

Metrology for Quantitative Cell Biology

Cell-based assays are utilized extensively in the biotechnology and pharmaceutical industries during multiple phases of product and drug development. Despite the widespread use of these assays, there has been little effort in developing metrics and standards to validate cell lines and the culture conditions before their use in experimental settings. The absence of these validation procedures can cause complications when intra- or inter-laboratory data comparisons are required to fully interpret experimental results. The NIST research in quantitative cell biology has three dimensions: 1) development of indicator cells that express green fluorescent protein (GFP) that report a cellular response, 2) development of highly reproducible extracellular matrix protein thin film substrates for use as reference cell culture substrates, and 3) automated fluorescence microscopy and image analysis methodologies for quantifying a cellular response.

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Two aspects of the NIST research program in quantitative cell biology are described here. First, we identified optimized procedures for fixing cells that retain the morphological features of cells and the intracellular protein content. Second, we have identified the material properties required to fabricate the thin films of fibrillar collagen on polystyrene dishes commonly used in cell culture studies.

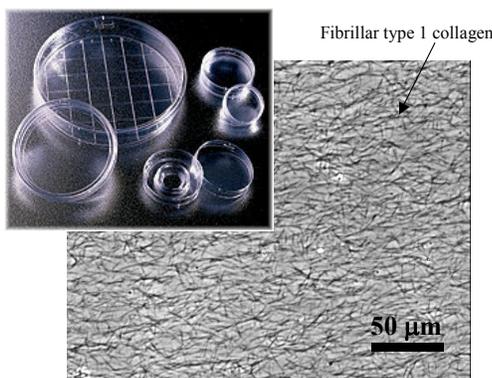
During the last processing step in many cell-based assays, the cells are often treated with fixatives to preserve the physiological state of the cells. The use of a fixative increases the stability of the sample and thus significantly reduces handling before the sample is analyzed by microscopy techniques. The most common fixative used in biology is formaldehyde, but several studies have shown that the use of formaldehyde does not always preserve the structures and intracellular protein concentrations within cells. We demonstrated this fact by using a novel *in-situ* assay in which cells that express GFP are fixed while cellular GFP fluorescence is measured in real time. The intracellular GFP acts as a representative intracellular protein and the fluorescence measurements during *in-situ* fixation allow one to quantitatively determine which steps of the fixation process result in a reduction in intracellular GFP during preservation. Our results indicate that commonly used fixation procedures that utilize 1% to 4% formaldehyde result in up to a 60% loss in intracellular soluble GFP

concentration. We further used the assay to evaluate several cross-linkers and fixation buffers to identify an optimal fixation procedure that best preserves the intracellular protein concentration. Our results indicate that the use of a maleimidobenzoic-N-hydroxysuccinimide ester cross-linker with a microtubule stabilizing buffer provides up to 95% preservation of intracellular GFP concentrations within fixed cells. The assay developed for this work and the results from the comparative fixation study provide a quantitative basis for optimizing procedures for preserving biological information within cells after fixation processes.

A second major focus of our efforts has been the development of highly reproducible fibrillar collagen thin films that can be used as cell adhesion substrates and thus ensure reproducible tissue culture conditions. These protein films are prepared by adsorbing type 1 collagen under polymerizing conditions to self-assembled alkanethiol monolayers on gold films. Comparative studies with vascular smooth muscle cells indicate that cells respond to these thin films similarly to how these cells respond to collagen gels. The

thin films are significantly more robust than collagen gels and exhibit significantly better optical properties for microscopy studies. Because these collagen thin film substrates are fabricated with a step-by-step procedure, each step of the fabrication can be characterized by surface analytical procedures to ensure reproducibility during preparation. Although these collagen-based substrates provide a method to ensure identical culture conditions between cell culture experiments, the construction of these substrates on thin films on gold/alkanethiol monolayers is not convenient for

use in most conventional biological laboratories. We have recently performed systematic studies to identify the surface properties that govern the formation of fibrillar collagen films. Our results indicate that materials with hydrophobic smooth surfaces such as bacterial grade polystyrene (BPS), routinely used in biological laboratories allow the reproducible formation of thin films of fibrillar collagen (see Figure). The development of procedures to form thin films of fibrillar collagen on BPS may facilitate standardization of tissue culture conditions for intra-laboratory and inter-laboratory experiments. These materials will also be beneficial for controlling the cell adhesion matrix in cell experiments analyzed with automated microscopy and high-content analysis procedures that are commonly used in pharmaceutical drug screening assays.



This figure shows a phase microscopy image of the thin film on collagen fibrils formed on bacterial-grade polystyrene.