Analyte affinity capture by surface-immobilized diagnostic agents is a routinely employed assay format for profiling numerous medically and technologically important target analytes. These assays suffer from numerous performance limitations, including sensitivity and rapidity. Assay miniaturization is advocated to improve surface-capture performance, specifically exploiting the inverse relationship between analyte flux and capture feature size under mass transfer-limiting capture conditions that characterize many such assay formats. Reduced capture feature sizes, e.g., microarrays, are proposed to overcome mass transfer limitations, yet this is difficult to achieve across several size scales. This study validates certain advantages advocated for capture spot miniaturization using a rationale to understand surface capture miniaturization strategies. Experimentally derived immobilized ligand and target capture densities as a function of microspot size for DNA oligomers immobilized on model gold substrates are compared directly with theoretical analysis, validating the hypothesis that miniaturization yields many practical assay advantages. Specifically, results show that transitions from assay mass transfer limiting to kinetically limiting conditions as feature size decreases identify an optimal microspot size range for a specific bioassay system. Analytical advantages realized from such assay miniaturization are more uniform target-spot coverage and substantially increased rate of capture (hybridization), increasing assay signal and rapidity.

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