Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplemental Information for "Screening of nutritional and genetic anemias using elastic light scattering"

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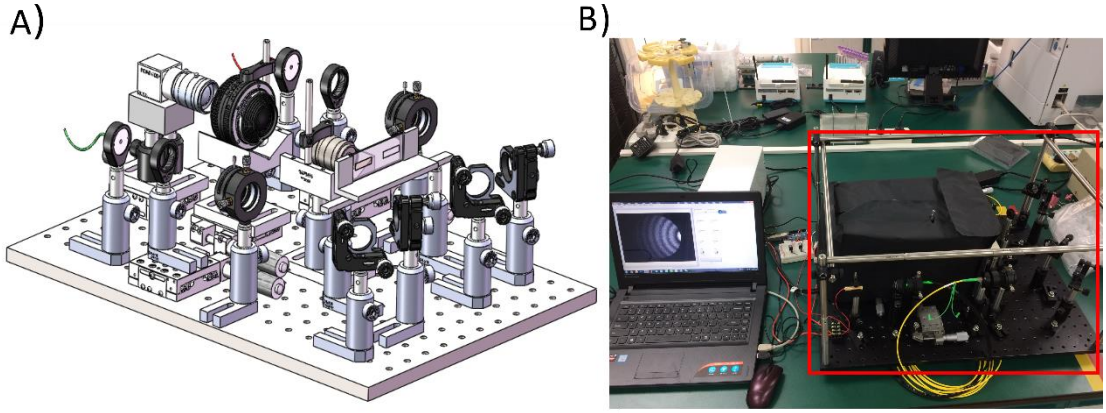

Fig. S1 (A) 3-D rendering of system, (B) Physical prototype(red region) in the clinical laboratory of the Children's Hospital of Chongqing Medical University.

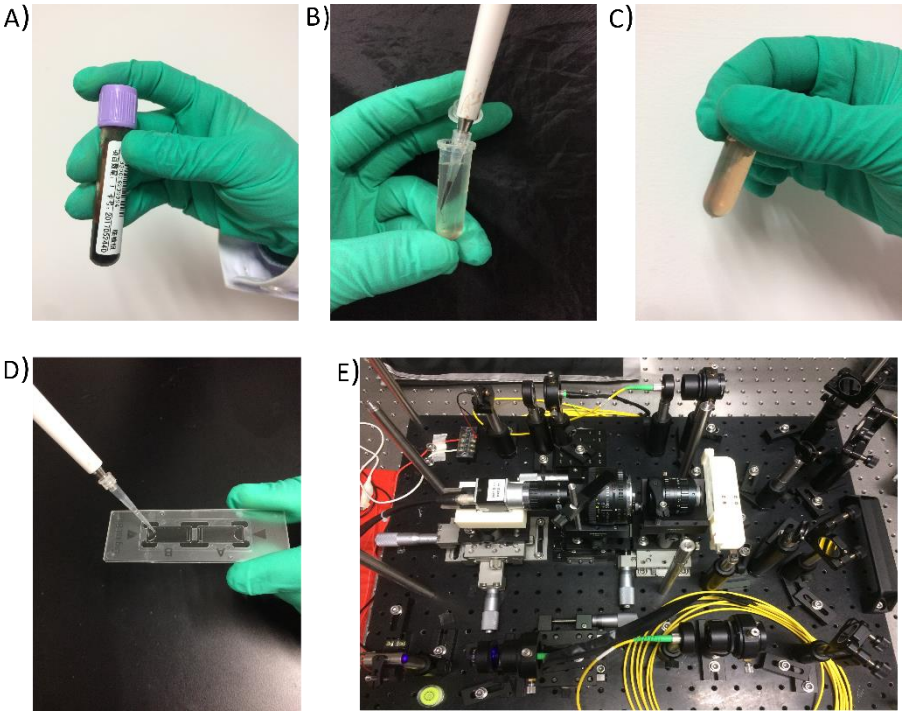

Fig. S2 (A) Blood is collected from a finger stick or venous draw. (B) 10 uL is pipetted into a preprepared PBS+SDS solution and (C) mixed. (D) the diluted and sphered blood is transferred to a disposable sample chamber and (E) placed in the system for measurement.

