Super Resolution Light Microscopy

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Nobel Prizes for Microscopy Developments

Richard Zsigmondy invented the **ultramicroscope**.
Nobel Price 1925

Frits Zernike invented the **phase-contrast microscope**.
Nobel Price 1953

Maria Goeppert-Mayer described the **two-photon excitation** fluorescence.
Nobel Price 1963

Ernst Ruska build the first **electron microscope**.
Nobel Price 1986

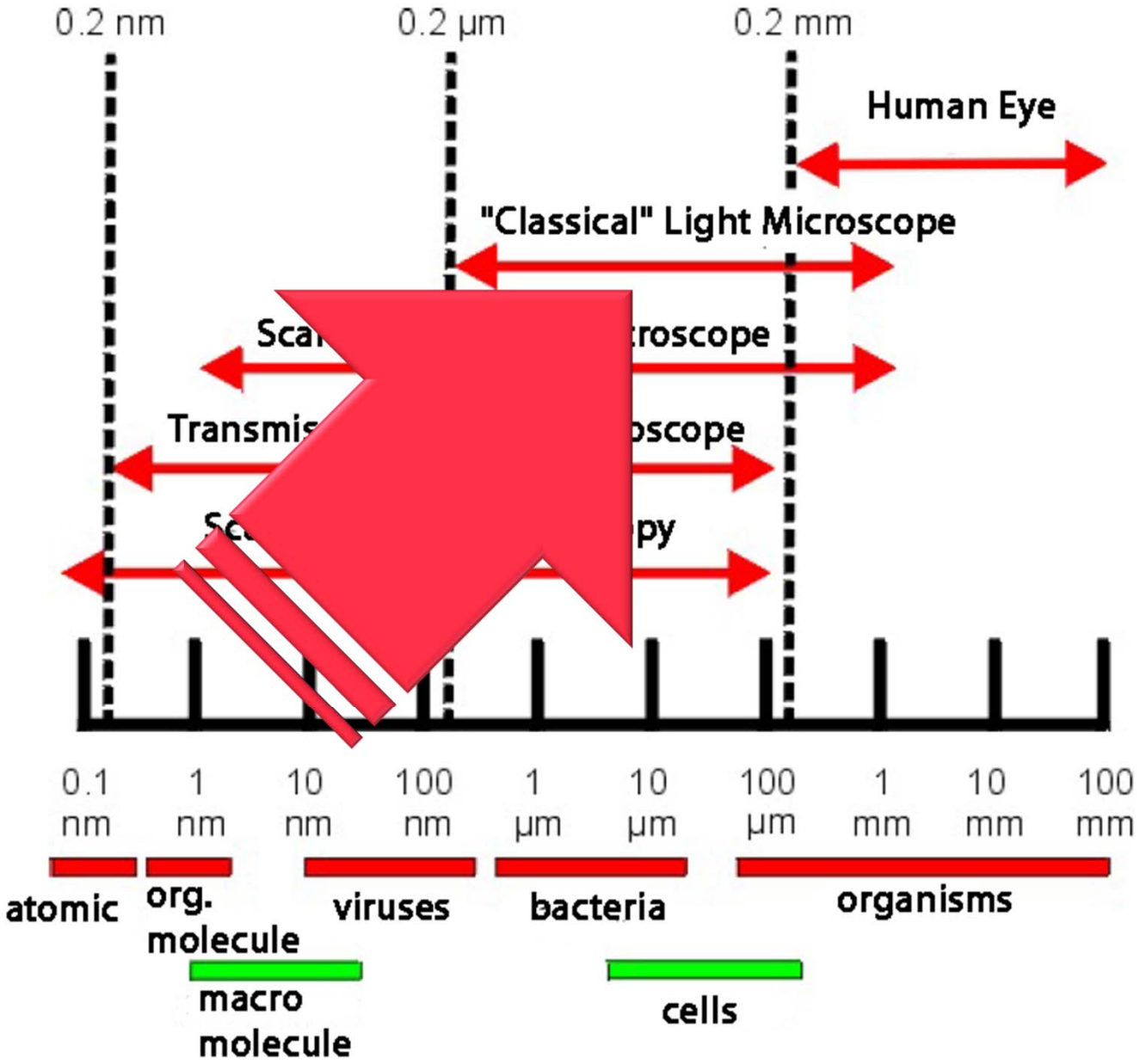
Gerd Binnig designed the **scanning tunneling microscope** (STM).
Nobel Price 1986

