An automated image cross-correlation analysis to quantify the real-time dynamics of mast cell signaling.

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Mast cells and basophils are immune system cells primarily responsible for the allergic immune response. These cells express on their surface a high affinity receptor FcϵRI that binds IgE. Crosslinking of receptor-IgE complexes by a cognate multivalent antigen initiates an intracellular signal cascade that leads to the release of pharmacological mediators of allergies. We have investigated the spatio-temporal dynamics of early events in this signal cascade using real-time multicolour confocal fluorescence microscopy. Living RBL-2H3 mast cells expressing fluorescent protein chimeras of various signaling proteins were imaged during stimulation with multivalent antigen. The experiments were carried out at physiologically relevant temperatures and images were acquired with a time resolution of 3s. A fluorescent label on the multivalent antigen allowed us to visualize the plasma membrane distribution of crosslinked receptors. We developed an automated image analysis scheme to efficiently quantify the translocation of fluorescently tagged intracellular proteins to the plasma membrane, and their colocalization with crosslinked receptors as measured by a cross-correlation between the plasma membrane distribution of the two fluorophores. In this talk I will present the details of this approach and describe results of this analysis used to characterize the stimulated interactions of some key proteins in the FcϵRI signaling pathway.