FINAL DEGREE PROJECT

**Degree in Mechanical Engineering**

# EXPERIMENTAL MEASUREMENTS AND NUMERICAL ANALYSIS OF VOLUME CHANGES IN LIVING CELL AFTER MECHANICAL DEFORMATION.

## Report and Annexes

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# Resumen

Las fuerzas mecánicas intervienen en el funcionamiento normal de las células, tanto en el funcionamiento vital de los órganos, como el corazón o los pulmones, o en el caso de las enfermedades, como cuando una célula cancerosa migra y se deforma para hacer metástasis.

Este Proyecto de Fin De Carrera se centra en la investigación de los cambios de altura y volumen usando imágenes a partir de experimentos realizados en el laboratorio con fibroblastos humanos. Se comparará si la célula puede cambiar tanto la altura como el volumen celular al ser tratada con el fármaco Cytochalasin D, que inhibe las fuerzas mecánicas que la célula es capaz de ejercer para adherir un material. Esta comparación se obtendrá utilizando un método novedoso como la microscopía de desenfoque sobre perlas fluorescentes adheridas tanto al sustrato como a la membrana celular. Esta técnica tiene como objetivo permitir inferir las deformaciones 3D a partir de imágenes 2D. A partir de los resultados obtenidos de la comparación, se muestran los cambios de altura y volumen en las células.

Finalmente, se creará un programa con MatLab para automatizar todo el análisis de los datos asociados a las imágenes experimentales desenfocadas.

# Resum

Les forces mecàniques intervenen en el funcionament normal de les cèl·lules, tant en el funcionament vital dels òrgans, com el cor o els pulmons, o en el cas de les malalties, com quan una cèl·lula cancerosa migra i es deforma per fer metàstasi.

Aquest Projecte de Fi De Carrera se centra en la investigació dels canvis d'alçada i volum utilitzant imatges a partir d'experiments realitzats al laboratori amb fibroblasts humans. Es comparará si la cèl·lula pot canviar tant l'altura com el volum cel·lular en ésser tractada amb el fàrmac Cytochalasin D, que inhibeix les forces mecàniques que la cèl·lula és capaç d'exercir per adherir un material. Aquesta comparació s'obtindrà utilitzant un mètode innovador com la microscòpia de desenfocament sobre perles fluorescents adherides tant al substrat com a la membrana cel·lular. Aquesta tècnica té com a objectiu permetre inferir les deformacions 3D a partir d'imatges 2D. A partir dels resultats obtinguts de la comparació, es mostren els canvis d'altura i volum en les cèl·lules.

Finalment, es crearà un programa amb Matlab per automatitzar tota l'anàlisi de les dades associades a les imatges experimentals desenfocades.

# Abstract

Mechanical Forces are involved in the normal functioning of cells, whether in the vital functioning of organs, such as the heart or lungs and in the case of disease, or during disease, such when a cancer cell migrates and deforms to metastasise.

This Final Degree Project focuses on the investigation of changes in lab cultured human fibroblasts height and volume. A comparison was made to find out whether the cell can change both cell height and volume when treated with the drug Cytochalasin D, a cell force inhibitor. This comparison was obtained using a novel method, defocusing microscopy, applied on 2D images of fluorescent beads adhered to both the substrate and the cell membrane. This technique aims to infer 3D deformations from 2D images. Results obtained from the comparison showed detectable changes in cell height and volume, demonstrating the feasibility of the proposed method.

Finally, a program was created with MatLab to automate the whole data analyses associated to the defocused experimental images.

# Acknowledgements

First of all, I would like to thank the directors of this TFG, Andrea Malandrino and José Javier Muñoz, to research a topic with significant repercussions for future biomechanics.

I would also like to thank them for their constant dedication, support and effort, which was of great help in carrying out this TFG.

Last but not least, I would like to mention all the colleagues I have met during these years of my degree. They have been a great help during the ups and downs that one tends to have during the years of the degree.

Furthermore, focusing on a more closed circle, I would like to thank my parents, Pepi and Juan José, for having instilled in me values such as effort and perseverance in achieving any objective.

# Index

# 1. INTRODUCTION

## 1.1 Cell Mechanics

Variations of animal cell shape is of fundamental importance. Cell shape changes are involved in the normal functioning of different organs under compression/stretching, such as the lungs as they inflate or the heart as it beats. Cell shape changes are also involved in the migration and deformation of cancer cells to give rise to metastasis. [1,2,3]

Cell deformation, in response to mechanical stimuli exerted on cells, is vital because it informs us of cellular structural changes, under the submission of a force [4].

The cytoskeleton is the main structure responsible for determining cellular mechanical properties and changes in cell morphology and is also believed to be the most influential structure in terms of cell stiffness [5,6].

The cytoskeleton is mainly composed of subcellular elements such as the actin cytoskeleton, protein fibres, microtubules and intermediate filaments. These cellular sub-elements enable basic processes for cell survival, e.g. mitosis.

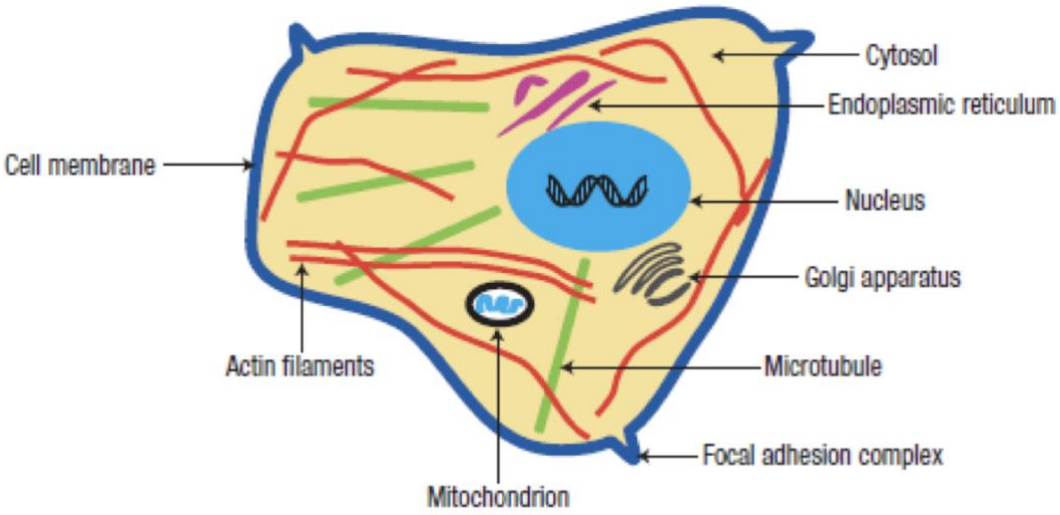


**Figure 1.** *Diagram by Bao and Suresh[5]*. Components of the cell cytoskeleton (e.g.actin filaments, microtubules) are also depicted.

From a biological point of view, changes in cell volume and deformation can be the consequence of altering the mechanical properties of cells. For example, it has been shown that in diseases such as asthma [7], arthritis [8] or malaria [9,10], there is an increase in cellular stiffness. In contrast, in other diseases such as cancer, it is known that cancer cells tend to have properties of greater volume deformability and a lower degree of stiffness and are therefore more permissive to penetration into different tissues than those located in the tumour core. [10,11,12,13].

Variations in cell morphology are related to external forces, both compressive, or shear stresses, through mechanotransduction.
Mechanotransduction refers to those processes that initiate when the cell membrane captures an external mechanical stimulus. Once initiated, the signal propagates trough the focal adhesions and into different internal structure of the cell. These intracellular signaling affects in the turn the remodelling of actin filaments, the filaments that allow the cell to exert tension in response to stimuli, causing stretching and deformation of the cell's shape [14-16].

As such, the cell volume variation can arise as an adaptation of the cell after exerting a force, or from the uptake of external stimuli. In response to these stimuli, extracellular deformations or osmotic perturbations, cell exhibit changes in the influx/efflux of fluid flow, ultimately causing variations in cell volume. It is known that homeostatic mechanisms regulate cell volume by acting in concert to establish an appropriate intracellular ionic framework when external osmotic challenges arise [17,18].
For instance, external osmotic challenges can lead to increased regulatory volume (RVI), ion flux into the cell, defloration/contraction caused by decreased intracellular hydraulic pressure, and, after a decrease in regulatory volume (RVD), ion flow will be directed outwards from the cell. As such, swelling will occur as there has been an increase in intracellular hydraulic pressure. Furthermore, the cytoskeleton is connected to protein complexes that regulate the opening and closing of mechanosensitive ion channels (Figure 2).

It is thus known how osmotic perturbation affect the internal cell hydraulic balance through water influx/efflux. However, much less is known on how or if the changes in tensional cell state, i.e. the forces that cell build adhere to the extracellular material, can also, determine changes in volume.

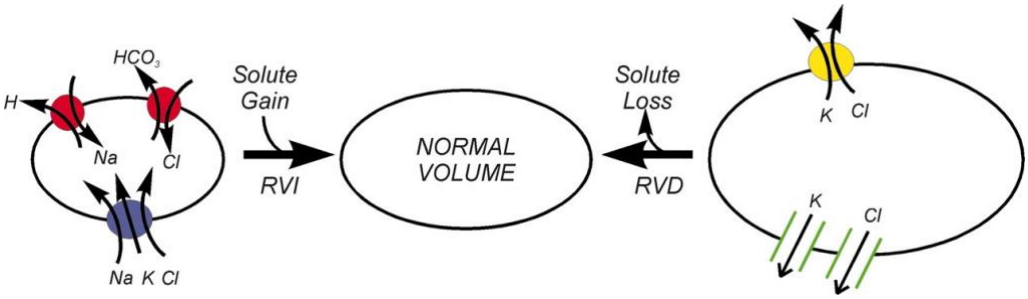


***Figure 2. Schematic by K.Strange [19].*** The loss and accumulation of volume-regulating electrolytes are governed by variations in the activity of transporters and membrane channels. Activation of these transport pathways follows volume perturbation. This indicates that channel and transporter proteins reside in the cell membrane or are rapidly inserted from a pre-existing cytoplasmic pool.