Nobel Prizes for using Microscopy as a Primary Scientific Instrument

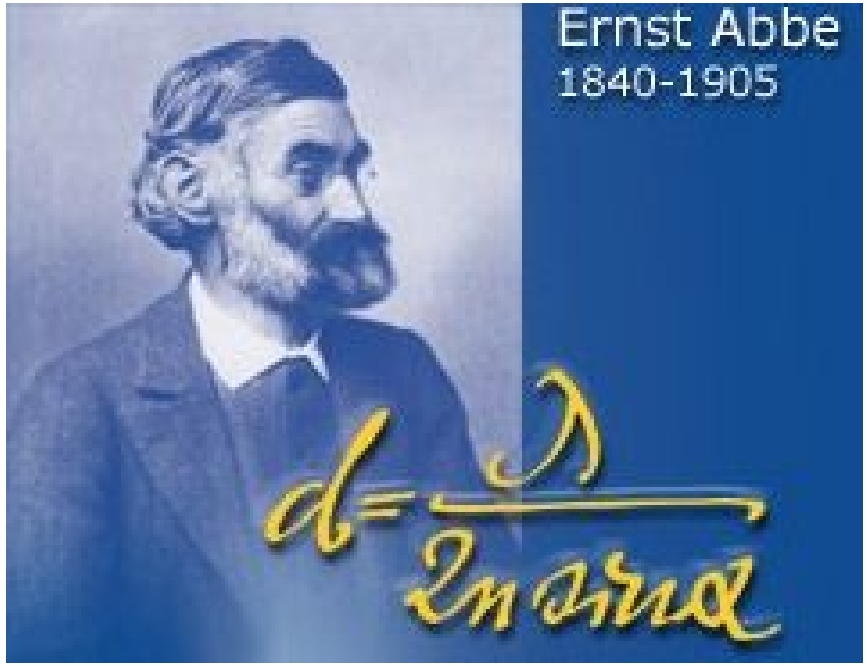
More than 10 Nobel Prizes, especially in the last 10 years

including Harald zur Hausen, **Nobel Prize for Physiology or Medicine, 2008**
(1983 to 2003 DKFZ chairman).

Scale Issue


0.2 nm
0.2 µm
0.2 mm
Human Eye
"Classical" Light Microscope
Scan
roscope
Transmis
oscope
Sc
py
0.1 nm
1 nm
10 nm
100 nm
1 µm
10 µm
100 µm
1 mm
10 mm
100 mm
atomic
org. molecule
viruses
bacteria
organisms
macro molecule
cells

