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14. ABSTRACT Report developed under Topic#CBD10-110, contract W911NF-12-C-0112. A modified configuration for a THz stage has been designed, fabricated and assembled with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The new stage accommodates a modified optical mounting system that focuses THz radiation on micro/nanofluidic chips with reduced size of 1.5 mm x 3.0 mm for the active region, encompassing 10 sample channels in parallel.					
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Report Title

Development of a Biosensor Nanofluidic Platform for Integration with Terahertz Spectroscopic System

ABSTRACT

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A modified configuration for a THz stage has been designed, fabricated and assembled with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The new stage accommodates a modified optical mounting system that focuses THz radiation on micro/nanofluidic chips with reduced size of 1.5 mm x 3.0 mm for the active region, encompassing 10 sample channels in parallel. Another important improvement to the stage is modified linear positioning of the microdetector with a probe relative to the microfluidic chip in a near field configuration. A significant change in the software for computer controlled operation has also been made. We continued protocol development for spectroscopic characterization of biomaterials using micro/nanofluidic chips. The algorithm for amplitude and frequency shift corrections has been investigated and tested.

The possibility to further improve spectral resolution has been demonstrated.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

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Number of Papers published in peer-reviewed journals:

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Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Paper

TOTAL:

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(d) Manuscripts

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TOTAL:

Patents Submitted

Patents Awarded

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Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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FTE Equivalent:

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Total Number:

Names of Under Graduate students supported

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This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

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Names of Personnel receiving masters degrees

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Names of personnel receiving PHDs

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Total Number:

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
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Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

A modified configuration for a THz stage has been designed, fabricated and assembled with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The results will be used for optimization of design and technology during the Phase II periods. The new stage accommodates a modified optical mounting system that focus THz radiation on micro/nanofluidic chips with reduced size of 1.5 mm x 3.0 mm for the active region, encompassing 10 sample channels in parallel.

We continued protocol development for spectroscopic characterization of biomaterials using micro/nanofluidic chips. The algorithm for amplitude and frequency shift corrections has been investigated and tested. Although the needed corrections are usually not big, the correction procedure can be important, since it improves the sensitivity limit and reduces the risk of possible artifacts. As a result of using the procedure for amplitude and frequency corrections described above, we have recently characterized transmission spectra of artificial DNA crystals from the group of professor Michael Norton (Marshall University) and of a single cancer cell obtained in the laboratory of professor Amir Jazaeri at UVA School of Medicine. These results confirm the potential for sub-THz vibrational spectroscopy using our spectroscopic sensor with a high spectral and spatial resolution as a sensitive label free and reagent free optical resonance technology for detecting and identifying bio-chemical threat agents.

Technology Transfer

FINAL PROGRESS REPORT

Report performance period September 18, 2012 – December 17, 2012

Contract W911NF -1-2C-0112

Proposal Number 62126ELCBD

Contractor's name and address: Vibratess, LLC 104 Chaucer Road, Charlottesville, VA 22901

Title of the project: Development of a Biosensor Nanofluidic Platform for Integration with Terahertz Spectroscopic System

Contract performance period: September 18, 2012 – December 17, 2012

Authors: Tatiana Globus, Aaron Moyer, Jerome Ferrance, Igor Sizov, Tatyana Khromova

Abstract: *A modified configuration for a THz stage has been designed, fabricated and assembled with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The new stage accommodates a modified optical mounting system that focuses THz radiation on micro/nanofluidic chips with reduced size of 1.5 mm x 3.0 mm for the active region, encompassing 10 sample channels in parallel. Another important improvement to the stage is modified linear positioning of the microdetector with a probe relative to the microfluidic chip in a near field configuration. A significant change in the software for computer controlled operation has also been made. We continued protocol development for spectroscopic characterization of biomaterials using micro/nanofluidic chips. The algorithm for amplitude and frequency shift corrections has been investigated and tested. The possibility to further improve spectral resolution has been demonstrated.*

Subject terms: THz sensor, micro/nanofluidic spectroscopic platform, biological material sensing, amplitude and frequency shift corrections

I. The Optional Phase Project Objectives.

Two Objectives in the project Contract W911NF -1-2C-0112 (Proposal Number 62126ELCBD) have been identified.

