

Supplementary information for:

A low-cost smartphone fluorescence microscope for research, life science education, and STEM outreach

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1. Supplementary Figures

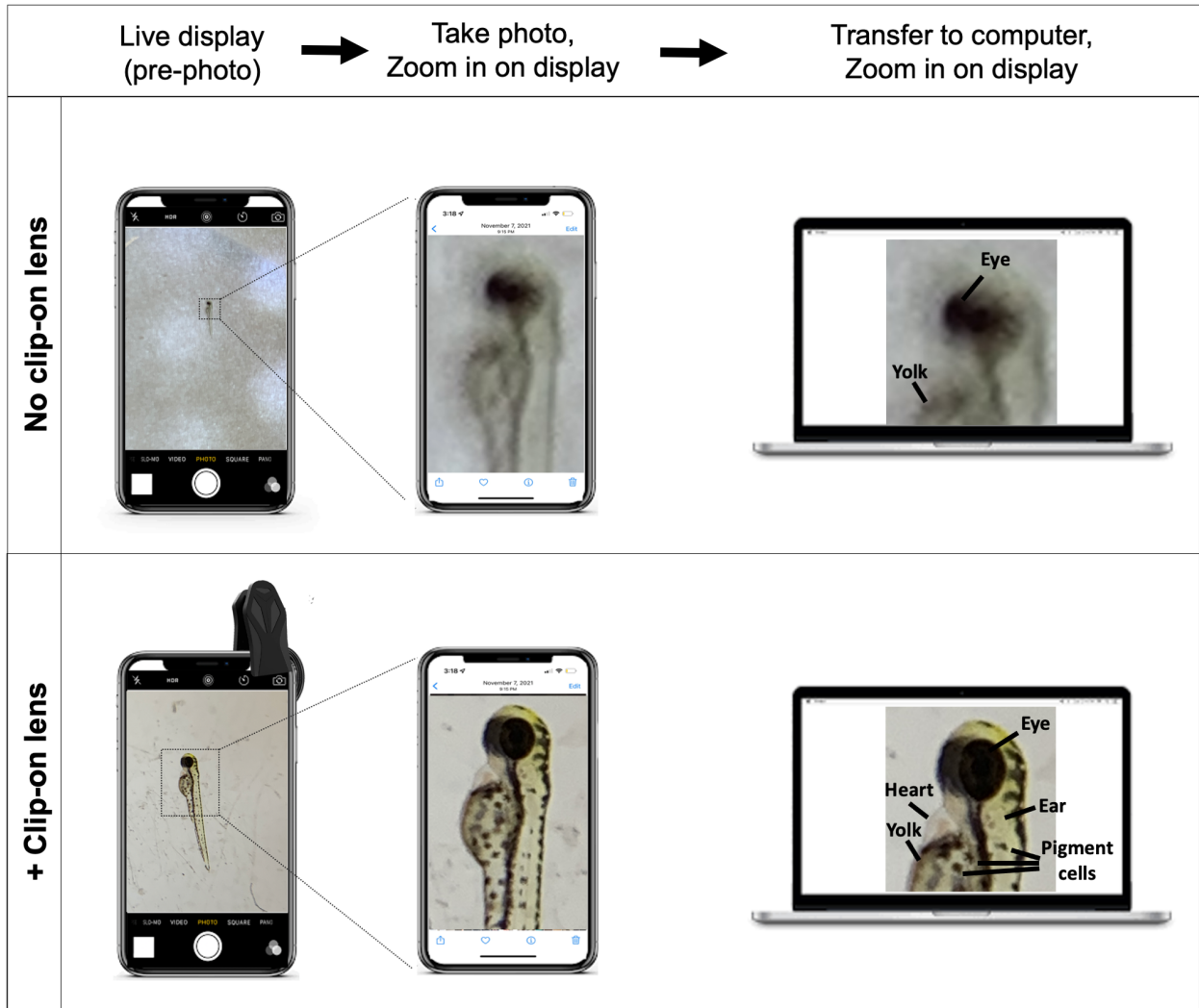


Figure S1. Photo capture and image stretching provides further magnification and anatomical detail. All images show 3 dpf zebrafish embryos (lateral view). Upper and lower images show the effect of taking and zooming in on photos with and without the clip-on lens, respectively. Note the benefits of digital magnification (image stretching) after image capture were most beneficial when the clip-on lens is used. Images were acquired using an Apple iPhone 12 Pro with 1x (middle magnification) lens, 6x digital zoom.

