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

Peter Van Osta, MD
Senior Scientist
JRF, LSBI, Biological Imaging Laboratory

JANSSEN RESEARCH FOUNDATION

- **Light microscopy**
- **Image structure**
- **Linear scale space**
- **Spatial color model**

JANSSEN RESEARCH FOUNDATION

Light microscopy

High throughput microscopy

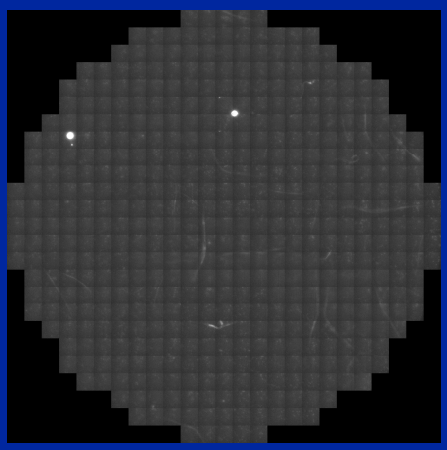
JANSSEN RESEARCH FOUNDATION

Image Acquisition Setup

ZEISS Axiovert 135 inverted microscope with SGI O2 workstation, Maerzhauser motorised stage, dual CCD camera setup

JANSSEN RESEARCH FOUNDATION

Multi well plate scan C. elegans



Circular pattern scanned - view on one well

- Central 60 wells of 96 well plate
  - 40x magnification (dry)
  - 473 512x512 pixel images / well
  - 20x focus / well (1200x / plate)
  - 28380 images in less than 4 hours
    0.5 seconds / image
  - Mosaic of 12800 x 12800 pixels

JANSSEN RESEARCH FOUNDATION

Tissue scan

Tissue scan on ZEISS Axiophot, SONY 950P 3CCD RGB color camera

- Toluidin blue stained rabbit heart tissue, Epon 2 ❍ semi thin slice
  - 63x, immersion oil
  - 1300 512x512 images
    (0.75 Gb)
  - 433x autofocus
  - 40 minutes scan for mosaic
  - 51x66=3366 image tiles
  - 26112 x 33792 pixels
    (1.9 Gb)
  - Automatic tissue edge detection