Objective 1: To design and build an optical setup to evaluate the possibility of focusing THz radiation onto a small microfluidic chip with the area of $\sim 1.5 \times 2 \text{ mm}^2$ that is close to the diffraction limit in our spectral region, This geometry is instead of 20 mm^2 chip in the existing THz stage that required a parallel beam. This Objective corresponds to the Task 3 of the Phase II Contract: Layout and implementation of experimental system prototype. Integrating components for building a spectroscopic system. The results will be used for optimization of design and technology during the Optional and Phase II periods.

Objective 2. To study artifacts in measured transmission spectra from biosamples and to develop an approach to mitigate the problem of the possible frequency mismatch in measured background and sample spectra. This Objective corresponds to the Task 4a) of the Phase II Contract: Protocol development for spectroscopic characterization of biomaterials using Micro/Nanofluidic chips.

II. Work performed and Results obtained

Objective 1 (Task 3 of the Phase II Contract). Layout and implementation of experimental system prototype. Integrating components for building a spectroscopic system.

A modified configuration for a THz stage has been designed with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The new design accommodates a modified optical mounting system that focus THz radiation on micro/nanofluidic chips with reduced size of 1.5 mm x 3.0 mm for the active region, encompassing 10 sample channels in parallel (Fig. 1). This size is comparable with a minimal spot of sub-THz radiation dictated by the wavelength of radiation and at the same time is enough to locally enhance the intensity of radiation interacting with sample materials.

Mechanical components have been designed and fabricated. An adapter device/sample holder for holding nano/microfluidic system and integrating with a spectroscopic instrument has been designed and fabricated. There is the option of apertures with different dimensions in the holder (Fig.2).

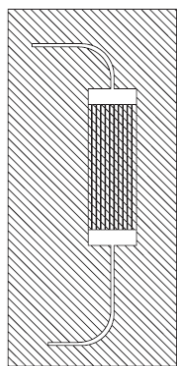


Figure 1. Reduced size microfluidic chip (the active region of 10 channels- 1.5 mm x 3.0 mm)

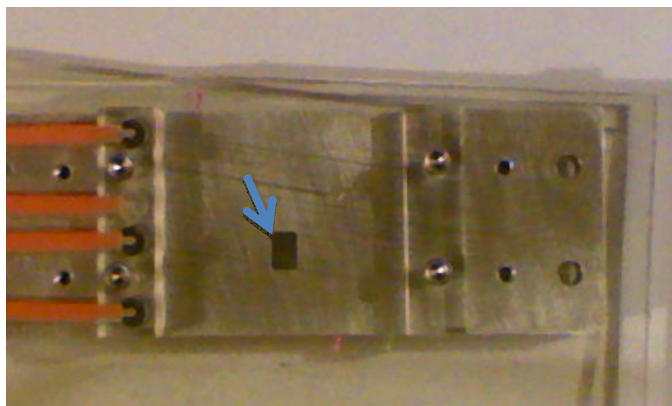


Figure 2. A microfluidic chip holder assembly

Fig 3 demonstrates the schematic of the new optical mirror set-up that was built. As compared with previously assembled spectroscopic systems, a smaller mirror with half the diameter (only 25 cm) is used that potentially will permit reductions in the overall size and the cost of a sub-THz spectroscopic sensor system.

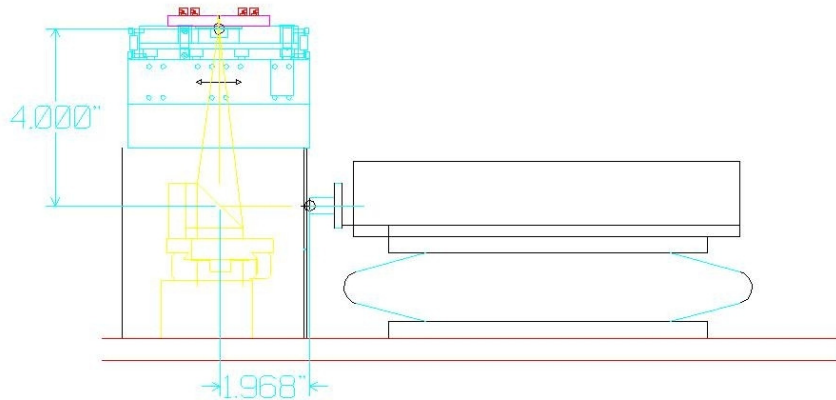


Fig. 3. Drawing of a new optical setup built in Optional Phase. THz radiation is focused on a microfluidic chip .Adjustment of a focal point is possible in all three directions.

