Molecular Lock-In:

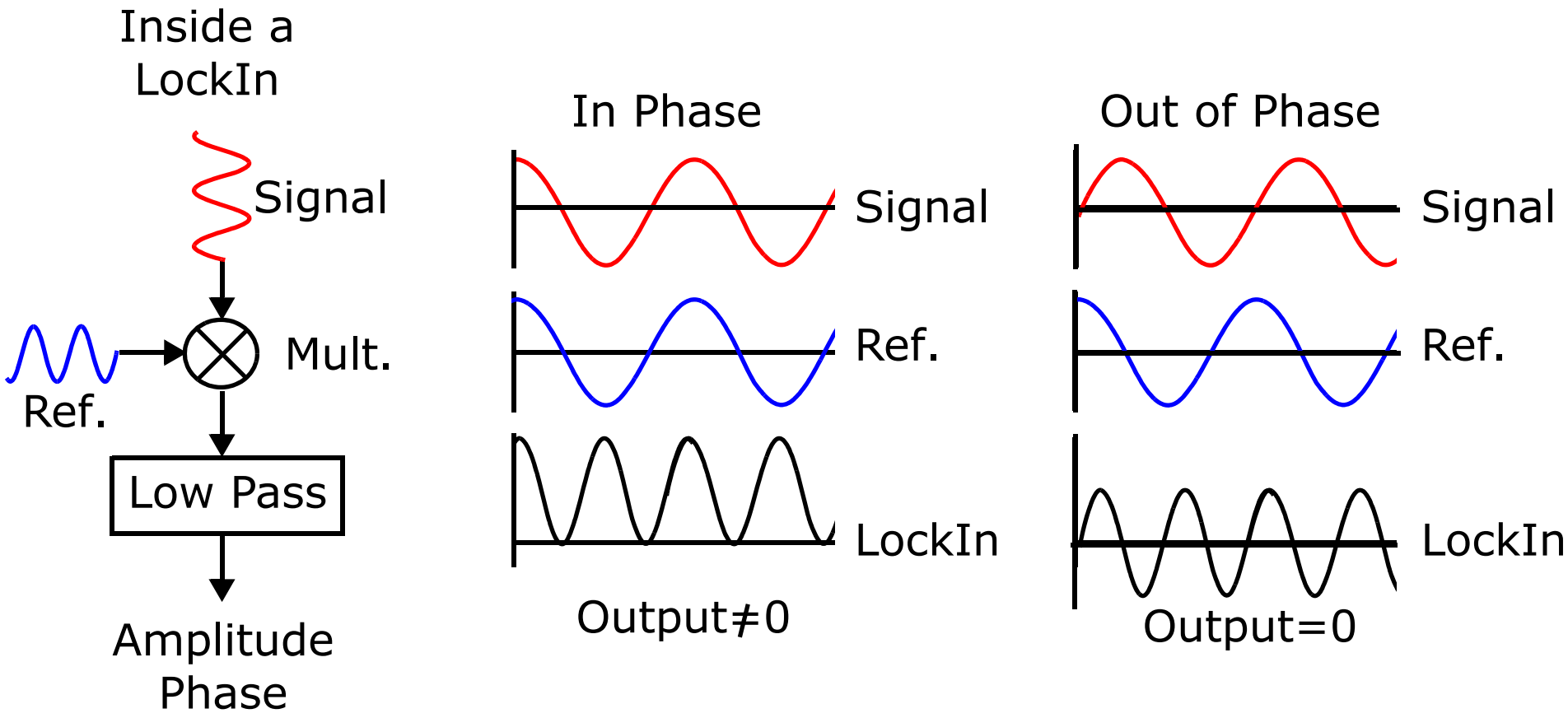
Imaging kinetics of a DNA hairpin in frequency space for each pixel of a CCD

Lock-In

- Signal Recovery
- Spectrum Analyzer
- Phase Meter
- Noise Measurement

Dieter Braun, www.dieterb.de

How a Lock-In works



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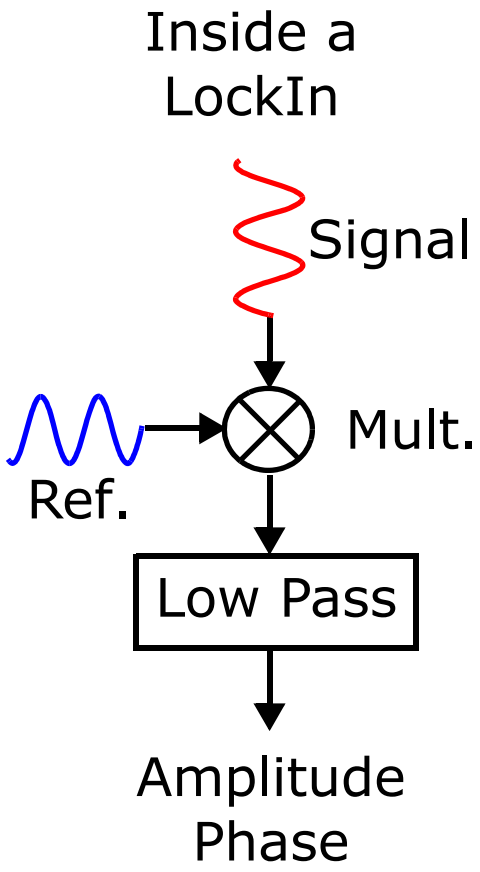
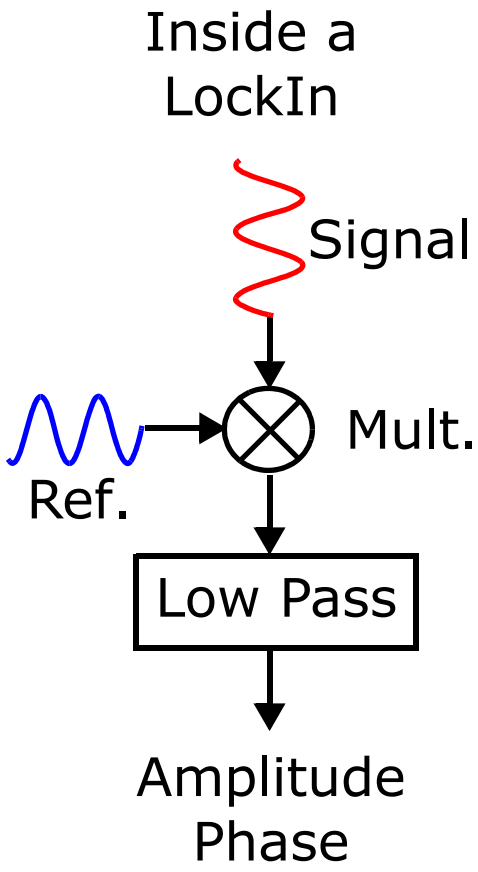
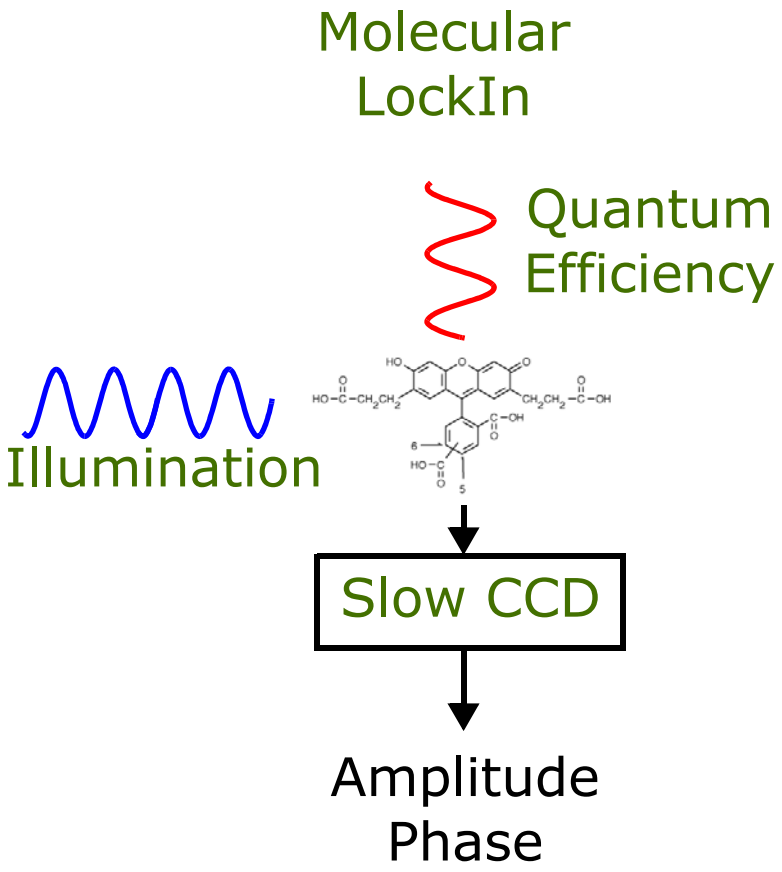
Molecular Lock-In

