Low-cost illumination module for multi-channel fluorescence microscopy

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Abstract: Here we present a design to build a low-cost illumination module that combines illumination from three different color LEDs and costs less than a 100 dollars. We provide open-source designs and a tutorial on how to build one. This design uses an X-cube used in commercial projects to combine the light, instead of dichroic mirrors commonly used. This reduces the cost significantly. We provide spectral and power analysis of the combined light, and demonstrate its use in a simple home-built multi-channel fluorescence microscope.

1. Introduction

The fluorescence microscopy has been widely used in the field of biological imaging, such as fluorescence confocal scanning microscopy, which uses lasers as sources of fluorescence excitation lights. Such a highly functional integrated microscope is always very expensive, and is not open source. It's therefore difficult to adapt the acquisition procedure to the experimenter's own requirements, such as the use of phase contrast illumination [1], or the use of different angles of illumination, like Fourier Ptychography Microscopy(FPM) [2].Here we designed a low-cost fluorescent microscope, whose fluorescence excitation light illumination system is completely self-assembled by single LED, X-cube prism for collecting lights and 3D printed modules. Other parts of the self-assembled microscope include two cameras; an LED array that can generate different illumination modes including bright-field, dark-field and phase-contrast illumination of different wavelengths, which can be freely controlled by a Arduino single-chip; a motorized platform; and optical elements such as objective lenses and light filters. The cost of self-built microscope is much lower than that of integrated fluorescent microscope, and LEDs as excitation light source has the advantage of being used at any time without preheating. The whole image acquisition process is automatically controlled by the custom MATLAB program, which has high operability.

2. Design

2.1. Overall design of the fluorescent microscope

The optical path diagram of a standard multi-channel fluorescent upright microscope is shown in Figure.1, in which the X-cube part is our design of the multi-channel excitation light illumination module. The X-cube prism fixed in the 3D-printed bracket can focus maximum three light beams emitted by LEDs of different spectral bands from different directions to the same direction as the excitation light of the sample. When the excitation light of a plurality of wavelength bands is used, the corresponding excitation light filter and emission light filter of a plurality of wavelength bands are needed.

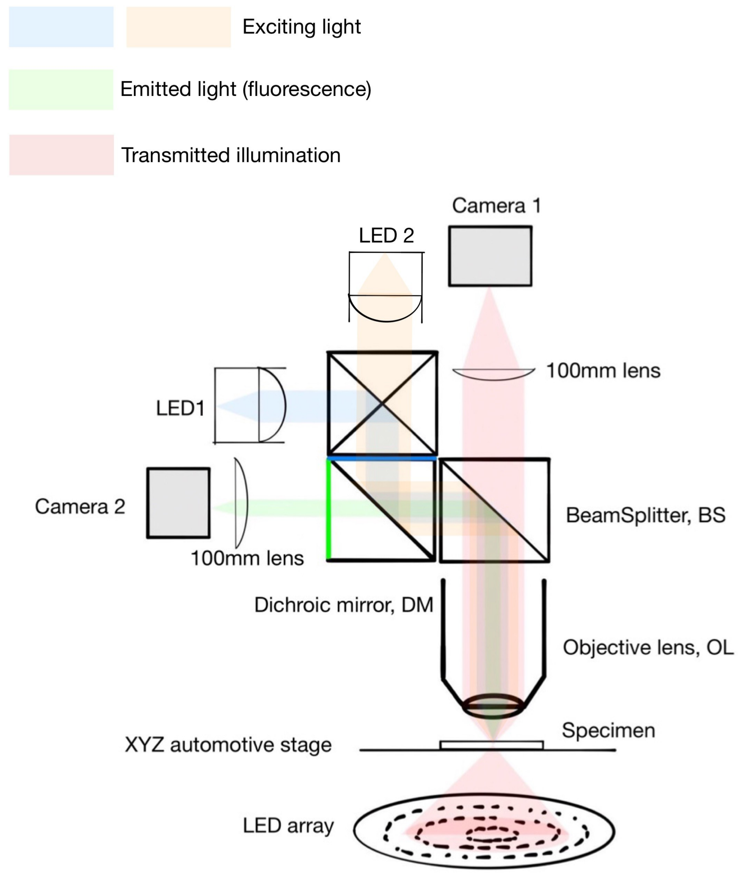


Fig. 1. Light path diagram of the self-built fluorescent microscope. The whole microscope apparatus can be used to acquire transmitted illumination images and fluorescent signal images of different regions of a fluorescent specimen.

2.2. *Design of excitation light illumination module*

In the multi-channel excitation light illumination module in Figure.1, we design to use a prism called X-cube, which is commonly used to scatter light in commercial decoration, to focus the excitation light of various wavelengths together. Light beams emitted by LEDs of different colors in different directions are converged by the X-cube and then incident into the objective lens from the same direction to illuminate the sample to be excited. we assembled LEDs of different colors with heat sinks and so on, and fixed them together with X-cube on the microscope by our self-designed 3D printed elements(Figure.2).

