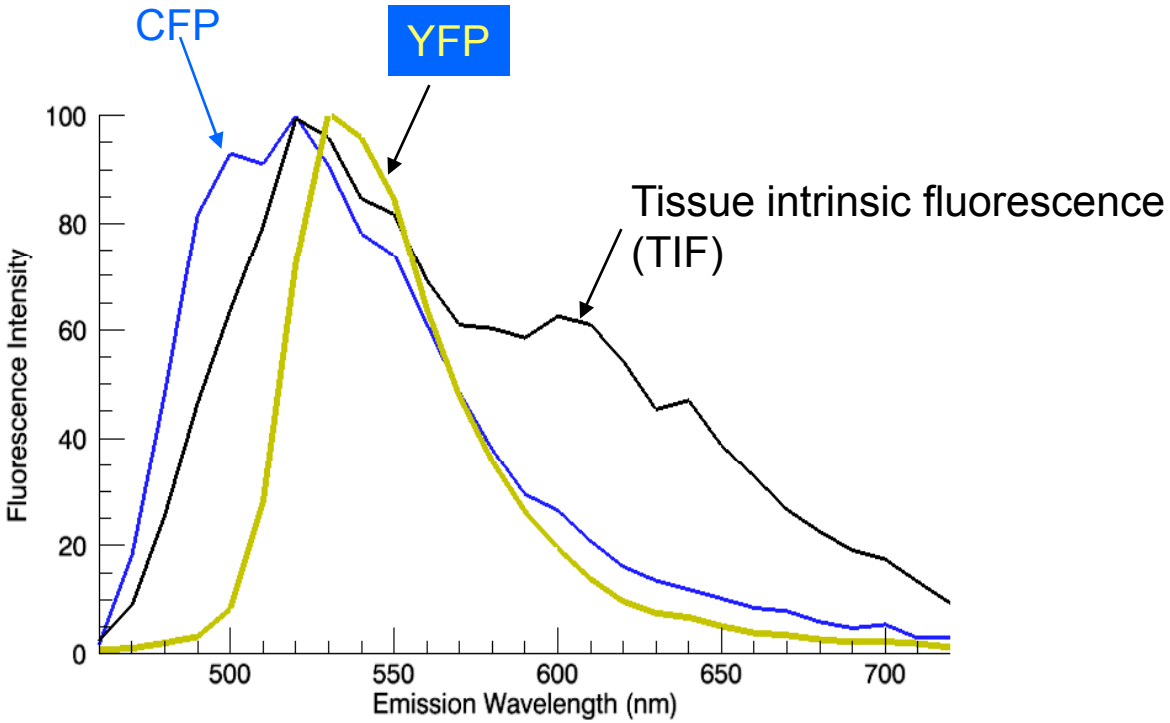

Fig. 1S. Normalized reference spectra for spectral un-mixing. The CFP and YFP spectra were obtained from agarose beads that bound either recombinant CFP or YFP. The tissue intrinsic fluorescence spectrum was obtained from the pixels representing the wall of a wild-type artery, that did not contain the exMLCK biosensor molecule. The spectra were derived by the CRI software from 'image cubes' consisting of 26 images, spanning the range, 470 to 720 nm, each of bandwidth, 20 nm. Excitation wavelength was 436/10 nm with an additional long-pass excitation barrier filter centered at 470 nm.

