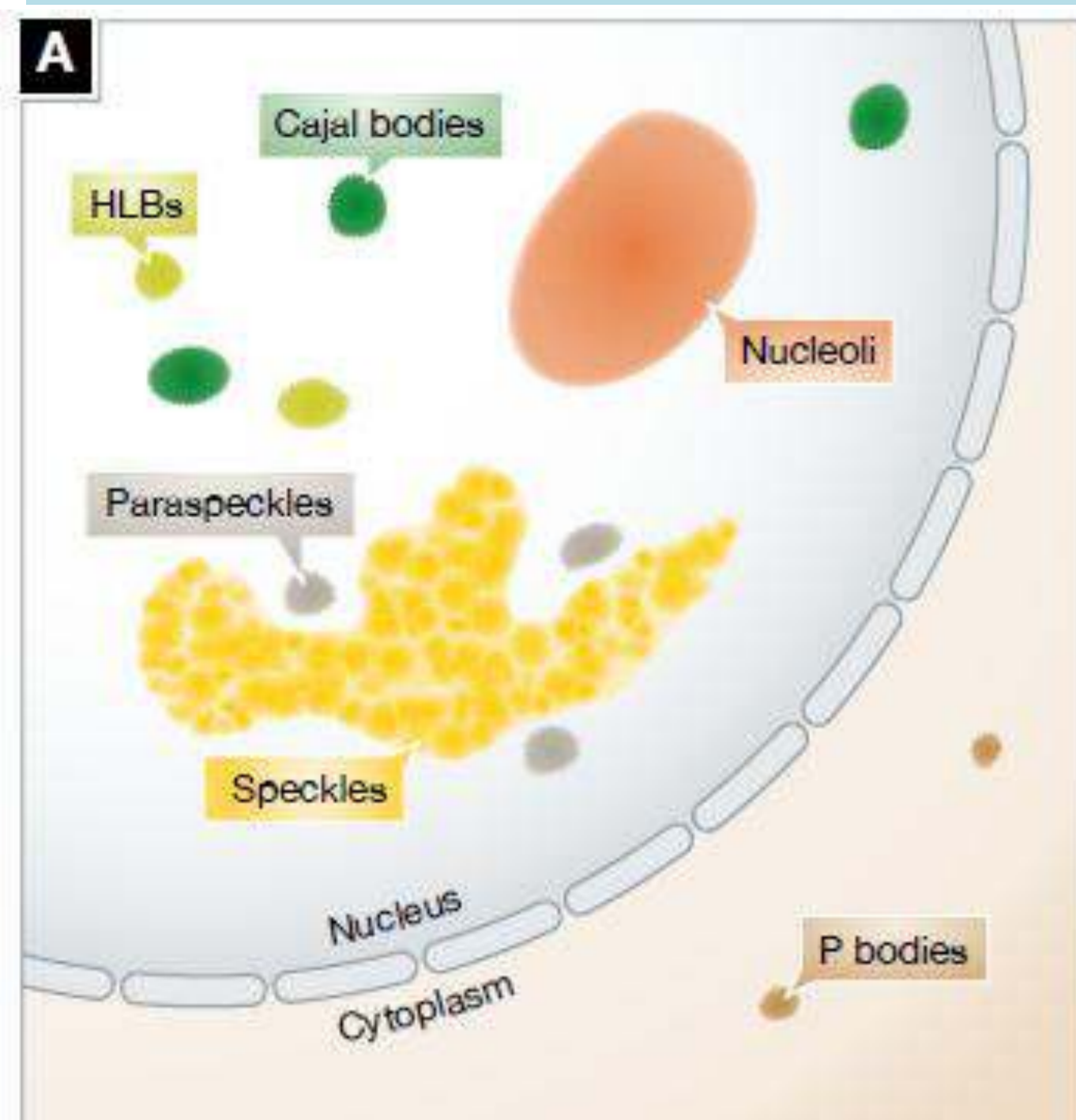


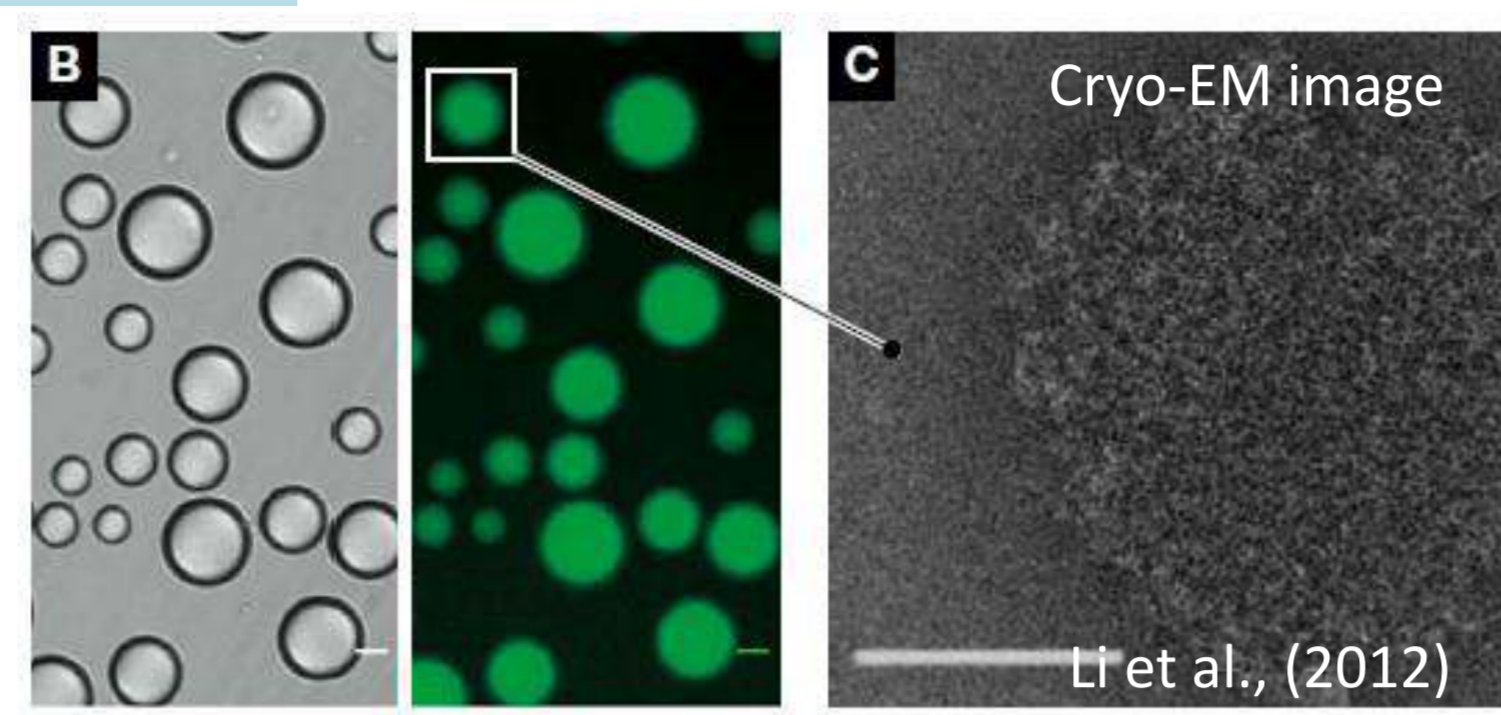
# Surfaces of nuclear bodies visualized by low-voltage scanning electron microscopy

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## Nuclear bodies represent liquid droplets demixing from surrounding nucleoplasm



