Microscopi

Components & Assembly Manual

Model Microscopi-β2

CONTENTS

1. SPECIFICATIONS

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-
- Dimensions (235 mm x 235 mm base; 250 mm height)
- Weight **1.5 Kg** (without optional Lithium battery pack)
- XY flexure stage travel **3 mm** in X and Y
- Z lifter range **4 mm**
- Onboard processor running Python 3 (Raspberry Pi B4)
- CMOS camera, Sony IMX219 8-megapixel sensor (Pi-camera V2)
- Power: **5V**, < **3.0 A**
- Portable battery run time approximately **10 h**
- Optical path 160-DIN lens compatible, 45 mm parfocal distance

All printed components have been tested using PLA (polylactic acid) which is recylable but unfortunately is not widely recycled. In addition, it may be composted under commercial conditions, however these are also not currently widely accessible. Small electrical components are widely recycled although you may have to go to a household waste recycling centre. Li-ion rechargeable batteries are not recycled in the UK, however, such batteries are collected at UK recycling centres under the EU Batteries Directive. Do not dispose of batteries in household waste. Li-ion batteries are potentially hazardous when damaged due to the risk of fire, damaged batteries must be disposed of as hazardous waste, check local authority rules.

1.1 Safety Considerations

Microscopi is intended to be an easy to use accessible instrument for use outside the science lab environment and by the public. However, the LED used for fluorescence illumination is relatively bright so staring into the beam should be avoided. To prevent accidental exposure, the lid should be fitted with an interlock which disables the fluorescence LED when opened. Microscopi is modular, therefore, for some users we recommend only installing the bright-field 8x8 white LED, LED ring-light and optional flexilamp.

Microscopi being used in the field

When not in use or during transport the instrument should be packed safely in its protective carrying case. Note that the optical rail and Z-lifter assembly should be secured before packing away the instrument.

Do NOT store the Lithium power pack portable power supply with the instrument. Always follow the manufactures recommendations on charging and storing of Lithium power packs.

When operating microscopi, protect internal components from water. In case of accidental exposure, disconnect from the power, blot excess water, disassemble and allow to dry thoroughly before attempting to start the instrument.

Objectives for use with the system should be stored safely to avoid dirt and scratches ideally in plastic cases:

2. PREPARATION

A full listing of all commercially obtained parts may be found in Table 1 and all printed parts Table 2. Microscopi components are classified into the five types listed below. We suggest allowing a week to assemble the instrument allowing a couple of hours per day.

3D Printed	Custom components 3D printed from PLA
Generic Hardware	Widely available e.g. M6 screws
Optics	Sourced from specialist suppliers
Generic Electronics	Widely available e.g. 12 power supply
Custom Electronics	Assembly required from generic components / Sourced from specialist suppliers

2.1 Tools Required

A full list of tools and materials required for assembly of the unit may be found in Table 2 at the end of this document. 3D printers for generating the custom 3D printed components are becoming more widespread and easily available commercially or to build yourself (http://3dhobbyist.com/3d-printers-for-hobbyists/). Alternatively, many companies offer 3D printing services.

2.2 3D Printed Components

Components of Microscopi to be 3D printed were designed in the freely available OpensCAD software (https://www.openscad.org). Other free CAD packages are available, for example, the entry level TinkerCad (https://www.tinkercad.com) or the more comprehensive design software FreeCAD (https://www.freecadweb.org/). Designs were exported as .STL format files, all of which may be found online at https://micronoxford.com/microscopi. Components were printed on various 3d printers, including a Lulzbot Taz 5 and an Ultimaker S5, one of the latest FDM (fused deposition modeling) printers. STL format files were imported into the printer software, for example the Ultimaker Cura software, the print parameters set (see table below) and the resulting toolpath file exported as a .gcode or equivalent file. We recommend using PLA filaments: PLA is similar in strength to ABS but less prone to shrinking during the printing process. Nylon in comparison is much tougher and more durable but requires desiccation and is slower to print. After printing, parts generally require “finishing” using a scalpel, needle files set and

sandpaper to remove minor printing imperfections and to smooth interlocking surfaces.

2.3 3D Printer parameters

Different FDM 3D printers will have different print parameters and settings. Critically, the size of parts which can be printed is determined by the build area or volume, for the Ultimaker S5 the build volume is 330 x 240 x 300 mm. Generally, print quality is determined by the nozzle diameter and filament deposition thickness I.e. the layer height or line thickness. The upper and lower bounds of the layer thickness is limited by the print nozzle selected (https://ultimaker.com/en/resources/22225-how-to-print-with-ultimaker-pla). The typical range for FDM printers is 0.050-0.4 mm, with 0.2 mm being typical. Extruder speed and XY nozzle speed are usually optimised by the printer for the filament material.

Suggested Print Parameters:

Material ---------- PLA (Polylactic acid)
Print Nozzle size---------- 0.4-0.5 mm nozzle
Layer size ---------- 200 micron
Shell Thickness ---------- Default
% Infill pattern/density ---------- Default
Print Speed---------- Default

Safety Note: printing PLA can emit a low level of gases and particles, therefore, it is recommended to 3D print in a well-ventilated area or house your printer in an enclosure with a filtration system.

