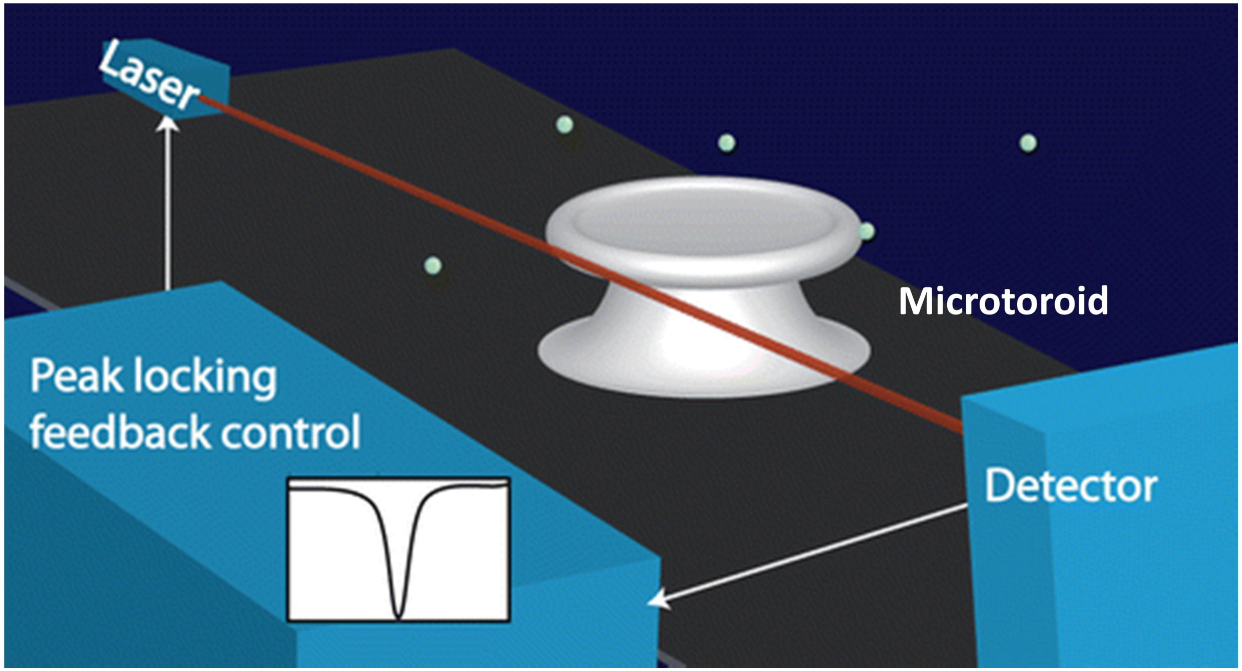
Biosensing with optical resonators

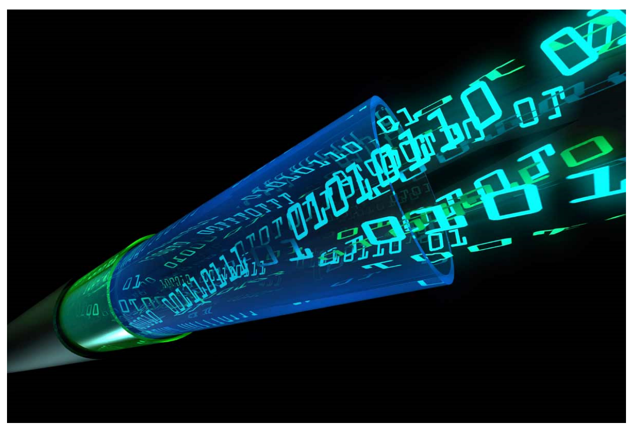


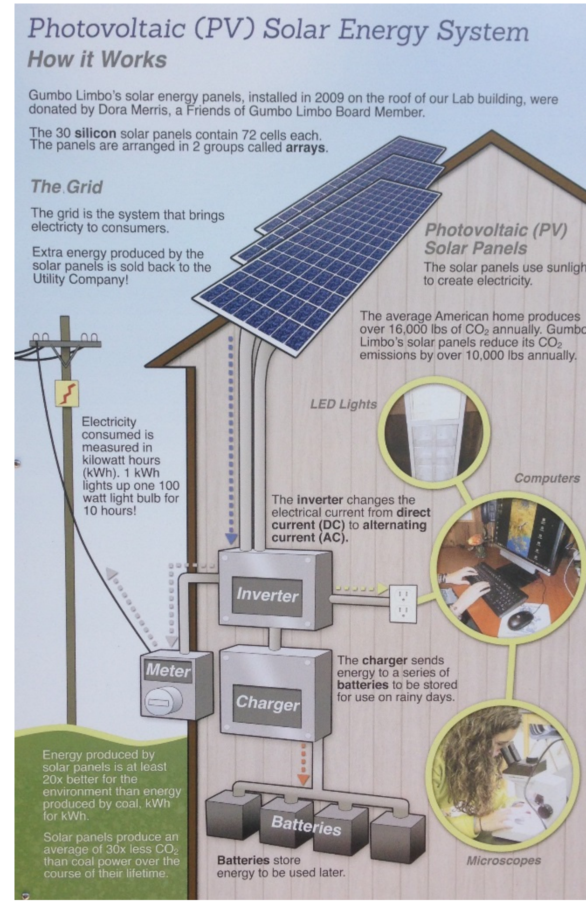
Judith Su
College of Optical Sciences, University of Arizona
University of Arizona Winter School
1/05/16

Why is optics exciting and important?

- TV display
- Solar panels
- Internet
- Fiber optics communication

http://cdn2.bigcommerce.com/n-d57o0b/tvhc2xod/product_images/uploaded_images/solar-panels.jpg?t=1416860323



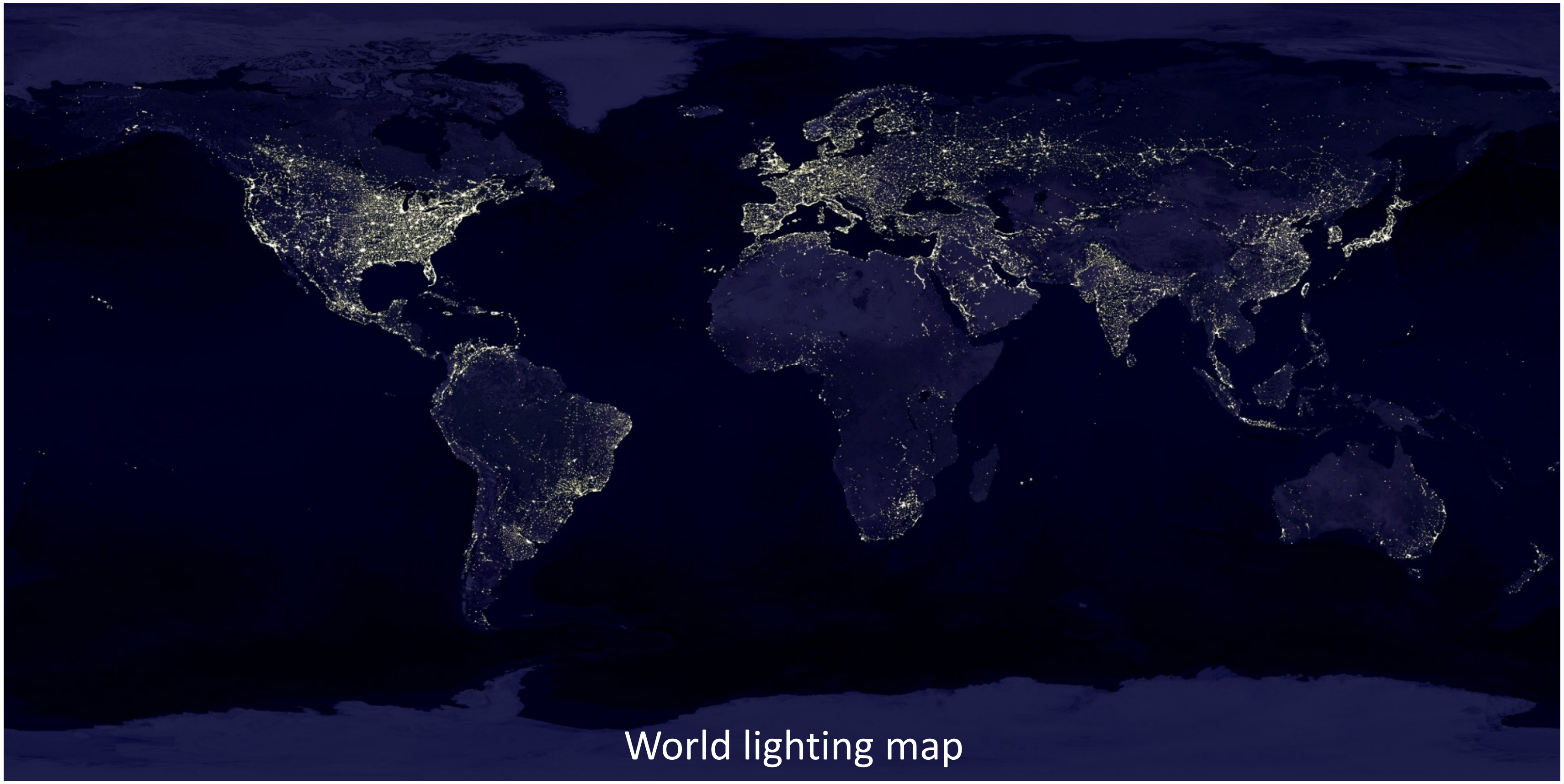


http://www.ethiopianopinion.com/wp-content/uploads/2014/08/7259d071.jpg

Humanity's Top 10 problems for the next 50 yrs (R. Smalley, Rice University)

1. Energy
   - Solar panels
2. Water
   - Optical sensors for water quality monitoring
3. Food
   - Optical sensors for food quality monitoring
4. Environment
   - Optical sensors for air quality monitoring
5. Poverty
   - Solar panels and LEDs reduce dependence on fossil fuels
   - Optical tools such as light bulbs and computers assist in education which can lift you out of poverty

Humanity's Top 10 problems for the next 50 yrs (R. Smalley, Rice University)



More light = More prosperity

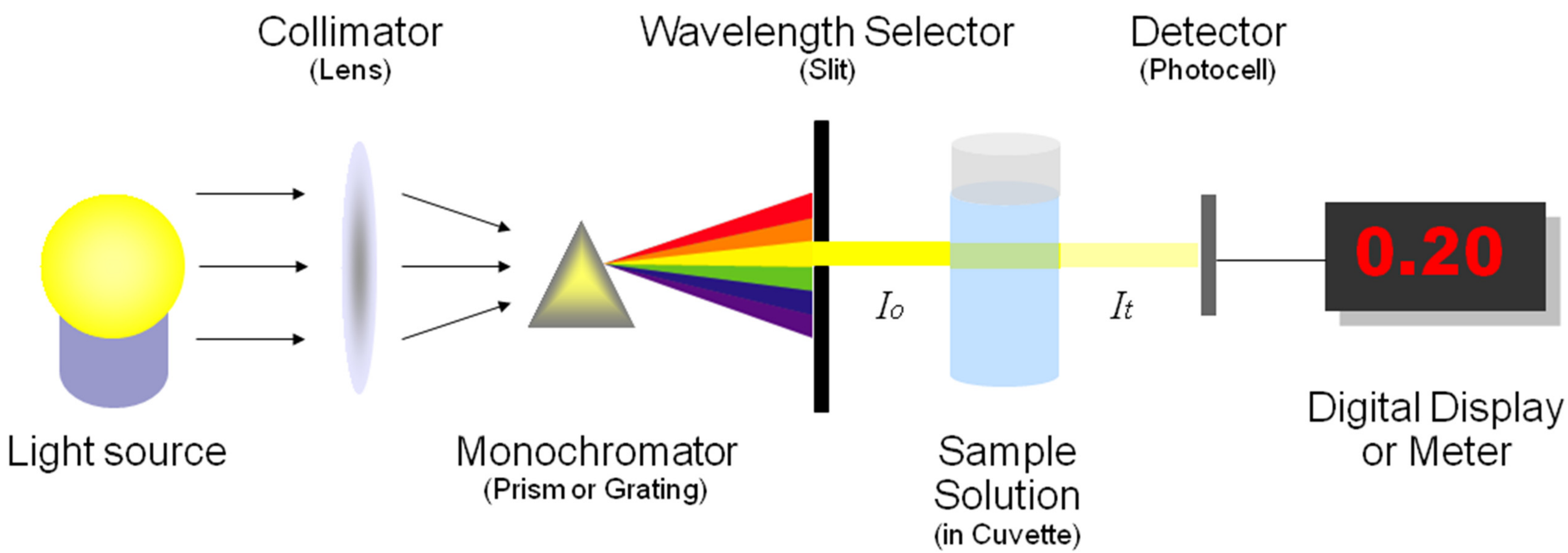
http://socialearth.org/wp-content/uploads/2012/12/electric-lightening-at-night-around-the-world-earth-at-night1.jpg

Humanity's Top 10 problems for the next 50 yrs (R. Smalley, Rice University)

6. Terrorism & War
   - Optical sensors for pathogens
7. Disease
   - Optical sensors for early detection of disease
8. Education
   - Computers, smartphones
9. Democracy
   - Voting machines
10. Population
    - Medical devices for healthy aging: mobile health technologies, MRI

