

# **Elemental Analysis of Sunflower Cataract in Wilson Disease**

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The authors have no financial interest in the subject matter of this e-poster

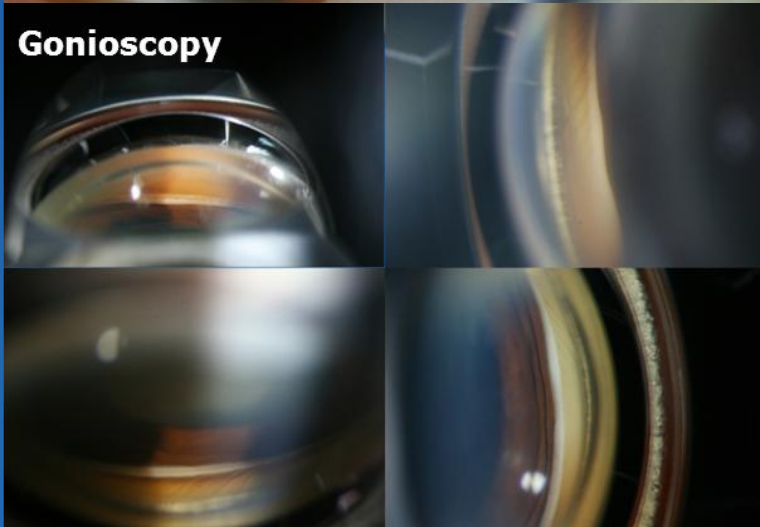
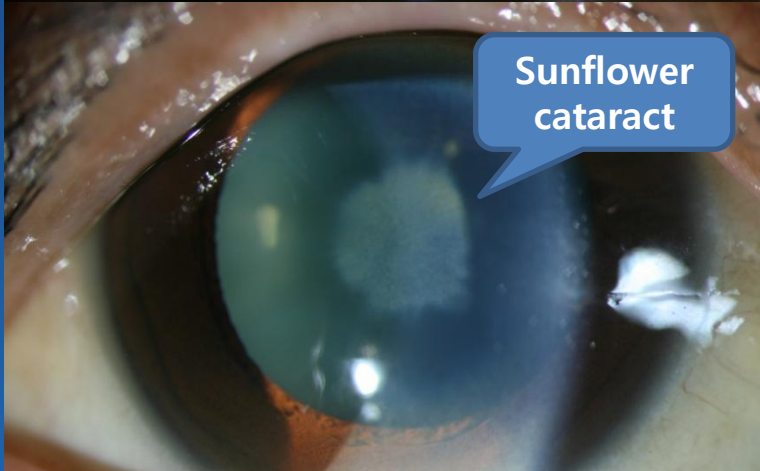
## ◆ Introduction

- The ophthalmic characteristics with Wilson's disease (WD) have been known as diagnostically important manifestations.
- In previous studies, copper accumulation in liver with WD had been proved.
- However, in case of the sunflower cataract, one of the rare diagnostic signs in WD, **there was no study that has demonstrated copper accumulation in lens capsule** of sunflower cataract.
- Transmission electron microscopy (TEM) with energy dispersive X-ray spectroscopy (EDS)
  - : Elemental microanalysis method widely applied in various fields
  - : Capable of identifying and quantifying all elements in the periodic table except H, He, and Li with sub-Angstrom ( $\text{\AA}$ ) resolution

## ◆ Purpose

- To investigate the nanostructure and elemental composition in the lens capsule of sunflower cataract in Wilson's disease.

