Super-resolution imaging with Dronpa and its mutants

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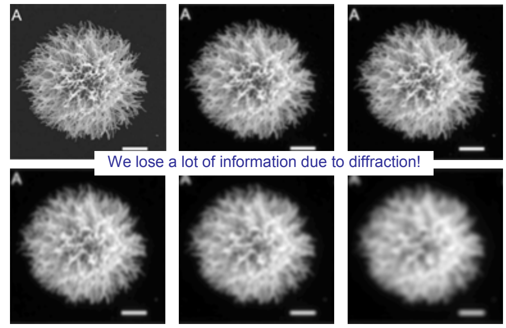
Resolution



Van Duin *et al. Antimicrob. Agents Chemother.* 48:2014-22 (2004)

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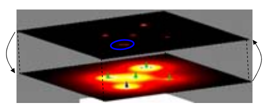
Two approaches for super-resolution based on photoswitching

1) Photoactivation-localization microscopy



2) Donut mode fluorescence depletion microscopy

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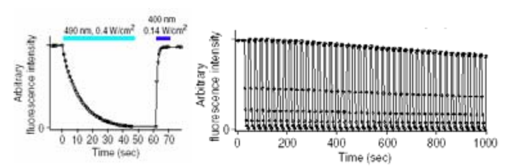
Dronpa

- Dronpa is a monomeric **GFP-like protein** from coral.
- It shows **reversible photoswitching** upon irradiation with 488 and 405 nm light.



Ando et al, Science 2004

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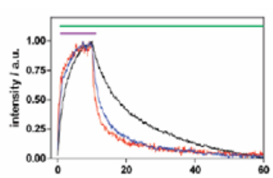
Dronpa mutants with faster switching



	Dronpa[a]	Dronpa-2	Dronpa-3
$\epsilon/M^{-1}\cdot cm^{-1}$	95 000	56 000	58 000
λ_{abs}^{max}/nm	503	486	487
λ_{fluo}^{max}/nm	518	513	514
Φ_F	0.85	0.28	0.33
Φ_{SW}[b]	3.2×10^{-4}	5×10^{-2}	5×10^{-3}
τ_F/ns[c]	3.6	2.1 (47%) 0.97 (42%) 0.09 (11%)	2.2 (66%) 1.1 (20%) 0.02 (14%)

R. Ando *et al., Biophys J* 92:L97–L99

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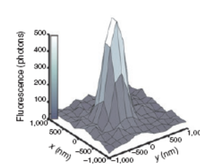
Photoactivation-localization microscopy (PALM)

➤The **position** of single object can be determined at **nanometer resolution**.

$$\left\langle (\Delta x)^2 \right\rangle = \frac{s^2 + a^2/12}{N} + \frac{8\pi s^4 b^2}{a^2 N^2}$$



➤If we could measure the positions of the fluorescence molecules **one by one**, we could make the **map** of them at **nanometer resolution**.

➤We can take advantage of **photoactivation** to achieve **separation in time** of fluorescence emission

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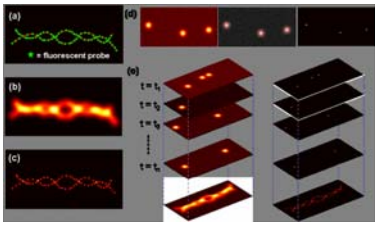
Photoactivation-localization microscopy (PALM)

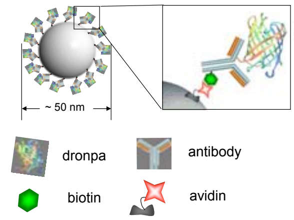
Number of activated molecules <=1 in diffraction limited area

Determination of center with nanometer resolution

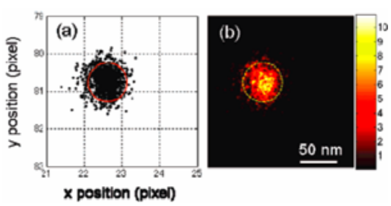


Betzig et al, *Science* 2006 Rust et al, *Nat. Methods*, 2006 Hess et al, *Biophys. J.* 2006

