

# **A possible case of exogenous ochronosis in the examination of a mummified head of the Medical and Forensic Anthropology Museum of the Complutense University of Madrid.**

Moissidou, Despina, Labajo González, Elena, González Arema, Ángel, Sánchez Sánchez, José Antonio, Gorgoulis, Vasilios, Chavaki, Sofia, Benito Sánchez, María y Perea Pérez, Bernardo.

Cita:

Moissidou, Despina, Labajo González, Elena, González Arema, Ángel, Sánchez Sánchez, José Antonio, Gorgoulis, Vasilios, Chavaki, Sofia, Benito Sánchez, María y Perea Pérez, Bernardo (2015). *A possible case of exogenous ochronosis in the examination of a mummified head of the Medical and Forensic Anthropology Museum of the Complutense University of Madrid. XIII Congreso Nacional de Paleopatología. Asociación Española de Paleopatología (AEP), Écija (Spain).*

Dirección estable: <https://www.aacademica.org/elenalabajogonzalez/97>

ARK: <https://n2t.net/ark:/13683/pcQr/efb>



Esta obra está bajo una licencia de Creative Commons.  
Para ver una copia de esta licencia, visite  
<https://creativecommons.org/licenses/by-nc-nd/4.0/deed.es>.



XIII Congreso Nacional de Paleopatología

Paleopatología y bioarqueología.

Contextualizando el registro óseo

Écija, 1 - 4 de octubre - 2015

# A possible case of exogenous ochronosis in the examination of an mummified human head of the Medical and Forensic Anthropology Museum of the Complutense University of Madrid

Moissidou D<sup>1</sup>, Labajo-González E<sup>2</sup>, González-Arema A<sup>2</sup>, Sánchez-Sánchez JA<sup>2</sup>,  
Gorgulis V<sup>1</sup>, Chavaki S<sup>1</sup>, Benito-Sánchez M<sup>2</sup>, Perea-Pérez B<sup>2</sup>

(1) Department of Histology and Embriology, Medical School of Athens, National Kapodistrian University of Athens

(2) Escuela de Medicina Legal y Forense, Universidad Complutense de Madrid

Organizan:



Sociedad  
Española de  
Paleopatología



Excmo.  
Ayuntamiento  
de Écija

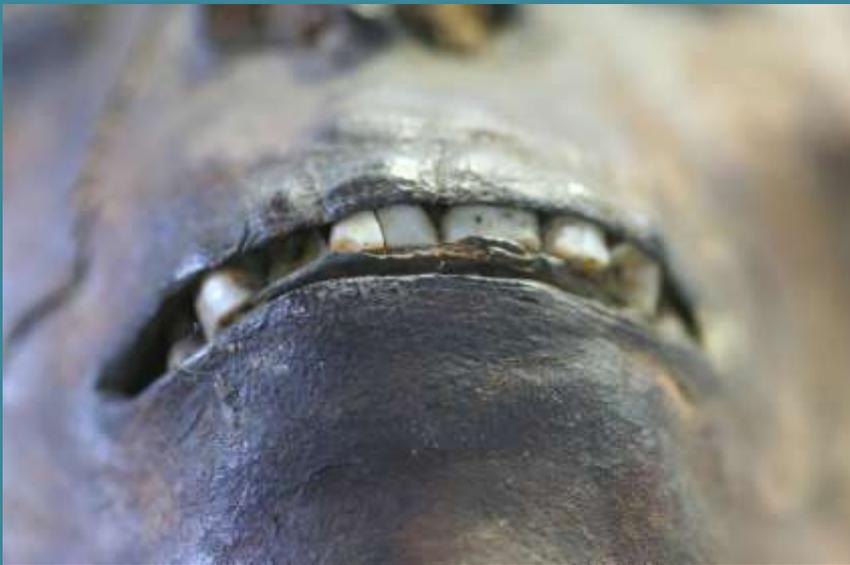
# Egyptian mummified head (ME 006)



# Historical Background

- Female of 20-25 years of age.
- First half of XVIII Dynasty, during the reigns from Aa-Kheper-en-Re Thutmose II to Men-Kheper-Ra Thutmose III, New Kingdom.
- From the Theban area.
- Transferred to the Cairo Museum some time between 1.898 to 1.930.
- Sold to the Spanish collector and banker Ignacio Bauer
- Donated the piece to the Real Sociedad de Arqueología, Antropología y Prehistoria in Madrid (1930).

# Egyptian mummified head (ME 006)



- Plugged nose and ears with linen wrappings.
- Protruding tongue, possible as part of the Opening of the Mouth ritual.

# Egyptian mummified head (ME 006)



Macroscopic examination revealed papulo-nodular lesions, and the diagnosis suggests skin inflammation due to possible use of cosmetics.

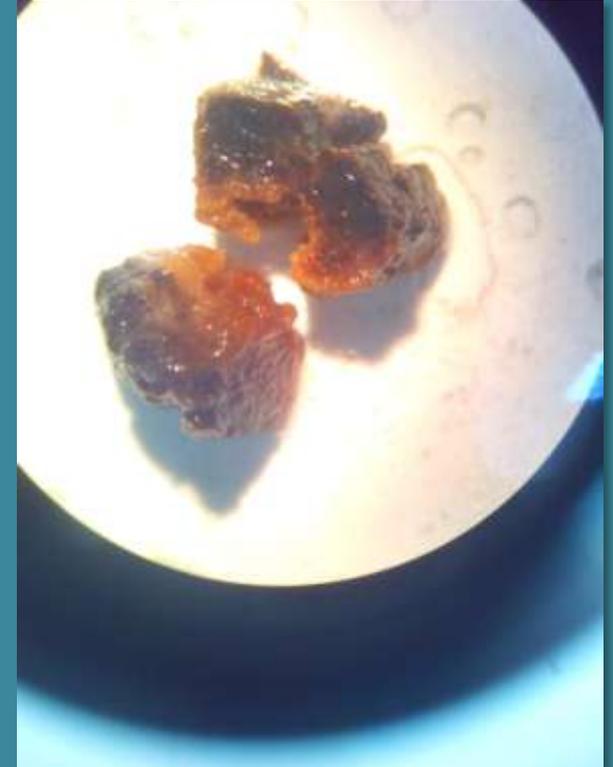
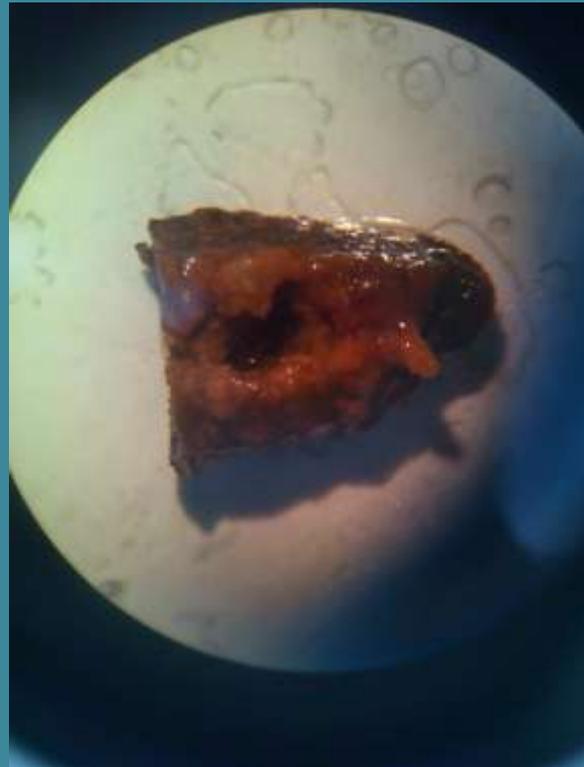
# Egyptian mummified head (ME 006)



Samples were retrieved from the base of the neck.



# Stereoscopic view of the samples



- Leathery appearance.
- Not heavily resinated.
- Difficult to cut into smaller pieces.

# Raman spectroscopy

- Portable DeltaNu Rockhound portable Raman Spectrometer (785nm).

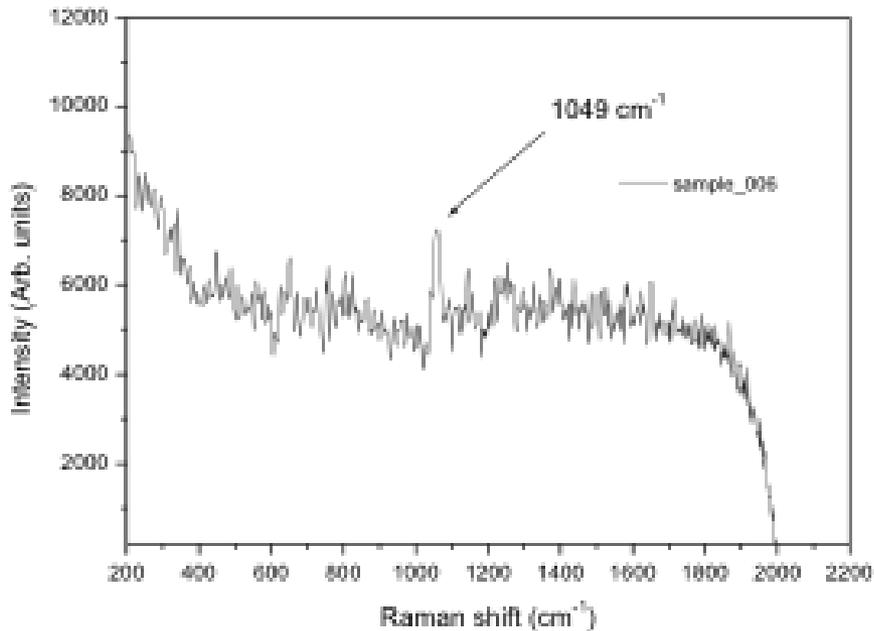


- Fluorescence is a common problem in Raman Spectroscopy. Under conditions where fluorescence is generated, it may be intense and can overshadow the Raman features.
- The Advantage of 785 nm reduces competing fluorescence interference in compounds through this process. It uses a 785nm excitation laser to reduce the fluorescence signature in paintings that show strong fluorescence at shorter wavelengths.

