Lecture 2:

Electromagnetic Spectrum and Biomedical Measurements

From Photons to Diagnostics

Ho-min Park

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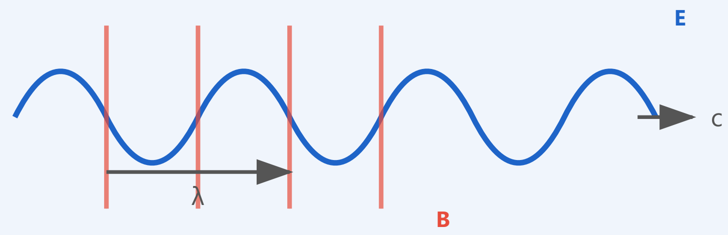
Lecture Contents

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

$k = 2\pi/\lambda$ (wave number)
$\omega = 2\pi\nu$ (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

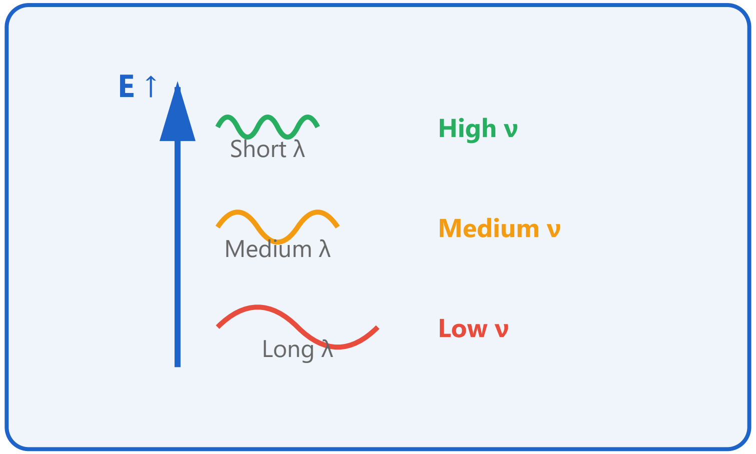
Energy, Wavelength, Frequency Relationships

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

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

🌊 Frequency Relation

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

✨ Photon Flux

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

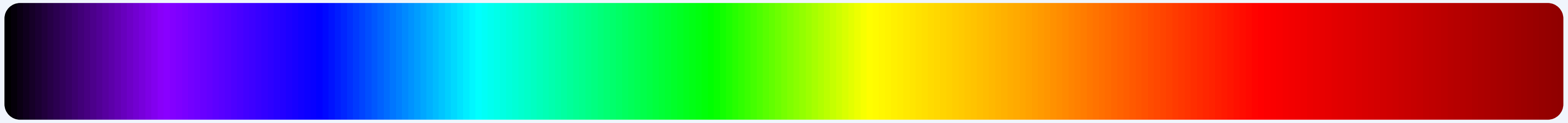
photons per second

⚡ Biological Energy Scales

~2 eV	~0.1 eV	~4 eV	~25 meV
Visible light photosynthesis	IR vibrations molecular bonds	UV damage DNA breaks	kBT at 25°C thermal energy

Electromagnetic Spectrum Overview

Full Electromagnetic Spectrum



Radio | Microwave | IR | **Visible** | UV | X-ray | Gamma

← Lower Energy | λ: 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

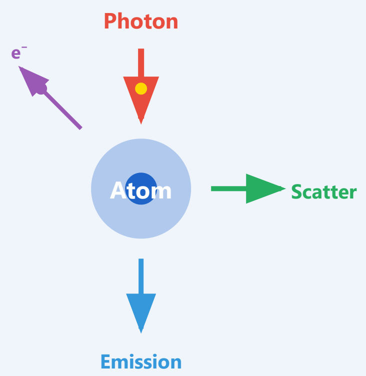
Medical Imaging Regions

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



Different interaction pathways

● 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 > 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, ...)

