

## **IQB HW ASSIGNMENT - 8**

### **Group Members --**

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### **Question - 1:**

Process of cell death is called apoptosis. There are two types: Type 1 apoptotic pathway and type 2 apoptotic pathway. In type 2 apoptotic pathway, a second initiator caspase 9 is needed for signal amplification and whose activation is controlled by pre and post- mitochondrial regulatory mechanisms. By binding with caspase 9, it inhibits their pro - apoptotic activity. In this pathway, Fas-DISC starts a feedback loop that spirals into increasing release of proapoptotic factors from mitochondria and the amplified activation of caspase 8. And we know that factors like Fas promotes apoptosis, thus kills cancer cells, otherwise insufficient amount results in uncontrolled cell proliferation such as cancer.

### **Question - 2:**

#### **Basic steps of kinetic Monte Carlo simulation of the apoptotic cell death pathway--**

Pick a random molecule and perform following moves in proportion to probability rate constants and specified probabilities. This model is probabilistic rate constant based kinetic Monte Carlo simulation (implicit free energy).

- a) Diffusion
- b) Reaction

In Diffusion, diffusion is take place with one of the neighbouring nodes, four for membrane bound molecules and six for cytosolic molecules, only if neighbouring site is free and not occupied with any other molecule.

In Reaction, molecule undergo a proteolysis activation reaction by either binding with another molecule (forming a complex) or dissociate into its constituent free molecules.

Now, repeat above mentioned reactions for N molecules given in the system and given number of time intervals T.

Thus, MC simulations helps us in capturing the dynamics of cell death activation based on molecular concentrations, constants and biochemical reaction networks.

### **Question - 3: Code to generate groups of MC runs using bootstrapping based on given data :**

```
folders = {'CancerCell_Type1', 'CancerCell_Type2', 'HealthyCells'};
for i = 1:3          % Loop to iterate over the folders
    celldeaths = []; % Cell-death array to store no. of dead cells
```

```

for j = 1:16      % Loop for 16-MC runs
    for k = 1:16  % Loop to iterate over 16 randomly selected files
        apoptosis = 0;
        for m = 1:16 % Loop to select a file randomly

```

**Question - 4: Code to decide between cell survival and death depending upon the Caspase 3 activation threshold:**

```

        if (tokens(6) > 80)      % Decide if the cell survives or dies depending upon the
caspase-3 activation threshold (= 80)
            apoptosis = apoptosis + 1;
        else
            survival = survival + 1;
        end

```

**Question - 5: Code to estimate the number of cell death in each group and generate a histogram from such estimates:**

```

apoptosis = 0;
survival = 0;
for m = 1:16 % Loop to select a file randomly
    filenum = randi(64);
    file = strcat('output', num2str(filenum));
    filename = fullfile(folders{i}, file);
    fileID = fopen(filename, 'r');
    lastline = "";
    offset = 1;
    fseek(fileID, -offset, 'eof');
    newchar = fread(fileID, 1, '*char');
    while (~strcmp(newchar, char(10))) || (offset == 1)
        lastline = [newchar lastline]; % Add the character to a string
        offset = offset + 1;
        fseek(fileID, -offset, 'eof'); % Seek to the file end, minus the offset
        newchar = fread(fileID, 1, '*char');
    end
    tokens = str2num(sprintf(lastline));
    if (tokens(6) > 80)      % Decide if the cell survives or dies depending upon the
caspase-3 activation threshold (= 80)
        apoptosis = apoptosis + 1;
    else
        survival = survival + 1;
    end
    fclose(fileID);

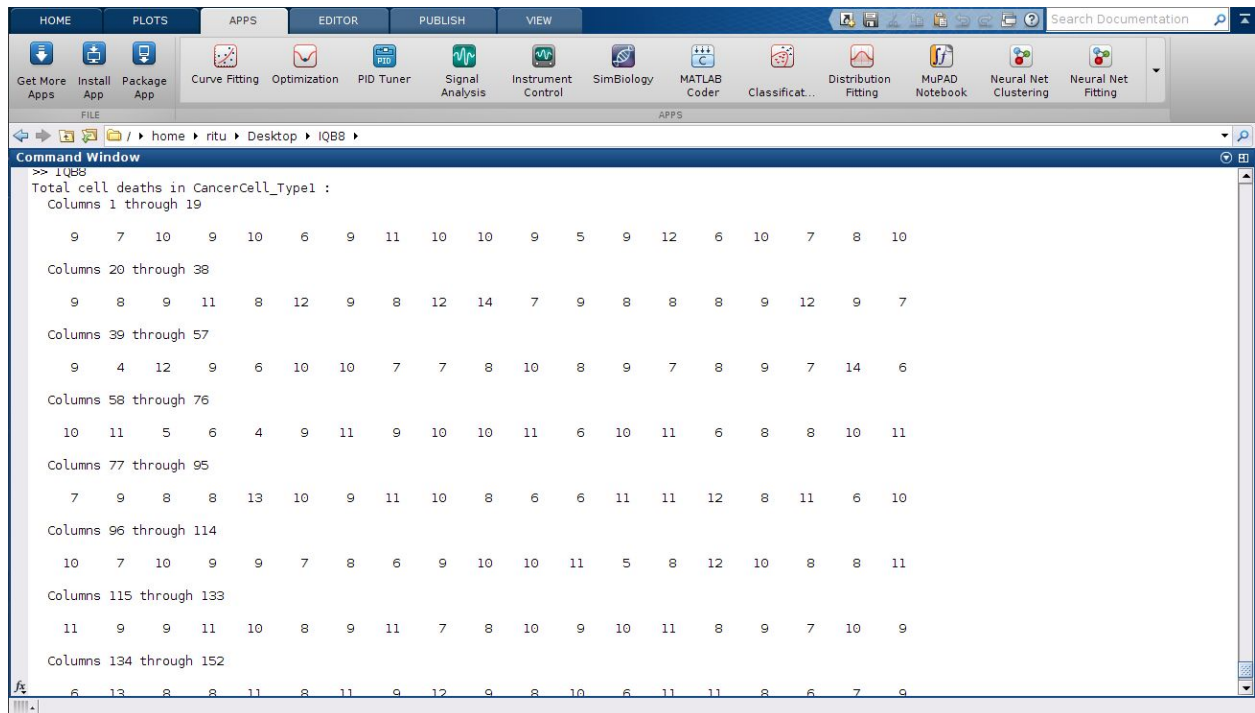
```

