

Lecture 2:

# **Electromagnetic Spectrum and Biomedical Measurements**

From Photons to Diagnostics

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# Lecture Contents

**Part 1:** EM Spectrum Fundamentals

**Part 2:** Spectroscopy Methods

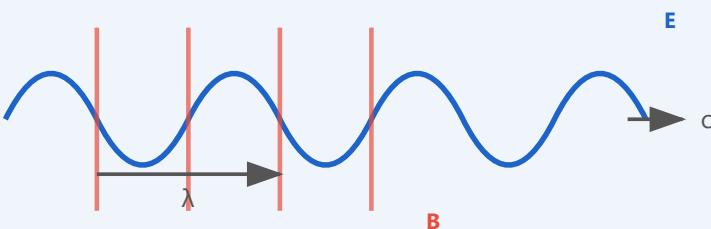
**Part 3:** Biological Applications

**Part 1/3:**

# **EM Spectrum Fundamentals**

- 1.** Light as wave and particle
- 2.** Energy scales in biology
- 3.** Photon-matter interactions
- 4.** Absorption and emission principles
- 5.** Scattering phenomena
- 6.** Fluorescence foundations

# Electromagnetic Wave Properties



Electric (E) and Magnetic (B) fields oscillate perpendicular to each other

$$E(x, t) = E_0 \cos(kx - \omega t + \varphi)$$

Wave equation describing electric field oscillation

$$k = 2\pi/\lambda \text{ (wave number)}$$

$$\omega = 2\pi\nu \text{ (angular frequency)}$$



## Wavelength ( $\lambda$ )

Distance between wave crests

$$c = \lambda\nu$$



## Frequency ( $\nu$ )

Oscillations per second

Measured in Hertz (Hz)



## Speed of Light (c)

$3 \times 10^8$  m/s in vacuum

Reduced in media:  $c/n$



## Polarization

Direction of E-field oscillation

Linear, circular, elliptical



## E and B Fields

Perpendicular oscillating fields

Energy transport mechanism



## Coherence

Phase relationship maintenance

Critical for interferometry

# Energy, Wavelength, Frequency Relationships

Planck-Einstein Relation

$$E = h\nu = hc/\lambda$$

$h = 6.626 \times 10^{-34} \text{ J}\cdot\text{s}$  (Planck constant)

