Temperature non-uniformity detection on dPCR chips and temperature sensor calibration (Supplementary data)

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Supplementary Section A: The melting curves and its derivative after amplification from a commercial PCR thermal cycler

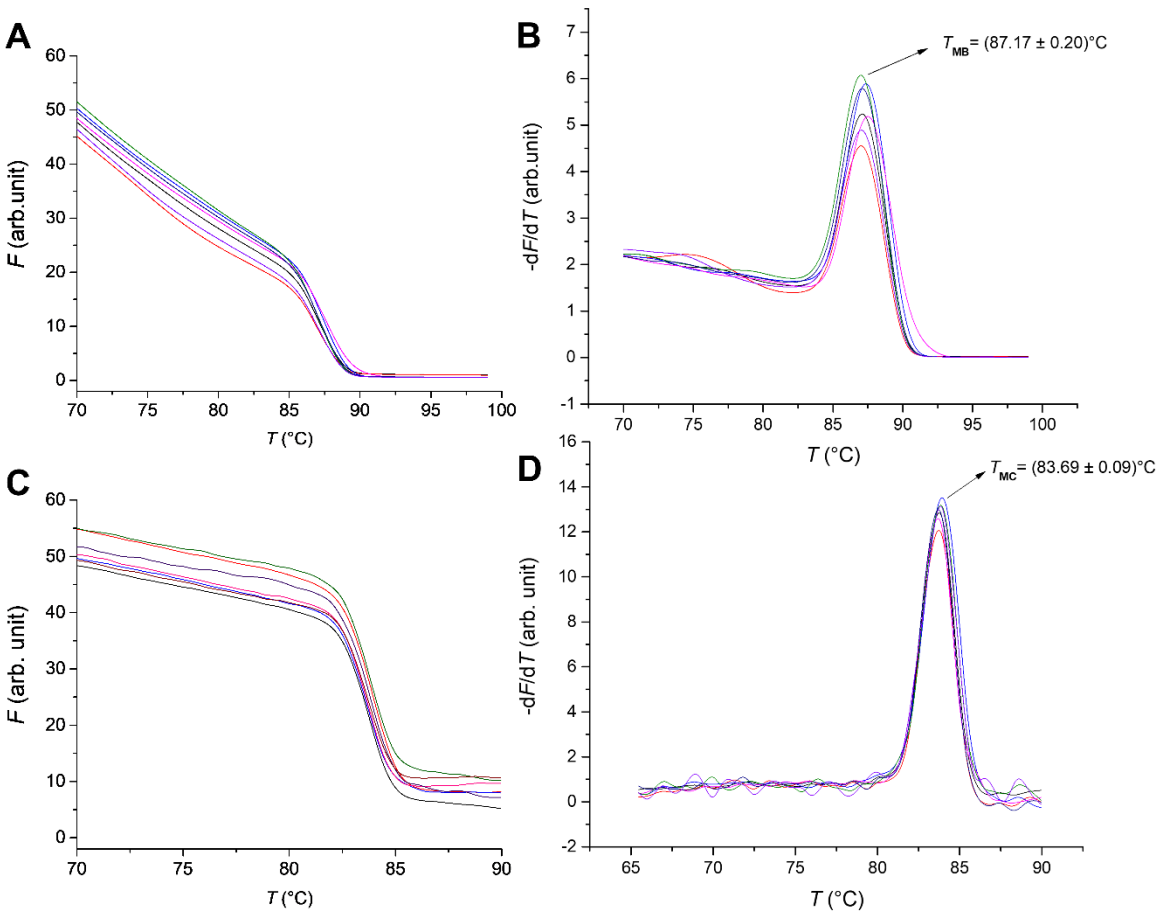

Figure S1: The qPCR MCA measurements. (A)Seven melting curves of HBV gene and (B) its –d*F*/d*T with mean value of* T_{MB}*. (C)Seven melting curves of Chr21 gene and (D) its* –d*F*/d*T with mean value of* T_{MC}.

