

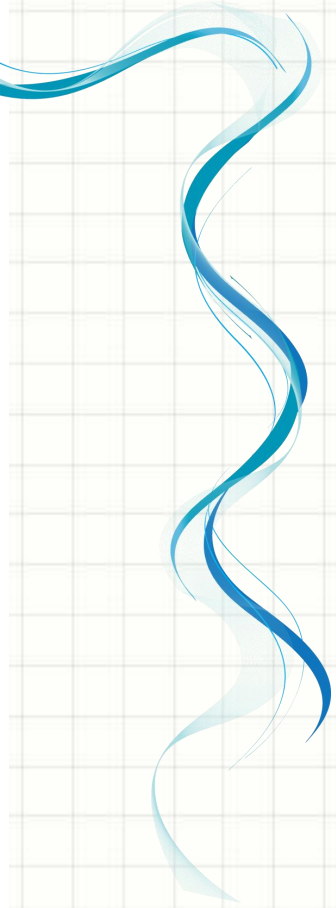
PRS
CO-RESIDENT OBJECTIVE
MEASURE OF
IHC STAIN PERFORMANCE FOR
PROCESS QC AND DIAGNOSTIC
AID

Frederick Husher
Jee Shum





Successful IHC Diagnosis Starts With an Effective Process QC

A decorative graphic on the left side of the slide, consisting of several overlapping, wavy blue lines that create a sense of movement and depth. The lines are in various shades of blue, from light to dark, and are set against a light gray grid background.

For decades, The College of American Pathologists and their European counterparts have waited for a valid product to perform certifications of the Laboratory and the interpreting Pathologist.

While daily stand alone QC offers minimal assurance of IHC processing efficacy, the only 100% solution is an on-slide QC test for every slide processed.

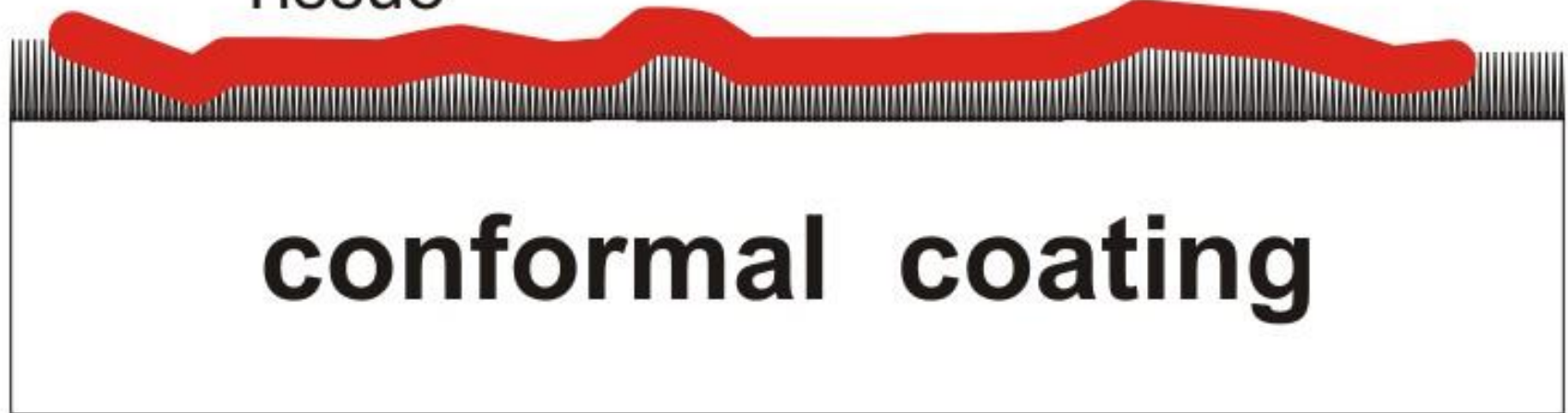
Only the PRS offers this process verification with a permanent co-resident record.

Adhesive Coated Slide Structure

Tissue



Tissue



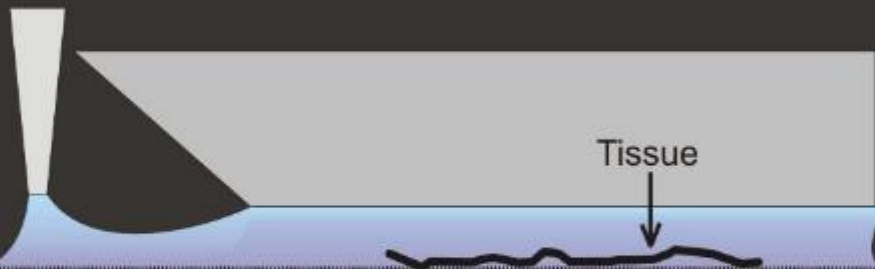
Impact of reagent Exchange in closed capillary gap vs. coating wettability