# Raman spectroscopy results

- Presence of sodium carbonate, sodium bicarbonate and impurities of chloride and sulfate as its major elemental components.
- Probably from the natron extracted at Wadi Natrun.
- The characteristic Raman peak at 1045  $\text{cm}^{-1}$  belongs to the mineral Trona  $[\text{Na}_3(\text{HCO}_3)(\text{CO}_3)\cdot 2\text{H}_2\text{O}]$  which is the main component of the natural substance from the Desert of Wadi Natrun (Egypt). Edwards et al (2007)
- The further Raman examination uncovers the other materials of organic nature, such as amide and proteins.
- The mummy was embalmed by natron, which absorbs water and is also mildly antiseptic, in the way used by ancient Egyptians.

# Raman spectroscopy results



Raman spectrum (RockHound 785 DelatNu spectrometer) was recorded in the range 2000–200  $\text{cm}^{-1}$ . We have examined the optical properties of the samples using Raman Spectroscopy, as can be seen in Fig.1 and 2, with a high resolution of  $10 \text{ cm}^{-1}$  and low signal to noise.



# Histological Processing of ME006 samples

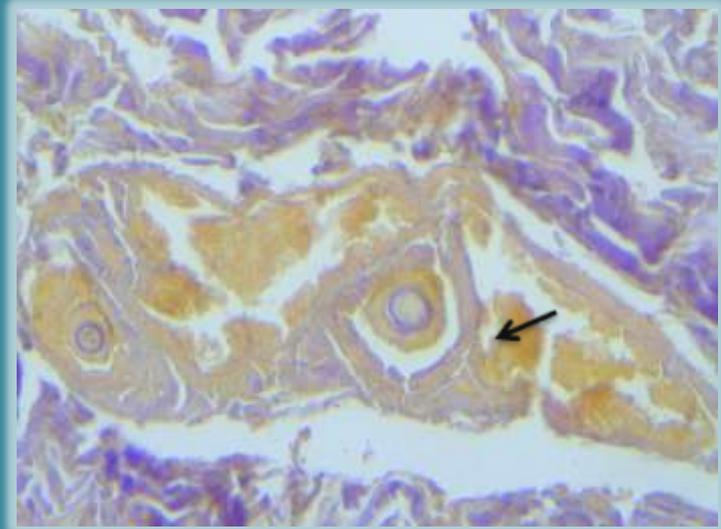
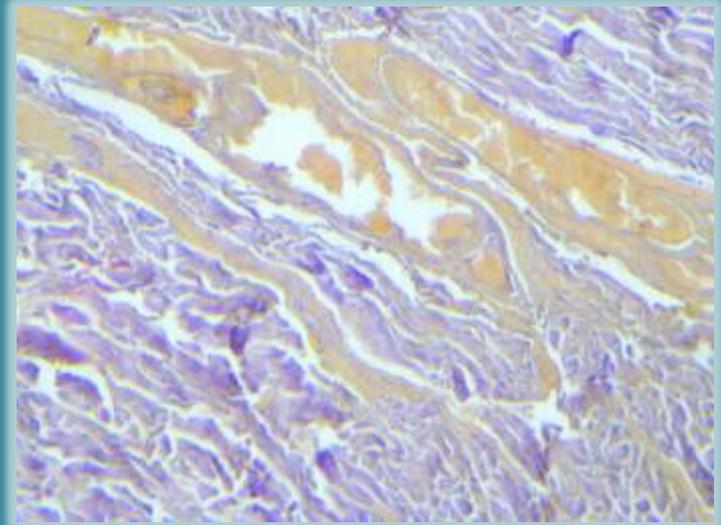
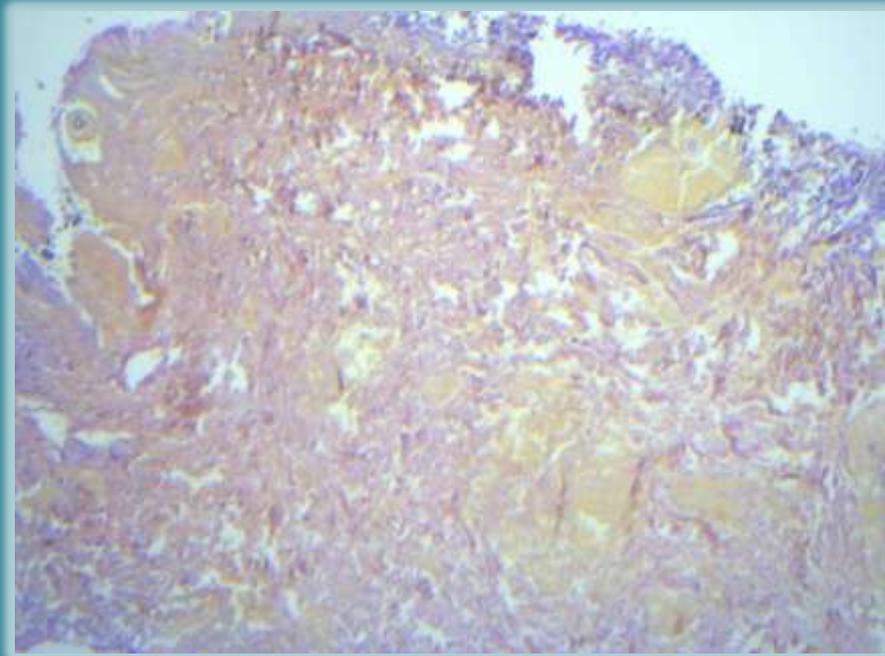
- Partially elastic tissue texture on the exterior, quite hard on the inside.
- Removal of embalming residues with the use of HistoClear (citric acid product, used for fixation).
- Softening and rehydration of the tissue samples (app. 1 week):
  - Comfort 1 % Buffer Solution.
  - Sandison's Solution.
- Tissue was placed in cassettes, processed overnight and put into paraffin blocks – tissue was cut in microtome.

# Histological Processing of ME006 samples

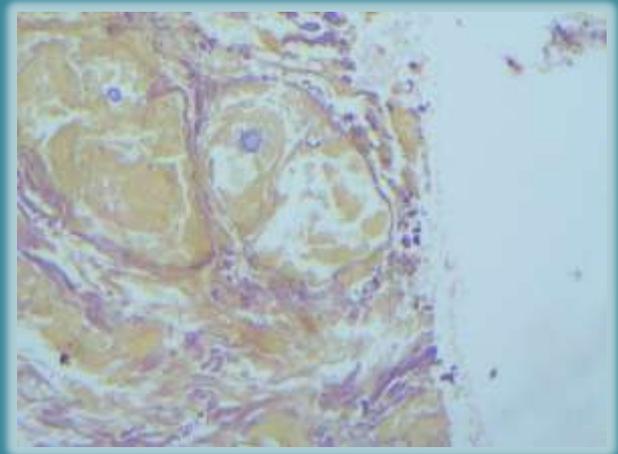
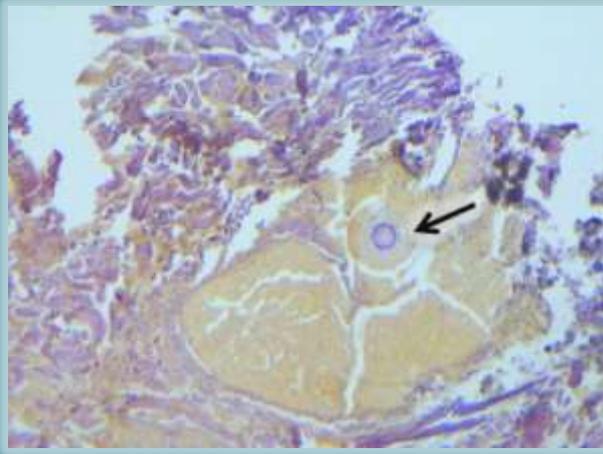
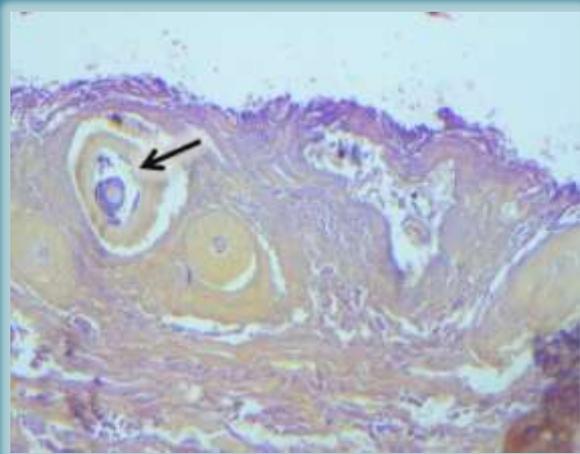
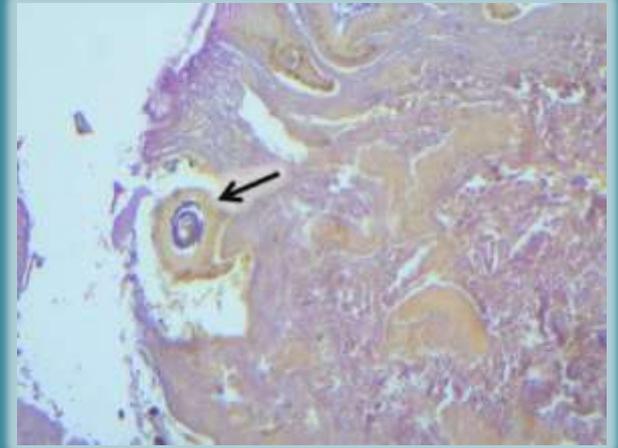
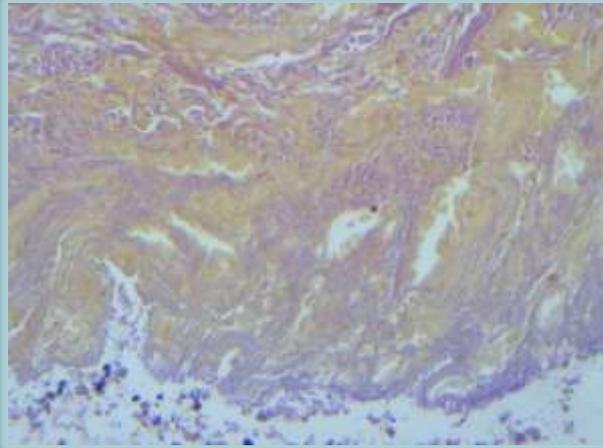
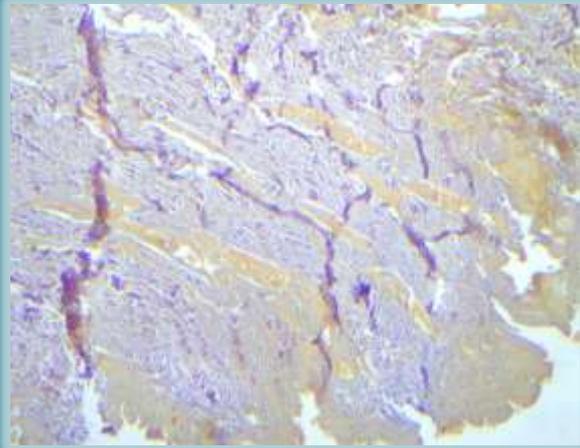
- Histochemical techniques:
  - Haematoxylin & Eosin.
  - Masson's Trichrome.
  - PAS (Periodic Acid Schiff).
- Immunohistochemistry:
  - CD3, CD8, CD20, CD34, CD44, CD68, NOX4.
- Transmission Electron Microscopy TEM:
  - Standard protocol followed/ gradual aldehydes.
  - Softening/rehydration process not performed.