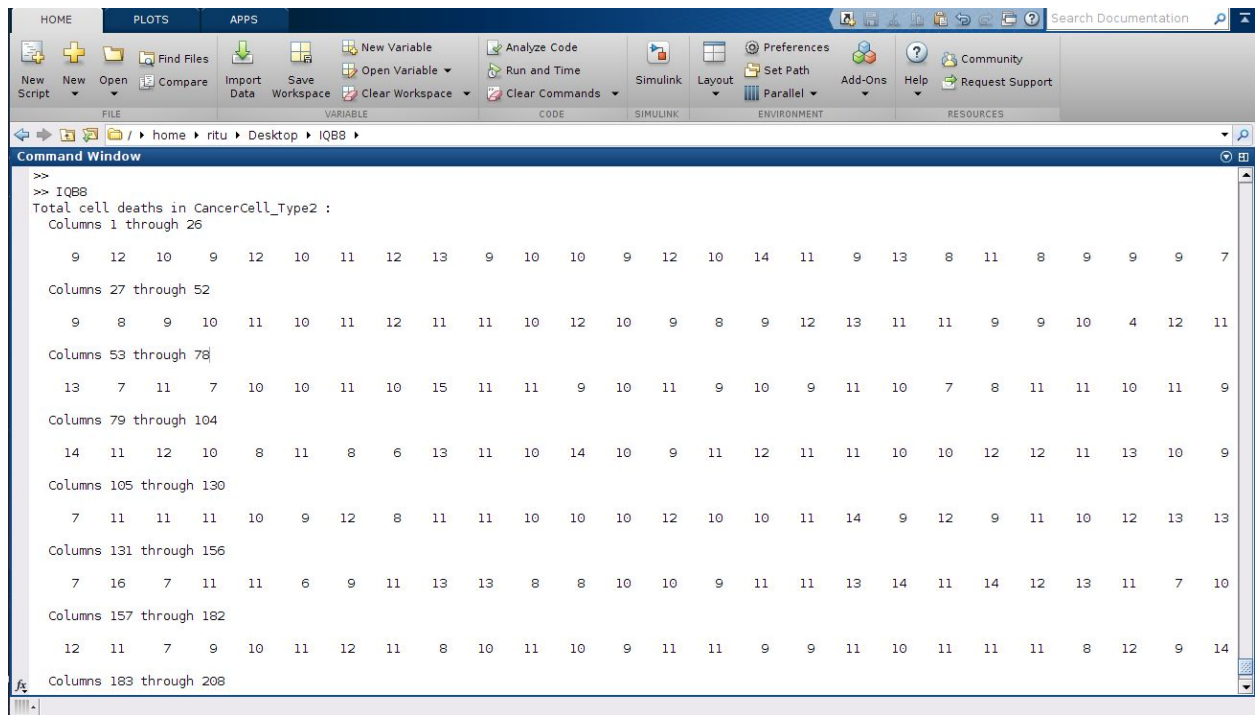
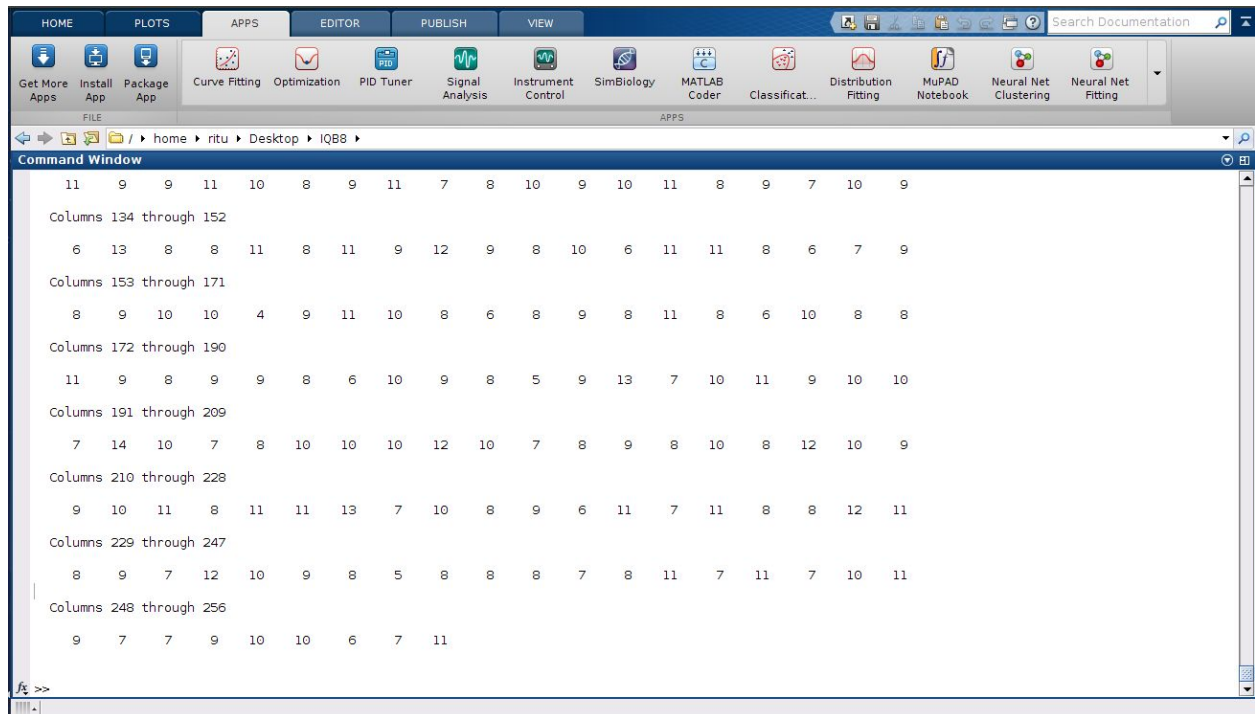
```

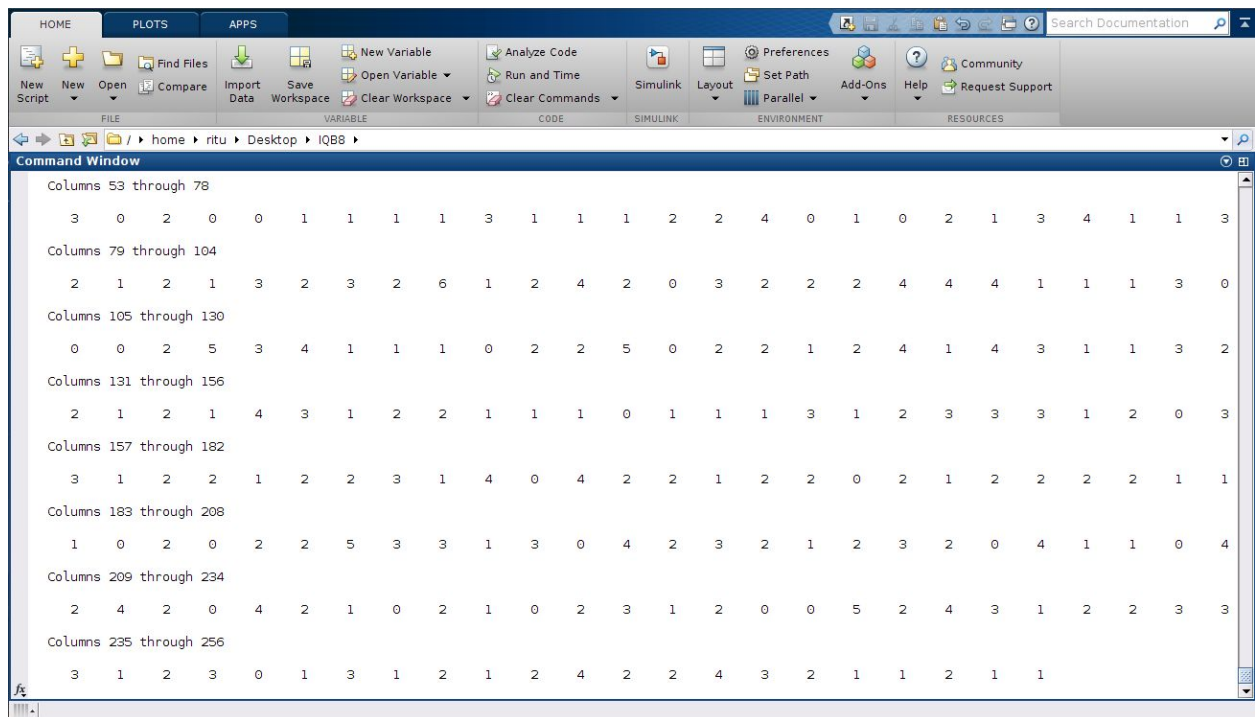
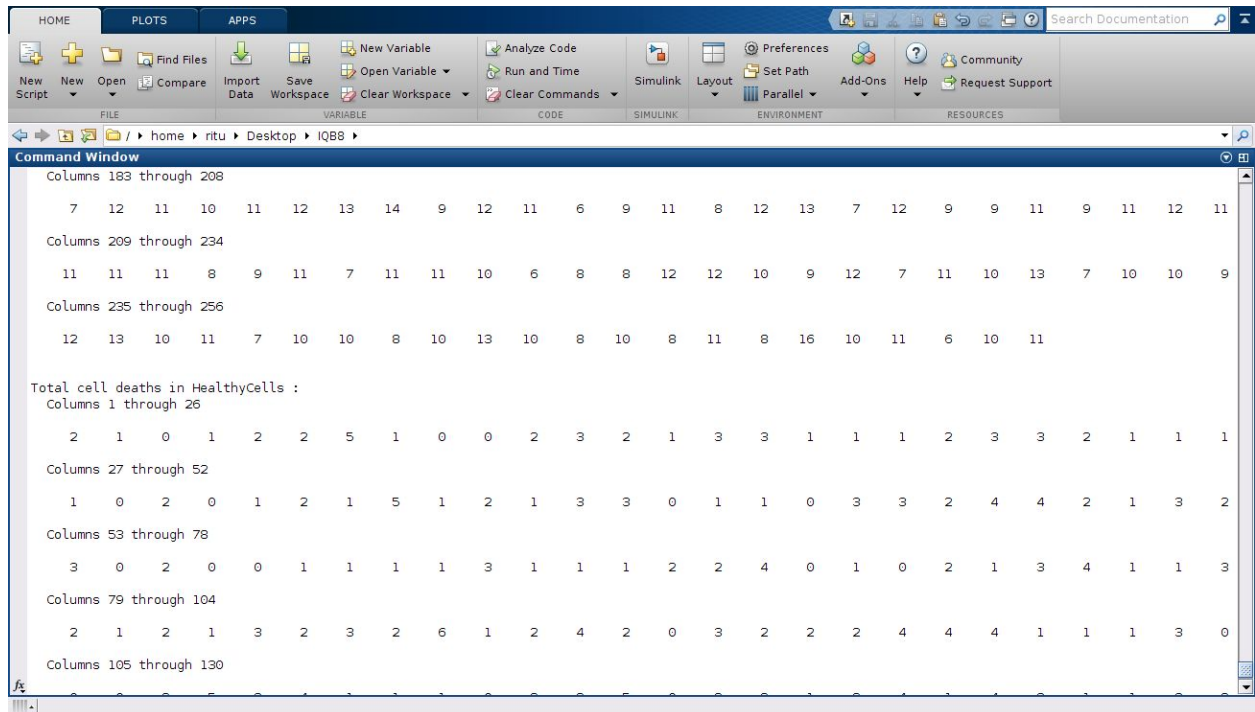
        end
        celldeaths = [celldeaths, apoptosis];
    end
end
fprintf('Total cell deaths in %s :\n', folders{i});
disp(celldeaths);
subplot(1, 3, i);
histogram(celldeaths);
title(folders{i});
xlabel('No. of Cell Deaths');
ylabel('Frequency');
fprintf('\n');
end