Supplementary Section B: A fluorescent images of dPCR chip

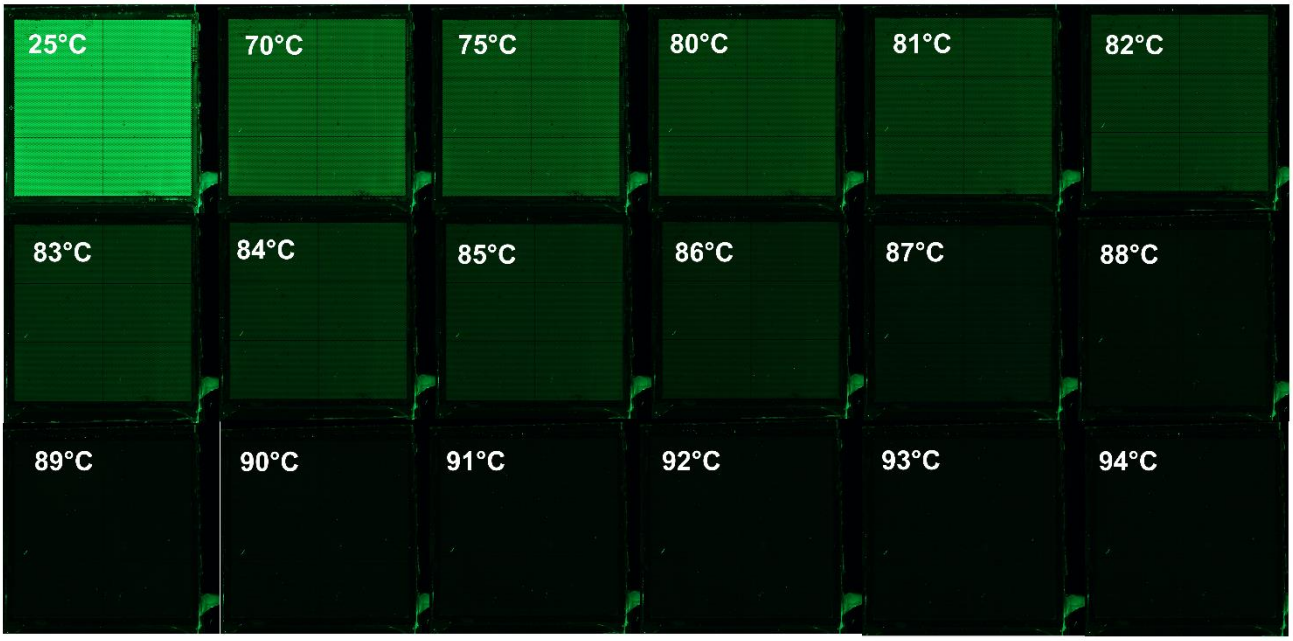

Figure S2: The fluorescence images of dPCR chip in the T_S range from 25℃ to 94℃ loaded with the PCR amplicons of HBV target gene covered with mineral oil and a cover glass.

