A Multistate Single-Connection Calibration for Microwave Microfluidics

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Abstract—With emerging medical, chemical, and biological applications of microwave-microfluidic devices, many researchers desire a fast and accurate calibration that can be achieved in a single connection. However, traditional on-wafer or coaxial calibrations require measurements of several different artifacts to the data prior to measuring the microwave-microfluidic device. Ideally, a single artifact would be able to present different impedance states to correct the vector network analyzer data, minimizing drift and eliminating artifact-to-artifact connection errors. Here, we developed a multistate single-connection calibration that used a coplanar waveguide loaded with a microfluidic channel. We then used measurements of the uncorrected scattering parameters of the coplanar waveguide with the channel empty, filled with deionized water, and filled with 30 w% (30 grams per liter) of saline to construct an eight-term error model and switch-term correction. After correction, the residuals between measured scattering parameters and with the literature-based finite-element simulations were below −40 dB from 100 MHz to 110 GHz. This multistate single-connection calibration is compatible with both wafer-probed and connectorized microwave-microfluidic devices for accurate impedance spectroscopy and materials characterization without the need for multiple device measurements.

Index Terms—Calibration, microfluidics, microwave, scattering parameters, vector network analyser (VNA).

I. INTRODUCTION

MICROWAVE-MICROFLUIDIC devices integrate microwave circuits with microfluidics for quantitative electrical measurement of fluids [1]–[5]. This emerging field has the potential to advance industrial applications of impedance spectroscopy, including point-of-care diagnostics and quality assurance for pharmaceutical and chemical manufacturers [4], [6]. For these commercial applications to be realized, it is important to accurately and quickly correct the electrical measurements of microwave-microfluidic devices for the attenuation and phase shift of the measurement leads and the standing waves between the fluid-under-test and the vector network analyzer (VNA) [7]–[11].

Due to its basis in circuit theory, the on-wafer multiline thru-reflect-line (TRL) calibration algorithm [12] is the most accurate VNA calibration algorithm. However, like other calibration algorithms—e.g., load-reflect-match [13], [14], series-resistor [15], and short-open-load-thru [16]—the multiline TRL calibration [17], [18] requires more than one calibration artifact. For on-wafer measurements, this requirement means one must move the wafer probes to contact different artifacts. For connectorized measurements, this problem is even worse, because the disconnect between artifacts can occur behind the reference plane of the microfluidic channel. Moving the probes or exchanging calibration artifacts has been shown to introduce connection errors between different measurements of the scattering (S-) parameters [19], which ultimately increases the measurement uncertainty [20]. Increasing the measurement uncertainty has the potential to overwhelm sample-to-sample differences or result in false-positive statistics, which may limit the motivating applications for microwave microfluidics. Hence, a fast and accurate calibration algorithm is needed; one that can be done on the microwave-microfluidic device itself [21], [22] in a single connection without separate calibration artifacts. Such a multistate single-connection calibration would facilitate testing of sources of uncertainty that limit the signal-to-noise ratio, the measurement drift of the VNA, and even the drift of the fluid sample itself.

Our approach for calibrating microwave-microfluidic devices is to use fluids of known electrical properties to access different impedance states, and then use those artifacts to build an error model (Fig. 1) as in the series-resistor calibration algorithm [15]. In lieu of the series resistor, we developed an algorithm for correcting the S-parameters of a microwave-microfluidic transmission line that uses known fluids as the calibration artifacts. Here, we show that a microfluidic channel filled with different known fluids enables a multistate single-connection calibration with the reference plane directly adjacent to the channel. This proposed calibration requires only two known fluids to correct the S-parameters of a microwave-microfluidic device with an unknown fluid. The single-connection calibration results in S-parameter errors like those obtained with a microfluidic-multiline TRL.
calibration, which uses fluid-loaded transmission lines that have different lengths. Compared to the microfluidic-multiline TRL calibration, the multistate single-connection calibration greatly reduces measurement time and simplifies impedance spectroscopy.