```

### **Numerical Output:**

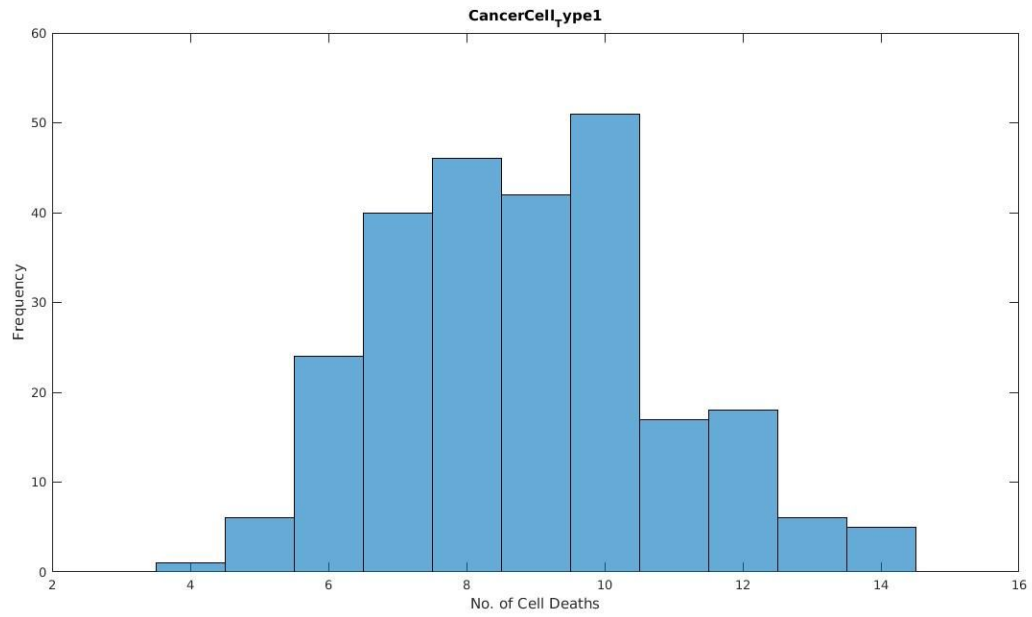




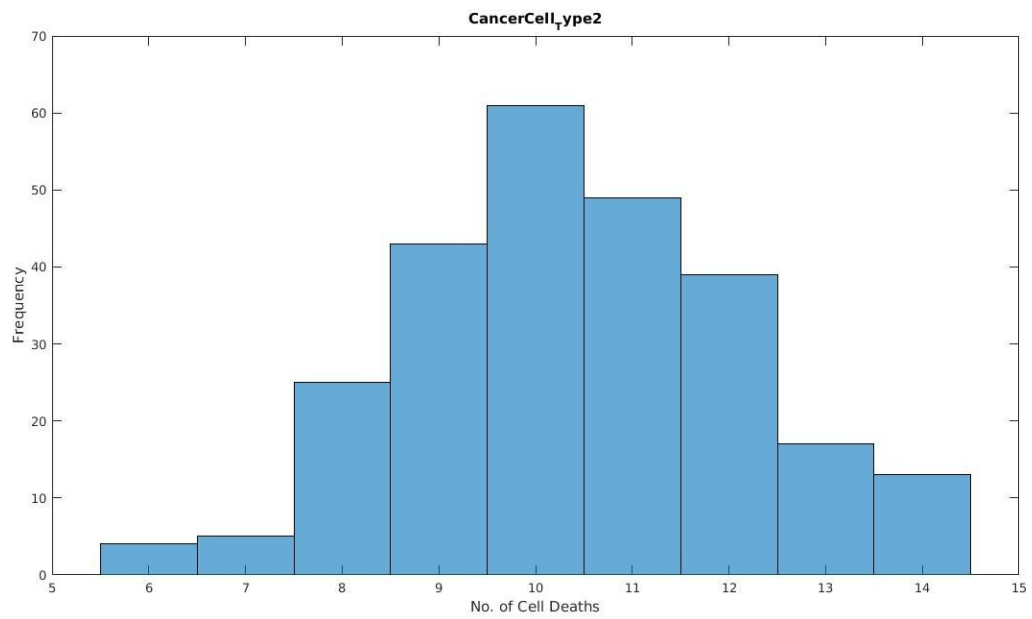


## Histograms Generated:

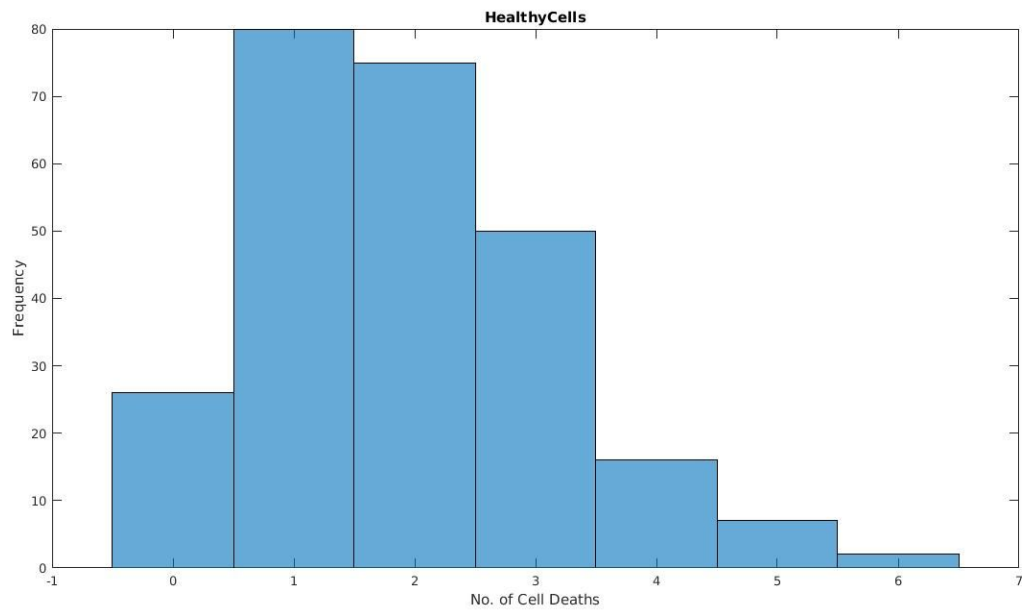
### 1. For Cancer Cell Type-I :



