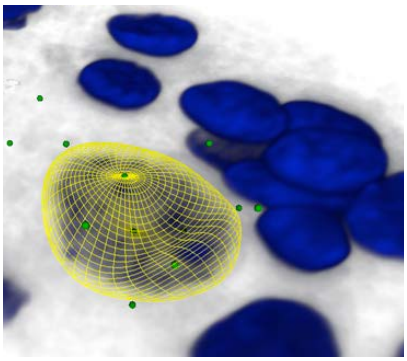
ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Challenges and Opportunities in Biological Imaging



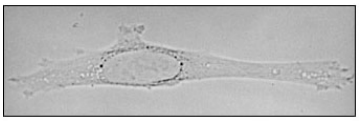
Michael Unser

Biomedical Imaging Group
EPFL, Lausanne, Switzerland

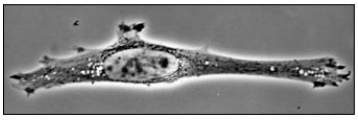
Plenary talk, *IEEE Int. Conf. Image Processing* (ICIP), 27-30 September, 2015, Québec City, Canada.

Cellular microscopy & matters of contrast

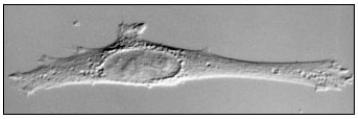
The good old days (much of the 20th century)



Bright-field microscopy



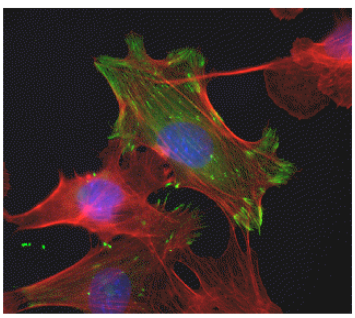
Phase contrast
[Zernike, circa 1940]



Differential Interference Contrast
[Normarski, 1955]

Zeiss/Abbe microscope 1880

The current state (colored revolution)

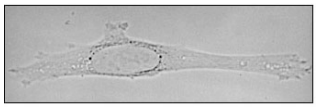


Confocal microscopy workstation

Cellular microscopy: the key (r)evolutions

Traditional light microscopy

Transparent specimen, flat (2D), static, qualitative



Modern light microscopy

Colored (highly specific), 3D, dynamic (time-lapse), quantitative

1. Video microscopy: 2D + t

2. Fluorescence

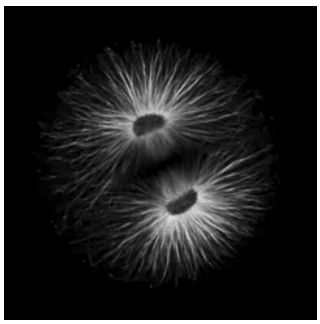
- Labeling of specific proteins (multispectral)

3. Optical sectioning & localization

- Confocal microscopy
- Super-resolution microscopy

4. Signal processing

- **Digital optics**
- **Bioimage informatics**



tubulin

Related list of Nobel laureates

Frits Zernike

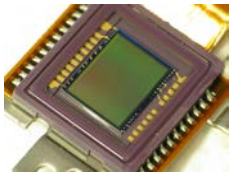
1953 Nobel Prize in Physics

"for the invention of the phase contrast microscope".

Osamu Shimomura, Martin Chalfie, and Roger Y. Tsien,

2008 Nobel Prize in Chemistry

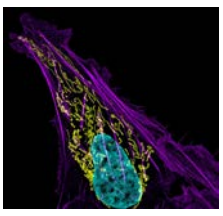
"for the discovery and development of the green fluorescent protein, GFP".



Willard S. Boyle, George E. Smith

2009 Nobel Prize in Physics

"for the invention of an imaging semiconductor circuit – the CCD sensor"



Eric Betzig, Stefan W. Hell, William E. Moerner

2014 Nobel Prize in Chemistry

"for the development of super-resolved fluorescence microscopy"

Nobelprize.org

OUTLINE

- Part 1: Basics of fluorescence microscopy

  Functional imaging of living cells

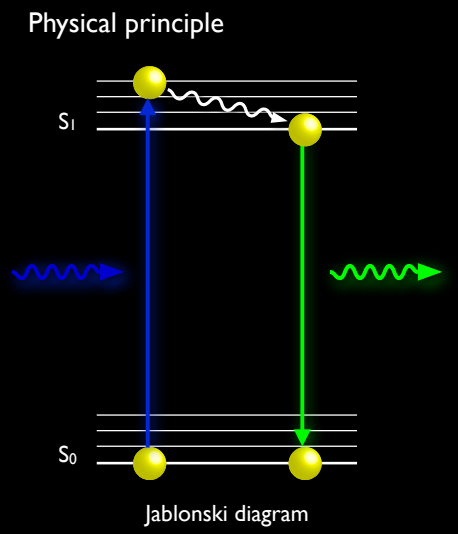
- Part 2: Mathematical Imaging

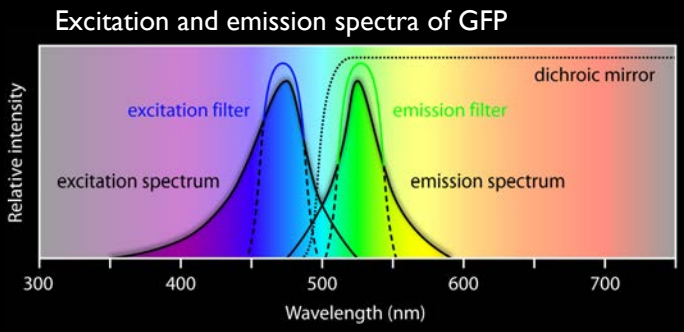
  The emergence of "digital optics"

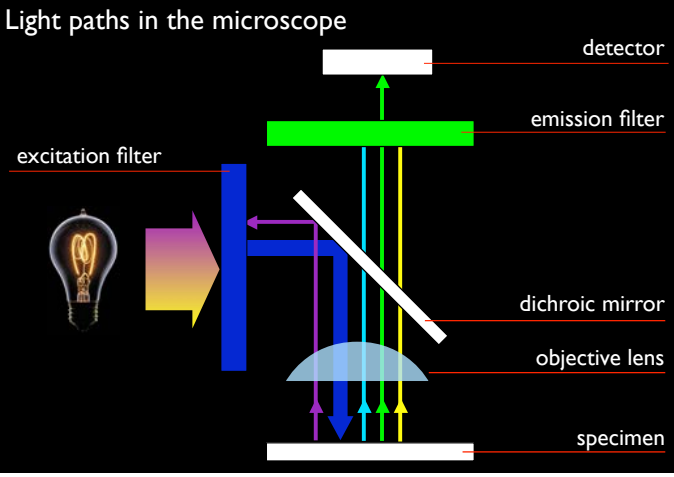
- Part 3: Tools for bioimage analysis

  The nascent field of "bioimage informatics"

Physical principle of fluorescence

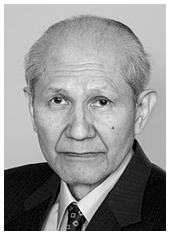




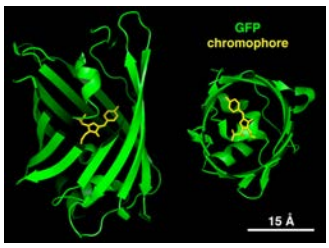


Green fluorescent protein (GFP)

Naturally occurring in jellyfish *aequorea victoria*

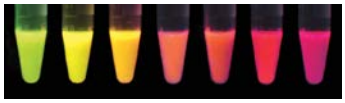


Osamu Shimomura

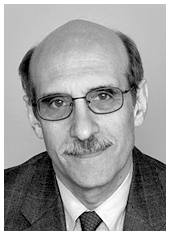


GFP variants engineered to fluoresce in different colors

Roger Y. Tsien

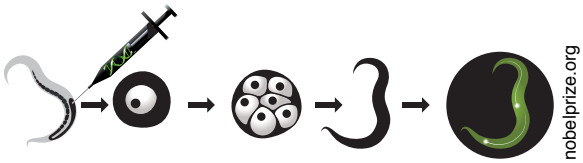


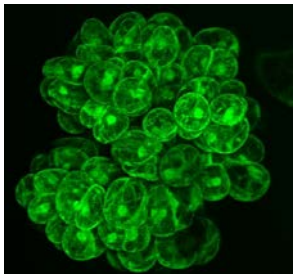
Shaner, N.C. *Nat. Biotech.* **22**(12), 2004

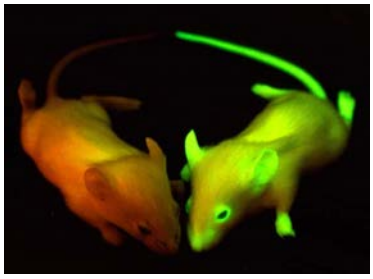


Martin Chalfie

GFP cloned and expressed in *C. elegans*

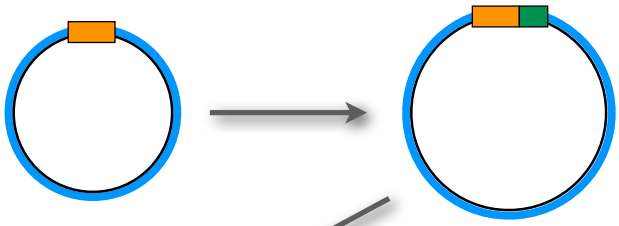






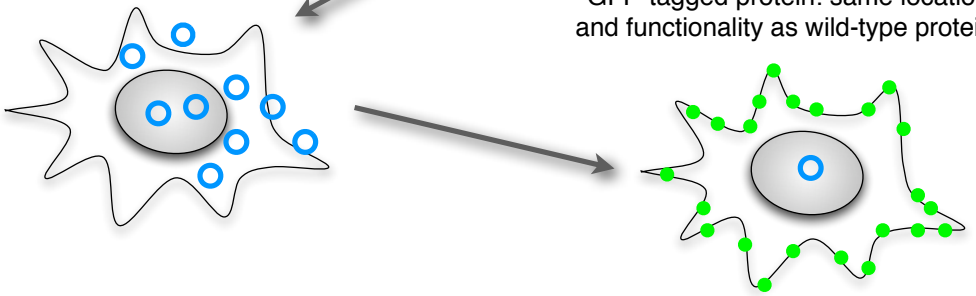
Making proteins of interest glow

STEP 1. Cloning of **GFP-sequence** in frame with the **gene** of the protein of interest in an expression vector (e.g., a plasmid)



