Application of C-dots for Cellular Imaging

Ali Utku Özen
Supervisors: Prof. Dr. Serdar Özçelik, Dr. Iroda Saydullaeva

İzmir Institute of Technology, Department of Chemistry, Urla 35430, Izmir

Introduction

Carbon dots (CDs) are small (below 20 nm) carbon nanocrystals suspended in aqueous or any suspensions. Core attribute of CDs is fluorescent applications. They are biocompatible, safe to environment, have a high quantum yield, chemically stable, and have a high solubility in water.

The **A549 cell line** is the line which obtained from epithelial carcinoma of 58-year-old male lung cancer patient. In imaging of these cells with carbon dots, observation of fluorescent spots mostly around cytoplasmic area and cell membrane. However, there are still weaker signals in cell nucleus region still existed.

In this study, it is aimed to show imaging process of interaction between AuCD and A549 cells.

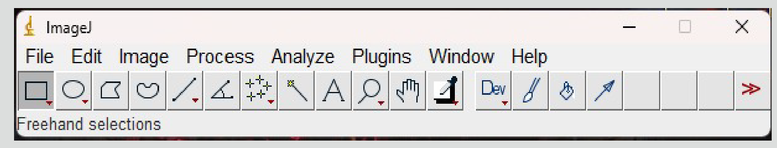
Methods

Preparation of A549 cells

- A549 cell lines which were cultured using DMEM/F-12 medium enriched with 10% FBS, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 mg/mL).
- Cells were cultivated in a humidified incubator with 5% CO2 at 37 °C for all experiments. The T-25 flask was used to culture the A549 cells.
- On the day before treatment, 200.000 cells per well in a 6-well plate were seeded on glass coverslips. An optical microscope was used to confirm cell density the night after seeding.
- Then the cells were treated for 6 hours with 50 g/mL c-dot and c-dot@AuNP solutions. As a positive control, AuNP is used. Cells on coverslips were washed with PBS before being fixed in cold methanol for 20 minutes at 4 °C.
- Coverslips were then picked up and hung to dry. To stain nuclei, 10 L of 1g/mL DAPI was deposited on a coverslip and placed on a slide.
- After that, the cells on coverslips are immediately photographed using a Zeiss Axiozoom V.16 microscope with DAPI, green, and red channels, as well as bright-field and dark-field contrast techniques.

Imaging Process

ImageJ is the image processing program developed with java which allows us to analyse our samples, merge them and processing image. ImageJ can also evaluate colocalization which defines that distance between dyes or stains. It analyses and gives information about, molecules and proteins that used are bound or interacting. One of the most used colocalization coefficient is Pearson's correlation coefficient. In this method +1 value is the perfect correlation and 0 value for no correlation while -1 value perfect anticorrelation. Any noise might shift the value towards zero.



