

Lecture 3:

Biomedical Imaging Technologies

From Molecules to Organs
Clinical Impact Through Imaging Science

Introduction to Biomedical Datascience

Imaging across scales visualization

Lecture Contents

Part 1: Microscopy Fundamentals

Part 2: Medical Imaging Modalities

Part 3: Computational Image Analysis

Part 1/3:

Microscopy

- Resolution limits
- Contrast mechanisms
- Live vs fixed imaging
- 3D reconstruction

Light Microscopy Principles

Köhler illumination

Uniform field illumination technique

Numerical aperture

Light gathering power of objective

Abbe diffraction limit

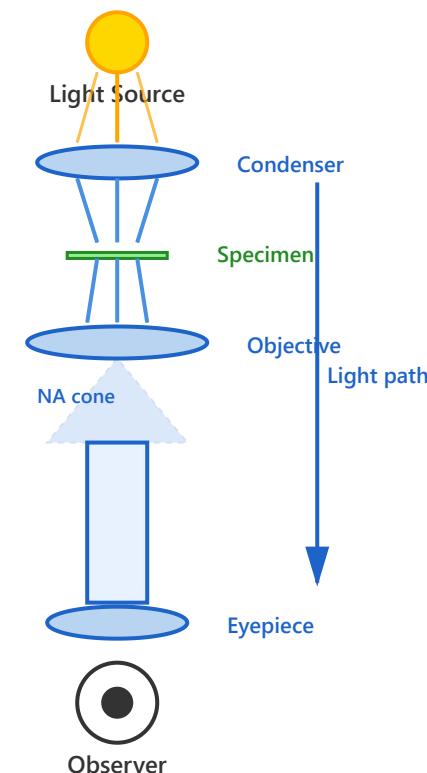
$$d = \lambda / (2 \cdot NA) \approx 200 \text{ nm}$$

