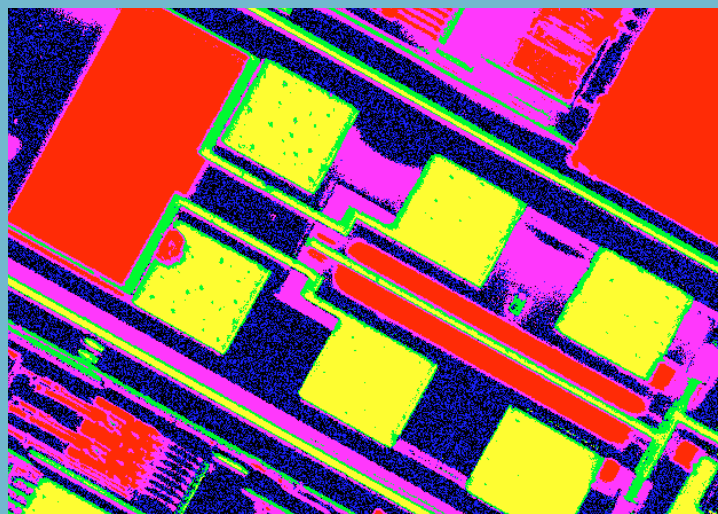


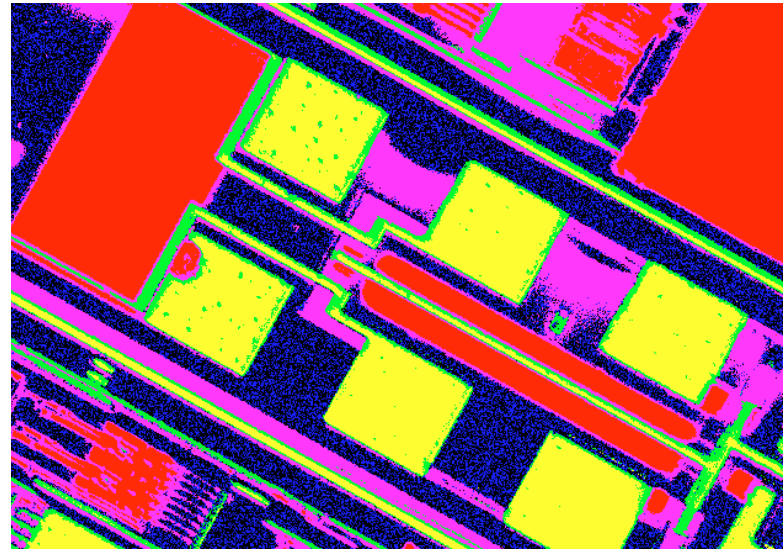


Application Note:

Hyperspectral Microscopy



In this application note we demonstrate a hyperspectral microscope by combining some of our products. Physically, it's an imaging reflectance spectrometer: at each point of the sample a reflectance spectrum is generated.



1 Segmented image of a semiconductor chip

Introduction

Hyperspectral and Multispectral Imaging (HSI, MSI) have seen increasing interest in recent years due to the availability of improved hardware that facilitates hyperspectral data generation for a variety of applications. HSI and MSI are being applied very successfully in the geosciences using satellites equipped with appropriate cameras. By this, it is possible to monitor vegetation, soil or humidity which is useful for agriculture and climate research. More recently, it has become popular to use HSI with drones in agriculture. This is possible now due to the development of light and compact HSI cameras.

Other important applications include quality control in food processing, security, medical applications, materials sciences, or waste recycling. The general concepts of HSI and MSI have been known for long as spectral imaging or imaging spectroscopy, and may be realized in many different technical ways, depending on the application. Examples are Infrared and Raman Microscopy, Imaging Polarimetry, Roentgen or Terahertz scanners on airports etc.

