

NANOPHOTONICS: ENABLING TECHNOLOGY FOR BIOSENSING, SPECTROSCOPY, BIOIMAGING

Prof. Hatice ALTUG

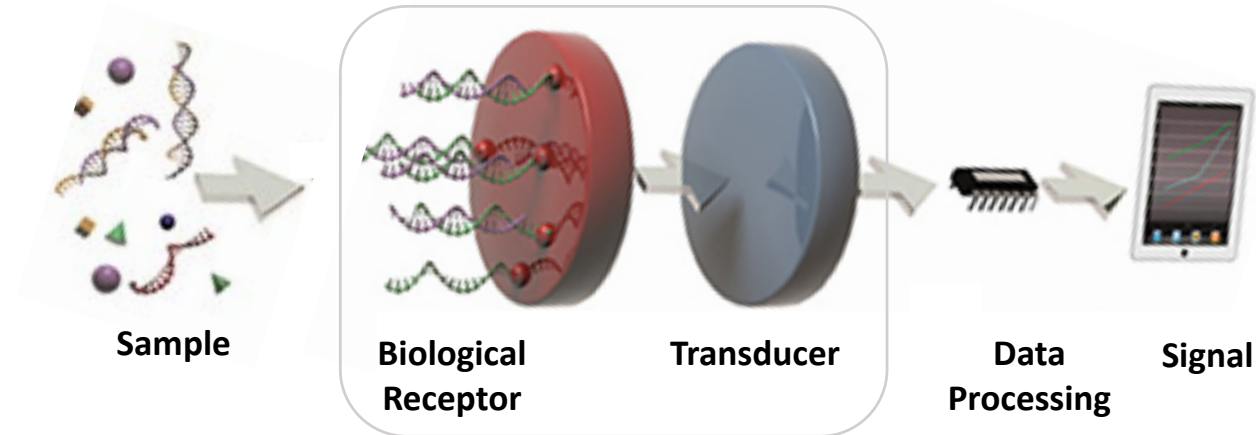
BioNanoPhotonic Systems Laboratory

École Polytechnique Fédérale de Lausanne, Switzerland

EPFL

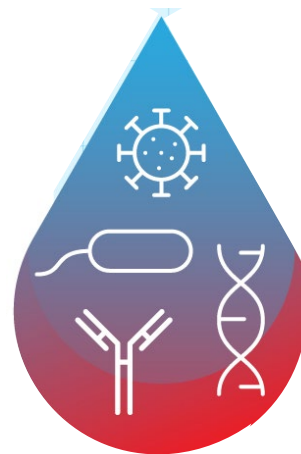
What is a biosensor?

Biosensor is an analytical device that can detect and analyze biochemical analytes



Detected Analytes:

- Small molecules (i.e. drugs)
- Biomolecules (i.e. enzyme, hormones)
- Proteins, antibodies
- Nucleic acids (DNA, RNA, miRNA)
- Extracellular vesicles (exosomes)
- Pathogens (virus, bacteria)
- Cells ...

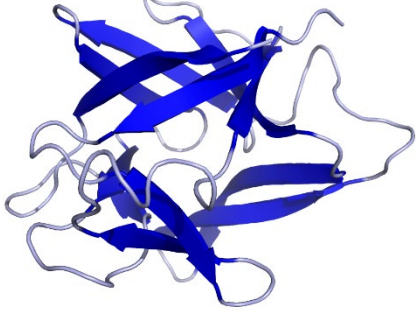


Test/Device Response:

- Yes/No indicator
- Quantitative detection
- Analyze interactions & kinetics
- Extract chemical/conformational information ...

Biosensors have a wide range of applications

Life Sciences



Pharma



Diagnostics



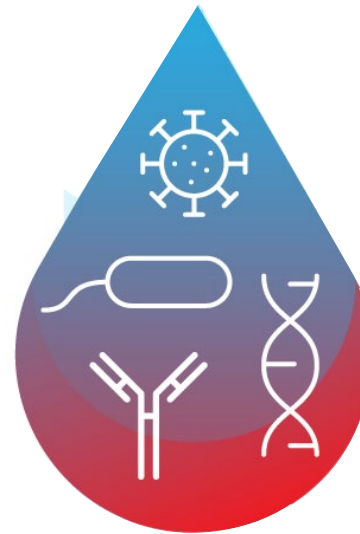
Enviromental



Food Safety



Security



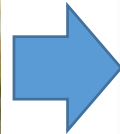
Detected Analytes:

- Small molecules (i.e. drugs)
- Biomolecules (i.e. proteins, antibodies)
- Nucleic acids (DNA, RNA, miRNA)
- Extracellular vesicles (exosomes)
- Pathogens (virus, bacteria)
- Cells

BIOSENSORS in DIAGNOSTICS

- Diagnostics is the **FIRST** step in medical care
- Follow-up treatments are based on diagnosis

PCR Technology



Advantageous:

- Provides accurate results
- High-throughput

Limitations:

- Time consuming
- Centralized labs
- Expensive instrumentation
- Trained personnel
- Not available to everyone – *resource-poor settings*

COVID-19 EXPERIENCE!

Rapid Tests (Lateral Flow Assays)



Advantageous:

- Point-of-care technology
- Low-cost
- Used by anyone
- Provides rapid results

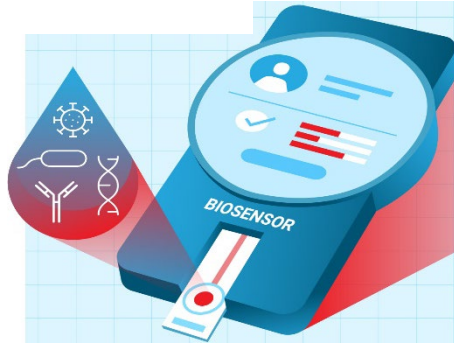
Limitations:

- Not accurate/sensitive
- Not quantitative
- Limited multiplexing capability

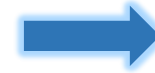
Diagnostics - Tomorrow



sample



Bioanalytical device



treatment



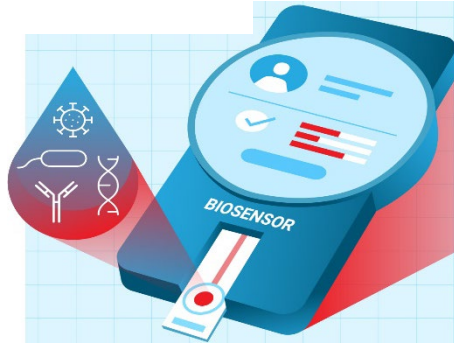
Global health care
Point-of-care diagnostics
Personalized medicine



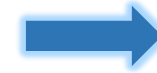
Diagnostics - Tomorrow



sample



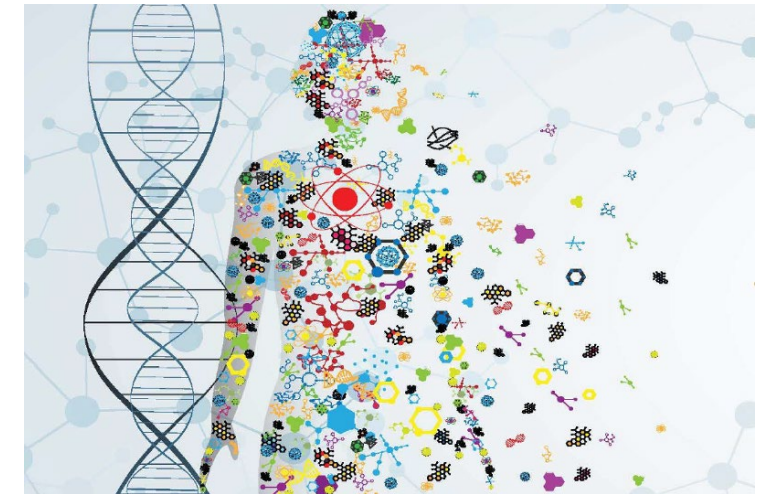
Bioanalytical device



treatment

- ☐ Sensitive
- ☐ Throughput
- ☐ Multiplexed
- ☐ Real-time
- ☐ Low-cost
- ☐ Rapid
- ☐ Integrated
- ☐ Portable
- ☐ Intelligent
- ☐ Autonomous

Global health care
Point-of-care diagnostics
Personalized medicine



Next-generation biosensors will be everywhere!

- ❑ Sensors that can measure everything and everywhere will serve at the nodes of the “Internet-of-body” & “internet-of-things”
- ❑ ... connected devices enable **smarter systems**

REVIEW ARTICLE

NATURE NANOTECHNOLOGY

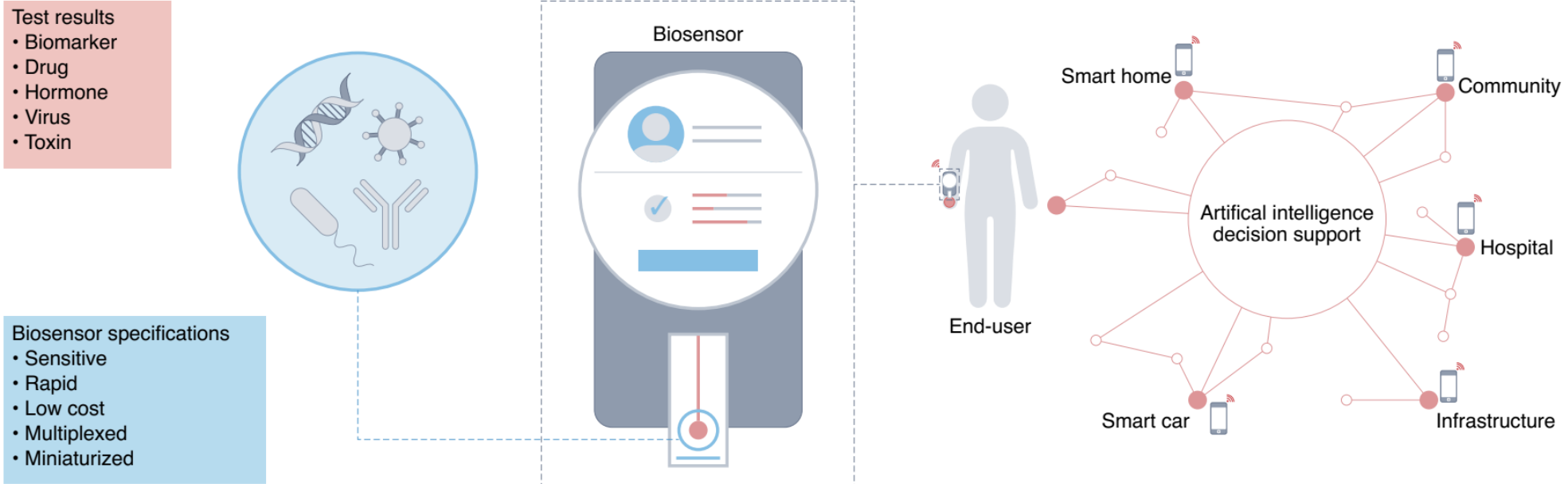
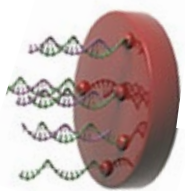


Fig. 1 | Future of biosensors. Biosensors detect a broad range of analytes in a variety of settings and are connected within the Internet of Things.

MAIN BIOSENSOR PARAMETERS

- ☐ **Response:**
...Yes/No or Quantitative

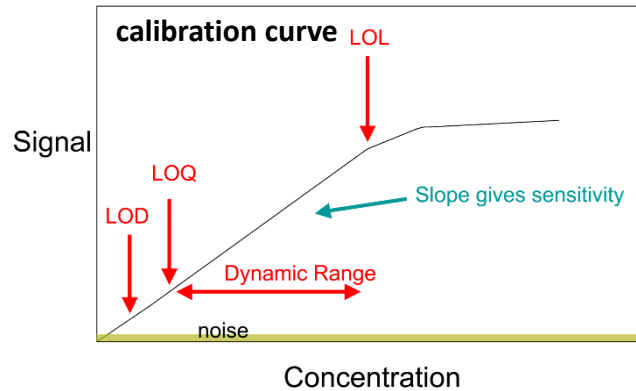


- ☐ **Selectivity**
Affinity based
(surface functionalization)

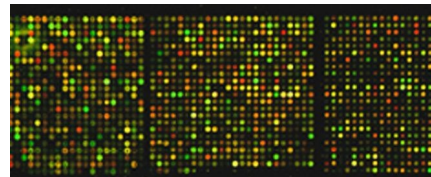
- ☐ **Reliability (Accuracy)**

Sick	Healthy
TRUE POSITIVES (TP)	FALSE POSITIVES (FP)
FALSE NEGATIVES (FN)	TRUE NEGATIVES (TN)
Sensitivity $\frac{TP}{TP + FN}$	Specificity $\frac{TN}{TN + FP}$

- ☐ **Limit of Detection (LoD) & Dynamic Range**
fg/mL, pg/mL, ng/mL, mg/mL..



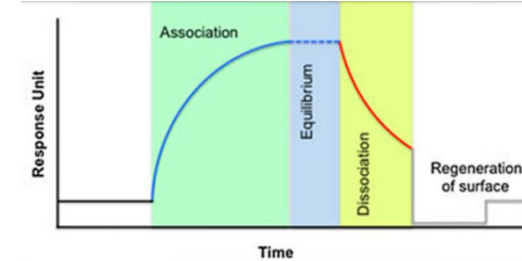
- ☐ **Multiplexing**
Microarray



- ☐ **Throughput**
How many samples per test?

- ☐ **Cost**
Reader, cartridge

- ☐ **Time Resolution & Response Time**
End point, Real-time (Kinetics) or Continuous



- ☐ **Sample Volume**
... pL, nL, uL ...

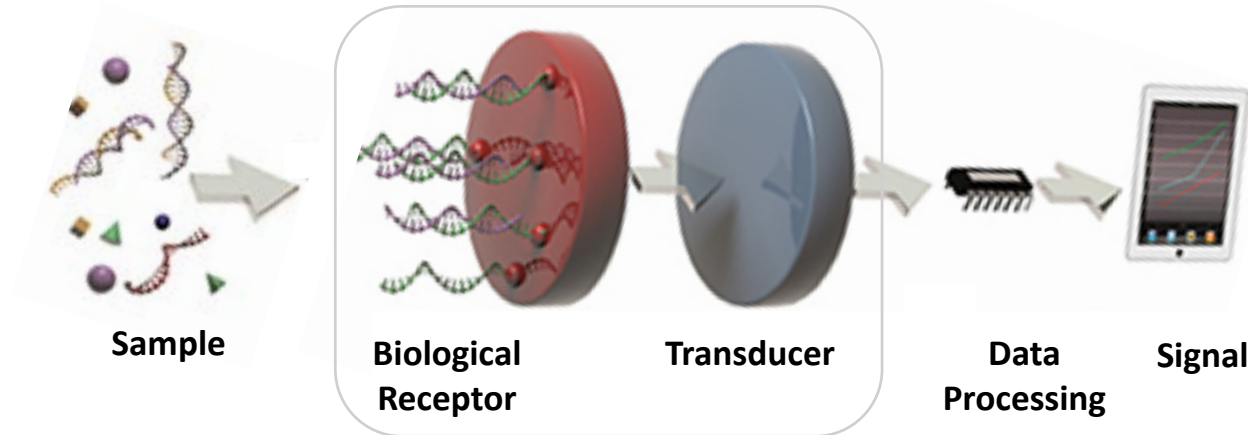
- ☐ **Automation & Sample Handling**
Microfluidics ..

- ☐ **Single-Use (Disposable) or Multiple Use**
Cartridge? Fixed Reader?

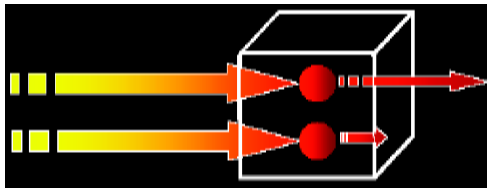
- ☐ **Footprint & Integration**
Bench-top, Portable, Wearable..

Optical Transduction Mechanisms

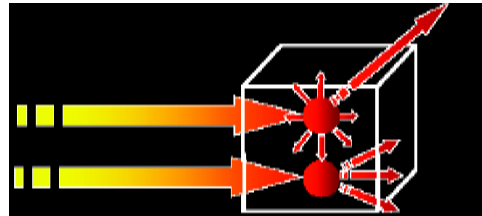
Biosensor is an analytical device that can detect and analyze biochemical substances



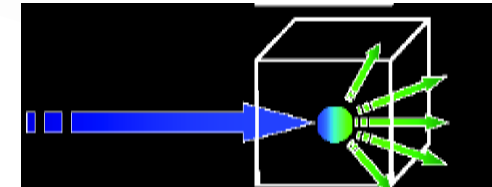
absorption



scattering



fluorescence



UV-Vis absorption

IR absorption

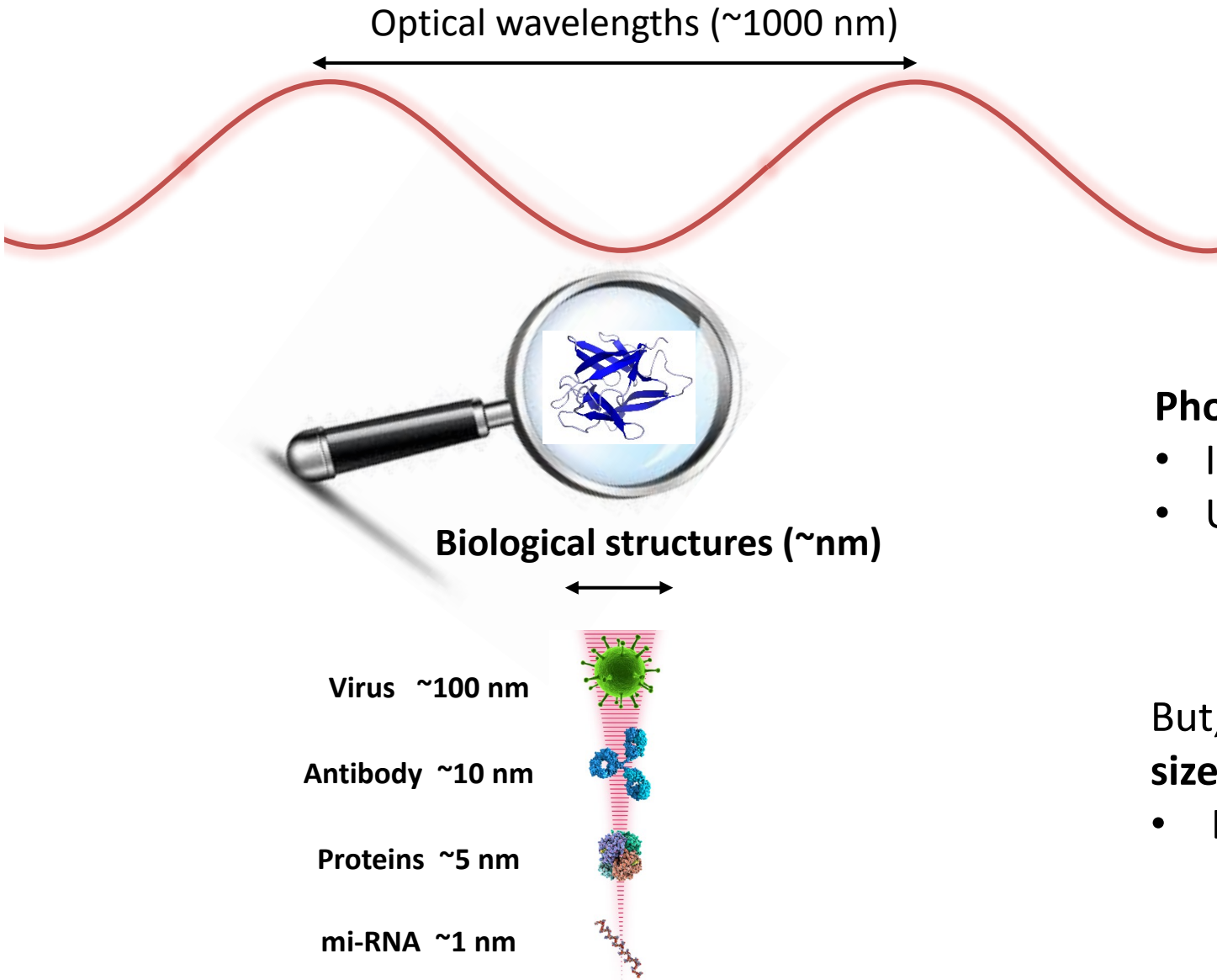
Raman scattering

Bioimaging & Sensing

Refractometric detection

Hyperspectral imaging

WHY NANO-PHOTONICS ?



Photonics provides:

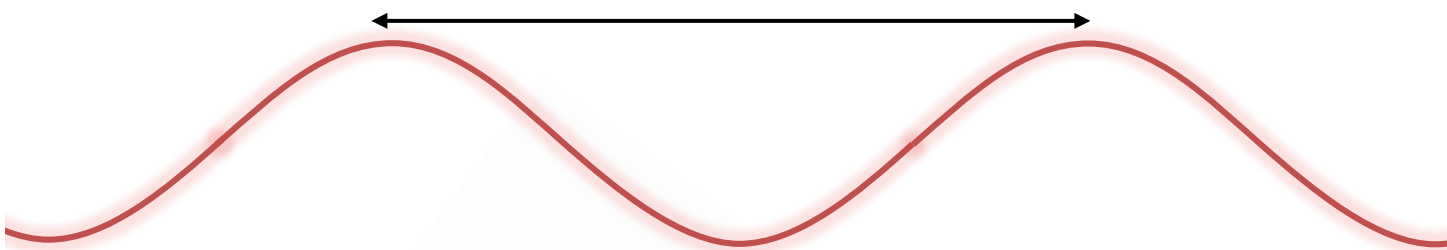
- Immunity to electromagnetic interferences
- Ultra-wide bandwidth ...

But, micron long optical wavelengths introduces **size mismatch between** light & nanometric samples:

- Lower performance

WHY NANO-PHOTONICS ?

Optical wavelengths (~ 1000 nm)



Biological structures ($\sim$ nm)



Virus ~ 100 nm

Antibody ~ 10 nm

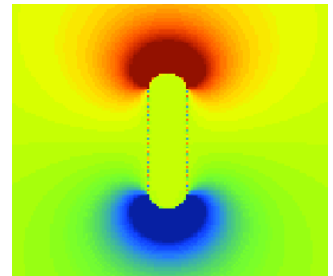
Proteins ~ 5 nm

mi-RNA ~ 1 nm

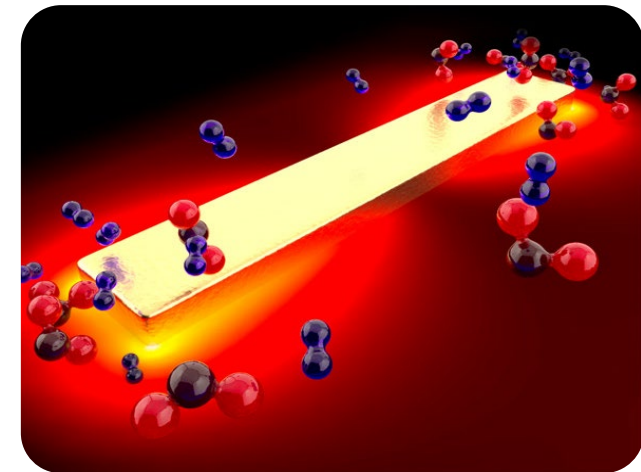


Optical nano-resonators

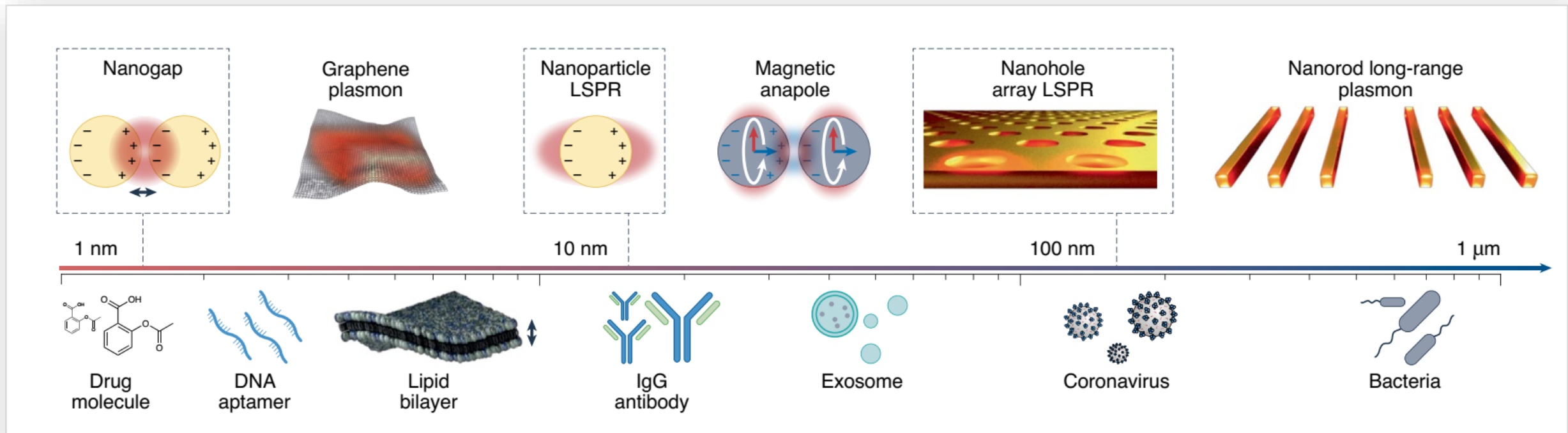
- Focus light into nanoscale volumes
- Enable strong light-matter interaction
- Ideal to achieve high sensitivity, small footprint, multiplexed integration, ...



Eg: Metal nanorod



Localization of E&M field in nanophotonic structures

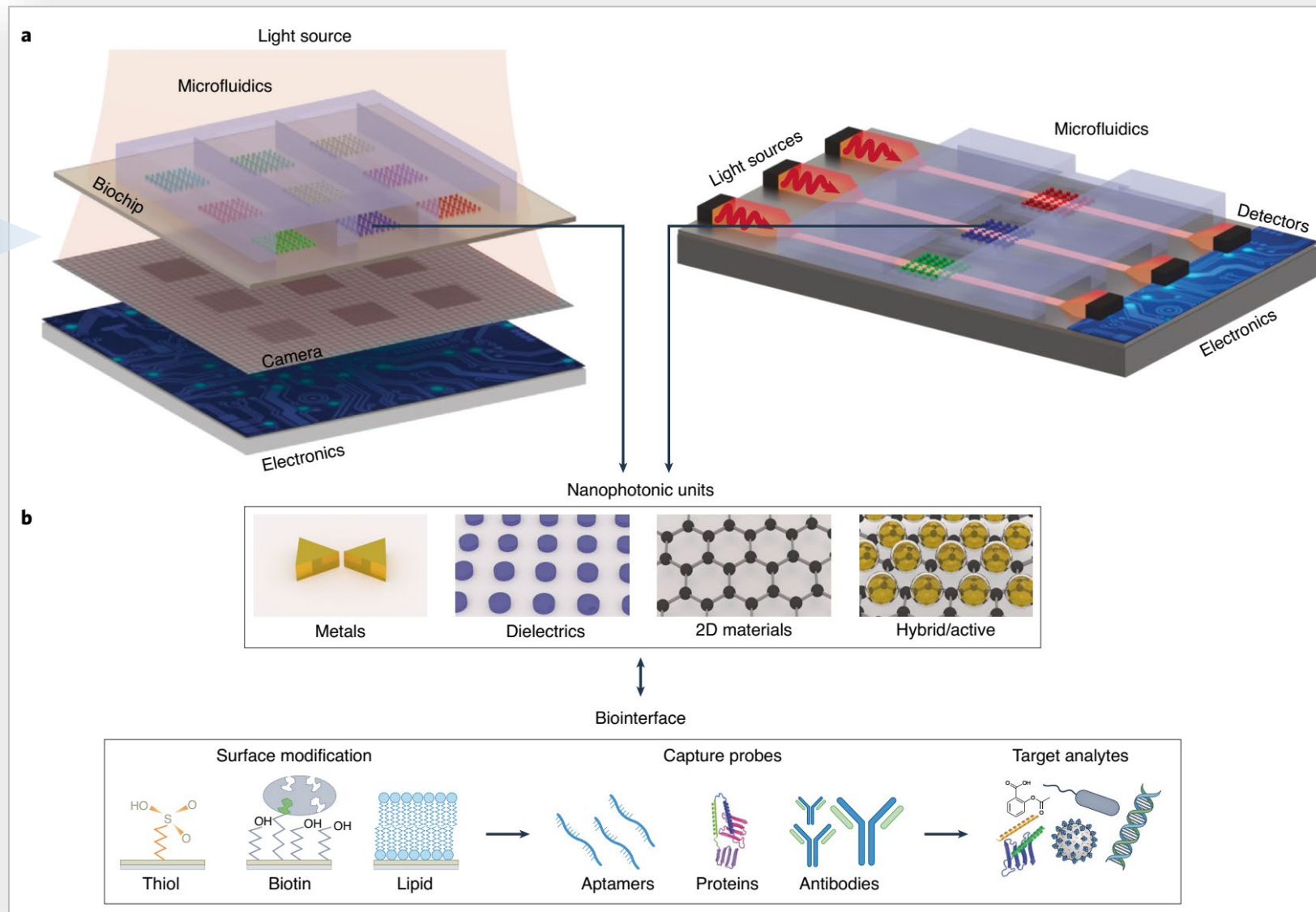


Size comparison between common biological analytes and the decay length of the evanescent field in various nanophotonic structures

Integration Schemes

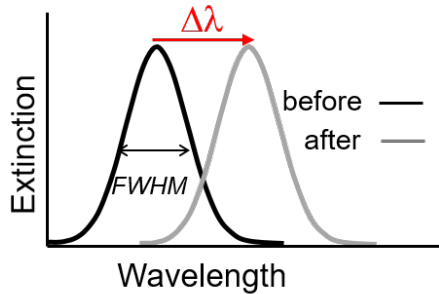
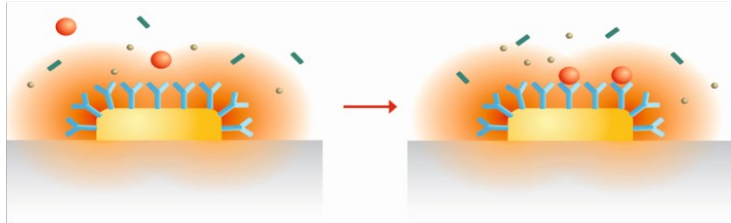
Vertical
Integration

Planar
Integration



Refractometric affinity sensing

receptor analyte



Refractive index sensitivity

$$S_{\text{bulk}} = \Delta\lambda / \Delta n$$

$$S_{\text{surface}} \sim S_{\text{bulk}} [1 - \exp(-2t/l_d)]$$

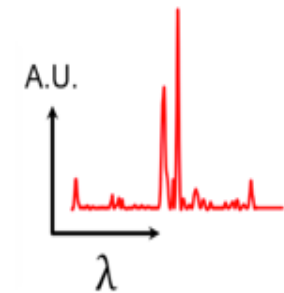
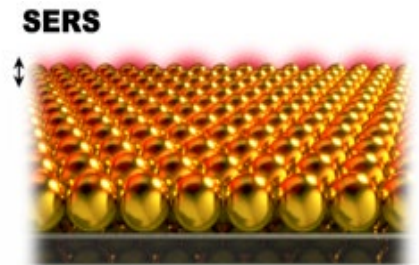
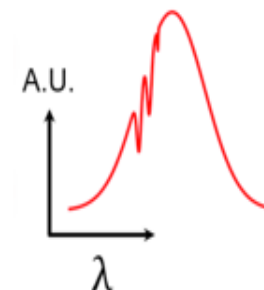
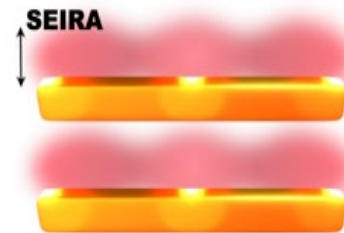
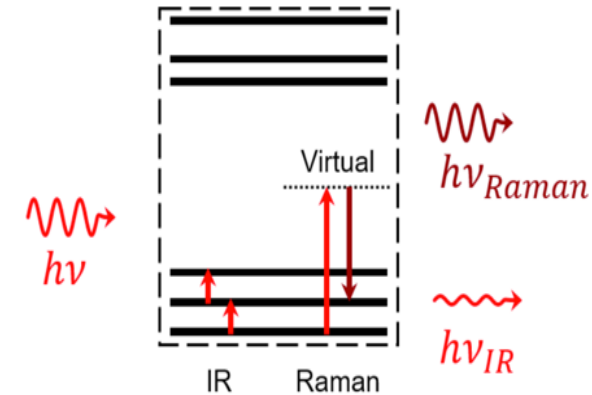
$$\text{FOM} = S / \text{FWHM}$$

$$Q = \lambda_R / \text{FWHM}$$

Resonance engineering

- High field enhancement
- Spatial overlap between analyte & enhanced near-fields
- Q factor
- Resonance wavelength

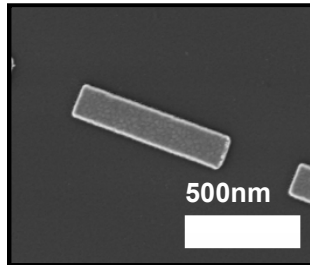
Surface enhanced spectroscopic sensing



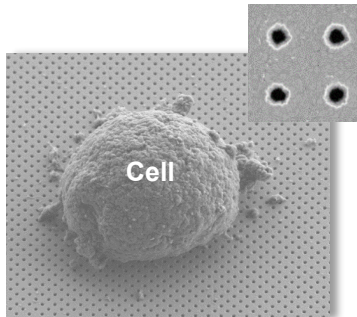
Control optical response and functionality of nano-resonators with:

- Material choice
 - Metals, 2D materials, dielectrics, ...
- Shape, size & arrangement of nanostructures

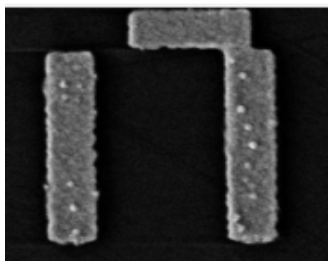
Metallic Plasmonics



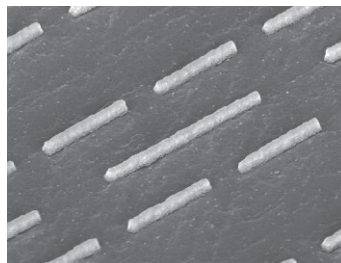
PNAS 2009



Small 2018

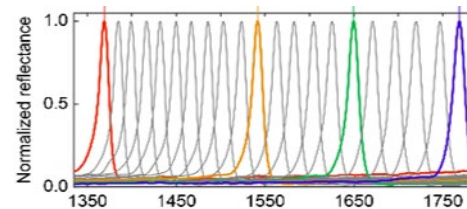
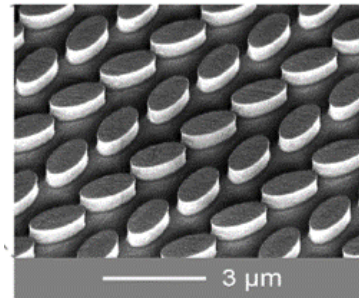


Nature Materials 2012



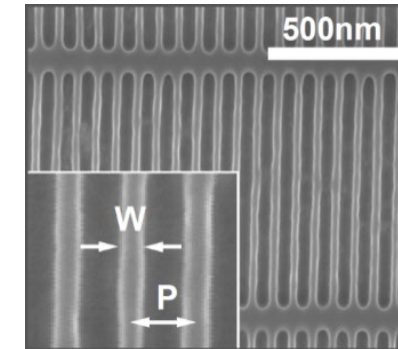
Nature Comm. 2018

Dielectric Metasurfaces

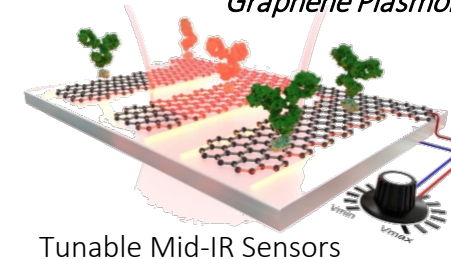


Science 2018,
Science Advance 2019
Nature Photonics 2019
Nature Nanotechnology 2019

2D Quantum Materials

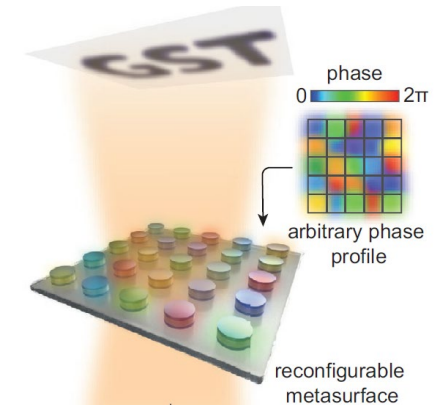


Graphene Plasmons



Tunable Mid-IR Sensors
Science 2015
Light Science & App 2017

Hybrid System



*Combine
different materials & systems*

Advanced Functional Materials 2020
Nano Letters 2020

BIOS: Research Directions

Point-of-Care Diagnostics



- Low-cost, portable, rapid & multiplexed microarray technologies.
- New device schemes including lens-free microscopy, hyperspectral imaging ...



PNAS 2010 ..

Light Science and Technology 2014 & 2018

ACS Photonics 2015, patent granted

ACS Nano 2018

Nature Photonics 2019

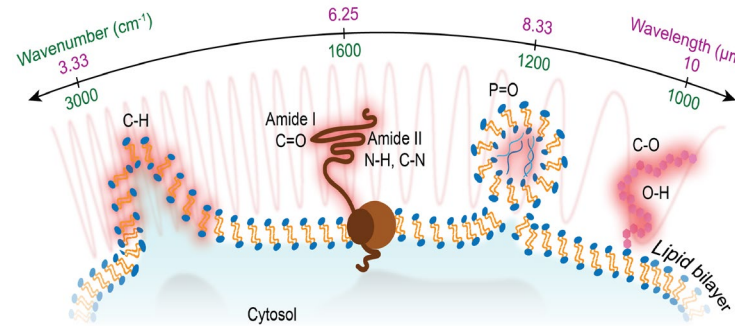
Small 2020

Nature Communications 2021 ...

