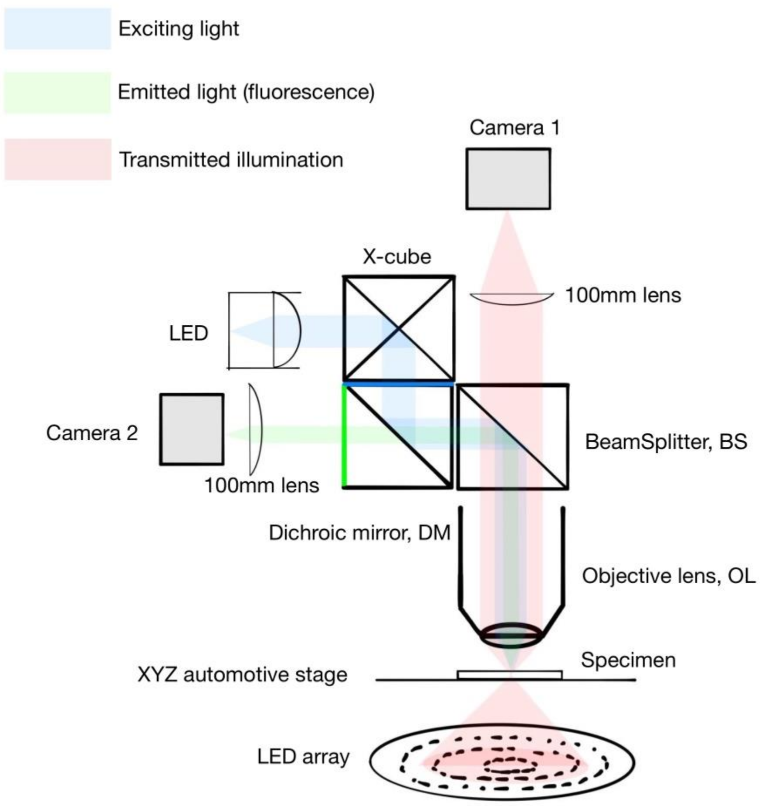
Instructions to build a Multi-color Fluorescent Microscope

This document provides instructions to build a low-cost electric platform fluorescent microscope, which can realize multi-color fluorescent excitation and various modes of transmission imaging, such as different angle single point illumination, bright field illumination, dark field illumination, and phase contrast illumination.

Ⅰ.Introduction

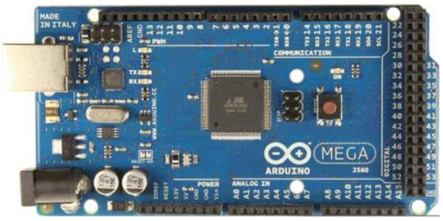
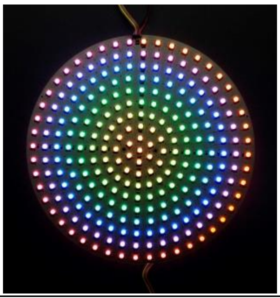


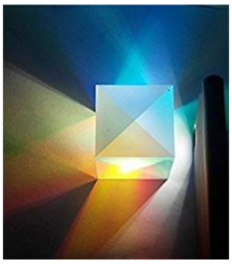
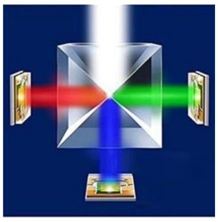
Supplementary Figure 1. Schematic diagram and elements' names of the optical path design of the fluorescent microscope.

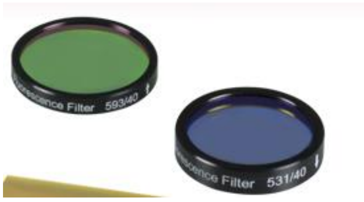
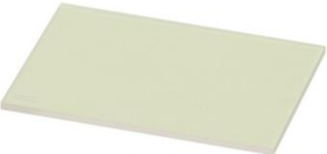
This fluorescent microscope is designed for basically scanning the whole sample and collecting a large number of transmitted light illumination images of fluorescent biological samples and fluorescent images of the corresponding positions, which will be used for subsequent machine learning tasks to predict multi-color fluorescence images of biological samples from their transmitted light illumination images.

One of the aims when building this microscope was to build a very low-cost multi-color fluorescent microscope using only off-the-shelf components with minimal external components. Designs for the parts were made using OpenSCAD open source CAD software and printed with 3D printer.

