BERN
03. November 2010

T. Lasser & Laboratoire d'Optique Biomédicale
Ecole Polytechnique Fédérale de Lausanne

Structure - Function

Complementary parts of life

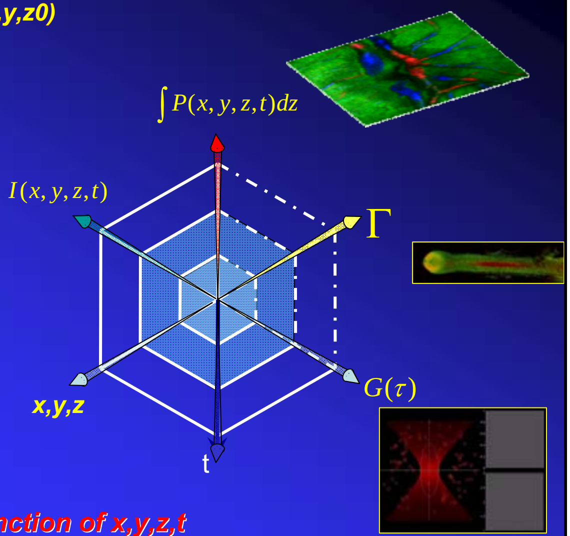
structural Imaging $\Rightarrow$ I (x,y,z0)

functional Imaging

function

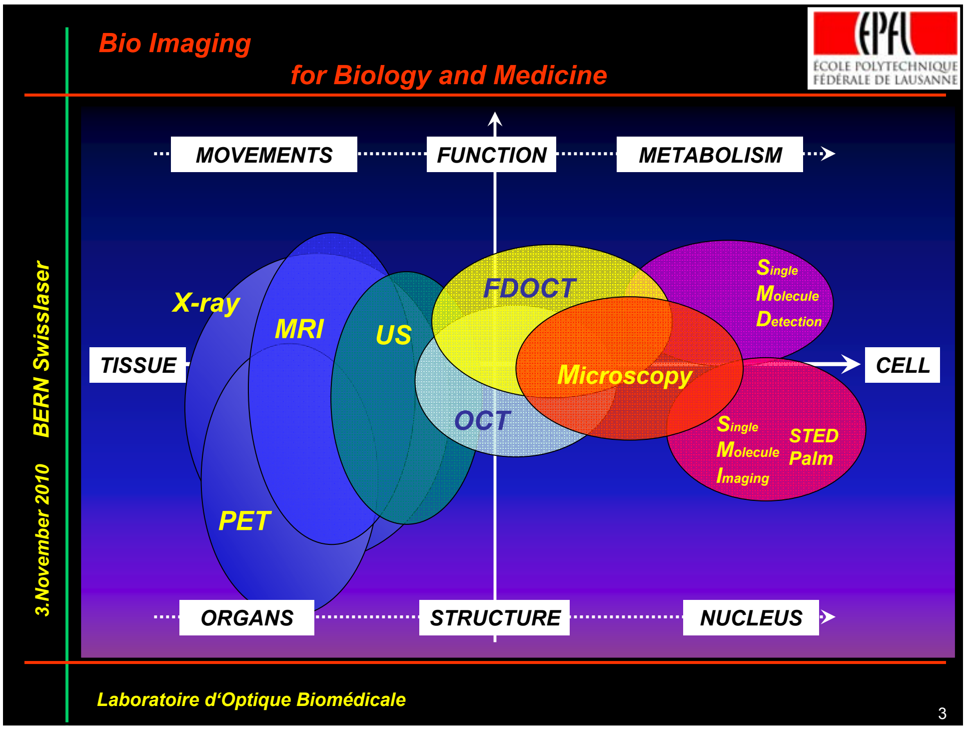
more dimensions

- Lifetime Γ
- Correlation $G(\tau)$
- Concentration c_i
- chemical Cinetics



information

but all as… a function of x,y,z,t


Bio Imaging
for Biology and Medicine
ÉCOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE
MOVEMENTS
FUNCTION
METABOLISM
X-ray
MRI
US
FDOCT
Single Molecule Detection
TISSUE
Microscopy
CELL
OCT
Single Molecule Imaging
STED
Palm
PET
ORGANS
STRUCTURE
NUCLEUS
3.November 2010 BERN Swisslaser
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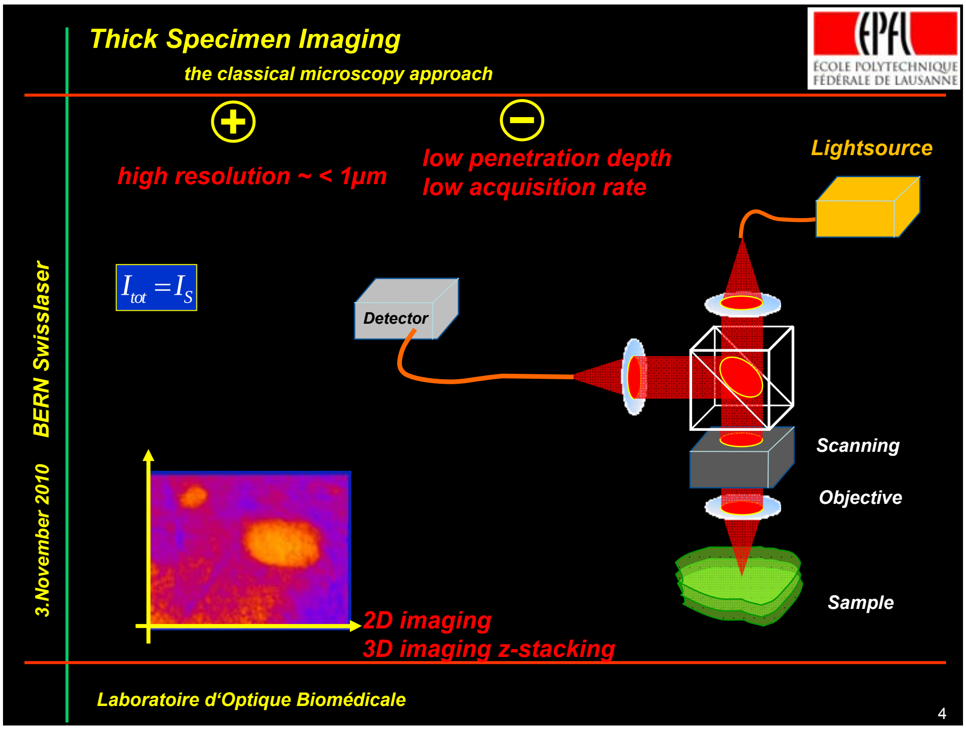
3


Thick Specimen Imaging
the classical microscopy approach
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high resolution ~ < 1µm
low penetration depth
low acquisition rate
Lightsource
$I_{tot} = I_S$
Detector
Scanning
Objective
Sample
2D imaging
3D imaging z-stacking
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What to do?