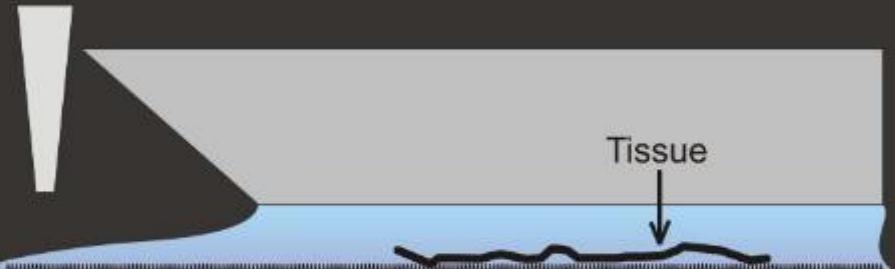
hydrophobic coating



hydrophobic coating



hydrophilic coating

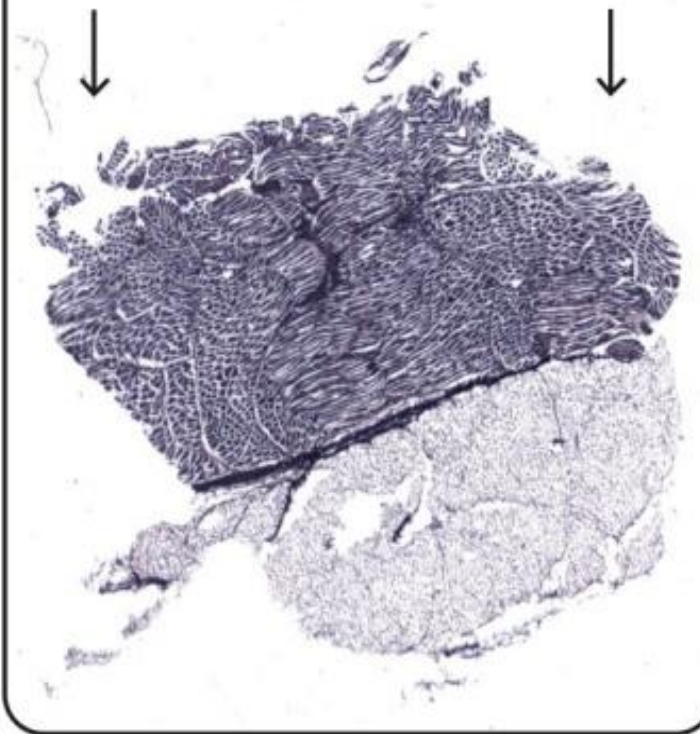


hydrophilic coating

Damage caused by HIER process

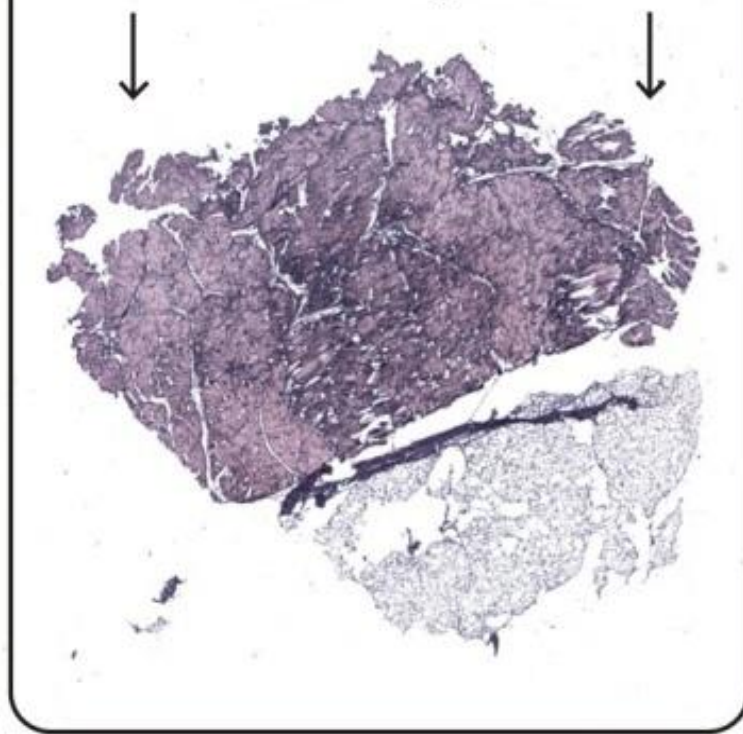
dewax no HIER- H&E stain

reagent flow



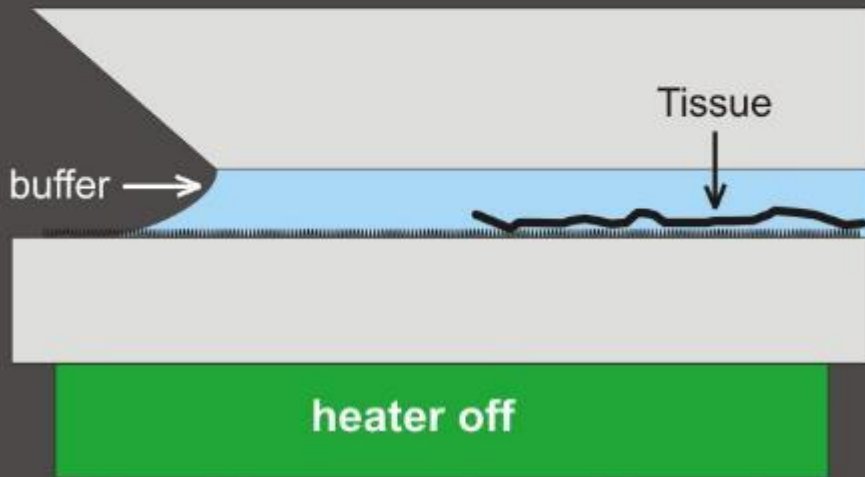
