Z-project: Neuropathology

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# What can we offer ?

Expertise
- full range of histopathological analysis for both human and mouse tissue samples of CNS, muscle and nerve,
-   assistance in planning of projects, analysis and interpretation of results

Tissue processing, embedding, cutting:
-Paraffin embedding of formalin-fixed tissue
-Plastic embedding for EM
-Paraffin sections
-Frozen sections
-Vibratome sections
-Ultrathin plastic sections
-Generation of tissue microarrays

Stainings:
-standard histological stains
-Automated immunohistochemistry and immunofluorescence including antibody establishement
-in situ hybridisation
-TUNEL...
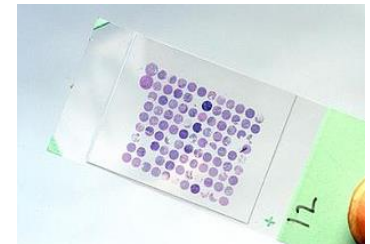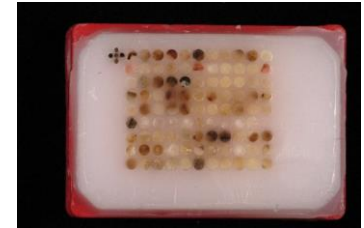
Microscopes:
- Light and fluorescence microscopes with CCD cameras

- Confocal laser scanning microscope:
LEICA TCS SP8 with white light laser (470-670nm); live cell imaging module

- SpectraCube system for spectral imaging and linear unmixing

Spectral imaging SpectraCube system (Applied spectral imaging ASI)
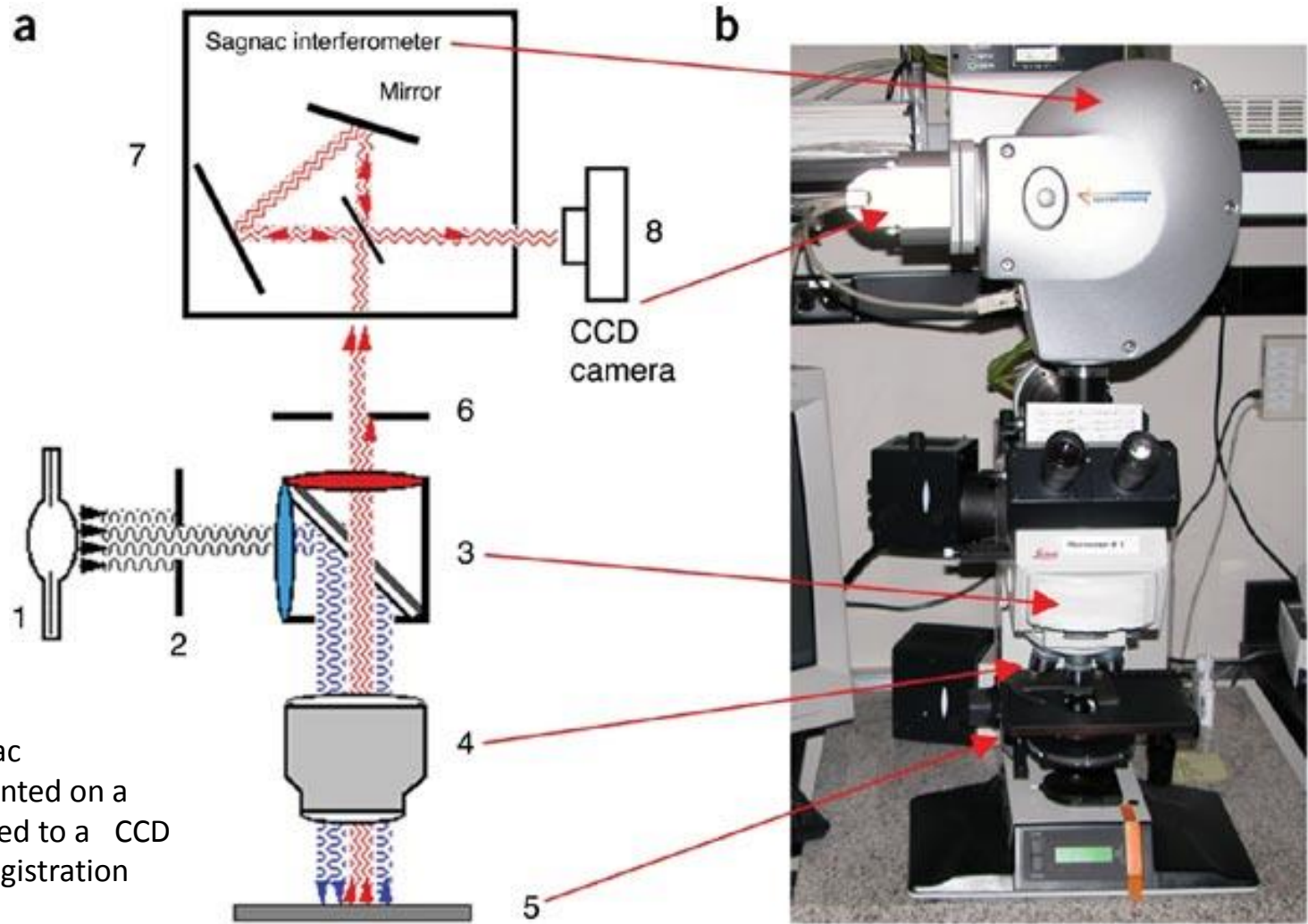
common path Sagnac interferometer mounted on a rotatable disk coupled to a CCD camera for image registration

