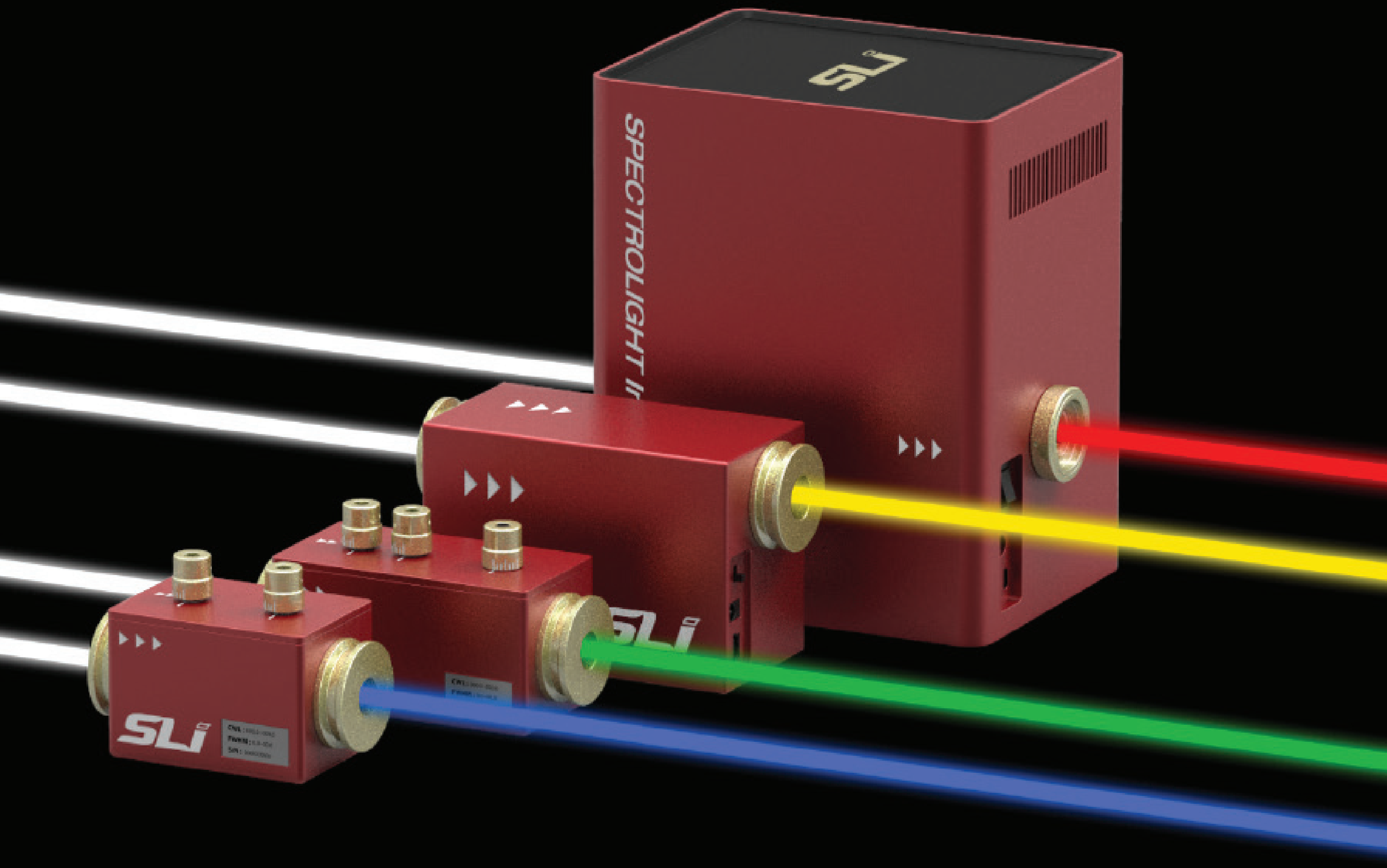
Innovating the tunable light source

How the tunable laser system is applied in fluorescence microscopy

A white paper by
SPECTROLIGHT Inc.



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Innovating the tunable light source - How the tunable laser system is applied in fluorescence microscopy

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Suppose you have a basic light microscope consisting of white light, a series of objective lenses (4X, 10X, 20X, 40X, etc.), and a charge coupled device (CCD). This microscope only gives you bright-field images. However, you would like to conduct fluorescence experiments with mammalian cells or fluorescent molecules.