In the following, Section II-A discusses a multistate single-connection calibration based on the measurement of a microwave-microfluidic device with known fluids (see Fig. 2). Section II-B describes the use of the multistate single-connection calibration to extract the error boxes that de-embed the microfluidic channel. The design and fabrication of the microwave-microfluidic device is introduced in Section III. We then compare and validate the multistate single-connection calibration with a co-fabricated microfluidic-multiline TRL calibration and finite-element simulation in Sections IV-A and IV-B, respectively. Section IV-C provides data for different numbers of known fluid artifacts. Finally, we summarize our findings in Section V and offer a perspective on how the multistate single-connection calibration can be applied.

II. THEORY

A. Artifacts

Like [23], this microwave-microfluidic device had five regions [Fig. 1(a)], which are modeled as uniform distributed transmission-line segments. We labeled each segment
per the material above: air, SU-8, and fluid. As shown in Fig. 1(b), the microwave-microfluidic device consisted of two air segments, two SU-8 segments, and a fluid segment. The air segments had length $l_a$, characteristic impedance $Z_a$, and propagation constant $\gamma_a$; the SU-8 segments had length $l_s$, characteristic impedance $Z_s$, and propagation constant $\gamma_s$; and, the fluid segment had length $l_f$, characteristic impedance $Z_f$, and propagation constant $\gamma_f$. The reference impedance for the model [Fig. 1(b)] was $Z_o$. The models for the calibration artifacts $A$ [Fig. 1(c)] were simply the microwave-microfluidic device with different known fluids.

The calibration artifact models ($A$) require $\gamma_f$, $Z_f$, $l_f$, transmission (T-)-matrix model of the transmission line, and impedance transformers [24]. From the telegrapher’s equations [25], we wrote $\gamma_f$, and $Z_f$ as

$$\gamma_f = \sqrt{(R_f + i\omega L_f)(G_f + i\omega C_f)}$$

and

$$Z_f = \frac{(R_f + i\omega L_f)}{(G_f + i\omega C_f)}$$

where $R_f$, $L_f$, $C_f$, and $G_f$ were the distributed resistance, inductance, capacitance, and conductance per unit length of the transmission line loaded by the fluid. The parameters $R_f$, $L_f$, $C_f$, and $G_f$ are dependent on frequency, which we omitted for clarity.

There were several approaches to obtain $R_f$, $L_f$, $C_f$, and $G_f$ for the known fluid samples, including finite-element simulation [23], direct measurement [23], and analytical calculation [26]. If the materials used to fabricate the microwave-microfluidic transmission lines (including the fluid) are nonmagnetic, then $R_f$ and $L_f$ depend solely on the metallic conductors [27]. In this nonmagnetic case, both finite-element simulations and analytical calculations [23], [28] could be used to obtain $R_f$ and $L_f$ from the cross-sectional dimensions of the transmission line. Then, either finite-element simulation or conformal mapping can be used to obtain geometrical factors, $m$ and $n$, that relate $C_f$ and $G_f$ to the complex permittivity of the fluid. The dielectric constant extraction method for different fluids at the full frequency band from 100 MHz to 110 GHz can be found in [23]. For a known fluid of complex permittivity $\tilde{\epsilon}_f$, the $C_f$, and $G_f$ are

$$G_f + i\omega C_f = \frac{\tilde{\epsilon}_f}{m} + \frac{\tilde{\epsilon}_q}{n}$$

where $\tilde{\epsilon}_q$ is the complex permittivity of the substrate. Note that (3) assumes that the contribution of the fluid is in parallel with that of the substrate, which is only true for a coplanar waveguide (CPW) on a dielectric with a uniform fluid on top covering both gaps.

After computing $R_f$, $L_f$, $C_f$, and $G_f$ for the known fluid artifacts, we obtained $\gamma_f$ and inserted it into the T-matrix model of the transmission-line segment of length $l_f$

$$T_{l_f} = \begin{bmatrix} e^{-\gamma_f l_f} & 0 \\ 0 & e^{\gamma_f l_f} \end{bmatrix}.$$  

Then $Z_f$ could be used to construct the T-matrix model of the impedance transformer [24] that transitioned from $Z_o$ to $Z_f$

$$Q_{Z_f}^{Z_o} = \frac{1}{2Z_o} \left[ \frac{\text{Re}(Z_f)}{\text{Re}(Z_o)} \right] \left[ \frac{Z_o + Z_f}{Z_o - Z_f} \right].$$

The calibration artifact models were

$$Q_{Z_f}^{Z_o} = \frac{Z_f}{Z_o}$$

Then we multiplied the left and right sides of (4) by $Q_{Z_f}^{Z_o}$ and $Q_{Z_f}^{Z_o}$, respectively, to obtain the T-matrix model relative to $Z_o$ as

$$T_{l_f}^{Z_o, Z_f} = Q_{Z_f}^{Z_o} T_{l_f}^{Z_f} Q_{Z_f}^{Z_o}.$$  

In the following section, we develop the multistate single-connection calibration algorithm based on (6) and switch to a more conventional simplified notation, where $T_{l_f}^{Z_o, Z_f} = A$.

