

Figure 2 Single-molecule FISH (smFISH) used to characterize mRNA copy-number statistics. (a) Gene expression level (mRNA per cell) from the P_{lac} promoter as a function of inducer (isopropyl β-D-1-thiogalactopyranoside, IPTG) concentration. The mean mRNA number per cell as measured by smFISH (average of two independent experiments) is shown, as well as the results of quantitative PCR (qPCR; average of two independent experiments; normalized by the mean smFISH level) and β-galactosidase activity assay, as reported in the literature²⁷ (normalized by the mean smFISH level). Error bars represent the standard errors from duplicate experiments. The good agreement between the three assays, over approximately three orders of expression level, shows the accuracy and dynamic range of the smFISH method. (b) Typical images of smFISH-labeled cells at different induction levels. An overlay of the phase contrast (grayscale) and smFISH probes targeting the lacZ gene (red) is shown. Each image corresponds to the expression level designated by the horizontal arrow. (c) lacZ mRNA copy-number histograms obtained from smFISH at different induction levels. The experimental data (red) and the fit to a negative binomial distribution (black) are shown, as well as the estimated values for mean mRNA number $\langle n \rangle$ and standard deviation σ in that sample. Each plot corresponds to the expression level designated by the horizontal arrow. See Online Methods for more information.