Understanding the principles of digital PCR and why Ruby Chip is the most flexible digital PCR consumable.

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About This Technical Note

In this technical note, we explore and compare the performance of Stilla's Ruby and Sapphire chips through both theoretical modeling and experimental data. Starting with the core principles of digital PCR (dPCR), we introduce the concept of **relative uncertainty (CI 95%)**—a useful metric for evaluating the intrinsic precision of a dPCR assay. We then examine analytical performance across a series of comparisons between chip types and pooling strategies.

This document is designed for multiple levels of readership: whether you're seeking a high-level overview or interested in deeper technical insights, we've included both summary explanations and optional deep dives into the underlying mathematical models to suit your needs.

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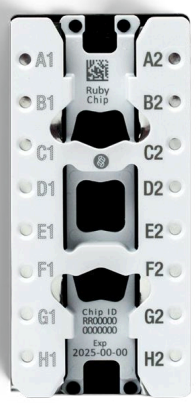


Ruby Chip

- 16 chambers/chip
- 5 µL/chamber*
- 17,000-136,000 droplets/chamber
- ~5 logs dynamic range**

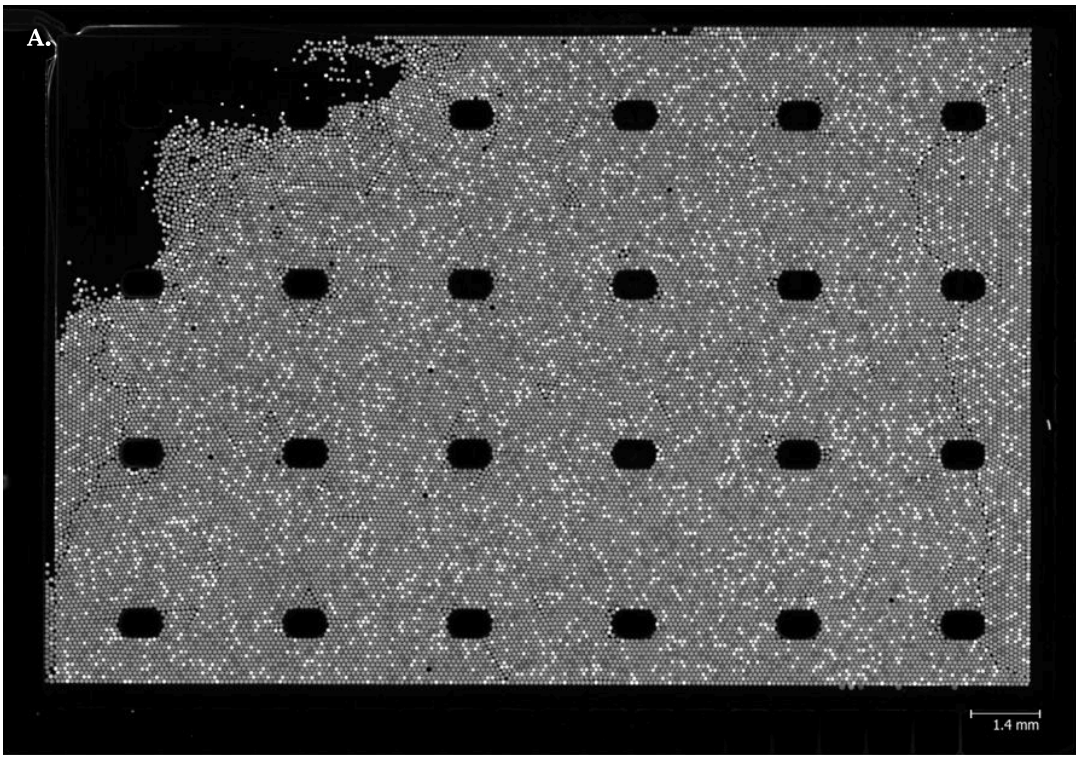
*Load up to 40 µL by pooling chambers.
**Analytical specs are assay-dependent.

Highlights

- Digital PCR results should be interpreted within the context of statistical confidence, specifically the Poisson confidence interval. This means that if the sample is tested repeatedly under the same conditions, 95 out of 100 times, the confidence interval (obtained from the measurement) will contain the true value. This range reflects the inherent statistical precision of dPCR, not accounting for external experimental variabilities like pipetting errors.
- Ruby Chip offers superior precision at higher concentrations (above 2000 cp/µL), and performs comparably to the Sapphire Chip in the mid-range (120–2000 cp/µL).
- Applying a pooling strategy with Ruby Chip chambers (two to three chambers), users can significantly enhance analytical performance, while reducing costs—particularly in high-concentration scenarios where Sapphire Chip's dynamic range is stretched.

Introduction

The Ruby and Sapphire chips ensure droplet crystal formation for Crystal Digital PCR®. The droplet crystal is a monolayer of droplets of the same size, self-arranged in a crystalline or “honeycomb” configuration. In the following, the microfluidic characteristics refer to both the droplet size and droplet number of a droplet crystal. In such a droplet crystal, droplet size and number are controlled by the physical dimensions of the chambers of the chips, and of the microchannels through which the reaction mix is injected into the chamber **(Fig. 1)**. Because of their different dimensions, both chips lead to the formation of different droplet crystals, with different droplet sizes and numbers.



