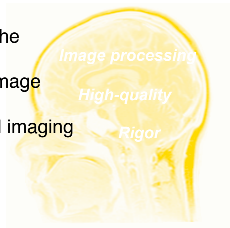
Recent advances in image processing for bio-photonics

Michael Unser

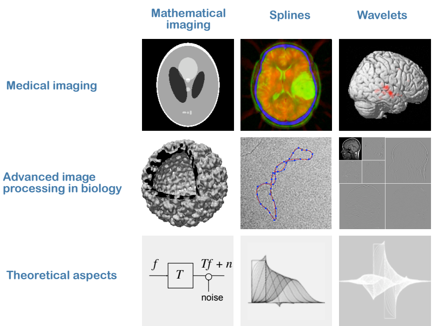
Biomedical Imaging Group
Institute of Imaging and Applied Optics
EPFL, Lausanne, Switzerland

Biomedical Imaging Group (BIG)

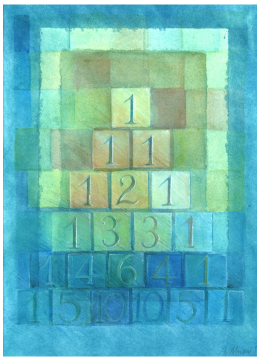
- **Institute of Imaging and Applied Optics (IOA)**
- **BIG**: 10-15 people
- **Activities**
  - Image processing tools for the biomedical community
  - Mathematical methods for image processing
  - Digital optics, computational imaging
- **Research**
- **Teaching**



Research strategy



RESEARCH: Fundamental aspects



Splines and wavelets

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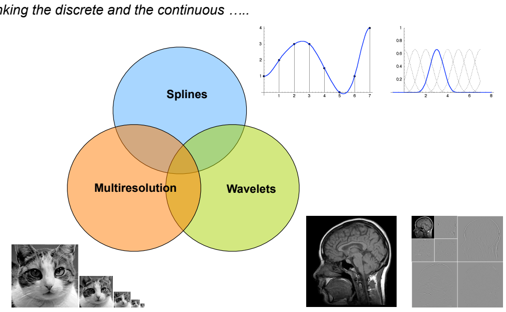
Splines: a unifying perspective

Linking the discrete and the continuous …..



RESEARCH: Image processing in biology

Structural and cellular biology
Optical and electron microscopy

Molecular biology
Fluorescence
Micro-arrays

Image analysis
- background correction
- particle detection/ tracking
- shape and motility

Computed imaging

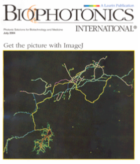
Multi-modal imaging

Visualization

IMAGE ANALYSIS

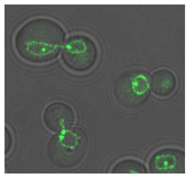
- Particle tracking (*D. Sage*)
  - Collaboration with S. Gasser

- Neuron tracing (*E. Meijering*)
  - Collaboration with H. Hirling, J.-C. Sarria

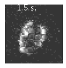


Single particle tracking

- Study of yeast nuclear dynamic
  - Goal: analysis of the movement of a tagged chromosomal locus within the nucleus
  - GFP Labeling



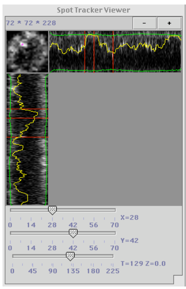
  - Time-lapse microscopy: 1 frame per 1.5 s
  - Nuclear ∅ ≈ 2 μm



Data: Prof. S. Gasser
Dept. Molecular Biology
University of Geneva

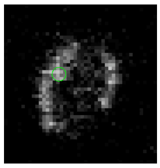
Single particle tracking: the solution

- Problem-specific constraints
  - Particle signature: bright, round spot
  - Smooth trajectory
  - Movement constrained to within the nucleus
- Algorithmic solution
  - Global optimization (DP) : past + future
  - Cost function trade-offs
    - Favors bright (or spot-like) structures
    - Imposes continuity constraints
    - Penalizes large jumps
    - Penalizes proximity to the nuclear boundary
  - Automatic or semi-automatic mode
    - Can accept user-constraints
    - Editing of solution



