

ThermoFisher
SCIENTIFIC

***QuantStudio™ 3D Digital PCR System:
Data Troubleshooting***

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General Flag Definitions

- Touchscreen of QuantStudio® 3D displays **Red**, **Yellow**, **Green** Flags for each reporter dye



Re-scan or repeat the experiment, visually inspect chip, **possibly not worth transferring to AnalysisSuite™**



Inspect chip **in AnalysisSuite™**



Chip passed all screening criteria

- Note that even if chip has **Green** flag, further analysis in AnalysisSuite™ is recommended

Flags in AnalysisSuite™ Software: For Chip Inspection

- AnalysisSuite™ displays **Green**, **Red** or **Yellow** Flags
 - By default, FAM dye is populated, hence Flag is for FAM (if VIC Dye was run, then need to change define chip settings)
 - If **dual reporter**, then **WORST** flag is shown of two dyes, user should inspect chip
 - “**Broken Flag**” indicates that automated analysis has been manually overridden 

Chip	Assay	Sample	Rare Dye	Dilution	Run date	Flag
151215_153917_C01LJ7.eds	CNV	Sample_1		1	Dec 15, 2015 06:39 PM	
gc_151215_154642_C01I6B.eds	CNV	Sample_3		1	Dec 15, 2015 06:46 PM	
jg_151215_154741_C01K4F.eds	CNV	Sample_4		1	Dec 15, 2015 06:47 PM	
jh_151215_154102_C00MGF.eds	CNV	Sample_5		1	Dec 15, 2015 06:41 PM	
lz_151215_154438_C01KFY.eds	CNV	Sample_6		1	Dec 15, 2015 06:44 PM	
ma_151215_154536_C01GNN.eds	CNV	Sample_7		1	Dec 15, 2015 06:45 PM	

Table view of “Review Data” Tab (AnalysisSuite™ v3.0.3)

Further detail on Flag definitions



Data meets all quality thresholds



- The separation between positives and negatives is not “clean”
- The distribution of positives on the chip is not uniform
- The concentration is outside of the range that we are confident in the chip quality value metrics (200-2000 copies/ μ l)

Suggestions:

1. Review histogram & adjust threshold if needed. If not bimodal, or a high concentration or low concentration that is mostly monomodal, reject chip. If double peak, you can view in scatter plot view to determine if clusters are separated in this view.
2. Look at chip image view to see if positives & negatives are randomly distributed. If not randomly distributed, reject chip.
3. You can try to raise the QV threshold to see if the histogram gets more bimodal and the positive/negative distribution gets more uniform.

Further detail on Flag definitions



Re-image. If still red, reject the chip. Look for specific failure modes that are mostly visible on the consumable (leak, large bubble, liquid on lid if dropped, cracked chip etc.)

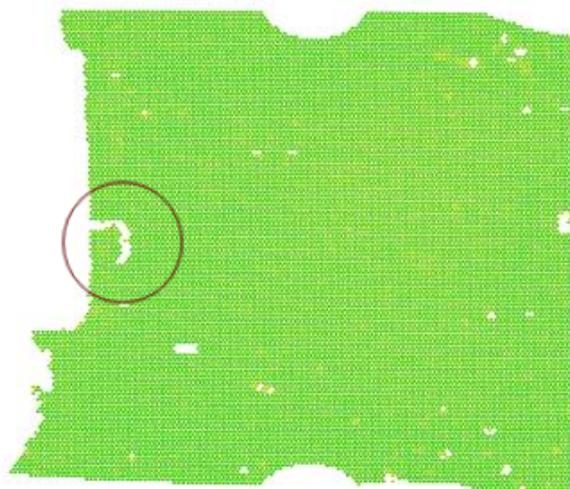
Successful Load



- This a good example of a successful load

Scenario 1: *Debris on chip*

Chip view



Possible Cause

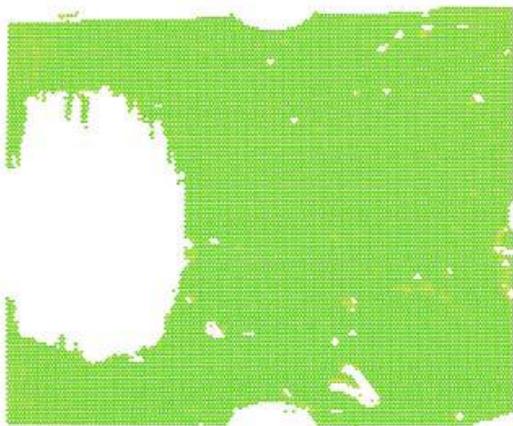
- Dust or other debris are present on the Chip Sealant during imaging.

Action

- No action required.
- The AnalysisSuite™ Software can compensate for small quantities of dust and debris on the Digital PCR 20K Chip.

