Measuring laser intensity underwater in a sample chamber with an open-hardware solution

Freya Whiteford, Chas Nelson
School of Physics and Astronomy, University of Glasgow, UK

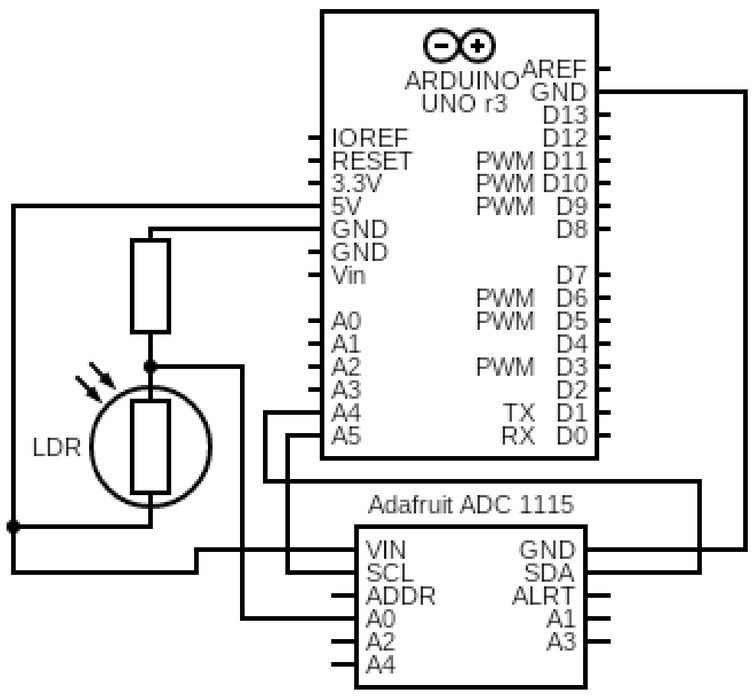
Abstract. Phototoxicity, photodamage and photobleaching (the 3Ps) are the bane of in vivo light sheet fluorescence microscopy. Not only do they affect our science but they are incredibly difficult to report and correct for (see [1]). Here we represent our attempts to create a simple solution for measuring the light intensity at the sample, inside the chamber and underwater.

By using off-the-shelf components and the readily available and easy to use Arduino systems, we have created a power meter that can be used to provide an idea of light intensity at the sample's position and under identical conditions. Use of such a tool could help unify reporting of laser parameters and light intensity in the field.

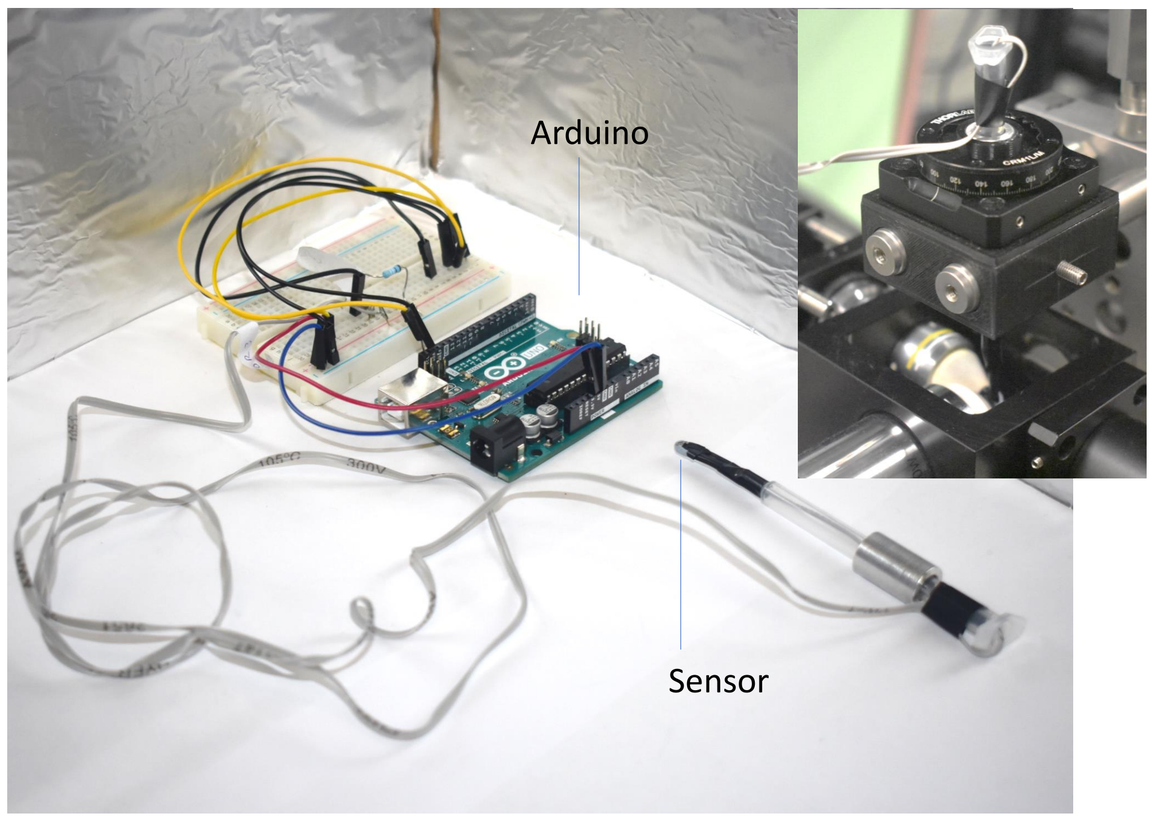
Here we present step-by-step instructions on how to build the device, provide details of the codes used and example results from our own light sheet microscope. This design was based on [2].