STEP 2. Insertion of expression vector in “host” cell (“transfection” or “transduction”)

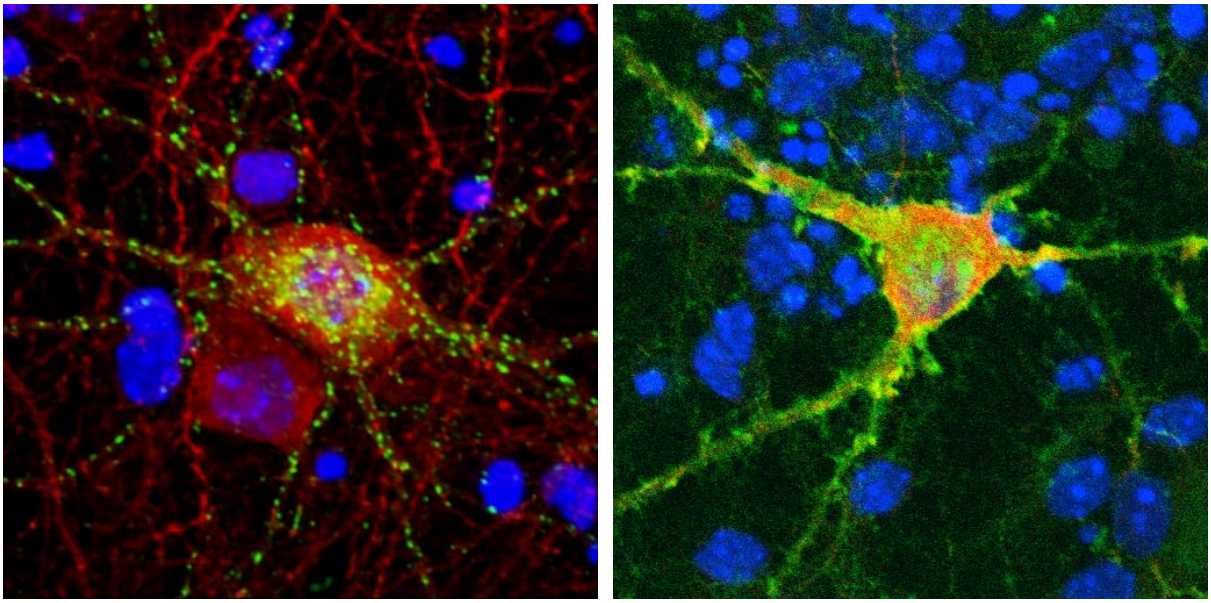
STEP 3. Expression of GFP-tagged protein: same location and functionality as wild-type protein



example: GFP-tagged membrane receptor

Examples: Images of neurons

(images courtesy of Scherrer et al., IGBMC, Illkirch, France)



Genetic (GFP): specific receptor protein (delta opioid)

Immunostaining (red): neuro-receptor (GABA)

Dye (DAPI blue): DNA in cell nuclei

OUTLINE

- Part 1: Basics of fluorescence imaging ✔
- **Part 2: Mathematical Imaging**

  The emergence of "digital optics"
- Part 3: Tools for bioimage analysis

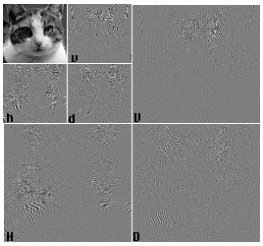
Part 2: Mathematical Imaging

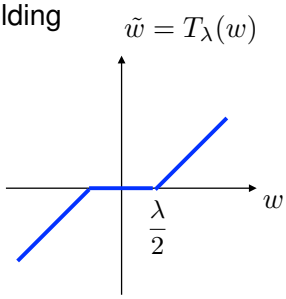
- Restoration/reconstruction algorithms with the aim of:
  - Faster acquisition (less photons)
  - Signal enhancement and noise reduction
  - Improving spatial resolution
- The new frontier: **Digital optics**

Denoising by wavelet thresholding

- Basic idea
  - Orthogonal WT: white noise $\rightarrow$ white noise
  - Signal is concentrated in few coefficients, while noise is spread-out evenly

$\Rightarrow$ Noise attenuation is achieved by simple wavelet shrinkage/thresholding





(Weaver et al. *Magnet. Reson. Med.* 1991; Donoho *IEEE Trans. Inf. Theory*, 1995)

- Improved scheme (state-of-the-art in microscopy)
  - MMSE-optimized threshold (SURELET)
  - Use of redundancy
  - Explicit modelling of Poisson noise (PURELET) with autocalibration

2009 Best Paper Award IEEE SPS

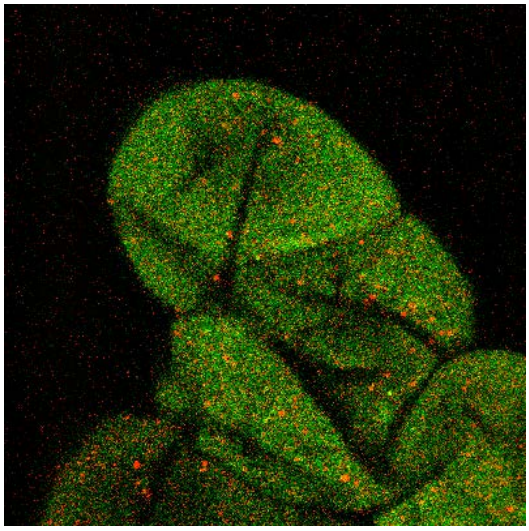
IEEE

(Luisier et al. *IEEE Trans. Im. Proc.* 2007 & 2010)

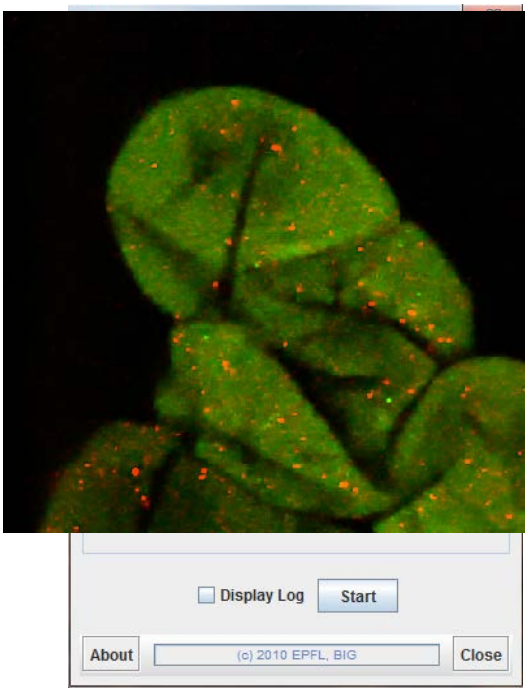
PureDenoise (plugin for ImageJ)

SURE-LET denoising

(Poisson + Gaussian noise, UWT)



Ground truth
(average over 500 acquisitions)

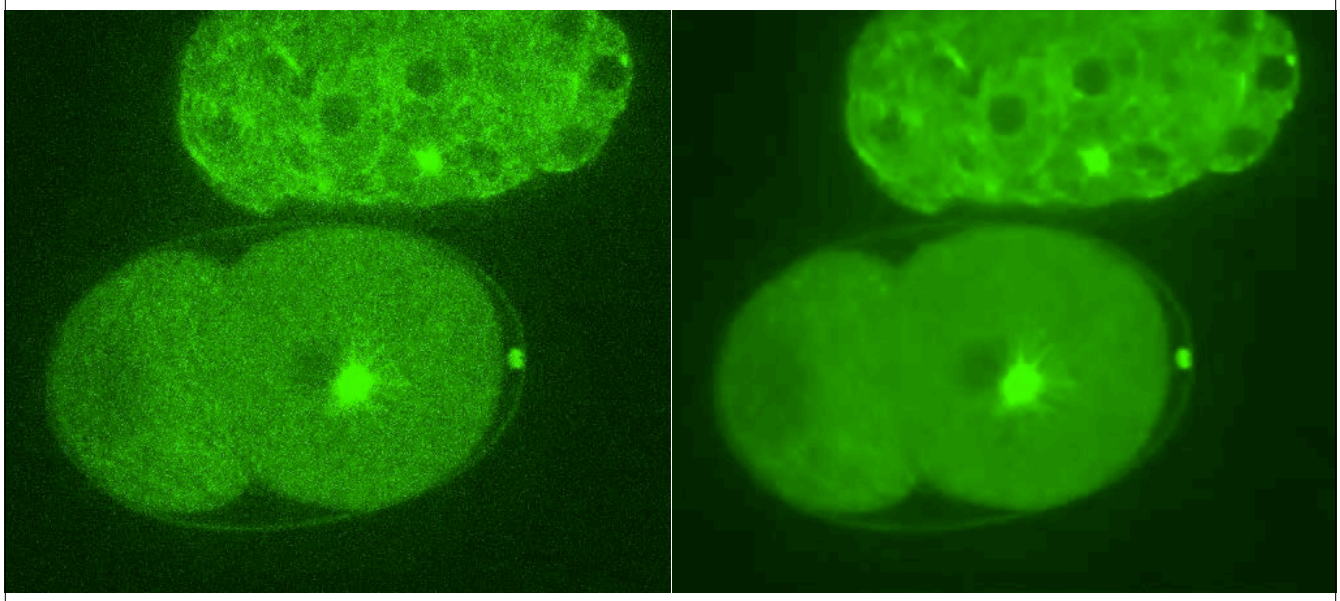


(Luisier, Blu, U., *Sig. Proc.* 2010)

http://bigwww.epfl.ch/algorithms/denoise/

State-of-the-art wavelet denoising in Poisson noise

2D + time SURE-LET denoising (DWT): C-elegance embryo

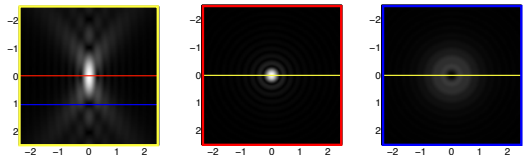


(Luisier et al. *IEEE Trans Imag Proc* 2011)