dewax HIER 95°C pH9 20min H&E stain

Air knife / reagent flow

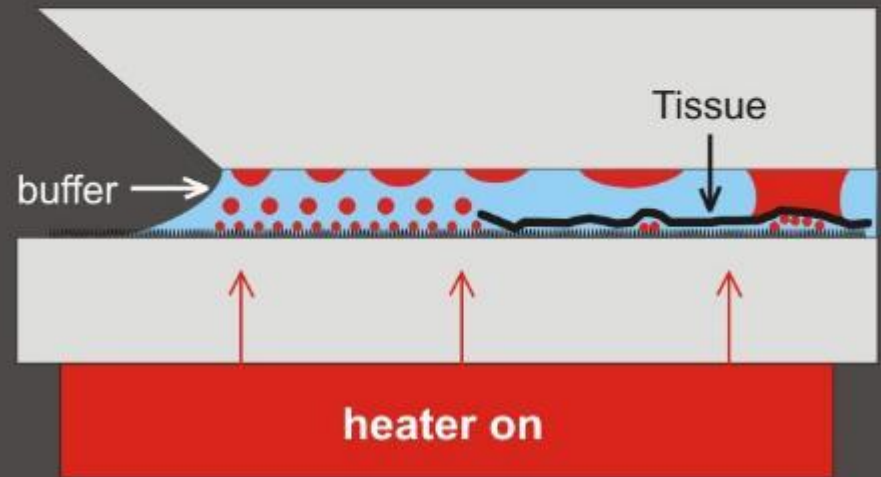


Heat Induced Bubble Formation

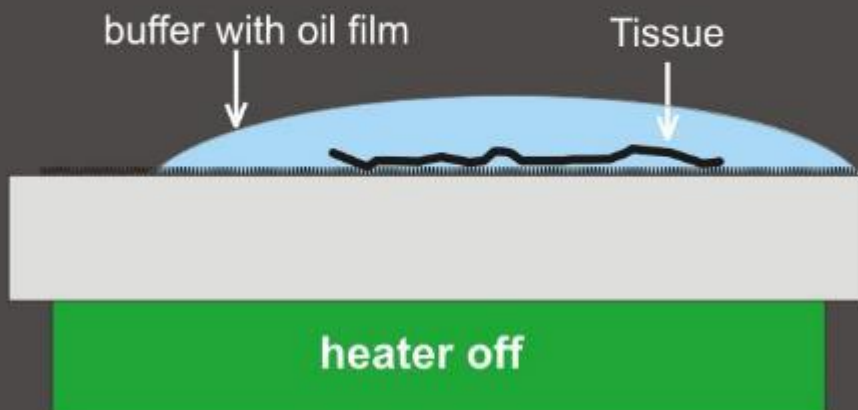
close capillary gap



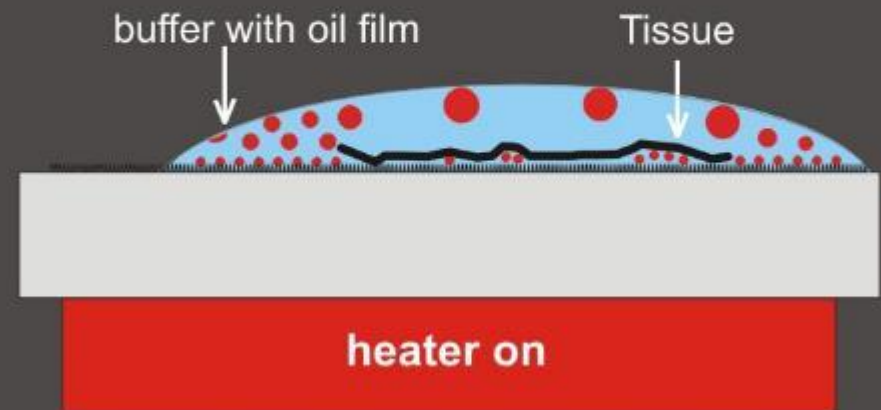
close capillary gap



open capillary gap

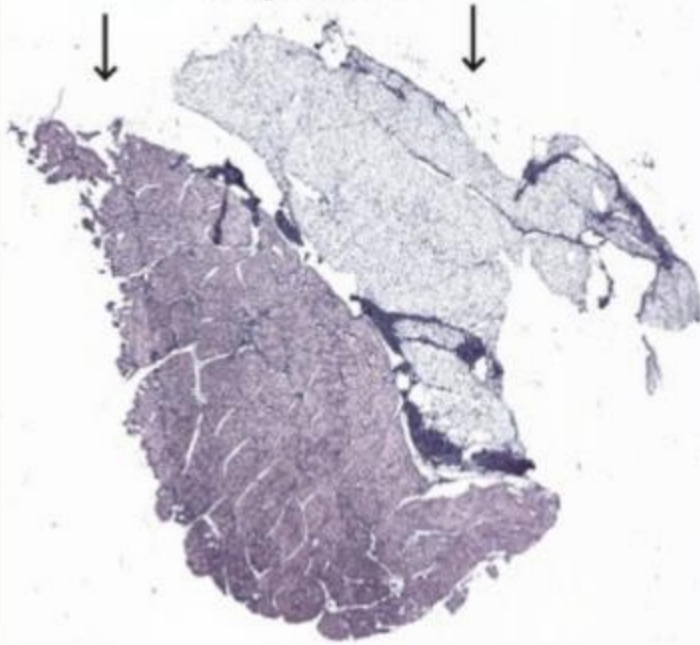


open capillary gap