## ◆ Material and Methods

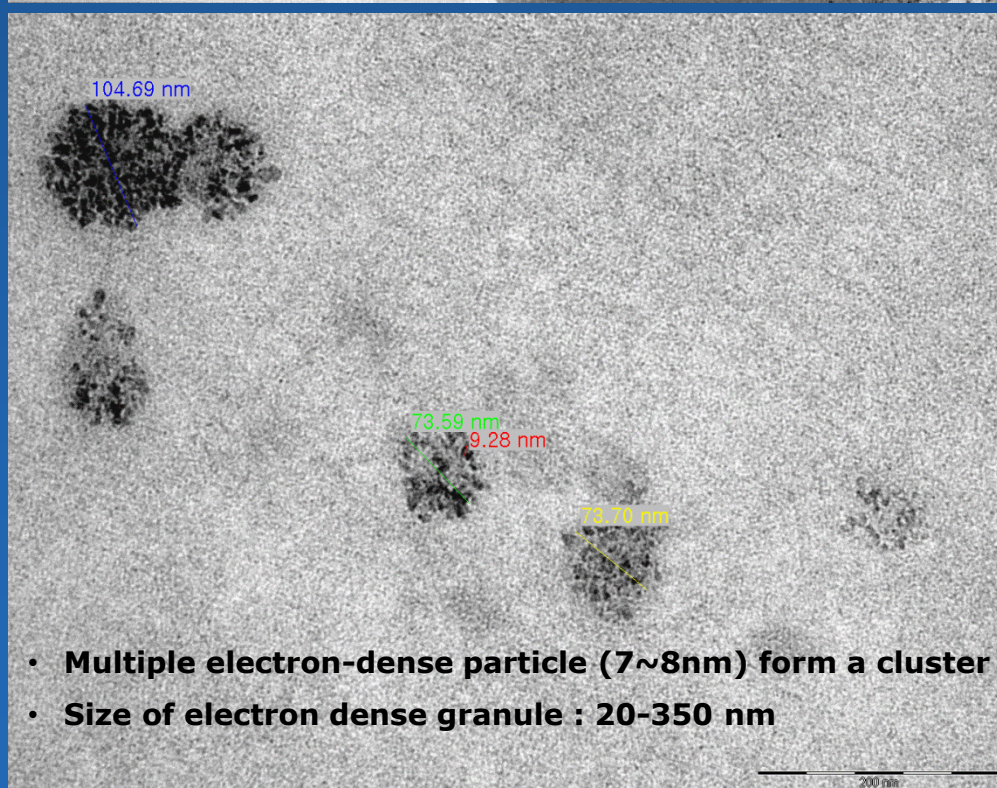