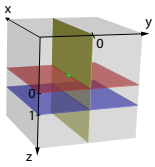
3D-deconvolution fluorescence microscopy

- Physical model of a diffraction-limited microscope

$$g(x, y, z) = (h_{3\mathrm{D}} * s)(x, y, z)$$



3-D point spread function (PSF)



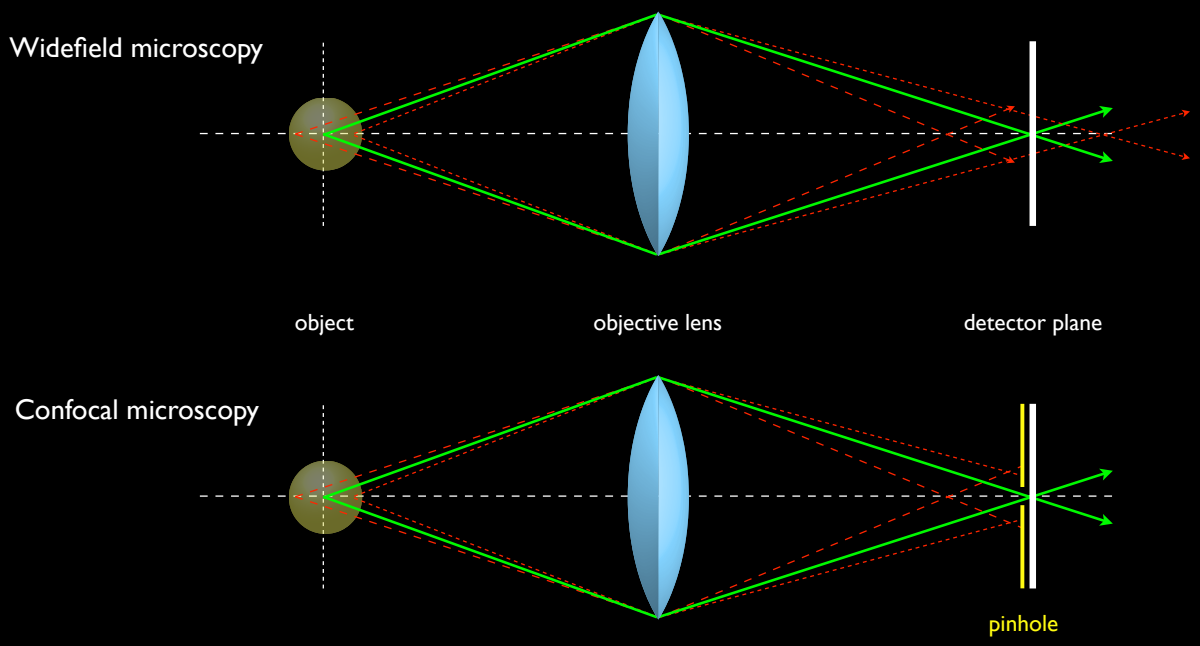
$$h_{3\mathrm{D}}(x, y, z) = I_0 \left| p_\lambda \left(\tfrac{x}{M}, \tfrac{y}{M}, \tfrac{z}{M^2} \right) \right|^2$$

$$p_\lambda(x, y, z) = \int_{\mathbb{R}^2} P(\omega_1, \omega_2) \exp\left(\mathrm{j}2\pi z \frac{\omega_1^2 + \omega_2^2}{2\lambda f_0^2} \right) \exp\left(-\mathrm{j}2\pi \frac{x\omega_1 + y\omega_2}{\lambda f_0} \right) \mathrm{d}\omega_1 \mathrm{d}\omega_2$$

Optical parameters

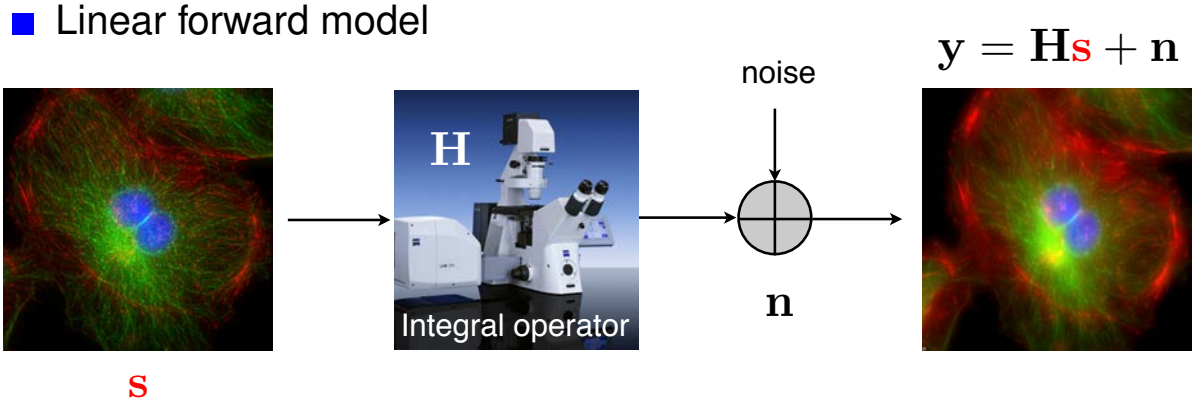
- λ: wavelength (emission)
- M: magnification factor
- f_0: focal length
- $P(\omega_1, \omega_2) = \mathbb{1}_{\|\boldsymbol{\omega}\| < R_0}$: pupil function
- $\mathrm{NA} = n \sin\theta = R_0 / f_0$: numerical aperture

Image formation: widefield vs confocal



Variational formulation of image reconstruction

- Linear forward model



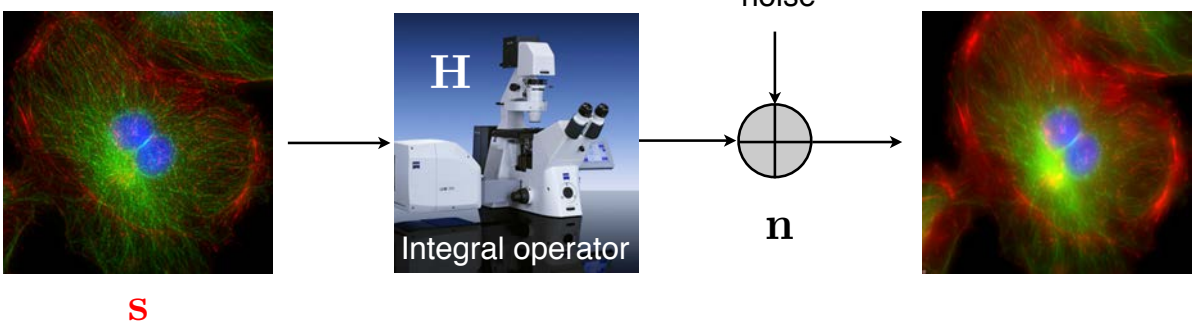
Ill-posed inverse problem: recover $\mathbf{s}$ from noisy measurements $\mathbf{g}$

- Reconstruction as an optimization problem

$$\mathbf{s}_{\text{rec}} = \operatorname{argmin} \underbrace{\|\mathbf{y} - \mathbf{Hs}\|_2^2}_{\text{data consistency}} + \underbrace{\lambda \mathcal{R}(\mathbf{s})}_{\text{regularization}}$$

Variational formulation of image reconstruction

- Linear forward model



Ill-posed inverse problem: recover $\mathbf{s}$ from noisy measurements $\mathbf{g}$

- Reconstruction as an optimization problem

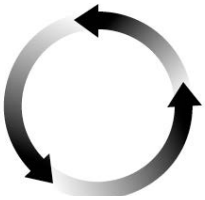
$$\mathbf{s}_{\text{rec}} = \arg\min_{\mathbf{s} \in \mathbb{R}^K} \left(\frac{1}{2} \|\mathbf{y} - \mathbf{Hs}\|_2^2 + \sigma^2 \sum_n \Phi_U\left([\mathbf{u}]_n\right) \right) \quad \text{subject to } \mathbf{u} = \mathbf{Ls}$$

promotes sparsity of $\boldsymbol{u}$

Alternating direction method of multipliers (ADMM)

$$\mathcal{L}_{\mathcal{A}}(\mathbf{s},\mathbf{u},\boldsymbol{\alpha}) = \frac{1}{2}\|\mathbf{g}-\mathbf{H}\mathbf{s}\|_2^2 + \sigma^2\sum_n \Phi_U([\mathbf{u}]_n) + \boldsymbol{\alpha}^T(\mathbf{L}\mathbf{s}-\mathbf{u}) + \frac{\mu}{2}\|\mathbf{L}\mathbf{s}-\mathbf{u}\|_2^2$$

