Immunocytochemistry

Definition: The use of labeled antibodies as specific reagents for the localization of tissue components (antigens) "*in situ*".

This technique bridges anatomy, immunology, biochemistry, and molecular biology.

It completely relies on antibodies specific to their antigen.

Best method largely determined by:

1) type of information desired

2) nature of antigen

3) effect on antigen of fixation and embedding

4) subcellular distribution

Requirements for ICC

1. Preservation of Antigens

a. insoluble

b. antigenic sites available without alteration of the tertiary structure

c. Preservation of the surrounding tissue architecture so can interprete ICC reaction:

Chemical fixatives
Frozen sections
Pre-/post embedding
Freeze substitution
Embedding resins
Pretreatment of sections

2) Specific Staining

a) "background"
endogenous peroxide
cross-reactivity

b) Polyclonals

c) Monoclonals

3) Well Characterized Antibody

a) high affinity

b) avidity (binding strength)

c) titor or concentration of $1^{0}\alpha$

4) Easily Visible Label

Choose the best method by considering:

1) preparation of material being studied

2) the marker

3) linking molecule (s)

History

1) Fluorescent-labeled Probes

1941-50 Conns, etal

Problems: LM only
Impermanence
Poor morphology

2) Ferritin Labeled Antibody

Singer and Schick (1961)

Advantage: visible in EM
Disadvantage: small
hard to see via LM
Prep of conjugate difficult
Penetration difficult
Aggregates

3) Enzymes (1966)

Primarily HRP, Avidin-biotin

Advantages: Stable
- available in pure form
- Variety of chromagens
- See in LM and EM
- Pre-embed-can penetrate

Disadvantages:
- DAB carcinogenic
- HRP e- density not high
- Can over develop
- More steps to process

4) Radioactive Tags

incubate with radioactive amino acids (P^{32}, I^{125}, H^{3}

Disadvantages:
- difficult to localize label
- long developments

5) Colloidal Gold

Advantages:

a) visible in LM & EM
b) simple
c) stable
d) Conjugate preps easy
e) can use double label

Disadvantages:

a) poor penetration
b) surface label
c) less efficient than enzymes

Gold particle Size	Mean Number Particles/μm^2	Standard Deviation
5nm	746	±105
10nm	390	± 84
15nm	227	± 40
20nm	141	± 26
30nm	167	± 18

Variations:

1) IgG - bridge >> PAP complex

react with DAB in presence of H_2O_2

2) IgG conjugated to Colloidal Gold

different sizes

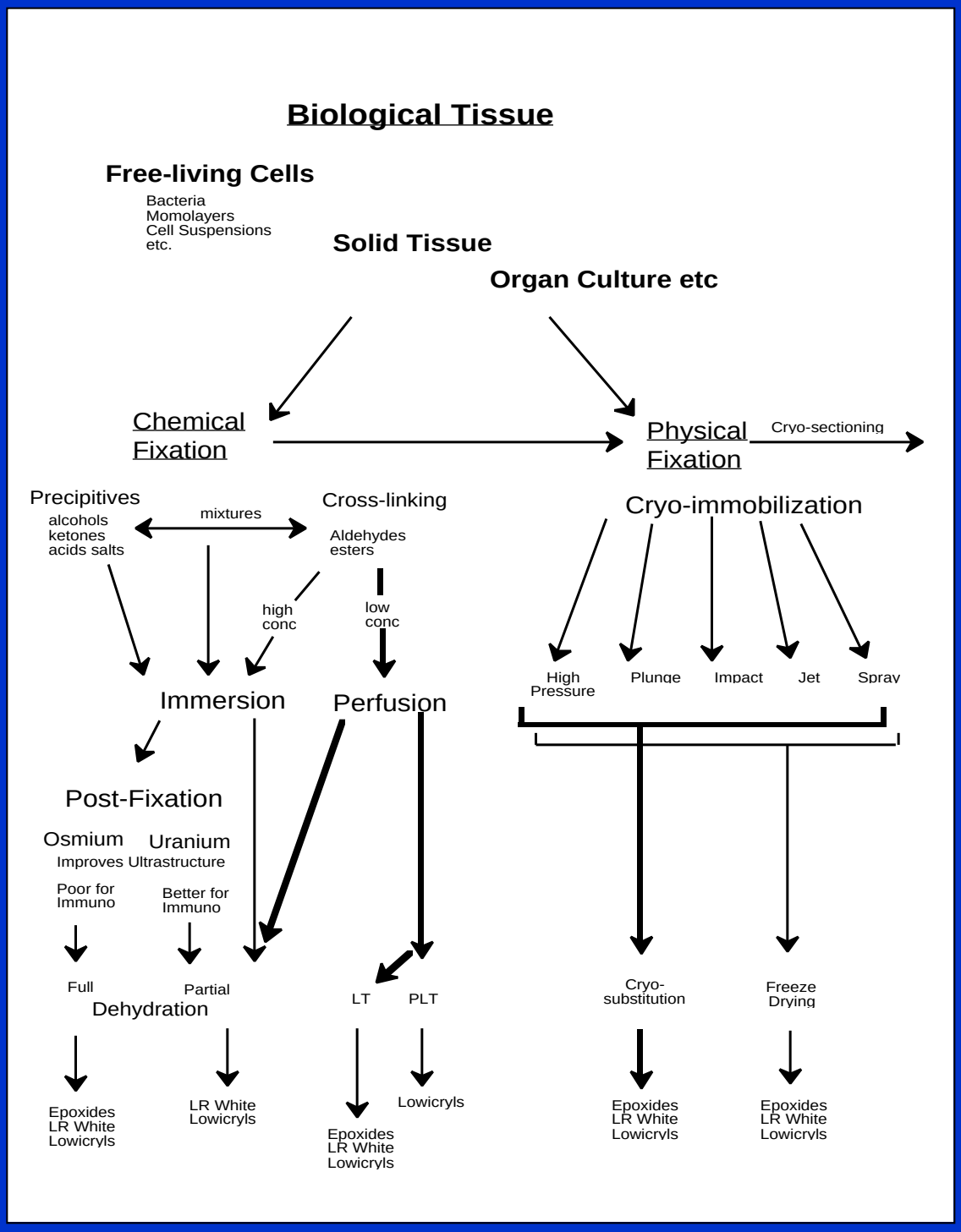
3) IgG conjugated to ferritin

4) Protein A from bacterium *Staphylococcus aureus*

small (42,000 mw)….not species specific
conjugates to all known markers without loss of activity

5) Avidin-Biotin

use biotinylated IgG as bridge
use avidin conjugated to peroxidase complex with biotin to attach HRP marker
develop with DAB for dark brown ppt.


Biological Tissue
Free-living Cells
Bacteria
Momolayers
Cell Suspensions
etc.
Solid Tissue
Organ Culture etc
Chemical Fixation
Physical Fixation
Cryo-sectioning
Precipitives
alcohols
ketones
acids salts
mixtures
Cross-linking
Aldehydes
esters
high conc
low conc
Cryo-immobilization
High Pressure
Plunge
Impact
Jet
Spray
Immersion
Perfusion
Post-Fixation
Osmium
Uranium
Improves Ultrastructure
Poor for Immuno
Better for Immuno
Full Dehydration
Partial Dehydration
LT
PLT
Cryo-substitution
Freeze Drying
Epoxides
LR White
Lowicryls
LR White
Lowicryls
Epoxides
LR White
Lowicryls
Lowicryls
Epoxides
LR White
Lowicryls
Epoxides
LR White
Lowicryls

Fixation

Ultrastructure

1º fix **3-4% GLutaraldehyde**

Excellent cross-linker

Karnovsky's Fix:

Mix of Formaldehyde (penetration)
and Glutaraldehyde (x-linker)

