

# Observe Cell Morphology and Culture Cell

BY YUEJIAN MO(11510511), WENHAO ZHANG

Cell Biology Experiment Class I

October 8, 2018

## 1 Introduction

Cell is basic unit of living thing, study of cell is a import subjective. But it is difficult to do cell experiment *in vivo*, we need to culture cell *in vovi* and do the experiment. First, cell are essiential to isolated from origism body. Nowsday, here are many difference cell lines we can use in laborator. Such as **C**hinese **h**amster **o**vary(CHO) cells, tobacco **by**ellow **2** (BY2) cells, **h**uman **e**mbryonic **k**idney (HEK) cells, **H**enrietta **L**acks (HeLa) cells and etc. Cell lines share some simiar morphology, but some of mophology are enough difference for us to differ it, which can statify diverse experiment requirment. Cell in culture can be divided into three basic categories based on their shape and appearance, including fibroblastic(or fibroblast-like) cells, epithelial-like cells, lymphoblast-like cells.

To study the begin the study of cell morphology, we should be familly with two technology, phase-contrast microscope and cell counting. Phase-contrast miscroscope provides higher contrast image of transparent speicmen than brigh field microscope. Most important priciple is that it converts light phase contrast when light out from specimen to the intensity, which differ the backgroud and specimen efficiently. We will use an invert phase contrast microscopy to observe mophology of 3 type of cell lines. It also helps us to determine the healthy condition of cells.

Counting cells with a hemocytometer is an easy, fast, and effcient way to determine the density of a cell culture. Using hemocytometer is an application of **random sampling**. Here are 3 type of and total 5 counting chambers in hemocytometer. The ceteral lattice chamber is used to count small cells, whilemean the 4 corner lattice with 16 medium lattice are used to count larger cells. After injecting cell suspension(should be diluted to 25-250cells/1mm<sup>2</sup> square) and counting cell inside one type of chamber, we can find out the original cell concentration based on the relationshiop of volume. We will use hemocytometer to count the cell density and viability of CHO-K1 cell.

To begin a long or continue cell experiment, we need to culture cell. **Cell culture** refers to the transfer of cells from an animal or plant and their subsequent growth to a favorable artificial environment. Cells some directly from tissue are **primary culture**. After the first subculture, the primary culture becomes known as **cell line or subclone**. Cell culture need suitable environment. First, strict aseptic techniques are used to avoid contamination and potential loss of valuable cell lines. Culture conditions vary widely consists of a suitable vessel, medium that supplies essential nutrients (animo acids, acarbohydrates, vitamins, minerals), growth factors, hormones, and gases(O<sub>2</sub>, CO<sub>2</sub>), and regulates the physicochemical environment(pH, osmotic pressure, temperatures). We will learn to maintain and subculture monolayer CHO-K1 cell.

## 2 Materials & Methods

### 2.1 Observe morphology

Under Olympus inverted phase contrast microscope, we observed **CHO-K1 cell**, **NIK/3T3 cell**, **U-937 cell** in 60mm tissue culture dish with different objective. Different objective paired with correct condenser annulus.

### 2.2 Determine CHO-K1 cell density and viability

Firstly, we removed the cell culture medium from a 60mm tissue culture dish(CHO-K1 cell) with Pasteur pipette. Then we added 2ml Ca<sup>2+</sup> and Mg<sup>2+</sup> free PBS(Phosphate Buffered Saline: dissolve NaCl 8g, KCl 0.2g, Na<sub>2</sub>HPO<sub>4</sub> 1.44g and KH<sub>2</sub>PO<sub>4</sub> 0.24g completely in 800ml ddH<sub>2</sub>O, adjust pH to 7.4 by HCl, add ddH<sub>2</sub>O to 1L, sterilized by autoclave at 121°C for 20min, pre-warmed to 37°C) to wash cells by gently swirling PBS over the layer of adherent cells. PBS were removed and 1ml trypsin-EDTA(0.25% trypsin and 0.2% EDTA in PBS, balanced to RT) were added. Cells were digest at room tempure at about 2min until 80% cell turn round. We added 2ml fresh Ham's F-12K supplied with 10% FBS(fetal bovine serum,pre-warmed to 37°C), and mix gently and thoroughly to avoid making bubbles. Almost compelely dissociated cells on dish were rinsed and transferred to a new 15ml centrifuge tube. Cell suspension were centrifuged(Eppendf R450) for 3min at 800rpm, and removed medium over cell pellet. 1ml PBS were added to resuspend.