Sequential minimization



$$\mathbf{s}^{k+1} \leftarrow \arg\min_{\mathbf{s}\in\mathbb{R}^N} \mathcal{L}_{\mathcal{A}}(\mathbf{s},\mathbf{u}^k,\boldsymbol{\alpha}^k)$$

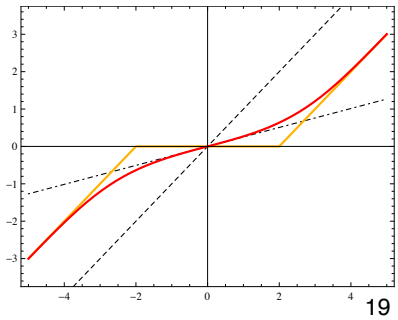
$$\boldsymbol{\alpha}^{k+1} = \boldsymbol{\alpha}^k + \mu\left(\mathbf{L}\mathbf{s}^{k+1}-\mathbf{u}^k\right)$$

$$\mathbf{u}^{k+1} \leftarrow \arg\min_{\mathbf{u}\in\mathbb{R}^N} \mathcal{L}_{\mathcal{A}}(\mathbf{s}^{k+1},\mathbf{u},\boldsymbol{\alpha}^{k+1})$$

Linear inverse problem: $\mathbf{s}^{k+1} = \left(\mathbf{H}^T\mathbf{H}+\mu\mathbf{L}^T\mathbf{L}\right)^{-1}\left(\mathbf{H}^T\mathbf{y}+\mathbf{z}^{k+1}\right)$

with $\mathbf{z}^{k+1} = \mathbf{L}^T\left(\mu\mathbf{u}^k-\boldsymbol{\alpha}^k\right)$

Nonlinear denoising: $\mathbf{u}^{k+1} = \mathrm{prox}_{\Phi_U}\left(\mathbf{L}\mathbf{s}^{k+1}+\frac{1}{\mu}\boldsymbol{\alpha}^{k+1};\frac{\sigma^2}{\mu}\right)$

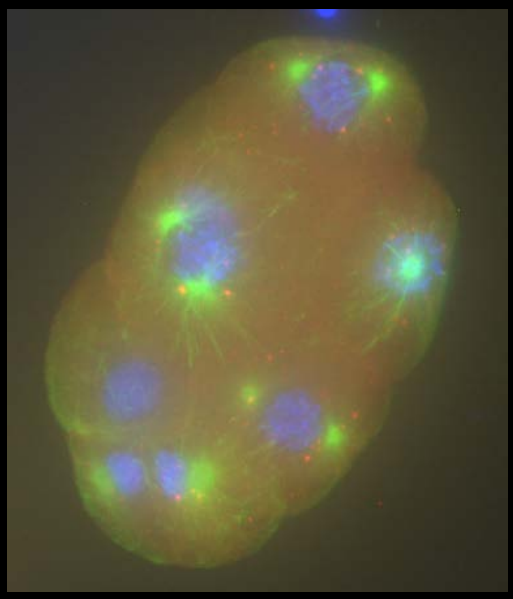


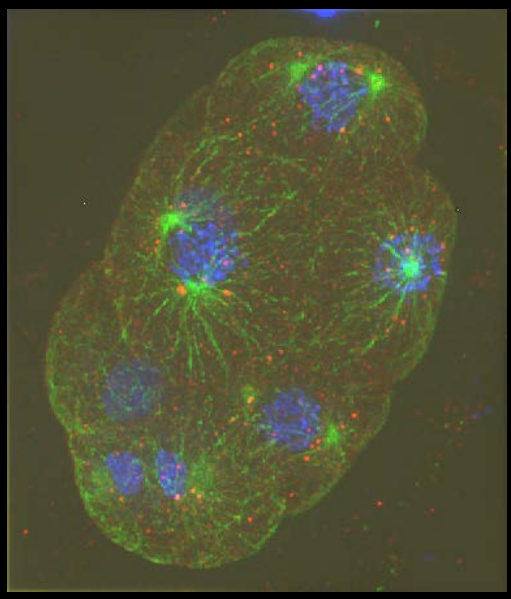
- Proximal operator taylored to potential function

$$\mathrm{prox}_{\Phi_U}(y;\lambda) = \arg\min_u \frac{1}{2}|y-u|^2 + \lambda\Phi_U(u)$$

(Bostan et al. *IEEE Trans. Im. Proc.* 2013)

3D deconvolution of widefield stack





Maximum intensity projections of 384×448×260 image stacks;
Leica DM 5500 widefield epifluorescence microscope with a 63× oil-immersion objective;
C. Elegans embryo labeled with Hoechst, Alexa488, Alexa568;
each channel processed separately; computed PSF based on diffraction-limited model;

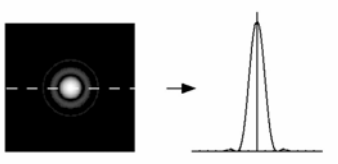
(Vonesch et al. *IEEE Trans. Im. Proc.* 2009)

21

22

Resolution limit(s) in fluorescence microscopy

- Lateral resolution



Abbe's lateral resolution: $d_{XY} = \frac{\lambda}{2\mathrm{NA}}$

Practical limits: 180 nm (confocal) – 250 nm (brightfield)

Can this be improved ?

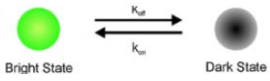
- With deconvolution: 250 nm → 180 nm
- With structured illumination: ~100 nm
- With multiplexing in time and/or **localization** (PALM or STED) ~10-55 nm

- Axial resolution

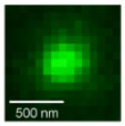
Abbe's axial resolution: $d_Z = \frac{2\lambda}{\mathrm{NA}^2}$

Practical limits: 500 nm (confocal) – 1000 nm (brightfield)

Single-molecule localization microscopy

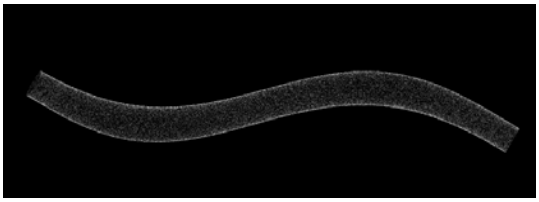


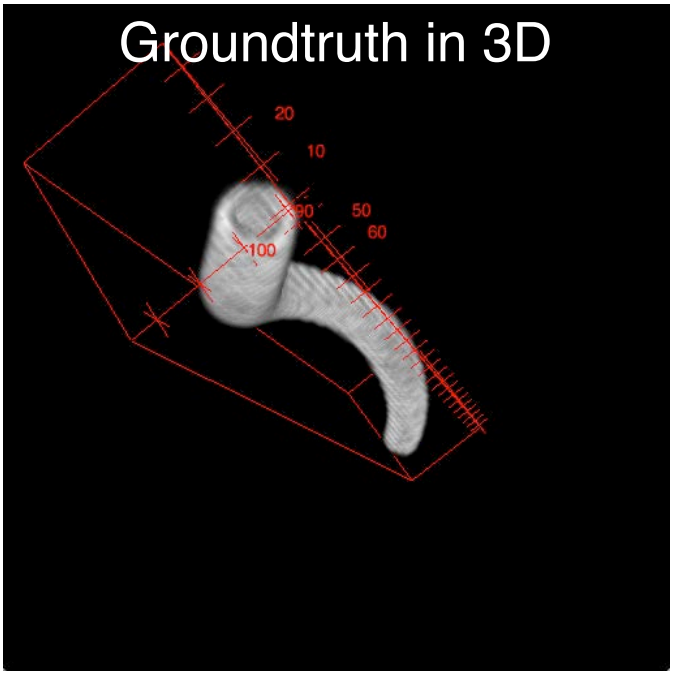
Activation of a single molecule:



= PSF

Simulated PALM reconstruction

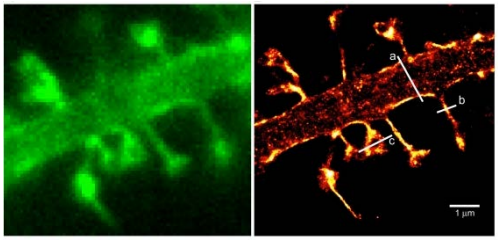




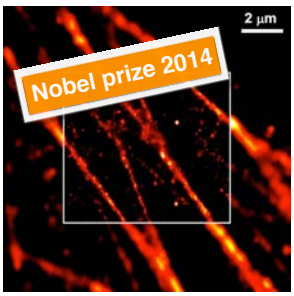
Real data

Conventional fluorescence

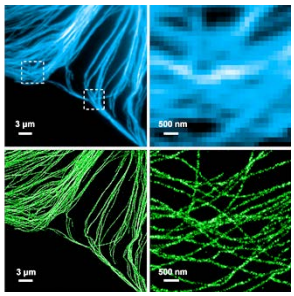
PALM super-resolution



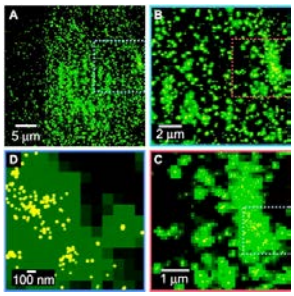
Method of the Year 2008, Nature Methods



PALM (*Eric Betzig*)
Photo-Activation
Localization Microscopy



STORM (*Xiaowei Zhuang*)
Stochastic Optical
Reconstruction Microscopy

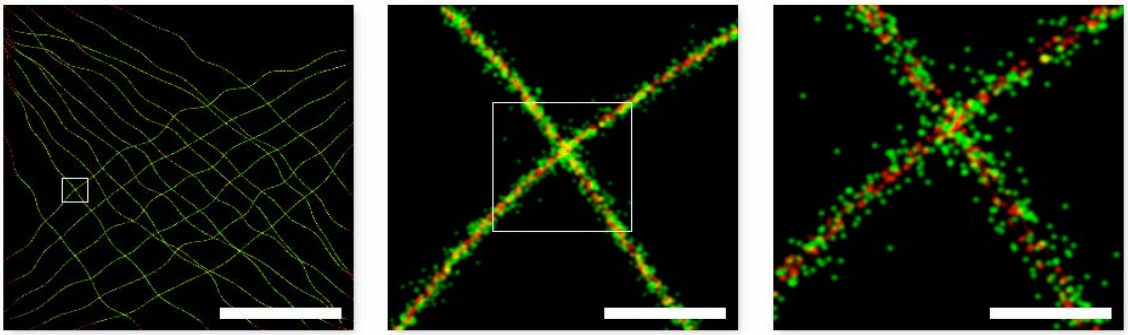


FPALM (*Sam Hess*)
Fluorescent Photo-Activation
Localization Microscopy

Benchmarking of SMLM Software

- ***Grand Challenge ISBI 2013***
  - More than 30 participants
  - Run by the authors on the same datasets
  - Assessment using the ground-truth
  - Multiple decision criteria analysis

D. Sage, H. Kirshner, T. Pengo, N. Stuurman, J. Min, S. Manley and M. Unser, "Quantitative evaluation of software packages for single-molecule localization microscopy," ***Nature Methods*** 12, 2015.



