The Biophysicist

4f Koehler Transmitted Illumination Condenser for Teaching and Low-Cost Microscopic Imaging

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ABSTRACT Transmitted light imaging is an important tool in biophysics for applications that include sample analysis, recording samples whose viability is compromised by high levels of illumination (e.g., live cell tracking), and finding regions of interest in a sample. Koehler transillumination is a powerful illumination method used in commercial microscopes; yet commercial Koehler condensers are expensive, are difficult to integrate into tabletop systems, and make learning the concepts of Koehler illumination difficult because of their closed-box nature. Here, we show a protocol to build a simple 4f Koehler illumination system that offers advantages with respect to commercial condensers in terms of simplicity, cost, and compatibility with tabletop systems, such as open-source light sheet fluorescence microscopes. We include step-by-step instructions that can be followed by advanced undergraduate or graduate students without experience in optics on how to align and assemble the illuminator, along with a list of the necessary parts for assembly. We also include supplemental material that describes 4 supporting educational activities students can conduct with the apparatus and helps in the understanding of key concepts relevant to Koehler illumination and optics. The performance of the system is comparable to that of commercial condensers and significantly better, in terms of illumination homogeneity and depth of field (optical sections are possible), than that of LED flashlights, such as those found in low-cost diagnostic devices and tabletop systems.

KEY WORDS bioengineering; biomedical engineering; laboratory exercises; teachers and students of graduate and upper-level undergraduate courses in biophysics and related sciences; optical microscopy and imaging

I. INTRODUCTION

Transmitted illumination is one of the most commonly used light microscopy techniques. It is also used as a complementary method to find samples in other kinds of microscopy, such as fluorescence microscopy, thanks to its gentleness with the samples and the complementary information it provides. August Koehler's introduction of his illumination system in the late 1800s radically improved the attainable quality of microscopic imagery (1). Before Koehler's system, samples were unevenly illuminated, both spatially and angularly, typically under critical illumination, in which the lamp is placed at the back focal plane of a condenser lens. This resulted in conjugate images of the lamp on the sample, a strong spatial dependence of resolution, the projection of shadows, and glare from the illumination source inside the detection objective, affecting image contrast.

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Koehler illumination significantly improves illumination quality in several aspects, including illumination homogeneity, glare, and resiliency with respect to dust or imperfections on the condenser lens. Moreover, it provides control of the angular profile of the illumination by changes in the numerical aperture (NA) of the condenser. In transmitted illumination, resolution and depth of field depend on the numerical aperture of both the detection objective and the illumination condenser (2); thus, a condenser with a large enough NA will make optical sectioning possible because of the resulting shallow depth of field.

These advantages are highly beneficial even in systems that are not mainly dedicated to transmitted illumination imaging, such as light sheet fluorescence microscopy (LSFM). For instance, when imaging complex 3-dimensional (3D) specimens that have features at different depths, the possibility of adjusting contrast and depth of field with changes in the NA may facilitate navigating through the sample or acquiring complementary views for anatomical reference. From the perspective of low-cost microscopy, Koehler illumination provides several advantages. First, in difficult environments such as those found outside research and diagnostic laboratories, the effect of dust or imperfections in the condenser lens are minimized, reducing image degradation. Additionally, as detailed below, homogeneous illumination makes it easier to analyze samples, especially for automated analysis (3, 4) in the context of low-cost diagnostics (5). Although postprocessing may improve image quality, it is not enough to compensate for limited quality in the recorded data (6). Nonuniform illumination, saturated regions, fluctuating brightness, and excessive cluttering are examples of factors limiting the quality of acquired images (7, 8) and the reproducibility of quantitative analyses made from them (9). The system resulting from this protocol addresses these limitations by ensuring uniform illumination and reduced cluttering from increased NA, which ensures sectioning capability.

From the perspective of microscopes built on optical tables, transillumination condensers are difficult to integrate, and they are relatively costly because they are not fundamental for the core function of the microscope. For example, in our experience and that of others, few labs that do LSFM use proper Koehler illumination for transmitted light viewing (10), because most data comes from fluorescence despite this making it more difficult to correlate anatomy in samples with fluorescence. Sometimes LSFM uses transmitted light advantageously, such as optical tomography (11) and optical projection tomography (12, 13), but the first requires another water immersion objective, making it more difficult to implement, and the second is not commonly used in nonspecialist labs. Finally, in the area of low-cost microscopy, many systems work with a flashlight to illuminate the sample (4, 14–16), limiting the potential of those systems in terms of image quality. Our proposed illuminator provides a simple solution to overcome these limitations in custom-built microscopes.

Here, we show a protocol to build a 4f Koehler illumination setup that can be made for applications in teaching, low-cost diagnostics, and LSFM. The 4f implementation simplifies alignment and the understanding of the method. This system is highly intuitive, illustrating the need for even illumination while simplifying its design and operation. The protocol can be followed by inexperienced users as they build and align the illuminator.

Briefly, the system consists of a light-emitting diode, whose light is collected and collimated with an aspheric lens. Two convex lenses in a 4f configuration relay the focal plane of the aspheric onto the focal plane of the detection objective of the microscope, where the sample is placed. Irises at the common focal planes of the lenses act as field and aperture diaphragms, as shown in Figure 1.

