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Paul N. Patrone, Amy Q.H. Li, Gregory A. Cooksey, Anthony J. Kearsley

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Measuring Microfluidic Flow Rates: Monotonicity, Convexity, and Uncertainty

Paul N. Patrone[a], Amy Q. H. Li[b], Gregory A. Cooksey[c], Anthony J. Kearsley[a]

[a] *Applied and Computational Mathematics Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*
[b] *Department of Mathematics, University of Texas at Austin, Austin, TX 78712, USA*
[c] *Microsystems and Nanotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*

Abstract

We consider a class of non-linear integro-differential equations describing microfluidic measurements. We prove that under reasonable conditions, solutions to these equations are convex functions of a flow-rate parameter ξ used in metrology. The key elements of our analysis are: (i) elevation of ξ to an independent variable through reformulation of the problem as a partial-differential equation (PDE); and (ii) extension of techniques from the theory of ordinary differential equations to the PDE setting.

1. Introduction

Several of us recently demonstrated that robust and accurate tools for measuring flow rates v_v down to 5 nL/min can be developed from physically informed scaling laws [1, 2]. As shown in Fig. 1, the underlying experiments advect fluorophores into laser light, which causes them to fluoresce before eventually bleaching (i.e. deactivating). The measurement procedure for v_v is guided by the intuition that lower flow rates decrease the distance that fluorophores survive into the region illuminated by the laser.

This setup is well-described by the system of non-linear integro-differential equations

$$\mathcal{I} = \int_\Omega \mathrm{d}\mathbf{r}\ F(c(\mathbf{r}), \mathbf{r}) \tag{1}$$

$$\frac{\mathrm{d}c}{\mathrm{d}z} = -g(\xi)B(c(\mathbf{r}), \mathbf{r}) \tag{2}$$

where Ω is a compact domain in $\mathbb{R}^3$ corresponding to the laser profile, $\mathbf{r} = (x, y, z) \in \Omega$, c is the concentration of fluorophores, F is a fluorescence rate, and g and B characterize the dependence of bleaching on the laser light strength p and fluorophore concentration;[1]

Email addresses: paul.patrone@nist.gov (Paul N. Patrone), amyqli@math.utexas.edu (Amy Q. H. Li), gregory.cooksey@nist.gov (Gregory A. Cooksey), anthony.kearsley@nist.gov (Anthony J. Kearsley)

[1] B may be a non-local function of $c(\mathbf{r})$ if fluorophores absorb an appreciable amount of laser light.

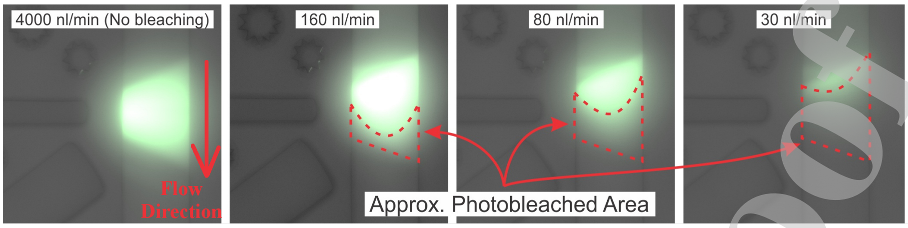

Figure 1: A microfluidic device measuring four different flow rates v_v. Excitation light (not visible) emitted in the horizontal direction causes a dye to fluoresce green. As $v_v \to 0$, the fluorescence efficiency decreases monotonically, since most of the dye is destroyed before traversing the excitation region.

B, F, and g are assumed to be known, while p can be experimentally controlled. The fluorescence efficiency $\mathcal{I}$, (total fluorescence per input laser light) is a functional of c, while the dosage $\xi = p/v_v$ is the characteristic radiation received by a fluorophore. *Notably, $\mathcal{I}$ is a function of ξ via its dependence on c.* The measurement procedure uses this dependence to determine v_v via its relation to $\mathcal{I}$, since the latter is directly observable.