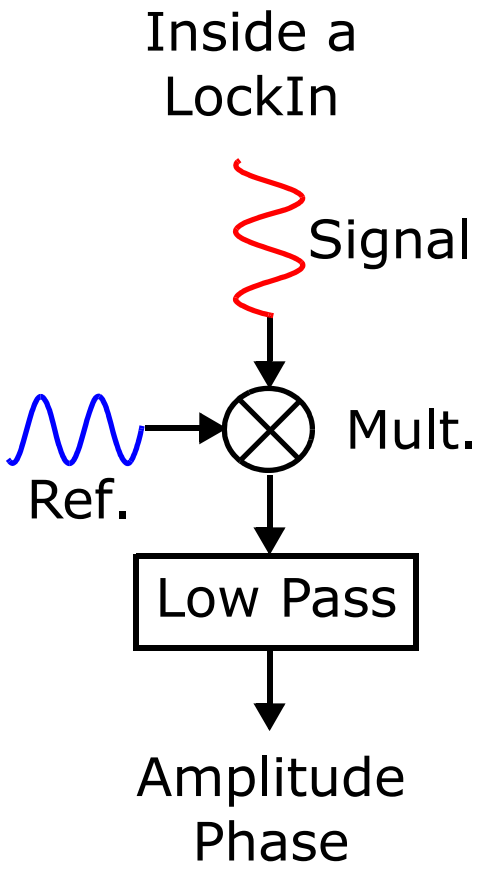


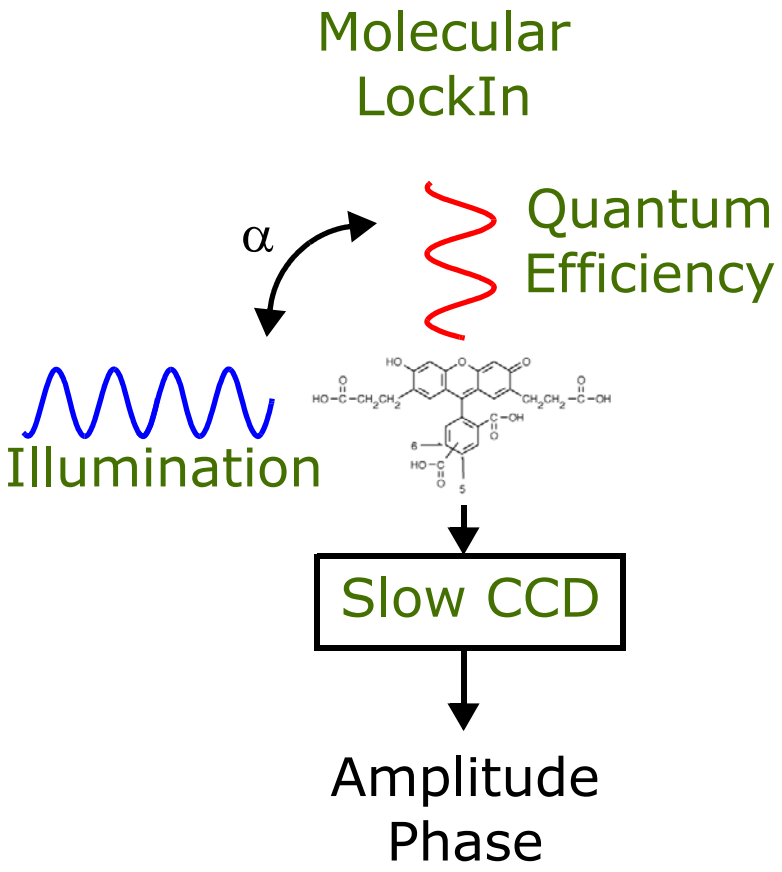
Molecular Lock-In





Molecular Lock-In





$$Amplitude \cdot e^{iPhase} = \frac{4}{\pi}\left[\frac{I_{0°} - I_{180°}}{I_{0°} + I_{180°} - 2I_{back}} + i\frac{I_{270°} - I_{90°}}{I_{270°} + I_{90°} - 2I_{back}}\right]$$

