The Biophysicist

Microscope in Action: An Interdisciplinary Fluorescence Microscopy Hands-on Resource for Schools

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ABSTRACT Fluorescence microscopy is a ubiquitous technique in the life sciences that uses fluorescent molecules to visualize specific components of biological specimens. This powerful tool has revolutionized biology, and it represents a perfect example of the advancements enabled by biophysical research and technology development. However, despite its central role in contemporary research, fluorescence is hardly covered in typical secondary school curricula, with few hands-on "entry-level" materials available for secondary school teachers to introduce this important method to their students. Furthermore, most commercially available fluorescence microscopes are prohibitively costly and often appear as "black boxes." To address this gap, we introduce here an experimental, research-grade fluorescence microscopy kit and educational resource targeted at secondary school students and teachers. Microscope in Action is an interdisciplinary resource based on active learning that combines concepts from both optics and biology. The students assemble a functional microscope from basic optical, mechanical, and electronic parts, thereby testing and understanding the function of each component "hands-on." We also present sample preparation and imaging activities that can be incorporated to enable an exploration of biological topics with the assembled microscope and exercises in which students actively learn and practice scientific thinking by collecting and analyzing data. Although the resource was developed with secondary schools in mind, the variety of available protocols and the adjustable module lengths make it suitable for different age groups and topics, from middle school to PhD level, from short workshops to courses spanning several days.

KEY WORDS microscopy; fluorescence; active learning; secondary school

I. INTRODUCTION

Fluorescence is a phenomenon ubiquitous in nature that occurs when molecules (fluorophores) absorb light of a specific wavelength and emit light at a different (typically longer) wavelength. By staining cellular structures of interest with fluorescent dyes of distinct colors, fluorescence microscopy enables the visualization of molecules with a high degree of specificity. This powerful tool has completely revolutionized biology, and it represents a perfect example of the advancements enabled by biophysical research, as recently highlighted by the 2014 Nobel Prize in Chemistry for the development of super-resolution microscopy (1). A key advantage of fluorescence microscopy is that it enables quantitative measurements in cells, in tissues, and in vivo. For example, using appropriate calibrations and

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Received: 23 October 2020
Accepted: 28 June 2021
Published: 7 October 2021

corrections, the fluorescence emission can be used to estimate amounts of a specific protein or mRNA, in addition to their cellular localization. Temporal dynamics can also be extracted at high precision, for example, by using appropriate single-molecule tracking techniques.

Despite its key role in contemporary life science research, fluorescence is hardly covered in secondary school curricula, and learning resources to introduce this important topic are lacking. Most commercial fluorescence microscopes are presented as "black boxes," whose inner workings are unexplained, and the few hands-on kits available are often too advanced and expensive to be used effectively in schools. New optics curricula incorporating fluorescence are now being introduced; however, they are mostly targeted at more advanced undergraduates (2). To address this gap, we present here Microscope in Action (MiA), an inquiry based, multidisciplinary learning resource that enables students to understand concepts from both optics and biology while building and operating a fluorescence microscope. This paper focuses on the implementation of MiA in the classroom and also discusses a selection of additional educational settings on the basis of our pilot workshops conducted with around 330 participants in three different countries. Although the resource has been developed with secondary school teachers and students in mind, the activities are modular and highly versatile, and have been adapted successfully to a variety of audiences and formats.

II. SCIENTIFIC AND PEDAGOGIC BACKGROUND

A. Approach

The MiA resource bridges the gap between research and schools by bringing a research-grade fluorescence learning microscope to the classroom. It offers a safe and innovative learning experience for students, who assemble a fully functional microscope from its individual components and use it to carry out various experiments. MiA engages and inspires students by showing them how technology enables scientists to study the fascinating details of life and how rewarding exploration of the unknown can be.

1. Design principle

The resource has been designed to be very flexible with regard to learning level, length of modules, setting, and topic areas and currently offers complementary content for physics, biology, technology, chemistry, and visual arts for ages 14–19+. The driving principle of the resource is to present a research-grade instrument and real-life research experience to students in informal and formal learning environments. MiA enables students to understand the role of each microscope component and operate a high-end research instrument that they build themselves. The modular structure enables teachers to adjust the resource easily for various needs. The experimental protocols are customizable and leave ample room for new ideas and applications. The resource can also be used as a training tool for undergraduate, graduate, and doctoral students or other scientific training programs.

Students assemble the microscope and conduct inquiry-based experiments to understand the application of fluorescent staining and imaging in life sciences research and beyond. They work in small groups and need to collaborate closely to be able to address technical and scientific challenges. The resource aims to provide students with various access points, regardless of their background knowledge and preferred learning styles. Whereas the main activities of the three learning modules stimulate kinesthetic, interpersonal, and logical learning through collaborative hands-on engagement, the diverse set of additional learning materials, including reading resources, videos, and assignment options, engage visual, auditory, and verbal learners.

The provided teaching and learning materials give teachers the freedom to adjust the MiA resource to their teaching methodology and to choose between an inquiry-based approach or a more guided approach when using the resource. MiA makes students aware of the connections between research, technology,

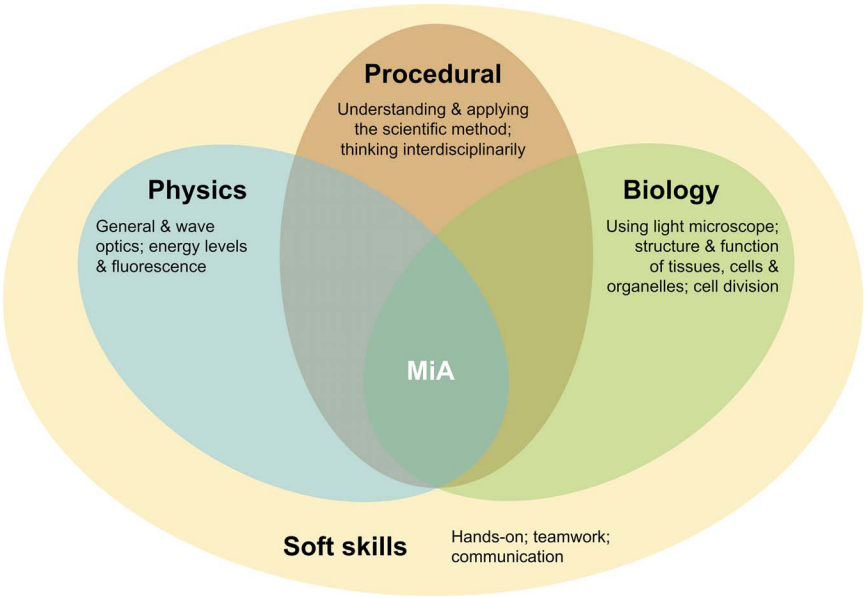

Fig 1. Scheme highlighting the content knowledge and competencies in the subjects of biology and physics, procedural knowledge, and non–subject-related "soft" skills of the MiA resource.

and applied sciences. It facilitates the development of interdisciplinary and scientific thinking and, most importantly, stimulates participants' curiosity and interest toward research and the science, technology, engineering, arts, and mathematics subjects.

