

The Adams-Levin whole-animal optogenetics rig comprises five interconnected machines. The centerpiece is a Nikon AZ100 diascopic dissecting microscope with epifluorescence optics. The standard stage has been replaced with a Ludl MAC6000 computer controlled stage that provides automated X-Y movement. The fluorescence port at the top rear of the scope is used for the input of a fiber optic cable, using a custom built adapter, coming from a Spectra4 LED light source providing light of wavelengths 390 nm, 500 nm, 560 nm, and 630 nm. The diameter of the beam is controlled by an interchangeable aperture contained within the adapter unit, and the beam is transmitted through the 5X objective lens, thereby being reduced another 5X in diameter. This light is used for illuminating specific points within a sample. In addition, a second light source is used for illuminating large areas to simultaneously activate or de-activate all of the channels simultaneously. The illumination source for this spot light is an Intesilight with a mercury bulb, connected via a fluid field light guide to a custom built holder attached to the microscope stand. This holder offers infinite degrees of freedom, allowing this light to be aimed. Interchangeable filters are screwed into place in front of the light to set the wavelength. Brightfield illumination is provided by a Volpi LED light with two gooseneck light-guides; this light is also controlled by computer via a DAQ board connection. There is also a transmitted light port at the back rear of the scope. All components are controlled by NIS Elements©.

To use the device for imaging over long periods of time, a microfluidic sample-holding device was constructed (Zhu, et al., 2015) that allows fluid flow past the samples via tubes connected to a mechanically-isolated peristaltic pump.

To use the live animal optogenetics device, beam size is chosen and the correct aperture inserted into the LED-input adapter. If the background light is to be used, the correct filter is screwed in place. The sample should be anesthetized, if necessary to prevent movement, and placed within a transparent holder appropriate for that organism. The holder is then stabilized on the stage plate using modeling clay. The gooseneck light guides are then positioned to provide brightfield illumination to the sample, and the background light is aimed to provide the correct area and direction of illumination. Software is then used to focus, set exposures and illumination schedules (for repeated stimulation), overall duration, and the point or points within the sample, or samples, to be illuminated. The program then carries out the protocol.