What you need is Spectrolight's FWS-Poly. If you use our FWS-Poly, you can convert a light microscope into an excellent fluorescence microscope without drastic modification of the existing one. Only a few optical parts are required to create a fluorescence microscope. Once you construct a fluorescence microscope with our FWS-Poly, you can perform various experiments since the FWS-Poly enables wide applications.

A commercial fluorescence microscope uses a tungsten lamp, mercury lamp, or LED as a light source, and it is connected to the microscope through a light guide or fixed directly to the body. In the case of a confocal microscope, lasers in the visible range are usually included, such as 405 nm, 488 nm, 561 nm, and 640 nm, and as the number of lasers increases, the size of the entire microscope system and the price increases.

In a fluorescence microscope using a lamp, light is selected from a filter cube in a turret. Excitation filters are needed to select the excitation light of the desired wavelength from the light source from all areas of visible light and part of IR. Dichroic beam splitters are required to separate the excitation light reflected or scattered along with the fluorescence from the sample, and emission filters are required to select only the desired fluorescence range and have it sent to the CCD.

To obtain a clearer fluorescence image, an additional filter wheel is installed in front of the CCD. The fluorescent filter system has the advantage of being inexpensive and having a relatively simple configuration. However, since the spectral range is quite wide due to the intrinsic properties of the filter, the number of filters that can be used in the visible range is limited, which means that the available fluorophores are also limited. In addition, there is a high risk of damage to the filter if a strong excitation light source is used due to the damage threshold problem.

*Spectrolight's FWS-Poly is an innovative wavelength selector that **can be used for both excitation and emission** wavelength selection. With this product, you can convert a very basic light microscope into an effective fluorescence microscope.*

All light sources in general, can be used as light sources in fluorescence microscopy. If FWS-Poly (385 ~1015 nm) or FWS-Poly-SWIR (1015 ~ 1650 nm) is connected, any wavelength can be selected and used as excitation light. (Figure 1). FWS-Poly products have a minimum bandwidth of 3 nm and can be widened to a maximum of 15 nm. This conversion is entirely automatic and can quickly adjust wavelength and bandwidth in real-time through the software provided.

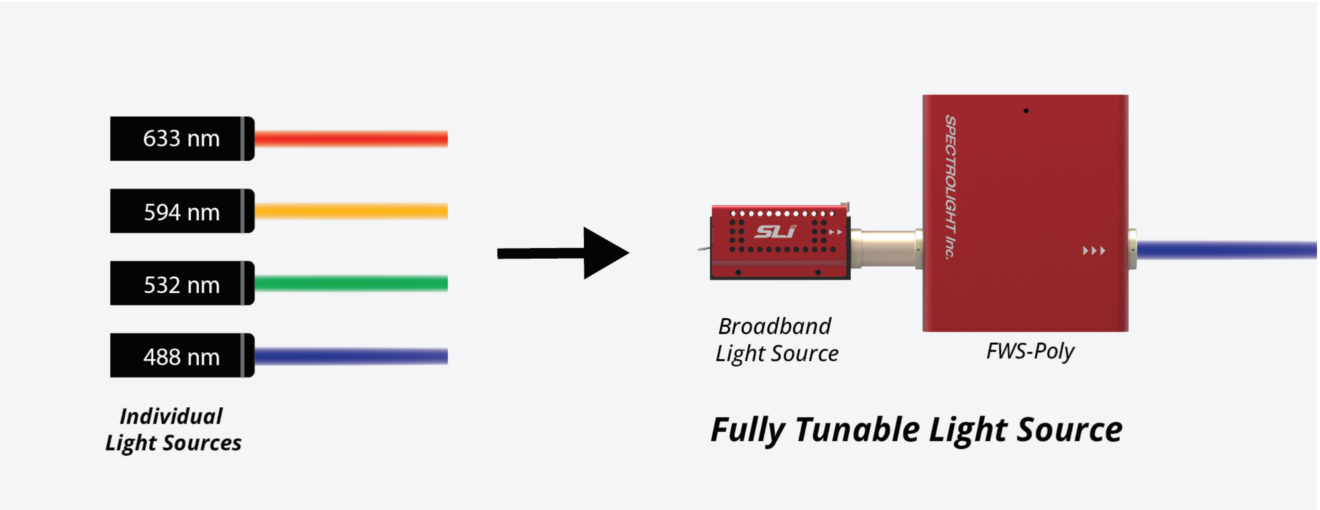

Figure 1. FWS-Poly with broadband light sources can be used as a tunable light source (TLS) instead of a bundle of individual light sources.