2. Learning goals

The learning content of the MiA resource fosters scientific literacy and aligns with various national science frameworks for secondary schools. The PISA 2018 Science Framework defines scientific literacy by three key competencies: explaining phenomena scientifically, evaluating and designing scientific inquiry, and interpreting data and evidence scientifically (3). According to this definition, a scientifically literate person draws on three types of knowledge: content knowledge, procedural knowledge, and epistemic knowledge. Many national science education frameworks correspond to this definition of science literacy and define their educational standards accordingly. The MiA resource facilitates learning in all these areas and directly links to national secondary school curricula in physics, biology, interdisciplinary natural sciences, and technology (see Fig 1 for an overview and Supplemental Material 2 for a table that details the intersections of the MiA resource with the curricula of several sample countries). For example, in the United States, the United Kingdom, and Germany, standards for lower secondary schools (11–15 yr) include knowledge of basic cell biology and geometric optics, the use of a light microscope, and the application of science through experimentation. In upper secondary school (15–19 yr) standards are extended to advanced cell biology, geometric optics, and more complex experimentation. Across the curricula, students are taught aspects of procedural knowledge and competencies, including scientific and interdisciplinary thinking, as well as understanding and applying the scientific method.

After completing the full MiA course, students can describe the basic concepts of light and fluorescence microscopy and are able to build a basic fluorescence microscope from its individual components with little to no support. They are also able to illustrate some applications of fluorescence microscopy in life sciences; become aware of the link between technology, research, and applied sciences; and possess a clear perception of scientific research. Besides teaching subject-specific conceptual and procedural knowledge and competencies, MiA also supports deeper learning and the development of 21st century skills (4), aiming to foster the domains of cognitive, interpersonal, and intrapersonal competencies. MiA is a tool to create a collaborative learning environment that pro-

vides an excellent opportunity for students to share both the learning experience and the earned knowledge with peers, teachers, and other facilitators. Its flexibility in structure and content allows MiA teachers to adjust learning goals to their needs or extend them. For example, MiA can be used to refresh or deepen previously acquired knowledge or serve as a preview tool to introduce aspects of topics the students will learn in later years.

B. Assessment Schemes

To assess the quality of MiA during its development, we adapted an iterative evaluation approach in which we collected feedback from teachers and students after completion of the various pilot workshops and teacher training courses. We then used this information to further improve the next development stages of MiA. A set of qualitative and quantitative tools were used to evaluate the learning experience and effectiveness of MiA as a teaching and learning tool.

To evaluate the resource as a learning activity, students who took part in the pilot workshops were provided with a post-workshop survey containing qualitative and quantitative questions to assess how accessible, enjoyable, and useful they found the resource. Additionally, for two of the three pilot workshops, the teacher conducted an oral debrief discussion with the class to gain a deeper understanding of the students' perception of the activity. To assess the effect on learning goals, students took part in a written in-class assignment assessing their conceptual knowledge related to the workshop topic. This in-class assignment was introduced at a later stage of the MiA development and only ran in the third pilot. The assignment was taken as part of the regular lesson and comprised questions on concepts and applications related to the topics of "imaging optics and fluorescence" and "fluorescence microscopy."

To assess MiA as a teaching tool, teachers who participated in a pilot workshop provided feedback to the developers in one-on-one debriefings and semistructured post-experience interviews. Topics addressed included learning goals set by teachers and how those were evaluated, the outcome of the learning goals, and the teachers' perception of the usefulness of the resource as a teaching tool. Teachers who took part in the teacher training provided feedback in a post-training survey containing qualitative and quantitative questions and a post-training group discussion. Those tools aimed at getting a deeper understanding on the level of difficulty for implementation of the resource and learning content, the strengths and weaknesses of MiA as a teaching tool, and the learning environments in which teachers would apply MiA. The outcomes of the iterative evaluations are discussed in the Results and Discussion section.

III. MATERIALS AND METHODS

A. Experimental equipment

1. Microscope

The main component of the learning resource is a portable microscope-building kit. Our goal was to employ research-grade, easy-to-assemble, and durable parts that are ideally suited for repetitive use by inexperienced users. All the components needed are shown in Figure 2: the majority of optomechanical parts are from the photonics company Thorlabs (Newton, NJ; used by permission and not meant to endorse a particular vendor), but any equivalent components could be used. A detailed list of components with their indicative cost and potential substitutions is provided in Supplemental Material 3.

Figure 3 shows an overview of the assembled microscope and its light path. Because we designed the kit to be usable in a variety of contexts, including classrooms with only basic equipment, a compact 20 × 20-cm breadboard is used as a platform to build the microscope instead of an optical table. It was important to design the microscope to be light-tight, given that it might not be possible to guarantee dark conditions for imaging; therefore, we decided to connect several parts with tubes that are adjustable in length and can be connected to

Fig 2. Components of the microscope building kit. (1) Screwdriver, (2) cage cube and dichroic mirror, (3, 4) cage plate and assembly rods, (5) Allen wrench, (6) adjustable lens tube, (7) collimator lens, (8) excitation filter, (9) post, (10) locking ring, (11) coupler, (12) emission filter, (13) post holder, (14) LED, (15, 16) tube lenses, (17) LED power supply, (18) objective lens and adaptor, (19) screws, (20) XYZ stage, (21) extension tube, (22) LED driver, (23) breadboard, (24) camera and connecting cable.

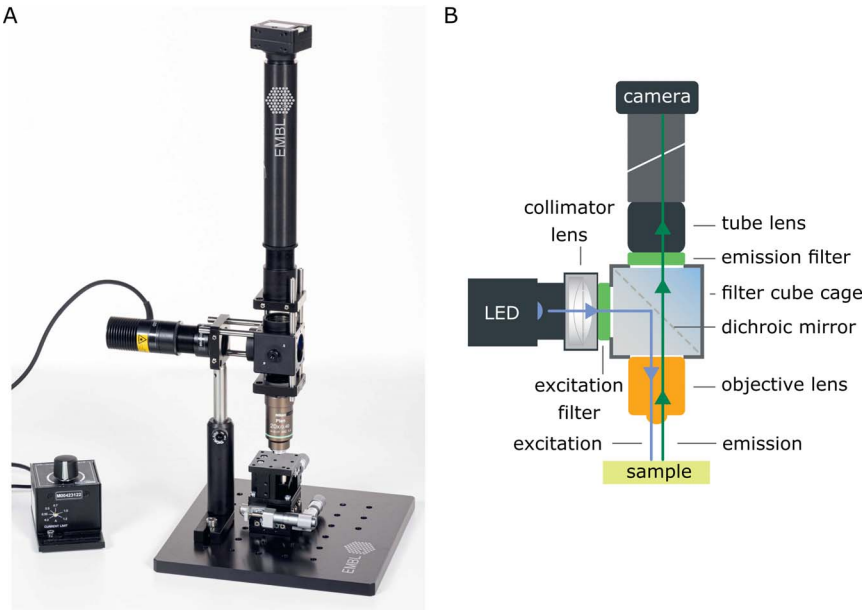

Fig 3. Overview of the educational fluorescence microscope. (A) Picture of the assembled microscope. (B) Scheme of the microscope light path.

appropriate adaptors (e.g., to the camera). To ensure safe assembly conditions, we recommend that most of the steps be performed horizontally on a table, with the microscope lifted and mounted vertically only at the final step.

We opted for a cage assembly system: in this way, most of the light path components can be assembled light-tight, and the dichroic mirror is safely housed inside a cage cube, allowing the students to manipulate its orientation without risk of damage (as described in the Results and Discussion section).