To realize this in practice, we leverage a lemma stating that $\mathcal{I}$ is a strongly monotone decreasing function of ξ when B, F, and g are non-negative and monotone increasing in c and/or ξ. This implies that the correspondence between v_v and $\mathcal{I}$ is a bijection; i.e. measuring the latter uniquely determines the former (given p). The usefulness of this result arises from not needing to know the precise forms of B, F, and g, which are hard to determine. Specifically, the function $\mathcal{I}(\xi)$ can be *experimentally constructed* by fixing a known flow-rate v_0, measuring $\mathcal{I}_n = \mathcal{I}(p_n/v_0)$ for a discrete set of laser powers p_n, and interpolating the resulting data. Uncertainty in subsequent measurements for v_v is dominated by the accuracy with which $\mathcal{I}$ can be measured, uncertainty in v_0, and the data analysis used to infer the underlying bijection; see Fig. 2 [2].

This letter provides a result that can reduce uncertainty due to data analysis. As Fig. 2 shows, interpolation errors are decreased if, in addition to being monotone, $\mathcal{I}(\xi)$ is convex ([3]). We show that this stronger property holds under physically reasonable conditions. Our approach is to elevate Eq. (2) to a second-order PDE in terms of z *and* ξ to show that $c_{\xi\xi}$ exists and is non-negative. This implies our main result through the dependence of $\mathcal{I}$ on ξ via c. Our main analytical tool is an extension of the Picard existence theorem. Key steps in our analysis are to: (i) recast the PDE in terms of an integral equation; and (ii) identify conditions that guarantee boundedness of solutions.

2. Monotonicity

Because x and y are parameters in Eq. (2), we can use z in place of $\mathbf{r}$ and consider $\Omega = [0, Z]$ for some $Z > 0$.

Lemma 1. *Let $c(0,\xi) = c_0$ for some $c_0 > 0$ and assume that: (i) $F(c,z) \geq 0$, with equality only when $c = 0$, be summable and strongly monotone increasing in c for fixed z; (ii) $B \in C^2([0,\infty) \times \Omega)$, satisfying $0 \leq B \leq B_m$ for some positive maximum B_m and $B(c,z) = 0$ only when $c = 0$; and (iii) $g(\xi) \geq 0$ is strongly monotone increasing with equality achieved only when $\xi = 0$. Then*

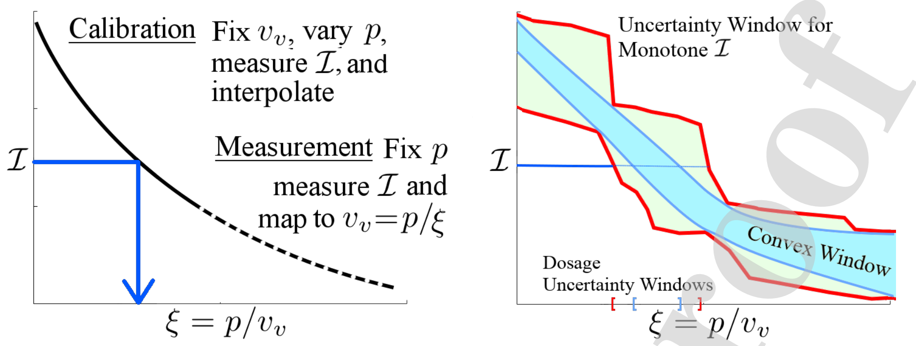

Figure 2: *Left*: Process by which the device in Fig. 1 is calibrated and used. First, $\mathcal{I}$ is measured at a known v_v and discrete set of p. Interpolation yields $\mathcal{I}(\xi)$. Then, v_v is changed to an unknown value (and p possibly lowered). Measuring $\mathcal{I}$ then yields v_v. In practice, $\mathcal{I}(\xi)$ has uncertainty due to interpolation. *Right*: Schematic of monotone uncertainty bounds (red) versus convex uncertainty bounds (blue). For the latter, the set of admissible $\mathcal{I}(\xi)$ is smaller, yielding lower measurement uncertainty.

(A) there is a unique $c(z;\xi)$ that solves $c_z = -g(\xi)B(c,z)$, is differentiable on $\Omega = [0,Z]$ for any $Z > 0$, satisfies the bounds $0 < c(z) \le c_0$ on Ω, and is strictly monotone decreasing in ξ;

(B) the integral $\mathcal{I} = \int_\Omega F(c,z)$ is a strictly monotone decreasing function of ξ.

Reference [2] provides a proof relying on the observation that $c(z;\xi)$ is differentiable and monotone decreasing in z, thereby implying monotonicity in ξ. The physical interpretation is straightforward: increasing radiation dosage decreases concentration.

