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# Optics beyond the Abbe Limit:

# “Molecular Constellations“

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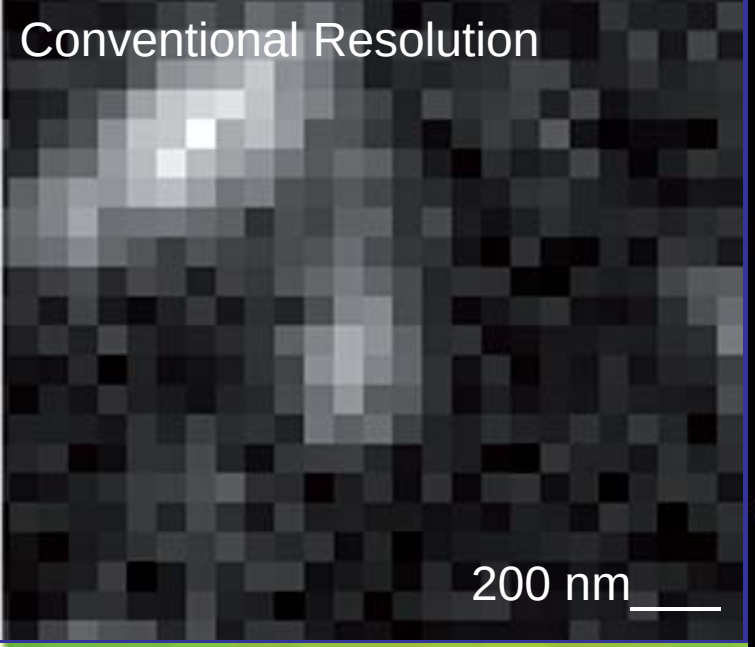
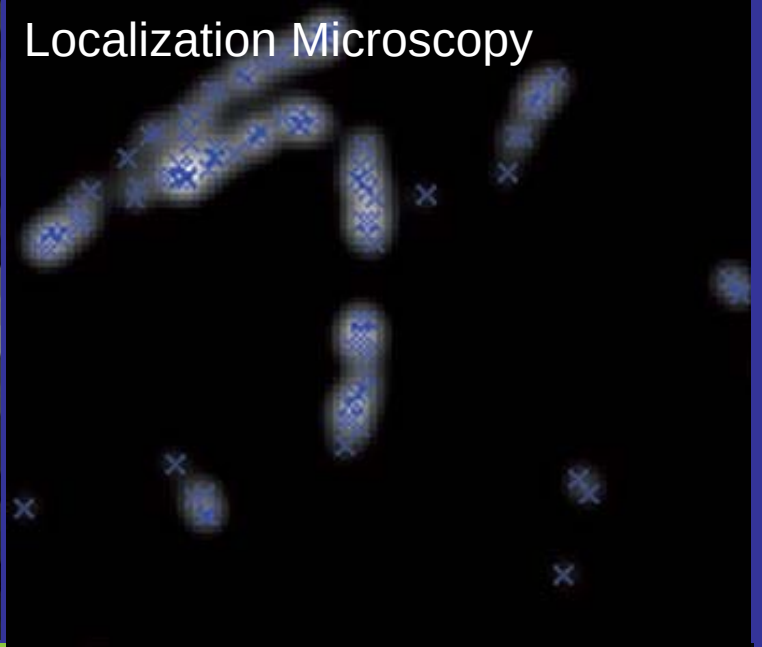
***For more information see www.imb-mainz.de***



