- Brain cells coordinates the activities of the body (Sembulingam and Sembulingam, 2012).

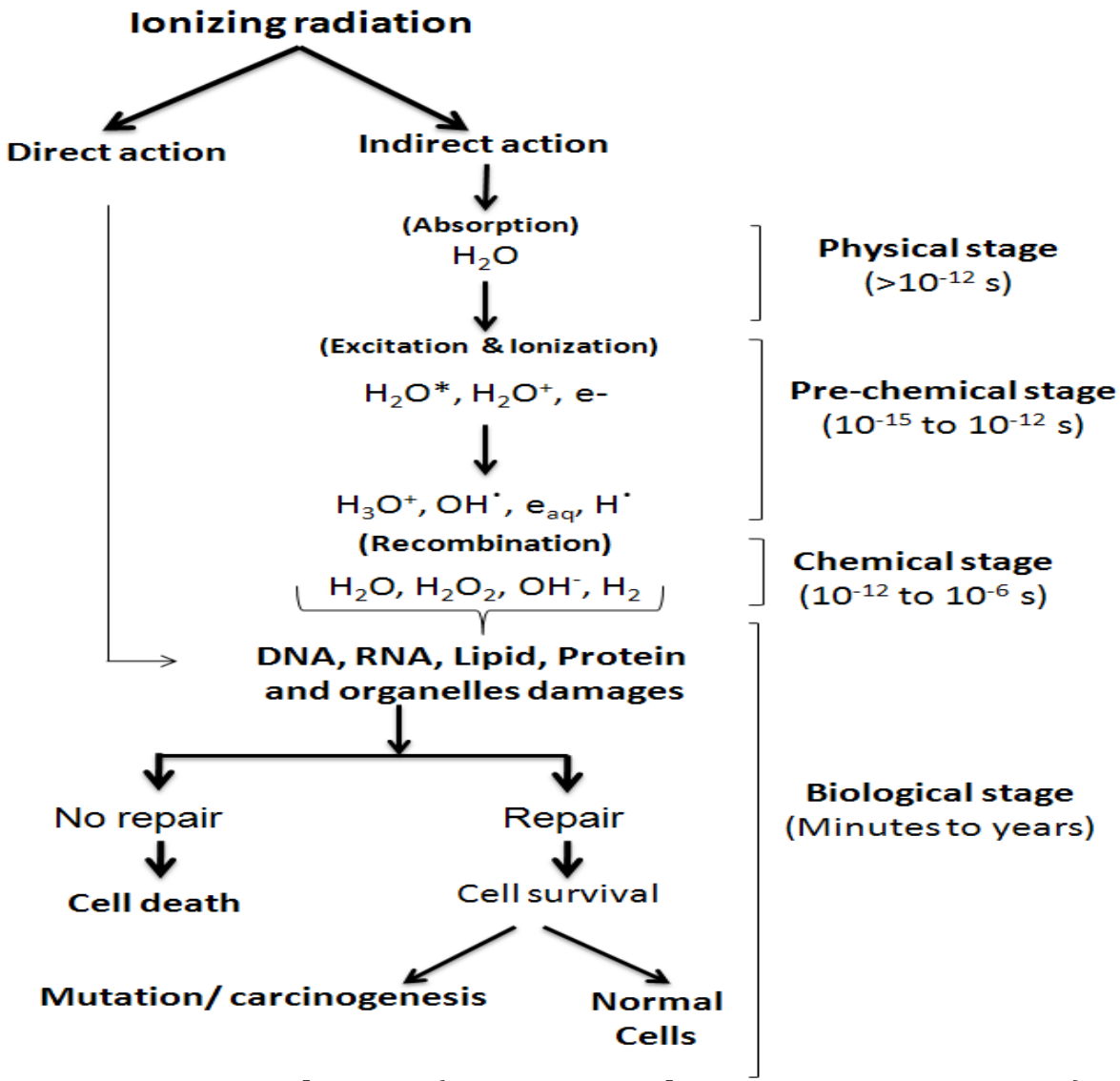
- Radiation used for (Hall and Giaccia, 2006):
  - Imaging (neuronal degeneration/damage)
  - Radiotherapy (brain tumour/cancer)
  - Palliative



- Radiation can have detrimental effects
  - Radiation burn- Tissue effects
  - Chromosomal aberrations/DNA breaks- Molecular changes
  - Inflammatory responses/Oxidative stress- Biochemical changes

- *C. longa* reported to have anti-oxidant properties (Essien *et al.,* 2015; Oyemitan *et al.,* 2017).

- Radiation effects with 'cell-interaction' prevention basically biochemical (Podgorsak, 2006).



Radiation Damage Pathway: (Maurya and Devasagayam, 2011).

Background …

- Cells are complex in nature with different inherent properties.
- Clinical use of tissue stiffness have been part of medicine (Wang and Thampatty, 2006).
- No known/limited clinical assessment of radiation effects incorporating other cell inherent properties.
- Vital to understand the biomechanical changes (Franze *et al.*, 2013).
- Supportive models for radiobiology will improve management.

Statement of Research Problem

- The use of radiation for brain imaging and therapy has been on the increase.

- Biochemical studies, over a few decades, have however shown that the practices result in detrimental effects through the production of free radicals that induce oxidative stress within cells with little or no evidence on the cellular biomechanical communications of such detriments.

- Requisite information on the effects of locally produced scavengers on the biomechanical properties of irradiated brain cells has become imperative.

Aim

Investigate the radioprotective potentials of *Curcuma longa* (turmeric) extract on gamma radiation-induced changes in different types of brain cells using:

1. Biochemical techniques (Cell viability and ROS assays).

2. Stiffness characterization of established brain cell lines.

Objectives

The objectives of this research are to

- evaluate the free radical scavenging activities of the *C. longa* extract *in vitro*;
- determine the cell viability in the presence and absence of the extract;
- quantify the levels of reactive oxygen species (ROS) following gamma radiation in the presence and absence of extract; and
- characterize the stiffness properties brain cells.

Materials & Methods


Turmeric


Safety cabinet


Γ-source


Plate reader


Co_2 incubator

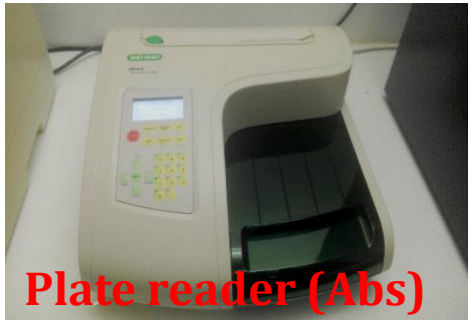
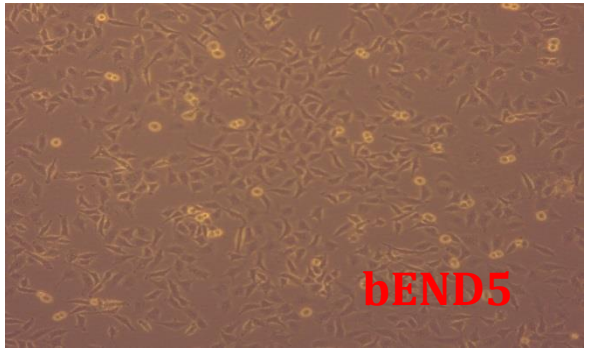
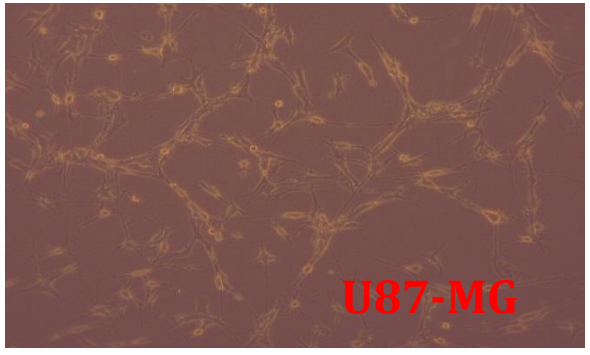


Plate reader (Abs)

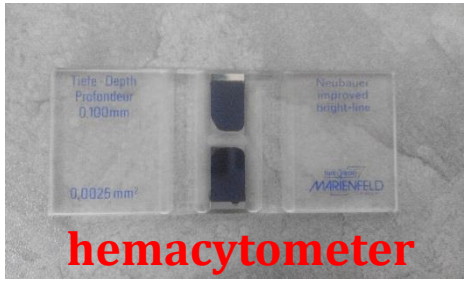

bEND5


U87-MG


ZEISS Microscope

Reagents & consumables


centrifuge



hemacytometer


Flow cytometer


Countess


Veeco 3100D

Methods

- Extract Production.
- *In vitro* Anti-oxidant Assay.
- Viability Assay after radiation exposure.
- Fluorescence Measurement.
- Cell Indentation.

Extract Production

- 250 g dried plant material + 500 ml dH_20 + 2000 ml MeOH = macerated for 24 hrs.
- Filtration using cotton plug + Whatman No. 1 filter paper = crude extract.
- Concentrated (*in vacuo)* with rotary evaporator at 40^oC to dryness.
- Stored at 4^oC until further use.

