Object Detection for C. elegans Population Analysis

Matthew Adner 11th Grade

The goal of this study was to reduce the amount of time and effort required to observe and record the various developmental stages of C. elegans. Two supervised machine learning solutions to this problem were compared: Apple Core ML and the YOLOv5 Object Detection system. These models were provided with identical training and testing data sets consisting of images of wild type C. elegans worms at various developmental stages gathered by a microscope mounted camera. The machine learning platforms were asked to detect, count, and categorize the worms at various developmental stages. The Apple Core ML model (originally reported by this author at AAAS/AJAS 2020) had a loss of 0.98 after 12,000 iterations and was capable of detecting 80% of the worms in the testing data set. This slightly outperformed the manual efforts of a moderately experienced C. elegans researcher. The YOLOv5 Object Detection system, run using Google Colaboratory's GPU runtime, had a loss of 0.02 and was able to identify 94% of the worms it was shown in the testing data set. The YOLOv5 model was more transparent in its learning process, showing the change in weights of the neural network over epochs, but required a substantial amount of coding expertise. Apple CoreML provides the user with a graphical user interface, but the machine learning process is obscured. The Apple CoreML model took just under five hours to finish training, while the YOLOv5 model took less than two hours. It was found that the YOLOv5 system has more potential for a custom-tailored machine learning model, which could allow researchers to adjust the algorithm in specific and deliberate ways in which an easier to use platform like Apple CoreML cannot adjust. The interface of the YOLOv5 Object Detection system could be made more intuitive for the C. elegans researcher to use through the development of a desktop or web application that would remove much of the current technical complications. If this algorithm could be reliably implemented, it could automate visual observations and substantially reduce the extensive labor of C. elegans researchers.

SARS-CoV-2 Infection in the Zoonotic Reservoir of MERS-CoV

Trace Barnhart 12th Grade

The goal of this study was to better understand the similarities and differences in the viral entry of the coronaviruses responsible for Middle Eastern Respiratory Syndrome (MERS) and COVID-19. The known zoonotic reservoir of MERS-CoV (the MERS virus) is the dromedary camel (C. dromedarius). MERS-CoV has been found to enter cells via interactions between the viral spike protein and the DPP4 receptor. The zoonotic reservoir of SARS-CoV-2 (the COVID-19 virus) is unknown. SARS-CoV-2 cell entry is mediated by interactions between the viral spike protein and the ACE2 receptor. In their March 19, 2020 publication in Biochemical and Biophysical Research Communications, Junwen Luan and coworkers reported that C. dromedarius ACE2 is unable to bind with the SARS-CoV-2 spike protein. This was solely based on their analysis of the protein sequence of the receptor binding domain. To further understand the mechanism of this binding prevention and provide a potential path towards COVID-19 mitigation, we calculated and compared the binding affinity of the ACE2 of H. sapiens (humans) and C. dromedarius to the SARS-CoV-2 spike protein. This began with the acquisition of folded models of each of the proteins. As the folded structure of C. dromedarius ACE2 has yet to be experimentally determined, it was calculated from its amino acid sequence using the server Robetta. The two ACE2 sequences were aligned, then docked with the spike protein. These docked models were analyzed thermodynamically to determine binding affinity and strength of binding. The ACE2 of C. dromedarius has a Gibbs free energy of binding (ΔG) of -11.9 kcal/mol with the spike protein of SARS-CoV-2, whereas the ACE2 of *H. sapiens* has a ΔG of -15.8 kcal/mol. This suggests that the binding of the spike protein to the ACE2 of camels is not only possible, but it is more favorable than to the ACE2 receptor of humans. The same reaction also had a lower dissociation constant with the ACE2 of C. dromedarius than with the ACE2 of H. sapiens, suggesting that at a given concentration of spike protein, more camel will be bound than human. No part of the binding process prevents the ACE2 of *C. dromedarius* from binding to the SARS-CoV-2 spike protein.

Computational Analysis of Llama Nanobodies as Therapeutics for SARS and COVID-19

Eden Anne Bauer 11th Grade

SARS-CoV-2 causes the pandemic disease COVID-19. One treatment in development uses the variable domain of heavy chain camelid antibodies, known as nanobodies. Nanobodies can bind to and inhibit viruses on their own, despite their small size. This study explored the structural interactions underlying the potential use of Lama glama (Ilama) nanobodies in the development of COVID-19 therapeutics. Protein modeling was used to reveal how nanobodies might inhibit the binding of SARS-CoV-2 and SARS-CoV spike proteins to human ACE2 receptors and compare that inhibition to the efficacy of the nanobodies in llamas themselves. The ACE2 receptors of the related alpaca (Vicugna pacos) were used in place of llama ACE2 receptors as neither the llama proteome nor the llama genome has been sequenced. A folded model of the alpaca ACE2 receptor was calculated by Robetta, using the human ACE2 as a template. Both SARS-CoV and SARS-CoV-2 spike proteins were docked with human and alpaca ACE2, and the Gibbs free energies (Δ Gs) of binding and dissociation constants (K_ds) were compared to those of llama nanobody bound SARS-CoV and SARS-CoV-2 spike proteins. Almost all of the nanobody bound viral spike protein combinations of the SARS-CoV and SARS-CoV-2 spike glycoproteins, human ACE2 receptor and alpaca ACE2 receptor, and VHH-72 and H11-D4 llama nanobodies had lower ΔG values than those unbound by nanobodies, except the H11-D4 nanobody bound SARS-CoV-2. The H11-D4 nanobody bound SARS-CoV-2 spike protein bound to alpaca ACE2 had a slightly higher ΔG of binding than the structure without the nanobody, indicating that the binding of the nanobody inhibits the interaction of the SARS-CoV-2 spike protein with the alpaca ACE2 receptor structure. The other nanobody bound spike proteins had slightly higher Kd's and lower AGs of binding than the unbound spike proteins. These results indicate that the nanobodies caused the coronavirus spikes to be slightly more likely to bind to and bind more tightly to the ACE2 receptors. As there is in vitro evidence of nanobodies preventing infection, these findings suggest that the methods utilized in this experiment did not effectively model how these nanobodies function. Further in vitro experimentation, or more powerful in silico analysis, will be needed to fully understand how nanobodies prevent viral entry. The results of this study suggest that the underlying structural mechanism of nanobody inhibition of coronavirus viral entry is more complex than the simple interruption of the interactions between the spike protein and the ACE2 receptor.