3. Convexity

To motivate the conditions under which $I(\xi)$ is convex, formally differentiate Eq. (1):

$$\mathcal{I} = \int_\Omega \mathrm{d}z\ F(c,z) \implies \mathcal{I}_{\xi,\xi} = \int_\Omega \mathrm{d}z\ F_{cc}(c,z)c_\xi^2(z) + F_c(c,z)c_{\xi,\xi}(z). \tag{3}$$

If, in addition to the assumptions of Lemma 1, $F_{cc} \ge 0$ and $c_{\xi\xi} \ge 0$, then $\mathcal{I}$ is convex in ξ. Assuming regularity of F, our main goal amounts to determining when c is convex in ξ. This is accomplished rigorously by explicitly constructing $c_{\xi\xi}$ and identifying the conditions on g, F, and B that lead to the desired inequality.

Formally differentiating Eq. (2) with respect to ξ yields a candidate expression for c_ξ

$$c_{z,\xi}(z,\xi) \overset{?}{=} -g'(\xi)B(c,z) - g(\xi)c_\xi B_c(c,z), \tag{4}$$

where the symbol $\overset{?}{=}$ indicates Eq. (4) is a conjecture. We have recast Eq. (2) as a PDE by elevating ξ to an independent variable. An expression for c_ξ is obtained by an integrating factor; viz, taking $c_\xi(0,\xi) = 0$ (consistent with the initial data), one finds

$$c_\xi(z,\xi) \overset{?}{=} -g'(\xi) \int_0^z \mathrm{d}s\ B(c,s)e^{-g(\xi)\int_s^z \mathrm{d}t\ B_c(c,t)}, \tag{5}$$

where $g'(\xi)$ is the derivative with respect to ξ. Omitting certain independent variables for brevity, Eq. (5) suggests a conjecture useful for establishing $c_{\xi\xi} \geq 0$, namely,

$$c_{\xi\xi} \stackrel{?}{=} \int_0^z \mathrm{d}s\, e^{-g(\xi)\int_s^z \mathrm{d}t\, B_c(c,t)} \left[-g'' B - g' B_c c_\xi + B \int_s^z \mathrm{d}t' g'^2 B_c(c,t') + g' g B_{cc}(c,t') c_\xi \right]. \quad (6)$$

While the right-hand side (RHS) of Eq. (5) is well defined, we do not know if it equals the required derivative. Integrating in ξ and observing that $c(z,0) = c_0$ yields

$$c(z,\xi) \stackrel{?}{=} c_0 - \int_0^\xi \mathrm{d}\zeta\, g'(\zeta) \int_0^z \mathrm{d}s\, B(c,s) e^{-g(\zeta)\int_s^z \mathrm{d}t\, B_c(c,t)}. \quad (7)$$

We must: (i) validate that Eq. (7) has a unique solution on the desired domain; and (ii) demonstrate that this solution is identical to the c solving Eq. (2). Equation (6) provides sufficient conditions for convexity: in addition to being differentiable, g and B must be concave in ξ and c, respectively.

Remark 1. One can show that $u_z(z,\xi) = c_z(z;\xi)$ when c solves Eq. (2) taking $u(z,\xi)$ be the RHS of Eq. (7). However, $u_z = -g(\xi)B(c,z)$ does not imply that $u = c$ or $u_z = -g(\xi)B(u,z)$, since, for example, u and c can differ by a function of ξ alone. One can view u as the solution to a PDE, whereas c is the solution to an ODE.

Theorem 1. *Assume the conditions of Lemma 1 and that $B_{cc} \leq 0$, $B_c \geq 0$, and $g \in C^2[0,\Xi]$ is bounded from above, with $g_{\xi\xi} \leq 0$. Let $F \in C^2([0,c_0] \times \Omega)$ be bounded, with $F_{cc} \geq 0$ and set $c(0,\xi) = c(z,0) = c_0 > 0$, then*

(I) Equation (7) has a solution $c(z,\xi) \in C[0,Z] \times C^2[0,\Xi]$ that is convex in ξ;

(II) this $c(z,\xi)$ is the unique solution to the ODE $c_z = -g(\xi)B(c,z)$, $c(0) = c_0$; and

(III) $\mathcal{I}(\xi) \in C^2[0,\Xi]$ as defined by Eq. (1) is a convex function of ξ.

