DEVELOPMENT OF ULTRASONICALLY LEVITATED DROPS AS MICROREACTORS FOR STUDY OF ENZYME KINETICS AND POTENTIAL AS A UNIVERSAL PORTABLE ANALYSIS SYSTEM

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Abstract

Development of microfluidics has focused on carrying out chemical synthesis and analysis in ever-smaller volumes of solution. In most cases, flow systems are made of either quartz, glass, or an easily moldable polymer such as polydimethylsiloxane (Whitesides 2006). As the system shrinks, the ratio of surface area to volume increases. For studies of either free radical chemistry or protein chemistry, this is undesirable. Proteins stick to surfaces, biofilms grow on surfaces, and radicals annihilate on walls (Lewis et al. 2006). Thus, under those circumstances where small amounts of reactants must be employed, typical microfluidic systems are incompatible with the chemistry one wishes to study. We have developed an alternative approach. We use ultrasonically levitated microliter drops as well mixed microreactors. Depending on whether capillaries (to form the drop) and electrochemical sensors are in contact with the drop or whether there are no contacting solids, the ratio of solid surface area to volume is low or zero. The only interface seen by reactants is a liquid/air interface (or, more generally, liquid/gas, as any gas may be used to support the drop). While drop levitation has been reported since at least the 1940's, we are the second group to carry out enzyme reactions in levitated drops, (Weis; Nardozzi 2005) and have fabricated the lowest power levitator in the literature (Field; Scheeline 2007). The low consumption aspects of ordinary microfluidics combine with a contact-free determination cell (the levitated drop) that ensures against cross-contamination, minimizes the likelihood of biofilm formation, and is robust to changes in temperature and humidity (Lide 1992). We report kinetics measurements in levitated drops and explain how outgrowths of these accomplishments will lead to portable chemistry/biology laboratories well suited to reconnaissance, preparation of the battlespace, force protection, and similar tasks in the asymmetric battlefield environment and in sustainment operations.

1. The Levitated Drop Microreactor

Common laboratory surfaces are anything but passive. If anionic, they adsorb metal ions. If cationic, they adsorb chloride, sulfate, and other common anions. If neutral, hydrocarbons and other hydrophobic substances stick to the equipment. In most cases, biofilms form over time (Lewis et al. 2006). For large reactors, such behavior has a small, even vanishing effect on experiments; reactions in bulk solution dominate surface behavior. If a container has characteristic length L, then surface area to volume ratio scales as 1/L. This means that microfluidic systems have large surface-area-to-volume ratio, so that surface chemistry, whether or not desirable, may be of overwhelming importance (Noyes 1951).

A number of approaches can ameliorate the role of surface reactions in altering concentration, reactivity, and reactant mobility. One may coat walls with passivating agents (Fiorini; Chiu 2005; Jenkins et al. 2004; Kamande et al. 2005; Lenghaus et al. 2003; Sweryda-Krawiec et al. 2004) or use fluorocarbons to separate aqueous samples from the walls (Roach et al. 2005; Zheng et al. 2004). Clearly, simply avoiding solid/liquid interfaces is an alternative. Use of levitated drops is an approach that has gained favor in recent years (Field; Scheeline 2007; Laurell et al. 1999; Leiterer et al. 2008; Omrane et al. 2004; Petersson et al. 1998; Priego-Capote; de Castro 2006; Santesson; Nilsson 2004; Santesson et al. 2004; Santesson et al. 2000; Santesson et al. 2003a; Santesson et al. 2003b; Weis; Nardozzi 2005; Westphall et al. 2008). Here, we describe three aspects of the development of levitated drop reactors that open new measurement opportunities: reducing the power and size of drop levitators, use of drop levitators for kinetics measurements, and integration of electrochemical and optical microsensors with drop levitators to allow portable use of such devices while minimizing the logistical tail sometimes associated with one-use microfluidic devices. We envision battery-powered analytical laboratories that are small, light, and reliable enough for field use in the battlefield environment and under other challenging condi-

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
DEC 2008	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Development Of Ultrasonically Levitated Drops As Microreactors For Study Of Enzyme Kinetics And Potential As A Universal Portable Analysis System	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Department of Chemistry, University of Illinois at Urbana-Champaign; Urbana, IL 61801**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
See also ADM002187. Proceedings of the Army Science Conference (26th) Held in Orlando, Florida on 1-4 December 2008, The original document contains color images.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**UU**	**7**	

tions. The inherent rapid mass transfer between gas and liquid phases will be particularly useful for sensing airborne species.

