IHC and Molecular Morphology in the Era of Precision Medicine 8.30am Jan 5, 2018

40 min.

Clive R Taylor MD. Dphil.
MRCP(Ir) , MRCPath (UK).

Precision Medicine requires Precision Diagnostics.

Many of the targets for personalized therapy are proteins

IHC is, in theory, an ideal method for their detection and measurement

-----but we need to improve **IHC performance, choice of controls and interpretation of results.**

Disclosures; CRT –has consulting arrangements with for Philips, Agilent, PerkinElmer, Optra

Theme
Pathology is technology driven

“The Age of the Microscope”

180 years ago the microscope changed everything in medicine

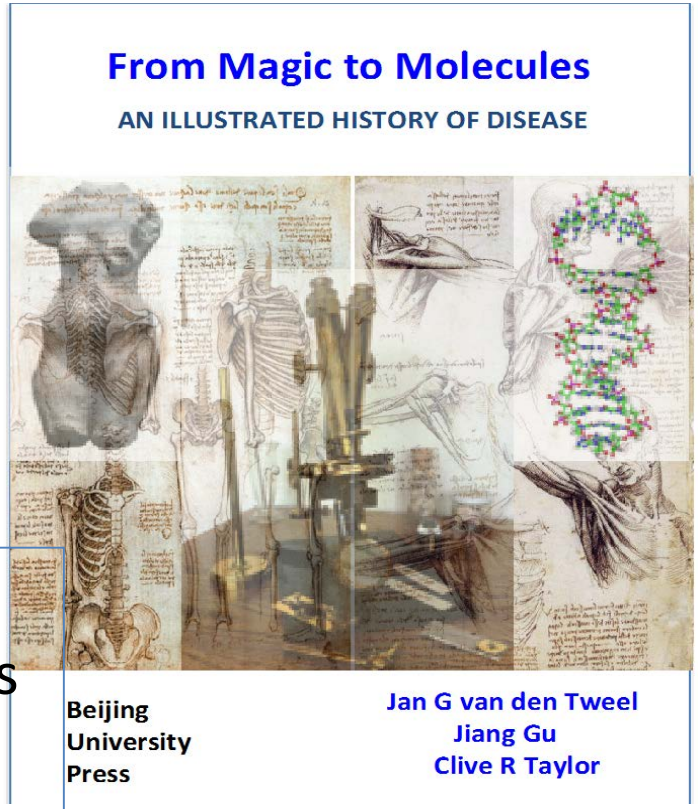
“The Age of the Intelligent Microscope”

NOW - change of comparable magnitude is occurring today

– driven by two technologies

Molecular
(genetic) methods

Digital
computer analysis



From Magic to Molecules: An Illustrated History of Disease

Van den Tweel et al. 2016

Circa - 1700
Microscope invented

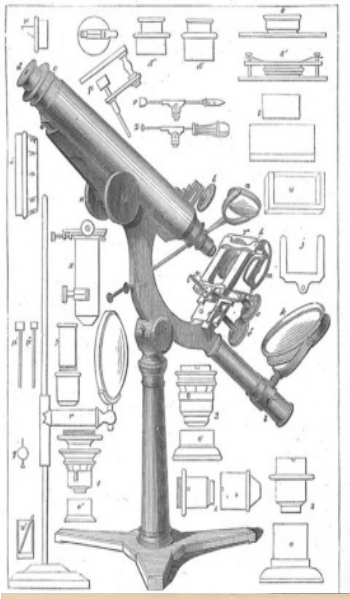
Technology drives everything

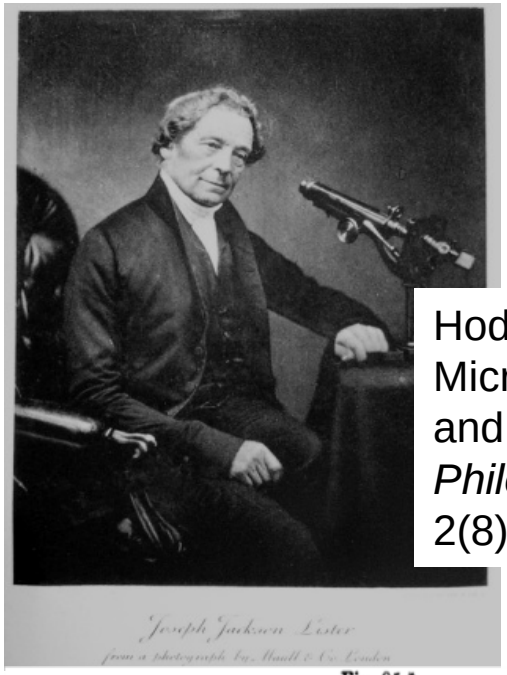
Better quality
Lower cost
Wider availability

Circa - 1840
The microscope 'invents' Surgical Pathology

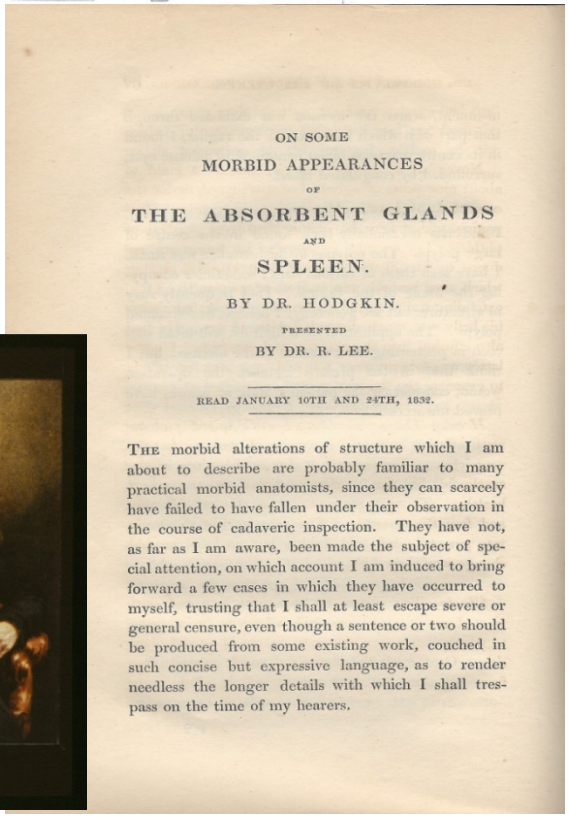
The microscope- medical adoption was slow due to poor resolution and cost

Joseph Jackson Lister





Hodgkin T, Lister J J. Notice of Some Microscopic Observations of the Blood and Animal Tissues. *Philosophical Magazine*, 1827. 2(8), 130-138 pp131-132



ON SOME
MORBID APPEARANCES
OF
THE ABSORBENT GLANDS
AND
SPLEEN.

BY DR. HODGKIN.

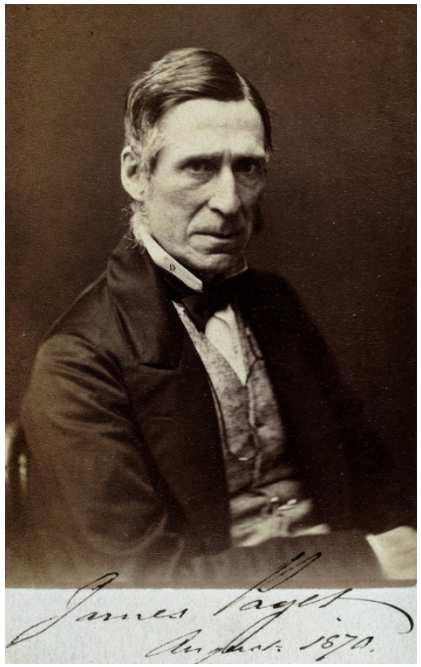
PRESENTED
BY DR. R. LEE.

READ JANUARY 10TH AND 24TH, 1832.

THE morbid alterations of structure which I am about to describe are probably familiar to many practical morbid anatomists, since they can scarcely have failed to have fallen under their observation in the course of cadaveric inspection. They have not, as far as I am aware, been made the subject of special attention, on which account I am induced to bring forward a few cases in which they have occurred to myself, trusting that I shall at least escape severe or general censure, even though a sentence or two should be produced from some existing work, couched in such concise but expressive language, as to render needless the longer details with which I shall trespass on the time of my hearers.


Fig. 85.*

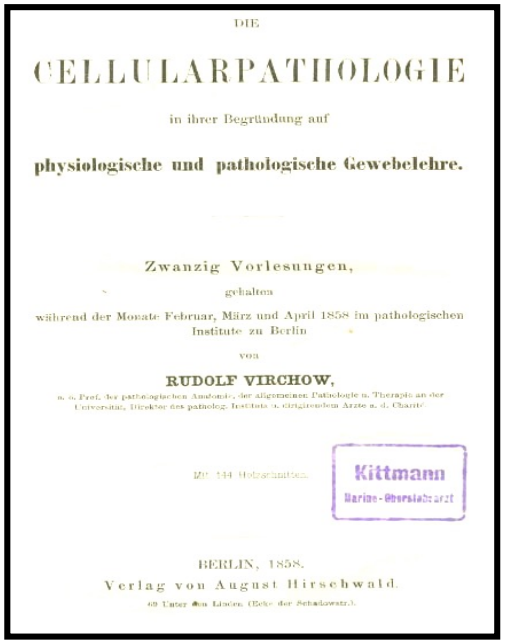
The First Pathology Microscopy Texts



Sir James Paget. 1854

'Lectures on Surgical Pathology', based on a series of 36 lectures given at the College of Surgeons 1847-1852.

Rudolf Virchow 1858



DIE

CELLULARPATHOLOGIE

in ihrer Begründung auf

physiologische und pathologische Gewebelehre.

Zwanzig Vorlesungen,

gehalten

während der Monate Februar, März und April 1858 im pathologischen Institute zu Berlin

von

RUDOLF VIRCHOW,

Kittmann
Marine-Oberstabsarzt

BERLIN, 1858.

Verlag von August Hirschwald.

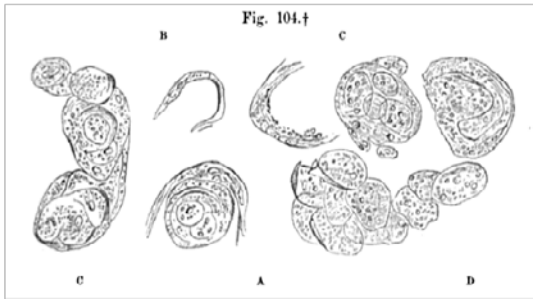


Fig. 104.†

B C

C A D

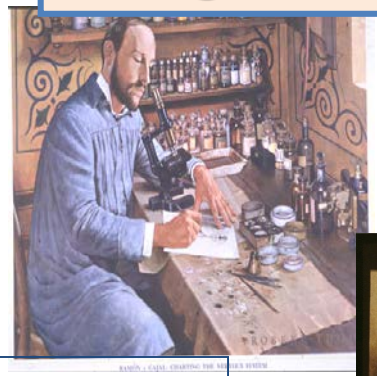
Both
Classified cancers
Depicted cancer cells

Krebs und Cancroid. 429

Der eigentliche Krebs hat auch Elemente von epithelialem Habitus, und Sie brauchen nur eben solche Punkte im Körper zu suchen, wo sich die Epithelzellen unregelmässig entwickeln, z. B. an den Harnwegen (Fig. 15), so werden Sie dieselben sonderbaren, mit grossen Kernen und Kernkörperchen versehenen Bildungen antreffen, welche man als die specifischen, polymorphen Krebszellen schildert. Der Krebs, das Cancroid oder Epitheliom, die Perlgeschwulst oder das Cholesteatom, ja vielleicht das Dermoid, welches Haare, Zähne, Talgdrüsen producirt, wie sie im Eierstock so häufig vorkommen, alle

Fig. 112.

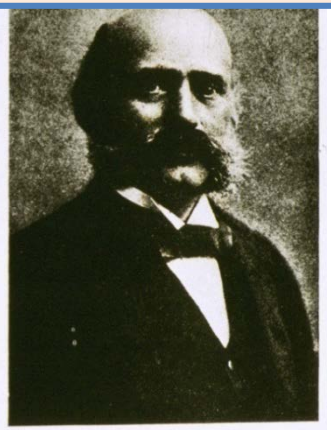
Ushered in the Age of the Microscope - for 150 years
THE Diagnosis was by H&E
- image analysis by mind and microscope !!! 1850 - 2017



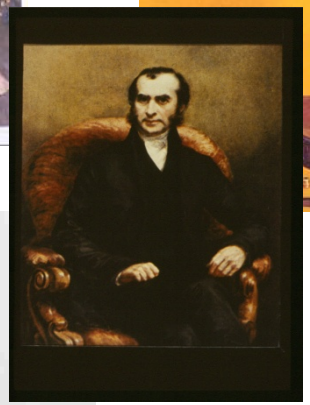
Cajal

Maximow

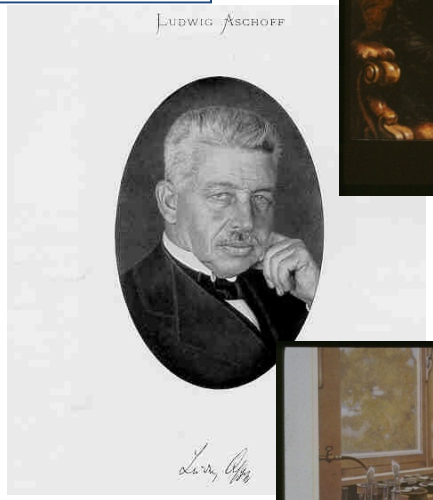
Weigert



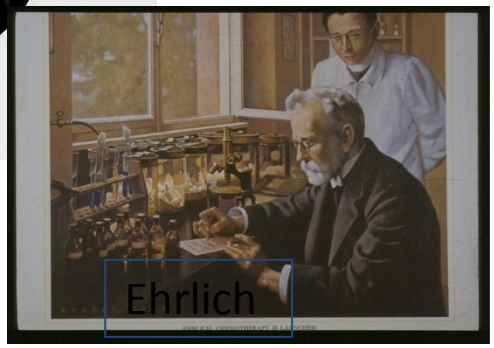
Carl Weigert (1845–1904).



Hodgkin

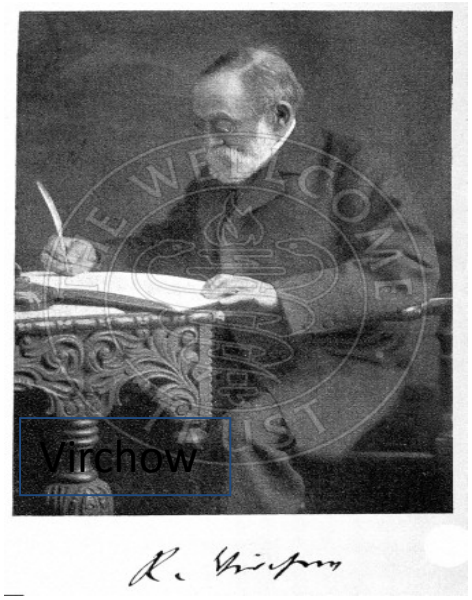


Aschoff



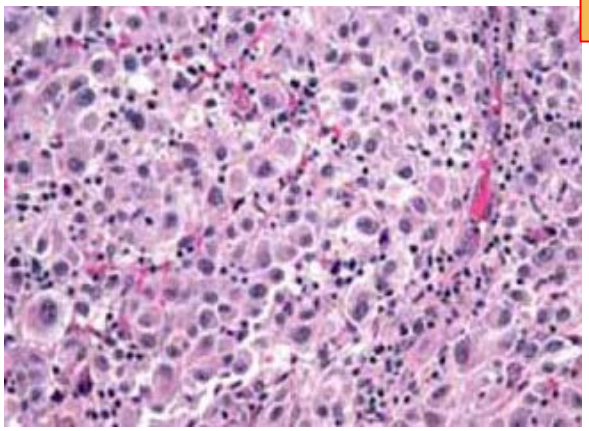
Ehrlich

Lukes

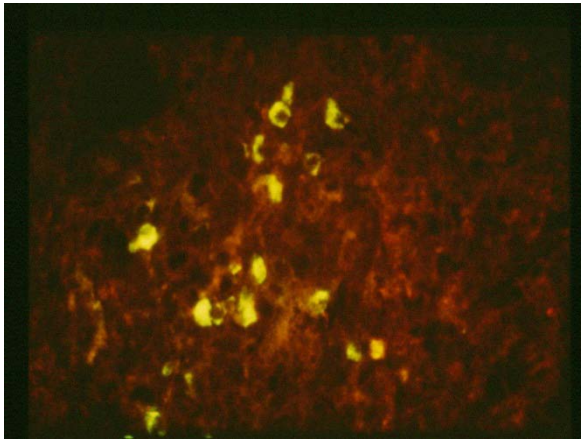


Virchow

H&E became the GOLD STANDARD Diagnosis

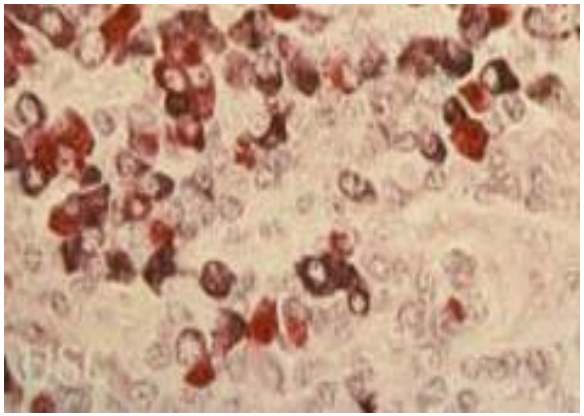


For 150 + yrs - H&E - formalin paraffin section
–diagnostic opinion by a pathologist
STILL TRUE IN 2017



80 years ago.
Immunofluorescent labeling – on frozen sections
Albert Coons, Astrid Fagraeus and others

Limited use in AP As Flu method loses GOLD STANDARD of morphology



40 + years ago.
IHC on FFPE tissue added in 1974
Taylor, Burns, Mason et al Oxford
Combined immunology with morphology
note also first ‘multiplex’ IHC stain

From 1974 - 1998 IHC was just a 'special stain'

DAKO Corporation
6392 Via Real
Carpinteria, California 93013

SEP 25 1998

Re: P980018
DAKO HercepTest
Filed: May 18, 1998
Amended: June 2, June 4, August 4, August 10, August 18, August 24, August 31 and September 25, 1998.