In vitro radical scavenging assay:

Diphenyl-1-picrylhydrazyl (DPPH) assay

- 1 ml Extract + 1 ml 0.3 mM DPPH (constituted in MeOH) = Mixed
- Incubated in dark = 30 minutes
- Absorbance @ 517 nm vs DPPH + 1 ml MeOH (blank)

Hydroxyl radical scavenging assay

- 1 ml OH^- reagent + 1 ml extract = incubated at 37^oC for 1 hr
- 1 ml TBA + 1 ml TCA = heated in boiling H_20 for 20 minutes
- Absorbance @ 532 nm

Ferric reducing Antioxidant Power (FRAP)

- 1 ml working FRAP reagent + extract.
- Incubated for exactly 10 minutes.
- Absorbance read at 593 nm.

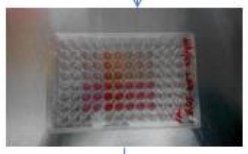
Cell viability after γ-radiation

DMEM+ 10%FBS+ 1%P/S = U87-MG

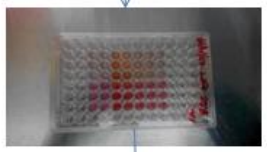
RPMI + 10 % FBS + 1% P/S = bEND5

Plating 70 % confluence

Extract 24 hours



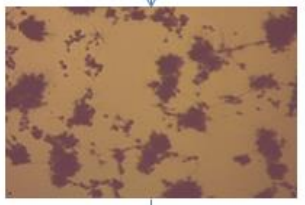
Radiation 24 hours



MTT Assay

10 µl MTT Reagent

4 hours



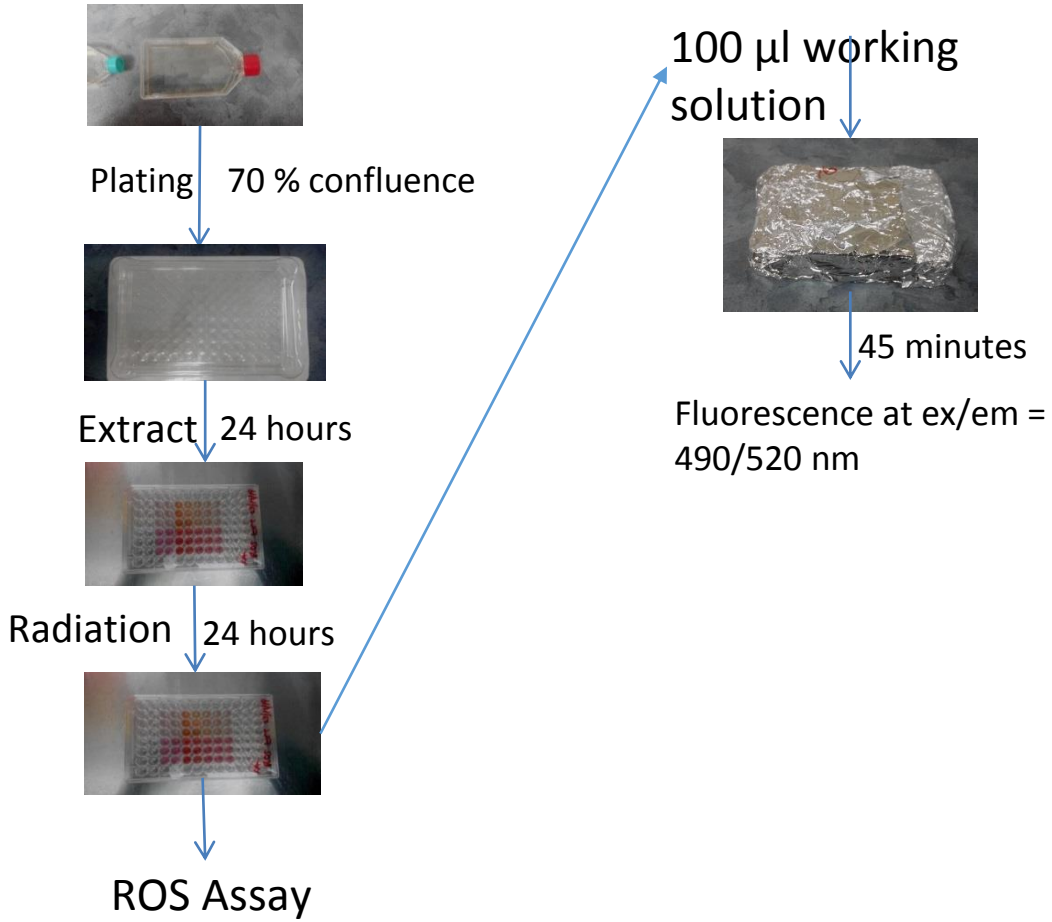
Detergent + 2 hours

Absorbance @ 450 nm

MTT experiment timelines

ROS measurement (extract + γ-radiation)

- The fluorescence is a function of the amount of ROS present.
- Cells grown as earlier described.



ROS measurement using DCFDA dye.

AFM indentation

- Cells grown on 22 mm glass coverslips placed in 6- well plates.
- Measurements taken in air within the shortest possible time.
- Coverslips were kept in growth medium until measurement time.
- Tapping mode used.
- Parameters are adjusted until satisfying data are obtained.
- Extract concentration and radiation dose used based on technical possibilities and previous study.
- $F = \frac{4\sqrt{R}E}{3(1-\nu^2)}\delta^{3/2}$
- R=sphere radius,

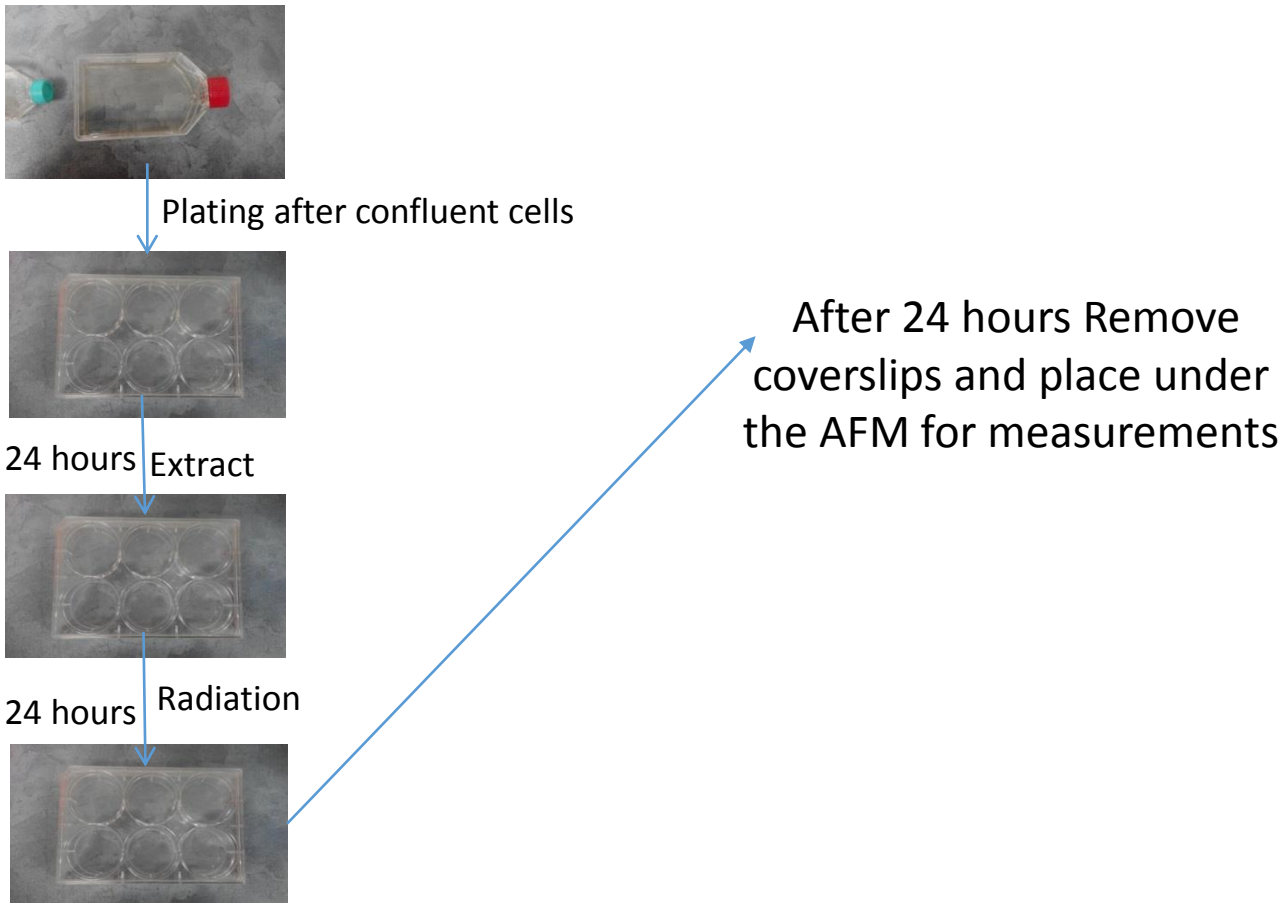
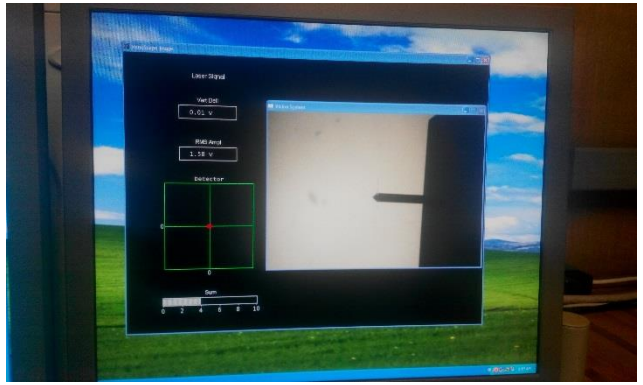
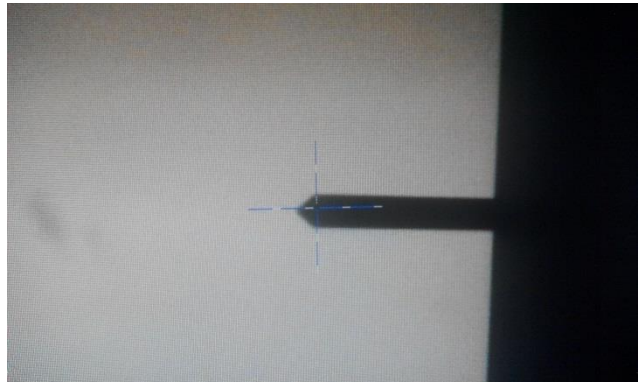