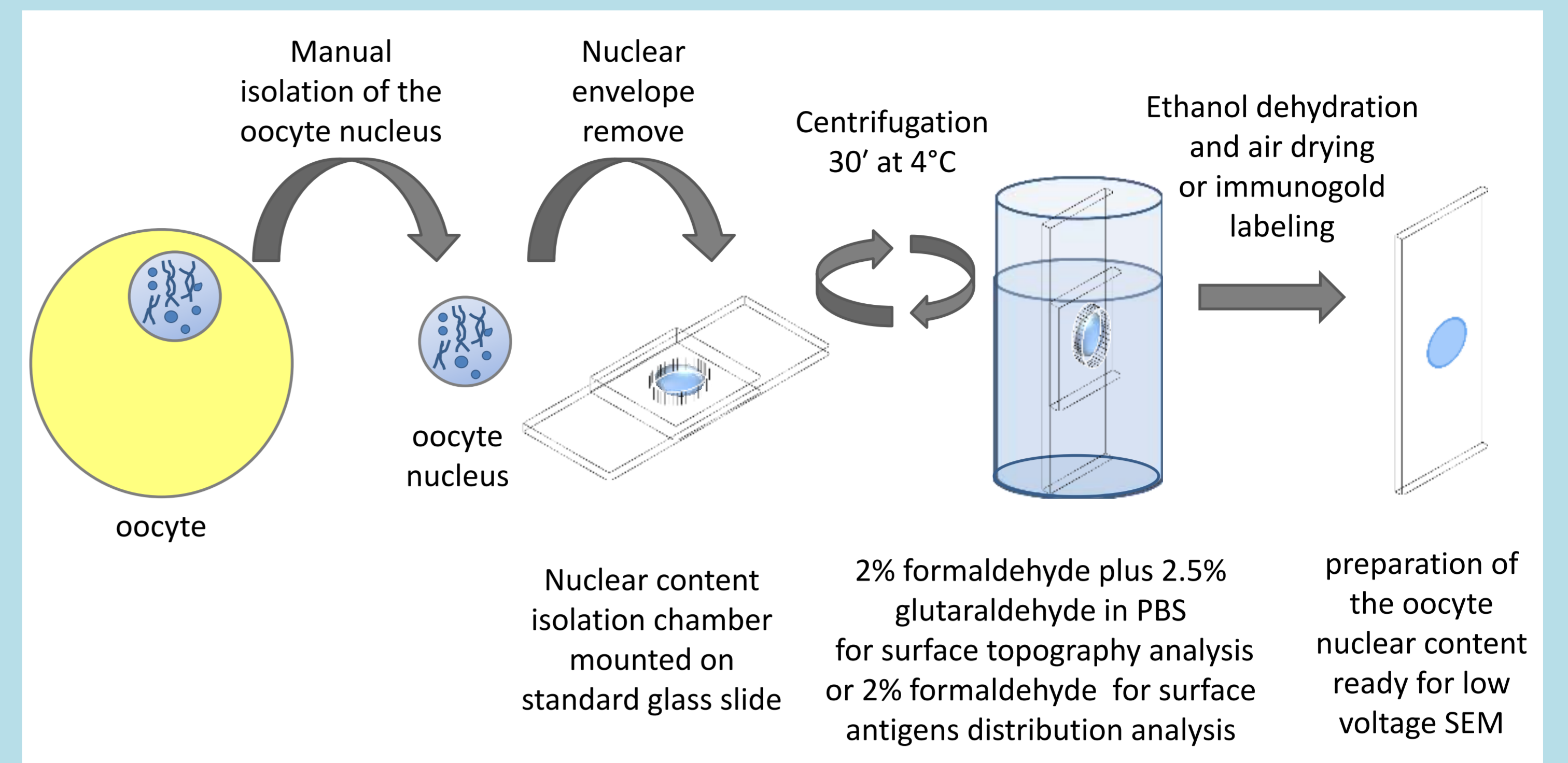
Courchaine, Lu & Neugebauer, 2016



Analogous dynamics and fluid properties are obtained when a purified RNA-binding protein with a low-complexity region is incubated with RNA and observed over time in vitro (Courchaine et al., 2016).

Thus nuclear body surface represent liquid-liquid phase border

## Specimen preparation workflow



## Low voltage scanning electron microscopy



Scanning electron microscope Zeiss Merlin

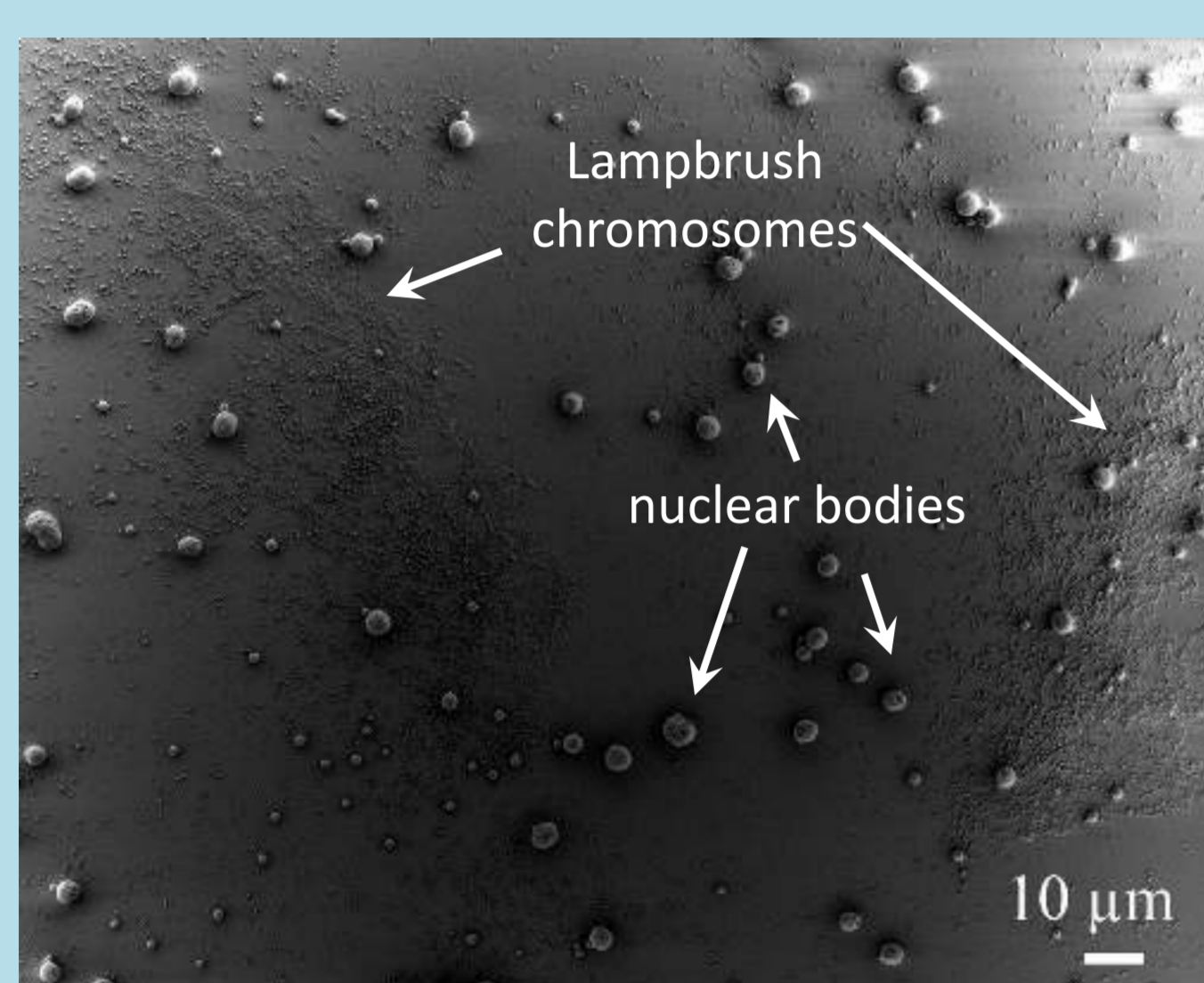
Preparations were analyzed at low voltage regime 0.1–0.4 kV

**Secondary electrons detector** was used for both imaging of the surface topography and detection of colloidal gold particles

Acceleration voltage of 0.1–0.2 kV was used for the imaging surface topography and 0.4 kV for gold nanoparticles.