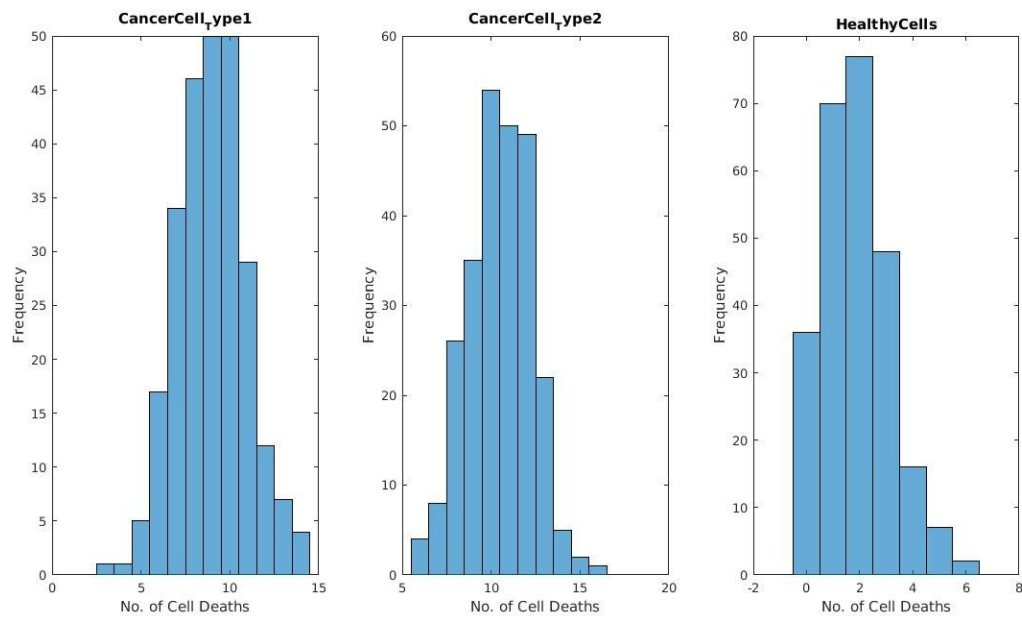
## 2. For Cancer Cell Type-II :