2º fix **1-2% Osmium tetroxide**

Cross-linker, Contrast

Immunocytochemistry

1º fix **% PAF + 0.5% Glutaraldehyde**

Good penetration

Poor cross-linking

2º fix **No Secondary**

Resin Embedding

- **Ultrastructure**

  Spurr's Resin

  Epoxy resin mixture

  Low viscosity

  Polymerize at 60°C

- **Immunocytochemistry**

  LR White

  Acrylic monomer

  Low viscosity

  Hydrophilic - polar

  Polymerize at 55°C

  Lowicryl - HM 20

  Low viscosity

  Hydrophobic - nonpolar

  UV-polymerized (360mm long-wavelength)

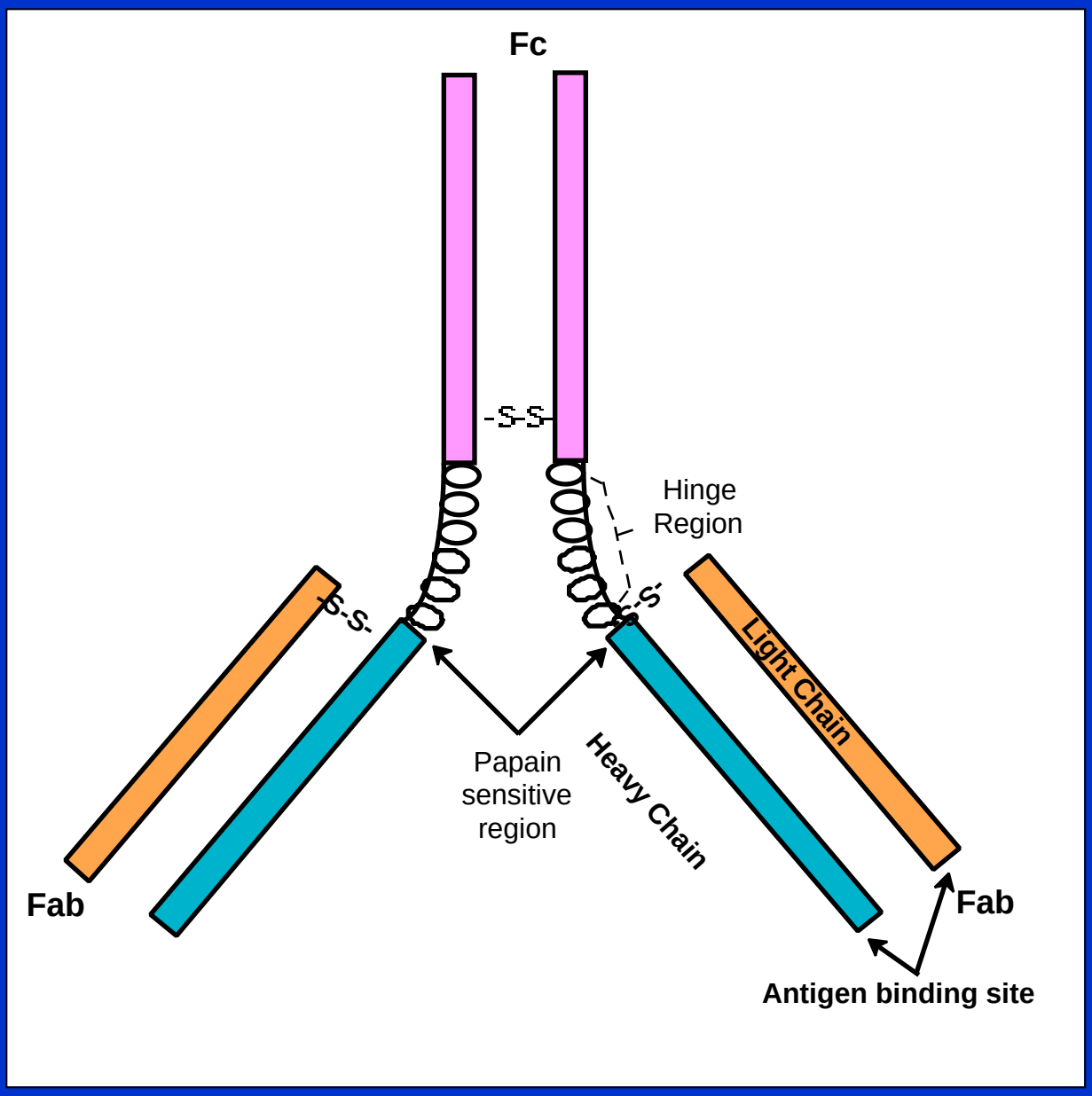
  Polymerize at low temperature:
  (+4°C > -20°C)

