

# Chromofy – New Dye For Ultrasensitive High-Throughput qPCR



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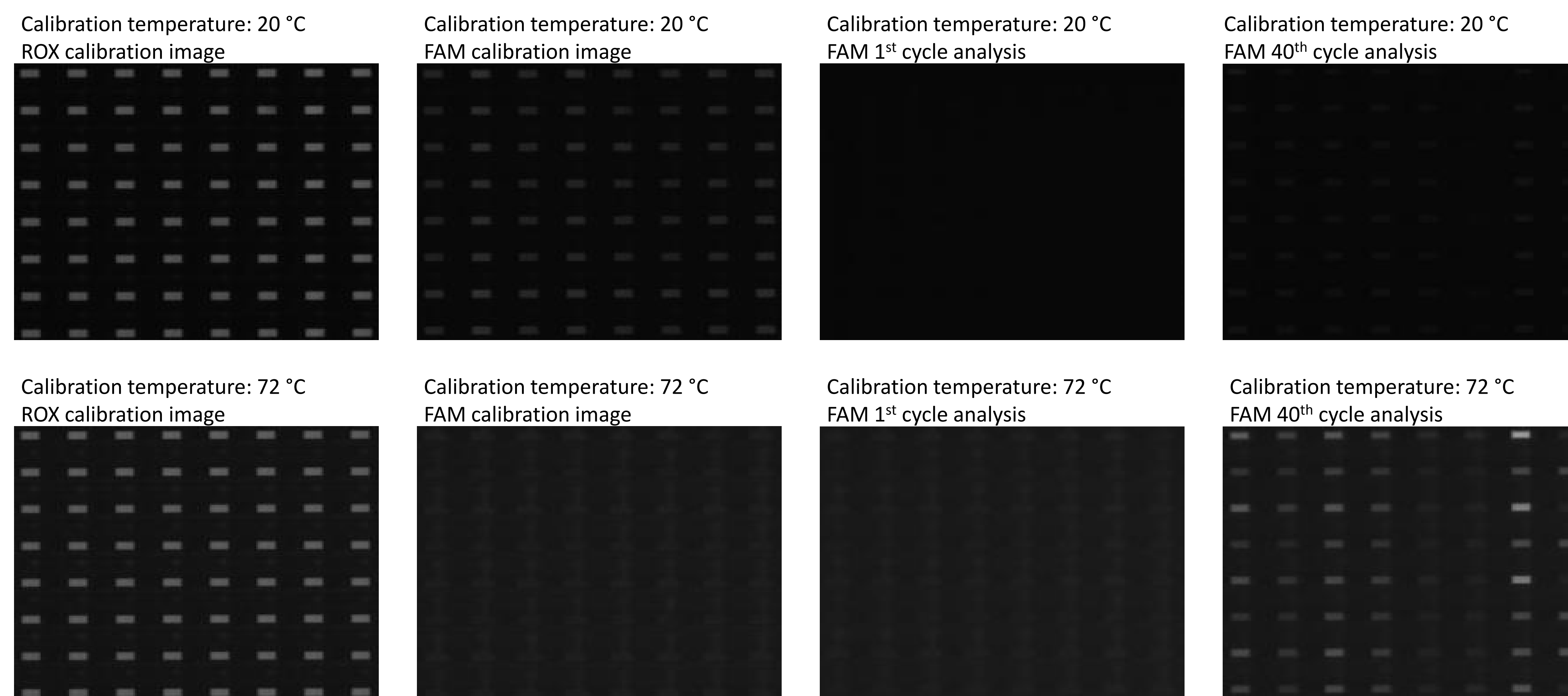


