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# Fluorescence Calibration Tools

## The challenge

Many IVD assays and devices rely on fluorescence emission and detection. This is particularly true in the field of molecular diagnostics, where real-time PCR and other instruments that detect and process fluorescent dye signals are used every day in both research and clinical laboratories. Calibration of fluorimeters in in vitro diagnostic (IVD) devices is essential to ensuring repeatable, reproducible, and accurate fluorescence measurements and test results.

## The solution

Volpi combines decades-long experience in fluorometer manufacturing and unrivaled expertise in the use of fluorescence dyes to develop this new fluorescence calibration tool for manufacturing quality assurance, calibration services and other uses.

Volpi has developed a new 96-well fluorescence calibration plate with fluorescence standards for five commonly used fluorescence dyes. These dyes are the most commonly used in assays today. The dyes are embedded in a solid matrix in the wells of a standard test plate to ensure long-term stability and reusability.

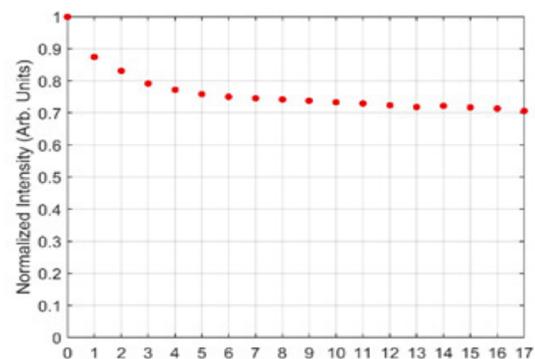
During fluorometer manufacturing or at the site of use of the diagnostic device, Volpi's new fluorescence calibration tools can be used...

- To calibrate for signal non-linearities
- To calibrate the dynamic range of a detector for different fluorescence dye conversion efficiencies

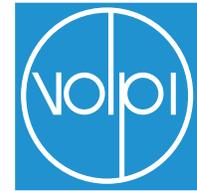
- For calibration of the detector sensitivity
- For calibration of the signal dependency in response to sample volume dependencies
- To detect fluorescence bleed-through compensation in multiplex assays
- Recognize inter-position variances within the system
- For blank signal subtraction



Fluorescence reference dyes immobilized in standard multi-well plate



Fluorescent signal level upon continuous irradiance with 100mW/mm<sup>2</sup> @ 460nm



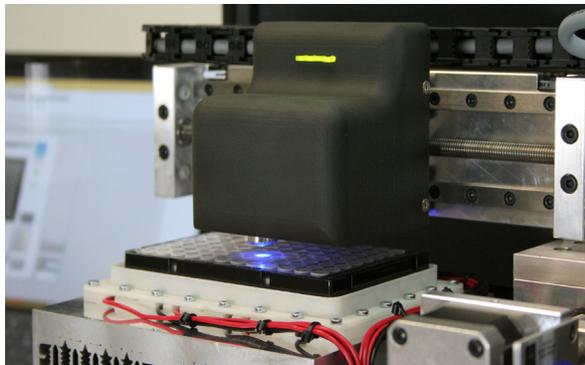
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## Fluorescence Calibration Tools

The dyes are embedded in a solid matrix, allowing for room-temperature storage with no concentration change and undiminished high mechanical robustness. The calibration tool is used by simply placing in the fluorimeter and running it manually.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dye 440 Conc. 1	Dye 440 Conc. 2	Dye 440 Conc. 3	Dye 440 Conc. 4	Dye 440 Conc. 5	Matrix	Dye 440 Conc. 1	Dye 440 Conc. 2	Dye 440 Conc. 3	Dye 440 Conc. 4	Dye 440 Conc. 5	Empty
B	Dye 520 Conc. 1	Dye 520 Conc. 2	Dye 520 Conc. 3	Dye 520 Conc. 4	Dye 520 Conc. 5	Matrix	Dye 520 Conc. 1	Dye 520 Conc. 2	Dye 520 Conc. 3	Dye 520 Conc. 4	Dye 520 Conc. 5	Empty
C	Dye 580 Conc. 1	Dye 580 Conc. 2	Dye 580 Conc. 3	Dye 580 Conc. 4	Dye 580 Conc. 5	Matrix	Dye 580 Conc. 1	Dye 580 Conc. 2	Dye 580 Conc. 3	Dye 580 Conc. 4	Dye 580 Conc. 5	Empty
D	Dye 620 Conc. 1	Dye 620 Conc. 2	Dye 620 Conc. 3	Dye 620 Conc. 4	Dye 620 Conc. 5	Matrix	Dye 620 Conc. 1	Dye 620 Conc. 2	Dye 620 Conc. 3	Dye 620 Conc. 4	Dye 620 Conc. 5	Empty
E	Dye 710 Conc. 1	Dye 710 Conc. 2	Dye 710 Conc. 3	Dye 710 Conc. 4	Dye 710 Conc. 5	Matrix	Dye 710 Conc. 1	Dye 710 Conc. 2	Dye 710 Conc. 3	Dye 710 Conc. 4	Dye 710 Conc. 5	Empty
F	Dye 780 Conc. 1	Dye 780 Conc. 2	Dye 780 Conc. 3	Dye 780 Conc. 4	Dye 780 Conc. 5	Matrix	Dye 780 Conc. 1	Dye 780 Conc. 2	Dye 780 Conc. 3	Dye 780 Conc. 4	Dye 780 Conc. 5	Empty
G	Mix Conc. 1	Mix Conc. 2	Mix Conc. 3	Mix Conc. 4	Mix Conc. 5	Matrix	Mix Conc. 1	Mix Conc. 2	Mix Conc. 3	Mix Conc. 4	Mix Conc. 5	Empty
H	Mix Conc. 1	Mix Conc. 2	Mix Conc. 3	Mix Conc. 4	Mix Conc. 5	Matrix	Mix Conc. 1	Mix Conc. 2	Mix Conc. 3	Mix Conc. 4	Mix Conc. 5	Empty

Dye filling pattern in 96 well plate



Breadboard test system with full functional integration of optics and thermal sample control for qPCR



This calibration tool features long-term, stable fluorescence dyes with high temperature stability and resistance to photobleaching, caused by an alteration of the fluorescent dye that inhibits its ability to fluoresce.

The plate is comprised of five dilutions of each of the single dyes that emit between 440-780 nm, and dilutions of dye mixtures and blank positions. All single dyes, mixtures and blank samples are present in replicate to determine inter-position variances and to validate the system. As part of the development process the 96-well fluorescence dye plate was used for testing a six-fluorescence-channel fluorescence scanner. With the availability of this fluorescence calibration tool, Volpi continues to expand its capabilities in the development, manufacturing, and life-cycle management of IVD devices.

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