Ultra small Immunogold labeling
&
Optimizing Signal-noise ratios.

Jan L.M. Leunissen[§¶] and Hong Yi[*]

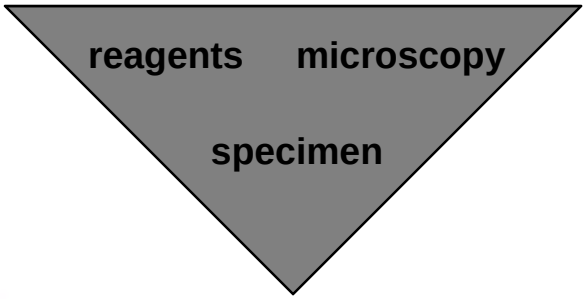
[§]**Aurion Immuno Gold Reagents**, Costerweg 5, 6702 AA Wageningen, The Netherlands
[¶]**Dept Anatomy & Structural Biology**, University of Otago, PO Box 913, Dunedin, New Zealand

[*]**Emory School of Medicine Microscopy Core**, Emory University.
6215 Woodruff Memorial Research Building, 101 Woodruff Circle, Atlanta, GA 30322

Unravel the principles

reagents

- Specificity (all reagents)
- Sensitivity
- Contrast/Detectability
- Transparency
- Resolution



microscopy

- Suitable resolution
- Sensitivity/Detectability
- Should allow interpretation
- Matching label characteristics

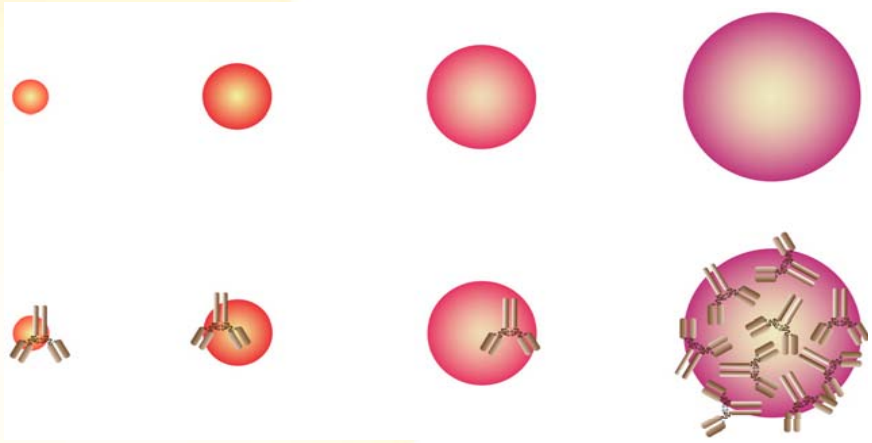
specimen

- Physiological state
- Recognition of the antigen
- Access of reagents
- (Ultra)structure

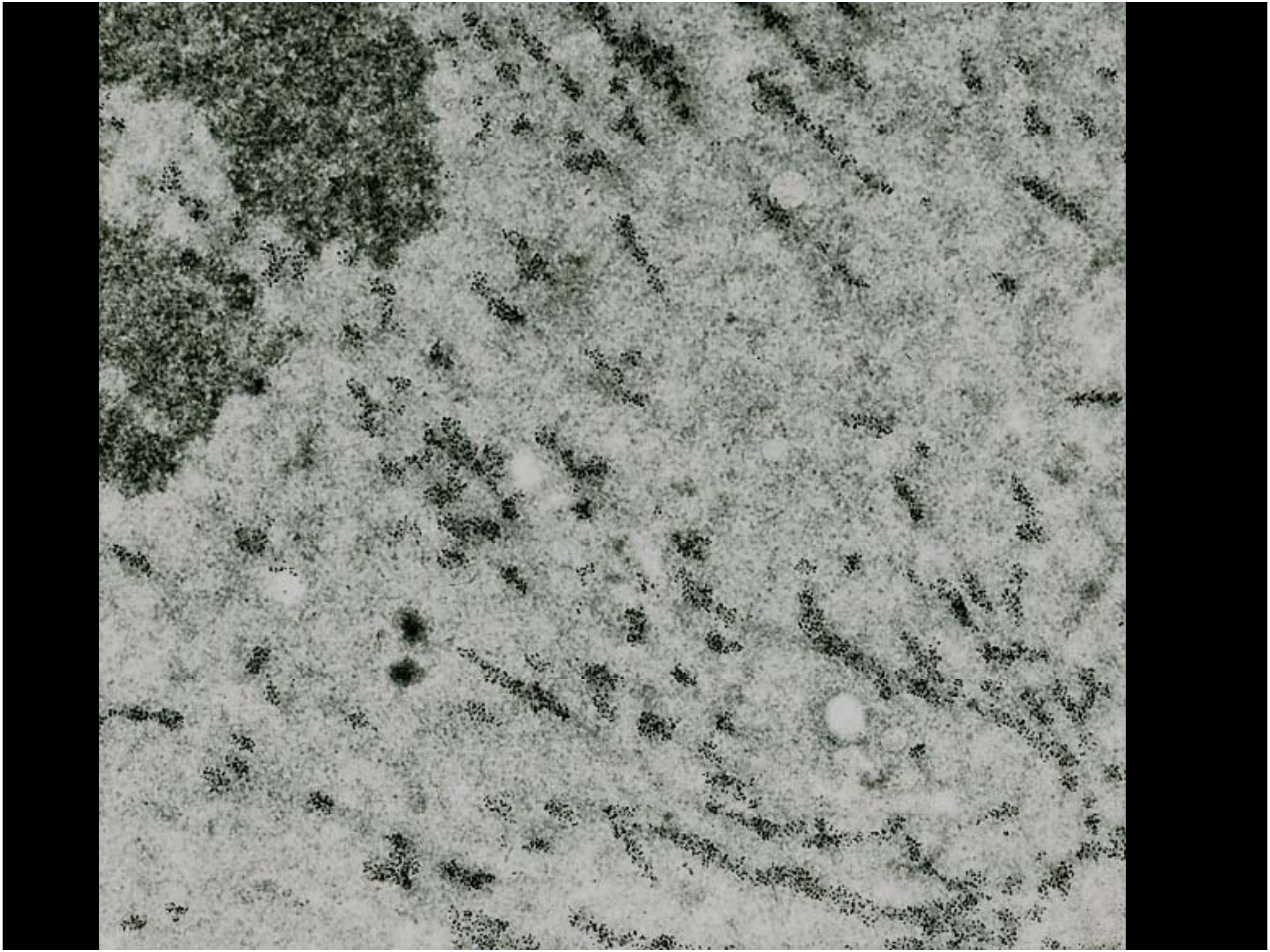
AURION

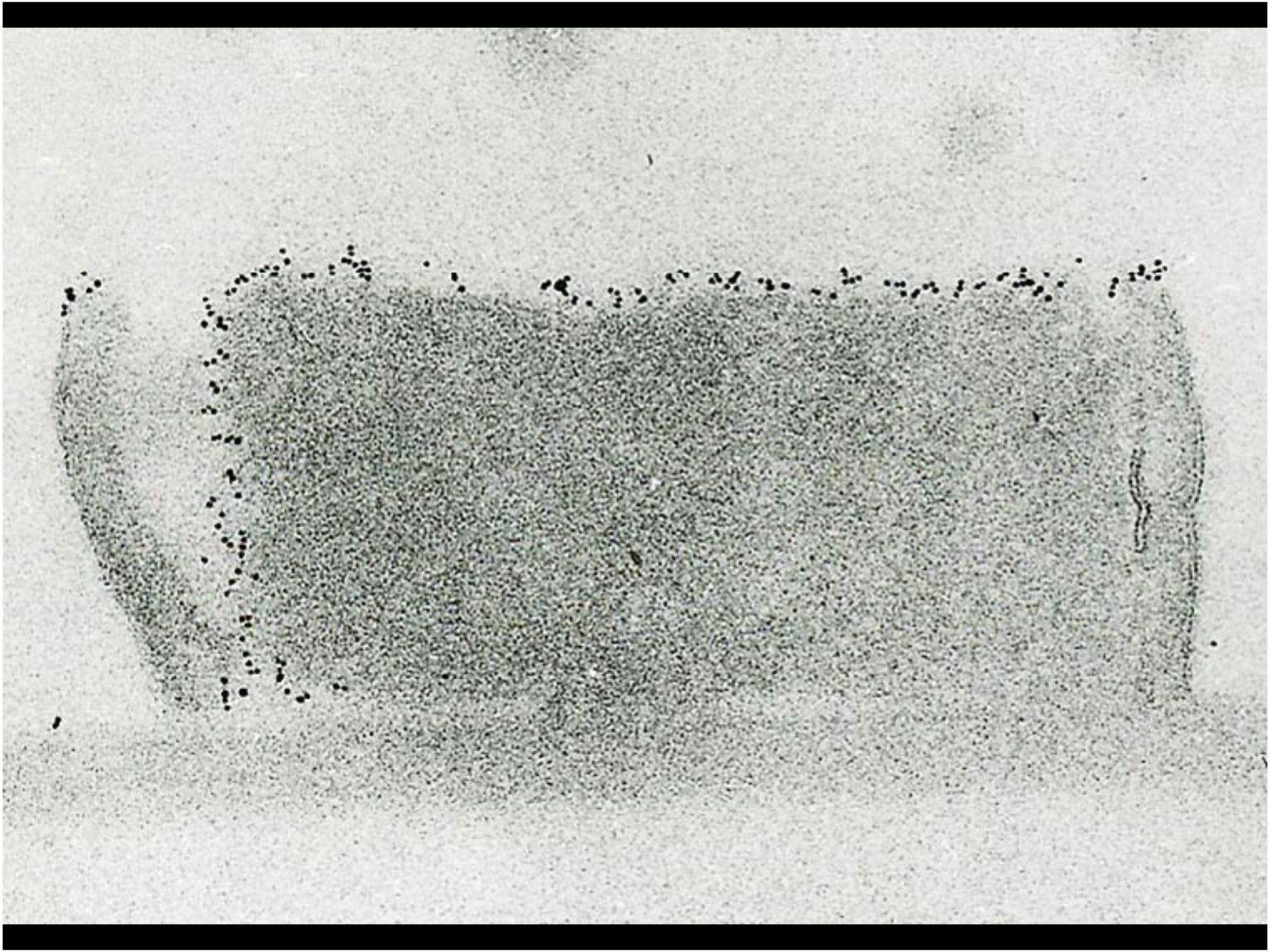
Physical characteristics of Colloidal Gold

