Measurement of Blood Dilution during Lancet-Free Blood Sampling

Michael S. F. Hoffman, *Member, IEEE*, James W. McKeage, *Member, IEEE*, Bryan P. Ruddy, *Member, IEEE*, Poul M. F. Nielsen, *Member, IEEE*, Andrew J. Taberner, *Senior Member, IEEE*

***Abstract*— In this paper, we report on a fluorescent and colorimetric system for measuring the dilution of capillary blood released by a needle-free jet injector. Jet injection uses a high-speed liquid jet to penetrate tissue, and in the process can release capillary blood that can be collected for performing blood tests. In this way, blood sampling can be performed without the use of a lancet. However, any injectate that mixes with the collected blood dilutes the sample and may significantly impact subsequent analyses. By adding the fluorescent marker indocyanine green to the injected liquid, the fraction of injectate mixed into the collected blood can be measured. The incorporation of colorimetry allows our system to also correct for the impact of hematocrit on fluorescence. The results from this system show that it can determine the dilution of blood that has been diluted by up to 10 %, the upper limit of dilution typically observed in lancet-free blood sampling via jet injection.**

Clinical Relevance— Blood samples can be collected by jet injection without significant dilution, avoiding the need for lancing.

I. Introduction

Many people living with diabetes must take multiple measurements of their blood glucose concentration every day. Meeting this requirement typically involves performing multiple finger pricks throughout the day with a lancet, which can lead to chronic pain in the fingertips and ultimately poor compliance with insulin therapy [1].

Jet injection involves developing a liquid into a thin high-speed liquid jet that can penetrate tissue to depths of up to 20 mm. Jet injection is usually used for drug delivery, but has recently been used as an alternative to lancing for capillary blood collection [2]. This study found that the volumes of blood collected following a jet injection of isotonic saline were sufficient for conducting a glucose test using commercially available test strips. However, colorimetric measurements of hematocrit in the extracted samples indicated that blood collected after jet injection was diluted relative to blood collected by lancing. It remained unclear whether the dilution was primarily by the injectate that was used to pierce the skin, or by interstitial fluid. It is thus important to measure and understand the dilution of samples collected in this way to understand any impact on the glucose concentration measured.

To precisely distinguish the dilution of a blood sample with injectate from dilution by interstitial fluid, the exogenous fluorescent marker indocyanine green (ICG) can be added to the injectate. The concentration of ICG in a mixture with blood can then be determined using a fluorimeter [3]. While the fluorescent signal in a fluorimeter is predominantly determined by the concentration of the fluorophore, the signal can also be significantly influenced by light scattering from erythrocytes (red blood cells) within the solution [4]. This phenomenon suggests that the quality of a fluorescent measurement of blood dilution could be improved by incorporating a measurement of hematocrit to compensate for the concomitant scattering from erythrocytes.

In this paper, we propose a combination of colorimetry and fluorimetry to measure the dilution of blood samples collected by needle-free jet injection. Colorimetry is used to provide a measurement of the hematocrit present in a blood sample, similar to previous methods [2]. The measurement of hematocrit is then used to correct for the effect of scatter from red blood cells on fluorescence in the samples collected.

The goal that motivates this work is to quantify the extent to which human blood samples collected by needle-free jet injection are diluted by injectate. The dilution measurement system was validated on porcine blood before being applied to data from human blood collected in a study of suction-enhanced blood release following needle-free jet injection [5] (full results under review). Knowledge of the extent to which blood samples collected by needle-free jet injection are diluted will help guide the development of an all-in-one system capable of collecting a blood sample, performing an assay, and delivering a therapeutic drug.

II. Methods

A. Fluorimeter configuration

A fluorimeter was modified (Figure 1) to measure the concentration of the fluorescent marker (ICG) in blood samples collected by needle-free jet injection. A 780 nm laser diode (US-lasers D7805I) was collimated (Thorlabs, C330TMD-B) and split in a 50/50 beam splitter. Half of the incident laser intensity was diverted to a photodiode (UDT-455) to measure the laser power. The remaining fraction was focused to a point within the blood sample, which was held in

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M. S. F. Hoffman, J. W. McKeage, B. P. Ruddy, P. M. F. Nielsen and A. J. Taberner are with the Auckland Bioengineering Institute, University of Auckland, New Zealand. A. J. Taberner and B. Ruddy, and P. Nielsen are also with the Department of Engineering Science, University of Auckland, New Zealand.