## 3. For Healthy Cells :

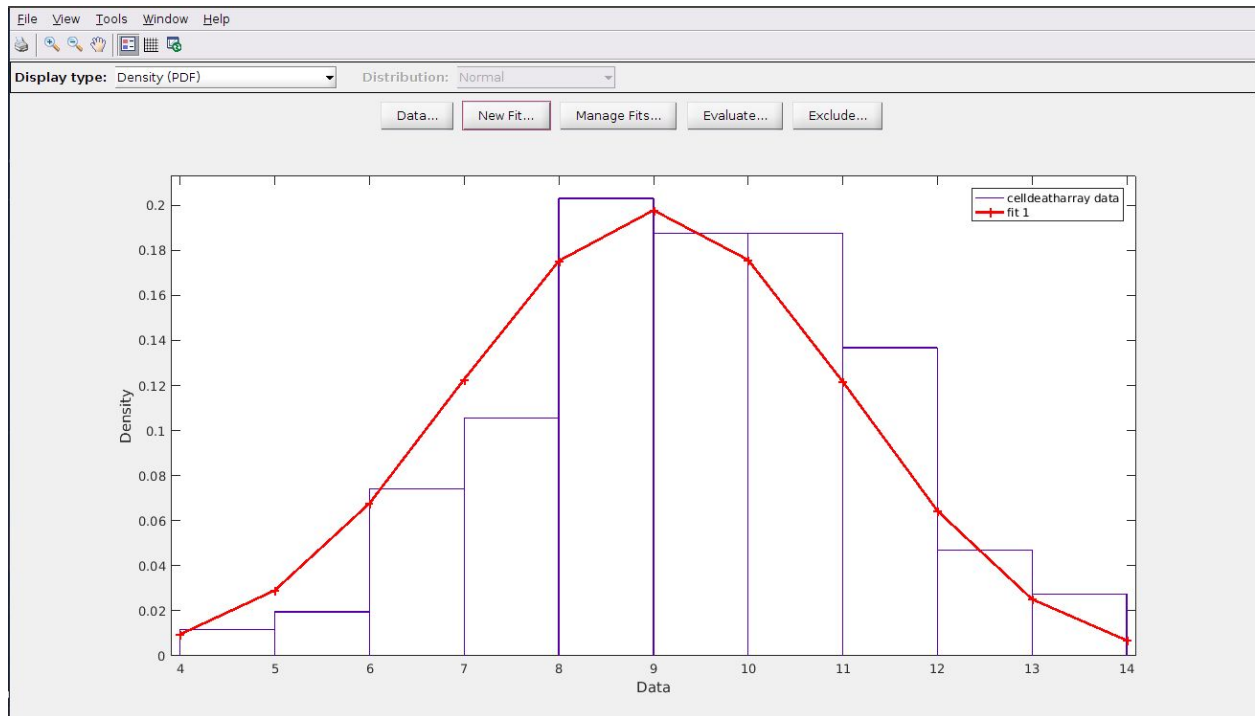


**Merged plots :**

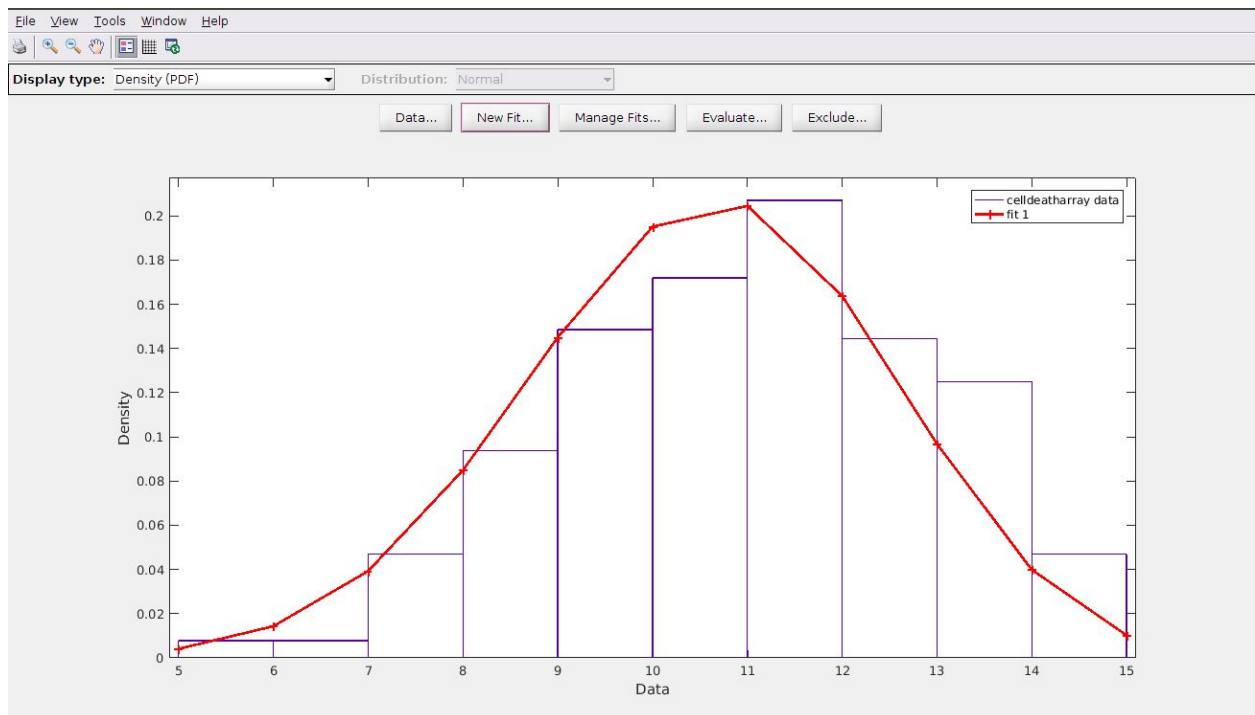


**Question - 6: Binomial Distribution:**

**1. For Cancer Cells Type-I :**

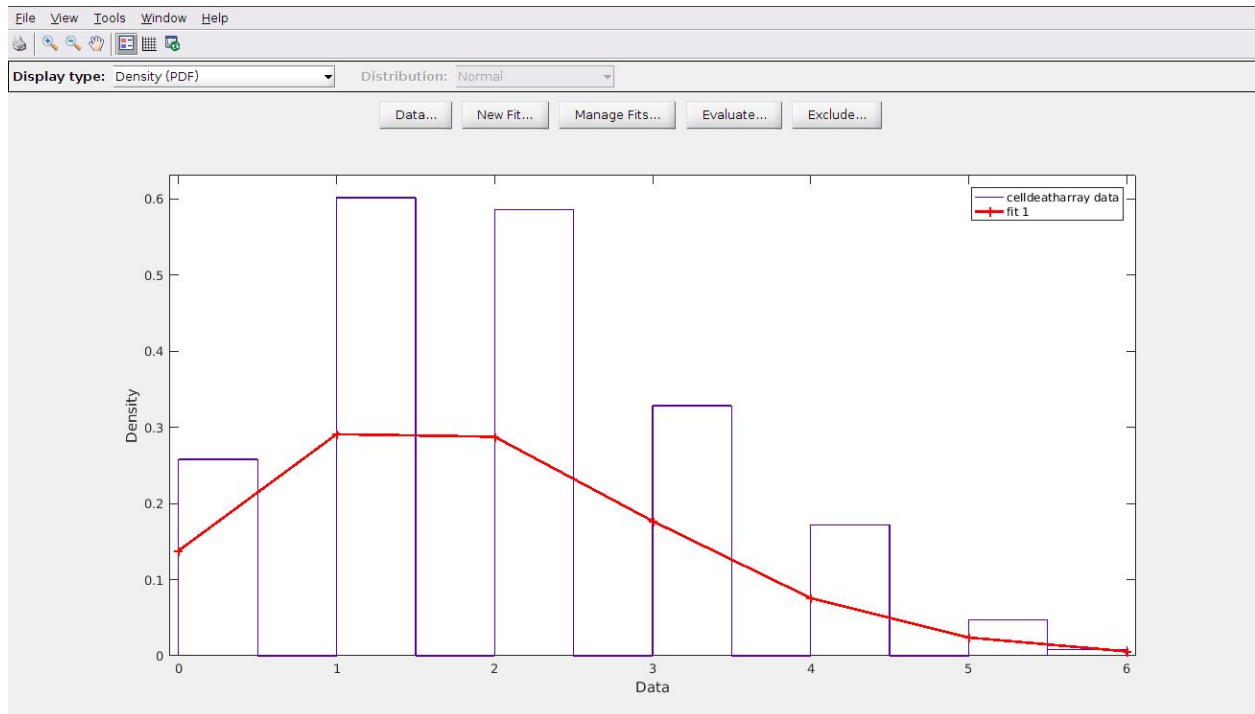


## 2. For Cancer Cells Type-II :



## 3. For Healthy Cells :





**Question - 7: Code for selective activation of cell death in cancer cells based on single cell data analysis :**

```

folders = {'CancerCell_Type1', 'CancerCell_Type2', 'HealthyCells'};
prob = [];
mean = 0;
for i = 1:3          % Loop to iterate over the folders
    celldeaths = []; % Cell-death array to store no. of dead cells
    probability = 0;
    for j = 1:16      % Loop for 16-MC runs
        for k = 1:16  % Loop to iterate over 16 randomly selected files
            apoptosis = 0;
            survival = 0;
            for m = 1:16 % Loop to select a file randomly
                filenum = randi(64);
                file = strcat('output', num2str(filenum));
                filename = fullfile(folders{i}, file);
                fileID = fopen(filename, 'r');
                lastline = "";
                offset = 1;
                fseek(fileID, -offset, 'eof');
                newchar = fread(fileID, 1, '*char');
            end
        end
    end
end

```

```

while (~strcmp(newchar,char(10))) || (offset == 1)
    lastline = [newchar lastline];    % Add the character to a string
    offset = offset + 1;
    fseek(fileID,-offset,'eof');      % Seek to the file end, minus the offset
    newchar = fread(fileID,1,'*char');
end
tokens = str2num(sprintf(lastline));
if (tokens(6) > 80)                    % Decide if the cell survives or dies depending upon the
caspase-3 activation threshold (= 80)
    apoptosis = apoptosis + 1;
    probability = probability + 1;
else
    survival = survival + 1;
end
fclose(fileID);
end