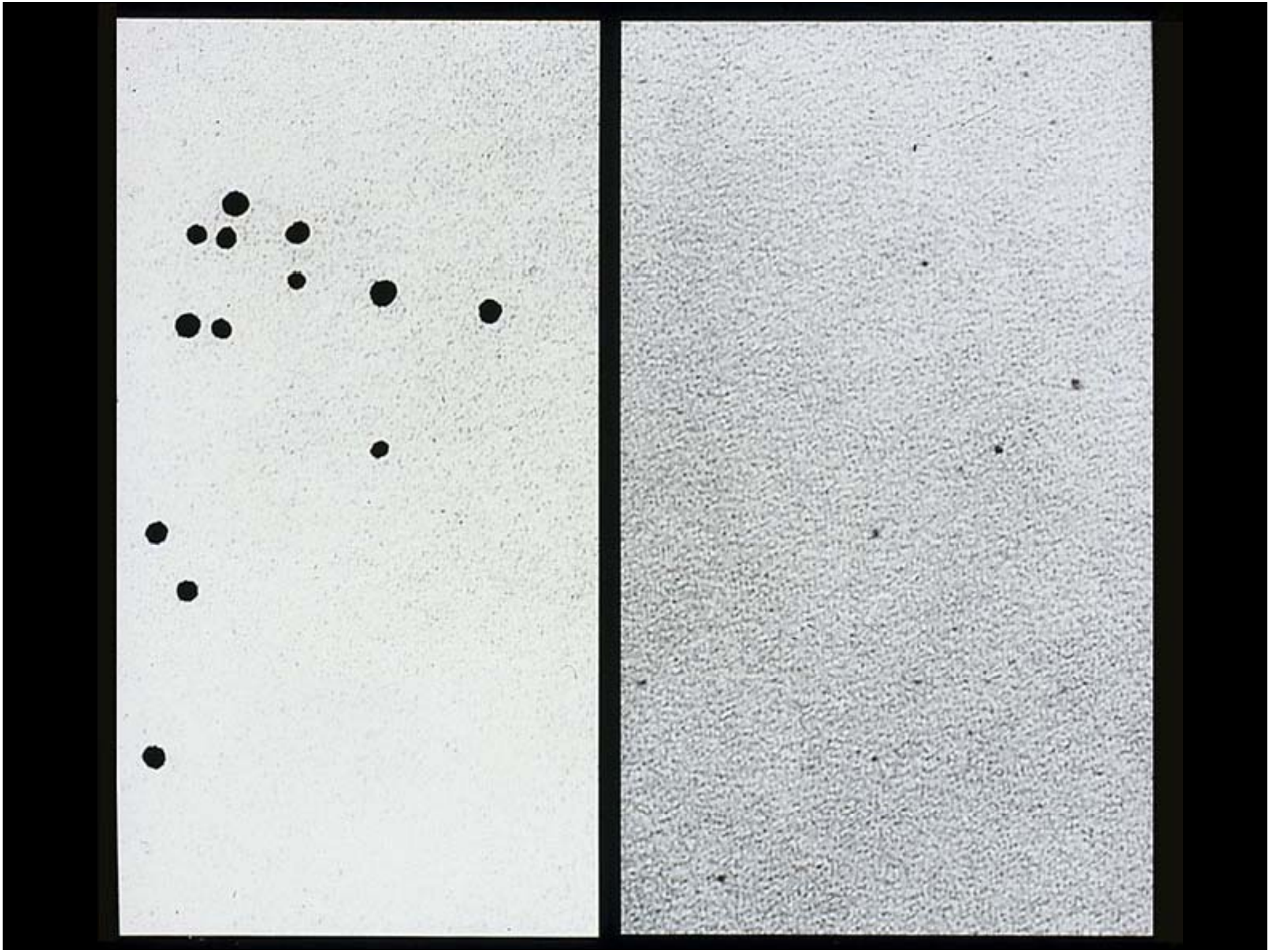
Particle diameter	± #Au atoms	± MWt. (daltons)	± Surface (nm2)	± Volume (nm3)	± # particles /ml	± # Ab (/part.)
6	6500	1.3*10e6	113	113	2.4*10e13	1-2
10	30*10e3	6*10e6	315	525	5*10e12	7-12
15	100*10e3	20*10e6	710	1770	1.5*10e12	25-40
25	470*10e3	92*10e6	1970	8200	3.3*10e11	115-180