Scale bar: 6600 nm
Rendering pixelsize: 66 nm/pixel
FWHM: 10.0 nm

Scale bar: 500 nm
Rendering pixelsize: 5 nm/pixel
FWHM: 8.0 nm

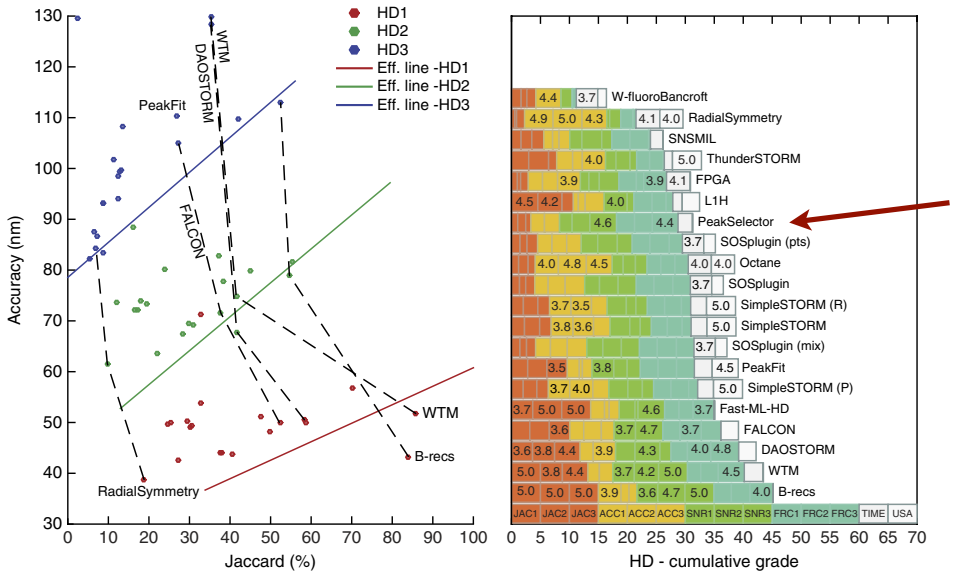
Scale bar: 200 nm
Rendering pixelsize: 2 nm/pixel
FWHM: 4.0 nm

Benchmarking of SMLM Software

- ***6 criteria of assessment***
  - **Accuracy** (nm) of the localizations
  - **Jaccard index** (%) on the localizations
  - SNR (dB): comparison of rendering images
  - Resolution FRC (Fourier ring correlation)
  - Usability of the software (grade)
  - Computation time (grade)

High-density data

1. B-recs, Janelia Farm
2. WTM, Hamamastu
3. DAOSTORM, University of Oxford



Challenges for digital optics

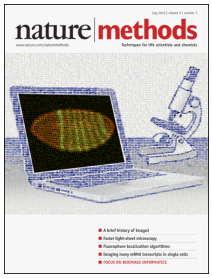
- Realistic physical models (space-varying?)
  - Relating the PSF to the refractive index of the specimen
  - Identification & calibration
- Better problem formulation
  - Semi-blind deconvolution
  - Regularization/sparsity based on statistical modeling
  - Beyond MAP and variational formulation

    ... *belief propagation* ...
- New inverse problems
  - Space-varying deconvolution
  - Refractive-index tomography (with holography)
  - Alternative mode of acquisition (structured light, multi-spectral, multi-modal)

OUTLINE

- Part 1: Basics of fluorescence imaging ✔
- Part 2: Mathematical Imaging ✔
- **Part 3: Tools for bioimage analysis**

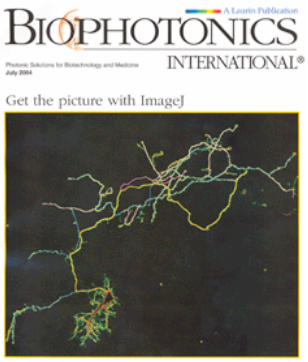
The nascent field of "bioimage informatics"



Part 3: Tools for bioimage analysis

Making microscopy quantitative, handling large data sets in 3D + time ...

- Particle tracking
  - Study of yeast dynamics (*D. Sage*)
- Cells: shape and motility (*R. Delgado-Gonzalo*)
- *Filaments*
  - Neuron tracing (*E. Meijering*)
- Extraction of gene expresion profiles



EDITORIAL

Nascent field of bioimage informatics

The quest for quantitative microscopy

With the aid of informatics, microscopy is in the midst of a crucial evolution into a more quantitative and powerful technique.

Microscopy has historically been a qualitative technique, but the transition to digital microscopy and advances in camera technology, coupled with new labeling and imaging methods, are making it easier to extract meaningful quantitative data from images. Computational techniques are central to this process. The transition of microscopy into a more quantitative technique will bring important scientific benefits in the form of new applications and improved performance and reproducibility.

Current limitations in bioimage-informatics techniques are preventing sophisticated optical methods from realizing their full potential. For example, the algorithms necessary to localize individual fluorophores in super-resolution microscopy data are still in their infancy, and the lack of tools to automatically reconstruct neuronal networks from 3D image stacks is hindering progress in neuroscience.

biologists in user-friendly packages.

Encouragingly, some institutions are devoting substantial resources in support of major open-source software tools. Funders are also making efforts: the US National Institutes of Health runs a 'Continued Development and Maintenance of Software' program, and the US National Science Foundation recently announced the 'Software Infrastructure for Sustained Innovation' program.

With sufficient support for bioimage informatics, we expect that the days of manually chosen 'representative' images are numbered. Not only will such images be replaced by quantitative measures based on the underlying image data, but even the example images shown in research articles will be either computed representations or computationally chosen representative images. As a result, the level of trust placed in imaging results should increase.

Why bioimage informatics matters

Gene Myers

Driven by the importance of spatial and physical factors in cellular processes and the size and complexity of modern image data, computational analysis of biological imagery has become a vital emerging sub-discipline of bioinformatics and computer vision.

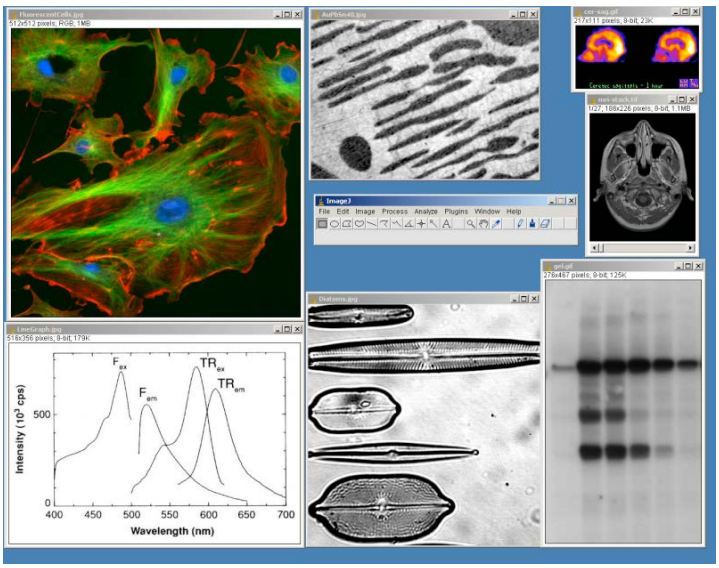
Making algorithms available to biologists

- Public-domain, open-source, platform independent
- Beautiful, widely-accepted software: ImageJ (thanks to Wayne Rasband)
- Crucial component of scientific imaging projects (quantitation, analysis)
- Committed population of developers
  JAVA + interoperability
- Huge, growing community of users

NIH Image to ImageJ: 25 years of image analysis

Caroline A Schneider, Wayne S Rasband & Kevin W Eliceiri

For the past 25 years NIH Image and ImageJ software have been pioneers as open tools for the analysis of scientific images. We discuss the origins, challenges and solutions of these two programs, and how their history can serve to advise and inform other software projects.