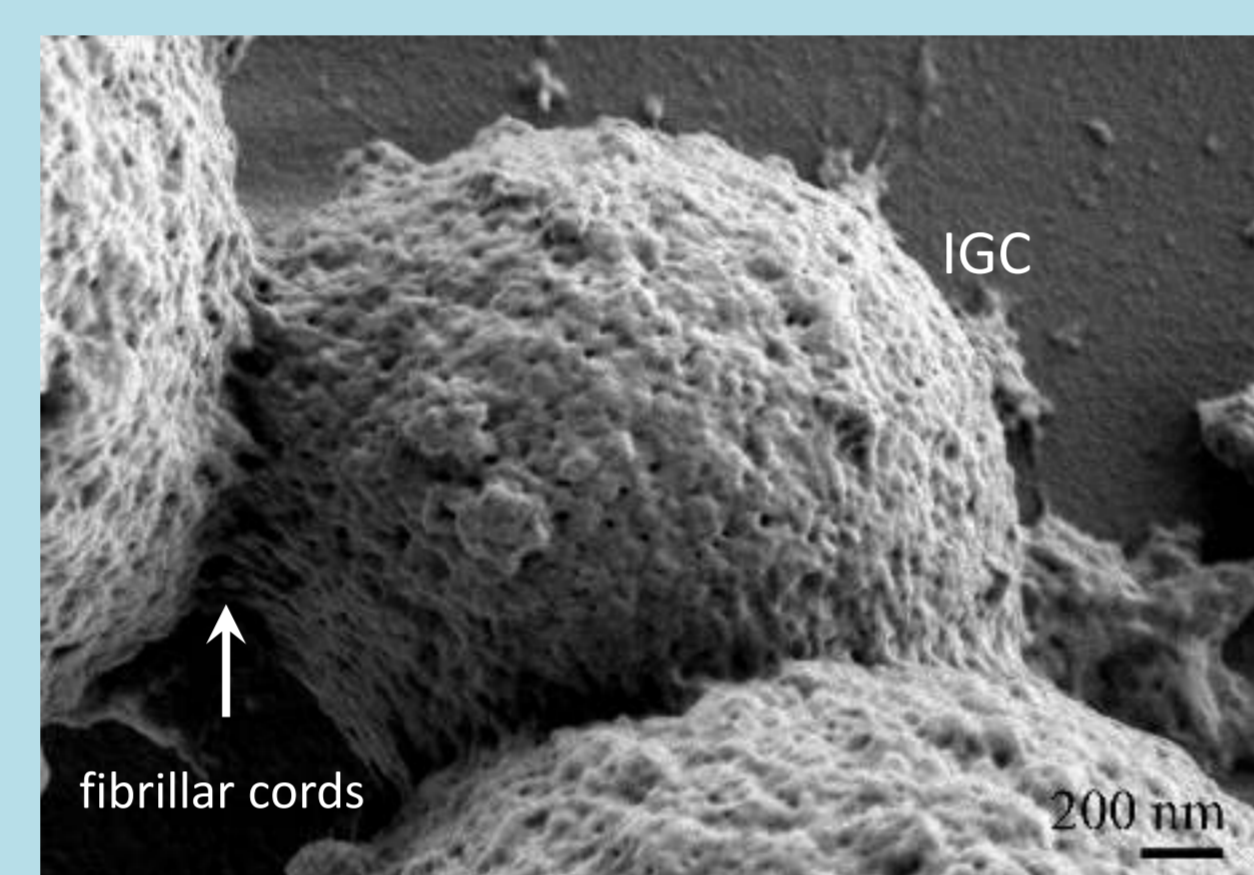
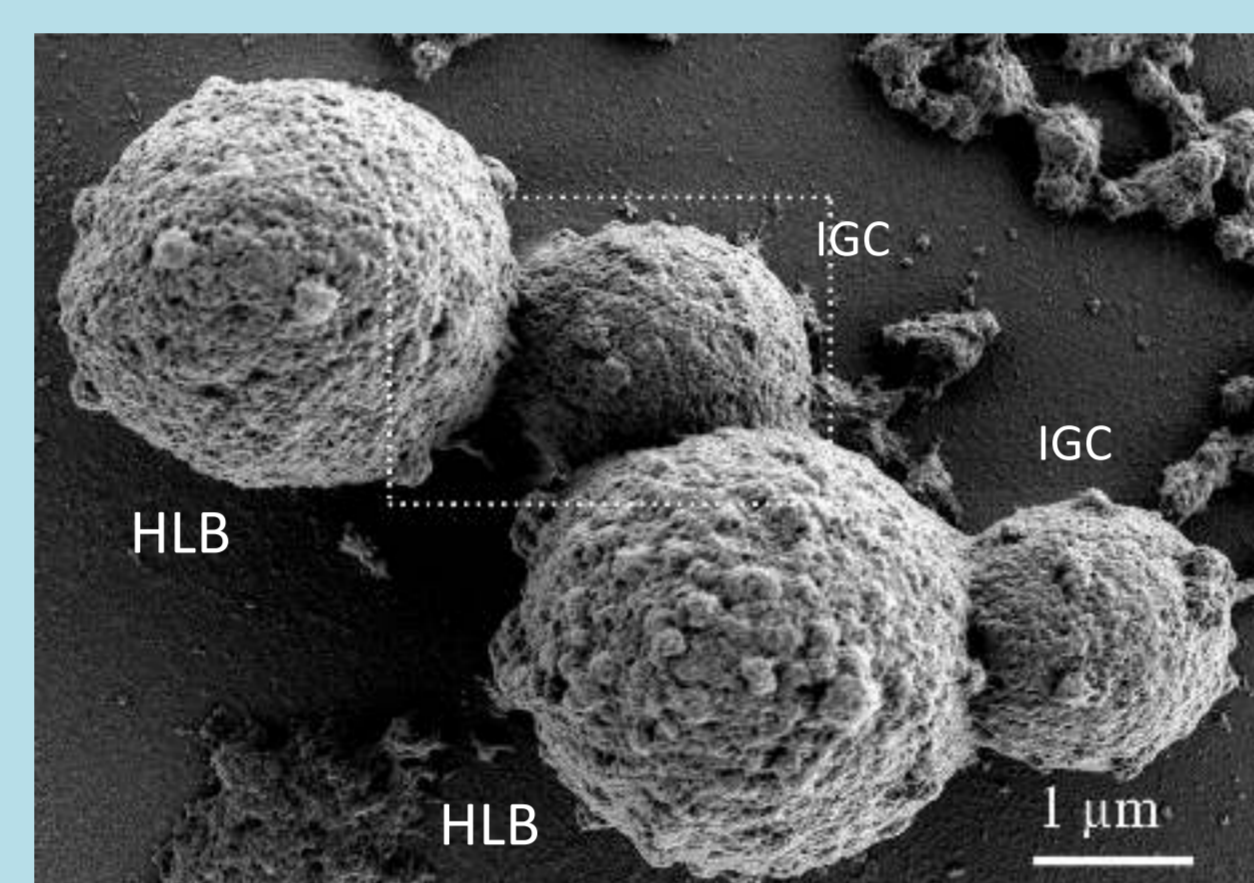
To validate presence of colloidal gold particles In-column detection of back-scattered electrons was used.

## Nuclear bodies from *Xenopus* oocytes

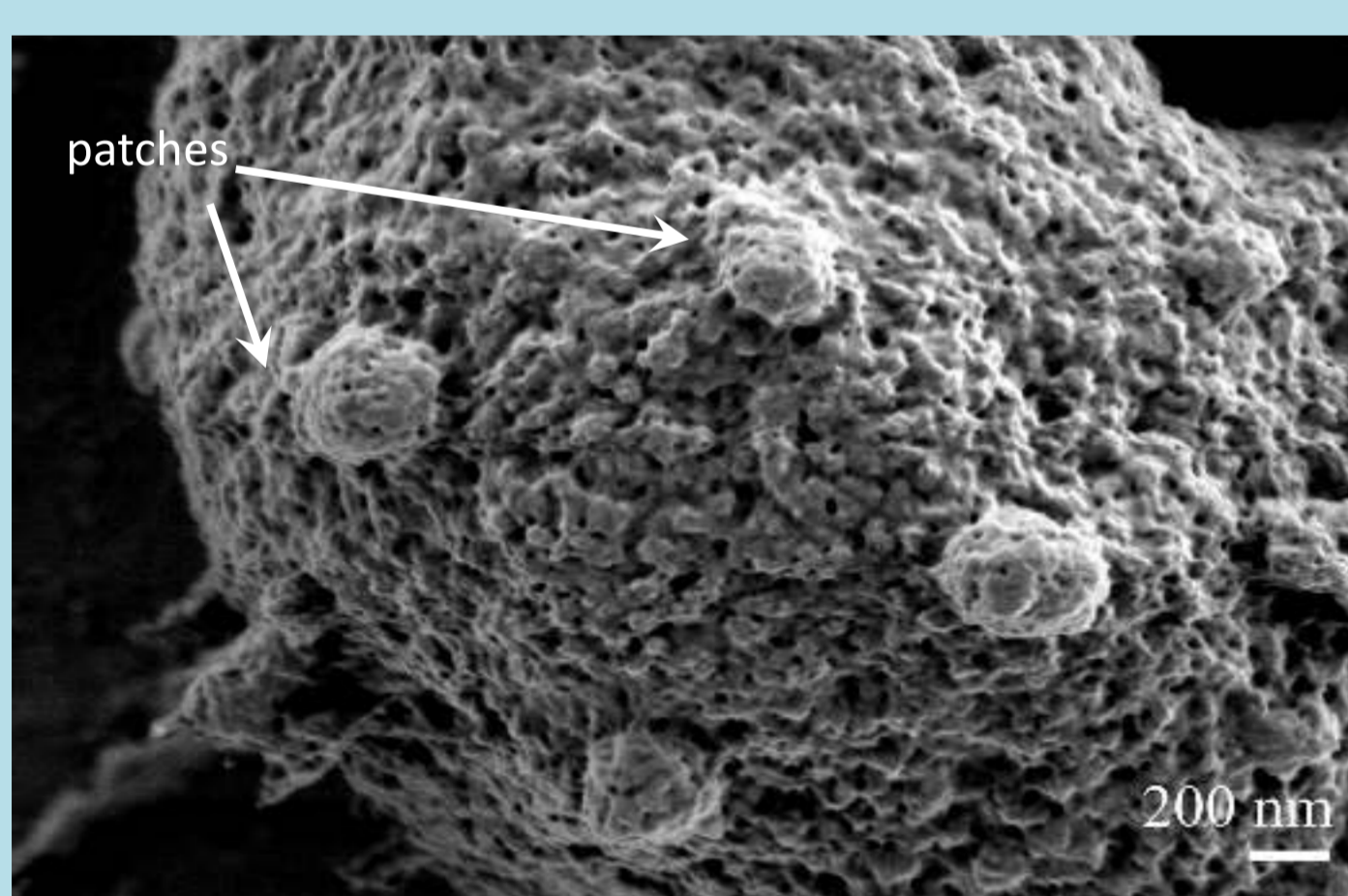
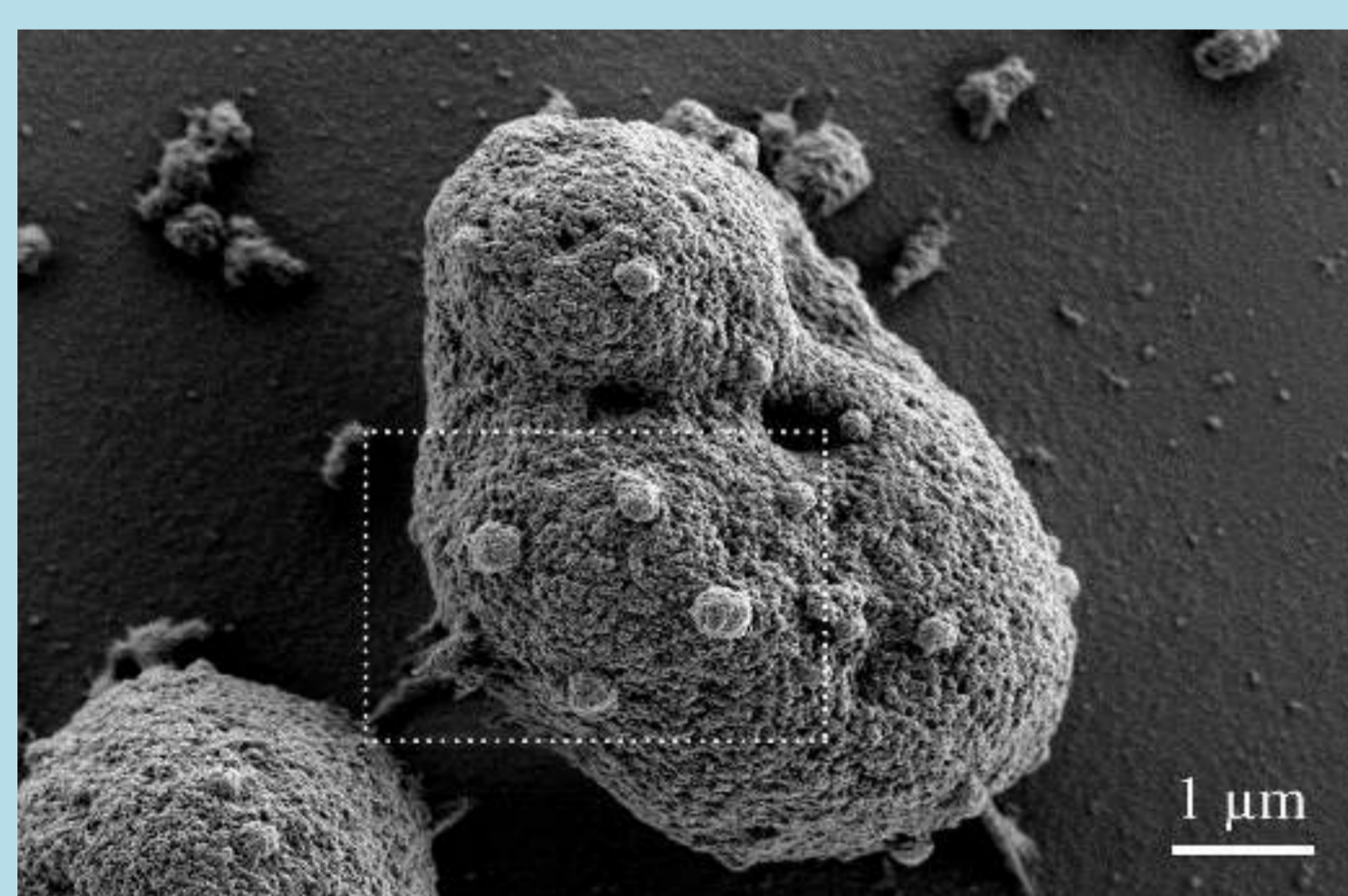