AURION







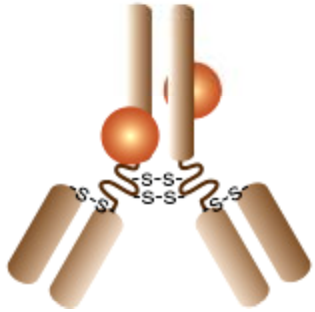
Ultra Small Probes: concept

Reduced overall size

Labeled molecule -vs- coated gold particle

Reduced steric hinderance: higher sensitivity

Improved penetration: ‘new’ applications

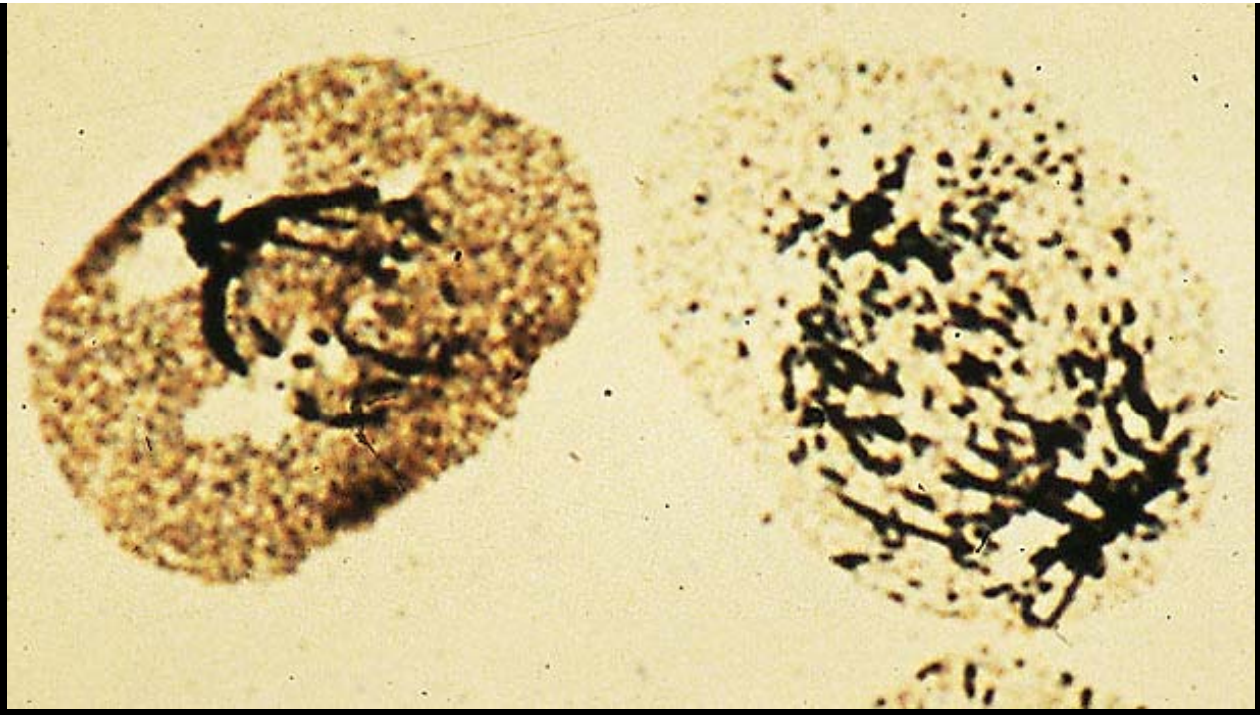


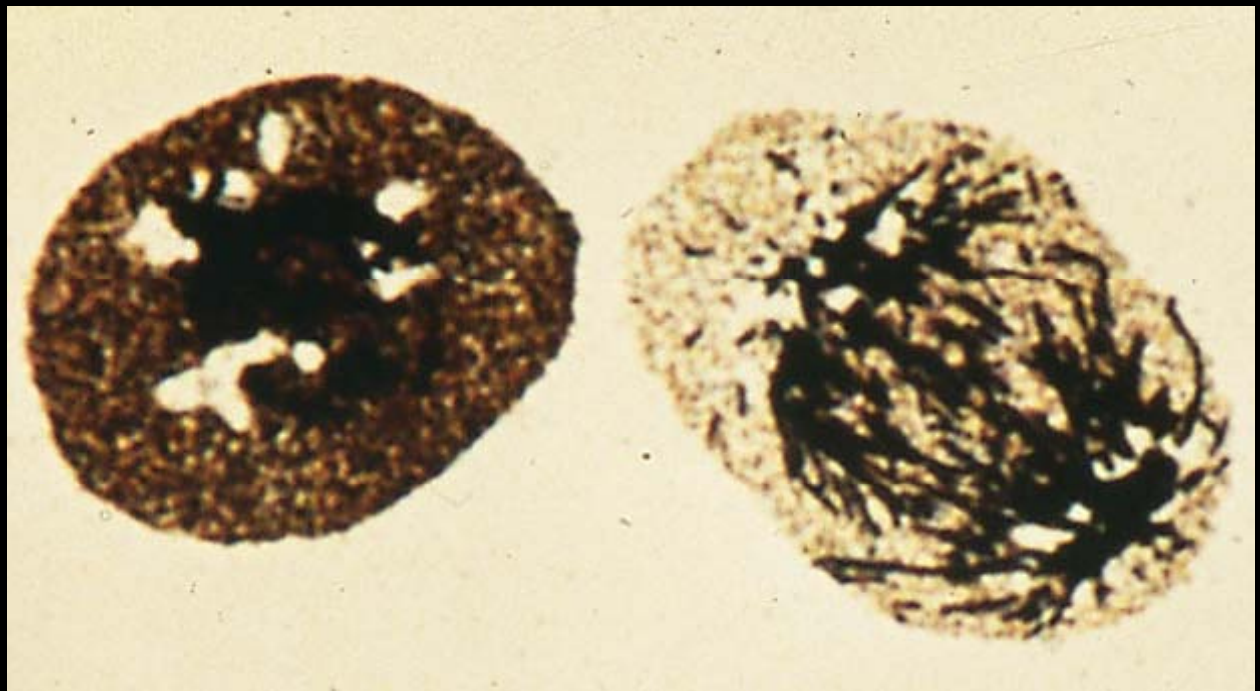
One conjugate....Correlative Microscopy

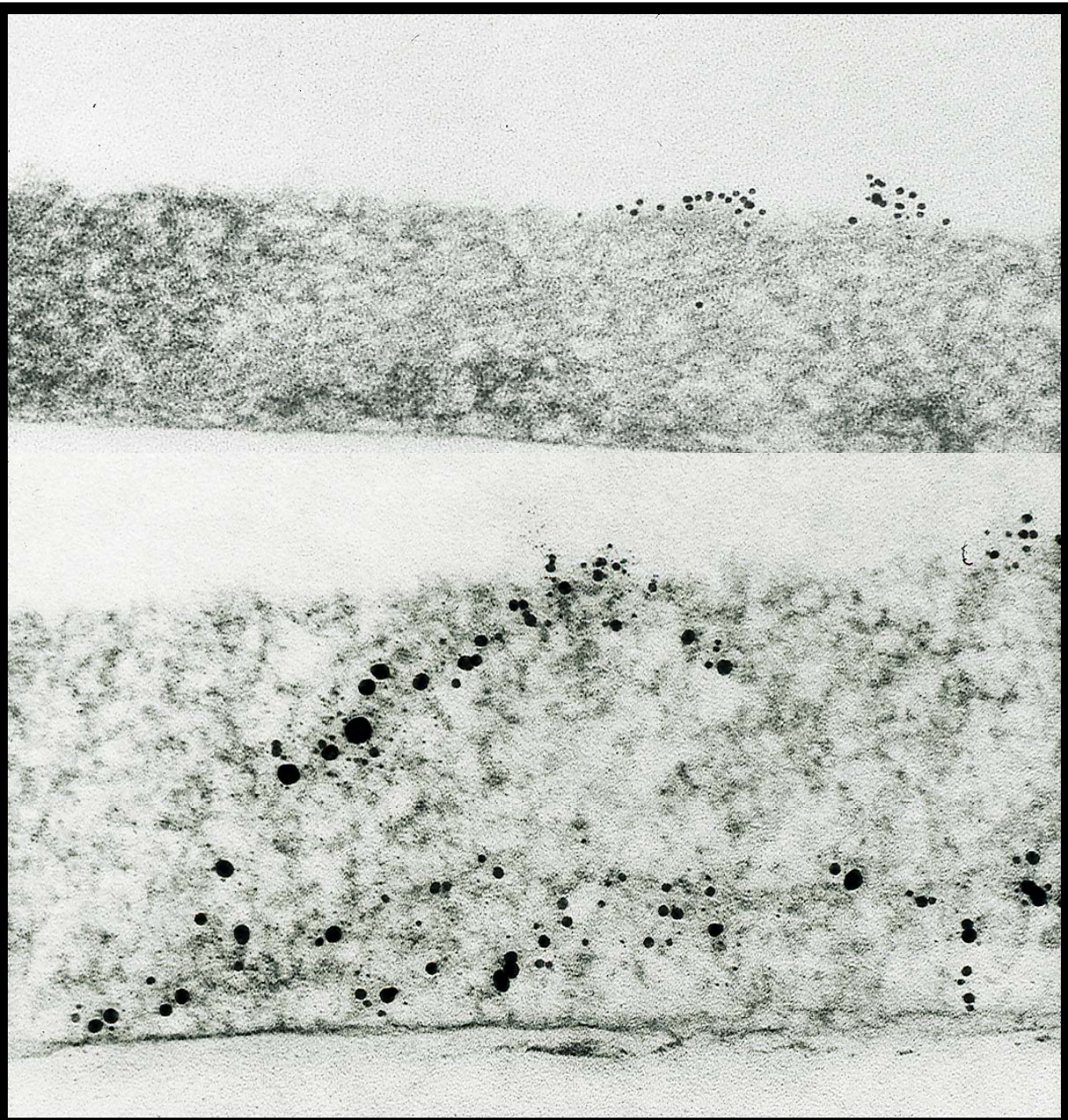
- **Electron Microscopy**
  - **Hydrated Specimens**
  - **Embedded Specimens**
  - **Single and Double Labeling**
- **Light Microscopy**

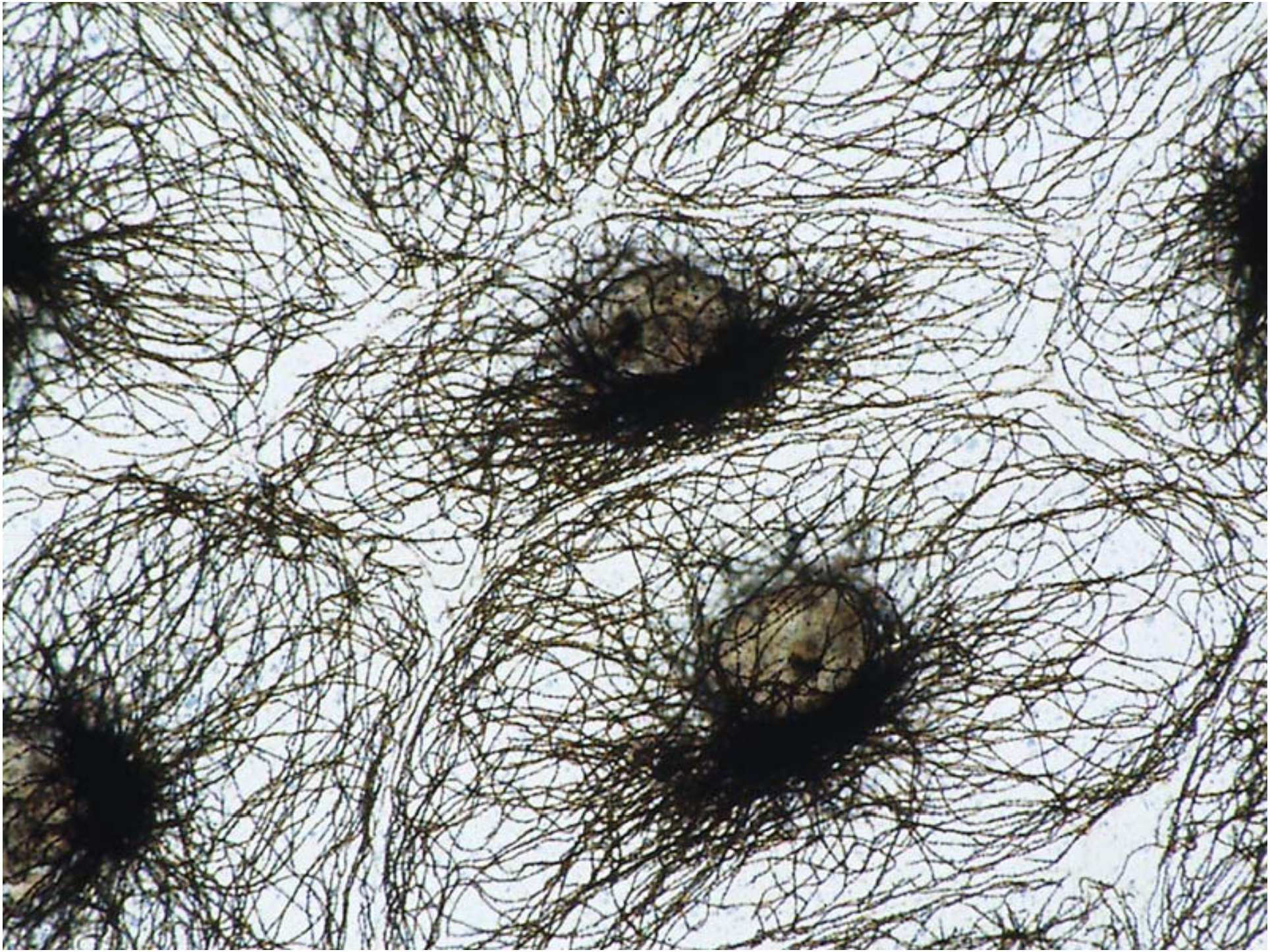
- **Assays**
- **Blotting applications**

