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Estimating postvoid residual volume without measuring residual bladder volume during serial cystometrograms

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[1]*Department of Biomedical Engineering, Duke University, Durham, North Carolina;* [2]*Department of Neurobiology, Duke University, Durham, North Carolina;* [3]*Department of Surgery, Duke University, Durham, North Carolina; and* [4]*Department of Electrical and Computer Engineering, Duke University, Durham, North Carolina*

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Danziger ZC, Grill WM. Estimating postvoid residual volume without measuring residual bladder volume during serial cystometrograms. *Am J Physiol Renal Physiol* 311: F459–F468, 2016. First published April 20, 2016; doi:10.1152/ajprenal.00516.2015.—The postvoid residual volume (PVR) is a common urodynamic parameter used to quantify the severity of lower urinary tract dysfunction. However, the serial cystometrograms that are typically used to assess bladder function in animal models make measuring PVR very difficult. Current approaches are to either remove PVR after each void to measure it, which is disruptive to the bladder, or to neglect the unknown contribution to PVR from ureter flow, which results in inaccurate estimates. We propose a procedure to estimate PVR during a serial cystometrogram that requires only a single measurement, rather than measuring after each void. Moreover, this measurement can occur at the end of the experiment such that it does not affect the bladder during data collection. We mathematically express PVR for all voids during a serial cystometrogram using a linear recurrence equation and use this equation to build an estimation procedure for PVR. Using in vivo measurements in urethane anesthetized rats and computer simulations we show that the estimation procedure is at least as accurate in determining PVR as the traditional method of measuring PVR after each void. Furthermore, we demonstrate the adverse effects of repeated PVR measurements in a common animal model of cystitis. Using the proposed procedure can increase the efficiency and accuracy of determining PVR for a serial cystometrogram and is less disruptive to the system under study. This, in turn, allows the calculation of other urodynamic parameters that are critical for research studies, including voiding efficiency and bladder capacity.

voiding efficiency; urodynamics; underactive bladder; rat; ureter; recurrence equation

THE POSTVOID RESIDUAL VOLUME (PVR) is one of many common urodynamic measures obtained from in vivo cystometric animal studies used to describe bladder filling phase and voiding dynamics. PVR is also used to assess urinary retention and other urinary tract dysfunctions in animal models. In a clinical setting, PVR, along with other urodynamic measures, is used to help assess and manage patients with a variety of lower urinary tract symptoms (1–3, 7, 13, 14). When expressed as a fraction of bladder capacity, PVR quantifies how efficiently the lower urinary tract expels the bladder contents during a void. Voiding efficiency (VE) is commonly used to determine the effectiveness of bladder emptying (8, 10, 15, 17) and is written as

$$VE = V_V/(V_V + V_r) \quad (1)$$

where V_v is the volume voided and V_r is the PVR. VE is the fraction of total bladder volume expelled during the void and is bounded between zero and one. Therefore, VE is intrinsically normalized to each animal's bladder capacity, which makes it an ideal metric to evaluate bladder function in a heterogeneous population or across differing experimental conditions. Furthermore, PVR allows one to calculate the starting and ending volumes of each voiding event. This is critical information needed to determine the bladder volume as a function of the measured bladder pressure throughout the filling process, which can then be used to estimate bladder tension and compare cystometrograms through time (4, 18).

Unfortunately, PVR is difficult to measure directly in animal experiments. It is common in animal studies to perform a serial cystometrogram by inserting a catheter through the dome of the bladder and continuously infusing fluid to observe lower urinary tract function over repeated voiding events (11). This approach is useful for quantitative descriptions of bladder function and increasing statistical power, but it is difficult to obtain the PVR of each void in the series. During a serial cystometrogram, volume enters the bladder through the catheter, but concurrently volume enters via the ureters at an unknown rate. Therefore, measuring the voided volume alone is insufficient to determine the PVR. To handle this problem the experimenter is forced to choose among three unsatisfactory options. The first is to remove the PVR after each void to measure it, which is undesirable because removing the PVR does not correspond to the natural physiological conditions the experiment is designed to study. The second is to neglect the unknown renal contribution to bladder volume, which results in a systematic underestimation of PVR. The third is simply to forego any measurement of PVR and all the parameters whose calculation requires PVR.

To address the difficulties in measuring PVR during serial cystometrograms, we propose a procedure that estimates all residual volumes in the series using only a single measurement of PVR. Moreover, this single measurement is obtained at the end of the experiment, such that it does not interfere with the system during data collection. We derive the procedure from a mass balance approach and establish its accuracy by comparing traditional measurements with those obtained using the proposed estimation procedure in both in vivo rat experiments and in computer simulations.

METHODS

All animal care and procedures were reviewed and approved by the Institutional Animal Care and Use Committee at Duke University. Female Sprague-Dawley rats (0.267 ± 0.015 kg, mean ± SD, $n = 7$) were initially anesthetized with 3% isoflurane, followed by two

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subcutaneous injections of urethane totaling 1.2 g/kg (dissolved as 0.2 mg/ml in 0.9% saline solution). A catheter (PE50) with a heat-flared tip was inserted into the bladder dome and secured with suture. The animal lay supine on a heated water blanket to maintain body temperature. Heart rate and blood oxygenation were monitored with a pulse oximeter on the hindpaw. Following the experiment the animal was killed with an intraperitoneal injection of 500 mg of Euthasol.

Room temperature 0.9% saline solution was infused via syringe pump into the bladder until a void occurred. Once the void was completed the pump was stopped and PVR was measured by withdrawing the bladder volume manually through the catheter using a 1-ml Luer Lock syringe. Following this measurement, the residual volume was slowly reinserted into the bladder using the same syringe. Voided volume was then measured and the pump was restarted. In three animals, following serial cystometrograms and data collection, the ureters were clamped. The bladder was emptied and a known amount of fluid was inserted into the bladder. A second experimenter, blinded to the volume, attempted to withdraw and measure the bladder contents. A total of 65 such measurements were performed, and the standard deviation of the difference between the inserted and measured volumes was used to determine the expected error in PVR (see left edge of the white rectangle in Fig. 2*A*) and $\xi(V_r)$ (see Table 2). In two animals the serial cystometrogram protocol could not be completed successfully because the bladder contracted, generating voiding, on reinsertion of the residual, and all data from these animals were excluded from analysis.

A second series of experiments were conducted to test the effects of invasive PVR measurements on urodynamic outcomes. Fifteen animals were surgically prepared as described above (0.275 ± 0.014 kg). In these animals we infused acetic acid dissolved to 0.25% concentration in 0.9% saline solution at room temperature as an acute model of cystitis. In addition, we recorded external urethral sphincter electromyographic activity using fine platinum iridium wire electrodes placed near the urethra, and we applied Vaseline to the skin near the meatus to prevent cutaneous irritation by the acetic acid. The bladder was filled continuously at 2.5 ml/h for four to six voiding cycles following surgery to allow the urinary tract to acclimate. In all animals we performed four separate serial cystometrograms of seven voids each at a fill rate of 2.5 ml/h, separated by 20-min quiet periods without filling (see Fig. 3*A*). Within each of the four serial cystometrograms, we obtained the PVR for all voids in one of two ways: *1* stopping the infusion pump after each void and withdrawing (and not replacing) the bladder contents through the suprapubic catheter, or *2* only measuring the last PVR directly and using our estimation procedure to determine the other PVRs in the serial cystometrogram. The estimation and measurement procedures were alternated for each serial cystometrogram in the crossover design, and the measurement type of the first block was randomized (see Fig. 3*A*). One of the 15 animals exhibited extreme sensitivity to acetic acid and voided immediately when the infusion pump was started. Data could not be collected for this animal and it was excluded from all analysis.