Single particle tracking: results

- Features
  - Ease-of-use: 1 to 5 clicks
  - Automatic alignment
  - 5 seconds to track a spot over 300 images
  - Tuning of the cost function
- Advantages
  - Reduction of work load
  - Reproducibility
- Extensions
  - 3D tracking
  - Multiple particles



Sage et al., *IEEE Trans. Image Processing*, submitted

Tracing neurons…

- Semi-automatic approach: "livewire"
  - *Optimal path between A and B (mouse clicks)*
  - *Minimization of a cost function*
  - *Dijkstra shortest path algorithm*

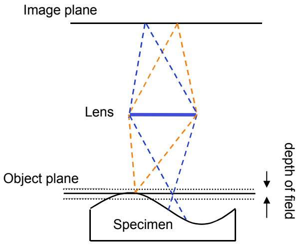
Collaboration with H. Hirling, EPFL

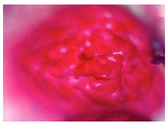
Meijering et al., *Cytometry*, 2004.

MATHEMATICAL IMAGING

- Extended depth-of-field (*B. Forster*)
  - Collaboration with ISREC
- Super-resolution microscopy (*F. Aguet*)
  - Collaboration with N. Garin
- Super-resolution OCT (*T. Blu, R. Langoju*)
  - Collaboration with S. Bourquin, R. Salathé

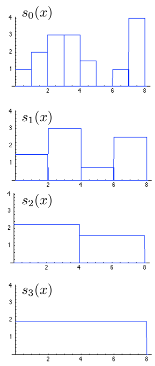
Limited depth-of-field: the problem





Focal series (*z*-stack)