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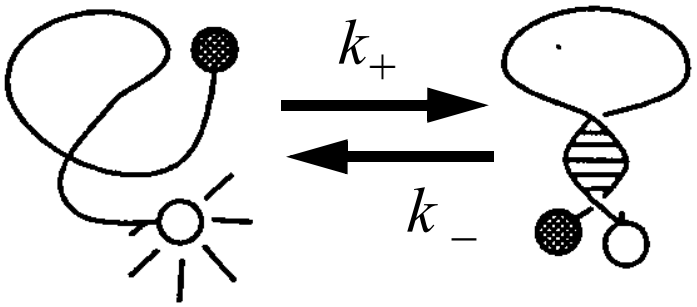
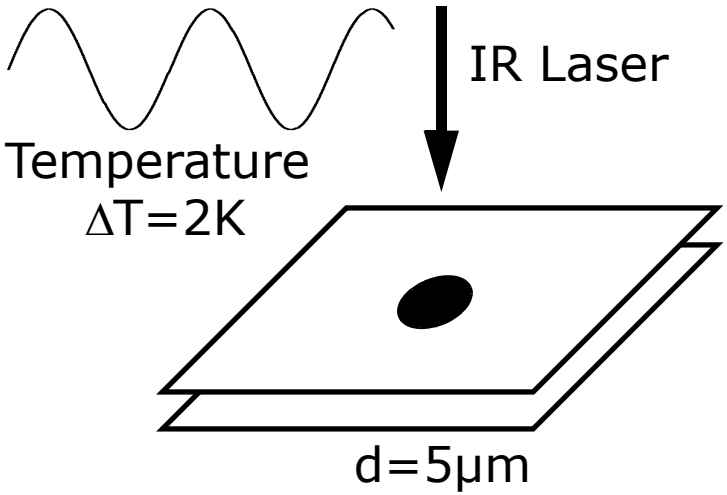
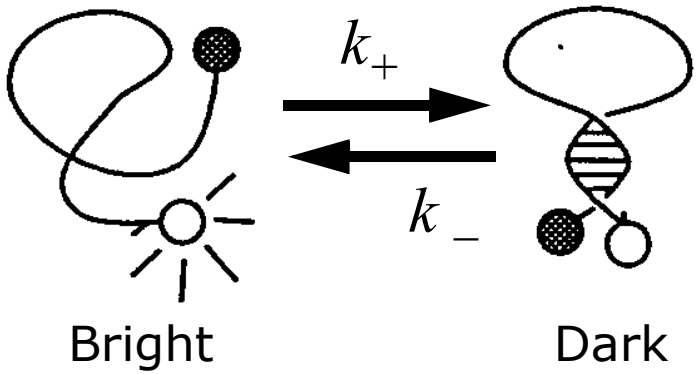
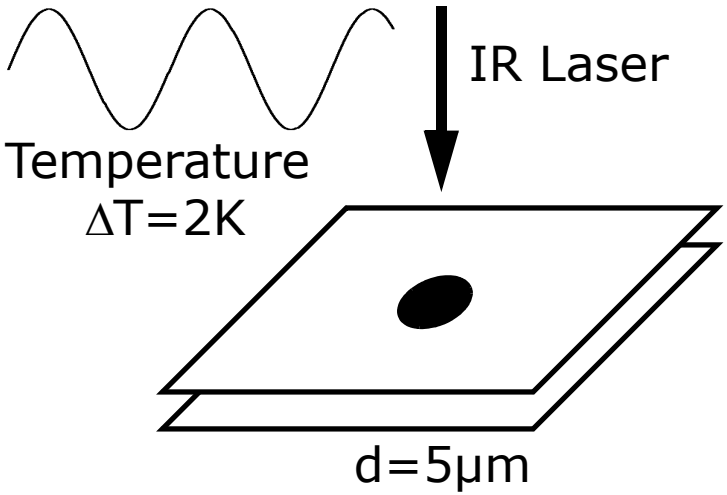
Molecular Lock-In