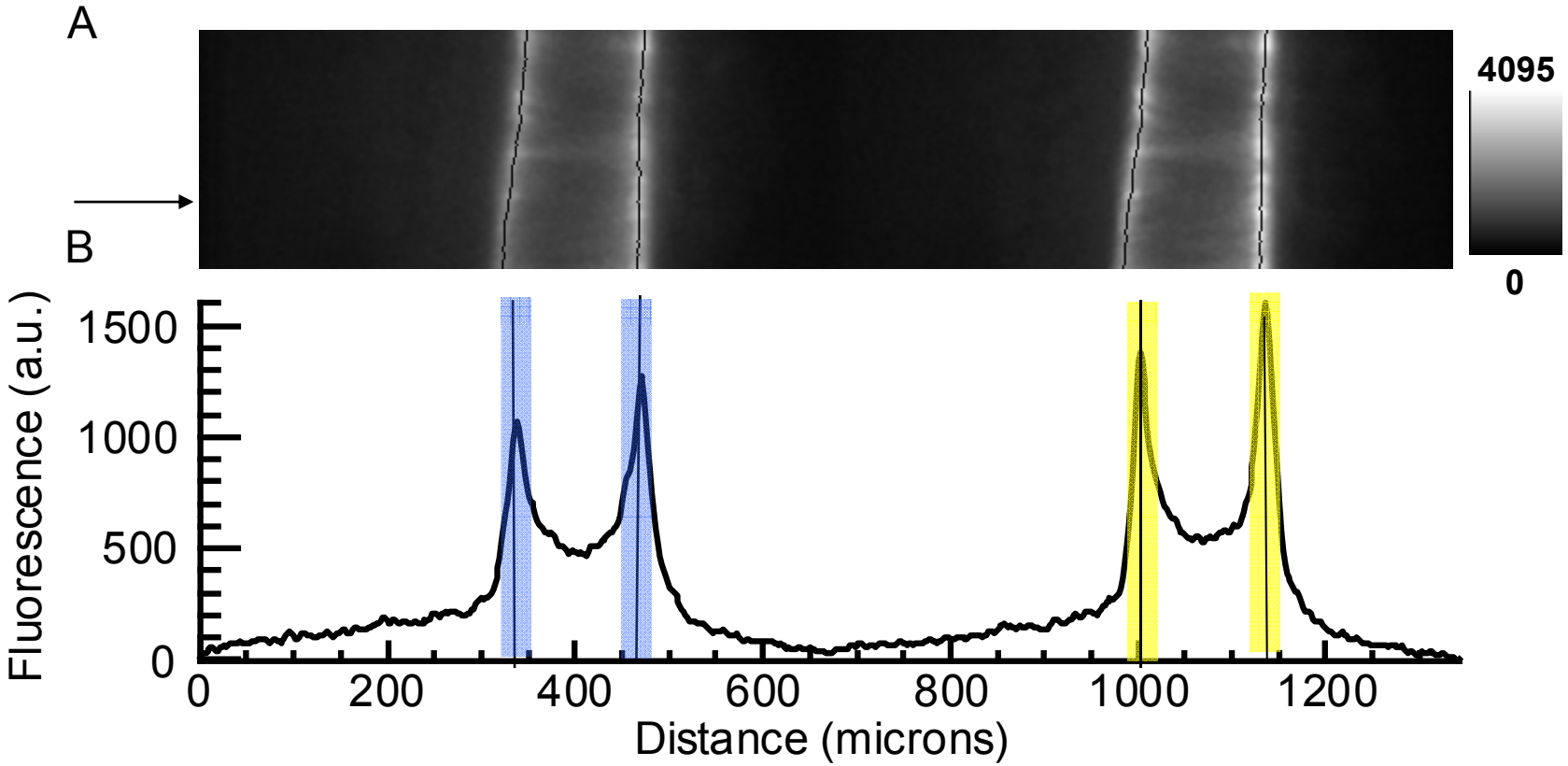

Fig. 2S. Methods for defining artery walls. (A) Unprocessed images from the image-splitter. CFP channel on left, YFP channel on right. The black lines running down the walls of the arteries are at the peak fluorescence in each video line of each image of the sequence (consisting of up to 1200 frames or 1200 s). Arrow indicates one video line displayed in (B). The artery walls were then defined as regions, 10 to 20 μm wide, around the peaks, as shown schematically with the blue and yellow shaded regions. Fluorescence within these regions was summed and used in further processing (correction for spectral overlap, ratioing). This method was devised to exclude from analysis any fluorescence that did not arise from the artery walls.

