Development and Setup of a Light-sheet Microscope

The aim of the workshop is to discover the optical sectioning by selective plane illumination (SPIM) and to build a compact microscope that makes use of illuminating a sample with a thin light-sheet from the side. The workshop teaches how a complex three-dimensional setup can be created with only a few steps using 3D printed modules.

The biological aspect of this workshop will stimulate your creativity and encourage you to find your own samples, prepare them and image them with the microscope. The digital reconstruction of the acquired image will be done using open source software tools such as Fiji.

Benedict Diederich
René Richter
Barbora Maršíková
Xavier Uwurukundo
Alejandra Zegarra
Rainer Heintzmann

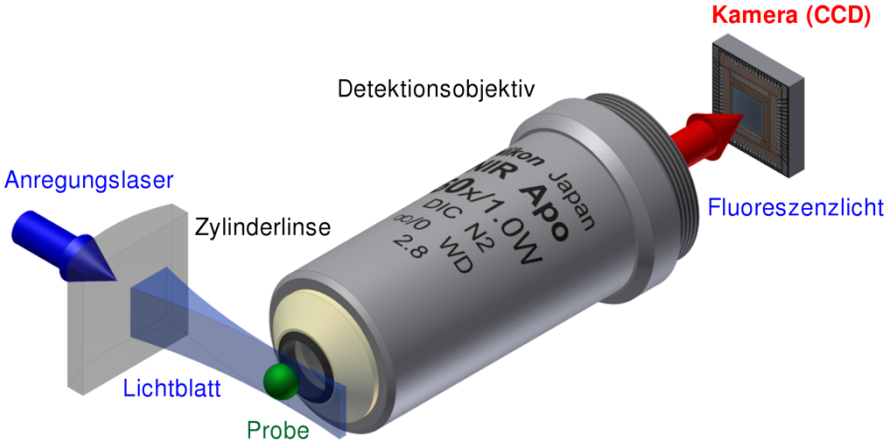
V0; 15.05.2019

Table of contents

Motivation

Key Elements of the workshop

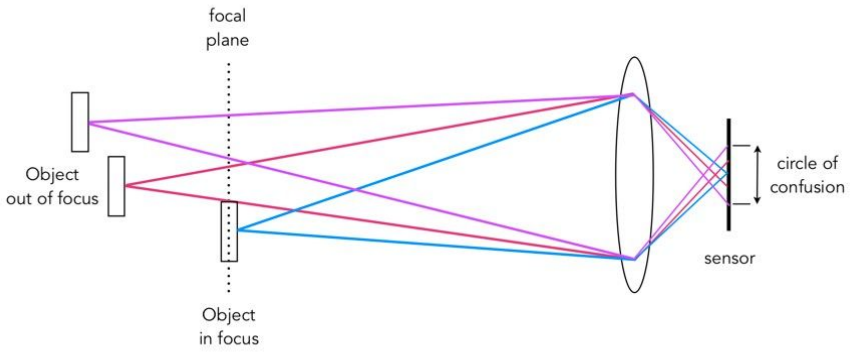
- What does selective plane illumination means?
- How can we build a light-sheet using off-the-shelf components?
- What is the cylindrical lens good for?
- How can we acquire 3D data?
- What is fluorescent Imaging?
- Getting familiar with the Raspberry Pi camera.



Abstract

Further information can be found in the wikipedia article:
https://en.wikipedia.org/wiki/Light_sheet_fluorescence_microscopy

The basic idea of lightsheet microscopy is to make the sectioning along optical axis independent from the imaging objective. This effect is mostly known from photography, where people try to have only a very thin place “in-focus”. Large lenses can produce a nice “Bokeh” which is mostly visible in portraits, where the eyes are in focus and the rest is out-of-focus. The effect can be explained using ray-optics. The lens produces an image on a detector - which is usually represented by a pixelated camera sensor. Only a limited range of rays from a sample (e.g. eye) are passing the entrance diameter of any lens. Imaging a 3D sample which is extended along the optical axis, is getting projected on the 2D sensor, therefore information beyond and after the actual focus is captured by the imaging-system. The amount of out-of-focus blur is governed by the diameter of the imaging lens - which is finite. The larger the opening diameter (sometimes also measured as the opening angle - sinus of the opening aperture with respect to the focal-length), the more light-rays can transmit the system and the better the z-sectioning capability of the system will be.



https://www.google.com/url?sa=i&source=images&cd=&cad=rja&uact=8&ved=2ahUKEwiLpLiMh4_jAhUSJVAKHfsFD2cQjhx6BAgBEAM&url=https%3A%2F%2Fcraftofcoding.wordpress.com%2F2017%2F10%2F23%2Foptical-blur-and-the-circle-of-non-sharpness%2F&psig=AOvVaw18BEE7k9xYu4urkjybjXCp&ust=1557511852549689

The same holds true not only in photography, but also in microscopy. When imaging biological sample, many people nowadays rely on fluorescent imaging. Pushing the optical resolution limit using super-resolution microscopy followed in techniques to capture fine structures of biological samples - at a price of biological hazardous chemicals which alter the living organism of the cell most of the time. To achieve high z-resolution, the concept of ultra-microscopy or in new terminologies light-sheet microscopy was invented. Here a thin light sheet illuminates a sample from the side (i.e. 90° degrees w.r.t. the optical axis) and therefore illuminates only parts which lay inside the volume of the sheet. The objective lens - perpendicular to the illuminating sheet - images the scattered or fluorescent part. The sectioning capability of the microscope is now governed by the thickness of the light-sheet and not depending on the opening angle of the microscopic objective which is usually proportionally to its price.

Advantages over classical Brightfield Microscopy

- Illumination and detection path is separated
- Very good optical sectioning => very thin planes from the 3D microscopic sample can be observed
- Measurements deep in the tissue are possible
- Low-cost setup makes the technique widely affordable and available

Goals

At the end of the workshop, the participants will be able to

- understand the working principle of light-sheet microscopy
- understand the mechanism of fluorescence in imaging
- develop a concept of light-sheet microscope
- get familiar with the open-source toolbox UC2
- get experience in the following fields:
    - Designing 3D CAD parts using 3D CAD software
    - Print the designed parts using 3D printers
    - Soldering electronics
    - Aligning optical setups
    - Programming hardware-control-systems for moving samples/lenses
    - Code an app which makes controlling the hardware through an GUI possible
- Putting the prototype into operation and testing it
- Evaluate and Document the work
- Contribute to further development of UC2 toolbox by providing feedback

General Time-Plan

Topic	When	What	Who	Where
Introduction	14:15-15:05	Reception by OSA/SPIE	SPIE, OSA	Foyer
		Registration, Badge, Pizza-Form, Legal Form	SPIE, OSA	Foyer
Talks 'n' Safety	15:00	Introduction, Schedule	René, Barbora	SR1 (left Seminar room)
	15:10	Talk about Lightsheet Microscopy	Rainer	SR1 (left Seminar room)
	15:25	Talk about UC2	René	SR1 (left Seminar room)
	15:40	Defining Goals & What do we have already? (=Start)	Benedict	SR1 (left Seminar room)
	15:55	Safety Instructions (get signature from participants again)	Benedict	SR1 (left Seminar room)
	16:05	Forming Teams	Barbora	SR1 (left Seminar room)
Grouped Work-phase	16:15	Go to the different stations/workshops	Benedict, Barbora, René, Alejandra	Workstations
	16:25-19:30	Work at different Stations	Benedict, Barbora, René, Alejandra	Workstations
Break (Dinner)	19:30-20:15	Pizza (time for SPIE/OSA to do advertisement)	SPIE, OSA	Food-Stand @ Foyer, Tables @ SR2 (small glass room)
Measuring Together	20:15 - 21:00	Put everything together	Barbora, Benedict, René	Room with optical table
	21:00	Assembly and interplay with electronics	Barbora, Benedict, René	Room with optical table
	21:00	Mount Sample, Acquire images	Alejandra	Room with optical table
Survey	21:30	Online Survey about the Workshop	Barbora	SR1 (left Seminar room)

Presentation	21:40	Final Presentation of the Group's results (5min per Group)	Groups	SR1 (left Seminar room)
	22:10	Closing remarks	René	SR1 (left Seminar room)
Clean-Up (coarse & fine)	until 23:00	Clean-up all rooms, Lichtwerkstatt etc (next day are lectures!)	all	all
Security	23:00	Close all rooms and gather keys	René	all

Time-Plan per Station

Topic	When	What	Who	Where
CAD/Printing	15:30-19:00	Intro to 3D printing	Barbora	Computer Pool
		Intro to 3D CAD Software (Inventor /Fusion 360)		
		Design 1-2 parts and print them (beamexpander/lens holder)		
		Print the parts		Lichtwerkstatt (Printing Room)
		Assembly the parts (Magnets, Screws, etc.)		
		Mount Telescope		
		Mount Lenses		
Programming	15:30-15:50	Short: Intro to Raspberry Pi programming (SD card, images, ...)	René	SR1
		install apps (Aduino IDE, ?)		
		Define goal: Shoot-move-shoot-move…		
	15:50-18:00	Intro to MQTT		
		1st MQTT circuit: controling ESP32-LED with button		
		2nd MQTT circuit: control motors via ESP32		
	18:00-19:30	Intro to Raspberry Pi camera (Shell)		
		Play with camera; find out how to efficiently manipulate camera		
		if time: Python-script to read-in parameter file and take image OR small (interactive) shell gui (e.g. Where to store image?, change settings?, ...)		

	???	Optional: Introduction to GUI-programming with kivy (Python->Raspi) or ReactNative(Android/iOS->Smartphon)		
Optics/Electronics	15:30-19:00	Solder Wires to ESP32+Motor (Z+X-stage)	Benedict	Workshop (Electronic)
		Test Motors with ready-to-use programm for ESP		
		give motors to René		
		Mount Laser		
		Start Assembling the entire setup		Lichtwerkstatt (Room with optical table)
		Wait for Barbora's Assembly & Get Motors from René		
		Put everything together		
		Align Optical path together	Benedict+Barbora	
Sample-Preparation + Documentation	15:30-19:00	Introduction in Sample Preparation	Alejandra	SR2 (Aquarium-room)
		Create Sample		
		Prepare sample for 3D printing people		
		Let them document everything (Fotos of other group-members work, note down steps; prepare final presentation)		
		Twitter @openUC2 & #openUC2		
		Teach them to use Fiji for reconstruction (install; easy example; ...)		

STRUCTURE OF THE MICROSCOPE (PRACTICAL PART)

In the following part, we will briefly explain what are the individual components that are used in the setup and how they relate to each other. At the end a light-sheet microscope is to be developed, which can be built completely.

FINISHED CONSTRUCTION

Figure 1 - This is what the finished microscope looks like when all parts come together.