Higher frequency → Higher energy  
Shorter wavelength → Higher energy



## 💡 Energy in eV

$$E (\text{eV}) = 1240 / \lambda (\text{nm})$$

## ⚠️ Wavelength Conversion

$$\lambda (\text{nm}) = 10^7 / \nu (\text{cm}^{-1})$$

## ⌚ Frequency Relation

$$\nu (\text{Hz}) = c / \lambda (\text{m})$$

## 🌟 Photon Flux

$$\Phi = P / (h\nu)$$

photons per second

~2 eV

Visible light  
photosynthesis

~0.1 eV

IR vibrations  
molecular bonds

~4 eV

UV damage  
DNA breaks

~25 meV

$kT$  at 25°C  
thermal energy

## ⚡ Biological Energy Scales

# Electromagnetic Spectrum Overview

## Full Electromagnetic Spectrum



Radio

Microwave

IR

**Visible**

UV

X-ray

Gamma

← Lower Energy

$\lambda$ : km → pm

Higher Energy →

## Spectrum Range

Radio waves → Microwaves → IR → Visible → UV → X-rays → Gamma rays

Frequency:  $10^3$  Hz to  $10^{20}$  Hz | Wavelength: km to pm



### Biological Windows

**Visible:** 400-700 nm

Vision, photosynthesis

**NIR:** 700-1000 nm

Deep tissue penetration

**UV-A:** 320-400 nm

Minimal DNA damage



### Atmospheric Transmission

**Transparent:** Visible light, radio waves

**Absorbed:** Most UV, IR, X-rays

**Ozone layer:** Blocks harmful UV-C radiation



## Medical Imaging Regions

**X-ray:** 0.01-10 nm

Radiography, CT scanning

**Gamma:** <0.01 nm

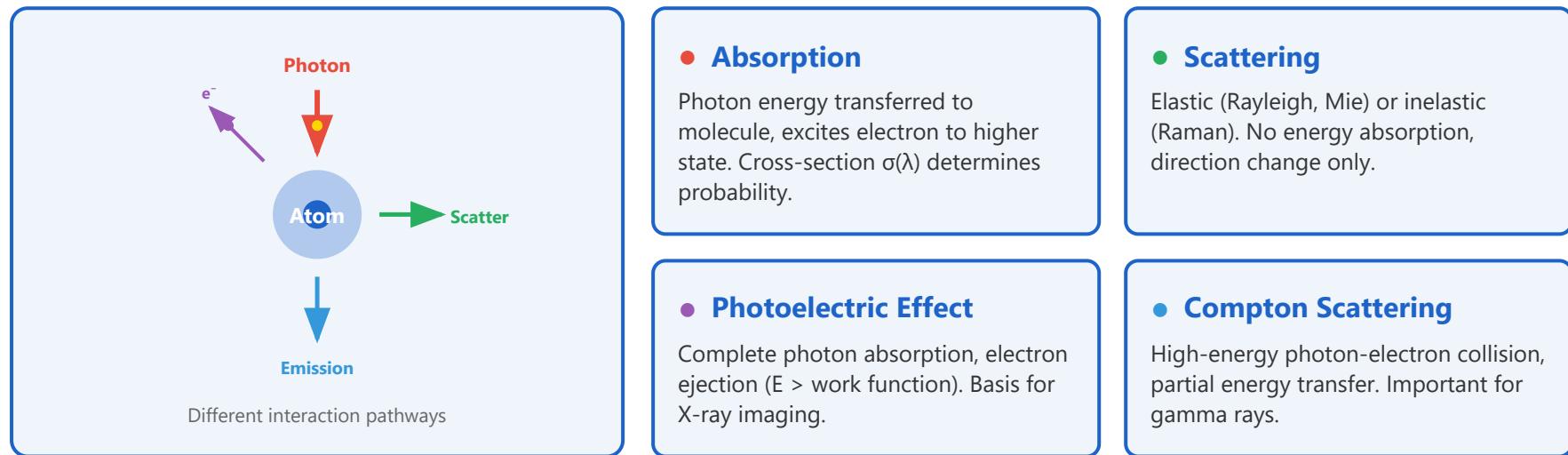
PET, SPECT imaging

**Optical:** Microscopy, endoscopy

## Interactive Applications

- Spectral databases for reference
- Wavelength calculators
- Interactive spectrum explorers
- Energy conversion tools

# Photon-Matter Interactions



## ⚠ Biological Damage Thresholds

**UV:** DNA damage, thymine dimers (<320 nm)

**Visible/NIR:** Generally safe, but high intensity causes thermal damage

**Ionizing (X-ray,  $\gamma$ ):** Direct DNA breaks, ROS generation

**Photobleaching:** Fluorophore destruction limits imaging time

# Absorption and Emission

## Electronic Transitions

Ground state ( $S_0$ ) → Excited states ( $S_1, S_2, \dots$ )

$$\Delta E = E_{\text{excited}} - E_{\text{ground}} = h\nu$$

Allowed transitions follow selection rules

## Vibrational Modes

Molecular vibrations (stretching, bending)

IR absorption region

Fine structure in spectra

Characteristic frequencies for bonds

## Selection Rules

**Allowed:**  $\Delta l = \pm 1$  (dipole transitions)

**Spin:**  $\Delta S = 0$  (singlet-singlet)

**Symmetry:** Determines intensity

**Forbidden:** Weak but observable

## Stokes Shift

$\lambda_{\text{emission}} > \lambda_{\text{excitation}}$

Energy loss to vibrations

Typically 20-100 nm shift

Enables fluorescence detection

## Quantum Yield ( $\Phi$ )

$\Phi = \text{photons emitted} / \text{photons absorbed}$

Range: 0-1 (0-100%)

High  $\Phi \rightarrow$  bright fluorophores

GFP:  $\Phi \approx 0.79$

# Scattering Phenomena

## Rayleigh Scattering

Particles  $<< \lambda$  (air molecules)

Intensity  $\propto 1/\lambda^4$

Why sky is blue

Used in DLS for size measurement

## Mie Scattering

Particles  $\approx \lambda$  (cells, bacteria)