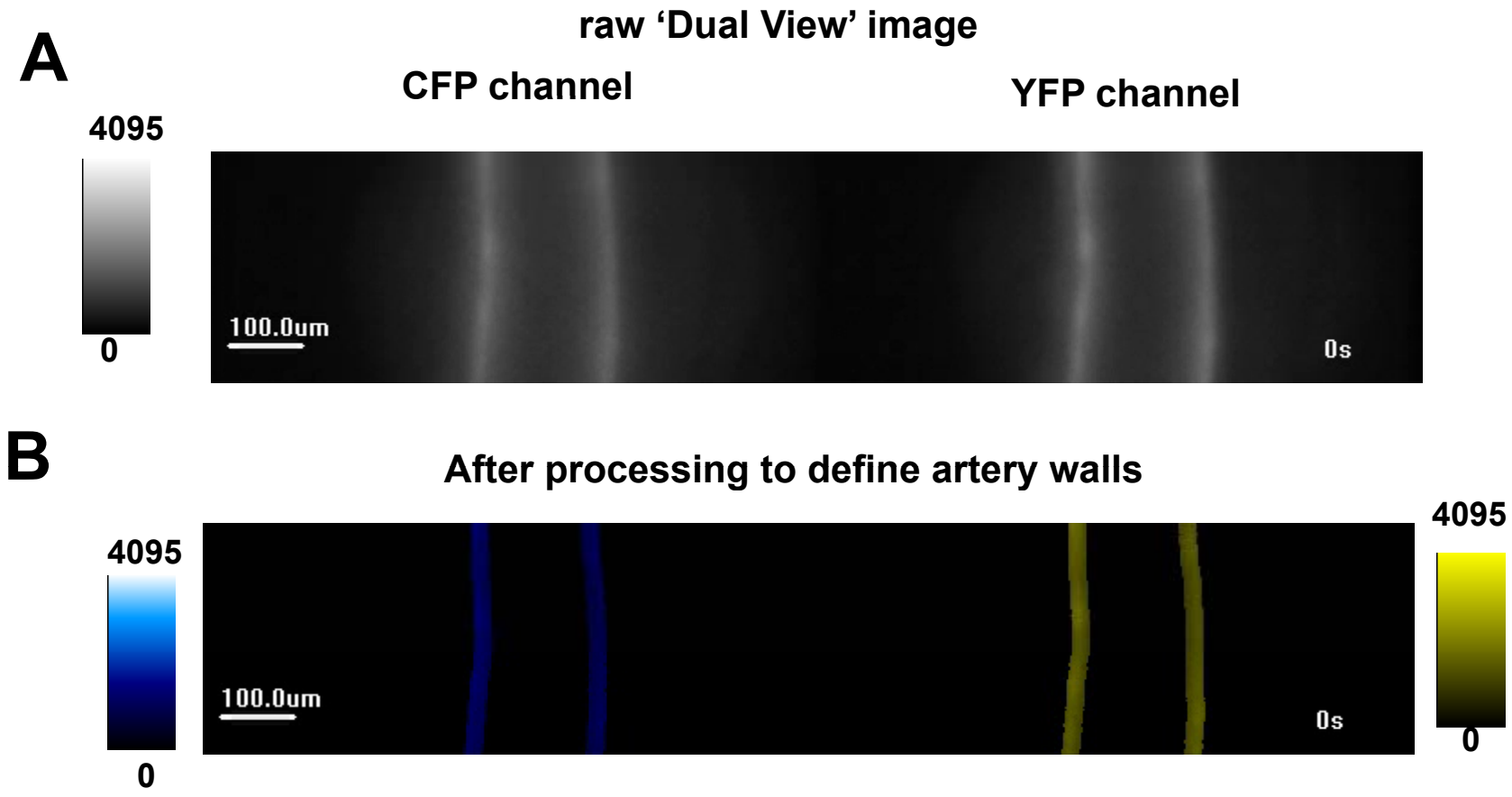

Fig. 3S. Video clips of a cremaster artery contracting in response to 10 μM PE (Fig. 2 of Article). Video clips are intended to show the contraction and the result of processing to define the artery walls. (A) Unprocessed images from the image-splitter. CFP channel on left, YFP channel on right. (B) The artery walls, defined as described previously, are shown.