Optical biosensors

Spectrometer

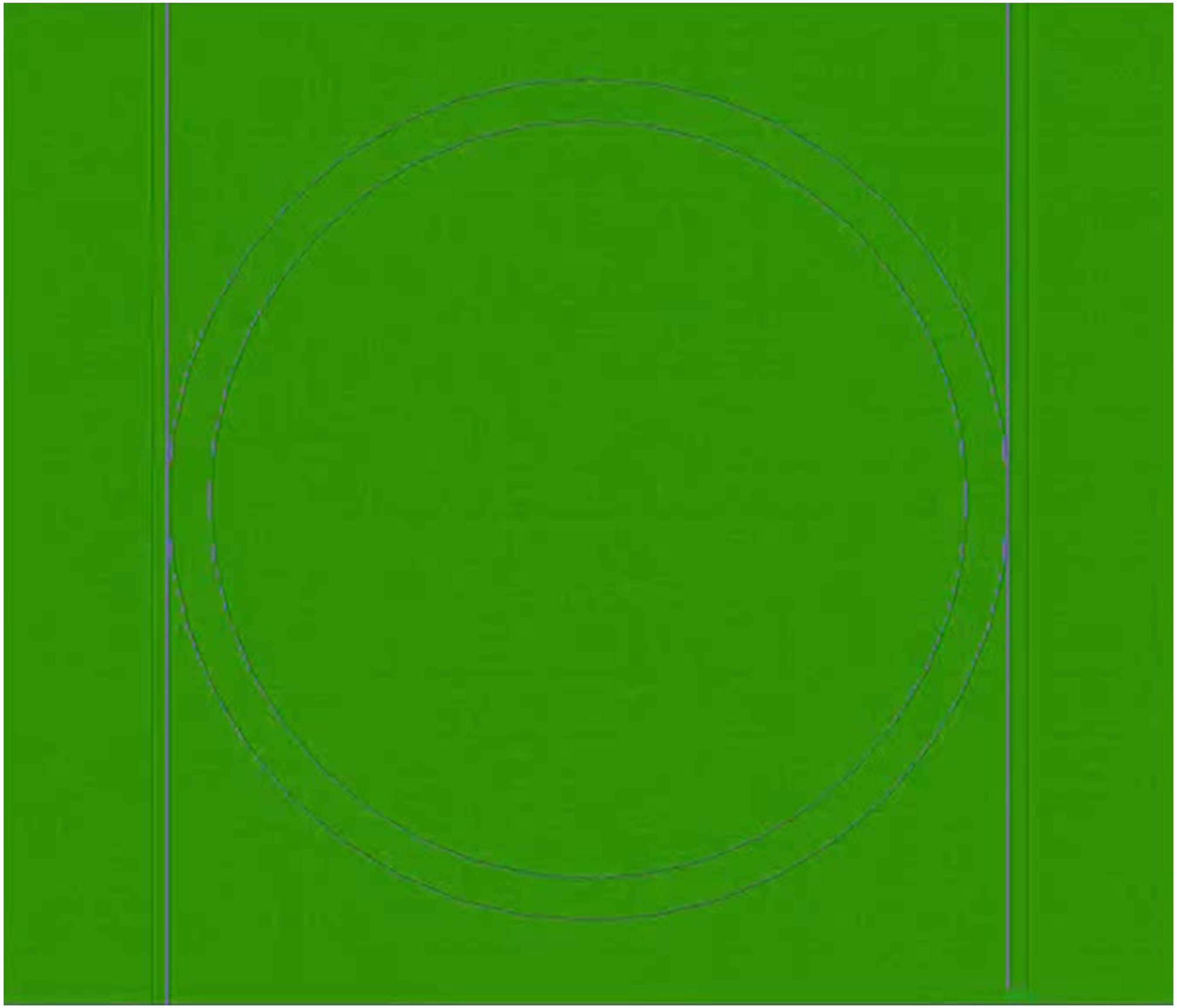


http://chemwiki.ucdavis.edu/Physical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetcs/Spectrophotometry

Beer-Lambert Law: $A = \epsilon l c$

A = absorbance
c = concentration
l = path length
ϵ= molar absorptivity

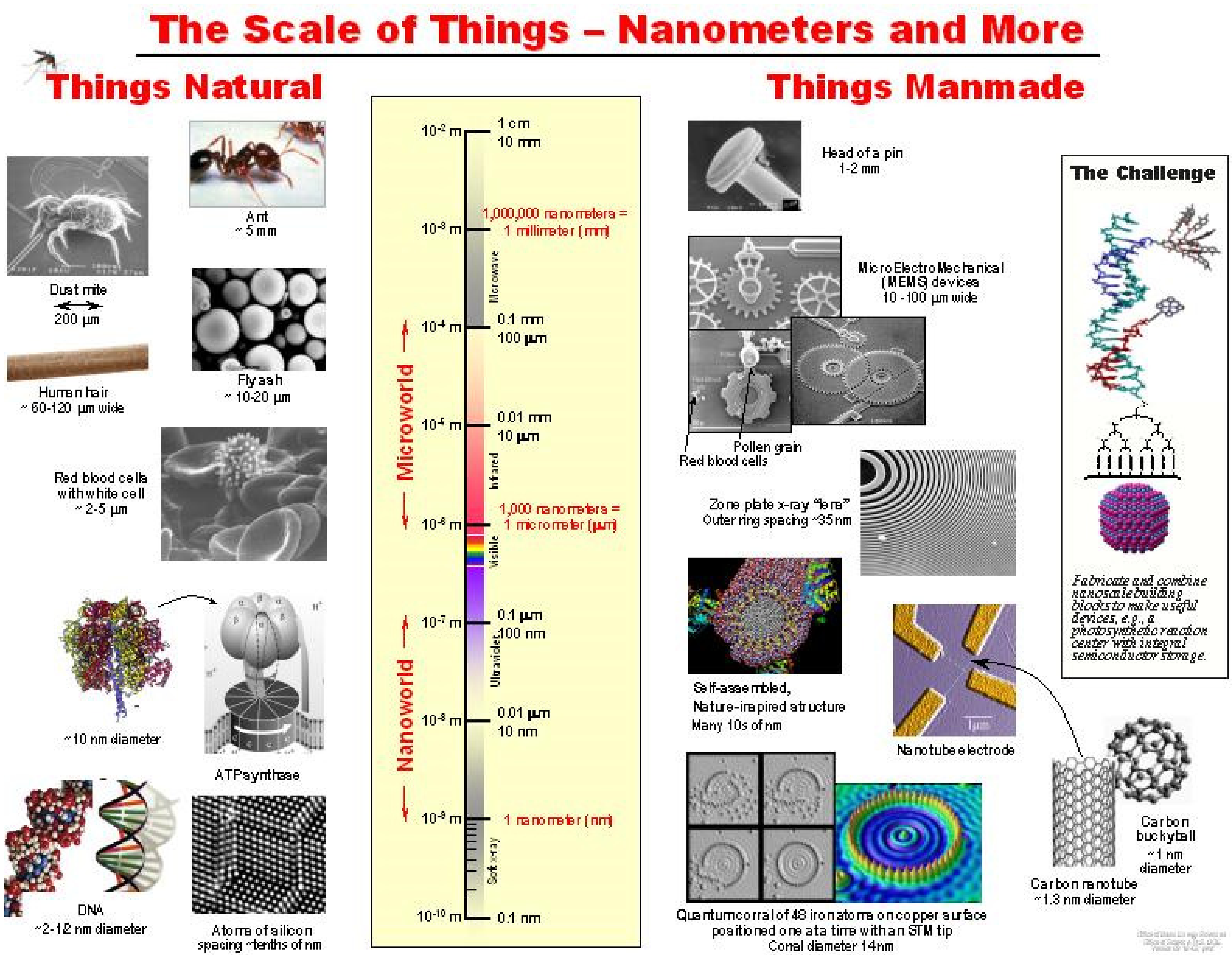
Optical resonator



https://www.youtube.com/watch?v=Df0TEtmQzw4

Sensing applications for optical resonators

- Homeland security
- Nanoparticle detection (air quality)
- Virus assembles inside a host
- Virus detection
- Basic science (protein folding)
- Medical diagnostics (early detection of disease)
- Drug design
- Food and water quality monitoring

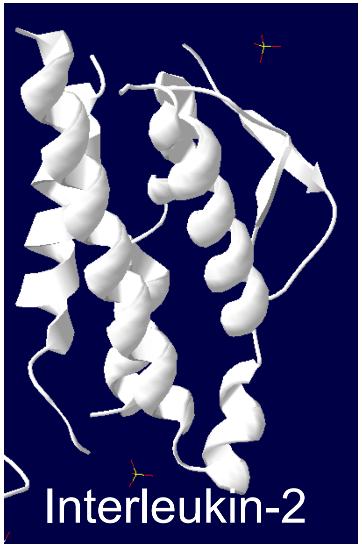


https://nanohub.org/resources/13842

Importance of single molecule detection

Fundamental studies:

1. Transient states of protein folding
2. Motor protein (myosin & kinesin) movement and step size



Applied studies:

1. Trace detection of tumor-specific cancer antigens
2. Public health detection of bacteria and/or viruses

Why single molecule approaches are useful:

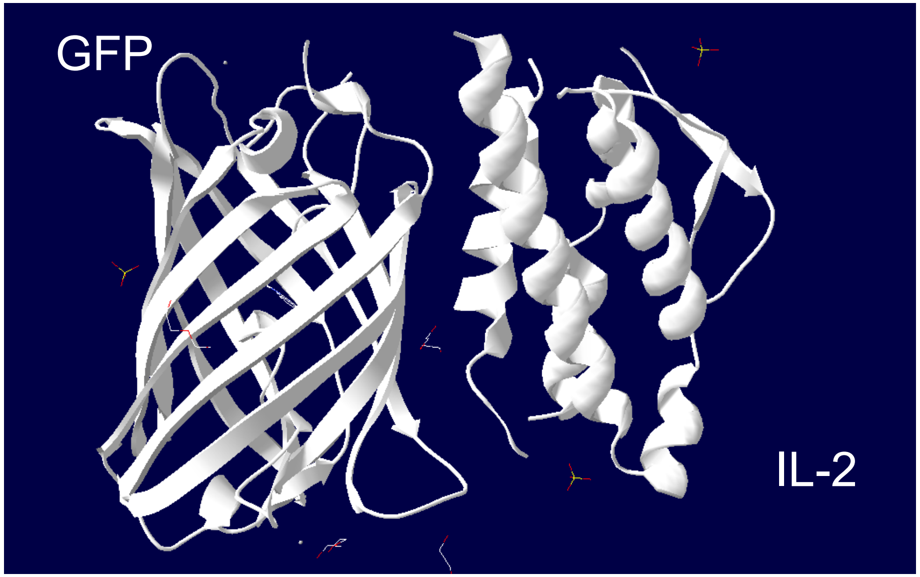
1. Direct measurements of molecule properties (vs. model-dependent inferences from bulk methods).
2. Populations of state-switching molecules need not be synchronized
3. Vastly reduced analyte required (and $$$)

Why is a label-free approach important?

Importance of a label-free approach

Labels are tags that make single molecules easier to detect. Common examples are:

1. Fluorescent markers
2. Radioactive tags
3. Enzymatic labels
4. Quantum dots



However, they can be:

1. Expensive
2. Difficult to generate
3. Disruptive (may perturb molecular events and introduce artifacts due to bleaching and blinking)
4. Complicated (studies often require multiple tags)

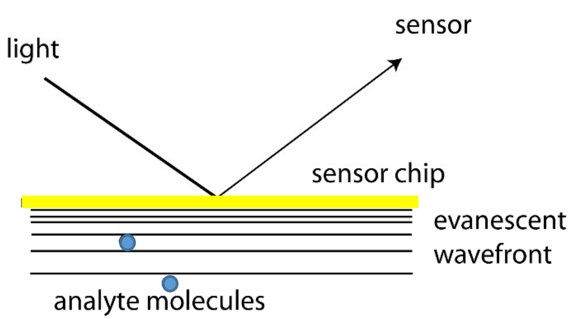
The microtoroid can eliminate the need for labels

What’s currently available?

