



# Innovations in Diagnostics - Looking Beyond The Microscope: Molecular Diagnostics and Digital Pathology

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# Disclosures

- ▶ No conflicts of interest to disclose



# Objectives

- ▶ Understand what molecular pathology and digital pathology encompass
- ▶ Explore clinical examples and relevance to patient care
- ▶ Understand the future of data-driven diagnostics



# (Brief) History of Pathology

- ▶ 18<sup>th</sup> Century: Organ-Based Pathology
- ▶ 19<sup>th</sup> Century: Cellular Pathology
  - ▶ Cell Theory, Germ Theory
- ▶ Early 20<sup>th</sup> Century: Diagnostic Pathology
  - ▶ 1900s - Development of Histopathology
  - ▶ 1928 - Development of Pap Smear
- ▶ Mid-Late 20<sup>th</sup> Century: Technological Expansion
  - ▶ Electron Microscopy
  - ▶ Immunopathology
  - ▶ Flow Cytometry

Humors → Organs → Cells → Molecules → Data & AI

- ▶ Late 20<sup>th</sup> Century: Molecular Pathology
  - ▶ 1983 - Polymerase Chain Reaction (PCR)
  - ▶ 1990s - Cytogenetics and Fluorescent In-situ Hybridization (FISH)
- ▶ 21<sup>st</sup> Century: Precision and Digital Pathology
  - ▶ 2000s - Genomic and Molecular Profiling
    - ▶ NGS
  - ▶ 2010s - Digital Pathology
    - ▶ Whole-slide imaging
  - ▶ 2020s - AI and Computational Pathology

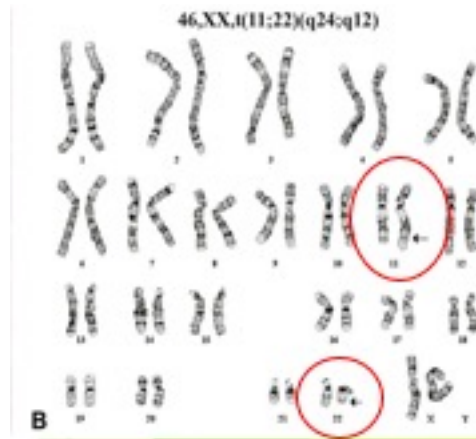
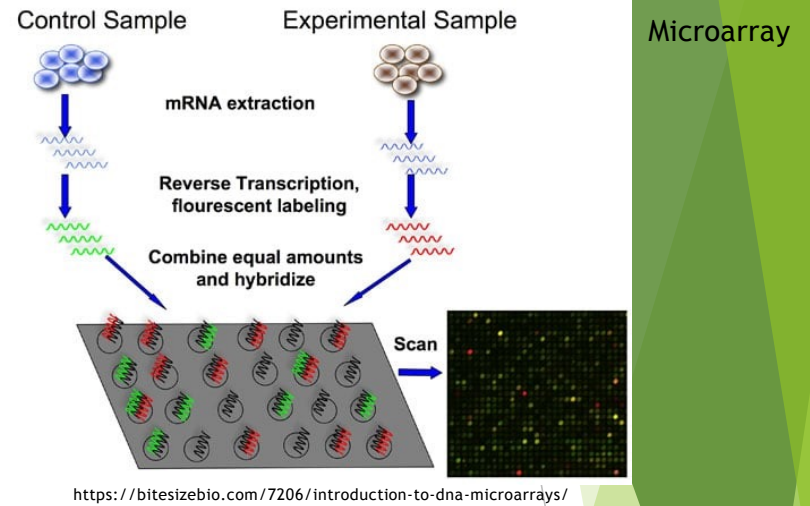


# Molecular Pathology

- ▶ Analyzing DNA and other genetic material (RNA, proteins) within tissues or bodily fluids for the purpose of diagnosing or monitoring disease
- ▶ Molecular Pathology plays a role in all clinical steps:
  - ▶ Disease Screening
  - ▶ Diagnosis (“Molecular Diagnostics”)
  - ▶ Prognosis
  - ▶ Treatment
    - ▶ Pharmacogenomics
    - ▶ Disease Monitoring
- ▶ Personalized (Precision) Medicine
  - ▶ An approach that tailors the healthcare process – from diagnosis to treatment – to the individual patient to improve effectiveness and reduce side effects
  - ▶ Customizes medical treatments based on an individual’s unique genetic makeup

# Molecular Pathology Tools

- ▶ Cytogenetic Analysis/Karyotyping
- ▶ Fluorescence in-situ hybridization (FISH)
- ▶ Polymerase Chain Reaction (PCR)
- ▶ Sanger Sequencing
- ▶ Next Generation Sequencing (NGS)
- ▶ Microarray analysis