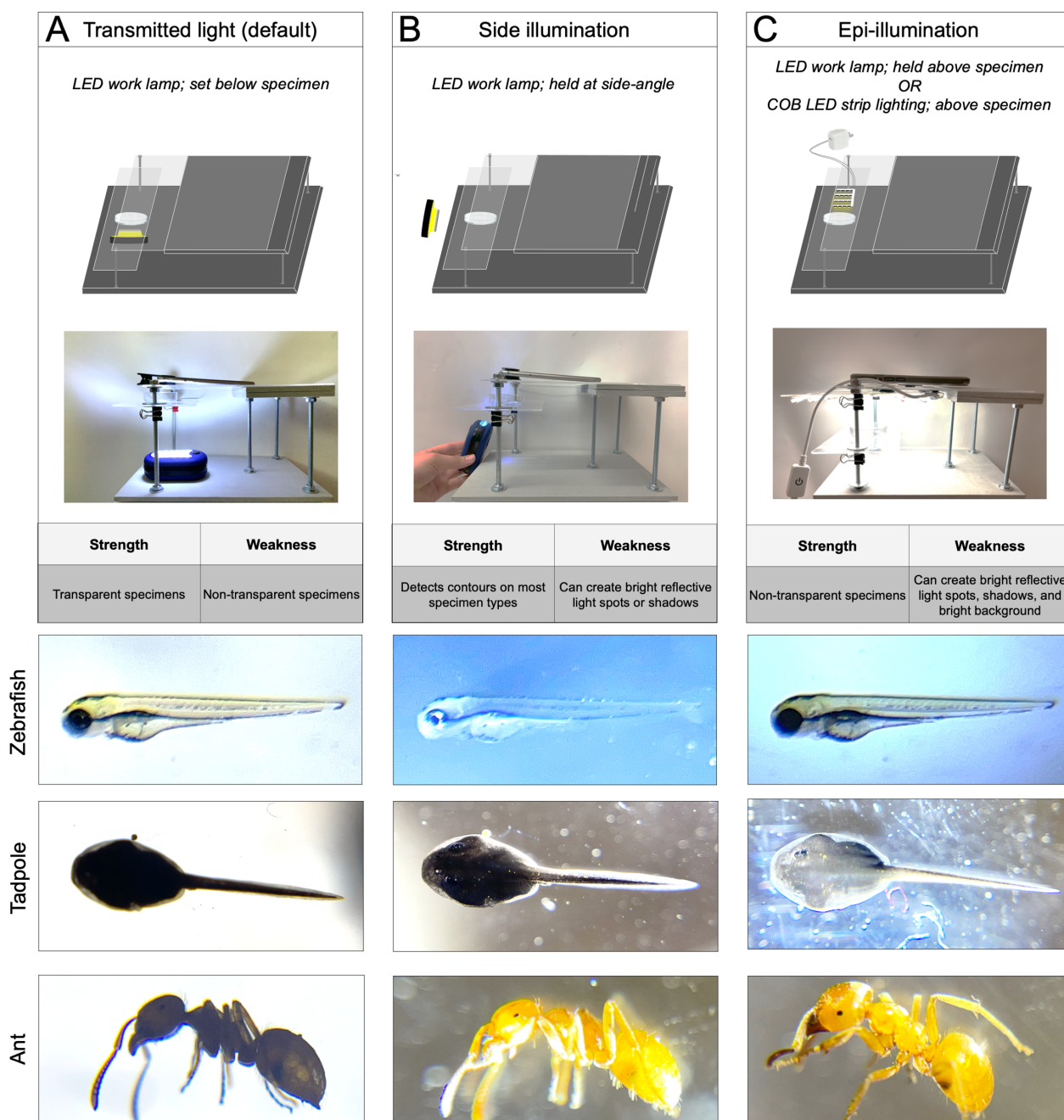
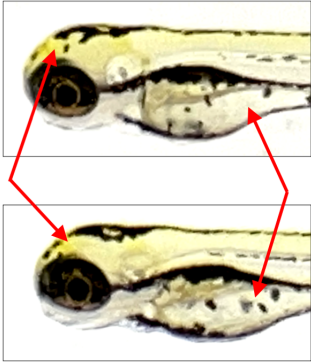


Figure S2. Optimized lighting for non-fluorescent viewing. Upper panels show the configuration, strengths, and weaknesses for (a) transmitted light, (b) side illumination, and (c) epi-illumination. Lower representative images show the same specimen viewed using each of the three lighting methods. Images shown were acquired using an Apple iPhone 12 Pro, 1x (middle magnification) lens, 6x digital zoom, with the additional clip-on macro lens.

A

Next Generation Science Standards, Grade 1:	Individuals of the same kind of plant or animal are recognizable as similar but can also vary in many ways (1 – LS3-1; LS3.B)
Student learning activity:	Students use glowscopes (non-fluorescent) to observe differences between zebrafish larvae (siblings)

Skin pigmentation



Body length



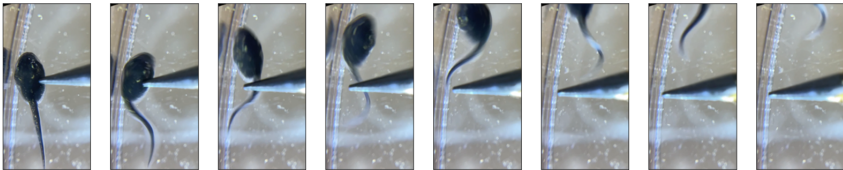
Student table: observations

<u>Similarities</u>	<u>Differences</u>
Eye size	Body length
Ear size	Pigmentation
Head size	

B

Next Generation Science Standards, Grade 1:	Adult plants and animals can have young. In many kinds of animals, parents and the offspring themselves engage in behaviors that help the offspring to survive. (1–LS1-1, 1-LS1-2; LS1.B)
Student learning activity:	Students use a pencil to gently touch zebrafish larvae, frog tadpoles, or caterpillars. While doing so, students observe touch-escape response behaviors that help offspring to survive.