Optical Resolution (lateral) for Light Microscopy: Abbe Limit



Source: Abbe School of Photonics, Jena

Numerical **A**perture = n ·*sin α*

Today b*est available objective:* **NA = 1.49**

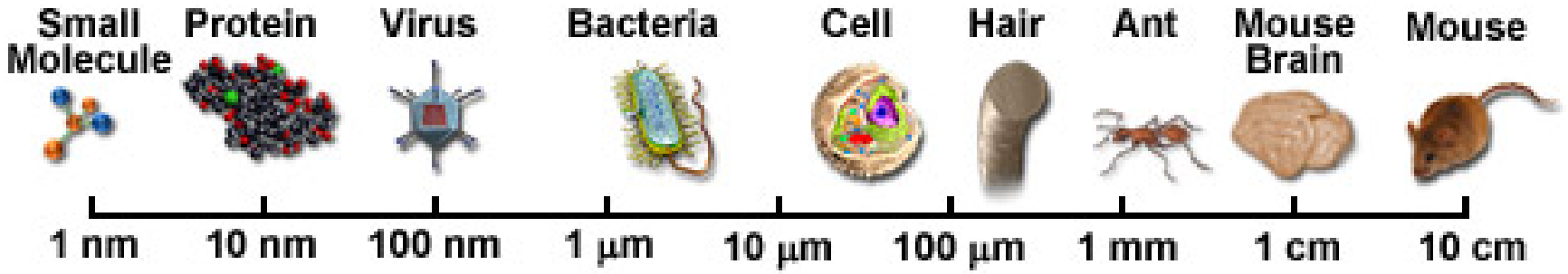
Source: Olympus

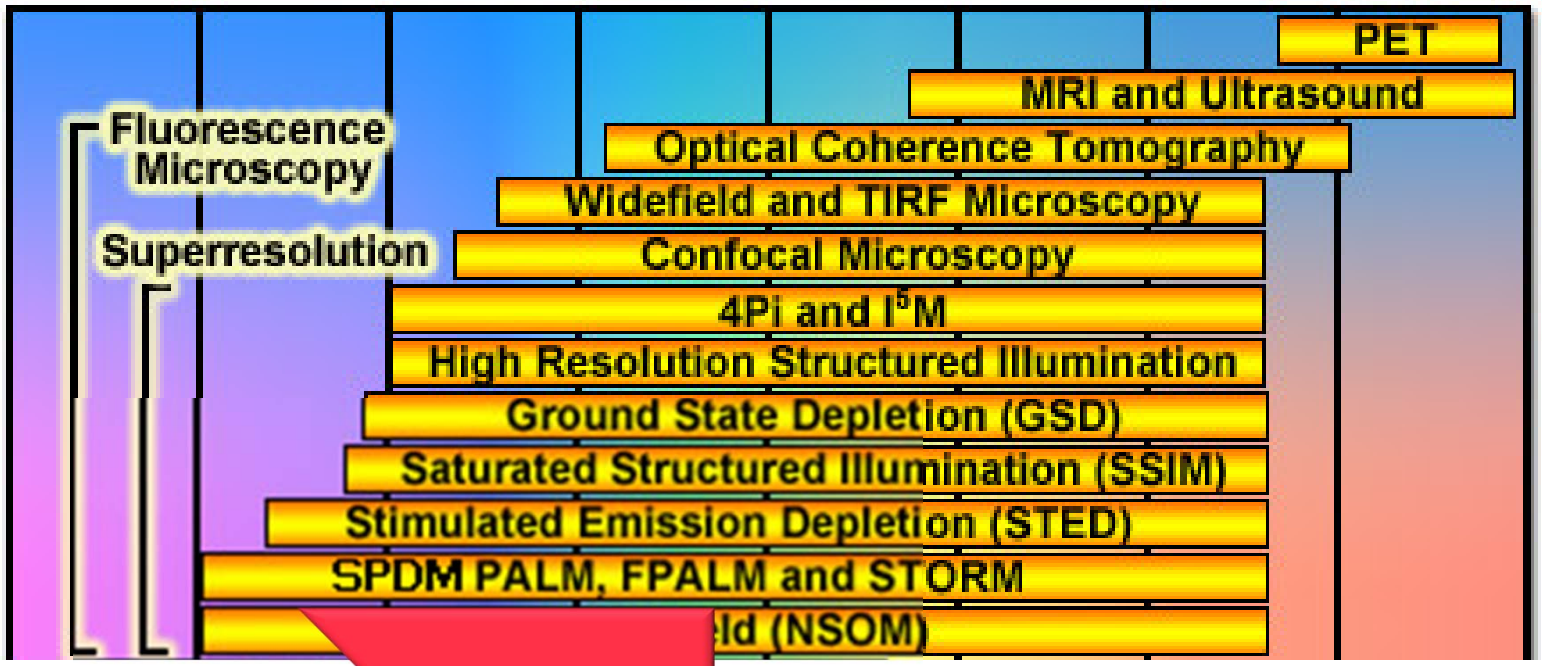
$$d = \frac{\lambda}{2 * 1,49} = 0,34 * \lambda$$

λ(Red Light) = 600nm

~210 nm Resolution Limit

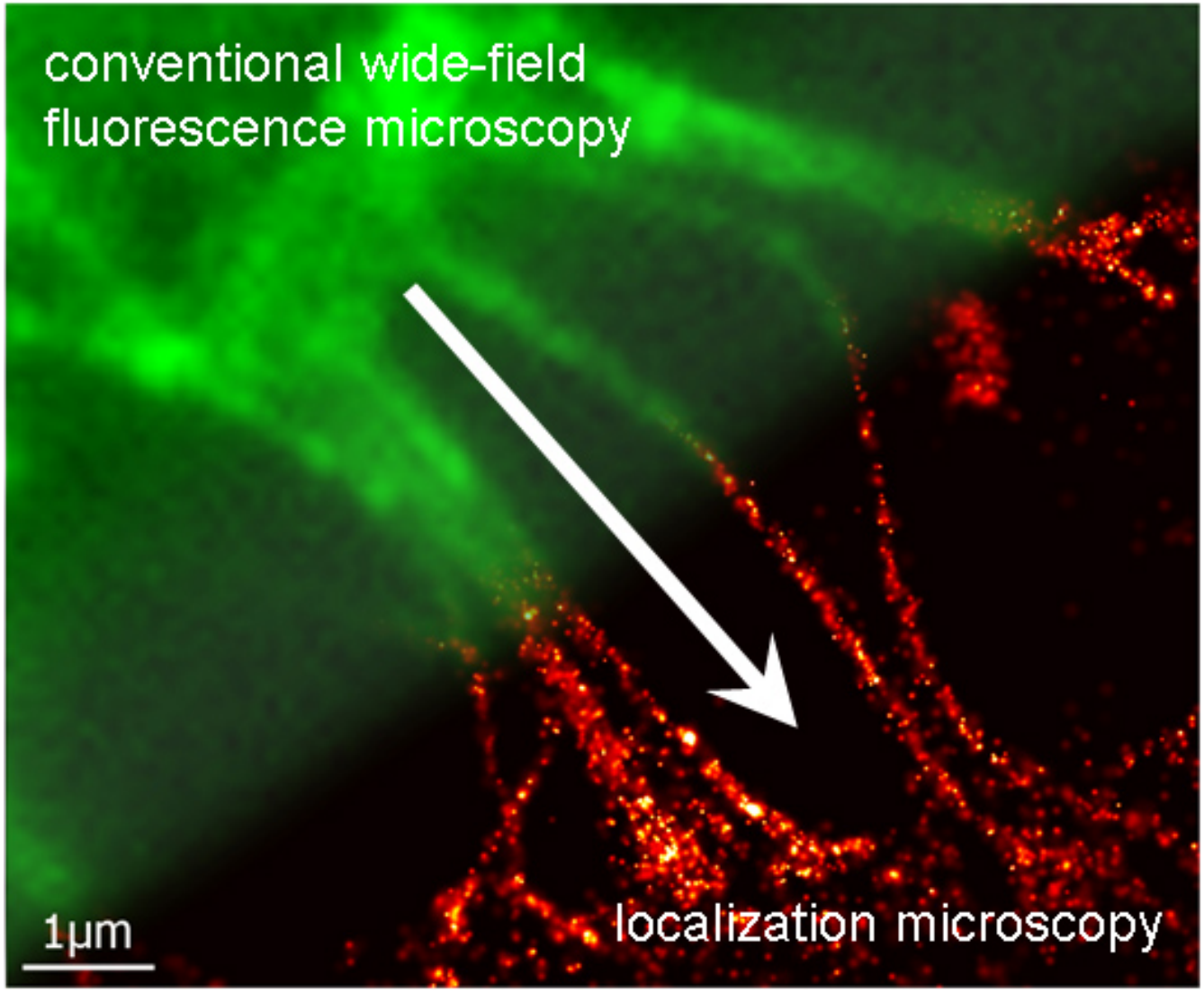
Spatial Resolution of Biological Imaging Techniques



