The Origins of Immunocytochemistry

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Disclaimers

- Current
  - Freelance consultancy
    - Diaceutics
    - Discovery Life Sciences
    - Agilent
- Recent past
  - Bristol Myers Squibb
  - Merck Sharpe Dohme
  - Roche

Definition & Aim of Presentation

- Immunohistochemistry

  - Art and science of microscopic visulaisation of biomolecules in context their cell/tissue localisation and function using antibodies linked to fluorescent or chromogenic detection system

- Aim of Presentation

  - To describe the rationale and people behind the advent of the key methodological inventions and applications of immunohistochemistry
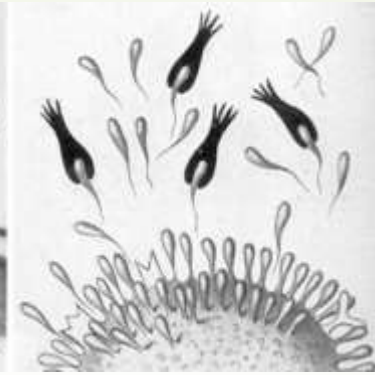
Content

- Advent of Immunofluoresence Technique
- Advent of Immunoperoxidase Methodology
- Advent of Diagnostic Immunohistochemistry
- Advent of Automated Immunohistochemistry
- Advent of Predictive Immunohistochemistry
- Advent of Multiplex Computational Immunohistochemistry

Advent of Immunofluorescent Method

*1890 Emil von Behring - Concept of antibodies – anti-diphtheria toxin*
1897 R Kraus - Concept of antibody-antigen complexing using precipitin reaction
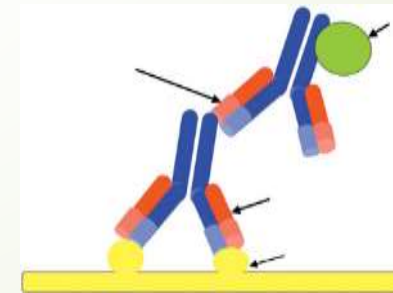*1896 et seq Paul Ehrlich - Concept of tissue staining using antibody-antigen complexing*
1920s Michael Heidelberger – Azo dye-antigen conjugates to detect serum antibodies
1934 John R Marrack – Red azo dye- antibody conjugates to detect protein antigens
1941 Albert Hewett Coons - Fluorescent Ab-conjugates for Ag detection in tissue sections
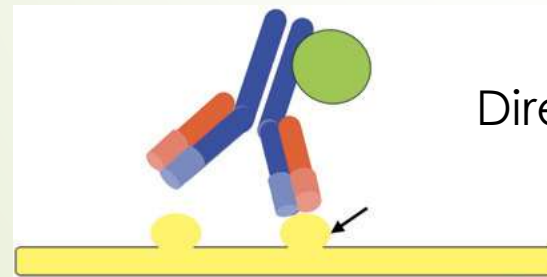
Direct

Indirect

Immunofluorescence Techniques

Advent of Immunoperoxiase Method

Main drawbacks of Immunofluorescence Method
1. Could not be applied for ultrastructural examination of antigen
2. Autofluorescence

Images adapted from Gwen Childs History of Immunohistochemistry In Pathology of Human Disease 2014 pp3775-3796