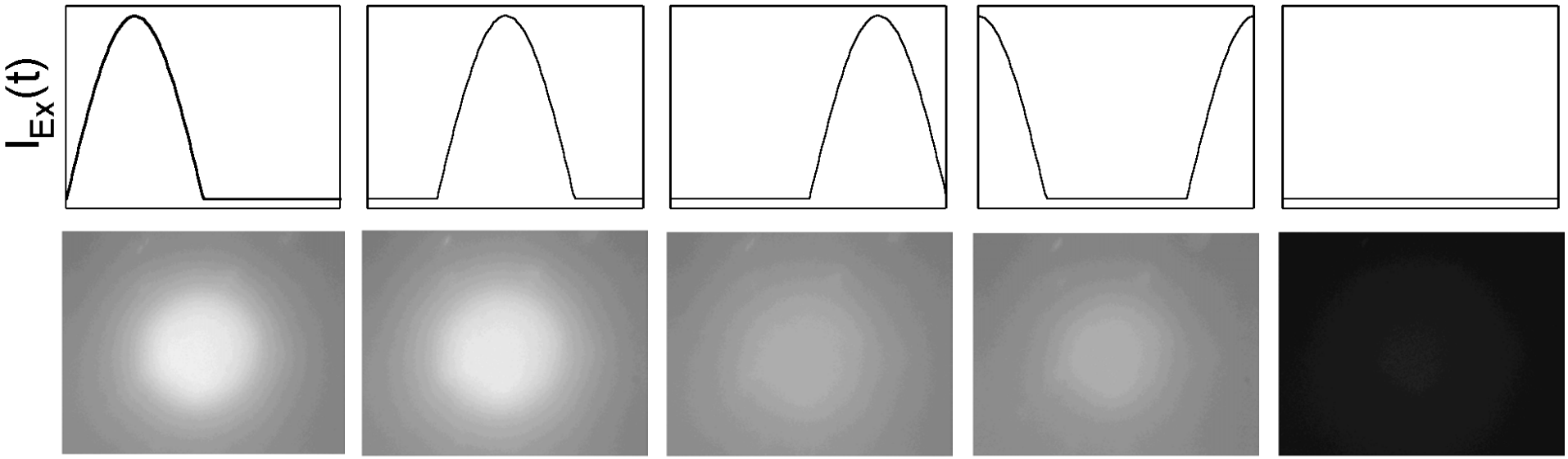




Molecular Lock-In







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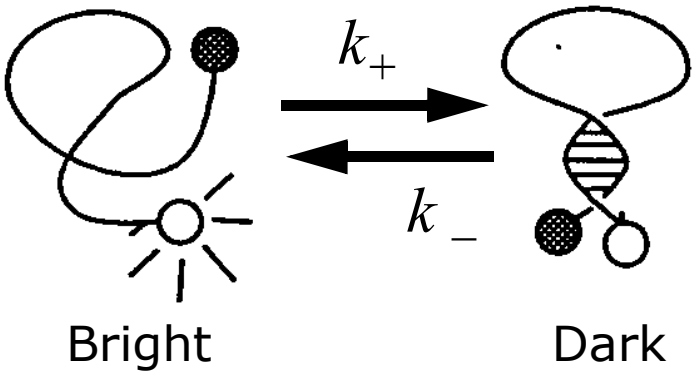
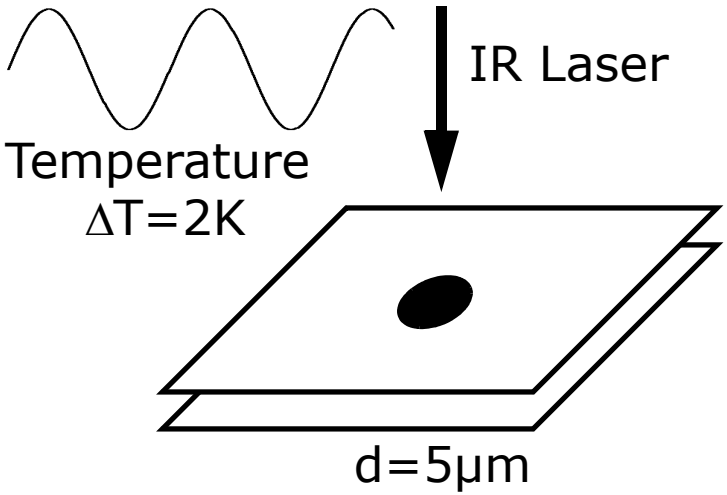
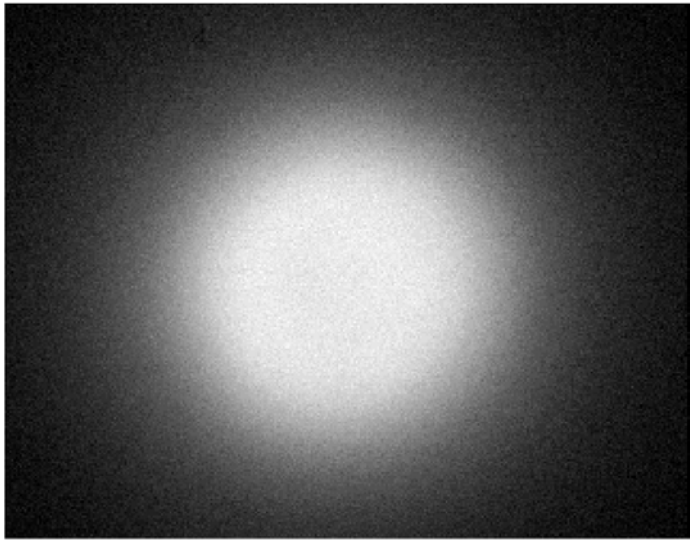
Molecular Lock-In

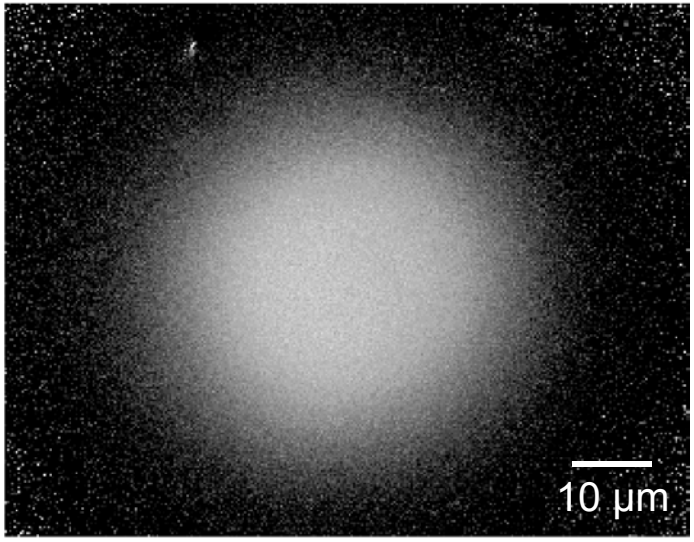




Amplitude

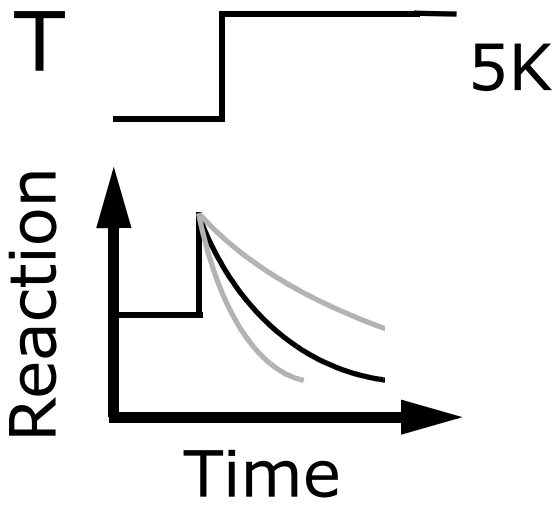


Phase



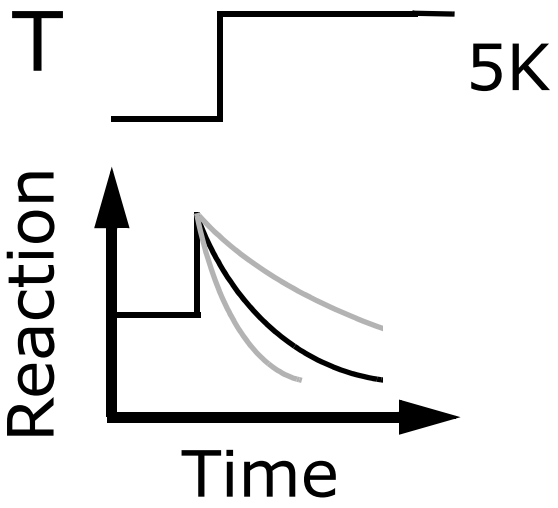
Applied Physics Letters 83:5554-5556 (2003)

Lock-In Approach to Kinetics $A \rightleftharpoons B$

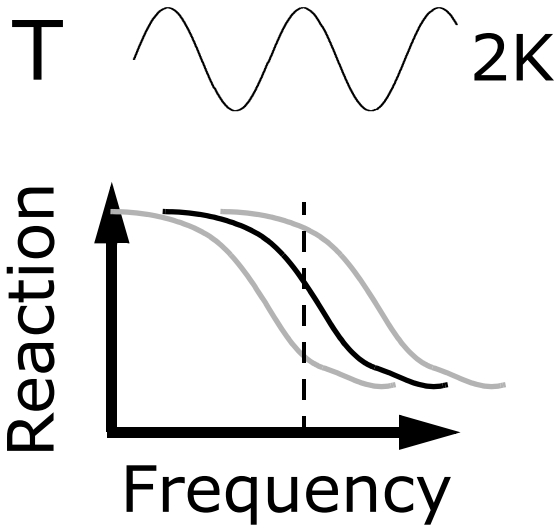


Temperature Jump

Lock-In Approach to Kinetics $A \rightleftharpoons B$



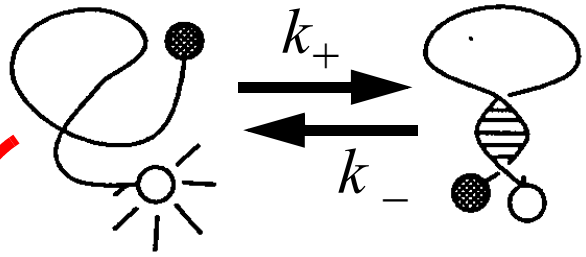
Temperature Jump



Temperature Oscillation

- o No dead time
- o Fluorescence Microscopy
- o High S/N from Lock-In

Kinetics of DNA Hairpins

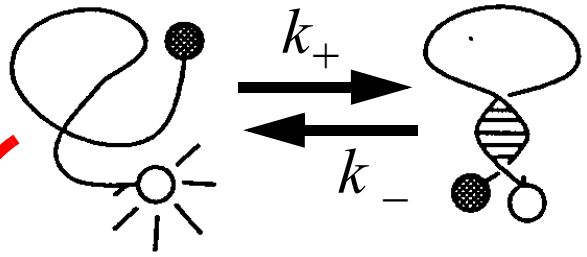


Bright Dark

$$\frac{\frac{\Delta F}{F}}{\frac{\Delta T}{T}} = \frac{\beta/\alpha - 1}{1 + K\beta/\alpha} \frac{K}{1 + K} \frac{\Delta H}{RT} + \chi T$$

