A New Detection Modality for Label-Free Quantification of DNA in Biological Samples Via Superparamagnetic Bead Aggregation

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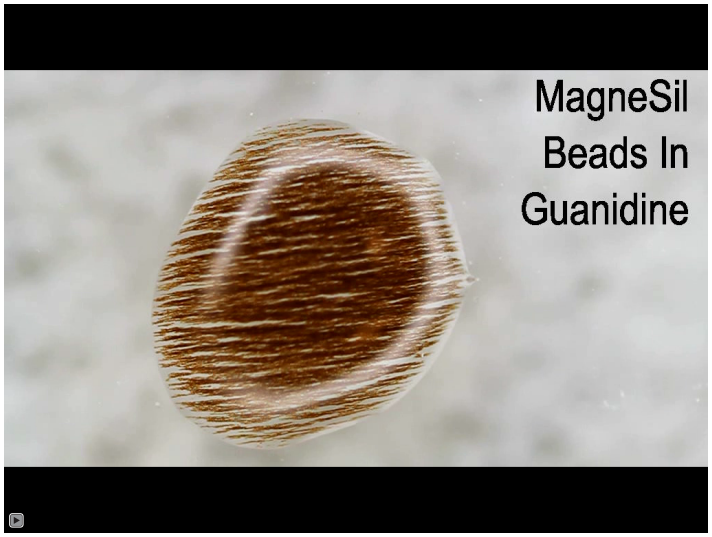
SUPPORTING INFORMATION

Supporting Information

Video 1 - Video demonstrating the pinwheel effect for visual detection of DNA with superparamagnetic microparticles. A 3 µL droplet (3 mm in diameter) of chaotropic DNA-binding solution (6 M guanidine hydrochloride) containing ~10^5 beads silica-coated magnetic beads is placed on a piece of glass and exposed to a rotating magnetic field (RMF) starting at the video time of 3 sec. A nanogram of lambda phage genomic DNA is added (9 sec) and multiple, visible pinwheel aggregates immediately form. Over time (44 secs) these smaller aggregates coalesce into a single large aggregate (by 52 sec). This visually striking transformation is reversible upon termination of the RMF (53 sec),replacement of the DNA-binding solution (59 sec) with DNA-eluting solution (1X Tris-EDTA) (60 sec), and re-initiation of the RMF (61 sec), leading to rapid dis-aggregation of the pinwheels.



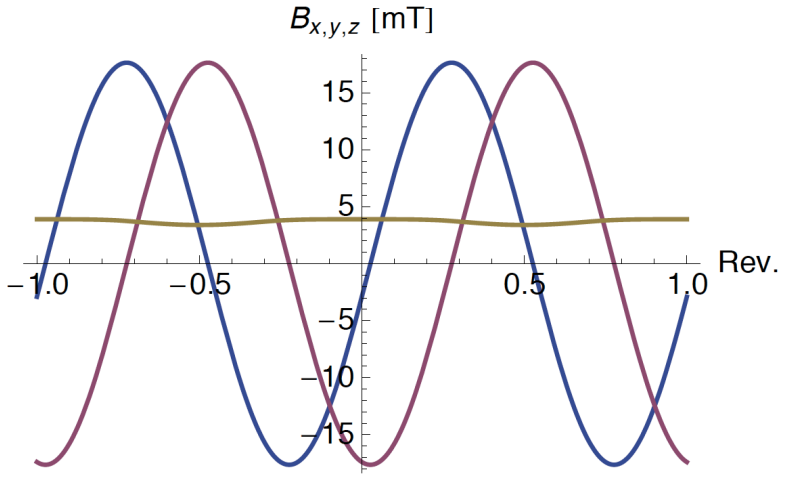


Figure S1. Hall sensor measurement of the x, y, and z components of the magnetic field in the sample well as the magnetic field is rotated.