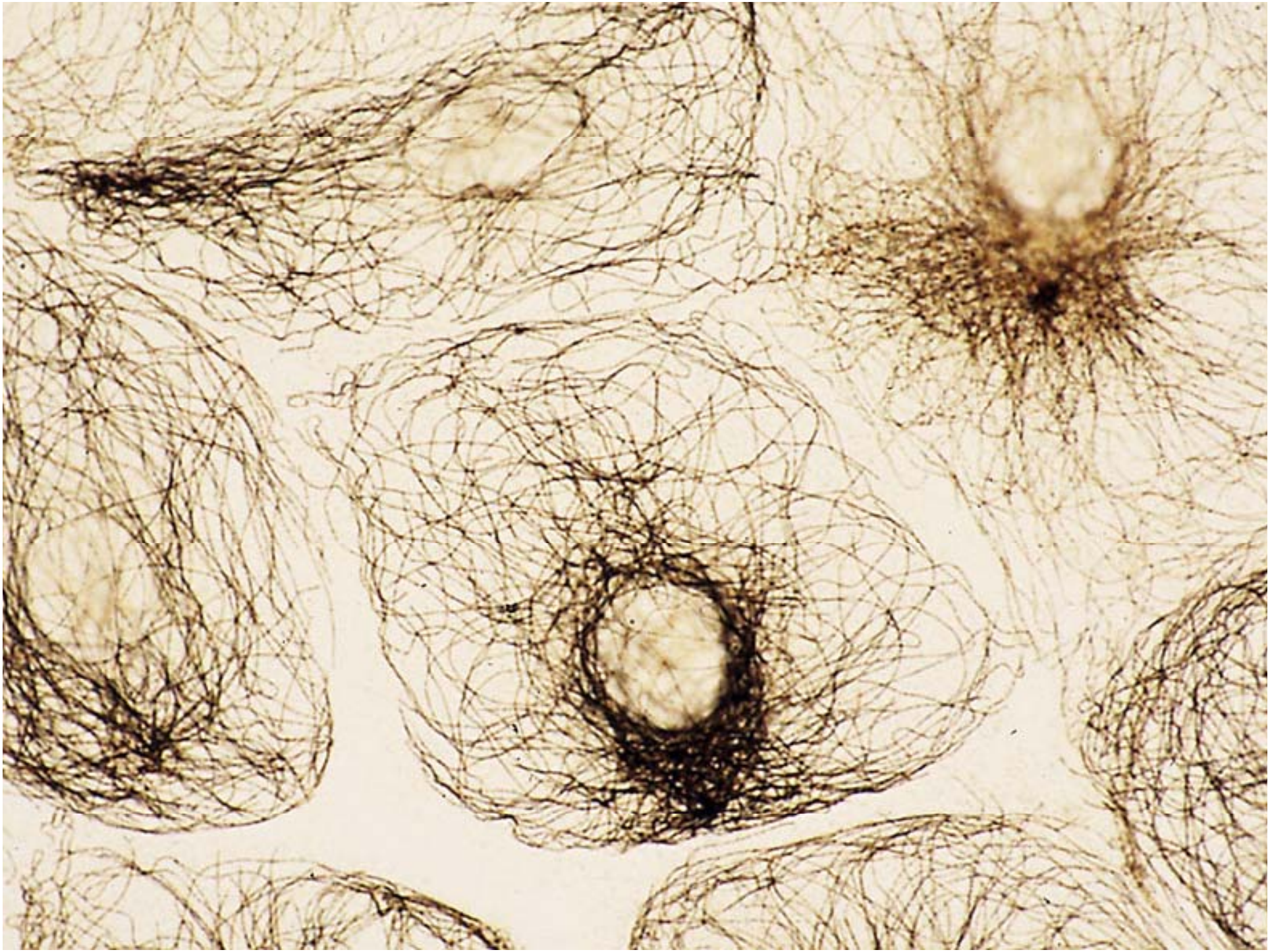
AURION

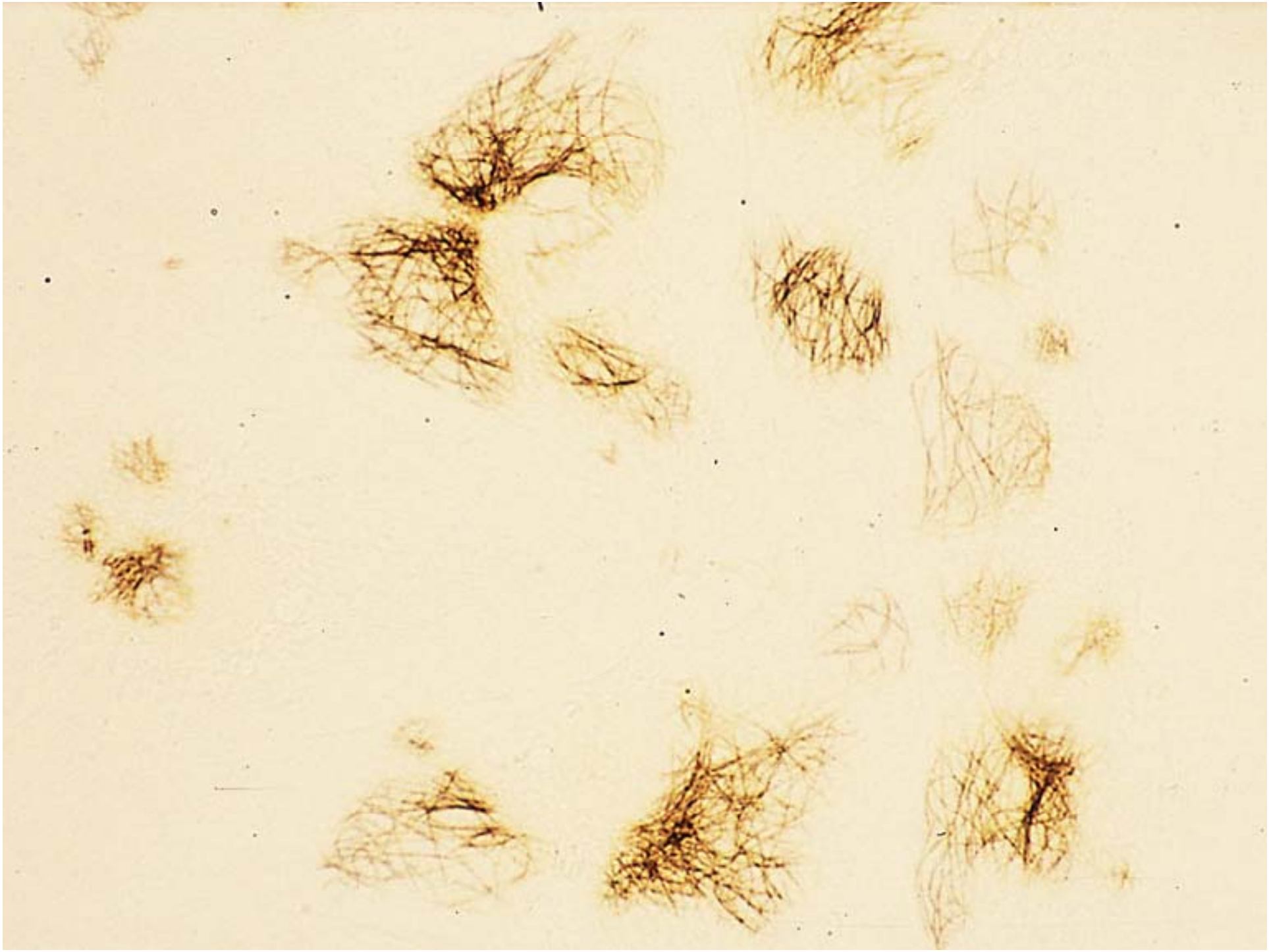


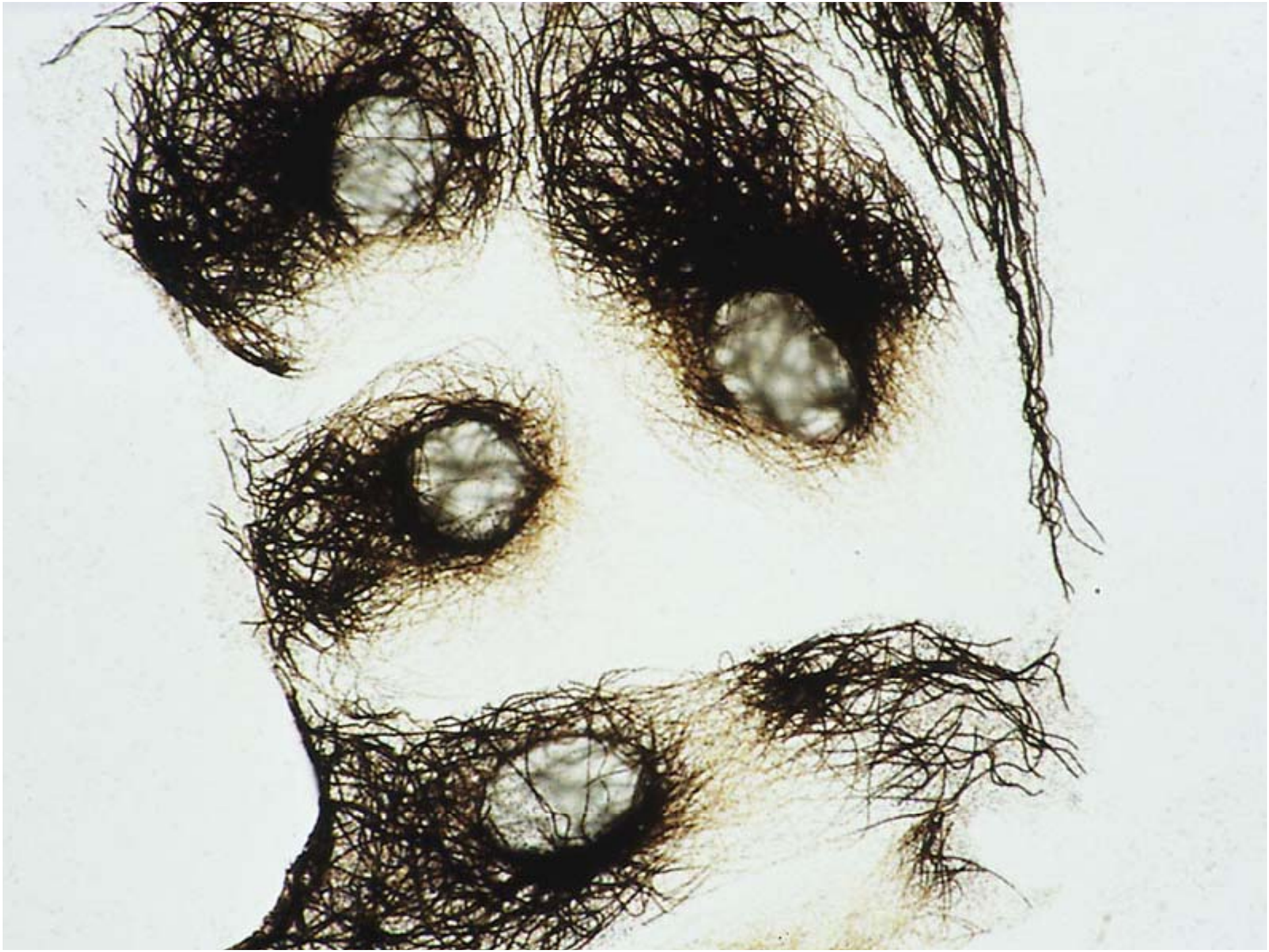






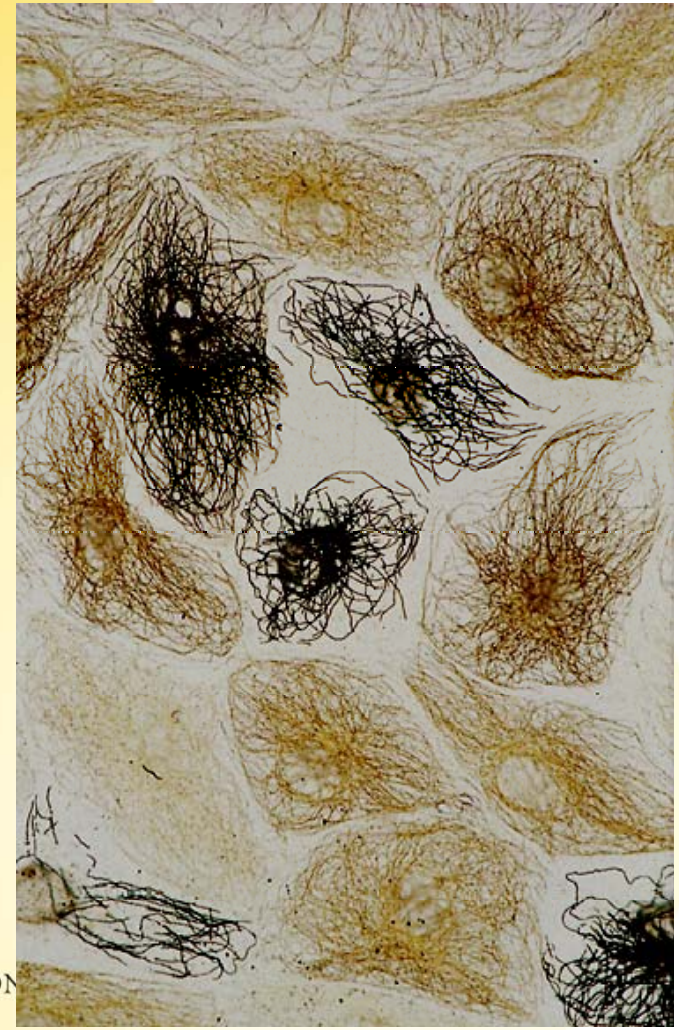