Current gold standard: Biacore surface plasmon resonance

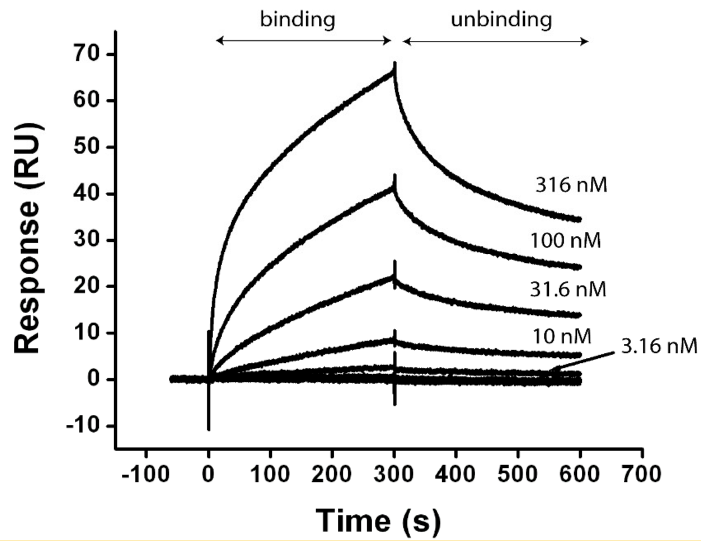
http://biobest.com.au/bioweb/images/stories/biacore_3000.jpg

Sensing approach



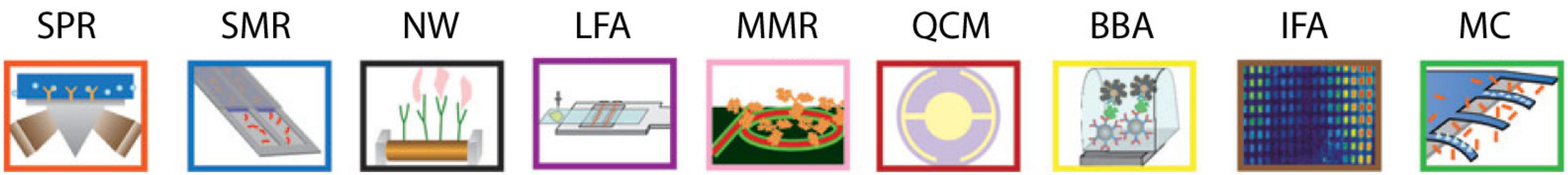
Biacore surface plasmon resonance

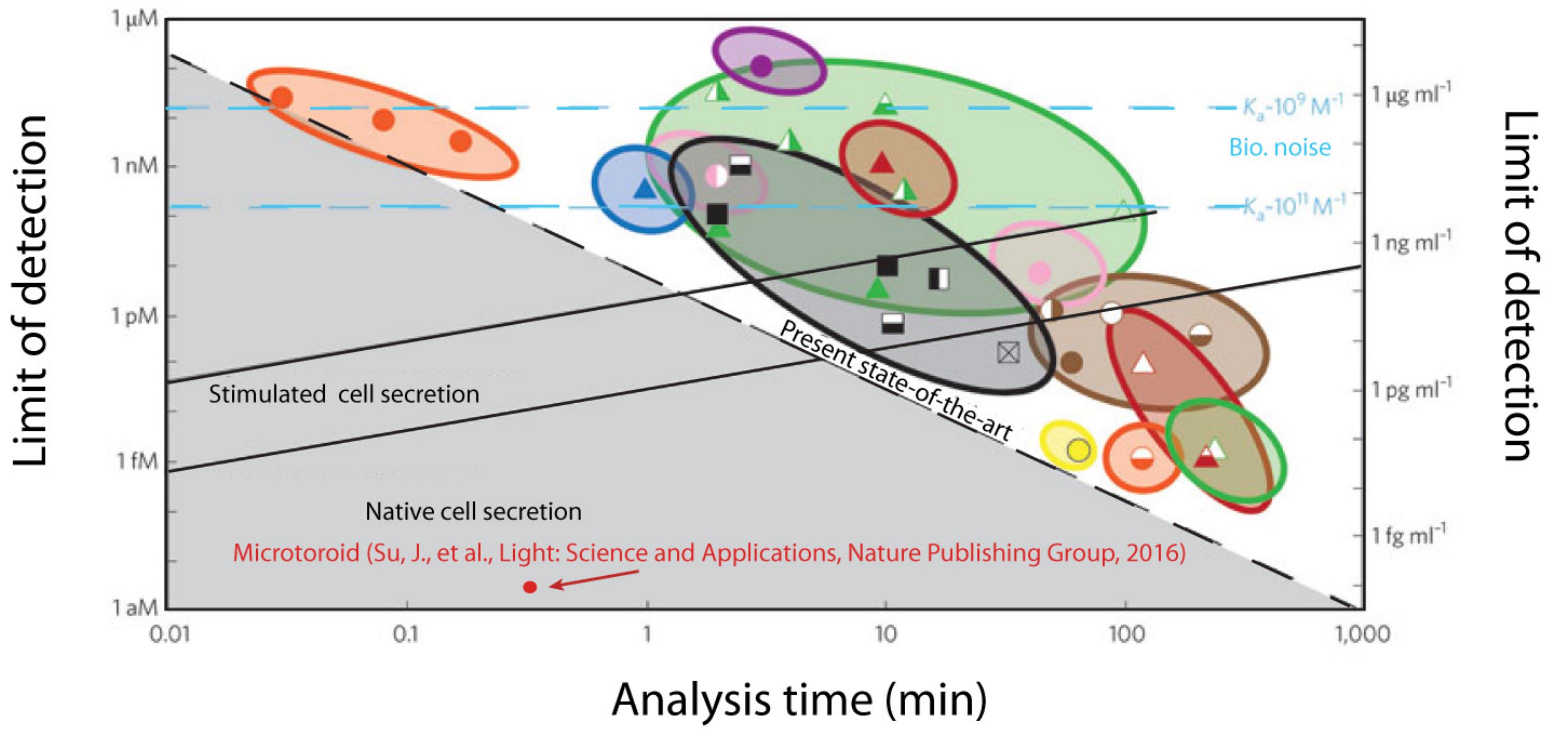
Finding the limit of detection



The Biacore has a lower limit of detection on the order of nM. Concentrations lower than this are necessary for single cell proteomics.

Design goal





Arlett J.L., Myers E.B., Roukes M.L., Comparative advantages of mechanical biosensors. Nature Nanotechnology 6(4), 203-15 (2011).

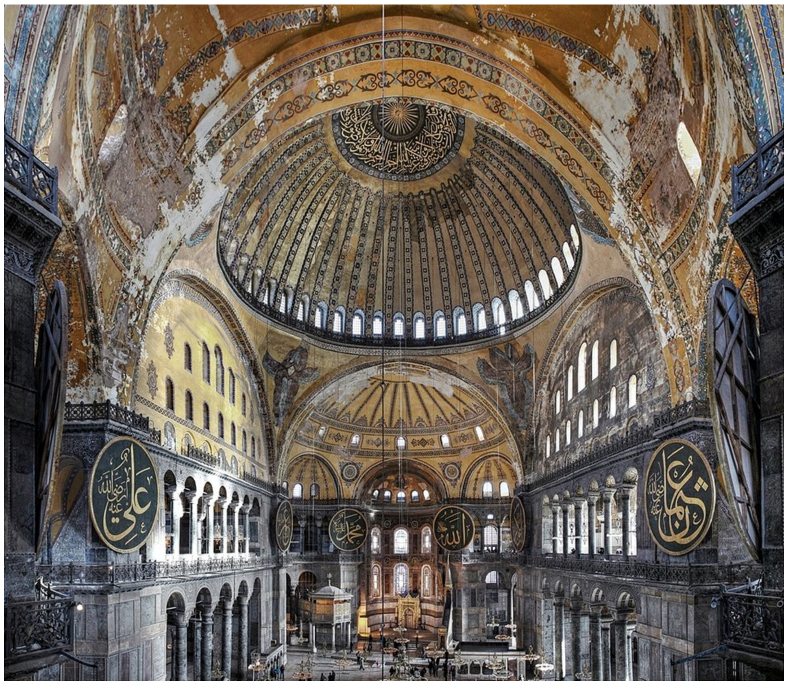
How do we achieve our goal?

Outline

- Microtoroid optical resonators
- Detection principles
- Previous work
- Frequency-Locked Optical Whispering Evanescent Resonator (FLOWER)
- Results: Beads from 100 nm – 2.5 nm radius, variety of bioparticles
- Clinical Application: Minimally invasive tumor biopsy
- Conclusions

Acoustic whispering gallery

Hagia Sophia, Istanbul



http://th07.deviantart.net/fs71/PRE/f/2012/001/8/0/under_the_dome_of_hagia_sophia_2_by_erhansasmaz-d4kx0j1.jpg

St. Paul's Cathedral, London

http://media.npr.org/assets/img/2012/04/20/53041002-st-pauls-cathedral-london_custom-9395d26199fc1bf96b463b9be27e035d760e665a-s6-c30.jpg

Whispers at one end of the dome can be heard at the other as sound skirts along the edges with low loss.

Explained by Lord Rayleigh for the first time (Philos Mag 20 1001-1004, 1910)

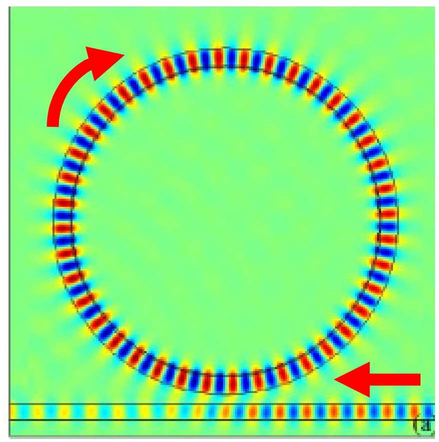
Toroids are the optical analog of this…

Optical whispering gallery mode resonators

Microsphere

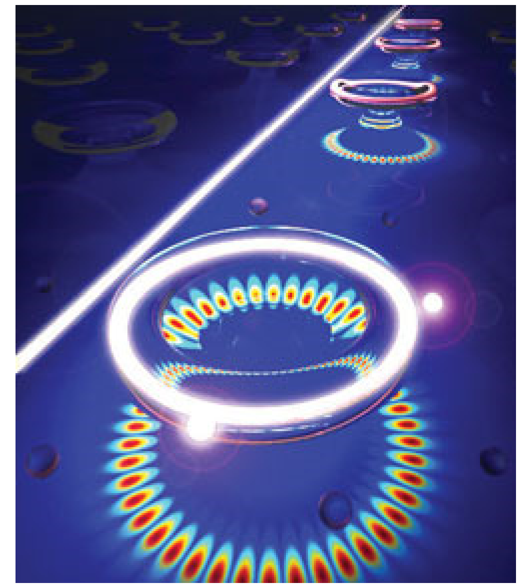
http://k-lab.epfl.ch/files/content/sites/klab/files/figure/Big_cover.jpg

Microring



http://ej.iop.org/images/1612-202X/10/1/015901/Full/lpl453706f7_online.jpg

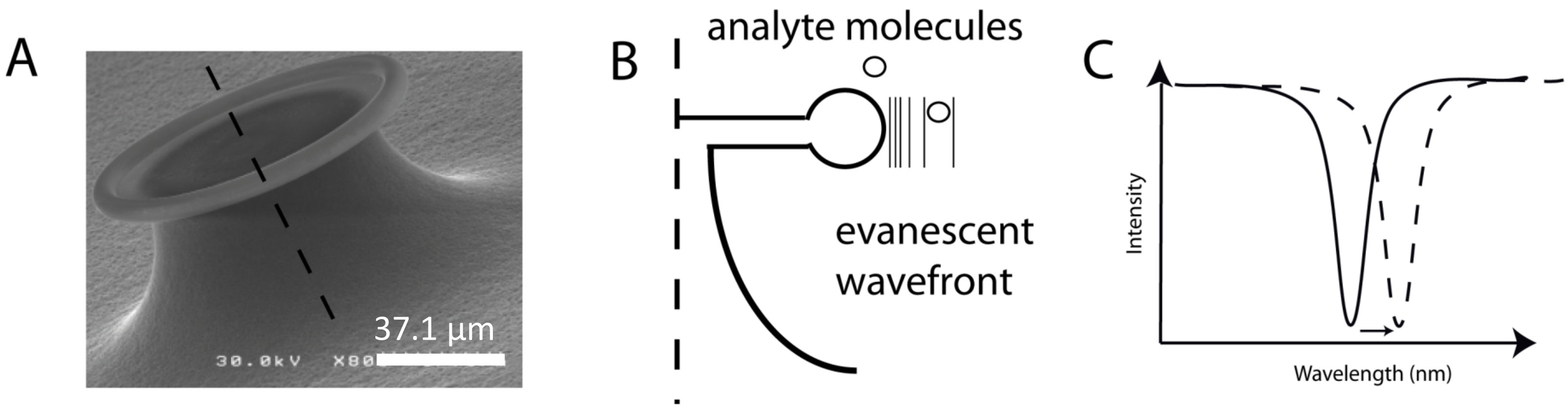
Microtoroid



http://spie.org/Images/Graphics/Newsroom/Imported 2010/003078/003078_10_fig1.jpg

At resonance a standing wave forms and transmission through the optical fiber drops due to interference. This “drop” enables a means of identifying the resonant wavelength of the device.

Detection principles



Microtoroid scanning electron micrograph

Microtoroid cross-section

Molecular binding is detected by shifts in the resonance frequency

The toroid has a photon lifetime of 270 ns. In this time, light travels around the toroid **270,000x** or **68 meters**. Therefore, a single photon interacts with analyte molecules multiple times, thereby increasing sensitivity.

Why does the resonance wavelength shift?

Reactive sensing principle (RSP)

Wavelength shift upon particle binding is proportional to the polarizability of the particle:

$$\frac{\Delta\omega}{\omega} = -\frac{\frac{\mathrm{Re}[\alpha_{ex}]}{4} E_0^2(\boldsymbol{r}_s)}{\frac{1}{2}\int \varepsilon(\boldsymbol{r}_c)\, E_0^2(\boldsymbol{r}_c)\, dV} = -\frac{\mathrm{Re}[\alpha_{ex}]\, E_0^2(\boldsymbol{r}_s)}{2\int \varepsilon(\boldsymbol{r}_c)\, E_0^2(\boldsymbol{r}_c)\, dV}$$

Arnold, S., Khoshsima, M., Teraoka, I., Holler, S. & Vollmer, F. Shift of whispering-gallery modes in microspheres by protein adsorption. *Optics Letters* **28**, 272-274 (2003).

