Microscopy Metadata Checklist

Microscope stand and Motorized components

☐ Microscope Stand manufacturer and Model *Thorlabs*

☐ Illumination Shutter Manufacturer and Model *None*

☐ Stage Manufacturer and Model  *MPositioning MS-02XY 100x100mm travel*

☐ Linear encoded stage? *DIY: https://github.com/SonyCSLParis/Motorized-stage*

☐ Focusing device manufacturer and model *Thorlabs NFL5DP20/M*

☐ Focusing device type *piezo Thorlabs KPZ101*

☐ Hardware-based Focus maintenance device manufacturer and model *None*

☐ Software based focusing maintenance set up (wavelength, range, step size, algorithm) *470nm, 75um range, 10 um step followed by 1.5um step Laplacian variance*

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

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

Illumination

☐ Light source manufacturer and model *blue: Lumileds LXZ1-PB01, purple: Lumileds LHUV-405*

☐ Light source type *LED*

☐ \*\*\*Light source power output *1W*

Wavelength selection

☐ Filter manufacturer and product number  *blue: Semrock FF01-479/40-25, purple: Chroma 405/20x*

☐ Filter center wavelength and bandwidth (FWHM), cut on or cut off wavelength *479/40; 405/20*

☐ Filter coating method *hard-coated*

☐ \*\*\*Additional filters manufacturer and model *Neutral Density OD1*

Optics

☐Light injection *LEDs are injected in a 400 µm core optical fiber using a 20× objective (Nikon, NA = 0.75) to further homogenize the light beams. Light at the output of the fiber is collimated with a 10× objective (Olympus, NA = 0.5)*

☐ Objective manufacturer *Zeiss*

☐ Objective correction  *Infinity Color Corrected System (ICS), flatness*

☐ Objective magnification *10x*

☐ Objective numerical aperture *0.50*

☐ Specified immersion medium  *Air*

☐ Optovar or relay lens magnification  *Thorlabs, f = 150 mm lens AC254-150-A*

Detection

Dichroic filter: *FF506-Di03*

Fluorescence filter: *Semrock FF525-30*

☐ Camera manufacturer and model *IDS UEye 3060CT-M*

☐ Camera type *CMOS*

☐ Binning *None*

☐ Bit Depth and associated gain *12 bit gain 100*

☐ EM gain (if EMCCD used) *NA*

☐ \*\*\*Pixel size *5.86 x 5.86 um*

☐ \*\*\*Chip size *1936 x 1216*

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

☐ \*\*\*If sCMOS, rolling or global shutter  *Global*

Acquisition software

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

☐ If custom, Author and appropriate citation *Aliénor Lahlou, to be released in microscope-specific publication*

☐ State of the shutter during acquisition *NA*

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

☐ If custom macro *to be released in microscope-specific publication*

☐ Time interval *333ms*

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) Poly-L-lysine coated coverslips

☐ 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)**

**ACQUISITION CONDITIONS**

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| **figure** | **light source** | **emission filter** | **dichroïc filter** | **fluorescence filter** | **Detection** | **camera framerate** | **camera exposure** |
| **Figure 2a-i** | *LXZ1-PB01* | *FF01-479/40-25* | *FF506-Di03* | *FF525-30* | *IDS UEye 3060CT-M* | 3Hz | *50ms* |
| **Figure 5** | *LXML-PWN1-0080* | *694/SP* | *FF665-*  *Di02* | *775/140- BP* | *IDS UEye 3060CT-M* | NA - single shot | *800ms* |