3. ASSEMBLY

3.1 Commercially Sourced Parts

Table 1: Listing of all commercially sourced parts

Item	Part #	Category	Subcat	Maker	Part no.	Supplier	Supplier ref	Web link
Black Water Resistant Case with Foam Insert - 569 x 425 x 215mm	**CASE**	Hardware	General	Duratool	D03134	Farnell	SG33494	https://uk.farnell.com/duratool/waterproof-case-20-5/waterproof-case-569x425x215mm/dp/2500551?st=WATERPROOF%20CASE
Steel specimen platform (175 mm square x 0.5 mm / central 35 mm hole)	**P005**	Hardware	Custom	University of Oxford	/	/	/	/
nylon hinge	**P006**	Hardware	General	RS Pro	770-3006	RS	770-3006	https://uk.rs-online.com/web/p/hinges/7703006/
steel baseplate (dimensions 265 mm square x 2 mm thick)	**P018**	Hardware	Custom	University of Oxford	/	/	/	**/**
Optional “stick-on” feet	**P010**	Hardware	General	Hammond	1421T9BK	Farnell	1876525	https://uk.farnell.com/hammond/1421t9bk/feet-stick-on-pk24/dp/1876525
Foam sheet 265 mm square x 50 mm	**P011**	Hardware	General	/	/	/	/	https://wecutfoam.co.uk/foam-sheets/packaging-grey-foam-sheet-half-sheet-234cm-x-1015cm-025
Eclipse 3mm Neodymium Magnetic Disc	**P019**	Hardware	General	Elipse	N835RS	RS	909-3632	https://uk.rs-online.com/web/p/neodymium-magnets/7924559/
Eclipse 4mm Neodymium Magnetic Disc	**P007**	Hardware	General	Elipse	N836RS	RS	909-3641	https://uk.rs-online.com/web/p/neodymium-magnets/9093641/
Eclipse 5mm Neodymium Magnetic Disc		Hardware	General	Elipse	N837RS	RS	903-3644	https://uk.rs-online.com/web/p/neodymium-magnets/9093644/
Eclipse Neodymium Magnets (15.4 mm)		Hardware	General	Elipse	N832RS	RS	873-4997	https://uk.rs-online.com/web/p/neodymium-magnets/8734997/
Aluminium Flexible Motor Coupling Bore 5 / 5mm	**P027**	Hardware	General	/	/	Reprap World	FLEXCOUPLING5M5	https://reprapworld.co.uk/products/motors_fans/couplers/flexible_motor_coupling_5_mm_5_mm/
M6 socket screws, 12mm	**P008**	Hardware	Nuts, Bolts etc	RS-Pro	874-1030	RS	874-1030	https://uk.rs-online.com/web/p/socket-screws/8741030/

M5 Pan Head, 60mm Stainless Steel Slot A2 304	**P026**	Hardware	Nuts, Bolts etc	RS-Pro	914-1605	RS	914-1605	https://uk.rs-online.com/web/p/machine-screws/9141605/
M5 Hex nut, stainless steel	**P029**	Hardware	Nuts, Bolts etc	RS-Pro	189-585	RS	189-585	https://uk.rs-online.com/web/p/hex-nuts/0189585/
M3 socket screws, 8mm		Hardware	Nuts, Bolts etc	RS-Pro	280-997	RS	280-997	https://uk.rs-online.com/web/p/socket-screws/0280997/
M3 socket screws, 12mm		Hardware	Nuts, Bolts etc	RS-Pro	187-1229	RS	187-1229	https://uk.rs-online.com/web/p/socket-screws/1871229/
M3 socket screws, 20mm	**P012/ P028**	Hardware	Nuts, Bolts etc	RS-Pro	293-319	RS	293-319	https://uk.rs-online.com/web/p/socket-screws/0293319/
M3 socket screws, 30mm	**P025**	Hardware	Nuts, Bolts etc	RS-Pro	293-331	RS	293-331	https://uk.rs-online.com/web/p/socket-screws/0293331/
M3 Hex nut	**P010**	Hardware	Nuts, Bolts etc	RS-Pro	560-293	RS	560-293	https://uk.rs-online.com/web/p/hex-nuts/0560293/
M3 cap screw hardware kit		Hardware	Nuts, Bolts etc	Thorlabs	/	Thorlabs	HW-KIT5/M	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=248
M3 Pan Head, 8mm Stainless Steel Slot A2 304	**P009**	Hardware	Nuts, Bolts etc	RS-Pro	9141582	RS	9141582	https://uk.rs-online.com/web/p/machine-screws/9141582/
M3 Pan Head, 12mm Stainless Steel Slot A2 304		Hardware	Nuts, Bolts etc	RS-Pro	526-962	RS	526-962	https://uk.rs-online.com/web/p/machine-screws/0526962/
M2.5 Pan Head, 12mm Stainless Steel Slot A2 304	**P038**	Hardware	Nuts, Bolts etc	RS-Pro	526-934	RS	526-934	https://uk.rs-online.com/web/p/machine-screws/0526934/
brass threaded inserts M3 (5.7 mm)	**P030**	Hardware	Nuts, Bolts etc	TR FASTENINGS	M3-UHBRHESF	Farnell	12474921	https://uk.farnell.com/tr-fastenings/m3-uhbrhesf/brass-insert-unheaded-m3/dp/2474921
"Hot Glue" sticks	**/**	Hardware	Consumable	Power Adhesives	239-12-300-DRP-TP16-RS	RS	896-2271	https://uk.rs-online.com/web/p/glue-sticks/8962271/
Superglue	**/**	Hardware	Consumable	Locktite	577093	RS	330-4002	https://uk.rs-online.com/web/p/cyanoacrylate-adhesives/3304002/
Wet&Dry paper in various grades	**/**	Hardware	Consumable	Norton	63642535107	RS	779-087	https://uk.rs-online.com/web/p/products/0779087/?tpr=1
Hot-glue gun [TEC305 110 240V Hot Melt Glue Gun]	**/**	Hardware	Tool	Power Adhesives	TEC 305	RS	781-5045	https://uk.rs-online.com/web/p/products/7815045/?tpr=2]
Small slotted head screwdriver (3mm)	**/**	Hardware	Tool	RS-Pro	459-4082	RS	459-4082	https://uk.rs-online.com/web/p/screwdrivers/4594082/
Small Phillips screwdriver	**/**	Hardware	Tool	RS-Pro	125-3084	RS	125-3084	https://uk.rs-online.com/web/p/screwdrivers/1253084/
Hex screwdrivers or Allen keys – M2 (2.5), M3 (2.5), M6 (4)	**/**	Hardware	Tool	Allen	56615G	RS	297-1566	https://uk.rs-online.com/web/p/hex-keys-sets/2971566/
Standard needle file set	**/**	Hardware	Tool	RS Pro	123-0930	RS	123-0930	https://uk.rs-online.com/web/p/file-sets/1230930/
Small needle-nose pliers	**/**	Hardware	Tool	Gedore	772-4217	RS	VDE 8132-160 H	https://uk.rs-online.com/web/p/long-nose-pliers/7724217/