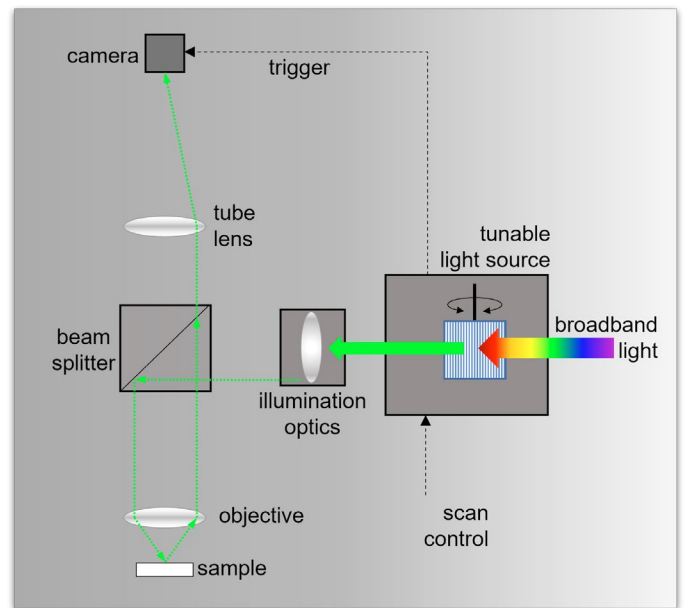
The term *hyperspectral* is being used to describe the generation of images of a scene at a multitude of continuous, narrow spectral bands ("channels"). When the number of spectral bands is lower and at discrete wavelengths, often the term *multispectral imaging* (MSI) is being used instead ¹. The so obtained (hyperspectral) *data cube* is then processed with dedicated algorithms. Typically, the goal is to segment the image into areas of the same composition or material.

A hyperspectral data cube provides a spectrum at each pixel of the image. Thus, instead of the red, green, and blue image planes of a color camera image, one gets in the order of 10^6 spectra. If these spectra are recorded in a suitable, sensitive wavelength range, it is obvious that such a huge amount of data allows a much more detailed analysis of composition, material, density, surface texture or other structural parameters of the objects observed.

¹ https://en.wikipedia.org/wiki/Hyperspectral_imaging#Distinguishing_hyperspectral_from_multispectral_imaging

The Hyperspectral Microscope

One way to upgrade a conventional optical microscope to hyperspectral operation is by adding a tunable light source providing narrow-band illumination. The data cube is generated by scanning over a wavelength range while collecting images of the sample. This straight-forward approach is possible with static samples where one can afford a relatively slow sequential acquisition of the images. In other applications, like airborne HSI from drones using natural sunlight, the spectral dispersion usually happens in front of the camera with special optics. Snapshot hyperspectral cameras are available, providing instant spectral imaging, yet at the cost of resolution. Advantages of the wavelength scanning approach are the overall simplicity, the flexibility to change the number of wavelength channels, and the possibility to work at the full resolution of a monochrome camera.



2 Scheme of a wavelength scanning HSI microscope



Your contacts at lightsource.tech:

+49 (0) 551/270765-0
info@lightsource.tech

A scheme of the concept of our microscope is shown above: on the left side, a microscope with collinear bright-field illumination is shown. A beam splitter directs light from a source towards the sample, which is partly reflected there and imaged onto a camera using microscope optics. Usually, this is constructed of an infinity corrected objective lens and a tube lens.

Instead of white light, we use narrow band, "monochromatic"² light. To get this, the broadband light from a source is spectrally dispersed by a grating monochromator. The illumination optics block ensures that the light is properly introduced into the microscope (see below).

A computer interface and software allow automatically scanning the wavelengths.

² bandwidth < 10 nm FWHM

Hardware details

Instead of the refractive optics shown above, we have used a reflective objective (our LD 10x/0.25 modified Schwarzschild objective). A reflective objective lens exhibits no chromatic distortion. This means that focus and image magnification remain constant over a much wider wavelength range than with refractive glass lenses. This is advantageous if we want to collect hyperspectral images, especially if UV or NIR images shall be captured. The LD 10x/0.25 works in a range of 200 – 2000nm, which in principle allows to collect spectral information in the DUV-UV-VIS-NIR range. Note that this device is normally attached to a vacuum or processing chamber. Here, we use it on an improvised stand for benchtop operation in the lab.