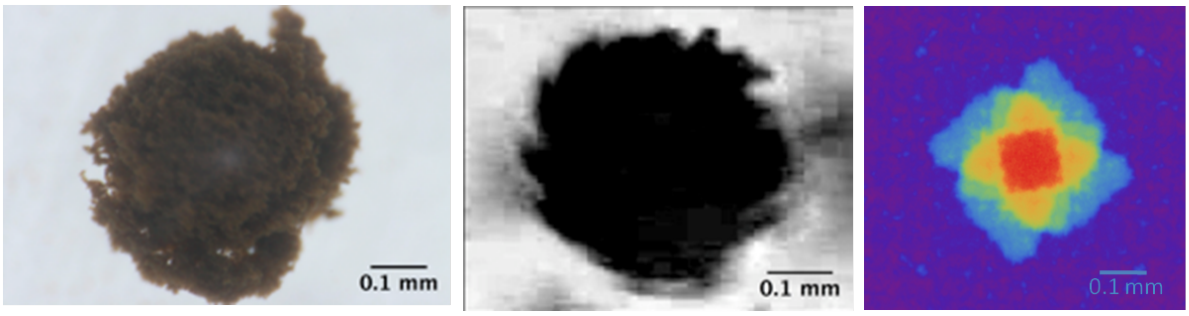


Figure S2. Photograph of pinwheel (A) formed by aggregation of superparamagnetic silica-coated microparticles (8 um average diameter) with addition of 1.6 ng of lambda phage genomic DNA. Photograph of pinwheel in motion under same conditions (B) and false color overlay of 4 photographs of the pinwheel in motion (C), clearly demonstrating the pinwheel shape.

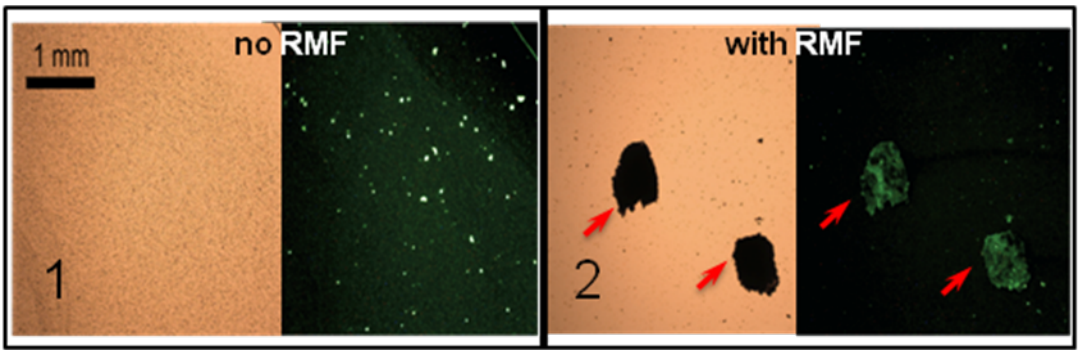


Figure S3. Further confirmation that DNA is critical to the formation of pinwheel aggregates in the presence of a RMF is demonstrated by exploiting amine-modified superparamagnetic particles interacting electrostatically with fluorophore-labeled DNA in the absence of chaotropic salt (fluorescence of common DNA intercalators (e.g., ethidium bromide) is quenched by guanidinium). In the absence of a RMF, the particles and labeled DNA are broadly distributed throughout the well (1). Once the RMF is applied, dense aggregates with labeled DNA form (2). Bright field (left) and fluorescence micrographs (right) of beads with EvaGreen staining without (1) and with RMF (2).