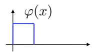
Wavelets: Haar transform revisited



Signal representation

$$s_0(x) = \sum_{k \in \mathbb{Z}} c[k] \varphi(x-k)$$

Scaling function



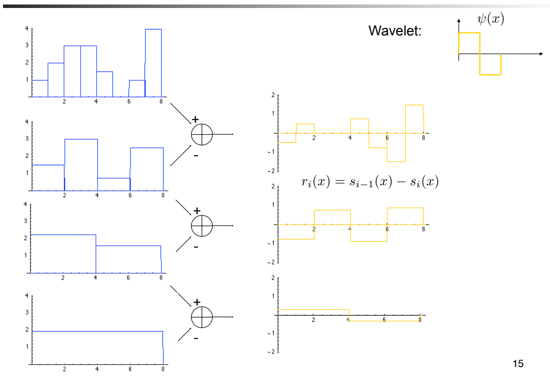
Multi-scale signal representation

$$s_i(x) = \sum_{k \in \mathbb{Z}} c_i[k] \varphi_{i,k}(x)$$

Multi-scale basis functions

$$\varphi_{i,k}(x) = \varphi\left(\frac{x - 2^i k}{2^i}\right)$$

Wavelets: Haar transform revisited



Wavelets: Haar transform revisited

$$r_1(x) = \sum_k d_1[k] \psi_{1,k}$$

+

$$r_2(x) = \sum_k d_2[k] \psi_{2,k}$$

+

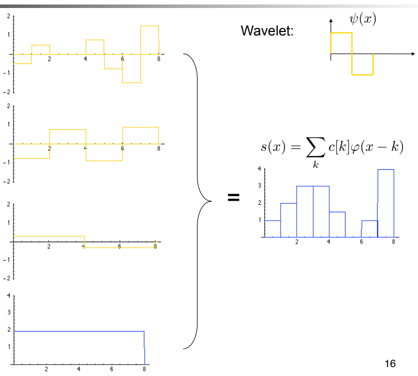
$$r_3(x) = \sum_k d_3[k] \psi_{3,k}$$

+

$$s_3(x) = \sum_k c_3[k] \varphi_{3,k}$$

=

$$s(x) = \sum_k c[k] \varphi(x-k)$$



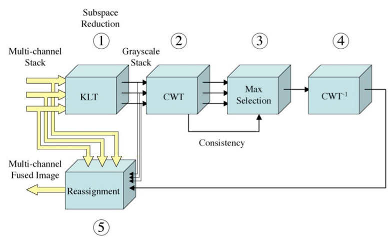
Wavelet transform for images

and iterate…

Wavelet transform

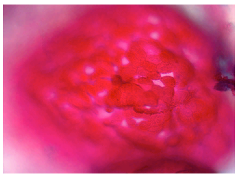
Wavelet-based extended depth-of-field

- Simple, wavelet-domain EDF algorithm

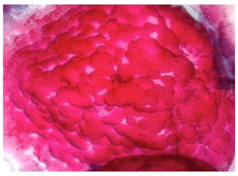


Forster et al., *Microscopy Research and Technique*, 2004

Extended depth-of-field: results

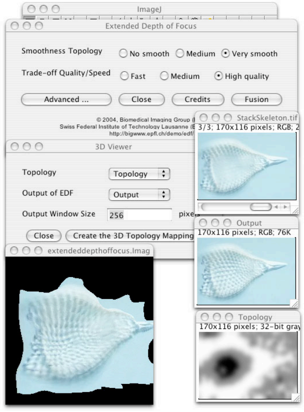


Focal series (z-stack)



In-focus image composite

ImageJ plugin



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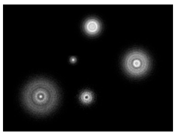
Super-resolution particle localization



Objective:

An efficient approach for locating particles in 3D space

Main challenge:

Axial localization

Characteristics of sub-resolution fluorescent particles

- Diffraction patterns appear in acquired images as particles move out of focus
- Image of a particle: point spread function (PSF) at the corresponding defocus distance

Can the axial position be recovered from out-of-focus acquisitions ?

Solution

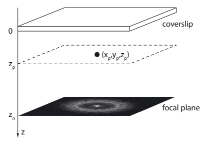
Exploit diffraction patterns by fitting acquisitions to a theoretical model

Defining an image formation model

Principal source of noise:

Statistical variation in photon arrival rate on CCD

- Follows a Poisson distribution



Acquisition model:

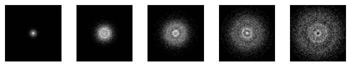
Theoretical PSF with Poisson statistics

Probability of measuring q photons

$$P\left(q(x,y,z_n|z_p)\right) = \frac{e^{-\bar{q}(x,y,z_n|z_p)}\bar{q}(x,y,z_n|z_p)^{q(x,y,z_n|z_p)}}{q(x,y,z_n|z_p)!}$$

$\bar{q}$: expected photon count, proportional to theoretical PSF intensity:

$$\bar{q}(x,y,z_n|z_p) = c\, PSF(x,y,z_n|z_p)$$



Position estimation

Fitting the experimental data to a theoretical model

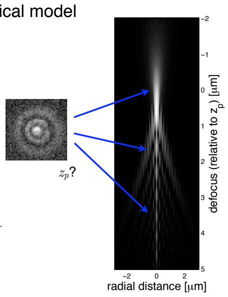
Possible criteria:

- Least squares
- Maximum likelihood

Optimal solution:

- Iterative, based on maximum likelihood

$$\hat{z}_p^{(k+1)} = \hat{z}_p^{(k)} - \frac{\sum_{n=1}^{N}\sum_{(x,y)\in\mathcal{S}}\left(\frac{\partial\bar{q}}{\partial z_p}\left(\frac{q}{\bar{q}}-1\right)\right)}{\sum_{n=1}^{N}\sum_{(x,y)\in\mathcal{S}}\left(\frac{\partial^2\bar{q}}{\partial z_p^2}\left(\frac{q}{\bar{q}}-1\right)-\left(\frac{\partial\bar{q}}{\partial z_p}\right)^2\frac{q}{\bar{q}^2}\right)}$$



$\bar{q}$: expected photon count (theoretical)

q : observed photon count (experimental)

'Resolution' limits

How can the maximal precision of the estimation be determined ?