Ⅱ.Component list

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

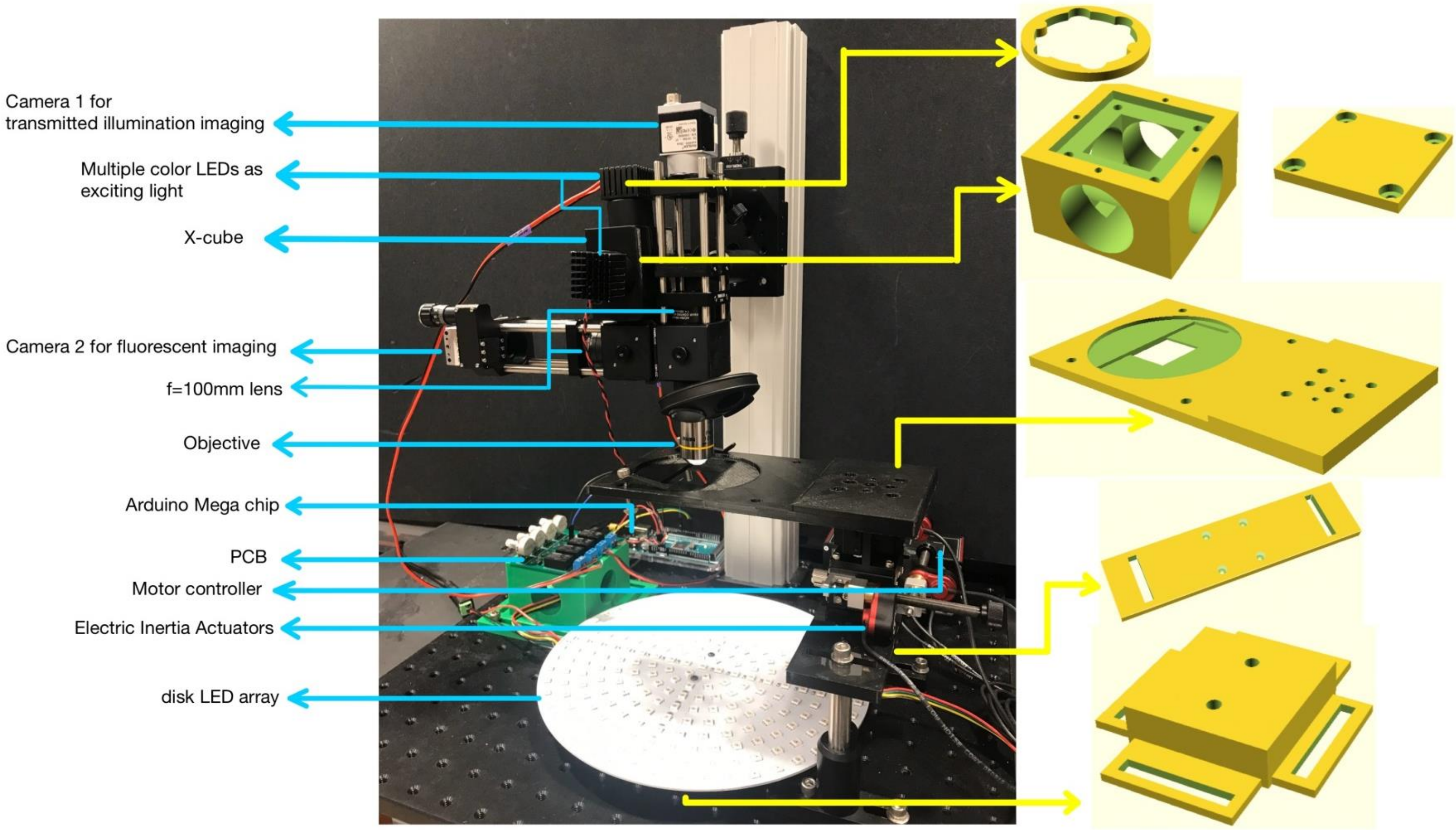
Aluminum Base Plate Panel PCB Circuit Board Substrate 20mm Star DIY Heat sink Cooling for COB High Power LED Chips 	3	Connecting the single LEDs with wires	Chanzon
30x30x30mm Optical Glass RGB Dispersion Prism X-Cube  	1	Reflecting or transmitting three light beams in red, green, and blue, and emitting white light from the remaining surface	CynKen
KIM101 - Four-Channel K-Cube Inertial Motor Controller 	1	Controlling Piezoelectric Inertia Actuators to automatically move sample stage	Thorlabs
Piezoelectric Inertia Actuators 	1	automatically move sample stage	Thorlabs

Olympus PLN 10X Objective 	1	Imaging the sample at the focal plane	Edmund Optics
Excitation, Emission Filters for Imaging Applications 	2	Filtering out light except excitation and fluorescence bands	Thorlabs
Dichroic Filters 	1	the excitation light and its associated back reflection are reflected while the longer wavelength fluorescence signal is transmitted	Thorlabs
Beamsplitter 	1	Dividing the transmission light and fluorescent light into two paths	Thorlabs
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

Plano-Convex Lens, f = 20.0 mm, Uncoated 	3	Converting the point excitation light from single LED into collimated light	Thorlabs
The CM1-DCH Dichroic Cage Cube 	2	Holding a Rectangular Mirror	Thorlabs
Microscope Objective Lens Turret, SM1-Compatible  	1	holding objectives securely	Thorlabs
Constant Current LED Driver  	3	The RCD series is a step-down constant current source designed for driving high power LEDs.	RECOM
Other optical path support elements(e.g. Multi-Axis Stages，Hardware Kits)		Supporting the whole setup	Thorlabs

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

Ⅲ.Design

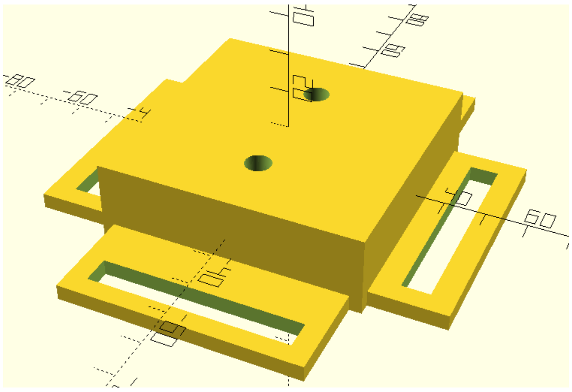


Supplementary Figure 2. Photographs of the multi-color fluorescent microscope.

Supplementary Figure 2 shows the assembled setup together with 3D printed parts required. It was built using components described in Supplementary Table 1.

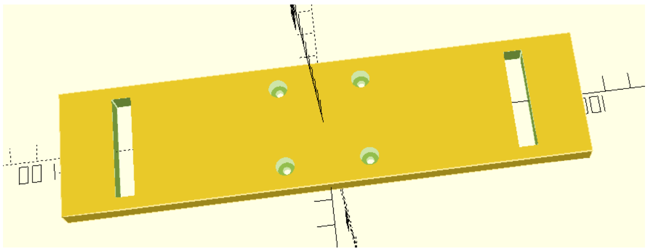
Design 3-D print components

1. LED array Base



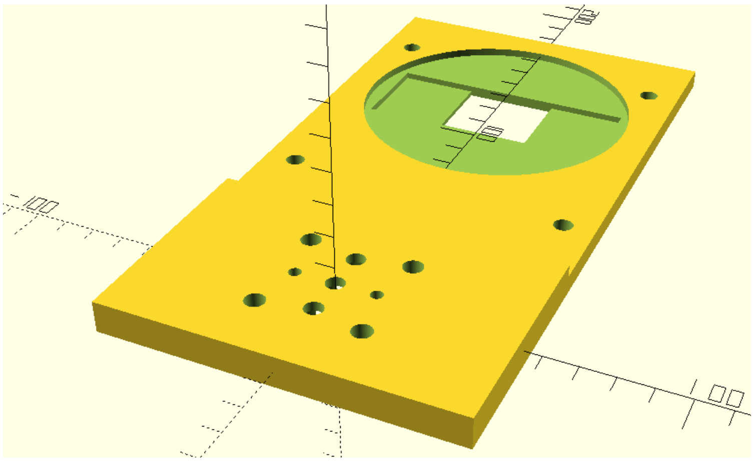
The plastic LED array base was printed such that the DotStar RGB LED Disk could be screwed onto it. The base itself can be screwed to the optical bench. It was designed to provide higher stability, to align the center of LED disk and the objective, and to prevent the LED disk from contacting the optical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