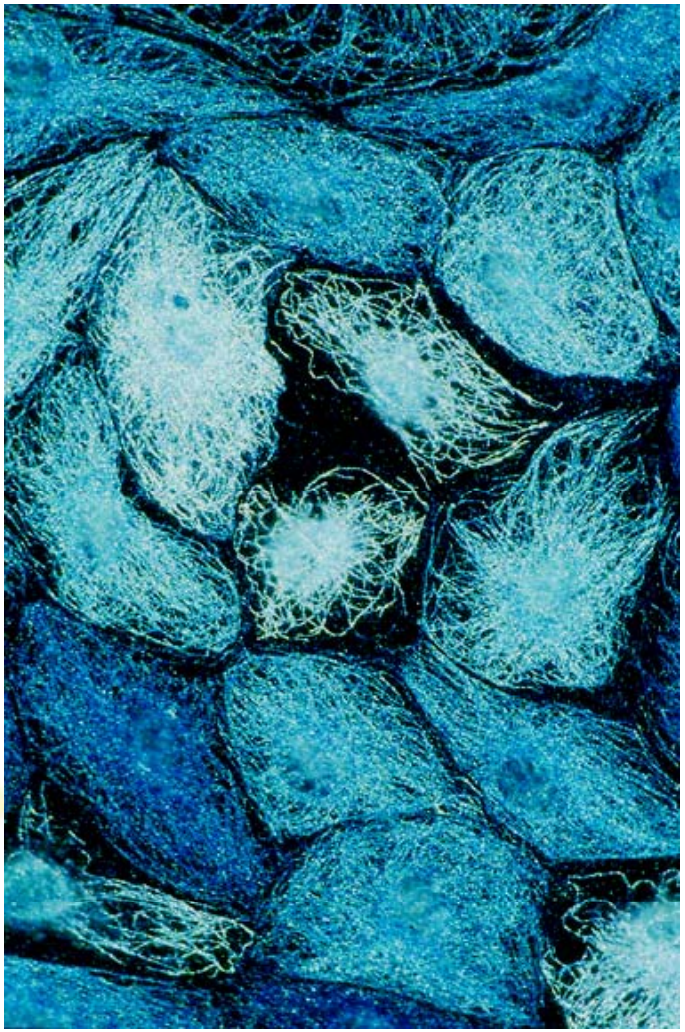




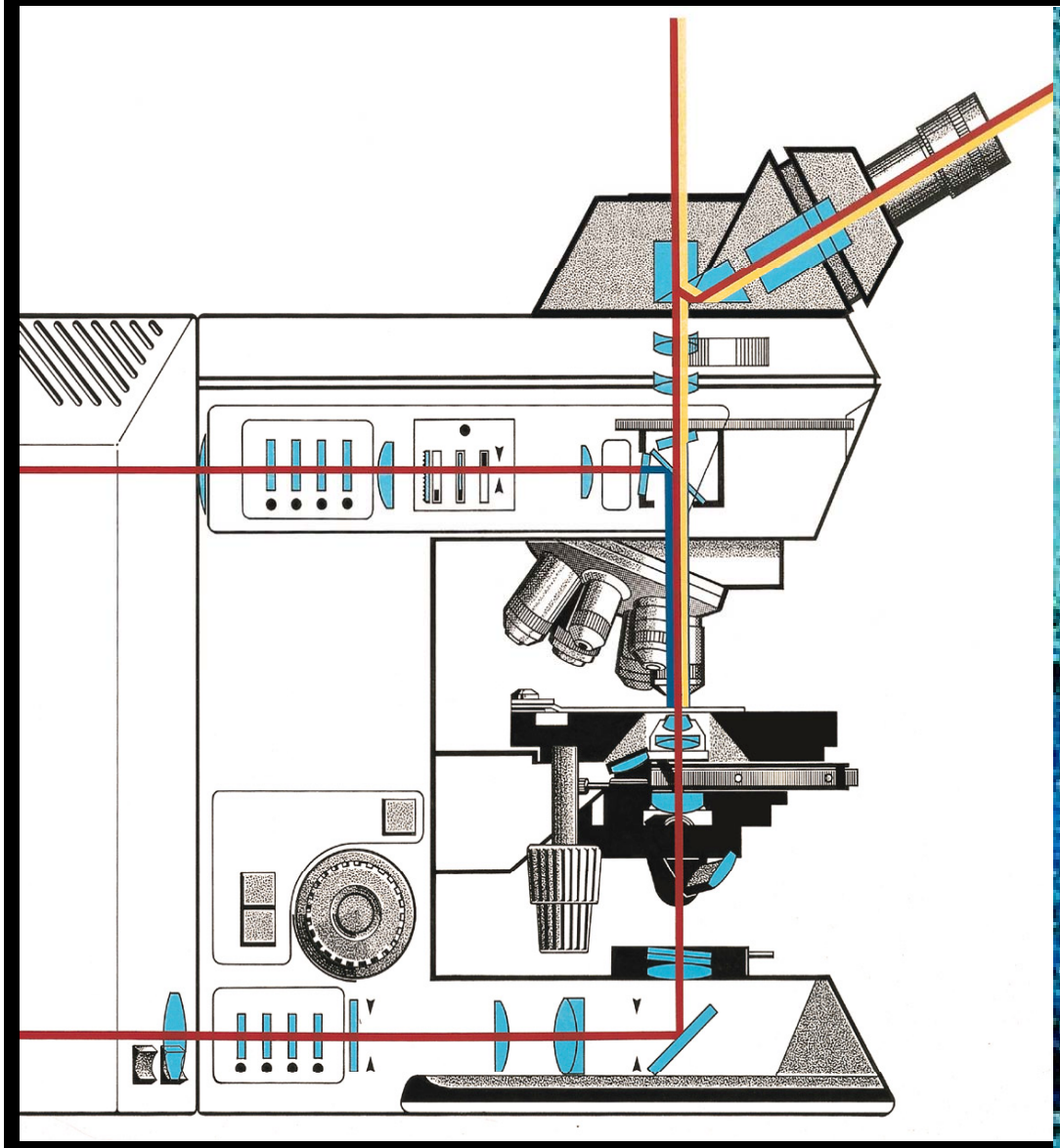
IGSS

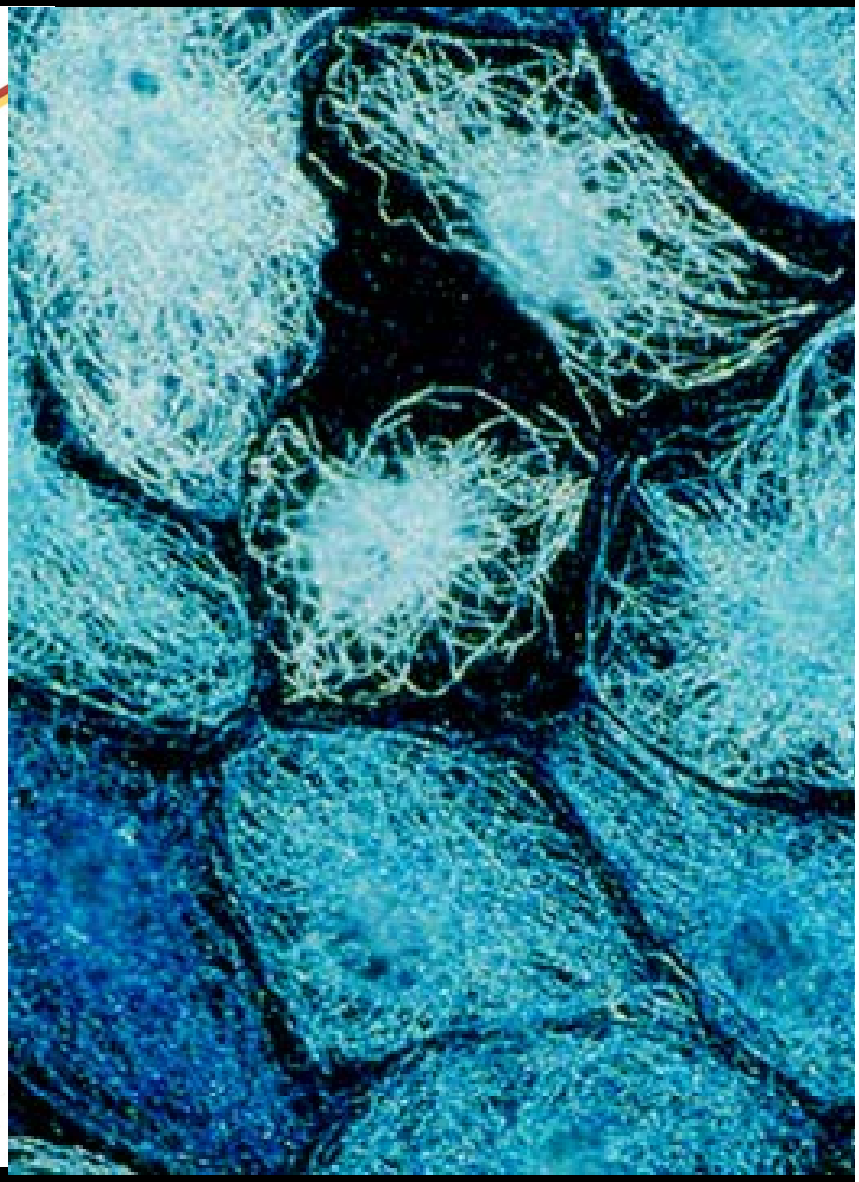
Bright field and epi-polarization microscopy



