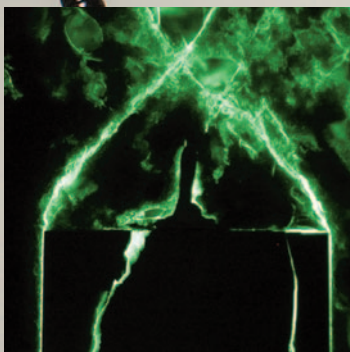
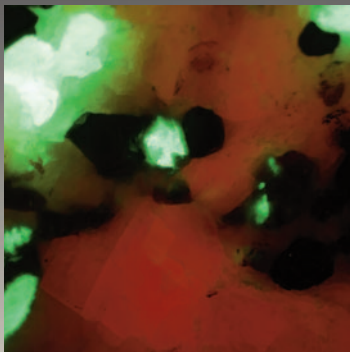
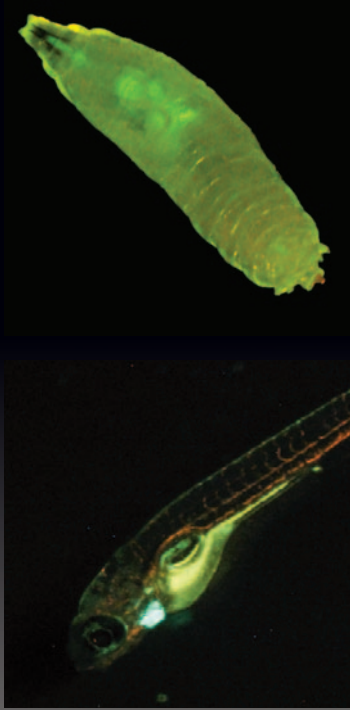


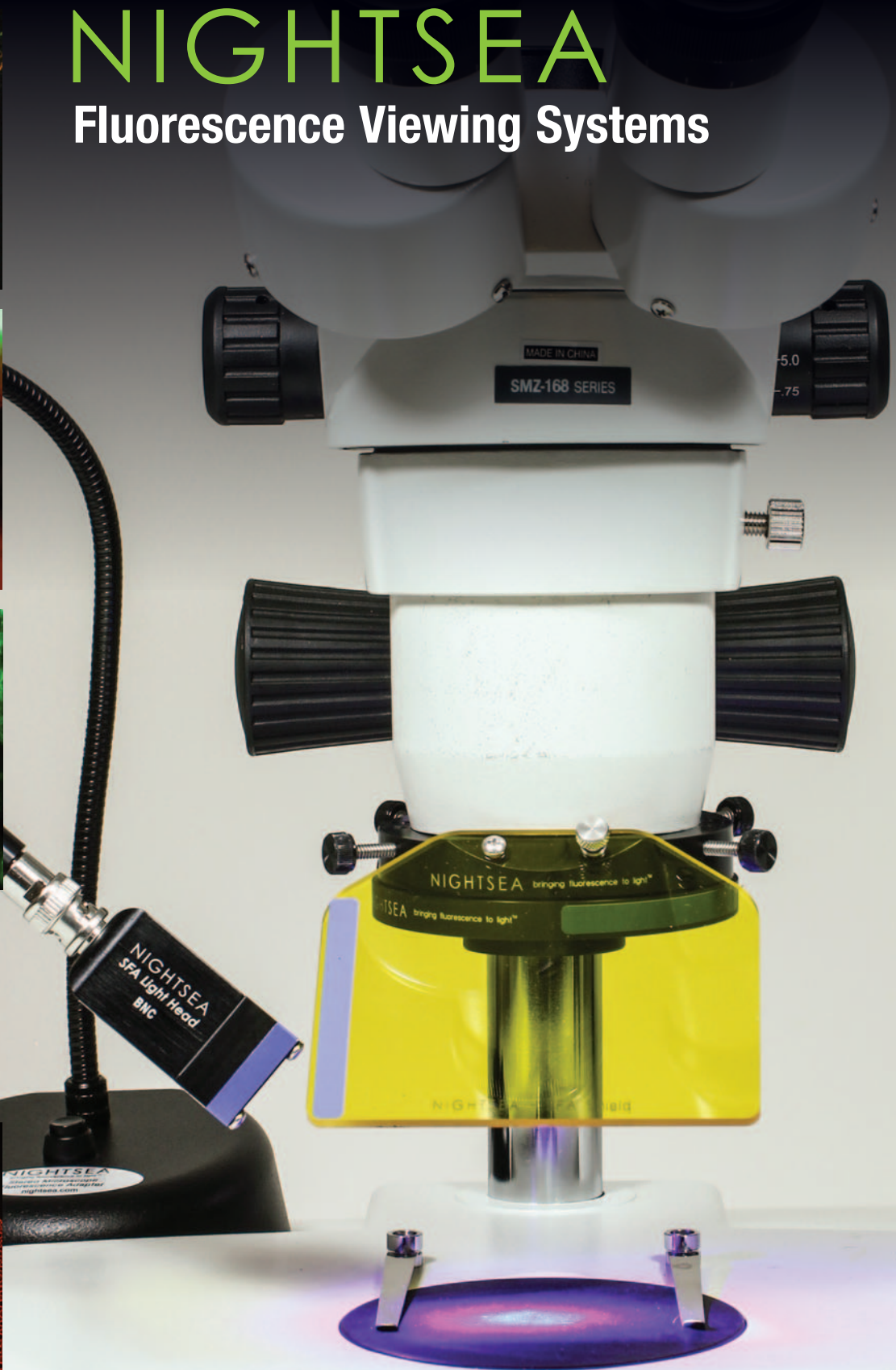
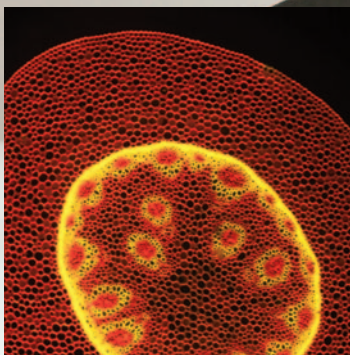
Practical and economical solutions for viewing
and photographing fluorescence

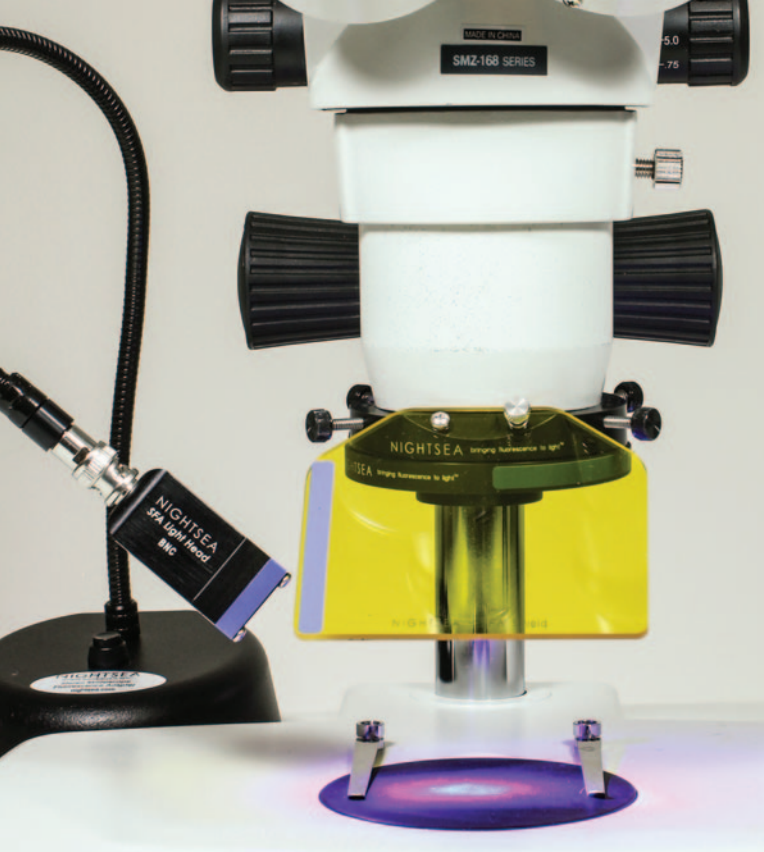
NIGHTSEA

Fluorescence Viewing Systems



**Electron
Microscopy
Sciences**





NIGHTSEA Stereo Microscope Fluorescence Adapter

Adapt your existing lab stereo microscopes for fluorescence

Overview

The NIGHTSEA Stereo Microscope Fluorescence Adapter adapts just about any stereo microscope (dissecting microscope) for fluorescence with no modification to the microscope itself. The modular design lets you easily switch between several different excitation/emission combinations to work with a variety of fluorescent proteins and other fluorophores. There are now **six** different excitation/emission combinations available, plus white light.

Applications

This simple system is excellent for:

- Quick screening of your fluorescent genotypes - *Drosophila*, zebrafish, *C. elegans*,...
- Genotype sorting
- Fluorescence-aided dissection, injection, or micromanipulation
- Pre-screening sample preps for confocal or other high-resolution imaging
- Freeing up your research-grade fluorescence microscopes for more demanding work
- New faculty start-up budgets
- Bringing fluorescence into the teaching laboratory
- Coating and failure analysis, circuit board work, defect location, food safety, paper analysis, and more

The Stereo Microscope Fluorescence Adapter system consists of:

- Flexible gooseneck lamp base with power supply
- Adapter for microscope
- Light head
- Barrier filter
- Filter shield

The light head, barrier filter, and filter shield are interchangeable so that you can easily switch between excitation/emission light+filter combinations.

The microscope mounting adapter fits up to 67mm to work with the majority of stereo microscopes. An oversize adapter and an adapter for the Leica EZ4 series are also available.



Fluorescence isn't just for research microscopes anymore...

- Now sort on your laboratory-level stereos
- Use to facilitate micromanipulation and dissection
- Expand from your research lab to your classroom

Modular...

- Installs in seconds - just clicks into place
- Interchangeable excitation/emission combinations
- Move from microscope to microscope
- No modification to your microscope needed

Economical — More Glow for the Dough...

- Stretch your lab budget
- Inexpensive enough for classroom use

Grows as your lab grows...

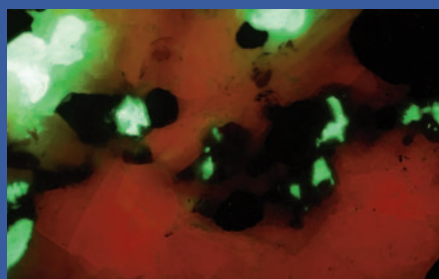
- Buy just what you need now (up to 6 different wavelength sets)
- Add more as your needs expand

Here are samples of what you can see:



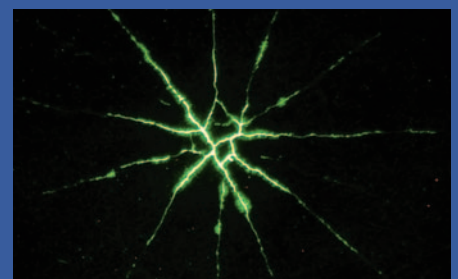
Fluorescent Proteins

GFP-tagged *Drosophila* larva



Geology

Calcite and willemite fluorescence



Industry

Electronic component failure analysis

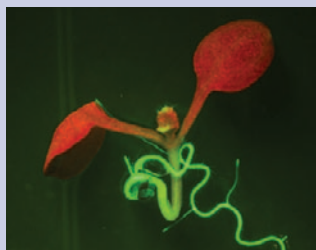
Once you are set up for one excitation/emission wavelength combination, additional combinations can be added by purchasing a kit that consists of a light head, barrier filter, and viewing shield. These three elements can be removed and replaced in seconds, and color coding ensures that you are using the right combination. The barrier filter clicks on to the ring adapter magnetically, so it is easy to remove it to switch back to white light viewing.

Wavelength Sets

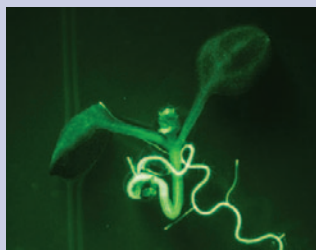
Designation	Excitation	Emission	Fluorophores
UV – Ultra Violet	360-380nm	415nm LP	DAPI, ...
VI – Violet	400-415nm	450nm LP	CFP, ...
RB – Royal Blue	440-460nm	500nm LP	GFP, eGFP, fluorescein...
RB-GO – Green Only	440-460nm	500-560nm BP	GFP, eGFP, fluorescein...
CY – Cyan	490-515nm	550nm LP	YFP, Venus, Lucifer Yellow...
GR – Green	510-540nm	600nm LP	DsRed, dTomato...

Green-Only Barrier Filter

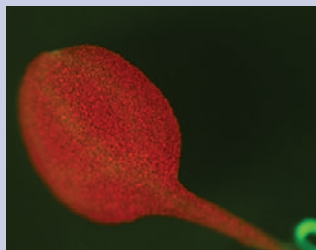
The Green-Only (GO) Barrier Filter isolates the green part of the spectrum and is for use with the Royal Blue excitation source. While our other barrier filters are longpass filters this filter is a bandpass, transmitting from approximately 500 to 560nm. The longpass filter has served well for most users who need to visualize green-fluorescent protein (GFP), and if you are exploring fluorescence in nature it is preferable. The primary motivation for adding the green-only filter to the line-up was for the benefit of researchers using GFP in plants such as *Arabidopsis thaliana*, a common research model. Plants contain chlorophyll, which has a distinctive red fluorescence that can sometimes mask the GFP emission, making it harder to see and photograph.



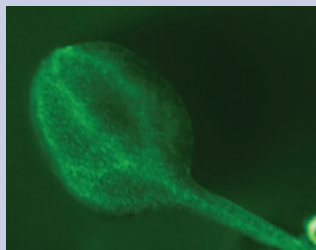
Arabidopsis fluorescence imaged with longpass filter



Arabidopsis fluorescence imaged with bandpass filter



Arabidopsis fluorescence imaged with longpass filter



Arabidopsis fluorescence imaged with bandpass filter

We tested this new barrier filter with *Arabidopsis* supplied by Dr. Chip Celenza (Department of Biology, Boston University). These plants express GFP in the roots and vasculature. The images above show examples of plants photographed with the longpass filter (left) and green-only filter (right). There is no chlorophyll in the roots so the GFP is evident there in both images, but the weaker expression in the leaves is much more apparent in the images on the right.

