The aim of this project is to investigate the structural dynamics of the molecular motor of muscle, the myosin heads that cross-link the myosin and the actin filaments, responsible for the generation of force/shortening in muscle. The investigation is made combining fast mechanics and X-ray diffraction in single fibres isolated from the frog skeletal muscle, where the molecular mechanism of contraction can be studied in the native system. Thanks to the collimation of the X-ray beam at ID2, ESRF, we can exploit the X-ray interference between the two arrays of myosin heads in the thick filament, to measure the motion of the myosin heads with sub-nanometre resolution. The experiments during SC-2264 are aimed at defining structural differences in the myosin heads and in the myosin and actin filaments among the resting state, the active state attained at the plateau of the isometric contraction (T0) and the active zero force state attained during steady unloaded shortening that prevents isometric force development.

In an intact fibre from frog muscle at sarcomere length ~2.2 μm the length of the actin and myosin filaments increases upon activation and tension development by an amount that is quite larger than that expected from the instantaneous compliance of the myofilaments. Previously it was found that during the rise of isometric tetanus the ~1.5% increase in spacing of the myosin based meridional reflections has different time courses and different sensitivity to mechanical maneuvres for the M3 and M6 reflections, indicating that, in the active muscle, the M3 originates mainly from actin attached myosin heads, while the M6 originates mainly from other periodical structures along the myosin filament (Brunello et al., 2006). During the unloaded shortening imposed at T0, within the time adequate to attain the steady state mechanical response, there is a slow partial recovery of the resting spacing of M3 and M6 reflections. To find the steady state values, in this visit we applied unloaded shortenings of amplitudes 20% of the fibre length, using a new loudspeaker motor able to impose length changes (complete within 0.5 ms) up to ±1.5 mm. Camera length was set at 2.5 m to collect up to the 6^{th} and 7^{th} actin based layer line reflections. Experiments were made on fibres isolated from the tibialis anterior muscle of the frog Rana esculenta. 10 ms time frames were collected at different times during ~20 % shortening (from 2.55 to 2.15 μm sarcomere length) imposed either at the start of tetanic stimulation or at the tetanus plateau. Control frames were also taken at rest and at the tetanus plateau. The results indicate that maintaining zero force with unloaded shortening , the spacing of M3 reflection stays at the resting value, while the spacing of the M6 reflection goes to an intermediate value between that in the resting state and that at plateau of the isometric contraction.