Surface Enhanced Mid-IR Spectroscopy



- Chemical-specific detection
- Conformational sensitive



PNAS 2009 ...

Nature Communications 2013, patent granted

Science 2015 & 2018

Nano Letters 2016, 2018

Light Science & Applications 2016 & 2017

Nature Communications 2018

ACS Sensor 2018

ACS Photonics 2018

Advanced Materials 2021 & 2022

Science Advances 2019 & 2023 ...

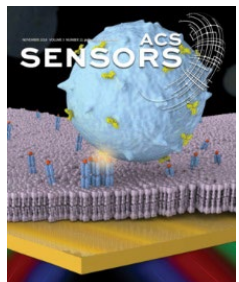
Live Cell & Single-Cell Technologies



- Cell screening & profiling, e.g. for immunoengineering applications
- Real-time & label-free secretion analysis from single cells
- Cell-cell interactions & incorporation of biomimetic surfaces



Lab on a Chip



Lab-on-a-Chip 2017

ACS Sensors 2018

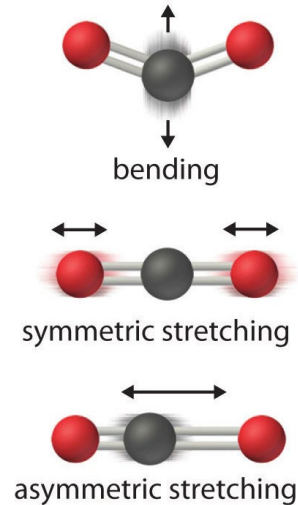
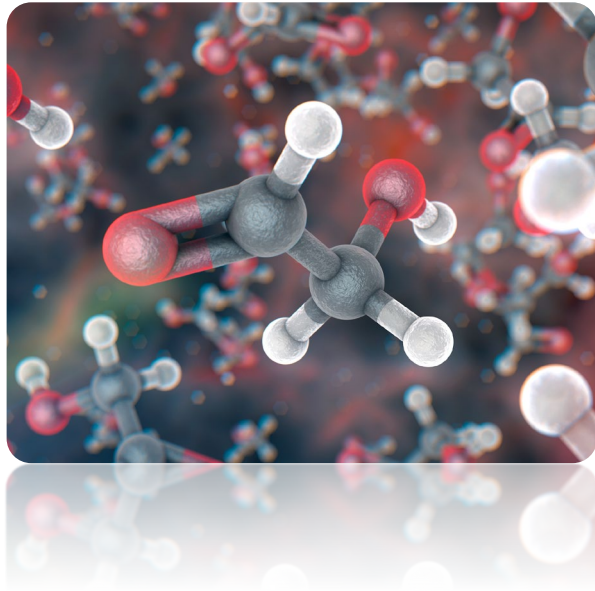
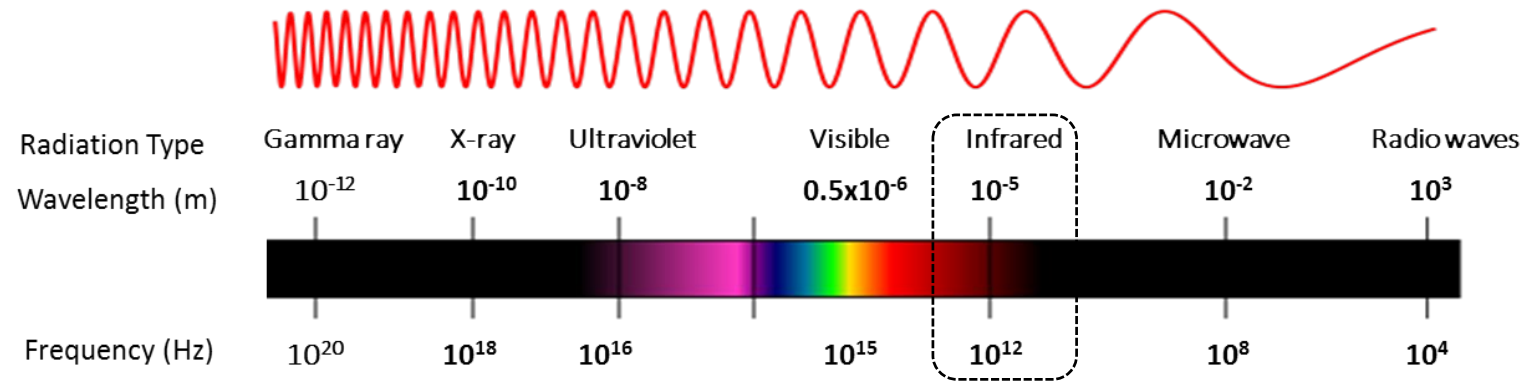
Small 2018

Science Advances 2021

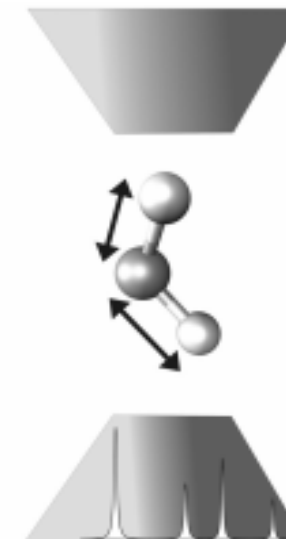
Biosensors and Bioelectronics 2022

Nature Biomedical Engineering 2023 ...

Infrared spectroscopy provides chemical specific identification



Characteristic vibration motions of molecules

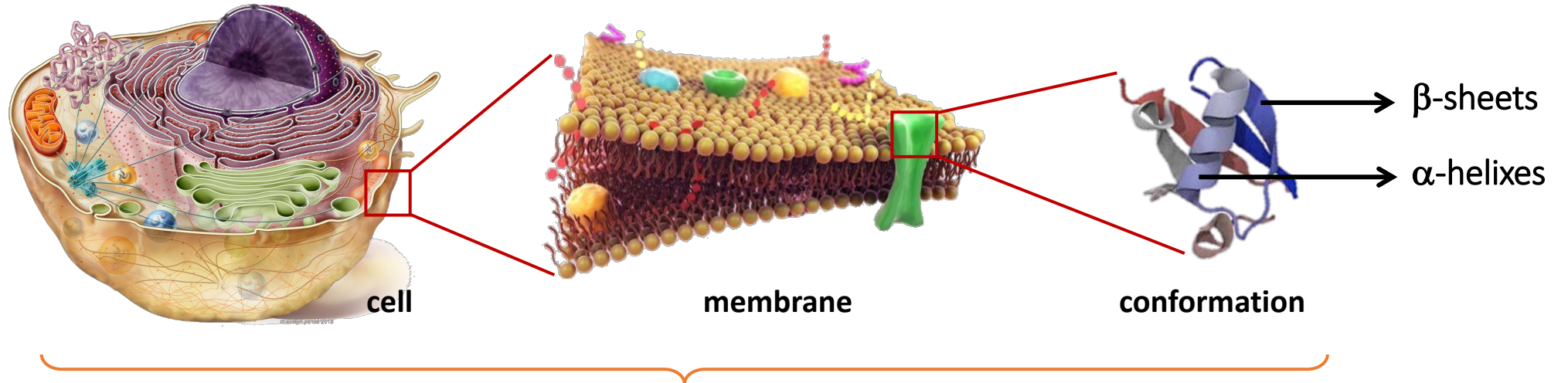


detected via absorption of infrared light

molecular fingerprints

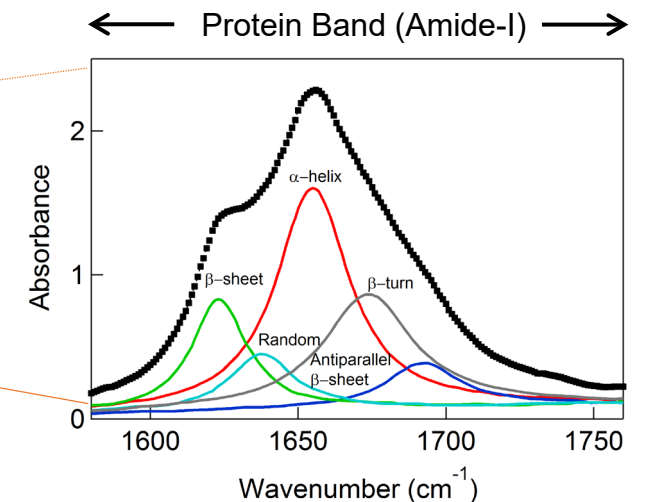
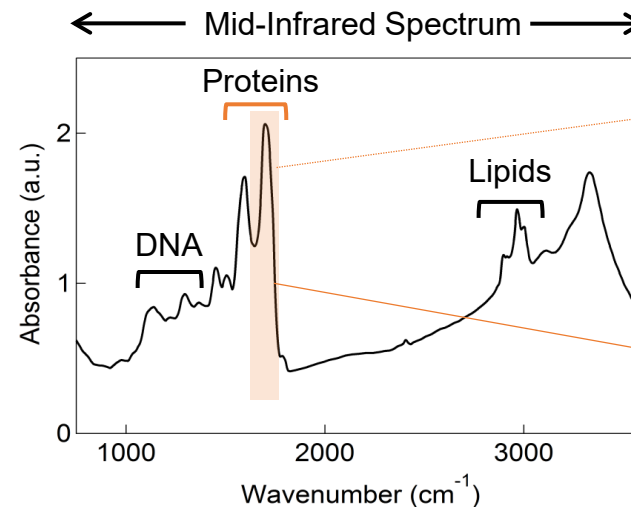
- Chemical specific
 - Label-free

Mid-infrared sensing for biology



non invasive access to
molecular vibrations

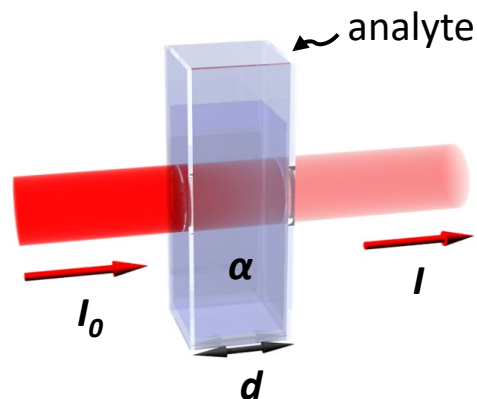
- signal intrinsic to molecular structure
- Resolves secondary structure from amide I band shape



low sensitivity

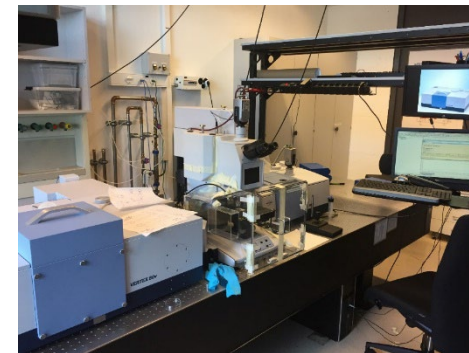
Beer's law $T = \frac{I}{I_0} \sim \exp(-\alpha d)$

- Prohibits measurements from monolayers with nanometer thickness
- Requires extremely high concentrations



bulky & expensive instrumentation

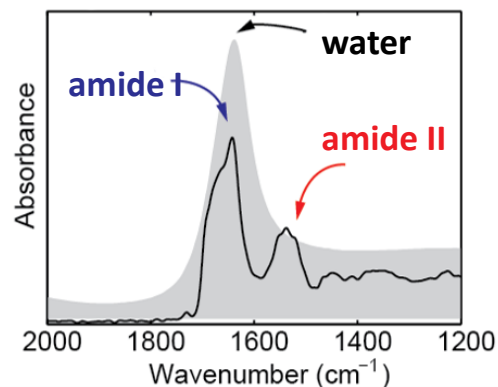
- State-of-the-art is the table-top FTIR



water absorption

Water bands **overlap** with the **vibrational signatures of biomolecules**

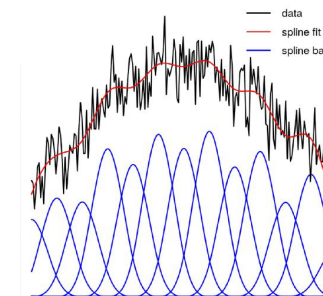
- Requires sample drying or D₂O which are not compatible with living systems (i.e. cells), resolving kinetics, conformational changes ...



* Adato et al., *Nat. Comm.* **4**, 2154 (2013)

spectral overlap

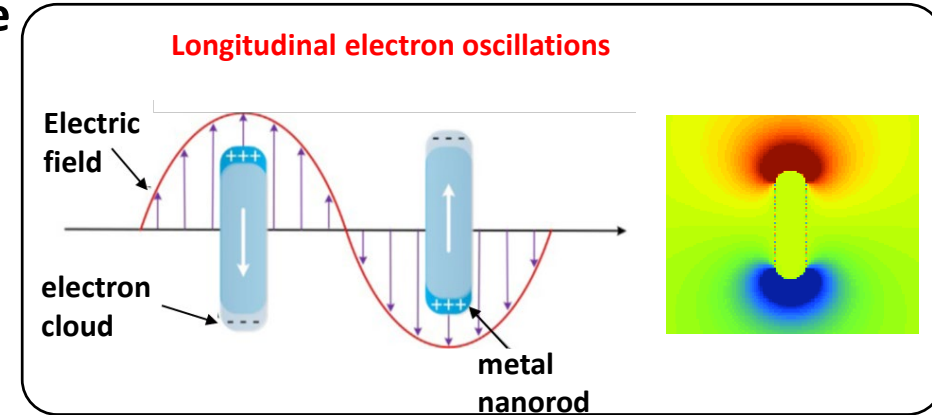
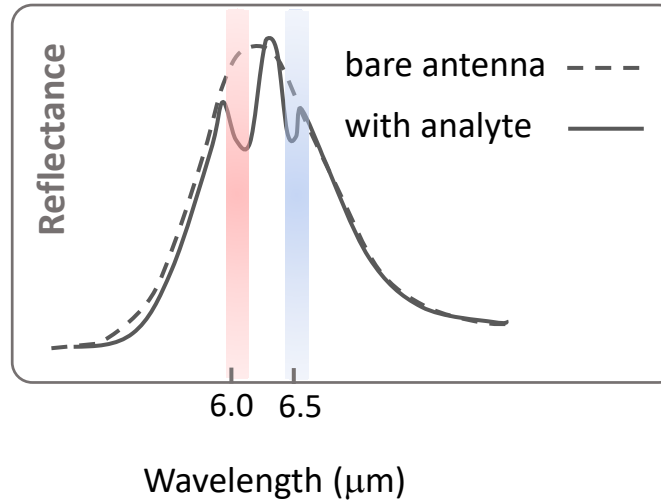
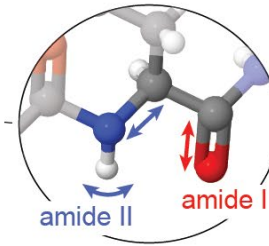
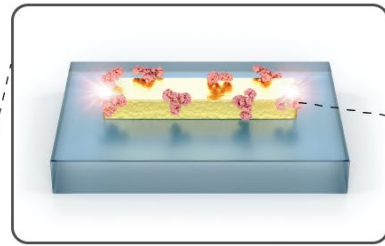
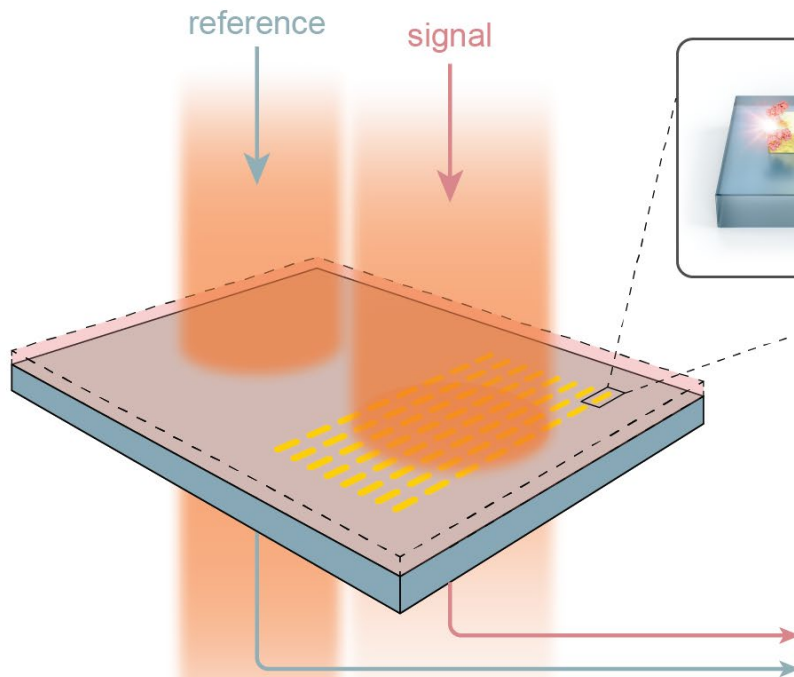
- Complex sample analysis require chemometrics



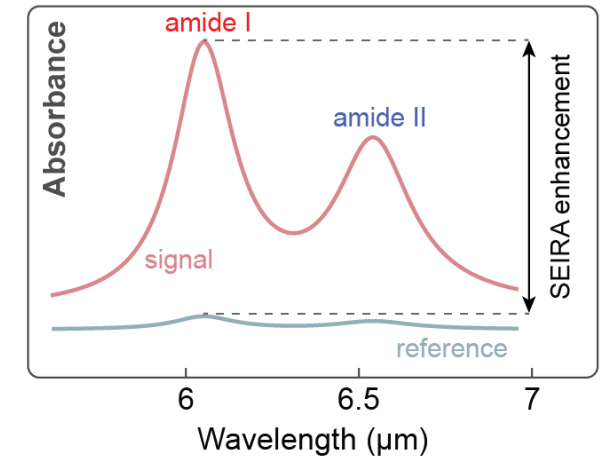
Wilson, M., 2021. 85(1).

Surface-enhanced IR absorbance spectroscopy (SEIRA)

- IR absorbance $\propto$ Oscillator strength of molecular bond $\times |\text{Electric field}|^2$
- Optical nanoantennas can be used to **boost** IR absorbance

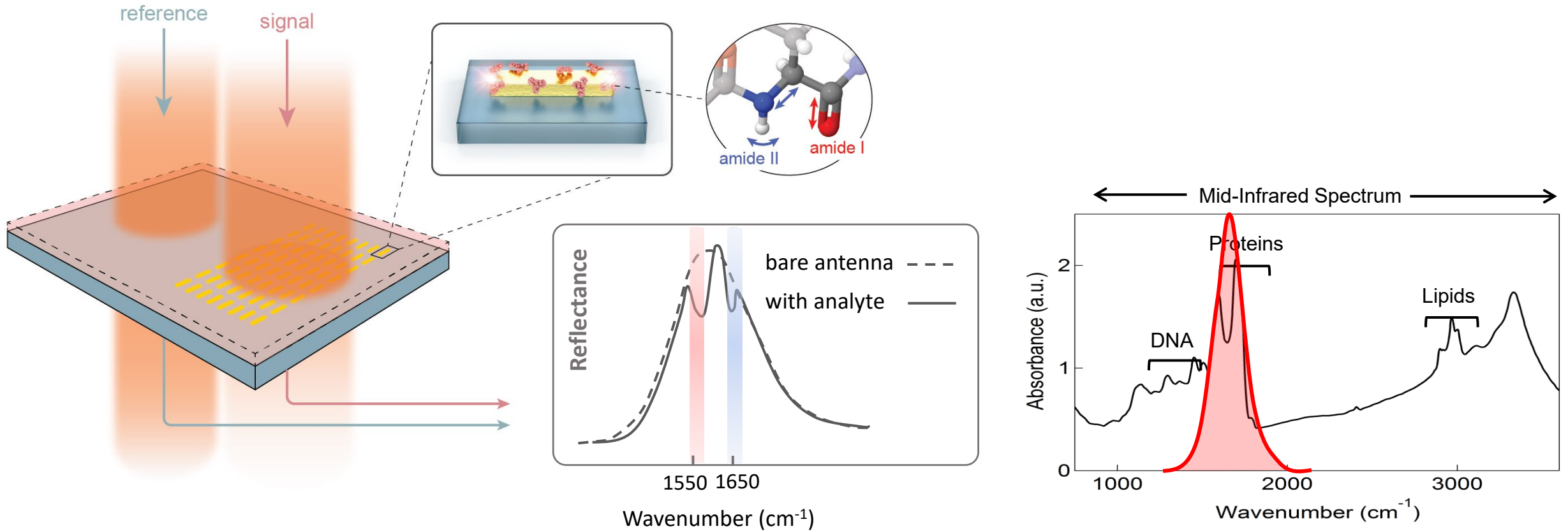


Signal processing



Surface-enhanced IR absorbance spectroscopy (SEIRA)

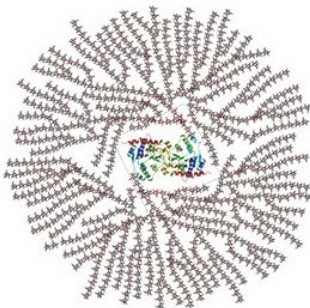
- IR absorbance $\propto$ Oscillator strength of molecular bond $\times$ |Electric field|²
- Optical **nanoantennas** can be used to **boost IR absorbance**.



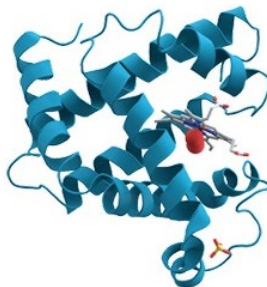
- Mid-IR spectroscopy is broadband & provides rich information
- If a single/narrow resonance is used, collected spectral information will be limited

Life is formed by Biomolecules from Four Major Classes

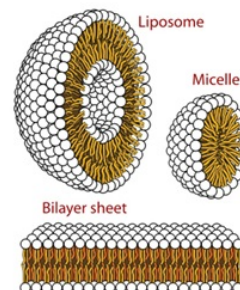
Carbohydrates



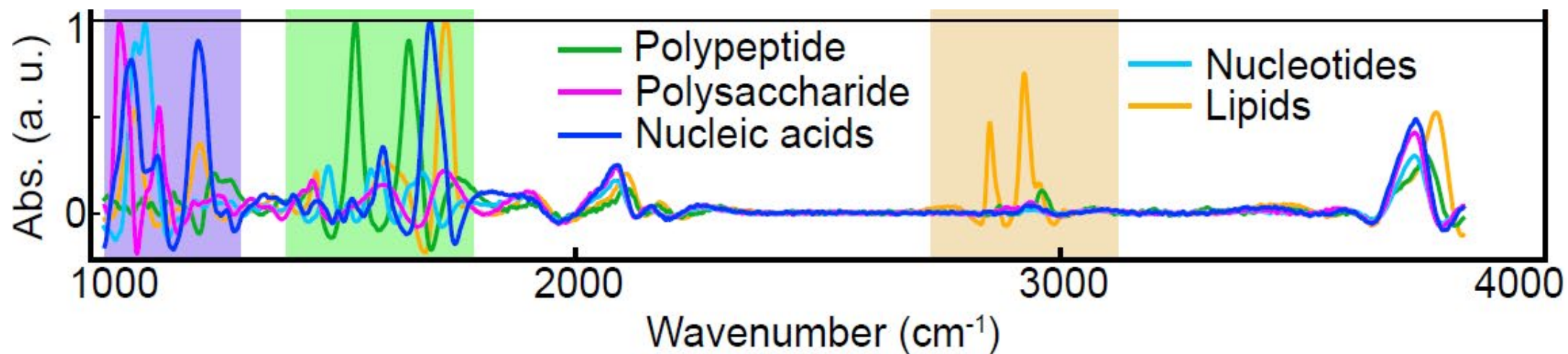
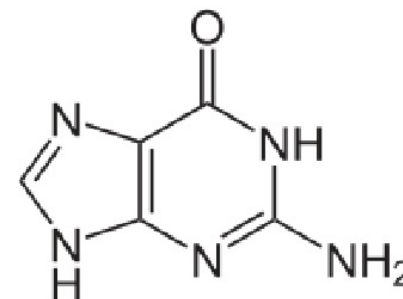
Proteins



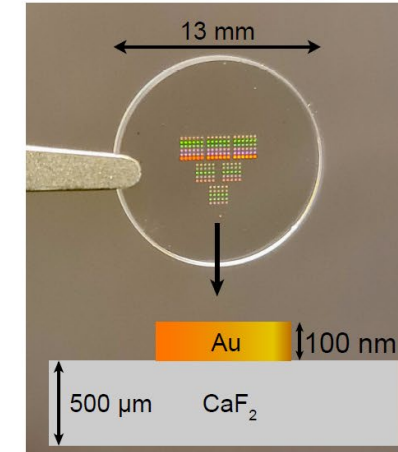
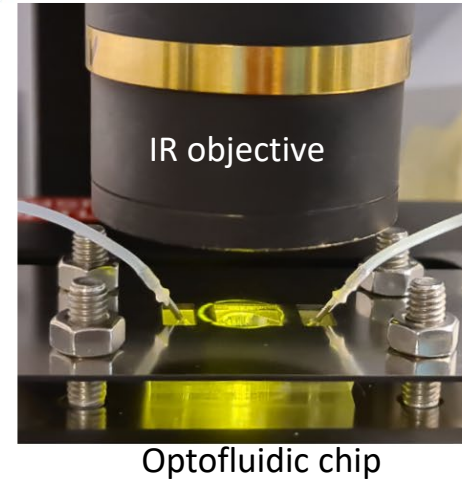
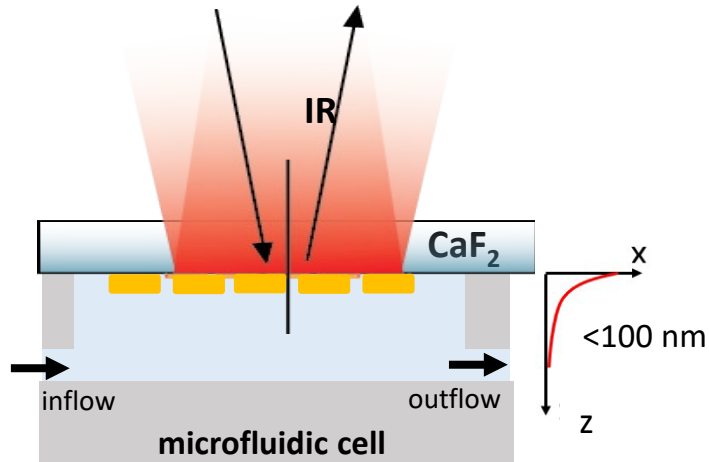
Lipids



Nucleic Acids

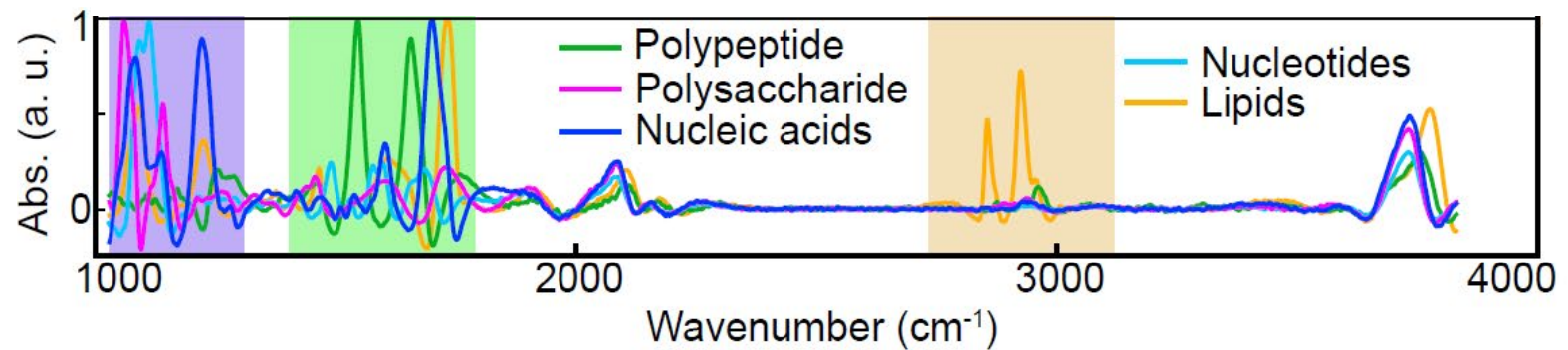
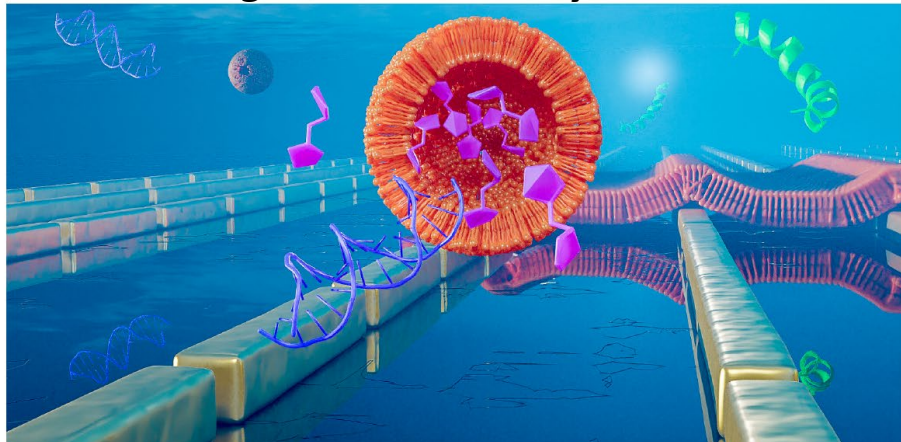


Real-time SEIRA measurements in aqueous media - nanoplasmonic metasurfaces

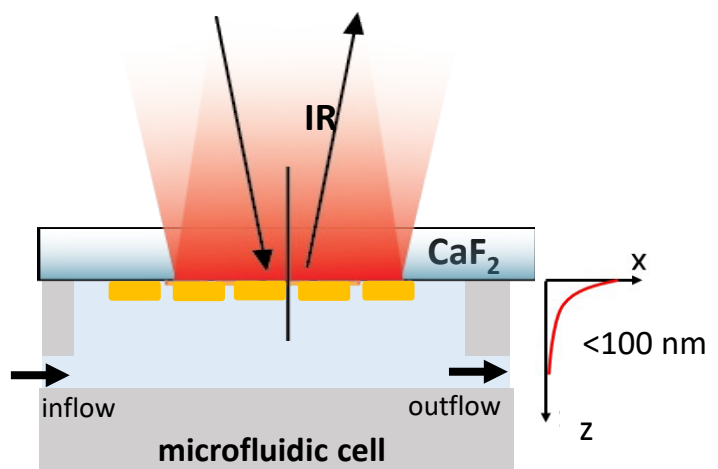


Nature Communications 2013, patent granted
Nano Letters 2016
Nature Communications 2018
Advanced Materials, 2021

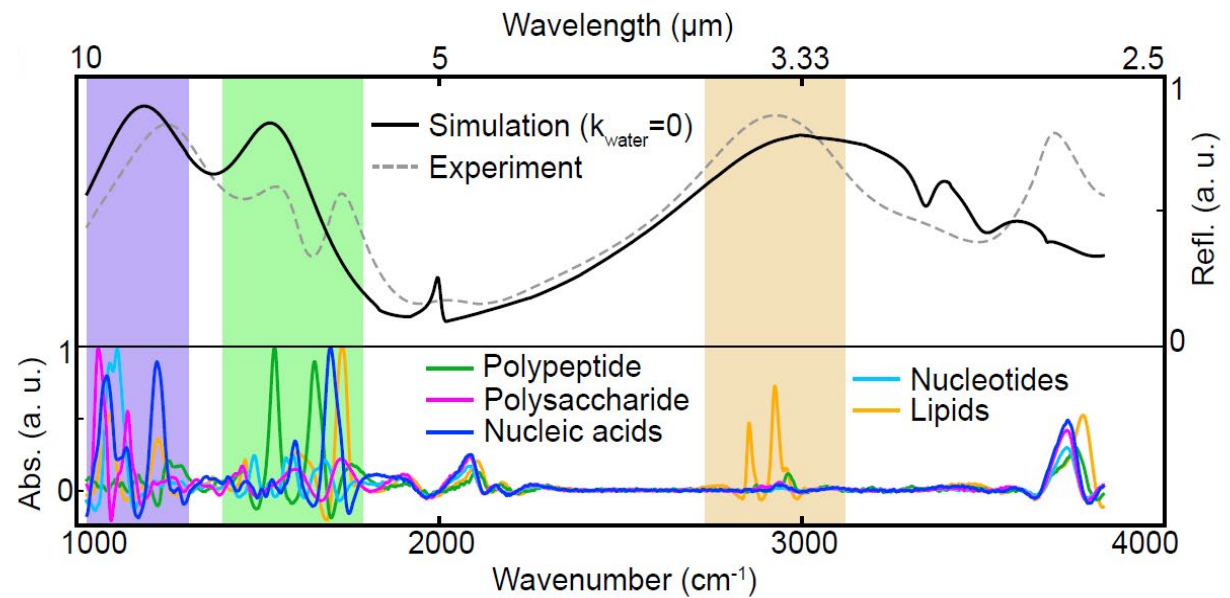
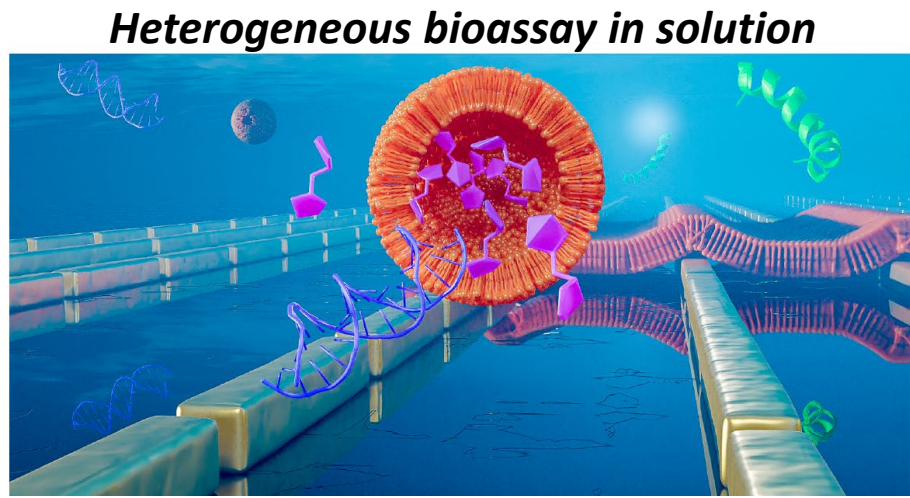
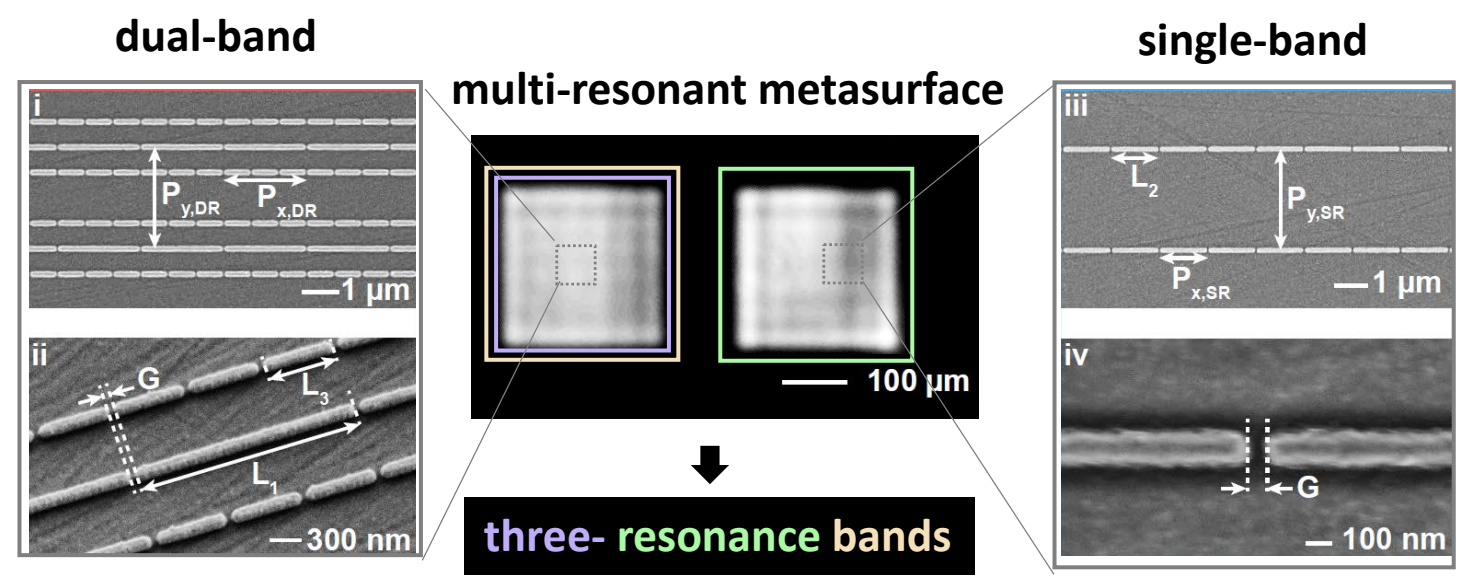
Heterogeneous bioassay in solution