## Abstract

Analysis of gene expression is key component of functional genomics. The general trend in the field is towards higher throughput of less complex samples, for example, analyzing hundreds of genes in a large number of a single cell type. When getting down on single cell level, high-throughput qPCR with its broad dynamic range, high sensitivity and high versatility outperforms techniques such as microarrays or massive parallel sequencing. Until now, microfluidic high-throughput qPCR assays were mainly based on probe for detection. Dyes could not be used because of poor reproducibility due to problems related to surface adsorption. We have screened new dyes for their performance as reporters that can be detected in the SYBR/FAM channel, and that are also suitable for high-resolution melt analysis (HRM). We found the new dye Chromofy from TATAA Biocenter ([www.tataa.com](http://www.tataa.com)) to be most suited as qPCR reporter in the microfluidic qPCR Biomark platform. We also found that the performance depends strongly on the sample temperature at CCD camera calibration. Under optimized experimental conditions data quality is as good or even higher that obtained with probe based assays. The advantages of dye based assays is the less complicated assay design, lower cost and the possibility to perform melting curve analysis or HRM.

## Calibration

8x8 wells in the top left corner of the chip were analyzed. Two chips are compared. Sigma Jump start master mix is used. Each column of reactions represents one gene, samples are in rows.

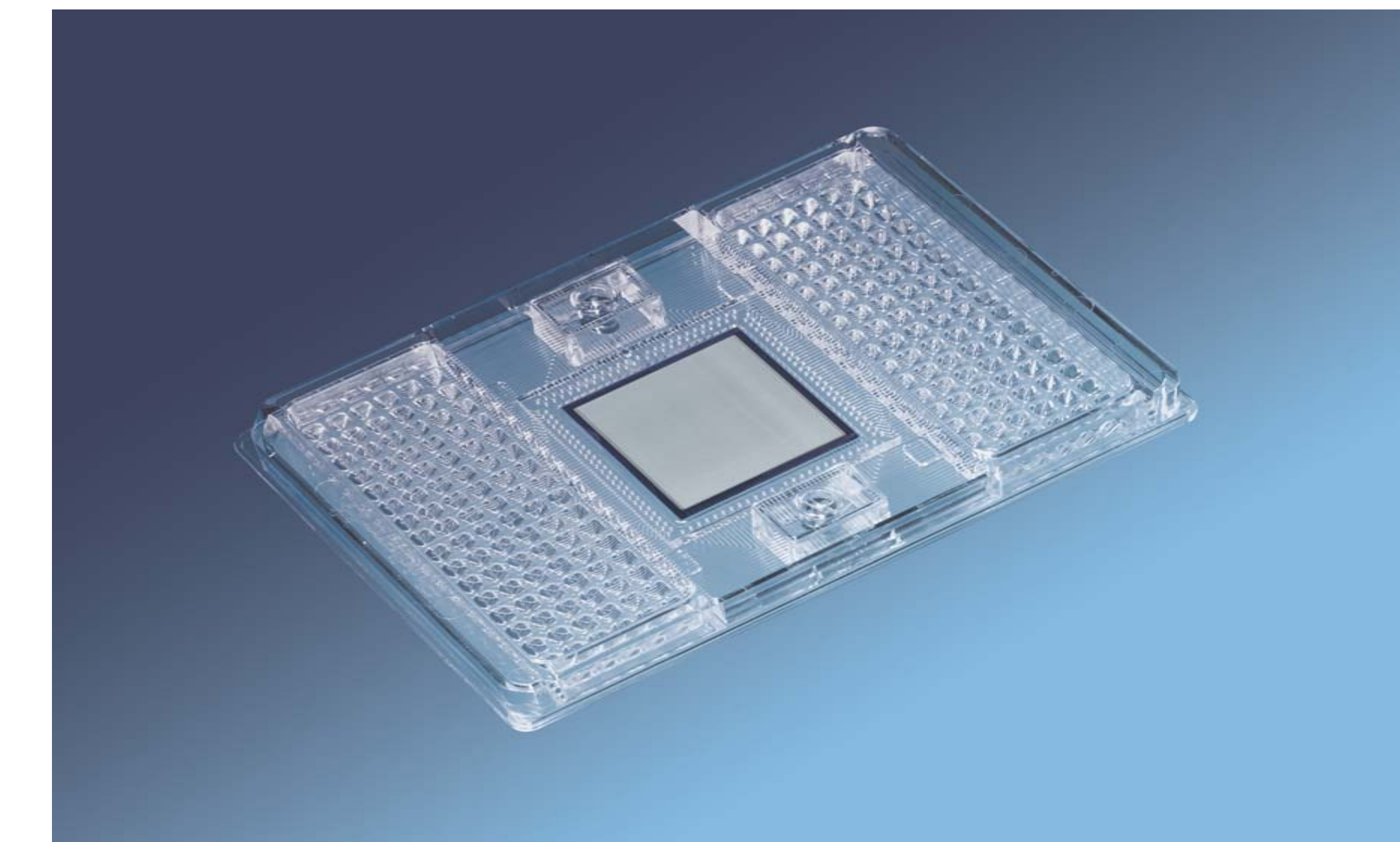
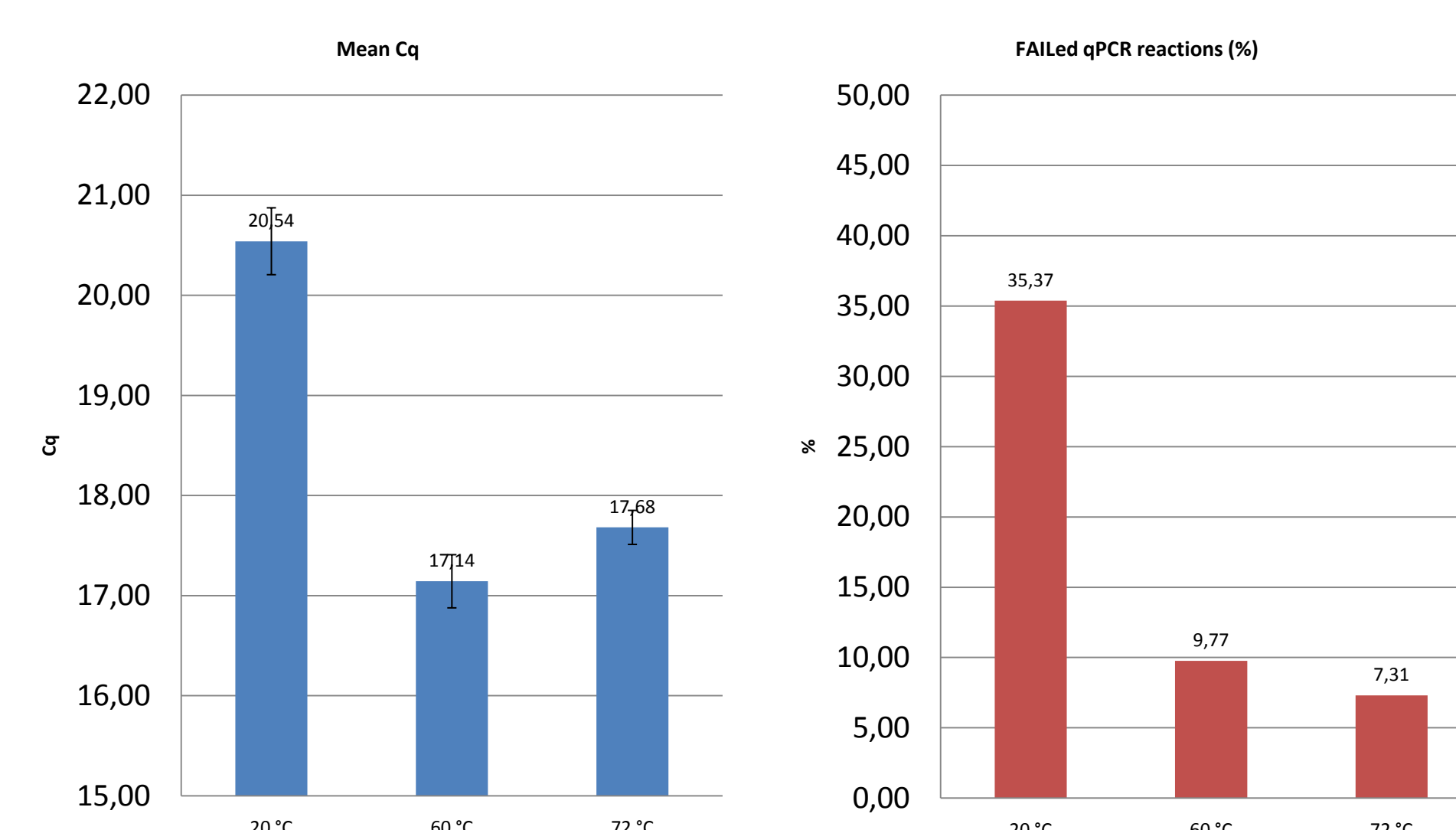