Tadpole



Caterpillar



Student observation tables:

<u>Behaviors (tadpole)</u>
Touch reflex (respond to outside world)
Swim away from predator (avoid being eaten)

<u>Behaviors (caterpillar)</u>
Crawl away from predator (avoid being eaten)
Coil up (protect self; play dead)

Figure S3. Grade 1 NGSS student learning activities using non-fluorescent glowscope. (a) Viewing of sibling zebrafish embryos to address how individuals of the same kind of animal are similar but can vary in many ways. Arrows on left point to skin pigment cells, which may represent melanocytes, xanthophores, or iridophores. Note that their position and patterns on the body are variable from animal to animal. **(b)** Use of a pencil touch demonstrates offspring behaviors that aid their survival. Images from left to right are a time-series of the escape response. Images in panels **a-b** were acquired using an Apple iPhone 12 Pro.

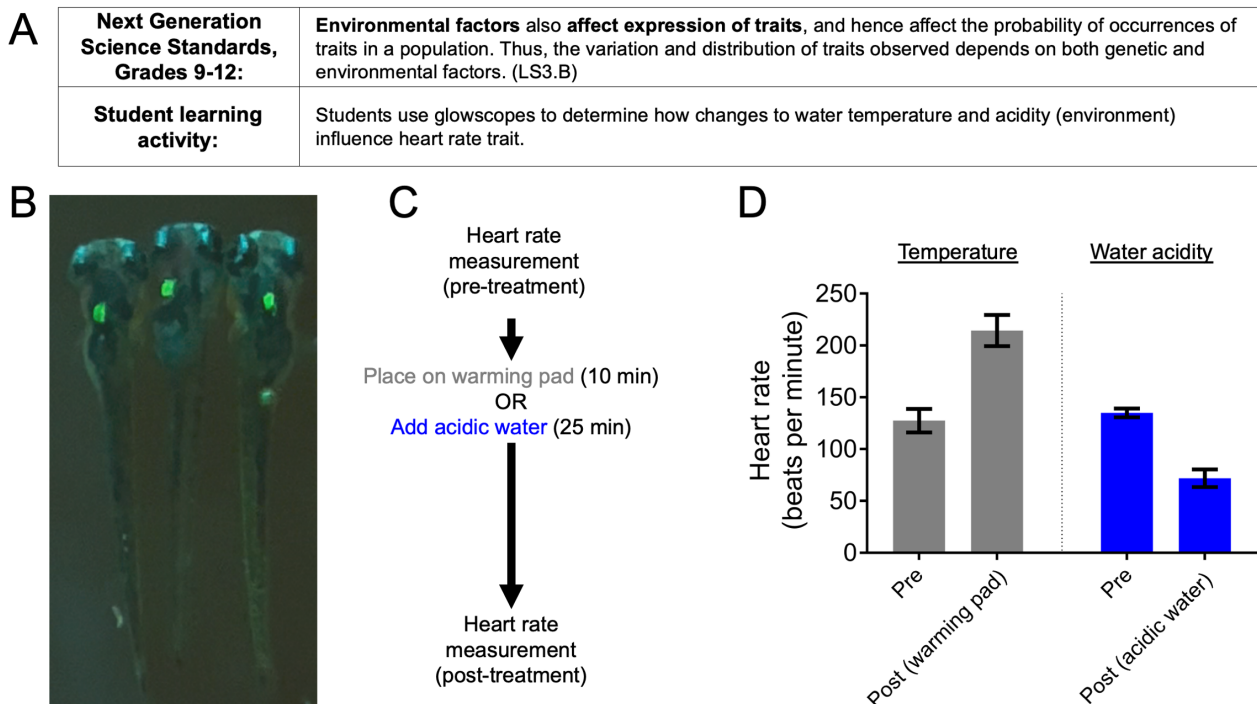


Figure S4. Grades 9-12 student learning activities addressing NGSS core ideas of how environment affects expression of traits. (a) Description of NGSS standard and proposed student activity. (b) Representative glowscope fluorescence image of 4 dpf *Tg(myf7:EGFP)* zebrafish larvae used for treatments and heart rate determination. (c) Experimental design for the influence of environment on heart rate trait. (d) Graph shows the effect of treatments on heart rate. Heart rate measurements on the same fish both prior to and after treatment, $n = 4$ larvae (temperature), $n = 2$ larvae (water acidity); bars show the mean $\pm$ standard deviation. Image shown in panel b was acquired using an Apple iPhone 12 Pro. Videos available in Supplementary Video S2.

2. Supplementary Video Legends

Video S1. Glowscope detection of drug-induced cardiac arrhythmia. Video corresponds to Fig. 6. Video playback is in real time.