Scenario 2: *Bubble in the Sample Loading Blade*

Chip view



Possible Cause

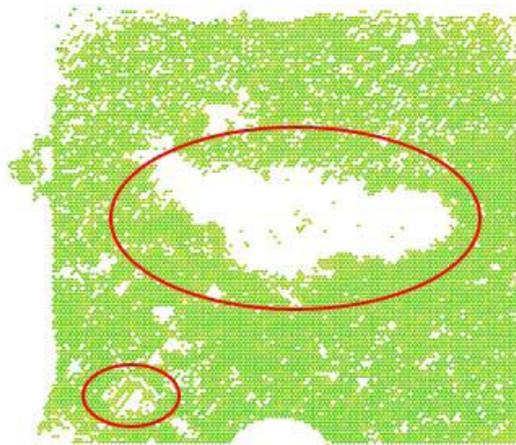
A bubble was present in the Sample Loading Blade when it was used to apply the dPCR reaction to the Chip.

Action

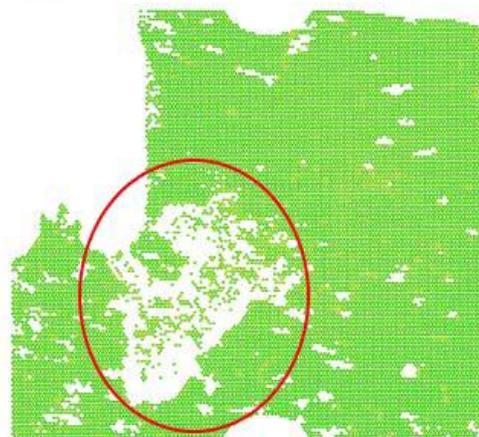
- If possible, use the AnalysisSuite™ Software to filter the low quality data points, **or** discard the chip and run the sample again.
- When filling the Loading Blades:
 - If you are using a manual pipette, pipette to the first stop.
 - If you are using an electronic pipette, decrease your pipetting speed.
 - If a bubble does form in the Sample Loading Blade, gently tap it to remove bubble before loading.

Scenario 3: *Bridging*

Chip view



Chip view

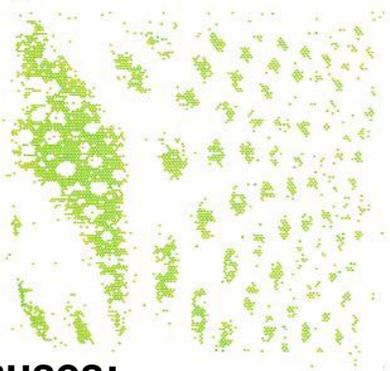


Possible Cause

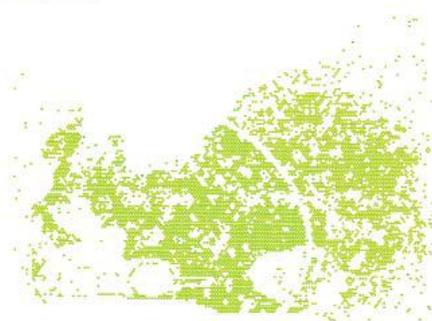
- Excess dPCR volume was present on the Digital PCR 20K Chip after loading it with the Sample Loading Blade.
- The Sample Loading Blade was drawn across the chip too quickly or at an angle shallower than 70-80°.

Scenario 4: *Major Leakage*

Chip view



Chip view



Possible Causes:

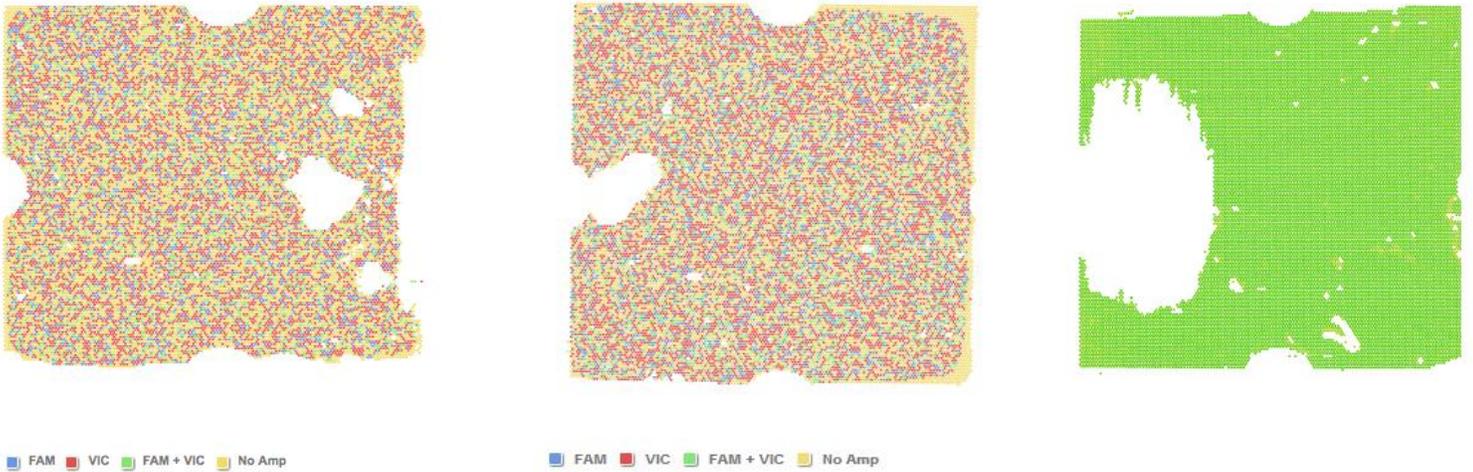
- The Digital PCR 20K Chip leaked during thermal cycling or imaging.
- A very large bubble was present in the chip (insufficient Immersion Fluid).
- Immersion Fluid was not applied to the chip immediately after loading (evaporation of the PCR reaction).
- Excess Immersion Fluid is present on the Chip Case Lid (impeding proper imaging).
- Fill port not sealed adequately.