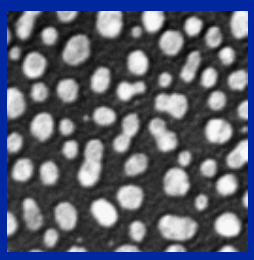
JANSSEN RESEARCH FOUNDATION

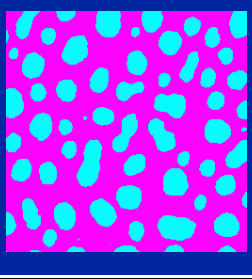
Image structure

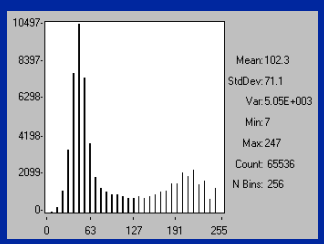
Threshold
Image landscape

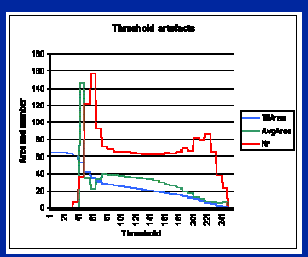
JANSSEN RESEARCH FOUNDATION

Conventional threshold segmentation





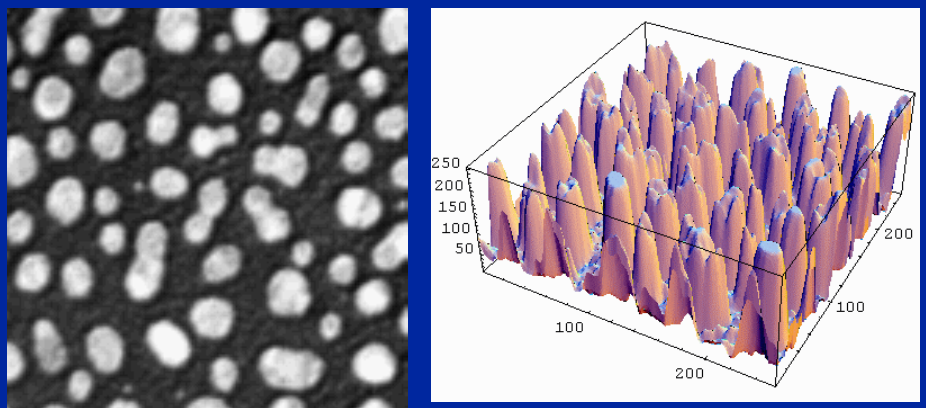




Threshold on gray value 128 - bright foreground versus dark background

JANSSEN RESEARCH FOUNDATION

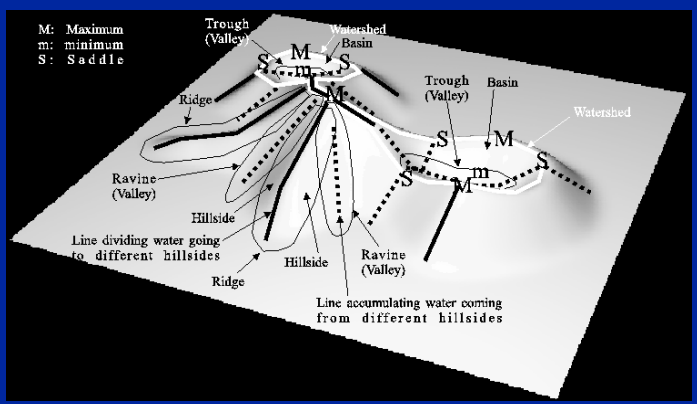
Image landscape



A 2D grayscale image landscape seen as a 2D function

JANSSEN RESEARCH FOUNDATION

Geometric patterns



An image landscape

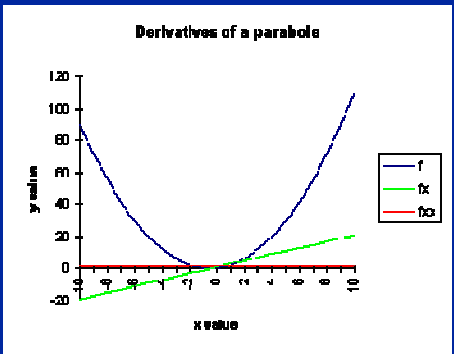
JANSSEN RESEARCH FOUNDATION

Linear scale space

Differential geometry
Scale

JANSSEN RESEARCH FOUNDATION

Differential Geometry 1- dimensional

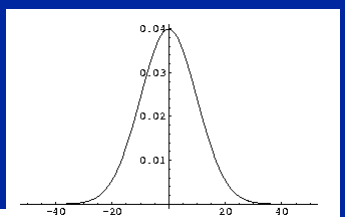


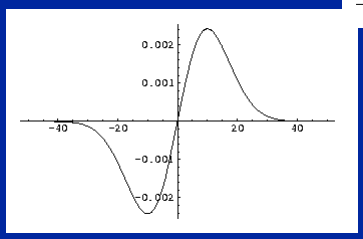
- f_x=dy/dx = 1st order derivative (the slope of the function)
  f_{xx}=d^2y/dx^2 = 2nd order derivative (the curvature of the function, or the rate of change of the slope)
- At each point **x** at which the function **f(x)** is **decreasing** the slope of the tangent is negative -- that is, f_x is negative.
- At each point **x** at which the function **f(x)** is **increasing** the slope of the tangent is positive -- that is, **f_x** is positive.
- At each point **x** which is the **top** of a mountain or the **bottom** of a valley the tangent is horizontal, so its slope is zero -- that is, **f_x = 0**.