Video S2. Impact of environment on the expression of heart rate trait. Video corresponds to Supplementary Fig. S4. Video playback is in real time.

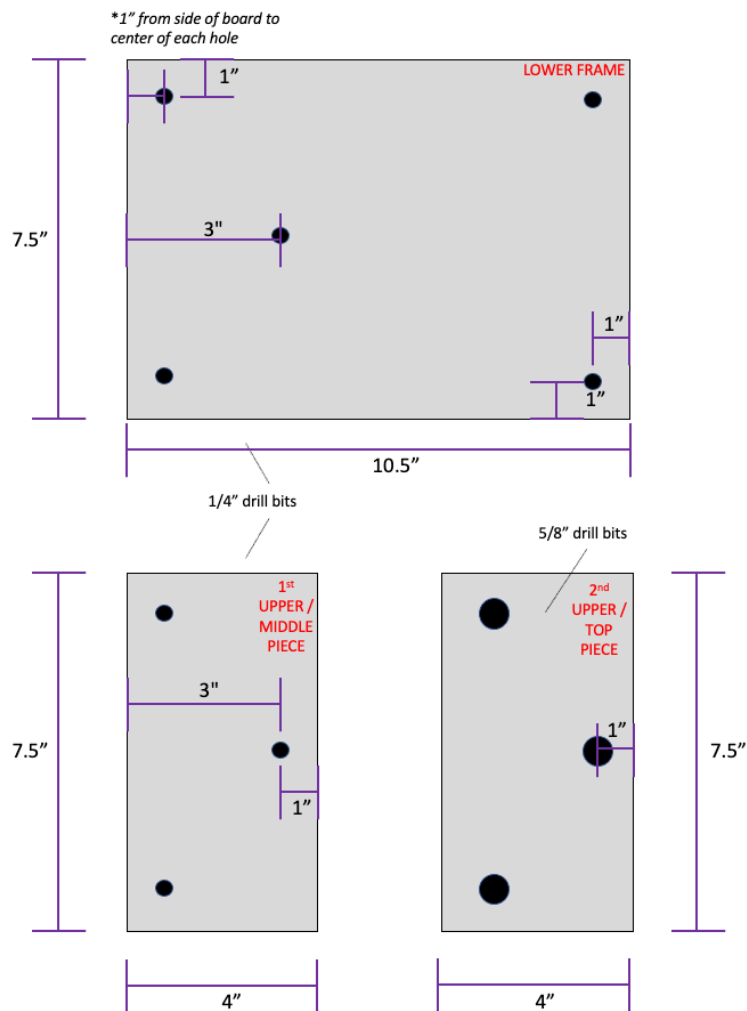
3. Supplementary Methods

Wood or composite board frame preparation

1. Plywood or composite board lower frame. Cut to 7.5" x 10.5". Drill five holes with a 1/4" drill bit based on the design coordinates below.
2. Plywood or composite board upper pieces (these support the plexiglass top). Cut two pieces to 7.5" x 4".
 - a. In the first upper piece, which will eventually be the middle piece, drill with a 1/4" drill bit (design coordinates below, lower left image).
 - b. In the second upper piece, which will eventually be the top piece, drill holes (design coordinates below, lower right image) using a 5/8" drill bit.

**the center of each drilled hole should be 1" from each side.*

Design coordinates for frame preparation



Plexiglass preparation

Use of an oscillating tool with plastic or wood compatible blade is best. Alternatively, a glass or plexiglass scoring tool (sharp like a knife), when used along with clamps and straightedge, can be used to score and then snap the plexiglass.

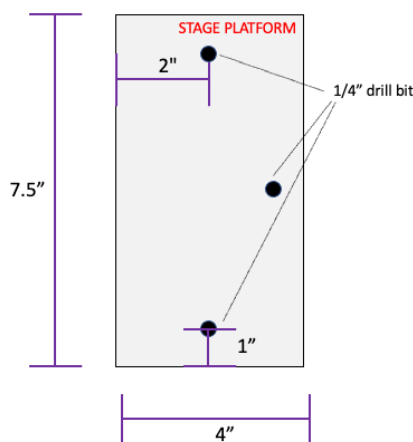
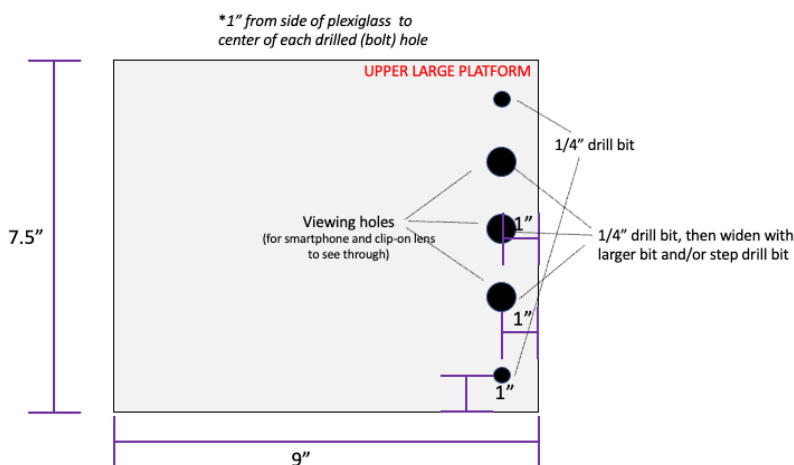
After cutting or snapping, a bench grinder (grinding wheel) can be useful to smooth imperfections or round sharp corners.

