

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

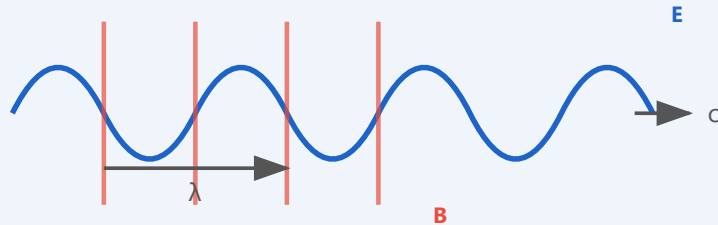
Part 3: Biological Applications

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

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

Wave equation describing electric field oscillation

$$k = 2\pi/\lambda \text{ (wave number)}$$
$$\omega = 2\pi\nu \text{ (angular frequency)}$$



Wavelength (λ)

Distance between wave crests

$$c = \lambda \nu$$



Frequency (ν)

Oscillations per second
Measured in Hertz (Hz)



Speed of Light (c)

3×10^8 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

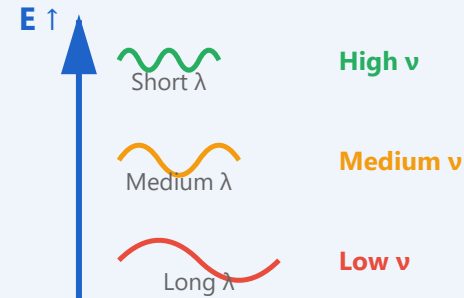
Energy, Wavelength, Frequency Relationships