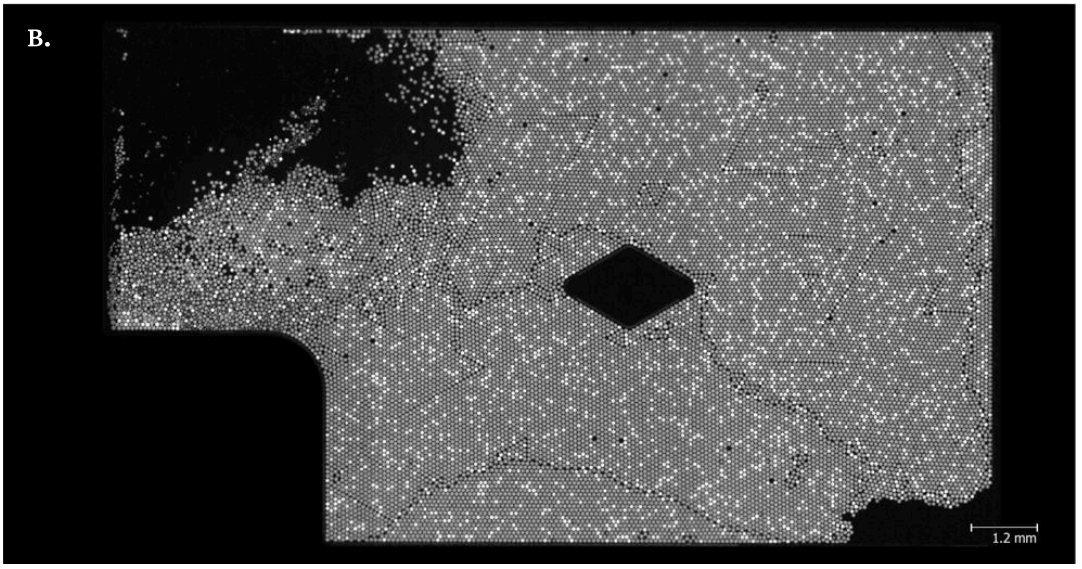


Figure 1:

A. Droplet Crystal obtained in the Sapphire Chip: the droplets are formed in the space between the chamber and the outlet of the microcapillaries in which the PCR mix flows, at the bottom and on the side of the chamber. All metrics discussed in the following are computed using 22,000 droplets of 0,62 nL for Sapphire Chip chambers.

B. Droplet Crystal obtained in the Ruby Chip: the droplets are formed in the space between the chamber and the outlet of the microcapillaries in which the PCR mix flows, on the side of the chamber. All metrics discussed in the following are computed using 15,000 droplets of 0.22 nL for Ruby Chip chambers.

These microfluidic characteristics, in turn, determine the range of concentrations that can be measured, i.e. the dynamic range, with each chip, and the associated precision on the measurement. In the following, the analytical performances refer to the dynamic range together with the precision of the measurement throughout this range.

Introduction (continued)

This note explains how analytical performances are linked to microfluidic characteristics of the chip and offers a theoretical and experimental comparison of the analytical performances between Sapphire Chip and Ruby Chip. It provides tools for Crystal Digital PCR® users to tailor their Ruby Chip experiments parameters and meet their own application analytical performances requirements. To make the note accessible to all readers, two reading levels are presented: the key takes away messages and a concise mathematical background for readers who want to go deeper in the understanding of the underlying principles of digital PCR.