To use entire range of visible light (or sometimes near IR), users have to use a strong light source with a few Watt outputs. To change wavelength for various fluorophores, the filters and dichroic beam splitters in the filter turret should be replaced every time, or another laser line should be installed. This is cumbersome and cost-ineffective. However, a tunable light source clearly solves this problem. Generally, a tunable light source is a unit that can manually or automatically tune wavelength and bandwidth. Recently, many fully-automated products have been developed, enabling fast, accurate, and precise adjustment. As a result, one can use any kind of fluorophores without replacing filter cubes and spectral overlap.

Spectrolight Inc. provides two types of tunable light sources, the Tunable Laser System (TLS) and the Tunable Mighty Light (TML). The difference between TLS and TML is that TLS uses a pulsed laser, and TML uses a continuous wave (CW) light source. (Figure 2). As a result, the output beam from TLS is coherent, whereas that from TML is incoherent. Thus, users can choose light sources according to their applications. If a supercontinuum laser is used, a pulse width of 100 ps can be obtained, which can be used for time-correlated single photon counting (TCSPC), enabling fluorescence lifetime imaging (FLIM).

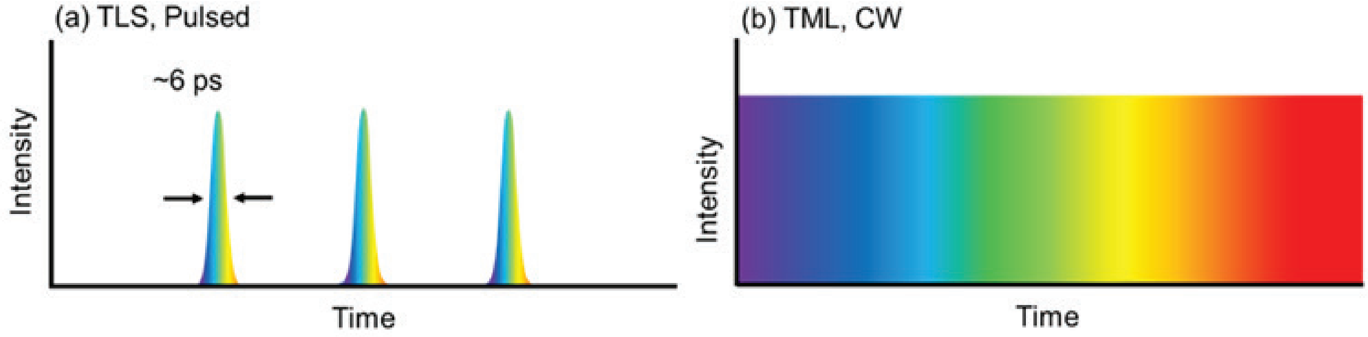

Figure 2. (a) Tunable light source (TLS) as a tunable pulse laser (b) Tunable mighty light (TML) as an imaging light source.

FWS-Poly acts as an effective detection filter when installed in front of the detection module. In the case of a commercial microscope, various emission filters are mounted on a cube inside the turret and used in a rotating form. Since FWS-Poly can freely select the detection wavelength, it is easy to detect the signal of the desired wavelength without a turret. Therefore, a filter system for detection can be configured with a simple connection without using a separate filter wheel. (Figure 3).

