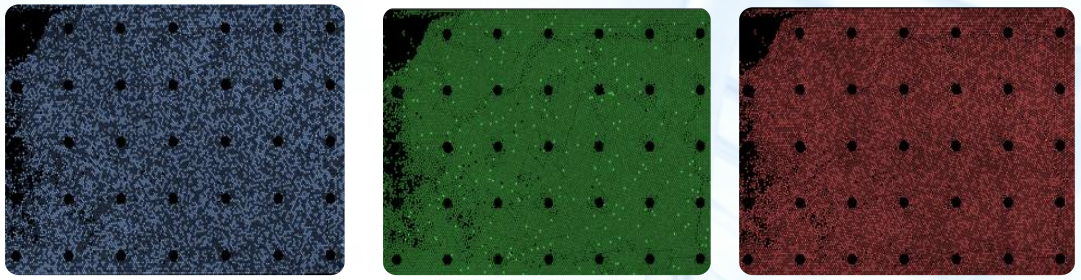
STILLA

Crystal Digital PCR™: a high-plex solution for fast and simultaneous target quantification

Overview

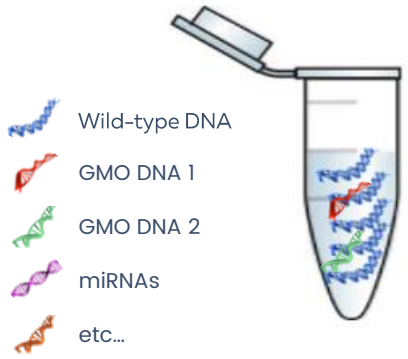
1	3-color Crystal Digital PCR™ and the Naica™ System Workflow
2	Announcing 6-color Crystal Digital PCR™
3	6-color Lung Cancer Panel for *EGFR* mutation quantification
4	6-color Crystal Digital PCR™ Breast and Rectal cancer assays
5	6-color Crystal Digital PCR™ assays for Food Testing applications
6	Conclusions

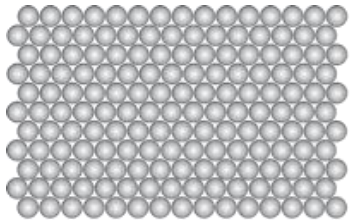
1 3-color Crystal Digital PCR™ and the Naica™ System Workflow



Principles
of Crystal Digital PCR™

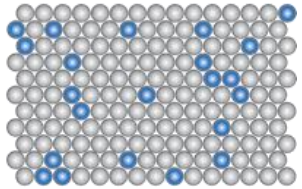
PARTITIONING

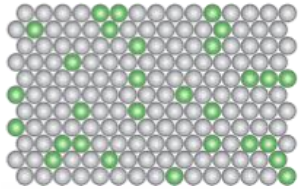


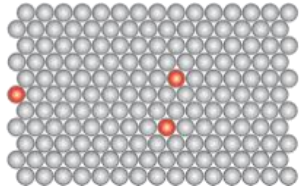


Droplet crystal:
Self-assembled
array of droplets

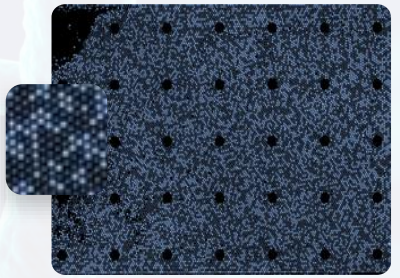
PCR

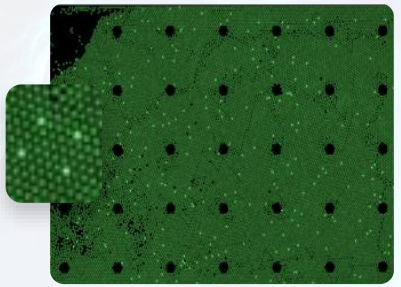


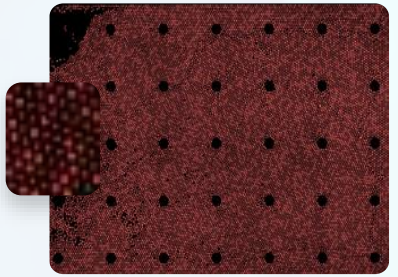




READING & ANALYSIS







Blue

Ex: 415-480 nm
Em: 495-520 nm
FAM...

Green

Ex: 530-550 nm
Em: 560-610 nm
ROX, HEX...

Red

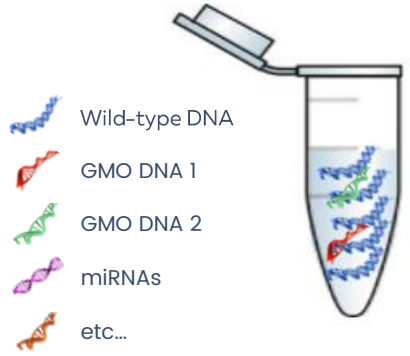
Ex: 615-645 nm
Em: 655-720 nm
Cy®5...

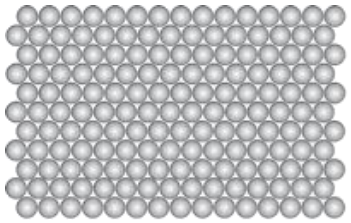
2 Parameters for good quantification in dPCR:

- Number of droplets
- Size of the droplets

Principles
of Crystal Digital PCR™

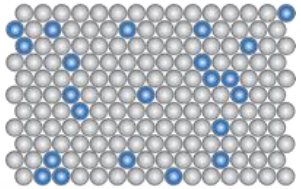
PARTITIONING

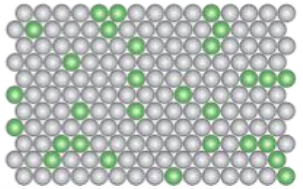


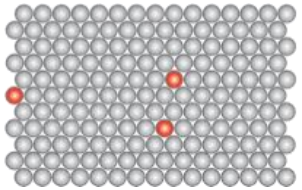


Droplet crystal:
Self-assembled
array of droplets

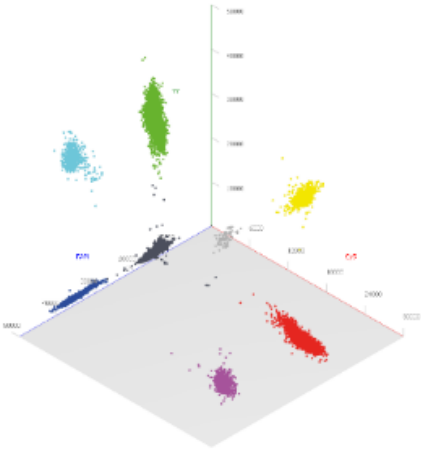
PCR







READING & ANALYSIS

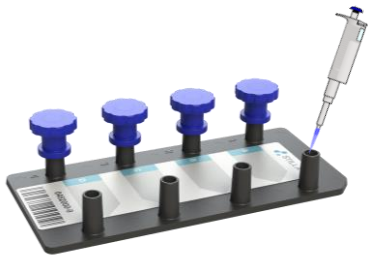
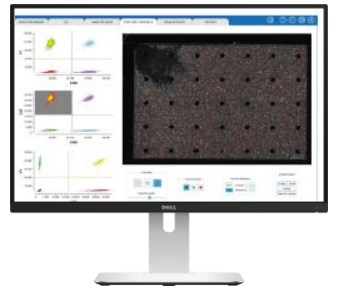


C = 102 cp/µL
C = 152 cp/µL
C = 8 cp/µL

2 Parameters for good quantification in dPCR:

- Number of droplets
- Size of the droplets

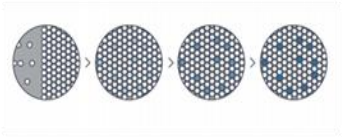
Perform Crystal Digital PCR™ using the Naica™ system in 2h30 with Minimum Hands-on Time

				
DESCRIPTION	Pipette 25 µL of PCR mix into the Sapphire Chips and seal with cap	Place Sapphire Chips into the Naica Geode and launch the combined partitioning and thermocycling program	Read Sapphire Chips with Naica™ Prism3 using three fluorescent detection channels	Analyze results using our intuitive Crystal Miner software
PROCESS TIME 2H30	**5 min**	**2h10 min**	**10 min**	**5 min**
HANDS-ON TIME 5 min	**5 min**	**15 s**	**15 s**	

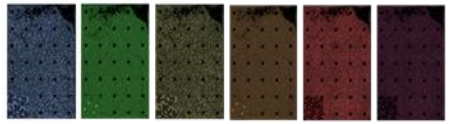
2 Announcing 6-color Crystal Digital PCR™

Workflow

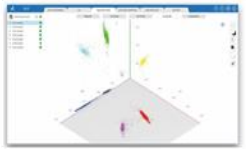
Sapphire Chips



Naica™ Geode



6-Color Reader



Introducing
6-color Crystal Digital PCR™

Examples of compatible fluorophores:

Channel	Fluorophores
1	FAM
2	YY®
3	Atto 550
4	ROX
5	Cy®5
6	Atto 700

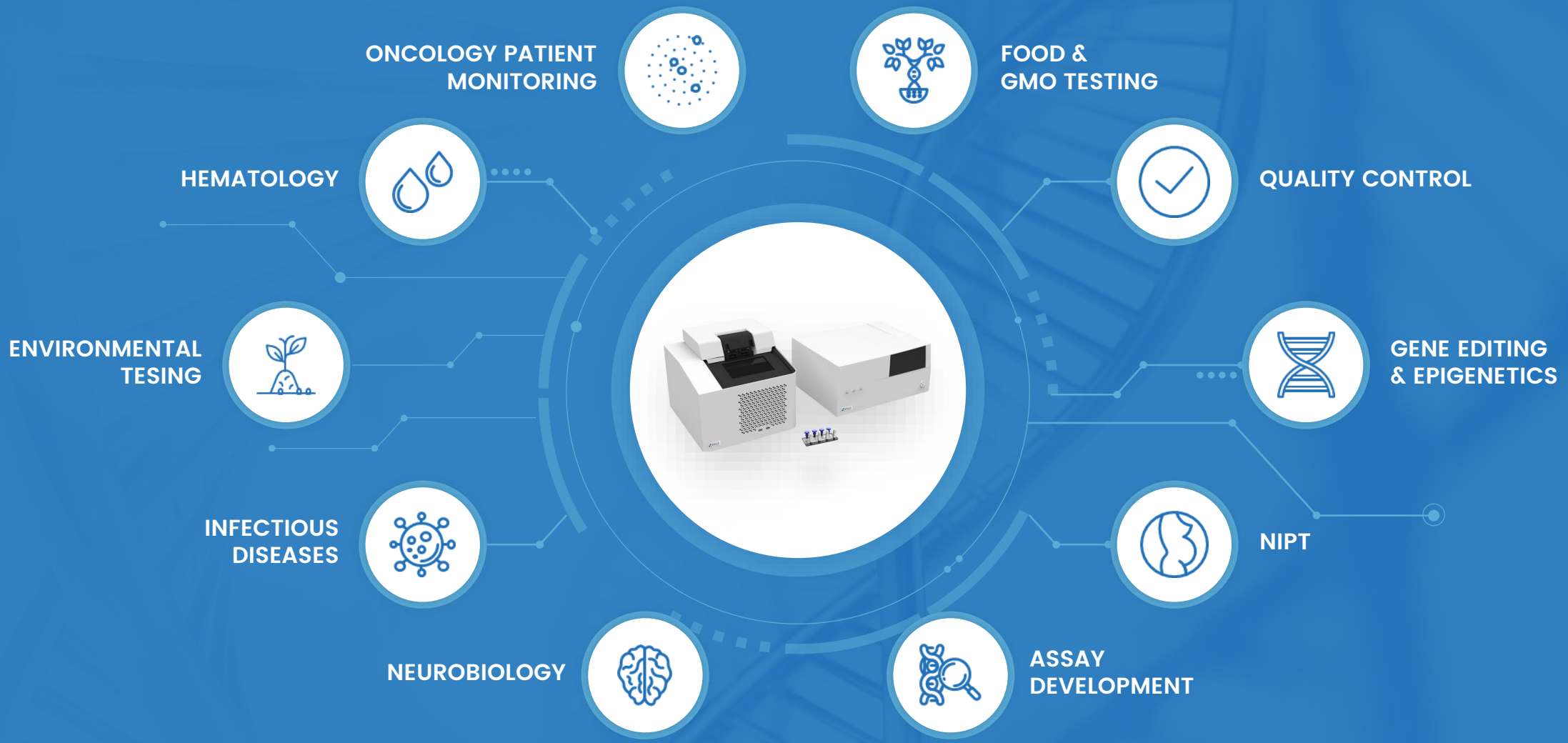
6-Color Reader

Chip Compatibility

- Sapphire chips (36 samples per day per 8h shift)
- Opal chips (144 samples per day per 8h shift)

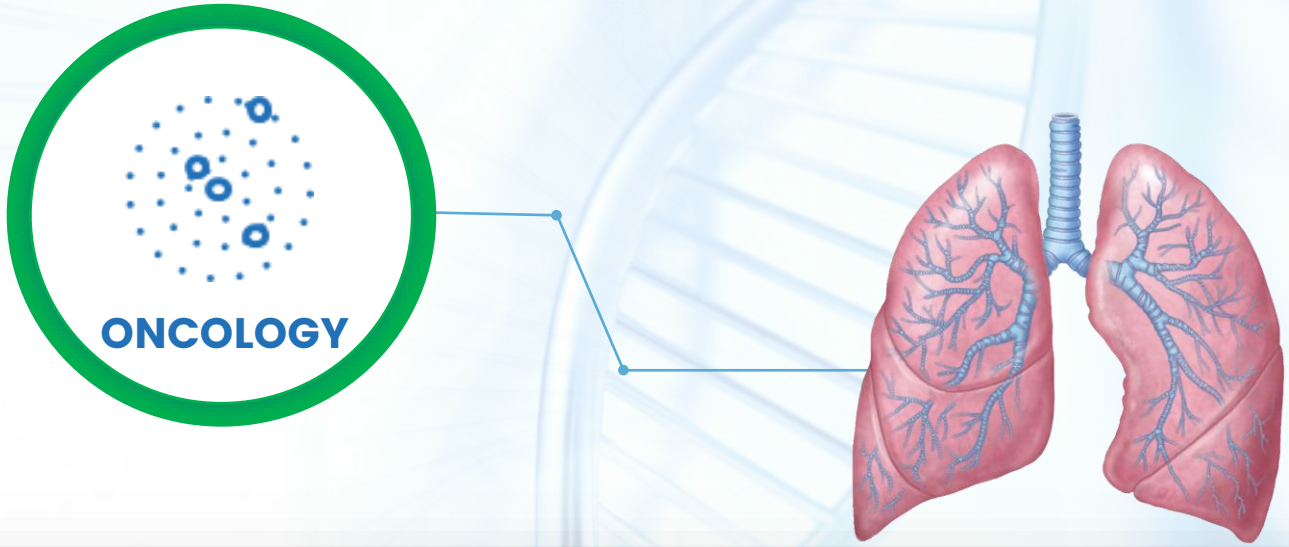
- 3 chips per run
- Time-to-result: ≤ 3 hours, for 6 channels

The Naica™ System Applications Across Life Sciences & Translational Research



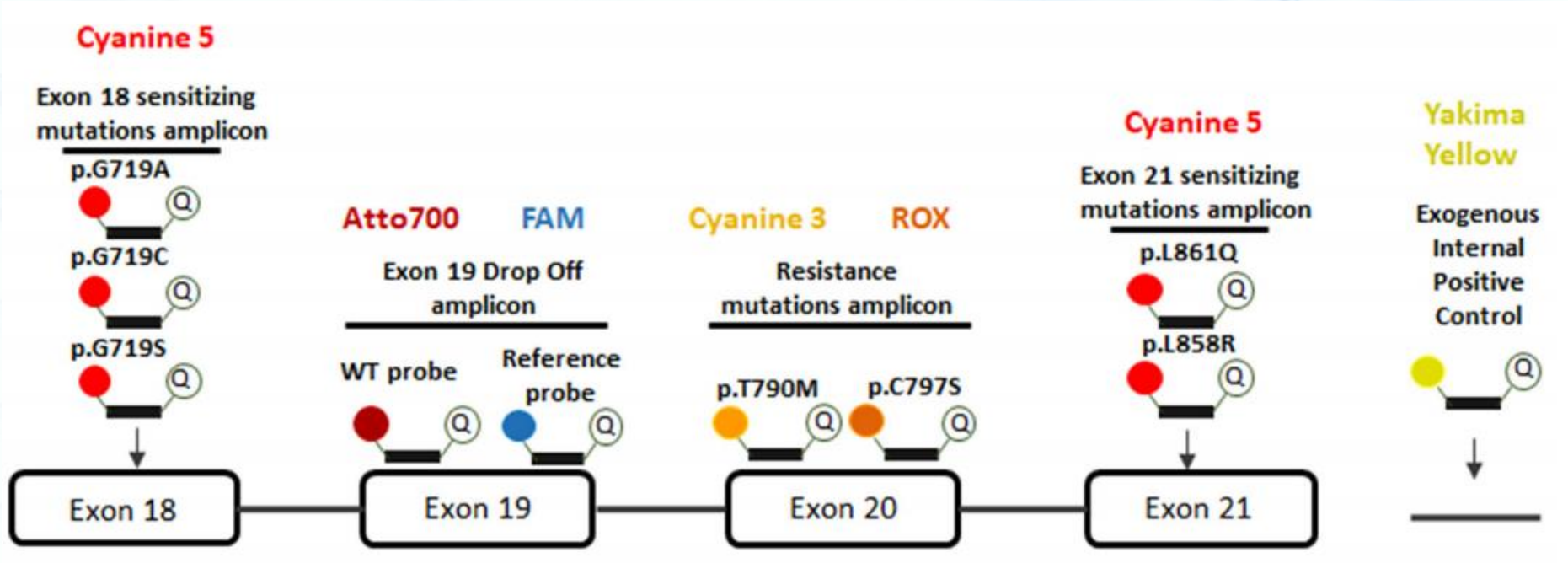
6-color Detection Channels : Proof of concept

3	6-color Lung Cancer Panel for *EGFR* mutation quantification



Workflow

6-color Crystal Digital PCR™ quantifies > 90% of known *EGFR* mutations in a single assay



- **Quantifies 19** of the most prevalent TKI sensitizing and resistance *EGFR* mutations
- **Maximize** the use of your precious sample
- **Minimize** time to results

Madic et Jovelet et al., 2018 Oncotarget

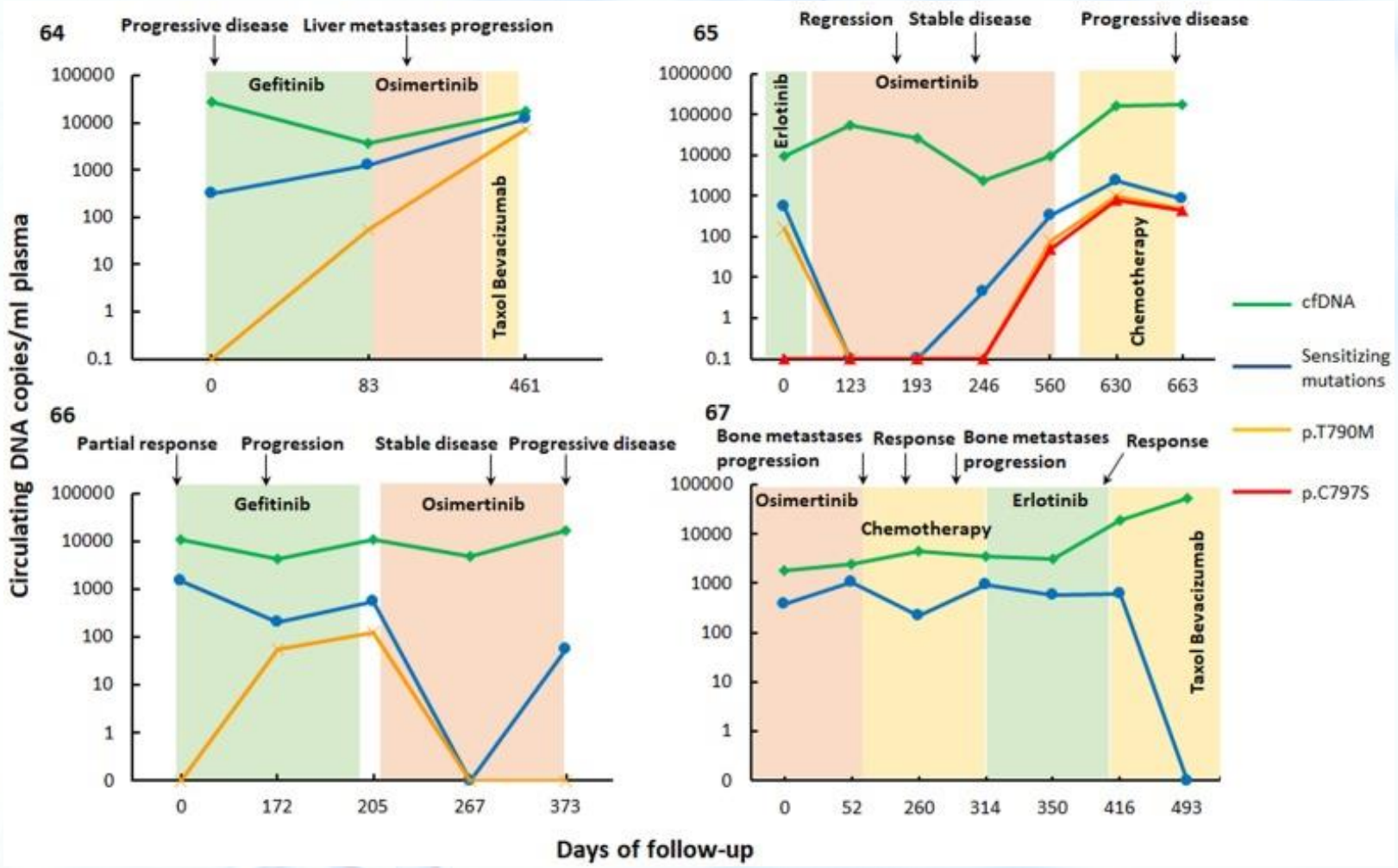
6-color Detection of the most prevalent sensitizing and resistance *EGFR* mutations in NSCLC