Portability and Cost Analysis:

As seen in Figure S1, and Figure S2(E), the prototype system is still relatively large. Its volume is about 1 cubic foot, although as Figure S2(E) makes clear, the majority of this is empty space. Therefore, substantial miniaturization is expected in future versions of the instrument. Nevertheless, the system is small enough to be physically transported to remote locations, as we did when we moved it from our laboratory in Hefei to our field location in Chongqing.

The cost-effectiveness of our instrument can be evaluated based on the cost per instrument and cost per test. For the cost per instrument, this can be compared with US$50,000 - US$150,000 for standard clinical instrumentation to perform a CBC. For our instrument, the total cost for our one-off prototype is US$3,550. The largest costs are the camera and optical lenses used in our system, these sum to US$1,400. These could potentially be further optimized (using a lower quality camera, discounts due to volume production, etc.), but represent the limiting factors in the cost of our device. The remaining US$2,150 comes from the optical mounting hardware, including precision translation mounts and other

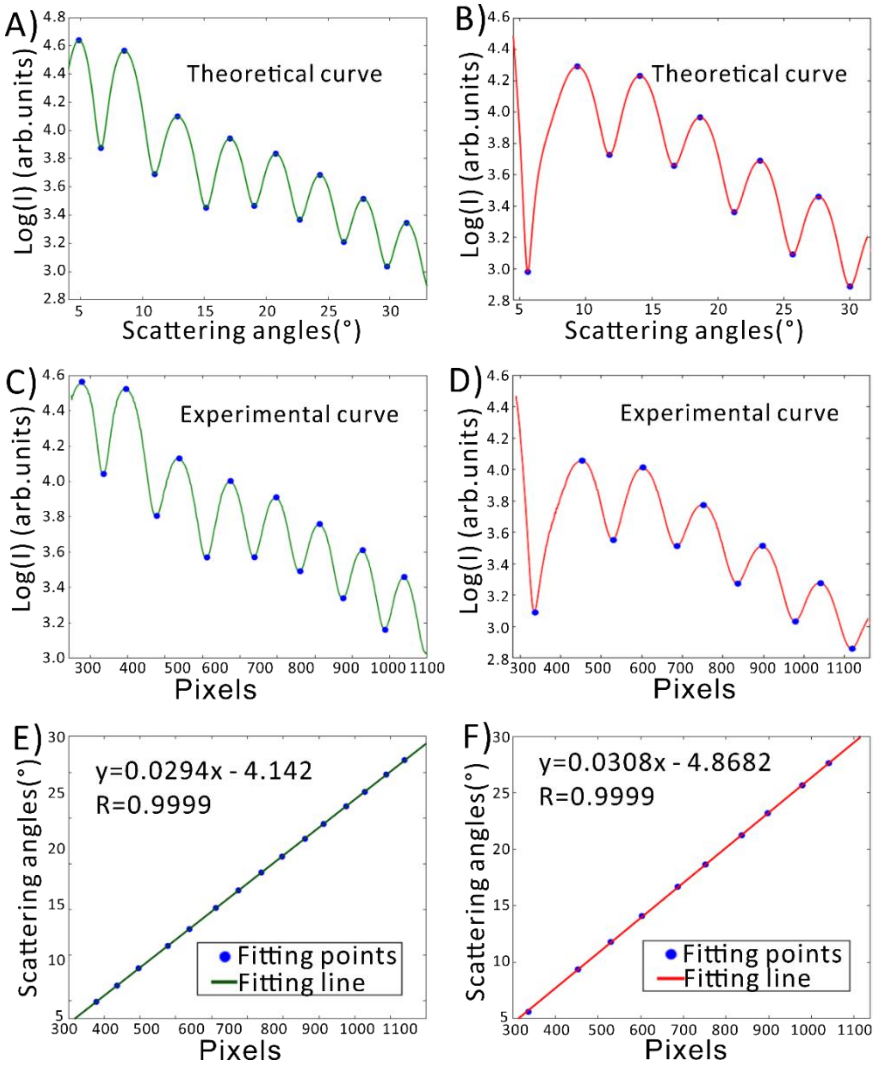

Fig. S3 Pixel to angle calibration. (A) and (B) are theoretical curves for 7 micron polystyrene beads following the manufacturer provided size information, and illuminated by green and red lasers, respectively. Blue points represent angular peak locations for each fringe. (C) and (D) Experimentally measured green and red scattering curves for the same polystyrene sample. Blue points represent pixel locations of fringe maxima. (E) and (F) show angular locations vs. corresponding pixel values for the blue points in (A)-(D). Linear fits provide a pixel-to-angle calibration for all pixels.