Planck-Einstein Relation

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

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

Higher frequency → Higher energy
Shorter wavelength → Higher energy



💡 Energy in eV

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

📐 Wavelength Conversion

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

🌐 Frequency Relation

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

✨ Photon Flux

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

photons per second

⚡ Biological Energy Scales

~2 eV

Visible light
photosynthesis

~0.1 eV

IR vibrations
molecular bonds

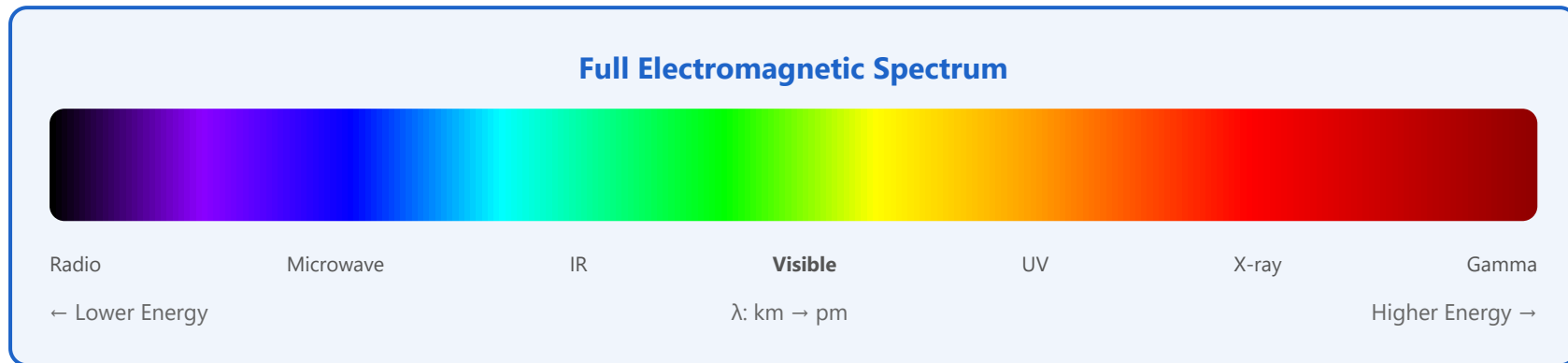
~4 eV

UV damage
DNA breaks

~25 meV

kBT at 25°C
thermal energy

Electromagnetic Spectrum Overview



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

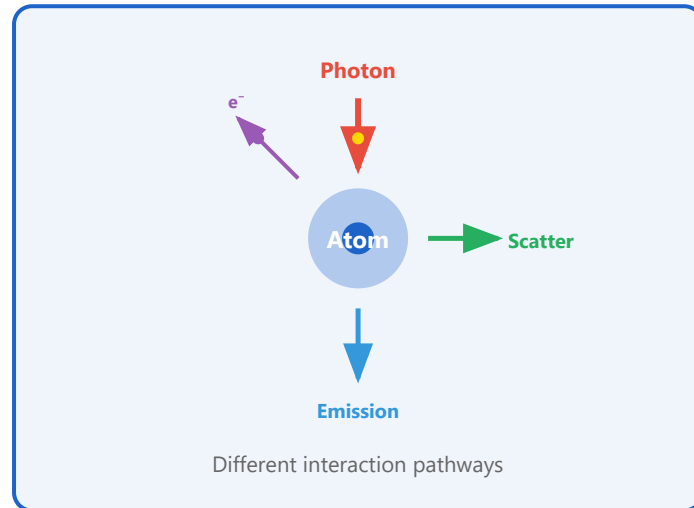
PET, SPECT imaging

Optical: Microscopy, endoscopy

Interactive Applications

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

Photon-Matter Interactions



● Absorption

Photon energy transferred to molecule, excites electron to higher state. Cross-section $\sigma(\lambda)$ determines probability.

● Scattering

Elastic (Rayleigh, Mie) or inelastic (Raman). No energy absorption, direction change only.

● Photoelectric Effect

Complete photon absorption, electron ejection ($E > \text{work function}$). Basis for X-ray imaging.

● Compton Scattering

High-energy photon-electron collision, partial energy transfer. Important for gamma rays.

⚠ Biological Damage Thresholds

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

Ionizing (X-ray, γ): Direct DNA breaks, ROS generation

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

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 (Φ)

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

Range: 0-1 (0-100%)

High Φ → bright fluorophores

GFP: $\Phi \approx 0.79$

Scattering Phenomena

Rayleigh Scattering

Particles $\ll \lambda$ (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

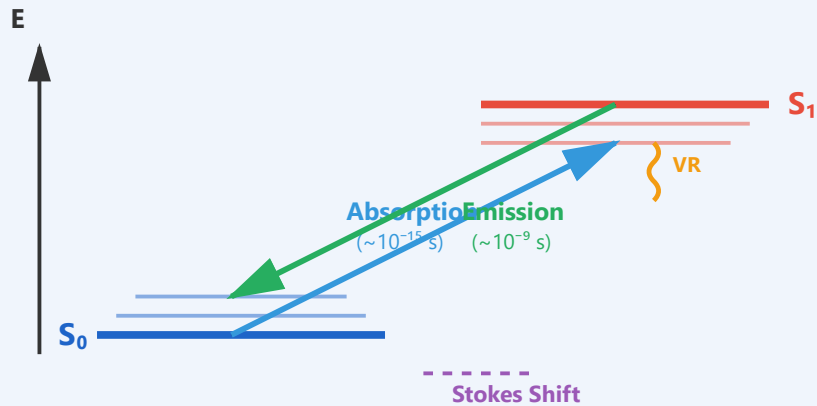
Cell sorting: Forward/side scatter in flow cytometry

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)
 \downarrow 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: τ (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

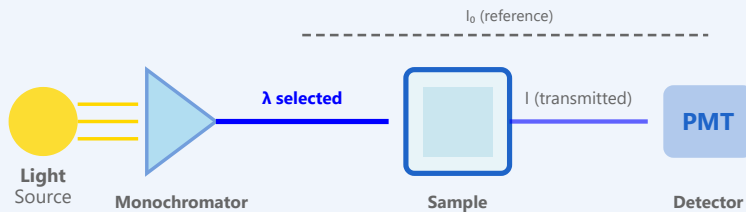
Instrumentation • Detection Principles • Quantitative Analysis

UV-Vis Spectroscopy

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

ϵ : 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

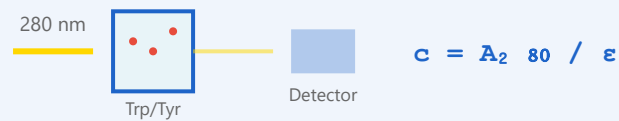
$A = 0.1$ - 1.0 optimal | Beyond $A=2$: non-linear | Dilute if necessary

Light Sources

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

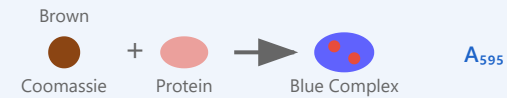
Protein Concentration Measurement

A280 Method



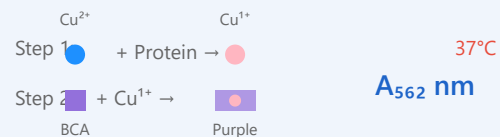
Direct, fast, needs pure protein. ϵ calculated from Trp/Tyr content.