...or how to do better

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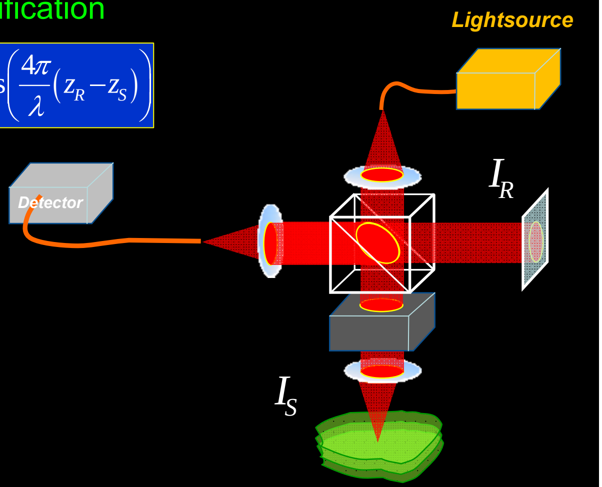
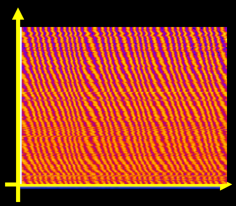
Coherent Amplification

$$I_{tot} = I_R + I_S + 2\sqrt{I_R I_S}\cos\left(\frac{4\pi}{\lambda}(z_R - z_S)\right)$$





What to do?

Fourier Domain Optical Coherence Tomography

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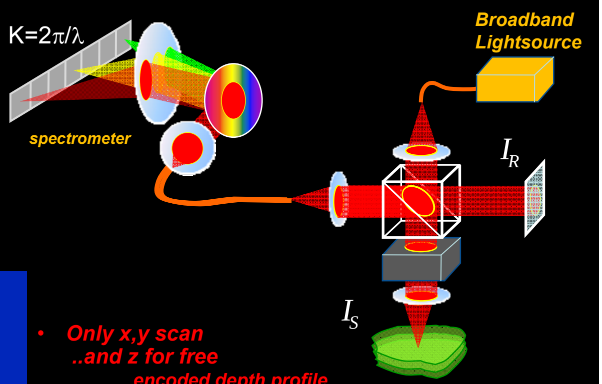
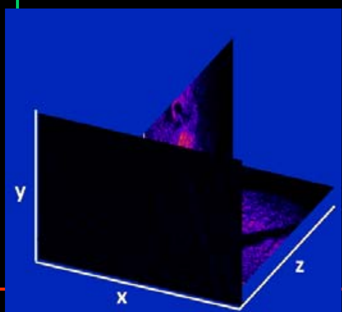
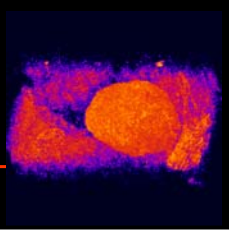


Fourier transform From x,y,k to x,y,z

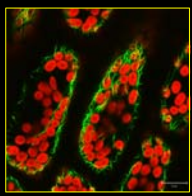


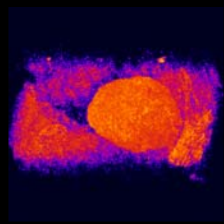
- *Only x,y scan ..and z for free encoded depth profile*
- *high sensitivity >100dB*
- *in-vivo 3D*
- *high resolution ~ 5 – 8 µm*
- *high penetration depth*



Let‘s do...

Functional imaging...and FDOCT



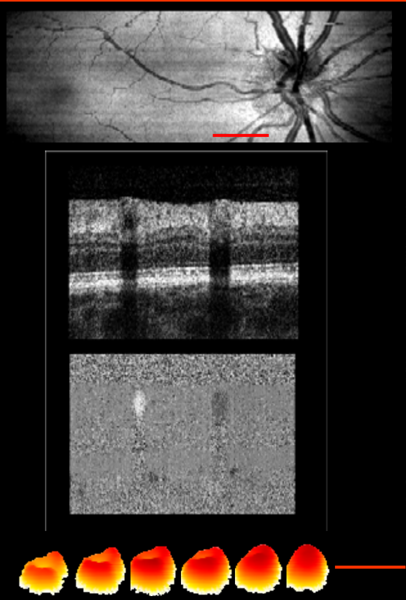


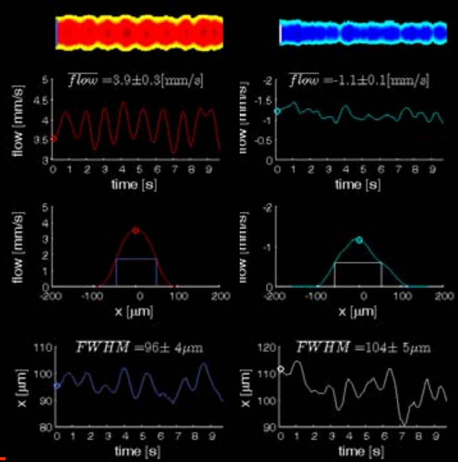
Langerhans’ Islets
Wild mouse

- **Molecular contrast**
  - fluorescence
- **2D imaging**
  - 3D needs z-stacking
- **low penetration depth**
- **high resolution ~ < 1µm**

- **label free imaging contrast**
  - refraction index > intrinsic sample property
- **high sensitivity**
  - > 100 dB
- **Parallel depth probing**
  - high speed 3D, time lapse imaging
- **Availability of phase information**
  - functional imaging
- **Isotropic resolution (ca. 5 µm)**
  - 3D-microstructure of biological samples

Functional Imaging – Doppler flow





R. Michaely et al.
J. Biomedical Optics

FDOCT & microscopy

a combination….??

+ coherent amplification
  sample signal
  high contrast images

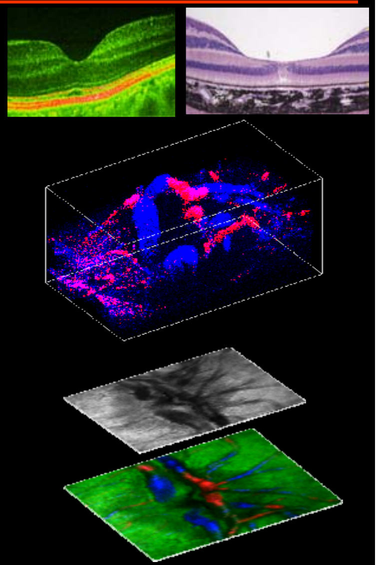
$$S_{OCT} \propto \sqrt{I_{ref} I_{sample}}$$

+ availability of phase information
  -> phase contrast schemes
  -> functional imaging (nm resolution)

+ optical sectioning > coherence gating
  along optical axis (broad band source)

+ imaging speed of FD OCT methods
  -> high temporal resolution (30µs)
  accessing physiological processes

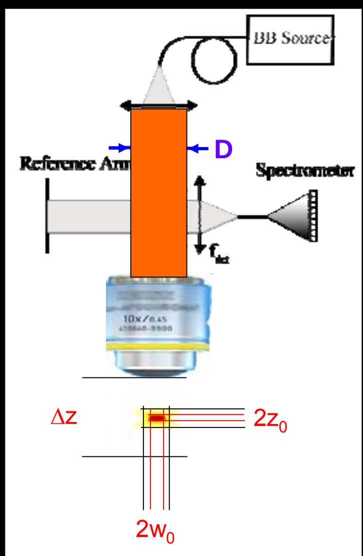
+ direct access to depth resolved
  spectral sample properties
  (molecular contrast OCT,
  diff spectroscopic OCT,…)



…but what about resolution
lateral and axial

OCM

an obvious contradiction...?