Hesed M Padilla-Nash, Linda Barenboim-Stapleton, Michael J Difilippantonio & Thomas Ried
Nature Protocols **1**, 3129 - 3142 (2007)

# Spectral imaging SpectraCube system (Applied spectral imaging ASI)

Underlying principle is the simultaneous measurement of the detailed spectrum of every point of a given area (i.e. of each pixel of a given CCD array),
Able to record fluorescence or brightfield spectra, such as absorption, transmission, or reflection spectra.
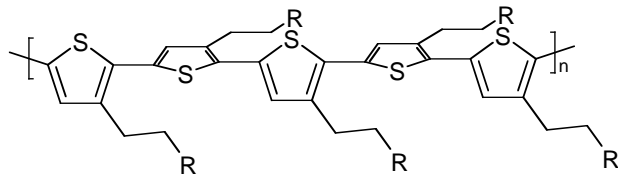Spectral range 450-800 nm
spectral resolution 5nm at 400 nm, <20nm at 780 nm,
                            completely visible as well as the near infrared
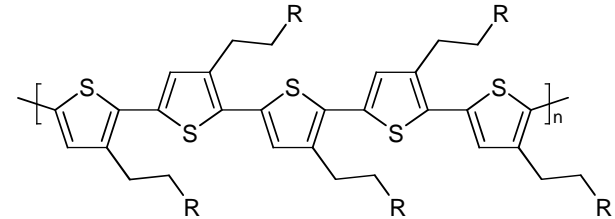