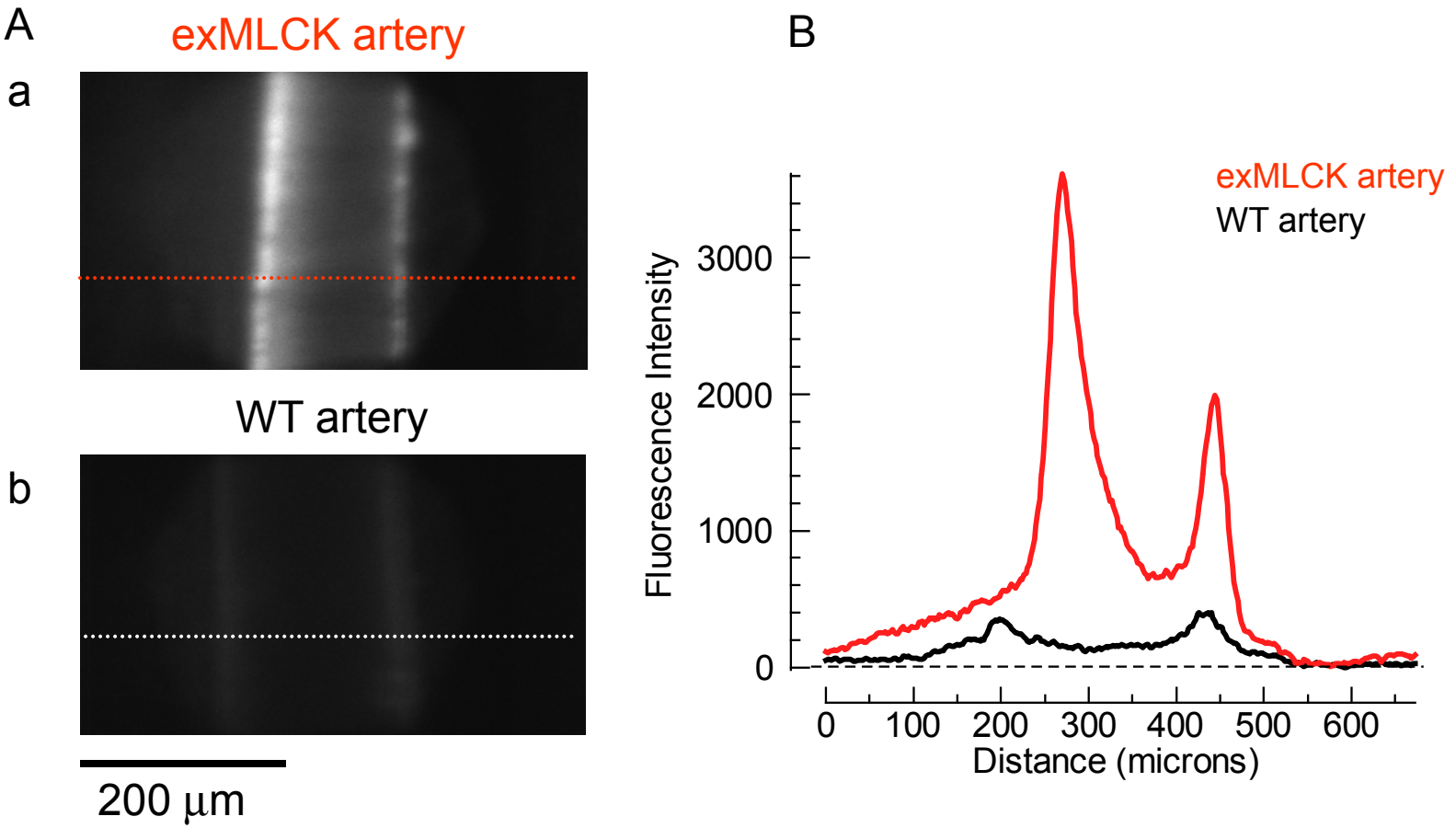

Fig. 4S. Analysis of tissue intrinsic fluorescence (TIF). (A) Images of an exMLCK femoral biosensor artery (a) and wild-type femoral artery (WT, did not contain exMLCK) under basal conditions, *in vivo*. Fluorescence excitation intensity and all recording conditions are exactly the same for the two arteries. The two images (a and b) of the two arteries are displayed with exactly the same display parameters. The WT artery, which had a slightly larger basal diameter than the biosensor artery, is barely visible in image (b). (B) Representative line plots from the two images, as indicated by the red and white dotted lines. Although TIF of the WT animal is barely visible, it is appreciable compared to the CFP (and YFP) fluorescence. The method of accounting for TIF is described in Fig. 4S (this Supplement).