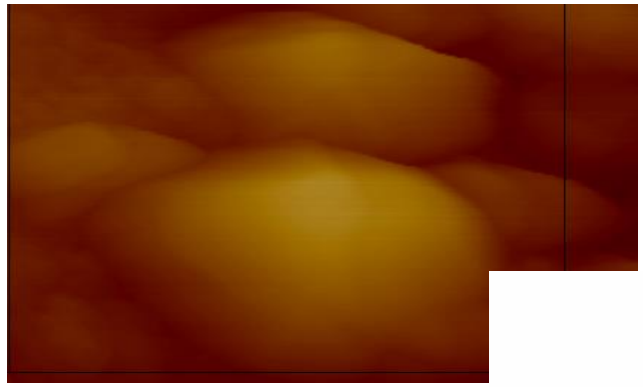
Diagram shows timelines of indentation experiment.

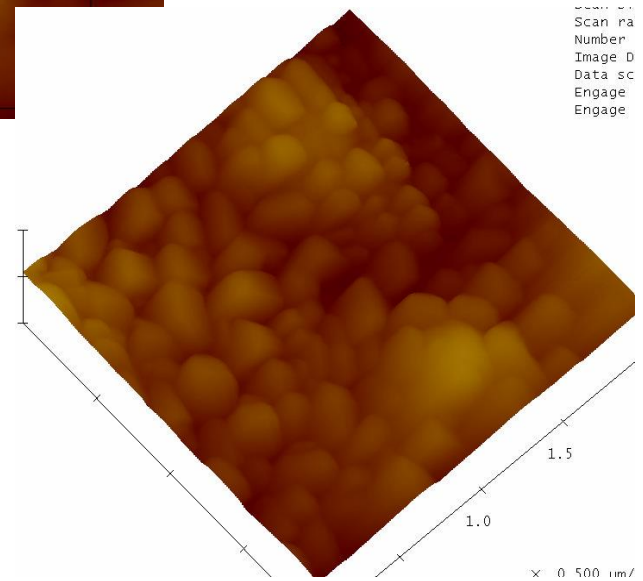





Laser Signal
Vert Defl
-0.02 V
RMS Ampl
2.48 V
Detector
Sum


Dual Trace
Scope Trace
Height
81.51 nm/div
TMR
0.17 nm
First Trace
Scan
24.99 ms/div, 0.30 µm/div
Amplitude
88.75 mV/div
TMR
62.56 mV
Second Trace
Phase
9.50 °/div
TMR
3.39 °
Third Trace
Extended
Trace
Retrace

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Number
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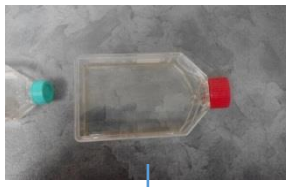
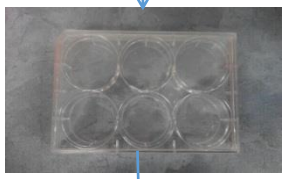
Data sc
Engage
Engage
1.5
1.0