Wire snips	/	Hardware	Tool	Rs Pro	606-490	RS	606-490	https://uk.rs-online.com/web/p/cable-cutters/0606490/
Wire stripper	/	Hardware	Tool	Rs Pro	613-044	RS	613-044	https://uk.rs-online.com/web/p/wire-strippers/0613044
Crimping tool	/	Hardware	Tool	Rs Pro	499-2313	RS	499-2313	https://uk.rs-online.com/web/p/products/4992313/?tpr=1
Retractable craft knife	/	Hardware	Tool	Rs Pro	232-9123	RS	232-9123	https://uk.rs-online.com/web/p/scalpels-craft-knives/2329123/
Dremmel or equivalent multi-tool	/	Hardware	Tool	Dremmel	F0133000JR	RS	769-0338	https://uk.rs-online.com/web/p/miniature-power-tool-kits/7690338/
1/2" square protected silver economy mirror, 3.2mm thick	**P041**	Optics	General	Thorlabs	ME05S-P01	Thorlabs	ME05S-P01	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=890&pn=ME05S-P01#4645
Adapter with External SM1 Threads and Internal RMS Threads	**P031**	Optics	General	Thorlabs	SM1A3	Thorlabs	SM1A3	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1524&pn=SM1A3#3229
Ø1" Fresnel Lens, f = 25 mm (fluorescence condensor)	**P053**	Optics	General	Thorlabs	FRP125	Thorlabs	FRP125	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1222&pn=FRP125#1222
Ovjective x4/0.1	**ObjX04**	Optics	General	/	/	Edmundoptics	43-902	http://www.usedmicroscopes.co.uk/objectives.html
Objective x10/0.25	**ObjX10**	Optics	General	/	/	Edmundoptics	43-903	http://www.usedmicroscopes.co.uk/objectives.html
Objective achro 20x/0.45	**ObjX20**	Optics	General	/	/	Microthek	5.0120.22	http://www.usedmicroscopes.co.uk/objectives.html
Objective achro 40x/0.45	**ObjX40**	Optics	General	/	/			
SM1 Lens Tube Without External Threads, 1" Long, Two Retaining Rings Included	**P052**	Optics	General	Thorlabs	SM1M10	Thorlabs	SM1M10	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=3307&pn=SM1M10#3387
Polarising filter LPVISA100 Ø25.0 mm Unmounted Linear Polarizer, 480 - 550 nm	**P043**	Optics	General	Thorlabs	LPVISA100	Thorlabs	LPVISA100	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=752&pn=LPVISA100#4316
Royal Blue (455 nm) LED on Metal-Core PCB, 1000 mA, 900 mW (Min)	**P049**	Optics	General	Thorlabs	M455D2	Thorlabs	M455D3	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=6071&pn=M455D3
Self-adhesive heatsink for LED		Electornics (optics)	General	Adafruit	/	Adafruit	3082	https://www.adafruit.com/product/3082
Adafruit Small 1.2" 8x8 Ultra Bright Square White LED Matrix + Backpack	**P056**	Optics	General	Adafruit	ADA1857	PiHut	/	https://thepihut.com
Adafruit NeoPixel Ring - 16 x 5050 RGBW LEDs w/ Integrated Drivers	**P057**	Optics	General	Adafruit	ADA2856	PiHut	/	https://thepihut.com
SPDT-NO/NC Hinge Lever Microswitch, 100 mA @ 30 V dc	**P032**	Electronics	General	ZF	DB3C-B1LC	RS	517-488	https://uk.rs-online.com/web/p/microswitches/0517488/

