**FP7-INFRASTRUCTURES-2012-1**

# Enabling Science and Technology through European Electron Microscopy

**Project Acronym: ESTEEM2**

**Grant Agreement n°: 312483**

## Deliverable 6.2

## Report on protocols and standards developed in ESTEEM2

**REPORT OF ACTIVITIES BY TU GRAZ**

**A coffee-based staining alternative for biological samples**

## A coffee-based staining alternative for biological samples

For contrasting biological samples, osmium tetroxide, **uranyl acetate**, lead citrate and phosphorus tungstate are often used either individually or in combination. Hereby, uranyl acetate offers several advantages for biological sample preparation in electron microscopy such as easy application and fast time-to-result, allowing to examine the sample within a few minutes after treatment.
The downsides are chemical toxicity and slight radioactivity, so extra care has to be taken to avoid cumulative effects by long term exposure.

In the light of these limitations, other more unconventional reagents for a suitable non-toxic and non-radioactive alternative to uranyl acetate have been investigated. Attempts by Sato et al. [1,2] and Miller et al. [3] with Oolong tea already rendered equivalent contrast results to uranyl acetate. Following this idea, a comparative study of the contrast effect of substances contained in coffee, as a substitute for uranyl acetate, on animal and plant cells was conducted.

## Sample Preparation

The animal and plant cells were first fixed with glutaraldehyde (3%) and 0,1mol sodium cacodylate buffer solution, and subsequently rinsed with distilled water. The first opacification was obtained with aqueous $OsO_4$ (2%) for 4h. After rinsing, a dehydration series (increasing the ethanol concentration) of the samples was carried out, and the samples were embedded in Agar100 (also by slowly increasing the Agar100 content), concluding the first preparation step. An Ultramicrotome UC6 from Leica Microsystems, equipped with an 35° diamond knife (Diatome), was then used to cut sections with a thickness of about 75nm. These cuts were applied onto copper/palladium grids covered by a Pioloform film.

Both the animal cells and the plant cells were then stained with an aqueous uranyl acetate (2%) solution for 15 minutes or the coffee reagent for 30 minutes.

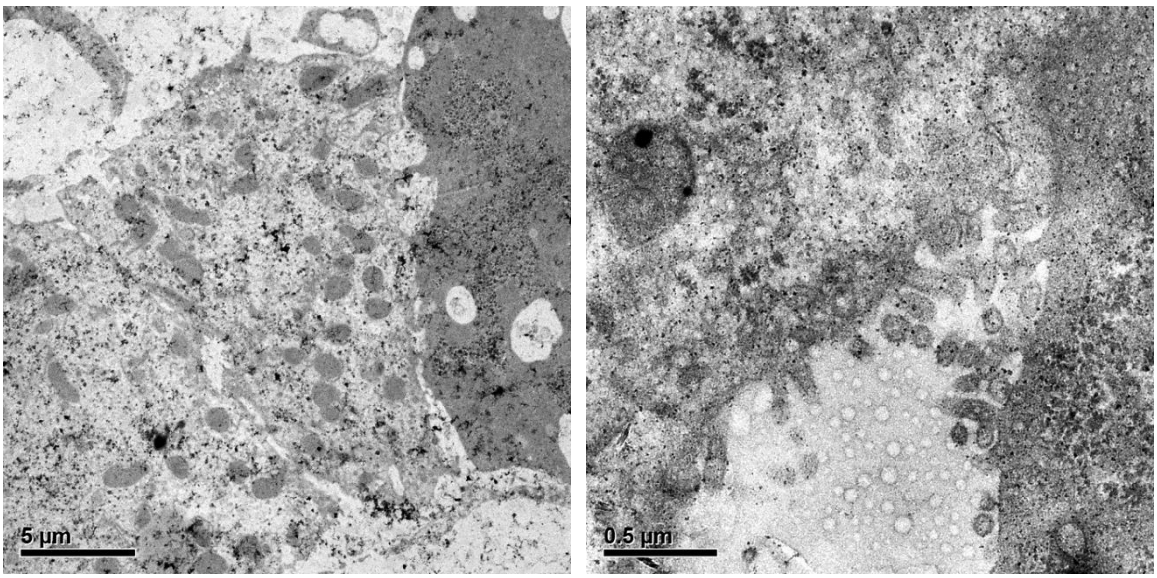
Production of the coffee reagent:
1g ground coffee (“Eduscho Gala” ! ;-) was mixed with 100ml double distilled water and boiled for 30 minutes to extract the coffee ingredients. Missing water was added back to this concentrate to obtain a 1% solution after filtration.