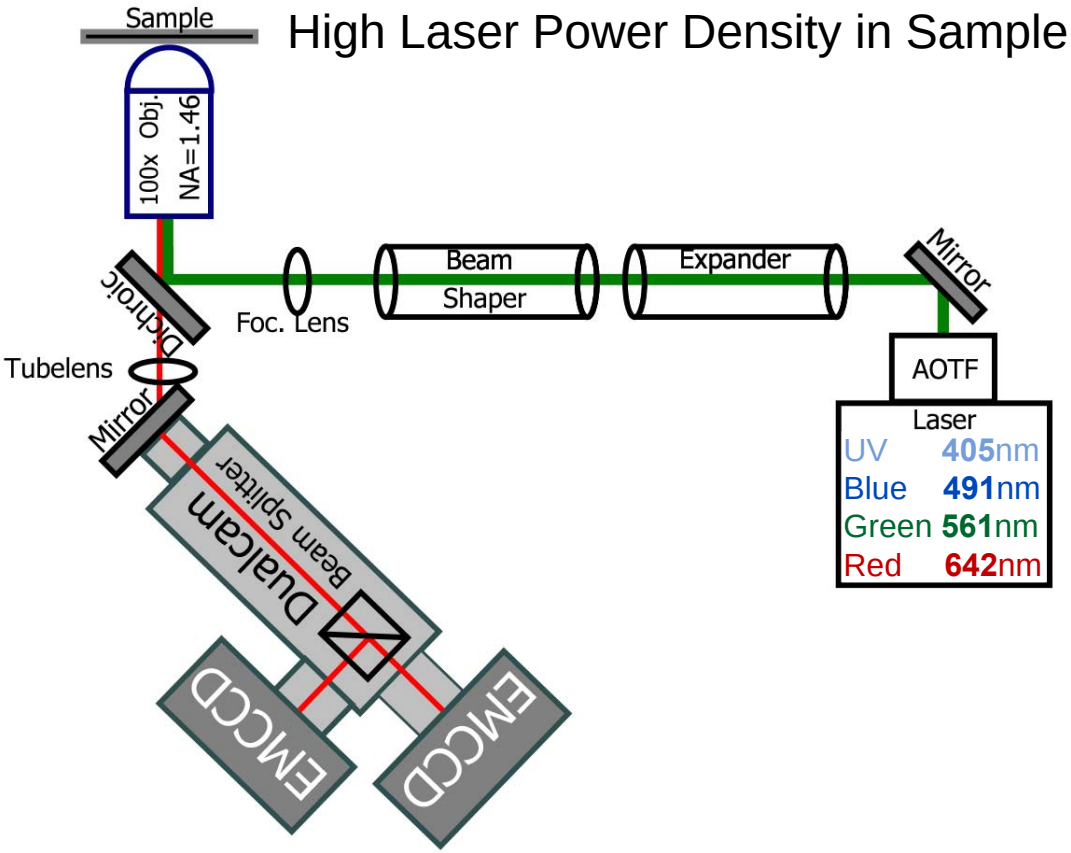


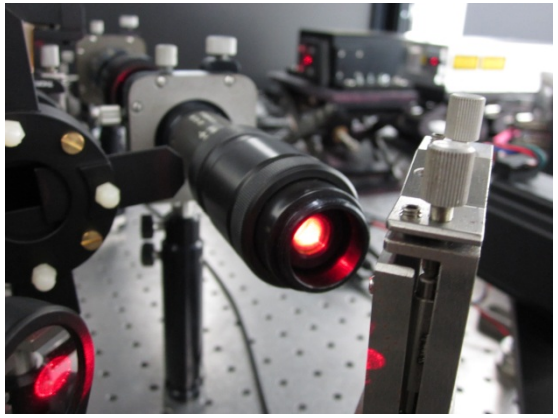
http://zeiss-campus.magnet.fsu. /palm/practicalaspects.html