B. Algorithm

After we used (6) to form the T-matrices of different known fluid artifacts, we derived the multistate single-connection calibration algorithm that solved for the error boxes $X$ and $\tilde{Y}$ [Fig. 1(c)], which included everything between port 1 of the VNA to the fluid and from the fluid to port 2 of the VNA, respectively. In this case, any measurement ($M$) could be expressed as

$$M = XA\tilde{Y}.$$  

For an artifact $A_a$ and measurement $M_a$, $\tilde{Y}$ could be solved as

$$\tilde{Y} = A_a^{-1} X^{-1} M_a.$$  

Inserting (8) in (7), we derive

$$M = X A (A_a^{-1} X^{-1} M_a).$$

Following [15], we could either set $X$ or $Y$ to be reciprocal without losing the generality of the error model. We chose $X$ to be reciprocal with form

$$X = r \begin{bmatrix} 1 & a \\ b & c \end{bmatrix}.$$  

where $r = (c - ab)^{-1/2}$. This eliminated one unknown in $X$. We then solved for the unknown complex parameters $a$, $b$, and $c$ by measuring another fluid artifact. Above (7)–(10) are all taken from [15].

At least two artifacts are required to solve (9) for the unknown complex parameters $a$, $b$, and $c$. We chose to use measurements of the microwave-microfluidic device filled with air ($M_a$), and deionized (DI) water ($M_w$). The corresponding models were $A_a$, and $A_w$ for air and water, respectively. We inserted $M_a$, $M_w$, $A_a$, and $A_w$ into (9), which imposed
four conditions on the $a$, $b$, and $c$. These conditions were
\[
-\left[A_{11}^{w}(A_{11}^{a})^{-1} + A_{22}^{w}(A_{22}^{a})^{-1}\right]a + (M_{w}M_{a}^{-1})_{12}b \\
= A_{11}^{a}(A_{11}^{a})^{-1} + A_{12}^{a}(A_{21}^{a})^{-1} - (M_{w}M_{a}^{-1})_{11}
\]
\[
\left[(M_{w}M_{a}^{-1})_{11} - A_{11}^{w}(A_{11}^{a})^{-1} - A_{12}^{w}(A_{21}^{a})^{-1}\right]a + (M_{w}M_{a}^{-1})_{12}c \\
= A_{11}^{a}(A_{11}^{a})^{-1} + A_{12}^{a}(A_{22}^{a})^{-1} \\
\left[(M_{w}M_{a}^{-1})_{22} - A_{21}^{w}(A_{11}^{a})^{-1} - A_{22}^{w}(A_{22}^{a})^{-1}\right]b \\
-\left[A_{21}^{w}(A_{11}^{a})^{-1} + A_{22}^{w}(A_{22}^{a})^{-1}\right]c \\
= - (M_{w}M_{a}^{-1})_{21}
\]
\[
(M_{w}M_{a}^{-1})_{21}a - \left[A_{11}^{w}(A_{12}^{a})^{-1} + A_{12}^{w}(A_{22}^{a})^{-1}\right]b \\
+ \left[(M_{w}M_{a}^{-1})_{22} - A_{21}^{w}(A_{12}^{a})^{-1} - A_{22}^{w}(A_{22}^{a})^{-1}\right]c \\
= 0
\]
where $A_{ij}$ is a matrix element of $A$. Since (11)–(14) overdetermined $a$, $b$, and $c$, we used a least-squares algorithm [29] to obtain the complex values of $a$, $b$, and $c$.