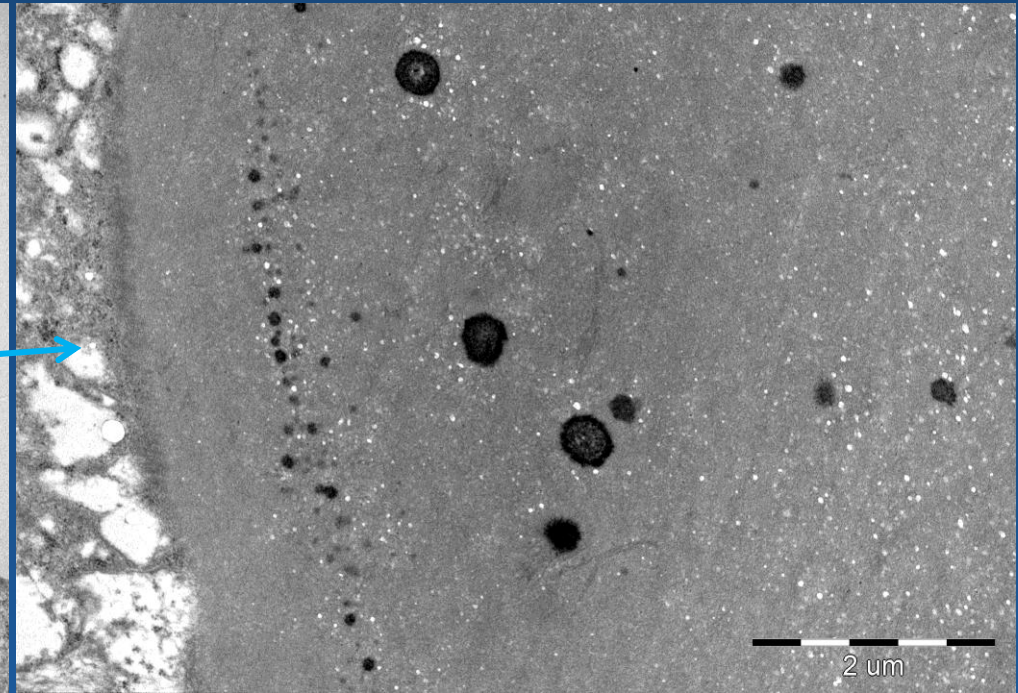
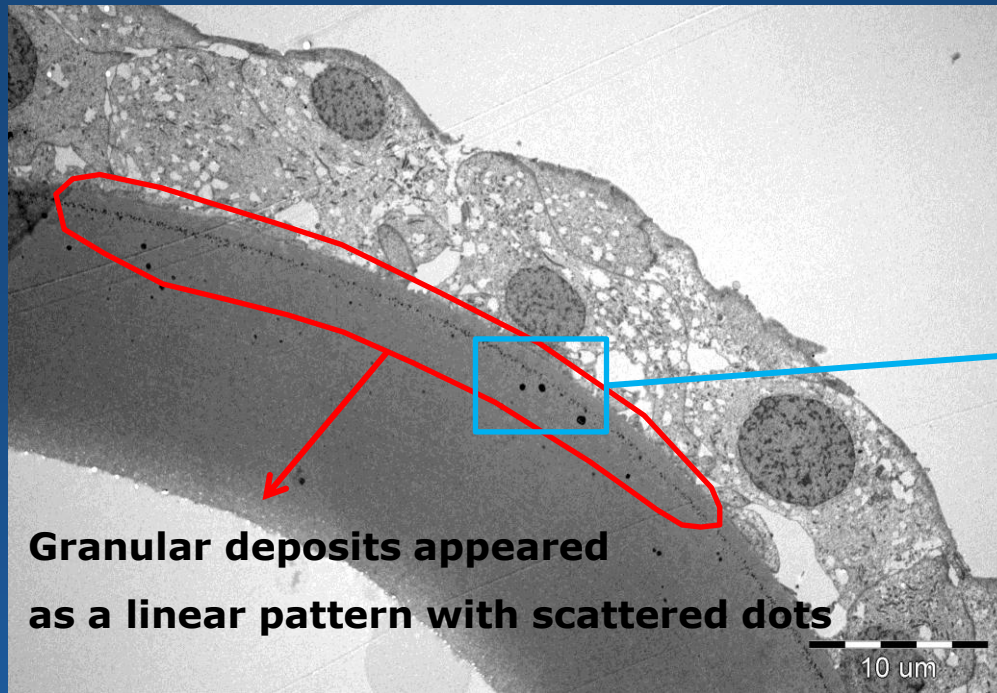


