Cavity-Enhanced Microscopy (CEMIC)

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ABSTRACT
Many biological and physical samples cause minimal absorption/scattering with a refractive index closely matching their environment. Examples range from organelles in cells, to molecular monolayers, trace gasses, and laser-induced plasmas. In other cases, the sample must not be disturbed by the imaging process, e.g. non-destructive imaging of quantum gases. Cavity-Enhanced Microscopy (CEMIC) is a novel imaging technique, which increases the optical cross-section of the samples by many orders of magnitude, whilst at the same time measuring the refractive thickness of extremely optically-thin samples. A first demonstration of this novel technique is presented and applications in Surface Science, Biology, Medicine, and Quantum Physics outlined.

Keywords: Microscopy; Non-Destructive Imaging; Quantum Technology; Label-Free Imaging;

1. INTRODUCTION

Much of the knowledge that we have from our world stems from the analysis of images. Using optics and cameras, we can map how much light a sample absorbs or how by how quickly the light propagates in a medium (both together are described by the complex refractive index). From these spectral properties, we can then deduce the chemical composition and/or physical makeup of the sample. Examples are many and wide-reaching, from astronomy to trace chemicals in the atmosphere, from monolayers on semiconductor surfaces, to biological cells and their inner workings. Absorption or refractive index measurements are used in cancer detection, as cell morphology and growth in cell biology, malaria and anaemia disease diagnosis in haematology, and cancer cell and circulating tumour cell detection in pathology.[1,2]
There is a large number of techniques, which focus on different aspects of imaging. One of the most important one is the imaging of live cells. Recent years have seen enormous progress, e.g. in fluorescence and super-resolution microscopy. One of the main challenges is still the label-free imaging of the constituents of a living cell. The confined functional units of cells, the organelles, are the cogwheels of life. By size, they could often be nicely resolved by optical microscopy (a few µm). Unfortunately, optically they differ very little from the surrounding cell. The refractive index of E. Coli. is 1.401-1.403 for the cell liquid and the one of its Cytosol is ~1.375 and its Nucleus ~1.385, [1] which gives rise to a difference in the refractive index of only 0.02. A very large nucleus is a few µm in diameter resulting in a single pass phase delay of only 20 nm or 2% of the wavelength of light.

2. STATE OF THE ART

There are very few imaging techniques that can measure very small differences in refractive index, such as Hilbert phase microscopy, Phase-shifting interferometry and Tomographic bright-field imaging. These techniques have a resolution in the refractive index of about 10^{-2} when measured across the whole of the cell [1]. Clearly, these methods do not have access to details within the internal structure of the cells.
One method to increase the sensitivity of measurements is to let the light pass multiple times through the sample. Recently, researchers at Stanford University demonstrated this by placing two objectives between semi-transparent mirrors [6] thus imaging the object back onto itself, forcing the light to pass the same spot multiple times. A modest enhancement by a factor of 2.7 was observed. The main limitation of this method is its large optical complexity: In their design, the light passes through a minimum of 18 surfaces per round trip, thus severely limiting the maximum number of round-trips that can be achieved.
A scanning cavity microscope was demonstrated in 2015, which in analogy to its electronic counterpart (STEM) slowly moves a single detecting spot across the image [5].
Clearly, there is no practical technique yet that exploits the enormous enhancement possible by imaging highly transparent specimens inside optical cavities.

3. BREAKTHROUGH CHARACTER OF THE PROJECT

CEMIC has demonstrated for the first time intra-cavity microscopy with simultaneous access to the absorption *and* phase information across a large image plane yielding **an image of the full complex refractive index** of the sample under investigation.

CEMIC is based on an optical cavity where light travels many times back and forth between its mirrors. The light then passes many times through any transparent object placed inside the cavity. Any amount of absorption or any difference in refractive index is then amplified by the number of passes of the light, which can be more than 10^5 times. This principle has been the key for example to the detection of tiny amounts of absorption in cavity-ringdown spectroscopy. Usually, cavities have many different transverse modes, which interact differently with the object thus resulting in a completely scrambled image at the output of the cavity[1].