Dear Dr. Murray:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the DAKO Herceptest. This device is a semi-quantitative immunohistochemical assay to determine HER2 overexpression in breast cancer tissues routinely processed for histological evaluation. HercepTest is indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered (see Herceptin package insert). We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

SUDDENLY THINGS CHANGED

-The 'quality' of IHC was no longer sufficient

-Quantification was at best an estimate

1998 – saw the first Companion Diagnostic
It marked the beginning of Precision Medicine.

A targeted therapeutic - is a 'drug' that----

- targets a specific molecule on a cell/tumor
- need to identify which patients respond

eg. HERCEPTIN therapy

eg. HER2

Detects

COMPANION DIAGNOSTIC

- **is a 'classifier'**

RESPONDERS V NON-RESPONDERS

KEY - Linked to defined therapeutic by data

eg. HERCEPT test

Response data

To use in this way IHC must be more than just a stain

FDA

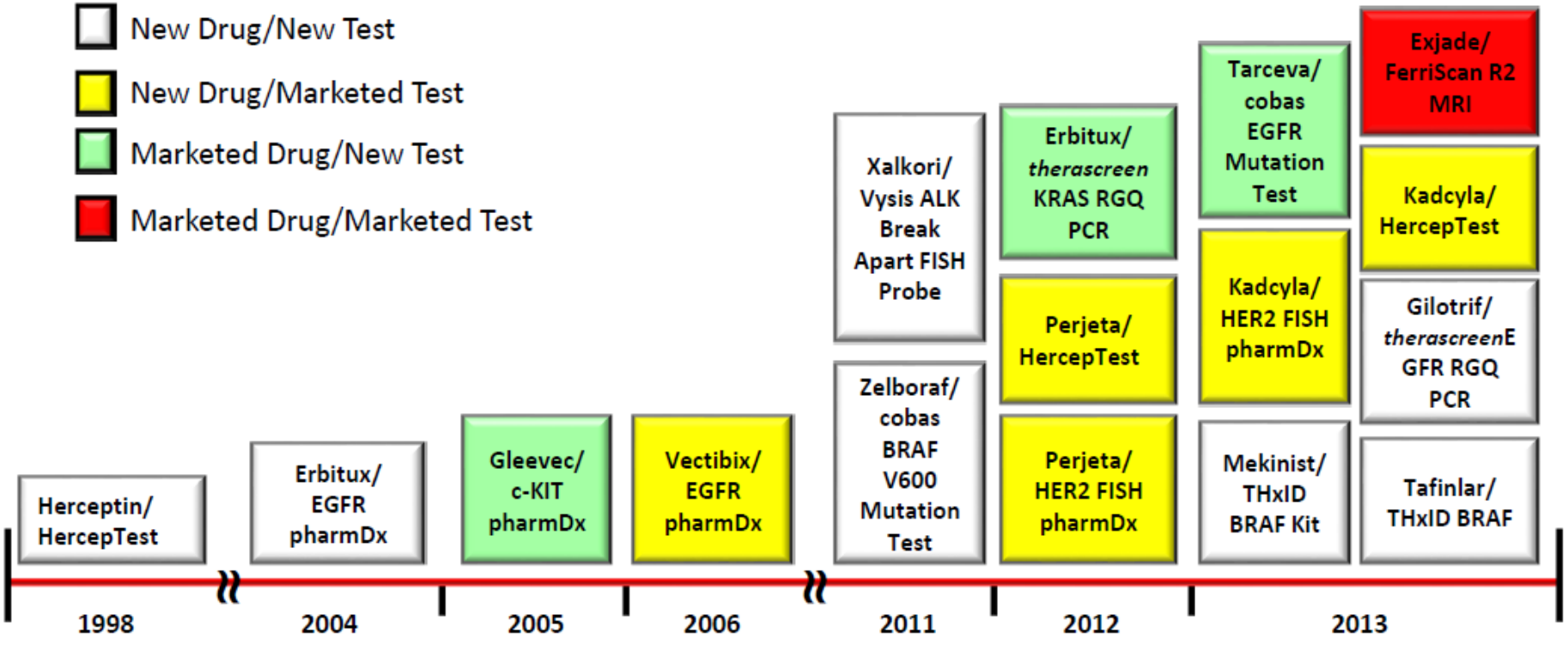
CE

Prognostic versus predictive value of biomarkers in oncology
Oldenhuis et al; 44, 946, 2008

How many companion diagnostics??? **Industry says "A LOT"**

Progress of Companion Diagnostics

Approved US Drug and CDx Combinations



Companion diagnostics partnerships grew from 8 in 2008 to 90+ in 2014.

Bristol-Myers Squibb

How many will we need ? 'BUSINESS' also says "A LOT"

Industry recognizes the opportunity and are willing to work with *anyone*

WSJ.com THE WALL STREET JOURNAL. ONLINE

January 23, 2008

Roche to Acquire Ventana for $3.4 Billion

By JEANNE WHALEN
January 23, 2008; Page A20

After trying for seven months to win over **Ventana Medical** Systems Inc., Swiss drug giant **Roche Holding** AG said it reached an agreement to acquire the U.S. diagnostics company for $3.4 billion.

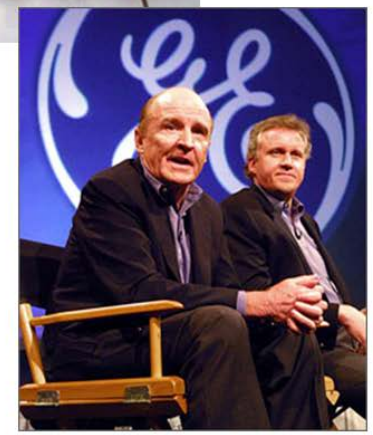
The deal advances Roche's aim of diversifying more into machines and other tools that help diagnose and monitor disease. Ventana's board approved the deal after Roche raised its offer by 19%. Ventana m… of infectious disease. Roche believes such tools will take up a greater share of health-care spending in the future.

Roche has mov… signs of trouble in the pharmaceutical industry have mounted. Sales of prescription drugs are growing at their slowest pace in years, and … medicines.

Roche's prescription-drug sales have fared better than those of many rivals, but the company is still hedging its bets by expanding more into diagnostics. In addition to Ventana, Roche in the past year has said it would acquire two other diagnostics companies: closely held NimbleGen Systems Inc., of Madison, Wis., for $272.5 million, and Bioveris Corp., of Gaithersburg, Md., for $600 million.

Are diagnostics the *new* wonder drug on Wall Street?

Franz Humer

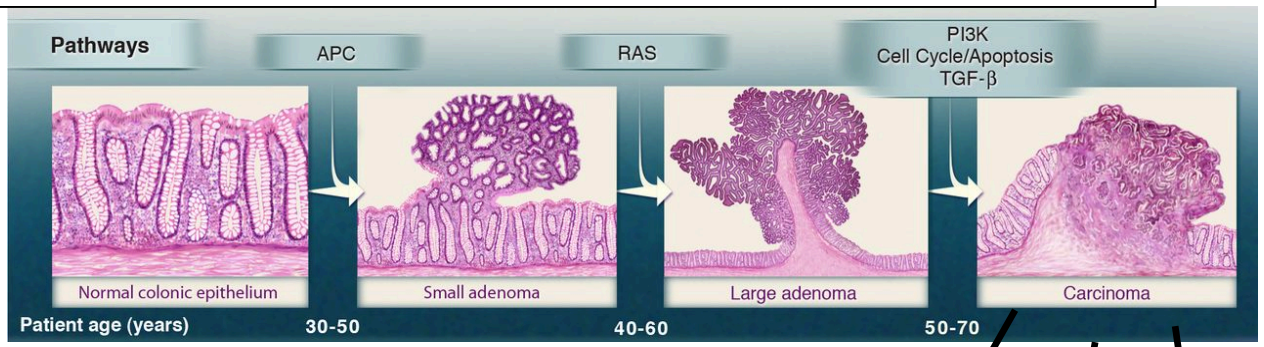


FEEDING FRENZY - but concerns at many levels

How many companion diagnostics??? **Science also says "A LOT"**

Science
AAAS

B Vogelstein et al.
Science 2013;
339:1546-1558



'MULTI – HIT' process

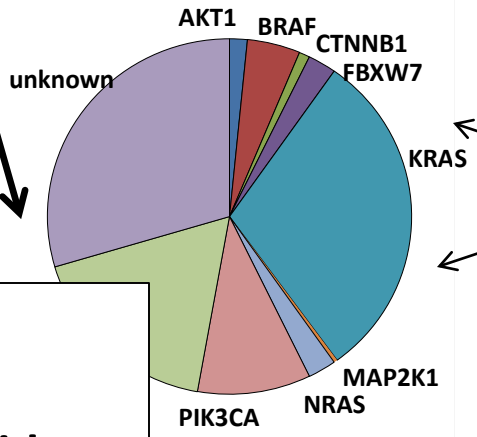
COMPANION DIAGNOSTICS
detect these 'target'molecules

HOW WILL WE DO IT?

IHC
FISH
PCR
NGS

ANY WAY WE CAN!!
Immunohistochemistry
Fluorescent in situ hybrid
Polymerase chain reaction
Next generation sequencing

Molecular classification of colon adenocarcinoma



Different cases of colon cancer

different mutated 'driver' genes

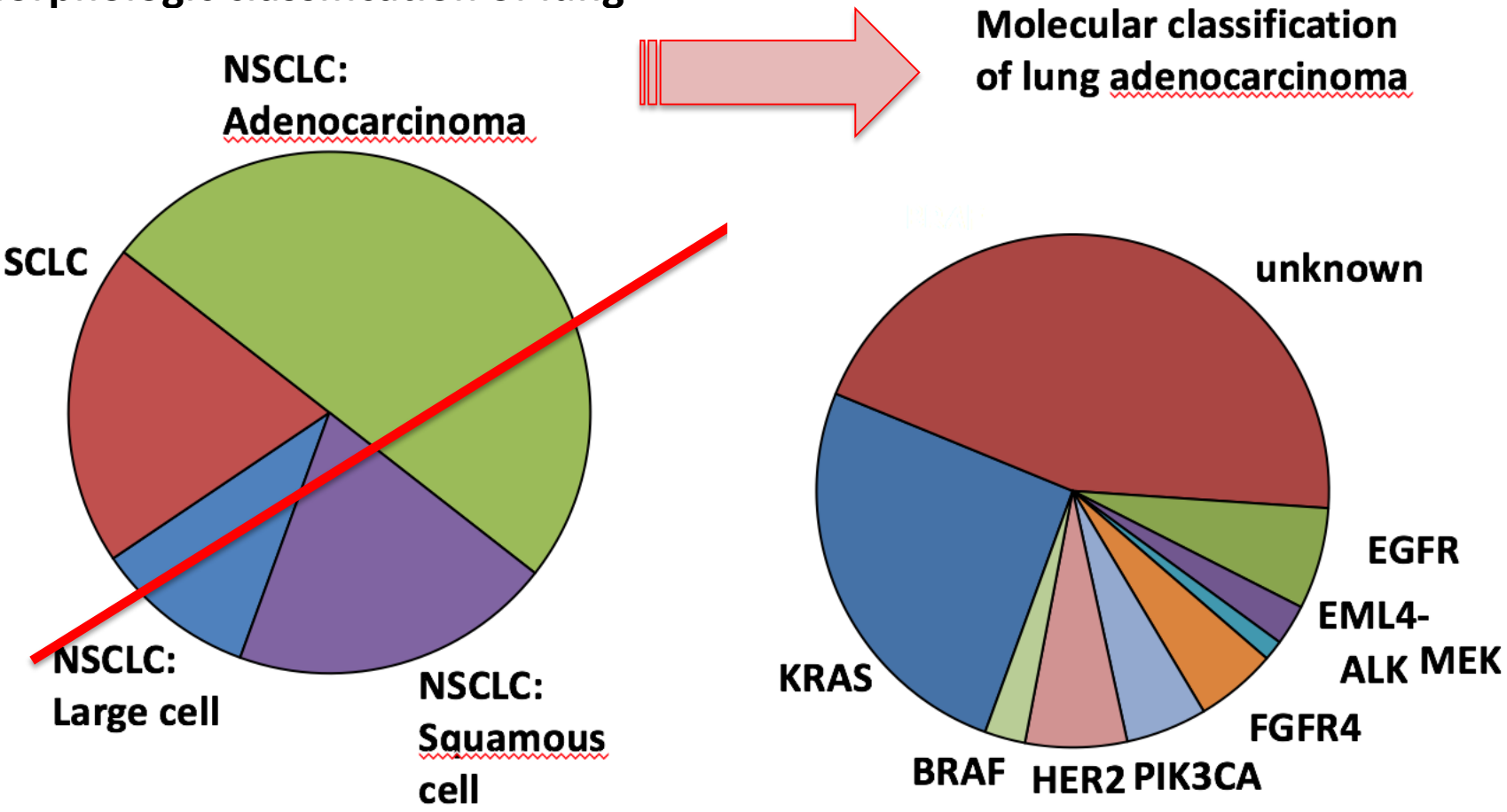
Different molecules targets for therapy

As a result - the role of pathology has changed

YEAR 2010 **4 types**

Morphologic classification of lung

TODAY **10+ types**



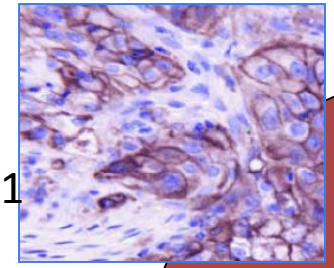
~~SCLC~~

~~NSCLC: Adenocarcinoma~~

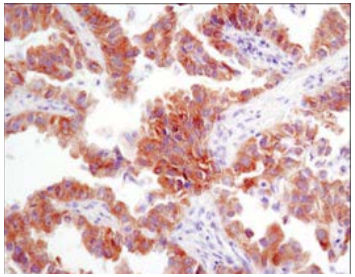
~~NSCLC: Squamous cell~~

~~NSCLC: Large cell~~

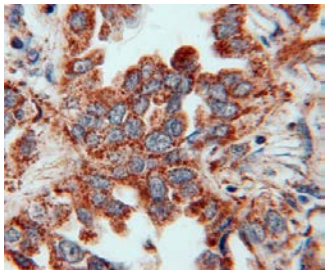
CANNOT do this by H&E
Thus in lung cancer alone –
Many Companion Dxs needed for molecular classification.



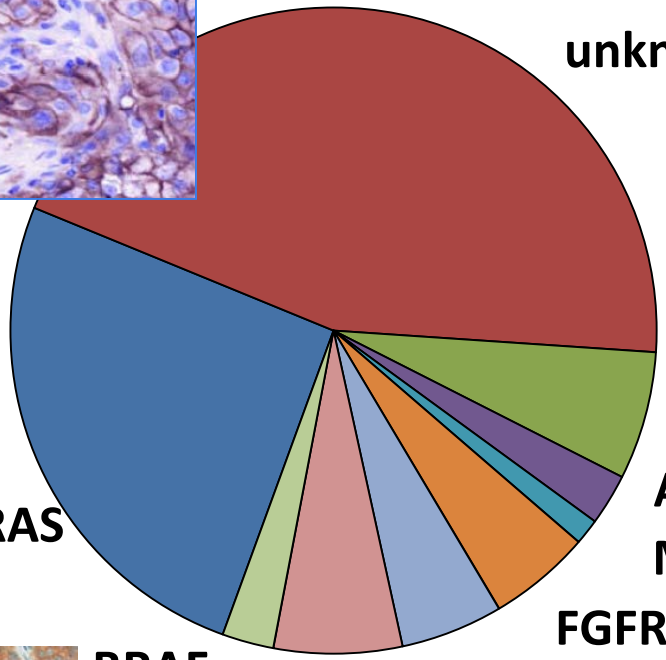
PD-L1

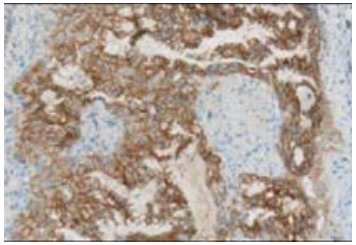


ALK

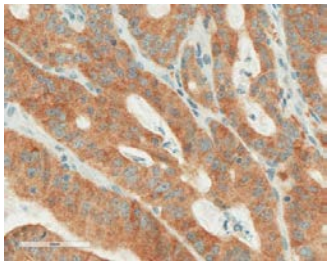


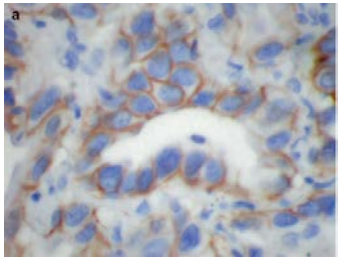
KRAS





MET





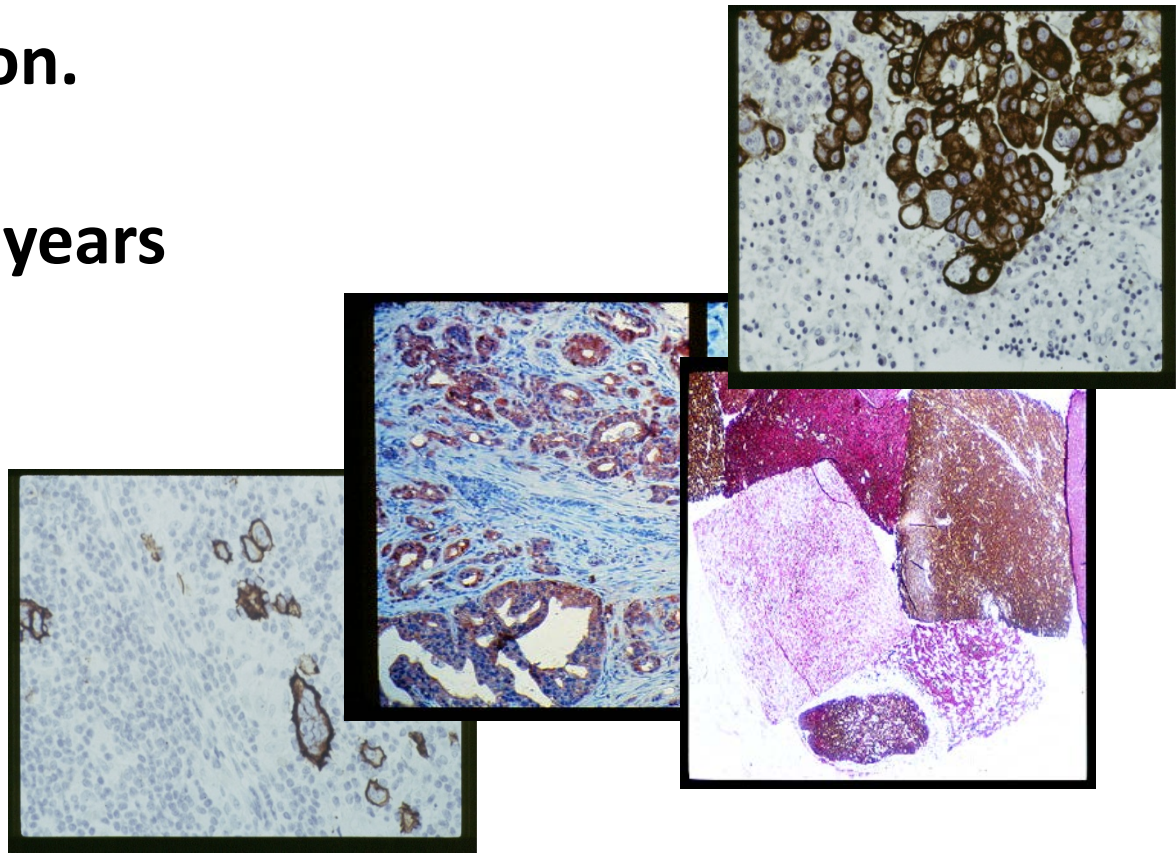
Proteintech, Ventana, Dako, Biosource, CRT