- M/37 with Wilson's disease
- On his ophthalmic examination,
  - : **K-F ring** on slit lamp examination & gonioscopy
  - : **Central disk-shaped & radiating spoke-like golden brown colorations** on the anterior lens capsules

### ◆ TEM & EDS preparation

1. Right eye underwent phacoemulsification with a CCC
2. Anterior capsule preparation for TEM.
  - Samples were embedded in Epon812
  - Ultrathin sections (70nm thickness)
  - Mounted onto formvar-carbon film coated nickel (Ni) grid
  - Stained with uranyl (U) acetate and lead (Pb) citrate
3. Cross section image
  - Titan™ 80–300 (FEI company, Netherlands) at 80kV
4. Elemental analysis by Scanning TEM with EDS
  - Using both point and mapping spectroscopy
  - EDS data analysis by automatic peak identification software, ES vision (Emispec, Beaverton, USA)

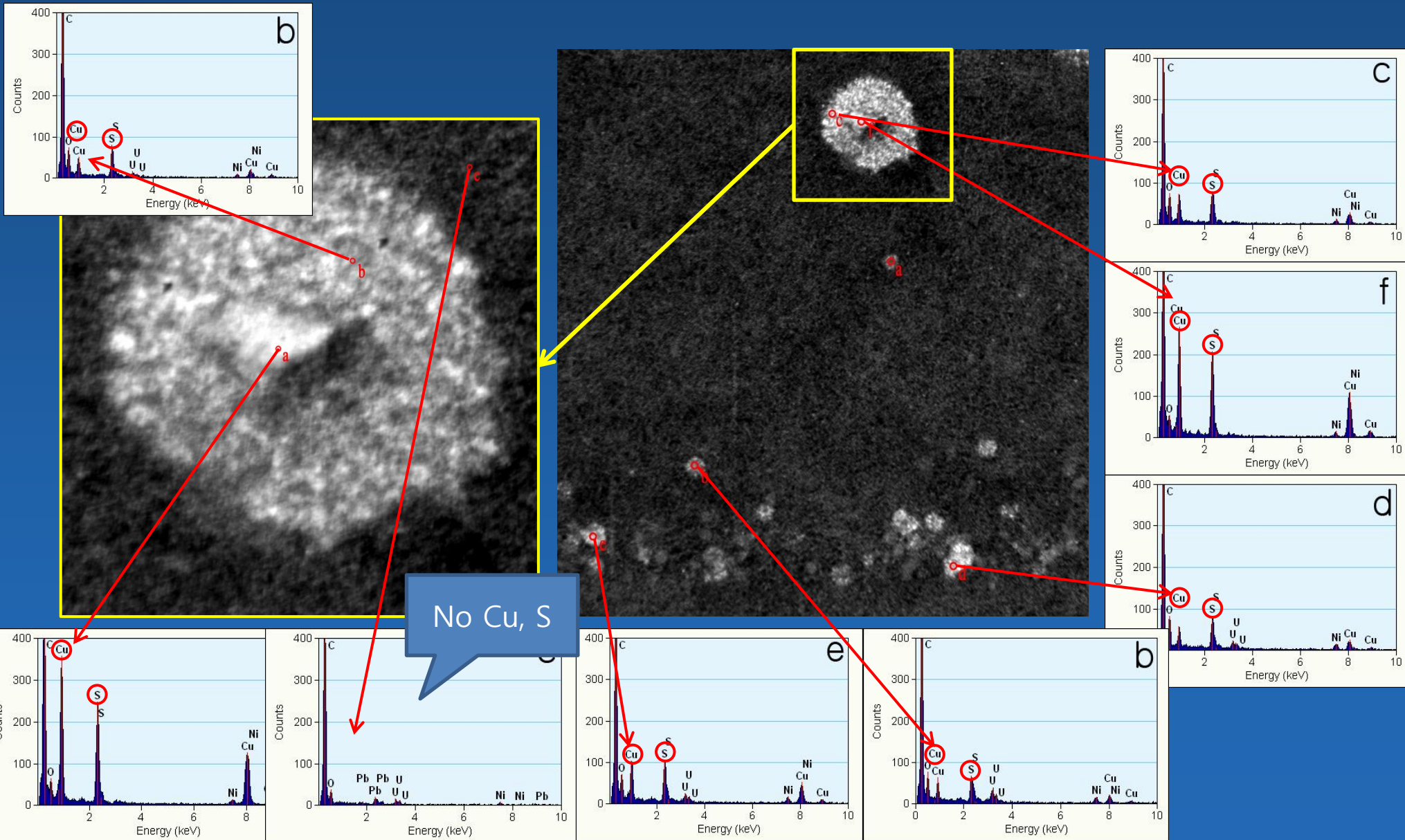
## ◆ Results (TEM image)



- The majority of granular deposits presented at the posterior one third of the anterior capsule
- The deposits were appeared as a linear pattern with scattered dots.
- A few of them were seen in the anterior two third.
- There was no electron dense particles in the epithelial cell layer of the lens.

# ◆ STEM-EDS analysis

- An **EDS point analysis** of electron dense granular deposits from anterior capsule revealed **consistent peak for copper (Cu) and sulfur (S) in all electron dense granule in all magnification view**
- STEM image showed higher peak intensities with the brighter electron dense granule