3. Results

3.1. *The physical map of the self-built fluorescence microscope*

Most of the optical and mechanical parts of the assembled microscope can be ordered from the websites of companies such as Olympus, Thorlabs, etc. The parts that we used on the self-assembled microscope are listed in Table.1. According to the size of each module, we also designed 3D-printed elements for fixation, and assembled them into the multi-channel

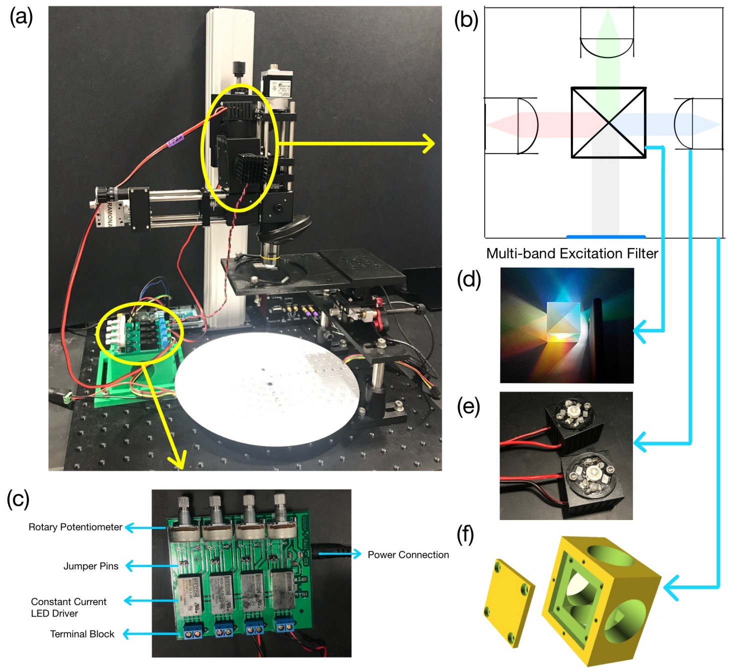


Fig. 2. The introduction of each part of the excitation light illumination module in the picture of the self-built fluorescent microscope. (a) The real picture of the self-built fluorescent microscope. (b) Multi-channel excitation light convergence module. (c) Self- designed and soldered LED control circuit board, on which there are electronic components such as jumper pins which can accept the digital signal output from Arduino and knobs which can adjust the LEDs' intensity. (d) Low-cost X-cube that can diverge or converge up to three light beams. (e) Self-soldered and assembled LED illumination module, including LEDs with positive and negative wiring, heat sinks and 3D-printed fixing rings. (f) 3D-printed parts for fixing, the designs of which are drawn by the OpenSCAD software.

fluorescent microscope as shown in Figure.3, in which the three-dimensional movement of the sample platform can be controlled by the electric motors. The transmission illumination module, fluorescence excitation illumination module, sample platform and data acquisition module can be automatically controlled by MATLAB combined with Arduino microcontroller.

3.2. Measurement of the excitation light module parameters

In Figure.4 we measured the spectral distribution of the red, green and blue bands dispersed from a beam of white light by x-cube. In order to compare the effect of LEDs of different colors and powers as excitation light, we measured the spectral distribution(Table.2)of several commercially available and inexpensive LEDs refracted from different sides to the converging side by the x-cube. By referring to this table, the experimenter can conveniently select the excitation light band and corresponding multi-channel bandpass filter according to the specific experimental requirements.

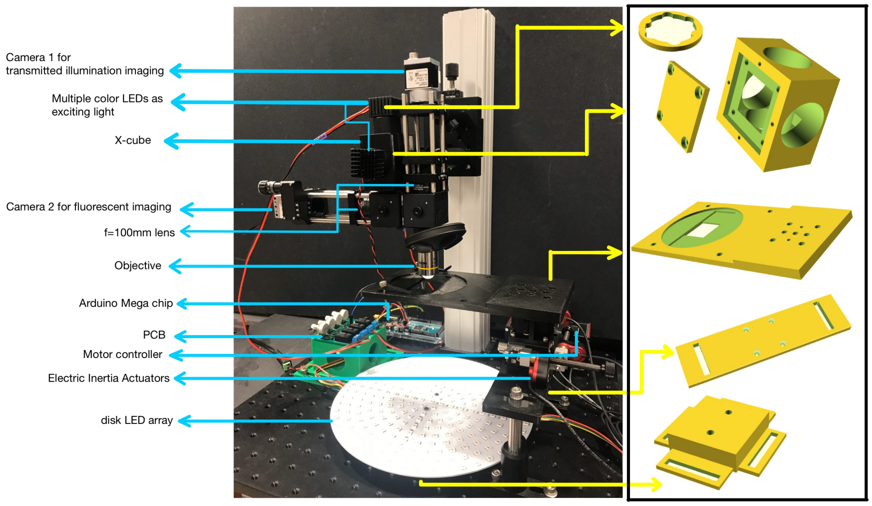


Fig. 3. Description of each part of the self- assembled fluorescent microscope and all the 3D-printed parts designed for supporting. The microscope was built using components described in Table.1, and the 3D-printed parts' designs are drawn by the OpenSCAD software.