33 Tumor samples (21 Frozen, 12 FFPE)

- 24 *EGFR* sensitizing anomalies (73%)
- 13 T790M and 5 C797S resistance mutations (54%)
- 9 WT

49 cfDNA samples

- 35 *EGFR* sensitizing anomalies (71%)
- 14 T790M and 3 C797S resistance mutations (35%)
- 14 WT



Madic et Jovelet et al., 2018 Oncotarget

4 6-color Crystal Digital PCR™ Breast and Rectal cancer assays

6-color Monitoring of Breast & Rectal Cancer Mutations in two Clinical Studies

Motivation

- **4 year** EU-funded LIMA project led by Philips
- **Goal:** Combine liquid biopsy monitoring & MRI scans to predict and monitor cancer therapy response

Challenge

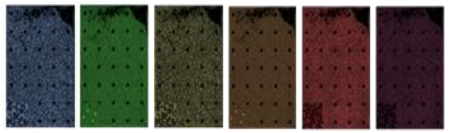
- **100 patients per trial**
  - Breast: 10 samples per patient - *1000 samples*
  - Rectal: 4 samples per patient - *400 samples*
- **Reliable, rapid and cost-effective quantification** of at least 6 targets from a single blood sample

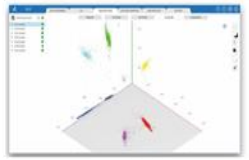
Workflow

Sapphire Chips

Naica™ Geode

6-Color Reader

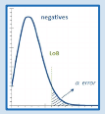
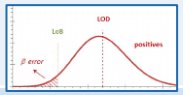
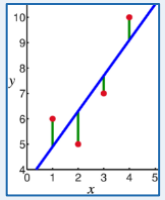




Clinical Trial 6-color Crystal Digital PCR™ Panels

		RECTAL Cancer Panel patient coverage: 10% - 30%	**BREAST Cancer Panel** patient coverage: 25% - 35%
Channel	Fluorophores	Target	Target
1	FAM	***PIK3CA* H1047R**	***ERBB2* (*HER2* amp.)**
2	YY®	***PIK3CA* H1047 WT**	***PIK3CA* H1047 WT**
3	Atto 550	***PIK3CA* E542K**	***MRM1* (Polysomy 17 Ref.)**
4	ROX	***PIK3CA* E545K**	***PIK3CA* Mut (H1047R / E542K / E545K)**
5	Cy®5	***PIK3CA* H1047L**	***TSN* (Amplification Ref.)**
6	Atto 700	**PhiX (Int. Ctrl.)**	**PhiX (Int. Ctrl.)**

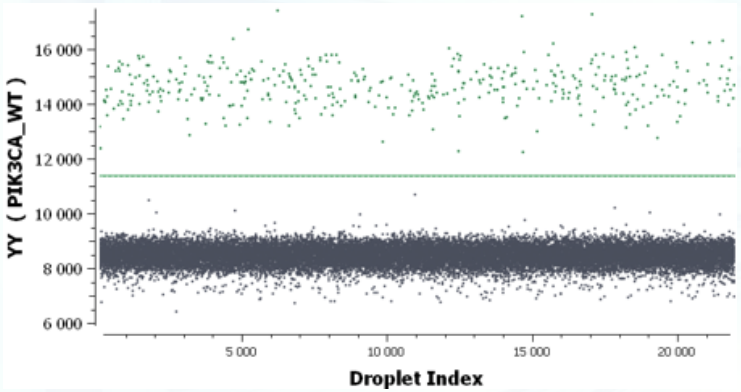
Key Results – Low limit of blank, low limit of detection and high linearity

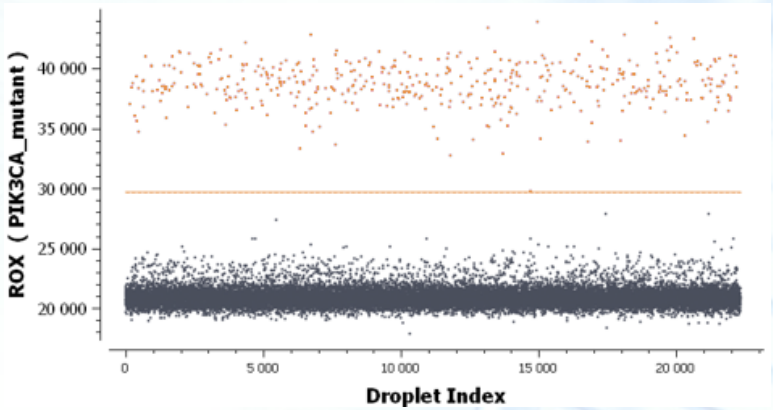
Performance criterion	Nb of observations	RECTAL cancer	BREAST cancer
Limit of Blank LOB 95% (in 1 chamber) 	30	• *PIK3CA* H1047R : 4 droplets • *PIK3CA* H1047L : 3 droplets • *PIK3CA* E542K : 4 droplets • *PIK3CA* E545K : 3 droplets	• *PIK3CA* (H1047R or E542K or E545K) : 6 droplets
Limit of Detection LOD 95% (in 1 chamber) 	Theoretically derived from LOB	• *PIK3CA* H1047R : 0.77 cp / µL • *PIK3CA* H1047L : 0.65 cp / uL • *PIK3CA* E542K : 0.77 cp / uL • *PIK3CA* E545K : 0.65 cp / uL	• *PIK3CA* (H1047R or E542K or E545K) : 0.8 cp / uL
Control Ratio	33	N/A	• *HER2* ratio ([*ERBB2*] / [*TSN*]) : 1.00
Linearity R^2 	6-point dilution range 3 replicates / point	• *PIK3CA* H1047R : R2 = 0.996 (20 - 0.5 cp / uL) • *PIK3CA* H1047L : R2 = 0.993 (20 - 0.5 cp / uL) • *PIK3CA* E542K : R2 = 0.985 (20 - 0.5 cp / uL) • *PIK3CA* E545K : R2 = 0.995 (20 - 0.5 cp / uL)	• *PIK3CA* H1047R : R2 = 0.996 (20 - 0.5 cp / uL) • *PIK3CA* E542K : R2 = 0.986 (20 - 0.5 cp / uL) • *PIK3CA* E545K : R2 = 0.995 (20 - 0.5 cp / uL) • Ratio [*ERBB2*] / [*TSN*] : R2 = 0.922 (ratio 0.9-2.4)

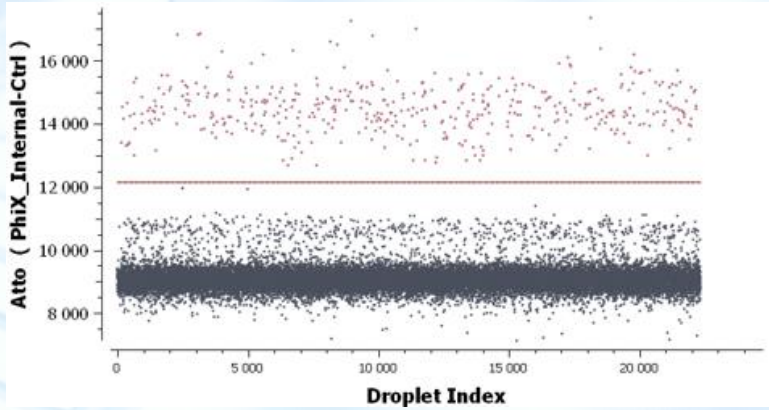
6-color Breast Cancer Panel

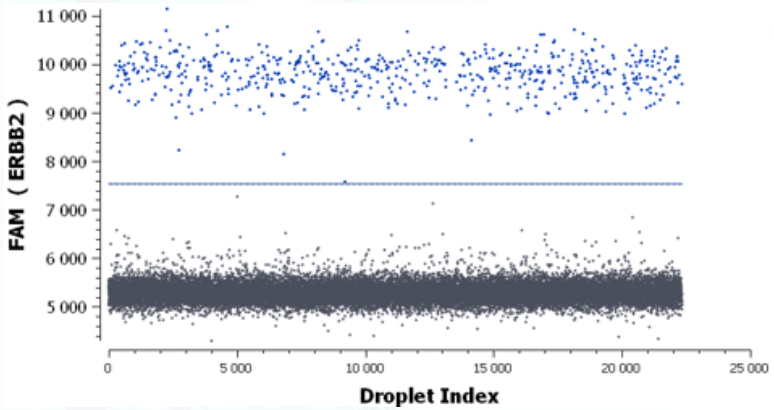
Crystal Miner 1D plots

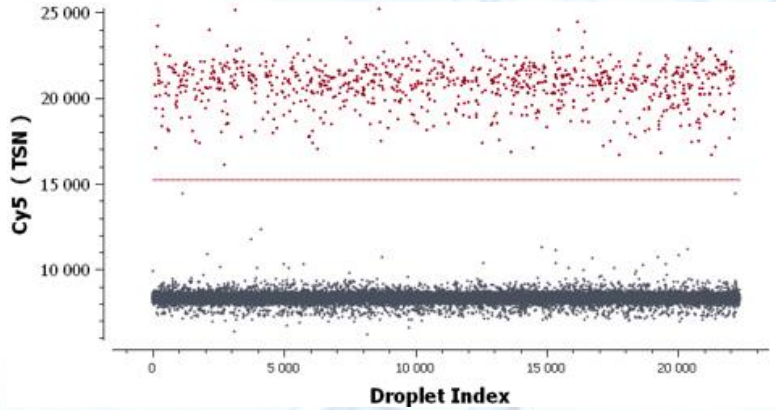
All 6 detection channels showed robust positive and negative cluster separability, crucial for correct and reliable copy number determination