Gu, Taylor
AIMM 2014

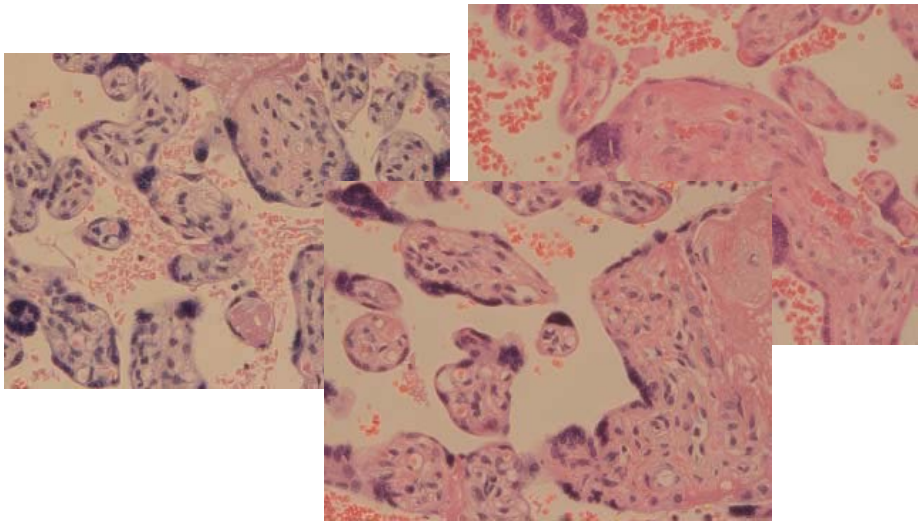
Up to this time IHC was used to produce 100 s of 'special stains' on FFPE tissues

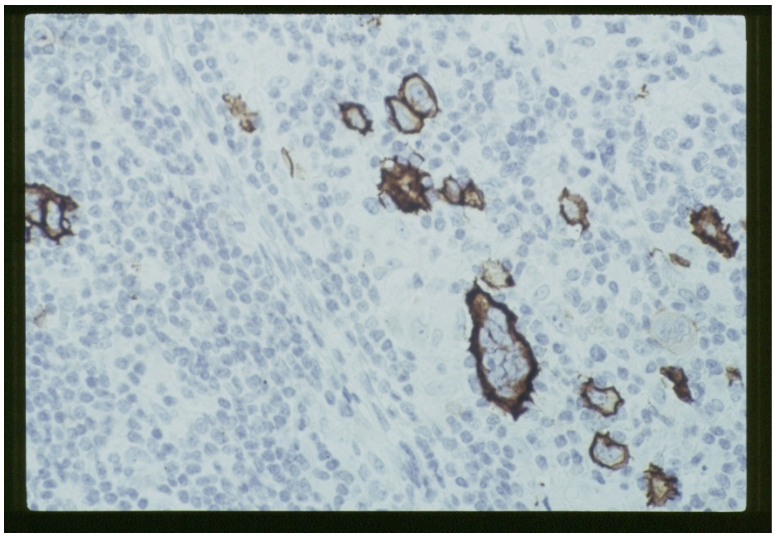
Same rationale as for any other stain, --to produce a different color to assist cell / tissue recognition.

But over the past 100 years this approach has produced some very bad habits



Routine stain H & E **'Special Stain' - IHC CD30**





No controls

The result is adjusted to 'please the pathologist'

MUST have positive and negative controls

Should **not** be adjusted to 'please the pathologist'

REPRODUCIBILITY IS POOR

run to run
day to day
lab to lab

Result

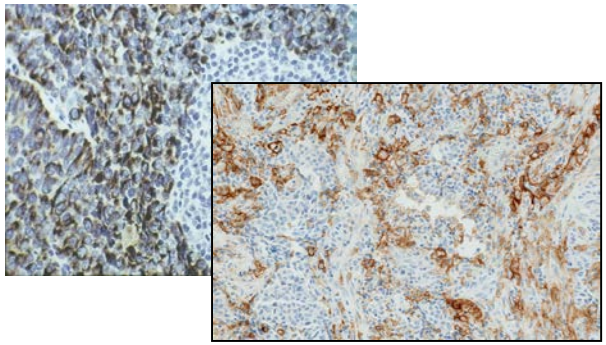
- IHC quality poor and variable

--- quantification not possible

So - what is the problem?

IHC detects targeted protein –

BUT - IHC is just a stain -

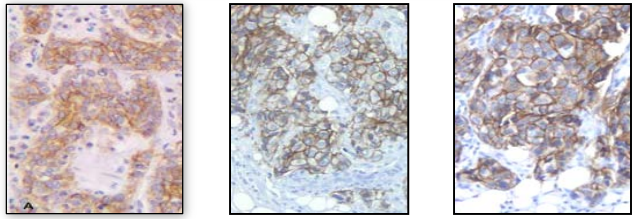


Can we achieve
--- consistency and the quality
to turn IHC into a
'quantitative' assay??

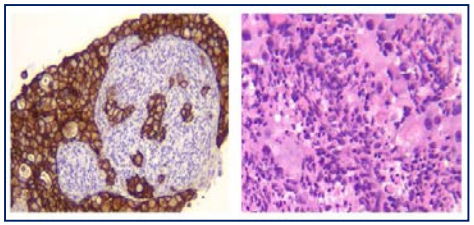
To convert a 'stain' to an 'assay'
Validation & Controls & interpretation must be more rigorous

Her2 result – current

+ *++* *+++*



Her2 result - quantitative
mean value surface expression
100 1000 10,000
attograms/cancer cell



In Situ Proteomics – ISP
Measuring protein per cell

Our IHC Problem

IHC

Right now IHC has elements of witchcraft -
-labs 'do their own thing'

1. Require a detailed strict protocol with controls

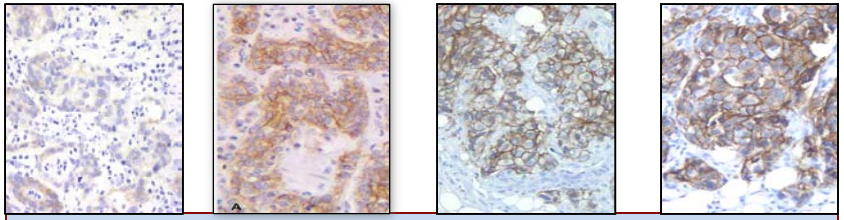
Can we achieve
-improved quality?
-true quantification?

2. Require that we follow the protocol exactly

3. Require BETTER controls to assure that we are doing it

The problem is in detail---
Can we 'control' the UNCONTROLLED VARIABLES?

"scoring" Predictive Markers is crude



0, 1+, 2+ 3+

IS THE SCORE 'REAL' ??

Is the variation 'real' = biology?
Or is it due to --

1. **'Poor sample preparation' - variable fixation**
2. **Variable section thickness**
3. **Variable IHC/AR Protocol / different labs**
4. **Variable chromogen development**
5. **Section heterogeneity**
6. **Variation in pathologist scoring / subjective**

Many 'uncontrolled' assay variables
How can we improve??
Better controls would be a good start

Taylor, Becker, AIMM 2011
Taylor AIMM 2014, CTR 2015

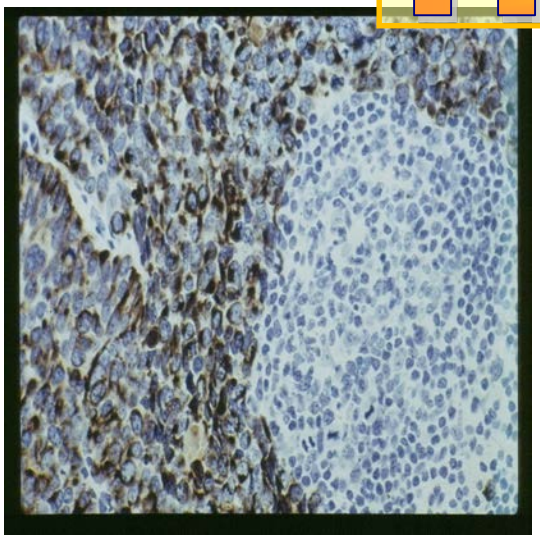
Part Solution ---- turn the Anatomic Pathology lab into a Clinical Lab

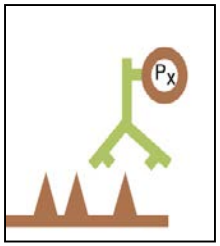
Clinical Lab
- Highly automated
- Strict protocols
- Validated reagents
- Rigorously controlled
- Universal reference standards

Regular AP lab
- none of these

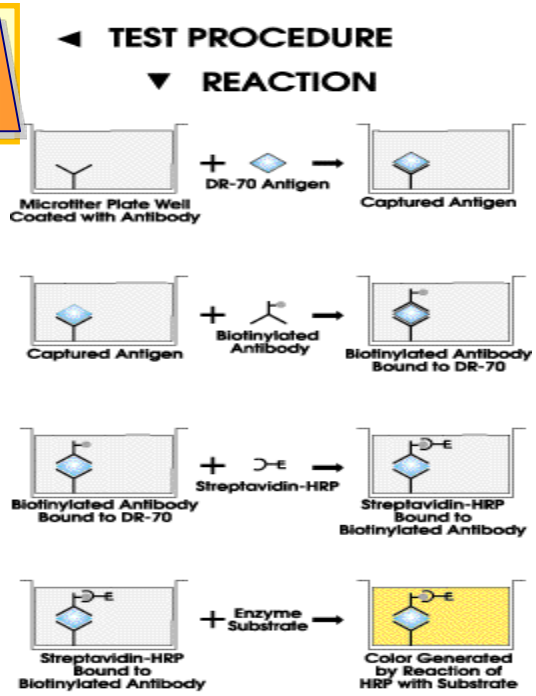
Multiple manual steps of IHC 'stain' are difficult to reproduce manually

I H C = ELISA





SAME REAGENTS
SAME PRINCIPLES



Immunohistochemistry = Enzyme Linked ImmunoSorbent Assay

Sample prep uncontrolled	←	Sample preparation controlled
Partly automated	←	Fully automated
No universal reference standard	←	Universal reference standard
Poor reproducibility	= = = = = = →	**Excellent reproducibility**
Not quantifiable	= = = = = = →	**Strictly quantitative**

True we cannot control everything – but there are some possible approaches to improvement of IHC

The model - Convert IHC to an ELISA type approach on tissue
- turn a 'qualitative stain' into a 'quantitative immunoassay'

1. **Consider all phases of IHC - THE TOTAL TEST**
2. **PRE-ANALYTIC - Control or Qualify Sample Preparation**
3. **ANALYTIC - Use same control materials in all IHC labs**
4. **ANALYTIC - Produce a Quantifiable Reference Standard for calibration**
5. **POST–ANALYTIC - Score Predictive Markers by digital analysis**

Within Lab and from Lab to Lab - Sample Preparation one of the biggest problems

PRE-ANALYTIC VARIABLES.

WARM ISCHEMIA – surgery, vessels clamped

COLD ISCHEMIA - (transport, fix?, gross schedule)

GROSSING – block size – penetration reagents

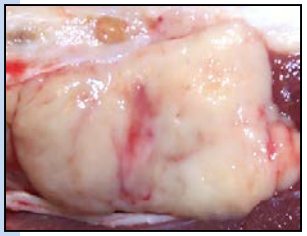
FIXATION – type, (formalin) freshness, pH, TOTAL TIME

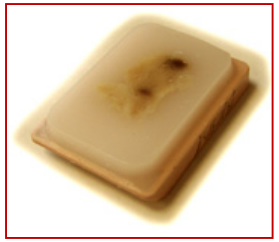
PROCESSING, - alcohol stages, xylol (TIME) paraffin temp

STORAGE - as block

CUTTING – thickness, evenness, tears

TIME LAPSE to staining

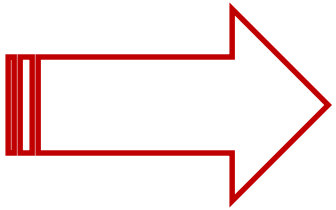




THE TOTAL TEST Standardization & Quantification in IHC
The Road to In Situ Proteomics.

Immunohistochemistry (IHC) to in situ proteomics (ISP)

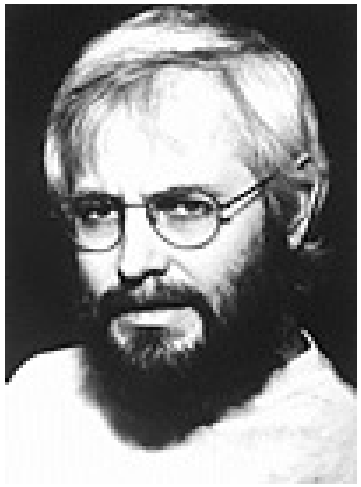
a 'stain' → a measurement



STANDARDIZE & CONTROL EVERYTHING

Pre-analytic	Sample acquisition Fixation, processing, cutting
Analytic	Retrieval Reagents, Protocols, Basic controls,
Post-analytic	Interpretation, scoring Reporting

Analytic - No Shortage of Reagents



Max Planck Institute For Immunobiology, Freiburg.

Georges J. F. Köhler César Milstein
Nobel Prize in Physiology and Medicine
1984

HYBRIDOMAS

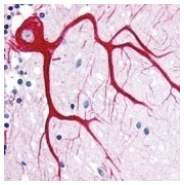
Monoclonal antibodies

British medical Council Laboratory for Molecular Biology, Cambridge

Or detection methods
Or retrieval methods
Or automated platforms
Or opinions

But there is a shortage of VALIDATION : including lack of validated controls

Huge number of antibodies AVAILABLE



But - great VARIATION AMONG ANTIBODIES

ADVERTISEMENTS

"XXXX Abs Inc" (USA) has increased the number of validated IHC antibodies available in its catalog to more than **3,500. IHC antibodies**.
- extensively tested
against formalin-fixed paraffin-embedded (FFPE)human tissues.

Immunohistochemistry
can detect any protein encoded by the 21,000 genes in the human genome."

Catalog includes
83,400 monoclonal and polyclonal Abs to 13,000 targets.
USA

IHC Collection– "YYYY Abs Inc" (Taiwan) IHC collection of
8600+ antibodies targeting human genes,
(tissue microarray for novel biomarker discovery),
and 400+ antibodies in **Pathology** research.

NordiQC & UK data - NO SHORTAGE OF PROTOCOLS

UK NEQAS SURVEY RUNS

Run 96 - 365 participants	Run 97 - 365 participants
Markers SMA , CK	Markers SMA , CD34/CD31
Retrieval Heat - 297 labs; 76% acceptable results Enzymatic - 146 labs; 32% acceptable Retrieval reagents Mostly pH6 or 9	**Retrieval** Heat - 336 labs; 83% acceptable results Enzymatic - 32 labs; 29% acceptable Retrieval reagents Mostly pH6 or 9
Primary Antibodies* SMA – 18 antibodies from 10 suppliers CK – 26 antibodies from 16 suppliers	**Primary Antibodies*** SMA – 20 antibodies from 9 suppliers CD34/CD31 – 25 antibodies,11 suppliers
Detection Reagents 26 different detection reagents from 13 suppliers	**Detection Reagents** 23 different detection reagents from 11 suppliers
Autostainers 17 different instruments from 7 suppliers	**Autostainers** 17 different instruments from 7 suppliers
Chromogen+ Great majority used DAB from 19 suppliers	**Chromogen+** Great majority used DAB from 11 suppliers

What about the controls??

CONTROLS

To assure quality

MORE STANDARD CONTROLS

INTERPRETATION

AUTOMATION HELPS ACHIEVE THIS

PROTOCOL

- **Fixation**
- **Primary ab**
- **Secondary ab**
- **Label**
- **Chromogen**
- **Method**
- **AR**
- **Background**

National Institute of Standards & Technology Reference Standard (control) - Requirements

Table 5 Summary of required characteristics of any reference standard that would provide a basis for accurate quantification of IHC on FFPE tissue

Immunohistochemical reference standard: requirements
It must be subjected to all of the same rigors of sample preparation (ischemia, transport, fixation) as the "test" tissue
It must be integrated into all steps of the test (assay) protocol, including evaluation of the result
It should contain a known amount of the reference standard protein
It should be universally available to all laboratories performing the assay
It should be inexhaustible and inexpensive

Taylor CR. **Quantitative In Situ Proteomics--**Cell Tissue Res. 2015; 360:109-120.

So what are the possibilities???

