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Wide Band Frequency Measurements of Fungal Species Using Laser Patterned Finger Electrodes on LTCC

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Abstract—High frequency measurements at 50 MHz–10 GHz were performed for the first time using interdigitated electrodes on a low temperature co-fired ceramic substrate to analyze fungal spores. Wet and dry spore generation methods were evaluated and tested with two different fungal species. The dry generation method was found feasible for RF measurements, since the component capacitance increased 14–21% in the 2–6 GHz range, but for the wet generation method the capacitance decreased only slightly ($< 1\%$). Based on these initial results the RF measurements have the capacity to evaluate the quantity of fungal spores but not to identify their species.

1. INTRODUCTION

Indoor mould growth due to moisture issues or water damage is an important health concern, and exposure to airborne mould particles and toxins is responsible for many adverse health effects observed among occupants of such buildings. Therefore, methods to detect mould in buildings are important both for preventive purposes and for surveillance in connection with moisture damage. For example, a mould detection method could be integrated with the air conditioning system.

The conventional methods used to detect the presence of mould in indoor environments include visual inspection and sampling of air on surfaces followed by cultivation on growth media or microscopic evaluation. These methods are laborious and time consuming. Microbial cell wall agents such as ergosterol or β-glucan have been used for fungal species recognition. Recently, real-time devices with optical and laser induced fluorescence characterization have been employed [1]. In addition, trained dogs have been used for mould detection. These dogs are fast and accurate and thus economical in locating sites of mould growth.

Organic material measurements and electromagnetic spectral analysis are known methods for the study of living cell material [2, 3] Interdigitated Electrodes (IDEs) have proven to be suitable in the detection of various particulate matter and cells by applying conductometric or capacitive sensing mechanisms [4]. In this paper, laser patterned IDEs on Low Temperature Co-fired Ceramic (LTCC) have been used to evaluate detection possibilities of Aspergillus versicolor and Penicillium brevicompactum fungal species using a high frequency measurement setup to detect any capacitance change due to the presence of spores.

2. EXPERIMENTAL

IDEs were prepared using a slightly modified LTCC process. The pattern shown in Fig. 1 was screen printed with Ag based conductive paste (6142D, DuPont, USA) through a stainless steel mesh. After

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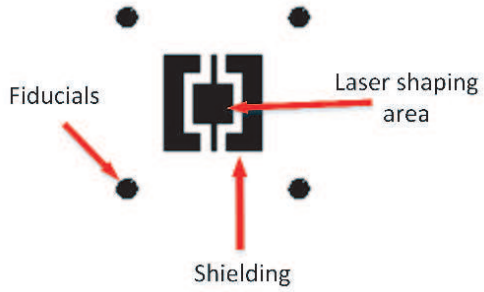


Figure 1. Screen printed pattern for laser modified IDE. Laser shaping area is 1.5 mm × 1.5 mm. Fiducials are used for precise optical alignment.

