SOLUS | SMART OPTICAL AND ULTRASOUND DIAGNOSTICS OF BREAST CANCER

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Deliverable 4.2: Definition of protocols for system characterization

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Abbreviations

BIP	Basic Instrument Performance
CNR	Contrast to Noise Ratio
CV	Coefficient of Variation
DO	Diffuse Optics
DOT	Diffuse Optical Tomography
DTOF	Distribution of Times of Flight
fNIRS	functional Near-Infrared Spectroscopy
FWHM	Full-Width at Half Maximum
IEC	International Electrotechnical Commission
IRF	Instrument Response Function
ISO	International Organization for Standardization
TD	Time-Domain
US	Ultrasound

1. Introduction

The aim of this deliverable is the definition of a Protocol for performance assessment of DOT and combined DOT-US systems. The formulation of standardized protocols is a key issue to be addressed in order to ensure the possibility to compare and evaluate the performance of a system in a measurable way, thanks to the definition of the relevant figures of merit, which are related to the main requirements of the instrument final application. Additionally, such figures of merit provide a common basis for comparing different systems to evaluate the effect of the different technologies employed, of the different measurement techniques and of the different data analysis methods. Similarly, they permit to quantify the effect of technical interventions or upgrades on a system. Finally, they guarantee the consistency of data taken during clinical validation campaigns or large clinical trials, which is of the utmost importance to ensure day by day the reliable operation of the instrument.

In the field of diffuse optics, some standard protocols have already been developed and widely adopted, reaching a broad consensus at international level. These are: *i)* BIP: for the characterization of an instrument at a basic level by measuring its main intrinsic features without any measurement medium; *ii)* MEDPHOT: for the characterization of the instrument performance in recovering optical properties of homogeneous scattering media; *iii)* NEUROPT: for assessing the instrument capability in measuring localized changes in absorption. All these existing Protocols are highly relevant within SOLUS being suitable to evaluate performances of components, sub-systems and of the whole optode before the final performance assessment of the SOLUS system. Therefore, they will be applied to achieve the goals of the Deliverable D4.5 (Performances assessment of optode components, M24) and of the Deliverable D4.6 (Performance assessment of the single optode, M30). This process also ensures standardized procedures to verify the achievement of Milestone MS5 (Smart optode validated in laboratory, M30).

However, since existing protocols are not specified for tomography, within SOLUS there is also the need to make an additional step in this standardization process, which, together with previously developed protocols, in the longer run could lead to the definition of new International Industrial Standards (ISO/IEC).

This document therefore proposes the main figures of merit that have been identified as relevant within SOLUS. These have been defined to address three specific problems that are significant when dealing with tomography: *i)* the ***sensitivity*** (i.e. the capability to detect small optical perturbations buried inside a diffusive medium); *ii)* the ***localization*** (i.e. the capability to correctly retrieve the position and dimension of a perturbation inside a diffusive medium); *iii)* the ***quantification*** (i.e. the capability to quantify the optical properties of a localized perturbation inside a diffusive medium).

The practical Implementation of the Protocol depends on the specific purposes of the different tasks where it will be applied and on the composition of the phantom kit it will be delivered at M18 (D4.4 Provision of the multi-modal phantom kit). Therefore, measurements conditions can not be specified here (e.g. number of measurements, integration time, number of phantoms, etc). Still, this document already provides a relevant support for the project by allowing the evaluation of: performance of simulated tomographic reconstructions (WP2) and of preliminary phantom measurements (WP4) by standardizing the interpretation of the simulation/measurement outcome. These simulations and preliminary measurements can then support the optode development (WP1) and the design of the SOLUS prototype (WP3). Therefore, we follow here the same methodology used in the proposition of the BIP, MEDPHOT, and NEUROPT Protocols, where the definition of the problem, the assumptions and the relevant figures of merit (Protocol) were separated from the specifications of the practical experimental conditions needed to perform the test as well as of the phantom in use (Implementation). This approach grants higher flexibility in applying the Protocols to diverse clinical problems.

