FACILE SINGLE-MOLECULE LIGHT-SHEET MICROSCOPY USING AN AFM CANTILEVER: OBSERVING G-QUADRUPLEX FORMATION IN LIVING CELLS

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Abstract: Light-sheet microscopy has revolutionised high-speed fluorescence imaging in living cells. This technique offers a substantial reduction in out-of-focus fluorescence and photobleaching that is particularly useful for single-molecule imaging. A main obstacle preventing the wide-spread use of light-sheet microscopy has been the complexity involved with introducing light to the sample, which often requires the use of dipping objectives or manufacturing custom made sample holders. We have created a simple solution to this problem [1] that can easily be implemented on standard inverted microscopes. Inspired by single-objective selective plane illumination (soSPIM) microscopy, we have developed a method that introduces a reflective AFM cantilever on top of the sample to enable light-sheet microscopy (Fig. 1). This offers a number of advantages including: simple implementation, compatibility with standard coverslips and petri dishes, compatibility with multiwell plates and the ability to use high NA objectives that are suitable for single-molecule imaging.

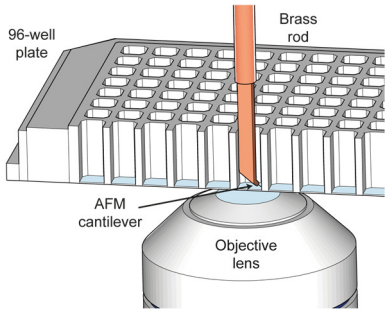

Fig. 1: Light-sheet microscopy in a 96-well plate

We have applied light-sheet microscopy to the study of G-quadruplex (G4) formation in living cells. Guanine-rich nucleic acid sequences can fold into G4 structures, which is a process that is believed to occur in human cells. These structures are important as they are thought to be involved in active transcription and replication processes and they have been identified as promising markers for cancer therapy [2]. Nevertheless, there are currently few studies demonstrating the existence of G4s in living cells and these studies have all been limited to bulk measurements of G4s. Consequently, we have developed novel fluorescent probes that, when combined with light-sheet microscopy, enables us to visualise individual G4s in living cells for the first time (Fig. 2). We show that G4s dynamically fold/unfold in cells on a timescale of tens of seconds and that these structures are directly linked to transcription and replication.

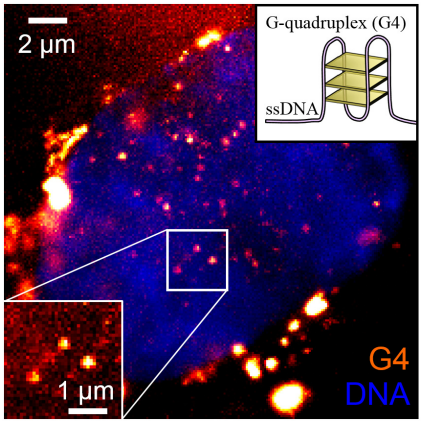

Fig. 2: Single-molecule imaging of nuclear G4s in U2OS cells

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