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Charge Contrast Imaging of Human Enamel

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Mature human enamel is a tissue consisting mostly of mineral (96%) with 3% water and ~ 1% organic matrix [1]. Being both a mineral and a human tissue makes it an interesting object for charge contrast [2] observation: while there were no reports of charge contrast observation of human tissue until now, some minerals were observed utilizing charge contrast [3]. Charge contrast usually can be observed on non-conductive not coated specimens with help of variable pressure/environmental scanning electron microscope (ESEM). Crystals of mineral (hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, with multiple substitutions) in human enamel have size of 20-80 nm in cross section and length of hundreds, if not thousands, microns. These thin long crystals are organized in bundles, so called "enamel rods," of about 4-8 microns in diameter. Orientation of rods is of significant importance in dental research, since many enamel properties depend on it.

Ten human third molars were collected according to the protocol #03-06e approved by the University of Missouri – Kansas City Adult Health Sciences institutional review board. Teeth were sectioned, embedded in fast setting acrylic resin and polished. Charge contrast images were obtained from all teeth. Figure 1 present micrographs obtained from one of the teeth; microhardness impression was used as a place locator for specimen reposition. Rods boundaries (sheaths regions) were outlined, direction of rods was obvious (in this case they were orthogonal to the specimen surface) on image obtained from non-coated enamel in ESEM at 1 Torr pressure, 15 kV accelerating voltage, 10 mm working distance with wide field gaseous secondary electrons detector (Fig. 1a). Charge contrast increased with increasing voltage (Fig. 1b) and decreased with increasing pressure (Fig. 1c), which corresponds to published results [3]. Rods were invisible on image obtained at the same conditions with backscattered electron detector (BSE, Fig.1d). Rods are also invisible when observing non-coated specimen in high vacuum (conventional) SEM mode at 1 kV (Fig. 1e). Conductive coating prevents charge trapping, and no contrast was observed after carbon coating (Fig. 1f) at the same conditions as were used in obtaining micrograph presented on Fig. 1a. While exact mechanism of charge contrast is still under discussion, it is widely accepted that it depends on local variations in charge trapping which represent microstructural information [4]. For enamel rods such variations may include variations of distribution of organic phase across the rods [5], or crystals orientation change on the rods boundaries.

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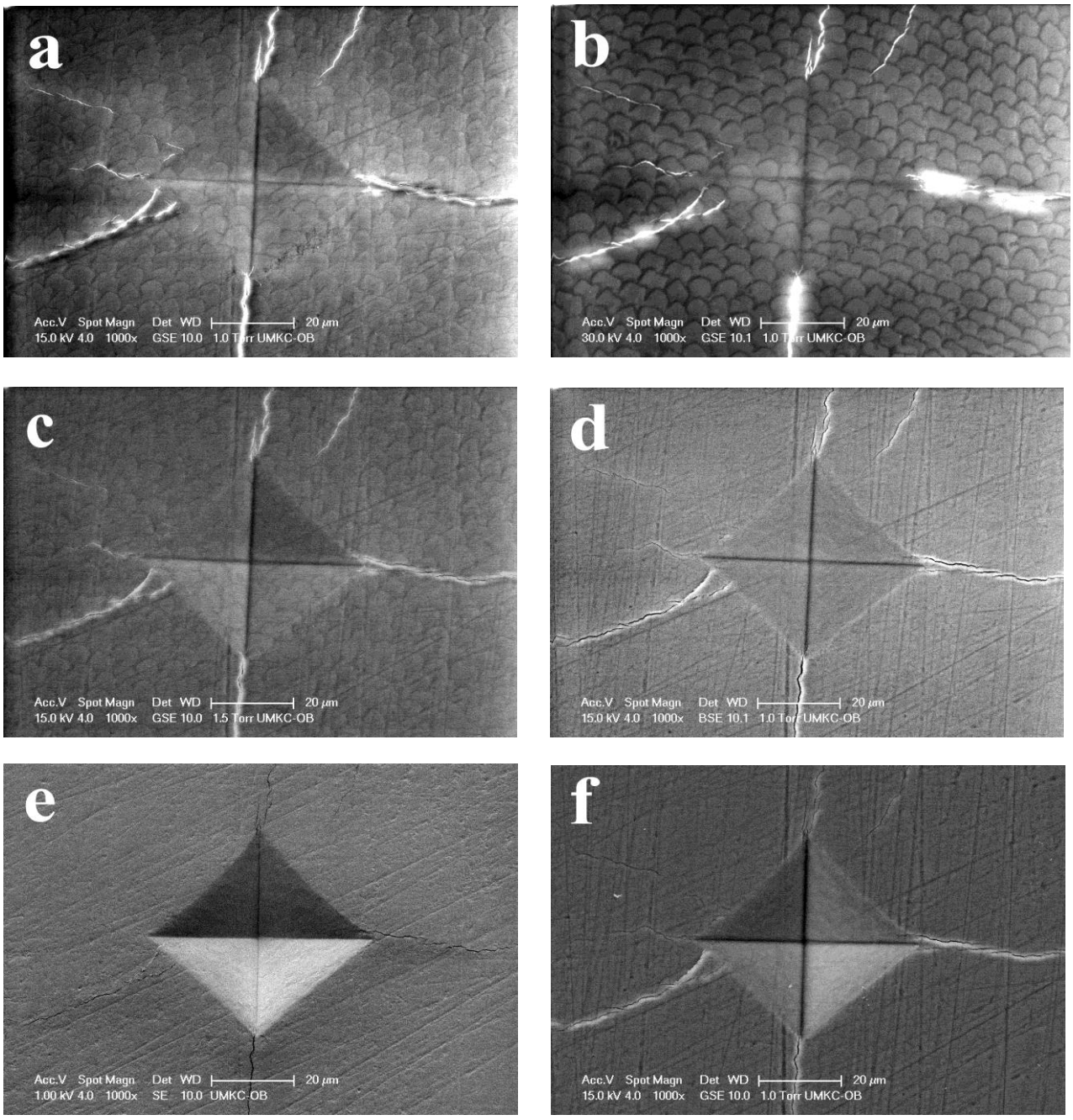

Figure 1. All micrographs were obtained from the same field of view; all pictures but picture **e** were obtained in variable pressure mode; all but **f** – from not coated specimen. Bar 20 microns.

a. 1.0 Torr pressure, 15 kV, wide field gaseous SE detector
b. 1.0 Torr pressure, 30 kV, wide field gaseous SE detector
c. 1.5 Torr pressure, 15 kV, wide field gaseous SE detector
d. 1.0 Torr pressure, 15 kV, BSE detector
e. High vacuum, 1 kV, SE detector
f. 1.0 Torr pressure, 15 kV, wide field gaseous SE detector, specimen coated with carbon

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