Scenario 4: *Major Leakage*

Action

- If possible, remove excess Immersion Fluid from the chip lid and image the chip again.
- If possible, use the AnalysisSuite™ Software to filter the low quality data points.
- Make sure to apply Immersion Fluid to each chip immediately after loading it with PCR reaction (prevent evaporation).
- **To minimize leakage**, when sealing each Digital PCR 20K Chip:
 - Wear correctly fitted gloves to prevent the glove material from snagging during lid application.
 - Make sure that the Chip Case Lid is correctly aligned to the Chip Case.
 - Firmly press all four corners when applying the Chip Case Lid.
 - *****Be wary of getting excess immersion oil on the adhesive areas of the chip lid, on the chip case where lid will be applied, or on the Immersion Fluid fill port*****

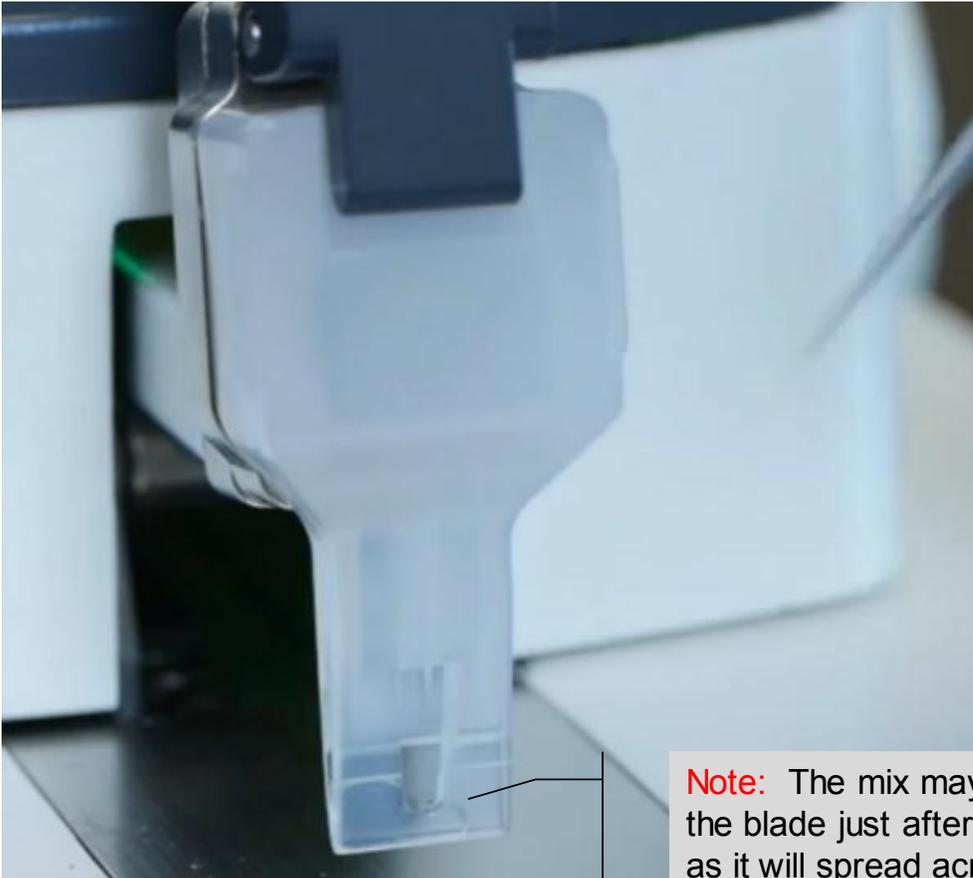
Bubbles in dPCR Reaction Mix



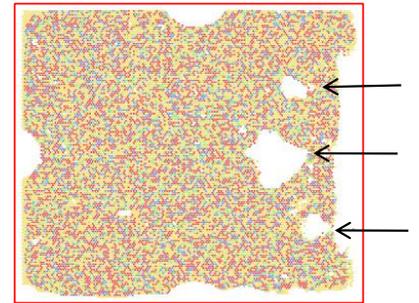
To prevent this from happening:

- Check reaction in tube before pipetting
- Pipette to the first stop when using a manual pipette
- Pipette very carefully
- Decrease pipetting speed

Ensure that there are no bubbles in the loaded mix

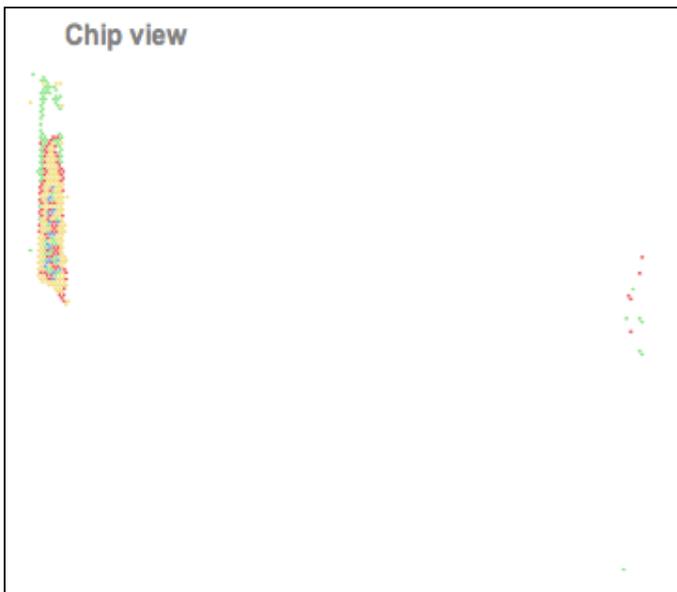


Bubbles in the blade will show up as bubbles on the chip – wells will not be loaded

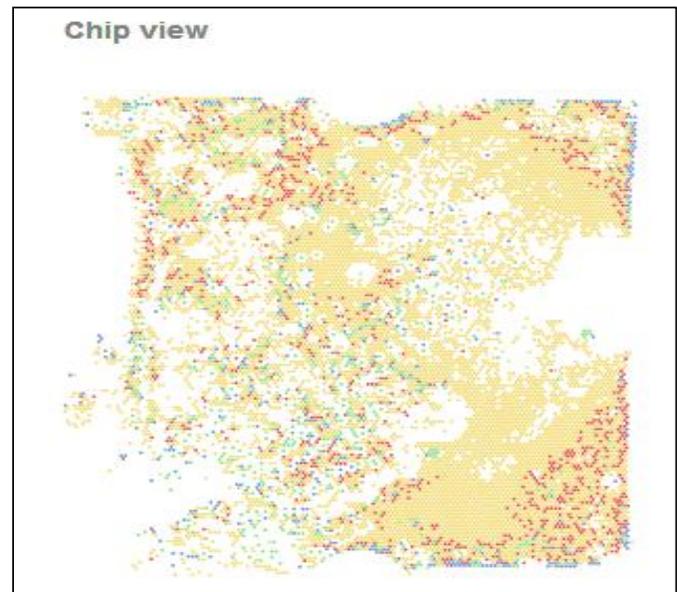


Note: The mix may not be evenly distributed in the blade just after loading. This should be fine, as it will spread across itself

Dropped Chip



Chip dropped before PCR



Chip dropped after PCR

Don't drop your chip

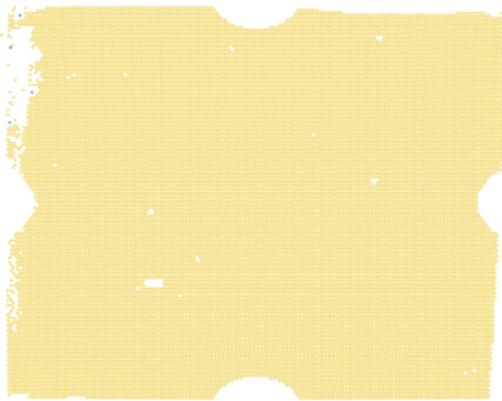
Data obtained from dropped chips should be discarded