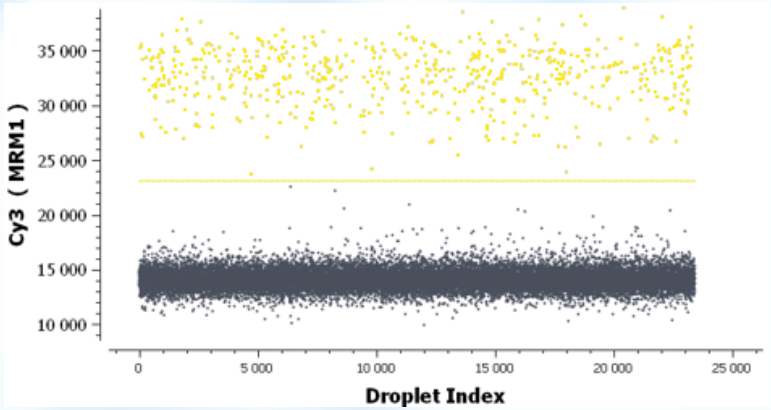






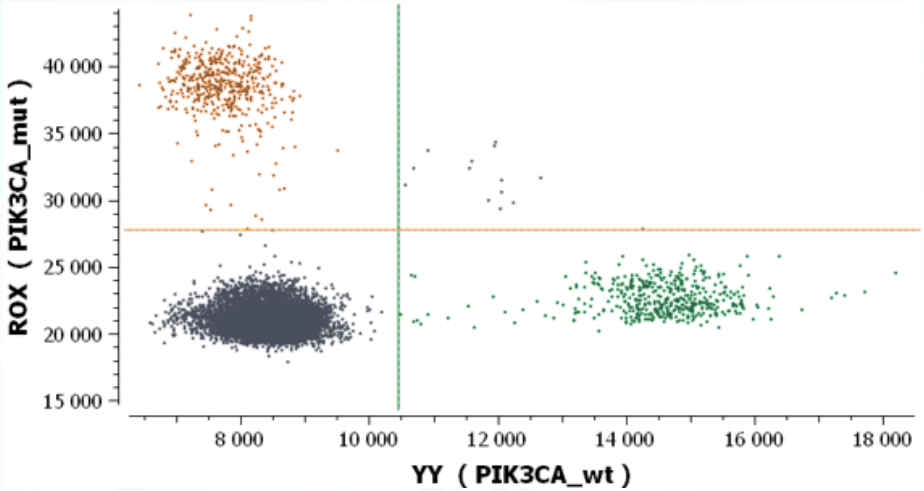


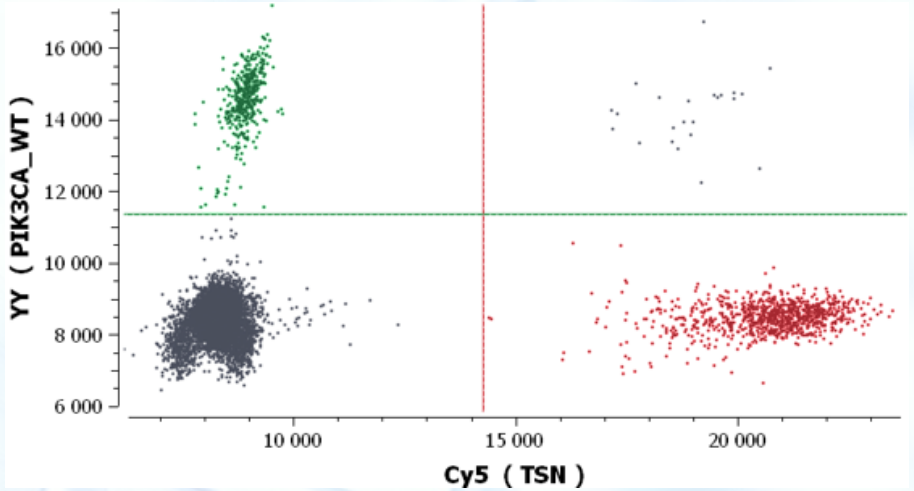


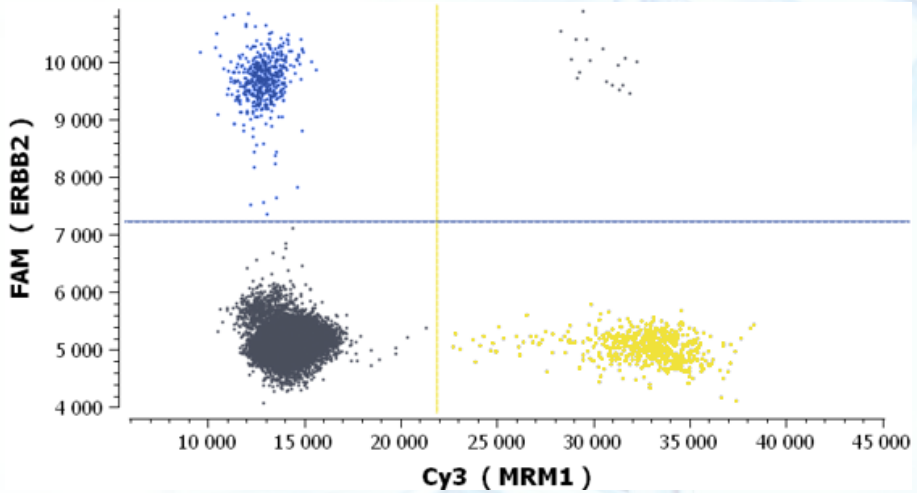


6-color Breast Cancer Panel

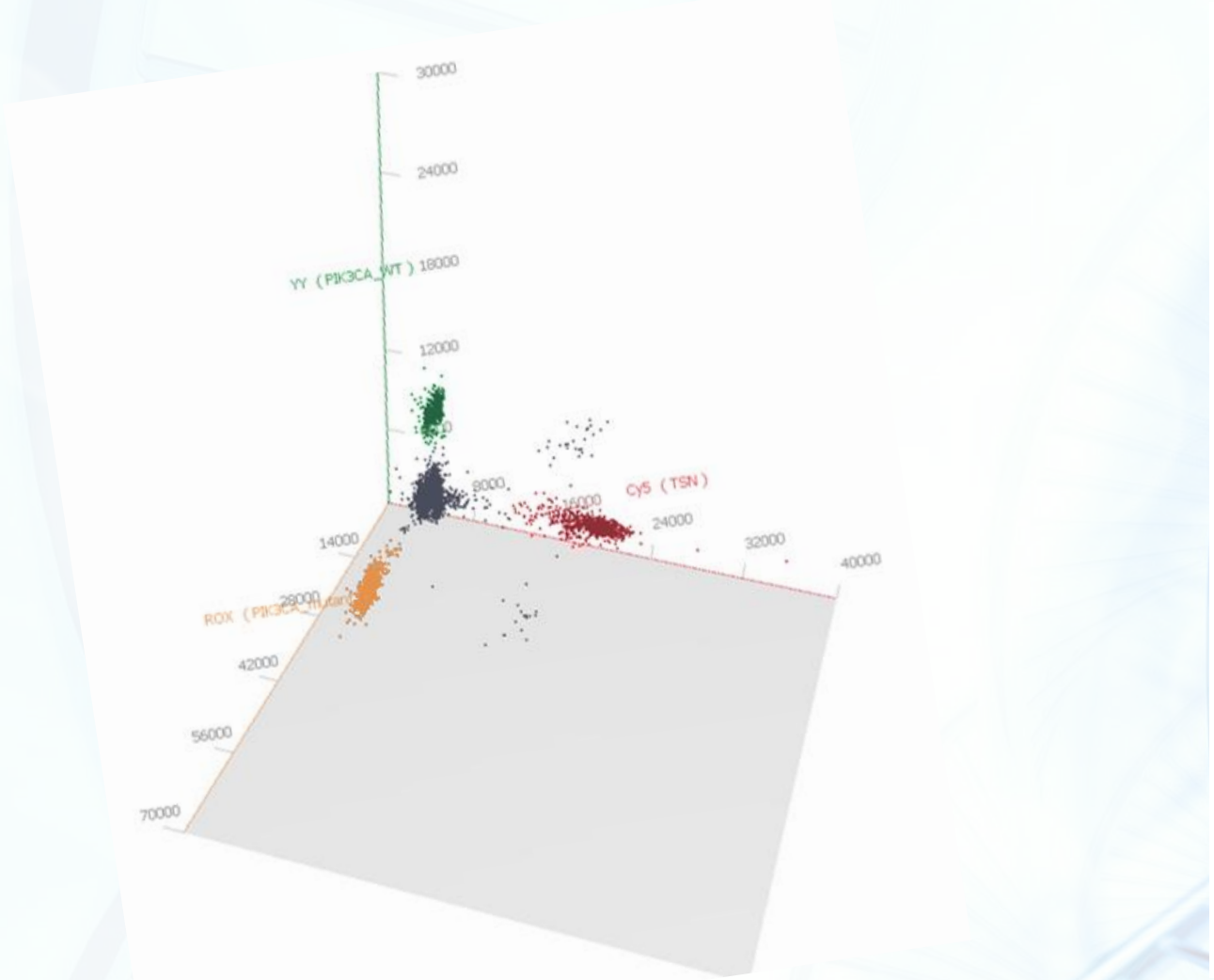
Crystal Miner 2D plots

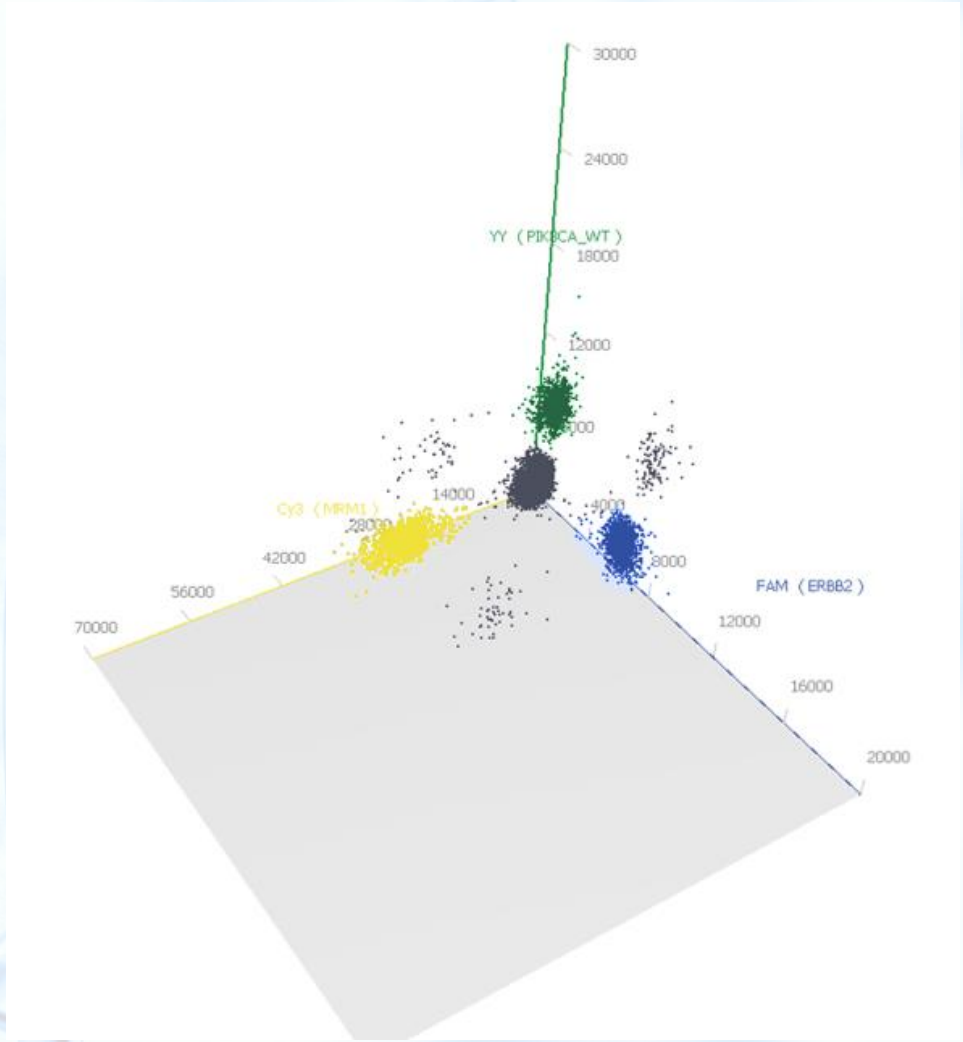




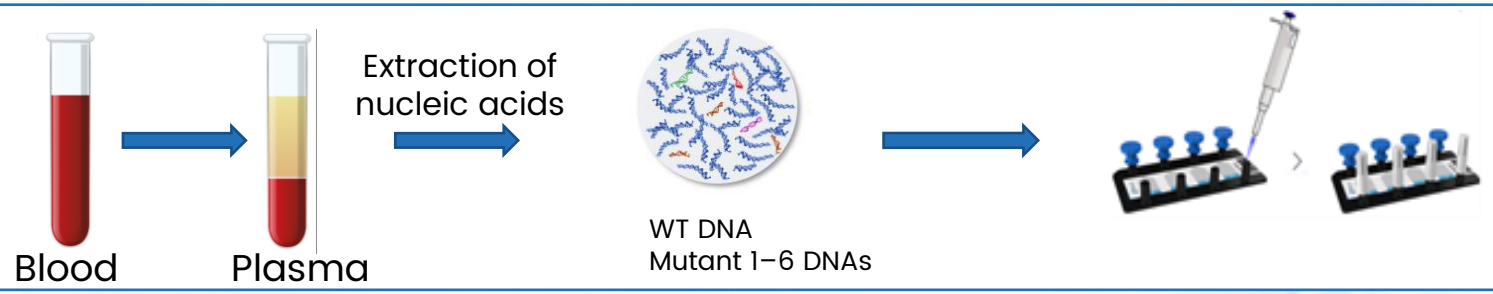


6-color Breast Cancer Panel
Crystal Miner 3D plots



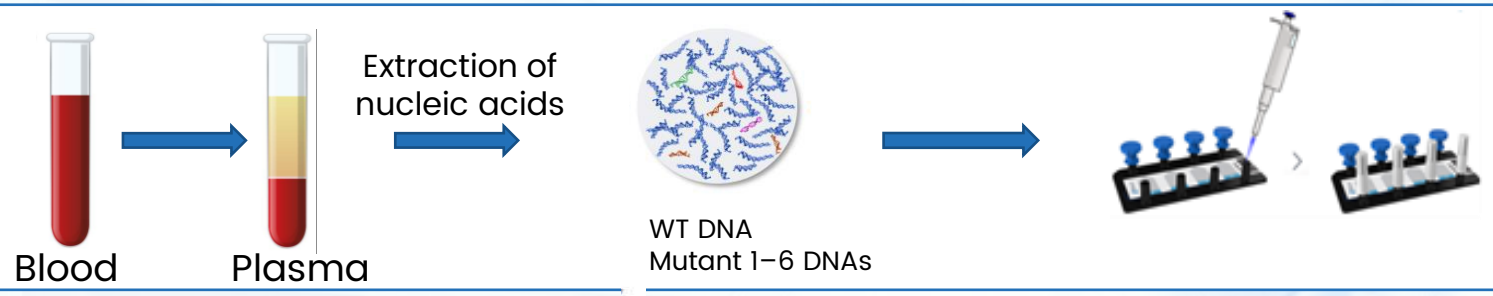


6-color Crystal Digital PCR™ analysis of Breast Cancer patient cfDNA



6-color Crystal Digital PCR™ workflow

6-color Crystal Digital PCR™ analysis of Breast Cancer patient cfDNA



6-color Crystal Digital PCR™ workflow