# Histochemistry – Haematoxylin & Eosin

- Standard techniques, used for daily diagnosis (nuclei, cytoplasm).
- Visible fibres and connective tissue.
- Hair follicles.
- No nuclei present.

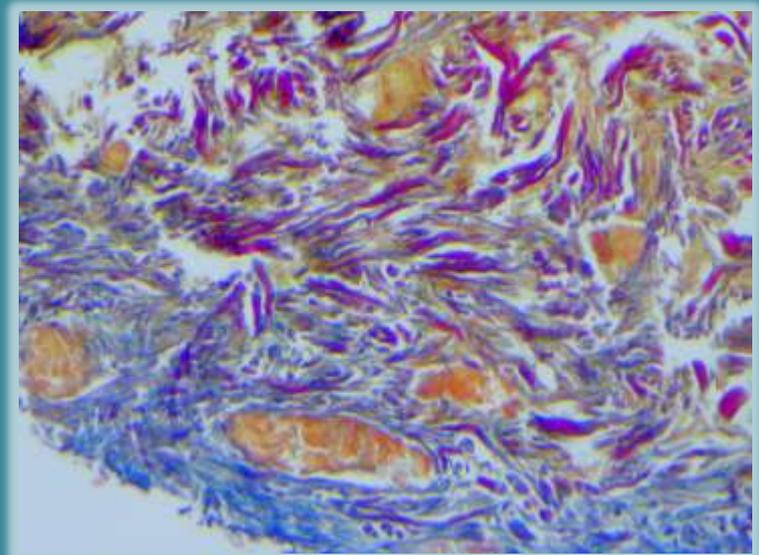
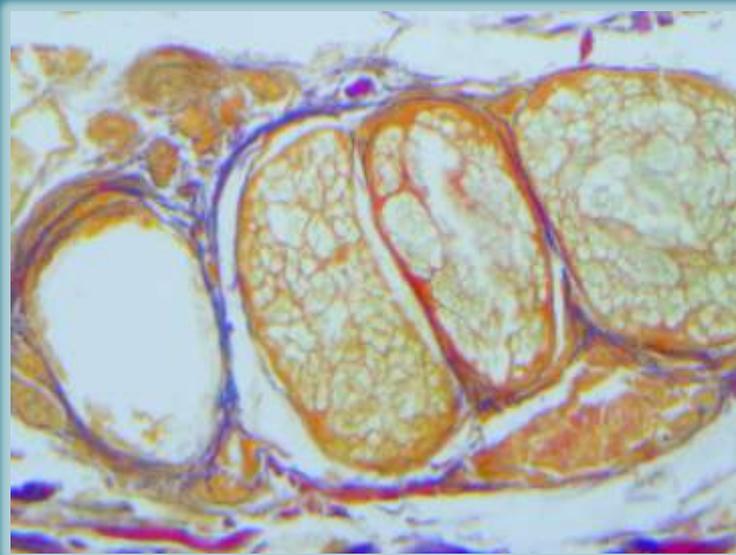
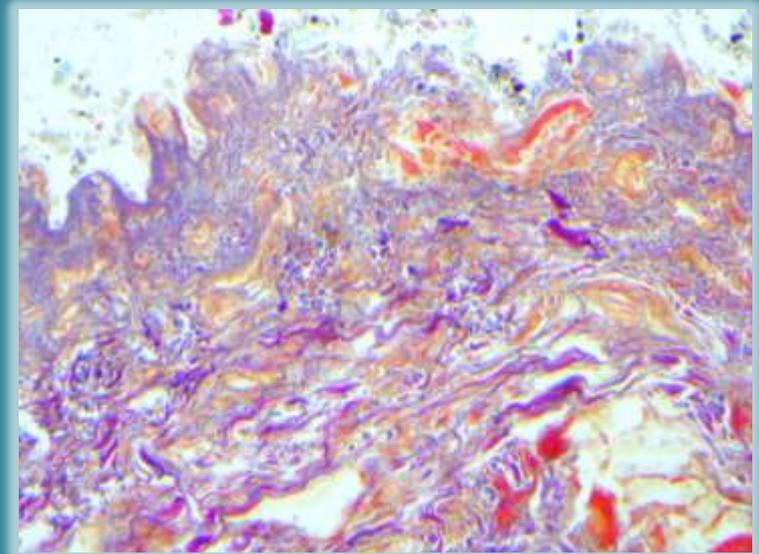


- Keratinised areas in the dermis and around the hair follicle area.
- Most probable skin inflammation.

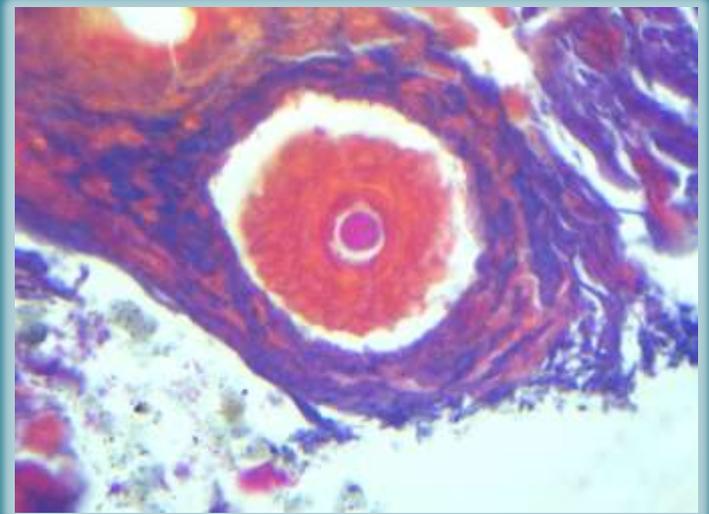
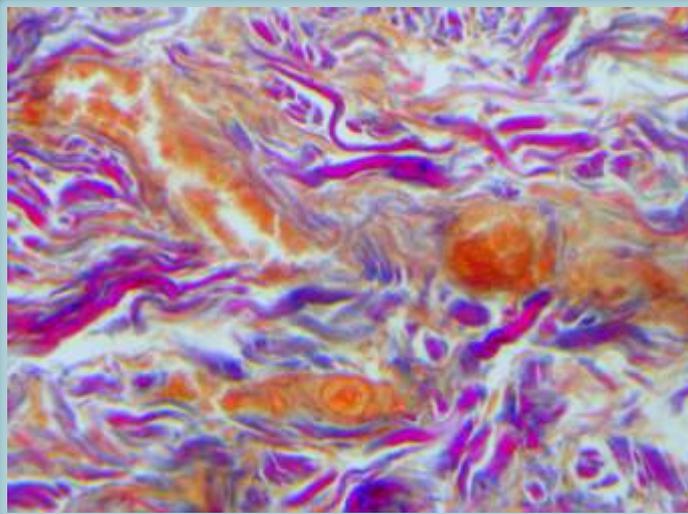
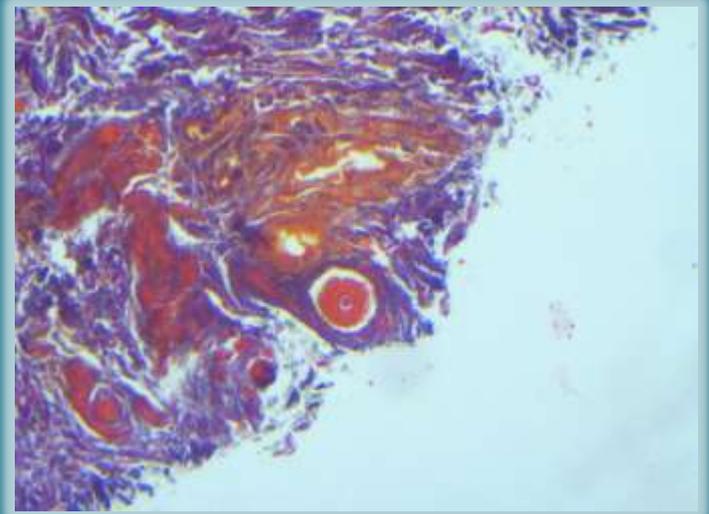
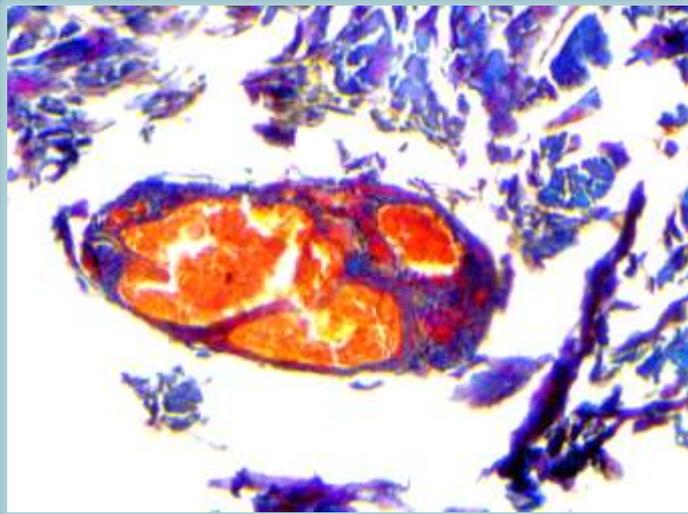