But this is something that hasn't been tested at the molecular level…

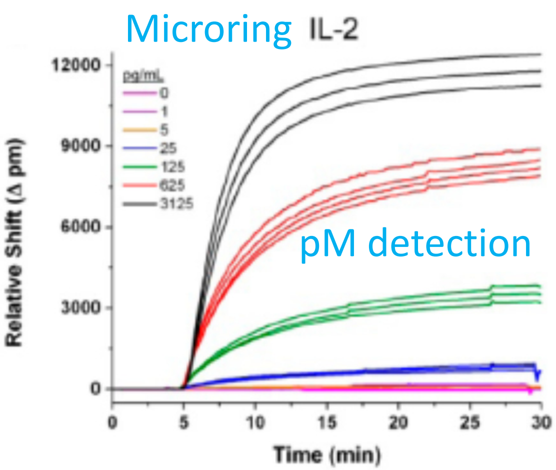
What makes detection difficult?

- Signal strength scales as r^3 (particle volume)
- To detect a 2.5 nm radius particle, you need to resolve wavelength shifts < 0.006 fm
- $\frac{\Delta\lambda}{\lambda} = \frac{1}{10^{12}}$

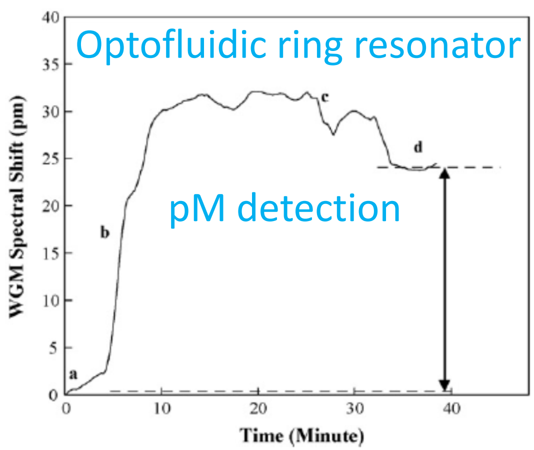
This should be possible…

Some recent advances

Dilute biomolecules:

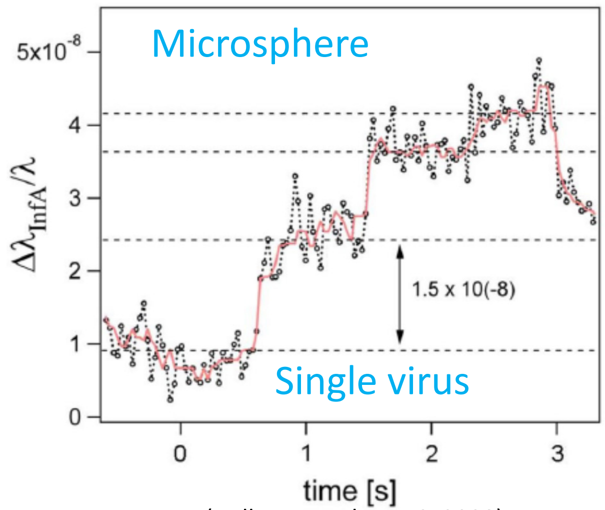


(Kindt, et al, Anal. Chem, 2013)

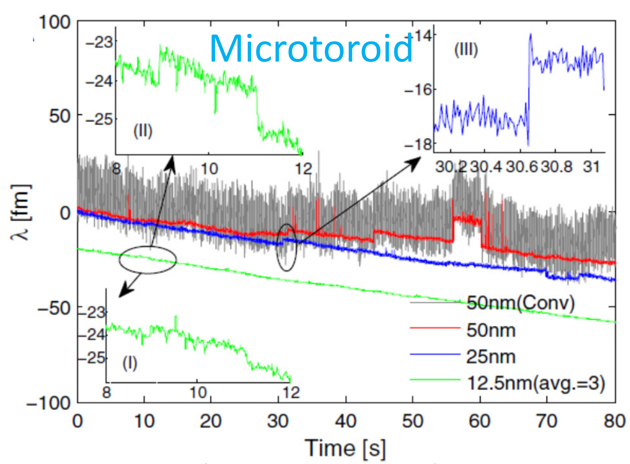


(Gohring, et al, Sens. & Act. B, 2010)

Single particles:

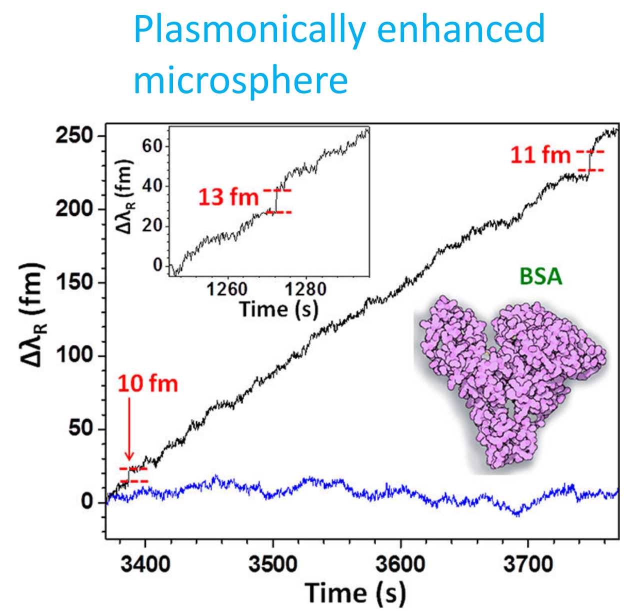


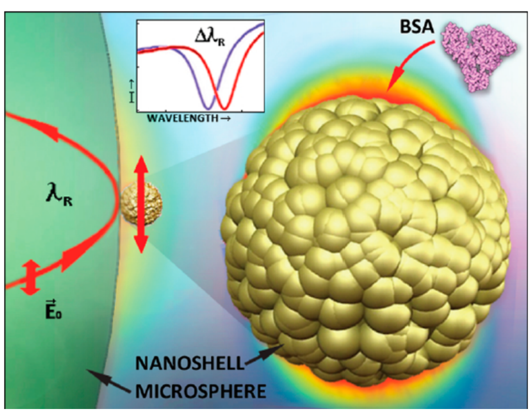
(Vollmer, et al, PNAS, 2008)



(Lu, et al, PNAS, 2011)

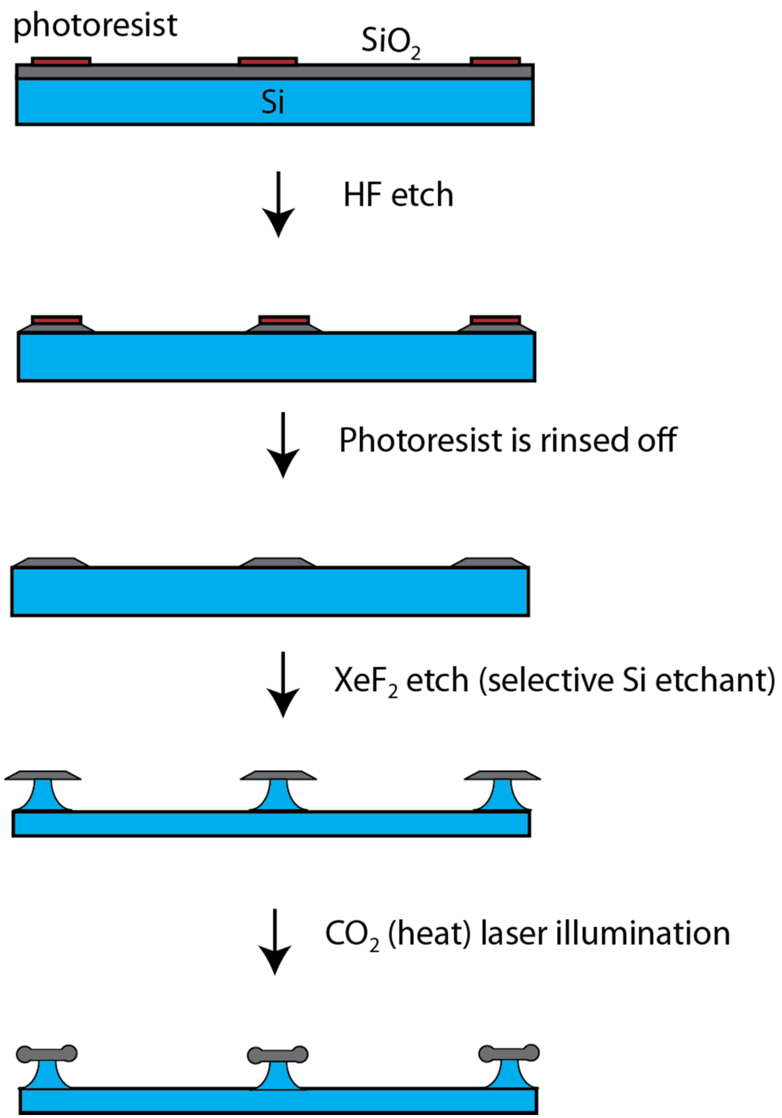
Single biomolecules:





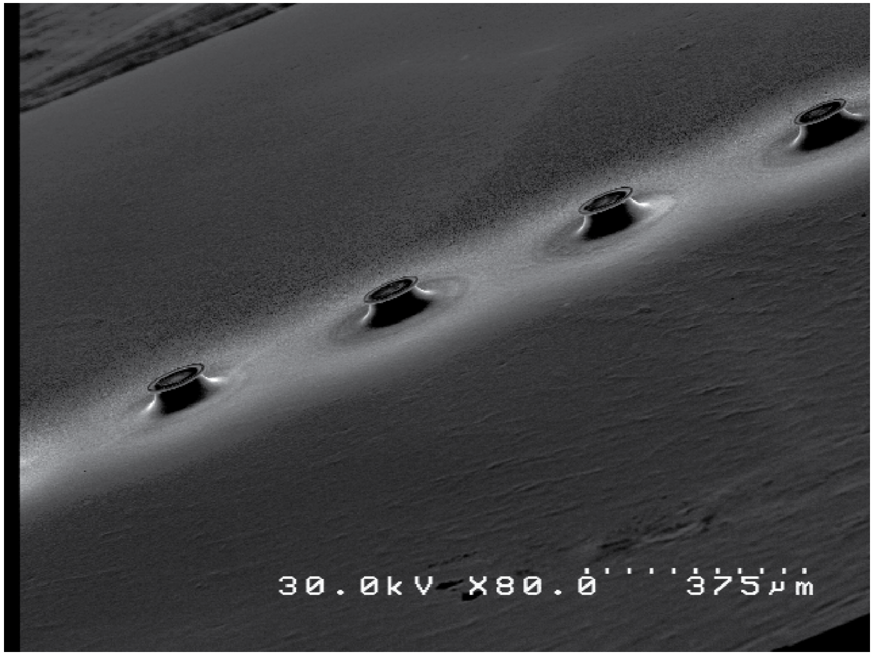
(Dantham, et al, Nano Lett., 2013)

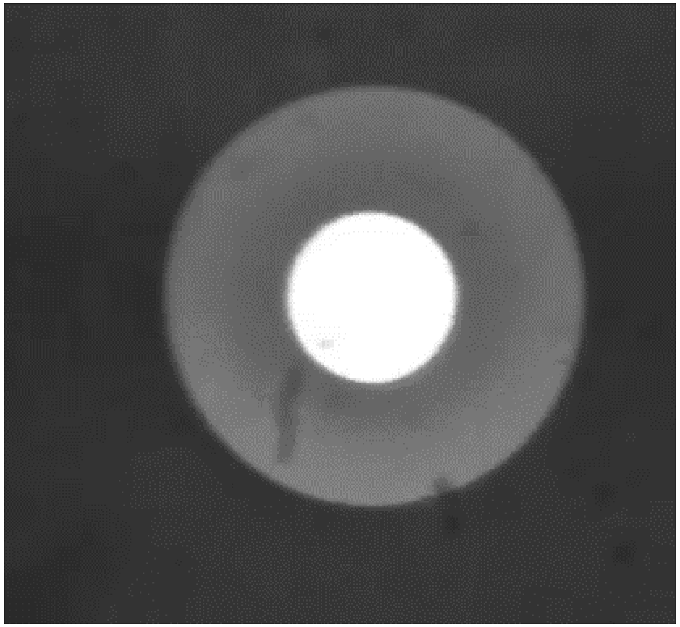
Microtoroid fabrication



Microtoroids are fabricated in a combination of lithography and etching steps. Wafer-based fabrication allows for the potential of multiplexing

Microtoroids



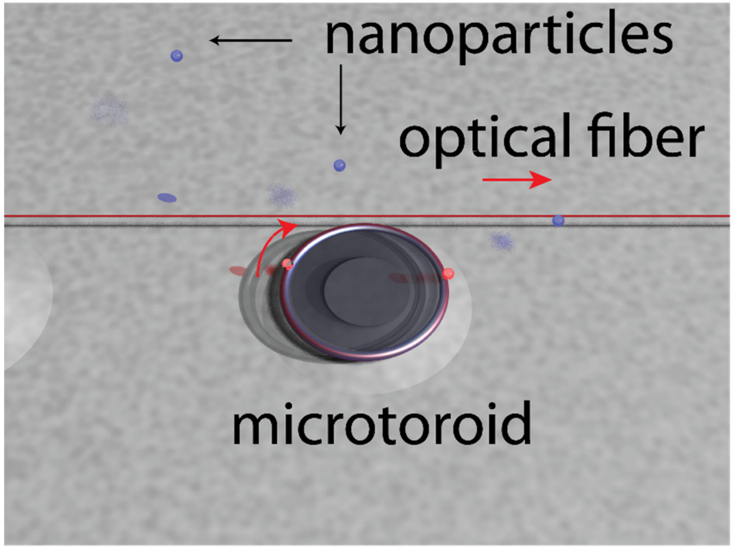


Su, J. Label-free Single Molecule Detection Using Microtoroid Optical Resonators. *J. Vis. Exp.* (106), e53180, doi:10.3791/53180 (2015).