*Xenopus* oocyte nuclei contain lambrush chromosomes and numerous and diverse extrachromosomal bodies: amplified extrachromosomal nucleoli, histone locus bodies (HLB) and interchromatin granule clusters (IGC) or B-snurposomes.

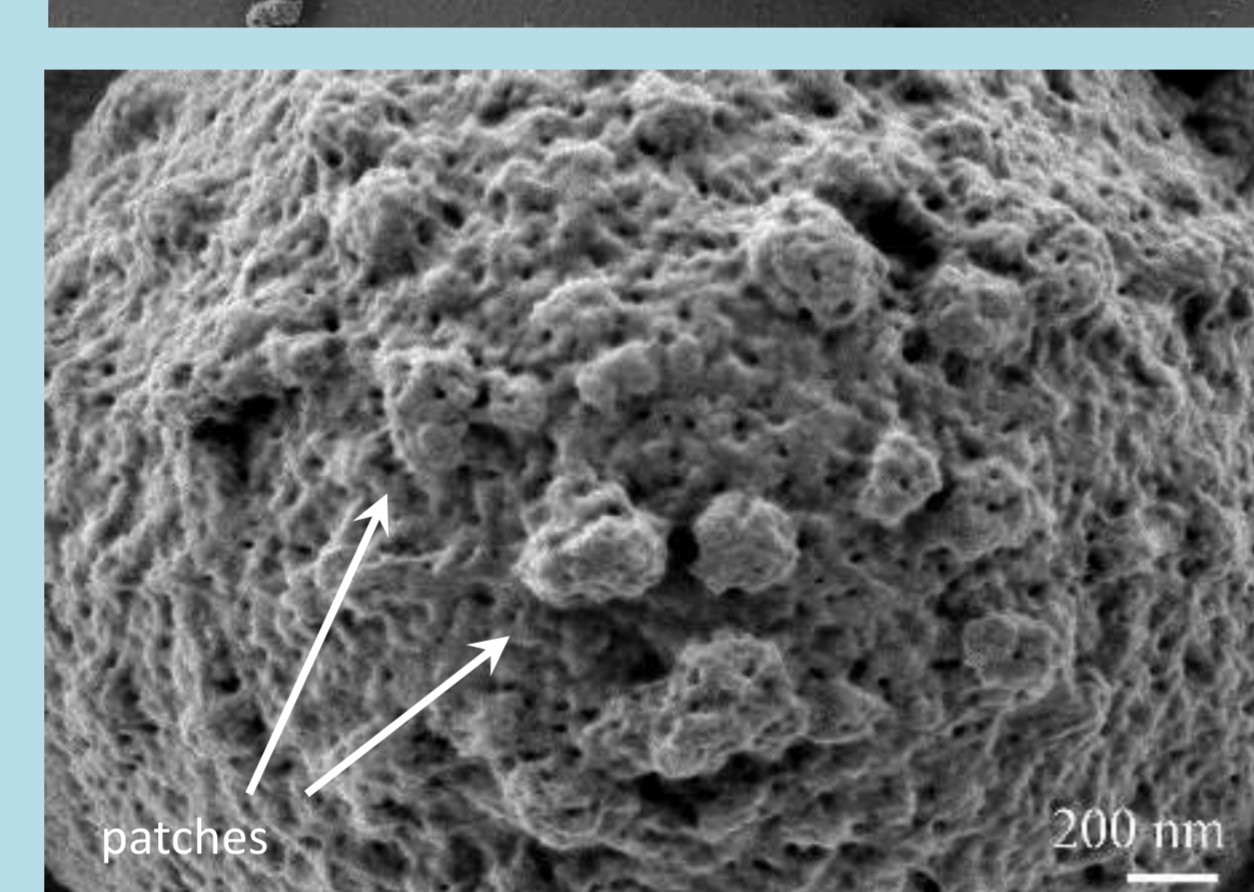
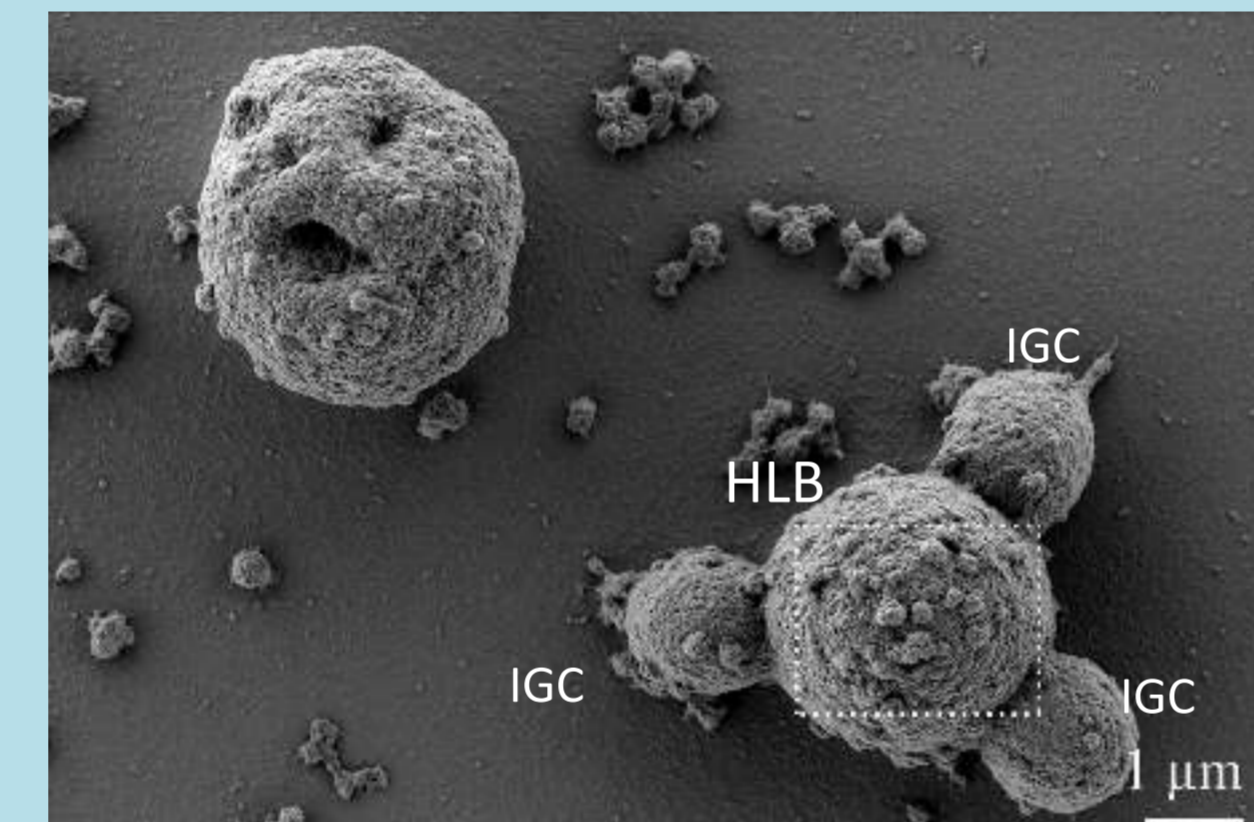
## Interchromatin granule clusters (IGC) or B-snurposomes