This report is organized as follows: Section 2 reports an overview of the Protocols presently available for the standardized characterization of diffuse optics systems; Section 3 introduces the proposed new Protocol for the characterization of both pure DOT or combined DOT-US systems; finally, conclusions are discussed in Section 4.

2. Overview of Protocols for Diffuse Optics

This section provides an overview of the 3 protocols defined in the last decade for the performance assessment of diffuse optics instruments. They have been elaborated thanks to international collaborations between different institutions, in particular at the European level in the framework of previous EU projects.

BIP

The Basic Instrument Performance (BIP) Protocol has been proposed to test the main hardware specifications of Time-Domain (TD) Diffuse Optics (DO), which could have a great impact on the outcome of a measurement [1]. It was defined as a joint effort by 7 institutions and preliminary tested on 8 DO instruments. The BIP Protocol focuses the attention on 3 instrument parts: *i)* the laser source; *ii)* the whole detection chain; *iii)* the whole system without measuring media. For each part, different measurables are considered:

- LASER SOURCE
  - *Average power available at the laser output*
  - *Average power available at the sample* (i.e. after passing through lenses, fibers, etc.)
  - *Injection area at the sample*
  - *Central wavelength and bandwidth*
- DETECTION CHAIN
  - *Responsivity* (i.e. the overall collection efficiency of diffused - Lambertian - light)
  - *Differential nonlinearity*
- ENTIRE SYSTEM
  - *Instrument Response Function (IRF) Full-Width at Half Maximum (FWHM) and shape*
  - *Afterpulsing ratio* (i.e. a signal-dependent background noise contribution)
  - *IRF stability over time*

The main focus of the BIP protocol was on optical brain imagers, but the same set of tests can be applied also to other TD instruments. Indeed, even if not dealing with the problem of tomography, this Protocol is relevant also within SOLUS, being suitable to evaluate the basic components, sub-systems and optode (i.e. a fully operative TD DO system) performances in reliable comparison with previously developed and tested bulkier instruments (i.e. the present state of the art).

MEDPHOT

The MEDPHOT Protocol aims at evaluating the performances of an instrument in recovering optical properties of homogeneous scattering media [2]. It was defined as a joint effort by 5 institutions and preliminary tested on 8 DO instruments. In this case the instrument is considered just a black box (instrument + analysis of TD waveform to retrieve optical properties) providing just 2 numbers, which are the main measurables: the absorption coefficient and the reduced scattering coefficient. Over these numbers, five assays are foreseen to estimate:

- *Accuracy*, i.e. difference between retrieved and expected optical properties. The proposed figure of merit to evaluate this property is the relative error.
- *Linearity*, i.e. deviation of the measurand from the linear behaviour when increasing absorption or reduced scattering coefficients of the sample under investigation.
- *Noise*, i.e. the uncertainty over the measured values of optical properties. The proposed figure of merit to evaluate this property is the Coefficient of Variation (CV), which is the ratio between the standard deviation of different repeated measurements and its average value.
- *Stability*, i.e. short and long-term drift/instabilities of the measured values of optical properties.

- *Reproducibility*, i.e. consistency of measurements repeated in different days. The proposed figure of merit to evaluate this property is again the CV.

A kit composed by 32 solid phantoms combining 8 absorption values with 4 scattering values was fabricated for the Implementation of this Protocol [2]. This kit has been circulated and requested many times all over the world.

As the BIP Protocol, the MEDPHOT Protocol is relevant within SOLUS, being suitable to evaluate the single optode performance in recovering optical properties of homogeneous scattering media, to compare such miniaturized TD system with many other previously developed and tested bulkier instruments.