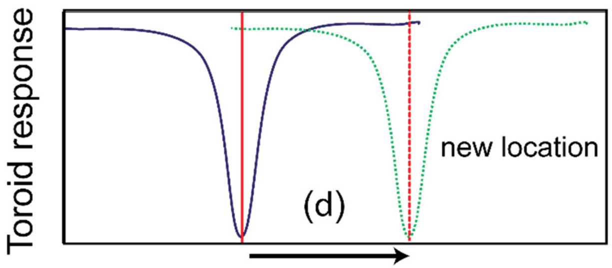
How do we do experiments with these?

Experimental setup

3D experimental schematic

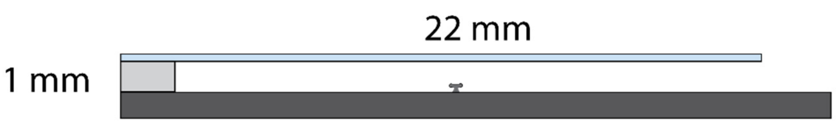


Active tracking of resonance

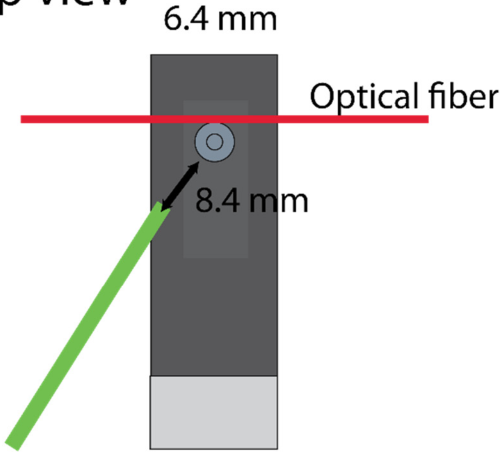


We measure the amount the laser shifts to stay on resonance (d)

Side view

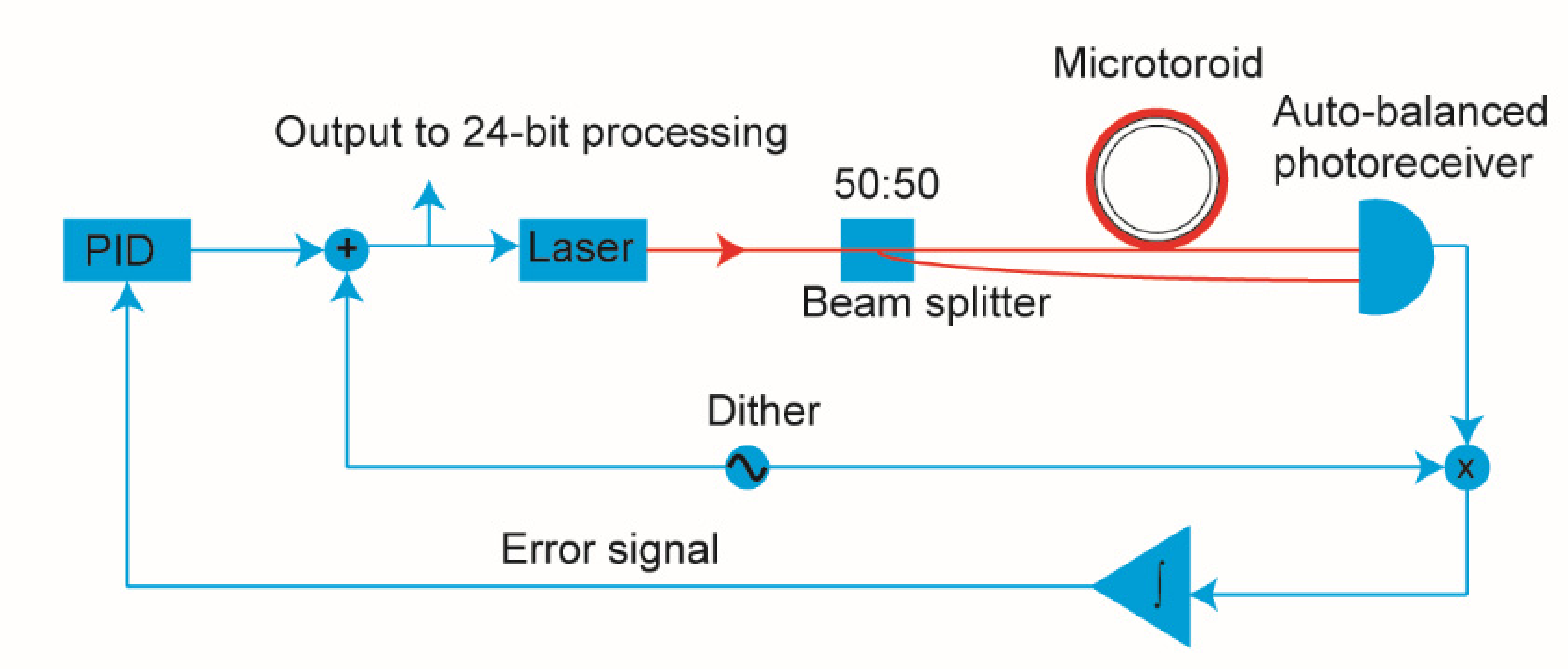


Top view



Frequency Locking Optical Whispering Evanescent Resonator (FLOWER)

Laser-locked microcavity with auto-balanced photo-receiver

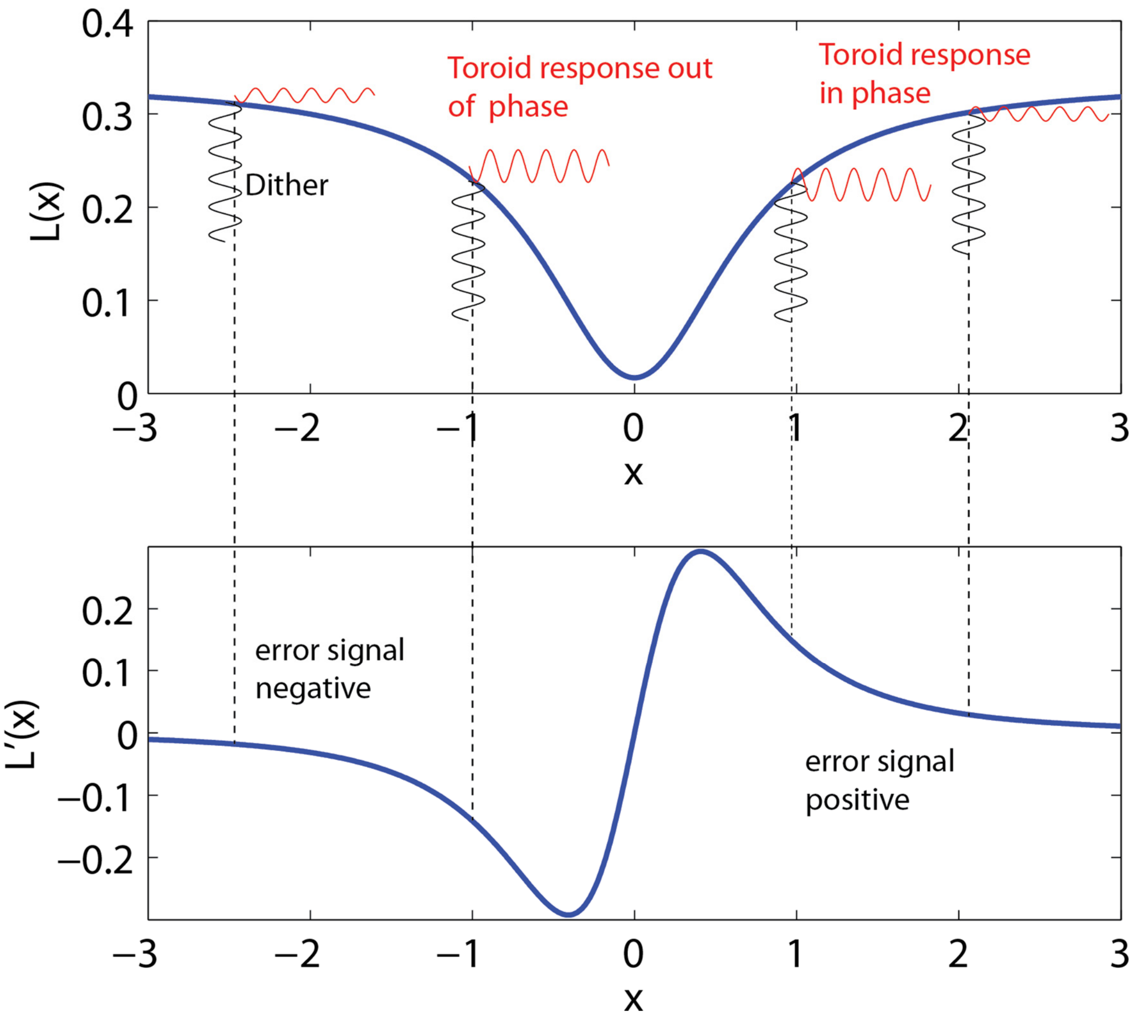


- **Continuously stay on resonance**
- **Sample more points per second**

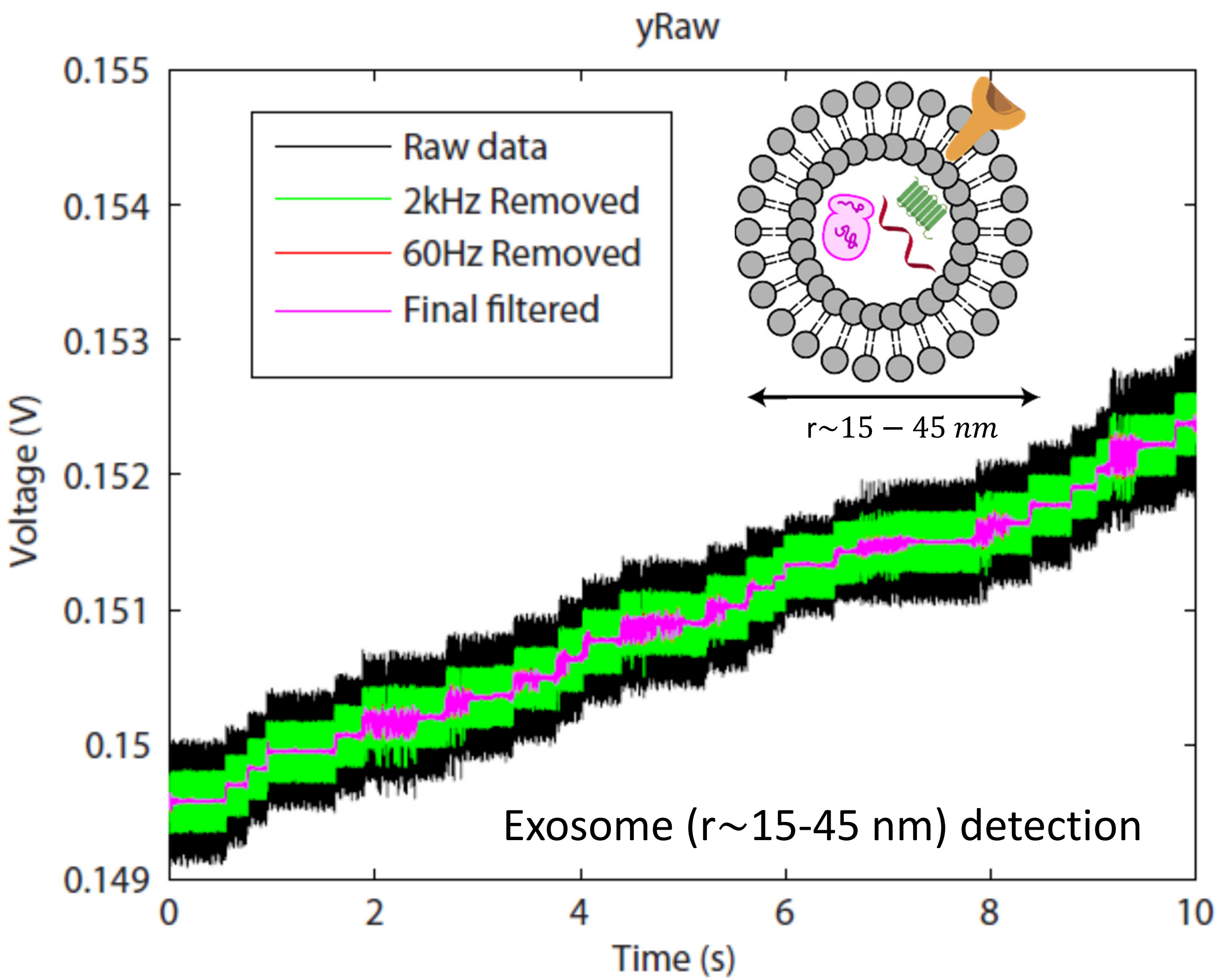
J. Su, et al., *Light: Science & Applications, Nature Publishing Group*, (**2016**) **5**, e16001; doi:10.1038/lsa.2016.1

J.Su, United States and PCT Non-Provisional Patent Application Filed, 61/953,695 (2015)

How does frequency locking work?

