

2024 Human Performance and Biosystems Program Review

Dr. Patrick Bradshaw | October 22-24, 2024 | Arlington, VA

FA9550-23-1-0282 (08/2023 – 08/2027)

Precision Nanosurgery and Multimodal Intracellular Monitoring of Cellular Networks by Biomimic Nano-optoelectrode Arrays

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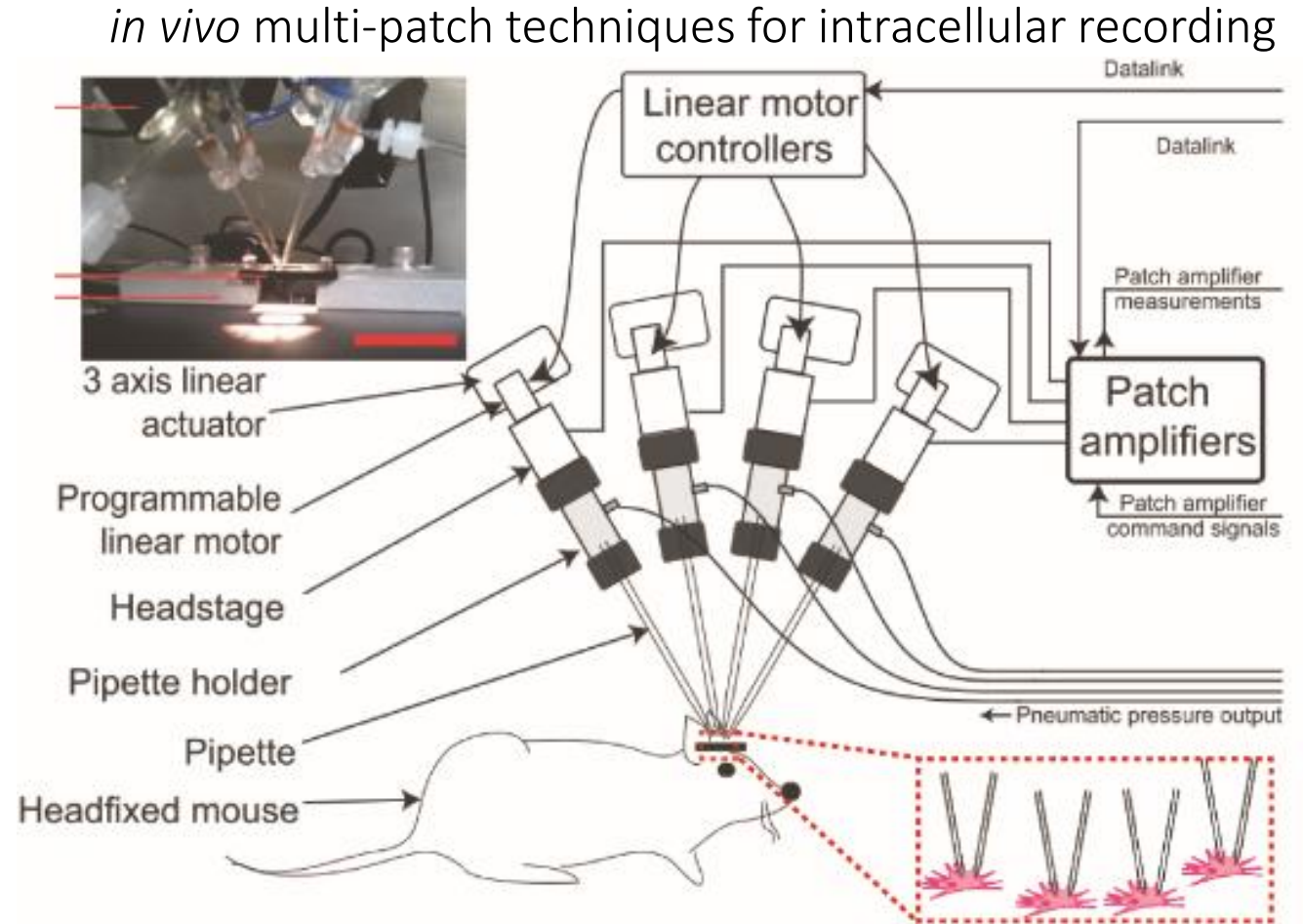


Gap: *in vivo*, Scalable, Intracellular, Multimodal Bioinformation Conversion is Challenging

Multi-patch intracellular recording can provide *in vivo* data to link subthreshold synaptic physiology, cell identity, and circuit functions.

Gaps:

- (i) Invasiveness and poor chronic stability
- (ii) Difficult to scale up over 4 intracellular recording channels *in vivo*
- (iii) Limited to electrical recording modality



Kodandaramaish, S.B. et al. Elife 7 (2018).

Nanopillar Nanoelectrode: Biomimetic Nanotopology Solution

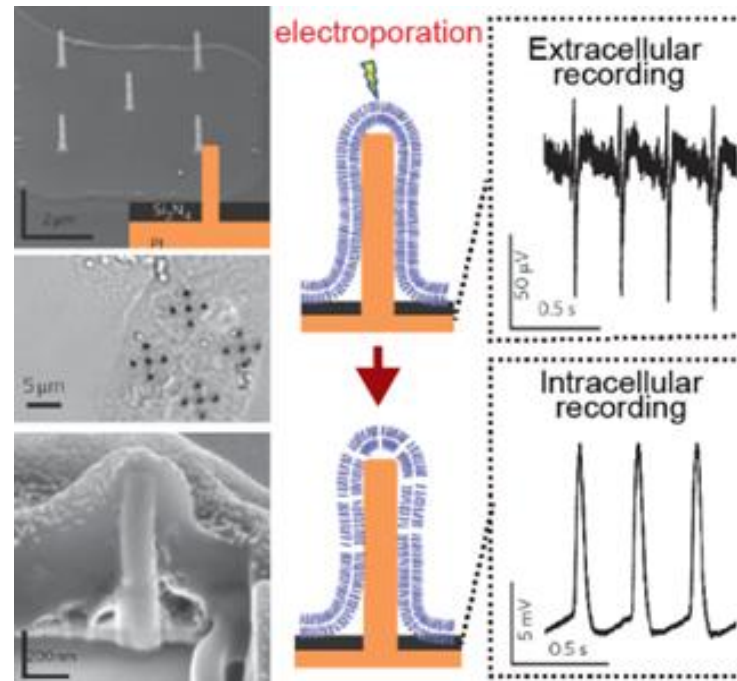
Nanopillar nanoelectrode:

- Reduced interfacial impedance for high S/N recording,
- Nanopillar topology to induce cell engulfment for high sealing resistance and effective electroporation.

Gaps:

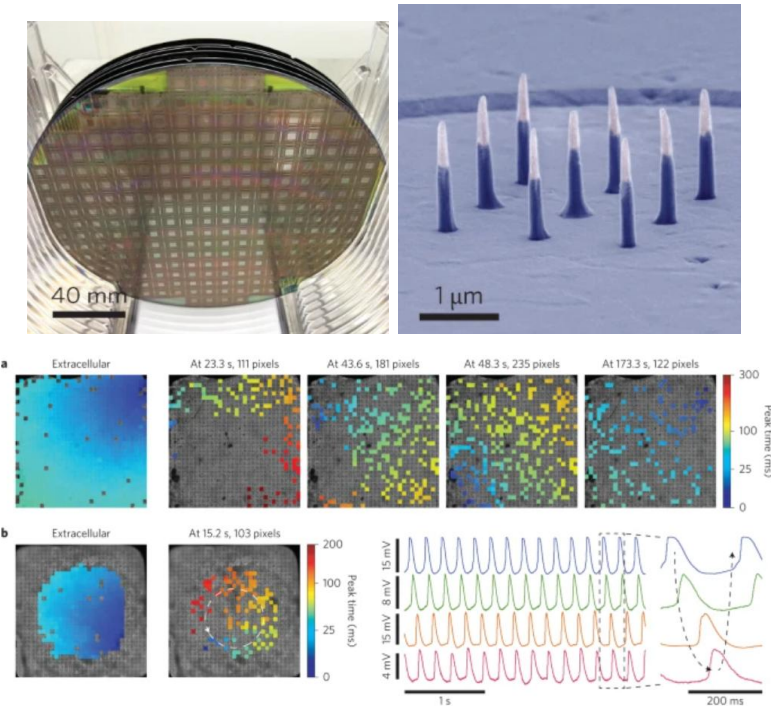
- (i) Still limited to *in vitro* systems.
- (ii) Pulsed volumetric ionic currents lead to invasiveness to 3D cell populations for *in vivo* electroporation
- (iii) Limited to electrical modality

in vitro intracellular recording
by nanopillar nanoelectrodes



Xie, C., Cui, B.X. et.al. Nat Nanotechnol 7 (2012).

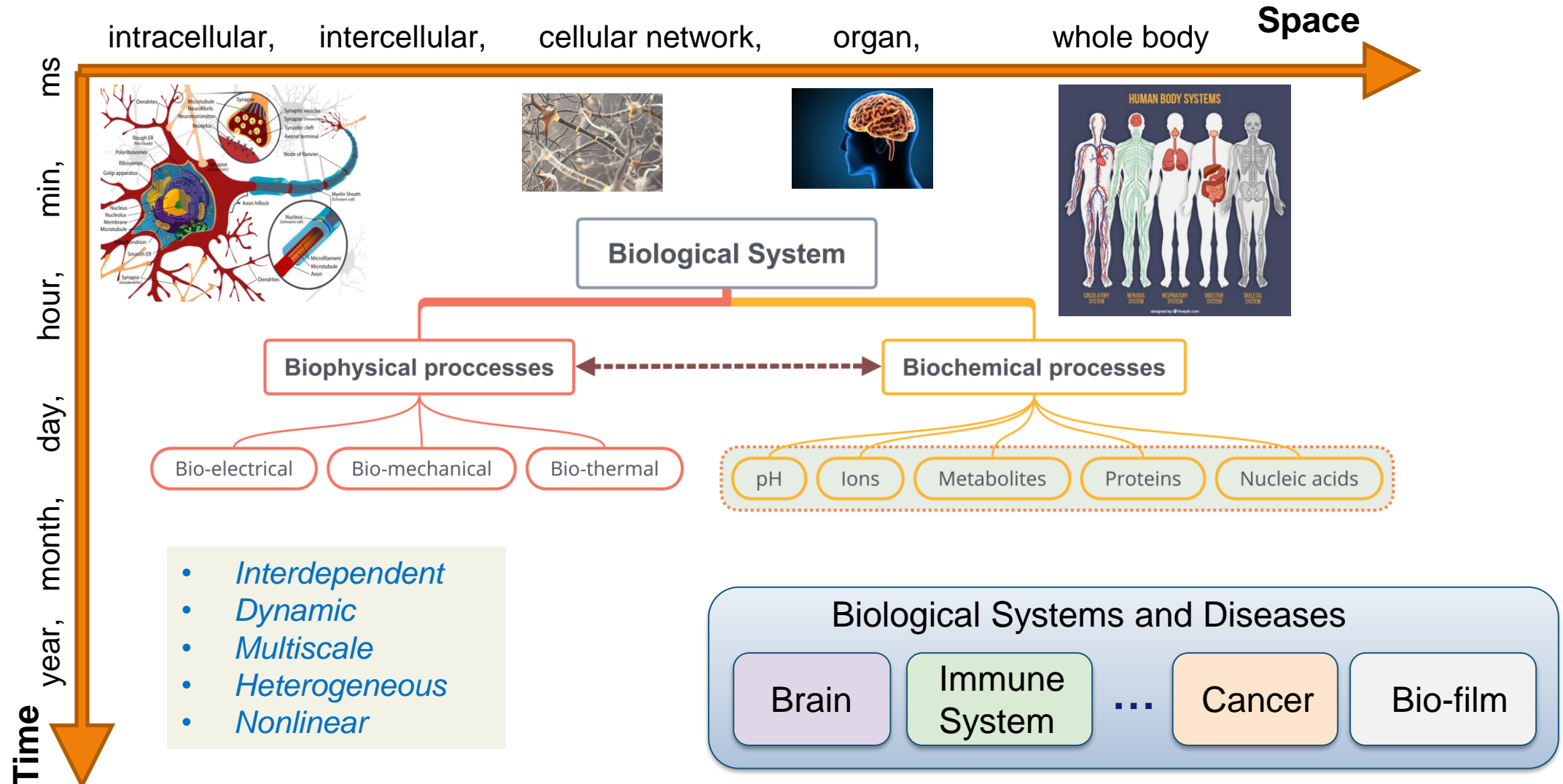
in vitro intracellular imaging by
nanopillar nanoelectrode arrays



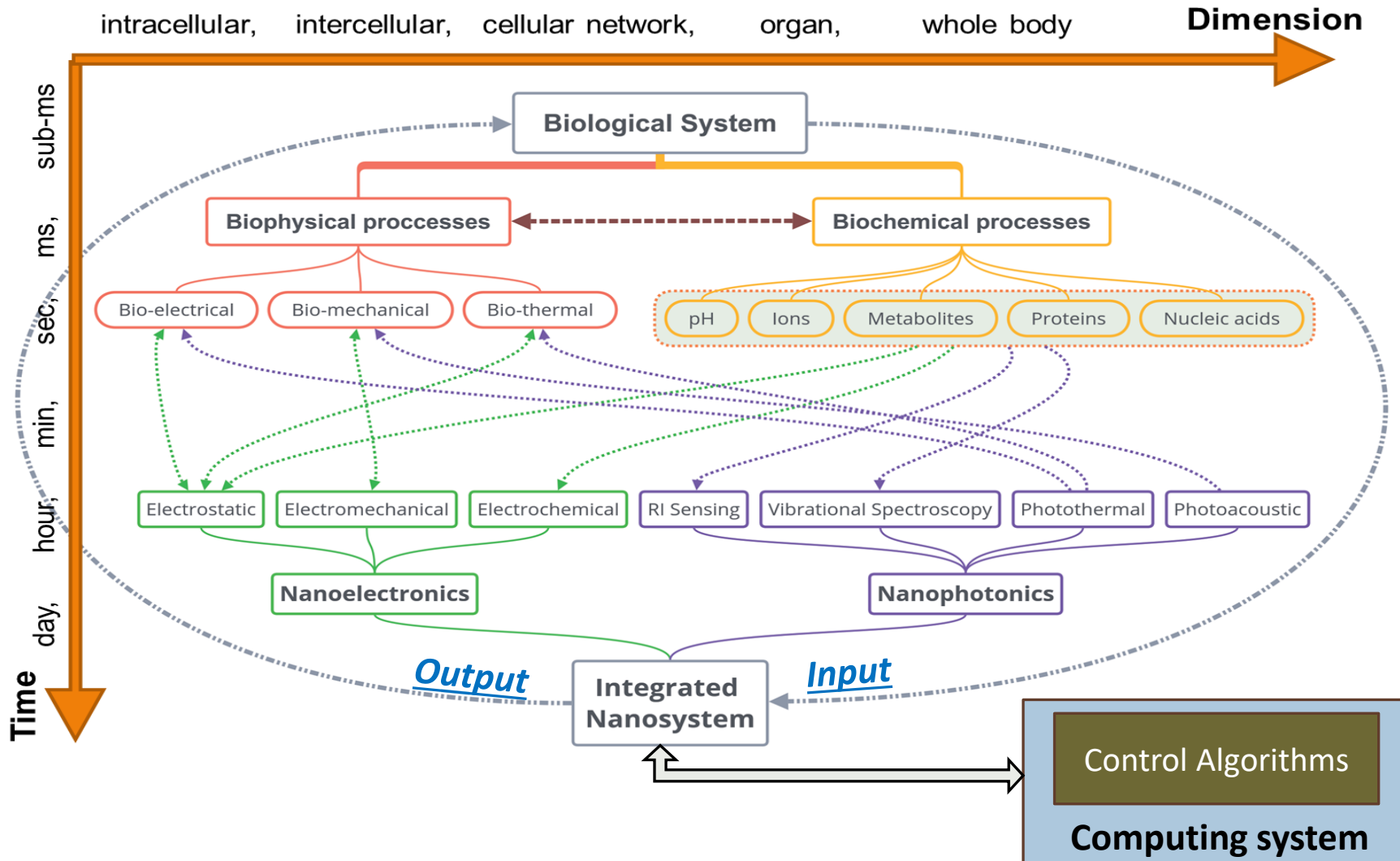
Abbott, J., Park, H. et.al. Nat Nanotechnol 12 (2017).

Shen, C. et al. Translational opportunities and challenges of invasive electrodes for neural interfaces. Nature Biomedical Engineering 7 (2023)

How to Realize Multiscale, Multimodal, Spatiotemporal Information Conversion at Bio-Machine Interface?



The Vision: Scalable, Multifunctional Bio-Interfaced Nanosystem



Aim 1: Nanodevice-level multifunctionality:

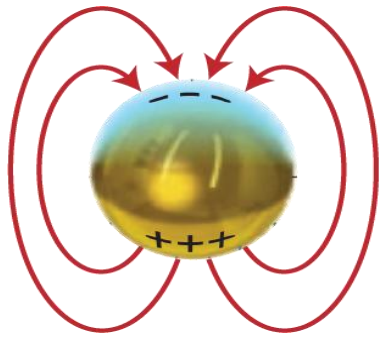
- Fuse electronics and photonics at nanoscale
- Allow multi-scale multi-physics sensing and actuation operations at nanoscale

Aim2: System-level scalability and versatility:

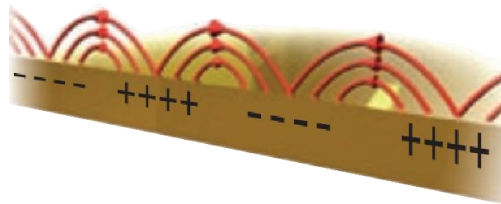
- High-throughput top-down nanofab process
- Flexible, hierarchical micro-nano architecture
- Compact instrumentation

Plasmonic Nanophotonics Modalities at Bio-Interface

Plasmonic Nanocavity



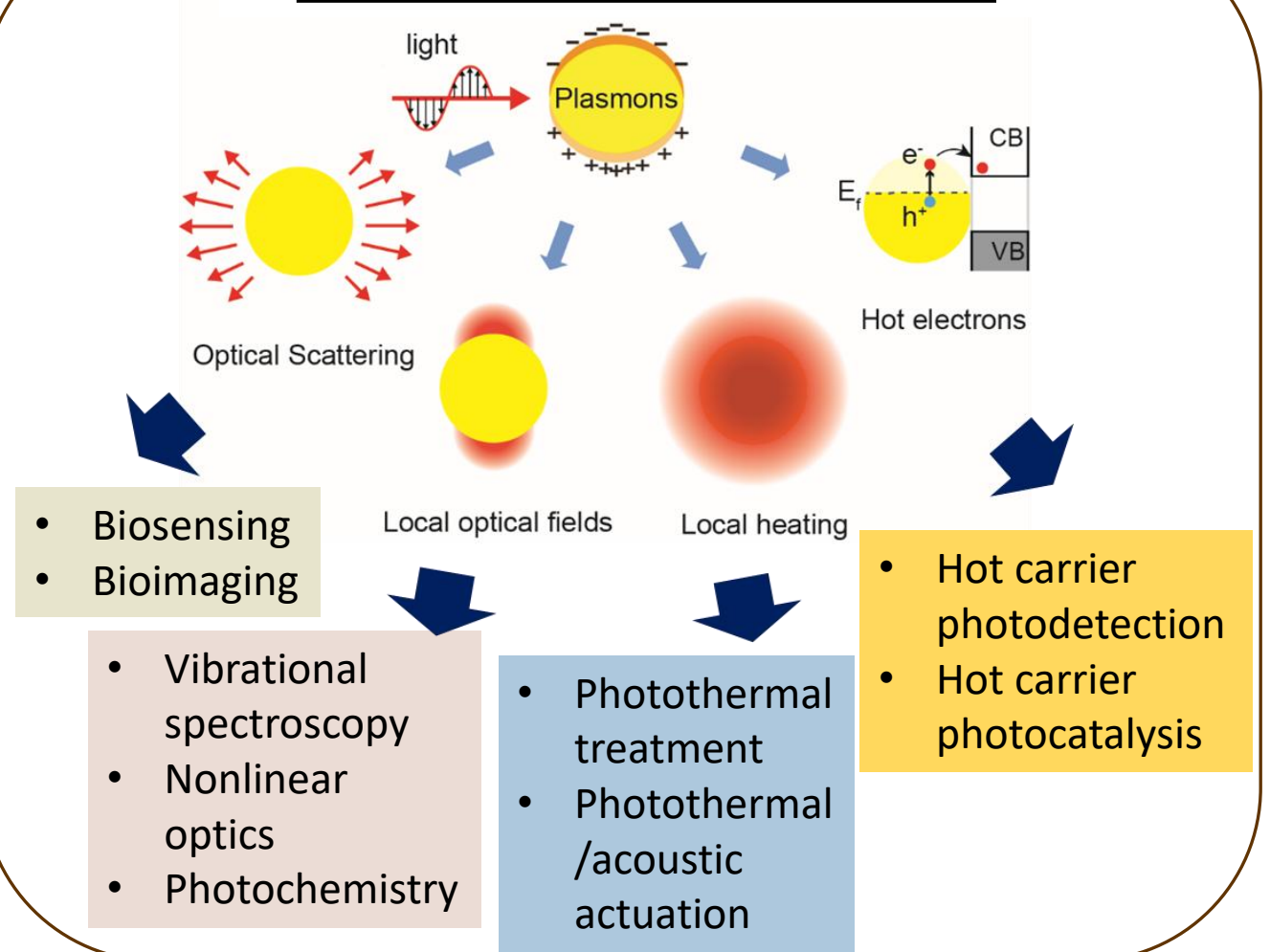
Localized Surface Plasmons (LSPs)



Surface Plasmon Polaritons (SPPs)

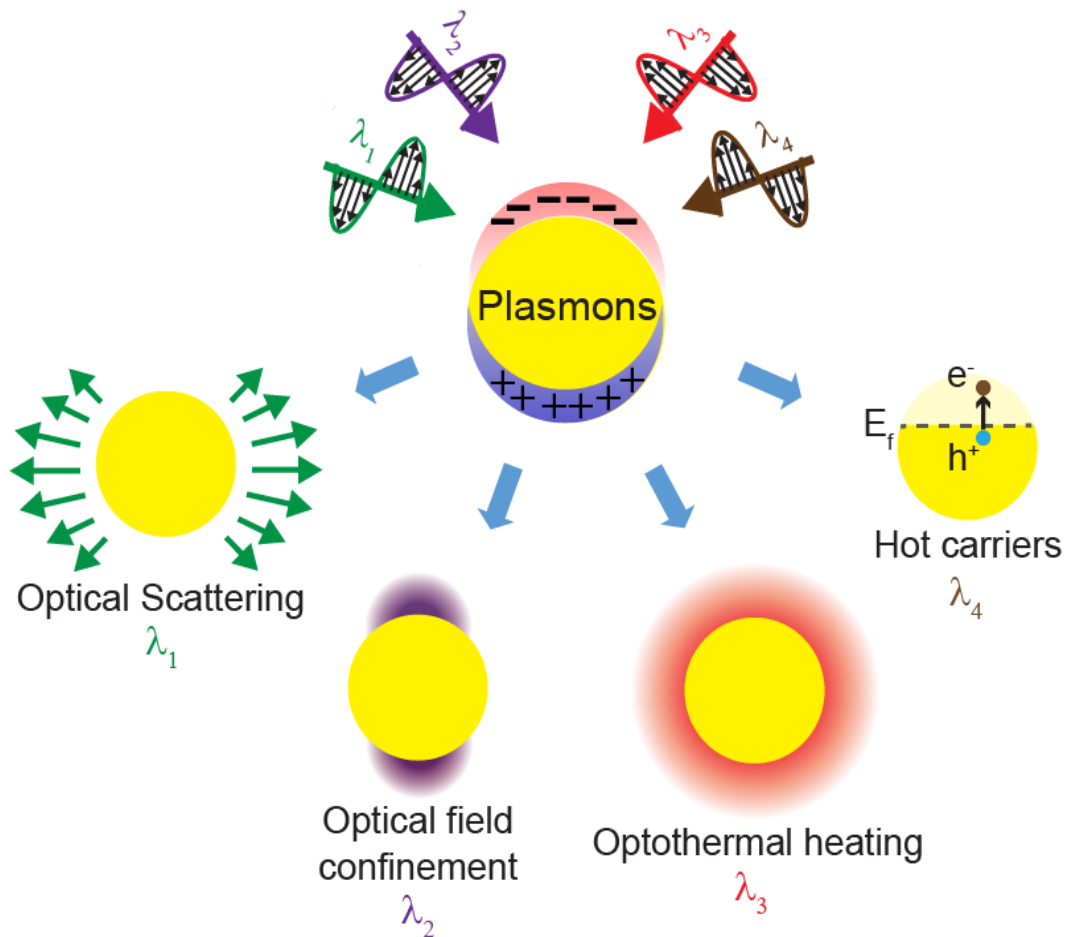
- ❑ Enhance nanoscale light-matter interaction
- ❑ Conventional plasmonics
 - ❑ Single-resonant operations
 - ❑ Individual functions