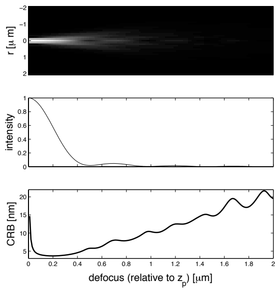
Statistical tool: Cramér-Rao bound

- Theoretical lower bound on the variance of any unbiased estimator
- In short: the performance of the best estimator

$$\mathrm{Var}(\hat{z}_p) \geq 1 \Big/ \sum_{n=1}^{N}\sum_{(x,y)\in\mathcal{S}} \bar{q}(x,y,z_n|z_p)^{-1}\left(\frac{\partial}{\partial z_p}\bar{q}(x,y,z_n|z_p)\right)^2$$

- Depends on the theoretical PSF model, thus on acquisition parameters
- Key factor: presence of noise (high amount implies lower precision)

CRB properties



XZ-section of the PSF

Axial intensity profile

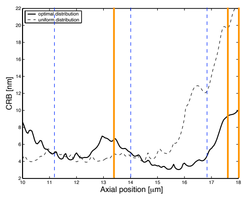
Cramér-Rao bound

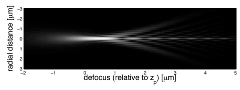
➡ Out-of-focus acquisitions yield better results !!

CRB: Beyond the theoretical aspects

Optimal experimental acquisition settings can be deduced

- Hypothesis: uniform distribution of particles within a specific section of specimen
- What distribution of focal positions (acquisitions) will yield the lowest CRB ?





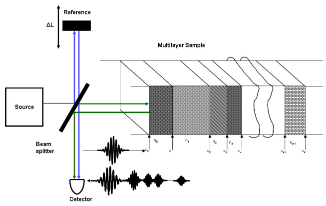
Example

- Interval: [10,18] μm into specimen
- 3 acquisitions (3rd at 18 μm)

Conclusions

- Higher precision can be reached from out-of-focus acquisitions
- Optimal acquisition positions are non-trivial

Optical coherence tomography (OCT)



- Model of OCT signal

$i(t) = I_0 + 2\mathrm{Re}\{(h * g)(t)\}$

I_0 : constant offset

$h(t)$: impulse response of object

$g(t)$: coherence function of light source

Super-resolution OCT

- Multi-layered model (K interfaces)

$$H(\omega) = \sum_{k=1}^{K} a_k e^{j b_k \omega}$$

Parameter vector: $\mathbf{p} = (a_1, \cdots, a_K, b_1, \cdots, b_K)$

(Absorption negligible) $\Longrightarrow$ $i_{\mathrm{model}}(t; \mathbf{p}) = I_0 + 2\sum_{k=1}^{K} a_k g(t - b_k)$

- Estimation method

$$\mathbf{p}_{\mathrm{opt}} = \arg\min_{\mathbf{p}} \left\{ \|i(t) - i_{\mathrm{model}}(t; \mathbf{p})\|^2 \right\}$$

  - Non-linear Least Squares (Marquardt-Levenberg)
  - Subspace method (direct)

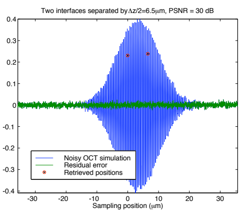
Super-resolution OCT: results

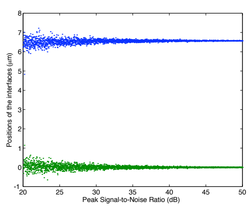
Two layer experiment:

$n_{\mathrm{film}} = 1.65$

$4\,\mu m$

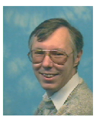
Sub-resolution: $\Delta b = \Delta z/2$





MULTI-MODALITY IMAGING AND VISUALIZATION

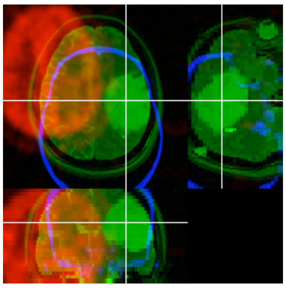
- Image registration (*P. Thévenaz*)
  - Generic problem: multi-modal and time laps sequences
- 3D Rendering (*P. Thévenaz*)
  - High-quality, spline-based isosurfaces



