Introduction
- As part of our adaptive immune system, CD4+ T cells differentiate into specific phenotypes based on type of infection detected
- Correct phenotype is important to fighting infections, as phenotypes determine the defense mounted by these cells
- Traditionally thought to specialize based on signals from other cells (antigen-presenting cells — APCs) and remain differentiated
- Still, recent research suggests “swarm-like” behavior among CD4+ T cells and plasticity and variability in their phenotypes

Objectives
The primary objective is to investigate whether CD4+ T cells exhibit “swarm-like” behavior (i.e., collective decision-making) rather than specializing into a particular subset based on APC signaling. In addition, this research will also seek to investigate CD4+ T cell flexibility and plasticity and their influence on the efficacy of decision-making.

Methods
- Isolate naïve CD4+ T cells from mice spleens
- Perform polarization protocol to differentiate T cells into two phenotypes — Th1 and Th2
- Visualize CD4+ T cells using confocal microscopy
- Analyze photos of differentiated CD4+ T cells over time to observe their interactions and responses to APCs

Results (Summer 2018)
- After isolation and polarization of the cells into a particular phenotype (Th1), cells were imaged using confocal microscopy
- Yellow fluorescence indicates cells differentiated into the Th1 phenotype
- Red fluorescence indicates cell death.
- Cells with neither yellow nor red fluorescence are alive but not Th1 differentiated
- Graphs depict the successful isolated of CD4+ T cells from mouse spleens
- Analysis of purity using flow cytometry indicates 96% purity, confirming protocol’s efficacy
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- Analysis of purity using flow cytometry indicates 96% purity, confirming protocol’s efficacy

Discussion
- Differentiated (fluorescent) cells after 18 hours provide evidence suggesting successful polarization
- Use of images from confocal microscopy also allow for clear distinction between different cells and their fluorescence
- Results are indicative that confocal microscopy may continue to be a useful tool for tracking cells and their changes in phenotype

Moving Forward
- Will begin to use MatLab to process images and track cell movement and clustering
- Will introduce APCs into the experimental design
- Will examine impact of APCs on T cell phenotype using confocal microscopy
- In observing T cell responses to APCs polarized towards the opposite phenotype, we can examine whether the T cells can collectively resist change in phenotype or adjust according to APCs signal

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Reference