No template added to dPCR reaction

Results in monomodal data with a “No Amp” peak

B1RFY8_130716_155720.eds Data exceeding quality threshold colored by calls

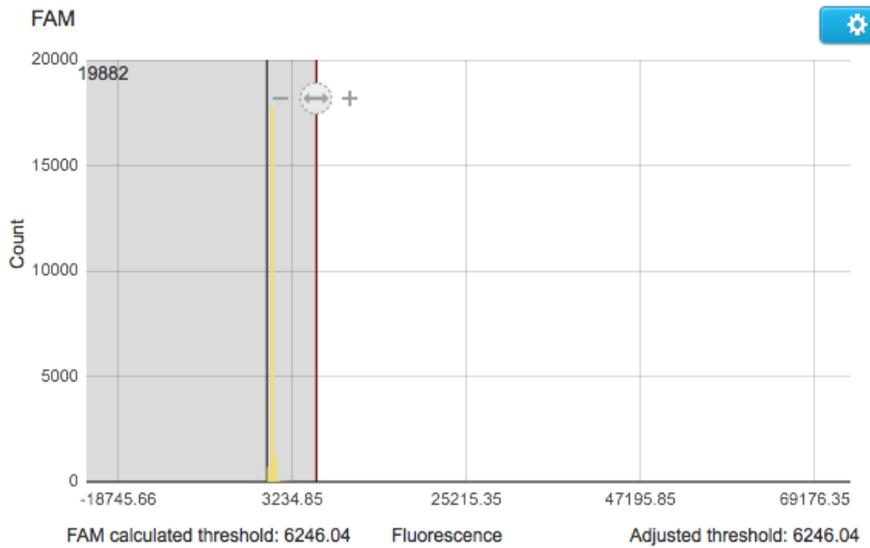
Chip view



FAM No Amp

Color by quality
 Color by calls

Histogram view

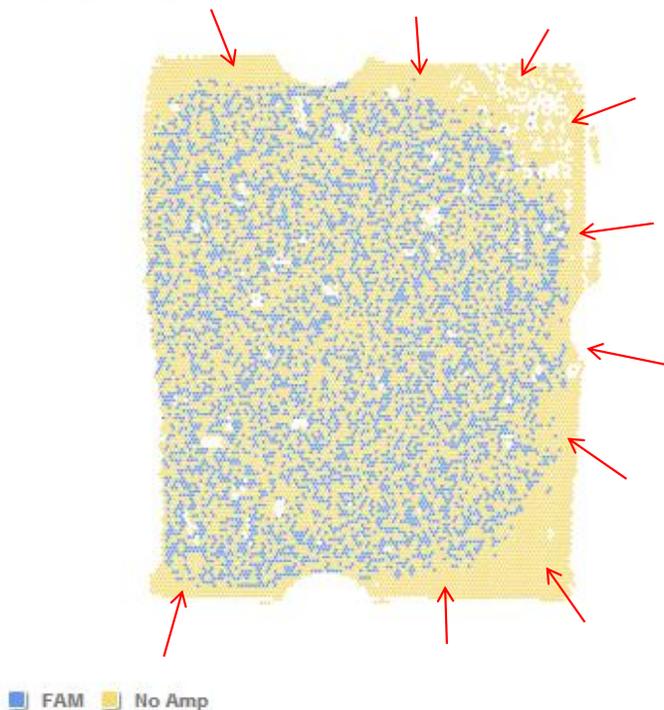


Quality threshold Data points above threshold
0.62 19,886 of 20,052

Omit this chip from results

Evaporation around edges

Chip view



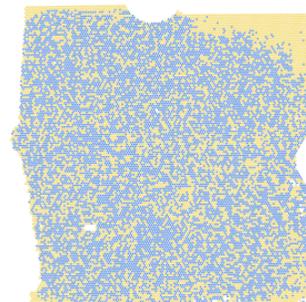
- Chip was not completely covered with immersion fluid (while still in chip nest on chip loader)
- When using the chip loader, as soon as the loading blade is off of the chip, start to add immersion fluid

Apply immersion fluid immediately after loading



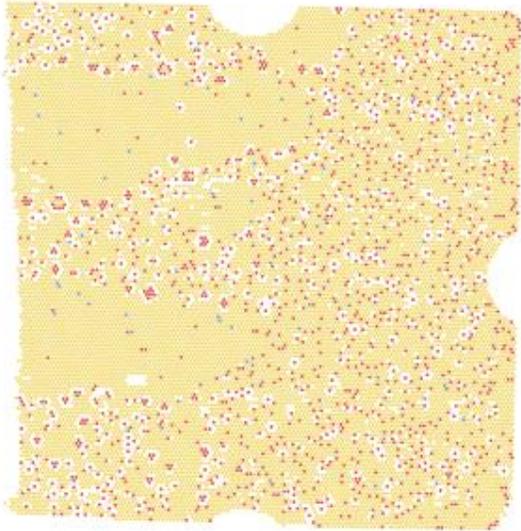
Ensure that entire chip is covered with immersion fluid, even the edges to prevent evaporation