Multi-modal image registration

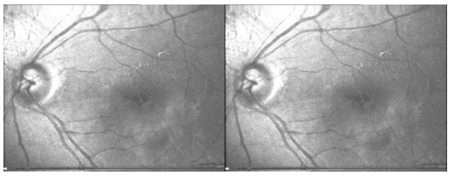
Specificities of the approach

- Criterion: mutual-information
- Cubic spline model
  - high quality
  - sub-pixel accuracy
- Multiresolution strategy
- Marquardt-Levenberg like optimizer
  - Speed
  - Robustness



Thévenaz and Unser, *IEEE Trans. Imag Proc*, 2000

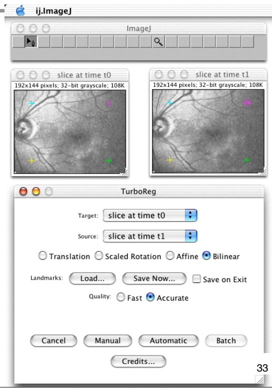
Alignment of image sequences



Software distribution: ImageJ pluggins

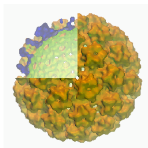
- Software development in JAVA
  - Teaching
  - Student projects
  - Research

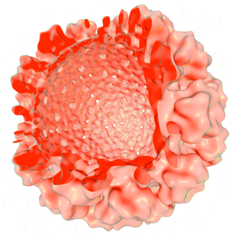
http://bigwww.epfl.ch/



Visualization

- High-quality methods
  - *Splines, …*



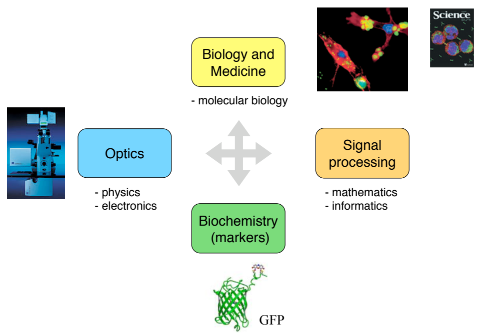


Collaboration with B. Trus, NIH, USA

CONCLUSION

- On-going challenges for bio-imaging
  - Quantitative image analysis
  - Computed imaging: reconstruction, deconvolution, ...
  - 3D + time data: storage, processing and analysis
  - Parametric imaging
- Bio-photonics and signal/image processing
  - Imaging software is becoming part of modern systems
  - Digital optics
- Making algorithms available
  - Plateform independence (Java)
  - Web, ImageJ plugins

Global, integrative view of bio-imaging



Acknowledgments

- **Biomedical Imaging Group**

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  - Cedric Vonesch

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  - Michael Liebling (Caltech)

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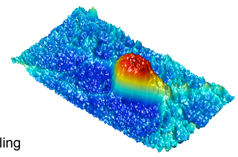
  - Floyd Sarria
  - Harald Hirling, Ph.D.
  - Prof. John Maddocks

  ISREC
  - Nathalie Garin, Ph.D. (MIME)
  - Claude Bonnard, Ph.D.

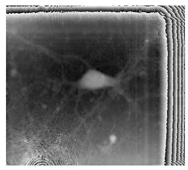
  Uni GE / F. Miecher Institute
  - Prof. Susan Gasser
  - Frank Neumann, Ph.D.
  - Florence Hediger, Ph. D.

Image reconstruction and restoration

- 3D reconstruction from projections
- Deblurring, noise reduction
  - *Wavelets, correlation-averaging*
- Digital holography microscopy



M. Liebling



Project MICRODIAG, EPFL/UNIL

Biomedical image processing

Neurology and neuro-sciences
Functional imaging, fMRI

Structural and cellular biology
Optical and electron microscopy

Molecular biology
Fluorescence
Micro-arrays

Image analysis

Computed imaging

Multi-modal imaging

Visualization

Radiology
X-ray tomography
MRI

Nuclear medicine
SPECT, PET

Cardiology
Ultrasound, image sequences