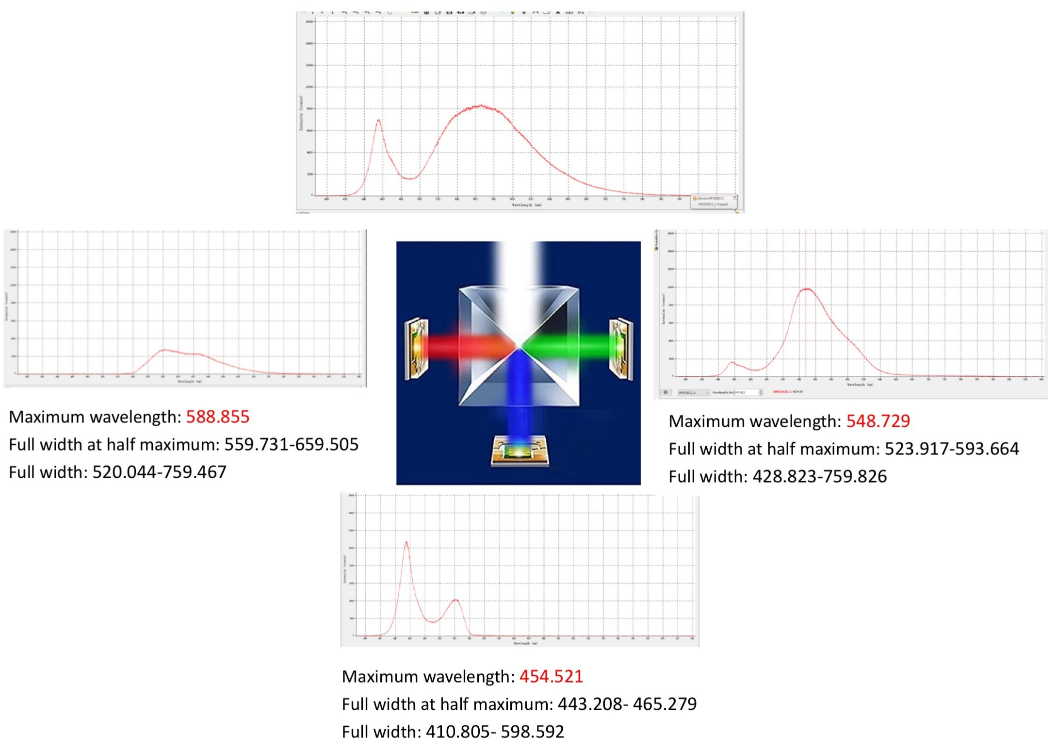


Fig. 4. The white light incident from one side of the x-cube is dispersed into red, green and blue. The spectral distribution of the three sides are measured respectively with spectrometer.

3.3. Microscope parameters measurement

The magnification of a microscope is determined by the focal length of the objective and the focal length of the condenser lens. The actual magnification of a microscope can be accurately calibrated with the acquired image of the standard sample, such as the USAF resolution chart. For example, we calculate the magnification of the microscope by using the image of a standard sample(Figure.5) imaged by the 10X objective and collected by the camera with known pixel size, which is very close to the theoretical value of the magnification of the 4F imaging system.

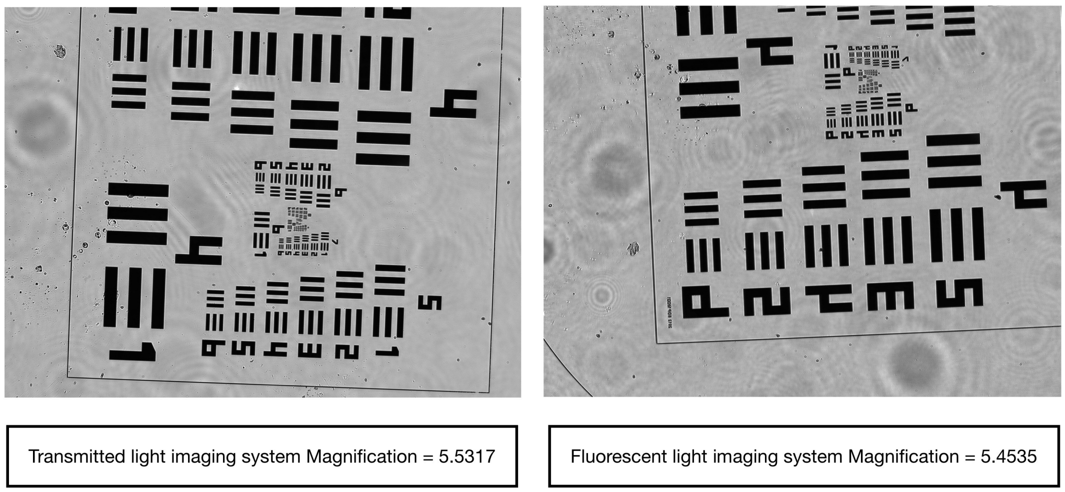
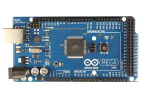
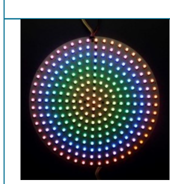
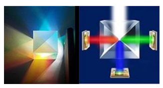


Fig. 5. Images of USAF resolution chart from Camera 1(transmitted illumination system) and Camera 2(fluorescent illumination system).

