Screenshot 1

1.1. Click on the **readout mode** button for each camera and set it to **CONV 3 Megahertz**. Check that the temperature of each camera is -80 degrees Celsius and that the camera fan is off **[1]**.

1.1.1. SCREEN: To be provided by the authors: readout mode button got clicked then talent setting it to CONV 3 MHz.

1.1.2. SCREEN: To be provided by the authors: Camera temperature setting displaying -80 degrees

Screenshot 2

1.2. Turn on the **reflected** camera **[1]**. Under **Lights**, choose **ambient** and under **linkam**, click on **condenser [2-TXT]**. Click on the **Video mode** button **[3]**.

1.2.1. SCREEN: To be provided by the authors: Reflected camera turned on.

1.2.2. SCREEN: To be provided by the authors: Under the Lights option, Talent selecting ambient and then under linkam clicking on condenser. **TEXT: exposure 20 ms**

1.2.3. SCREEN: To be provided by the authors: Video mode button clicked

Screenshot 3

1.3. In the **Mosaic view** window, zoom out to see the grid outline **[1]**. Click on **Find stage** if it cannot be seen. Center the grid by double-left-clicking in the middle of the circle **[2]**.

1.3.1. SCREEN: To be provided by the authors: Mosaic view window is opened and talent zooming out to see the grid line

1.3.2. SCREEN: To be provided by the authors: Talent double clicking on the middle of the circle

1.4. Focus the sample until the grid support film or any other relevant sample feature is in focus, using the **up** and **down** keys **[1]**. Use the **9** and **3** keys on the numerical pad to change the z-step. Set it to 100 micrometers for initial focusing **[2]**.

1.4.1. SCREEN: To be provided by the authors: Sample focusing using up and down keys

1.4.2. SCREEN: To be provided by the authors: 9 and 3 keys are used to change the Z-step

Screenshot 4

2. Brightfield mosaic acquisition

2.1. Once the stage is centered, turn off **video mode**. Collect a visible light mosaic by clicking on **Run mosaic** in the **Mosaic view** to produce tiles of visible light images that spiral outward from the center.

2.1.1. SCREEN: To be provided by the authors: Turning off the video mode

2.1.2. SCREEN: To be provided by the authors: Run mosaic clicked

2.2. Save the view by clicking on **Save mosaic**.

2.2.1. SCREEN: To be provided by the authors: Save mosaic clicked

Screenshot 5

3. Identification of areas of interest

3.1. Inspect the brightfield mosaic alongside any previous fluorescence "map" images by turning off the **ambient light** and **condenser** as well as the **video mode** [1]. Turn on the required excitation laser and choose the corresponding **camera** and **filter**, initially at 50 milli Watt, for 50 milliseconds exposure time [2].

3.1.1. SCREEN: To be provided by the authors: Talent turning off the ambient light, condenser and video mode

3.1.2. SCREEN: To be provided by the authors: Talent turning on the relevant excitation laser and choosing the camera and filter at relevant settings

3.2. Press **0** to snap an image and **star** to auto-contrast. Alternatively, manually adjust the contrast by using the slider at the bottom of the image [1].

3.2.1. SCREEN: To be provided by the authors: talent adjusting the contrast

3.3. Once biologically interesting cells with suitable fluorescence have been found, mark their positions using the **Mark site** button in **Mosaic view** [1].

3.3.1. SCREEN: To be provided by the authors: Mark site button clicked in Mosaic View

3.4. Continue marking all potential sites before beginning image acquisition. Re-save the mosaic with the marked sites by clicking on **Save sites to file** [1].

3.4.1. SCREEN: To be provided by the authors: Talent marking the sites and clicking Save site to file option

4. Screenshot 6

4.1. To stitch the mosaic images together using the StitchM software, drag and drop the **.txt mosaic** file into the StitchM file with the extension **.bat** and save the combined tiff image of the mosaic tiles in the same folder [1].

4.1.1. SCREEN: To be provided by the authors: Talent dragging and dropping .txt file into StitchM file with extension .bat. **TEXT: developed inhouse at beamline B24**

4.2. To save an image with the marked sites, drag and drop the **mosaic.txt** file and the **markers.txt** file into the icon at the same time.

4.2.1. SCREEN: To be provided by the authors: Talent dragging and dropping mosaic.txt and markers.txt file into the icon at the same time.

Screenshot 7

5. Data collection

5.1. Set the laser exposure time based on the counts in the dynamic range in the fluorescence image at the bottom of the camera view window [1]. Choose which filter to apply and optimize the settings for each wavelength of excitation light to be used,

turning each laser on separately [2].

5.1.1. SCREEN: To be provided by the authors: Talent setting the laser exposure time

5.1.2. SCREEN: To be provided by the authors: Talent choosing which filters to apply

5.2. Click on both **cameras** to turn them on. Return to one of the marked sites and focus on the desired depth again [1].

5.2.1. SCREEN: To be provided by the authors: Talent choosing which filters to apply

5.3. Once in focus in an area of interest, move out of focus using the up arrow key in the **XY** window on the **Macro Stage** to choose the height of the z stack to acquire, and click on **Save top** [1]. Move out of focus using the down arrow key, click on **Save bottom** and then on **Go to the centre**. Verify that the image is still in focus [2].

5.3.1. SCREEN: To be provided by the authors: Talent clicking on up arrow key in XY window of Macro stage and then clicking on Save top

5.3.2. SCREEN: To be provided by the authors: Talent clicking on down arrow key, then clicking on Save top and then clicking on Go to the center

5.4. In the **Cockpit** window, select **Single-site experiment**. From the dropdown list, select **Structured Illumination** [1]. Alter the **Stack height** so that it equals the z-height plus 1 micrometer [2].

5.4.1. SCREEN: To be provided by the authors: Talent clicking on Single site experiment in Cockpit window and then selecting Structured illumination

5.4.2. SCREEN: To be provided by the authors: Talent altering the stack height

5.5. Enter the exposure times in milliseconds for the 405 nanometer and 488 nanometer lasers in the upper row for the reflected camera and the exposure times for the 561 nanometer and 647 nanometer lasers in the lower row for the transmitted camera [1].

5.5.1. SCREEN: To be provided by the authors: Talent entering the exposure time for relevant wavelengths for the reflected and transmitted camera

5.6. Input a file name and click on **Update** to produce a new file containing the date and time without overwriting previous files. Then, click on **Start** [1].

5.6.1. SCREEN: To be provided by the authors: Talent inputting a file name, clicking on update and then clicking on start

5.7. If the liquid nitrogen dewar refills the cryo-stage during image acquisition, abort the process by clicking on the **Abort** button in the **Cockpit** software [1].

5.7.1. SCREEN: To be provided by the authors: Talent clicking on Abort in Cockpit software

Screenshot 8

5.8. At each position, collect a z stack using visible light by Switching off the lasers, and switching on **Ambient light** and **condenser** [1]. Under **Single-site experiment**, select **Z-stack**, set the **Ambient light** to 20 millisecond exposure, maintaining the z height. Repeat this process for all marked sites [2].

5.8.1. SCREEN: To be provided by the authors: Talent switching off the lasers and switching on ambient light and condenser

5.8.2. SCREEN: To be provided by the authors: Talent selecting Z-stack under Single-site experiment and setting the Ambient light to 20 millisecond exposure