NEUROPT

The NEUROPT Protocol was devised to assess the performance of TD brain imagers in detecting, localizing and quantifying spatially-confined absorption changes, like the ones occurring in the brain during functional activations [3]. It was defined as a joint effort by 6 institutions and preliminary tested on 8 DO instruments. Again (as in MEDPHOT), the instrument is considered just a black box, providing in this case time-resolved waveforms (not optical properties). Six tests are foreseen, in order to address three main issues:

- *Sensitivity*, i.e. capability to detect a small localized absorption perturbation in the diffusive medium, with the following figures of merit:
    - Contrast, i.e. relative effect of the perturbation on the number of counts within a given time window on the time-resolved waveform;
    - Contrast to Noise Ratio (CNR), i.e. the ratio between the variation of the number of counts produced by the perturbation within the time window on the time-resolved waveform and the intrinsic fluctuation of the number of counts (due to e.g. Poisson noise or other instabilities);
- *Spatial Resolution*, i.e. capability to localize the absorption perturbation in the diffusive medium, with the following figures of merit:
    - Depth selectivity, i.e. the capability to distinguish between absorption changes occurring in different layers of the head. It is quantified by means of the ratio between the variation of the number of counts in the time-window due to a small absorption change in the lower layer and the same variation due to the same change in the upper compartment;
    - Lateral spatial resolution, i.e. the FWHM of the spatial point spread function produced by laterally moving a point-like absorption perturbation in the diffusive medium;
- *Quantification*, i.e. absolute and relative quantification of localized absorption changes in the diffusive medium, with the following figures of merit:
    - Accuracy, i.e. the relative error in the quantification of the absolute value of absorption perturbations with respect to the nominal values;
    - Linearity, i.e. the deviation from the linear behaviour in the retrieved value of the absorption perturbation when increasing the nominal value of such absorption perturbation.

The first phantom proposed for the Implementation of this Protocol was a hybrid solid-liquid phantom [4]. Afterwards, a new, more practical and versatile solid-solid phantom was developed, based on switchable perturbations [5].

As the BIP and the MEDPHOT Protocols, the NEUROPT Protocol will be applied within SOLUS to evaluate the single optode performance in detecting, localizing and quantifying spatially-confined absorption changes, to compare the new device with many other previously developed and tested state-of-the-art instruments.

3. The SOLUS Protocol

INTRODUCTION

The SOLUS Protocol is meant to characterize the performances of generic DOT systems, combined US-DOT systems or even DOT systems combined with other techniques that can provide morphological information like magnetic resonance imaging systems. Within the SOLUS project, the Protocol will be applied for the validation in laboratory settings of the SOLUS prototype, in particular addressing the case of a DOT system combined with US, which provides the morphological information.

A substantial work was taken in the initial phase of SOLUS to translate the clinical problem into a physical paradigm. A great role was played here by clinical partners in transferring their clinical expertise on practical cases and also in translating the clinical needs into measurable quantities. This activity led to the Deliverable D2.1 (Definition of paradigms representing exemplary breast lesions cases), where the geometrical paradigm was identified in lesions with a round shape of the inclusion as a first approximation, with a minimum diameter of 1 cm. For the minimum dimension, the centre of the inclusion could be located at a typical depth of 1.0 ± 0.5 cm, while for bigger sizes the top of the inclusion could be located at a typical depth of 1.5 ± 0.5 cm. An overview of optical properties for both lesion and surrounding tissues was reported in D2.1. Hence, the targeted paradigm is a heterogeneous problem, similarly to the case addressed by the NEUROPT Protocol. It is therefore possible to draw inspiration from there, but taking into account the main differences between the brain activation paradigm and the breast lesion paradigm.

A first difference is that the brain paradigm is often managed as a 2-layer or multilayer problem: the head is modelled as a layer of scalp, skull and cerebrospinal fluid overlaying the grey matter, where the activation has to be detected. Hence, the NEUROPT Protocol was conceived to quantify the contrast in the inner layer. Therefore, it was not devised to deal with the performance assessment of tomographic reconstructions. Vice versa, within SOLUS, the breast nodule problem is approached by DOT.