The mirror assembly has been designed to mount on an optical rail. Further modifications to the commercially available rail components enable repeatable registration of the mirror's position after removal and reinstallation on the THz fixture (see Fig. 4). The ability to remove the assembly will allow it to be easily reconfigured such that the parabolic mirror's focal point is directed in two different orientations (Fig. 5). One orientation places the focal point to the side while the other one directs the focal point upward.

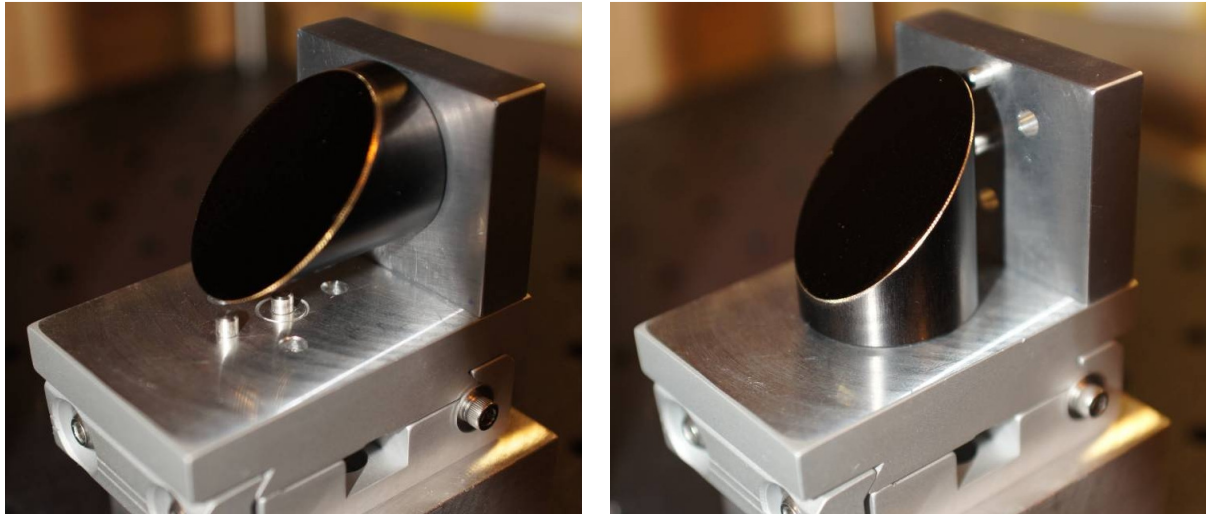


Fig. 4. Two positions of the mirror mounting for two possible configuration of a radiation beam: a parallel or a focused beam on a microfluidic chip.

One more modification of the spectrometer that was done in the Optional period is a significant change of software for computer controlled operation.

Results obtained for Objective I:

All components of a modified THz stage have been fabricated and the complete set up has been assembled. All wiring of this stage has been completed (Figure 5). Testing of the entire stage has identified a problem in reading the signal caused by the presence of a parasitic signal that masks the useful signal. The problems of this kind can usually be eliminated by proper grounding components and a system, and installing antireflection insulation along the optical path of a radiation. The origin of this problem is currently under investigation and it will be corrected in the Phase II contract.

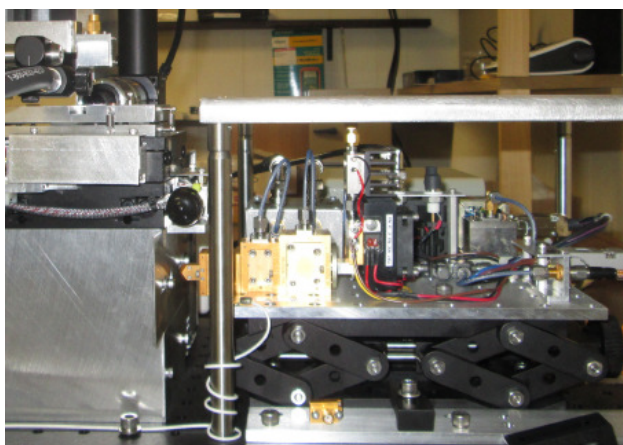


Fig.5. A modified THz stage with an experimental optical setup that permits two possible configuration of a radiation beam: a parallel or a focused beam on a microfluidic chip.

Objective II. Task 4 a) of the Phase II Contract. Protocol development for spectroscopic characterization of biomaterials using Micro/Nanofluidic chips.