4. Methods

4.1. List of parts required for microscope assembly

components pictures	components descriptions	Quantity	Purpose	Suppliers
	Arduino Mega 2560 Microcontroller Board	1	Controlling the disk LED array and LEDS for fluorescent excitation light	Arduino [3]
	acA4024-29um - Basler ace	2	Acquiring the transmitted light image and fluorescent image	BASLER [4]
	DotStar RGB LED Disk - 240mm diameter	1	Providing transmitted light illumination of different colors and positions	adafruit [5]
	Chanzon High Power Led Chip (3W)	3	multi-color fluorescent excitation lights	Chanzon [6]

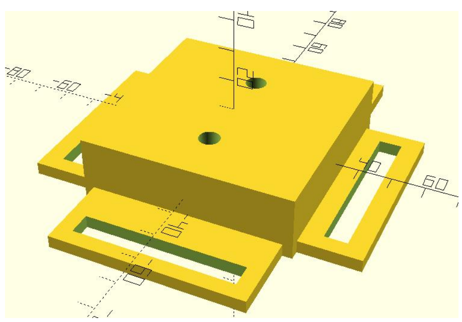
	Aluminum Base Plate Panel PCB Circuit Board Substrate 20mm Star DIY Heat sink Cooling for COB High Power LED Chips	3	Connecting the single LEDs with wires	Chanzon [7]
	30x30x30mm Optical Glass RGB Dispersion Prism X-Cube	1	Reflecting or transmitting three light beams in red, green, and blue, and emitting white light from the remaining surface	CynKen [8]
	KIM101 - Four-Channel K-Cube Inertial Motor Controller	1	Controlling Piezoelectric Inertia Actuators to automatically move sample stage	Thorlabs [9]
	Piezoelectric Inertia Actuators	1	automatically move sample stage	Thorlabs [10]
	Olympus PLN 10X Objective	1	Imaging the sample at the focal plane	Edmund Optics [11]
	Excitation, Emission Filters for Imaging Applications	2	Filtering out light except excitation and fluorescence bands	Thorlabs [12]
	Dichroic Filters	1	the excitation light and its associated back reflection are reflected while the longer wavelength fluorescence signal is transmitted	Thorlabs [12]
	Beamsplitter	1	Dividing the transmission light and fluorescent light into two paths	Thorlabs [12]
	Mounted Achromatic Doublets, f = 100.0 mm, AR Coated: 400 - 700 nm	2	Converging the parallel light onto the photosensitive surface of the camera	Thorlabs [12]
	Plano-Convex Lens, f = 20.0 mm, Uncoated	3	Converting the point excitation light from single LED into collimated light	Thorlabs [12]

	The CM1-DCH Dichroic Cage Cube	2	Holding a Rectangular Mirror	Thorlabs [12]
	Microscope Objective Lens Turret, SM1-Compatible	1	holding objectives securely	Thorlabs [12]
	Constant Current LED Driver	3	The RCD series is a step-down constant current source designed for driving high power LEDs	RECOM [13]
	Other optical path supporting elements (Multi-Axis Stages, Hardware Kits, etc.)		Supporting the whole setup	Thorlabs [12]

Table 1. List of the main off-the-shelf components required to build the microscope. Several links for the products are provided in the reference at the end of the document.

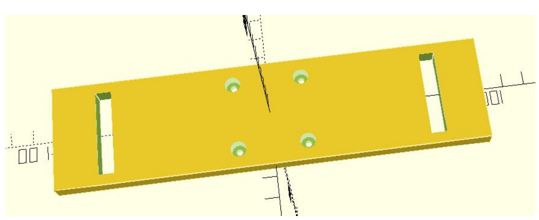
4.2. Design of 3D-printed components

4.2.1. LED array Base



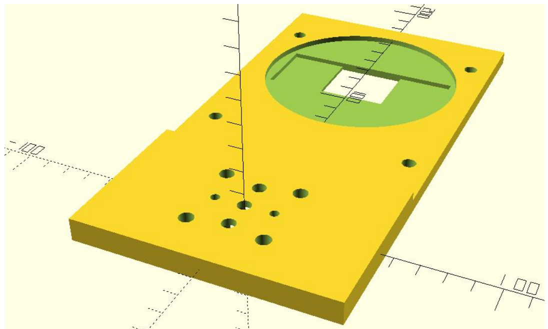
The plastic LED array base was printed such that the DotStar RGB LED Disk could be screwed onto it. The base itself can be screwed to the optical bench. It was designed to provide higher stability, to align the center of LED disk and the objective, and to prevent the LED disk from contacting the optical bench and shorting.

4.2.2. Stage holder



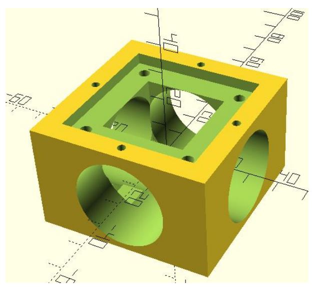
The stage holder was designed to hold the Multi-Axis Stages, on which is mounted a sample stage located at the focal plane of the objective lens.

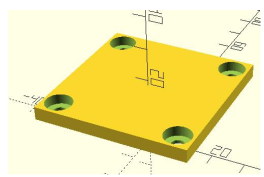
4.2.3. Sample stage



The sample stage was designed to be mounted on the Multi-Axis Stages with the sample on top. The four screw holes around the sample is designed for mounting the pressing clamps.