Describing the shape and evolution of a function by means of differential calculus

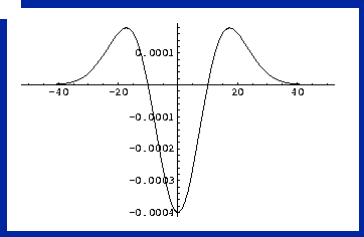
JANSSEN RESEARCH FOUNDATION

Gaussian derivatives





First order derivative

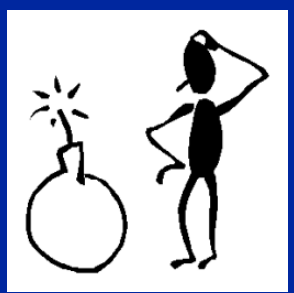


Second order derivative

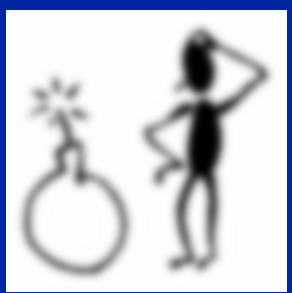
1D Gaussian, scale sigma 10

JANSSEN RESEARCH FOUNDATION

Convolution with a Gaussian kernel



Original 256x256 pixels image



Zero order Gaussian
just blurring

First order Gaussian
x-derivative

Differentiating a 2D image by convolution with the derivative of a Gaussian kernel

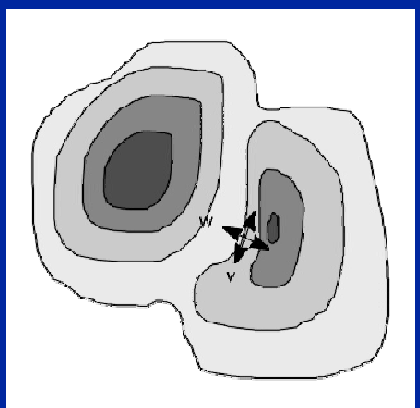
JANSSEN RESEARCH FOUNDATION

Scale

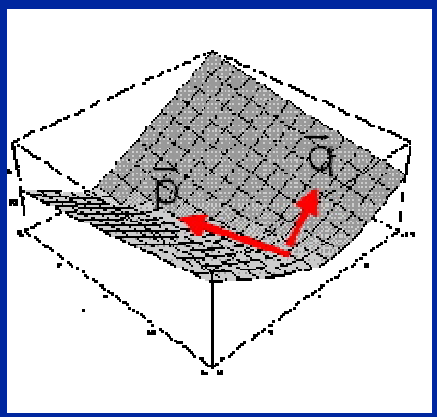
Intensity gradient Lw at scale (sigma) 1, 5 and 10
camera noise elimination and size selection
Robust Autofocusing in Microscopy, J-M Geusebroek et al.,
Cytometry 39:1-9 (2000)

JANSSEN RESEARCH FOUNDATION

Gauge coordinates



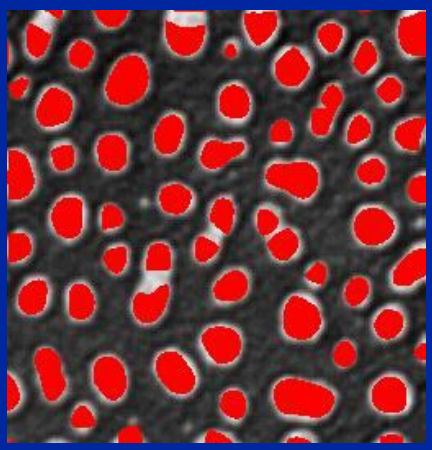
gradient gauge
{v,w} are gradient gauge coordinates,
w along and v perpendicular on the
gradient



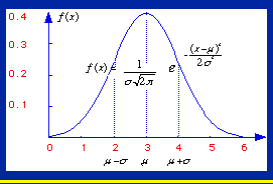
curvature gauge
{p,q} are the Hessian gauge coordinates,
minimal and maximal directional
second order derivatives