Complex angular distribution

Flow cytometry application

Forward/side scatter

## Dynamic Light Scattering

Measures Brownian motion

Hydrodynamic radius determination

Protein aggregation studies

Nanoparticle characterization

## Raman Scattering

Inelastic scattering

Molecular fingerprinting

Label-free chemical analysis

Surface enhancement (SERS)



## Biological Applications

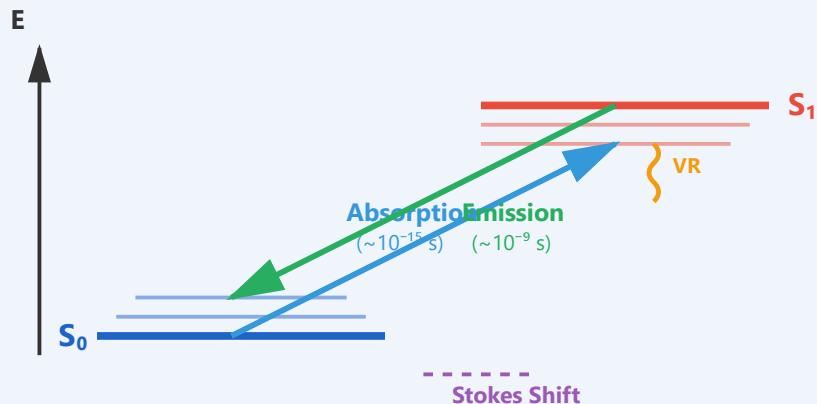
**Cell sorting:** Forward/side scatter in flow cytometry

**Protein analysis:** DLS for aggregation and stability

**Tissue imaging:** Raman microscopy for cancer detection

# Fluorescence Principles

## Jablonski Energy Diagram



## Jablonski Diagram

$S_0 \rightarrow S_1$  (absorption)  
↓ vibrational relaxation  
 $S_1 \rightarrow S_0$  (emission)

### Timescales:

Absorption:  $\sim 10^{-15}$  s  
VR:  $\sim 10^{-12}$  s  
Emission:  $\sim 10^{-9}$  s

### Stokes Shift:

$\lambda_{\text{emission}} > \lambda_{\text{excitation}}$

## Excitation/Emission Spectra

Mirror image relationship due to vibrational structure  
Stokes shift separation enables detection  
Peak wavelengths for filter optimization  
Spectral overlap considerations for multicolor imaging

## Fluorophore Properties

**Brightness:**  $\epsilon \times \Phi$  (extinction  $\times$  quantum yield)  
**Lifetime:**  $\tau$  (1-10 ns typical)  
**Stokes shift:** 20-100 nm  
**Photostability:** varies widely between fluorophores

## Photobleaching

Irreversible fluorescence loss over time  
Reactive oxygen species (ROS) mediated damage  
Antifade reagents help preserve signal  
Limits long-term imaging duration

## FRET Basics

**Förster Resonance Energy Transfer**  
Distance-dependent (2-10 nm range)  
Requires donor-acceptor pair  
Molecular ruler for protein interactions

**PART 2/3**

# **Spectroscopy Methods**

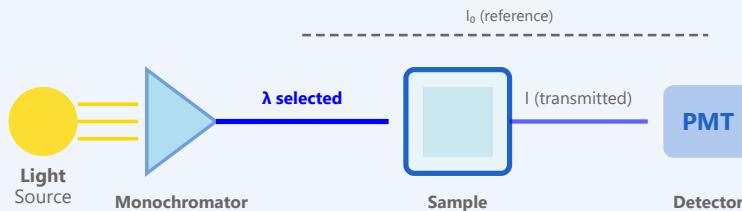
Instrumentation • Detection Principles • Quantitative Analysis

# UV-Vis Spectroscopy

$$A = \epsilon bc = -\log_{10}(I/I_0)$$

$\epsilon$ : molar absorptivity ( $M^{-1}cm^{-1}$ ) | b: path length (cm) | c: concentration (M)

## Spectrophotometer Design



## Chromophores in Biology

**Proteins:** Trp, Tyr (280 nm)

**DNA/RNA:** 260 nm

**Heme:** Soret band (420 nm)

## Cuvette Selection

**Quartz:** UV region

**Glass/Plastic:** Visible only

**Standard:** 1 cm path length

## Applications

Protein quantification | DNA/RNA purity | Enzyme kinetics | Drug screening

## Baseline Corrections

Buffer blank essential | Scatter correction for turbid samples | Temperature control