As a wavelength-tunable light source we added our fiber-to-fiber Monochromator-F, equipped with either a laser-driven plasma lamp (ISTEQ XWS30 or Energetiq EQ99-X with our fiber coupler) or our LS-WL1 / LS-BB1 light sources.

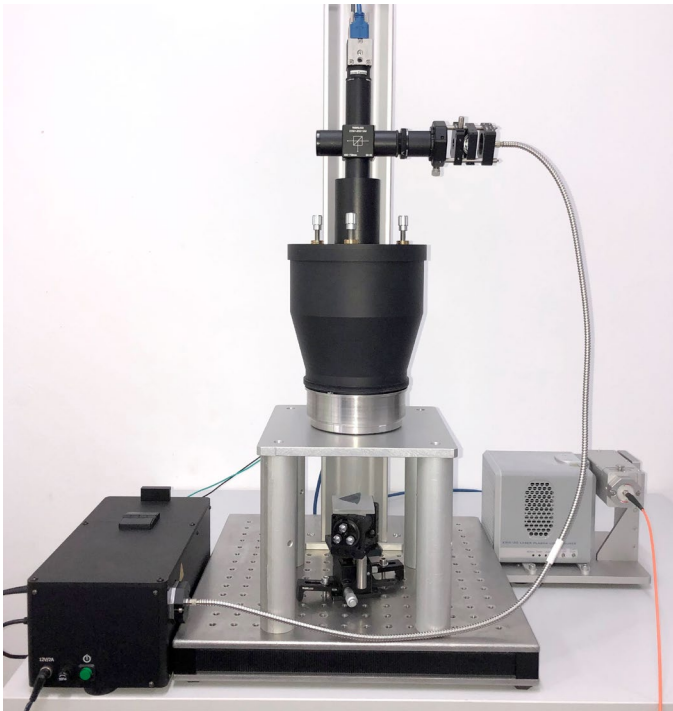
Depending on the chosen monochromator grating and diffraction order, a bandwidth of the light of 3-6nm FWHM is used in our setup. In all cases, the fiber coupled sources provided convenient attachment to the microscope and act as point sources with high luminance.

Why are we not shining the light from the side onto the sample, like in a normal stereo microscope? That would work for diffusely reflecting samples. But for glossy samples like semiconductor chips, this would constitute darkfield illumination: only the scattering from edges etc. would enter the objective. We want to image the glossy areas, too, so we need brightfield illumination with a common path of illumination and detection.

Additionally, we want to be able to perform quantitative spectroscopy on the samples. This means that the cone of incoming light rays (i.e., the angle distribution) should be roughly the same for each object point. Thus, it is important to establish Köhler illumination³ (or nearly...) with appropriate optics on the entrance port.

A challenge for very broadband HSI is in the availability of suitable beamsplitter cubes (BSC). Polka-dot types of BSC in principle work in the full range of the reflective objective. However, in practice we have seen imaging artifacts then, so we use a standard VIS BSC for our setup. In a more advanced setup one could switch between UV-, VIS- and NIR-optimized BSC.

For the demo setup presented here we have used a standard CMOS camera, limiting us to ca. 330 -1000nm. More advanced designs could use back-illuminated sCMOS cameras, covering 200-1100nm and InGaAs cameras for 900-1700+ nm. To speed up the wavelength scanning process we used the trigger-out option of the Monochromator-F to trigger frame capture immediately after each wavelength step.