## Extrachromosomal nucleolus

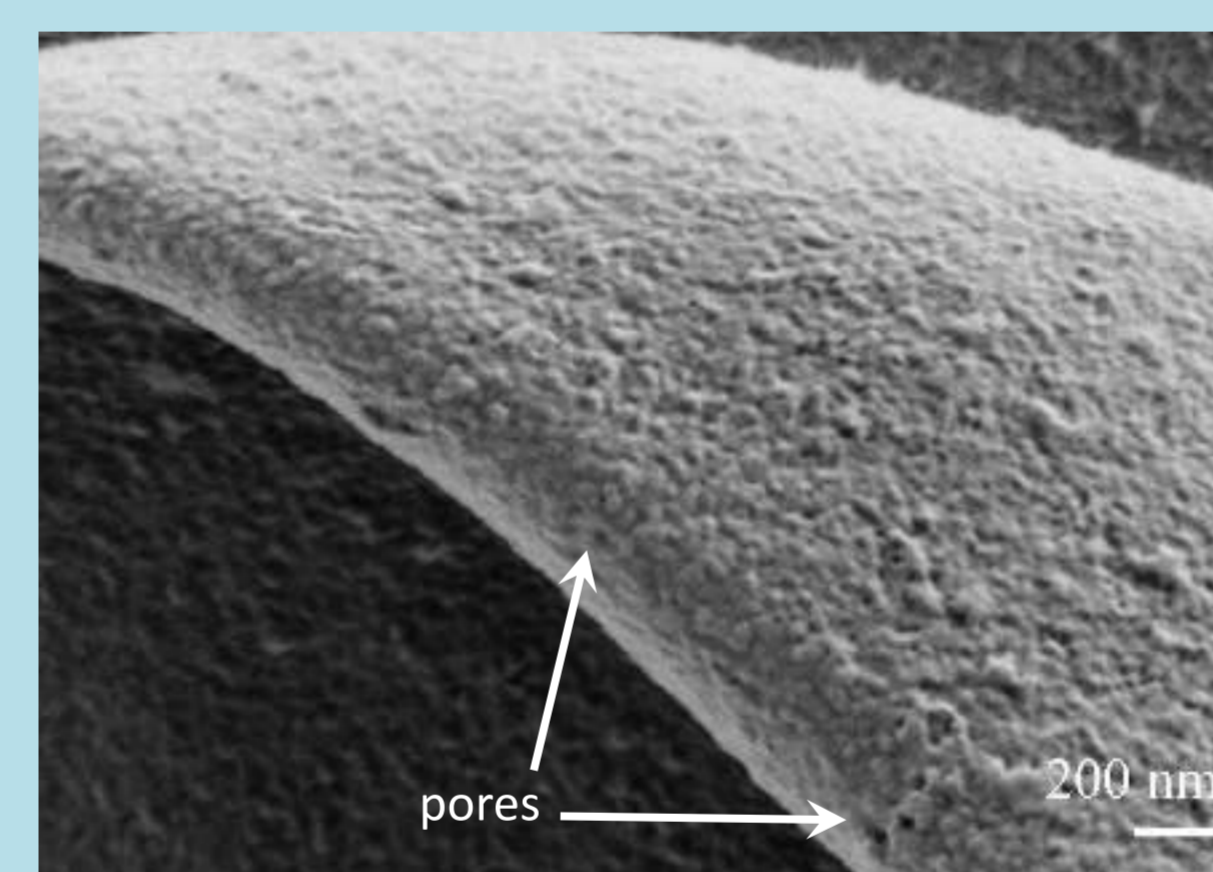
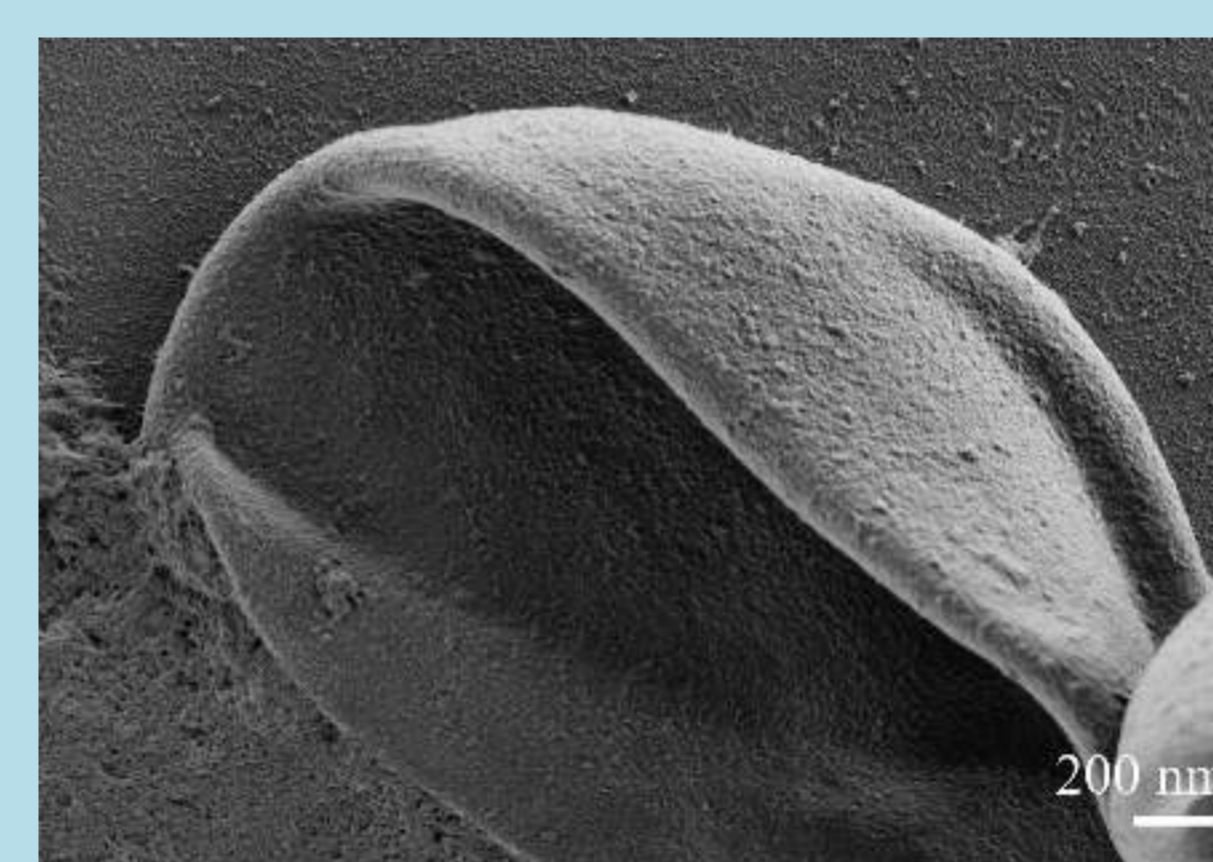
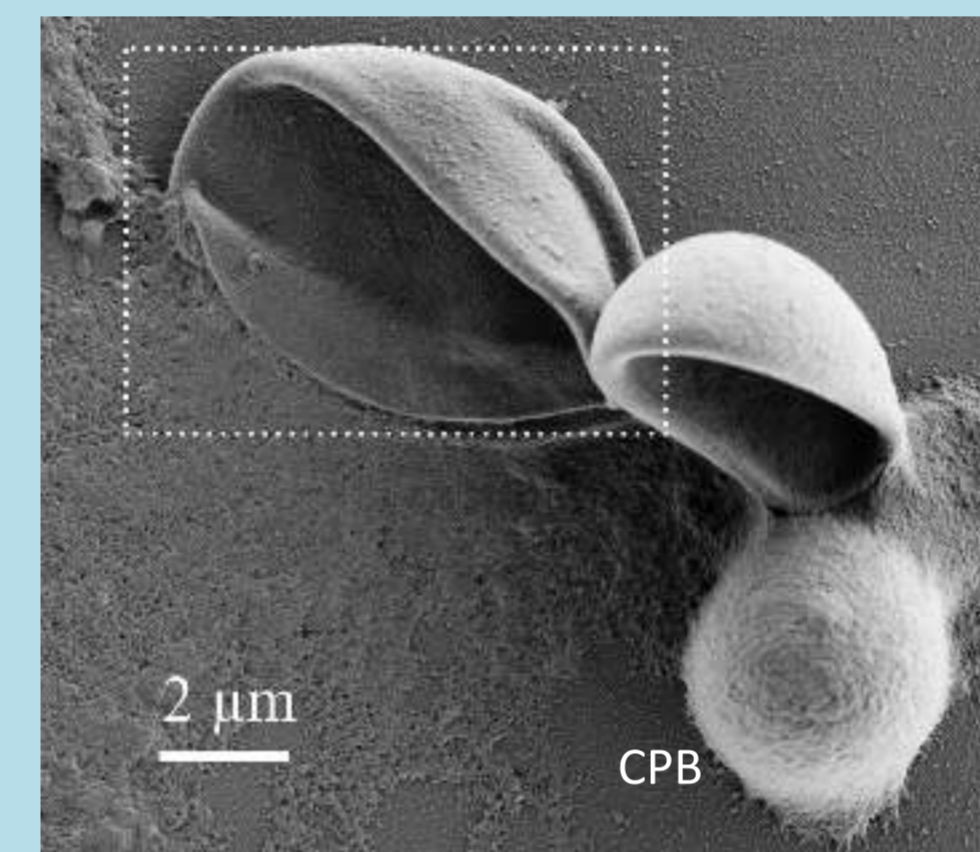


