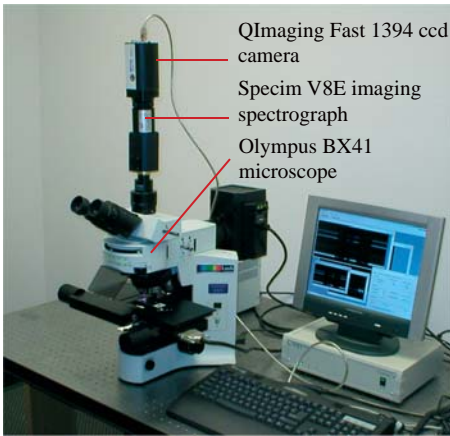


HYPERSPECTRAL MICROSCOPY

Hyperspectral microscopy is utilized in biosample research, developed by Iowa University Ophthalmology Center (U.S.A.) and Kestrel Corporation (U.S.A.). Utilized technology: SPECIM's ImSpector V8E imaging spectrograph, Olympus BX41 microscope and high quality CCD camera.



Measurement set-up

IMAGING OF CROSS SECTIONS OF MOUSE RETINA

University of Iowa is currently involved in a research project where hyperspectral microscope images are taken from a mouse retina. In this application the ImSpector V8E spectrograph is combined with Olympus BX41 microscope and high quality CCD camera to form a hyperspectral measurement system. Illumination is realized using modified halogen and cold-cathode fluorescence lamps.

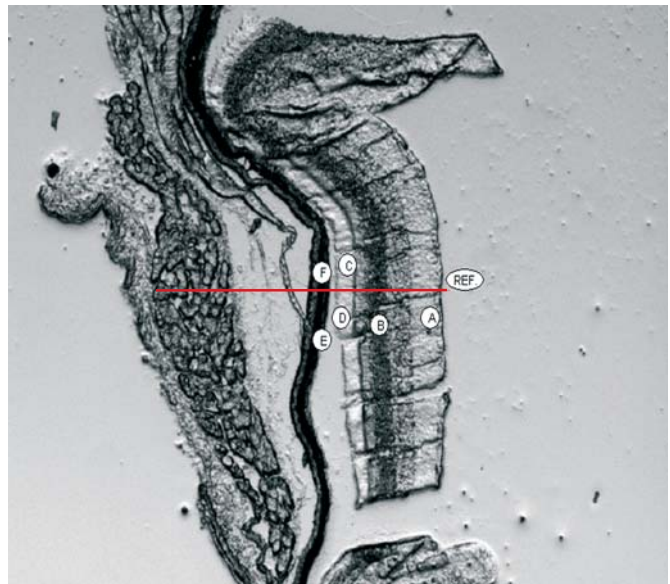
The CCD camera manufacturer QImaging is making CCD cameras that are well suited for low light level applications like fluorescence microscopy and live cell imaging. The camera has 12 bit Firewire (IEEE1394) output with up to 200 fps non-binned readout capability. The camera also has Peltier cooling to reduce thermal noise in long exposure times.

Sample image specification

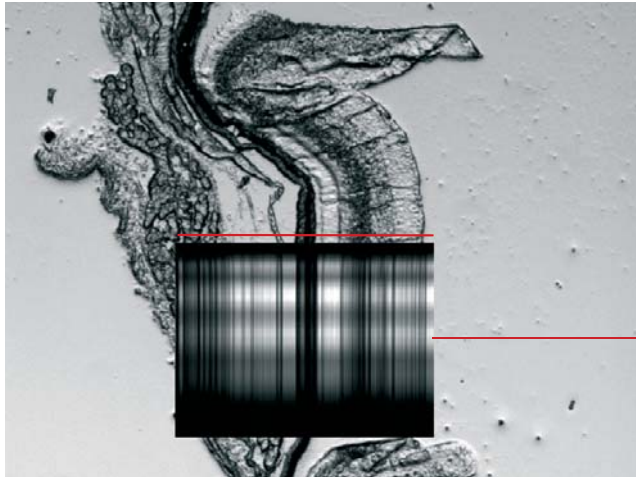
Shown hyperspectral images were taken at 10x magnification leading to a scan line length of 0.645mm and a width of 30um. Typical integration time is 140ms when camera is 2x2 pixel binned.

Label description:

- A = Inner Plexiform
 - B = Outer Nuclear
 - C = PhotoReceptors, Outer Segs
 - D = Outer Seg/PC + RPE *
 - E = Bruch's + Choricappilaris*
 - F = Choroid
- *approximation and probable mixed pixels



Processed regions with labels

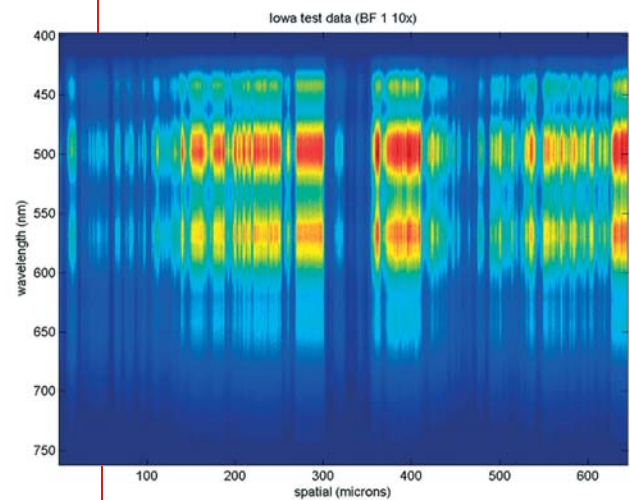


Scene image overlaid with the hyperspectral image for sample. Red line indicates the position where the spatial/spectral images is taken.

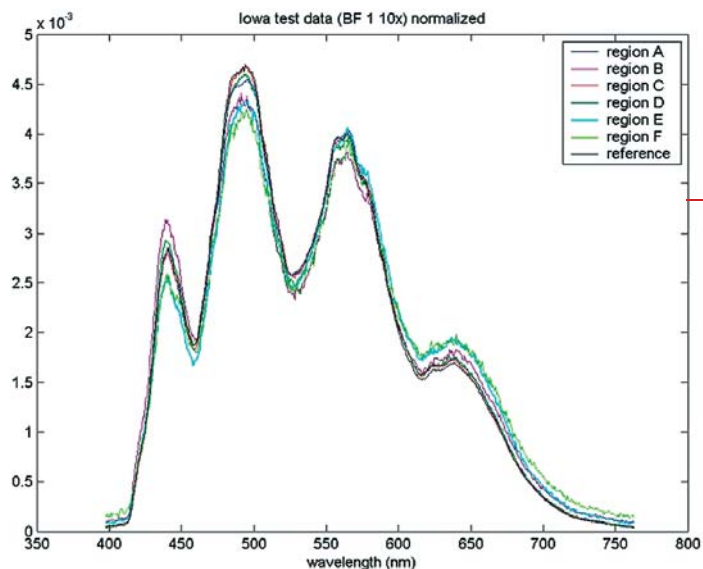
MEASUREMENT PROCESS

1. After assembly and adjustment of the system the spectral axis is calibrated using HgA and/or HgNe calibration lamps.
2. Before sample measurement also the spatial image axis is calibrated to verify the size and position of the images target line.

3. Selected ROI columns raw data are averaged and plotted.
4. Selected ROI curves are normalized and re-plotted.
5. Normalized ROI curves are subtracted from the normalized reference.
6. The resulting ROI curves are compared.



Scaled hyperspectral image



Normalized sample spectra