Lamp Base Light Control Options

The SFA lamp base is available in three versions: Standard, DIM, and PULSE. Standard lamp bases have a simple OFF/ON operation. DIM lamp bases feature an OFF/ON/DIM switch to change the intensity of the light. PULSE lamp bases incorporate a BNC connector that accepts a voltage signal to control the excitation source ON/OFF.

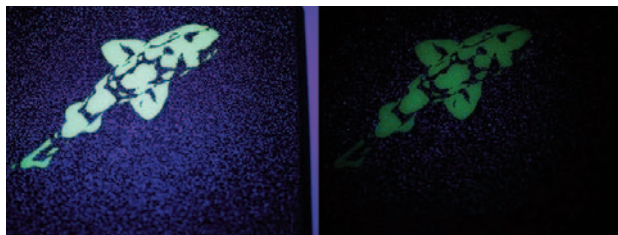
Only one control option is available per base. The DIM and PULSE options can be purchased as part of a new system or in a stand-alone lamp base purchased a la carte. The PULSE option can be retrofitted to an existing base.

NIGHTSEA DIM Option

The DIM option adds a combined switch and dimmer control. When you first turn the unit on it is at full power. As you rotate the switch the intensity decreases, reaching about 30% power at the minimum setting. You have finer control in the brighter portion of the adjustment range. An intensity indicator (1-10) makes it easy to record and repeat preferred settings.



Dimmer switch option on SFA



Target at max and min excitation

NIGHTSEA PULSE Option

The PULSE option adds a BNC connector on the rear of the base that accepts a user-supplied voltage input to turn the light on and off. Some possible applications are:

- External control (computer, function generator, etc.) for precise illumination timing for photoactivation, behavioral experiments
- PWM input for light dimming



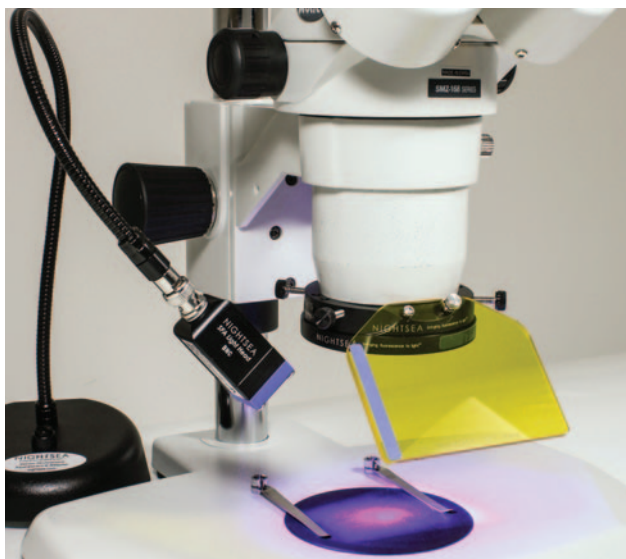
Voltage Input	2.8 - 6.0 VDC for ON, <0.6VDC OFF
Maximum Operation Frequency	10kHz
BNC cable not included	

See how it works... Learn how to do it...

We've added video content to our website to help you get to know our latest products even better!

Stop by and see what it's all about.

Ordering Information: Full Systems



Stereo Microscope Fluorescence Adapter, Full System

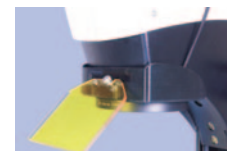
Full system with one illumination color consisting of:

- Lamp Base with Power Supply and International Plug Set
- Light Head — Ultraviolet, Violet, Royal Blue, Cyan, or Green
- Microscope Mounting Adapter
- Barrier Filter
- Viewing Shield
- Padded Travel Case

Cat. No.	Description	Qty.
Standard Lamp Base		
SFA-UV	Full System with Ultraviolet, Standard Lamp Base	each
SFA-VI	Full System with Violet, Standard Lamp Base	each
SFA-RB	Full System with Royal Blue, Standard Lamp Base	each
SFA-RB-GO	Full System with Royal Blue, Green-Only Barrier Filter, Standard Lamp Base	each
SFA-CY	Full System with Cyan, Standard Lamp Base	each
SFA-GR	Full System with Green, Standard Lamp Base	each
DIM Lamp Base		
SFA-UV-DIM	Full System with Ultraviolet, DIM Lamp Base	each
SFA-VI-DIM	Full System with Violet, DIM Lamp Base	each
SFA-RB-DIM	Full System with Royal Blue, DIM Lamp Base	each
SFA-RB-GO-DIM	Full System with Royal Blue, Green-Only Barrier Filter, DIM Lamp Base	each
SFA-CY-DIM	Full System with Cyan, DIM Lamp Base	each
SFA-GR-DIM	Full System with Green, DIM Lamp Base	each
PULSE Lamp Base		
SFA-UV-PULSE	Full System with Ultraviolet, PULSE Lamp Base	each
SFA-VI-PULSE	Full System with Violet, PULSE Lamp Base	each
SFA-RB-PULSE	Full System with Royal Blue, PULSE Lamp Base	each
SFA-RB-GO-PULSE	Full System with Royal Blue, Green-Only Barrier Filter, PULSE Lamp Base	each
SFA-CY-PULSE	Full System with Cyan, PULSE Lamp Base	each
SFA-GR-PULSE	Full System with Green, PULSE Lamp Base	each



Leica EZ4 Mount Adapter



Full System with Leica EZ4 Adapter

The Leica EZ4 Mounting Adapter enables you to use the NIGHTSEA Stereo Microscope Fluorescence System with the Leica EZ4 series of stereo microscopes, with or without integrated camera. Easily attach the barrier filter magnetically and hold the filter shield in place with a thumbscrew. The adapter is available for purchase separately to quickly move between the Leica EZ4 and other stereo microscopes.

Full system with one illumination color consisting of:

- Lamp Base with Power Supply and International Plug Set
- Light Head — Ultraviolet, Violet, Royal Blue, Cyan, or Green
- Leica EZ4 Mounting Adapter
- Barrier Filter
- Viewing Shield
- Padded Travel Case

Cat. No.	Description	Qty.
Standard Lamp Base		
SFAZ-UV	Full System with Ultraviolet, Standard Lamp Base	each
SFAZ-VI	Full System with Violet, Standard Lamp Base	each
SFAZ-RB	Full System with Royal Blue, Standard Lamp Base	each
SFAZ-RB-GO	Full System with Royal Blue, Green-Only Barrier Filter, Standard Lamp Base	each
SFAZ-CY	Full System with Cyan, Standard Lamp Base	each
SFAZ-GR	Full System with Green, Standard Lamp Base	each
DIM Lamp Base		
SFAZ-UV-DIM	Full System with Ultraviolet, DIM Lamp Base	each
SFAZ-VI-DIM	Full System with Violet, DIM Lamp Base	each
SFAZ-RB-DIM	Full System with Royal Blue, DIM Lamp Base	each
SFAZ-RB-GO-DIM	Full System with Royal Blue, Green-Only Barrier Filter, DIM Lamp Base	each
SFAZ-CY-DIM	Full System with Cyan, DIM Lamp Base	each
SFAZ-GR-DIM	Full System with Green, DIM Lamp Base	each
PULSE Lamp Base		
SFAZ-UV-PULSE	Full System with Ultraviolet, PULSE Lamp Base	each
SFAZ-VI-PULSE	Full System with Violet, PULSE Lamp Base	each
SFAZ-RB-PULSE	Full System with Royal Blue, PULSE Lamp Base	each
SFAZ-RB-GO-PULSE	Full System with Royal Blue, Green-Only Barrier Filter, PULSE Lamp Base	each
SFAZ-CY-PULSE	Full System with Cyan, PULSE Lamp Base	each
SFAZ-GR-PULSE	Full System with Green, PULSE Lamp Base	each
SFA-FOOT	ON/OFF Foot Switch option	each
Leica Adapter Only		
SFAZ-AD	Leica EZ4 Adapter only	each

Add-On Light and Filter Sets

Each add-on excitation/emission set consists of:

- Light Head
- Barrier Filter
- Viewing Shield
- Padded Storage Box



Ultraviolet



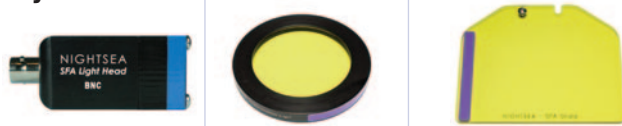
Cat. No.	Description	Qty.
SFA-LFS-UV	Add-On Light and Filter Set, Ultraviolet	each

Violet



Cat. No.	Description	Qty.
SFA-LFS-VI	Add-On Light and Filter Set, Violet	each

Royal Blue



Cat. No.	Description	Qty.
SFA-LFS-RB	Add-On Light and Filter Set, Royal Blue	each

Green-Only



Cat. No.	Description	Qty.
SFA-LFS-RB-GO	Add-On Light and Filter Set, Green-Only	each

Cyan



Cat. No.	Description	Qty.
SFA-LFS-CY	Add-On Light and Filter Set, Cyan	each

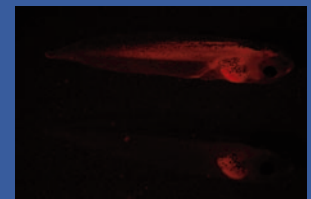
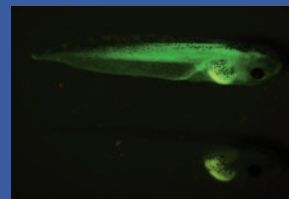
Green



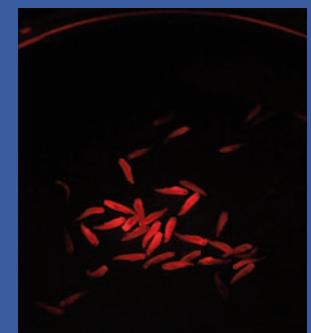
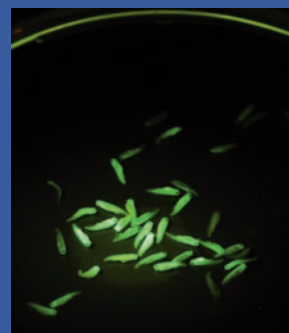
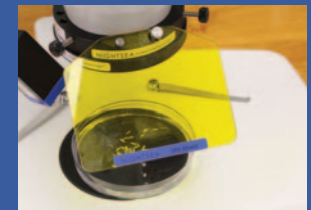
Cat. No.	Description	Qty.
SFA-LFS-GR	Add-On Light and Filter Set, Green	each

The NIGHTSEA Stereo Microscope Fluorescence Adapter in Action...

...at the National Xenopus Resource (NXR) at the Marine Biological Laboratory in Woods Hole, MA — special thanks to NXR Director and Bell Center Scientist Dr Marko Horb and his postdoctoral scientist Dr Matthew Salanga. The fluorescence adapter system worked great for visualizing all of the fluorescence, both injected and transgenic, in the specimens. The pictures below show both positive- and negative-expressing Stage 46 *X. laevis* under white light, Royal Blue excitation (green fluorescence), and Green excitation (red fluorescence).



In addition to seeing the fluorescence through the eyepieces, you could easily distinguish presence/absence and relative strength of expression just by looking through the filter shield.



Collection of Stage 37-38 *X. laevis*, messenger RNA injected ubiquitous GFP and membrane RFP viewed through filter shields for green and red fluorescence.

NIGHTSEA Stereo Microscope Fluorescence Adapter Accessories

Light Head Hangers and Cables

The Light Head Hanger system is an accessory for the NIGHTSEA Model SFA Stereo Microscope Fluorescence Adapter. It is a combination of power extension cables and a custom made holder that enables you to take the fluorescence excitation light heads off the gooseneck and mount them directly on the SFA adapter ring. In addition, it provides the capability to drive two light heads from one lamp base.

The extension cables plug into the gooseneck lamp base and can bring power to either one or two light heads.



Light head hangers configured for LWD (left) and SWD microscopes (right)

The Light Head Hanger mounts to the end of any of the thumbscrews on the SFA adapter ring. It can be configured for use with either long or short working distance microscopes.

- Long working distance (LWD) – 6.5 cm (2.56") and greater
- Short working distance (SWD) – 6.5-4.6 cm (2.5-1.8")

There are two main potential benefits with this accessory.

- 1) Increase excitation intensity without buying a second lamp base. Without this system, if you wanted to increase the fluorescence excitation intensity of the SFA, you would need to buy a second light head and lamp base. This would take up additional real estate on your laboratory bench. With the Light Head Hanger system you can power two light heads from one base and mount them both directly to the NIGHTSEA adapter ring.
- 2) Reduce the chance of accidentally bumping the light. Taking the light head off the gooseneck and mounting it directly to our microscope adapter ring reduces the chance of the light head being bumped so that it is not illuminating the subject on the microscope stage to best effect. This might be especially valuable if you are using the system for outreach demonstrations and multiple people will be working around the microscope.

Switch Box Kit

The Switch Box Kit enables you to mount two different wavelength excitation light heads on your microscope and switch between them easily. You will still need to manually change the barrier filter, but it eliminates the need to swap out light heads on the gooseneck.

The Switch Box Kit is available only for SFA systems that use the BNC light head connector, and not the older V1 connector.

Cat No.	Description	Qty.
SFA-SWK	Switch Box Kit, includes BNC switch box, 3 BNC cables, 2 LH Hangers	kit



Two SFA Light Heads mounted to adapter ring with Light Head Hangers

“We had some issue detecting red fluorescent proteins with some of our weaker transgenic zebrafish lines, but by shining two LED lights onto the same embryo, most of our weakest GFP and mCherry transgenic lines can now be detected. One light head was fine for reasonably bright specimens, and with two lights, the fluorescence is nearly the same as traditional stereomicroscopes priced \$15-20K.”



Single light head and cable



Dual light head cable serial adapter

Cat No.	Description	Qty.
SFA-LHC	Single Light Head Cable	each
SFA-DLHC	Dual Light Head Cable	each
SFA-LHH	Light Head Hanger	each
SFA-HK	Hanger Kit for Single Light Head, includes Single Light Head Cable and one Light Head Hanger	each
SFA-DHK	Hanger Kit for Dual Light Heads, includes Dual Light Head Cable and two Light Head Hangers	each

Note: If you are ordering a Dual kit to power two light heads at once for increased intensity, you will need to have two light heads of the same color. Above prices do not include the cost of an additional light head.

The default configuration is for systems with the BNC connector on the lamp base and the light head. Cables are available for the pre-2015 V1 connector on request.



White Light Head

This is an extremely convenient option for general illumination and as a focusing aid for fluorescence imaging. Now if you are using the NIGHTSEA system for fluorescence you do not need a separate white light source. Just exchange the fluorescence excitation light head module for the white-light module in a matter of seconds.