Insulated Crimp Receptacle, 2.8 x 0.5mm, 0.5mm² to 1.5mm², 22AWG to 16AWG, Tin Plated		Electronics	Consumable	RS Pro	459-799	RS	459-799	https://uk.rs-online.com/web/p/crimp-receptacles/0459799/
2.1mm DC power jack		Electronics	General	CLIFF	FC681473	Farnell	2353931	https://uk.farnell.com/cliff-electronic-components/fc681473/connector-receptacle-dc-power/dp/2353931
5V 2.5A Step-Down Voltage Regulator D24V22F5		Electronics	General	Pololu	D24V22F5	Hobbytronics	/	http://www.hobbytronics.co.uk/
QX 15Ah 5V Power Bank Portable Charger		Electronics	General	RS Pro	136-6475	RS	136-6475	https://uk.rs-online.com/web/p/power-banks/1366475/
5V Relay SPDT 10A		Electronics	General	/	/	Hobbytronics	RELAY5V10A	http://www.hobbytronics.co.uk
25HK-AB-120A250-D56-FR - AC/DC Power Supply, ITE, 1 Output, 30 W, 12 V, 2.5 A		Electronics	General	Ideal Power	25HK-AB-120A250-D56-FR	Farnell	2630905	https://uk.farnell.com/
DRV8834 Low Voltage Stepper Motor Driver Carrier		Electronics	General	Pololu	/	Cool Components	SKU 1134	https://coolcomponents.co.uk/products/drv8834-low-voltage-stepper-motor-driver-carrier?_pos=1&_sid=8268beb33&_ss=r
Raspberry Pi 4		Electronics	General	Raspberry Pi	Raspberry Pi 4 2G Model B	RS	182-2095	https://uk.rs-online.com/web/p/processor-microcontroller-development-kits/1822095/
Raspberry Pi Camera Board Replacement Cable - 300mm		Electronics	General	Pro Signal	CTLCAMCABLEASSY-300MM	CPC-Farnell	SC13282	https://cpc.farnell.com/pro-signal/ctlcamcableassy-300mm/cable-for-pi-camera-300mm-formed/dp/SC13282
RPI 8MP Camera Board	**P037**	Electronics	General	Raspberry Pi	RPI 8MP CAMERA BOARD	RS	913-2664	https://uk.rs-online.com/web/p/video-modules/9132664/
Stepper Motor, 512 Step, 5V dc - 858	**P024**	Electronics	General	Adafruit	858	CPC-Farnell	MC02058	https://cpc.farnell.com/adafruit/858/stepper-motor-512-step-5vdc/dp/MC02058?st=tepper%20Motor,%2048%20Step,%205V%20dc
NEMA 14 internal captive motor (includes leadscrew)	**P020**	Electronics	General	Wantai	39BYGL215A	ebay	/	https://www.ebay.co.uk/itm/181874647638
CP30200G - HDMI Connector		Electronics	General	CLIFF	CP30200G	Farnell	2491558	https://uk.farnell.com/cliff-electronic-components/cp30200g/adaptor-hdmi-a-19pos-jack-panel/dp/2491558
40-way ribbon cable		Electronics	General	RS Pro	289-9975	RS	289-9975	https://uk.rs-online.com/web/p/flat-ribbon-cable/2899975/
IDC connector		Electronics	General	Assmann	AWLP 40/3,2-T	RS	674-1312	https://uk.rs-online.com/web/p/idc-connectors/6741312/
PVC insulated hookup wire, copper multifilament (2A)	**/**	Electronics	General	RS Pro	285-7662	RS	285-7662	https://uk.rs-online.com/web/p/hookup-equipment-wire/2857662/
Crimp Terminals, 22-30 AWG	**/**	Electronics	Consumable	Molex	08-50-0032	CPC-Farnell	CN09629	https://cpc.farnell.com/molex/08-50-0032-pack-100/crimp-terminal-22-30-awg/dp/CN09629?st=Molex%20KK%20crimp%20terminal%2022-30%20AWG

Transcend 16 GB MicroSDHC Card Class 10, UHS-1 U1		Electronics	Consumable	Transcend	TS16GUSDCU1	RS	124-9640	https://uk.rs-online.com/web/p/micro-sd-cards/1249640/
Solderless Breadboard 400 tie point		Electronics	General	Sunhayato	SAD-101	RS	189-2277	https://uk.rs-online.com/web/p/breadboards/1892277/
Breadboard Jumper Wire Kit, 10 piece (male/female pin)		Electronics	General	Mikro Elektronika	MIKROE-512	RS	791-6454	https://uk.rs-online.com/web/p/products/7916454/?tpr=1
Breadboard Jumper Wire Kit, 10 piece (male/male pin)		Electronics	General	Mikro Elektronika	MIKROE-513	RS	791-6463	https://uk.rs-online.com/web/p/products/7916463/
(optional) Bespoke PCB		Electronics	Custom	University of Oxford	/	/	/	/
LED Spotlight with adjustable neck		Electronics	General	Thorlabs	PSX-501	Thorlabs	PSX501	https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=1921

Table 2: Listing of All Printed Components

Printed Part Description	PART #	Suggested Print Quality			Part Type*
		LOW	MED	HIGH	
Main enclosure	**P001**	X			support
Lid	**P002**	X			support
Stage platform	**P003**		X		support
Illumination port cover	**P004**				support
XY flexure insert	**P013**	X			moving
Sample holders for slides	**P014**			X	support
Sample holders for 35 mm dishes	**P015**			X	support
XY motor mounting plate	**P016**		X		support
Lifter stand & Rail	**P021**			X	moving
Lifter-insert	**P022**			X	moving
Objective lifter platform	**P023**			X	support
Optics rail	**P033**			X	moving
Camera mount	**P034**			X	support
Sliding rail mount for optics	**P035**			X	support
Optics path cover	**P036**	X			support
Illumination module housing	**P044**		X		support
Illumination module alignment ring	**P045**			X	moving
Alignment screw end caps	**P046**			X	moving
LED mounting-tube	**P047**			X	moving
Pi mounting base plate	**P050**		X		support

* Parts are classified as either having a structural role (support) or acting as a moving part, which requires a higher print quality and a greater degree of “finishing”.