# Histochemistry – Masson's Trichrome

- Collagen fibers in blue.
- Elastin and muscle fibers in dark red.
- Keratinised fibers in yellow confirming inflammation.



# Histochemistry – Masson's Trichrome



# Exogenous Ochronosis

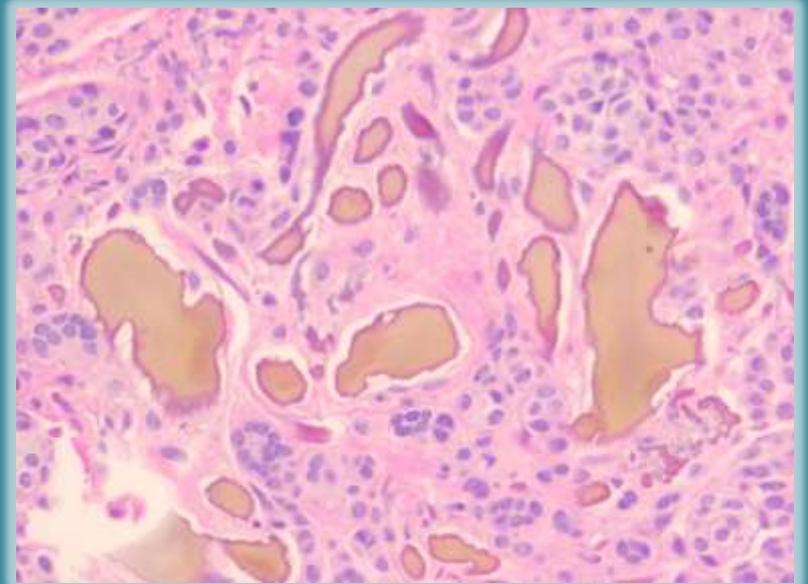
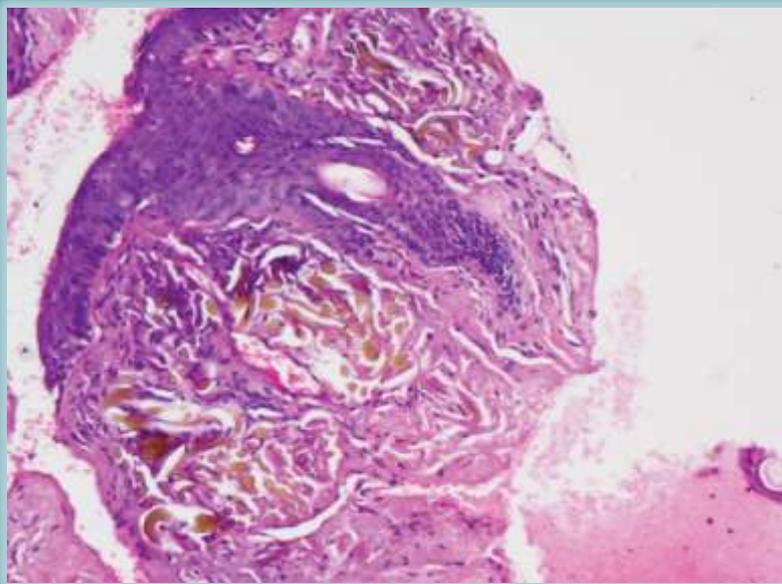
**Exogenous ochronosis:** skin disorder due to the use of “hydroquinone” a substance used as a bleach to lighten the skin colour (common in Asian and Black people)

The embedded pigments also form cross-linkages with pigment depositions in adjacent fibers, stabilizing and reducing the elastic recoil of the fibers. This results in hardening of elastic structures increasing their rigidity and brittleness. Once ruptured, the exposed pigments cause a foreign body reaction and inflammation. This pigment deposition also invokes deposition of **hydroxyapatite**, the mineral responsible for bone calcification, further hardening the connective tissue.



# Exogenous Ochronosis

Modern cases of exogenous ochronosis with keratinised deposits and pigmented areas under the epidermis.



# Cosmetics in ancient Egypt



- Ancient Egyptians regarded the use of cosmetics both for aesthetic purposes as well as magical and religious ones.
- Cosmetic pigments were used on a daily basis.
- Royals had to look like gods.
- Many of the cosmetics used by Egyptians, included lead as a basic component, which can cause skin inflammation, as well as malignancies.

# Immunohistochemistry

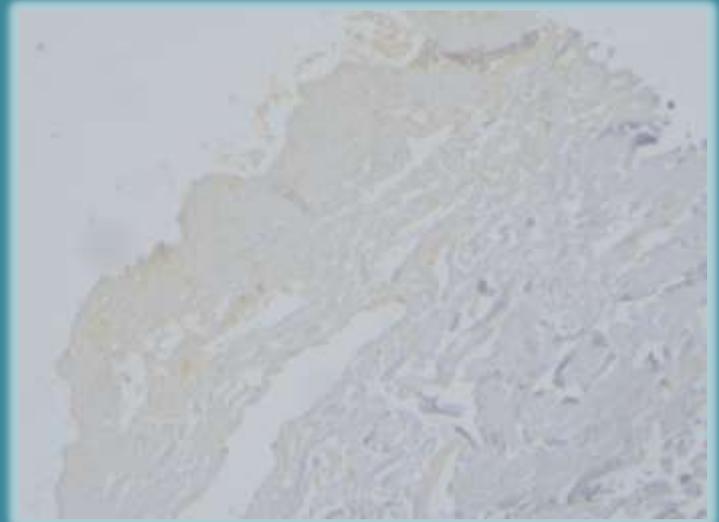
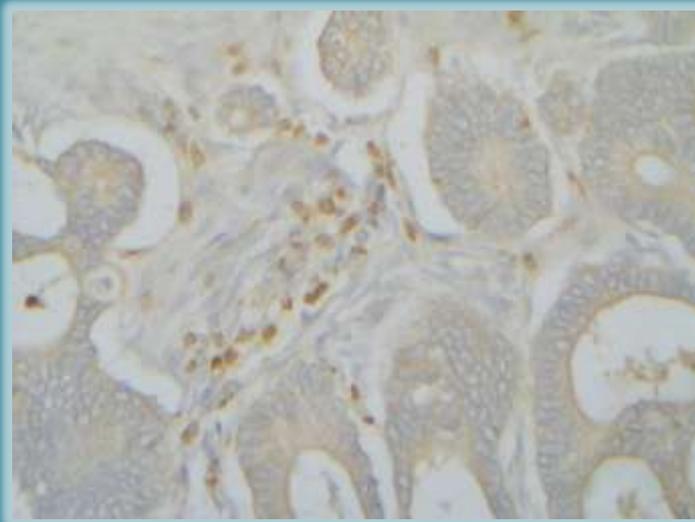
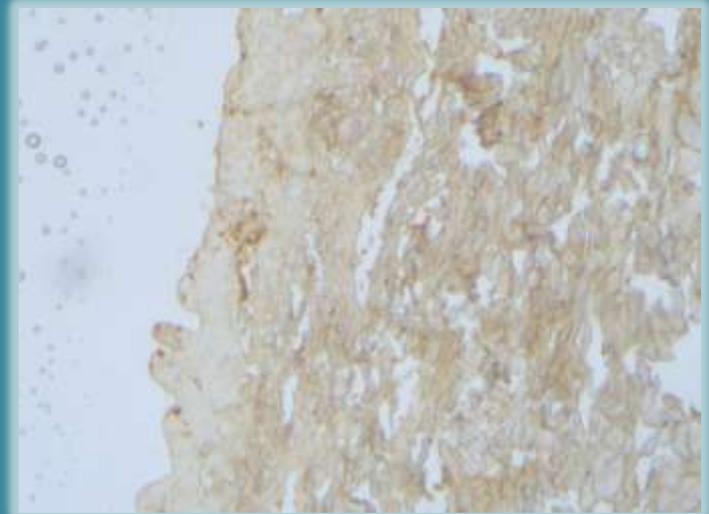
- Various markers (antibodies) for inflammation and malignancies (skin disease diagnosis).
- Standard protocol with variations in preparation time (with regards to normal tissue).
- Master control from normal samples.
- Negative control from the mummified sample (for invalid staining).