Point spread function

3D light distribution pattern

Optical aberrations

Spherical, chromatic distortions



Resolution and Magnification

Rayleigh criterion

Minimum resolvable distance

Empty magnification

Magnifying beyond resolution limit

Nyquist sampling

2× sampling above highest frequency

Digital resolution

Pixel size vs optical resolution

Super-resolution preview

Fluorescence Microscopy

Filter cube design

Excitation, dichroic, emission filters

Multichannel imaging

Multiple fluorophores simultaneously

Autofluorescence

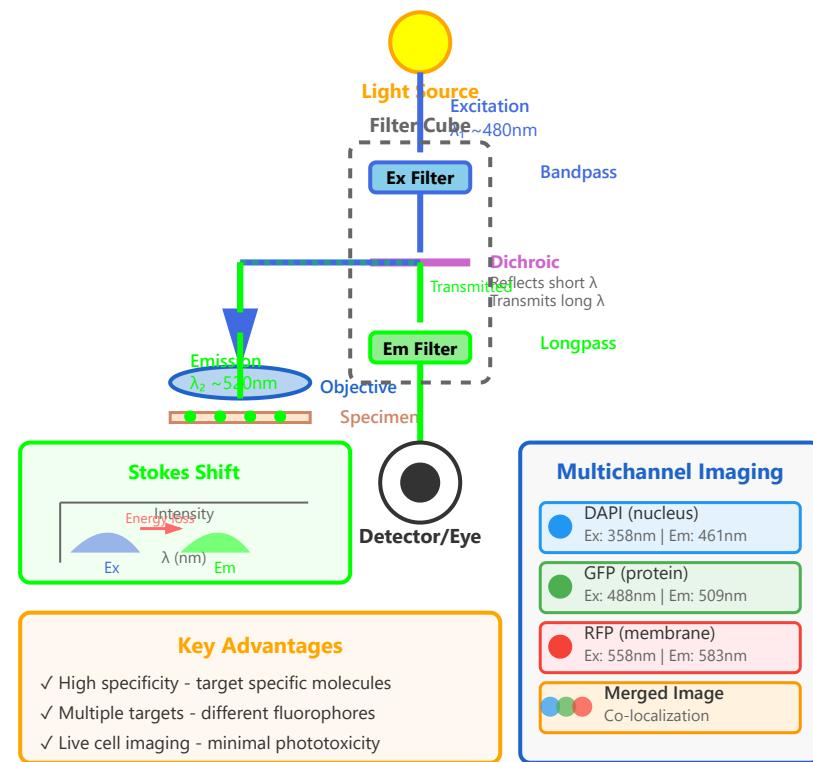
Background from endogenous molecules

Phototoxicity

Cell damage from light exposure

Live cell considerations

Environmental control requirements



Confocal Microscopy

Pinhole principle

Rejection of out-of-focus light

Optical sectioning

Thin optical slices through sample

Laser scanning

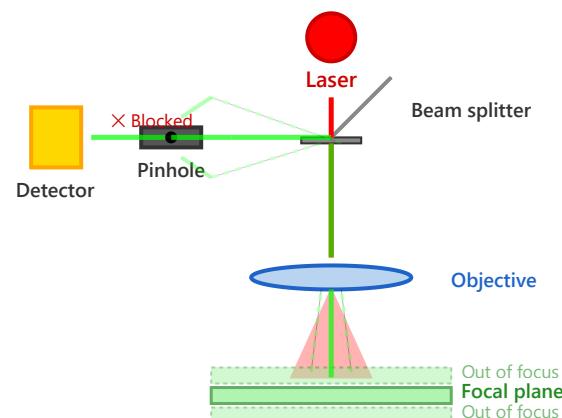
Point-by-point image acquisition

Z-stack acquisition

Series of optical sections

3D rendering

Volumetric visualization from stacks



Pinhole Principle

- ✓ In-focus light passes through pinhole
- ✗ Out-of-focus light blocked by pinhole
 - High axial resolution (~500 nm)

Two-Photon Microscopy

Nonlinear excitation

Two photons absorbed simultaneously

Deeper penetration

Up to 1mm in tissue

Reduced photobleaching

Excitation only at focal point

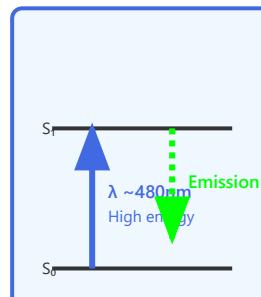
In vivo imaging

Live animal brain imaging

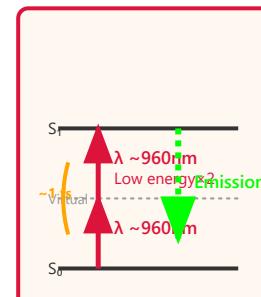
SHG imaging

Second harmonic generation for collagen

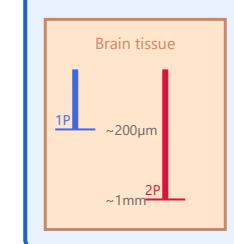
One-Photon



Two-Photon



Penetration Depth



Excitation Volume



Advantages

- Deep imaging
- Less photobleach
- Lower phototoxicity
- Intrinsic sectioning
- NIR light scatters less

Clinical & Research Applications

In vivo brain imaging • Deep tissue microscopy • Neuroscience studies
Intravital microscopy • Tumor microenvironment • Long-term live imaging

Super-Resolution Techniques

STORM/PALM principles

Single molecule localization (20-30 nm)

STED microscopy

Stimulated emission depletion (~50 nm)

SIM principles

Structured illumination (~100 nm)

Resolution comparisons

10× improvement over diffraction limit

Sample requirements

Special fluorophores and preparation

Electron Microscopy (SEM/TEM)

Electron sources

Wavelength ~0.004 nm vs light ~500 nm

Sample preparation

Fixation, dehydration, coating

Contrast mechanisms

Electron density differences

Cryo-EM revolution

Near-atomic resolution of proteins

Correlative microscopy

Combining light and electron microscopy

Part 2

Medical Imaging

- Clinical modalities overview
- Contrast agents
- Radiation considerations
- Multi-modal imaging