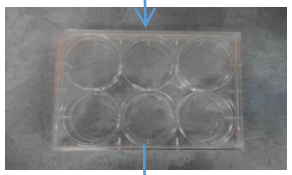
Atomic Force Microscopy



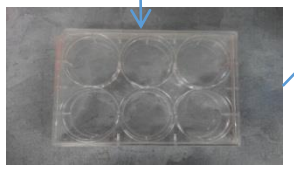
Plating after confluent cells



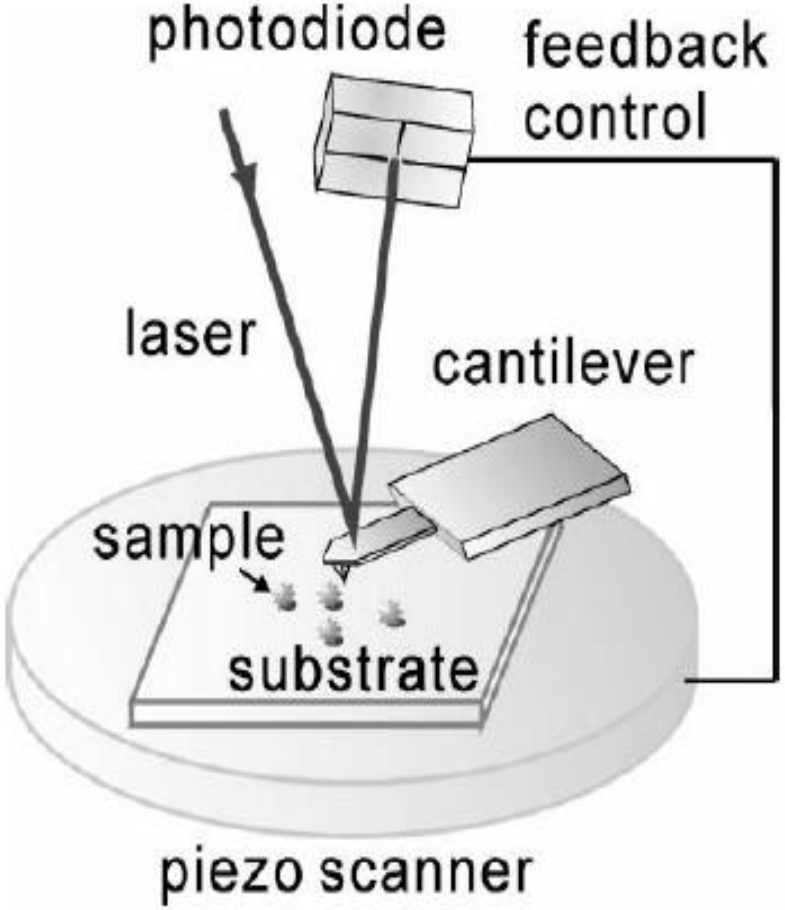
24 hours Extract



24 hours Radiation



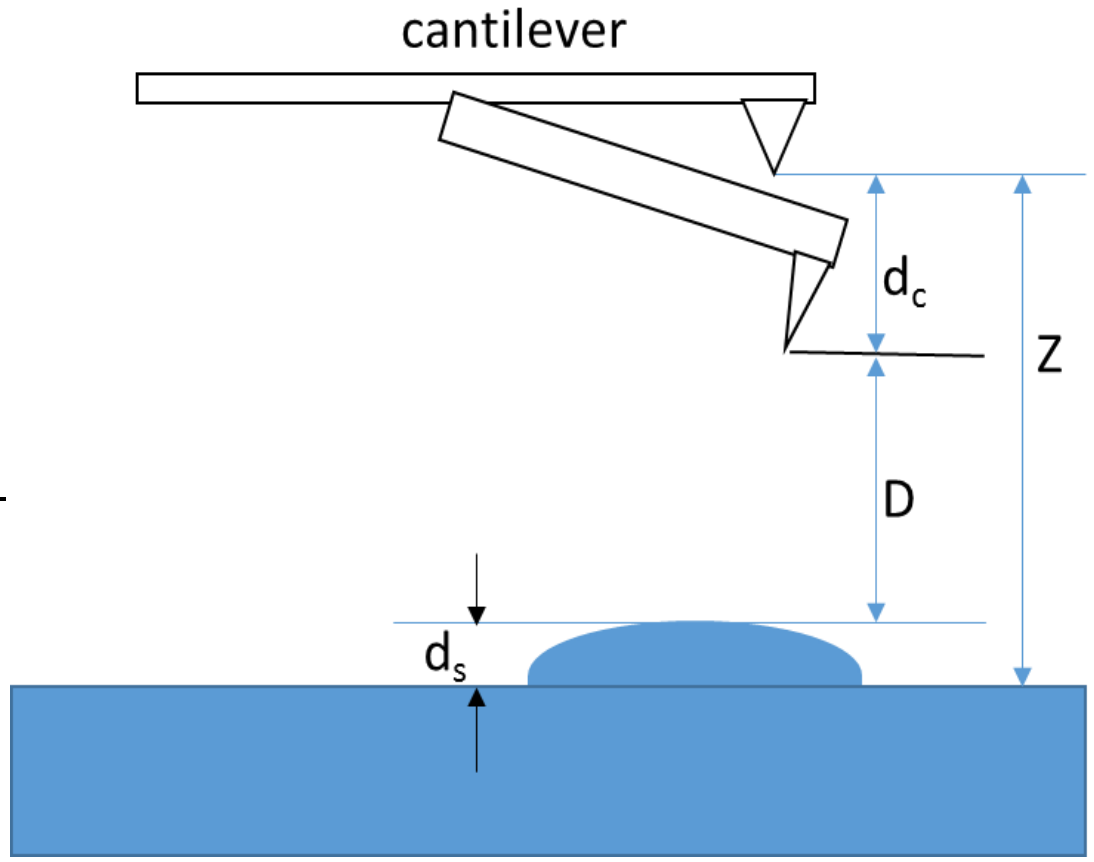
After 24 hours Remove coverslips and place under the AFM for measurements



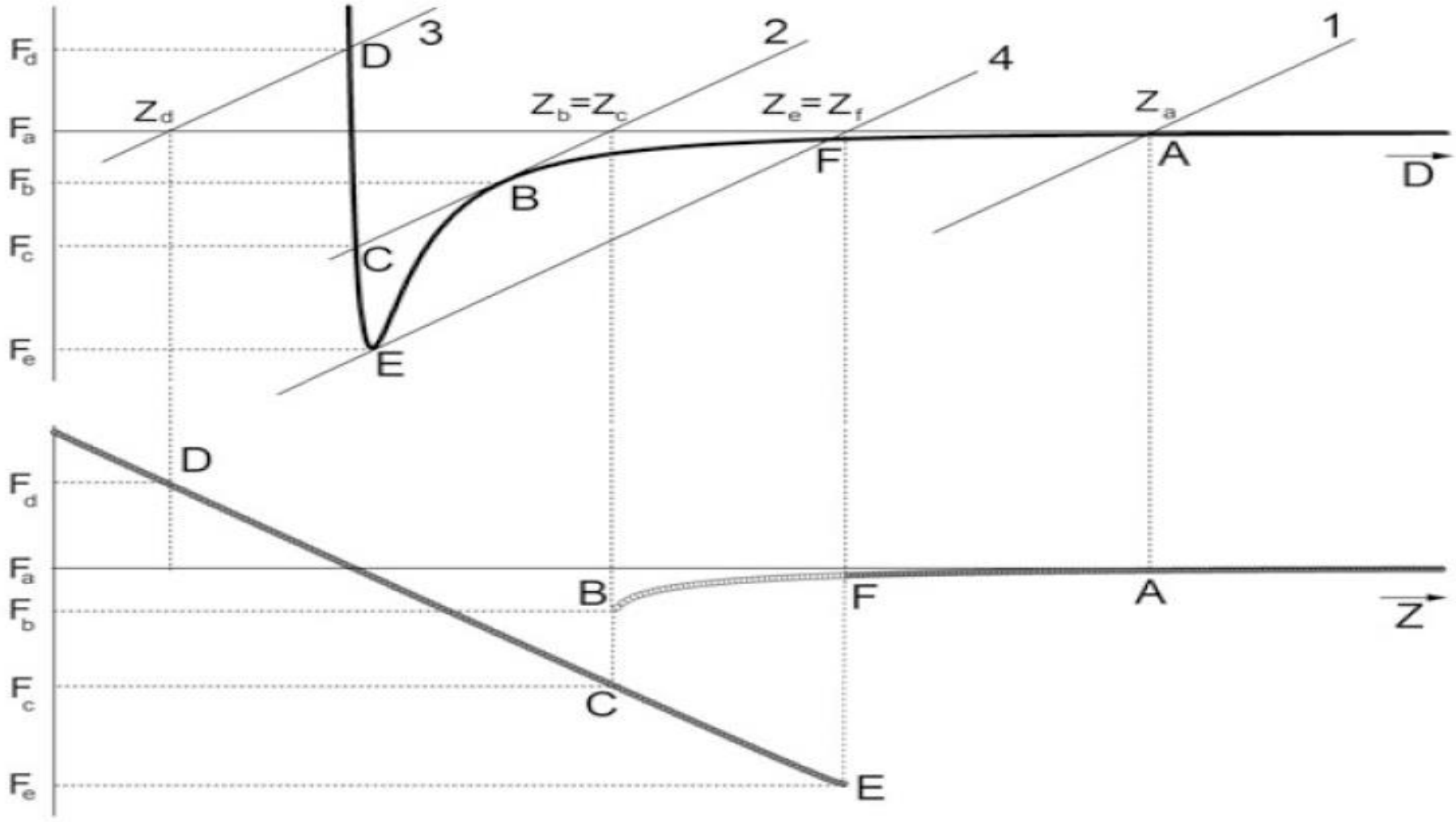
Takeyasu, 2014

Force distance curve acquisition principle

- $\boldsymbol{dc\ vs\ Z\ curves == raw\ data}$
  - $D = Z - (d_c + d_s)$
  - $F = -k_c * d_c$ (Hooke's law)
  - $d_c = S * \Delta V$
  - $F(\delta) = \frac{4\sqrt{R}}{3(1-\nu^2)} E_{Hertz} \delta^{\frac{3}{2}}$
- $d_c = cantilever\ deflection, D = tip - sample\ distance, S = sensitivity\ factor, \Delta V = photodiode\ signal, R = radius\ of\ indentation\ tip, d_s = sample\ indentaion, E_{Hertz} = Young's\ Modulus$



Data Processing Method (Cappella, 2016)

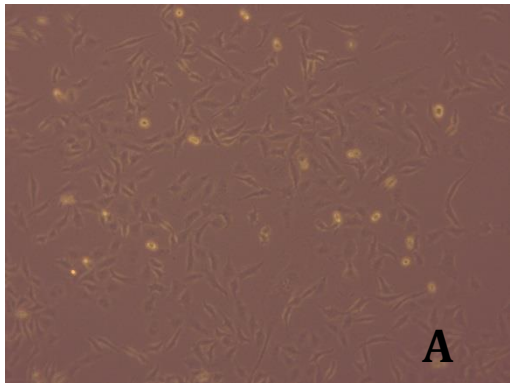


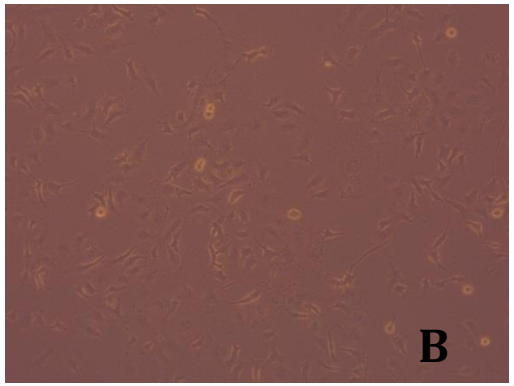
$$F = -K_c (Z - Z_i) + F_i$$

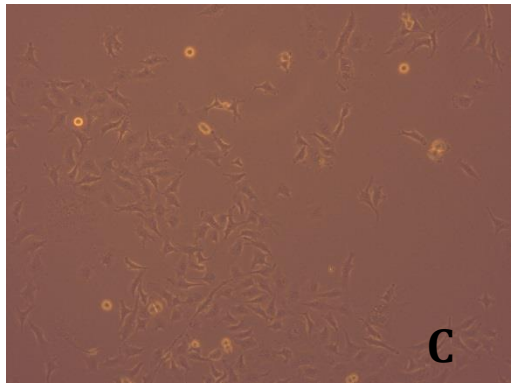
Results

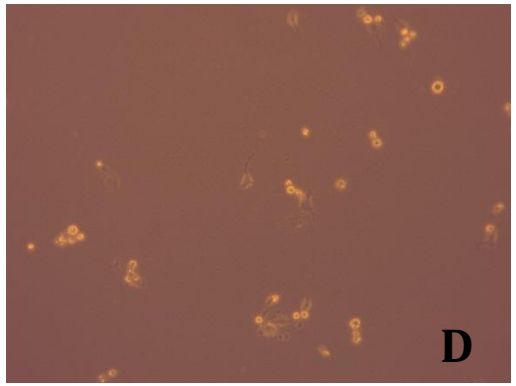
- *In vitro* Anti-oxidant Assay.
- Viability Assay after radiation exposure.
- Fluorescence Measurement
- Cell Indentation