1.1 Reducing Power Requirements

Use of ultrasound to levitate drops dates to the 1940s (Lee et al. 1994; Liu; Dasgupta 1996; Santesson; Nilsson 2004; Santesson et al. 2000; Trinh; Robey 1994; Trinh et al. 1996). A common design for drop levitators is a Langevin resonator (Langevin 1921) coupled to an acoustic resonant cavity. Figure 1a shows the design of the electronics powering the piezoelectric transducer used to excite the resonator and cavity. Designs used prior to our work typically required a 200-W amplifier feeding an impedance matching transformer. This high power and mass equipment was too large for a practical field-deployable system. However, our group recently reported (Field; Scheeline 2007), as illustrated in Figure 1b, that it is feasible to reduce the power to less than 10 W by directly driving the piezoelectric transducer using an operational amplifier, optimized to the actual electrical impedance of the transducer (e.g. OPA548T, Texas Instruments). Servo-controlling the air-filled resonator gap as a function of temperature and gas composition, and using a concave reflector to focus acoustic power, thus minimizing losses, are additional critical features of such power reduction. The transducer/air resonator assembly is shown in Figure 2.

The size of the resonator is inversely proportional to the drive frequency. While we have worked at 20.6 kHz, convenient operation in air can be achieved at 60 kHz, shrinking all dimensions by a factor of 3, and thus apparatus mass by over one order of magnitude. Operating at greater than 60 kHz may present problems because a) amplifier gain and efficiency decrease, b) mechanical losses increase, and c) sound wavelength becomes inconveniently short. At 20.6 kHz, wavelength at 25ºC and 760 torr is 1.675 cm (Lide 1992). Because maximum levitatable drop diameter is $< \lambda/2$, (Oran et al. 1980) 60 kHz can sustain only drops 2.75 mm or less in diameter. Further, small-amplitude vibration or small drop displacement due to gas flow makes stable levitation more tenuous at high frequency.

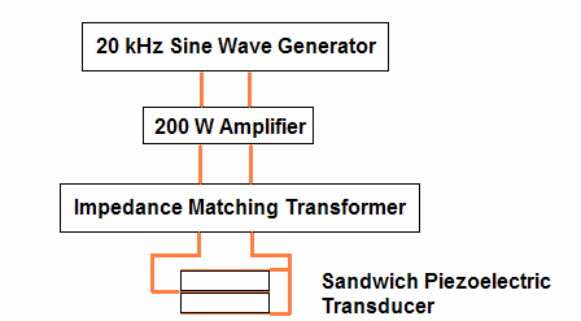


Fig. 1a. Electronics driving typical Langevin resonator/levitator.

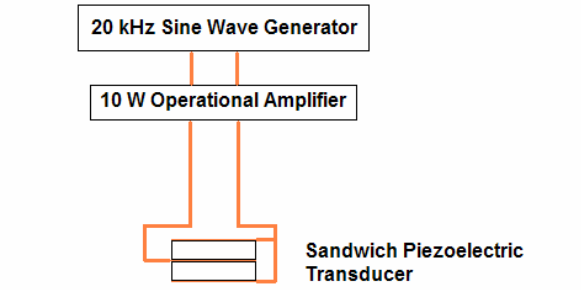


Fig. 1b. Electronics driving low-power Langevin resonator/levitator.