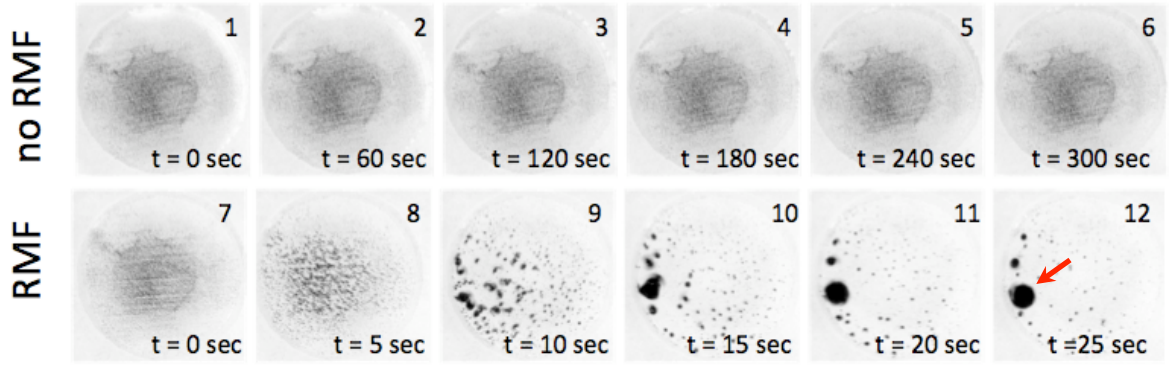


Figure S4. Lambda phage genomic DNA (50 ng) was added to silica-coated super-paramagnetic particles (MagneSil) in a microwell (5 mm diameter) filled with a chaotropic, DNA-binding solution (6 M guanidine hydrochloride) above a stationary magnetic field at t = 0 s [1]. No pinwheel or aggregation is observed over 5 min [2-6]. The same conditions are repeated with a rotating magnetic field, and aggregation of particles starts to form within 5 seconds [7]. Within 25 s [8-12], distinctive, circular pinwheels are readily apparent (arrow).

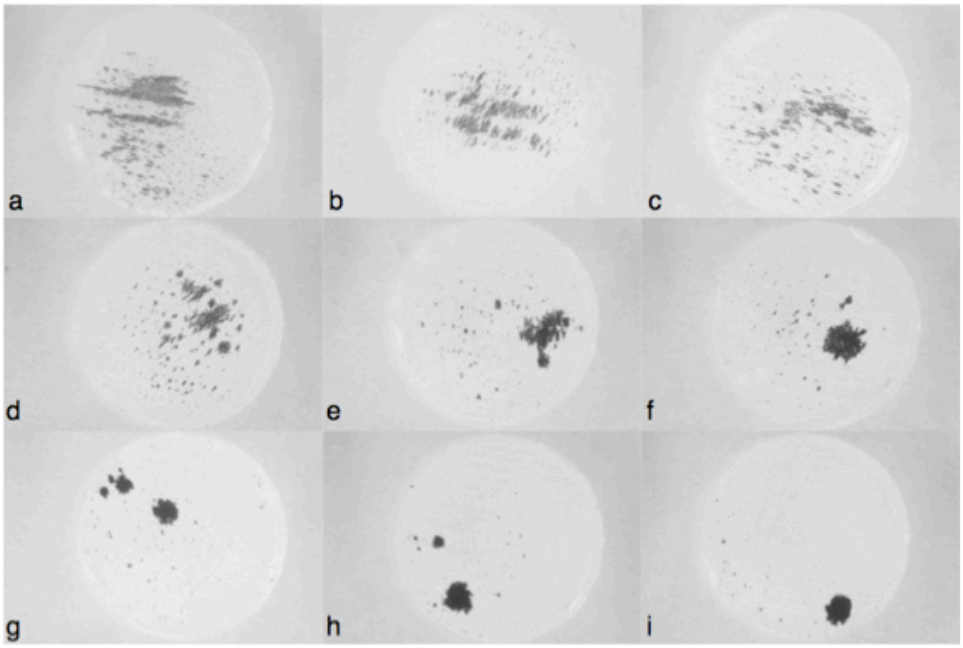


Figure S5. Images of increasing amounts of lambda phage genomic DNA added to silica-coated superparamagnetic particles in chaotropic solution (6 M GnHCl) after 5 min on a rotating magnetic field. Added DNA concentration was 0 (a), 5 (b), 10 (c), 15 (d), 20 (e), 30 (f), 40 (g), 60 (h), and 80 (i) pg/μL.