- It's OK for the oil to overflow into the chip base, but use caution to make sure it does not get on the sides on the chip or that there is too much oil



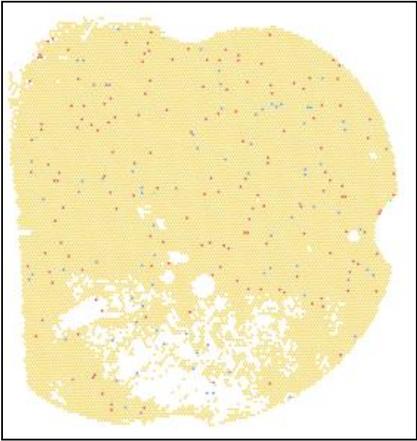
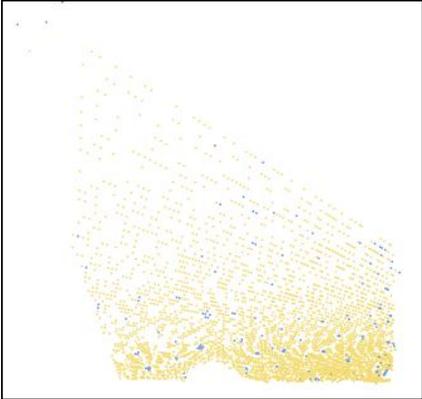
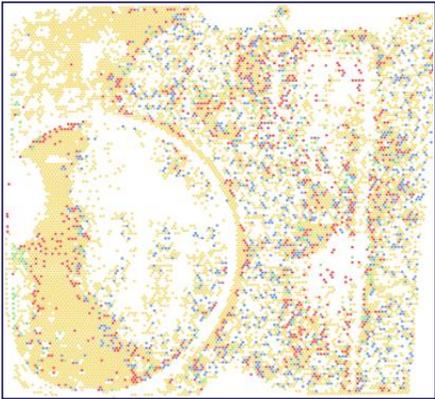
Yellow around the edges is a strong indication of evaporation

Evaporation



- Immersion fluid was not added to chip before applying chip lid, but was added after lid was applied.

Leaked Chips: Examples



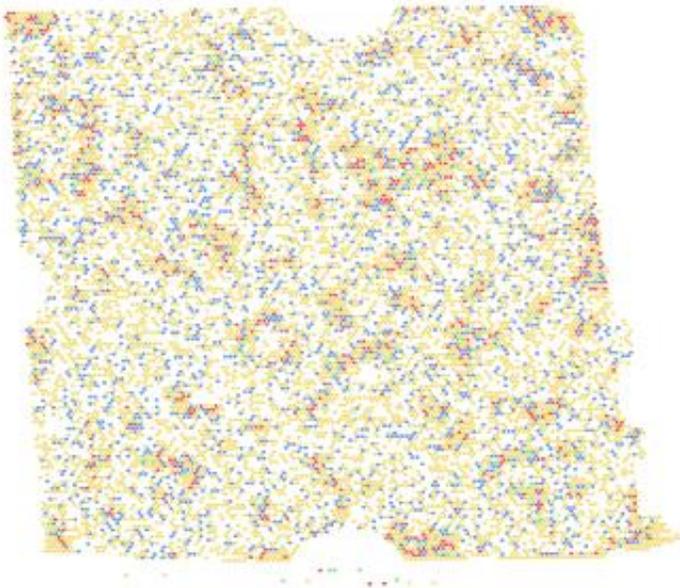
Leaking



Most leaks are from manually loading chips. The chip loader significantly decreases chance of leaks