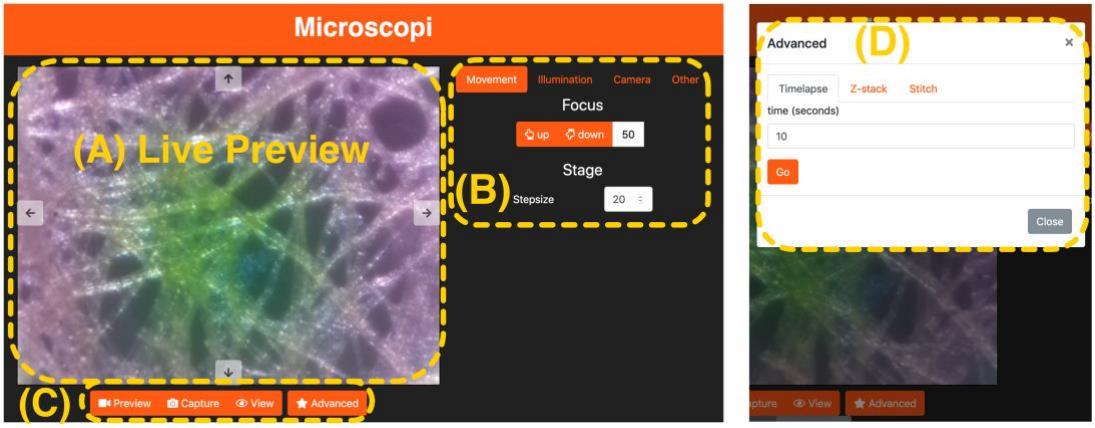
4. OPERATING INSTRUCTIONS

Microscopi is designed to be operated from a mobile device such as a laptop computer or smartphone, however it is possible to directly connect the instrument to a HDMI compatible monitor, keyboard and mouse via the ports on the back panel. Please refer to the "Components & Assembly Manual" for additional technical details including all part numbers.

(1) Plug an appropriate 5V power supply into the instrument via the power jack port on the rear panel, bottom right. If you lift the enclosure lid you should be able to see the lights flickering on the onboard raspberry-Pi.

(2) After about 30s the instrument should broadcast its own Wi-Fi signal. On your mobile device, navigate to the Wi-Fi settings and view the networks available. When the network "microscopi" pops up, join it. You may now connect to the instrument from your mobile device via an internet browser using any web address of the form: http://*anytxt*. You may want to bookmark the address http://microscopi for your convenience. You will have to enter a password, the default is "changeme".

(3) You should now see the Microsocopi user interface (GUI) displayed on your device:



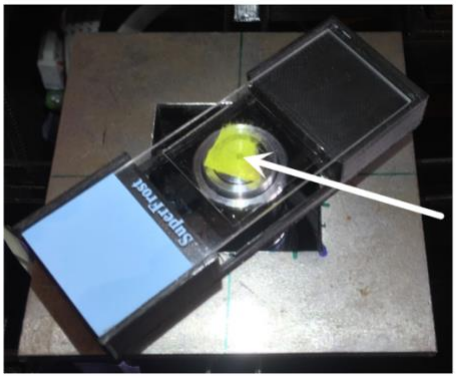
(4) The display (shown above) comprises: (A) the live preview window which, when the "movement control tab is selected, incorporates the four way "XY" movement controls that appear as arrows; (B) movement controls dialogue with the focus Up/Down controls above and the XY stage step size dialogue below; (C) illumination controls which allow you to save and preview saved images and includes the "Advanced functions" tab showing the Timelapse, Z-stack and Stiching options. (D) Pop-up menu from the Advanced tab. Additional pop-up menus are available from the main menu (top of

(B) for controlling the illumination and camera settings. Controls are accessed from the touch screen interface of your smartphone or tablet, or via the mouse and keyboard of your laptop.

(5) When first using Microscopi, we suggest using the lens tissue sample slide described in **Section 5.1**. We also suggest that when first starting, use a low mag (x4) objective to facilitate finding the specimen.