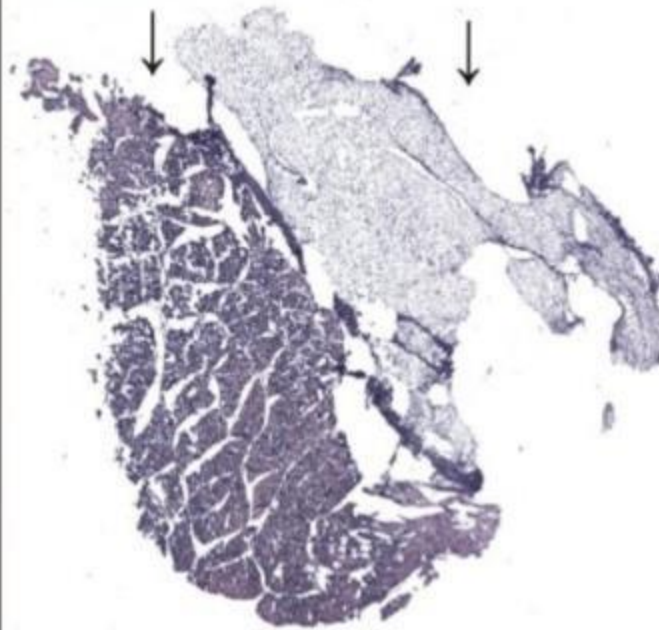


Contemporary stainers performance

dewax HIER 95°C pH9 20min H&E
use Close Capillary Gap stainer
reagent flow



dewax HIER 95°C pH9 20min H&E
use Open Capillary Gap stainer
air knife / reagent flow



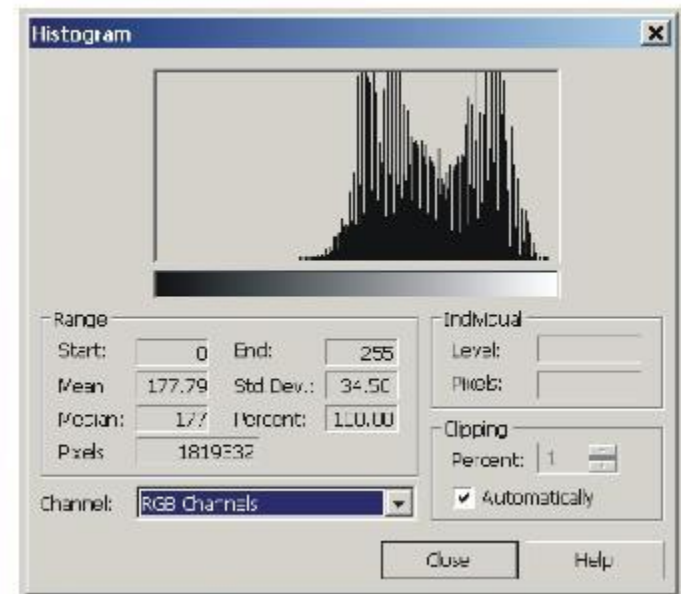
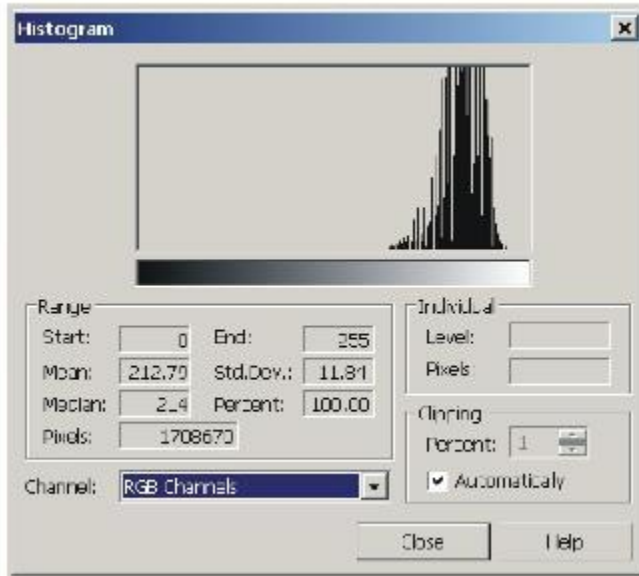
3D conformal slide coatings