Supplementary Section C MATLAB codes

Analysis.m

```
%%  Read image.



clear;clc;close all;warning off;
%%
% load centers_final;

img=imread('25.JPG');
img_double=double(rgb2gray(img))./255;
figure;imshow(img_double);hold on;
leftup=[1435,648];rightup=[3398,733];
leftdown=[1353,2595];rightdown=[3314,2679];
Coor_Real=StandardChip(0,47.6314,1);
Lenx=max(Coor_Real(:,1))/2;
Leny=max(Coor_Real(:,2))/2;
Coor_Real=Coor_Real-[Lenx,Leny];

hold on;
basepoints=[leftup;rightup;leftdown;rightdown];
inputpoints=[-Lenx,-Leny;Lenx,-Leny;-Lenx,Leny;Lenx,Leny];
tform=fitgeotrans(basepoints,inputpoints,'projective');
centers_final=zeros(26448,2);
for i=1:1:26448
    Coor_Grid=Coor_Real(i,:);
    centers_final(i,:)=transformPointsInverse(tform,Coor_Grid);
end
```

```
scatter(centers_final(:,1),centers_final(:,2),5,'g','filled');
save centers_final centers_final


%%
% load results;
Temp=["25","70","75","80","81","82","83","84","85","86","87","88","89","90","91
","92","93","94"];
len=numel(Temp);
load centers_final;
Fluo=zeros(26448,len);
centers_round=round(centers_final);
Rad=1;
model_orig=getnhood(strel('disk',Rad));
model_orig=double(model_orig);
for i=1:1:len
   str_temp=char(strcat(Temp(i),'.JPG'));
   img=imread(str_temp);
   img_final=double(rgb2gray(img))./255;
   figure;imshow(img_final); hold on;
   scatter(centers_round(:,1),centers_round(:,2),5,'r','filled');title('Detected circles');
   for j=1:1:26448
      cir_temp=img_final(centers_round(j,2)-
Rad:centers_round(j,2)+Rad,centers_round(j,1)-Rad:centers_round(j,1)+Rad);
      cir_temp=cir_temp.*model_orig;
        Fluo(j,i)=mean(mean(cir_temp));
   end
end
Fluo=Fluo';
Temp=(str2double(Temp))';
```

```
load chirp
save results Fluo Temp
sound(y,Fs)
%%
img=imread('25.JPG');
img_final=double(rgb2gray(img))/255;
img_show=cat(3,img_final,img_final,img_final);

Fluo_diff=-diff(Fluo);
filter1=Fluo_diff(1,:)>0;

Jud=Fluo_diff<0;
filter2=sum(Jud(1:end-1,:))<3;

filter=filter1&filter2;
index_fill=find(filter);
num_fill=numel(index_fill);
%%
Fluo_filtered=Fluo(:,index_fill);
xi=60:0.1:90;
Temp_melt_ind=zeros(num_fill,1);
yi_all=zeros(numel(xi),num_fill);yi_diff_all=zeros(numel(xi)-1,num_fill);
wa=waitbar(0,'Please wait....');
for i=1:1:num_fill
    yi=interp1(Temp,Fluo_filtered(:,i),xi,'PCHIP');
    yi_diff=-diff(yi);

    yi_all(:,i)=yi';
```

```
    yi_diff_all(:,i)=yi_diff';

    [~,Temp_melt_ind(i)]=max(yi_diff);
    waitbar(i/num_fill);
end
close(wa);
Temp_melt=xi(Temp_melt_ind);
%figure;plot(xi,yi_all);title('Fluo as function of temperature');
%figure;plot(xi(2:end),yi_diff_all);title('dF/dT as function of temperature');
axis([65,90,0,inf]);
figure;histogram(Temp_melt);
%%
rad=5;
model_orig=getnhood(strel('disk',rad));
model_orig=double(model_orig);
centers_round=round(centers_final);
col=jet(255);
for k=1:1:num_fill
    col_temp=round((Temp_melt(k)-80)/15*255);
    %col_temp=round((Temp_melt(k)-64)/20*255);
    try
        img_show_temp=img_show(centers_round(index_fill(k),2)-(rad-
1):centers_round(index_fill(k),2)+(rad-1),...
        centers_round(index_fill(k),1)-(rad-1):centers_round(index_fill(k),1)+(rad-
1),:);
        img_show(centers_round(index_fill(k),2)-(rad-
1):centers_round(index_fill(k),2)+(rad-1),...
        centers_round(index_fill(k),1)-(rad-1):centers_round(index_fill(k),1)+(rad-
1),:)=...
```

```
cat(3,model_orig.*col(col_temp,1),model_orig.*col(col_temp,2),model_orig.*col(c
ol_temp,3))+cat(3,~model_orig,~model_orig,~model_orig).*img_show_temp;
    catch
    end
end
figure;imshow(img_show);title('Distribution of melting Temperature');
%colorbar('Ticks',[0,0.25,0.5,0.75,1],...

%'TickLabels',{'70','75','80','85','90'});
colorbar('Ticks',[0,0.2,0.4,0.6,0.8,1],...
        'TickLabels',{'80','83','86','89','92','95'});
gcf
saveas(gcf,'result.png');
imwrite(img_show,'result.tif');
Show3DMap(Temp_melt',index_fill)
```