Corresponding author: Michael S. F. Hoffman
Email: Michael.hoffman@auckland.ac.nz

a borosilicate capillary tube (part #3524, 0.2 mm by 4 mm by 30 mm, VitroCom, NJ). The intensity of the fraction passing through the sample was measured at PD2. The fluorescent emission from the sample was filtered (FB830-10, Thorlabs) and measured using a photomultiplier tube (Hamamatsu H7422).

B. Instrumentation

The 780 nm laser was sinusoidally modulated (787 Hz) to allow the fluorescence signal to be extracted from external noise. A tone search algorithm (LabVIEW 2017) was used to extract the amplitude of the signals measured by PD1, PD2 and PMT at 787 Hz. A data acquisition system (National Instruments cDAQ9174, NI9223, NI9263) was used for synchronous signal output and acquisition, performed at 20 kHz. The measurements made from PD2 (transmission) and the PMT (fluorescence) were normalized to the signal measured at PD1.

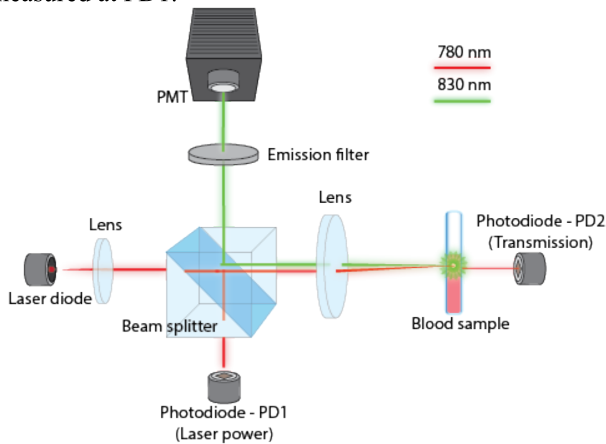

Fig. 1: Optical configuration of a 780 nm fluorimeter during a measurement.

C. Colorimetry

Transilluminated images of the specimen in the collection capillary were taken with a digital microscope (Digitech QC3199, Digitech industries, Sheung Wan, Hong Kong)[2]. The blood-containing region of the images was segmented in Python (v2.7.9) using the OpenCV-Python library (v4.6.0). The arithmetic mean of the intensity from each color channel in the segmented region was calculated. A ratiometric measurement of transmission was calculated by dividing the average intensity of each color intensity from the same region of a reference image without the capillary present.

D. Porcine blood collection and preparation

Porcine blood was collected in accordance with the University of Auckland Code of Ethical Conduct for the Use of Animals for Teaching and Research. Blood was mixed with 2.5 % of Heparin (5000 IU/mL). The blood was then centrifuged (7000*g*) for 30 minutes to separate the red blood cells from plasma. A range of blood hematocrits were prepared by reconstituting the red blood cell and plasma fractions in differing proportions.

Injectate prepared for use with porcine blood was diluted in isotonic saline from a stock ICG solution in methanol (5 g/L). Hematocrit was measured in diluted blood by centrifuging a blood sample at 12,000 rpm (7500*g*) for 3 minutes (ZipCombo, LW Scientific, Inc). The red blood cell fraction was then measured using the provided reading card.

E. Human blood collection and preparation

Human capillary blood was drawn from the fingertips of 15 participants as the part of a study approved by the Northern B Health and Disability Ethics Committee of New Zealand (2021 FULL 11035, registration: ACTRN12621001572853, www.anzctr.org.au). This study was focused on investigating how suction increases the volume of blood release [5]. Samples were collected into 24 µL borosilicate capillary tubes. Measurements were taken immediately following blood collection.

A stock Indocyanine Green (ICG) (Verdye, Diagnostic Green) solution was mixed with isotonic saline solution to give a final ICG concentration of 10 mg/L for human administration.

F. Glucose measurement

The glucose concentration of human blood samples was measured using a glucometer (CareSens N Premier; i-SENS, Inc, Seoul, Korea). This device has a rated accuracy of ±15.0 mg/dL (0.83 mmol/L) for glucose concentration <100 mg/dL (5.55 mmol/L).