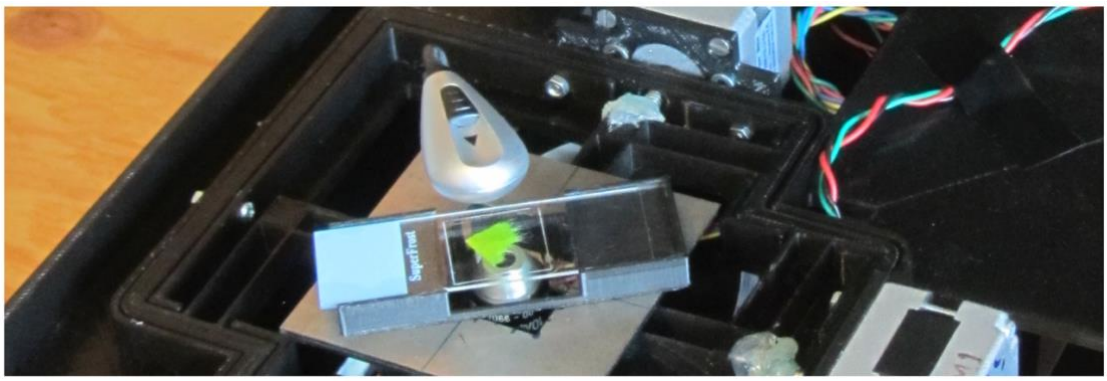
(6) Open the enclosure lid and check that the XY flexure stage is in the "neutral position" i.e. the flexures are not under tension. This should be checked before loading every new specimen. If the flexures are bent, reposition with the XY controls (arrows to the top, bottom left and right of the live preview window, A).

(7) To load a specimen onto the instrument, first fit your slide or small Petri-dish to the sample holder (**P014** / **P015**), illustrated below left:



(8) Place the specimen holder onto the stage plate, illustrated above right. Ensure that the part of the specimen to be imaged lies directly above the centre of the objective, indicated by the white arrow.

(9) The simplest way to get an initial bright-field image is to use the optional flexi-lamp illuminator. Make sure the light is above the stage and positioned over the centre of the sample while observing the "preview" image in the image display window:



(10) It is likely that the slide will initially be out of focus. If the image is slightly out of focus, set a value of between 50 and 200 on the “up down” steps range and observe the effects of moving up and down a few steps. If the image sharpens, modify your step size and move again to find the sharpest focus. Avoid physical vibrations as this will make the image appear blurred even in focus. If the “home” microswitch is installed and set up, selecting the “home” function should drive the focus to approximately the correct focus which can then be refined.

(11) If the home microswitch is not fitted or not calibrated and If the sample is far from focus so that no image is visible, try the following: manually move the sample side-to-side while observing the preview image for moving shadows of the out-of-focus image. If a moving shadow is seen, this can be used to home in on. A step of 500 may be needed if the sample is far from focus. If no shadow is visible, try to determine whether the lens is too high or too low: first remove the slide from the holder and place it directly onto the stage checking for improving focus – this means that the lens it too low and the Z-focus needs to be raised (**up** step); if there is still no focus, hold the lens-tissue sample level and steady then slowly raise it, viewing the preview. How far the sample needs to be raised before an image comes into focus indicated how much the lens needs to be lowered from the sample (**down** step). The different lenses should be parfocal (when one is in focus they should all be at the same Z-position) but the front element of teach lens will be a different distance from the specimen, with experience it becomes easier to judge.

(12) To capture an image of the current view, select the “**capture**” icon under the active display window (panel C), this saves a single frame snap to the working memory. Saved snaps may be reviewed from the “**view**” mode, again, under the active display window. From the view mode snapped images can be saved or deleted. Note that if the power is turned off or the browser crashes, then snapped imaged that have not been saved will be lost.

(13) Remember to return to the live “**preview**” mode when you want to resume taking snapshots.

(14) More advanced functions: time-lapse, Z-stack and XY tiling modes, are accessed from the “Advanced” button on the GUI.

(15) To collect a Z-stack, ensure that you first move to the bottom of the desired focus range and enter the step size and number of steps for your desired Z-stack. Take care that you are entering

reasonable values so that you are operating within the specified 4 mm range of Z- lifter.

(16) To collect a time-series, specify the time interval and number of frames. Remember not to exceed the image storage capacity of the raspberry pi when collecting time-lapse series.

(17) To collect a tiled XY grid, we recommend that you first check that you are within the XY travel range of the flexure stage. You may need to reset the XY flexures to their central (unstrained) positions before carrying out tiling imaging sets. To achieve this you will first have to reposition the motors and then reposition the sample manually by sliding it sample holder on the stage to bring the field of interest within the full travel range. The tiling grid is a “snakes-by-rows” pattern where the starting position is the bottom left tile of the array. In the advanced settings Stich menu (D) set your grid size e.g. 3x3 or 5x5. Displacements in X and Y must be set correctly for the magnification of the objective in use to ensure an ~30% tile overlap to facilitate image stitching. For example, a x10 objective requires a 30 step displacement left/right (X direction) and a 20 step displacement up/down (Y direction).

(18) To use the different illumination modes: Matrix LED, Fluorescence LED or reflection ring-light, first ensure that the required illumination module is installed and the cable connected. Select the appropriate illumination mode from the GIU main menu (B) and access the associated functions.

(19) To achieve the desired illumination of the specimen, the adjustable illumination mount in the lid allows the light source to be positioned above the specimens at different distances (sliding up and down) and centred in XY over the lens (using the three centring screws). Patterned LED illumination is particularly sensitive to distance from the lens in dark field mode, while the fluorescence illumination is particularly sensitive to alignment in XY.

(20) With fluorescence imaging, extra care should be taken to avoid directly looking into the with the bright LED illumination light. This mode requires the long-pass emission filter (**P042**) to be in place on the emission path rail (**P033**). It is also important to ensure the emission path is covered (**P036**) to protect the detector from stray light.