The last opacification step with carried out with lead citrate according to Reynolds [4].

[1] Sato, S., Sasaki, Y., Adachi, A. et al., Med. Electron. Microsc. 36 (2003) 179
[2] Sato, S., Adachi, A., Sasaki, Y., & Ghazizadeh, M., J. Micr. 229 (1) (2007) 17
[3] Miller, A.A., & Simakova, A.V., Cell Tiss. Biol. 4 (2010) 109
[4] Reynolds, E.S., J. Cell. Biol. 17 (1963) 208

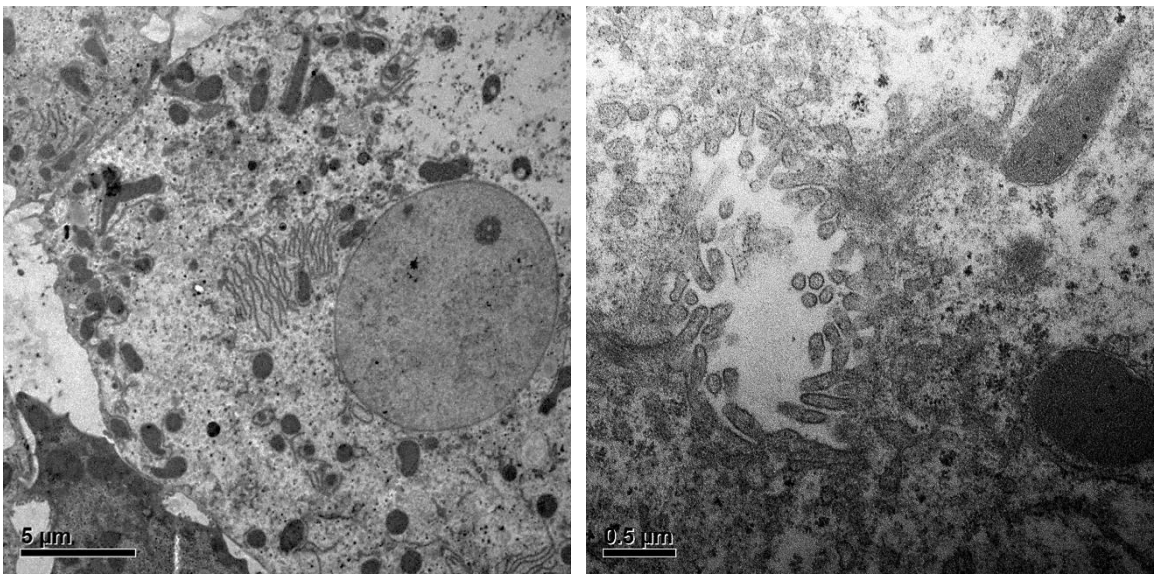
## Animal cells

Classical staining with $OsO_4$ / **uranyl acetate** / lead citrate according to Reynolds [4]