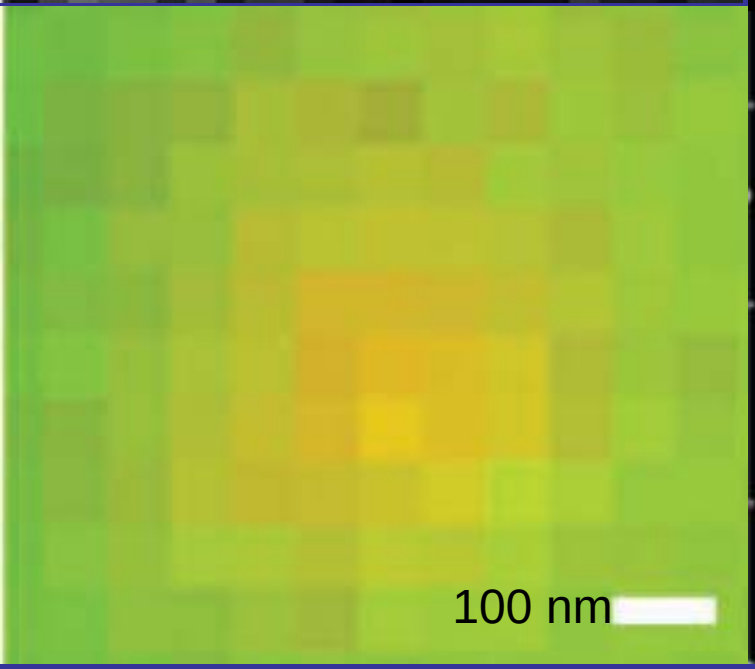



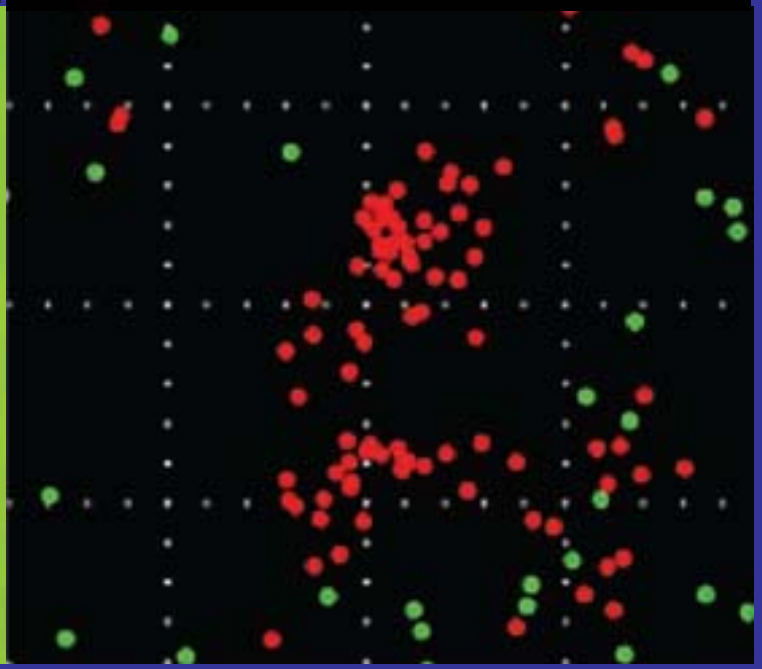

From:

C. Cremer et al.,

Biotechnology J.

6 (2011)



From:

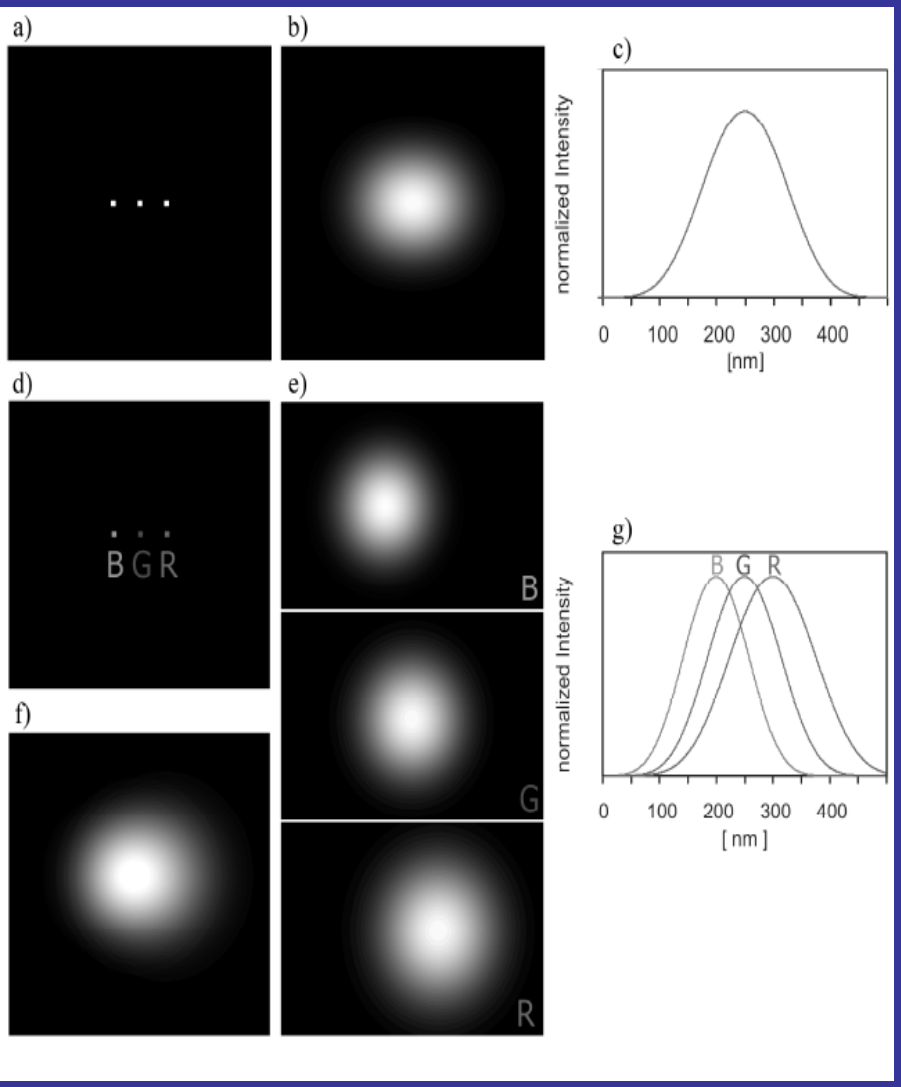
Y. Markaki et al.,

CSH Symposia

75 (2011)

Above: Viruses; Below: 2 Protein types within a human cell nucleus. The crosses/circles denote positions of single molecules (exciting wavelength 488 nm)

# Principle of Spectrally Assigned Localization Microscopy (SPDM): Optical Isolation of Diffraction Images of individual Molecules: Determination of Gravity Centers




*Cremer et al. 1996, 1999, 2010, 2011*

**3 point-like objects in x,y plane with next neigbour distances 50 nm**

**a) Labelling with same spectral signature**

**b) Labeling with different unique spectral signatures B,G,R**

Computation (scalar Theory):

NA = 1.4, $\lambda_{exc}$ = 488 nm

**Optical Isolation by the "Supernova" Mode: Induction of stochastically distributed short flashes of light emission by individual molecules**

***Present State of Superresolution Far Field Light Microscopy realized @ C. Cremer Lab (December 2011)***

- **Optical Resolution OR (resolvable distance):**

**OR ~ 5 nm (~ 1/100 $\lambda_{exc}$, from localization precision)**

- **Structural Resolution (imaging capability):**

**● Mean (2D) distance between individual molecules actually detected: ~ 6 nm ( ~ 1/80 $\lambda_{exc}$)**

**● Density of individually detected Molecules : ~ 2,8 · $10^4$/µm$^2$**

**- Multicolor- Localization Microscopy:**

**● 2 – 3 different molecules types**

**● 3D single Molecule Resolution inside cells (best values): 3D Observation Volume about 1*$10^4$ times smaller (i.e. 3D resolution $10^4$ times better) than in conventional Light Microscopy (“Abbe-limit”)**

# Some recent references (CremerLab):


C. Cremer, Optics far Beyond the Diffraction Limit: From Focused Nanoscopy to Spectrally Assigned Localization Microscopy. Springer Handbook of Lasers and Optics, 2nd edition (F. Träger, Edit.), in press.

Y. Markaki et al. (2011) Functional nuclear organization of transcription and DNA replication: a topographical marriage between chromatin domains and the interchromatin compartment. Cold Spring Harbor Symposia on Quantitative Biology 75: 1–18. doi:10.1101/sqb.2010.75.042.

R. Kaufmann et al. (2011) Analysis of Her2/neu membrane protein clusters in breast cancer cells using localisation microscopy. J. of Microscopy **242**: 46–54.

G. Best et al. (2011) Structured illumination microscopy of autofluorescent aggregations in human tissue. Micron **42**: 330–335.

C. Cremer et al. (2011) Superresolution imaging of biological nanostructures by spectral precision distance microscopy, Biotechnology **6**: 1037–1051.

C. Cremer (2011) Lichtmikroskopie unterhalb des Abbe-Limits, Physik in Unserer Zeit **42**: 21 – 29.