**Figure 1: (top)** Using 20 °C auto-exposure, the signal from SYBR is detected easily in the wells in the calibration step. The same camera setting is then used to measure at 72 °C during the qPCR. In the 1st cycle no signal is detected. Even after 40 cycles the signal is very weak. **(bottom)** Using instead 72 °C auto-exposure the signal from SYBR is weak, but sufficient for the wells to be distinguishable. The same camera setting is then used during the qPCR, This time the signal after the first 1 cycle is essentially identical to the calibration image. After 40 cycles strong signals develop. Because of the much clearer signals the signal to noise ratio is substantially increased.

## qPCR data extraction success

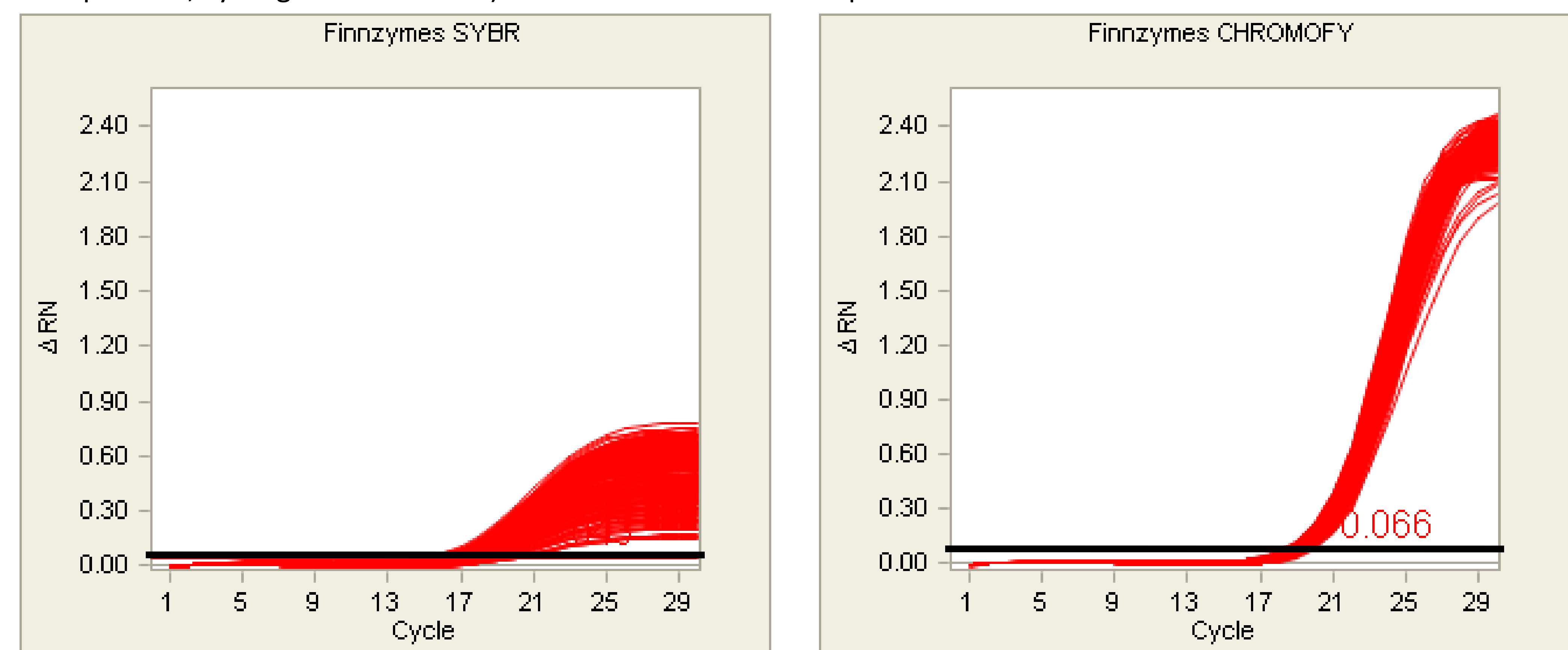
Three chips with the same assays and same kind of samples were compared. Average Cq of all the wells was calculated, as well as the standard deviation of all technical triplicates, which was averaged afterwards. We used the BioMark software to select the FAILED reactions.