TEM bright-field images of a mouse liver cell

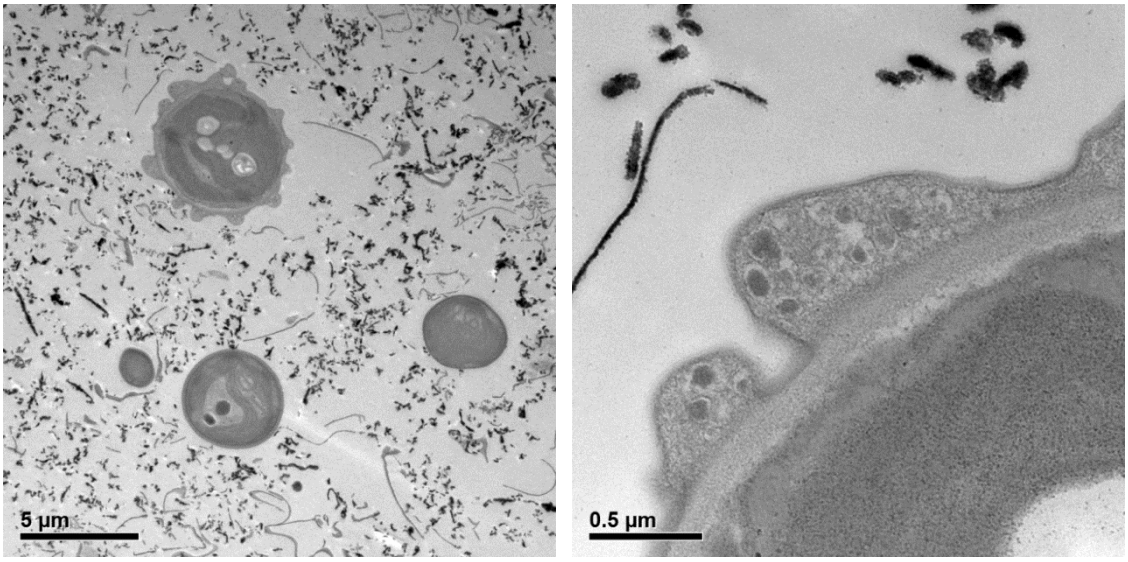
Alternative staining with $OsO_4$ / **coffee** / lead citrate according to Reynolds [4]




TEM bright-field images of a mouse liver cell

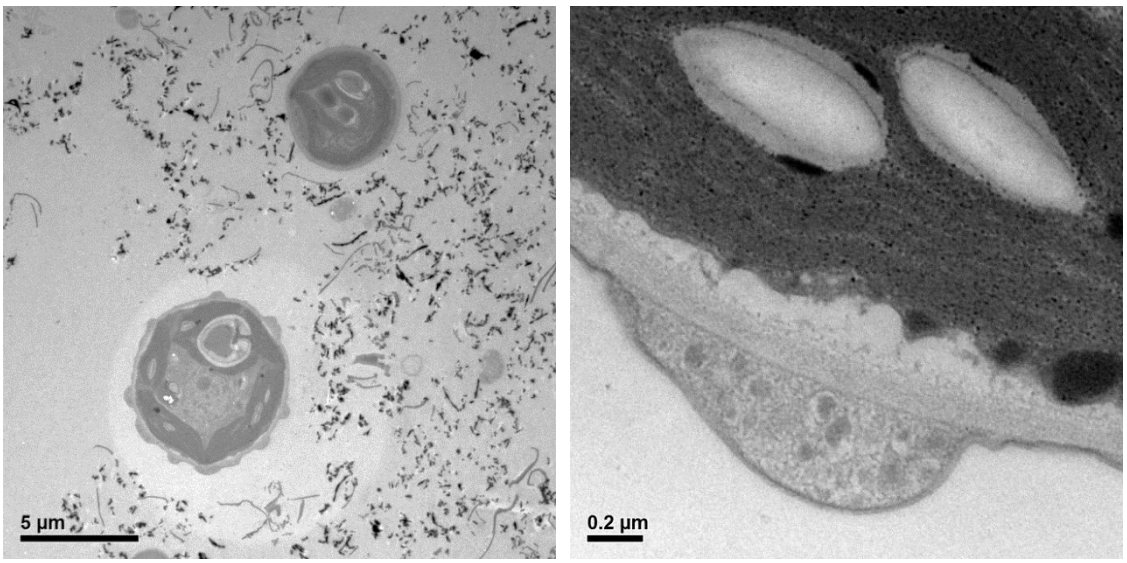
## Plant cells

Classical staining with $OsO_4$ / **uranyl acetate** / lead citrate according to Reynolds [4]




TEM bright-field images of an algae cell

Alternative staining with $OsO_4$ / **coffee** / lead citrate according to Reynolds [4]




TEM bright-field images an algae cell