Optical Engineering with Economical 3D Printed Components

Joseph Blackburn 11th Grade

Optical systems used for research and education require high-precision mechanical and optical components. For high school education and STEM research, as well as for amateur enthusiasts. access to optical equipment is often severely limited by the associated cost of optical components. This financial barrier has motivated low-cost alternatives, ranging from the use of LEGO blocks to custom-designed plastic mounts. This was the context of this author's work titled "Creating an Economical Interferometer Through CAD Engineering" (AAAS/AJAS 2020). A Michelson interferometer was implemented to demonstrate the feasibility of replacing conventional optomechanical components with 3D printed alternatives. The continuation of this project was primarily focused on two goals: 1) replace costly kinematic mounts with 3D printed parts without compromising precision, and 2) custom design 3D printed adapters to incorporate less expensive second-hand optical components seamlessly into the optical system. Mechanical deficiencies of existing designs were addressed, including improvements of the mechanical stability and precision of optical mounts. The newly designed kinematic mount can tilt mirrors in two axes, which is pivotal for the alignment of the interferometer. For all optical components, including mirrors, lenses, beam splitter, and laser diode, second-hand and low-cost alternatives were selected, keeping the total cost of the optical system below 50 USD. All printed mechanical parts were designed with Fusion 360 and Cura. These designs were printed with a Lulzbot Taz 6 Pro 3D printer. All parts were mounted on an optical table. The kinematic mirror mount was designed as an assembly of three 3D printed parts: the base plate, mirror mount, and adjustable stage. The mount is designed to be manually tilted relative to the optical table. The mount itself is pressed against the mirror holder by a set of springs and adjusted by two knurled thumbscrews. A 3D printed hemisphere forms the pivot for the tilt in both axes. The function of the kinematic mount was assessed qualitatively by the ease with which the interferometer could be aligned to observe interference fringes. The successful implementation of a functional optical system exclusively built with 3D printed parts and low-cost optical components suggests the possibility to implement optical systems considerably more complex than a generic interferometer. Current efforts investigate the long-term stability of the system by measuring the time until the system must be realigned to observe interference.

Using DNA Sequencing to Analyze Genetic Diversity in Cypripedium reginae

Jane Cowie 9th Grade

The showy lady's slipper orchids (Cypripedium reginae) are critically endangered in much of the northeastern United States. Increased genetic diversity correlates with an organism having a more sustainable population through the potential of the population to adapt to a changing environment. Due to the endangered status of Cyp. reginae, genetic diversity in a local population would indicate that the lady's slippers are thriving and that some of the population will likely be maintained within that location, even with potential external stresses. Cyp. reginae can reproduce by cloning or through seed production in fens, which have the moist soil that lady's slippers need. When the lady's slippers reproduce through cloning, those plants are all the same genetically. In the summer of 2019, our lab tested microsatellite primers developed for another Cypripedium species on Cyp. reginae. Microsatellites are genomic regions of DNA repeats, which tend to accumulate mutations. Such regions are useful for genetic analysis because they may show sequence differences even between closely-related individuals and increasing differences in more distantly-related individuals. This experiment extends the progress made in 2019, using the successful primers from that study on DNA extracted from samples harvested in the Eshqua Bog in Woodstock, Vermont in June 2020. Leaf samples were collected from three plants in each of three clusters in order to compare genetic diversity of closer and more distant plants. DNA was extracted from each of the nine samples and amplified with both microsatellite primer sets shown previously to work with Cyp. reginae. The resulting DNA was checked by gel electrophoresis and then sent to an external lab for sequencing. The sequences received were compared to each other to determine whether individuals were possibly identical or genetically diverse. The sequencing chromatograms were also analyzed for heterozygous positions in the DNA that could be compared between individuals. Based on our data, there are mutations present within and between clusters of plants within the Eshgua Bog, indicating a level of genetic diversity in the population, rather than the plants being largely clones. More work will need to be done to confirm the mutations observed here and to investigate more genomic regions as well as a larger sample size of plants. However, our preliminary work to assess genetic diversity in the Eshgua Bog indicates that the plants will continue to thrive in that fen.

Imaging with Active Illumination

Molly DellaValla 9th Grade

An imaging system with active illumination consists of a camera and a dedicated light source to acquire one or more images of an object and extract information otherwise not accessible. This work describes the hardware of a low-cost camera system to record images with active illumination. The system is developed with the long-term perspective to support projects conducted at the STEM Lab of the New Hampshire Academy of Science (NHAS) in the context of environmental monitoring and microscopy. The system can either be operated in multi-spectral imaging mode, where a series of frames with different source colors are recorded, or in a structured illumination mode, where the angle of the incident light is changed between frames. The system consists of a RaspberryPi with a 5MP NOIR camera for recording images, and a 16 Red Green Blue (RGB) Neopixel array arranged in a ring around the camera lens. Brightness and color of each Light Emitting Diode (LED) is controlled independently with an Arduino microcontroller via a serial connection. The system was tested with two model objects. Model 1 consisted of a set of color filters taped to a piece of white cardstock to test the ability of the camera to separate color information. Model 2 consisted of white acrylic paint on a piece of cardstock to demonstrate the response of the camera to surface texture. Photos of the two models were recorded in a dark room to avoid any interference of ambient light with the active illumination. While the targeted extraction of object information via image processing was outside the scope of the project, the basic function of the camera system was confirmed. The results obtained with model 1 indicate that the three RGB colors provided by the Neopixel LEDs and the color channels of the camera are sufficiently different to identify 7 distinct color channels for the overall system. Images obtained with model 2 show a brightness change as a function of the LED location which can be used to extract the surface gradient. The basic function of the active illumination system was evaluated qualitatively by extracting color and texture information of the object not present in pictures recorded with ambient light only. Ongoing developments include the integration of the camera and the active illumination with a microscope setup, as well as the automation of image acquisition and processing.