elements not needed in a production model. While this cost is significant in a prototype stage, we expect these costs to be substantially reduced were the instrument to actually be commercialized for widespread clinical use. For example, our prototype is built with standard optical posts, post holders, and a relatively heavy optical breadboard, none of which would be expected to remain in a production model, substantially lowering weight and cost.

For the cost per test, in our test this amounts only to the cost of the chambers used to hold the samples, which are US$1.16 per test (2 tests per slide). This can be compared with a cost, at the Children's Hospital of Chongqing Medical University, of US$3 for a CBC measurement and US$10 for gel electrophoresis, which constitute the standard screening tests performed for anemia in that hospital's clinical practice. Thus, our method represents at least a factor of 10 reduction in both cost per test and instrument cost compared to the clinical standard. Further, in addition to their substantial costs, standard clinical tests cannot be performed outside of a centralized clinical laboratory.

Table S1 Baseline Values in HC Group , IDA Group and TT Group (μ±σ)

Variable	**HC**	**IDA**	**TT**
No. of samples	195	49	24
Age (years)	5.75±3.77	1.94±2.89[a]	3.03±3.16[ab]
Age group(years)			
0~0.5（N,%）	4(2.1)	7(14.3)	6(25.0)
0.5~2（N,%）	51(26.2)	33(67.3)	9(37.5)
2~6（N,%）	56(28.7)	5(10.2)	4(16.7)
6~15（N,%）	84(43.1)	4(8.2)	5(20.8)
Females（N,%）	77(39.5)	14（28.6）	7(29.2)
Males（N,%）	118(60.5)	35（71.4）	17(70.8)
MCV (fL)	85.50±3.35	70.98±7.79[a]	63.15±6.56[ab]
MCHC (g/L)	32.72±0.73	31.61±1.91[a]	31.02±0.93[a]
RDW (%)	13.02±0.78	16.44±3.25[a]	16.72±2.04[a]

[a]Significantly different compared with HC group, $p<0.05$; [b]Significantly different compared with IDA group, $p<0.05$.

Note: MCV, erythrocyte mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

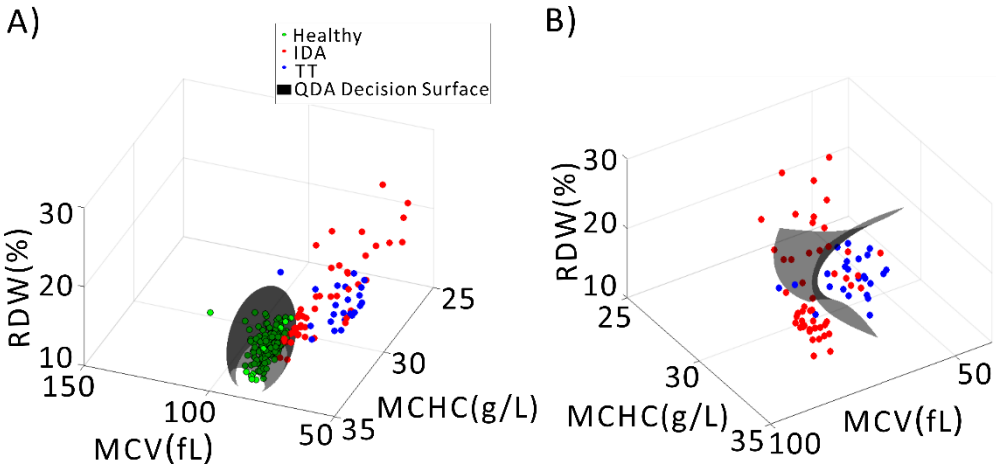

Fig. S4 Classifying samples into healthy, IDA, and TT groups using QDA based on clinical values of MCV, MCHC, and RDW. (A) QDA decision between Healthy and anemia. (B) QDA decision between IDA and TT.

