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

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

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

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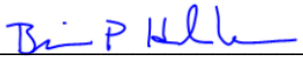
Approved  Date _05/11/2022_

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General Research Problem

How has rising meat consumption introduced growing health and environmental burdens in the last 100 years?

Much of human evolution can be attributed to meat consumption. Chimpanzees, our closest extant primate ancestors, hunted and shared monkey meat to reinforce social bonds and, among males, to attract females. Carnivorousness throughout history was considered a sign of success and could be used to bridge cultural groups together. Economic modernization has facilitated rising meat consumption by painting it as an integral dietary component. However, we face diverse agricultural, economic, nutritional, and environmental consequences as the trend continues (Smil, 2002). Sociotechnical change is necessary to avoid irreversible health and environmental damage.

Technical Research Problem

How can a developed workflow help optimize image analysis of epithelial cells subjected to Toxoplasma gondii *parasitic invasion?*

This project is in collaboration with fellow student Emma Wolcott and advisor Dr. Brian Helmke in the Department of Biomedical Engineering. Our goal is to use automated segmentation to accurately label nuclei and parasites against a background in epithelial cells a) grown on varying substrate stiffnesses and b) uniaxially stretched on membranes. Applying this workflow to images from the parasite invasion assay can then quantify the concentration of cells successfully invaded.

Traditional manual segmentation is time and labor-intensive, so an automated system will increase efficiency even if a dataset contains hundreds of images (Englbrecht et al., 2021). The

main goal of this project is to efficiently label nuclei with a high level of accuracy. Constraints include needing ideal elastic moduli of soft, medium, and stiff polyacrylamide gels and achieving accurate stretch profiles for frequency and percentage of stretch. These factors must be consistent and within a certain range in order for the invasion assays to be comparable. Once consistency is achieved, the segmentation workflow will be applied to images of assays to determine whether or not the detection of nuclei is accurate.

Automated segmentation programs do already exist that use watershed separation, machine learning algorithms, and neural networks, but these programs are limited to detecting nuclei (Sharma & Aggarwal, 2010). The scope of this project is more microscopic, which is why a novel approach is needed to be able to detect those smaller objects. Another method used ImageJ, a Java-based image processing program, to segment and count parasites following an invasion assay. Researchers then verified the accuracy of segmentation by hand (Leung et al., 2017). This method also poses limitations both in the experimentation specification as well as in the verification phase. The assays were performed on glass, which is easier to image than the gel and polydimethylsiloxane (PDMS) surfaces that will be used in this experiment, and so the images will be inherently more difficult to analyze in our case. Also, the experiments will produce many images, in the order of hundreds, so manual verification would be nearly impossible.

The experiment is divided in three parts: surface preparation, invasion assay, and imaging. The first part involves plating epithelial cells on gels with varying stiffnesses and on PDMS membranes that will be stretched. For the substrate stiffness experiment, soft, medium, and stiff gels are formulated in 6-well plates. The cells are plated on top of these gels and then inoculated with *T. gondii*. For the cell stretching experiment, epithelial cells are plated onto

PDMS membranes, and the membranes are subjected to two hours of cyclic uniaxial stretch through a stretching device and then plated with burp the parasite. After both aspects are complete, the automated or semi-automated segmentation procedure is applied to all images taken after the invasion assay.

Step one is to create a ground truth dataset for each type of stained image: DAPI (labels cell nuclei), FITC (labels parasites green), and TRITC (labels parasites red). The dataset will train an object classifier to detect and label both parasites and cells. The results of the classifier will be manually confirmed for a subset of the images to verify accuracy. If the accuracy does not reach a threshold value, the workflow will be modified with the software's parameters such as threshold intensity, object size exclusion, and more. We hope that this workflow will provide insight into the poorly understood mechanism of *Toxoplasma gondii* invasion in human hosts as well as improve upon existing segmentation methods.

The American Meat Industry: Promoting Consumption while Promising Sustainability

In the U.S. how has the meat industry promoted and sustained meat-intensive diets, and limited the extent of regulation to which it is subject, while striving to build a reputation for sustainability?

In the United States, an average adult eats 50 grams of protein per day (SCL Health, 2019). For a population of nearly 330 million people in the US, this means at least 16.5 billion grams of protein are consumed daily. To promote demand and to expand supply, meat industries engaged in livestock overproduction, a dangerous, environmentally damaging, and unsustainable practice supported by government subsidies and competition (Rust et al., 2020). Here, livestock overproduction refers to farmers using large-scale breeding techniques to optimize livestock

reproduction rates. Livestock contribute to ammonia and methane emissions, which can lead to the acidification of ecosystems (Dopelt et al, 2019).

Participants include meat-eating Americans, meat packing companies and providers, and the US Environmental Protection Agency (EPA). An Ipsos survey polled a wide range of Americans to determine meat-consumption patterns in the US (Berg & Jackson, 2021). The survey found that 59% of the population considers eating red-meat an "American way of life," and 52% percent sees eradicating red-meat consumption as an attempt "to control what Americans eat." According to Reuters, four companies control the US beef industry today: Cargill, Tyson Foods Inc, JBS SA, and National Beef Packing Co (Reuters, 2021). On its "Sustainability" page, Tyson's Food Inc. claims "three key ambitions: empowering people, customers, and community, conserving natural resources and protecting our planet, [and] innovating smart, responsible agriculture" (Tyson Foods, n.d.). Finally, under the EPA page for "Meat and Poultry Products Effluent Guidelines," a supporting document sets the standards for facilities that slaughter and process poultry (US EPA, 2015).

Participants also include environmental sustainability advocacies like Good Food Institute. To promote sustainability, GFI's director of policy, Jessica Almy, recommends substituting plant-based proteins for meat (Ipsos, 2018). Earth's resources are finite. Almy warns: "Either the world is going to have to cut back dramatically" on meat consumption "or we are going to have to find a way to produce meat" independent of animals. Researchers are working to create plant-based products that mimic meat. If they succeed, meat eaters may forego real meat without difficult dietary adjustments. Beyond Meat is a company seeking to develop plant-based products that are healthier, better for the environment, and unimplicated in animals' suffering. It claims its products generate "90% fewer Greenhouse Gas Emissions" than their

quarter-pound U.S. beef counterparts (Beyond Meat, 2021). If a giant meat company, such as Tyson's Food Inc., follows in Beyond Meat's footsteps, the movement toward more sustainable diets may accelerate.

Researchers have found that worldwide, "820 million people have insufficient food and…global food production is the largest pressure caused by humans on Earth, threatening local ecosystems," (Willett, 2019). The pollution effects of meat production are impossible to measure precisely, in part because the pollutants can seldom be reliably traced to their widely scattered resources. Additionally, farms across the country are plentiful and scattered. Because regulating and inspecting food producers is expensive and labor intensive, farms are under little direct pressure to comply with safe animal harvest standards (Treich, 2021). Because trends in developing countries often follow U.S. trends, efforts to reduce meat overconsumption in the U.S. must accelerate. Using science to their advantage, biomedical engineers are applying "antibody protein therapeutics, cell-based therapies, and regenerative medicine" to food production to yield tissue-engineered meat without the slaughter of actual animals. Lab-produced meat can relieve the environmental strain that meat consumption imposes and promote human health. Poore and Nemecek contend that "dietary change can deliver environmental benefits on a scale not achievable by producers," (qtd. in Specht et al., 2018). Lab-produced meat appears to offer relief without difficult social change. Yet, social change is also necessary.

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