X-ray Physics and Imaging

X-ray production

High energy electrons hit metal target

Attenuation principles

Absorption varies with tissue density

Digital detectors

CR and DR systems replace film

Dose considerations

ALARA principle (As Low As Reasonably Achievable)

Image quality metrics

Contrast, resolution, noise tradeoffs

CT Scan Principles

Tomographic reconstruction

Multiple X-ray projections create 3D volume

Hounsfield units

Standardized tissue density scale

Spiral/helical CT

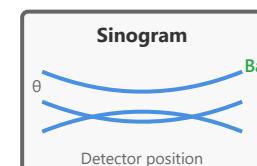
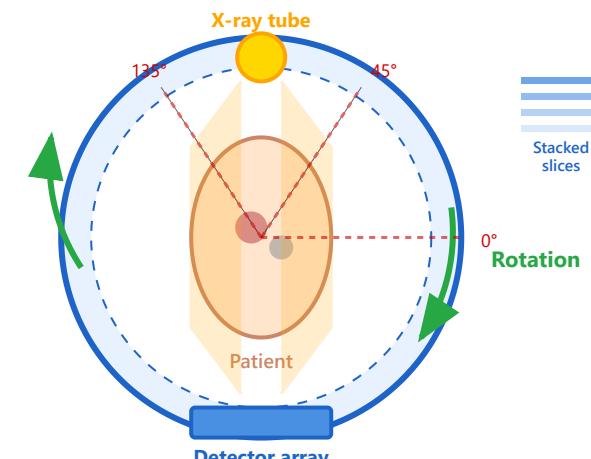
Continuous rotation and table movement

Dose reduction strategies

Iterative reconstruction, tube modulation

Contrast protocols

IV contrast timing for specific applications



Hounsfield Units	
Air:	-1000
Fat:	-100
Water:	0
Bone:	+1000

MRI Physics Basics

Nuclear magnetic resonance

Hydrogen protons align in magnetic field

Gradient fields

Spatial encoding of signal

K-space

Frequency domain data representation

Relaxation times (T1, T2)

Tissue-specific signal recovery

Signal equation

$S \propto \rho \cdot (1 - e^{(-TR/T1)}) \cdot e^{(-TE/T2)}$

No Magnetic Field



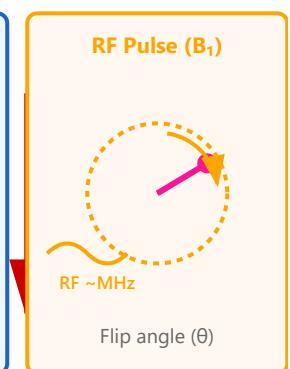
Random orientation

B₀ Field Applied

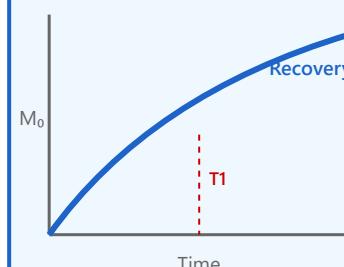


Net magnetization M₀

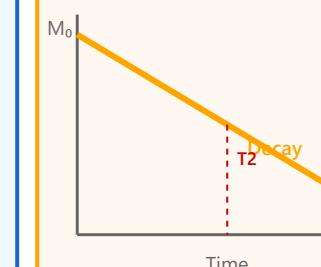
RF Pulse (B₁)