JANSSEN RESEARCH FOUNDATION

Differential Geometry
Elliptic patch detection

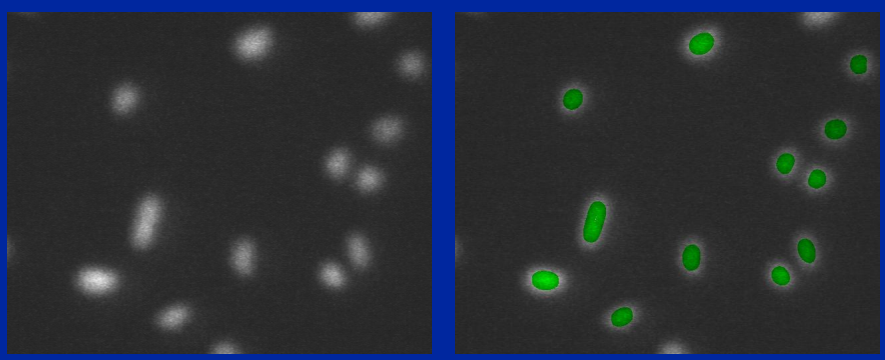


- Detecting bright elliptic regions on a dark background
- Object size selection with sigma of Gaussian convolution kernel
- det. Hessian = $|H_f| = f_{xx}f_{yy} - f^2_{xy}$
  - deviation of flatness: magnitude and direction
- $f_{xx} < 0$ and $f_{xx}f_{yy} - f^2_{xy} > 0$
- $Lxx < 0$ and $LxxLyy - Lxy^2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



JANSSEN RESEARCH FOUNDATION

Differential Geometry
Elliptic patches



$Lww < -0.0004$ and $LvvLww - Lvw^2 > 0$, sigma 9.0
40x, Hoechst stained nuclei, Photonic Science ISIS-3 intensified CCD camera
Courtesy of Kris Ver Donck

JANSSEN RESEARCH FOUNDATION

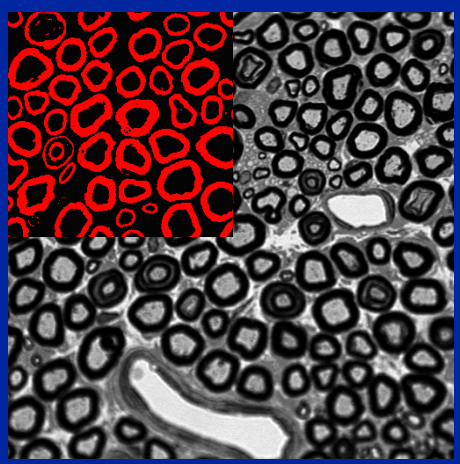
Differential geometry
Line detection in neurite tracing

Line detector for dark ridges: Lpp > 4.0 * threshold / s^2

Scale (s,♦) 2.0, threshold 1.0

JANSSEN RESEARCH FOUNDATION

Differential Geometry - Myelin



Line detection on myelinated axon myelin sheats.

Sample preparation:
Toluidin blue stained, 1 µm Epon embedded sections

Scanning:
40x immersion oil, autofocus every 3 images

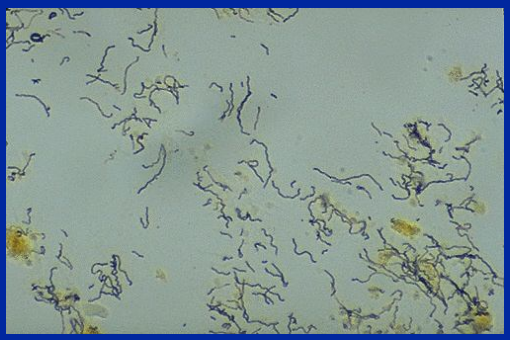
Line detector for dark ridges:
Lxx+Lyy-0.5*sqrt((Lxx-Lyy)^2+4Lxy^2) > 0

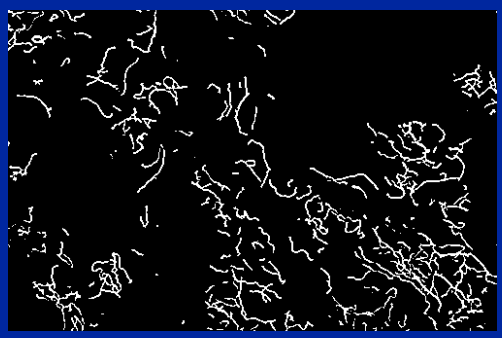
Gaussian scale (♦) 1.5

Courtesy of Jos Van Reempts

JANSSEN RESEARCH FOUNDATION

Differential Geometry - Spirochetes





Spirochetes are seen with a Warthin-Starry silver stain.
inv_ridges , sigma 1.0, dark ridges

