The Hidden Power of Coffee

An Exceptional Staining Replacement for Uranyl Acetate

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Introduction

The use of uranyl acetate as a staining agent for biological samples before investigating in the TEM is strictly regulated by law and in many labs even banned. In recent years alternatives with similar staining results have been developed, however these alternatives are not equally effective for all biological structures. Examples are Uranyl Acetate Replacement (UAR), Uranyless (UL) and Oolong tea extract (OTE) [1,2,3,4]. In this work it has been shown that coffee may also be a serious candidate for staining purposes. We performed contrasting experiments on zebrafish tissues with household coffee as well as pure chlorogenic acid (CGA), which is an essential component of coffee, and compared the contrasting results with those of UA and the aforementioned commercially available alternatives UAR, UL as well as OTE. This work also describes how a subjective impression of good or bad contrast can be converted into an objective and thus comparable numerical value.

TEM - Results

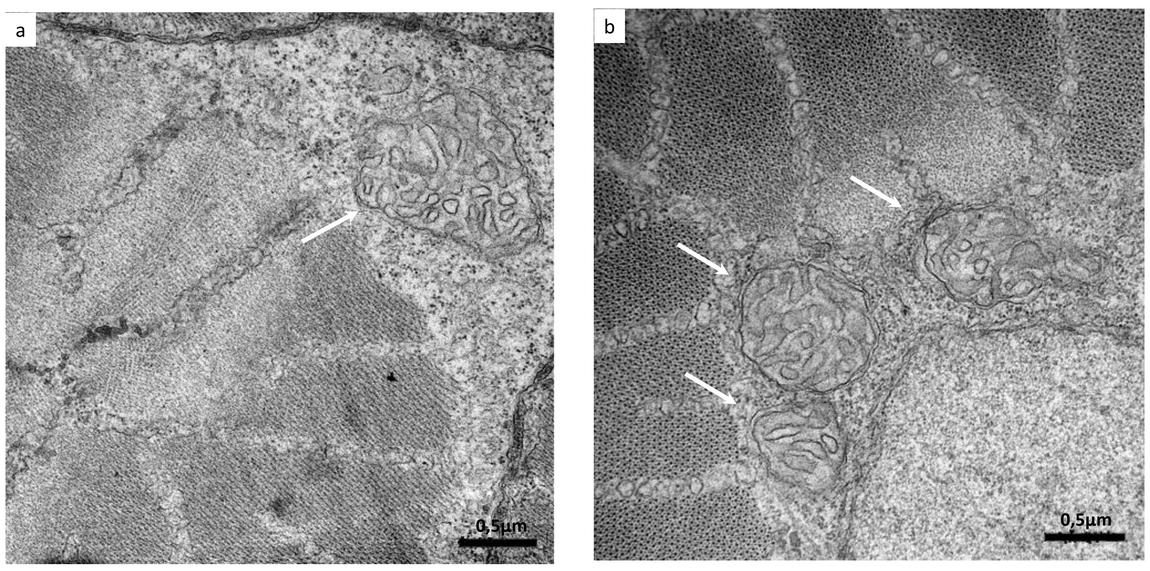
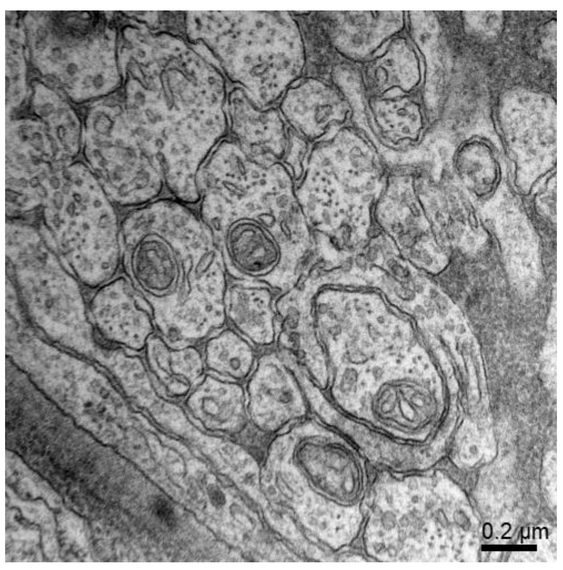


Fig. 1: Both TEM images show mitochondria (white arrows) in a zebrafish tissue. In a) the tissue is stained with UA, in b) the used staining agent is coffee. Both images show similar regions of the zebrafish, mitochondria within muscle cells. The membranes are visible very well, but having a closer look, there are certain impurities in a) whereas in b) the staining of the sample looks more homogeneous.



Instruction:

- ground coffee : water = 1 : 9
- simmer at 70°C for 3 hours
- filter twice through a 0.2 µm syringe filter
- 20 min application time
- final treatment with lead citrate [1] for 2 min
- washed in 0.02M NaOH solution [1]

Successful testing!

