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Optical Mammography in the Time Domain up to 1060 nm: From Tests on Healthy Women to Initial Data for Monitoring Neoadjuvant Chemotherapy

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Abstract: We present *in vivo* tests on healthy women through our optical mammograph in preparation for a clinical validation on neoadjuvant chemotherapy monitoring, and we report preliminary data on the first patient enrolled in the study.

1. Introduction

Now-a-days, major efforts are made to optimize and enrich breast cancer diagnostic and monitoring tools, being the most widespread tumor among women [1]. Optical mammography tries to weave its way in this scenario. Its strong suit is the ability to characterize the breast constituent concentrations exploiting a multi-wavelength approach. This makes it suitable also for tracking changes in the constituent distribution and quantity due to different factors: age, metabolism, presence of (benign and malignant) lesions and undergoing therapies [2].

In this work, we focus on the study of physiological effects of chemotherapy on tumor-bearing breasts. In fact, we aim at exploring a possible correlation between response to neoadjuvant chemotherapy and specific variations in the lesion and breast tissue composition by means of our time-resolved multi-wavelength optical mammograph, as already suggested in literature (*e.g.*, [3,4]). The purpose is to monitor and eventually predict the therapy outcome.

With this goal, we are currently carrying out a clinical study at the San Raffaele Hospital, in Milan, by implementing multiple optical measurements at different strategical stages of chemotherapy.

We now present how we prepared the instrument before going to clinics and, as an example of how the study will be carried out, we illustrate some preliminary results about the first patient under neoadjuvant chemotherapy engaged in our clinical trial.

2. Implementation

2.1. Instrument

Following the light path, our optical mammograph emits picosecond pulses at seven wavelengths (635, 680, 785, 905, 933, 1060 nm), that investigate the compressed breast following a raster pattern. Light is collected on the opposite surface by an 8-SiPM (Silicon PhotoMultipliers) detector and, after amplification, the signal is processed by a Time-to-Digital Converter (MultiHarp 150 8N, PicoQuant, Germany). Finally, data are acquired by a PC [5].

2.2. Goal of preparatory and clinical measurements

Before going to clinics, *in vivo* rehearsals have been performed in laboratory as the last step of a thorough validation protocol [5]. They are essential to verify whether the optical mammograph is ready to begin the clinical trial, paying particular attention to the breast intrinsic heterogeneity and inter-subject variability.

We implemented a protocol similar to the one conceived for the clinics: 4 views (cranio-caudal and oblique views of both breasts) replicated on different days. There were 3 main differences with respect to the clinical study:

1. women were healthy (*i.e.* no tumor nor therapies);
2. consequently, we did not search for variations in the breast constituent concentrations, rather we wanted to assess the instrument reproducibility;
3. optical measures were not scheduled in conjunction with specific medical events (*e.g.* breast tumor clipping or specific chemotherapy sessions), but on close days so as to avoid strong physiological variations.

In other words, in clinics we aim at registering the variations induced over time in hemoglobin, water and lipids concentrations by neoadjuvant chemotherapy [3]. Ranging our optical mammograph from 635 to 1060 nm, we are interested also in collagen behavior, as it is involved in tumor onset and may be a target of chemotherapy [6]. In order to appreciate such variations, it is important to previously verify the instrument reproducibility *in vivo*, by performing measurements on healthy women on different days.

3. Data analysis

At this stage of the instrument validation, measurements on both healthy women and the patient are analyzed by applying the homogeneous model of the diffusion approximation of radiative light transport in turbid media, with extrapolated boundary conditions. A spectrally constrained approach allows better outcome stability when fitting multiple wavelengths simultaneously [7]. Mean breast constituent concentrations have been retrieved by averaging over a squared area enclosing a hundred of pixels. In the case of the patient, the square delimits the tumor area. The use of the homogeneous model is restricted to this preliminary evaluation; we plan to apply a perturbative approach to analyze all patient data from the clinical study.