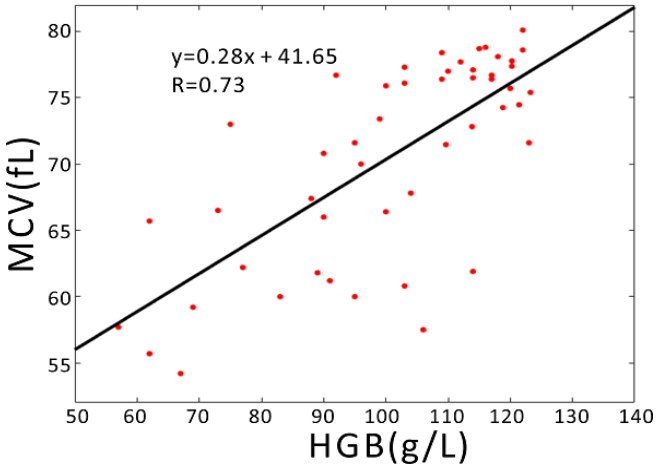

Fig. S5 Linear fitting result between the HGB and the MCV for anemic patients.

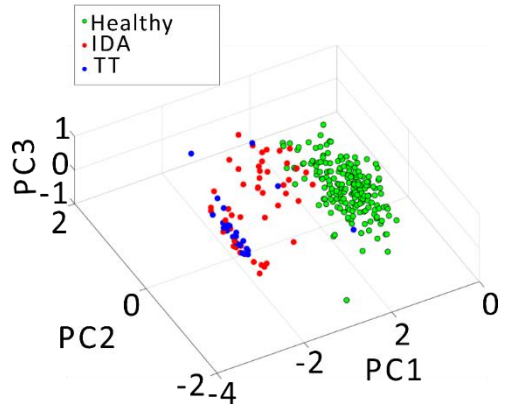

Fig S6 First three principal components of raw scattering data, color-coded by healthy, IDA, or TT.

Dependence of Machine Learning Models on Model Parameters:

In order to mitigate the potential for over-fitting and over-performance of the model, our chosen algorithms (PLS, QDA, and SVM) have few hyperparameters to set, and the results are relatively insensitive to parameter choice, highlighting the robustness of the data and lending more confidence to our conclusions. For the QDA algorithm, the hyperparameters were not optimized, but kept the same as in our prior modeling of clinical data, which include using a quadratic decision surface and Euclidean distance metric. For SVM, we also did not optimize hyperparameters, but used the radial basis function with standardized variables, as driven by prior experience on multivariate Raman spectroscopy data. Therefore, for each method (PLS-QDA and PCA-SVM), the only hyperparameter varied was the PLS model rank, or how many PCA components to feed to the SVM algorithm.

To explore the sensitivity of our cross-validation results to hyperparameter choice, in Figure S7 we show how the Youden's Index for discriminating Healthy vs. Anemia (blue) and IDA vs. TT (red) varies as the hyperparameter is changed for our PLS-QDA and PCA-SVM models. As we can see from the plots, the choice of hyperparameter does not lead to a substantial variation in the Youden's Index, particularly for the PCA-SVM model. The shaded regions represent the standard deviation among 20 runs of the CV, as the data partitioning changes. Instability caused by the relatively small size of the TT group would be expected to decrease substantially in a full-scale trial with a large (>100) population of TT patients.