Generally, the more artifacts included in the calibration, the more conditions there are on $a$, $b$, and $c$. Any two artifacts imposed four conditions on $a$, $b$, and $c$. Hence, $n$ artifacts would impose $4 \times (n(n-1)/2)$ conditions on $a$, $b$, and $c$. This means that the number of conditions increases quadratically with the number of artifacts. We expect that increasing $n$ would improve the worst case error comparison to the microfluidic-multiline TRL calibration.

III. FABRICATION OF THE MICROWAVE-MICROFLUIDIC DEVICE

In this section, we discuss the fabrication of the microwave-microfluidic device, the microfluidic-multiline TRL test set, and a companion dry reference wafer with conventional on-wafer artifacts. All of them were co-fabricated to reduce the effect of fabrication tolerances. We chose quartz (fused silica) as the substrate for all the devices due to its low dielectric loss and homogenous dielectric constant. Devices were fabricated on a 76.5-mm-diameter and 0.5-mm-thick quartz wafer, and the dimensions of quartz and other layers were labeled in Fig. 2(c). A commercial stepper that used projection lithography was used to pattern each layer. The stepper had layer-to-layer alignment better than 250 nm [30].

Each wafer was fabricated in five layers for resistor, pads, conductors, SU-8, and polydimethylsiloxane (PDMS), respectively. The resistor, pad, and conductor layers were deposited by electron-beam evaporation and lifted off via a two-layer resist process [31]. First, we deposited a 1-nm Ti adhesion layer followed by 10 nm of PdAu for the resistor [32]. Next, a 10-nm Ti adhesion layer was deposited followed by 100 nm of Pd for the pads. Later, we deposited a 10-nm Ti adhesion layer followed by 650 nm of Au for the conductor. The CPW had a 50-μm-wide center conductor with 5-μm gaps from 200-μm-wide ground planes [see Fig. 2(c)].

We added the microfluidics onto the wafer with the SU-8 [33] sidewalls and PDMS roof (see Fig. 2). We first spin-coated the wafer with SU-8 to a thickness of 60 μm. Second, we patterned it with the stepper, using an exposure dose of 200 mJ/cm², a postexposure-bake at 55 °C for 1 h, and developed it to define the microfluidics. To remove crazing, we performed a postdevelop bake to 150 °C for 5 min, then let it cool to room temperature on the hot plate. After the SU-8 layer, we diced the wafer into individual 12 mm × 12 mm dies. The PDMS layer was patterned following a procedure outlined in [34], which produced the PDMS layer’s microfluidic channels. Finally, we placed the PDMS onto the SU-8 layer under a microscope and sealed the completed microfluidic-microfluidic with a clamp.

The completed microfluidic-microfluidic device (Fig. 2) had inlet and outlet for the microfluidic channels. The device consisted of a full microfluidic-multiline TRL test set with four transmission lines of lengths (0.5, 0.85, 1.55, and 3.314) mm and a 0.25-mm offset short-circuit reflect. We selected the 0.85-mm line to demonstrate the multistate single-connection calibration [see Fig. 2(b)]. The companion dry reference wafer had identical conductor cross sections to the microfluidic-microfluidic device. On this wafer, we fabricated a 10-μm series resistor, a 10-μm series capacitor, a short-circuit reflect, and seven bare CPW transmission lines with lengths (0.420, 1.000, 1.735, 3.135, 4.595, 7.615, and 9.970) mm. Each lumped element artifacts had a 0.21-mm offset, which was half the length of the 0.42-mm thru.

IV. METHODOLOGY

A. Measurement

We measured the S-parameters of the microwave-microfluidic device, the microfluidic-multiline TRL test set, and the dry calibration artifacts on the companion reference wafer, using an intermediate frequency bandwidth of 50 Hz and a power level of −20 dBm for the Anritsu MS4647A VNA with extender heads. The small input power is used to ensure that the extender heads were linear. However, one must take care when performing microwave-microfluidic measurements, as some fluids may absorb microwave energy. The measurement setup is probe-based and the S-parameters were measured from 100 MHz to 110 GHz in 512 logarithmic steps. The measurements were performed on a temperature-controlled probe station at 28.5 ± 0.5 °C. The quality of the calibration is dependent on the temperature dependence of the microwave-microfluidic device, and the temperature sensitivity of the states. We tested the temperature dependence of our microwave-microfluidic device and its associated calibration by varying the temperature dependence of the model and recalculating the difference between microfluidic-multiline TRL and our approach. The result of this test proved that our microwave-microfluidic device was insensitive to deviations on the order of 1 °C. And 1 min is also ample amount of time to assume thermal equilibrium between injected fluids and the probe station which we measured directly. After measuring the dry calibration artifacts on the companion reference wafer, we placed the microwave-microfluidic device onto the temperature-controlled probe station. Then the raw S-parameters of the device were measured with the microfluidic channel filled with air, DI water, and (30 and 3 %) saline solutions. We first flushed out the channels with air followed by DI water three times to make sure that the
channels were clean, and then injected new samples. Since the time to clean the channel is a function of the fluid flow rate and the total channel volume, it takes roughly 0.01 s to completely change fluids. A microfluidic switch could be used to automatically control and change artifacts during the calibration, which would reduce the time between measurements and minimized the effect of measurement drift along with the time.

