Actin Gels dynamics

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CHAPTER

ONE

BACKGROUND

Todo

- Even in mitosis for big cell, actin is needed to assemble chromosoms [Lenart, Bacher, Daigle, et al. 2005]
- Rapid change in actin structure [Vasilev, Chun, Gragnaniello, et al. 2012], timing is also important (exposition to inomycine disrupt cortex functionality)
- F-actin network cabable of supporting mechanical load [Feric, Brangwynne, 2013]
- Presence of f-Actin meshwork meshsize ~0.5µm [Feric, Brangwynne, 2013]
- This actin network can wistant repetitive compression [Feric, Brangwynne, 2013]
- F actin network might be linked to the lamin (a kind of IF) cortex around the nucleus [Feric, Brangwynne, 2013]
- Such a network woudl only need to sutain a pressure on the order od 0.01 PA [Feric, Brangwynne, 2013], and is essential to fight agains gravity

1.1 Introduction

Cells are the basic component of living organism, understanding their individual behavior and the way they function is a key step into understanding how they interact with their environment and other organism. One of the key component to most of organism is Actin, a protein which is highly conserved across the species and play a important role in cell mechanics, from cell migration to cell differentiation and division. It plays also a non negligible in most mechanical properties of the cell and how it interacts with its environment. In particular actin is the main component of the actin cortex : the part of the cell cytoskeleton below the plasma membrane mostly responsible for cell mechanical properties. The properties of this actin cortex is drive by the mechanics of the properties of its main component : a dynamic actin network. Understanding this actin network is hence a key piece to learn how the actin cortex behave.

The properties of an actin network highly depend on it's structure. The structure itself depends on many parameters that influence how the network is formed. Network structure and formation can be not only influence by its physical and chemical environments but also by the variation of this parameters with time and space.

Cells are complex systems that adapt their shape, mechanical properties and biochemical conditions permanently. The spacial repartition of theses properties is also variable as the cell regulate the concentration of proteins all across its body. To well study the effect of each components independently, it is crucial to study actin network in a controlled environment.

Biomimetic systems allow to respond to most of these concern, they provide a well controlled environment where biochemical condition can be well controlled both in space and time. Theses systems keep their biological relevance, as they can mimic *in vivo* phenomenon. Biomimetic systems are also well adapted to the tools available and the approach from a physics point of view. The optical trap will allow us to study local mechanical properties of actin network with a high time resolution which could allow to get insight into the variation of theses properties as a function of time.

During my PhD, my work has mainly be to study the mechanics of branched actin network polymerizing on optically trapped polystyrene beads. Such network was studied before [Kawska, Carvalho, Manzi, et al. 2012] but have suspected to be highly inhomogeneous, the use of optical trap allowed to probe mechanics of part of the network unaccessible before.

- time resolution
- network dynamics
- Move to liposome,
- study in OOCyte :
- Basically from purely biiomimetic to real cells

1.2 Living Cells

Todo

Todo

We can see in plants that actin, also known as microfilament [Iwabuchi, Takagi, 2010] is used to move nucleus away from

Cells are the smallest living component which are present from unicellular plants to multicellular animals. Thus, cells should cover a huge range of behavior going from extremely specific on multicellular organism, to all the function that are needed to survive and reproduce for bacterial colony. Multicellular organism will grow specialized cell from neurone to osteoblast going through germinal or muscle cells. In the other hand, unicellular organisms are made of cell that are responsible for all the function of the organism, from motility to reproduction, passing through absorption of nutriment to replication of the cell.

Cell are hence able to adapt to their environment as a function of time, and also have function and behavior that depends on time, and a small change of timing and/or biochemical conditions can highly injure the development of an organism [Lenart, Bacher, Daigle, et al. 2005], it has also been observe that the mechanical properties of substrate can govern the differentiation of cell [Engler, Sen, Sweeney, Discher, 2006] .