III. Results

Fluorescence in porcine blood

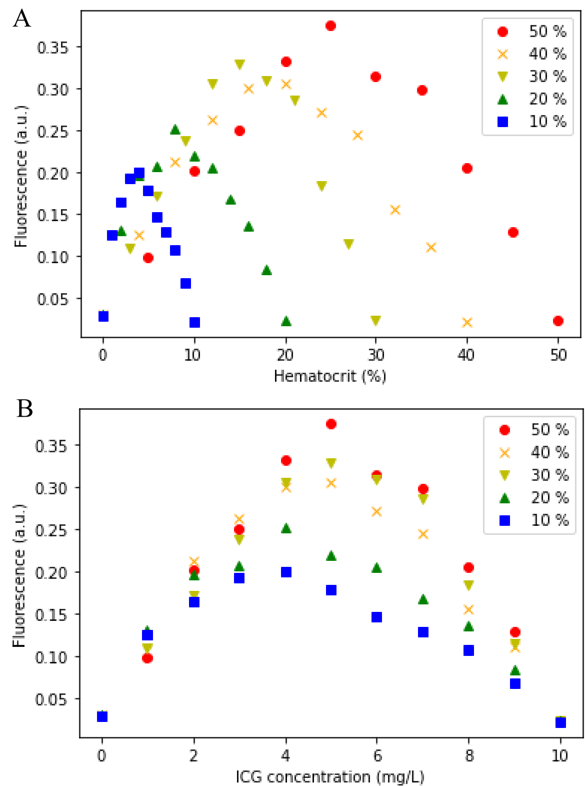

Fig. 2: A—Data collected from in vitro experiments diluting porcine blood with injectate containing 10 mg/L of ICG. Each color series denotes a different initial hematocrit (10 % to 50 %). B—The same experimental data plotted against the hematocrit (Hct) of the sample for each dilution series. Data are grouped by initial haematocrit: ● 50 %, x 40 %, ▲ 30 %, ▼ 20 %, ■ 10 %.

The fluorescence response of porcine blood samples to progressive dilution is presented in Figure 2. The results show that fluorescence does not follow a monotonic relationship with ICG concentration. The maximum fluorescence is observed between 4 mg/L to 5 mg/L for all hematocrit series. This result demonstrates the dependence of fluorescence on scattering due to hematocrit.

Due to the nonlinear relationship between fluorescence, ICG concentration and hematocrit, the following model was fit

$$F = a(ICG - b * Hct)^2 + c \qquad (1)$$

where *F* is the fluorescence, *ICG* is the concentration of ICG, *Hct* is the hematocrit and *a, b, c* are fit parameters (Figure 3A). The relative root mean square error of the model in Figure 3A is 1.2 %.

Each dilution series was fit individually, and the fit parameters were plotted against hematocrit, as shown in Figure 3B.

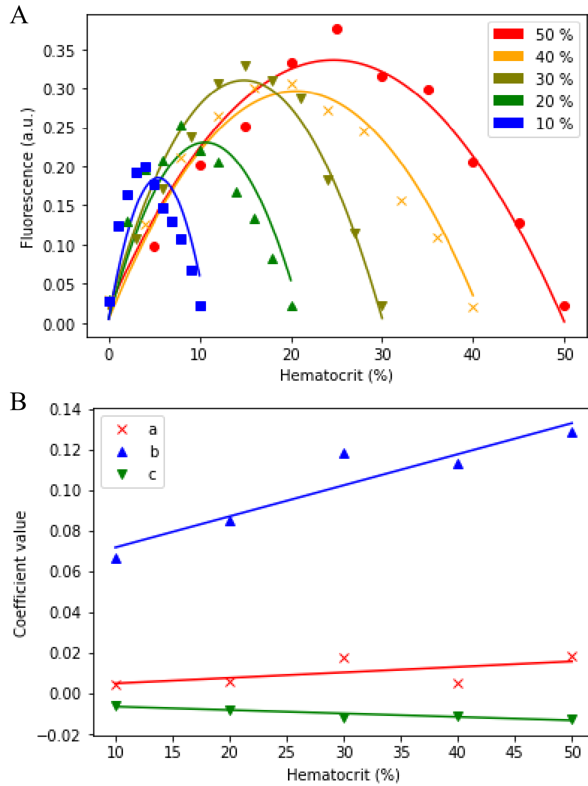

Fig. 3: A—Model performance (solid line) compared to measured values. Data are grouped by initial haematocrit: ● 50 %, x 40 %, ▲ 30 %, ▼ 20 %, ■ 10 %. (x markers). B—Fitting the model parameters (a, b, c from (1)) with a linear regression as a function of hematocrit.

Hematocrit measurement

The intensity of the green transmission showed a good correlation to hematocrit over the entire range of hematocrits (Figure 4B). Transmission of 780 nm light only showed a good correlation to hematocrit for low values of hematocrit when samples had high levels of dilution (Figure 4A). In order to predict hematocrit, an exponential was fitted to the colorimetric data of the form

$$Hct = xe^{-G/y} + z \qquad (2)$$

where *Hct* is hematocrit, *G* is the measured transmission of the green intensity and *x*, *y*, and *z* are fit variables. Fitting the exponential relationship (2) with the measurements resulted in the fit parameters of $x = 46.6$ %, $y = 0.31$ %, and $z = 4.1$ %. The RMS error of this model is 2.4 %.