-Focus light on sample

-Effective Numerical Aperture: $NA = \frac{D}{2f}$

-Waist: $w_0 = \frac{\lambda}{\pi NA}$

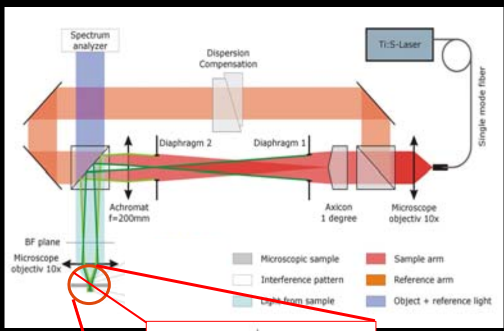
-Rayleigh-Range: $z_0 = \frac{\lambda}{\pi NA^2}$

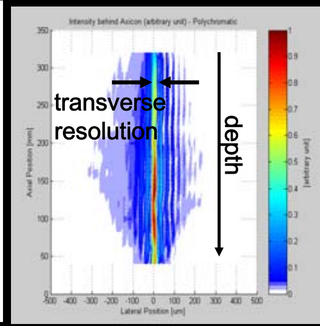
-Depth of Field: $\mathrm{DOF} = 2z_0$

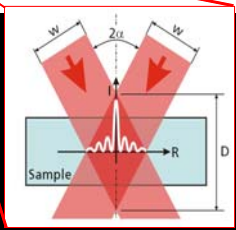
-Lateral resolution and Depth of Field (DOF) depend both on NA

-Loss of resolution and signal outside DOF

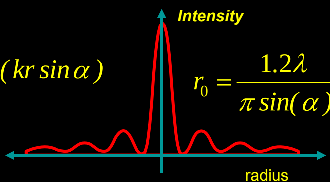
xf FDOCM - Experimental Setup







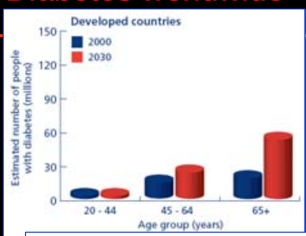
$$I(r) \propto J_0^2(kr\sin\alpha)$$



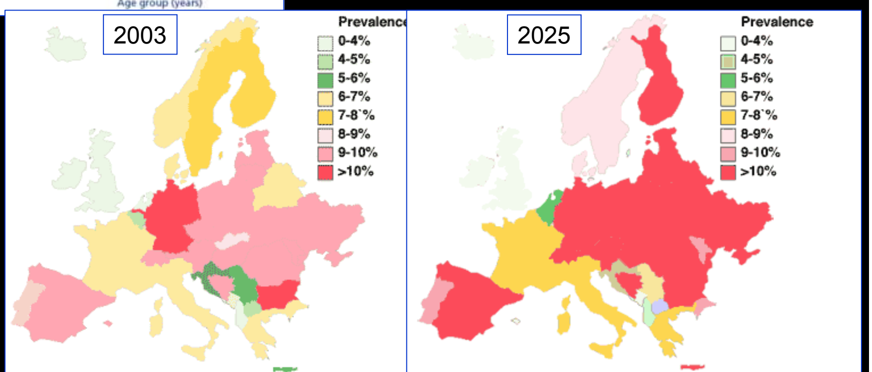
$$r_0 = \frac{1.2\lambda}{\pi\sin(\alpha)}$$

R. Leitgeb et al.
OPTICS LETTERS / Vol. 31, No. 16 / August 15, 2006

Diabetes worldwide

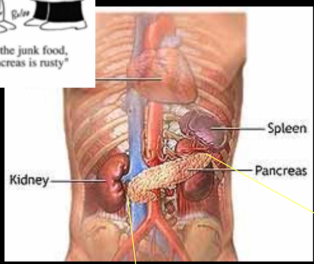


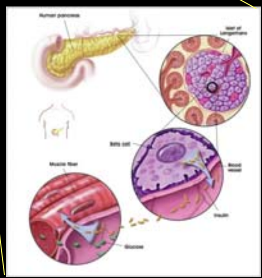
diabetes	*2000*	*2030*
World	*171,000,000*	*366,000,000*

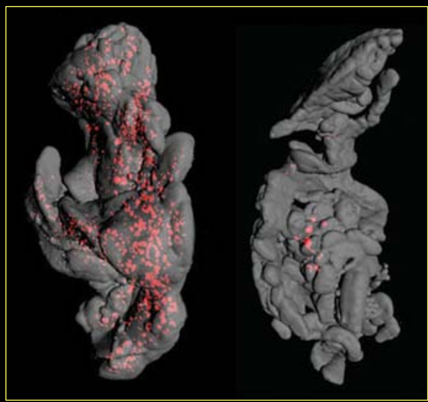


Optical Projection Tomography

ex vivo imaging





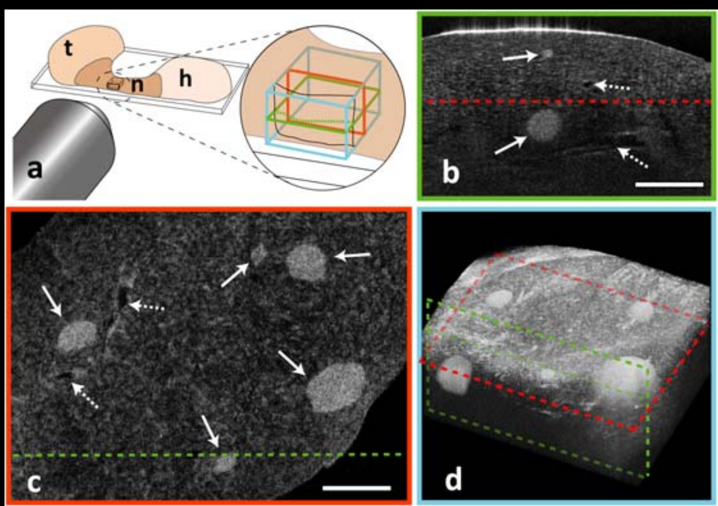


OPT adult mouse pancreata
wild-type (left) diabetic NOD mouse (right)
insulin labeled Islets of Langerhans (red).
size approx. 1.3 x 0.7 cm.

U. Ahlgren, Nature Methods, Jan;(1):31-3, 2007

islet Imaging

In vitro



Scale bar: 250 µm

islet Imaging

In vitro

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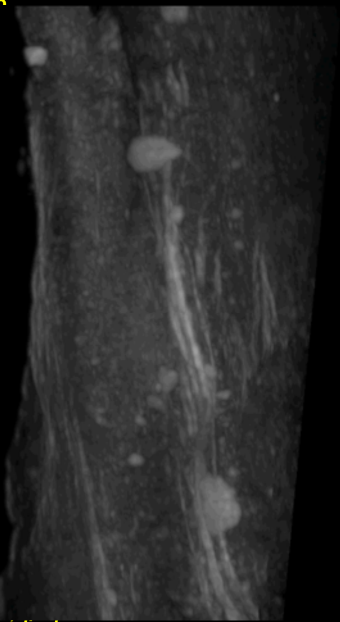