‘in house’ control tissue (block)

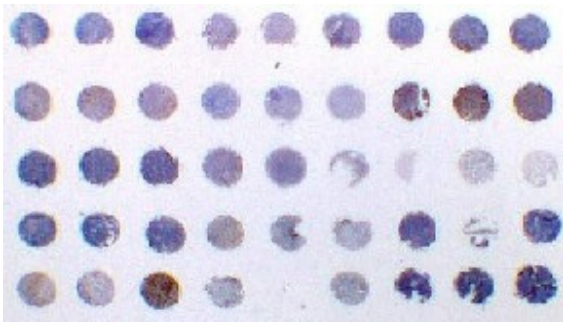
–is in fact an ‘external’ tissue control

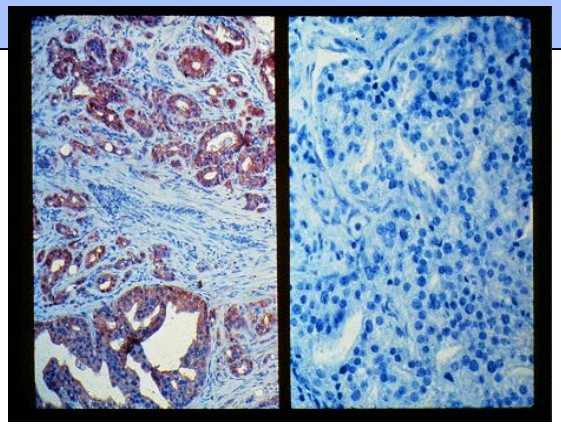
-- has similar but not same FFPE as test tissue

-- a finite amount of control tissue block(s)

- so a lab must make **new controls all the ti**
- and **every lab in fact has different control**

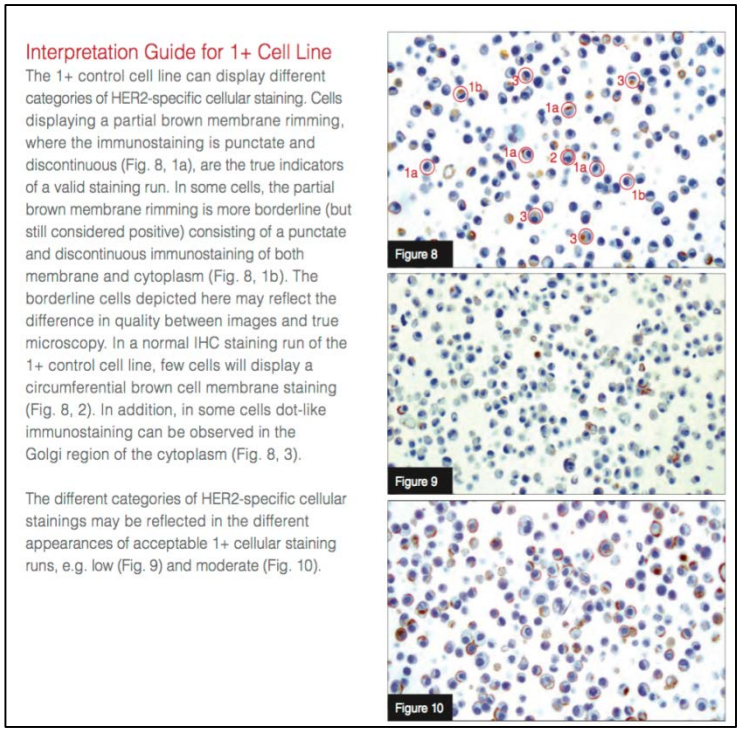
-- therefore not quantifiable





1998. HercepTest – included
Cell lines as ‘RUN’ controls
To assure greater consistency
- in labs
- and among labs

POTENTIAL NEW CONTROLS
Cell line controls should be validated in the context of their use
Note – do not control pre-analytic phase



Interpretation Guide for 1+ Cell Line

The 1+ control cell line can display different categories of HER2-specific cellular staining. Cells displaying a partial brown membrane rimming, where the immunostaining is punctate and discontinuous (Fig. 8, 1a), are the true indicators of a valid staining run. In some cells, the partial brown membrane rimming is more borderline (but still considered positive) consisting of a punctate and discontinuous immunostaining of both membrane and cytoplasm (Fig. 8, 1b). The borderline cells depicted here may reflect the difference in quality between images and true microscopy. In a normal IHC staining run of the 1+ control cell line, few cells will display a circumferential brown cell membrane staining (Fig. 8, 2). In addition, in some cells dot-like immunostaining can be observed in the Golgi region of the cytoplasm (Fig. 8, 3).

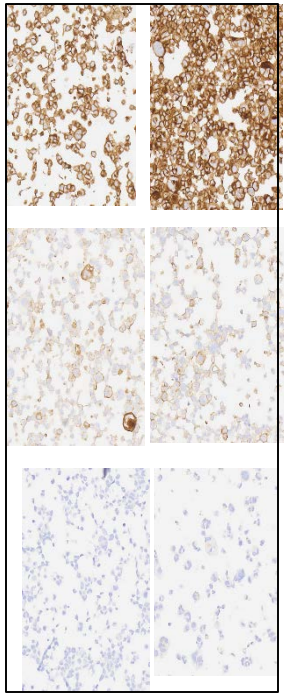
The different categories of HER2-specific cellular stainings may be reflected in the different appearances of acceptable 1+ cellular staining runs, e.g. low (Fig. 9) and moderate (Fig. 10).

Figure 8

Figure 9

Figure 10

HercepTest
Interpretation manual
Dako



Bioengineered Cell lines. PDL-1 by IHC

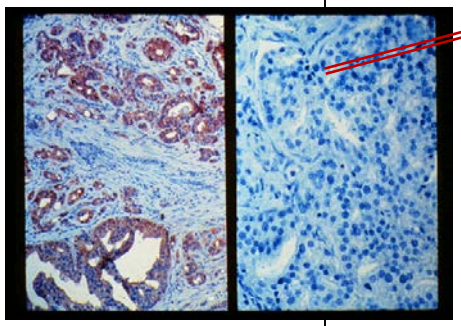
High

medium

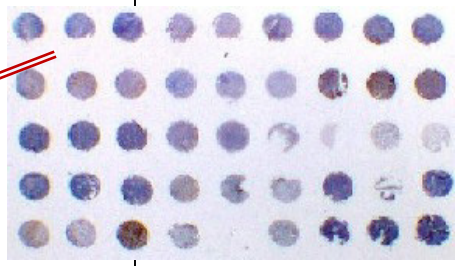
neg

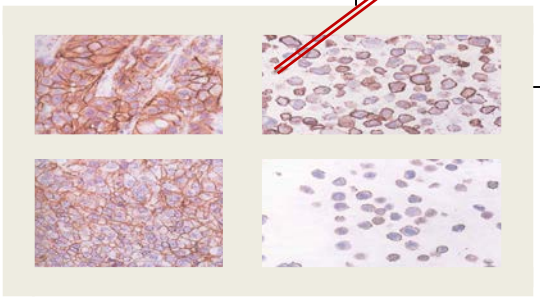
Courtesy Farah Patell-Socha
Horizon Discovery,
Cambridge, UK.

WHAT CHOICES do we have? -- Existing types of 'controls'

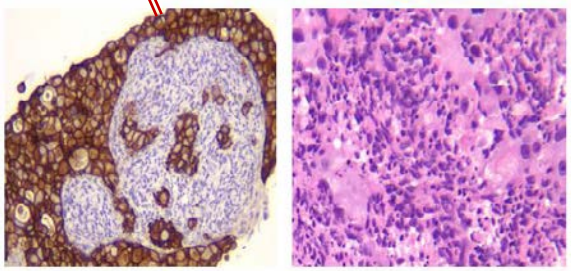


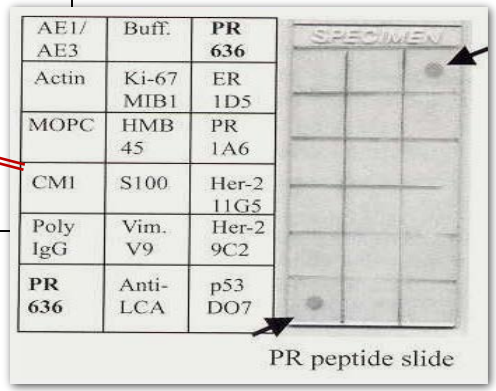
Tissue block - 'known'
Sausage block
Micro-tissue array
Cell line cytoprep.
Cell line block
Peptide 'dots'
Faux Tissue





(Rhodes A, et al.
AJCP 118:408-417,2002)





(Sompuram SR, et al.
J. Histochem. Cytochem.
50:1425-1433,2002)

All provide some control of assay
--- But limited control of sample prep
--- Cell lines, and 'spots' –potentially quantify

Histoid MC7 + FSF

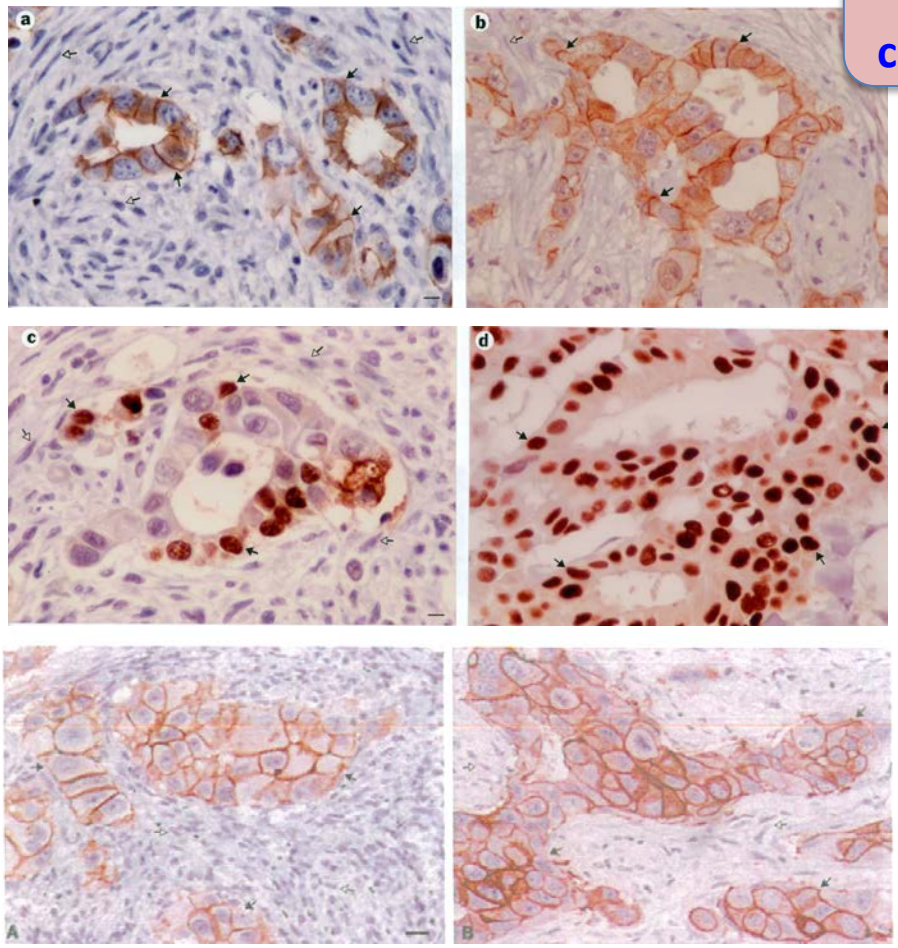
Breast ca section

POTENTIAL NEW CONTROLS
Improved validated cell line controls – retaining morphology

E cadherin

ER

HER 2 Ki-67



3D. FAUX TISSUE
Mimics morphology
Potentially -
-- Quantifiable
-- universal

Courtesy Dr A Imam
StatLabs, Texas.

Journal of Histochemistry & Cytochemistry 59(12) 1087–1100
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DOI: 10.1369/0022155411423680
http://jhc.sagepub.com
SAGE

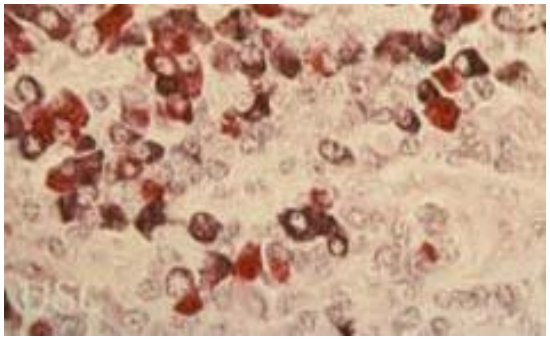
JHC

Human Breast Cancer Histoid: An In Vitro 3-Dimensional Co-culture Model That Mimics Breast Cancer Tissue

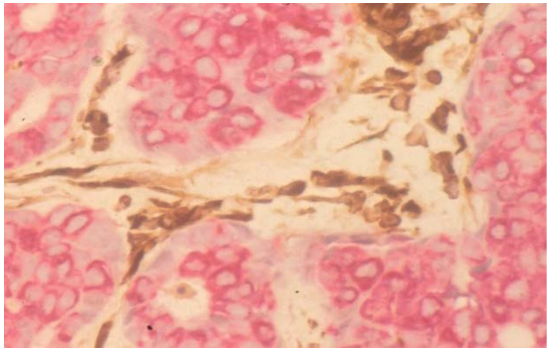
Pavinder Kaur, Brenda Ward, Baisakhi Saha, Lillian Young, Susan Groshen, Geza Techy, Yani Lu, Roscoe Atkinson, Clive R. Taylor, Marylou Ingram, and S. Ashraf Imam

Molecular Pathology Program (PK,BS,SAI) and In Vitro System/Tissue Engineering Program (BW,GT,MI), Huntington Medical Research Institutes, Pasadena, California, Departments of Pathology (LY,RA,CRT) and Preventive Medicine (SG), University of Southern California Keck School of Medicine, Los Angeles, California, and Department of Population Sciences (YL), City of Hope, Duarte, California

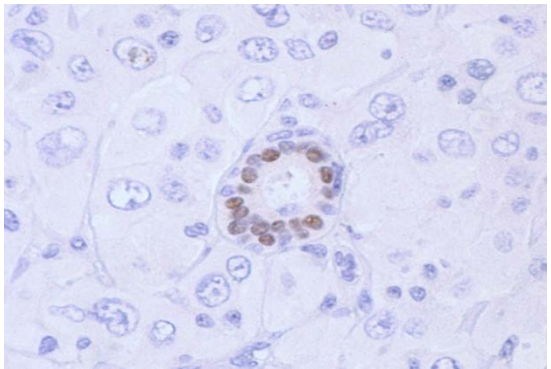
POTENTIAL NEW CONTROLS
Tissue Internal Controls have been used for years to inform on 'quality' – but we can do better



Plasma cells Ig , K, L
Internal controls
Taylor and Burns, 1974



VIMENTIN
Used as fixation guide
Batttifora et al



Estrogen Receptor

ER on residual normal breast

Serves as internal fixation and method control

Taylor
AIMM 2014

Applied Immunohistochemistry and Molecular Morphology.
The Control series: how to optimize use of current controls

Torlakovic et al. **Standardization of Negative Controls in Diagnostic Immunohistochemistry:** Recommendations from the International Ad Hoc Committee. Applied Immunohistochem Mol Morph. 2014; 22:241-252.

Torlakovic et al. **Standardization of Positive Controls** ----- in Diagnostic Immunohistochemistry: Recommendations from the International Ad Hoc Committee. Applied Immunohistochem Mol Morph. 2015; 23:1-18.

Cheung. CC et al. **Evolution of Quality Assurance of Clinical Immunohistochemistry in the Era of Precision Medicine - Part 1: Fit-for-purpose Approach to Classification of Clinical Immunohistochemistry Tests**. Applied Immunohistochem Mol Morph.2017; 25: 4-11. Online.Publish ahead of print.

Torlakovics et al. **Evolution of Quality Assurance of Clinical Immunohistochemistry in the Era of Precision Medicine - Part 2: Immunohistochemistry Test Performance Characteristics.** Applied Immunohistochem Mol Morph.2017; 25: 79-85. Online.Publish ahead of print.

Torlakovics et al. **Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine - Part 3: Technical Validation of Immunohistochemistry (IHC) Assays in Clinical IHC Laboratories.** From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path). Applied Immunohistochem Mol Morph.2017;25:151-159.. Online Publish ahead of print.

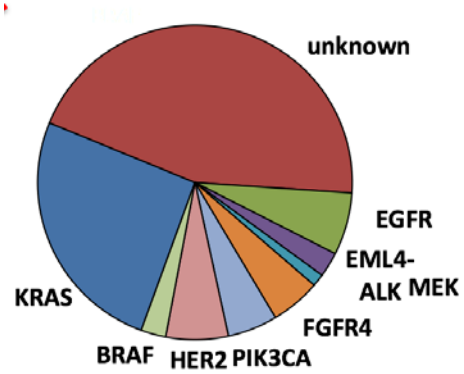
Cheung, Torlakovics et al.. **Evolution of Quality Assurance for Clinical Immunohistochemistry in the Era of Precision Medicine - Part 4: Tissue Tools for Quality As Immunohistochemistry**. From the International Society for Immunohistochemistry and Molecular Morphology (ISIMM) and International Quality Network for Pathology (IQN Path). Applied Immunohistochem Mol Morph. 2017; 25: 227-230. Online. Publish ahead of print.