The Effect of The Wilder Dam on the Health of the Connecticut River

Kai Doron 10th Grade

Dams, such as the Wilder Dam which spans the gap between West Lebanon, NH, and Wilder, VT on the Connecticut River, often have negative effects on the surrounding ecosystems. Dams cause water stagnation leading to temperature fluctuation, eutrophication, and increased bacterial growth. The impact of these effects on the Connecticut River near Wilder Dam was investigated by comparing the quality of the water upstream, at, and downstream of the Wilder Dam. The concentrations of dissolved oxygen, ammonia, phosphate, microplastics, coliform bacteria, and total dissolved solids were measured along with temperature and pH over the course of three days in July 2020. The data collected indicates that the average water temperature increases by 1°C as the Connecticut River approaches the Wilder Dam, and the location of the dam correlates with a spike in coliform growth by 250 CFU/100 mL. The concentration of microplastics in the size range of 0.075 mm to 0.355 mm was determined to be 3.25 ppm upstream of the dam, 3.65 ppm at the dam, and 3.15 ppm downstream of the dam. The Wilder Dam appeared to have no consistent impact on the other test parameters, as they were all within a standard, healthy range for recreational surface water. However, it is possible one may have emerged with increased testing. The changes seen indicate that the Wilder Dam could be damaging the surrounding ecosystem through the elevation of water temperature, microplastic concentrations, and coliform bacteria counts. All of these could have a detrimental effect on the local ecosystem.

Testing the Detection Limits of GMOs Through PCR and Gel Electrophoresis

Anika Eastman 12th grade

Legislation regarding the labeling of genetically modified organisms (GMOs) has been introduced only recently, around 2016. This legislation placed regulations on what determines whether a food contains GMOs, and how the presence of GMOs would be disclosed to the consumer. Specifically, the USDA requires food with more than 5% GMO content to be labeled as bioengineered. This project aimed to test the detection limits of GMOs using Polymerase Chain Reaction (PCR) followed by gel electrophoresis, and to determine if the 5% labeling limit outlined by US legislation was arbitrary or based on a reasonable detection limit. Given that current detection limits determined by agencies such as the NIH are as low as 0.1% with advanced PCR techniques, this project tests the hypothesis that, even with traditional PCR and gel electrophoresis methods, samples with less than 5% GMO DNA will be detectable. The experiments conducted utilized three main steps: DNA extraction, PCR, and gel electrophoresis. GMO and non-GMO corn and sov leaf samples were obtained and verified using PCR and gel electrophoresis. These corn and soy leaf samples were mixed to produce varying percentages of GMO content, ranging from 1.25% to 25%, and DNA was extracted from these mixtures. PCR was used to amplify the DNA using four different primer sets, two sets specific to GMOs and two control sets that amplify plant DNA. Gel electrophoresis was then used to analyze results. Initial testing indicated GMOs were only detectable at 25% and 10% concentrations. However, control tests showed evidence that an element in the reaction had been contaminated with GMO DNA. Continued testing and the replacement of the primers and molecular grade water used in the PCR reactions resolved this issue. Further testing showed evidence that GMO content is detectable in 5%, 2.5%, and 1.25% concentrations in the corn variety tested, but the soy variety showed weaker evidence of the GMO DNA being detected below a 5% concentration. Despite the variability in these results, the data suggests that the 5% cutoff limit can be refined without loss of acuity using a simple PCR and gel electrophoresis assay.

A Conservational and Thermodynamic Study of DAF-16/FOXO

James Eiler 12th Grade

FOXO is a transcription factor in the human insulin signaling pathway that influences diabetes, cancer, and life expectancy. DAF-16 is the C. elegans ortholog of human FOXO. The FOXO protein binds to promoters pivotal to the IGF-1/insulin signaling pathway. The DAF-16 protein is similarly important to the insulin-like signaling pathway. There are several paralogs of FOXO proteins in humans. In C. elegans, there are several isoforms of DAF-16. Isoforms are similar proteins produced by the same gene. This investigation analyzed the protein sequence conservation of DAF-16 and FOXO and thermodynamic binding affinities of two different isoforms of DAF-16 (DAF-16a and DAF-16h) and two paralogs of FOXO (FOXO1 and FOXO3) for their promoters. The conservational study reveals the importance of DAF-16/FOXO across lifeforms. The thermodynamic study reveals the specificity of the different isoforms/paralogs of DAF-16/FOXO. The protein sequences of DAF-16 in C. elegans and FOXO in humans, along with the orthologous proteins in six other species (zebrafish, bovine, pig, chicken, African clawed frog, and rat), were compared using Clustal Omega. While the whole sequence percent identities between DAF-16a, DAF-16h, FOXO1, and FOXO3 ranged from 30 to 37%, indicating high variance, the binding domains were well conserved. Comparisons of the 6 other species also showed a lack of similarity in the non-binding areas and higher similarity in the binding domains. Conservation of the binding domain sequences suggests those areas are crucial to the function of the protein whereas the remainders of the proteins are less crucial to the conserved function. The server Robetta was used to calculate the DAF-16 structures from protein sequences, as those structures have yet to be experimentally determined. The binding affinities of DAF-16a and DAF-16h, and FOXO1 and FOXO3, to their promoters, were determined by preDBA. The Gibbs free energies of binding were very similar: FOXO3 was -12.9kcal/mol, FOXO1 was -14.0kcal/mol; DAF-16a was -18.7kcal/mol, and DAF-16h was -14.3kcal/mol. This suggests that the isoforms of DAF-16 and the paralogs of FOXO are somewhat interchangeable in function and that the small differences between their binding domains do not have a substantial impact on that interaction. The consistency among the binding affinities of the isoforms and paralogs suggests that only the binding domains have a considerable impact on binding affinity and that the different isoforms and paralogs can fulfill similar functions.

Residual Effects of the Elizabeth Mine on the West Branch of the Ompompanoosuc River