Partlist

Bill of Material

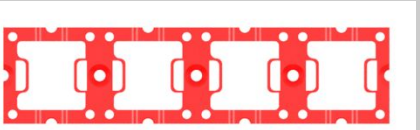
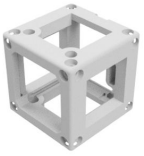
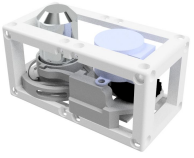
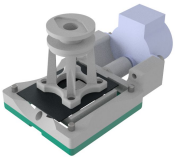
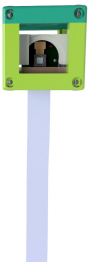
Amount	Setup Group	Title	Vendor	Price
1	Control (Raspi etc)	Anpro ESP32 NodeMCU Modul WLAN WiFi Entwicklungsboard Development Board, EINWEG	Amazon	6,99
1	Control (Raspi etc)	Ersatz Flexkabel 100 cm für Raspberry Pi Kamera -> 20Stk	Amazon	2
1	Control (Raspi etc)	Raspberry Pi Display 7"	Amazon	74,96
1	Control (Raspi etc)	Logitech K400 Plus Touch Wireless Tastatur schwarz (QWERTZ, deutsches Tastaturlayout)	Mindfactory	28,43
1	Control (Raspi etc)	Goobay 56746 Micro USB Ladegerät 3,1 A	Pollin	9,3
1	Control (Raspi etc)	Drahtbrücken	Pollin	3,59
1	Control (Raspi etc)	Raspi Case 7"	Pollin	14
1	Control (Raspi etc)	Raspberry Pi Noir Kamera-Modul V2	Pollin	26,95
1	Control (Raspi etc)	MicroSDXC Card, 64 GB, CLASS10, SANDISK Ultra	Pollin	12,95
1	Electronics	Z-Focus	Aliexpress	2,5
1	Electronics	XY-Stages	Aliexpress	3
2	Electronics	C 5V Schrittmotor Regler + ULN2003 Treibermodul Board 28BYJ-48 TE119	Amazon	3,398
1	Housing	500 Stück Inbus Zylinderkopfschrauben DIN 912 10.9 verzinkt M3x8	Amazon	21,7
1	Housing	Scotchtape	Amazon	10
0	Housing	IXO screwdriver	Amazon	42,37
1	Housing	Modelling Mass, Tesa, PAD	Böttcher	14
1	Housing	Avistron 1kg ABS 1.75mm 3D Drucker Filament innovative Verpackung (1.75, Schwarz)	Filamentworld	30
8	Housing	5x50 Rods, Alumiium, Workshop	IPHT Workshop	2
1	Housing	100x Kugelmagnet, 5mm, Neodym	Magnetmax	5
1	Illumination	450nm laserpointer, 5mW	Laserlands	16

1	Illumination	LED Torch	Pollin	6,95
1	Optics	Mikroskop Objektiv 10x, 0.3NA	Aliexpress	16
1	Optics	Mikroskop Objektiv 4x, 0.1NA	Aliexpress	12
1	Optics	Polarizing Film	Aliexpress	9,61
1	Optics	Comar, Excitation filter, GFP (?), 454 IY 116	Comaroptics	21
1	Optics	63 YE 25, cylindrical lens 63, coated	Comaroptics	40
1	Optics	iPhone 5 camera/lenses	Ebay	6,99
1	Optics	Plankonvex Linse 20/12,7	Optikbaukasten	4,95
1	Optics	Bikonvex Linse 50/12,7	Optikbaukasten	4,95
1	Optics	Plankonvex Linse 40/25,4	Optikbaukasten	4,95
1	Optics	PF10-03-P01 - Ø1" Protected Silver Mirror	Thorlabs	47,29
0	Safety	Lasersafety - Goggles (borow them?) -> Maybe not necessary (weak laser used)	Laserlands	0
1	Sample	Spritzenset 2ml (25x), 5ml (25x) , 10ml (10x), 20ml (10x) + Box	Amazon	15
1	Sample	Fluorescenz Marker yellow	Böttcher	0,77
1	Control (Raspi etc)	Raspberry 1373331 Pi 3 Modell B+ Mainboard, 1GB	Amazon	36,5
1	Control (Raspi etc)	AmazonBasics - Geflochtenes HDMI-Kabel, 3 m	Amazon	8,99

List of 3D printed parts

All parts with detailed assembly/print description can be found in the UC2 github repository:

https://github.com/bionanoimaging/UC2-GIT/tree/master/CAD

Anzahl	Bezeichnung	Bild	Preis
1	Grundplatte (4x4)		1€
7	Cube (1x1), 2 Teile		1€
1	Cube (2x1) + Z-Stage (Microscope Objective)		1€
1	Cube (2x1) + Z-Stage (Sample)		1€
1	Cube - Raspi-Camera Adapter		1€

In the following we first describe each component briefly before we go into details about the optical system which produces the light-sheet and later the image of a given sample.

A detailed description on how to assemble everything can be found on our github repository under CAD -> and then each module individually.

- https://github.com/bionanoimaging/UC2-GIT/tree/master/CAD/

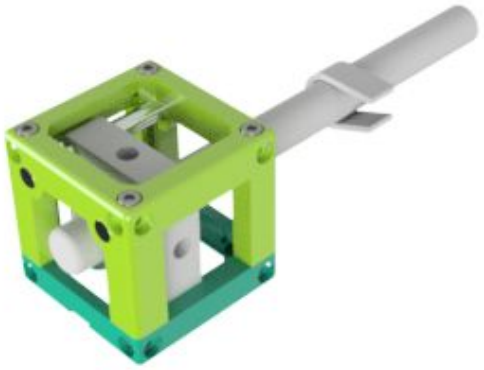
LASER mount cube

This is the repository for the Laser Mount Cube.

The stl-files can be found in the folder STL.

Purpose

It adapts a laser-pointer to the to the UC2 system.



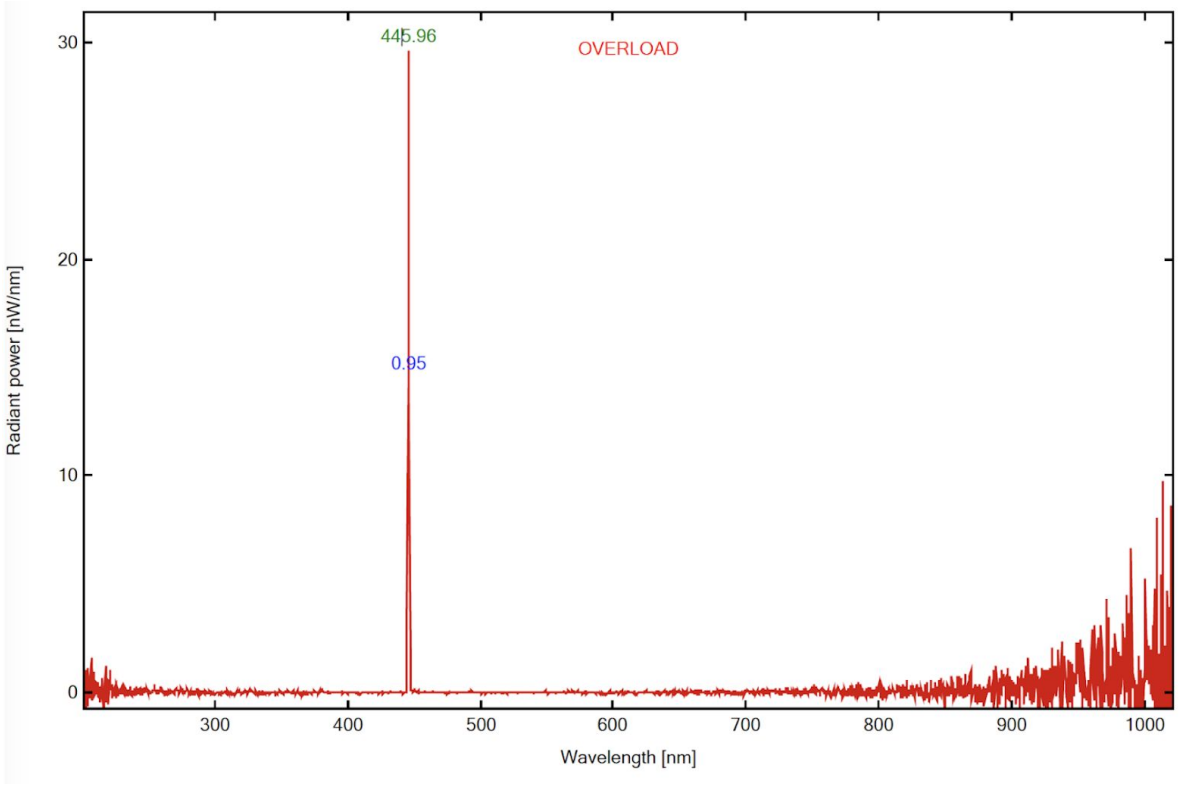
The laser-pointer is permanently switched on/off using a 3D printed clamp. It is inserted in a Thorlabs-like adapter which centers the laser on the optical axis. Using a set of rods, this adapter can be mounted inside the base-cube. Having two of these adapters makes the design very robust!

Properties

- design is derived from the base-cube
- the adapter for the laser can be adjusted to individual laser-pointer diameters
- the 4 screws make centering of the laser w.r.t. the optical axis easy
- Diode-laser, Multimode lineprofile, Beam
- Peak-Wavelength: ***446 nm ***

Laser Spectrum

The measured spectrum from the 450nm laser pointer we used for the Lightsheet setup can be found below:



Laser Power

We measured a mean power of ***0.546 mW*** in continuous mode. We used new batteries.

Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)
- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Laser Mount Adapter** which holds the laser and adapts it to the base cube (INLET)
- **The Laser Clamp** allows switching the laser permanently (clamp)

Additional parts

- 8x DIN912 M3*12 screws (non stainless steel)
- Laserlands 450 nm laser-pointer
- 2x Rod's (50mm*6mm Diameter)

Remarks and Tips

3D Printing:

- No support required in all designs
- Carefully remove all support structures (if applicable)

Assembly

- Prepare the laser-pointer by inserting the batteries
- Insert the 4 screws in the Laser Mount Adapter and screw them just so far that they are not visible in the inner hole
- Insert the Laser-pointer inside the adapter
- Fix the laser by precisely rotate all 4 screws so that the laser-pointer is centered
- Insert the rods on one side of the cube
- Put the Laser Mount Adapter inside the cube and mount them on the rods by sliding the rods through the holes
- Add the lid to the cube and fix it with the 4 M3 screws
- Done!

Safety

Never (!) look into the laser pointer! It will damage your eye immediately!

- ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY
- NEVER SWITCH ON THE LASER WITHOUT INTENDED USE
- BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!

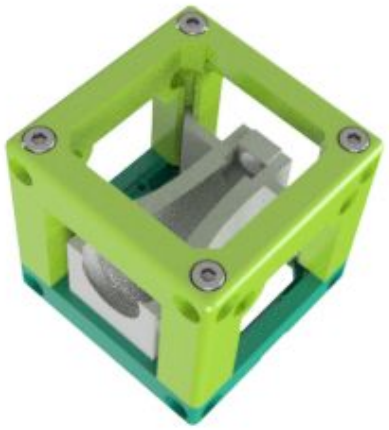
Integrated Telescope/Beam-Expander Cube

This is the repository for the Integrated Telescope Cube.

The stl-files can be found in the folder STL.

Purpose

It adapts a very small beam-expander to the UC2 system.



