|  |  |  |  |  |  |  |  |  |  |  |  |  |
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| **Table1: Confocal microscope settings for acquisition of presented representative images** | | | | | | | | | | | | |
|  | **Parameters** | **Fig3A** | **Fig4A** | **Fig4B** | **Fig5A** | | **Fig5B** | **Fig5C** | **Fig6A** | **Fig6b** | **Fig7A** | **Fig7B** |
| **Acquisition** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Objective | Plan-Apochromat 63×, 1.0 NA water immersion | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil |
|  | Scan mode | Frame | Frame | Frame | Frame | | Frame | Frame | Frame | Frame | Frame | Frame |
|  | Frame size | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:513 |
|  | Speed | 7 | 8 | 8 | 8 | | 8 | 8 | 8 | 7 | 7 | 7 |
|  | Pixel dwell | 3.55µs | 2.55µs | 2.55µs | 2.55µs | | 2.55µs | 2.55µs | 2.55µs | 3.15µs | 3.15µs | 3.15µs |
|  | Scan Time | 1.97 s | 781.96 ms | 1.56 s | 1.56 s | | 3.13 s | 1.56 s | 4.69 s | 3.87 s | 15.49 s | 7.75 s |
|  | Averaging Number | 1 | 1 | 1 | 1 | | 1 | 1 | 1 | 1 | 2 | 1 |
|  | Bit Depth | 8 bit | 8 bit | 8 bit | 8 bit | | 8 bit | 8 bit | 8 bit | 8 bit | 8 bit | 8 bit |
|  | Direction | Uni | Bi | Bi | Bi | | Uni | Bi | Uni | Uni | Uni | Uni |
|  | Zoom | 2 | 0.5 | 0.5 | 0.5 | | 2 | 0.5 | 0.5 | 1.9 | 2.2 | 2.5 |
| **Channels** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Laser | Gr:514nm (488)nm | Rd:555nm | Gr:488nm Rd:555nm | Gr:488nm Rd:555nm | | Gr:488nm Rd:555nm | Gr:488nm Rd:555nm | Gr:488nm Rd:555nm | Gr:488nm Rd:555nm | Gr:639nm Rd:555nm Wt:488nm | Gr:639nm Rd:555nm Wt:488nm |
|  | Pinhole (Optical Slice) | Gr:15 µm | Rd:2.0 µm | Gr:2.0 µm Rd:2.0 µm | Gr:2.4 µm Rd:2.4 µm | | Gr:1.7 µm Rd:1.7 µm | Gr:1.2 µm Rd:1.2 µm | Gr:2.3 µm Rd:2.3 µm | Gr:1.5 µm Rd:1.5 µm | Gr:2.0 µm Rd:2.1 µm | Gr:1.5µm Rd:1.5µm |
|  | Gain (Detector) | Gr:700 | Rd:600 | Gr:700 Rd:600 | Gr:700 Rd:600  Ct:600  Ce:700 | | Gr:700 Rd:700 | Gr:700 Rd:600 | Gr:700 Rd:733 | Gr:700 Rd:733 | Gr:700 Rd:600 Wt:544 | Gr:700 Rd:705 Wt:700 |
|  | Power (Laser) | Gr:100% | Rd:1.0% | Gr: 1.0% Rd:1.0% | Gr: 2.0% Rd:1.0% | | Gr: 2.0% Rd:1.0% | Gr: 2.0% Rd:1.0% | Gr: 1.0% Rd:9.5% | Gr: 1.0% Rd:9.8% | Gr:15.0% Rd:1.0% Wt:3.5% | Gr:17.1% Rd:20.0% Wt:2.2% |
| **Z Sectioning** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Z Interval | 2 | 2 | 2 | 2 | | 2 | 2 | 2 | 2 | 1 | 2 |
| **Light Path** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Switch Track | Frame | line | line | line | | line | line | line | line | Frame | Frame |
|  | Filters | Gr:SP555 | Rd:LP560 | Gr: SP555 Rd:LP560 | Gr:SP555 Rd:LP560  Ct:LP560  Ce:SP555 | | Gr:SP555 Rd:LP560 | Gr:SP555 Rd:LP560 | Gr: SP555 Rd:LP560 | Gr:SP555 Rd:LP560 | Gr:None Rd:None  White:None | Gr:BP555-610+LP650 Rd:BP420-475+VP500-16  Wt::BP55-610 + LP650 |
| **Time Series** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Interval Time | 10 secs |  |  |  | |  |  |  |  |  |  |
| **Photobleaching** |  |  |  |  |  | |  |  |  |  |  |  |
|  | Laser Power | 488nm, 100% |  |  |  | |  |  |  |  |  |  |
|  | Repeat Bleach after # scans | 5 |  |  |  | |  |  |  |  |  |  |
|  | Iterations | 50 |  |  |  | |  |  |  |  |  |  |
| Footnotes: Green: Gr Rd: Red White:Wt Cross Talk: Ct Cross Excitation: Ce BL: Blue | | | | | | | | | | | | |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Parameters** | **Figure 9A** | **Figure 9B** | **Image 9C** | **Figure 7C** | **Image 9D** |
| **Acquisition** |  |  |  |  |  |  |
|  | Objective | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil | Plan-Apochromat 40x/1.4 Oil |
|  | Scan mode | Frame | Frame | Frame | Frame | Frame |
|  | Frame size | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 | X:512 Y:512 |
|  | Speed | 8 | 7 | 8 | 8 | 8 |
|  | Pixel dwell | 2.55µs | 3.15µs | 2.55µs | 2.55µs | 2.55µs |
|  | Scan Time | 6.25 sec | 3.87 | 6.25 sec | 6.25 sec | 3.13 sec |
|  | Averaging Number | 1 | 1 | 1 | 1 | 1 |
|  | Bit Depth | 8 bit | 8 bit | 8 bit | 8 bit | 8 bit |
|  | Direction | Uni | Uni | Uni | Uni | Uni |
|  | Zoom | 1.3 | 1 | 1.0 | 1.0 | 1.0 |
| **Channels** |  |  |  |  |  |  |
|  | Laser | Wt:488nm  Bl:405nm | Wt:488  Bl:405 | Gr:488nm  Rd:555nm | Gr:488nm  Rd:555nm | Gr:488nm  Rd:555nm |
|  | Pinhole (Optical Slice) | Wt:79μm  Bl: 81μm | Wt:79  Bl:81 | Gr: 36μm  Rd: 36μm | Gr: 79μm  Rd: 79μm | Gr: 90μm  Rd: 90μm |
|  | Gain (Detector) | Wt:700  Bl:700 | Wt:700  Bl:639 | Gr:701  Rd:700 | Gr:701  Rd:700 | Gr:701  Rd:733 |
|  | Power (Laser) | Wt:1.5%  Bl: 2.8% | Wt:5.0%  Bl:3.5% | Gr: 0.3%  Rd: 0.5% | Gr: 0.3%  Rd: 2% | Gr: 1.0%  Rd: 2.0% |
| **Z Sectioning** |  |  |  |  |  |  |
|  | Z Interval | 2 |  | 0.46 |  |  |
| **Light Path** |  |  |  |  |  |  |
|  | Switch Track | Frame | Frame | Line | Line | Line |
|  | Filters | Wt:SP555  Bl: BP420-475 | Wt:SP555  Bl:SP490 | Gr: 415-735  Rd: 415-735 | Gr: 415-735  Rd: 415-735 | Gr: SP555  Rd: LP560 |
|  | Footnotes: Green: Gr Rd: Red White:Wt Bl: Blue Cross Talk: Ct Cross Excitation: Ce | | | | | |