Digital PCR principles

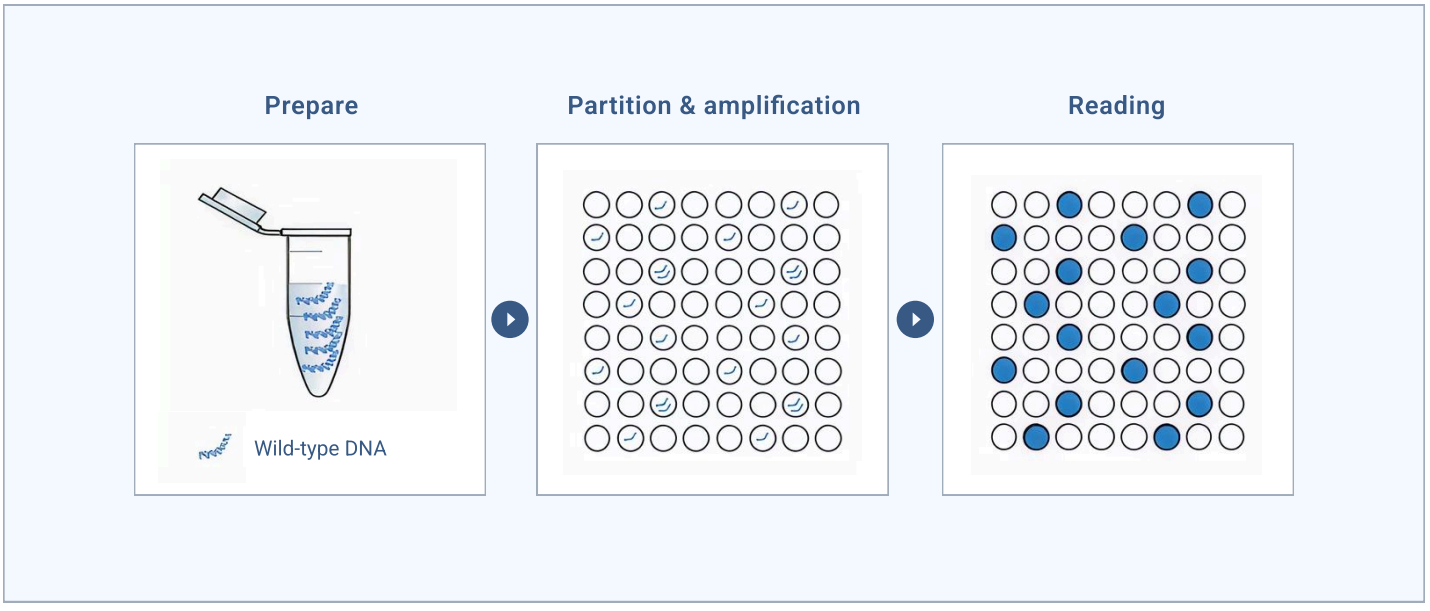
1. Accurate concentration estimation

The Poisson distribution is a discrete distribution for the probability of a given number of events occurring within a fixed interval, usually a time interval, but it can also be a 'space' interval, i.e. a fixed volume. In the case of Crystal Digital PCR®, the aqueous reaction mix is emulsified in an oil phase. The event occurring is the encapsulation of a target molecule in a droplet of reaction mix, and the interval (or fixed volume) is the droplet volume. For this distribution to accurately describe what happens in dPCR, two assumptions are required: that all the droplets are of equal volume and that target molecules are randomly distributed across droplets (i.e. that an encapsulation event is not influenced by the previous encapsulation events).

In Sapphire Chip and Ruby Chip, partitioning occurs without oil flow and the droplet size is determined by the physical dimensions of the microchannels in which the PCR mix flows. This clever design ensures that the abovementioned assumptions are met.

Once the target sequences are randomly distributed within the droplet crystal, PCR occurs independently in each drop containing at least one of them and leads to a fluorescent (i.e. positive) droplet. Ensuring that the abovementioned assumptions are met allows to **accurately estimate the concentration** of the target by counting the number of positive droplets out of the total number of droplets **(Fig. 2)**.

Figure 2: Once assembled, the PCR mix is loaded in the chip where it is partitioned in thousands of droplets. The target sequences are randomly distributed in the droplets, leading to a positive fluorescent signal after PCR amplification.



Going deeper with the mathematical model:

Let X be the number of target molecules encapsulated in a droplet. According to the Poisson distribution, the probability, $P(X=j)$, for a droplet to encapsulate j target molecules is given by Eq. 1.

Eq. 1

$$P(X = j) = \lambda^j * \frac{e^{-\lambda}}{j!}$$

where λ is the average number of target molecules per droplet.

The readout for a droplet in dPCR is either positive or negative, there is no way of counting how many target molecules were encapsulated in a given droplet. In negative droplets on the other hand, the number of encapsulated target molecule(s) is known and equal to 0. The probability, $P(neg)$, for a droplet to be negative is given by Eq. 2.

Eq. 2

$$P(neg) = P(X = 0) = e^{-\lambda}$$

Conversely, the probability, p, for a droplet to be positive is given by Eq. 3.

Eq. 3

$$p = 1 - P(neg) = 1 - e^{-\lambda}$$

In a Crystal Digital PCR® chamber, one can observe a drawing of N droplets where each partition has a probability p of being positive. This is a binomial process with probability p. An estimator of p, $\hat{p}$, is Eq. 4.

Eq. 4

$$\hat{p} = \frac{k}{N}$$

with k number of positive droplets observed. This estimator is unbiased and follows a normal distribution when N is large.

From this observation we can estimate the average number of target molecules per droplet, $\hat{\lambda}$, using Eq. 5.

Eq. 5

$$\hat{\lambda} = -\ln(1 - \hat{p}) = -\ln\left(1 - \frac{k}{N}\right)$$

If c is the target molecule concentration per volume unit (e.g. in cp/µL), λ is simply $c * v$, where v is the droplet volume. The target molecule concentration can thus be estimated with Eq. 6.

Eq. 6

$$\hat{c} = -\frac{1}{v} * \ln(1 - \hat{p}) = -\frac{1}{v} * \ln\left(1 - \frac{k}{N}\right)$$

2. Measurement uncertainty

It is important to understand that for a given target molecule concentration, each Crystal Digital PCR chamber is a random drawing of a given number of positive droplets among the total number of droplets, from a binomial distribution. Reciprocally, that means that when a drawing of this number of positive partitions is observed in a chamber, this particular drawing can stem from a range of target molecule concentrations. This range is reflected in the confidence interval at a given confidence level, typically 95%, provided together with the concentration estimation. It is often denoted as the Poisson confidence interval. The result of a digital PCR experiment should be read as ***"if I test the exact same sample a great number of times, 95 times out of a hundred, the true value will be within this Poisson confidence interval"***.

It is very important to note that this interval does not include experimental sources of variance among reactions, such as pipetting errors.

In the rest of this note, we define an uncertainty coefficient, the relative uncertainty ***(CI95%)***, as the relative half width of the 95% confidence interval, and use it as an indication of the intrinsic precision of dPCR as a measurement technic. The ***CI95%*** thus reflects the best precision (with 95% confidence) that can be achieved with dPCR.

To go deeper with the mathematical model:

To estimate how far the observed $\hat{c}$ might be from the true c, a confidence interval can be built. Since $\hat{p}$ follow a normal distribution when N is large, the 95% confidence interval, $[\hat{p}_{min} ; \hat{p}_{max}]$, for $\hat{p}$ is given by Eq. 7.

Eq. 7

$$\hat{p}_{min} = \hat{p} - 1.96\sqrt{\frac{\hat{p}(1-\hat{p})}{N}}$$

$$\hat{p}_{max} = \hat{p} + 1.96\sqrt{\frac{\hat{p}(1-\hat{p})}{N}}$$

From which the confidence interval on $\hat{c}$, $[\hat{c}_{min} ; c_{max}]$, is easily derived as Eq. 8.