2. Detection and Diagnostics of Chemistry in Levitated Drops

Optical detection is the simplest approach to sensing species in drops (Priego-Capote; de Castro 2006; Rohling et al. 2000; Santesson; Nilsson 2004; Santesson et al. 2003b). Other approaches include electrophoresis (Petersson et al. 1998), mass spectrometry (Westphall et al. 2008), liquid chromatography (Bogan; Agnes 2004; Petersson et al. 1998), and (potentially) electrochemistry. All the "obvious" approaches have been employed: focusing radiation into the drop, continuous flow of reactants through the drop and into a collection capillary, using capillary action to wick the drop into an otherwise empty collection capillary, and placing sensors into the drop. Our emphasis here is on contactless optical methods due to their flexibility, comparative simplicity, and freedom from cross-contamination or the need for inter-sample decontamination.

2.1 Kinetics in Levitated Drops

Study of reaction kinetics in ~ microliter drops requires a means to initiate reaction with temporal precision, high signal-to-noise ratio observations to allow fine time division of observed signals, and careful control of drop positioning to avoid signal arti-

facts due to drop jitter. Two geometries to feed and drain drops have been used: capillaries and ballistically injected, free-floating drops. As the former was first employed, we describe it in some detail, even though such a system is not completely free of solid/liquid interfaces. Such a system provides worthwhile information, and provides a basis for comparison to the clean interface free-floating drop version of the system.

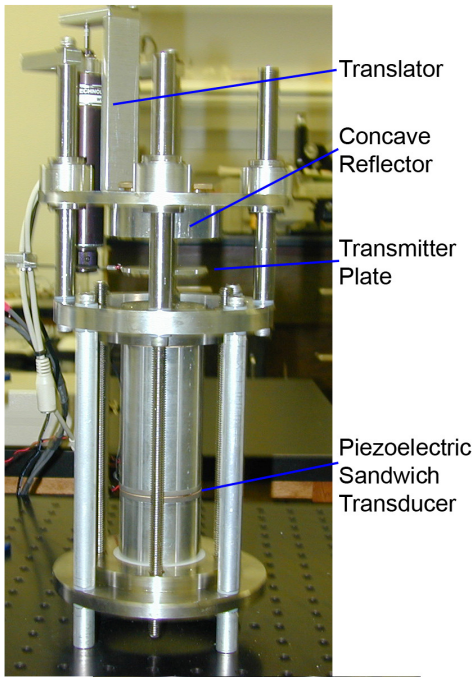


Fig. 2. Drop levitator assembly

Capillary-fed levitated drops are subject to the no-slip boundary condition at the gas-liquid interface of all fluidic interfaces. Thus, the acoustic waves in the support gas set the drop in motion, spinning, oscillating in modes related to spherical harmonics, and vibrating as a pendulum. Fluid is fed with head pressure of 40 psig; typical valve open times for each feed channel are ~ 1 s currently, generating drops of total volume 5 µL. Upon experiment completion, the drop is withdrawn through a capillary linked to vacuum. Capillary geometry and surface treatment are critical to experimental success. As shown in Figure 3, the two feed capillaries must be horizontally adjacent, with the efflux capillary below the feeds and centered. Even a few degrees misorientation results in drops wicking along the sides of the capillary. Assisting in minimizing wicking is a coating of CYTOP® ultrahydrophobic fluorocarbon. The capillary was carefully aligned within 0.5 mm of a sonic nodal plane, ensuring that levitation, not hydrophilicity, suspended the drop. For experiments using enzyme, protein adsorption on unprotected capillary walls was found to be a serious problem. Coating the capillary inner wall with poly-L-lysine (Kamande et al. 2005) gave the wall a positive charge, effectively preventing protein adsorption. The drop assumed an oblate form, with elongated axis parallel to the capillary axis.

Detection for both chemiluminescence and enzyme kinetics used an R928 photomultiplier (Hamamatsu), biased so that peak sensed photocurrent was ~ 10 µA. The photomultiplier output was converted to a voltage using an OP43 operational amplifier with 1 MΩ feedback resistor with the output digitized using a Measurement Computing Inc. DAS1602 analog-to-digital converter. Data were collected at 1 kHz.