## 1.2 State of the Art.

Numerous studies have established relationships between mechanical responses and biological functions of various organs and even tissues, such as the heart, lungs, bones, cartilage, etc. [20,21,22].

Thanks to these studies, it has been possible to improve the diagnosis and treatment of cardiovascular, respiratory or orthopedic diseases [39]. However, further research into deformation, structural dynamics and transduction in living cells is still needed.

Many mechanobiology studies have highlighted that living cells undergo significant deformations [23].

To quantify mechanical deformations, studies have often considered that the composite forming the cells is like a hydrogel, where a fluid part, an aqueous medium, and a solid polymeric network, coexist. As such, deformations

that the cells will experience in response to mechanical stimuli must involve the knowledge of cell material properties.
Considering the cell as a porous hydrogel suggests that the mechanical state of the cell will depend on the rate at which the fluid can flow and redistribute through the pores of the solid network, thus allowing volumetric deformations in the form of swelling or contractions. [24,25,26].

This behaviour can be modelled considering the intracellular material as poroelastic, and changes in volume or height can be corroborated as a response to osmotic changes [27-29].

In principle, similar to osmotic perturbations, the own cell contraction (force) that cells build to adhere to a substrate can result in hydraulic/intracellular reorganization and ultimately in shape and volume changes.

These examples suggest that the adaptation and survival of cells to deformation is of important magnitude for understanding both physiological and pathological processes. Recently, different models of cell shape coupling and cell deformation have been proposed. [30,31,32,33]
For instance, by reducing the volume, cells increase molecular crowding from osmotic pressures. Alternatively, it has also been studied that depending on the substrate stiffness, ionic interactions can lead to volumetric deformations and produce variations in cell deformation. All these studies are based on inferring the volume with indirect method, but few studies directly measure these morphological variations due to the difficulty of understanding the 3D nature of volume/height changes and connect them to substrate/ 2D deformations [34,35].

## 1.3 Limitations on cell deformation investigations.

3D representation is a priority because it gives a much more comprehensive picture of the changes occurring in cellular structures and physiological functions.
With a 3D representation, we can obtain measurements of how the cell undergoes a readaptation of its volume after a stress stimulus. Although this 3D rendering technique is very sophisticated compared to 2D, it requires a considerable increase in research and instrumentation.

## 1.4 Objectives, methodology and scope.

This project will mainly target an innovative imaging method to obtain volume and height measurements of the cell deformation and the substrate.

***Measure cell heights and volume variations form 2D images.***

This method will consist of obtaining a heightmap from a 2D image. These images will show the morphology of the cell in a normal adhered state with forces built up by the cell (Pre situation) and in a state in which the cell has been treated with a drug, Cytochalasin D, which with only a 15 minute treatment will relax the cell from any forces exerted to adhere to the substrate, by depolymerizing the actomyosin cable that propagate this force (Post situation). Once these images have been analysed, it will be possible to obtain a map of the heights and the cell outline to see the difference between the normal state and the one treated with the drug.

Because in the Pre situation the cell is in it is normal, contracted, and adhered state, and the Post situation the cell is in a relaxed state, the volume changes that we will observe will be indicative of a volume variation associated with the forces that the cell use to normally adhere to the substrate.

Thanks to this methodological advance, we can determine if and how the morphology varies with Cytochalasin D.

***Relate and height changes during cell contractility***

We have introduced experimentally both fluorescent beads to the substrate to obtain a reference height for the substrate underneath the cell and another family of fluorescent beads which are bound to the cell membrane (external surface) that will provide a height measurement (compared to the reference beads) even with a single image, thus allowing us to know from a 2D image its 3D representation.

***Code development for measurements of beads heights***

Finally, a computer program was created with MATLAB software to represent this experimental process and to obtain these height variations and volume modifications in an automated way.

# 2. EXPERIMENTAL MATERIALS AND METHODS.

This section of the Final Degree Project aims to explain the experimental procedure that has been followed to find out the responses that the cell can experience after relaxing significantly cell forces through the drug Cytochalasin D.

## 2.1 Cells cultures and sample preparation.

The cells used in all experiments are Human Lung fibroblasts (from Lonza), transfected with LentiBrite GFP Control Lentiviral Biosensor (from Sigma) to provide cell with live fluorescent emission in the green spectrum (around 510 nm wavelenght) after being excited with a 488 nm laser line.

The cells are seeded on top of a polyacrylamide gel in the shape of a thin disk and incubated for 2 hours before being subjected to imaging. At the incubation period, they shall be maintained at a low concentration in order not to form aggregates or clusters, and immersed in DMEM culture medium supplemented with antibiotics and fetal bovine serum (FBS).

Cells respond differently to substrate stiffness. For instance, mesenchymal cells cultured in 0.25 KPa stiffness behave more like brain cells, whereas in 100 KPa behaving more like bone cells [36]. To vary substrate stiffness, researcher have used polyacrylamide, a transparent and versatile hydrogel for microscopy, with the advantage of tunable stiffness.

It has to be noted that in this project, we have used a single polyacrylamide formulation that provide an intermediate substrate stiffness of around 5 KPa because changing the substrate stiffness was outside the scope of this project.

Briefly, for the polymerization of polyacrylamide hydrogels, a series of crosslinkers are added to a mesh of polymeric chains (Acrylamide) together with a saline solution (PBS).

Due to the versatility and optical properties of polyacrylamide fabrication, it is possible to produce thin transparent disk for cell culture, suitable for microscopic investigation within the range of working distances allowed by the most standard confocal and epifluorescent microscopes used in cell biology. In the present study, we have produced disks of polyacrylamide with 80 mm thickness, compatible with the working distance of the microscope used in this project, and 18 mm of diameter a sufficiently large area for a large number of cells to adhere to the top of the disk in one single experiment. The polyacrylamide is coated with fibronectin to promote stable adhesions of the cell and thus obtain a single-cell configuration per image.

To promote adhesions of the beads to cell membrane, the fluorescent beads (Green Fluorescent 1μm in diameter, from Sigma) are coated with collagen and incorporated into the cell medium 1 hour prior the experiment (referred as membrane beads). Also, the same beads are used in their carboxylate manufactured state to promote attachment to the top surface of the polyacrylamide disk (referred as reference beads).

## 2.2 Microscopy

### 2.2.1 Experimental preparation for measuring heights.

Images of our cell culture were extracted using the epifluorescent laser scanning technique, performed with a ZEISS LSM 800 microscope. We used the x40 magnification objective in oil immersion. This objective moves on the XY axis thanks to its motorised XYZ stage. To extract the images, layers (z-stacks) are acquired and limited to a few microns above and below the cells. This z-stack is acquired ensuring a precise distance of 0.25 μm in z between subsequent images of the stack.

### 2.2.2 Imaging from defocusing.

Defocusing microscopy has been used to track the fluorescent beads attached to both the cell membrane and the substrate (Figure 3), in both an untreated situation and in a situation where cells are treated with the drug Cytochalasin D on the stage of the microscope for a time of ~15 minutes.
The bead appears as a set of concentric rings in the image plane (figure 4 a,b.c), and the shape and size of the rings will depend on the diffraction pattern of the bead and the point spread function of the imaging system. This is because images have been obtained outside the plane of the bead itself.

Considering the back-calculation from defocused diameters and the precision in the z distance between images of a z-stack, the resolution of defocusing microscopy is relatively higher than confocal microscopy, allowing us to calculate small changes in cell heights that may have arisen due to treating the cell with Cytochalasin D.

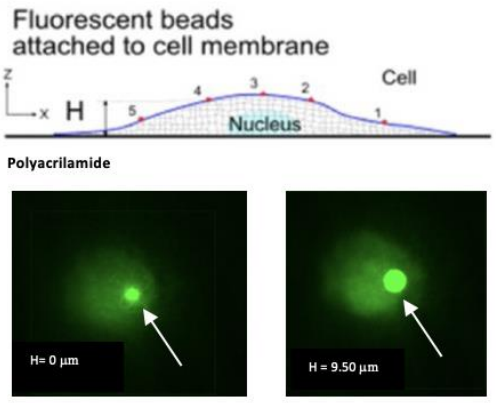


**Figure 3.** Beads adhered to the cell membrane.

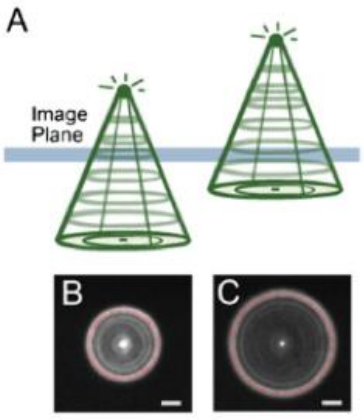


**Figure 4.** Different defocuse planes of the beads.

### 2.2.3 Image processing to determine changes in height from diametric projections.

First of all, the fluorescent beads to be studied must be chosen. When choosing the fluorescent bead, it is taken into account that there are no anomalies such as agglomerations with other beads or signs of vibration, i.e. beads must be tied to the substrate, if reference beads, or the cell membrane, if membrane beads. Once the estimated position of the centre of the bead of interest has been visually selected, the centroid coordinates of the chosen bead (Xc, Yc) are estimated.

Fluorescent beads are excited with a green laser at 405 nm, and their emission is recorded in images. Therefore, the degree of fluorescence emitted can be measured from a *plot profile*, which plot these emissions as fluorescent intensity units along a desired direction. The intensity profile for a defocused beads shows a characteristic shape with a significant peak in the centre of the bead (centroid) and two smaller peaks that coincide with the defocused ring diameter. Therefore, to estimate such diameter, the coordinates corresponding to the smaller peaks are taken (Figure 5).

