Engineering Research Express

PAPER

A label-free sensing of creatinine using radio frequency-driven lab-on-chip (LoC) system

Andleeb Zahra[1,2], **Swarnim Sinha**[1], **Alimpan Modak**[2], **Imran Siddiqui**[3], **Syed Azeemuddin**[4], **Prabhakar Bhimalapuram**[2], **Tapan Kumar Sau**[2], **Pawan Kumar**[5] **and Zia Abbas**[1,*]

[1] Center for VLSI and Embedded Systems Technology (CVEST), International Institute of Information Technology (IIIT), Hyderabad, India
[2] Center for Computational Natural Sciences and Bioinformatics (CCNSB), International Institute of Information Technology (IIIT), Hyderabad, India
[3] Department of Biochemistry, ESIC Super Speciality Hospital, Hyderabad, India
[4] Department of Electrical and Computer Engineering, Behrend College, Penn State University, Erie, PA, United States of America
[5] Centre for Security, Theory & Algorithm Research (CSTAR), International Institute of Information Technology (IIIT), Hyderabad, India
[*] Author to whom any correspondence should be addressed.

E-mail: andleeb.zahra@research.iiit.ac.in, swarnim.sinha@research.iiit.ac.in, alimpan.modak@research.iiit.ac.in, dr.imran.siddiqui@esic.nic.in, syed@psu.edu, prabhakar.b@iiit.ac.in, tapan.sau@iiit.ac.in, pawan.kumar@iiit.ac.in and zia.abbas@iiit.ac.in

OPEN ACCESS

RECEIVED
19 March 2024

REVISED
17 July 2024

ACCEPTED FOR PUBLICATION
1 August 2024

PUBLISHED
14 August 2024

Keywords: interdigitated capacitor (IDC), lab on chip (LoC), creatinine, phosphate buffer (PB), assayed chemistry control (ACC) solution, radio frequency (RF), high-frequency structure simulator (HFSS)

Abstract

This paper presents a promising avenue of Radio Frequency (RF) biosensors for sensitive and real-time monitoring of creatinine detection. Knowing creatinine levels in the human body is related to its possible association with renal, muscular, and thyroid dysfunction. The detection was performed using an Inter-Digitated Capacitor (IDC) made of copper (Cu) metal over an FR4 substrate. To demonstrate our methodology, we have chosen Phosphate Buffer (PB) as our solvent for making the creatinine solutions of different concentrations. Moreover, Assayed Chemistry Control (ACC), a reference control consisting of human serum-based solutions has been mixed with the different concentrations of creatinine in a ratio of 1:9 to spike the creatinine value in the ACC solution. The sensor has been designed using a High-Frequency Structure Simulator (HFSS) tool with an operating frequency of 2.53 GHz. Then the design is fabricated over the FR4 printed circuit board (PCB) and tested using a Vector Network Analyzer (VNA). However, the sensitive area of the IDC is introduced to grade 4 Whatman filter paper for the Sample Under Test (SUT) handling unit. The main advantage of using Whatman filter paper is that the uniform spreading of liquid reduces experimental error, and less volume is required for testing the sample. The principal idea implemented in the biosensor design is to track the shift in the operating frequency in the presence of different concentrations of creatinine mix in ACC solution with Phosphate Buffer (PB) solution as a reference.

1. Introduction

The increasing demand for biomedical diagnostic methods in research requires low-cost, fast, highly sensitive, and easy-to-use detection methods [1–4]. Among the different chemical compounds in the human blood and urine, creatinine (2- amino-1-methyl-5H-imidazol-4-one) $C_4H_7N_3O$ plays an important role in kidney function [5–8]. Creatinine is a key biomarker for evaluating renal health and diagnosing conditions such as chronic kidney disease (CKD). High levels of creatinine in the blood may indicate impaired kidney function, as the kidneys may struggle to filter and excrete it effectively [9]. Therefore, regular monitoring of creatinine levels is essential in clinical practice to diagnose and manage conditions related to renal function. The typical range of serum creatinine for adult men is 0.74 mg dL^{-1} to 1.35 mg dL^{-1}, and for adult women is 0.59 mg dL^{-1} to 1.04 mg/dL. The level of creatinine reaches >1000 μM in serum during renal, thyroid, and kidney dysfunction or muscle disorder [5–8]. Various methods are used for creatinine detection such as the Jaffe method [9],

enzymatic methods, chromatographic methods, immunoassays, colorimetric methods [7–11], and other methods. However, these methods have some drawbacks such as being time-consuming, the requirement of sample pre-treatment, high-cost instrumental set-up, and the skilled person requirement to operate. Additionally, they are often not reusable and require expensive fabrication and synthesis methods. Moreover, creatinine biosensors can be classified into electrochemical sensors (viz., amperometric, potentiometric, conductometric biosensors, etc), immunosensors, chemical sensors, nanomaterials-based biosensors, and enzyme-based biosensors [11–13] as tabulated in table 1. However, such methods require expensive setup, chemical preparation, labeling, etc. Therefore, there is a need to explore possible alternatives that can tackle the challenges faced by these traditional techniques. The development of an ideal creatinine biosensor is challenging for the medical industry. Different approaches have been used to construct creatinine biosensors.

Ongoing research aims to address the limitations of existing methods and develop new techniques for creatinine detection. In order to find alternative methods for diagnosis purposes, RF-based sensors also show potential applications due to their unique properties. The principle of RF sensors is based on the interaction of electromagnetic waves with matter. RF biosensors allow for real-time monitoring of SUT, enabling continuous measurement without extensive sample preparation. Moreover, the response time of RF-based sensors is very fast (almost instantly), and the detection process is label-free. The basic principle lies in the dielectric features of many samples, which are unique across the microwave spectrum and offer a wealth of possibilities for analysis techniques [26–36].

Various RF and Microwave sensors have been developed for bio-sample detection, broadly categorized into resonant and non-resonant methods [37, 38]. Planar sensors, a subset of resonant RF sensing methods, particularly stand out due to their straightforward and compact design [39]. Planar resonant sensors such as IDCs are chosen for sensing and quantitative analysis of bio-liquids because of their high-quality factors and easy fabrication. When a bio-sample is placed over the IDC, its effective capacitance changes which shows the change in resonance frequency value [40–43]. The successful development of RF biosensors has the potential to revolutionize diagnostic procedures, providing rapid and accurate detection of biomolecules with implications for early disease diagnosis and monitoring. Furthermore, the miniaturization and integration of these biosensors into portable devices hold promise for point-of-care applications, enabling decentralized healthcare solutions. Despite the significant progress made in RF biosensor technology, challenges and opportunities for further improvement persist [44–46].