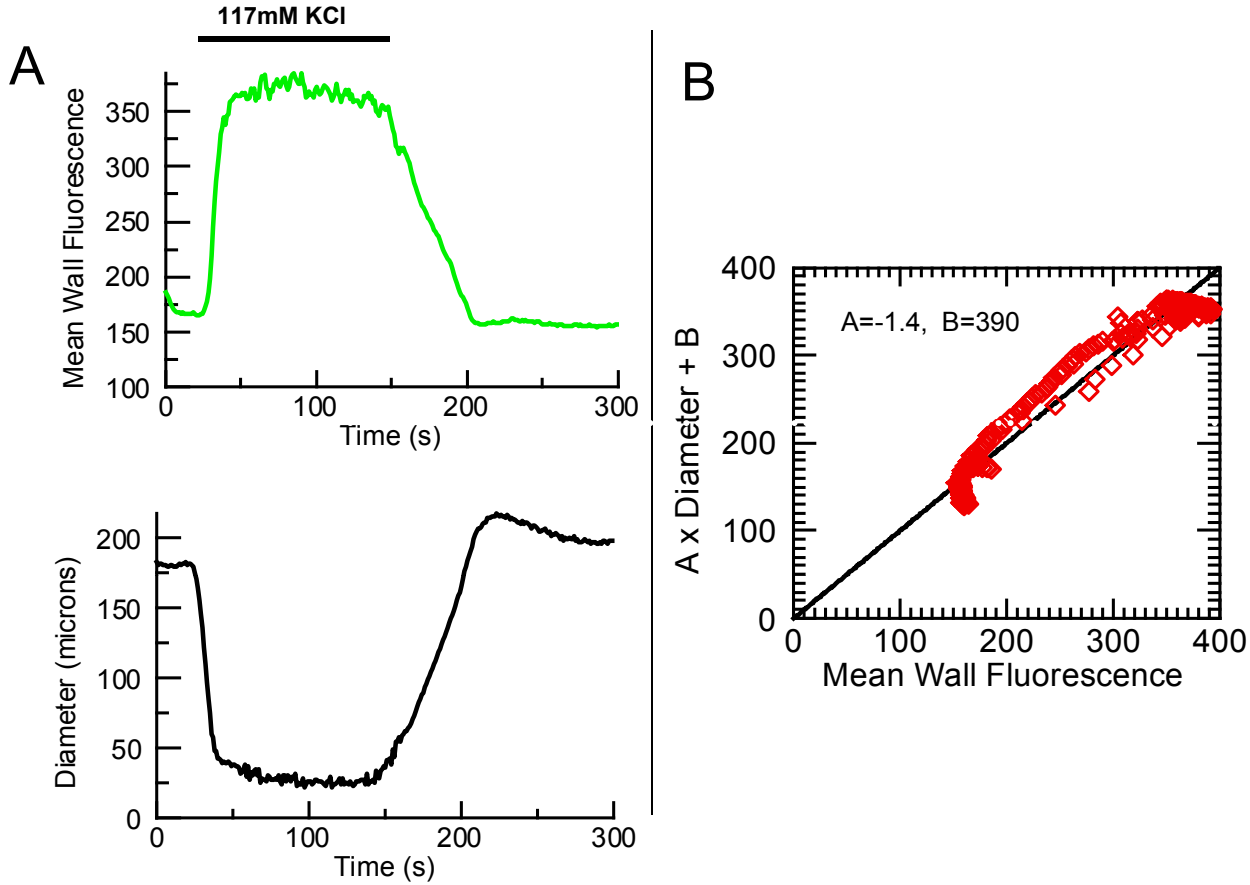

Fig. 5S. Analysis of change in TIF during contraction. A WT artery (did not contain exMLCK) in Ca^{2+}-free solution was exposed to the high $[K^+]_o$ solution and the mean wall fluorescence (TIF) and diameter were recorded (A). Artery diameter thus spanned the possible range that might be encountered physiologically. The intrinsic fluorescence and diameter were approximately linearly related (B), by the empirical equation, TIF (mean wall fluorescence) = -1.4 * Diameter (μm) + 390. Thus, TIF can be predicted from the diameter and subtracted from the CFP and YFP fluorescence over the time course of the recording. In practice, this made little difference in the exMLCK FRET ratio, because TIF is small compared to the CFP and YFP fluorescence. For example, if artery diameter is 150 μm, then TIF is predicted to be 180. Suppose that actual CFP and YFP are 1000 and 769, respectively. Then, the actual FRET ratio is 1.30, and the uncorrected ratio is 1.24. This difference, of 0.06, is small compared to the dynamic range of exMLCK, in which FRET ratio varies between 1.2 and 2.0 (R_{min} and R_{max}). Note that the constants A and B depend on the illumination intensity and other recording conditions. Thus, application of this correction to biosensor arteries requires that the experimental conditions be exactly the same as used for the WT artery.