Cheung CC, Taylor CR, Torlakovics EE. **Audit of Failed Immunohistochemical Slides in the Clinical Laboratory: The Role of On-Slide Controls**. Applied Immunohistochem Mol Morph. 2017; 25: 308-312. Online Nov 2015

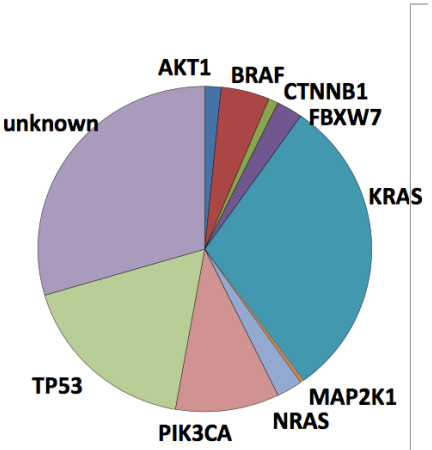
Torlakovic et al. **Getting controls under control - the time is now for immunohistochemistry.** J Clin Path. 2015: 0; 1-4Online 10.1136/jclinpath- 2014-202705

Having improved the IHC method - the biggest challenge remains - performing multiple Companion Diagnostics for many (all) cancers and SCORING THEM

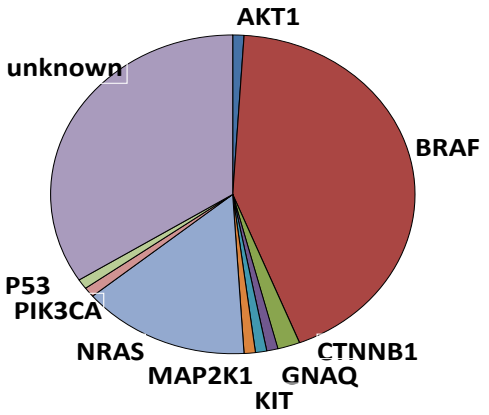
Molecular classification of lung adenocarcinoma



Molecular classification of colon adenocarcinoma



Molecular classification of melanoma



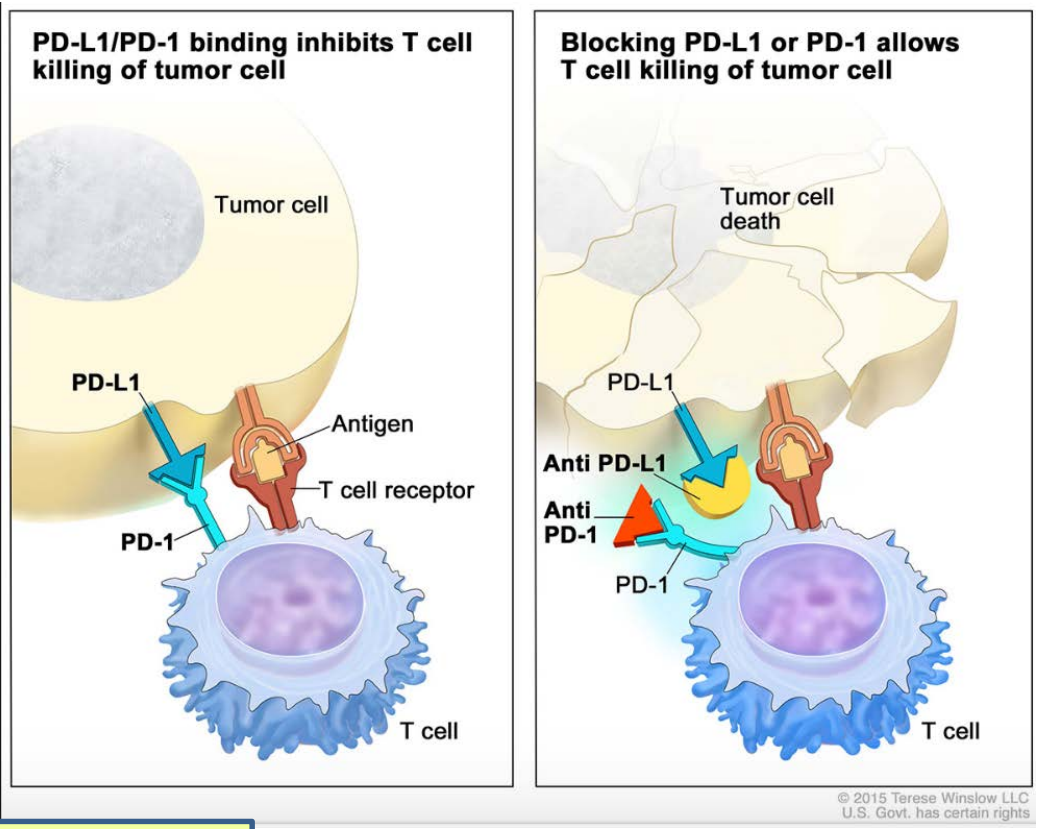
COMPANION DIAGNOSTICS HOW WILL WE DO IT?

IHC	Immunohistochemistry – retains morphologic cell ID
FISH	-----which is lost in
PCR	Polymerase chain reaction
NGS	Next generation sequencing

--- it is not just the expressed proteins
---- but also Immune cells
- lymphocytes/macrophages and their activation

Gu, Taylor
Applied Immunohistochem Mol Morphology Jan, 2014

MANY different cancers
Many different drugs
EACH REQUIRING DIFFERENT
approved TEST
- or different LDT



PD-1 inhibitors: Examples of drugs that target PD-1 include:

- **Pembrolizumab (Keytruda)**
- **Nivolumab (Opdivo)**

Melanoma, NSC lung cancer, colon cancer,
Kidney, bladder, head and neck cancer
Hodgkin and NH lymphomas

PD-L1 inhibitors: Examples of drugs that target PD-L1 include:

- **Atezolizumab (Tecentriq)**
- **Avelumab (Bavencio)**
- **Durvalumab (Imfinzi)**

HUGE PROBLEM for LABS

Assessment for Targeted Therapy Testing in Cancer: Urgent Need For Realistic Economic and Practice Expectations.

Yaziji, Taylor AIMM 2017

IN US approved Class III IHC based tests

AIMM September, 23;541 2015
Open Access
Applied Immunohistochem Mol Morph

RESEARCH ARTICLE

OPEN

Development of an Automated PD-L1 Immunohistochemistry (IHC) Assay for Non–Small Cell Lung Cancer

Therese Phillips, MA, Pauline Simmons, BS,* Hector D. Inzunza, MD, PhD,† John Cogswell, PhD,† James Novotny, Jr, PhD,† Clive Taylor, MD, PhD,‡ and Xiaoling Zhang, PhD**

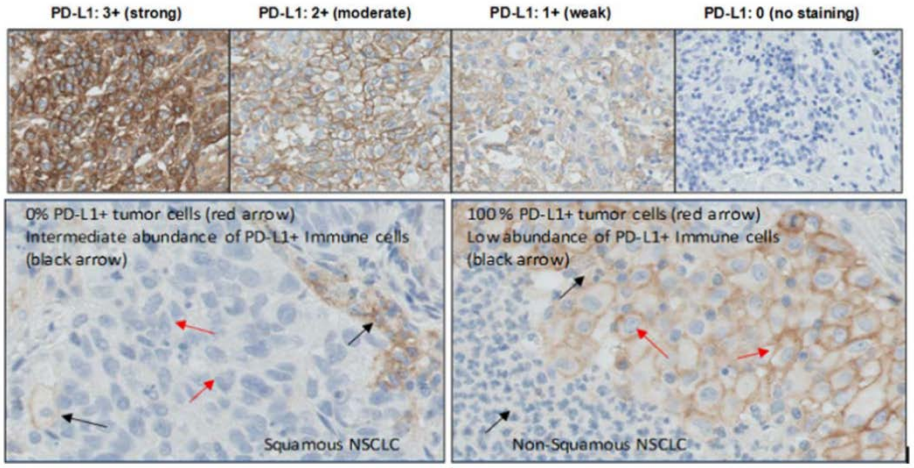


FIGURE 1. Positive PD-L1 membrane staining in NSCLC tumor tissues illustrating intensity grades (top, ×20) and PD-L1 tumor scores (bottom, ×40). NSCLC indicates non–small cell lung cancer; PD-L1, programmed cell death 1 ligand 1.

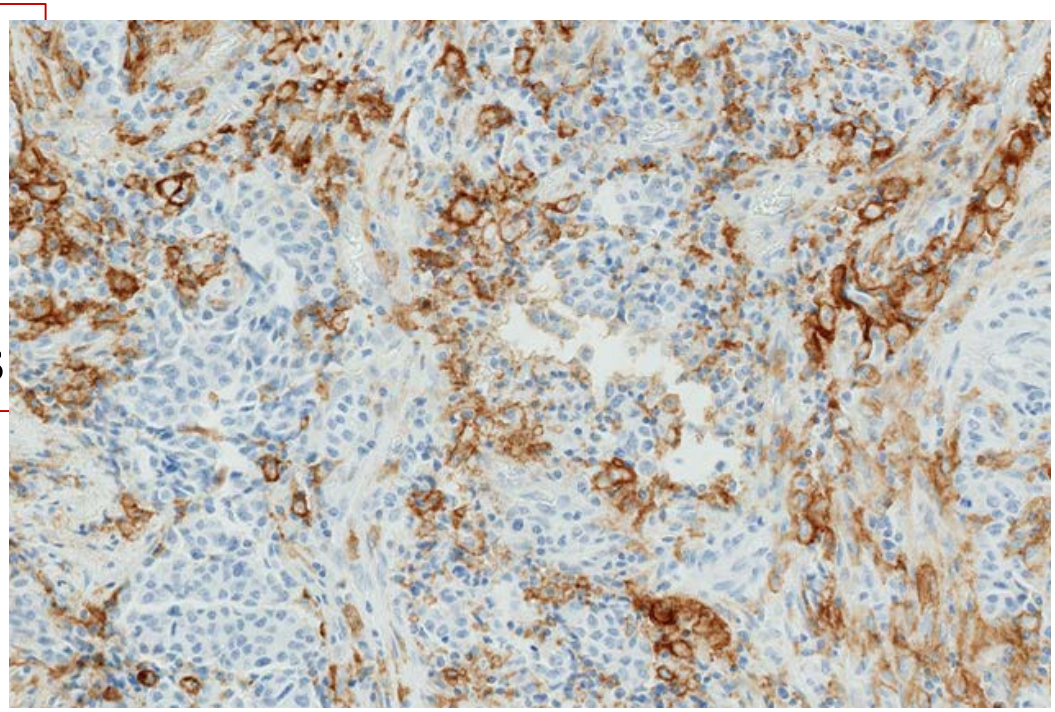
An approved Companion DX test – is validated vs clinical outcome

An FDA approved PDL-1 assay requires -
Validated method, reagents, controls & scoring – (manual in this case).

Challenges -
--Identification and scoring of cancer cells
--in some tests - Identification and scoring of immune cells
--Reproducibility

Look at the problem in the context of PD-L1

Many different antibodies
Many different approved tests



‘scoring systems’ - very complex

-differs among tests
-Is a cell positive?– threshold intensity
-Score Percentage positive?
--Semi-quantitative at best
-May include other difficult tasks
such as **presence of immune cells**

Scoring system - MUST be reproducible

First problem – percentage requires 'counting' the number of cancer cells that show 'positive staining' & TOTAL cancer cells by eye

X 4.
diam 5mm

X 10.
diam 2.0mm

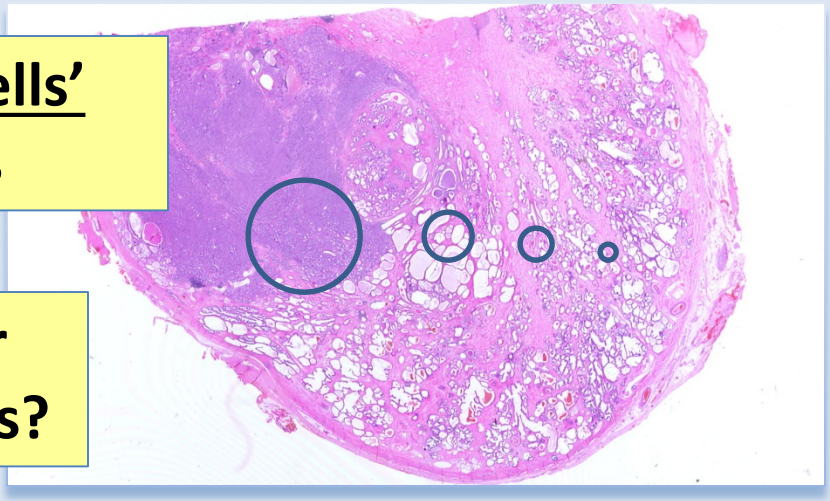
X 20.
diam 1 mm

X 40.
diam 0.5mm = 500µm

tissue (or whole slide image –WSI) 3 x 2 cms.

% = 'positive cancer cells' / total cancer cells

We put down a number -but really we just guess?



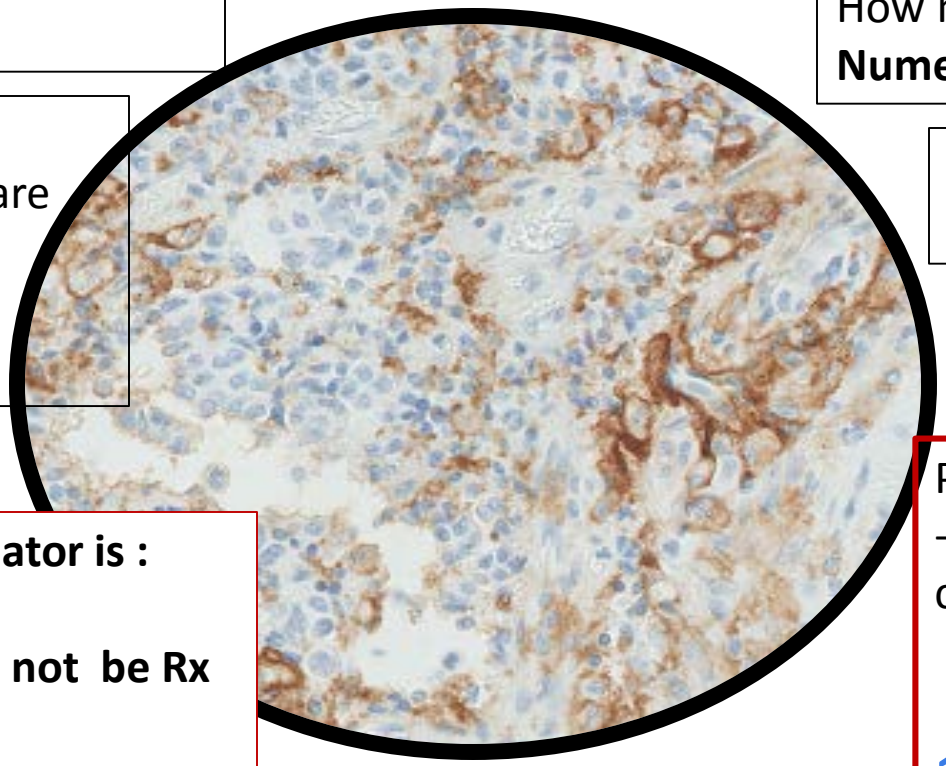
How many cancer cells per section? - --- up to 2,000,000 total cells per section
or per field (magnification)? - calculate πr^2 if tumor cell 20u diameter
then = 10,000 tumor cells per x 10 field
= 600 cells per x 40 field (varying with cell size, mix of tumor versus stroma)

What is the 'score'?
PD-L1 Threshold - 5%
Does the patient get treated or not?

ONE HIGH POWER X40 FIELD
Percentage positive = numerator: +ve cancer cells
denominator: total ca cells

How many total cancer cells
Denominator ?

Cannot count – so estimate that half are cancer cells ?
- 600 X ½
-- about 300



How many positive Ca cells?
Numerator ?

% = positive cell count / 300

But IF the denominator is :
- 330 (not 300)
- then '15' should not be Rx
or if 270
– then 14 should be Rx

But note -- we have only 'scored' 600 cells among maybe 2,000,000
or < 0.0003%

PDL-1 membrane stain
-- so count the positive cancer cells
15 cells= 5 % threshold

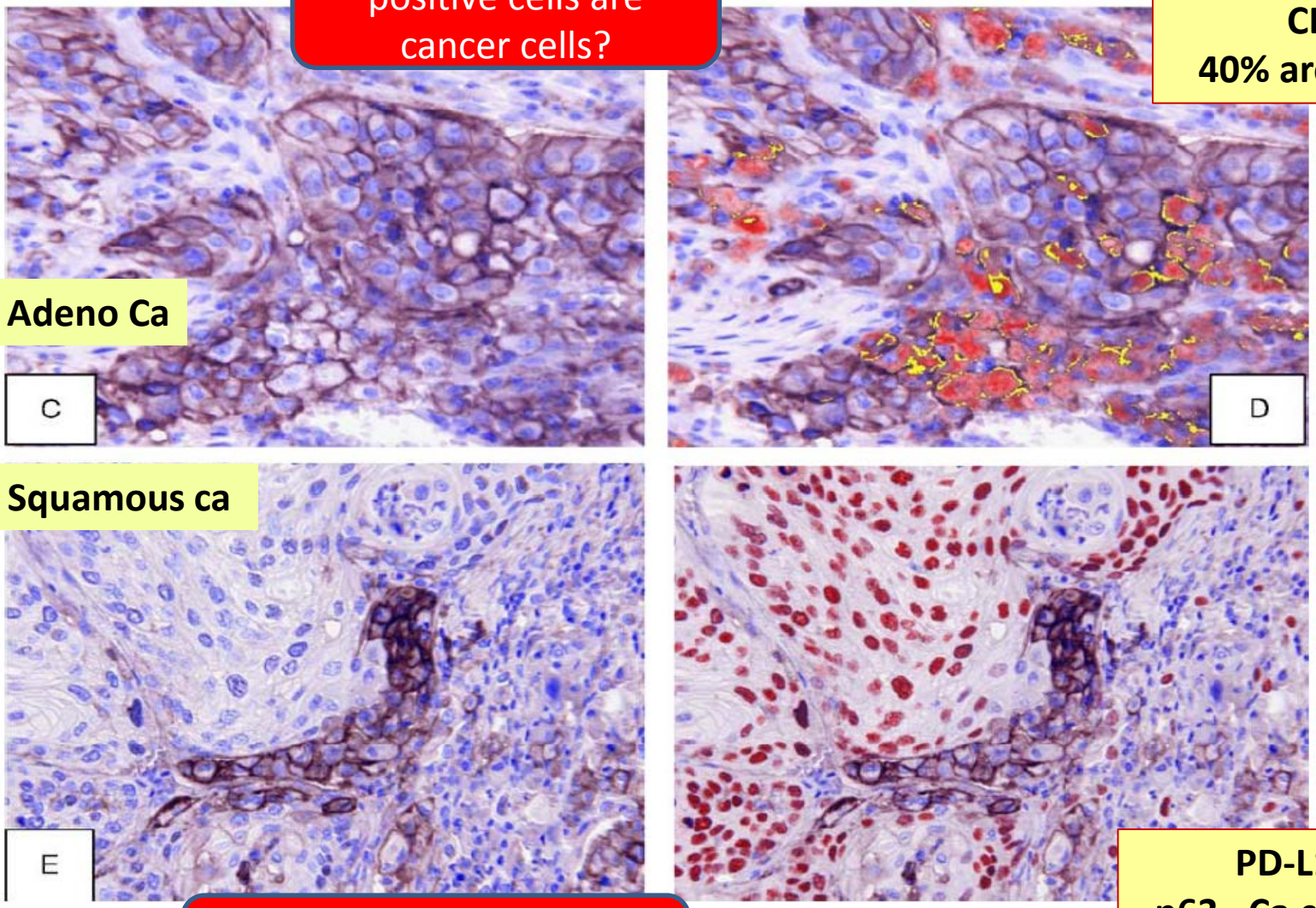
14 -no treatment
15 -- $100,000 Rx

Second problem- distinguish cancer cell from immune cells - by eye

How many of the positive cells are cancer cells?

PD-L1 - brown
CD 68 – red
40% are macrophages

Adeno Ca



Squamous ca

Is this a positive test?
>1%? > 5% ?