1966 Morris J Karnovsky - Horseradish peroxidase (HRP) as a neurone tracer using diaminobenzidine deposits as a marker visible under EM
1966 Stratis Avrameas - glutaradehyde Ab-HRP conjugates for immunoperoxidase labelling at EM level
1967 Paul Nakane – Improved activity Ab-HRP conjugates for EM
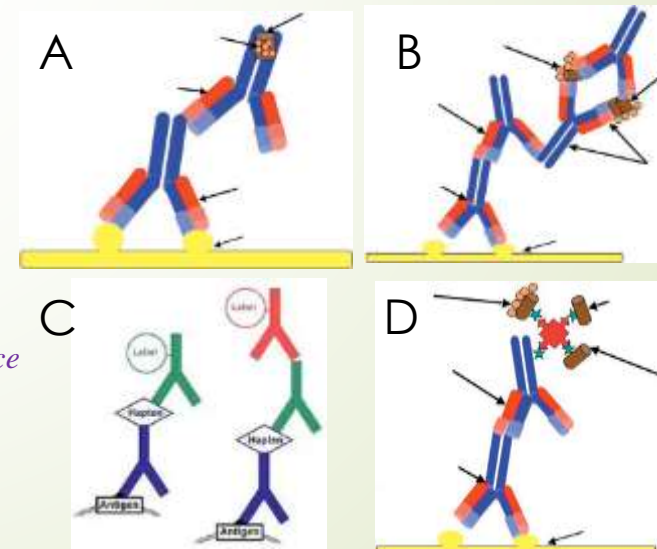*Improved Sensitivity*
1970 Ludwig Sternberger Peroxidase-Anti-Peroxidase Method (PAP)
Gwen Childs 1972 – Tipping Point for Immuno-Enzyme Technology Gordon Research Conference
Improved Specificity
1980 Bharat Jasani et al DNP hapten sandwich staining DHSS method
1982 Su-Ming Hsu Avidin Biotin Sandwich Method

A Indirect Labelled & **B** Unlabelled & **C** Hapten & **D** Avidin Biotin Sandwich Immunoperoxidase Methods

DNP-Hapten Sanwich Staining (DHSS) Method

- Localisation of TSH, IgE, EGFR & ER Receptors at LM/EM level

  - Jasani B et al Dinitrophenyl (DNP) hapten sandwich staining (DHSS) procedure. A 10-year review of its principal reagents and applications. J Immunol Methods. 1992 Jun 24;150(1-2):193-8.

- Silver Enhancement of Diaminobenzidine Product

  - Newman, G.R., Jasani, B. Silver Development in Microscopy and Bioanalysis: a New Versatile Formulation for Modern Needs. *Histochem J* **30**, 635–646 (1998). https://doi.org/10.1023/A:1003404128497

- Non-Deleterious Inhibition of Endogenous Peroxidase

  - Andrew SM, Jasani B. An improved method for the inhibition of endogenous peroxidase non-deleterious to lymphocyte surface markers. Application to immunoperoxidase studies on eosinophil-rich tissue preparations. Histochem J. 1987 Aug;19(8):426-30.
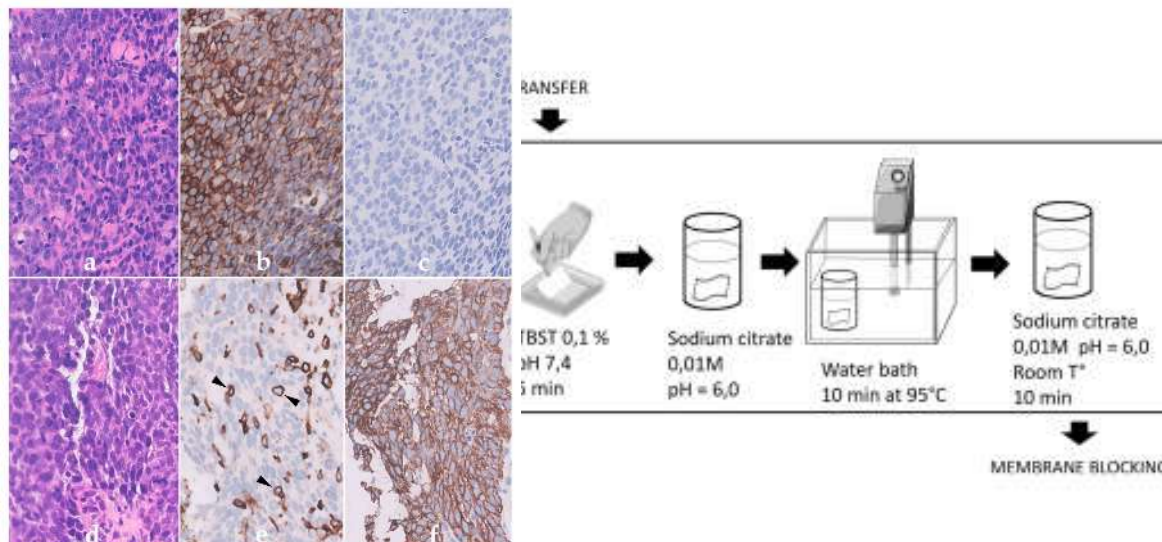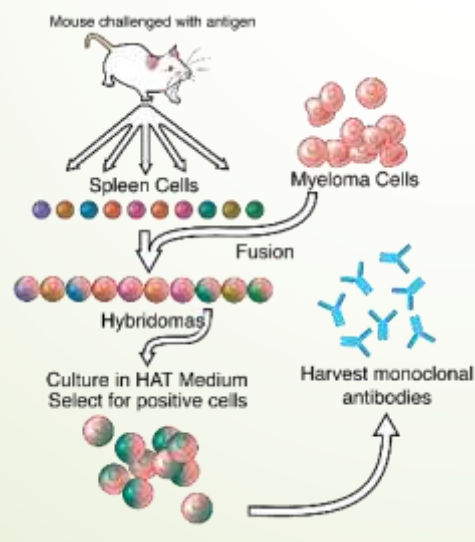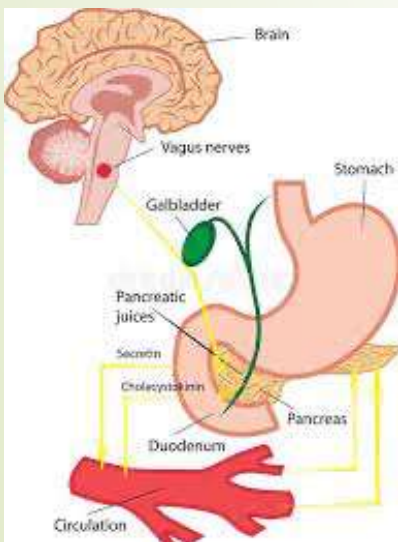
Advent of Diagnostic Immunohistochemistry
Clive Taylor Editorial Milestones in Immunohistochemistry and Molecular Morphology