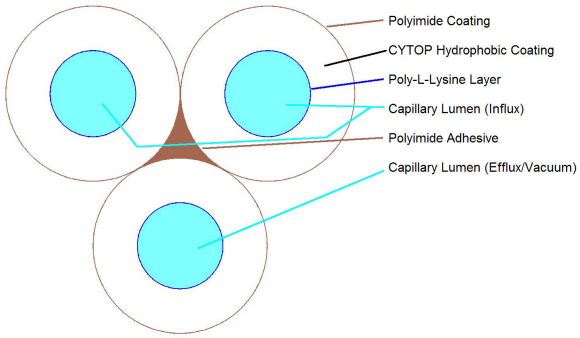


Fig. 3. Capillary bundle cross-section. Capillary inside diameter 160 µm, outside diameter 382 µm.

Typical chemiluminescence data are shown in Figure 4. The reactions are:

Cu^{2+} + luminol ⇔ Cu^{2+}luminol
Cu^{2+}luminol + H_2O_2 → Cu^{2+} + 4-aminophthalate*
4-aminophthalate* → 4-aminophthalate + *hv*

Solutions were maintained at alkaline pH with a buffer of 3 M Na_2CO_3, 0.5 M $NaHCO_3$, and 0.05 M $(NH_4)_2CO_3$. Cu^{2+} stock was 0.015 M $CuSO_4$. Luminol stock was 0.07 M. 10% H_2O_2 was prepared by dilution of commercial 30% H_2O_2 with 18 MΩ water. A 2.5 µL drop of $CuSO_4$/luminol/buffer was grown, after which 2.5 µL of H_2O_2 solution was injected. Mixing occurred from a combination of convection from the infused solution interacting with the pre-existing drop, diffusion, and circulation incited by the levitating ultrasound. Video camera images (not shown) and the data in Figure 4 show that mixing is essentially complete within 1 s after reactant influx is stopped. Strategies for reducing dead time are under development.