Sometimes one need to increase the diameter of an illuminating beam. This is necessary for the Light-sheet microscopy setup for example. Here we have a laser-pointer which comes with a relatively small beam-diameter of about 2mm. By using a telescope, this can be magnified by a factor of e.g. 8 which results in a beam-diameter of 16 mm. This is necessary to overfill the aperture of the following illumination objective lens.

In order to achieve this, we first need to focus the beam with a low focal length lens (e.g. cellphone lens, f'=3mm) and then re-collimate the lens with a second lens with a larger focal length e.g. f'=25 mm.

We designed a telescope where an iPhone 5 lens and an ordinary 25mm lens can be inserted in an adapter, which finds its place inside an ordinary UC2-cube as visualized in the image above.

Properties

- design is derived from the base-cube
- the adapter for the telescope can be adjusted for different magnifications and lenses
- the beam height can be adjusted by sliding the the telescope along the axis
- the degree of collimation can be adjusted beforehand * very cost-efficient beam-expander at a fairly good quality as the cellphone lens is diffraction limited (overall costs ~15€)
- Magnification : m=f*tl*/*f*ep = 25/3 = 8.33

Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)

- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Telescope** which holds the two lenses and adapts it to the base cube (TELESCOPE)

Additional parts

- 4x DIN912 M3*12 screws (non stainless steel)
- iPhone 5 lens (separated from an iPhone camera spare part), f'=3mm
- biconvex/plano-convex lens, f'=25mm, diameter=20mm, thickness=4mm

Remarks and Tips

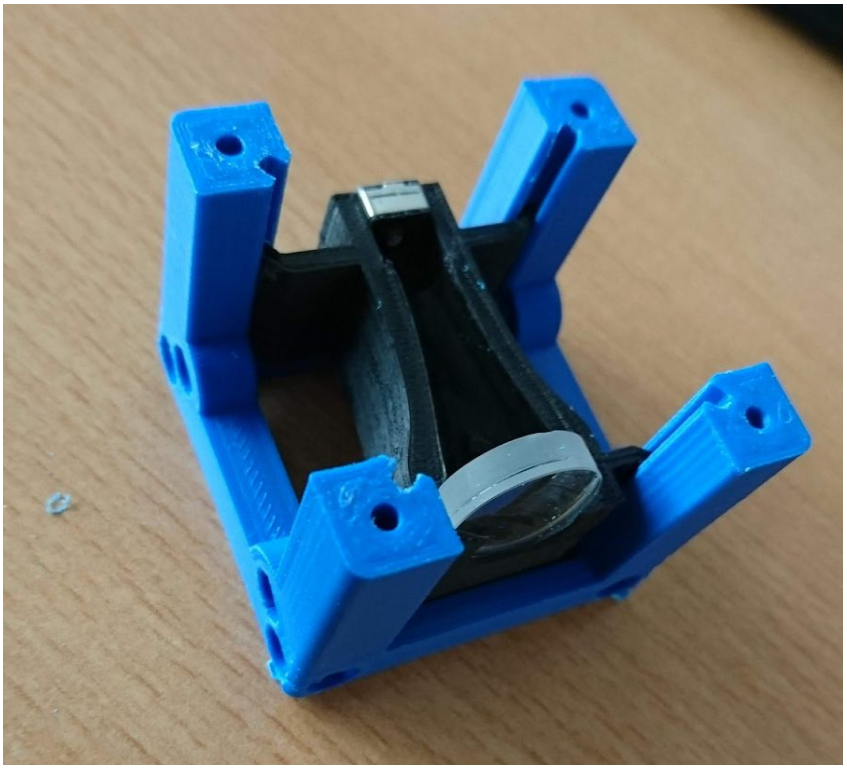
3D Printing:

- No support required in all designs
- Carefully remove all support structures (if applicable)
- You may choose to design your own telescope inlet

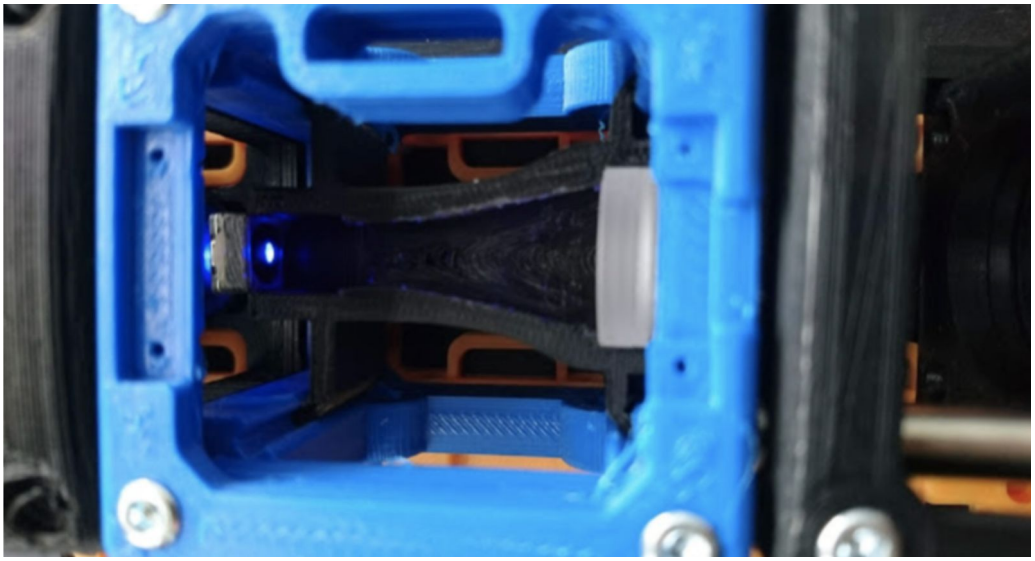
Assembly

- Remove the iPhone lens from the camera (a dedicated tutorial can be found in the UC2 Tutorial-Section.
- Insert the lenses inside the telescope adapter (orientation of the iPhone has to be the way, that the small aperture - hole - has to face the other bigger lens as indicated by the photo)

- Take the laser-pointer
- Point the laser towards the iPhone Lens
- Adjust the distance between the two lenses, so that the beam is collimated (=the beam diameter right after the telescope should not change over any distance)
- Put the telescope inside the cube by sliding it along the slides



- Add the lid to the cube and fix it with the 4 M3 screws
- Done!



Safety

Attention, don't cut your fingers while removing the lens from the iPhone sensor!

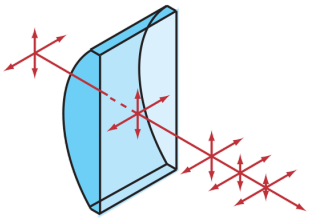
Never (!) look into the laser pointer! It will damage your eye immediately!

- ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY
- NEVER SWITCH ON THE LASER WITHOUT INTENDED USE
- BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!

Cylindrical Lens Holder

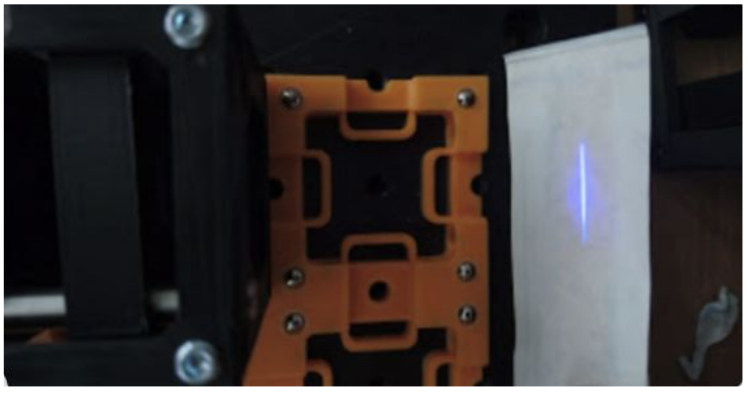
Purpose

- Like any other lens, a cylindrical lens focuses the incoming light. In case of a positive focal length, the focal spot can be found right after the lens inside the back focal plane (BFP). In case of plano-convex lens, the distance from the lens to the focal spot is measured as the focal length of the lens. In case of a cylindrical lens, the focus spot is not a single point as in a rotationally symmetric lens, but rather a line like focus. This is because an incoming parallel beam gets focussed only in one direction. In the eye this sometimes happens and hinders clear eyesight by introducing astigmatism (greek point-less)
- More information: https://www.edmundoptics.com/resources/application-notes/optics/what-are-cylinder-lenses/



Properties

- We rely on a set of 3 different lenses with a clear aperture/diameter of 25mm. The focal length varies from 40mm, 63mm and 100mm to have variety of different configurations.



Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)
- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Custom Lens Adapter** (holder and a C-ring) which fits onto the rails inside the cube and holds the lens in it position

Additional parts

- 4x DIN912 M3*12 screws (non stainless steel)
- Comar Optics, 63 YE 25, cylindrical lens f' = 63 mm, coated (alternatively 40 YE 25 or 100 YE 25 with focal lengths 40 mm and 100 mm respectively)

Remarks and Tips

3D Printing:

- No support required in all designs
- Carefully remove all support structures (if applicable)
- You may choose to design your own cylindrical lens holder that allows for easy rotation of the lens

Assembly

- The lens simply goes inside the Lens Holder, where the Ring is press fit in the hole to lock the lens's position. Be careful and don't touch the surfaces of the lens!
- Safety:

Safety

Don't touch the lens's surface!

Never (!) look into the laser pointer! It will damage your eye immediately!

- ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY
- NEVER SWITCH ON THE LASER WITHOUT INTENDED USE
- BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!

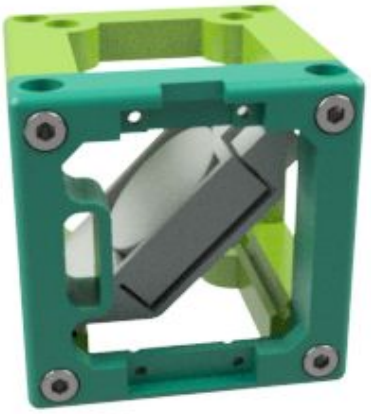
Adjustable Mirror Holder Cube

This is the repository for the Adjustable Mirror Holder Cube.

The stl-files can be found in the folder STL.

Purpose

It adapts a 1 inch fold-mirror to the UC2 system.



Due to limited space, we need to fold the beam using a mirror. This is done by reflecting the incoming light under an angle of 45°. It follows in a change of the optical axis by 90°

Properties

- design is derived from the base-cube
- the adapter holds a 1 inch circular mirror (e.g. Thorlabs part) at 45 degrees in a UC2 base cube
- the angle of the mirror can be varied in a limited range (e.g. +/- 5°-10°) using a flexure bearing driven by a screw and nut + spring to apply pre-force
- the here used mirror has the following parameters:
    - Diameter: 25,4mm
    - Reflectance
    - Surface Flatness: (Peak to Valley) λ/10 @ 633 nm
    - Substrate Fused: Silica
    - Thickness: 6.0 mm (0.24")

Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)
- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Adjustable Mirror Holder** which holds a 1 inch Mirror and adapts it to the base cube (LENSHOLDER)

Additional parts

- 5x DIN912 M3*12 screws (non stainless steel)
- 1x M3 Nut
- 1x Thorlabs PF10-03-P01 - Protected Silver Mirror

Remarks and Tips

3D Printing:

- No support required in all designs
- Carefully remove all support structures (if applicable)

Assembly

- Remove any support and clean the part
- Insert the screw and nut in the appropriate place
- Add a spring between the part which gets bended and the back (non-moving part).
- Insert the mirror in the appropriate hole
- Slide in the assembled Mirror part into the Cube-Base
- Add the lid and fix it using a set of M3 screws
- Done!