Plasmonic Effects at Nanoscale

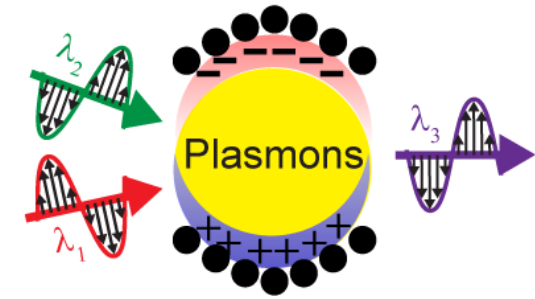
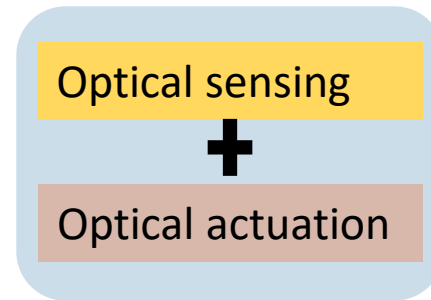


Our Innovations: Broadband Multiresonant Plasmonics

Wavelength-Multiplexed Multifunctionality

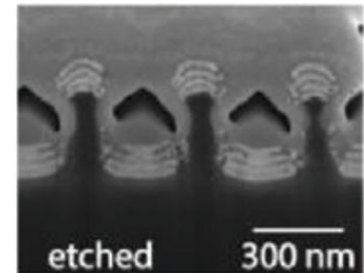


Multi-Photon Nonlinear Nano-optics



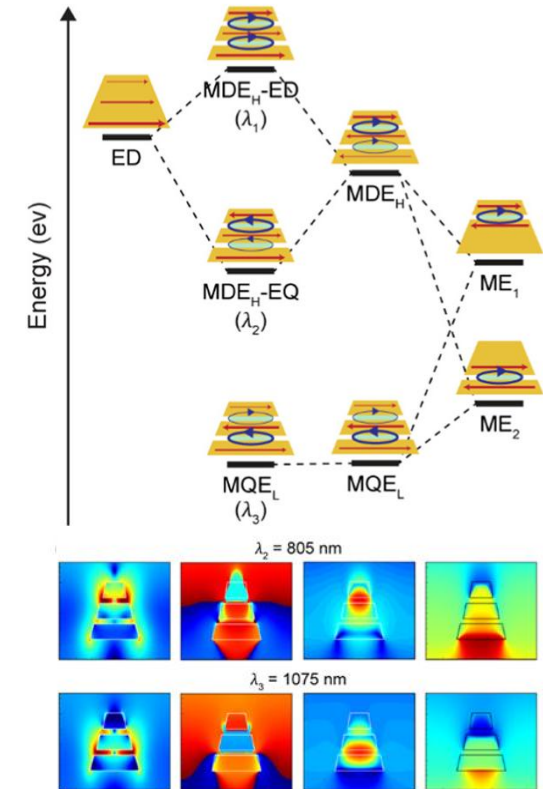
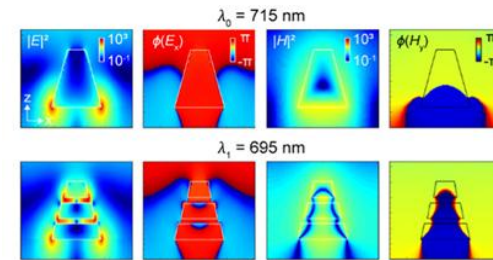
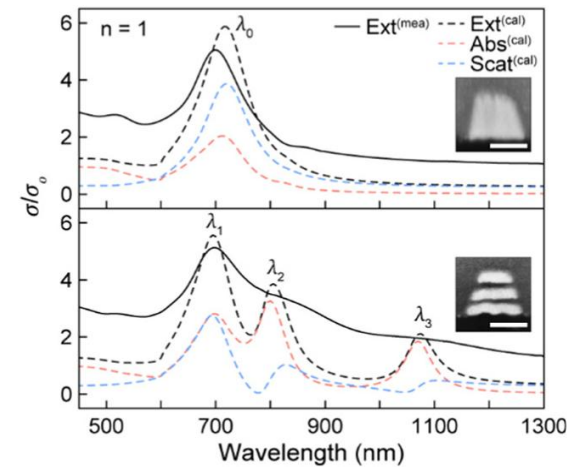
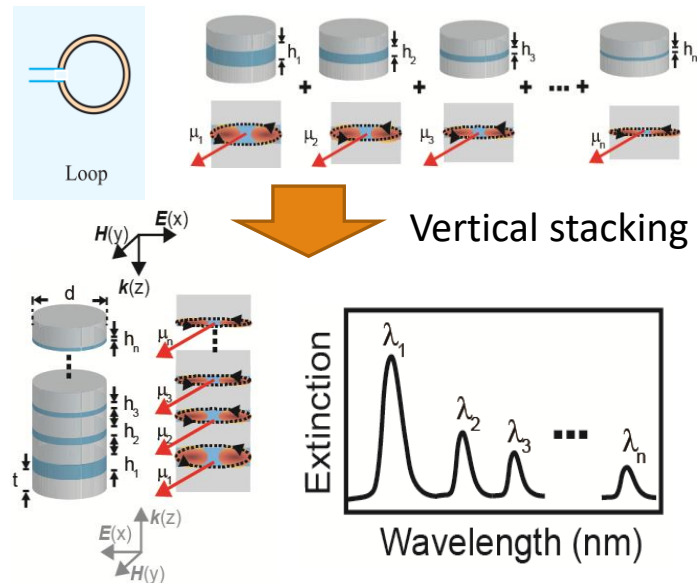
- *Nanoscale **multiresonant** design for wavelength-multiplexed or nonlinear multi-photon operations.*
- ***Co-design** together with nanoelectronics, biomimetic topology, and biomaterials.*

Multiresonant Nanopillar Nano-Optoelectrodes



Nanolaminate Multiresonant Plasmonic Mode Engineering

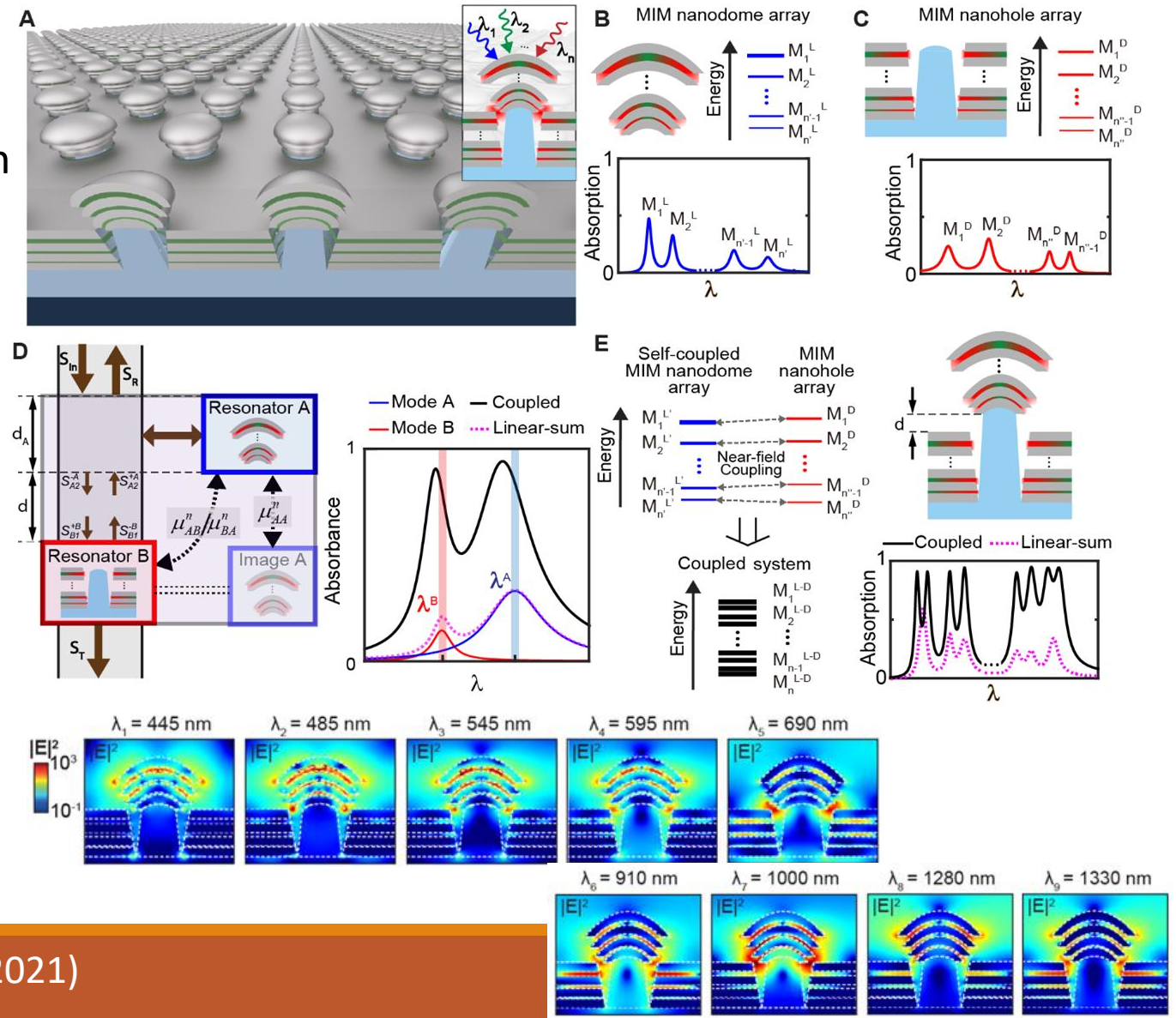
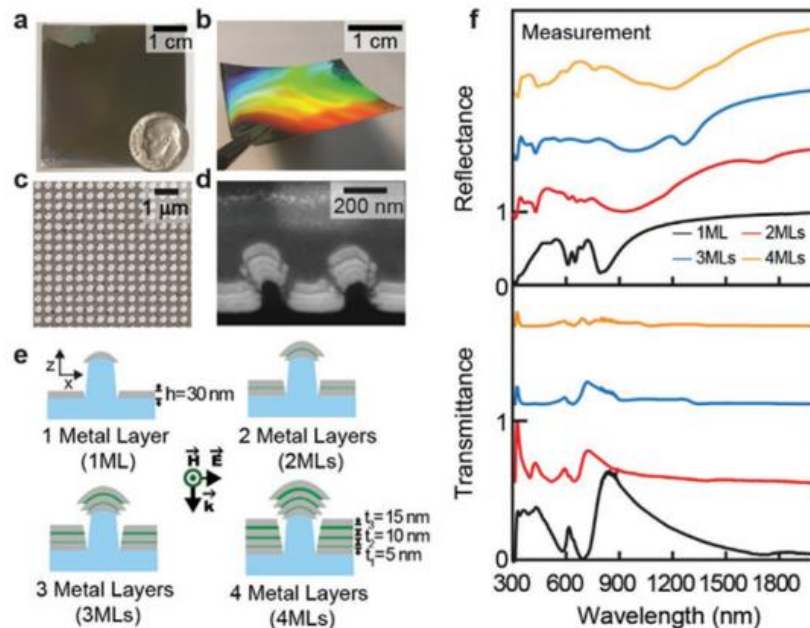
- Metal-insulator-metal nanocavity supports magnetic dipole-based localized surface plasmon mode.
- Multiresonant response via magnetic inductive coupling-assisted mode hybridization.



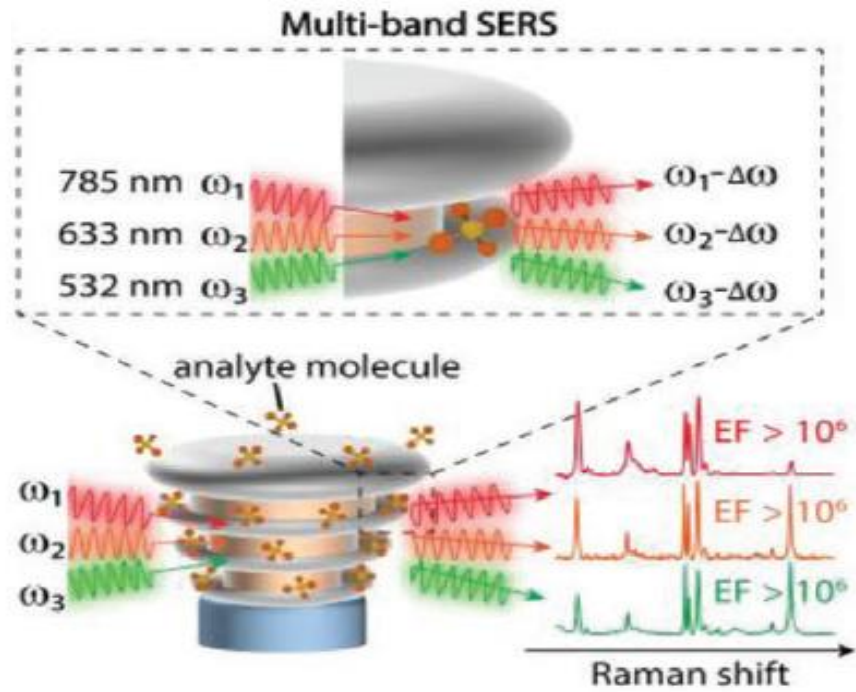
- Nanocavity geometry with broken symmetry can allow efficient excitation of multiple multipolar plasmon resonances with high spatial mode overlap.

Nanolaminate Multiresonant Plasmonic Mode Engineering

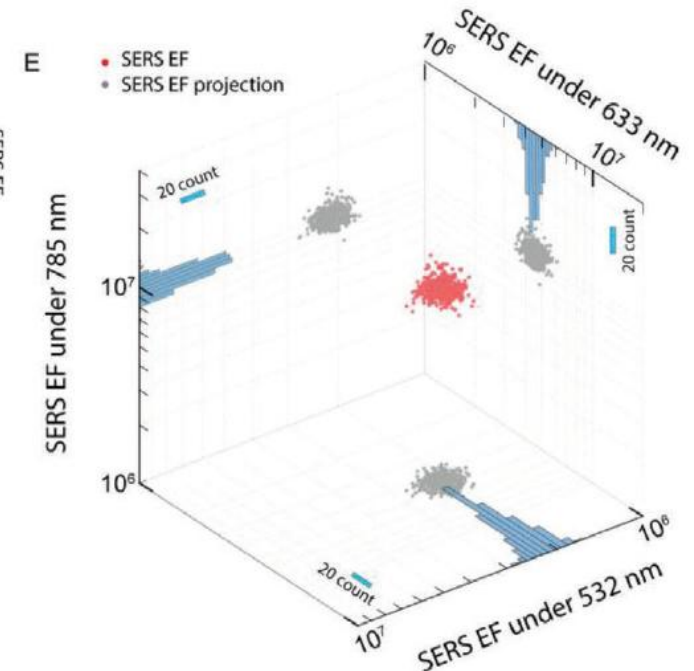
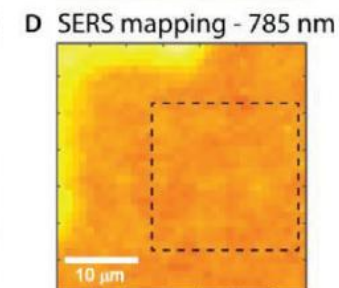
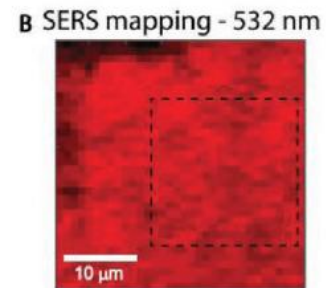
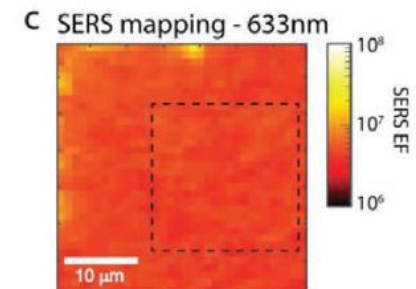
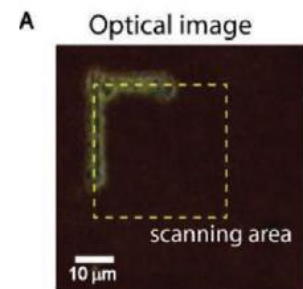
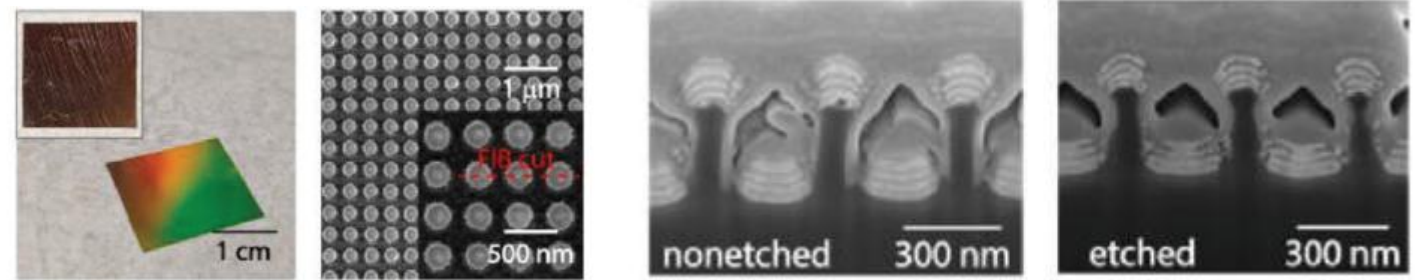
- 2-tier mode hybridization process between localized and delocalized modes
- Support ~ 10 multipolar plasmonic modes with spatial mode overlaps and high excitability across broadband (400 – 1500 nm)



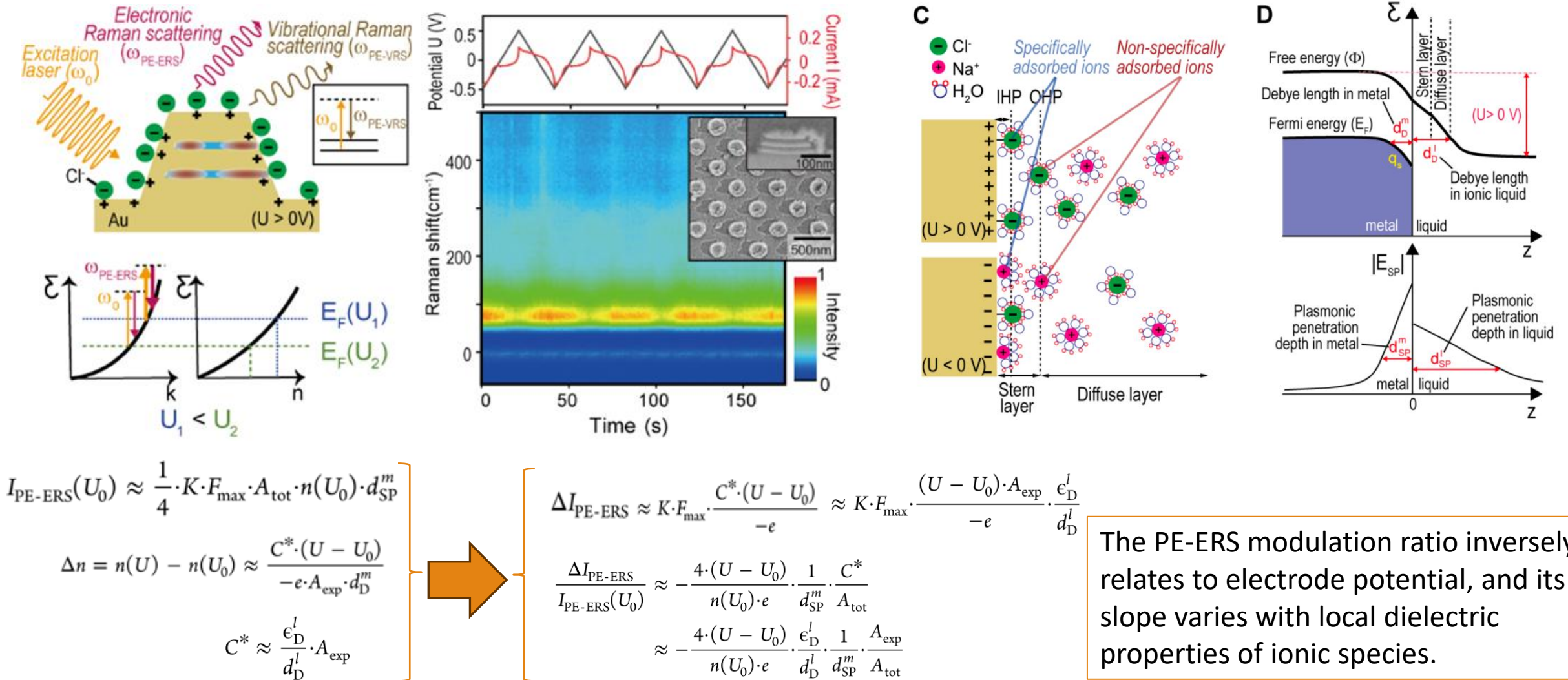
Multiband Plasmon-enhanced Vibrational Raman Scattering



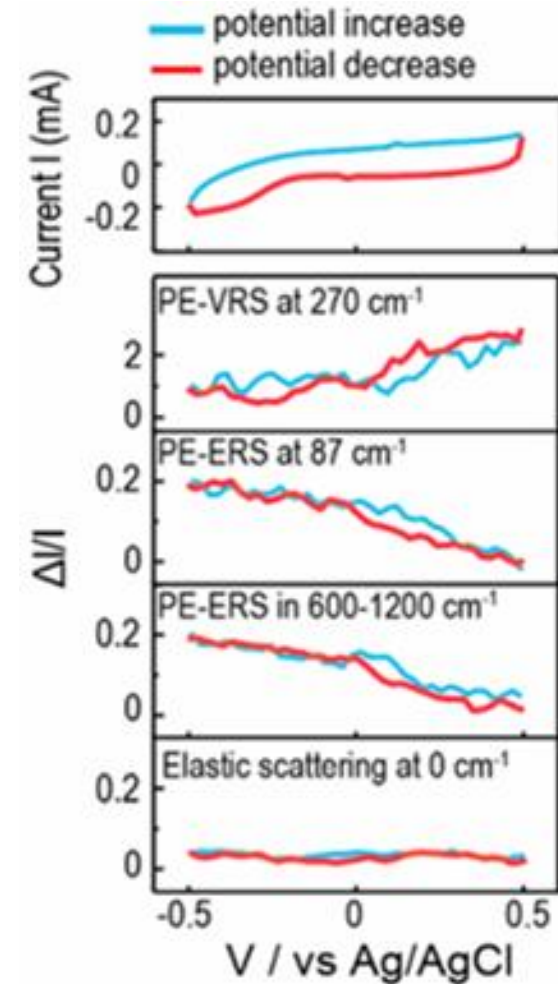
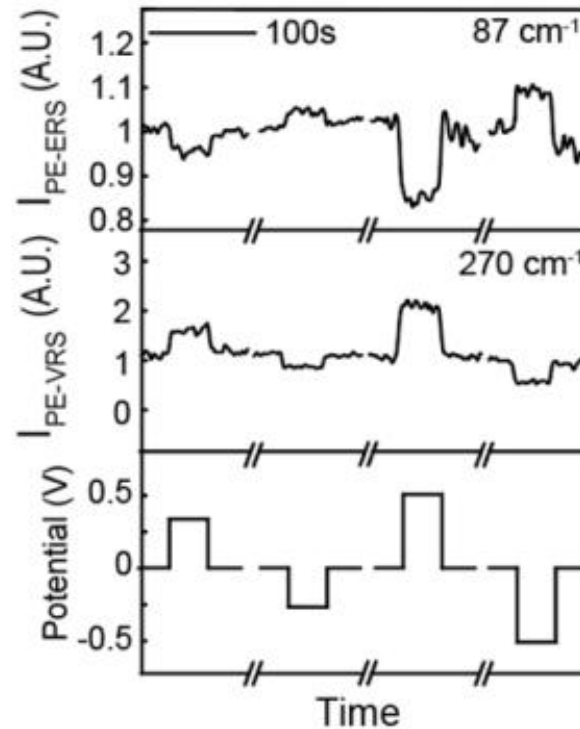
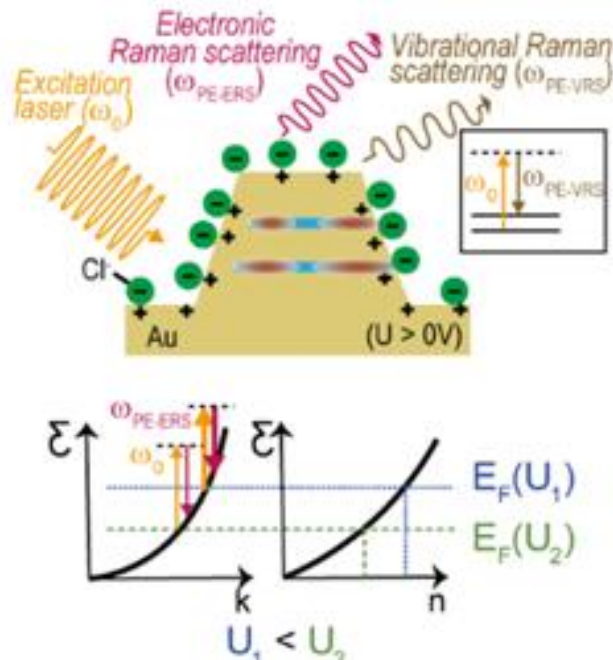
Selectively etching of dielectric layer in MIM nanocavity demonstrates multiband Raman enhancement under different laser excitation wavelengths.