Xia Gillespie 12th Grade

The Elizabeth Mine, in South Strafford, VT, was a copper and copperas mine in the 19th and 20th centuries. After closing in 1958, it was found that acid mine drainage leaked into the West Branch of the Ompompanoosuc River (OR) via Copperas and Lord Brooks. This continued until Elizabeth Mine was classified as a Superfund site in 2001. This investigation explored the health and recovery of the aquatic ecosystem through evaluation of dissolved oxygen (DO), ammonia, phosphates, copper, iron, total dissolved solids (TDS), conductivity, salinity, pH, and turbidity. Macroinvertebrates were used as an indicator of the effect of pollution on the ecosystem. Nine locations were tested, including Lord and Copperas Brooks, as well as a drainage site and the stream above it. In all test locations, ammonia levels were below the limit of detection (LOD) of 0.25 ppm, phosphate levels were below the LOD of 0.1 ppm, and DO levels were above the standard for healthy aquatic life. Levels for all water quality tests were similar for all OR test locations. The average conductivity for the OR, above the drainage site, and Lord Brook were all below harmful levels. Both Copperas Brook and the drainage site had average conductivity levels above the standard for aquatic life with 1408±12 µS/cm for Copperas Brook and 940±8 µS/cm for the drainage site. Results were similar for TDS and salinity, with the OR test locations, above the drainage site, and Lord Brook averaging below harmful levels. The average TDS and salinity readings were above safe levels for freshwater at Copperas Brook and the drainage site. Copperas Brook had an average TDS reading of 982±8 ppm and salinity of 0.667±0.005 ppt. The drainage site averaged 664±6 ppm for TDS and 0.400±0.003 ppt for salinity. The pH of the OR locations was slightly high, averaging 8.85±0.02. The most acidic pH reading of 5.75±0.01 came from the drainage site. Copper concentrations in the OR, above the drainage site, and in Copperas Brook were below the 0.1 ppm LOD, and minimal iron was detected. The drainage site was found to have toxic copper concentrations averaging 0.367±0.04 ppm, and harmful iron concentrations, averaging 78.1±1.1 ppm. These results correlate with the macroinvertebrates gathered. While the OR had both mayflies and stoneflies (species with little tolerance to pollution), Lord Brook only had mayflies, and neither Copperas Brook nor the drainage site appeared to have mayflies or stoneflies. When compared to data collected by the EPA, these results show improvement in the overall quality of the OR.

Development of an Experimental Platform for Recording Multispectral Images

Sarah Hall 10th Grade

Multispectral imaging systems record images of objects or scenes for a set of different colors. As compared to conventional cameras that record only the red, green, and blue colors (RGB), they typically record many separate optical wavelength bands spanning from the ultraviolet to the near infrared. Multispectral imaging is used to identify material properties not accessible with standard monochrome or RGB cameras. Important applications include monitoring the health of plants as well as food safety. The high cost of commercial camera systems prohibits their utilization in high school STEM education and research. The goal of this study is to develop a low-cost, experimental platform for recording multispectral images. In this project, a NOIR Raspberry Pi camera was used to record images. The RGB channels of the camera were combined to emulate a monochrome camera. To record a set of color images, consumer-grade photographic filters of various colors were, in turn, placed in front of the camera lens during the recording of individual frames. The test object was a set of light-emitting diodes (LEDs), each with a different wavelength spectrum ranging from the ultraviolet to the infrared. Pivotal for a successful postprocessing of the recorded images is the mechanical stability of the camera system. To minimize the relative movement of the camera and the object during the recording of all color frames, the mounts for the camera and filters were mechanically separated. The data measured with this system consisted of a set of gray images, each representing the object at a different color. Image postprocessing was performed with the open-source program for image analysis, ImageJ. The basic function of the camera system was demonstrated and confirmed by combining several color frames to highlight individual LEDs. This included a systematic characterization of the camera system by inspecting the responses of all LEDs for all the color filters. The successful development of a low-cost camera system for multispectral imaging will help to introduce this imaging modality to high school STEM education. The experimental platform developed in this project will be used for future projects in the environmental sciences conducted at the New Hampshire Academy of Science STEM Lab. Ongoing work is aimed at a quantitative characterization of the camera system, the automation of image acquisition, and the development of a 3D printed housing with improved sturdiness.

Effects of Cannabidiol Oil on Serotonin Levels of Caenorhabditis elegans

Esme E. Kimber 9th Grade

Serotonin (5-hydroxytryptamine or 5-HT) is a neurotransmitter that regulates a variety of neurological processes in animals, including humans. The FDA has approved cannabidiol (CBD) as a treatment for severe seizure disorders, substantiated by CBD's ability to block GPR55 receptors that trigger seizures in humans. Despite the FDA limited medical use approval for CBD, there are concerns about the safety and medicinal claims because of its increasing use in the general public. The goal of this research was to add to existing information about CBD and to determine if CBD has a significant effect on serotonin levels. We hypothesized that CBD would decrease serotonin levels in the model organism Caenorhabditis elegans since many cases of epilepsy are caused by heightened serotonin levels and cannabidiol is used to successfully treat epilepsy in humans. In this study, C. elegans were exposed to CBD and then placed in 5-HT. When exposed to a surplus of 5-HT, C. elegans become paralyzed, allowing for an assay of the baseline quantity of serotonin in the worms. If there is more baseline serotonin present, less exogenously added serotonin is needed to instigate paralysis. The C. elegans were exposed to three different concentrations of 5-HT to observe a possible dose response: 60mM, 80mM, and 100mM. The same method was used for a control group of C. elegans exposed to MCT coconut oil, the vehicle in the CBD oil. The C. elegans treated with CBD and exposed to the lowest concentration, 60mM, of 5-HT had similar percentages paralyzed to the MCT exposed worms (Rounds 1, 2, 3 p>0.05, t-test). The C. elegans treated with CBD and exposed to 80mM of 5-HT had significantly different paralysis percentages from the MCT exposed worms, however only in Round 3 (Rounds 1, 2 p>0.05; Round 3 p=0.00235); the CBD worms had lower paralysis percentages. The C. elegans treated with CBD exposed to the highest concentration, 100mM, of 5-HT had similar paralyzed percentages to the MCT exposed worms (Rounds 1, 2, 3 p>0.05). There was no significant difference in paralysis of worms exposed to CBD compared to those exposed to MCT except for Round 3 of the 80mM concentration. This could indicate that either CBD does not have a significant effect on serotonin levels in C. elegans, or the experiment needs to be repeated and modified to further analyze the effects of CBD, possibly by testing different levels. This experiment is important as it explores the credibility of CBD medicinal claims and adds to the existing research on this new drug.