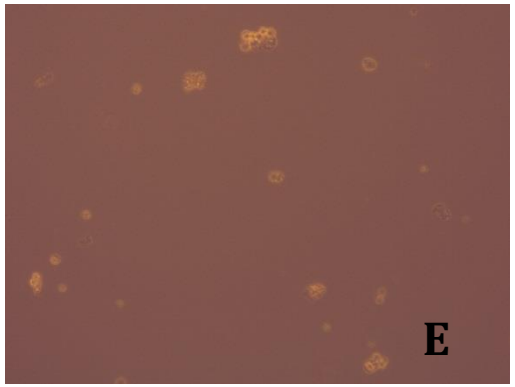
Safety Profile (Extract+ 24hrs): bEND5

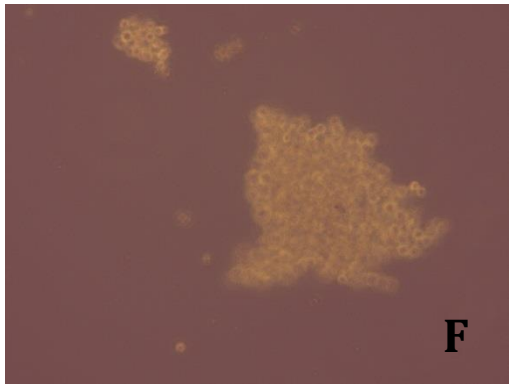


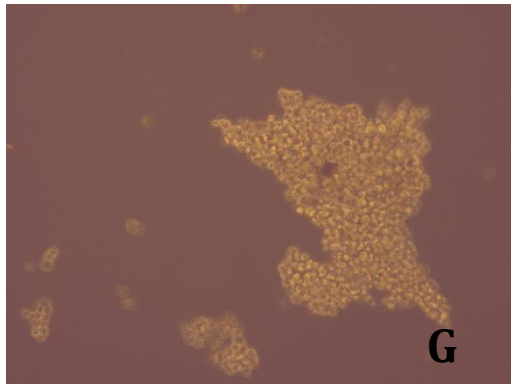






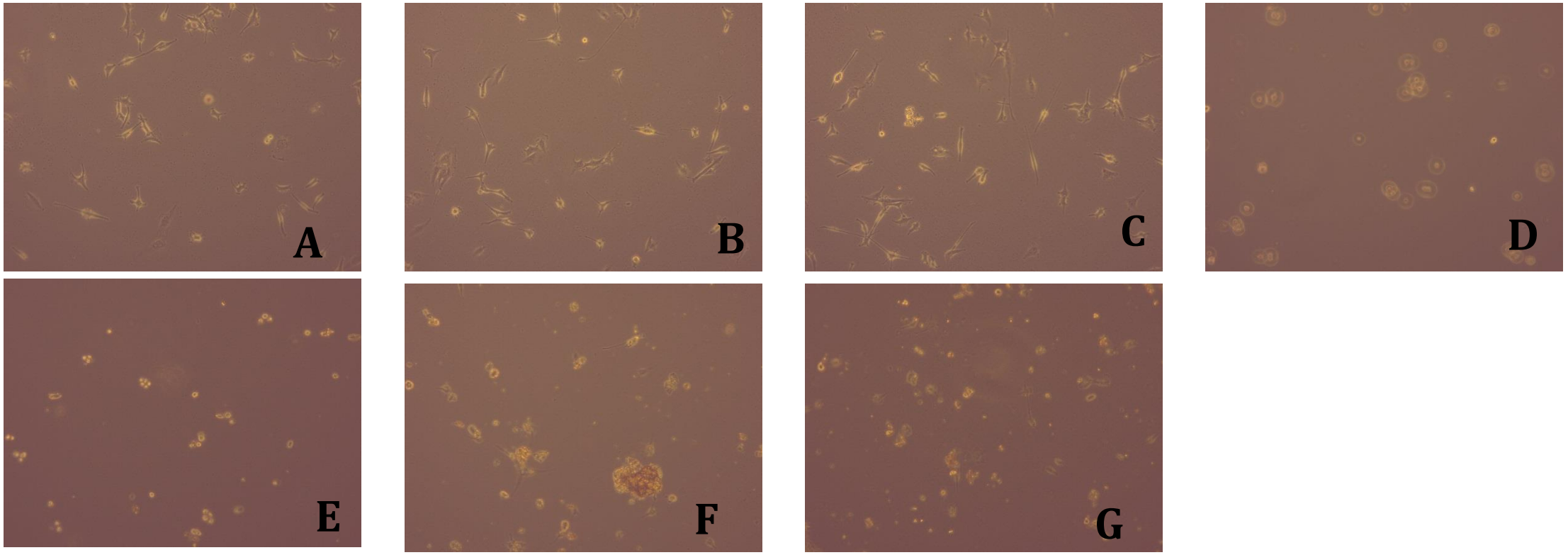




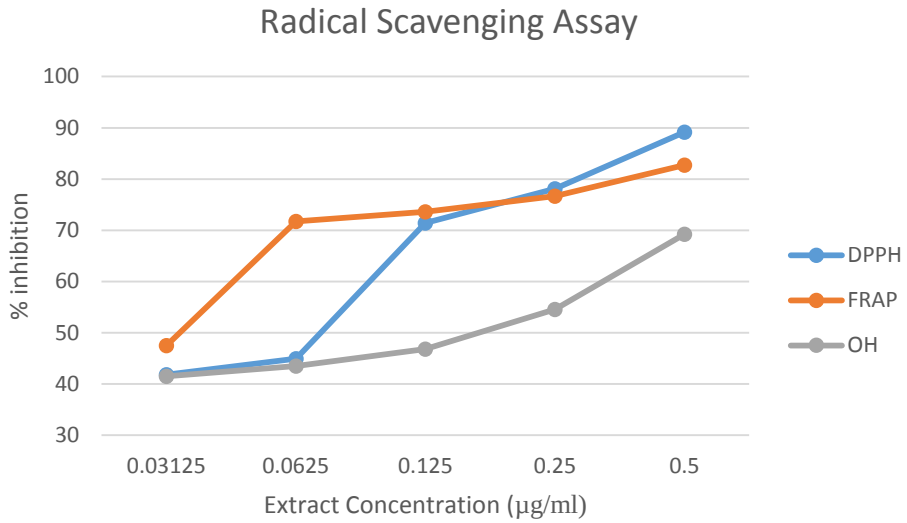


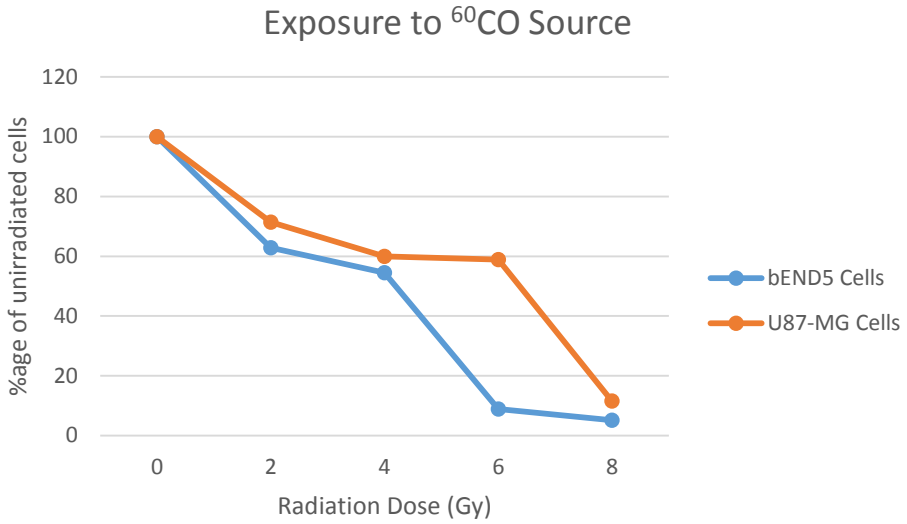
A= 0 µg/ml; B= 10 µg/ml; C= 20 µg/ml; D= 50 µg/ml; E= 100 µg/ml; F= 200 µg/ml; G= 500 µg/ml

