Supporting information to the article titled “Controlling Binding Site Densities on Glass Surfaces”

Joshua R. Wayment and Joel M. Harris*

Department of Chemistry
University of Utah
315 South 1400 East
Salt Lake City, UT 84112-0850

Contents: This Supporting Information contains images of distributions of spots prepared by a random number generator, which can be compared with the spatial distributions of amine groups bound to glass surfaces and imaged by single-molecule fluorescence microscopy. In addition, a magnified intensity profile of an immobilized gold particle imaged with bright-field illumination is provided.

Random Distributions of Spots in Two Dimensions. Arrays of 120-point random number pairs between zero and one were accumulated using a program written MATLAB (Mathworks). Plots of two of these arrays are provided below, at a spot density that is close to that observed in the single-molecule fluorescence images. These arrays of random numbers exhibit ‘patterns’ of spots in curved lines with large open areas between them, like the binding sites imaged by single-molecule fluorescence microscopy. One can only conclude that these ‘patterns’ are simply characteristic of a random distribution of spots in two dimensions. See Figure 1.
Figure 1. Plots of 120-point arrays of random-number pairs in two dimensions.
**Bright-field Image of a Streptavidin-Conjugated Gold Particle Immobilized on Glass.**

The measured bright-field images of gold particles exhibit an Airy disk pattern, the size of which is limited by the numerical aperture of the bright-field illuminator. The central, dark region of each spot exhibits a readily detected change in intensity relative to the bright background and provides a reliable area to threshold and count the gold particles bound to the glass surface.

![3-D projection of the bright-field image an immobilized gold particle on glass.](image)

**Figure 2.** 3-D projection of the bright-field image an immobilized gold particle on glass.