The Behavioral Effects of JUUL Menthol E-Liquid and Its Components on C. elegans

Leanna Kish 12th Grade

E-cigarettes and other electronic nicotine delivery devices have become extremely popular in the past decade due to wide availability, a variety of flavors, and marketing as alternatives to traditional cigarettes. In 2019-2020, the FDA has been adding some regulations on these products, but manufacturers are still not required to list their ingredients. One of the most prolific E-cigarette brands is JUUL. A previous study we conducted investigated the effects of different JUUL flavors on a stress-resistant mutant strain of *C. elegans*. This study aimed to determine the effects of JUUL E-liquid and its individual components on the behavior of wild type C. elegans. We hypothesized that the whole JUUL product would have a greater effect on the behavior of the worms than its components. We used chemotaxis and divided the worm plates into four concentric sections, with the test solution placed in the center. We chose to use chemotaxis to measure the worms' like or dislike of the product because often, if an organism senses something to be harmful to themselves, they will avoid it. We interpreted the worms' avoidance of the product as their dislike for it and worm death was also measured. JUUL menthol and its currently known individual components (benzoic acid, propylene glycol, glycerol, nicotine, and menthol) were tested. A labmade mock JUUL solution was also made using a recipe that was representative of what is found in a JUUL brand pod. The concentrations tested for each were 1%, 25%, 50%, and 100%, made by diluting the substances in sterile tap water. Chemotaxis and survival were monitored for 5 hours. For results, neither benzoic acid, propylene glycol, glycerol, nor menthol noticeably caused the behavior of the C. elegans to deviate from the controls. Our lab-made mock JUUL solution had a similar effect, not varying from the control much. The nicotine solution and the whole JUUL product both had negative effects on the worms in that the worms avoided the area of the plate with the test solution. These data suggest that the undisclosed ingredients used to flavor JUUL pods are having a negative effect on the worms since our lab-made mock JUUL solution did not cause the worms to stray from the center of the plate as much as the official JUUL brand product. However, a confounding result is that the nicotine alone shows a strong negative effect while our lab-made mock JUUL, which contains the same percent of nicotine, does not. Further experimentation is needed to determine if these results are accurate and explain the discrepancy.

Prototype of a Low-Cost Spectrometer for STEM Research

Alexander Low 11th Grade

The goal of this project is to build a low-cost optical spectrometer able to take quantitative measurements over the range of visible wavelengths and be sufficiently accurate for use in demonstration experiments, education, and STEM research. Compared to many similar efforts to implement a community spectrometer, the present project also includes a quantitative characterization of the instrument in terms of its spectral sensitivity and resolution. The evaluation of the instrument and its components will provide the necessary information to improve the design rather than accept the performance limits of the most generic setup. The spectrometer consists of an entrance slit, a diffraction grating, an imaging lens and a CMOS camera sensor. The spectrometer setup is housed in a metal project box with a size of W x H x L = 105mm x 55mm x 150mm. The entrance slit, made of two manually aligned razor blades, was placed at the center of one of the front faces of the box. A 2MP USB camera with a 12mm camera lens attached was placed about 100mm behind the slit to form a sharp image of the slit on the camera sensor. An inexpensive diffraction grating with 1,000 lines/mm was placed directly in front of the camera lens. The grating deflects each wavelength with a different angle. On the camera sensor, this causes a wavelength dependent shift of the slit image, effectively recording the light spectrum in the horizontal direction. The diffraction grating and the camera are placed in a 3D printed mount attached to the base of the project box. The camera is connected to a standard PC to capture and process the spectrum. The cost of materials for the entire spectrometer setup is about 100 USD. A discharge lamp was used to confirm the function of the spectrometer qualitatively and record discrete spectral lines throughout the visible spectrum. The slit width was measured to be 111.5um +/- 1um across the entire length of the slit. The ability to assemble slits manually with sufficient accuracy is a pivotal result to reduce the cost of the instrument, since a commercial spectrometer slit would approximately double the cost for the instrument. The spectrometer developed for this project confirms the potential for building low-cost community spectrometers for STEM research and education. The ongoing quantitative characterization of the instrument is expected to determine the range of potential applications.

Analysis of Microsatellite Regions within the Cyp. reginae of Strafford, VT

Liam Markey 10th Grade

Critically imperiled in New Hampshire and vulnerable in Vermont, Cypripedium reginae, or the showy lady's slipper orchid, has an unknown level of genetic diversity within local populations. Without sufficient diversity, these populations could risk extinction if faced with a new stress. Cyp. reginae reside in a type of wetland known as a fen and can reproduce clonally or through sexual reproduction. In this experiment, two variable regions in the Cyp. reginae genome were amplified using PCR, confirmed using gel electrophoresis, and then sent to an external lab for DNA sequencing. Sequencing results were used to determine the genetic diversity of a population of Cyp. reginae in a fen located in Strafford, VT. Leaf samples were taken from three plants in each of four clusters from the fen, allowing for the comparison of individuals growing close together as well as those growing farther apart. Clusters were at least 3 meters apart from one another. These sequences, amplified from the variable regions, can be compared between individuals to assess whether the population is largely identical or more genetically diverse by examining differences in DNA sequence and the presence of any heterozygous positions in the genomic regions. The resulting sequences were aligned and showed a fair amount of diversity within and between clusters of plants, suggesting the population in the Strafford fen is genetically diverse and may be in a good position to survive environmental stress. However, more research is needed to confirm these results. Future research will test more genomic regions and more individual plants.

Minimizing Microplastics: Using Biofilms to Degrade Microplastic Contamination

Shreya Nagri 12th grade

Microplastics are a growing problem in the world's oceans and the threat is mounting with increases in global plastic waste. Most plastic contamination is caused by polyethylene terephthalate, or PET, which is used in single-use water bottles and containers. The bacteria Ideonella sakaiensis has the ability to break down PET plastic using an enzyme called PETase. The gene that codes for this enzyme has been isolated and engineered into a plasmid that can be transformed into Escherichia coli K12 to allow them to express the enzyme. This study explores the use of biofilms, or collaborative communities of bacteria, in the treatment of microplastic contamination in water. Biofilms are hardier than bacteria not in biofilms and were therefore hypothesized to be more effective in breaking down PET plastic. Trials were conducted using liquid cultures and biofilms of wild type and transformed E. coli K12 to test their efficacy in breaking down PET plastic from a single-use bottle. The area of PET plastic was measured over time using NIH ImageJ software at different time points over the course of the experiments without disturbing the biofilms. Results obtained from ImageJ analysis of a series of pictures show that a PETasetransformed biofilm accelerated the degradation of PET, but future studies are needed to verify this finding. When comparing a biofilm of transformed bacteria and liquid culture of transformed bacteria, results also suggest that the biofilm was more effective. Modeling of the biofilm's growth over time as measured using safranin to visualize the biofilm showed that biofilms form at a regular pace of around 4.167*10⁻⁵ m² per hour. Future work includes repeating the experiments over a longer period of time to observe the long-term degradation of PET when exposed to the PETase bacteria in biofilms and liquid culture.