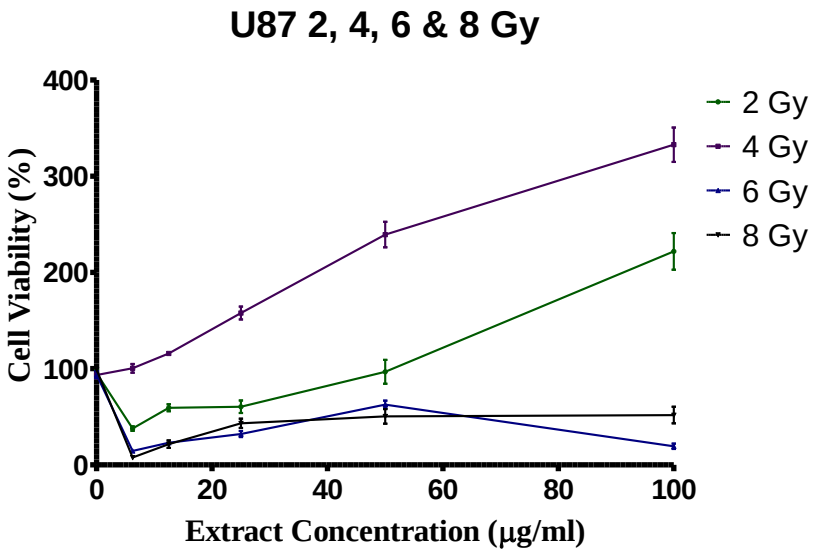
Safety profile (Extract+ 24hrs): U87-MG

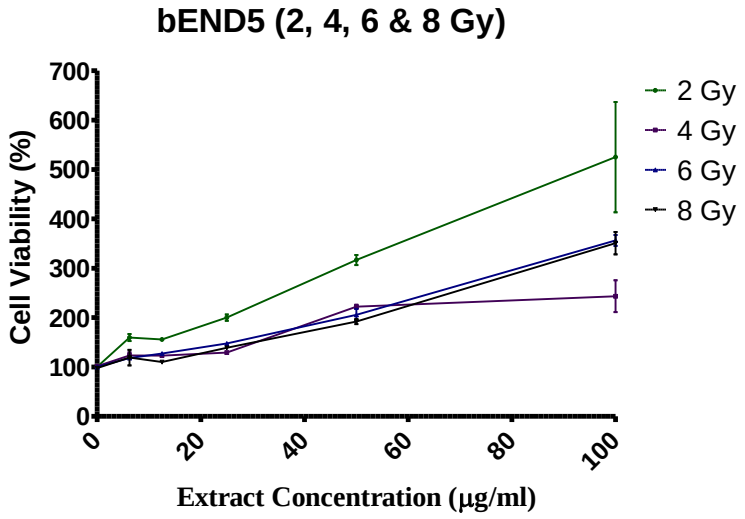


A= 0 µg/ml; B= 10 µg/ml; C= 20 µg/ml; D= 50 µg/ml; E= 100 µg/ml; F= 200 µg/ml; G= 500 µg/ml


Radical Scavenging Assay
100
90
80
70
60
50
40
30
% inhibition
0.03125
0.0625
0.125
0.25
0.5
Extract Concentration (µg/ml)
DPPH
FRAP
OH

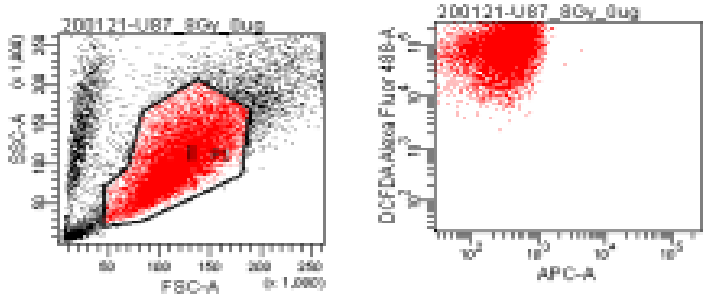

Exposure to 60CO Source
120
100
80
60
40
20
0
%age of unirradiated cells
0
2
4
6
8
Radiation Dose (Gy)
bEND5 Cells
U87-MG Cells

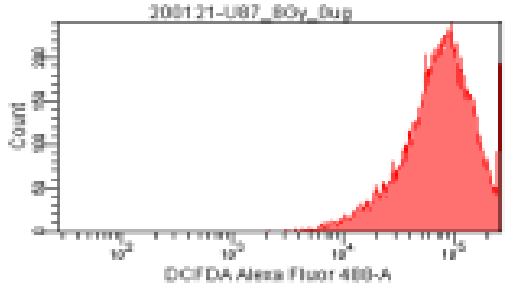

U87 2, 4, 6 & 8 Gy
400
300
200
100
0
Cell Viability (%)
0
20
40
60
80
100
Extract Concentration (µg/ml)
2 Gy
4 Gy
6 Gy
8 Gy


bEND5 (2, 4, 6 & 8 Gy)
700
600
500
400
300
200
100
0
Cell Viability (%)
0
20
40
60
80
100
Extract Concentration (µg/ml)
2 Gy
4 Gy
6 Gy
8 Gy

Flow Cytometry methods

FACSDiva Version 6.2

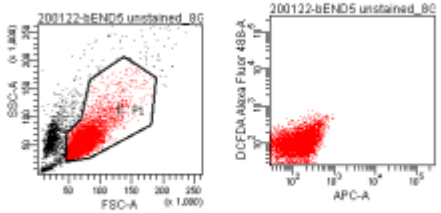


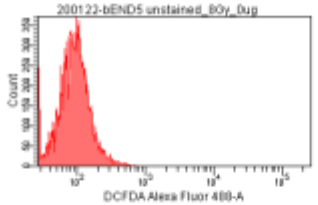


Experiment Name:	190114_radiation
Specimen Name:	200121
Tube Name:	U87_80y_0ug
Record Date:	Jan 21, 2020 3:52:44 PM
$OP:	Administrator
GUID:	32f46489-8580-494c-9c69-ad0e373e7ce2

Population	#Events	%Parent	DCFDA Alexa Fl.. Mean
P1	10,000	68.8	91,244

FACSDiva Version 6.2

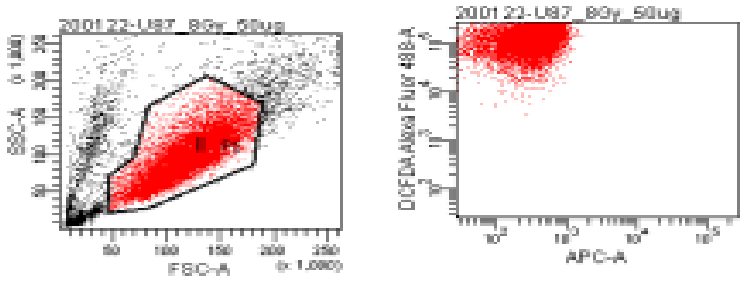


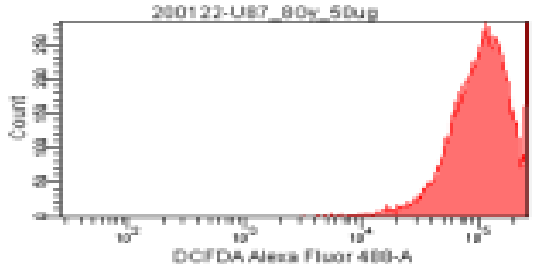


Experiment Name:	190114_radiation
Specimen Name:	200122
Tube Name:	bEND5 unstained_80y_0ug
Record Date:	Jan 22, 2020 11:55:50 AM
$OP:	Administrator
GUID:	5a5e29d1-a2c8-432d-84f2-7ccf2dfe78d0