Show3DMap.m

```
function [Map_fit_show] = Show3DMap(Matrix_new,Ind)
%UNTITLED7 ´Ë´¦ÏÔÊ¾ÓÐ¹Ø´Ëº¯ÊýµÄÕªÒª
%     ´Ë´¦ÏÔÊ¾ÏêÏ¸ËµÃ÷
Coor_Real=StandardChip(0,47.6314,1);
Lenx=max(Coor_Real(:,1))/2;
Leny=max(Coor_Real(:,2))/2;
Coor_Real=Coor_Real-[Lenx,Leny];
Lenx_ex=Lenx+25;Leny_ex=Leny+25;
len1=1000;x_grid=linspace(-Lenx_ex,Lenx_ex,len1);
len2=1000;y_grid=linspace(-Leny_ex,Leny_ex,len2);
[X,Y]=meshgrid(x_grid,y_grid);
%%
Map_interp=scatteredInterpolant(Coor_Real(Ind,:),Matrix_new,'linear','linear');
%Map_interp=scatteredInterpolant(Coor_Real(Ind,:),Matrix_new(Ind,:),'linear','lin
ear');
ft=fittype('poly22');
Map_fit=fit(Coor_Real(Ind,:),Matrix_new,ft,'Normalize','on');
%Map_fit=fit(Coor_Real(Ind,:),Matrix_new(Ind,:),ft,'Normalize','on');


Map_interp_show=zeros(len1,len2);
Map_fit_show=zeros(len1,len2);


wait=waitbar(0,'Constructing 3D Map, Please wait for seconds..');
for i=1:1:len1
   for j=1:1:len2
      Map_interp_show(i,j)=Map_interp(x_grid(i),y_grid(j));
      Map_fit_show(i,j)=Map_fit(x_grid(i),y_grid(j));
```

```
    end
    waitbar(i/len1);
end
close(wait);
Map_interp_show=imgaussfilt(Map_interp_show,30);
%%
figure;
% subplot(1,2,1);
s1=surf(X,Y,Map_interp_show);s1.LineStyle='none';
title('3D distribution Map of Tm');
xlabel('X-axis  Uint:um');
ylabel('Y-axis  Uint:um');
zlabel('Melting temperature (Tm)');
% axis([-Lenx_ex Lenx_ex -Leny_ex Leny_ex 0.5 1.5]);
%%
figure;
% subplot(1,2,2)
s2=surf(X,Y,Map_fit_show);s2.LineStyle='none';
title('Fitted 3D-Map');
xlabel('X-axis  Uint:um');
ylabel('Y-axis  Uint:um');
zlabel('Melting temperature (Tm)');
% axis([-Lenx_ex Lenx_ex -Leny_ex Leny_ex 0.5 1.5]);

end
```

StandardChip.m

```
function [Coor] = StandardChip( x,y,bili)
ori1=[];
ori2=[];
for i=0:1:75
   for j=0:1:28
      temp=[55*i,95.262794*j];
      ori1=[ori1;temp];
   end
end
ori2(:,1)=ori1(:,1)+27.5;
ori2(:,2)=ori1(:,2)-47.6314;
deltax=4205;
deltay=2792;
OneBlock=[ori1;ori2];

Coor1=OneBlock+([deltax*0;deltay*0]*ones(1,4408))';
Coor2=OneBlock+([deltax*0;deltay*1]*ones(1,4408))';
Coor3=OneBlock+([deltax*0;deltay*2]*ones(1,4408))';
Coor4=OneBlock+([deltax*1;deltay*0]*ones(1,4408))';
Coor5=OneBlock+([deltax*1;deltay*1]*ones(1,4408))';
Coor6=OneBlock+([deltax*1;deltay*2]*ones(1,4408))';
Coor=[Coor1;Coor2;Coor3;Coor4;Coor5;Coor6]./bili;
Coor=Coor+[x,y];

end
```

Supplementary Section D: The melting curves and its derivative extracted from five partitions of the dPCR chip