00:00:00.000

islet Imaging

In vivo

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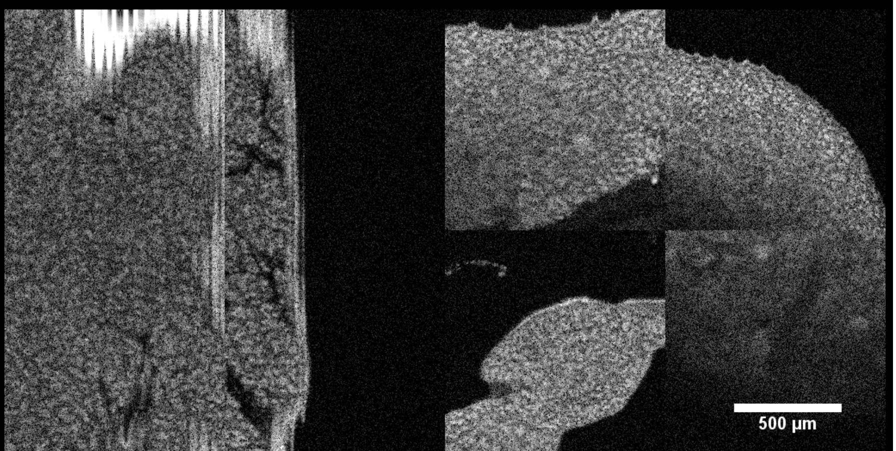


Induced diabetes

streptozotocin

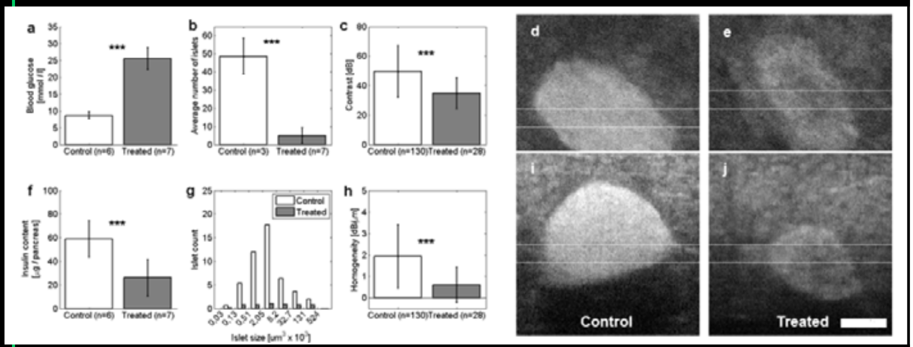
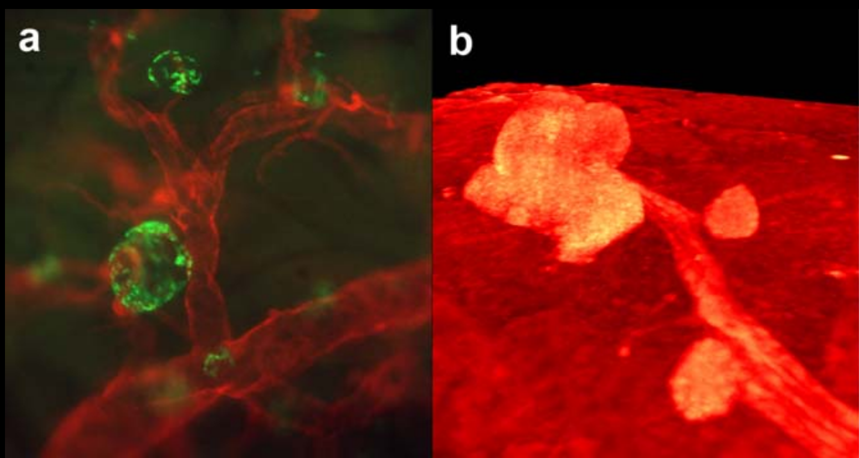


Figure 5: Exposure of C57B6 mice to streptozotocin (solid bars) resulted in a marked increase in blood glucose levels (**a**) and in a parallel decrease in the insulin content of the pancreas (**f**) compared to the control group (white bars). The selective beta cell death induced by the drug also decreased the number of islets found in the analysed regions of interest (**b**, screened tissue volume of 2.5mm^2 per animal). **g** The logarithmic distribution of the islet size exhibits a characteristic pattern for the control islets that is lost in the case of the remaining islets of treated animals. The few remaining islets (only islets >1000 µm^3 taken into account) likewise show a reduced contrast (**c**) and homogeneity (**h**). (a,b,c,f and h show means ± SE, ***$p<0.001$).



a Immunostained whole mount samples imaged with fluorescence wide field microscopy
Green: insulin, Red: endothelial cells.
b Rendered xfOCM tomogram, islets and vessel structure
on the background of the exocrine tissue.

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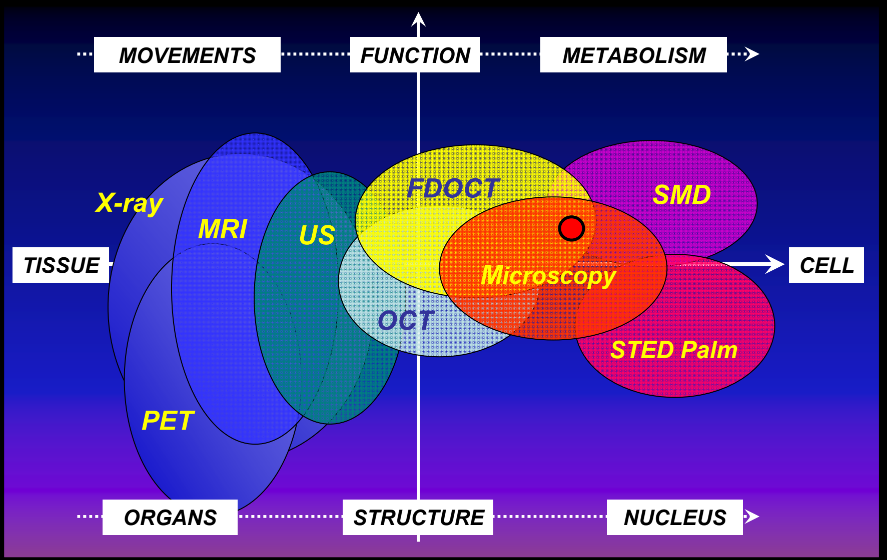
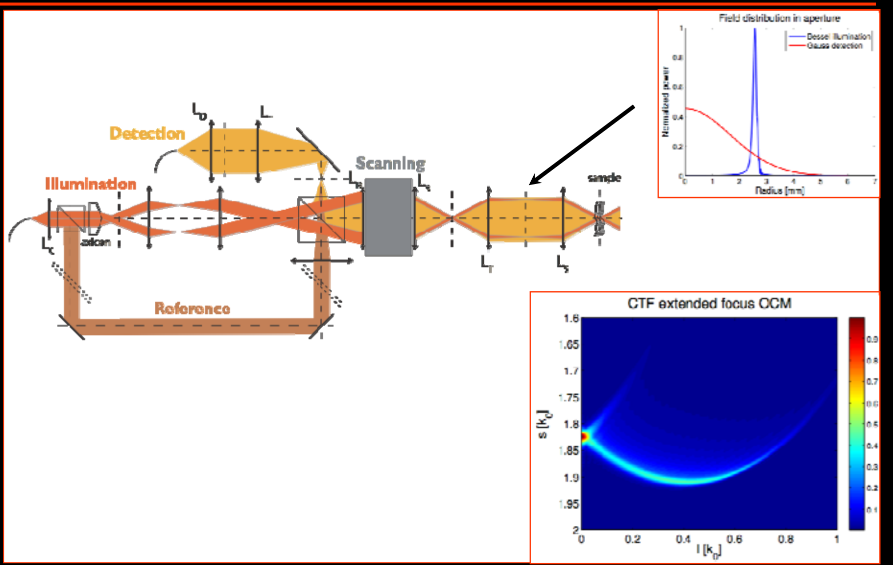
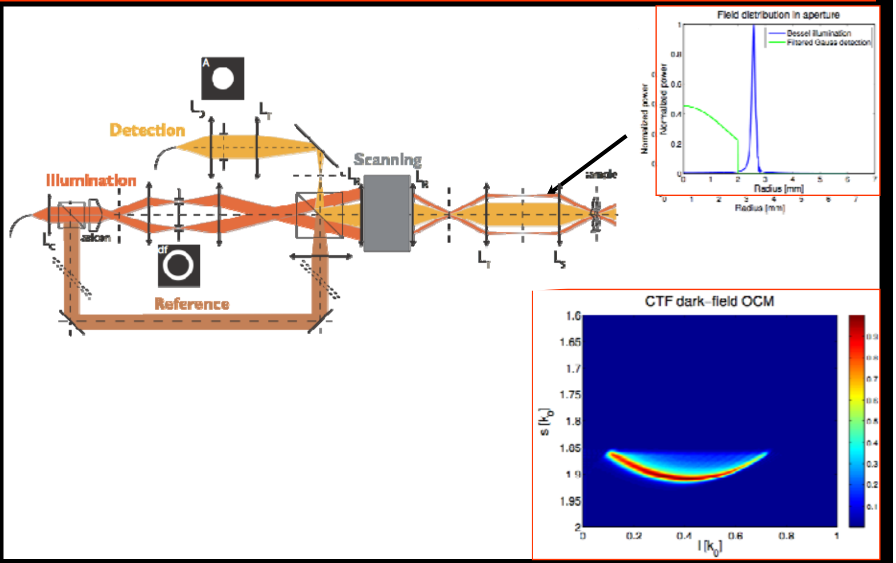