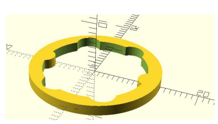
4.2.4. LED Combiner Block





The LED combiner block was designed to combine the excitation light of different color from different directions to enter the system from the same position and are finally converged and irradiated on the sample. The cap was designed to be screwed on after the RGB Dispersion Prism X-Cube was placed inside the center of the block to keep it from falling out. Three convex lenses with a focal length of 20mm inside tubes are inserted into the three circular holes respectively, to collimate the three light beams in red, green, and blue, respectively the base light. A tube with an excitation light filter inside it is inserted into the remaining hole to fix the block and let the excitation light through the system.

4.2.5. Single LED holder



The single LED holder was designed to align the single LED and the tube holding it to keep the LED facing the center of the lens.

4.3. Soldering Printed Circuit Board

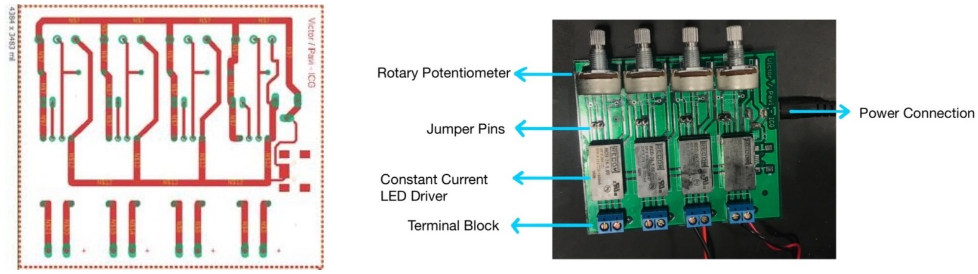
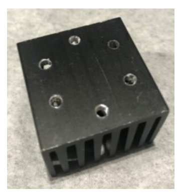


Fig. 6. Photographs and Design drawings of the printed circuit board.

The Printed Circuit Board was designed to control the light intensity of the LED through the rotary potentiometer and to control the LED on/off by connecting the Jumper Pins to the Arduino chip.

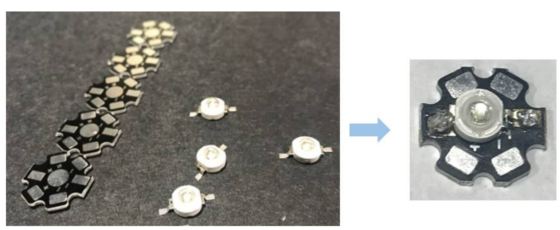
4.4. Assemble single LED module

4.4.1. Drilling holes in the heat sink



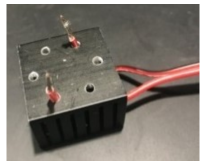
The single LED's power is 5W and light output power can be up to 650mW, so a heat sink is needed to be connected to dissipate heat.

4.4.2. Soldering the single LED to the circuit connecting piece



4.4.3. Applying thermal paste between heat sink and LED piece

4.4.4. Soldering the wire onto the circuit connecting piece through the holes



4.4.5. Placing the single LED holder and secure with screws

4.4.6. Aligning the LED

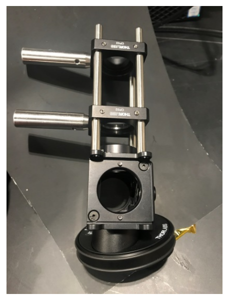
Fig. 7. Photograph of different colors of self-assembled LEDs

In order to make the collimated light enter the x-cube, we need to concentrate the point light source emitted by the LED with a lens (f=20mm). We use a 30 mm Lens Tube to fix the LED and hold the lens. By changing the lens' position back and forth to form a clear image of the LED at a far image plane.

4.5. *Microscope assembly steps*

4.5.1. Assemble the support column

4.5.2. Align the camera 1 for transmission light imaging

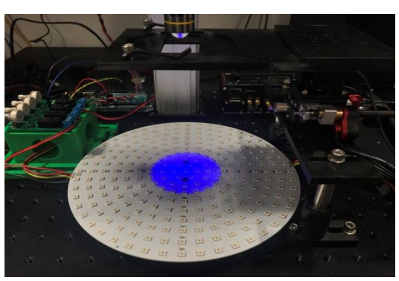


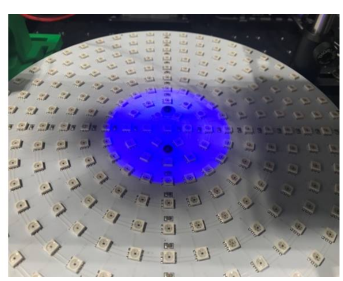
To align Camera 1, you can hold this towards a distant (collimated) light source, move the camera position back and forth to get the sharpest image.

4.5.3. Fix the Dichroic Filters and Beamsplitter

4.5.4. Screw on the objective

4.5.5. Mount the LED array

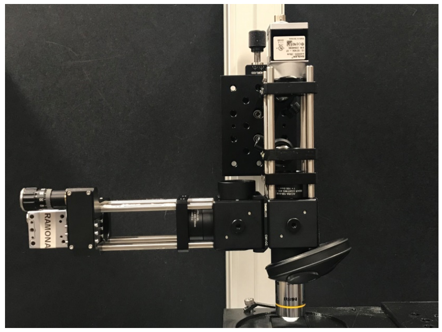




Turn on the fluorescent excited light. It will be focused through the objective and form a circle at the LED disk. Adjust the position of disk LED to make its' center LED coincides with the center of the objective.

