Application of linear scale space and the spatial color model in microscopy

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Summary

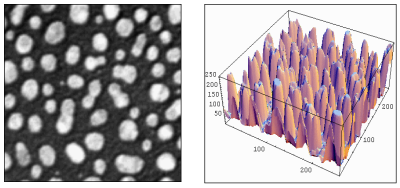
Structure and color are powerful cues in human vision to distinguish features in light microscopy. Taking structural features and color into consideration in machine vision often enables a more robust segmentation than based on intensity thresholding. Linear scale space theory and the spatial color model provide a framework for feature extraction in microscopy images. Differential geometry is applied in image analysis by convolving the image with Gaussian derivatives of the appropriate scale (σ) for the objects of interest.

Grayscale microscopy images acquired with a B/W camera contain structural features for which grayscale scale space provides a robust detection tool[1]. Feature detectors can be constructed based on differential invariants, which are relatively insensitive to changes in illumination condition and signal to noise ratio (SNR). This is an important advantage in light microscopy[2].

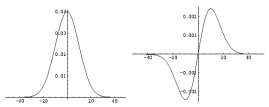
For color light microscopy we use the spatial color model to select different colored regions and objects. The visible spectrum is probed with a Gaussian kernel (E) and its first (E_λ) and second order derivative ($E_{\lambda\lambda}$) relative to wavelength (λ), with sigma (σ) 55 nm. and λ_0 centered on 520 nm[3]. Differential invariants can be constructed which are insensitive to changes in illumination color temperature and illumination intensity[4].

Methods

Linear scale space

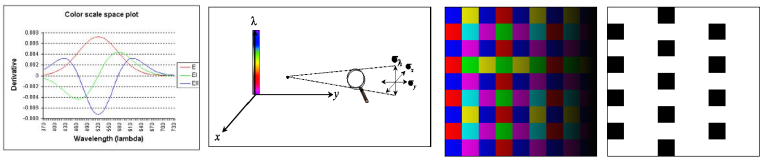


Detecting bright elliptic regions on a dark background
Object size selection with sigma (σ) of Gaussian convolution kernel
det. Hessian = $|H_i| = f_{xx}f_{yy} - f^2_{xy}$
deviation of flatness: magnitude and direction
$f_{xx} < 0$ and $f_{xx}f_{yy} - f^2_{xy} > 0$
$Lxx < 0$ and $LxxLyy - Lxy^2 > 0$
$Lww < 0$ and $LvvLww - Lvw^2 > 0$



A 2D grayscale image landscape represented as a 3D landscape - Gaussian derivatives

Spatial color model

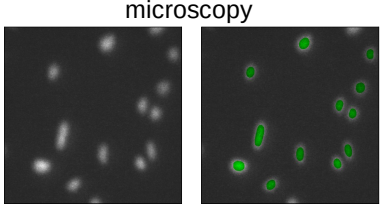


The visible spectrum is probed with a Gaussian kernel (E) and its first (E_λ) and second order ($E_{\lambda\lambda}$) derivative.

$E_\lambda > 0$ and $E_{\lambda\lambda} > 0$ zero crossing intensity invariance intra- and inter scene illumination intensity change

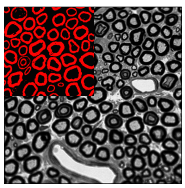
Applications of linear scale space

Cell nuclei in fluorescence microscopy



Bright elliptic patches in images with a low SNR.
$Lww < 0$ and $LvvLww - Lvw^2 > 0$, sigma (σ) 9.0
40x, Hoechst 33342™ stained nuclei,

Myelin sheath detection



Line detector for dark ridges:

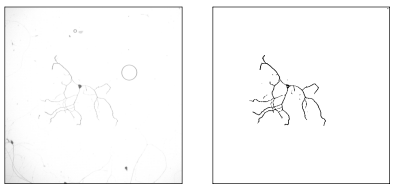
$$Lxx + Lyy - \frac{1}{2}\sqrt{(Lxx - Lyy)^2 + 4Lxy^2} > 0$$

Gaussian scale (σ) 1.5

Myelinated axon sheaths of densely packed rat tibial nerve.

Toluidin blue stained, 1 µm Epon embedded sections (40x imm. oil)

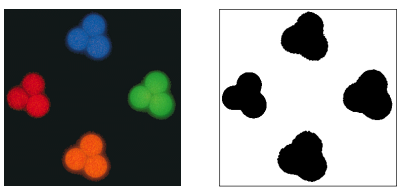
Neurite tracing



Line detector for dark ridges: $Lpp > 4.0 * \text{threshold} / \sigma^2$
Scale (σ) 2.0, threshold 1.0

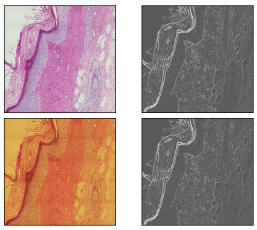
Applications of the spatial color model

Fluorescence microscopy



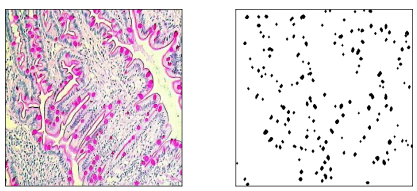
Red: $E_\lambda > 0$ and $E_{\lambda\lambda} > 0$, $E_\lambda - E_\lambda < 0$, Green: $E_\lambda > 0$ and $E_{\lambda\lambda} < 0$
Blue: $E_\lambda < 0$ and $E_{\lambda\lambda} - E_\lambda > 0$, Orange: $E_\lambda > 0$ and $E_{\lambda\lambda} > 0$ and $E_\lambda - E_{\lambda\lambda} > 0$
Scale sigma (σ) is 1.0
TetraSpeck 4.0 µm beads photographed using optical filter sets appropriate for DAPI, fluorescein, rhodamine and Texas Red dye.
Courtesy of Molecular Probes

Skin tissue section



Color invariant edge detection. Influence of illumination color temperature on edge strength, scale (σ) is 3.0 .
HE stained skin tissue section illuminated by a halogen bulb at 4000K (top) and 2600K (bottom) color temperature.

Goblet cells



Polysaccharides stain magenta and are elliptic patches
$Lww > 0$, $LvvLww - Lvw^2 > 0$, $E_{\lambda\lambda} - E_\lambda > 0$, scale sigma (σ) is 2.0
PAS stain for polysaccharides (goblet cells, gut)
Courtesy of Department of Pathology & Microbiology, University of Bristol, UK.

Conclusion

Scale space and the spatial color model provide the scientist with a powerful and intuitive tool for the detection of structure and color in images for quantitative microscopy.

References:

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