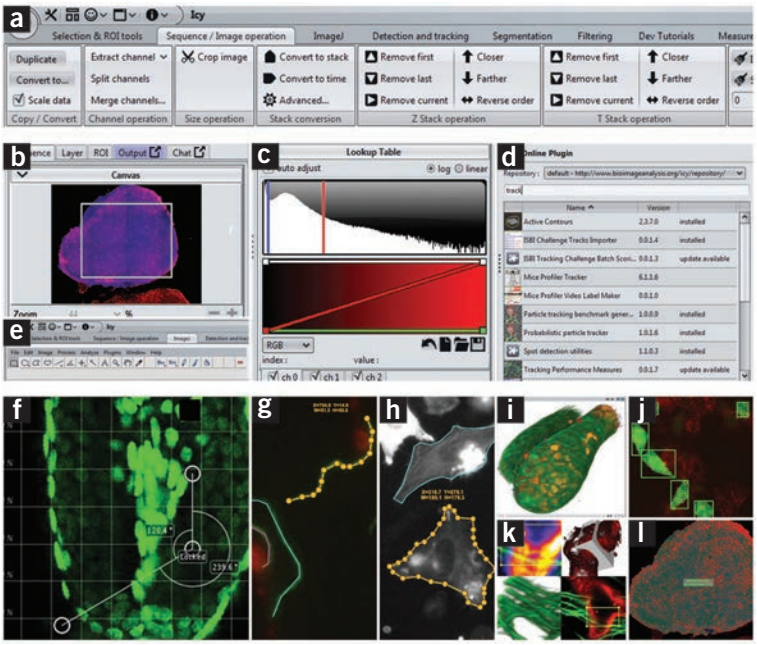


NATURE METHODS | VOL.9 NO.7 | JULY 2012 | 673

http://rsb.info.nih.gov/ij/

New kid on the block

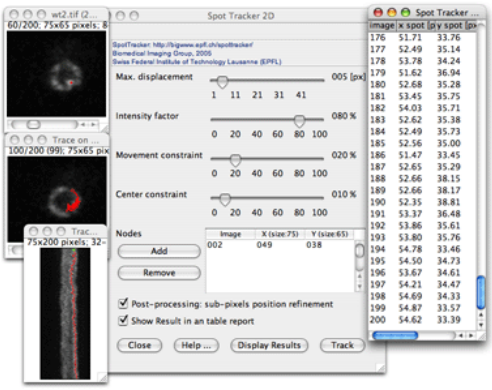
icy

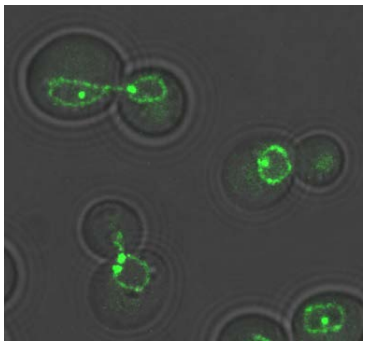


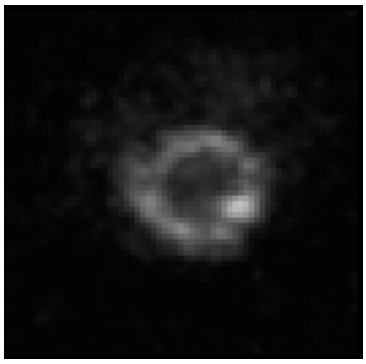
SpotTracker

Single particle tracking over noisy image sequence

- Study of yeast nuclear dynamic
- Global optimization (DP): past + future
- Cost-function tradeoffs:
  - Favors bright (or spot-like) structures
  - Imposes continuity constraints and penalizes large jumps
- Automatic or semi-automatic mode





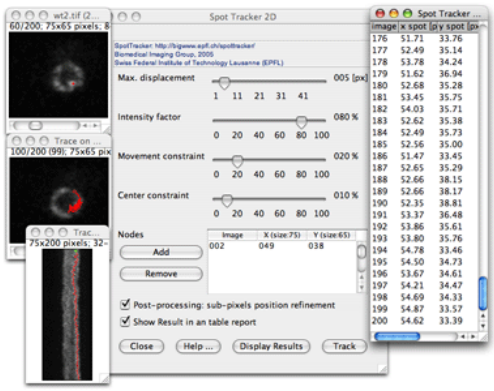


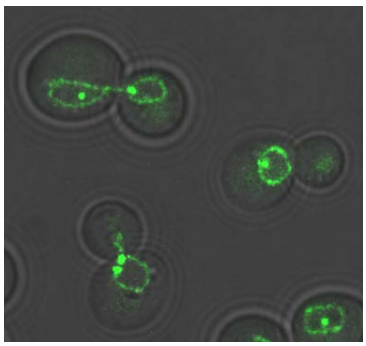
Data: Susan Gasser

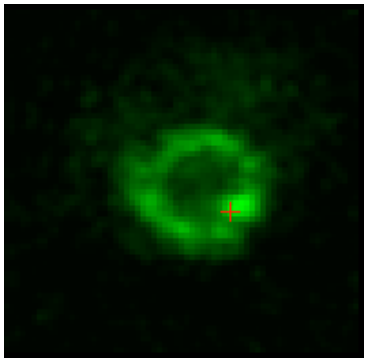
SpotTracker

Single particle tracking over noisy image sequence

- Study of yeast nuclear dynamic
- Global optimization (DP): past + future
- Cost-function tradeoffs:
  - Favors bright (or spot-like) structures
  - Imposes continuity constraints and penalizes large jumps
- Automatic or semi-automatic mode

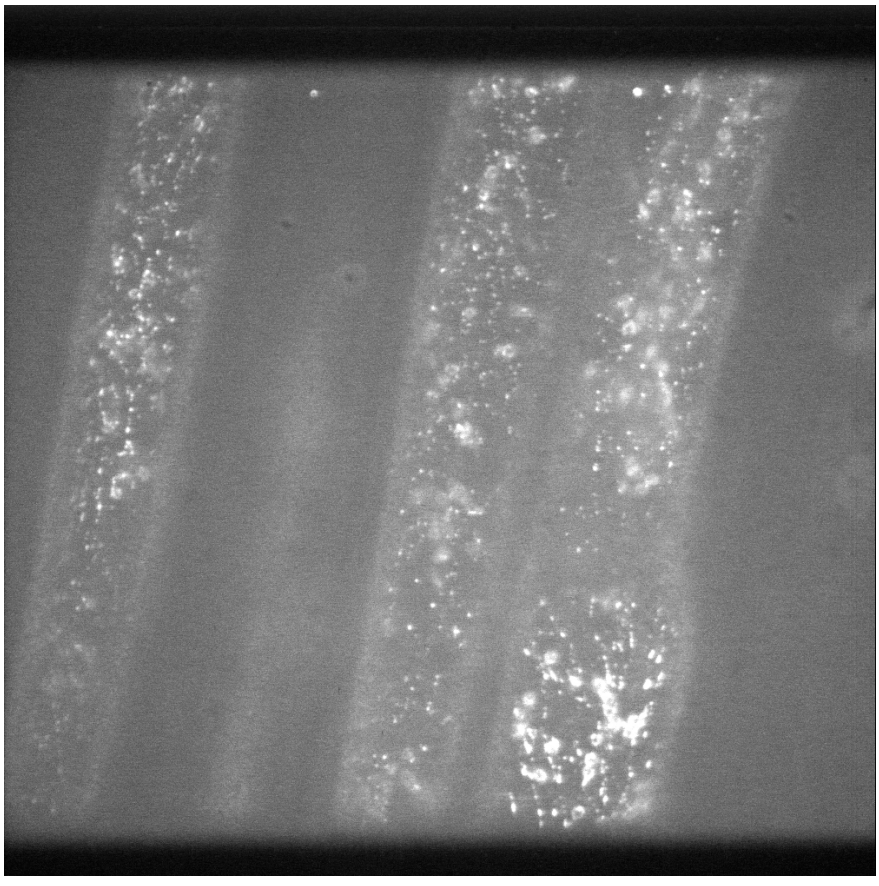






Data: S. Gasser, Dept. Molecular Biology, University of Geneva

Multi-particle tracking challenge



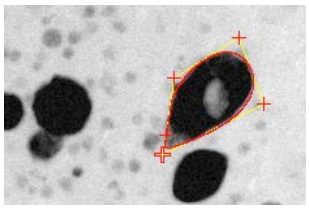
Data courtesy of
E. Crowell, INRA

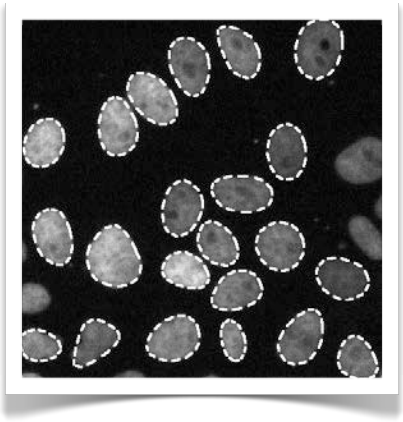
When E-splines meet snakes: active cells

control points

number of control points

$$\mathbf{r}(t) = \sum_{k=-\infty}^{\infty} \mathbf{c}[k]\, \varphi\left(M\, t - k\right) \qquad t \in [0, 1]$$



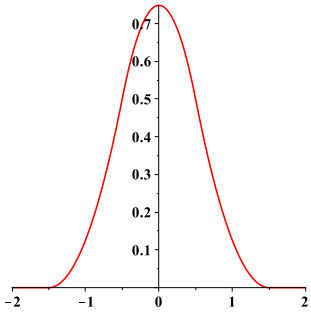


(Delgado-Gonzalo et al. *IEEE Trans. Image Processing* 2012)

Ellipse-reproduction B-spline

Shortest basis function (size $N = 3$) satisfying:

- Partition of unity (affine invariance)
- Riesz basis (stability and unicity)
- Reproduction of ellipses
- Continuity (+ differentiable twice)