From Taylor AIMM 2014

PD-L1 - brown
p63– Ca cell nuclei - red
Negative test
Positive Ca cells = 0

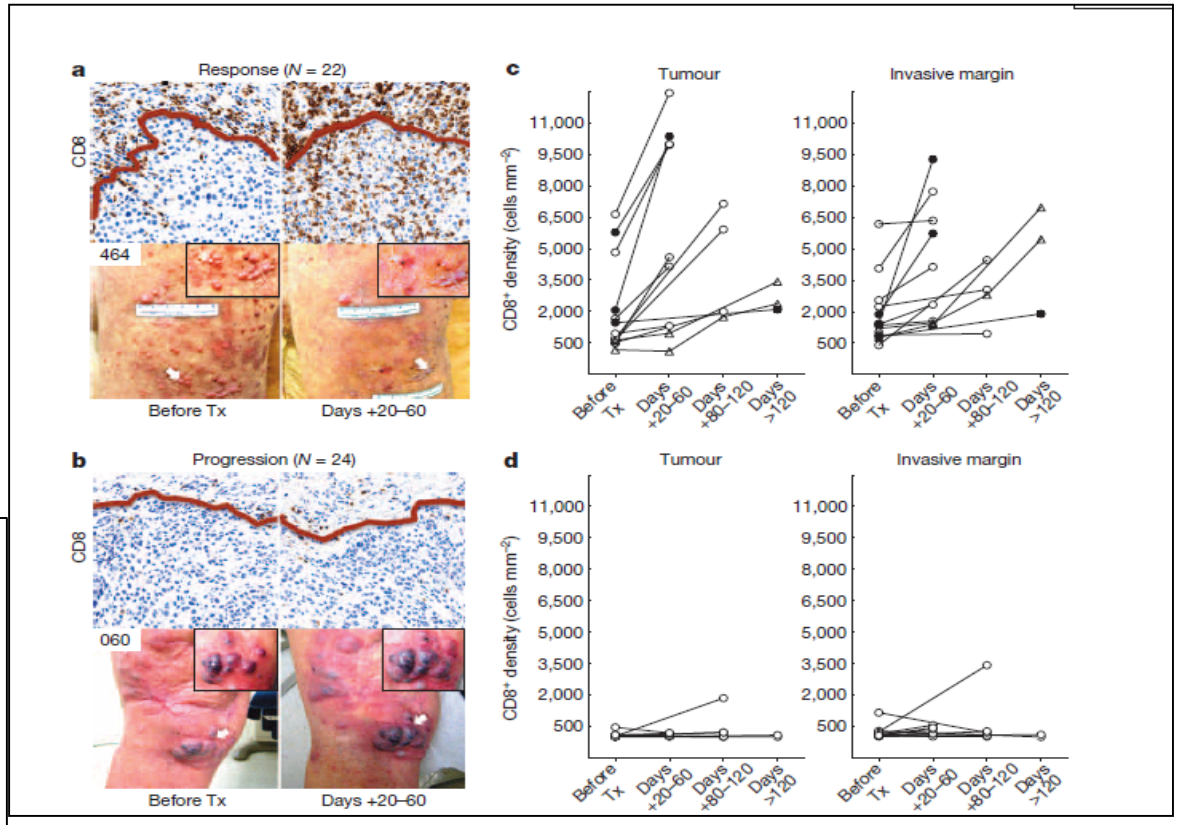
Third problem
- also need to evaluate immune cells by type and number

Cases with CD8 cells do well with PD-L1 Rx

Identify immune cells by phenotype

determine location in relation to tumor

How do we detect them?
MULTIPLEX IHC is effective
Include fluorescent methods
gives DIRECT INFORMATION



Tissue Extract methods
LOSE SPATIAL information

NGS **RNA**

PCR **Proteomics**

Tumeh P et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515 568 2014

Leads to notion of two categories of cancer

-- require very different therapeutic approaches

Immunogenic
'inflamed'
Response suppressed

non immunogenic
'silent'
Immune cells absent

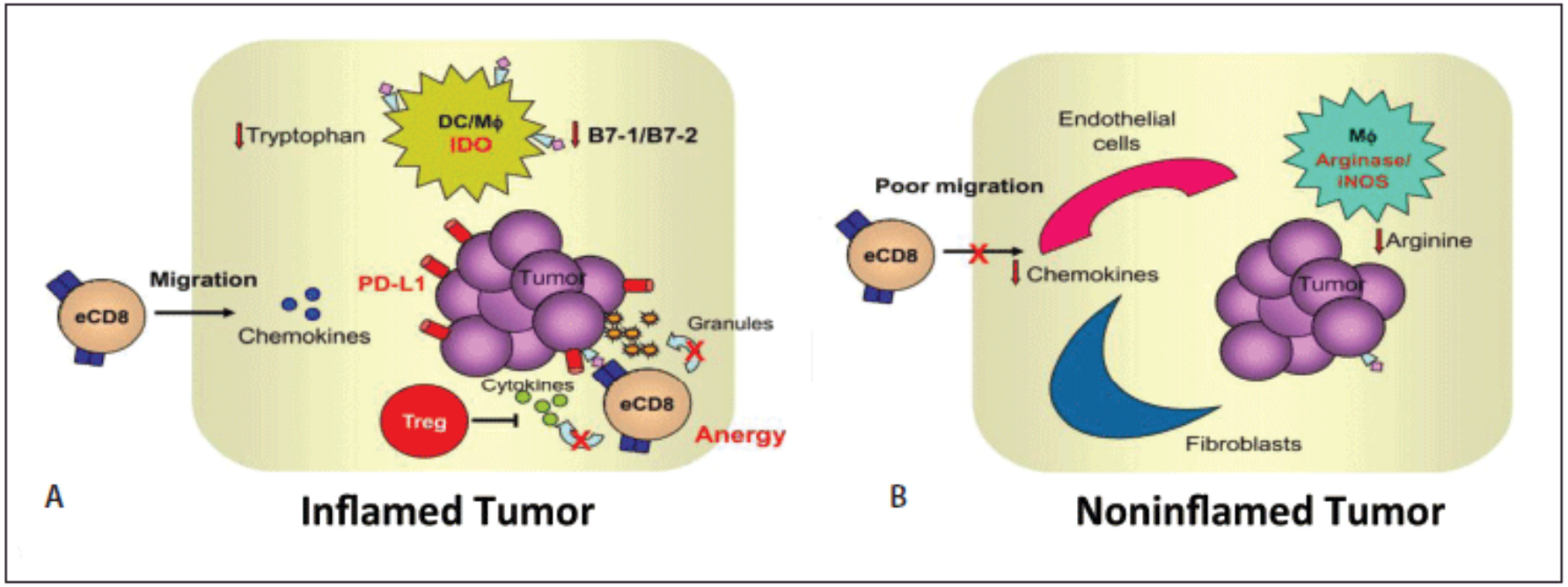


Figure 1: Two Distinct Mechanisms of Immune Resistance—**(A)** Inflamed tumors express high levels of pro-inflammatory innate and adaptive immune signals, such as chemokines for T-cell recruitment, but negative immune regulators dominate, including Foxp3+ T-regulatory cells, programmed death 1 ligand (PD-L1), and IDO; **(B)** non-inflamed tumors express few chemokines and have few tumor-infiltrating lymphocytes, due to poor effector cell trafficking. They have high expression of vascular markers and abundant macrophages and fibroblasts. From Gajewski et al. Curr Opin Immunol. 2011.[9] Used with permission.

Inflamed vs 'silent' cancers
Pathologists need to make the distinction - **HOW??**

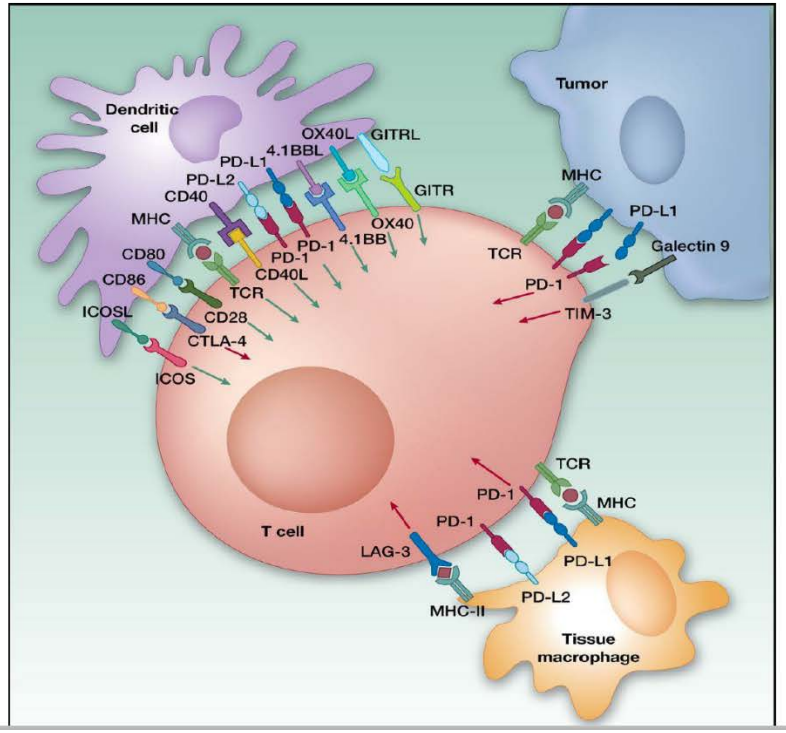
REQUIRES MULTIPLE MARKERS

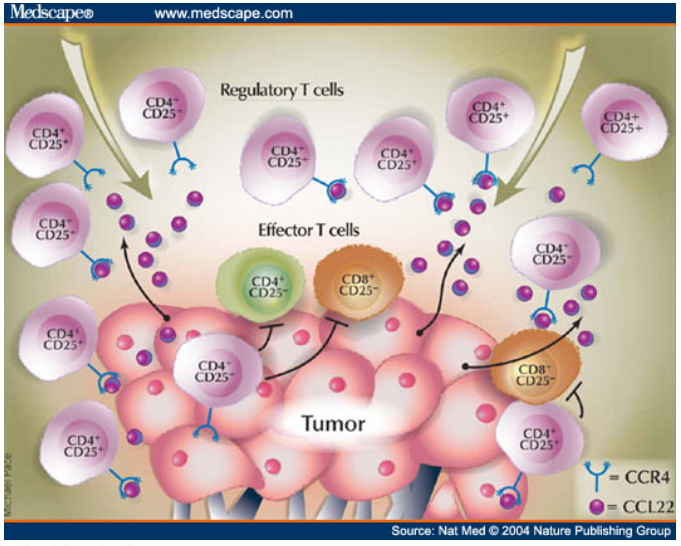
Tissue Section
Digital multiplex IHC FISH

Ligand pairs
PD 1 PDL 1
CD40 CD40L
CTLA-4 CD 86
OX40 OX40L
GITR GITR L

Retains ---
Tumor cell ID
Cell relationships
Cell numbers

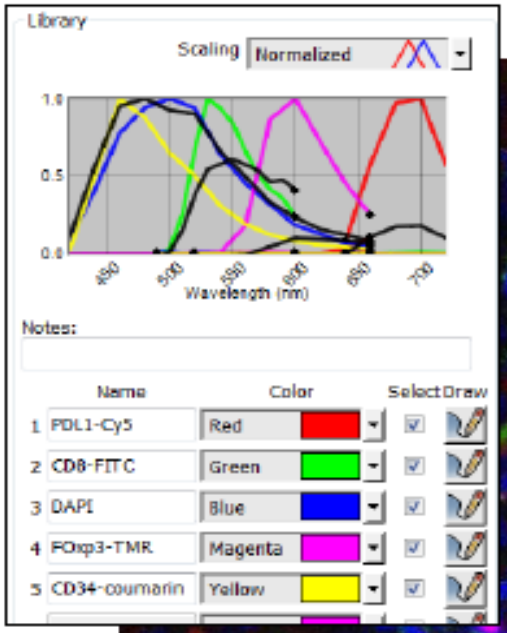
Immune Cell phenotypes
CD3 CD4 CD8 CD25
CD20 CD68 FoxP1 etc





Patrick A. Ott et al.
Clin Cancer Res 2013;19:5300-5309

Multiplex IHC may help solve these problems
because it can do all of these things at the same time



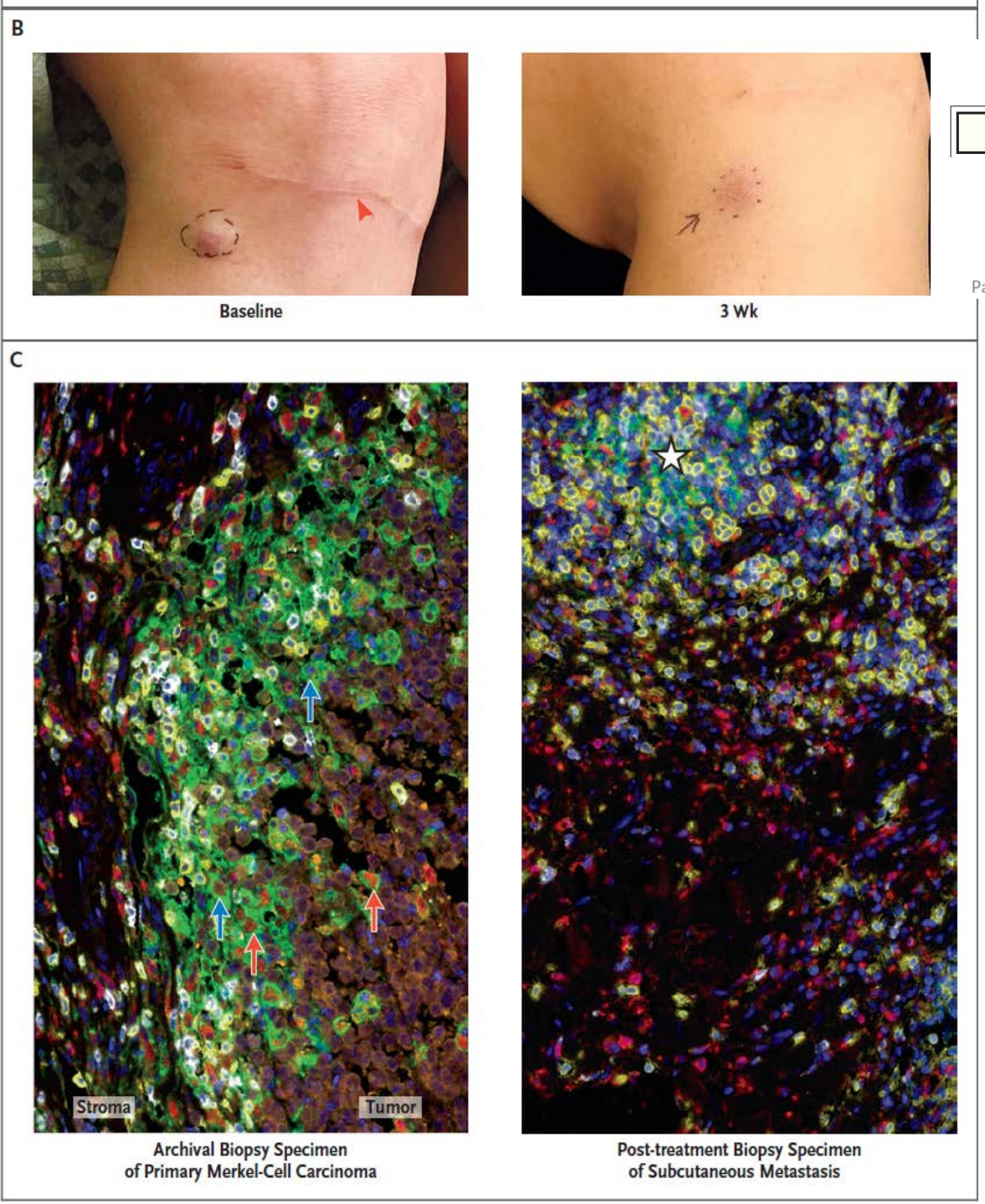
a. **Detect BIOMARKER expression**

b. **Achieve better cell ID**
Immune cell phenotyping

c. **quantification= counting**
Accurate scoring

d. **Quantification = amount**
--comparing intensity versus internal standard

Courtesy - Cliff Hoyt
PerkinElmer, 2015



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

PD-1 Blockade with Pembrolizumab in Advanced Merkel-Cell Carcinoma

Paul T. Nghiem, M.D., Ph.D., Shailender Bhatia, M.D., Evan J. Lipson, M.D.,

Merkel CA cells – orange-nse
PDL1 - green
CD8 T cells - yellow
CD68 macrophages – red

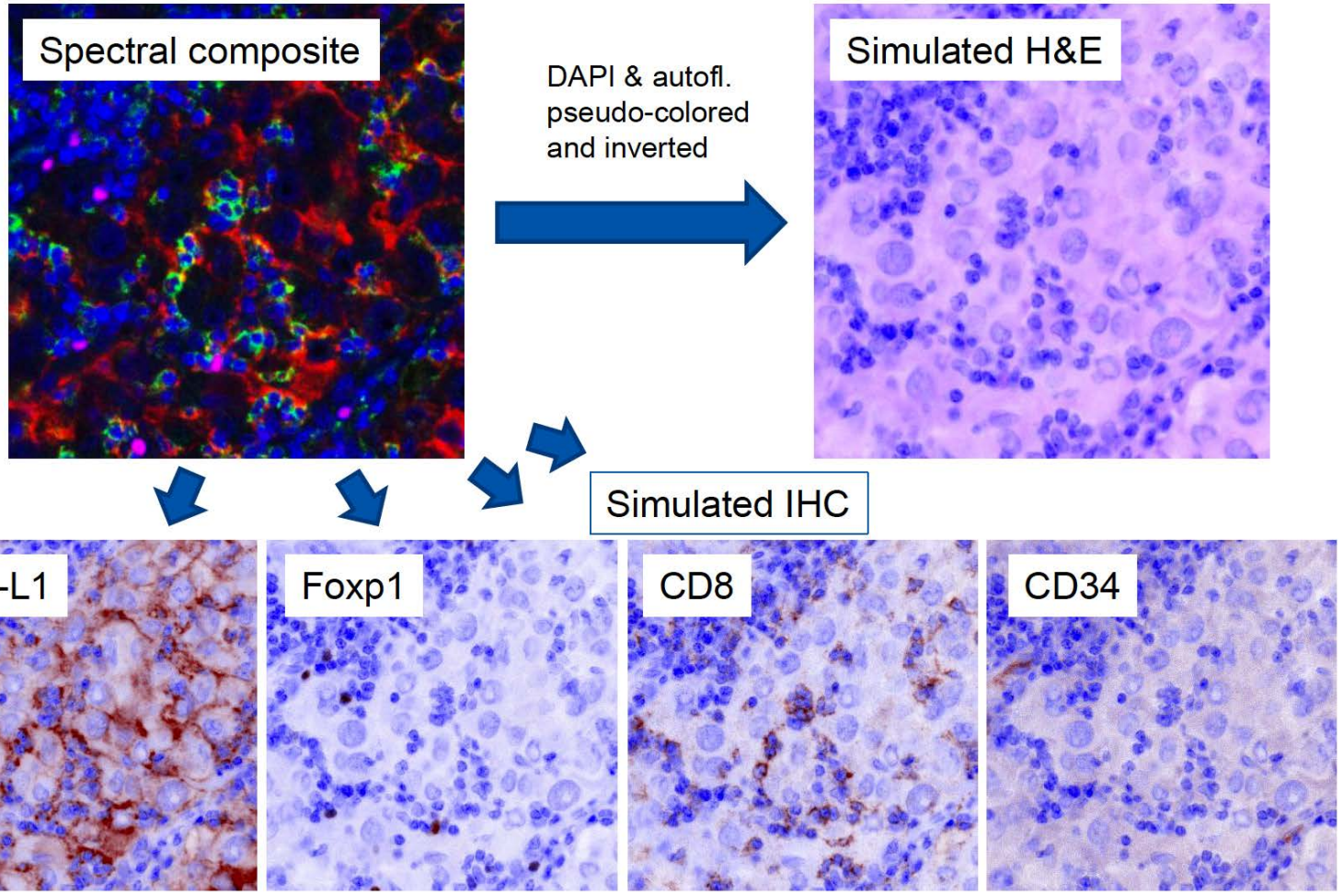
Post Rx in responder
Tumor reduced
PDL-1 reduced
CD 8 increased

