



**Figure 3.** Comparative illustration of traditional visual presentation of 2D data and absolute quantification by combination of MRM, protein quantification, and image analysis. Data for four proteins involved in central carbon metabolism [triose phosphate isomerase (TpiA), phosphoglycerate kinase (Pgk), succinate dehydrogenase flavoprotein subunit (SdhA), 2-oxoglutarate dehydrogenase E1 subunit (OdhA/SucA)] of *B. subtilis* (left) and *S. aureus* (right) are exemplarily illustrated to demonstrate the power of the analysis. Appearance of the corresponding protein spots on 2-D gels is illustrated in spot tiles showing enlarged sections of image overlays. False color overlays are utilized to visualize the trend in protein regulations. The sample corresponding to exponential growth was colored green, and all others are red. Thus, shades of yellow indicate almost equal amounts in the samples compared, whereas shades of green and red indicate overrepresentation during exponential phase or stationary phase, respectively. Additionally, the absolute concentration in  $\text{ng } \mu\text{g}^{-1}$  of total protein and in molecules per cell (rounded) are given for the four proteins in growing cells ( $t_0$ ) as well as cells in early ( $t_1$ ) and late ( $t_2$ ) stationary phase.