The light source is a 470-nm light-emitting diode (LED) equipped with a variable power supply to adjust the illumination intensity. We selected the 470-nm wavelength because it is the most applicable for biological samples (i.e., suitable for many available "green" fluorescent proteins and stains, and often a relevant range of autofluorescent samples), but alternative wavelengths could be used according to preference.

The design includes a 20× air objective lens, which proved suitable for imaging a variety of biological samples while avoiding the inconvenience and potential hazards of an immersion objective. Objectives with lower magnification factors can also work when a larger field of view is required, (e.g., to observe a whole insect). However, higher magnification factors (e.g., 40×, 60×) are not recommended because of their very small field of view and working distance. Under this circumstance, fine adjustment tools and experienced operators are essential. We also decided to include an adjustable XYZ stage; although, in principle students could move the microscope up and down to focus and move the slide by hand, the use of a stage is much safer because, once mounted upright, the microscope structure does not need to be further adjusted, enabling the user to easily explore the field of view (e.g., to look for cells with specific properties). Further details are described in the Results and Discussion section.

Instead of designing a microscope with an eyepiece to allow direct observation by eye, we decided to use a camera for imaging: this setup maximizes the portability of the kit and introduces the students to the concept of "seeing" with a camera, which is the standard in modern microscopy. Additional advantages of acquiring digital images is that they can be projected for the whole group to observe and can be saved and used in follow-up activities, such as image analysis, quantification, and calibration. We chose a small and inexpensive

Table 1. Recommended sample preparation materials.

General materials
Microscopy slides
Microscopy coverslips
Micropipettes
Micropipette tips
Gloves
Permanent markers
Water
Paper towels
Materials for fluorescent bead protocols
Microbeads (5 μm diameter)
Phosphate-buffered saline (PBS)
Vortexer
Eppendorf tubes (1.5 ml)
Eppendorf tube holder
Refrigerator
Autofluorescent biological samples
Flower parts (e.g., lily pollen)
Fern sporangium
Onion, apple
Pinus sp. branch
Materials for staining
Turmeric
Ethanol
Beaker
Miscellaneous materials
Food coloring
Well plate (96 wells)
Scalpel
Falcon tube holder
Toothpicks
Tweezers
Highlighter markers

complementary metal oxide semiconductor (CMOS) camera that can be easily connected to a laptop through a USB port and has sufficient resolution and sensitivity for imaging most biological samples. The camera is controlled with a software provided by the vendor that can be easily installed on most computers or, alternatively, with the open-source software μManager (5). We also recommend installing the open-source ImageJ software (6) that can be used to open, manipulate, and analyze the images. The entire microscope building kit fits in a portable case (40 × 25 × 25 cm), where all components can be safely housed and transported. The only additional requirement to operate the setup is the availability of a laptop and an electrical socket to power the LED.

2. Materials for sample preparation activities

As part of the MiA experience, we recommend that users prepare samples of different specimens that can then be imaged with the assembled microscope. Within our student and educator guides, we have included a series of sample preparation protocols, designed to make them as accessible as possible. Thus, the sample preparation protocols are largely based on common household goods and basic wet lab materials (listed in Table 1).

We note that, as an alternative to biological sample preparation activities, educators can access premade sample slides from various manufacturers. Examples for suitable sample slides are autofluorescent materials (i.e., plant tissues) as well as slides stained with acridine orange or Etzold stain.

B. Print and digital educational materials

The MiA resource is accompanied by print and digital materials. To introduce students to the MiA experience, we provide three recorded talks by European Molecular Biology Laboratory (EMBL) scientists that discuss the basic principles of fluorescence microscopy, application in basic research, and advanced microscopy techniques. The student guide leads students through the MiA experience without information about microscope assembly. The majority of the guide is dedicated to the student lab manual section. This section includes protocols for five sample preparation exercises, five imaging exercises, and two extension exercises. Within the protocols section, both the print and digital versions of the guide allow the students to answer questions, display images, and describe what they have learned.

Educators are supported by an assembly guide and assembly video that walk users through the steps of building the microscope, a PowerPoint presentation on fluorescence microscopy and an educator support section that includes tips and tricks for use of the MiA resource. The educator guide includes all aspects of the student guide. Additionally, the educator guide covers suggested time schedules for implementing the resource in class, a

Module	Activity	Learning Goals
Microscope Assembly	Assembly of illumination subsystem	Understanding collimation
	Assembly of imaging subsystem	Understanding image formation and focal lenght
	Assembly of filter cage cube	Understanding splitting of light in a fluorescence microscope
Sample Preparation	Dilution activity	Use of a micropipette, understanding dilution calculations
	Autofluorescence activity	Preparation of sample slide, understanding autofluorescence
	Staining activity	Preparation of sample slide, understanding concept of cell/tissue staining
Sample Imaging	Calibration activity	Understand and perform image calibration using image analysis software
	Observing samples	Operate microscope to focus on samples and take images, scientific observation
	Analyzing samples	Use image analysis software to extract more information from images (intensity, size)

Fig 4. Main modules in the MiA resource and corresponding learning goals.

dedicated teacher support section that offers safety and student protocol reminders, and answer keys for the student post-experience assessment. All print and digital educational materials can be accessed on the MiA web page on registration (7).

C. Methods

1. Overview of classroom activity schedule

The setup of the classroom activity is designed to facilitate learning progress and the development of respective transferable skills. To this end, the activity encompasses three main modules: microscope assembly, sample preparation, and sample imaging. An overview of key activities included in the modules and the corresponding learning goals is provided in Figure 4.

To create a deeper understanding of the physical processes within a fluorescence microscope, students ideally assemble the microscope before the imaging module. If not enough microscope sets are available, the class can be divided in two groups, with group 1 starting from microscope assembly and group 2 focusing on sample preparation first. This was the configuration chosen in the three pilot studies we have conducted so far (see Fig 5 for an overview of the module arrangement). In addition to the three main modules described above, flexible "introductory sessions" were used at the beginning of each lesson to introduce or review relevant concepts, discuss the schedule for the day, and address any questions.

Our pilot workshops were offered as a four-lesson unit, with each lesson having a 1.5-h duration, for a total of approximately 6 h in the classroom. On average, there were 18 students per lesson, one teacher, and supporting staff (up to three science education specialists and two scientists). Throughout the three pilot workshops, we have expanded the collection of support materials to enable the implementation of the activity with little or no external classroom support. A detailed schedule, including a breakdown of the time required for both in-class activities and teacher preparation, is provided in Supplemental Material 4.