Finally, 10 $\mu$ l PBS/cell suspension and 10 $\mu$ l 0.4% trypan blue(0.4% trypan blue in PBS) to a new 1.5ml centrifuge tube. 10 $\mu$ l mixture was added to hemocytometer and counted the cells. When cells were touching a line, we only counted the cells that touch the top and left lines of each square. We counted blue cells as dead cells.

The cell density is calculated as:

$$\text{Cell Density} = \frac{\text{cells counted in 4 large lattice}}{4} \times 10^4 \times 2$$

The Cell viability is calculated as

$$\text{Viability} = \frac{\text{number of liveing cells}}{\text{total number of cells}} \times 100\%$$

### 2.3 Subculture cell

Main procedure are same to above. But we didn't add trypan blue and used automatic cell counter. According the cell density( $1.5 \times 10^6$  cell/ml), 400 $\mu$ l cell suspension and 3.6ml F-12K were added to new cell culture tissue dish. Finally, subculture cell were incubate at 37°C, 5%CO<sub>2</sub> for 20 hr in incubater().

## 2.4 Cell freeze and recover

Main procedure are same to 2.2. We continued in the first time centrifugation. After discarded medium, we added 1ml cryoprotective medium(90%(v/v)FBS and 10%(v/v) DMSO, stored in 4°C) to resuspend. 1ml mixture were transfer to 1.8 ml cryovial. We labeled and put the vial in rate cryo-freezer(Thermofisher) and put in -80°C freezer overnight. Incubate overnight, then vials were transferred to liquid nitrogen storage freezer.

# 3 Results

## 3.1 3 type of Cell morphology

NIH/3T3 cells are fibroblast-like cell with bipolar, elongated shape, which attach to substrate(**Figure 1**). CHO-K1 cells are epithelial-like cell with more regular polygonal shape, which also attach to substrate(**Figure 2**). U-197 cells are lymphoblast-like cell with spherical shape, which grown in suspension(**Figure 3**).

## 3.2 CHO-K1 cell density and viability

The number of cells/ml in original culture is  $\frac{442}{4} \times 10^4 \times 2 = 2.24 \times 10^6$ . And the viability is  $\frac{210}{442} \times 100\% = 47.5\%$

## 3.3 Subculture CHO-K1 cell

No pollution, CHO-K1 cells grown in suitable and uniform density(**Figure 4**). Most of cells attached to substrate with polygonal edge. Some of cells were round and suspended, which were almost dead(**Figure 5**).

## 3.4 Freeze and Recover CHO-K1 cell

1.8ml CHO-K1 cell with cryoprotective medium were store in liquid nitrogen storage freezer at 2018-09-30.

# 4 Discussion

*How the cell are able to attach substrate on dish?* When cells grow in vivo, they interact and attach to neighbouring cells through specialised molecules of cell surface, whose process is called cell adhesion[1]. When researchers first time cultured cell in glass or plastic surface, they found that cell were poor to attach surface to grow up. Based on the research of cell adhesion, to enhance the adhesion between cell and dish, surface of dish are covered of some materials. A variety of biological materials are coated on dish or flask surface, including extracellular matrix, attachment and adhesion proteins, such as collagen, laminin and fibronectin, and mucopolysaccharides, such as heparin sulfate, hyaluronidate and chondroitin sulfate, both individually and as mixtures[2]. Some special materials can be coated on surface from the different requirements in research activities.