celldeaths = [celldeaths, apoptosis];
end
end
prob = [prob, probability];
mean = mean + probability;
fprintf('Total cell deaths in %s :\n', folders{i});
disp(celldeaths);
subplot(1, 3, i);
histogram(celldeaths);
title(folders{i});
xlabel('No. of Cell Deaths');
ylabel('Frequency');
fprintf('\n');
end
disp(prob/mean);

```

### **Outputs:**

```

probability(cancer_cells_type1) = 0.4090
probability(cancer_cells_type2) = 0.4882
probability(healthy_cells) = 0.1028

```

Since probability of cell death for cancer cells is significantly higher than that for healthy cells, selectivity in cancer cells targeting has been achieved.

### **Question - 8:**

#### **Challenges involved in activating the type 2 pathway in cancer cells --**

C1 -- High over - expression level of XIAP makes the type 2 pathway particularly resistant.

C2 -- Type 2 activation in cancer cells exhibits its characteristics all-or-none type behavior with large cell to cell variability.

### **Remedies --**

R1 -- Low expression level of death receptors and high cFLIP level may preferentially activate the type 2 pathway in a cancer cell.

R2 -- Type 2 to Type 1 transition seems to allow a stochastic to deterministic change in apoptotic activation.

### **Complete Code:**

```
folders = {'CancerCell_Type1', 'CancerCell_Type2', 'HealthyCells'};
prob = []; % Probability array
mean = 0;
for i = 1:3 % Loop to iterate over the folders
    celldeaths = []; % Cell-death array to store no. of dead cells
    probability = 0;
    for j = 1:16 % Loop for 16-MC runs
        for k = 1:16 % Loop to iterate over 16 randomly selected files
            apoptosis = 0;
            survival = 0;
            for m = 1:16 % Loop to select a file randomly
                filenum = randi(64);
                file = strcat('output', num2str(filenum));
                filename = fullfile(folders{i}, file);