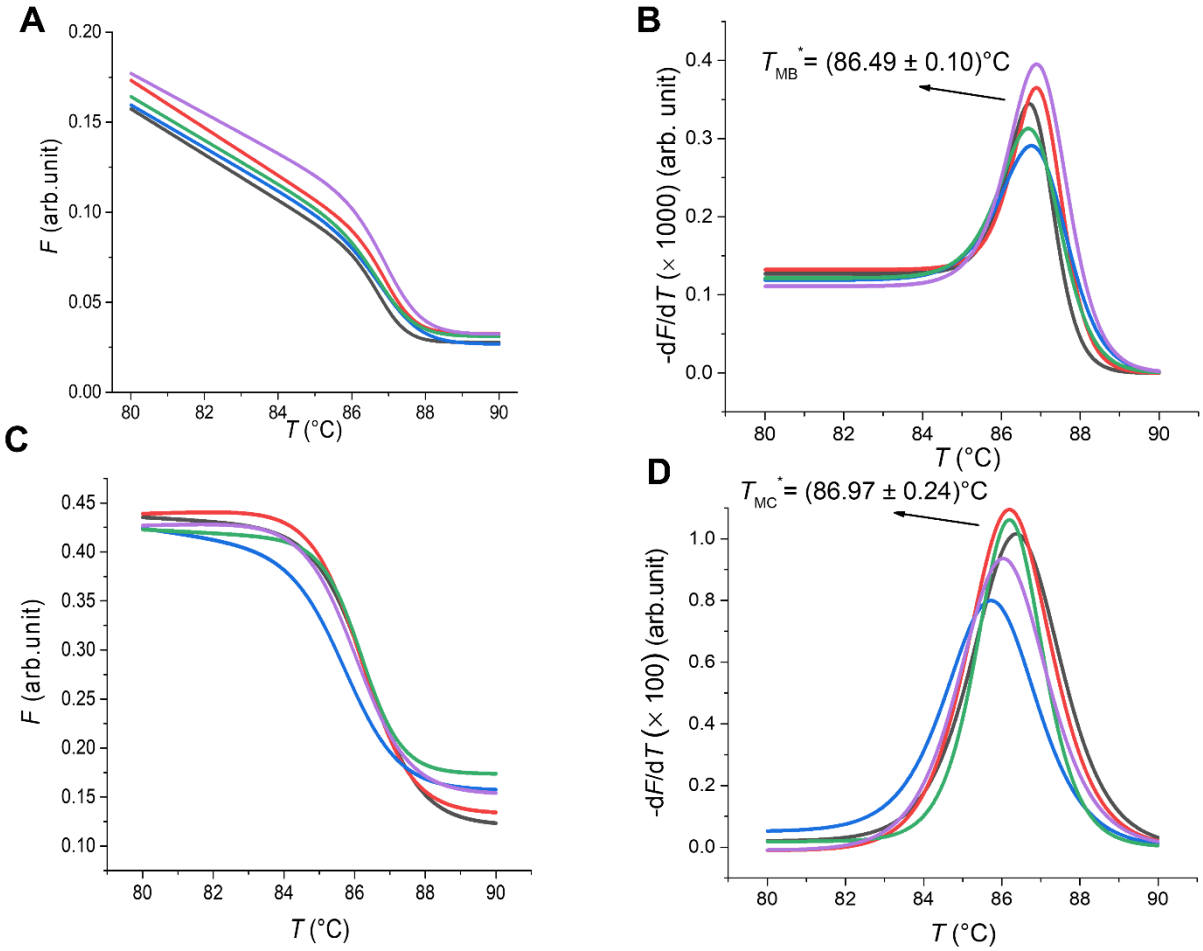

*Figure S3:MCA data from five partitions of a dPCR chip filled with (A) HBV target gene and (B) –*d*F/*d*T as function of T and its mean values of* T_{MB}^{*}*, (C) Chr21 target gene and (D) –*d*F/*d*T as function of T and its mean values of* T_{MC}^{*}*.*

Supplementary Section D: 2D distribution map of T_M^*

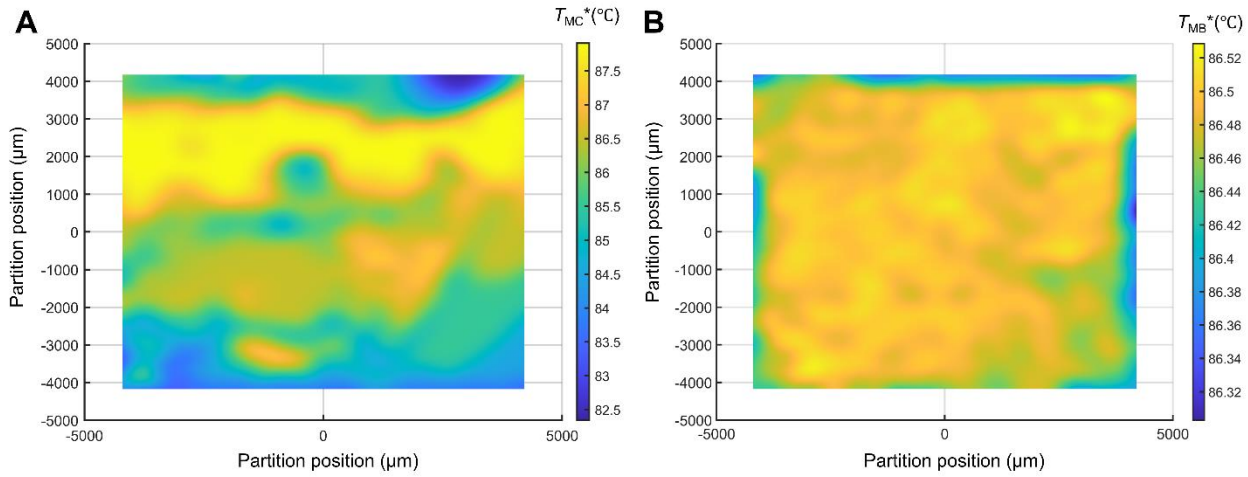

Figure S4:2D distribution map of (A)T_{MC}^* measured by setup A and (B) T_{MB}^* measured by setup B for better visualization of temperature distribution across the dPCR chip area.