

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

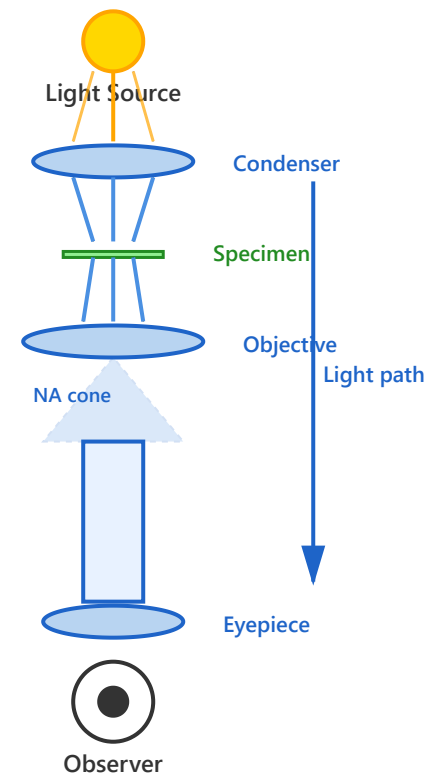
$$d = \lambda / (2 \cdot \text{NA}) \approx 200 \text{ nm}$$

## Point spread function

3D light distribution pattern

## Optical aberrations

Spherical, chromatic distortions



# Resolution and Magnification

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## **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**



## Filter cube design

Excitation, dichroic, emission filters

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Excitation, dichroic, emission filters

### Multichannel imaging

Multiple fluorophores simultaneously

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## Autofluorescence

Background from endogenous molecules

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**Phototoxicity**  
Cell damage from light exposure

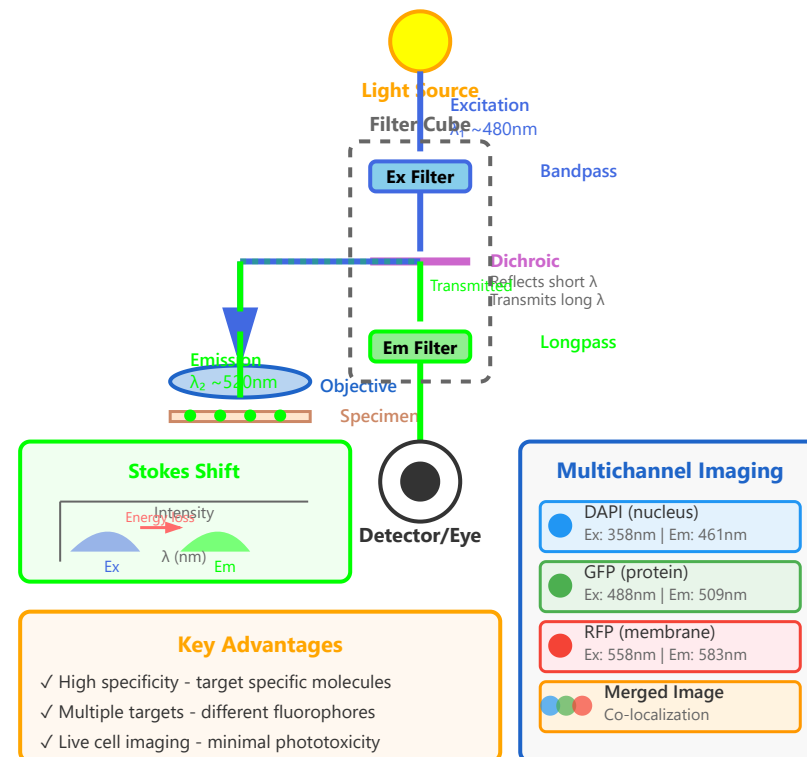
**Phototoxicity**  
Cell damage from light exposure