Genetic Variation in Microsatellite Regions of Cypripedium reginae and parviflorum

Roxane Park 8th Grade

Of the approximately 47 species of the Cypripedium genus that are widely distributed around the world, many are becoming increasingly endangered. We suggest that a central variable for ensuring the preservation of species in this genus is to confirm that the plants have retained enough genetic diversity to survive potential environmental stresses. Genetic diversity was assessed by investigating microsatellite regions of the genome, sections of repetitive DNA where short strings are repeated, typically 5-50 times. We accomplished this by repurposing primers developed by another group for Cypripedium tibeticum. One goal of this study was to test the efficacy of these primers on the Cyp. parviflorum, or yellow lady's slipper, to determine which primers successfully bind to the DNA of Cyp. parviflorum. Primers that amplify regions in the Cyp. parviflorum samples can be used for sequencing to assess genetic diversity among Cyp. parviflorum plants. Previous work from our lab tested many of the Cyp. tibeticum primers on Cyp. reginae, or showy lady's slippers, and found two primer sets that worked well in that species. The other goal of this study was to use those two primer sets to amplify and sequence microsatellite regions from Cyp. reginae samples taken from a fen in Norwich, Vermont to begin to assess genetic diversity in that fen. Because of the extremely small lady's slipper population there, only three samples were collected. For all samples, DNA extraction, PCR, gel electrophoresis, and DNA cleanup procedures were performed. The purified samples were then sent to an external lab for sequencing. Of the ten primer sets tested on Cyp. parviflorum, nine primer sets produced successful results with each tested on two Cyp. parviflorum samples. Of the six Cyp. reginae sequencing reactions performed, only three were successful, which were not enough samples to draw reliable conclusions about genetic diversity of individuals in the Norwich fen. However, many of the pairs of sequences acquired using the same primers on two different Cyp. parviflorum samples were nearly identical. Future work will use all nine primer sets on nine Cyp. parviflorum samples acquired from a single stand in a Strafford, VT forest to assess genetic diversity and will follow up on the Cyp. reginae work to attain more usable sequences, possibly by using additional aenomic regions.

Spike Protein Binding During Viral Entry of MERS-CoV, SARS-CoV, and SARS-CoV-2

Saia Patel 11th Grade

Over the past year, the Severe Acute Respiratory Syndrome Coronavirus 2 [SARS-CoV-2] has caused widespread death globally. Although much has been learned since its emergence. considerably more is left to uncover regarding the mechanism of action of the virus. The aim of this investigation was to illuminate the binding of the ACE-2 and DPP4 human receptors with the spike proteins of the epidemic coronaviruses SARS-CoV, SARS-CoV-2, and MERS-CoV to inform the development of effective therapeutics and vaccines. The binding affinities between the viruses and receptors were compared by computationally docking each of the viruses to each receptor. Modeling and analysis were done using free computational resources including, UCSF Chimera, Clustal Omega, Sublime Text, HADDOCK, and PRODIGY. The results received from Prodigy included the K_d, or the dissociation constant, of all three viruses when paired with both human receptors at 25°C. When paired with ACE-2, the dissociation constants of SARS-CoV, MERS-CoV, and SARS-CoV-2 were found to be 0.019 pM, 0.15 pM, and 5.9 pM, respectively. When paired with DPP4, the dissociation constants of SARS-CoV-2, SARS-CoV, and MERS-CoV were found to be 1.6 nM, 3 nM, and 45 nM, respectively. Through analysis of the calculated binding affinities, it was established that SARS-CoV binds most tightly with ACE-2, while SARS-CoV-2 binds most tightly with DPP4. It was also found that the viruses had lower dissociation constants when binding with ACE-2 compared to binding with DPP4. This suggests that the SARS-CoV, SARS-CoV-2, and MERS-CoV viruses were more easily able to bind with ACE-2 than DPP4. These results are notable in that MERS-CoV has been more closely associated with DPP4 than ACE2 while SARS-CoV-2 has been more closely associated with ACE2 than DPP4. Protein sequence analysis suggests that a large amount of DPP4 binding residues were well conserved across the three viral spike proteins, while a significant amount of ACE-2 interfacing residues are not conserved. These findings suggest that the weaker, well conserved interactions between coronavirus spike proteins and DPP4 may be a better target for general coronavirus therapeutics than the stronger, more variable spike protein-ACE-2 interactions.

Binding of Animal ACE2 Receptors with the SARS-CoV-2 Spike Protein

Sora Shirai 10th Grade

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus responsible for the current COVID-19 pandemic with over 63.3 million confirmed cases and over 1.47 million deaths worldwide as of December 1, 2020. SARS-CoV-2 is currently thought to have a zoonotic origin from intermediate horseshoe bats (Rhinolophus affinis), but the range of zoonotic hosts of SARS-CoV-2 is still unknown. In this study, the known receptor of the SARS-CoV-2 spike glycoprotein, Angiotensin Converting Enzyme 2 (ACE2), from various animals, including humans, was compared. In addition, the binding interactions of the various ACE2 receptors with the SARS-CoV-2 spike protein were compared and analyzed using multiple free web platforms (Chimera, Clustal Omega, HADDOCK 2.4, Prodigy). The animal ACE2 receptors were compared to the human ACE2 receptor through protein sequence analysis, binding pocket characterization, and comparison of the Gibbs free energy of binding. Results showed that the Felis catus (cat) ACE2 protein sequence was most similar to that of the human ACE2 while the Danio rerio (zebrafish) ACE2 was the least similar, with percent identities of 85.69% and 60.69%, respectively. For similarity to the human ACE2 receptor at the binding interface, both Gallus gallus (chicken) and Paguma larvata (masked palm civet) had the highest number of amino acid contacts at the interface in common with the human ACE2. G. gallus had the highest number of interfacial residue interactions that were identical to those seen in the human ACE2/spike protein complex at 46 identical interactions. Both Rhinolophus sinicus (Chinese rufous horseshoe bat) and F. catus have been clinically determined to be susceptible to SARS-CoV-2, and the results from this experiment found that their ACE2 receptors bind to the spike glycoprotein with high affinity demonstrated by their calculated Gibbs free energies of binding of -13.8 kcal/mol and -13.1 kcal/mol, respectively. All other animals tested, excluding D. rerio, were found to have more favorable Gibbs free energies of binding, which could suggest an even higher susceptibility to SARS-CoV-2 than seen in F. catus and R. sinicus.

