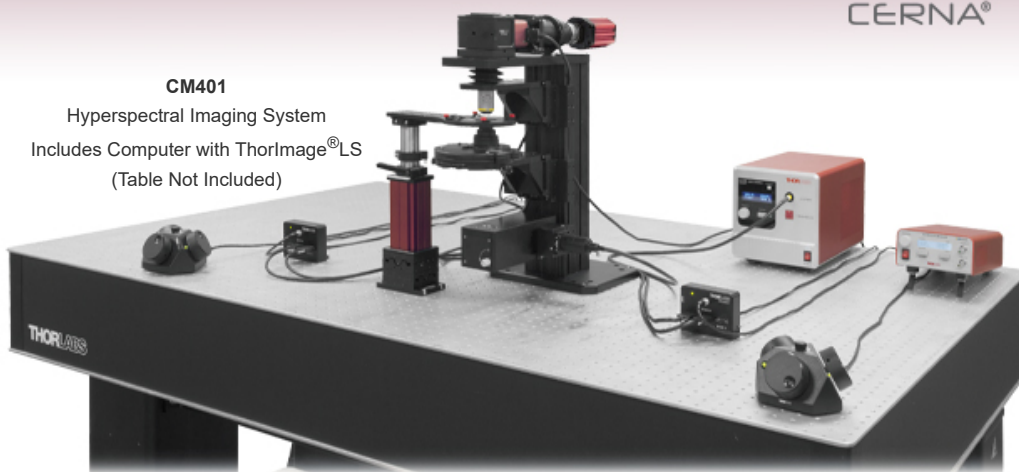# HYPERSPECTRAL IMAGING SYSTEM

► **Complete Hyperspectral Imaging Rig Based on Liquid Crystal Tunable Filter**

► **Built on Our Cerna® Microscope Platform**

CM401
Hyperspectral Imaging System
Includes Computer with ThorImage®LS
(Table Not Included)

Features

- Image at Discrete Wavelengths to Detect Multiple Co-Located Features
- Thorlabs' Kurios® Liquid Crystal Tunable Bandpass Filter for Vibrationless Wavelength Switching from 420 to 730 nm
- Low-Noise 12.3 Megapixel Monochrome Scientific CMOS Camera
- Complete System Including Computer with ThorImage®LS Software for Image Acquisition

Thorlabs' Hyperspectral Imaging Cerna Rig provides a platform for studies of samples with co-located features by rapidly acquiring signals at different wavelengths. Traditional color cameras represent the entire spectral range of an image by using three relatively wide spectral channels: red, green, and blue. In contrast, our hyperspectral imaging system uses a tunable bandpass filter that allows narrow spectral regions to be imaged separately with a monochrome camera. The result is an image with quantitative spectral data for each pixel.
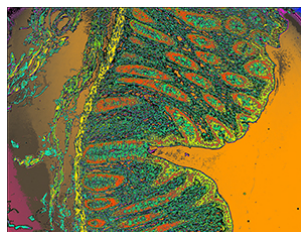
Our hyperspectral imaging system offers a number of advantages. Unlike approaches that rely upon angle-tunable filters or manual filter swapping, our Kurios filters use no moving parts, enabling vibrationless wavelength switching on millisecond timescales. Because the filter is not moved or exchanged during the measurement, the data is not subject to "pixel shift" image registration issues. Our system features fully automated wavelength switching and image capture using the included DAQ and ThorImage®LS software.

The features and specifications of our brightfield hyperspectral rig are listed on the *Specs* tab. To address a wide range of experimental parameters, Thorlabs offers several Cerna microscope configurations. In addition, we can work with you to configure a microscope that meets your unique needs.
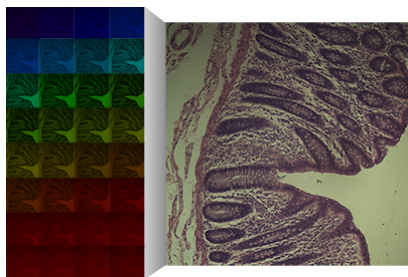

Click to Enlarge

The hyperspectral imaging system uses our Kurios tunable filter to record an image stack at discrete wavelengths over a user-selectable range. The tile images on the left show the images acquired at each wavelength (we have colored them during processing to make them easy to identify). The right image shows the reconstructed full-color image of the human glandular epithelium. For more information on hyperspectral imaging, please see the *Technique* tab.