Population	#Events	%Parent	DCFDA Alexa Fl.. Mean
P1	10,000	83.5	102

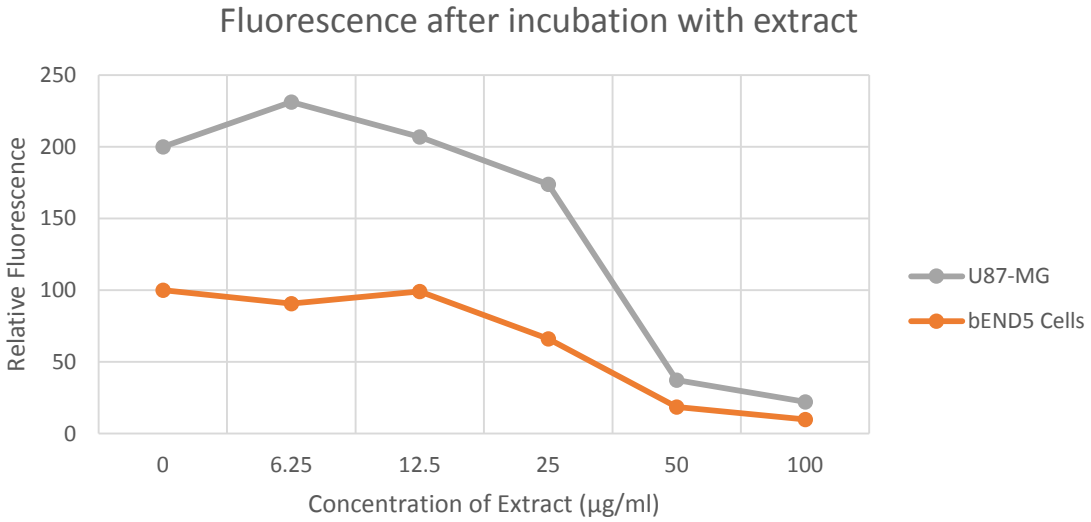
FACSDiva Version 6.2

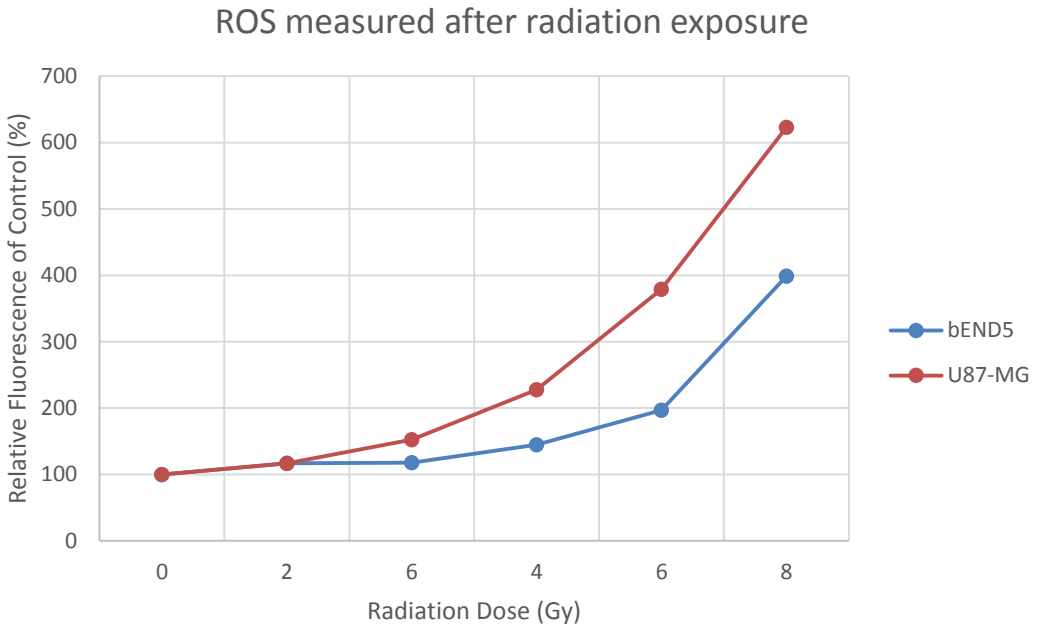


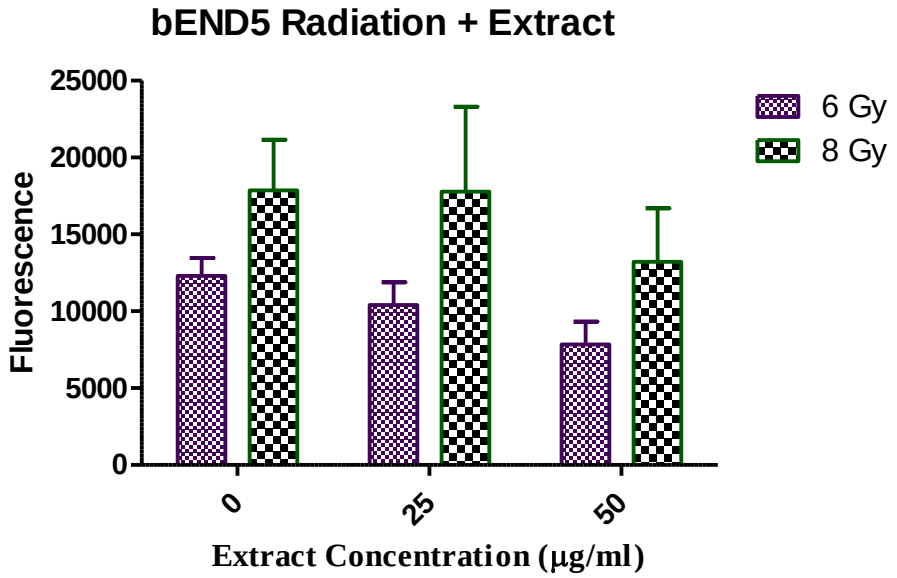


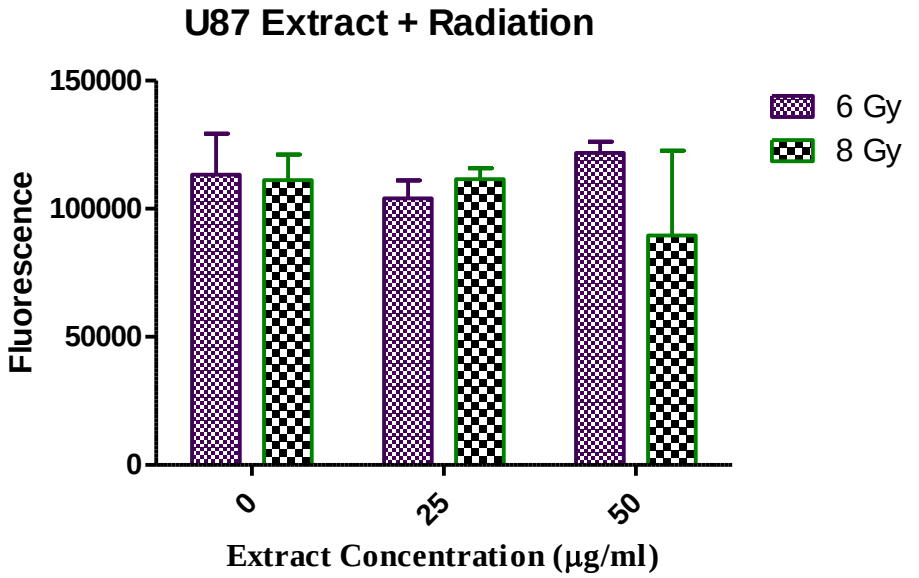
Experiment Name:	190114_radiation
Specimen Name:	200122
Tube Name:	U87_80y_50ug
Record Date:	Jan 22, 2020 12:16:58 PM
$OP:	Administrator
GUID:	cb581df2-5173-47f7-9012-7384a42714e6

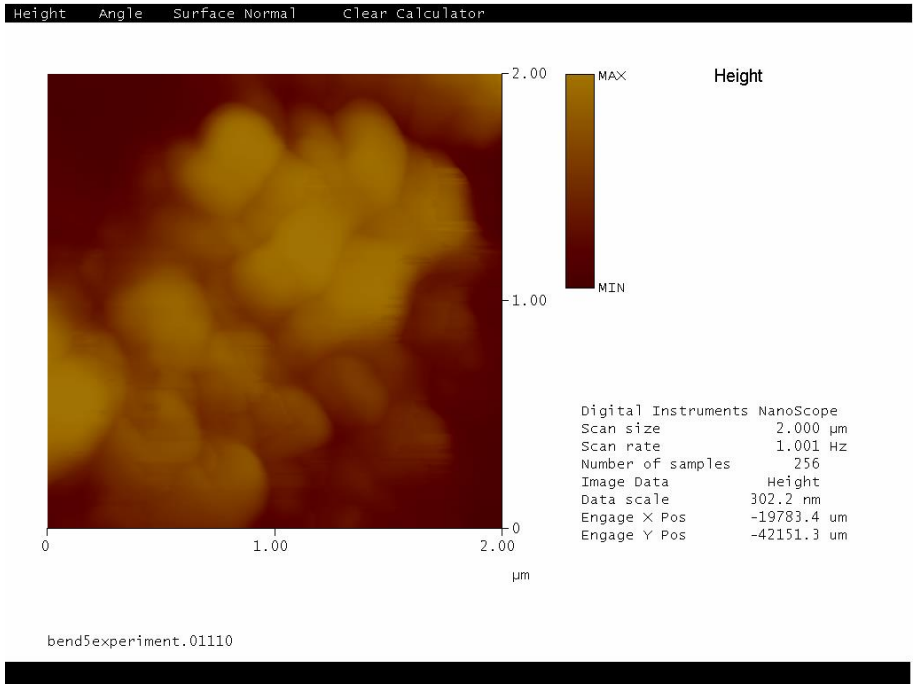
Population	#Events	%Parent	DCFDA Alexa Fl.. Mean
P1	10,000	78.9	121,087


Fluorescence after incubation with extract
Relative Fluorescence
250
200
150
100
50
0
0
6.25
12.5
25
50
100
Concentration of Extract (µg/ml)
U87-MG
bEND5 Cells

