**Microscopy Metadata Checklist**

Macroscope stand and Motorized components

☐ Microscope Stand manufacturer and Model *Thorlabs[[1]](#endnote-1) and 3D printed (design: Ian Coghill)*

☐ Stage Manufacturer and Model *Thorlabsi (XR50P) and 3D Printed*

☐ Focusing device type *Manual – mechanical*

☐ \*\*\*Commercial/commercial modified, *Custom*

☐ \*\*\*Upright or inverted *Inverted*

Illumination

☐ Light source manufacturer and model *LXZ1-PB01 (Lumileds[[2]](#endnote-2))*

☐ Light source type *LED*

☐ \*\*\*Light source power output *N/A*

Wavelength selection

☐ Filter manufacturer and product number *Chroma[[3]](#endnote-3) (ET470/40X)*

☐ Filter center wavelength and bandwidth (FWHM), cut on or cut off wavelength *470nm: 40nm*

☐ Filter coating method  *Hard-coated*

Optics

☐Light injection *LED light is injected into a light pipe (67-401, Edmund Optics[[4]](#endnote-4))*

☐ Objective manufacturer  *Nikon[[5]](#endnote-5)*

☐ Objective correction  *Plan and Achromatic*

☐ Objective magnification *1X*

☐ Objective numerical aperture *Not provided by manufacturer*

☐ Specified immersion medium *Air*

☐ Optovar or relay lens magnification  *Camera objective: Nikkor 50 mm f/1.4 D (Nikonv)*

Detection

Fluorescence filter:  *Edmund Opticsiv (33-331, 550nm: 93nm)*

☐ Camera manufacturer and model *iDS[[6]](#endnote-6) (UI-3060CP-M-GL)*

☐ Camera type *CMOS*

☐ Binning *None*

☐ Bit Depth and associated gain *12 bit*

☐ EM gain (if EMCCD used) *NA*

☐ \*\*\*Pixel size *5.86 x 5.86 um*

☐ \*\*\*Chip size *11.345 mm x 7.126 mm (1936 x 1216)*

☐ \*\*\*Pixel readout rate  *30 MHz - 480 MHz*

☐ \*\*\*Rolling or global shutter  *Global*

Acquisition software

☐ Software manufacturer, name, and version  *UEye Cockpit 4.94*

☐ If custom, Author and appropriate citation *Ian Coghill and Aliénor Lahlou, to be released in later publication*

☐ State of the shutter during acquisition *NA*

☐ Order of experimental acquisition *No stage movement, no shutter*

☐ If custom macro *To be released in later publication*

☐ Time interval *166.67 ms*

Sample preparation

☐ Sample holder type, manufacturer and product number *3D printed design*

☐ Coverslip grade *Epredia 24x60 #1 cover slips*

☐ Coverslip coating (type, concentration, detailed protocol) *None*

☐ Detail protocol: fixitive, concentration of fixitive, fixation conditions (buffers, time, temperature), blocking, binding and hybridization buffer composition, Ab manufacturer, lot number, concentration, probe concentration, binding or hybridization conditions (time, temperature, sequential/simultaneous)  *see supplementary materials*

☐ Mounting/imaging medium name, manufacturer and product number *NA*

☐ Specific fluorescent protein variant *Dronpa2*

☐ Transfection reagent, concentration or other method of expression *see supplementary materials*

**Microscopy Metadata Checklist Generator (MicCheck) developed by Rebecca Senft (2021)[[7]](#endnote-7)**

**ACQUISITION CONDITIONS**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Figure** | **Source** | **Excitation Filter** | **Dichroic Filter** | **Emission Filter** | **Detection** | **Camera Framerate** | **Camera Exposure** |
| **Figure 2j-l** | *LXZ1-PB01* | *470nm: 40nm* | *Not required* | *550nm: 93nm* | iDSvi (UI-3060CP-M-GL) | 6 fps | *50ms* |

1. Thorlabs Inc., Newton, New Jersey, United States. [↑](#endnote-ref-1)
2. Lumileds Holding B.V., Schiphol, Netherlands. [↑](#endnote-ref-2)
3. Chroma Technology Corp, Bellows Falls, Vermont, USA. [↑](#endnote-ref-3)
4. Edmund Optics, Barrington, New Jersey, USA. [↑](#endnote-ref-4)
5. Nikon, Minato City, Tokyo, Japan. [↑](#endnote-ref-5)
6. IDS Imaging Development Systems GmbH, Obersulm, Germany. [↑](#endnote-ref-6)
7. Montero Llopis, P., Senft, R. A., Ross-elliott, T. J., Stephansky, R., Keeley, D. P., Koshar, P., Marqués, G., Gao, Y., Carlson, B. R., Pengo, T., Sanders, M. A., Cameron, L. A., & Itano, M. S. (2021). Best practices and tools for reporting reproducible fluorescence microscopy methods. Nature Methods, 18(12), 1463-1476. [↑](#endnote-ref-7)