This study shows the application of an Inter Digitated Capacitor (IDC) sensor in detecting creatinine concentrations. We have chosen Phosphate Buffer (PB) as our solvent, as most bio-samples are dispersed in them to avoid chemical degradation [47]. Several concentrations of creatinine in the medical interest range of the human body, i.e., 0.8 mg dL^{-1} to 2 mg/dL have been prepared in PB solution. Moreover, Assayed Chemistry Control (ACC), a reference control consisting of human serum-based solutions has been mixed with the different concentrations of creatinine in a ratio of 1:9 to spike the creatinine value in the ACC solution (provided by ESIC Hospital, Hyderabad India). The sensitive region of the IDC has been covered with a grade 4 Whatman filter paper [48] for handling the fluid i.e. SUT. The advantage of using Whatman filter paper is that the uniform spreading of liquid reduces experimental error, and less volume is required for testing the sample. However, the sensor has been connected to the VNA and the SUT has been poured directly over the Whatman filter paper through a micro-pipette. Finally, the shift in the resonance frequency of different concentrations has been observed.

2. Materials and methods

2.1. Design and fabrication of an interdigitated capacitor

IDC has interdigitated fingers-like electrodes as two ports of the capacitor, as shown in figure 1. Unlike uniformly distributed electric fields in parallel plate capacitors, the electric field of an IDC starts from one group of signal electrodes having higher potential, coming up and penetrating the material under test (MUT), then down to another group of ground electrodes. To calculate the capacitance of IDC conformal transformation technique is used in which the coplanar geometry can be transformed into parallel plate geometry [41, 57].

The capacitance of IDC (C_{total}) can now be calculated as

$$C_{total} = nl(C_{ins} + C_{sub} + C_{mut})\,\mathrm{nF} \tag{1}$$

where, 'l' is the length of the IDC finger and 'n' is the total number of fingers, C_{sub}, C_{ins} and C_{mut} are the line capacitance of the coplanar strip in the air due to the substrate, capacitance due to the insulating layer of epoxy resin, and capacitance due to dielectric change of Material Under Test (MUT) respectively.

$$C_{ins} = 2\varepsilon_0 \frac{K_1'(K_1)}{K_1(K_1)} \tag{2}$$

Table 1. Various methods for creatinine detection.

Creatinine detection methods		Advantages	Limitations
Jaffe method (or Jaffe's reaction), the most common method for measuring creatinine levels in serum and urine, is based on the interaction of creatinine with picric acid in an alkaline medium and changes the colour. The colour change can be calibrated and estimated easily [9].		The Jaffe method, which was presented by Jaffe 130 years ago, is still used for its simplicity.	Sample access, interference, reaction time, linear/calibration range, response time, sample volume, large laboratory apparatus, etc.
Chromatography, particularly high-performance liquid chromatography (HPLC) and gas chromatography (GC) are commonly used in detecting creatinine [14–16].		Chromatography has high sensitivity and accuracy. It can analyze different types of samples (urine, blood, serum, etc) with appropriate sample preparation techniques. Chromatography separates creatinine from other compounds in complex biological matrices, reducing interference and improving specificity.	Requires specialized equipment and trained personnel, sample preparation, and time-consuming.
Enzymatic assays for creatinine detection are analytical methods used to measure creatinine levels in biological samples, such as blood or urine, by leveraging the activity of specific enzymes. In the case of creatinine, enzymatic assays can be designed to specifically measure creatinine without interference from other substances in the sample [17].		Enzyme-based assays can be very sensitive, allowing for the detection of low concentrations of creatinine in biological samples. It is important in clinical settings where accurate measurement of creatinine levels, especially at the lower end of the range, is crucial.	Expensive; may require specific sample preparation steps.
Nanomaterials-based methods exploit various unique characteristics of nanomaterials such as high ability of surface reaction, large surface-to-volume ratio, high absorption ability, improved catalytic properties, etc. Some of the nanoparticles used in the electrochemical and colorimetric methods to detect creatinine are gold nanoparticles (AuNPs), carbon nanotubes (CNTs), magnetic nanoparticles (MNPs), silica nanoparticles, etc [18, 19].		Nanomaterials-based methods of creatinine determination offer significant advantages in terms of sensitivity and specificity (if functionalized or imprinted specifically)	Involves complex and costly preparation and implementation methods and specialized equipment and personnel. Non-specific interactions and interference (if not functionalized or imprinted specifically) affecting accuracy and reliability.
Electrochemical methods: In electrochemical methods, the analyte is usually converted to a measurable substance.	Amperometric biosensors, for creatinine detection, are innovative tools that utilize enzymatic reactions to convert the presence of creatinine into an electrical signal [20, 21].	Amperometric biosensors for creatinine detection offer advantages in terms of sensitivity, rapid response, miniaturization, and selectivity.	Cost, complexity, potential interference, durability, and the need for rigorous validation and calibration processes.
	Potentiometric biosensors for creatinine detection utilize changes in electrical potential to measure the concentration of creatinine in a sample. These biosensors typically involve ion-selective electrodes (ISEs) that respond to specific ions or molecules, such as creatinine, in a sample solution [22].	Potentiometric biosensors offer advantages such as high sensitivity, selectivity, real-time monitoring capability, and long-term stability.	Dynamic range, potential interference, electrode conditioning requirements, response time, and cost.
	Conductometric biosensors for creatinine detection are based on measuring changes in the electrical conductivity that occur when creatinine interacts with the sensor surface or affects the conductive properties of the solution [23, 24].	Conductometric biosensors offer advantages such as high sensitivity detection, real-time monitoring capability, and simplicity of operation.	Potential interference limited dynamic range, electrode stability, response time, and sensitivity to environmental factors, etc.
Luminescence-based sensors exploit light emission due to a chemical reaction (Chemiluminescence) or radiative decay from the photon-induced excited state of matter to the ground state of the material (Photoluminescence) in the context of creatinine detection. These sensors typically rely on the enzymatic conversion of creatinine into a product that can either directly produce light or interact with a chemiluminescent substrate to emit light [24, 25].		Luminescence-based sensors offer advantages such as high sensitivity, wide dynamic range, quantitative analysis capability, rapid detection, and automatability.	Complexity in the synthesis of material to be used for the method, cost, potential interference, and specialized instrumentation requirements.