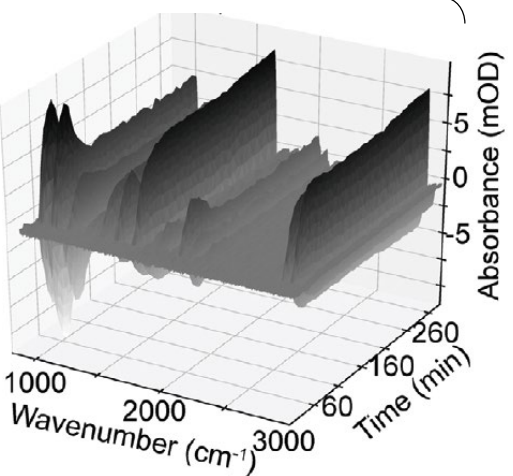
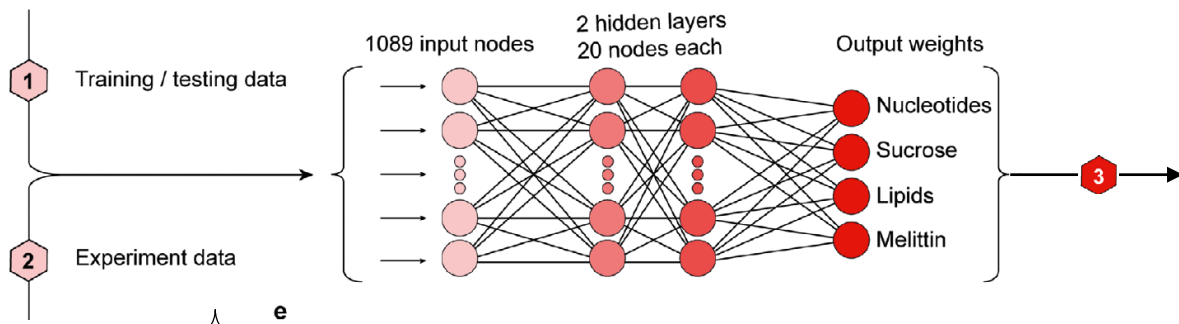
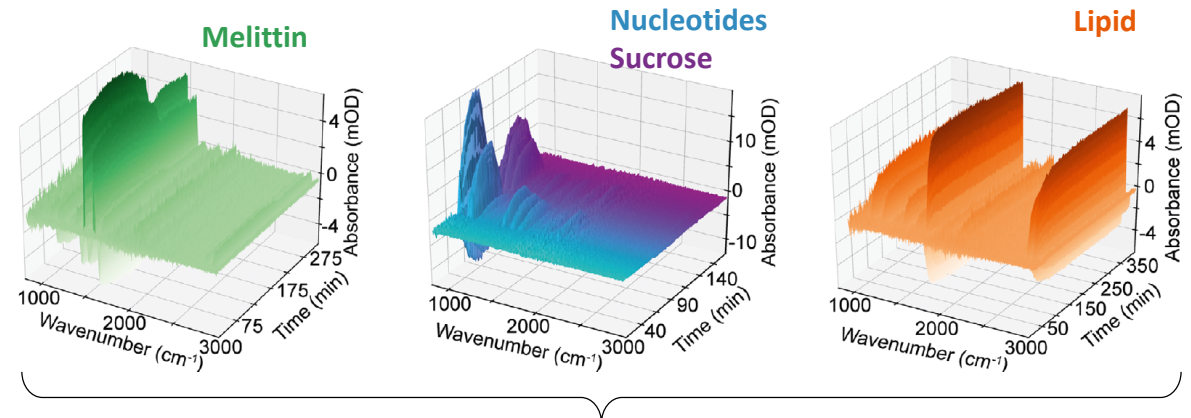
Multi-resonant metasurfaces tuned for lipid, peptide, nucleic acids, polysaccharide



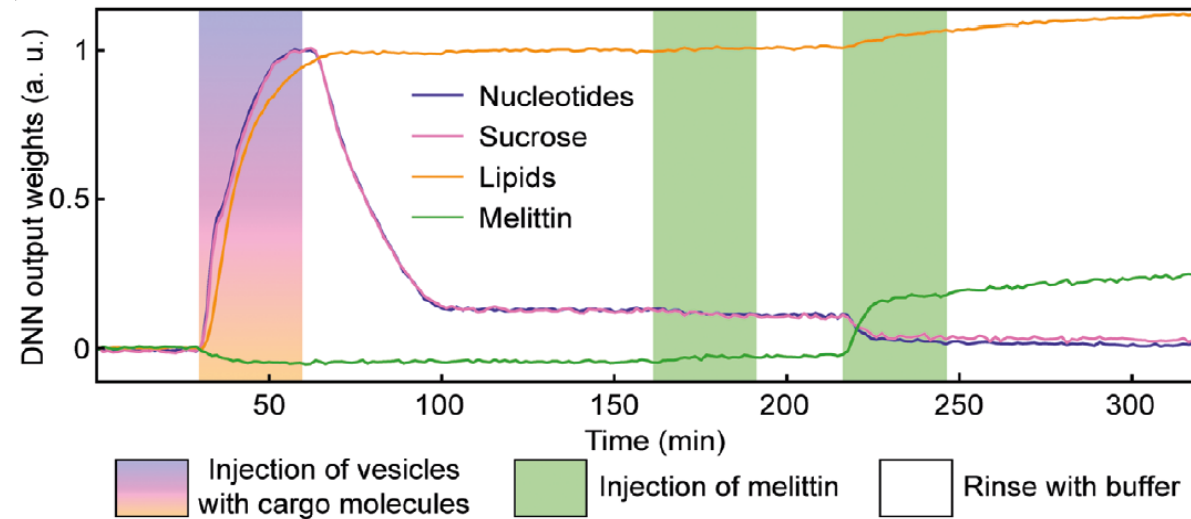
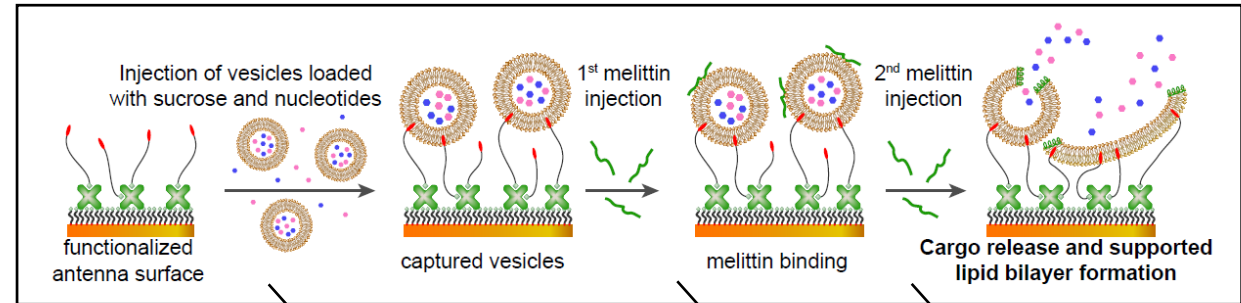
Nature Communications 2013, patent granted
Nano Letters 2016
Nature Communications 2018
Advanced Materials, 2021



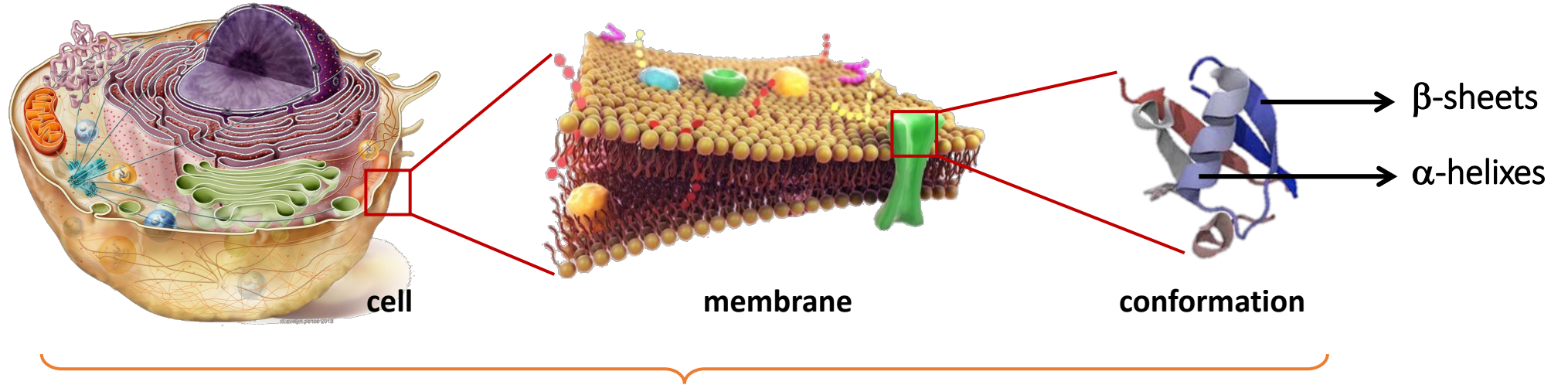
Real-time & label-free observation of vesicle-peptide interactions & cargo release



- DNN data set comprises 3,325,806 spectro-temporal data points obtained from real-time measurements.
- DNN models are built using the Sequential model of Python's Keras library, which uses Tensorflow as backend.

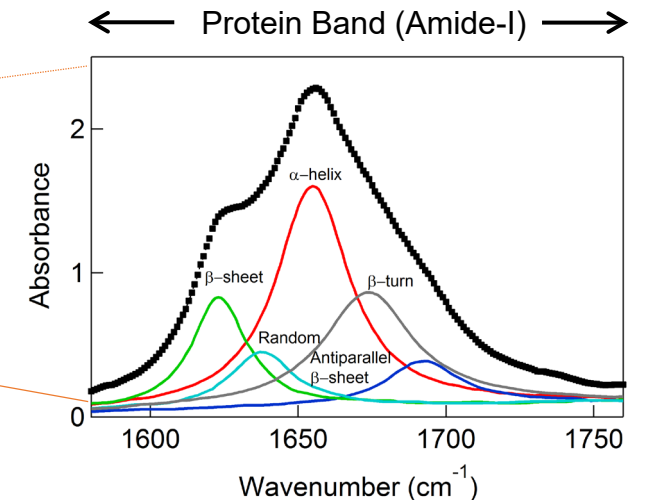
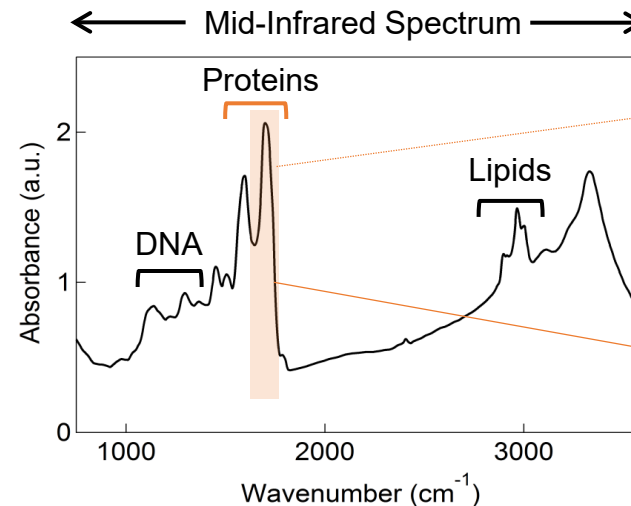


Reminder: Mid-infrared sensing for biology



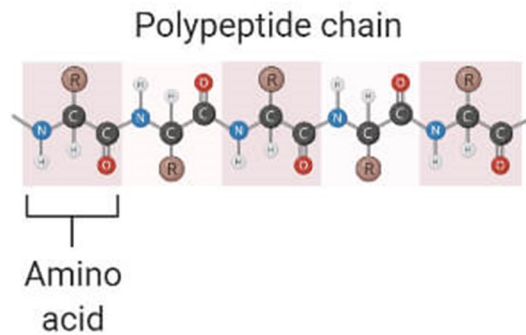
non invasive access to
molecular vibrations

- signal intrinsic to molecular structure
- Resolves secondary structure from amide I band shape



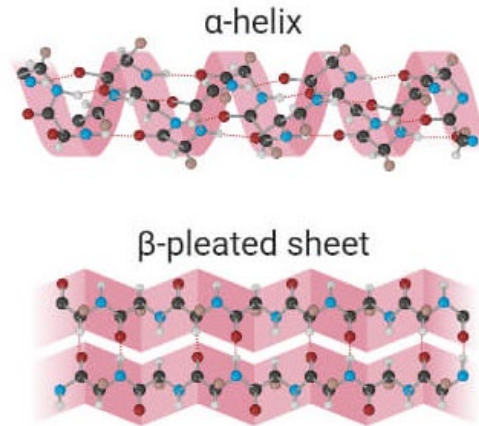
Protein structure

Primary structure



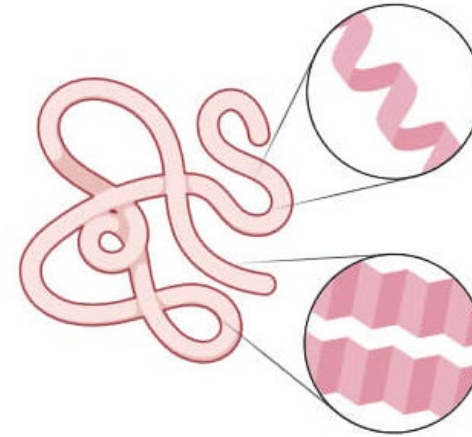
- Simple sequence of amino acids

Secondary structure



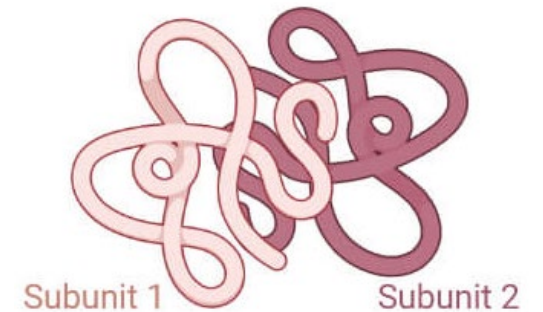
- Dictates protein folding and functions

Tertiary structure

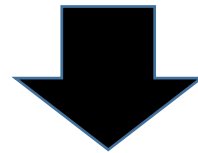


- 3D combination of different secondary structures

Quaternary structure

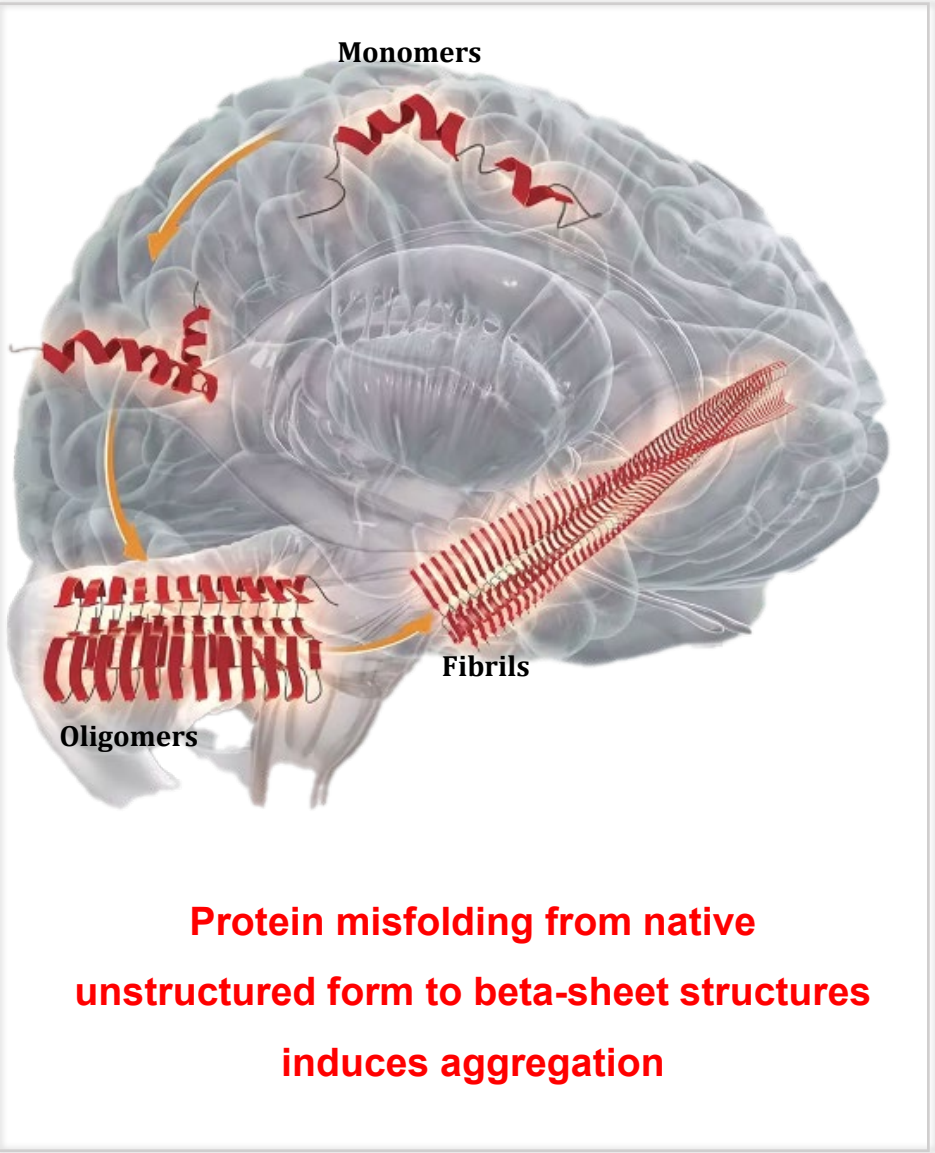


- Combination of multiple tertiary structures

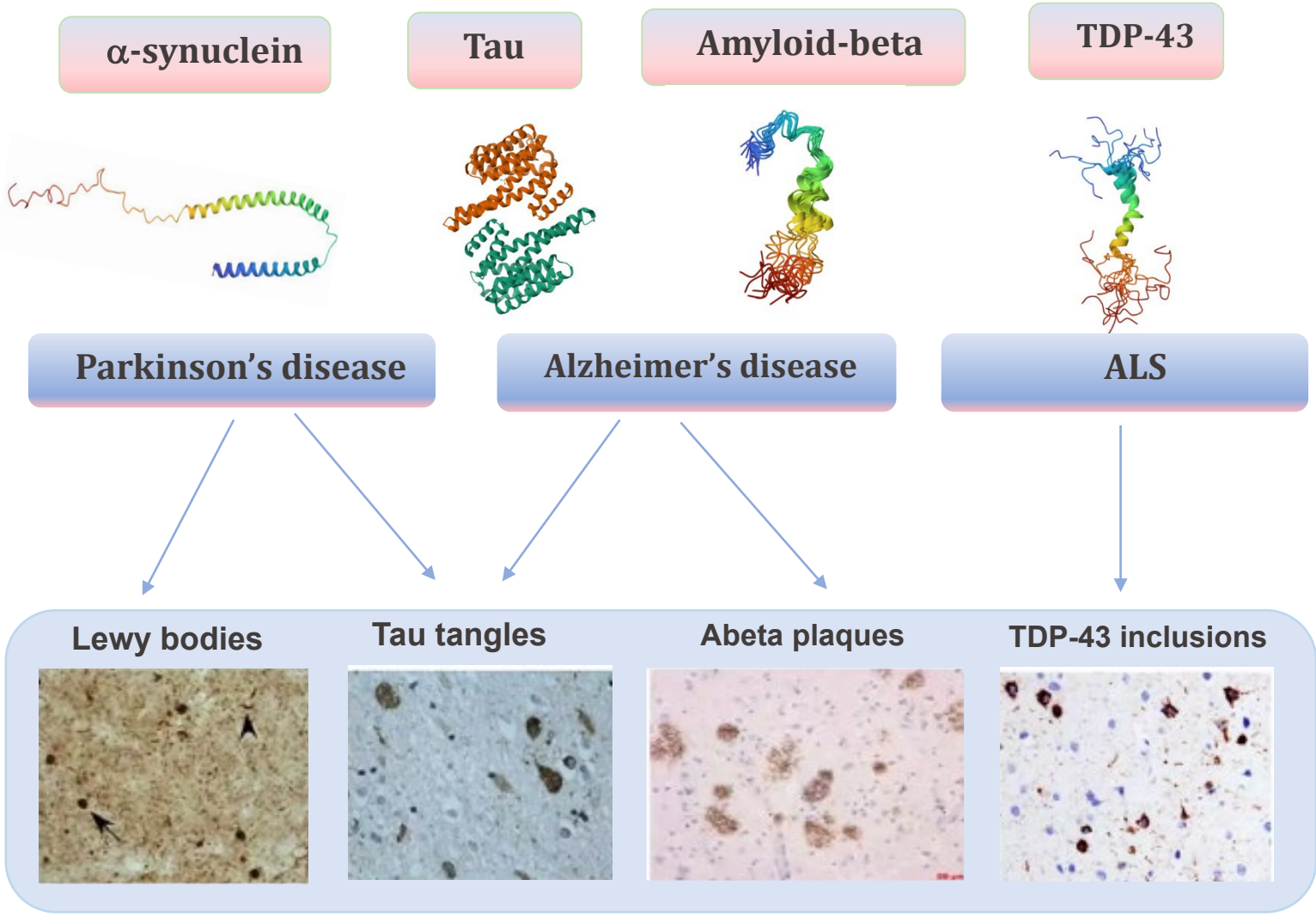


Misfolding disrupts protein function & lead to diseases

Protein misfolding – a common mechanism in neurodegenerative disorders

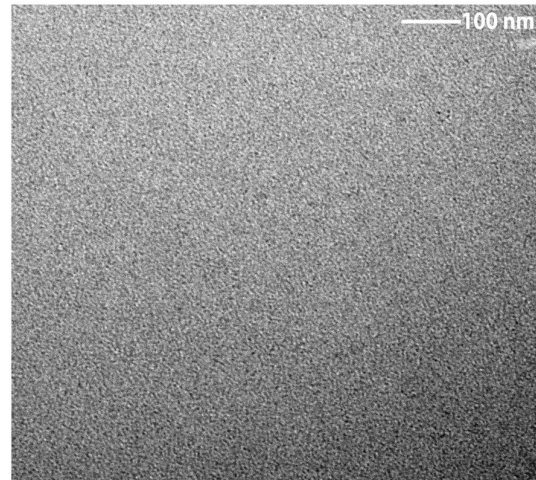


AC immune
RSCB PDB

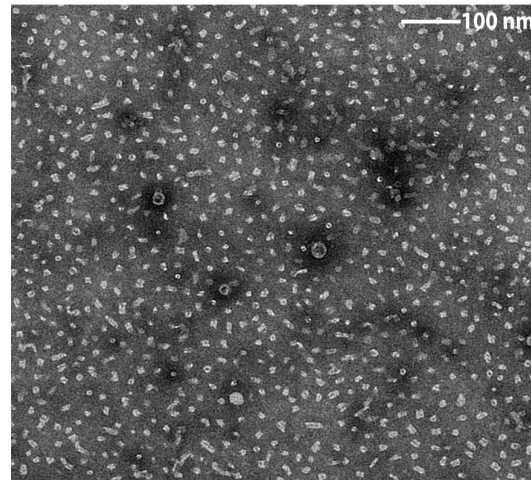


ALS - Amyotrophic lateral sclerosis

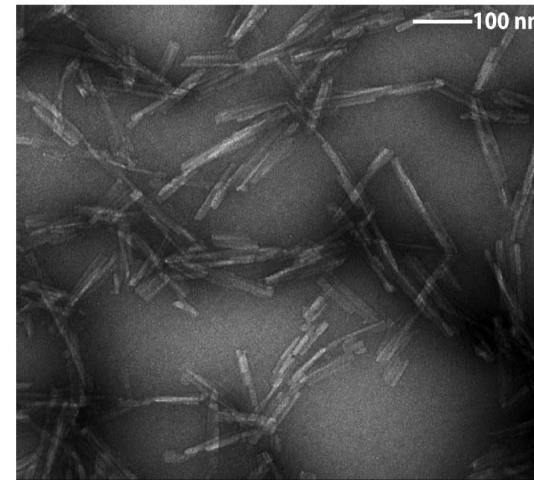
Aggregation pathway of alpha-synuclein protein



Monomers

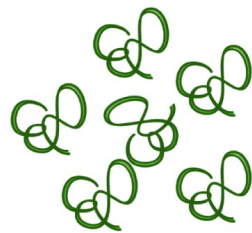


Oligomers



Fibrils

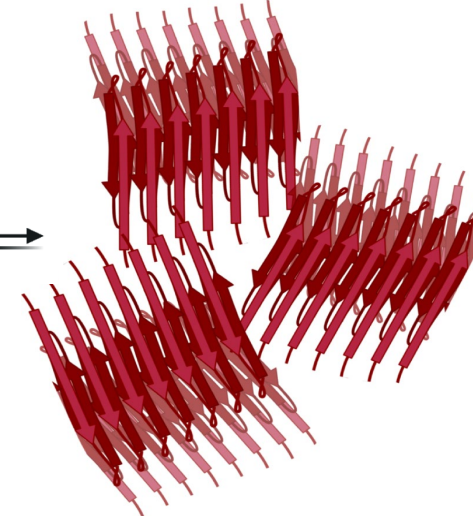
- TEM (Transmission Electron Microscope) images of 3D structure



- Random disordered



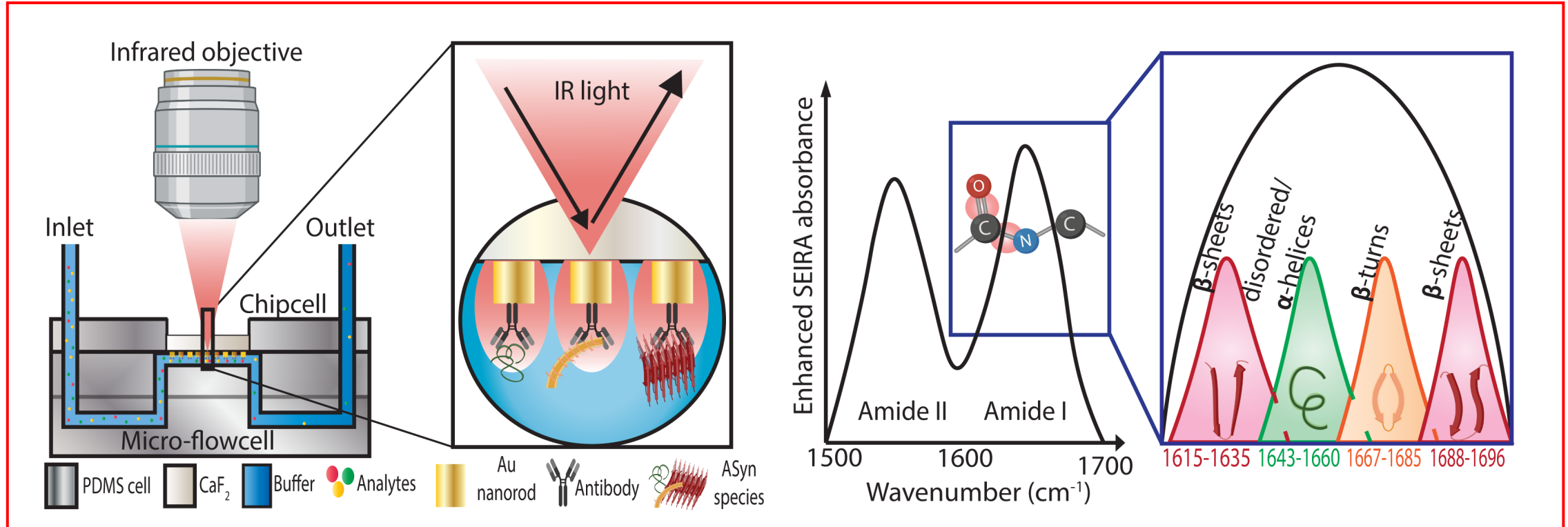
- Beta-sheet enriched



- Secondary structure

ImmunoSEIRA for protein structure analysis

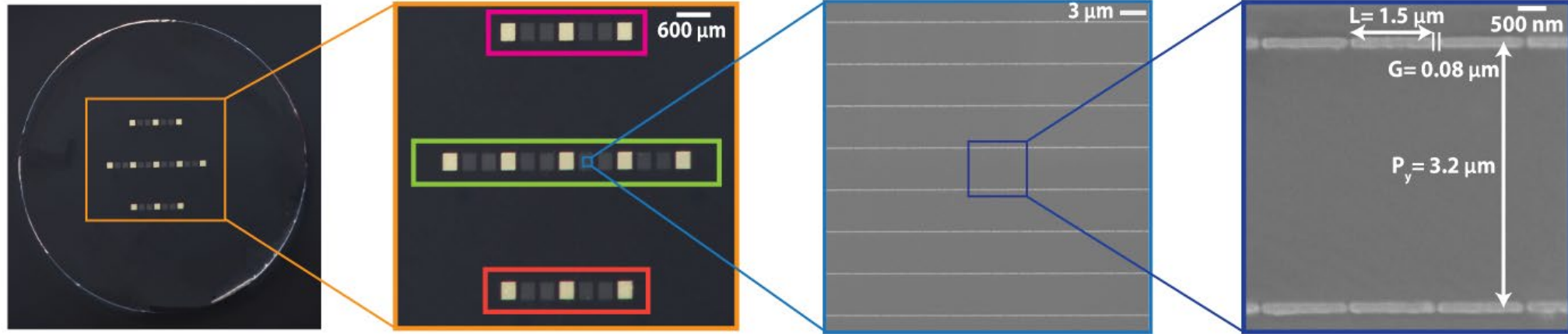
■ Sensing scheme



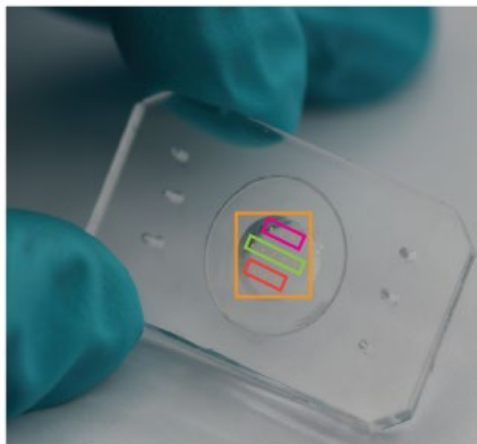
- Real time label-free optofluidic setup
- Immunoassay captures target proteins : α - syn, with highest specificity irrespective of their conformation using antibodies
- From the Amide I absorption bands (1600-1700 cm⁻¹) of the α - syn species, their distinct secondary structures are retrieved

Optofluidic configuration

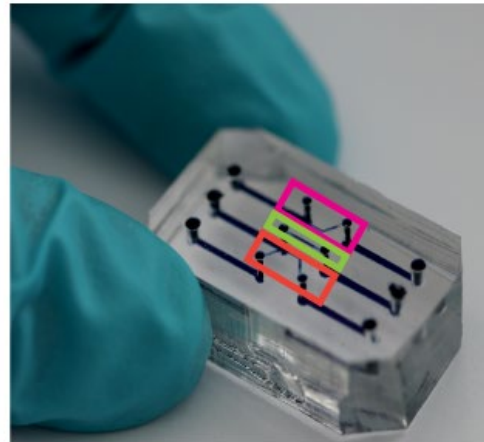
- 2D microarray SEIRA design



- Small sample volume microfluidics

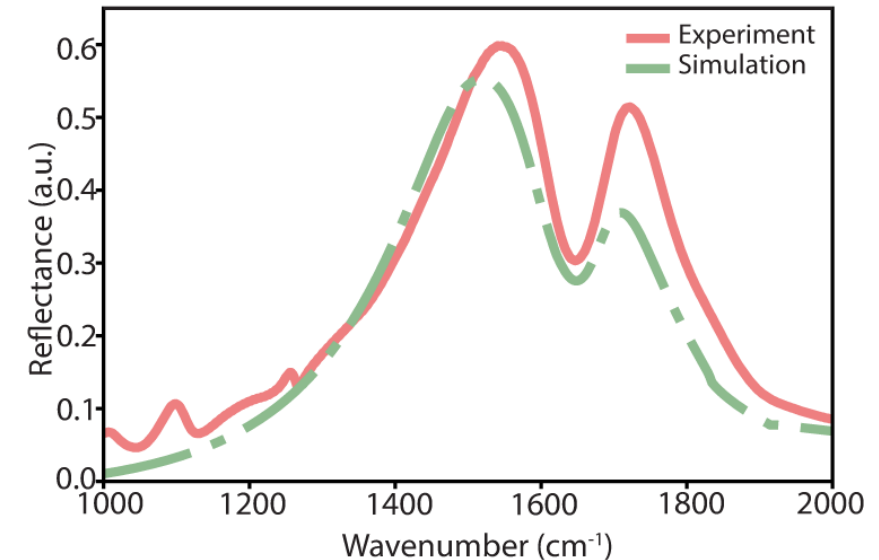


Chipcell

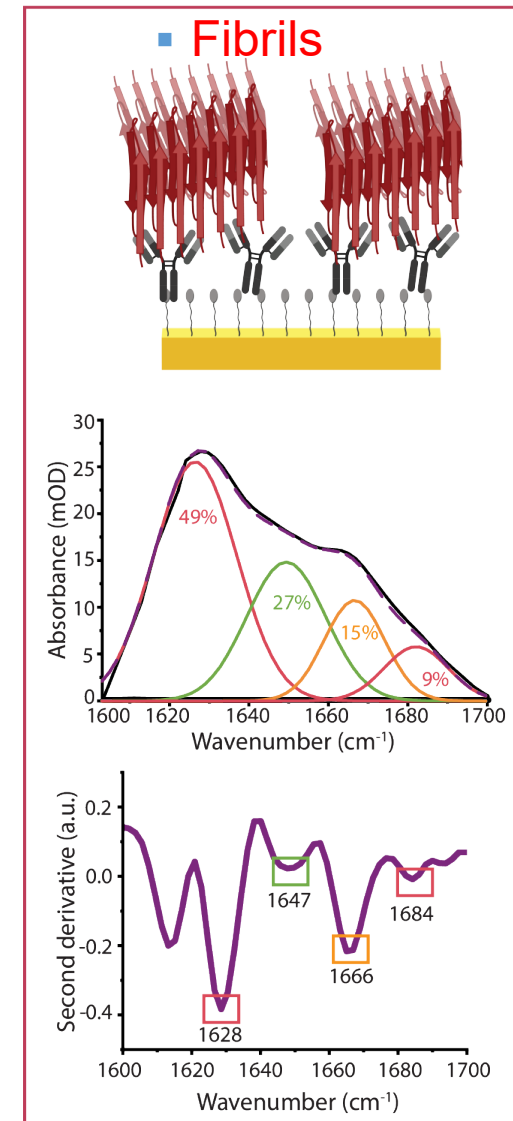
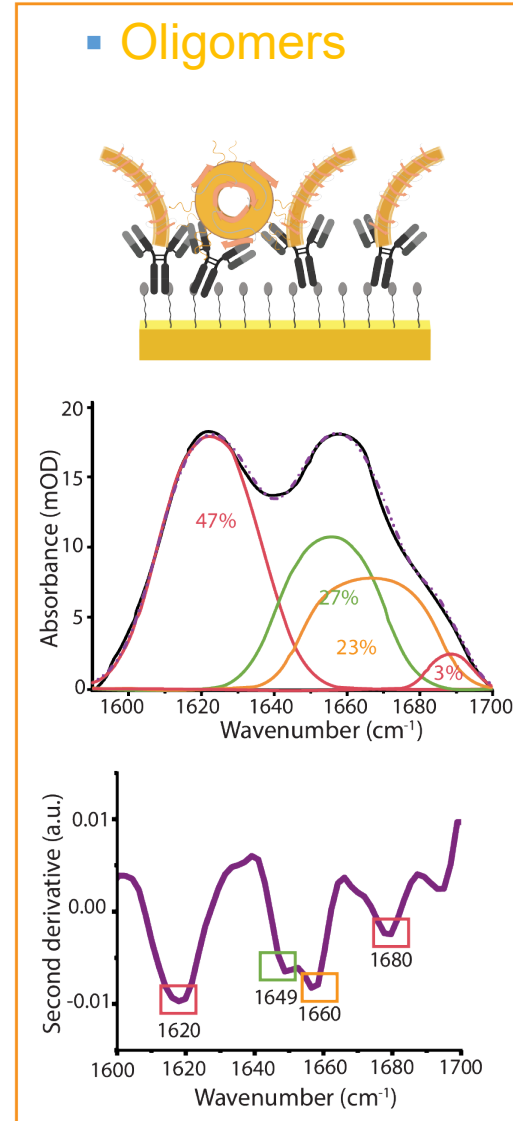
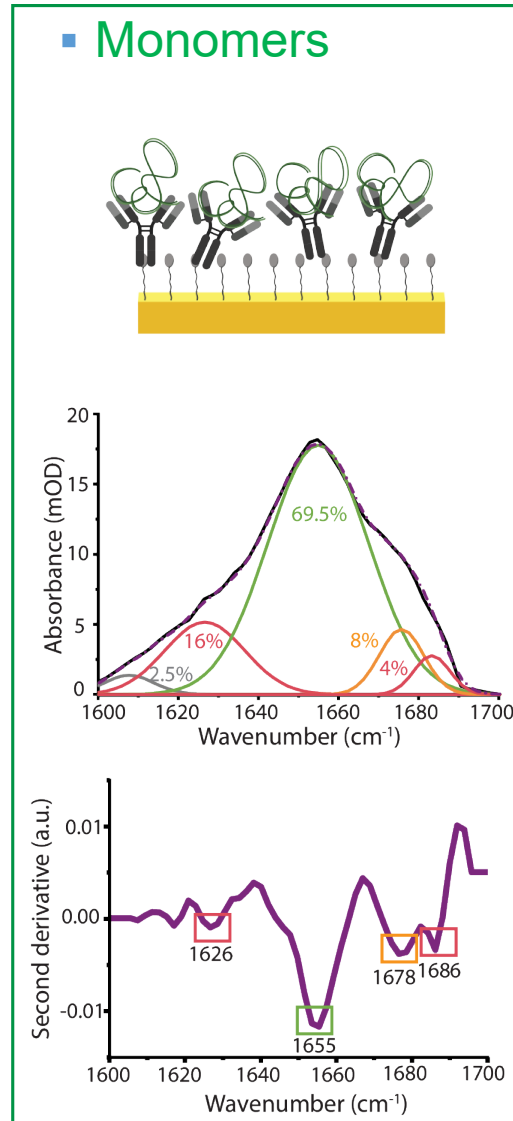