II. SCIENTIFIC AND PEDAGOGICAL BACKGROUND

A great deal of biophysical science developments have depended on good-quality microscopic images and, more recently, on the development of new microscopy methods or

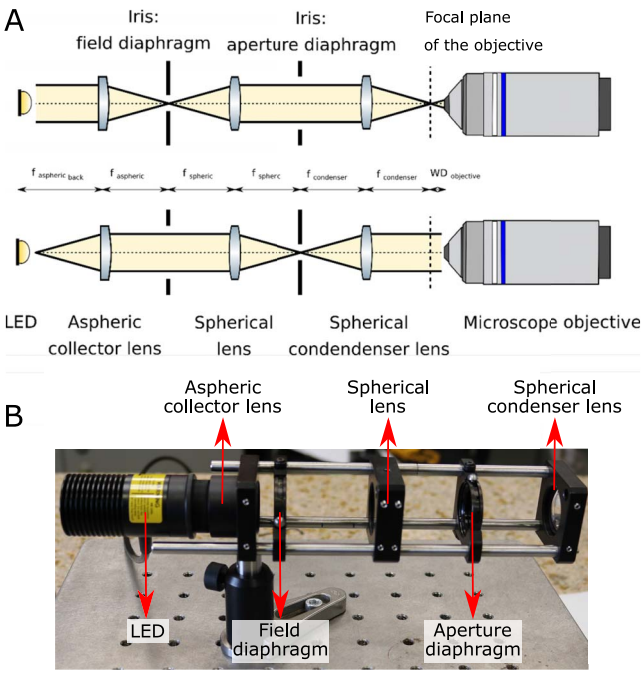

Fig 1. (A) Schematic and (B) photograph of the optical setup. The system is all configured in 4f, conjugating 2 sets of planes. The upper section of the diagram illustrates the conjugation of the sample plane with the field diaphragm and (not shown in the schematic) with the image plane on the camera sensor. In these planes the image of the LED is lost after having been Fourier transformed by the collector lens and spatially homogenized by the diffusing surface.

the adaptation of microscopes for specific experiments. One can argue that students and researchers in biophysics should at least have a basic understanding of the different methods in microscopy, as well as some experience with them. Knowledge and skills in microscopy help biophysicists not only because they can make a better use of microscopes, but also because it enables them to choose the right commercial tools to solve a scientific question, or even build their own microscope to cut costs, or to develop novel solutions and methods to answer specific scientific questions. An initiation in optical microscopy at the undergraduate level or the early graduate level can be useful for this purpose. The protocol we present here can be used as an activity to apply the theory of Koehler illumination, a fundamental concept in microscopy.

From the teaching perspective, in our experience and that of others (17), Koehler illumination is one of the teaching challenges in introductory microscopy courses for students at the advanced undergraduate level. Commercial Koehler illumination condensers have drawbacks for teaching: because of their closed-box nature, it is difficult to see what goes on inside; additionally, the optical complexity required to make them compact requires ray-tracing that is difficult to perform on paper, obscuring the working principles of the condenser.

This tutorial can be used in undergraduate optics courses or graduate microscopy courses. In the latter, students with backgrounds in biology and engineering do not need previous experience with optical systems and should be able to follow the guide. Moreover, the design of the proposed illuminator illustrates the concepts of plane conjugation, field homogeneity, illumination aperture, and depth of focus, which are key in microscopic imaging and its biophysics applications.

III. MATERIALS AND METHODS

Table 1 presents a list of materials by Thorlabs (Newton, NJ), but note that other manufacturers such as Qioptiq (Linos Microbench), Edmund Optics (Optical Cage System), Newport (Optics Cage Plus with a flange-mounted iris and an adapter), and OptoSigma (Optical Cage Systems) offer similar products. Additionally, it is possible to use a rail system, making sure to mount the rails on a support that allows for vertical alignment. In this case, the rods would be exchanged by a rail, with compatible lens mounts, irises, and an LED.

A. Assembly and alignment protocol

1. Collimate the LED light source by placing a diffusive aspheric lens in front of a mounted LED.
   (a) Mount the aspheric collector lens in an adjustable-length lens tube. Make sure the rounded face points away from the LED in order to maximize light collection efficiency; this position minimizes the angles at which the light rays from the LED enter the lens. In turn, small angles reduce the spherical aberration of the

Table 1. List of parts.