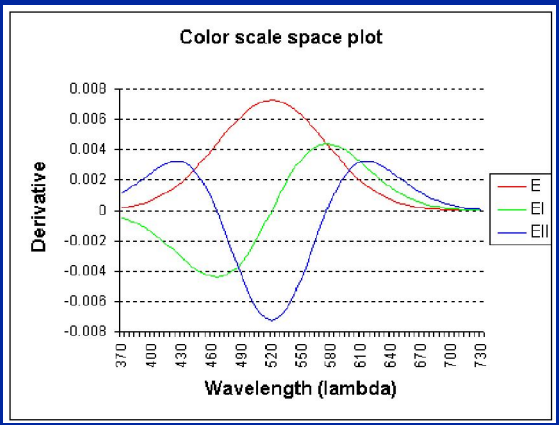
JANSSEN RESEARCH FOUNDATION

Spatial color model

Color and spatial extent

JANSSEN RESEARCH FOUNDATION

Spatial color model

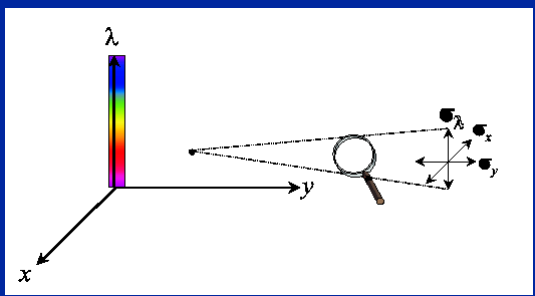


Color derivatives

The spectrum is probed with a Gaussian, centered on 520 nm and with sigma 55.0.