$$C_{sub} = 2\varepsilon_0(\varepsilon_{sub} - 1)\frac{K_2'(K_2)}{K_2(K_2)} \tag{3}$$

$$C_{mut} = 2(\varepsilon_{mut} - 1 \quad)\frac{K_3'(K_3)}{K_3(K_3)} \tag{4}$$

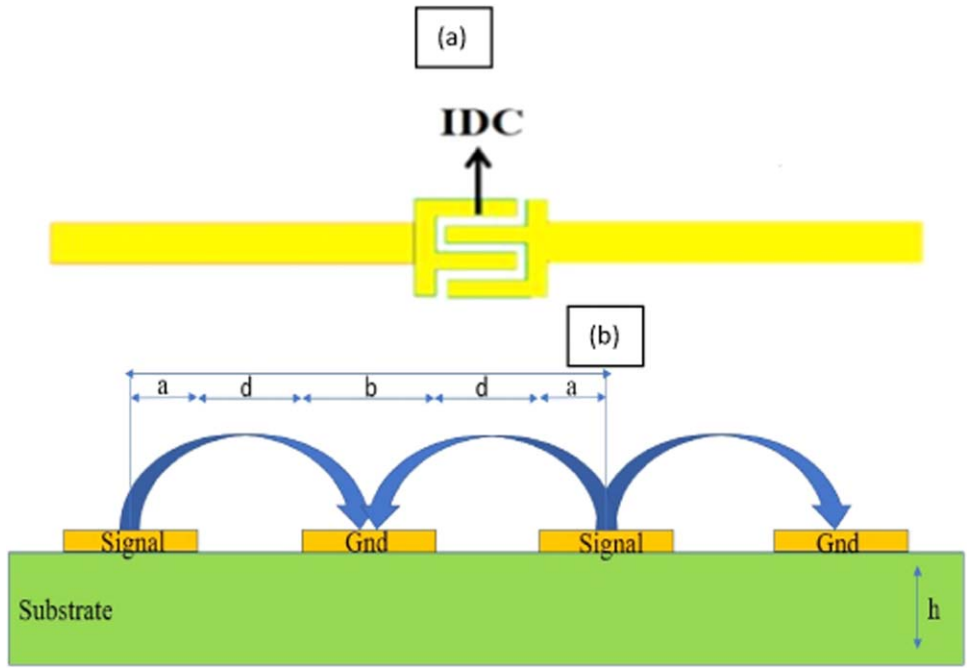

Figure 1. (a) Top view of IDC (b) E-Field distribution across IDC.

Here, $'\varepsilon_0'$ is the free space permittivity ($8.854 \times 10^{-12} Fm^{-1}$), $'\varepsilon_{sub}'$ is the dielectric constant of the substrate used and ε_{mut} is the dielectric constant of the samples, i.e., MUT used over the IDC.

The conformal mapping technique helps to find the capacitance of the IDC. The width of a plate is $K(K_1')$, where $K_1' = \sqrt{1 - K_1^2}$. $K(K_1)$, is the complete elliptical integral of the first kind with modulus K_1, and $K(K_1') = K'(K_1)$.

$$K_1 = \frac{x_3}{x_2}\sqrt{\frac{x_2^2 - x_1^2}{x_3^2 - x_1^2}} \tag{5}$$

where, $x_1 = \frac{b}{2}$, $x_2 = \frac{b}{2} + d$, and $x_3 = \frac{b}{2} + d + a$, '$a$' is half of the finger's width, 'b' is the finger's width,' d' is the gap between the fingers as seen in figure 1(b).

Also, the resonant frequency of IDC is given by:

$$f = \frac{1}{2\pi\sqrt{L_m \times C_{total}}} \tag{6}$$

where, 'L_m' is the inductance due to the trace strip of the IDC.

The sensor is designed using HFSS and operates at 2.5300 GHz the simulated resonance frequency plot with S_{11} is shown in figure 2. It is built using FR4 PCB as a substrate material of 1.6 mm thickness, with a Cu coating of 0.035 mm at the bottom acting as a ground. The IDC sensor on top of the radiating patch is made in 0.035 mm of Cu thickness. The IDC has four fingers each finger is 26 mm long, and 4 mm wide with a gap of 2 mm between them. The simulated structure has been fabricated over the FR4 substrate having a length of 126 mm and a width of 48 mm. The calculated C_{total} is 19.05 nF. and is L_m 0.000206 nH. The simulated structure has been fabricated over the FR4 substrate and covered with a green mask of epoxy resin to protect the Cu from oxidation leaving the contact 4 mm from each side for connections as shown in figure 3.

2.2. Sample preparations

To demonstrate our methodology, we have chosen Phosphate Buffer (PB) as our solvent and prepared the different concentrations of creatinine solutions. The spiking of creatinine of various concentrations in ACC solution (SUT) has been done. The brief description is given below-

2.2.1. Phosphate buffer solution (PBS)

3.4 g of Phosphate Buffer salt (purchased from SRL®) is dissolved thoroughly in 100 ml HPLC grade water in a reagent bottle. Then the reagent bottle is wrapped in aluminium foil and PBS is sterilized in an Autoclave at 121 °C, 15psi for 15 min. After cooling at room temperature, the PBS is stored at (2–8) °C.

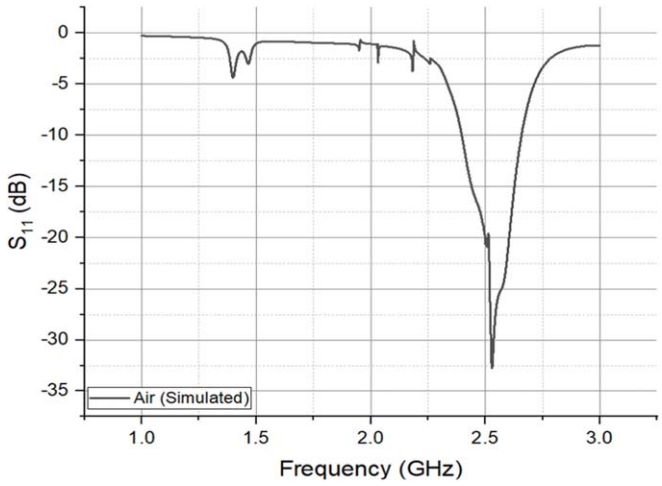

Figure 2. Reflection coefficient (S_{11}) versus operating frequency of simulated IDC structure.