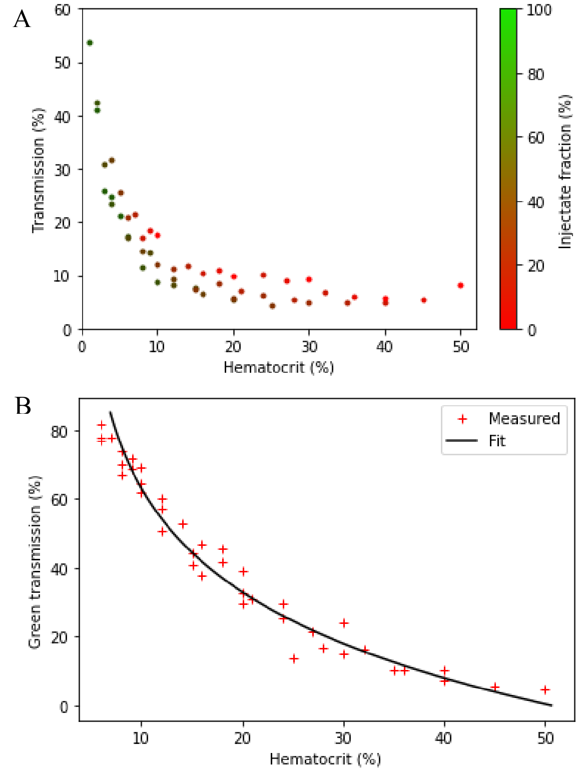

Fig. 4: A—Transmission measurements taken of porcine blood samples with varying proportions of injectate. B—Colorimetric measurements of transmission of green intensity values in porcine blood.

Dilution measurement

Using the model established in (1), an inverse solution can be derived to calculate the expected ICG concentration as follows:

$$ICG = \sqrt{\frac{F-c}{a}} + b * Hct. \qquad (3)$$

With the additional knowledge of the concentration of ICG present in the injectate, the dilution (percentage of injectate present in blood sample) can be quantified.

The measurements collected from human blood samples (Figure 5) show that the extent of dilution in jet injected samples is consistently low. The mean dilution measured in lancet samples was 0.26 % ± 0.19 %. The mean dilution measured in jet injection samples was 1.79 % ± 1.4 %

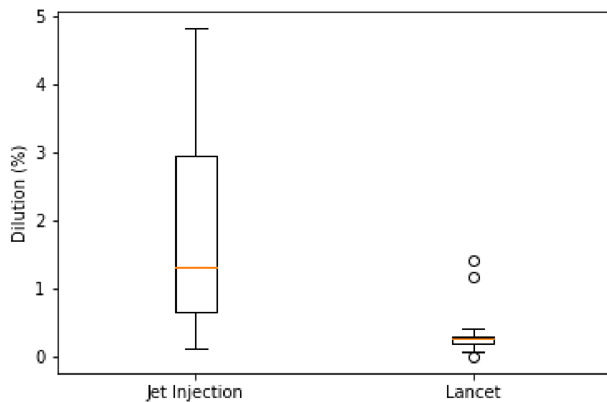

Fig. 5: The dilution of samples collected by needle-free jet injection under suction as compared to the measured dilution of samples collected by lancing.

IV. Discussion

Hematocrit has an important influence on measurements of the fluorescence response of ICG in blood (Figure 2). The effects of hematocrit are able to be compensated for by using colorimetry to determine the hematocrit of the blood collected. By incorporating both fluorescence and colorimetry we able to resolve dilutions of less than 10 % in blood volumes as low as 4 µL, although no samples measured in this study were diluted by more than 5 %. The average dilution of needle-free jet injection samples was 1.79 % with a standard deviation of 1.40 %.

The addition of colorimetry to our fluorimeter system provided estimates of hematocrit within 2.4 %. More sophisticated optical measurements systems are able to measure hematocrit within an absolute value of 0.7 % [6]. It is conceivable that colorimetric information may be included in other image-based hematocrit measurement strategies, such as with lateral flow tracking [7].

The low correlation of 780 nm transmission with hematocrit is surprising (Figure 4A), and may be due to the additional absorbance at 780 nm from the ICG introduced by the injectate. The sensitivity of the system can be seen in the change in transmission with ICG concentration at a given hematocrit. The lowest 780 nm transmission value measured was observed in a diluted sample with a hematocrit of 25 % rather than at the highest hematocrit.