- **There are a number of important advantages to 3D coatings:**
 - **More than one binding moiety**
 - **Conformal to tissue surface**
 - **Promotes fast transport of water**
- **Wettability can be set at time of production.**
- **There is one used in common IHC usage**
- **We too have a suite of such coatings**

Adhesive Slide Coating Uniformity tests



Here Are Two Currently Produced Amino-silane Coated Slides



Heat Induced Epitope Retrieval

- **The HIER process uses a buffer ranging between pH 6 to 9**
- **Heat is applied at about 95°C for 10-15 minutes**
- **Damage can cause feature loss and non-specific staining**

THE PRS TARGETS

- ❖ **Protein mixtures**
 - **Secondary stain targets**
 - **Primary Antigen stain targets**
- ❖ **Special Stain targets**
- ❖ **Black and white pigment targets**



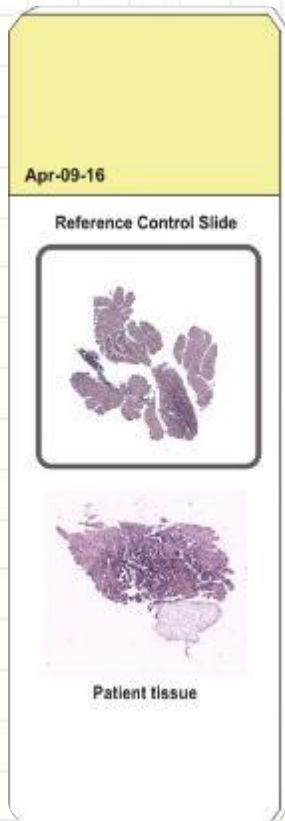
Traditional Subjective Biopsy Slide



- **Possible Errors:**
 - Human error
 - Machine failure
 - Cumulative processing variation
 - Effect the final stained result
 - Lack of QC and process control
 - Subjective diagnostic interpretation
 - False negative result

Control Slides Now Used

Known tissue
sausage



Multi-tissue sausage that is fixed and embedded for sectioning, whose members react with one of more primary antibodies.

There is no control over the antigen density so the QC is only pass/fail at best

Peptide on
Compressed
polymer



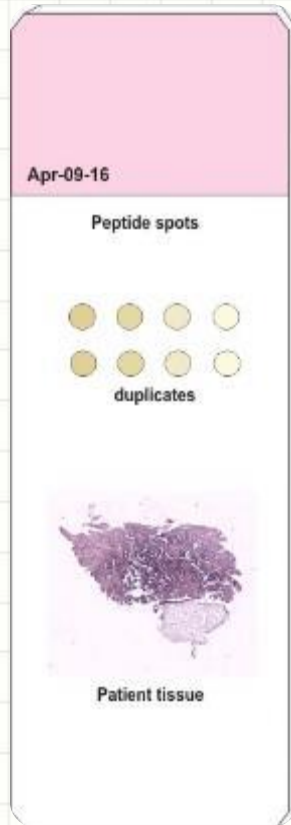
Primary peptides attached to a polymer backbone that is compressed, embedded, and sectioned.

Typically, only one primary peptide per section.

Very difficult to form consistent antigen concentration density targets.

Thus, the results do not produce objective measurement results.

Peptides on beads



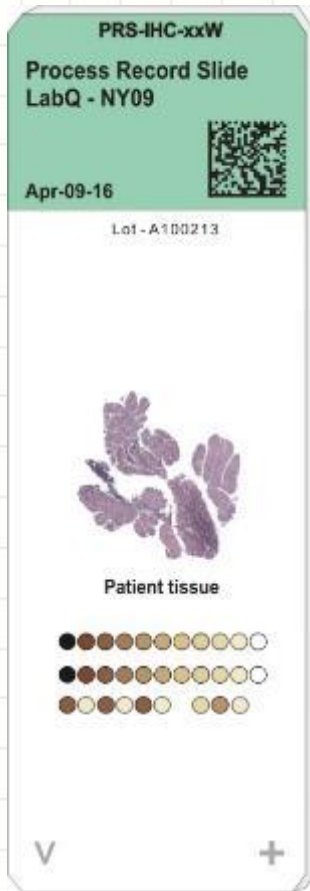
Primary peptides attached to coated beads, which are deposited in a monolayer slurry.

The slurry can contain different peptide concentration beads as well as known density colored beads.

