

**CBER Standards Recognition Program for Regenerative Medicine Therapies
Standards Recognition Summary (SRS)**

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SDO Name/Designation: ASTM F3294

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Title: Standard Guide for Performing Quantitative Fluorescence Intensity Measurements in Cell-based Assays with Wide Field Epifluorescence Microscopy

Scope:

1. This guidance document has been developed to facilitate the collection of microscopy images with an epifluorescence microscope that allow quantitative fluorescence measurements to be extracted from the images. The document is tailored to cell biologists that often use fluorescent staining techniques to visualize components of a cell-based experimental system. Quantitative comparison of the intensity data available in these images is only possible if the images are quantitative based on sound experimental design and appropriate operation of the digital array detector, such as a charge coupled device (CCD) or a scientific complementary metal oxide semiconductor (sCMOS) or similar camera. Issues involving the array detector and controller software settings including collection of dark count images to estimate the offset, flat-field correction, background correction, benchmarking of the excitation lamp and the fluorescent collection optics are considered.
2. This document is developed around epifluorescence microscopy, but it is likely that many of the issues discussed here are applicable to quantitative imaging in other fluorescence microscopy systems such as fluorescence confocal microscopy. This guide is developed around single-color fluorescence microscopy imaging or multi-color imaging where the measured fluorescence is spectrally well separated.
3. Fluorescence intensity is a relative measurement and does not in itself have an associated SI unit. This document does discuss metrology issues related to relative measurements and experimental designs that may be required to ensure quantitative fluorescence measurements are comparable after changing microscope, sample, and lamp configurations.
4. 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.
5. This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

Extent of Recognition: Complete Recognition

Rational for Recognition: This standard is relevant to regenerative medicine therapies and is not in conflict with FDA regulations or policy.

Standards Development Organization: <https://www.ASTM.org>

Please note that this standard may also be recognized under the Center for Devices and Radiological Health's (CDRH) Recognized Consensus Standards Database for Medical Device, found here: <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfStandards/search.cfm> .