CA2+ FLUCTUATIONS LIGHT THE PATH TO THE EGG - IMAGING DYNAMIC CA^{2+} CHANGES IN THE FLAGELLA OF SWIMMING SEA URCHIN SPERM WITH A NOVEL LED-BASED EPIFLUORESCENCE IMAGING SYSTEM.

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Sperm motility is essential for the successful union of male and female gametes and generation of a new individual. Sperm from a diverse range of organisms, from bracken ferns, many marine species and mammals, follow chemical cues in their journey to locate and fertilise the egg or oocyte, called chemotaxis. In sea urchins, short sperm-activating peptides (SAPs) diffuse away from the outer egg investments to activate sperm metabolism and modify sperm motility. Ca2+ ions regulate flagellar bending patterns and are required for chemotaxis in all sperm studied. We have devised an LED-based stroboscopic imaging system capable of recording spatially resolved fluorescence images at >300 frames per second, and recorded SAP-stimulated Ca2+ changes in beating sea urchin sperm flagella. We found that SAPs induce trains of Ca2+ fluctuations in the flagella, and that each individual fluctuation is associated with a transient increase in flagellar asymmetry that induces the sperm to turn. We also found that pharmacologically extending the duration and amplitude of the Ca2+ fluctuations also increased the size and duration of the sperm turns, and that such manipulations inhibit chemotaxis. We propose a new model for the SAP signalling pathway based on these results. The LED-based fluorescent imaging system used in this study is a low-cost, simple and durable alternative to conventional epifluorescence microscopy, particularly suited to high-speed and long-term imaging experiments. It is most appropriate for applications where phototoxic imaging artefacts (fluorophore bleaching and cytotoxicity) common with arc lamp-based fluorescence imaging are problematic.