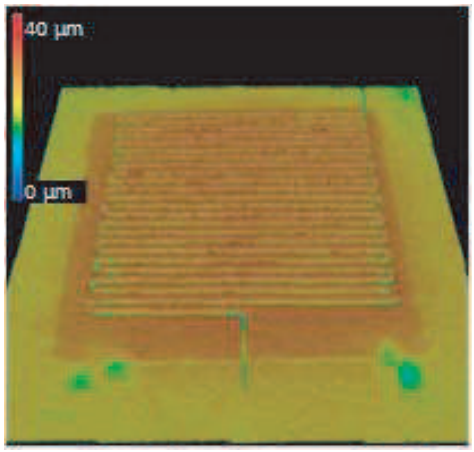
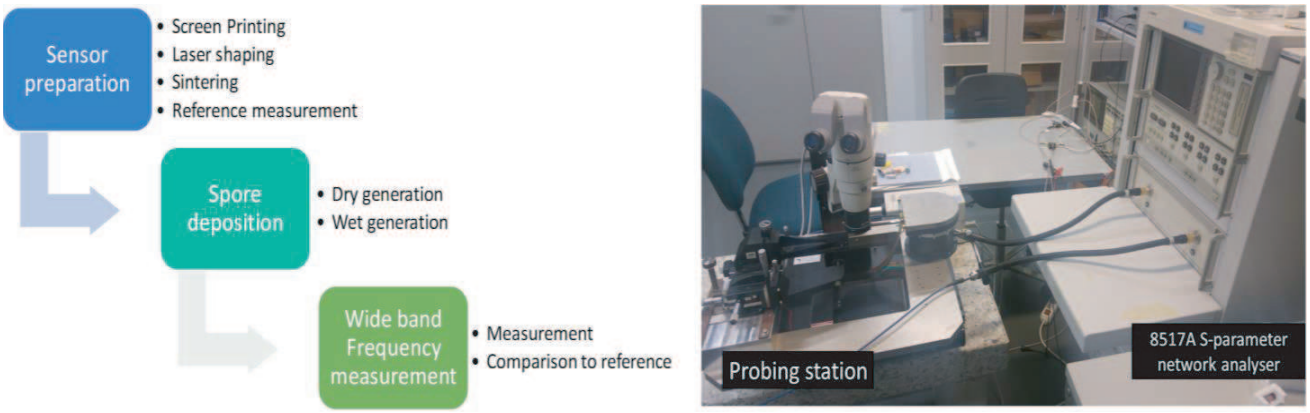


Figure 2. Laser profilometer picture of laser shaped IDE. Total height of the fingers is 11 µm, finger width is 32 µm, distance between fingers is 35 µm, total area of IDE is 4 mm^2.



(a) (b)

Figure 3. (a) The experiment consisted of 3 separate steps: sensor manufacturing; spore deposition; measurements. (b) Measurement setup.

printing the pattern on non-sintered tapes (951, DuPont, USA) it was modified by a UV pulsed laser with a beam diameter of 35 µm (LPKF, Germany). The original printing was scribed with the laser leaving an IDE pattern with a feature/gap ratio of 32/35, as presented in Fig. 2.

After printing and laser patterning the LTCC green sheets were laminated and sintered using standard parameters suggested by the vendor. Reference measurements were made with optically and electrically inspected IDE samples using an 8517A *S*-parameter network analyser (Agilent, USA) in the frequency range from 1 GHz to 10 GHz. Finally, the IDE structures were exposed to fungi spores and measured again using the same setup. The experiment setup and a photo of measurement device are presented in Fig. 3.

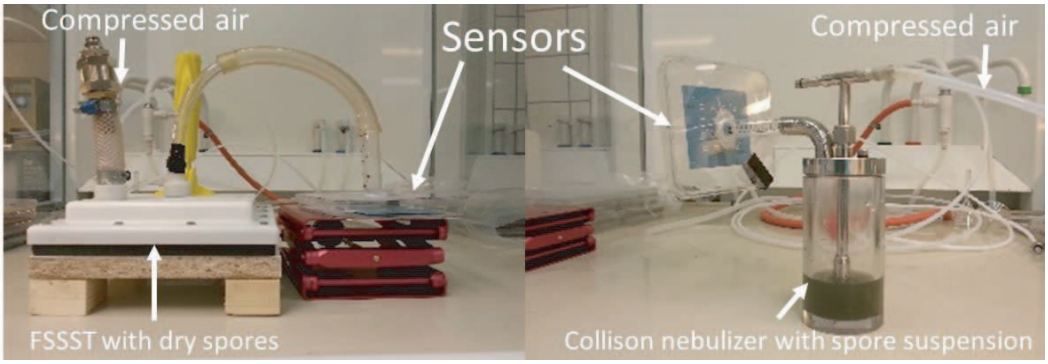
Fungal particle preparation: Two fungal species, Aspergillus versicolor (Culture collection of the Institute for Health and Welfare, Finland: HT31) and Penicillium brevicompactum, (American Type Cell Collection: ATCC 58606) were used in the experiments. These species are common in indoor air worldwide [5]. The fungal strains were grown on malt extract agar (ME) (LabM, Lancashire, UK) and incubated for at least two weeks in order to produce enough spores. Prior to aerosolization, 1 gram of glass beads (Ø = 425–600 µm; Sigma-Aldrich Co., Saint Louis, MO) was applied on each growth plate.