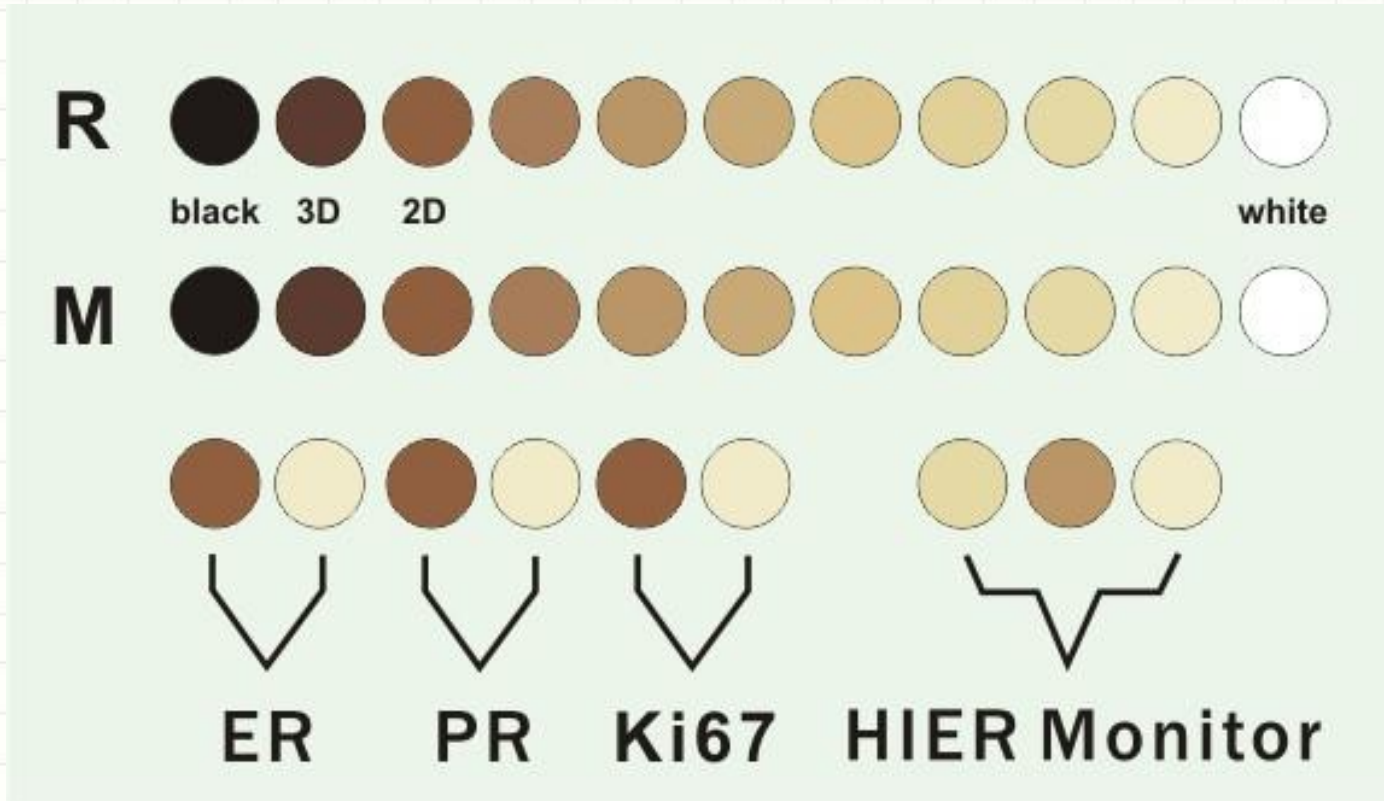
Requires each bead to be imaged. However, since the bead does not present a level surface to the imaging system and light source.

Introducing the new solution for IHC

Process Record Slide



The Process Record Slide Targets



The Process Record Slide Targets

There are three lines of protein deposits all covered by a Paraffin film layer

Two secondary gradient density arrays of mouse & rabbit each following a square law curve

Primary gradient density arrays for each primary antigen each following a square law curve

While dot pairs are shown, we will be using 3-dot arrays to better map onto the secondary array

A HIER monitor to capture the behavior of the antigen recovery process

All with the same protein blend but differing formaldehyde cross-linking densities

The QC Use of The PRS

The PRS is broken into three basic regions of operation:

- **Mouse & Rabbit 2D secondary stain reactive gradient density array**
- **One each of Mouse & Rabbit 3D secondary stain reactive targets**
- **One of more 2D primary antigen gradient density arrays**
- **Black and white reference targets**

How the Secondary Stain Reagent Kit performance is Captured in the Mouse & Rabbit Arrays



- **Mouse & Rabbit arrays use verified concentrations of IgG proteins**
- **All proteins are impacted the same by the antigen retrieval process**

How the Primary Antibody Reagent performance is Captured in the Mating Antigen array

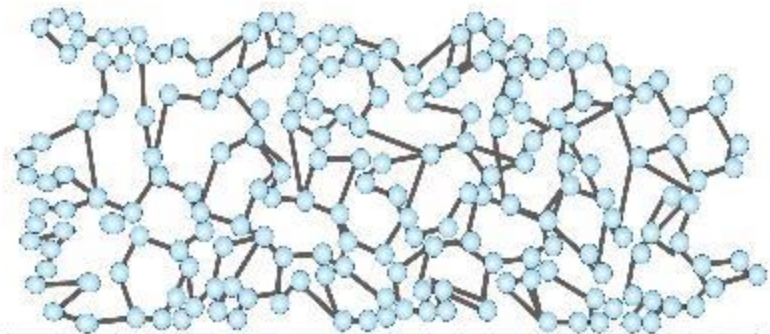


- **Primary antigen carrier proteins use verified concentrations**
- **All proteins are impacted the same by the antigen retrieval process**