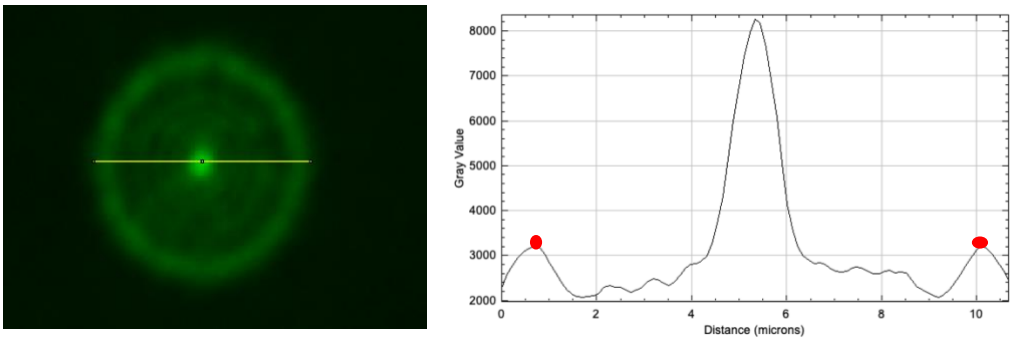


**Figure 5.** Measuring the diameter of beads from a figure plot (ImageJ) and the plot profile shows a profile with the fluorescence intensity of the ring. The two peaks marked in red indicate the references when estimating the diameter of the bead ring.

In defocusing microscopy, a defocusing ring width is proportional to the vertical distance of the focal plane of the image taken and the vertical position of the bead (Figure 6). As such, a defocusing cone can be defined, where the apex indicates the plane where the bead is located. The formula governing the relationship between changes in height and diameters is thus as follows:

$$\Delta Z = \Delta D \cdot c \qquad (1)$$

Where:

$\Delta Z = Z_1 - Z_2$ Variation of heights between different layers of the same cell.

$\Delta D = 2 \cdot R_1 - 2 \cdot R_2$ Variation of the experimental defocusing diameters of the beads.

**c:** Conicity.

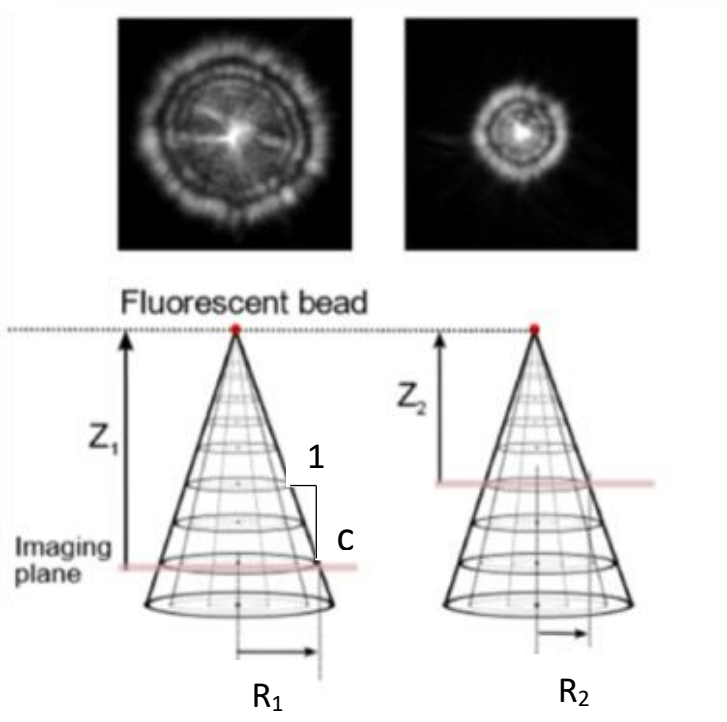


**Figure 6.**Defocusing in different planes of the bead.

To obtain the conicity, two readings from the same bead are needed. For instance, in the situation when the cell not treated with the drug, given that:

$\mathbf{Z_{pre1}}$; $\mathbf{Z_{pre2}}$: specifies the height of the image plane (1,2) containing the measured rings.
$\mathbf{D_{pre1}}$; $\mathbf{D_{pre2}}$: Experimental defocus diameter of the bead in the Pre-situation. It reads:

$$c^i = 2 \cdot \frac{Z_{pre1} - Z_{pre2}}{D^i_{pre1} - D^i_{pre2}} \qquad \textit{For a number of beads i=1, 2 … 6.} \qquad \textbf{(2)}$$

Once we obtain the cone ratio for each bead in the Pre-situation, we normalise this parameter by averaging:
**n**: is the number of beads to be analysed.

$$\bar{c} = \frac{\sum c^i}{n} \qquad \textit{For a number of beads i = 1, 2, … 6.} \qquad \textbf{(3)}$$

Once the normalised c has been obtained, two Pre-Situation values will be calculated for each bead and normalised to extract a value in the experimental Pre-Situation:

$\mathbf{D^i_{pre}}$: Experimental blur diameter of the bead in Pre-situation.
$\mathbf{Z^i_{pre1,2}}$: Experimental height of the bead for each layer of the stack.

$$Z^i_{pre1} = Z_{pre1} - \frac{D^i_{pre1}}{2} \cdot \bar{c} \qquad Z^i_{pre2} = Z_{pre2} - \frac{D^i_{pre2}}{2} \cdot \bar{c} \qquad \textbf{(4 - 5)}$$

The average was calculated to have a normalised measurement for the height in the Pre-situation.

$\mathbf{Z^i_{pre}}$: Experimental height of the bead for the Pre-situation.
$\mathbf{Z^i_{post}}$: Experimental height of the bead for the Post-situation.

$$Z^i_{pre} = \frac{1}{2} \cdot (Z^i_{pre1} + Z^i_{pre2}) \quad \textbf{(6)}$$

Next, we calculate the height in the post situation (cell treated with Cytochalasin D) and then calculate the difference to determine if there has been a variation after the treatment:

$\mathbf{Z_{post}}$: height at which the image plane containing the beads will be.
$\mathbf{D_{post}}$: Diameter of the highlight of the bead.
$\mathbf{Z^i_{post}}$: Experimental height of the bead for the post situation.

$$Z^i_{post} = Z_{post} - \frac{D^i_{post}}{2} \cdot \bar{c} \quad \textbf{(7)}$$

Finally, the variation in cell height after treatment reads:

$$\Delta Z^i = Z^i_{pos} - Z^i_{pre} \quad \textbf{(8)}$$

## 2.3 Computational method for determining changes in height.

To computationally measure the height changes, a Matlab program was created. The program reads as input the position parameters of each bead (Xc, Yc), the experimental defocused ring diameters, and the height parameters of the stack layers where the rings are measured. From these inputs, the program calculates the heights at which the beads are located (apex of the cone) and, through triangular representation plots, allows to visually see if the cell has experienced height variations when the drug Cytochalasin D is applied, i.e. the cell is relaxed. In addition, such a representation can show an outline of the cell to check if there have been differences in cell morphology.

To represent the heights in both the Pre and Post situations, we add a script called **cell_X** (X refers to the number of the cell), where we will have all the parameters described above for each bead. From the function ***MainBeads(),*** we compute an optical cone wherefrom the function ***GetCone()*** will reference the relationship between the diameter and the height.

We verified that a linear relationship is maintained between the height of the layer and the diameter of each bead. As shown in Figure 7, for both the Pre and Post situation, the slope of the lines did not change significantly.

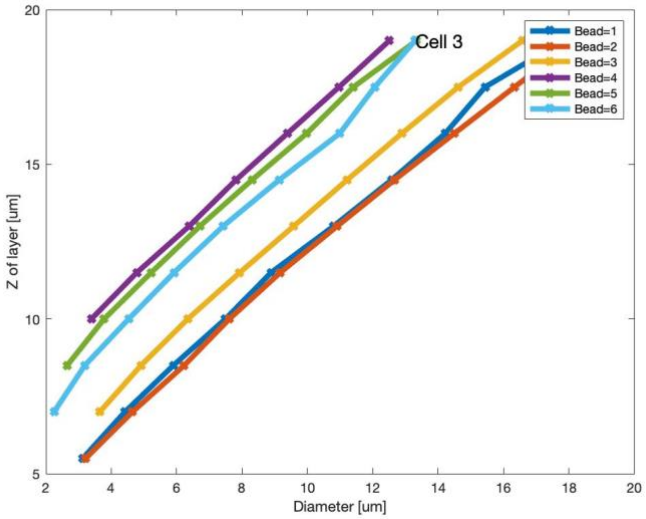


**Figure 7.** Diagram showing the linear relationship between layer height and bead diameter.

To obtain the representations of the heights of each bead, we use the function ***GetAllBeads()***, which, through a nodal triangulation plots the heights of each bead. To make the distribution of the beads more visual, the outline of the cell has been added to this plot to visualize both the inner (membrane bound) and outer (reference substrate) beads. This has been done using the ***GellCellCoord()*** function ( see example in Figure 8-9).

