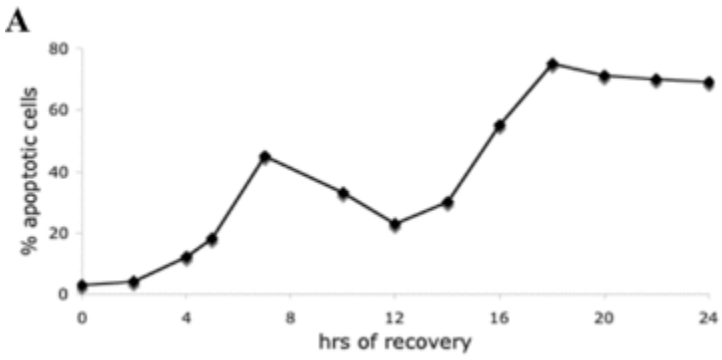
PROOF OF CONCEPT IN 9 DAYS

THE FIGURE



The figure to the left shows apoptosis due to hydrogen peroxide exposure over time. Hydrogen peroxide theoretically induces apoptosis by breaking down DNA (first peak) and inhibiting pore formation in mitochondria (second peak). The authors of that paper used fluorescence microscopy with Hoechst dye and a hemocytometer to count the number of cells that underwent apoptosis. These methods evaluate DNA breakage and nuclear shape, respectively; two trusted indicators of apoptosis[1]. If we can reproduce these two peaks using some stripped-down version of our method, we have proof-of-concept, and further research will only improve accuracy.

METHODS: CELL CULTURE

Note: Our cell line is HepG2, a human liver carcinoma cell line, growing in low-glucose DMEM with 10% FBS inoculated with pen/strep.

1. Grow enough cells to split
2. Treat cells for 1 hour with 1 mmol/L H_2O_2
3. Wash and resuspend in fresh medium
4. Plate 5 samples in 96-well plate, incubate
5. Every 1 hour for 24 hours, remove from incubator and image each well

METHODS: ANALYSIS

The airy disk is a pattern of concentric rings of high intensity light, caused by the diffraction of light after collimation through a circular or elliptical aperture. In the far field, the Frauenhofer approximation states that the positions of the first few minima of the pattern are given by

$$a = 1.22\frac{\lambda \mathrm{L}}{d}$$

[1] C Cerella, S Coppola, V Maresca, M De Nicola, F Radogna, L Ghibelli. Multiple mechanisms for hydrogen peroxide-induced apoptosis. *Annals of the New York Academy of Sciences* 2009, 1171: 559-563

where λ is the wavelength, d is the aperture diameter, and L is the distance between aperture and image (detection) plane[2].

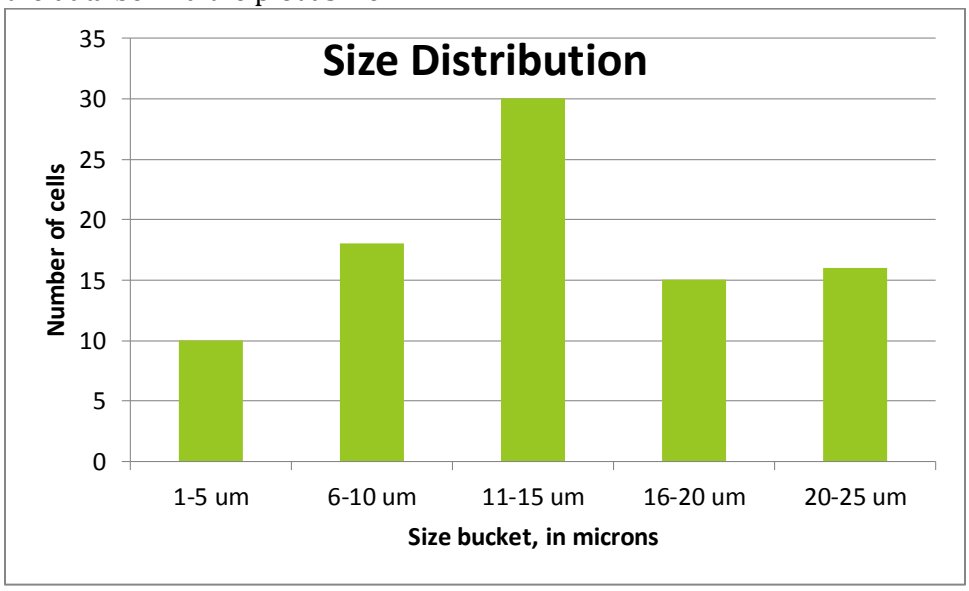
It is known that for partially coherent light, small particles act as an aperture, and the particle size compared to the scattering distance lets us take advantage of far field effects. Now we can relate minima of diffraction pattern a to particle size d, since we know wavelength λ and set the distance L.

Cell size may be a provisional marker for apoptosis. When cells undergo apoptosis, one of the first steps is volume loss via formation of a contractile cortical cortex which essentially pulls in parts of the membrane. The beginning of volume change in fact precedes and affects membrane blebbing. In early stages of apoptosis, the cell loses about 20-40% of its volume, and an additional 20-40% is lost later[3]. Experimentally, a 50 percent reduction of cell volume prior to complete disassembly has been found in epithelial cells in particular[4].

Therefore I hypothesize that the change in location of the first minima of a raw airy disk over time will give us a proof-of-concept estimate of apoptosis.

The development needed for analysis:

1. Remove background from image given raw image from sensor.
2. Locate and store the x-y coordinate of the center of many diffraction patterns. Hint: intensity should be higher in the center than in the rings.
3. Compute the radius a of the first few concentric circles for each diffraction pattern.
4. For each image, plot a distribution of cell size using the Frauenhofer approximation. I'm going to provisionally say that bucket size and number of buckets is up to you. Please store the data behind the plot as well.



[2] Y Wang. Experimental investigation of far-field diffraction by means of normally and non-normally illuminated elliptical apertures of wavelength dimension. *Optical Engineering* 1994, 33.2: 692-696.
[3] R Nunez, S Sancho-Martinez, J Novoa, F Lopez-Hernandez. Apoptotic volume decrease as a geometric determinant for cell dismantling into apoptotic bodies. *Cell Death and Differentiation* 2010, 17: 1665-1671.
[4] A Khmaladze, R Matz, T Epstein, J Janesky, M Holl, Z Chen. Cell volume changes during apoptosis monitored in real time using digital holographic microscopy. *Journal of Structural Biology* 2012, 178: 270-278.

5. Before further development (i.e. sorting) we should visually inspect the curves. Then we'll have a better idea of how we can build a simple classifier.
6. (optional, time dependent) You will be given a set of images that may either exhibit (a) entire image shifting or (b) a small amount of cell movement. It would be cool to see the progress of a single cell over time. Bonus: be able to tell when a cell is no longer visible, with confidence (i.e. it's not a failure of detection method but rather the cell is gone).

CRITICISM

Here are some assumptions I am making.

1. The resolution we are getting off the raw sensor is adequate to resolve a change, given a large enough cell/sensor distance L (I'll calculate this.)
2. Far field approximation is good for our setup.
3. HepG2, an epithelial line, will respond to hydrogen peroxide similar in the predicted way.
4. Cell movement is negligable.