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REAP: revealing drug tolerant persister cells in cancer using contrast enhanced optical coherence and photoacoustic tomography

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Abstract

Despite chemotherapy, residual tumors often rely on so-called drug tolerant persister (DTP) cells, which evade treatment to give rise to therapy-resistant relapse and refractory disease. Detection of residual tumor cells proves to be challenging because of the rarity and heterogeneity of DTP cells. In the framework of a H2020 project, REAP will gather researchers and engineers from six countries, who will combine their expertise in biology, chemistry, oncology, material sciences, photonics, and electrical and biomedical engineering in the hope of revealing DTPs in cancer using contrast enhanced multimodal optical imaging. Laser sources for photoacoustic microscopy, photoacoustic tomography, and optical coherence tomography will be developed to enable the design of a two-photon laser scanning optical coherence photoacoustic microscopy system and an optical coherence photoacoustic tomography system. Furthermore, novel photoacoustic detectors using micro-ring resonator will be designed and fabricated, granting improved sensitivity and easier integration of multiple optical imaging modalities into a single system. Innovative algorithms will be developed to reconstruct and analyze the images quickly and automatically. With successful implementation of this four-year project, we can not only gain insight into the mechanisms governing DTPs, but also significantly advance the technology readiness level of contrast agents, lasers, sensors, and image analysis software through joint efforts.

1. Introduction

REAP is a newly funded research project under the call of H2020-ICT36-2020 Disruptive Photonics Technologies. The overall objective of REAP is to reveal drug tolerant persister (DTP) cells in breast cancer both *in vitro* and *in vivo* by contrast enhanced multimodal optical imaging of relevant preclinical models. To

Table 1. Abbreviations of technical terms.

Abbreviation	Full name
DTP	Drug tolerant persister
2PLS-OC-PAM	Two-photon laser scanning optical coherence photoacoustic microscopy
OC-PAT	Optical coherence photoacoustic tomography
PAI	Photoacoustic imaging
PAM	Photoacoustic microscopy
OPO	Optic parametric oscillator
PAT	Photoacoustic tomography
OCT	Optical coherence tomography
SESAM	Semiconductor saturable absorber mirror
MRR	Micro-ring resonator
NEP	Noise equivalent pressure

Table 2. Abbreviations of names of REAP partners.

Abbreviation	Full name
MUW	Medical University of Vienna
AIT	AIT Austrian Institute of Technology GmbH
USC	Universidade de Santiago de Compostela
PICO	Picophotonics Oy
TAU	Tampere University
PoliTO	Politecnico di Torino
IN	InnoLas Laser GmbH
LVBT	LaVision BioTec GmbH, a Miltenyi Biotec Company
LXI	LioniX International BV

accomplish this goal, nine partners from six countries will join forces. The Medical University of Vienna (MUW) will coordinate a consortium consisting of the AIT Austrian Institute of Technology GmbH (AIT), the Universidade de Santiago de Compostela (USC), Picophotonics Oy (PICO), Tampere University (TAU), Politecnico di Torino (PoliTO), InnoLas Laser GmbH (IN), LaVision BioTec GmbH (LVBT), and LioniX International BV (LXI). Officially starting on 1 January 2021, the project will last four years. Four universities, a research institute, and four industrial partners will bring complementary expertise in multiple fields together to push forward respective photonics technologies. Two tables of abbreviations are given below for all technical terms (table 1) and the partner names (table 2), respectively.