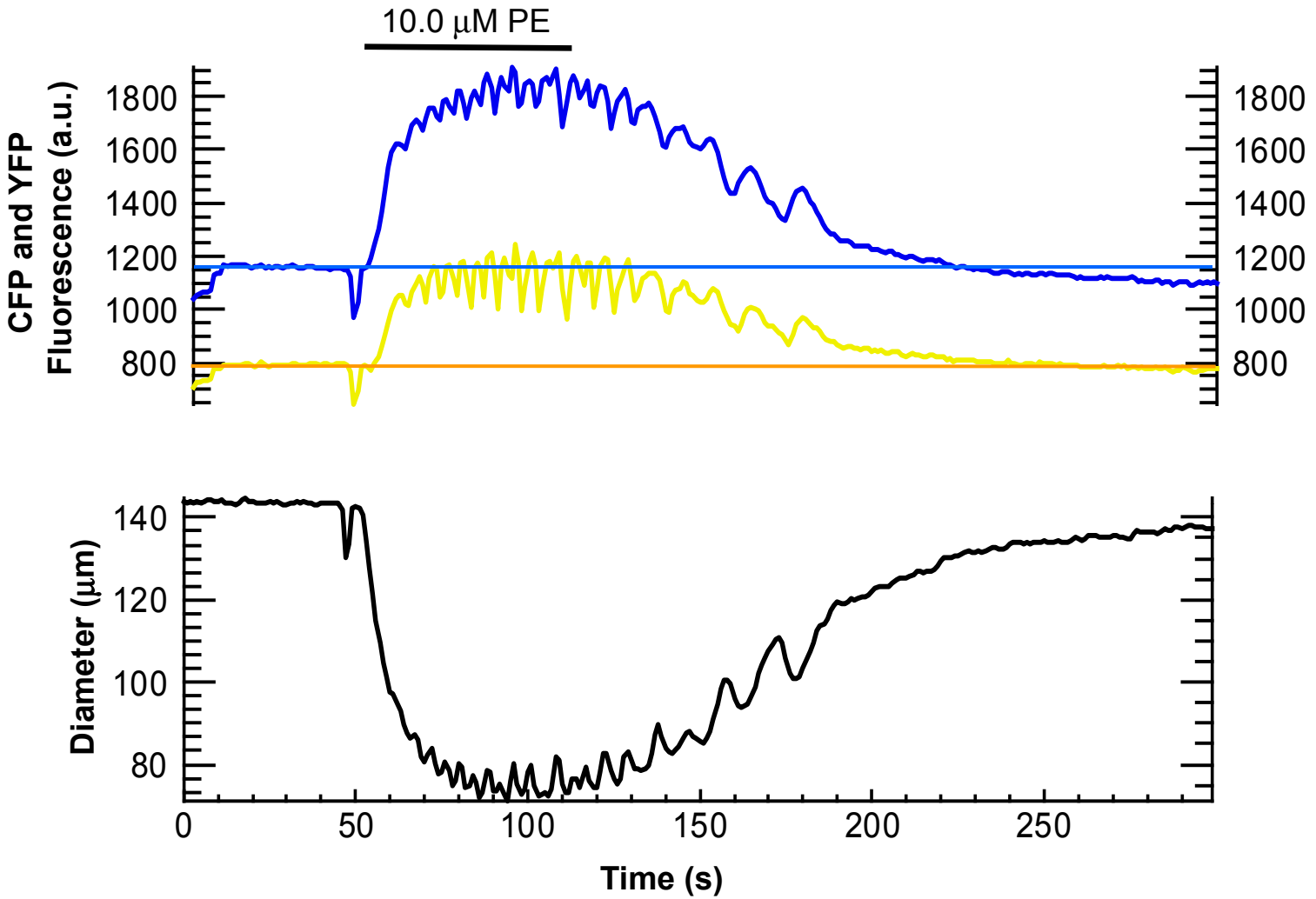

Fig. 6S. Photobleaching of YFP is not detectable, and photobleaching of CFP is small (~5%) over 300 s of recording. The horizontal blue and orange lines indicate the initial levels of CFP (blue trace) and YFP (yellow trace) fluorescence. We assume that the artery returns to its initial condition by the end of the recording. Same experiment as shown in Fig. 2 of this article.