## Linear Range

$A = 0.1-1.0$  optimal | Beyond  $A=2$ : non-linear | Dilute if necessary

## Light Sources

Deuterium (UV) | Tungsten-halogen (Visible) | Xenon flash lamps

# Protein Concentration Measurement

## A<sub>280</sub> Method



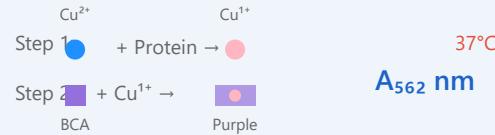
Direct, fast, needs pure protein.  $\epsilon$  calculated from Trp/Tyr content.

## Bradford Assay



Coomassie dye binding. Sensitive (1-100 µg/mL). Detergent interference.

## BCA Assay



Cu<sup>2+</sup> reduction. Compatible with detergents. 20-2000 µg/mL.

## ⚠ Interference Factors



Buffer composition critical for accurate measurements.

# DNA/RNA Quantification

## A260/A280 Purity Ratios

Pure DNA: ~1.8 | Pure RNA: ~2.0 | Protein contamination: <1.8

### Absorbance (NanoDrop)

Fast, 1-2 µL. A260/280, A260/230 ratios. Contamination detection.

### Fluorometric (Qubit)

Selective dyes. More accurate, less contamination sensitivity.

# Infrared Spectroscopy

## Molecular Vibrations

Stretch, bend, rock, wag, twist modes. Each unique to molecular structure.

## IR Regions

4000-2500: O-H, N-H | 2000-1500: C=O, C=C | 1500-400: Fingerprint

## ATR-FTIR

Attenuated Total Reflectance. No sample prep required.

## Water Interference

Strong O-H absorption. Use D<sub>2</sub>O or dry samples.

# FTIR for Biomolecules

## Amide Bands

- Amide I ( $1600\text{-}1700\text{ cm}^{-1}$ ): C=O stretch, secondary structure sensitive
- Amide II ( $1510\text{-}1580\text{ cm}^{-1}$ ): N-H bend, C-N stretch
- $\alpha$ -helix: 1650-1658 |  $\beta$ -sheet: 1620-1640, 1680-1690 | Random: 1640-1650

## Lipid Analysis

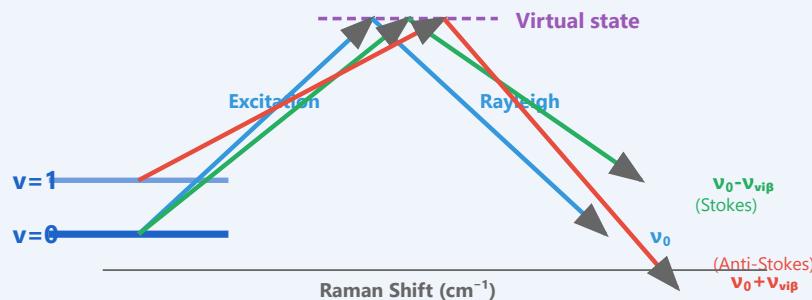
C-H stretch  $2800\text{-}3000\text{ cm}^{-1}$ . Membrane fluidity studies.

## Spectral Deconvolution

Fourier self-deconvolution resolves overlapping bands.

# Raman Spectroscopy

## Raman Scattering Energy Diagram



$$\Delta\nu = \nu_0 - \nu_s \text{scattered}$$

**Raman Shift:** Chemical fingerprint without labels

**Stokes:** Energy loss  
(molecule gains vibrational energy)

**Anti-Stokes:** Energy gain  
(less intense, temperature dependent)

Provides molecular vibrational information

## Biological Applications

- Cell imaging:** Label-free analysis
- Drug distribution:** Tissue mapping
- Cancer diagnostics:** Tissue characterization
- Protein structure:** Secondary structure analysis

## SERS (Surface-Enhanced)

- Enhancement:**  $10^6$ - $10^{14}$  fold
- Metal nanoparticles (Au, Ag)
- Single molecule detection possible
- Biosensing applications

## Raman Imaging

- Spatial mapping of molecular composition
- Confocal Raman microscopy
- Chemical maps of cells/tissues
- Sub-micron resolution

