# Multi-parametric standards for performance assessment and quality control of fluorescence molecular imaging and endoscopy systems

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## ABSTRACT

Fluorescence molecular imaging (FMI) and endoscopy (FME) are technologies with great potential for image-guided surgical or diagnostic interventions. However, FMI and FME still present challenges that can confound real-time decision making for disease management and/or treatment. Importantly, the markedly different systems hurdle the repeatability of measurements, the unbiased readout interpretation, and the wide clinical acceptability of FMI and FME. Herein we present different multi-parametric standards to perform quality control and performance assessment of FMI and FME systems. Moreover, we discuss examples illustrating how data analysis and the design of fluorescence standards influence performance assessment outcomes, potentially affecting comparisons between systems or studies. We, also, show the first standard tailored to the requirements of FME and demonstrate its use for quality control of a fiberscope-based FME system. The discussed performance assessment and quality control framework can accelerate the clinical translation of fluorescence molecular imaging and endoscopy and steer further developments in the field.

**Keywords:** Fluorescence standards; performance assessment; quality control; fluorescence imaging

## 1. INTRODUCTION

Fluorescence molecular imaging (FMI) and endoscopy (FME) are emerging as technologies with great potential to guide surgical and endoscopic interventions and to provide earlier, faster, and personalized diagnosis in oncology. In tandem with the recent advances in the development of novel tracers, their clinical validation is currently assessed under numerous clinical trials worldwide. The recent approvals by the US Food and Drug Administration of approximately 20 fluorescence-guided clinical imaging systems, as well as three tracers for surgical guidance are a promising result of the ongoing efforts[1].

However, FMI and FME still present challenges that can confound real-time decision making for disease management and/or treatment. Importantly, the markedly different systems hurdle the repeatability of measurements, the unbiased readout interpretation, and their wide acceptability as "red flag" techniques for cancer detection[2]. To that end, the first efforts for standardization of systems and procedures start to appear in literature and guidelines are suggested by different study groups[3-5]. Herein we discuss some of these efforts and guidelines, with reference to the work implemented by our group for the development of multiparametric, composite standards to perform quality control and performance assessment of FMI and FME systems.

## 2. METHODS

The FMI and FME fluorescence standards were built with transparent polyurethane (WC-783 A/B, BJB Enterprises, Tustin, United States) as the main material of the matrix and hardener. To simulate scattering, $TiO_2$ nanoparticles (Titanium IV Oxide; Sigma Aldrich, St. Louis, MO, USA) were used, while absorption was enabled by alcohol-soluble Nigrosine (Sigma Aldrich) in the matrix and bovine hemin (≥90% pure; Sigma Aldrich) in the different wells. Finally, organic quantum dots (QDot 800 ITK, Thermofisher Scientific, Waltham, MA, USA) were used for fluorescence due to their excellent stability. The components of the two standards are shown in Figure 1, while they were manufactured following the guidelines proposed previously by our group[6, 7].

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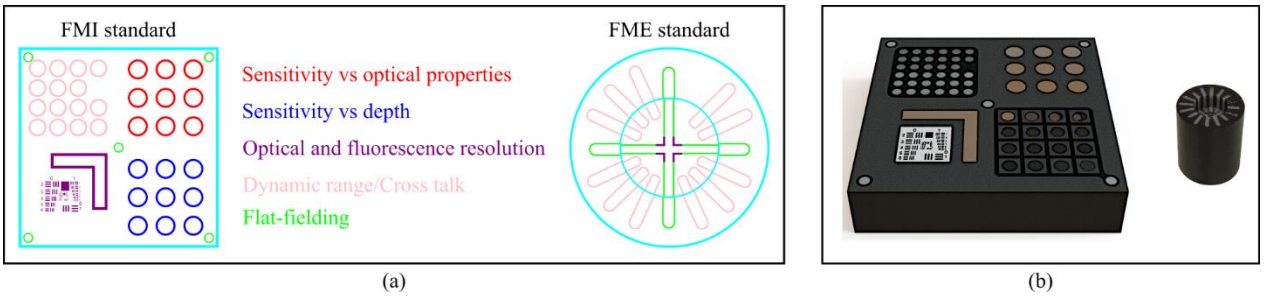


Figure 1. The FMI and FME fluorescence standards. (a) The different camera performance metrics that can be quantified by each standard. (b) Designs of the FMI (left) and FME (right) standards.