Sample ID	Experimental detection of *PIK3CA* Mutation	Theoretical presence of *PIK3CA* mutation	Experimental positive *ERBB2 / TSN* ratio	Theoretical *ERBB2 / TSN* ratio	Experimental positive *MRM1 / TSN* ratio	Theoretical *MRM1 / TSN* ratio
Sample 1	++	++	-	-	-	-
Sample 2	-	-	-	-	-	-
Sample 3	-	-	-	-	-	-
Sample 4	-	+ (<LOD*)	-	-	-	-
Sample 5	-	-	+	+	-	-
Sample 6	-	-	+	+	-	-
Sample 7	+	+	-	-	-	-
Sample 8	-	-	+	+	-	-

*LOD 0.8cp/µL

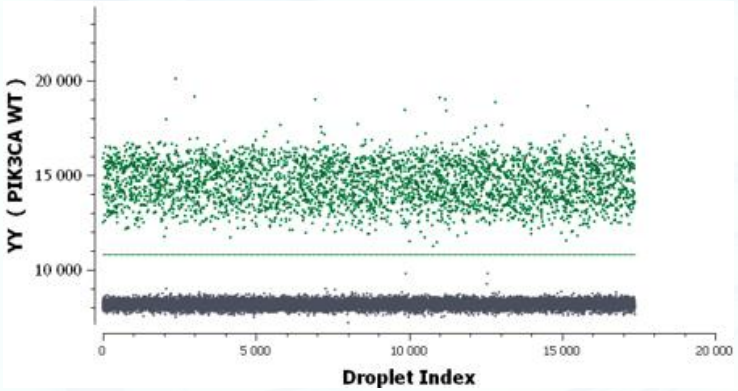
ERBB2 Amplification

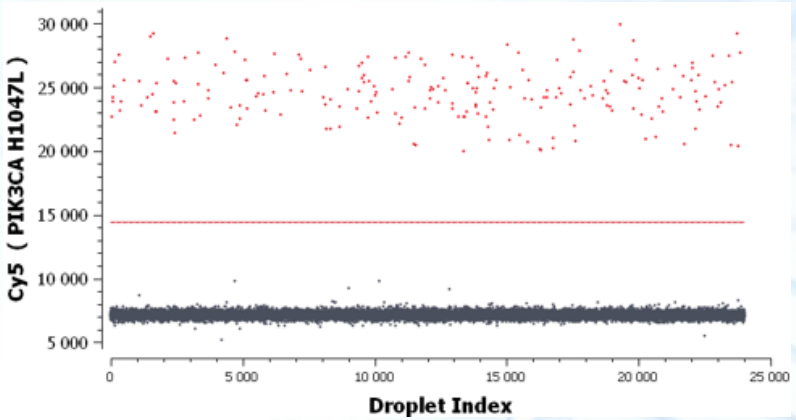
Polysomy chr 17

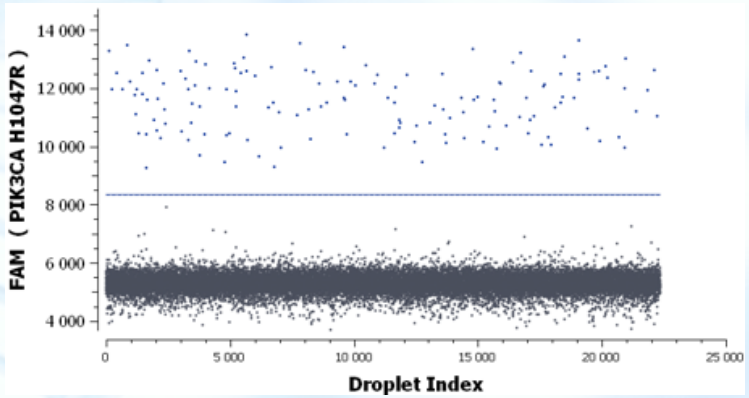
6-color Rectal Cancer Panel

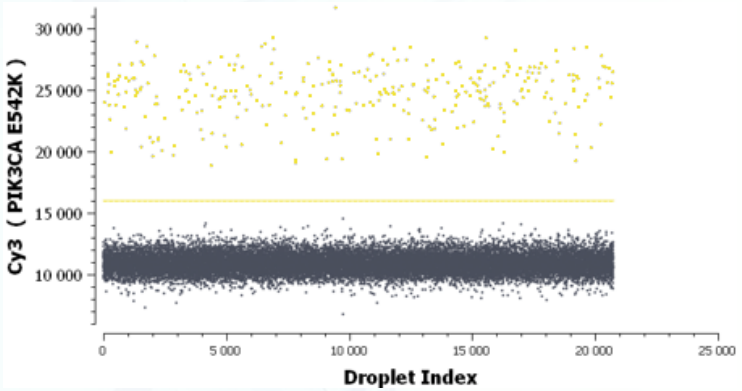
Crystal Miner 1D plots

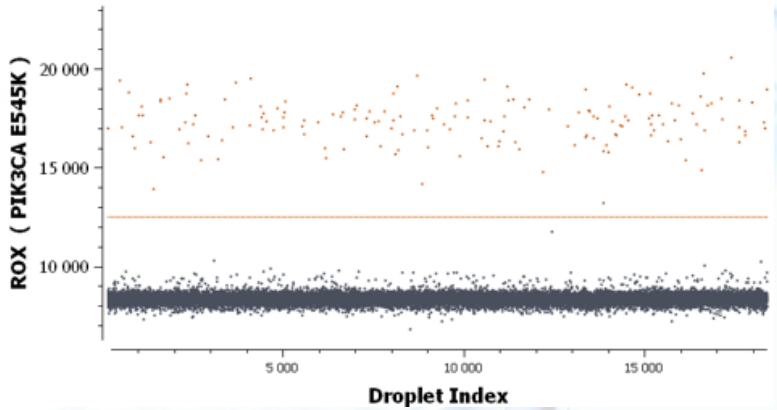
All 6 detection channels showed robust positive and negative cluster separability, crucial for correct and reliable copy number determination