Construction Rationale.

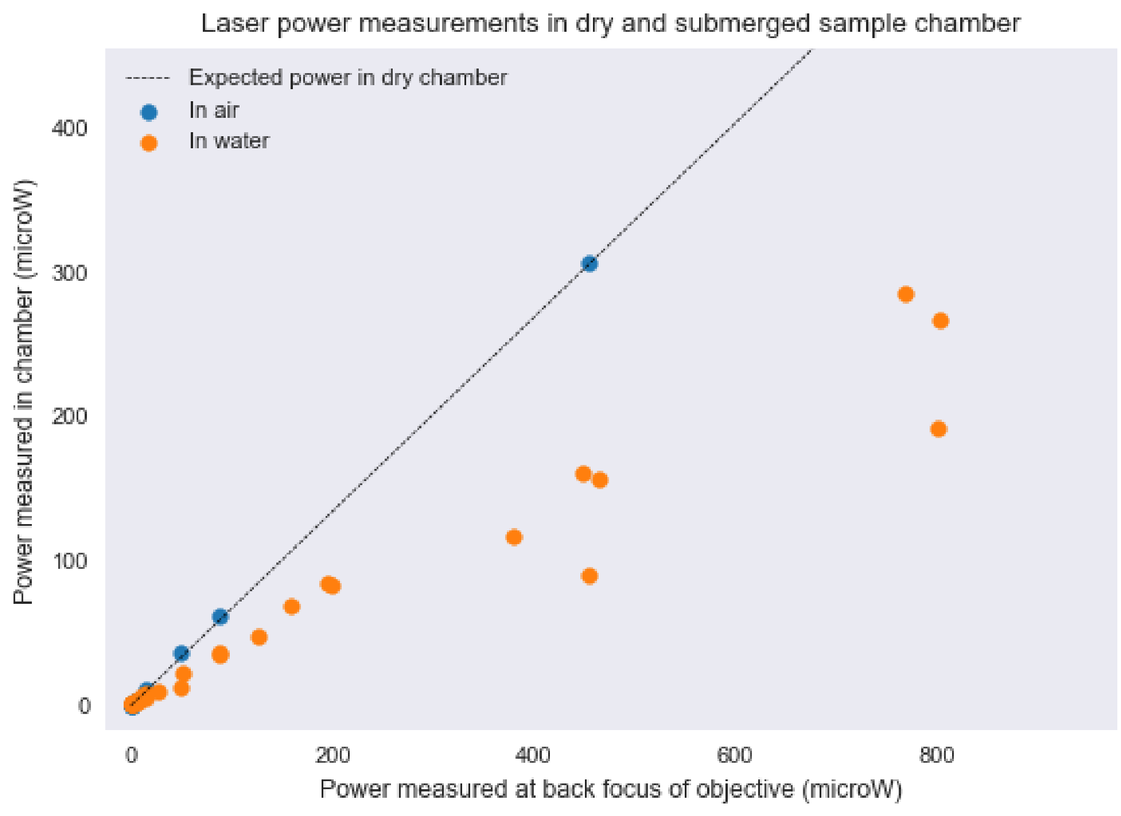
- Analogue-to-digital converter provides broader dynamic range for measurement compared to what can be detected with Arduino alone.
- The light-dependent resistor used is threaded through a syringe and the exposed wires waterproofed with tape allowing the resistor to measure intensity exactly at submerged sample position.
- To operate the device, the LDR must first be calibrated against incident laser beams of known intensity - a polynomial curve is fitted to correspond incident intensity with an estimate of power.



Circuit diagram of Arduino with light-dependent resistor and external analogue-to-digital converter.



Assembled components of the Arduino and sensor in-situ. The Arduino is mains-powered with its USB B port, but could equivalently run on 5V battery power.



Graph of comparison of dry-chamber and wet-chamber power measurements, demonstrating loss of intensity from submersion. Calibration performed at sample position provides the most reliable indication of incident intensity.

Advantages.

- System is easily adaptable to differing microscope setups, such as proprietary Zeiss Z.1 – just place at sample position as you would a normal syringe
- Ability to measure underwater intensity provides more consistent reporting of risk of 3Ps to biological samples when imaging

Limitations.

- Although the setup is modular – for example, the LDR can be swapped out for differing peak sensitivity wavelengths, it must be recalibrated for best results when doing so as no two LDRs are exactly the same.
- Incident laser power can be measured while underwater, though LDRs can struggle to measure low incident powers in this way and can vary in sensitivity depending on incident angle. A more robust sensor such as a photodiode may be more reliable while still maintaining component simplicity, and would likely not require the nonlinear polynomial fitting method of the light-dependent resistor.

[1] Laissue, P., Alghamdi, R., Tomancak, P. et al. Assessing phototoxicity in live fluorescence imaging. Nat Methods 14, 657–661 (2017) doi: 10.1038/nmeth.4344

[2] Dormann D.. IntensityCheck – The light measuring app for microscope performance checks and consistent fluorescence imaging. PLOS ONE 14(3): e0214659 (2019). doi: 10.1371/journal.pone.0214659

For further detail contact chas.nelson@glasgow.ac.uk

Resources for this project can be found at https://github.com/Glasgow-ICG/waterproof-intensity-meter or through this QR code:

University of Glasgow
IMAGING CONCEPTS GROUP
UKRI Engineering and Physical Sciences Research Council