

2 Results (outline)

2.1 Validating the laser modules

For every laser in the setup (561 nm, 640 nm, and 775 nm), I measured the polarisation state of the light at different points in the beam path and at the sample plane, by measuring the power transmitted by a linearly polarising filter at different angles relative to the beam. At the sample location, the 640 nm beam is quite well-polarised when set to a linear polarisation. When rotating a polariser, the transmitted power drops to about one fifth of the maximum. At the circular setting, though, the light is quite elliptical, with a minimum power transmitted through the polariser being about half as bright as the maximum, see Figure 4. The quality of the 561 nm polarisation is higher. When set to a linear polarisation, the power transmitted through a polariser drop from about 25 μ W to a value almost equal to background levels around 0.1 μ W, while the amplitude variations of the circularly polarised light are within 14% of the mean, see Figure 5. In conventional STED, the depletion beam is circularly polarised in order to achieve polarisation-independent quenching, so I also measured its polarisation state, see Figure 6. The minimum power transmitted is about 63% of the max. (Calculate ellipticity angle, or introduce the E_{max}/E_{min} characteristic in the beginning.) (Put this stuff into a table?)

There are two other things we can take away from those figures: the power of the 640 nm laser is not very constant. This can be explained by a slow ramp up to the set power every time the laser is turned on. Secondly, the noise on the signal from the 640 laser is higher. I believe this is due to the same effect. Therefore, I also measured the power profiles of the different lasers, see (figure). The conclusion from those measurements is that the response of the lasers to the desired power set in software is not linear at low powers. If experiments need to be done at low power, a neutral density filter is required. This is luckily not the case for biological specimens with a low density of fluorophores.

Finally, we also measured the PSFs of the different lasers as a sanity check. This is shown in Figure 7. This data looks good. (Why does it look good?) (Mention how we got this data: PMT and gold beads.)

2.2 Validating the detection module

First off, I checked the polarisation dependence of the APDs. To do so, I ensured the light coming into the APDs was unpolarised by illuminating a sample of TetraSpec beads with circularly polarised 561 nm light, since that was the closest to circular that we could get. Then I put a polarising filter after the detection waveplates and measured the signal from the APD as a function of the incoming polarisation, see Figure 8. For both APDs, there is a difference of about 4%.

To do:

- Should I do min/max like before? That would also be closer to a G factor, I guess.
- APD2 and power meter pol sensitivity.
- Correct for ellipticity of 561 polarisation

Second, I tested the effect of the detection waveplates on the polarisation state of incoming light. Linearly polarised input light was achieved by putting a polariser (P1) in the sample location and illuminating it with the microscope's top lamp. Then the intensity was measured by the APDs, which was dependent on the angle of a polariser located after the last waveplate (P2). Using Jones calculus, we know that two QWPs and a HWP can form a proper polarisation rotator (ref methods), but the default calibration (a function that maps the total rotation angle to the angles the waveplates have to take on) is unable to do so. I determined that a better calibration sets the (non-fixed) QWP to an angle of 50° and the HWP to half the rotation angle. The angle of the QWP was chosen such that it is aligned with the fixed QWP at the microscope end. The behaviour of both calibrations is shown in Figure 1. The new calibration works as expected.

Finally, I also measured the polarisation characteristics of the POL cube. I generated linearly polarised light as in the previous paragraph, but left out P2. Then I rotated the polarisation using the detection waveplates and measured the intensity of light reflected and transmitted by the POL cube into APD1 and APD2 respectively. Apparently, the degree to which the POL cube can split the signal is strongly dependent on the polarisation of the light at the sample (the angle of P1), Figure 2. The cause of this effect is not immediately obvious, but is most likely due to some birefringence present in the POL cube. (Is that true?)

