1. Plating murine macrophage cell line RAW264.7 onto 8 well Lab-Tek dishes

2. Transfection of 32 cDNA-YFP fusion vectors onto cells. cDNAs were taken from a library constructed at the DKFZ/EMBL or provided by the RZPD (Simpson et al., 2009) and in previous experiments, their products showed to be tyrosine phosphorylated (R. Pepperkok, unpublished data).

3. Preparation of samples for functional analysis
   3.1 Addition of:
   - IgG coupled beads (20min.)
   - Infection (5 and 60 min. and using a M.O.I of 100:1) with:
     - M. smegmatis mc2 (ATCC 1055)
     - M. avium (ATCC 25291)
   3.2 Cells were fixed in 3% paraformaldehyde, permeabilized in Triton X-100 (0.1%) and incubated with Cy5.5/PY2.

4. Analysis of samples by high content screening microscopy
   4.1 Illumination control (cells imaged were selected visually by conventional wide-field fluorescence microscopy, based on their high YFP fluorescence level).
   - Calibration procedure using reflective sample (foil)
   - Cell array matrix definition.

Fluorescence lifetime measurements were performed by wide-field frequency-domain FIM, using an instrument described in detail elsewhere (Squire et al., 2000).

Figure 2