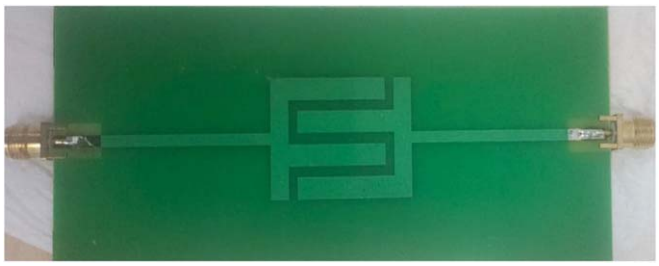

Figure 3. Fabricated inter digitated capacitor (IDC) sensor.

2.2.2. Creatinine in PBS

40 mg of Creatinine (purchased from SRL®) powder is dissolved in 10 ml of PBS to make the Creatinine Stock 400 mg dL^{-1} (cs400). 500 μl of cs400 is then diluted in 9.5 ml of PBS to make 10 ml of 20 mg/dL Creatinine Stock (cs20). cs20 is further diluted in PBS to make the target concentrations of creatinine. All the Stocks are stored at (2–8) °C.

2.2.3. Assayed chemistry control (ACC) solution

To the whole content of Assayed Chemistry Control 5 ml of HPLC grade DI water is added for preparing the Standardised Control Serum Solution. The solution is stored at −20 °C.

2.2.4. ACC solution spiked with creatinine

200 μl of ACC solution is added to 1800 μl of different concentrations of Creatinine in PBS so that the ACC solution is diluted in a ratio of 1:9. A pictorial representation of steps for SUT is shown in figure 4.

3. Experimental setup

The sensor has been connected to VNA via SMA cable as shown in figure 5. Whatman filter paper of grade 4 is placed over the sensitive region of IDC properly to cover all the fingers of IDC. The Whatman cellulose filter papers are manufactured from high-quality cotton liners and are treated to achieve an α-cellulose content > 98%. It has an extremely fast filtering with excellent retention of coarse particles having a pore diameter of 25 μm and a specific thickness of 210 μm [48]. Moreover, with the uniform distribution and less use of sample volume, the grade 4 paper has added an advantage in SUT handling and reducing the experimental error. However, the paper must be minutely cut by a precise CO_2 laser cutting and engraving machine for the specific area. Additionally, to fix and hold the sensor, a PCB holder of PLA material was also fabricated using a 3D printer to achieve stable readings. Finally, the sensor has been connected to VNA as shown in figure 5. like many capacitors, IDC has sensitive performance to temperature changes. In applications with extensive temperature ranges, the capacitance value alters, which might be a drawback. To avoid this issue, we strictly maintained the room temperature where the experiment was performed. When the SUT is dropped, ensure that high accuracy is maintained throughout the experiment the entire setup provides exceptional stability.

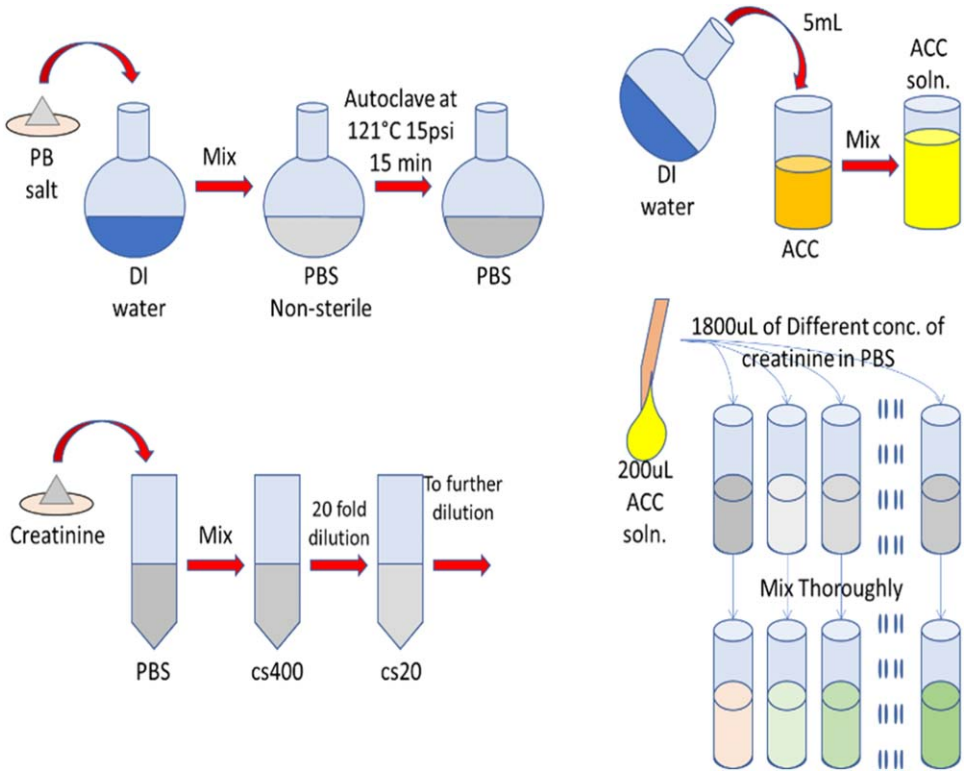

Figure 4. Schematic sample preparation protocol for various concentrations of creatinine in ACC solution.