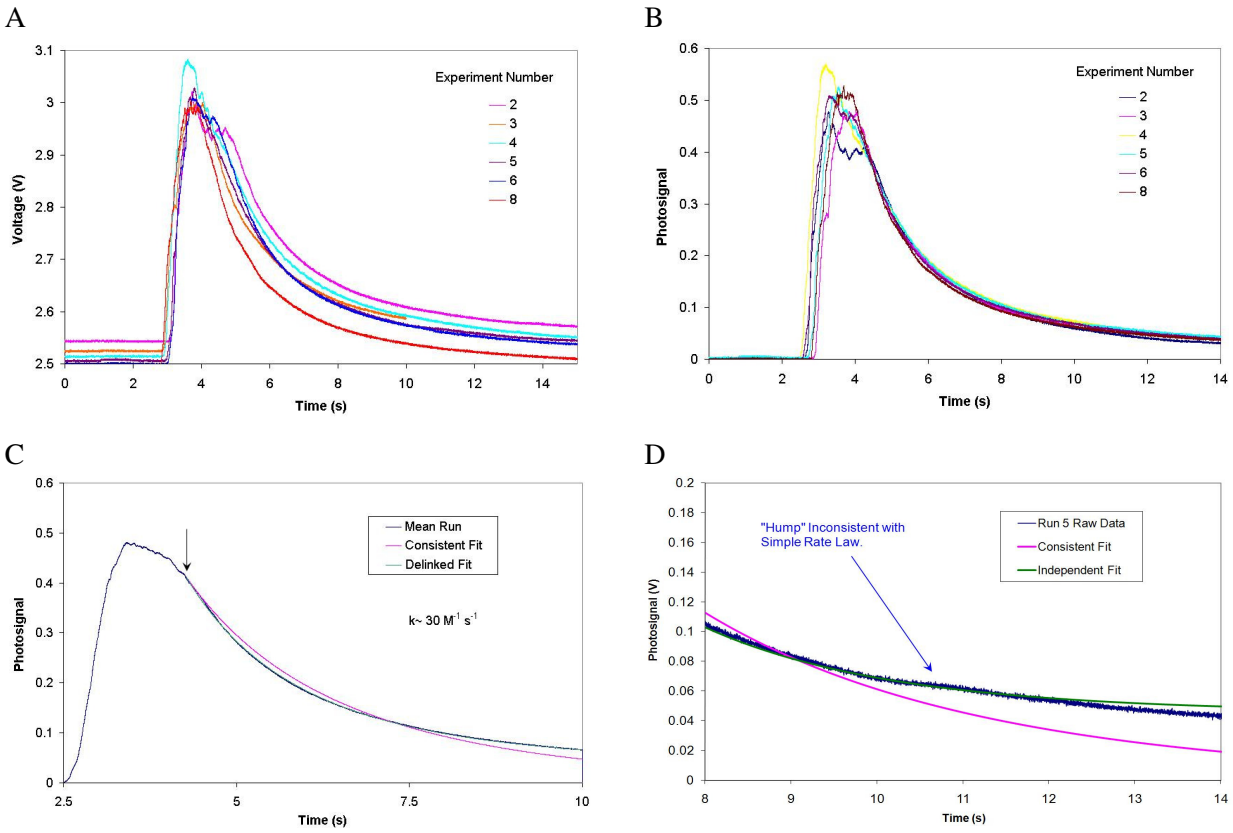


Fig. 4. Chemiluminescence kinetics in levitated drops. A. Six of eight raw chemiluminescence transients. B. Data from A, baseline and time-offset. C. Average of data in B. Fit to second-order rate law is "Consistent Fit." Allowing [luminol]/[H_2O_2] to be fit independently from [luminol] – [H_2O_2] gives "Delinked Fit." D. Scale expansion of fits in C. Raw data shows evidence of dynamics other than simply mass-action kinetics.

The chemiluminescence transient shows baseline offset from one run to the next that is trivially corrected. While valve switching is precise to better than 0.1 s, mixing time varies over a ~ 1 s range. Inset B of Figure 4 shows each trace offset to a common baseline, with transients shifted in time so that all decays go through 0.4 µA simultaneously. The reproducibility following these zero-point offsets can be seen to be a few percent. Averaging all the traces in B, one obtains Inset C. If the decay were a pure second-order reaction between luminol and H_2O_2 with the effect of Cu^{2+} limited to increasing the rate constant k_2, the fluorescence intensity would follow a time course,

$$I = \frac{I_0 kAB(B-A)^2 e^{(B-A)kt}}{\left(Be^{(B-A)kt} - A\right)^2} \qquad (1)$$

where A = [luminol]$_0$, B = [H_2O_2]$_0$, I_0 is an arbitrary scale factor dependent on observation system and reaction quantum yield, and k is an effective rate constant, dependent at least on pH, ionic strength, and [Cu^{2+}]. By design, $B>A$. As expressed here, (B-A) can either be computed from the initial values of A and B or used as a separate fitting parameter. When it is separately optimized, one obtains the best possible fits. Obviously, an alternative interpretation is that the rate law governing chemiluminescence is more complicated than pure second order. Yu *et al.* postulate a mechanism that would be at least third order or involve a pre-equilibrium (Yu et al. 1988). The point here is not to elucidate the mechanistic details but rather to demonstrate that such elucidation is plausible. Figure 4 inset D shows that some small signal modulation inconsistent with a pure mass-action, monotonic approach to equilibrium is observable. Whether this "hump" is due to drop inhomogeneity, dust, or some other source has not been determined.

Attempts to study the well-known reaction of NADH with pyruvate to form lactate, catalyzed by lactate dehydrogenase (LDH) presents additional

difficulties. The hydrophobic protein enables gas bubbles to form in the drop. The drop spins at ~ 300 Hz under the conditions used (~2 W ultrasound at 20.6 kHz, concave reflector). An example is shown in Figure 5, inset A, where the total amount of pyruvate was 1/5th that of NADH. Signal is from NADH fluorescence. After an initial transient, the system goes to steady state. Inset B shows otherwise similar circumstances, but with no pyruvate present.

Conditions for the enzyme work include: solvent is pH 7 PBS buffer. 1 µL of lactate dehydrogenase (0.25 units per mL) was mixed with 4 µL of 0.15 mM NADH + ≤ 1 mM pyruvate in the levitate drop. Reactions were at room temperature (23ºC).