T1 Relaxation



T2 Relaxation



MRI Sequences and Contrast

Spin echo

180° refocusing pulse, high SNR

Gradient echo

Faster acquisition, T2* weighting

T1/T2/PD weighting

Tissue contrast manipulation

DWI/DTI

Diffusion imaging for stroke and white matter

Functional MRI basics

BOLD signal reflects brain activity

Ultrasound Imaging

Piezoelectric transducers

Convert electrical to acoustic energy

Acoustic impedance

Tissue resistance to sound propagation

Reflection and refraction

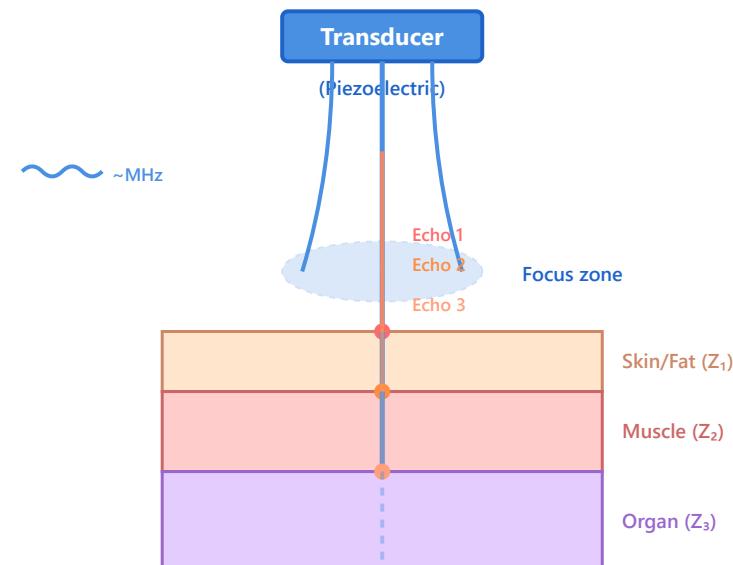
Interface properties determine echoes

Beamforming

Focusing and steering ultrasound beam

Harmonic imaging

Higher frequencies improve resolution



Acoustic Impedance

$$Z = \rho \times c$$

ρ = density, c = sound speed

Reflection Coefficient

$$R = (Z_2 - Z_1)^2 / (Z_2 + Z_1)^2$$

Larger $\Delta Z \rightarrow$ Stronger echo

Doppler Ultrasound

Doppler shift principle

Frequency change with moving blood

Color flow mapping

Direction and velocity visualization

Power Doppler

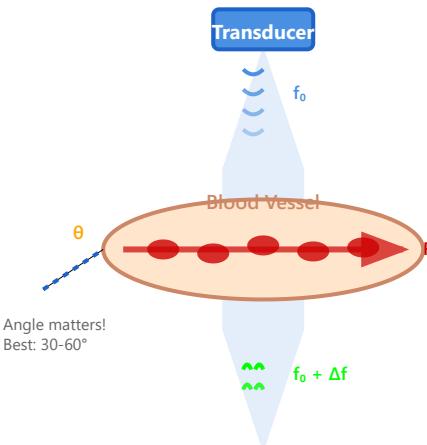
More sensitive to low flow

Spectral analysis

Velocity vs time waveforms

Clinical applications

Vascular, cardiac, obstetric imaging



- Applications**
- Cardiac valves
 - Carotid stenosis
 - Fetal heart rate
 - Portal vein flow
 - DVT detection

Doppler Equation

$$\Delta f = 2 \cdot f_0 \cdot v \cdot \cos \theta / c$$

Δf = frequency shift
 v = blood velocity, θ = angle

Color Doppler Mapping



Spectral Doppler (Velocity vs Time)



PET Imaging

Positron annihilation

511 keV photons in opposite directions

Coincidence detection

Simultaneous detection localizes source

Radiotracers (FDG, etc.)