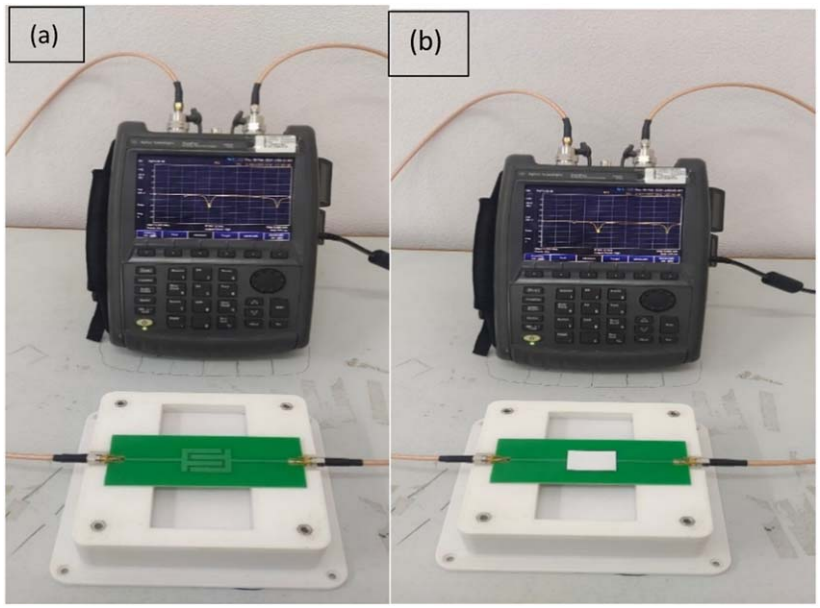

Figure 5. Experimental setup (VNA model N9918A, Keysight Technologies). (a) without filter paper (b) with Whatman filter paper over the IDC fingers.

4. Results and discussion

- It has been observed that there is little difference between the resonance frequency peaks of Air (simulated) at 2.5300 GHz and Air (Experimental) at 2.5475 as shown in table 2, which may result from fabrication-related variations.
- The shift in the resonance frequency has been noted down in the air and with Whatman filter paper, which shows the graph of resonance frequency with S_{11} almost the same in both cases as shown in figure 6. It clearly

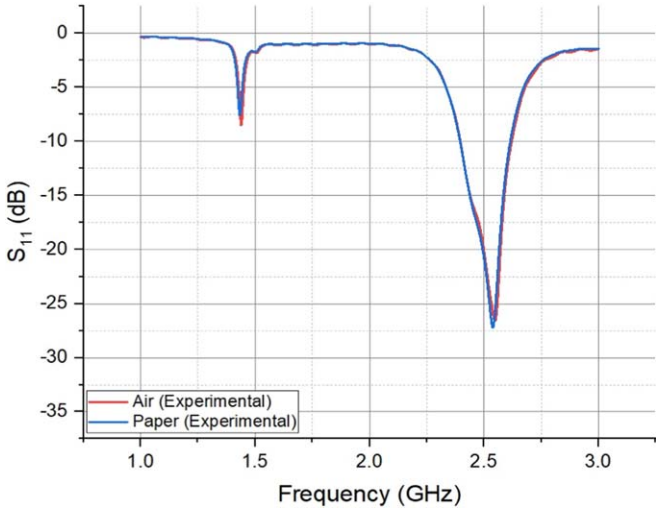

Figure 6. Resonant frequency of sensor with and without paper.

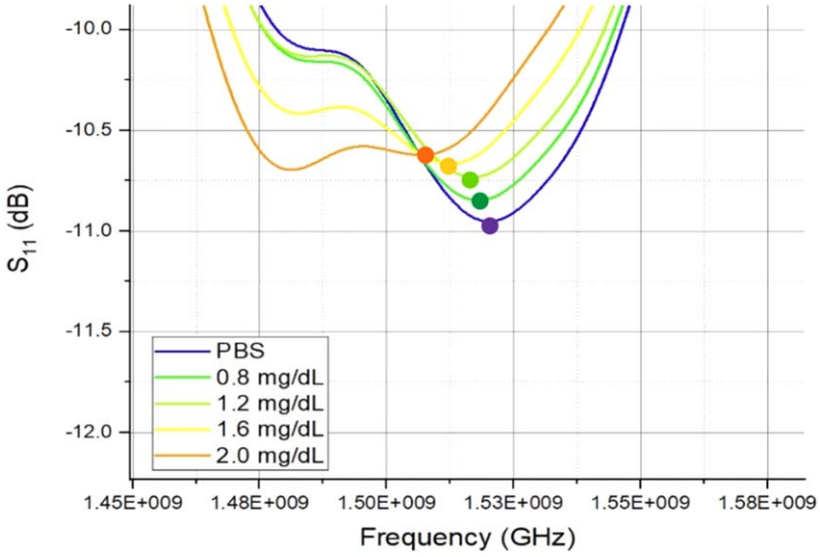

Figure 7. S_{11} versus operating frequency of various concentrations of creatinine with PBS as a reference solution.

Table 2. Resonance frequency peak values for air, pbs, and different concentrations of creatinine.

Sample	Frequency (GHz)	S_{11} (dB)
Air (Simulated)	2.5300	−32.7418
Air (Experimental)	2.5475	−26.6280
Paper	2.5380	−27.2070
PBS	1.5202	−20.9549
0.8 mg dL^{-1}	1.5177	−10.8544
1.2 mg dL^{-1}	1.5165	−10.7339
1.6 mg dL^{-1}	1.5123	−10.6799
2.0 mg/dL	1.5068	−10.6184

shows that the Whatman filter paper will add the additional advantage of liquid-based SUT handling without any hinders.

- The 500 μl solution of each concentration of creatinine was taken through a micropipette and dropped directly over the Whatman filter paper which is attached over the sensing region of the IDC. This volume is

Table 3. Comparison of other methods of creatinine detection with this work.