Nonetheless, even with all theses different behavior and phenotype, the cells all have a common structure. They are constituted by a membrane which is responsible form separating the cytoplasm from the outside

of the cell. The cytoplasm contains organelles, genetic material, and number of proteins that the cell use to accomplish its functions. Cells are of course not completely isolated, and have numbers of mechanism to exchange and communicate with the outside. Communication with the outside are either chemical or mechanics. To sens their mechanical environment, cell use adhesion complexes to attach to the medium, and integrins as trans-membrane protein will transfer the force to the cell cytoskeleton situated inside the cell.

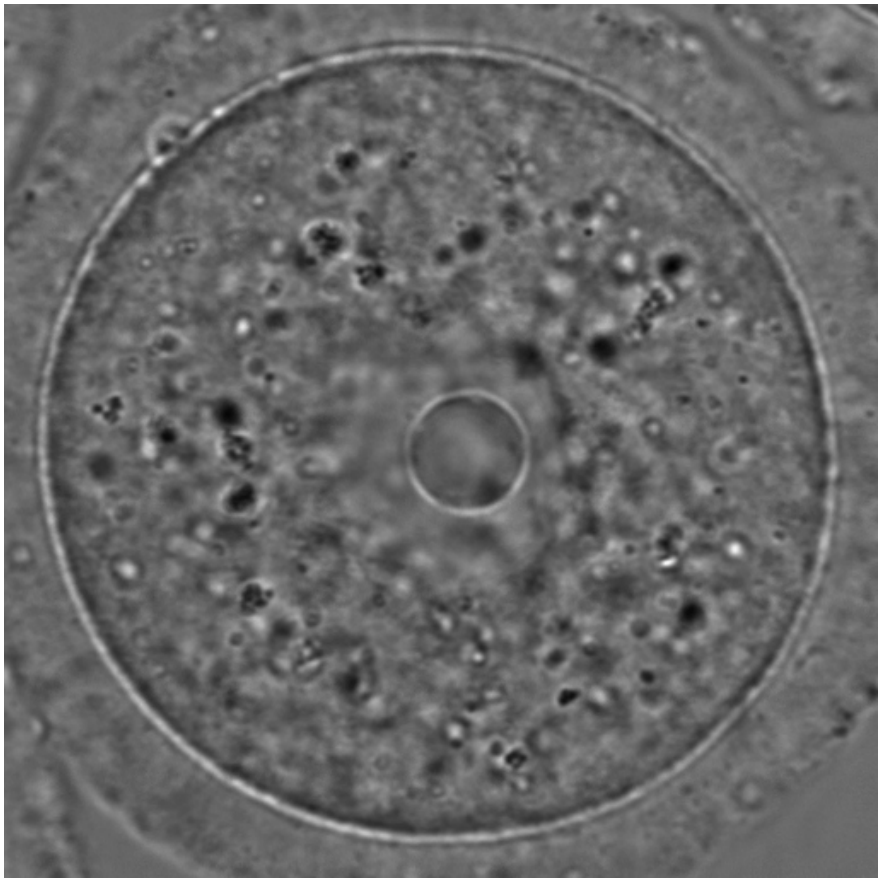

Figure 1.1: Bright field image of a mouse oocyte before meiosis. Cell diameter is of 80 µm. The nucleus can be clearly seen at the center of the cell. Image Credit to Maria Almonacid from Collège de France.

This structure, which is situated just below the cell membrane, is named the actin cortex,

1.2.1 The Cell Cytoskeleton

The cytoskeleton, literally skeleton of the cell, is the structure which give it shape to a cell. As for other multicellular animals that possesses skeleton, its shape is often a hint on how a organism move. As feet, fins and wigs are characteristics that will tell you whether a animal does more preferably prefer land, see or air, the cytoskeleton is will tell you many things a bout a cell.

In the other hand, unlike (exo)-Skeleton of animals which is ridged and unchanging, the cytoskeleton of cell is a highly dynamic structure that keep remodeling itself on a short time scale compared to the speed at which a cell move. That's through this dynamics that the cytoskeleton can achieve its functions. As mammals

skeletons are necessary to transmit force from one part of the body to another, the cell cytoskeleton is responsible to not only transmit the force the cell is exerting, but also to generate theses force. Thats through its cytoskeleton that a cell can be connected to its environment, both mechanically and biochemically.