Extended focus OCM



Dark-field OCM



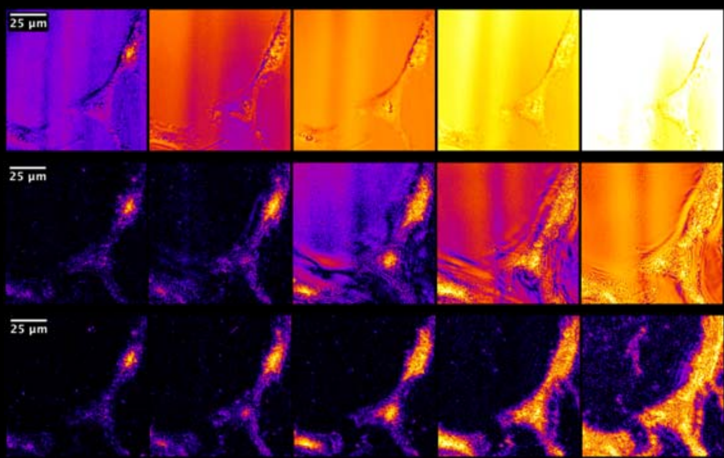
Comparison on living cells

- Through focus stack of living NIH-3T3 cells observed in up-right configuration. Axial sampling: 2 µm

OCM
NA 0.42

xf-OCM
NA 0.68

df-OCM
NA 0.68



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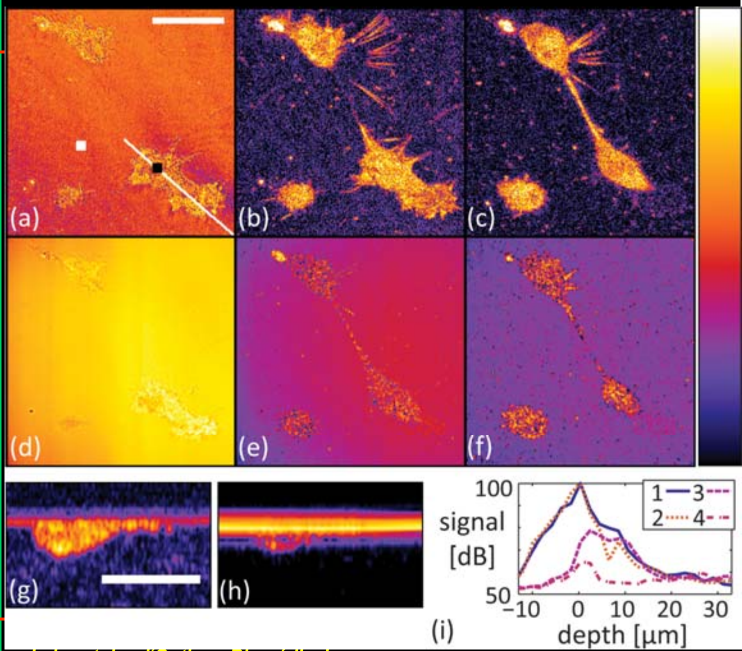

darkfield OCM

darkfield OCM

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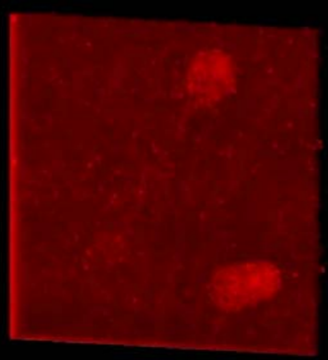
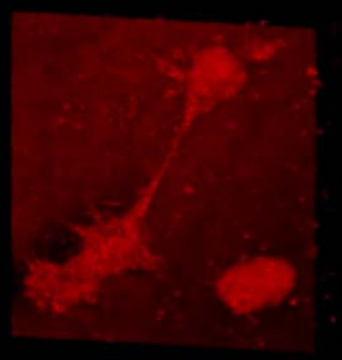
OCM

NIH-3T3 cells



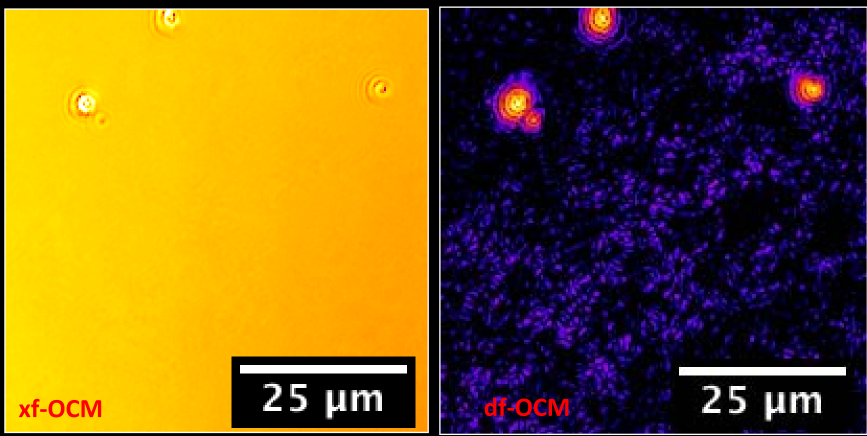


images dark-field OCM\cellClassicalOCM2010.mov

images dark-field OCM\celldfOCM2010.mov

Outlook (2)

- Nanoparticles labeling
  - down to 6 nm gold colloids
  - en face images of 30 nm gold nanoparticles