## Live cell considerations

Environmental control requirements

## 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

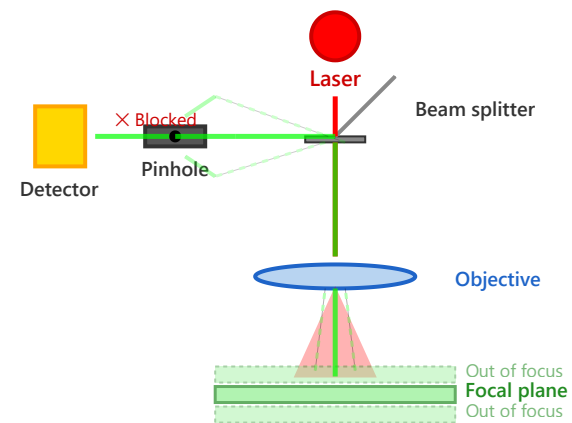
Point-by-point image acquisition

## 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

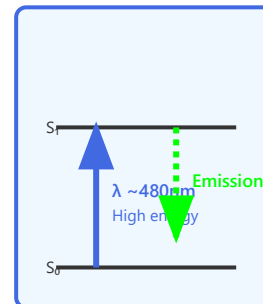
## In vivo imaging

Live animal brain imaging

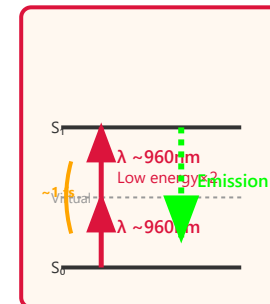
## SHG imaging

Second harmonic generation for collagen

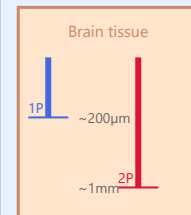
### One-Photon



### Two-Photon

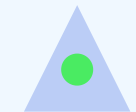


### Penetration Depth



### Excitation Volume

#### One-Photon



Entire cone excited

#### Two-Photon



Only focal point

Pulsed Ti:Sapphire laser  
~100 fs pulses, 80 MHz

### 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

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## **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)

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## Electron sources

Wavelength  $\sim 0.004$  nm vs light  $\sim 500$  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

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## **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