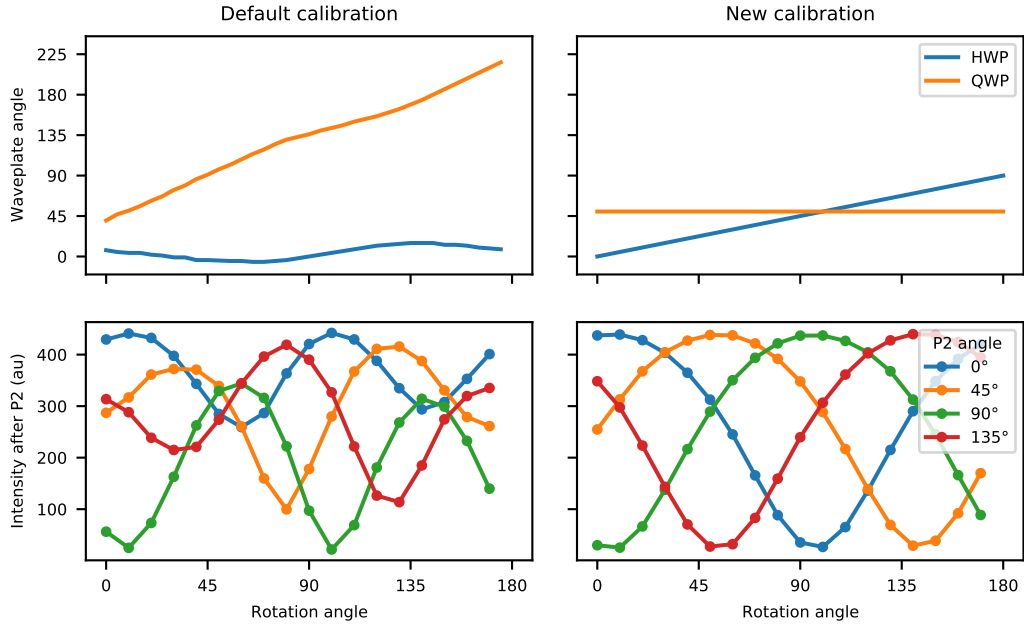


Figure 1: text

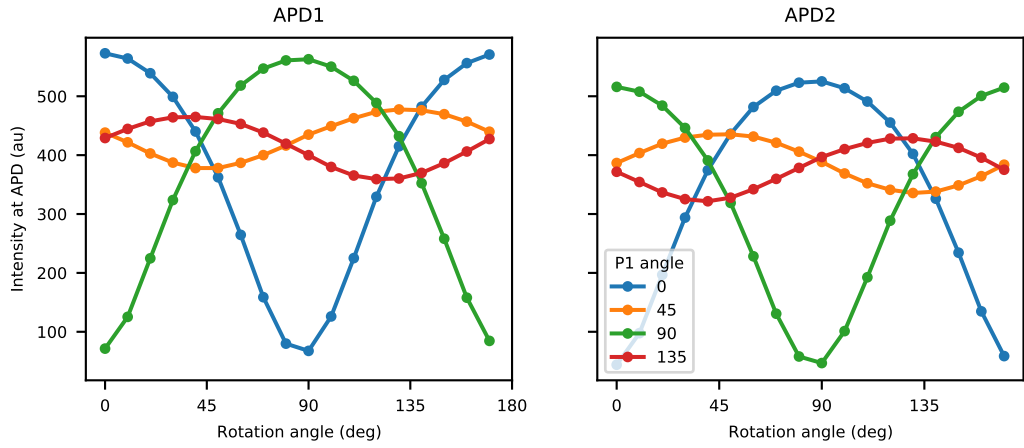


Figure 2: The POL cube distorts the polarisation state of incoming light. APD should measure the vertical component of the light (reflected by the POL cube), and APD2 should measure the horizontal component (transmitted). The detection waveplates were used to rotate incoming light, which was polarised along an angle shown in the legend.

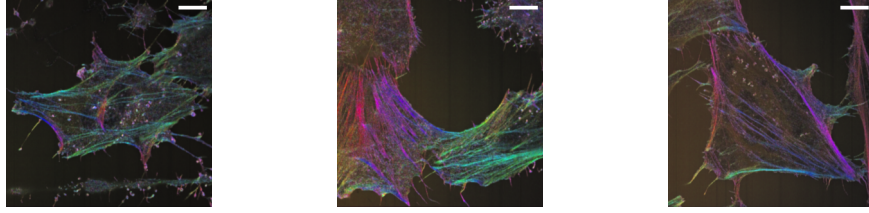


Figure 3: Polarisation microscopy images of three different regions. Scalebars $10\,\mu\text{m}$.

2.3 Demonstration of conventional polarisation microscopy

To do:

- Colour wheels in the images
- sSTED + polarisation
- Can I get some quantitative results?