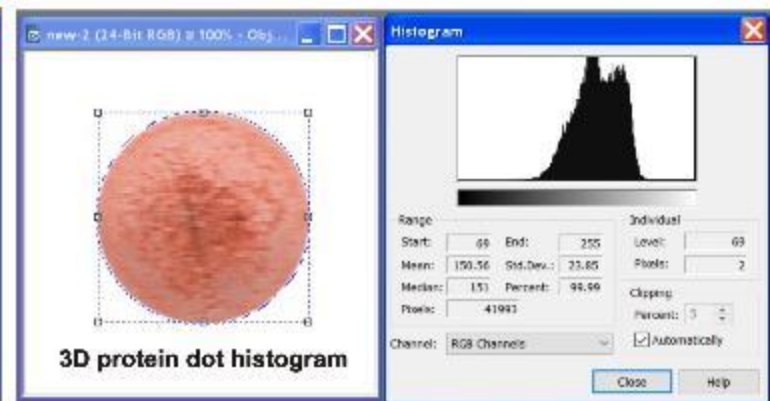
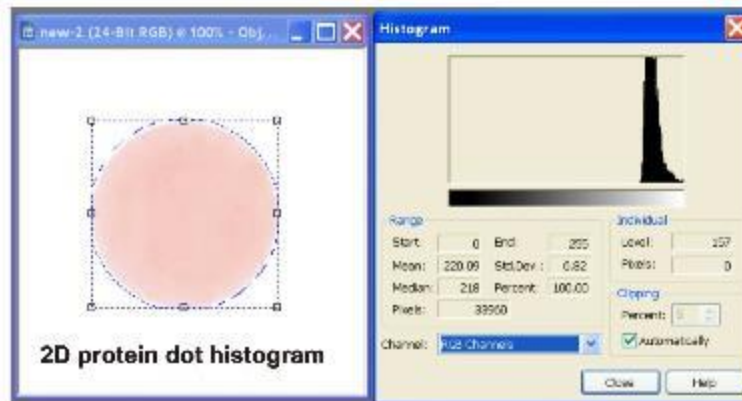
2D vs. 3D Same Protein Mixture Targets



2D Planar Protein Deposit



3D Protein Infused Scaffold



Primary Antigen Density Ruler Construction



86
Invasive, metastatic



106
Tumor-Like



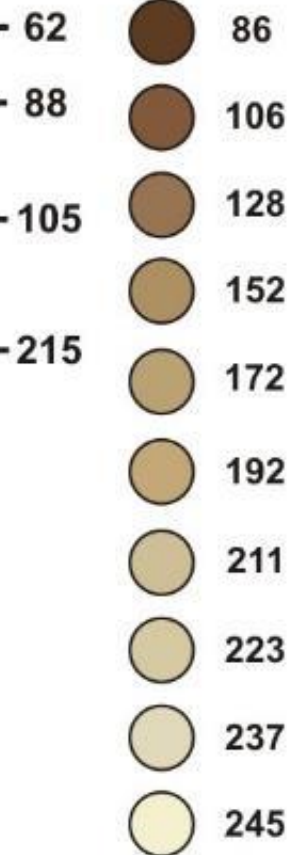
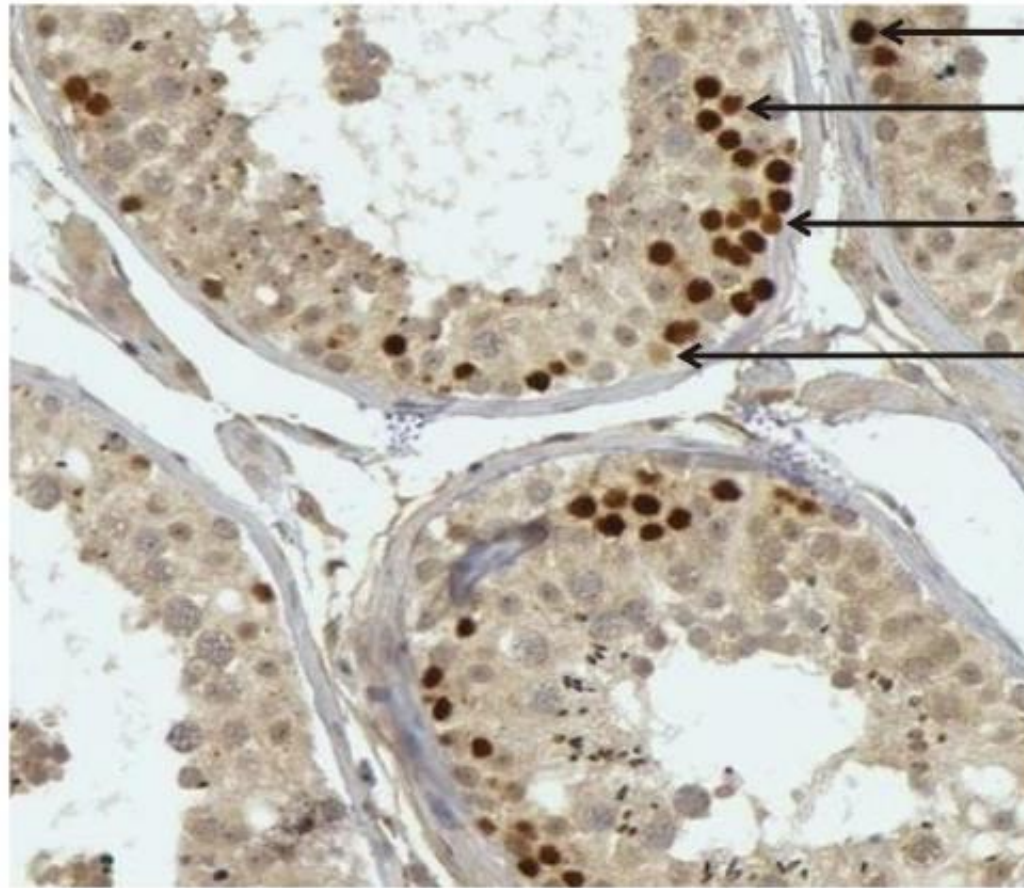
128
Hyperplastic