2. Stage holder



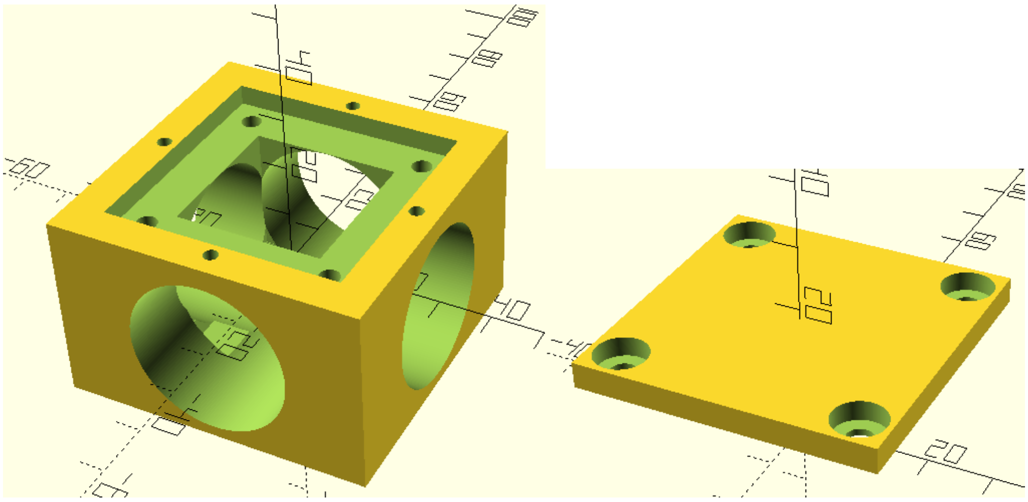
The stage holder was designed to hold the Multi-Axis Stages, on which is mounted a sample stage located at the focal plane of the objective lens.

3. Sample stage



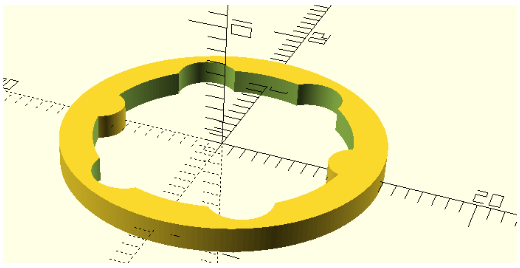
The sample stage was designed to be mounted on the Multi-Axis Stages with the sample on top. The four screw holes around the sample is designed for mounting the pressing clamps.

4. 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.

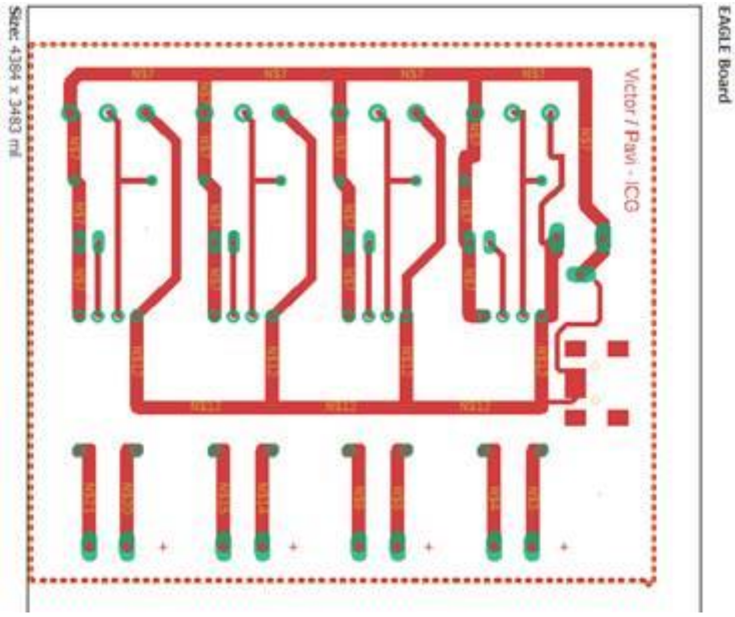
5. Single LED holder

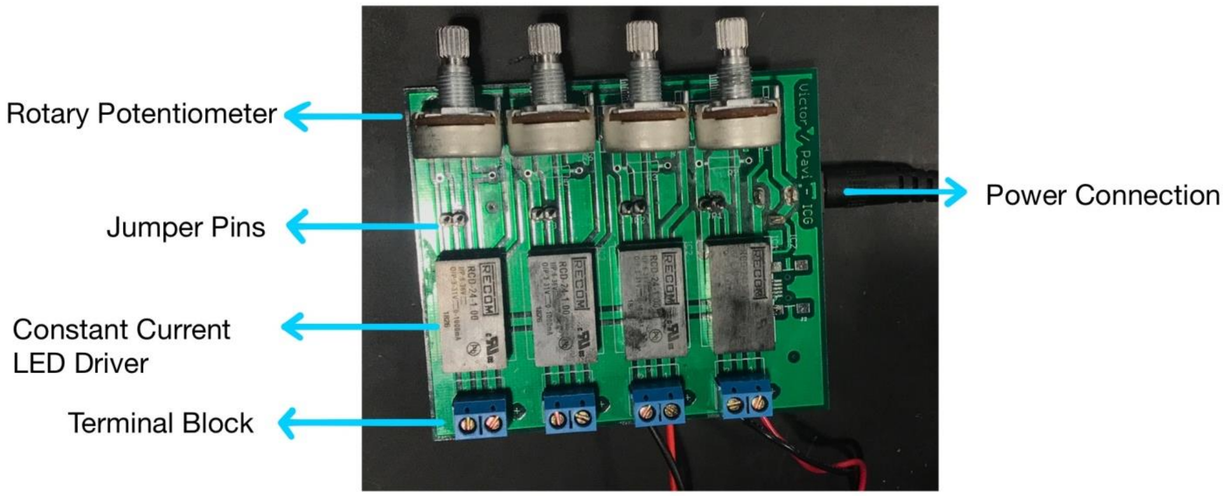


The single LED holder was designed to align the single LED and the tube holding it to keep the LED facing the center of the lens.