Detection method		Response time per sample	Materials used	Limitations	Cost effectiveness	Calibration range	References
Jaffe method (or Jaffe's reaction)		15 min (reaction time) + 30 min (data acquisition time)	alkaline picrate (Jaffe's reagent)	require high sample volume, reaction time, interferants	low to moderate	0.2–38.7 mg dL^{-1}	[9]
Chromatography	high-performance liquid chromatography (HPLC)	5–60 min	HPLC grade solvents (mobile phase)	requires trained personnel for operation, response time	high operational and instrumentation cost	0–500 mg $/l^{-1}$, 0.01–0.5 mg/dL	[14, 16]
	gas chromatography (GC)	up to an hour	GC grade pure inert gases (mobile phase)	requires trained personnel for operation, response time	high operational and instrumentation cost	20–10000 μM	[15]
Enzymes based assays		few minutes to hours	immobilized enzyme membrane, poly(y-methyl-L-glutamate)	highly specific sample preparation	high fabrication cost	1–10 mg dL^{-1}	[17, 49]
Spectrophotometric methods		10–30 min	Gold nanoparticles (AuNPs)	reaction time, response time, interferants, the high limit of detection as compared to the clinical range	moderate to high instrumentation, fabrication cost	1.5–4.0 mg/dL	[50, 51]
Electrochemical methods	Amperometric	few seconds to a few minutes	(a) ZnO-NPs/CHIT/c-MWCNT/PANI composite film	rigorous calibration, potential interference, complexity, electrode functionalization	moderate to high instrumentation, fabrication cost	(a) 10–650 μM	[20, 21]
			(b) c-MWCNT/PANI			(b) 10–750 μM	(a) [52], (b) [53], (c) [54]
			(c) AuNPs/MWCNT/Teflon			(c) 3–1000 μM	
	Potentiometric	few minutes—30 min	β-cyclodextrin (βCD)/poly-3,4-ethylene dioxythiophene (PEDOT)/ glassy carbon electrode (GCE) (noncovalent)	rigorous calibration, potential interference, limited dynamic range, electrode functionalization, response time	moderate to high instrumentation, fabrication cost	0.1–100 mM	[22, 55]
	Conductometric	few minutes	(a) solid-state contact ammonium-sensitive PVC–NH2 membrane	potential interference, limited dynamic range, electrode stability	moderate to high instrumentation, fabrication cost	(a) 1–10^5 μM	(a) [23]
			(b) PVA/PEI/CD. TPrA/Ni-NCs@BSA/MIP@GO-Fe_3O_4/ITO electrode			(b) 10–300 μM 1–10^5 μM	(b) [56] [24]

Table 3. (Continued.)

Detection method		Response time per sample	Materials used	Limitations	Cost effectiveness	Calibration range	References
Luminescence based methods	Chemiluminescence based methods	15 min (reaction time) + 10–30 min (data acquisition time)		electrode functionalization, high fabrication cost	moderate to high instrumentation, fabrication cost		
	Photoluminescence based methods	5–30 min (reaction time + 5–15 min (data acquisition time)	FCP-Pd	reaction time, response time, high fabrication cost	moderate to high instrumentation, fabrication cost	1–300 μM	[25]
RF-based sensing method		few milliseconds (almost instantly)	Not Applicable (label-free sensing)	calibration	low operational cost, low to moderate instrumentation cost	0.8–2.0 mg/dL	This Work

sufficient to cover all fingers of IDC. For each concentration check different paper was used and the sensor was washed & dried properly before each reading.

- As the concentration is raised, the resonance frequency peak for creatinine shifts to the left side with PBS buffer as a reference as shown in figure 7. The numerical values for PBS and various concentrations of creatinine are shown in table 2.
- The results clearly show the resonant frequencies downshift with increasing creatinine concentrations. It is accredited to the fact that a higher concentration of creatinine molecules increases the effective capacitance of IDC which results in the downfall of the resonance frequency values. The results investigate the utility of RF biosensors for creatinine detection.
- A comparison of this work's results with available methods for creatinine detection is shown in table 3. This shows the advantages of RF-based sensors in terms of short response time, label fee, and no need of extensive chemical preparation. Moreover, the RF-based sensors are reusable

5. Conclusion

This study investigates the use of an IDC-based RF biosensor to detect creatinine in the medical interest range of 0.8 mg dL^{-1} to 2 mg dL^{-1}. However, the detection range can be further improved by designing a more sensitive RF sensor. The phosphate buffer solution has been used as a solvent to prepare the various concentrations of creatinine. Moreover, an ACC solution was used in which we spiked the different concentrations of creatinine in the ratio of 1:9. Finally, the sensor was connected with the VNA, and the sensitive area of IDC was covered with the Whatman filter paper of grade 4 for fluid handling, which makes a complete system very stable. 500 μl of each concentration has been tested over the sensor and the shift in resonance frequency for various concentrations of creatinine has been observed. We have observed the resonant frequencies downshift with increasing creatinine concentrations because higher concentrations of creatinine molecules increase the effective capacitance of IDC which results in the downfall of the resonance frequency values. It essence, the RF-based sensor emerges as a promising technology for creatinine detection, with implications for early diagnosis and monitoring of renal function. As advancements in sensor technology continue the integration of such devices into clinical practice holds the potential to improve patient outcomes and contribute to the broader landscape of personalized healthcare. RF biosensors represent a cutting-edge technology with immense potential for advancing our capabilities in biosensing and diagnostics. As researchers continue to push the boundaries of innovation the integration of RF biosensors into mainstream applications holds the promise of transforming the landscape of healthcare and environmental monitoring in the years to come.

Acknowledgments

The work was supported by Kohli Challenge Program (Endowed by TCS Foundation) at IIIT Hyderabad, and in part by the Department of Science and Technology (DST), Government of India, through PURSE grant under Grant SR/PURSE/2022/119(G).

Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

ORCID iDs

Andleeb Zahra https://orcid.org/0000-0003-1853-7060
Alimpan Modak https://orcid.org/0000-0003-2046-8041
Syed Azeemuddin https://orcid.org/0000-0002-5974-3584
Tapan Kumar Sau https://orcid.org/0000-0002-0505-9357
Zia Abbas https://orcid.org/0000-0002-3747-3640

References

[1] Zahra A, Caputo D, Nascetti A, Petrucci G, Lovecchio N, Scipinotti R and de Cesare G 2015 Thermally actuated microfluidic system for lab on chip applications *2015 XVIII AISEM Annual Conference (Trento, Italy, 03-05 February 2015)* 1–4

[2] Zahra A, Scipinotti R, Caputo D, Nascetti A and de Cesare G 2015 Design and fabrication of microfluidics system integrated with temperature actuated microvalve *Sensors Actuators*A **236** 206–13

[3] Dutse S W and Yusof N A 2011 Microfluidics-based lab-on-chip systems in DNA-based biosensing: an overview *Sensors* **11** 5754–68

