**Supplementary File 2: ZEN software steps for SHG image acquisition**

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|  | Description | Icon in ZEN software | Notes: |
| 1 | In **Light Path**: Select the **Non Descanned** imaging mode. The transmission path corresponds to the bottom part of the diagram. Tick below the desired detection filter symbol, in this case **SP485**.  Main beam splitter for invisible light: select **MBS 760+**  Reflector revolver: select **Rear**. |  | This way the capture of the weak SH signal of tubulin will be optimized. |
| 2 | Turn on the laser in the software from **Laser**:  Chameleon, select **On** |  | This will open the shutter. NOTE: Make sure the laser was previously turned on from the pump laser module |
| 3 | In **Channels**, select the chameleon wavelength to **810 nm**. Select 10% to 20% chameleon laser power in the software |  | In the system described, this corresponds to 13 to 26 mW measured at objective BFP |
| 4 | In **Acquisition Mode**, select imaging speed 5, which corresponds to pixel dwell times of 12.6 us |  | Total acquisition time will be defined only after selecting averaging (step 5) |
| 5 | Select averaging 2 | With these settings, this will determine a total image time of about 15 sec. |
| 6 | Select 25x objective from the software | Oil should be added to the objective lens before selecting the objective, if the sample is already in place. The objective can be selected in alternative ways. |
| 7 | Use the **Live** mode for the initial visualization. Adjust acquisition parameters (gain, offset) with the **Continuous** mode. |  | **Live** reduces exposition |
| 8 | When ready, take an image with **Snap** |  |
| 9 | Repeat imaging (step 8) with bandpass filter inserted |  | This will clean the image from any autofluorescence |