Micro-flowcell

- Optofluidic SEIRA signal



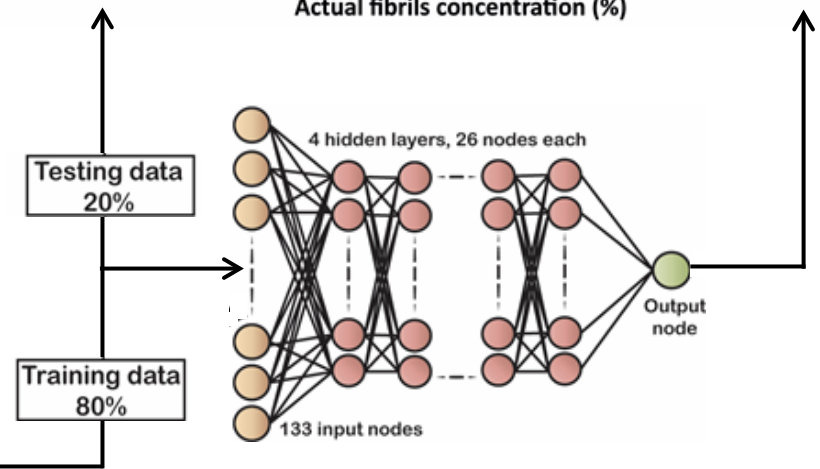
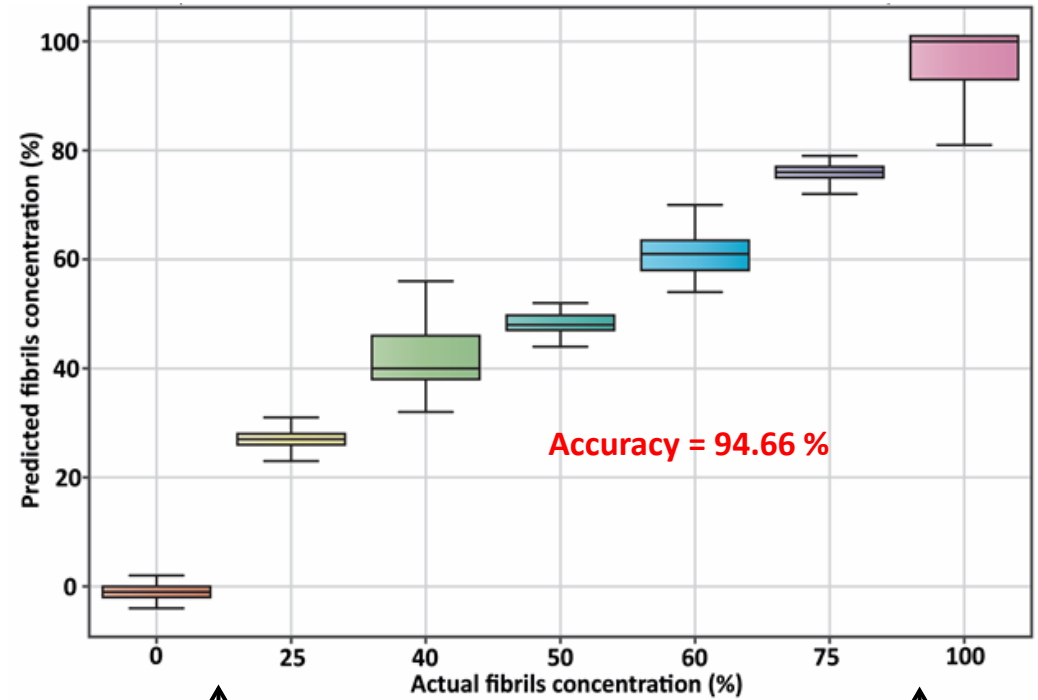
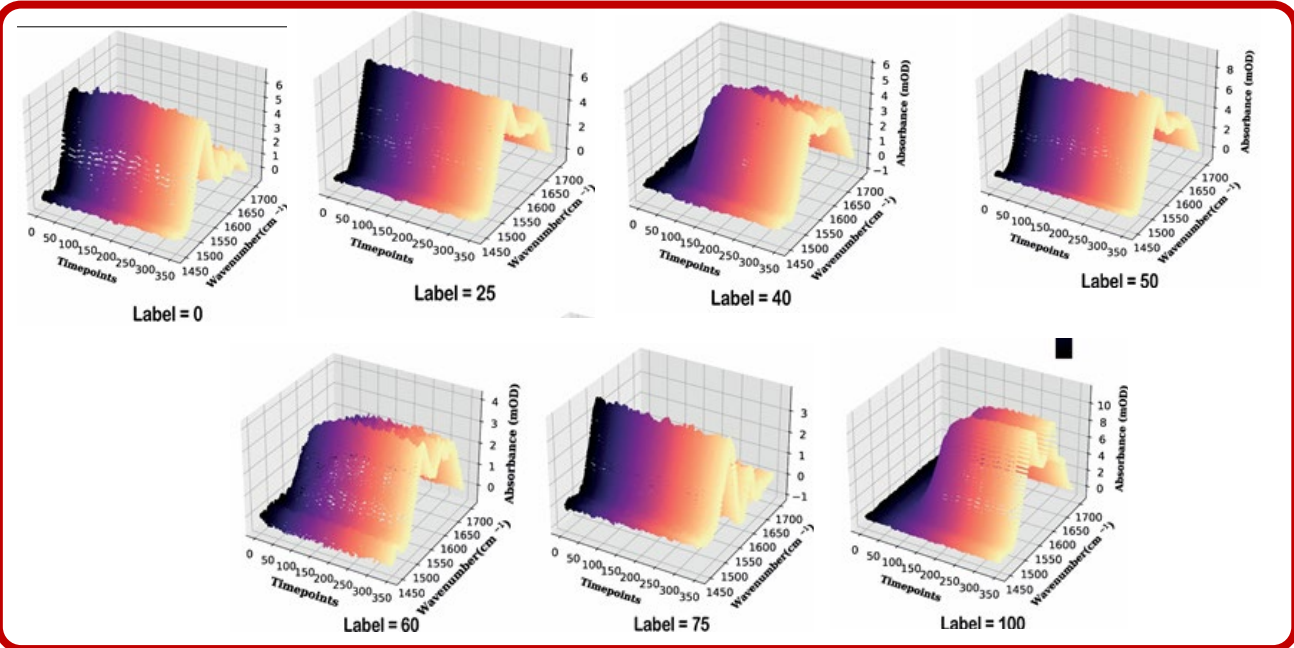
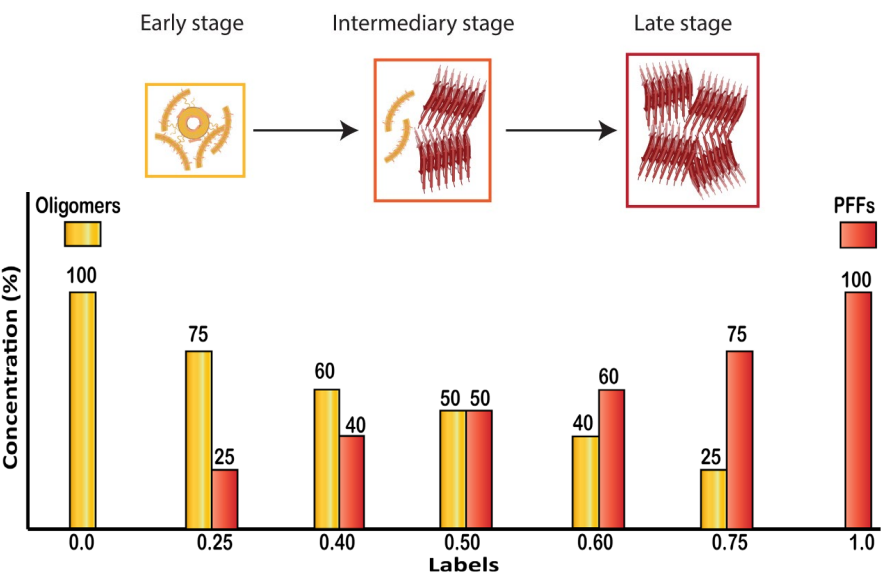
Conformational profiling of different alpha-synuclein protein forms



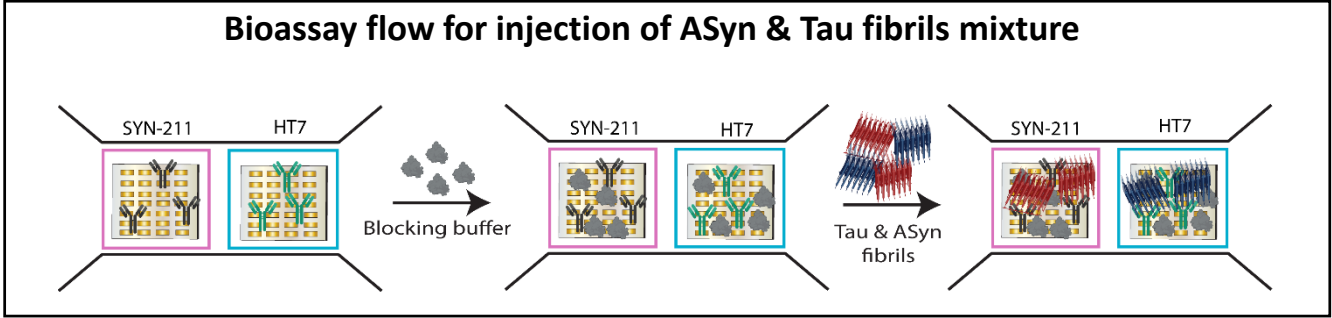
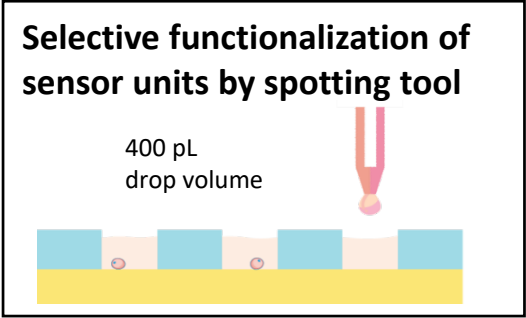
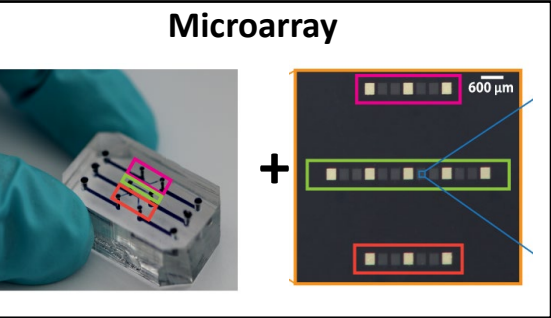
— Absorbance
 Absorbance fit
 — β-sheets
 — α-helix/disordered
 — β-turns

But, how can we quantitatively separate the pathological aggregates-oligomers and fibrils in a mixed sample?

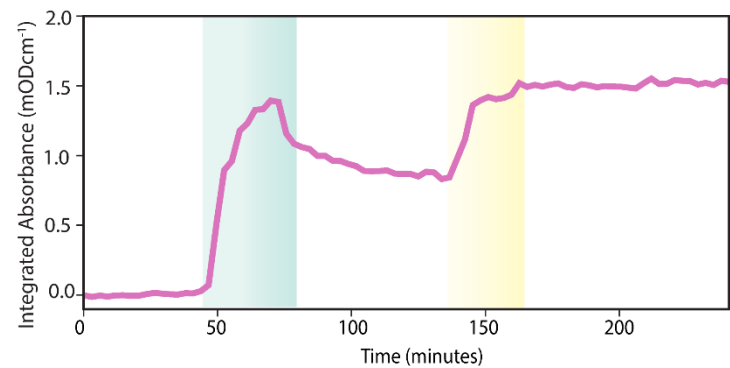
AI-Boosted Immuno-SEIRA for quantitative differentiation of aggregate mixture



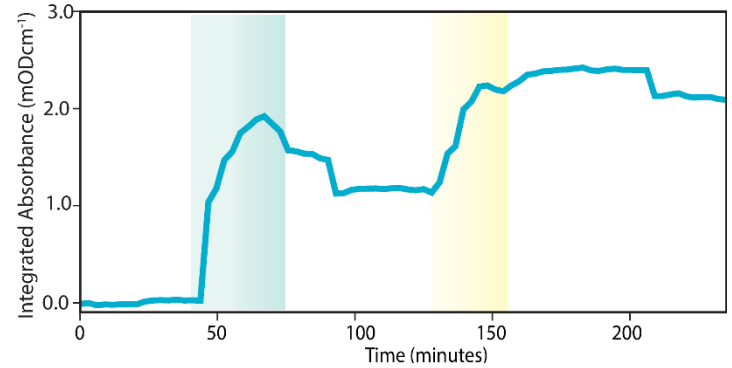
Multiplexed Detection of Structural Biomarkers with Immuno-SEIRA Microarray



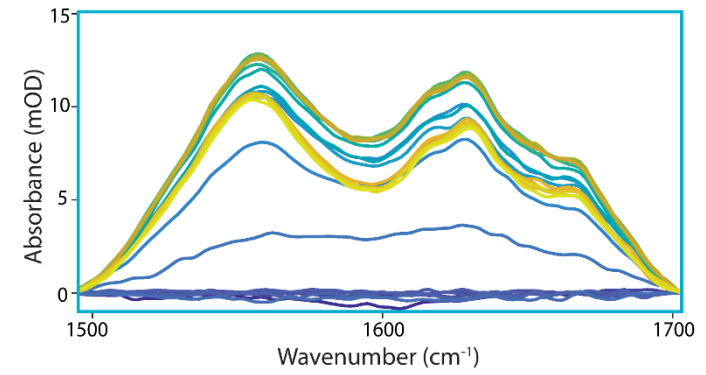
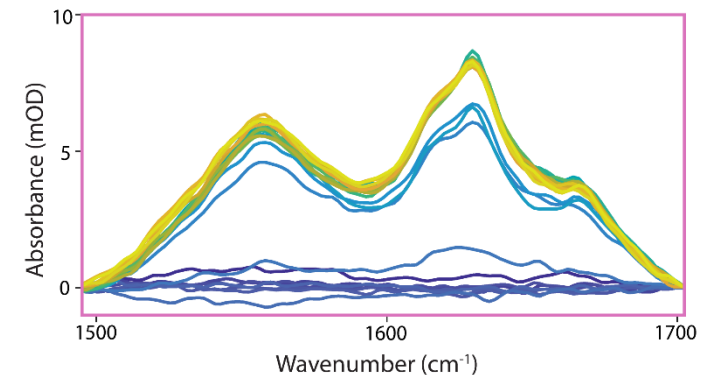
Signal obtained on **SYN-211** antibody functionalized unit



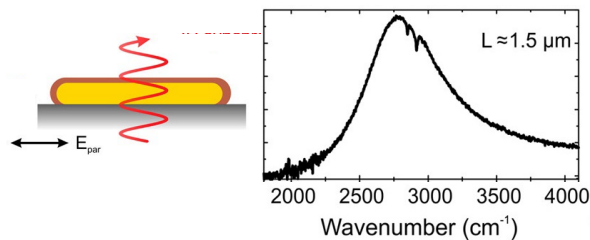
Signal obtained on **HT7** antibody functionalized unit



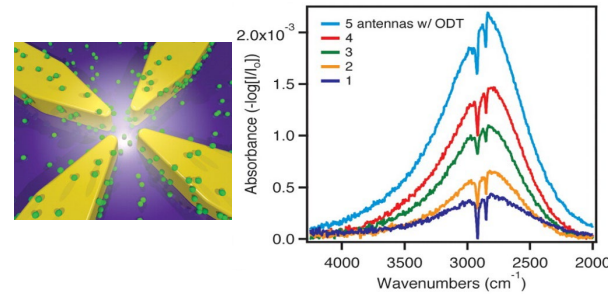
- Buffer wash
- Blocking wash
- Tau & ASyn fibrils injection



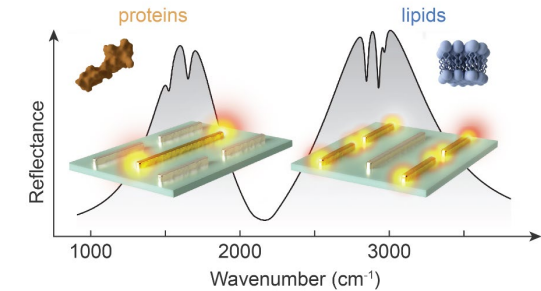
How to use high-Q factor Mid-IR antennas?



PRL **101**, 157403 (2008)



J. Am. Chem. Soc. **135**, 3688–3695 (2013)

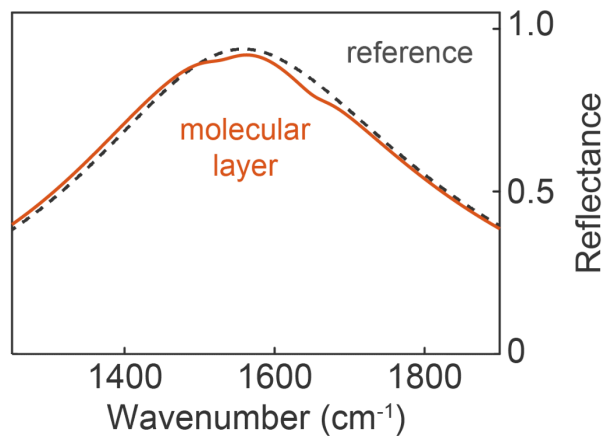


Nat. Commun. **9**, 2160 (2018)

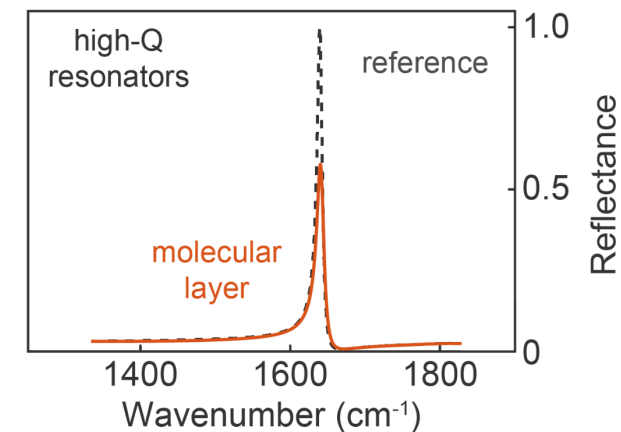
State-of-the art in SEIRAS - plasmonic antennas



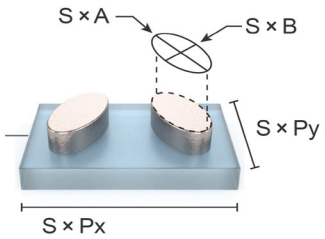
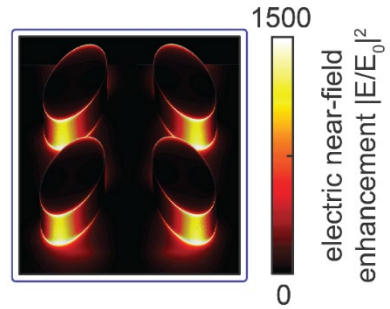
limited by broad resonances



- What can we do differently with sharper resonances?
- How can we harness them for new functionalities?



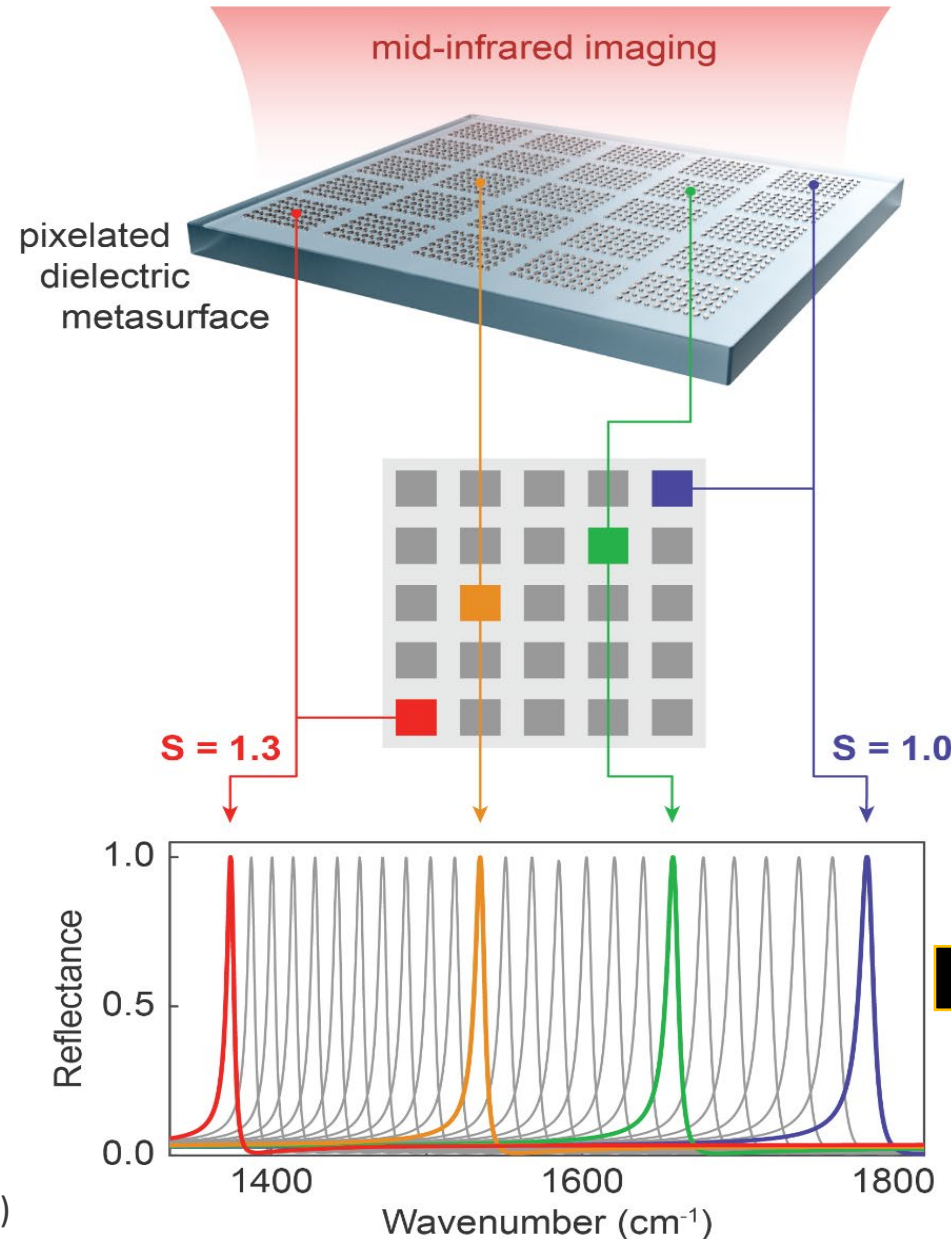
Mid-IR imaging biosensor with high-Q dielectric metasurfaces



Symmetry-broken
high-Q silicon resonators

Resonance tunability by
scaling factor

1-to-1 correlation
between spectrum &
chip position

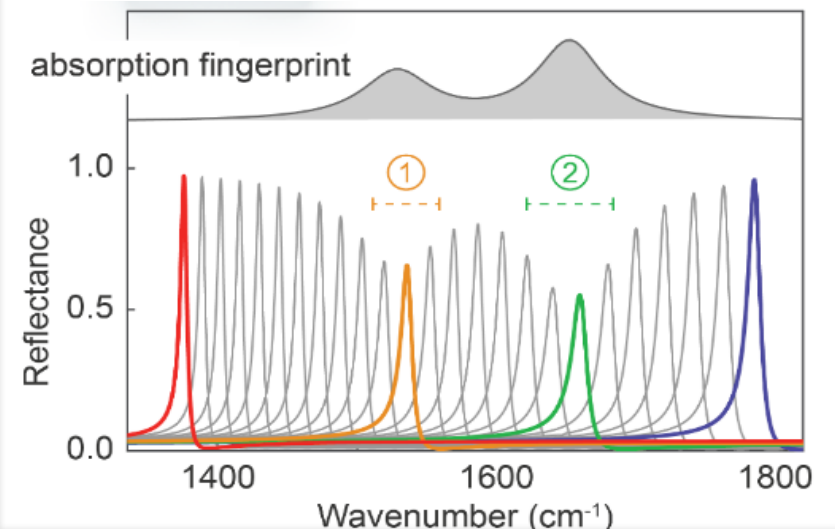


With protein
monolayer

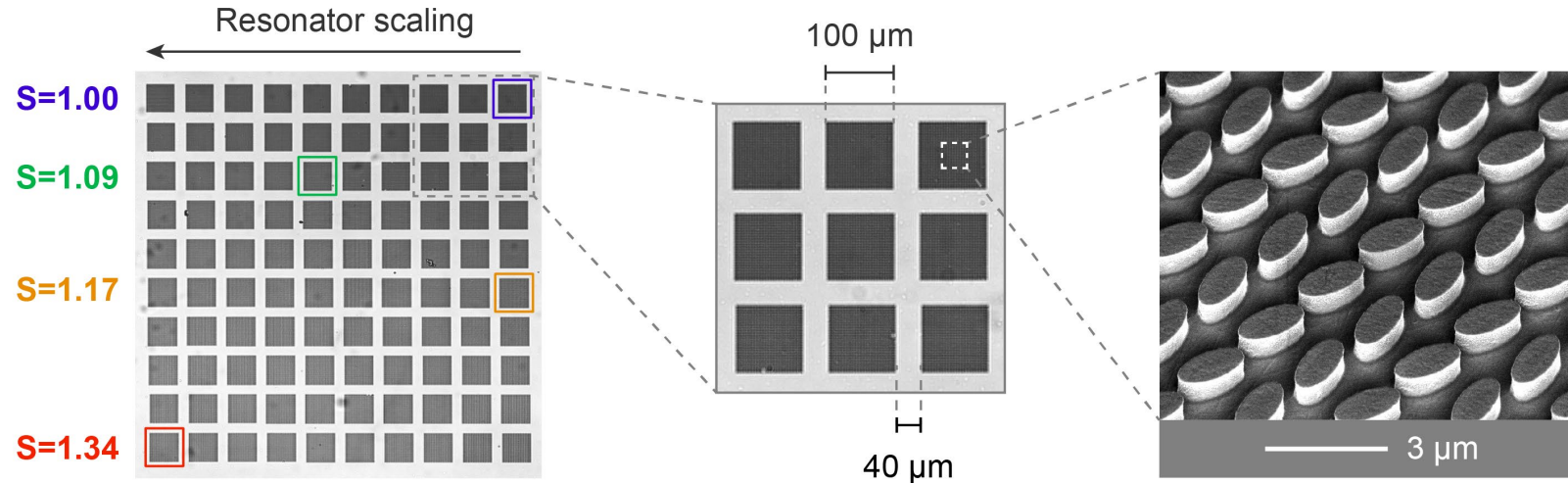


Two-dimensional
Molecular barcode

Imaging based
chemical
fingerprinting



Nanofabrication of pixelated dielectric metasurfaces



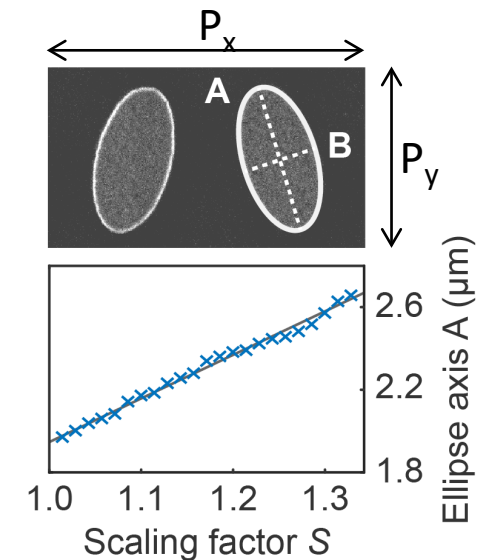
Fabrication method:

- hydrogenated amorphous **silicon** layer deposition via PECVD
- MgF₂ substrate
- electron-beam lithography
- fluorine-based dry plasma etching

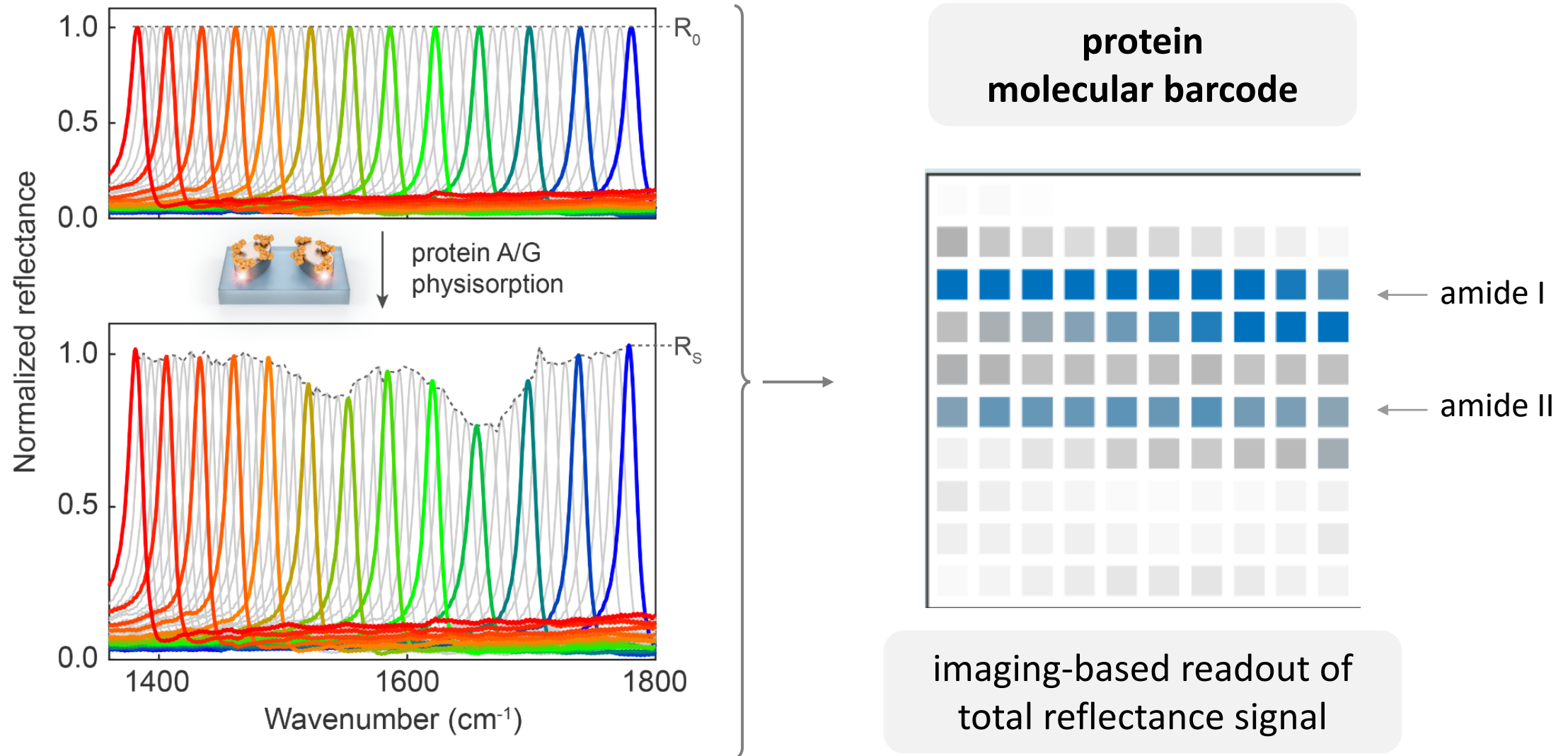
linear scaling of
resonator size, S

$$\begin{aligned} A &= 1.96 \text{ } \mu\text{m} \\ B &= 0.96 \text{ } \mu\text{m} \\ P_x &= 3.92 \text{ } \mu\text{m} \\ P_y &= 2.26 \text{ } \mu\text{m} \end{aligned}$$

Fixed height = 0.7 μm
Fixed tilt angle $\theta = 20^\circ$

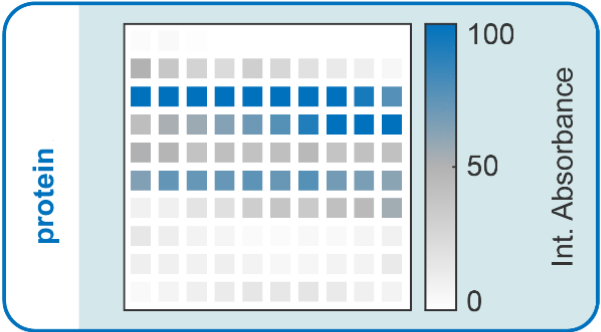
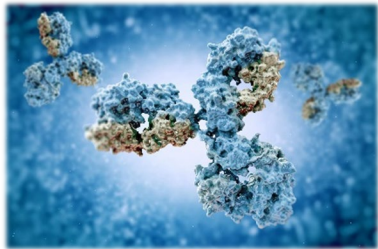


Broadband & Imaging-Based Spectral Detection : Molecular Barcodes

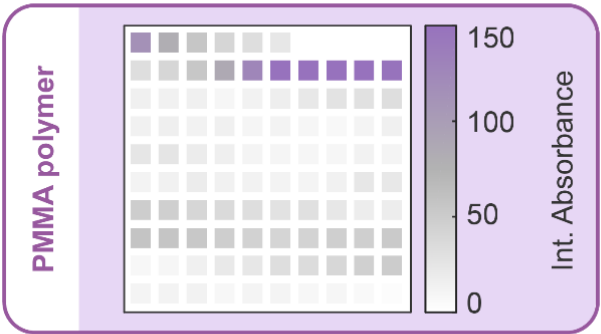
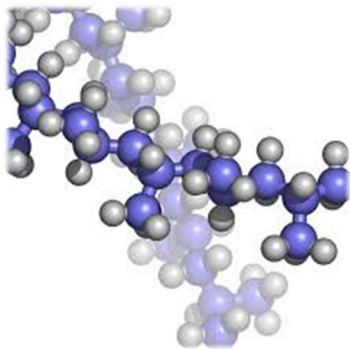


Chemical-specific barcodes: image based identification

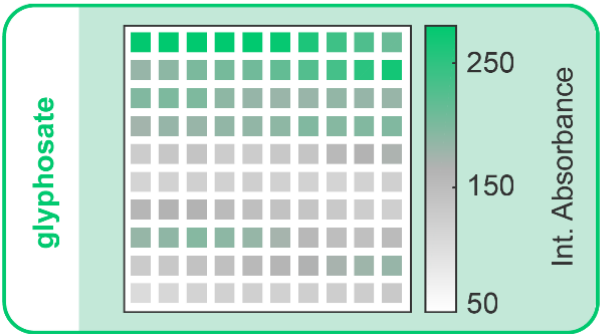
Biosensing & diagnostics



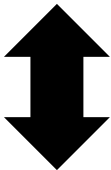
Materials science



Environmental monitoring



Barcode images are
unique & different



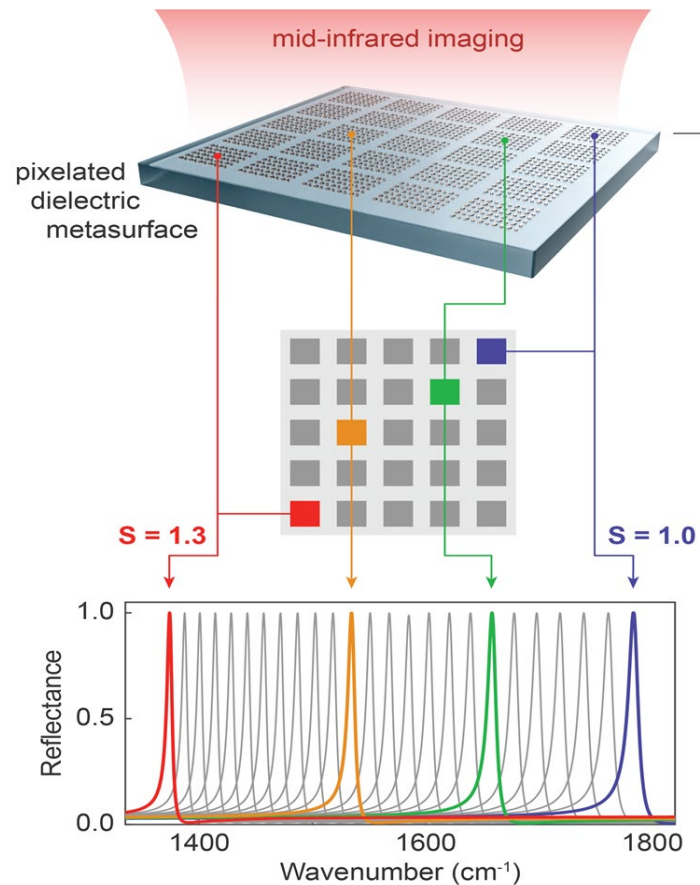
Absorption spectrum of
each sample is distinct

image-based pattern matching

Broadband Mid-IR Metasurfaces for SEIRA Spectroscopy & BioSensing

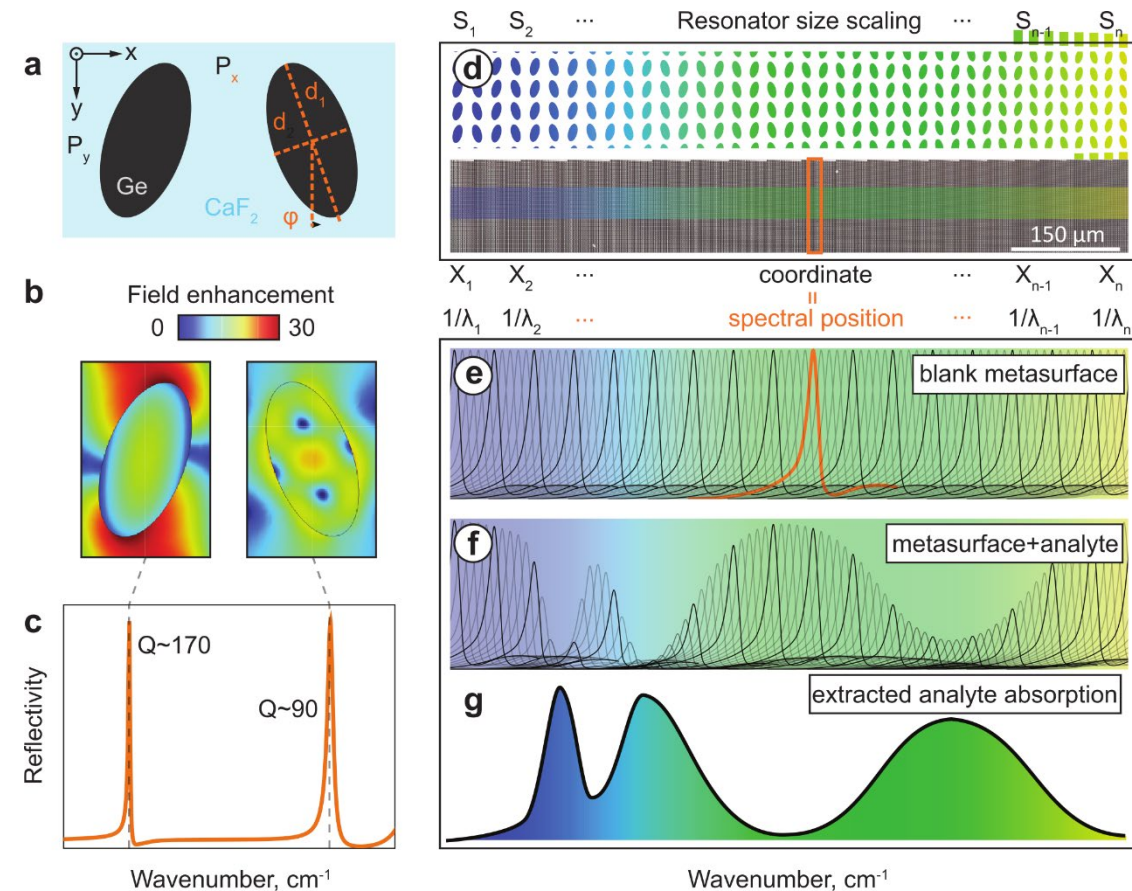
▪ Pixelated Metasurfaces:

- pixel size $> 100\mu\text{m}$
- discrete spectral sampling
- large area required

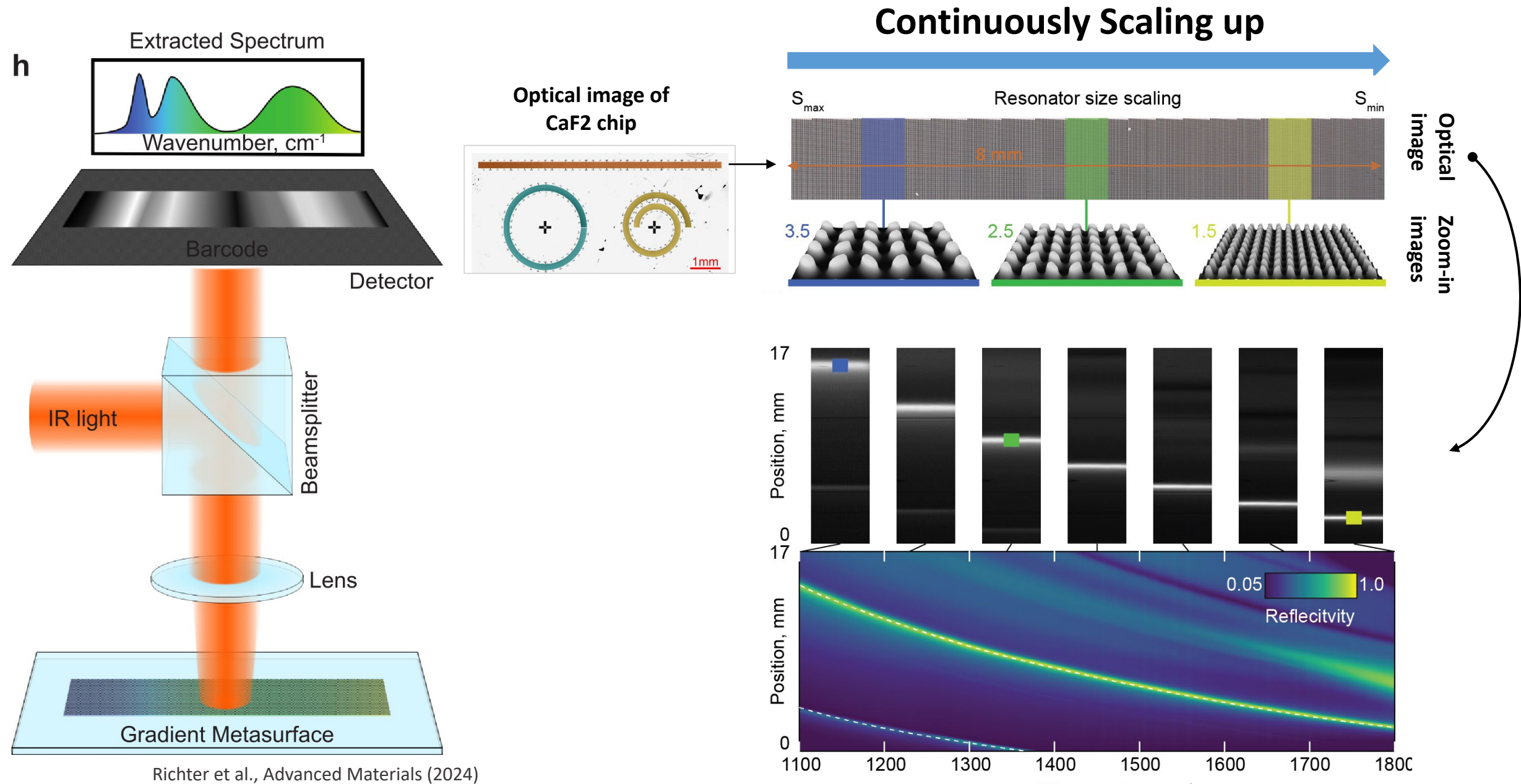


▪ Gradient Metasurface Concept:

- resonances tolerate perturbation
- scale every column slightly
- continues spectral coverage
- much less area required



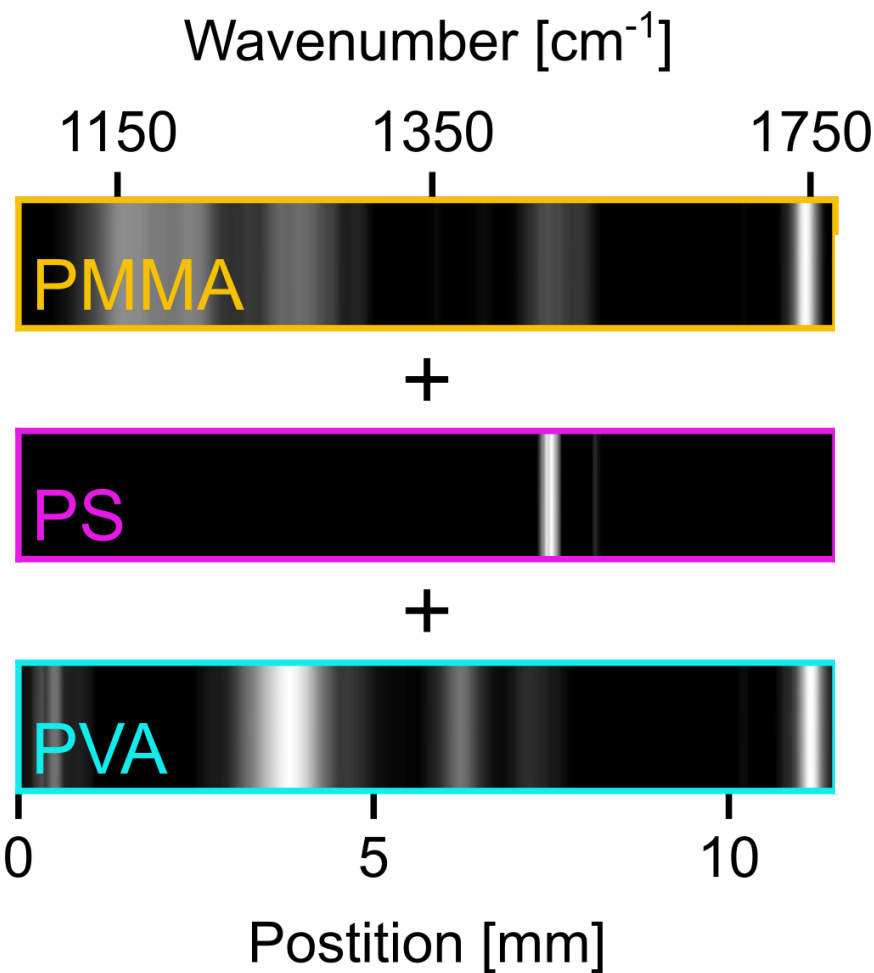
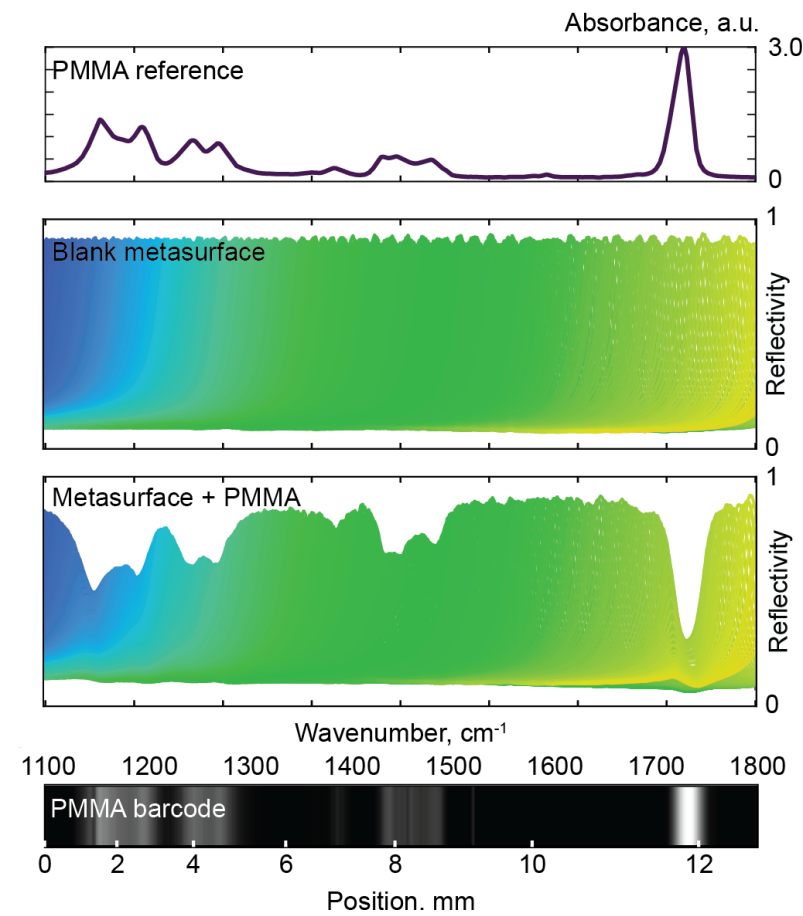
Broadband Gradient Mid-IR Metasurfaces



Broadband Gradient Metasurface for SEIRA Spectroscopy & BioSensing

Single-Analyte: Detection

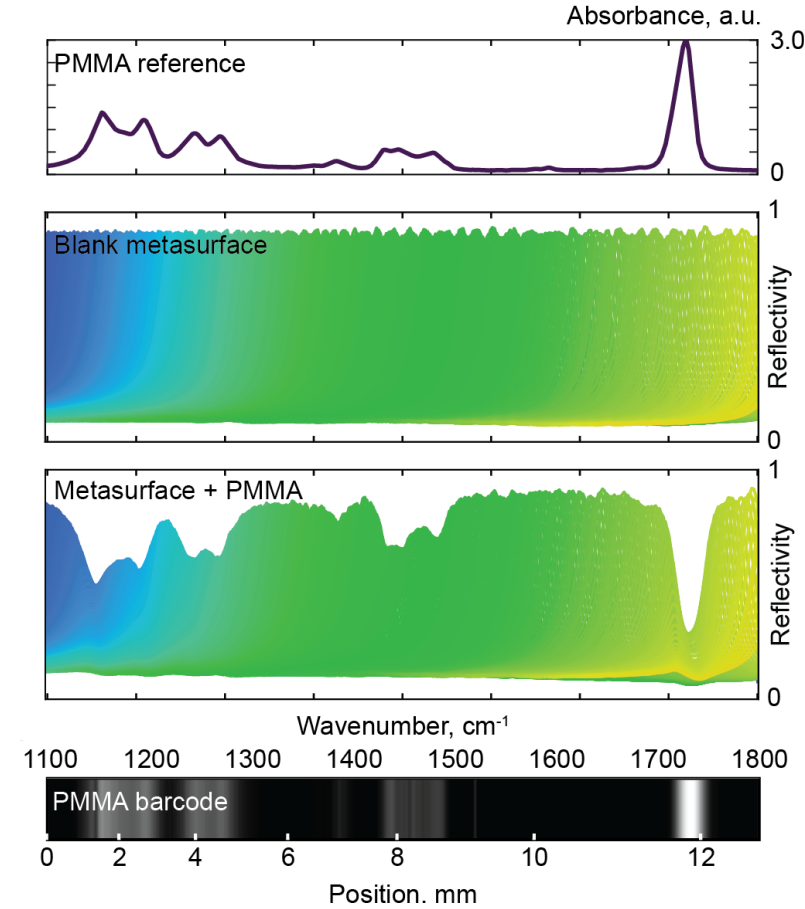
Metasurface is coated with a polymer, such as PMMA, PS or PVA



Broadband Gradient Metasurface for SEIRA Spectroscopy & BioSensing

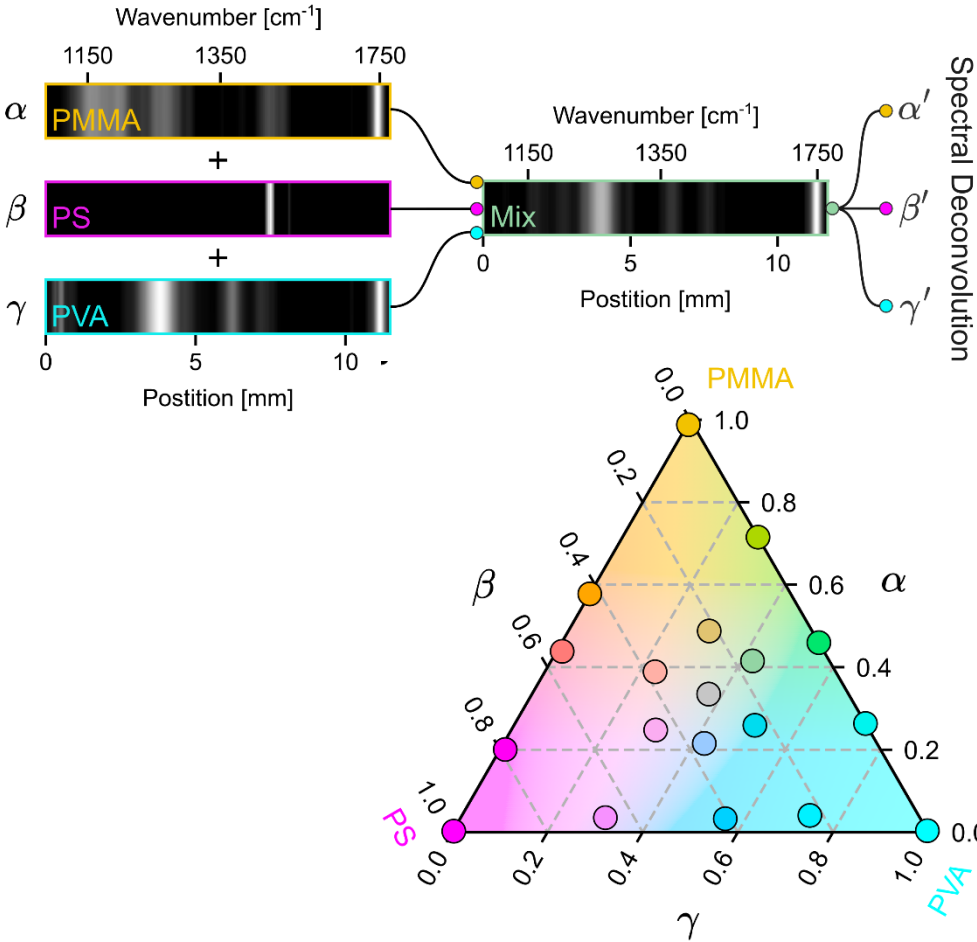
Single-Analyte: Detection

Metasurface is coated with a polymer, such as PMMA, PS or PVA

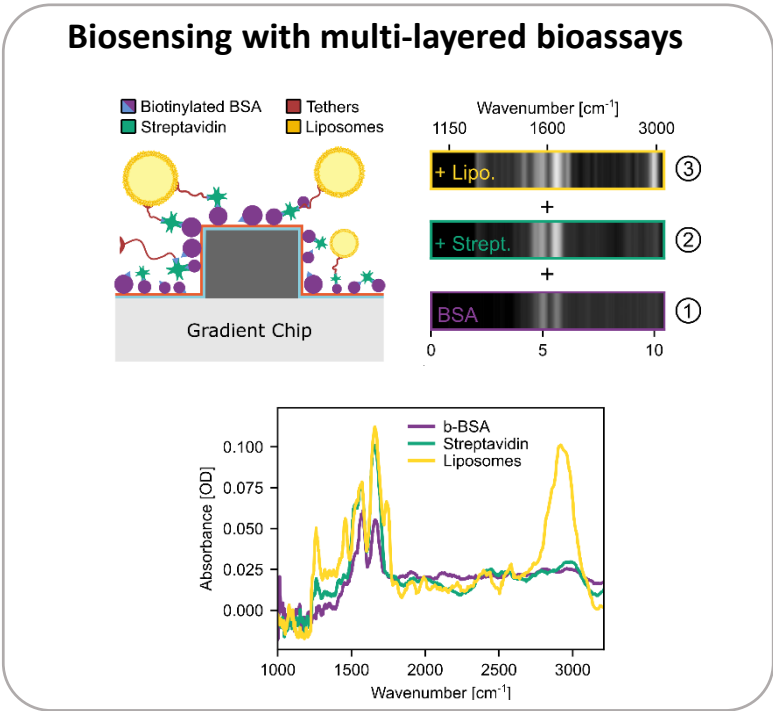
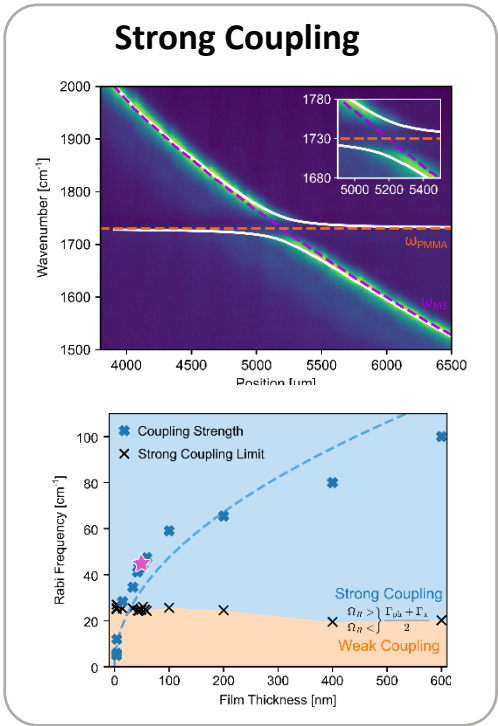
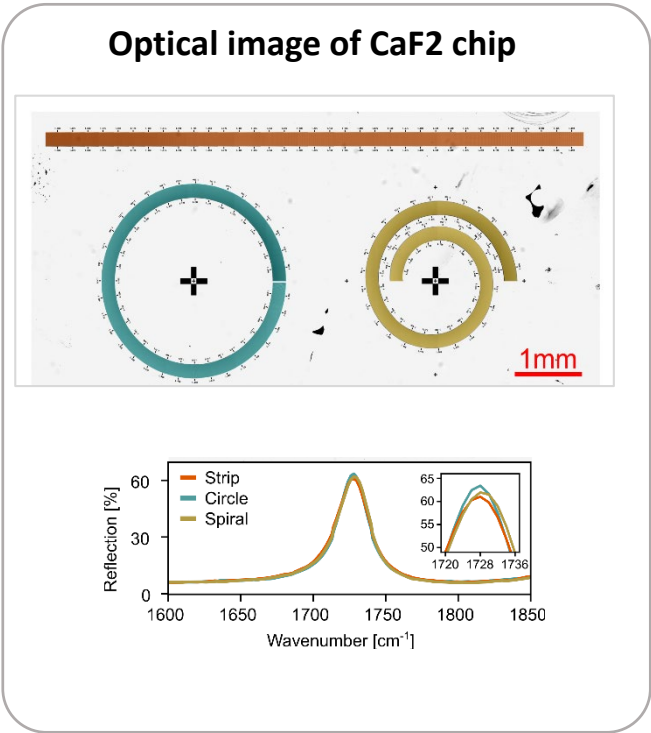
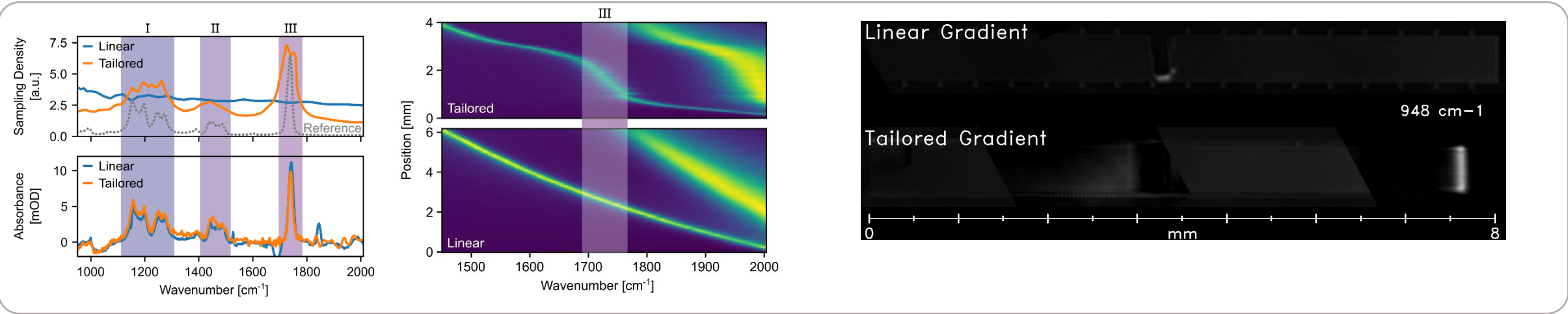


Multi-Analyte: Decomposition

Metasurface is coated with a polymer mixture

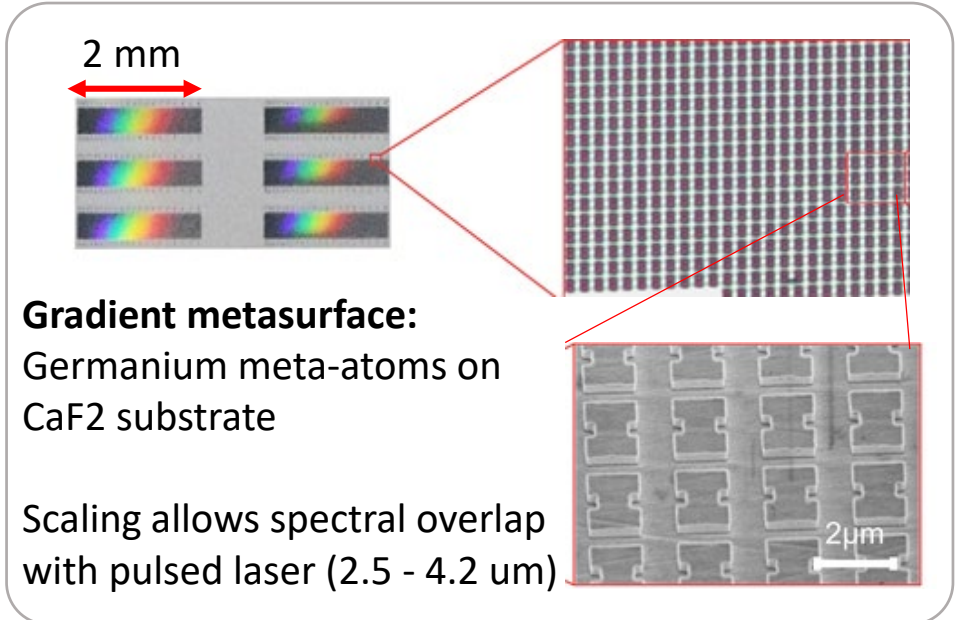
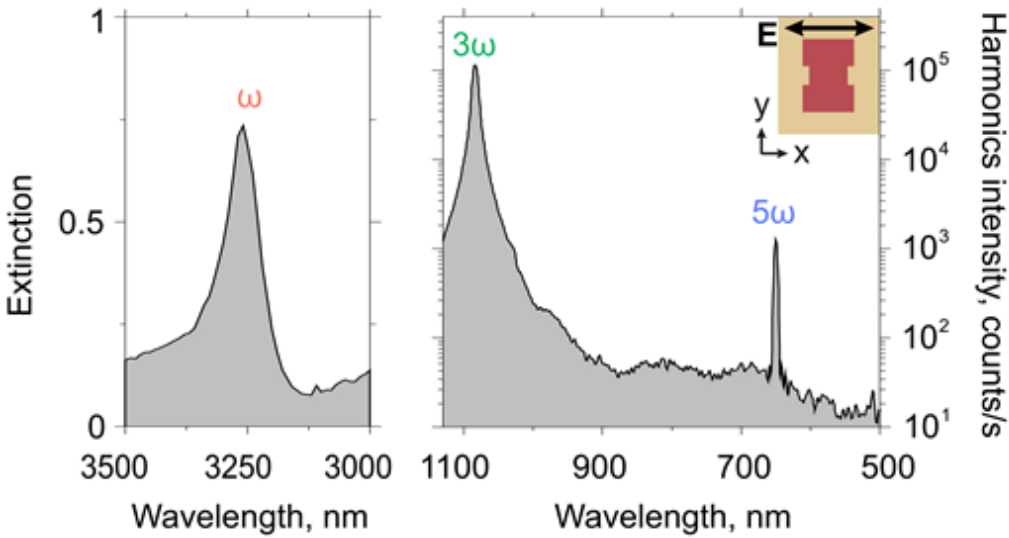
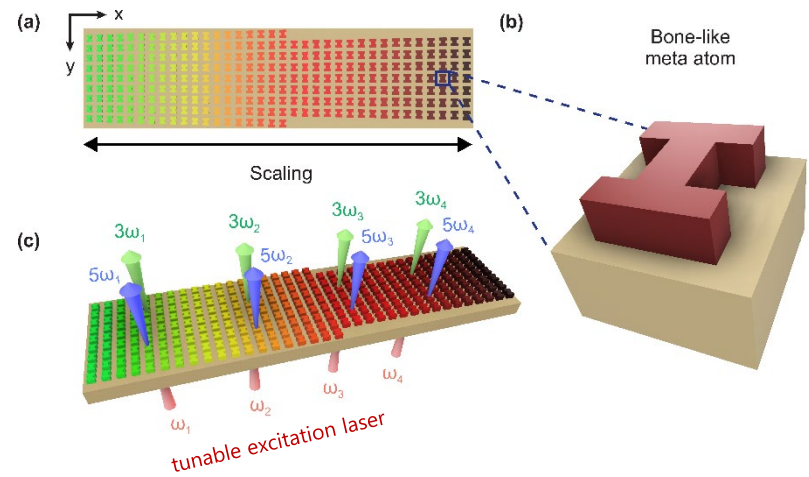


Flexibilities Offered by Gradient Mid-IR Metasurfaces

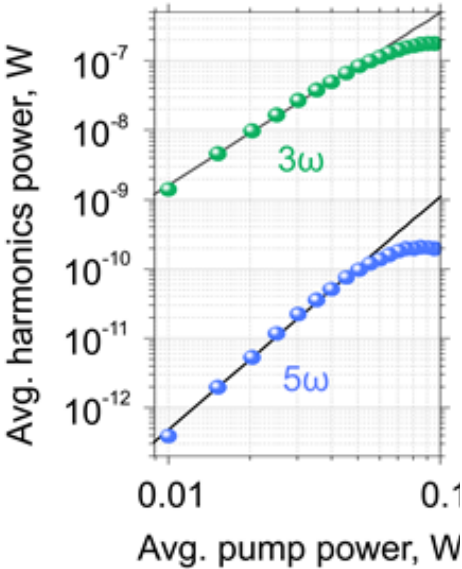


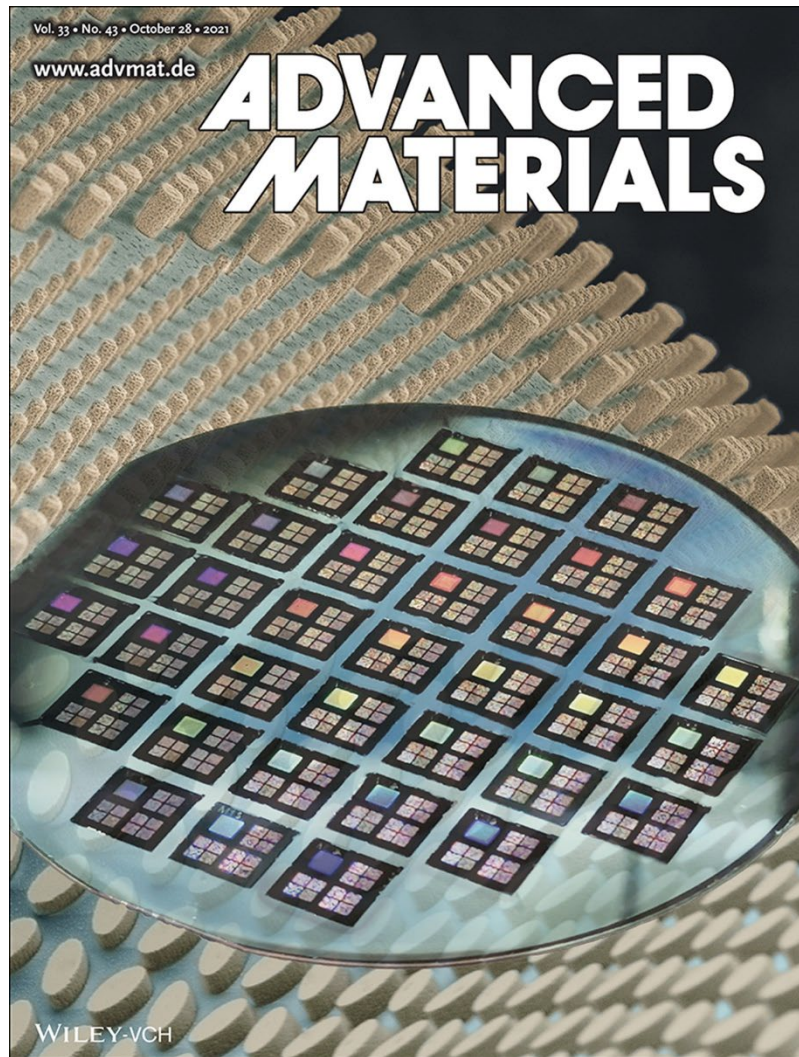
High Harmonic Generation: Nonlinear Optics with Gradient Broadband Metasurfaces

In collaboration with Yuri Kivshar's
Group at ANU

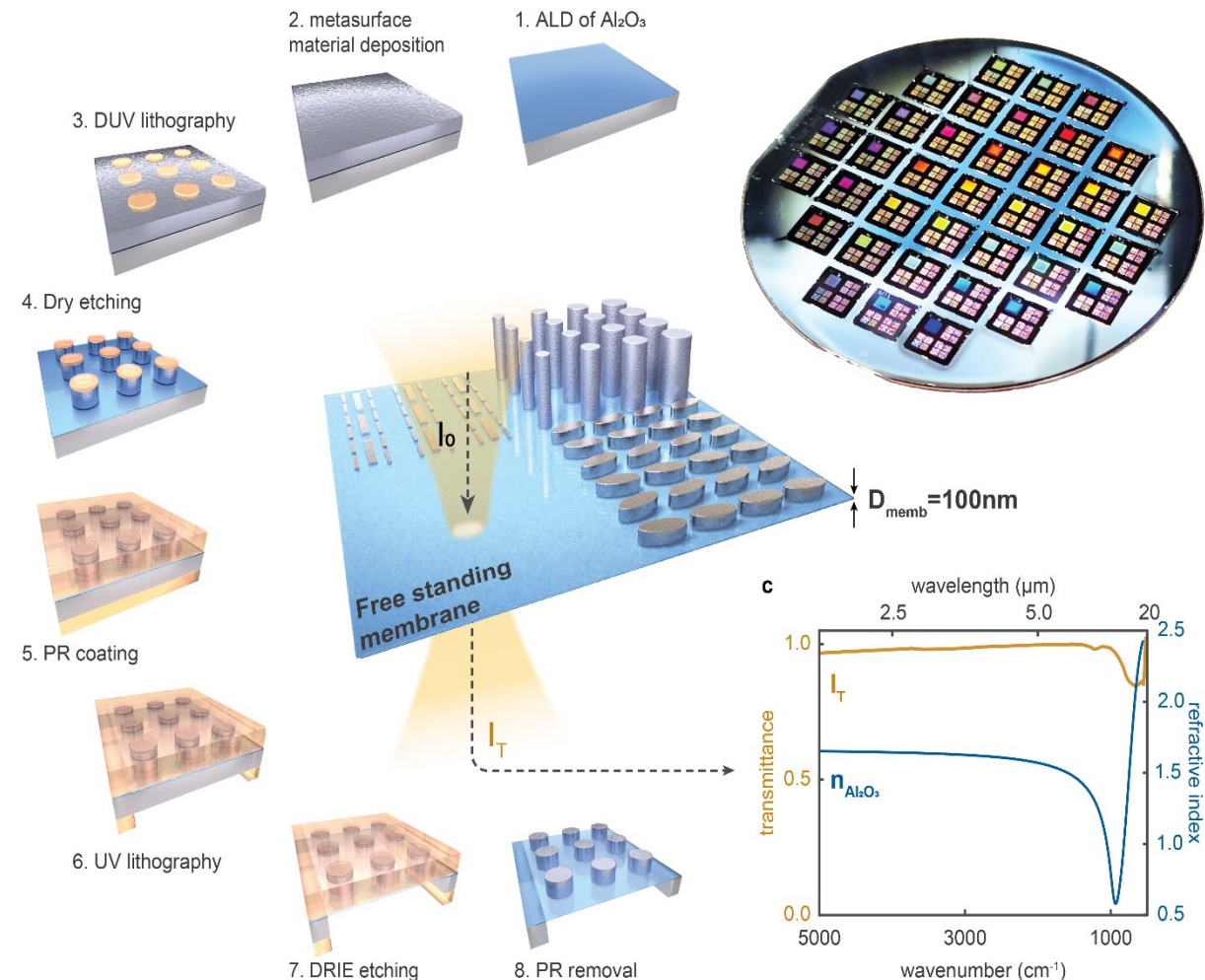


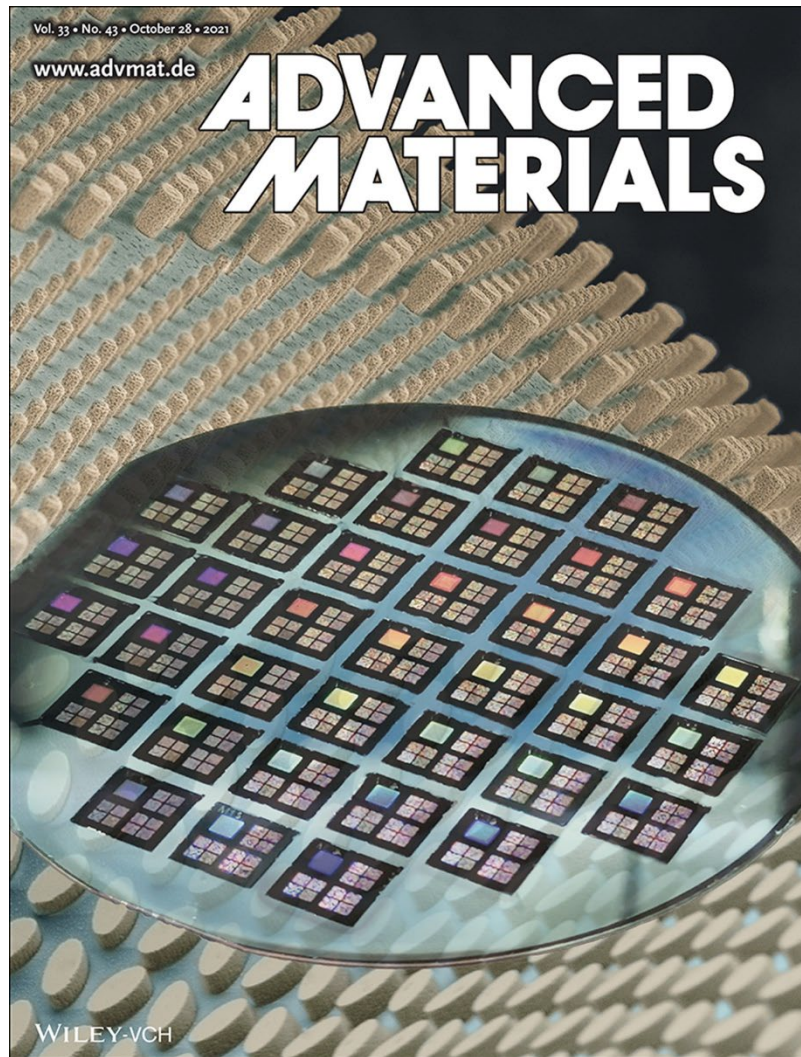
Jangid & Richter et al.,
Advanced Materials (2023)





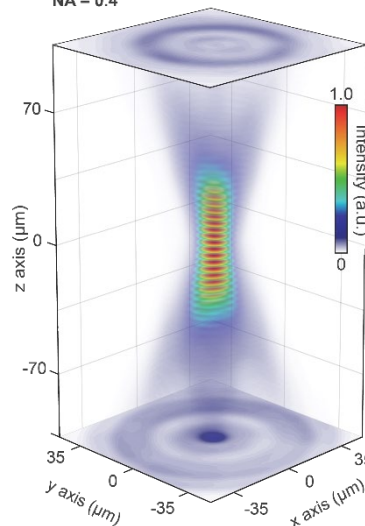
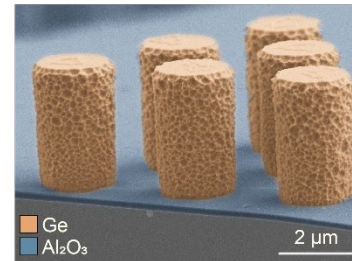
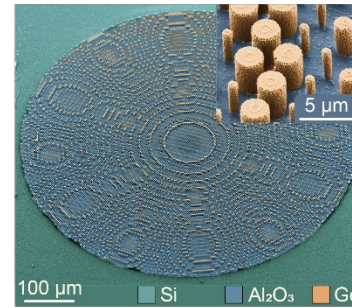
- 90% transmission over an ultra-broadband spectrum [from 2 to 20 μm]
- Ultra-low effective refractive index down to 1.04



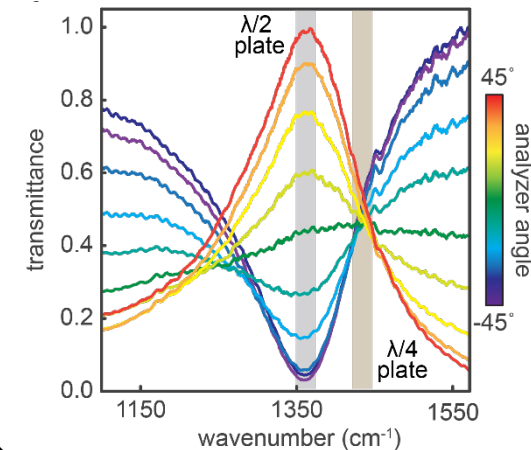
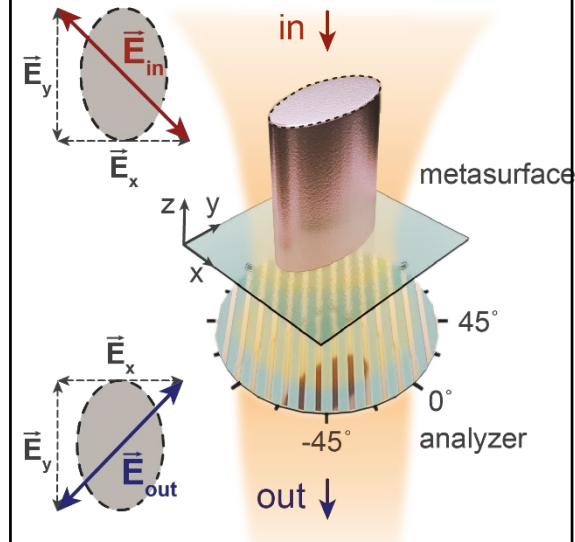


A. Leitis et.al., *Advanced Materials*, 2102232 (2021)

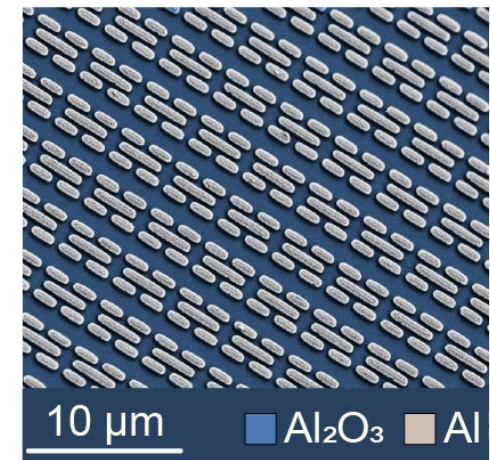
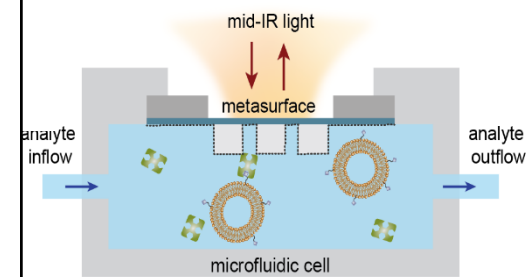
Large-area metalens



Birefringent metasurfaces



Aluminum Plasmonics



Ongoing Research Directions



Live Cell & Single-Cell Technologies

- Cell screening & profiling, e.g. for immunoengineering applications
- Real-time & label-free secretion analysis from single cells
- Cell-cell interactions & incorporation of biomimetic surfaces



Since Lab-on-a-Chip 2017 ...