## Histone locus bodies

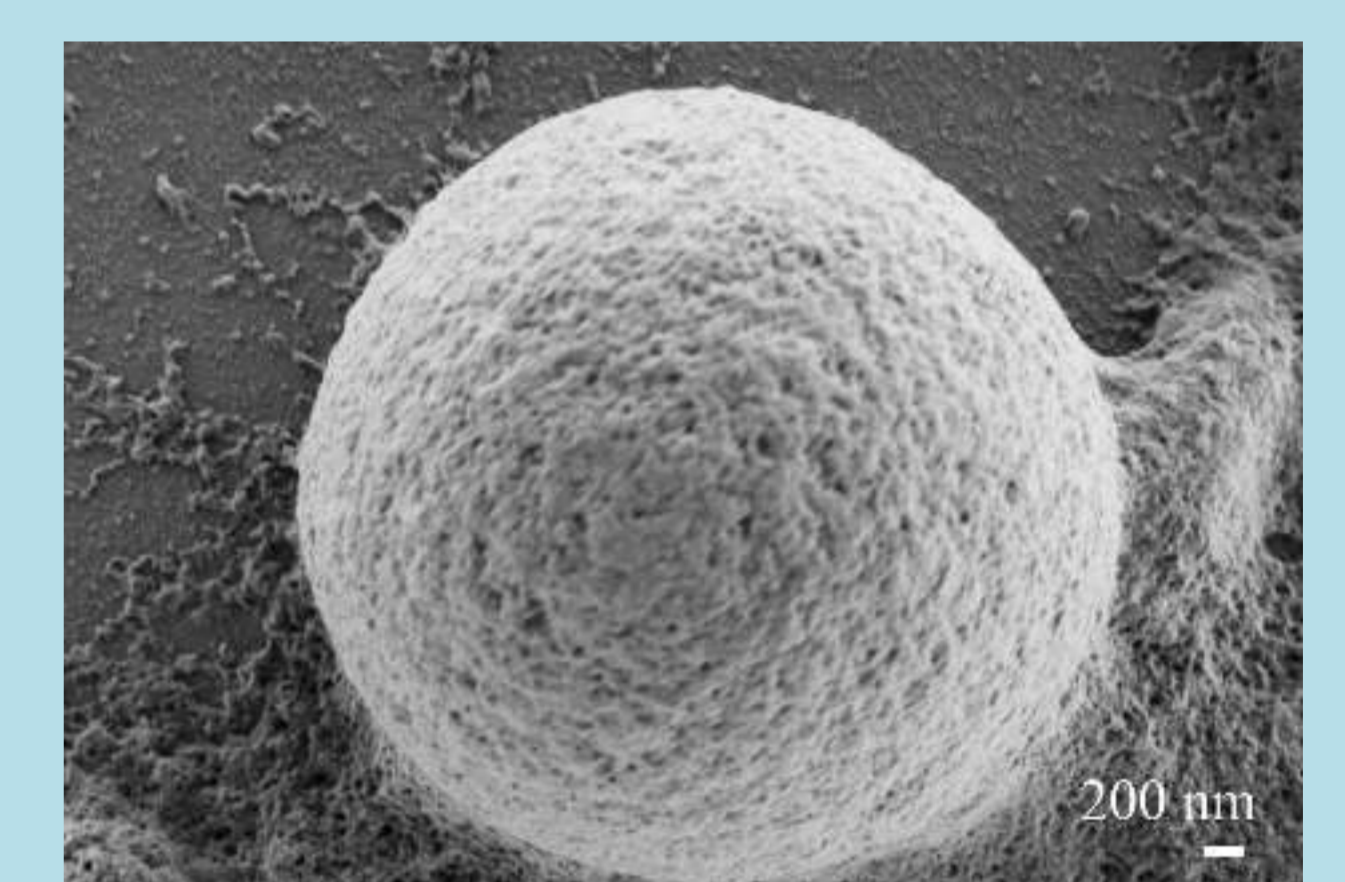
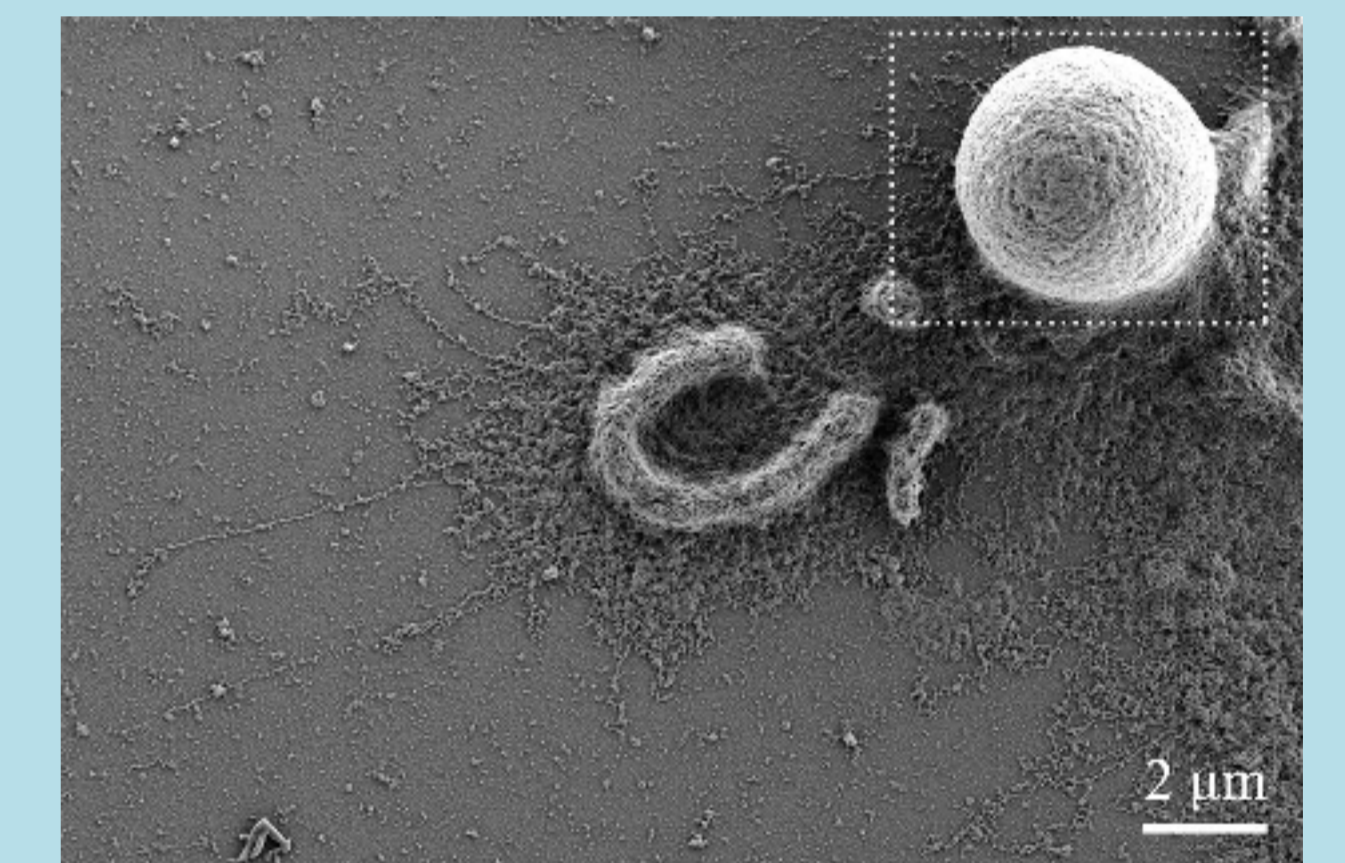