Different methods developed:

1) Direct - markers bound directly to primary antibody

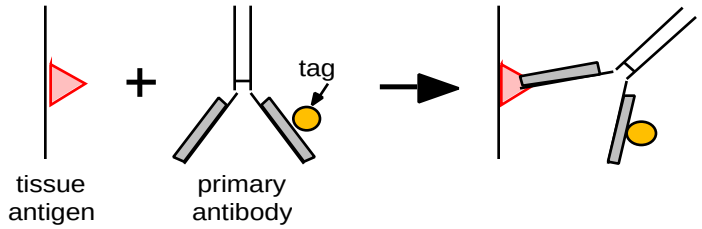
2) Indirect - linker molecules uses between primary Antibody and marker

Structure of the IgG Molecule

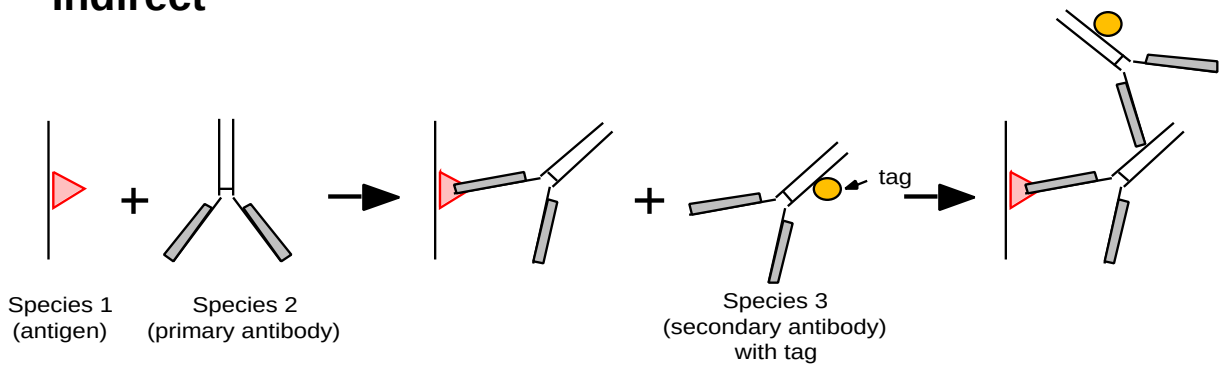


Immunocytochemical Methods

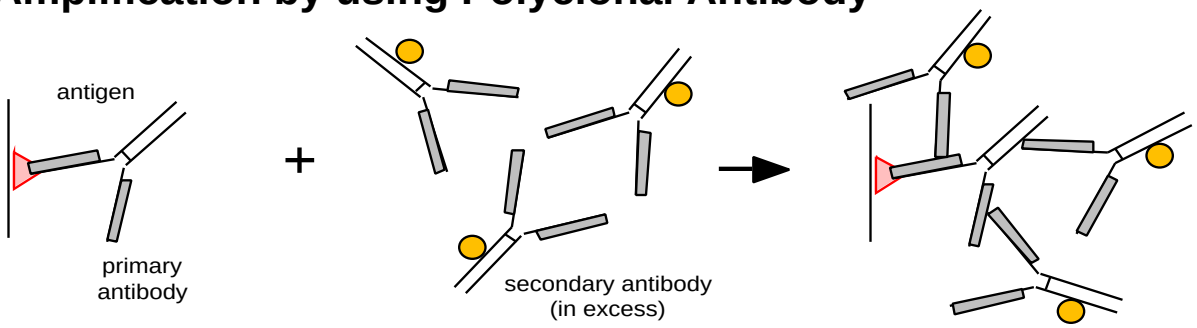
Direct



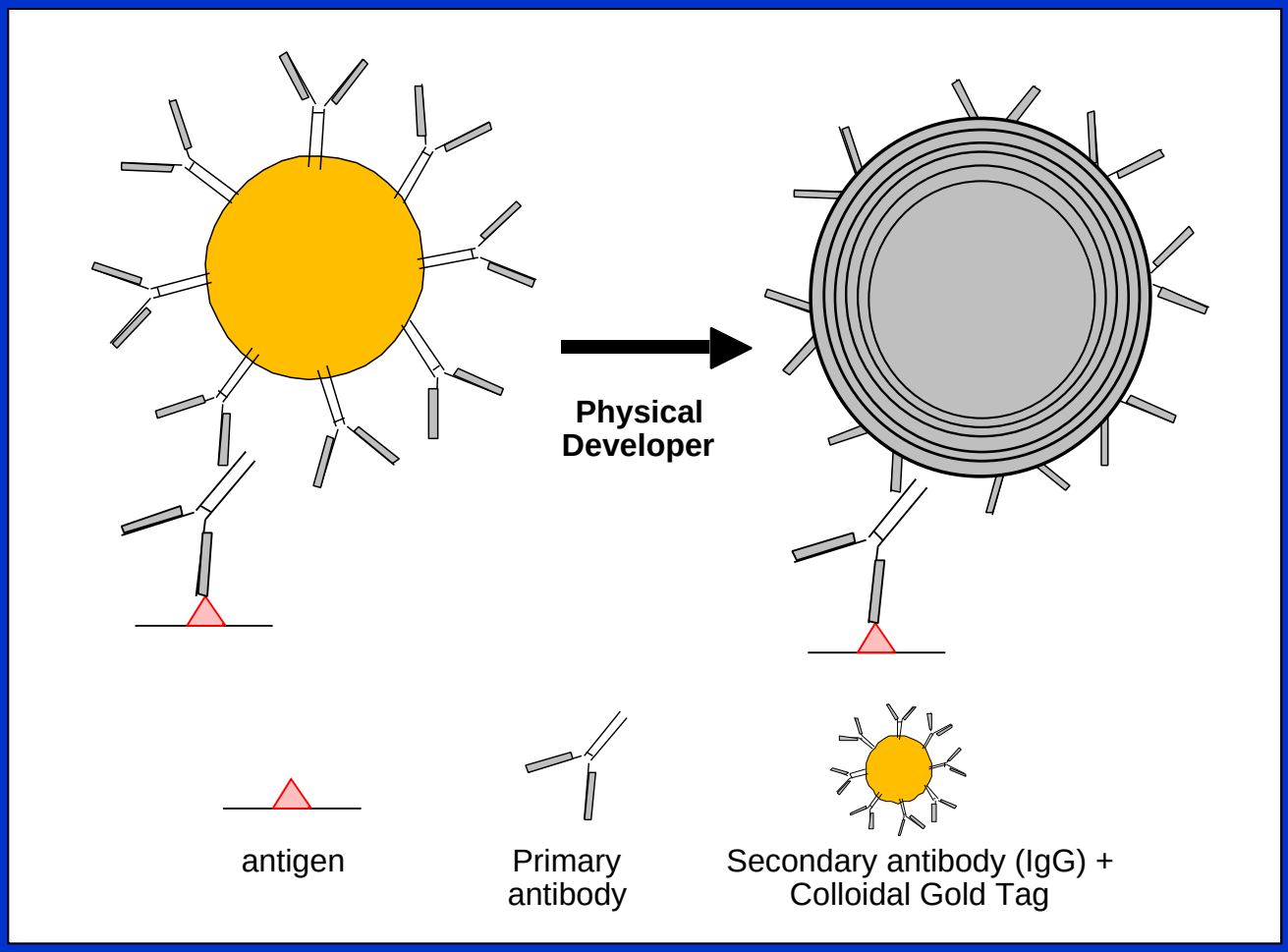
Indirect