Cat No.	Description	Qty.
SFA-LH-WH	Modular White Light Head	each

Infrared Light Head

Researchers sometimes need an infrared light source for non-intrusive observation of behavior. And sometimes the subjects are small and you need to make the observations with a stereo microscope. The SFA-LH-IR850 is an 850nm light source that is compatible with the NIGHTSEA Model SFA Stereo Microscope Fluorescence Adapter. The light head plugs directly into the lamp base supplied with the SFA system. You can acquire the light head as a supplement to the SFA system that you already own for fluorescence observation, or you can acquire it in combination with a lamp base as a stand-alone device.



850nm IR Light Head

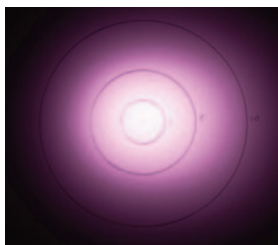
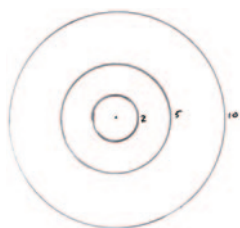


850nm IR Light Head on SFA Lamp Base

The light head incorporates a high power 850nm LED and a medium beamwidth diffusing lens to create a smooth illumination area. The output is centered at 850nm with a FWHM (full width at half maximum) of approximately 50nm. There is virtually no emission at wavelengths shorter than 750nm.

We recommend that you use the DIM base with the LH-IR850 in order to have finer control of illumination intensity.

A test target with circles of 2, 5, and 10cm diameter.



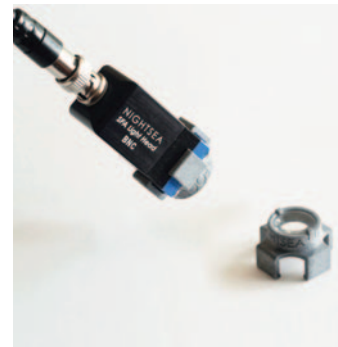
The photo on the left above was taken with a conventional camera under normal room light, and with the IR light head illuminated at full power, directed at the target center from a distance of approximately 10cm (4 in.). The illumination spot is not visible either to this camera or to the naked eye. The photo on the right was made under the same lighting conditions, but with a camera that had been modified to image only infrared light.

Please note that infrared light is not visible to the human eye and to many cameras. You will need an appropriate camera to record the observations.

Cat No.	Description	Qty.
SFA-LH-IR850	IR Light Head	each
SYS-IR850	IR Light Head plus NIGHTSEA DIM Base	each

Focus Lens for SFA Light Head

The focus lens is an add-on for the SFA light heads to produce a higher illumination intensity! This is a simple adapter that slips over the front of your existing NIGHTSEA SFA light head and grips with a friction fit. It is available with two options for the focal distance: 30mm or 50mm.



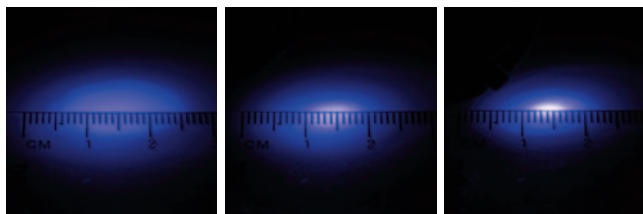
No lens – illumination spot approximately 15mm diameter with the light head positioned 50mm from the surface.

50mm lens – central spot approximately 5mm diameter, intensity increase ~2x.

30mm lens – central spot approximately 2mm diameter, intensity increase ~3.5x.



The images below show the focus lens add-on and the effect. The three illumination spot images were made in manual exposure mode and are indicative of the relative intensity.



No Focus Lens

50mm Focus Lens

30mm Focus Lens

Considerations for lens choice:

To gain the benefit of the 30mm lens it is necessary to position the light head very close to the subject. This may be challenging in the tight space beneath a microscope objective. It might be more suited for photoactivation, where a high light intensity dose in a small area is often needed.

The 50mm lens provides a trade-off – brighter fluorescence, easier to work with.

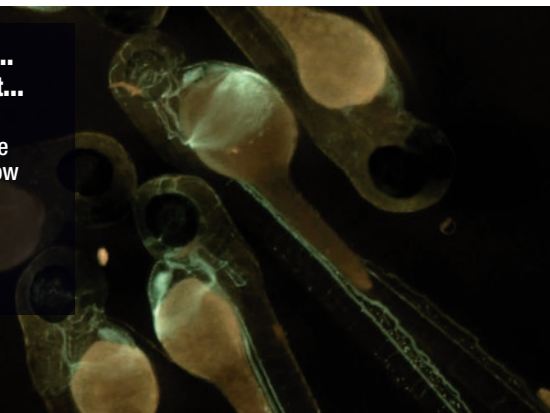
Note – If the light head has a snoot installed that will have to be removed to use the focus add-on.

Cat No.	Description	Qty.
SFA-FL-30	Focus Lens for SFA Light Head with 30mm Lens	each
SFA-FL-50	Focus Lens for SFA Light Head with 50mm Lens	each

See how it works... Learn how to do it...

We've added video content to our website to help you get to know our latest products even better!

Stop by and see what it's all about.



Battery and Charger



Compact battery pack that can run the Stereo Microscope Fluorescence Adapter (SFA) all day long. The battery enables truly portable operation so that you can set up demos anywhere or explore fluorescence in the field, without having to worry about plugging into the power grid.

Just plug the battery into the SFA base instead of the usual power connector. The battery has been tested at over 8 hours of continuous operation, and it will last even longer if you turn the system off when you don't need it. Plug it into the included charger overnight to recharge.

Specifications

Battery Type	Nickel Metal Hydride (NiMH)
Capacity	12V, 3.8 Ah
Dimensions	11.4 x 8.9 x 5.6 cm (4.5 x 3.5 x 2.19 in)
Weight	0.7 kg (1 lb 9 oz)
Operation Duration	8 hours continuous
Charge Time	Overnight
Charger	50/60 Hz, 110/220V (US type plug)

Cat No.	Description	Qty.
SFA-BATT	Battery and Charger	each

- SFA**
- + Eclipse MicroTent**
- + Tru-Block Eye Shields**
- + Battery**

= Fluorescence Everywhere

Combine the battery with the Eclipse MicroTent and Tru-Block Eye Shields and you not only don't need a place to plug in, you don't even need to be in the dark! We have used this combination to do fluorescence microscopy at a beach in the middle of the day, in the desert at high noon, and more. Fluorescence can be found everywhere, and now you have the tools to go there.

Eclipse MicroTent™

The patented (US Pat. No. 10,175,467) Eclipse MicroTent™ is a unique product for fluorescence microscopy that provides local darkness around conventional laboratory stereo microscopes. Fluorescence microscopes are kept in dark rooms for good reason - fluorescence can be weak and in many cases it can be difficult to see well if there is any ambient light. Microscopes may be on lab benches in shared spaces, near windows, or in other difficult-to-darken locations such as in the field. Turning off overhead lights can help but inconveniences others.

The Eclipse MicroTent™ creates local darkness around a microscope while still providing easy access to the sample stage and the focus and zoom controls. It is designed for stereo microscopes but could potentially be used with many varieties of compound microscopes.

Features

- Opening for the microscope oculars with elastic sleeve to minimize light entry
- Large front flap provides easy access to the sample stage and can fasten open
- Arm slots on sides to provide access to focus and zoom controls
- User-customizable feedthrough patches to provide additional penetrations for camera port, power cords, CO₂ lines, or other features as you need
- Tru-Block™ Eye Shields included with every Eclipse MicroTent™
- Folds flat for storage

Dimensions: 46 x 30 x 50 cm (18 x 12 x 20 in.)

Cat No.	Description	Qty.
SFA-TENT	Eclipse MicroTent™	each



Eye Shields

Light entering your eyes from the side can interfere with what you want to see in microscopy in general, and fluorescence microscopy in particular. Eye cups are available, but the standard ones don't extend far from the microscope and don't do a good job as ambient light increases. Our soft, molded rubber high-sided microscope eye shields are the answer. The tall wings extend up far enough to truly shield your eyes from any level of ambient light and eliminate distractions so that you can see your subject better. Two pairs (one Standard, one Compact) are included with every Eclipse MicroTent™, and you can also purchase them separately.

Tru-Block Eye Shields are available in two sizes:

Standard: fits 36 - 45mm (1.45 - 1.75")

Compact: fits 28 - 37mm (1.10 - 1.46")



Eye Shields, up for use



Eye Shields, folded down

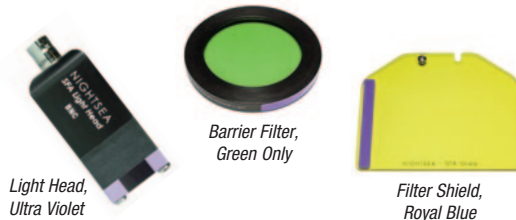


Using the microscope with the eye shields

Cat No.	Description	Qty.
SFA-EYE-S	Tru-Block Eye Shields - Standard	set
SFA-EYE-C	Tru-Block Eye Shields - Compact	set

SFA a la carte

Need an extra, not a set? Order from here:



Light Head, Ultra Violet

Barrier Filter, Green Only

Filter Shield, Royal Blue

Cat No.	Description	Qty.
SFA Light Heads:		
SFA-LH-UV	Light Head, Ultra Violet	each
SFA-LH-VI	Light Head, Violet	each
SFA-LH-RB	Light Head, Royal Blue	each
SFA-LH-CY	Light Head, Cyan	each
SFA-LH-GR	Light Head, Green	each
SFA Barrier Filters:		
SFA-BF-UV	Barrier Filter, Ultra Violet	each
SFA-BF-VI	Barrier Filter, Violet	each
SFA-BF-RB	Barrier Filter, Royal Blue	each
SFA-BF-RB-GO	Barrier Filter, Green Only	each
SFA-BF-CY	Barrier Filter, Cyan	each
SFA-BF-GR	Barrier Filter, Green	each
SFA Filter Shields:		
SFA-SH-UV	Filter Shield, Ultra Violet	each
SFA-SH-VI	Filter Shield, Violet	each
SFA-SH-RB	Filter Shield, Royal Blue	each
SFA-SH-RB-GO	Filter Shield, Green Only	each
SFA-SH-CY	Filter Shield, Cyan	each
SFA-SH-GR	Filter Shield, Green	each
SFA Adapters:		
SFA-AD	Standard Adapter	each
SFAZ-AD	Leica EZ4 Adapter	each
SFA-XL-AD	Oversize Adapter	each
SFA Bases:		
SFA-BASE	Standard Base	each
SFA-BASE-DIM	DIM Base	each
SFA-BASE-PULSE	PULSE Base	each

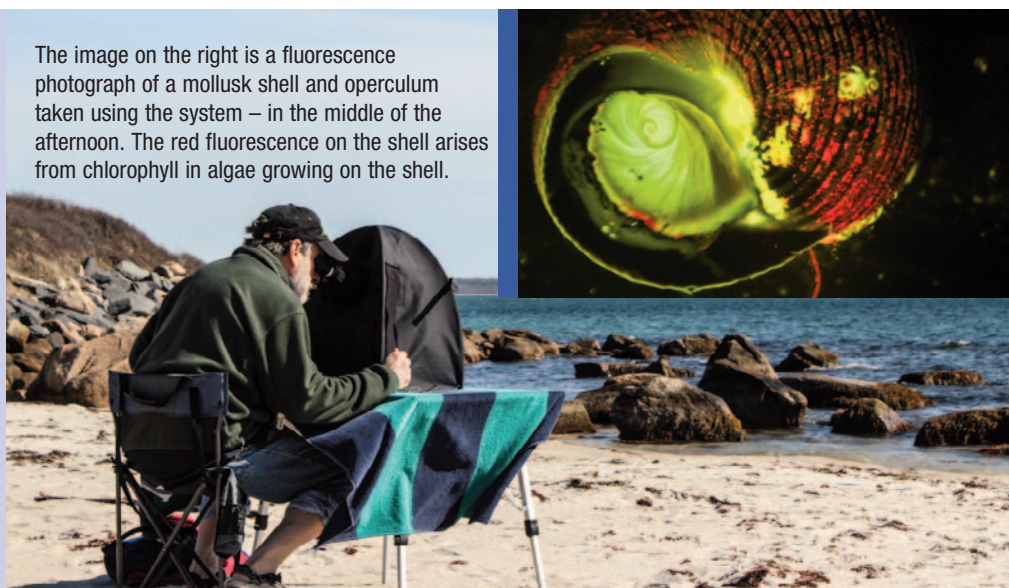
PULSE Retrofit Option

The PULSE option for the base (see page 3) can be retrofitted to existing Model SFA Stereo Microscope Fluorescence Adapters.

Cat No.	Description	Qty.
SFA-BASE-PULSE-R	PULSE Option — Retrofit	each

The combination of Tru-Block Eye Shields and the Eclipse MicroTent™ give you the freedom to do *Fluorescence Everywhere™*. The photograph on the left below shows NIGHTSEA founder Charles Mazel using the Stereo Microscope Fluorescence Adapter, powered by a battery, in combination with the tent and the Tru-Block Eye Shields to do fluorescence microscopy on Nobska Beach in Woods Hole, Massachusetts.

The image on the right is a fluorescence photograph of a mollusk shell and operculum taken using the system – in the middle of the afternoon. The red fluorescence on the shell arises from chlorophyll in algae growing on the shell.



NIGHTSEA Model SFA Fluorescence Adapter for Keyence VHX Series Digital Microscopes

Overview

The NIGHTSEA Model SFA Fluorescence Adapter system adds a versatile fluorescence imaging capability to the Keyence VHX series of digital microscopes. The system is being used successfully by a growing number of Keyence owners for a variety of applications. Visit our website for a full gallery of images made with the NIGHTSEA adapter and the Keyence microscope.

Fluorescence solutions for most Keyence lenses

Our adaptations to the Keyence system are concerned with the lens, not the microscope model (VHX-5000, -6000, -7000 etc.). We offer variants of our fluorescence adapter system that work with the most commonly used Keyence lenses – the VH-Z00, -Z20, -Z50, -Z100, -Z500, -ZST, and with two of the lenses incorporated in the VHX-7100 Fully Integrated (FI) head (VHX-E20 and -E100). Our system is not recommended for use with the higher magnification lenses in the FI head.