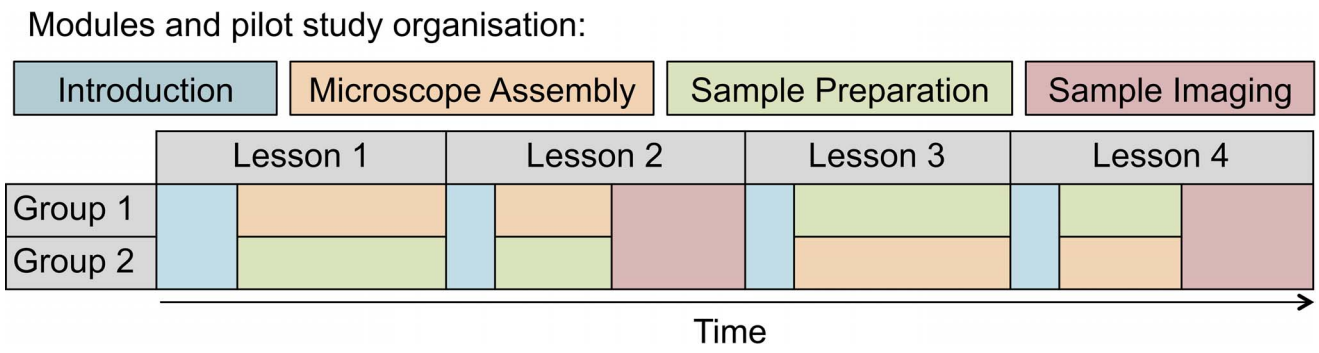

Fig 5. Scheme illustrating how the different MiA modules were structured in pilot studies. In this example schedule, the classroom is divided into two groups and activities are organized over four lessons. Each color-coded block of time indicates the module on which students worked, with the length of each block proportional to the duration of each activity.

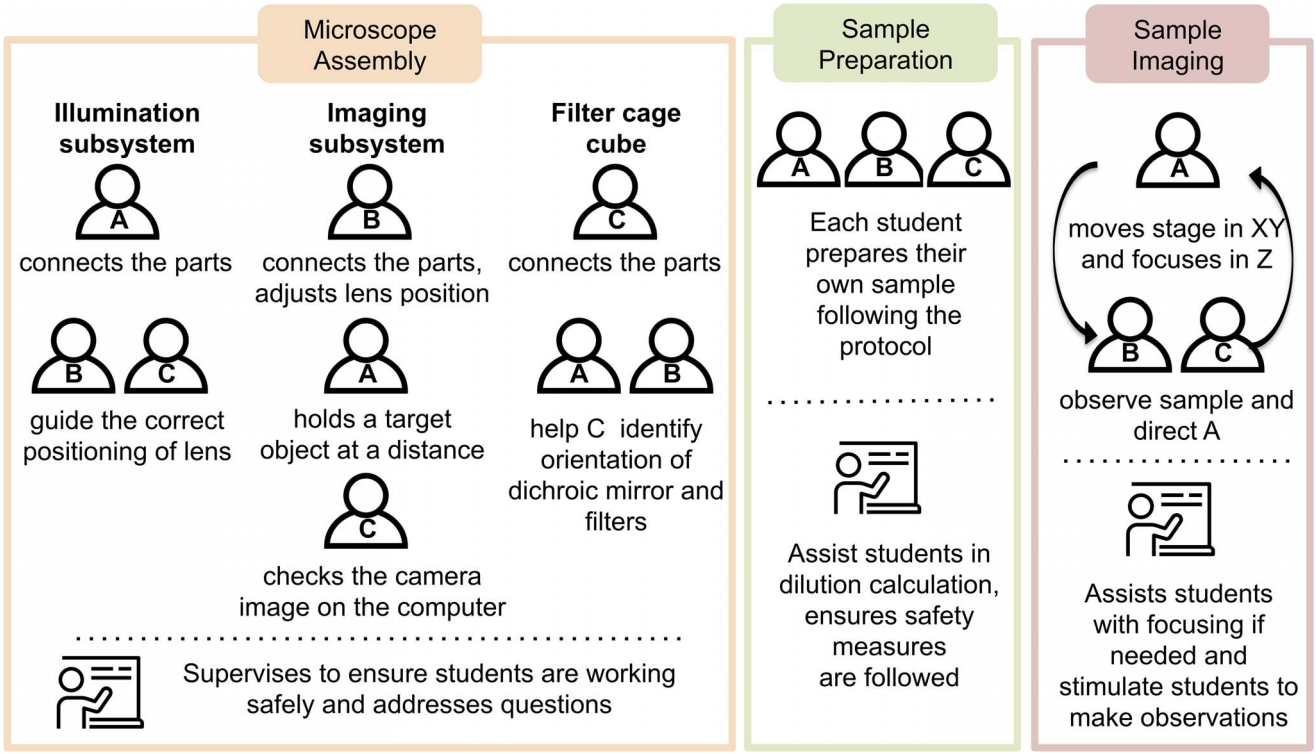

Fig 6. Diagram showing how students work both as a team and individually throughout the different MiA modules. The bottom row indicates the educator roles for each module.

We have found that working in smaller groups can be beneficial for both the students' learning progress and the development of their transferable skills, because all students will get to work with the resource but have to make an effort as a team to fulfill the tasks. Students in groups 1 and 2 were further split into subgroups of three students: Figure 6 illustrates how students in each subgroup A, B, and C work both as a team and independently in the different modules.

During the assembly module, by trial-and-error and tinkering, students figure out the precise function of the different microscope subsystems and how they come together. Depending on the time frame and learning context, the amount of guidance offered to the students can be adjusted. Dividing the assembly into three sections (subsystems), with each one illustrating a key concept from optics and fluorescence microscopy, helps the students approach the task in a structured manner. In the sample preparation module, students learn to use a micropipette and mount different samples on a slide for imaging. They also learn the concepts of autofluorescence and cell and tissue staining, as well as dilution calculations. Finally, the imaging module is an opportunity for students to explore many aspects of the scientific inquiry process hands-on: from using the microscope they assembled to inspect the samples, to performing calibrations and quantifications with image analysis software.

The human data used in this paper came from publicly accessible databases. According to our laboratory's internal policy and our institution's formal policy on the use of human biological material, utilizing publicly available human data is an acceptable practice. Consequently, the project did not require official approval by the laboratory's Bioethics Internal Advisory Committee or our larger institution.

2. Considerations on timing

Given the significant hands-on component of the activity, it is to be expected that the timing of the student tasks might need to be adapted. For example, during pilot studies, some sample preparation activities could not always be completed within lesson 1 and were therefore

postponed to the next lesson. The opposite might also be the case. For example, we noticed that the activities were completed faster after groups 1 and 2 "swapped" roles at lesson 3, likely because of an increased efficiency of both students and educators with the tasks at hand. To help buffer these potential time gap uncertainties, we recommend preparing additional backup activities (e.g., extension sample preparation protocols available in the print materials) or short presentations by the educator (e.g., in-depth discussion of cellular structures). Similar extension activities can also be used when subgroups complete their activities in slightly different amounts of time. Alternatively, the educator could invite the students to delve deeper into the recommended external article and video links and complete the student post-experience assessment or the student survey.

IV. RESULTS AND DISCUSSION

A. Preparation phase

When developing the MiA resource, one of our major aims was to unveil the black box of fluorescence microscopes, enabling students to explore concepts surrounding fluorescence microscopy and its use in the life sciences. However, for the majority of our target audience and some educators, these are new concepts. Thus, we have produced a series of introductory materials for both students and educators (see section III.B).

Before using MiA, we recommend that teachers first familiarize themselves with the educational materials provided as part of the resource. Educators should also practice microscope assembly, a process that can be supported by watching the assembly video, reading the assembly guide, attending a dedicated teacher training live event, or employing a combination of these steps (7). Additionally, to recap their own subject knowledge, we recommend educators review the "Fluorescence Microscopy" PowerPoint presentation and the recorded talks. One of the final preparatory steps for educators is determining which protocols they will offer to students and gather the corresponding supplies. A detailed table illustrating the different modular activities included in MiA and the corresponding preparation time and resources is provided in Supplemental Material 4. We believe that students will gain a deeper understanding of fluorescence microscopy if they watch the video about the basic principles of fluorescence microscopy before their MiA experience. Finally, the educator should review the related safety information with their students.