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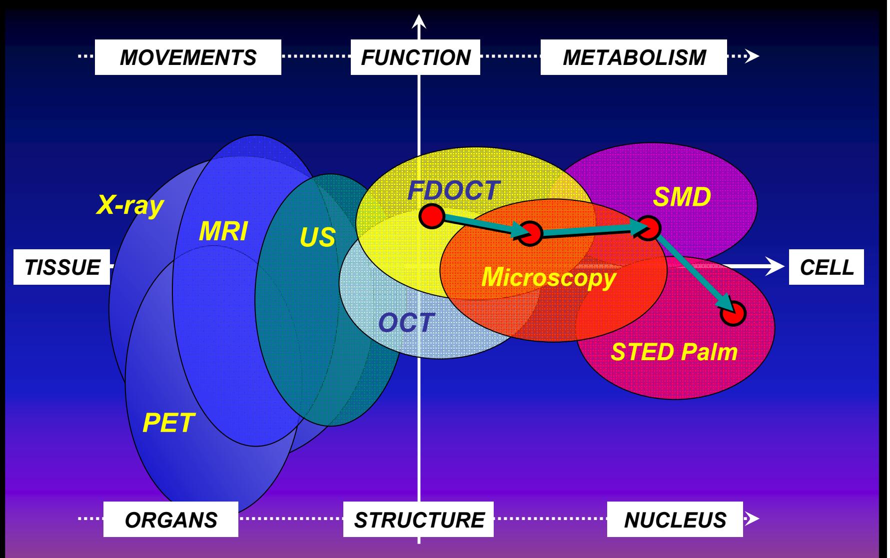
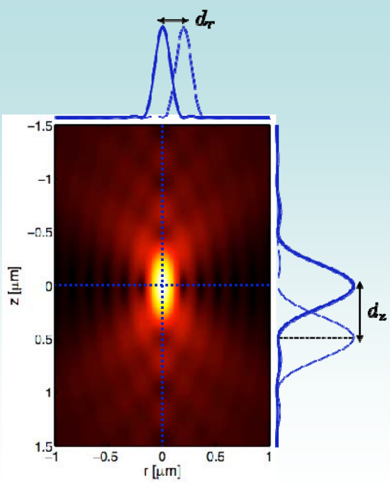


Resolution in microscopy
overcoming the limitation



- Lateral resolution

$$h(r,0;\mathbf{p}) = \left|\int_0^1 J_0(k_0 r \mathrm{NA}\rho)\rho\,\mathrm{d}\rho\right|^2 = \left(\frac{J_1(k_0 r\mathrm{NA})}{k_0 r\mathrm{NA}}\right)^2$$

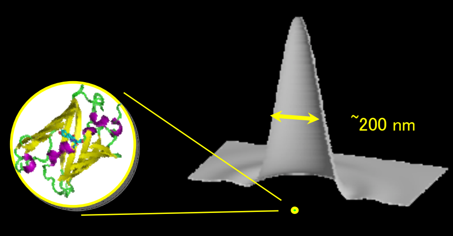
- Axial resolution

$$h(0,z;\mathbf{p}) = \left|\int_0^1 e^{ik_0 z\frac{\mathrm{NA}^2}{2n_i}\rho^2}\rho\,\mathrm{d}\rho\right|^2 = \frac{2n_i^2}{k_0^2 z^2 \mathrm{NA}^4}\left(1-\cos\left(\frac{k_0 z\mathrm{NA}^2}{2n_i}\right)\right)$$

$$d_r = \frac{0.61\lambda}{\mathrm{NA}} \qquad d_z = \frac{2\lambda n_i}{\mathrm{NA}^2}$$

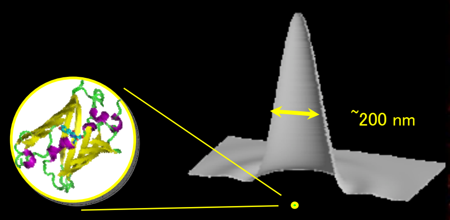
λ = 450 nm
NA = 1.4
n = 1.515
d_r = 200 nm
d_z = 700 nm

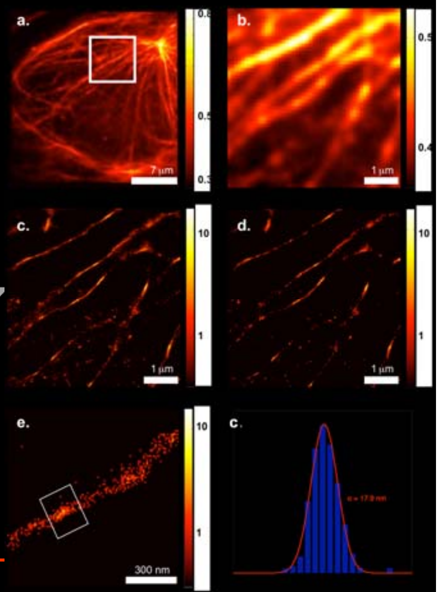
The fundamental limit
imaging beyond the diffraction limit



Goal:
optical imaging (sensing) at
nanometer/subnanometer scales

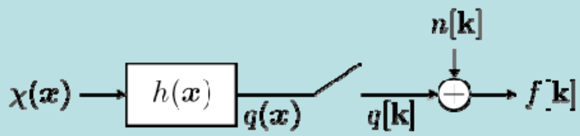
The fundamental limit
imaging beyond the diffraction limit

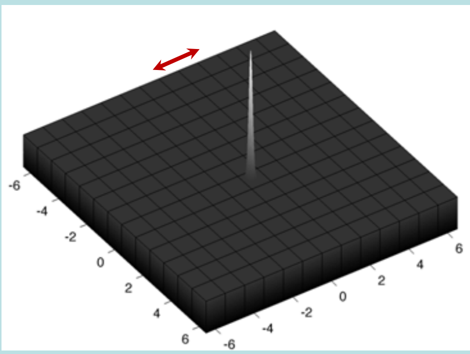




Goal:
optical imaging (sensing) at nanometer/subnanometer scales

"Super-resolution" through localization





- Average photon count at detector: $\bar{q}(x) = c \cdot s(x)$
- Shot noise

$$P_{q(x;x_p,\tau)}(q) = \frac{e^{-\bar{q}(x;x_p,\tau)}\,\bar{q}(x;x_p,\tau)^q}{q!}$$

- Read-out noise: $n[\mathbf{k}] \sim \mathcal{N}(\mu_r, \sigma_r^2)$
- Shift b: background signals + σ_r^2

$$s(x;x_p,\tau) = \int_{\mathbb{R}^3} o(t)\,h(x;t)\,\mathrm{d}t + b$$

F. Aguet EPFL Thesis 2009

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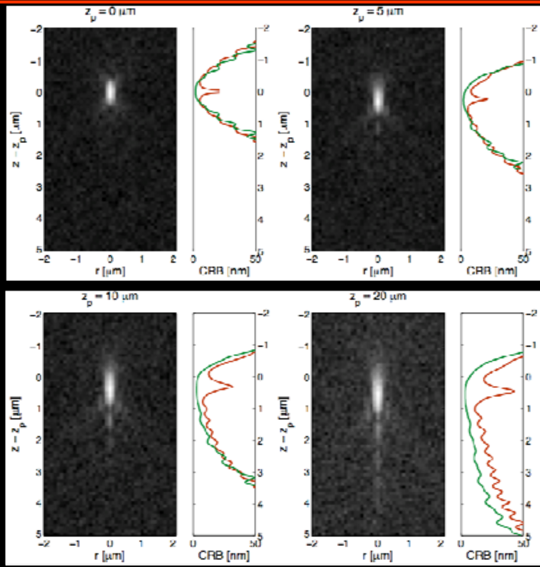
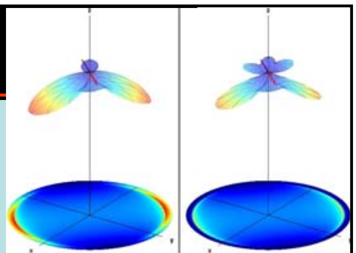
Theoretical limits on localization accuracy