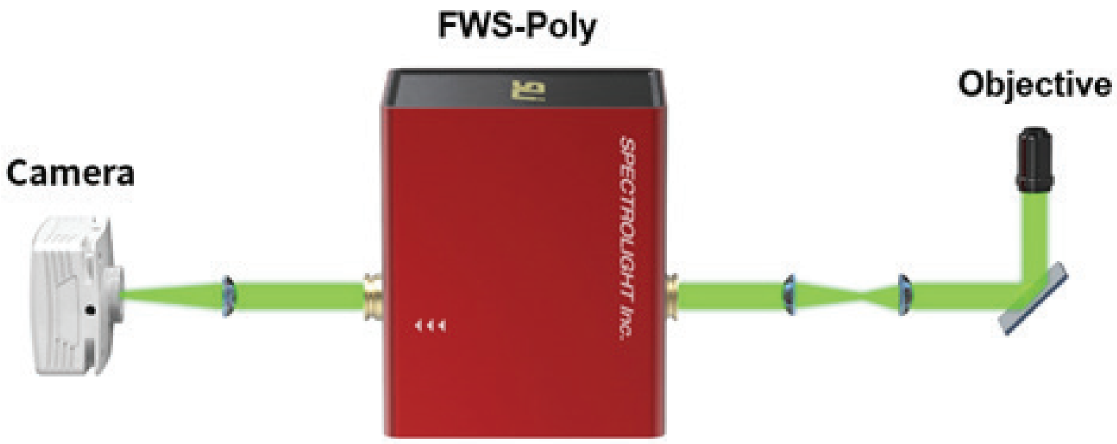

Figure 3. An example of configuration for fluorescence detection system

Since the light passing through the FWS-poly reaches the sample by a light guide, it can be used without the need for an excitation filter. Similarly, the fluorescence from the sample and the reflected excitation light passes through another FWS-Poly, and only the fluorescence in the required range can be selected and sent to the CCD, no emission filter or filter wheel is required. Figure 4 shows the FWS-Poly fully installed with a basic microscope's excitation and emission parts.

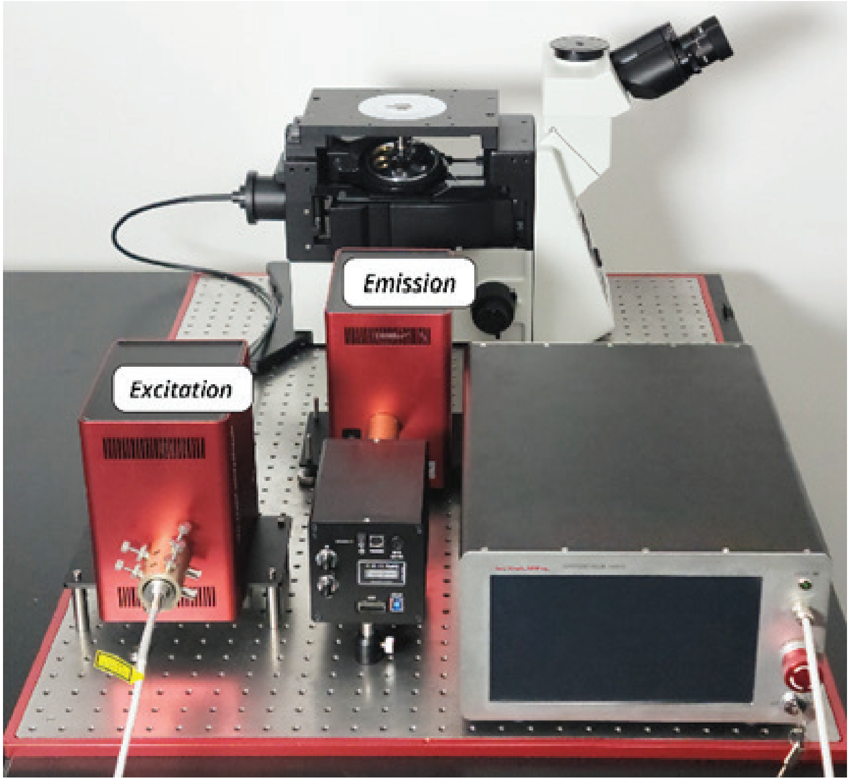

Figure 4. A picture of installed FWS-polys to our basic microscope. One is for the excitation coupled with the supercontinuum laser, and the other is for the emission selection.

These systems can be very useful for multi-color imaging of multiple labeled fluorophores. After labeling, different parts of cells with different fluorophores can be imaged simultaneously using FWS-Poly. Figure 5 shows fluorescence images of fixed HeLa cells labeled with DAPI (nucleus), CMFDA green (cytosol), and Mitotracker deep Red FM (mitochondria), respectively. Simultaneous three-color imaging can be done using the FWS-Poly instead of three different filter sets or time-consuming scanning by a monochromator.