B. Analysis

The analysis is divided into two parts: 1) the microfluidic-multiline TRL test set and 2) the microwave-microfluidic device. To analyze the S-parameters of the microfluidic-multiline TRL test set, we performed a two-tier calibration [18], which used the S-parameters of the multiline TRL calibration artifacts on the companion reference wafer to extract error boxes between the VNA and the probe tips. This first-tier calibration also extracted the propagation constant of the CPW without the microfluidics, which we assumed was equal to the microwave-microfluidic CPW with air in the channel. In this step, we also corrected the data for the switch terms [35]. Next, we transformed the reference impedance to 50 $\Omega$ using the resistor, and empirically computed the capacitance per unit length of the CPWs on the quartz substrate ($C_q = (\varepsilon_q/m) \cdot G_q \approx 0$) [23]. Having obtained $C_q$ [air, Fig. 3(c) and (d)], we used the propagation constant to obtain $R_f$ [Fig. 3(a)] and $L_f$ [Fig. 3(b)]. It is true that most the frequency dependence of loss is consistent with the classical skin effect, but the geometrical effects can also have a significant role for the CPW configuration [28], [36]. We then corrected the S-parameters of the microfluidic-multiline TRL test set to 50 $\Omega$, and performed a second-tier calibration to obtain $\gamma_f$ and the second-tier error boxes. And we calculated $C_f$ and $G_f$ from $\gamma_f$ using $R_f$ and $L_f$, which allowed us to transform the second-tier error boxes to 50 $\Omega$. This enabled us to verify that the modeled values of $C_f$ and $G_f$ were consistent with the measurements and the literature [23]. Finally, we cascaded the first-tier error boxes with the second-tier error boxes to construct the total error boxes that extended from the microfluidic channel to the VNA in 50 $\Omega$.

In addition to extracting $R_f$, $L_f$, $C_f$, and $G_f$ from measured S-parameters, we calculated these parameters (solid
Fig. 5. Demonstration of the multistate single-connection calibration. (a) As-measured reflection and transmission coefficients from 100 MHz to 110 GHz for the microwave-microfluidic device filled with air (red triangles), water (blue squares), and 30-w% saline (yellow dots), respectively. (b) Models used to generate the multistate single-connection calibration (black dotted lines) and microfluidic-multiline TRL-corrected results (dots). The models used to compute the multistate single-connection calibration agree with the microfluidic-multiline TRL results to within the measurement uncertainty.

