**Automatic Counting of Apoptosis in HCT116 human colon adenocarcinoma cell**

**Liu Hai-ling**

**Shin Young-suk**

### Background

Apoptosis is a complex, tightly regulated and active physiological process whereby individual cells are triggered to undergo self-destruction. As cancer cells frequently have acquired the ability to avoid apoptosis and continue to multiply in an unregulated manner, induction of apoptosis is an important factor in cancer therapy. Many anticancer drugs attempt to achieve the effect by inducing the cancer cell to apoptosis. So the quantitative analysis of apoptosis is required not only to understand the mechanism of this esoteric, quaint process but also to quantitatively evaluate the effect of anticancer drugs. Currently counting apoptosis in vivo is generally carried out manually and may consist of estimates of number of cells stained with a particular cell marker. But this method is extremely time-consuming and labor-intensive. Also manually counting is subjective and difficult to achieve high-throughput. There is a compelling necessity for developing an automatic cell counting method.

### Cell Culture and Drug Treatment

- **HCT116 human colon adenocarcinoma cell line** obtained from American Type Culture Collection was grown in RPMI 1640 medium (Invitrogen, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen) and 1% penicillin-streptomycin in a 37°C humidified incubator in an atmosphere of 5% CO₂.
- Drug treatment of cells was performed by adding 50 μM oxaliplatin and incubating for 48 h.
- The cells were incubated with 1 μg/ml Hoechst 33342 (HO3) for the final 10 min of drug treatment in the 37°C incubator and then, both floating and attached cells were collected by centrifugation. The pooled cell pellets were washed with ice-cold phosphate-buffered saline (PBS), fixed in 3.7% formaldehyde on ice, washed again with PBS, and a fraction of the suspension was centrifuged in a cytopipetter (Thermo Shandon, Pittsburgh, PA).

### Image Acquisition

The slides were air dried, mounted in an anti-fade solution, and images were analyzed using a DVM5000 fluorescence microscope (Leica, Germany) at excitation/emission wavelengths of 340/425 nm. For images capture, microscope connected Leica DFC800 camera and Application Suite software were used. The image size is 2560 × 1920.

### Image Preprocessing

Noise reduction: Median filter
Contrast enhancement: contrast-limited adaptive histogram equalization
Image before preprocessing (Left) and after preprocessing (Right)

### System Framework

- **Object Detection**: Detection of objects in the image
- **Object Grouping**: Group objects into meaningful clusters
- **Feature Extraction**: Extraction of features from each object
- **Feature Selection**: Selection of relevant features
- **Classification**: Classification of objects based on features

### Results and Conclusions

- **Size Distribution Estimation**
  - Method: Grey scale granulometry
  - Normal cell size (Left) and normal apoptotic bodies size (Right)

- **Contact Information**
  - 375 Saeseok-dong, Dong-gu
  - Gwangju 501-759, Korea
  - Tel: +82-62-230-7011
  - Fax: +82-62-230-7011
  - Email: yogin0757.12@chonnam.ac.kr
  - ysshin@chosun.ac.kr

- **Apoptosis Identification**
  - Target Objects identification. This step includes single objects identification and clumped objects separation. Single objects can be identified by thresholding. Clumped objects are firstly identified by the size distribution of nuclei and apoptotic bodies and then separated by region growing method and watershed algorithm. L-OHP-H (Upper Left) and objects identified in L-OHP-H (Upper Right)
  - Feature extraction. This step extracts six features as the basis of identification of apoptosis.
  - Apoptosis identification. This step uses neural networks classifier to identify apoptotic bodies. (Bottom Left)
  - Grouping apoptosis: This step group apoptotic bodies into an intact cell with the normal cell size obtained before. (Bottom Right)