## FRC-QE: A robust and comparable 3D microscopy image quality metric for cleared organoids

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## Abstract Text

Three-dimensional stem-cell-derived organoids are a powerful tool for studying cellular processes in tissue-like structures, enabling in vitro experiments in an organ specific context. While organoid research has been closely linked to advances in fluorescent microscopy, capturing cellular structures within their global context in an organoid often remains challenging due to the organoid's dense structure and opacity. The development of optical clearing methods has solved this issue for fixed organoids, but optimizing clearing protocols for a given sample type can be challenging. Importantly, quantitative measures for assessing clearing efficiency are missing. Here, we propose the Fourier Ring Correlation Quality Estimation (FRC-QE) as a new metric for automated 3D image quality estimation in cleared organoids. We observe that multi-view lightsheet acquisitions outperform established confocal microscopy for whole-organoid single-cell resolved imaging. We further show that FRC-QE reliably captures differences in clearing efficiency within organoids that have been imaged with multi-view light-sheet microscopy. Finally, we apply the metric across microscopy modalities, replicates and different clearing protocols, highlighting the method's capability for identifying the best clearing protocol for a given sample. FRC-QE is open-source and provided as an easy-touse and macro-scriptable plugin for the popular Fiji software. We therefore envision FRC-QE to fill the gap of providing a reliable quality metric for testing, optimizing and comparing optical clearing methods.