Google images

1971 Julia Polak – IF detection of peptide hormones in carboimide fixed paraffin embedded tissue sections
1974 Clive Taylor – IP detection of immunoglobulins in formalin-fixed paraffin-embedded tissue sections
1975 Georges Kohler & Cesar Milstein - Production of antigen specific monoclonal antibodies, mAbs
1987 David Mason & Kevin Gatter – Introduction of mAbs to diagnostic immunohistochemistry
1991 Shi SR et al Antigen retrieval in FFPE for widespread use of mAbs in immunohistochemistry

Advent of Automated Immunohistochemistry
(Google images)

Semi-Automation – Dewax & HIER manual

Manual Method
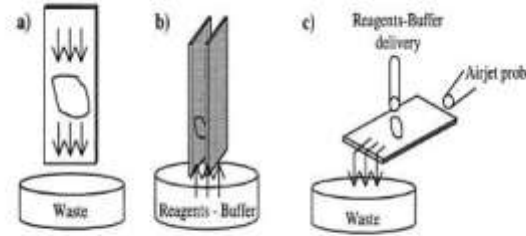
Autostainer Dako (Classic, Plus, 48Link)

a) Cadenza b) Techmate c) ES Ventana

Fig. 1. Automation of IHC – Principles (a) top-down capillarity, (b) ascendant capillarity, (c) flat immunohistolabelling.

Dr. Tom Grogan, MD
Ventana Founder

1988 David Brigatti - Techmate 500
1992 Tom Grogan - ES Ventana
1996 Dako's first autostainer

Full Automation – Dewaxing to Counterstaining

Bond Max

Bond-III

Benchmark XT &
Ultra Plus

Omnis

Advent of Predictive Immunohistochemistry (IHC)