Safety

Don't touch the silver surface!

Never (!) look into the laser pointer! It will damage your eye immediately!

- ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY
- NEVER SWITCH ON THE LASER WITHOUT INTENDED USE
- BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!

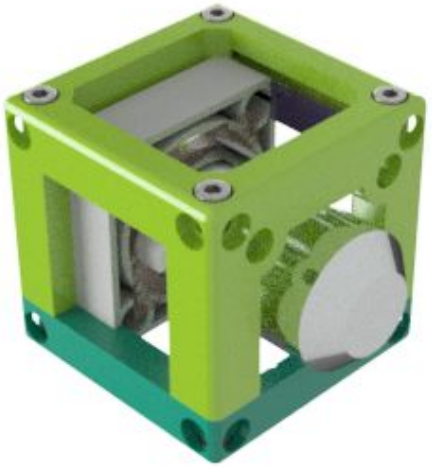
Objective-/Lens-holder Cube

This is the repository for the Objective/Lens-holder Cube.

The stl-files can be found in the folder STL.

Purpose

It adapts a RMS-threaded objective lens or any lens with a diameter of ~18-25 mm to the UC2 system.



The Objective lens images the light-sheet created by the cylindrical lens. This means, that its size can be decreased, thus being beneficial for the optical sectioning of the device. According to the formula d=lambda/sin(alpha)=lambda/NA, where lambda corresponds to the central wavelength of the illuminating rays and NA=sin(a) is given by the opening angle of the imaging lens, the minimal diameter d of a spot inside the focus of the lens is smaller, the smaller the NA of the lens is. Here we use a lens with an opening aperture of NA=0.1 which generates a thinner lightsheet, thus allowing better sectioning.

Properties

- design is derived from the base-cube
- the adapter can hold a large variety of different lenses (different diameters/threads)
- the spiral automatically centers the lens to the optical axis
- the here used objective lens has the following parameters:
    - Thread: RMS
    - Magnification: 4x
    - NA: 0.1
    - Finite Corrected Optics

Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)
- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Objective/Lens Holder** which holds a lens with varying diameter and adapts it to the base cube (LENS-HOLDER)

Additional parts

- 4x DIN912 M3*12 screws (non stainless steel)
- Microscopic Objective lens, 4x, 0.1NA, RMS-Thread, Finite corrected
- 2x Rods, 50*6mm, steel/aluminium

Remarks and Tips

3D Printing:

- No support required in all designs
- Carefully remove all support structures (if applicable)

Assembly

- Unscrew both of the caps of the objective lens to be left with the objective lens only in its mount
- Widen the 4 holes of the RMS adapter, so that it fits on the rods using a 4mm drilling tool (not too loose, not too stiff)
- Insert the 4x objective lens into the RMS-Adapter
- Insert the rods on one side of the cube
- Put the lens mount adapter inside the cube and mount it on the rods by sliding the rods through the holes
- Add the lid to the cube and fix it with the 4 M3 screws
- Done!

Safety

Never (!) look into the laser pointer! It will damage your eye immediately!

- ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY
- NEVER SWITCH ON THE LASER WITHOUT INTENDED USE
- BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!

Z-Stage (Sample) Cube

This is the repository for the Z-Stage (Sample) Cube.

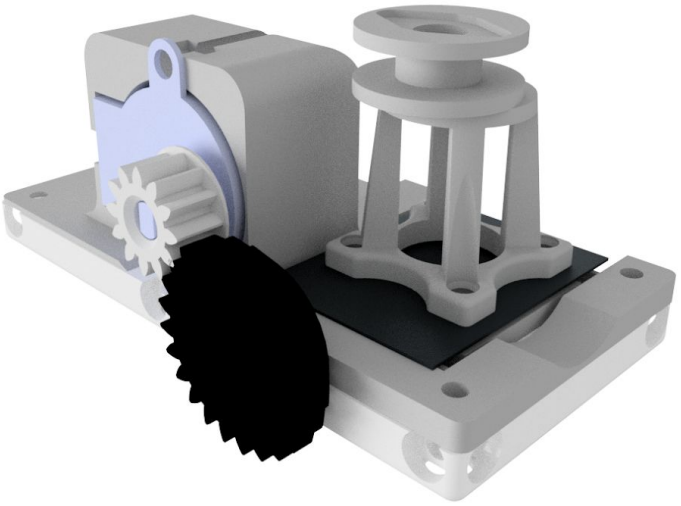
The stl-files can be found in the folder STL.

Purpose

In light-sheet microscopy one often needs the ability to move the sample through the illuminating light-sheet in order to capture the 3D information from the sample. This can either be done by scanning the light-sheet and focusing the objective lens simultaneously or by simply moving the sample along the optical axis w.r.t. the detection objective lens.

To keep the setup as simple as possible we decided to mount the sample (e.g. polen in agarose prepared in a syringe) on a stage which linearly moves it back and forth. Therefore, the light-sheet and objective lens once aligned can always stay in the same position.

The mechanism is as follows: A stepper motor (28-BYJ) drives a small gearbox which rotates a screw. On the screw, there is a nut which acts as a worm-drive. The conversion of the rotational into linear movement pushes/pulls a small table which is formed by a set of flexure-bearings. The syringe can be placed on a dedicated stand which is mounted using magnets on a ferromagnetic metal plate itself glued to the moving table.



For an updated version, please have a look at the Version 2:

- https://github.com/bionanoimaging/UC2-GIT/tree/master/CAD/CUBE_STAGE_Z-Sample_v2

Properties

- theoretically no play due to the use of flexure bearings
- moving range around +/- 10mm
- very low cost by relying on off-the-shelf components

Parts

3D printing parts

The Part consists of the following components.

- **The Lid (Special)** where the Arduino + Electronics finds its place (LID)

- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Syringe Holder - I** which holds a Syringe (Syringe Holder - I)
- **The Syringe Holder - II** which holds a Syringe (Syringe Holder - II)
- **The Z-Stage and Motor Holder** which moves the sample and holds the stepper motor (Z-Stage)
- **The Gear (large)** which drives the wormdrive - borrowed from BOWMAN'S flexurescope (gear (large))
- **The Gear (small)** which drives the wormdrive - borrowed from BOWMAN'S flexurescope (gear (small))

Additional parts

- 4x DIN912 M3*12 screws (non stainless steel)
- 3x M3 Nut
- 1x M3 Screw, 26 mm
- 1x M4 Screw, 16 mm + M4 Nut
- 1x ferromagnetic plate ~ 30x40 mm, 1mm thickness
- 1x 28-BYJ stepper motor
- 1x Driving electronic
- 1x ESP32 for controlling the motor
- 1x USB Micro Cable

Remarks and Tips

3D Printing:

- **The Z-Stage and Motor Holder is printed with support, so is the Syringe holder**
- Carefully remove all support structures (if applicable)

Assembly

- Detailed description coming soon
- Add motor and small gear, fix it with M4 screw
- Add the M3 nut in the dedicated hole close to the moving stage
- Add M3x26mm screw with mounted large gear at one end and insert it into the hole.
- rotate the M3x26 screw so that it pushes the moving z-stage
- Wire the motor, test it
- Done!

Safety

Be careful! The mechanism can be fragile, manipulate it carefully in order not to break it.

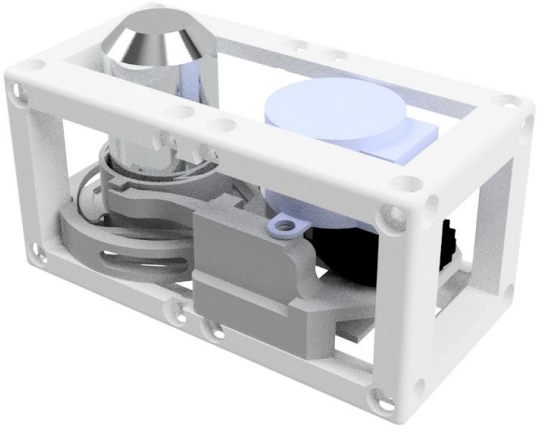
Z-Stage (Objective) Cube

This is the repository for the Z-Stage (Objective) Cube.

The stl-files can be found in the folder STL.

Purpose

In microscopy one often needs the ability to move the objective along the optical axis in order to refocus a given 3D sample. In order to automate this, we designed a very simple z-stage itself relying on flexure bearings also known from Bowman's flexurescope. The main difference here is, that we rely on a spiral-design which avoids the parallel-shift of the objective lens and makes the entire design very robust.



The mechanism is as follows: A stepper motor (28-BYJ) drives a small gearbox which rotates a screw. On the screw, there is a nut which acts as a worm-drive. The conversion of the rotational into linear movement pushes/pulls a small level-arm which is connected to the spiral spring-like linear actuator. It can thus move up-and down around the resting position. Another spiral-like spring can hold an objective lens with varying sizes. It also allows coarse z-focusing.

Properties

- theoretically no play due to the use of flexure bearings
- moving range
    - fine: around +/- 8mm
    - coarse: around +/- 20 mm (shifting the objective lens inside the spiral-like spring
- very low cost by relying on off-the-shelf components

Parts

3D printing parts

The Part consists of the following components.

- **The Lid (2x1)** where the Arduino + Electronics finds its place (LID)
- **The Cube (2x1)** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Z-Stage and Motor Holder** which moves the objective and holds the stepper motor (Z-Stage)
- **The Gear (large)** which drives the wormdrive - borrowed from BOWMAN'S flexurescope (gear (large))
- **The Gear (small)** which drives the wormdrive - borrowed from BOWMAN'S flexurescope (gear (small))

Additional parts

- 8x DIN912 M3*12 screws (non stainless steel)
- 3x M3 Nut
- 1x M3 Screw, 26 mm
- 1x M4 Screw, 16 mm + M4 Nut
- 1x 28-BYJ stepper motor
- 1x Driving electronic
- 1x ESP32 for controlling the motor
- 1x USB Micro Cable

Remarks and Tips

3D Printing:

- Print with support
- Carefully remove all support structures (if applicable)

Assembly

- Detailed description coming soon
- Add motor and small gear, fix it with 2 M4 screws
- Add the M3 nut in the dedicated hole close to the moving stage
- Add M3x26mm screw with mounted large gear at one end and insert it into the hole.
- rotate the M3x26 screw so that it pushes the moving z-stage
- Wire the motor, test it
- Done!

Safety

Be careful! The mechanism of the stage can be a bit fragile, make sure you don’t break it while removing the support.

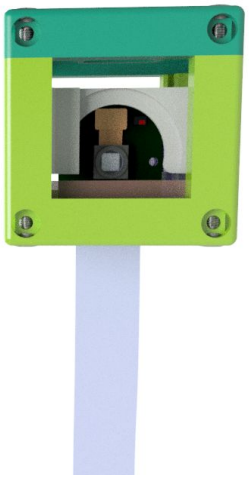
Camera Cube

This is the repository for the Camera Cube.