5. COMISSIONING

Once building is completed, going through the acceptance tests below will verify that your system is correctly assembled and performing within expected parameters. You should compare your imaging results to the examples provided here (Figures XX), and for problems, please refer to the notes in the corresponding “Trouble Shooting Guide”. These tests assume that the unit is powered from a mains supply. For electrical components it can be helpful to use a test circuit to verify that the components are functioning.

5.1 Lens Tissue Test Slide

Qty.	Description	Part#
1	Pack glass slides	
1	Pack 22x22 no.1 coverslips	
1	Sample slides (pack)	
pack	Lens tissue	
1	Fluorescent marker pen (yellow)	
1	Clear nail varnish	

Preparing a lens tissue sample slide:

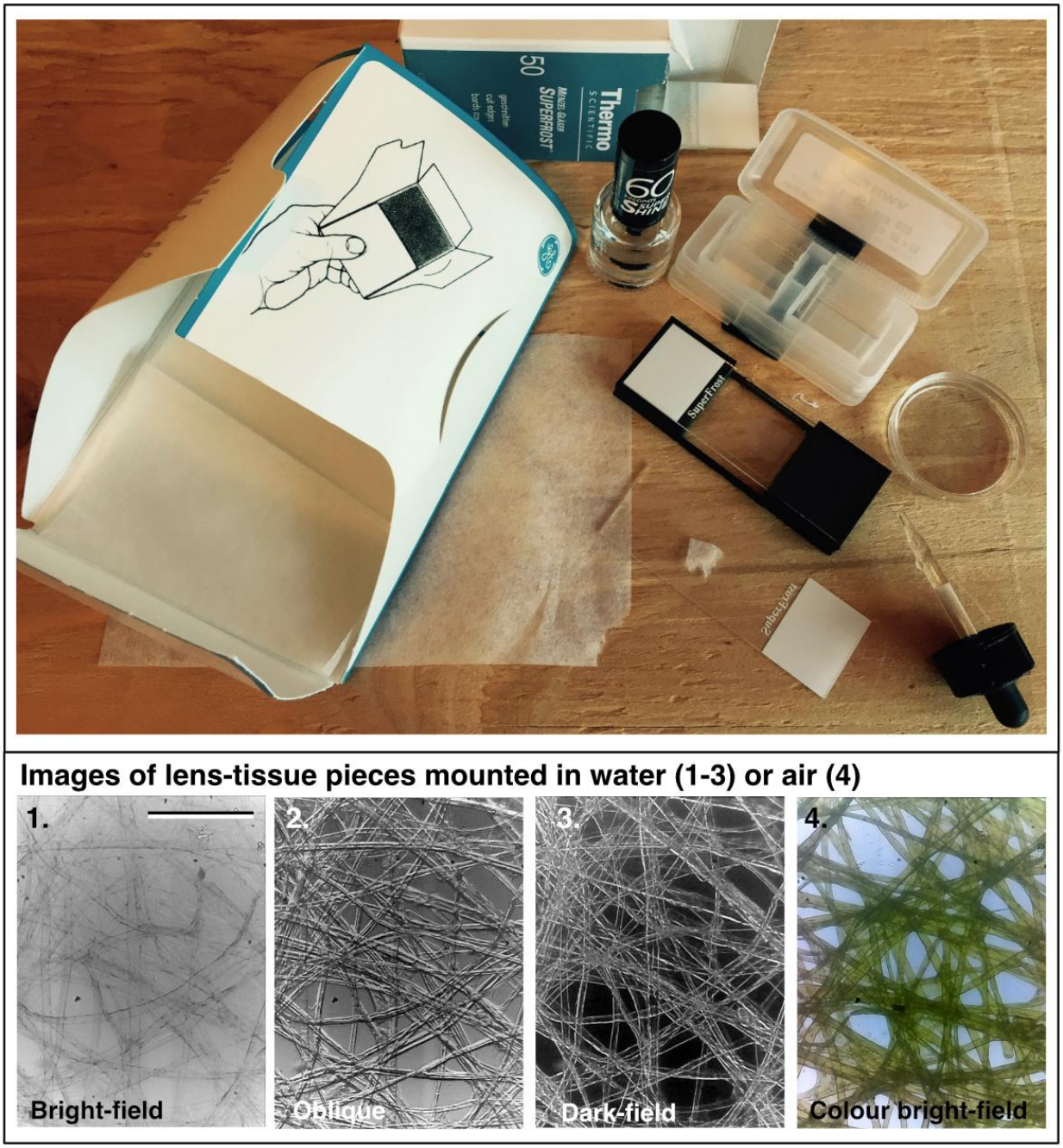
(21) Take a few sheets of lens tissue, place them over a glass slide and colour approximately 15 mm square patches with the fluorescent marker pen. Gently dabbing with the marker will preserve the sparse fibre structure of the lens tissue. Ensure that you keep the lens tissue on top of the glass as you colour.

(22) Once dry, cut or tear out the coloured sections of lens tissue and mount dry in the centre of a clean a glass slide. Press the tissue flat and then cover carefully with a coverslip and gently flatten down. Make slides with coloured and uncoloured sections of lens tissue.

(23) Apply clear nail varnish to the edges of the slide to seal it down, wait a few minutes for it to dry and then re-apply for a longer lasting finish.

(24) The lens tissue fibres in this prep should provide an excellent test sample for both bright-field, contrast enhancing bright-field and fluorescence imaging modes.