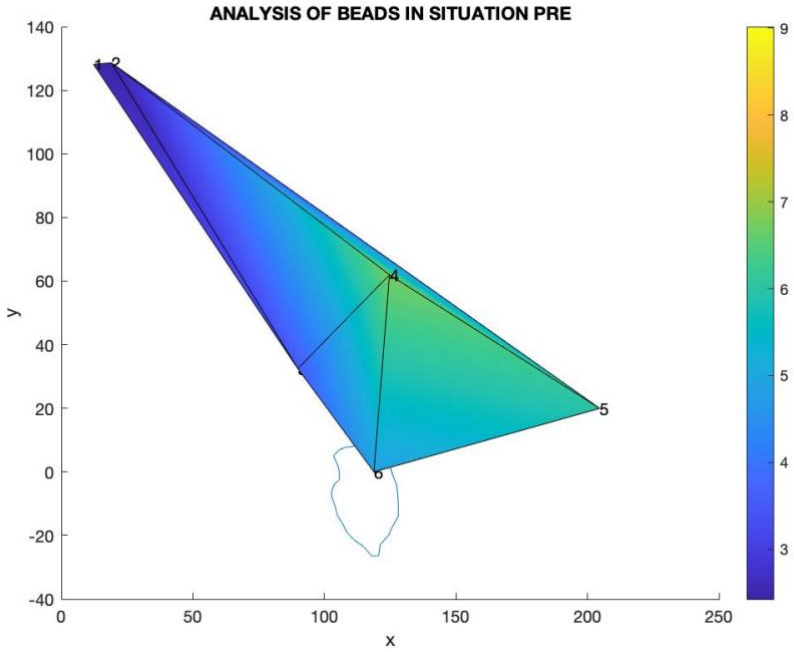


**Figure 8.** Representation of the heights of the situation beads Pre

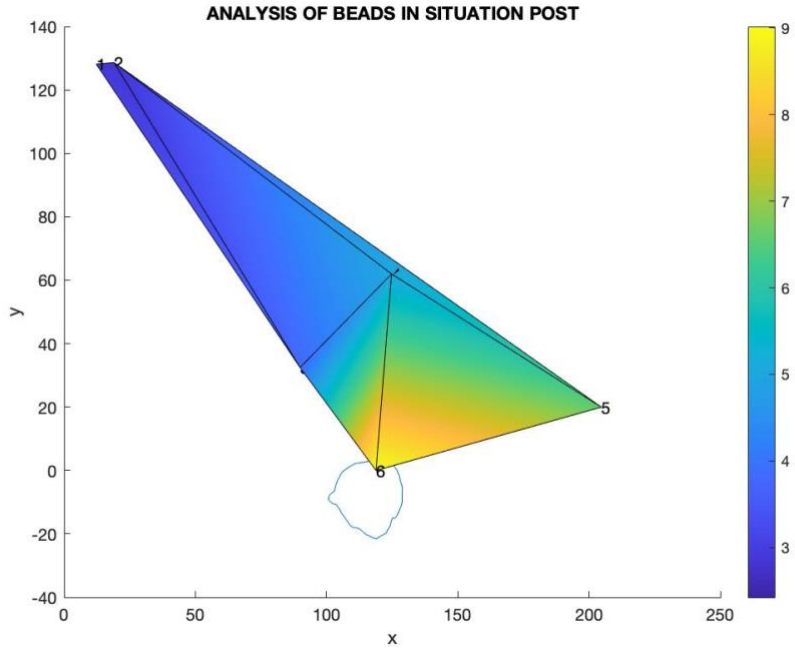


**Figure 9.** Representation of the heights of the situation beads Post.

# 3. RESULTS AND DISCUSSIONS

This section aims to show the results obtained from the cell deformations experienced by various Human Lung Fibroblasts cells when treated with the Cytochalasin D drug.

It has been shown that at high concentrations, Cytochalasin D causes a decrease in mechanical stability. This loss of stability leads to changes in the actin filaments involved in the strength of the cell. Also, small deformations are related to volume variation. Thus, while the drug is acting, a change in the morphological behaviour of the cell is observed [38].
Using the method developed in this project, we have found that the treated cells can change their height upwards (figure 10) and downwards (figures 11 and 12).

In cell 1, it can be seen that when measuring the inner and outer beads, the presence of Cytochalasin D has caused a decrease in height in the Post situation with respect to the Pre situation. Despite the changes in height, it can be seen that the cell has undergone a change in volume as specified by previous theories. These height changes were measured because the polyacrylamide gel holds the beads in place, making it much more visual to detect changes in the cells.

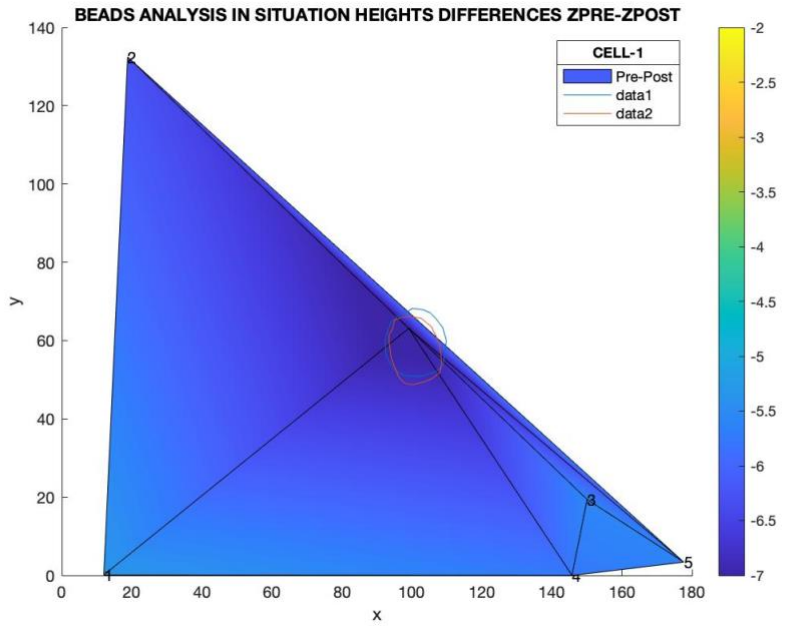


**Figure 10.** Differences Heights Pre-Post of cell-1.

On the other hand, in cells 2 and 3, we can see a variation in heights so that the cell in the post-drug situation can experience greater heights for its standard shape. In this case, as in cell 1, we only obtain changes in the beads inside the cell. Furthermore, concerning volume, it can be seen that there is a compression in the cell volume which will lead to molecular stiffening.

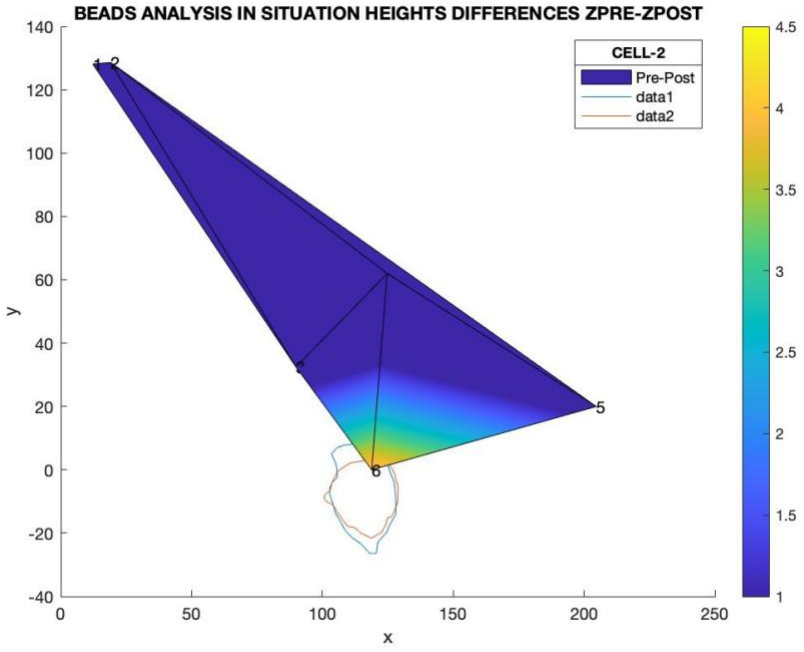


**Figure 11.** Differences Heights Pre-Post of cell-2.

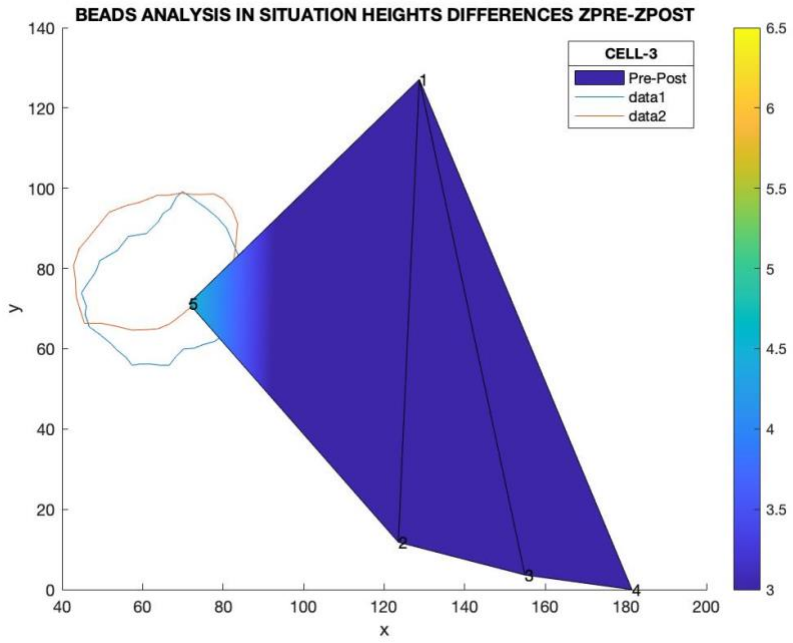


**Figure 12.** Differences Heights Pre-Post of cell-3.

As can be seen, this measurement method based on defocusing can be effective, as reading 2D images and interpreting them as 3D represents a significant advance in the analysis of standard epifluorescent images in cell biology. However, because the limitations in the number of experimental samples we can only speculate if our numerical analysis procedure can produce a significant advance in terms of analysis time. Moreover, the low number of samples prevent us from establish with statistical significance a definitive answer on how the volume variation and the distribution of the cell will evolve during the deformation.

# 4. CONCLUSIONS

In conclusion, we have produced a robust analysis pipeline associated with a microscopy strategy to detect small changes in cell morphology. We have assessed that a treatment with a cytoskeletal drug in the order of minutes, not only perturb cell forces, as well known in the field, but also presumably cause intracellular mechanical redistributions that ultimately perturb the cell volume. This result using defocusing microscopy is novel and promising and can be but in context and compared with other mechanobiology investigations in the future.

# 5. ECONOMIC ANALYSIS

In this section, the cost of this research work is specified.
For this study, in terms of instrumentation, a MatLab licence was required. The annual licence was chosen for teaching use at a value of €250/year. We have also considered the use of the electricity network for which we have assumed a cost of 0.24906 €/KWh. This value is general and represents the cost of KWh in Spain according to the regulated market. [40]

As for the study of the hours to which this work has been devoted, they have been calculated based on an average of 5 hours per day during the academic period of four months.

| **CONCEPT** | **HOURS WORKED** | **PRICE** |
|---|---|---|
| MatLab Programm | ———————— | 250 € |
| Electricity | 440h | 112,262 |
| Time Worked | **440 h** | ———————— |
| | | |
| **TOTAL** | | **362,262€** |

Therefore, this work has had a cost in hours of 440 hours and 362,262 €.