Name of material/ equipment	Company	Catalog no.	Comments/description
Mounted white LED	Thorlabs (Newton, NJ)	MWWHL4	LED illumination.
LED controller	Thorlabs	LEDD1B	LED illumination.
Power supply for LED controller	Thorlabs	KPS101	LED illumination.
SM1 lens tube	Thorlabs	SM1L03	LED illumination.
Adjustable lens tube	Thorlabs	SM1V05	LED illumination.
SM1 external retaining ring Ø1″	Thorlabs	SM1RR	LED illumination.
Diffusive aspheric condenser lens; $f = 20.1$ mm; collector lens	Thorlabs	ACL2520U-DG6	LED illumination. It is important to use an aspheric lens to limit the effects of increased spherical aberrations at short focal lengths. The short focal length (FL) ensures a high collection efficiency.
Lens mount (cage plate)	Thorlabs	CP02/M	Koehler optics. 2× (buy 2). If you work with imperial parts, buy the non-"/M" version.
Cage plate (lens mount) thick	Thorlabs	CP02T/M	Koehler optics. Thick for better support of the assembly on the optical post. If you work with imperial parts, buy the non-"/M" version.
Iris (for cage system)	Thorlabs	CP20S	Koehler optics. 2×.
Rods, 8 inches	Thorlabs	ER8	Koehler optics. 3×.
50 mm FL, achromatic lens	Edmund Optics (Barrington, NJ)	47-637	Koehler optics. 2×. Achromats ensure better alignment of the focal lengths for different colors in the spectrum of the white light LED and have reduced spherical aberrations. Alternatively, you can use nonachromatic lenses for a penalty in image quality, at lower cost.
Optical post	Thorlabs	TR50/M	System support. If you work with imperial parts, buy the non-"/M" version.
Optical post holder	Thorlabs	PH40E/M	System support. If you work with imperial parts, buy the non-"/M" version. If you do not have an optical table, we recommend you get 2 post holders and 2 optical posts for better support. If the table is made of steel you can use magnetic post holders to keep the system in place without bolting it to the table.
Translation stage	Thorlabs	PT1B/M	System alignment. Optional. Buy 2 or even 3 (PT3/M) for simpler alignment with the microscope. Buy a nonmetric ("/M") version if your optical table is in inches.
Clamping fork	Thorlabs	CF125	System attachment to optical table.
M6 × 1.0 stainless steel cap screw, 12 mm long, pack of 25	Thorlabs	SH6MS12	Buy a nonmetric (imperial) version if you buy an imperial stage and have an imperial table.

system and the reflections that increase with angle at interfaces. If it were backward, reflections would be present outside the central region. Hold the lens in position with the retaining ring.

(b) Attach a lens tube to the LED mount. This tube makes it easier to adjust the distance between the LED and the collector lens.

(c) Connect the LED to the power supply and turn it on. Adjust the distance between the collector lens and the LED by rotating the mounts with respect to the other until the least-diverging, non-converging beam is achieved, as shown in Figure 2. Such a beam should not decrease in size at large distances (approximately tens of centimeters to 1 m) from the lens, but its size should increase as little as possible.

(d) Fix this distance with a 1-inch external retaining ring. Because the LED is a diverging and relatively large light source, it is not possible to collimate the beam completely. Do not worry if you see an increase in size, but do try to minimize it. It is possible to do some fine-tuning at the end of the protocol.

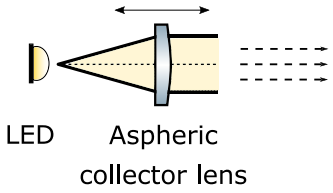

Fig 2. Collimation of the LED. Move the collector lens until you collimate the LED, so the light does not diverge too much. You should not have an image of the LED. If you do, you are not collimating the beam but focusing it.

(e) Mount the LED on a lens mount and insert rods in this plate.

2. Insert the field diaphragm.
   (a) Insert an iris in the cage rods.
   (b) Close the iris's diaphragm to its minimal aperture.
   (c) With the help of a photodiode power sensor placed on the other side of the aperture, move the iris to the position of maximum intensity, as shown in Figure 3. Alternatively, you can use the instructions in section III.B.2 to place a camera in front of the iris to measure (in arbitrary units) the light intensity or use a light meter app on your smartphone.
   (d) Fix the iris in this position with the cage screws.
3. Insert the first lens.
   (a) Place the first spherical lens (f = 50 mm achromat) in a lens mount.
   (b) Insert the lens mount in the rods. Make sure that the rounded face of the lens faces away from the LED light source to reduce the spherical aberration of the system by reducing the incidence angles of the light rays onto the lens, reducing the deviations from the paraxial approximation for spherical lenses. See Supplemental Activities S1 through S4 for more details.

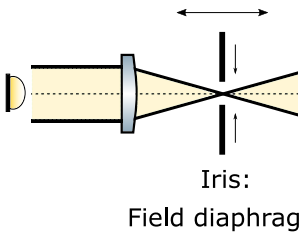

Fig 3. Placement of the field diaphragm (iris) at the focal plane of the collector lens. Using a power meter or a light meter on your phone, move the iris until you find the maximum light intensity, which should be the same as the focal length of the condenser lens.

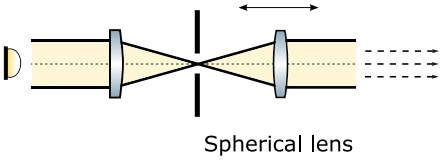

Fig 4. Once again, you want to collimate the beam with this lens. Because the closed diaphragm is more similar to a point source than the original LED, it should be easier to collimate than in the first step.