Eq. 8

$$\hat{c}_{min} = -\frac{1}{v} * \ln(1-\hat{p}_{min})$$

$$c_{max} = -\frac{1}{v} * \ln(1-\hat{p}_{max})$$

The relative uncertainty ***(CI95%)*** is defined as the relative half width of the 95% confidence interval (Eq. 9).

Eq. 9

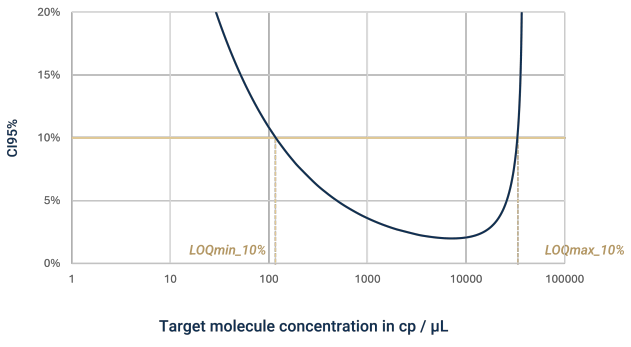
$$CI95\% = \frac{\ln(1-\hat{p}_{max}) - \ln(1-\hat{p}_{min})}{2 * \ln(1-\hat{p})}$$

3. U-curves

The relative uncertainty, ***(CI95%)***, associated with Crystal Digital PCR® measurement depends on the droplet volume, number of droplets, and target molecule concentration. For a given droplet volume and a given number of droplets per chamber, plotting the ***(CI95%)*** as a function of target molecule concentration yields a U-shaped curve that we denote as the U-curve, U standing for uncertainty **(Figure 3)**.

The U-curve can be used to find the target molecule concentration range for which the uncertainty of the dPCR measurement remains below a certain level, *x%* (e.g. 10%). The bounds of this range are the limits of quantification LOQ_{min} *x%*, the smallest concentration for which the is below *x%*, and LOQ_{max} *x%*, the highest concentration for which the ***(CI95%)*** is below *x%*.

Figure 3: Example of a U-curve for a Ruby Chip chamber containing 15,000 droplets of 0.22 nL on next page →



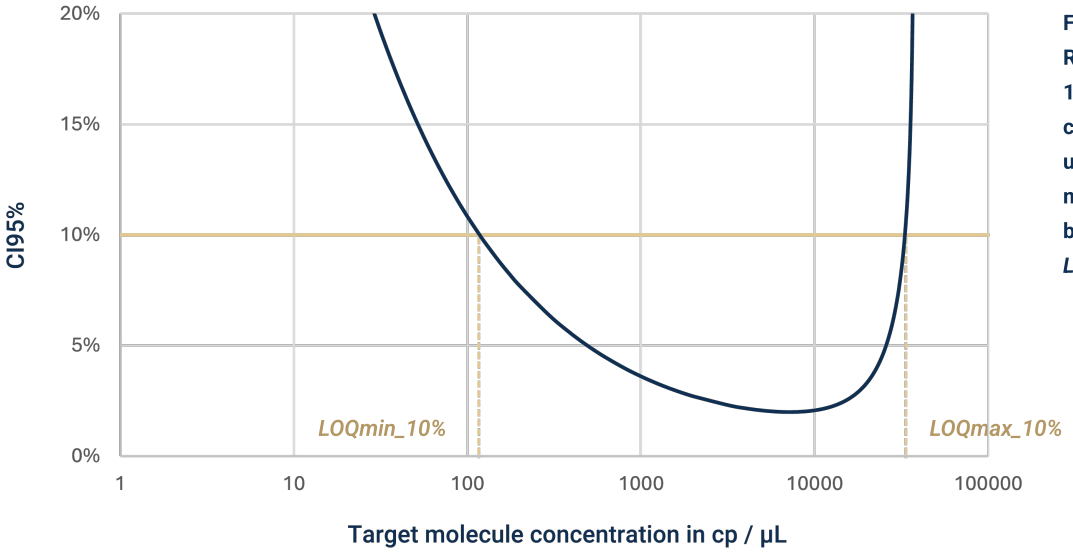


Figure 3: Example of a U-curve for a Ruby Chip chamber containing 15,000 droplets of 0.22 nL. In this case, the range for which the uncertainty of the dPCR measurement remains below 10% is between $LOQ_{min}10\%$ = 116 cp/µL and $LOQ_{max}10\%$ = 33,200 cp/µL.

4. Limit of Detection (LOD) min and max.

The theoretical minimum detection limit, $LOD_{min}95\%$, is defined as the smallest concentration for which the probability of detecting at least one positive partition is equal to or above 95%.

The theoretical maximum detection limit, $LOD_{max}95\%$, is defined as the highest concentration for which the probability of detecting at least one negative partition is equal to or above 95%.

To go deeper with mathematical model:

The formula for the $LOD_{min}95\%$ (Eq. 10) shows that it is inversely proportional to the analyzed volume.

with V the analyzed volume $(V = N * v)$.

The formula for $LOD_{max}95\%$ is given by Eq. 11.

Eq. 10

$$LOD_{min}95\% = \frac{3}{V}$$

Eq. 11

$$LOD_{max}95\% = -\frac{1}{v} * \ln(1 - 0.05^{1/N})$$

Analytical performances

1. Sapphire Chip VS Ruby Chip – theoretical comparison based on microfluidic characteristics

Fig. 4 shows the U-curves for a Sapphire Chip chamber (orange) and a Ruby Chip chamber (green).