B. Microscope assembly module

During the assembly module, the students need to apply their previously acquired basic knowledge about optics and fluorescence microscopy to build their own research-grade fluorescence microscope. After an introductory talk and a safety briefing, the students receive a microscope building kit, which contains all the necessary tools and components. By trial and error, the students can then start assembling. We noticed that most students enjoy figuring out the assembly themselves and do not need much support, but it can help to have supervisors present to offer instruction and explanations where needed. To facilitate supervision and tracking of the students' progress, we advise that the educators split up the project according to the subsystems, as described in Figure 4.

1. Assembling the illumination subsystem

The illumination subsystem includes all optical parts needed to achieve an even illumination of the sample with the excitation beam. For this purpose, the MiA kit uses an LED light source with a collimator lens in front. During the safety briefing, the educators should explicitly mention that the LED is very bright and looking directly into the light might harm the students' eyes (as an additional safety measure, the maximum setting could be limited through a small screw). At the start of the assembly, students need to connect the collimator lens to the LED. The lens should already be incorporated in an adjustable lens tube holder, which can be connected to the lens tube in front of the LED. This adjustable lens tube holder enables the students to adjust

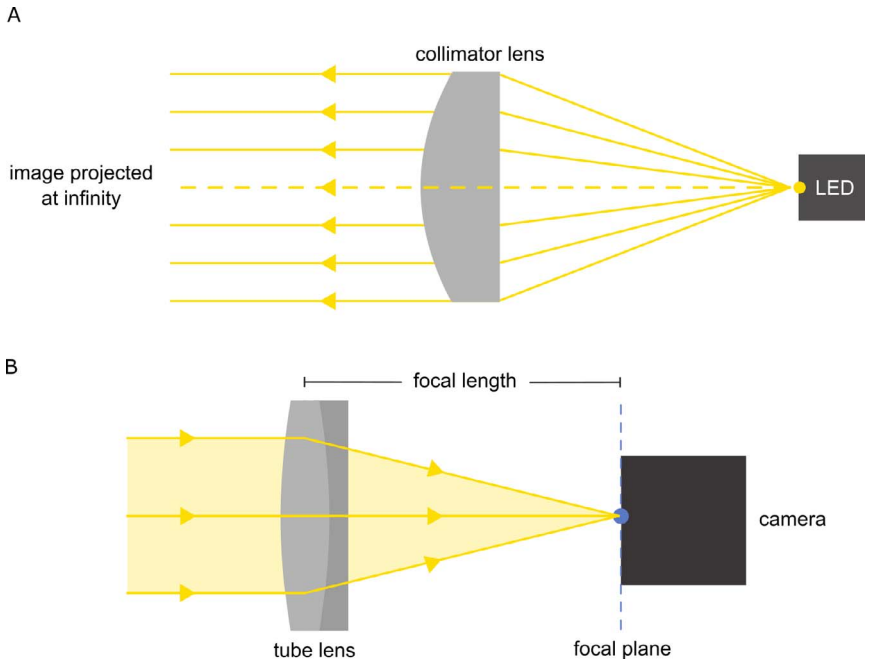

Fig 7. Schematic depiction of two key concepts that need to be applied during the microscope assembly: the processes of collimation (A) and focusing the camera at infinity (B).

the distance between the light source and the collimator lens, which will be crucial during the next steps. To turn the light source on, students then connect the LED to the LED driver and set it to continuous wave illumination. The students should realize that the light intensity can be adjusted by the knob, because this function will become important during the imaging module.

To achieve an even illumination of the sample and thereby successfully completing the assembly of the illumination subsystem, the concept of collimation should then be applied: the LED has a small emitting surface, which can almost be approximated as a point source. Therefore, light rays will travel from it radially, making the light coming directly from the LED unsuitable for uniform illumination. The collimator lens alters the direction of the light in such a way that the light rays proceed in parallel and illuminate the sample evenly. To observe the collimation, the students can direct the LED light onto a white wall or piece of paper at a distance of about 30 cm. By rotating the adjustable lens tube holder, the lens should then be moved closer or further away from the LED until a uniform illumination on the wall is achieved. At this point, the position of the collimation lens relative to the LED should be fixed by turning the locking ring on the adjustable lens tube holder. The educator can support the students' understanding of the collimation step by providing a diagram that illustrates the path of the light rays from a point source and through a collimator lens (see Fig 7A).

Instructor tip: It can be challenging to observe and adjust the collimation with the naked eye, especially in well-lit settings. Starting with the adjustable lens tube at one extreme or the other may help.

2. Assembling the imaging subsystem

The next step is the assembly of the imaging subsystem, where light is focused onto a camera to form an image of the sample. The subsystem's two main components are a tube lens and a camera, which the students have to connect with an extension tube and an adjustable lens tube holder.

Sharp images can only be obtained if the distance between the tube lens and the camera chip equals the focal length of the lens, so the results of the imaging section critically depend on the precise assembly of the imaging

subunit. This concept represents the main goal of the imaging subsystem assembly and is achieved by letting the students observe the concept "in action" during a hands-on exercise. The adjustable lens tube holder can be screwed in and out of the extension tube, giving students the opportunity to adjust the distance between the tube lens and camera and figure out the focal length themselves. This part is called "focusing at infinity" because, ideally, the light rays on the side of the lens not facing the camera chip should travel in parallel, and parallel lines would only converge at infinity.

To achieve a focused image, the camera needs to be connected to a computer so that the camera images can be viewed with the use of Point Grey Research FlyCapture (Teledyne FLIR, Wilsonville, OR) or similar software. Then, the assembled imaging subsystem has to be aimed at an "infinitely" far away object (e.g., a student on the other side of the classroom holding up a piece of paper with writing or a tree across the street). While turning the adjustable lens tube holder, the students can observe the image becoming blurrier or sharper on their screen. When the sharpest image possible is reached, the distance between the camera chip and the tube lens equals the focal length. Then, to fix the focal length distance, the students can carefully turn the locking ring on the adjustable lens tube holder. A diagram similar to the one shown in Figure 7B can be used to illustrate the light path.

Instructor tip: Getting objects into focus can be challenging, so we suggest a few steps that might help. (a) Students can position the imaging subsystem horizontally on a table to prevent the image from being too shaky. (b) To check whether everything is turned on and connected properly, a student can wave a hand in front of the camera. (c) The students can start by turning the adjustable lens tube holder to an extreme position (e.g., entirely screwed in) and slowly turn in one direction until focused. (d) If no objects at an "infinitely far distance" can be used, objects closer by can be used, too (e.g., the text on a poster). (e) If the camera keeps disconnecting, checking the cables and restarting the software might help.

3. Assembling the filter cage cube

The filter cube cage is the central connecting piece of the fluorescence microscope and is often the most challenging component for students to understand. The dichroic mirror, mounted inside the filter cage cube, is responsible for splitting the excitation and emission light according to their different wavelengths, which are then further refined by two optical filters (excitation and emission filters) that exclude all wavelengths other than the excitation and emission wavelengths.

