

Non-Thermal Effects of Microwaves Enhancing Fixation of Biological Samples

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The use of microwave radiation in the processing of samples for both light and electron microscopy is now well established and has been used for almost two decades. Commercial microwave processing instruments are available from several manufacturers that give improved control over the microwave environment, including variable wattage and pulsing of the microwave radiation. It has been shown that all steps of the processing procedure can be assisted by the use of microwaves.

Most work on the use of microwaves to assist in sample processing for electron microscopy have concentrated on the ability of microwave radiation to speed up the process. From taking days using conventional methods, full sample processing has been reduced to a matter of hours [1,2]. Each step of a processing protocol can be reduced in time substantially by the use of microwave radiation.

There are two major effects of the microwave radiation that expedite and improve the processing of samples. The first is the ability to increase the rate of diffusion of solutions through tissues. All processing solutions penetrate into samples more quickly under the effect of microwave radiation. The second is the ability to enhance reactions and so promote processes like fixation, staining and polymerisation. With microwave assisted fixation biological samples exhibit superior morphological preservation compared to those fixed with conventional processing methods [3,4]. The improvement in fixation can be quite marked (Figure 1). Even samples that show considerable damage to the morphology when fixed by conventional methods can show excellent morphological preservation when processed with the same chemicals but when assisted by microwave radiation (Figure 2).

There has been an ongoing debate for many years about whether all these effects are a result of microwave heating or whether there are some non-thermal microwave-specific processes also involved. In commercial processing microwave instruments with the ability to reduce the wattage (<150W), the use of chilled water loads and shortening the times plus pulsed bursts of microwave radiation the temperature rise is very slight and yet there is an obvious and marked effect on processes such as sample fixation. Also temperature effects must be very minimal as animals such as zebrafish embryos, fly larvae and adults show no obvious ill effects when they are subjected to the same microwave radiation used for fixation, even surviving 10 minutes of radiation at 150W in a pulsed form of 1-minute bursts with 1-minute breaks between. So it definitely appears that there is a non-thermal microwave effect that is enhancing fixation in the microwave.

The aim of this presentation is to look at the possible mechanisms for the effect of microwave radiation showing how it affects the penetration of solutions into biological tissue and how it enhances the reactions occurring during fixation [5].

References:

- [1] Giberson RT *et al*, *Ultrastruct Pathol* **27** (2003), p. 187.
- [2] Schroeder JA *et al*, *Micron* **37** (2006), p. 577.

[3] Wendt KD *et al*, *J Microsc* **214** (2004), p. 80.

[4] Harahush BK *et al*, *Microsc Res Tech* **75** (2012), 1218.

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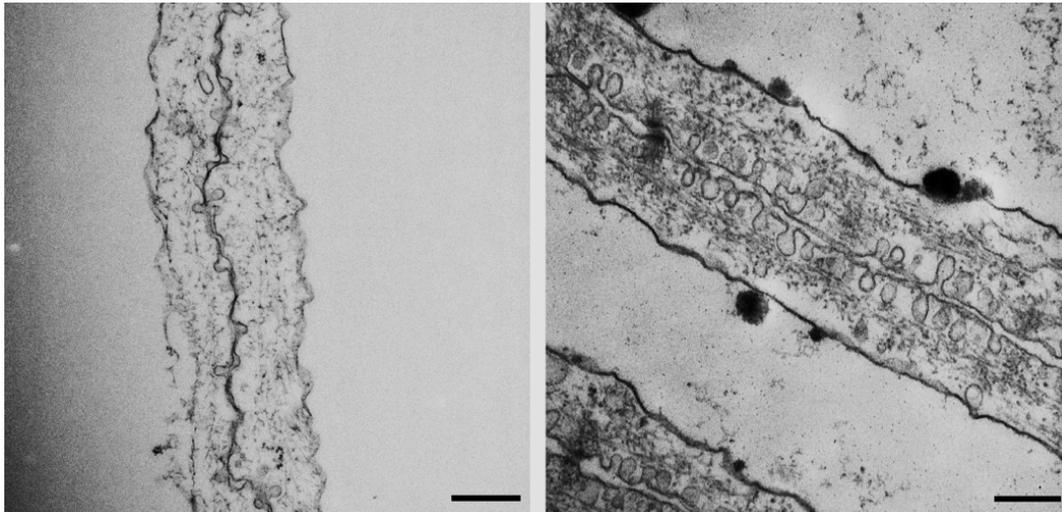


Figure 1. Figure 1. Notochord of a zebrafish embryo fixed using A. conventional methods and B. using microwave-assisted processing. Bar 500nm.

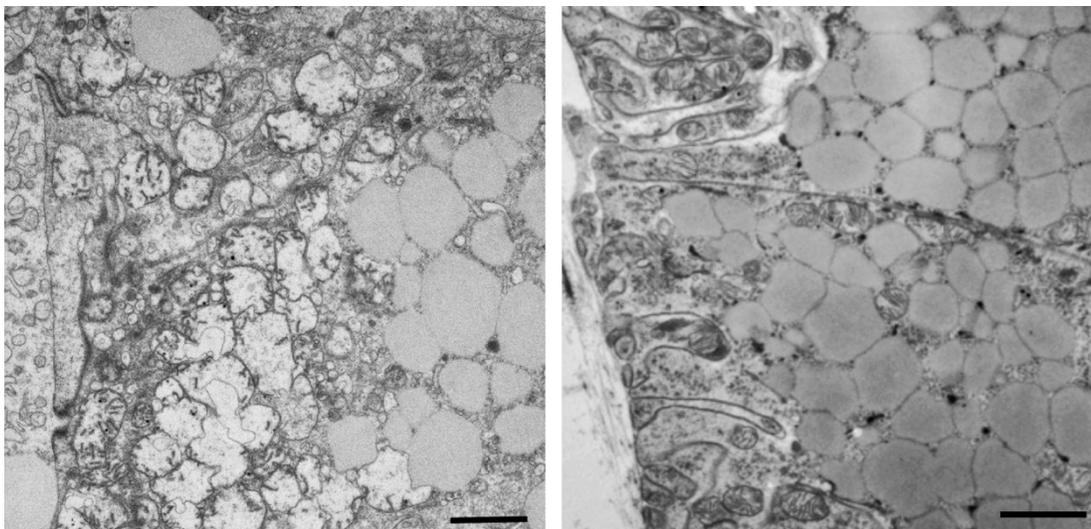


Figure 2. Hepatopancreas of the Mud Whelk *Pyrazus ebeninus*, fixed using A. conventional methods and B. using microwave-assisted processing. Bar 500nm.