

Supplementary Information for

Multiplexed imaging of high density libraries of RNAs with MERFISH and expansion microscopy

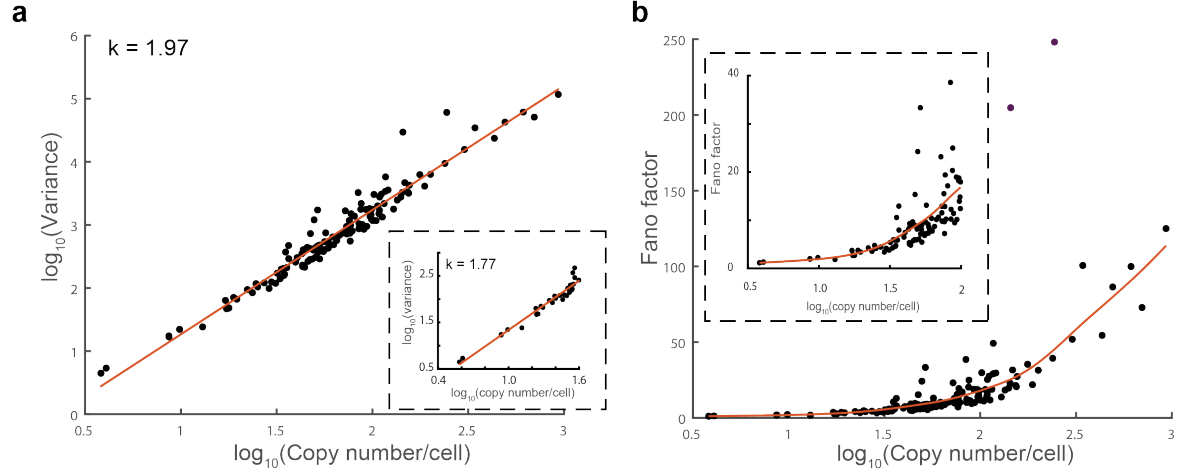
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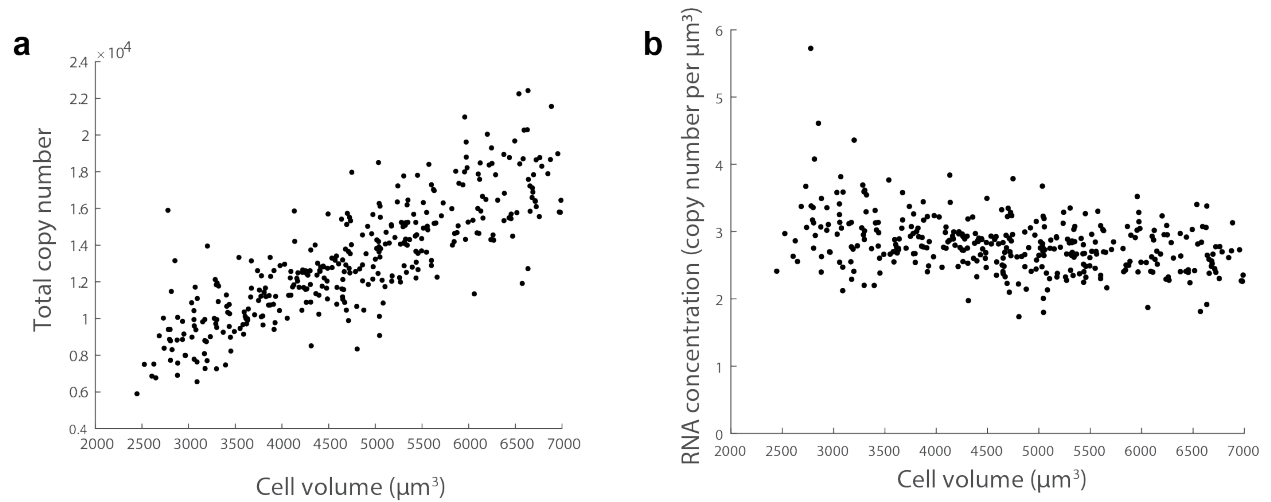
Supplementary Information includes:

Supplementary Figure 1

Supplementary Figure 2



Supplementary Figure. 1. Cell-to-cell variation in the RNA copy number as a function of the RNA expression level for the ~ 130 genes measured in this work. **(a)** The variance in RNA copy number as a function of the RNA copy number for an expression range of $10^{0.5} - 10^3$ measured in this work. The red line is a power-law fit $\sigma_{RNA}^2 \propto \mu_{RNA}^k$ with the scale exponent $k = 1.97$. Inset shows the data in the expression range of $10^{0.5} - 10^{1.6}$ copies per cell, and the power-law scaling coefficient in this range is slightly smaller, $k = 1.77$. This k value is similar to that derived previously from measuring the expression of one or two reporter genes incorporated into the chromosomes of T cells, which express RNAs in the same abundance range^{25,26}. **(b)** The Fano factor, defined by the variance over the mean RNA copy numbers, as a function of the RNA expression level. The red line is the cubic spline fit of the data for a guide for the eye (Inset: zoom in to show the data in the expression range of $10^{0.5} - 10^2$ copies per cell. The trend observed is similar to previously published results^{26,27}).



Supplementary Figure. 2. The total RNA copy number for the ~130 genes measured in each cell correlates linearly with the cell volume. **(a)** Scattered plot of the total RNA copy numbers of the 129 genes measured in single cells versus the cell volume. Cell volume was calculated by computing the volume of a 3D convex hull covering all RNAs in a cell. **(b)** Scatter plot of the RNA concentration, defined as total copy number per cell divided by the cell volume, versus the cell volume.