



# French Firm Stilla Technologies Unveils Three-Color 'Crystal Digital' PCR Platform

Mar 16, 2016 | [Madeleine Johnson](#)

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NEW YORK (GenomeWeb) – French firm Stilla Technologies last week unveiled a digital PCR system that uses a unique method of droplet formation and is the first to have three-color capabilities.

The core workflow, called Crystal Digital PCR, uses a consumable called a Sapphire Chip, which is run on a droplet partitioning and amplification system called the Naica Geode and read out on a separate instrument called the Naica Prism3.

Stilla unveiled the Naica system at Cambridge Healthtech Institute's Molecular Medicine Tri-Conference last week in San Francisco.

"What is unique about our system is that we have been able to integrate the three steps – droplet formation, amplification, and readout – on a single consumable," Rémi Dangla, president and founder of Stilla, told GenomeWeb in an interview following the conference.

In the Crystal Digital workflow, a sample is partitioned into a large, self-assembled, 2-D array of droplets.

"We call these arrays 'droplet crystals' because the droplets self-arrange into a hexagonal mesh that looks like a crystal when you zoom in," Dangla explained.

Each droplet on the system can contain a multiplex PCR reaction. "We are the only system that allows three-color detection," Dangla said. Three images are captured in different fluorescence channels during analysis, with one target per color, allowing the system to distinguish between targets.

The system generates between 25,000 to 30,000 droplets, which Dangla said targets about 99 percent of digital PCR applications. At this number of droplets, "You already have about six logs

of quantification range [and] your LOD is comparable to having millions of droplets," he said. Increasing the number of droplets may increase precision, but Dangla said that current publications indicate there is rarely a need for more droplets.

"We felt that by offering 25,000 to 30,000 droplets we already have an interesting product for most, if not all, digital PCR users."

Furthermore, the Crystal Digital system uses standard hydrolysis probe-based chemistries, so "for someone who is already doing qPCR, it's very easy to transfer a multiplex qPCR assay onto our system," Dangla said.

In fact, the firm has already worked with unnamed outside groups to develop triplex assays for liquid biopsies in oncology, including an assay for three different EGFR mutations relevant to non-small cell lung cancer treatment response monitoring.

Stilla also plans on increasing the multiplexing capacity by adding detection channels to future products.

"Three-plex digital PCR has never been done before, and it raises a number of questions in terms of data analysis — you now have 3-D dot-plots to analyze, and it gets tricky to visualize for the user," Dangla said. But with the knowledge the company is building from three channels, Dangla said the firm's second version will be able to have five or six colors while keeping the data analysis simple enough for the end user.

The system is built upon technology that Dangla and collaborators developed at École Polytechnique and described in [PNAS](#) in 2013. According to that study, droplet generation is achieved without relying on fluid flow, but instead using height variations to subject the immiscible interfaces of oil and liquid sample to "gradients of confinement" which can guide or trap droplets via microfabricated grooves.

This concept "was really the beginning of the story for Stilla and the Naica system," Dangla said, and was part of his graduate work at École Polytechnique.

Dangla was wrapping up his studies around the time that the first digital PCR products were coming onto the market. "I knew at the time that we had a microfluidic approach that could greatly improve the digital PCR workflow," he said, so once it had proof-of-concept chemistries and instrumentation, the firm incorporated in the fall of 2013 and began product development.

Stilla has been financed by a number of technology start-up grants from France, and is also backed by confidential private investors. The firm has a patent portfolio protecting innovations like the microfluidic chip, with some patents fully owned by Stilla and some exclusively-licensed from École Polytechnique.

"Because we have this unique microfluidic technology allowing us to make droplets on chip, we can integrate the whole digital PCR workflow in a single chip and offer users what we think is going to be the simplest and fastest digital PCR solution on the market," Dangla said.

Current commercially available droplet-based digital PCR systems — in which PCR is performed in microdroplets separated by oil — include the QX100 and QX200 Droplet Digital PCR systems from Bio-Rad and the RainDrop ddPCR platform from RainDance Technologies. Digital PCR performed in plate-based formats include the OpenArray and QuantStudio platforms from Thermo Fisher brand Life Technologies and Fluidigm's Biomark HD featuring integrated fluidic circuit technology, as [previously described](#). Most of these generate thousands or tens of

thousands of individual reaction volumes, except RainDance's product, which can generate millions of droplets.

The Crystal Digital workflow differs from the droplet-based systems in the droplet generation method as well as the analysis.

"From the very beginning our goal was to simplify the digital PCR workflow as much as possible," Dangla said. Currently, the process is too cumbersome compared to qPCR, he said. Stilla's users are people who are using qPCR, and are used to loading a microtiter plate with a pipette, placing it in single machine and getting results in two hours, so "this is where we're trying to take digital PCR in terms of simplicity and speed."

The Crystal Digital workflow also has a single pipetting step. "You only have to pipette your qPCR mix into the Sapphire Chip," since it is pre-filled with oil.

Running the workflow requires two machines. Stilla's Geode instrument performs droplet formation, and it's also a thermal cycler so will run the PCR step as well. The Prism3 readout instrument then performs the analysis using a software system called Crystal Miner.

"Compared to current solutions, where you have at least two consumables and three machines, we've considerably simplified the whole workflow," claimed Dangla.

The company announced the commercial launch of the system in October, is currently selling it in Europe for research use, and is "early in the process of building up commercial sales," Dangla said. It has had beta test users in Paris and a few users since launch. The firm is currently signing its first sales, "so it's off to a great start for us in Europe," he said.

The system is priced below \$100,000. "It's a competitive solution for digital PCR, because we also know that the instrument price is a barrier to entry to users [because] qPCR machines sell for \$20,000 to \$30,000."

The firm is currently focused on expanding its user base in the research market, where it believes there is a strong opportunity. But it is also interested in taking the product through CE-IVD certification and 510(k) clearance in a few years.

While Stilla is an instrument provider, not an assay development company, it is currently working with collaborators to develop assays using the full capacity of three-color digital PCR. "We are initially helping our users, because we know it is a bit challenging to increase to three colors in the beginning," Dangla said.

"In the long term we'll provide the system to any research lab and we're confident that, because it's so easy to use, our user will be able to develop assays in the field of liquid biopsy or any field where PCR is appropriate."

Dangla noted that the firm will now begin attending large conferences to meet users and potential industrial partners, and hopes to have products available for sale in the US by the end of the year.

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