Temperature Dye

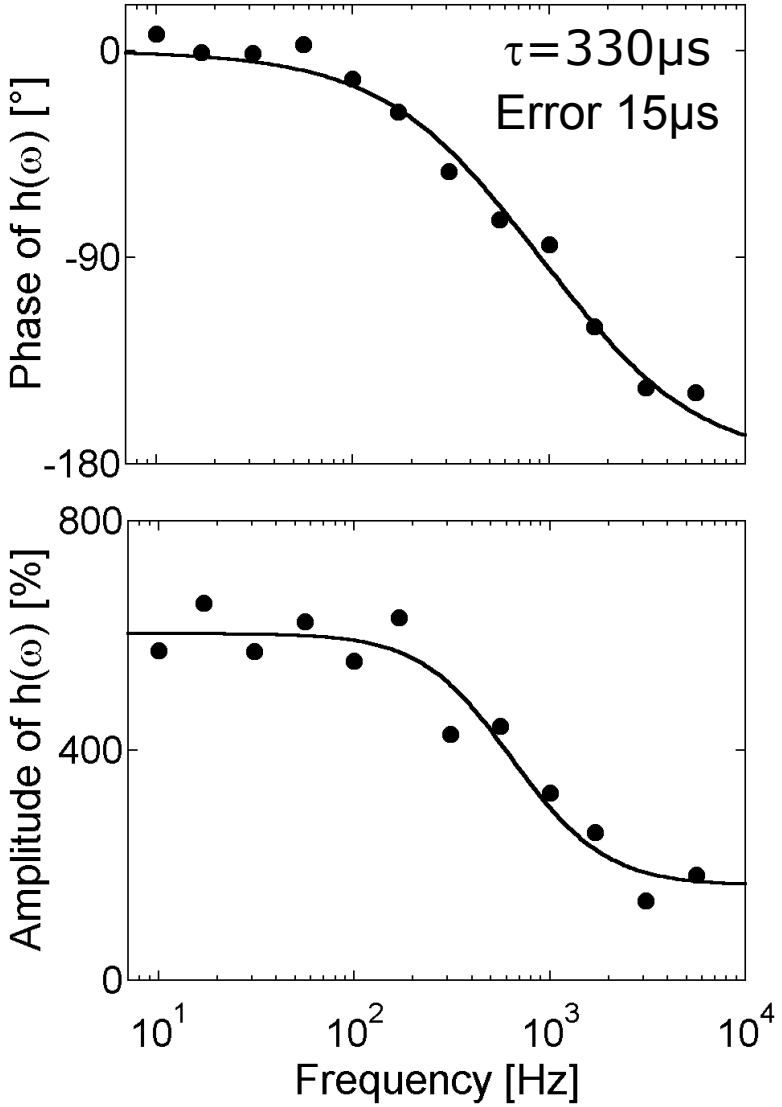
Kinetics of DNA Hairpins



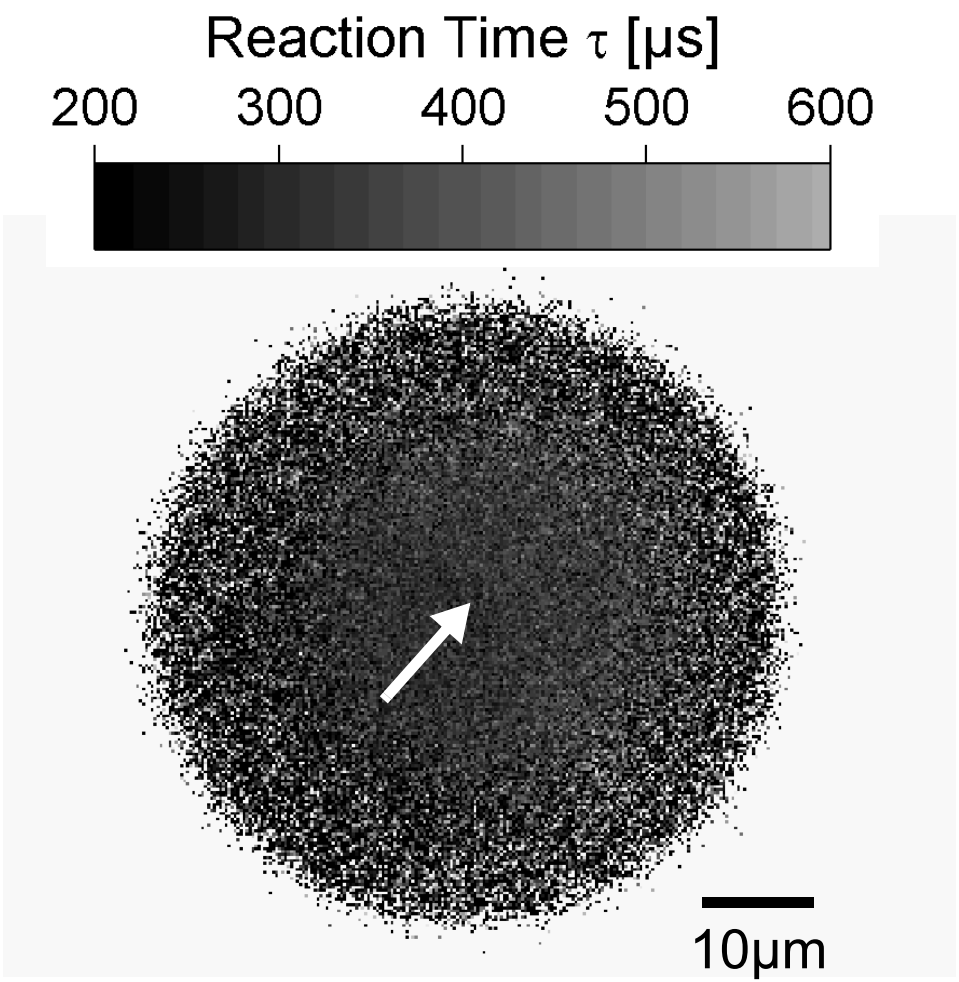
Bright Dark

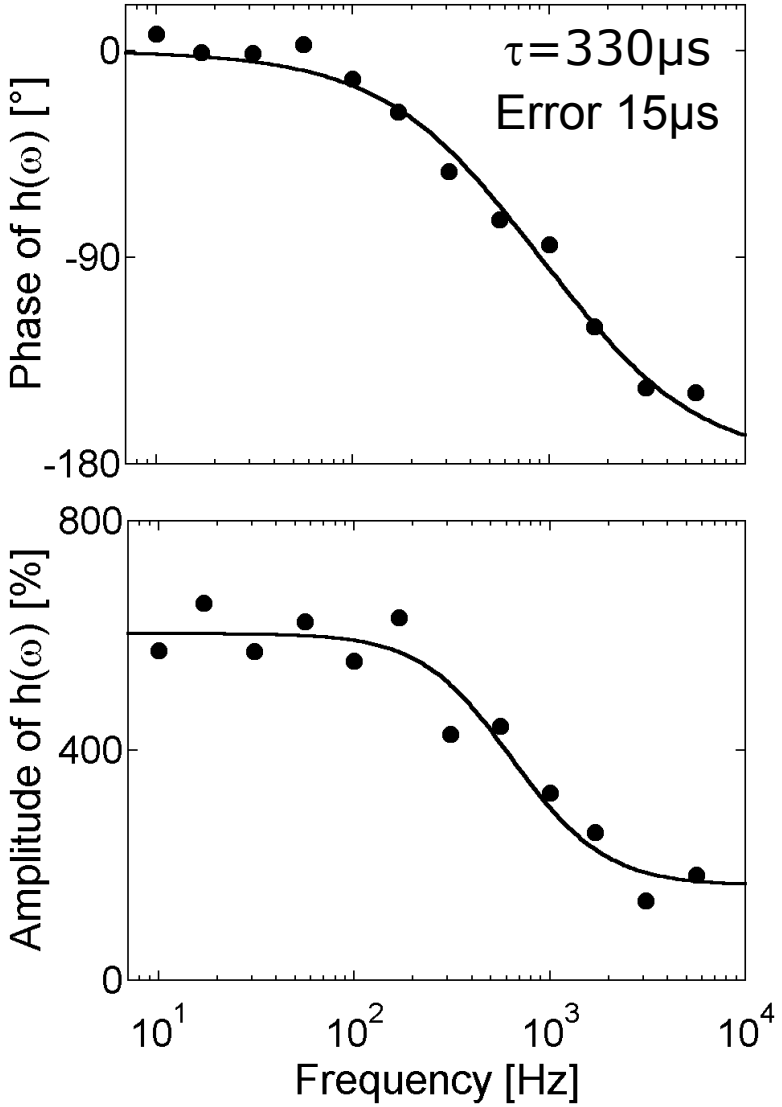
$$\frac{\frac{\Delta F}{F}}{\frac{\Delta T}{T}} = \frac{\beta/\alpha - 1}{1 + K\beta/\alpha} \frac{K}{1+K} \frac{\Delta H}{RT} + \chi T$$

Temperature Dye



Kinetics for Each Pixel





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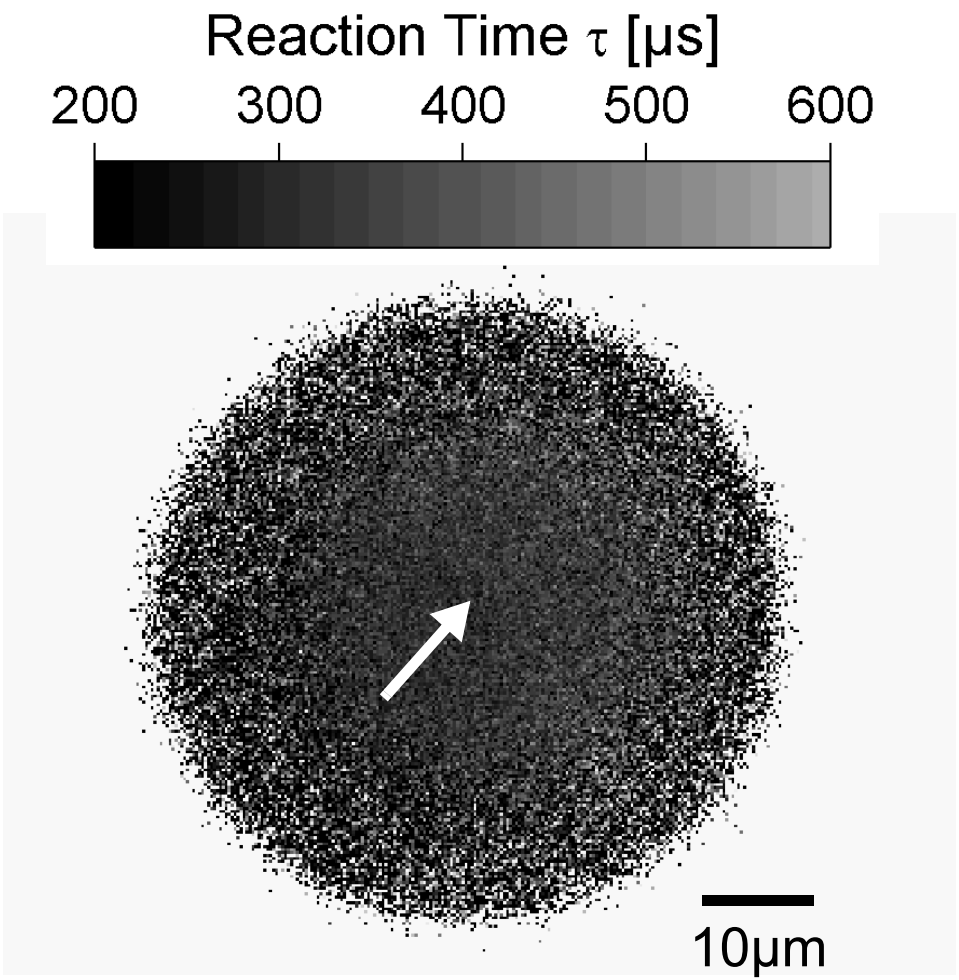
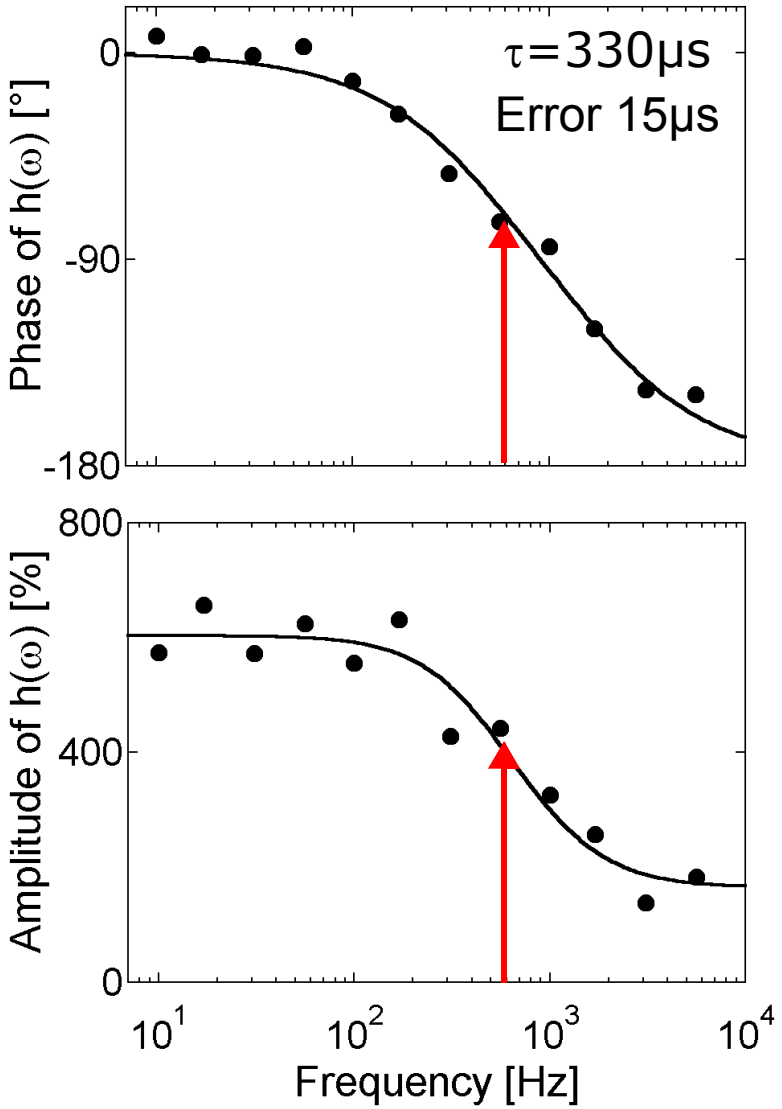
Kinetics for Each Pixel