   (c) While keeping the field diaphragm closed, as shown in Figure 4, adjust the distance from the achromat to the iris until a collimated beam is achieved. This distance should be about the focal length of the achromat. One way to check the collimation is to take a piece of paper and check how much bigger the beam becomes as you move the paper away from the lens to a larger distance of about 1 m. As with the collector, you want the size of the beam to increase as little as possible.
   (d) Fix the position of the lens with the cage screws on the cage plate.
4. Insert the aperture diaphragm.
   (a) Open the field diaphragm to its full aperture.
   (b) Insert an iris after the first spherical lens.
   (c) Close the second iris (the aperture diaphragm) to its minimal aperture.
   (d) Analogous to section III.A.2, adjust the position of the aperture diaphragm, as shown in Figure 5, until achieving the maximal intensity on the photodiode power sensor.
   (e). Fix the iris in position as before.
5. Insert the condenser lens.
   (a) Keep the field diaphragm open and the aperture diaphragm closed.
   (b) Insert the second spherical lens, which will work as a condenser lens (f = 50 mm achromat) in the lens mount. This time, the rounded face of the lens should face toward the light source. As before, this reduces the incidence angles of the light rays that illuminate the sample on the

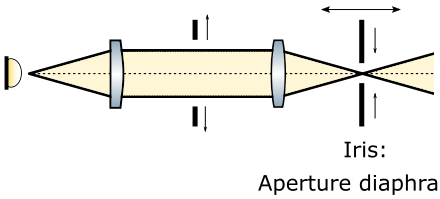

Fig 5. Placement of the aperture diaphragm at the back focal plane of the condenser lens. Once again, move the iris, which will work as the aperture diaphragm, to about 1 focal length of the spherical lens. Make sure you open the other iris (field diaphragm) and move the second iris until you maximize the intensity on your power meter or phone.

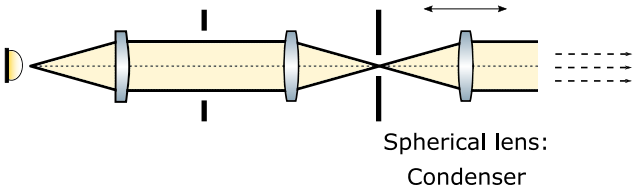

Fig 6. Placement of the condenser lens. Keep the field diaphragm open and the aperture diaphragm closed. Insert the second spherical lens, with its curved face pointing toward the light source, in the cage plate and adjust its position until a collimated beam is achieved. As before, do not expect perfect collimation, but try to achieve the least diverging beam.

second lens, reducing the spherical aberration of the illumination and helps to make a smaller focal spot, reducing the depth of field.

(c) As in section III.A.3, adjust the position of the lens until a collimated beam is achieved, as shown in Figure 6. Note that there is a position in which you can focus on the diaphragm of the condenser (project its image on the wall), but that is not the correct position. This imaging of the diaphragm will happen when the lens is between f and 2f (assuming the wall is more than 2f away from the system, as it should be) and means you need to get the lens a little closer to the iris.

(d) Fix the position of the lens mount as before. The Koehler transilluminator is now ready.

B. Illuminating the sample

1. Finely align the illumination LED for improved illumination homogeneity.
   (a) With the system in place or a camera in front of the condenser lens at its focal length, use a digital camera to get a profile of the intensity across the field. A live profile can be obtained by jointly using μManager (UCSF, San Francisco, CA) and ImageJ (NIH, Bethesda, MD) (19); draw a line across the field of view, plot the intensity profile, and set it to live mode (ImageJ > Analyze > Plot Profile > live).
   (b) Adjust the position of the LED diode as in section III.A.1 by rotating the lens tube until you achieve the flattest intensity profile and then lock the lens tube in place with the outer rings.
2. Align the illumination system with the rest of the microscope.
   (a) Attach a 12.7-mm optical post to the lens mount containing condenser lens and mount the assembly on a post holder. Make sure the height of the illuminator is the same as the detection subsystem of your microscope.
   (b) Partially close the field diaphragm until a shadow of the blades of the diaphragm appears on the camera or eyepiece (this is the first step of Koehler alignment). You want to focus and center the diaphragm, while keeping the optical axis of both systems aligned (i.e., the illuminator is parallel to the detection objective), as shown in Figure 7. Carefully move the illuminator along the optical axis until you are able to focus on its blades; then, progressively close the blades and follow their edge by adjusting the position of the illuminator in the 2 directions perpendicular to the optical axis until it is closed and centered as seen by the camera or eyepiece. The image of the field diagram should appear centered and in focus (see Supplemental Fig S3). Optionally, place the illumination system on an XY or XYZ translation stage to make these alignments easier and more precise.
   (c) Open the diaphragm until it is just at the outer edge of the field of view in the camera or the eyepiece of the micro-

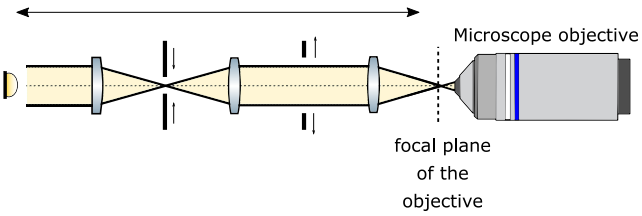

Fig 7. Coupling the illumination to the rest of the microscope. Close the field diaphragm and open the aperture diaphragm. Move the illuminator perpendicularly to the optical axis of the microscope until an image of the field diaphragm appears on the camera. Move the illuminator along the optical axis (shown as a horizontal dotted line) until the image of the field diaphragm appears sharp, and center it by translating the illuminator. Having the setup on a micrometric translational stage makes this step easier, but this can also be achieved manually with some patience.

scope. This reduces stray light in the system with respect to a fully open diaphragm.

(d) Fix the illumination system into position by placing a fork on the post holder and tightening the screw to a hole on the optical table.