3. Upper large platform. Cut or snap to 7.5" x 9". Drill the two corner holes using the design coordinates below using a 1/4" bit. Then, drill the desired number and size of camera viewing holes between these two corner holes. See images.

Use of a step drill bit (1/4" – 3/4" cone shaped bit") can be useful to open up the viewing holes in a way that will look perfectly circular and smooth.

4. Lower stage platform. Cut or snap to 7.5" x 4". Drill two holes using the design coordinates below using a 1/4" bit.

Design coordinates for plexiglass preparation



Assembly

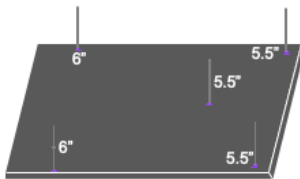
5. Assemble the frame parts based on the illustration below.

**Use of a ratcheting wrench made accelerates assembly*

Assembly illustration

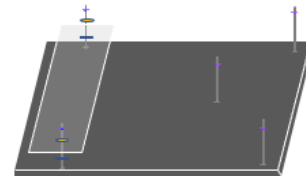
A. Assemble frame base

1. Insert carriage bolts through holes in board
2. Per bolt: one standard 1/4" washer and 1/4" hex nut, tighten



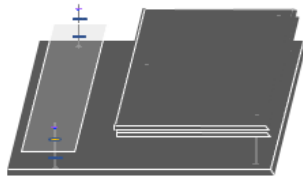
B. Attach stage, prep for top pieces

1. 1/4" Fender washer (below stage)
2. Plexiglass stage piece
3. 1/4" Fender washer (above stage)
4. Standard 1/4" hex nut near top of each 5.5" bolt
5. 1/4" jam nut (low profile nut) near top of each 6" bolt
6. Standard 1/4" washer above each nut



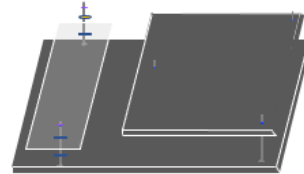
D. Add upper top piece

1. Add 'upper top piece' (wood/composite).
- *This piece just rests atop and jam nuts should be even with or just lower than the top of this 'upper top piece'. The jam nut and carriage bolt end should not protrude above this 'upper top piece' or they will scratch and break the plexiglass in next steps.*



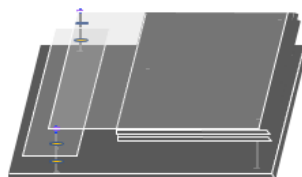
C. Attach upper middle piece

1. Add 'upper middle piece' (wood/composite)
2. Add a 1/4" jam nut above the piece onto each of the three 5.5" bolt. Tighten down.



E. Add, adjust, and tighten down plexiglass upper large platform

1. Insert pre-drilled plexiglass platform (2 holes) over and onto the two 6" carriage bolts (above stage)
2. Add a standard 1/4" washer on each side
3. Adjust the nuts below plexiglass platform, along with the wooden platform, to make the upper plexiglass platform as level as possible.
4. Add a standard 1/4" hex nut onto the top of each 6" carriage bolt (above plexiglass and washer). Tighten gently. (over-tightening may crack the plexiglass).



Stage positioning and use

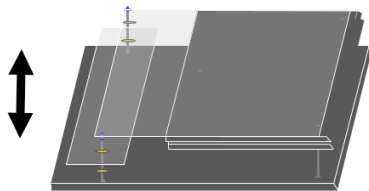
- Slide the stage up and down to the desired position. The use of fender washers and clamps both above and below the stage holds it into position and reduces its wobble. This will reduce the frequency that the specimen / dish slides or falls off the stage. See the illustrations below.

We've found that when using the extra small binder clamps (15 mm size) and a hex jam nut (low profile) underneath the upper plexiglass platform, pushing the stage as high as possible works perfectly with the clip-on macro lenses we tested.

Stage positioning illustration

A. Position the stage

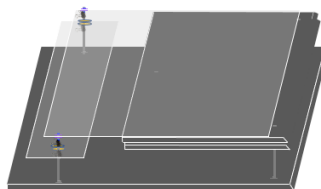
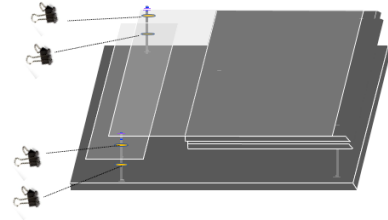
- Slide the stage to the desired position (up or down)



B. Use paper clips to sandwich the plexiglass stage with fender washers

- Use four extra small paper clips (lower two may be larger)
- Place one below each lower fender washer and one above each upper fender washer.
- Re-position stage, if desired.