JANSSEN RESEARCH FOUNDATION

Spatial color model

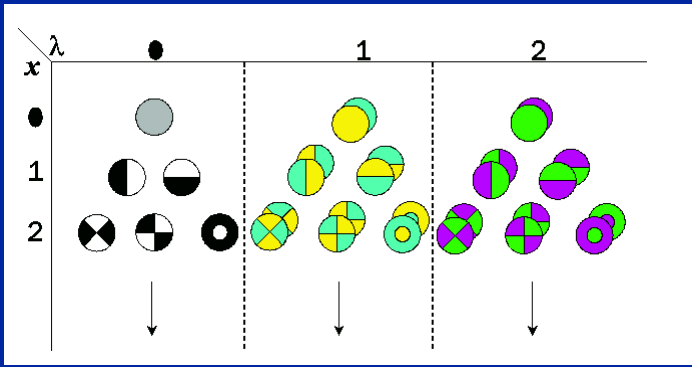


Color derivatives

Spectral and spatial probe

JANSSEN RESEARCH FOUNDATION

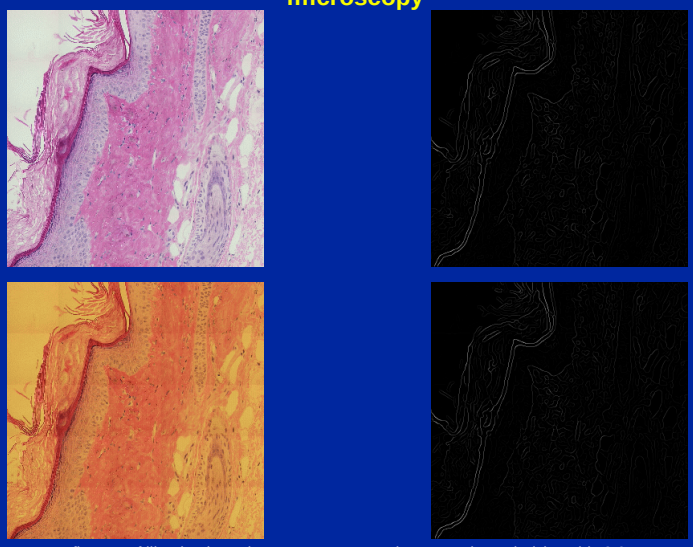
Spatial color model



Color derivatives
Spectral and spatial derivatives

JANSSEN RESEARCH FOUNDATION

Spatial color model and tracing color edges in microscopy

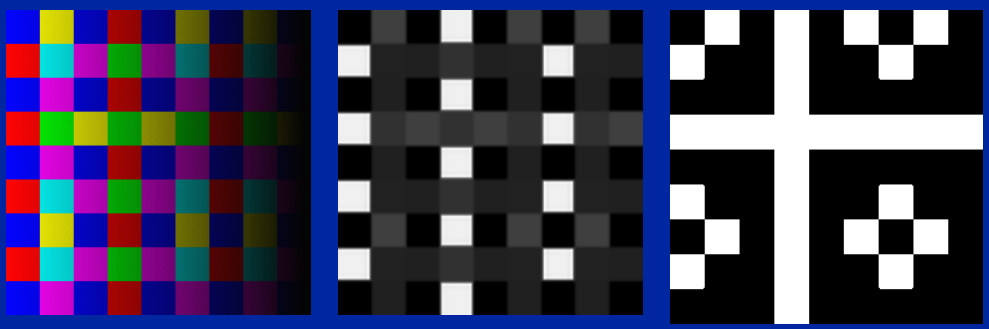


Influence of illumination color temperature on edge strength, scale (sigma) is 3.0 .

Skin tissue section illuminated by a halogen bulb at 4000K (top) and 2600K (bottom) color temperature.

JANSSEN RESEARCH FOUNDATION

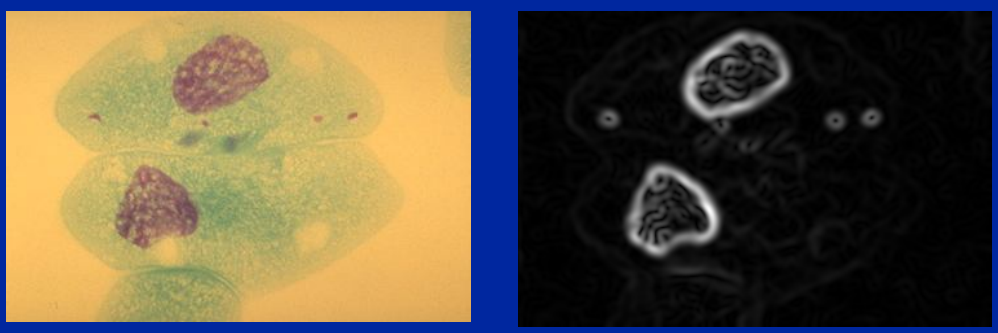
Intensity invariance



EI > 0, zero crossing intensity invariance
intra- and inter scene illumination intensity change

JANSSEN RESEARCH FOUNDATION

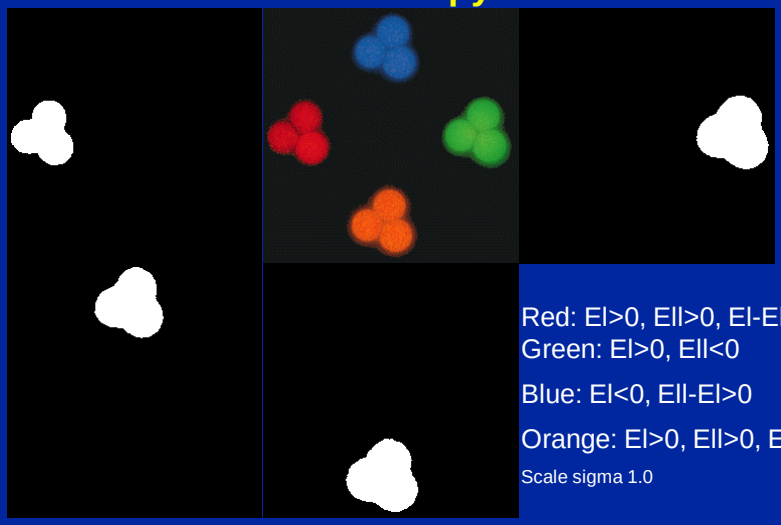
Spatial color model - Feulgen stain



Paramecium caudatum, Feulgen and Fast green stain
Color canny, red-green normalized edges, scale 3