Electrically Connected Multiresonant Nanocavities: Voltage-Sensitive Plasmon-enhanced Electronic Raman Scattering

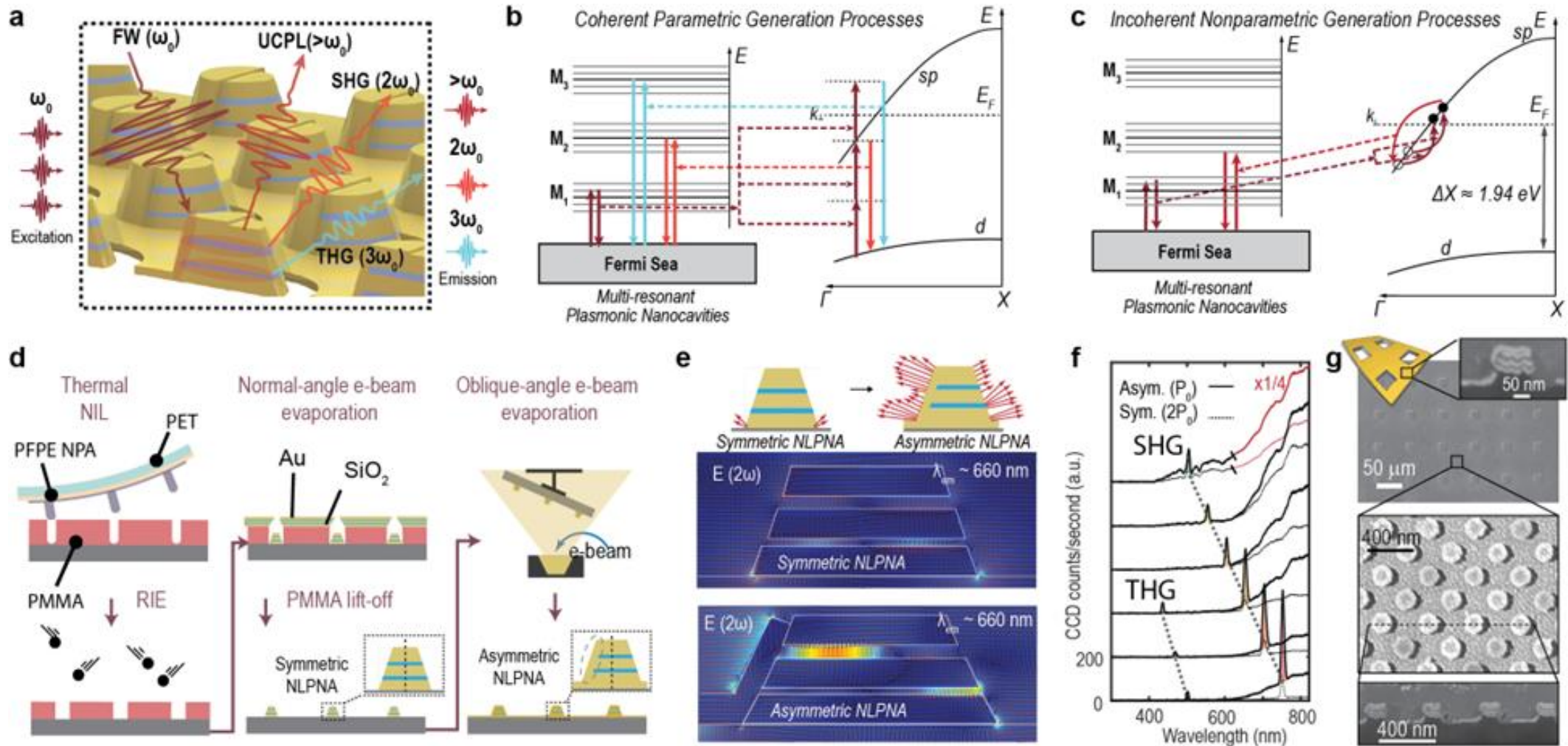


Dynamic Response and Implications for Voltage Sensing

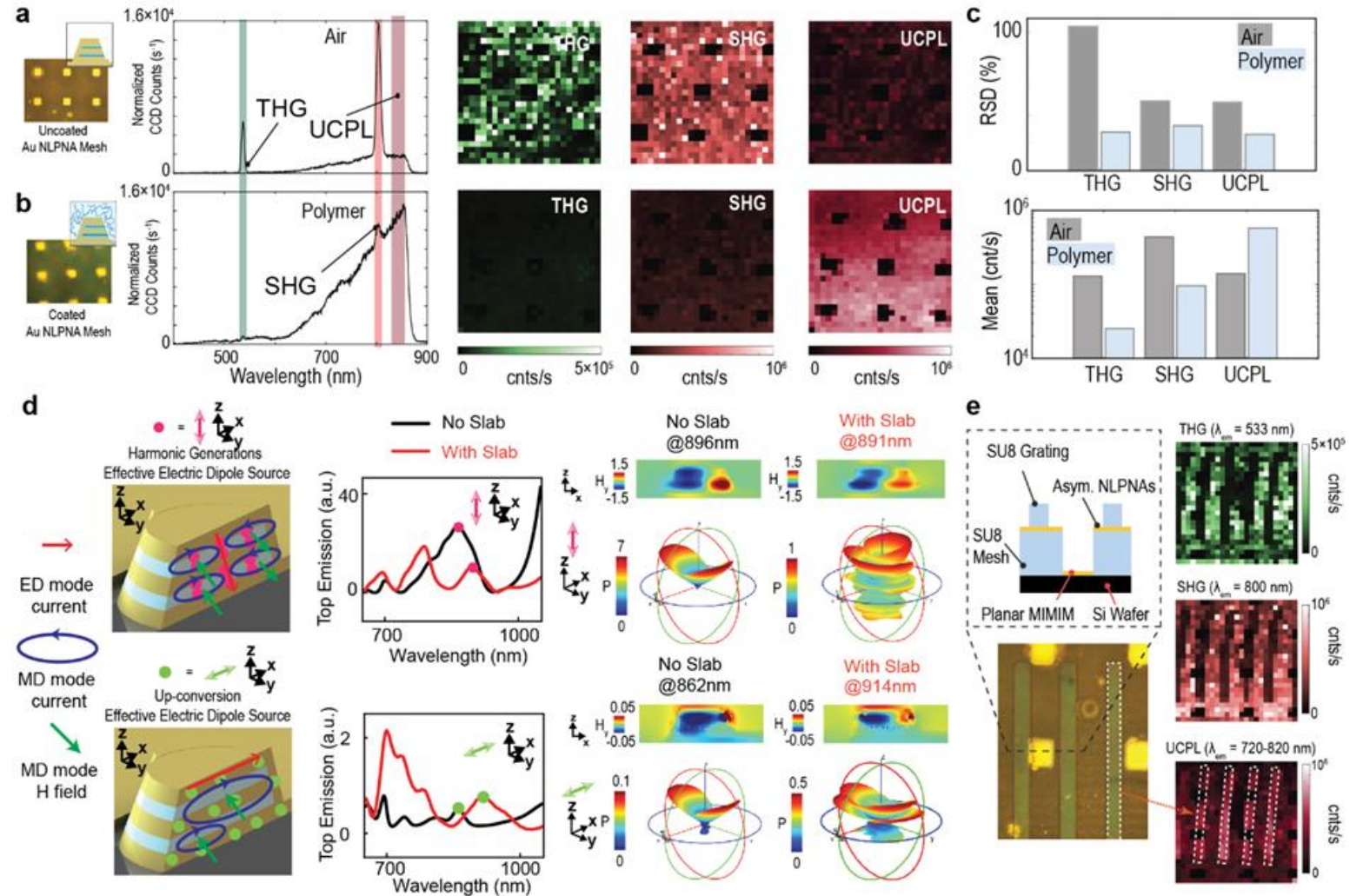
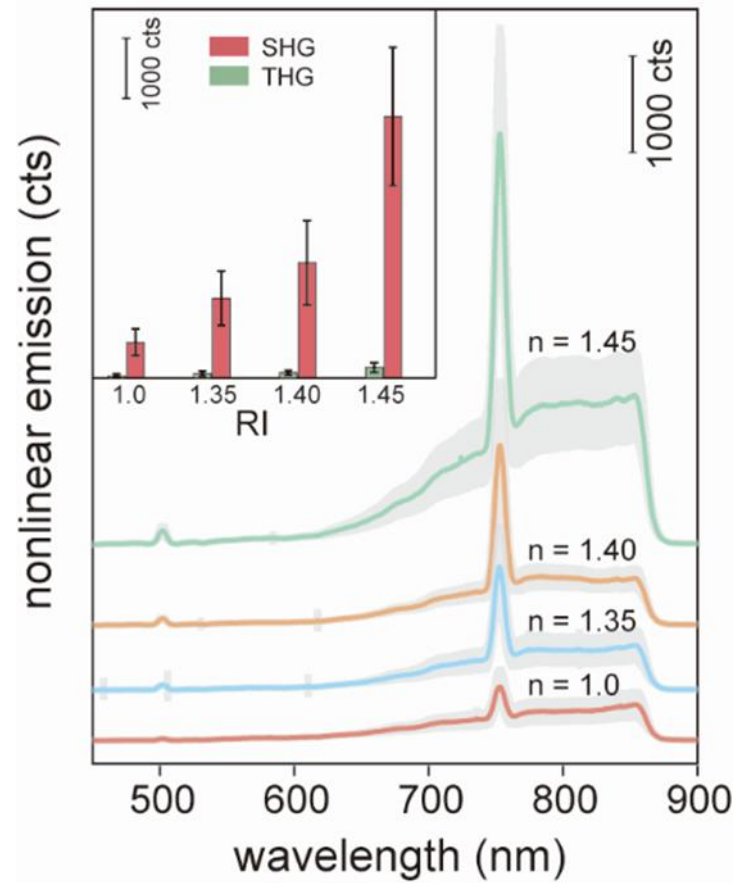


- Substantial 30%/V voltage-modulation of PE-ERS
- Label-free, NIR operation for voltage sensing

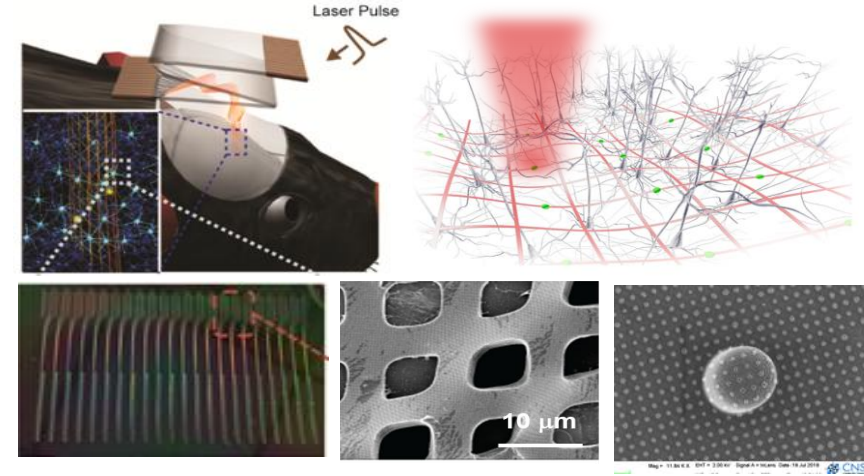
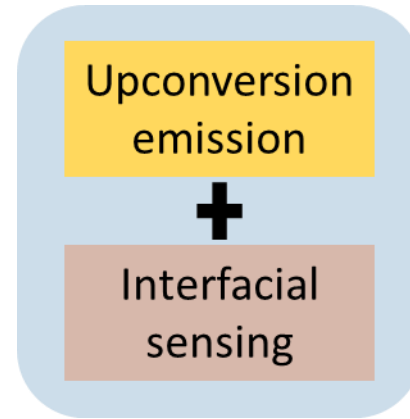
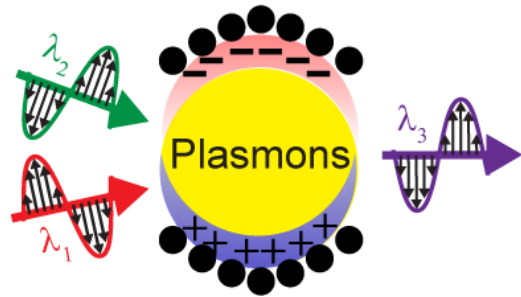
Multi-pathway Upconversion Emission in Symmetry-Broken Multiresonant Nanocavities



Multi-pathway Upconversion Emission with Interfacial Refractive-Index Sensitivity



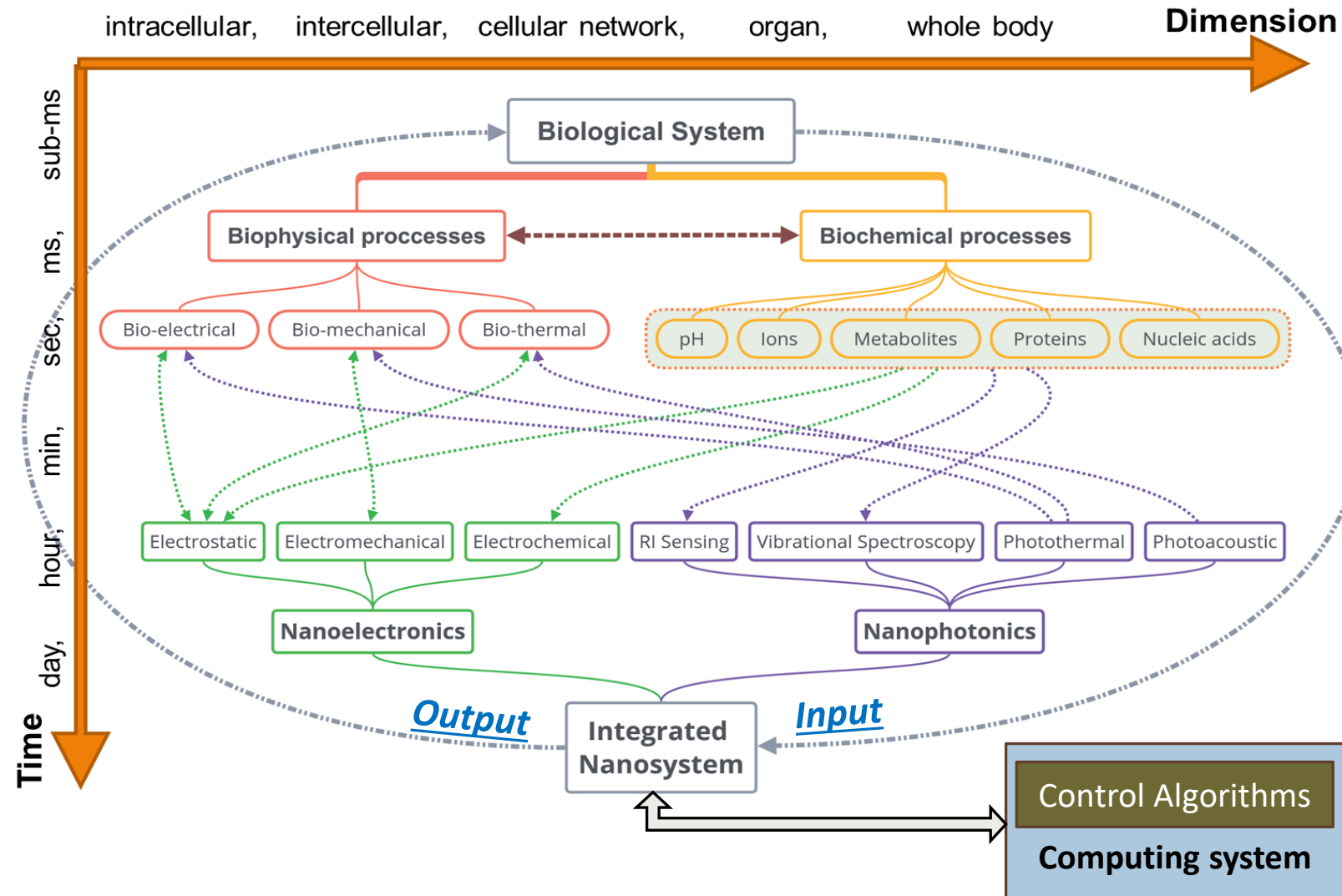
Motivation: Why Metal Nanocavity Light Upconversion?



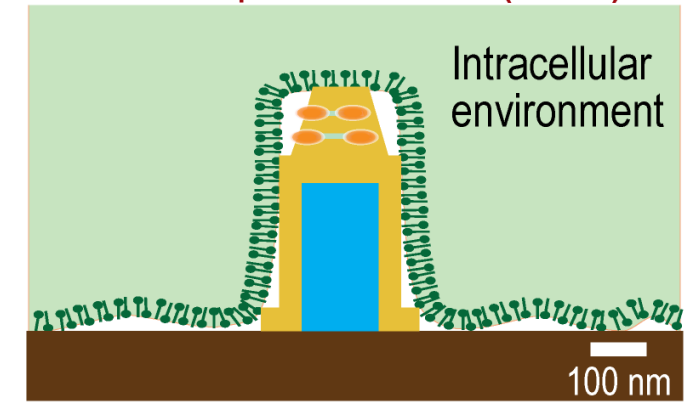
Multiresonant plasmonic nanocavities can serve as **up-conversion luminescence nanosensors** for **deep tissue biosensing/bioimaging** applications.

- High interfacial sensitivity (e.g., molecule, voltage, temperature, etc.)
- Plasmon-enhanced multiphoton multi-pathway up-conversion luminescence
- Photostability, mechanical stability, biocompatibility, and manufacturing scalability

Bio-Interfaced Hybrid Optical-Electrical Nanotransducer: Multiresonant Nanopillar Nano-Optoelectrode



Cell Engulfed Nanopillar Nano-Optoelectrode (NOE)



Sensing modalities:

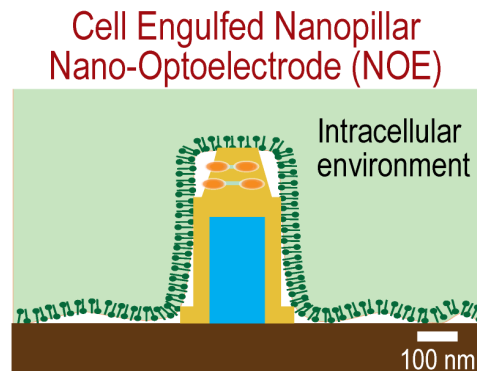
- Molecular Raman fingerprinting,
- Bioimpedance monitoring,
- Membrane potential recording, etc.

Actuation modalities:

- Electrokinetics,
- Electrochemistry,
- Thermo-/acousto-plasmonics,
- Plasmonic photochemistry, etc.

Research Objective

Explore the use of *multiresonant nanopillar nano-optoelectrode (NOE)* arrays for scalable and *precise optoporation nanosurgery*, aiming to facilitate *multimodal optical-electrical monitoring of intracellular processes* in cell networks.