The stl-files can be found in the folder STL.

Purpose

It adapts a standard Raspberry Pi Camera (v1, v2) to the UC2 system.



The sensor (w/wo lens) is put into an adapter which holds the camera in the center of the cube. The height can be varied by sliding the adapter along the slides. It is designed to eventually hold (fluorescent) filters. The camera needs to be fixed with a set of screws. M2x10mm in combination with nuts work best.

Properties

- design is derived from the base-cube
- camera adapter can be adjusted to individual needs

Parts

3D printing parts

The Part consists of the following components.

- **The Lid** where the Arduino + Electronics finds its place (LID)
- **The Cube** which will be screwed to the Lid. Here all the functions (i.e. Mirrors, LED's etc.) find their place (BASE)
- **The Camera Adapter Inlet** which holds the camera and makes it adaptable to the base-cube (INLET)

Additional parts

- 4x DIN912 M3*12 screws (non stainless steel)
- Raspi Camera (v1, v2)
- 2x M2*10 screws (best: plastic)
- 2x M2 nuts (best: plastic)

Remarks and Tips

3D Printing:

- No support required in all designs

Assembly

- Mount the flex cable to the raspi-cam
- Mount the camera board to the Camera-Inlet using the 2 M2 screws
- Fix the position by mounting the 2 nuts. Take care to not destroy the camera'S printed circuit board (PCB)
- Take the mounted camera adapter inlet and slide it into the base-cube
- Take the cube lid and mount it using the 4 hex screws
- Done!

Safety

Be careful with the camera's PCB. It's sensible to electronic static discharge!

CONSTRUCTION OF 3D PARTS AND 3D PRINTING

The construction of the 3D objects will be done with Autodesk Inventor 2017. Alternatively, it would be possible to use any newer version of Inventor, Autodesk Fusion 360 or other 3D designing software. Each component can be created and saved as a Part (.ipt) and then exported for 3D printing as an .stl file (3D object). These STL files can then be inserted into the 3D printing software (e.g. Cura), arranged and exported to an SD card for 3D printing. For a multi-part component, created and Assembly (.iam), place the chosen parts and arrange them together.

Remember:

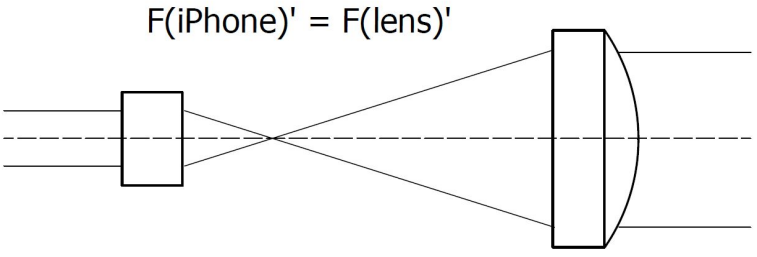
- 3D printed parts, especially holes and slits will always be a little smaller when you print them, compared to your design

Parameters for printing:

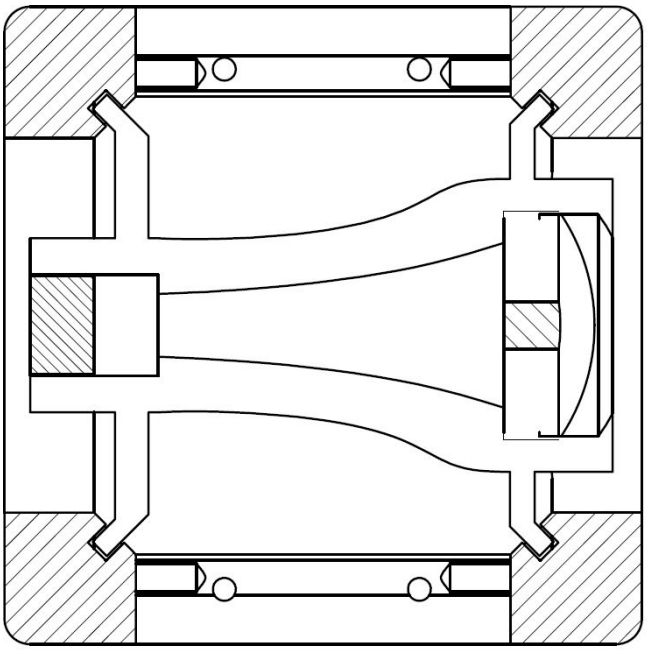
- Cura -> Prusa i3 Mk2
- Material:
- Nozzle:
- Layerheight:
- Infill:
- Support structure:
- Build plate adhesion:

You can choose to design one or both of the following parts

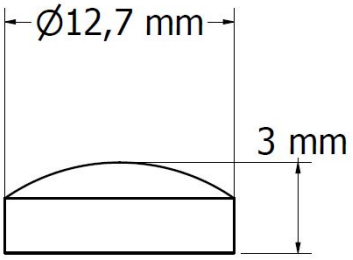
1. Beam expander
    - a telescope to expand the laser beam diameter
    - two lenses - an iPhone lens (f'=3mm) and a plano-convex lens (f'=20mm)
    - to create a collimated laser beam, the focal planes of the lenses must coincide (hint: 21 mm distance from surface to surface for these particular lenses)



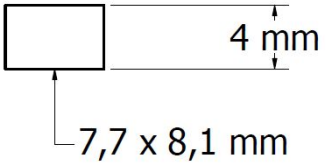
    - design a holder for the two lenses that will
        - ensure they're always in the correct mutual position
        - fit stiffly inside a single cube
        - the beam won't be limited in its diameter more than necessary
    - check out the STL file for dimensions: https://github.com/bionanoimaging/UC2-GIT/tree/master/CAD/CUBE_Pretty-Beamexpander/STL

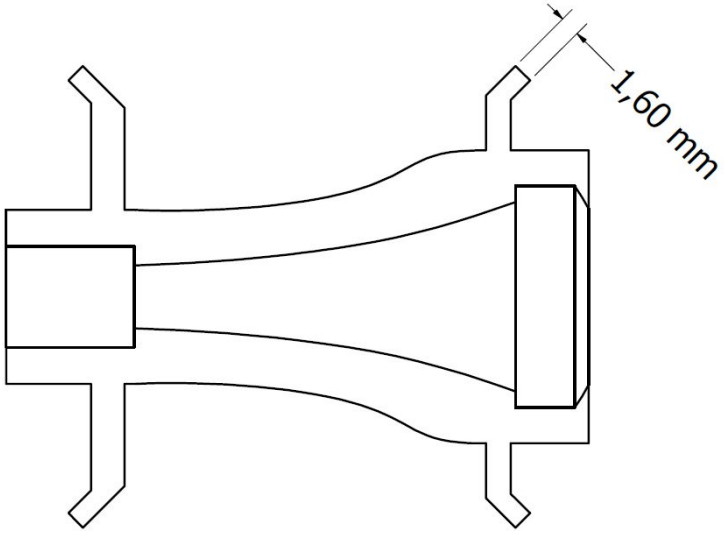


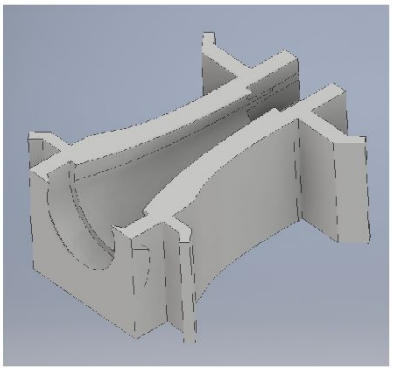
Lens f'=20 mm



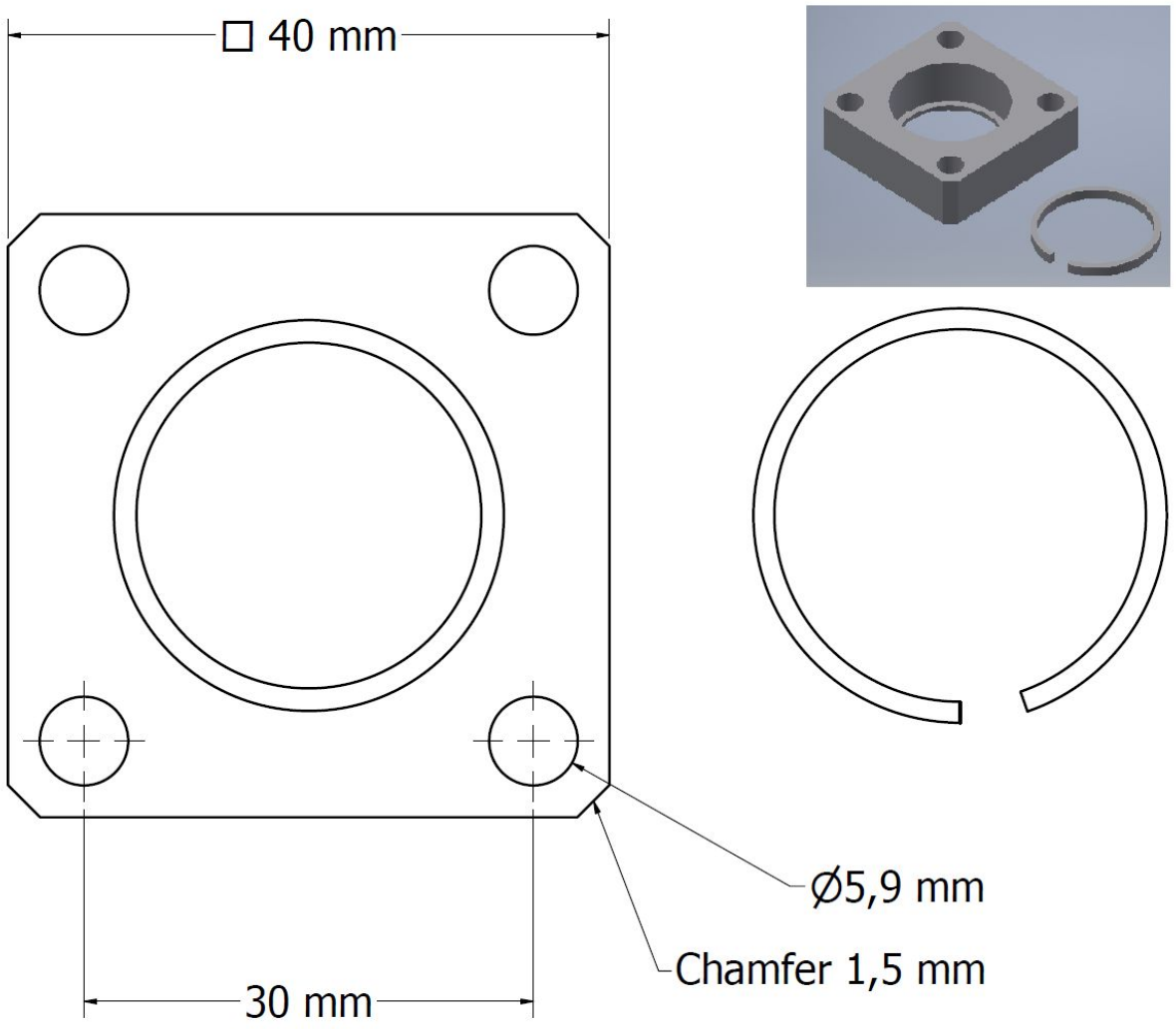
iPhone lens f'= 3 mm







2. Cylindrical lens holder
    - the current holder does not allow for easy rotation of the lens, therefore alignment of the light-sheet is difficult
    - design a holder that will
        - fit the rail system inside a cube, so the holder can be easily shifted
        - enable the lens to be rotated in such a way that the light sheet can be easily aligned to be really perpendicular to the detection path, even after the cube has been assembled
    - you may start from the current design and add an inlet that will press-fit in the holder but will also rotate freely



Code for the XY-STAGE

The electronics part of this project is not complicated. We only need two motors which drive the Z-stages for the microscope objective lens and the sample stage. We rely on the well-known 28BYJ-48 Stepper motor which operates at 5V and has up to 4096 steps per revolution. It comes with a motor driver (TI ULN2003) which converts a 4-wire input signal into the 5-wire output signal necessary for the bipolar stepper motor. Further resources can be found here.