The breakthrough idea of CEMIC is that we have found a novel simple cavity configuration based on a pair of specially shaped mirrors, which preserves the image inside the cavity during an unlimited number of round trips and allows the resulting intensity distribution (image of the full complex refractive index) to be transferred to a camera without requiring any additional computation.

The spatially dependent absorptivity (A) of the object will result in a reduction in the light detected on the camera by a factor of $(1-A)^n$, where n is the number of round-trips the light does in the cavity, which is roughly the finesse of the cavity, which for state of the art mirrors[5] can reach 10^5.

The spatial dependence of the refractive index leads to a spatially dependent shift of the cavity resonance. For a given distribution this will result in bright contour lines of constant optical delay. The resolution power in terms of optical delay is again the finesse of the cavity, i.e. spatial variations in the refractive index or sample thickness of the order of 10^{-5} can be resolved with CEMIC using state of the art mirrors [3]. By scanning either the cavity length or the wavelength of the light, we can then move these lines resulting in a full refractive index map.

CEMIC does so using only two reflective surfaces and no internal optics (lenses) as opposed to [2] where the light interacts with 18 surfaces per round trip. CEMIC is the first practical optical microscopy method capable of very high resolution simultaneously in space, absorptivity, and refractive index.

[1] This is one reason why e.g. high-finesse Fizeau interferometry cannot be used for high resolution imaging.

Figure 2 shows a proof-of-principle demonstration of CEMIC. It shows a low-resolution image of a step of 100 nm optical thickness. These represent the very first images of a refractive index measurement inside a stable optical cavity. These images were taken using a free-space cavity (finesse of $f = 10$) with the samples being placed in its center (Figure 2).

4. PROJECT RESULTS

The key result is that we have demonstrated the feasibility of the basic ideas of CEMIC and have constructed a technology demonstrator. Unfortunately, due to patent restrictions much of the details cannot be included in this public document prior to the final filing of the patent.

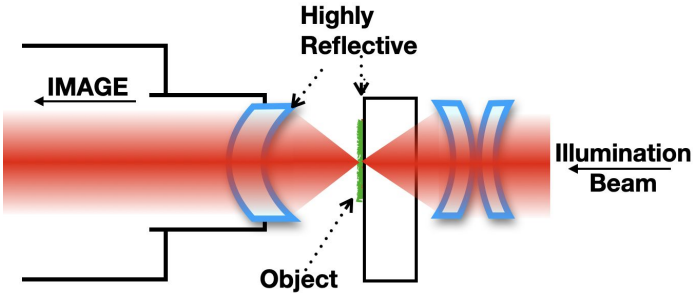

Fig. 1. A sketch of the cavity imaging design. The light enters from one side into the cavity, within which the picture builds up in up to 10^3 round-trips, just amplifying both the refractive index and absorption patterns. The image is then transferred into a standard microscope setup. The wavelength of the illuminating laser light is tuned in order to tune the sensitivity of the setup to different optical depths.

A careful analysis of the imaging capability of a CEMIC was carried out. The resulting limitations are close to the one of a standard optical microscope, albeit with reduced numerical aperture. The prototype apparatus was designed for a resolution of 4-6 micrometers. The main challenge is to maintain the high degree of resolution power inside the cavity without being reduced due to the multiple passes through the effective imaging system.