The key elements of any fluorescence system are:

- A light source that produces sufficient energy in the appropriate wavelength range to excite fluorescence in the sample of interest
- A barrier filter in the viewing path that blocks reflected excitation light while transmitting the fluorescence emitted by the sample

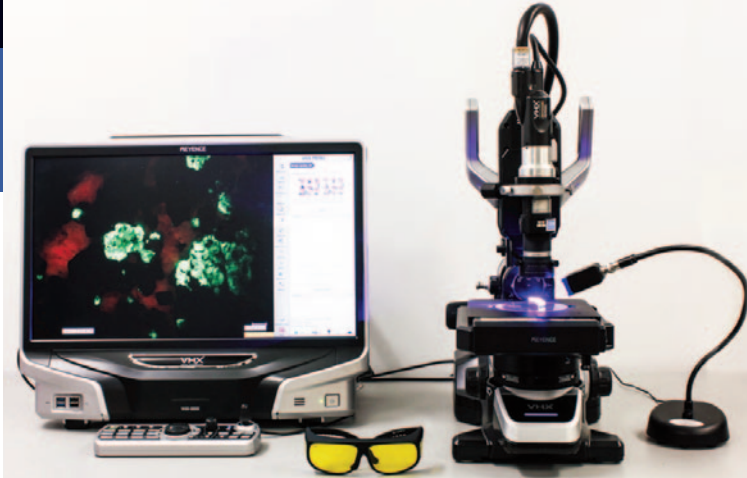
NIGHTSEA implements these for the Keyence system with:

- high intensity LED light sources available in five excitation wavelength ranges (see list on next page)
- emission barrier filters that can be added to the Keyence lenses easily and non-invasively

The components of a NIGHTSEA system that adds a single excitation/emission wavelength combination on a Keyence microscope are:

- Flexible gooseneck lamp base with LED drive circuit and intensity control;
- Universal power supply (120/240VAC, 50/60Hz) with international plug set
- Excitation light source
- Emission barrier filter
- Barrier filter glasses for fluorescence viewing and eye safety
- Padded carrying case

Additional wavelength sets are implemented by adding a matched set (light plus filter set) consisting of a light source, barrier filter, and filter glasses.



Fluorescent mineral sample imaged with the Keyence + NIGHTSEA systems

Comprehensive Solution Kit

We have configured Keyence fluorescence adapter kits that include the three most useful excitation wavelengths for varied industry applications, and that are suited to various combinations of the supported Keyence lenses. The kit price is significantly less than purchasing the items separately.

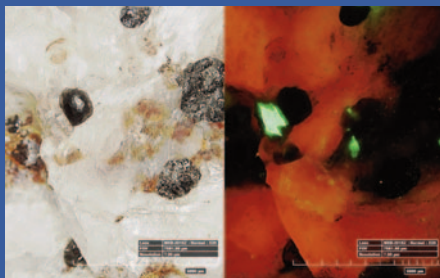


Custom case for 3-color system for Keyence

Each Industry Kit includes the following items, or a subset that is suited to the particular Keyence lenses you want to outfit:

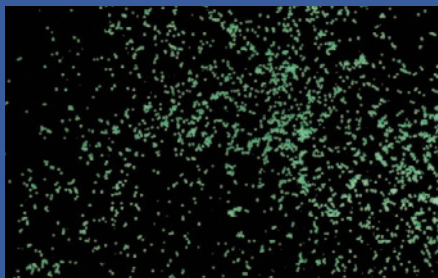
- Three excitation light heads – Ultraviolet (UV), Violet (VI), and Royal Blue (RB) This recommendation is based on our experience with varied applications. Read the explanation below.
- Three barrier filters, of one or more types depending on the lens(es) to be supported
- Matching barrier filter glasses for each excitation wavelength
- Accessory hardware for mounting the NIGHTSEA light head over the lens' fiber optic input port (only with VH-Z50, -Z100, -500, and -ZST)
- Gooseneck lamp base with dimming control
- Universal power supply – 120/240VAC, 50/60Hz, with international plug set
- Packing/carrying case with custom-cut foam

Here are samples of what you can see:



Naturally fluorescent mineral

This mineral sample, collected at the Sterling Hill Mine in Ogdensburg, New Jersey, contains willemite (green fluorescence), calcite (red fluorescence) and franklinite (black-no fluorescence).



Fluorescent-dosed plastic particles

38 – 45 microns. Image made with ultraviolet excitation. Image courtesy of an explosives detection company.



Epoxy on motor shaft,

VH-ZST lens, Ultraviolet excitation, 20x

Lens-specific solutions

The Keyence lens you are using (not the microscope model) determines how the NIGHTSEA light source and barrier filter will be added to the system. The light source will either be placed to the side of the microscope stage or mounted over the Keyence light source input port. The barrier filter will either be slipped over the bottom of the lens or inserted in the Analyzer slot. The options are summarized below.

Lens	Excitation Source		Barrier Filter	
	Mounted	Side	Inserted	Below
FI Head – E20 or E100		X	X	
VH-Z500	X		X	
VH-Z50, -Z100	X	X	X	
VH-ZST – high mag	X		X	
VH-ZST – low mag		X	X	
VH-Z00, -Z20		X		X

VH-Z00, -Z20

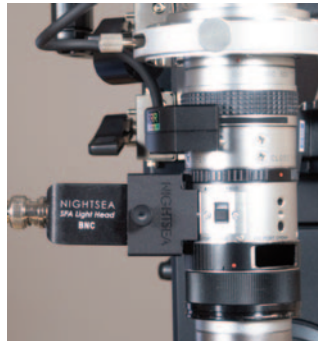
Light source – placed to the side of the microscope stage

Barrier filter – slips over the bottom of the lens and attaches with thumbscrews. **See at right.**

VH-Z50, -Z100

Light source – placed to the side of the microscope stage or mounted over the Keyence light source input port

Barrier filter – inserts in the Analyzer slot at the top of the lens



VH-Z100 lens light input port with NIGHTSEA adapter and light head.



LHM-Z100
Light Head Mount

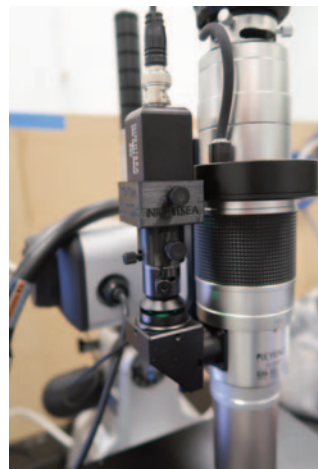


Z100, Z500, and ZST lenses – the barrier filter inserts in the Analyzer slot at the top of the lens.

VH-Z500

Light source – mounts over the Keyence light source input port

Barrier filter – inserts in the Analyzer slot at the top of the lens



VH-Z500 fiber optic port with NIGHTSEA adapter and light head



Z00 and Z20 lenses – the barrier filter slips over the bottom of the lens.

VH-ZST

• 20-200 lens

Light source – placed to the side of the microscope stage

Barrier filter – inserts in the Analyzer slot at the top of the lens

• 200-2000 lens

Light source – mounts over the Keyence fiber optic input port (except for UV) providing enhanced performance at high magnification

Barrier filter – inserts in the Analyzer slot at the top of the lens

NOTE – The Ultraviolet (UV) light source does NOT work well when mounted over the Keyence fiber input at either high or low magnification. Consider our Violet (V) light source as an alternative for many applications.



Keyence VH-ZST lens, fiber optic port with NIGHTSEA adapter and light head

VHX-7100 Fully Integrated (FI) Head

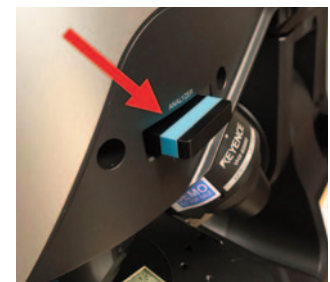
• VHX-E20 and -E100

Light source – placed to the side of the microscope stage

Barrier filter – inserts in the Analyzer slot at the right side of the FI head

• VHX-E500 and -E2500

Use with these lenses is likely not practical due to short working distance



FI head – the barrier filter inserts in the Analyzer slot at the side of the assembly

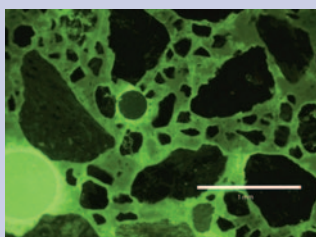
Concrete Thin Section Fluorescence

Fluorescence is a valuable tool in the concrete petrographic world, especially for the examination of thin sections (on the order of 20µm). There are a variety of techniques for introducing the fluorescence to the sample, most commonly by impregnating the sample under vacuum with an epoxy containing a fluorescent dye, or by replacing the water in the sample with a fluorescently marked ethanol.

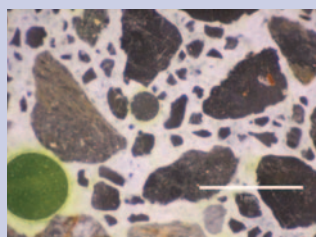
Fluorescence can make features of interest stand out in high contrast, or can be compared to a reference to make quantitative measurements. Among the characteristics that can be studied are: *Pore size and location, Water to cement ratio, Microstructure, Fractures and cracks*

Once the fluorescence has been introduced to the sample it is generally examined under a microscope. The NIGHTSEA Model SFA fluorescence adapter system is a simple and economical way to add a versatile fluorescence viewing capability to existing microscopes that operate at a wide range of scales. The dyes used in the concrete examination process are very strongly fluorescent and are excited well by ultraviolet (UV) or blue light. (Note – while the provider of the dye recommends use of UV, our experience is that our Royal Blue light source plus filter combination provides superior results for this and many other fluorescent indicators used in this and related applications.)

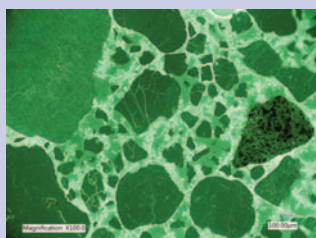
The images of concrete thin section fluorescence below were made with a stereo microscope (top row), and a Keyence VHX series digital microscope with the VH-Z100 lens (rows two and three). NIGHTSEA's Royal Blue excitation was used for all images.



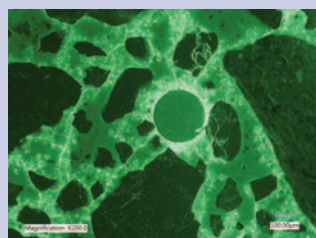
Concrete thin section under blue light excitation



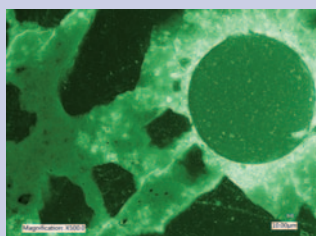
Concrete thin section, white light excitation



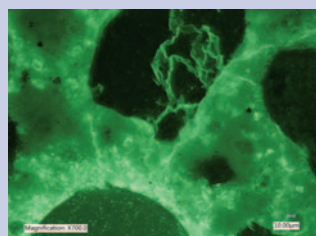
100x



200x



500x



700x

We are grateful to the Department of Mineralogy and Geochemistry, Institute of Geoscience and Geography, University Halle-Wittenberg, Germany for providing the sample for testing.

Why we recommend a set of three excitation wavelengths for industry needs

We have found that the combination of the Ultraviolet, Violet, and Royal Blue excitation/emission sets addresses a wide variety of industrial imaging challenges. The most common misconception we encounter is that fluorescence is uniquely associated with ultraviolet (UV) light and many customers immediately ask for that wavelength. However, we have found in several cases – even some in which UV was specifically recommended by a dye manufacturer – that either Violet or Royal Blue provided superior performance. For example:

A prospective customer wanted to image concrete thin sections impregnated with fluorescent dye. The dye supplier recommended UV, and it is true that UV will make the dye fluoresce nicely. We found that our Royal Blue excitation produced significantly brighter fluorescence.

A prospective customer sent us a sample of material that was known to fluoresce under UV. The goal was to see the distribution of non-fluorescent particles in a fluorescent matrix. While both UV and Royal Blue excited fluorescence in the sample, the best imaging contrast was achieved with Violet excitation.

We achieve enhanced performance with the ZST lens by mounting the light head on the lens's fiber optic port, but that path does not transmit UV well. In many cases, for example to inspect conformal coating, Violet is an excellent alternative to UV.

Acquiring the three-color system will provide the highest chance of success and will equip you with a versatile toolkit to address new fluorescence imaging challenges as they arise. Contact us if you would like to discuss your application further.

Wavelength Sets

Color	Designation	Excitation	Emission
Ultraviolet	UV	360 - 380nm	415nm longpass
Violet	VI	400 - 415nm	450nm longpass
Royal Blue	RB	440 - 460nm	500nm longpass
Cyan	CY	490 - 515nm	550nm longpass
Green	GR	510 - 540nm	600nm longpass

Performance Expectations

The microscopes in the Keyence VHX series were not designed for fluorescence. That said, the NIGHTSEA adapter system adds a fluorescence capability that is proving to be valuable for a growing number of users. Several companies have purchased multiple adapters so that they could replicate the performance across multiple microscopes. In at least one case the customer only purchased their Keyence system because of the added NIGHTSEA fluorescence capability that enabled a critical analysis function for them.

There is a direct correlation between the fluorescence intensity of the sample and the imaging magnification that can be achieved. With some of our solutions – for the -Z00, -Z20, and the FI head – the excitation light from our freestanding source is directed in from the side. It is not focused onto the target area by the Keyence optics, with the result that the greater the magnification (the smaller the area you are looking at), the less of the light emitted by the fluorescence there is to collect. Fortunately many industrial applications make use of highly fluorescent dyes, and imaging at 500 – 800x has been achieved. With other lenses – the -Z50, -Z100, -Z500, and -ZST – the excitation light source can be mounted over the Keyence light source fiber optic input port, resulting in a brighter spot on the subject. We have a growing body of experience with a number of fluorescence applications with the Keyence VHX series digital microscopes and associated lenses, as shown in the image gallery. This includes a biological application, looking at mosquito midguts immunostained with a fluorescent antibody.