In order to let the motor spin in both directions with different speed, we need to generate a signal which has a specific order of high/low pulses for each channel. Therefore we use a microcontroller (ESP32 DEV) which can conveniently be programmed in the Arduino IDE (see programming section).

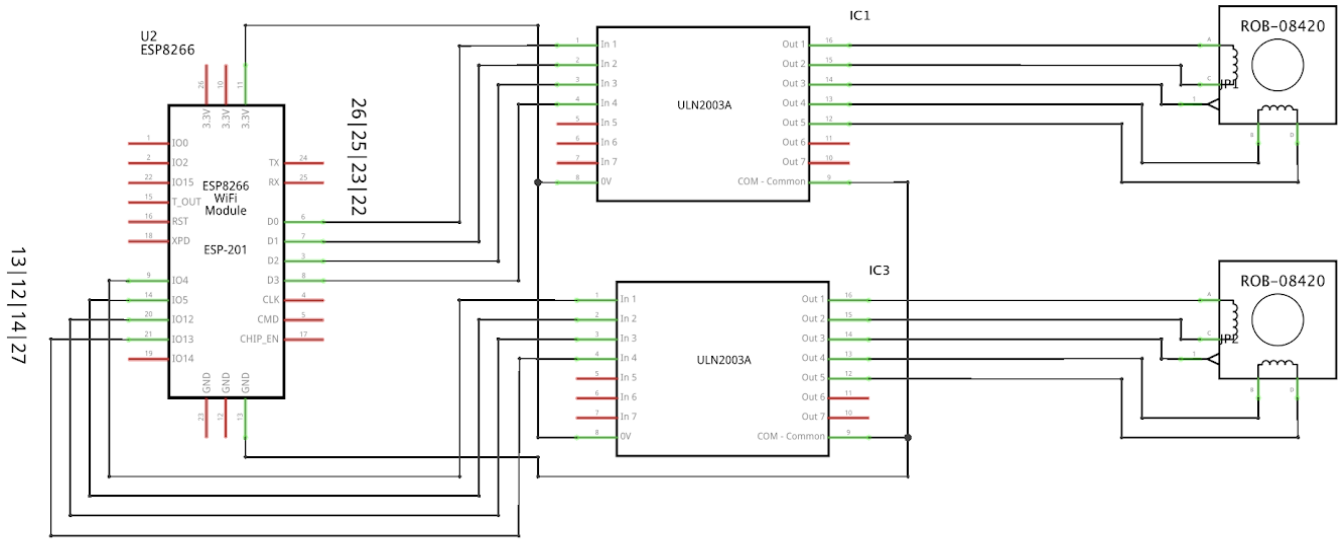
Wiring

The wiring of the electronic setup is done with only a few wires. We just need to connect each input-channel of the motor-controller board to an output of the ESP microcontroller. Additionally we need to connect the power of both controllers (i.e. +5V, GND). In general we could use any of the output-pins of the ESP32, but here we use the following:

Motor 1 = 13,12,14,27

Motor 2 = 26,25,23,32

They connect to the IN1, IN2, IN3, IN4 of the motor controller as indicated below:



Soldering

The process of connecting the motors to the ESP is again done with less effort. One only needs to solder a set of wires from the output-pins of the ESP to the input pins of the ULN2003 motor controller. Therefore one should follow these steps:

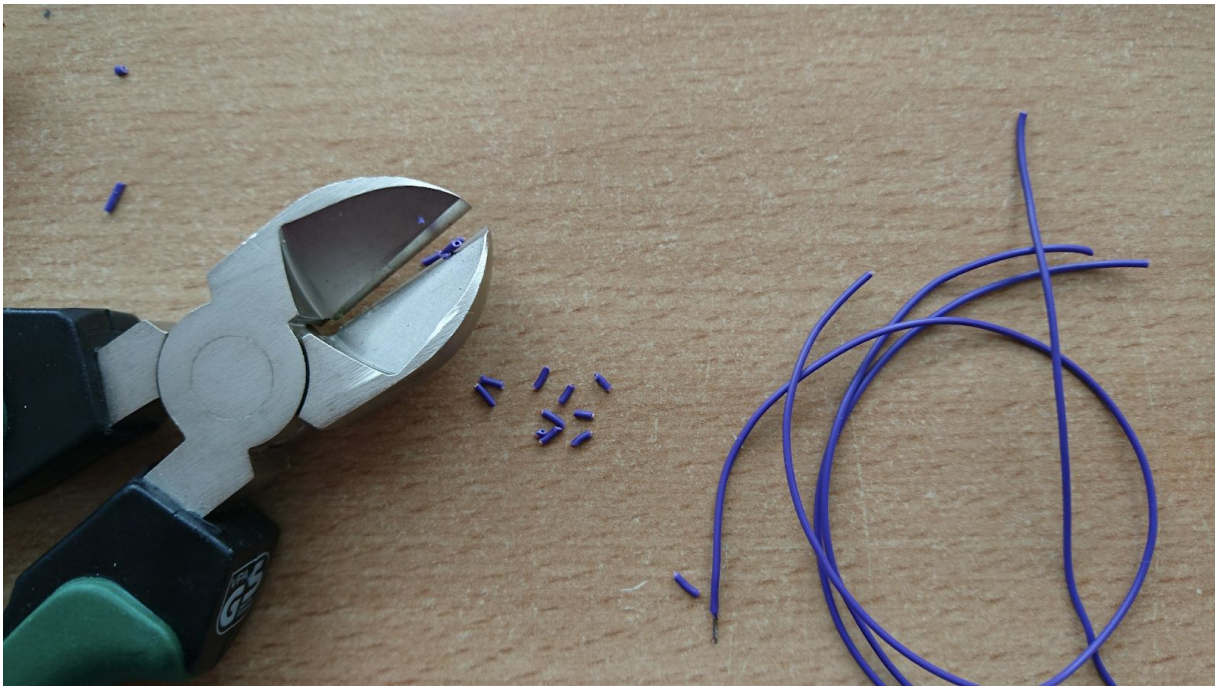
- Taking 10 wires and cut them to around 100 mm
- Remove the isolation of each wire at both sides
- Add some lead to both sides using the solder iron
- Solder the wires to the electronic parts

NOTES

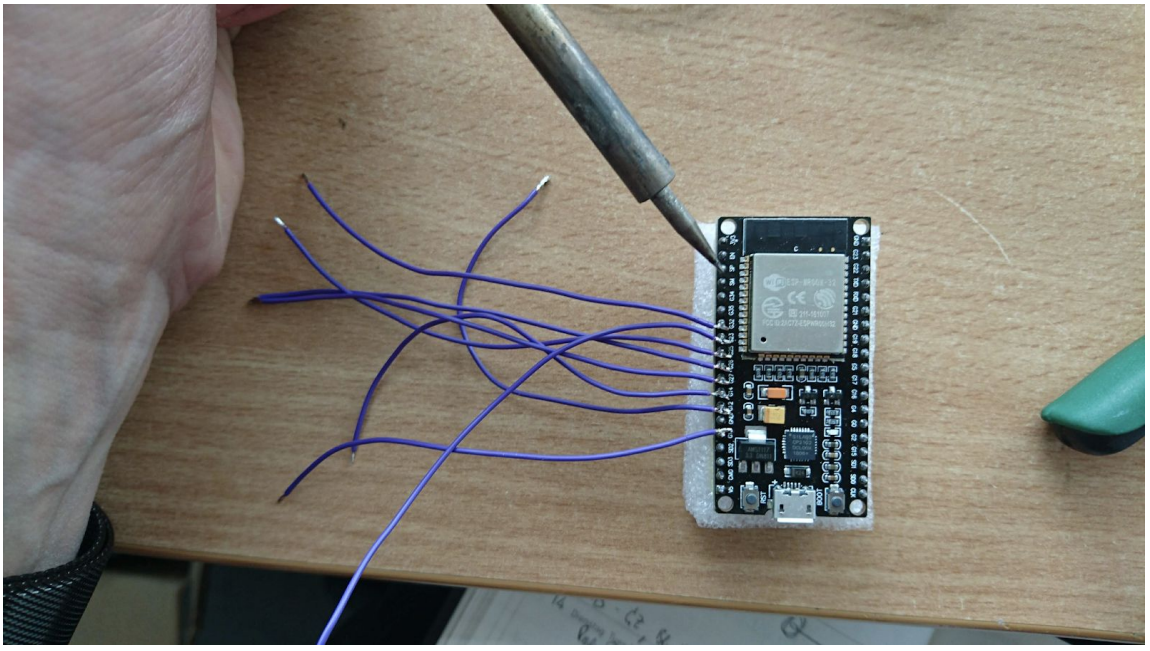
Make sure you're releasing the Motor after usage, otherwise it can get quiet hot!

If everything went correct, the entire circuit should look like the lower photograph.