[4] Dolai J, Modak A and Jana N R 2023 Nanocomposite with imprint of cholesterol for its nonenzymatic, visual, and smartphone-based detection *ACS Appl. Eng. Mater.* **2023** 428–35

[5] Jose H and Salazar 2014 Overview of urea and creatinine *Laboratory Medicine* **45** 19–20

[6] Hsu R K *et al* 2020 Research-based versus clinical serum creatinine measurements and the association of acute kidney injury with subsequent kidney function: findings from the chronic renal insufficiency cohort study *Clinical Kidney Journal* **13** 1

[7] Park J, Mehrotra R, Rhee C M, Molnar M Z, Lukowsky L R, Patel S S, Nissenson A R, Kopple J D, Kovesdy C P and Kalantar-Zadeh K 2013 Serum creatinine level, a surrogate of muscle mass, predicts mortality in peritoneal dialysis patients *Nephrology Dialysis Transplantation* **28** 2146–55

[8] Bąchor R, Konieczny A and Szewczuk Z 2020 Preparation of isotopically labelled standards of creatinine Via H/D exchange and their application in quantitative analysis by LC-MS *Molecules* **25** 1514

[9] Delanghe J R and Speeckaert M M 2011 Creatinine determination according to Jaffe-what does it stand for? *Nephrol Dial Transplant* **4** 83–6

[10] Lad U, Khokhar S and Kale G M 2008 Electrochemical Creatinine Biosensors *Anal. Chem.* **80** 7910–7

[11] Pundir C S, Kumar P and Jaiwal R 2019 Biosensing methods for determination of creatinine: a review *Biosens. Bioelectron.* **126** 707–24

[12] Narimani R, Esmaeili M, Rasta S H, Khosroshahi H T and Mobed A 2021 Trend in creatinine determining methods: conventional methods to molecular-based methods *Anal. Sci. Adv.* **2** 308–25

[13] Ullah H *et al* 2022 Anodic SnO_2 nanoporous structure decorated with Cu_2O nanoparticles for sensitive detection of creatinine: experimental and DFT study *ACS Omega* **46** 42377–95

[14] Hewavitharana A and Bruce H 2003 Simultaneous determination of creatinine and pseudouridine concentrations in bovine plasma by reversed-phase liquid chromatography with photodiode array detection *J. Chromatogr.* B **784** 275–81

[15] Nasrallah F, Feki M, Briand G and Kaabachi N 2010 GC/MS determination of guanidinoacetate and creatine in urine: a routine method for creatine deficiency syndrome diagnosis *Clin. Biochem.* **43** 1356–61

[16] Fraselle S *et al* 2015 Development and validation of an ultra-high performance liquid chromatography-tandem mass spectrometry method to measure creatinine in human urine *J. Chromatogr.* B **988** 88–97

[17] Killard A J and Smyth M 2000 Creatinine biosensors: principles and designs *Trends Biotechnol.* **18** 433–7

[18] Du H, Chen R, Du J, Fan J and Peng X 2016 Gold nanoparticle-based colorimetric recognition of creatinine with good selectivity and sensitivity *Ind. Eng. Chem. Res.* 2016 **55** 12334–40

[19] Patel A K, Sharma P S and Prasad B B 2008 Development of a creatinine sensor based on a molecularly imprinted polymer-modified sol-gel film on graphite electrode *Electroanalysis* **20** 2102–12

[20] Nieh C-H, Tsujimura S, Shirai O and Kano K 2013 Amperometric biosensor based on reductive H2O2 detection using pentacyanoferrate-bound polymer for creatinine determination *Anal. Chim. Acta* **767** 128–33

[21] Do J-S, Chang Y-H and Tsai M-L 2018 Highly sensitive amperometric creatinine biosensor based on creatinine deiminase/Nafion®-nanostructured polyaniline composite sensing film prepared with cyclic voltammetry *Mater. Chem. Phys.* **219** 1–12

[22] Pandey P C and Mishra A P 2004 Novel potentiometric sensing of creatinine *Sensors Actuators*B **99** 230–5

[23] Isildak I, Cubuk O, Altikatoglu M, Yolcu M, Erci V and Tinkilic N 2012 A novel conductometric creatinine biosensor based on solid-state contact ammonium-sensitive PVC–NH2 membrane *Biochem Eng* J **62** 34–8

[24] Babamiri B, Salimi A, Hallaj R and Hasanzadeh M 2018 Nickel nanoclusters as a novel emitter for molecularly imprinted electrochemiluminescence based sensor toward nanomolar detection of creatinine *Biosens. Bioelectron.* **107** 272–9

[25] Pal S, Lohar S, Mukherjee M, Chattopadhyay P and Dhara K 2016 A fluorescent probe for the selective detection of creatinine in aqueous buffer applicable to human blood serum *Chem. Commun.* **52** 13706–9

[26] Boybay M S and Ramahi O M 2012 Material characterization using complementary split-ring resonators *IEEE Trans. Instrum. Meas.* **61** 3039–46

[27] Kim N-Y, Adhikari K K, Dhakal R, Chuluunbaatar Z, Wang C and Kim E-S 2015 Rapid, sensitive and reusable detection of glucose by a robust radiofrequency integrated passive device biosensor chip *Sci. Rep.* **5** 1–9

[28] Munoz-Enano J, Coromina J, Velez P, Rasero, Su L, Gil M, Casacuberta P and Martın F 2021 Planar phase-variation microwave sensors for material characterization: a review and comparison of various approaches *Sensors* **21** 1542

[29] Mehrotra P P, Chatterjee B and Sen S 2019 EM-wave biosensors: a review of RF, microwave, mm-wave and optical sensing *Sensors* **19** 1013

[30] Mazumder A, Azeemuddin S, Sau T K and Bhimalapuram P 2020 Role of the shape of gold nanoparticles in sensing biomolecules using radio frequency-based sensors *IEEE Sensor (Rotterdam, Netherlands, October 25-28 2020)* 1–4 (https://doi.org/10.1109/SENSORS47125.2020.9278747)

[31] Grenier K, Dubuc D, Poleni P, Kumemura M, Toshiyoshi H, Fujii T and Fujita H 2010 Resonant-based microwave biosensor for biological cells discrimination *IEEE Radio and Wireless Symposium (RWS) (New Orleans, LA, USA, 10-14 January 2010)* (IEEE) 523–6