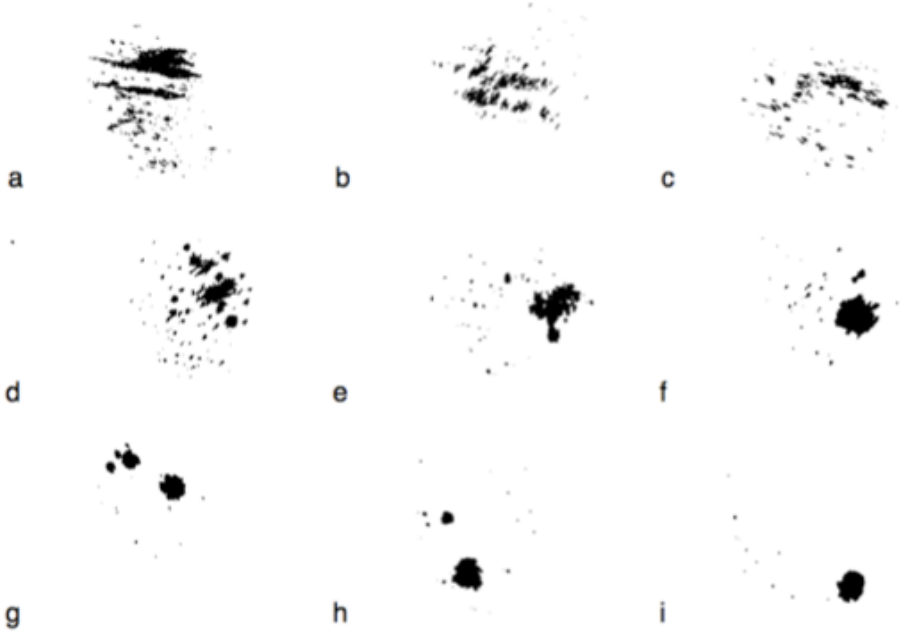


Figure S6. Processed, binarized images of increasing amounts of lambda phage genomic DNA added to silica-coated superparamagnetic particles in chaotropic solution (6 M GnHCl) after 5 min on a rotating magnetic field (from Fig. S2). Threshold was set in Mathematica software by the isodata algorithm. Added DNA concentration was 0 (a), 5 (b), 10 (c), 15 (d), 20 (e), 30 (f), 40 (g), 60 (h), and 80 (i) pg/μL.

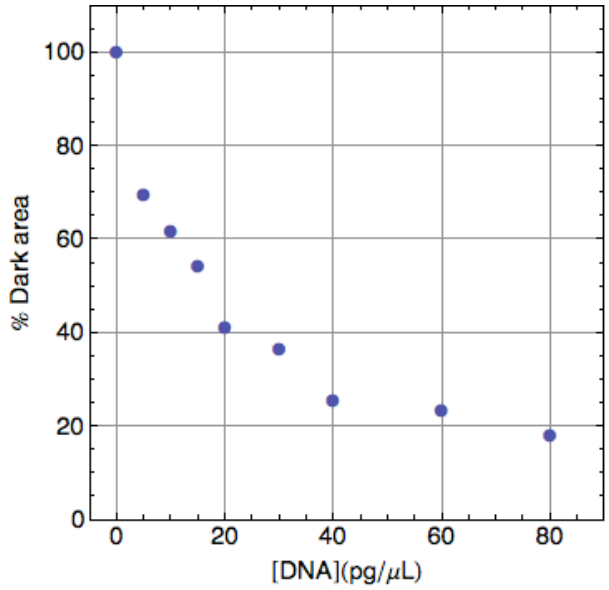


Figure S7. Plot of the dark area from the binarized images in Fig. S3 normalized to the 0 pg/μL data point vs. the lambda phage genomic DNA concentration added. A significant decrease in % dark area is seen with addition of DNA.