*Why CHO-K1 cell grow monolayer in our experiment?*

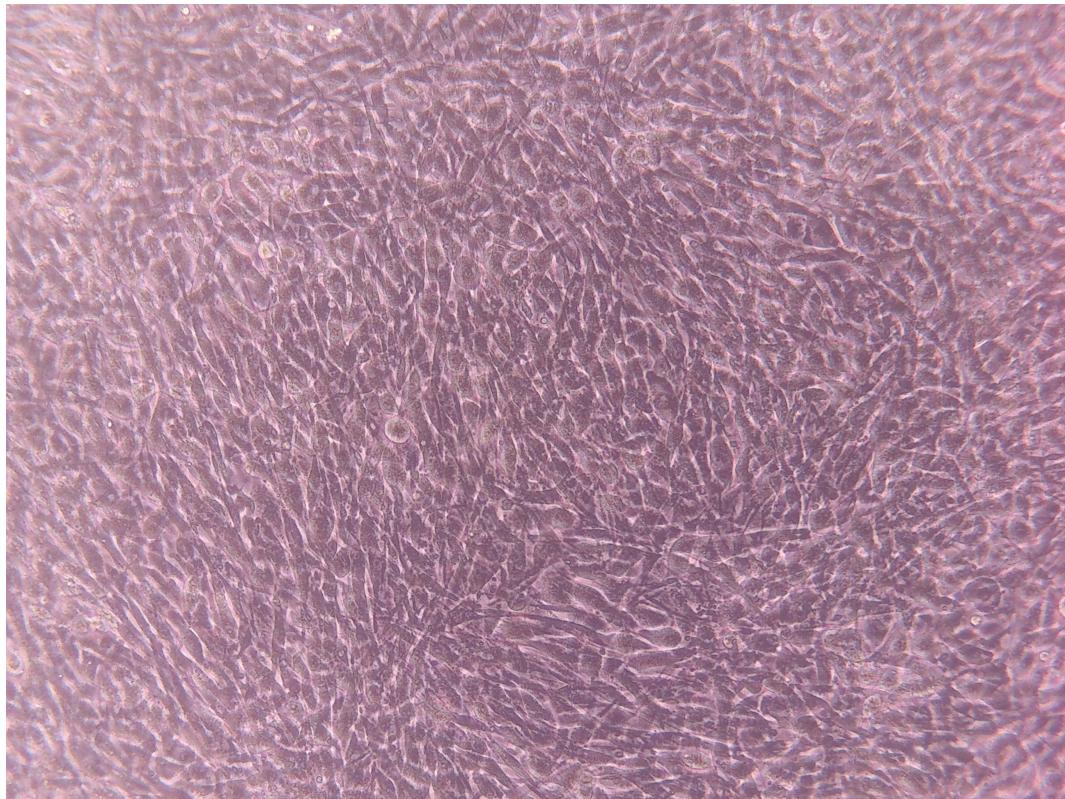
## 5 References

1. Cell Adhesion . Wikipedia. 2018-10-07  
[https://en.wikipedia.org/wiki/Cell\\_adhesion](https://en.wikipedia.org/wiki/Cell_adhesion)
2. Evolution of Cell Culture Surfaces. Sigma-Aldrich. 2018-10-07. <https://www.sigmaaldrich.com/china-mainland/zh/technical-documents/articles/biofiles/evolution-of-cell.html>.