4.5.6. Set up the sample holding stage

4.5.7. Assemble and align the camera 2 for fluorescent imaging



To align Camera 2, you can do the same as Camera 1, or you can set it up first, use the green transmitted illumination to form a clear image on Camera 1, and then adjust the Camera 2 to form the same clear image.

4.5.8. Fix and connect Arduino and PCB

For disk LED array, we choose Arduino board pin 4 as data signal input pin and pin 5 as clock signal input pin. For PCB connecting with single LEDs, we choose pin 8,9,10 as different colors LED input pins. We need to connect the corresponding Arduino output pins to the PCB Jumper pins. We also need to connect the GND and POWER pins of Arduino with LED array and PCB.

4.5.9. Shading the setup.

4.6. Softwares and Codes

Software

Before operating the microscope, you need to download several softwares for controlling the hardwares: Arduino , Matlab , APT Software (Thorlabs Motion Control Software).

Arduino code controlling disk LED array

We control the disk LED array by custom Arduino code matlab arduino control LEDs.ino. The code contains several functions to achieve different modes of illumination(as shown in Figure.8), such as different angle single point illumination, multiple points illumination, bright field illumination, dark field illumination, and phase contrast illumination.Before the use of microcontroller, it's necessary to update the control code into the board and it's unnecessary to update again for the next use unless you'd like to modify the code.

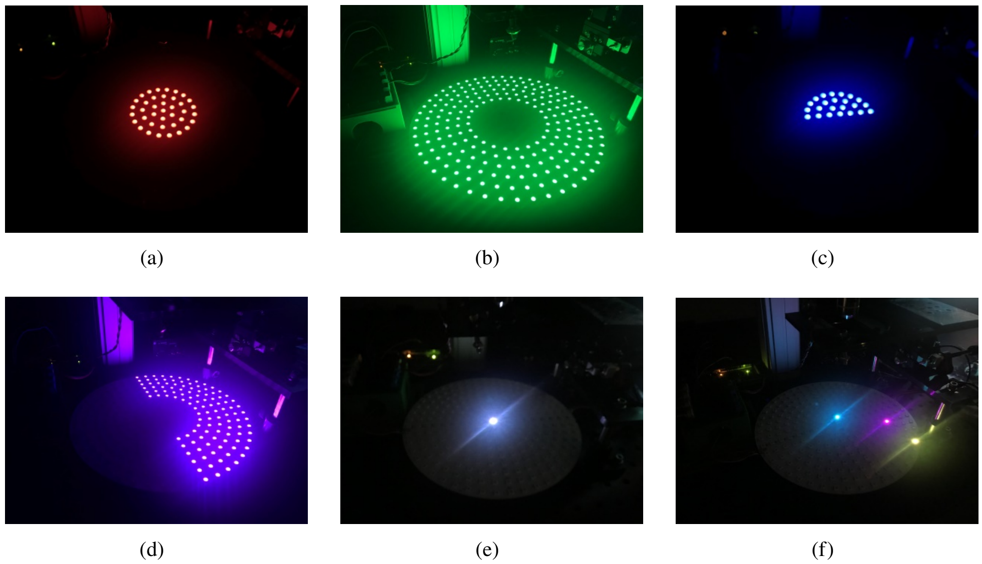
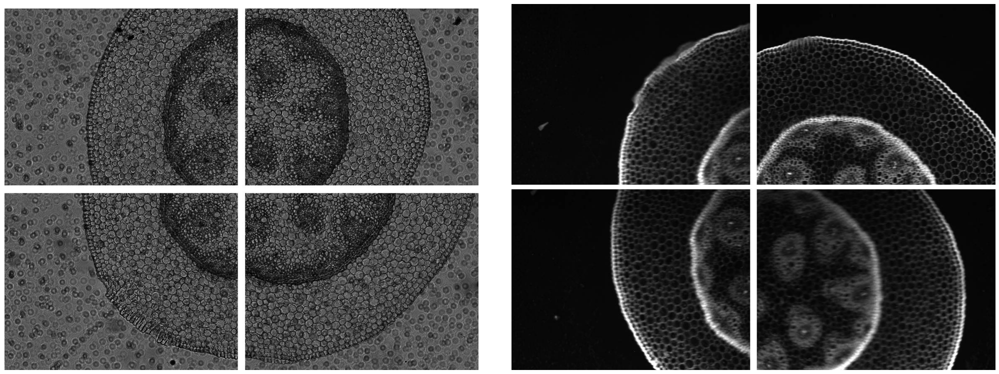


Fig. 8. Transmitted illumination system achieved by disk LED array. You can set any color. (a) bright field illumination. The diameter of the field is automatically calculated by the N.A. and distance parameters set in the code. (b) dark field illumination.(c) phase contrast illumination (bright top). (d) phase contrast illumination (dark right).(e) single point illumination. (f) multiple point illumination.