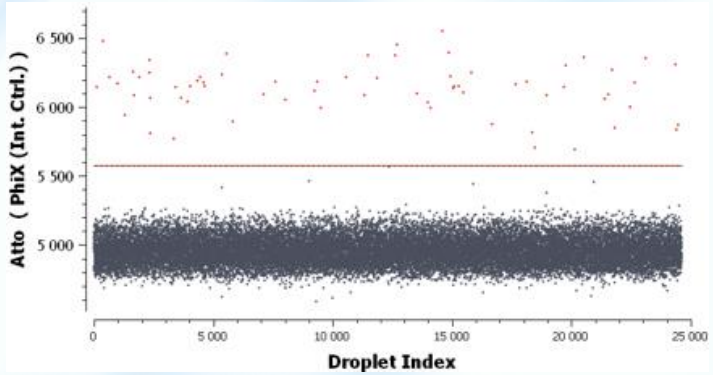




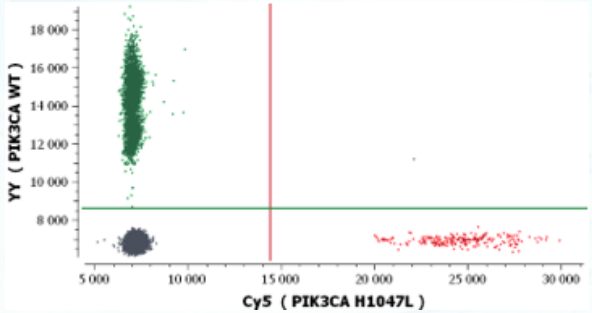


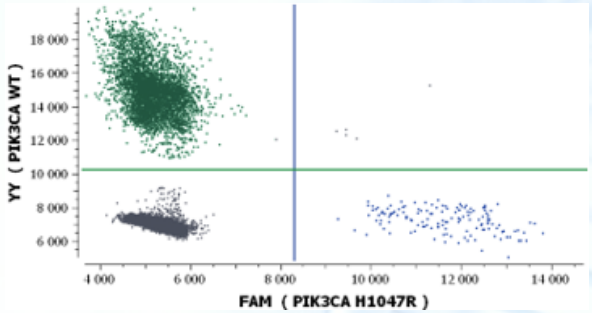


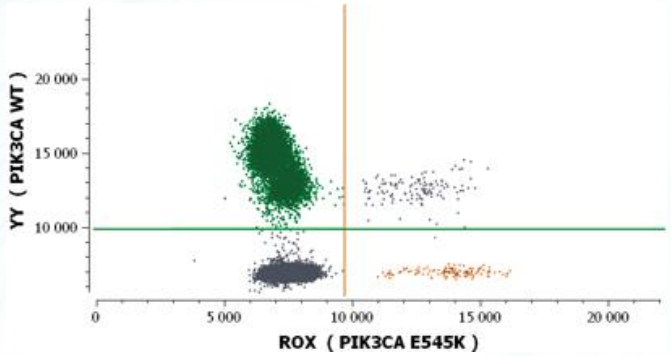


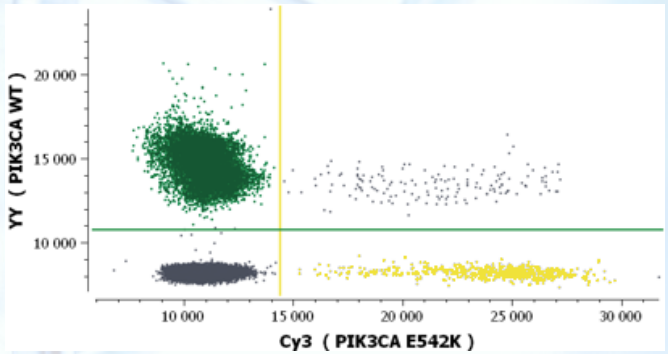


6-color Rectal Cancer Panel
Crystal Miner 2D plots









6-color Crystal Digital PCR™ analysis of Rectal Cancer patient cfDNA

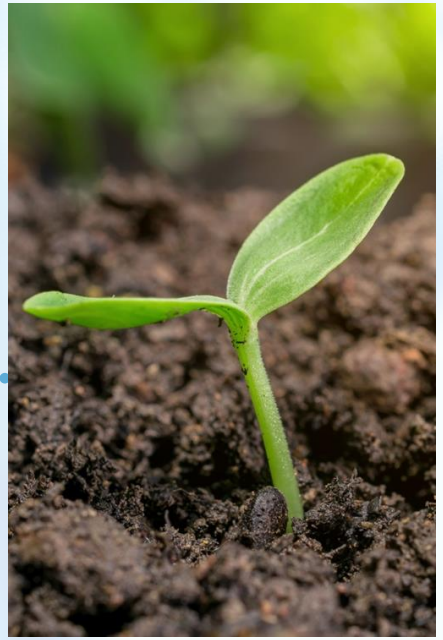
6-color Crystal Digital PCR™ workflow

Sample ID	Measured *PIK3CA H1047R*	Measured *PIK3CA H1047L*	Measured *PIK3CA E542K*	Measured *PIK3CA E545K*	Theoretical *PIK3CA* mutation
Sample 1	0	0	0.4%	0	0.4% *E542K*
Sample 2	0	0	0	0	Wild type
Sample 3	0.4%	0	0	0	0.5% *H1047R*
Sample 4	0	0	0	1.3%	1.2% *E545K*
Sample 5	0.7%	0	0	0	0.8% *H1047R*
Sample 6	0	0	0	0	Wild type

5 6-color Crystal Digital PCR™ assays for Food Testing applications

6-color Soybean GMO Crystal Digital PCR™ Assay

FOOD TESTING



6-color Crystal Digital PCR™ for Quantification of Genetically Modified Soybean

Motivation

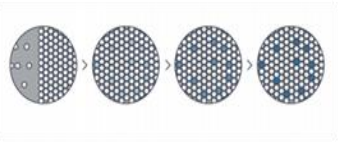
- Quantitative real-time PCR (qPCR) is the gold standard for detecting and quantifying GMOs
- With the rise in the number of EU-authorised GMOs, a strategy for faster, more cost-effective quantification is needed

Challenge

- GMOs have diversified and many new lines contain none of the most common GM elements
- Increase the number of GM lines that can be quantified in a single reaction

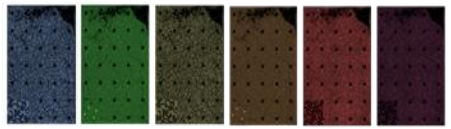
Workflow

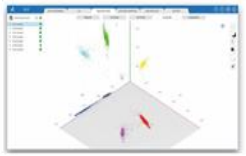
Sapphire Chips



Naica™ Geode

6-Color Reader

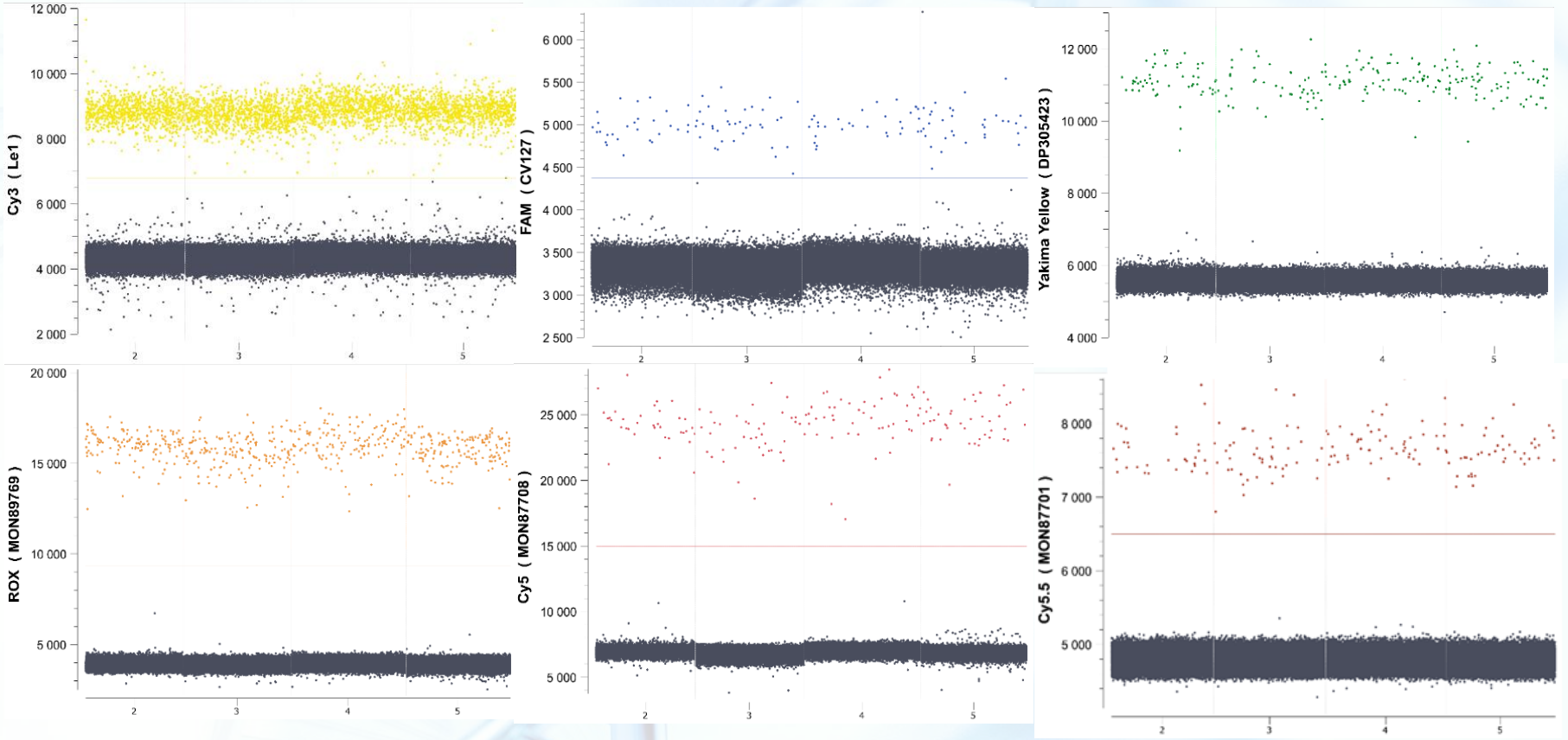




6-color Crystal Digital PCR™ for Quantification of Genetically Modified Soybean

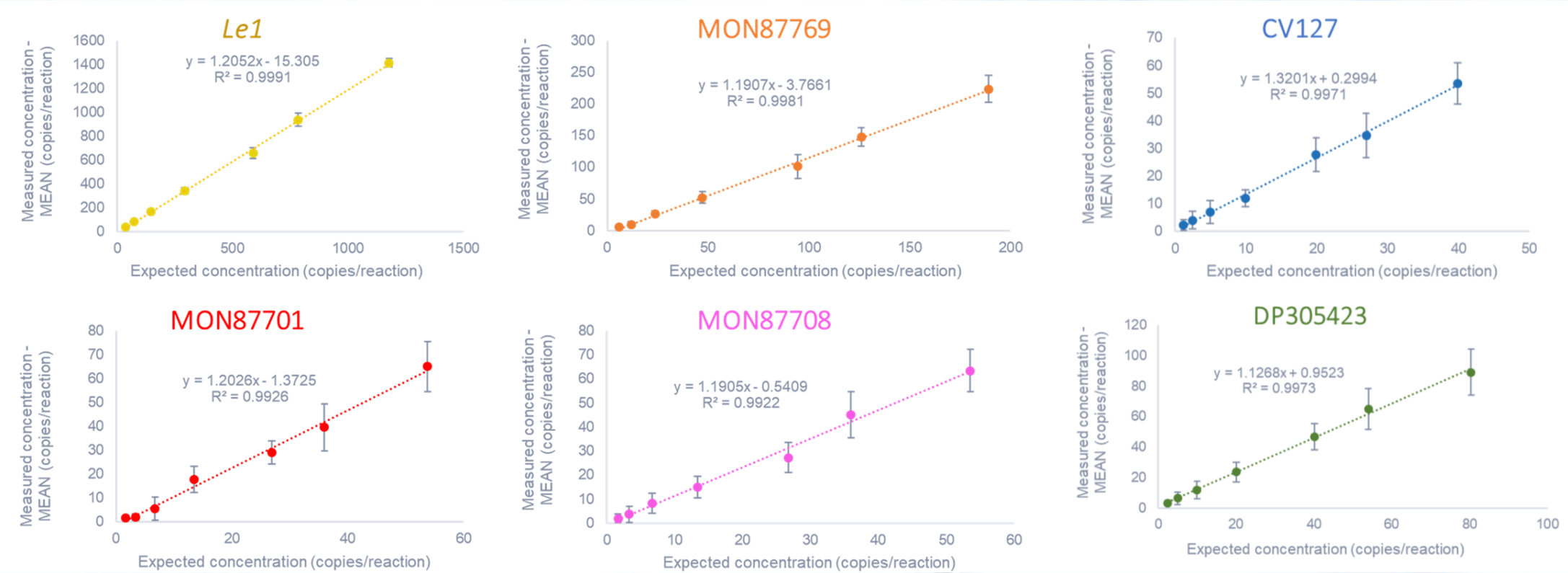
5 GM soybean lines and 1 soybean endogene quantified in a single reaction