- Localization limits for x and z are very close
- CRB is linked to 'information' in the diffraction pattern
- Off-focus imaging leads to higher localization accuracy
- Optimal experimental settings (focus positions) can be derived from CRB



3-D steerable dipole filters

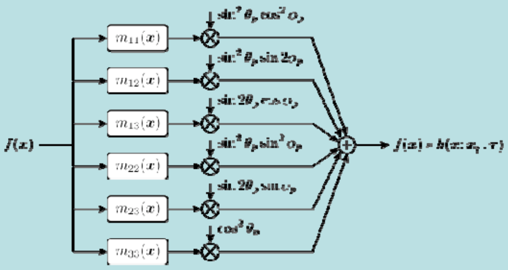


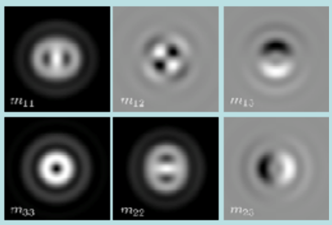
- Richards-Wolf integrals decouple from the dipole moment

$$\mathcal{E} = -i \begin{bmatrix} I_0 - I_2\cos(2\phi_d) & I_2\sin(2\phi_d) & -2iI_1\cos(\phi_d) \\ I_2\sin(2\phi_d) & I_0 - I_2\cos(2\phi_d) & -2iI_1\sin(\phi_d) \end{bmatrix} \mathbf{p}$$

- 3-D intensity distribution in image space is steerable

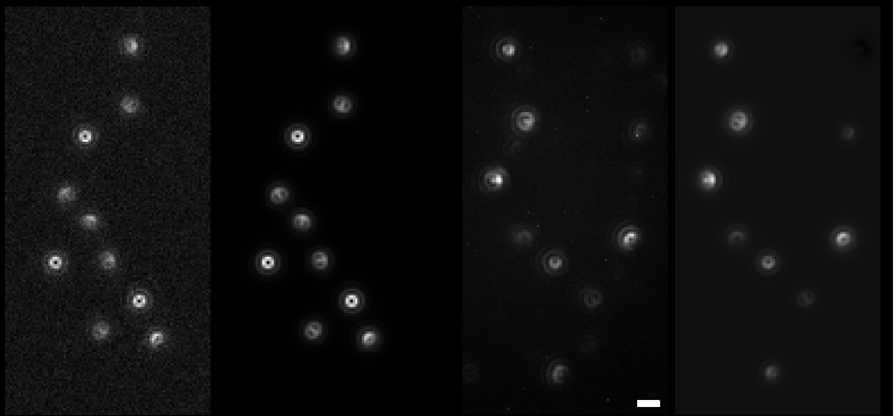
$$h_{\theta_p,\phi_p}(\boldsymbol{x}; \boldsymbol{x}_p, \tau) = \mathcal{E}^*\mathcal{E}$$
$$= \sin^2\theta_p\left(|I_0|^2 + |I_2|^2 + 2\cos(2\phi_p - 2\phi_d)\Re\{I_0^* I_2\}\right)$$
$$- 2\sin(2\theta_p)\cos(\phi_p - \phi_d)\Im\{I_1^*(I_0 + I_2)\} + 4|I_1|^2\cos^2\theta_p$$
$$= \mathbf{p}^T\mathbf{M}\mathbf{p}$$





Experimental results

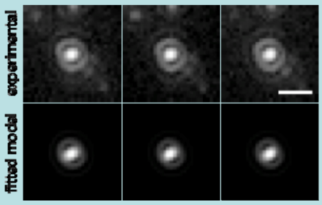
Cy5 dipoles at an air/glass interface



Measurement | Recalculation | Measurement | Recalculation

Experimental validation

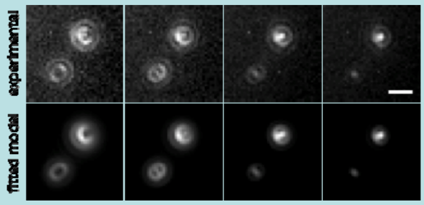
Localization reproducibility on a single dipole



	x_p	y_p	θ_p	ϕ_p	z
μ	1.42 μm	1.37 μm	85.45°	298.68°	214.53 nm
σ	3.00 nm	6.04 nm	1.05°	2.14°	10.72 nm

Relative values consistent with CRB

Accuracy of the model/fit in 3-D



	$x_p^{(1)}$	$y_p^{(1)}$	$\theta_p^{(1)}$	$\phi_p^{(1)}$
μ	2.99 μm	1.38 μm	86.46°	223.77°
σ	33.16 nm	28.62 nm	1.98°	5.30°

	$x_p^{(2)}$	$y_p^{(2)}$	$\theta_p^{(2)}$	$\phi_p^{(2)}$
μ	1.40 μm	2.46 μm	67.05°	293.26°
σ	21.48 nm	41.98 nm	4.45°	1.61°

- Estimation accuracy
  - Orientation: ~ 2°
  - Position: ~ 6 nm
  - Z-position ~ 11 nm

Bio Imaging
for Biology and Medicine

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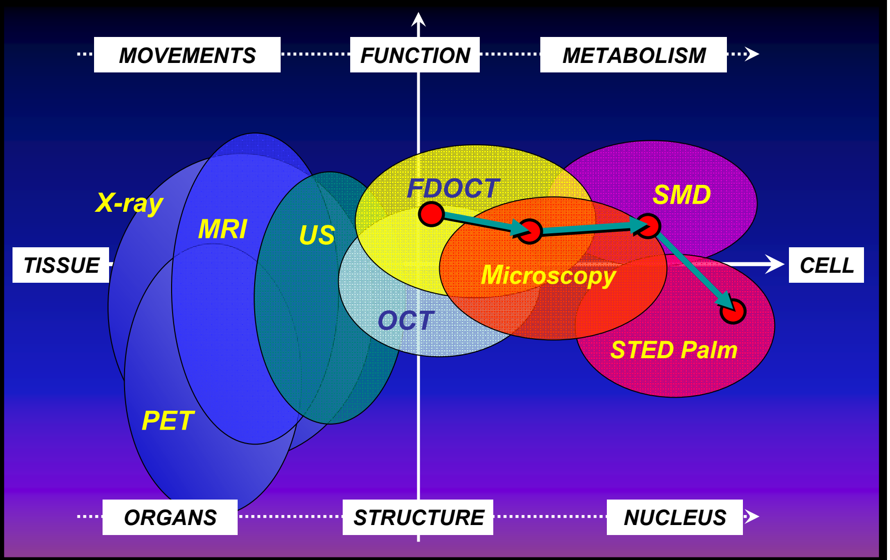