2.4 Polarisation-resolved STED microscopy

4 Supplemental figures

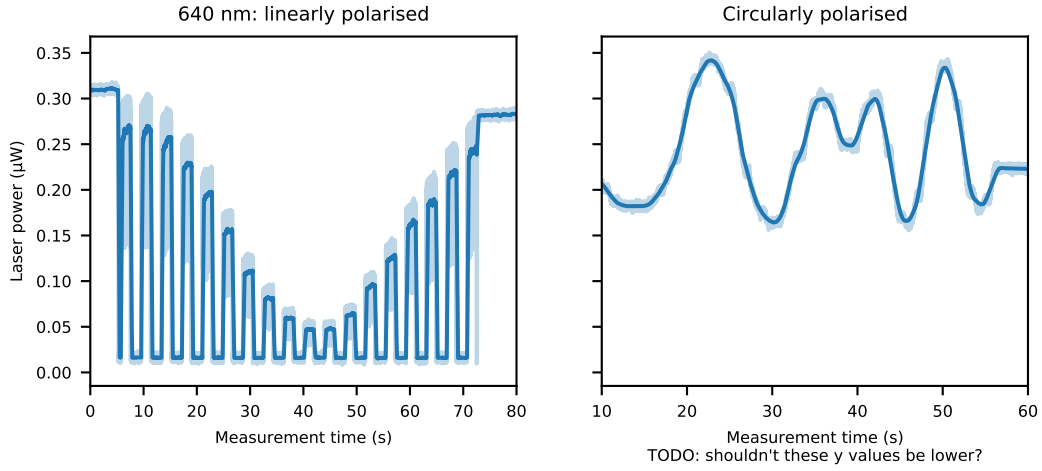


Figure 4: Polarisation characteristics at the sample plane of the 640 laser. **Left:** power transmitted through a stationary polariser aligned to maximise transmission of the 640 laser set to an polarisation of 0° (vertical in the sample plane), while the laser beam rotates. **Right:** power transmitted through a manually rotating polariser, while the beam is set to circular polarisation.

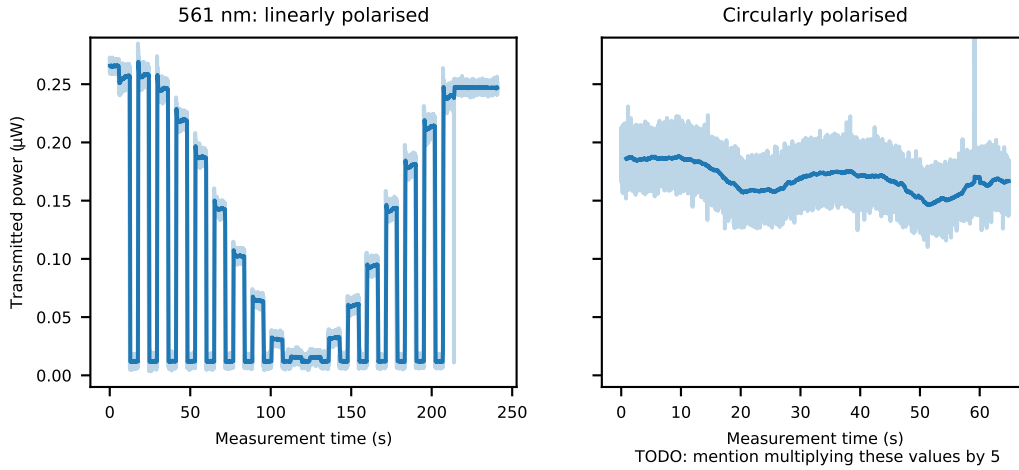


Figure 5: Polarisation characteristics at the sample plane of the 561 laser. Left and right panes are the same as Figure 4. (As the data on the left was acquired at 50% laser power to increase the signal at minimum transmission, but the data on the right was acquired at 10% laser power for safety reasons, the data on the right has been multiplied by a factor of 5 to allow comparison between the two figures. This also increased the noise.)