211
Nonmalignant



237
Normal Cell



How does the PRS aid the Whole Slide Imaging (WSI)

- **Black and white baseline targets to set illumination level**
- **Slide background: washed out or truncated**
- **PRS incorporates black and white targets**
- **Represents working range on slide**
- **3D target builds antigen scale to apply to tissue**

Time to Modernize to the revolutionary Process Record Slide



- The PRS incorporates co-resident patient tissue and control targets which:
- Records the IHC staining experience
- Target data provides objective QC evaluation of the processing experience
- Target data forms the basis of an antigen concentration ruler
- The ruler provides digital imaging baseline for pre-screening
- The ruler supports imaging adjustment for best diagnostic interpretation by the viewing Pathologist
- 2nd opinion possible as observer knows what was done to the PRS & tissue section

Subjective Result



Objective Result



Does the PRS Require FDA Approval?

The PRS has been granted Class I approval by the FDA. It is important to note that the PRS does not act on the patient tissue section in any way. It can aid in the veracity of the diagnostic interpretation by validating the processing experience and provide process control feedback.

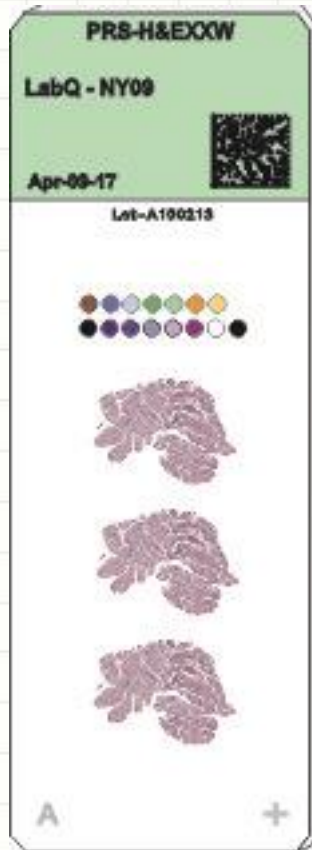
Additionally it can be use with digital imaging to adjust the stained result to a normalized presentation.

Commercialization of PRS

The PRS is coming into commercial product reality via a joint venture with the Hong Kong Productivity Council.

The mass production line incorporates our protein printer technology that supports 2D and 3D printing of biomaterials and reagents.

PRS-H&E Slide



The basic PRS concept has been extended to support H&E plus Special Stains

PRS-PAP Slide



**We have also
extended the
PRS-H&E to
support Pap
smears**



Development of a 5th Generation IHC Stainer with Integrated Antigen Retrieval

- **Using the primitives of the PRS to make test slides**
 - We have characterized all the current IHC stainers
 - Created critical path models of each system
 - Supports observation and measurement to take place
 - Used this in the development of our slide coating adhesives
- **This led to our stainer technology that is much easier on the tissue section**
- **The first model is all manual and low cost to support use with field clinics and medical students. It supports IHC and H&E staining.**
- **The second model supports the demand for Stat processing**


PRS: Use The Right Tool For The Right Job In The Right Way

Subjective Result




Objective Result





The PRS technology enables many divergent applications to become realized for the medical and R&D arenas.

The present Pathology QC applications address the only remaining area in medical diagnostics not covered with process controls and standards.



The PRS technology can also be used in process control, clinical studies, and R&D testing of new drugs opening new pathways for repeatable and objective analysis.

Additional derivatives from the PRS development will be forthcoming in the near future to continue the goals of better diagnostic support tools.

Bringing the PRS into commercial reality will significantly improve global health through objective diagnostic efficacy.

Thank You For Your Interest and Attention

**You can find more information
on our web site**

www.IHC-PRS.com