Dronpa-coated nanospheres



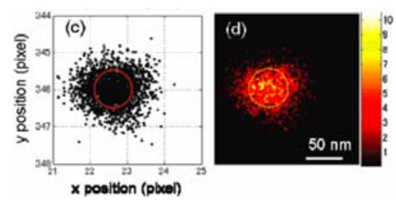
Dronpa-PALM



Integration time 40 ms
Pixel size 48 x 48 nm^2
I^{491} = 0.5 kW/cm^2
I^{406} = 4 – 30 W/cm^2
for 20 ms @ 5 Hz

- Max. ~170 Dronpa molecules per bead yielded > 1000 positions => reversibility
- Localization precision ~ 15 nm
- Total measurement time for 3000 frames: 2 minutes

Dronpa-3-PALM

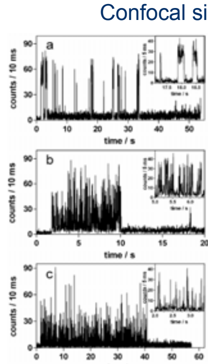


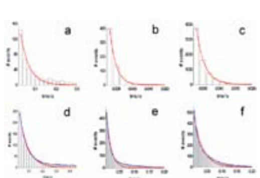
Integration time 40 ms
Pixel size 48 x 48 nm^2
I^{491} = 0.5 kW/cm^2
I^{406} = 4 – 30 W/cm^2
for 20 ms @ 5 Hz

Identical measurement conditions to Dronpa!

How can we improve this data?

Confocal single-molecule studies





On average a single molecule of Dronpa is on for 42 ms

A single molecule of Dronpa-3 is on for 6 ms

With integration times of 40 ms we record noise 85% of the time!

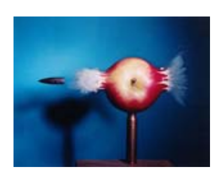
Solution: stroboscopic PALM

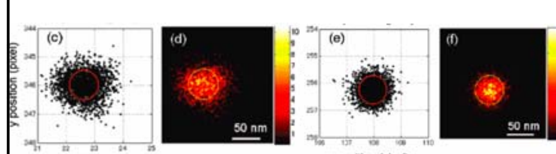
Time the irradiation to coincide with the on-times of the fluorophores

Also take into account possible (not-photoswitched) dark state relaxation

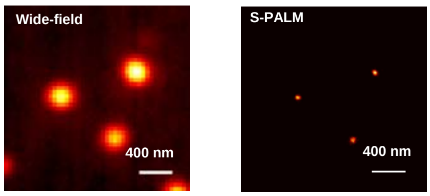
E.G. Dronpa-3: irradiate each frame with the lasers for 6 ms only, even though the integration time is 40 ms

… or buy a faster camera…



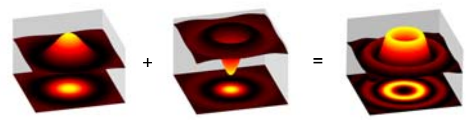


Comparison of wide-field and S-PALM images



C. Flors *et al.*, J. Am. Chem. Soc. 2007, 129, 13970

Donut mode fluorescence depletion microscopy



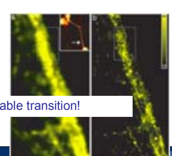
Peter Dedecker

Donut mode fluorescence depletion microscopy

Reversible, Saturable Transitions

Demonstrated using stimulated emission: STED

+ Impressive results.
- Requires high intensities to deplete the fluorescence within the excited-state lifetime (ps–ns)

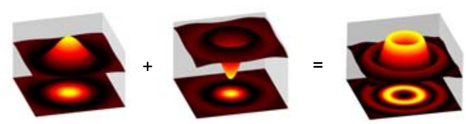


The Dronpa photoswitching is a reversible and saturable transition!

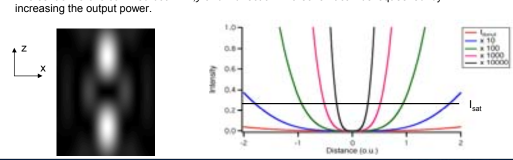
G. Donnert, PNAS 103:11440 (2006) and other papers.
S. Hell, *Science* 316:1153 2007

Donut modes

Phase shifting the central part of a laser beam by π radians leads to the generation of donut modes at the focus point.