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# 7. ANNEXES

## 7.1 *MainBeads Function.*

```
% Measurements
% NaN = not available
%  B    Z    X        Y       D       Zm
function Error=MainBeads()
% General Settings
Set.PlotCones=true;
Set.PlotSlopes=true;
Set.FS=14;
Set.LW=4;
Set.Transform=true; % false: Do not inverse y axes
Set.Trisurf=false; % true: one color /triangle. false: one color per
 node
Set.Tri=true; % =true. Plot 2D triangles. false: plot dots
Set.Lines=true; % =true. Plot 3D dots with lines. false: no lines
 between dots
Set.sc=1.5; % Synthetic value for Xpost
Set.Cell=3;
%
% Compute optical cone
%
nBeads=5;
c=zeros(nBeads,1);
if Set.PlotCones
    figure(1);clf;
end
Legend=cell(nBeads,1);
for Bead=1:nBeads
    [Xpre,Xpost]=GetDataCones(Bead,Set);
    if isempty(Xpre)
        break;
    end
    if Bead==1
        Xpre1=Xpre;
    end
    if Set.PlotCones
        figure(1)
        D=Xpre(:,end-1);
        Zm=Xpre(:,end);
        plot(D,Zm,'x-','LineWidth',Set.LW)
        hold on
    end
    c(Bead)=GetCone(Xpre);
    Legend{Bead}=sprintf('Bead=%i',Bead);
end
if Bead<nBeads
    nBeads=Bead-1;
    c(nBeads+1:end)=[];
    Legend(nBeads+1:end)=[];
end
legend(Legend);
% Plots
if Set.PlotCones
```

```
    xlabel('Diameter [um]')
    ylabel('Z of layer [um]')
    text(Xpre1(end,end-1),Xpre1(end,end),'Cell 1','FontSize',Set.FS)
    if ~isempty(Xpre)
        text(Xpre(end,end-1),Xpre(end,end),'Cell 2','FontSize',Set.FS)
    end
    hold off
end
if Set.PlotSlopes
    figure(2)
    plot(1:nBeads,c,'o-')
end
xlabel('Bead')
ylabel('Cone Slope')
%
% Compute Height/Diameter of each bead
%
c=mean(c(~isnan(c)));
[Xpre,Xpost]=GetAllBeads(Set);
% Zi=Zi-Di*c
Zpre=Xpre(:,end)-Xpre(:,end-1)*c;
Zpost=Xpost(:,end)-Xpost(:,end-1)*c;
Xpre=[Xpre Zpre];
Xpost=[Xpost Zpost];
% Plot Deformations
Set.LastFigure=2;
[Error,yMax]=PlotBeads(Xpre,Xpost,Set);
% Plot cell contour
[XCellPre,XCellPost]=GetCellCoord(Set);
[EAD,EHD]=AreaGellCell(XCellPre,XCellPost);
fprintf('Cell number %i\n',Set.Cell)
fprintf('Area Difference=%e\n',EAD)
fprintf('Height Difference=%e \n',EHD)
% Make same trasnformation asin Beads
if Set.Transform
    XCellPre(:,2)=yMax-XCellPre(:,2);
    XCellPost(:,2)=yMax-XCellPost(:,2);
end
figure(Set.LastFigure+1); % Pre
hold on
plot(XCellPre(:,1),XCellPre(:,2))
% axis equal

figure(Set.LastFigure+2); % Post
hold on
plot(XCellPost(:,1),XCellPost(:,2))
hold off
%Plot differences
figure(Set.LastFigure+4); % zpost-zpre
hold on
plot(XCellPre(:,1),XCellPre(:,2))
% axis equal
hold on
plot(XCellPost(:,1),XCellPost(:,2))
```

```
hold off
end

function c=GetCone(X)
% Searches correlation between diameter (D) and height (Zm)
% c=Height/Diameter
D=X(:,end-1);
Zm=X(:,end);
n=length(D);
A=[n sum(D)
    sum(D) sum(D.^2)];
b=[sum(Zm)
    sum(Zm.*D)];
c=A\b;
c=c(2);
end

% Calculation of Areas.
function [EAD,EHD] = AreaGellCell(XCPre,XCPost)


% Punto cPre = (116.00,141.76)
XCPre =  [XCPre zeros(size(XCPre,1),1)];
% Punto cPost = (116.68, 142.67)
XCPost =[XCPost zeros(size(XCPost,1),1)];
cPre = 0;
for i = 1:size(XCPre())-1
    cPre = cPre + cross(XCPre(i,:),XCPre(i+1,:));
end
APre = 1/2*sum(cPre);
ResultAPre = abs(APre)

cPost = 0;
for i = 1:size(XCPost())-1
    cPost = cPost + cross(XCPost(i,:),XCPost(i+1,:));
end

APost = 1/2*sum(cPost);
ResultAPost = abs(APost);

% Estimation of area differences

EAD = APre/APost;

% Estimation of heights differences
% Previously calculated messurements
% HPre = z cells
% HPost = z cells
HPre = 7.00
HPost = 11.50
EHD = HPre/HPost;
end
```

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# *7.2 PlotBeads Function.*

```
function [Error,yMax]=PlotBeads(Xpre,Xpost,Set)
% Plots diameters at different heights and computes z levels fom Xpre
 and Xpost
% Each Xpre-Post contains for each column:
%    Bead   x    y     D     Zm   Zi
% X=[                                  ]
% x = x coord. of bead
% y = y coord. of bead
% D = Diameter
% Zm= height of micorsopcy while reading
% Zi= computed height of bead
xi=2; % column of x coord
yi=3; % column of y coord
zi=size(Xpre,2);
xpre=Xpre(:,xi);
ypre=Xpre(:,yi);
zpre=Xpre(:,zi);
xpost=Xpost(:,xi);
ypost=Xpost(:,yi);
zpost=Xpost(:,zi);
yMax=max([max(ypre) max(ypost)]);
if Set.Transform
    ypre=yMax-ypre; % Reverse y-axis
    ypost=yMax-ypost; % Reverse y-axis
end
% Compute cone ratio
Error=0;
% Set.c=ComputeConeRatio(Xpre1, Xpre2);
n=size(Xpre,1);
% Pre data
if sum(isnan(xpre))==0 && sum(isnan(ypre))==0
    Set.clim=[min([zpre]) max([zpre])];
    figure(Set.LastFigure+1)
    clf
    Set.Title=' PRE';
    Error=PlotHeight(xpre,ypre,zpre,Set);
end
% Post data
if sum(isnan(xpost))==0 && sum(isnan(ypost))==0
    Set.clim=[min([zpost]) max([zpost])];
    figure(Set.LastFigure+2)
    clf
    Set.Title=' POST';
    Error=PlotHeight(xpost,ypost,zpost,Set);
end
% Plot Height Differences
if sum(isnan(xpost))==0 && sum(isnan(ypost))==0
    Set.clim=[min([zpre-zpost]) max([zpre-zpost])];
    figure(Set.LastFigure+4)
    clf
    Set.Title=' HEIGHTS DIFFERENCES ZPRE-ZPOST';
    Error=PlotHeight(xpost,ypost,zpost-zpre,Set);
```

1

```
    legend('Pre','Post')
end
% Plot heights
if length(xpre)==length(xpost)
    figure(Set.LastFigure+3)
    clf
    plot(1:n,zpre,1:n,zpost)
    legend('Pre','Post')
    xlabel('Bead')
    ylabel('Z_{bead}')
end
end
%
function Error=PlotHeight(xi,yi,zi,Set)
%    Bead x        y     D     Zm   Zi
% X=[                                  ]
%
Error=0;
hold on
if nargin==1
    Set.Title='';
end
dx1=max(xi)-min(xi);
dx2=max(yi)-min(yi);
if dx1<eps || dx2<eps
    warning ('Bead coordinates are too joined.')
    Error=NaN;
else
    DT=delaunay(xi,yi);
    if Set.Trisurf
        trisurf(DT,xi,yi,zi);
    end
    % triplot(DT,X(:,1),X(:,2)) % Shows triangulation only
    if Set.Tri
        if ~Set.Trisurf
            for i=1:size(DT,1)
                x=xi(DT(i,:));
                y=yi(DT(i,:));
                d=zi(DT(i,:));
                patch(x,y,d,d)
            end
        end
        for i=1:length(xi)
            x=xi(i);
            y=yi(i);
            text(x,y,zi(i),num2str(i));
        end
        colorbar
    else
        for i=1:length(xi)
            x=xi(i);
            y=yi(i);
            d=zi(i);
            plot3(x+2,y+2,d,'o');
```

```
            text(x,y,d,num2str(i));
        end
        if Set.Lines
            for i=1:size(DT,1)
                t=[DT(i,:) DT(i,1)];
                x=xi(t);
                y=yi(t);
                d=zi(t);
                plot3(x,y,d)
            end
        end
        plot3(0,0,0)
    end
    title(strcat('BEADS ANALYSIS IN SITUATION ',Set.Title))
    xlabel('x')
    ylabel('y')
    zlabel('Height')
    caxis(Set.clim);
    hold off
end
end
```