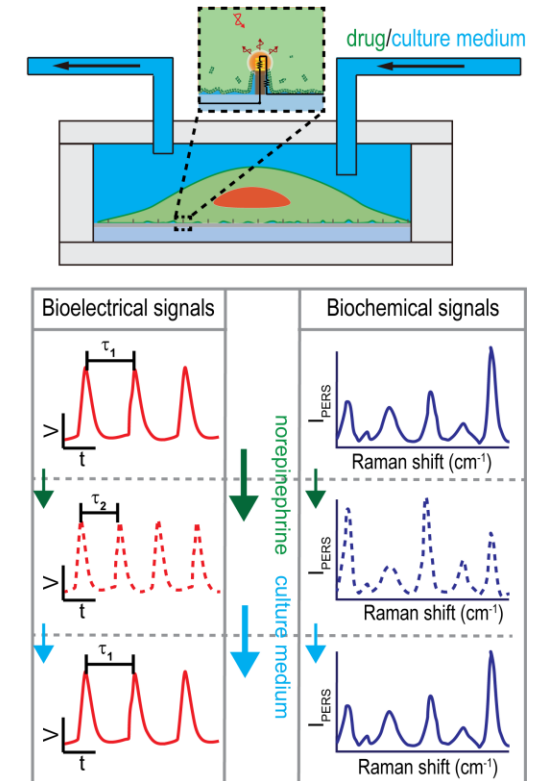
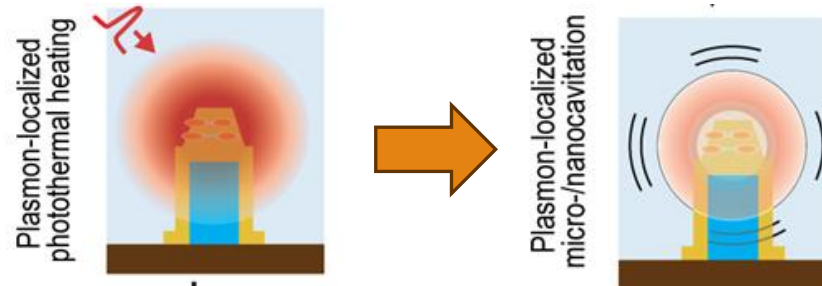
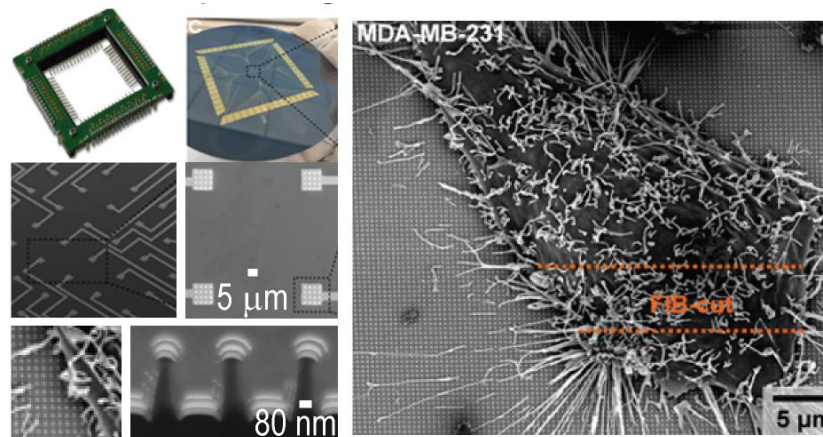


Sensing modalities:

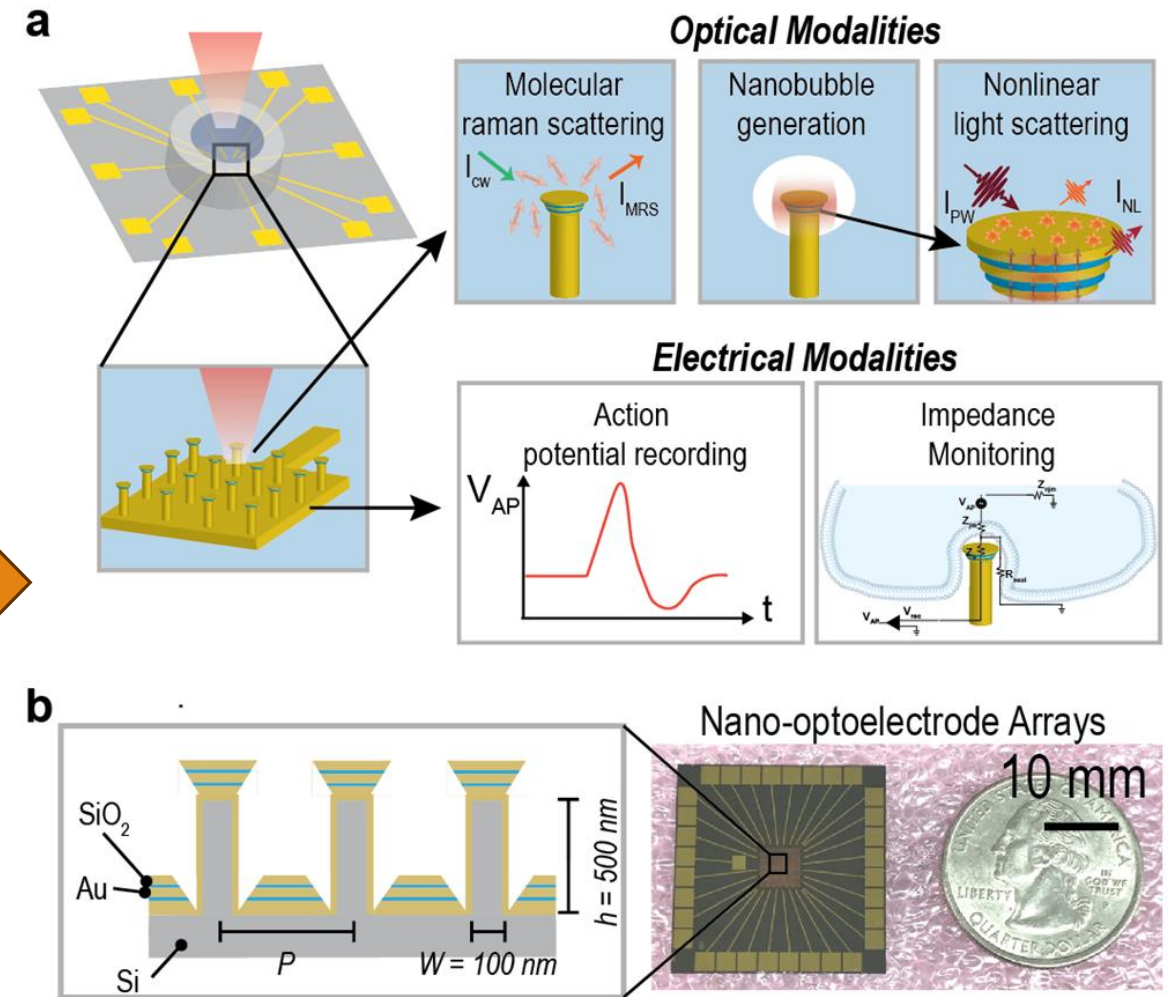
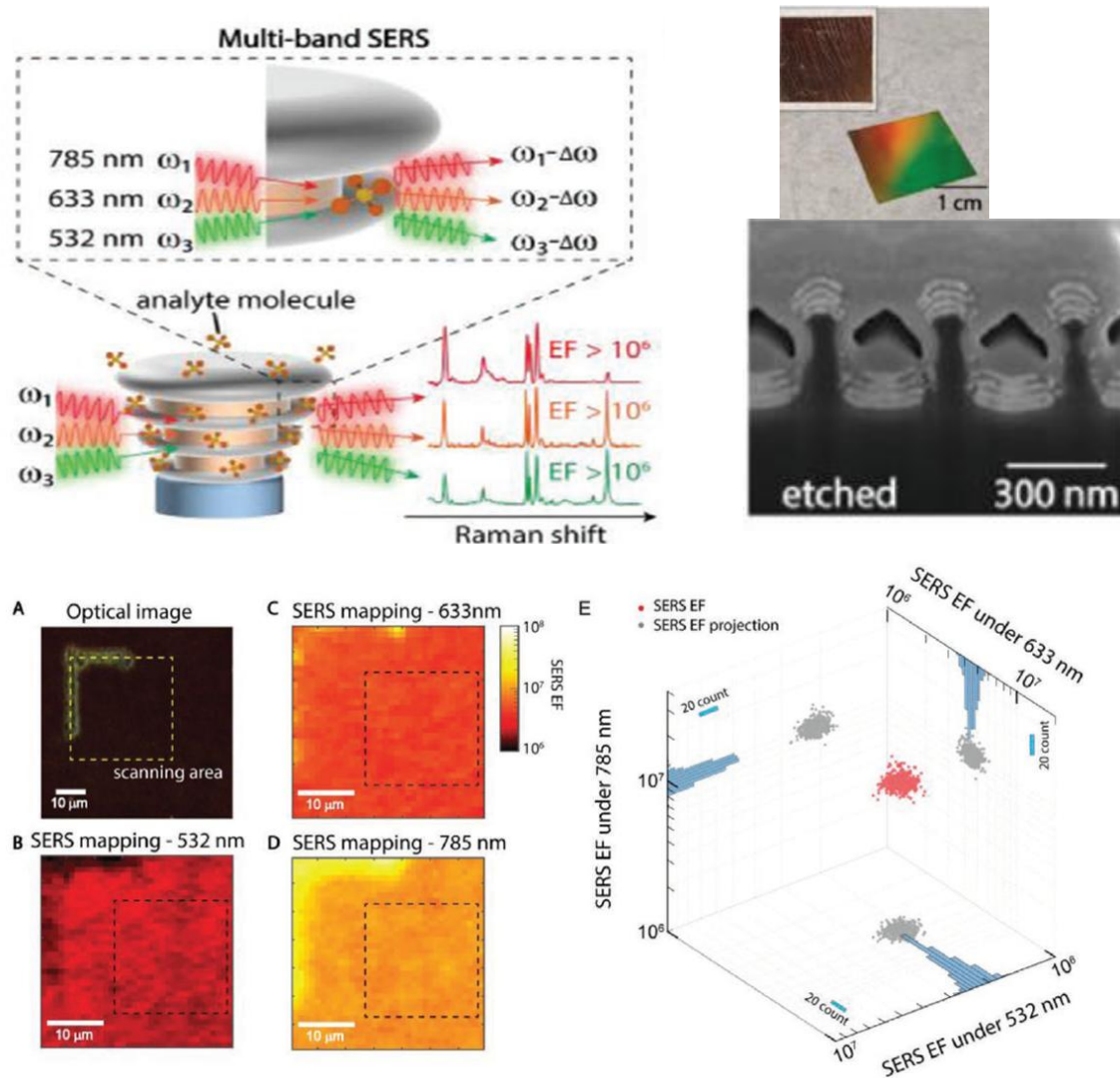
- Molecular Raman fingerprinting,
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Actuation modalities:

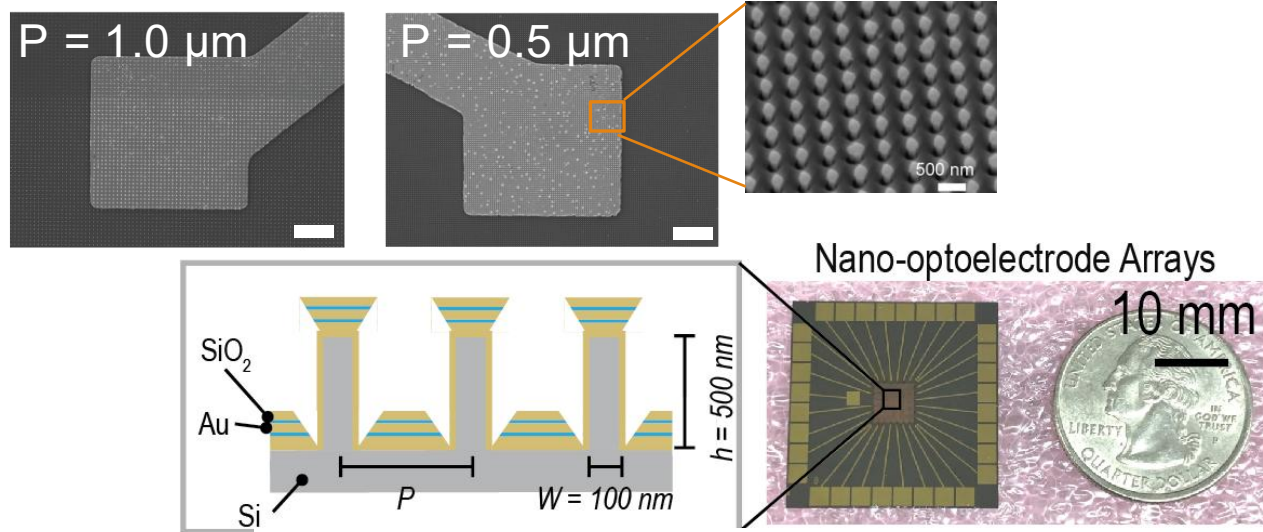
- Electrokinetics,
- Electrochemistry,
- Thermo-/acousto-plasmonics,
- Plasmonic photochemistry, etc.



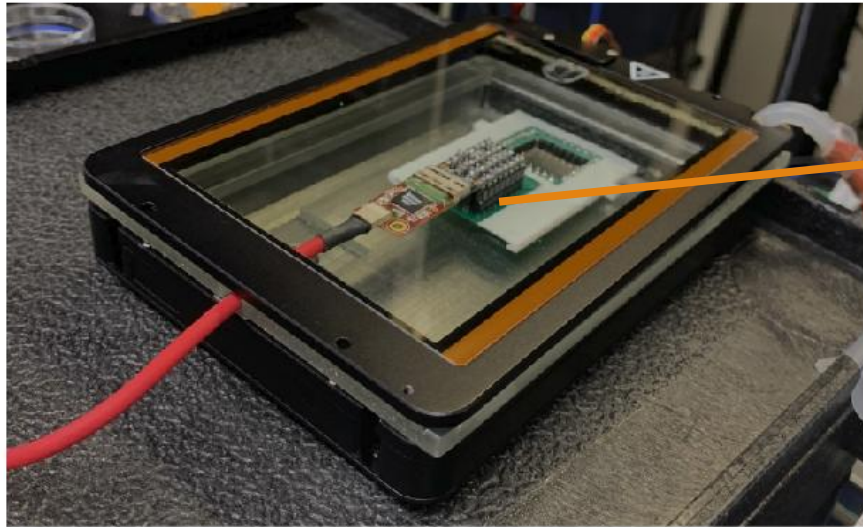
Multiresonant Nanopillar Nano-optoelectrode Biochips



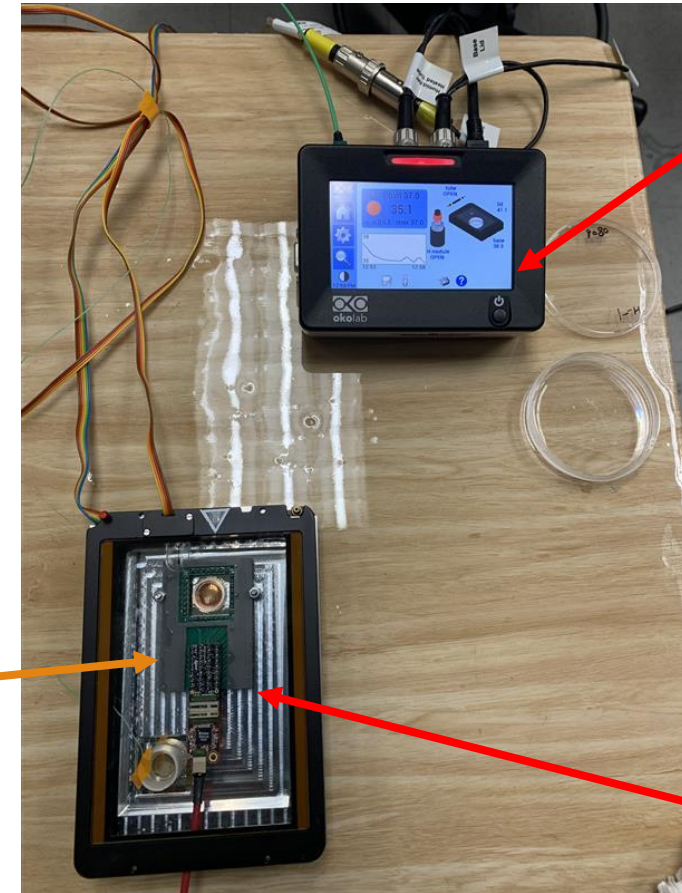
Instrumentation for Multimodal Optical-Electrical Measurements at the Nano-bio Interface



Mini-incubator



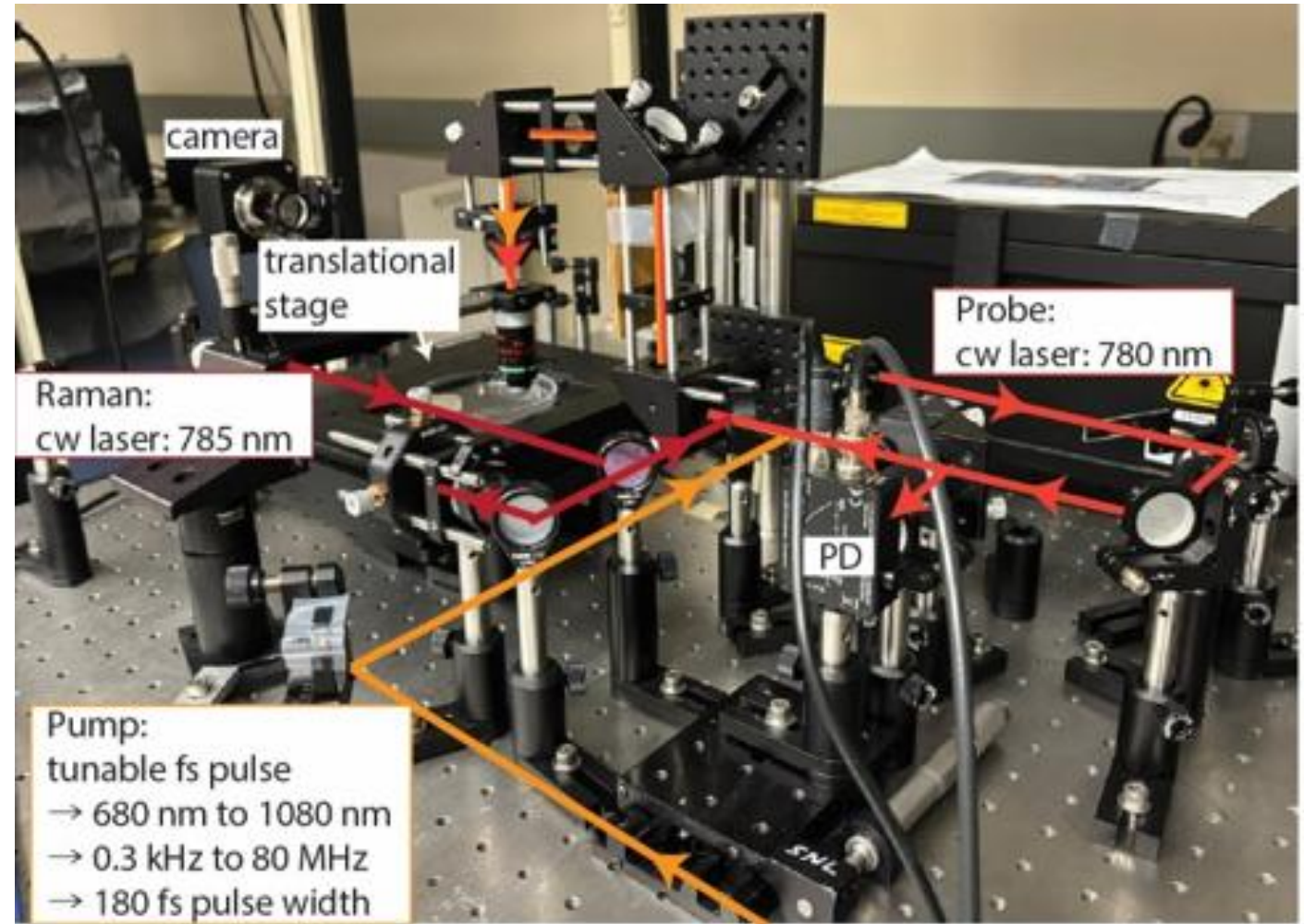
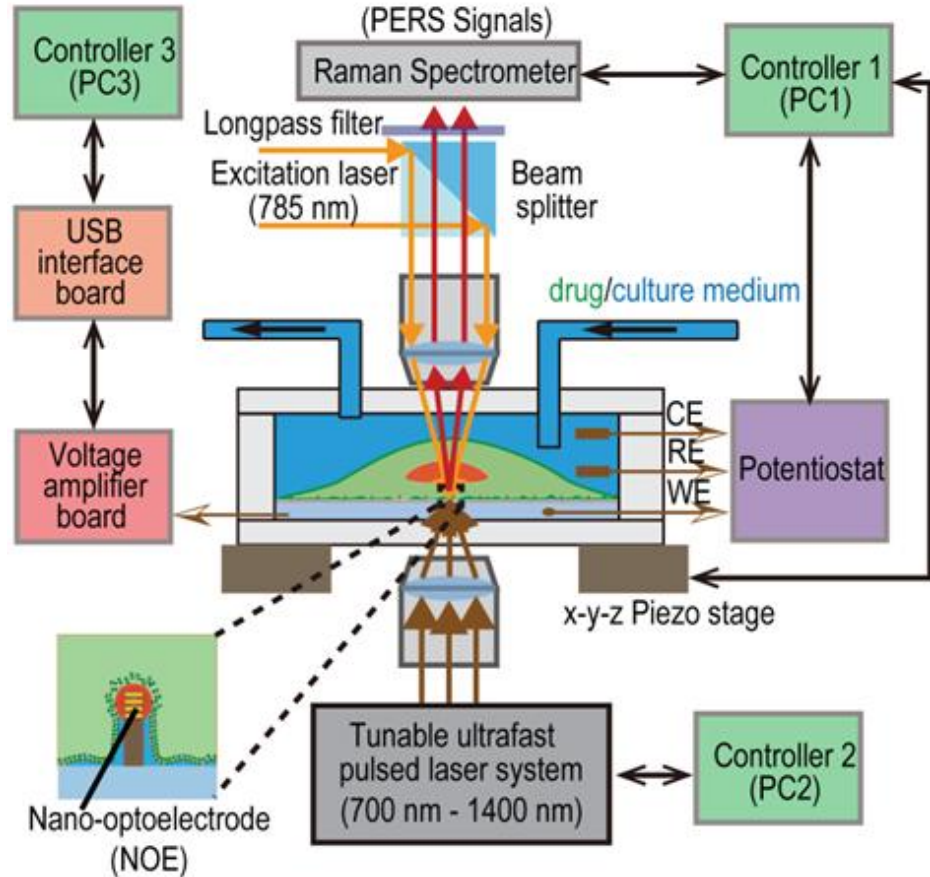
Base metal holder for heat control



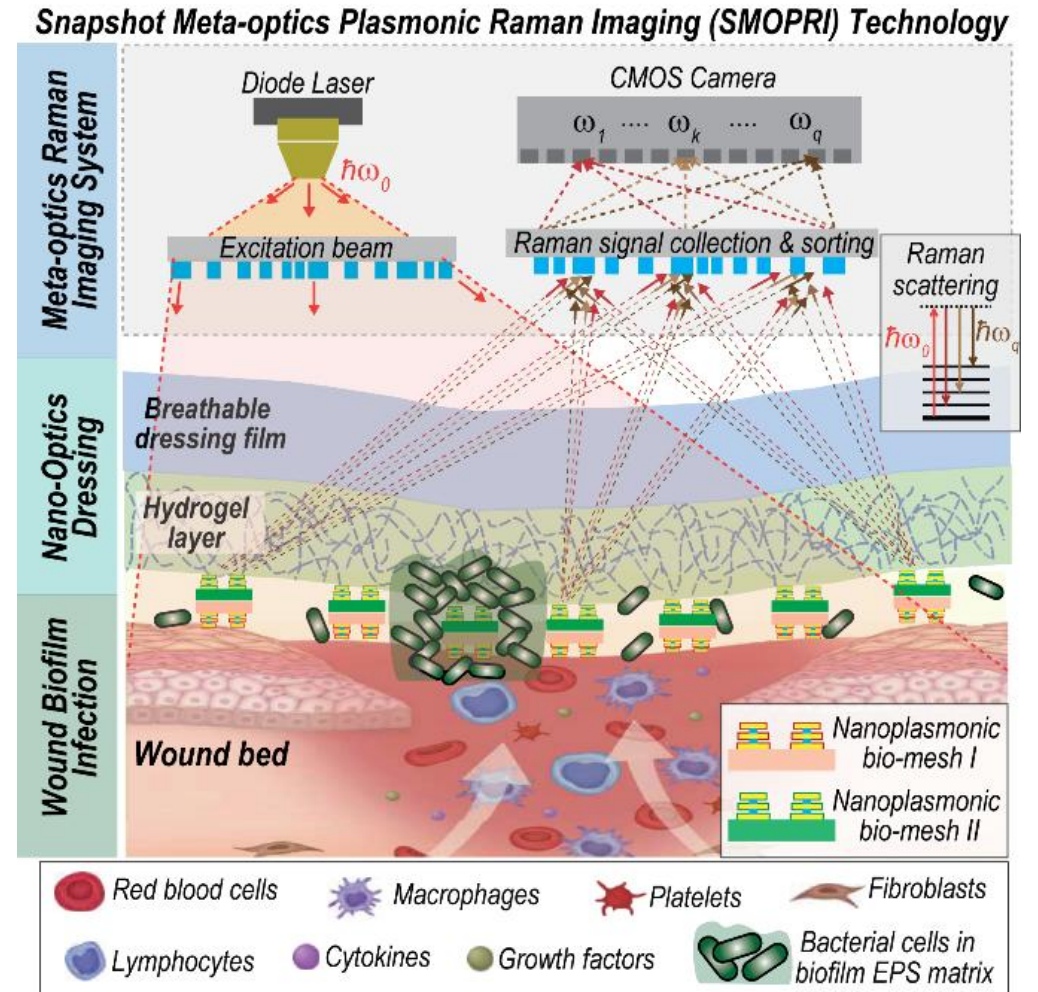
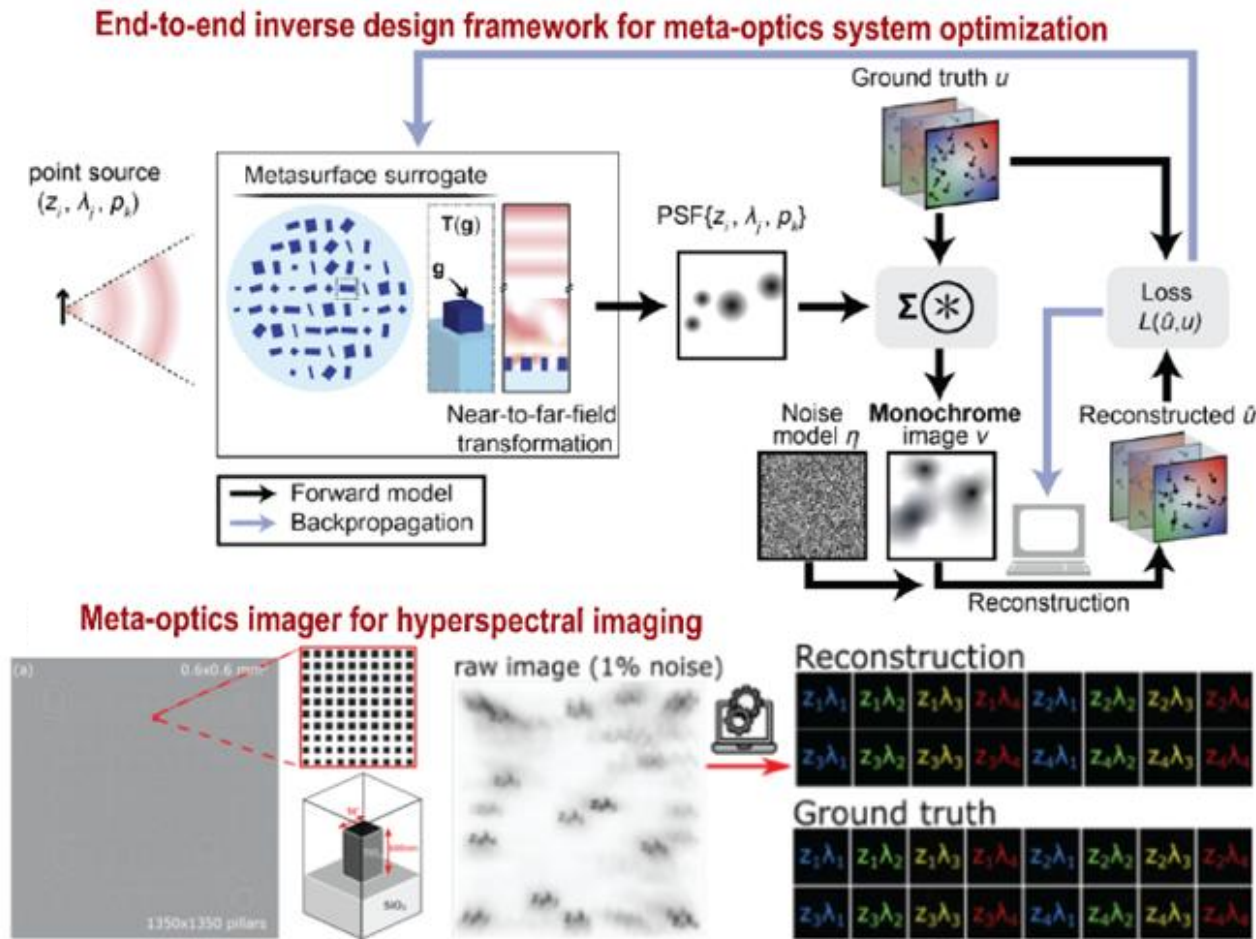
Temp and gas controllers