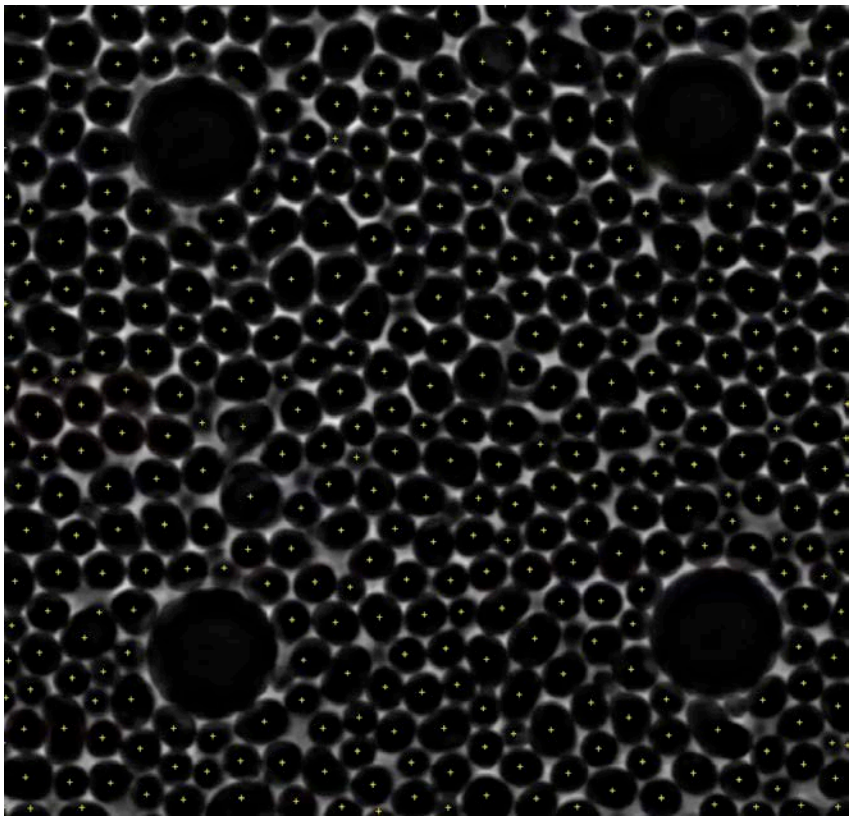
Exponential spline with parameter $\alpha = (0, \mathrm{j}\,\frac{2\,\pi}{M}, -\mathrm{j}\,\frac{2\,\pi}{M})$

$$\varphi(t) = \frac{1}{1 - \cos\frac{2\,\pi}{M}} \begin{cases} \cos\frac{2\,\pi\,|t|}{M}\,\cos\frac{\pi}{M} - \cos\frac{2\,\pi}{M} & 0 \le |t| < \frac{1}{2} \\ \left(\sin\frac{\pi\,(3/2 - |t|)}{M}\right)^2 & \frac{1}{2} \le |t| < \frac{3}{2} \\ 0 & \frac{3}{2} \le |t| \end{cases}$$

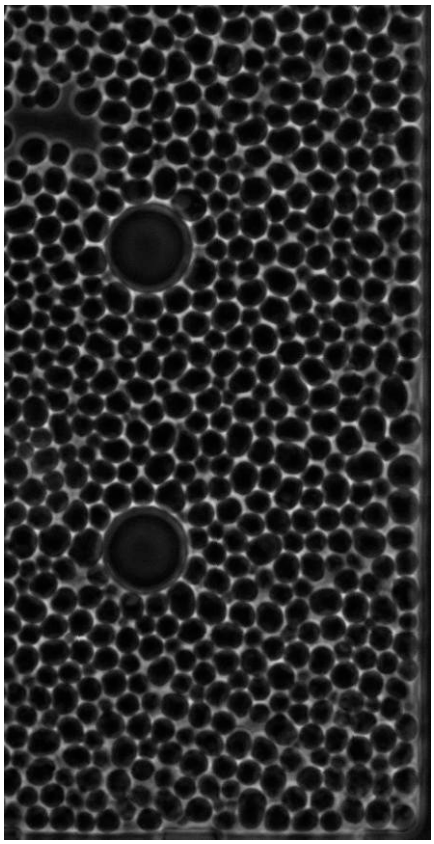
(Delgado-Gonzalo *et al.* CAGD 2012)

Active cells - Icy demo

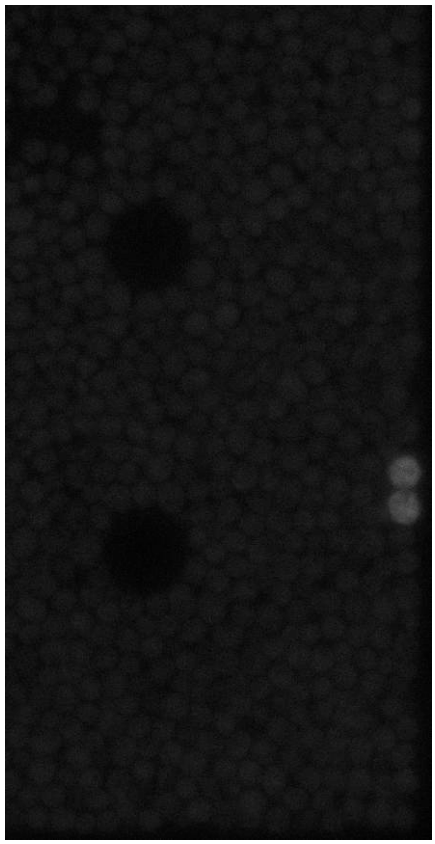
Tracking of cell crowds



Data courtesy of Prof. Sebastian Maerkl (EPFL)