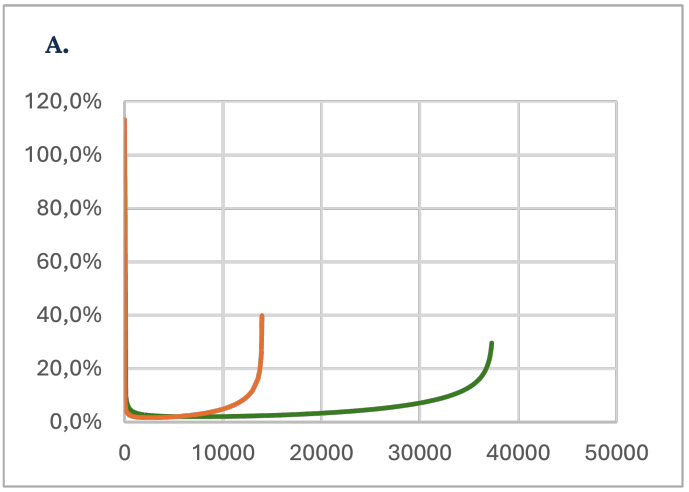
The Ruby Chip dynamic range extends further than the Sapphire Chip to higher concentrations **(Fig 4A)**, with an $LOD_{max}95\%$ above 38000 cp/µL. At about 0.22 cp/µL the Sapphire $LOD_{min}95\%$ is lower than the one of the Ruby chip, at 0.91 cp/µL. Overall the dynamic ranges are identical in amplitude, with uncertainty levels a bit higher with the Ruby chip for low concentrations, and a bit higher for the Sapphire chip on the high concentrations **(Fig 4B and 4C)**. The concentration range for which the *CI95%* remains below 25% is 4.5 to 13870 cp/µL (3.5 logs) with the Sapphire Chip, and 18.6 to 37140 cp/µL (3.3 logs) with the Ruby Chip.

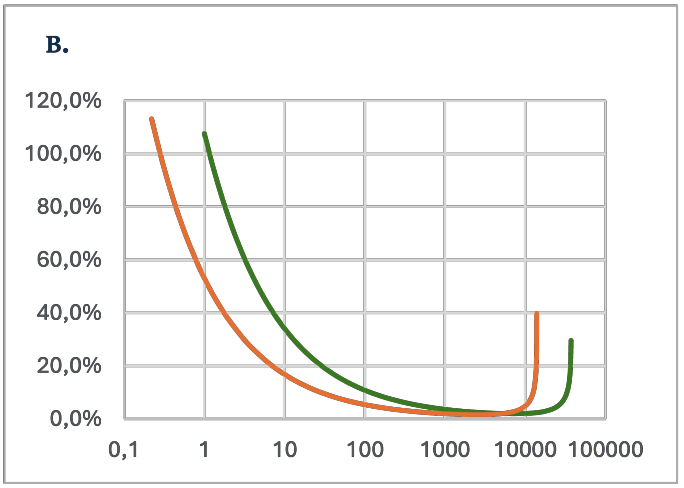
2. Sapphire Chip VS Ruby Chip – experimental comparison

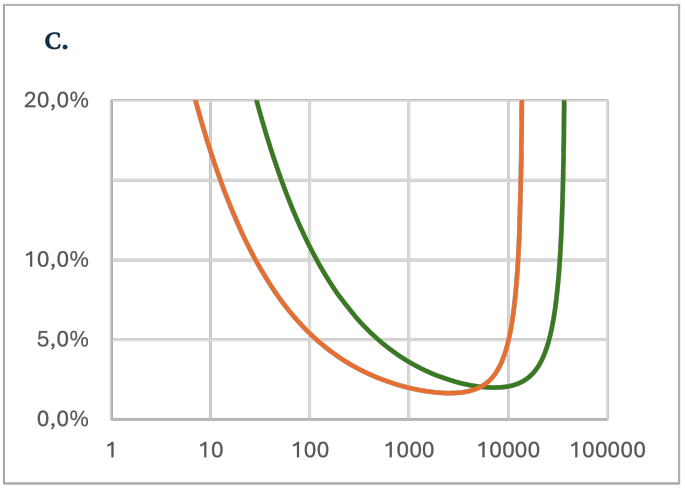
The precision obtained on Sapphire Chip was measured and reported in a previous technical note (https://www.stillatechnologies.com/digital-pcr/naica-system-analysis/1300/). Briefly, target concentrations of 200, 2000, and 10000 cp/µL for each of three different targets were measured with a triplex assay, using 12 replicates per concentration. For each concentration targeted, one single reaction mix was loaded in the 12 replicate chambers.

The coefficients of variation *(CV)* were measured for each target at each concentration level. These correspond to the entire system (Sapphire chip, Geode, Prism3) total measurement variability, but do not encompass variability from pipetting the sample several times as the exact same reaction mix (already containing the sample) was distributed in the replicate chambers.

Figure 4: U-curve of a Sapphire Chip chamber (orange) and a Ruby Chamber (green) plotted with (A) a linear concentration scale focusing on the high end of the dynamic range, (B) a logarithmic concentration scale focusing on the low end of the dynamic range and (C) zooming on the points for which CI95% remains below 20%.