Software to perform V_r and ureter flow rate estimation is provided in the Supplemental Material (MATLAB 2015a and Microsoft Excel 2013; Supplemental Material for this article is available online at the Journal website). Statistical analysis, including repeated-measures ANOVA, was done using the statistics toolbox (MATLAB 2015a). Boxplot edges mark the 25th and 75th percentiles of the data, the centerline shows the median value, and whiskers extend to cover the central 99.3% of normally distributed data. Data points are superimposed over the boxplot with horizontal jitter for clarity.

RESULTS

We describe a procedure to estimate postvoid residual (PVR) for a serial cystometrogram without the need to measure multiple residual volumes. We first present an overview of the procedure, describe how to use the method, and highlight its limitations. A full mathematical derivation is provided in APPENDIX 1. We then evaluate the effectiveness of the procedure using an in vivo experiment and quantify its accuracy using numerical simulations. In an additional set of in vivo studies, we compare the biological impact of our proposed procedure to that of traditional measurements.

Overview of the estimation procedure. During serial cystometry, volume is infused into the bladder from a catheter and, simultaneously, volume enters the bladder through the ureters. During a void, the bladder contracts to expel a portion of the accumulated volume through the urethra and retains the rest as a residual volume. This description accounts for the entirety of the volume passing through the bladder during a serial cystometrogram, and we express these relations in *Eq. 2*.

$$V_r + V_v = V_u + V_i + V_r^{(\text{previous})} \quad (2)$$

The equation states that, for any void in the serial cystometrogram, the postvoid residual volume (V_r) plus the voided volume (V_v) must equal the volume entering the bladder through the ureters (V_u) plus the volume infused through the catheter (V_i) plus the residual volume from the previous void in the series [$V_r^{(\text{previous})}$]. Generally, the experimenter will know V_i because it is under their control and will know V_v because it is easy to measure accurately and noninvasively.

We are interested in the value of V_r after each void; however, *Eq. 2* cannot be solved for V_r (in terms of known quantities) because V_u is unknown. There are two common approaches to this problem. The first method is to neglect V_u (assume it is zero; Refs. 5, 9, 19), which results in an underestimate of V_r. The second method is to remove V_r after each void to measure it directly (6, 12, 16). However, this can irritate the bladder and is a departure from the physiological conditions that the experiment aims to study (i.e., PVR normally remains in the bladder after voiding). Our proposed estimation procedure allows us to compute V_r without removing bladder volume during a serial cystometrogram, which we explain in the remainder of this section.

Equation 2 allows us to express V_r for each void as the net accrual of bladder volume from the previous void. Meaning, V_r for the last void is the difference between the incoming and outgoing volume of the last void plus the second to last residual. Likewise, the second to last residual is the net volume of the second to last void plus the third to last residual. This recursion continues until we reach the first void in the series. Therefore, the only unknown quantities in this recursive expression for the last residual volume are the last residual itself [$V_r^{(\text{last})}$], the initial bladder volume that cannot be expressed in terms of a previous void [$V_r^{(\text{1st})}$], and the volume contribution from the ureters (V_u).

However, when the catheter is initially inserted into the bladder all of the fluid can be withdrawn through the catheter or pushed out by gently expressing the bladder. This will set the initial bladder volume to zero, and eliminate $V_r^{(\text{1st})}$ as an unknown. Additionally, once the serial cystometrogram is over and the experiment completed, the final residual volume can be measured without interfering with subsequent trials. Therefore, the only remaining unknown is the volume contribution from the ureters. If we assume that volume enters the bladder via the ureters at an unknown rate that is the same throughout the serial cystometrogram, we can use this recursive form of *Eq. 2* to solve for the constant unknown ureter flow rate. Finally,

using this estimate of the ureter contribution [and the fact that we know $V_r^{(last)}$ and $V_r^{(1st)} = 0$], we can use *Eq. 2* to compute all of the residual volumes throughout the serial cystometrogram.

Thus this estimation procedure allows us to compute the residual volumes of each void in a serial cystometrogram without measuring them explicitly and without neglecting the volume contribution from the ureters. Below, we also discuss the accuracy of assuming a constant ureter flow rate, quantify the loss of accuracy if this assumption is not true, and provide approaches without this assumption. A practical user's guide to implementing this procedure is provided in APPENDIX 4.

Comparison of estimation with experimental measurements. To assess the similarity of PVR values obtained from the proposed estimation procedure and traditional PVR measurements we performed six serial cystometrogram experiments in five rats. The bladder was filled through a suprapubic catheter until a void occurred. Voided volume and PVR were measured and PVR was subsequently reinserted into the bladder, allowing us to measure PVR after each void and also to emulate an experiment where PVR was not removed from the bladder for measurement. As outlined in the description of the estimation procedure, we used the final measured PVR in the serial cystometrogram to estimate all other PVR in the series (*Eqs. 7* and *8*, APPENDIX 1). We then used either the estimated or measured PVR to compute voiding efficiency (VE, *Eq. 1*), which is a within animal normalized metric that allowed us to compare results across animals with different bladder capacities. There was close agreement between the estimated and measured VEs (Fig. 1). The magnitude of the difference between estimated and measured VEs for the serial cystometrograms in Fig. 1 was 0.037 ± 0.014. Parameters for each serial cystometrogram are listed in Table 1.

Determining estimation accuracy using computer simulations. It is not possible to determine the accuracy of the traditional method of measuring VE or our estimation procedure from the data presented in Fig. 1; it is only possible to show the difference between the values because the true VE for each void is obscured by measurement noise in PVR (V_r) and the voided volume (V_v). Therefore, we performed a computer simulation of serial cystometrograms to quantify the accuracy of the estimation procedure and traditional measurements in a case where the true VE was known. We performed the simulation as a set of successive volume balances defined by *Eq. 2* (and therefore, we did not explicitly model bladder pressure). The value of each parameter was drawn from a normal distribution (if variability was added) with mean and standard deviation listed in Table 2 (simulations deviate from these nominal values as indicated in Fig. 2).

To evaluate the difference in accuracy between the proposed procedure for estimating VE and measuring VE in the traditional way, Gaussian noise was applied to the true values of V_v and V_r (Table 2, ξ). We used these noise-corrupted values to calculate VE using both the traditional measurement method and the new estimation procedure to emulate experimental conditions. The accuracy loss, L, of using the estimate rather than measuring VE for a full serial cystometrogram of N voids was expressed with the following metric

Table 1. *Cystometrogram parameters*

Fig. 1	Rat ID	Infusion Time, min	Bladder Capacity, ml	Infusion Rate, ml/h
A	*1*	7.60 ± 1.69	0.56 ± 0.09	4
B	*2*	7.32 ± 3.33	0.28 ± 0.08	1.85
C	*3*	5.88 ± 1.90	0.36 ± 0.10	3
D	*6*	4.18 ± 2.54	0.26 ± 0.06	2
E	*7*	4.47 ± 1.62	0.35 ± 0.07	2
F	*7*	9.58 ± 13.6	0.34 ± 0.10	2 ± 1

Data are means ± SE and correspond to Fig. 1, *A–F*.

