**Checklist settings Sp8x confocal microscope**

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| **General** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| Objective/immersion | eg 40x, water |  |  |
| Z position knob | nm, eg -188nm |  |  |
| Z position Galvo | nm |  |  |
| Tile scan | Yes / No |  |  |
| Multiple positions | Yes / No |  |  |
| Sequential scan | Yes / No |  |  |
| Autofocus | Yes / No |  |  |
| Bidirectional scan x | Yes / No |  |  |
| Frame size | Pixels, eg 1024x1024 |  |  |
| Scan speed | Hz, eg 400 Hz |  |  |
| Zoom factor |  |  |  |
| Line average |  |  |  |
| Line accumulation |  |  |  |
| Frame average |  |  |  |
| Frame accumulation |  |  |  |
| Pinhole (Emission) | AU (λ) |  |  |
| Fluo / Reflection | AOBS |  |  |

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| **Excitation** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| Exc 405UV | Yes / No intensity % |  |  |
| Exc WLL: λ 1 | nm -%, eg 488 nm 1.2% |  |  |
| Exc WLL: λ 2 | nm -%, eg 540 nm 3.0% |  |  |
| Exc WLL: λ 3 | nm -%, eg 555 nm 3.0% |  |  |
| Exc WLL: λ 4 | nm -%, eg 594 nm 3.0% |  |  |
| Exc WLL: λ 5 | nm -%, eg 650 nm 3.0% |  |  |
| Exc WLL: λ 6 | nm -%, eg 733 nm 3.0% |  |  |
| Exc WLL: λ 7 | nm -%, eg 647 nm 3.0% |  |  |
| Exc WLL: λ 8 | nm -%, eg 670 nm 3.0% |  |  |
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| **Emission** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| **HyD 1** |  |  |  |
| HyD 1 Range | nm–nm, eg 496-549nm |  |  |
| HyD 1 Mode | Standard/Bright/Photon |  |  |
| HyD 1 Gain |  |  |  |
| HyD 1 Gating | Yes – No, From –To |  |  |
| HyD 1 pseudocolor | Eg Cyan |  |  |
| **PMT 1** |  |  |  |
| PMT 1 Range | nm-nm, eg 530-600m |  |  |
| PMT 1 Gain-Offset | Volt, %, eg 712.2V, 0.5% |  |  |
| PMT 1 pseudocolor | eg Green |  |  |
| **HyD 2** |  |  |  |
| HyD 2 Range | nm–nm, eg 496-549nm |  |  |
| HyD 2 Mode | Standard/Bright/Photon |  |  |
| HyD 2 Gain |  |  |  |
| HyD 2 Gating | Yes – No, From –To |  |  |
| HyD 2 pseudocolor | Eg Cyan |  |  |
| **PMT 2** |  |  |  |
| PMT 2 Range | nm-nm, eg 530-600m |  |  |
| PMT 2 Gain-Offset | Volt, %, eg 712.2V, 0.5% |  |  |
| PMT 2 pseudocolor | eg Green |  |  |
| **PMT Trans** |  |  |  |
| PMT Trans Range | nm-nm, eg 530-600m |  |  |
| PMT Trans Gain-Offset | Volt, %, eg 712.2V, 0.5% |  |  |
| PMT Trans pseudocolor | eg Gray |  |  |

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| **Z-stacks** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| Begin | nm |  |  |
| End | nm |  |  |
| Z-position center | nm |  |  |
| Z size | nm |  |  |
| Stack direction | Upward/Downward |  |  |
| Nr steps |  |  |  |
| Z-stepsize | nm |  |  |
| Z compensation |  |  |  |

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| **Time lapse** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| Time interval | h:min:sec:ms |  |  |
| Duration | h:min:sec:ms |  |  |
| Number frames |  |  |  |

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| **Sequential** |  |  |  |
| **Parameter** | **Unit/example** | **Own settings** | **Remarks** |
| **Seq 1** |  |  |  |
| Seq 1 exc λ | nm |  |  |
| Seq 1 HyD/PMT | Eg HyD 1 |  |  |
| Seq 1 emission λ | nm |  |  |
| Notes | | | |
| **Seq 2** |  |  |  |
| Seq 2 exc λ | nm |  |  |
| Seq 2 HyD/PMT | Eg HyD 1 |  |  |
| Seq 2 emission λ | nm |  |  |
| Notes | | | |
| **Seq 3** |  |  |  |
| Seq 3 exc λ | nm |  |  |
| Seq 3 HyD/PMT | Eg HyD 1 |  |  |
| Seq 3 emission λ | nm |  |  |
| Notes |  |  |  |