Proof. Our approach is a variation of the existence and uniqueness theorem based on Picard iteration in Ref. [4]. Specifically we define a sequence of iterates

$$c_{n+1}(z,\xi) = c_0 - \int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\, G(c_n, z, s, \zeta) \quad (8)$$

$$G(c_n, z, s, \zeta) = g'(\zeta) B(c_n, s) e^{-g(\zeta)\int_s^z \mathrm{d}t\, B_c(c_n,t)}, \quad (9)$$

with the goal of showing that $c_n \to c$ in the limit that $n \to \infty$.

We first identify a compact domain D containing each element of the sequence $\{c_n\}$. An extension of B is needed. Specifically, for $c < 0$, let $B(c,z) = -B(-c,z)$ be the odd extension. Note that this extension has a Lipschitz-continuous derivative in c also satisfying $B_c \geq 0$. Next, fix finite, positive values of Z and Ξ and let $\lambda = Z\Xi$. By boundedness of g' and B, there exists $M > 0$ such that

$$|c_n(z,\xi) - c_0| \leq M\lambda. \quad (10)$$

Thus $(z,\xi,c_n) \in D := [0,Z] \times [0,\Xi] \times [c_0 - M\lambda, c_0 + M\lambda]$ for all n.

Omitting certain independent variables for brevity, we next note that

$$|G(c,z,s,\xi)-G(\tilde{c},z,s,\xi)| \leq M_1|B(c)-B(\tilde{c})| + M_2\left|e^{-g\int_s^z \mathrm{d}t B_c(c)} - e^{-g\int_s^z \mathrm{d}t B_c(\tilde{c})}\right| \quad (11)$$

for some constants M_1 and M_2, where we have used the boundedness of g and B along with the triangle inequality. Since the exponents appearing in Eq. (11) are always negative, we can use Lipschitz continuity of e^x on the domain $(-\infty, 0]$ to determine that

$$\left|e^{-g\int_s^z \mathrm{d}t B_c(c)} - e^{-g\int_s^z \mathrm{d}t B_c(\tilde{c})}\right| \leq |g|\int_0^z \mathrm{d}t\ |B_c(c)-B_c(\tilde{c})|, \quad (12)$$

and thus

$$|G(c,z,s,\xi)-G(\tilde{c},z,s,\xi)| \leq M_3|c(s,\xi)-\tilde{c}(s,\xi)| + M_4\int_0^z \mathrm{d}t\ |c(t)-\tilde{c}(t)|, \quad (13)$$

where we have used Lipschitz continuity of B and B_c. This implies

$$\left|\int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\ G(c,z,s,\zeta)-G(\tilde{c},z,s,\zeta)\right| \leq \Lambda\int_0^\xi \mathrm{d}\zeta\int_0^z \mathrm{d}s\ |c(s,\zeta)-\tilde{c}(s,\zeta)| \quad (14)$$

for a constant Λ depending on the domain. Here we have leveraged the fact that the integral on the RHS of Eq. (13) is independent of s (thus the necessity that Z be finite).

To prove that $c_n \to c$ as $n \to \infty$ (again omitting independent variables), consider

$$|c_1 - c_0| \leq \int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\ |G(c_0,z,s,\xi)| \leq Mz\xi \leq M\lambda \quad (15)$$

$$|c_2 - c_1| \leq \int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\ |G(c_1)-G(c_0)| \leq \Lambda\int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\ |c_1-c_0| \leq \Lambda M\frac{\xi^2 z^2}{(2!)^2} \quad (16)$$

...

$$|c_{n+1}-c_n| \leq \Lambda^n M\frac{\xi^{n+1}z^{n+1}}{[(n+1)!]^2} \leq \frac{M}{\Lambda}\frac{(\Lambda\lambda)^{n+1}}{[(n+1)!]^2} \quad (17)$$

Thus, it is clear that

$$c_n = c_0 + (c_1 - c_0) + (c_2 - c_1) + ... + (c_n - c_{n-1}) \quad (18)$$

converges uniformly (i.e. in the sup-norm) to a limit $c(x,\xi)$, since the series is bounded above by the Taylor series for an exponential function. Likewise, Eq. (13) implies the sequence $G(c_n,z,s,\xi)$ converges uniformly to $G(c,z,s,\xi)$, which is continuous. Since all of the limits converge uniformly, we have that

$$\begin{aligned} c(z,\xi) = \lim_{n\to\infty} c_n(z,\xi) &= c_0 - \int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s \lim_{n\to\infty} G(c_n,z,s,\xi) \\ &= c_0 - \int_0^\xi \mathrm{d}\zeta \int_0^z \mathrm{d}s\ G(c,z,s,\xi). \end{aligned} \quad (19)$$