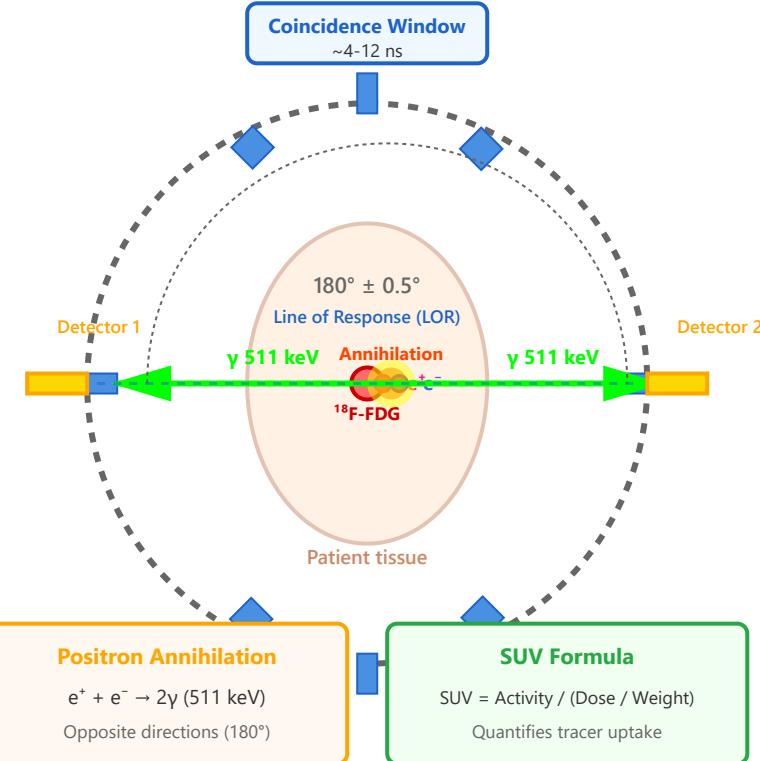
FDG shows glucose metabolism

SUV calculations

Standardized uptake value quantification

PET/CT integration

Functional and anatomical fusion



SPECT Imaging

Gamma camera principles

Scintillation crystal detects photons

Collimator design

Determines sensitivity and resolution

SPECT tracers

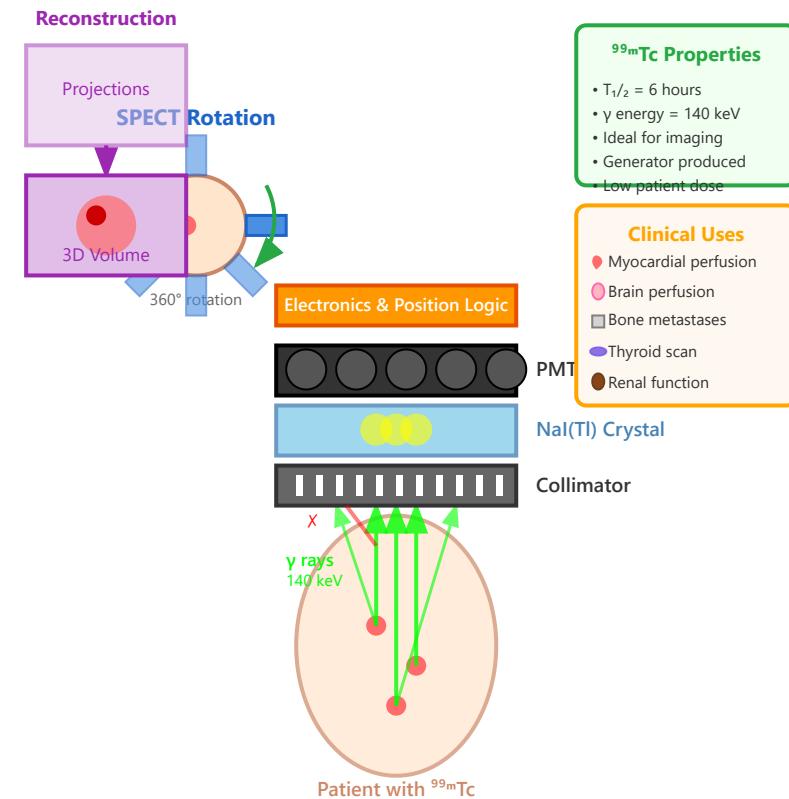
Tc-99m most common radionuclide

Cardiac applications

Myocardial perfusion imaging

SPECT/CT

Attenuation correction and localization



Part 3

Image Analysis

- Digital image fundamentals
- Processing pipeline
- Quantification methods
- AI integration

Digital Image Basics

Pixel and voxel concepts

2D picture elements, 3D volume elements

Bit depth

8-bit (256 levels), 16-bit (65,536 levels)

File formats

TIFF, PNG (lossless), JPEG (lossy)