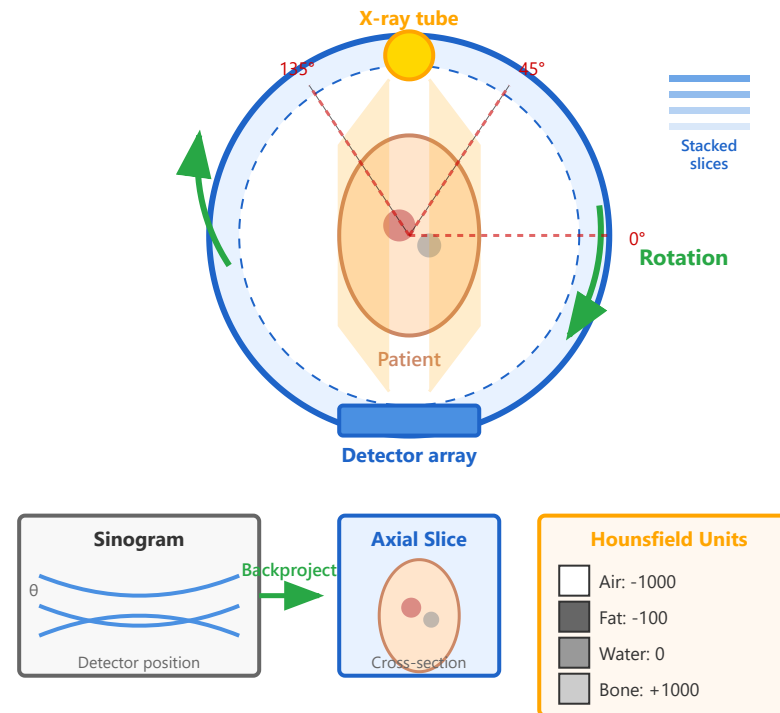
Continuous rotation and table movement

## Dose reduction strategies

Iterative reconstruction, tube modulation

## Contrast protocols

IV contrast timing for specific applications



# MRI Physics Basics

## Nuclear magnetic resonance

Hydrogen protons align in magnetic field

## Gradient fields

Spatial encoding of signal

## K-space

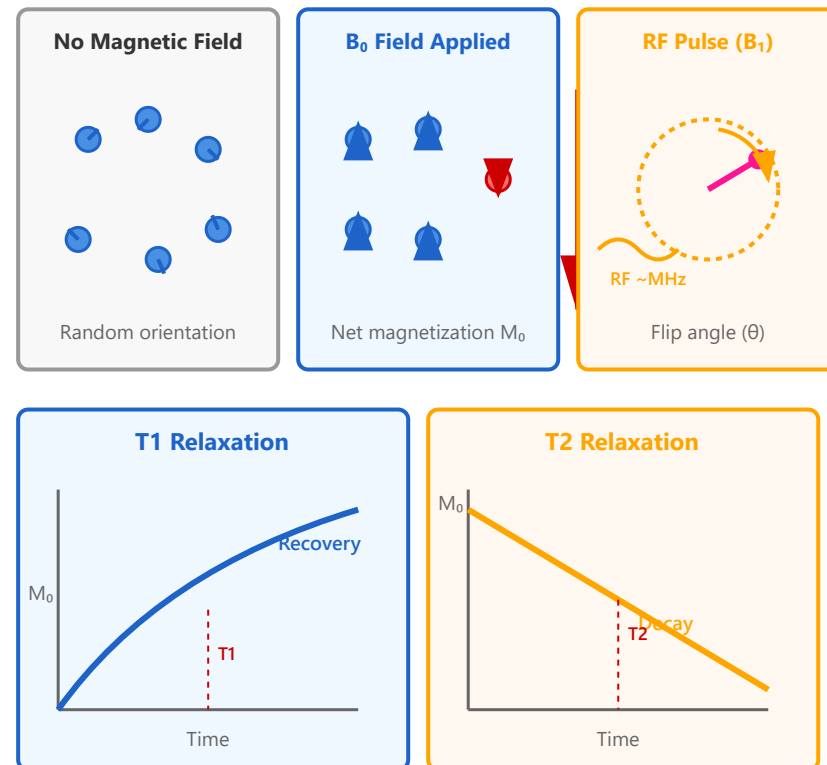
Frequency domain data representation

## Relaxation times (T1, T2)

Tissue-specific signal recovery

## Signal equation

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



# MRI Sequences and Contrast

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## **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

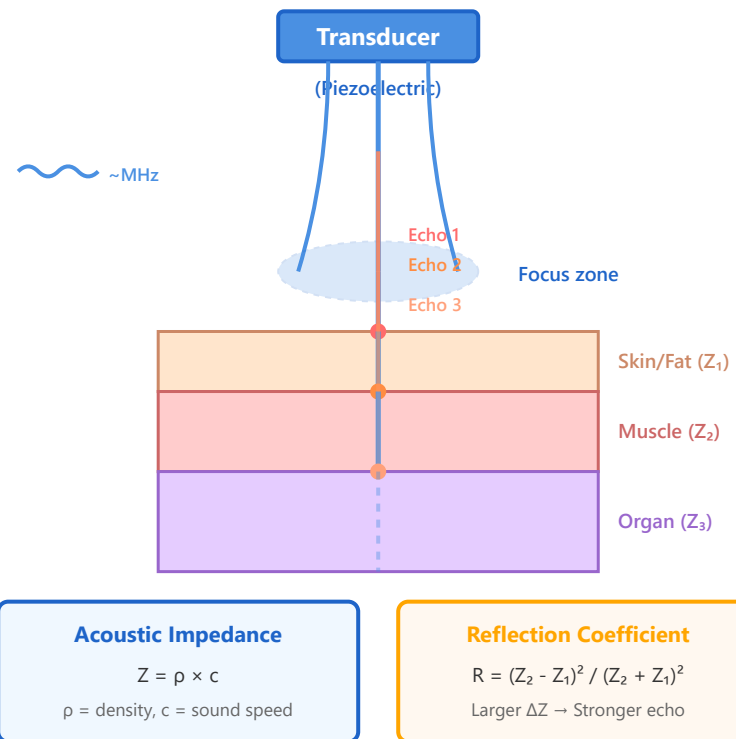
Interface properties determine echoes

## Beamforming

Focusing and steering ultrasound beam

## Harmonic imaging

Higher frequencies improve resolution



# Doppler Ultrasound

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## **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