NEJM April 19, 2017,
DOI: 10.1056/NEJMoa1603702

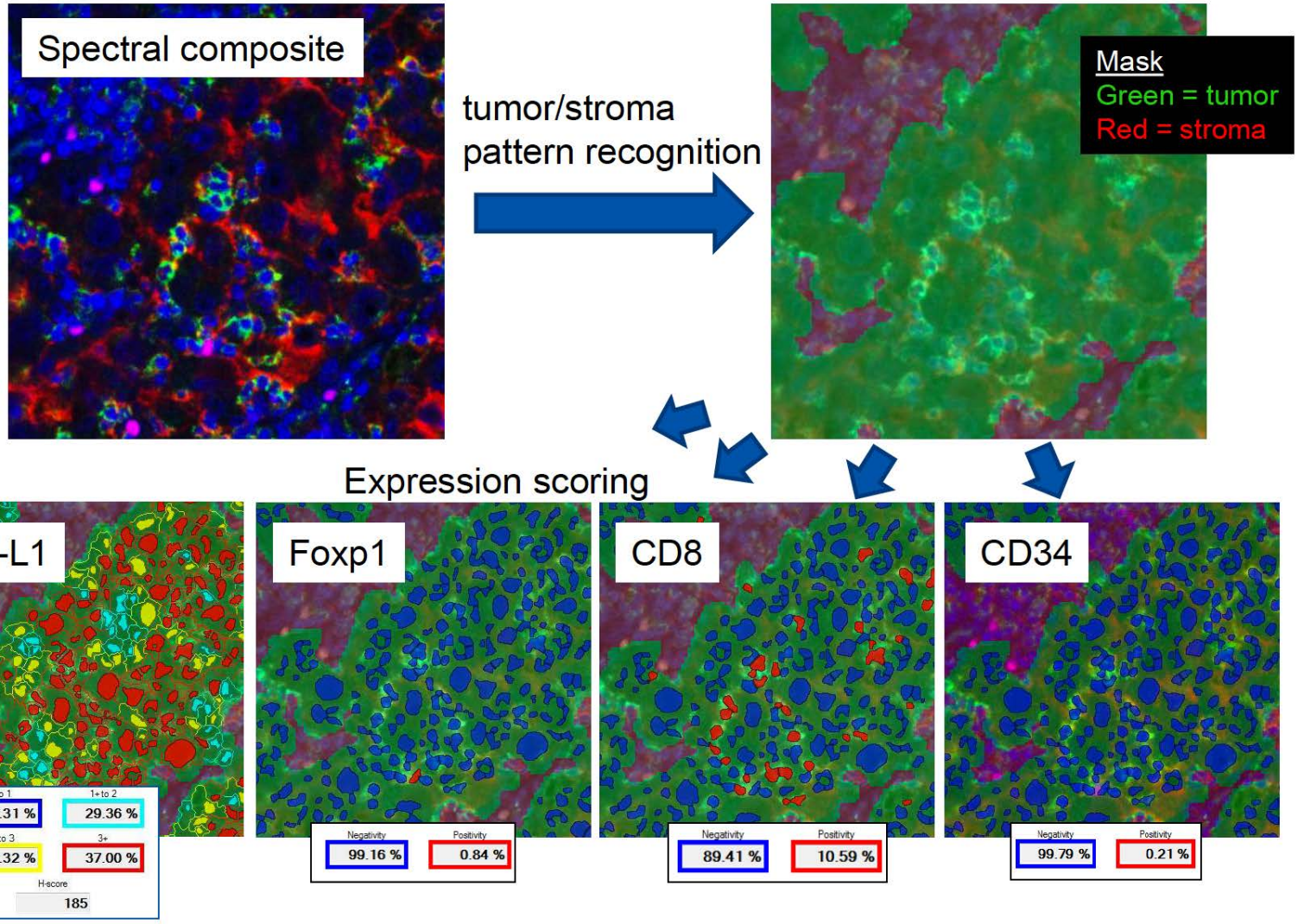
DIGITAL PATHOLOGY - BIOMARKERS
What NEW THINGS are possible?
'rehabilitate' fluorescence by restoring morphology – virtual H&E

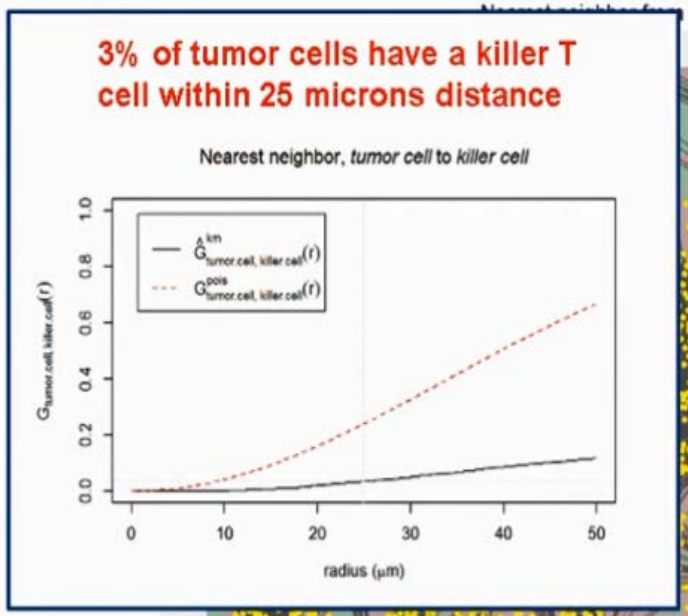
Displayed in familiar ways . . .

PerkinElmer
For the Better



Analyzed quantitatively . . .





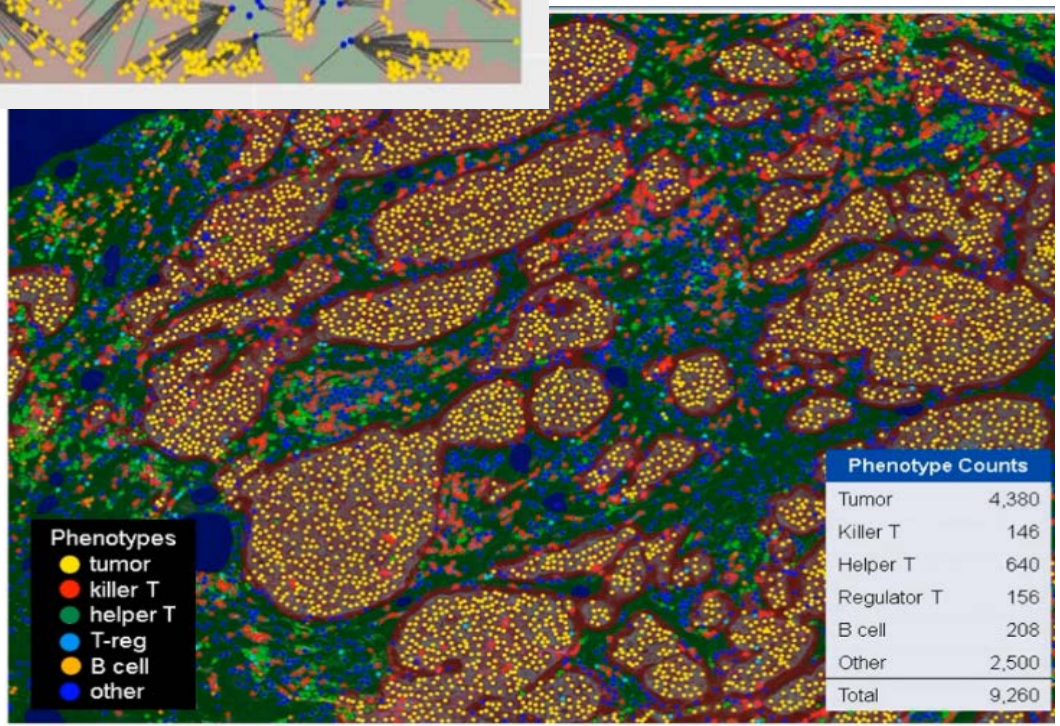
Multiplex IHC

-- phenotype ID multiple cell types

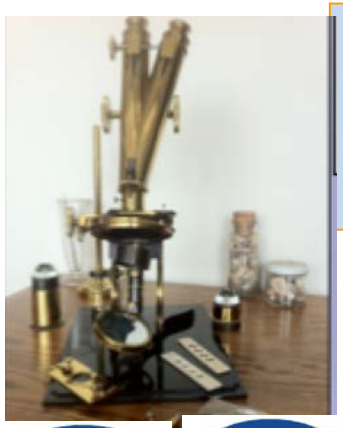
---"score ' them

--assess spatial relationships

at the same time

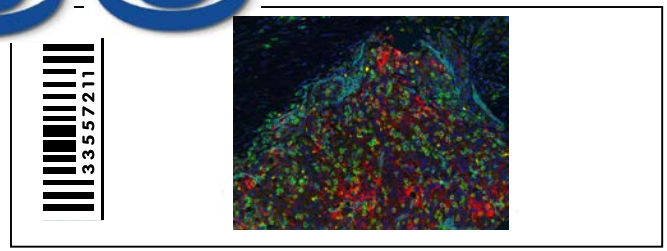


Courtesy - Cliff Hoyt
PerkinElmer, 2015



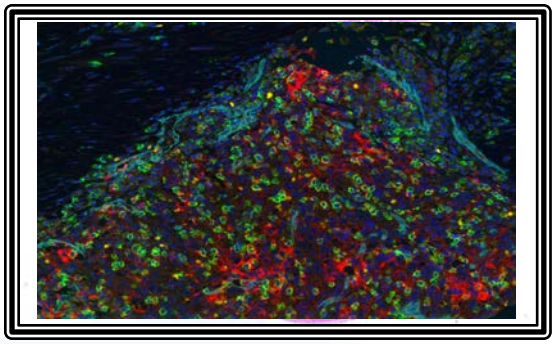
YOU CANNOT READ THESE SLIDES

-Microscope
-– glass slide



THIS CAN !! with your help

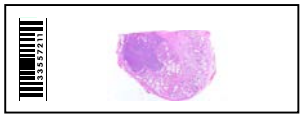
--Computer
-– WSI



Obstacles to digital pathology

RESOLUTION
SCANNING (acquisition, display) SPEED
IMAGE (file) STORAGE / SHARING /VIEWING
Apps for scoring(counting), quantification, analysis, metrics Acceptance by pathologists HARDWARE COSTS SOFTWARE costs- access
REGULATORY and REIMBURSEMENT

DIGITAL PATHOLOGY - MAJOR MILESTONE
- approval for PRIMARY DIAGNOSIS US –PHILIPS submitted to FDA



Compared microscope to WSI
607 cases - re-diagnosed v consensus 'gold standard'

=

Conclusions. **---diagnostic review by WSI was not inferior to microscope slide review.**

Validation of Whole Slide Imaging for Primary Diagnosis in Surgical Pathology

Thomas W. Bauer, MD, PhD; Lynn Schoenfield, MD; Renee J. Slaw, MBA; Lisa Yerian, MD; Zhiyuan Sun, MS; Walter H. Henricks, MD

Press Information

April 13, 2017

Philips receives FDA clearance to market Philips IntelliSite Pathology Solution for primary diagnostic use in the US

minor
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and alternate cases were scanned at original magnification of ×20. Each pathologist reviewed his or her cases using either a microscope or imaging application. Independent pathologists identified and classified discrepancies; an

d study
design, diagnostic review by WSI was not inferior to microscope slide review ($P < .001$).
(*Arch Pathol Lab Med.* 2013;137:518–524; doi: 10.5858/arpa.2011-0678-OA)

A Large Multicenter, Retrospective Non-Inferiority Study to Evaluate Diagnostic Concordance between Optical vs Digital Microscopic Diagnoses in 2000 Surgical Pathology Cases

Michael Feldman[1], Brian Rubin[2], Christopher Moskaluk[3], Nicolas Cacciabeve[4], Guy Lindberg[5], Mischa Nelis[6], Clive Taylor[7]

PA, [2]Cleveland Clinic, Cleveland OH, [3]University of Virginia, Charlottesville VA, [4]Advanced Pathology Assoc., Rockville MD,
, [6]Philips Digital Pathology Solutions, Best, The Netherlands, [7]University of Southern California Los Angeles, CA

April 2017
FDA approved
For Primary Diagnosis

Whole slide imaging (WSI), also known as digital pathology is an emerging technology. It involves the acquisition of images from stained tissue sections on glass slides. The images are converted into digital images that can be viewed on a monitor. Digital pathology can elevate collaboration between labs/specialists and allows easy consultation across distances. The digitized images can be archived and accessed in a moments time. The digital pathology platform (Philips Intellisite Pathology Solution) used in this study is intended for in vitro diagnostic use as an aid to the pathologist to view, review and diagnose digital images of surgical pathology slides. Before substituting the time-honored, familiar and versatile microscope with digital microscopy, several valid concerns need to be addressed. The most critical issue is whether pathologic diagnoses rendered using WSI are comparable to (i.e., non-inferior to) pathologic diagnoses made by optical microscopy. Although several studies comparing digital vs optical microscopy in diagnosis have been conducted, these studies have been single or small multicenter studies, sampling a single organ or lacking central adjudication. This large multi center non-inferiority study compares microscopy to WSI reads of 2000 surgical pathology cases from 20 different organ systems (54 subtypes) with 16 reading pathologists from 4 institutions.

OBJECTIVES

Primary objective:
- Demonstrate that diagnosing surgical pathology slides with a digital pathology platform was non-inferior to using an optical microscope.

Secondary objective
- Comparison of Manual Digital (MD) and Manual Optical (MO) discordance rates for organs, subtypes and pathologists.

METHODS

Retrospective blinded randomized non-inferiority study comparing MD to MO for primary diagnosis in surgical pathology.

Acceptance criteria
Digital microscopy would be declared non-inferior to the optical microscope if the upper bound of the 95% two-sided confidence interval for the overall MD – MO difference in major discordance rate (compared to the main diagnosis) was less than 4%.

Major discordance
A difference in diagnosis that would be associated with significant difference in patient management

CLINICAL STUDY DESIGN

- 4 Clinical sites
- 27 Pathologists for:
  - Case enrollment (4)
  - Validation (4)
  - Reading (16)
  - Adjudicating (3)
- 2,000 Cases
- 16,000 Reads:
  - 4 Pathologists per case
  - Read MO and MD
  - Wash out 4 weeks

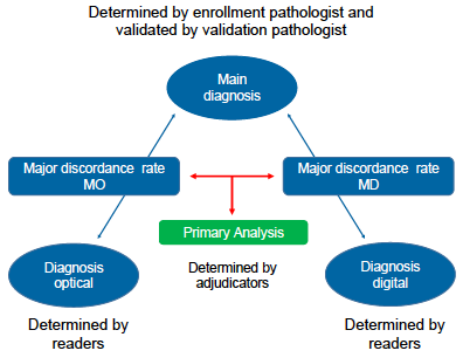


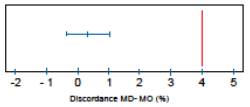
Figure 1: Study design

Table 1. Cases included in the study by organ system

Study Site	Organ system	No of cases
1	Colorectal	150
	Urinary bladder	99
	Gynecologic	150
	Liver/bile duct, neoplastic	49
	Brain	60
	Total (site 1)	508
2	Prostate	299
	Lymph node	100
	Endocrine	100
	Kidney, neoplastic	50
	Salivary	50
	Total (site 2)	599

Study Site	Organ system	No Cases
3	Gastroesophageal junction	115
	Skin	177
	Hernial/peritoneal	7
	Gallbladder	10
	Appendix	10
	Soft tissue tumors	21
	Anus/perianal	50
	Total (site 3)	390
4	Breast	299
	Lung/bronchus/larynx/oral cavity/nasopharynx	95
	Stomach	99
	Total (site 4)	495
	Total for all four sites (full analysis set)	1992

RESULTS



Left CI	Average	Right CI
-0.31	0.35	1.00

Figure 2: Difference in major discordance rate digital - optical (%)