**Figure 2: (right)** Assuming we have random and representative distribution of samples in all three chips, the increased calibration temperature makes it possible to measure lower Cq with higher reproducibility. Lower percentage of FAILED reactions suggests higher sensitivity.



## Comparison of dyes: performance, uniformity, reproducibility

Finnzymes Dynamo mastermix with either SYBR or with Chromofy was used for the uniformity test on two 48x48 chips. One concentration of standard human cDNA was used as a template for the whole chip. One gene only was analyzed in all 48 assay inlets (GAPDH). cDNA concentrations between chips were different so the Cq values are not comparable. 4 technical replicates of each sample were loaded (total 192 Cq values were measured). All other conditions (master mix composition, cycling conditions etc.) were identical for both chips.



**Figure 3: (top)** Comparison of two 48x48 chips – 192 qPCR's are shown in each graph. Technical sample tetraplicates were performed. Pools of human cDNA were used as substrate. To evaluate the uniformity of sample/assay loadings 48 assay inlets were loaded with GAPDH primers. Left: Finnzyme's Dynamo SYBR mix. ΔRN (normalized fluorescence) is 0.2-0.8. Mean Cq value on the left side of the chip was 15.4, and on the right side of the chip it was 19.2 cycles. Right: Finnzymes Dynamo mix with Chromofy. ΔRN is 1.9-2.4. Mean Cq on the left side of the chip was 19.4 and on the right side it was 19.7.

	SYBR	CHROMOFY
mean SD sample 4plicates	0.30	0.30
min SD sample 4plicates	0.08	0.11
max SD sample 4plicates	0.60	0.45
SD global (192 Cq values)	1.04	0.31
mean Cq (192 Cq values)	17.04	19.46
min (192 Cq values)	14.74	18.76
max (192 Cq values)	20.02	19.99
ΔCq left/right side of the chip	-4.80	0.47

**Figure 4: (right)** Descriptive statistics of the data in Figure 3. Note, Cq values between chips are not comparable. cDNA conc. in SYBR chip was considerably higher.

## Conclusions

Dye background fluorescence is strongly temperature dependent. Room temp. calibration leads to underexposure of subsequent images collected at 72°C during elongation steps. This results in uncertain and even incorrect readouts. Compared to more common dyes, Chromofy from TATAA Biocenter exhibits much lower surface adsorption in the BIOMARK microfluidic matrix leading to substantially higher and much more robust fluorescence signals.