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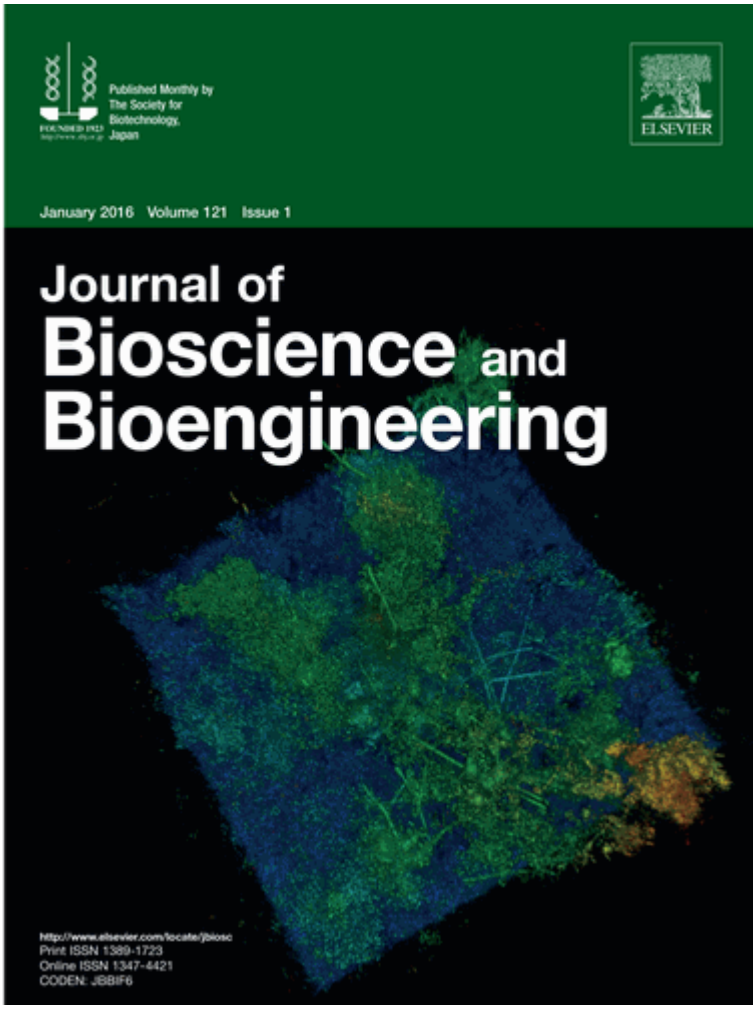

3D image of the hydroxyapatite (HA) disk and mixed-species oral biofilm visualized by continuous-optimizing confocal reflection microscopy (COCRM). Oral biofilm was produced by mixed-species oral bacteria cultured in TSB medium (1% saliva) on an HA disk. The reflection microscopy-based method COCRM uses reflected light, instead of fluorescence in confocal laser scanning microscopy, as a signal. Thus, COCRM does not depend on fluorescence and permits three-dimensional visualization of biofilms without genetic transformation or fluorescent probing. The COCRM technique can sequentially visualize intact biofilms and reveal their basal materials (in this case, on HA disks). Furthermore, a combination of COCRM and fluorescent proteins or staining can be used to visualize the localization of the subpopulation in the biofilm.

The image was taken by Dr. Tomohiro Inaba at Prof. Nobuhiko Nomura laboratory, Faculty of Life and Environmental Sciences, University of Tsukuba (**http://www.envr.tsukuba.ac.jp/~microbio/**).

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