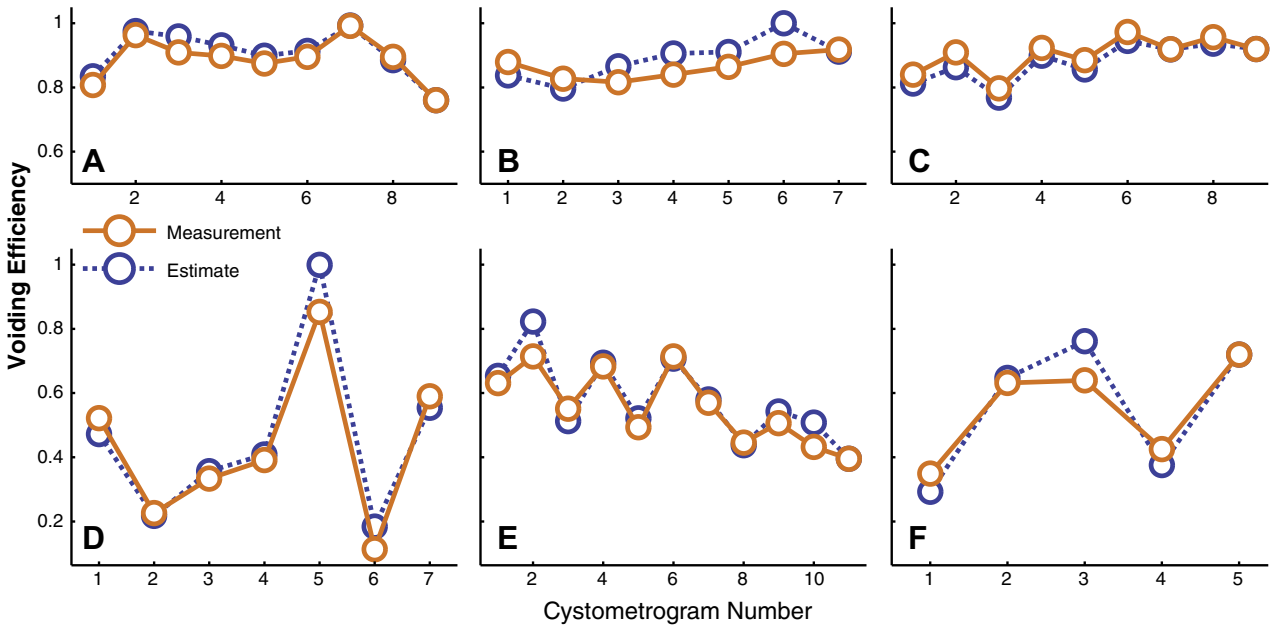

Fig. 1. The proposed estimation procedure produced voiding efficiency (VE) estimates that were similar to the VEs measured during serial cystometrograms in anesthetized rats. *A–F*: the estimated (dashed blue) and measured (solid orange) VEs are shown for each voiding event in the serial cystometrogram. The estimation procedure was performed using the measurement of the last V_r in the series. Parameters for each serial cystometrogram are given in Table 1.

Table 2. *Nominal cystometry simulation parameters*

Parameter Name	Symbol	Value
Ureter flow rate	$\dot{V}_u$	0.20 ml/h
Ureter flow rate SD	$\sigma(\dot{V}_u)$	Variable ml/h
Residual volume	V_r	0.10 ml
Residual volume	$\sigma(V_r)$	0.04 ml
Residual measurement noise	$\xi(V_r)$	Variable ml
Intervoid interval	t_i	8 min
Intervoid interval SD	$\sigma(t_i)$	1 min
Infusion rate	$\dot{V}_i$	4 ml/h
Void measurement noise	$\xi(V_v)$	0.005 ml
Number of consecutive trials	N	10

The parameters $\dot{V}_u$, V_r, and $\sigma(V_r)$ were the averages of measured values from the trials shown in Fig. 1. The voided volume, V_v, is omitted from table 1 because it is determined by specifying the other parameters in the simulation. We assumed that t_i was known exactly. t_u Was equal to t_i for all simulations.

$$L = \frac{1}{N}\sum_{k=1}^{N}\left(\left|VE_e^{(k)} - VE^{(k)}\right| - \left|VE_m^{(k)} - VE^{(k)}\right|\right) \quad (3)$$

where $VE^{(k)}$ is the true voiding efficiency for the kth void, VE_e and VE_m are the estimated and measured voiding efficiency, respectively, and |.| is the absolute value.

The accuracy loss, L, as a function of the variability in ureter flow rate, $\sigma(\dot{V}_u)$, and noise in the residual volume measurement, $\xi(V_r)$, is plotted in Fig. 2*A*. The vertical axis can be interpreted as the degree to which the assumption of constant ureter flow rate is violated, because $\sigma(\dot{V}_u)$ is the variability of $\dot{V}_u$ across voiding events, and as expected, L increased with $\sigma(V_u)$.

The left edge of the overlaid white rectangle corresponds to the expected value of $\xi(V_r)$ based on experimental measurements (-0.01 ± 0.05 ml, see METHODS). This value is likely to be an underestimate of $\xi(V_r)$ because measurements were performed by an expert in vivo experimentalist (12 yr of experience) with the rat supine and the bladder exposed; typical measurement conditions would be more difficult. The top edge of the white rectangle is the expected value of $\sigma(\dot{V}_u)$ calculated from the experimental measurements (Fig. 1, *A–F*). Meaning, the ureter flow rate was calculated algebraically from the measurements of each V_r, V_v, and infused volume using *Eq. 2*, and the standard deviation was computed. The resulting value is likely an overestimate of $\sigma(\dot{V}_u)$ because $\dot{V}_u$ was computed for each void using noisy measurements, which artificially inflate $\sigma(\dot{V}_u)$. Therefore, the rectangle enclosed by the two white lines is the region where we expect the true values $\sigma(\dot{V}_u)$ and $\xi(V_r)$ for our in vivo experiments shown in Fig. 1.

The black region of Fig. 2*A*, where L is approximately zero, represents a frontier of equivalence between the estimation and measurement methods for calculating VE. To the right of the black band (the equivalence frontier) L is negative, indicating that, remarkably, the estimation method is more accurate than the measurements. This occurs because when computing all $VE^{(k)}$ in a serial cystometrogram using measurements, every calculation incorporates a different $V_r^{(k)}$, which all have associated uncertainty. When estimating $VE^{(k)}$ with *Eqs. 7* and *8*, only one residual measurement is used, $V_r^{(k=N)}$, and the calculation is performed using the far less uncertain $V_v^{(k)}$, and the very precise $\dot{V}_i^{(k)}$, $t_i^{(k)}$, and $t_u^{(k)}$.

The effects on the equivalence frontier of varying key parameters are shown in Fig. 2, *B–D*, where each line represents the equivalence frontier for the noted parameter change. The estimation method is more accurate for smaller numbers of serial voids, because VE is estimated recurrently and error is propagated through the estimates of all $V_r^{(k)}$ (Fig. 2*B*). For small residual volumes, the estimation method becomes less accurate relative to standard measurements (Fig. 2*C*). This occurs because when adding measurement variability to small residuals in the simulation, the resulting V_r may be negative, and when this occurred we set V_r to zero. This effectively reduced $\sigma(V_r)$ for small V_r, which is more beneficial for the direct measurement method than the estimation method. This

