

## Automated Quantification of Apoptosis and DNA Damage

### Clinical Need

Cellular apoptosis is known to be a major factor in tissue remodeling, cancer elimination, aging, and age-related diseases. Determining the level of apoptosis and DNA damage in a given sample of cells is an important diagnostic for genotoxicity and health status. Current techniques in apoptosis evaluation suffer from a variety of functional issues, such as requiring large numbers of cells, insensitivity to low levels of apoptosis, artifact labeling, loss of detection of some cell types, and false positive results. New techniques to analyze apoptosis and DNA damage need to be inexpensive, simple, fast, and give accurate, reliable results.

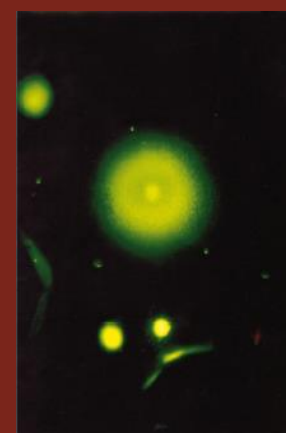
### Bioengineering Solution

The DNA diffusion assay developed by Dr. Narendra Singh is a low cost, highly sensitive technique for quantifying apoptosis of cell populations at the single cell level<sup>1</sup>. However, long processing and analysis times are involved with the technique and others similar to it. Several years ago, Dr. Singh collaborated with Dr. Sayan Pathak to develop an algorithm for automatic analysis of DNA diffusion assay images. The Singh lab now looks to expand this algorithmic approach and use it to revamp and standardize a DNA diffusion assay optimized for image processing and quantifying both apoptosis and DNA damage at the individual cell level. A complete software program for analysis and interpretation of data will be created to implement the algorithms developed by Dr. Pathak, as well as new algorithms for DNA damage assessment. These aims will have the effect of reducing the time and cost involved in DNA damage and apoptosis evaluations, while maintaining sensitivity, requiring few cells, and achieving higher specificity for desired cell types. This project will be of value for various areas of medicine.

### Current Status and Results

In 2006, the Fast Halo Assay (FHA) was implemented for the detection of DNA damage<sup>2</sup>, and has recently been modified<sup>3</sup>. This technique has the potential to be one of the most sensitive simple, fast, and accurate assessment of DNA damage at the single cell level. The Singh Lab believes that this assay can be further modified and expanded upon to yield improved results.

Drs. Singh and Pathak found the automated image analysis algorithms then developed to be effective in quantifying the apoptotic index using the DNA diffusion assay. This preliminary work showed a 95% confidence level of no significant difference between the automated and manual image analyses. In addition, they found only marginal errors in misdetection and false positives.



A typical apoptotic cell  
(Singh 2000)

“In the recent past, extensive efforts have been made to develop simpler assay methodology for detection of apoptosis”

- Suman *et al.* (2012)

### Personnel

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### Funding

UW BIOE RCR

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Concept Sheet