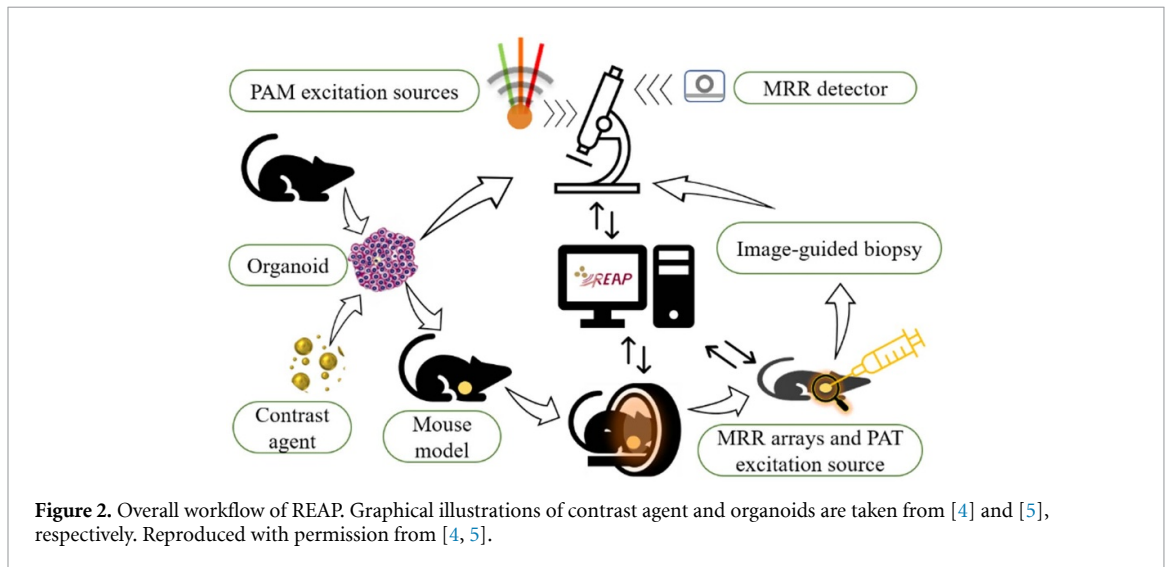
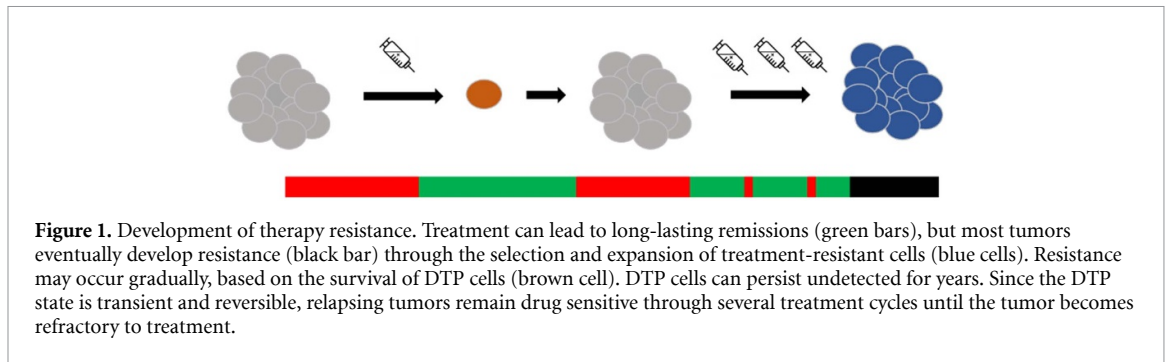
1.1. Motivation

According to WHO, breast cancer is the most common cancer in women both in developed and developing countries. From the WHO Cancer Regional Profile 2020, the incidence rate of breast cancer is leading all other cancers in European countries at around 12.3% [1]. Despite the increasing awareness of this disease and the efforts in early diagnosis and screening, it is still the second leading cause of cancer related death in women. Breast cancer is a heterogeneous disease, and various treatment strategies including endocrine therapy, anti-HER2 therapy, chemotherapy and immunotherapy exist according to molecular subtype. However, many patients develop therapy resistance over time. While early-stage, non-metastatic disease is curable in ~70%–80% of patients, advanced breast cancer is considered incurable with currently available therapies [2].

Therapy resistance may occur either through the selection of preexisting cancer cell populations or through the acquisition of mutations during therapy. However, in several instances, relapsing tumors remain sensitive and continue to respond to the initial therapy (figure 1). This may occur over and over again, until mechanisms causing stable drug resistance emerge and the tumor becomes refractory to treatment [3]. In these cases, the cellular origin of relapse is a critical reservoir of DTP cells. Thus far, no available methods can detect the tiny population of DTP cells effectively, making the investigation of these cells extremely challenging for cancer researchers. REAP proposes a complete imaging toolbox using multimodal two-photon laser scanning optical coherence photoacoustic microscopy (2PLS-OC-PAM) and optical coherence photoacoustic tomography (OC-PAT) to reveal the DTP cells in a preclinical setting at multiple scales.