GM line	Fluorescent dye
Le1	Cy3
CV127	FAM
DP305423	Yakima yellow
MON89769	ROX
MON87708	Cy5
MON87701	Cy5.5



Robust Performance

- Target sequences were detected with a 95% confidence level in serial dilutions (8 replicates per dilution)



Although assays were tested at the lower end of the dynamic range, the linearity was still very high (R^2>0.99)

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6 Conclusions

- Crystal Digital PCR™ is compatible with a wide range of samples (ex: frozen tissue, FFPE, liquid biopsy, plant and food material)

- Multiplex oncology panels display high clinical utility :
  - Lung (>90% of known *EGFR* mutations in NSCLC)
  - Breast (25-35% patient coverage)
  - Rectal (10-30% patient coverage)

- Maximize information output of your precious samples while minimizing time to results

STILLA

THANK YOU FOR YOUR ATTENTION!

Come and see us at **Booth 521** to discuss your future 3- and 6-color dPCR applications

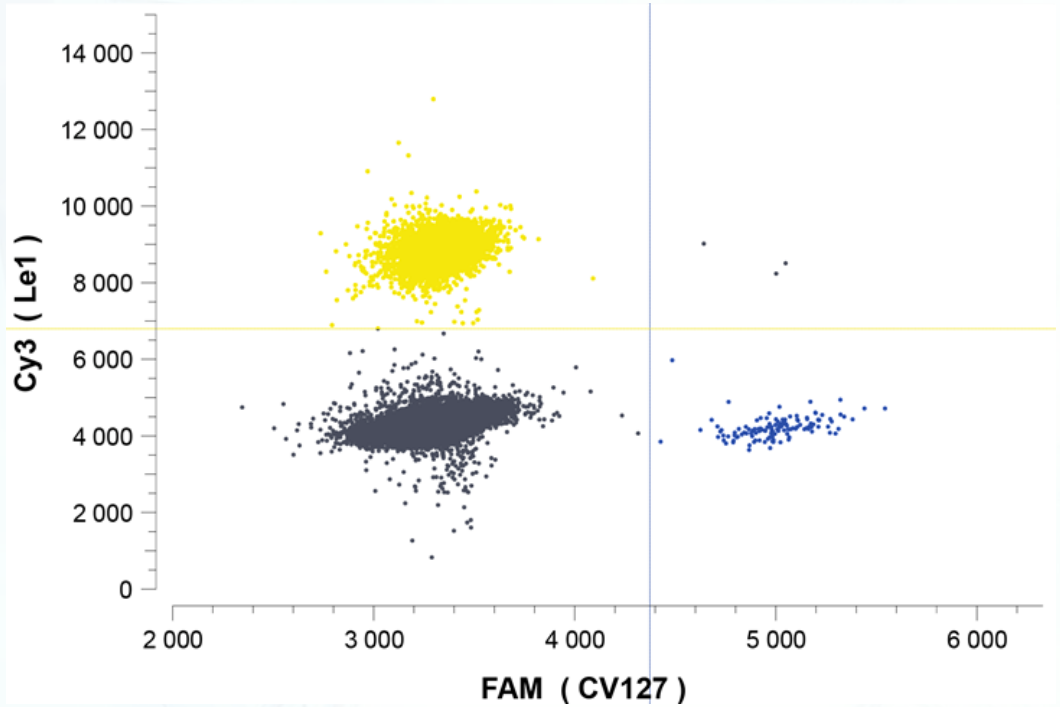
For more information on product and workflow, visit our website at

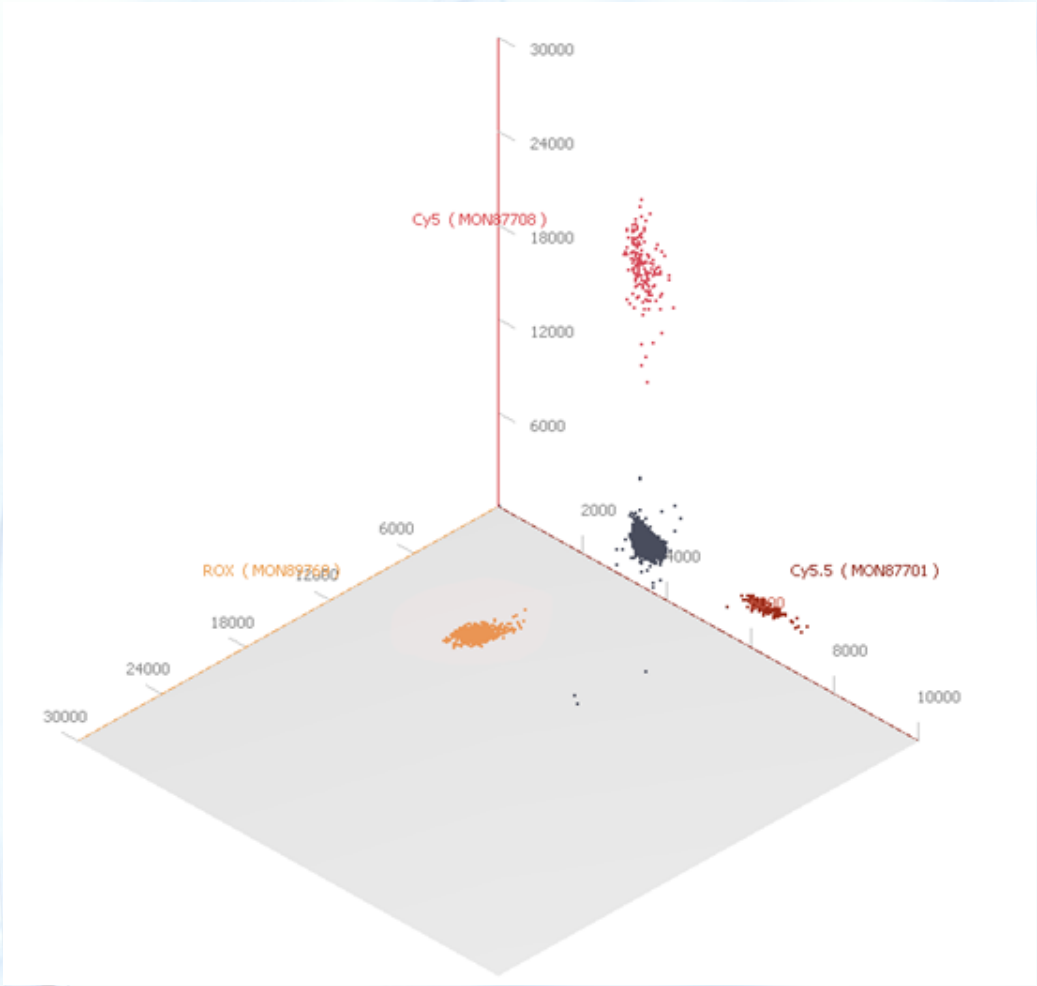
www.stillatechnologies.com

To learn more about Digital PCR, visit
www.Gene-Pi.com

6-color Soybean GMO Crystal Digital PCR™ Assay

2D and 3D plots to visualize robust separability of clusters



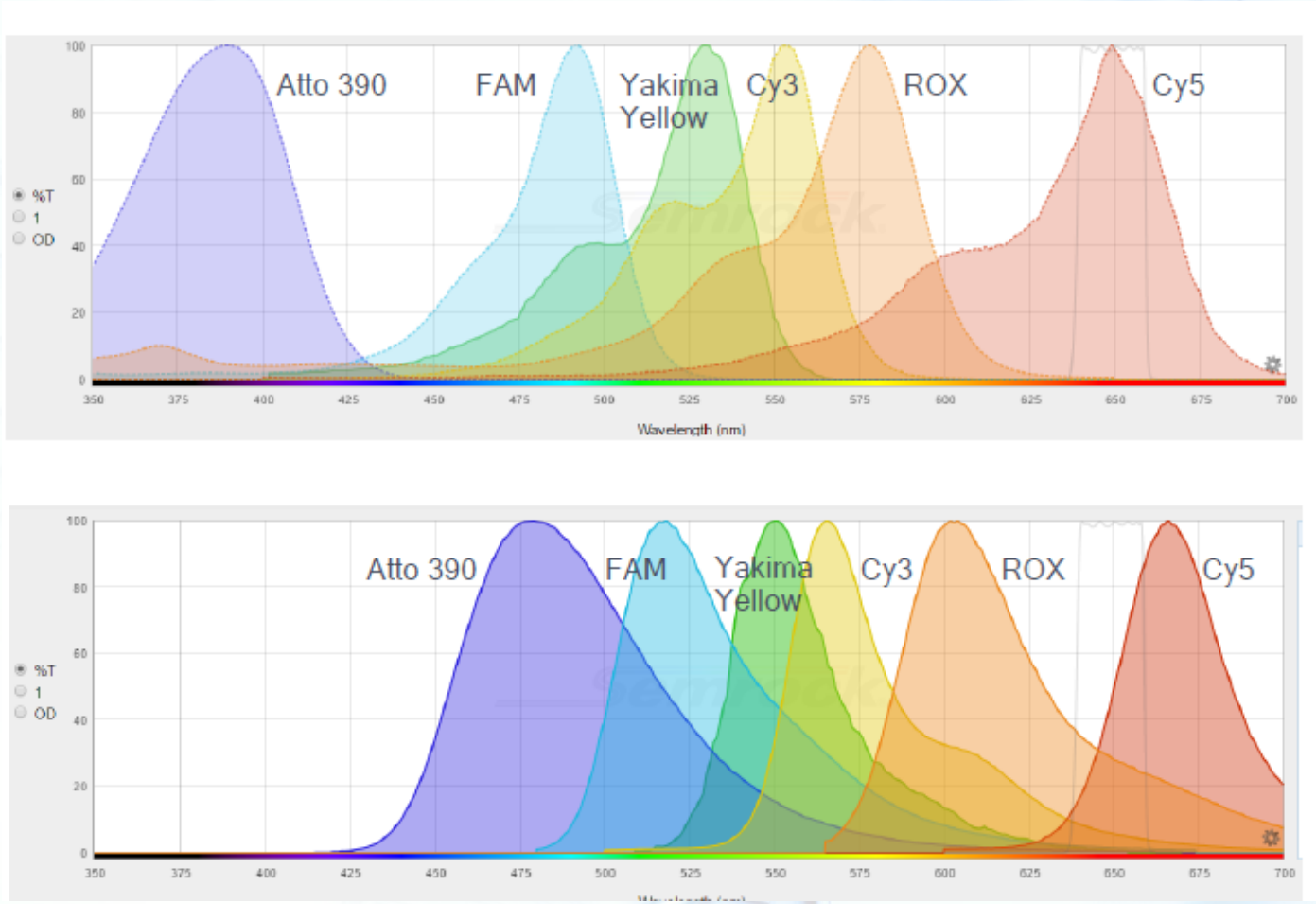


6-color Soybean GMO Crystal Digital PCR™ Assay

Crystal Digital PCR™ is a fast and cost-effective strategy for reliable simultaneous quantification of multiple GM soybean lines