Todo

trouver des ref pour ci dessous

The cytoskeleton is mainly composed of three type of filaments. The microtubules, intermediate filament and actin filament, also known as microfilament.

Microtubules

Microtubules are the wider with a diameter of 20nm and [un article où on voit le diameter] the stiffer of the three kinds of filament with a persistence length in the order of millimeter, which is much longer than the size of the usual cell. Microtubules are extensively studied [cite some reviews ...]. Microtubules form polar (oriented) filament that can be walked on by molecular motors that can be decomposed in two families – kinesins and dyneins – depending on the end toward which the motor preferably walk. Microtubules are mostly known for their action during the cells mitosis where they will form majority of the mitotic spindle that drive the segregation of the chromosomes in two groups, each group ending in one of the daughter cells.

We will not be interested directly into the effect and behavior of microtubules in this manuscript.

Intermediate filaments

Intermediate filaments are of medium diameter in the order of around 10nm, in between actin and microtubules filament, hence their name. Unlike microtubules and actin filament, intermediate filaments are composed by several sub-families of proteins and are non-polar.

Actin

Actin, is the third component of the cytoskeleton, the one we will focus most of our effort. Actin can forms actin filament, the thinest of the three kind that form the cytoskeleton. Actin is produced in the cell as a globular protein of ~40 kDa that once associated with ATP or ADP polymerise into helicoidal filament with a diameter between 7 and 9nm. The formed actin filament are polar, which both extremity respectively called the plus (+) or barbed end, and the minus (-) or pointed end. The polarity of the actin filament is of importance as this give rise to a proved direction for most processes that can happen on the filament.

The actin protein is highly conserved across species, and is know to directly interact with hundreds of proteins [dRemedios, Chhabra, Kekic, et al. 2003]. As hint before it can in particular bind to ATP, that can hydrolyse into,

Dynamic of actin polymerisation

The assembly mechanism that allow to go from singles monomers of actin (also refer to as G-actin in solution) to actin filament (also refer as F-actin) need to be well understood to explain the different structure

of network actin filament can give once put in presence of other proteins.

The polymerisation of ATP/ADP actin monomer to form an actin filament need to go through the step of forming a actin proto-filament which is constituted of at least 3 actin monomers. This will most of the time be the kinetically limiting step. Once proto-filaments are present in solution, single monomers can be freely added or removed on both end of the filament.

We now need to distinguish between the dynamic of adding or removing on both ends of the filament. Indeed it has been show that the association and dissociation rate are differing between the pointed (-) and barbed (+) end. More particularly, the association rate at the barbed rate is higher that on the pointed end, and same goes for the dissociation rate which has a bigger constant on the minus end of actin filament. This lead to in imbalance of actin (de)-polymerisation on both ends, which leads to actin filament preferably growing on the barbed end and preferably shrinking from the pointed end.

The equations that drive the polymerisation can thus be written as follow

$$\frac{dC_b}{dt} = k_{+,b}.[monomers] - k_{-,b}$$
$$\frac{dC_p}{dt} = k_{+,p}.[monomers] - k_{-,p}$$

Where *b* and *p* designate respectively the barbed and pointed end, and k_+ and k_- are the polymerisation and depolymerisation rate. The concentration in barbed and pointed end denoted by $C\_$. By assuming that the number of pointed end is equal to the number of barbed end, one can derive the steady state which give rise to the critical monomer concentration below which a actin filament cannot grow: $[monomers]_c$.

The rate constant of elongation of actin have been determined to also depend of whether the monomer was bound to ADP or ATP [Pollard, 1986]. We should now consider the fact that ATP-bound actin will hydrolyse to ADP-Pi then release the inorganic phosphate, and thus with a rate that also depend on whether the monomer is part of a filament or in solution.

It should be noted that the in stationary state the length of each actin filaments statistically constant because the speed of polymerisation on the barbed end is compensated by the depolymerisation on the pointed end. The filament is hence in a threadmilling state. If we follow a single actin monomer bound to an ATP molecule, it will be incorporated at the + end of the filament and progressively move toward the minus end, eventually hydrolysing it's ATP into ADP before detecting from the filament on the pointed end.