Instructor tip: Before starting the assembly, remind the students to wear powder-free gloves and to handle the components with care. Because of its fragility, the dichroic mirror is already mounted into the filter cage cube and does not need to be handled directly. However, the filter cube needs to be oriented correctly to split the light as desired. To facilitate this, it is essential to familiarize the students with the concepts of fluorescence emission and light splitting first. In our experience, this has often been challenging for the students. Providing supporting visual aids similar to those in Figure 3B and offering guidance during this process is therefore highly recommended. The coated side of the dichroic mirror must be directed toward the excitation source on the bottom side of the 45° angle.

Instructor tip: Checking whether the dichroic mirror is oriented correctly at the end of this assembly step is recommended to avoid having to disassemble the microscope at a later stage. To do so, the students can shine the LED into the mirror and check whether the light is reflected downward toward where the sample will be placed. Please remind the students that the LED is very bright and they should not look directly into it.

Once the students figure out the correct orientation in the final set-up, they can assemble the excitation and emission filters into the correct positions on the filter cage cube. The excitation filter must be directed toward the illumination subsystem and the emission filter toward the imaging subsystem. Afterwards, three rods need to be mounted onto the sides of the filter cage cube housing

Table 2. Overview of all sample preparation protocols categorized according to the main activity.

Dilutions
Pipette art
Diluting colored water
Diluting fluorescent microbeads
Autofluorescent samples
Pollen (e.g., *Lilium longiflorum*, *Eustoma* spp.)
Other plant samples (e.g., fern sporangia, *Pinus* sp. branches, stalk of an apple)
Extension: Everyday objects (e.g., sticky notes, highlighter ink)
Staining
Turmeric staining
Samples used: carrots, apples, and pears
Extension: highlighter ink on nonfluorescent samples

the excitation and emission filter and the objective lens. The rods will later be used to connect the subsystems: we recommend the use of just three rods, which are sufficient to provide a stable connection but allow easier access in case the filters need to be unmounted or adjusted.

4. Connecting the subsystems and mounting on a breadboard

For the final microscope assembly step, the students need to connect the three subsystems to each other and mount the objective lens. With the use of a connector tube, the illumination and imaging subsystems each need to be connected to one cage plate, which can then be slid onto the rods at the respective positions on the filter cage cube. The screws on the cage plates should only be tightened gently (finger tight). Then, the students can carefully unpack the objective lens from its case and connect it to the third cage plate with an adaptor ring. Sliding the cage plate onto the last free set of rods until it touches the filter cage cube completes the assembly of the fluorescence microscope.

To mount the microscope vertically on the breadboard, the XYZ linear translation stage and post holder need to be screwed onto the breadboard. If time is limited, educators can prepare the breadboards before this step of the project by mounting these two components ahead of time. The post can now be attached to the cage plate of the illumination subunit and carefully lowered into the post holder, with a second person tightening the post knob to secure the setup. To protect the sensitive objective lens, the students should very slowly lower the post into the post holder, with the objective lens positioned either next to the XYZ stage or with a piece of foam between the two. Because the last step needs to be conducted very carefully, the educator should offer as much guidance as possible.

C. Sample preparation module

The sample preparation module gives students the opportunity to prepare their own sample slides and thereby experience the laboratory work that precedes research with fluorescence microscopes. In addition to learning how to create sample slides that they can image during the imaging module, the primary goals of this module are to teach the students how to work with a micropipette, deepening relevant biological and chemical knowledge (e.g., cell biology, staining techniques, autofluorescence), and how to design an experiment. We recommend that the educator choose at least two to three sample preparation exercises, according to duration (see Table 2 and Supplemental Material 4), available supplies, and relevant learning goals. Before starting the sample preparation module, the teacher and students should review the safety information together (see Supplemental Material 1).

1. Dilutions

The sample preparation protocols include a ''micropipette art warm-up'' exercise that introduces the students to micropipetting. The activity allows educators to correct any errors in micropipetting technique at this stage before moving on. In the pilot studies, we found this to be a critical activity because many of the students had never handled a micropipette before and they needed an opportunity to build their skills before the upcoming lab activities. The resource also includes dilution activities that, step-by-step, lead the students through volume calculations and give students the opportunity to familiarize themselves with volume adjustments when using a micropipette. During the pilot studies, we found the dilution activities were beneficial because the

students were given an opportunity to refresh the math skills required for such dilution calculations, which we initially identified as a challenging task for the students. The use of materials employed in scientific laboratories, such as micropipettes and microbeads, was designed to give the students an impression of working in a scientific research environment.

2. Autofluorescence

The resource offers two protocols that are directly related to the concept of autofluorescence. To ensure that the students understand the theoretical background of these protocols, we recommend that educators give a short introduction into autofluorescence before working on these protocols. Depending on the learning goals, different autofluorescent samples can be prepared from materials easily sourced from the students' immediate environment (e.g., *Lilium*, *Eustoma* spp., *Narcissus* spp. [daffodil], fern sporangia, and *Pinus* spp. branches) (8). Additional examples for everyday objects that can be imaged under a fluorescence microscope are highlighter ink, adhesive paper strips, and white paper. This protocol connects the abstract concept of autofluorescence with the students' everyday life in an interactive, curiosity-driven way.

3. Staining

As an alternative to working with autofluorescent samples, nonfluorescent samples can be stained for imaging under the fluorescence microscope. This section of the protocols is suitable to introduce the students to the concept of cell staining in a safe way, without the use of hazardous stains that are frequently used in professional staining methods. The easiest protocol lets the students color everyday objects with highlighter ink. The students' knowledge of staining can be extended further by introducing the concept of specific labeling, such as labeling a specific cell organelle or protein, which can be brought into practice by letting students stain plant samples with turmeric, a natural and safe histological stain that makes the cell walls and other cell parts visible (9, 10).

D. Imaging samples

After preparing samples and building the microscope, the students are invited to use the microscope connected to a computer and its imaging software to capture images of the specimens that they have prepared. Protocols that guide them through this module are advised, because not all software functions are self-explanatory. The most obvious goal of the imaging protocols is that students be able to use the microscope to image and see the samples they have prepared. Imaging with a camera connected to a display (e.g., overhead projector) has the advantage that educators can point out details more easily than under a microscope with a regular eyepiece. Additionally, during imaging, students will use image processing software to learn about the importance of being able to store images and perform image processing functions such as applying a scale bar and measuring intensity. The imaging module can be custom-designed by educators according to the samples that have been prepared and on how strong the focus on image processing should be.

1. Calibrating the microscope

Before using the microscope to image samples, it is advisable to let the students explore the functioning of the microscope first and calibrate it by setting a scale bar, which should be used on all images throughout the imaging module. For this purpose, the camera's software (e.g., FlyCapture2) may be used to take an image of a calibration grid, which has lines of a specific length etched into it. Opening this image in image processing software like ImageJ allows one to set a scale bar corresponding to the length of the lines on the calibration grid. This scale bar can be added to all images taken throughout the project, which will familiarize the students with the standards of thoroughly executed experiments in research.