$\Delta E = E\_excited - E\_ground = h\nu$

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

$\lambda\_emission > \lambda\_excitation$

Energy loss to vibrations

Typically 20-100 nm shift

Enables fluorescence detection

Quantum Yield (Φ)

Φ = photons emitted / photons absorbed

Range: 0-1 (0-100%)

High Φ → bright fluorophores

GFP: $\Phi \approx 0.79$

Scattering Phenomena

Rayleigh Scattering

Particles << λ (air molecules)

Intensity $\propto 1/\lambda^4$

Why sky is blue

Used in DLS for size measurement

Mie Scattering

Particles ≈ λ (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

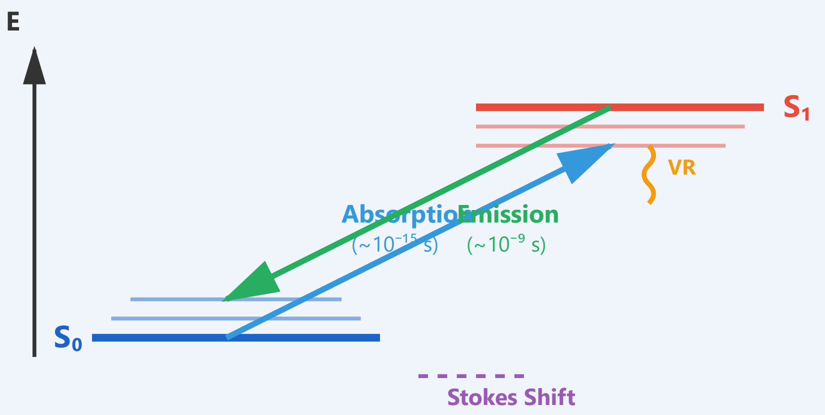
Cell sorting: Forward/side scatter in flow cytometry

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:
λ_emission > λ_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: ε × Φ (extinction × 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

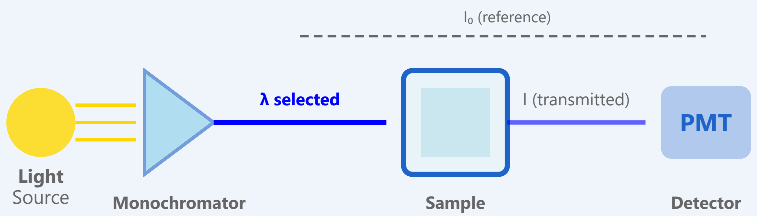
Instrumentation • Detection Principles • Quantitative Analysis

UV-Vis Spectroscopy

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

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.

$c = A_{280} / \varepsilon$

Bradford Assay

Coomassie dye binding. A_{595}. Sensitive (1-100 μg/mL). Detergent interference.

BCA Assay

Cu^{2+} reduction. A_{562}. Compatible with detergents. 20-2000 μg/mL.

⚠ Interference Factors

DTT, β-ME affect BCA. SDS affects Bradford. Buffer composition critical.

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_2O or dry samples.

FTIR for Biomolecules

Amide Bands

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

Lipid Analysis

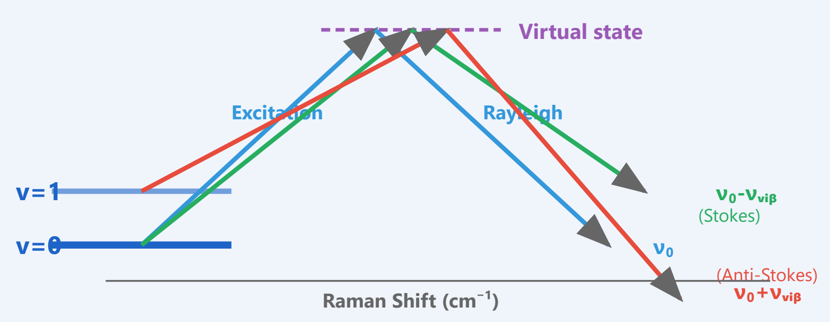
C-H stretch 2800-3000 cm^{-1}. Membrane fluidity studies.

Spectral Deconvolution

Fourier self-deconvolution resolves overlapping bands.

Raman Spectroscopy

Raman Scattering Energy Diagram



$\Delta\nu = \nu_0 - \nu_s$cattered

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, Quadrupole, Orbitrap, Ion Trap

Tandem MS (MS/MS)

Fragment ions for structural elucidation. Proteomics workflow.

