

Microscopi

Operating Instructions



Model Microscopi-β2



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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 recyclable 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.

2. CARE & SAFETY

2.1 Care and 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:



3.TEST SLIDE

3.1 Materials Required to Prepare a Fuorescent Test Slide:

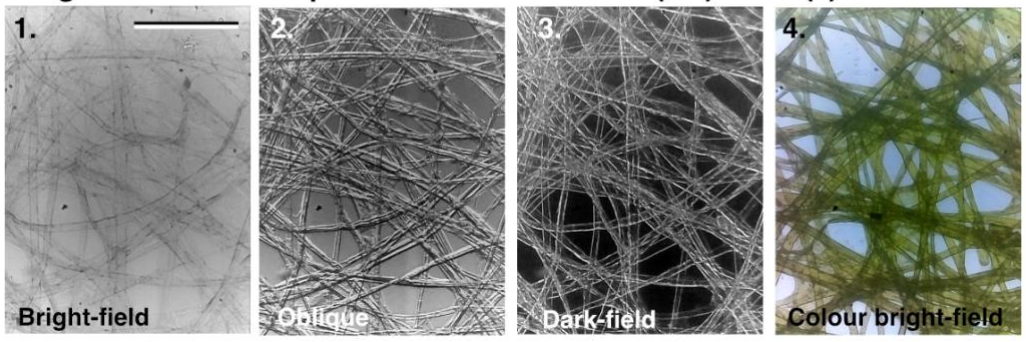
Qty.	Description	Part#
1	Pack glass slides	
1	Pack 22x22 no.1 coverslips	
1	Sample slides (pack)	
pack	Lens tissue	
1	Fluorescent marker pen (yellow)	
1	Clear nail varnish	

* See figure below

Lens tissue test sample



Images of lens-tissue pieces mounted in water (1-3) or air (4)



3.2 Preparing a Fluorescent Lens Tissue Sample Slide:

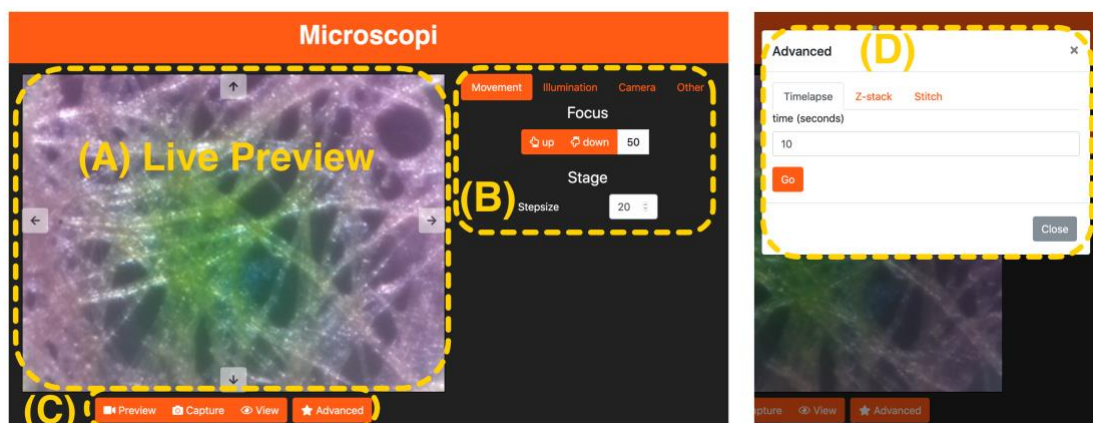
- (1) Take a few sheets of lens tissue, place them over a glass slide and colour approximately 15 mm square patches with the fluorescent marker pen. Gently dabbing with the marker will preserve the sparse fibre structure of the lens tissue. Ensure that you keep the lens tissue on top of the glass as you colour.
- (2) 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.
- (3) 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.
- (4) 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.

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.

4.1 Step-by-Step Operating Instructions

- 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 Microscopi user interface (GUI) displayed on your device:



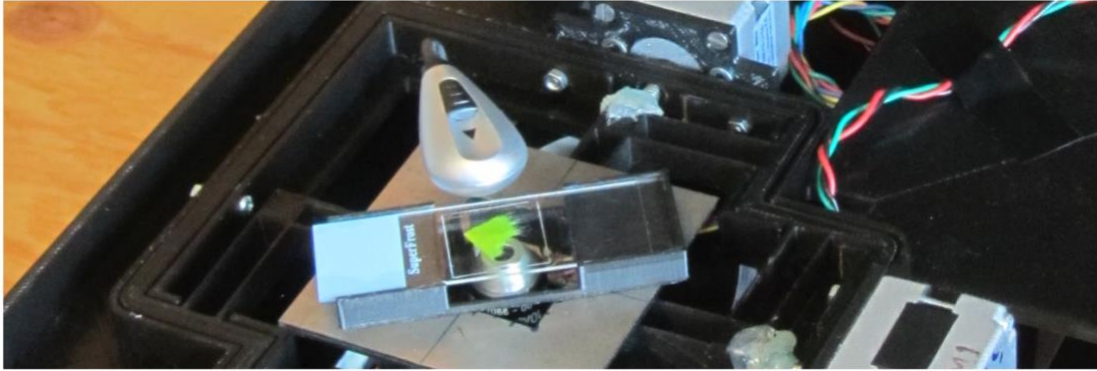
- 4) The display (shown above) comprises: (A) the live preview window which, when the “movement control tab is selected, incorporates the four way “XY” movement controls that appear as arrows; (B)

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 3**. 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 is 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 each 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 images 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. TROUBLESHOOTING

5.1. Troubleshooting guide:

- (1) **No WIFI.** Plug a USB mouse and keyboard and HDMI monitor into the Raspberry 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.