Soldering PCB

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.



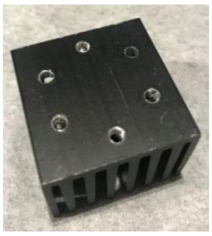


Supplementary Figure 3. Photographs and Design drawings of the printed circuit board.

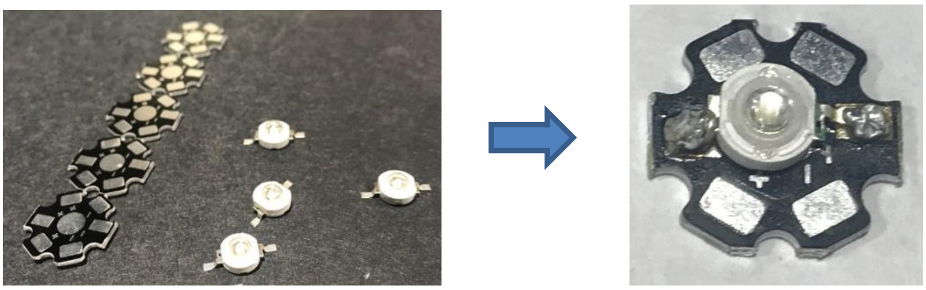
Assemble single LEDs

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.

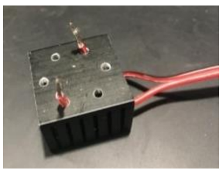


2. Soldering the single LED to the circuit connecting piece

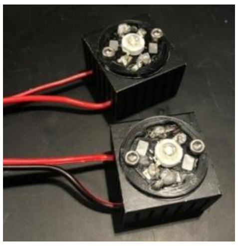


3. Applying thermal paste between heat sink and LED piece

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



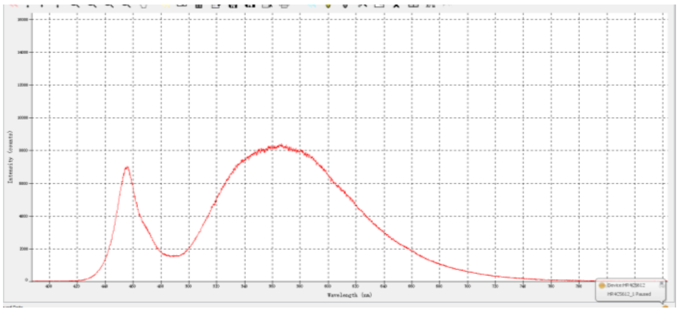
5. Placing the single LED holder and secure with screws

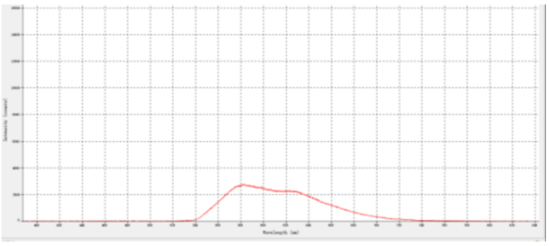


6. Aligning the LED

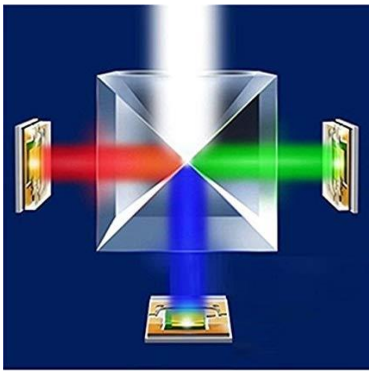
In order to make the collimated light enter the x-cube, we need to concentrate the point light source emitted by the LED with a lens (f=20mm). We use a 30 mm Lens Tube to fix the LED and hold the lens. By changing the lens' position back and forth to form a clear image of the LED at a far image plane.

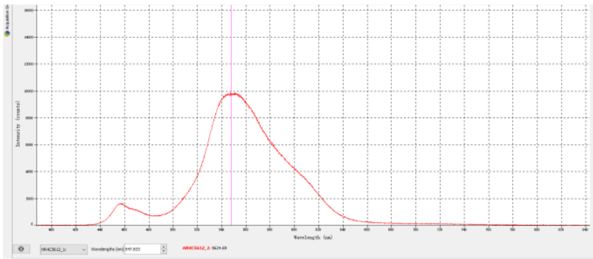
Multi-color fluorescent illumination system



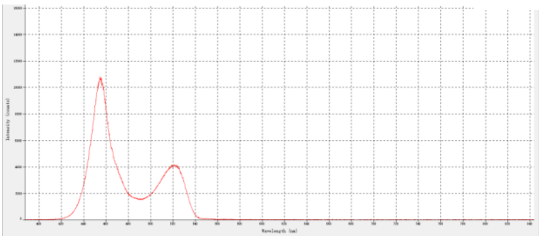


Maximum wavelength: 588.855
Full width at half maximum: 559.731-659.505
Full width: 520.044-759.467