The central zero is confined both in *x,y* and *z* direction. Moreover it can be 'squeezed' by increasing the output power.



J. Hotta, Optics Express 14:6273 (2006)

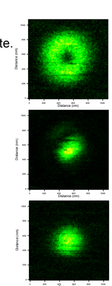
High Resolution Microscopy with Dronpa

Using the Dronpa photoswitching with 'donut' modes requires the use of 3 beams:

Dump — Switches Dronpa to the photoswitched (dark) state.

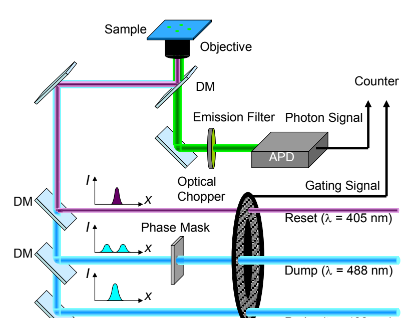
Probe — Collects fluorescence from the Dronpa that remain in the bright state.

Reset — Restores the bright state.



P. Dedecker *et al.*, J. Am. Chem. Soc. *In press*

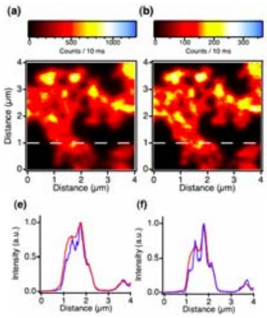
Experimental Setup



P. Dedecker *et al.*, J. Am. Chem. Soc. *In press*

Results

Layered-double hydroxide (LDH) crystals coated with Dronpa-2



Proof-of-principle!

But it's still limited

P. Dedecker *et al.*, J. Am. Chem. Soc. *In press*

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Conclusions of this part

- We demonstrated PALM with Dronpa
- We optimized the performance of Dronpa-3 in PALM by studying its photophysics and adapting the measurement (S-PALM)
- We demonstrated spatial-depletion superresolution microscopy with Dronpa

Fancy techniques, but limited by the photophysics of the fluorophore

To get optimal results it is important to study the single-molecule photophysics

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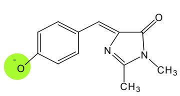
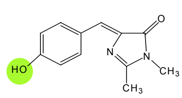
Collaborative Dronpa-related projects

1) Nonlinear optical properties (Second harmonic generation):



pH = 8



840 nm

(880 nm)

pH = 4 β_{HRS} **= 952 × 10^{-30} esu**
β_{zzz} = 2300 × 10^{-30} esu

β_{HRS} **= 226 × 10^{-30} esu**
β_{zzz} = 550 × 10^{-30} esu

(similar to model EGFP)

Photoswitched β_{HRS} **= 386 × 10^{-30} esu**
β_{zzz} = 933 × 10^{-30} esu

No measurable signal for the synthetic chromophore HBDI

Dr. Inge Asselberghs, Prof. Koen Clays (group 6 INPAC)

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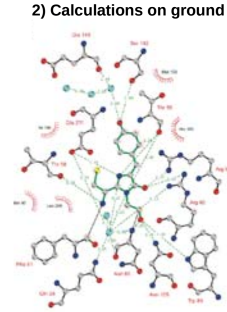
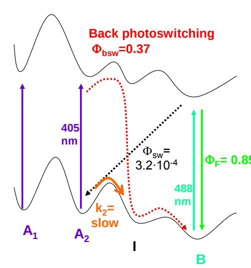
Collaborative Dronpa-related projects

2) Calculations on ground state recovery from dark to bright state





S. Michielssens , S. Moors, Prof. A. Ceulemans (group 2)

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Acknowledgments

KUL
Peter Dedecker, Jun-ichi Hotta, Michel Sliwa, Hiroshi Uji-i
Profs. J. Hokens, M. Van der Auweraer (Group 3 INPAC)
I. Asselberghs, Prof. K. Clays (Group 6 INPAC)
S. Moors, S. Michielssens, Prof. A. Ceulemans (Group 2 INPAC)

Brain Science Institute, RIKEN, Japan
R. Ando, H. Mizuno, Prof. A. Miyawaki

€ from INPAC!!

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