Recent highlights:

ACS Sensors 2018

Small 2018

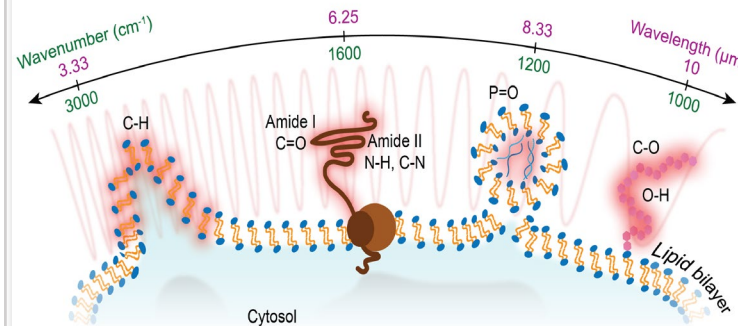
Science Advance 2021

Biosensors and Bioelectronics 2022

Nature Biomedical Engineering 2023 ...

Surface Enhanced IR Spectroscopy

- Chemical-specific & fingerprint detection
- Monitor conformational/structural changes
- Real-time measurements in aqueous solution



Since PNAS 2009 ...

Recent highlights:

Nature Communications 2013 → patent granted

Science 2015

Nature Communications 2018

Science 2018

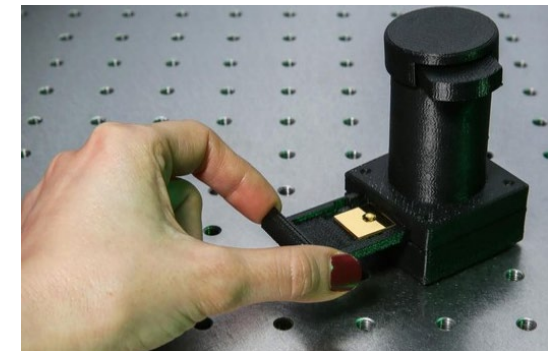
Science Advance 2019

Advanced Materials 2021 & 2022

PNAS 2023 ...

Point-of-Care Diagnostics

- Low-cost, portable, rapid & multiplexed microarray technologies.
- New device schemes including lens-free microscopy, hyperspectral imaging ...



Since PNAS 2011 ...

Recent highlights

Light Science and Technology 2018

ACS Photonics 2015 → patent granted

ACS Nano 2018 → patent filed

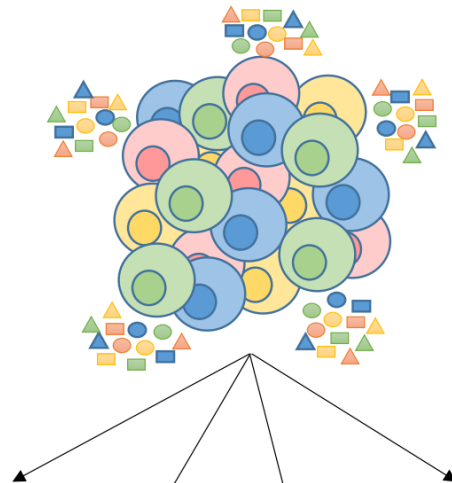
Nature Photonics 2019

Small 2020

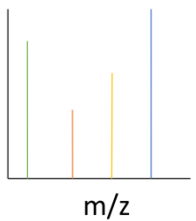
Nature Communications 2021 ...

Importance of heterogeneity

Cells present heterogeneity in various characteristics



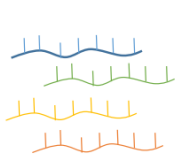
Metabolic markers



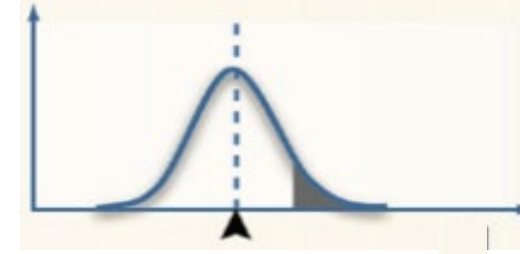
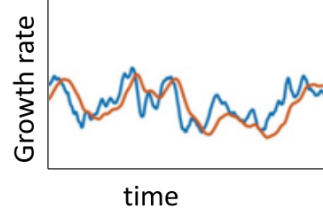
Protein markers



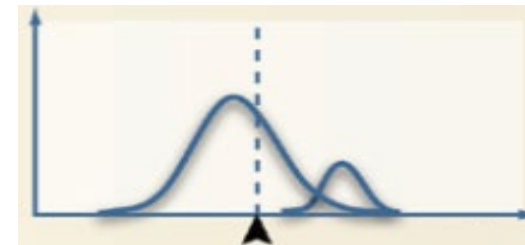
Genetic markers



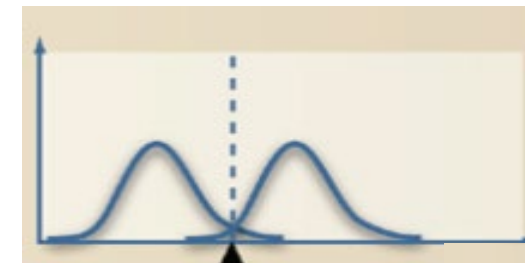
Phenotypic heterogeneity



Homogenous population



Heterogeneous population

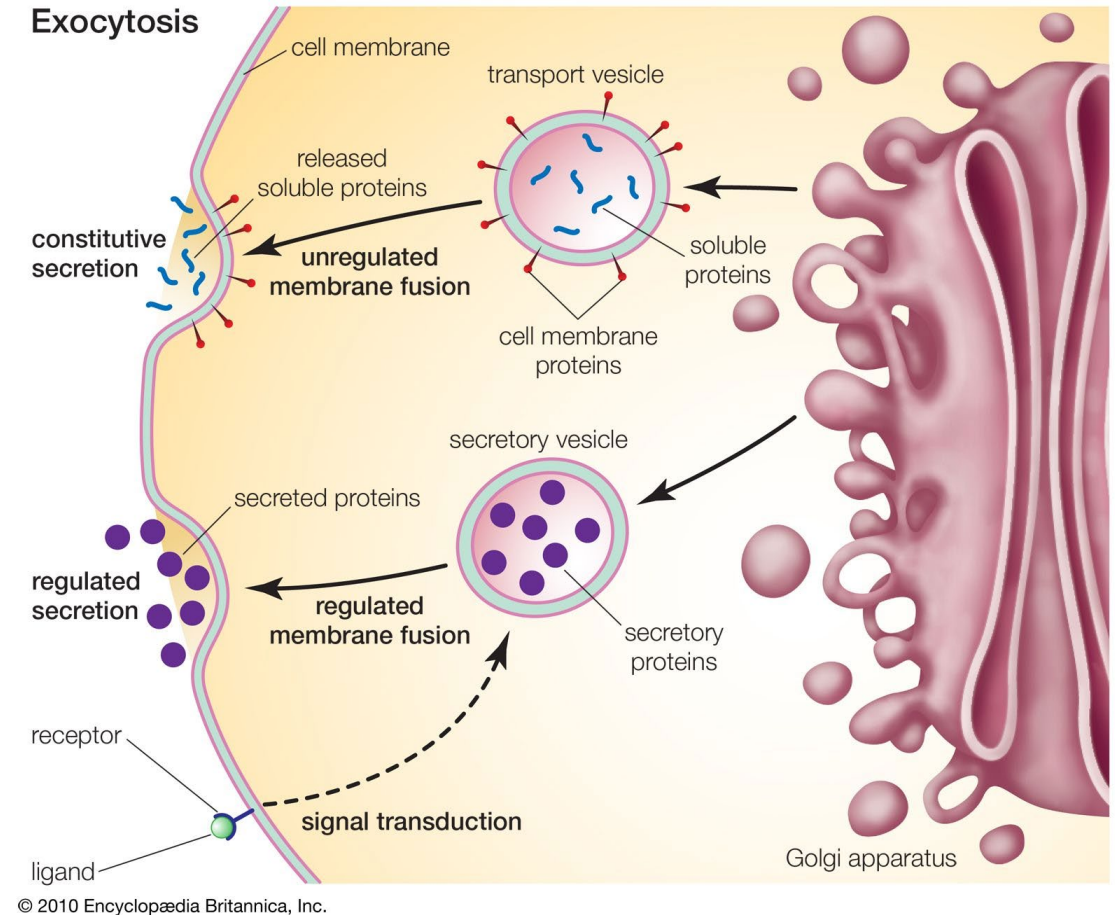


Highly heterogeneous population

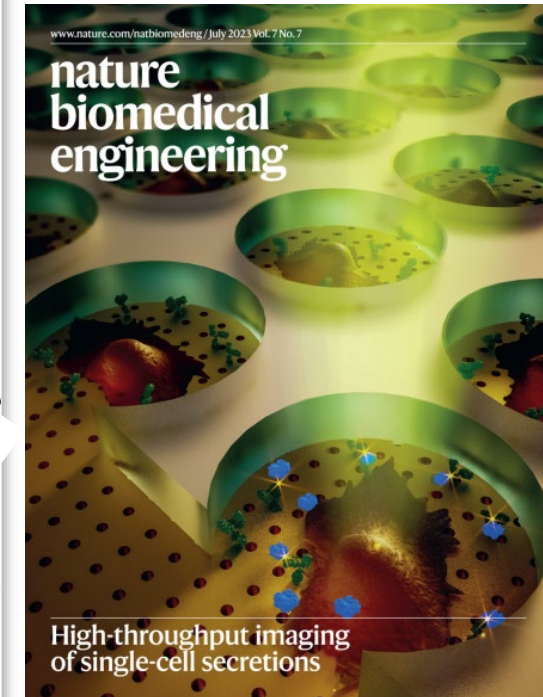
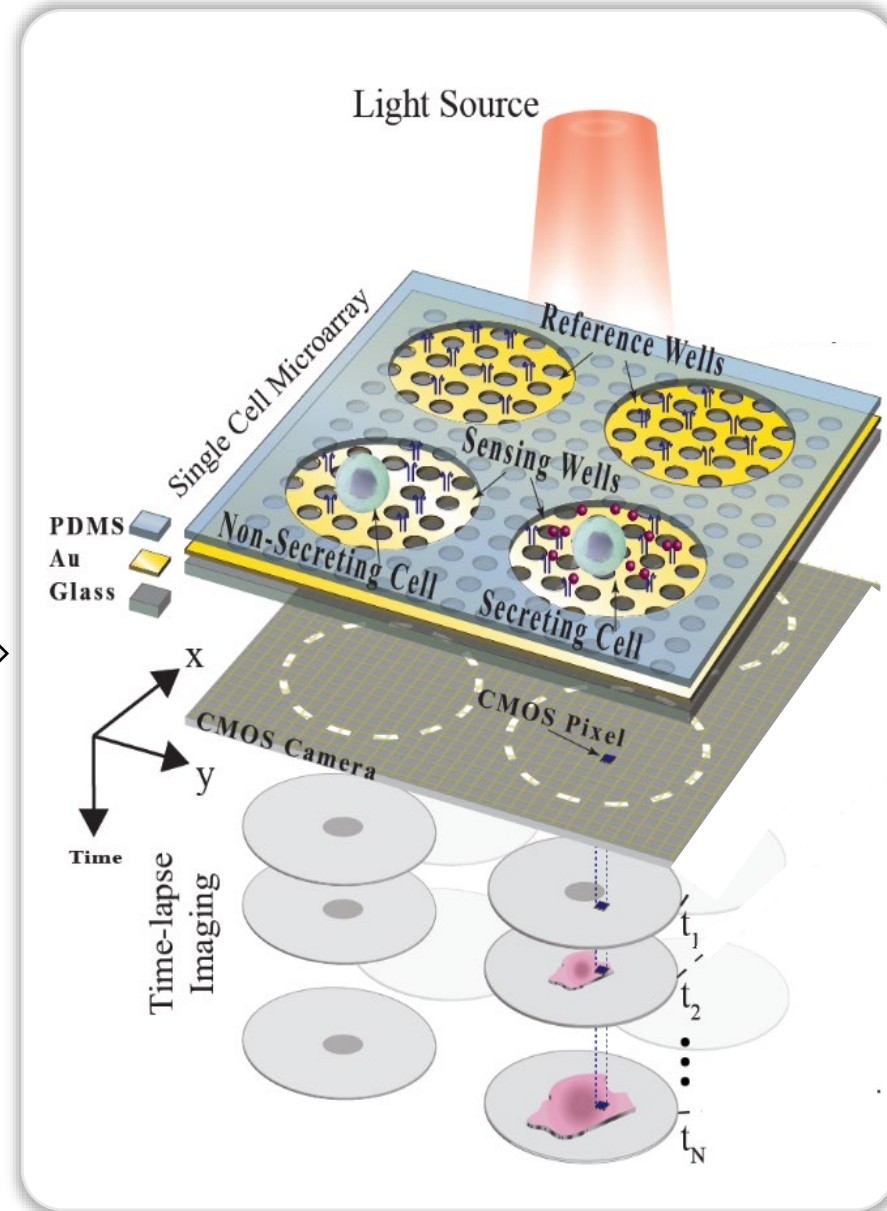
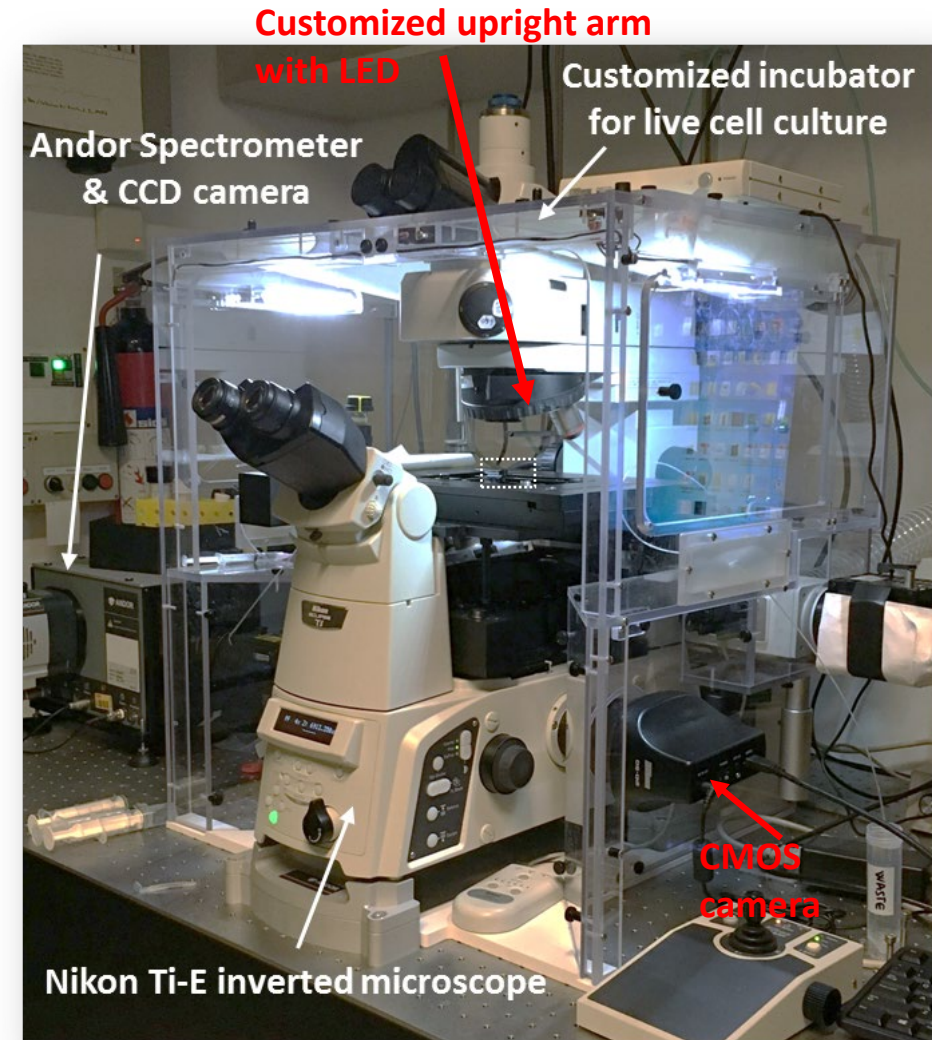
Ensemble measurements can mask the variations in responses

Protein secretion from cells

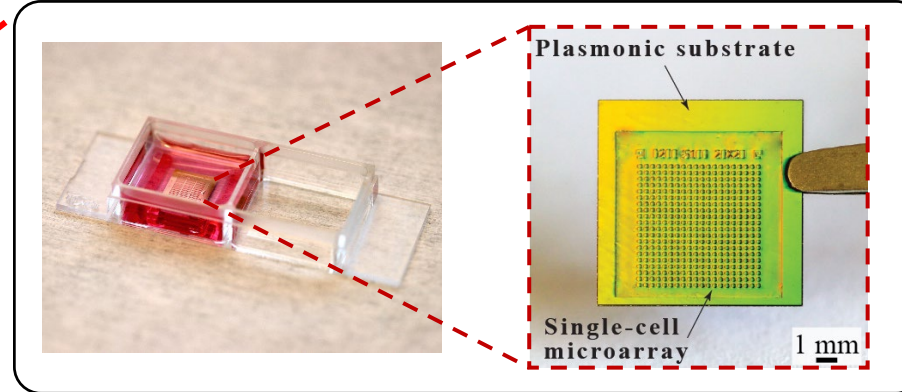
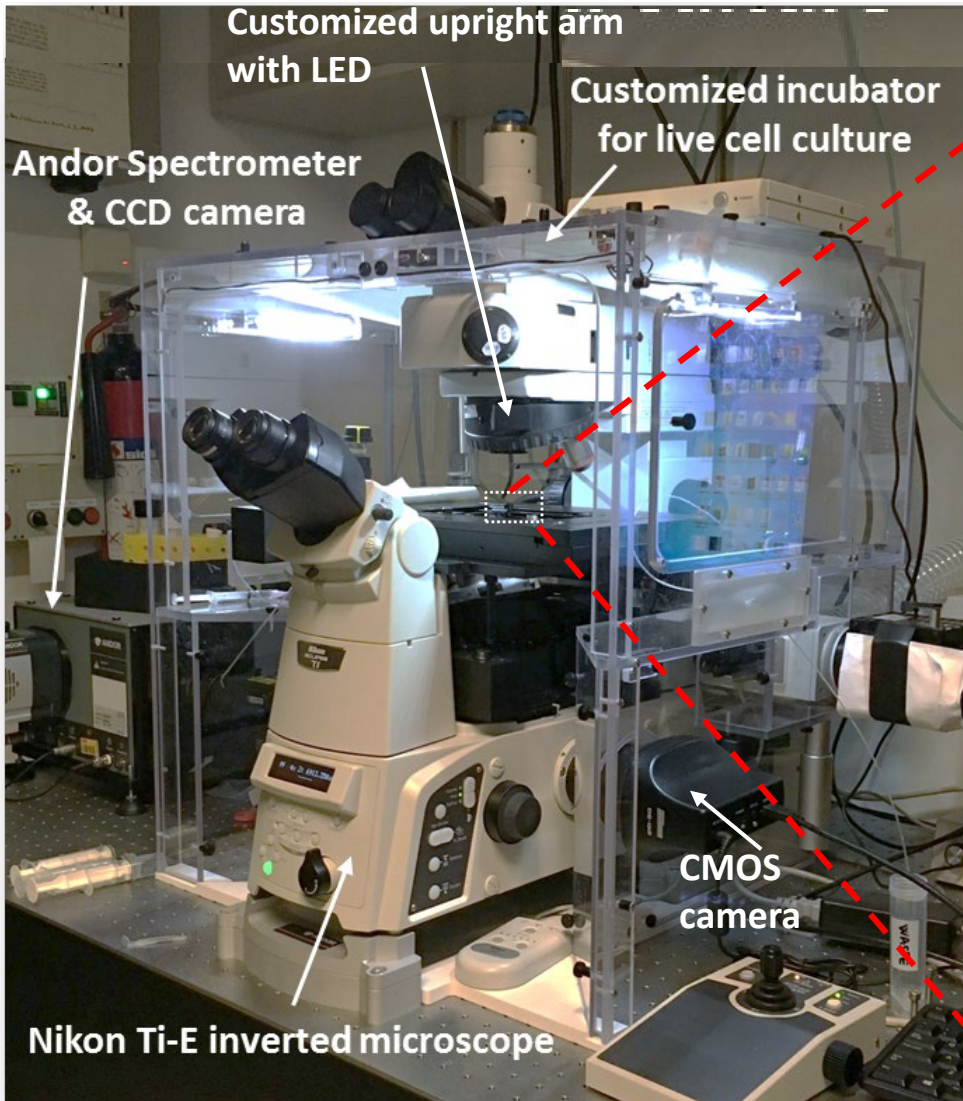
- Closely related to physiology and disease progression
- A precisely regulated dynamic process
 - External stimulation
 - Gene expression
 - Exocytosis
- Involved in biological functions, interactions & communications
- A variety of secreted substances:
 - Cytokines
 - Antibodies
 - Extracellular vesicles ...



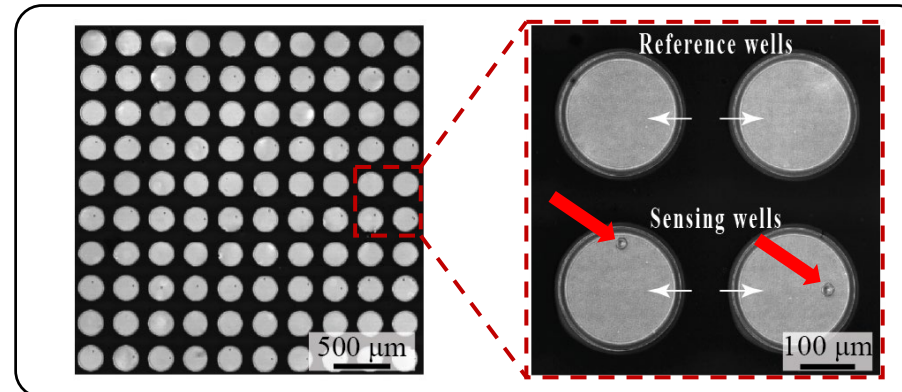
Single-Cell Microarray for “**Spatio-temporal**” Secretion Analysis



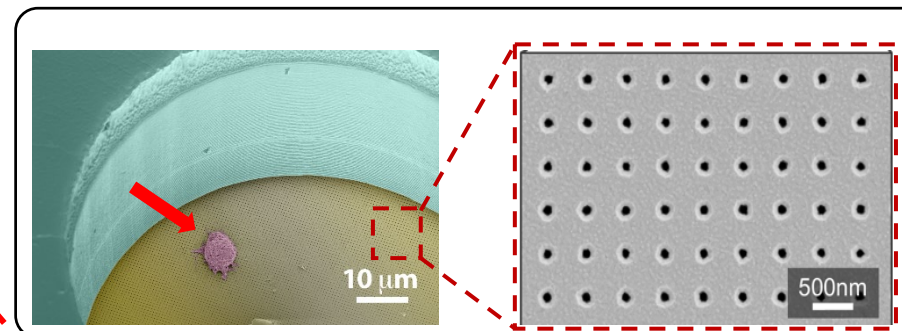
Nanoplasmonic single-cell microwell arrays



Integrated biochip:
- Maintains live cells



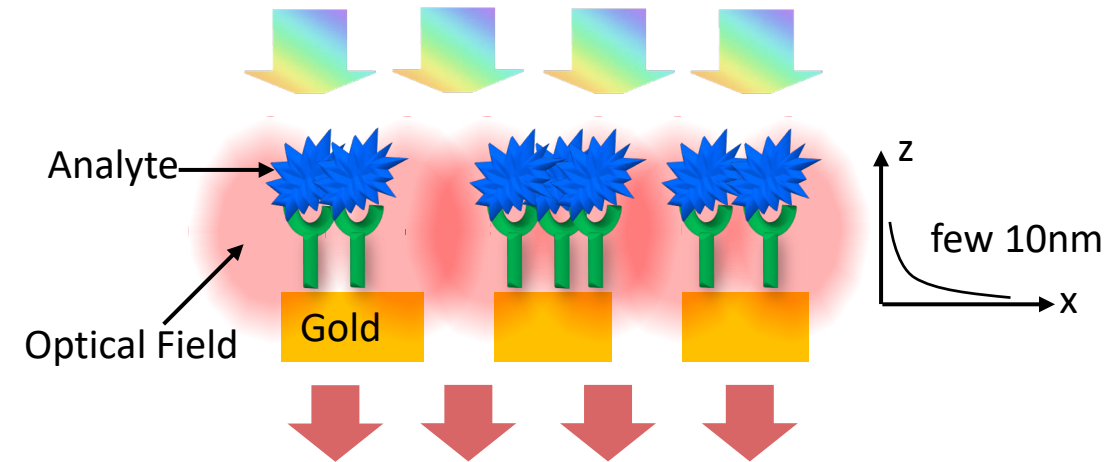
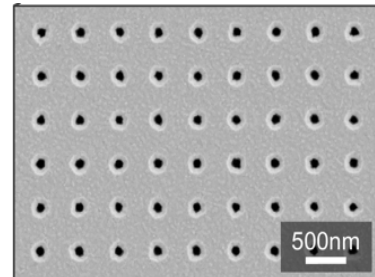
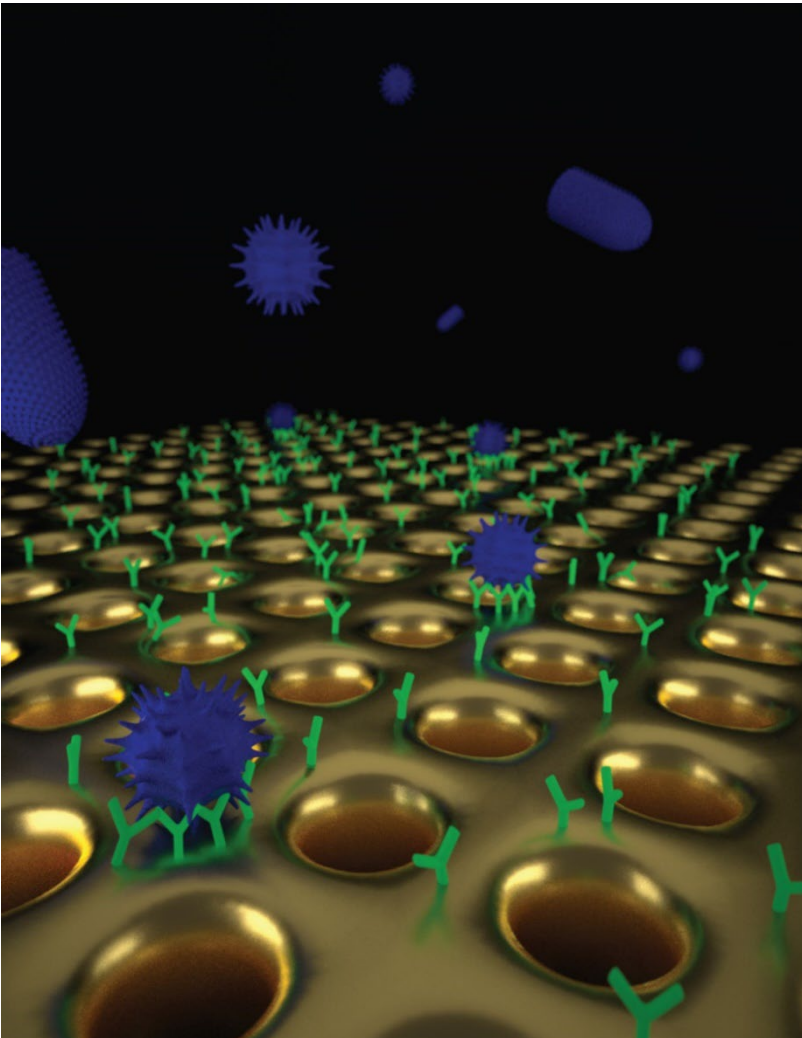
PDMS microwells:
- Compartmentalize single cells



Plasmonic nanoholes
- Enables label-free detection

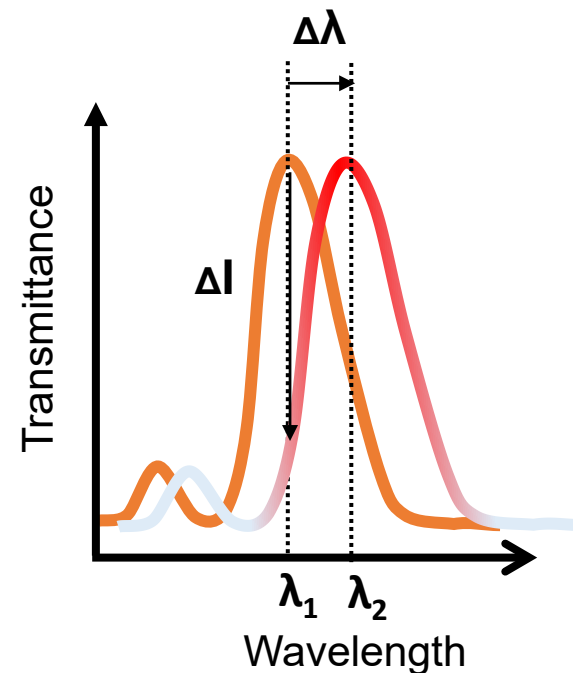
Label-free Detection Principle - NanoPlasmonics

Plasmonic Gold Chip with Nanohole Arrays

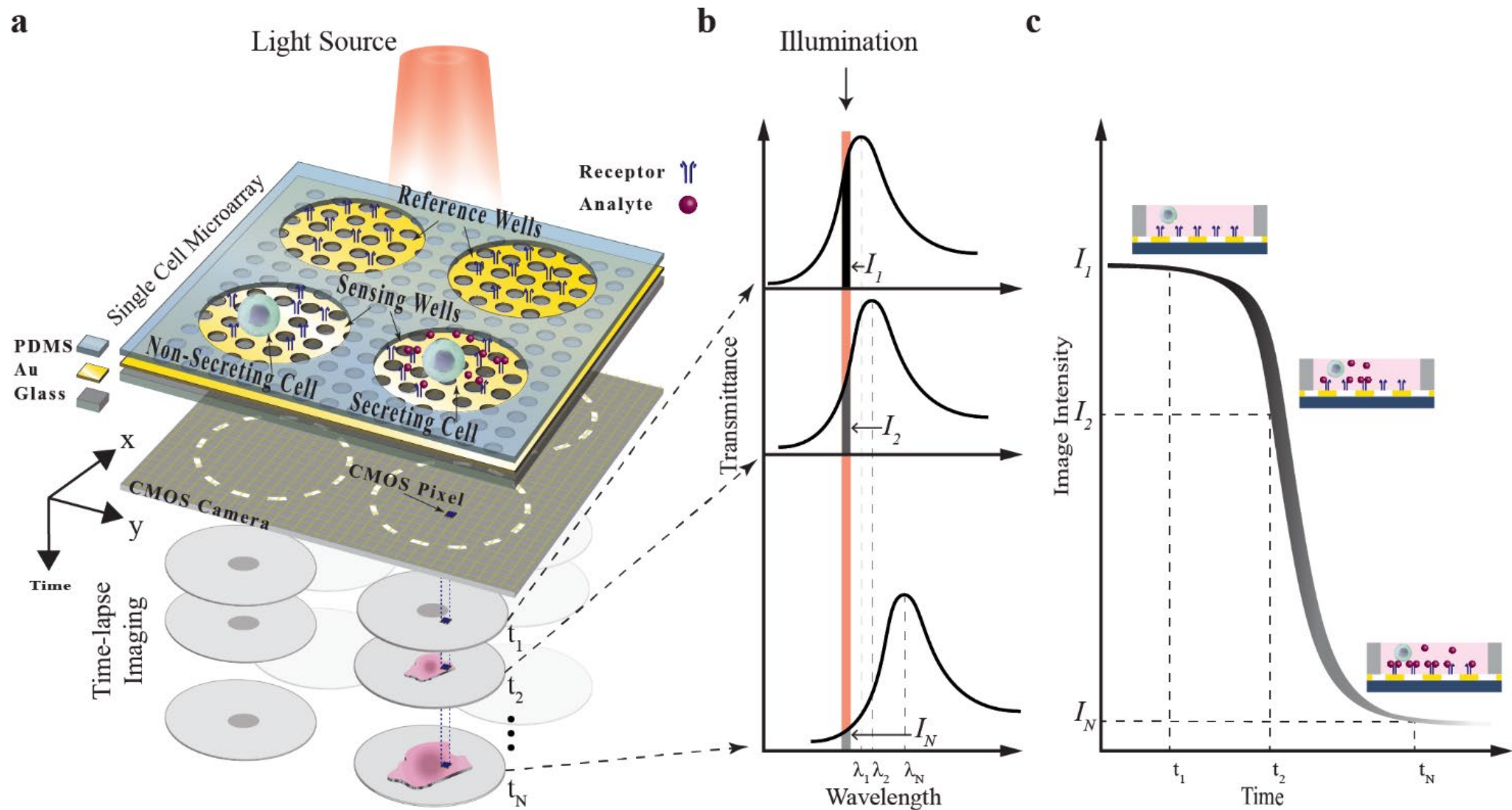


Confined near-fields within <50 nm from surface enables:

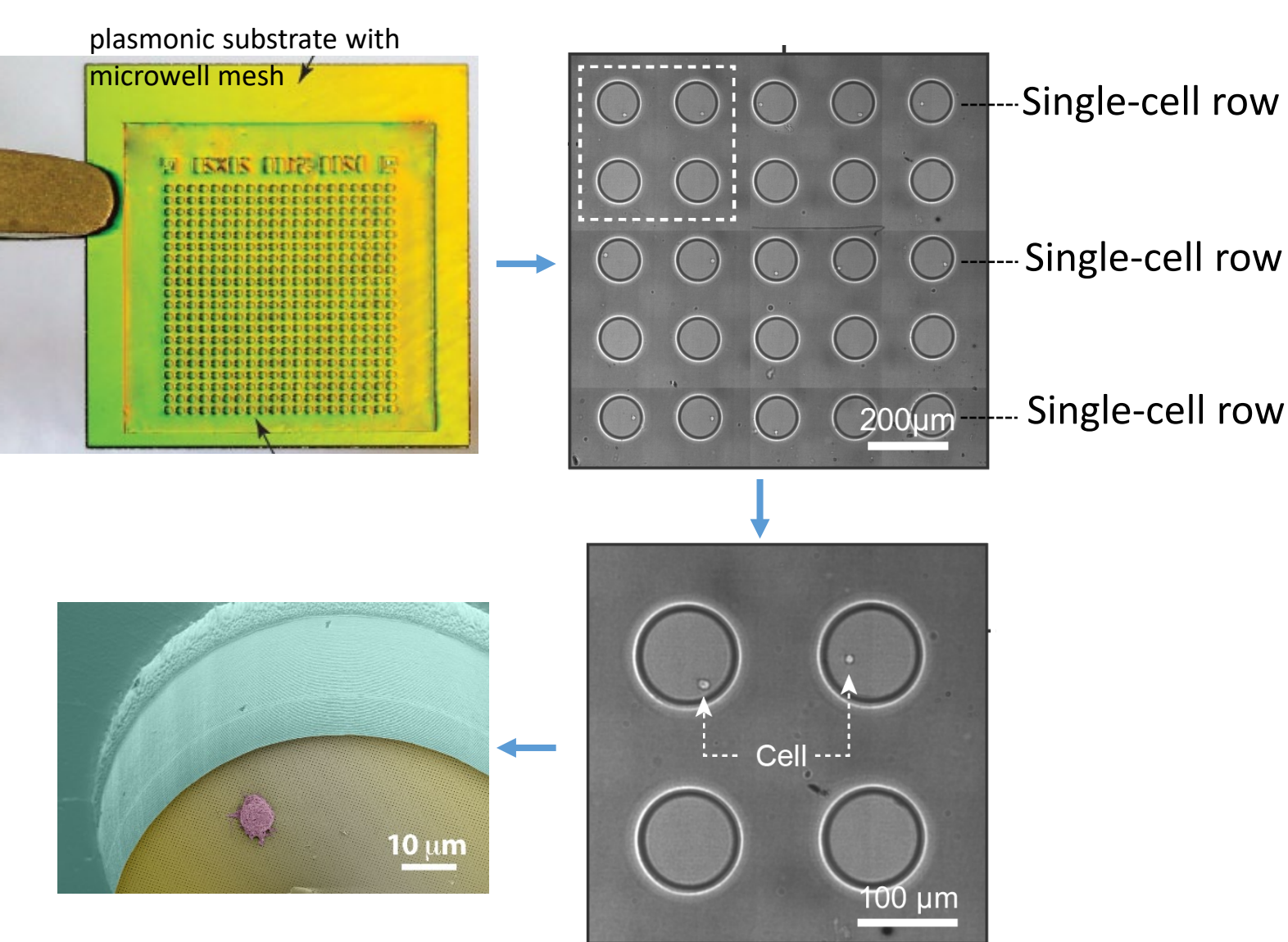
- Strong analyte-light overlap
- High sensitivity



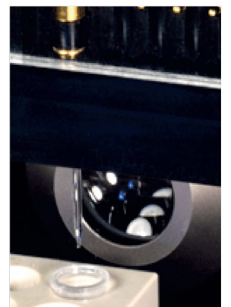
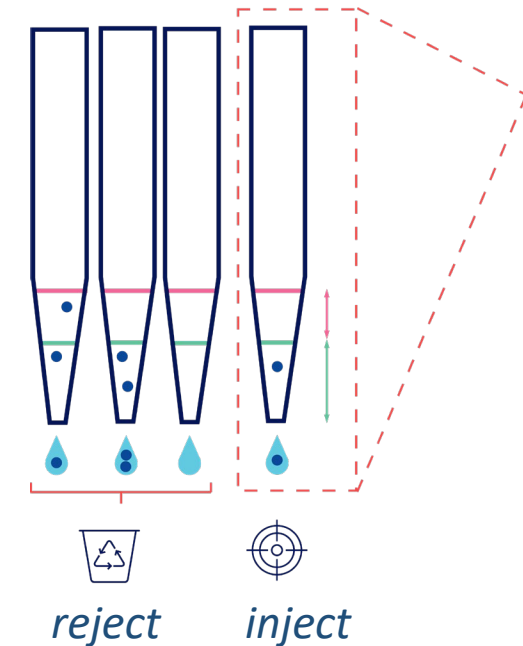
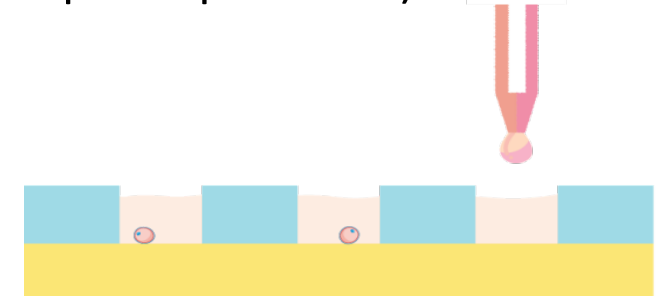
High-throughput spatiotemporal monitoring of single-cell secretions



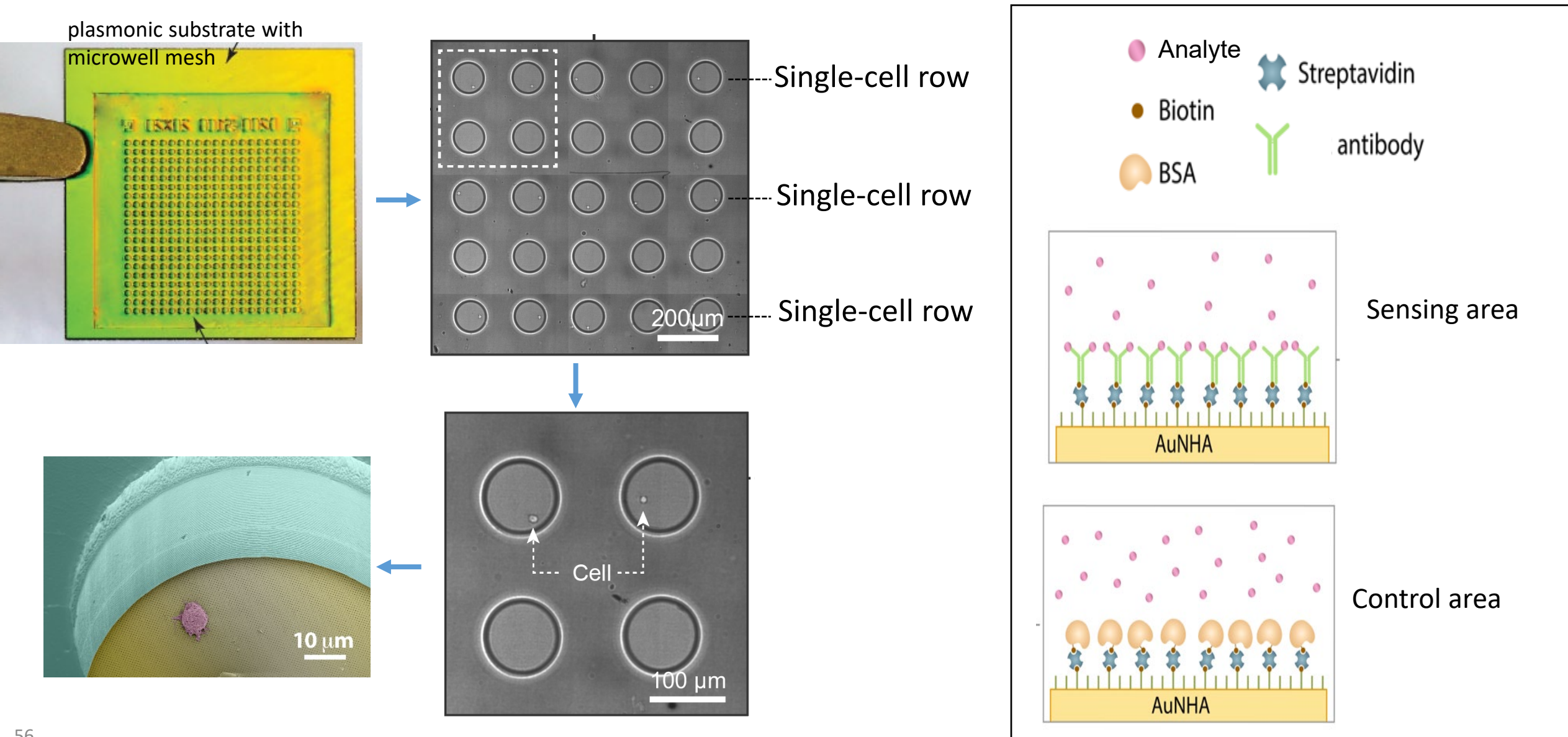
Deterministic single-cell loading in the microarray



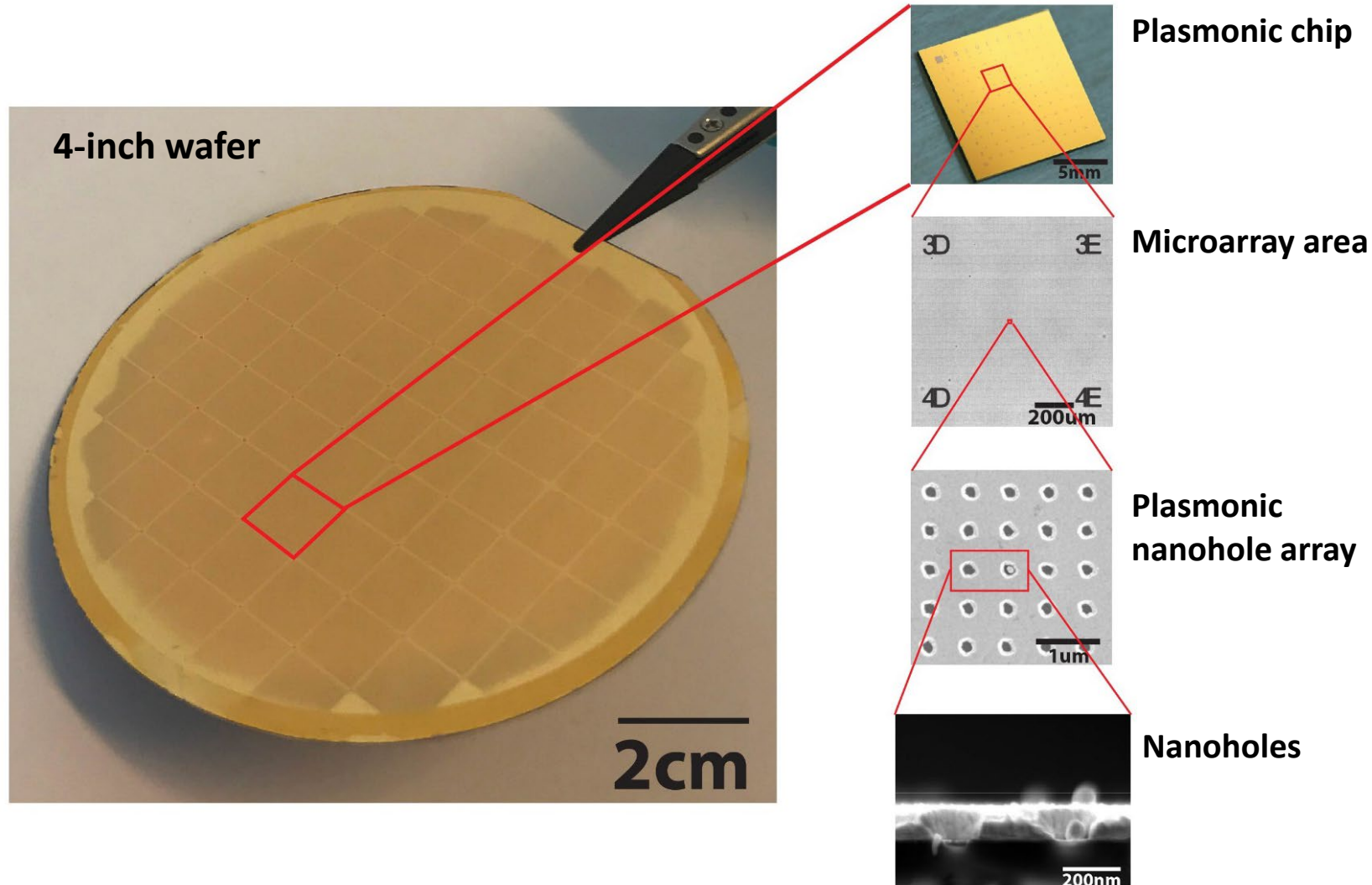
Non-contact & low volume dispenser
(400 pL drop volume)



Specific detection of target analyte – surface functionalization

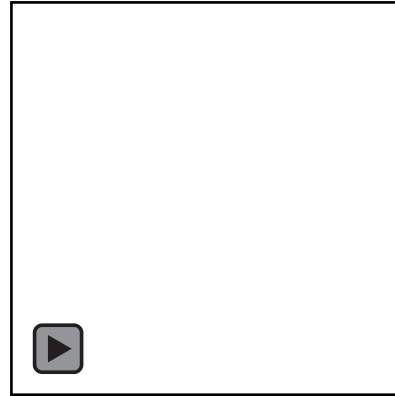
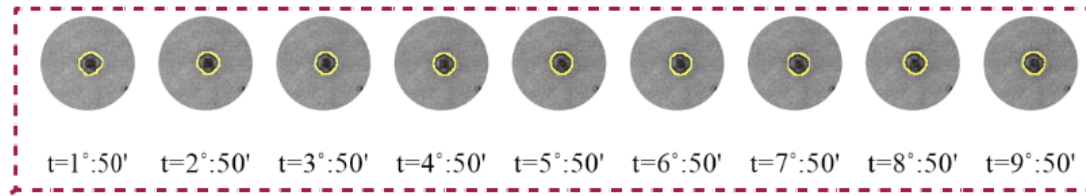
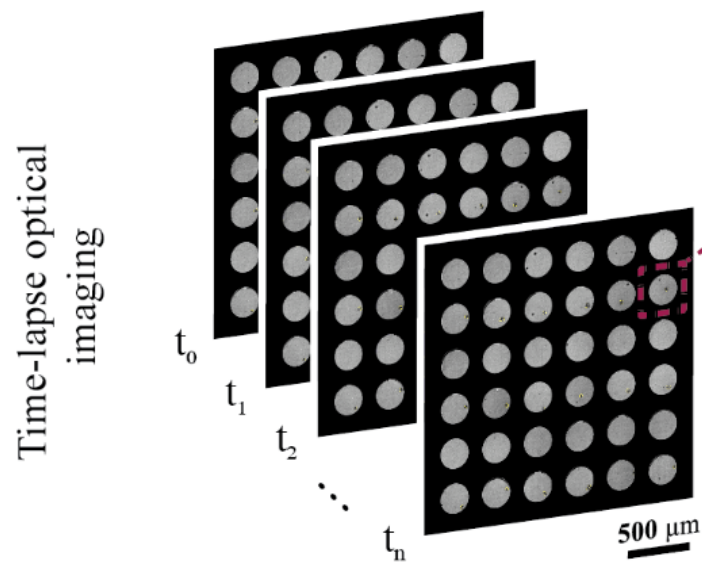


Wafer-scale manufacturing of nanoplasmonic sensors with DUVL process

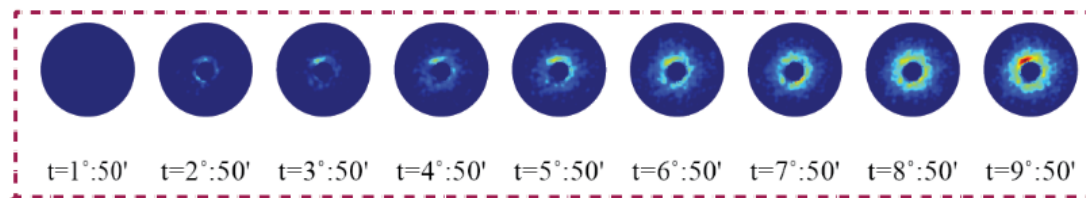
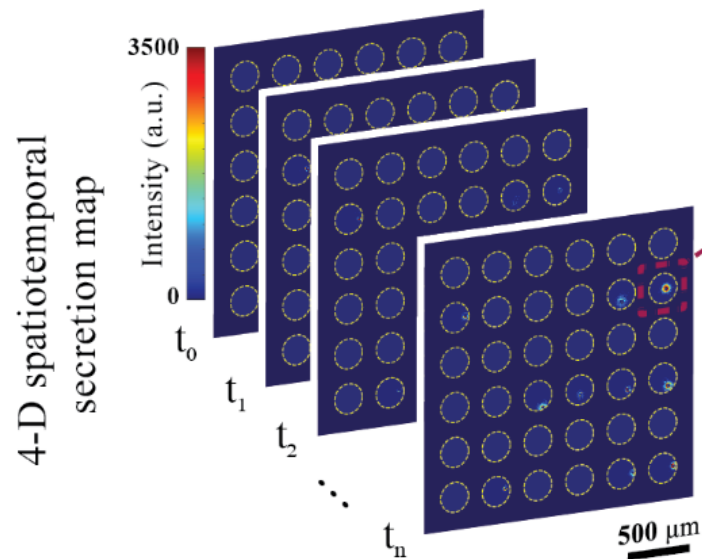


- Substrate is typically glass or silicon
- This example is based on glass:
 - Coated with 100 nm thick gold film
 - ~50 1x1 cm² nanoplasmonic chips

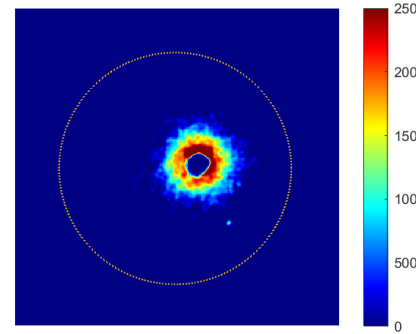
Simultaneous monitoring of cell secretion and morphology



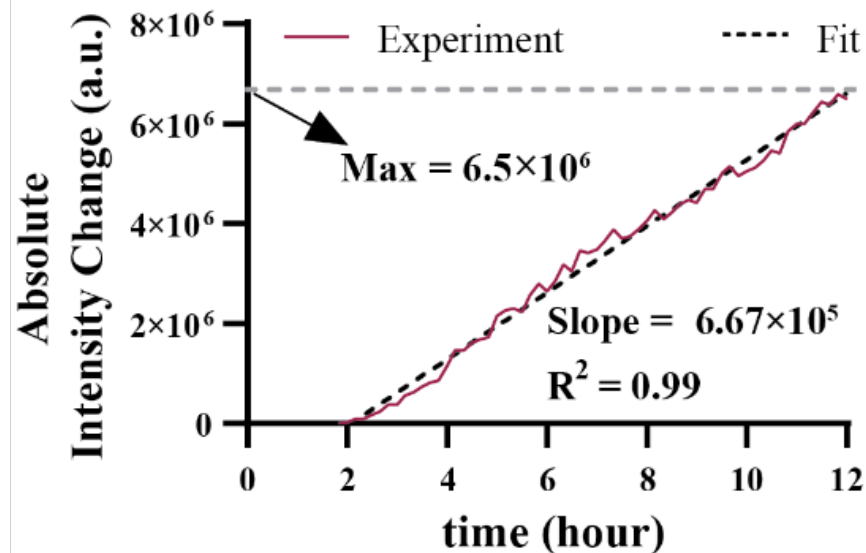
- IgG from hybridoma cells
- Cell area excluded with machine learning-assisted image processing
- 4D spatiotemporal map
 - X, Y, intensity, time



Spatio-temporal visualization of extracellular secretion from single cells



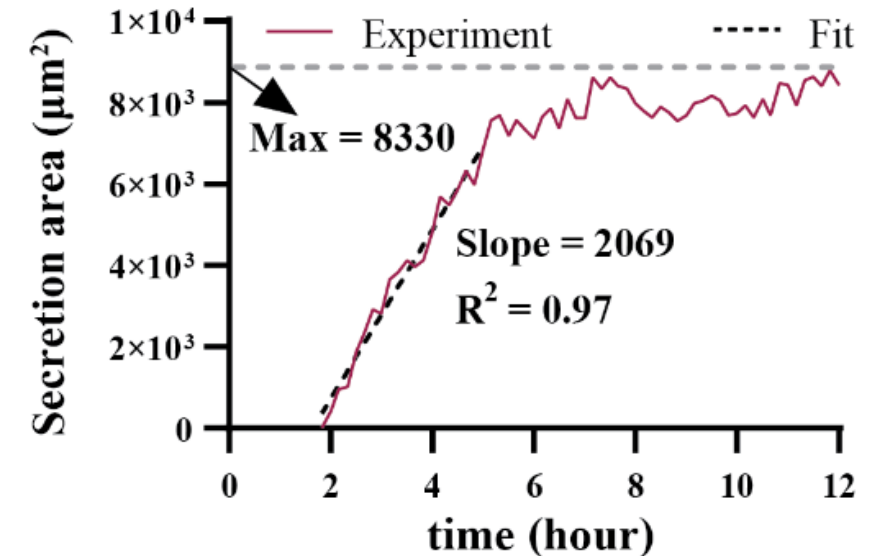
Temporal analysis



Max $\equiv$ **Maximum amount of secretion**

Slope $\equiv$ **Secretion rate**

Spatial analysis

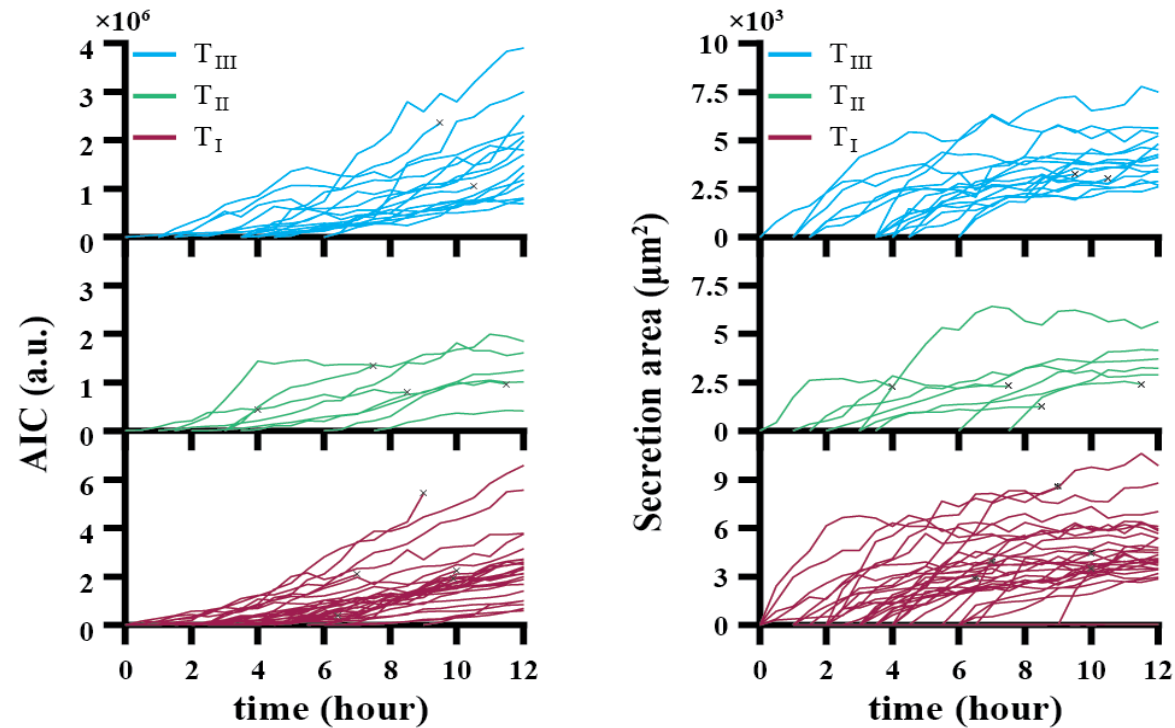


Max $\equiv$ **Maximum areal coverage**

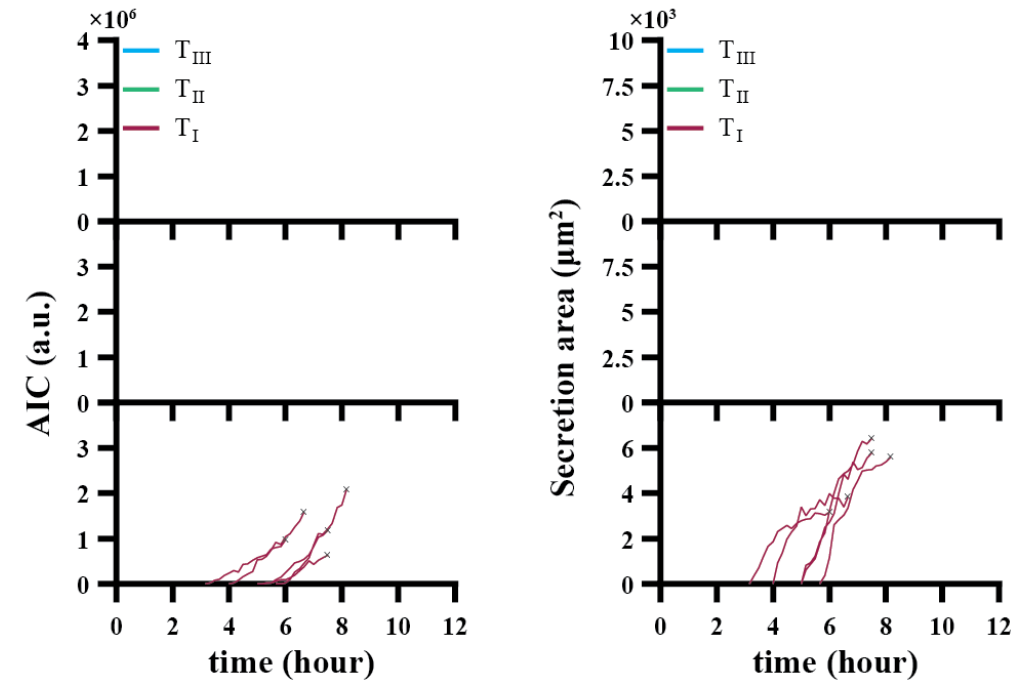
Slope $\equiv$ **Adsorption rate**

Statistical analysis of a population with single-cell microarray

Hybridoma

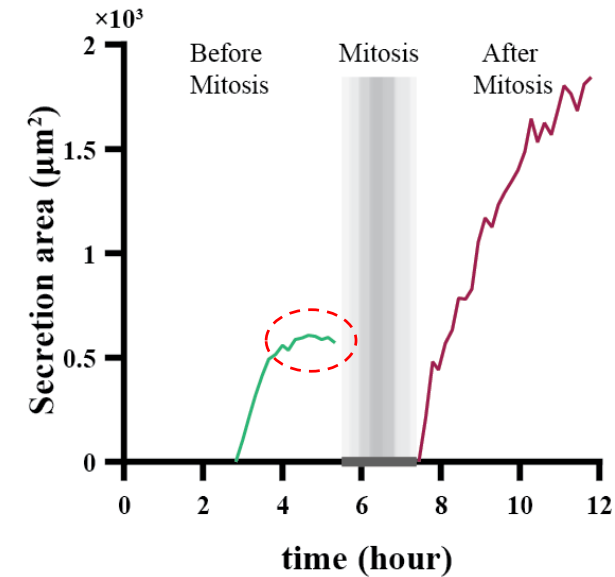
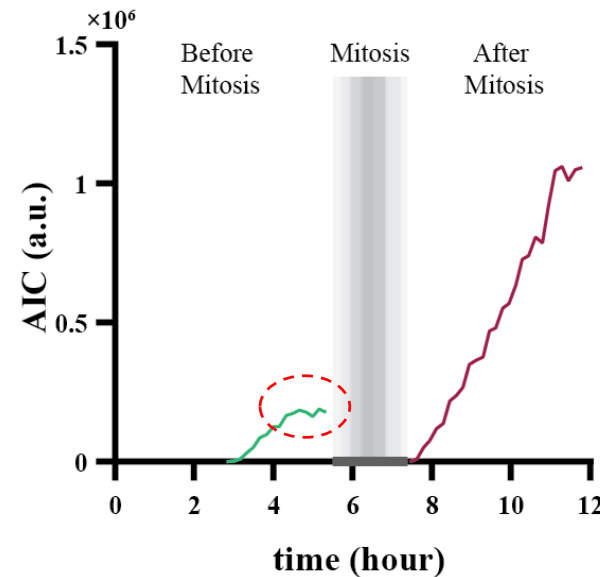
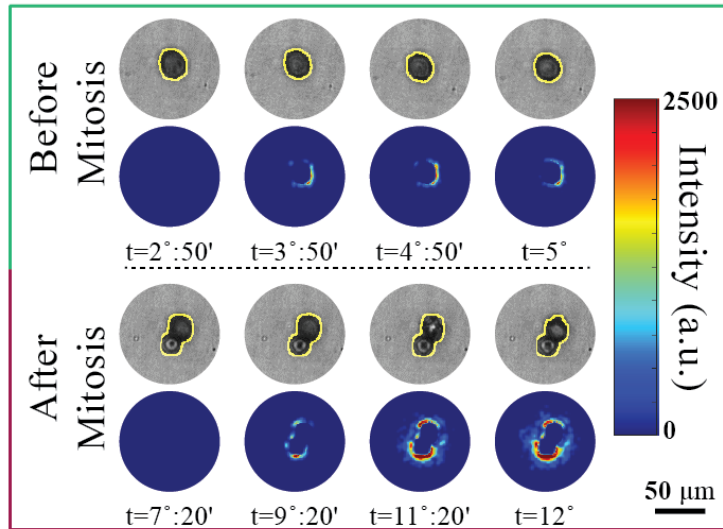
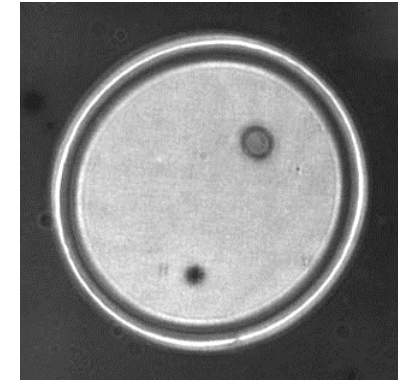


Protein transport inhibited (PTI) hybridoma



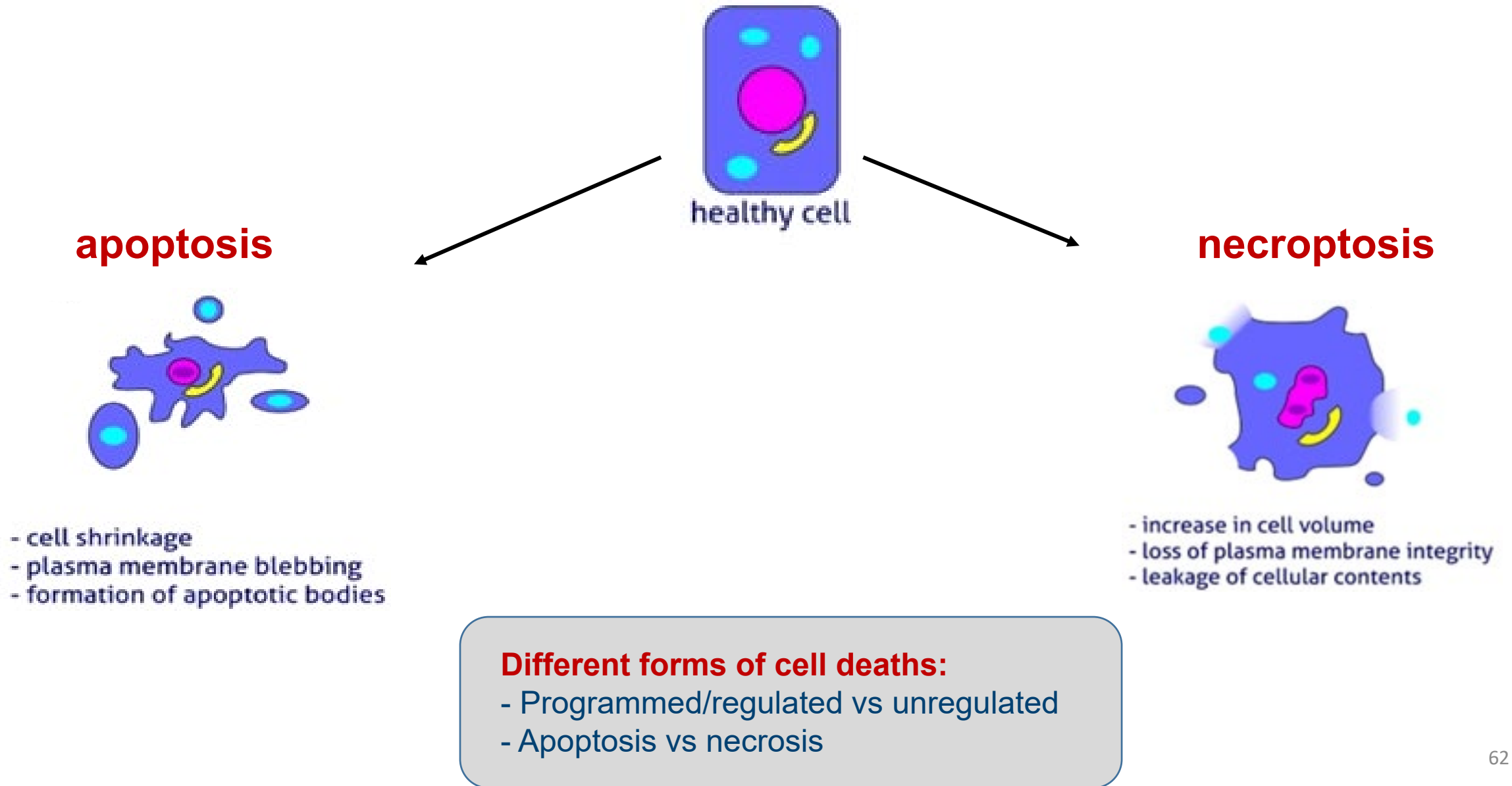
Cell secretion during mitosis

- Non-destructive monitoring of secretion during cell division

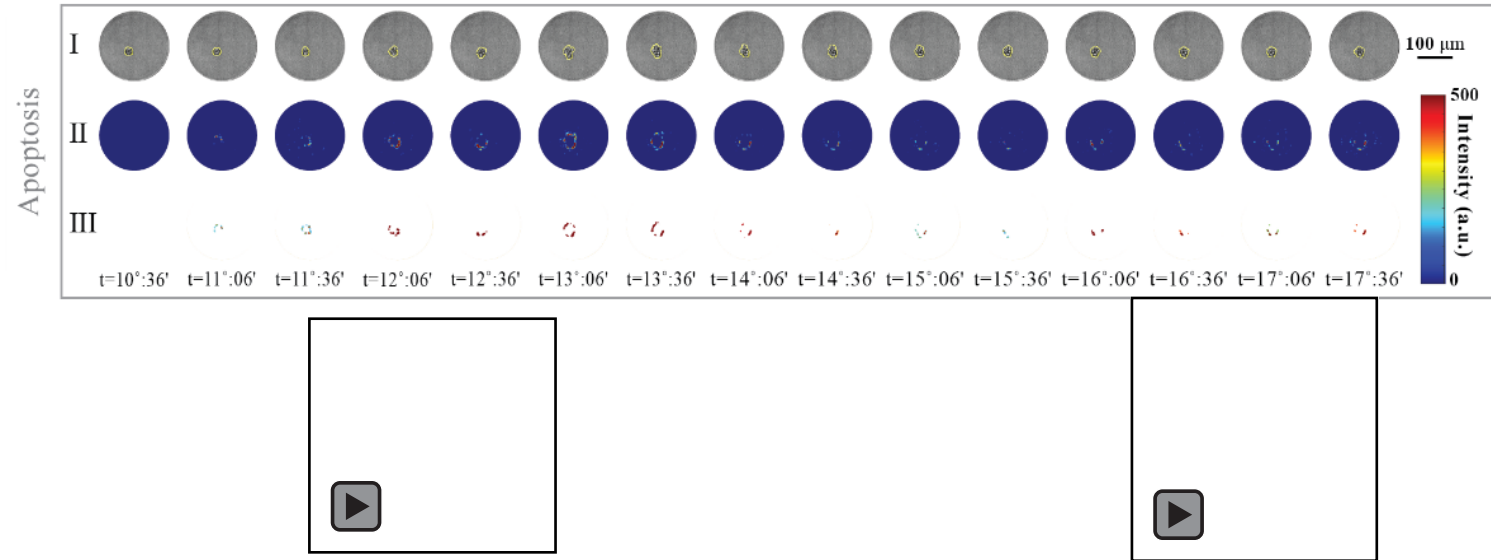


Downregulation of the ER-Golgi pathway before mitosis

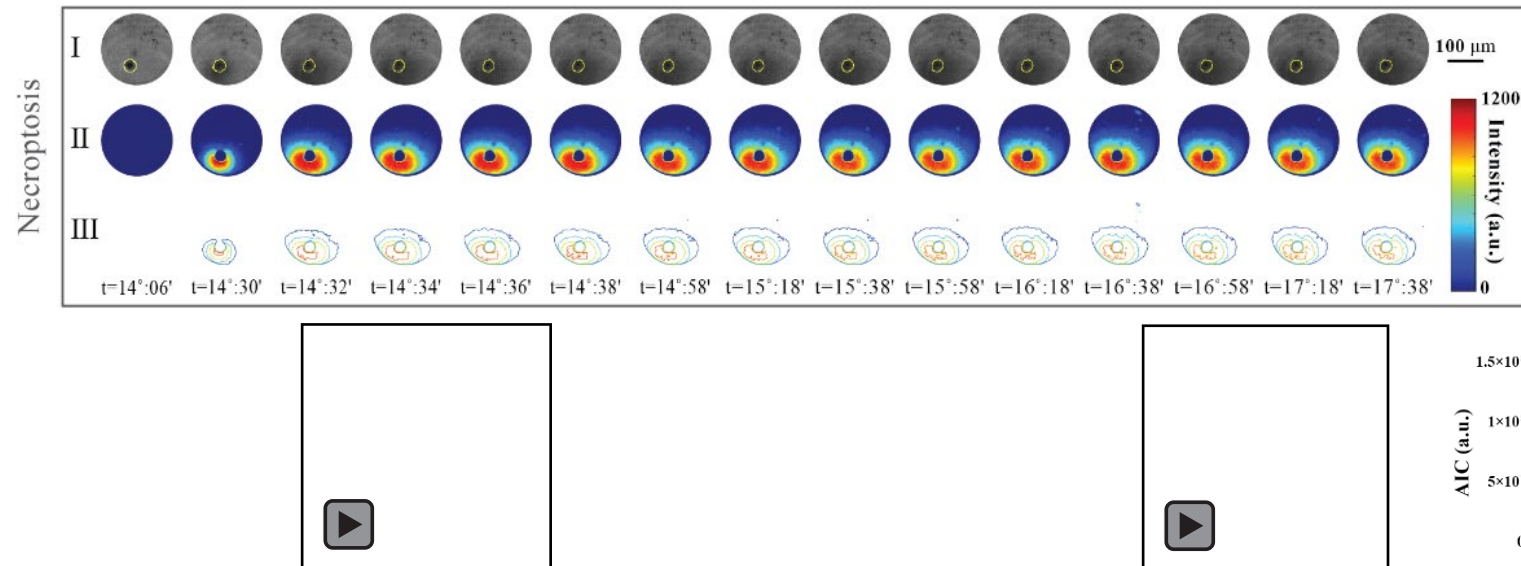
Cell content release during cell death



Cell content release during apoptosis and necroptosis

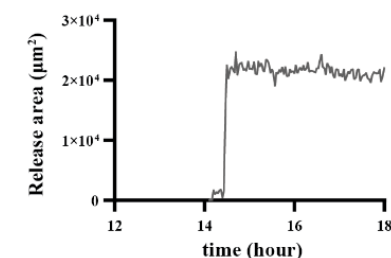
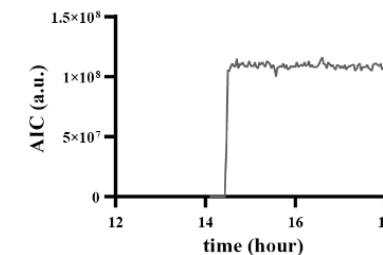