# Immunohistochemistry

## CD3

A protein complex , expressed in cell proliferation.

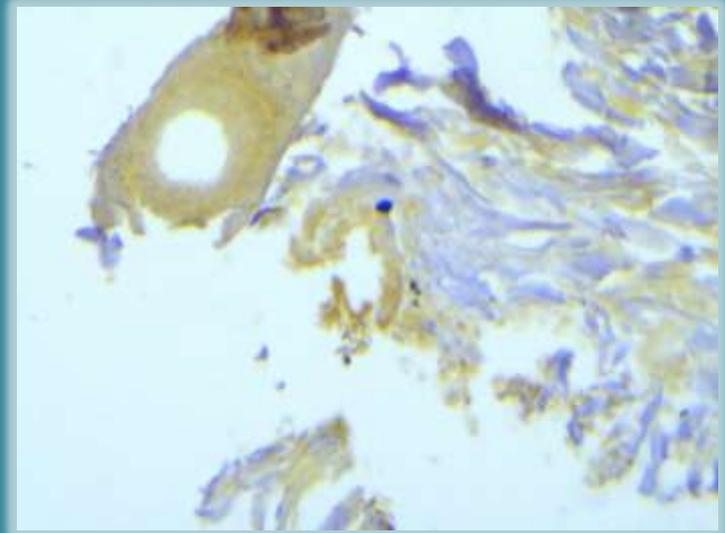
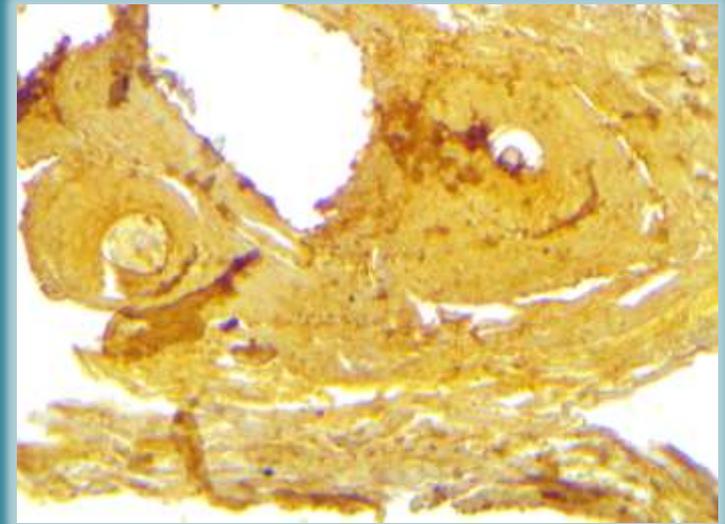
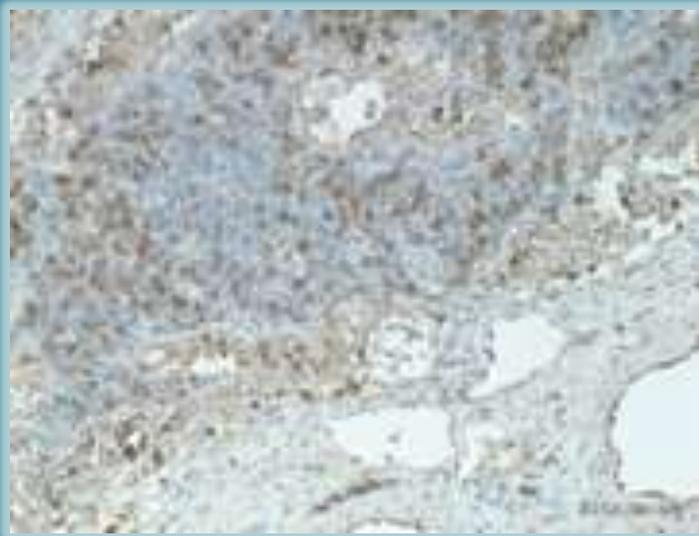
Used as a cytoplasmic marker for malignancies and inflammation.



# Immunohistochemistry

## CD8

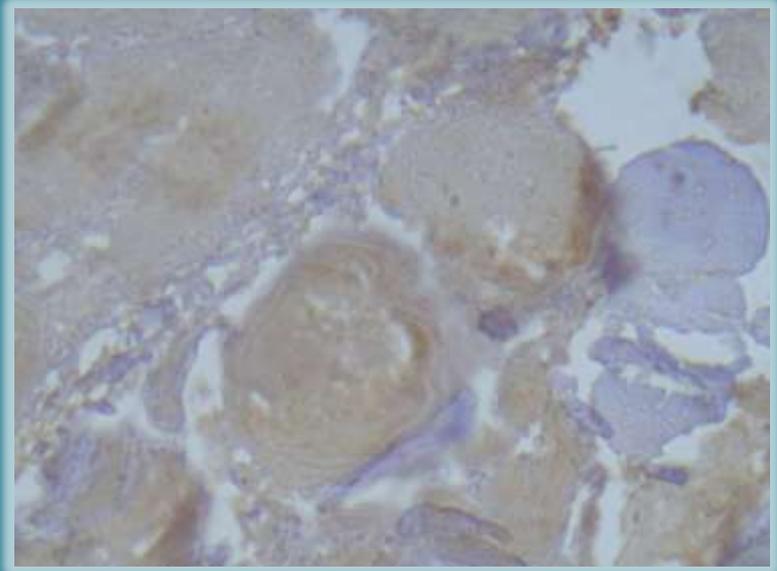
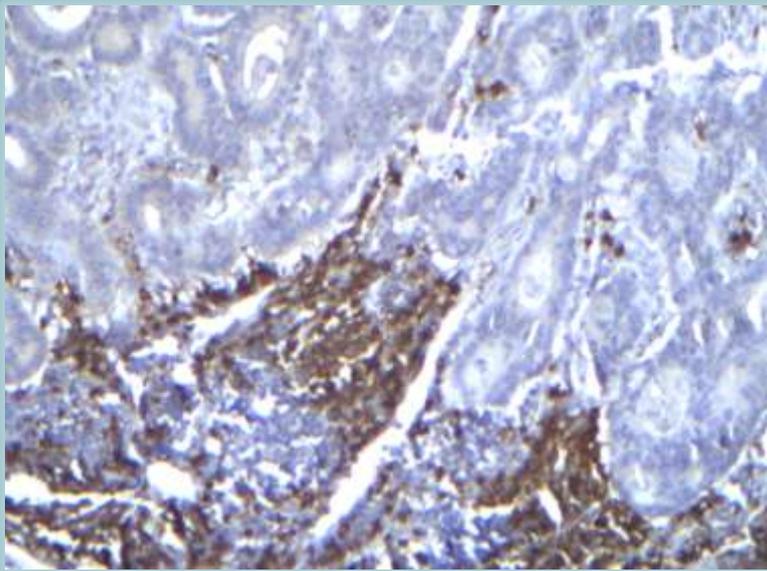
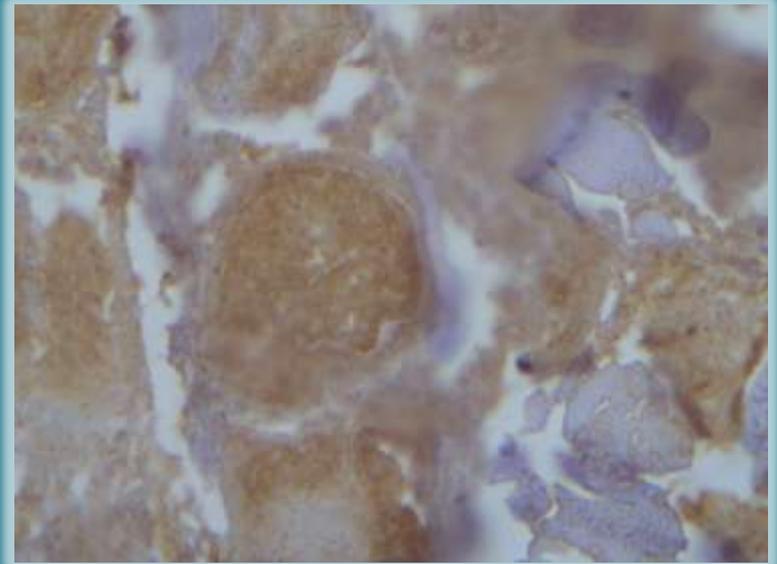
- A glycoprotein expressed in T-lymphocytes (T-cells), natural killer cells (NK), and macrophages.
- Commonly used for skin inflammation.