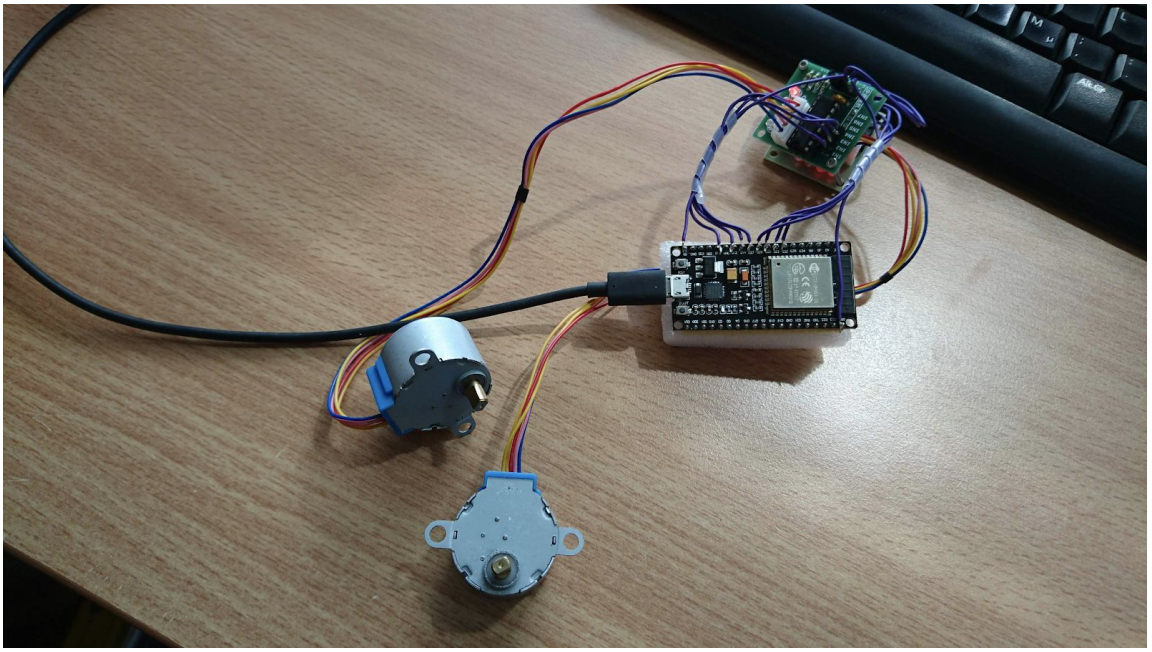
Step 1: Remove the isolation of the wires



Step 2: Add some lead to the tips of the short wires and solder them to both sides of the ESP and ULN2003.



Stept 3: Finish the setup using and flash the example program to the ESP so that both motors move back-and-forth



Code

The code can also be found in the folder code.

The code needs to flashed using the Arduino IDE using the ESP32 library. Further information can be found in this handy tutorial for integrating the ESP in the Arduino environment: Arduino ESP Tutorial:

https://randomnerdtutorials.com/getting-started-with-esp32/

Diverse species of pollen provided by Andreas Kleiber from IPHT, were embedded in agarose.

To prepare agarose gel, 400 mg of standard agarose were dissolved in 20 ml of water stirring at room temperature. Afterwards, the temperature was increased to 160 °C. When the solution becomes transparent, the temperature is reduced to 100°C and 0.5 ml of water dissolved polen is added. After 1 minute of steering, the solution is collected with a B. Braun 1 ml syringe (Figure Xb.) and submerged in water for cooling down. When the solution solidifies, the tip of the syringe is remove with a scalpel (Figure Xc.). Finally, the solid solution is partially ejected, therefore the syringe will be used as a sample holder for the microscope (Figure Xd.).

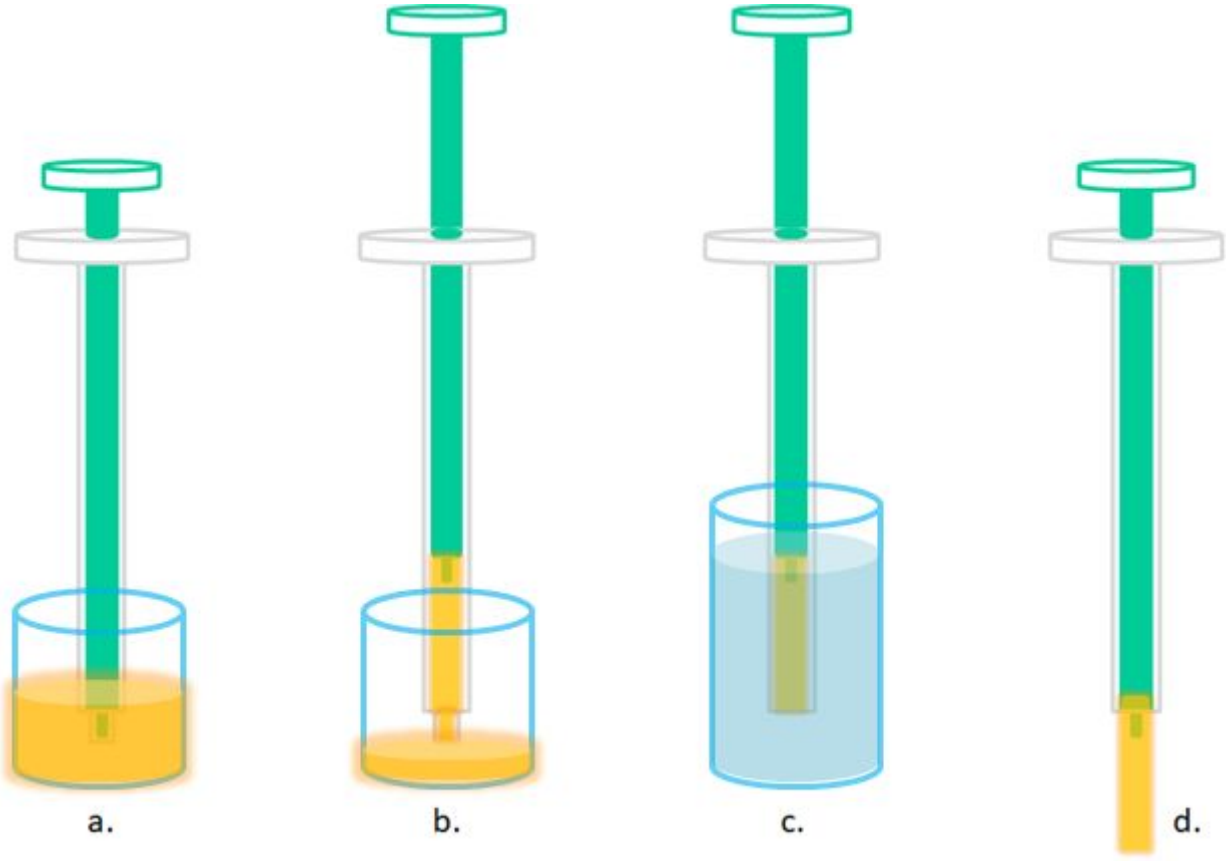


Figure X. (a) The syringe is submerged into the agarose embedded polen (in yellow) and **(b)** it is extracted and cooled down into room temperature water until the coagulation. **(c)** The tip of the syringe is cutted and **(d)** expelled to be analysed in the light-sheet microscope.

Light-sheet Setup (Workshop)

This is the manual for the Lightsheet Microscope used for the International Day of Light (IDoL) workshop.

The stl-files can be found in the folder STL.

Purpose

Produce 3D images with better sectioning.

Properties

- design is derived from the base-cube

Parts

3D printing parts

- Please find all parts inside the folder STL

Additional parts

- Please have a look at the bill of materials

Remarks and Tips

3D Printing:

- Look at each part individually
- Carefully remove all support structures (if applicable)

Assembly and Alignment of the optical path

Here we briefly give a step-by-step tutorial on how-to align the beam-path.

The illumination path is independent from the detection path - the order how you align it is up to you.

Assemble the illumination path (lightsheet)

Here we try to form the lightsheet.

1. Mount the pre-assembled laser cube

- Place a white screen (e.g. paper) perpendicular to the laser (so that the beam-spot actually hits the screen) at a distance of 20 cm behind the base plate
- Switch on the laser with the clamp
- Make sure the Laser is correctly centered
- Place the cube on the lattice as indicated by the Animation below
- ATTENTION: !Don't hit your or anybody's eyes!
- If you make a break or do something else than aligning at the moment, always switch off the laser to make sure you don't endanger anybody's eyesight!

2. Align the telescope

- Place the pre-assembled telescope on the grid right after the laser
- Shift the iPhone lens inside the rail back and forth so that a parallel (i.e. collimated) beam is created

- This can be measured by simply comparing the beam at a distance of 2 cm and 20 cm right after the telescope; The diameter should not change
- ATTENTION: !Don't hit your or anybody's eyes!

3. Add the pre-mounted fold-mirror

- Place the pre-assembled 45° fold mirror on the grid after the telescope with one place-holder position
- ATTENTION: !Now the laser beam is going to a perpendicular direction to the one before. Make sure to block the light with i.e. a cardboard, so it can't leave you setup and hit somebody's eyes!

4. Add the pre-mounted objective lens

- Place the pre-assembled 4x objective lens on the grid perpendicular to the fold mirror
- Tune the focus of the illumination so that it is in the center of the following block-position
- Tuning the objective lens can be done by shifting it back and forth in the spring-loaded spiral bearing
- Make sure the metal housing is removed from the objective by threading it apart (eventually apply some force; we need additional working distance)

5. Add the sample mount cube

- Place the pre-assembled sample mount cube on the grid right after the 4x objective lens
- The motor must be pointing over the side of the baseplate

6. Add the cylindrical lens

- Place the pre-assembled lens-mount on the grid right after the telescope and before the fold-mirror
- Observe the focus inside the sample holder
- The line-focus (i.e. lightsheet) should be in the center of the sample holder stage

Assemble the detection path

Here we try to form the compound microscope.

1. Mount the pre-assembled z-stage

- Place the Z-stage perpendicular to the illuminating light sheet (see figure below)