In the future, spherical errors will need to be compensated by a more complex mirror shape. Special care was given to the structure of the modes inside the cavity. The interference between the individual modes is what gives rise to the enhancement factor of absorption in the CEMIC cavity. We have demonstrated that the enhancement can approach the finesse of the cavity, which can be as high as 10^5 for commercially available mirrors. In our initial test system, we chose an enhancement of only a factor of 100, which is large

enough to demonstrate the principles, but small enough to obtain an undistorted image.
Fig. 1 shows the basic layout of the cavity-enhanced microscope. The sample object is placed onto a highly reflecting substrate and placed within the cavity. It is then illuminated from behind using a coherent light source. The light then cycles inside the imaging cavity. In every roundtrip part of the light exits the cavity into a standard microscope setup. The light from different numbers of round-trips interferes to form a preliminary image, which is then detected on a CCD camera. After some numerical processing, both the absorption and refractive index is determined separately for every pixel. Figure 2 shows one such image, with the left side of the image containing a bio film with the stripe on the right hand side being an artificial height and absorption marker. The color-scale indicates the degree of enhancement of the absorption in AU.
We have clearly demonstrated an enhancement of the absorption by a factor of about two orders of magnitude, which was limited by the round-trip losses of the optics and can be increased by at least one order of magnitude by using improved coatings. The resolution in optical height is ten nanometer without any post-processing. A digital image deconvolution and multiview fusion of the absorption and phase images further enhances the optical microscopy images [4].

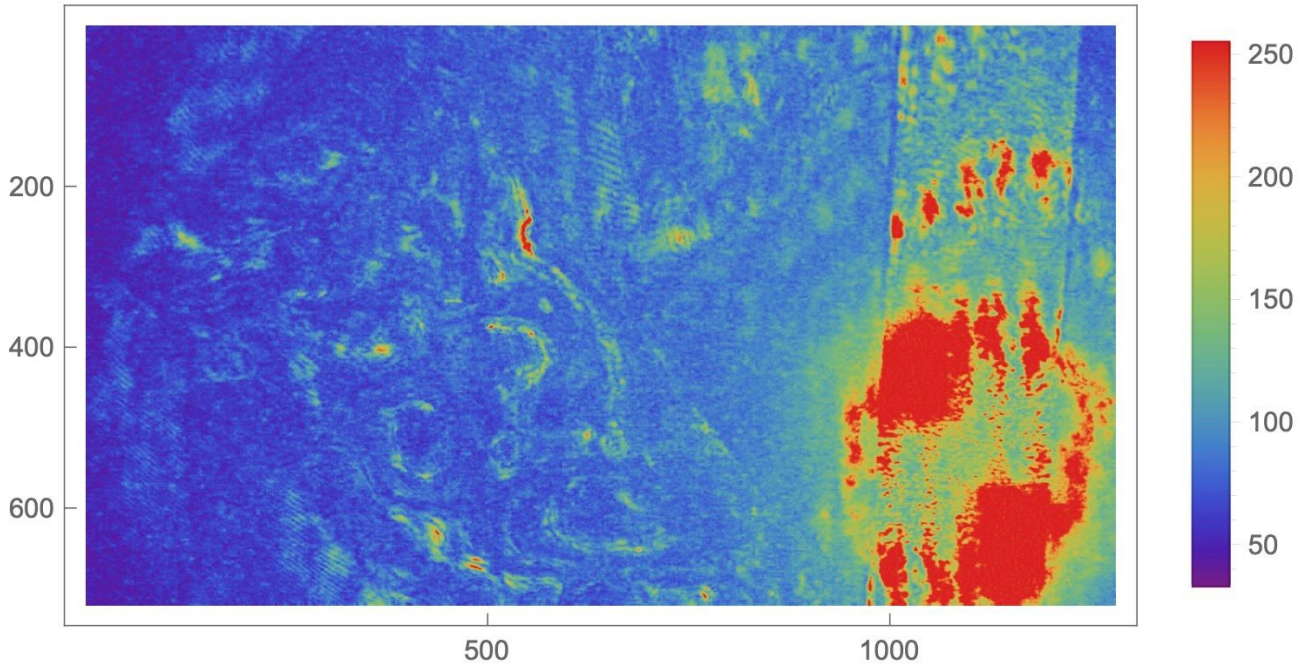

Fig. 2. A false color image of a biofilm. The strip visible on the right side of the image is a thickness marker. The resolution in the vertical direction is about 100 nm.