Fig. 2: TEM image of the central nervous system of the zebrafish. Not only the mitochondria, but also the nervous system can be excellently visualized by using coffee as a staining agent.

Conclusion

In the search for a substitute for uranyl acetate, the contrasting potential of coffee and one of its constituents, chlorogenic acid, was investigated on zebrafish sections. Regions free of artifacts over wide areas and convincing values for the interference contrast achieved give hope that both CGA and coffee will be able to completely replace UA for other biological tissue samples in the future.

Acknowledgements

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Matlab: Algorithm & Results

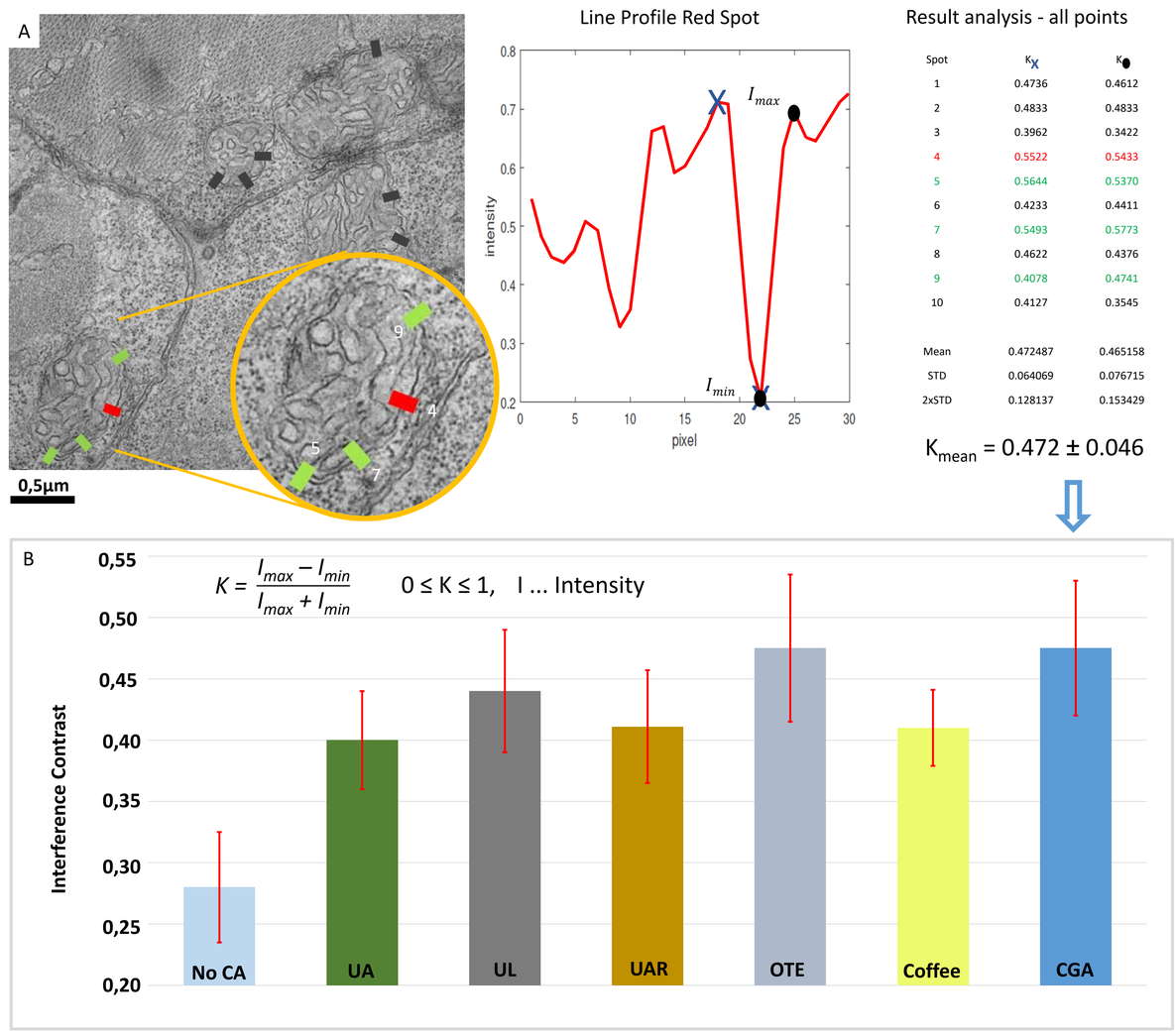

Fig. 3: The magnified section in (A) shows a mitochondrion whose outer membrane was used to determine the interference contrast via a Matlab algorithm [5]. First, a line profile is determined at a defined position (center), which is the basis for calculating the interference contrast values (right). (B) shows the interference contrast values with error bars obtained for the different staining agents. Relative to UA, which has a value of 0.40, coffee and especially CGA have higher values of 0.41 and 0.48, respectively. Note the lowest standard deviation for coffee indicating a homogeneous staining result.

References

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