**Appendix V**

There are two approaches that will lead to proper ablations, but these need to be tested and optimized before conducting the actual experiments. These are: I, Performing ablations using small numbers of iterations and higher transmissions of the two-photon laser; II, Using more iterations and less laser power. Based on our experience, we found results using method II more reproducible than using method I. Therefore we recommend method II.

*Note: For method I, we found, that higher laser power and less iterations leads to settings that are more sensitive to day-to-day variations leading to more variability between results acquired on different microscope sessions. For method II, day to day variability is reduced, although it imposes a limit on how fast after ablation a frame can be acquired.*