Single Phase Image => Kinetics !

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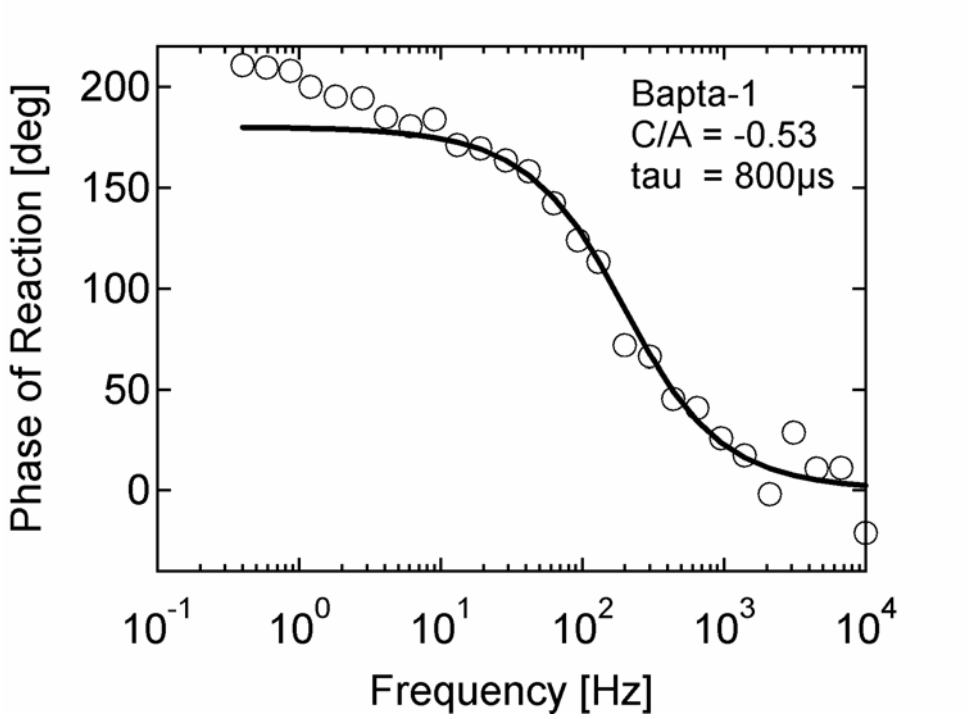
Ca Binding Kinetics (Bapta-1)