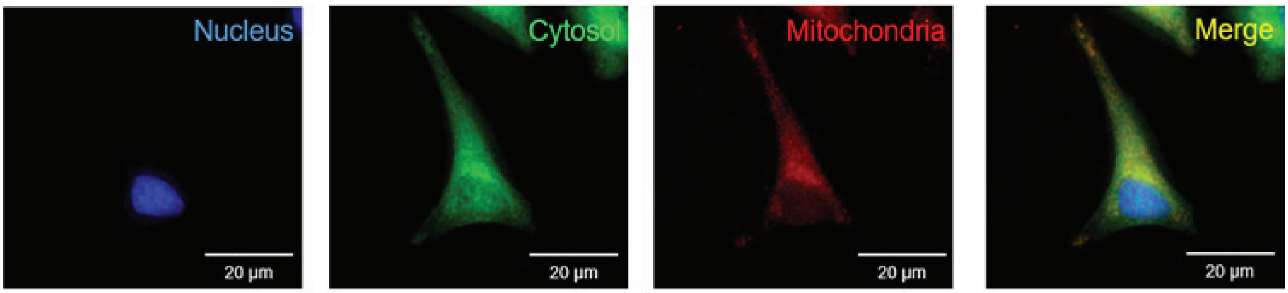

Figure 5. HeLa cells labelled with DAPI, CMFDA green, and Deep Red showing localization of the nucleus, cytosol and mitochondria, respectively. The images were obtained by using an epi-fluorescence microscope equipped with the 60X objective lens.

Using FWS-Poly widens the pool of the available fluorophore and provides flexibility in the adjustment of wavelength range and bandwidth. In addition, it is possible to create a high-performance fluorescence microscope on a lower budget than when purchasing a commercial fluorescence microscope. If you are hesitating to purchase a fluorescence microscope due to a limited budget, the FWS-Poly system from Spectrolight Inc. can be an excellent choice.

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FWS-Poly

- Transmission: > 75% (in proportion to an input light power / FWHM > 10 nm)
- Spectral range : 255 ~ 1650 nm
- Center wavelength accuracy : See table below
- FWHM range
  - 255 ~ 890 nm : 3 ~ 15 nm
  - 880 ~ 1500 nm : 5 ~ 15 nm
  - 1475 ~ 1650 nm : 7 ~ 13 nm
- FWHM resolution : 1 nm
- Out-of-band blocking : OD 6 (0.00001 % transmission)

Item Number	Spectral Range	C.W.A
FWS-Poly-SC	385 ~ 1015 nm	0.5 nm
FWS-Poly-UV	255 ~ 400 nm	0.5 nm
FWS-Poly-VIS	350 ~ 890 nm	0.5 nm
FWS-Poly-NIR	615 ~ 1015 nm	0.5 nm
FWS-Poly-SWIR	1005 ~ 1650 nm	1 nm
FWS-Poly-IR Plus	615 ~ 1650 nm	1 nm
FWS-Poly-Custom	Custom range	0.5 / 1 nm

**Note : For the optimal performance of Wavelength Selectors, the incident light should be collimated.*
**Note : FWS-Poly-UV can block up to 600 nm.*
**C.W.A = Center Wavelength Accuracy*

FWS-Mono

- Transmission: > 75%
(in proportion to an input light power / FWHM 10 nm)
- Spectral range : 255 ~ 1650 nm
- Center wavelength accuracy : See table
- FWHM range
  - 255 ~ 890 nm : 3 ~ 15 nm
  - 880 ~ 1500 nm : 5 ~ 15 nm
  - 1475 ~ 1650 nm : 7 ~13 nm
- FWHM resolution : 1 nm
- Out-of-band blocking : OD 6 (0.00001 % transmission)

Item Number	Spectral Range	C.W.A
FWS-Mono-F00	255 ~ 290 nm	0.5 nm
FWS-Mono-F01	280 ~ 310 nm	0.5 nm
FWS-Mono-F02	310 ~ 350 nm	0.5 nm
FWS-Mono-F03	348 ~ 390 nm	0.5 nm
FWS-Mono-F04	385 ~ 435 nm	0.5 nm
FWS-Mono-F05	430 ~ 490 nm	0.5 nm
FWS-Mono-F06	485 ~ 550 nm	0.5 nm
FWS-Mono-F07	545 ~ 620 nm	0.5 nm
FWS-Mono-F08	615 ~ 700 nm	0.5 nm
FWS-Mono-F09	690 ~ 790 nm	0.5 nm
FWS-Mono-F10	775 ~ 890 nm	0.5 nm
FWS-Mono-F11	880 ~ 1015 nm	1 nm
FWS-Mono-F12	1000 ~ 1150 nm	1 nm
FWS-Mono-F13	1140 ~ 1310 nm	1 nm
FWS-Mono-F14	1300 ~ 1500 nm	1 nm
FWS-Mono-F15	1475 ~ 1650 nm	1 nm

**Note : For the optimal performance of Wavelength Selectors, the incident light should be collimated.*
Note : F00-F02 models can block up to 600 nm.
**C.W.A = Center Wavelength Accuracy*