3 HSI microscope, comprising:

- a) LD 10x/0.25 process microscope (normally attached to a vacuum chamber)
- b) Monochromator-F
- c) XWS30 lamp with fiber coupler
- d) Brightfield illumination optics
- e) Standard CMOS camera
- f) makeshift stand for the microscope
- g) sample on support
- h) beam splitter cube

³ https://en.wikipedia.org/wiki/K%C3%B6hler_illumination

Software and Control

Software for basic operation of the microscope has been developed using LabVIEW™ and coupled with the existing Monochromator-F GUI, offering the following functionality:

Editing sequences for wavelengths scans: overall range and wavelength step; as the light power and camera sensitivity varies a lot with wavelength, the camera's exposure time and gain may be changed from frame to frame.

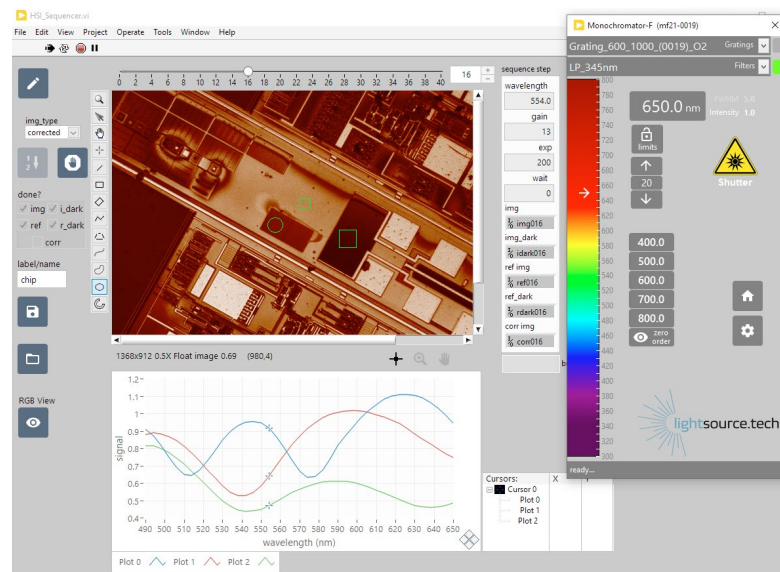
Capture and display of the acquired frames. Histogram-based automatic tuning of exposure time and gain to maintain good signal (i.e., image brightness) during wavelength scans.

Dark image subtraction (light source shutter closed) to get rid of ambient false light.

Reference sample: to be able to get reflection spectra in meaningful physical quantities (instead of grey level count), a reference sample can be measured. This not only allows to reference the camera signal to a [sample with known reflectivity](#), but also corrects for inhomogeneous field illumination. The reference sample is measured slightly out-of-focus to blur dust or other small particles. The output quantity of our software is thus the relative spectral reflectance at each point, and the image data cube is of floating-point type. Using the known reflectance of the reference sample, it would then be possible to translate this into absolute reflectance by simple multiplication.

However, the reflectance is not being measured at a single angle-of-incidence. Instead, a cone of light is incident on the sample, depending in the numerical aperture of the objective.⁴ Also polarization comes into play, especially for reflective objectives and at higher aperture. A careful analysis of the spectra could take that into account⁵.

Graph for ROI spectra: selecting multiple regions-of-interest (ROI) on the image cube and display the averaged spectra for those regions.



4 Screenshot of the control software, with the standard Monochromator-F GUI on the right.

For displaying and analysis, additional functions have been implemented:

Visualisation tool: a false color viewer, assigning selected wavelength planes to an RGB image.

Clustering of the data into classes of similar spectral content (i.e., same material) with standard clustering algorithms

For a demonstration of the software, short videos are [found here](#):