Click to Enlarge

Our CM401 hyperspectral imaging system records a spectrum at each pixel. Since the spectrum is indicative of the sample's materials, areas of an image with the same spectrum indicate that it is composed of the same materials. This photo shows hyperspectral image stack of a human glandular epithelium. We used the Scyven software package to give each unique spectral signature a different false color to indicate which parts of the sample contain the same materials. For more information, please see the *Software* and *Technique* tabs.

| Specifications	
Microscope	Cerna® Series Microscope
Objective	Nikon 10X Plan Fluorite Objecitve (Item # N10X-PF)
Condenser	0.9 NA Air Condenser (Item # CSC1002)
Tunable Filter Specifications	
Item #	KURIOS-VB1
Wavelength Range	420 - 730 nm
Bandwidth, CWL = 550 nm[a]	Narrow: 10 nm FWHM Medium: 18 nm FWHM Wide: 32 nm FWHM
Minimum Incremental Step Size	1 nm
Tuning Accuracy	±FWHM/10
Switching Speed[b]	Narrow: <230 ms Medium: <150 ms Wide: <100 ms

Tunable Flter Performance Plots	Setting		
	Narrow	**Medium**	**Wide**
Transmission Spectrum			
Bandwidth			
Transmission at Center Wavelength			

Scientific Camera Specifications	
Item #	CS126MU
Sensor Type	Monochrome CMOS
Number of Active Pixels	4096 x 3000 (Horizontal x Vertical)
Pixel Size	3.45 µm x 3.45 µm
Imaging Area	14.131 mm x 10.350 mm (Horizontal x Vertical)
Host PC Interface	USB 3.0
Vertical Digital Binning[c]	Continuous Integer Values from 1 to 16
Horizontal Digital Binning[c]	Continuous Integer Values from 1 to 16
Exposure Time	0.028 ms to 14700.9 ms in ~0.014 ms Increments
Region of Interest (ROI)	**Frame Rate**
Full Sensor (4096 x 3000)	21.7 fps
Half Sensor (2048 x 1500)	46.1 fps
~1/10th Sensor (410 x 300)	206.1 fps
Min ROI (260 x 4)	914.4 fps

Light Source Specifications	
Item #	HPLS343 (Previous-Generation)
Type	High-Power Plasma Light Source and Ø3 mm Liquid Light Guide (LLG)
Wavelength Range	350 - 800 nm
Optical Output Power[c]	4.0 W (Typical) 3.5 W (Minimum)

Automated Stage Specifications	
1" (25.4 mm) Motorized Z-Axis Travel Nosepiece and Condenser Holders	
1" (25.4 mm) Motorized XY Travel Sample Holder	
Bidirectional Repeatability	1 µm
Backlash	1 µm
Minimum Achievable Incremental Movement	100 nm
Minimum Repeatable Incremental Movement	200 nm
Included Software	
ThorImageLS 3.1 with Lifetime Support	

- The bandpass width increases linearly with wavelength. See the Performance Plots for details.
- The switching speed depends upon the bandpass width setting and the initial and final wavelength. See the Performance Plots below for details.
- Measured at the output of the liquid light guide, when bulb and LLG are at start-of-life.

Hyperspectral Imaging

In hyperspectral imaging, a stack of wavelength-separated, two-dimensional images is acquired. This technique is frequently used in microscopy, biomedical imaging, and machine vision, as it allows quick sample identification and analysis.