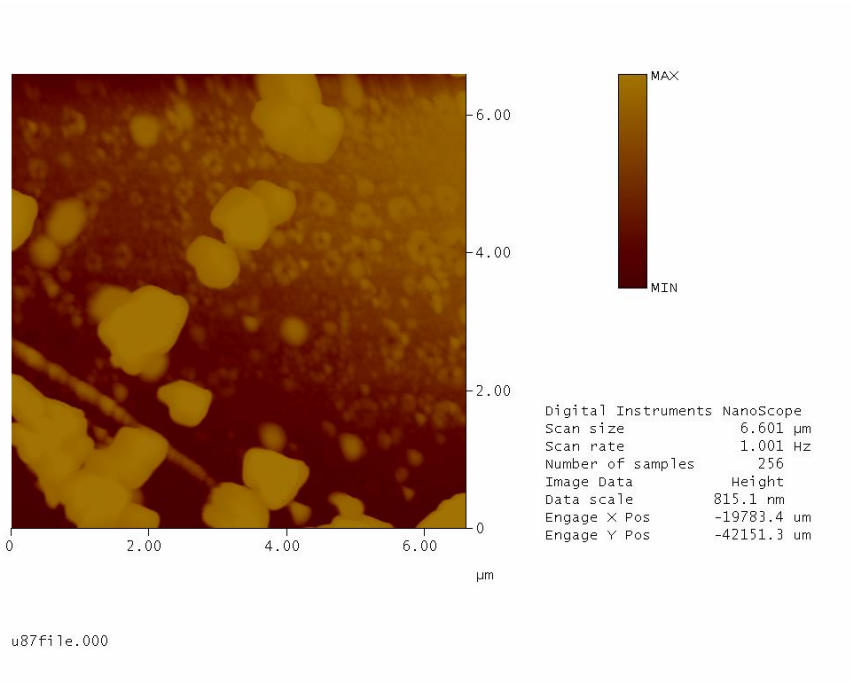

ROS measured after radiation exposure
Relative Fluorescence of Control (%)
700
600
500
400
300
200
100
0
0
2
6
4
6
8
Radiation Dose (Gy)
bEND5
U87-MG


bEND5 Radiation + Extract
Fluorescence
25000
20000
15000
10000
5000
0
0
25
50
Extract Concentration (µg/ml)
6 Gy
8 Gy

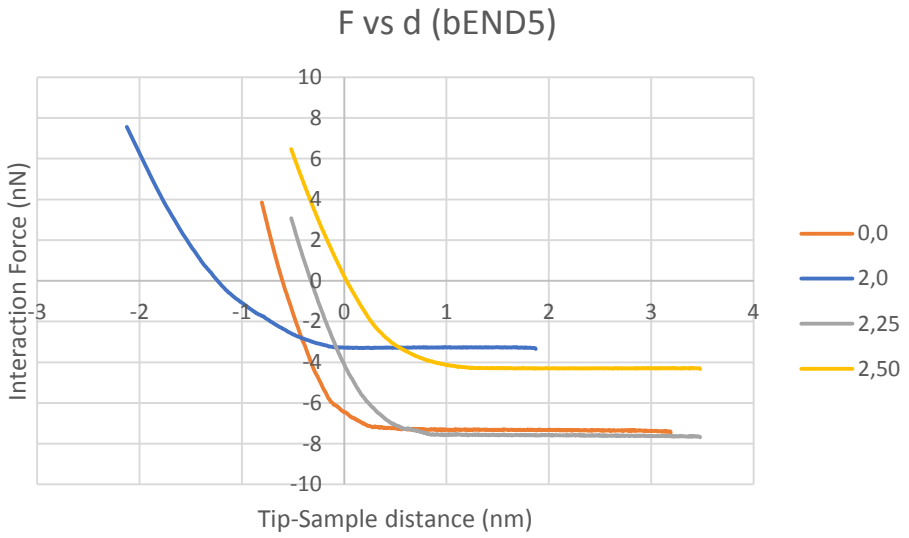

U87 Extract + Radiation
Fluorescence
150000
100000
50000
0
0
25
50
Extract Concentration (µg/ml)
6 Gy
8 Gy

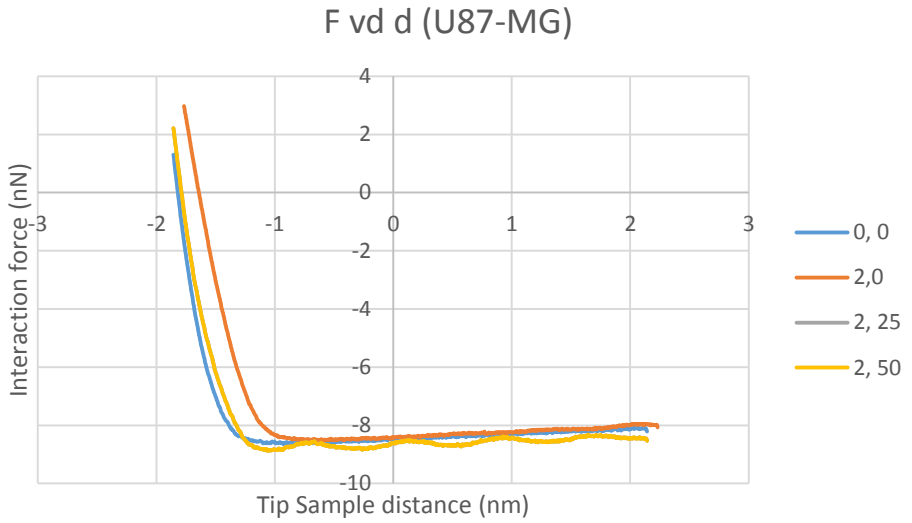


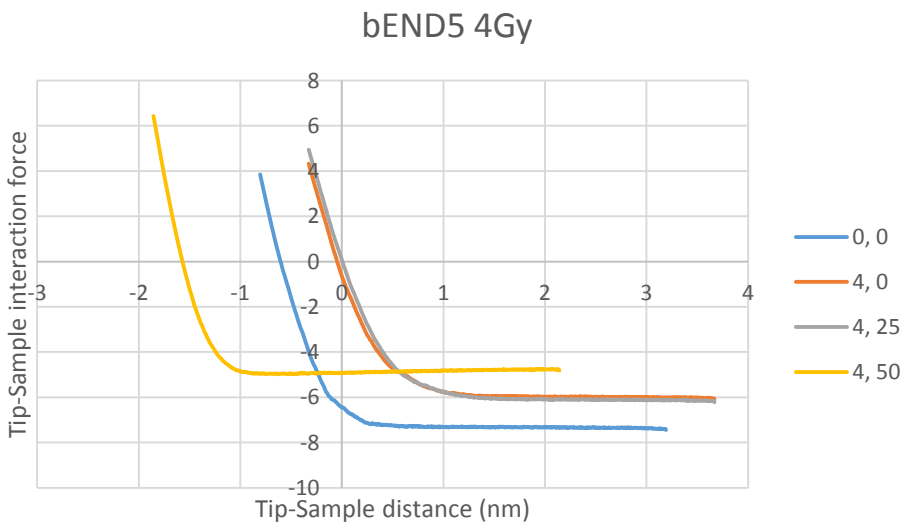
AFM Image of bEND5 cells

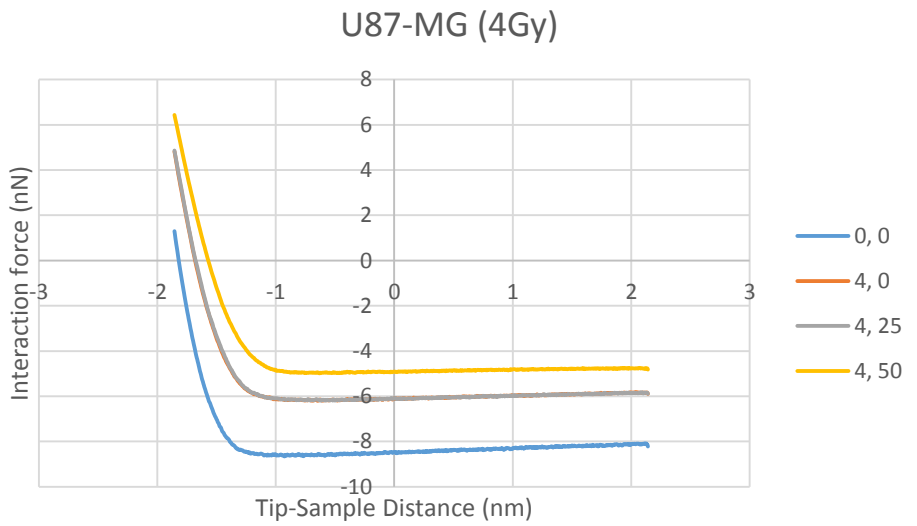


AFM Image of U87-MG


F vs d (bEND5)
Interaction Force (nN)
Tip-Sample distance (nm)
0,0
2,0
2,25
2,50


F vd d (U87-MG)
Interaction force (nN)
Tip Sample distance (nm)
0, 0
2,0
2, 25
2, 50


bEND5 4Gy
Tip-Sample interaction force
Tip-Sample distance (nm)
0, 0
4, 0
4, 25
4, 50


U87-MG (4Gy)
Interaction force (nN)
Tip-Sample Distance (nm)
0, 0
4, 0
4, 25
4, 50

Summary of Results

- *In vitro* inhibition of the extract varies as the concentration used.
- U87-MG cells were more resistant to radiation than bEND5 cells.
- Stiffness of cells increased with extract concentration.
- Radiation induced stiffness changes in the cells used.

Further Studies

- Indentation experiment under standard growth conditions.
- Stiffness/Adhesion of Malaria infected blood sample.
- AFM study of erythrocyte from sickle cell patients in Nigeria.
- Biophysical characterization of cancer biopsy tissues.

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Appreciation

- My Supervisors
- Heads/Members of laboratories used.
- ASP
- Colleagues
- Friends

Thank you for your attention