3. Place a sample in the microscope. After conjugating the field diaphragm with the image plane of the microscope objective, the illumination should be spatially homogeneous. Once the sample is inserted, however, the position of the illuminator will have to be adjusted along the optical axis to correct for the additional optical path of the microscope slide or sample holder.
   (a) Place the sample in the optical path and open the field diaphragm.
   (b) Focus the sample with the eyepiece or camera by moving it along the optical axis. The sample should be mounted on a micrometric stage to allow for fine, repeatable movements, and if it is mounted on a slide and coverslip, the coverslip side should be oriented toward the microscope.
   (c) With the sample in focus, close the field diaphragm. It should appear slightly blurry because of the introduction of the microscope slide in the optical path.
   (d) Move the illuminator along the optical axis until both the field diaphragm and the sample are simultaneously in focus. When this is achieved, the sample plane and the field diaphragm plane will be conjugated. Open the field diaphragm again to the edge of the field of view. The sample is now illuminated by Koehler illumination.

IV. RESULTS

A. Field homogeneity

We evaluated the homogeneity of the illumination by placing a camera at the focal plane of the condenser lens to eliminate the effects from the apodization of the detection objective (decreasing intensity toward the edges). To avoid saturation, we reduced the exposure of the camera to 30 ms and placed a neutral-density filter (optical density [OD] = 2) at an intermediate plane between the 2 spherical lenses. This intermediate plane did not coincide with the focal planes of the lenses to avoid projecting images of possible imperfections on the neutral-density filter. Figure 8 compares the homogeneity of the illumination over a field of 1300 μm × 1300 μm, which corresponds to the field of view achieved with a large-chip scientific CMOS camera and a 10× objective. The 4f system achieves a more homogeneous field, with differences in intensity of up to 5%, compared with ∼20% for the LED flashlight in direct illumination. Smaller fields of view will result in better homogeneity of illumination.

B. Depth of field

In many cases, high–numerical aperture illumination is desirable, because it shortens the depth of field, reducing image clutter and making optical sectioning possible for somewhat thick samples. We compared the 4f system against a commercial system, both at their highest NA (∼0.2 and 0.52, respectively) and with an LED flashlight (whose NA is not adjustable). We imaged the petals of an orchid (*Phalaenopsis amabilis*) at 10× magnification with a detection NA of 0.3 at different depths, as shown in Figure 9. Under these conditions, it is possible to distinguish features in some planes that are not visible in other planes (e.g., the nuclei and fibers that are observable