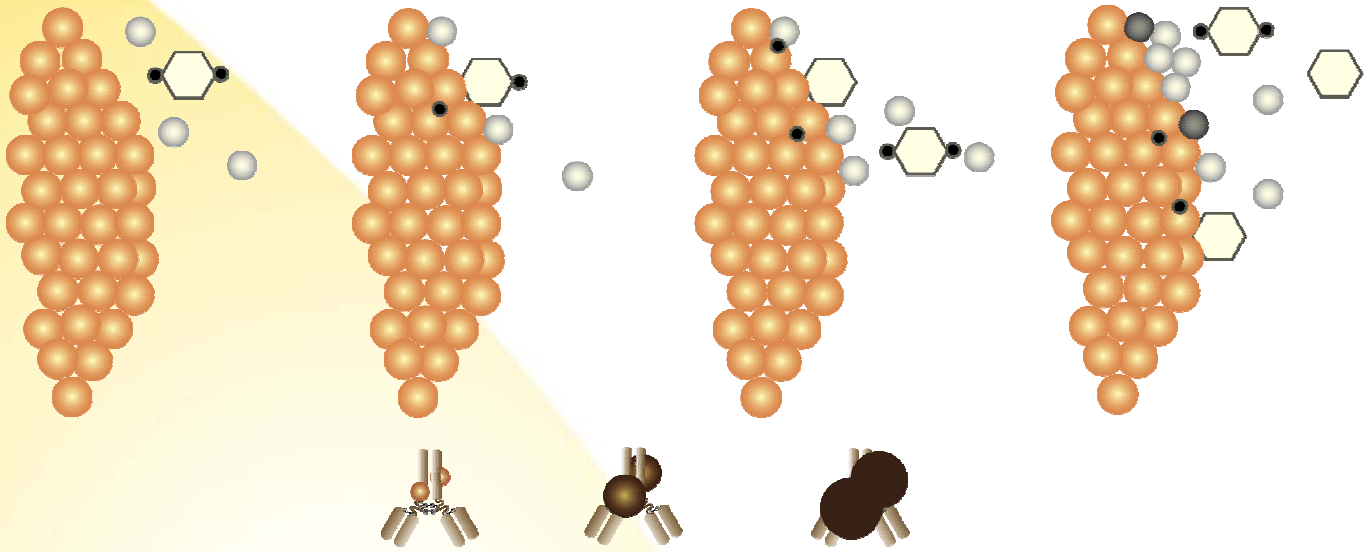


AURION

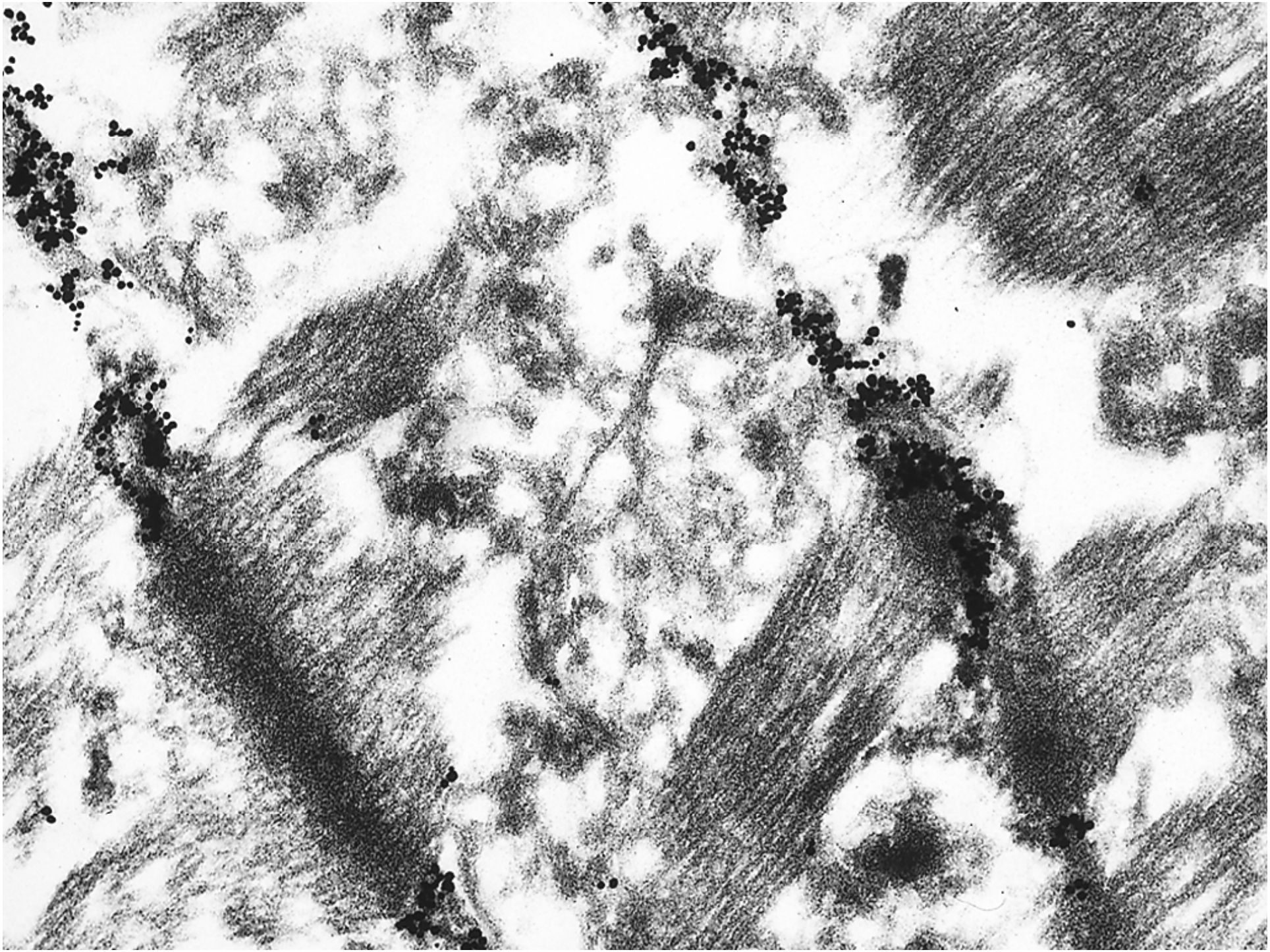




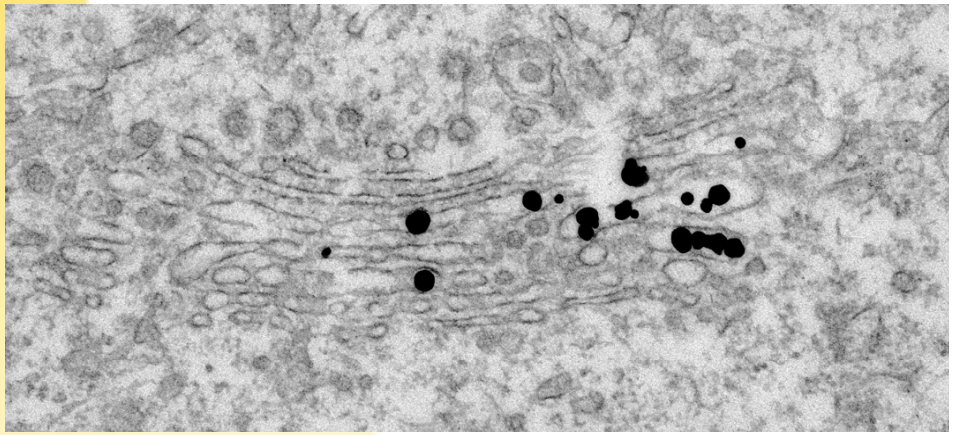
Silver Enhancement (IGSS)

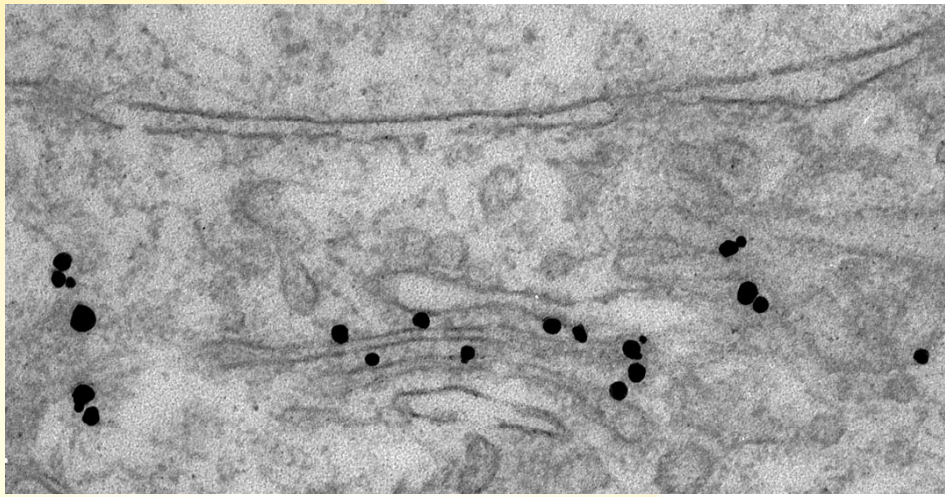


AURION



Reduction of reagent size using smaller proteins
Sfab (55k)





