Monitoring subcellular Ca\textsuperscript{2+}-signaling using temporal unmixing

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For a plethora of cellular processes the mobilization of Ca\textsuperscript{2+} ions from intra- and extracellular sources is essential for intracellular signaling. A thorough understanding of the complex interplay of the Ca\textsuperscript{2+}-profiles in the various cellular organelles requires simultaneous monitoring of Ca\textsuperscript{2+}-levels in these different compartments. Such a recording however demands high temporal and spatial resolution combined with a need for organelle-specificity.

Using antigen-induced Ca\textsuperscript{2+}-signals in B cells as a model system, we measure subcellular Ca\textsuperscript{2+}-profiles of various organelles simultaneously by combining fast time-lapsed confocal microscopy with an algorithm to unmix superimposed fluorescence signals. Our real time imaging approach directly visualizes the molecular hierarchy of BCR-induced Ca\textsuperscript{2+} signaling events and moreover allows for the characterization of individual Ca\textsuperscript{2+} profiles in different subcompartments of the live B cell. This approach enabled us to provide evidence that SLP65 recruitment to the plasma membrane precedes Ca\textsuperscript{2+} mobilization (Fig. 1). Moreover, we identified distinct spatiotemporal Ca\textsuperscript{2+} profiles for the cytosol, the Golgi apparatus and the mitochondria during BCR-induced activation (Fig. 2). The developed experimental setup provides a useful tool to resolve the spatiotemporal dynamics in cellular signaling systems.

Figure 1: Simultaneous measurement of cytosolic Ca\textsuperscript{2+} and citrine-tagged protein SLP65 (1A) from individual lymphocytes reveals that membrane recruitment of SLP65 precedes Ca\textsuperscript{2+} influx (1B) during activation.

Figure 2: Temporal unmixing of Ca\textsuperscript{2+}-signals from a single lymphocyte provides temporal (2A) and spatial (2B) profiles of the distinct Ca\textsuperscript{2+}-fluctuations in various organelles during activation of the B-cell.