**Record of instrument details during image acquisition**

**Experiment**

Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Project: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sample mounting medium: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Coverslip thickness: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Sample position: On the slide On the coverslip

**Equipment: Leica DMI6000B**

Light Source

Type: Broadband source (EL6000 (Leica), Mercury short-arc reflector lamp, OSRAM HXP-R120W/45C VIS)

Objective(s)

*(highlight the ones you used for the experiment)*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Manufacturer** | **Magnification** | **Numerical Aperture (NA)** | **Immersion medium** | **Optical Aberration Correction** | **Working distance (mm)** |
| Leica | 20x | 0.40 | Air (dry) | HC PL FLUOTAR L | 7.5 |
| Leica | 63x | 1.30 | Glycerin | HC PLAPO CS2 | 0.3 |
| Leica | 100x | 1.40 | Oil | HCX PL APO CS | 0.09 |
| Leica | 63x | 0.90 | Water | HC APO L U-V-I | 2.20 |
| Leica | 100x | 1.25 | Oil | N PLAN | 0.14 |

Immersion oil: Type F Immersion Oil (Leica) – with refraction index of 1.518

Available filters

*(highlight the ones you used for the experiment)*

|  |  |  |  |
| --- | --- | --- | --- |
| **Filter cube** | **Excitation wavelength (nm)** | **Dichroic mirror** | **Emission wavelength (nm)** |
| A4 (DAPI) | 340 – 380 | 400 | 450 – 490 |
| GFP | 450 – 490 | 495 | 500 – 550 |
| Y3 | 530 – 560 | 570 | 572 – 648 |
| Y5 | 590 – 650 | 660 | 662 – 738 |
| YR2\* | Fast Filter Wheels: GFP and mRFP | 495; 595 | Fast Filter Wheels: GFP and mRFP |
| CG1\* | Fast Filter Wheels: CFP and  YFP | 440; 520 | Fast Filter Wheels: CFP and YFP |

\* These filter cubes have only the dichroic mirror. So they need to be used with the Fast Filter Wheels

|  |  |  |
| --- | --- | --- |
| **Fast Filter Wheels** | **Excitation wavelength (nm)\*\*** | **Emission wavelength (nm)\*\*** |
| GFP | 470/40 | 520/40 |
| mRFP | 572/35 | 632/60 |
| CFP | 427/40 | 472/30 |
| YFP | 504/12 | 542/27 |

\*\* The wavelength reported corresponds to the ET/bandpass. As an example, 470/40 means 470 ± 20

Power densities (as of 8.9.21; lamp running time 1100h, sensor S175C)

|  |  |  |  |
| --- | --- | --- | --- |
| **Filter cube** | **Power density (W/cm2)** | | **Measurement location** |
|  | 100x oil, NA 1.4, FN 25 | 63x oil, NA 1.3, FN 25 |  |
| A4 (DAPI) | 0.160 | 0.081 | objective focal plane |
| GFP | 0.713 | 0.485 | objective focal plane |
| Y3 | 0.917 | 0.728 | objective focal plane |
| Y5 | 0.489 | 0.364 | objective focal plane |

Detector

Camera model: DFC 365 FX Manufacturer: Leica

**Sensor model: ICX285 AL (CCD B/W) Manufacturer: Sony**

Pixel size: 6.45 µm

Dynamic range: 12 bit

Imaging frequency (at full frame and 40MHz pixel clock): 21 fps

C-mount Adaptor: 0.7x

Binning: \_\_\_\_\_\_\_\_\_\_\_ (default: 1 x 1)

Resolution: \_\_\_\_\_\_\_\_\_\_\_ (at default binning: 1392 X 1040)

Offset: *(default value not determined; change of settings not possible)*

**Acquisition/detection parameters**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Target** | **Fluorophore** | **Channel/Filter** | **Exposure time** | **Gain** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |

**3D recording**

Z-size: \_\_\_\_\_\_\_\_\_\_ µm

Z-step size: \_\_\_\_\_\_\_\_\_\_ µm (system optimized = 0.21)

Number of steps: \_\_\_\_\_\_\_\_\_\_\_

**Time series recording**

Pixel clock: \_\_\_\_\_\_\_\_\_ (default: 40 MHz)

T steps/frequency: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Duration/frames: \_\_\_\_\_\_\_\_\_\_\_\_\_ ms

**Reporting your methods**

**(In the sample preparation/immunofluorescence section)**

The samples were prepared on 12 mm Ø glass coverslips (thickness 0.13-0.16 mm), mounted on glass microscope slides using VECTASHIELD® Antifade Mounting Medium with DAPI (Vector Laboratories, USA), and sealed with nail polish.

**Microscope configuration and image acquisition**

Images were acquired using a DMI6000B widefield microscope (Leica Microsystems, Germany) with a HCX PL APO CS objective (100x, NA = 1.4, Leica Microsystems, Germany) and Type F Immersion Oil (refractive index = 1.518, Leica Microsystems, Germany). Samples were illuminated with a Leica EL6000 Mercury short-arc reflector lamp (HXP-R120W/45C VIS, OSRAM, Germany). Excitation light was selected by using Y3 (530-560 nm), GFP (450-490 nm), and A4 (340-380 nm) bandpass filter cubes (Leica Microsystems, Germany). The power density (measured at the objective focal plane) was respectively 0.160, 0.713, 0.917 W/cm2 for the three filters. Emitted light was collected at ranges of 572-648 (Y3), 500-550 nm (GFP), and 450-490 nm (DAPI) respectively. The individual exposure times and camera gains were as follows: Y3, 400 ms (gain = 1.7); GFP, 200 ms (gain = 1.0); DAPI, 100 ms (gain = 1.0). Differential interference contrast (DIC) was used to visualise cell morphology. 3D recording of each field of view was obtained using 50 Z-slices (step size = 0.21 µm). Fields of view were selected in the DIC channel in order to blind the user to the fluorescence signal and subjectively select for cells with optimum morphology. Images were captured using a black-and-white CCD sensor (ICX285 A, pixel size = 6.45 µm, Sony, Japan).