



Light. Insight. Life.

Volpi's Offering in the Field of OEM qPCR-Measurement Systems

The challenges

Quantitative polymerase chain reaction or qPCR, sometimes called real-time PCR, simultaneously detects and measures the amount of a specific DNA target in a sample by amplifying that target during cycles of heating and cooling the sample. Since its development in the early 1990s, qPCR has become an essential part of many molecular diagnostic workflows.

Sample prep is an important part of performing qPCR in a clinical laboratory. It involves extracting and purifying the DNA sample from the patient's blood, saliva or other sources. Sample prep is the first step in a clinical laboratory process that involves preparation of the diagnostic assay, performance of the testing, and the analysis of the testing results. Today's qPCR sample prep, testing and analysis are performed in different compartments of the same instrument.



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

Precisely controlling the temperature during qPCR thermocycling is crucial for accurate results and assay reproducibility. Temperature control is strongly dependent on good thermo-mechanical contact between the wells of a multiwell plate that hold the DNA sample and the heat transfer unit of the qPCR instrument.

Even with solid thermo-mechanical contact, changes in the surface energy of the wells and other temperature-dependent liquid wetting properties create variations in the signals generated by the qPCR reaction.

In top-reading architectures, these variations affect the instrument's optical interface. Through this interface, the liquid sample receives the excitation light that generates the fluorescent signal from the products of the qPCR reaction exiting the sample through the same interface. The fluorescent light then transported by the detection optics and converted at the detector into an electrical signal used in the analysis of the qPCR reactions.

There are other factors that can affect the read-out geometry on the optical interface. These factors include strong changes in the concentration-dependent light absorption and emission properties of the reaction solutions used in each plate well during the qPCR reaction. These changes influence the penetration-depth of the light and the reabsorption properties of emitted light and by this, they affect the measured strength of the fluorescent signal generated by the qPCR reaction when the DNA is present in the sample.



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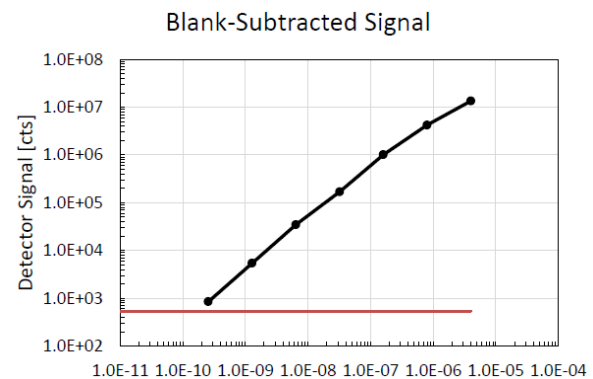
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Because of these and other factors, a tolerant optical read-out scheme is a prerequisite for the highly reliable measurement systems that customers demand in qPCR systems today.

The solution

Volpi is a trusted, reliable partner of major in-vitro diagnostic companies. For more than a decade, Volpi has supplied qPCR components to some of the largest IVD companies in the world as a single-source supplier of Class 3 devices cleared by the U.S. Food and Drug Administration. This work has included developing and manufacturing optical components that are used in the best PCR instruments in the world.

Based on its breadth of experience and its international expertise, Volpi has developed optical detection modules with tolerant optical interfaces for on-deck qPCR thermocyclers. These solutions ensure that the fluorescent signals generated during the qPCR reaction are captured accurately and can be transferred for further analysis. The optical detection modules created by Volpi can be used in instruments that use qPCR in NGS workflows for cancer diagnostics and rare congenital disease and fast assays for viral and immune diagnostics, among other applications.



Typical sensitivity performance achieved with Volpi's OEM measurement heads in qPCR architectures

Volpi's development of these new optical detection modules is based on its thorough understanding of the design challenges associated with OEM qPCR-measurement solutions. The company invested in the internal development of demonstrators and sub-system platforms for its work on these new lead architectures.