Computational Analysis of SARS-CoV-2 Spike Glycoprotein Bound to ACE2 and DC-SIGN

Lian Snow 11th Grade

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a strain of coronavirus that emerged in Wuhan, China, in December 2019. As of December 1, 2020, the virus has infected over 63 million people and has killed over 1.4 million worldwide. It is critical to learn as much as possible about SARS-CoV-2 to direct successful vaccine design and development of therapeutics. Research suggests that SARS-CoV-2 binds with the angiotensin converting enzyme 2 (ACE2), a cell membrane receptor and a key component in the renin angiotensin aldosterone system. It is suspected that SARS-CoV-2 uses a variety of other receptors as well, including the dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN, or CD209), a receptor found on dendritic cells that recognizes and binds with a variety of pathogens enabling contact between dendritic cells and T lymphocyte cells. Computational analyses were conducted to determine the Gibbs free energy of binding, measure the binding affinity, and characterize the binding sites involved in the initial binding interaction between the spike glycoprotein of SARS-CoV-2 and DC-SIGN and ACE2. The binding affinity of the spike protein and each receptor was also calculated at 25°C, 37°C, and 40°C to mimic a fever. This was achieved using the UCSF Chimera, Protein Data Bank (PDB), HADDOCK 2.4, Clustal Omega, UniOmegat, PDBePISA, and PRODIGY. Two models of DC-SIGN docked with SARS-CoV-2 were created to show binding variations at the different regions of the carbohydrate recognition domain. The Gibbs free energy of binding at 25°C for ACE2 with the spike protein was found to be -11.9 kcal/mol ACE2, while that of the spike protein with DC-SIGN was -14.1 kcal/mol, for both models. This is likely due to the 30-40 additional interfacial amino acid contacts involved in binding of the spike protein by DC-SIGN compared to SARS-CoV-2. At all temperatures sampled, the binding affinity of DC-SIGN to SARS-CoV-2 spike protein exceeds that of ACE2. In all cases, binding affinity decreased at higher temperatures. This study suggests SARS-CoV-2 may bind more tightly to DC-SIGN than ACE2, emphasizing that the disease mechanism of COVID-19 is varied and complex. The primary function of DC-SIGN is to recognize and bind with pathogens in the body. If DC-SIGN is hindered, the body could have increased potential for co-infection with pathogenic bacteria and viruses, resulting in a decreased immune response.

Generational Thermotolerance in *C. elegans* with Increased Microbiome Diversity

Brynne Spaeth 10th Grade

The gut microbiome has been shown to influence stress in a number of animals. Caenorhabditis elegans (C. elegans) is a microscopic roundworm and a popular model organism. In the lab, the worms are often fed only a single strain of E. coli bacteria, OP50. In their natural habitat, C. elegans eat a wide variety of bacteria found in rotting vegetation. In this investigation, sylvatic nematodes that were morphologically characteristic of C. elegans were isolated from rotting vegetation. Bacteria were isolated from the guts of these sylvatic nematodes. Bacteria isolated from sylvatic nematodes (SB) or OP50 were fed to the sylvatic worms and laboratory (N2 wild type) worms over multiple generations. The impact of microbiome diversity on the C. elegans stress response, with the stressor being heat, was measured by death rate during heat stress over three generations. The results suggest that sylvatic worms in generations 2 and 3 have an increased death rate when fed a diet of OP50 compared to sylvatic worms fed a diet of SB. Correspondingly, the death rate of N2 worms fed SB was less than that of N2 worms fed OP50 in all generations. These results were not significant (p≥0.05) for all three generations. This may be due, at least in part, to the small sample size used in this experiment. Further experimentation with a larger sample size will help determine whether these preliminary data reflect a significant change that the microbiome has on thermotolerance in both sylvatic worms and N2 wild type C. elegans. It will also be helpful to perform species-specific genetic analysis on the sylvatic nematodes to confirm that they are C. elegans.

Does Non-GMO Branded Sweet Corn Contain Genetically Modified DNA?

Anna Tovchigrechko 11th Grade

The primary goal of this study was to determine whether certain processed and unprocessed sweet corn products labelled as non-GMO or organic contained genetically modified content, as evidenced by genetically modified DNA. The secondary goal of this study was to determine whether products labelled neither as non-GMO nor GMO contained genetically modified content. All experiments were performed in a home setting. DNA extraction was used to obtain DNA from multiple samples of sweet corn products labelled as non-GMO, organic, or not labelled, as well as GMO and non-GMO maize plant controls. That DNA was amplified using PCR (polymerase chain reaction) and the presence of control and GMO DNA markers was assessed using gel electrophoresis. After many rounds of PCR and gel electrophoresis, the controls and samples were repeatedly contaminated with genetically modified DNA, making our data unreliable. The results are so far inconclusive due to this contamination, although the contamination shows the differences of working in a home lab versus in a professional setting. The experiment will need to be repeated, preferably in an uncontaminated lab environment, in order to obtain conclusive results to answer the questions posed in this study.

Testing Edamame Products for GMOs Using PCR

Cayden Van Dolah 9th Grade

Genetically modified organisms (GMOs) have DNA that has been synthetically altered in a laboratory so that it contains combinations of genes that do not occur in nature. The goals of this study were to determine whether edamame products marketed as non-GMO contained genetically modified material and to determine the relative amounts of genetically modified material in purportedly non-GMO products using Polymerase Chain Reaction (PCR). PCR was employed to amplify the GMO DNA, if present, and a control non-GMO DNA sequence using two primer sets: chloroplast and 35S. These primers determine the regions to be amplified, with the chloroplast primers amplifying a gene common to all plants and the 35S primers amplifying a promoter commonly used to express genes in GMOs. Three edamame samples from three different vendors were tested. Two of the three edamame products tested were labeled as verified non-GMO,' while the third product was labeled 'organic.' Three of the four tests showed that all of the edamame samples contained GMO DNA. A test with no sample DNA added was performed to determine if the primers or other reagents were contaminated with DNA. They were not, which suggests that all of the samples contained GMO material. Because of this, the second component of the original research plan could not be completed since there was no confirmed non-GMO material. A possible source of error could have occurred when the DNA was extracted from the edamame. It is possible that there was cross-contamination between the edamame products or perhaps through the gloves worn during experimentation. These results suggest that it is very hard to find non-GMO edamame products, even if they are labeled as non-GMO. In the future, I plan to continue this research with the original research plan by testing more samples to ensure a non-GMO sample can be identified.