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## 7.3 *GetDataCones Function*

```
function [Xpre,Xpost]=GetDataCones(Bead,Set)
% Gets data on bead location.
% Output:
% Xpre = before deformation
% Xpost = after deformation
% Each column contains:
%  B   Z   X       Y      D      Zm
% with,
% B   =Bead
% Z   =layer
% X.Y = x.y-coordinates
% D   = Diameter
% Zm  = height of layer in um
%
% Data 20/4/2021
% Cell 2
% Bead 1

%-------------------------------------------------------------
% BEADS Experiment 13_May.
%-------------------------------------------------------------
% CELL 1. PRE-Situation
%-------------------------------------------------------------
if Set.Cell == 1
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
  Xpre=[15 12.03 137.45 3.76 7.00
        18 12.03 137.45 5.13 8.50
        21 12.03 137.45 6.45 10.00
        24 12.03 137.45 8.00 11.50
        27 12.03 137.45 9.67 13.00
        30 12.03 137.45 11.32 14.50
        33 12.03 137.45 13.32 16.00
        36 12.03 137.45 15.09 17.50
        39 12.03 137.45 16.89 19.00
        42  12.03 137.45  19.03   20.50];
elseif Bead==2 % Bead 2.
   Xpre=[15 18.84 5.11 3.14 7.00
        18 18.84 5.11 4.52 8.50
        21 18.84 5.11 5.86 10.00
        24 18.84 5.11 7.24 11.50
        27 18.84 5.11 8.93 13.00
        30 18.84 5.11 10.54 14.50
        33 18.84 5.11 12.40 16.00
        36 18.84 5.11 14.08 17.50
        39 18.84 5.11 15.90 19.00
        42  18.84 5.11    17.90   20.50];

elseif Bead==3 % Bead 3
  Xpre=[21 150.05 118.15 3.87 10.00
        24 150.05 118.15 5.05 11.50
        27 150.05 118.15 6.40 13.00
```