Sapphire Chip chamber

Ruby Chip chamber

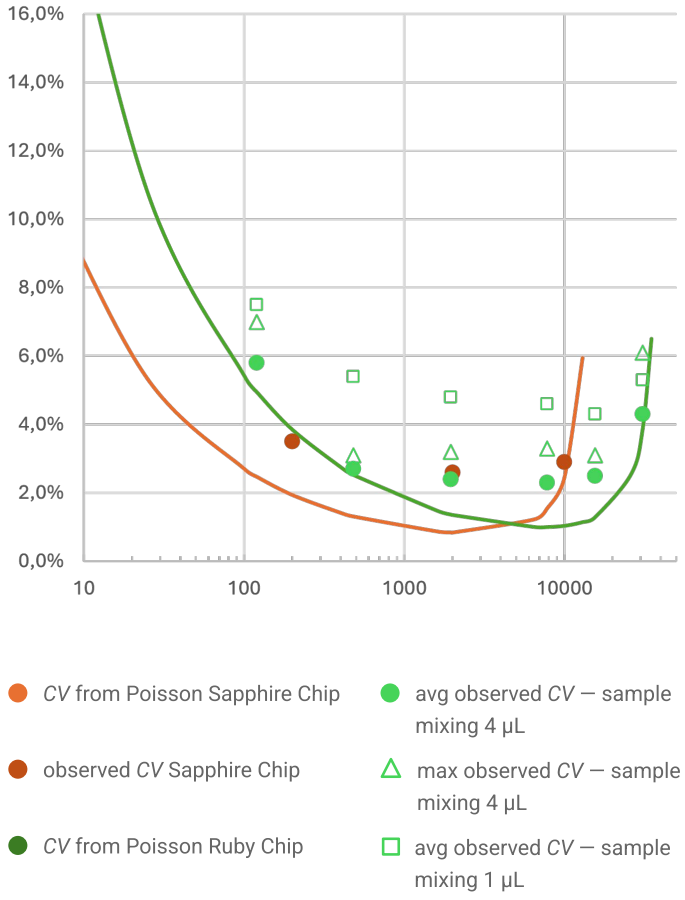
The *CV* values observed are on average twice as large as the *CV* values predicted by the Poisson confidence interval (for a normally distributed random variable, the *CI95%* is equal to twice the *CV*, hence, assuming the quantification estimation is normally distributed, we obtain: ***Poisson predicted CV = CI95%/2***) **(Fig 5).**

The precision obtained on Ruby Chip was measured and reported in a previous technical note (https://www.stillatechnologies.com/digital-pcr/naica-application-notes/automation-of-the-sample-preparation-workflow-from-mix-assembly-to-ruby-chip-loading-with-nio/). Briefly, a reaction premix (naica® multiplex PCR MIX, primers and probes, water) was prepared with the naica® IQ/OQ Kit (ref R30001) and dispensed in 42 wells of a 96-well plate (3.5 µL of premix per well). A sample containing 6 targets each at a different concentration from 120 to 30000 cp/µL was prepared and 4 µL of sample were dispensed into each of the 42 wells. The reaction mixes (42 replicates) were then loaded in Ruby Chip. Reaction premix preparation, dispensing, sample dispensing, and chip loading were carried out automatically with an Opentrons OT-2 liquid handling robot.

The *CV* were measured for each target. These correspond to the entire system (Ruby Chip, Nio® Digital PCR) total measurement variability, and do encompass variability from pipetting the sample independently in each replicate well, contrary to the Sapphire Chip experiment described above. To compare with the Sapphire Chip data, *CV* values were computed on groups of 12 replicates (31 groups made, by taking 12 contiguous chambers as they appeared in the results table of the analysis software) and not all 42 replicates. The worst *CV* (CV_{max}) out of the 31, and the average of the 31 *CV* (CV_{avg}) were analyzed. The observed *CV* values, both CV_{max} and CV_{avg}, are closer to the Poisson predicted CV for Ruby Chip than for Sapphire Chip **(Fig 5)**.

For high concentrations, the observed precision is better on Ruby Chip with CV_{avg} values at 2.3% and 2.5% for target concentrations of 7800 and 15500 cp/µL respectively, while Sapphire Chip's *CV* is at 2.9% for 10000 cp/µL. Note that on Sapphire Chip, the observed *CV* will soar for concentrations going above 10000 cp/µL up until the LOD_{max} *95%* slightly above 14000 cp/µL, while with the Ruby Chip, the observed CV_{avg} remains at 4.3% even at 30800 cp/µL **(Fig 5).**

Figure 5: Poisson predicted *CV* for Sapphire Chip (orange line) and for Ruby Chip (green line) compared to observed CV for Sapphire Chip (orange) and Ruby chip (green).
The observed CV_avg with 4 µL of sample input on Ruby is comparable to the observed CV on Sapphire. The observed CV_avg with 1 µL of sample input on Ruby is higher than the one with 4 µL of sample input, highlighting the importance of other experimental factors in the overall precision. Curves for Poisson predicted CV were computed using 22,000 droplets of 0.62 nL for Sapphire Chip chambers and 15,000 droplets of 0.22 nL for Ruby Chip chambers.



For low concentrations, on Ruby Chip the CV_{avg} for 120 and 480 cp/µL are 5.8% and 2.7% respectively, i.e. only about 20% and 10% higher than the Poisson predicted *CV* values of 4.9% and 2.5%. The Sapphire Chip *CV* for 200 cp/µL is at 3.5% **(Fig 5)**. Extrapolating the Ruby Chip data to get a comparison at 200 cp/µL, with a Poisson predicted *CV* of 3.8%, the worst estimate would be an observed *CV* 20% higher than that, i.e. 4.6%.