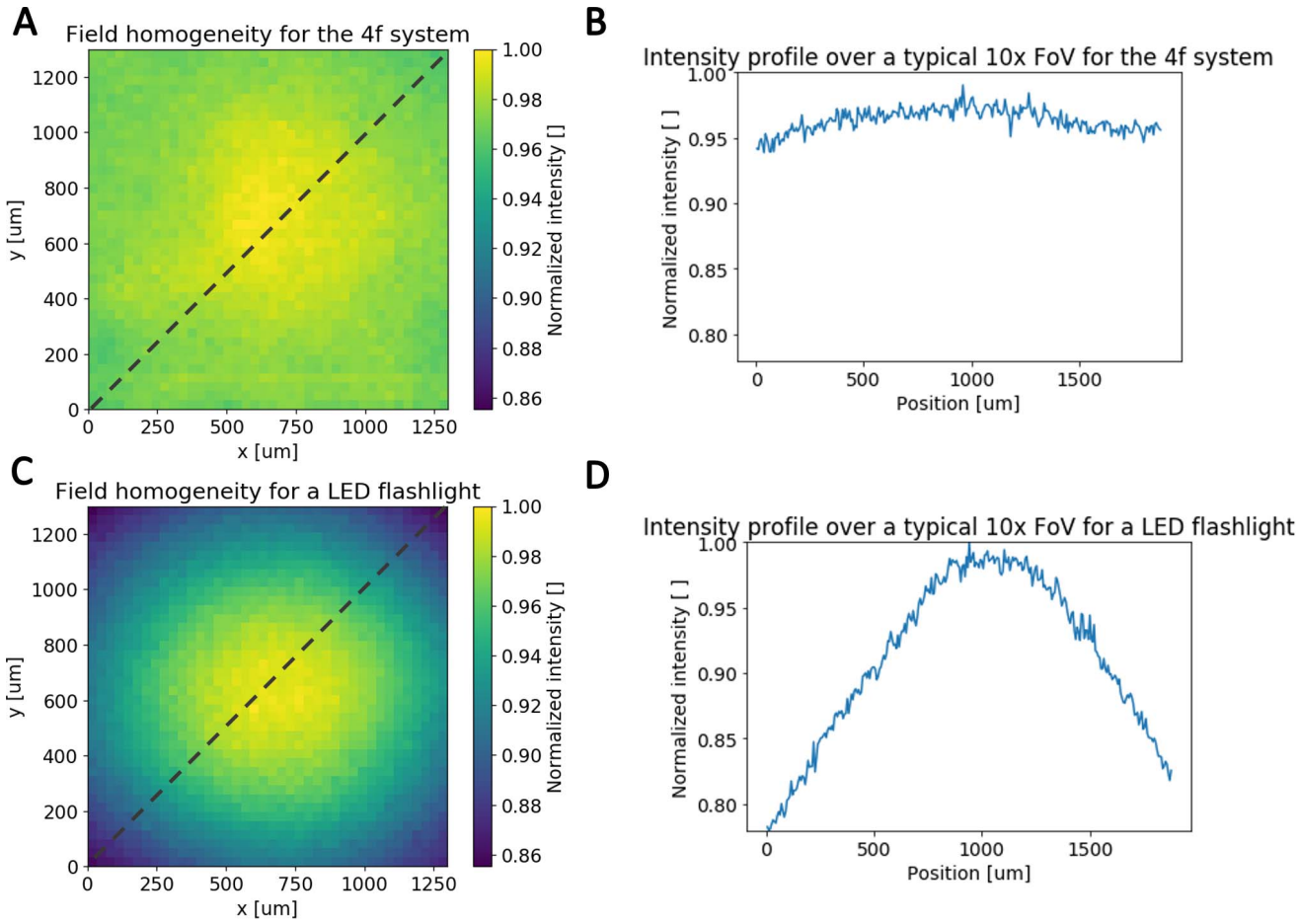

Fig 8. Field homogeneity. (A) Relative intensity profile for the system presented here over a typical field of view with 10× magnification. (B) Intensity profile along the dotted diagonal in panel A. (C, D) Analogous to panels A and B, but for a single LED flashlight in direct illumination.

at $z = 0$ μm and $z = 20$ μm are not observable in the other planes) when illuminating with the 4f and the commercial systems. When illuminating with the LED flashlight, all features appear overlain, making it impossible to distinguish specific structures such as nuclei and cell walls, among others. This is essentially because of the tradeoff between contrast and depth of field, as shown in Supplemental Figure S1. Similarly, when imaging zebrafish, it becomes possible to distinguish muscle fibers despite the presence of additional structures at other depths, as shown in Figure 10. When imaging bacteria, it becomes easier to single out individual bacteria (Supplemental Fig S2). This system achieves an NA comparable, although not as high, as that of the commercial system, which is evidenced in the higher contrast and longer depth of field of the views in the top row of Figure 9.

V. DISCUSSION

We presented a brightfield 4f Koehler illumination system for microscopic imaging and a protocol for its assembly that can be followed by undergraduate and graduate students in sciences and engineering. The protocol makes use of cage optics to simplify assembly, as well as optical alignment and alignment with a microscope. We examined the spatial homogeneity of the illuminator and showed that over regions of 1300 μm × 1300 μm the field is homogeneous; intensity varies less than 5%. Additionally, the reduced depth of field of the resulting assembly compares well against a commercial system at magnifications such as 10× in terms of image quality and sectioning, while significantly outperforming an LED flash-