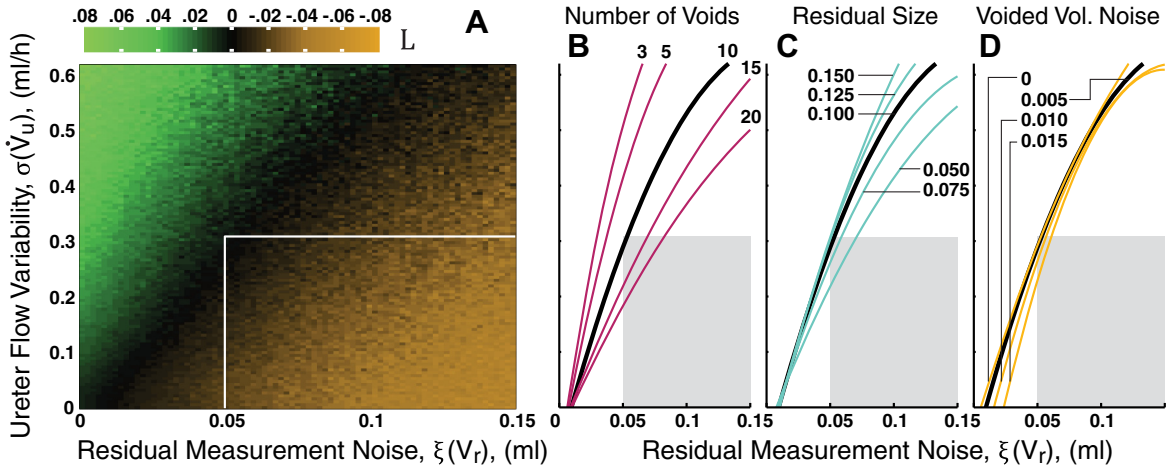

Fig. 2. Simulations comparing accuracy of VE calculations using the estimation procedure and standard measurements. *A*: heatmap of the loss in accuracy, L (*Eq. 3*), when estimating rather than measuring VE. Negative L (orange) indicates parameter combinations where the estimation procedure outperformed standard measurements. The white rectangle encloses the region where the true vales of $\sigma(\dot{V}_u)$ and $\xi(V_r)$ for the experiments conducted in Fig. 1 are likely to be located. Simulation parameters are listed in Table 2. *B–D*: the effects of varying a parameter from the nominal values used to generate the plot in *A*. Each line represents the $L = 0$ equivalence frontier for the listed parameter change, and the black line is the equivalence frontier from *A*. Parameters examined were the number of voids in the serial cystometrogram (N), the residual volume (V_r), and the amount of noise corrupting the measurements of the voided volume [$\xi(V_r)$]. The gray region corresponds to the white rectangle in *A*. Values of L for *A–D* were determined by averaging across 100 repetitions of the simulated serial cystometrogram at each parameter combination.

effect was also present in experimental measurements where a negative V_r is never recorded. In practice, this non-negativity constraint causes measurements of low V_r to have lower $\sigma(V_u)$, because it limits the degree of underestimation of V_r. Finally, the effects of changing $\xi(V_v)$ are shown in Fig. 2*D*. There is far less uncertainty in measuring V_v than V_r because all expelled volume can be readily collected. Therefore, we set the nominal value of $\xi(V_v)$ to 0.005 ml, which is the margin of error for our measurement device (a 1-ml syringe with 0.01 ml demarcations). The simulation indicates that the equivalence frontier is very robust to increases in $\xi(V_v)$ because both the estimation and the measurement methods incorporate all $V_v^{(k)}$ to compute VE.

The effect of residual volume measurement in an acetic acid model of cystitis. In an additional set of experiments we performed four blocks of serial cystometrograms with 0.25% acetic acid and alternated between traditional invasive measurements of PVR and our estimation procedure in each block (Fig. 3*A*). The animals were pseudorandomly assigned to a measurement sequence such that there were an equal number in each group. We focused our analysis on bladder capacity because it is the most critical and commonly investigated urodynamic outcome in cystitis and computing bladder capacity in a serial cystometrogram requires knowledge of PVR.

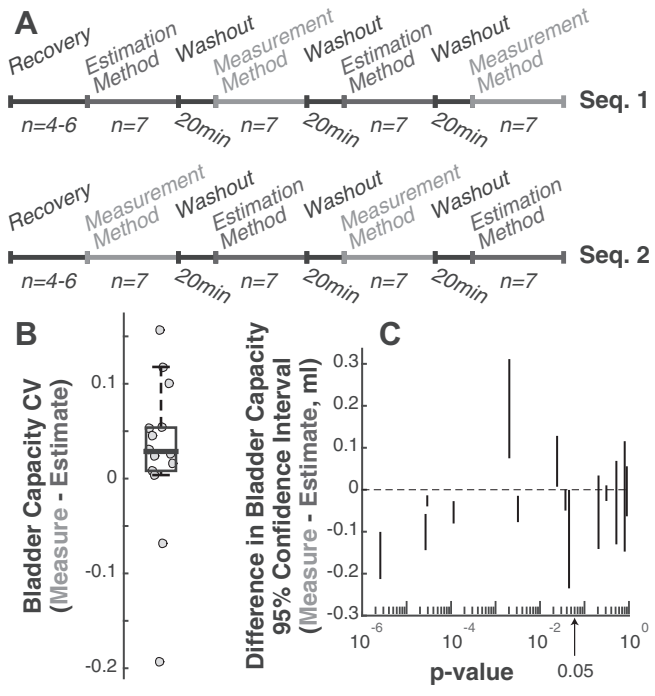

Fig. 3. Comparison of traditional measurement method to the proposed estimation procedure to calculate PVR during a serial cystometrogram with 0.25% acetic acid. *A*: following catheter implantation, 4–6 fill and void cycles were performed in each animal to allow recovery of normal voiding. Four blocks of serial cystometrograms were performed containing seven voids each. Each block was alternated between invasive measurement of PVR, by withdrawing volume through the catheter, and the noninvasive estimation procedure. A 20-min period where no filling occurred elapsed between each serial cystometrogram. The 14 animals were equally distributed between *sequences 1* and *2*. *B*: the coefficient of variation of bladder capacity is significantly higher when removing PVR for direct measurement than with the noninvasive estimation procedure. *C*: 95% confidence intervals over the difference in bladder capacity when using the direct measurement or estimation procedure for measuring PVR. The data show many more significant changes in bladder capacity than would be expected by chance.

The direct measurement method increased the void-by-void variation in bladder capacity. A paired *t*-test revealed a significant increase in the variability of bladder capacity within each animal when removing PVR for measurement (Fig. 3*B*, $P = 0.026$) compared with the estimation procedure. Variation in bladder capacity was quantified using the coefficient of variation (CV) because overall bladder capacity varied substantially between animals (0.25 ± 0.17 ml) and the CV normalizes variation to the mean. This increase in variability is likely to be an artifact of the direct measurement method, and may be caused by irritation associated with repeatedly removing PVR for measurement.

A repeated-measures ANOVA with sequence as a between subject factor and PVR measurement type as a within subjects factor failed to detect a significant effect of sequence on bladder capacity [$F(1,12) = 2.54$, $P = 0.14$]. This indicates that the order in which the measurement types were implemented did not affect bladder capacity. No significant effect of measurement type on bladder capacity was detected [$F(1,12) = 1.38$, $P = 0.26$] and no interaction was detected between sequence and measurement type ($P = 0.57$).

Although no difference in bladder capacity was found between the measurement types when grouping all animals, there were many individual experiments where there were large significant differences in bladder capacity. In 12 of 14 animals bladder capacity was lower when using the direct measurement method, with 7 of those animals having differences significant at the $\alpha = 0.05$ level. Figuare 3*C* shows 95% confidence intervals of the difference in bladder capacity between the measurement types for each animal. Nine of 14 animals showed a significant difference in bladder capacity (in either direction) between the two measurement types, whereas if there were no effect of measurement type on bladder capacity we would expect only 1 in 20 animals to show significance at this level. The ANOVA failed to detect an overall difference between measurement types because one animal displayed a very large effect in the opposite direction of the trend (5th from the left, Fig. 3*C*). For example, if that animal were removed as an outlier, the ANOVA detects a significant effect of measurement type ($P = 0.02$). Due to interanimal variation, a much larger study is required to determine if the two animals that showed lower bladder capacity during the estimation procedure are the result of natural variation, are outliers, or represent a subpopulation of animals that consistently respond in this way.