Hyperspectral imaging obtains images with significantly better spectral resolution than that provided by standalone color cameras. Color cameras represent the entire spectral range of an image by using three relatively wide spectral channels—red, green, and blue. In contrast, hyperspectral imaging systems incorporate optical elements such as liquid crystal tunable bandpass filters or diffraction gratings, which create spectral channels with significantly narrower bandwidths.
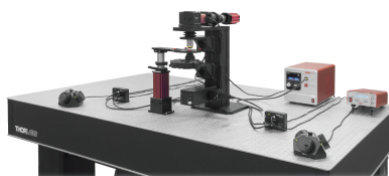
Click for Details
Our CM401 hyperspectral imaging system is built on Thorlabs' Cerna Microscopy Platform. Key components include the
KURIOS-VB1 Tunable Bandpass Filter, the CS126MU Monochrome Scientific Camera, and our previous-generation HPLS343 High-Power Plasma Light Source.
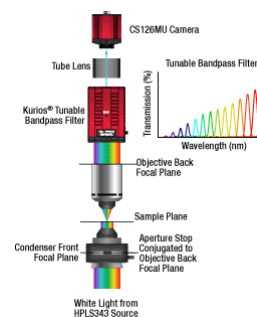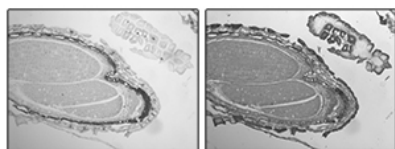
Click to Enlarge
Schematic of the Hyperspectral Imaging Microscope

We have adapted our Cerna® microscopy platform, Kurios® tunable filters, and scientific-grade cameras to build a rig specifically designed for hyperspectral imaging.

Example Image Stacks

The data in the images and video below demonstrate the hyperspectral imaging technique. Figure 1 depicts two images of a mature *capsella bursa-pastoris* embryo (also known as shepherd's-purse) taken with the tunable filter set to center wavelengths of 500 nm and 650 nm. These two images show that an entire field of view is acquired at each spectral channel. Figure 2 is a video containing 31 images of the same sample, taken at center wavelengths from 420 nm to 730 nm in 10 nm steps. (10 nm is not the spectral resolution; the spectral resolution is set by the FWHM bandwidth at each wavelength.) In Figure 3, images from each spectral channel are used to determine the color of each pixel and assemble a color image. Figure 3 also demonstrates that a broadband spectrum is acquired at each pixel, permitting spectroscopic identification of different sample features within the field of view.

Kurios tunable filters offer a number of advantages for hyperspectral imaging. Unlike approaches that rely upon angle-tunable filters or manual filter swapping, Kurios filters use no moving parts, enabling vibrationless wavelength switching on millisecond timescales. Because the filter is not moved or exchanged during the measurement, the data is not subject to "pixel shift" image registration issues. Our filters also include software and a benchtop controller with external triggers, making them easy to integrate with data acquisition and analysis programs.
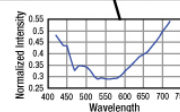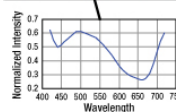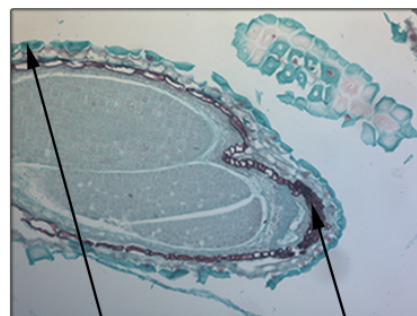
500 nm   **650 nm**

Click to Enlarge
Figure 1: Two images of a mature *capsella bursa-pastoris* embryo taken at different center wavelengths. The entire field of view is acquired for each spectral channel.

Click to Enlarge
Figure 3: A color image of the mature *capsella bursa-pastoris* embryo, assembled using the entire field of view acquired in each spectral channel, as shown in Figure 1. By acquiring across multiple channels, a spectrum for each pixel in the image is obtained.

Identifying Component Compounds Using the Scyven Software Package

Since spectral data is saved for every pixel, an image can be processed to show regions with similar spectra in a false color. Since the spectra should be indicative of the compounds present in that region of the image, these images indicate which regions of the sample contain the same materials. We recommend the Scyven software package for this analysis; more details about software for acquiring and analyzing hyperspectral image stacks can be found on the *Software* tab.