The relationship between fluorescence intensity and hematocrit is inconsistent with previous findings[3], [8]. This is likely due to the combination of measuring backward fluorescence and using a capillary with a short path length (0.2 mm).

Although the porcine blood used for *in vitro* validation of the system was heparinized, while human blood is not, this step is not likely to produce a significant error in measurement [9]. Human and porcine blood samples with no ICG present showed a small fluorescent response to the excitation laser that is indicative of autofluorescence, consistent with previous observations [3].

V. Conclusion

We have presented a system that integrates colorimetry and fluorescence to determine the dilution of blood samples collected following needle-free jet injection. Colorimetry provides sufficient information to correct for the effect of hematocrit on fluorescence. The system was able to determine the dilution of samples within 1.2 % across hematocrit levels of 0 % to 50 %. In a human trial the system determined that the average dilution of samples extracted following needle-free jet injection was 1.79 % ± 1.40 %. This indicates that for most measurements, the use of needle-free jet injection to collect a blood sample will not introduce a significant error. The low levels of dilution observed in these needle-free samples is encouraging for the development of jet injection for minimally invasive blood sampling methods. In future, blood extraction may become a function incorporated into jet injection devices, enabling an all-in-one system for both measurement and drug delivery.

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References

[1] L. Heinemann, “Finger Pricking and Pain: A Never Ending Story,” *Journal of diabetes science and technology (Online)*, vol. 2, no. 5, p. 919, 2008, doi: 10.1177/193229680800200526.

[2] J. Xu, J. W. McKeage, B. P. Ruddy, P. M. F. Nielsen, and A. J. Taberner, “Jet-Induced Blood Release From Human Fingertips: A Single-Blind, Randomized, Crossover Trial,” *J Diabetes Sci Technol*, Oct. 2021, doi: 10.1177/19322968211053895/ASSET/IMAGES/LARGE/10.1177_19322968211053895-FIG4.JPEG.

[3] B. Madadkhahsalmassi, J. McKeage, B. Ruddy, and A. Taberner, “Blood dilution measurement by a dual laser fluorimeter,” *I2MTC 2020 - International Instrumentation and Measurement Technology Conference, Proceedings*, May 2020, doi: 10.1109/I2MTC43012.2020.9128472.

[4] N. A. Giauque, C. E. Flowerday, and S. R. Goates, “Enhanced Fluorescence in a Scattering Medium,” *Appl Spectrosc*, vol. 75, no. 12, pp. 1461–1464, Dec. 2021, doi: 10.1177/00037028211029312/ASSET/IMAGES/LARGE/10.1177_00037028211029312-FIG1.JPEG.

[5] M. S. F. Hoffman, J. W. McKeage, B. P. Ruddy, P. M. F. Nielsen, and A. J. Taberner, “Needle-free Jet Injection with suction to Measure Glucose Concentration in Capillary Blood for Diabetes,” *Australian New Zealand Clinical Trials Registry [Internet]*, 2022. https://www.anzctr.org.au/ACTRN12621001572853.aspx (accessed Jan. 20, 2023).

[6] S. Oshima and Y. Sankai, “Improvement of the Accuracy in the Optical Hematocrit Measurement by Optimizing Mean Optical Path Length,” *Artif Organs*, vol. 33, no. 9, pp. 749–756, Sep. 2009, doi: 10.1111/J.1525-1594.2009.00891.X.

[7] E. Frantz, H. Li, and A. J. Steckl, “Quantitative hematocrit measurement of whole blood in a point-of-care lateral flow device using a smartphone flow tracking app,” *Biosens Bioelectron*, vol. 163, p. 112300, Sep. 2020, doi: 10.1016/J.BIOS.2020.112300.

[8] J.-M. I. Maarek and D. P. H. M.D., “Estimation of indocyanine green concentration in blood from fluorescence emission: application to hemodynamic assessment during hemodialysis,” *https://doi.org/10.1117/1.3233652*, vol. 14, no. 5, p. 054006, Sep. 2009, doi: 10.1117/1.3233652.

[9] R. Bamberg, T. Gwyn, J. Miller, M. Thompson, and P. Transou, “The Effects of Over-anticoagulated Blood on Hematocrit Values by the Microcentrifuge Method,” *CLINICAL LABORATORY SCIENCE*, vol. 21, 2008, Accessed: Nov. 24, 2022. [Online]. Available: http://hwmaint.clsjournal.ascls.org/