Double Spread

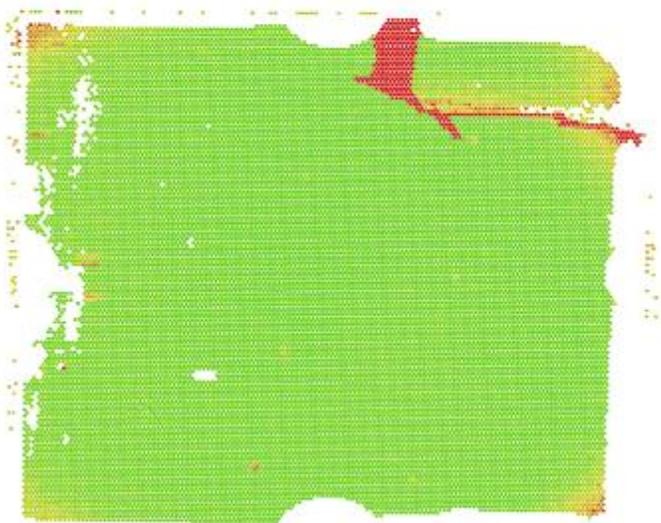
Chip view



Cracked Chip

B1070M_130719_141047.EQS Data exceeding

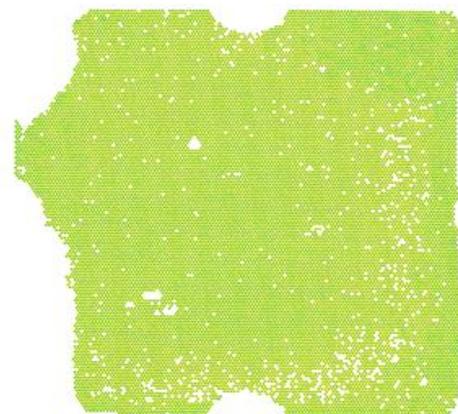
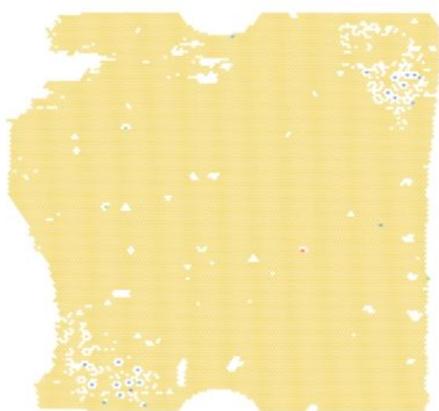
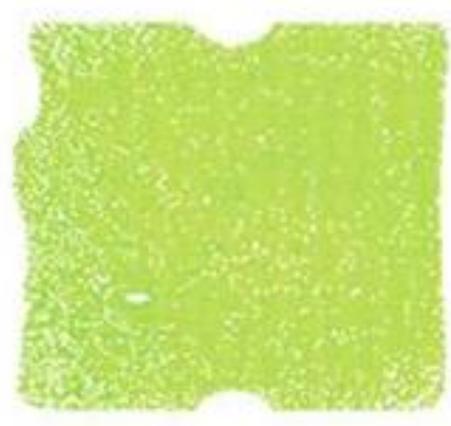
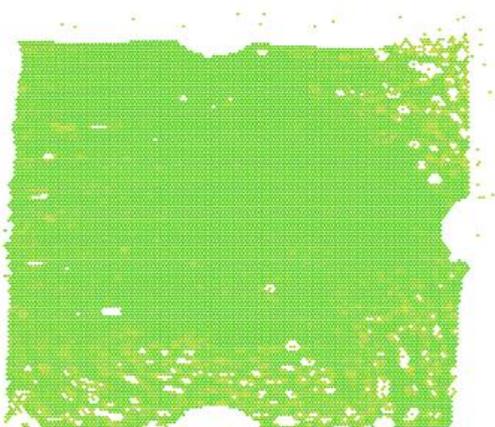
Chip view



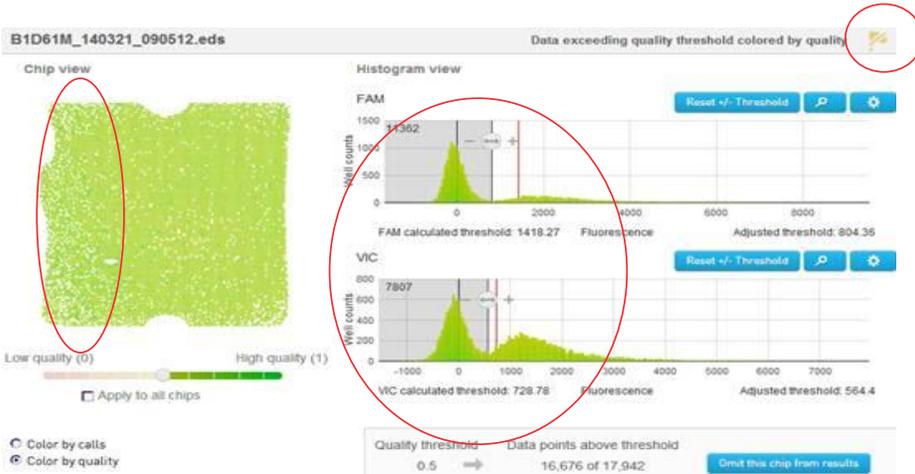
Condensation

Allow chips to warm to room temperature after being held at 10 deg C on thermal cycler.

Note: Sometimes condensation is not obvious.