# Immunohistochemistry

## CD20:

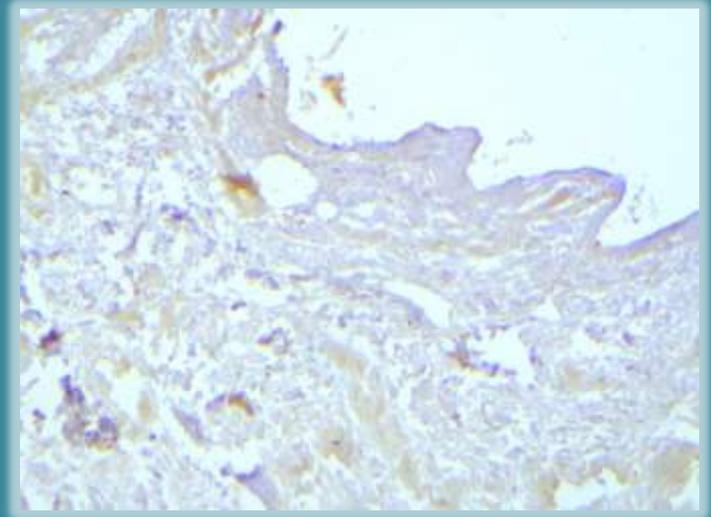
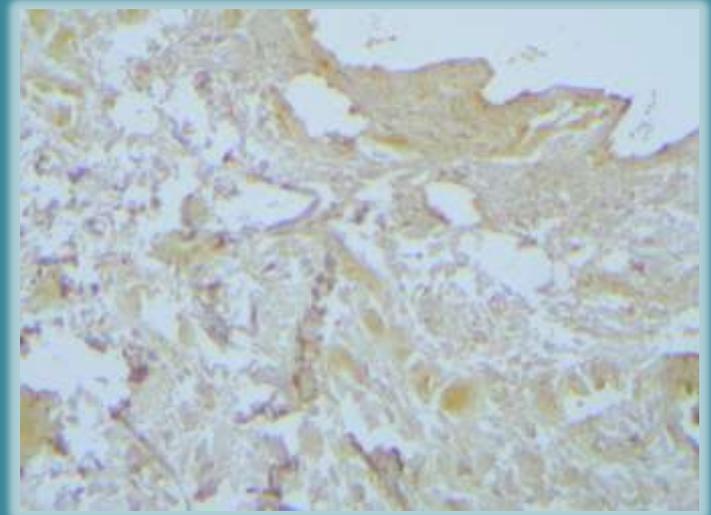
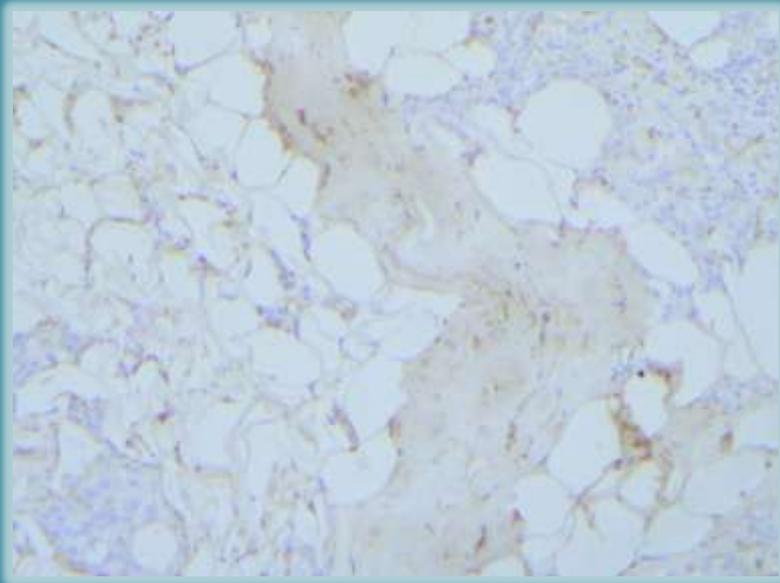
- A phosphoprotein which reacts with B cells (production of antibodies).
- A marker for melanoma cancer stem cells.



# Immunohistochemistry

## CD34

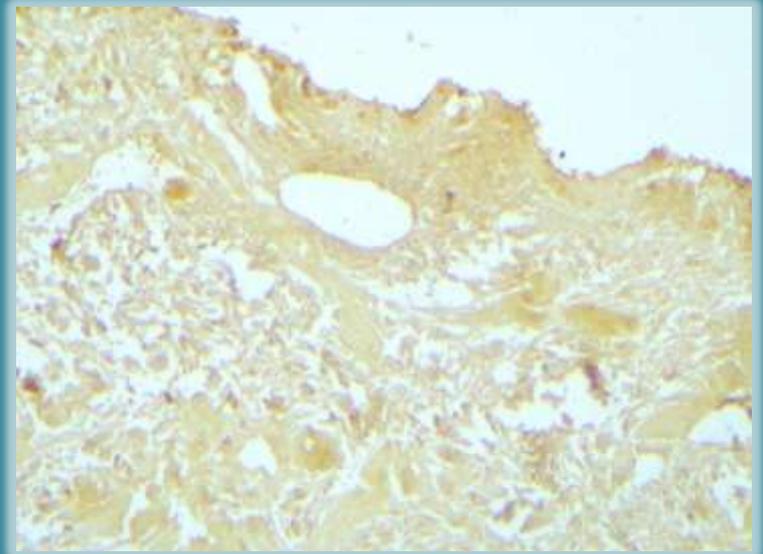
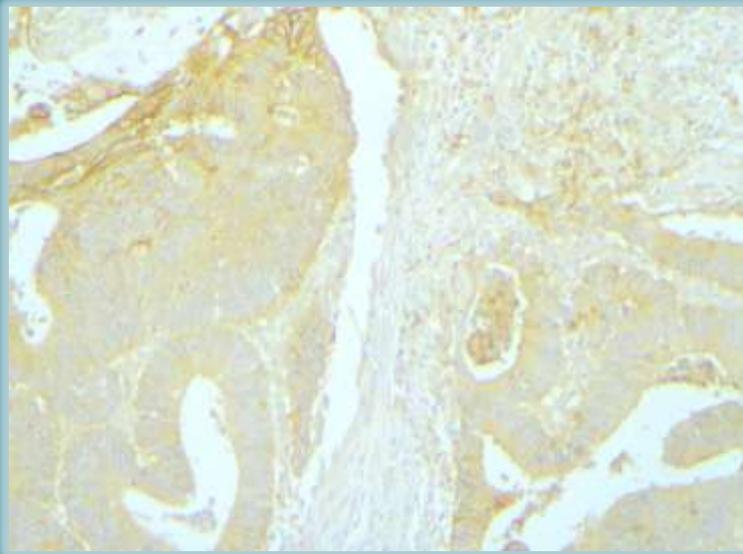
- A glycoprotein used for endothelial cells.
- A marker where inflammation occurs.



# Immunohistochemistry

## CD44

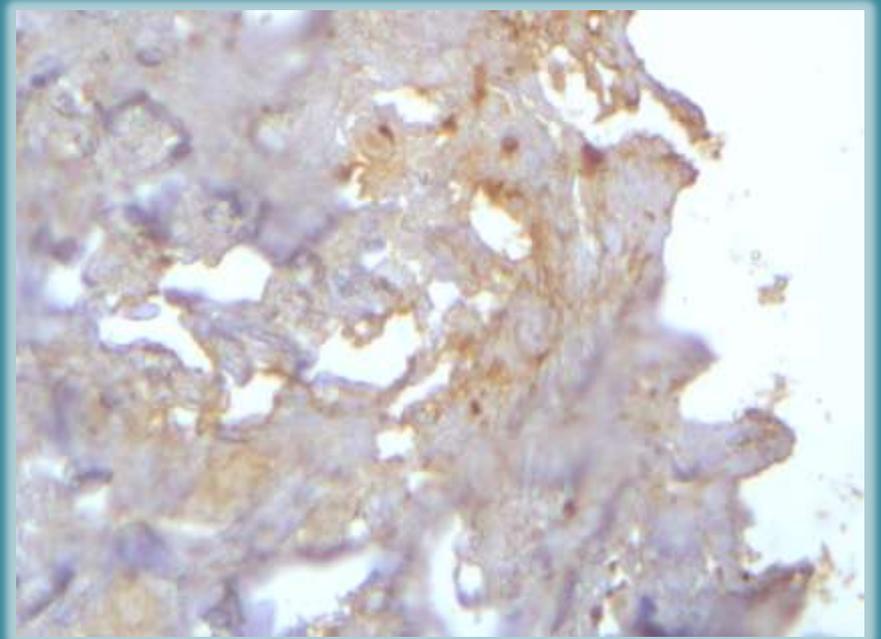
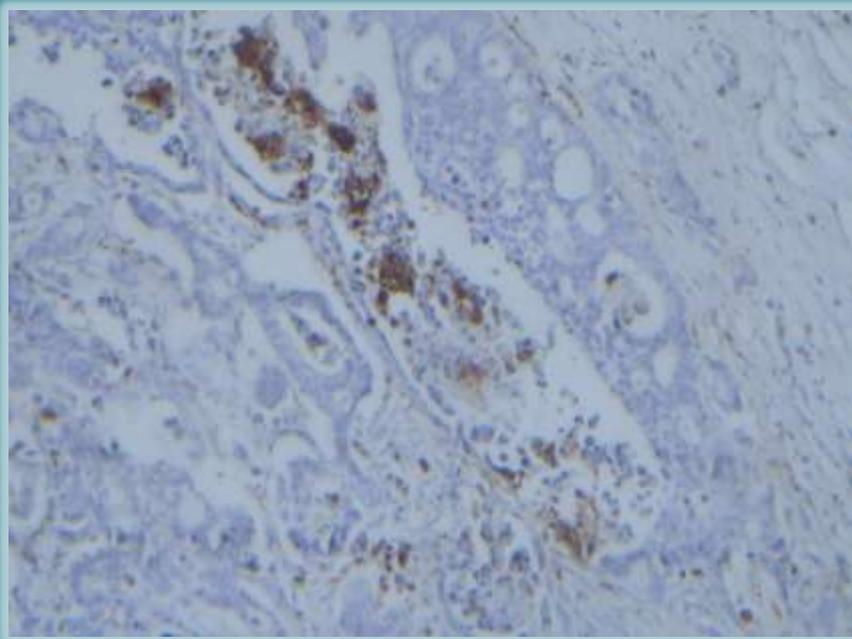
- (Cell-surface glycoprotein), used for subacute dermatitis.
- Also wound healing in inflammatory response.