Amplifiers and recording controllers

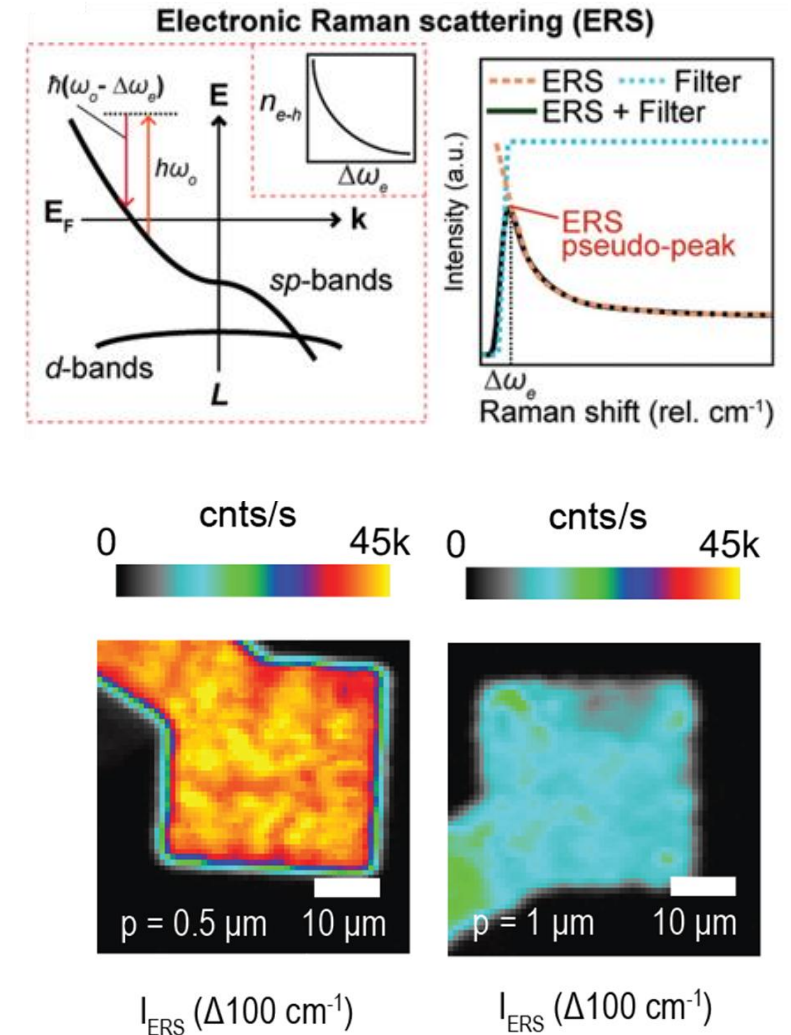
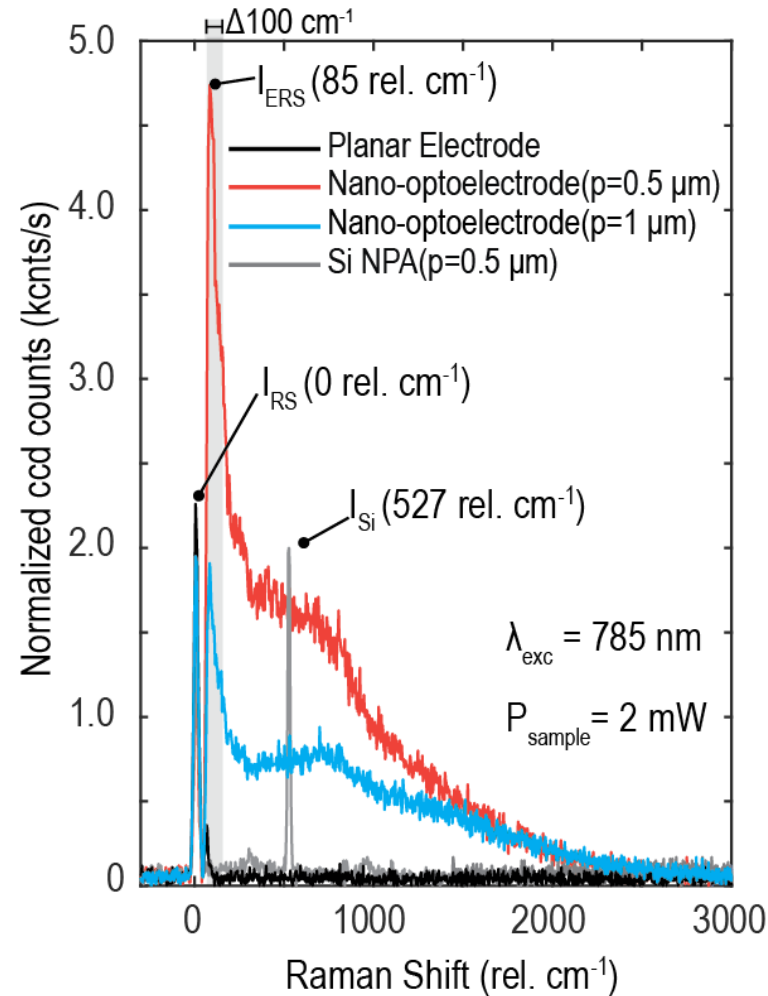
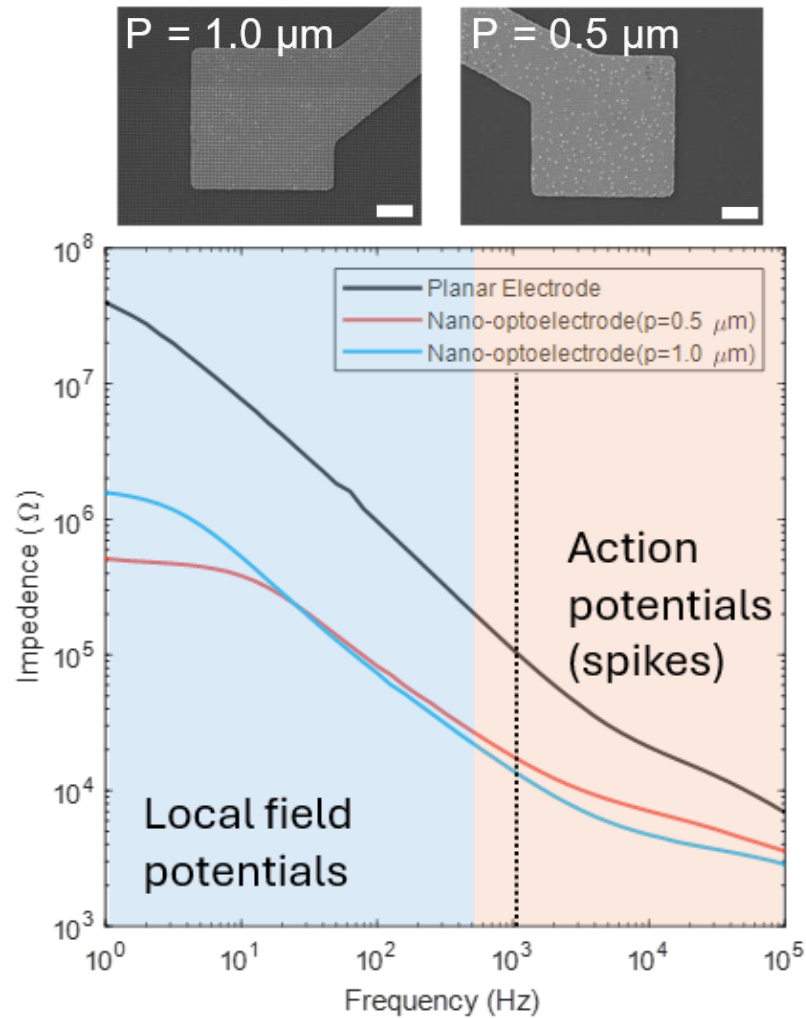
Instrumentation for Multimodal Optical-Electrical Measurements at the Nano-bio Interface



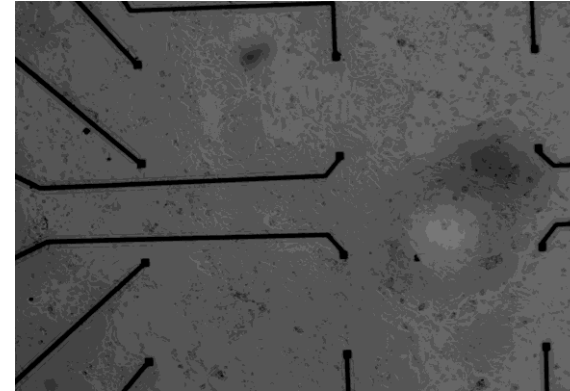
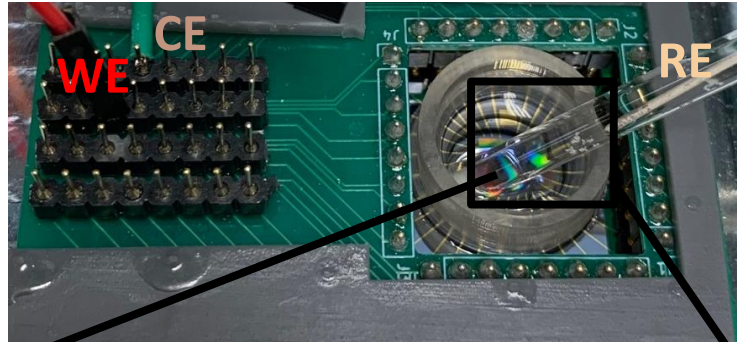
Compact Meta-optics Hyperspectral Imaging System: Toward Point-of-Care Optical Nano-bio Interfacing



Electrical and Optical Characterization

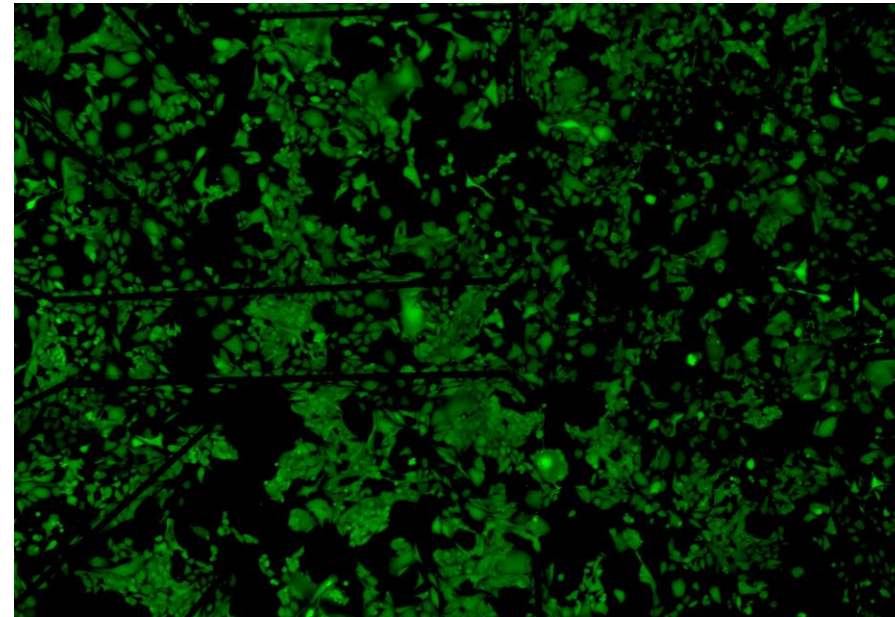
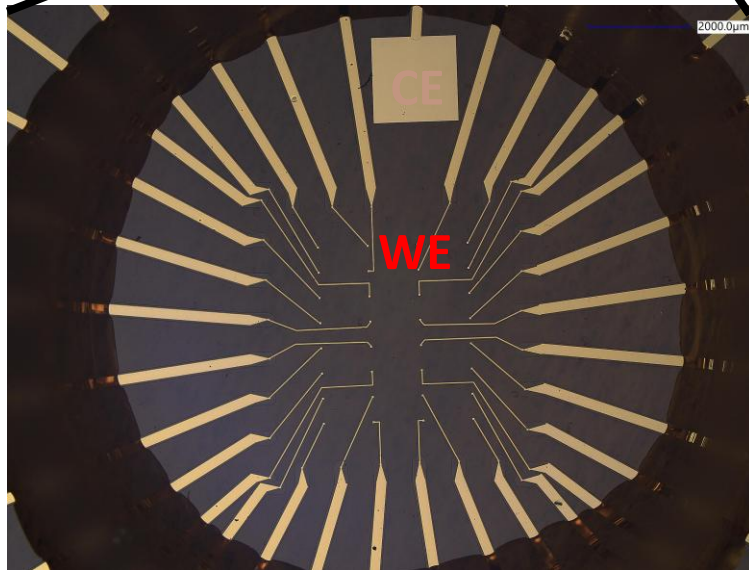


Biocompatibility and Electrical Recording (ongoing)



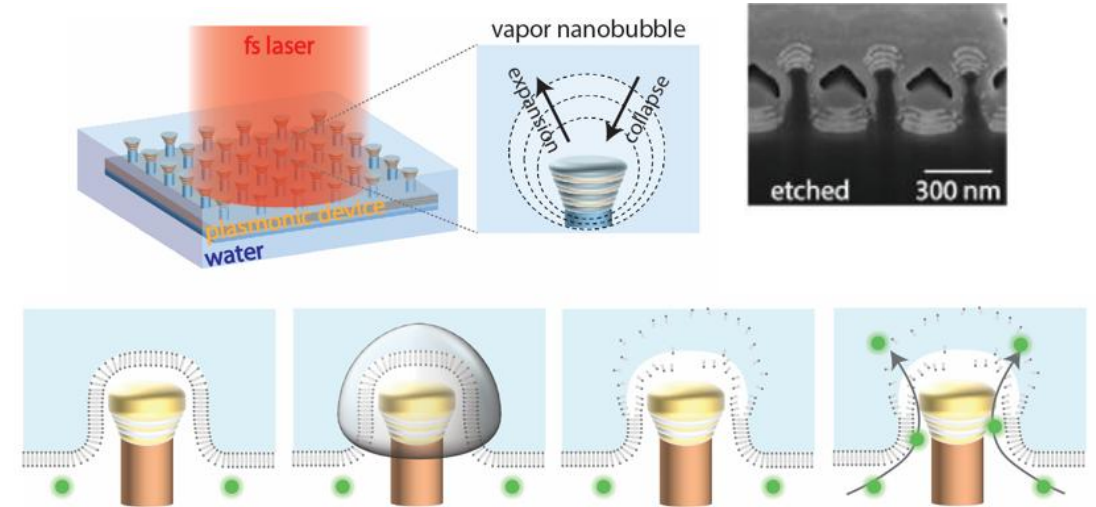
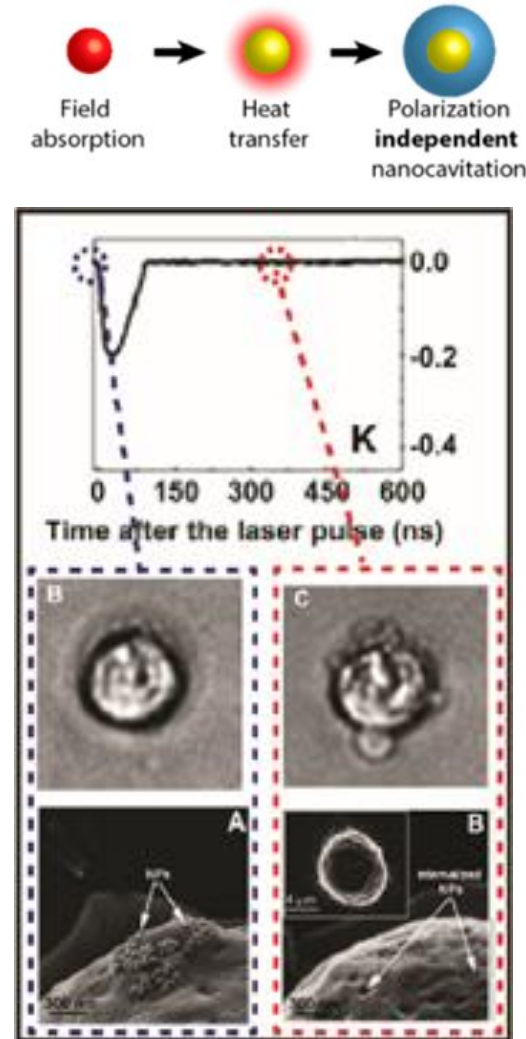
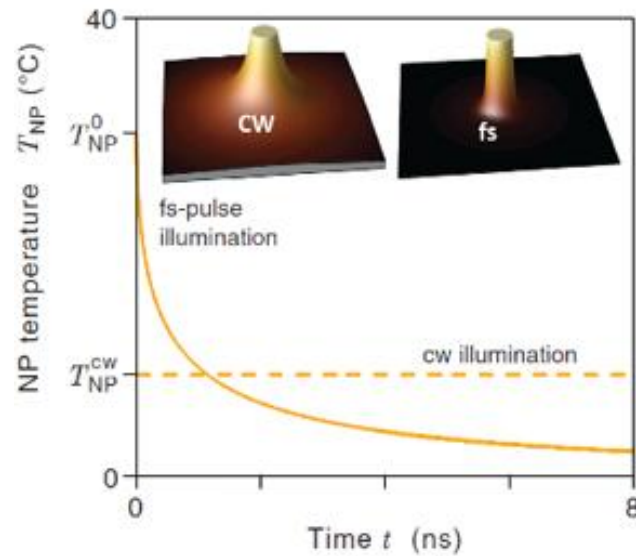
Bright-field image

Cell line: HL1 cells



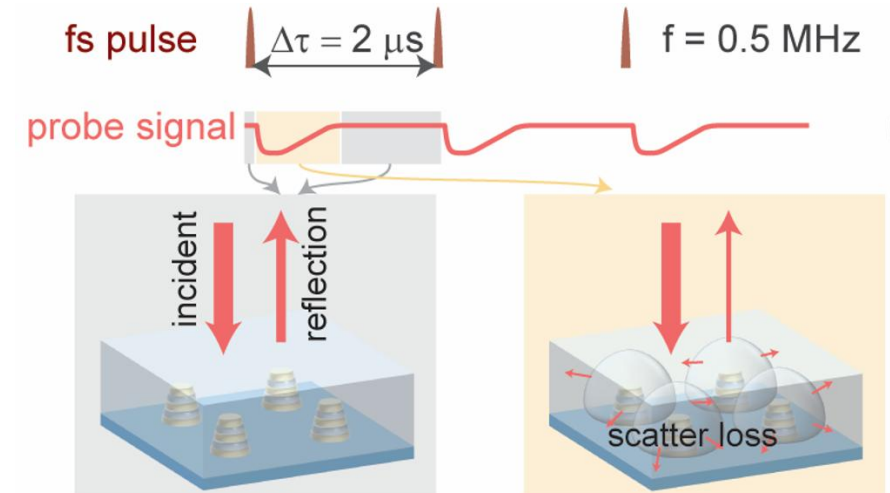
Live/Dead Assay

Ultrashort Laser Pulse-Triggered Plasmonic Nanocavitation for Optoporation and Intracellular Access

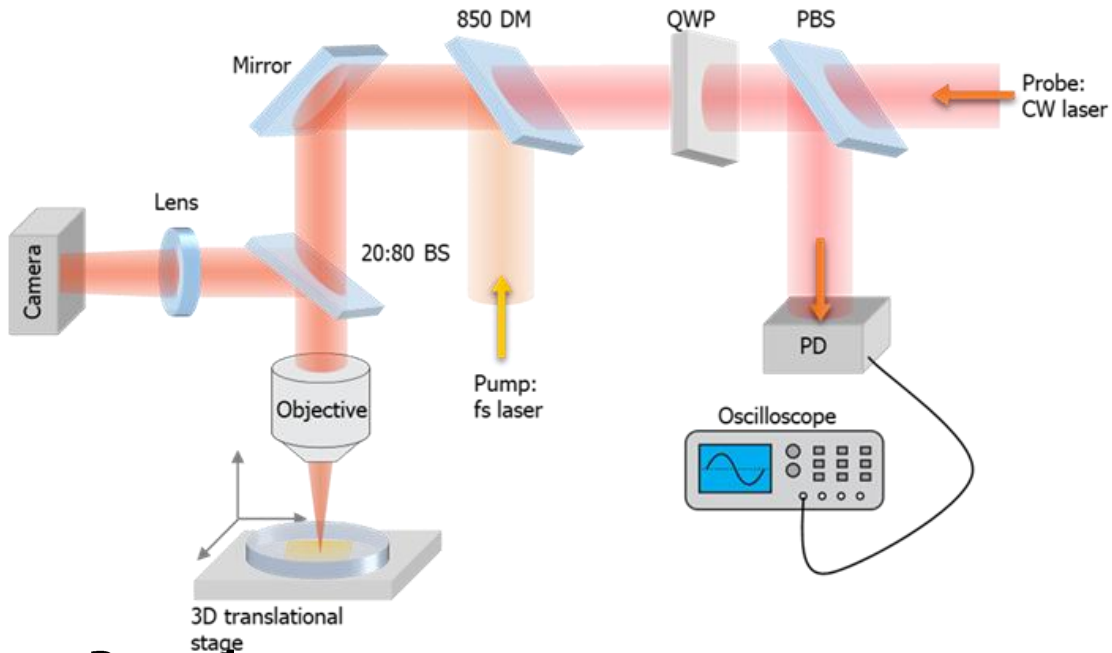


Issues:

- Unbounded NPs not suitable for chronic bio-studies
- Single-band operation
- Single modality
- Limited understanding