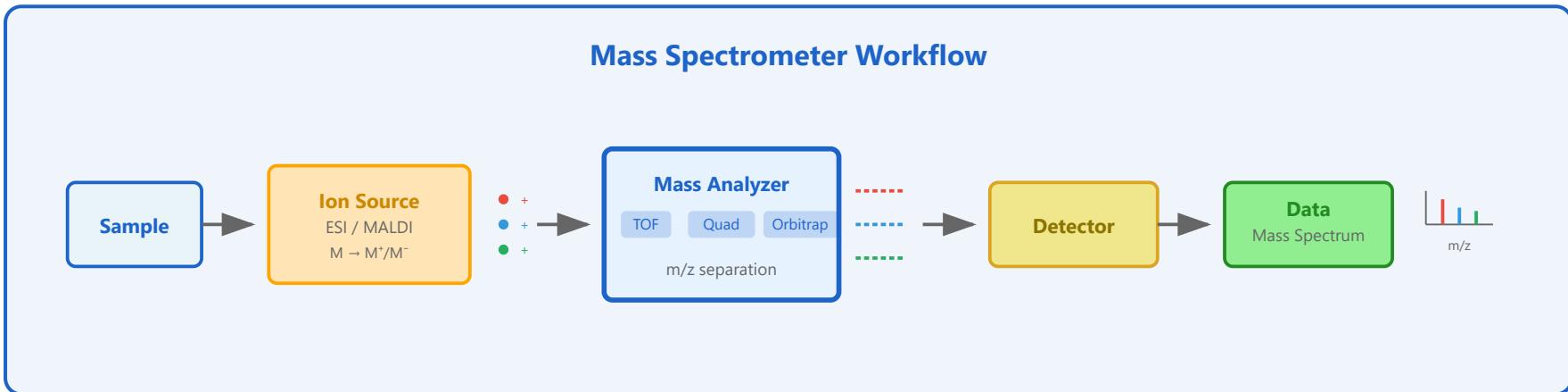
## Label-free Analysis

- No fluorophores needed
- Native state biomolecules
- Non-destructive measurement
- Real-time monitoring possible

 **SERS Enhancement:  $10^6$  to  $10^{14}$  Signal Amplification**

Enables ultra-sensitive detection for biosensing and single-molecule studies

# Mass Spectrometry Basics



## Ionization Methods



## Mass Analyzers

**TOF:** Time-of-flight, fast  
**Quadrupole:** Selective, robust  
**Orbitrap:** High resolution  
**Ion Trap:** MS<sup>n</sup> capability

## Tandem MS (MS/MS)



Proteomics workflow essential.

## Resolution & Accuracy

**Resolution:** Distinguishes isotopes  
**Accuracy:** <5 ppm for Orbitrap  
**Dynamic range:** 10<sup>4</sup>-10<sup>6</sup>

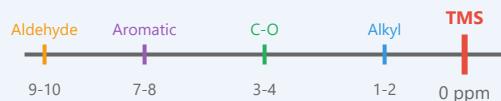
# NMR Fundamentals

## Nuclear Spin in Magnetic Field



$^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{31}\text{P}$  nuclei have spin  $1/2$

### Chemical Shift ( $\delta$ )



Electronic environment. ppm scale.

### J-coupling



Spin-spin splitting. Connectivity information.

### 2D NMR



→ Structure determination

### Biomolecular NMR



Solution state  
Dynamics info

**PART 3/3**

# **Biological Applications**

Translation to Diagnostics • Clinical Implementation • Point-of-Care  
Devices

# Fluorescent Proteins and Tags

## GFP Family

GFP, YFP, CFP, RFP. Ex/Em: 395/509nm (GFP). Nobel Prize 2008.

## Genetic Encoding

Fusion proteins. Minimal perturbation. Live cell compatible.

## Multicolor Imaging

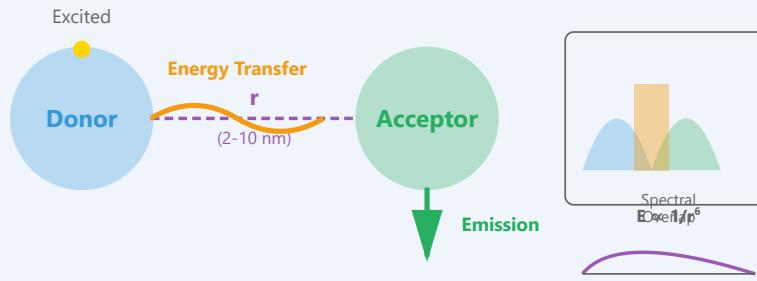
Simultaneous tracking. mCherry, mTurquoise, mVenus.