C. Validation

With three sets of error boxes from the microfluidic-multiline TRL calibration, consisting of multistate single-connection calibration with two known fluids (air, water), and multistate single-connection calibration with three artifacts (air, water, and 30-w% saline), respectively, we used each set of error boxes to correct the as-measured S-parameters of the microwave-microfluidic device filled with an “unknown” fluid (3 w% saline, in reality). The corrected S-parameters agreed with microfluidic-multiline TRL calibration [green triangles, Fig. 6(a)] and the multistate single-connection calibrations (two artifacts: blue circles; three artifacts: red squares). Both the reflection [left-axis, Fig. 6(a)] and transmission [right axis, Fig. 6(a)] agreed between calibration methods up to 20 GHz. Above 20 GHz, both reflection [left-axis, Fig. 6(a)] and transmission [right axis, Fig. 6(a)] deviated from the microfluidic-multiline TRL results. The deviation from microfluidic-multiline TRL results was much larger for the two artifact case [blue circles, Fig. 6(a)] compared to the three artifact case [red squares, Fig. 6(a)]. For frequencies above 60 GHz, the three artifact case also disagreed with the microfluidic-multiline TRL result. We hypothesize that additional artifacts would place more constraints on \(a\), \(b\), and \(c\) in (11)–(14), which would further improve the agreement between the multistate single-connection calibration and microfluidic-multiline TRL calibration. The method to extract permittivity of saline solution over such broad frequency ranges, Fig. 3) for the water and air cases based on finite-element simulations of the microwave-microfluidic device (Fig. 4), using the measured dc resistivity of gold \((\rho = 2.57e^{-8} \text{S/m})\), relative permittivity of substrate \((\varepsilon_r = 3.83)\), and the literature values for the permittivity of air and water \((\varepsilon = 76.39, \tau_1 = 7.39 \text{ ps}, \varepsilon_2 = 5.75, \tau_2 = 0.9 \text{ ps}, \text{ and } \varepsilon_{\infty} = 4.6)\) [37]. We optimized the mesh of the finite-element simulation with a 1% convergence on the calculated admittance. After confirming that the simulated \(R_f, L_f, C_f, \text{ and } G_f\) agreed with the microfluidic-multiline TRL corrected result to within the measurement uncertainty, we used the finite-element simulations to construct S-parameter models of the microwave-microfluidic device filled with air \(A_a\), water \(A_w\), and 30 w% saline \(A_s\) based on (1)–(6). We then used these models and the measured raw S-parameters \(M_a, M_w, \text{ and } M_s\) [Fig. 5(a)] in multistate single-connection calibration based on (11)–(14). Note that the discontinuity around 30 GHz in raw data is purely due to the specific VNA using extender heads, which give rise to this discontinuity. These multistate single-connection model \(A_a, A_w, \text{ and } A_s\) [solid line, Fig. 5(b)] compared well with microfluidic-multiline TRL-corrected results [circles, Fig. 5(b)]. In both cases, the reference planes of both the microfluidic-multiline TRL calibration and the single-connection calibration are at the planes of the interface between the SU-8 and fluid with a reference impedance of 50 \(\Omega\).
S-parameters and S-parameters, we calculated an error function \[ |S_{ij}| = \sqrt{\sum_{i=1}^{N} (S_{ij}^{\text{SCC}} - S_{ij}^{\text{TRL}})^2} \] (15)

where \( S_{ij}^{\text{TRL}} \) were microfluidic-multiline TRL corrected S-parameters and \( S_{ij}^{\text{SCC}} \) were multistate single-connection corrected S-parameters. This error function facilitates visualizing the difference between the two calibrations, as well as the effect of additional artifacts. As shown in Fig. 6(b), \( S_{err} \) was less than \(-60 \) dB below \( 20 \) GHz, but increased according to a power law above \( 20 \) GHz. Yet, even at \( 110 \) GHz, the \( S_{err} \) was less than \(-40 \) dB for the three artifacts case [red circles, Fig. 6(b)].

**V. CONCLUSION**

In this paper, we established a multistate single-connection calibration algorithm and technique for microwave-microfluidic devices, providing an accurate calibration at the reference planes of the microfluidic channel to a reference impedance of our choosing for frequencies up to \( 110 \) GHz. We demonstrated the single-connection calibration algorithm with microwave-microfluidic devices filled with air, water, and \( 30 \) w\% saline. We then used finite-element simulation and the literature values to construct models that we validated with microfluidic-multiline TRL calibration. Next, we applied the single-connection calibration using two or three known fluids, and compared the results to microfluidic-multiline TRL calibration. With three artifacts, multistate single-connection calibration produced the least-square error from the microfluidic-multiline TRL calibration below \(-30 \) dB from \( 100 \) MHz to \( 110 \) GHz.

Future work will include an uncertainty analysis on the multistate single-connection calibration to clearly define the error and repeatability of the experiment and test microfluidic techniques to achieve variable states. Two key questions remain: 1) how different do the impedance states of the artifacts need to be and 2) how increasing the number of states improves the calibration accuracy. In a word, we developed a multistate single-connection calibration algorithm that can be performed by simply measuring known fluids, which is essential for the commercialization of microwave-microfluidic devices. This calibration protocol could be easily extended to packaged devices by connectorizing the microwave microfluidics.

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