GM Soybean line	Rep	Expected GM%	Mean % measured
CV127	1	3.38	3.89
	2		
DP305423	1	5.73	6.89
	2		
MON89769	1	13.47	16.06
	2		
MON87708	1	3.8	4.7
	2		
MON87701	1	3.82	4.83
	2		

6-color Detection Channels : Proof of concept



Detection of the most prevalent sensitizing and resistance EGFR mutations in NSCLC patients

-33 Tumor samples (21 Frozen, 12 FFPE)

- 24 *EGFR* sensitizing anomalies (73%)
  - ✓ 14 Exon 19 DelIns
  - ✓ 9 L858R/L861Q mutations
  - ✓ 1 G719 mutation

  } Resistance mutations: 13 T790M (39%)
- 9 WT

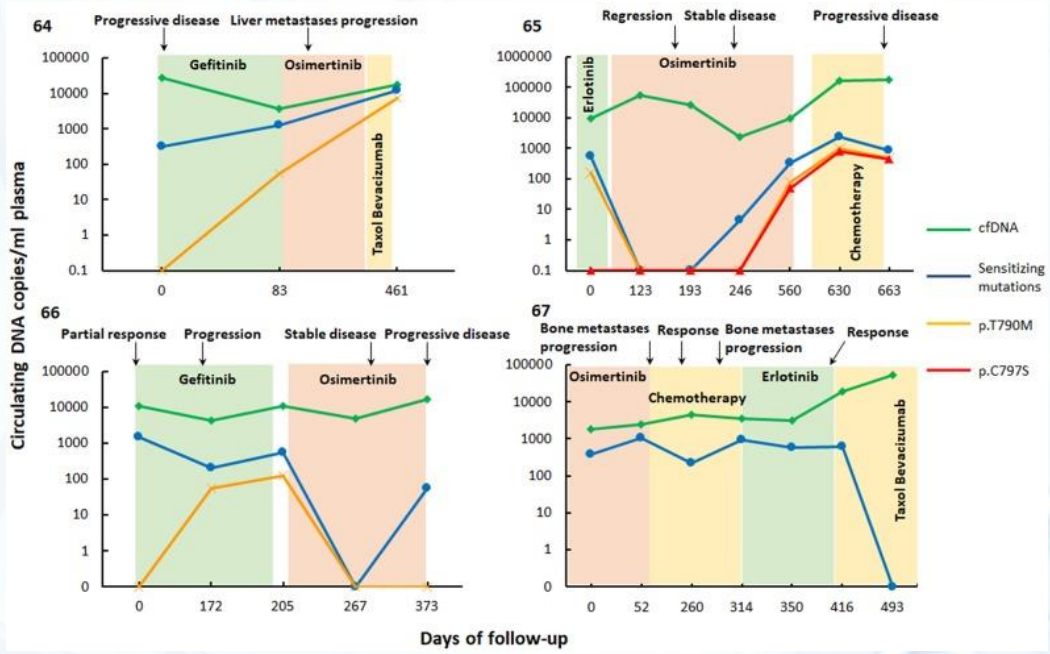
} Resistance mutations: 5 C797S (15%)

-49 cfDNA samples

- 35 *EGFR* sensitizing anomalies (71%)
  - ✓ 24 Exon 19 DelIns
  - ✓ 9 L858R/L861Q mutations
  - ✓ 2 G719 mutations

  } Resistance mutations: 14 T790M (29%)
- 14 WT

} Resistance mutations: 3 C797S (6%)



www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 100), pp: 37393-37406

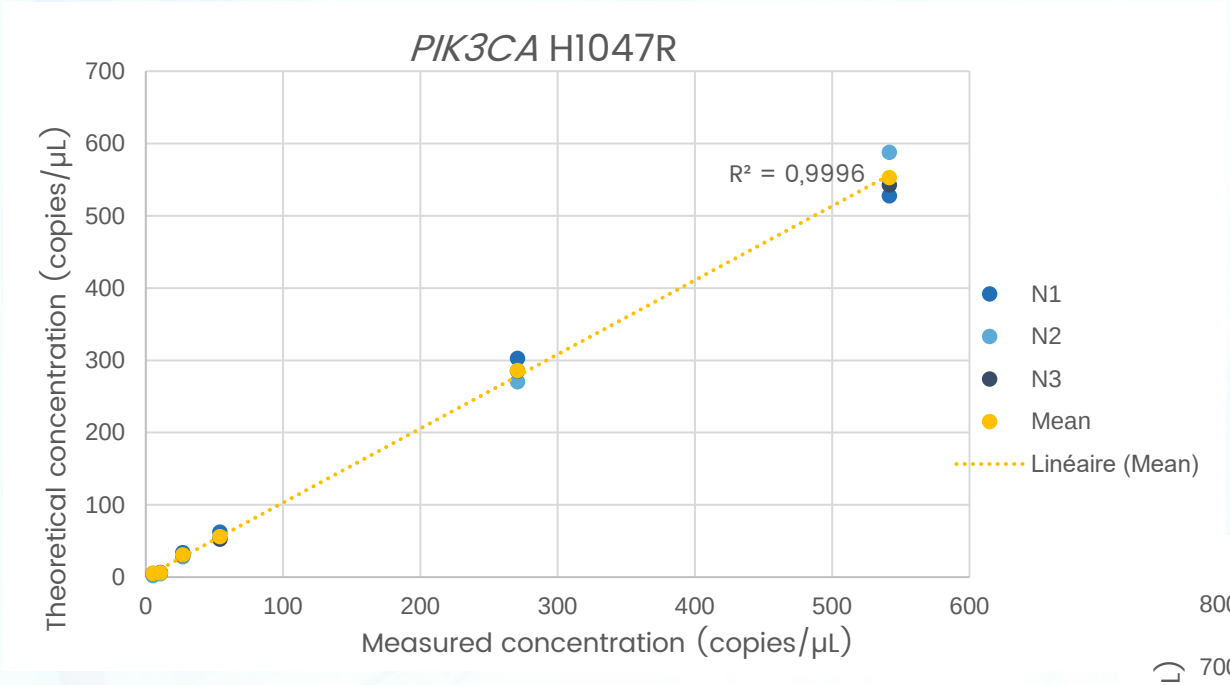
Research Paper

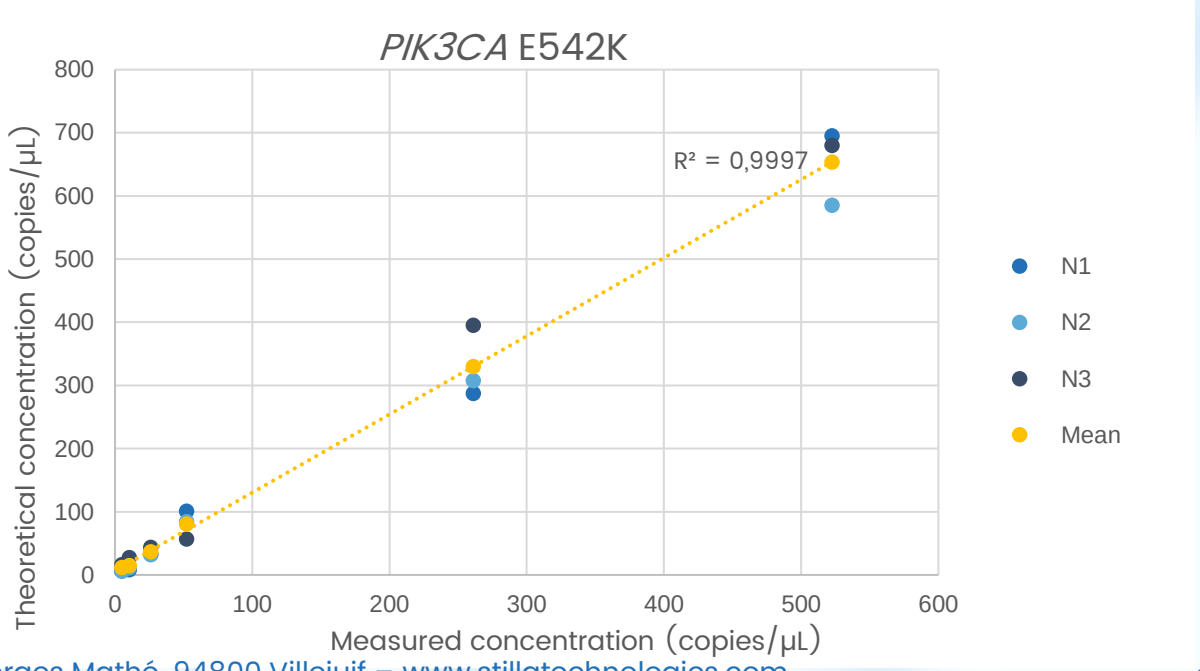
***EGFR* C797S, *EGFR* T790M and *EGFR* sensitizing mutations in non-small cell lung cancer revealed by six-color crystal digital PCR**

Jordan Madic[1,*], Cécile Jovelet[2,*], Julien Lopez[1], Barbara André[1], Jean Fatien[3], Isabelle Miran[2], Aurélie Honoré[2], Laura Lezquita[5], Benjamin Besse[5], Ludovic Lacroix[2,4,*] and Magali Droniou[1,*]

Figure: Follow-up of the sensitizing and resistance *EGFR* mutations and cfDNA levels in longitudinal plasma samples of metastatic NSCLC patients

Breast Cancer Assay





Measured concentrations versus theoretical concentrations