It is clear that $c(z,\xi)$ so defined solves Eq. (7) and is twice differentiable in ξ. Thus, Eq. (6) is well defined, and $c \in C[0,Z]\times C^2[0,\Xi]$ is convex in ξ, which proves assertion (I).

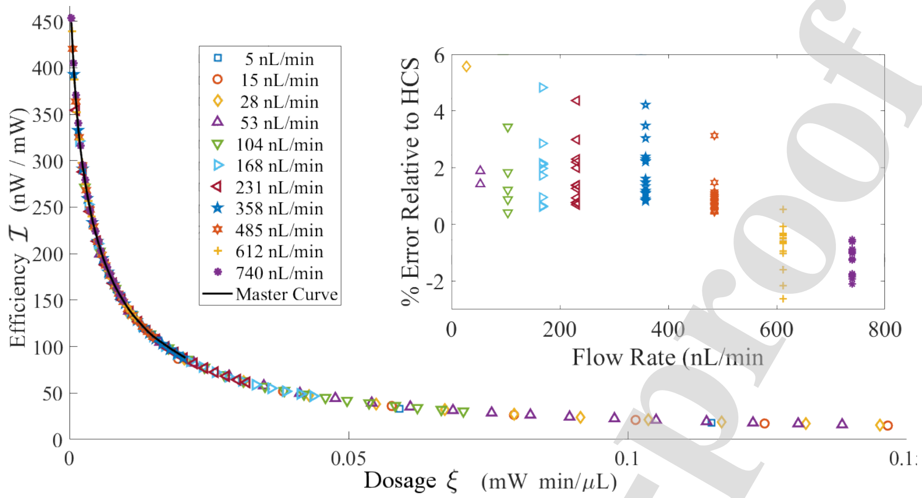

Figure 3: Comparison between experimental data and a calibrated estimate of $\mathcal{I}(\xi)$. Adapted from Ref. [2].

Moreover, invoking the Leibniz rule (or Fubini's theorem) for interchanging derivatives and integrals and using Eq. (5), one finds that

$$c_z(z,\xi) = -\int_0^\xi \mathrm{d}\zeta\ g'(\zeta)B(c,z) + g(\zeta)B_c(c,z)c_\zeta = -\int_0^\xi \mathrm{d}\zeta\ \partial_\zeta\left[g(\zeta)B(c,z)\right]$$
$$= -g(\xi)B(c,z). \quad (20)$$

Thus, c is the unique solution to $c_z = -g(\xi)B(c,z)$, since existence and uniqueness of the solution to the ODE are already established in Lemma 1. This proves assertion (II). Assertion (III) follows immediately from Eq. (3). □

4. Validity of the Assumptions

In Ref. [2], we used this result to motivate convex optimization for estimating $\mathcal{I}(\xi)$ (see also Ref. [5]). Figure 3 illustrates that experimental data is both convex and in excellent agreement with an $\mathcal{I}(\xi)$ obtained from optimization. See Refs. [2] and [1] for more details. The remarkable consistency of this data with our main result begs the question: what is the physical interpretation of the concavity assumptions, and why should they be valid? Taken with monotonicity, $g'' < 0$ and $B_{cc} < 0$ imply that bleaching rate increases more slowly with ξ and c. In experimental systems, fluorophores tend to block some light, so increasing c simultaneously yields more bleaching candidates and further decreases light transmission from the excitation source. Likewise, increasing ξ yields smaller increases in the rate of bleaching as flourophoer absorbtion of excitation photons is limited . Thus, the validity of these assumptions stems from *generic* properties of light matter interactions. The requirement $F_{cc} \geq 0$ is somewhat at odds with this argument, but we recognize that in Ref. [2, 1], the fluorescence light tends not to be re-absorbed by fluorophores; thus F is likely to be linear in c. Notwithstanding, the generic nature of these assumptions (and their physical interpretations) is fundamental to the robustness of the measurements in Ref. [2].

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