# ◆ STEM-EDS Analysis

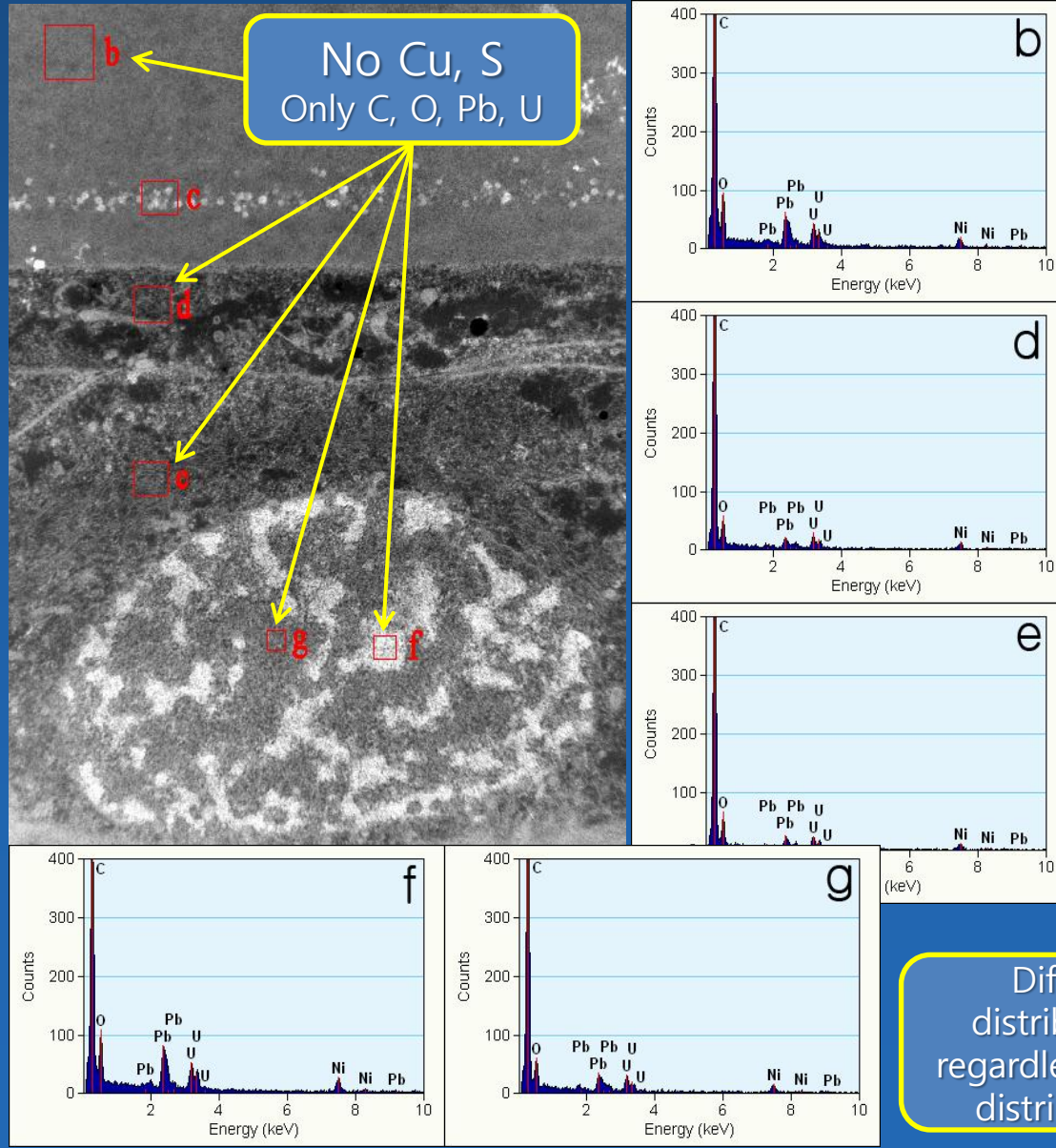
Whereas **peaks of Cu and S were absent in other parts**

including granule-free lens capsule and epithelial cell (b, d, e, f, g)