ThorImage®LS Software

**Comprehensive Imaging Platform for:**

- Hyperspectral Imaging (CM401 Hyperspectral Imaging System)
- Multiphoton Imaging (Bergamo® II)
- Confocal Imaging
- Multi-Modality Image Control

Advanced Software Functionality

- Multi-Column Customizable Workspace
- Image Acquisition Synced with Hardware Inputs and Timing Events
- Live Image Correction and ROI Analysis
- Multi-User Settings Saved for Shared Workstations

ThorImageLS is an open-source image acquisition program that controls Thorlabs' Cerna® based hyperspectral imaging system, as well as Bergamo II and confocal microscopes. It also controls supplementary external hardware. ThorImageLS provides an integrated, modular workspace tailored to the individual needs of the scientist. Its workflow-oriented interface supports single image, Z-stacks, time series, and image streaming acquisition, vizualization, and analysis. ThorImageLS is included with a Thorlabs microscope purchase and open source, allowing full customization of software features and performance. ThorImageLS also includes Thorlabs' customer support and regular software updates to continually meet the imaging demands of the scientific community. A summary of features used with the hyperspectral imaging system is below; for more information on the general features of ThorImageLS, please download our brochure.

Capturing Images
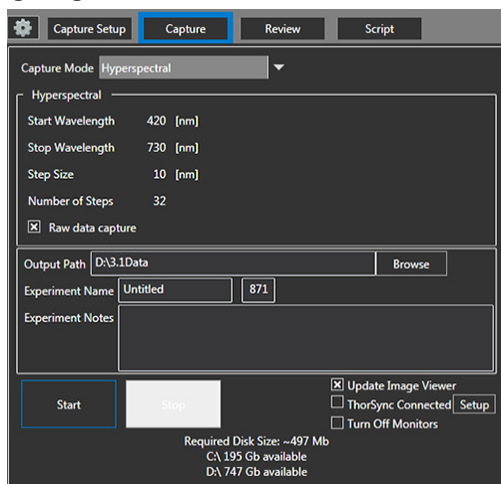
As is shown in the Capture tab screenshot, the Kurios tunable filter's starting wavelength, stopping wavelength, and wavelength step size can be set depending on the needs of the experiment.
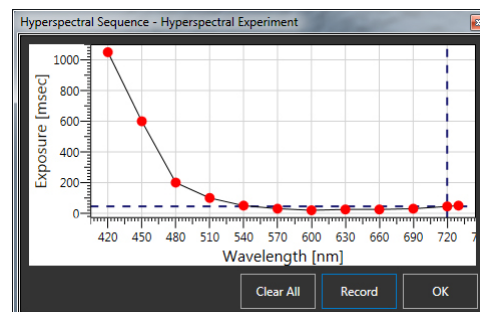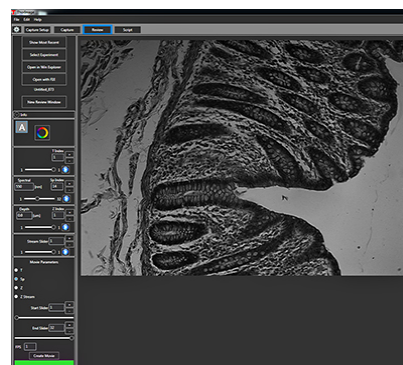

Since the tunable filter's transmission varies with wavelength, the software allows for a different exposure to be used at each wavelength step; the user can set several exposure levels manually as shown by the red dots in the plot; this curve is interpolated for the exposure values at intermediate wavelength steps.

Reviewing and Analyzing Results

ThorImageLS displays the recorded hyperspectral dataset in the review pane. Here, a slider in the control pane on the left of the screen shows the wavelength of the current image and allows the user to scroll across the set of wavelengths to see each monochrome image. The "info" panel allows for a quick review of all of the acquisition settings used for the experiment. These features make it easy to check images and confirm settings immediately after acquisition is complete.

Images can be saved in a TIFF stack for analysis with ImageJ or MATLAB. If the raw format is selected, then the data can be analyzed using Scyven, which is free software. Features of Scyven include the ability to view spectra for a pixel or region, detect the materials in a sample (see the *Technique* tab for more details), and classify samples using a user-defined spectral library.

Click to Enlarge
Review Tab

https://thorlabs.nt-rt.ru/ || tbe@nt-rt.ru