Evaluating the Impacts of Human Activity on the Mink Brook Watershed of Hanover, NH

David Viazmenski 8th Grade

The purpose of this study was to examine whether the water quality of Mink Brook in Hanover, New Hampshire deteriorates as it flows from its source to its output at the Connecticut River. As Mink Brook drains the largest watershed in Hanover, the quality of its water is critical to the health of the town's ecosystem. The health of the Mink Brook is evaluated every two years through the NH Surface Water Quality Assessment Program. By testing the water quality at four sites along the brook from the source to the output, this study examined the general impact on the brook from human activity due to residences, businesses, and traffic along the brook, and the specific impact of Mink Meadow Farm, through which the Mink Brook flows. To specifically evaluate the impact of Mink Meadow Farm, two of these four sites were chosen directly upstream and downstream of the farm. Over the course of two weeks in July, dissolved oxygen concentration, pH, temperature, salinity, conductivity, the concentration of total dissolved solids, and the concentration of coliform bacteria were measured on six different dates. Comparative analysis of the sites confirmed that the Mink Brook's water quality declines between its source and its output, and directly downstream of Mink Meadow Farm. From the source near Mill Pond to the output into the Connecticut River (a distance of roughly 8 miles), the mean difference in temperature was 6.9°C. the mean difference in conductivity was 795 µS/cm, and the mean difference in concentration of total dissolved solids was 558 ppm. The data collected between the farm sites, which lie an estimated 0.2 miles apart, showed a greater rate of change. The mean difference in conductivity for the farm sites was 62 µS/cm and for total dissolved solids was 40 ppm, indicating that the rate of change within the farm was more than three times higher than the rate of change along the whole waterway. An additional important finding of the data, however, was that the readings at the farm fell within ranges considered safe and allowable. This suggests that although the farm decreases water quality rapidly, farming of this nature, on a small scale in a rural neighborhood, may be tolerable for a brook of this size. This study does confirm a decline in water quality in the Mink Brook from its source to output. The localized decline in water quality due to agriculture validates the need for continued monitoring and stewardship of the Mink Brook to safeguard the local environment from the potentially deleterious impacts of activities and development along the waterway.

Comparing the Binding Interfaces of SARS-CoV-2 and SARS-CoV Complexed with ACE2

Miriam Viazmenski 12th Grade

The novel coronavirus SARS-CoV-2 was first identified in Wuhan, China in December 2019 and has since become responsible for the COVID-19 pandemic. Like the SARS-CoV virus, the virus implicated in the 2003 SARS pandemic, SARS-CoV-2 gains entry into cells via the angiotensin converting enzyme 2 (ACE2) receptor, found on many cell types throughout the body. The receptor binding domains (RBDs) of the spike proteins of the viruses interact with the N-terminal helix of the ACE2 receptor in order to enter cells. Researchers have turned to SARS-CoV to better understand SARS-CoV-2, due to their similarity in structure and function. Although the RBDs of both viruses bind with the ACE2 receptor, there is disagreement in the literature as to which virus binds ACE2 more tightly. This investigation sought to utilize sequence and structure alignment, affinity calculations, and analysis of residue interactions to further understand the similarities and differences between SARS-CoV-2 and SARS-CoV binding with ACE2. A sequence alignment of the RBDs using Clustal Omega showed a sequence identity of 72.78%, and a tertiary structure matchmaker alignment of the RBDs using UCSF Chimera indicated high structural similarity. Using experimentally solved structures of the virus RBDs bound to ACE2, the PRODIGY WebServer was utilized to calculate Gibbs Free Energy (ΔG) of binding, the dissociation constants (Kd), and non-interacting-surface residue percentages at both 37°C and 40°C. These values suggest slightly tighter binding between the RBD of SARS-CoV-2 and ACE2 than between the RBD of SARS-CoV and ACE2, with ΔG values of -11.9 kcal/mol and -10.8 kcal/mol, respectively. Sorting and analysis of the amino acid residues implicated in the interactions between the RBDs of each virus and ACE2 revealed that the largest proportion of ACE2 interactions take place between ACE2 and amino acid residues conserved between SARS-CoV-2 and SARS-CoV. This result provides insight into the degree of similarity in the ACE2 binding of the two viruses. Forty percent of all interactions with ACE2 were found to be with RBD residues that are identical across SARS-CoV-2 and SARS-CoV and 22.4% of interactions were found to occur with RBD residues of the same chemical type across the viruses. ACE2 binding remains a promising target for the development of antibodies and other therapeutics. Knowledge about the immunologic interactions of SARS-CoV can inform directions for further study of SARS-CoV-2.

Adapting a Micronucleus Assay for Use in C. elegans

Luke Young-Xu 12th Grade

Genotoxicity testing is used to evaluate a compound's potential to induce damage to genetic material, often for regulatory purposes. In vivo genotoxicity testing is often more accurate and reliable, but the cost and regulations involved with employing more biologically complex organisms limits its use. The generally cheaper and more efficient in vitro genotoxicity testing employs simpler models that frequently produce higher rates of irrelevant results due to the lack of certain metabolizing pathways and DNA repair responses. If using a simpler organism could produce comparable results to higher eukaryotes in *in vivo* genotoxicity testing, then *in vivo* tests could be conducted more frequently, with larger numbers and lower cost. The objective of this study was to develop a micronucleus assay procedure for use in *C. elegans* by isolating cells from worms and staining them to observe the DNA. The micronucleus test analyzes genotoxicity by evaluating the frequency of micronucleated cells. Micronuclei, small extra-nuclear bodies containing chromatin, form due to improper segregation of chromosomes during mitosis. commonly caused by DNA strand breaks or damage to cellular mechanisms involved in mitosis. Such problems can also lead to the formation of other nuclear abnormalities, such as blebbed nuclei or lobed nuclei. C. elegans is a potentially good candidate for a simpler organism to use in this test owing to their metabolic similarities to human systems and conserved pathways of DNA damage responses. Here, C. elegans were allowed to hatch and develop on agar pretreated with two genotoxic drugs: doxorubicin at 50µM and 25µM and vinblastine at 500nM and 100nM concentrations. Negative control plates used filtered water. Cells were isolated from the worms, stained with the nuclear stain DAPI, and observed under a fluorescence microscope. Exposure to both doxorubicin concentrations and vinblastine at 500nM induced significant increases in the frequency of nuclear abnormalities. Exposure to vinblastine at 500nM was also found to induce significant increases in the frequency of micronucleated cells. These preliminary results suggest that the procedure used successfully stains the nuclei and micronuclei of isolated C. elegans cells and thus can be used to identify micronuclei and other nuclear abnormalities to analyze the genotoxicity of target compounds. More research into increasing cell yield and reducing background noise for imaging cells is necessary, however, to develop a procedure that could reliably produce accurate and statistically significant results.