Amplification by using Polyclonal Antibody



Silver Intensification of Colloidal Gold Probes



SBMV virus

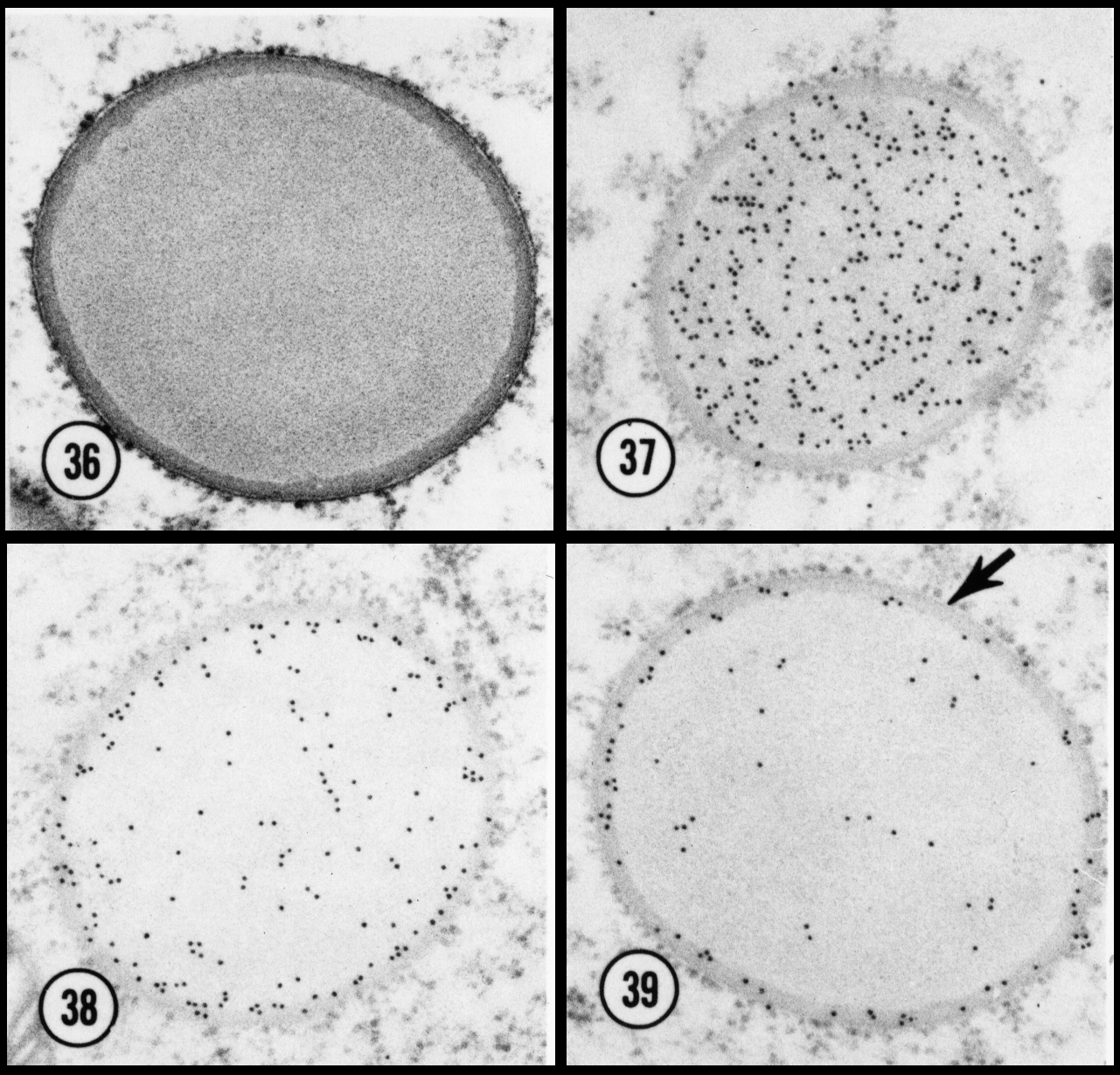
Virus + antibody

C. E. Bracker

