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INTRODUCTION

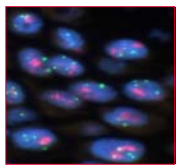
Classical monochrome or colour imaging and spectroscopy have independently proven to be valuable methods for a wide range of applications, including life sciences. While classical monochrome / colour images contain no to little spectral information, spectroscopy is severely limited when it comes to measuring inhomogeneous samples. **Spectral Imaging (SI)** combines both technologies, allowing to simultaneously study spatial and spectral features both qualitatively and quantitatively. The development of cost-effective imaging spectrographs, tune-able filters and both fast and highly sensitive cameras make these systems now available at competitive prices and thus form the basis for the practical implementation of high-performance SI-applications.

The two prevalently used principles to acquire optical "hyper-spectral images" are *wavelength scanning SI*, in remote sensing better known as "staring imagers", and *spatial scanning SI*, also known as "push-broom scanning". Both methods have their specific merits, and it depends very much on the application which principle is the preferable solution.

Thus, in particular when combined with advanced, fast and application-optimised statistical data evaluation algorithms it is nowadays possible to analyse a wide range of samples with full spectral and spatial resolution.

HYPER-SPECTRAL IMAGING SENSING

SAMPLES



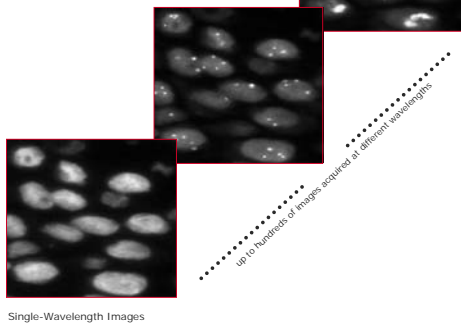
RGB image of an fluorescent *In Situ* Hybridization (FISH) preparation co-stained with three different fluorescent dyes

Spectral Image Acquisition

Simultaneous acquisition of two spatial dimensions, Sequential acquisition of the spectral dimension.

System Characteristics

- spectral resolution 7nm
- spatial resolution depends on the used optical components and the camera



(temporary) storage
SI DATA ACQUISITION

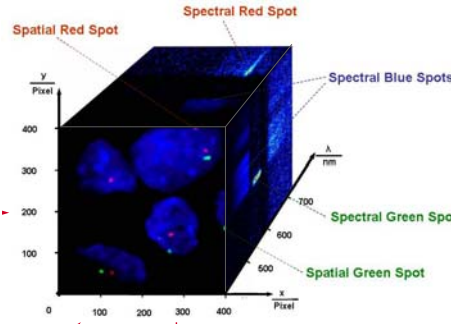


image stack
spectra extraction
SPECTRAL DATA PROCESSING

1) Pre-Processing

- ❖ **Standardisation**
 - dark current subtraction, dead pixel correction
 - reference scaling
- ❖ **Data Reduction**
 - redundant data binning (selective/global)
 - data correlation reducers (wavelet analysis, ...)
- ❖ **Data Preparation**
 - 1st/ 2nd derivative
 - normalisation (classification applications only)
 - PC dimensionality reduction

2) Spectral Data Evaluation

- Spectral Unmixing
- Spectral Classification

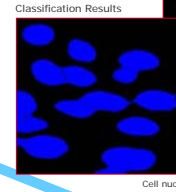
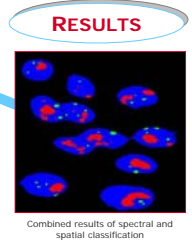


IMAGE POST-PROCESSING

Image Processing

- ❖ **RGB Image Generation**
 - virtual combination of subsequent SI sensor results
- ❖ **Data Reduction**
 - image segmentation
 - transformation to object level
- ❖ **Image Data Processing**
 - artefact removal
 - shape and pattern recognition
 - defect classification (defect size, distribution and frequency,...)
 - removal of object interferences (overlaps,...)



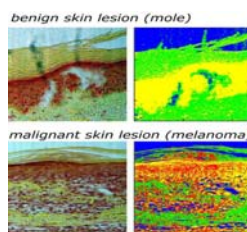
LIFE SCIENCE EXAMPLE APPLICATIONS

HYPER-SPECTRAL MICROSCOPY



Hyper-Spectral Fluorescence Microscope equipped with a liquid crystal tuneable filter (LCTF), a metal-halide fluorescence light source and an EMCCD camera

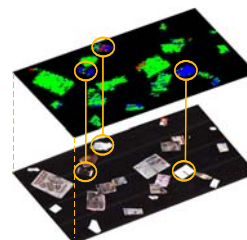
MEDICAL DIAGNOSTICS



Histology & tissue diagnostics: objectified detection of cancerous and pre-cancerous areas in tissue slide preparations; *VIS transmission*

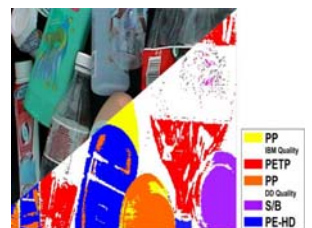
INDUSTRIAL EXAMPLE APPLICATIONS

PAPER RECYCLING



On-line classification and sorting: of different types of recovered paper on a conveyor belt; *NIR diffuse reflection*

POLYMER WASTE RECYCLING



On-line material-specific classification and sorting: automatic material sorter for plastic and/or paper waste; *NIR diffuse reflection*

CONCLUSION

Spectral Imaging has proven to be a valuable method for applications in both industry and life-sciences. The combined evaluation of spectroscopic and spatial information provides a wealth of information hardly accessible with other methods. Thus, SI allows a precise determination of materials or the measurement of parameters not (easily) measurable with classical imaging or spectroscopy systems. Targeted applications range from using NIR-SI for label-free investigation of tissues and tissue preparations to UV fluorescence imaging. The aim is now to establish these methods in practice and provide, together with partners active in these fields, systems that could be used e.g. in routine health screening.

OUTLOOK

Future applications of hyper-spectral imaging will go far beyond the systems in use today. Ongoing work focuses on applications and improved systems, in particular

- ❖ non-invasive in-vivo diagnostics
- ❖ quality control applications: foodstuff, pharmaceuticals, ...
- ❖ optimisation of spectral data pre-processing and evaluation routines
- ❖ implementation of advanced image processing methods
- ❖ extension of the spectral range towards UV / fluorescence and SW-MIR