4.7. Image acquisition workflow

- Put your sample on the sample stage and fix with the pressing clamps
- Plug the external power plug of the LED array, the power plug of the Motor Controller and the power plug of PCB into the power source.
- Connect the Arduino microcontroller to the USB port to communicate with MATLAB.
- Open the MATLAB data acquisition toolbox to preview.
- Focus the sample by two cameras using green fluorescence channel and green transmission channel , then close preview.
- Open the custom MATLAB script main control Thorlabs APT disk LED BASLER 10X.m, Run full data acquisition, which basically scans the whole sample(as shown in Figure.9).



(a) (b)

Fig. 9. (a) Scanning Transmitted central point illumination(green) imaging of convallaria. (b) Scanning fluorescent imaging of convallaria. The images have overlapping part. The scanning step size can be set in the custom MATLAB scripts main control Thorlabs APT disk LED BASLER 10X.m.

5. Conclusion

The self-built microscope has higher flexibility, which can adjust the hardware of each module according to individual experimental needs, and can also write open source programs to automatically acquire images. And the cost of self-assembled microscope is much lower than that of the integrated commercial fluorescent microscope. Our design provides a solution for experimenters who wish to use a low-cost microscope to perform the task of multi-field observation and image collection of fluorescent samples with multi-color channels.

Acknowledgments

Acknowledgments should be included at the end of the document. The section title should not follow the numbering scheme of the body of the paper. Additional information crediting individuals who contributed to the work being reported, clarifying who received funding from a particular source, or other information that does not fit the criteria for the funding block may also be included; for example, “K. Flockhart thanks the National Science Foundation for help identifying collaborators for this work.”

Disclosures

The authors declare no conflicts of interest.

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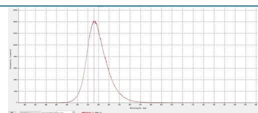
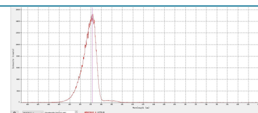
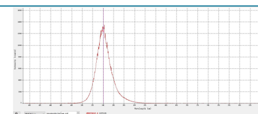
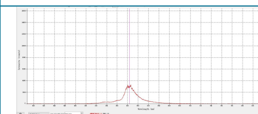
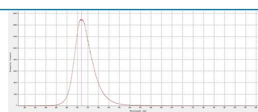
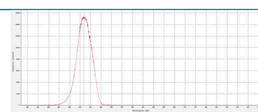
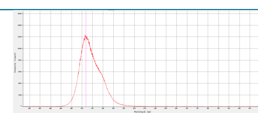
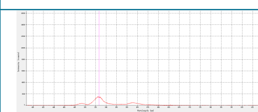
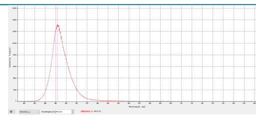
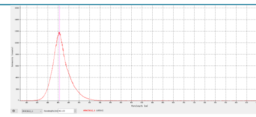
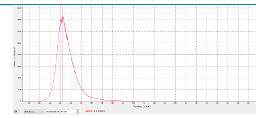
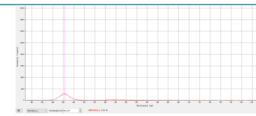
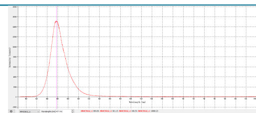
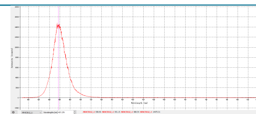
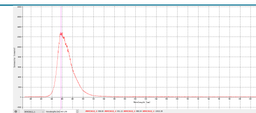
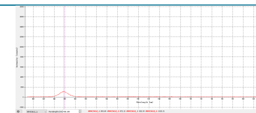
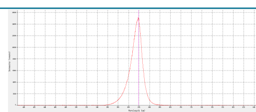
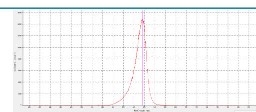
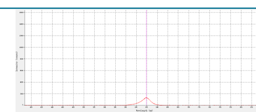
2. Liu, Z., Tian, L., Liu, S., and Waller, L., “Real-time brightfield, darkfield, and phase contrast imaging in a light-emitting diode array microscope,” J. biomedical optics **19(10)**, 106002 (2014).
3. Arduino software and hardware: https://www.arduino.cc/.
4. BASLER Area Scan Cameras: https://www.baslerweb.com/en/products/cameras/area-scan-cameras/ace/aca4024-29um/.
5. DotStar RGB LED Disk - 240mm diameter: https://www.adafruit.com/product/2477.
6. Chanzon High Power Led Chip (3000K-3500K / Input 600mA-700mA / DC 6V-7V / 5 Watt) Super Bright Intensity Light Emitter Components Diode 5 W Bulb Lamp Beads DIY Lighting: https://www.amazon.com/9000K-10000K-600mA-700mA-Intensity-Components-Lighting/dp/B01DBZJ9WK/ref=sr_1_5?dchild=1&keywords=Chanzon%2BHigh%2BPower%2BLed%2BChip%2B(3W)&qid=1604327514&sr=8-5&th=1.