A second difference is represented by the broadband wavelength range that is needed for retrieving the relative concentrations of the main tissue constituents. Indeed, as specified in Deliverable D1.1 (Specifications of smart optode components), each smart optode will embed laser dices with wavelengths between 600 and 1100 nm. Vice versa, functional Near-Infrared Spectroscopy measurements (fNIRS) on the brain usually rely on the use of two wavelengths, often chosen on opposite sides of the isosbestic point of hemoglobins (i.e. the wavelength at which the specific absorption of the oxygenated haemoglobin is equal to the one of the deoxygenated haemoglobin, around 790 nm). These two wavelengths could be therefore separated by e.g. 100 nm. Absorption in this spectral region is relatively flat, therefore the optical properties of the tissue around the perturbation (i.e. the region where the activation takes places) are supposed to be similar when the tissue is probed with these two wavelengths. Due to the much broader range required in SOLUS, there is the need to take into account the strong variation in the optical properties (both in absorption and reduced scattering coefficients) of the tissue surrounding the nodule. As an example, the specific absorption of water changes by two orders of magnitude in the range between 600 and 1100 nm, thus strongly affecting the amount of signal detected by each optode depending on the wavelength employed, with the main consequence to reduce the sensitivity of optical investigations performed at wavelengths where the absorption is higher.

The third main difference is represented by the nature of the perturbation. Indeed, using fNIRS, the brain activation can be reliably modelled as a change in the absorption spectrum in the region where the activation takes place, due to a change in the concentrations of oxy- and deoxy-haemoglobin, while the microstructure is not altered (thus not affecting the reduced scattering coefficient). Vice versa, the nature of breast lesions can be different, with also different microstructures. As an example, a cyst usually features a reduced scattering coefficient lower than the surrounding tissues. Hence, while the NEUROPT Protocol was meant for considering only absorption perturbations, the Implementation of the SOLUS Protocol should ensure a proper consideration also for the scattering ones, whose value is therefore relevant both to improve the quantification of the absorption coefficient and for lesion diagnostic purposes.

A forth difference is the relative change in optical properties over time in the brain activated area as compared to the initial resting state. In SOLUS the problem is substantially more difficult due to the lack of a reference unperturbed state. This is a major challenge which is being addressed in WP2 with the design of proper reconstruction strategies.

As in the NEUROPT Protocol, SOLUS relies on measurements performed in reflectance geometry (i.e. with sources and detectors on the same side of the tissue), since both sources and detectors for DOT are integrated in the same multimodal probe as the US transducer array. However, the Protocol can be easily extended also to the transmittance geometry.

PROTOCOL

As in the NEUROPT Protocol, to address the problem of breast lesions, we identified three main tests to be performed on the measurands: *1)* *Sensitivity* (to evaluate the capability of the system to detect a small perturbation embedded inside a diffusive medium), *2)* *Localization* (to measure the performance of the system in retrieving the correct position and size of the perturbation), and *3)* *Quantification* (to assess the absolute and relative quantification capability of the optical properties of the lesion).

Depending on the test considered, these tests are performed on: *1)* DTOF curves (*Sensitivity*); *2)* 3D maps of optical properties (*Localization*), and *3)* absorption coefficient and the reduced scattering coefficient inside the lesion (*Quantification*). Indeed, dealing with DOT, the output of a measurement is in general the 3D map of absorption and reduced scattering coefficients ($\mu_a(x,y,z)$ and $\mu_s'(x,y,z)$, respectively). Thus, the system (instrument + data analysis) will provide a couple of numbers for each voxel of the reconstruction mesh. However, to fully investigate the diagnostic potential of SOLUS (Task 5.5), the knowledge of the average lesion composition is certainly needed. Thus, for the *Quantification* test, further elaboration is required (e.g., the integral of the optical properties over a given volume). Being an additional processing of data that can be performed with different criteria, this step should pertain to the data analysis process instead of to the Protocol, thus leaving to the characterization Protocol only the role of computation of some basic figures of merit.

1) *Sensitivity*

Within SOLUS, the problem of sensitivity to small objects is shared between US and DOT. Indeed, the perturbations should be visible to US to extract the geometry information to improve the performance of DOT, but also DOT should be sensitive to the perturbation to be able to provide its optical properties. Therefore, the problem of sensitivity can be faced as in the NEUROPT Protocol with the two following figures of merit: *1a*) *Contrast* (C) and *1b*) *Contrast-to-Noise Ratio* (CNR).