1.2. Concept

The overall strategy of REAP is shown in figure 2. The major technological development in REAP involves contrast agent development, laser development, detector development, and system integration as well as



automatic image analysis software development. Breast cancer organoids that are considered as *in vitro* 3D tumor correlates will be used in combination with chemotherapy for disease and DTP modelling. Contrast agents will be developed and engineered along with genetic engineering of organoids to target the cancer cells. The contrast agent labeled organoids will be directly studied *in vitro* using the 2PLS-OC-PAM system. Upon transplantation into mice, organoid derived tumors and their therapy-induced changes will be imaged *in vivo* with the OC-PAT systems. Both longitudinal monitoring of tumor development and image-guided biopsy can be performed using the *in vivo* imaging systems, and biopsied tissue can be evaluated with the 2PLS-OC-PAM system.

2. Methodology

REAP will develop three intricately connected multimodal optical imaging systems. Based on extensive experience and expertise, the project will promote significant innovations to bring the next generation of microscopes and tomographic imaging systems. The following subsections detail the innovative aspects in five subdivisions and figure 3 demonstrates the overall technological concept.

2.1. Contrast agents

AIT and USC will take the lead for the contrast agent related tasks. Biofunctionalized nanoparticles will serve as contrast agent to selectively target breast cancer cells. The contrast agent will feature tunable absorption peaks specifically designed to enhance the contrast in photoacoustic imaging (PAI). The contrast agents will also have a great colloidal stability and good targeting abilities to ensure reduced toxicity and proper performance both *in vitro* and *in vivo*.

2.2. Laser technology

A triple wavelength photoacoustic microscopy (PAM) excitation laser will be developed with high repetition rate (100 kHz), high output energy (1 μ J), low cost, and compact size (15 \times 15 \times 5 cm). An optical parametric oscillator (OPO) will be developed for photoacoustic tomography (PAT) excitation with exceptionally high repetition rate (\geq 1 kHz) and energy (\geq 10 mJ). Two dual-purpose lasers will be developed

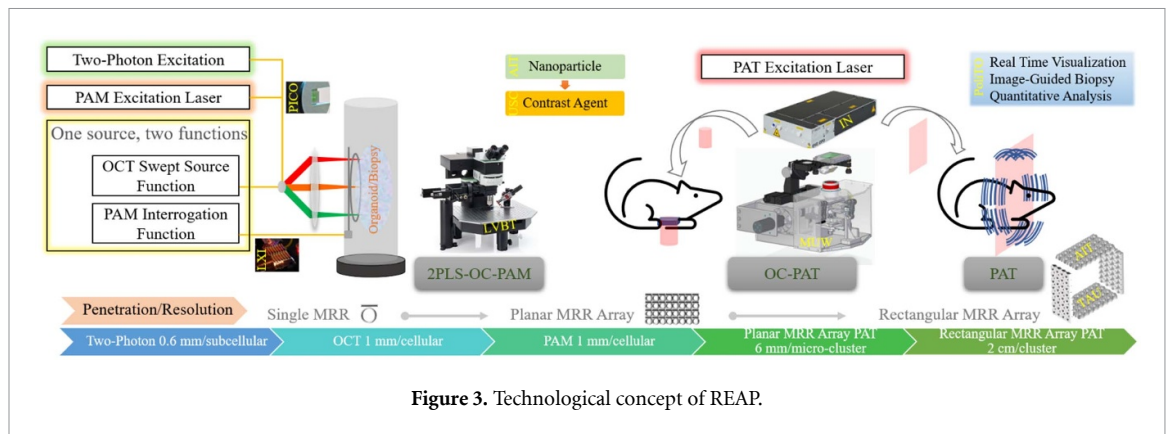


Figure 3. Technological concept of REAP.

for both all-optical detection photoacoustic interrogation and optical coherence tomography (OCT) at 780 and 1310 nm with wide bandwidth, narrow linewidth, and stable phase.

2.2.1. PAM excitation

The PAM excitation source will provide 532, 767, and 1064 nm outputs. The laser system will exploit a semiconductor saturable absorber mirror-based μ -chip laser technology platform and will address the need for high repetition rate pulses with high energies. The μ -chip laser can be processed on wafer-scale and assembled with automated tools, hence enabling large volume and low-cost production. Additionally, based on the nonlinear effect [6, 7], extended excitation wavelength range will be enabled using the individual wavelengths as seed wavelengths. The start-up company PICO will lead the development of the PAM excitation sources.