## Nuclear bodies from pigeon oocytes

### Cajal body (CB)-like bodies



### Centromere protein bodies (CPB)

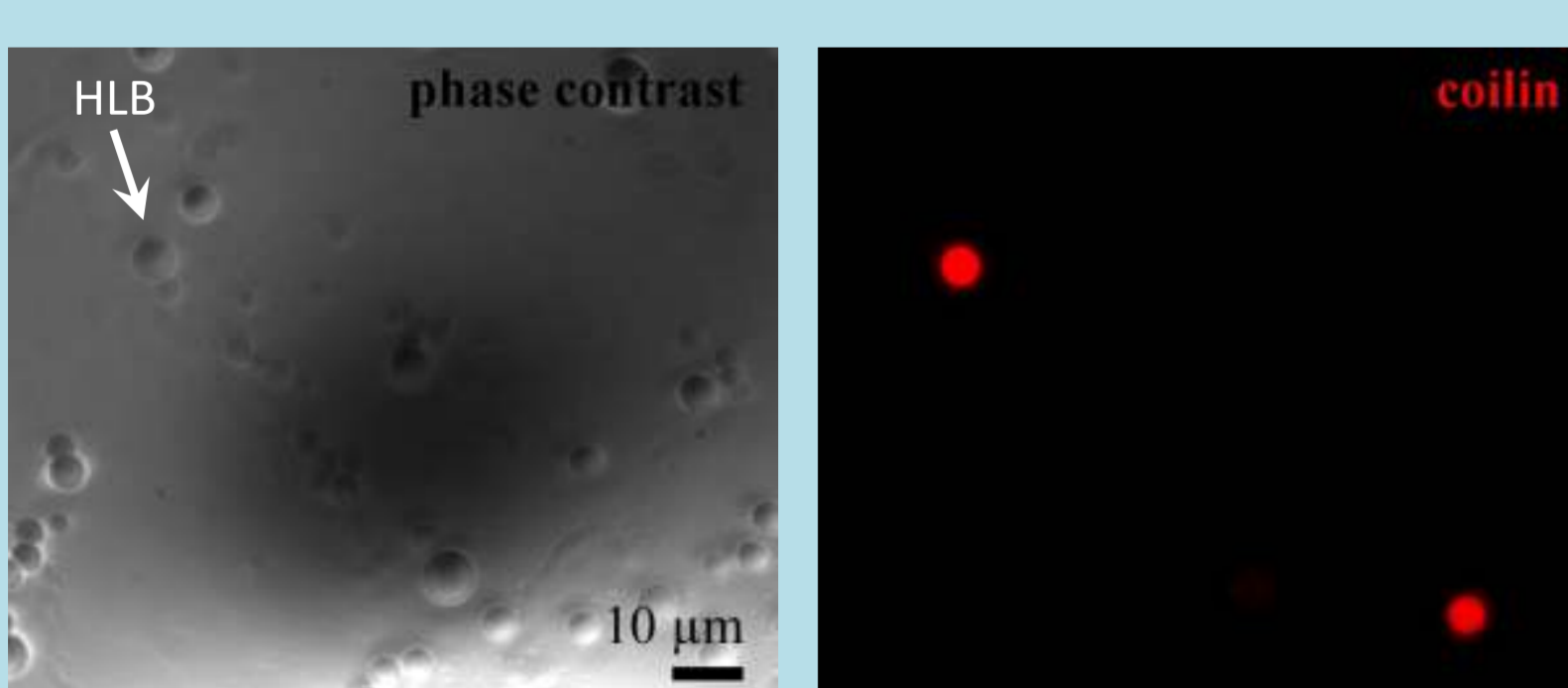


Proteinaceous centromere associated nuclear body enriched with topoisomerase II and cohesion proteins

- RNP-containing (nucleolus, HLB, IGC) and proteinaceous nuclear bodies (CPB) have similar ultrastructural surface morphology;
- Coilin-rich nuclear bodies in amphibian and avian oocytes have significantly different ultrastructural surface morphology;
- Nuclear bodies with a dynamic traffic and biogenesis of the macromolecules probably have more complex and loose surface organization, while nuclear bodies involved only in RNP storage have more solid and homogeneous surface ultrastructure.

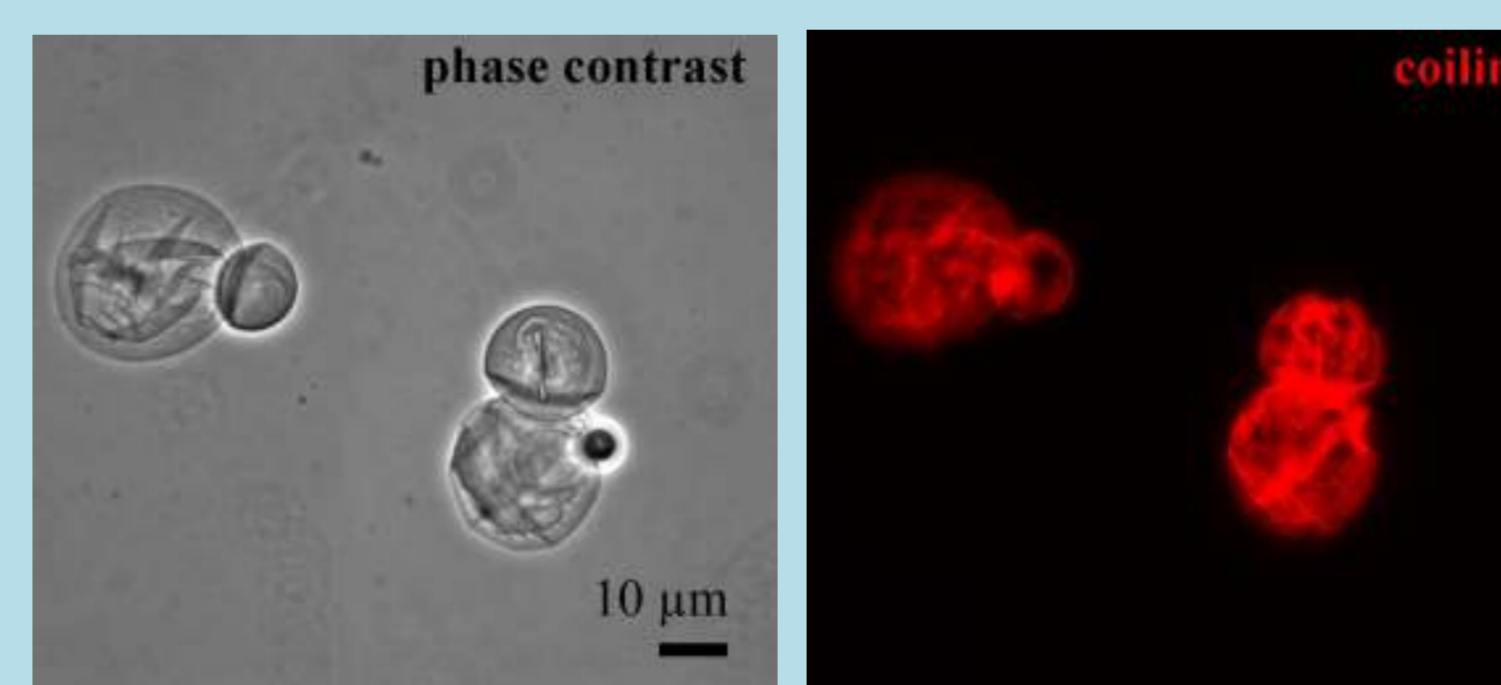
## Relative distribution of coilin and snRNA on the surfaces of coilin-rich nuclear bodies from *Xenopus* and pigeon oocytes