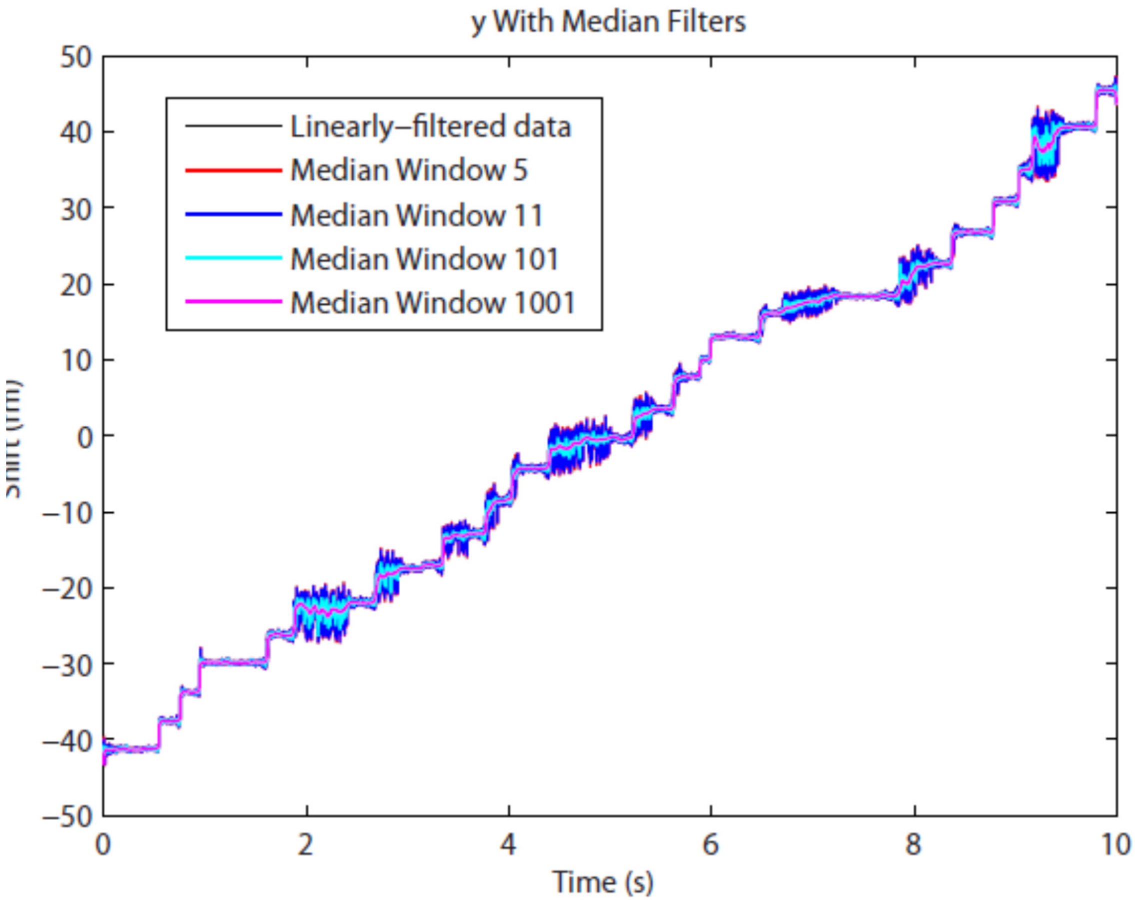


Filtering further improves signal to noise



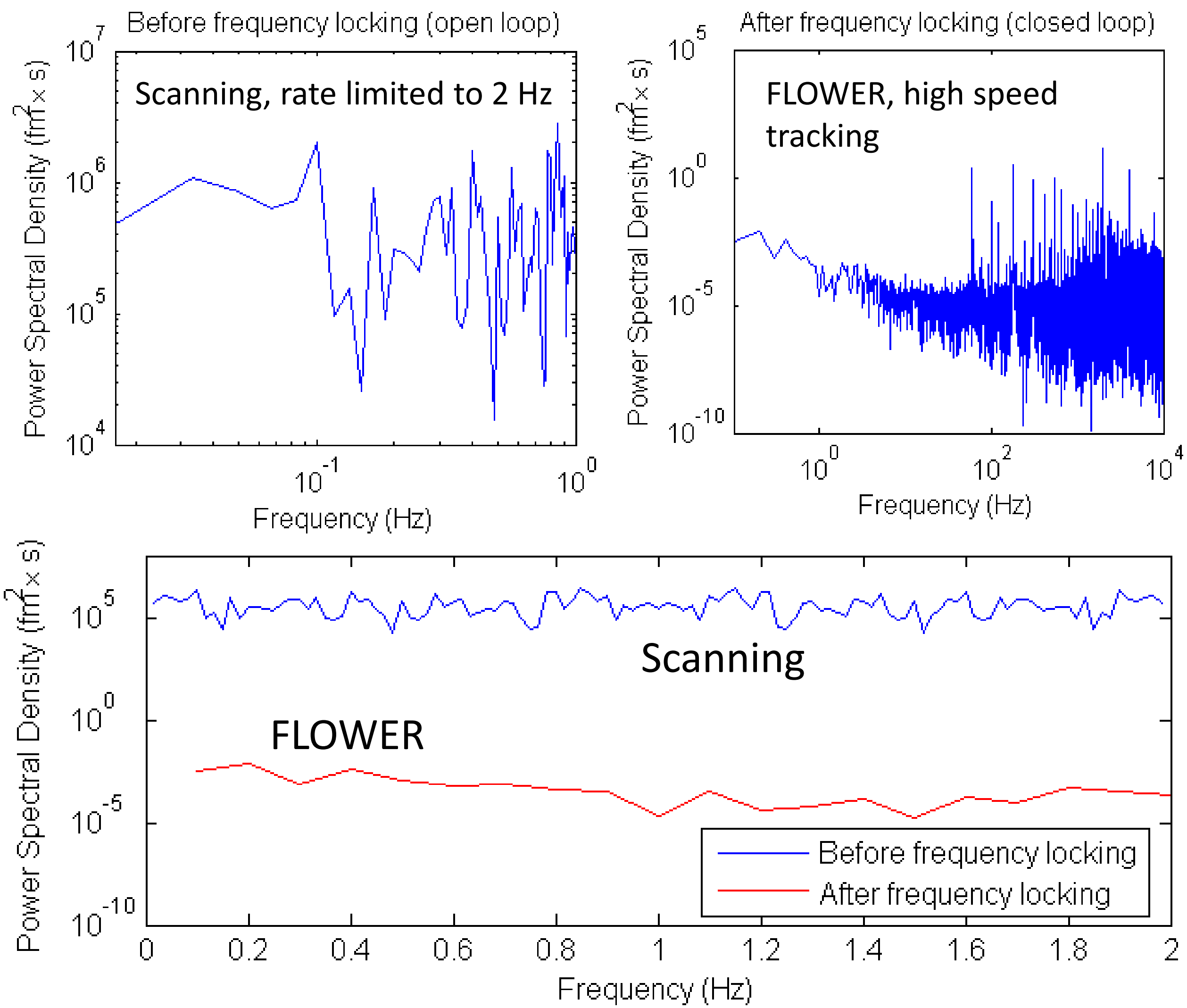
A low-pass filter is computationally applied to the data, as well as a 60 Hz notch filter.

Median filtering is performed



After Fourier filtering, a median filter is applied

Noise level determination



After frequency locking, the noise is significantly reduced

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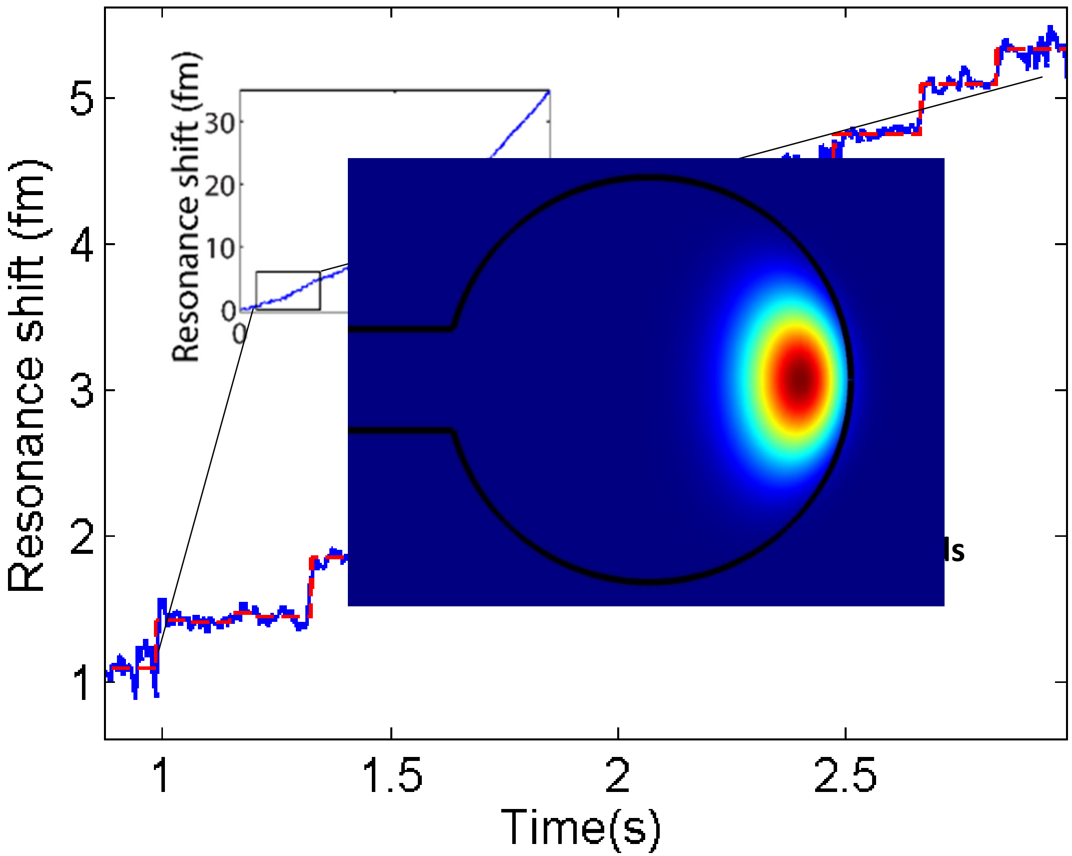
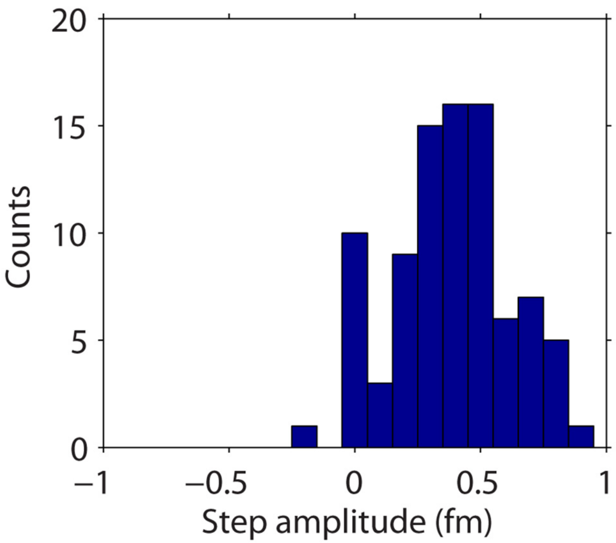
- Applications: Non-invasive tumor biopsy
- Conclusions