PVR errors caused by neglecting ureter inflow. An alternate approach to estimate PVR and bladder capacity is to neglect the contribution of filling from the ureters. If the only source of bladder filling were the experimenter-infused volume, then PVR could be calculated algebraically using measurements of the voided volume (*Eq. 2*). However, because the volume entering from the ureters is nonzero, neglecting it will result in errors in PVR and bladder capacity estimates.

We empirically determined the magnitude of error caused by neglecting ureter flow by comparing the bladder capacity in the acetic acid trials obtained using our estimation procedure with what would have been obtained had we neglected the ureter flow (setting $\dot{V}_u = 0$). The error, as a percent of bladder capacity, is plotted in Fig. 4*A*, where each point is the error averaged over all voids in one acetic acid serial cystometrogram. We restricted this analysis to blocks where the estimation method was used because when neglecting ureter flow to

compute PVR no volume would be removed from the bladder. A student's *t*-test rejected the hypothesis that the error caused by neglecting ureter flow was zero ($P < 10^{-5}$).

In Fig. 4*B* we show the amount of volume in the bladder through time, as obtained using our estimation procedure (blue dashed) and from neglecting ureter flow (green solid) for three examples. These traces show that neglecting ureter flow results in an underestimation of PVR that compounds through time as the total neglected volume continues to grow. Moreover, as error accumulates from neglecting ureter flow, estimates of bladder volume become negative because there is insufficient catheter infusion to account for all the measured voided volume.

More generally, we can compute the error in estimating bladder capacity caused by neglecting ureter flow for any given void (for a constant ureter flow rate) as the difference between total volume passing through the bladder and the infused volume,

$$V_{err} = t\left(\dot{V}_i + \dot{V}_u\right) - t\dot{V}_i = t\dot{V}_u \quad (4)$$

where $\dot{V}_i$ is the infusion rate, $\dot{V}_u$ is the ureter flow rate, and t is the elapsed time of the entire serial cystometrogram (not the time since the previous void). *Equation 4* states that the bladder capacity estimation error increases the longer the serial cystometrogram lasts and the larger the ureter flow rate. The error as a percentage of bladder capacity is shown as a function of time and ureter flow rate in Fig. 4*C* using *Eq. 4*. The black circles denote the median ureter flow rates and times for the acetic acid serial cystometrograms, indicating 5.9% error at the time of the first void and a 41% error at the seventh. This plot illustrates the high levels of error caused by neglecting the volume entering via the ureters. To maintain an estimation error of <10% of the bladder capacity when neglecting ureter flow, the serial cystometrogram time and ureter flow rate combination must lie to the left of the white curve.

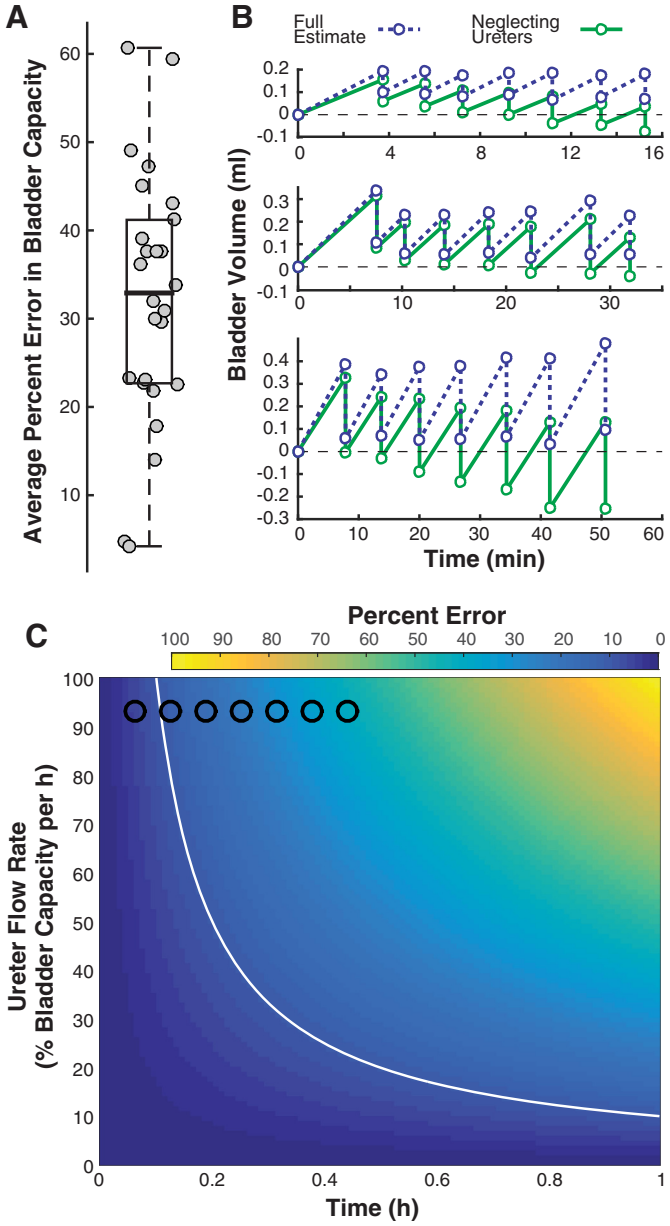

Fig. 4. Errors in estimation of bladder capacity caused by neglecting ureter flow. *A*: the error as a percentage of bladder capacity caused by neglecting ureter flow for the estimation procedure blocks in the acetic acid experiments. *B*: bladder volume as a function of time as calculated using the estimation procedure (dashed) and by algebraic computation neglecting ureter flow (solid). These examples show the accumulation of underestimation error through time. *C*: theoretical heatmap (*Eq. 4*) of the error (as a percentage of bladder capacity) incurred when computing PVR by neglecting ureter flow, as a function of time and ureter flow rate. Median experimental values from the acetic acid serial cystometrograms are overlaid as the black circles, and the white curve is the 10% error isocline.

DISCUSSION

We developed and validated a procedure to estimate postvoid residual volumes (and thereby also voiding efficiency and bladder capacity) for a serial cystometrogram that requires only a single measurement of residual volume at the end of the series. Our method shows excellent agreement with the experiment (Fig. 1), and simulations demonstrate that, for common experimental parameters, our estimation will be at least as accurate as traditional measurements that require removing fluid from the bladder after each void to determine the postvoid residual (Fig. 2). APPENDIX 4 includes a practical user's guide on how to implement the estimation procedure, and the Supplemental Material contains a template to perform the basic calculations in Excel as well as MATLAB code to run the more sophisticated estimations described in APPENDIXES 1–3.

The value of our estimation procedure is that it outperforms the alternative methods for obtaining PVR. The first alternative is to neglect ureter flow to compute PVR. However, we demonstrated that this results in high levels of error, especially for longer cystometrograms (Fig. 4). The second alternative is to remove the PVR after each void for measurement. In addition to forcing each void cycle to start from zero volume and collapsing the bladder when withdrawing the PVR (neither of which are physiological), we showed that this method causes increased variability of bladder capacity (in an in vivo cystitis model, Fig. 3*B*) compared with our noninvasive procedure. Furthermore, our estimation procedure is more accurate than either alternative method under typical experimental conditions (Fig. 2*A* and Fig. 4*C*).