It was recently demonstrated in our experimental characterization of biological materials using the Vibratess THz stage that mismatch between background and sample measurements can lead to artifacts in their transmission/absorption spectra. The analyses of experimental results received so far identified two different sources of possible artifacts. These are 1) change of the signal with time between the background and sample measurements and 2) a frequency mismatch between background and sample signal measurements. Although the absolute changes are very small in both cases, they can lead to incorrect signature patterns. Experimental results also show that improper amplitude values of signal happened more often than the frequency shift, and lead to erroneous transmission values above 1 (above 100%) in some regions of a spectrum where transmission of a sample is high due to a small thickness, material concentration or low absorption. This type of error is especially important for characterizing small amounts of less absorbing materials and for frequencies where intensity of background radiation is low (see Fig. 6). This effect limits the sensitivity of characterization. There are several possible explanations for this effect. The soft plastic substrate can slightly change its form when sample material is added to a channel thus modifying the optical path of THz beam. We have changed the chip technology in this project by using more robust substrate material, reduced the area of a substrate that extends beyond its support, and used additional frames to enhance the substrate robustness. Temperature change can be another reason and we reduce optical illumination intensity used in the instrument

for visualization of the probe and channel to minimize heating. These measures have significantly reduced the effect. Very simple correction can be achieved by multiplying measured transmission by a constant coefficient to correct the remainder.

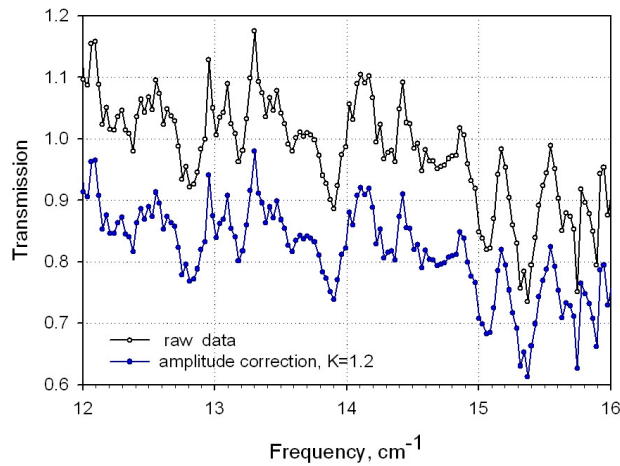


Fig. 6. Transmission spectra of cancer cells sample with trace amount of material. Raw data (black) and results with an amplitude correction

After the sample spectrum is corrected for amplitude, it can be compared with the background spectrum for the frequency mismatch correction. Such mismatch can be caused by variety of reasons. The change of a dielectric constant inside the channel when it filled with biological materials can be one of these reasons.

During the Optional period the problem of correction for the frequency mismatch between background and sample spectra was analyzed. The value of frequency shift, δ , can be roughly estimated by experimentally determining the average value of the apparent shifts δ_1 and δ_2 on two sides of a peak-like spectral feature (see Fig. 7). Transmission has to be constant in this frequency sub-range to ensure that there is no shift caused by an absorption change. This approach was used to correct raw data. The results are shown in Fig. 8.

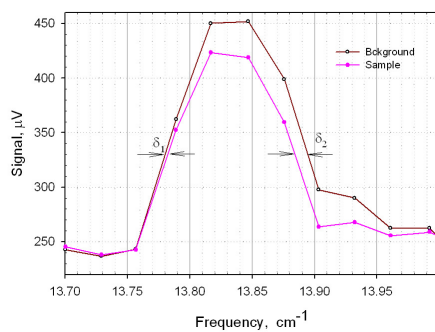


Fig. 7. The value of frequency shift, δ , estimated from experiment is averaged value between apparent shifts δ_1 and δ_2 on two sides of a peak-like spectral feature. Transmission has to be constant in this frequency sub-range to ensure that there is no shift caused by absorption change.

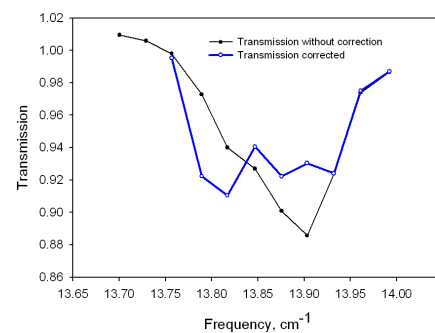


Fig. 8. Calculated transmission before (black) and after (blue) correction.