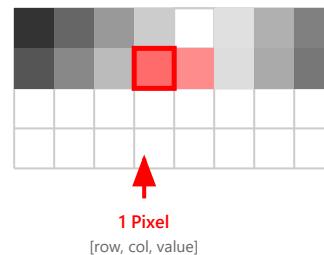
Compression methods

Lossless vs lossy tradeoffs

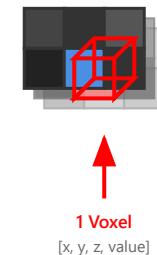
Metadata standards

EXIF, OME-TIFF for scientific imaging

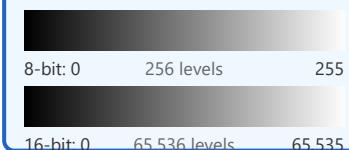
Pixel (2D)



Voxel (3D)



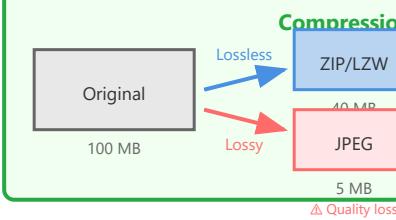
Bit Depth



File Format Comparison

Format	Compression	Use Case
TIFF	Lossless	Scientific
PNG	Lossless	Web/Analysis
JPEG	Lossy	Web/Display
DICOM	Both	Medical

Compression Methods



Metadata Standards

- EXIF: Camera settings
- OME-TIFF: Microscopy
- DICOM: Patient info

Image Preprocessing

Noise reduction

Gaussian, median, bilateral filtering

Contrast enhancement

Stretching, adaptive methods

Histogram equalization

Uniform intensity distribution

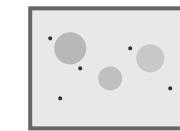
Morphological operations

Erosion, dilation, opening, closing

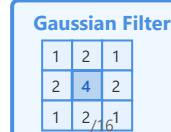
Registration basics

Aligning multiple images

Preprocessing Pipeline



Original (Noisy)



Gaussian Filter



Median Filter

Values:
3, 5, 7, 9, 12, 15, 21, 22, 25
Median
Output: 12



Bilateral Filter



Histogram Eq.

Erosion

Shrinks

Dilation

Expands

Opening

Separates

Closing

Connects

Complete Pipeline Example



Each step targets specific image quality issues

Segmentation Methods

Thresholding techniques

Global, adaptive, Otsu's method

Region growing

Seed-based similar pixel grouping

Watershed algorithm

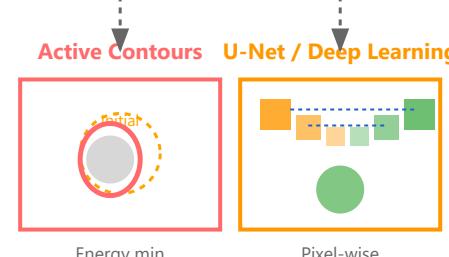
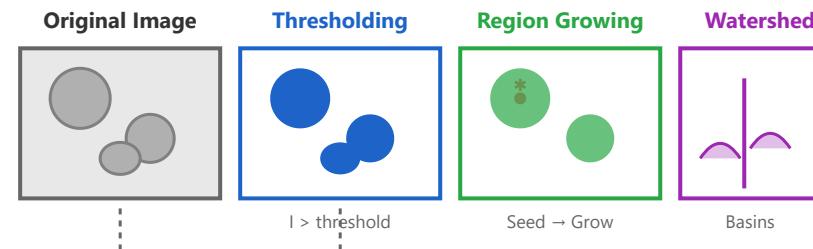
Treating image as topographic surface

Active contours

Energy-minimizing snakes

Machine learning methods

U-Net, Mask R-CNN for segmentation



Clinical Applications
Tumor delineation • Cell counting
Organ segmentation • Lesion detection

Method	Speed	Accuracy
Threshold	Fast	Medium
Region	Medium	Medium
Watershed	Medium	High
Snakes	Slow	High
Deep Learn	Slow	High

Key Considerations:

- Threshold: Simple, fast, manual tuning
- Region: Good for homogeneous areas
- Watershed: Handles touching objects
- DL: Best accuracy, needs training data