Ordering Information

Cat. No.	Description	Qty.
Best Value Full System Kit for Z00/Z20 Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-KZB-IND	Best Value Full System	kit
Single Wavelength Full Systems for Z00/Z20 Lens		
SFA-KZB-UV	Z00/Z20 Single Wavelength Full System, UV	kit
SFA-KZB-VI	Z00/Z20 Single Wavelength Full System, Violet	kit
SFA-KZB-RB	Z00/Z20 Single Wavelength Full System, Royal Blue	kit
SFA-KZB-CY	Z00/Z20 Single Wavelength Full System, Cyan	kit
SFA-KZB-GR	Z00/Z20 Single Wavelength Full System, Green	kit
Light + Filter Sets for Z00/Z20 Lens		
SFA-KZB-LFS-UV	Z00/Z20 Light + Filter Set, UV Excitation	each
SFA-KZB-LFS-VI	Z00/Z20 Light + Filter Set, Violet Excitation	each
SFA-KZB-LFS-RB	Z00/Z20 Light + Filter Set, Royal Blue Excitation	each
SFA-KZB-LFS-CY	Z00/Z20 Light + Filter Set, Cyan Excitation	each
SFA-KZB-LFS-GR	Z00/Z20 Light + Filter Set, Green Excitation	each
Barrier Filters for Z00/Z20 Lens		
SFA-KZB-BF-UV	Z00/Z20 Barrier Filter, UV	each
SFA-KZB-BF-VI	Z00/Z20 Barrier Filter, Violet	each
SFA-KZB-BF-RB	Z00/Z20 Barrier Filter, Royal Blue	each
SFA-KZB-BF-CY	Z00/Z20 Barrier Filter, Cyan	each
SFA-KZB-BF-GR	Z00/Z20 Barrier Filter, Green	each

Best Value Full System Kit for Z50/Z100 Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-KZ100-IND	Best Value Full System	kit
Single Wavelength Full Systems for Z50/Z100 Lens		
SFA-KZ100-UV	Z50/Z100 Single Wavelength Full System, UV	kit
SFA-KZ100-VI	Z50/Z100 Single Wavelength Full System, Violet	kit
SFA-KZ100-RB	Z50/Z100 Single Wavelength Full System, Royal Blue	kit
SFA-KZ100-CY	Z50/Z100 Single Wavelength Full System, Cyan	kit
SFA-KZ100-GR	Z50/Z100 Single Wavelength Full System, Green	kit
Light + Filter Sets for Z50/Z100 Lens		
SFA-KZ100-LFS-UV	Z50/Z100 Light + Filter Set, UV Excitation	each
SFA-KZ100-LFS-VI	Z50/Z100 Light + Filter Set, Violet Excitation	each
SFA-KZ100-LFS-RB	Z50/Z100 Light + Filter Set, Royal Blue Excitation	each
SFA-KZ100-LFS-CY	Z50/Z100 Light + Filter Set, Cyan Excitation	each
SFA-KZ100-LFS-GR	Z50/Z100 Light + Filter Set, Green Excitation	each
Barrier Filters for Z50/Z100/Z500/ZST Lens		
<i>see ordering information at right..</i>		

Best Value Full System Kit for Z500 Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-KZ500-IND	Best Value Full System	kit
Single Wavelength Full Systems for Z500 Lens		
SFA-KZ500-UV	Z500 Single Wavelength Full System, UV	kit
SFA-KZ500-VI	Z500 Single Wavelength Full System, Violet	kit
SFA-KZ500-RB	Z500 Single Wavelength Full System, Royal Blue	kit
SFA-KZ500-CY	Z500 Single Wavelength Full System, Cyan	kit
SFA-KZ500-GR	Z500 Single Wavelength Full System, Green	kit
Light + Filter Sets for Z500 Lens		
SFA-KZ500-LFS-UV	Z500 Light + Filter Set, UV Excitation	each
SFA-KZ500-LFS-VI	Z500 Light + Filter Set, Violet Excitation	each
SFA-KZ500-LFS-RB	Z500 Light + Filter Set, Royal Blue Excitation	each
SFA-KZ500-LFS-CY	Z500 Light + Filter Set, Cyan Excitation	each
SFA-KZ500-LFS-GR	Z500 Light + Filter Set, Green Excitation	each
Barrier Filters for Z50/Z100/Z500/ZST Lens		
<i>see ordering information at right..</i>		

Accessories		
SFA-LHC	Single Light Head Cable	each



Various excitation light heads and corresponding barrier filters.

Cat. No.	Description	Qty.
Best Value Full System Kit for ZST Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-KZST-IND	Best Value Full System	kit
Single Wavelength Full Systems for ZST Lens		
SFA-KZST-UV	ZST Single Wavelength Full System, UV	kit
SFA-KZST-VI	ZST Single Wavelength Full System, Violet	kit
SFA-KZST-RB	ZST Single Wavelength Full System, Royal Blue	kit
SFA-KZST-CY	ZST Single Wavelength Full System, Cyan	kit
SFA-KZST-GR	ZST Single Wavelength Full System, Green	kit
Light + Filter Sets for ZST Lens		
SFA-KZST-LFS-UV	ZST Light + Filter Set, UV Excitation	each
SFA-KZST-LFS-VI	ZST Light + Filter Set, Violet Excitation	each
SFA-KZST-LFS-RB	ZST Light + Filter Set, Royal Blue Excitation	each
SFA-KZST-LFS-CY	ZST Light + Filter Set, Cyan Excitation	each
SFA-KZST-LFS-GR	ZST Light + Filter Set, Green Excitation	each
Barrier Filters for Z50/Z100/Z500/ZST Lens		
SFA-KZX-BF-UV	Z50/Z100/Z500/ZST Barrier Filter, UV	each
SFA-KZX-BF-VI	Z50/Z100/Z500/ZST Barrier Filter, Violet	each
SFA-KZX-BF-RB	Z50/Z100/Z500/ZST Barrier Filter, Royal Blue	each
SFA-KZX-BF-CY	Z50/Z100/Z500/ZST Barrier Filter, Cyan	each
SFA-KZX-BF-GR	Z50/Z100/Z500/ZST Barrier Filter, Green	each

Best Value Full System Kit for FI Head, VHX-7100 Fully Integrated Head		
Includes three wavelengths (UV + VI + RB)		
SFA-KFI-IND	Best Value Full System	kit
Single Wavelength Full Systems for VHX-7100 FI Head		
SFA-KFI-UV	FI Single Wavelength Full System, UV	kit
SFA-KFI-VI	FI Single Wavelength Full System, Violet	kit
SFA-KFI-RB	FI Single Wavelength Full System, Royal Blue	kit
SFA-KFI-CY	FI Single Wavelength Full System, Cyan	kit
SFA-KFI-GR	FI Single Wavelength Full System, Green	kit
Light + Filter Sets for VHX-7100FI Head		
SFA-KFI-LFS-UV	FI Light + Filter Set, UV Excitation	each
SFA-KFI-LFS-VI	FI Light + Filter Set, Violet Excitation	each
SFA-KFI-LFS-RB	FI Light + Filter Set, Royal Blue Excitation	each
SFA-KFI-LFS-CY	FI Light + Filter Set, Cyan Excitation	each
SFA-KFI-LFS-GR	FI Light + Filter Set, Green Excitation	each
Barrier Filters for VHX-7100 FI Head		
SFA-KFI-BF-UV	FI Barrier Filter, UV	each
SFA-KFI-BF-VI	FI Barrier Filter, Violet	each
SFA-KFI-BF-RB	FI Barrier Filter, Royal Blue	each
SFA-KFI-BF-CY	FI Barrier Filter, Cyan	each
SFA-KFI-BF-GR	FI Barrier Filter, Green	each
Minus Blue Illumination Filter (see more information on page 14)		
KFI-IF515	Minus Blue Illumination Filter for Keyence VHX-7100 FI Head	each

Photoresist Inspection with the Keyence VHX Microscope Series

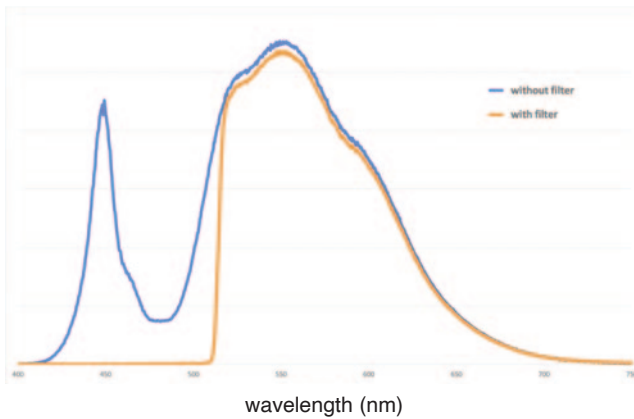
NIGHTSEA offers solutions for inspecting photoresist with the Keyence VHX series of digital microscopes. The photoresist used in electronics manufacturing is designed to react when exposed to light in the ultraviolet and blue parts of the spectrum. Once the resist is applied the materials are only handled in rooms in which these wavelength ranges are absent (typically yellow light). Microscopic inspection of the photoresist layers must similarly be done with light sources that will not cause premature exposure.

Our line of fluorescence adapters for Keyence microscopes provides an opportunity for us to also offer solutions for photoresist inspection tailored to the most commonly used lenses. Note that the solutions are specific to the lenses and not to the microscope model (e.g. VHX-6000, -7000, etc.).

VHX-7100 Fully Integrated (FI) Head



We designed a custom filter insert that slips into the polarization slot on the left side (as you are facing the microscope) of the FI head. This removes the shorter wavelengths from the built-in Keyence light source. The image on the left below shows the filter insert. The graph shows the spectral output of the light before (blue line) and after (orange line) inserting the NIGHTSEA filter.



Spectral output of the light before (blue line) and after (orange line) inserting the NIGHTSEA Custom Light Source Filter for Keyence VHX-7100.

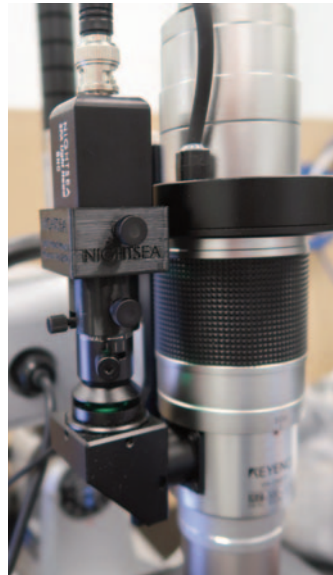
Cat. No.	Description	Qty.
KFI-IF515	Minus Blue Illumination Filter for Keyence VHX-7100 FI Head	each

VH-Z00/VH-Z20

With the VH-Z00 and VH-Z20 lenses there is no provision to replace the Keyence light source with our excitation sources. For fluorescence our light source is placed to the side of the microscope and a barrier filter is placed beneath the lens, as shown in the photograph below. For fluorescence the Keyence light source would be turned off. If the Keyence light source is operated with a barrier filter in place the light has to pass through that filter to reach the subject. Use of an appropriate barrier filter removes UV and blue wavelengths. For photoresist inspection we recommend the Cyan (CY) barrier filter, which is a longpass filter with cutoff at around 550nm. This would be all you need to safely inspect photoresist with these lenses.



Cat. No.	Description	Qty.
SFA-KZB-BF-CY	Z00/Z20 Barrier Filter, Cyan	each



Light head mount for VH-Z500 lens



Light head mount for VH-ZST lens



Light head mount for VH-Z100 lens

VH-ZST/VH-50/VH-Z100/VH-Z500

With the VH-ZST, VH-50, VH-Z100, and VH-Z500 there is no provision to introduce a filter in the light path after the Keyence light source as there is with the FI head. For fluorescence applications NIGHTSEA developed adapters that enable the user to mount our excitation light heads in place of the Keyence fiber optic source on these lenses. In this way the excitation light is channeled through the Keyence optics to illuminate the sample from above. We offer an amber LED light source that takes advantage of this adapter to illuminate the sample with light that contains no UV or blue wavelengths.

There are two scenarios for adding the photoresist illumination capability to Keyence microscopes with these lenses:

Scenario 1 – You already own a NIGHTSEA system for fluorescence work with one of these lenses.

You only need to purchase the amber light head:

Cat. No.	Description	Qty.
SFA-LH-AM	Light Head, Amber	each

Scenario 2 – You do not own any NIGHTSEA equipment

You will need:

- lamp base with power supply
- lens-specific mount
- light head
- light head cable (for operating the light head remote from the lamp base)

These are packaged as complete systems:

Cat. No.	Description	Qty.
SFA-PR-ZST	VH-ZST Full System for Photoresist	each
SFA-PR-Z100	VH-Z50/Z100 Full System for Photoresist	each
SFA-PR-Z500	VH-Z500 Full System for Photoresist	each

Imaging Tips for Keyence Microscopes

Recommendations for imaging fluorescence with the Keyence systems are primarily driven by the fact that **the fluorescence emitted by your sample tends to be weaker than the reflected white light that the system is designed to image**. This means that you will want to adjust things to either get more light to the camera, or do more with the light you have.

Exposure Adjustment

Aperture

If your lens has an aperture control set this to Open. You want to let as much light in as you can.

Brightness Control

In the Lighting section go to Brightness and take control of your integration time (shutter speed). Click Manual and move the slider to the right, or for even more exposure click Super-charge and adjust there. Depending on the brightness of the subject we have found it not unusual to get 100's of milliseconds of exposure time. You can also go to the Gain section, select Manual, and increase the gain. Some of these adjustments can also be made from the console controls.

HDR and Texture

In some cases we have seen it helpful to use HDR and Texture modes, but in other cases this has not worked well. Experiment as needed.

Subject Selection

Fluorescence is often not bright and, depending on the subject, may be restricted to a small area. If your exposure time (shutter speed / integration time) is in the hundreds of milliseconds there will be a noticeable image lag when you move the sample. Combine these factors and it can at times be difficult and annoying to try to make large sample movements in fluorescence mode. If you know generally the area you want to examine it can be more convenient to first use white light (with its short integration times) to home in on the area of interest and then switch over to fluorescence mode.

Focusing

When you are imaging fluorescence you need a barrier filter that blocks reflected excitation light and transmits the fluorescence of interest. This introduces an extra optical element and some accompanying refraction of the light, causing a shift in the lens-to-subject distance for optimal focus. If you use white light to find an area of interest and focus on it, then switch to fluorescence and add the barrier filter you will need to refocus by a small amount. An alternative is to insert the barrier filter before doing your white-light sample area selection and focusing, and the image will still be in focus when you switch to the fluorescence excitation source.

Artifact Detection

Another byproduct of the long integration time needed to image fluorescence is the potential to have non-fluorescent artifacts in the image. These arise from ambient room light reflected from the sample. A lot of this can be avoided by turning off room lights or providing something to shade the sample area from light sources. You can easily determine if a feature in an image is true fluorescence or not by turning off the excitation light source. Any true fluorescence will disappear immediately, while artifacts will be unaffected.

