Typical operation

Ensure all lasers, APD’s etc are on before opening software

Turn on lasers in the omicron software, set to digital modulation so the acquisition software can control the ALEX cycle. If you need a laser to be on for alignment/focussing, set to CW mode first.

Add immersion oil to the objective, and carefully place a coverslip avoiding bubbles. We use 631-0124 22x22mm coverslips, however as you are imaging in solution rather than on the surface the quality of the glass should not matter so long as it is free of dirt and dust.

Add a solution to the coverslip, initially to focus, so water will work. 10-100 ul works fine.

Use the focusing software to get the detection volume into the solution as described

Next add your fluorescent sample, usually at ~10-100 pM, aiming for approximately 1 burst per second. More concentrated than this will result in coincident bursts which will limit the quality of your data, less concentrated will require more acquisition time. Due to the very low concentrations invovled, we typically include 0.1 mg/ml of BSA to solutions as a passivating agent to prevent loss of molecules to the coverslip surface throughout the experiment.

Once happy with concentration, begin acquiring data. We typically use 80/15% power on the green and red lasers respectively with an ND 1 filter to attenuate, when using bright dyes like Cy3B, Atto550, Atto647N etc. However for less bright dyes more laser power is required and so we work without the ND filter. ALEX cycles used are typically 45 µs on, then 5 µs off for each laser.

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