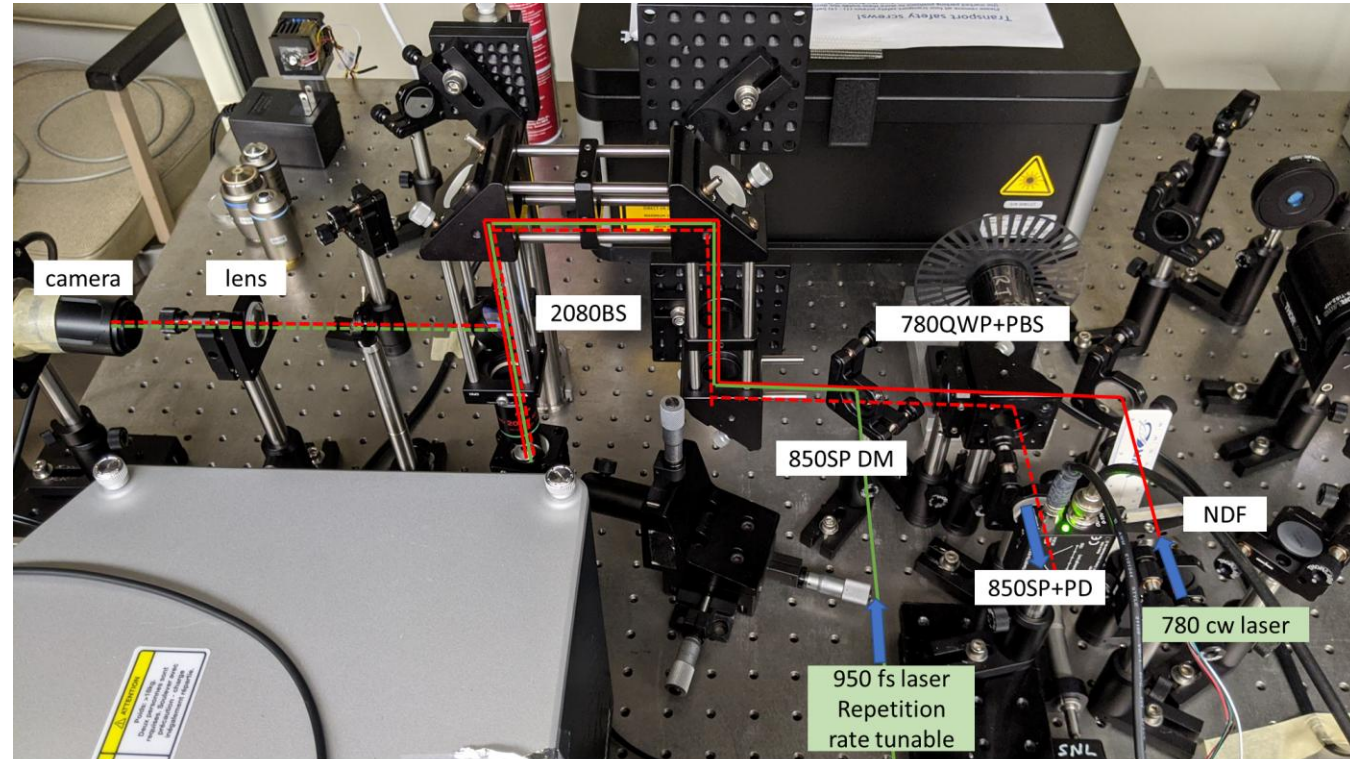


Experimental Setup for Monitoring Nanocavitation Dynamics



Pump laser:

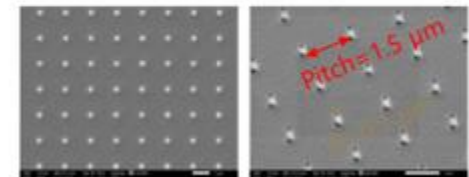
- fs laser wavelength: 950 nm
- fs laser pulse width: ~ 150 fs
- Power: 0.5-1 mW
- Beam size: $\sim 13 \mu\text{m}^2$
- Pulse repetition rate: 0.5 MHz (δt : 2 μs)
- Pulse energy: 1-2 nJ ($0.5\text{-}1.0 \times 10^{10}$ photons)



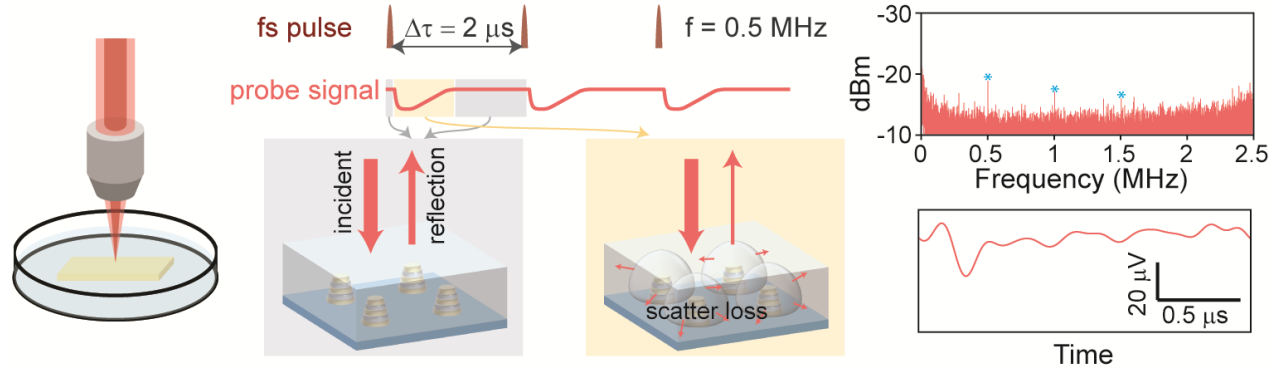
Probe laser (Raman laser):

- cw laser wavelength: 780 nm
- Power: 0.3 mW
- Beam size: $\sim 10 \mu\text{m}^2$

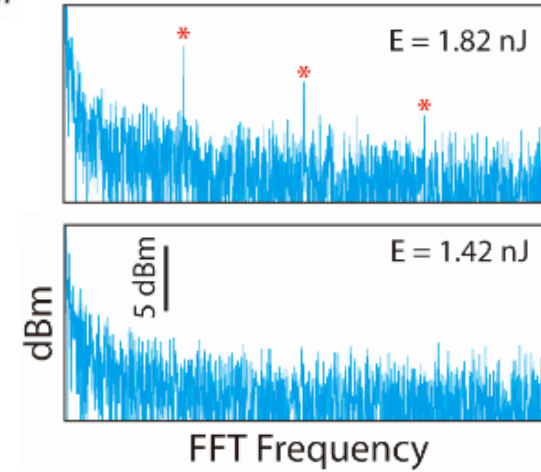
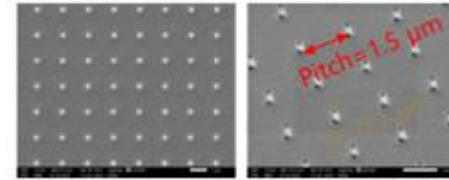
Substrate 1: 1.5 μm pitch sample with MIM



Nanocavitation Dynamics Detection and Signal Processing



Substrate 1: 1.5 μm pitch sample with MIM



fs triggered and
cw probed
bubble
generation

Probe signal
acquisition by
oscilloscope

Fast Fourier
Transform (FFT)

Temporal data: FFT
filtering, iFFT,
averaging

Criteria for nanocavitation

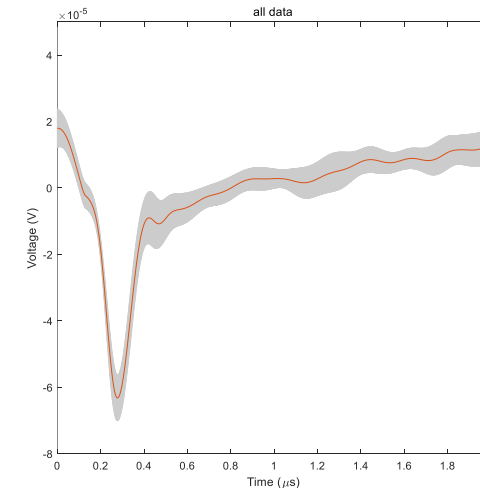
- FFT peaks at $N \times 0.5 \text{ MHz}$
- YES: above the threshold
- NO: below the threshold

Temporal shape

- Timescale of the process
- Bubble size estimation

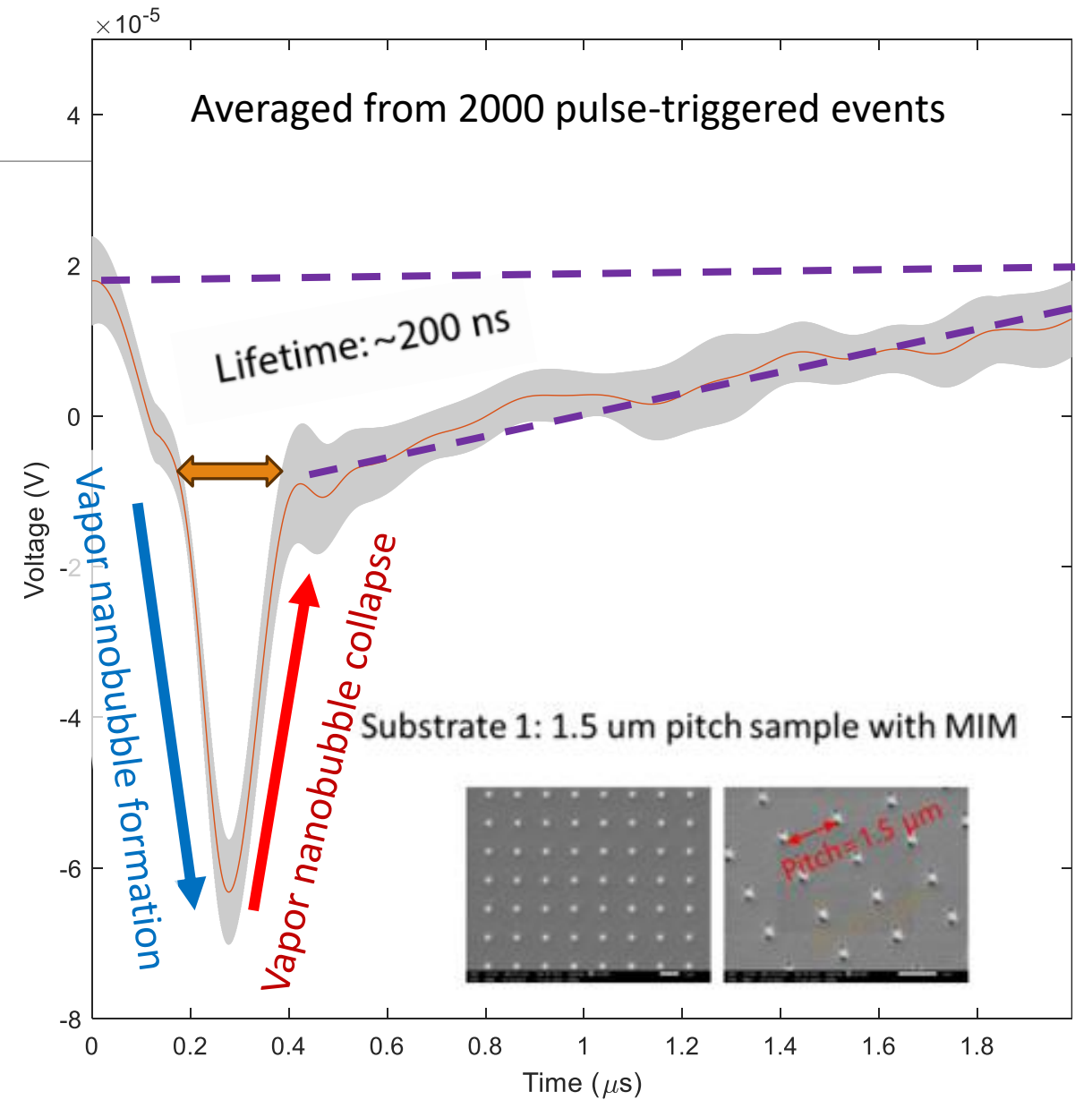
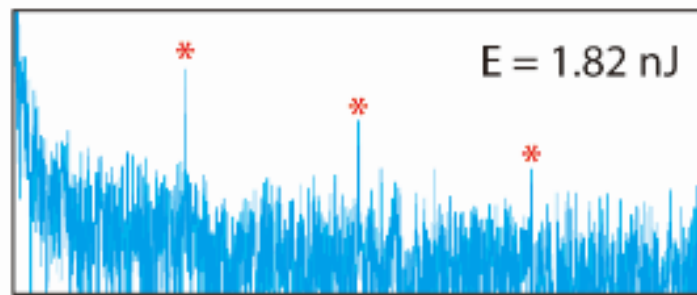
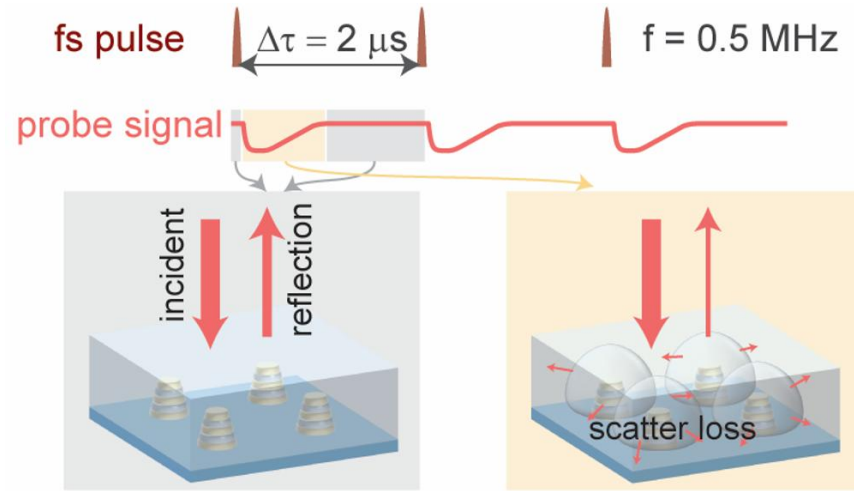
Oscilloscope recording:

- 1 ns temporal resolution
- 4 ms time trace

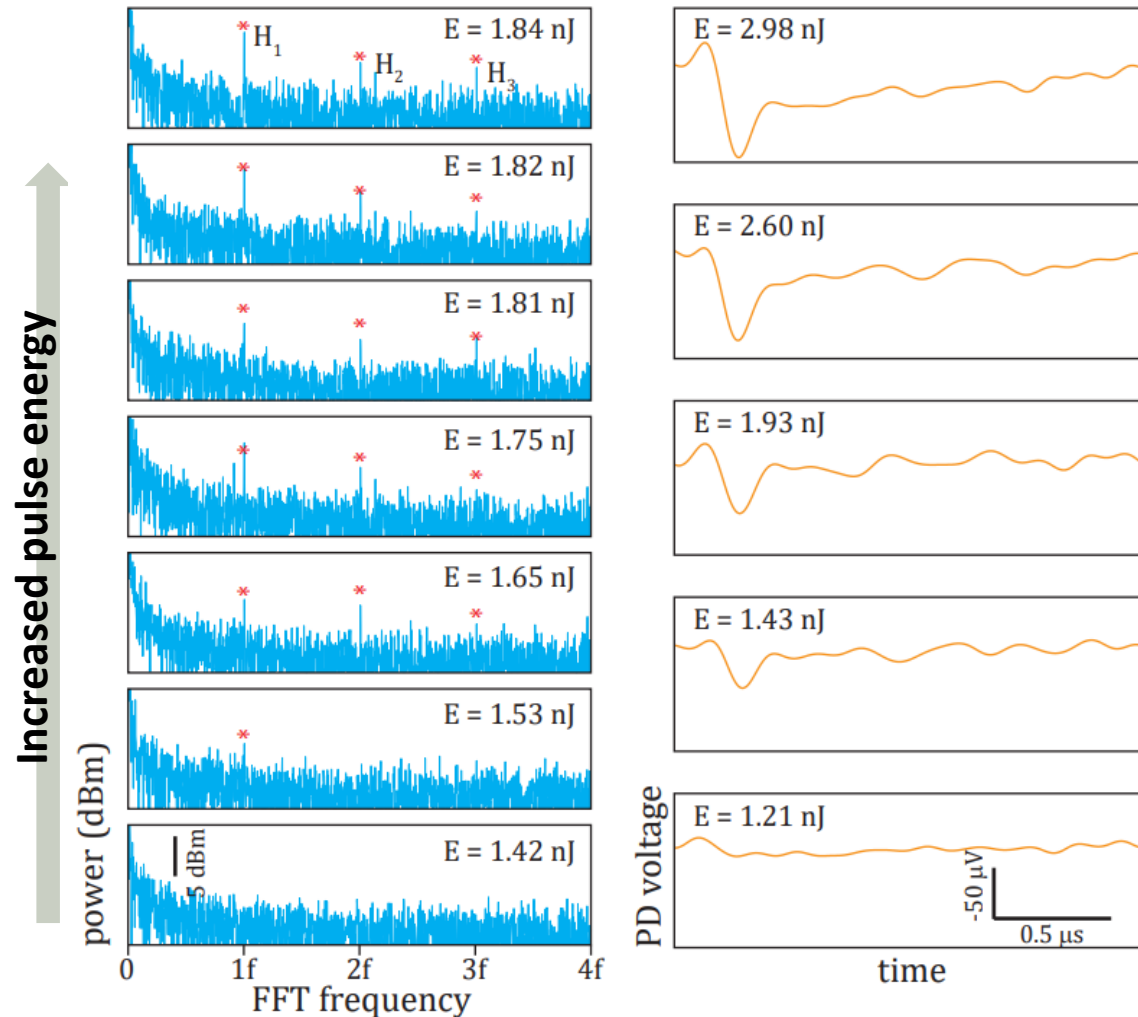


Pulse train: $f = 0.5 \text{ MHz}$ (2 μs between neighboring pulses)

Nanocavitation Dynamics

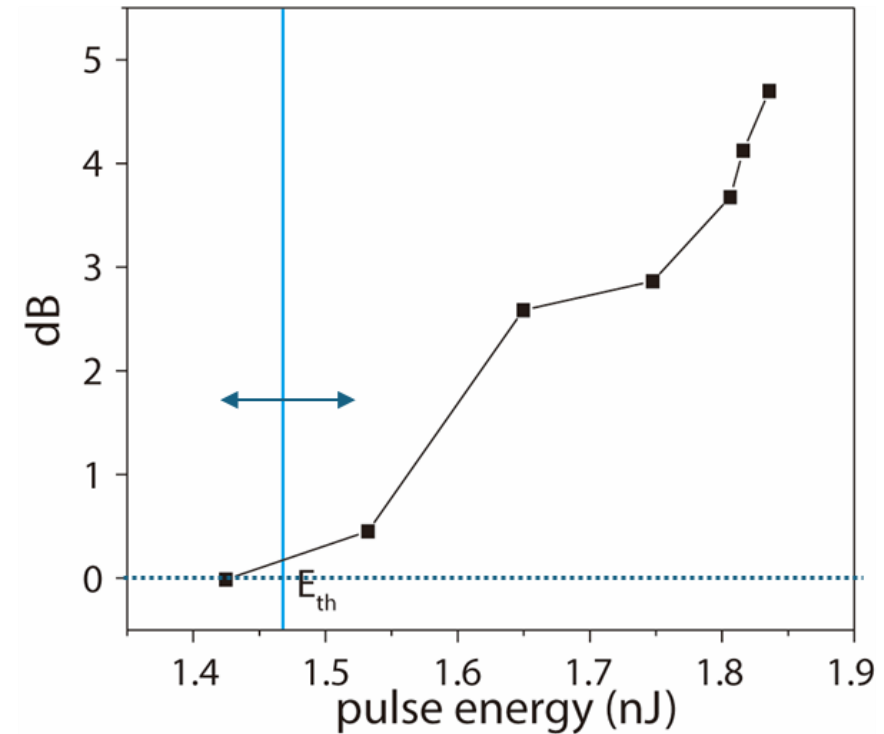


Nanocavitation Dynamics: Power Dependence



Threshold:

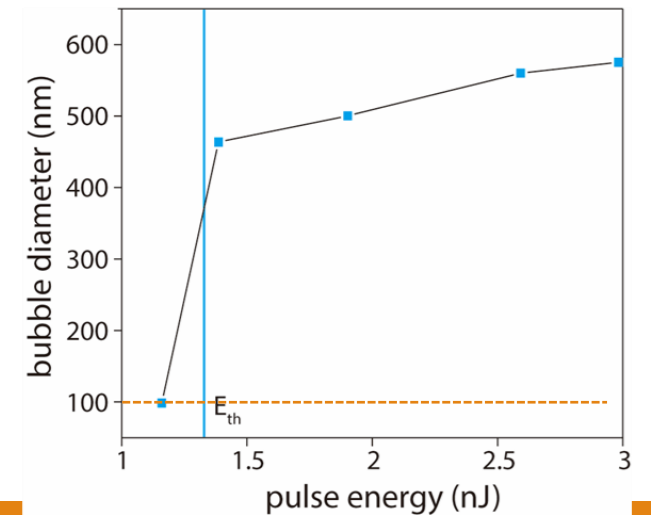
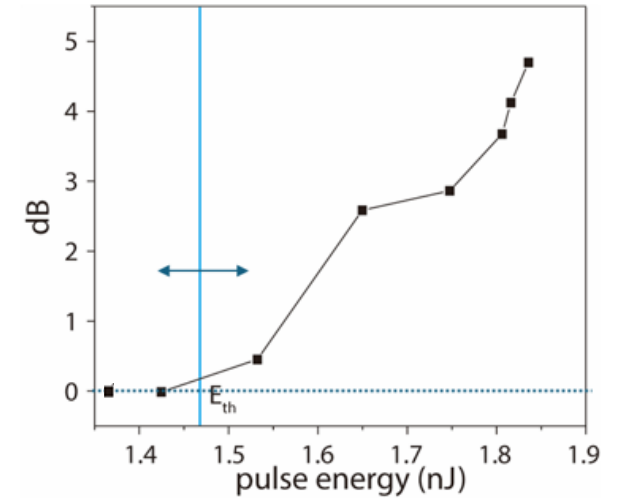
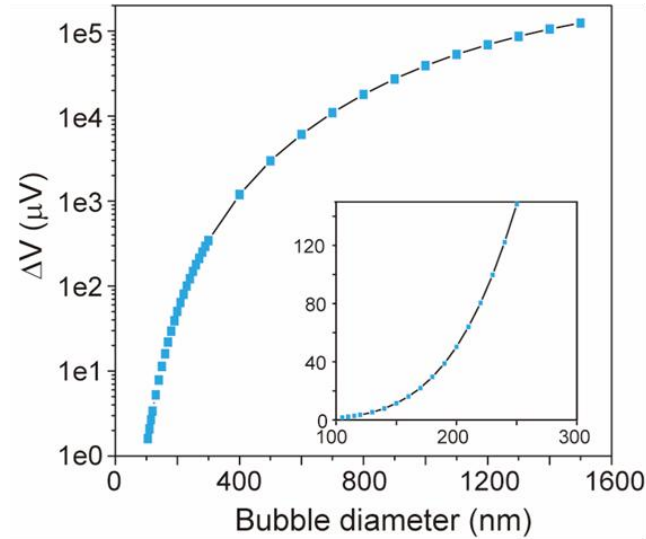
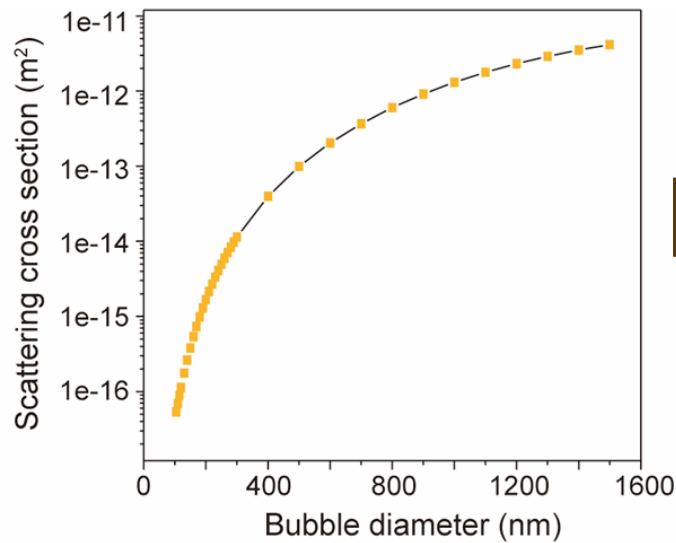
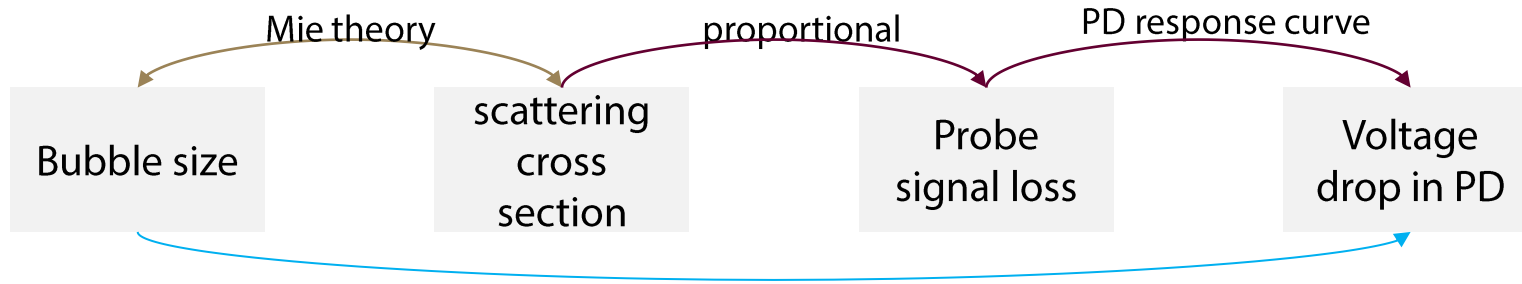
Beam size: $\sim 13 \mu\text{m}^2$