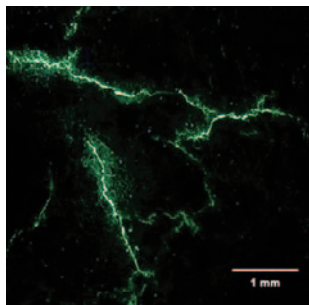
Note that all techniques may not function equally well with all of the Keyence lens options. Experiment to find the best solution for your particular equipment configuration. If you are having problems imaging, contact your local Keyence sales rep or NIGHTSEA.

Crack and Failure Analysis with Fluorescence

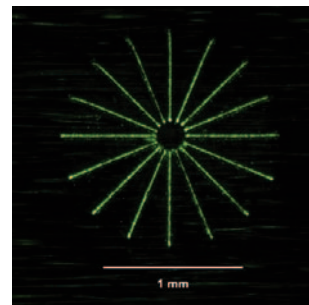
Fluorescence is a valuable tool for failure analysis, helping investigators see what they might otherwise miss. Fluorescence indicators of various types – including fluorescent penetrants, magnetic particles, and other fluorescent dyes – are commonly used to highlight cracks or defects that would otherwise be difficult or impossible to see. The fluorescence makes them stand out in high contrast. The NIGHTSEA fluorescence adapter systems can be used with microscopes at a variety of scales for detailed examination of these features. Here we show images made with a stereo microscope and with a Keyence digital microscope.

Note that all of the images below were made using the Royal Blue excitation/emission option. While this kind of analysis is normally associated with Ultraviolet, we find that the Royal Blue option is an excellent choice for working with a wide variety of fluorescent indicators.

First, some small cracks highlighted with fluorescent penetrants.

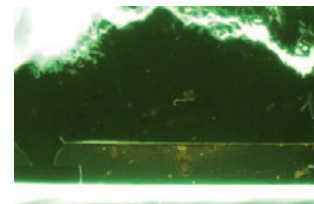
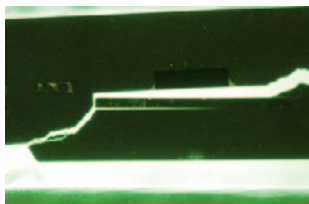


Cracks in Sonaspection test plate, fluorescent penetrant



TAM panel starburst with fluorescent penetrant

Next we show some cross sections of electronic components that were embedded in epoxy, cross-sectioned, and polished, after which indications were highlighted with an epoxy-like dye with Morton Fluorescent Yellow G as the fluorescent ingredient.



Circuit Board Conformal Coating Inspection

The conformal coating used on electronic circuit boards fluoresces under excitation by either Ultraviolet or Violet light. The fluorescence is a powerful tool for checking coating integrity.



Microscope Magnification: X50

NIGHTSEA Model SFA Fluorescence Adapter for Hirox Digital Microscope

Overview

The NIGHTSEA Model SFA Fluorescence Adapter system can add a versatile fluorescence imaging capability to the Hirox digital microscope. Visit our website for a full gallery of images made with the NIGHTSEA adapter and the Hirox microscope.

Our adaptations to the Hirox system are concerned with the lens. We now offer variants of our fluorescence adapter system that work with the HR-2500E and HR-1020E lenses. (We may be able to support older lenses – contact us for more information.)

Fluorescence solution

The key elements of any fluorescence system are:

- A light source that produces sufficient energy in the appropriate wavelength range to excite fluorescence in the sample of interest
- A barrier filter in the viewing path that blocks reflected excitation light while transmitting the fluorescence emitted by the sample

NIGHTSEA implements these for the Hirox system with:

- high intensity LED light sources available in five excitation wavelength ranges (see list on next page)
- emission barrier filters that can be easily added to the Hirox lenses
 - The filters for the HR-2500E and HR-1020E lenses simply insert into the Analyzer slot.

Comprehensive Solution Kit

The components of a NIGHTSEA system that adds a single excitation/emission wavelength combination on a Hirox microscope are:

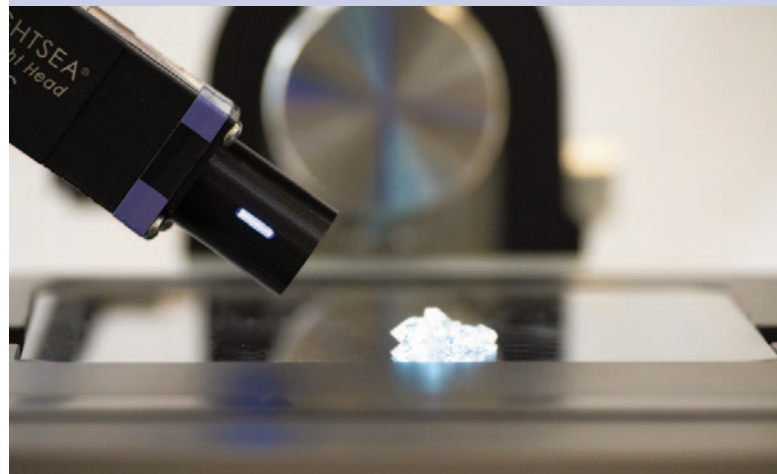
- Flexible gooseneck lamp base with LED drive circuit and intensity control;
- Universal power supply (120/240VAC, 50/60Hz) with international plug set
- Excitation light source
- Emission barrier filter
- Barrier filter glasses for fluorescence viewing and eye safety
- Padded carrying case

Additional wavelength sets are implemented by adding a matched set (light plus filter set) consisting of a light source, barrier filter, and filter glasses.

We are currently working on adapters for the full range of Hirox lenses, for use with all of our wavelength options. Please contact Hirox or NIGHTSEA to discuss your application and compatible solutions.



Hirox HR-1020E lens with NIGHTSEA UV illuminator



Detail of NIGHTSEA light head with Hirox microscope



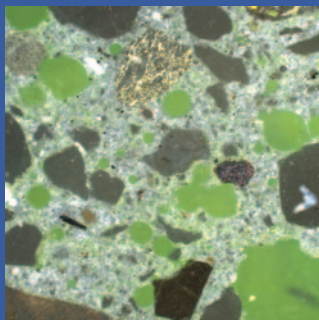
Royal Blue barrier filter for HR-2500E lens

Barrier filter installed in HR-2500E lens

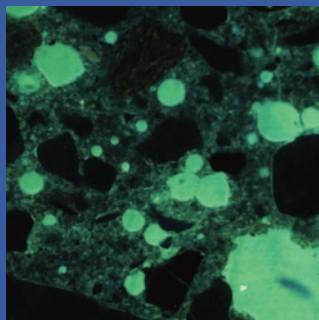


Here are samples of what you can see:

Concrete sample

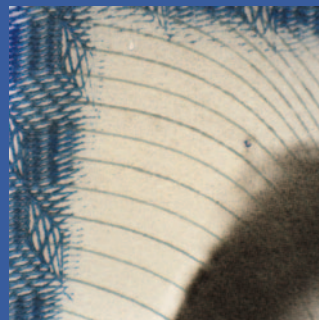


HR-2500E 140x ring light



HR-2500E 140x fluorescence with UV

Travel document



HR-2500E 30x ring light



HR-2500E 30x fluorescence with UV

Why we recommend a set of three excitation wavelengths for industry needs

We have found that the combination of the Ultraviolet, Violet, and Royal Blue excitation/emission sets addresses a wide variety of industrial imaging challenges. The most common misconception we encounter is that fluorescence is uniquely associated with ultraviolet (UV) light and many customers immediately ask for that wavelength. However, we have found in several cases – even some in which UV was specifically recommended by a dye manufacturer – that either Violet or Royal Blue provided superior performance. For example:

A prospective customer wanted to image concrete thin sections impregnated with fluorescent dye. The dye supplier recommended UV, and it is true that UV will make the dye fluoresce nicely. We found that our Royal Blue excitation produced significantly brighter fluorescence.

Similar to above, except that a fluorescence epoxy had been used to highlight surface cracks in a test sample. Royal Blue excitation yielded brighter fluorescence.

Acquiring the three-color system will provide the highest chance of success and will equip you with a versatile toolkit to address new fluorescence imaging challenges as they arise. Contact us if you would like to discuss your application further.

In addition to the Comprehensive Solution Kits, you can also purchase single-color full systems that provide everything you need to get started with one excitation/emission combination. Once you have a full system, additional wavelength sets can be ordered separately. These contain the new light head, microscope barrier filter, and barrier filter glasses.

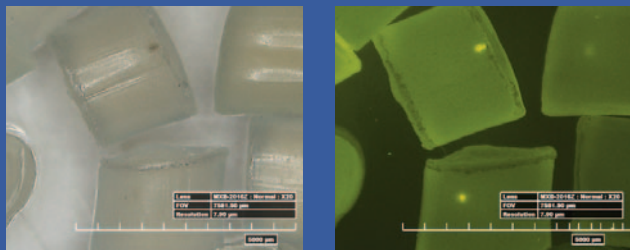
Wavelength Sets

There are five excitation/emission wavelength combinations available for the system.

Color	Designation	Excitation	Emission
Ultraviolet	UV	360 - 380nm	415nm longpass
Violet	VI	400 - 415nm	450nm longpass
Royal Blue	RB	440 - 460nm	500nm longpass
Cyan	CY	490 - 515nm	550nm longpass
Green	GR	510 - 540nm	600nm longpass

Gel defect in a nylon granule

Nylon 6,6 granules can manifest a process defect called “gel” and if there is too much of this in a production batch it can compromise downstream production. The gel shows up as a brighter fluorescent area within the fluorescing granule. Images made with Royal Blue excitation.



Nylon granules with gel defect, 20x, white light and fluorescence under Royal Blue excitation.

Ordering Information

Cat. No.	Description	Qty.
Best Value Full System Kit for HR-1020E Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-HR1020E-IND	HR-1020E Best Value Full System	kit
Single Wavelength Full Systems for HR-1020E Lens		
SFA-HR1020E-UV	HR-1020E Single Wavelength Full System, UV	kit
SFA-HR1020E-VI	HR-1020E Single Wavelength Full System, Violet	kit
SFA-HR1020E-RB	HR-1020E Single Wavelength Full System, Royal Blue	kit
SFA-HR1020E-CY	HR-1020E Single Wavelength Full System, Cyan	kit
SFA-HR1020E-GR	HR-1020E Single Wavelength Full System, Green	kit
Light + Filter Sets for HR-1020E Lens		
SFA-HR1020E-LFS-UV	HR-1020E Light + Filter Set, UV Excitation	each
SFA-HR1020E-LFS-VI	HR-1020E Light + Filter Set, Violet Excitation	each
SFA-HR1020E-LFS-RB	HR-1020E Light + Filter Set, Royal Blue Excitation	each
SFA-HR1020E-LFS-CY	HR-1020E Light + Filter Set, Cyan Excitation	each
SFA-HR1020E-LFS-GR	HR-1020E Light + Filter Set, Green Excitation	each
Barrier Filters for HR-1020E Lens		
SFA-HR1020E-BF-UV	HR-1020E Barrier Filter, UV	each
SFA-HR1020E-BF-VI	HR-1020E Barrier Filter, Violet	each
SFA-HR1020E-BF-RB	HR-1020E Barrier Filter, Royal Blue	each
SFA-HR1020E-BF-CY	HR-1020E Barrier Filter, Cyan	each
SFA-HR1020E-BF-GR	HR-1020E Barrier Filter, Green	each

Cat. No.	Description	Qty.
Best Value Full System Kit for HR-2500E Lens		
Includes three wavelengths (UV + VI + RB)		
SFA-HR2500E-IND	HR-2500E Best Value Full System	kit
Single Wavelength Full Systems for HR-2500E Lens		
SFA-HR2500E-UV	HR-2500E Single Wavelength Full System, UV	kit
SFA-HR2500E-VI	HR-2500E Single Wavelength Full System, Violet	kit
SFA-HR2500E-RB	HR-2500E Single Wavelength Full System, Royal Blue	kit
SFA-HR2500E-CY	HR-2500E Single Wavelength Full System, Cyan	kit
SFA-HR2500E-GR	HR-2500E Single Wavelength Full System, Green	kit
Light + Filter Sets for HR-2500E Lens		
SFA-HR2500E-LFS-UV	HR-2500E Light + Filter Set, UV Excitation	each
SFA-HR2500E-LFS-VI	HR-2500E Light + Filter Set, Violet Excitation	each
SFA-HR2500E-LFS-RB	HR-2500E Light + Filter Set, Royal Blue Excitation	each
SFA-HR2500E-LFS-CY	HR-2500E Light + Filter Set, Cyan Excitation	each
SFA-HR2500E-LFS-GR	HR-2500E Light + Filter Set, Green Excitation	each
Barrier Filters for HR-2500E Lens		
SFA-HR2500E-BF-UV	HR-2500E Barrier Filter, UV	each
SFA-HR2500E-BF-VI	HR-2500E Barrier Filter, Violet	each
SFA-HR2500E-BF-RB	HR-2500E Barrier Filter, Royal Blue	each
SFA-HR2500E-BF-CY	HR-2500E Barrier Filter, Cyan	each
SFA-HR2500E-BF-GR	HR-2500E Barrier Filter, Green	each

For other Hirox lenses...

Cat. No.	Description	Qty.
Best Value Full System Kit		
Includes three wavelengths (UV + VI + RB)		
SFA-H-IND	Hirox Best Value Full System	kit
Single Wavelength Full Systems		
SFA-H-UV	Hirox Single Wavelength Full System - UV	kit
SFA-H-VI	Hirox Single Wavelength Full System - Violet	kit
SFA-H-RB	Hirox Single Wavelength Full System - Royal Blue	kit
SFA-H-CY	Hirox Single Wavelength Full System - Cyan	kit
SFA-H-GR	Hirox Single Wavelength Full System - Green	kit
Add-on Single Wavelengths		
SFA-H-LFS-UV	Hirox Single Wavelength Add-On - UV	each
SFA-H-LFS-VI	Hirox Single Wavelength Add-On - Violet	each
SFA-H-LFS-RB	Hirox Single Wavelength Add-On - Royal Blue	each
SFA-H-LFS-CY	Hirox Single Wavelength Add-On - Cyan	each
SFA-H-LFS-GR	Hirox Single Wavelength Add-On - Green	each

NIGHTSEA Fluorescence Adapter for Dino-Lite Digital Microscope

Overview

The NIGHTSEA Model SFA Stereo Microscope Fluorescence Adapter can be used with the Dino-Lite series of digital microscopes. While Dino-Lite does offer some models with fluorescence built in, in some cases you can achieve improved performance by using the SFA excitation light sources in combination with matching barrier filters pre-mounted in a Dino-Lite endcap so that they just snap onto the front of the microscope.