The plates were then gently shaken back and forth to aid attachment of asexually produced spores onto the beads [6].

To evaluate the efficiency of the IDEs to detect fungal particles, two types of fungal particle generation were performed:

1) Dry fungal particles generation: This was achieved by placing the fungal plates with the glass beads directly in a Fungal Spore Source Strength Tester (FSSST) [7] and allowing filtered air to flow through it at a rate of 15 LPM (Liters per minute) to displace the fungal particles from the glass beads. The displaced particles were directed onto the LTCC surface.

2) Wet fungal generation: Glass beads with fungal particles were transferred into a tube containing 15 ml of 0.05% Tween 80. The fungal particles were suspended from the beads by shaking the tube and decanting the fungal suspension. The spores were counted with a hemacytometer (Fuchs-Rosenthal: Hirschmann EM Technicolor) and the concentration was adjusted to 1×10^6 spores/ml. The suspension was then aerosolized onto the LTCC surface using a Collison nebulizer (a liquid aerosolization device). Experimental setups for the two methods of creating aerosols of the fungal particles are shown in Fig. 4.]



(a) (b)

Figure 4. Experimental setup to create aerosol of fungal particles. (a) Dry generation and (b) wet generation.

3. RESULTS AND DISCUSSION

Results of the RF measurements of dry and wet generated particles are presented in Figs. 5 and 6. Both types of particles were deposited on three different IDEs (components 1–3), measured over a wide frequency band from 50 MHz to 10 GHz and three characteristic component resonances (one for each component) were observed in the data. In the dry processed components, the characteristic resonances of the IDE electrodes were measured around 2.5 GHz, 4 GHz and 6 GHz and the resonant shift induced by the fungal particles was 1–3% of the positive frequency range. The increase in the capacitance was calculated to be from 7 pF to 8.5 pF for component 1 (2 GHz), from 1.34 pF to 1.53 pF for component 2 (3 GHz) and from 1.29 pF to 1.49 pF for component 3 (5.3 GHz) (an increase of 1.5, 0.19 and 0.20 pF, respectively). The positive shifts of capacitances were in the range of 14–21 %. The fungal particles clearly increased the capacitance of all components, but the different species gave the same results and thus it was not possible to distinguish between them by this method. In the case of the wet processed components, the resonance shifts and capacitances induced by the fungal particles were in the slightly negative range and varying by less than 1%. The electromagnetic effect was not clear. The results did not reveal whether the phenomenon was caused by the generation method, e.g., moisture in the structure, or by the distribution, quantity and density of particles on the surface of the components. Both could be potential reasons for the results. Methods were not available to measure actual particle quantities on the surface. The method was too inaccurate to differentiate the fungal type based on the value of the loss component of capacitance.

In further research, the use of an open-ended coaxial cavity method for powdery materials could be an alternative method to determine differences between moulds [8, 9].

