The goal of our research is to study the relationship between the structure and electrophysiological activation of contraction in cardiac myocytes. In myocytes, calcium passes into the cell through L-type Ca channels and causes additional calcium release from the sarcoplasmic reticulum through release channels called Ryanodine receptors. The result is a transient rise in calcium, which activates contraction. Ryanodine receptors are closely opposed to L-type calcium channels in the t-tubules and this receptor-channel complex is known as a couplon. To establish the relationship between couplons and t-tubules we have begun to create 3D images of the t-system using confocal microscopy with dyes that label cellular structure and (dynamically changing) calcium concentration. For the initial rendering of these three-dimensional images, we have used BioImage, a BioPSE PowerApp.

T-tubules and local Ca release events or sparks produced by couplons. Simultaneous recordings of sulphorhodamine B which labels t-tubules and fluo-3 which detects sparks. (Left) red (sulphorhodamine B) and green (fluo-3) XY images. White horizontal lines in both images indicate the position of a confocal scan line. (Right) The averaged sulphorhodamine B and F/F₀ images were calculated from 23 red and green sequential line scan (XT) images.

Confocal images of stained t-tubule system in Guinea pig ventricular myocytes. The upper image shows an XY, the lower an XZ slice through a 3D dataset consisting of 512x512x96 volume elements.

3D point spread function represented by central XZ-, YZ-, and XY- slices. The point spread function shows anisotropic characteristics with a significant elongation in Z-direction and a tilt of the central Z-axis in X- and Y-direction.

3D reconstruction of the t-tubule system in Guinea pig ventricular cells using Richardson-Lucy deconvolution algorithms (MATLAB) and software for volume based visualization (SCIRun/BioImage). Tubules are spaced at approx. 2 μm. These tubules form junctions with the terminal cisternae of the sarcoplasmic reticulum.