# Immunohistochemistry

## CD68

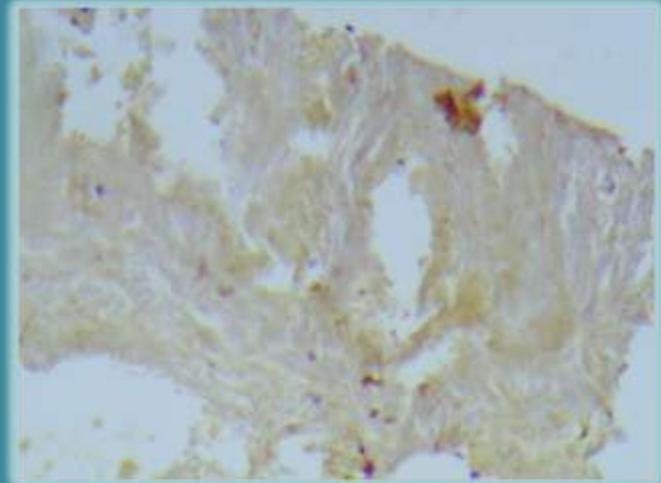
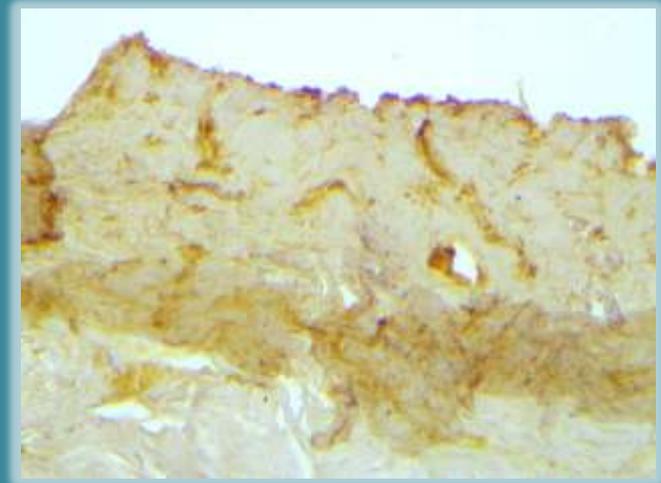
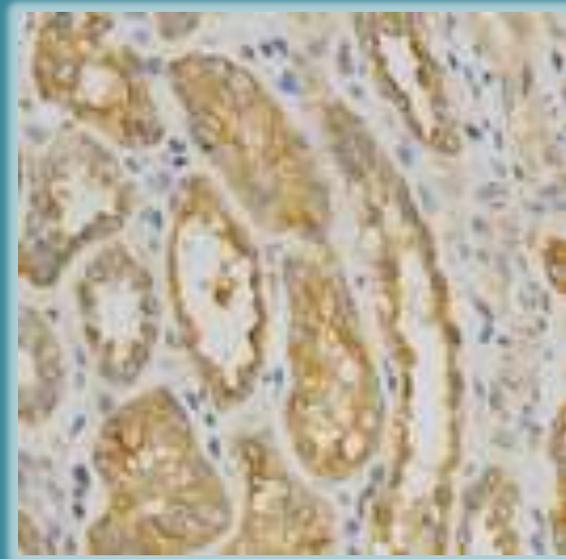
- A glycoprotein found in cytoplasmic granules of various macrophagocytic cells.
- For diagnosis relating to abnormality/proliferation of such cells.



# Immunohistochemistry

## NOX4

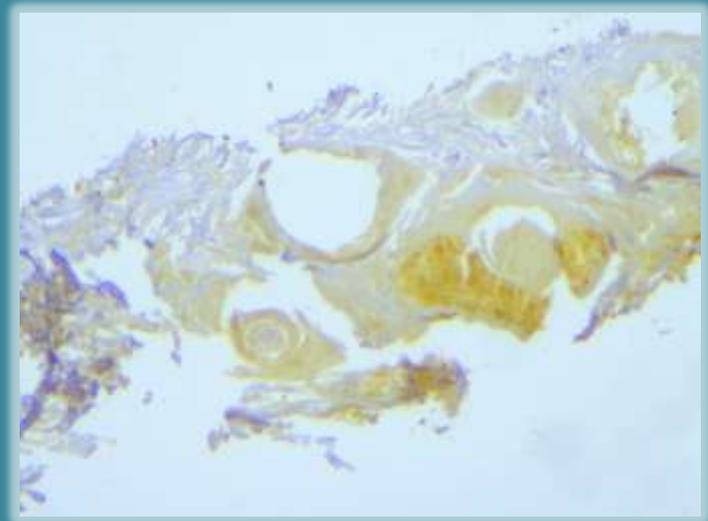
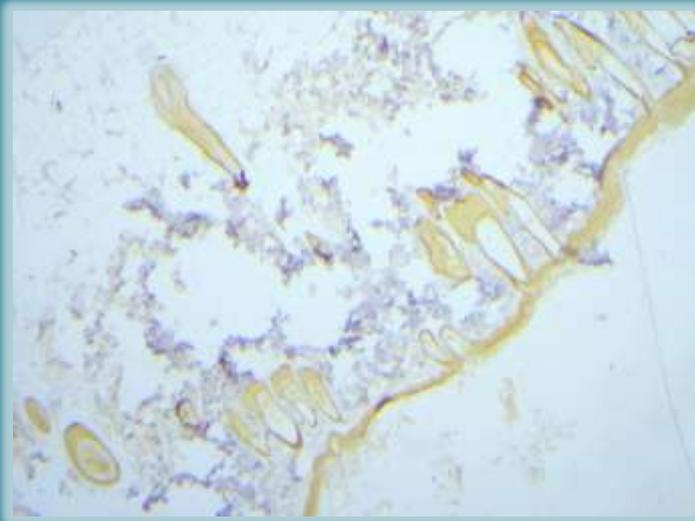
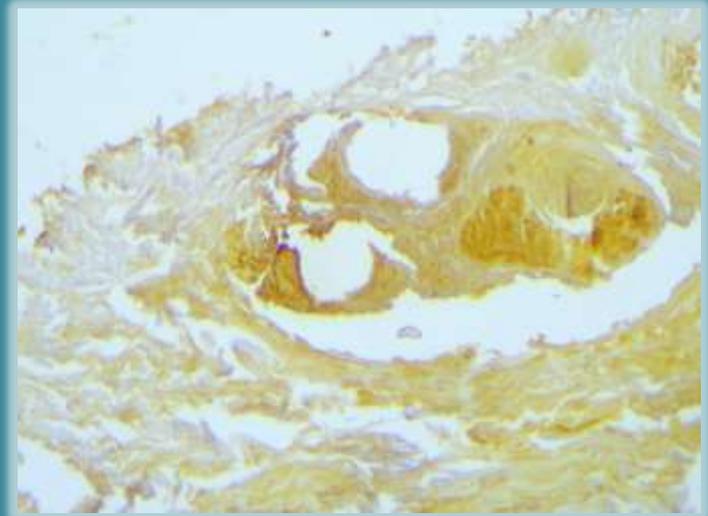
(Family of NADPH oxidases): enzyme which reacts to oxidative stress due to inflammatory response.



# Immunohistochemistry

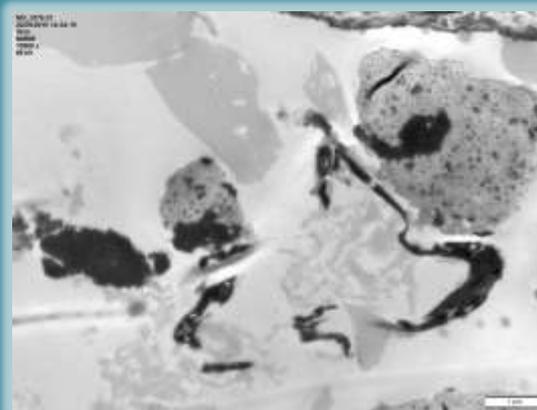
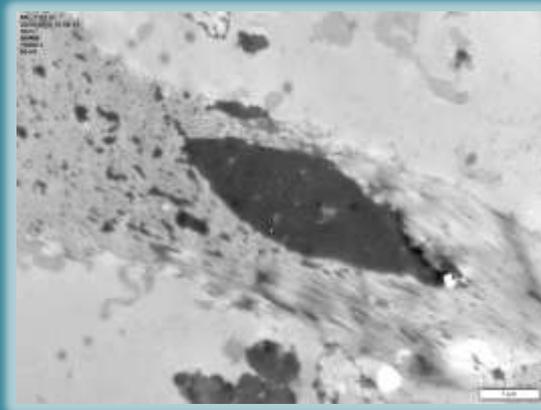
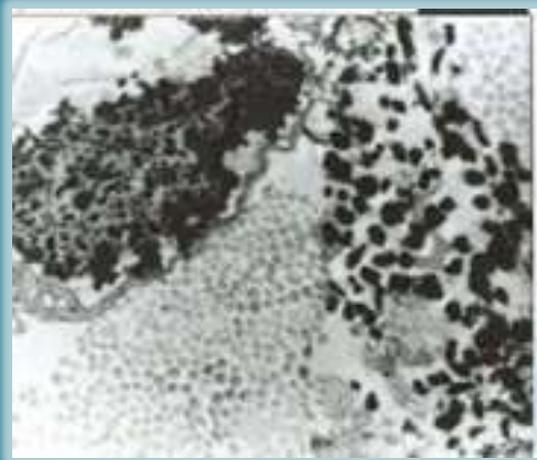
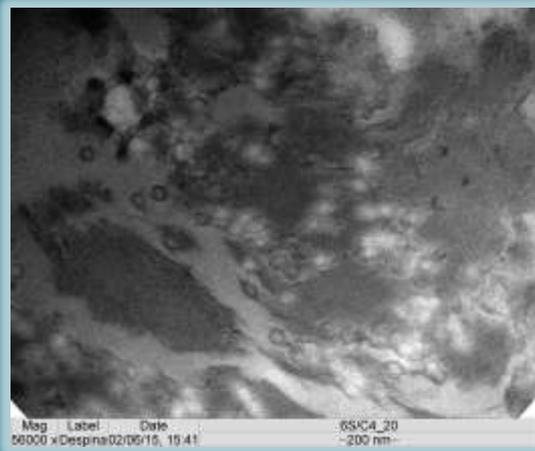
## PANKERATIN

- protein used for detection of amounts of normal or abnormal epithelial cells.
- used for skin disorders but also malignant tumors.