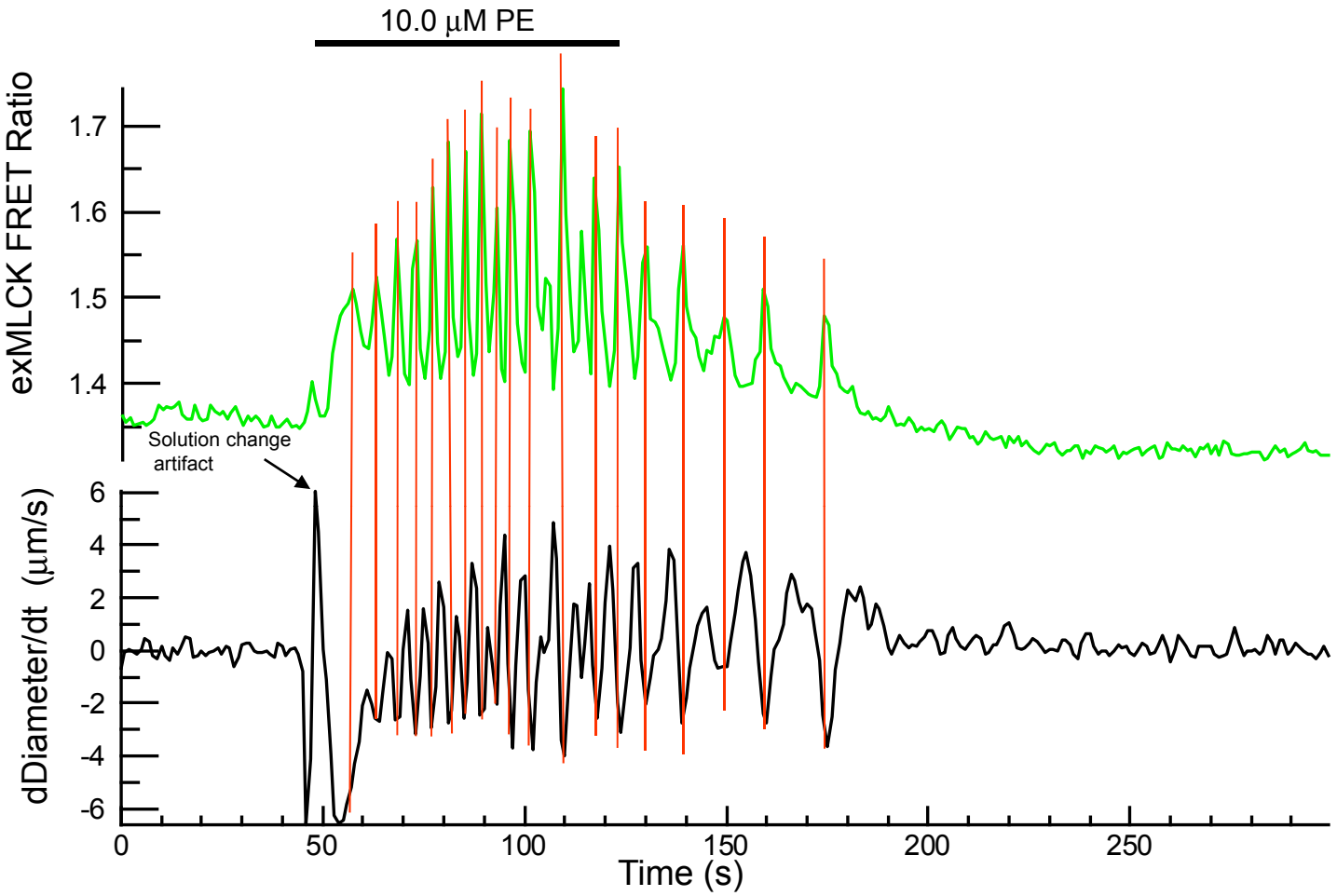

Fig. 7S. Relation between exMLCK FRET ratio and artery motion. Peaks in exMLCK FRET ratio coincide approximately with the fastest rates of decrease in diameter. The red vertical lines are positioned to intersect at the peak of the oscillations in FRET ratio, and are extended to the diameter derivative tracing. This is the expected result for a signal related to force generation, but not to a motion artifact. Errors in FRET ratio that might arise from motion of the artery would be expected to correlate with the diameter itself, not it's time derivative. Same experiment as shown in Fig. 2 of this article.