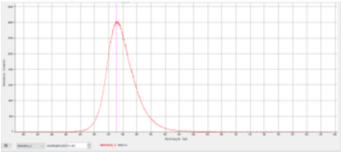
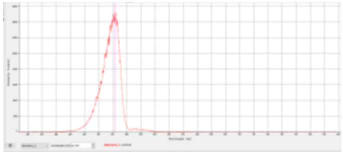
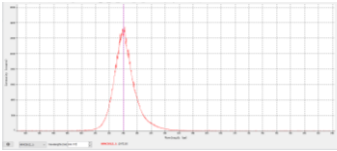
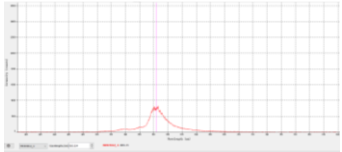
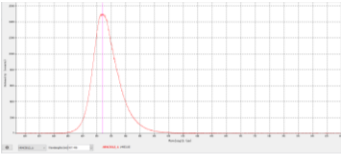
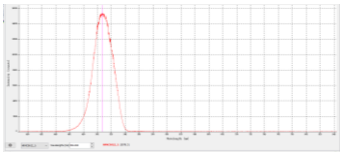
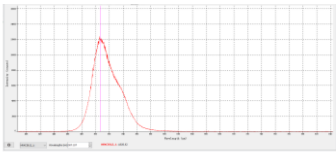
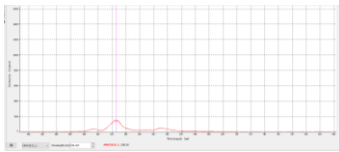
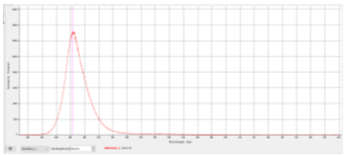
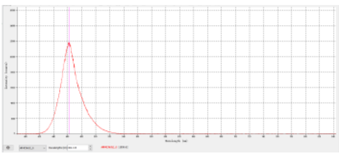
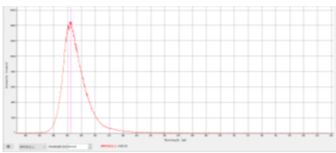
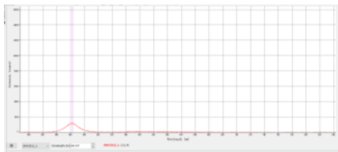


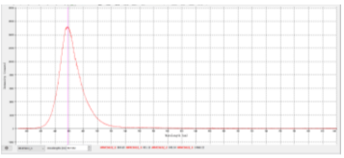
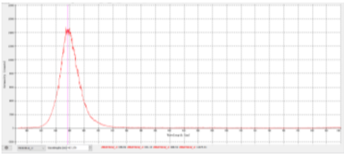
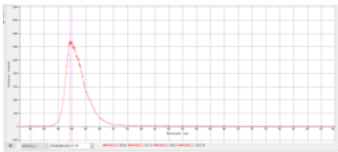
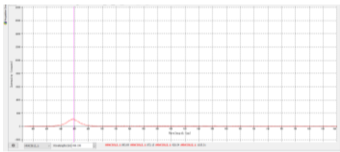
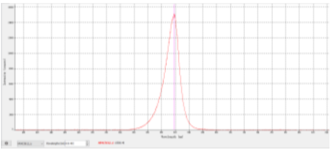
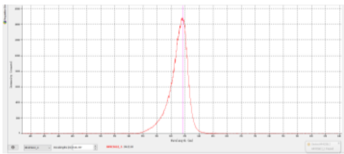
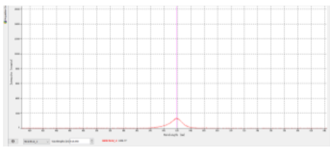
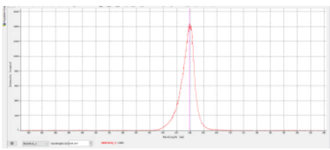
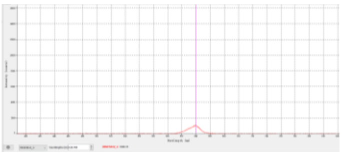
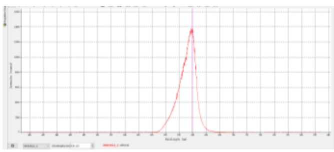
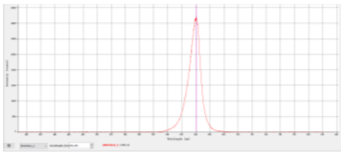
Maximum wavelength: 548.729
Full width at half maximum: 523.917-593.664
Full width: 428.823-759.826

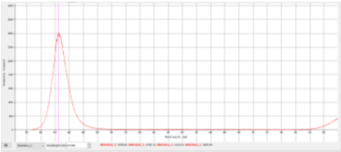
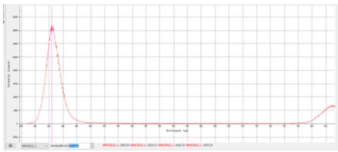
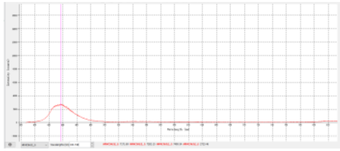


Maximum wavelength: 454.521
Full width at half maximum: 443.208- 465.279
Full width: 410.805- 598.592

Supplementary Figure 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.

Light name	Light color	Blue side	Green side	Red side
5W green	 Maximum wavelength: 531.263 Full width at half maximum: 515.134- 554.618 Full width: 467.113- 640.494	 Maximum wavelength: 522.497 Full width at half maximum: 504.644- 531.649 Full width: 458.198- 578.073	 Maximum wavelength: 540.777 Full width at half maximum: 526.109- 552.699 Full width: 484.882- 616.724	 Maximum wavelength: 583.024 Full width at half maximum: 571.715- 602.63 Full width: 506.848- 665.321
5W cyan	 Maximum wavelength: 507.755 Full width at half maximum: 492.825- 528.816 Full width: 454.259- 603.512	 Maximum wavelength: 506.848 Full width at half maximum: 491.264- 526.109 Full width: 445.71- 542.959	 Maximum wavelength: 507.107 Full width at half maximum: 493.996- 533.451 Full width: 459.117- 602.251	 Maximum wavelength: 526.367 Full width at half maximum: 514.746- 534.994 Full width: 478.097- 636.503
5W blue	 Maximum wavelength: 463.051 Full width at half maximum: 451.236- 482.013 Full width: 420.221- 580.232	 Maximum wavelength: 462.133 Full width at half maximum: 449.658- 474.701 Full width: 417.836- 535.637	 Maximum wavelength: 464.624 Full width at half maximum: 452.156- 480.578 Full width: 426.046- 569.806	 Maximum wavelength: 462.527 Full width at half maximum: 452.156- 471.301 Full width: 433.448- 493.996