5. FUTURE PROJECT VISION

The innovation potential for CEMIC lies in that it combines very large sensitivity to extremely small changes in refractive index or absorption at high spatial resolution. Having demonstrated the potential of CEMIC we will apply it to four different domains: Biology, Medicine, Surface Science, and Quantum Measurements.

In Biology, there is a great need for novel label-free imaging techniques for low-contrast images. We have already initiated a collaboration with Professor Tavernarakis of the Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas (IMBB), who is a microscopy specialist working on the C-Elegans[2] — a transparent worm which is exceedingly well studied and thus a perfect in-vivo model-system for CEMIC.
Similarly in **Medicine**, there are many cases, where staining is impractical or impossible. An obvious example is the human eye, where subtle variations in the refractive index of the lens can be precursors of a cataract [2]. Larger refractive index changes at lower resolution are already used in cancer detection, as cell morphology and growth in cell biology, malaria and

[2] Caenorhabditis Elegans, also known as Roundworm.

anaemia disease diagnosis in haematology, and cancer cell and circulating tumour cell detection in pathology [1]. The large increase in sensitivity of an imaging device is almost guaranteed to lead to many new discoveries. We are going to explore these, especially with respect to the human eye, with the Laboratory of Vision & Optics of the University of Crete.

In surface science, there are already some very powerful optical techniques to monitor, for example, the growth of (mono) layers on surfaces. By folding the optical path using a prism inside the cavity CEMIC will be able to study extremely minute changes in total internal reflection and thus be able to *image* growth at the level of single atomic layers. This work will be carried out in collaboration with Prof. Maria Vamvakaki of the Department of Materials Science and Technology of the University of Crete

Trace Gas imaging In combustion science, plant biology, or any gaseous spectroscopy such as localized gaseous emissions from plants will profit enormously from the ability to detect trace-gasses at much lower concentration with good spatial resolution even for gasses which do not exhibit fluorescence. An important example is the imaging the detection of gaseous hormone-like substances of plants (ethylene) and insects.

Finally, in the fundamental physics, **Quantum-Non-Demolition Imaging** and Heisenberg-limited quantum-state creation crucially depend on the light-matter interaction. We have recently demonstrated a 100 fold decrease of atom-number noise approaching the atom-shot noise (Heisenberg limit) using polarization rotation detection [7]. Using CEMIC this technique would for the first time allow the Heisenberg limited *imaging* of Bose-Einstein Condensates. This work will be carried out in the Quantum Technology group of Wolf von Klitzing at IESL-FORTH.

5.1. Technology Scaling

CEMIC will reach TRL3 at the end of this project with a high level TRL2 having been achieved earlier this summer. We have observed the basic principles and formulated, designed and tested the basic concepts. We are now in the process of constructing a demonstrator, where we can test the technology on relevant samples first in a laboratory setting (TRL5).

From this point on the main task will be to design CEMICs for specific applications in a relevant environment. For example, for the **biology laboratories**, we will design (and are in the process of doing so) CEMIC optics, which can be mounted directly into the ZEISS microscopes used there (TRL6). The **quantum information** experiments will require a more advanced design compatible with XHV/UHV conditions. The system already implemented is compatible with trace gas imaging under atmospheric pressure.

If selected for a second phase, we will design and test a CEMIC for use in biology research labs and test it there. Further work in a clinical/medical setting is being negotiated. A spin-off company will be started with the aim of working on a commercial product commencing in year 3 (assuming sufficient risk capital can be attracted in time)

5.2. Project Synergies and Outreach

The project is **extremely synergetic** by definition. It will take advantage of the presence at the Cretan research site at Heraklion of state of the art facilities in all relevant areas: Biology, Medicine, Surface Science, and Quantum Physics (both Quantum Optics and Quantum Matter Waves). Only the close proximity of these research facilities and already existing collaborations make a project of such diversity possible.