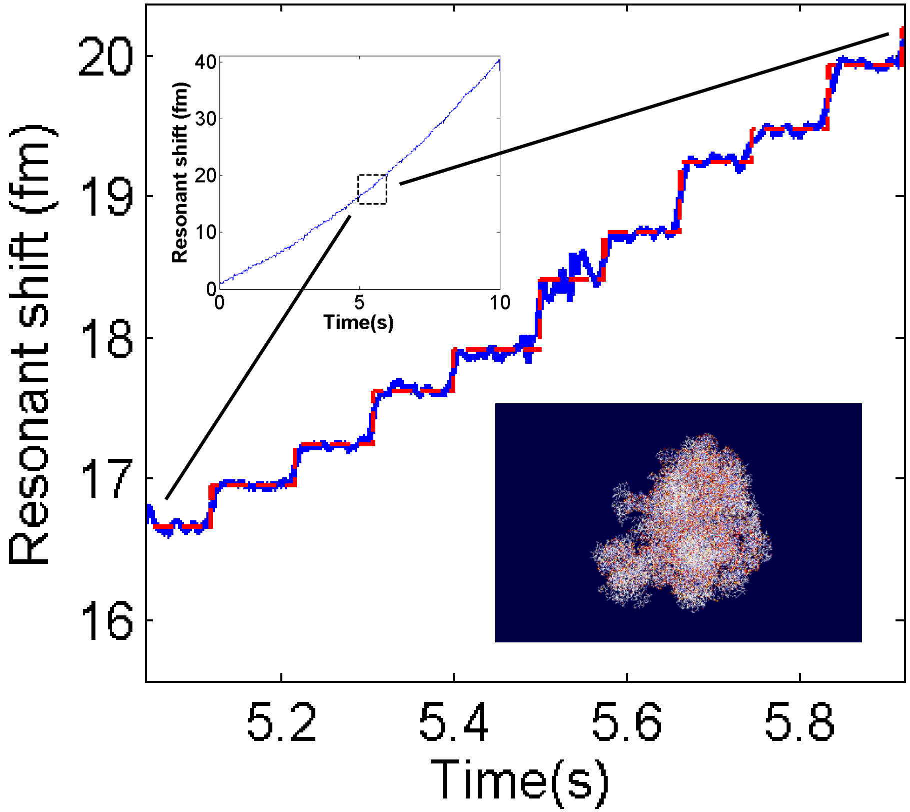
Experimental results

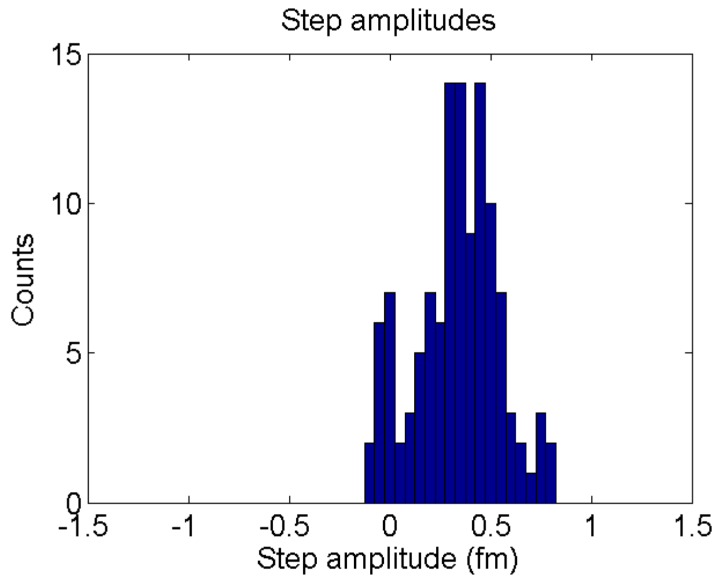


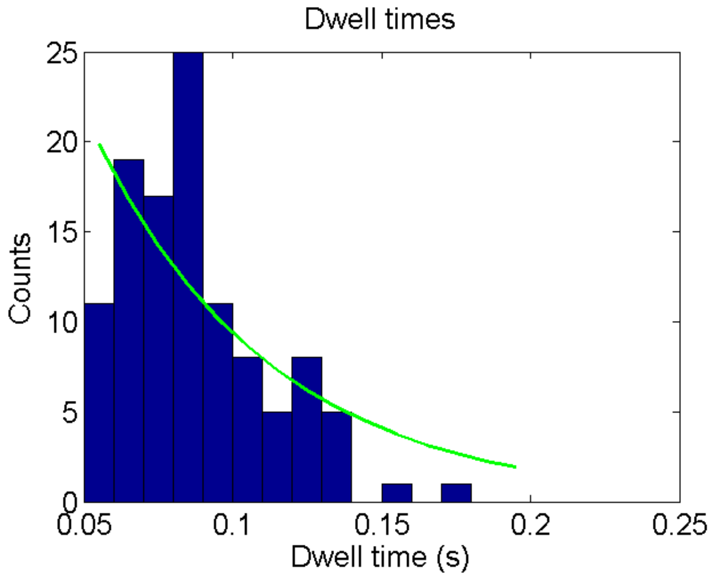


A maximum step amplitude of 0.9 fm corresponds to a RSP prediction of 10.5 nm.

Ribosome (r ~10-12.5 nm) detection

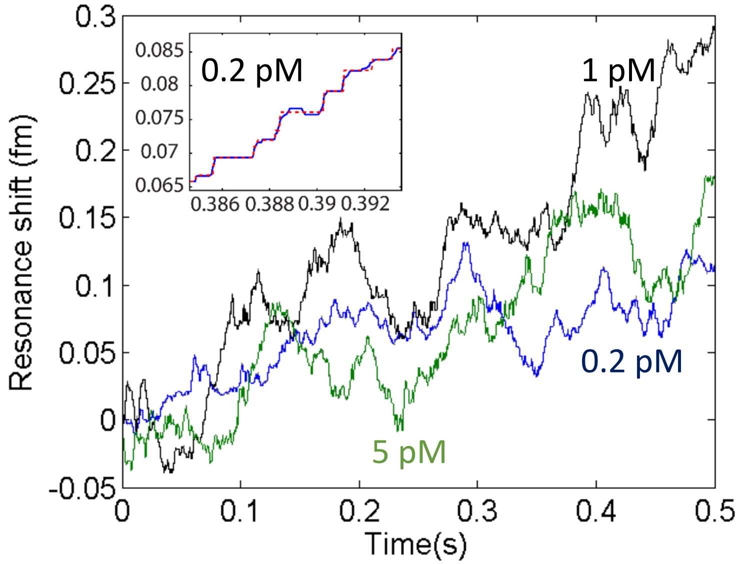


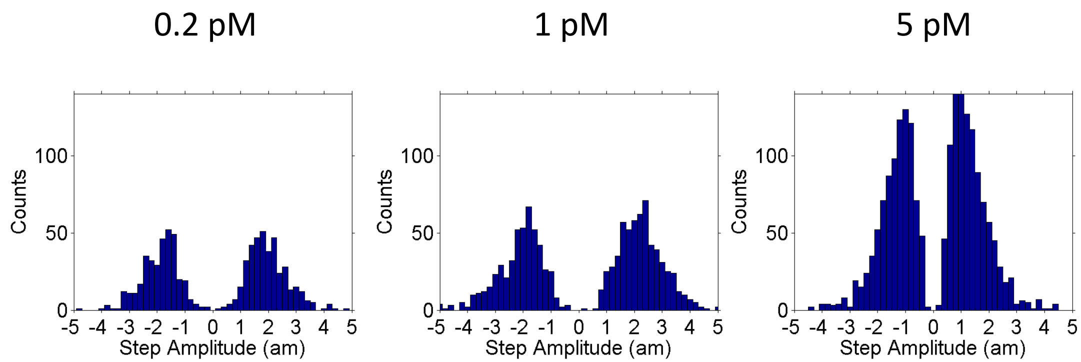




RSP prediction based on max step size = 12.6 nm

2.5 nm radius silica bead detection

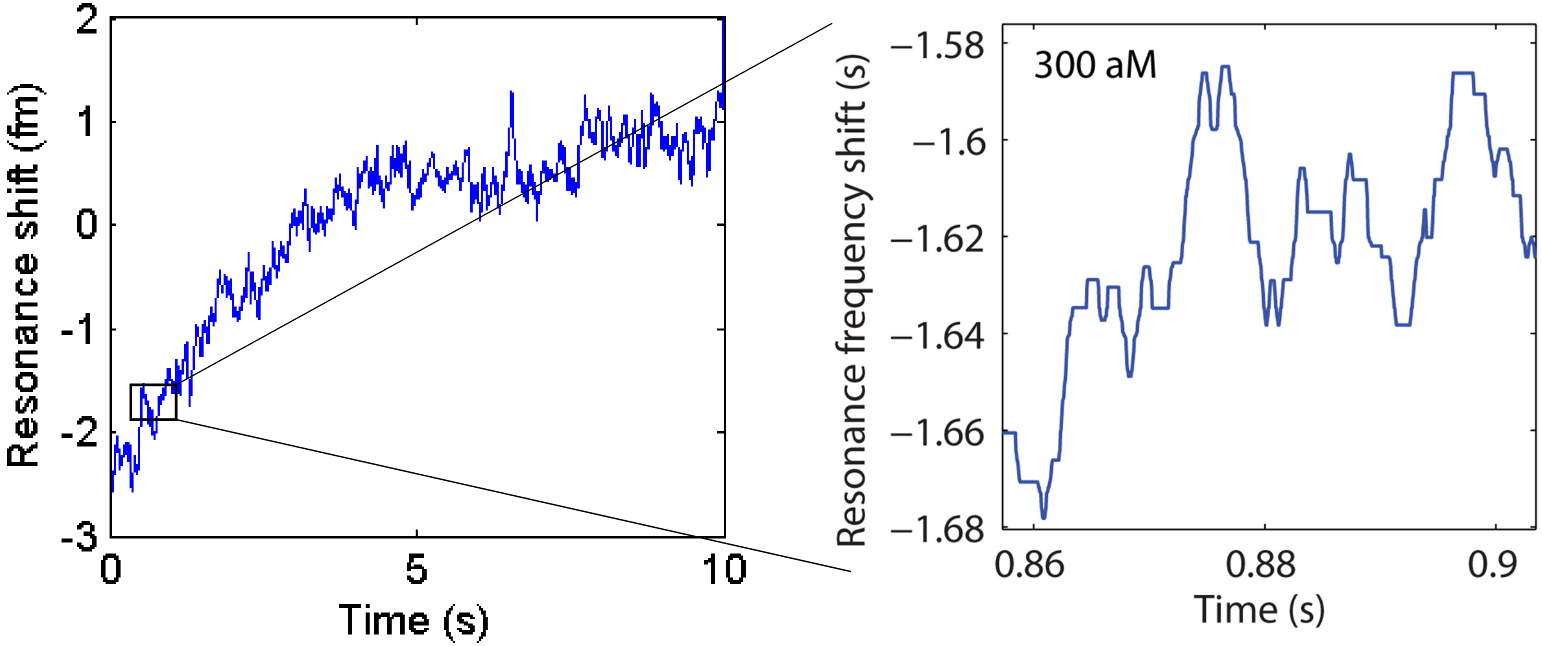




- Both up and down steps are observed
- Steps arrive more quickly than for larger particles

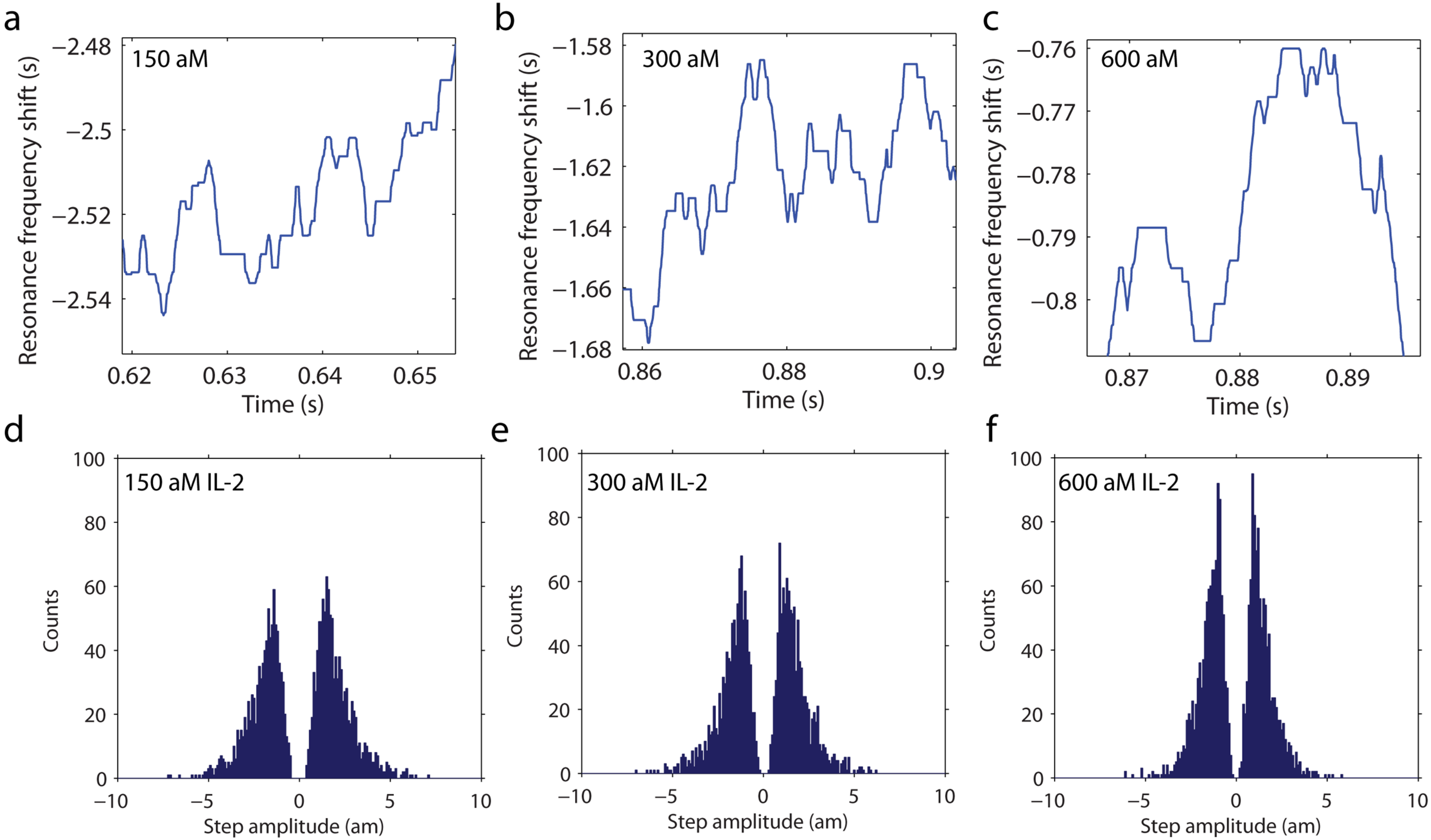
A maximum step amplitude of 5 am corresponds to a RSP prediction of 2.4 nm.