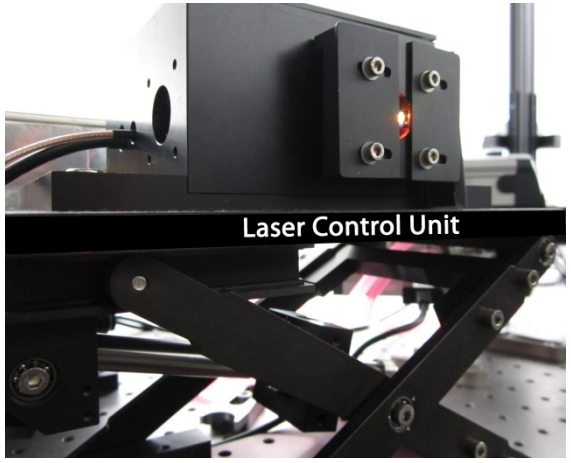
Localization Microscopy



Significantly Simplified Optical Setup for Localization Microscopy

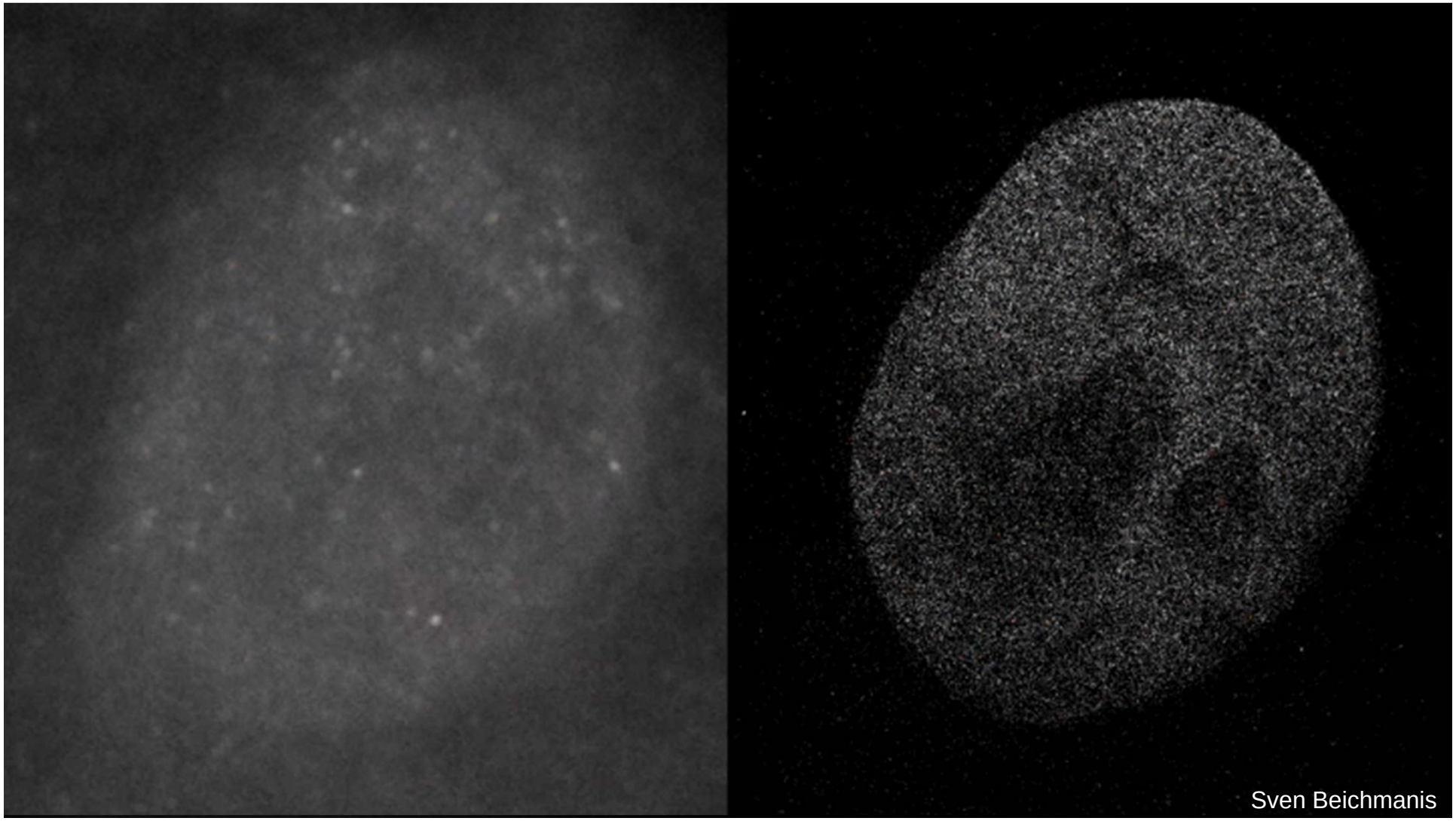






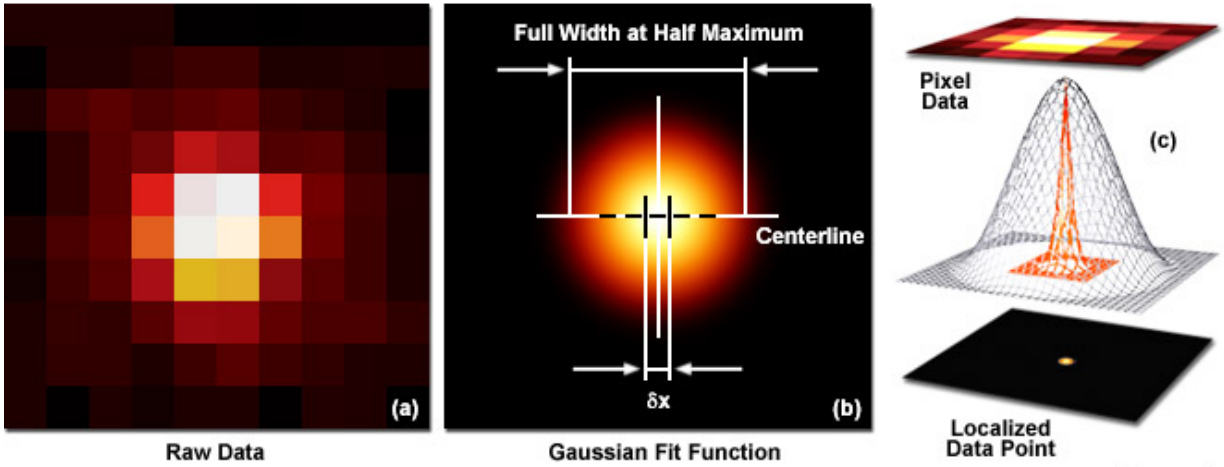
DKFZ Super Resolution Laboratory (was established by W. Schaufler)
at Central Microscopy Facility (Head: Dr. Felix Bestvater)

Localization Microscopy (SPDM)
(developed and patented in the Prof. Dr. Dr. Christoph Cremer Group)