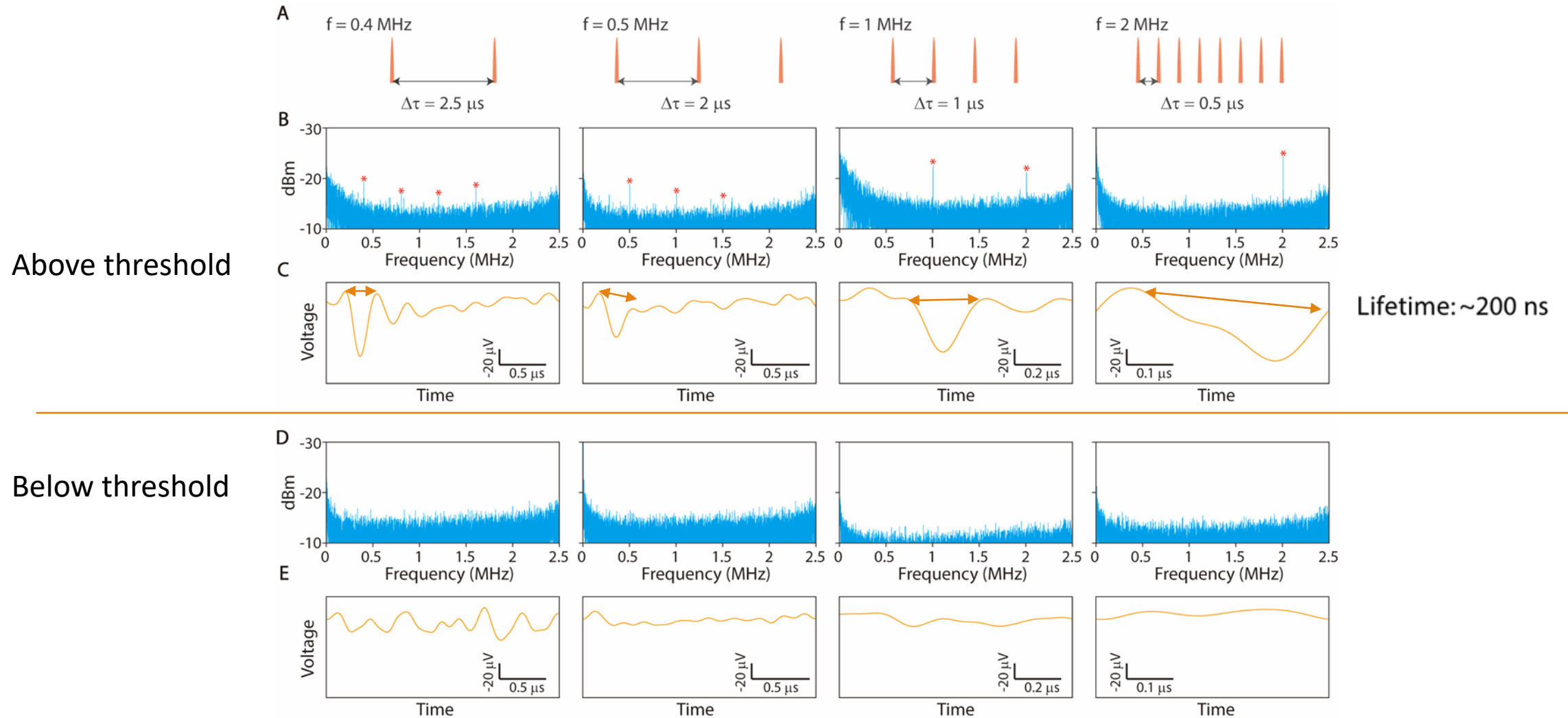
Lifetime: ~ 200 ns

$$\text{dBm} = 10 \log_{10} \frac{P}{1 \text{ mW}}$$

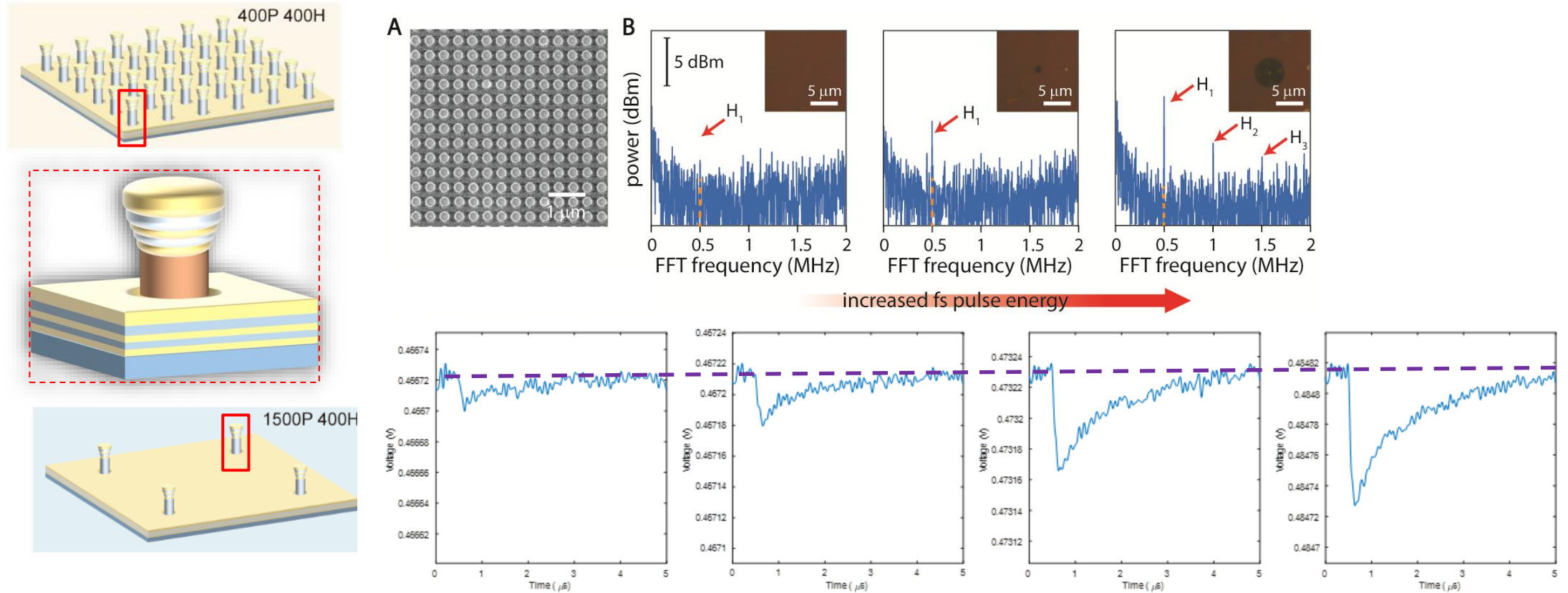
Bubble size estimation using Mie theory



Pulse repetition rate sweeping



Connected Nanocavitation in High-Density Arrays

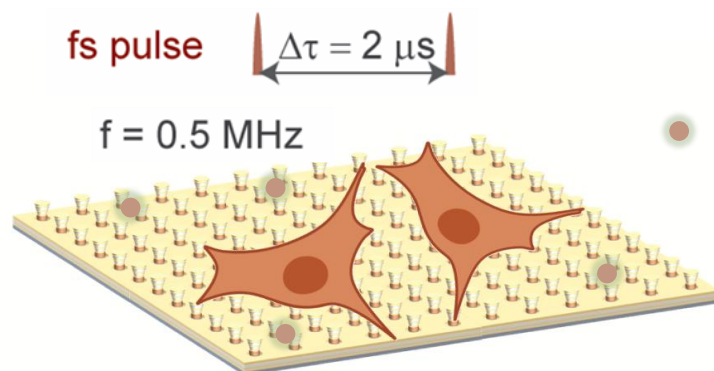
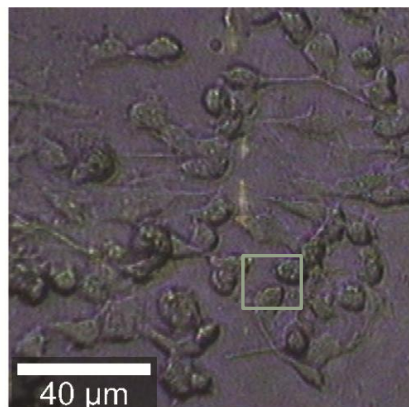


In the higher density array, the nanobubbles generated are close together and tend to merge into a connected microbubble sheet with a fs beam size $\sim 10 \mu\text{m}^2$.

□ Fast nanobubble generation process ($< 200 \text{ ns}$), and slow microbubble collapse process ($> 3 \mu\text{s}$).

Molecule Intracellular Delivery via Nanocavitation Optoporation

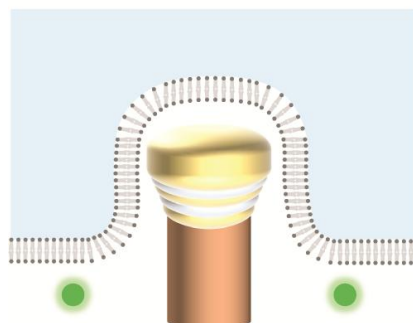
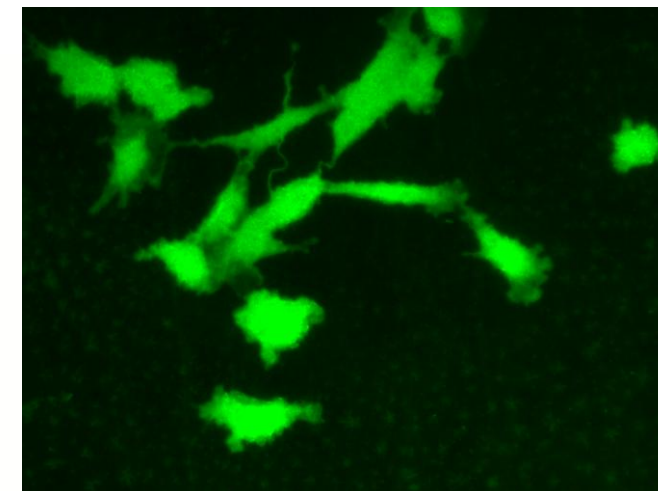
U251 cell line (malignant glioblastoma multiforme tumor cell)



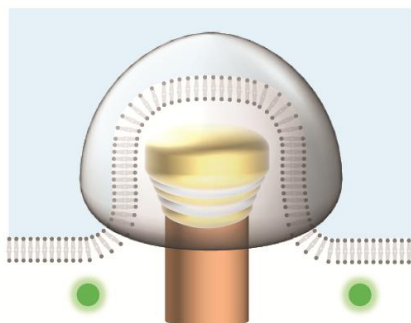
Cell non-permeable dye (FITC)

Excitation: 490 nm
Emission: 520 nm

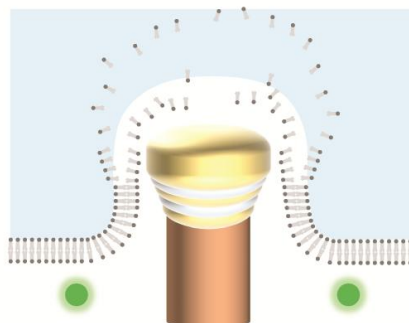
Above threshold



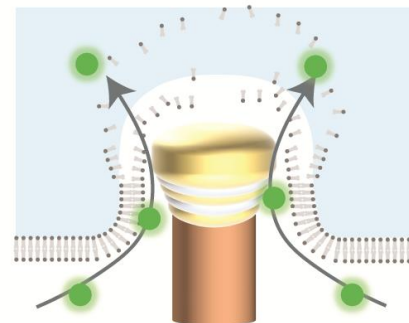
Dye molecules exist in extracellular environment.



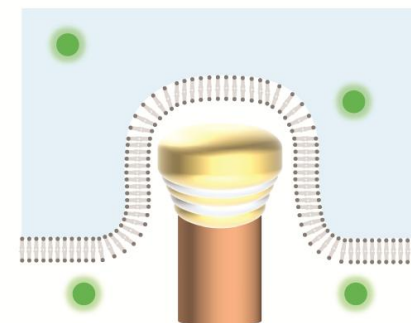
A transient nanobubble interacts with membrane in $\sim 200\ \text{ns}$.



Lipid bilayer deforms and membrane loses local integrity at nanoscale.



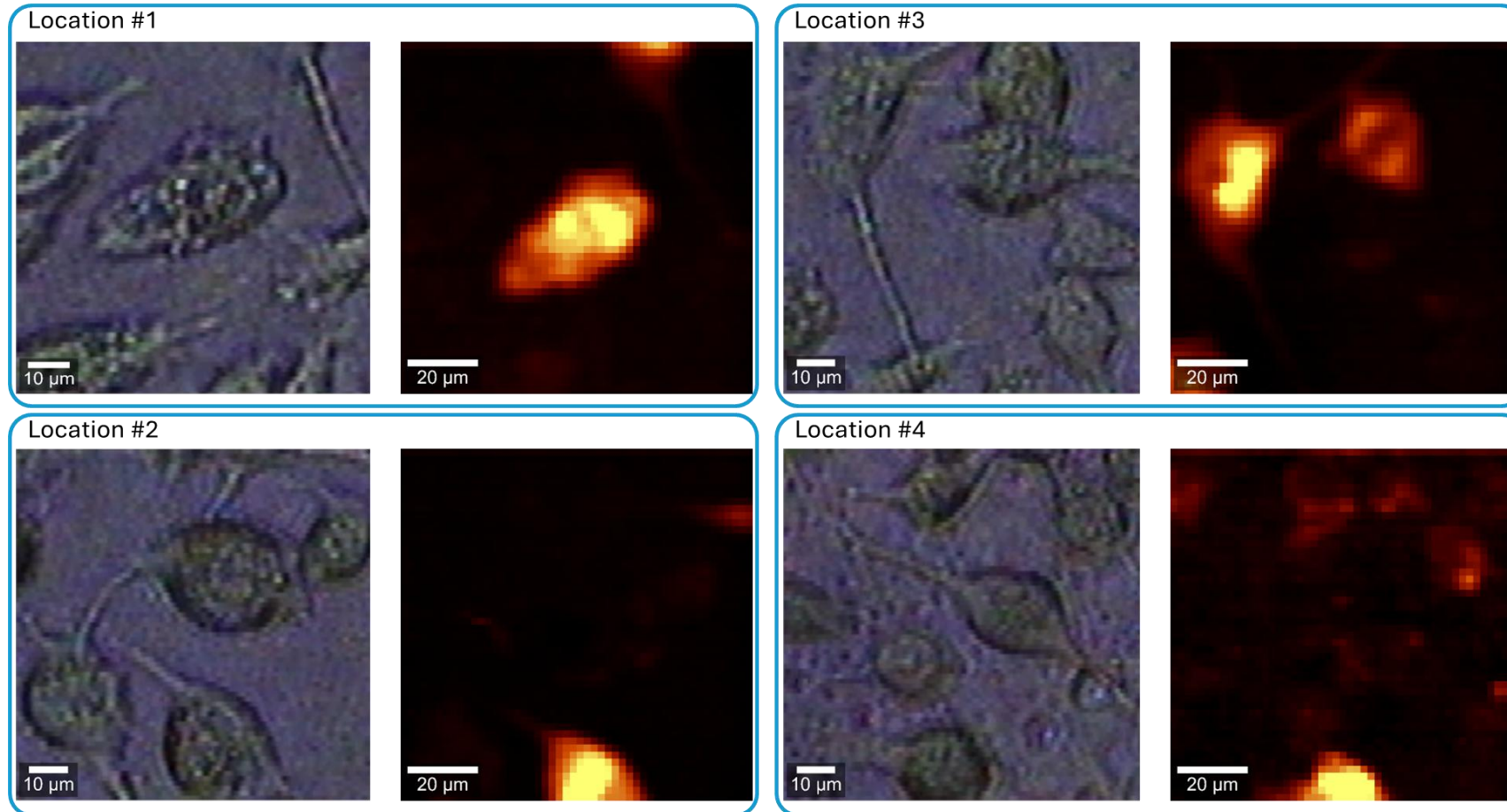
Dye molecules diffuse into intracellular environment.



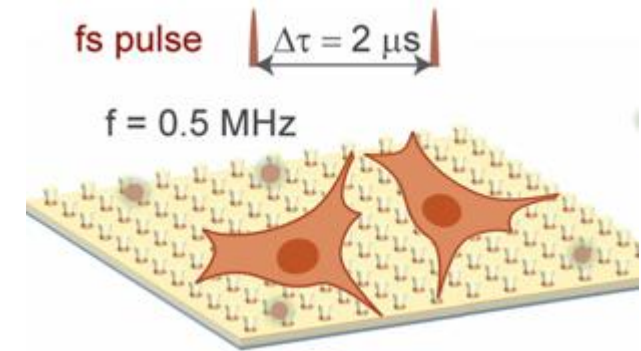
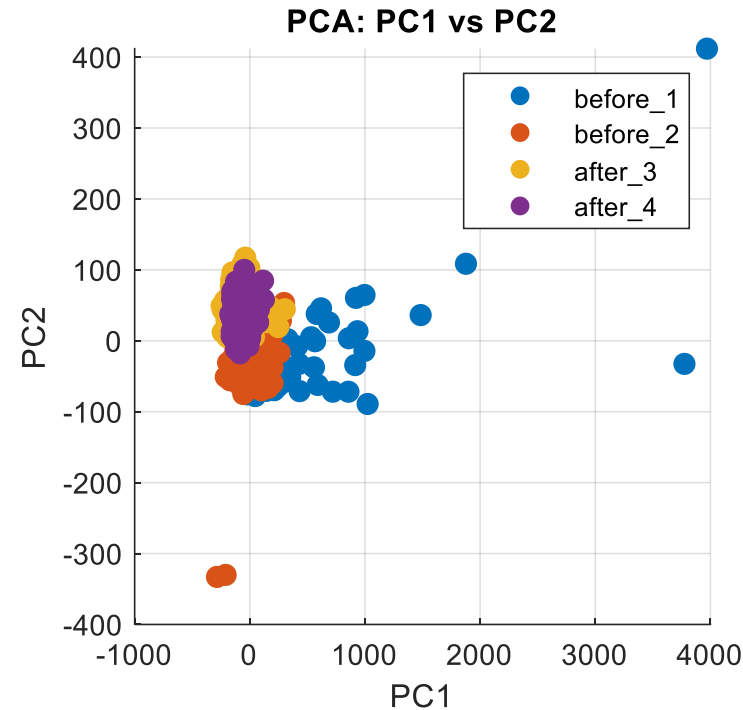
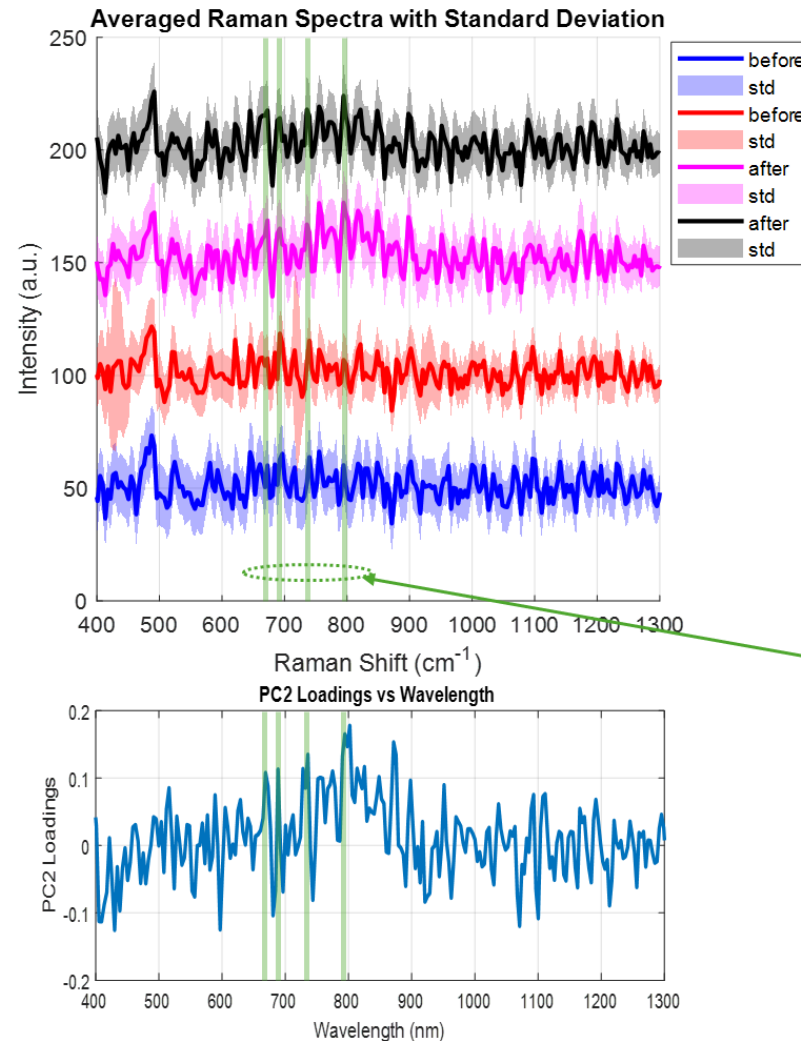
Lipid bilayer recovers and dye molecules stay inside.

Molecule Intracellular Delivery via Nanocavitation Optoporation

- PI: cell non-permeable dye. Ex/em: 535/617. Bind to double strand DNA to exhibit fluorescence.
- U251 cell line (brain tumor cells)



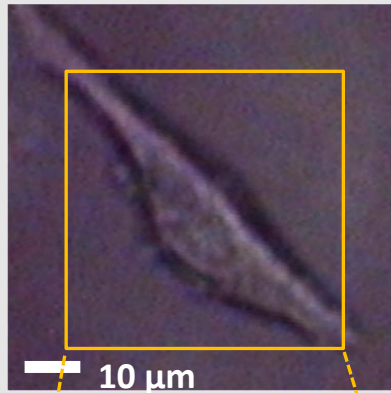
Raman Analysis before and after optoporation



- **Nucleic acids related Raman peaks** get increased after nano-cavitation treatment.
- The origin of other Raman peaks are under investigation.