- Apoptosis is immunologically **“silent”**

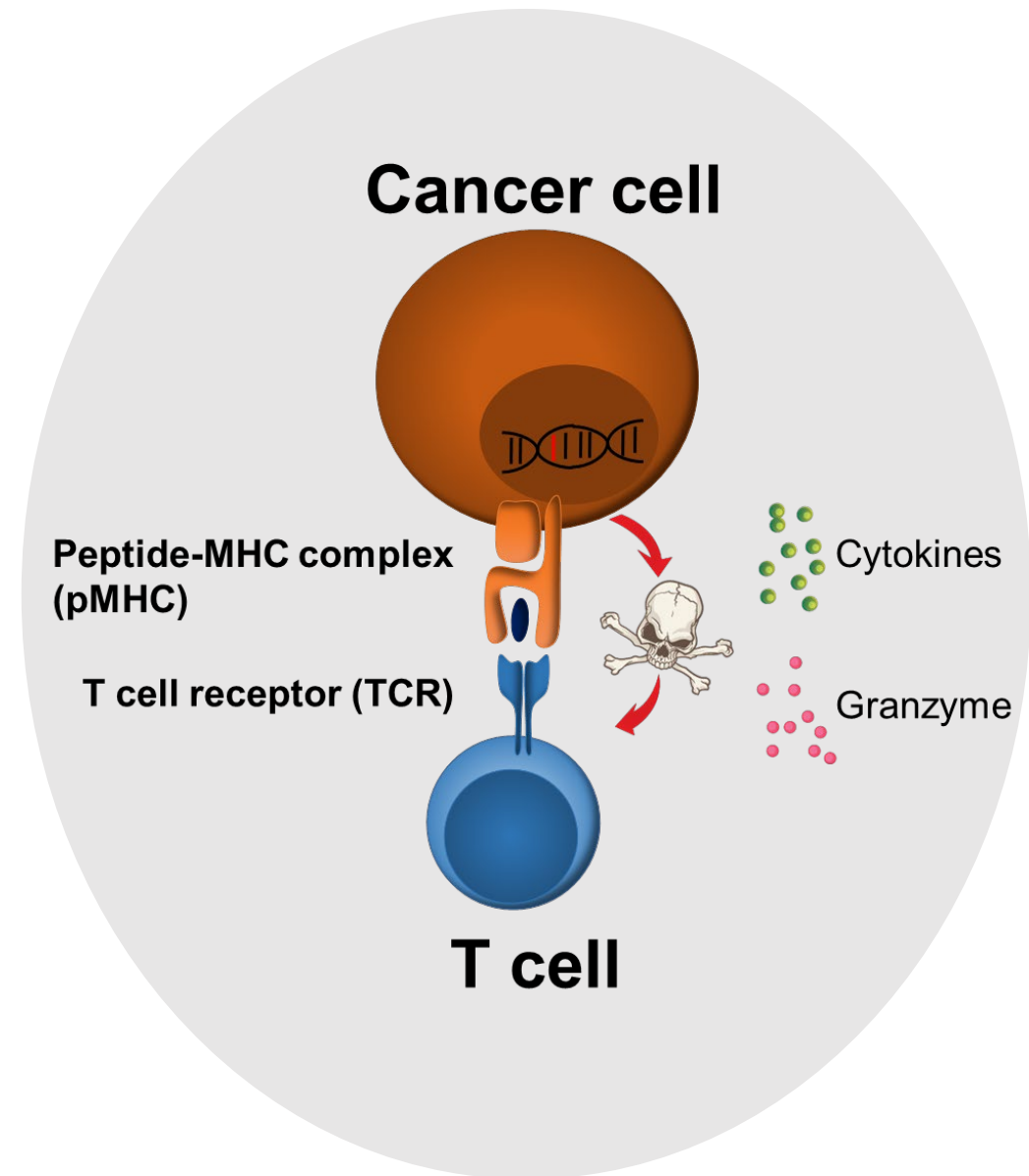


Bursting behavior of necroptosis could mediate:

- “long-distance”** cell communications
- “Higher immunogenicity”**

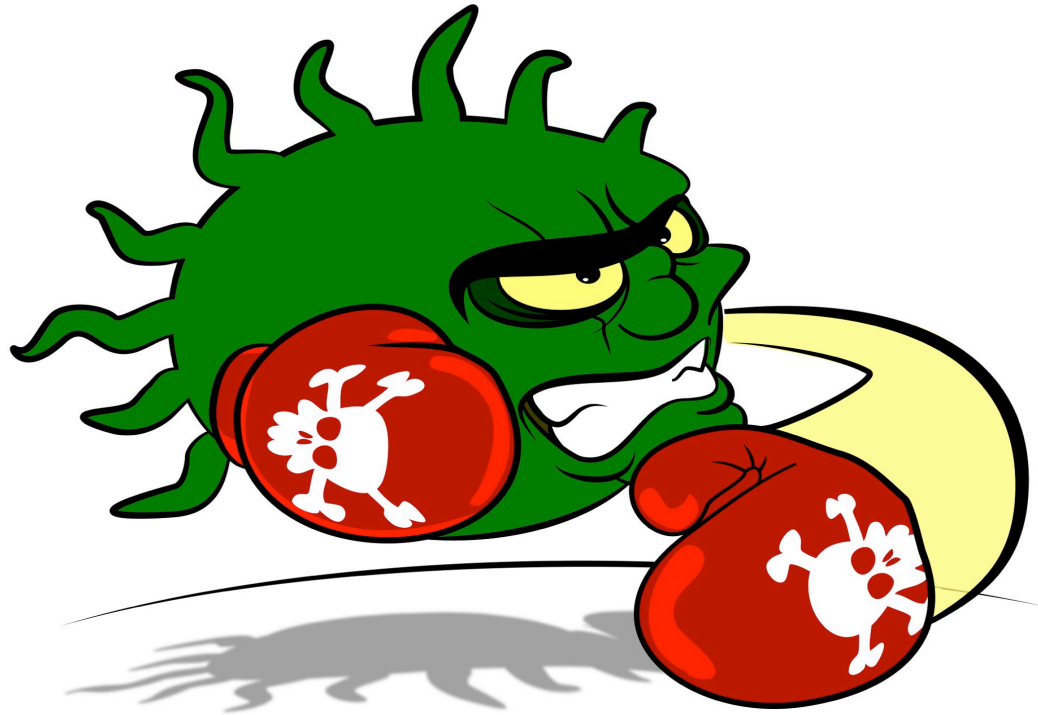


EPFL Photonic biosensors & bioimaging platforms for cell screening

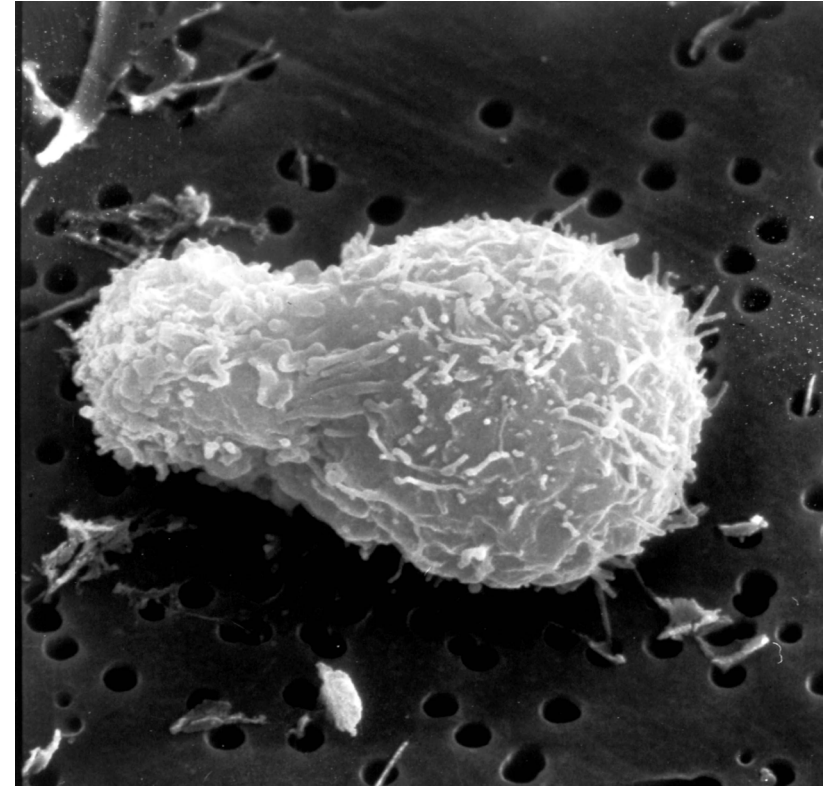


- Cell-cell interactions for **cytotoxicity analysis**
- Spatio-temporal **secretion analysis**

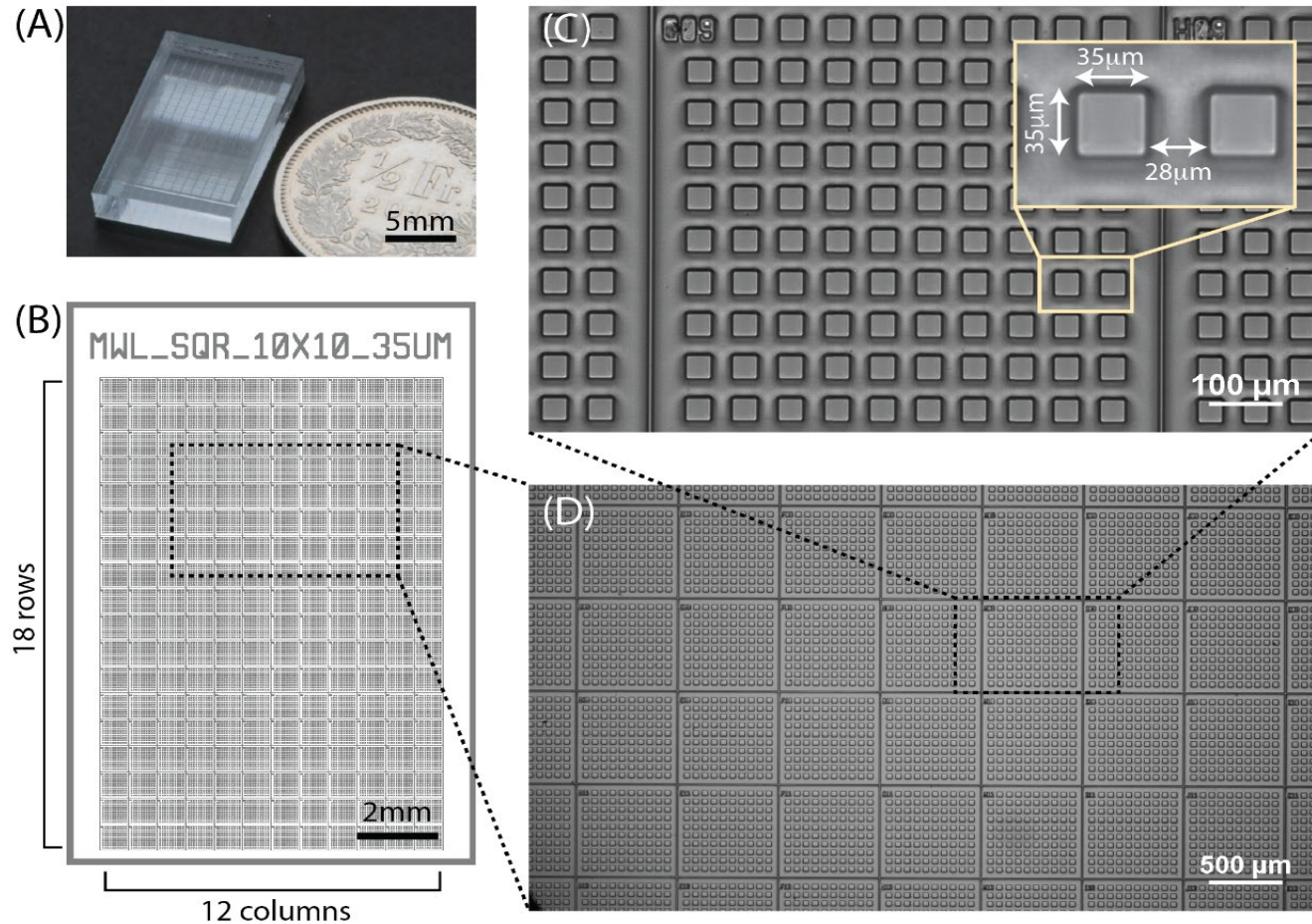
Cytotoxic T cells in anti-tumor immunity



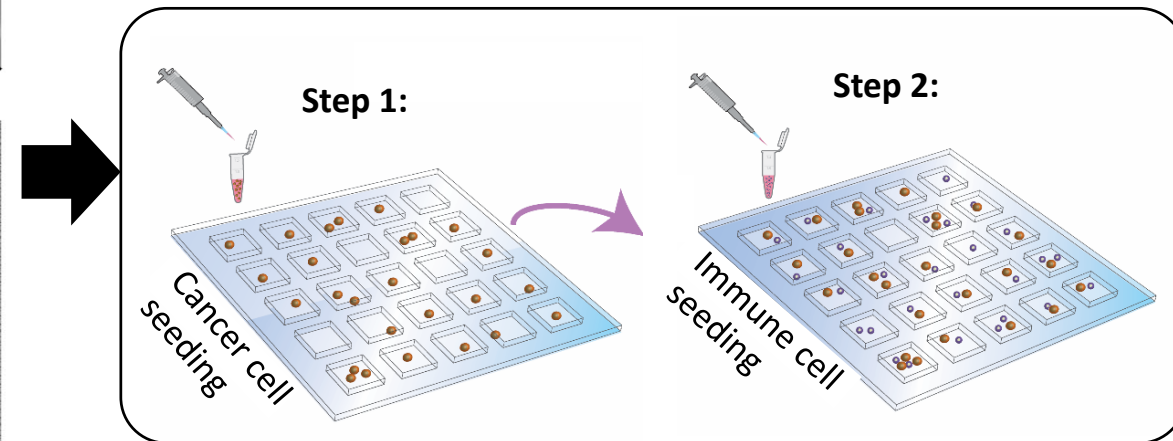
Cytotoxic T cells



High-throughput biochip for real-time analysis of cell-cell interactions



- Each PDMS chip contains 21'384 microwells
- Each well has only 65 picoLiter volume
- Confinement allows close interaction between T cells and tumor cells



Single Cell Microarrays For Monitoring of Cancer Cell Killings

Time-lapse imaging with
4 simultaneous channels
(1 bright field & 3 fluorescence)

Tumor-specific
T cells

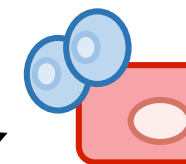
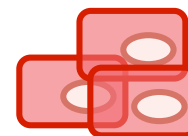
CFSE violet



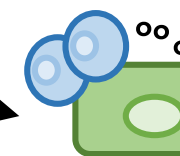
+

Tumor cells

PKH26

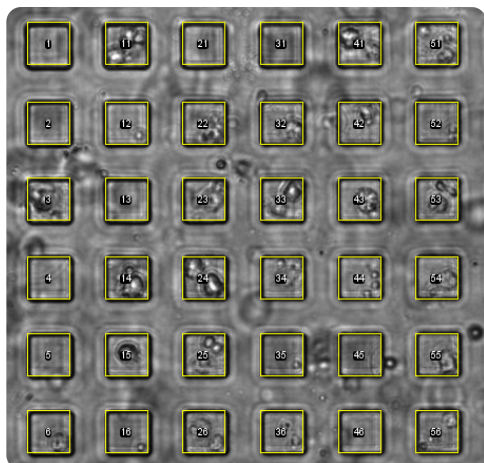


Absence of
“cell death”

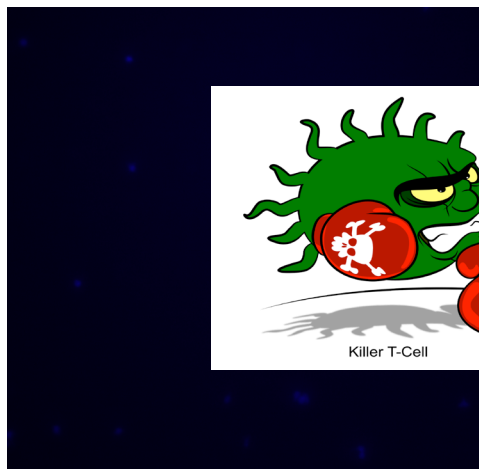


Specific “death”
*Caspase 3/7
pathway*

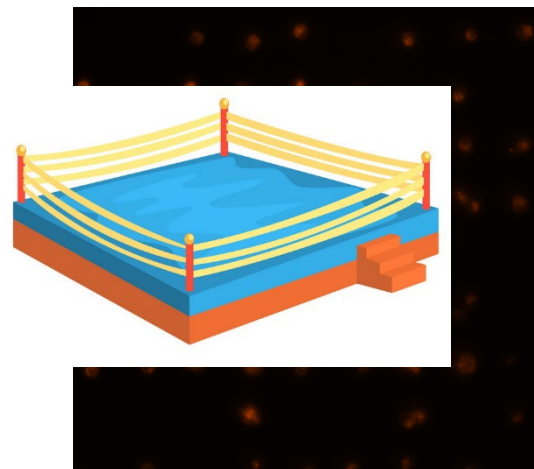
Bright field image



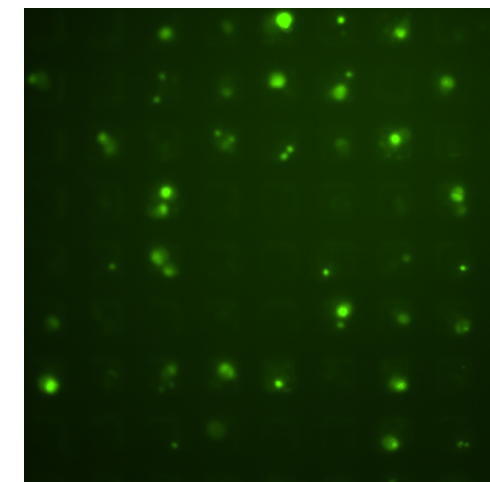
T-cells



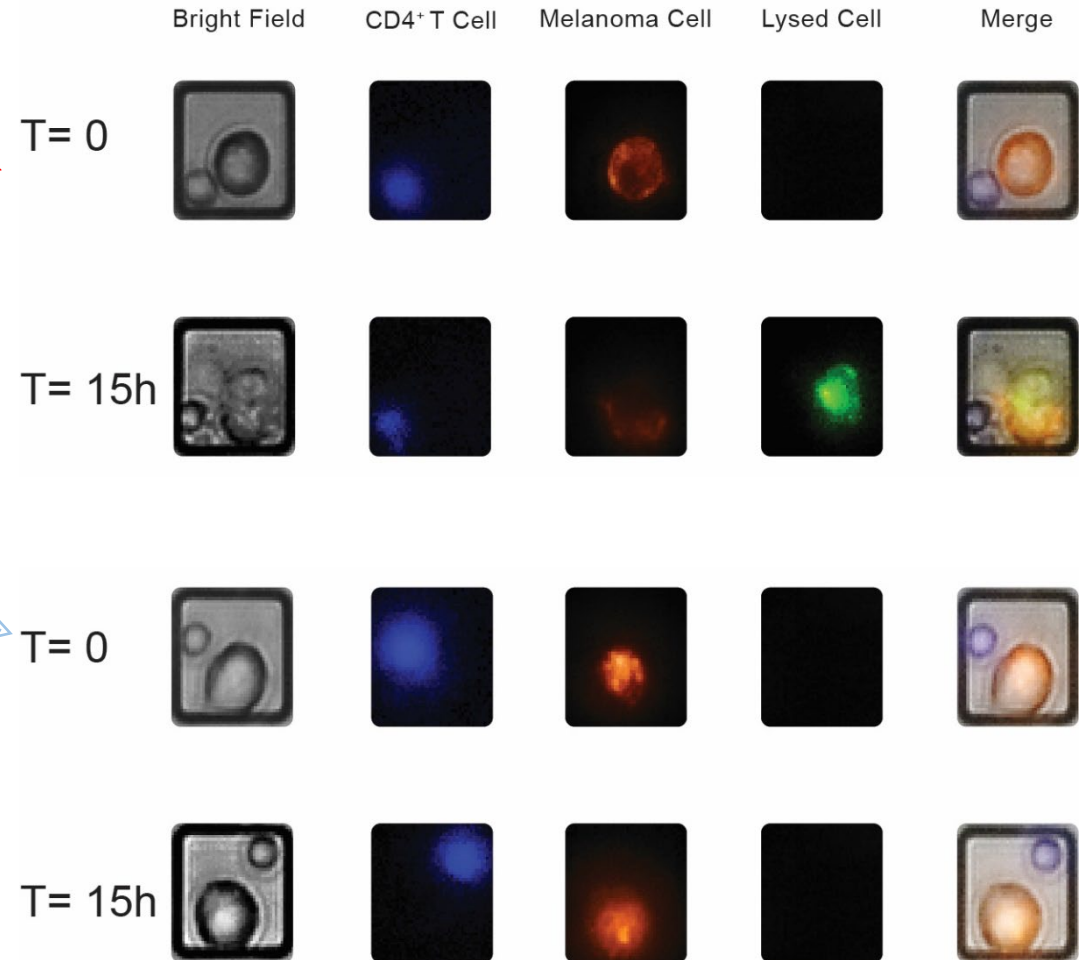
Tumor cells



Cell death (lysis)



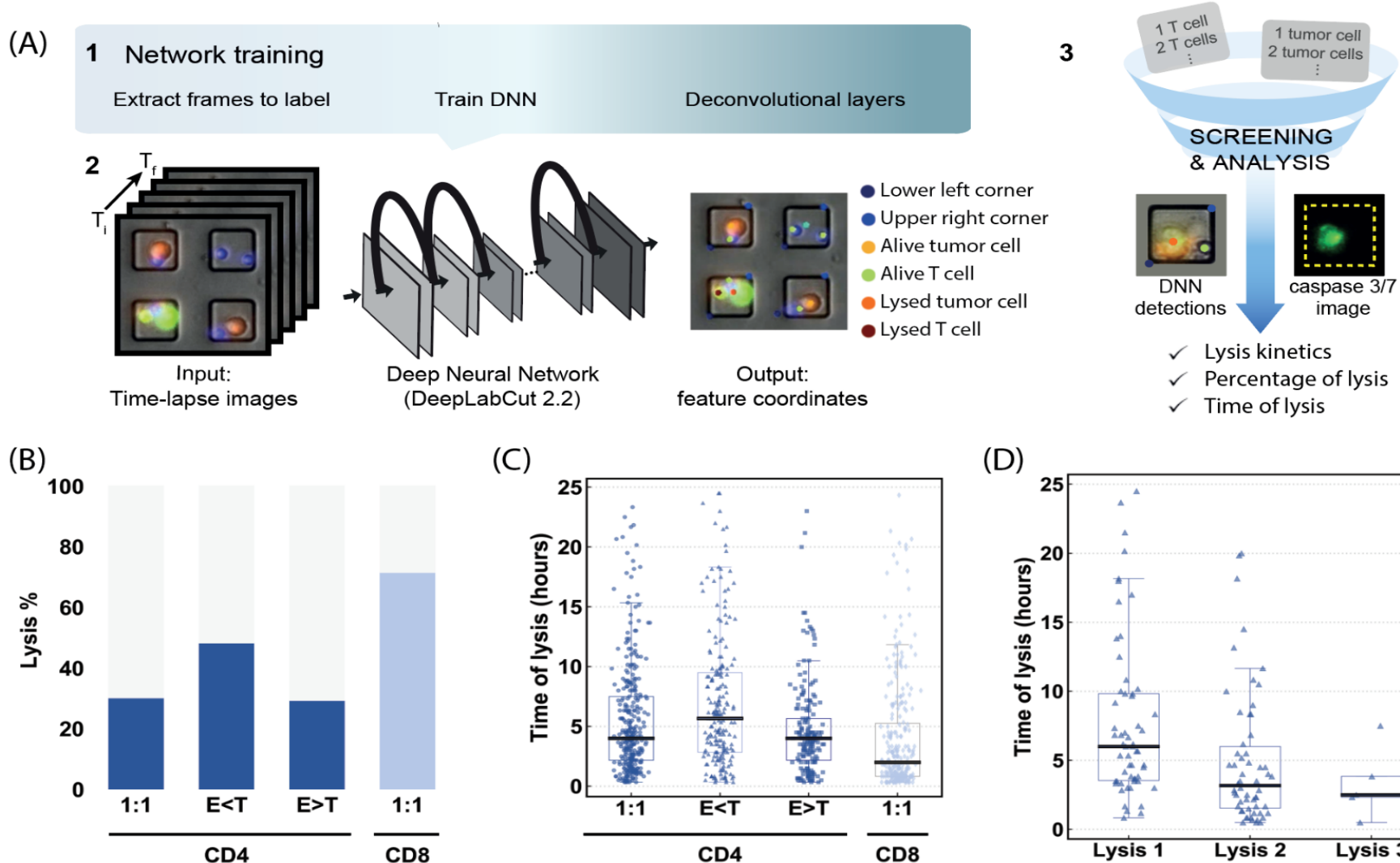
“Cytotoxicity” Effect of T Cells on Melanoma Cancer Cells



Technology generates a large set of spatially and temporally resolved data

➔ Images of each cell from multiple channels (bright-field & fluorescent channels) over a long time period (i.e. 24 hours with 15 min intervals)

Deep neural network: Rapid and quantitative analysis of single cell killing events at high-throughput

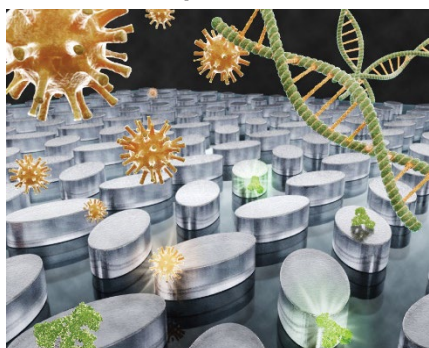


BIOS: BioNanoPhotonic Systems Labotatory

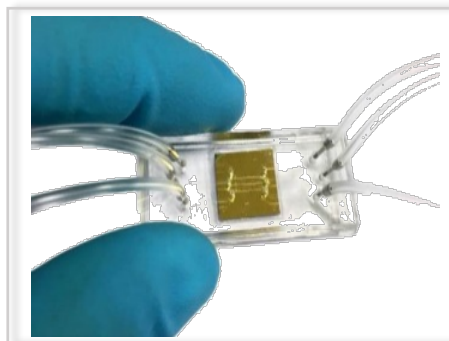
Mission

Develop *science and technology* for powerful *optical bioanalytical devices* that can *have impact* on areas ranging from basic research in life sciences to real-world applications such as disease diagnostics.

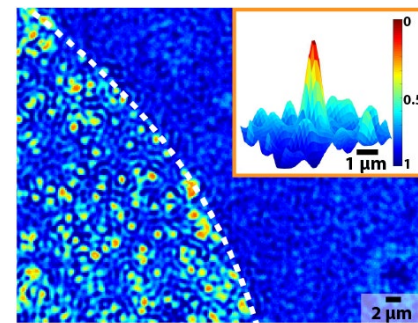
Nanophotonics



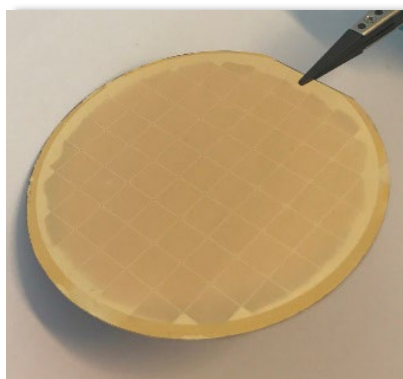
Microfluidics



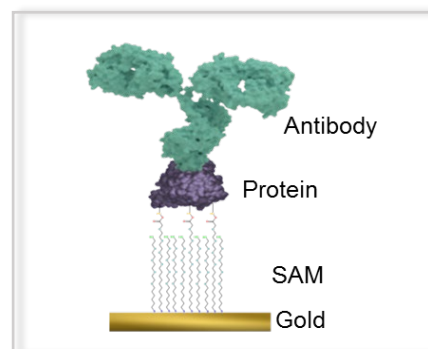
Imaging
Hyperspectral & Lens-free



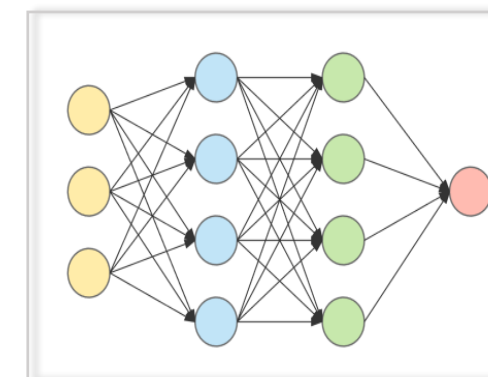
Nanofabrication



Surface Chemistry & Microarrays

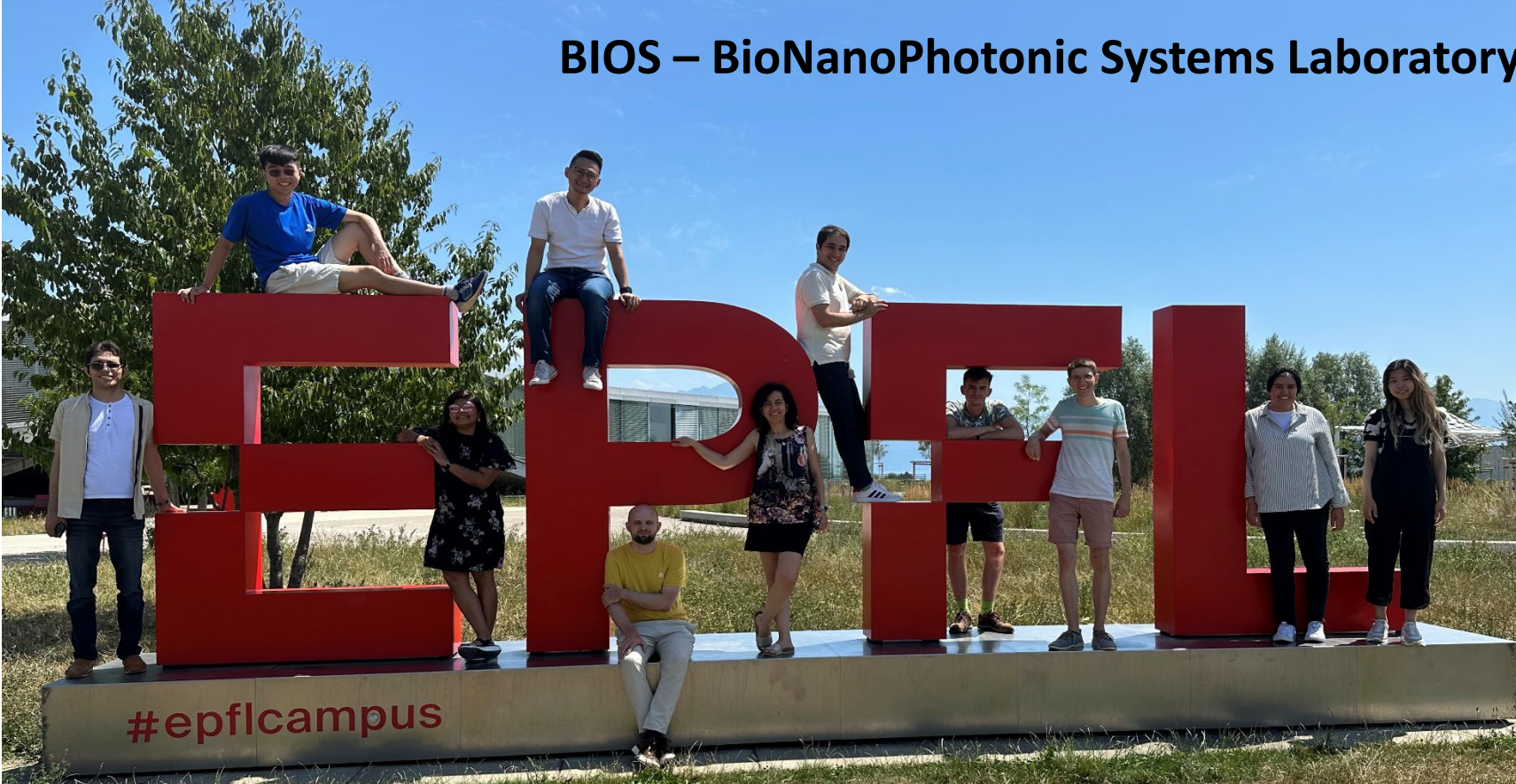


Data Science



Acknowledgements

BIOS – BioNanoPhotonic Systems Laboratory



Scientific Collaborators:

- Hilal Lashuel, EPFL
- Camilla Jandus, University of Geneva
- Alexander Mathis, EPFL
- Matthias Lutholf, EPFL & Roche
- George Coukos, Agora
- Yuri Kivshar, Australian National University
- Javier Garcia de Abajo, ICFO, Spain
- Sang-Hyun Oh, Univ. Minnesota
- ...and more

Open MS thesis projects !!

Check our website & send your CV
bios.epfl.ch
hatice.altug@epfl.ch

Open post-doc and PhD positions !!

Send your CV
bios.epfl.ch
hatice.altug@epfl.ch



Acknowledgements



- Swiss State Secretariat for Education, Research and Innovation (SERI)
 - under contract number 22.00081 for **MIRAQLS** project (#101070700) linked to European Union Horizon Europe Research and Innovation Programme
 - under contract number 22.00018 for **TWISTEDNANO** project (#101046424) linked to European Union Horizon Europe Research and Innovation Programme
- European Union's Horizon 2020 Research and Innovation Programme
 - under the Marie Skłodowska-Curie grant agreement No. 955623 (**CONSENSE** Project)
- Swiss National Science Foundation *Korean-Swiss* Science & Tech Programme
- Swiss National Science Foundation *Sinergia* Grant
- EPFL & ZEISS IDEAS Program

THANK YOU!

Prof. Hatice ALTUG

BioNanoPhotonic Systems Laboratory

École Polytechnique Fédérale de Lausanne, Switzerland

EPFL