Video: Hyperspectral Microscopy Demonstrator

Video: Hyperspectral Microscopy Demonstrator false color viewer

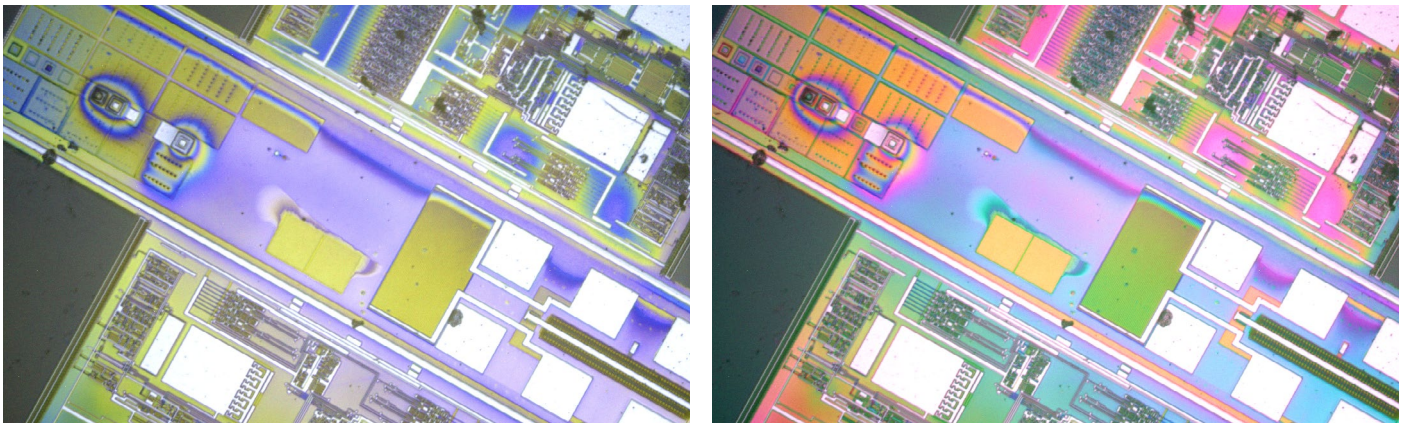
⁴ With Schwarzschild-type reflection objectives, the central obstruction of must be considered.

⁵ See for example DOI: 10.1063/1.4889879

Data Representation and Analysis

False Color Views

The 3D HSI data cube⁶ cannot be completely visualized in a single view. Instead, slices of the data along selected dimensions are shown. A simple, yet intriguing way is to visualize the spectral data in false color. To this end, an RGB color image is assembled from selected wavelength image planes. By that, features and details of the sample may become visible that are not seen in a standard RGB camera image.



5 False color image using image planes 502/506/534nm (left) and 582/502/534nm (right)

Clustering

One of the common important tasks in HSI is the **segmentation** of images by the similarity of point spectra: contiguous areas of uniform material or composition shall be identified and displayed.

All points in such an area will exhibit similar spectra. However, the question arises how to quantify such similarity. This is especially true in the presence of noise, gradients, and inhomogeneous illumination. By scaling the raw images with the help of reference images, as explained above, the latter artifacts are at least partly compensated for. Noise can partly be removed by downsampling or pixel binning.