Our estimation procedure is a versatile method that can accommodate all ranges of VE, PVR, voided volume, ureter flow rates, infused volumes, serial cystometrogram lengths,

and multiple or repeated measurements. For example, Fig. 1*F* shows a serial cystometrogram in which the bladder infusion rate was a random variable that changed with each fill in the series. The flexibility of this procedure makes it suitable for many types of experiments.

Our estimation procedure also provides a way to measure PVR (and by extension VE and bladder capacity) in chronic animal experiments, which is currently not possible. When an animal is first instrumented for a chronic study, the bladder can be catheterized via the urethra and drained. Thereafter, the time and volume of each void can be recorded using a metabolic cage. Periodically, the animal could be externally catheterized, and the residual volume removed and measured. This would constitute a set of serial cystometrograms, and the data required to estimate PVR for all intervening voids would be available. Similarly, our procedure is also effective for acute unanesthetized animal experiments because it requires only volume and time measurements to produce the estimates of PVR. Conscious animals tend to have smaller PVR; however, our simulations show that for the range of physiological parameters in healthy anesthetized rats, a PVR reduction of 50% results in an accuracy loss of at most ~2% (less with lower measurement noise, lower flow rate variability, or larger PVR) relative to measuring PVR directly (Fig. 2*C*). Therefore, this estimation procedure is also suitable for unanesthetized animal studies and other conditions with smaller PVR.

The primary limitation of the method is the assumption that the ureter flow is constant; however, our proposed method performs well in experiments (Fig. 1) and simulations (Fig. 2) with typical levels of ureter flow variability. If there is reason to believe that the ureter flow will vary substantially throughout the course of the experiment, the serial cystometrogram can be broken up into shorter segments where the within-segment variability in ureter flow rate is low. Alternatively, using an extension of the estimation procedure, additional measurements of V_r can be incorporated into the $\dot{V}_u$ estimate to increase the accuracy of V_r estimates (*Eq. 10*, APPENDIX 2). The increase in accuracy as more measurements are incorporated plateaus quickly (Fig. 5), and one additional measurement is likely to be sufficient to improve accuracy to acceptable levels in these cases. Finally, it is possible to solve *Eq. 7* for $\dot{V}_u^{(k)}$ without assuming a constant flow (see APPENDIX 3); however, this introduces substantial complexities. Because the estimation method functions well with the constant flow assumption across a very wide range of experimental parameters, it may not be necessary to introduce this more sophisticated procedure.

APPENDIX 1

Complete derivation of the PVR estimation method. The bladder is an input/output system where the law of conservation of mass applies (equivalent to conservation of volume under the physiological range of pressures and temperatures). The volume balance of the bladder for the *k*th void in a serial cystometrogram (where the residual volume after each void is not removed) can be stated as a first order linear recurrence equation

$$V_r^{(k)} = \dot{V}_u^{(k)} t_u^{(k)} + \dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} + V_r^{(k-1)} \qquad (5)$$

where V_r is the residual volume, V_v is the voided volume, $\dot{V}_i$ is the rate (denoted with the overdot) of volume infusion through the bladder catheter, t_i is the duration of the infusion, $\dot{V}_u$ is the rate of the ureters, and t_u is the duration of ureter flow. Superscripts denote the index of the void and are in parentheses to emphasize that they are not exponents. It is important to note that $t_u^{(k)}$ and $t_i^{(k)}$ need not be equal. The term $t_u^{(k)}$ represents the total time between voided volume measurements because the ureters contribute volume throughout the fill and void. The $t_i^{(k)}$ is the total amount of time the infusion pump is on and thereby accounts for periods when pump may be off during a void or measurement of a residual.

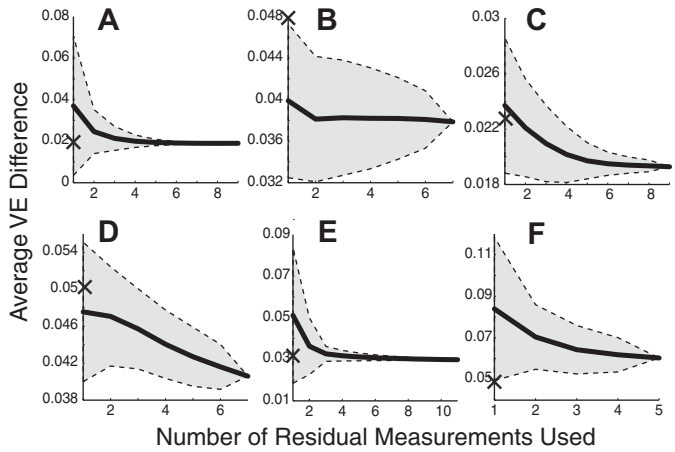

Fig. 5. The difference in estimated and measured VE for each voiding event was recalculated for each combination of k measurements of V_r used in the estimation procedure (horizontal axis) and averaged to produce the average (thick trace) and standard deviation (gray patch) of the difference. Therefore, the number of differences averaged for each point is $n!/[k!(n-k)!]$, where n is the total number of voiding events in the serial cystometrogram. The red "x" denotes the value when using the last measured residual in the series to estimate VE, as shown in Fig. 1, *A–F*. *A–F* corresponds to Fig. 1, *A–F*.

By recursively substituting the expression for $V_r^{(k-1)}$ into the volume balance for $V_r^{(k)}$ (*Eq. 5*) we obtain the nonrecursive expression for the residual volume of any void, n, in a series of voids

$$V_r^{(n)} = V_r^{(0)} + \sum_{k=1}^{n} \left(\dot{V}_u^{(k)} t_u^{(k)} + \dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \right) \qquad (6)$$

We wish to use this expression to compute any arbitrary residual volume. *Equation 6* includes $t_u^{(k)}$ and $V_v^{(k)}$ that are easily measured, and the terms $\dot{V}_i^{(k)}$ and $t_i^{(k)}$ that are typically known, independent experimental variables. Furthermore, $V_r^{(0)}$ can be set to zero (or any other known value) at the start of the serial cystometrogram.

$\dot{V}_u^{(k)}$ and $V_r^{(n)}$ are the last remaining unknowns in *Eq. 6*. The $\dot{V}_u^{(k)}$ terms are challenging to account for because they are not in the experimenter's control and cannot be measured in a noninvasive way during each cystometrogram. To estimate the $\dot{V}_u^{(k)}$ terms we begin by assuming that ureter flow rate is a constant unknown quantity [i.e., $\dot{V}_u^{(k)} = \bar{V}_u$] throughout each serial cystometrogram, the accuracy of which has been investigated above (Fig. 2). We then measure the residual volume once following the last void, N, in the serial cystometrogram (the residual can actually be measured after any void, but we recommend the final void so that the act of measuring has the least impact on the results of future cystometrograms). Because $V_r^{(n)}$ in *Eq. 6* does not depend on any other V_r in the serial cystometrogram [except the known $V_r^{(0)}$], we can solve for $\bar{V}_u$ when $n = N$ by substituting in the measured value of $V_r^{(n=N)}$. Using the assumption that $\bar{V}_u$ is constant, the solution is written as

$$V_r^{(N)} = \bar{V}_u \sum_{k=1}^{N} t_u^{(k)} + \sum_{k=1}^{N} \left(\dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \right) + V_r^{(0)} \qquad (7)$$

$$\bar{V}_u = \left(V_r^{(N)} - \sum_{k=1}^{N} \left(\dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \right) - V_r^{(0)} \right) \left(\sum_{k=1}^{N} t_u^{(k)} \right)^{-1} \qquad (8)$$

Finally, we achieve the goal of computing all PVRs in the series: the solution for $\bar{V}_u$ from *Eq. 8* is substituted back into *Eq. 6* (eliminating

the final unknown), thus allowing the calculation of any $V_r^{(n)}$ in the serial cystometrogram.

