Using Automatic Pattern Recognition to Study DNA Repair in U2OS Cells

Sijie Peng[1], Jonathan Xue[1], Yufei Zhang[1], Justin I. Chen[1], Tao Wu[1], Xiaohua Wu[1], Linda Shi[1]
[1]Institute of Engineering in Medicine, University of California San Diego, La Jolla, CA 92093, [2]Department of Molecular & Experimental Medicine, The Scripps Research Institute

Background

Rad52 is an essential protein for loading Rad51 on single-strand DNA during homologous recombination in lower eukaryotes, but mammalian cells have evolved other proteins to replace this function of Rad52. Under specific conditions such as R-loop accumulation, Rad52 will be recruited to the damaged site to process unexpected R-loops. Since SETX loss is related with R-loop level increase, we hypothesize that SETX-deficient cells will implement some novel proteins to repair DNA double-strand breaks, namely Rad52, XPF and XPG. The biophotonics lab at UCSD uses the laser damage to study the recruitment of Rad52 in the DNA repair mechanics. Biologists often spend lots of hours measuring the recruitment intensity using ImageJ. As high school students with a strong coding background, we proposed and implemented an automatic way to detect post-laser damage recruitment to speed up image processing.

Methods

Overall project goal of automating line detection process, with a focus on producing similar results to ImageJ in minimal time

1. Use CLAHE to enhance image for pattern recognition
2. Find the center of each cell and single them out
3. Binarize image and fill in holes
4. Use Hough Transform to detect lines in cells
5. Adjust the cell centroid and lines in each image
6. Calculate the line intensities for each cell in every image

Results

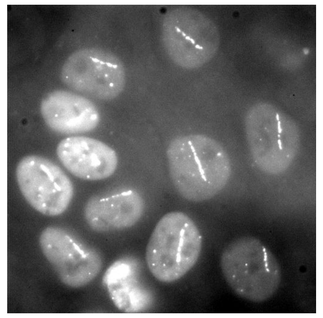


Figure 1:Initial image of U2OS cells after laser damage

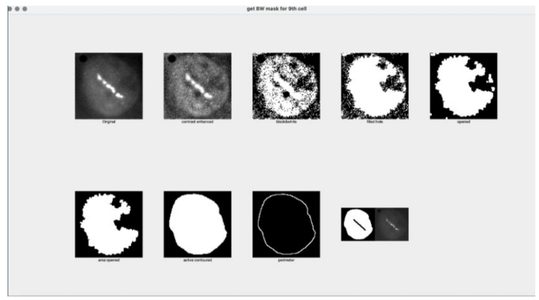


Figure 2: Process of centroid and line detection on the upper cell in Figure 1

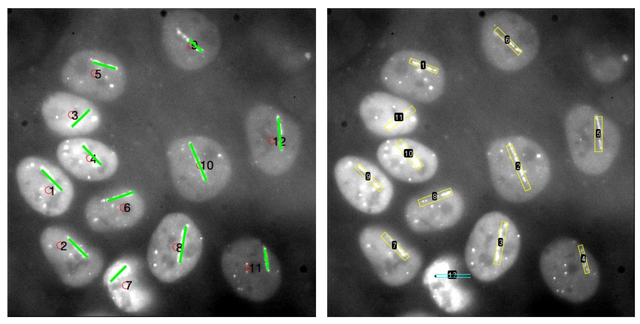
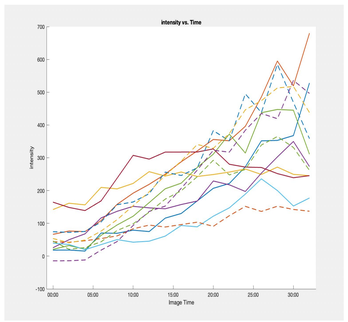


Figure 3: U2OS cells with MATLAB-detected centroids and lines, with the same lines manually traced on ImageJ (right). The difference in detected line location on cell 7 (left) is due to image brightness affecting the line detection

Results

a



b

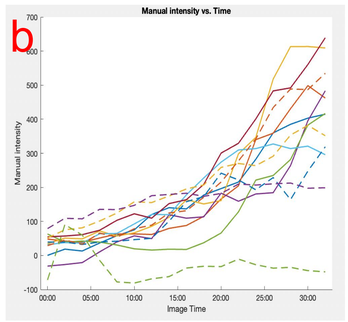


Figure 4: Average intensity of Rad52 recruitment: 4a Using proposed pattern recognition algorithm, 4b Using manual way in ImageJ. The graphs both show similar positive trends, intensity values, and shapes, indicating MATLAB's partial success in replicating ImageJ's pattern recognition results. Some variance is due to imperfections in the photograph limitations of the MATLAB program and human error using ImageJ.

Conclusions

1. MATLAB automatic pattern recognition yields results comparable to manual image analysis using ImageJ but can save lots of manual labor work and speed up the measuring process
2. Accuracy of MATLAB analysis highly dependent on quality of images, spherical shape of cells
3. If the pattern recognition program is refined and images are consistently high quality, could greatly expedite research on DNA repair in cells

References and Acknowledgements

1. He, J., Shi, L. Z., Truong, L. N., Wu, X., Berns, M. W., Shiloach, J., Negrete, A., Li, Y., Razavian, N., & Lu, C.-S. (2012). Rad50 Zinc Hook Is Important for the Mre11 Complex to Bind Chromosomal DNA Double-stranded Breaks and Initiate Various DNA Damage Responses. *Journal of Biological Chemistry*, *287*(38), 31747–31756.
2. You, Z., Shi, L. Z., Zhu, Q., Wu, P., Zhang, Y.-W., Basilio, A., Tonnu, N., Verma, I. M., Berns, M. W., & Hunter, T. (2009). CtIP Links DNA Double-Strand Break Sensing to Resection. *ScienceDirect*, *36*(6), 954–969.
3. Wang, H., Shi, L. Z., Wong, C. C. L., Han, X., Hwang, P. Y.-H., Truong, L. N., Zhu, Q., Shao, Z., Chen, D. J., Berns, M. W., Wu, X., Chen, L., & Yates, J. R. (2013). The Interaction of CtIP and Nbs1 Connects CDK and ATM to Regulate HR–Mediated Double-Strand Break Repair. *PLOS Genetics*, *36*(6).

This material is based upon work supported by a gift from the Beckman Foundation to M.W. Berns. The high school students (S. P., J. X., J. C.) participated in the Outreach Program for Advancing Learning in STEM (OPALS) in Institute of Engineering in Medicine at UCSD, Y. Z and J. C. participated the Mentor Assistant Program (MAP) at UCSD