## Photoswitchable

PALM, STORM superresolution. Dronpa, mEos.

# FRET and Molecular Interactions

## Förster Resonance Energy Transfer Mechanism



## FRET Efficiency

$$E = R_0^6 / (R_0^6 + r^6)$$

$R_0$ : Förster radius

$r$ : Donor-acceptor distance

When  $r = R_0$ ,  $E = 50\%$

**Highly distance-dependent:**

$1/r^6$  relationship makes FRET exquisitely sensitive to small distance changes

### $R_0$ Calculations

Förster radius typically 2-10 nm  
Depends on spectral overlap  $J(\lambda)$   
Quantum yield of donor  
Orientation factor  $\kappa^2$

### FRET Pairs

**Classic:** CFP-YFP  
**Red-shifted:** GFP-RFP  
**Organic dyes:** Alexa Fluor, ATTO  
**Quantum dots:** Semiconductor nanocrystals

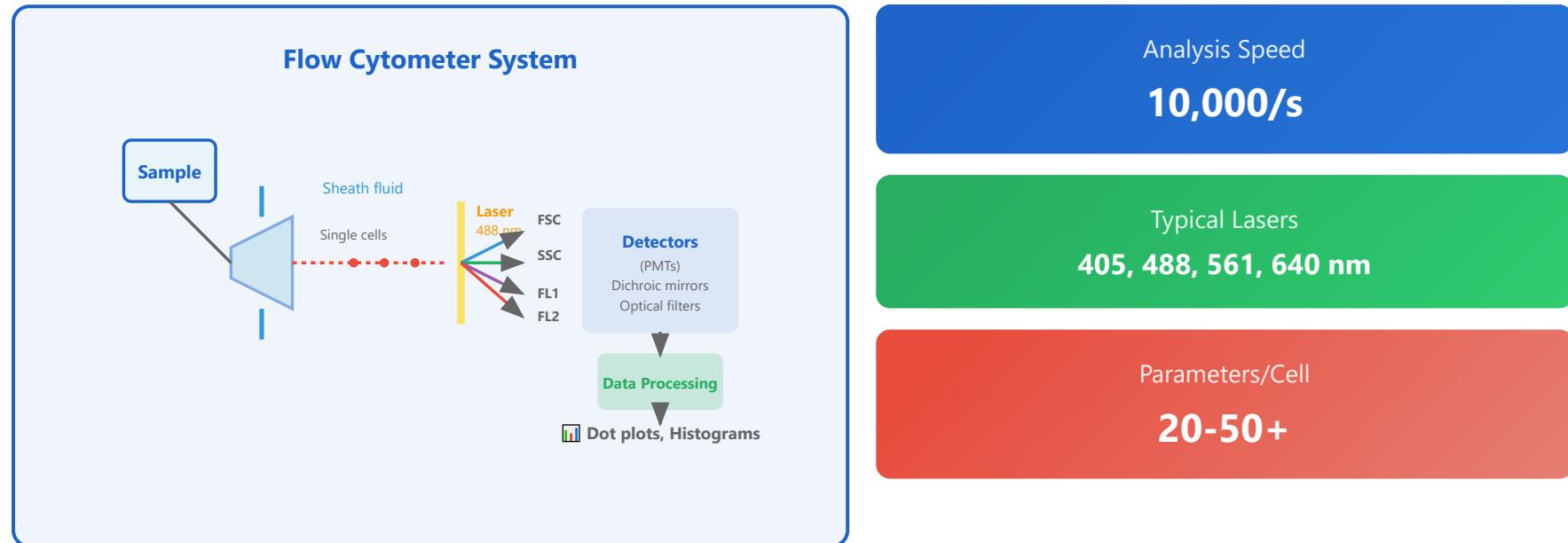
### Biosensor Design

Conformational change sensors  
**Examples:**  $\text{Ca}^{2+}$ , cAMP, kinases  
Protein-protein interactions  
Enzyme activity reporters

### Live Cell Applications

Real-time protein interactions  
Signaling pathway dynamics  
Molecular proximity measurements  
Drug screening assays