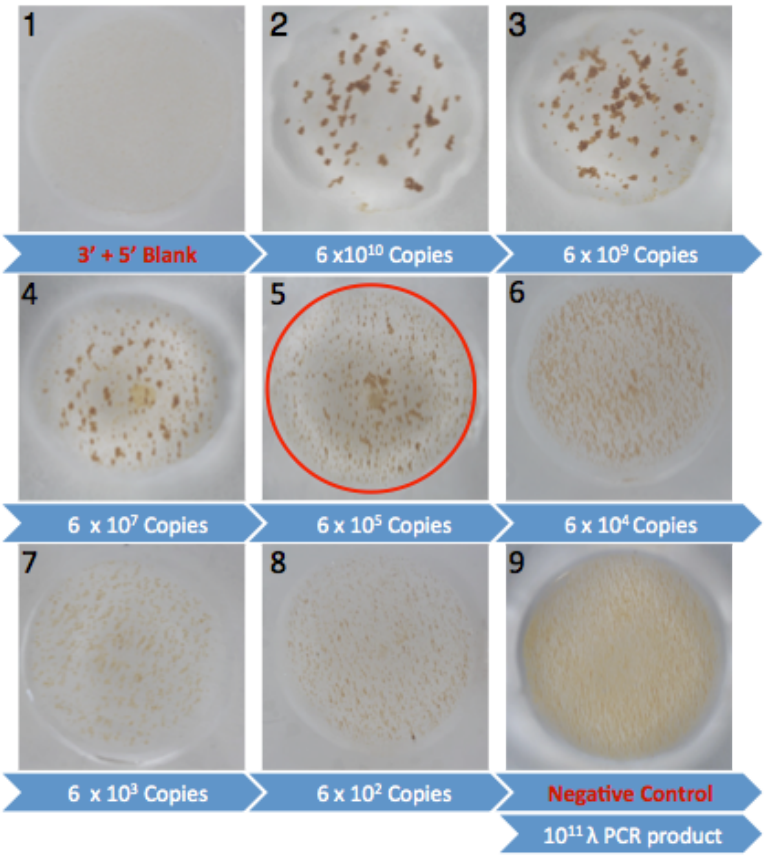


Figure S8. Photographs of sequence-specific DNA hybridization pinwheel assay for a 26-base ssDNA target (connector) binding to two populations of complimentary DNA-modified particles. No aggregation was observed in the absence of DNA with only DNA-coated superparamagnetic particles present (1). Aggregation was observed throughout a 10-fold serial dilution from 6×10^{10} copies of connector copies down to 600 copies (2-8). Less aggregation was observed in the presence 10^{11} copies of a 500-bp, non-complementary sequence (9). Red circle illustrates the LOD for a non-specific fluorophor-based detection system (Nanodrop).