Feature Extraction

Texture analysis

GLCM, LBP patterns

Shape descriptors

Area, perimeter, circularity, moments

Intensity statistics

Mean, std, min/max, histogram metrics

Haralick features

14 texture features from GLCM

Radiomics

High-throughput feature extraction

GLCM (Gray Level Co-occurrence)

Image:  GLCM Matrix:

4	2	1
2	3	2
1	2	0

Captures texture patterns

LBP (Local Binary Pattern)

Binary:  Pattern:

$$11011100_2 = 220$$

Rotation invariant texture

Shape Descriptors



Haralick Features (from GLCM)

Statistical: Correlation:

- Energy (uniformity)
- Entropy (randomness)
- Contrast (local variations)
- Homogeneity
- Correlation
- Dissimilarity

Higher-order:

- Cluster shade
- Cluster prominence
- IMC1, IMC2
- Max probability

Radiomics Pipeline

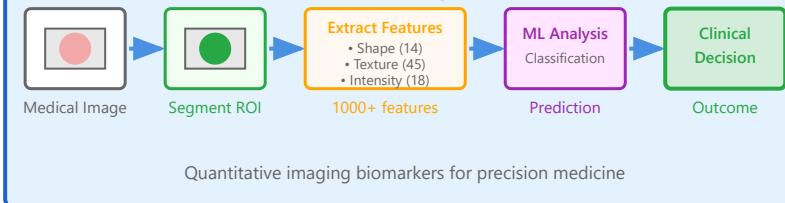


Image Registration

Rigid vs non-rigid

Translation/rotation vs deformation

Similarity metrics

Mutual information, correlation

Optimization methods

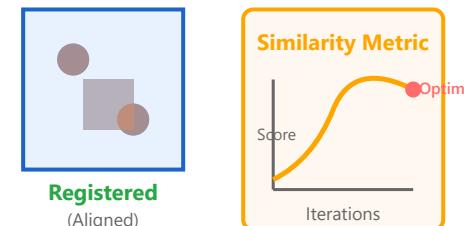
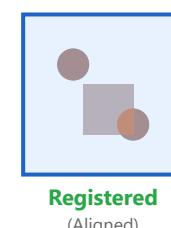
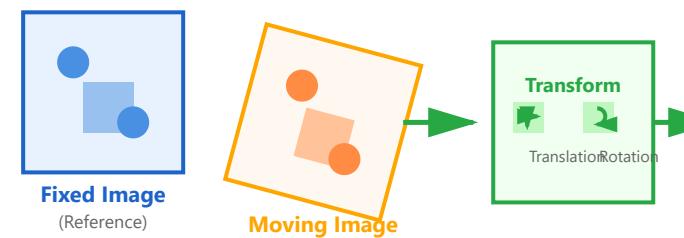
Gradient descent, genetic algorithms

Multi-modal registration

Aligning different imaging modalities

Validation approaches

Fiducial markers, Dice coefficient



Clinical Applications

- Multi-modal fusion (MRI + PET)
- Longitudinal analysis (tumor growth)
- Atlas-based segmentation
- Image-guided surgery planning

3D Reconstruction

Volume rendering

Ray casting through 3D data

Surface rendering

Isosurface extraction (Marching Cubes)