Lens tissue test sample



5.2 Acceptance Tests for Basic Functions:

(1) Power up, verify that you can receive the WIFI signal broadcasting the GUI on your mobile device: select WIFI network “microscopi”, open a browser and navigate to “https://microscopi”.

(2) XY movement. Check that the flexures of the XY stage are in the neutral flexure position i.e. not bent. In the main window and Movement menu set XY movement increment to 60. If required adjust the stage to neutral XY flexures position. Step 4 times in X, 4 times in Y. Step back to return to the start position and then from the Advanced Tab select the Stich menu and tile 3x3.

(3) Z movement. Set Z increment to between 200 and 500, step through Z until the insert reaches the top of the rails and return.
(4) Engage “Flexi lamp” illumination and note the response of the “live view” image on the GUI.
(5) Mount the standard tissue paper test slide in the slide holder, coverslip down, and place the slidegholder on the stage.
(6) In the Movement menu Set Z increment to 200, and step through Z up and down to achieve sharp focus on the tissue paper slide.
(7) Snap a single image and save. View saved image
(8) From the advanced menu select Tme-lapse set to 10 s and collect a time-lapse series.
(9) In the movment menu with the focus increment set to 200, go down 2 steps. In the Advanced menu, Z-stack, set Z increment to 50, and collect a Z-series of 15 frames.

5.3 Acceptance Tests for Advanced functions

(10) Patterned LED illumination module. Bend the flexi-lamp illuminator out of the way. Insert white light LED assembly into the lid and ensure it is tight. Connect cabling. With the lid open, set Power to low in the GUI. Verify illumination ON/OFF function in the GIU main menu Illumination tab. Verify power increments in the GUI.
(11) With the tissue paper slide in place, illuminate with the white light LED in bright field mode, focus as required while viewing the live image. The height of the LED array above the slide can be adjusted and centred for best effect. Snap a single image and save. Repeat with oblique illumination selected and compare the results.
(12) For dark-field imaging, the sample must be lit with a ring of light at the correct distance to provide a hollow cone of illumination. Select the “Dark-field” option from the GUI and view the result. While the tissue slide is in sharp focus, raise and lower the LED array relative to the slide and adjust the lateral positioning with the set screws to achieve the “inverted contrast” effect with the tissue paper fibres. Snap a single image and save.
(13) Fluorescence illumination module. Remove the Patterned LED illumination module and replace will the Fluorescence illumination module, seating securely and connecting the cabling. With the lid propped open slightly (an M6 bolt at the front will do), set Power to low in the GUI. Verify illumination ON/OFF function in

the GIU. Verify power increments in the GUI. The standard fluorescence illumination will be blue in colour. **Do not look directly into the excitation illumination** [**Hazard Warning**: 32 µW/mm2 (1445 mW) at maximum current; RG2 – Moderate Risk Group (standard IEC 62471:2006, www.thorlabs.com/contact].

(14) For fluorescence imaging, there must be an emission filter in the optical path after the microscope objective, between the sample and camera. Please ensure the correct emission filter is in place (refer to the assembly instructions, Section 3). Fluorescence emission from samples tends to be weak. We recommend using lens tissue “coloured” with yellow highlighter ink as a test sample. Using the Flexi lamp illumination, focus on a region of coloured tissue in bright-field. Snap a single image and save. Note the image will appear coloured due to the emission filter.

(15) Switch to the fluorescence illumination module. Verify as the illumination is turned on from the GUI that the LED light with the lid slightly open. Completely close the lid and check the live view. If an image is not immediately apparent, adjust the height and alignment of the illumination column in *slow increments* until a fluorescence image is observed. Snap a single image and save.

(16) Ring-light reflection imaging. This works best with the x3 objective. Ensure that the ring light is in place round the objective base and connected, test that it illuminates under control from the GUI. Mount a tissue paper slide and under illumination from the flexi-lamp, set the focus using the live view. Turn off the flexi-lamp, close the lid and then illuminate the ring light from the GUI and view the live image. Snap a single image and save.

6. TROUBLESHOOTING

6.1. Troubleshooting guide:

(1) **No WIFI.** Plug a USB mouse and keyboared and HDMI monitor into the Rasberry Pi. Verify that the system has booted. Check that the WIFI is being broadcast. Cannot connect to mobile device. Check in the device network preferences for the Microscopi network and enable. Restart your browser or try another browser and check the typing of http://microscopi.

(2) **XY stage does not move**. Check whether it is different for X and Y movements. Increase the step increment and reverse the attempted stage movement. Verify that the motor lead screws are turning and that they are in contact with the stage flexures, adjust as appropriate. Gently verify that the flexures are not beyond their travel range. Check the motor connections.

(3) **Z lifter does not move**. Remove the objective. Check the requested step size. Check that the lifter is not at the extent of its travel range up or down. Verify that the "coupler" is turning when focus is activated, ensure that the lead screw is in straight, tighten the grub screws as required. Check the motor connections.

(4) **No live view image on the GUI.** Restart the Pi and / or mobile device browser. Try an alternative browser. Move the flexi-lamp illumination from side to side to test whether the image is live.

(5) **Cannot save image/see saved images.** Restart the Pi. Restart the browser, clear the browser cache or try a different browser.

(6) **Illumination modules do not illuminate the sample**. Remove the module and check the connections. Remount modules and check GUI control.