Imaging Somatic Homolog Pairing in *D. Melanogaster*
Molly Moore '21

Gene regulation, which plays a critical role in the production of normally functioning proteins, can be influenced by the organization of genetic elements within the nucleus (Lewis 1954; Leiserson et al. 1994). The effects that interactions between chromosomes can have on gene regulation can be easily investigated in *Drosophila melanogaster*, commonly known as the fruit fly, due to a phenomenon called somatic homolog pairing. While the pairing of the two parental copies of the same chromosome, called homologous chromosomes, occurs in meiotic cells across many organisms, *D. melanogaster* and some other fly species experience a unique phenomenon whereby their homologous chromosomes pair in somatic cells, as well as in meiotic cells; geneticists have called this process somatic homolog pairing (Stevens 1908; Joyce et al. 2012). The pairing of homologs in *Drosophila* enables interactions between the two chromosomes. In particular, it is possible for the regulatory elements of one homolog to influence the transcription of a gene on the paired homologous chromosome (Lewis 1954; Leiserson et al. 1994). Given this regulatory effect of pairing, it is critical to better understand the effects and mechanism of somatic homolog pairing.

While the phenomenon of somatic homolog pairing was discovered in the early 1900s, the underlying mechanism remains poorly understood (Stevens 1908). Genes that influence pairing have been identified, but the biophysical mechanism through which pairing is initiated and maintained is still being investigated (Bateman and Wu 2008; Joyce et al. 2012; Rosin et al. 2018; Rowley et al. 2019). Currently, scientists favor a "button model" whereby certain locations on the chromosomes have high affinities for each other, prompting them to hold the chromosomes together at this location (Rowley et al. 2019; Viets et al 2019). The "buttons" along the length of the chromosome then randomly encounter each other and stick together such that the entire chromosomes are held together. We aim to further investigate this model by determining whether it is consistent with the distance between chromosomes over time. In order to investigate this broad question, we first needed to develop an image processing workflow that would allow us to make 3D measurements from 2D xy images stacked along the z axis, called "z-stacks" (Figure 1).

To better characterize the mechanism responsible for somatic homolog pairing, we have used DNA Fluorescent In-Situ Hybridization (DNA-FISH) and antibodies to visualize the location and proximity of chromosomes in *Drosophila* embryos. DNA-FISH is a technique that labels a specific region of a chromosome with a fluorescent tag, enabling the detection of chromosomes through fluorescence microscopy. Similarly, antibodies with fluorescent tags can be used to detect the location of specific chromosomal segments. These techniques allowed us to obtain fluorescent images on a confocal microscope, which were compiled into z-stacks that represent 3D structures. With these stacked images, we could measure parameters such as the distance between probes in various mutants to look for genes associated with changes in the pairing phenotype.

After obtaining images, we used software to process and analyze the images. We developed a processing pipeline in TANGO, an image processing software, to investigate whether there were differences in pairing between different fly lines. We anticipated that this comparison might help us identify genes involved in the observed pairing phenomenon. We found that there are existing differences in pairing between different fly lines, suggesting that some genes involved in pairing differ between these fly lines. After extensively testing the imaging processing software TANGO, we transitioned to using Ilastik and ImageJ 3D suite to enable greater accuracy and greater control over the steps of image processing. Ilastik, a segmentation program powered by machine learning, used in tandem with the ImageJ 3D Suite, allowed us to develop a process to produce our measurements of interest, including distance measurements, colocalization measurements, and other quantitative measurements of the structures within our images. We anticipate being able to use this workflow to process ongoing images of embryos from various mutants over the upcoming semester so that we can investigate the role of various genes in somatic homolog pairing. Broadly, this research will further inform our understanding of nuclear organization and its potential effects on gene regulation.

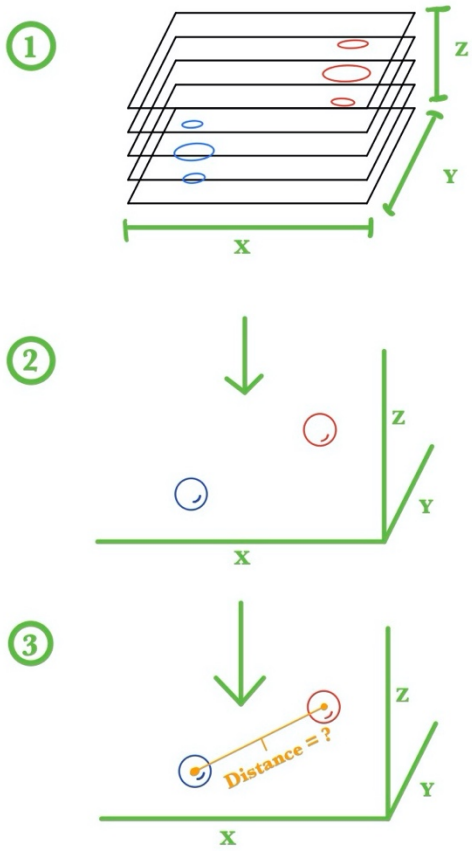

Figure 1. Schematic of image processing workflow. (1) Use confocal microscope to take xy images at different depths in z (2) Reconstruct image into 3 dimensions using image processing software such as TANGO or ImageJ 3D Suite (3) Use image processing software to measure center-to-center distances and percent overlap of signals; these measurements can be used to determine whether homologs are paired and unpaired in different mutants or at different time points

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