Since there is no provision for mounting a filter shield on the Dino-Lite, the SFA system for Dino-Lite will include a pair of barrier filter glasses for each wavelength purchased.

A one-color complete system consists of a gooseneck lamp base with power supply, light head, matching snap-on barrier filter, and barrier filter glasses, all packed in a convenient padded travel case. You can add additional modular excitation/emission wavelength sets at any time. These contain a light head, matching snap-on barrier filter, and barrier filter glasses.

Wavelength Sets

Wavelength sets are named and color coded for the color of the excitation light, not the color of the emitted fluorescence.

Designation	Excitation	Emission
UV – Ultra Violet	360-380nm	415nm longpass
VI – Violet	400-415nm	450nm longpass
RB – Royal Blue	440-460nm	500nm longpass
RB-GO – Green Only	440-460nm	500-560nm bandpass
CY – Cyan	490-515nm	550nm longpass
GR – Green	510-540nm	600nm longpass

With the Royal Blue (RB) excitation we offer two barrier filters – longpass and bandpass. Read our article on selecting the right passband option for your application.

Note: Wavelength sets are named and color coded for the color of the excitation light, not the color of the emitted fluorescence.

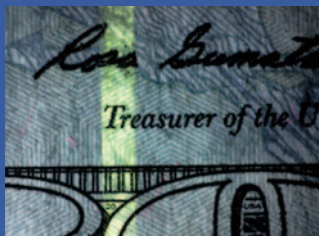


Dino-Lite digital microscope paired with NIGHTSEA fluorescence excitation light source

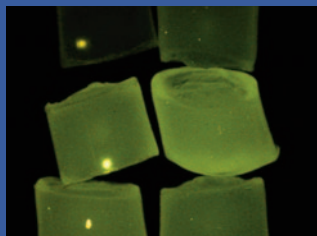


Dino-Lite digital microscope with array of NIGHTSEA barrier filters

A sampling of images we have made with the Dino-Lite/NIGHTSEA combination.



Fluorescent markings in currency. Dino-Lite + NIGHTSEA UV excitation



Gel defect in nylon granules. Dino-Lite + NIGHTSEA Royal Blue excitation



Red fluorescence from chlorophyll in a leaf. Dino-Lite + NIGHTSEA Royal Blue excitation

Dino-Lite Edge Series Digital Microscopes

The NIGHTSEA system works best with long working distance Dino-Lite Edge models. Higher magnification units have very short working distances, making it difficult to direct the NIGHTSEA light source onto the subject. Please see our website for more information and ordering.



Ordering Information

Cat No.	Description	Qty.
One-color complete setup		
SFA-DL-UV	Ultraviolet (360 – 380nm) excitation	pair
SFA-DL-VI	Violet (400 – 415nm) excitation	pair
SFA-DL-RB	Royal Blue (440 – 460nm) excitation with longpass filter	pair
SFA-DL-RB-GO	Royal Blue with Green-Only bandpass filter	pair
SFA-DL-CY	Cyan (490 – 515nm) excitation	pair
SFA-DL-GR	Green (510 – 540nm) excitation	pair
Modular excitation/emission sets		
SFA-DL-LFS-UV	Ultraviolet excitation	pair
SFA-DL-LFS-VI	Violet excitation	pair
SFA-DL-LFS-RB	Royal Blue excitation	pair
SFA-DL-LFS-RB-GO	Royal Blue excitation with Green-Only bandpass filter	pair
SFA-DL-LFS-CY	Cyan excitation	pair
SFA-DL-LFS-GR	Green excitation	pair

Xite Fluorescence Flashlight System

View fluorescence in the lab or the field!

The Xite™ Fluorescence Flashlight System includes single-wavelength fluorescence excitation flashlights available with any of the five wavelengths in the NIGHTSEA line, paired with matching barrier filter glasses for maximum viewing contrast. Genotyping your transgenic organisms, screening cell cultures, inspecting parts with fluorescent penetrants, finding fluorescing critters in the field, ... the Xite line provides what you need.

Features

- Tightly focused for a high intensity illumination spot
- Two power levels, plus unique flashing mode and battery status indication
- Add-on diffuser for a broader, smoother beam
- Each light paired with matching barrier filter glasses
- Compact and lightweight
- Rechargeable high capacity lithium ion battery

Included with each system

- Fluorescence excitation flashlight (your choice of one wavelength) with lanyard
- Matching barrier filter glasses with microfiber case for storage and cleaning
- Diffuser cap
- High capacity lithium ion battery
- Battery charger and USB charging cord
- Instruction sheet
- Padded foam shipping/carry/storage case



Flashing mode for enhanced detection in ambient light

As bright as fluorescence may appear when you use an intense light source to view it in conditions of darkness, in most cases it actually tends to be a relatively weak effect that is easily masked by other light. If you try to find fluorescence when there is moderate ambient light (room light, sunlight, etc.), any fluorescing subject will respond to the excitation, but the response may be too weak to notice easily.

But there's a trick - by making the excitation light source blink repetitively (a strobe effect), any fluorescence will blink at the same rate, while the illuminated background will not. This flickering increases the apparent contrast and thus increases detectability. With the Royal Blue (RB) excitation we offer two options for filter glasses - longpass and bandpass.



Diffuser

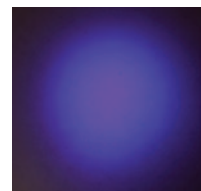
Add-on 15° diffuser cap provides a wider, softer beam.



NIGHTSEA Xite light with push-on diffuser



Xite illumination spot, no diffuser



Xite illumination spot, with diffuser

Flashlight Specifications

Dimensions	13.5 cm long x 3.2 cm diameter (5.3 in x 1.26 in)
Weight	255g (9.0 oz) with battery

Note - the Xite flashlights are not suitable for underwater use

Available Wavelengths

Wavelength sets are named and color coded for the color of the excitation light, not the color of the emitted fluorescence.

Designation	Excitation	Barrier Filter Glasses
UV - Ultraviolet	360 - 380nm	415nm longpass
VI - Violet	400 - 415nm	450nm longpass
RB - Royal Blue	440 - 460nm	500nm longpass
RB-GO - Royal Blue, Green Only	440 - 460nm	500 - 560nm bandpass
CY - Cyan	490 - 515nm	550nm longpass
GR - Green	510 - 540nm	600nm longpass

Ordering Information

Cat. No.	Description	Qty.
Xite-UV	Ultraviolet (360 - 380nm) excitation	each
Xite-VI	Violet (400 - 415nm) excitation	each
Xite-RB	Royal Blue (440 - 460nm) excitation with longpass filter glasses	each
Xite-RB-GO	Royal Blue with green-only bandpass filter glasses	each
Xite-CY	Cyan (490 - 515nm) excitation	each
Xite-GR	Green (510 - 540nm) excitation	each

NIGHTSEA Barrier Filter Glasses

The Royal Blue filter glasses are available in a choice of three styles. Styles 1 and 2 fit over eyeglasses, Style 3 does not. All other glasses colors are available in Style 2 only. Glasses meet ANSI Z87.1 impact standards for safety glasses. **NOTE:** Glasses Description refers to the excitation light source with which they are to be used.



FG-RB-1



FG-RB-2



FG-RB-3

Cat No.	Description	Qty.
FG-UV	Filter glasses, Ultra Violet	each
FG-VI	Filter glasses, Violet	each
FG-RB-1	Filter Glasses, Royal Blue, style 1	each
FG-RB-2	Filter Glasses, Royal Blue, style 2	each

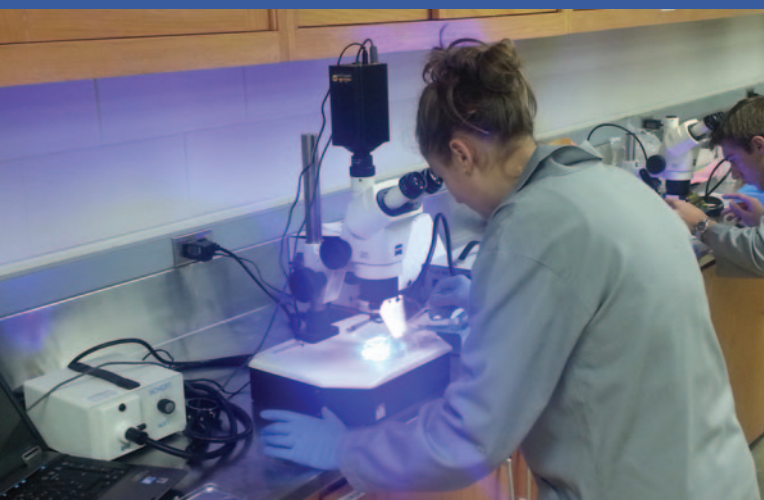
Cat No.	Description	Qty.
FG-RB-3	Filter Glasses, Royal Blue, style 3	each
FG-RB-GO	Filter glasses, Green Only	each
FG-CY	Filter glasses, Cyan	each
FG-GR	Filter Glasses, Green	each



SFA with microscope/camera for teaching



Observing fluorescent zebrafish under the microscope at BrainFest



SFA in a biology teaching lab

Fluorescence for Education and Outreach

“Now I can use fluorescence in my classes!”

We heard this over and over as soon as we introduced our Model SFA Stereo Microscope Fluorescence Adapter to the scientific community, and the message continues to resonate. Not only can it handle routine research tasks like sorting, screening, and dissection, but the price point and simplicity make it practical to add it to lab class stereo microscopes. Prior to the SFA the cost (easily \$20,000 and up) and complexity of conventional fluorescence stereo microscopes from the major manufacturers were virtually insurmountable barriers to incorporating fluorescence in routine undergraduate laboratory courses. Those high-end systems are terrific for research, but you can't buy lots of them and you are not going to turn a group of inexperienced undergraduates loose on them. At under \$1,100 per unit, simple to use, and rugged enough to stand up to repeated student handling, the NIGHTSEA SFA breaks through the cost and complexity barriers (and there is a discount for orders of 10 or more).

“The relatively low cost enabled us to purchase enough for our students, and they allow us to have students make observations of specimens that they would otherwise not be able to study.”

There are many disciplines – biology, marine science, forensic science, materials evaluation, and more – in which fluorescence is a key means to see what you need to see at the level of the stereo microscope. However, without a practical way to visualize fluorescence you can't take advantage of this.

“Due to their affordability, we were able to purchase 6 units for our developmental biology teaching lab! A great investment!”

The SFA is a near-universal modular system that attaches to the stereo microscopes that you already own at a price that can be 5% or less of the cost of a 'conventional' fluorescence stereo microscope. All you need is one excitation/emission wavelength combination to get started, with the option to add additional wavelength sets (we offer 5 options) at about half the cost of the original system.

The benefit is not just in the initial cost. The SFA attaches in seconds and is extremely rugged so students get a true hands-on experience.

“Our students were very excited to use them and they did not require any special training as the units adapt easily to the stereoscopes that we presently own and are very user friendly.”

“The NIGHTSEA system was extremely easy to set up, it can be moved to different microscopes and the support for the product has been fantastic. I would recommend this product to anyone who is looking for a quick and cheap way to add the ability to image fluorescence to their lab”

Using routine fluorescence to sort *Drosophila* larvae

The Challenge

Dr. Laura Reed (Dept. of Biological Sciences, University of Alabama, Tuscaloosa) was heading 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 required large numbers of larvae of each genotype. Her program involved testing 84 different genotypes and, for each genotype, 200 or more larvae. A special strain of fruit flies had been genetically engineered to express Green Fluorescent Protein (GFP) driven by an actin promoter (Figure 1). Only the flies without the mutations fluoresced. The clear difference between fluorescent and non-fluorescent larvae made them easy to sort.

For best results, the larvae needed to be collected, sorted, and frozen when at their largest, but before they pupated. However, they were at this stage for only about six hours. With 84 genotypes to be tested and 200+ larvae per genotype, sorting was a major challenge. While Dr. Reed had 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 the Stereo Microscope Fluorescence Adapter (SFA) system.

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.

For Dr. Reed, the Royal Blue excitation/emission set provided excellent results.

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 worked only with GFP (blue excitation/green fluorescence), she only needed to purchase one version of SFA. However, as the needs of her lab grew, additional sets could readily be added.

Finally, as demonstrated by Figure 2, the 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 did not need to be in complete darkness.

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



Figure 1. Non-mutant *Drosophila melanogaster* expressing GFP.



Figure 2. Larval sorting under ambient lighting.

Figure 3. Students sort larvae using NIGHTSEA's SFA in Royal Blue. Dr. Reed had shifts of two to four undergrads sorting in parallel.



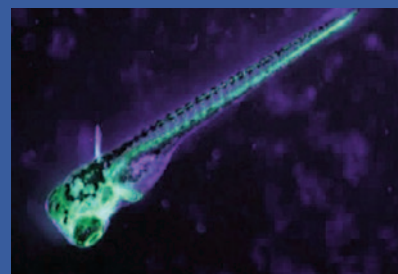
Fluorescing Zebrafish

Here are comments from a faculty member at Colgate University:

Students in Developmental Biology Lab were examining the effects of pharmacological agents on development of zebrafish embryos. In order to better visualize the development of the nervous system and vasculature, we used transgenic fish that expressed GFP either throughout their nervous system or in the developing vasculature. The NIGHTSEA system easily adapted to our dissection scopes and allowed students to observe the development of their fish at several different time-points. They could readily observe the transgene expression, and it helped solidify the phenotypes they were observing and allowed them to determine an optimal time to fix their fish for analysis under the compound microscope.

For quick screens it actually worked perfectly well in a bright room. For more intimate looking (more than presence/absence calls), we turned out the room lights. Worked better than I'd hoped it would.

These pictures of fluorescing zebrafish embryos and juveniles were taken using the NIGHTSEA Stereo Microscope Fluorescence Adapter.