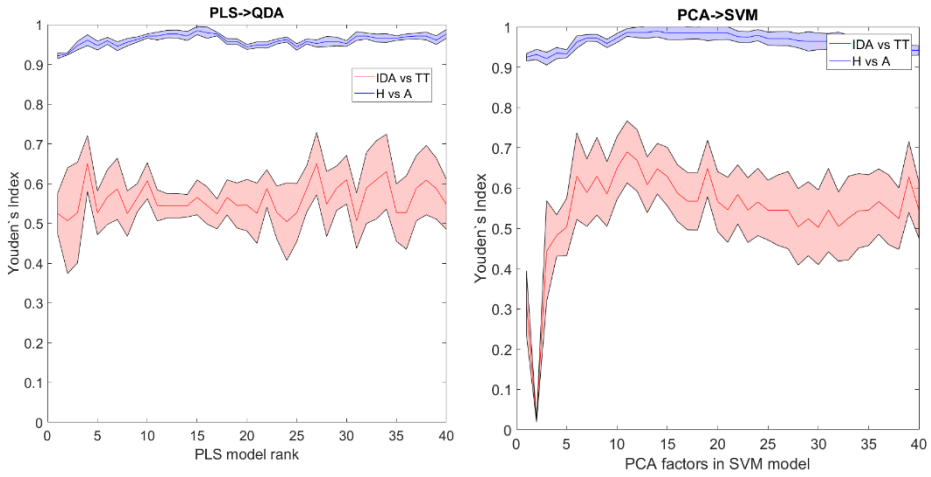

Fig. S7 – Youden's Index of models vs. hyperparameters. (Left) PLS-QDA or (Right) PCA-SVM for discriminating healthy and anemia, and IDA vs. TT.

To further highlight this point, we performed a similar exploration of hyperparameter space using half of the dataset in a 10-fold cross-validation model to find the optimum set of hyperparameters, while the other half was used as an independent validation set using this optimized hyperparameter set. These results are shown in Figure R2, where the lines and shaded areas are as in Figure R1 (except using only half of the dataset), while the black dots and triangles on the two graphs represent the independent validation using the optimized hyperparameters (and allowing the IDA-TT and Healthy-Anemia discrimination to each have their own hyperparameter values). We can see that the Youden's Index for the Healthy vs. Anemia discrimination suffers slightly due to the relative paucity of data with which to form a model. Yet, the results are largely quite consistent with those obtained using the cross-validation method shown in the main text. However, we emphasize that while we believe our cross-validated results from our modestly-sized dataset accurately represents expected future performance, the method still requires rigorous evaluation using a multi-instrument, multi-center trial with a test and validation set for model construction, followed by a set of totally novel inference data.

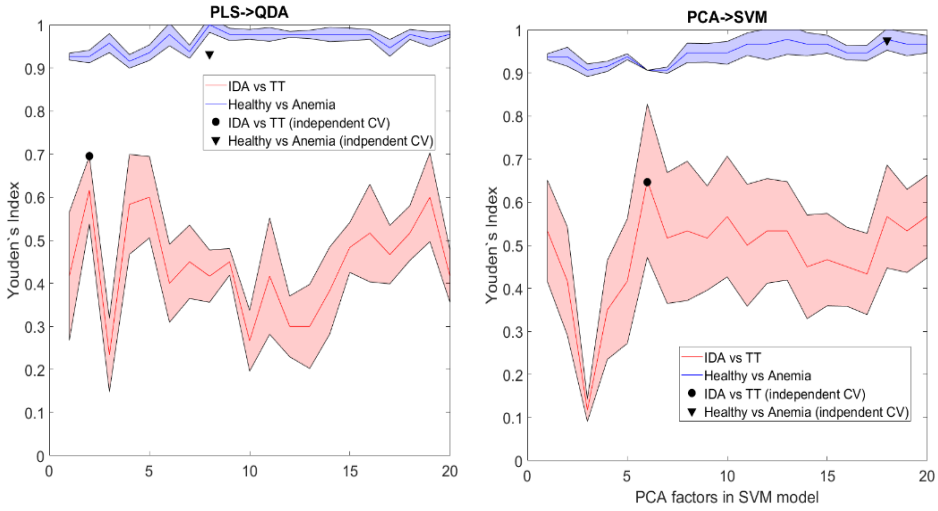

Fig. S8 – Youden's Index of models using an independent validation set. (Left) PLS-QDA or (Right) PCA-SVM for discriminating healthy and anemia, and IDA vs. TT.