5W royal blue	 Maximum wavelength: 457.542 Full width at half maximum: 444.393- 475.615 Full width: 408.946- 559.22	 Maximum wavelength: 457.279 Full width at half maximum: 444.393- 472.347 Full width: 409.212- 529.588	 Maximum wavelength: 457.279 Full width at half maximum: 449.658- 477.183 Full width: 426.972- 547.576	 Maximum wavelength: 458.198 Full width at half maximum: 449.658- 465.803 Full width: 432.523- 482.404
5W yellow	 Maximum wavelength: 618.482 Full width at half maximum: 603.512- 626.504 Full width: 553.595- 662.6	none	 Maximum wavelength: 616.347 Full width at half maximum: 601.999- 624.375 Full width: 552.059- 650.573	 Maximum wavelength: 618.858 Full width at half maximum: 609.307- 628.256 Full width: 580.232- 646.595
5W red	 Maximum wavelength: 639.247 Full width at half maximum: 628.256- 646.968 Full width: 590.374- 680.859	 Maximum wavelength: 639.871 Full width at half maximum: 628.256- 645.475 Full width: 611.823-659.753	 Maximum wavelength: 638.125 Full width at half maximum: 622.119- 645.102 Full width: 585.814- 677.29	 Maximum wavelength: 640.245 Full width at half maximum: 629.757- 646.968 Full width: 592.525- 675.689

5W UV	 Maximum wavelength: 424.988 Full width at half maximum: 415.715- 438.463 Full width: 388.175-501.661	 Maximum wavelength: 423.664 Full width at half maximum: 415.052- 435.824 Full width: 388.041- 486.315	 Maximum wavelength: 436.748 Full width at half maximum: 419.029- 459.379 Full width: 389.909- 501.401	none

Supplementary Table 2. Spectrum of different colors of LEDs ,which incident from different sides(red,green,blue side), measured at the white light side.

Supplementary Figure 5. Photograph of different colors of LEDs.

Ⅳ.Assembly instructions

Step-by –step instructions to assemble the microscope:

1. Assemble the support column

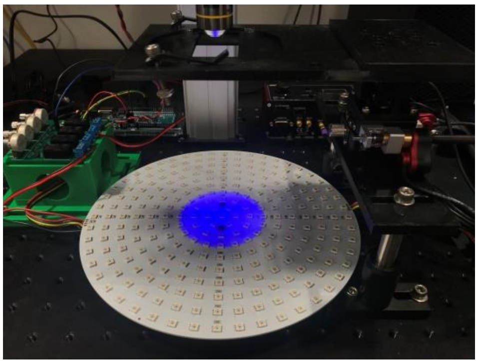
2. Align the camera 1 for transmission light imaging

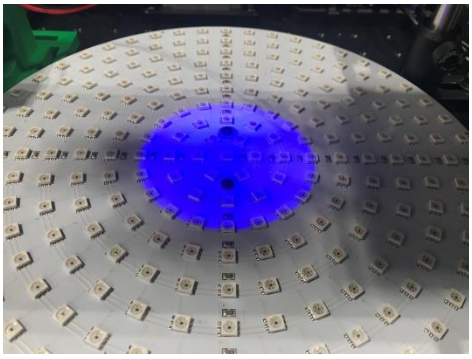
To align Camera 1, you can hold this towards a distant (collimated) light source, move the camera position back and forth to get the sharpest image.

3. Connect the Dichroic Filters and Beamsplitter

4. Screw on the objective

5. Mount the LED array

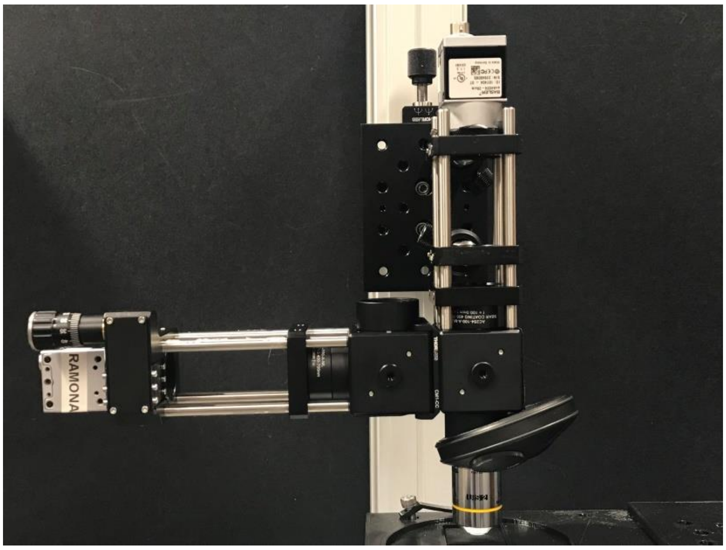




Turn on the fluorescent excited light. It will be focused through the objective and form a circle at the LED disk. Adjust the position of disk LED to make its' center LED coincides with the center of the objective.

6. Set up the sample holding stage

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.

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.

9. Shading the setup.

Ⅴ.Operating the Microscope

Software

Before operating the microscope, you need to download several softwares for controlling the hardwares:

Arduino

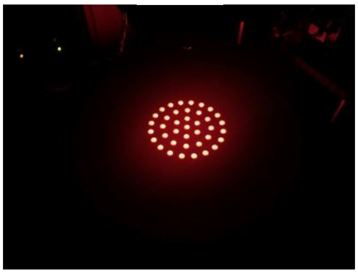
Matlab

APT Software (Thorlabs Motion Control Software)

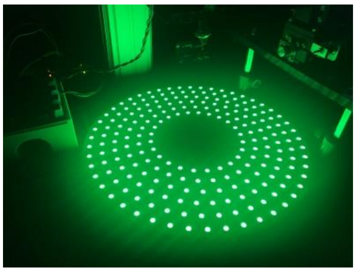
Arduino code controlling disk LED array

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, such as different angle single point illumination, multiple points illumination, bright field illumination, dark field illumination, and phase contrast illumination.