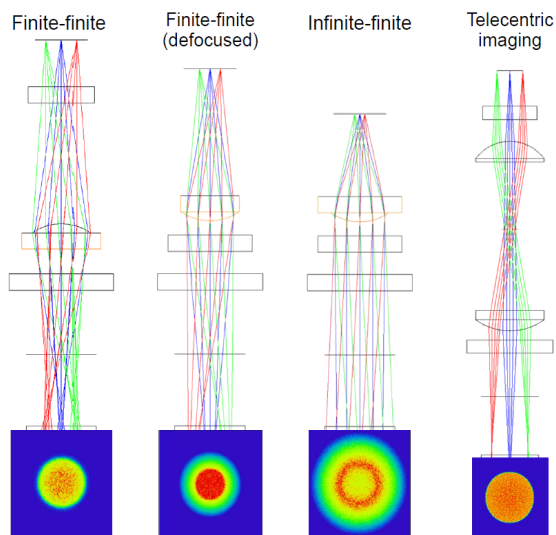
The focus of these efforts has been the development of applications that require a low-profile solution that can be integrated effectively with a general-purpose liquid-handling platform. Liquid-handlers can increase testing throughput and reduce user error, and are an integral part of current qPCR workflows. These applications typically place an extra constraint on the system design, namely z-height of cooler and optical detection module.



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Volpi's experience ensures that the z-dimensions of its solutions can be matched to the needs of almost all instruments they will be used in.



Different illumination / pick-up architectures

In addition to its work with solutions used in liquid handling, Volpi perfected two optical read-out methods for multiwell plate based instruments. Both of these methods can improve the detection limit and reading & processing speed of the fluorescent signal generated during the qPCR reaction.

Before beginning its work on these new methods, the company analyzed a number of different existing optical read-out concepts. This analysis led to the selection of the two contrary approaches.

The first solution involves spacial scanning free-space optics with beam splitters for color-channel multiplexing. These optics scan the x,y plane, provide high bandwidth for the successful transmission of an optical signal like those used in a qPCR system, and uses multiple colors or channels.



Illumination and detection solution applying spectral channel switching

The second solution involves spectral switching within the light engine for fiber bundle illumination whose multiple outputs generate the spatial multiplexing effect. This solution can switch the qPCR excitation wavelength very fast and uniform among other advantages.



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Illumination and detection solution applying spatial switching

During the development of both of these optical read out solutions, the optical couplings to the PCR plate wells were analyzed in detail to find the best balance of signal sensitivity and geometry tolerance. Both of the solutions that resulted from this careful analysis can improve the reading of qPCR reaction signals and, ultimately, the accuracy of the assay results.

Volpi's optical system reduces the effects of pipetting errors. This type of error in liquid handling can cause variations in the fill-height of reaction solutions in the different plate wells. They also can create meniscus variations, which influence the read-out signal strength. A varying meniscus has one concave surface with varying radius, generating an additional uncontrolled optical power in the system. This property affects the excitation light distribution in the sample and the pick-up efficiency of the detection system



by changing the acceptance angle of the optical system respectively the emission distribution of the fluorescent light.

Volpi has also developed a sample heat management solution that demonstrated outstanding temperature control in use in a qPCR instrument. Precise temperature control is critical to ensuring the performance of the qPCR assay and for minimizing variations in the read-out signals from the wells in the test plate. This heat management solutions complements the optical solutions that Volpi has developed.

All these new solutions have been prototyped and successfully integrated into qPCR instruments. The system, which includes optical, heat management and liquid-handling solutions, is controlled by a real-time controller that enables control of each of these solutions. The controller also communicates the results of the qPCR reactions through a controller area network (CAN) bus to application-level software that performs raw data processing, provides data storage and displays the qPCR reaction results.

Volpi's experience complements its expertise in the field of optical electronics and their application to qPCR instruments. This powerful combination allows Volpi to better serve its clients, including adapting new optical, heat management and liquid-handling solutions for qPCR instruments to meet the specific needs of individual clients.

Volpi AG

Wiesenstrasse 33, CH-8952 Schlieren, Switzerland
Tel. +41 44 732 43 43
mail@volpi-group.ch, www.volpi-group.ch

Volpi USA

5 Commerce Way, Auburn, NY 13021, USA
Tel. +1 315 255 1737
volpi@volpi-group.us, www.volpi-group.us