X protein labeled with alexa488 fluorescence marker, 50ms per frame

Fitting Single-Molecule Pixel Data to a Gaussian Function



http://zeiss-campus.magnet.fsu.edu/articles/superresolution/palm/practicalaspects.html

1) Amplitude(Photons),
2) x(nm),
3) y(nm),
4) Loc.Error⁺_x(nm),
5) Loc.Error⁺_y(nm),
6) FWHM*_x(nm),
7) FWHM*_y(nm),
8) Photonamount (Integral),
9) Framenumber
10) Asymetry dx/dy

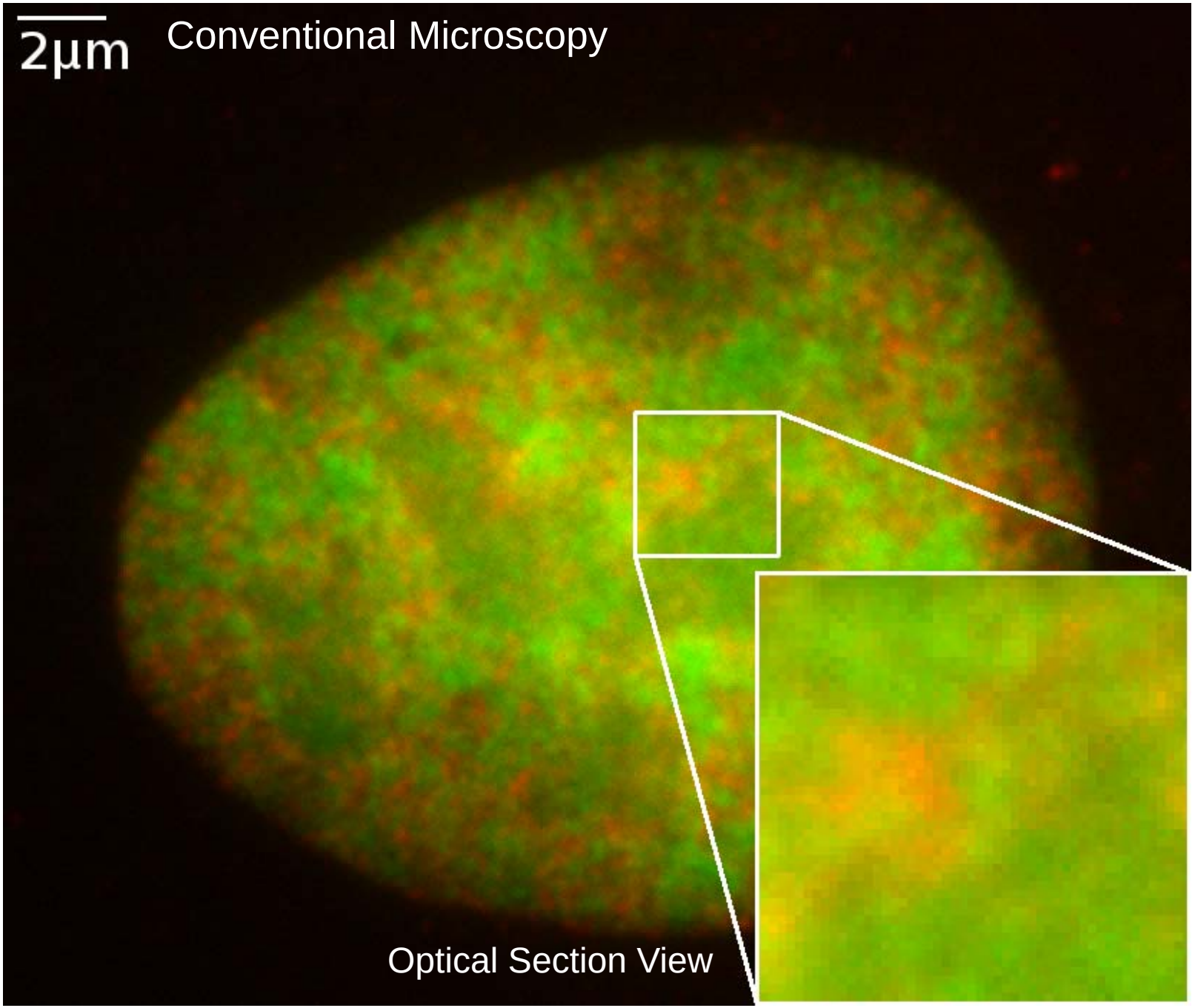
***FWHM** (*Full Width at Half Maximum*) ~ 200nm