; only lead (Pb) & uranyl (U) peaks observed weakly

; Carbon (C), oxygen (O) and nickel (Ni) peaks were from region of

the formvar-carbon supporting film nickel grid

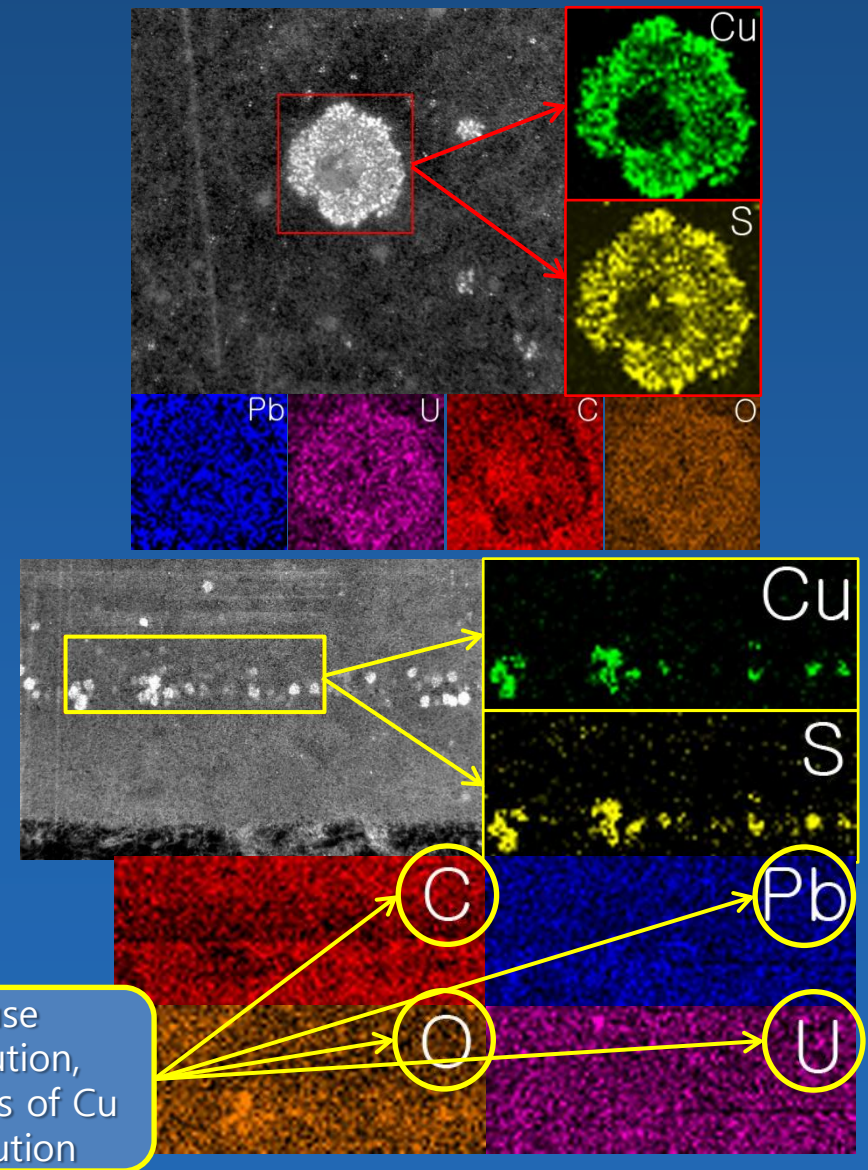


# ◆ Elemental Chemical Mapping

• Images presented in figures

: **Copper distribution overlapped with sulfur**

• Other background peaks associated element (C, O, Pb, U) showed diffuse distribution, regardless of copper distribution



## ◆ Discussion

- Our EDS study demonstrated
  - **Presence of Cu and S within electron-dense granular deposits** in lens capsule.
  - Elemental mapping showed **Cu distribution overlapped with S**.
  - Denser electron particles had higher intensity of Cu and S peak than relatively less dense electron particle.
- This results suggests
  - : The sunflower cataract is **not homogenous compound composed purely of copper and sulfur**, but **heterogenous compound composed of several material including Cu, S or copper binding proteins**
- We think that this electron dense granule is originated from **copper binding proteins such as metallothioneins**.
- We suggest how the granule composed of copper and sulfur accumulates in lens capsule close to lens epithelium as follow:
  - (1) In equatorial region, ionic copper uptake into lens epithelium occurs by diffusion.
  - (2) The sulfhydryl groups in the the metallothionein or other copper binding proteins could form a chelate complex with the ionic copper in cytoplasm.
  - (3) In long-term exposure to copper, there could be slow release of copper-thionein from lens epithelial cell to the lens capsule during the secretion of the basal lamina at the germinative area of the lens.

## ◆ Conclusion

- The sunflower cataract with WD is assumed the result from **accumulation of heterogenous compound** composed of several materials including **copper, sulfur or copper binding protein**.
- We suggest that accumulation of electron dense granule is related to both diffusion into and synthesis of lens capsule by lens epithelial cell.