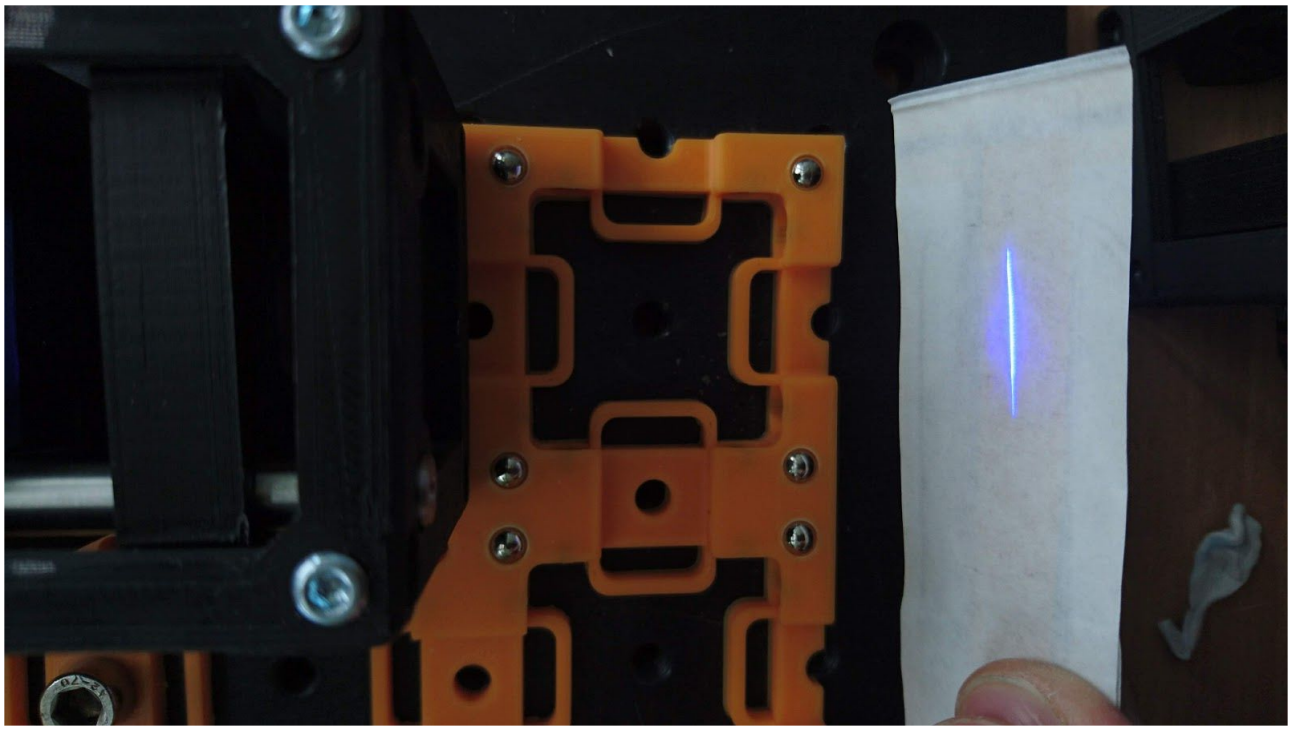
2. Place the camera cube

- Place the camera cube right behind the filter cube

3. Align the illumination path

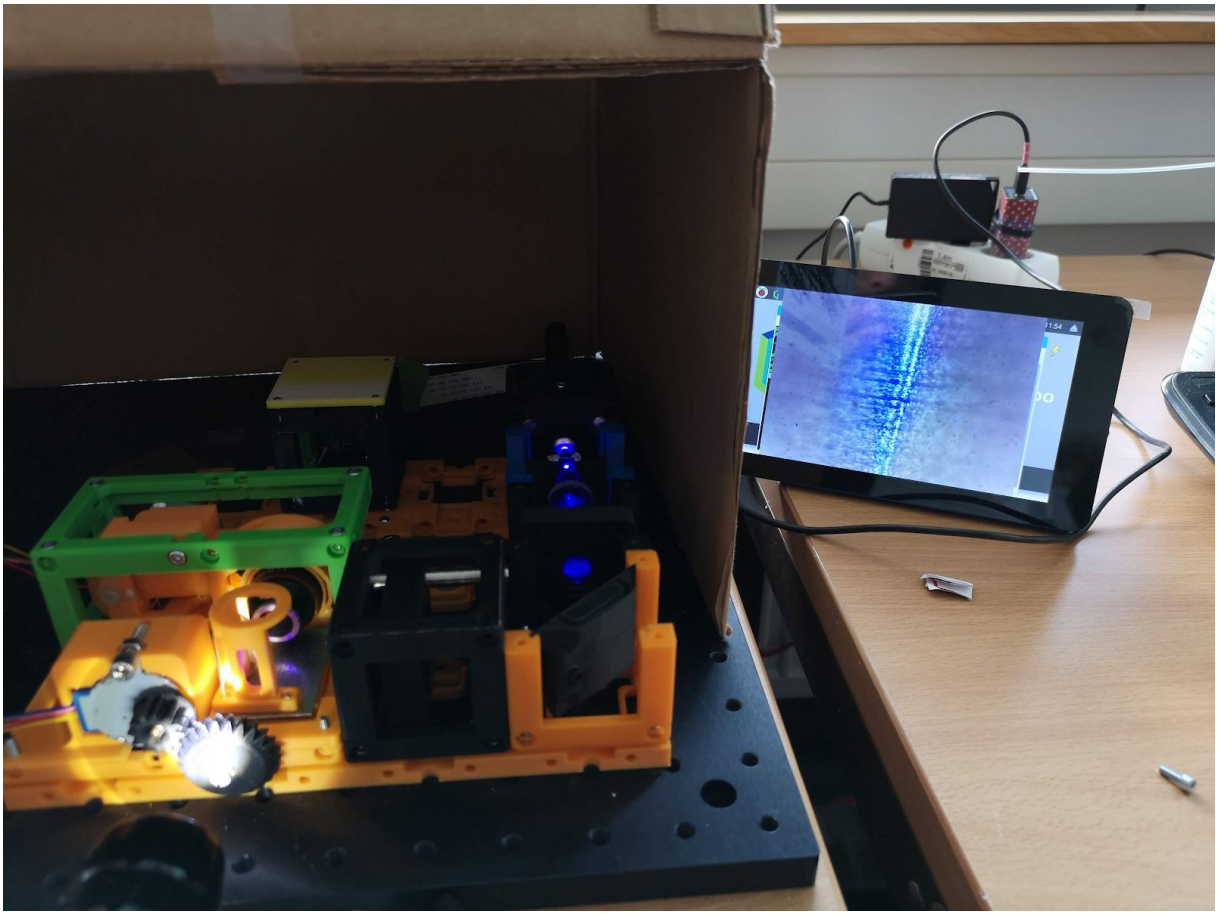
- Place a sample (ideally a sheet of paper tilted 45° w.r.t. the lightsheet/imaging path) so that one sees speckle on the Raspberry Pi camera
- The paper can be mounted using the magnetic sample mount; fixed with some sticky tape

- The line-profile of the illuminating lightsheet should be roughly in the position of the focus of the objective lens



4. Align the imaging path

- The focus of the objective lens (therefore of the light-sheet) can be varied coarsely by shifting the lens back and forth in the spiral mount
- Take a torch and illuminate the sheet of paper from the position of the motor
- Move the paper along the z-focus so that one sees the structure of the paper on the raspi-camera (i.e. screen)
- Once you see the pattern of the paper, align the lightsheet again
- Take a screwdriver and turn the screw of the tiltable/movable mirror - this mirror varies the angle of the lightsheet and the position where it hits the back focal plane of the 4x objective lens respectively



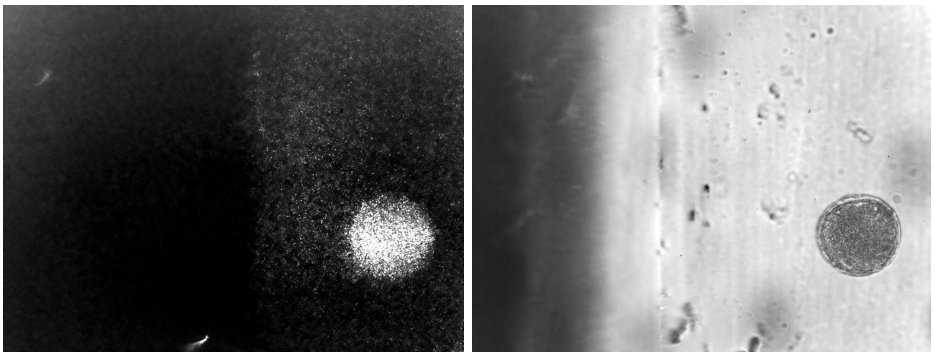
- Move it so that you see the light-sheet in-focus with the bright-field image

5. Use of filters

- When using a correct filter between the Z-stage and the camera, it's possible to observe a fluorescent image of the sample
- Here we omit the filter and therefore capture only the scattering image

The result could look like this:

Fluorescent and Brightfield Image (*Pollengrain in Agarose*)



Animation

Safety

Don't touch the optical surfaces of lenses and objectives!

Attention, don't cut your fingers while removing the lens from the iPhone sensor and the support material from 3D printed parts!

Never (!) look into the laser pointer! It will damage your eye immediately!

- **ATTENTION: NEVER LOOK DIRECTLY INTO THE LASER! EYE WILL BE DAMAGED DIRECTLY**
- **NEVER SWITCH ON THE LASER WITHOUT INTENDED USE**
- **BEAM HAS TO GO AWAY FROM ONESELF - ALWAYS!**

Software used during this Workshop

Laptop

For 3D-Designing our parts and preparing them for printing we will use:

- Autodesk Inventor 2017 or newer/ Autodesk Fusion 360
- Cura (https://ultimaker.com/en/products/ultimaker-cura-software) -> for Ultimaker Fused-Filament Printers
- Alternative with nice presets: Slic3r (https://slic3r.org) -> for Prusa i3 Mk2S Fused-Filament Printers

For easy image-processing and image-display we will use:

- Fiji (https://imagej.net/Fiji/Downloads)
- and might have a look into our self-written Fiji Plugin und Python Code (https://github.com/bionanoimaging/UC2-GIT/tree/master/WORKSHOP/INLINE-HOLOGRAMM).

Next, for remote programming on the Raspberry Pi we will use:

- a local Python distribution: Anaconda (Py3.6) (https://www.anaconda.com/download)
- VisualStudioCode (https://code.visualstudio.com/download)
- Arduino IDE (https://www.arduino.cc/en/Main/Software)
-

RaspberryPi

Here we need:

- Python alternative with easy maintainability and local environments: Miniconda (Py3.6) (https://docs.conda.io/en/latest/miniconda.html)
- Mosquitto (https://mosquitto.org/download/)

Smartphone

Preparing the Raspberry Pi

We have prepared an installation script which simplifies your work enormously. You can find the link with all important information here:

- https://github.com/bionanoimaging/UC2-GIT/tree/master/RASPBERRY-PI

This script installs:

- OpenCV 2.4.9.1 (Image-processing Library)

- kivy 1.10.1 (UI-Provider) based on Raspi-Standard Python2.7
- Cython 0.28.2 (C-Compiler for Python)
- numpy (Math library for Python)
- libmtdev (Touchscreen drivers)
- smbus-cffi (I2C-drivers for Python)
- hostapd (Host access point daemon)

and sets up the RaspberryPi to be an WiFi access point.
For the tutorial, we use an already prepared installation which has even all the features explained in the next chapter. In case you want to try for yourself we gathered some installation directives.

Appendix

Recording with Raspi camera

The camera is a central component of image acquisition. In order to do justice to the basic idea of the easy availability of the devices, we also fall back here on open source solutions.
The mini-computer Raspberry Pi is the most sold computer in the world and allows the control of hardware components and complex software. In this workshop we use the camera of the device, which is very easy to operate via the terminal, but also via the Python PiCam interface.
Further information can be found here:

- https://github.com/rwb27/openflexure_microscope/wiki/Camera-Options

Specifications

- Resolution: 2464x3280
- Pixel size: 1.12um
- Sensor size: 3.67x2.76mm
- Standard lens (focal length): 3.04mm

The sensor is very sensitive, the software allows a simple control of the shooting parameters (exposure time, etc.), as well as the storage and output of captured images. The new version (V2.1) is based on a Sony IM135 sensor, which can also be found in mid-range smartphones.

Please note that the lens must be removed from the camera model. The normally corrected shading correction, which compensates for lens errors, now overcompensates the images, resulting in a bright edge.

Recording images

It is important to connect the camera correctly to the Raspberry first. Please note the orientation of the ribbon cable. The power supply, a keyboard+mouse and a monitor should also be connected.

Take a torch to illuminate the sample. The program can always be killed by hitting “ctrl+c” on the keyboard!

To open a camera live-stream, enter the following: **raspistill -t 1000000000**

To create an image, enter the following: **raspistill -f test.jpg -t 10000**

To create a video, enter the following: **raspivid -f test.h264 -t 10000**

- raspistill is the program that opens the camera.
-f is a flag that displays the image seen on the screen.
- test.jpg is the file name for the information stored in the /home/pi folder.
- t 10000 is another flag which indicates the time of the display (10s or 10000 ms).

Multiple images with different file names can now be captured. Save them on a USB stick and reconstruct them with aFIJI plugin.