Maximum intensity projection

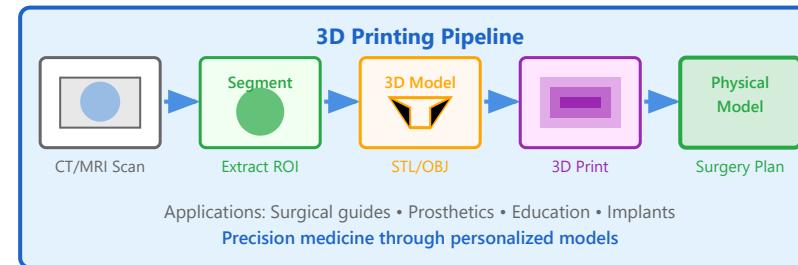
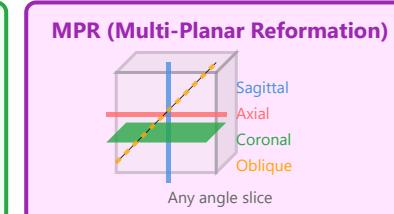
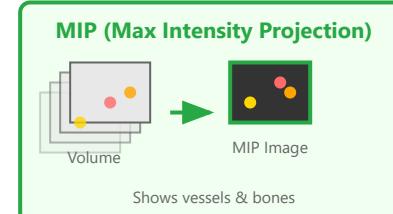
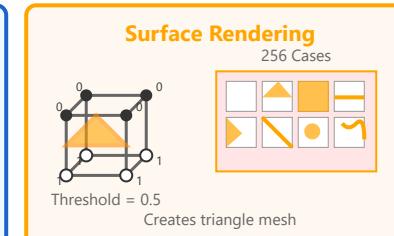
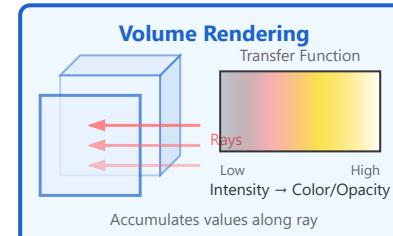
MIP shows brightest voxels

Multi-planar reformation

MPR creates arbitrary slice planes

3D printing applications

Surgical planning, prosthetics



DICOM Format

DICOM structure

Digital Imaging and Communications in Medicine

Tags and metadata

Patient info, acquisition parameters

PACS systems

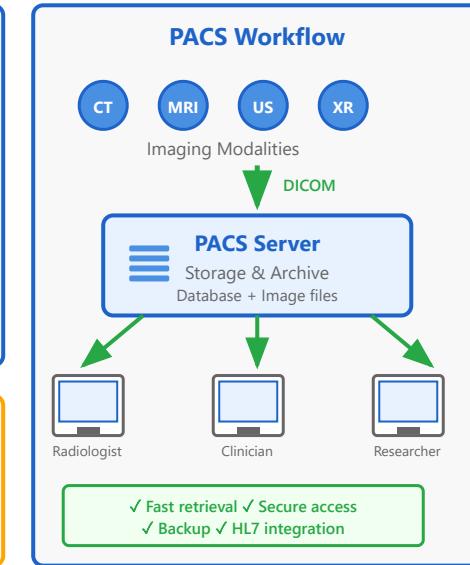
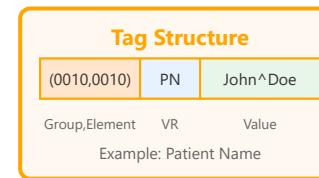
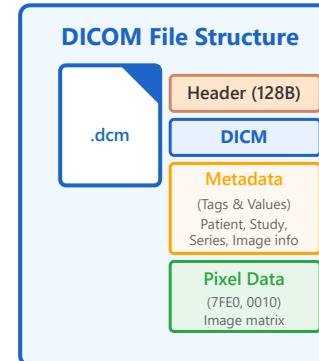
Picture Archiving and Communication Systems

Anonymization

Removing protected health information

Viewer software

Horos, 3D Slicer, RadiAnt





Hands-on: Medical Image Processing

SimpleITK tutorial

Python library for medical image analysis

Loading DICOM series

`sitk.ReadImage()` and `GetArrayFromImage()`

Basic operations

Filtering, thresholding, morphology

Segmentation example

Region growing and connected components

3D visualization

Integration with matplotlib and VTK



Hands-on: ImageJ and Python Imaging

ImageJ macro basics

Automating repetitive tasks

Python with scikit-image

skimage for scientific imaging

Batch processing

Processing multiple images efficiently

Custom plugins

Extending ImageJ functionality

Analysis workflows

Cell counting, intensity measurements

Thank You!

Clinical Impact Through Imaging Science

- ✓ Imaging breakthroughs enabling precision medicine
- ✓ Super-resolution microscopy revealing molecular structures
 - ✓ AI transforming medical image analysis
- ✓ Multi-modal imaging providing comprehensive diagnosis

Introduction to Biomedical Datascience