4. Results

4.1. In vivo rehearsals

Clinical trials should be preceded by propaedeutic *in vivo* tests. In light of this good practice, we recently performed laboratory measurements on 3 healthy women, that highlighted the importance of preparatory *in vivo* tests to comply with a clinical environment [5]. The results obtained for the 3 sessions dedicated to healthy volunteer #1 are depicted in Table 1. Reproducibility is assessed in terms of Coefficient of Variability (CV).

Table 1. Mean breast constituent concentrations for healthy volunteer #1. Cranio-caudal right view. Water, lipids and collagen are measured in mg/cm^3, total hemoglobin in μM, oxygen saturation and CV in percentage value.

	Session 1 Day 1	Session 2 Day 3	Session 3 Day 5	CV
Water	308	324	308	3
Lipids	560	536	546	2
Collagen	69	75	76	6
Total hemoglobin	16	15	14	4
Oxygen saturation	69	66	68	2

We can observe that CV values over the three sessions never exceed 6% for any constituents of interest, implying an adequate level of reproducibility for our purposes. In fact, higher variations are expected in the case of significant physiological effects of therapy [8]. Results on other subjects are consistent with these values.

4.2. Initial clinical measurements

The first patient had a 59 mm tumor between the upper quadrants of the right breast, as observed via ultrasound images. The tumor reduced to 35 mm after 3 weeks, during which she underwent 2 chemotherapy sessions, each one immediately after an optical measure. Other chemotherapy sessions and programmed optical measurements took place afterwards. Table 2 resumes data about optical events, where “Day 0” corresponds to the baseline session, when tumor clipping had not been performed yet and neoadjuvant chemotherapy was about to start.

Due to its size, the lesion cannot be ascribed as a small perturbation, thus allowing the use of the homogeneous model for a preliminary evaluation of this specific case.

Table 2. Mean breast constituent concentrations for patient #1. Cranio-caudal right view. Water, lipids and collagen are measured in mg/cm^3, total hemoglobin in μM, oxygen saturation and CV in percentage value.

	Session 1 Day 0	Session 2 Day 8	Session 3 Day 21	Session 4 Day 42	Session 5 Day 81	CV
Water	251	252	258	288	235	8
Lipids	356	528	570	576	589	18
Collagen	165	133	118	87	83	29
Total hemoglobin	21	17	14	10	10	32
Oxygen saturation	98	92	88	55	54	28

First of all, by comparing corresponding CVs in Table 1 (*i.e.* healthy woman) and in Table 2 (*i.e.* patient), we can immediately notice that variations are significantly higher in the case of the patient, meaning that effective neoadjuvant chemotherapy does affect the breast composition. We are able to detect such variations even using the homogenous model, also thanks to the big lesion size.

In fact, we can observe from Table 2 that lipids grow session after session (+65% from first to last session), while total hemoglobin content, oxygen saturation and collagen reduce over time (-51%, -45%, -50% respectively). On the contrary, water does not show a well-defined behavior.

These trends are consistent with previous findings of other groups: the fibrous tumor tissue decreases, in favor of a more adipose tissue [3,8]. In literature, this has been interpreted as a potential positive response to neoadjuvant chemotherapy. Such hypothesis seems supported by the marked reduction in the lesion size. Moreover, we can deduce that collagen might be a significant biomarker in neoadjuvant chemotherapy monitoring.

These are only preliminary results. Further future evaluations of the first patient response and the contribution of other treated patients that are presently being monitored by optical means will help us to derive stronger considerations.

5. Conclusion

In conclusion, preparatory measurements in laboratory on healthy women were helpful to establish our optical mammograph readiness to the clinical trial on neoadjuvant chemotherapy monitoring and prediction of therapy outcome, currently ongoing at the San Raffaele Hospital.

Measures on the first patient seem to suggest a possible correlation between therapy response and specific variations in the breast composition. In particular, in this first case, collagen seem to play a non-trivial role in tracking the therapy effects on the tumor composition. However, further confirmations from measurements on the next patients are required to better understand the potential role of optical measurements.

6. Acknowledgements

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