2.2.2. PAT excitation

IN will lead the development of an OPO featuring 1 kHz repetition rate with the capability to perform pulse to pulse diagnosis of the output. Efficient pumping of the OPO will be achieved by using high pumping energy without exceeding the damage threshold of the barium borate crystals. The output intensity distribution will also be accurately monitored for a more precise PAT reconstruction.

2.2.3. PAI interrogation and OCT

In the realm of all optical detection PAT, especially for the polymer film detector [8, 9], the interrogation speed is one of the bottlenecks limiting imaging the acquisition time. In this project, LXI will lead the development of two dual-purpose lasers featuring unprecedented phase stability, narrow linewidth, as well as fast tuning speed. These two lasers will be used both as the interrogation laser for the photoacoustic pulse detection, and as the swept source for OCT.

2.3. PAI sensor

A micro-ring resonator (MRR) based detector with $\leq 50 \mu\text{m}$ diameter and $\geq 160 \text{ MHz}$ bandwidth will be developed. Together with interrogation lasers mentioned in section 2.2.3, we target at sub-Pa noise equivalent pressure. MRR arrays will also be developed for real time PAT imaging. This proposed MRR will see a significant improvement compared with other PAI detection methods such as a kinetic detection [10, 11]. Another feature worth noting is that the MRR substrate is optically transparent, which permits easy implementation in multimodal optical imaging systems. AIT and TAU will take the lead to design and fabricate these MRR-based detectors.

2.4. Image reconstruction and analysis

Fast image reconstruction [12] and quantification [13, 14] algorithms are always an indispensable part for translational imaging systems. For REAP, PoliTO will develop real time image reconstruction algorithms for all the imaging modalities involved. A needle tracing algorithm will be developed for image-guided biopsy. 3D visualization and quantification algorithms will be developed to perform quantitative analysis of the tumor and the DTP cells. The tasks will be tackled using traditional image analysis and deep learning approaches.

2.5. System integration

The combination of OCT and PAI systems has been explored for more than a decade by various groups [15, 16].

In REAP, LVBT and MUW will lead the development of two novel multimodal imaging systems with superior performance. A horizontal 2PLS-OC-PAM system will be developed with subcellular resolution, deep penetration (≥ 1 mm from one side and ≥ 2 mm with sample rotation), multiple contrast channels (fluorescence contrast for two photon laser scanning microscopy, scattering contrast for OCT, absorption contrast for PAM), and fast imaging speed. A high-resolution OC-PAT system will be developed using planar MRR array for localized tumor imaging and image-guided biopsy. A rectangular-MRR-array-based PAT system will be developed for deep tissue (≥ 2 cm penetration depth) imaging and metastasis screening. The final integrated systems will also include the software developed in section 2.4.

3. Impact

REAP will bring innovations in biomedical engineering, laser technology, photoacoustic detector technology, computer science, and system engineering. With the envisaged advancement in various technologies enabled by REAP, Europe's industrial position in the biophotonics-related market for microscopes and research and development tools will be strengthened. For the industrial partners, the products developed in this project will reach higher technology readiness levels and several of the products will be immediately available for market launch after the project. The project also aims to contribute to standards that have long been missing for multimodal imaging. With the more than 6 million Euro budget, about 30 jobs will be created according to the work plan by all the partners, among them 12 jobs by the industrial partners.

During the project, the consortium will host four workshops in different countries with different themes. These workshops are meant to not only disseminate and communicate the project's outcomes to attendees, but also inspire all stakeholders for more innovative ideas. A LinkedIn page [17] and a website (www.projectreap.eu/) have been set up for this project already with a customized logo symbolizing the project. The communications and dissemination events will be constantly updated in the LinkedIn page and the project webpage.

4. Conclusion

With concerted efforts from academia and industry, REAP gives the possibilities to reveal the underlying mechanisms of treatment resistance development by DTP cells. Along the path of the project, several different types of lasers will be developed, as well as novel contrast agents and PAI sensors, which are by themselves also disruptive advancements in their respective fields.











Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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