

学際物質科学研究センター(TIMS)セミナー

題目: 『DNA Microarrays in Bioassays: What does Signal mean?』

講演者: Professor David W. Grainger

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日時: 7月2日(土曜日) 11:00-12:00

場所: 総合研究棟 B 0110 公開講義室

概要:

Nucleic acid microarrays are used with increasing frequency to assert specific presence or absence of biological markers of disease, biowarfare agents, and genetic changes. This would imply that their assay results are quantitative and reproducible. However, numerous challenges preclude sensitivity, specificity, direct assay from complex milieu, and direct abundance quantitation required for such performance. We have noted that reactive polymer-coated microarray substrates based on N-hydroxyl succinimide (NHS) chemistry lose their bio-immobilization reactivity to DNA probe nucleophiles over time, both in use and in storage, due to their intrinsic hydrolytic instability. Poor DNA and protein probe immobilization efficiency is often observed with routine microarray printing conditions, with accompanying reliability and stability variability under assay. We have described a one-step reaction to regenerate NHS-reactive chemistry in situ on these microarray polymer surfaces with straightforward reactions. Surfaces regenerated with this method perform equal to or better than freshly prepared slides in print-immobilization of amine-oligonucleotide probes. Assay performance on these printed commercial polymer-coated substrates is not significantly altered by surface protein adsorption for the milieu conditions tested. This is in contrast to DNA assays on SPR biosensors where proteins in the assay interfere with assay performance We have compared radio-metric (32P-DNA) and SPR signals for thiolated DNA on gold substrates versus XPS nitrogen signals and find good correlation of immobilized probe densities and target hybridization efficiencies from assays. Factors affecting assay performance will be discussed for these microarray formats.

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