A: MGP-160
B: Huntingtin Interacting Protein Interactor

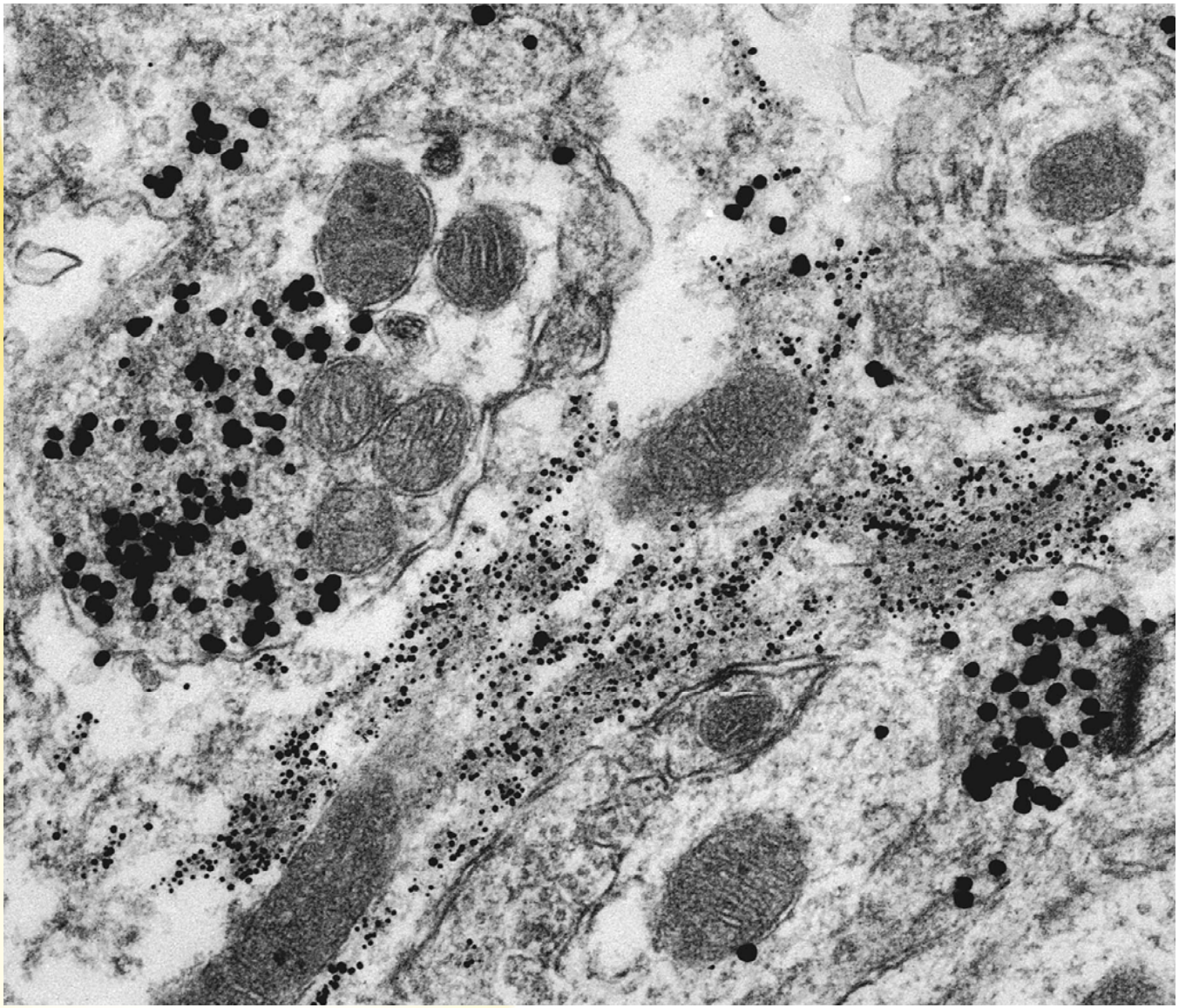
Fixative:
3% PF and 0.2% glut

Sample:
50 µm vibratome section

Permeabilization:
0.05% Triton-X-100,
30 min

Conjugate:
Aurion GAM & GARb Fab-US

AURION



AURION

Pre-embedding double immunogold labeling of synaptophysin (large particles) in axon terminals and GFAP (small particles) in glia processes

Future Developments

Select antibodies against active protein

Further reduction of protein size

- **Using smaller proteins:**
- **protein A/G (15-45k)**
- **Sfab (50k)**
- **engineered antibody fragments (15-20k)**

Further reduction of particle size

‘Active’ labels

Development of preparation techniques

Tomography

AURION

Optimized Immuno Labelling

Aurion Blocking Solutions
Aurion BSA-c™

Peter van de Plas

Jan Leunissen

AURION

N-Cadherin detection in heart muscle cells

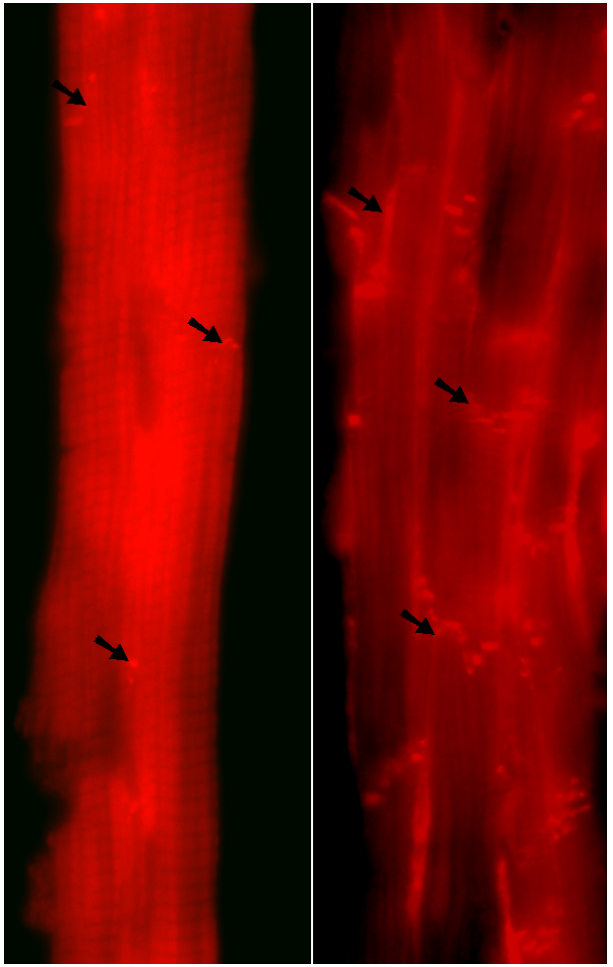
Immunofluorescence Alexa 568 labelled Fab Goat-anti-Mouse

Left hand panel:
Background using a commonly used protocol obscures sites of specific labelling

Right hand panel:
N-Cadherin immuno labelled areas obtained using Aurion Blocking Solution and BSA-c™ stand out with clear definition.

Courtesy of Lauren Hruby and John Harris
Dept. Physiology, University of Otago, Dunedin,

New Zealand



AURION

The Players

- Specimen - Antigen
- Antibodies - Labels

- Procedure

Labelling: result of interaction between specimen and antibodies as depending on the procedure

AURION

Specimen

- Fixation
  - Inactivation
  - Quenching
- Masking
  - Enzyme treatment
  - Antigen retrieval
  - Etching
- Endogenous ‘activity’
  - autofluorescence
  - peroxidase

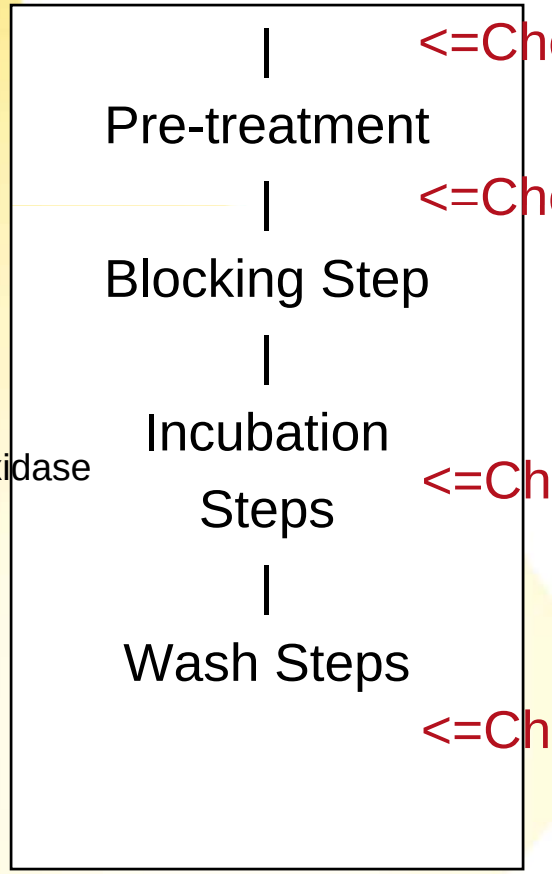
AURION

Antibodies - Label

- Fluorescent (analogue)
- Peroxidase (analogue)
- Particles
  - Gold/Enhancement (digital)
  - Quantum Dots (analogue/digital)

AURION

Procedure



<=Check point 1=>

Noise level 1
Initial Autofluorescence
Initial Endogenous enzyme activity

<=Check point 2=>

Noise Level 2
Residual Autofluorescence
Residual Endogenous enzyme activity

or

Pre-treatment endogenous peroxidase

<=Check point 2=>

<=Check point 3=>

Label control incl Noise Level 2
Specific result

AURION

General considerations

Two main streams

- Protein way
  - Protein block to cover sticky specimen areas
  - Protein additive during incubation
- Detergent way
  - Detergent as ‘specimen block’
  - Detergent additive during incubation

AURION

General considerations

Protein way

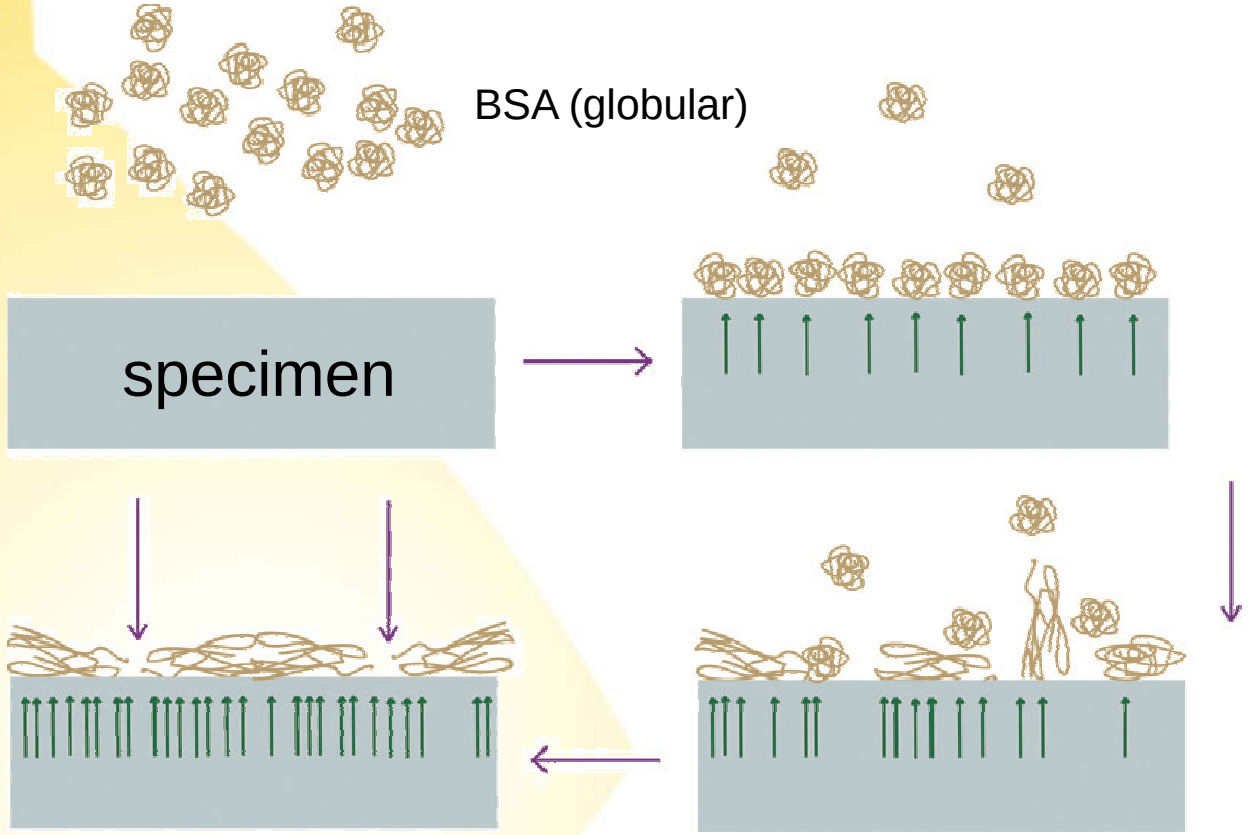
- **Positive:**
  - "Gentle" on specimen, antibodies and conjugates
  - Suited for LM as well as EM, delicate details preserved
  - Deals with hydrophobic and charge based background
- **Negative:**
  - Masking, slow