Todo

- cf fletcher 2010 review [Fletcher, Mullins, 2010] the cytoskeleton as 3 main functions :
  - organize cell in space
  - connect cell to external environment (biochemical and mechanical)
  - generate and coordinate force to allow cell to change shape.
  - some things on temporal and spacial effect of structures like "bud scar"
  - schema of branched Arp2/3 actin factor
- Loading history determines the velocity of actin-network growth [Parekh, Chaudhuri, Theriot, Fletcher, 2005] hence network can record history, single filament cannot.

Proteins influencing actin polymerisation

Despite the already complex process that is actin polymerisation and the numbers of parameter that we have already introduce, the formation of an actin network is a even more complex process that involve many other components. Especially, actin monomers and filament can interact with a high number of proteins that will effect previously established dynamics. We will present some categories of such preoteins.

Polymerase family The polymerase family as their name indicate will directly have effect on the polymerisation of actin. In the right condition, polymerase will increase the k_+ at one end of the actin filament for the same concentration of actin monomers. This can lead to an average longer filament length.

Formins Are one of those polymerase proteins that will increase the polymerisation rate at the barbed end. It has the particularity of being processive, meeting that it will stay bound to the barbed and while catalysing the addition on new monomers.

Crosslinkers

Stabilising proteins

Capping Protein

Todo

- more than 150 protein have been found to bind with actin.
- Wave complex,
  - Wasp, N-Wasp (need to cite *Machesky1999*)
- Not composed only by actin

  Should cite *Pollard2003*
  - Some network need actin, some other do not. (Fletcher review 2010)
  - NPF
  - Polymerase, (depolymerase severing),
  - crosslinker,
    - // like fascine
      - rotate like alpha-acitinnin
      - effect of cross linking distance [Morse20..]
  - stabilizing
  - Moleular motors.
  - interphase, cellule prepare for division
  - Mitosis : “DNA Segregating”

  - need to describe actin,
    * depending on the length scale semi-flexible polymers.
  - polymerisation barbed end pointed end, (directed)
    * form microfilement
  - cytoskeleton is dynamic
  - formed under the plasme membrane
  - ratchet nechanisme
  - use of Arp2/3 to branch
  - capping, protein, formin (OOcyte)
  - myosin, run on actin to barbed end/ processive/not processive.
    * stress fibres
  - troppomyosine

All the living kingdom is characterised by the fact that organism can reproduce,

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And

1.2.2 Cell Organelle

- Mitoncondria, ER (made to produce proteins), also serve in lacust
- nucleus en eucariotes cells, contains the chromosomes.
- Nucleus get moved by actin filement to the periclinal/anticlinal wall,
- centromere centriole,
- Organelles are supported by

1.3 The Role Of Actin Cytoskeletton

1.3.1 Cell Motility

1.3.2 The actin cortex

1.3.3 Organelle Positioning (actin cloud)

1.3.4 Nuclear positionning during miosis

1.4 In vitro reconstituted actin networks

1.5 Actin networks as viscoelastic material

1.6 Active and Passive microrheologie

1.7 Optical tweezer

1.8 Membrane Physics

1.9 Myosis (to move away)

Todo

- Asymetric division of oocyte,
- from diploid, to haploid, - spindle usually in mmithosis pulled by microtubule

CHAPTER

TWO

MATERIALS AND METHODS

2.1 Actin

2.2 Profiline

2.3 Arp2/3

[Goley, Welch, 2006]

2.4 Bead Motility

2.5 3D fitting

2.6 ?? ?? ??

CHAPTER

THREE

RESULTS

3.1 Actin Cloud

3.2 Doublets

3.3 Oocytes

CHAPTER

FOUR

DISCUSSION

CHAPTER

FIVE

OPTICAL TRAP SETUP

5.1 Preface

In order to manipulate the polystyrene beads that are used in the different experiments, I worked on a already build setup with time shared optical trap

To investigate the effect of different actin network on mechanics of cell behavior, Sykes team of curie institute is specialized in using biomimetics sytems. In particular, polystyrene actin bead covered with nucleator of actin polymerisation have been developped as a biomimetic system of listeria monocytogen. It is such a system that I have studied.