ER IHC
1973 William McGuire
1990 David Allred

HER2 IHC
1989 Dennis Slalom
1997 Michael Press

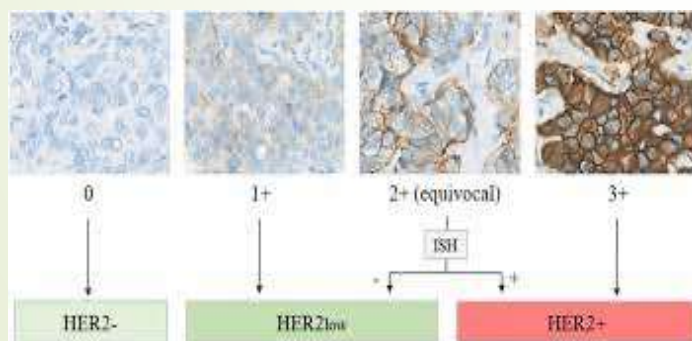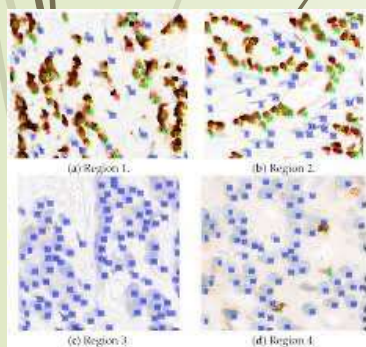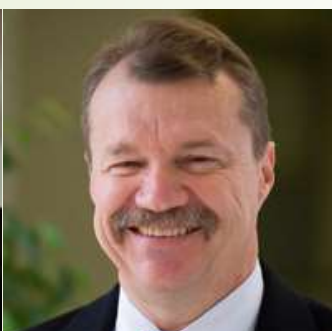EQA & Standardisation
1985 Keith Miller, UKNEQAS
2021 Emina Torlakovic, IQNPath

Ki-67 & PD-L1 IHC
2011 Mitch Dowsett
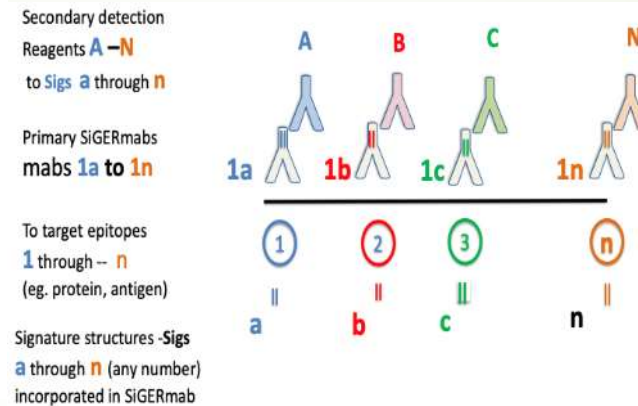2016 Kenneth Emancipator
2018 Debra Ann Hanks
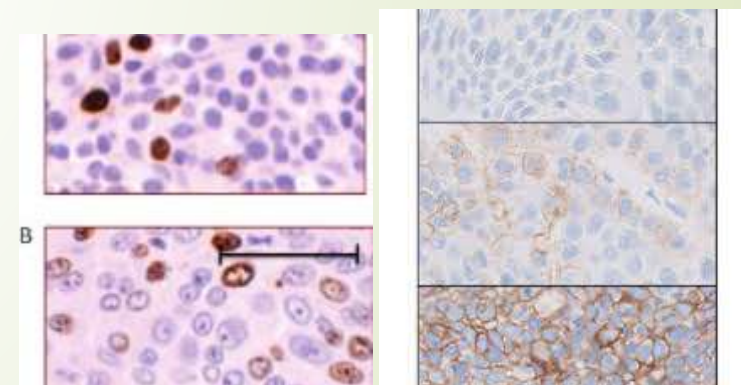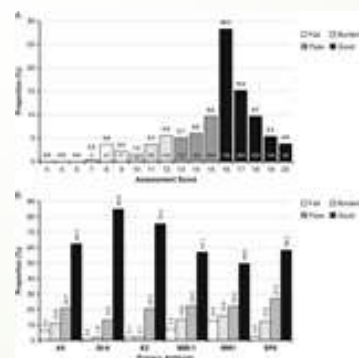
Advent of Multiplex Computational Immunohistochemistry