### Histone locus bodies from *Xenopus* oocyte

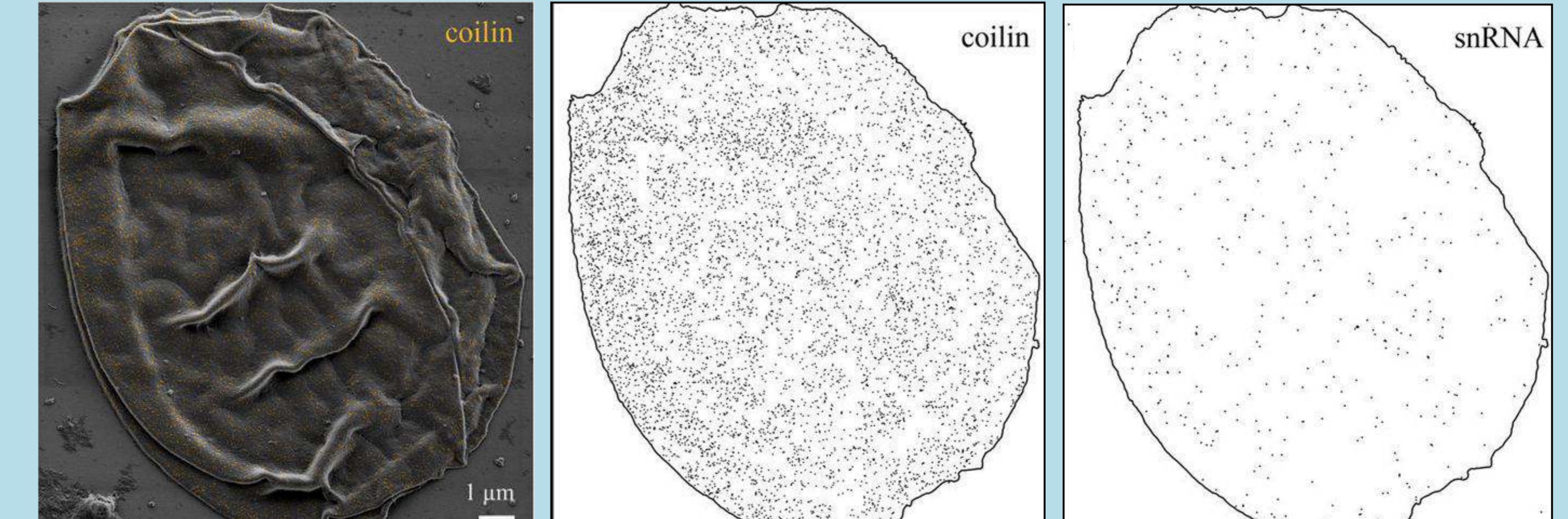
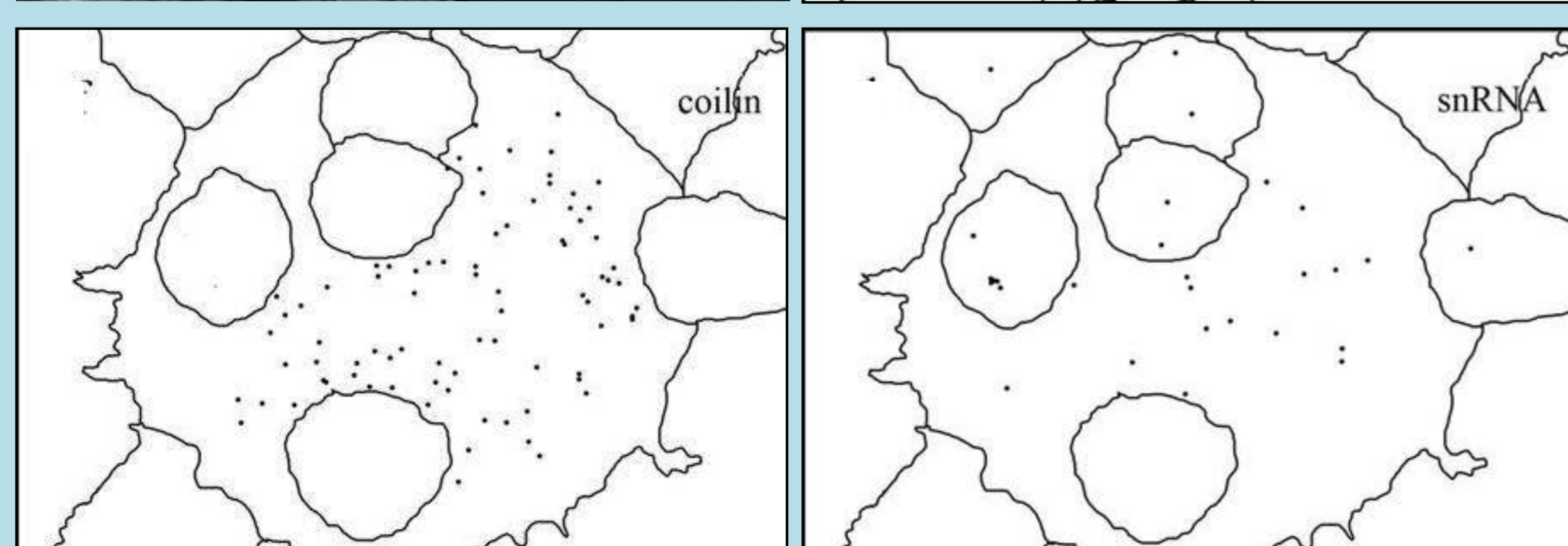
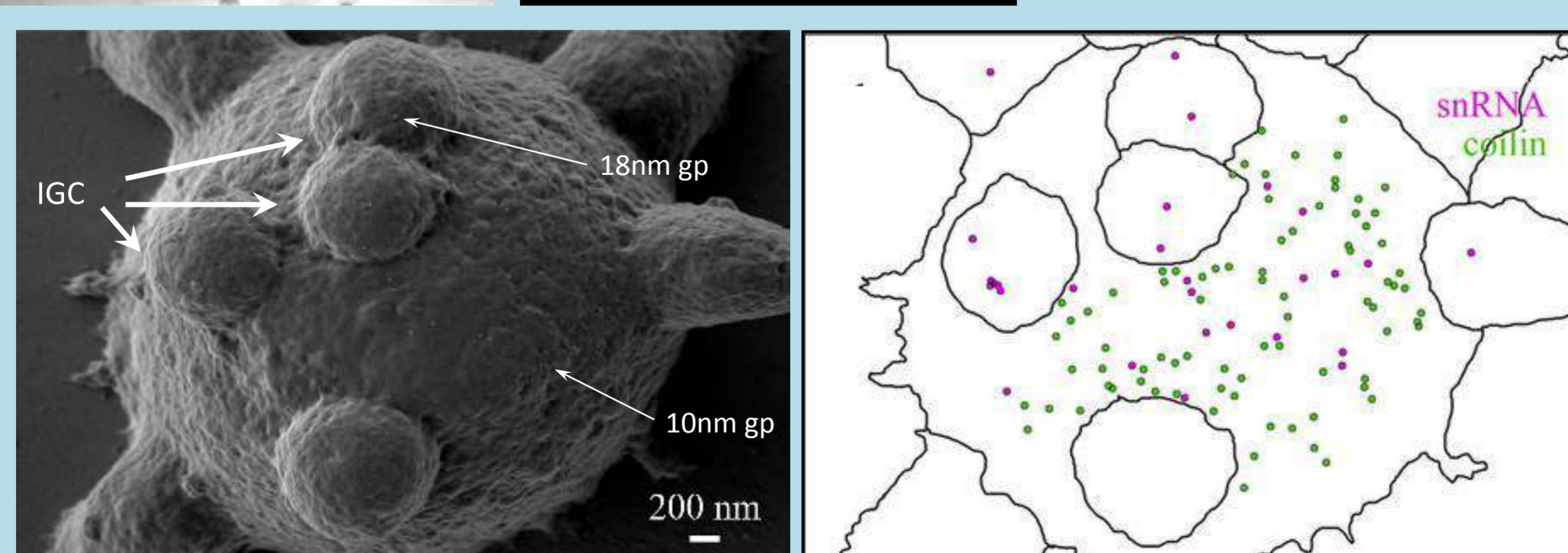


Histone locus bodies from *Xenopus* oocytes and Cajal body-like bodies from pigeon oocytes belong to heterogeneous group of nuclear bodies containing both coilin and splicing snRNPs.

### Cajal body-like bodies from pigeon oocyte



- Coilin and snRNAs did not co-localize on the surface of both *Xenopus* HLB and pigeon CB-like bodies;
- CB-like bodies from pigeon oocytes demonstrated denser superficial distribution of coilin than HLB from *Xenopus* oocytes;
- Density of superficial snRNAs is comparable for both types of nuclear bodies;
- Higher superficial concentration of structural protein coilin may reflect lower dynamics of CB-like bodies components.



Sequential double immunogold labeling with antibodies against TMG cap of snRNAs (K121) - 18 nm gold particles (gp) and coilin (H-300) - 10 nm gp reveals relative superficial distribution pattern of coilin and snRNA. Gold nanoparticles (gp) are pseudocolored with yellow.