**when using a clip-on macro lens, we find that positioning the stage as high as possible works perfectly.*



C. Clamp the stage to reduce its wobble

- Once the stage is positioned and clamps are in place, slide them together. This will squeeze the plexiglass stage between the fender washers, and by doing so, reduce stage wobble.

Supplementary Table S1

Next Generation Science Standards (NGSS)

Primary; Grades K-5

Grade Level	NGSS Disciplinary Core Ideas		Potential student activities	Recommended viewing devices	NGSS Science and Engineering Practices	
K -- LS1-1	Use observations to describe patterns of what plants and animals (including humans) need to survive.	Animals need food in order to live and grow (K-LS1-1).	Rear zebrafish (or tadpoles) to 1 month under different feeding conditions. Observe growth by comparing body length and size between groups.	None needed	Scientific Knowledge is Based on Empirical Evidence	Scientists look for patterns and order when making observations about the world. Use observations to describe patterns in the natural world in order to answer scientific questions. (K-LS1-1)
1 – LS1-1	Use materials to design a solution to a human problem by mimicking how plants and/or animals use their external parts to help them survive, grow, and meet their needs.	All organisms have external parts. Different animals use their body parts in different ways to see, hear, grasp objects, protect themselves, move from place to place, and seek, find, and take in food, water and air (LS1.A).	Observe zebrafish 3-7 day-old embryos or tadpoles and compare to insects.	Optional: Glowscope (without fluorescence)	Constructing explanations and designing solutions in K–2 builds on prior experiences and progresses to the use of evidence and ideas in constructing evidence-based accounts of natural phenomena and designing solutions.	Make observations (firsthand or from media) to construct an evidence-based account for natural phenomena.
		Adult plants and animals can have young. In many kinds of animals, parents and the offspring themselves engage in behaviors that help the offspring to survive (LS1.B). Animals have body parts that capture and convey different kinds of information needed for growth and survival. Animals respond to these inputs with behaviors that help them survive (LS1.D).	Observe and touch zebrafish 3-7 day-old embryos or tadpoles with a hair-tool. Construct an explanation for how the observed behavior helps them to survive, and the body parts that capture and convey this information.	None needed		
1 – LS3-1	Make observations to construct an evidence-based account that young plants and animals are like, but not exactly like, their parents.	Young animals are very much, but not exactly like, their parents. Individuals of the same kind of plant or animal are recognizable as similar but can also vary in many ways (LS3.A/B).	Use 3-7 day-old zebrafish embryos to observe any similar morphological features as well as potential variation in pigmentation traits. If available, include use of albino or casper mutant zebrafish strains.	Glowscope or Foldscope for high-magnification viewing of pigmentation		
3-LS3-1	Analyze and interpret data to provide evidence that plants and animals have traits inherited from parents and that variation of these traits exists in a group of similar organisms.	Many characteristics of organisms are inherited from their parents (LS3.A). Different organisms vary in how they look and function because they have different inherited information (LS3.B).	Use 3-7 day-old zebrafish embryos to observe any similar morphological features as well as potential variation in pigmentation traits. If available, include use of albino or casper mutant zebrafish strains.	Glowscope or Foldscope for high-magnification viewing of pigmentation	Analyzing data in 3–5 builds on K–2 experiences and progresses to introducing quantitative approaches to collecting data and conducting multiple trials of qualitative observations.	Analyze and interpret data to make sense of phenomena using logical reasoning. Use evidence (e.g., observations, patterns) to support an explanation.
3-LS3-2	Use evidence to support the explanation that traits can be influenced by the environment.	Other characteristics result from individuals' interactions with the environment, which can range from diet to learning. Many characteristics involve both inheritance and environment LS3.A). The environment also affects the traits that an organism develops (LS3.B).	Observe the body plan (morphology) and/or heart rate of 2-4 day-old zebrafish embryos. Consider altering the environment (egg water) by adding herbicide, pesticide, or fungicides (see Discussion). Acquire evidence to support an explanation for how the environment can influence traits.	Viewing of fluorescent (glowscope) or non-fluorescent heart (glowscope or Foldscope)	Constructing explanations and designing solutions in 3–5 builds on K–2 experiences and progresses to the use of evidence in constructing explanations that specify variables that describe and predict phenomena and in designing multiple solutions to design problems.	
3-LS4-3/4	Construct an argument with evidence that in a particular habitat some organisms can survive well, some survive less well, and some cannot survive at all. Make a claim about the merit of a solution to a problem caused when the environment changes and the types of plants and animals that live there may change.*	When the environment changes in ways that affect a place's physical characteristics, temperature, or availability of resources, some organisms survive and reproduce, others move to new locations, yet others move into the transformed environment, and some die (LS2.C). For any particular environment, some kinds of organisms survive well, some survive less well, and some cannot survive at all. (LS4.C). Populations live in a variety of habitats, and change in those habitats affects the organisms living there (LS4.D).	Observe the physical characteristics (growth, swim behavior, heart rate) and survival of 1-10 day-old zebrafish embryos. Consider altering the environment (embryo water) by altering the pH or temperature. Acquire evidence and analyze data to support an explanation for how the environment can influence traits and survival.	None needed. (Optional): view heart rate using glowscope		