# PET Imaging

## Positron annihilation

511 keV photons in opposite directions

## Coincidence detection

Simultaneous detection localizes source

## Radiotracers (FDG, etc.)

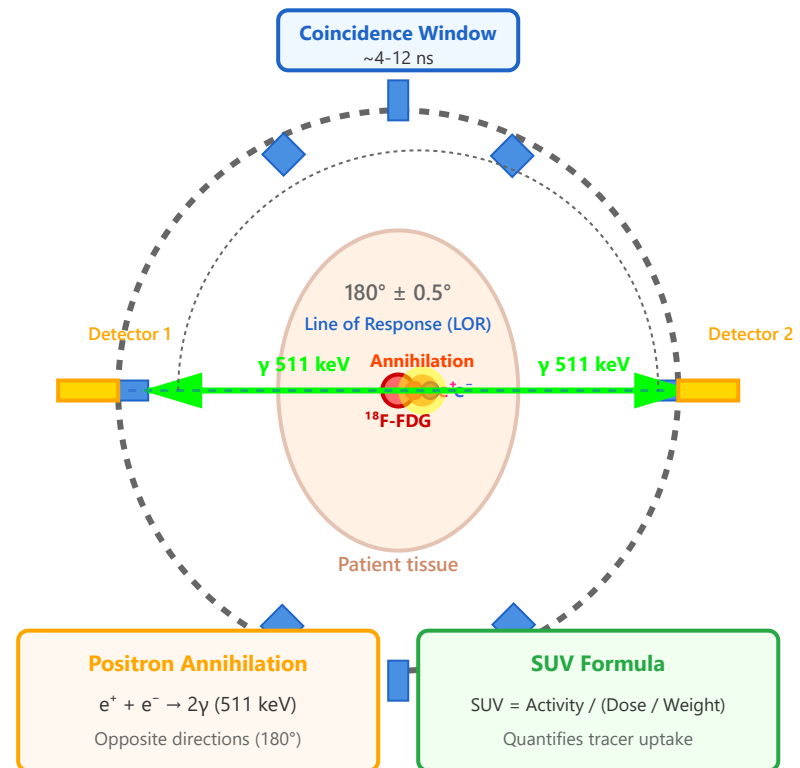
FDG shows glucose metabolism

## SUV calculations

Standardized uptake value quantification

## PET/CT integration

Functional and anatomical fusion



# SPECT Imaging

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## **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

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## **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

# Image Preprocessing

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## 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

# Segmentation Methods

## Thresholding techniques

Global, adaptive, Otsu's method

## Region growing

Seed-based similar pixel grouping

## Watershed algorithm

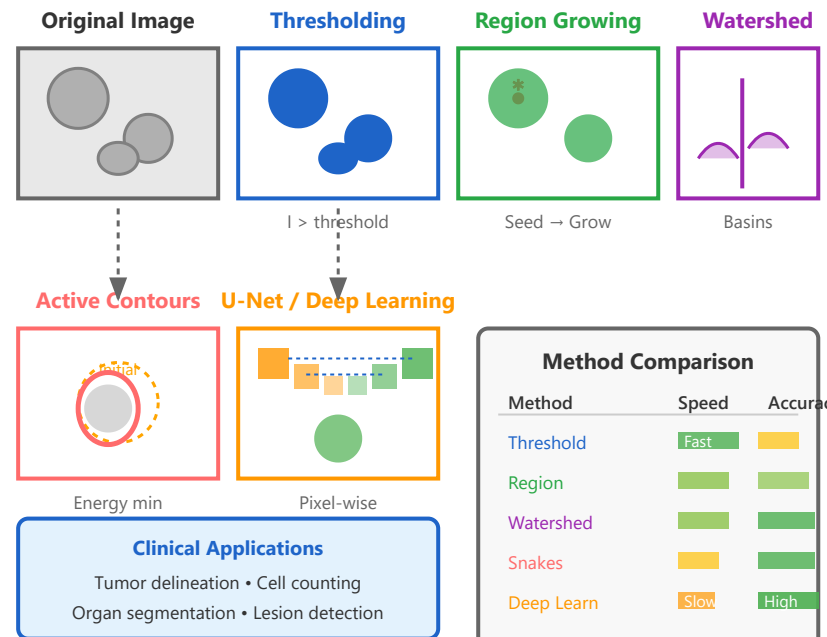
Treating image as topographic surface

## Active contours

Energy-minimizing snakes

## Machine learning methods

U-Net, Mask R-CNN for segmentation





# Feature Extraction

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## 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