By growing actin network in controlled condition I was able to reproductibly determine mechanical properties.

5.2 Choice of experimental tools

The choice of experimental tools, and experimental conditions is important to determine in the range of properties and parameters you can access to. Previous and ongoing studies at the time were focused on the properties of dense gel in comets tails, as well as the one around the polystyrene beads, as well as the one on the surface before symetry breaking.

CHAPTER

SIX

LIPOSOME DOUBLETS

In this chapter we study the comportment of what we will refer as “Doublets”; an biomimetic system that allow us to study quantitatively the tension on a membrane covered with a actin shell mimicking the cells actin cortex. Even if such a system has already been studied, we believe that the new technique we develop can allow the non-invasive measure of variation of tension on liposome.

Starting with a liposome solution containing biotinilated actin filament and streptavidine doublet will naturally occurs. Liposomes will either adhere before or after being covered with actin filament. In the rest of this chapter we are interested in studying only the case where both liposome adhere before being in coverd with actin. To increase the ratio of both kinds of doublet, the solution of liposome is gently centrifuged before adding the F-actin into the mix. As experiments were done using fluorescently labeled actin, the discrimination between the two kinds of doublets was easily done looking at the fluorescent intensity on the interface between the two doublets. Only doublet where no signal on the interface was visible were analysed.

6.1 Relating Doublets to Living cell

Determining contact angle in cell is the often use methods to determine effective tension of membrane linked to cythoskeleton. :cite:Leon Maitre:

6.2 Doublets geometric parameter

Using biomimetic system allow to more control over a system and decrease the number of parameter one have to study to better understand there influence. Unlike cell that are active and constantly shape changing, liposome have a shape that follow a smaller number of physical law. This translate in the right condition to liposome taking a spherical form. Knowing this information we derived a methods to determine the contact angle between liposome in a less experimentor biased way.

In this section we will show that the geometrical parameters of a doublet can be modeled by the combinaison of two intersecting sphere, simulate the fluorescent image that such a doublet would generate and show that we can optimise the parameters of the model to reflect the exerimental data thus determining the actual geometrical parameter of the doublets in an experimentor independant mater by also automatising the system.

In the following par we will restrain ourselves to example in a two dimensional space for easier visualisation and work in pixel for convenience