7. Chanzon LED Heat Sink Aluminum Base Plate Panel PCB Circuit Board Substrate: https://www.amazon.com/Chanzon-Aluminum-Circuit-Substrate-Heatsink/dp/B06XKTRSP7/ref=sr_1_1?dchild=1&keywords=Aluminum+Base+Plate+Panel+PCB+Circuit+Board+Substrate+20mm+Star+DIY+Heat+sink+Cooling+for+COB+High+Power+LED+Chips&qid=1604328905&sr=8-1.
8. CynKen 30x30x30mm Optical Glass RGB Dispersion Prism X-Cube: https://www.amazon.com/cynken-dispersi%c3%b3n-Physics-ense%c3%b1ar-Decoraci%c3%b3n/dp/B0742CCSHM?currency=USD&language=en_US.
9. Kinesis® K-Cube™ Piezo Inertia Actuator Controller: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=9790&pn=KIM101.
10. Piezoelectric Inertia Actuators: https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_ID=9776.
11. Olympus Objectives: https://www.olympus-lifescience.com/en/objectives/.
12. Optical components: https://www.thorlabs.com/.
13. constant current source: https://recom-power.com/en/rec-p-RCD-24-0.30.html?5.

Supplementary Information

LED type	LED color	Blue side of X-cube	Green side of X-cube	Red side of X-cube
green	 Maximum wavelength: 531.263 Width at half maximum: 515.134- 554.618 Full width: 467.113- 640.494	 Maximum wavelength: 522.497 Width at half maximum: 504.644- 531.649 Full width: 458.198- 578.073	 Maximum wavelength: 540.777 Width at half maximum: 526.109- 552.699 Full width: 484.882- 616.724	 Maximum wavelength: 583.024 Width at half maximum: 571.715- 602.63 Full width: 506.848- 665.321
cyan	 Maximum wavelength: 507.755 Width at half maximum: 492.825- 528.816 Full width: 454.259- 603.512	 Maximum wavelength: 506.848 Width at half maximum: 491.264- 526.109 Full width: 445.71- 542.959	 Maximum wavelength: 507.107 Width at half maximum: 493.996- 533.451 Full width: 459.117- 602.251	 Maximum wavelength: 526.367 Width at half maximum: 514.746- 534.994 Full width: 478.097- 636.503

blue	 Maximum wavelength: 463.051 Width at half maximum: 451.236- 482.013 Full width: 420.221- 580.232	 Maximum wavelength: 462.133 Width at half maximum: 449.658- 474.701 Full width: 417.836- 535.637	 Maximum wavelength: 464.624 Width at half maximum: 452.156- 480.578 Full width: 426.046- 569.806	 Maximum wavelength: 462.527 Width at half maximum: 452.156- 471.301 Full width: 433.448- 493.996
royal blue	 Maximum wavelength: 457.542 Width at half maximum: 444.393- 475.615 Full width: 408.946- 559.22	 Maximum wavelength: 457.279 Width at half maximum: 444.393- 472.347 Full width: 409.212- 529.588	 Maximum wavelength: 457.279 Width at half maximum: 449.658- 477.183 Full width: 426.972- 547.576	 Maximum wavelength: 458.198 Width at half maximum: 449.658- 465.803 Full width: 432.523- 482.404
yellow	 Maximum wavelength: 618.482 Width at half maximum: 603.512- 626.504 Full width: 553.595- 662.6		 Maximum wavelength: 616.347 Width at half maximum: 601.999- 624.375 Full width: 552.059- 650.573	 Maximum wavelength: 618.858 Width at half maximum: 609.307- 628.256 Full width: 580.232- 646.595

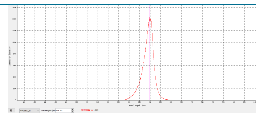
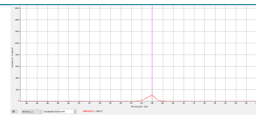
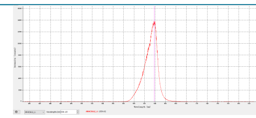
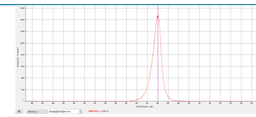
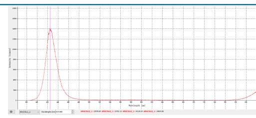
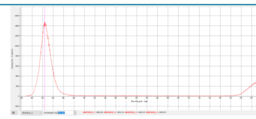
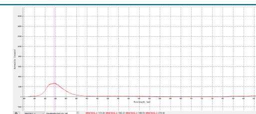
red	 Maximum wavelength: 639.247 Width at half maximum: 628.256- 646.968 Full width: 590.374- 680.859	 Maximum wavelength: 639.871 Width at half maximum: 628.256- 645.475 Full width: 611.823-659.753	 Maximum wavelength: 638.125 Width at half maximum: 622.119- 645.102 Full width: 585.814- 677.29	 Maximum wavelength: 640.245 Width at half maximum: 629.757- 646.968 Full width: 592.525- 675.689
UV	 Maximum wavelength: 424.988 Width at half maximum: 415.715- 438.463 Full width: 388.175-501.661	 Maximum wavelength: 423.664 Width at half maximum: 415.052- 435.824 Full width: 388.041- 486.315	 Maximum wavelength: 436.748 Width at half maximum: 419.029- 459.379 Full width: 389.909- 501.401	

Table 2. Spectrum of different colors of LEDs (5 Watt) and their Spectrum after incidenting from different sides(red side, green side ,blue side), measured at the white light side.