1

```
        30 150.05 118.15 7.95 14.50
        33 150.05 118.15 9.52 16.00
        36 150.05 118.15  11.04   17.50
        39 150.05 118.15 12.76 19.00
        42  150.05 118.15  14.89   20.50];
elseif Bead==4 % Bead 4
  Xpre=[21 145.73 137.56 3.65 10.00
        24 145.73 137.56 5.02    11.50
        27 145.73 137.56 6.50 13.00
        30 145.73 137.56 7.84 14.50
        33 145.73 137.56 9.58 16.00
        36 145.73 137.56 11.03 17.50
        39 145.73 137.56 12.77 19.00
        42 145.73 137.56 14.53 20.50 ];

elseif Bead==5 % Bead 5
  Xpre=[21 177.63 134.16 3.28 10.00
        24 177.63 134.16 4.63    11.50
        27 177.63 134.16 6.04 13.00
        30 177.63 134.16 7.50    14.50
        33 177.63 134.16 9.19 16.00
        36 177.63 134.16 10.54 17.50
        39 177.63 134.16 12.09 19.00
        42 177.63 134.16 14.09 20.50 ];
elseif Bead==6 % Bead 6 INSIDE CELL.
  Xpre=[36 99.09 74.46 3.72  17.50
        39 99.09 74.46 4.84 19.00
        42 99.09 74.46 7.64 20.50  ];
else
    %
    % Data 13/4/2021
    %
    warning('Bead Number %i not assigned',Bead)
    Xpre=[];
    Xpost=[];
end
if Bead<10 % Add bead number
    Xpre=[Bead*ones(size(Xpre,1),1) Xpre];
end

%-----------------------------------------------

%-----------------------------------------------
% CELL-1. POST-Situation
%-----------------------------------------------
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
  Xpost=[3 12.03 137.45 3.01 1.00
        6 12.03 137.45 3.95 2.50
        9 12.03 137.45 5.42 4.00
        12 12.03 137.45 6.82 5.50
        15 12.03 137.45 8.48 7.00
        18 12.03 137.45 9.97 8.50
        21 12.03 137.45 11.7 10.00
```

```
        24 12.03 137.45 13.53 11.50
        27 12.03 137.45 15.36 13.00
        30 12.03 137.45 17.20 14.50 ];
elseif Bead==2 % Bead 2.

 Xpost=[3 18.84 5.11 3.45 1.00
        6 18.84 5.11 4.65 2.50
        9 18.84 5.11 6.05 4.00
        12 18.84 5.11 7.60 5.50
        15 18.84 5.11 9.02 7.00
        18 18.84 5.11 10.79 8.50
        21 18.84 5.11 12.71 10.00
        24 18.84 5.11 14.41 11.50
        27 18.84 5.11 16.33 13.00
        30 18.84 5.11 18.38 14.50 ];
elseif Bead==3 % Bead 3
 Xpost=[9 150.05 118.15 3.39 4.00
        12 150.05 118.15 4.55 5.50
        15 150.05 118.15 6.04 7.00
        18 150.05 118.15 7.36 8.50
        21 150.05 118.15 8.95 10.00
        24 150.05 118.15 10.55 11.50
        27 150.05 118.15 12.28 13.00
        30 150.05 118.15 14.13 14.50 ];
elseif Bead==4 % Bead 4
  Xpost=[9 145.73 137.56 3.38 4.00
        12 145.73 137.56 4.51 5.50
        15 145.73 137.56 5.90 7.00
        18 145.73 137.56 7.40 8.50
        21 145.73 137.56 8.78 10.00
        24 145.73 137.56 10.58 11.50
        27 145.73 137.56 12.18 13.00
        30 145.73 137.56 13.85 14.50 ];

elseif Bead==5 % Bead 5
  Xpost=[9 177.63 134.16 2.84 4.00
        12 177.63 134.16 4.13 5.50
        15 177.63 134.16 5.53 7.00
        18 177.63 134.16 6.88 8.50
        21 177.63 134.16 8.35 10.00
        24 177.63 134.16 9.58 11.50
        27 177.63 134.16 11.35 13.00
        30 177.63 134.16 13.04 14.50 ];

elseif Bead==6 % Bead 6 INSIDE CELL.
  Xpost=[18 99.09 74.46 1.69 8.50
        21 99.09 74.46 2.93 10.00
        24 99.09 74.46 4.09 11.50
        27 99.09 74.46 5.36 13.00
        30 99.09 74.46 6.80 14.50 ];
end

if Bead<10 % Add bead number
    Xpost=[Bead*ones(size(Xpost,1),1) Xpost];
```

```
end
%-------------------------------------------------------------
% BEADS Experiment 13_May
%-------------------------------------------------------------
% CELL 2. PRE-Situation
%-------------------------------------------------------------
elseif Set.Cell==2
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
  Xpre=[12 12.26 5.67 3.13 5.50
        15 12.26 5.67 4.42 7.00
        18 12.26 5.67 5.90 8.50
        21 12.26 5.67 7.49 10.00
        24 12.26 5.67 8.89 11.50
        27 12.26 5.67 10.80 13.00
        30 12.26 5.67 12.58 14.50
        33 12.26 5.67 14.22 16.00
        36 12.26 5.67 15.44 17.50
        39  12.26 5.67    17.84   19.00 ];
elseif Bead==2 % Bead 2.
  Xpre=[12 19.07 5.22 3.22 5.50
        15 19.07 5.22 4.65 7.00
        18 19.07 5.22 6.23 8.50
        21 19.07 5.22 7.61 10.00
        24 19.07 5.22 9.17 11.50
        27 19.07 5.22 10.91 13.00
        30 19.07 5.22 12.67 14.50
        33 19.07 5.22 14.50 16.00
        36 19.07 5.22 16.34 17.50
        39  19.07 5.22    18.18   19.00 ];

elseif Bead==3 % Bead 3
  Xpre=[15 89.89 101.36 3.65 7.00
        18 89.89 101.36 4.91 8.50
        21 89.89 101.36 6.34 10.00
        24 89.89 101.36 7.93 11.50
        27 89.89 101.36 9.58 13.00
        30 89.89 101.36 11.22 14.50
        33 89.89 101.36 12.89 16.00
        36 89.89 101.36 14.62 17.50
        39  89.89 101.36  16.58   19.00 ];
elseif Bead==4 % Bead 4
  Xpre=[21 124.74 71.85 3.39 10.00
        24 124.74 71.85 4.78 11.50
        27 124.74 71.85 6.38 13.00
        30 124.74 71.85 7.82 14.50
        33 124.74 71.85 9.39 16.00
        36 124.74 71.85 10.97 17.50
        39  124.74 71.85   12.51   19.00 ];

elseif Bead==5 % Bead 5
  Xpre=[18 204.64 113.84 2.65 8.50
        21 204.64 113.84 3.78 10.00
        24 204.64 113.84 5.22 11.50
```

```
        27 204.64 113.84 6.71 13.00
        30 204.64 113.84 8.31 14.50
        33 204.64 113.84 9.98 16.00
        36 204.64 113.84 11.41 17.50
        39  204.64 113.84  13.34   19.00 ];
elseif Bead==6 % Bead 6 INSIDE CELL.
  Xpre=[15 118.83 133.82 2.25 7.00
        18 118.83 133.82 3.19 8.50
        21 118.83 133.82 4.53 10.00
        24 118.83 133.82 5.92 11.50
        27 118.83 133.82 7.42 13.00
        30 118.83 133.82 9.14 14.50
        33 118.83 133.82 10.99 16.00
        36 118.83 133.82 12.06 17.50
        39  118.83 133.82  13.30   19.00 ];
else
    %
    % Data 13/4/2021
    %
    warning('Bead Number %i not assigned',Bead)
    Xpre=[];
    Xpost=[];
end
if Bead<10 % Add bead number
    Xpre=[Bead*ones(size(Xpre,1),1) Xpre];
end
%--------------------------------------
%--------------------------------------
% For sinthetic Bead (Remove when measured)
% Cell 2
% Bead 1

%Xpost=Xpre;
%Xpost(:,4)=Xpost(:,4)-Set.sc;
%-----------------------------------------------
% POST-Situation
%-----------------------------------------------
% CELL-2. POST Situation
%-----------------------------------------------
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
 Xpost=[12 12.26 5.67 2.70 5.50
        15 12.26 5.67 4.11 7.00
        18 12.26 5.67 5.46 8.50
        21 12.26 5.67 7.11 10.00
        24 12.26 5.67 8.62 11.50
        27 12.26 5.67 10.04 13.00
        30 12.26 5.67 12.04 14.50
        33 12.26 5.67 12.94 16.00
        36 12.26 5.67 15.83 17.50
        39  12.26 5.67    17.62   19.00 ];

elseif Bead==2 % Bead 2.
 Xpost=[12 19.07 5.22 2.78 5.50
```

```
        15 19.07 5.22 4.21 7.00
        18 19.07 5.22 5.78 8.50
        21 19.07 5.22 7.29 10.00
        24 19.07 5.22 8.79 11.50
        27 19.07 5.22 10.37 13.00
        30 19.07 5.22 12.24 14.50
        33 19.07 5.22 14.11 16.00
        36 19.07 5.22 15.85 17.50
        39  19.07 5.22    17.88   19.00 ];

elseif Bead==3 % Bead 3
 Xpost=[15 89.89 101.36 3.35 7.00
        18 89.89 101.36 4.55 8.50
        21 89.89 101.36 6.03 10.00
        24 89.89 101.36 7.42 11.50
        27 89.89 101.36 9.04 13.00
        30 89.89 101.36 10.72 14.50
        33 89.89 101.36 12.20 16.00
        36 89.89 101.36 14.26 17.50
        39  89.89 101.36  16.18   19.00 ];

elseif Bead==4 % Bead 4
 Xpost=[18 124.74 71.85 3.61 8.50
        21 124.74 71.85 5.00 10.00
        24 124.74 71.85 6.48 11.50
        27 124.74 71.85 8.00 13.00
        30 124.74 71.85 9.65 14.50
        33 124.74 71.85 11.00 16.00
        36 124.74 71.85 12.89 17.50
        39  124.74 71.85  14.41    19.00 ];

elseif Bead==5 % Bead 5
 Xpost=[18 204.64 113.84 2.02 8.50
        21 204.64 113.84 3.52 10.00
        24 204.64 113.84 4.87 11.50
        27 204.64 113.84 6.12 13.00
        30 204.64 113.84 7.91 14.50
        33 204.64 113.84 9.47 16.00
        36 204.64 113.84 10.89 17.50
        39  204.64 113.84  13.02   19.00 ];

elseif Bead==6 % Bead 6 INSIDE CELL.
 Xpost=[24 118.83 133.82 2.60 11.50
        27 118.83 133.82 3.89 13.00
        30 118.83 133.82 5.25 14.50
        33 118.83 133.82 6.66 16.00
        36 118.83 133.82 8.23 17.50
        39  118.83 133.82  9.52    19.00 ];
end


if Bead<10 % Add bead number
    Xpost=[Bead*ones(size(Xpost,1),1) Xpost];
end
```

```
%-------------------------------------------------------------
% BEADS Experiment 13_May.
%-------------------------------------------------------------
% CELL 3. PRE-Situation
%-------------------------------------------------------------
elseif Set.Cell==3
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
  Xpre=[24 128.71 22.47 3.29 11.50
        27 128.71 22.47 4.63 13.00
        30 128.71 22.47 5.96 14.50
        33 128.71 22.47 7.52 16.00
        36 128.71 22.47 8.94 17.50
        39 128.71 22.47 10.64 19.00
        42  128.71 22.47   12.32   20.50];
elseif Bead==2 % Bead 2.
   Xpre=[24 123.49 137.79 3.92 11.50
        27 123.49 137.79 5.16 13.00
        30 123.49 137.79 6.54 14.50
        33 123.49 137.79 8.20 16.00
        36 123.49 137.79 9.75 17.50
        39 123.49 137.79 11.30 19.00
        42  123.49 137.79  12.95   20.50];

elseif Bead==3 % Bead 3
  Xpre=[24 154.93 145.96 3.32 11.50
        27 154.93 145.96 4.62 13.00
        30 154.93 145.96 5.94 14.50
        33 154.93 145.96 7.49 16.00
        36 154.93 145.96  9.02    17.50
        39 154.93 145.96 10.59 19.00
        42  154.93 145.96  11.16   20.50];
elseif Bead==4 % Bead 4
  Xpre=[24 181.49 149.59 2.98    11.50
        27 181.49 149.59 4.42 13.00
        30 181.49 149.59 6.22 14.50
        33 181.49 149.59 7.25 16.00
        36 181.49 149.59 8.66 17.50
        39 181.49 149.59 10.20 19.00
        42 181.49 149.59 11.76 20.50 ];

elseif Bead==5 % Bead 5
  Xpre=[24 71.50   78.31 3.02    11.50
        27 71.50   78.31 4.72 13.00
        30 71.50   78.31 5.52    14.50
        33 71.50   78.31 7.06 16.00
        36 71.50   78.31 8.51 17.50
        39 71.50   78.31 10.11 19.00
        42 71.50   78.31 11.65 20.50 ];

else
    %
```

```
    % Data 13/4/2021
    %
    warning('Bead Number %i not assigned',Bead)
    Xpre=[];
    Xpost=[];
end
if Bead<10 % Add bead number
    Xpre=[Bead*ones(size(Xpre,1),1) Xpre];
end

%------------------------------------------------

%------------------------------------------------
% CELL-3. POST-Situation
%------------------------------------------------
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
 Xpost=[24 128.71 22.47 2.91 11.50
        27 128.71 22.47 4.22 13.00
        30 128.71 22.47 5.40 14.50
        33 128.71 22.47 6.84 16.00
        36 128.71 22.47 8.31 17.50
        39 128.71 22.47 10.04 19.00
        42  128.71 22.47   11.95   20.50];
elseif Bead==2 % Bead 2.
 Xpost=[24 123.49 137.79 3.36 11.50
        27 123.49 137.79 4.70 13.00
        30 123.49 137.79 6.07 14.50
        33 123.49 137.79 8.01 16.00
        36 123.49 137.79 9.21 17.50
        39 123.49 137.79 10.64 19.00
        42  123.49 137.79  12.40   20.50];
elseif Bead==3 % Bead 3
 Xpost=[24 154.93 145.96 2.76 11.50
        27 154.93 145.96 4.07 13.00
        30 154.93 145.96 5.52 14.50
        33 154.93 145.96 6.80 16.00
        36 154.93 145.96 8.49 17.50
        39 154.93 145.96 9.84 19.00
        42  154.93 145.96  11.61   20.50];

elseif Bead==4 % Bead 4
 Xpost=[27 181.49 149.59 3,91 13.00
        30 181.49 149.59 5,15 14.50
        33 181.49 149.59 6,43 16.00
        36 181.49 149.59 8,03 17.50
        39 181.49 149.59 9,49 19.00
        42  181.49 149.59  11,37   20.50];

elseif Bead==5 % Bead 5 INSIDE CELL.
 Xpost=[33 71.50   78.31 2.95 16.00
        36 71.50   78.31 4.41 17.50
        39 71.50   78.31 5.77 19.00
        42 71.50   78.31 7.41 20.50 ];
```

```
end

if Bead<10 % Add bead number
    Xpost=[Bead*ones(size(Xpost,1),1) Xpost];
end

%-------------------------------------------------------------
% BEADS Experiment 13_May.
%-------------------------------------------------------------
% CELL 4. PRE-Situation
%-------------------------------------------------------------
elseif Set.Cell==4
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
  Xpre=[18 41.20 115.09 4.36 8.50
        21 41.20 115.09 5.77 10.00
        24 41.20 115.09 7.25 11.50
        27 41.20 115.09 8.94 13.00
        30 41.20 115.09 10.58 14.50
        33 41.20 115.09 12.15 16.00];
elseif Bead==2 % Bead 2.
   Xpre=[18 94.20 21.45 2.78 8.50
         21 94.20 21.45 3.80 10.00
         24 94.20 21.45 5.24 11.50
         27 94.20 21.45 6.68 13.00
         30 94.20 21.45 8.20 14.50
         33 94.20 21.45 9.85 16.00];

elseif Bead==3 % Bead 3
  Xpre=[21 129.73 5.90 3.46 10.00
        24 129.73 5.90 4.72 11.50
        27 129.73 5.90 6.26 13.00
        30 129.73 5.90 7.56 14.50
        33 129.73 5.90 9.29 16.00];
elseif Bead==4 % Bead 4
  Xpre=[21 186.03  90.35 2.63 10.00
        24 186.03  90.35 3.91    11.50
        27 186.03  90.35 5.30 13.00
        30 186.03  90.35 6.76 14.50
        33 186.03  90.35 8.42 16.00];

elseif Bead==5 % Bead 5
  Xpre=[21 176.04 146.07 2.70    10.00
        24 176.04 146.07 4.11    11.50
        27 176.04 146.07 5.53 13.00
        30 176.04 146.07 6.91    14.50
        33 176.04 146.07 8.62 16.00];
elseif Bead==6 % Bead 6 INSIDE CELL.
  Xpre=[18 94.20 21.45 3.03    8.50
        21 94.20 21.45 4.32    10.00
        24 94.20 21.45 5.57    11.50
        27 94.20 21.45 6.99 13.00
        30 94.20 21.45 8.62 14.50
        33 94.20 21.45 10.38 16.00  ];
```

```
else
    %
    % Data 13/4/2021
    %
    warning('Bead Number %i not assigned',Bead)
    Xpre=[];
    Xpost=[];
end
if Bead<10 % Add bead number
    Xpre=[Bead*ones(size(Xpre,1),1) Xpre];
end


%-------------------------------------------------

%-------------------------------------------------
% CELL-4. POST-Situation
%-------------------------------------------------
if Bead==1 % Bead 1.
    %     Z   X       Y      D      Zm
 Xpost=[18 41.20 115.09 4.13 8.50
        21 41.20 115.09 5.32 10.00
        24 41.20 115.09 6.92 11.50
        27 41.20 115.09 8.46 13.00
        30 41.20 115.09 10.28 14.50
        33 41.20 115.09 12.15 16.00
        36 41.20 115.09 13.60 17.50 ];

elseif Bead==2 % Bead 2.
 Xpost=[21 94.20 21.45 3.65 10.00
        24 94.20 21.45 4.82 11.50
        27 94.20 21.45 6.29 13.00
        30 94.20 21.45 7.94 14.50
        33 94.20 21.45 9.26 16.00
        36 94.20 21.45 11.01 17.50 ];

elseif Bead==3 % Bead 3
 Xpost=[21 129.73 5.90 3.29 10.00
        24 129.73 5.90 4.49 11.50
        27 129.73 5.90 5.86 13.00
        30 129.73 5.90 7.31 14.50
        33 129.73 5.90 8.92 16.00
        36 129.73 5.90 10.66 17.50 ];
elseif Bead==4 % Bead 4
  Xpost=[21 186.03  90.35 2.45 10.00
        24 186.03  90.35 3.73 11.50
        27 186.03  90.35 5.07 13.00
        30 186.03  90.35 6.54 14.50
        33 186.03  90.35 8.01 16.00
        36 186.03  90.35 9.71 17.50 ];

elseif Bead==5 % Bead 5
  Xpost=[24 176.04 146.07 3.78 11.50
         27 176.04 146.07 5.07 13.00
```

```
         30 176.04 146.07 6.60 14.50
         33 176.04 146.07 8.10 16.00
         36 176.04 146.07 9.81 17.50 ];

elseif Bead==6 % Bead 6 INSIDE CELL.
  Xpost=[21 94.20 21.45 3.25 10.00
         24 94.20 21.45   4.59 11.50
         27 94.20 21.45 5.80 13.00
         30 94.20 21.45 7.42 14.50
         33 94.20 21.45 8.86 16.00
         36 94.20 21.45 9.87 17.50 ];
end

if Bead<10 % Add bead number
    Xpost=[Bead*ones(size(Xpost,1),1) Xpost];
end

end % Set.Cell

end
```