Chitesomes decorated with Colloidal Gold

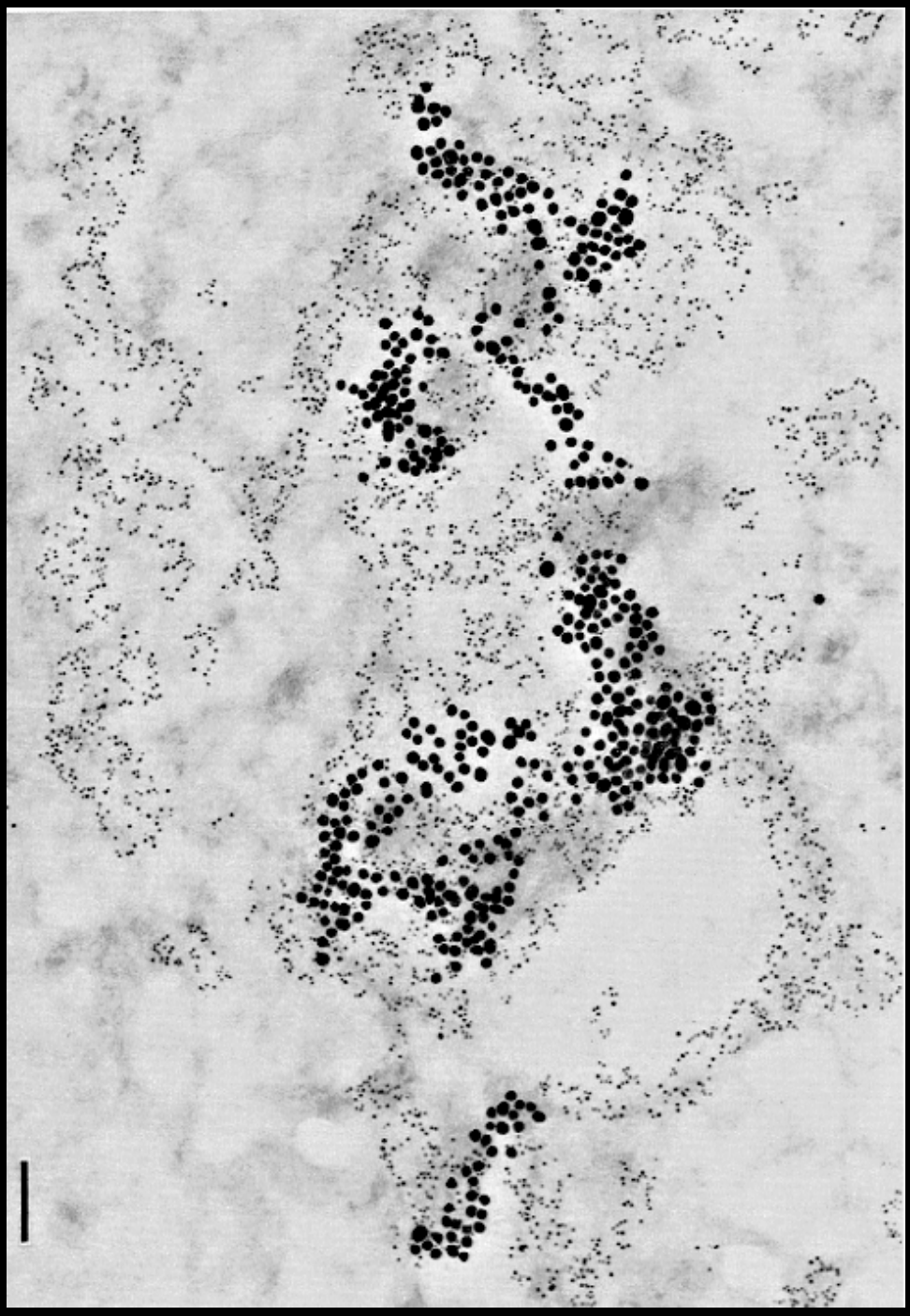
C.E. Bracker

Zein Distribution in Protein Bodies



Jeanette Shull

Double Labeling ICC Localization



Colloidal Gold labeling Artifact