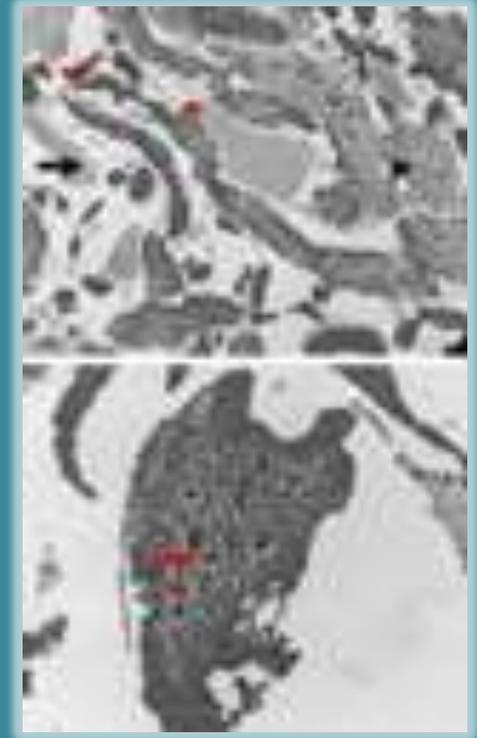
# Transmission Electron Microscopy of ME006

“Empty” collagen fibers, amorphous/abundant elastin, elastolysis, elastorrhexis.



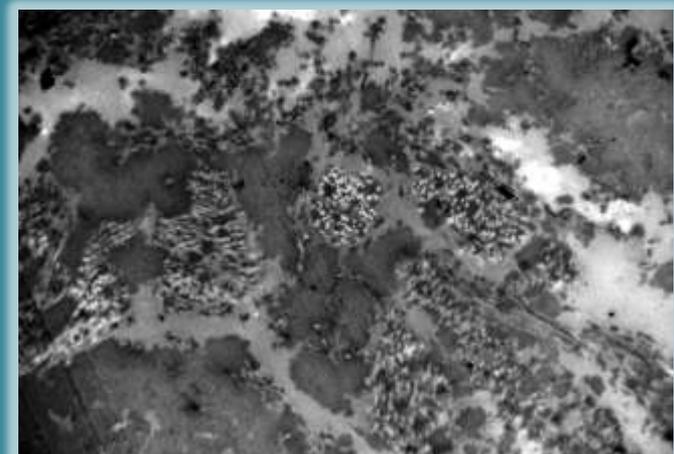
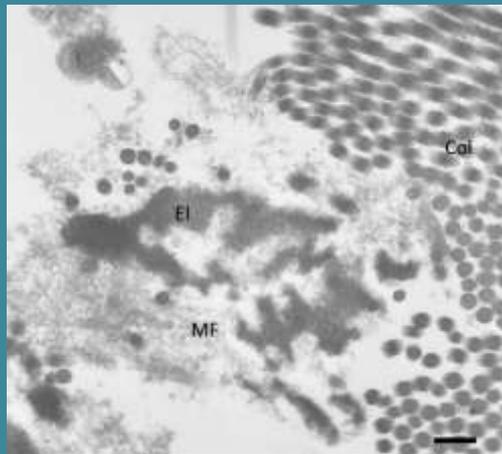
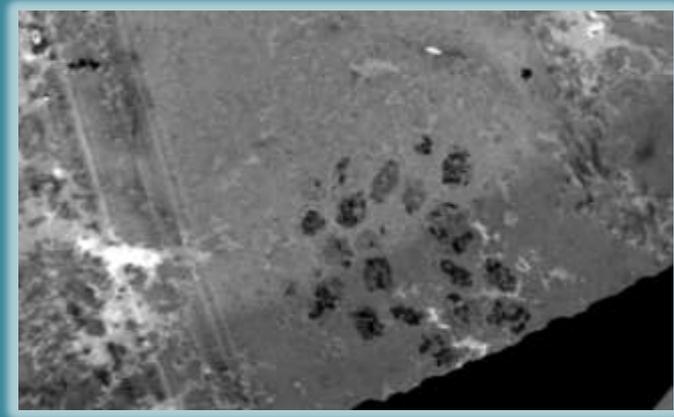
# Transmission Electron Microscopy of ME006

Electron microscopic image of exogenous ochronosis. A, Amorphous electron-dense structures deposited in the interstitium (black arrow). Elastolysis (black arrowhead), elastorrhexis (red arrow), and moth-eaten defects (red arrowhead) in the external outline of elastic fibers are noted. B, Deposition of the same electron-dense structures in the core of elastic fibers (red arrow).



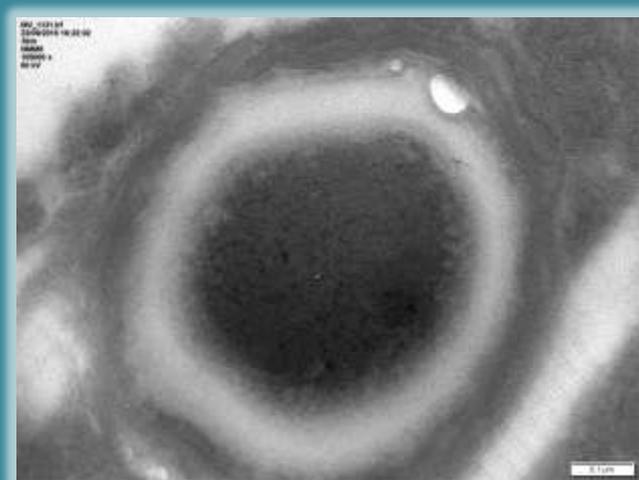
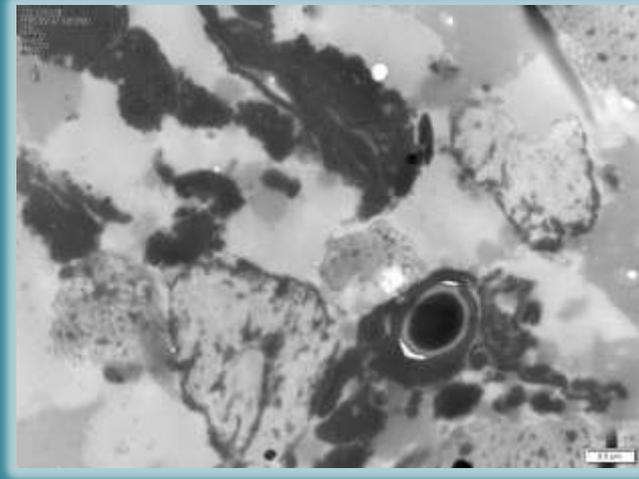
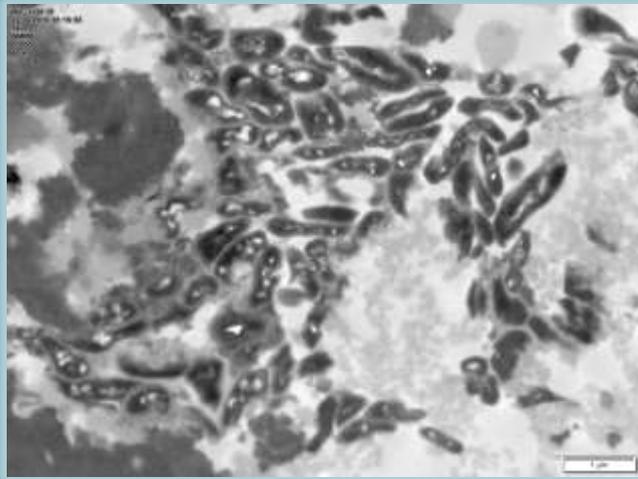
# Transmission Electron Microscopy of ME006

TEM on ME006: comparison of mummified sample with modern showing phagocytic melanocytes and collagen fibers with elastin structures.



## ...and some other findings

- Presence of bacterial colonies, possible mycobacteria.
- Presence of fungus of unknown species yet (looking similar to *Candida Albicans*) ?



# CONCLUSION

- Both confocal and electron microscopy show similarities with exogenous ochronosis.
- Immunohistochemistry confirms inflammation.
- Further investigation is needed (including bacterial/fungal presence).

# Acknowledgements

Dept. Histology/Embryology, Medical School, University of Athens

Mr. Plato Selemenakis

School of Legal and Forensic Medicine, University of Madrid

Mr. David Cáceres Monllor

Laboratory of Non-Destructive Analysis, Piraeus University of Applied Sciences.

Laboratory of Physicochemical Analyses, Byzantine and Christian Museum, Athens.

Prof. Thanos Ganetsos

Dr. Thomas Katsaros