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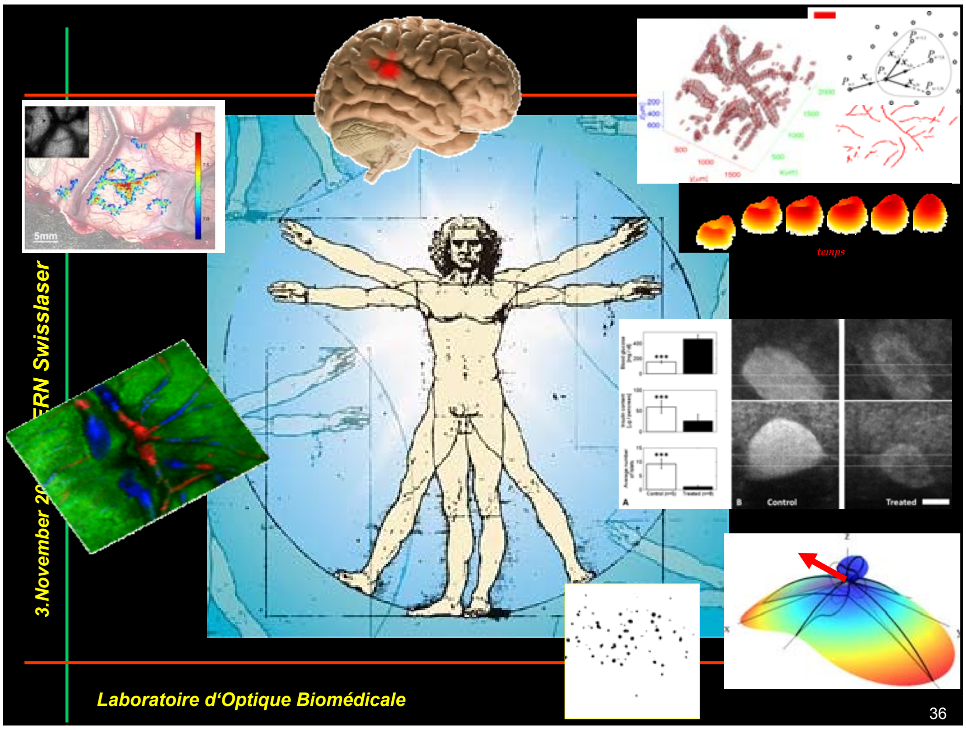

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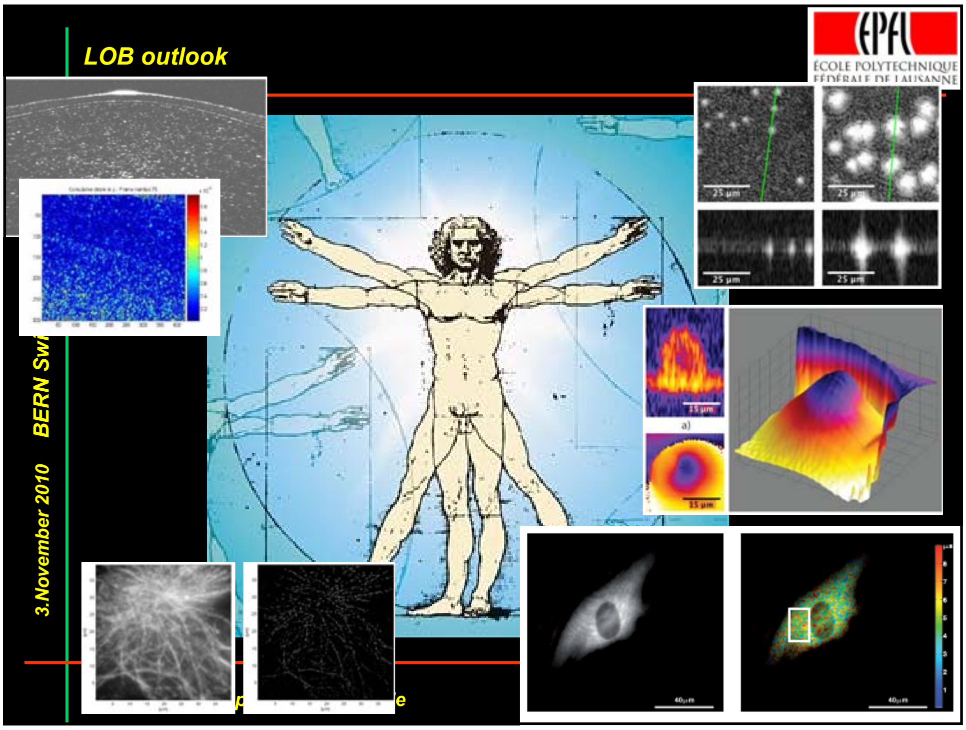

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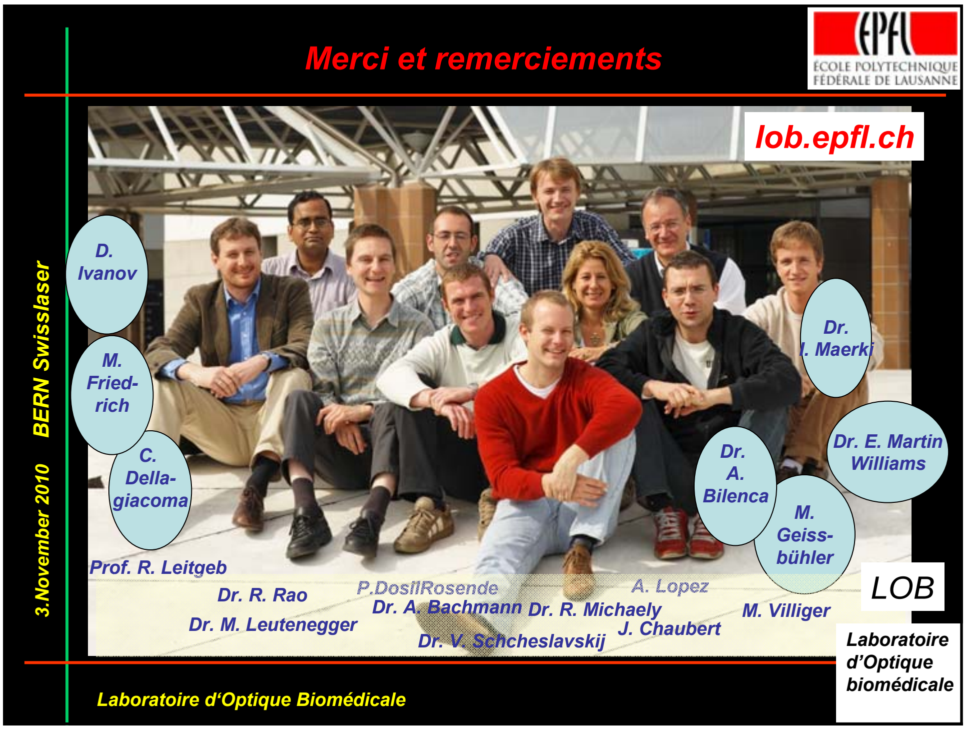

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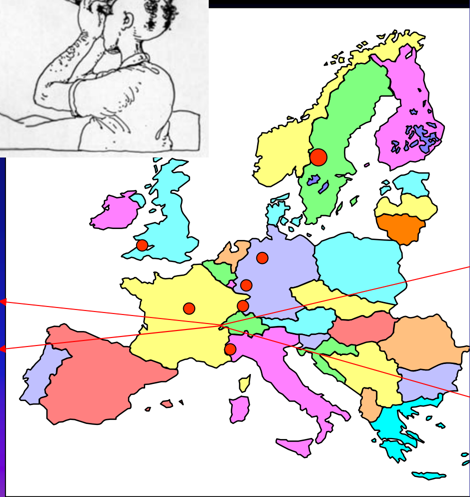

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