Bradford Assay



Coomassie dye binding. Sensitive (1-100 $\mu\text{g/mL}$). Detergent interference.

BCA Assay



Cu^{2+} reduction. Compatible with detergents. 20-2000 $\mu\text{g/mL}$.

⚠ 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 μ L. 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 D₂O 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
- α -helix: $1650\text{--}1658$ | β -sheet: $1620\text{--}1640$, $1680\text{--}1690$ | Random: $1640\text{--}1650$

Lipid Analysis

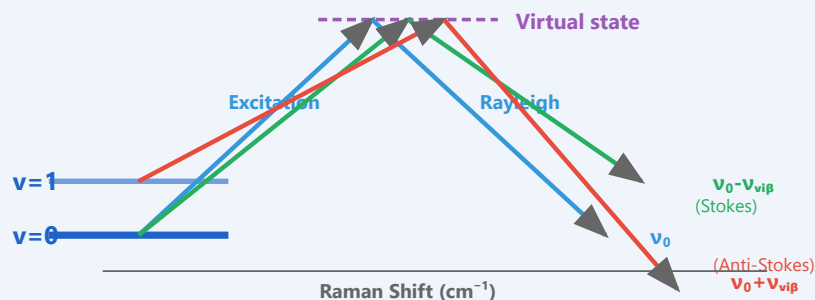
C-H stretch $2800\text{--}3000\text{ cm}^{-1}$. Membrane fluidity studies.

Spectral Deconvolution

Fourier self-deconvolution resolves overlapping bands.

Raman Spectroscopy

Raman Scattering Energy Diagram



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

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

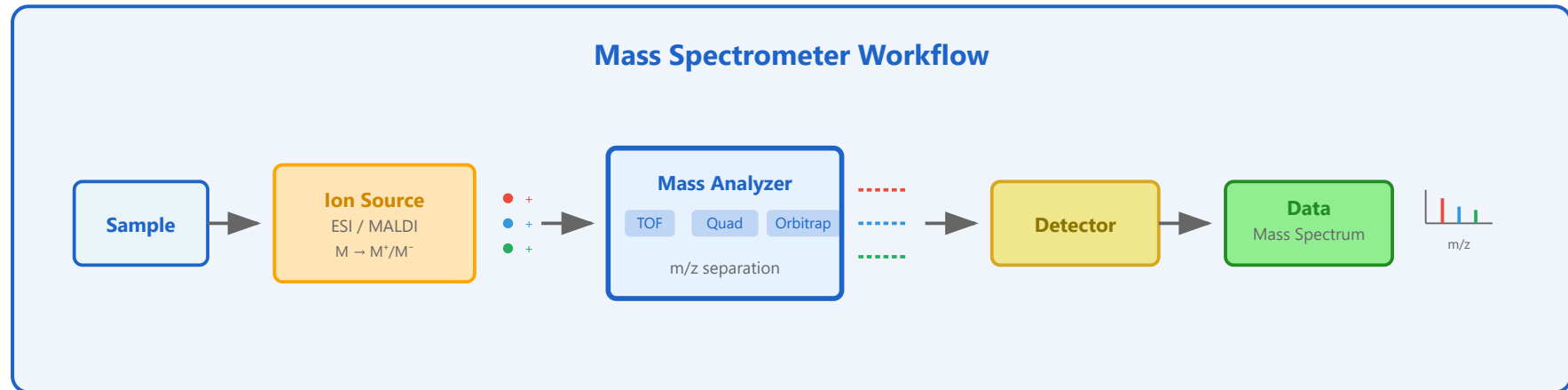
Real-time monitoring possible



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

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

Mass Spectrometry Basics



Ionization Methods

ESI
Soft, proteins

MALDI
Peptides, tissues

Mass Analyzers

TOF: Time-of-flight, fast

Quadrupole: Selective, robust

Orbitrap: High resolution

Ion Trap: MSⁿ capability

Tandem MS (MS/MS)

Precursor → **Fragment** → Structure elucidation

Proteomics workflow essential.