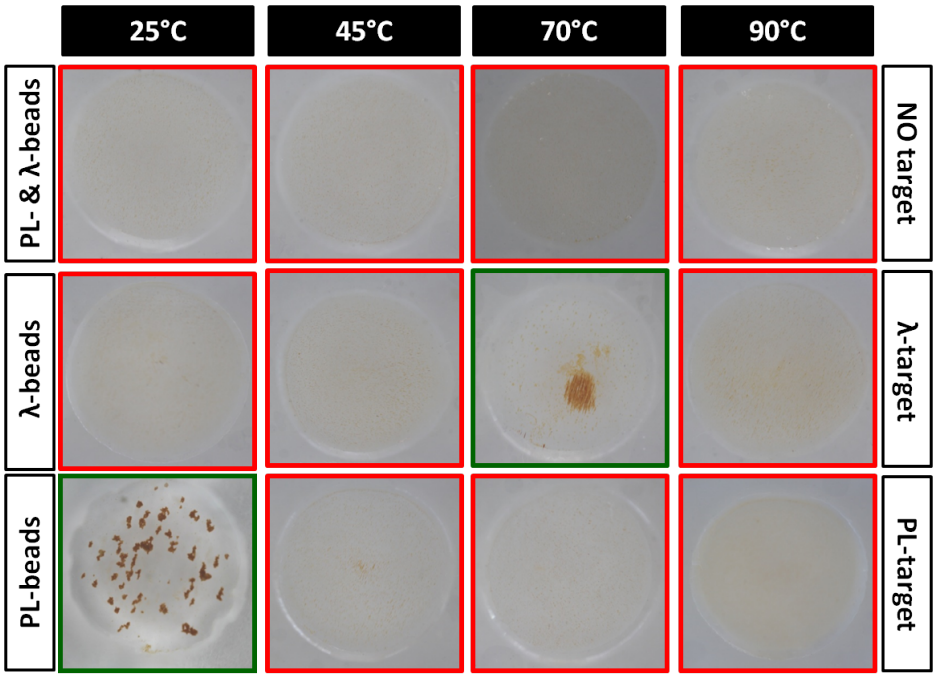


Figure S9. Photographs of sequence-specific DNA hybridization pinwheel assay illustrating specificity of PL-beads, λ-beads and a combination of both to aggregate in a target- and temperature-dependent manner. Top row shows that the beads do not aggregate without DNA. Middle row shows the λ-beads only pinwheel at the hybridization temperature expected for the sequences (75 °C), and the bottom row shows the PL-beads only pinwheel at the room temperature as expected for those sequences (25 °C).

The isodata algorithm written in Mathematica for image thresholding and binarization.

```
SetDirectory["Desktop/16"];
```

Set the directory to be the folder where the images are stored and the results will be saved.

```
func1[histodata_,threshold_]:=
  Module[{mean1,mean2,thr,greylevel,i},
   greylevel=Table[i,{i,256}];
   If[Total[Take[histodata,threshold]]==0,mean1=0,
    mean1=Round[Total[Take[greylevel*histodata,threshold]]/Total[Take
[histodata,threshold]]]];
   If[Total[Take[histodata,threshold-256]]==0,mean2=0,
    mean2=Round[Total[Take[greylevel*histodata,threshold-256]]/Total
[Take[histodata,threshold-256]]]];
   thr=Round[(mean1+mean2)/2]
   ];
```

Function 1 utilizes a histogram of an image and a threshold value as input. The threshold splits the histogram into two parts: below threshold and above threhshold. This function calculates the mean gray level below the threshold, and the mean gray level above the threshold, and then generates the average value of the two mean gray levels as output.

```
func2[filenames_]:=
  Module[{data1,data2,data3,threshold,darkarea,b},
   data1=ImageData[ColorConvert[ImageResize[Import[filenames],Scaled
[1/10]],"HSB"]];
   data2=data1 //. {a_,b_,c_}->c;
   data3=BinCounts[Flatten[data2],{0,1,1/256}];
   threshold=FixedPoint[func1[data3,#]&,128];
   darkarea=Total[Take[data3,threshold]]
   ];
```

Function 2 imports an image (Figure S5) in HSB (hue-saturation-brightness) color space, resizes it to 1/10 of the original, and transforms the brightness value into a histogram. Function 1 is then repeatedly applied to the histogram with a specified initial threshold value, one typically found between the extremes of the grey levels in the histogram, until the output no longer changes, and the result is the final threshold. The pixels in the images are now categorized into two groups by the threshold (Figure S6), and the black pixels, i.e. the ones below the threshold, represent the beads in the images. The dark area is defined as the total number of black pixels.

```
filenames=FileNames["*.JPG"];
filenumber=Total[Dimensions[filenames]];
results=Table[func2[filenames[[i]]],{i,filenumber}];
Export["result.xls",
  {Transpose[Join[{filenames},{results}]]}];
```

JPG files in the directory are recognized, imported, and analyzed with Function 2, and the results, i.e. dark area for each JPG file, are exported as a excel file. Normalized dark area values are calculated in the excel file and correlated with their corresponding DNA concentrations (Figure S7).

Sequences of Hybridization Induced Aggregation Probes

For room temperature hybridization probes (PL-probes) used:
Biotin-TTTTTTTATGTGGTCTATGTCGTCGTTCGCTAGTAGTTCCTGGGCTGCAC
TCGAGGCGTAGAATTCCCCCGATGCGCGCTGTTCTTACTCATTTTT-Biotin

Target DNA:
AAATACGCCTCGAGTGCAGCCCATTT

Reconfigured sequence used for competitive inhibition:
AAACAGAGGCCTACACCTCTGCTTTT

For lambda PCR product detection, probes used:
Biotin-TTTTTTGGTTATCGAAATCAGCCACAGCGCC
CCAGTTGTACGAACACGAACTCATCTTTTTT-Biotin