(a)



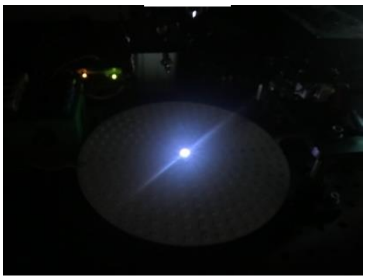
(b)



(c)

(d)

(e)



(f)

Supplementary Figure 6. 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 NA 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.

This is the Initial interface showed in the Serial Monitor of Arduino software.

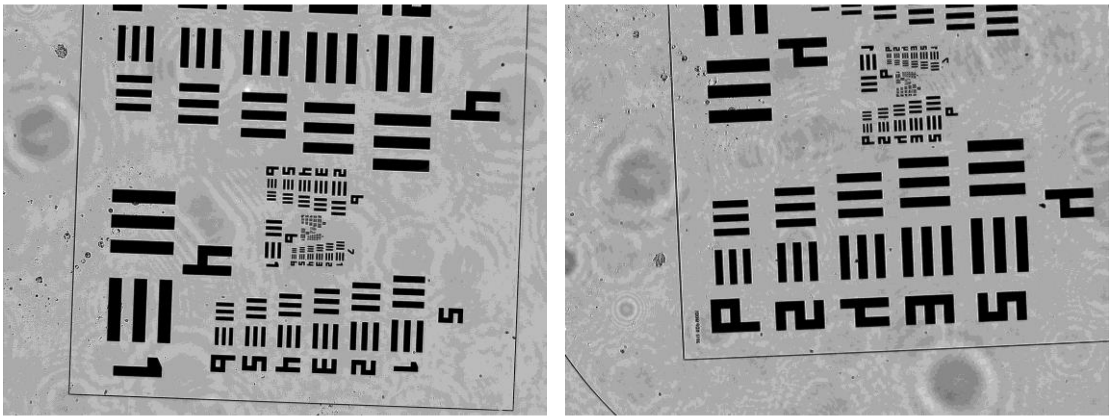
```
<Arduino is ready>
-----------------------------------
Command List:
-----------------------------------
COMMAND: Fluorescence excitation
SYNTAX:  fe/color_on/off/
EXAMPLE: fe/blue_on/
-----------------------------------
COMMAND: Bright Field
SYNTAX:  bf/(r,g,b)/
EXAMPLE: bf/(000,064,254)/
-----------------------------------
COMMAND: Dark Field
SYNTAX:  df/(r,g,b)/
EXAMPLE: df/(000,064,254)/
-----------------------------------
COMMAND: Phase Contrast
SYNTAX:  pc/(r,g,b)/field_direction(bt/bb/bl/br/dt/db/dl/dr)
EXAMPLE: pc/(000,064,254)/bl/
-----------------------------------
COMMAND: Multiple Points
SYNTAX:  mp/number(1~255)/(r,g,b)num1/(r,g,b)num2/.../
EXAMPLE: mp/3/(000,064,254)254/.../
-----------------------------------
COMMAND: Set Parameters
SYNTAX:  set/dist/num/
         set/na/num/
EXAMPLE: set/dist/90/
         set/na/0.5/
-----------------------------------
COMMAND: Turn Off All
SYNTAX:  x/
-----------------------------------
-----------------------------------
```

Matlab code controlling the whole image acquisition process

In the scripts, you can change the scanning step size of the Piezo motor, the exposure time for both illumination systems and the saving file folders' names.

Measuring the Magnification

We measure the magnification of the system (10X objective) by imaging the USAF resolution chart and calculate the magnification by custom Matlab function.



Trans_Magnification=5.5317

Fluo_Magnification=5.4535

Supplementary Figure 7. Images of USAF resolution chart from Camera 1(transmitted illumination system) and Camera 2(fluorescent illumination system).

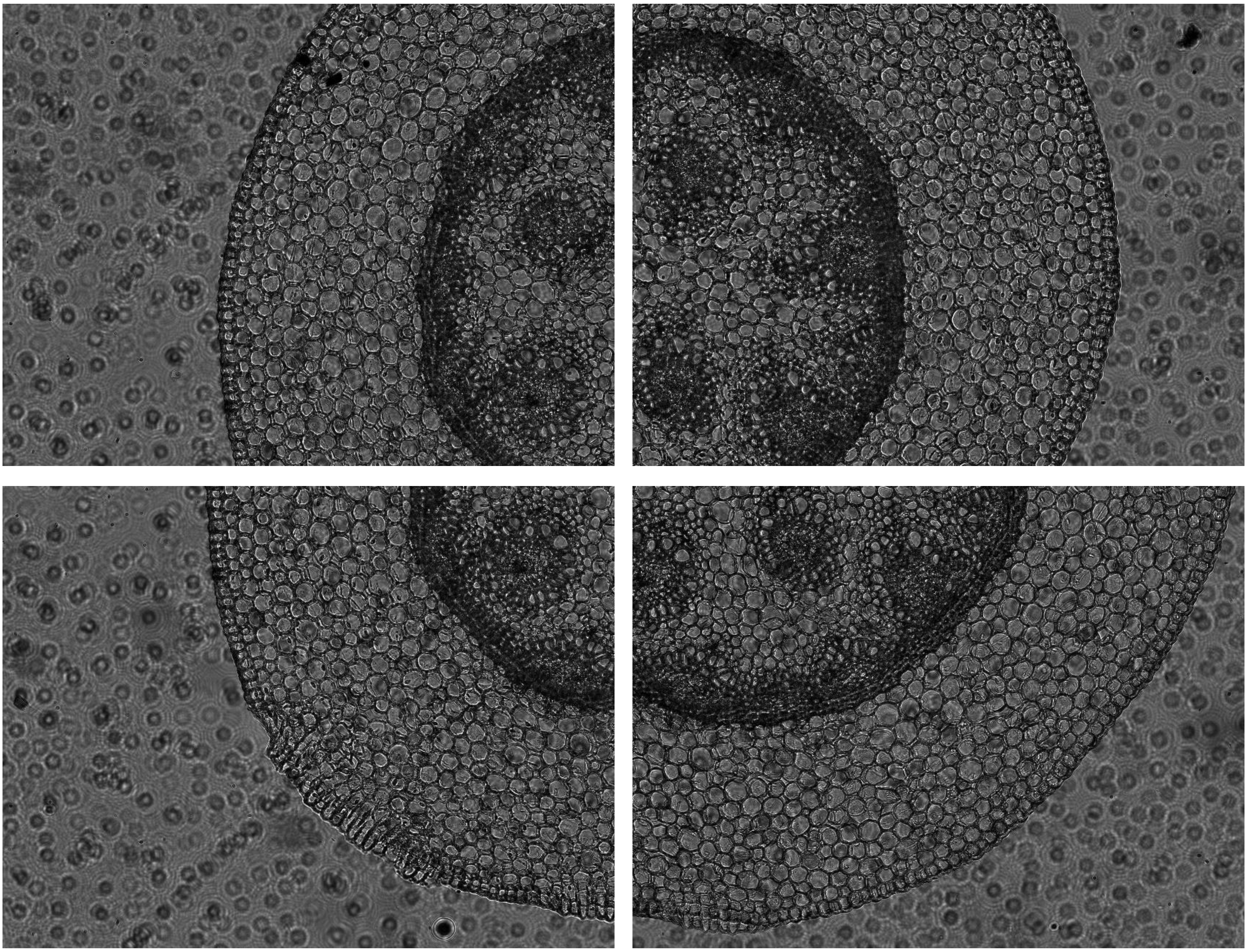
The theoretical magnification of this 4f system is $\frac{f_2}{f_1}$,which is really close to the experimental number.