Human IL-2 (2 nm detection)



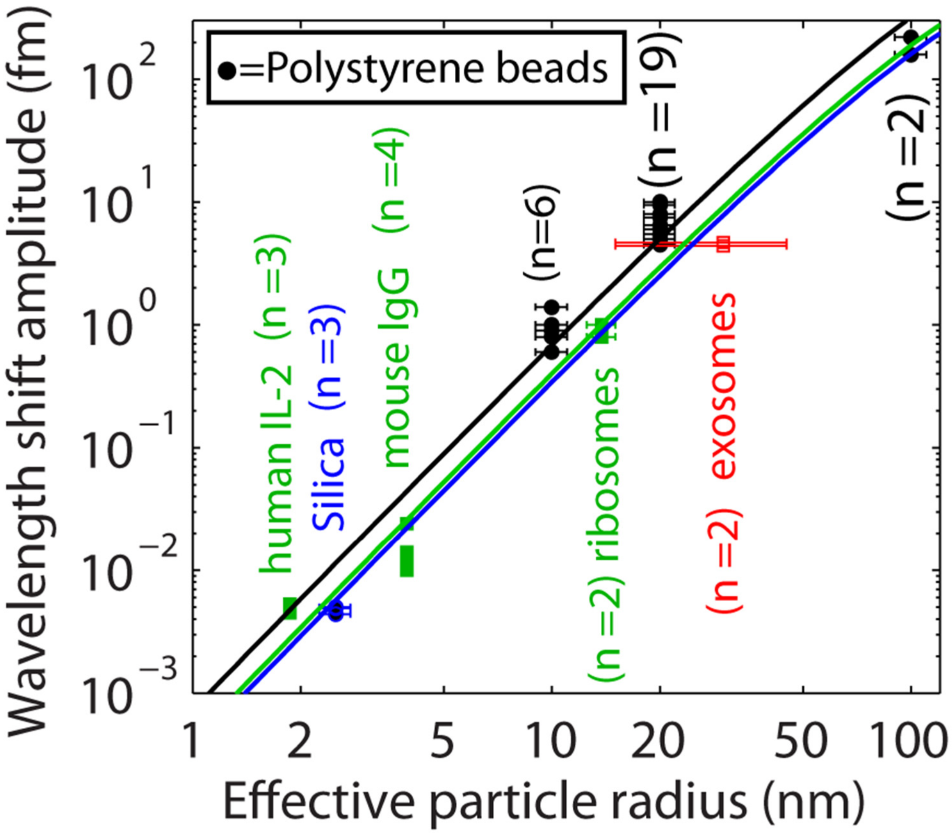
Zooming-in reveals a distribution of steps, whose maximum step amplitude corresponds well with that predicted by the RSP.

Human IL-2 (2 nm detection)



Human IL-2 histograms scale with concentration

Theory and Experiment

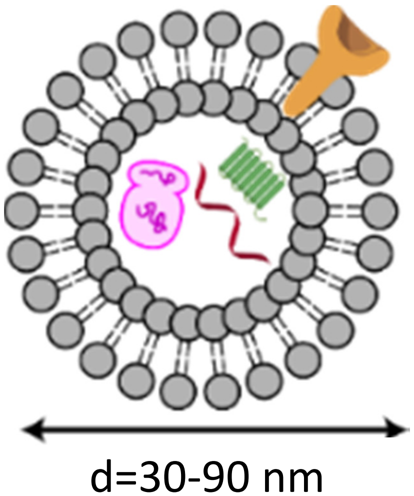


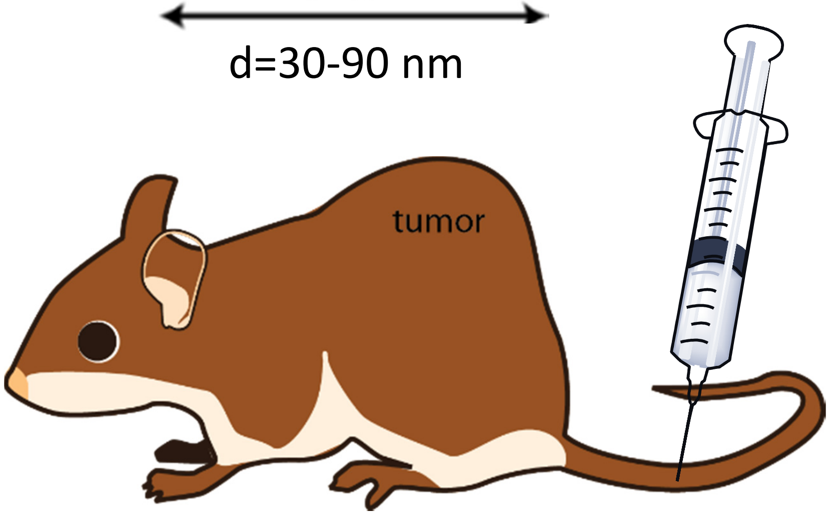
$$\left(\frac{\Delta\lambda}{\lambda}\right)_{\max} = \frac{D\,a^3}{2\,V_m}\,\frac{E_0^2(\boldsymbol{r}_e)}{E_{0,\max}^2}$$

$$D = 4\pi\, n_e^2\, \frac{n_p^2 - n_e^2}{n_p^2 + 2n_e^2}$$

Our results agree well for variously-sized particles with the RSP (solid line prediction).

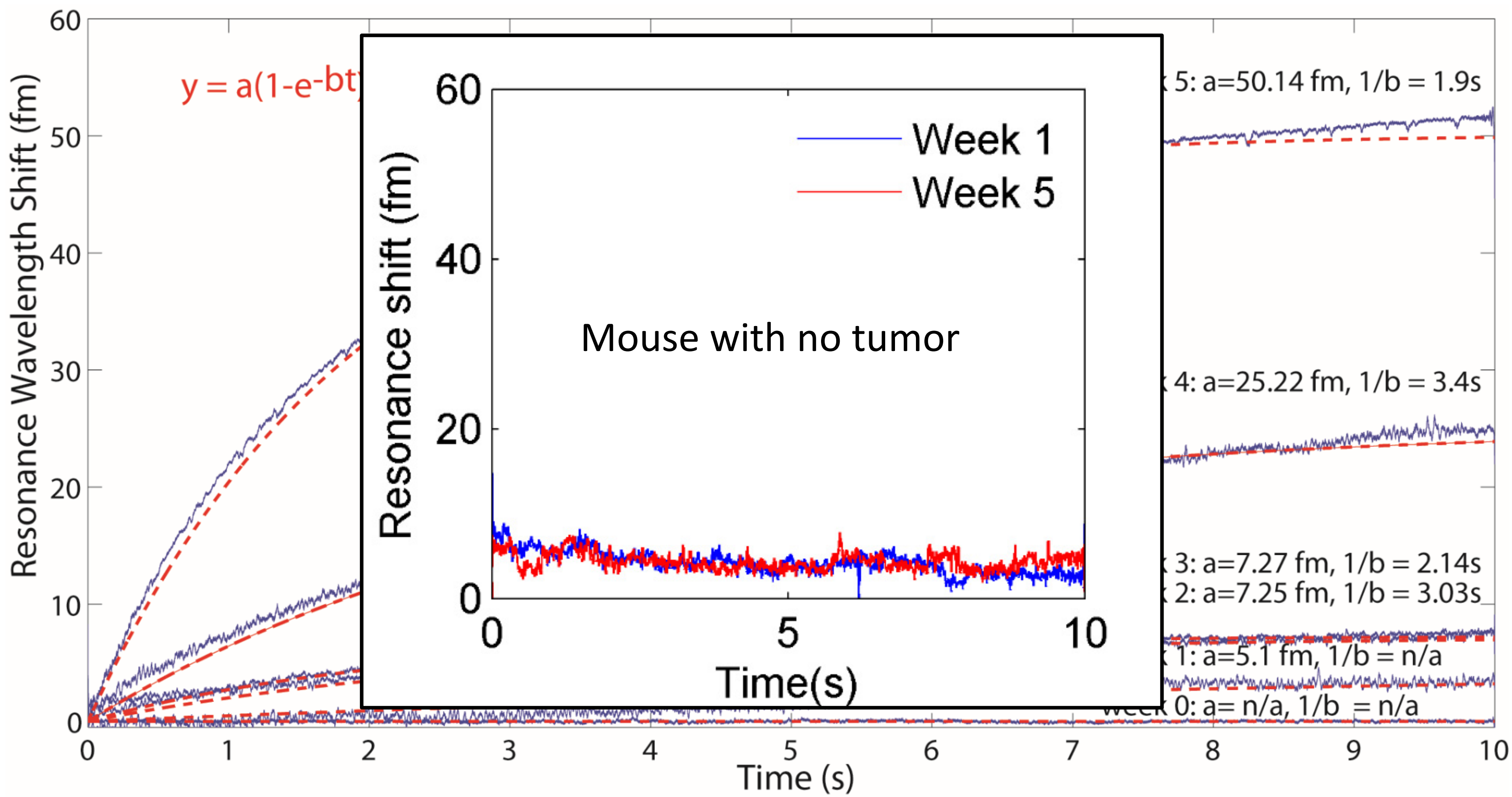
Application: minimally invasive tumor biopsy





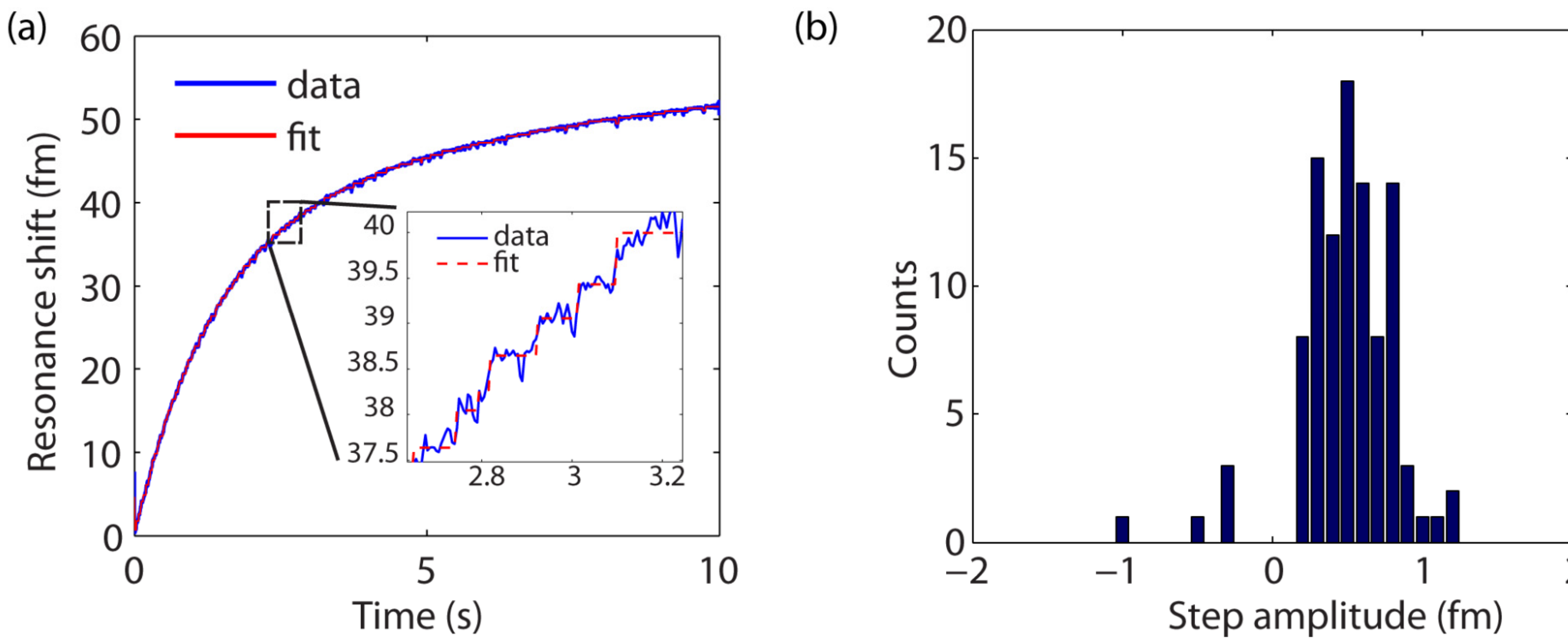
- Tumor cells secrete exosomes (small vesicles r~15-45 nm)
- These exosomes have cancer-specific antigens on them
- Quantifying exosome concentration allows one to detect tumor presence and growth without the need to find or access tumors.

Toroid wavelength shift increases as tumors grow



J. Su, *ACS Photonics* **2015** *2* (9), 1241-1245

Single exosome detection in serum ($1:10^6$ dilution in saline)



Maximum step amplitude size of 1.2 fm corresponds to a size of r~20 nm which corresponds with an independent measurement using field flow fractionation.

Conclusions

- Improved **SNR > 1000x** using frequency locking in combination with balanced detection (FLOWER).
- Demonstrated detection over a large range (**2.5-100 nm radius**) of particle sizes using beads
- Applications:
  (1) Demonstrated detection of **individual yeast ribosomes**
  (2) Developed a basis for a **non-invasive tumor biopsy system** by detecting human **exosomes** in serum and showing that their concentration scales with tumor progression
- Step amplitudes observed in human IL-2 and IgG solutions are consistent with the RSP prediction for **single-molecule detection**

Where do we go from here?

- Water safety
- Food safety
- Environmental monitoring
- Performance enhancing drug detection
- Early detection of disease
- Drug design
- Understanding basic biological functions
- Portable diagnostics

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