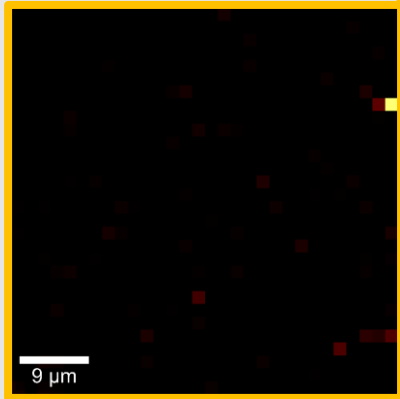
SERS analysis before and after optoporation

Before optoporation

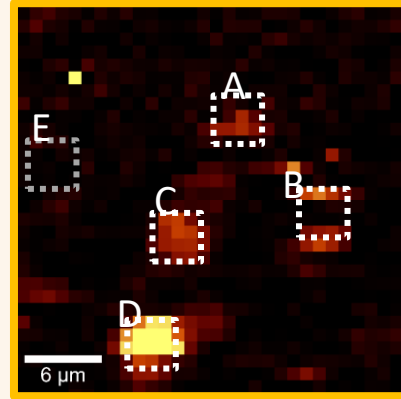
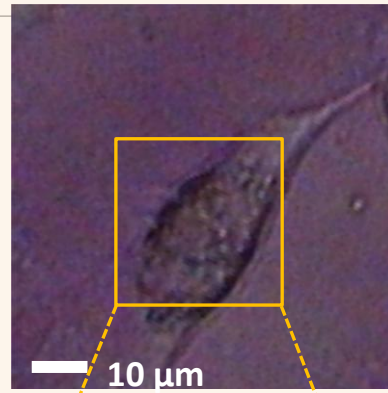


SERS mapping @1323 cm^{-1}

- ring mode of Adenine and Guanine

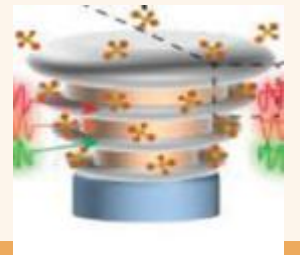
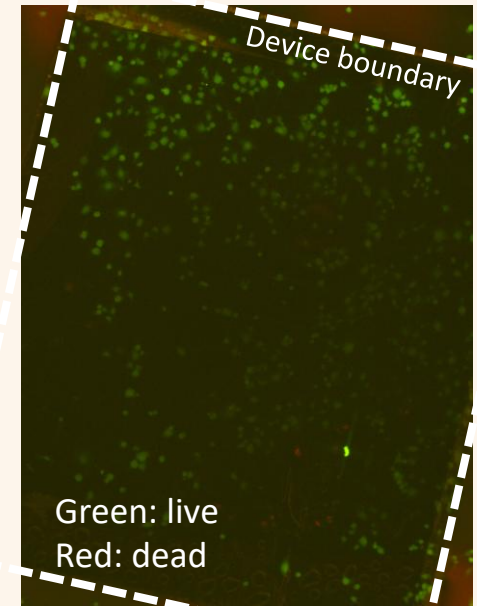
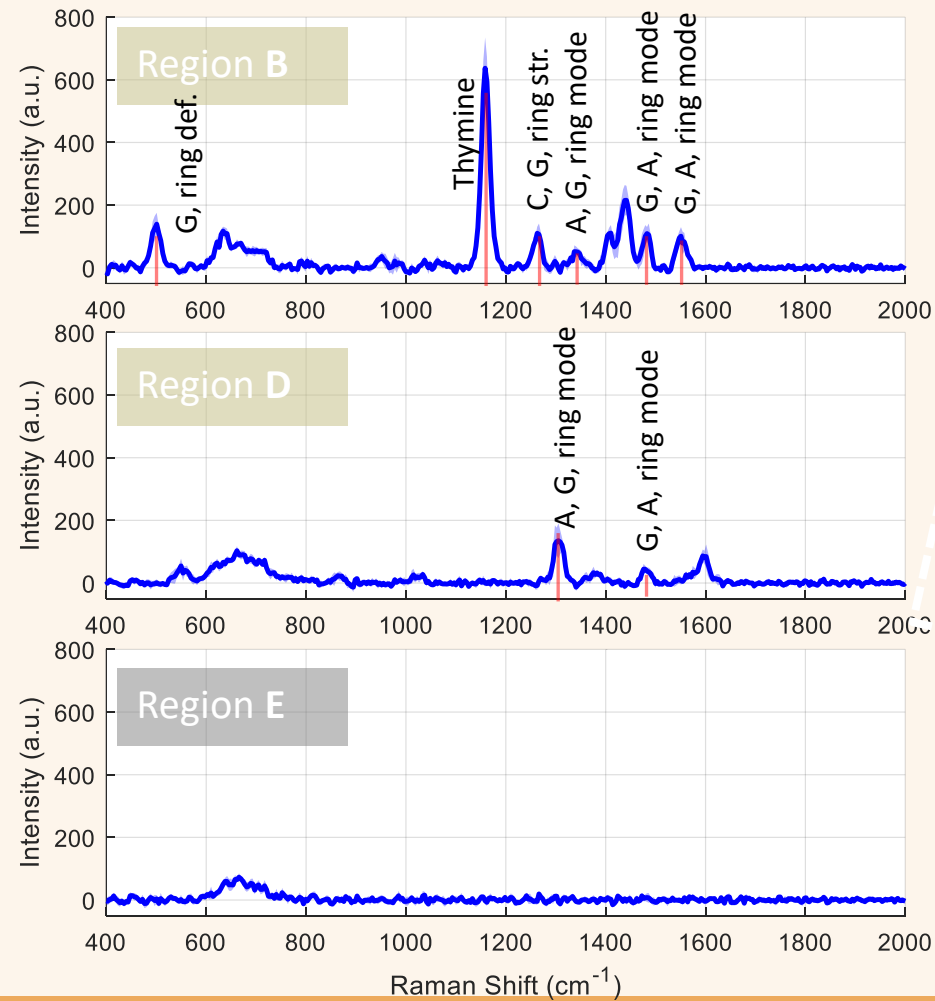


After optoporation



A-D: inside the cell
E: outside the cell

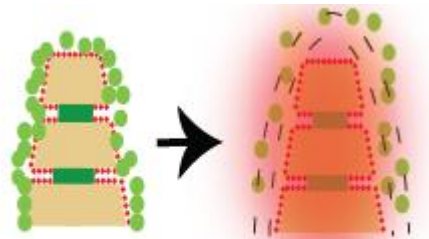
- Representative spectra in different locations
- End-point live/dead assay



Plasmonic Nano-Cavitation for Biosensing Interface Actuation

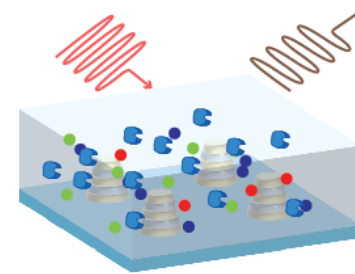
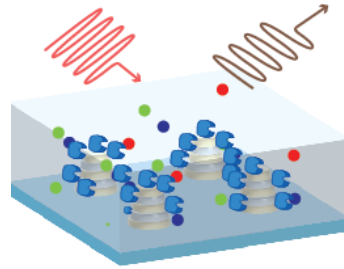
Nanodevice-level
multifunctionality:

- Wavelength-multiplexed optical multifunction
- Multi-physics sensing and actuation operations



Photoacoustic force for
hotspot regeneration

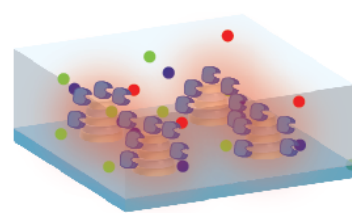
continuous wave (CW) laser



molecule 2
molecule 1 molecule 3

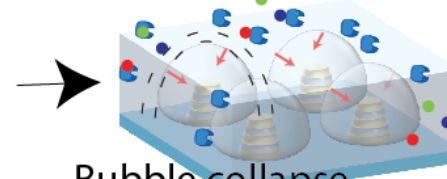
fs laser pulses

$\Delta\tau = 2 \mu\text{s}$ $f = 0.5 \text{ MHz}$



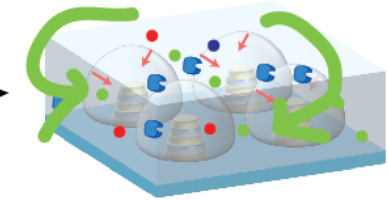
Local heating

shock waves



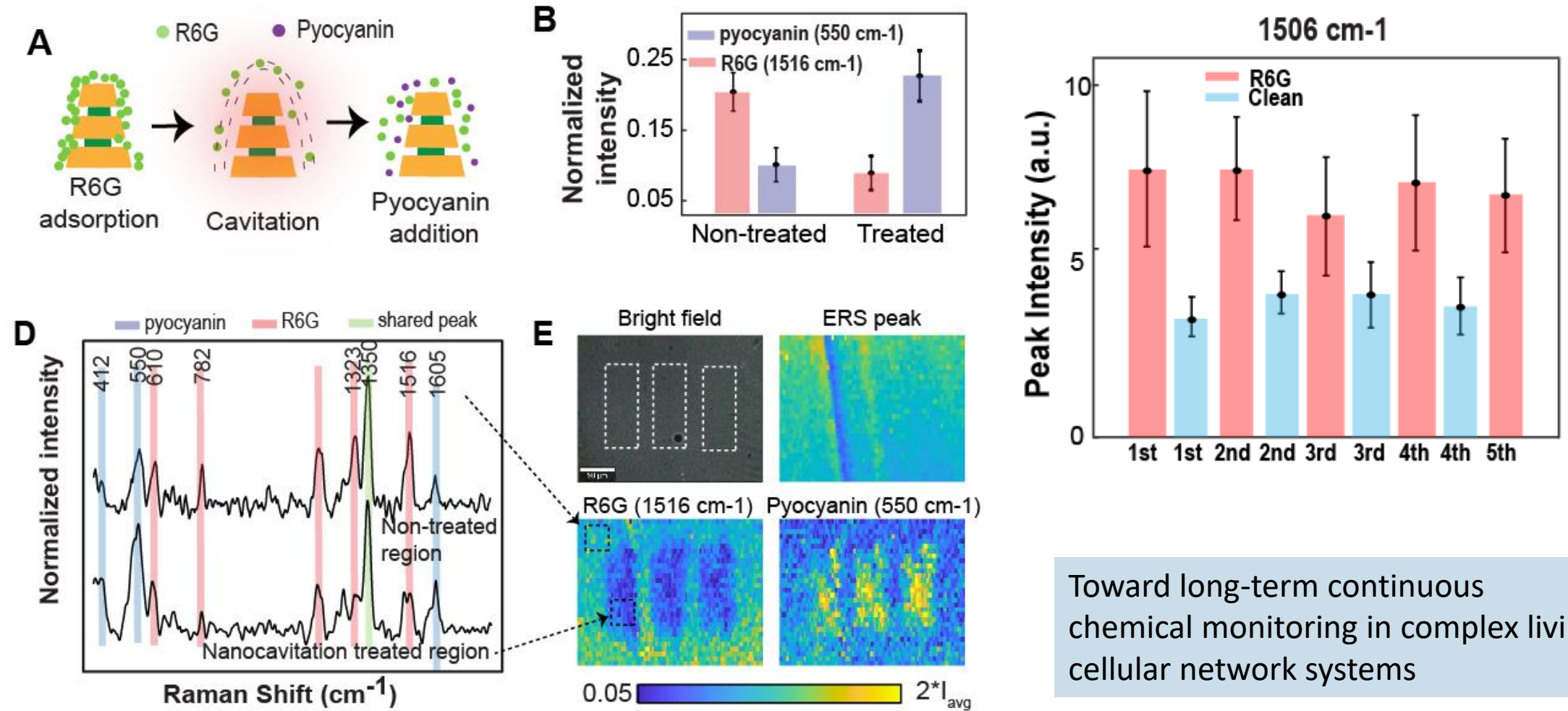
Bubble collapse

Foulant removal

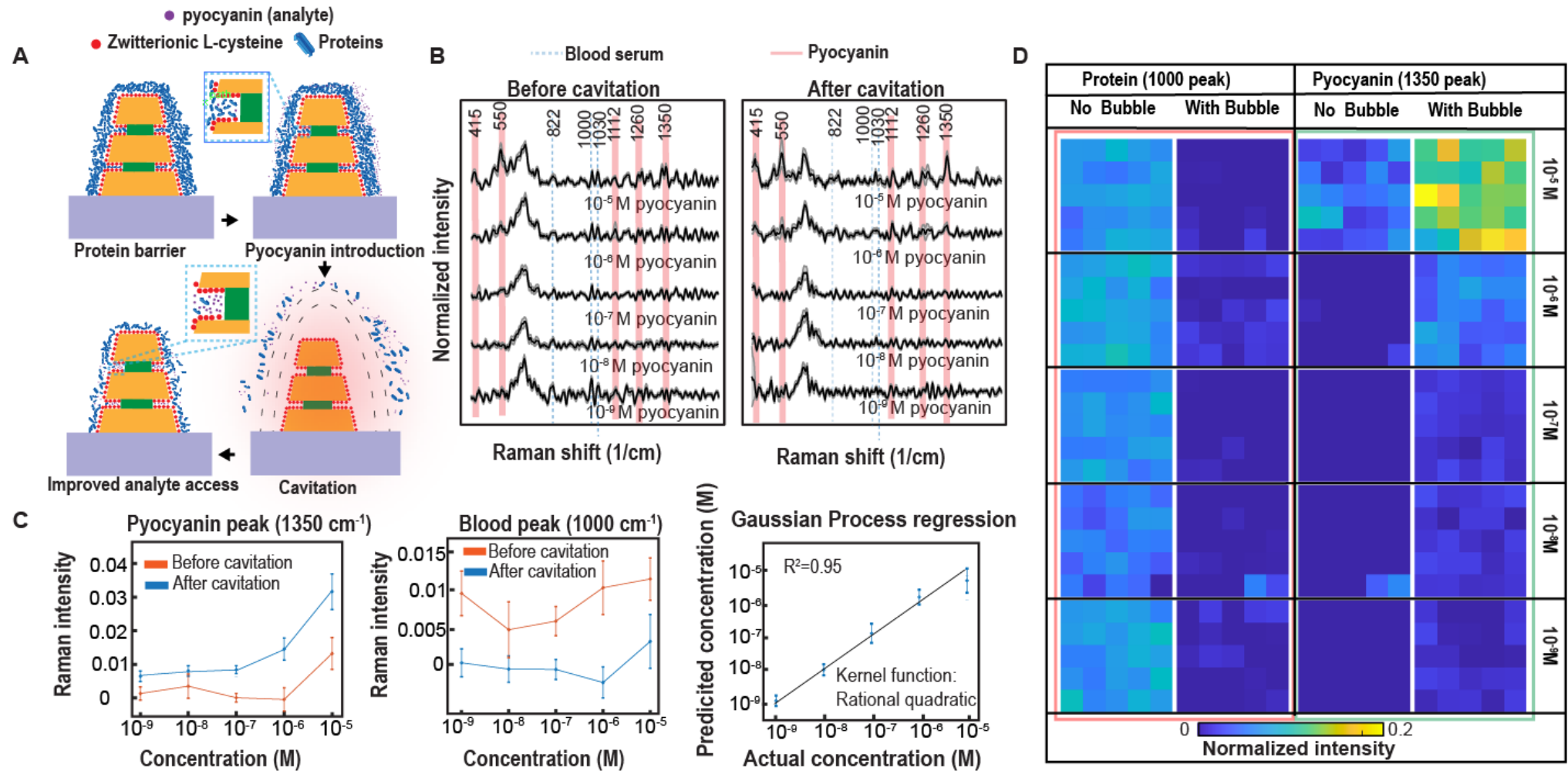


Fluid mixing

Nano-Cavitation for Raman Hotspot Regeneration

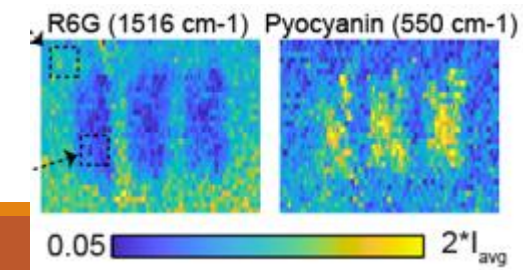
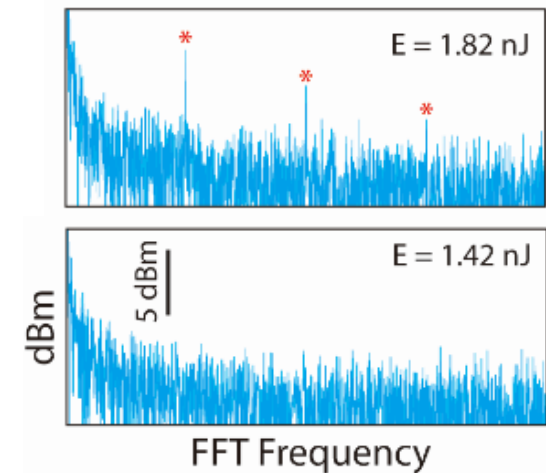
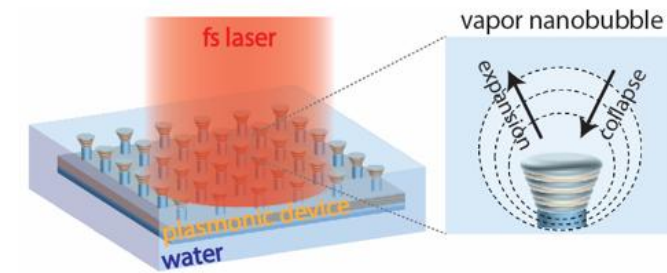
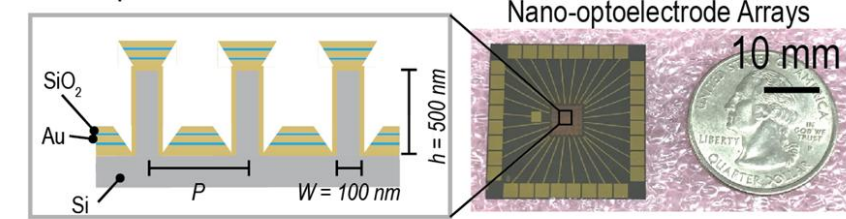


Ultrasensitive Chemical Mapping: Nano-Cavitation Hotspot Regeneration Combined with Passive Anti-fouling Coating



Key Takeaways

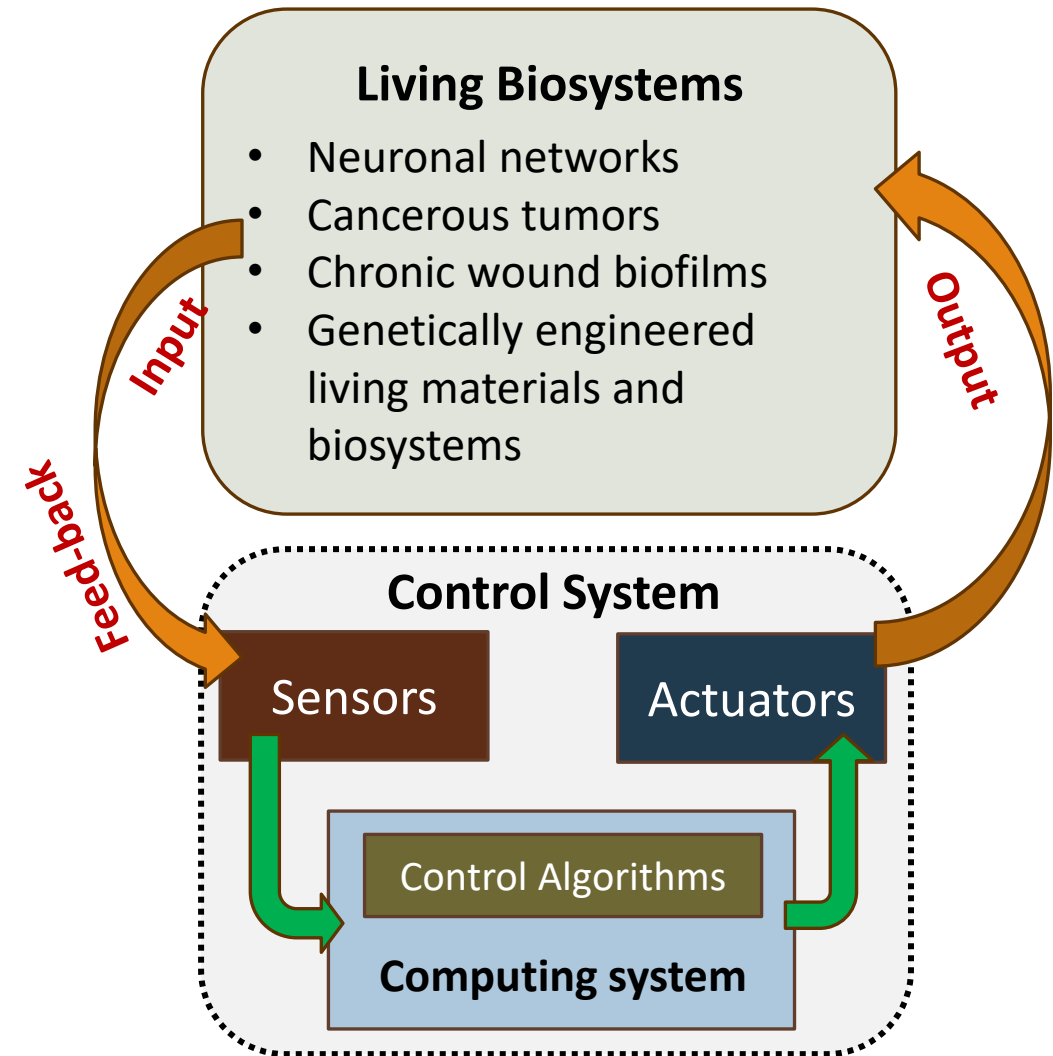
- Multiresonant plasmonic nanostructures can be integrated with micro-nanoelectrodes to create novel nano-optoelectrodes capable of both sensing and actuation.
- These multiresonant nano-optoelectrodes hold great promise as multimodal sensors and actuators, bridging the gap between cyber/physical and chemical/biological domains.
- Precisely controlled plasmonic nanocavitation, triggered by short laser pulses, enables optoporation nanosurgery with well-defined thresholds.
- This laser-induced nanocavitation can also be employed to regenerate plasmonic hotspot biosensing interfaces by removing molecular fouling.



Next Steps and Future Research

This work opens exciting avenues for future research, including:

- Instrumentation and biochip engineering
- Developing nanosurgery techniques for cells based on optoporation with improved precision and control.
- Investigating novel strategies for mitigating biofouling and enhancing the longevity of plasmonic biosensors.



Acknowledgment



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Yuming Zhao

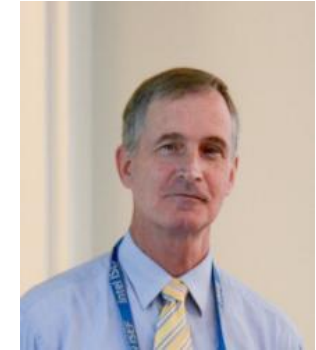
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Dr. Wenqi Zhu (NIST-PML)



Dr. Patrick Bradshaw



Drs. Nickolay V Lavrik,
Sujoy Ghosh, Bernadeta
Srijanto, Dayrl Briggs,
Dale K Hensley, Kevin C
Lester, and ...



Precision Nanosurgery and Multimodal Intracellular Monitoring of Cellular Networks by Biomimic Nano-optoelectrode Arrays

(FY24) First year report by Wei Zhou at Virginia Tech



Objectives:

- Nano-optoelectrode Array Design: Develop 2-tier nano-optoelectrode arrays optimized for tight nanodevice-cell coupling using a hierarchical modular design approach.
- Optical and Electrical Operation: Refine the design of 2-tier protruding nano-optoelectrodes to optimize their performance in multimodal optical and electrical operations.
- Extracellular Monitoring: Optimize bio-topology to enable effective multimodal extracellular monitoring of electrogenic cells.
- Nanobubble Generation: Establish optimal femtosecond (fs) laser pulse excitation conditions for reliable and controlled nanobubble generation.

Technical Approach:

- Optimized nanoantenna design: Developed tiny antennas for two types of nano-optoelectrodes (combining light and electrical sensing), enhancing both optical and electrical signals for improved biochemical detection and electrophysiology.
- Vapor nanobubble dynamics: Investigated the formation and behavior of vapor nanobubbles around a miniature metal structure when hit with a fast laser pulse, using a pump-probe laser setup and Fast Fourier Transform analysis.
- Nanoplasmonic heating simulation: Modeled how these tiny metal structures heat up when excited by extremely short laser pulses.

Accomplishments:

- Publications and IPs:
 - 2 peer-reviewed journal articles published.
 - 2 conference proceeding papers.
 - 3 journal papers in preparation.
 - 1 patent application filed.
- Presentations:
 - 4 invited presentations delivered at conferences and universities.
- Mentorship:
 - 1 PhD student graduated.

DoD Benefit: Hybrid electrical-optical nano-bio interfacing devices hold significant promise for the Department of Defense (DoD). These devices can be integrated into advanced closed-loop, multimodal bio-machine interface platforms to enhance human performance. By enabling simultaneous monitoring of both bioelectrical and biochemical activities within cells, this technology can provide crucial insights into cellular responses to environmental changes, such as those induced by chemical and biological weapons. Moreover, it can deepen our understanding of the intracellular and intercellular biochemical pathways underlying various diseases.