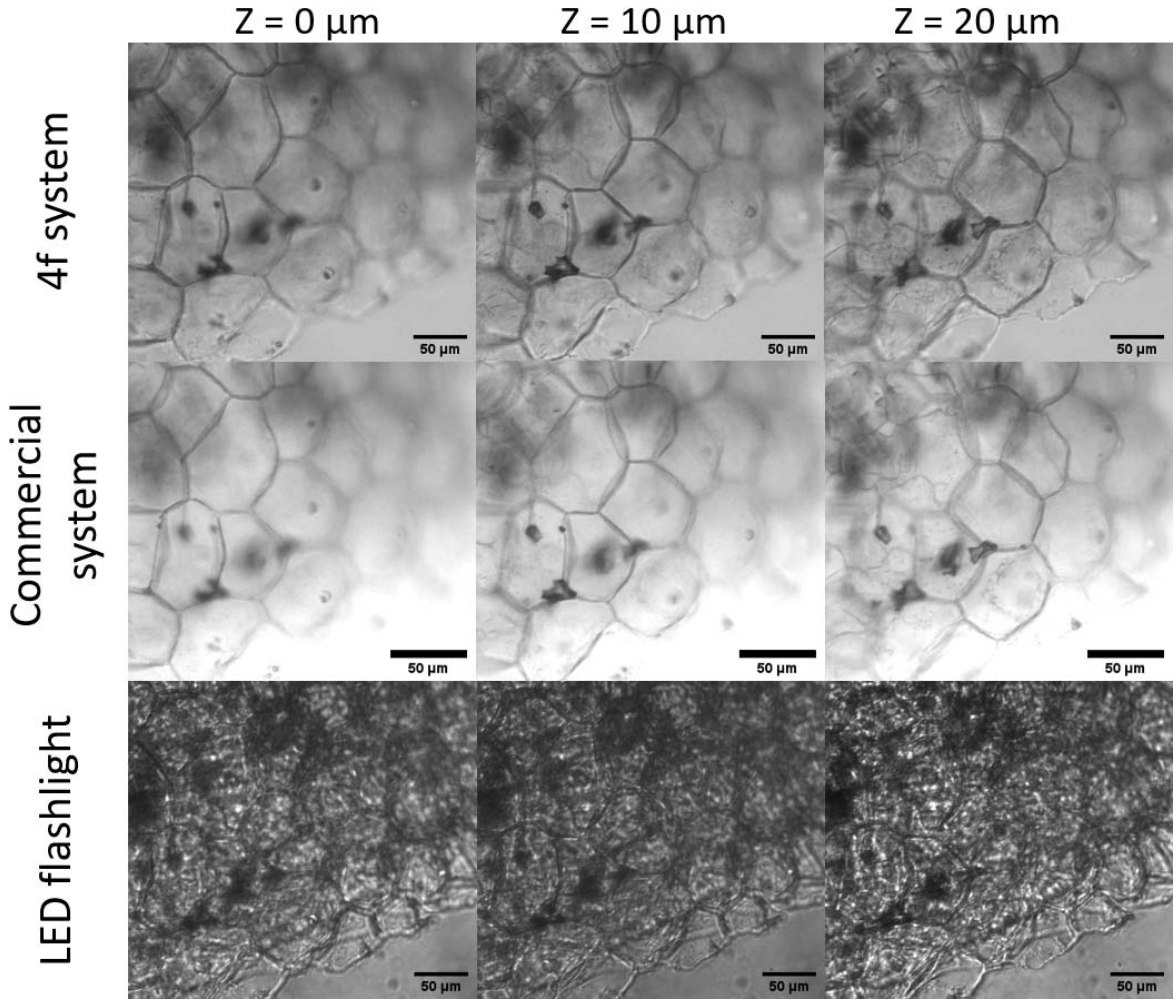

Fig 9. Sectioning capability of the 4f system with respect to a commercial condenser and an LED flashlight in direct illumination. Different optical planes (sections) of a petal from *Phalaenopsis amabilis*, captured with a 10× 0.3 numerical aperture (NA) air objective. (Top row) 4f system with the condenser aperture fully open (NA = 0.2). (Middle row) Commercial Koehler system with the condenser aperture fully open (NA = 0.52). (Bottom row) Single LED source. Scale bar 50 µm.

light–based system with 40× water immersion detection.

This illuminator can be used for teaching because it illustrates the inner functioning of Koehler illumination systems, which generally operate as a closed box and are obscure to many microscope users. Additionally, the 4f configuration used in the protocol makes it easier to align and understand the concept of conjugate planes and how illumination is controlled in the Koehler configuration (complementary activities). In a non-4f system, drawing the optics becomes more complicated, obscuring the inner workings of Koehler illumination. Building the condenser is a complementary laboratory activity to Koehler alignment that can be completed in 2–4 h and additionally gives students basic skills in optics. The condenser can then be used for biophysics experiments that require reduced depth of field (e.g., multiple cell layers, as shown in Figs 9 and 10) or for which critical illumination makes automated analysis difficult or impossible. For instance, the reduced depth of field and homogeneous illumination facilitates automated analysis during cell tracking experiments.

We include Supplemental Video S1 with detailed step-by-step assembly and alignment instructions, which should allow students with little or no experience with optical systems to build the illuminator and integrate it with a microscope in the course of a few hours,