2. Observing samples

After the microscope has been calibrated, the students can start imaging their own sample slides. To increase the learning effect of this section, each sample imaged can be

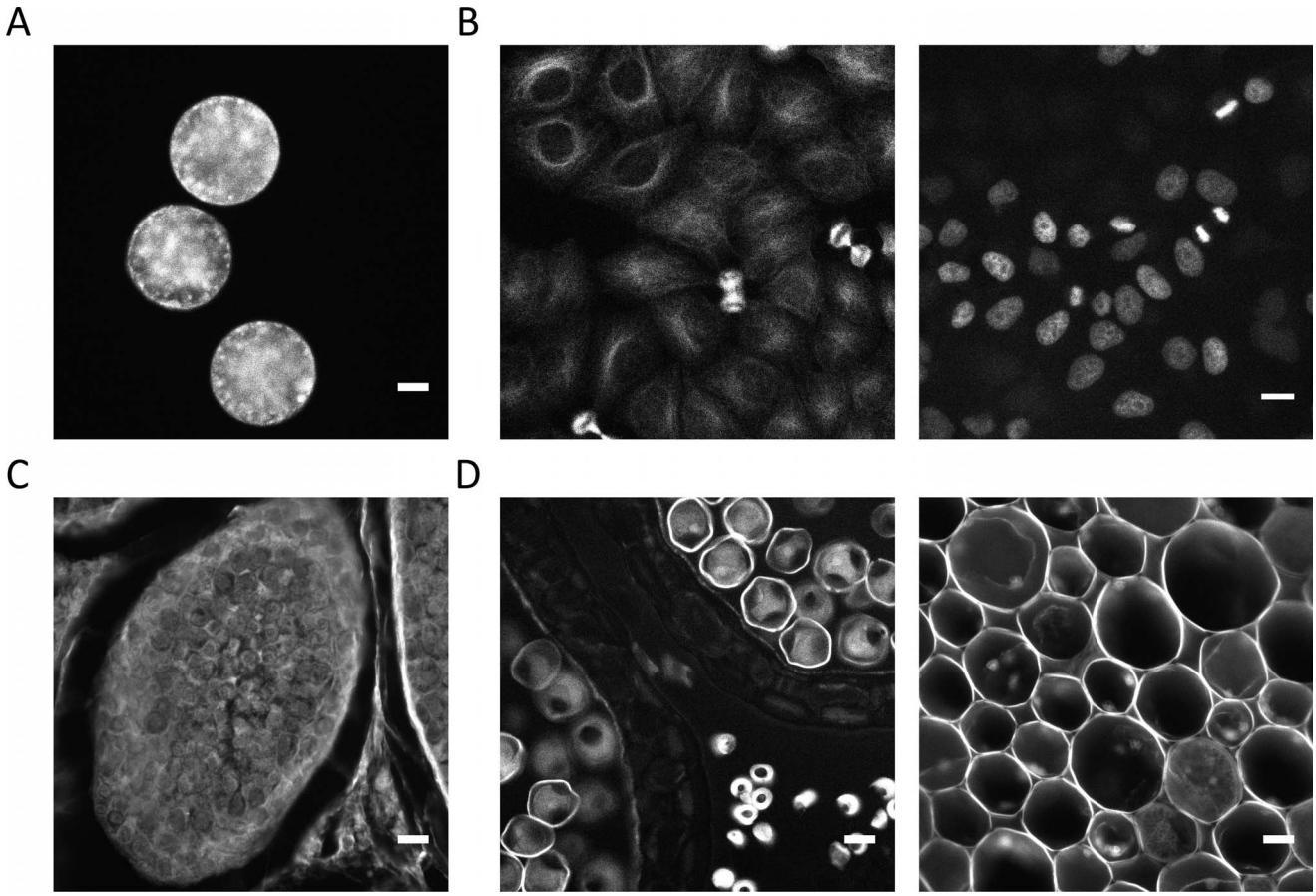

Fig 8. Example of samples imaged with the microscope (bars = 25 μm). (A) Fluorescent beads. (B) HeLa cells with different staining: tubulin (left) and histones (right), showing cells at different phases of the cell cycle. (C) Mouse testis tissue, stained with acridine orange. (D) Plant samples: *Corylus* male flower stained with Etzold stain (left) and *Convallaria* rhizome stained with acridine orange (right).

combined with the visualization of a key concept. Concepts that can be explained during this module include the importance of filters on the filter cage cube by comparing images of a sample taken with and without filters. To illustrate the functioning of specific labeling, samples prepared with turmeric during the sample preparation section can be imaged and stained areas identified. Educators can also reintroduce the concept of autofluorescence by letting the students observe the differences in intensity of different materials or areas of the sample (e.g., everyday objects or plant sample protocols). This exercise can be facilitated by adding color schemes that encode different light intensities. Consequently, the students can pose hypotheses about possible reasons for their observations and thus deepen the students' understanding of the concepts and train their critical thinking.

Instructor tip: To show students how specific labeling can look under a fluorescence microscope when using commercially available fluorescent dyes that target different cell structures, educators could also integrate free online resources, such as the Cell Staining Tool (11). This simulation tool allows students to use their computer to generate images of specifically stained cell parts.

3. Analyzing samples

The last category of imaging protocols focuses on the analysis of samples. Here, the image processing software is used to gather more information from the obtained images. Figure 8 shows examples of images acquired by students and educators with the assembled MiA microscope. In addition to the observations the students made when looking at the autofluorescent samples, they can now go a

step further by quantifying the intensity values. Furthermore, the image processing software offers the option of measuring sizes of specific structures or samples. When working with fluorescent microbeads or stained cells, the students can use the software to count how many of these are visible on their image or measure their size. Allowing the students to work with these quantitative measures will illustrate the importance of quantitation in supporting observations in scientific research.

E. Assessment of learning outcomes, student and educator experience

In the time between March 2018 and December 2020, three MiA pilot studies in the classroom took place. MiA was run as an integral part of lessons for the subject "natural sciences and technology" in a German secondary school. Students were aged 14–18 yr, with mode and average age being 14 yr in 2018 and 16 yr in 2019 and 2020. The class size was between 18 and 19 participants. The student survey data of the second pilot were excluded from evaluation because the survey was taken by students 2 mo after the respective workshop because of operational circumstances at the school. Full survey results of the latest pilot are provided in Supplemental Material 5.

In the student surveys, students rated their overall satisfaction with MiA as high (first and third pilot: 19 respondents indicated an average of 4.4 and 3.9 on a five-level Likert scale, with 1 indicating "very poor" and 5 indicating "excellent"), and the majority of students would recommend the resource to peers (first and third pilot: of 19/19 respondents, 18/14 chose "yes", 1/3 chose "maybe", 0/2 chose "no"). Students commented on the experience, for example, as being "a lot of fun" and "able to see things they would not normally see in school." Students regarded the microscope assembly, sample preparation, and imaging modules as well structured and the provided instructions as appropriate. They also rated the overall difficulty level of the resource as being appropriate to their knowledge and skills. In the debrief session with the teacher, some students noted that they appreciated the level of guidance and learning support they received and preferred this to a completely self-guided learning experience. Overall, this suggests that MiA was taken up well by students and indicates that the resource is equally suitable for students both at the age of 14 and 16. Our evaluation provides evidence that MiA strengthens subject-related conceptual knowledge and competencies, procedural knowledge and competencies, and non–subject-related transferable skills. Results from the in-class written assignment, which assessed conceptual knowledge acquired through MiA and was only run in the third pilot, showed a good understanding of concepts of lens optics, fluorescence, and fluorescence microscopy (third pilot: on average, students received 86% of full points on questions related to lens optics and 71% of full points on questions related to fluorescence and fluorescence microscopy).