# Image Registration

## Rigid vs non-rigid

Translation/rotation vs deformation

## Similarity metrics

Mutual information, correlation

## Optimization methods

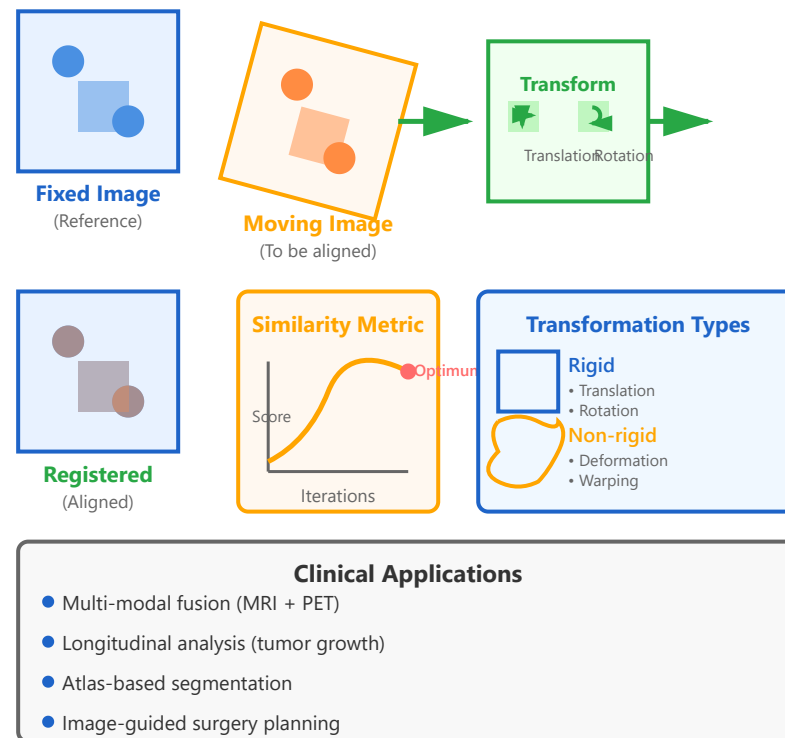
Gradient descent, genetic algorithms

## Multi-modal registration

Aligning different imaging modalities

## Validation approaches

Fiducial markers, Dice coefficient



# 3D Reconstruction

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## **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

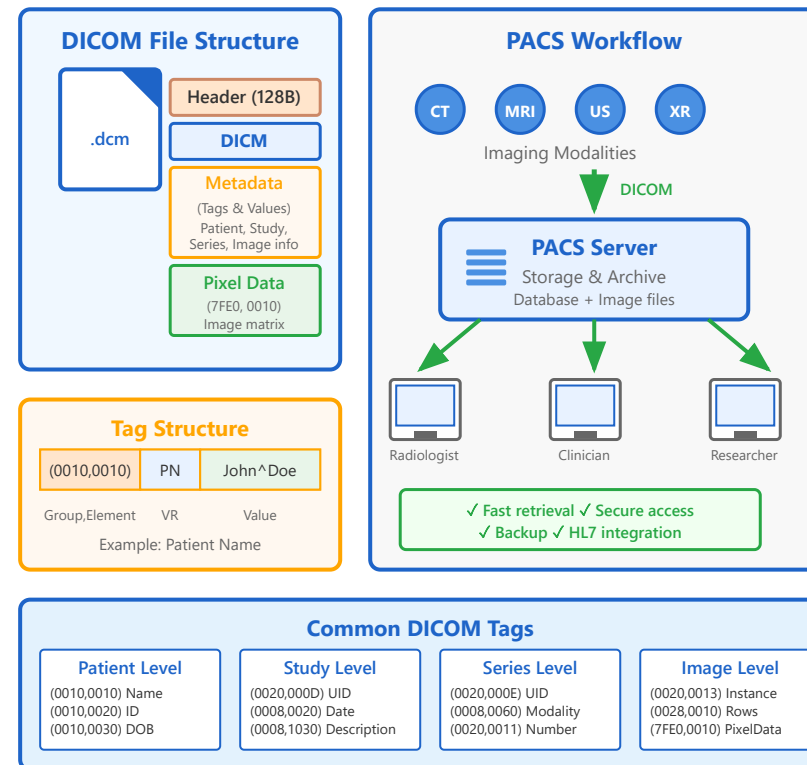
Picture Archiving and Communication Systems

## Anonymization

Removing protected health information

## Viewer software

Horos, 3D Slicer, RadiAnt





# Hands-on: Medical Image Processing

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## **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

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## **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