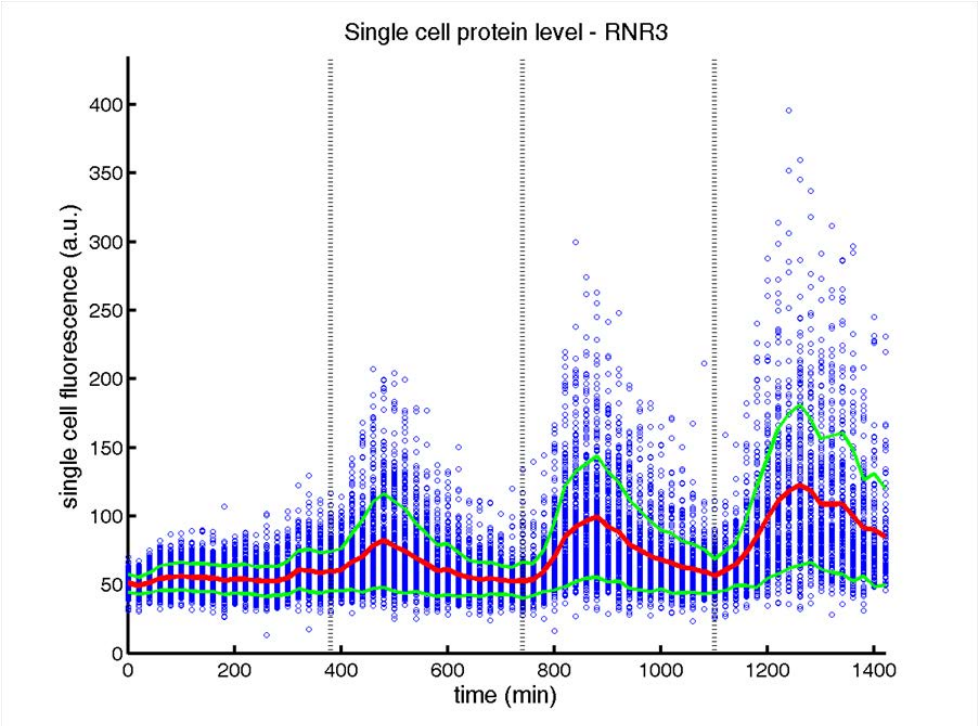


Phase contrast



Fluorescence

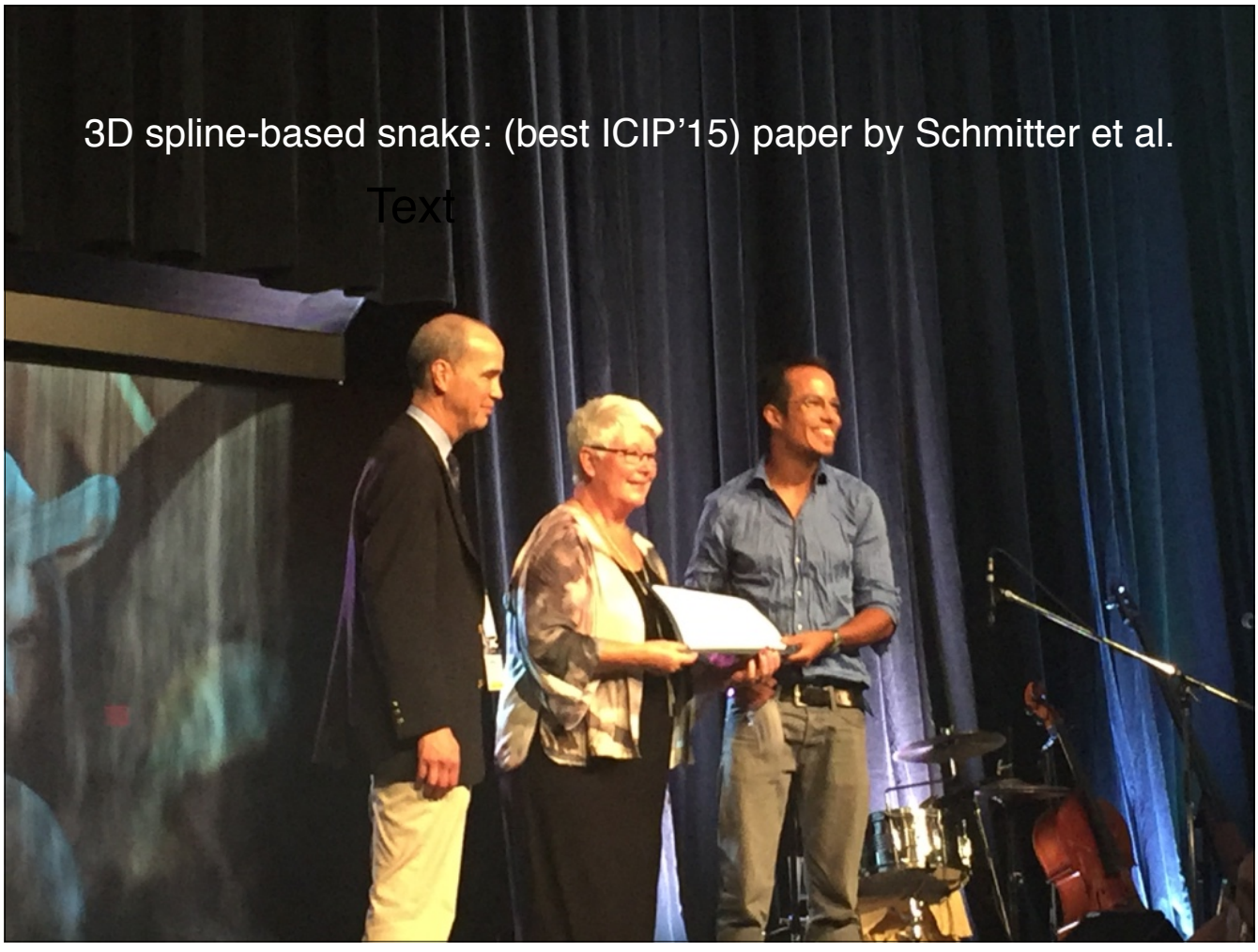
Gene expression profile



(Sage et al. *Cell Division* 2010)

Primary challenge for bioimage informatics

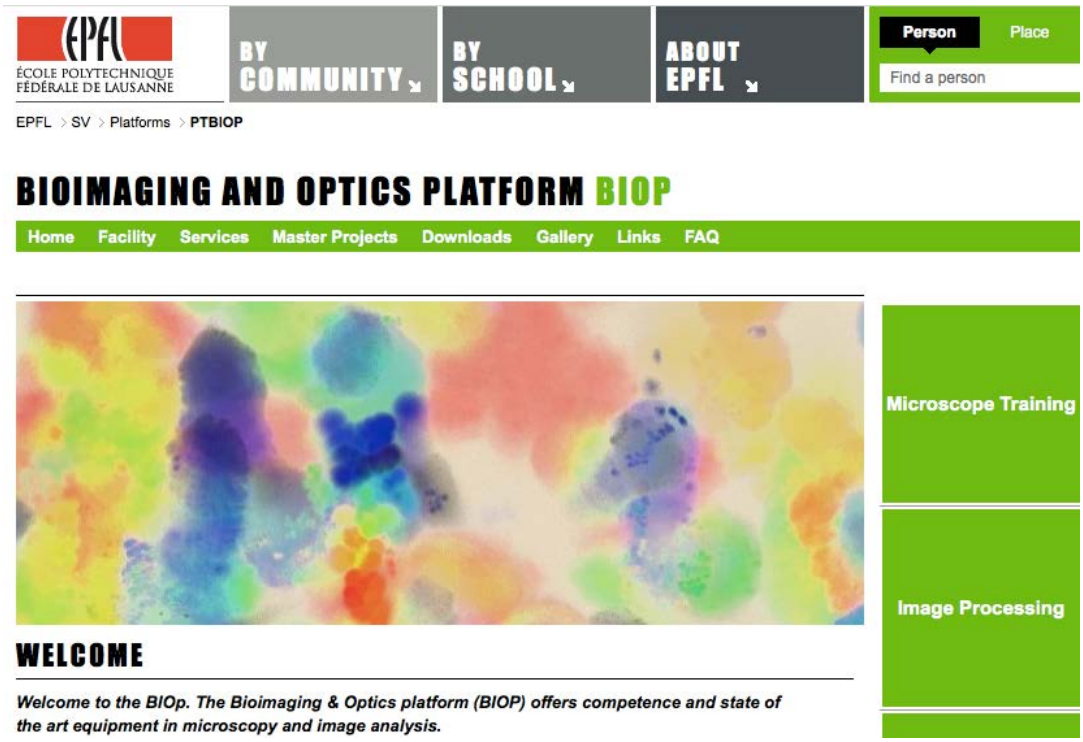
- Design of improved segmentation tools
  - Dealing with complicated shapes
  - Handling of cell division
  - Introducing models of time evolution
    ⇒ Global optimization in space + time
  - Crowded images, touching cells:
    ⇒ introducing repelling forces
  - High throughput constraint
    (huge numbers of cells and images)
  - Fast, reproducible and easy-to-use algorithms
  - Extension to 3D and 3D + time



CONCLUSION

- Invaluable role of fluorescence (GFP)
  - Plethora of experimental techniques
  - Molecular biology/biochemistry
- Advances in optics: confocal, localization, ...
  - Trend towards non-linear techniques
- Increasing role of signal processing
  - Deconvolution, imaging software
  - Digital optics
  - Quantitative image analysis

Creation of bioimaging centers



Shared resources, including specialists in image analysis

CONCLUSION (2)

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

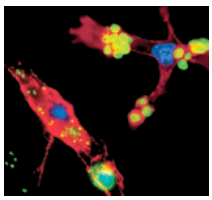
Global, integrative view of bioimaging

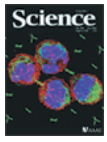
Optics

- physics
- electronics

Biology and Medicine

- molecular biology



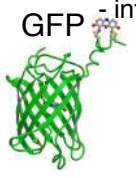


Signal processing

- mathematics
- informatics

Biochemistry (markers)

GFP



Special issues on topic:

IEEE Sig. Proc. Magazine, May 2006;
Nature Methods, July 2012;
IEEE Sig. Proc. Magazine, January 2015;
IEEE Selected Topics in Signal Processing, to appear 2016

Acknowledgments

FNSNF
FONDS NATIONAL SUISSE
SCHWEIZERISCHER NATIONALFONDS
FONDO NAZIONALE SVIZZERO
SWISS NATIONAL SCIENCE FOUNDATION

- Biomedical Imaging Group

Senior scientists & Post docs

- Philippe Thévenaz, Ph.D.
- **Daniel Sage**, Ph.D.
- Cédric Vonesch, Ph.D.
- **Ricard Delgado-Gonzalo**, Ph.D.

...

Ph.D. Students

- Emrah Bostan, Daniel Schmitter, Virginie Uhlmann, Anais Badoual, ...

Alumni

- Dr. François Aguet (Harvard)
- Prof. Thierry Blu (Chinese Univ., Hong Kong)
- Dr. Nicolas Chenouard (NYU)
- Prof. Brigitte Forster (Univ. Munich)
- Prof. Mathews Jacob (Univ. Iowa)
- Prof. Erik Meijering (Erasmus Univ.)
- Prof. Matthieu Querquin-Kern (ENSEA)
- Prof. Michael Liebling (UC Santa Barbara)
- Dr. Florian Luisier (Harvard)
- Prof. D. Van De Ville (EPFL
- ...

- Partial list of collaborators

- Dr. Arne Seitz
- Prof. Nikos Stergiopoulos
- Prof. Sebastian Maerkl
- Prof. John McKinney
- Prof. Viesturs Simanis
- Prof. Theo Lasser
- ....

erc

Selected references

- Introduction to fluorescence microscopy
  - C. Vonesch, F. Aguet, J.-L. Vonesch, M. Unser, "The Colored Revolution of Bioimaging," *IEEE Sig. Proc. Magazine*, vol. 23, no. 3, pp. 20-31, May 2006.
- Mathematical imaging
  - F. Luisier, T. Blu, M. Unser, "Image Denoising in Mixed Poisson-Gaussian Noise," *IEEE Trans Image Processing*, vol. 20, no. 3, pp. 696-708, March 2011.
  - C. Vonesch, M. Unser, "A Fast Multilevel Algorithm for Wavelet-Regularized Image Restoration," *IEEE Trans. Image Processing*, vol. 18, no. 3, pp. 509-523, March 2009.
  - E. Bostan, U.S. Kamilov, M. Nilchian, M. Unser, "Sparse Stochastic Processes and Discretization of Linear Inverse Problems," *IEEE Trans. Image Proc.*, vol. 22, no. 7, pp. 2699-2710, July 2013.
  - D. Sage, H. Kirshner, T. Pengo, N. Stuurman, J. Min, S. Manley, M. Unser, "Quantitative Evaluation of Software Packages for Single-Molecule Localization Microscopy," *Nature Methods*, vol. 12, pp. 717–724, 2015.
- Bioimage informatics
  - D. Sage, F.R. Neumann, F. Hediger, S.M. Gasser, M. Unser, "Automatic Tracking of Individual Fluorescence Particles: Application to the Study of Chromosome Dynamics," *IEEE Trans. Image Proc.*, vol. 14, no. 9, pp. 1372-1383, September 2005.
  - R. Delgado-Gonzalo, P. Thévenaz, C.S. Seelamantula, M. Unser, "Snakes with an Ellipse-Reproducing Property," *IEEE Trans. Image Processing*, vol. 21, no. 3, pp. 1258-1271, March 2012.
  - R. Delgado-Gonzalo, V. Uhlmann, D. Schmitter, M. Unser, "Snakes on a Plane: A Perfect Snap for Bioimage Analysis," *IEEE Sig. Proc. Mag.*, vol. 32, no. 1, pp. 41-48, January 2015.

http://bigwww.epfl.ch

TurboReg (Plugin for ImageJ)