A first effect of the presence of an optical perturbation inside a diffusive medium is a change in the number of photon counts N_P at the detector with respect to the homogeneous case (N_0, i.e. when the perturbation is not present). N_P and N_0 can be computed either by integrating the number of counts of the whole distribution of times of flight (DTOF) or (more typically) integrating the number of counts in different time-windows on the DTOF, thus highlighting the relation between the average photon pathlengths (encoded in the photon arrival delay at the detector) and the depth of the perturbation, which is one of the main advantages of the TD approach to DOT. The *Contrast* (*C*) can be calculated as:

$$C = \frac{N_P - N_0}{N_0}$$

where *C* is defined as a relative change in the number of counts.

It is worth noting that here the aim is to define the main figures of merit related to the new Protocol, without detailing the Implementation, as already discussed above, since the latter will depend on the kind of phantom kit it will be developed during the project. However, it is useful to already foresee the main

conditions affecting such figures of merit. Apart from the intrinsic characteristics of the system (e.g. IRF shape, afterpulsing, etc), the level of *Contrast* produced by a localized optical perturbation can strongly depend on many analysis, geometrical and optical factors. Among the analysis factors, for example, *C* can depend on the temporal position (t_w) of the time window on the DTOF curve used to compute N_P, as well as on its temporal width (Δt). The geometrical factors that can affect *C* are, for example, the source-detector separation (ρ), the depth (z_P) and volume (V_P) of the perturbation, and its lateral displacement (x_P, y_P) with respect to the centre between the source and the detector used to collect the DTOF. Finally, optical factors that can impact on the value of *C* are the optical properties of the bulk medium where the perturbation is placed (μ_a^{Bulk}, $\mu_s'^{Bulk}$) and the amount of the perturbation with respect to the bulk properties ($\Delta\mu_a$, $\Delta\mu_s'$). It is already clear that the investigation of all these dependences for *C* and also for the following figures of merit represents an excessive overburden. As the main purpose of the Protocol is the standardization of procedures for performance assessment to allow reliable comparison among different executions of the characterization Protocol (on different instruments, or after upgrades/modifications of the system, or on different methods for data analysis), its Implementation should be kept as slim as possible in order to simplify the process. For example, to keep the measurement matrix as small as possible, the size of the inclusion can be kept constant, selected as the smallest volume defined by the clinical protocol. Hence, most probably, not all these dependences will be investigated every time the Protocol will be implemented, but just the most significant ones. In particular, considering that operation *in-silico* are much easier, investigation of some of these dependences only with simulations can be considered.

The detectability of the inclusion is not only dependent on the relative change on N_0, since a small change over this can be hidden by random fluctuations. In particular, single-photon counting is a statistical process dominated by Poisson noise, where the intrinsic fluctuation over N_0 is given by the square root of N_0. By computing the theoretical $CV = \sigma_P(N_0) / \langle N_0 \rangle$ of repeated measurements over N_0, (where $\sigma(N_0)$ is the theoretical standard deviation of N_0 due to the Poisson statistics, i.e. the square root of N_0, and $\langle N_0 \rangle$ is the average value of N_0 over different repetitions) it is possible to verify that at least 10k photons are needed to keep the fluctuation due to Poisson noise below 1%. Moreover, real systems usually have some additional contributions of random fluctuations due to e.g. variability in the laser pulse shape or other instabilities. Hence, fluctuations can become very important, in particular when the amount of signal is low. To this purpose, an additional figure of merit has been identified, i.e. the *Contrast-to-Noise Ratio* (*CNR*), defined as:

$$CNR = \frac{N_P - N_0}{\sigma(N_0)}$$

where essentially the detected variation in the number of counts between the perturbed and unperturbed states (numerator) is compared with the fluctuation of the number of counts (denominator) computed as the standard deviation of a series of repeated measurements on the unperturbed case. *CNR* should be greater than 1 in order to be able to detect the inclusion (i.e. the information overcomes the noise). Being defined with the absolute *Contrast* at numerator, it is affected by all the dependences listed above for C, while, since the Poisson noise depends on the number of photons acquired, *CNR* also depends on the acquisition time (T_{acq}) and on the power injected into the tissue (*P*).