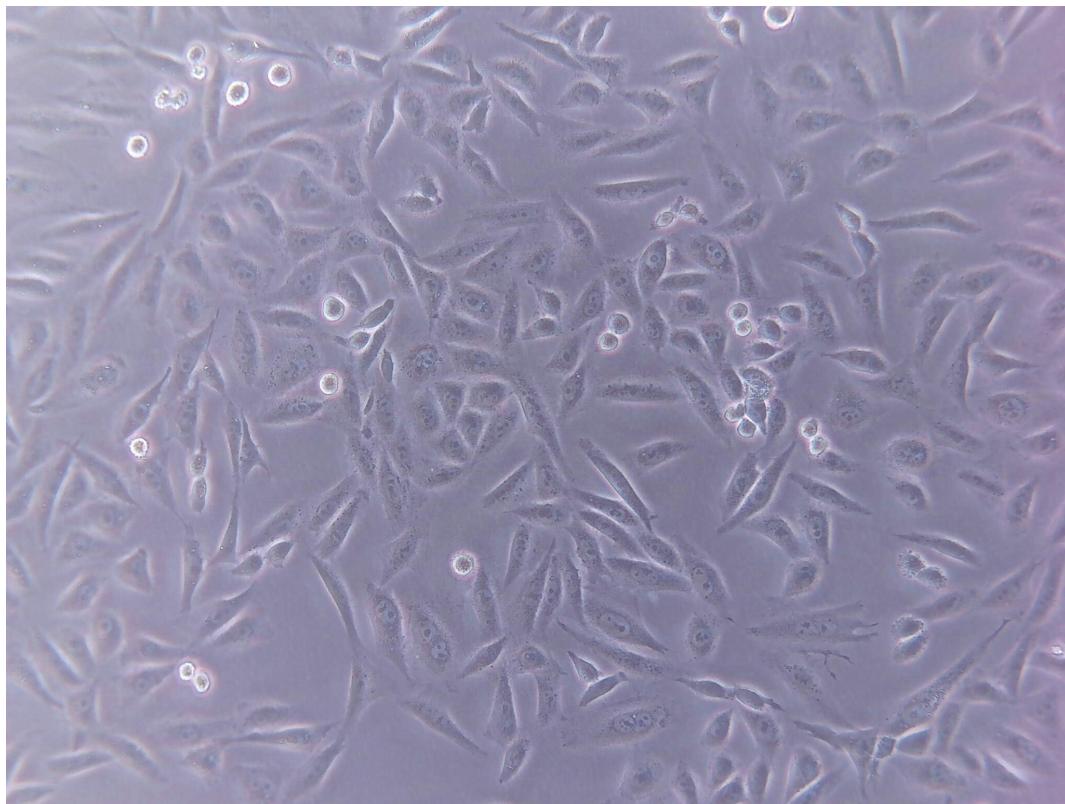
## 6 Contribution

Wenhao Zhang and I did the expeiment one time ereveryone. We helped and remined each other. Thanks to Dr. Zhao and Dr.Jia who explained pricniples andprocedures as well as helped us prepared cells and other materials.