Detergent way

- **Positive:**
  - Facilitates penetration, no masking, relatively fast
- **Negative:**
  - Destroys ultrastructure, loss of soluble components
  - Deals only with hydrophobic background aspects
  - Not suited for EM

AURION

Blocking Step



AURION

Incubation Solutions

Purpose

creating an environment favoring antibody-antigen binding

while

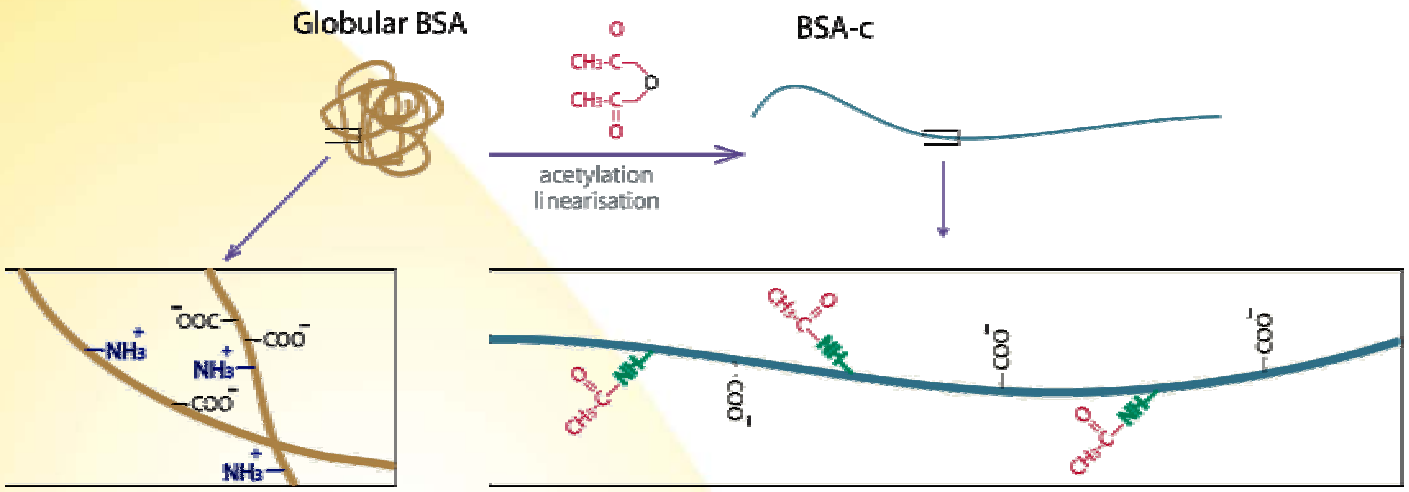
preventing background interactions

How?

by controlling the left-open spaces on the specimen surface using AURION BSA-c™

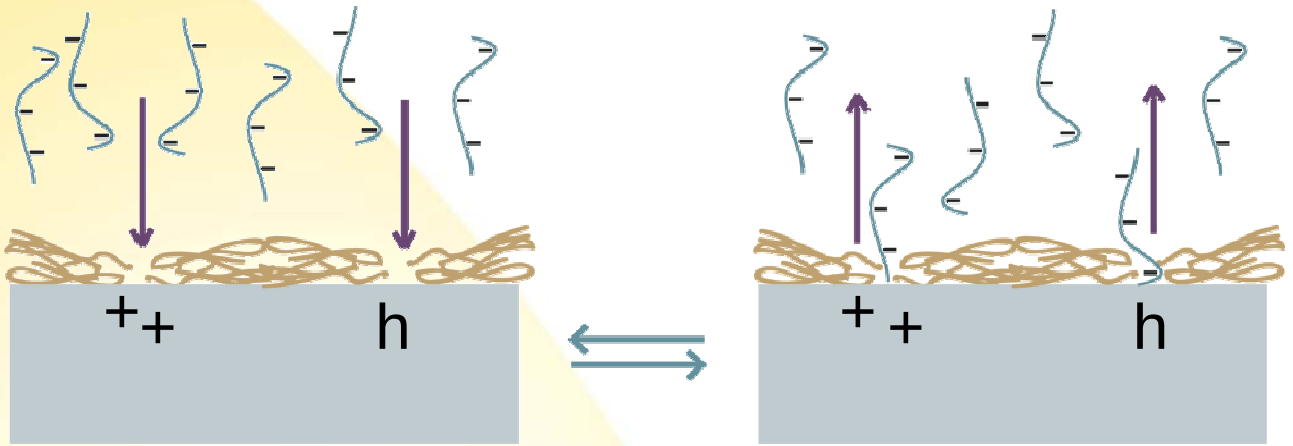
AURION

What is BSA-c™?



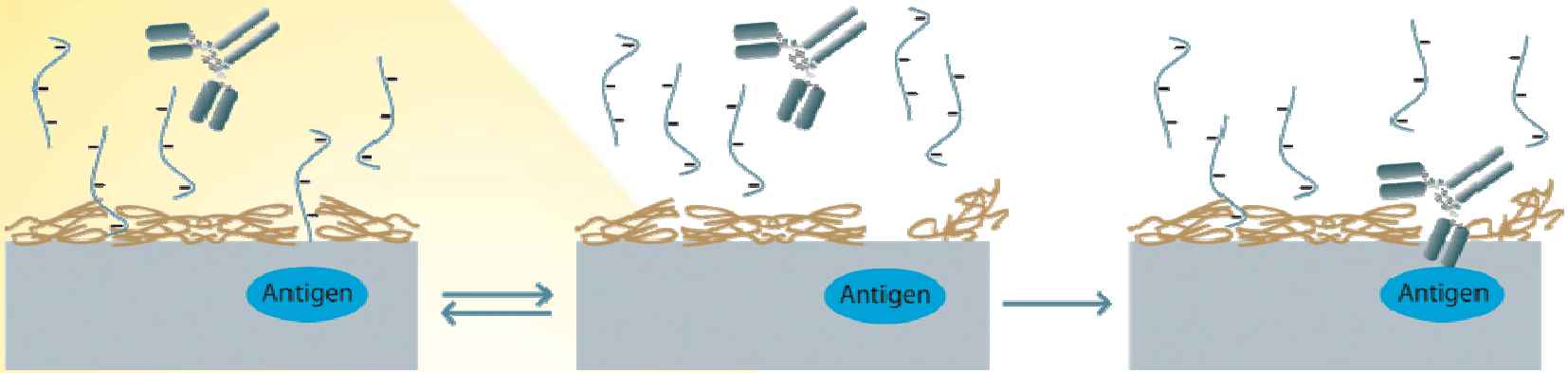
AURION

How does BSA-c™ act?



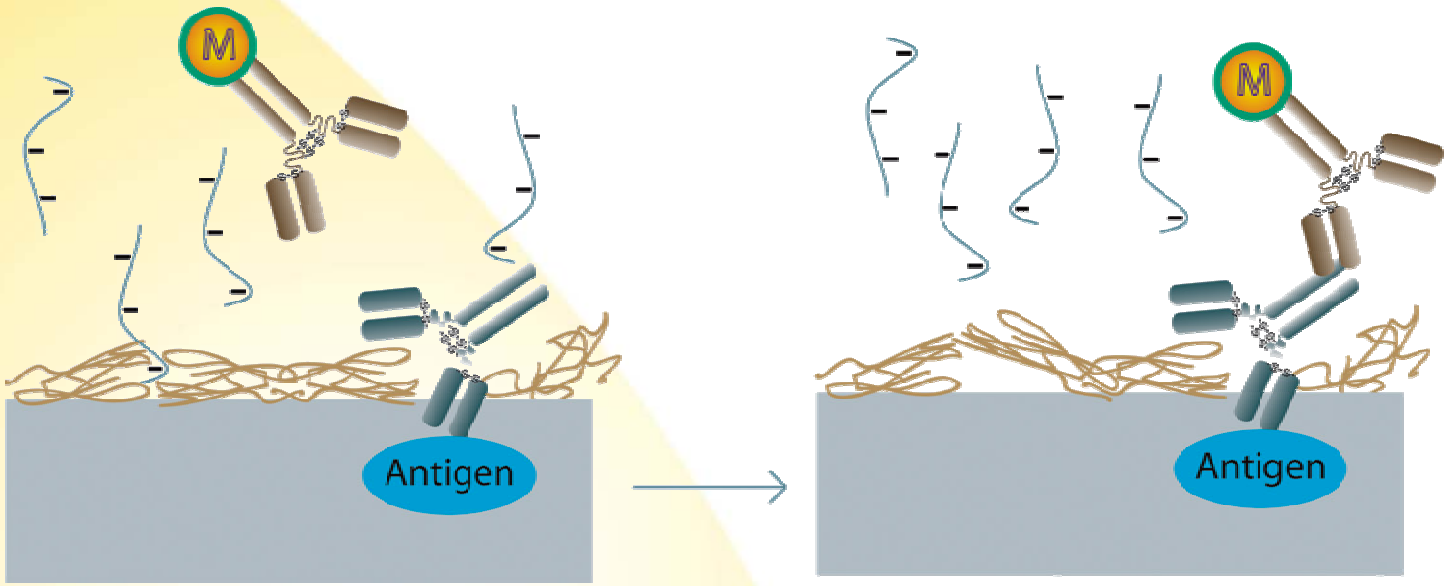
AURION

BSA-c™ favors specificity



AURION

Secondary Incubation



AURION

Summary

Blocking Relevant component: BSA	**Incubation** Relevant component: BSA-c™
Interaction: Dynamic and Long lived (multipoint) So needs applied only once	Interaction: Dynamic and Short lived So needs to be available all the time
*Flattened out globular protein *Interaction area large (multipoint) *Ka of individual point-to-point interaction lower than Ka AgAb interaction	*Linearized negatively charged protein *Interaction area small (‘oligo’point) *Ka lower than Ka AgAb interaction

AURION

Controlled Set-up

- Check antigen preservation/availability (dot-spot, cryostat sections)
  - Apply proven fixative / fixation protocol
- Check for endogenous noise (1,2) (before and after pre-treatment)
  - Apply or adjust pre-treatment
- Check for secondary/tertiary background (3)
  - Apply appropriate Blocking and Incubation

AURION

Thank you

Mrs Hong Yi - Emory University - Atlanta, GA
Mr Peter van de Plas - Aurion - The Netherlands

Stacie Kirsch - Electron Microscopy Sciences
1560 Industry Road, Hatfield, PA 19440
Email: sgkcck@aol.com
Web: http://www.emsdiasum.com/microscopy/
Tel #: 215-412-8400