In one-on-one debriefings and semistructured post-experience interviews, teachers noted that they observed improvements in practical and experimentation skills, as well as lateral thinking skills linking different scientific concepts across disciplines with each other. Teachers also noted an increase in learning motivation and engagement. Students who might not have shown interest in science before showed a high level of engagement in the practical aspects of MiA, particularly microscope assembly and imaging. Moreover, as reflected by teachers' observation and student reflections in the post-pilot debrief, students showed an increased motivation to learn more about science-related subjects and to deepen their experience in scientific inquiry. The notion that MiA positively drives students to become engaged with the scientific method is further substantiated by the results of the student survey of the first pilot in which the majority of students indicated that they would consider joining a student research project offered by a scientific organization. After the third pilot, the teacher reported a high number of students registering to take up advanced biology classes in the upcoming school year and directly attributed this to the science learning experi-

ence provided by MiA. In summary, our data suggest that MiA has a positive effect on students' science-related competencies and motivation.

MiA was evaluated positively by teachers throughout the development process. In post-training surveys, teachers rated the level of difficulty of all three modules as appropriate. All teachers who attended the training course either planned to use the resource or would consider using the resource in their teaching. Besides using MiA in science lessons, teachers identified extracurricular clubs, individual student research projects and student competitions as suitable learning environments for the resource. During post-experience one-on-one debriefings and semistructured interviews, teachers highlighted two aspects they valued most about the resource. First, teachers appreciated MiA's flexibility with respect to scientific subjects and learning goals. For example, whereas MiA could be used to teach topics directly relevant to the geometrical optics curriculum in physics or the cell biology curriculum in biology in one class, it may be applied to topics linked to more distantly related subject areas such as general optics in a different class. In this way, MiA may be used to revise content from previous years or to introduce new topics that will be covered in more depth later on. Second, teachers appreciated MiA's ability to visualize basic scientific concepts and embed this in a learning experience that links to real-life applications. The following quotes summarize the general sentiments teachers highlighted in one-on-one interviews: MiA allows "students to use high-end research technology while grasping the basic principles behind its function." MiA provides "cutting-edge research experience in a nutshell." MiA shows "care and effort to tune the educational resource to the needs of teachers, very versatile—an extremely helpful support of teaching."

F. MiA beyond secondary schools and future developments

Aside from having developed and refined the MiA resource for use in secondary schools, we have implemented the resource in a variety of other contexts at various locations worldwide, including science fairs, teacher workshops, and PhD-level practicums (see Supplemental Material 6 for an overview). We found MiA to be a versatile teaching and learning tool suited to different formal and informal learning environments.

In addition to the different formats in which the resource can currently be used, we are also developing new student protocols. The new classroom practicums will interlink fluorescence microscopy with the areas of bioinformatics, cell biology, and molecular biology. We believe that the diversity of protocols we offer will enable educators to apply MiA with different learning goals at different educational levels and settings. The current setup of the fluorescence learning microscope can easily be adapted to cater to other types of imaging protocols and support more advanced level imaging. The microscope may also be supplemented by an Arduino chip to control time-lapse imaging, as already tested in one of our activities. Moreover, future materials will include recommendations for setups that use MiA for different scales of magnification, staining protocols, sample handling, and imaging. We would like to encourage educators and other users to play an active role in shaping the development of the MiA resource. We envisage establishing a MiA community that facilitates the exchange of new user-produced protocols, experiences, and future ideas.

V. CONCLUSION

In this paper, we presented an interdisciplinary educational resource centered around a research-grade fluorescence microscopy kit that students assemble fully from individual components and use to image biological samples and make scientific observations. We have successfully used the resource in structured secondary school pilots, as well as in a variety of other contexts, including science fairs for the general public, PhD-level optics courses, and scientist training. Educators particularly appreciated MiA for its flexibility regarding learning goals and its ability to

create a learning environment that allows students to experience high-end technology with real-life applications. The equipment is relatively inexpensive (the whole microscope kit amounts to 1800–3000 euro [∼$1500–$2500], depending on the preferred configuration) and is ready-to-use with only minimal additional requirements (access to a laptop and electrical socket). Thanks to its design, it can be operated in different environments, without the need of an optical table or strict low-light conditions.

Recent years have seen the development of other inexpensive, easy-to-use microscope systems, such as the foldscope (12), a LEGO-based microscope (13), the Community Microscope Kit (14), and the more advanced Raspberry Pi–based FlyPi microscope (15). These systems are ideal for broad community use and massive scale detection purposes, providing direct instructions for assembly and immediate use. However, they are not designed for inquiry-based learning with respect to microscope assembly but rather focus on quick use. Recently, Kemp et al. (2) presented a hands-on optics curriculum for undergraduate students to teach fundamental concepts of optics and microscopy through a hands-on kit. This curriculum offers students the experience of learning from mistakes and contributes to training future innovators. In addition to adopting a similar hands-on, inquiry-based approach to learning, MiA focuses on the key role of fluorescence microscopy in life sciences research and offers an interdisciplinary educational resource that can already be used from the age of 14 yr. It is an excellent tool, both to teach academic knowledge and science-related competencies and to create a collaborative learning environment for learners with diverse backgrounds and interests. Its multidisciplinary nature ensures various access points for educators and students. MiA invites students to "experience cutting-edge research in a nutshell," helps learners understand the basic principles of scientific explorations, and relates these concepts to the application of scientific data and results beyond basic research. We feel that, especially in times of public health challenges, it is important to make young and older learners aware of the powers and challenges of scientific research and its implications on the future development of society.

SUPPLEMENTAL MATERIAL

All Supplemental Material is available at: https://doi.org/10.35459/tbp.2020.000171.s1.

AUTHOR CONTRIBUTIONS

GP, LK, DM, LW, RP, and AS designed and developed the educational resource. GP, EH, DM, LW, and AS were instructors in the Microscope in Action school pilot. EH, LK, and AS analyzed survey data. GP, EH, LK, DM, and AS wrote the manuscript. All authors reviewed and edited the manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Giulia Mizzon (EMBL) for her contributions to the educational resource development in the initial stages of the project and for supporting pilot workshops. Philipp Hoess and Sabine Reither (both EMBL) supported the development of learning materials and the pilot workshops. The authors acknowledge Benedikt Best, Iria Regueiro Carrera, Joshua Lindner, Miriam Reiss, Dmitry Richter, Lina Streich, and Jin Wang (all EMBL) for supporting pilot workshops and Joshua Lindner and Julianna Varga (all EMBL) for contributing to the development of learning materials. The authors acknowledge Shweta Gaikwad (EMBL) for contributing to resource development and to the analysis of survey data. The authors are grateful for the pupils and teachers at Internationale Gesamtschule Heidelberg who participated in pilot workshops and tested the educational resource. The authors thank the Content and Design teams of the Communications department, the Photolab, the Mechanical Workshop, and the Electronic Workshop at EMBL for their dedicated support. The authors acknowledge the Office of Resource Development of EMBL for fundraising support, and EMBLEM Technology Transfer GmbH for providing legal advice. The project was financially supported by the Dr. Rolf M. Schwiete Foundation, the Zukunft Metropolregion Rhein-Neckar e.V., EMBLEM Technology Transfer GmbH, and the Friends of EMBL program.

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