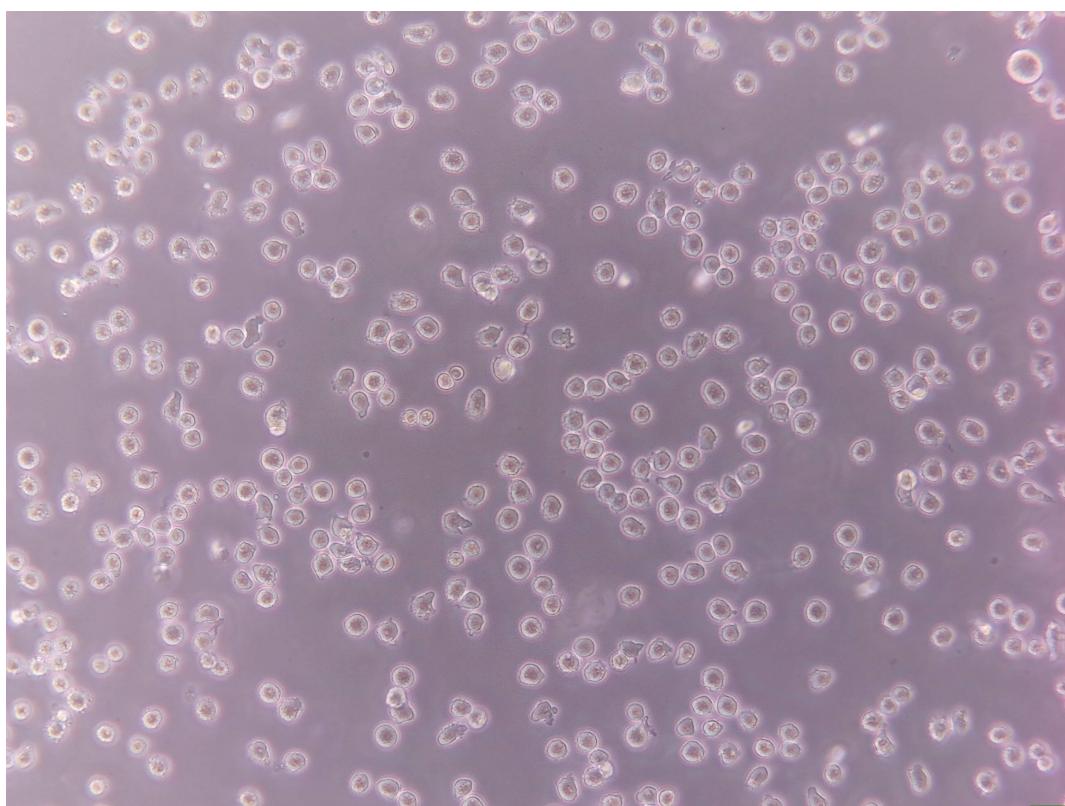
## 7 Figures & tables



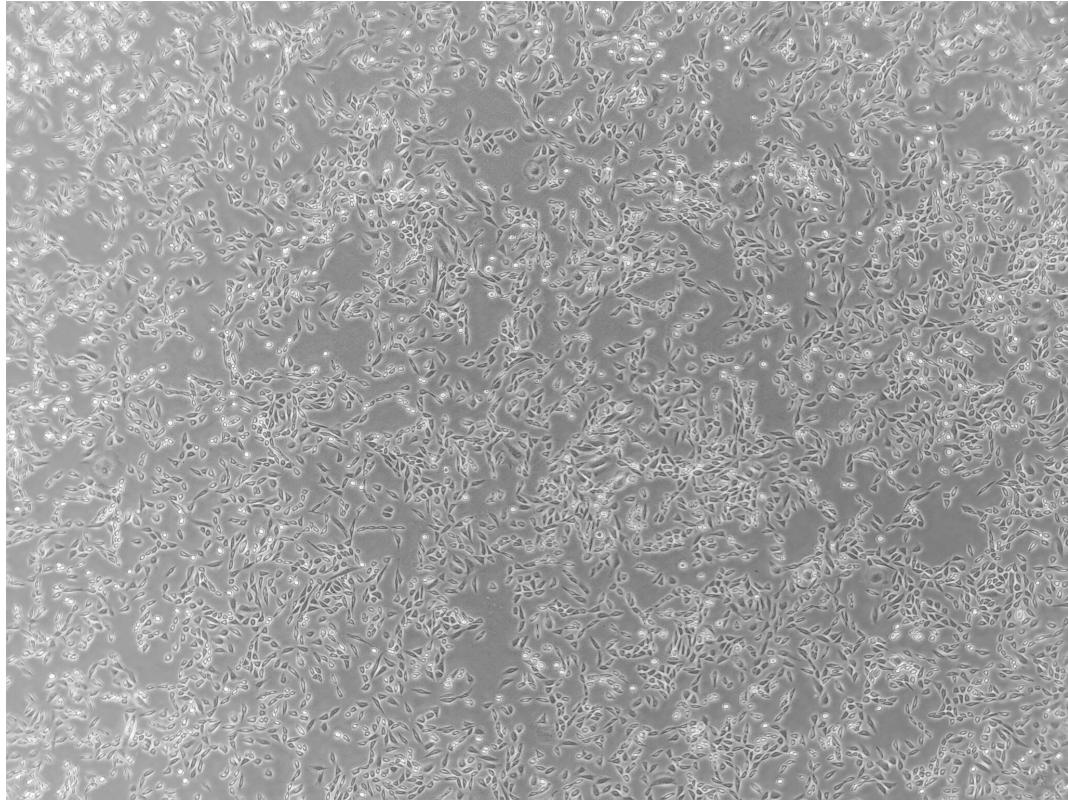
**Figure 1.** NIH/3T3 cells under inverted phase contrast microscope(20X).