The same Ruby Chip experiment was repeated but using only 1 µL of sample per well instead of 4 µL. For all target concentrations tested, the CV_{avg} for this experiment were higher than when 4 µL of sample were added per reaction **(Fig 5)**. In this case this difference is most likely explained by the decreased pipetting precision of the OT-2 robot for 1 µL compared to 4 µL (manufacturer specifications: 10% *CV* for 1 µL pipetted, 1.5% *CV* for 20 µL pipetted). This result highlights the importance of considering all potential sources of uncertainty when designing a test plan and when reporting results.

On the concentration range tested, with 4 µL of sample input per chamber, the Ruby Chip provides a better precision than the Sapphire Chip for high concentrations (above 2000 cp/µL), and a precision that nearly equaled the Sapphire Chip one for concentrations between 120 and 2000 cp/µL.

3. Sapphire Chip VS pools of several Ruby Chip chambers – theoretical comparison

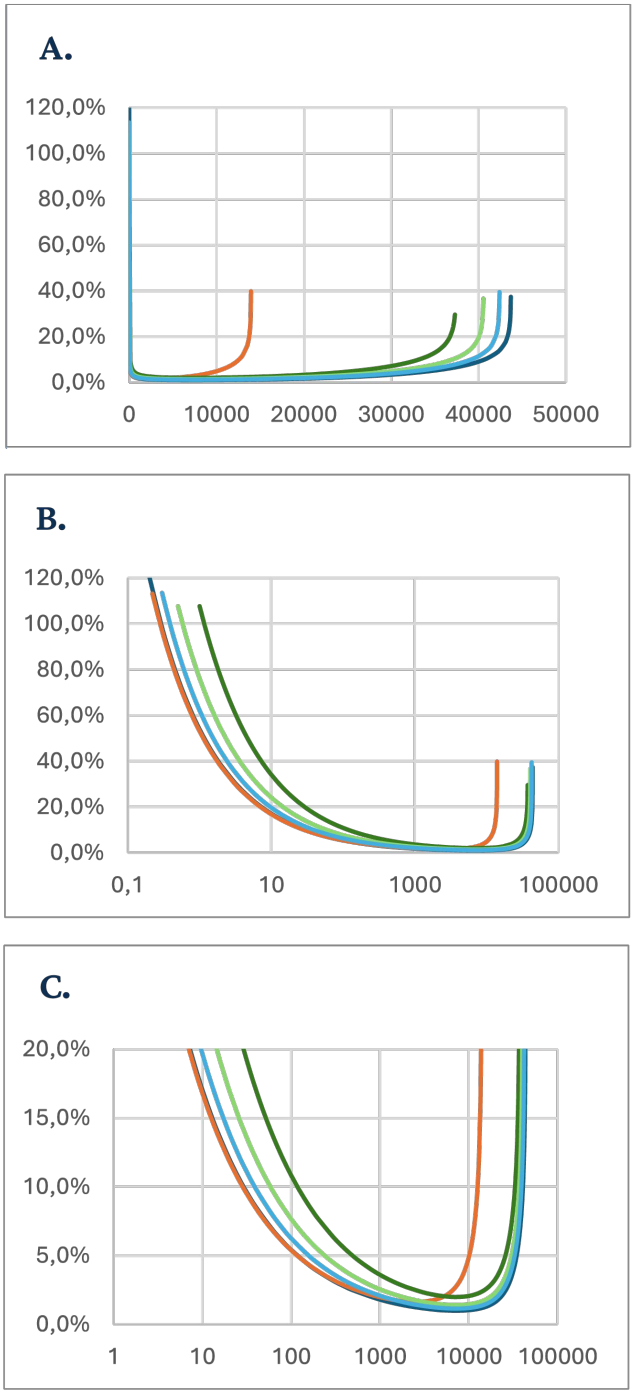
For applications requiring the highest precision on very low concentrations, and/or requiring the lowest *LOD* possible, the pooling feature allows to extend the Ruby Chip dynamic range and lower its uncertainty on the low concentration end of the U-curve **(Fig 6)**.

Over most of the dynamic range, the *CI95%* is decreased by a factor of around 1.4 (√2) when pooling two chambers compared to using only one chamber. This factor is around 1.73 (√3) for three chambers pooled, and around 2 (√4) for four chambers pooled. This rule does not hold as well close to the LOD_{max} *95%*.

As chambers are pooled, the LOD_{max} *95%* slightly increases, from around 38500 cp/µL with one chamber to 44800 cp/µL with four chambers.

Similarly, as chambers are pooled, the LOD_{min} *95%* decreases. The analyzed volume of n pooled chambers is simply n times the analyzed volume of one chamber, thus the LOD_{max} *95%* for n chambers is the LOD_{max} *95%* for one chamber divided by n. The exact values for n. from 1 to 4 are given in **Table 1**. For low concentrations, pooling chambers allows to decrease the Poisson predicted uncertainty **(Fig 6 B and 6 C)**.

Figure 6: U-curve of a Sapphire Chip chamber (orange) one Ruby Chip chamber (dark green), two Ruby Chip chambers pooled (light green), three Ruby Chip chambers pooled (light blue), and four Ruby Chip chambers pooled (dark blue), plotted with (A) a linear concentration scale focusing on the high end of the dynamic range, (B) a logarithmic concentration scale focusing on the low end of the dynamic range and (C) zooming on the points for which CI95% remains below 20%.



The extension of the dynamic range from LOD_{min} *95%* and LOD_{max} *95%* **(Table 1)**, and the general diminution of uncertainty brought by pooling chambers is reflected in the various dynamic ranges of interest, such as the range for *LOQ 10%* **(Table 2)** and the range for *LOQ 25%* **(Table 3)**. Of note, the ranges for one Ruby Chip chamber are slightly shorter than the range with one Sapphire Chip, but from two pooled chambers, the dynamic ranges with several Ruby Chip chambers exceed the dynamic ranges with one Sapphire Chip chamber.