Supplementary Table S2

Next Generation Science Standards (NGSS)

Intermediate; Middle School Level

Grade Level	NGSS Disciplinary Core Ideas		Potential student learning activities	Recommended viewing devices	NGSS Science and Engineering Practices	
MS-LS1-1	Conduct an investigation to provide evidence that living things are made of cells; either one cell or many different numbers and types of cells.	All living things are made up of cells, which is the smallest unit that can be said to be alive. An organism may consist of one single cell (unicellular) or many different numbers and types of cells (multicellular) (LS1.A).	Construct a model for how specialized cells with pigment granules, if spatially localized and distributed throughout the animal, could be responsible for organismal trait like stripes and coloration. Observe pigment cells on the 3-7 day-old zebrafish embryo to revise model .	Recommended: Foldscope or glowscope (non-fluorescent)	Developing and using models. Modeling in 6–8 builds on K–5 experiences and progresses to developing, using, and revising models to describe, test, and predict more abstract phenomena and design systems. Planning and Carrying out Investigations in 6-8 builds on K-5 experiences and progresses to include investigations that use multiple variables and provide evidence to support explanations or solutions. Constructing Explanations and Designing Solutions in 6–8 builds on K–5 experiences and progresses to include constructing explanations and designing solutions supported by multiple sources of evidence consistent with scientific knowledge, principles, and theories. Engaging in argument from evidence. Engaging in argument from evidence in 6–8 builds on K–5 experiences and progresses to constructing a convincing argument that supports or refutes claims for either explanations or solutions about the natural and designed world(s).	Develop and use a model to describe phenomena. AND Develop a model to describe unobservable mechanisms. Conduct an investigation to produce data to serve as the basis for evidence that meet the goals of an investigation. Construct a scientific explanation based on valid and reliable evidence obtained from sources (including the students' own experiments) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. Use an oral and written argument supported by evidence to support or refute an explanation or a model for a phenomenon or a solution to a problem. (MS-LS1-4)
MS-LS1-3	Use argument supported by evidence for how the body is a system of interacting subsystems composed of groups of cells.	In multicellular organisms, the body is a system of multiple interacting subsystems. These subsystems are groups of cells that work together to form tissues and organs that are specialized for particular body functions (LS1.A).	Construct a model for the specialized cells that form heart chambers and valves that are specialized for the functions they provide, and how these groups of cells behave differently than most somatic cell types (contractility). Observe the beating heart using the glowscope. Construct an explanation for how these structures and cell behaviors confer the function of the system (blood flow that is unidirectional). Plan and carry out an investigation using high dose anesthetic treatment (tricaine) to block observed heart contractility and draw comparisons of red blood cell movement through vasculature between groups. Use evidence to support or reject earlier models.	Recommended: Glowscope viewing of fluorescent heart. Foldscope viewing of blood cell movement through vasculature.		
MS-LS1-5	Construct a scientific explanation based on evidence for how environmental and genetic factors influence the growth of organisms.	Genetic factors as well as local conditions affect the growth of the adult plant or animal (LS1.B).	Plan and carry out an investigation to determine how the density of zebrafish (per dish) influences their growth. Interpret evidence and construct an argument to refute or support the claim that environment influences animal growth.	Recommended: Glowscope (non-fluorescent) and length measurements.		
MS-LS1-8	Gather and synthesize information that sensory receptors respond to stimuli by sending messages to the brain for immediate behavior or storage as memories.	Each sense receptor responds to different inputs (electromagnetic, mechanical, chemical), transmitting them as signals that travel along nerve cells to the brain. The signals are then processed in the brain, resulting in immediate behaviors or memories (LS1.D).	Construct a model for how sensory information (touch) is relayed to the brain. Plan and carry out an investigation to determine if 5-10 day-old zebrafish larvae learn to reduce their sensitivity and response to repeated touch, increase their swim behavior in response to the presence of food (paramecium), or decrease their sensitivity after being continuously provided food for a day or more.. Interpret evidence and construct an argument to refute or support the claim that the brain interprets touch sensation.	None needed		
MS-LS3-1	Develop and use a model to describe why structural changes to genes (mutations) located on chromosomes may affect proteins and may result in harmful, beneficial, or neutral effects to the structure and function of the organism.	Genes are located in the chromosomes of cells, with each chromosome pair containing two variants of each of many distinct genes. Each distinct gene chiefly controls the production of specific proteins, which in turn affects the traits of the individual. Changes (mutations) to genes can result in changes to proteins, which can affect the structures and functions of the organism and thereby change traits (LS3.A). In addition to variations that arise from sexual reproduction, genetic information can be altered because of mutations. Though rare, mutations may result in changes to the structure and function of proteins. Some changes are beneficial, others harmful, and some neutral to the organism (LS3.B).	Observe transgenic zebrafish and non-transgenic siblings. Construct a model for why some but not all display the phenotypic trait. After learning about the green/red fluorescent protein gene and protein, Plan and carry out an investigation to determine if may be genetically inherited. Use evidence to support or reject earlier models and predictions. Construct an argument that the trait is encoded by a heritable gene.	Glowscope (fluorescent)		
MS-LS3-1, 2	Develop and use a model to describe why asexual reproduction results in offspring with identical genetic information and sexual reproduction results in offspring with genetic variation.	Organisms reproduce, either sexually or asexually, and transfer their genetic information to their offspring (LS1.B). Variations of inherited traits between parent and offspring arise from genetic differences that result from the subset of chromosomes (and therefore genes) inherited (LS3.A). In sexually reproducing organisms, each parent contributes half of the genes acquired (at random) by the offspring. Individuals have two of each chromosome and hence two alleles of each gene, one acquired from each parent. These versions may be identical or may differ from each other (LS3.B).				