EDTA-Buffer: $\Delta[Ca^{2+}]$ upon ΔT

Phase

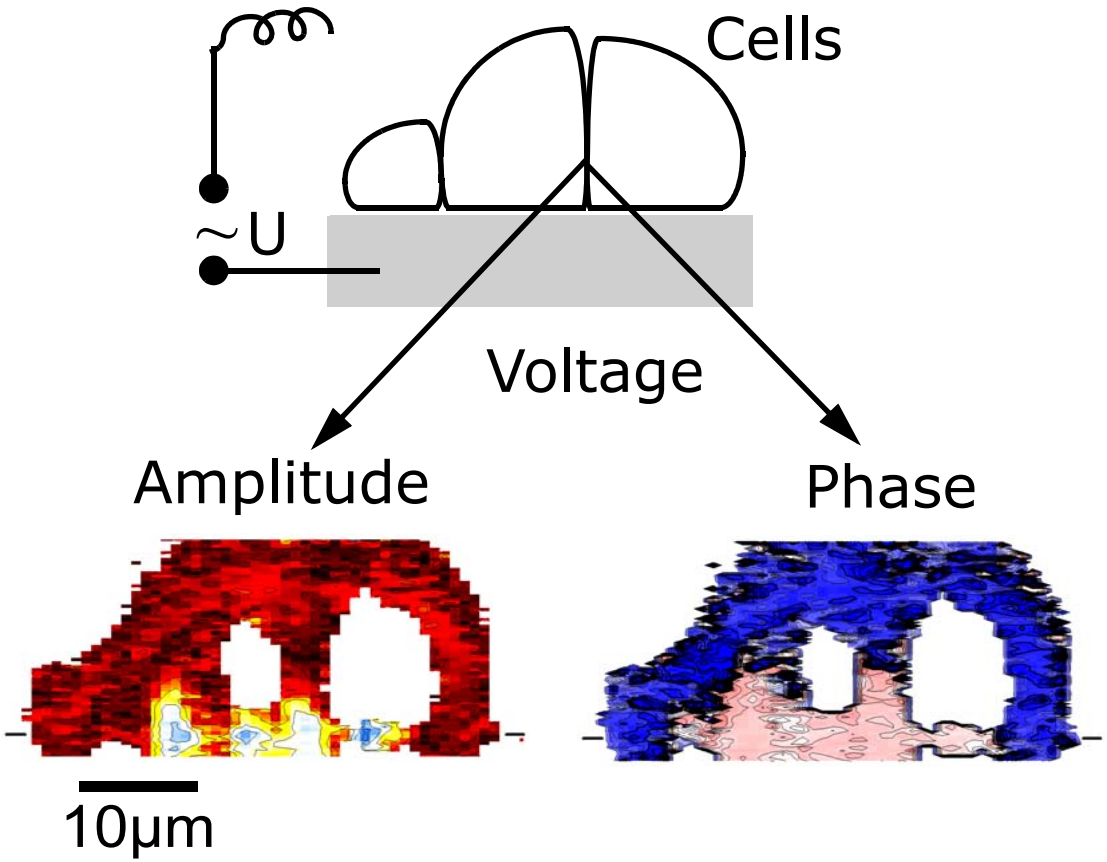


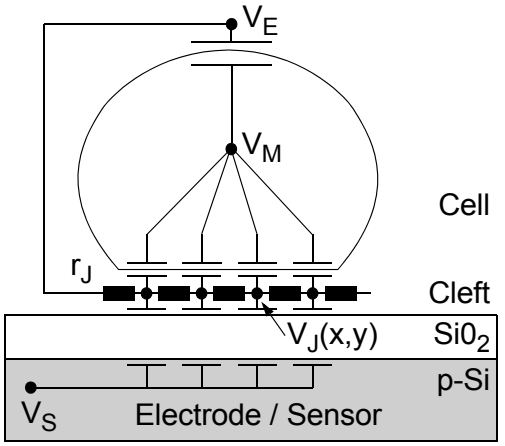
Time Constant 800µs

Cell-Electrode Contact

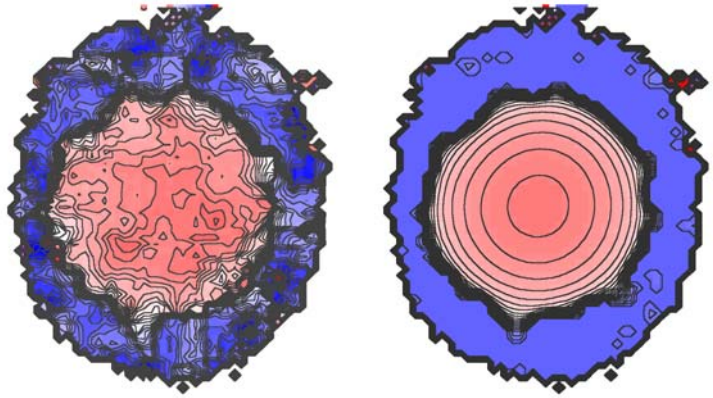
Reference: Electrical Modulation

Signal: Voltage-Sensitive Dye





Experiment Theory



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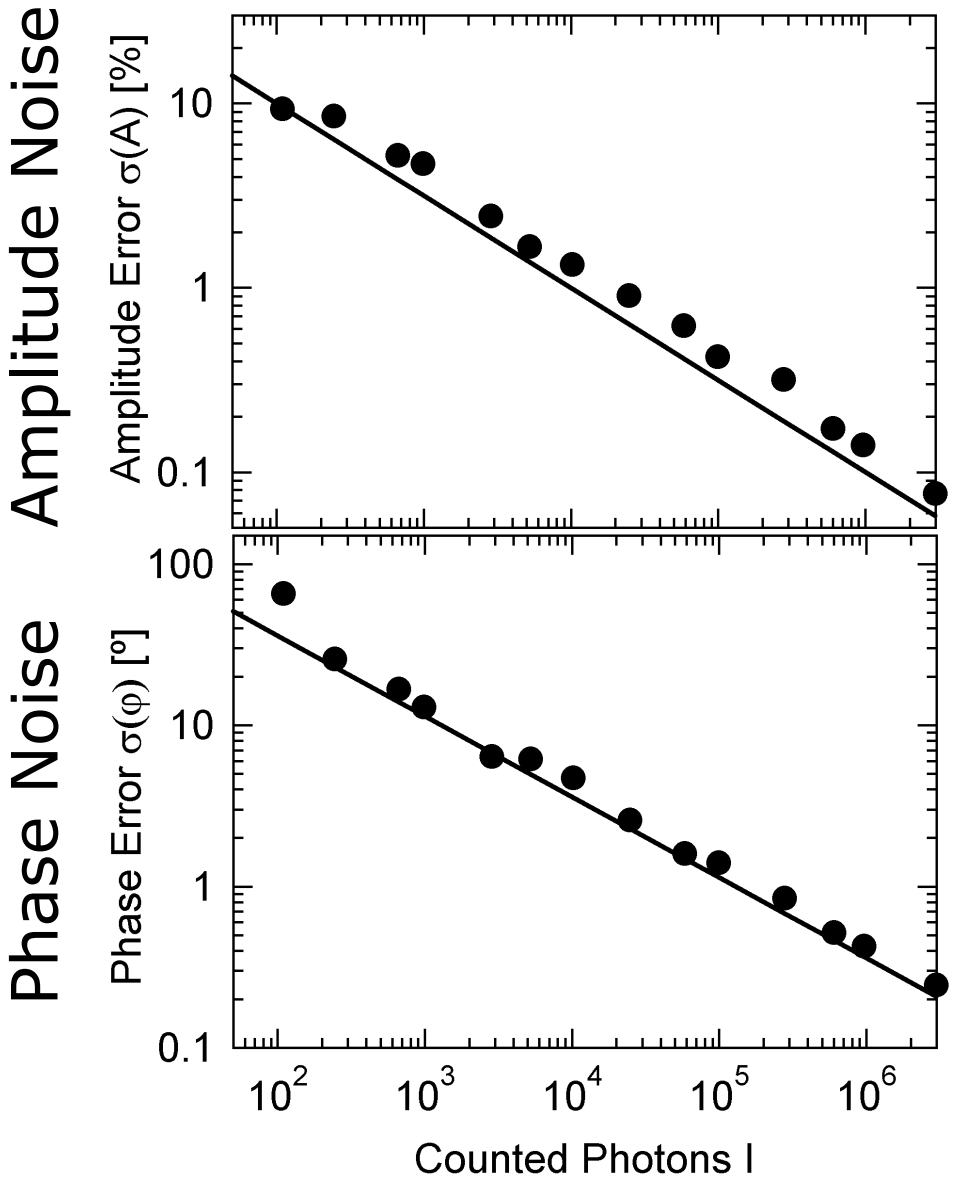
At the Limit of Photon Shot Noise



Error given by
Shot Noise Limit

$$\left.\begin{array}{l} Fluorescence \\ Amplitude \\ Phase \end{array}\right\} = \sqrt{\#\text{Photons}}$$

Optics Letters, 27:1418-1420 (2002)

Advantages

- Reduction of noise
- At shot noise limit
- Amplitude/Phase for each pixel
- Speed only limited by Illumination

Prospects

- Kinetics in single Cells
- High Throughput Screens
- Search for Collaborations

Acknowledgements: Emmy Noether Program, DFG