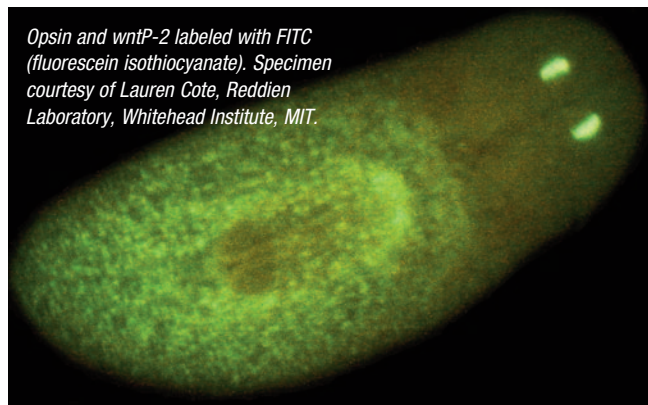
Zebrafish – GFP fluorescence



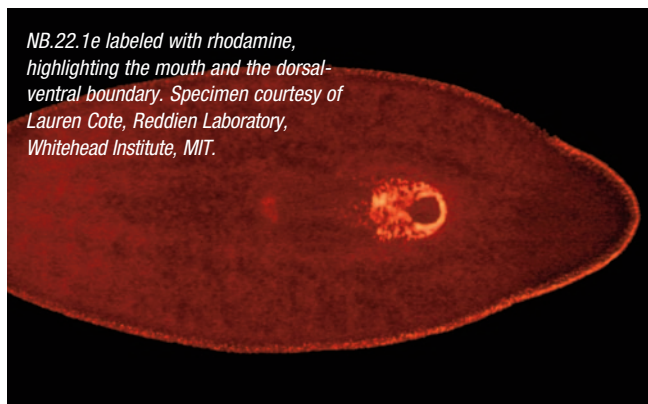
Zebrafish embryos – histone H2B-Dendra2

Screening FISH-Labeled Planarian (*Schmidtea mediterranea*)

FISH (fluorescence in situ hybridization) is routinely used to label features in planarians (*Schmidtea mediterranea*). The NIGHTSEA Model SFA Stereo Microscope Fluorescence Adapter can be added to just about any existing stereo microscope to create a practical system for screening samples for successful preparation prior to moving to higher resolution imaging techniques.



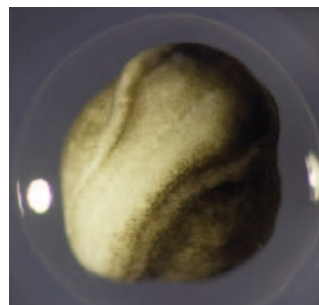
Opsin and wntP-2 labeled with FITC (fluorescein isothiocyanate). Specimen courtesy of Lauren Cote, Reddien Laboratory, Whitehead Institute, MIT.



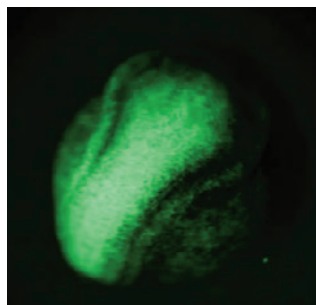
NB.22.1e labeled with rhodamine, highlighting the mouth and the dorsal-ventral boundary. Specimen courtesy of Lauren Cote, Reddien Laboratory, Whitehead Institute, MIT.

Fluorescent Axolotl

These pictures of GFP-expressing transgenic neurula stage axolotl (*Ambystoma mexicanum*) embryos were taken using the NIGHTSEA Stereo Microscope Fluorescence Adapter for illumination, with an iPhone 5 camera held up to the eyepiece. It's a convenient way for students to take photos during labs! Access to specimens courtesy of Dr. Kristi Wharton and Kathy Patenaude, Brown University.



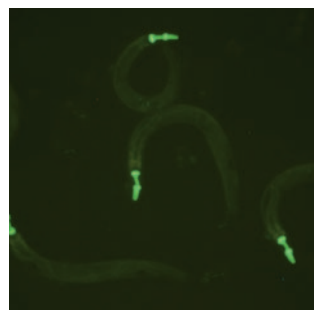
Axolotl (Ambystoma mexicanum), white light.



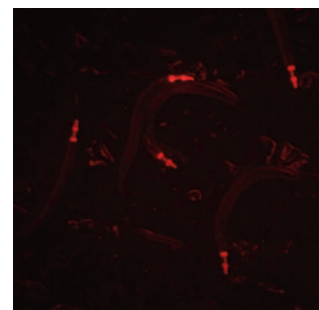
Axolotl (Ambystoma mexicanum), fluorescence.

Fluorescing *C. elegans*

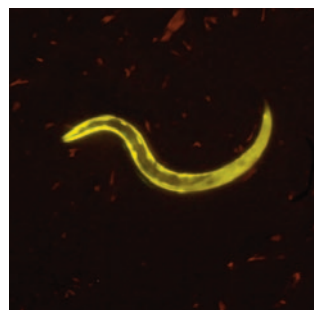
The pictures of fluorescing transgenic *C. elegans* in this gallery were all taken using the NIGHTSEA Stereo Microscope Fluorescence Adapter.



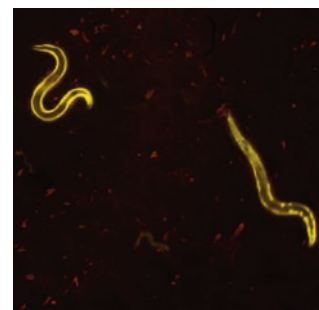
GFP C. elegans



mCherry C. elegans



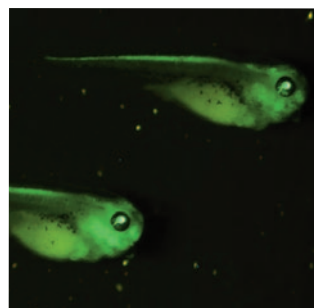
YFP C. elegans



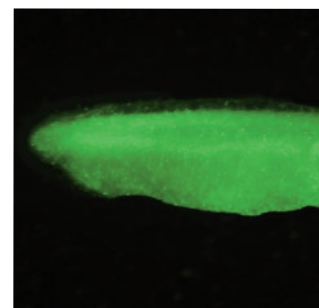
YFP C. elegans

Fluorescing *Xenopus*

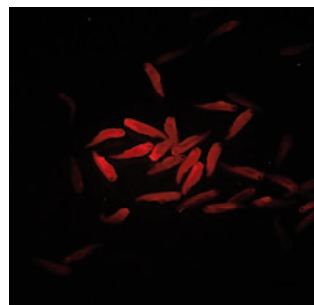
All of the specimen photographs below were taken with a Canon Rebel T2i camera mounted on a Motic trinocular stereo microscope with the NIGHTSEA Stereo Microscope Fluorescence Adapter for illumination and filtering.



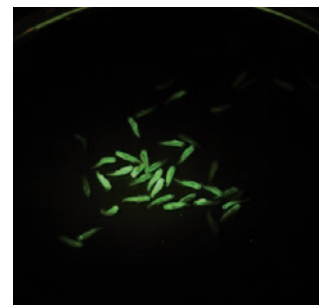
Stage 41 X. tropicalis, transgenic OTX-GFP eyes.



Stage 29-30 X. laevis, messenger RNA injected ubiquitous GFP and membrane RFP.



Collection of Stage 37-38 X. laevis, messenger RNA injected ubiquitous GFP and membrane RFP viewed through shield filter for sorting.



Collection of Stage 37-38 X. laevis, messenger RNA injected ubiquitous GFP and membrane RFP viewed through shield filter for sorting.

Pre-Screening Samples for Fluorescence

The NIGHTSEA Model SFA Stereo Microscope Fluorescence Adapter can turn your routine laboratory stereo microscope into a valuable tool for pre-screening your sample preparations for fluorescence before moving on to higher resolution systems.

The Challenge

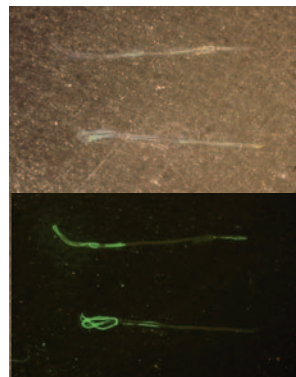
High resolution imaging of biological samples is heavily based on fluorescence techniques. Confocal, 2-photon, and high resolution compound fluorescence microscopes are almost always a limited resource. They are often located only in imaging core facilities and accessible on a scheduled, pay-per-use basis.

The processes for introducing fluorophores to specimens are not always successful. Staining, introduction of GFP-bearing plasmids to cells, immunohistochemistry – all are fallible. It is not unusual to spend time searching for fluorescence on a high end system when there is not even any there to be found.

The Practical Solution

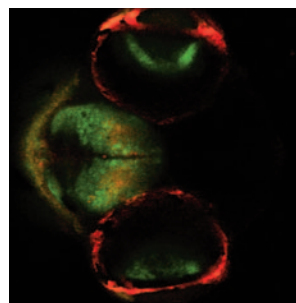
The NIGHTSEA SFA enables fluorescence pre-screening of specimens on a standard stereo microscope. The detail that you see is not important – the simple presence or absence and general location of fluorescence lets you know whether it is worth taking your specimen to the imaging core. Between the direct expense of the use fee and the time wasted to look at a non-fluorescent specimen it will not take many saved trips for the NIGHTSEA system to more than pay for itself.

One researcher's work requires staining rabbit psoas muscle fibers with Alexa Fluor 488 Phalloidin. There was some frustration with samples that did not take up the stain. After acquiring the SFA she wrote:



Rabbit psoas muscle fibers stained with Alexa Fluor 488 Phalloidin, in white light and fluorescence. Images made using NIGHTSEA's white LED (top) and the Royal Blue excitation/emission light+filter set. Samples courtesy of Dr. Beth Brainerd and Natividad Chen, Brown University.

"The NIGHTSEA fluorescence setup is a great way to quickly check whether the stain was successful before we try to image the muscle fiber at a higher magnification on the confocal."



Confocal image of brain of transgenic zebrafish (*Dania rerio*). Kaede protein – green is unconverted, red is photoconverted. Image courtesy of Robert Thorn, Creton Lab, Brown University.

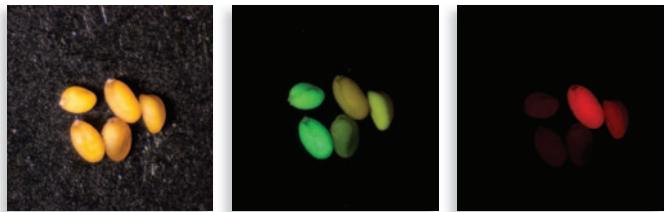
Another researcher uses zebrafish as a system to look at the way different toxicants (pharmaceuticals, pesticides, food additives, etc.) alter brain development. He writes:

"Before using NIGHTSEA to screen my samples, I would have to select samples to mount, go to the confocal and then hope that some of my samples were actually fluorescent. Now that I use NIGHTSEA to prescreen my samples I save both time and money by making sure the only samples I image are fluorescent."

Arabidopsis Seeds

Arabidopsis thaliana is a small flowering plant that is widely used as a model organism for a variety of genetic studies. Dr. Scott Poethig and colleagues at the University of Pennsylvania have developed a novel transgenic strain of *A. thaliana* that has chromosomal segments with eGFP on one end and dsRed at the other. The segments can be followed in genetic crosses and manipulated via recombination. The transgenic strains will enable a variety of experiments, including phenotypic analyses of mutations with weak or environmentally sensitive phenotypes. They are intended for use in both research and education.

Dr. Poethig was looking for a cost-effective way to sort the genetically modified seeds in a teaching setting. He learned about the new NIGHTSEA Stereo Microscope Fluorescence Adapter and sent a set of seeds for testing. There were five varieties - strong and weak green fluorescence, strong and weak red fluorescence, and non-fluorescent control. All of the variations were easy to see, even with the room lights on.

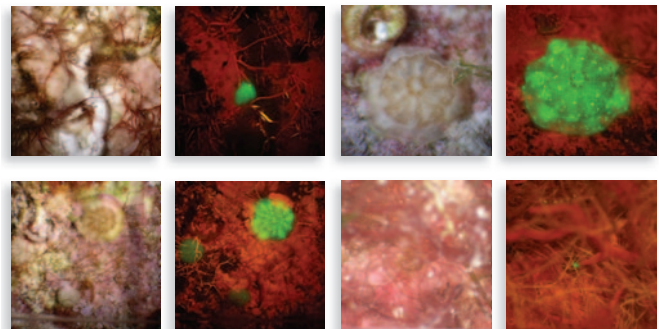


In the example above, the image on the left was taken with white light illumination, the image in the center with the Royal Blue excitation/emission combination, and the image on the right with the Green excitation/emission combination. Equipment - NIGHTSEA Stereo Microscope Fluorescence Adapter, Motic SMZ168 trinocular stereo microscope, Canon EOS Rebel T2i camera.

Coral Recruitment Through The Microscope

Fluorescence is a valuable tool for coral recruitment research and one of the ways to apply it is to use a stereo microscope to examine corals on settlement tiles or other surfaces. The NIGHTSEA Stereo Microscope Fluorescence Adapter is an economical system that adds fluorescence capability to existing stereo microscopes and is rugged enough for use in field laboratories in remote locations.

The images below are coral polyps viewed through a stereo microscope, with each pair, white-light (left) and fluorescence (right) showing the same area on settlement tiles. These were made by Dr. Alina Szmant (UNCW) during a research project with NIGHTSEA's Charles Mazel to develop fluorescence tools for coral recruitment research.



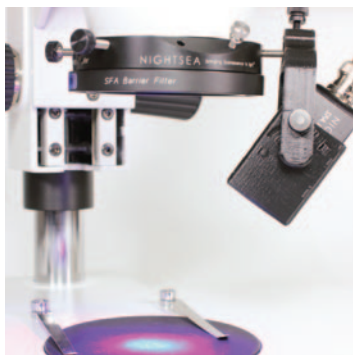
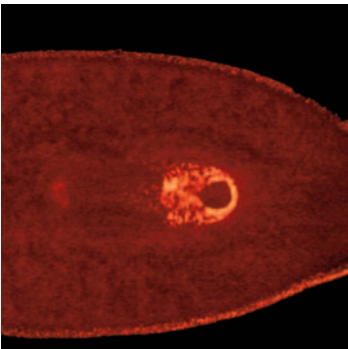
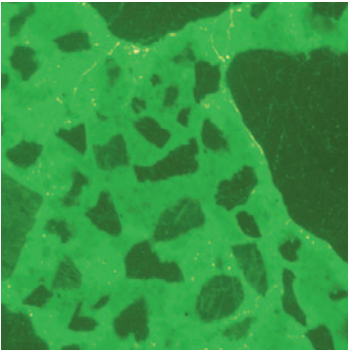


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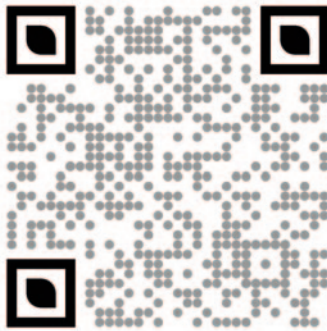
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