2) *Localization*

While in a pure DOT system the problem of localization is addressed thanks to the multiple light injection and collection points, solving then the inverse problem for retrieving tomographic information, within SOLUS this task is mainly ascribed to US imaging, thus increasing the spatial resolution and potentially improving the quantification capability of DOT. However, there is the need to quantify the performance of the segmentation algorithm used to extract information from B-mode US data. Additionally, here the scope is to provide a protocol that can be exploited also for the performance assessment of pure DOT systems, where the localization problem is usually a relevant issue.

The problem of localization can be addressed by quantifying the system precision in providing both position and size of the perturbation. It is therefore possible to assess these performances by computing: *2a) Displacement* and *2b) Broadening* of the reconstructed perturbation.

The *Displacement* error along the *i*-th dimension (ε_{x_i}) can be computed as:

$$\varepsilon_{x_i} = \tilde{x_i} - x_i$$

where $\tilde{x_i}$ is the retrieved position of the perturbation, while x_i is the nominal position. This error can be computed along the three dimensions: $x_i = x, y, z$.

The *Broadening* along the *i*-th dimension (σ_{x_i}) can be computed as:

$$\sigma_{x_i} = RMS(\tilde{x_i}) - \Delta x_i$$

where $RMS(\tilde{x_i})$ is the root mean square width of the reconstructed perturbation along the *i*-th dimension, while Δx_i is the nominal size of the inclusion in the same dimension. Again, this figure of merit can be computed along $x_i = x, y, z$.

As for the sensitivity, also here the analysis, geometrical and optical factors affecting the localization problem can be investigated. In particular, it is expected that a pure DOT system will be affected by the nominal position of the perturbation with respect to the center of the probe (x_P, y_P, z_P) and by μ_a^{Bulk} and $\mu_s'^{Bulk}$. On the contrary, when the DOT system is combined with another technique that can provide the morphology information (e.g. US), a much lower *Displacement* and *Broadening* is expected, with negligible dependence on geometrical and optical factors as long as the perturbation lays in the field of view of the US probe.

3) *Quantification*

Generally speaking, the "quantification" capability of the system is the main concern in SOLUS. Indeed, the aim here is to improve the discrimination of breast lesions that, on a previous US investigation, appear borderline between benign and malignant. To allow this, the key parameter is the ability of DOT to provide the composition of the tissue inside the lesion. In terms of performance assessment on phantoms, a precise characterization of the tissue constituents inside the lesion implies (among other requirements) a good quantification capability of optical properties at each wavelength inside the lesion, independently of the surrounding tissue properties.

Also the quantification problem can be divided into two assays, which are: *3a) Accuracy* and *3b) Linearity*.

Accuracy is defined as the system capability to retrieve values of optical properties inside the perturbation/lesion ($\tilde{\mu}_a^{Pert}$, $\tilde{\mu}_s'^{Pert}$) as close as possible to the true values (μ_a^{Pert}, $\mu_s'^{Pert}$). This figure of merit can be quantified by the definition of a relative error in the retrieved value for both the absorption (ε_a) and reduced scattering (ε_s) coefficients:

$$\varepsilon_a = \frac{\tilde{\mu}_a^{Pert} - \mu_a^{Pert}}{\mu_a^{Pert}}; \qquad \varepsilon_s = \frac{\tilde{\mu}_s'^{Pert} - \mu_s'^{Pert}}{\mu_s'^{Pert}}$$

Accuracy is relevant when absolute measurements are needed. However, *Linearity* of the measurement when changing μ_a^{Pert} and $\mu_s'^{Pert}$ can be more important in the scope of the SOLUS project. Indeed, the system capability to follow changes without distortions is crucial for spectroscopy to ensure that the shape of the absorption spectrum is preserved, thus allowing a correct estimation of the relative concentration of the main constituents.