Outreach is an important part of the DNA of IESL-FORTH and the University of Crete. Open-Lab days and researcher-nights combined with frequent visits to local schools have created a great awareness, appreciation and support in the local community for the science in the local community. We plan to demonstrate cavity enhanced imaging at the next public outreach event at FORTH.

5.3. Technology application and demonstration cases

Health: One of the main applications of CEMIC lies in the in vivo imaging of live cells by eliminating the need for fluorescence markers, which in many cases complicate the interpretation of results or make imaging of live cells impossible. The impact on any cell research is evident. Areas to impact immediately are eye diseases [2] and cancer research [1].

Biology (trace gas microscopy): Plant and insect hormones are becoming ever more important factors in agriculture as the pressure to produce more food with less resources increases. Our ability to understand the microscopic origins and interactions of these substances is a crucial factor in this. **Biology (label free microscopy):** one of the most exciting aspects of the follow-on project will be the application of CEMIC to the microscopy of minute spatial changes in absorption and refractive index—thus enabling the label-free microscopy of the inner workings of living cells with obvious impacts in biology and medicine. The demonstration case will be performed on C-Elegans

worms in collaboration with Prof Tavernarakis of IMBB.

Quantum-Measurements: One of the exciting aspects of CEMIC is its ability to enhance the cross-section of extremely faint objects, even down to single scattering events, and still detect the resulting shifts in optical phase. This will give us the ability to create a spatially resolved quantum non-demolition images of macroscopic quantum objects. Applications will include on the fundamental side a better understanding of the decoherence of macroscopic quantum states and new applications to quantum computation on the applied side as well as improved detection and state preparation for matterwave interferometers and atomic quantum sensors (e.g. for gravimetry)

The **impact on the Research Infrastructure Communities** in Europe and particularly in Greece is another important factor. Most of the research will take place in a synergetic fashion in research centres on Crete. IESL, IMBB, and the University of Crete are undisputed leaders in their fields in Greece and major players in Europe. Competitive grants, like this one, have allowed the creation of this Research Infrastructure Community and are crucial in fostering real economic activity emanating from this research results.

5.4. Technology commercialization

The direct application of CEMIC to biological research and medical diagnostics lend themselves directly to commercialisation. The Quantum (Imaging) Technology market, for example, is growing exponentially and the medical imaging market alone amounts to more than twenty billion Euro per year. Towards the second year of the eventual grant we plan to found a start-up company. We will rely heavily on our technology park, where FORTH operates a successful business incubator (Step-C) in collaboration with spin-off consultants (PRAXI) and an EU co-funded investment scheme. Crucial to this will be the patents currently under preparation.

5.5. Envisioned risks

The viability of the principles of CEMIC has been demonstrated. Its application to biological samples seems straight-forward. Some risks remain:

Complexity of design, making implementation difficult *likelihood:* small as shown in the present initial studies, *impact:* medium -- delays, *mitigation:* increased design efforts in collaboration with the University of Crete (Prof. Papazoglou).

Large parasitic scattering in some classes of biological samples *Likelihood:* moderate, *impact:* medium, *mitigation:* more targeted applications and refocusing on more transparent samples.

Slow Market Uptake would harm the commercialization *likelihood*: low, *impact:* medium, *mitigation*: additional initial research funding, diversification of portfolio (e.g. scientific equipment and quantum detection).

5.6. Liaison with Student Teams and Socio-Economic Study

CEMIC appointed an officer in charge of liaison with the MSc Level student teams. Some initial ideas about the societal impact of CEMIC were discussed and the special situation of a research infrastructure located geographically at the fringes of the EU was explored. Measure for increasing the human resources of the research base and the reduction and reversal of brain drain were explored. CEMIC is keen on taking part in any future exploration of such socio-economic dynamics.

6. ACKNOWLEDGEMENT

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