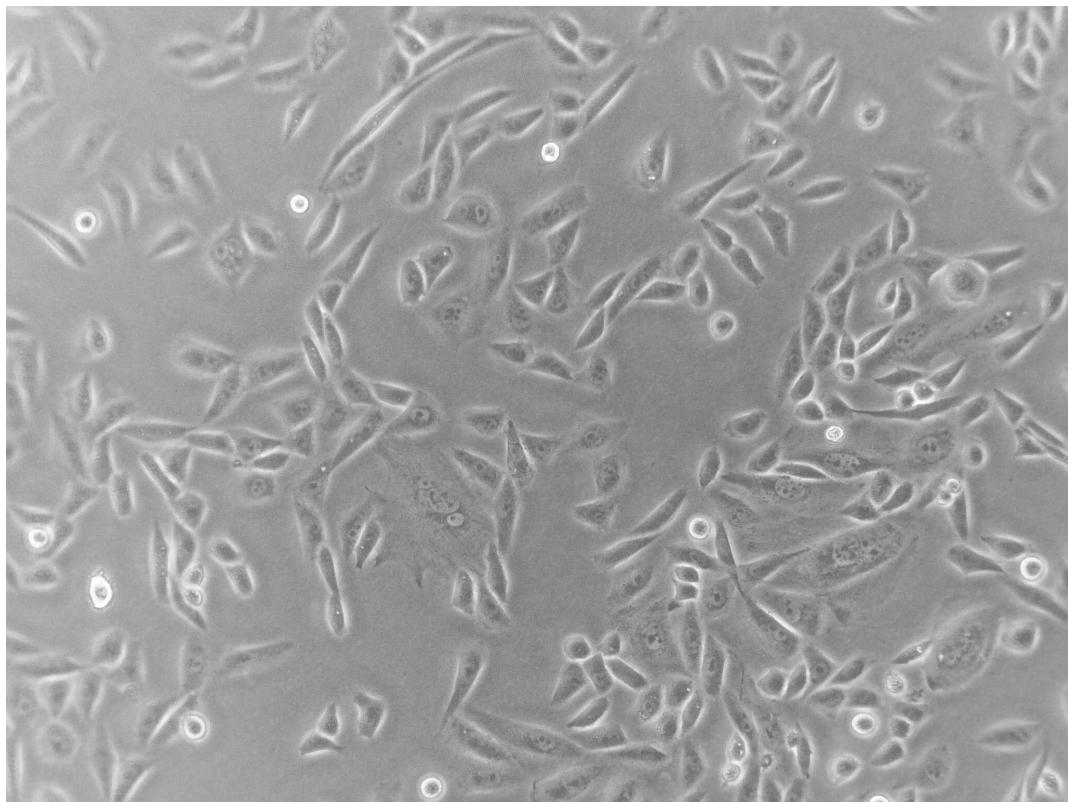
**Figure 2.** CHO-K1 cell under inverted phase contrast microscope(20X)



**Figure 3.** U-397 cell under inverted phase contrast microscope(20X)



**Figure 4.** Subculture CHO-K1 cell under microscope(20x). Most of cell attached to substrate with polygonal edge. Some cell were round and suspended, which were dead.



**Figure 5.** Subculture CHO-K1 cells under microscope(4x). No pollution, suitable density.