The Spectral Imaging Lambda Stack
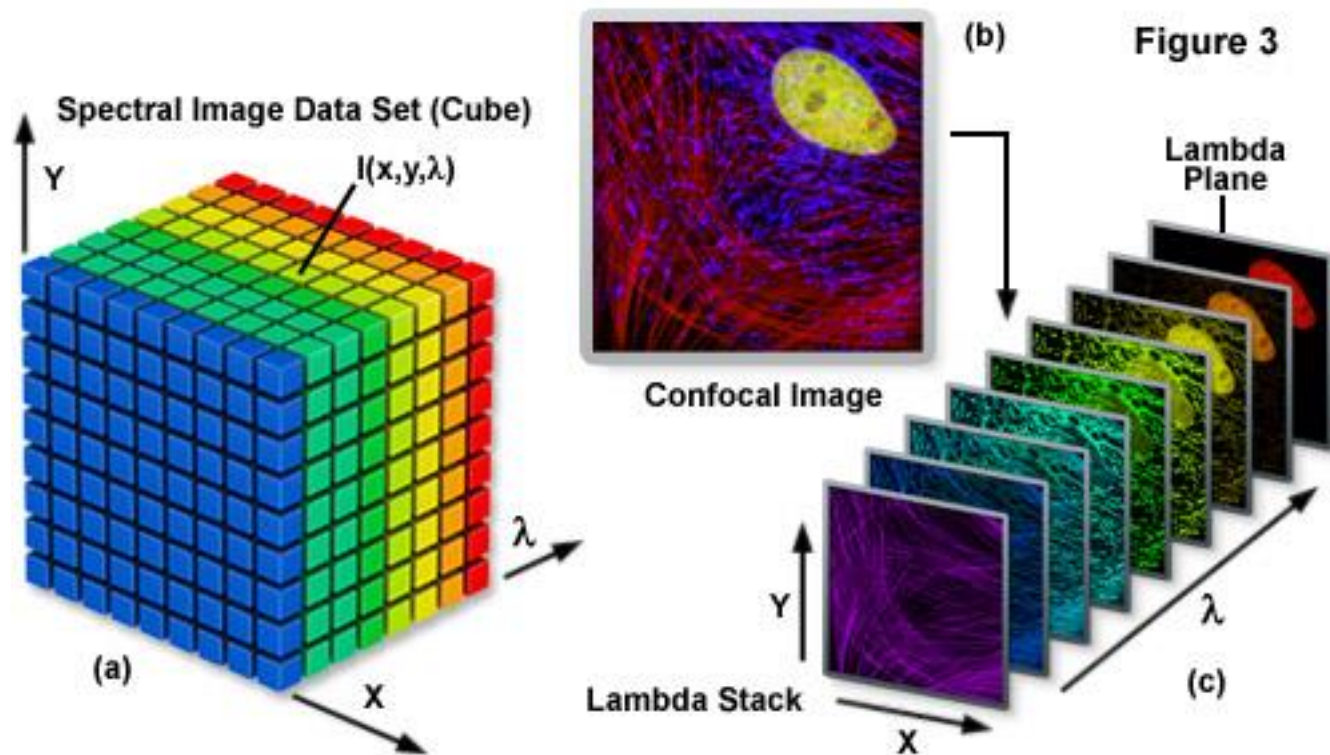
Luminescent conjugated polymers (LCPs)

- Molecules with rotationally flexible polythiophene chains

- Geometrical changes of the polymer chains gives changes in fluorescence
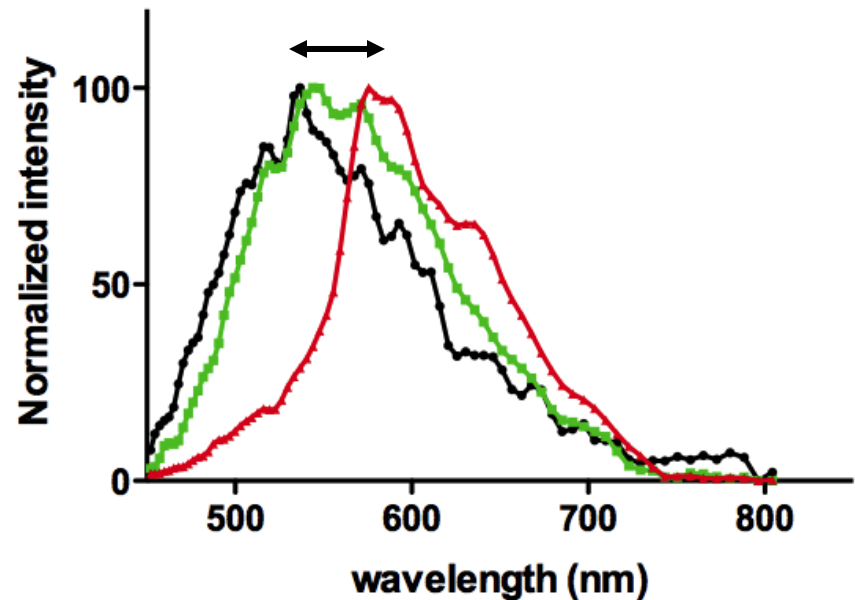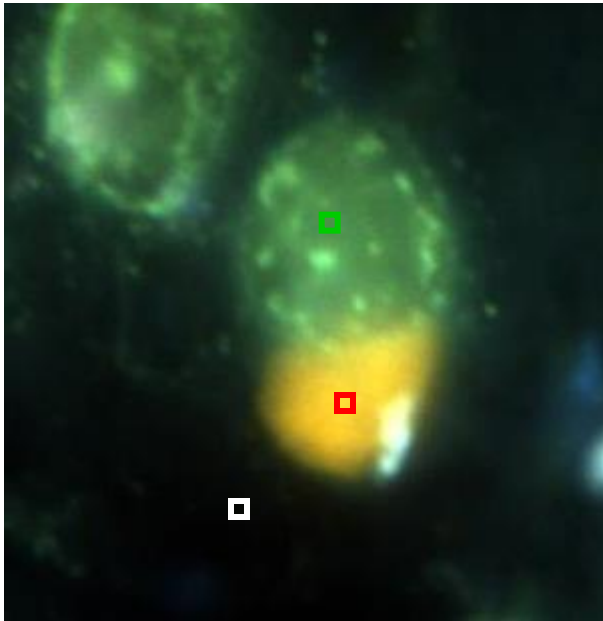