# Flow Cytometry Principles



## Fluidics System

Hydrodynamic focusing creates single-cell stream  
Sheath fluid (PBS) surrounds sample  
Laminar flow for precise alignment

## Laser Excitation

Multiple lasers for multicolor detection  
Common: 405, 488, 561, 640 nm  
Each excites different fluorophores

## Detection Channels

**FSC:** Forward scatter (cell size)  
**SSC:** Side scatter (granularity)  
**FL1-FLn:** Fluorescence PMTs

## Compensation

Corrects spectral overlap between fluorophores  
Single-color controls essential  
Software or hardware compensation

 Applications:

Immunophenotyping • Cell cycle analysis • Apoptosis detection • Rare cell identification • Biomarker expression

# FACS Sorting

## Fluorescence-Activated Cell Sorting



### Droplet Formation

High-frequency vibration creates uniform droplets. Poisson statistics ensure one cell per droplet.

### Charge Deflection

Electrostatic deflection:  $\pm 3000\text{-}5000\text{V}$ . Precise timing critical for accurate sorting.

### Purity vs Yield

### Index Sorting

Tradeoff in gating strategy. Purity mode: >99%. Yield mode: maximize recovery.

Link phenotype to well position. Enables single-cell sequencing correlation.

# Spectroscopy in Diagnostics

## Clinical Chemistry

Automated analyzers. Glucose, electrolytes, enzymes.

## Immunoassays

ELISA, CLIA. Antibody-based detection. High sensitivity.

## Molecular Diagnostics

PCR, qPCR, NGS. Pathogen detection, cancer markers.

## Validation

Accuracy, precision, sensitivity, specificity. FDA/CLIA requirements.

# Point-of-Care Devices

## Lateral Flow Assays

Pregnancy tests, COVID-19, Rapid diagnostics. Gold nanoparticles.

## Microfluidic Platforms

Lab-on-a-chip. Minimal sample volume. Integrated detection.

## Smartphone Readers

Camera-based detection. AI image analysis. Telemedicine integration.

## Colorimetric Tests

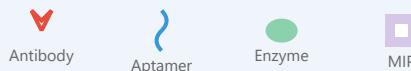
Visual readout. No instrumentation. Resource-limited settings.

# Biosensor Technologies

## Biosensor Architecture & Signal Transduction



### Recognition Elements



High specificity biorecognition layers

### Transduction Methods

- Optical: SPR, SERS, fluorescence
- Electrochemical: CV, DPV, EIS
- Piezoelectric: QCM, SAW
- Thermal: Calorimetric

### Surface Chemistry

SAMs: Self-assembled monolayers  
PEG: Anti-fouling coating  
Blocking: BSA, casein  
Minimize non-specific binding

### Signal Amplification

Enzyme cascades: HRP, ALP  
Nanoparticles: Au, Ag enhancement  
SERS:  $10^6$ - $10^{14}$  fold  
Improve LOD to fM-aM range

# Hands-on: Spectral Data Analysis

## Python/R for Spectral Analysis

- Libraries: NumPy, SciPy, Matplotlib, pandas
- Baseline correction: Polynomial, asymmetric least squares
- Peak fitting: Gaussian, Lorentzian, Voigt
- Multivariate: PCA, PLS-DA for classification
- Quality metrics: SNR, resolution, reproducibility

```
import scipy.signal as signal
peaks, _ = signal.find_peaks(spectrum, height=0.1)
```

# Hands-on: Python for Signal Processing

## Signal Processing Essentials

- FFT analysis: Frequency domain transformation
- Filtering: Low-pass, high-pass, band-pass, Savitzky-Golay
- Noise reduction: Moving average, Wiener filter
- Feature extraction: Peak detection, integration, moments
- Deconvolution: Separate overlapping signals

```
from scipy.signal import savgol_filter
smoothed = savgol_filter(data, window=11, polyorder=2)
```

## Thank You & Resources

# Thank You!

Questions?

### Resources

- Lakowicz: Principles of Fluorescence Spectroscopy
- Skoog: Principles of Instrumental Analysis
- Online simulators: PhET, Fluorophores.org
- Software: ImageJ/Fiji, Python (SciPy, scikit-learn)
- Next lecture: Advanced Imaging Techniques

## Thank You & Resources

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