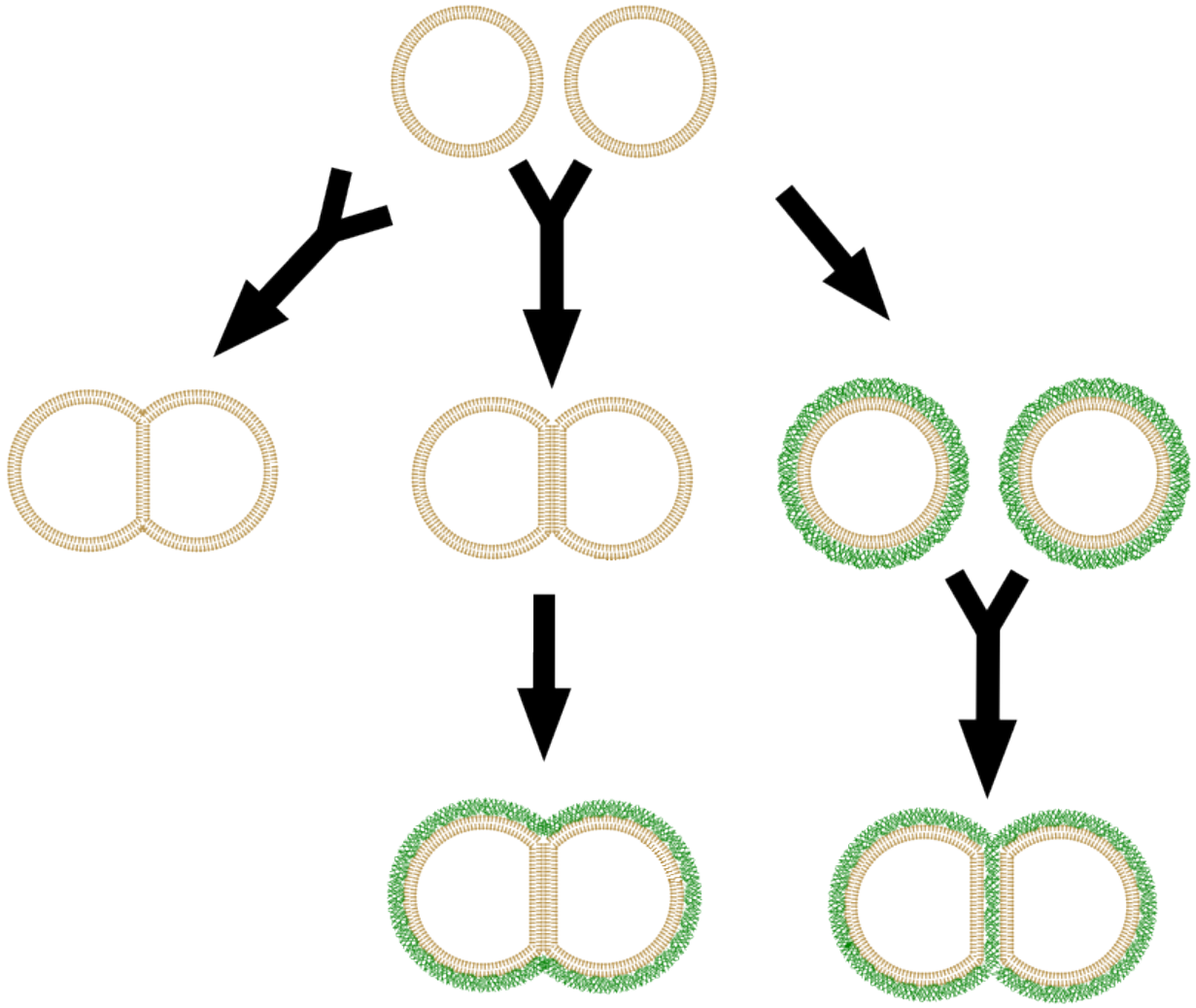


Figure 6.1: Single liposome can formxe a doublets with a single double double layer separating them, adhere with a double bi-layer, or cover with atin. Bare doublets can then get covered with actin and actin covered liposome can adhere together. The two kinds of doublets can be differentiated by using fluorescently labeled acctin.

6.2.1 Finding a single liposome

Experimentally liposomes are observed using fluorescently labeled component, in particular we used a GFP labeled actin and streptavidine that will be imaged using a inverted microscope. In the observation plane, the liposome formed using fluorescently labeled streptavine will form a bright ringi of given thickness. When imaging the actin shell, assuming the actinshell is of homogeneous thickness around the liposome will also manifest as a fluorecent ring.

In the case where the membrane is marked, the radius of liposome will be the median radius of the ring.

In the case of actin shell, when the thickness of the actin shell is bigger compared the resolution limit of our method, then the liposome radius should be taken as the inner raius of the ring