It's important to note that the values presented in **Tables 1 to 3** correspond to quantifications within the chamber. To obtain the corresponding concentrations in the sample working solutions, these values must be multiplied by the dilution factor associated with the sample input volume relative to the total reaction volume.

Since the Ruby Chip chemistry differs from that of the Sapphire Chip, it may be more resilient to inhibitors compared to Sapphire Chip. Therefore, increasing the sample input relative to the total reaction volume on the Ruby Chip can help reduce the dilution factor and further improve the *LOD* and *LOQ* values in the sample.

For example, using a 5 µL sample input in the 25 µL Sapphire Chip reaction mix resulted in an LOD_{min} *95%* of 0.22 cp/µL in the chamber, which corresponds to 1.1 cp/µL in the sample solution. Testing the same sample with a 2.5 µL input in the 5 µL Ruby Chip reaction volume led to an LOD_{min} *95%* of 0.91 cp/µL in the chamber, or 1.82 cp/µL in the sample solution. Pooling two Ruby Chip chambers further improved the LOD_{min} *95%* to 0.45 cp/µL in the chamber, equivalent to 0.90 cp/µL in the sample solution.

Table 1: *LOD 95%* dynamic range

	Min, cp/µL	Max, cp/µL	Range, log10
Ruby - 1 chb	0.91	38564	4.6
Ruby - 2 chb	0.45	41701	5.0
Ruby - 3 chb	0.30	43537	5.2
Ruby - 4 chb	0.23	44839	5.3
Sapphire - 1 chb	0.22	14358	4.8

Table 2: *LOQ 10%* dynamic range

	Min, cp/µL	Max, cp/µL	Range, log10
Ruby - 1 chb	115.94	33200	2.5
Ruby - 2 chb	57.97	37000	2.8
Ruby - 3 chb	38.65	39200	3.0
Ruby - 4 chb	28.98	40700	3.1
Sapphire - 1 chb	28.16	12580	2.6

Table 3: *LOQ 25%* dynamic range

	Min, cp/µL	Max, cp/µL	Range, log10
Ruby - 1 chb	18.55	37140	3.3
Ruby - 2 chb	9.28	40370	3.6
Ruby - 3 chb	6.18	42250	3.8
Ruby - 4 chb	4.64	43570	4.0
Sapphire - 1 chb	4.51	13870	3.5

Table 4: Reaction and analyzed volumes

	Reaction volume, µL	Analyzed volume, µL
Ruby - 1 chb	5	3.3
Ruby - 2 chb	10	6.6
Ruby - 3 chb	15	9.9
Ruby - 4 chb	20	13.3
Sapphire - 1 chb	25	13.6

Using a two-chamber pooling strategy on the Ruby Chip still provides higher throughput—8 samples per chip—compared to 4 samples per Sapphire Chip. In addition, the total reaction volume required is significantly lower: just 10 µL for two Ruby Chip chambers and 20 µL for four, versus 25 µL for a single Sapphire Chip chamber. This means that similar performance can be achieved while using a smaller volume of precious sample material **(see Table 4)**.

4. Sapphire Chip VS pools of several Ruby Chip chambers – experimental comparison

The previous section on U-curves and dynamic ranges are based only on microfluidic characteristics, i.e. they do not consider sources of uncertainty other than the Poisson uncertainty, and they do not consider biological noise coming from the assay and the samples (i.e. the limit of blank, *LOB*) used that lead to experimental *LOD* values higher than those predicted theoretically.

To assess how close the experiment is to the theory outlined above, LOD_{min} *95%* values obtained with the *EGFR* 6-color Crystal Digital PCR® Kit (ref R30006) were measured on Sapphire Chip and on Ruby Chip either without or with pooling 3 or 4 chambers.

Limit of Detection *(LOD)* was determined according to a method adapted from the CLSI EP17-A2 recommendations, as described in **the application note *How to characterize the Limit of Blank and the Limit of Detection in Crystal Digital PCR™***. (https://www.stillatechnologies.com/digital-pcr/naica-system-analysis/how-to-characterize-the-limit-of-blank-and-the-limit-of-detection-in-crystal-digital-pcr/).

34 Sapphire Chip chambers and 118 Ruby Chip chambers were processed for the *LOD* experiment. For the Ruby Chip, this allowed obtaining 38 pools of 3 chambers (containing the same sample) or 28 pools of 4 chambers.

Table 5: LOD_{min} *95%* for the *EGFR6-color Crystal Digital PCR® Kit (ref R30006), all values are in cp/µL.*

Chip ↓ Target →	N chambers	DEL19+	INS20+	L858R	L861Q, GG719X	T790M	C797S
Ruby - 1-chamber	118	1.27	1.55	0.97	1.63	1.64	1.29
Ruby - 3-chamber	38	0.75	0.91	0.65	1.05	0.92	0.69
Ruby - 4-chamber pooling	28	0.71	0.92	0.54	0.87	0.81	0.62
Sapphire chamber	34	0.51	0.77	0.52	0.86	0.65	0.34

This experience confirms that pooling chambers lowers the LOD_{min} *95%* values. With 3-chambers pooling, values are improved on all counts and values close to the Sapphire chip are reached for the L858R and INS20+ targets. With 4-chambers pooling, the LOD_{min} *95%* falls below 1 cp/µL on all targets, and a value similar to Sapphire Chip is reached for the L861Q,G719X target. **(Table 5)**