Twisted backbone –
blue/green fluorescence

Planar backbone –
red fluorescence

Conformation sensitive optical probes

© P. Nilsson

# PTAA binds to inclusions in ALS resulting in distinct emission spectrum

Ex: 405/30 long-pass filter
Em spectra: 450-800 nm

Spectral unmixing in multiple labeling experiments :

highly useful technique to untangle fluorescence spectral overlaps in cells and tissues labeled with synthetic fluorophores that would be otherwise difficult to separate (e.g. multi-labeling, life cell imaging separation of EYFP and EGFP)

Spectral Imaging and Linear Unmixing of Fixed Cells with Synthetic Dyes

(a)   (b)   Figure 1
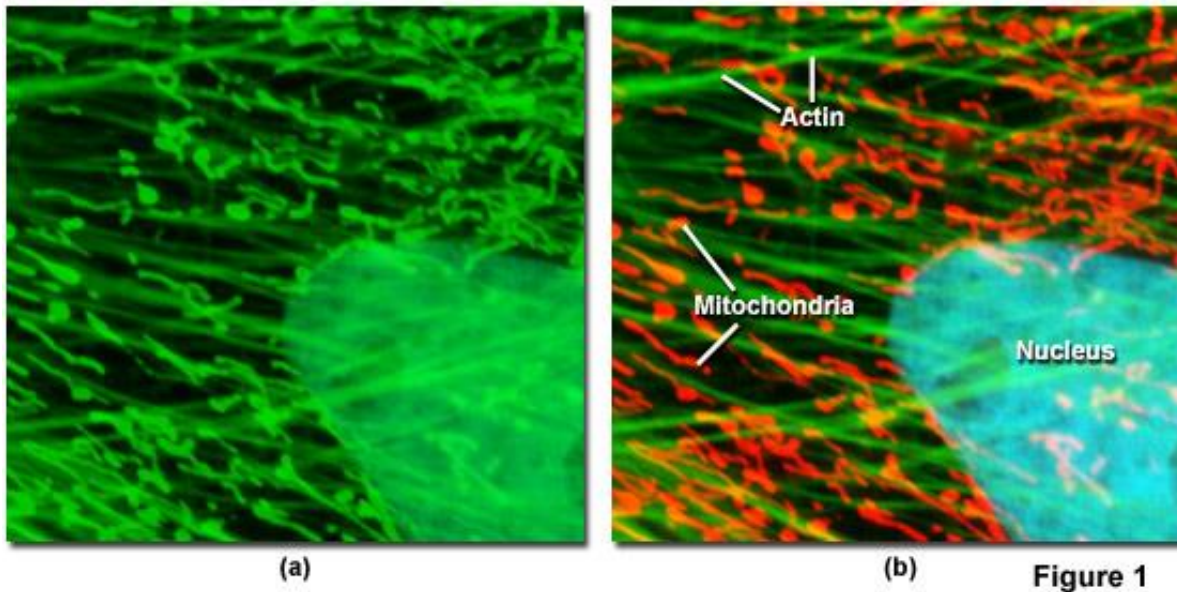
SYTOX Green =nucleus, emission maximum 523
Alexa Fluor 488 = actin, emission maximum 518
Oregon Green 514 = mitochondria, emission maximum 528
Standard FITC cube, spectral imaging combined with spectral unmixing.

Spectral unmixing in multiple labeling experiments :