*Published with MATLAB® R2020b*

## 7.4 *GetAllBeads Function*

```
function [Xpre,Xpost]=GetAllBeads(Set)
% create a plot where we make a profile with the values of the first
% layer of each bead and we can represent the height as a function of
% each bead.
if Set.Cell==1
%    Bead x        y     D     Zm
Xpre=[
      1 12.03 137.45 3.76 7.00
      2 18.84 5.11 3.14 7.00
      3 150.05 118.15 3.87 10.00
      4 145.73 137.56 3.65 10.00
      5 177.63 134.16 3.28 10.00
      6 99.09 74.46 3.72  17.50 ];

Xpost=[
      1 12.03 137.45 3.01 1.00
      2 18.84 5.11 3.45 1.00
      3 150.05 118.15 3.39 4.00
      4 145.73 137.56 3.38 4.00
      5 177.63 134.16 2.84 4.00
      6 99.09 74.46 1.69 8.50];

elseif Set.Cell==2
   % Bead x        y     D     Zm
Xpre=[
      1 12.26 5.67 3.13 5.50
      2 19.07 5.22 3.22 5.50
      3 89.89 101.36 3.65 7.00
      4 124.74 71.85 3.39 10.00
      5 204.64 113.84 2.65 8.50
      6 118.83 133.82 2.25 7.00 ];
Xpost=[
      1 12.26 5.67 2.70 5.50
      2 19.07 5.22 2.78 5.50
      3 89.89 101.36 3.35 7.00
      4 124.74 71.85 3.61 8.50
      5 204.64 113.84 2.02 8.50
      6 118.83 133.82 2.60 11.50];

elseif Set.Cell==3
   % Bead x        y     D     Zm
Xpre=[
      1 128.71 22.47 3.29 11.50
      2 123.49 137.79 3.92 11.50
      3 154.93 145.96 3.32 11.50
      4 181.49 149.59 2.98    11.50
      5 71.50   78.31 3.02    11.50 ];
Xpost=[
      1 128.71 22.47 2.91 11.50
      2 123.49 137.79 3.36 11.50
      3 154.93 145.96 2.76 11.50
      4 181.49 149.59 3.91 13.00
```

1

```
      5 71.50   78.31 2.95 16.00];

elseif Set.Cell==4
    % Bead x        y     D     Zm
Xpre=[
      1 41.20 115.09 4.36 8.50
      2 94.20 21.45 2.78 8.50
      3 129.73 5.90 3.46 10.00
      4 186.03  90.35 2.63 10.00
      5 176.04 146.07 2.70    10.00
      6 94.20 21.45 3.03    8.50];

Xpost=[1 41.20 115.09 4.13 8.50
       2 94.20 21.45 3.65 10.00
       3 129.73 5.90 3.29 10.00
       4 186.03  90.35 2.45 10.00
       5 176.04 146.07 3.78 11.50
       6 94.20 21.45 3.25 10.00];
end
end
```

*Published with MATLAB® R2020b*

# 7.5 *GellCellCoord Function*

```
% Get Cell coordinates
function [XCellPre,XCellPost]=GetCellCoord(Set)
if Set.Cell==1
    XCellPre =
 [100.221,103.417,105.385,106.898,108.790,109.849,109.546,
        108.941,107.806,105.914,103.266,100.315,98.196,96.153,94.640,

 93.732,92.673,92.446,92.446,92.975,94.110,96.229,98.196,100.221;

 69.349,69.746,70.578,72.016,74.210,77.464,80.717,83.290,84.879,

 85.787,86.619,86.695,86.544,85.787,84.652,83.366,81.398,79.280,
        77.085,75.421,72.772,71.183,70.351,69.349]';
    XCellPost
 =[98.745,97.780,96.191,95.283,94.148,93.467,93.467,94.375,
        95.056,96.986,99.256,102.661,105.612,107.314,108.222,108.563,
        107.995,105.839,103.796,101.866,100.277,98.745;
        88.644,88.360,86.771,84.728,82.231,77.918,76.102,74.740,
        72.356,71.675,71.335,71.902,74.059,77.237,80.301,83.593,
        85.636,87.225,88.019,88.473,88.814,88.644]';


elseif Set.Cell==2

   XCellPre =[113.273,115.827,118.551,120.934,122.977,124.112,125.247,
       126.155,127.404,127.858,128.085,128.085,125.701,124.566,121.2,

 120.594,117.983,114.692,111.400,108.563,106.974,104.931,103.909,

 102.774,102.888,103.796,105.725,105.612,105.271,103.455,105.498,
       108.109,110.038,113.273;

 125.304,125.815,126.609,126.950,128.198,130.468,132.965,135.235,

 137.732,140.797,143.748,147.834,151.239,153.509,156.687,160.205,

 160.205,157.141,155.325,152.828,150.217,147.493,144.202,141.591,

 139.435,137.732,136.257,133.873,132.171,128.766,126.950,126.042,
        125.928,125.304]';
    XCellPost
 =[118.948,121.842,124.037,125.399,126.609,127.744,128.274,

 128.879,128.879,128.804,128.804,128.274,127.442,126.836,126.155,

 125.096,124.566,124.415,123.961,123.280,122.750,121.540,120.480,

 120.026,119.118,118.059,117.075,116.092,115.108,114.049,113.216,

 112.61,111.400,110.644,109.660,108.676,107.995,106.709,105.574,

 104.893,103.607,102.169,100.580,101.110,103.001,103.682,104.439,
```

1

```
105.650,106.860,108.903,110.795,113.141,115.486,117.908,118.948;

129.276,129.749,130.733,132.171,133.457,135.576,137.619,139.056,

140.721,142.461,143.823,145.261,146.926,147.985,148.817,148.817,

149.952,150.785,151.617,152.525,153.509,154.114,154.492,154.795,

155.325,155.249,154.719,154.417,154.038,153.357,152.676,152.298,

151.844,151.844,151.617,150.785,149.877,148.439,147.153,145.942,

144.580,144.202,142.991,141.553,140.267,137.921,136.559,134.592,
       133.684,132.549,131.641,131.338,131.036,129.901,129.276]';


elseif Set.Cell==3

    XCellPre =
[70.143,68.270,66.606,64.487,62.066,59.342,57.374,55.861,

53.591,52.229,49.505,46.781,45.722,46.024,44.814,45.873,47.992,

48.748,49.354,53.742,56.466,61.006,63.882,65.092,66.908,68.422,

69.935,75.686,78.561,80.831,82.042,83.858,84.917,86.128,85.522,
       84.312,82.647,80.226,77.804,75.080,72.810,70.143;

89.665,91.576,93.694,93.694,93.392,93.392,93.694,91.727,89.911,

88.398,86.128,84.160,80.982,79.015,75.686,74.021,70.994,69.481,

67.665,65.092,61.612,60.855,57.980,55.861,54.650,51.775,50.413,

54.499,56.920,59.493,62.368,66.454,69.784,73.718,79.318,81.890,
       84.009,86.128,87.792,88.549,89.457,89.665]';
    XCellPost =
[55.956,58.623,60.893,63.617,66.341,69.405,72.470,74.740,

77.691,79.961,82.231,83.593,83.366,83.139,82.912,82.458,81.096,

79.620,78.031,75.875,72.470,69.746,66.795,63.844,61.006,57.374,

53.288,49.656,45.570,44.435,43.527,43.300,42.846,44.322,45.911,
       48.862,51.699,55.956;

53.913,53.288,52.494,51.359,51.359,50.791,51.132,51.132,51.018,

52.153,54.877,58.396,61.347,63.617,65.660,68.724,70.767,73.491,
       75.648,77.464,78.826,81.096,83.366,84.614,84.841,84.955,83.933

,83.252,83.252,79.734,76.669,72.016,68.838,64.525,62.709,59.077,
       55.558,53.913]';
```

2

```
elseif Set.Cell==4

    XCellPre = [94.432,98.007,101.072,103.682,106.293,106.747,106.860,
        105.952,103.796,102.093,100.845,99.142,96.872,94.262,90.970,

 88.360,86.884,84.728,81.663,80.642,80.301,80.869,82.231,83.139,
        85.295,87.565,91.084,94.432;

 91.141,91.538,93.581,95.283,98.915,103.115,106.406,109.130,111.287,

 113.216,114.238,114.919,115.259,115.827,115.827,114.578,113.443,

 111.741,109.130,106.860,103.115,99.142,96.986,95.851,92.900,91.992,
        90.630,91.141]';
    XCellPost =
 [93.410,97.667,102.661,106.974,106.747,104.250,101.753,

 99.256,95.624,90.857,86.090,83.366,80.869,79.507,79.507,80.188,
        81.550,84.728,89.949,93.410;
        89.779,91.084,94.262,99.029,105.158,109.471,112.876,114.919,

 115.827,116.054,114.238,111.287,108.563,104.704,101.072,96.986,
        94.489,91.992,89.949,89.779]';
end

end
```

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