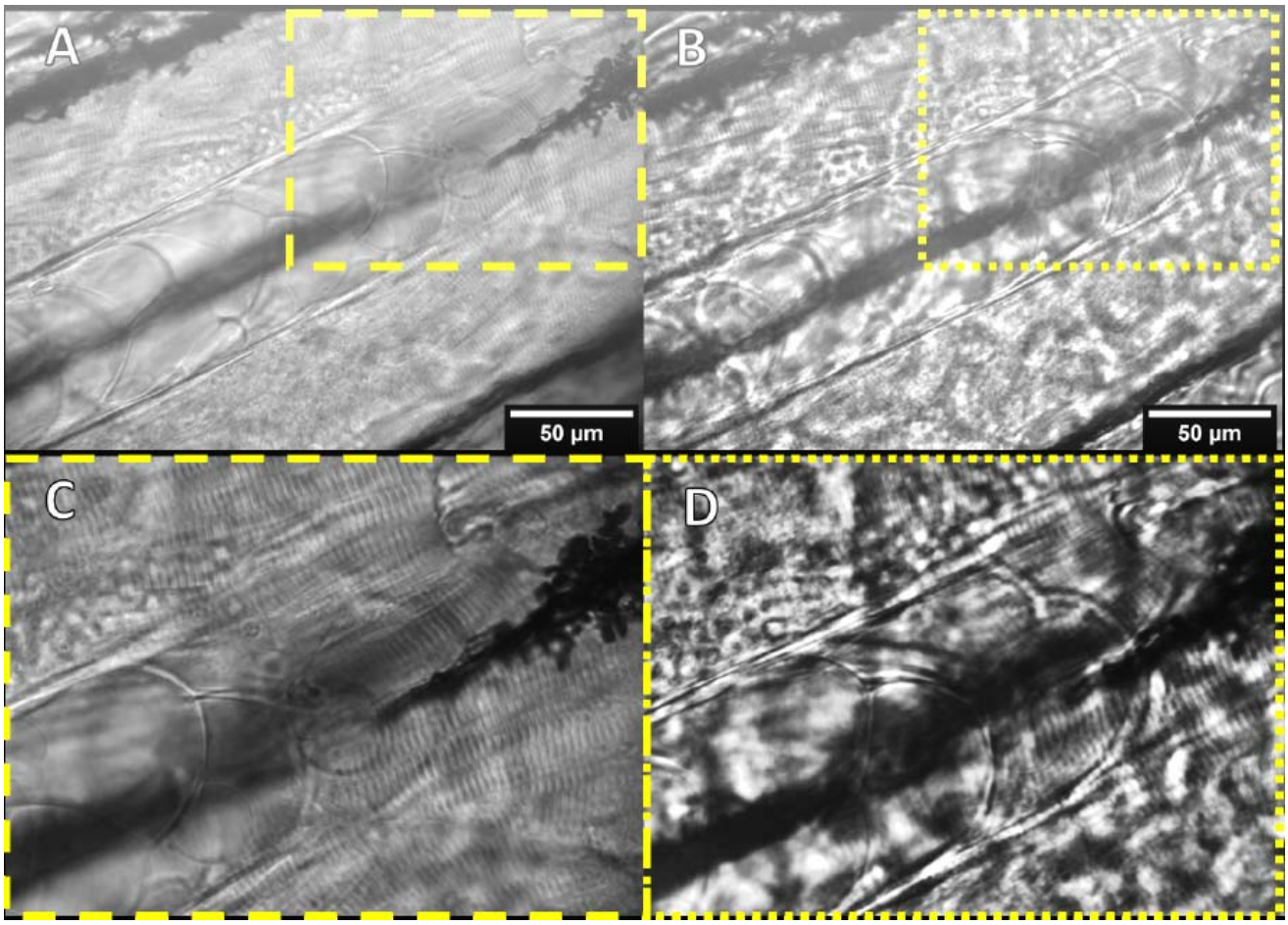

Fig 10. Biophysical application: Notochord of the zebrafish under Koehler and critical illuminations. The images were taken by light sheet fluorescence microscopy (LSFM) with a 40× 0.8 numerical aperture (NA) water immersion objective. (A) The 4f system with full NA. (B) An LED flashlight in direct illumination. (C, D) Regions within the dashed rectangle in panels A and B, respectively. Skeletal muscle fibers are distinguishable in panel C, but hardly in panel D.

namely one laboratory session. The video includes visualizations of the expected results at the intermediate steps, allowing the students to work with the setup autonomously.

We have also included the following additional activities with the condenser to improve their understanding of Koehler illumination: Supplemental Activity S1 illustrates the tradeoff between contrast and depth of field. Supplemental Activity S2 explores how misalignment affects image quality. Supplemental Activities S3 and S4 help understand the importance of Koehler illumination with respect to critical illumination by using the concept of reciprocal planes. Supplemental Activity S4 on lens orientation and spherical aberration further illustrates the reasoning behind lens orientation in optics.

Although the protocol presented here is simple to implement, there are limitations in terms of depth of field, cost, and size, addressed as modifications to the protocol as follows. The NA of the condenser is given by the focal length of the last lens, which in this case is 50 mm. The resulting NA is $n \times \sin[\arctan(10\ \text{mm}/50\ \text{mm})] = 0.2$, given that the maximum free aperture of the condenser lens is ∼20 mm (radius 10 mm). A commercial condenser will provide a larger NA, but for many applications, 0.2 provides sufficient sectioning capability (<15 μm). Changing the focal length of the condenser and relay lens is a relatively simple modification that will provide a reduced depth of field, if required, at the expense of reduced working distance. Note that it is important to change the focal length

of both to ensure the pupil of the condenser lens is full, and the focal length of both must be longer than that of the collector (20 mm here).

The approximate cost of this simple-to-implement version of the illuminator is USD$1500, and at this price point, it starts being comparable in optical quality and cheaper than a full-fledged commercial standalone condenser, whose cost, including a state-of-the-art LED, can easily be up to USD$7000 (see Supplemental Table S1 for details), but costlier than some integrated condensers. Depending on the available budget, know-how, and experimental requirements of a lab, it is possible to reduce the cost significantly. For example, in Supplemental Table S2 we include a list of parts to build the system for under USD$500, using off-the-shelf parts and a 3D-printed adapter to build the illumination, which is the main expense. This lower cost system requires basic electronics know-how, as well as the ability to operate a 3D printer and assemble the electronics of the illumination LED, tasks that undergraduate students with a basic electronics course can do.

The size of the illuminator is larger than that of most commercial condensers, potentially limiting its applications in setups with reduced space, mainly because of the 4f configuration. It is possible to modify the protocol with an additional step by adding a folding mirror after the condenser iris (see additional parts in Supplemental Table S1), and the focal lengths are calculated to allow for this without additional modifications for condenser lenses of f = 50 mm and greater.

The system presented here is compatible with tabletop experiments and instruments. As such, it can easily improve the transmitted light imaging quality in laboratories working with horizontally mounted tabletop microscopes, such as light sheet systems (20, 21), improving on low-cost direct illumination systems (10) in terms of image quality. Besides enhancing image quality in complementary transmitted light imagery, the system can help with sample navigation, provides anatomic context for fluorescent structures (11), and can also improve quantitative fluorescence by reducing the effect of attenuation artifacts (13).

We have shown a simple, yet competitive, transmitted light illumination system with even illumination and reduced depth of field for a relatively low cost. The 4f configuration makes it simple to align and easy to understand, which, in combination with the open nature of the protocol, should help the spread of proper illumination in teaching labs but also in low-cost diagnostics, microscopy, and even research tabletop systems.

SUPPLEMENTAL MATERIAL

A document containing supplementary figures, tables, and supporting activities for students is available at https://doi.org/10.35459/tbp.2019.000113.S1. A video is available at https://doi.org/10.35459/tbp.2019.000113.S2.

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AUTHOR CONTRIBUTIONS

JMW: investigation, visualization, writing (original draft preparation). MFS: conceptualization, funding acquisition, methodology, investigation, supervision, writing (original draft preparation).

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