Resolution & Accuracy

Resolution: Distinguishes isotopes

Accuracy: <5 ppm for Orbitrap

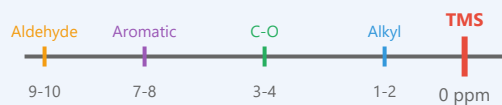
Dynamic range: 10⁴-10⁶

NMR Fundamentals

Nuclear Spin in Magnetic Field



Chemical Shift (δ)



Electronic environment. ppm scale.

J-coupling



Spin-spin splitting. Connectivity information.

2D NMR



\rightarrow 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

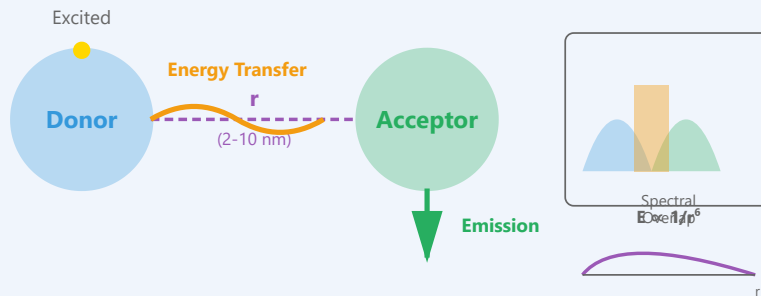
Simultaneous tracking. mCherry, mTurquoise, mVenus.

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 κ^2

FRET Pairs

Classic: CFP-YFP

Red-shifted: GFP-RFP

Organic dyes: Alexa Fluor, ATTO

Quantum dots: Semiconductor nanocrystals

Biosensor Design

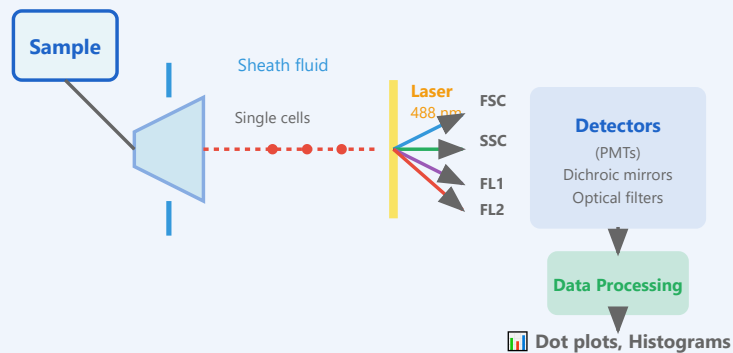
Conformational change sensors
Examples: Ca^{2+} , cAMP, kinases
Protein-protein interactions
Enzyme activity reporters

Live Cell Applications

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

Flow Cytometry Principles

Flow Cytometer System



Analysis Speed

10,000/s

Typical Lasers

405, 488, 561, 640 nm

Parameters/Cell

20-50+

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: ± 3000 - 5000 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

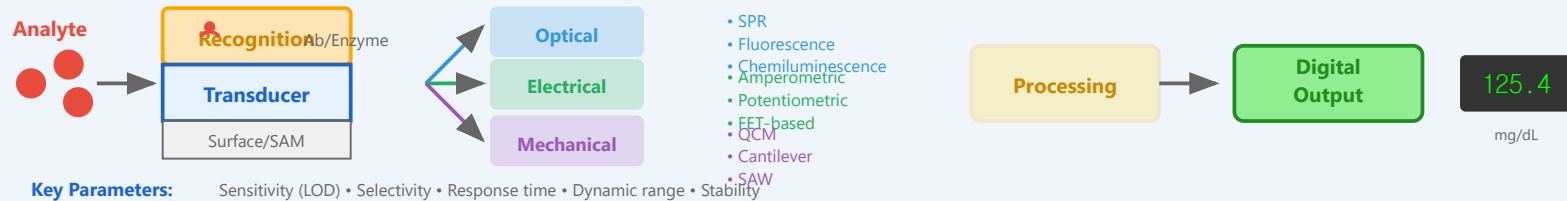
Camera-based detection. AI image analysis. Telemedicine integration.

Colorimetric Tests

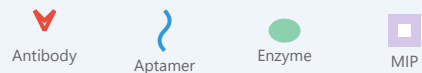
Visual readout. No instrumentation. Resource-limited settings.

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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