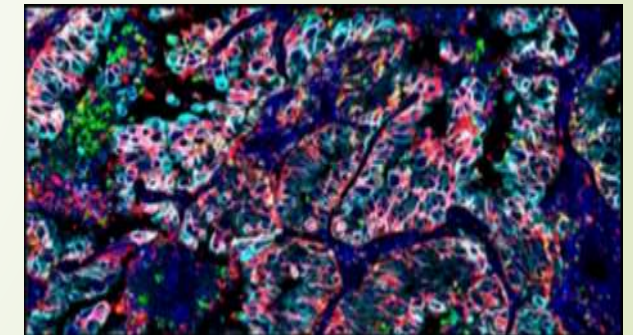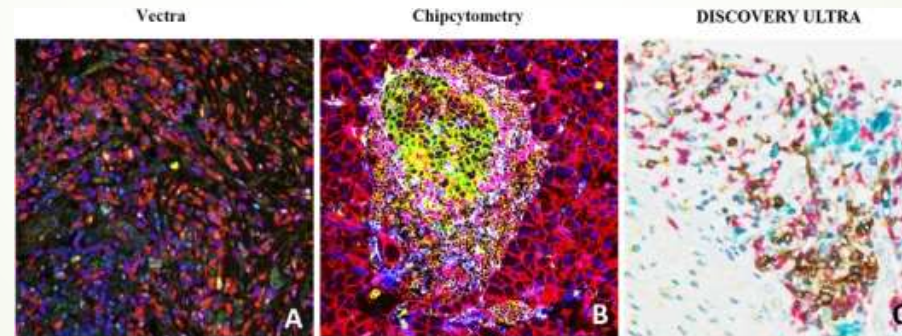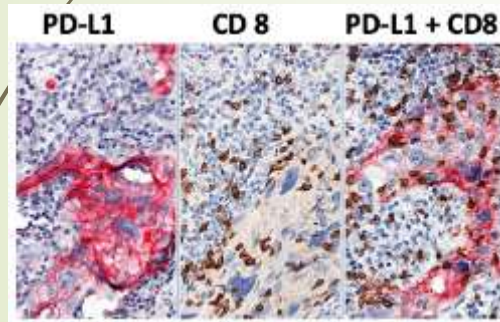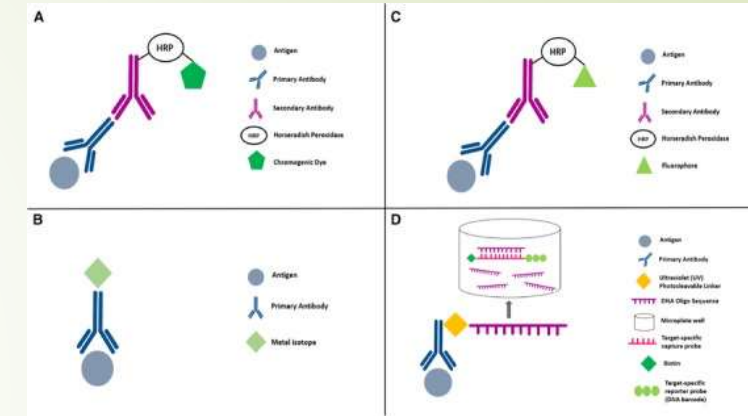(Adapted from Jasani, Huss & Taylor, Springer Nature, 2021)