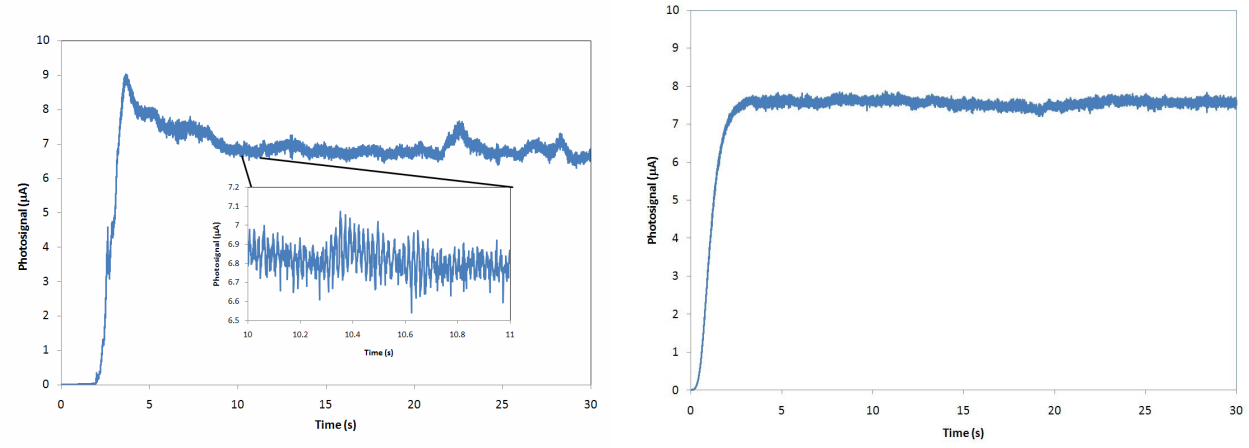
While not yet precise enough nor sufficiently artifact-free to employ routinely, the promise of measuring enzyme kinetics in levitated drops is clear.

3. Levitated Drop Reactors for Force Protection

Part of the attraction of microfluidics has been the promise that chemical and biological analyses could be carried out with portable instruments, few consumables, and a drastically shortened logistical tail compared to earlier laboratory systems. While this has in part been realized, micro flow channels can become contaminated, biofilms can grow on channel surfaces, dust can clog narrow channels, and the channels themselves must be disposed in a safe (and, in the field, discrete) manner. While no field-portable drop levitation analysis system has yet been devised, what characteristics might one have? First, sampling from gases would be trivial; the drop is exposed to the surroundings. Thus, extracting airborne analyte into a drop from a controlled airstream would allow analysis of bacteria, pollen, spores, and concentrated vapors without a separate preconcentration step (Abe et al. 2007). Secondly, if piezoelectric (MicroDrop Technologies), inductive (Nanodrop, Inc.), or pneumatic droplet generators can be integrated, ruggedized, and shrunk to be compatible with levitators, reservoirs of reactants could be encapsulated and maintained against severe environmental challenges. Clean droplets of reactants could be injected into the levitator as needed, the analyte and spent reactants (all 1-5 µL) wicked away upon assay completion, and no flow channel or other hardware disposed of except when reactants were exhausted. With efficient light-emitting diodes as spectral sources and solid-state detection, a logistics-optimized, reliable, flexible instrument can be realized. Increased understanding and control of the levitated drop dynamics and further system optimization for levitation efficiency and stability appear to be the main barriers to realizing this end.

ACKNOWLEDGMENTS

Support from the U. S. Department of Defense through the U. S. Army Construction Engineering Research Laboratory, Cooperative Agreement W91312T-08-2-0009, Research Corp., grant RA0333, and NIH-NIGMS grant 5 R01 GM067193-06 is gratefully acknowledged.



A B

Fig. 5. Lactate dehydrogenase catalyzed reduction of pyruvate to lactate in LDR. A. Initial transient relaxes to steady state with consumption of all pyruvate and ~ 10% of NADH. High frequency fluctuations correlate with spinning of drop. B. Transient in absence of pyruvate.

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