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Tunable Laser System (TLS)

**Tunable Laser System (TLS) consists of -*
Supercontinuum Laser light source, FWS-Poly, Software PC, All neccessary linker accessories

/ *TLS-Pico-B Specifications*

Total power : >8 W
Visible power : > 1 W
Spectral range : 430 ~ 1050 nm
FWHM range : 3 ~ 15 nm (above 880 nm : 5 ~ 15 nm)
Fundamental pulse width : ~ 100 ps
Internal repetition rate : 10 kHz - 80 MHz adjustable
Beam diameter and quality : ~ 2 mm@633nm;M2<1.1
Beam divergence (Half angle) : < 1mrad
State of polarization : Unpolarized

/ *TLS-Pico-M-B Specifications*

Total power : >7 W
Visible power : > 2 W
Spectral range : 410 ~ 1050 nm
FWHM range : 3 ~ 15 nm (above 880 nm : 5 ~ 15 nm)
Fundamental pulse width : ~ 6 ps
Internal repetition rate : 80 MHz
Beam diameter and quality : ~ 2 mm@633nm;M2<1.1
Beam divergence (Half angle) : < 1mrad
State of polarization : Unpolarized

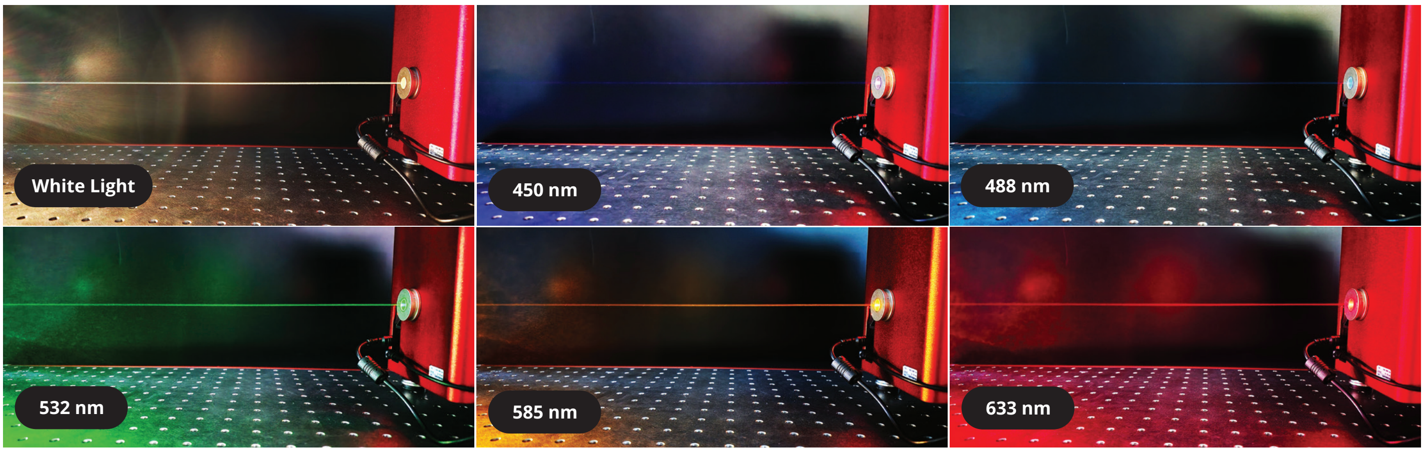
/ *TLS-Pico-C Specifications*

Total power : >8 W
Visible power : > 1 W
Spectral range : User defined (430 ~ 1650 nm)
FWHM range : 3 ~ 15 nm (above 880 nm : 5 ~ 15 nm)
Fundamental pulse width : ~ 100 ps
Internal repetition rate : 10 kHz - 80 MHz adjustable
Beam diameter and quality : ~ 2 mm@633nm;M2<1.1
Beam divergence (Half angle) : < 1mrad
State of polarization : Unpolarized

/ *TLS-Pico-M-C Specifications*

Total power : >7 W
Visible power : > 2 W
Spectral range : User defined (410 ~ 1650 nm)
FWHM range : 3 ~ 15 nm (above 880 nm : 5 ~ 15 nm)
Fundamental pulse width : ~ 6 ps
Internal repetition rate : 80 MHz
Beam diameter and quality : ~ 2 mm@633nm;M2<1.1
Beam divergence (Half angle) : < 1mrad
State of polarization : Unpolarized

Application Idea



Output result of the Tunable Laser System

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