[32] Wadhwani K, Hussaini S, Mazumder A and Syed A 2022 Solvent-based optimization of CSRR and IDC RF Bio-sensors *IEEE Sens. J.* **22** 5651–61

[33] Bao X, Ocket I, Crupi G, Schreurs D, Bao J, Kil D, Puers B and Nauwelaers B 2018 A planar one-port microwave microfluidic sensor for microliter liquids characterization *IEEE Journal of Electromagnetics, RF, and Microwaves in Medicine and Biology* **2** 10–7

[34] Ebrahimi A, Withayachumnankul W, Al-Sarawi S and Abbott D 2013 High-sensitivity metamaterial-inspired sensor for microfluidic dielectric characterization *IEEE Sens. J.* **14** 1345–51

[35] Govind G and Akhtar M J 2020 Design of an ELC resonator-based reusable RF microfluidic sensor for blood glucose estimation *Sci Rep.* **10** 18842

[36] Suster M A, Blackburn B, Gurkan U and Mohseni P 2014 An RF/microwave microfluidic sensor based on a 3D capacitive structure with a floating electrode for miniaturized dielectric spectroscopy *IEEE Sensors (Valencia, Spain, 02-05 November 2014)* (IEEE) Valencia, Spain 1784–7

[37] Ansari M A H, Jha A K and Akhtar M J 2015 Design and application of the csrr-based planar sensor for noninvasive measurement of complex permittivity *IEEE Sens. J.* **15** 7181–9

[38] Yue C P, Ryu C, Lau J, Lee T H and Wong S S 1996 A physical model for planar spiral inductors on silicon *Electron Devices Meeting* 1996 (IEEE) 155–8 IEDM'96 (https://web.ece.ucsb.edu/yuegroup/Publications/IEDM96.pdf)
[39] Dimitrov K C, Song S, Chang H, Lim T, Lee Y and Kwak B-J 2020 Interdigital capacitor-based passive LC resonant sensor for improved moisture sensing *Sensors* **20** 6306
[40] Truong T, Kim J-S and Kim J 2022 Printed wearable pressure sensor using interdigitated capacitor for application in smart textile *2022 IEEE/ACIS 7th International Conference on Big Data, Cloud Computing, and Data Science (BCD)* 08 *(Danang, Vietnam, 04-06 August 2022)* 376–81
[41] Kim J W 2008 Development of interdigitated capacitor sensors for direct and wireless measurements of the dielectric properties of liquids *Doctoral Thesis* The University of Texas at Austin (http://hdl.handle.net/2152/10565)
[42] Chen T, Dubuc D, Poupot M, Fourni´e J-J and Grenier K 2013 Broadband discrimination of living and dead lymphoma cells with a microwave interdigitated capacitor *IEEE Topical Conference on Biomedical Wireless Technologies, Networks, and Sensing Systems (Austin, TX, USA, 20-23 January 2013)* 64–6
[43] Hofmann M, Fischer G, Weigel R and Kissinger D 2013 Microwave-based noninvasive concentration measurements for biomedical applications *IEEE Trans. Microwave Theory Tech.* **61** 2195–204
[44] Chretiennot T, Dubuc D and Grenier K 2013 A microwave and microfluidic planar resonator for efficient and accurate complex permittivity characterization of aqueous solutions *IEEE Trans. Microwave Theory Tech.* **61** 972–8
[45] Lee H-J, Lee J-H, Moon H-S, Jang I-S, Choi J-S, Yook J-G and Jung H-I 2012 A planar split-ring resonator-based microwave biosensor for label-free detection of biomolecules *Sensors Actuators* B **169** 26–31
[46] Kim J, Babajanyan A, Hovsepyan A, Lee K and Friedman B 2008 Microwave dielectric resonator biosensor for aqueous glucose solution *Rev. Sci. Instrum.* **79** 086107
[47] Brooke D, Movahed N and Bothner B 2015 Universal buffers for use in biochemistry and biophysical experiments *AIMS biophysics* **2** 336–42
[48] Avantor 2023 (https://uk.vwr.com/store/product/36937920/grade-4-qualitative-filter-papers-whatmantm)
[49] Krishnegowda A, Padmarajaiah N, Anantharaman S and Honnur K 2017 Spectrophotometric assay of creatinine in human serum sample *Arabian J. Chem.* **10** S2018–24
[50] Kubo I and Karube I 1986 Immobilization of creatinine deiminase on a substituted poly (methyl glutamate) membrane and its use in a creatinine sensor *Anal. Chim. Acta* **187** 31–7
[51] Sittiwong J and Unob F 2015 Detection of urinary creatinine using gold nanoparticles after solid phase extraction *Spectrochim Acta*A **138** 381–6
[52] Yadav S, Devi R, Kumar A and Pundir C S 2011 Tri-enzyme functionalized ZnONPs/CHIT/c-MWCNT/PANI composite film for amperometric determination of creatinine *Biosens. Bioelectron.* **28** 64–70
[53] Yadav S, Kumar A and Pundir C S 2011 Amperometric creatinine biosensor based on covalently coimmobilized enzymes onto carboxylated multiwalled carbon nanotubes/polyaniline composite film *Anal. Biochem.* **419** 277–83
[54] Serafín V, Hernández P, Agüí L, Yáñez-Sedeño P and Pingarrón J M 2013 Electrochemical biosensor for creatinine based on the immobilization of creatininase, creatinase and sarcosine oxidase onto a ferrocene/horseradish peroxidase/gold nanoparticles/multi-walled carbon nanotubes/Teflon composite electrode *Electrochim. Acta* **97** 175–83
[55] Naresh Kumar T, Ananthi A, Mathiyarasu J, Joseph J, Lakshminarasimha Phani K and Yegnaraman V 2011 Enzymeless creatinine estimation using poly (3, 4-ethylenedioxythiophene)-β-cyclodextrin *J. Electroanal. Chem.* **661** 303–8
[56] Braiek M *et al* 2018 A conductometric creatinine biosensor prepared through contact printing of polyvinyl alcohol/polyethyleneimine based enzymatic membrane *Microelectron. Eng.* **187-188** 43–9
[57] Veyres C and Fouad Hanna V 1980 Extension of the application of conformal mapping techniques to coplanar lines with finite dimensions *Int. J. Electron.* **48** 47–56