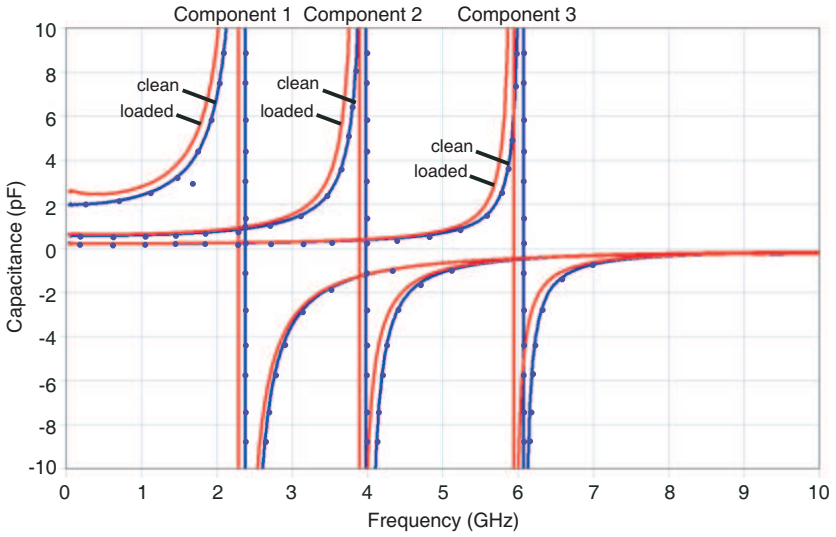


Figure 5. Results of 50 MHz–10 GHz frequency range capacitance measurement of three different IDE electrodes as clear and with dry generated fungal particles on the electrodes.

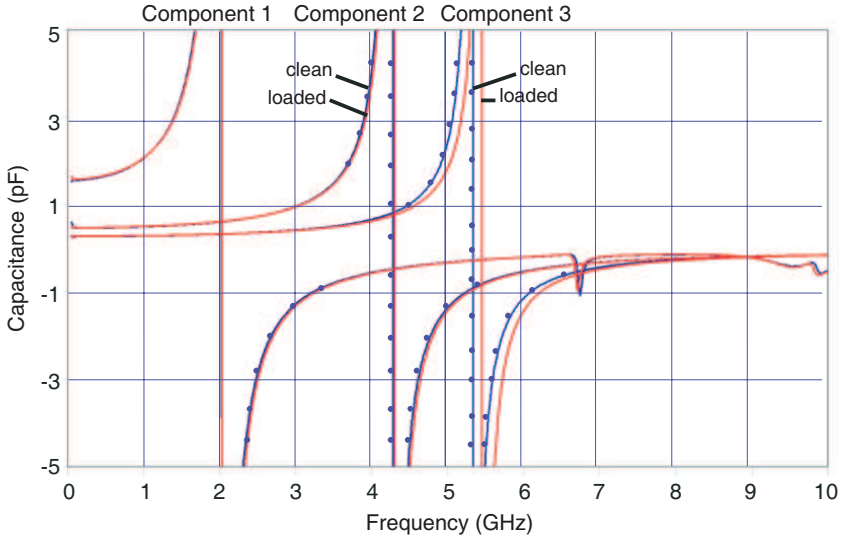


Figure 6. Results of 50 MHz–10 GHz frequency range capacitance measurement of three different IDE electrodes as clear and with wet generated fungal particles on the electrodes.

4. CONCLUSIONS

A new method has been tested to analyse fungal spores using high frequency 50 MHz–10 GHz measurements performed with IDE electrodes on LTCC substrates. Both wet and dry fungal generation methods of applying the spores to the sample surfaces were evaluated and tested for two different fungal species. For the dry generation method the capacitive component increased 14–21% in the 2–6 GHz range, showing the feasibility of these RF measurements to detect spores but the change in capacitance for the wet generation method was very poor ($< 1\%$). The results did not reveal whether the phenomenon with wet processing was caused by the generation method, e.g., by moisture in the structure, or by the distribution and quantity of particles on the surface of the components. Moisture and temperature influence could be countered by a reference sensor or conditioning the sampled air

before measurement. Similarly, obtaining dielectric spectra for different kind of spores would be the next step in our research as creating a reference library for the final sensor system would be crucial. With the presented RF measurement method, it is possible to evaluate the quantity of fungal particles, but improvement of the method is needed in order to specify the species of the mould. The method could potentially be utilized in multiple practical applications in the future such as in building construction areas, for example in air conditioning systems.

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