Resolution & Accuracy

High resolution distinguishes isotopes. ppm accuracy.

NMR Fundamentals

Nuclear Spin in Magnetic Field

^{1}H, ^{13}C, ^{15}N, ^{31}P nuclei have spin ½

Chemical Shift (δ)

Electronic environment. ppm scale. TMS reference.

J-coupling

Spin-spin splitting. Connectivity information.

2D NMR

COSY, NOESY, HSQC for structure determination

Biomolecular NMR

Protein structure in solution. Dynamics information.

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

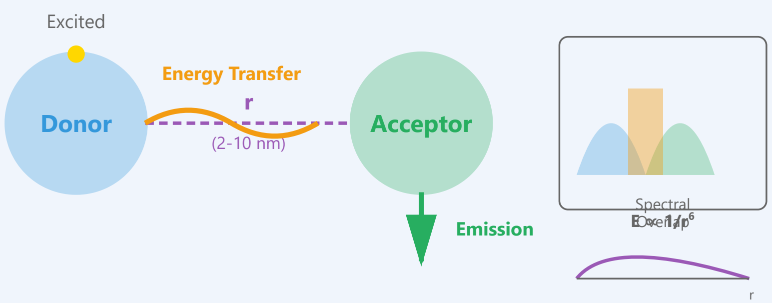
Simultaneous tracking. mCherry, mTurquoise, mVenus.

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

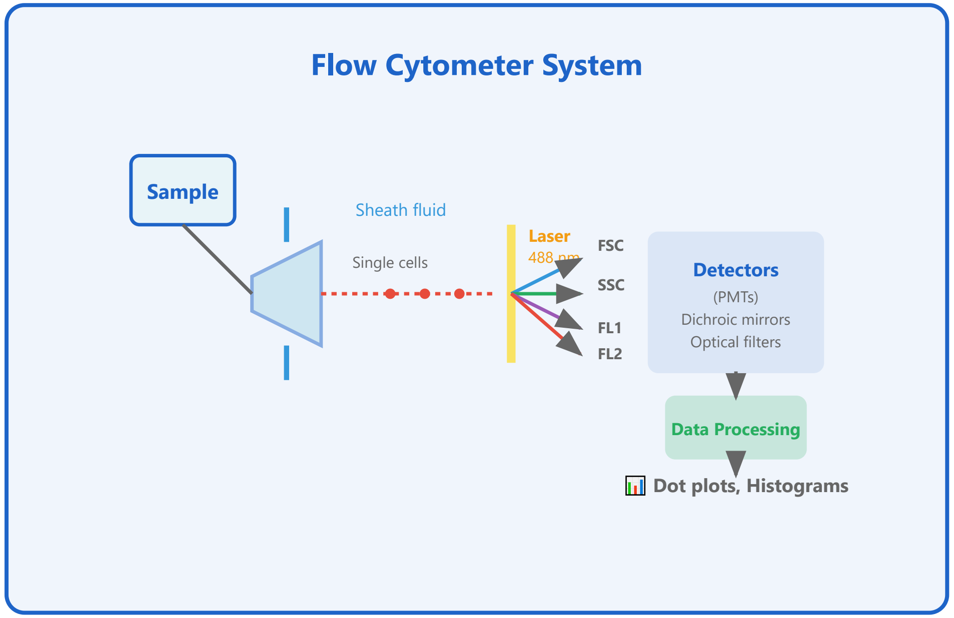
🔬 Biosensor Design

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



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 droplets. One cell per droplet.

Charge Deflection

Electrostatic deflection based on fluorescence signal.

Purity vs Yield

Tradeoff in gating strategy. >95% purity achievable.

Index Sorting

Link phenotype to plate location. Single-cell sequencing.

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

Recognition Elements

Antibodies, aptamers, enzymes, molecularly imprinted polymers

Transduction Methods

Optical, electrochemical, piezoelectric, thermal

Surface Chemistry

SAMs, PEG, blocking strategies. Minimize non-specific binding.

Signal Amplification

Enzyme cascades, nanoparticles, SERS. Improve sensitivity.

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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