⁺Localization Error ~ 5-25nm

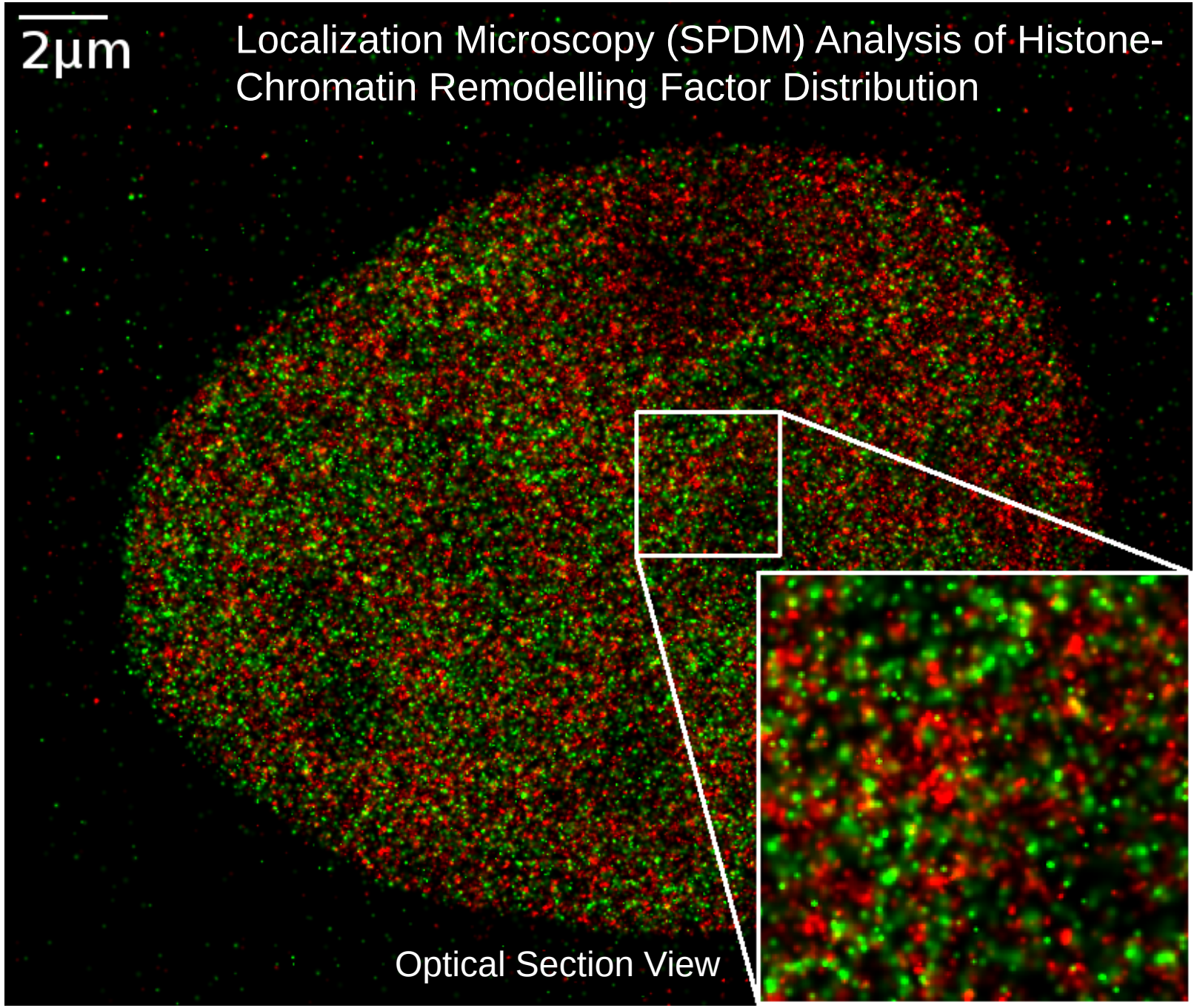
Table with Data for Each Single Fluorescence Labeled Molecule (Matlab)

Orte_02A568_cnap <212x12 double>

	1	2	3	4	5	6	7	8	9	10
1	61.2500	984.6322	1.3965e+03	8.6563	8.6464	94.5536	87.4841	840	9	0.9015
2	99.3750	2.0817e+03	2.7822e+03	6.6698	6.6324	86.2140	84.8601	1.2036e+03	9	0.9951
3	128.0172	287.4525	743.2350	5.2867	5.2673	83.5424	86.2618	1.6816e+03	10	0.9989
4	58.2672	980.8653	1.3836e+03	10.0753	9.4057	73.3987	74.7404	580.7946	10	0.9842
5	54.8075	1.0027e+03	1.3858e+03	9.6323	10.1535	72.4121	71.4834	518.9484	11	0.9736

