

Use of live cell microscopy to follow the temporal regulation of tenascin-C gene expression

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Quantitative measurements of dynamic processes in single cells are challenging and require the identification, segmentation and tracking of live cells. Collecting and storing live cell image data has been greatly facilitated by automated microscopy, but determining quantitative metrics of cell behavior using image analysis algorithms remains challenging. We quantified the fluorescence intensity from individual NIH-3T3 fibroblasts stably transfected with a tenascin-C promoter driving a destabilized eGFP reporter. Hundreds of individual cells were segmented and tracked both manually and by fully automated image analysis routines throughout the cell cycle during live cell imaging experiments lasting 62 hours. We observed that the GFP production in individual cells increased as they approached mitosis. On average the increase began when cells were approximately 60% through the cell cycle, suggesting that the tenascin-C promoter is more active at the end of the cell cycle. This work illustrates the application of live cell microscopy and automated image analysis of a promoter-driven GFP reporter cell line to measure the dynamics of single cell gene expression activity. Image analysis tools such as these for live cell image data will facilitate our understanding of long-lived phenotypic and gene expression changes that occur in cells due to chemical perturbation or spontaneous differentiation.