JANSSEN RESEARCH FOUNDATION

Spatial color model in fluorescence microscopy



Red: EI>0, EII>0, EI-EII<0
Green: EI>0, EII<0

Blue: EI<0, EII-EI>0

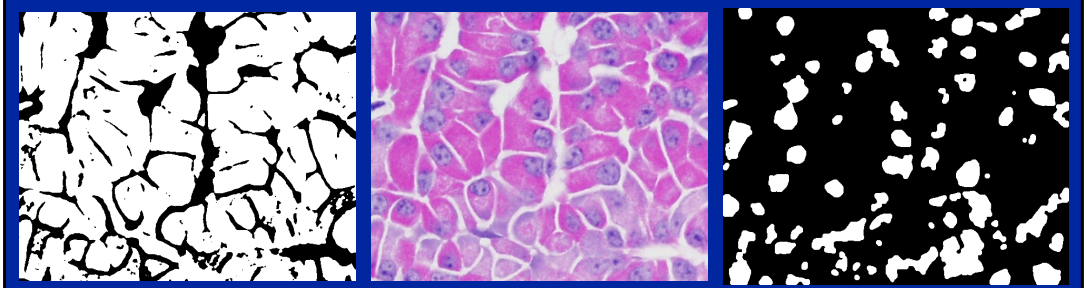
Orange: EI>0, EII>0, EI-EII>0

Scale sigma 1.0

JANSSEN RESEARCH FOUNDATION

TetraSpeck 4.0 µm beads photographed using optical filter sets appropriate for DAPI, fluorescein, rhodamine and Texas Red dye.

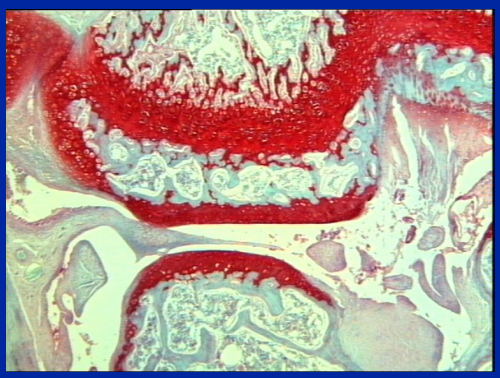
Spatial color model - Hematoxylin eosin stain



Pituitary gland, sheep, adenohypophysis 40x
Cell:EI<0, EII>0, scale 1.0
Nuclei:EI<0, EII>0, EI+EII<0, scale 3.0
additional constraint added to refine selection

JANSSEN RESEARCH FOUNDATION

Spatial color model - Safranin O stain

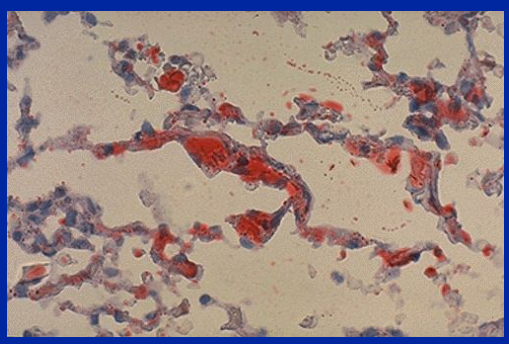


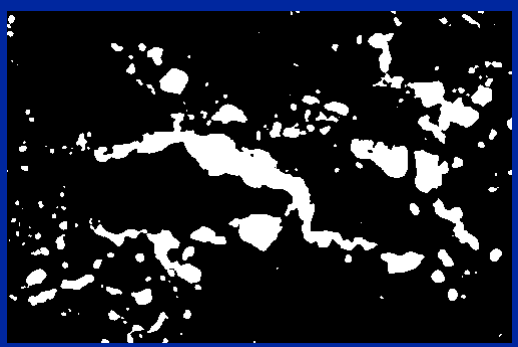
El>0, Ell>0, Scale sigma 1.0
Safranin O stain for proteoglycans (mouse knee joint)