Results and Discussion

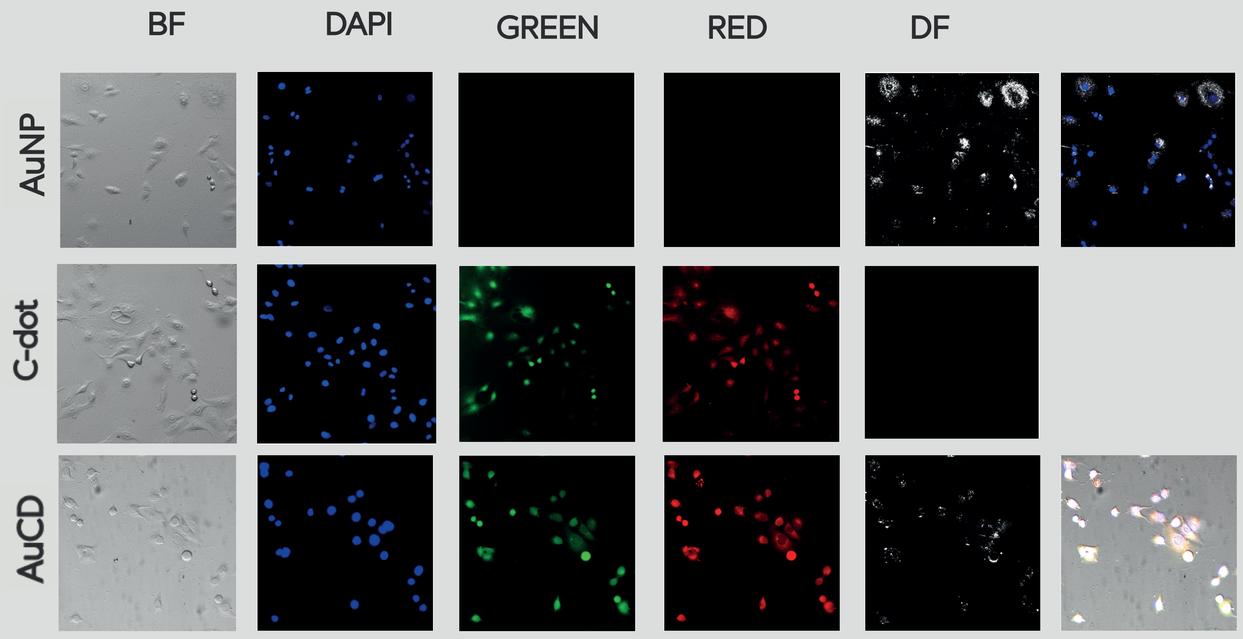
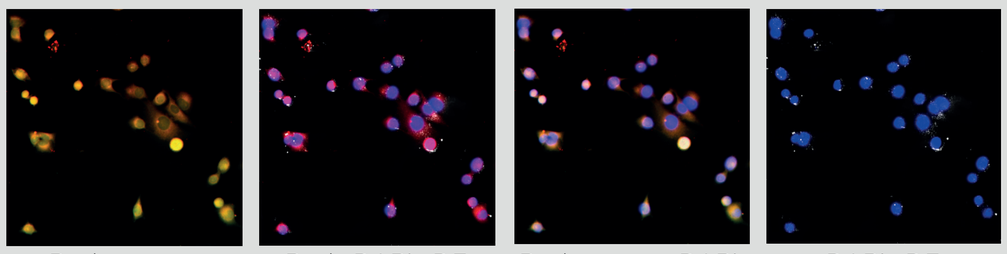


Image Processing for AuCD
Coloc: a) Pearson's R value, 0.43 Au_CD(red)
b) Pearson's R value, 0.36 Au_DAPI
c) Pearson's R value, 0.82/0.93 red_green
d) Pearson's R value, 0.80 red_DAPI

A) The code with 0.43 R value means this value DF-Red has a low rate of carrying Au
B) Au particles have a low relationship with the nucleus
C) When the red and green merge is made, this value and the yellow color in the merge image, that is, coincident, shows that the cdots are connected to specific points in the cell.
D) Since dapi direct dyes the nucleus, the red dapi ratio being close to 1 indicates that the red emitter is connected to the nucleus.

- Green-tag and red-tag linked to CDot are used to see if CDot is linked to the same locations in Cells. If the images of these two processes are merged through imagej, it can be shown that they bind to the same place with the overlapping points, and at this point, a yellow glow can be obtained and cdot application can be shown to bind to specific points in the cell, thus it can be observed that AuNP can be carried to the core.
- The DF image shows that AuNPs bound to Cdot are transported to the cell nucleus by Cdot in A549 cells treated with AuCd.
- Positive-control AuNP: In this part Dark field(Au) and DAPI(nucleus)– There is no image in red and green because there is no cdot.

Merges



Red_green | Red_DAPI_DF | Red_green_DAPI | DAPI_DF

Conclusion

It has been observed that AuNPs, which cannot reach the nucleus of the cell under normal conditions, reach the nucleus of the cell when they are attached to the carbondots and given to the environment. By looking at the Pearson values we received, we understand that the au-dapi difference, which is 0.36 for the colocalization value under normal conditions, increases to 0.80 when connected with cdots.

References

Jaiswal, S. S. Ghosh and A. Chattopadhyay, Chem. Commun., (2012). 48, 407–409.
Sheng-Tao Yang, Xin Wang, Haifang Wang, Fushen Lu, Pengju G. Luo, Li Cao, Mohammed J. Meziani, Jia-Hui Liu, Yuanfang Liu, Min Chen, Yipu Huang, and Ya-Ping Sun The Journal of Physical Chemistry C., (2009). 113 (42), 18110-18114
Song, Y., Zhu, S., & Yang, B. (2014). Bioimaging based on fluorescent carbon dots. RSC Advances, 4(52), 27184.

Contact : aliozen@std.iyte.edu.tr