Supplementary Table S3

Next Generation Science Standards (NGSS)

Secondary; High School Level

Grade Level	NGSS Disciplinary Core Ideas		Potential student learning activities	Recommended viewing devices	NGSS Science and Engineering Practices	
HS-LS1-1	Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins, which carry out the essential functions of life through systems of specialized cells.	All cells contain genetic information in the form of DNA molecules. Genes are regions in the DNA that contain the instructions that code for the formation of proteins, which carry out most of the work of cells (LS1.A).	Develop a model of genes, chromosomes, and DNA and their relatedness to proteins within cells. Observe zebrafish offspring from adults (various parental crosses) that produce varying ratios of offspring possessing the trait. Determine the proportion carrying the trait and match this to possible Punnett squares. More advanced: Observe zebrafish offspring from adults that carried the (trans)genes for green and red fluorescent traits. Plan and carry out an investigation , using mathematical analysis , for the ratio of offspring displaying one or both traits and whether they are linked. Use evidence to revise models , if needed.	Glowscope (green and red fluorescence)	Developing and Using Models Modeling in 9–12 builds on K–8 experiences and progresses to using, synthesizing, and developing models to predict and show relationships among variables between systems and their components in the natural and designed worlds. Planning and Carrying Out Investigations in 9–12 builds on K–8 experiences and progresses to include investigations that provide evidence for and test conceptual, mathematical, physical, and empirical models. Constructing explanations and designing solutions in 9–12 builds on K–8 experiences and progresses to explanations and designs that are supported by multiple and independent student-generated sources of evidence consistent with scientific ideas, principles, and theories.	Develop and use a model based on evidence to illustrate the relationships between systems or between components of a system. (HS-LS1-2), Use a model based on evidence to illustrate the relationships between systems or between components of a system. Plan and conduct an investigation individually and collaboratively to produce data to serve as the basis for evidence, and in the design: decide on types, how much, and accuracy of data needed to produce reliable measurements and consider limitations on the precision of the data (e.g., number of trials, cost, risk, time), and refine the design accordingly. Construct an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS1-1) Construct and revise an explanation based on valid and reliable evidence obtained from a variety of sources (including students' own investigations, models, theories, simulations, peer review) and the assumption that theories and laws that describe the natural world operate today as they did in the past and will continue to do so in the future. (HS-LS1-6)
HS-LS1-4	Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.	In multicellular organisms individual cells grow and then divide via a process called mitosis, thereby allowing the organism to grow. The organism begins as a single cell (fertilized egg) that divides successively to produce many cells, with each parent cell passing identical genetic material (two variants of each chromosome pair) to both daughter cells. Cellular division and differentiation produce and maintain a complex organism, composed of systems of tissues and organs that work together to meet the needs of the whole organism (LS1.B).	Observe early embryonic development 1-2-4-8 cell stage divisions of zebrafish eggs (or frog, sea urchin, or others available). Develop a model for the genetic material and whether/how it is passed from parental cell(s) to daughter cells within the organism.	Foldscope (or glowscope), non-fluorescent		
HS-LS3-2/3; HS-LS2-7	Make and defend a claim based on evidence that inheritable genetic variations may result from (1) new genetic combinations through meiosis, (2) viable errors occurring during replication, and/or (3) mutations caused by environmental factors AND Apply concepts of statistics and probability to explain the variation and distribution of expressed traits in a population. Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.	Environmental factors can also cause mutations in genes, and viable mutations are inherited. Environmental factors also affect expression of traits, and hence affect the probability of occurrences of traits in a population. Thus the variation and distribution of traits observed depends on both genetic and environmental factors (LS3.B). When evaluating solutions it is important to take into account a range of constraints including cost, safety, reliability and aesthetics and to consider social, cultural and environmental impacts (ETS1.B).	Design and investigation to test how environmental factors such as herbicides, pesticides, fungicides, drugs such as allergy pills with adverse side effects, or waste pharmaceuticals influence the display of traits (zebrafish heart rate). Use evidence to design a solution based on observed data and other constraints including cost, effects on agriculture, safety, social, cultural, and environmental impacts.	Glowscope		