Based on the fluorescence standards shown in Figure 1, we showcase examples for their integration into the standard clinical processes. A key factor for this integration is the identification of the various design elements through the color and fluorescence images. Following this identification, different approaches for quantification of the various performance metrics can be applied. Signal-to-noise ratio and contrast are two commonly used metrics for the sensitivity assessment of different fluorescence imaging systems, while the contrast transfer function is frequently used for the resolution assessment[3, 8]. We quantify all these metrics for different systems, with and without application of flat-fielding, i.e., correction of the non-uniform illumination spatial distribution. Finally, we show how all these approaches can be transferred to FME systems through the multi-parametric standard of Figure 1b[7].

## 3. RESULTS

The composite fluorescence standards of Figure 1 can be employed for the performance assessment and quality control of FMI and FME systems. The influence of the acquisition settings and the definition of different metrics on the system characterization can also be quantified through these fluorescence standards. One example is the impact of the excitation source spatial distribution, as shown in Figure 2a. Furthermore, these standards can be employed also for the definition of image fidelity assessment approaches, in order to enable visualization that is consistent with the biodistribution of the administered tracers and to allow for efficient interpretation of the acquired data[2].

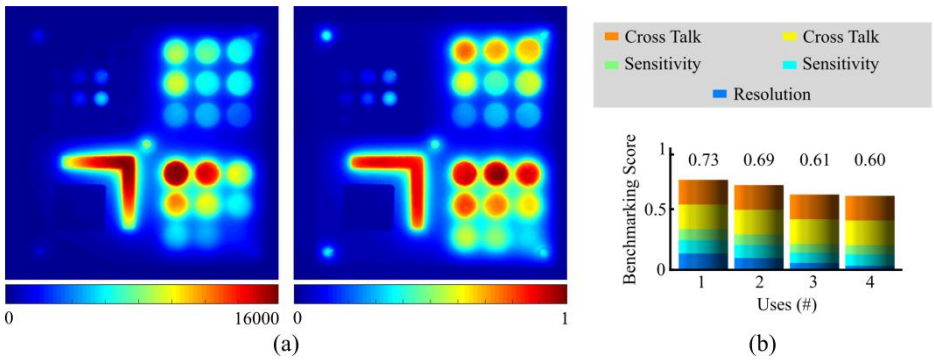


Figure 2. Exemplary uses of the FMI and FME fluorescence standards. (a) Application of the FMI fluorescence standard to correct for the excitation illumination spatial distribution. (b) Application of the FME fluorescence standard for quality control of the flexible fiberscopes as a function of the uses.

At the same time, we show the first FME performance assessment and quality control application that is in agreement with the recent guidelines from the American Association of Physicists in Medicine[3]. Importantly, the sterilization process and the repeated uses regularly degrade the performance of the employed fiberscopes. Up to now, this degradation was only assessed after the use of the fiberscope on the patient. With the proposed standard shown in Figure 1, and adopting the previously proposed benchmarking scores[8, 9], this degradation can be monitored before the endoscopic session (see Figure 2b) and if needed the fiberscope can be replaced in time to avoid interference with the standard clinical procedures[7].

## 4. CONCLUSIONS

In this study we present our most recent efforts to establish FMI performance assessment and quality control protocols through the use of composite multiparametric fluorescence standards. In addition, we propose a standard that is suitable for FME performance assessment and quality control. We describe its design and showcase its potential application. In contrary to intraoperative FMI, FME is based on optics that degrade as a function of uses and cleaning cycles, making the proposed phantom an essential tool for quality control and optimal data acquisition.

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