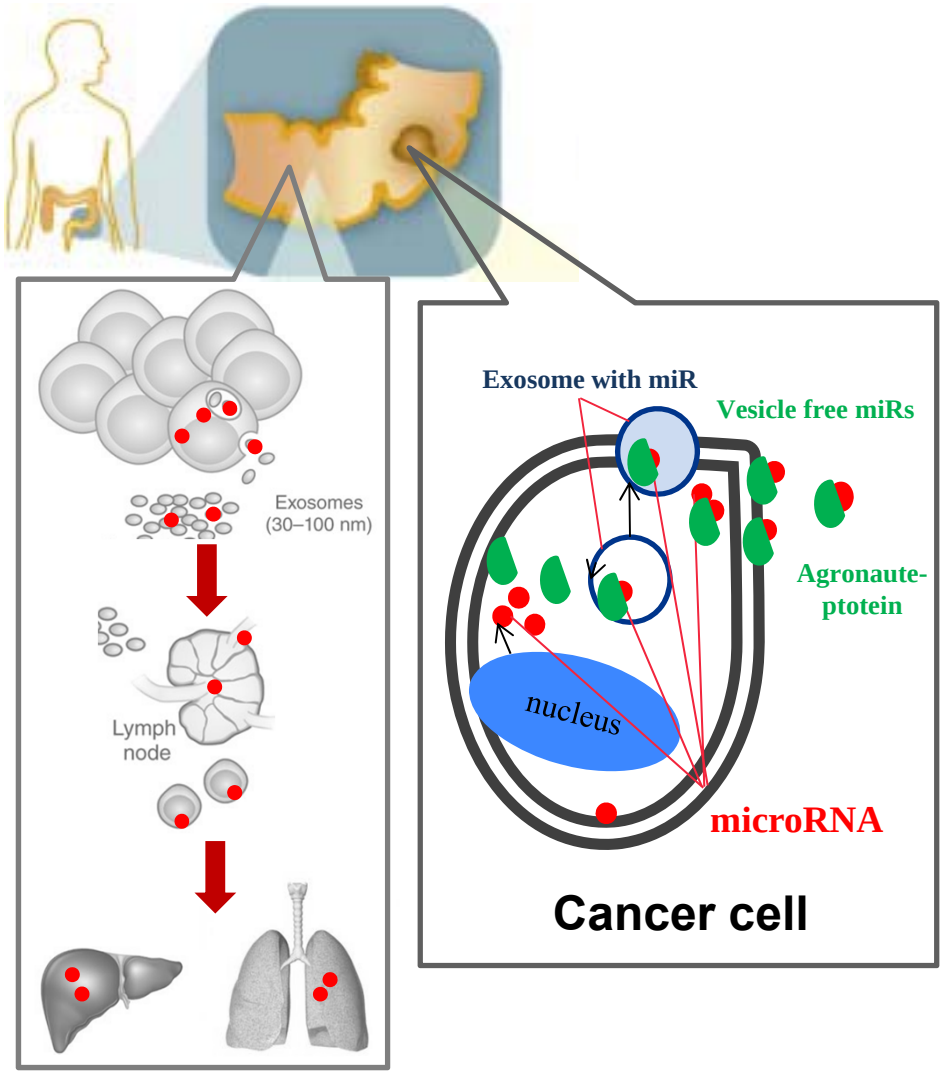


Gunkel et al. 2009



Gunkel et al. 2009

microRNAs promote metastasis



Nearly no data are available about miRNA distribution within cells or tissues

Our protocol

Seed of cultured cells on coverslips

↓

Labeling of metastasis-specific miRNA/s (transfection or FISH)

↓

Conventional microscopy

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Super Resolution Microscopy SPDM

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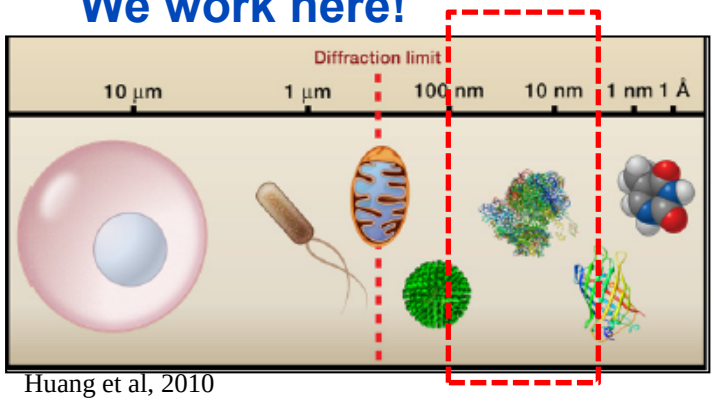
3D-recostruction&quantative analyse of spatial distribution and localization (home-made software)

We work here!



Huang et al, 2010

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