NIGHTSEA
Bringing fluorescence to light

Application Note #1: Using routine fluorescence to sort *Drosophila* larvae

Over 90% of research microscopes destined for biological labs are now purchased with fluorescence attachments. Clearly, fluorescence has become *the* tool of choice for studying many animal models on upright and inverted research stands.

New technology from NIGHTSEA now extends fluorescence to standard routine stereo microscopes, where its specificity and sensitivity provide an ideal assist for life science applications using bigger, more 3-dimensional biological entities such as *Drosophila* larvae.

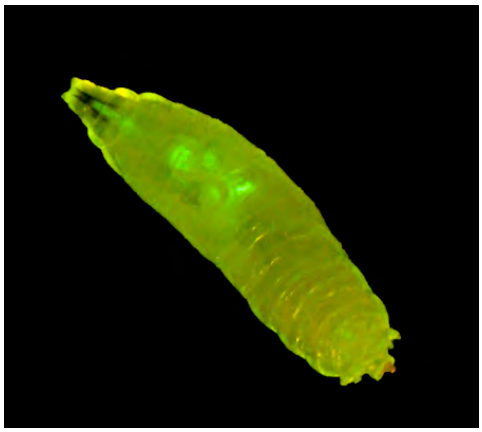

Figure 1. *Non-mutant Drosophila melanogaster expressing GFP (Image courtesy of C. Mazel)*

THE PROBLEM:

Dr. Laura Reed (Dept. of Biological Sciences, University of Alabama, Tuscaloosa) heads a research program to investigate whether mutations in specific genes in fruit flies, *Drosophila melanogaster*, affect triglyceride storage.

To gather sufficient material for analysis, Dr. Reed requires large numbers of larvae of each genotype. Her program involves testing 84 different genotypes and, for each genotype, 200 or more larvae. A special strain of fruit flies has been genetically engineered to express Green Fluorescent Protein (GFP) driven by an actin promoter (Figure 1). Only the flies *without* the mutations fluoresce. The clear difference between fluorescent and non-fluorescent larvae makes them easy to sort.

For best results, the larvae need to be collected, sorted, and frozen when at their largest, but before they pupate. However, they are at this stage for only about six hours. With 84 genotypes to test and 200+ larvae per genotype, sorting is a major challenge. While Dr. Reed has a large pool of undergraduates available for sorting, the greater challenge was that she only had access to borrowed time on another lab's research fluorescence stereo microscope.

THE PRACTICAL SOLUTION:

Dr. Reed visited the NIGHTSEA booth at the annual Drosophila Research Conference and tested our Stereo Microscope Fluorescence Adapter (SFA) system (Figure 2).

She immediately realized the potential of putting both her undergraduates and four of her existing lab-grade stereo microscopes to work. The SFA provided a practical, economical solution for her limited equipment.

Figure 2. *The NIGHTSEA SFA*

The SFA comes in three different excitation/emission combinations:

Excitation/ Filter application	***Excitation***	***Emission***
"Royal Blue" for GFP, eGFP, fluorescein	440-460nm	500nm
"Cyan" for YFP and similar fluorophores	490-515nm	550nm
"Green" for DsRed, dTomato	510-540nm	600nm

For Dr. Reed, "Royal Blue" provided excellent results (Figure 3, 4).

Figure 3. *(L) Katie Bray and (R) Dana Davis sort larvae using NIGHTSEA's SFA in Royal Blue. Dr. Reed now has shifts of two to four undergrads sorting in parallel.*

SFA ADVANTAGES

NIGHTSEA's Stereo Microscope Fluorescence Adapters offer a number of advantages. First, they require no modification to your existing microscope. They just click into place, making them easy to use and easy to exchange, either on one microscope or between different microscopes in the lab.

Secondly, SFAs are economical and expandable. Since Dr. Reed currently works only with GFP (blue excitation / green fluorescence), she only needed to purchase one version of SFA. However, as the needs of her lab grow, additional sets can readily be added.

Finally, as demonstrated by Nick Izor (Figure 4), SFA's bright illumination and excellent barrier filters allow many fluorescence experiments to be conducted under near-ambient lighting. In this case, the overhead lights were turned off and the blinds closed, but the room does not need to be in complete darkness.

As for Dr. Reed? Using NIGHTSEA's SFA, she routinely has shifts of two to four undergrads at a time, sorting *Drosophila* larvae in parallel. 84 genotypes? 200 larvae per experiment? Problem solved!

Figure 4. *Nick Izor demonstrates larval sorting under ambient lighting*