



# Guide for Using Fluorescence Microscopy to Quantify the Spread Area of Fixed Cells<sup>1</sup>

This standard is issued under the fixed designation F2998; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This guide describes several measurement and technical issues involved in quantifying the spread area of fixed cells. Cell spreading and the distribution of cell spread areas of a population of cells are the result of a biological response that is dependent on intracellular signaling mechanisms and the characteristics of cell adhesion to a surface. Cell spread area is a morphological feature that can be responsive to alteration in the metabolic state or the state of stress of the cells. Changes in cell spread area can also indicate an alteration in the adhesion substrate that may be due to differences in manufacturing of the substrate material or be in response to extracellular matrix secretions. High quality measurement of cell spread area can serve as a useful metric for benchmarking and detecting changes cell behavior under experimental conditions.

1.2 The measurement described in this document is based on the use of fluorescence microscopy imaging of fixed cells and the use of image analysis algorithms to extract relevant data from the images. To produce robust cell spread area measurements, technical details involved in sample preparation, cell staining, microscopy imaging, image analysis and statistical analysis should be considered. Several of these issues are discussed within this document.

1.3 This standard is meant to serve as a guide for developing methods to reliably measure the area to which cells spread at a surface. This surface can be conventional tissue culture polystyrene or sophisticated engineered biomaterial surfaces. An example of a detailed procedure to measure the spreading area of cells on a tissue culture polystyrene surface is provided in the appendix section.

1.4 Cell morphology features such as cell spreading area and perimeter are generally reported in units of length. For example, spreading area per cell (that is, cell spread area) is likely reported in units of  $\mu\text{m}^2$ . A spatial calibration standard is required to convert between numbers of pixels in a CCD camera image to  $\mu\text{m}^2$  as an SI unit.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.5.1 *Sodium azide is used as an anti-bacterial reagent in the slide mounting media. This preserves the integrity of the mounting media. The toxicity of this reagent (for example, MSDS) should be considered before use of this reagent in large scale slide mounting procedures.*

## 2. Referenced Documents

### 2.1 ASTM Standards:

E1488 [Guide for Statistical Procedures to Use in Developing and Applying Test Methods](#)

F2150 [Guide for Characterization and Testing of Biomaterial Scaffolds Used in Tissue-Engineered Medical Products](#)

## 3. Terminology

### 3.1 Definition of Terms:

3.1.1 *cell morphology*—the physical shape properties of a cell such as cell volume, cell spread area, and cell perimeter; and the non-unit measures of roundness and circularity.

3.1.2 *cell spread area*—the area that encompasses a 2-dimensional (2-D) projection of a 3-dimensional (3-D) adhered cell.

3.1.3 *cell object*—a single cell or two or more adhered cells adjacent to each other such that they cannot unambiguously be segmented from one another by cell edge detection techniques.

### 3.2 Definitions of Terms Specific to This Standard:

3.2.1 *segmentation*—the act of classifying pixels in an image as cell or non-cell areas and the grouping of adjacent cell pixels into a cell object.

## 4. Summary of Practice

4.1 The measurement of a cell morphology feature such as cell spread area in a population of cells in culture can be a quantitative characteristic of cell population behavior and cell population state. This document provides guidance on measuring the 2-D morphological property (that is, cell spread area) for a population of cells in culture on a material.

<sup>1</sup> This test method is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.46 on Cell Signaling.

Current edition approved Jan. 1, 2014. Published May 2014. DOI: 10.1520/F2998-14.