The frequency shift can be found more accurately via minimization of the sum of squared differences between the sample $s_{f+\delta}$ and background b_f signals at each frequency point f , as a function of shift δ .

$$\sum_f (s_{f+\delta} - b_f)^2$$

This identified frequency shift $\delta_{\min}$, which gives the minimal sum will be used to correct the frequencies in the measures spectrum from the sample material inside the microfluidic channel of the chip with a cover layer.

4 b) Further improvement of spectral resolution.

As it was found experimentally, better spectral resolution is required to resolve spectral lines. An approach to improve the spectral resolution from 0.75 GHz to 0.37 GHz by reducing the voltage step (to 10 mV) applied to YIG oscillator of the THz source, thus reducing the frequency step, has been tested. Fig. 9 demonstrates the background signal measured with this smaller frequency step. Although the spectral resolution was improved, this spectrum is not good. An additional study is required to improve the spectral distribution of radiation by eliminating reduced signal at 13.9 cm^{-1} and 14.5 cm^{-1} . This study will be performed in the Phase II Contract W911NF-12-C-0046 to correct the problem.

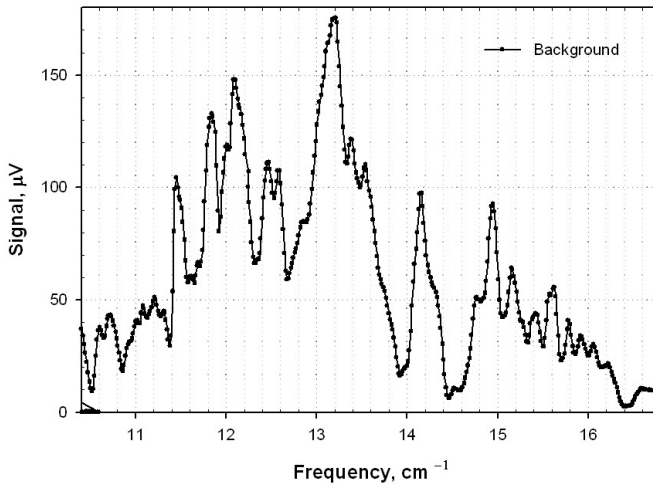


Fig 9. Background spectrum measured with a resolution of 0.37 GHz

III. Estimates of technical feasibility.

A modified configuration for a THz stage has been designed, fabricated and assembled with the final goal to combine the THz spectrometer with a microfluidic platform for characterization of biological samples in solutions. The results will be used for optimization of design and technology during the Phase II periods. The new stage accommodates a modified

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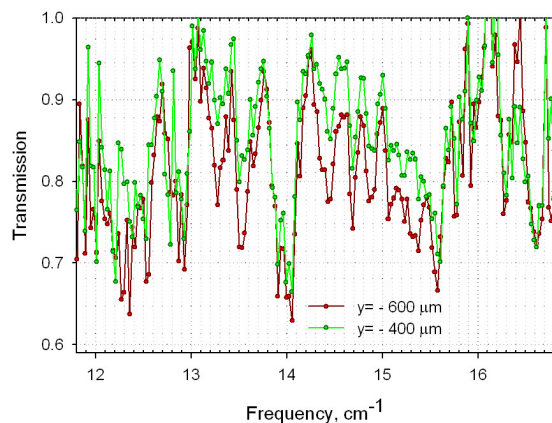


Fig. 10. Variability of transmission spectra of artificial DNA crystals (Group of Prof. M. Norton).

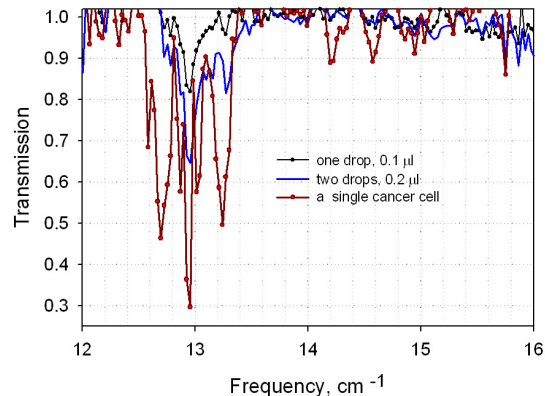


Fig. 11. Transmission spectra (sub-THz spectroscopic signature) of a cancer cell material (Prof. A. Jazaeri, UVA Medical School)

The possibility to further improve spectral resolution has been demonstrated that will further improve the technology discriminative capability.