

## A Novel Confocal FRAP technique for the Measurement of Long-term Actin Dynamics in Stress Fibres

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**INTRODUCTION:** The actin cytoskeleton has been implicated in many aspects of cell function. Actin is biphasic, with globular (G) actin contributing subunits for fibrous (F) actin, which forms microfilaments and organises into stress fibres. This dynamic relationship can be examined using fluorescence recovery after photobleaching (FRAP). However, previous studies failed to target FRAP to defined structures and account for inherent cytoskeletal movement<sup>1</sup>. This study utilises advances in confocal microscopy to develop a novel FRAP technique for quantifying F and G actin dynamics.

**METHODS:** Passage 5 cells derived from a mature chondrocyte cell line<sup>2</sup> (H5) were transfected using Amaxa Nucleofector technology (Amaxa, Germany) with a p-eGFP-actin vector (Clontech, BD Biosciences). GFP-Transfected cells were visualized at 37°C by confocal microscopy (TCS SP2, Leica). Photo-bleaching was confined to 3 x 1µm areas overlying regions of stress fibre and interfibre space (fig 1.). FRAP analysis comprised 1 pre-bleach, 1 bleach and 50 post-bleach image frames. The frame-rate was 11.64 seconds. Fluorescence intensity was recorded though multiple linear profiles bisecting the long axis of each bleached area. The lowest mean intensity value over data points of 3µm length was used to indicate the level of fluorescence recovery. FRAP curves were described by fitting a two-phase exponential.

$$y = YMAX_1(1 - e^{-k_1x}) + YMAX_2(1 - e^{-k_2x}) \quad (1)$$

**RESULTS:** The success of GFP transfection was confirmed by its conformity to rhodamine phalloidine stained actin filaments (data not shown). A comparison of FRAP in stress fibre (n=56) and interfibre space (n=13) (fig 2.), reveals two modes of recovery. Actin recovery was slower in the stress fibres compared to that in the interfibre space. Based on the curve fit, the mobile fractions within stress fibres and interfibre space comprised 45.9% and 69.4% respectively (YMAX<sub>1</sub>+ YMAX<sub>2</sub>).

**DISCUSSION:** The present study describes a powerful new FRAP technique for quantifying

protein dynamics in cytoskeletal networks. This technique overcomes many limitations of previous studies and has been used to illustrate differences in long-term actin dynamics in and around stress fibres. This technique may now be used to examine the effect of factors such as mechanical loading and surface topography.

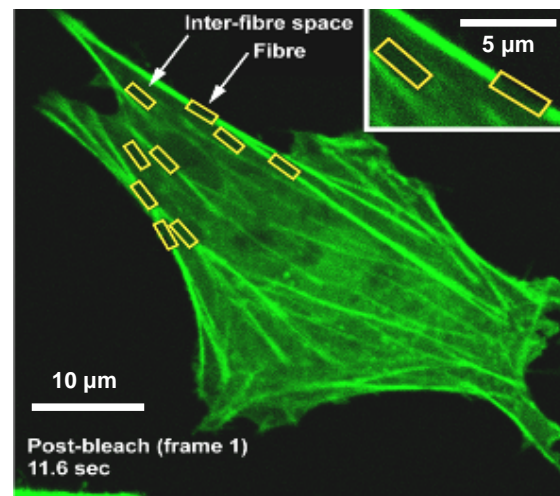


Fig. 1: Post-bleaching image indicating regions of FRAP.

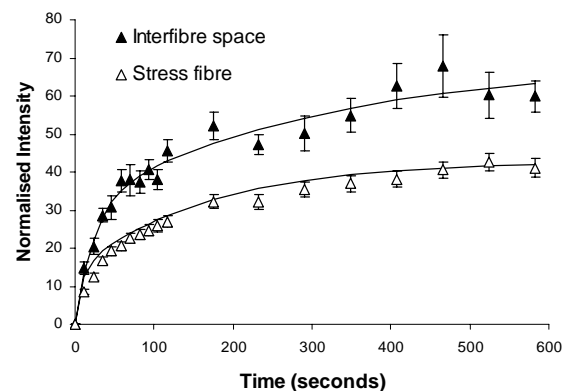


Fig. 2: Normalised FRAP curves, with two-phase exponential curve fit, showing temporal change in fluorescence intensity within stress fibres (n=56, R<sup>2</sup> 0.999) and interfibre space (n=13, R<sup>2</sup> 0.967). Error bars represent standard deviation.

**REFERENCES:** <sup>1</sup> T.E. Kreis et al. (1982) *Cell* **29**:835-45. <sup>2</sup>H.M. van Beuningen, et al (2002) *Osteoarthritis & Cartilage*.**10**:977-86.

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