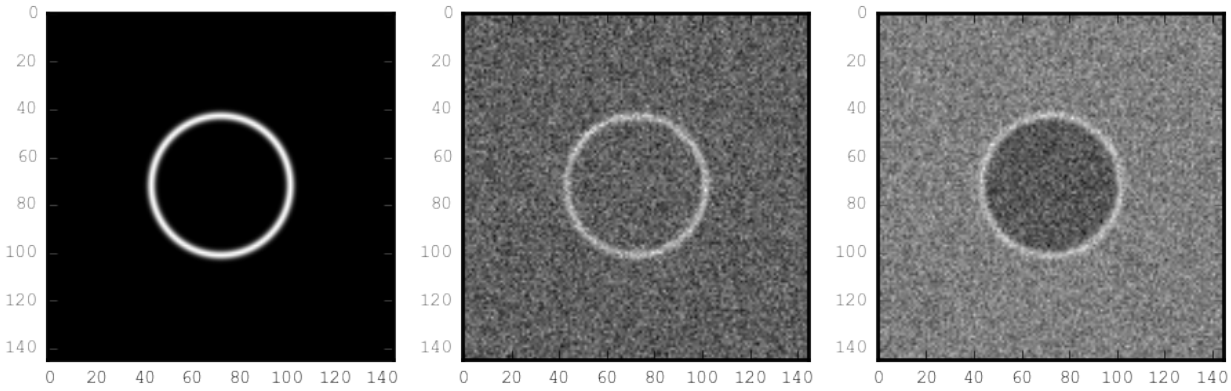
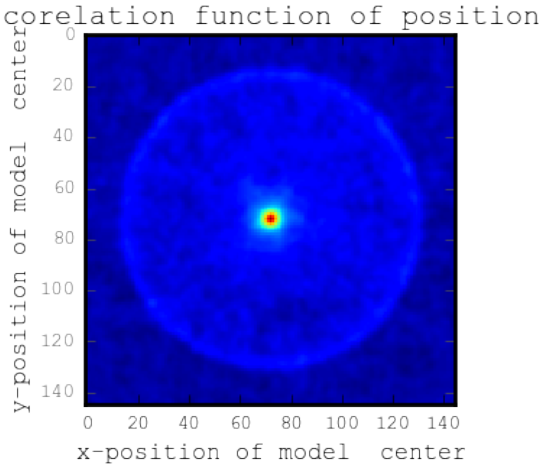


Figure 6.2: Left : A simulation of liposome fluorescent of an uniform shell or membrane. Middle: Same Image Adding gaussian noise to simulate a plane from a confocal Z-stack. Right: Fluorescently labelled Liposome in fluorescent External Buffer and non fluorescent medium.

- *search*

Note: You can Download the latest pdf version of this document.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y-position of model center
x-position of model center
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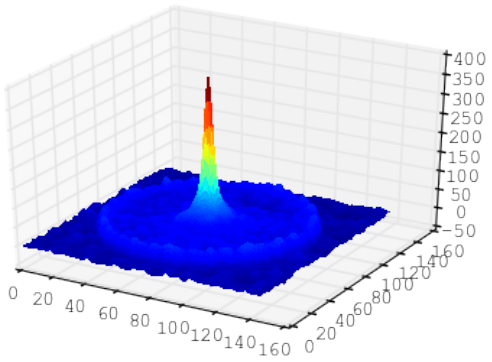
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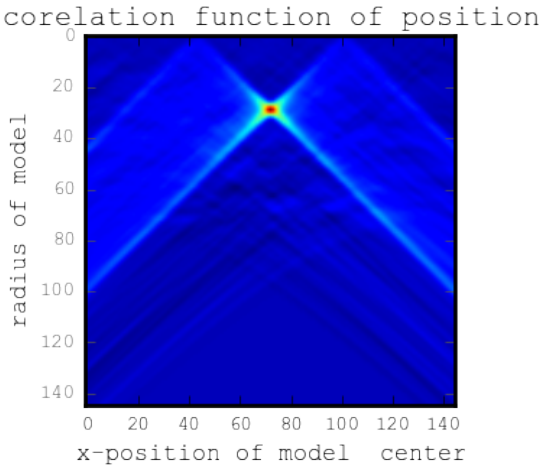
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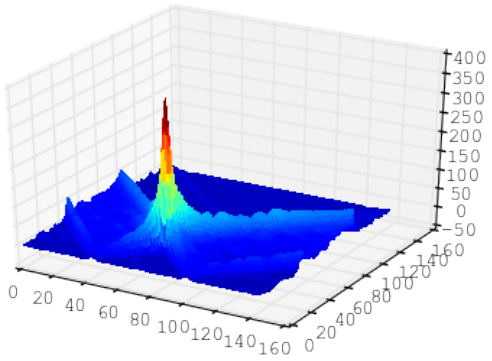
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CHAPTER

SEVEN

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