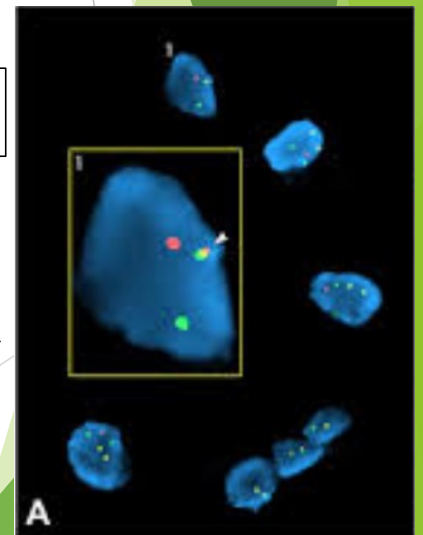


Pfeifer, J. (2010)

EWRS1/FLI1 fusion -  
Ewings sarcoma/PNET

← Conventional  
cytogenetics

Interphase FISH →



Liou, S et al. (2021)

# Molecular Pathology - Disease Screening

- ▶ Cancer Screening
  - ▶ Population-Based screening:
    - ▶ Cervical cancer screening
      - ▶ hrHPV DNA/RNA testing
    - ▶ Colorectal cancer screening
      - ▶ Stool DNA tests
      - ▶ Stool RNA tests
  - ▶ Hereditary disease screening:
    - ▶ BRCA1 and BRCA2 genes → breast and ovarian cancer
    - ▶ APC genes → colorectal cancer

# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

- ▶ Inheritable diseases
  - ▶ Newborn testing
    - ▶ Sickle Cell Disease
      - ▶ HPLC/IEF → molecular testing
    - ▶ Cystic Fibrosis
      - ▶ IRT → molecular testing
- ▶ Medical Microbiology
- ▶ Cancer Diagnostics
  - ▶ Hematologic malignancies
  - ▶ Solid tumors



# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

## ▶ Medical Microbiology

- ▶ Molecular testing has revolutionized medical microbiology by enabling rapid and precise detection of pathogens and rapid determination of antimicrobial resistance in bacteria
  - ▶ Allows doctors to choose appropriate treatment faster
  - ▶ Improves tracking and control of infections
- ▶ Virus and fungal identification
- ▶ Automated Bacterial identification
- ▶ Automated Antibiotic Susceptibility Testing
  - ▶ Automated alternative to disk diffusion method of determining antibiotic susceptibility of bacterial cultures
  - ▶ Plates with pre-filled antimicrobial agents at varying dilutions to determine the minimum inhibitory concentration (MIC) that prevents growth
- ▶ Antimicrobial Resistance Testing
  - ▶ Staph aureus → *mecA* gene detection → methicillin resistance
  - ▶ Klebsiella pneumoniae → KPC, NDM, OXA-48 gene detection → carbapenem resistance
  - ▶ Mycobacterium tuberculosis → *rpoB*, *katG* → rifampicin and isoniazid resistance



<https://www.bd.com/en-us/products-and-solutions/products/product-families/bd-phoenix-m50-instrument>

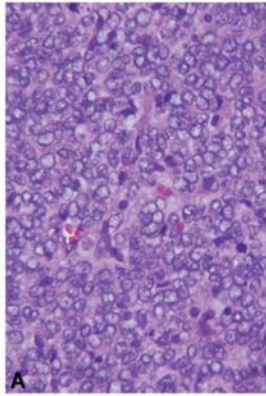
# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

## ▶ Cancer Diagnostics

- ▶ Diagnosis of tumors by detecting somatic or acquired DNA mutations limited to the disease process itself
  - ▶ Diagnosis
  - ▶ Prognostic classification
- ▶ Soft Tissue Tumors
  - ▶ Important as soft tissue tumors often look morphologically similar and have overlapping immunophenotypes
  - ▶ Many are now classified primarily by molecular features rather than histology alone

# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

| Tumor   | Key Molecular Abnormality                     | Testing Method                  | Diagnostic Role                                    | Clinical / Therapeutic Impact                                      |
|---|---|---------------------------------|--|--|
| <u>Ewing sarcoma</u>                                      | <i>EWSR1-FLI1</i> fusion (t(11;22))           | FISH, RT-PCR, NGS               | Confirms diagnosis in small round blue cell tumors | Directs Ewing-specific chemotherapy protocols                      |
| <u>Synovial sarcoma</u>                                   | <i>SS18-SSX1/SSX2</i> fusion                  | FISH, RT-PCR, NGS               | Distinguishes from other spindle cell tumors       | Ensures correct sarcoma classification and management              |
| <u>Liposarcoma</u> (well-differentiated/dedifferentiated) | <i>MDM2</i> amplification                     | FISH, PCR                       | Differentiates from benign lipoma                  | Determines need for wide excision and surveillance                 |
| <u>Gastrointestinal stromal tumor</u>                     | <i>KIT</i> or <i>PDGFRA</i> mutation          | PCR, NGS                        | Confirms diagnosis in KIT-equivocal cases          | Predicts response or resistance to imatinib                        |
| <u>Alveolar rhabdomyosarcoma</u>                          | <i>PAX3-FOXO1</i> or <i>PAX7-FOXO1</i> fusion | RT-PCR, FISH                    | Distinguishes from embryonal subtype               | Fusion-positive tumors have worse prognosis → risk-adapted therapy |
| <u>Dermatofibrosarcoma protuberans</u>                    | <i>COL1A1-PDGFB</i> fusion                    | FISH, RT-PCR                    | Confirms diagnosis in atypical cases               | Predicts response to imatinib in unresectable disease              |
| <u>NTRK-rearranged spindle cell neoplasm</u>              | NTRK gene fusion ↓                            | NGS, pan-TRK IHC + confirmation | Identifies distinct molecular subtype              | Eligible for TRK inhibitors (e.g., larotrectinib)                  |



Pfeifer, J. (2010)

# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

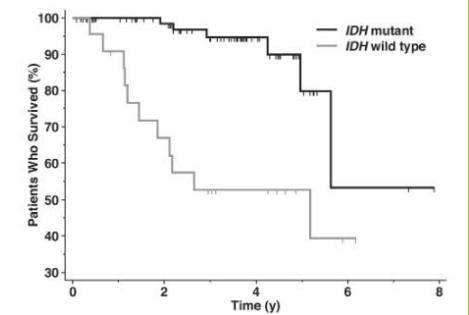
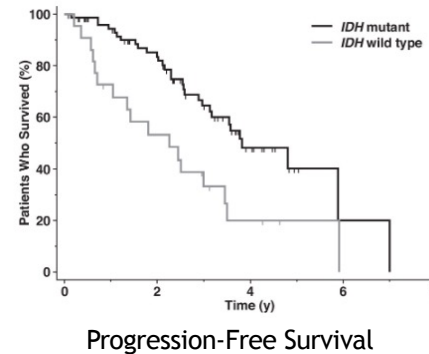
## ▶ Diffuse Gliomas

- ▶ Modern classification requires molecular testing
  - ▶ IDH mutation status
  - ▶ 1p/19q co-deletion

## ▶ Chronic Myeloid Leukemia (CML)

- ▶ Caused by BCR-ABL1 fusion gene (t(9;22) - Philadelphia chromosome))
- ▶ Mutation can be detected by conventional cytogenetics, FISH, or PCR

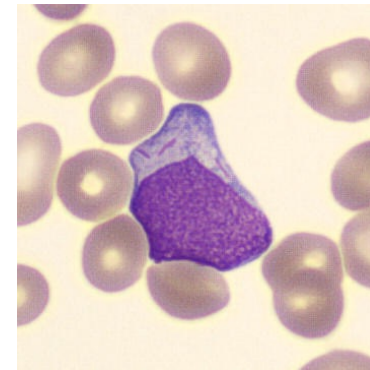
Grade II Diffuse Gliomas



<https://ajronline.org/doi/10.2214/AJR.17.18457>

# Molecular Pathology - Diagnosis of Disease (Molecular Diagnostics)

- ▶ Acute Myeloid Leukemia (AML)
  - ▶ Acute Promyelocytic Leukemia (APL)
    - ▶ Defined by PML-RARA fusion
      - ▶ Identified by FISH or PCR
    - ▶ Prone to thrombotic events
    - ▶ Targeted therapy dramatically improves survival
  - ▶ Prognosis of AML overall depends heavily on mutation profile:
    - ▶ FLT3-ITD mutation → adverse prognosis
    - ▶ NPM1 mutation (w/o FLT3-ITD) → favorable prognosis
    - ▶ TP53 mutation → very poor prognosis



Promyelocyte with Auer rods

# Molecular Pathology - Guiding Treatment

## ▶ Pharmacogenomics/Pharmacogenetics

- ▶ Understanding how a person's genetic makeup influences drug metabolism and response
- ▶ Examples:
  - ▶ BCR-ABL testing in CML → Gleevac
  - ▶ KRAS testing in colon cancer → resistance to certain anti-EGFR therapies
  - ▶ HER2 testing in breast cancer → Trastuzumab
  - ▶ EGFR testing in lung cancer → TKI

Molecular Pathology is just one field. driving pathology towards machine-based practice...