As defined in the MEDPHOT Protocol, a *Linearity* assay can be performed by measuring a set of phantoms combining N values for μ_a^{Pert} with M values for $\mu_s'^{Pert}$. If both are taken as measurands, four *Linearity* plots can be drawn, showing both the *Linearity* to μ_a^{Pert} and $\mu_s'^{Pert}$ changes, as well as possible couplings between the two retrieved values. Similar to what proposed in [6], if a sufficient number of points are available in the plots, *Linearity* curves can be fitted using a second order polynomial and evaluating the non-linear term. Hence, for example on $\tilde{\mu}_a^{Pert}$, using:

$$y = ax^2 + bx + c$$

where $y = \frac{\tilde{\mu}_a^{Pert}}{\gamma}$, $x = \frac{\mu_a^{Pert}}{\gamma}$, and γ = 0.1 cm^{-1} is used to have dimensionless coefficients. The a and b values are the fitting parameters. These coefficients can be used to compute the slope of the curve (i.e. $SL = \frac{\delta y}{\delta x} = 2ax + b$) at the desired x, and the fractional deviation from *Linearity* behaviour (i.e. $NL = \frac{2a}{b}\Delta x$) over the desired range of Δx (e.g. Δx = 1 if considering an absorption change of 0.1 cm^{-1}). It is therefore desirable to obtain a b value as close as possible to 1 to obtain the correct variation in $\tilde{\mu}_a^{Pert}$ with μ_a^{Pert}. Of course, if the *NL* is high, the *SL* will highly depend on the actual x value. A low b combined with *NL* ~ 0 indicates that the system is linear, but the absorption increase in underestimated. For b ~ 0 with *NL* ~ 0, the system is linear, but absorption variations cannot be detected. The c coefficient can only affect the accuracy. Hence, c = 0, combined with b = 1 and a = 0 means perfect accuracy.

As for previous assays, again there are different factors that can potentially affect the quantification capability. In particular, it can depend on x_P, y_P and z_P with respect to the centre of the probe, on the amount of the perturbation with respect to the bulk properties ($\Delta\mu_a$, $\Delta\mu_s'$) and on μ_a^{Bulk} and $\mu_s'^{Bulk}$. Also, it will be heavily dependent on the reconstruction strategy, and in particular on the a-priori information about size and position provided by the US and on the regularization parameters. A critical aspect will also be the definition of $\tilde{\mu}_a^{Pert}$. Since DOT provides a 3D map of μ_a, it is necessary to define how to extract a single number ($\tilde{\mu}_a^{Pert}$) out of the spatial distribution. This can be for instance, the maximum or the median value over the region of interest and is somehow incorporated in the reconstruction model. Therefore, the Protocol will assess the overall performances of the system (hardware + analysis software) since this is eventually the only needed information for the clinician.

4. Conclusions

In this document, we reported the 3 Protocols previously defined and already widely adopted in the scientific community for the performance assessment of diffuse optical instruments, which are: 1) BIP, 2) MEDPHOT and 3) NEUROPT Protocols. They will be used during the project to evaluate newly developed optode components, sub-systems and the final single-optode system.

Additionally, in agreement with the description of action, here we reported the definition of a Protocol which is suitable for performance assessment of both pure DOT systems and combined DOT-US systems. Three main assays have been identified, which are relevant in the SOLUS project: *1) Sensitivity* (i.e. the capability to detect a small optical perturbation buried inside a diffusive medium); *2) Localization* (i.e. the capability of a system to correctly retrieve the position and dimension of a perturbation inside a diffusive medium); *3) Quantification* (i.e. the capability to quantify optical properties of a localized perturbation inside the scattering medium). Each of these parameters is quantified through some figures of merit, for a total of 6 tests.

The Implementation of the Protocol will be defined in different project tasks and different stages of the project depending on the specific needs. In particular, the final standardized Implementation will depend on the phantom kit that will be delivered at M18 (D4.4 Provision of the multi-modal phantom kit). However, this document provides an important support to WP2 for the performance of simulated tomographic reconstructions and to WP4 itself for the evaluation of preliminary phantom measurements.

In conclusion, up to now WP4 has met all the goals in timely manner.

5. References

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