Thesis Project Portfolio

Automated counting method for analyzing the results of *T. gondii* invasion assays
(Technical Report)

The American Meat Industry: Promoting Consumption while Promising Sustainability
(STS Research Paper)

An Undergraduate Thesis

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Table of Contents

Sociotechnical Synthesis

Toxoplasma gondii is an obligate intracellular parasite that invades a wide variety of homeothermic hosts and can cause a disease called toxoplasmosis. The cost of this illness in the United States is estimated to be 3 billion dollars and an 11,000 quality-adjusted life year loss annually. Approximately 8-22% of people in the United States are infected, and in most cases, the infection is asymptomatic. However, if a pregnant woman contracts toxoplasmosis, the child could develop blindness and mental retardation, and the infection can even lead to death in immunocompromised people. Toxoplasmosis is acquired through ingestion of tissue cysts in uncooked or undercooked meat or through ingestion of cat feces containing the parasite oocysts. The parasite invades by pulling itself into the host's gut epithelial cells, but the proteins involved in this process have not fully been identified. Invasion assays are an *in vitro* method of quantifying the number of parasites that have invaded host cells, and by manipulating the substrate stiffness and stretch of the host cells, it is possible to learn more about the mechanisms of parasite invasion. The output of the experiment is fluorescent images which must be analyzed with a process that is both time-efficient and accurate. The goal of this project is to optimize quantitative image analysis of *T. gondii* invasion, which will lead to a better understanding of the method of invasion.

Invasion assays in this project will be performed on substrates of varying stiffnesses ranging from soft to stiff. These varying stiffnesses model the varieties of extracellular matrices (ECMs) that cells in the body interact with, and interactions with different ECMs can influence cell morphology and behavior. Polyacrylamide (PA) gels can be formulated to have different stiffnesses by using varying concentrations of acrylamide and bis-acrylamide. Human foreskin fibroblasts (HFFs) were plated on the prepared PA gels of varying stiffness and were cultured in growth medium until confluent. Once the cells had reached confluency, the invasion using

parasite *T. gondii* was performed on the gels as well as on an untreated glass dish as a control variable. Fluorescent images of the intracellular and extracellular parasites were taken, which we could feed into our automated cell counting program of choice, which was CellProfiler. The workflow we determined to most efficient and accurate involved firstly reducing noise in the image, then identifying fluorescent objects, filtering these objects by size and intensity, editing any mistakes manually, and then overlaying outlines of objects onto the final image. We found that the workflow identified correct objects with 5.5% error and, on average, took half the time to count objects than manual counting would require. This counting method will now be applied to real images of parasitic invasion and can perhaps allow for more efficient *T. gondii* research in the future.

As mentioned earlier, toxoplasmosis is a disease that can be contracted through consumption of undercooked meat. We might make an assumption that an increased rate of meat consumption might also increase the risk of contracting diseases like toxoplasmosis. If we compare meat consumption now and just a few decades ago, we see that global meat production has more than quadrupled. We could attribute this rise in meat consumption as a result of the rising global population since the 1960's. However, instead of the two rates increasing proportionally, we observe that meat production has been growing at a much faster rate than that of population growth. From these rising trends, not only can we expect a larger frequency of people consuming raw meat and being exposed to diseases like toxoplasmosis, but also a decline in the quality of arable land. As demand for meat production increases, producers will have to claim more and more land to dedicate to livestock production. Currently, there is no plan to slow down or entirely halt this progression.

This thesis will consolidate information from participants in the U.S. who play a role in either the progression or reversal of this phenomenon. These participants include government organizations like the Environmental Protection Agency (EPA), advocacy groups like Good Food Inc., companies like Beyond Meat, and America's meat-eating population. We will examine how each party plays a unique role in contributing to or combatting meat overproduction as well as what measures can be taken to prevent irreversible climate change.