Workflow

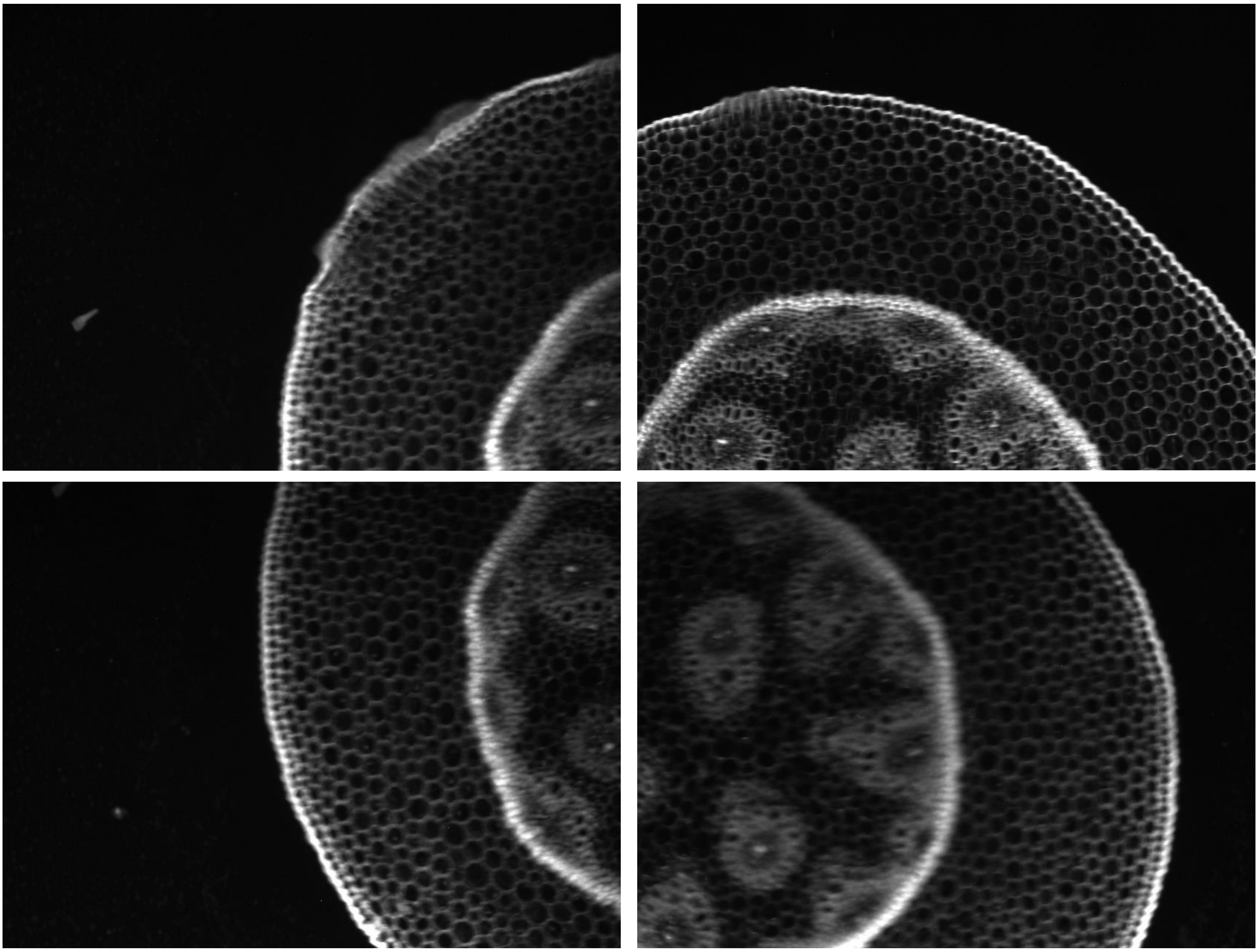
- Put your sample on the sample stage and fix with the pressing clamps
- Plug the external power plug of the LED array, the power plug of the Motor Controller and the power plug of PCB into the power source.
- Open the matlab data acquisition toolbox to preview.
- Focus the sample by two cameras using 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!



Supplementary Figure 8. Scanning Transmitted central point illumination(green) imaging of convallaria. The images has overlapping part. The scanning step size can be set in the custom MATLAB scripts *"main_control_Thorlabs_APT_disk_LED_BASLER_10X.m",*



Supplementary Figure 9. Scanning fluorescent imaging of convallaria. The images has overlapping part. The scanning step size can be set in the custom MATLAB scripts *“main_control_Thorlabs_APT_disk_LED_BASLER_10X.m”,*

Ⅵ.Links

Software download websites

Components ordering websites

Shared codes