Quantifying Cell-Response to Materials Through Population Analyses Enabled by High-Throughput Techniques

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Efforts in combinatorial materials science at NIST have evolved into three interwoven toolsets for exploring cell response to materials: material library preparation, high-throughput screening, and statistical treatment of population distributions. As the material library available for investigating cell-biomaterial interactions continues to expand, image acquisition and analysis have been exposed as bottlenecks in the high-throughput approach. Furthermore, sampling biases inherent to imaging techniques are amplified as gradient samples are used to facilitate the exploration of a large parameter space.

In order to remove sampling bias and strengthen the quantitative nature of image-based analyses, the following commercial systems have been integrated and establish a framework for streamlining the process of screening cell-material interactions:

1) A fully automated, multi-channel image acquisition system is operational and capable of z-stack compiling for auto-focusing or extended depth of field imaging.
2) A versatile image analysis software package with integrated macros reduces image files to data files.
3) A versatile graphing and analytical software package with integrated macros performs rigorous statistical analysis and facilitates data visualization.

At present, image files are reduced to data files that document general cellular information such as area, perimeter, roundness, and asymmetry. As more sophisticated cell-staining protocols are added to the existing arsenal, data files will include more specific information describing cell populations.

The power of the streamlined process manifests itself in that information from thousands of *adherent* cells can be documented, cell-by-cell in a reasonable amount of time. As sampled populations increase, representing data, managing data, and quantitative analysis can differ from that of traditional approaches to investigating cell response to materials. This has become evident from preliminary studies on sugar-containing polyesters. Figure 1 shows two distributions of cell areas measured after culturing 3T3 fibroblasts on two different films for 24 h in the presence of serum. Each distribution represents a population of approximately 1000 cells.

Data are presented as smooth population distributions only for the purpose of providing a qualitative visual aid for comparison. A kernel density estimator was used to produce the smooth curves. Defining the standard uncertainty in measurements of this type is complicated by the fact that the underlying population distribution contains inherent variations in cell area equal to or larger than the measurement error.

Quantitative comparisons are made using a variety of techniques, two of which are a t-test and a Kolmogorov-Smirov (KS) test. Surprisingly, each test results in a different conclusion. From the results of a KS-test, a null hypothesis of similarity cannot be rejected for the two samples described in Figure 1. From the results of a t-test, an alternative hypothesis of dissimilarity can be accepted with 95 % confidence.

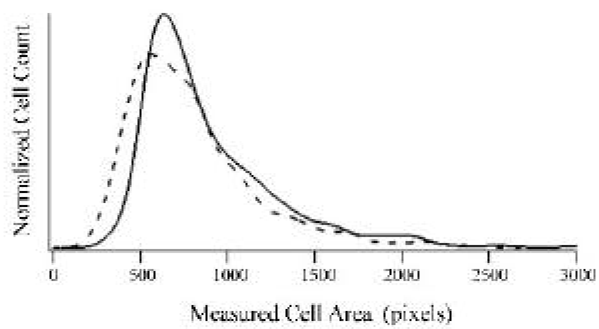

Figure 1. Distributions of cell areas measured after culturing on polyesters containing mol fractions of 12 % sorbitol (solid) and 30 % sorbitol (dashed).

The discrepancy in statistical comparisons arises from differing assumptions between the two tests. The t-test is a parametric test against the null hypothesis that two data sets have been randomly selected from the same underlying *normal* distribution. The KS test is a nonparametric test against the null hypothesis that two data sets have been randomly selected from the same underlying *general* distribution. As such, the t-test is more powerful if the underlying population is, in fact, normally distributed, while the KS test is more reliable if the assumption of normalcy is questionable.

High throughput image acquisition and analysis techniques are now being refined and applied to several material systems. We will report results from these investigations, including a rigorous statistical analysis comparing several parametric and nonparametric tests.