# Digital Pathology

- ▶ Digitization of glass slides into high-resolution interactive images for viewing, managing, and analyzing using computer screens as an alternative to traditional microscopes
- ▶ Opportunities:
  - ▶ Remote access
    - ▶ Telepathology (consultations)
    - ▶ Remote pathology work
  - ▶ Quicker image access
  - ▶ AI applications



# Digital Pathology

## ▶ Process:

- ▶ Specimen gross evaluation and tissue sampling
- ▶ Tissue embedding in paraffin
- ▶ Tissue block cutting and tissue application to slides
- ▶ Slide staining
- ▶ Scanning of slides
- ▶ Storage in servers
- ▶ Access to images through image management system (IMS) and image analysis software
- ▶ Pathologist examination
  - ▶ Additional staining studies (immunohistochemistry, special stains)
- ▶ Pathologist diagnosis



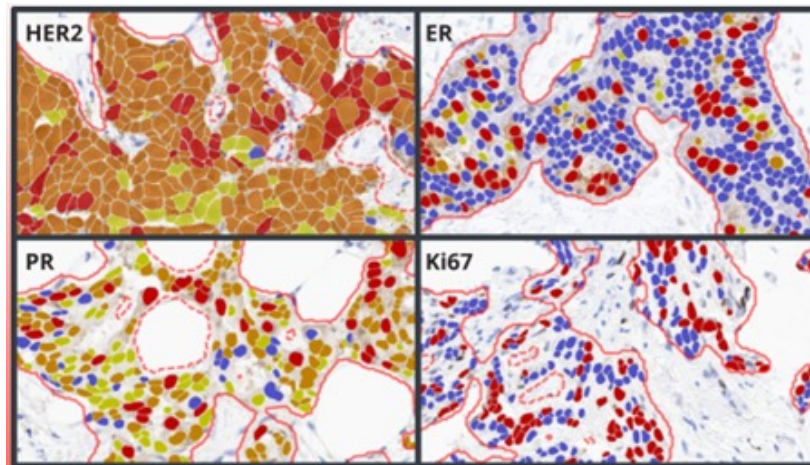
Leica GT450

# Digital Pathology - Where we are now

- ▶ Europe has been quicker to apply digital pathology than the United States
  - ▶ Structured, coordinated funding and policy support for DP
  - ▶ Regulatory environment less stringent than FDA
- ▶ Primary Diagnosis:
- ▶ In the U.S., the adoption of DP for primary diagnosis has been slow, and is currently used mainly at academic institutions and large conglomerate hospital systems
  - ▶ High initial costs
    - ▶ WSI scanner: ~\$50,000 - \$300,000
    - ▶ IT infrastructure and data storage
      - ▶ Single WSI slide file: 1-5 GB
        - ▶ Medium-sized lab: 100,000 slides/yr → hundreds of terabytes to petabytes
      - ▶ Redundant back-up systems
      - ▶ High-speed network upgrades
      - ▶ Ongoing data lifecycle management
    - ▶ Image management software
      - ▶ Updated required for AI applications
  - ▶ Uncertain reimbursement for DP services

# Digital Pathology - Where we are now

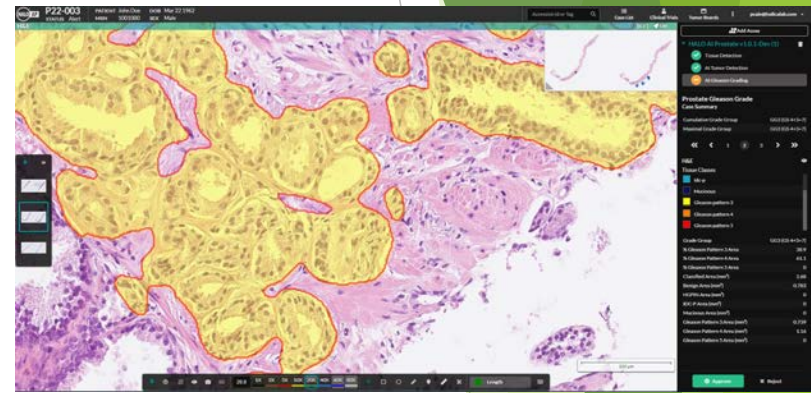
- ▶ AI applications:
  - ▶ Image analysis
    - ▶ IHC quantification
      - ▶ ER, PR, HER2, Ki-67 assessment in breast cancer (for prognosis and treatment)
      - ▶ Ki-67 quantification in GI neuroendocrine tumors (for grading)



Breast IHC AI - Indica Labs (<https://indicalab.com>)

# Digital Pathology - The Future of Digital Pathology

- ▶ AI Applications
  - ▶ To supplement pathologists' decisions rather than replace them
  - ▶ Opportunities
    - ▶ Workflow assistance
      - ▶ Prioritization of cases
    - ▶ Diagnosis enhancement
      - ▶ Grading of prostate cancer
      - ▶ Identifying and grading invasive breast cancer
- ▶ Expanded telepathology



Halo AI - Indica Labs (<https://indicalab.com>)



# Summary

- ▶ Both molecular and digital pathology are transforming the practice of pathology with opportunities to improve diagnostic accuracy, speed, and personalization in patient care



Questions?