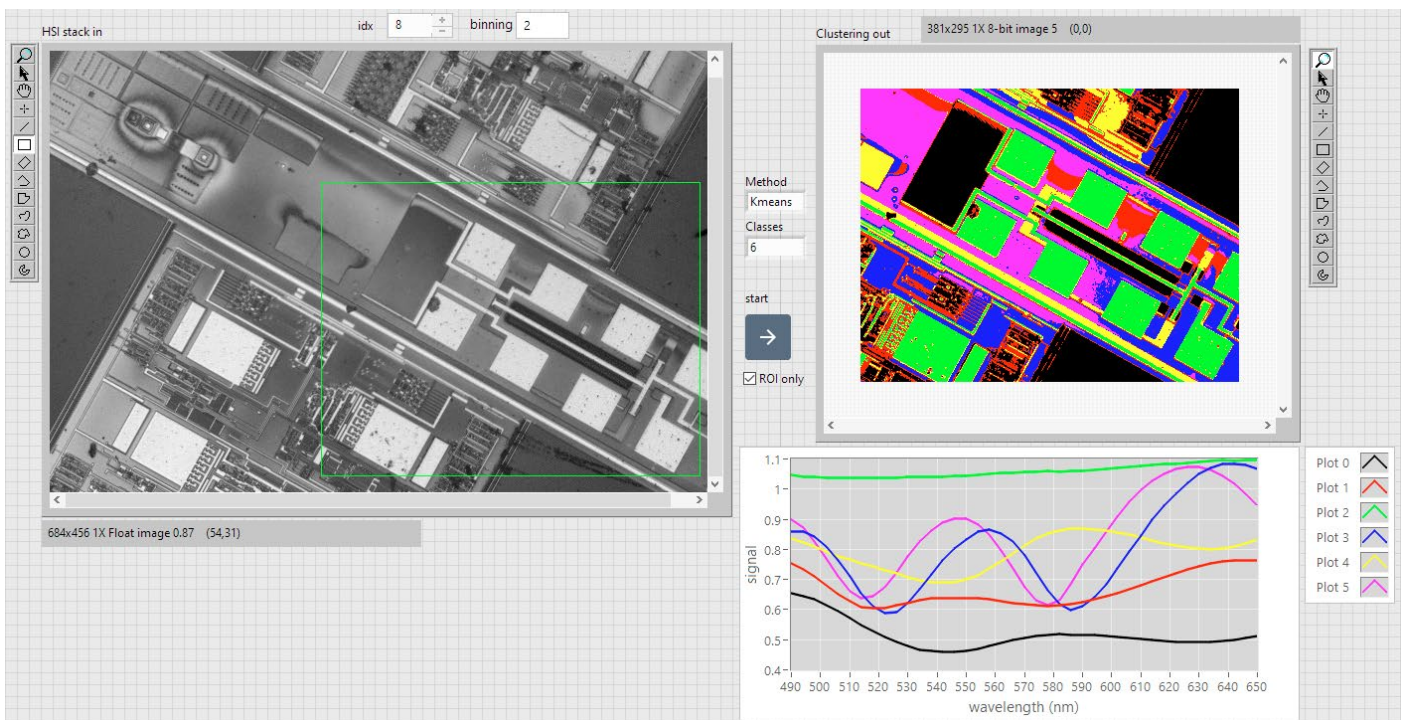
A great variety of methods and algorithms have been developed in recent years for the analysis of HSI data cubes to improve noise immunity, provide robust clustering, reduce dimensionality, and speed up calculation. Each spectrum can be seen as a vector of n elements, with n being the number of wavelength channels. The Euclidean distance between vectors is a first approach to measure similarity of spectra. However, it does not always lead to unambiguous results and a proper **clustering** of the data.

⁶ Or even 4D for HSI time series

For our demonstration we used the simple *k-means* algorithm⁷, using a predefined number *k* of classes. Each class represents a unique material and/or film thickness and is displayed in a different color. The associated averaged spectra are shown underneath. Ultimately, comparing such spectral fingerprints with known spectra from a data base or optical models would allow to identify the materials.

Conclusion

A model hyperspectral microscope for spectral investigation of small samples, e.g., processed semiconductor chips, has been assembled and basic capabilities have been demonstrated. Lightsource.tech offers the broadband tunable light sources needed for such applications. Technologie Manufaktur offers high performance imaging optics like the LD 10x/0.25 long distance microscope as well as complete integration services to build customized HSI or MSI solutions for research and industry.



6 Clustering of a region of the HSI data cube into 6 classes by *k-means*

Disclaimer for external links:

External links are being provided as a convenience and for informational purposes only. Lightsource.tech or Technologie Manufaktur bear no responsibility for the accuracy, legality, or content of the external site or for that of subsequent links. Contact the external site for answers to questions regarding its content.

⁷ https://en.wikipedia.org/wiki/K-means_clustering

Your contacts at lightsource.tech:

+49 (0) 551/270765-0
info@lightsource.tech



Dr. Dirk Hönig (left) – Specialist fields: optical and measurement technologies

Dr. Jan Thirase (right) – Specialist field: optical design and optical systems engineering