Effects of Condensation on Chip

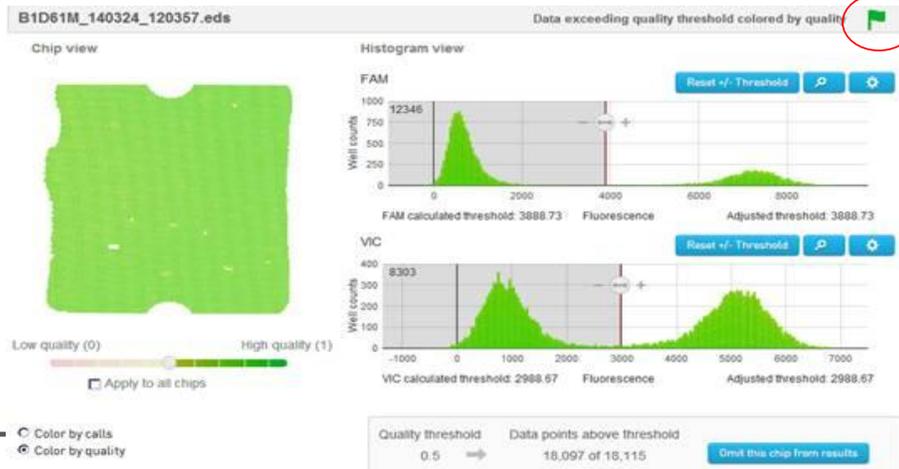


This is the **same chip**, read directly out of the thermal cycler, and then allowed to warm to room temp.

Warming up the chips increases the:

- data quality
- flag
- separation of the amplified vs non-amplified peaks.

Note: Condensation was **not** visible on the chip



Prevent Aqueous Condensation – QuantStudio™ 3D Digital PCR Chips

- Confirm thermal cycler run has completed, and is at the 10deg hold.
- Do not open heated lid

To stop the run (from a final hold):

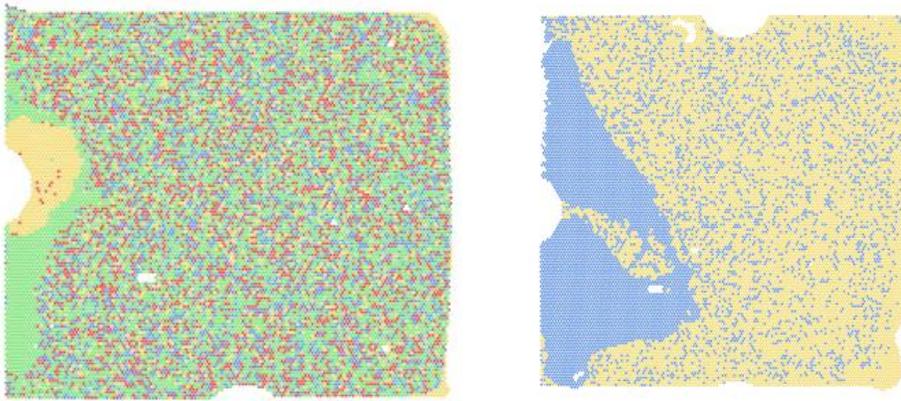
GeneAmp® 9700	Press STOP twice to stop the run, then Exit
Proflex™	Press Stop Run , then OK to Stop the current run



- With heated lid closed, allow thermal cycler to sit ~5 min
- Remove chips

Note: Any other condensation may be immersion fluid. Use lint-free wipe with Isopropanol or Ethanol to remove immersion fluid from lid

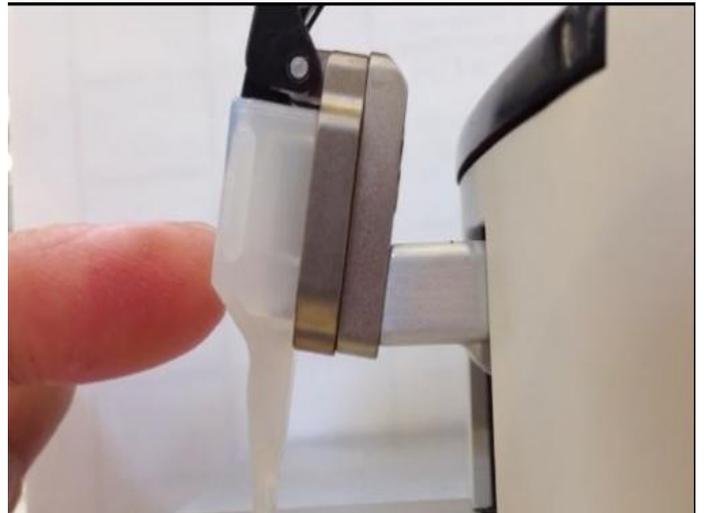
Reaction Pooling



- Too much sample left in loading blade; reaction mix accumulated on the chip.
- Load only 14.5 μl of reaction mix into loading blade.
- Possible cause: pipettes in need of recalibration – can try loading 14 μl instead.

Loading blade not flush; stuck at beginning of load

Make sure loading blade is flush / contacting metal surface



Questions



The QuantStudio® 3D Digital PCR System is For Research Use Only. Not for use in diagnostic procedures.

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