5. Portable confocal scanning optical microscopy of *Australopithecus africanus* enamel structure

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Abstract

The study of hominid enamel microanatomical features is usually restricted to the examination of fortuitous enamel fractures by low magnification stereo-zoom microscopy or, rarely, because of its intrusive nature, by high magnification compound microscopy of ground thin sections. To contend with limitations of magnification and specimen preparation, a Portable Confocal Scanning Optical Microscope (PCSOM) has been specifically developed

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for the non-contact and non-destructive imaging of early hominid hard tissue microanatomy. This unique instrument can be used for high resolution imaging of both the external features of enamel, such as perikymata and microwear, as well as internal structures, such as cross striations and striae of Retzius, from naturally fractured or worn enamel surfaces. Because there is veritably no specimen size or shape that cannot be imaged (e.g. fractured enamel surfaces on intact cranial remains), study samples may also be increased over what would have been possible before. We have applied this innovative technology to the study of enamel microanatomical features from naturally occurring occluso-cervical fractures of the South African hominid, *Australopithecus africanus* representing different tooth types. We present for the first time detailed information regarding cross striation periodicity for this species and, in addition, we present data on striae-EDJ angles in a large sample of teeth and crown formation time for a molar of *A. africanus*. Our results characterize a pattern of enamel development for *A. africanus*, which is different to that reported for the genus *Paranthropus*, as previously observed.

Introduction

Most fossils are either translucent or, if they are surface reflective, are not flat. In both cases, light interacts with the sample over a considerable vertical range and is reflected (or the fluorescent light emanates) from a thick layer. The challenge we face for the non-destructive examination of enamel is how to obtain research-grade images of microantomical features in the field setting from such surfaces and sub-surface volumes. We have found a solution in development of portable confocal microscopy for the evaluation of rare and unique early hominid fossils. Our ultimate objective is to image features, such as cross-striations and striae of Retzius, for the purpose of describing aspects of the hard tissue biology and the organismal life and evolutionary histories of our extinct ancestors.

The principle of the Portable Confocal Scanning Optical Microscope (PCSOM) is to eliminate the scattered, reflected, or fluorescent light from out of focus planes, allowing only light originating from the plane of focus of the objective lens to contribute to image formation. It does this at the several conjugate focal planes (each plane representing the image of the other, that is intermediate, eye point, and image recording device), and thus eliminates light coming from all out of focus planes. In practice, an illuminated spot in the plane of focus is scanned across the field of view and an image is compiled. Confocal scanning optical microscopy thus differs from conventional light microscopy, where light from the focus plane of the objective lens, as well as from all out of focus planes across the entire field of view, is observed. The history and various technical achievements in confocal microscopy are summarized in Boyde (1995).

There is much interest in obtaining details of hominid enamel microanatomy from fractured surfaces, but such surfaces are rarely giving of all the desired detail; amongst existing instruments, the resolving power, such as that of stereo-zoom microscopy, and detail from below the surface, limited as in scanning electron microscopy, has been wanting. However, the PCSOM provides Z-axis through focus imaging of topographically complex surfaces at relatively high magnifications revealing a plane view of enamel microstructure (e.g. striae of Retzius and cross striations). Further, with the employ of circularly polarized light, the PCSOM provides some sub-surface enamel crystallite orientation contrast as well (Bromage et al., 2005).

Beyond a simple description of the PCSOM, we report here initial studies using this technology to assess *Australopithecus africanus* crown formation time, cross striation periodicity, and variation on the enamel extension rate for selected teeth. The phylogenetic relationships of *A. africanus* with