Multiple Biomarker Target
Multicompartmental analysis
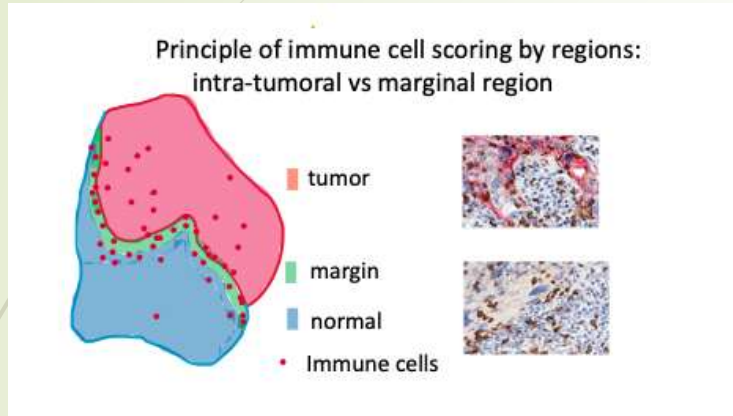
Simultaneous Application of
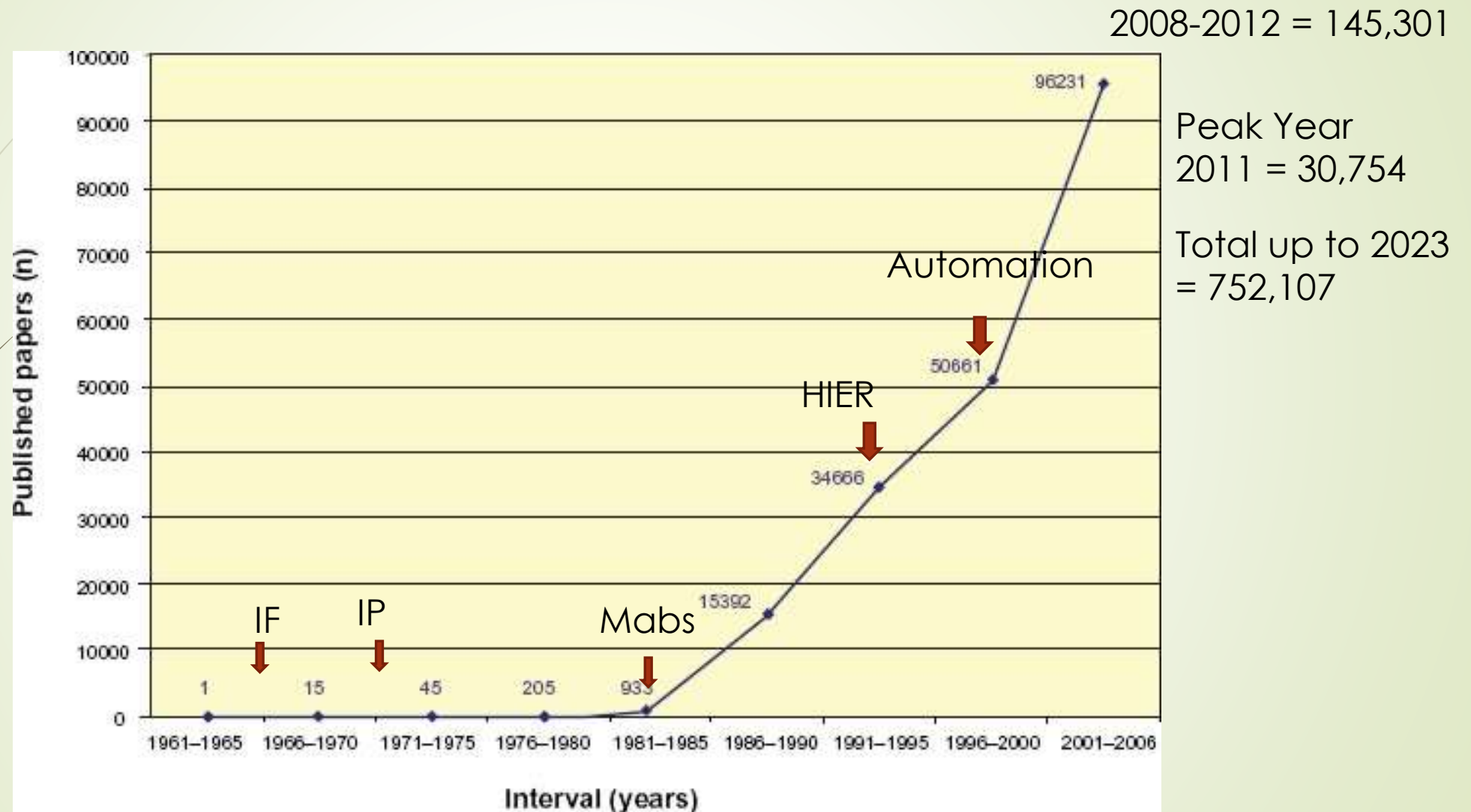Primary Ab/Detection Reagents

Multiple Reporter labels

Potential to provide comprehensive cellular spatial information, allowing greater insight into the pathogenesis of cancer and responsiveness to immunotherapy
Cost and time-ineffectiveness for routine diagnostic use and need for bioinformatic skills & experience

Tan et al Overview of multiplex immunohistochemistry/ immunofluorescence techniques in the era of cancer immunotherapy Cancer Communications. 2020;40:135–153.

Impact of Methodological Advances in Immunohistochemistry

2008-2012 = 145,301

Peak Year
2011 = 30,754

Total up to 2023
= 752,107

Adapted from Werner et al. J Bras Pathol Med Lab. 2005;41:353–64

Thank you for
Your
Attention