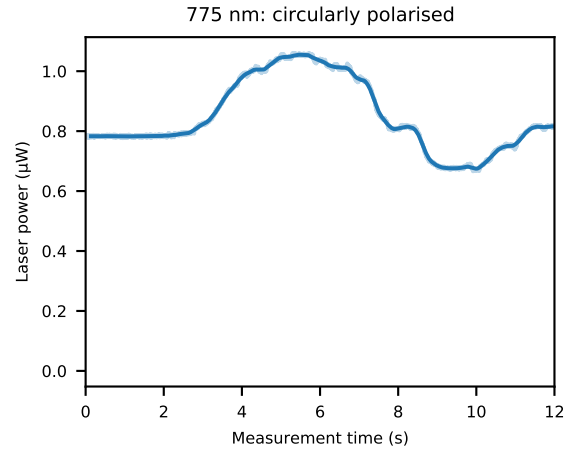


Figure 6: Polarisation characteristics at the sample plane of the depletion beam, without pSTED optics. As its polarisation state cannot be manipulated through the software, only circular polarisation is characterised by manually rotating a polariser at the sample plane and measuring the transmitted power.

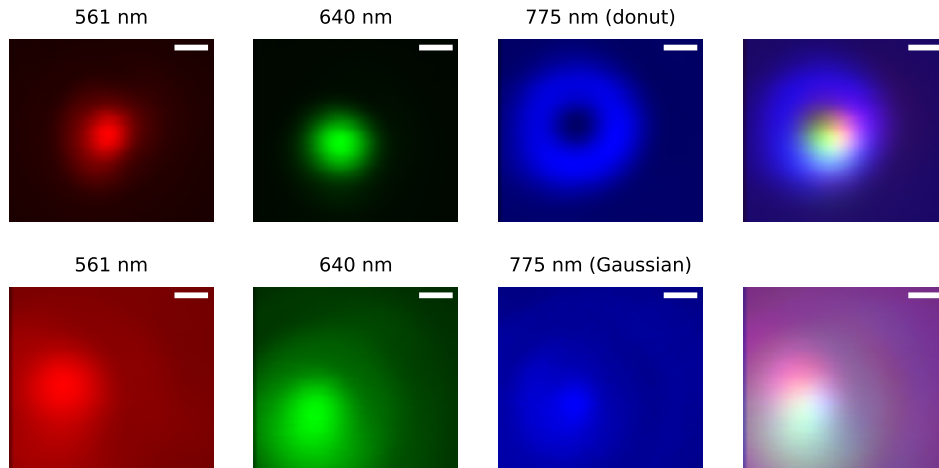


Figure 7: Point spread functions of the different lasers at different SLM configurations, by measuring the reflection from 100 nm wide gold beads. Scale bars 200 nm. [\(show better data from 26 march\)](#)

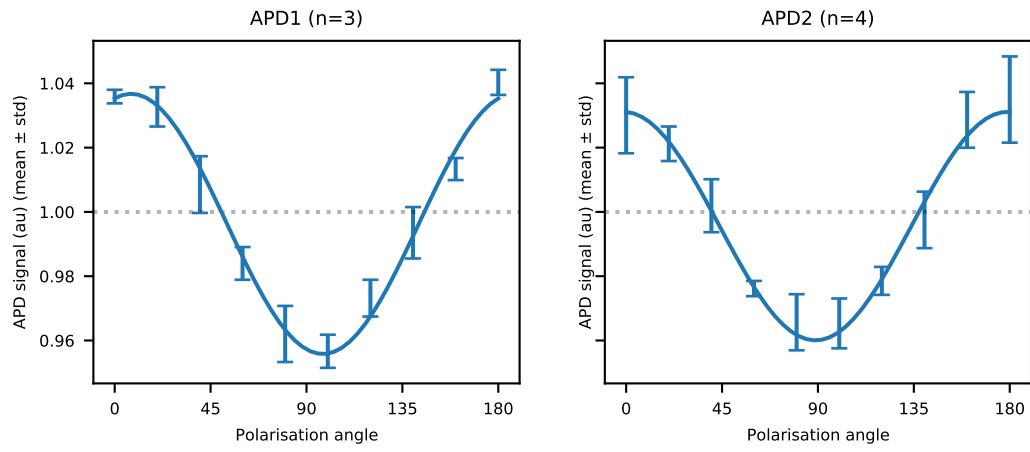


Figure 8: Dependence of the signal from APD1 on the angle of polarisation of incoming light.