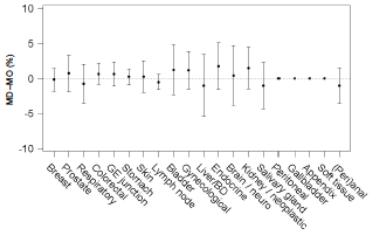


Figure 3: Difference major discordance rates (MD – MO) by organ

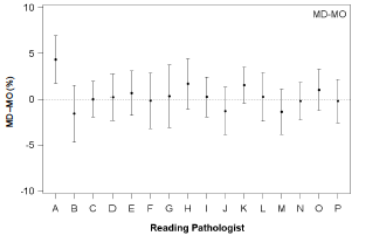


Figure 4: Difference major discordance rates (MD – MO) by reading pathologist

CONCLUSIONS

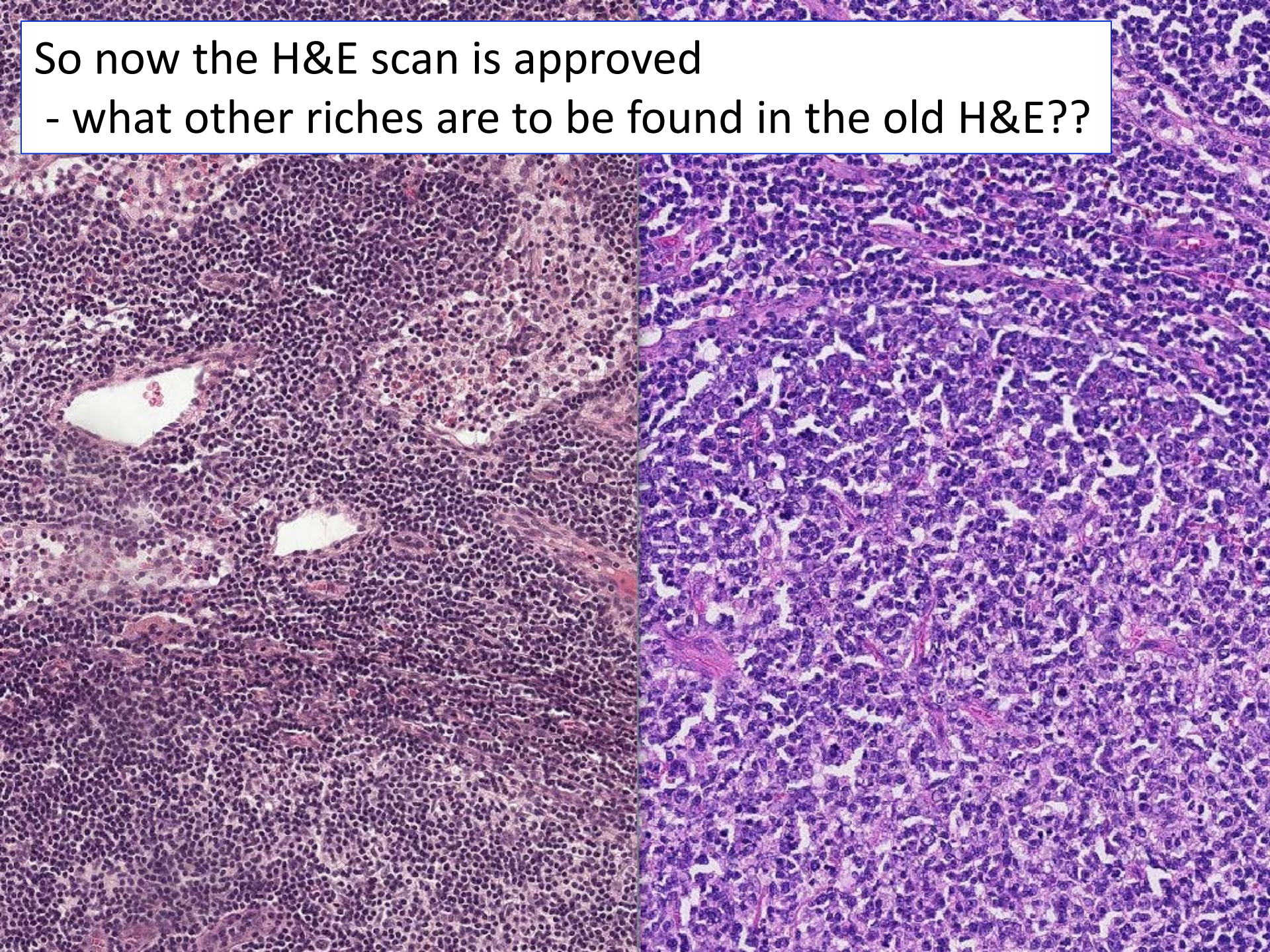
Manual Digital is non-inferior to Manual Optical for primary diagnosis in surgical pathology

Manual Digital is non-inferior to Manual Optical across a wide range of organ systems and pathologists

DISCLOSURE

This study was sponsored by Philips Digital Pathology Solutions

So now the H&E scan is approved
- what other riches are to be found in the old H&E??



THE TREND - “cloud based” --Optra

No special software; any hardware you like

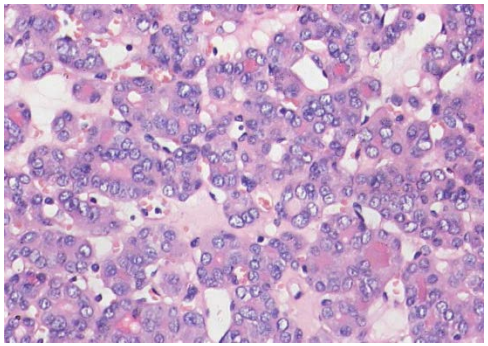
All you need is THE NET and a BROWSER

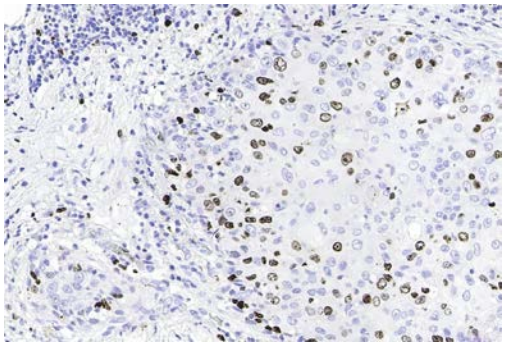
Camelyon 16 challenge data set
https://arxiv.org/pdf/1703.02442.pdf

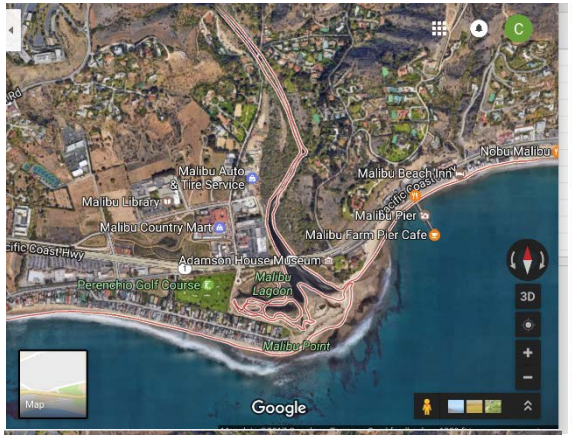
Like Google Maps

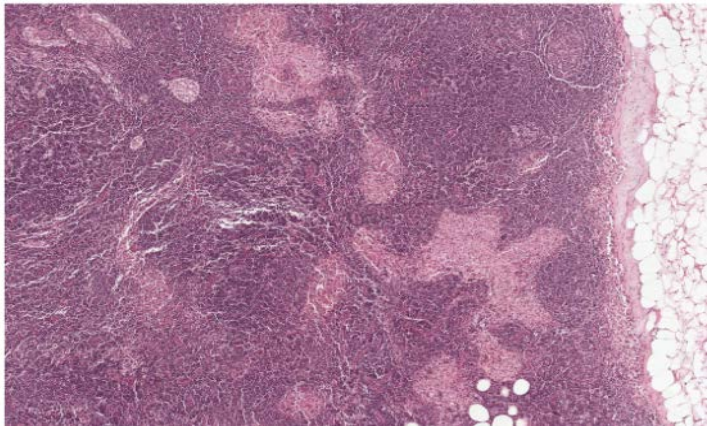
Where are the nearest restaurants?

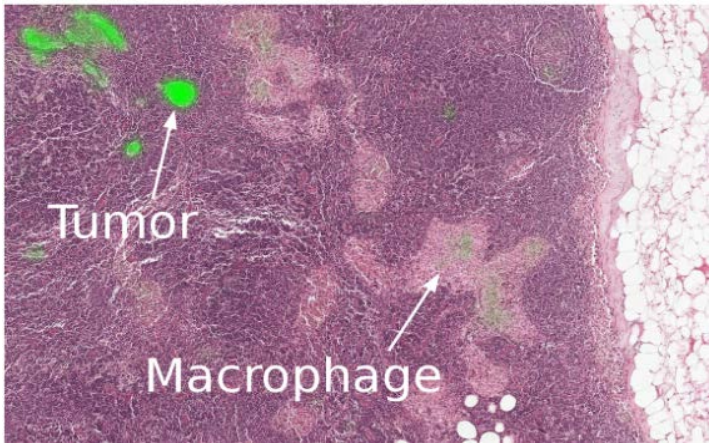
Where are the nearest Cancer cells?





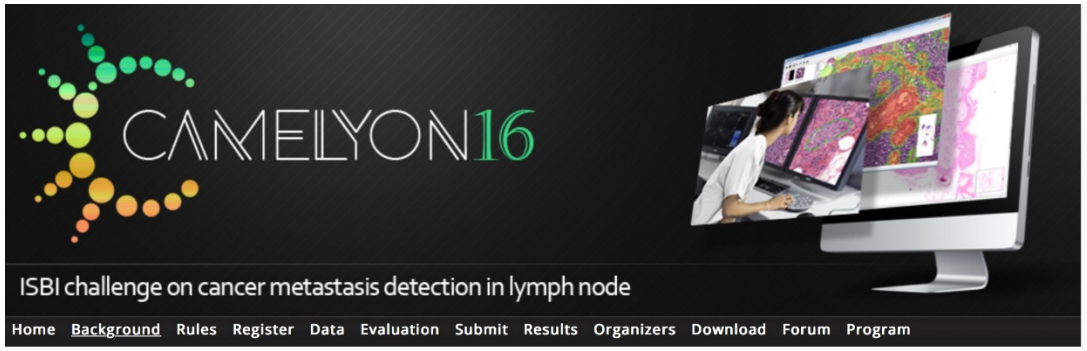






Courtesy Anagha Jadhav, OptraScan

Detecting Cancer Metastases on Gigapixel Pathology Images
Yun Liu et al Martin C. Stumpe. GoogleBlog 2017



Camelyon 16 challenge data set
https://arxiv.org/pdf/1703.02442.pdf

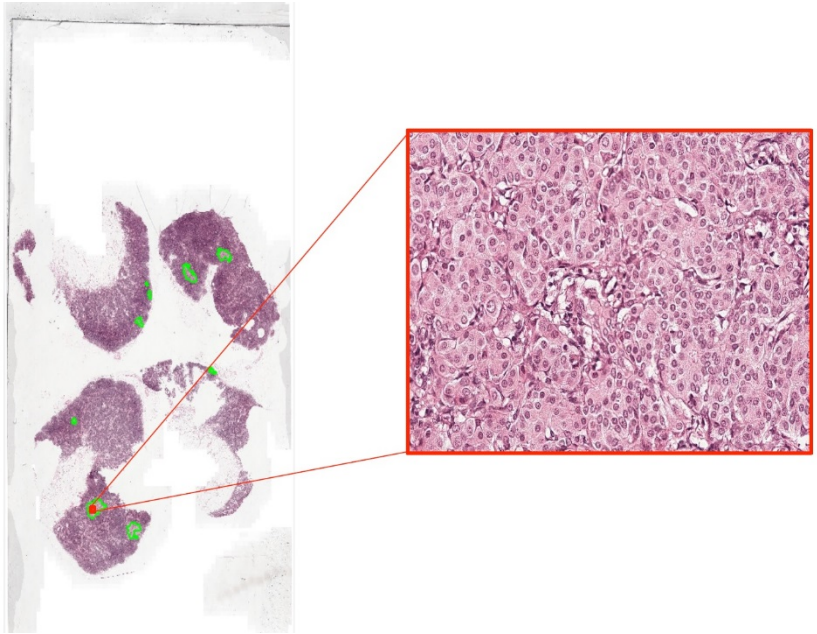
The CAMELYON16 challenge has ended in November 2016

PLEASE CHECK OUT CAMELYON17:

https://camelyon17.grand-challenge.org

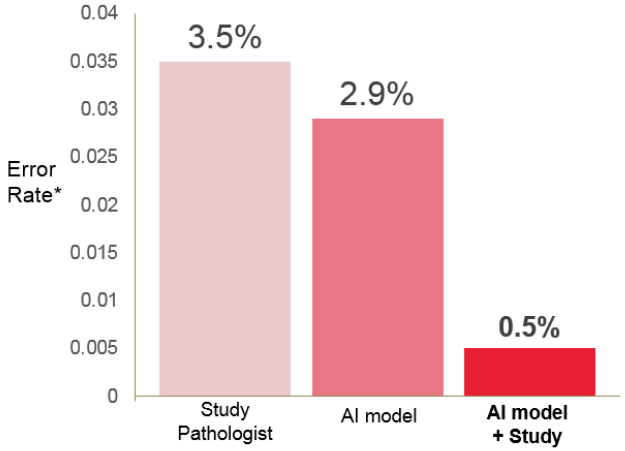
32 entries from 23 teams

Winner Andrew Beck et al Beth Israel
AI beat the pathologist standard



University of Warwick

(AI + Pathologist) > Pathologist



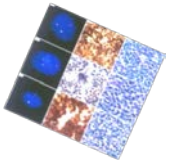
* Error rate defined as 1 – Area under the Receiver Operator Curve
** A study pathologist, blinded to the ground truth diagnoses, independently scored all evaluation slides.

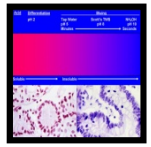
© 2016 PathAI

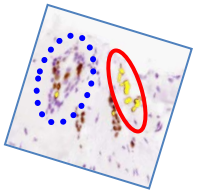
WHY is this important?

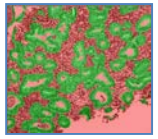
REVOLUTION

Just as 150 years ago









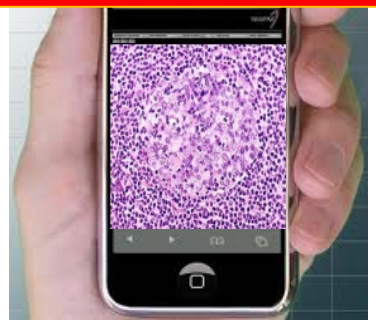
AND EVERYTHING WILL CHANGE

This revolution will affect us

Not just the hardware

But the software

Pathology ‘Apps’.

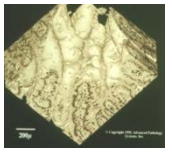


Horus.

Seeing Eye

Path PAD 2020

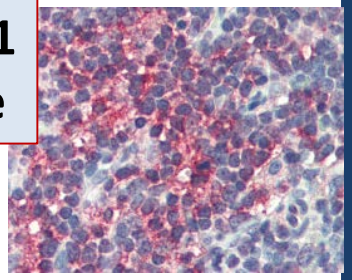
Apps

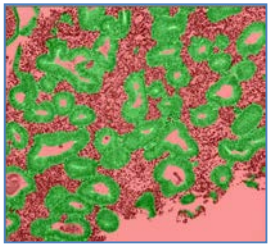


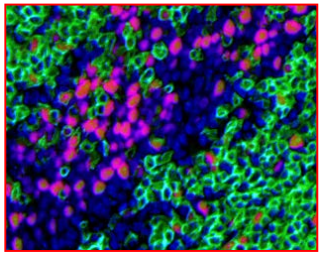
Digital assistance for pathologist

The 'intelligent' microscope

PDL-1 score

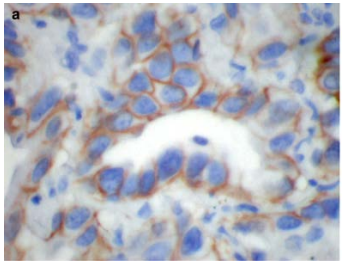


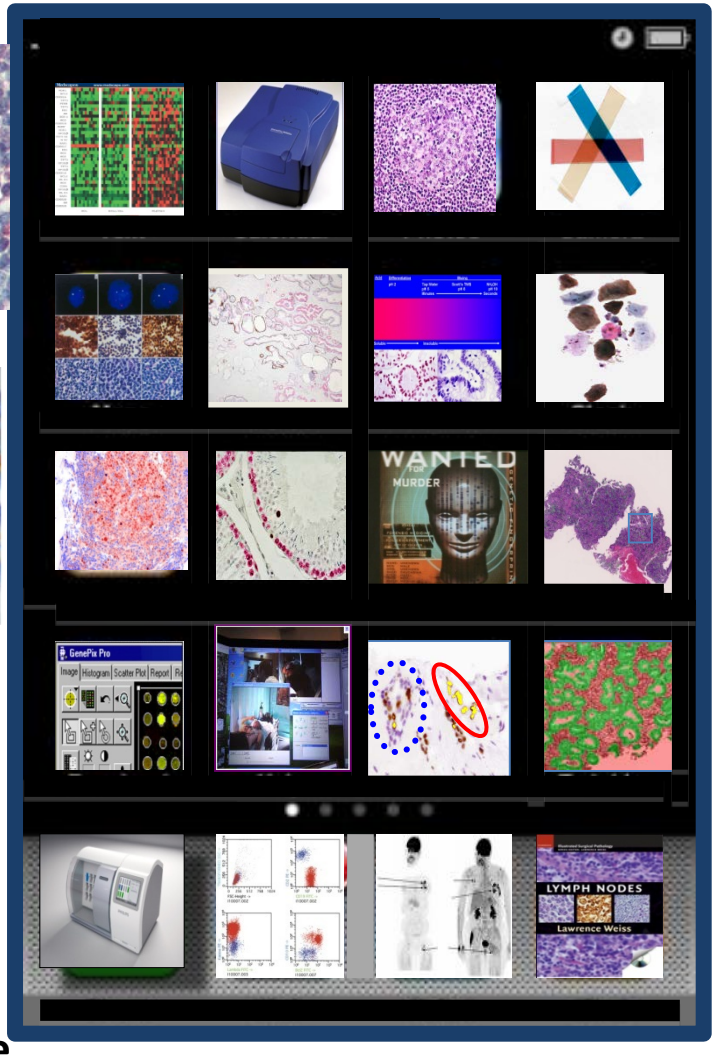




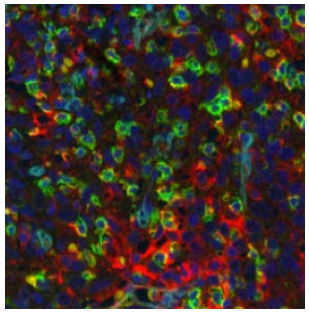
Ki67 score

Segment Cancer cells

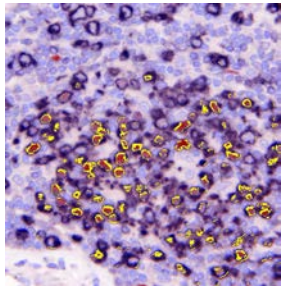




HER2 score



6-plex score



Co-localize

Summary - IHC - to improve quality and to quantify
- what must be done?

CONTROL - preparation-fixation (**qualify** tissues)
DEFINE - Analytes (protein targets)
VALIDATE - Reagents
VALIDATE / STANDARDISE - Total Method as a whole
DEVELOP - uniform 'shared' control systems
DEVELOP – quantitative internal reference standards
DEVELOP - standard interpretation/scoring by computer

and ALL OF THESE STEPS REQUIRE IMPROVED CONTROLS
and all require 'monitoring'

--Total Test Concept--

Companion Diagnostics and digital pathology
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