JANSSEN RESEARCH FOUNDATION

Courtesy of Koen Gijbels and Paul Stoppie

Spatial color model - Oil red O stain

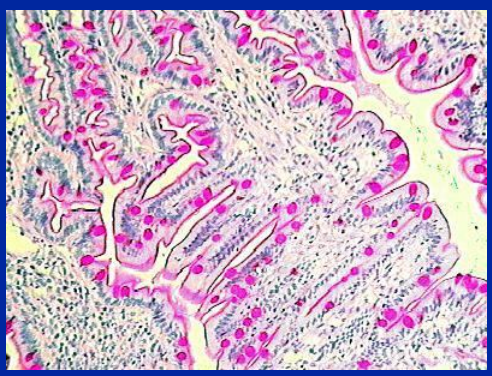


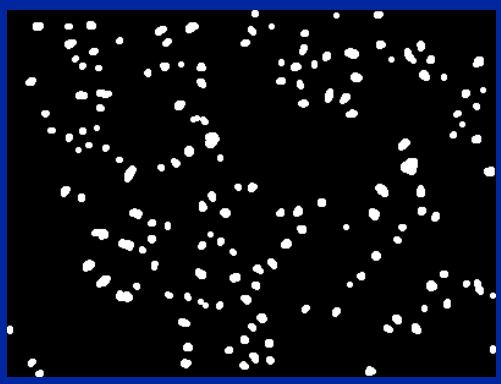


Oil red O stain of fat emboli in lung
El>0, Ell>0, scale 1.5
intensity invariant

JANSSEN RESEARCH FOUNDATION

Spatial color model - PAS stain





Lww>0, LvvLww-Lvw^2>0, Ell-El>0

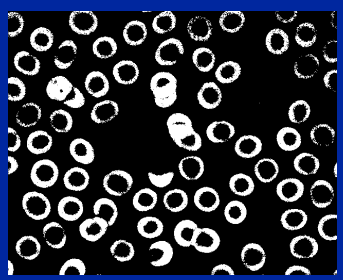
Scale sigma 2.0

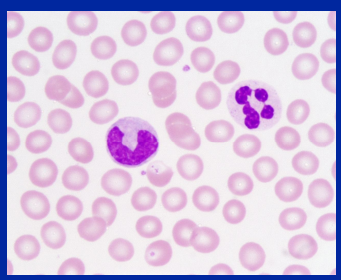
P.A.S. stain for carbohydrates (goblet cells, gut)

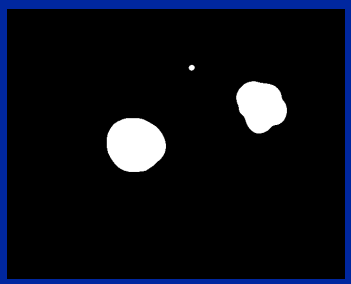
carbohydrates stain magenta - elliptic patches

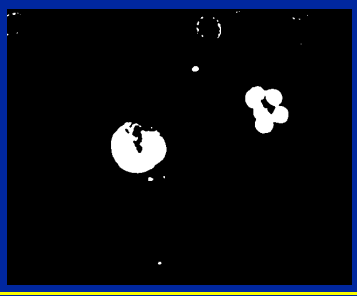
JANSSEN RESEARCH FOUNDATION

Spatial color model - Blood smear









Blood smear, Giemsa stain, 100x, JPEG compression

RBC: El>0, El+Ell>0, scale 0.5

Leucocytes: El<0, scale 12

Leucocyte nuclei: El<0, Ell>0, scale 3

JANSSEN RESEARCH FOUNDATION

Conclusions

- Linear scale space
  - Object selection based on structural features
  - Noise insensitive (scale, order of differential)
  - Intensity invariant
  - Robust autofocusing
  - Suitable for rapid development of high volume analysis
- Spatial color model
  - “Natural” color space
  - Robust for illumination color temperature
  - Robust for illumination intensity
  - Suitable for rapid development of high volume analysis

JANSSEN RESEARCH FOUNDATION

Acknowledgements

Jan-Mark Geusebroek

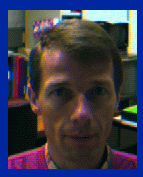
Bart ter Haar Romeny

Kris Ver Donck

JANSSEN RESEARCH FOUNDATION

Biological Imaging Team

- JRF, LSBI, Biological Imaging Laboratory
  - Frans Cornelissen
  - Peter Van Osta
  - Jan-Mark Geusebroek (currently UvA)



JANSSEN RESEARCH FOUNDATION