A useful additional step in estimating VE is to include the constraint that all estimated $V_r^{(n)}$ must be nonnegative. Negative residuals can be caused by underestimating the ureter inflow and occurred rarely in some permutations of our experimental data when voiding efficiencies were very high (Fig. 1). In these cases, we use the smallest $\dot{V}_u$ such that $V_r^{(n)} \geq 0$ for all k in the seires. Although this procedure requires only a single measurement of V_r to estimate PVR for all trials, combining multiple measurements of V_r can slightly improve the accuracy of the $\dot{V}_u$ estimate. An extension of our estimation procedure that can optimally incorporate multiple measurements of V_r is presented in APPENDIX 2.

APPENDIX 2

Estimation of PVR with multiple measurements of residual volume. Our estimation procedure can determine V_r of all voids in a serial cystometrogram using a single measurement of V_r. However, if V_r is measured (and reinserted into the bladder) after multiple voids in the series, these measurements can be combined to increase the estimation accuracy of the ureter flow rate, $\dot{V}_u$, thereby increasing the accuracy of each estimated V_r and VE.

The extended estimation procedure uses the method of weighted least squares (WLS), and is outlined below. If m residual measurements are obtained from voids $a \in \{a_1, a_2, ..., a_m\}$ we can construct the matrix form of *Eq. 7* containing all residual measurements

$$\vec{V}_r = \dot{V}_u \begin{vmatrix} \sum_{k=1}^{a_1} t_u^{(k)} \\ \sum_{k=1}^{a_2} t_u^{(k)} \\ \vdots \\ \sum_{k=1}^{a_m} t_u^{(k)} \end{vmatrix} + \begin{vmatrix} \sum_{k=1}^{a_1} \dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \\ \sum_{k=1}^{a_2} \dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \\ \vdots \\ \sum_{k=1}^{a_m} \dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)} \end{vmatrix} = \dot{V}_u \vec{t}_u + \vec{V}_q \tag{9}$$

where the arrows represent vector quantities. The WLS solution for $\dot{V}_u$

$$\dot{V}_u = \left(\vec{V}_r - \vec{V}_q\right) \vec{W} \vec{t}_u^{T} \left(\vec{t}_u \vec{W} \vec{t}_u^{T}\right)^{-1} \tag{10}$$

where $\vec{W}$ is the connonical diagonal weight matrix with elements equal to the inverse variance of the measurement noise of each $V_r^{(k)}$, and the T superscript denotes the transpose operation. If each $V_r^{(k)}$ has a different uncertainty (e.g., the measurements taken in the middle of the experiment are noisier than the final measurement when the bladder can be exposed), and variance (or relative variance) of the noise is known, then *Eq. 10* is the optimal solution for $\dot{V}_u$. WLS can also be used in the case where the noise variances are not known, in which case the weight matrix is set to the identity matrix. This solution supports any number of measurements. The software in the Supplemental Material is capable of handling multiple residual measurements for serial cystometrograms by using *Eq. 10*.

In our in vivo experiments, we found that incorporating multiple measurements has a relatively small effect on our estimates of VE (Fig. 5). To quantify this effect we plotted the average magnitude of the difference between the estimated and measured VE for the experiment data shown in Fig. 1, as a function of the number of measurements used to estimate VE (thick black lines in Fig. 5). Furthermore, we obtained a distribution of averages by repeating the estimation process using all combinations of any given number of V_r measurements for each serial cystometrogram (gray patches in Fig. 5). The data indicate only small improvements when incorporating additional measurements, which levels off quickly after two measurements. In all cases, the discrepancy between measurement and estimate decreased monotonically as more measurements were incorporated into the estimate.

All estimations shown in Fig. 1 were obtained using only the measurement of the final residual in the series; the average magnitude of the difference between the estimated and measured VE in Fig. 1 are shown as the "x"s in Fig. 5. As expected by random chance, some estimates using the final V_r measurement (Fig. 5, *A–F*) show better than average (*F*), worse than average (*B*), or are at the average (*C*) agreement with the measurements.

APPENDIX 3

Relaxing the constant ureter flow constraint on PVR estimation. A potential drawback of *Eq. 8*, the calculation of $\dot{V}_u^{(k)}$, is the assumption of a constant ureter flow rate for each cystometrogram in the series, $\dot{V}_u^{(k)} = \dot{V}_u$. As a matter of practice, however, we demonstrated both experimentally (Fig. 1) and theoretically (Fig. 2) that the estimation procedure is at least as accurate as measuring VE directly for typical ureter flow rate variability. Nevertheless, it is possible to solve for $\dot{V}_u^{(k)}$ without assuming it is constant. The main challenge with this case is that when solving for the N different $\dot{V}_u^{(k)}$, there are fewer measurements than ureter flow rates, which admits an infinite number of possible solutions. One solution among these must be chosen, and there are no guarantees that a given solution will be more accurate than the constant solution in *Eq. 8*.

For variable $\dot{V}_u^{(k)}$ the problem can be reformulated as follows. For any arbitrary number of measurements of V_r (repeated measures on the same cystometrogram or measurements on different cystometrograms)

$$\vec{V}_r = \left[r_1^{(a_1)}, r_2^{(a_2)}, \ldots, r_m^{(a_1)}\right], \quad a_i \in \{1, 2, \cdots, N\} \tag{11}$$

where the vector $\vec{V}r$ contains the m residual measurements, the superscript is the index of the voiding event in the series (following the convention in the main text), and N is the total number of cystometrograms in the series. *Equation 7* can then be conveniently expressed in matrix form as

$$\vec{V}_r = \left[\dot{V}_u^{(1)}, \dot{V}_u^{(2)}, \ldots, \dot{V}_u^{(N)}\right] \cdot \begin{bmatrix} t_u^{(1)} & t_u^{(1)} & \cdots & t_u^{(1)} \\ t_u^{(2)} & t_u^{(2)} & \cdots & t_u^{(2)} \\ \vdots & \vdots & \cdots & \vdots \\ t_u^{(a_1)} & t_u^{(a_2)} & \cdots & t_u^{(a_1)} \\ 0 & 0 & \cdots & 0 \\ \vdots & \vdots & \cdots & \vdots \end{bmatrix} + \left[\sum_{k=1}^{a_1} \left(\dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)}\right), \ldots, \sum_{k=1}^{a_1} \left(\dot{V}_i^{(k)} t_i^{(k)} - V_v^{(k)}\right)\right] + V_r^{(0)} \tag{12}$$

Each column in the (N by m)t_u matrix is constructed by concatenating all values of $t_u^{(k)}$ up to cystometrogram a_i, followed by $(N - a_i)$ zeroes. In this form, *Eq. 12* can be solved readily for all $\dot{V}_u^{(k)}$ (where each element is potentially different, and therefore, a constant ureter flow rate is not enforced) with the same procedure used to solve *Eq. 9*. The software supplied in the Supplemental Material is also able to compute this solution.

If $m < N$, that is, if there are fewer measurements than there are cystometrograms (which is almost certain to be the case), *Eq. 12* is underdetermined. This results in the so-called minimum norm solution for $\vec{V}u$, and there will be no weight matrix as in *Eq. 10*. The minimum norm solution has very little to do with the physiological realities of the ureter flow rate, and we should not expect this solution to outperform the solution constrained to constant ureter flow.