                fileID = fopen(filename, 'r');
                lastline = "";
                offset = 1;
                fseek(fileID, -offset, 'eof');
                newchar = fread(fileID, 1, '*char');
                while (~strcmp(newchar, char(10))) || (offset == 1)
                    lastline = [newchar lastline]; % Add the character to a string
                    offset = offset + 1;
                    fseek(fileID, -offset, 'eof'); % Seek to the file end, minus the offset
                    newchar = fread(fileID, 1, '*char');
                end
                tokens = str2num(sprintf(lastline));
                if (tokens(6) > 80) % Decide if the cell survives or dies depending upon the
                    caspase-3 activation threshold (= 80)
                        apoptosis = apoptosis + 1;
                        probability = probability + 1;
                    else
                        survival = survival + 1;
                    end
                end
            end
        end
    end
end
```

```

        end
        fclose(fileID);
    end
    cellDeaths = [cellDeaths, apoptosis];
end
end
prob = [prob, probability];
mean = mean + probability;
fprintf('Total cell deaths in %s :\n', folders{i});
disp(cellDeaths);
subplot(1, 3, i);
histogram(cellDeaths);
title(folders{i});
xlabel('No. of Cell Deaths');
ylabel('Frequency');
fprintf('\n');
end
for p = 1:3
    fprintf('Probability of cell death for %s :\n', folders{p});
    disp(prob(p)/mean);
end

```

---

[references] :

- 1)<https://en.wikipedia.org/wiki/Apoptosis>
- 2)<https://www.omicsonline.org/open-access/in-silico-approach-to-find-an-optimal-strategy-in-selective-targeting-ofcancer-cells-jcsb-1000228.pdf>
- 3)<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3994981/>
- 4)[https://file.scirp.org/pdf/OJApo\\_2015012113442617.pdf](https://file.scirp.org/pdf/OJApo_2015012113442617.pdf)