Future work may expand *Eq. 12* to include a regularization term that constrains the solution. Potential regularization candidates in-

Table A1. *Example formatted SCMG data table*

Flow Rate, ml/s	Voided Volume, ml	Flow Time, s	Ureter Time, s
x_1	v_1	t_2-t_1	t_3-t_0
x_2	v_2	t_5-t_4	t_5-t_3
x_3	v_3	t_6-t_5	t_6-t_5
x_4	v_4	t_7-t_6	t_8-t_6

Conceptual example of formatting data from the synthetic four-void serial cystometrogram (SCMG) in Fig. 6.

clude the magnitude of the standard deviation of $\vec{v}u$. This constraint would favor solutions with less overall ureter flow variations, which is reasonable because we observe relatively low variations throughout serial cystometrograms. Another possibility is to use the vector magnitude of the successive differences in $\vec{v}u$. This would not penalize overall variability but rather would penalize large changes in flow rates for adjacent cystometrograms. This may be a more realistic constraint if, for instance, ureter flow rate is slowly declining throughout a long serial cystometrogram. Such regularized solutions will perform better the more that is known (and can be incorporated into the regularization) about the structure of ureter flow variability. Regardless of the regularization, we expect only modest gains over the procedure presented in the main text because it was demonstrated to be quite accurate for typical experimental conditions.

APPENDIX 4

Practical usage guide to implement the estimation procedure. To implement the PVR estimation procedure, we created a data table for each serial cystometrogram (SCMG). The table has four columns: *1*) the infused volumetric flow rate into the bladder, *2*) measured voided volume, *3*) the amount of time the infusion pump was running, and *4*) the time between voids (i.e., the duration ureters added volume to the bladder since the last measurement). Place data from each voiding cycle in the SCMG in successive rows in the table. Lastly, record the measured residual volume of the final void cycle in the SCMG.

We illustrate how to build the data table and compute the intervoid interval (ureter time) and the amount of time the infusion pump was running (flow time) in Table A1, based on the synthetic SCMG example in Fig. A1. After the data table is constructed, the basic estimation method (*Eq. 8*), and subsequent calculation of residual volume, is performed automatically using the template Excel spreadsheet provided in the Supplemental Material. The template file, DataExample.xlsx sheet 2 labeled "Excel Calculation," contains properly formatted data used in Fig. 1 and the implementation of the equations needed to calculate PVR. The user is responsible for ensuring consistent units.

The more sophisticated ureter flow estimations described in APPENDEXES 1–3, such as including nonnegativity constraints on PVR, optimal integration of multiple PVR measurements (*Eq. 10*), or ureter flow estimation without the constant flow assumption (*Eq. 12*), cannot be performed in Excel. The MATLAB code in the Supplemental Material will import a formatted Excel data table, accept an input indicating which method to use, and perform the computations. The Supplemental Material contains an Excel file formatted for use with the MATLAB code (DataExample.xlsx, sheet 1 labeled "Data"), as well as a script to run these computations and reproduce Fig. 1*A* (main.m). Documentation for the MATLAB code is provided in the Supplemental Material.

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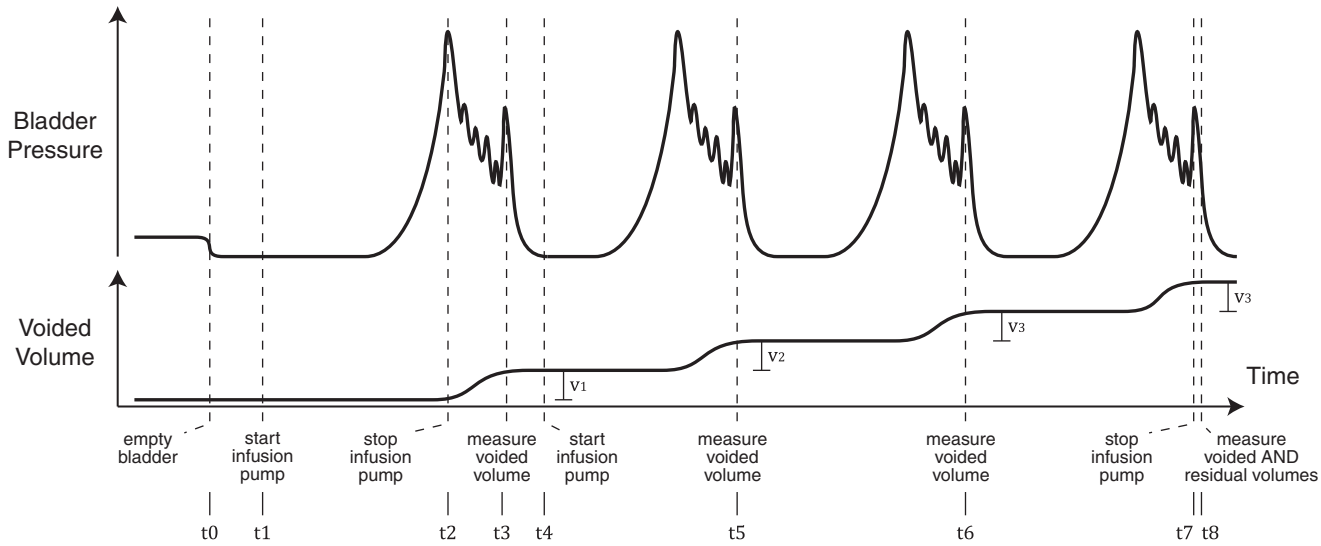

Fig. A1. Schematic of a synthetic four-void serial cystometrogram (SCMG) illustrating how data are formatted for the estimation procedure (not real data). The SCMG is initiated when the bladder is completely emptied at t_0. Infusion of volume into the bladder begins at t_1. The infusion pump is stopped just prior to the first voiding event at time t_2, and following the completion of the void the voided volume (v_1) is measured at t_3. (This is done as an example and, in general, infusion need not be stopped.) Therefore, the total amount of time the ureters spend contributing volume to the bladder for the first void cycle is t_3-t_0, whereas the total amount of time spent infusing volume into the bladder is only t_2-t_1 because the pump is not active for the entire duration of the voiding cycle. Note that there are no limitations on the time between emptying the bladder and starting the infusion pump, and experimental preparations can continue long after the initial emptying, if needed. Should the animal void after emptying but before the start of the first infusion trial, treat it as the first void cycle in the SCMG with an infusion rate of zero, and record the voiding time and volume in the data table as normal. The ureter flow time for the second voiding cycle is the span of time between the end of *void 1* and *void 2*, t_5-t_3. The infusion pump is left running for voiding *cycles 2* and *3* of the SCMG, and because the pump is active for the entire duration of the third voiding cycle the ureter and flow times are equal. The residual bladder volume is measured following the final void in the SCMG. The infusion pump is turned off at t_7 so that no new volume is infused into the bladder as the residual is being removed for measurement at t_8. The infusion flow rate into the bladder is x1, x2, x3, and x4 and the voided volumes are v_1, v_2, v_3, and v_4 for void *cycles 1* through *4*, respectively. Data from this example SCMG are compiled into Table A1. and formatted for use in the estimation procedure software.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Z.C.D. and W.M.G. conception and design of research; Z.C.D. performed experiments; Z.C.D. analyzed data; Z.C.D. interpreted results of experiments; Z.C.D. prepared figures; Z.C.D. drafted manuscript; Z.C.D. and W.M.G. edited and revised manuscript; Z.C.D. and W.M.G. approved final version of manuscript.

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